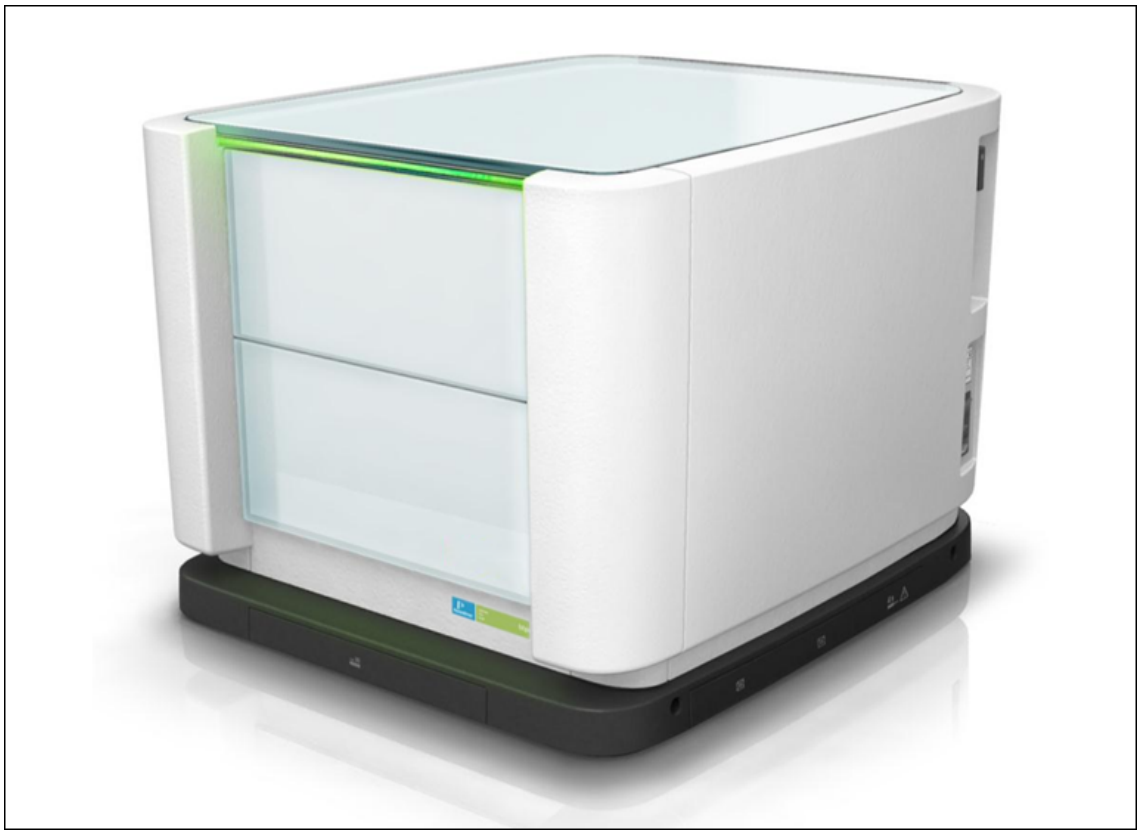
MULTIMODE DETECTION

EnSight™



User Manual

PerkinElmer®
For the Better

Release History

Revision	Publication Date
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Documented software version: Kaleido 3.0

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Contents

1 Safety Symbols

The following signal words are used in this manual:

Danger!

Indicates a hazardous situation which, if not avoided, can result in death or irreversible injury.

Warning!

Indicates a hazardous situation which, if not avoided, can result in severe but normally reversible injury.

Caution!

Indicates a hazardous situation which, if not avoided, can result in pain or minor injury.

Notice

Failure to observe may result in invalid measurement results or damage of the instrument.

Specific symbols are used which show you the type of hazard:

Biohazard!

Risk of crushing!

Failure to observe may result in injury or damage to the system.

Concentrated laser beam!

Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Optical radiation!

Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Signal words used to warn about optical radiation have a slightly different definition (according to IEC 62471):

- **Caution:** Does not pose a hazard due to aversion response to bright light or thermal discomfort.
- **Warning:** Hazardous even for momentary exposure.

Electrical shock!

Direct and indirect electrical contact.

Hot surface!

Touching these elements could burn your fingers.

2 Safety Instructions

2.1 Usage of the EnSight system

Caution!

Use only as intended.

- *The EnSight system, including the original accessories may only be used in conjunction with the methods described in the provided manuals. PerkinElmer does not assume any liability for any other applications or procedures, including use of individual subassemblies or components for other purposes.*
- *The manufacturer does not assume any liability for any other kind of application, including individual subassemblies and the addition of individual components. This exclusion of liability also applies to all service or repair work which was not carried out by authorized PerkinElmer service personnel.*

For research use only. Not for clinical diagnostic use.

The EnSight is an automated multimode plate reader for use in biological and pre-clinical research only. The EnSight is particularly suited for cellular and biochemical research applications.

2.2 Power Requirements

- Mains voltage: 100-240 V, 50/60 Hz
- Power consumption: Max. 300 VA

2.3 Operating and Maintenance Staff

- The EnSight may only be operated by qualified staff who have been specially trained and are familiar with the contents of this manual and the operating instructions of the device.
- The system must only be operated when it is in a fully assembled and installed condition.
- Unauthorized intervention or tampering inside the machine is prohibited.
- Corrective maintenance work and service may only be performed by the PerkinElmer Service. Operational and maintenance procedures described in the provided manuals are excepted and may be carried out by the user.
- Additional safety instructions and warnings must be observed by service personnel during maintenance or repair. Such information is *not* part of this manual.
- The manufacturer can accept no liability for this product if the provisions of this documentation are not complied with.

2.4 Environmental Conditions

The instrument has been designed to be safe under the following conditions:

- Indoor use only
- Altitude: up to 2000 m
- Operating conditions:
  +15 °C to +30 °C, relative humidity 10 - 80%
- Operating conditions for Alpha technology:
  +20°C to +25°C, relative humidity < 80%
- Operating conditions for Label-free technology:
  23 °C ± 3 °C, relative humidity < 70 %, non-condensing
- Installation category (overvoltage category): II, according to IEC 60664-1 (see note)
- Pollution degree: 2 according to IEC 60664-1 (see note)

Notice

- ***Installation category*** *(overvoltage category) defines the level of transient overvoltage which the instrument is designed to withstand safely. It depends on the nature of the electricity supply and its overvoltage protection means. For example in CAT II which is the category used for instruments in installations supplied from supply comparable to public mains such as hospital and research laboratories and most industrial laboratories the expected transient overvoltage is 2500 V for a 230 V supply and 1500 V for a 120 V supply.*
- ***Pollution degree*** *describes the amount of conductive pollution present in the operating environment. Pollution degree 2 assumes that normally only non-conductive pollution such as dust occurs with the exception of occasional conductivity caused by condensation.*
- *Both of these affect the dimensioning of the electrical insulation within the instrument.*

2.5 Indication of Hazards and Danger Zones

Before operating the EnSight system for the first time please read this manual carefully to avoid incorrect operation of the EnSight system.

2.5.1 Mechanical Hazard

2.5.1.1 General

Caution!

Parts inside the instrument can cause hand injury.

- *Do not reach into the instrument through the plate loading door when it is open.*

Caution!

The glass plate on top of the instrument is not fixed. If the glass plate is lifted, an interlock mechanism will switch off all light sources (except light source for Label-free technology) and disable the upper measure head mover. Running measurements will be canceled and the instrument needs to be initialized.

- *Do not remove the glass plate on top of the instrument. User access to this area is not required.*

2.5.1.2 Optional Stacker

Caution! (For stacker option only)

Moving parts within the sample loading area: The plate lift and the rods in the stacker will move up during operation and initialization and can cause hand injuries.

- *Keep your hands away from the sample loading area.*
- *The magazines have to be removed for loading/unloading plates.*
- *Please note that the stacker mechanics will shortly move up and down during initialization even if the magazine table is not installed and the stacker is not used.*

Please see section 4.13 “Stacker”, page 58 for detailed instructions.

2.5.2 Laser Radiation

Danger!

Use of this instrument other than specified in the user instructions may result in exposure to hazardous laser radiation.

Classified according to standard IEC 60825-1:2014.

With Alpha technology only:

Danger!

Laser radiation – Eye injury

Class 3B laser radiation may be present inside the EnSight's protective housing during operation. Since the instrument's lid and loading door are protected by laser safety interlocks, no harmful radiation will become accessible when they are opened.

- *Nominal wavelength: 680 nm (red)*
  Nominal output power: 400 mW
- *Do not defeat the safety interlocks!*
- *Do not remove any housing components!*
- *AVOID DIRECT EXPOSURE TO BEAM!*
- *Service tasks that require access to the instrument while the interlocks are defeated may only be carried out by qualified service personnel.*

With Label-free technology only:

Danger!

Invisible laser radiation – Eye injury

Invisible Class 3R laser radiation may be available behind the plate loading door. The light source of the Lable-free module is not blocked by the interlock system if the plate door is opened. Insertion of reflecting parts may result in laser radiation to be reflected out of the instrument.

- *Nominal wavelength: 832 nm (infrared)*
  Nominal output power: 0.6 mW
  Max. output power: 3 mW (in case of error)
- *Do not insert any reflecting tools through the plate door!*
- *Do not remove any housing components!*
- *AVOID DIRECT EXPOSURE TO BEAM!*
- *Service tasks may only be carried out by qualified service personnel.*

With Imaging technology only:

Danger!

Invisible laser radiation – Eye injury

Invisible Class 3B laser radiation may be present inside the EnSight's protective housing during operation. Since the instrument's lid and loading door are protected by laser safety interlocks, no harmful radiation will become accessible when they are opened.

- *Nominal wavelength: 850 nm (invisible)*
  Nominal output power: 10 mW
  Max. output power: 50 mW (in case of error)
- *Do not defeat the safety interlocks!*
- *Do not remove any housing components!*
- *AVOID DIRECT EXPOSURE TO BEAM!*
- *Service tasks that require access to the instrument while the interlocks are defeated may only be carried out by qualified service personnel.*

2.5.3 Optical Radiation

Xenon flash lamp:

Visible and invisible optical radiation – Risk of eye injury

Visible and invisible optical radiation (see below for details) may be present inside the EnSight's protective housing during operation. Since the instrument's lid and loading door are protected by safety interlocks, no harmful radiation will become accessible when they are opened.

- *Do not defeat the safety interlocks!*
- *Do not remove any housing components!*
- *Service tasks that require access to the instrument while the interlocks are defeated may only be carried out by qualified service personnel.*

Warning!
Actinic UV hazard, 180 - 400 nm

Risk Group 3, High-Risk. Hazardous even for momentary exposure.

- *Avoid eye and skin exposure to the light.*

Caution!
Blue Light Radiance, 300 - 700 nm

Risk Group 2, Moderate-Risk. Does not pose a hazard due to aversion response to bright light or thermal discomfort.

- *Do not stare into the light, may be harmful to the eye.*

Warning!
Retinal Thermal Hazard, 380 - 1400 nm

Risk Group 2, Moderate-Risk. Does not pose a hazard due to aversion response to bright light or thermal discomfort.

- *Do not stare into the light, may be harmful to the eye.*

Warning!
IR Radiation, 780 - 3000 nm

Risk Group 2, Moderate-Risk. Does not pose a hazard due to aversion response to bright light or thermal discomfort.

- *Do not stare into the light, may be harmful to the eye.*

With Imaging technology only:

Visible and invisible LED radiation – Risk of eye injury

Visible and invisible optical radiation (see below for details) may be present inside the EnSight's protective housing during operation. Since the instrument's lid and loading door are protected by safety interlocks, no harmful radiation will become accessible when they are opened.

- *Do not defeat the safety interlocks!*
- *Do not remove any housing components!*
- *Service tasks that require access to the instrument while the interlocks are defeated may only be carried out by qualified service personnel.*

Warning!
UVA hazard, 315 - 400 nm

Risk Group 3, High-Risk. Hazardous even for momentary exposure.

- *Avoid eye and skin exposure to the light.*

Caution!
Blue Light Radiance, 300 - 700 nm

Risk Group 2, Moderate-Risk. Does not pose a hazard due to aversion response to bright light or thermal discomfort.

- *Do not stare into the light, may be harmful to the eye.*

Warning!
Retinal Thermal Hazard, 380 - 1400 nm

Risk Group 3, High-Risk. Hazardous even for momentary exposure.

- *Do not look at the light, eye injury may result.*

2.5.4 Electric Shock

2.5.4.1 General

The EnSight operates with up to 240 V AC. Highly dangerous electric voltages occur in a number of places throughout the system.

Danger!

Direct electrical contact – Electrical shock.

- *Use only the provided power cable which has a mains plug with earthing contact. The mains plug must only be inserted in a socket outlet with earthing contact.*
- *No user serviceable parts inside the housing. Service has to be carried out by qualified PerkinElmer service personnel only!*
- *Turn main power off and unplug from mains for a complete disconnection.*
- *Check the power cable for damages before switching on the EnSight. Do not use the instrument with damaged power cable.*
- *In case of failure pull out the power cable at the instrument to disconnect it from the mains immediately. Easy access to that location must be ensured at all times.*
- *In case of a damaged power cable contact PerkinElmer to request a new cable.*

2.5.4.2 Replacing a Fuse

If one of the two fuses of the EnSight has blown, it can be exchanged by the user.

Danger!

Direct electrical contact – Electrical shock.

- *Unplug from mains before replacing fuses.*
- *For continued protection against risk of fire, replace only with certified fuse of same type and rating: T4.0 H / 250 V (T = time delay; 4.0 = 4 Ampere; H = high breaking capacity).*

Please see section 6.3 “Replacing a Fuse”, page 259 for detailed instructions.

2.5.5 Hot Surface

Caution!

The heating elements inside the instrument can be hot.

- *Do not reach into the instrument through the plate loading door when it is open.*
- *Do not touch any elements of the sample chamber.*

2.5.6 Spilling of Liquids

Danger!

Direct electrical contact - Electrical shock.

- *Do not handle large amounts of liquids near or above the EnSight.*
- *If liquids should be spilled into the instrument accidentally, switch off the EnSight immediately, i.e. unplug the power cable.*
- *Contact PerkinElmer Service to let them check the instrument and repair the damages, if necessary.*
- *Service and repair may be carried out by qualified PerkinElmer service personnel only!*

2.5.7 Cleaning

Warning!

Ethanol or other cleaning solvents may cause fire or injury if used in great amounts.

- *Observe the cleaning instructions.*
- *Keep the concentration of dangerous solvents as low as possible.*
- *Do not use organic solvents.*
- *Ensure the area is well ventilated.*

Please see section 6.2 “Cleaning”, page 258 for detailed cleaning instructions.

2.5.8 Process Liquids or Substances

Danger! Biohazard!

It is within the responsibility of the user to attach a biohazard label (included in delivery) as soon as potential infective substances are used.

If this instrument is to be operated in a Biosafety Level 3 or Level 4 environment, it is the responsibility of the user to establish adequate decontamination procedures for the instrument, as required for safe operation and maintenance of the BSL facility. PerkinElmer cannot guarantee the effectiveness of specific decontamination procedures established by the user, nor the long-term stability of the instrument against specific decontamination agents.

If you are interested in field service for an instrument installed in a Biosafety Level 3 or 4 environment, please contact your PerkinElmer representative to discuss service terms and conditions.

2.5.9 Ventilation

Caution!

Covered ventilation openings may cause malfunctions or damage to the equipment.

- *Make sure that none of the ventilation openings of the instrument (in the back and bottom) are obstructed.*
- *Keep a distance of at least 5 cm (2 in) to walls and other objects.*

The air filters of the instrument need to be checked regularly. See section 6.4 “Changing Air Filters”, page 260 for detailed instructions.

2.5.10 Transport

Warning!

The instrument is heavy (75 kg / 165 lb) and the glass plate at the top is not fixed.

- *At least two persons are required to transport the instrument.*
- *Do not try to lift or transport the instrument without all transport handles mounted correctly.*
- *All four transport handles have to be screwed in completely. Do not use inadequate force or tools for this manual process, otherwise the handle threads may be damaged.*
- *Keep the instrument in upright position, otherwise the glass plate could fall down.*
- *An instrument with optional stacker is heavier (+9 kg, without magazine table and magazines) and the instrument’s center of gravity is closer towards the front.*

Please see section 6.5 “Transport”, page 262 for detailed transport instructions.

2.5.11 Disposal

Caution!

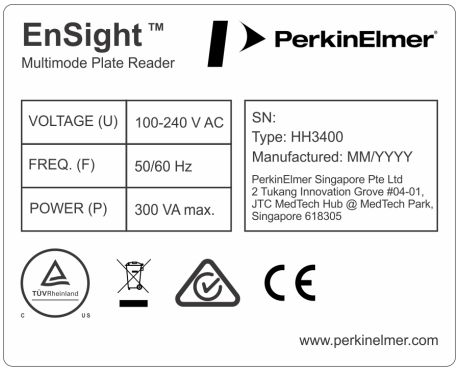
Device may be contaminated by hazardous substances.

- *Before disposal, ensure that the device is decontaminated.*

The user is responsible for protecting the environment against any hazardous substances used in the process, in particular the environmentally appropriate disposal of process residues. Relevant local regulations must be observed. See also section 9.2 “WEEE Instructions”, page 283.

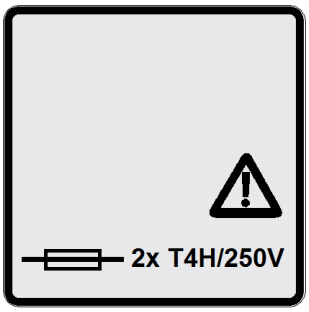
2.6 Location of Warning Signs and Labels

On the back of the instrument



Classified according to standard IEC 60825-1:2014.

On the side of the instrument



CAUTION:
For continued protection against risk of fire, replace only with same type and rating of fuse.

- 2x T4H/250V

Behind plate loading door (visible if plate door is opened)

On stacker magazine table (for optional stacker only)

DO NOT PUT YOUR FINGERS INTO THE SAMPLE LOADING AREA

3 Servicing Information

This chapter contains laser warnings and protective procedures which have to be observed by service personnel when opening the housing in case of service or repair (according to IEC 60825-1 {Ed3.0}, 8.2 b). This information is not intended for the user and it is not part of any user maintenance!

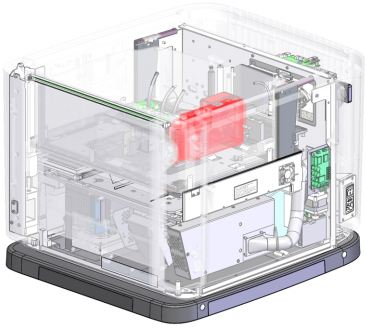
Caution!

There are no user-serviceable parts inside the instrument.

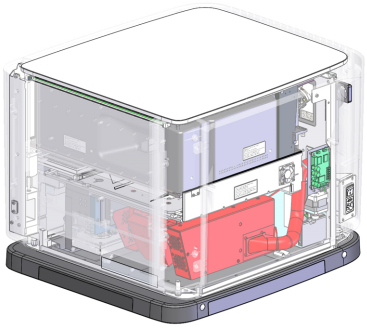
- *Repair and maintenance (excluding the user maintenance tasks described in chapter 2) may only be performed by specially trained and authorized persons (e.g. PerkinElmer Service).*

3.1 Laser Light Sources

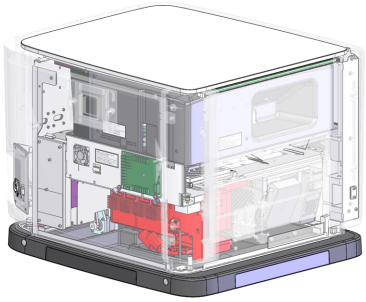
Positions of the laser light sources inside the instrument (highlighted in red). These components are only present if the corresponding technology option is installed.



Alpha laser



Label-free module



Imaging module

3.1.1 Alpha Laser

The Alpha laser belongs to Laser Class 3B. The Alpha laser is automatically disabled by the interlock system when the upper measure head moves into the open position or the plate door is open.

- There are no serviceable parts within the Alpha laser.
- There are no service adjustments for the Alpha laser except for the scan table position which is calibrated within the instrument, without access to laser radiation above Class 1.
- There are no service procedures for the Alpha laser other than the replacement of the complete unit.
- There are no precautions required, the Alpha laser is automatically disabled by the interlock system and there is no possible exposure to laser radiation above Class 1.
- There is no maintenance required to keep the product in compliance.
- There are no controls or procedures to increase accessible emission levels of radiation.
- There are no protective procedures required, because the AlphaLaser is automatically disabled.

3.1.2 Label-free Module

The Label-free module belongs to Laser Class 3R. If the module is installed in the instrument the laser radiation is not accessible and therefore the Label-free module is not controlled by the interlock system.

- There are no serviceable parts within the Label-free module.
- There are no service adjustments for the Label-free module except for the scan table position which is calibrated within the instrument, without access to laser radiation above Class 1.
- There are no service procedures for the Label-free module other than the replacement of the complete unit.
- As precaution to avoid possible exposure to laser radiation above Class 1 always turn off the instrument before removing or installing the Label-free module.
- There is no maintenance required to keep the product in compliance.
- There are no controls or procedures to increase accessible emission levels of radiation.

3.1.3 Imaging Module

The autofocus laser of the Imaging module belongs to Laser Class 3B. The laser and the LED light sources are automatically disabled by the interlock system when the upper measure head moves into the open position or the plate door is open.

- There is no maintenance required to keep the product in compliance.
- There are no controls or procedures to increase accessible emission levels of radiation above Class 3B.

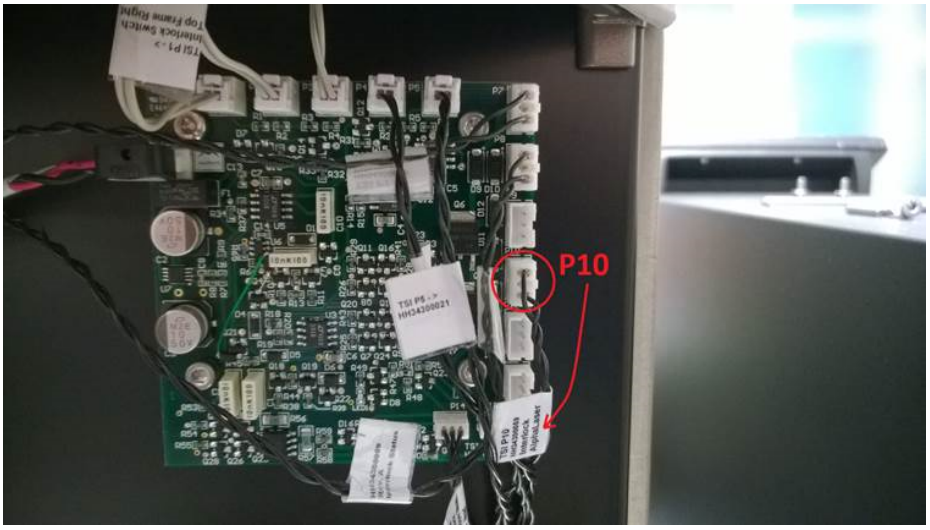
The following service procedures and service adjustments require access to the Imaging module with enabled laser sources:

Alignment of the Brightfield LED

1. Remove the side panel and the safety cover.
2. Close the plate door and move the upper measure head to close position to enable the light sources.

The autofocus laser is not accessible during this alignment. If the Alpha laser option is installed in the same instrument, dangerous laser radiation may be accessible after removing the safety cover if the fiber of the Alpha laser is damaged or broken. In order to avoid any hazard, the following precautions must be observed:

- Before removing the safety cover disable the Alpha laser by disconnecting the cable at the TSI-Board, P10, interlock Alpha laser (see photo).



- Reinstall the safety cover before you enable the Alpha laser.

Alignment of the Camera (in case of an exchange of the Camera)

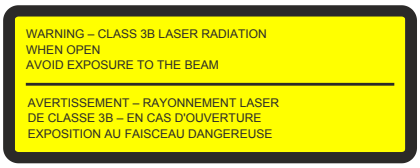
For the alignment of the camera the Imaging module has to be removed from the instrument and has to be used with enabled light sources outside of the protective housing of the instrument. During this operation dangerous laser radiation of the autofocus laser may be accessible through the microscope objective. To block this radiation a special target tool has been designed. In order to avoid any hazard, the following precautions must be observed:

- Turn off the power before removing the Imaging module from the instrument.
- Make sure that the target tool is undamaged (no broken glass etc.).
- Place the target tool over the microscope objective.
- Never operate the Imaging module with the light sources enabled outside the instrument without the target tool in place.

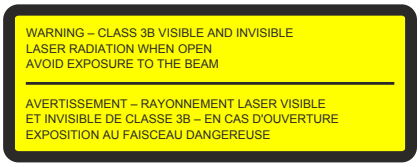
3.2 Internal Warning Labels

The following laser warning labels are placed inside the instrument if the corresponding option is installed. If the component or housing part bearing such a label is opened or removed, there will be access to laser radiation above Class 1. The exact nature of the hazard and corresponding instructions are stated on the warning label.

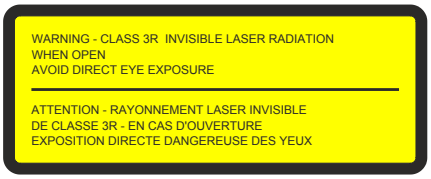
Label 1 (if the Alpha laser is installed)



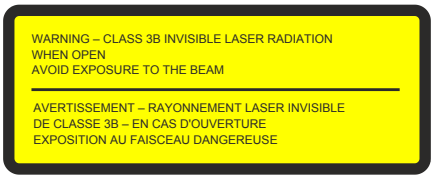
Label 2 (if Alpha laser, Imaging module or Label-free module are installed)



Label 3 (if Label-free module is installed)

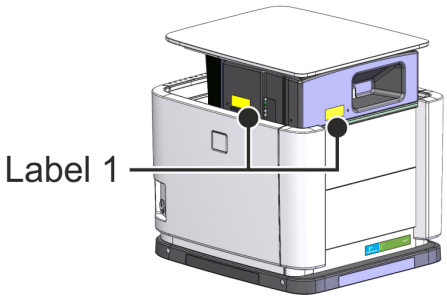


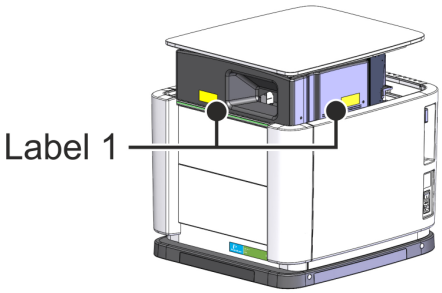
Label 4 (if Imaging module is installed)



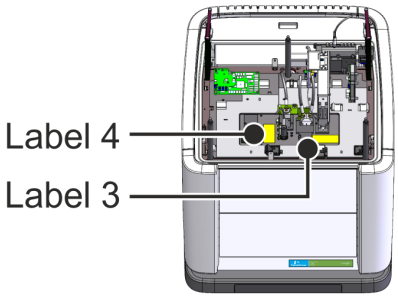
The exact label positions are shown in the following chapters. The numbers used in these views correspond to the label number (see list above).

3.2.1 Access from Above



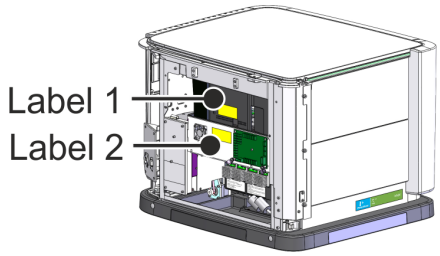


Upper measure head moved up

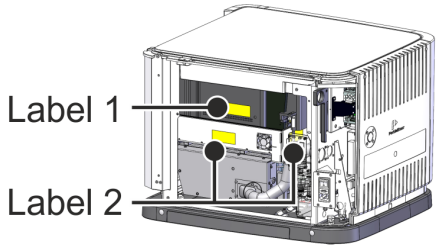


Safety cover removed

3.2.2 Removing Side Panels

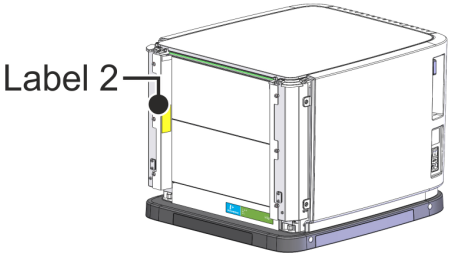


Left panel removed



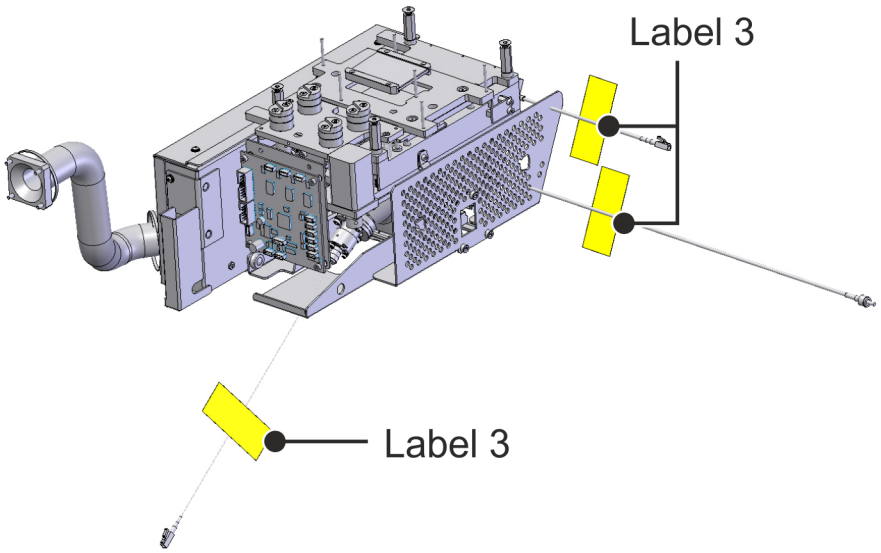
Right panel removed

3.2.3 Removing Plate Door Assembly



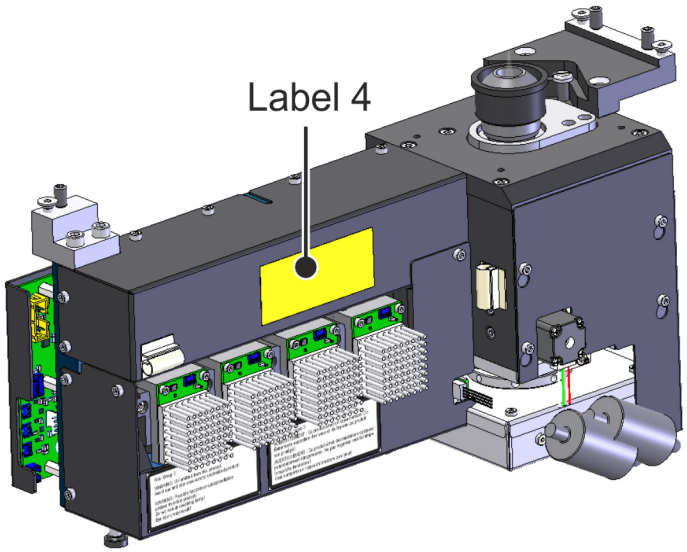
Housing corners removed

3.2.4 Label-free Module



Light guide fibers

3.2.5 Imaging Module



4 Instrument Description

The EnSight Multimode Plate Reader from PerkinElmer is an easy-to-use platform for quantitative detection of light-emitting or light-absorbing markers in research and drug discovery applications. EnSight consists of a base unit and optional modules according to customer needs. The measurement technologies are based on proven EnVision® and EnSpire® technology.

In the basic version, EnSight can read absorbance (filter-based). Optional technologies include all standard technologies such as monochromator-based absorbance, luminescence and monochromator-based fluorescence (top and bottom). Additionally, the instrument can be equipped with time-resolved fluorescence (TRF), laser-based Alpha, label-free and imaging technologies. It comes with modern control software Kaleido with data analysis capabilities. Detailed descriptions can be found in section 4.5 “Technologies”, page 34.

The technologies can be used in different measurement modes (see section 5.2.1.3 “Measurement Modes”, page 112) like single point, on-the-fly, kinetic, well area scan, wavelength scan (excitation/emission) etc., depending on the technology. Single point reading with extremely fast and accurate mechanical movement allows reading of plates with up to 384 wells.

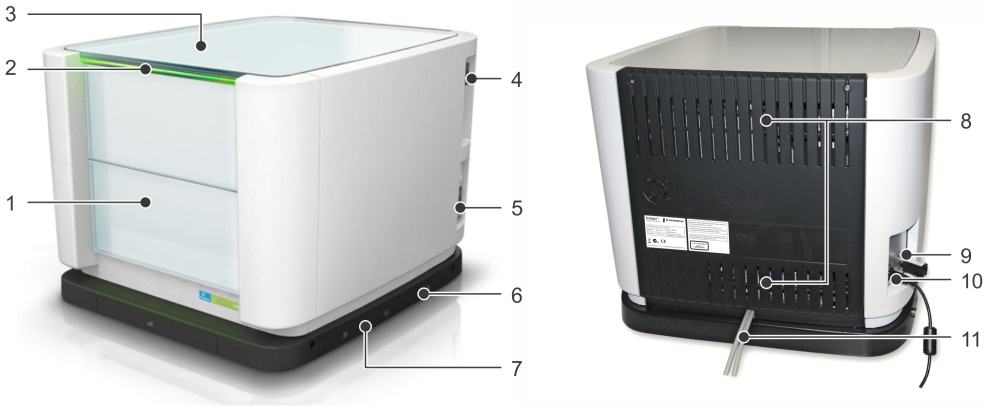
EnSight is a very compact, small footprint bench top unit with features such as temperature control, shaking, scanning and plate barcode reading. Barcodes can be read from the left side (or optionally from any of the four sides) of the plate. The optional stacker module allows you to measure multiple plates automatically.

The Kaleido software is easy to use and provides a clear view of all relevant information on the screen. Protocols and results are stored in a database. There is a protocol explorer for quick access and editing of protocols. Example protocols are included as a starting point for users to create their own application-specific protocols. Result files can be exported in multiple formats.

EnSight can be used independently as a stand-alone instrument and manually loaded system or as a robot-controlled subsystem in an automated laboratory. Different kinds of robots can be used as the main system in an automated laboratory. Data generated by EnSight can be transferred to other systems via network.

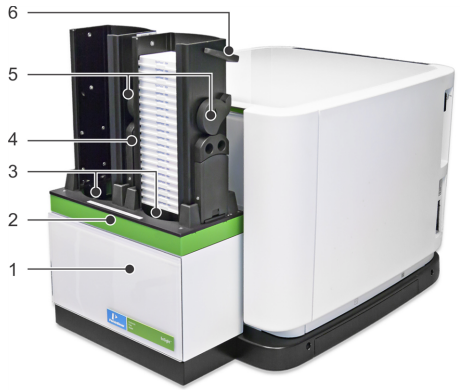
4.1 Overview

EnSight without stacker



1. Plate loading door
2. Status light
3. Upper measure head / glass plate
4. Filter wheel (excitation filters)
5. Power switch / fuses / mains connection
6. Storage for transport handles (behind cover)
7. Central air filters (behind cover in base plate)
8. Ventilation slots (rear air filters)
9. CAN bus
   CAN terminator must always be connected (unless the port is used for future upgrade components).
10. USB
    Connection to control PC
11. FireWire (with Imaging option only)
    Connection between camera and control PC

EnSight with optional stacker



1. Stacker main unit
2. Magazine table (removable)
3. Sample loading area
4. Magazines (removable)
   - Right position: input
   - Left position: output
5. Release mechanism
6. Handles

4.2 Status Light

The status light of the EnSight indicates the instrument status.

Signal	Description
Light Off	**Off / Service Mode** This signal is used for the following states: • Instrument is switched off. • Instrument is in service mode.
Green  	**Idle** This signal is used for the following states: • Instrument is idle and measurements can be started. • During general temperature adjustment (configured in general settings, not triggered by protocol). Measurements can be started, even if the temperature has not been reached yet.
Soft Pulsation of Green  	**Busy** The instrument is busy (in a correct and expected way). This signal is used for the following states: • Initialization of instrument • Load / unload plate • Scan filter wheel • Measurement running • During entire stacker run (also during plate handling) • Restack plates (stacker) • Unlock magazines (stacker) • Shut down instrument • Preparation for instrument transport. When this process is finished, the status light will change to orange (see below).
Orange  	**Alert / Error** The instrument is in alert/error condition, no protocol can be started. This signal is used for the following states: • Run of protocol stops during a measurement and instrument is in error condition. Depending on the error, a restart of software and instrument is necessary. This also contains *Fatal Execution Errors* especially caused by a stacker run. • Initialization of the instrument failed and instrument is in non-defined condition, restart of software and instrument is necessary. • Filter wheel holder is open or no filter wheel inserted. • Lid is open (glass plate of upper measure head). • Preparation for instrument transport is finished.

Signal	Description
Soft Pulsation of Orange  	**User Action Needed** The instrument is waiting for the user's action. This signal is used for the following states: • Plate carrier has been moved out for loading a plate. • The currently processed protocol has reached a delay operation (waiting time). Manual dispensing is possible now.

4.3 Plate Loading Door

The plate door is software-controlled. Please use the **Load/Eject** function to insert or remove a sample plate (see section 5.1.5 “Load/Eject”, page 66). As soon as the door is opened, all light sources (except Lable-free light source) are switched off immediately by safety interlocks. Furthermore, the upper measure head mover is disabled.

For loading plates using the optional stacker please see section 4.13 “Stacker”, page 58.

Caution!

Parts inside the instrument can cause hand injury.

- *Do not reach into the instrument through the plate loading door when it is open.*

Caution!

The heating elements inside the instrument can be hot.

- *Do not reach into the instrument through the plate loading door when it is open.*
- *Do not touch any elements of the sample chamber.*

Only with Label-free technology:

Danger!

Invisible laser radiation – Eye injury

Invisible Class 3R laser radiation may be available behind the plate loading door. The light source of the Lable-free module is not blocked by the interlock system if the plate door is opened. Insertion of reflecting parts may result in laser radiation to be reflected out of the instrument.

- *Nominal wavelength: 832 nm (infrared)*
  Nominal output power: 0.6 mW
  Max. output power: 3 mW (in case of error)
- *Do not insert any reflecting tools through the plate door!*
- *Do not remove any housing components!*
- *AVOID DIRECT EXPOSURE TO BEAM!*
- *Service tasks may only be carried out by qualified service personnel.*

4.4 Upper Measure Head

Caution!

The glass plate on top of the instrument is not fixed. If the glass plate is lifted, an interlock mechanism will switch off all light sources (except light source for Label-free technology) and disable the upper measure head mover. Running measurements will be canceled and the instrument needs to be initialized.

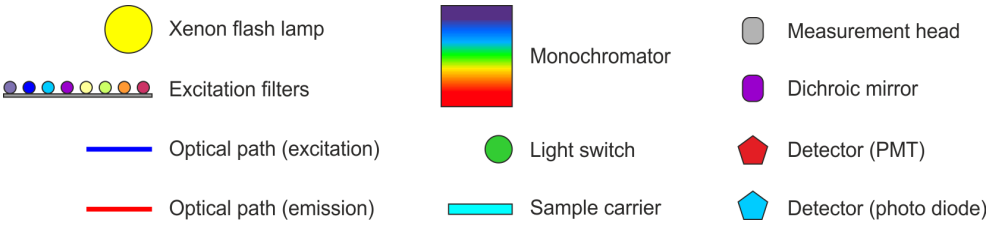
- *Do not remove the glass plate on top of the instrument. User access to this area is not required.*

The glass plate on top of the instrument covers the upper measure head. It holds optical and electrical components for the measurement technologies operating from above the sample plate. The measure head is software-controlled and can be moved in vertical direction to adjust the desired measurement height.

4.5 Technologies

EnSight offers up to seven different measurement technologies. The technologies are described in more detail in the following sections.

The following symbols are used to illustrate the optical setups:



4.5.1 Fluorescence Intensity With Quad-Monochromator

The light source is a Xenon flash lamp. The polychromatic light is directed into the excitation monochromator unit where there are two diffraction gratings. These separate the incident polychromatic beam into its constituent wavelength components, sending each wavelength in a different direction so that a narrow band of wavelengths can be collected. Wavelength selection is performed by turning a diffraction grating to the desired position with a stepper motor. The excitation wavelength is selectable from 230 nm to 835 nm. The excitation light is then directed into the sample.

The fluorescence from the sample enters the emission monochromator. This has a similar structure to that of the excitation monochromator module. The emission wavelength is selectable from 245 nm to 850 nm.

Although monochromators relieve you of the need to have filters for every label, a broad waveband cut-off filter is still required in order to block harmonic multiple orders of the wavelength chosen. Three cut-off filters cover the entire range of wavelengths supported by the instrument. The software automatically ensures that the correct filter is used for each wavelength.

The light from the monochromator passes through the appropriate cut-off filter on the filter wheel. A side-on photomultiplier tube is used as a detector and it is located so as to maximize the efficiency. The detector is used in gated analog mode with optimized gain (high voltage) setting.

A signal from a reference photodiode is always read after every flash. The reference signal is then compared to the original reference value and the results are corrected for the same excitation energy. See also section 5.14.4 “Reference value of Xenon flash lamp”, page 252.

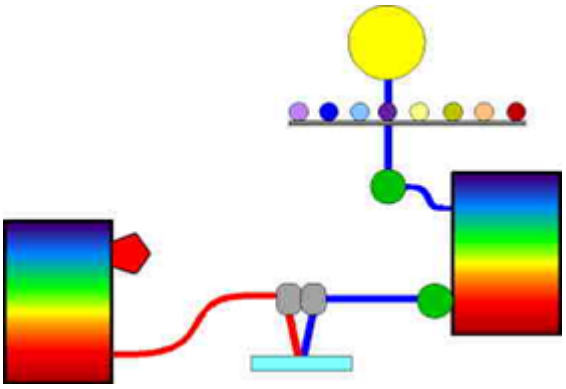
Fluorescence intensity readings can be taken from above or below the plate:

- **FI Top:** Excitation and emission from **above** the plate; most efficient way when no seal is used because no plastic surface has to be penetrated.

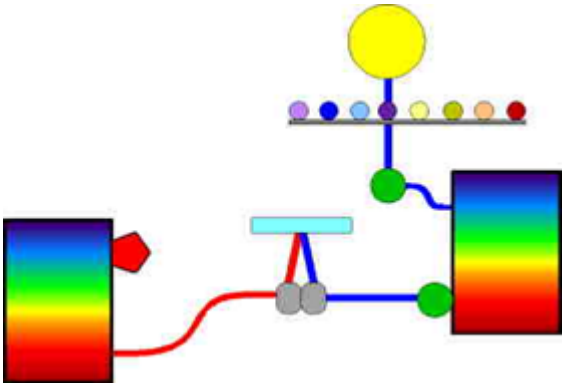
- **FI Bottom:** Excitation and emission from **below** the plate (requires optional detector below plate); for adherent cells and lidded plates, reading from below provides superior efficiency.

Switching between reading from above to reading from below and vice versa is controlled via the software and both FI Top and FI Bottom can be used in the same run.

Note: The symbols are explained in a legend in section 4.5 “Technologies”, page 34.



FI measurement from the top with the quad-monochromator



FI measurement from the bottom with the quad-monochromator

The FI measure head consists of two channels, one for the excitation light and another for the emission light. This measurement head base can be moved up and down in order to set the focus height for FI Top excitation and emission. For FI Bottom the focus height is fix.

4.5.2 Absorbance Technology

4.5.2.1 Double-Monochromator-Based

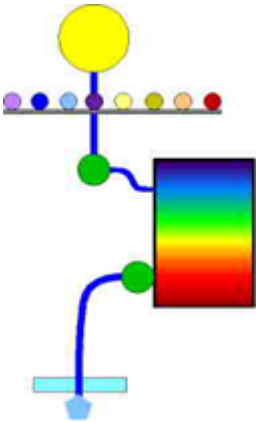
For absorbance measurements (230-1000 nm) the same Xenon light source is used as for FI measurements.

Using the monochromator option, light passing into the sample comes from the excitation double monochromator.

The light is directed from the top and measurement made from below.

The intensity of the light directed through the sample is measured using a reference photodiode. The light is then focused into the sample. The focal plane is the same as for FI measurements.

Note: The symbols are explained in a legend in section 4.5 “Technologies”, page 34.



Absorbance measurement with the double-monochromator

The Absorbance measurement head focuses the excitation light into the sample. This measurement head, can be moved up and down controlled by the software in order to set the focus height inside the sample.

The intensity of the light is first measured without the sample (I_0) and then the samples in one plate are measured.

The light intensity is measured by a photodiode placed at an optimal position directly below the plate. The light path for absorbance measurements is thus different than for FI measurements.

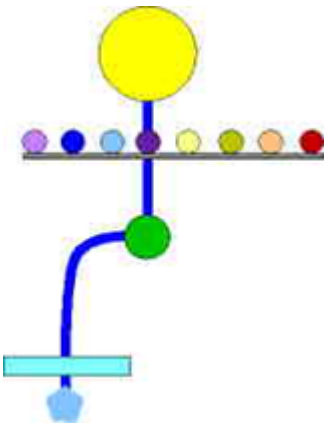
The absorbance value is calculated by the equation

$$A = -\log (I/I_0)$$

where I_0 is the light intensity without any sample and I is the intensity after an absorbance.

4.5.2.2 Filter-Based

Note: The symbols are explained in a legend in section 4.5 “Technologies”, page 34.



Absorbance measurement using filters

The same excitation light source is used as for the monochromator model. The wavelength of the light is selected by an optical filter placed in the filter wheel and it can be in the range 230-1000 nm. Several absorbance filters are available.

The light intensity is measured by a photodiode.

The intensity of the light is first measured without any sample (I_0) and with the samples in the light path.

The absorbance value is calculated by the equation

$$A = -log\ (I/I_0)$$

where I_0 is the light intensity without any sample and I is the intensity after an absorbance.

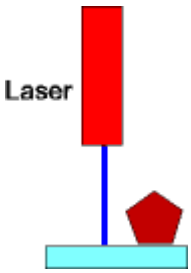
4.5.3 Alpha Technology

The Alpha technology enables very high sensitivity measurements with very low background and very high signal to background ratio. The Alpha technology uses a semiconductor laser to excite the sample. This produces high optical power at 680 nm.

The Alpha technology donor beads are excited by the laser beam. A photosensitizer in the donor bead converts ambient oxygen to a more excited singlet state. These oxygen molecules diffuse to the bound acceptor bead where they react with a thioxene derivative generating chemiluminescence at 370 nm. This activates fluorophores in the bead which emit fluorescence light in the range 450 to 645 nm. The long half-life of the signal permits the measurement to be time-resolved to reduce the contribution of background fluorescence. The fact that the excitation wavelength is longer than the emission, further reduces the background, as does the fact that wavelength itself is long.

The detector is a very high sensitivity photomultiplier (PMT). The PMT is located right above the sample and reads the well adjacent to the one excited by the laser. Light passes through an aperture and into the detector. This aperture is fixed in size.

Note: The symbols are explained in a legend in section 4.5 “Technologies”, page 34.



Alpha technology measurement with laser excitation from above and adjacent well reading from above.

Both excitation and emission occur from the top of the sample.

Only one measurement per well is recommended because the sample is partially bleached by the excitation light.

Depending on the assay and type of sample carrier cross talk effects (cross excitation and bleaching of adjacent samples) can become an issue. We recommend to use Alpha Plates to avoid these effects and thus improve the signal/background properties of your assay.

4.5.4 Luminescence Technology

Note: The symbols are explained in a legend in section 4.5 “Technologies”, page 34.

Luminescence measurement

Luminescence uses a very high sensitivity luminescence PMT as detector. It has extremely low background, high dynamic range and spectral response from 450 nm up to 645 nm. The emission light is collected directly from the top of the well in order to maximize the efficiency.

The detector can be lowered so that it is just above the plate, thus reducing the crosstalk between wells. The detector has an aperture to define the area of the plate it can view. There is one aperture size: for 384 well plates but which can be used with 96 well plates as well. The aperture is optimized to give the highest possible signal and minimize crosstalk between wells for 384 well plates.

There is a sensor which allows automatic precise plate height determination to allow the aperture to come very close to the plate without touching it.

4.5.5 Label-free Technology

In Label-free mode, the established Corning® Epic® label-free technology can be used to characterize cellular signaling mechanisms and to understand the complexity of multiple signaling pathways. In addition, the technology can also be used to study biochemical interactions. Label-free detection delivers a unique orthogonal perspective, providing information about both cellular and biochemical assays,

pathway unbiased analysis of both endogenously and recombinantly expressed targets, non-invasive, more physiologically relevant data, and the ability to study difficult targets (e.g. Gi-coupled receptors) or weak biological interactions.

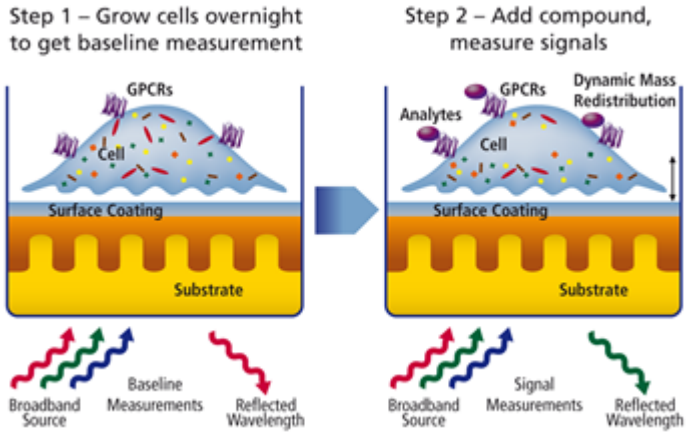
Notice

For dedicated purposes application-oriented documentation is available. Please contact your PerkinElmer specialist for more information.

Application and Technical Notes are published on our web page on a regular basis. Please visit www.perkinelmer.com/ensight.

Cell-based Assays

Cellular assay label-free microplates offer flexibility for many cell types including adherent and suspension cells, mammalian and primary cells.



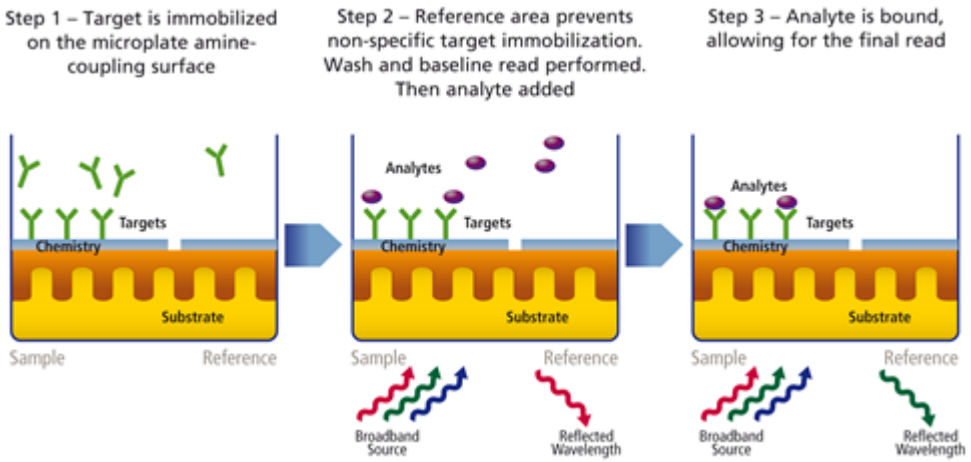
Label-free cell-based assay

Label-free technology measures changes in light refraction resulting from dynamic mass redistribution (DMR) within the cell. This occurs in response to receptor activation or deactivation in a zone within the cell's monolayer. The change is indicated by a change in wavelength.

Biochemical Assays

Biochemical assay label-free microplates incorporate patented dual-sensor self-referencing technology for protein/ligand assays, ensuring that only one true analyte binding is reported.

1. Target is immobilized on the microplate amine-coupling surface.
2. Reference area prevents non-specific target immobilization. Then washing is performed.
3. Analyte is bound, allowing for the final read.



Label-free biochemical assay

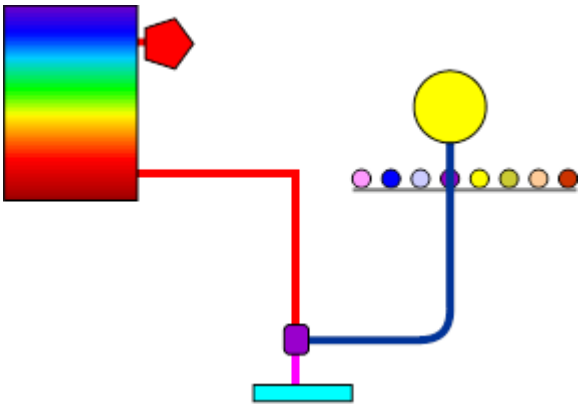
Label-free biochemical assays measure changes in the index of refraction upon a binding event. As in cellular assays, the change is indicated by a shift in wavelength.

4.5.6 Time-resolved Fluorescence (TRF) Technology

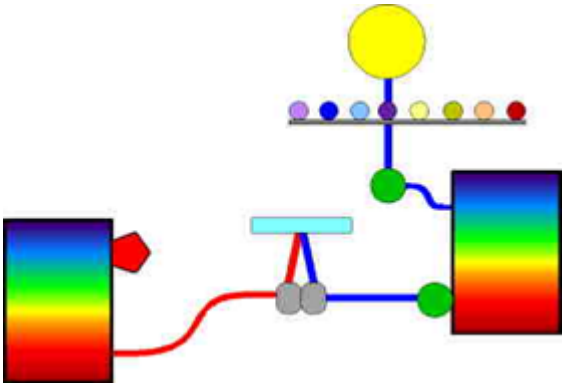
The same excitation light source is used as for FI measurements.

- For **TRF Top** measurements, the excitation wavelength of the light is selected by an optical filter placed in the filter wheel (excitation filter 320 or 340 nm). The excitation wavelength can be in the range of 230-385 nm.
  The excitation light is directed to the TRF measurement head, employing a dichroic mirror with cut-on wavelength at 400 nm. Excitation light below that wavelength is reflected into to the sample.
- For **TRF Bottom** measurements, the excitation monochromator will be selected (excitation wavelength range 230-835 nm). The same measurement head and the same detector will be used as for Fluorescence Intensity (FI Bottom).

Note: The symbols are explained in a legend in section 4.5 “Technologies”, page 34.



TRF measurement from the top with excitation filter and emission monochromator



TRF measurement from the bottom with excitation and emission monochromators

With both TRF Top and Bottom, the fluorescence from the sample enters the emission monochromator (emission wavelength range Top: 400-850 nm, Bottom: 245-850 nm). The same side-on photomultiplier tube detector is used as in Fluorescence intensity measurements. In TRF measurements it is operated in digital mode, i.e. every single photon emitted by the sample will be counted. Only those photons detected after a user-defined time delay and within one or multiple defined time windows contribute to the TRF signal.

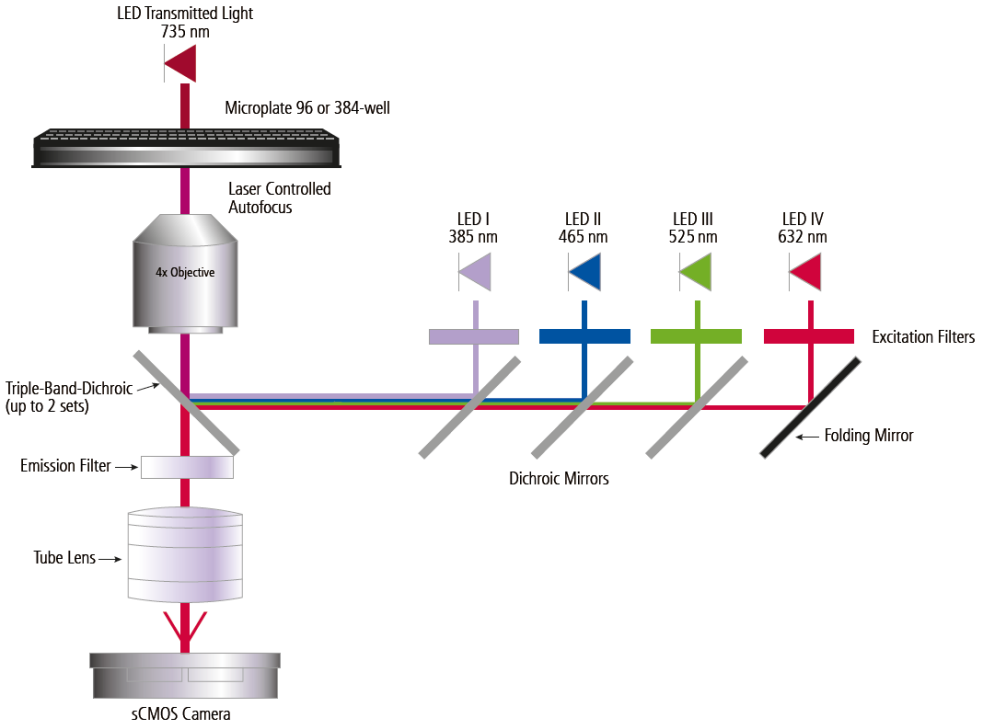
4.5.7 Imaging Technology

The imaging module acts as an inverted optical (fluorescence) microscope. It is designed for well imaging with cellular resolution using a 4 x microscope objective and a low-noise sCMOS camera. Up to 4 different high power LEDs are used to excite fluorescence, additionally a near IR LED located above the sample carrier can be used for transmission (brightfield) and digital phase imaging.

Notice

For dedicated purposes application-oriented documentation is available. Please contact your PerkinElmer specialist for more information.

Application and Technical Notes are published on our web page on a regular basis. Please visit www.perkinelmer.com/ensight.



Light path of the imaging module

To separate (fluorescence) excitation and emission light multi-band dichroic mirrors / emission filters avoid the time consuming need to switch between different filters. In combination with a fast laser-based autofocus mechanism high throughput can be achieved even for multi-color applications.

The large field of view allows to image almost a complete well of a 96 well microtiter plate while the resolution of ~3 µm per pixel supports also analysis of small cell types.

In addition to the pre-defined image evaluation methods a growing collection of algorithms including customized methods can be included as they become available. Intended applications include cell counting, cell viability, analysis of confluency, cell migration, Mitosis and many others.

Imaging can be combined with all other technologies (e.g. Luminescence or Label-free) to allow the analysis of various aspects of cellular assays.

4.6 Applications

EnSight supports several kinds of assays.

4.6.1 Reporter Gene Assays

When you need to measure either the level of expression or the functional effect of a drug candidate in terms of transcriptional activity of cells, EnSight provides the features you need for reliable detection of reporter gene expression.

The instrument has comprehensive and versatile scanning and kinetics capabilities. It supports GFP assays with reading from below.

4.6.2 Enzyme Assays

Kinase, Protease, Helicases or Caspase assays, are examples of enzyme assays that can be run on EnSight. The design of the plate conveyor enables these applications to be run in stabilized conditions. The instrument's kinetics facility allows you to work fast and effectively.

4.6.3 Receptor Ligand Binding Assays

One of the most common molecular targets for drug discovery are G protein-coupled receptors (GPCR). Fast-reading EnSpire is ideal, for example, for B2-Bradykin, MC3, MC4 and MC5.

4.6.4 Cellular Assays

Reading from below, scanning and kinetics are some of the features that make EnSight the ideal tool for cellular assays such as cAMP, Ca2+ or any ADME/tox assays.

Using fluorescence imaging in combination with automated image analysis various cellular assays become available including cell counting, confluency analysis, cell toxicity / apoptosis, cell migration and many more.

Label-less brightfield imaging can be used for quality control, cell normalization and phenotypic cellular assays.

Label-free cellular assays allow analysis for intra-cellular events without interfering with the cellular behavior by using fluorescence stains or without need for cellular engineering.

Label-less imaging and label-free measurement technology are thus ideally suited for the analysis of stem and primary cells as well as for assays with unknown or very complex cellular pathways or orphan receptors.

4.6.5 Genotyping Assays

A feature of single nucleotide polymorphisms (SNPs) research is the need for fast results. With its detector configuration, plate barcode reader, as well as factory set protocols to cover all labels and plates, EnSight provides a complete facility for fast detection of SNPs.

Phenotype assays analyse general cellular reaction without being limited to a certain marked pathway of the cells. Label-free as well and bright field imaging can detect intra-cellular reactions as well as morphological changes of cells and make the EnSight an ideal tool for this type of cellular application.

4.6.6 Alpha Technology Assays

The Alpha technology is an ideal tool for screening a broad range of targets. The technology provides an easy and reliable means to determine the effect of compounds on biomolecular interactions and activities. The Alpha technology offers the possibility to assay many biological interactions including low affinity interactions as well as enzymes, receptor-ligand interactions, second messenger levels, DNA, RNA, proteins, peptides, sugars and small molecules.

4.6.7 Label-free Assays

Label-free mode offers rich, physiologically relevant information from both recombinantly and endogenously expressed targets. The integrated cellular response resulting from label-free detection gives you the ability to characterize pathways that otherwise could be affected by biased agonism, dimerization and allosterism. Label-free mode also provides a complementary and orthogonal assay format that can be ideally used for cellular GPCR and Receptor Tyrosine Kinase (RTK) screening, orphan receptor screening, receptor panning, and ion channel screening. Use of label-free cellular assays ultimately leads to pathway identification and validation.

Label-free mode also allows the advantage of recording actual binding events, including difficult targets or weak biological interactions, rather than the downstream functional effect of binding associated with other assays. Label-free mode also provides screening binding strength (KD) assays that complement Surface Plasmon Resonance (SPR) technology. Direct biochemical interactions such as protein/protein interactions, protein/small molecule interactions, protein/antibody interactions, DNA/RNA interactions, and protease interactions, can be detected.

4.6.8 Time resolved Fluorometry Assays

In time resolved fluorometry, lanthanides are used as measurement technologies to give a long decay time and a large Stokes shift. There are two types of time-resolved fluorescence: DELFIA, which involves enhancement and washing steps, and LANCE, a homogeneous assay. In the most common form of LANCE the light excites the donor molecule which, after a delay, transfers the energy to the acceptor molecule which then emits light. Two measurements must be defined for LANCE, one for the donor and the other for the acceptor. The EnSight also supports other TRF chemistries such as HTRF®.

4.6.9 Imaging

Imaging with single cell resolution gives access to a large variety cellular properties. Analysis of different fluorescence stains within the cells allows for several types of cellular assays including cell counting, live-dead and toxicity assays, proliferation, cell migration, transfection rate analysis etc.

Brightfield (transmission mode) and digital phase imaging allow quantification of single cells as well as of confluent cellular layers without interfering cellular activities by applying stains.

4.7 Light Sources

EnSight employs a high stability, Xenon short-arc flash lamp as a light source in measurements for the following measurement technologies:

- Fluorescence intensity
- Absorbance with monochromators or filters
- Time-resolved fluorescence

The high-efficiency light source has a high repetition rate for high throughput applications and it allows you to perform faster multi-flash measurements. You can select the number of flashes used. To ensure both the long-term and short-term stability of measurements, the excitation energy is monitored after every flash using a reference photodiode.

- In the case of Alpha technology a high power semiconductor laser emitting light at wavelength of 680 nm is used.
- The imaging module for the imaging technology is equipped with up to four different high power LEDs and an additional LED (735 nm) for transmission (brightfield) and digital phase imaging.
- In the case of Label-free technology a superluminescent diode (832 nm) is used.

The instrument has an interlock mechanism switching off all light sources (except Lable-free light source) immediately if the plate door is opened or the glass plate on top of the instrument is lifted.

Only with Alpha technology:

Danger!

Laser radiation – Eye injury

Class 3B laser radiation may be present inside the EnSight's protective housing during operation. Since the instrument's lid and loading door are protected by laser safety interlocks, no harmful radiation will become accessible when they are opened.

- *Nominal wavelength: 680 nm (red)*
  Nominal output power: 400 mW
- *Do not defeat the safety interlocks!*
- *Do not remove any housing components!*
- *AVOID DIRECT EXPOSURE TO BEAM!*
- *Service tasks that require access to the instrument while the interlocks are defeated may only be carried out by qualified service personnel.*

Only with Label-free technology:

Danger!

Invisible laser radiation – Eye injury

Invisible Class 3R laser radiation may be available behind the plate loading door. The light source of the Lable-free module is not blocked by the interlock system if the plate door is opened. Insertion of reflecting parts may result in laser radiation to be reflected out of the instrument.

- *Nominal wavelength: 832 nm (infrared)*
  Nominal output power: 0.6 mW
  Max. output power: 3 mW (in case of error)
- *Do not insert any reflecting tools through the plate door!*
- *Do not remove any housing components!*
- *AVOID DIRECT EXPOSURE TO BEAM!*
- *Service tasks may only be carried out by qualified service personnel.*

4.8 Filter Wheel

The excitation filter wheel has eight filter positions.

- In filter absorbance measurements, bandpass filters are placed in the wheel.
- Automatically controlled cut-off filters are used for monochromator-based absorbance and fluorescence intensity measurements. Filters provided with the monochromator must be placed into the wheel.
- For TRF top measurements, filters are used to select the excitation wavelength.

EnSight can use the same filters as EnSpire® and EnVision®. There is a large collection of filters to choose from. If you cannot find a suitable filter from the existing selection, please contact your local PerkinElmer sales representative. He can help you to order a custom filter specially designed for your application.

You can easily change the filters by pulling down the handle on the right-hand side of the EnSight. This will expose the filter wheel so that you can take it out and change filters. Please see section 6.1 “Change Excitation Filters”, page 257 for detailed instructions.

4.9 Control PC

The control PC is directly connected to EnSight via network cable. The Kaleido software running on that computer is used to control the instrument and analyze the measured data.

4.10 Focus Point Adjustment

Measurement Height

The focus point for the incident light is adjustable via the parameter **measurement height** (0-20 mm) for the following technologies:

- Absorbance
- Fluorescence Intensity (Top)
- Time-resolved Fluorescence (Top)

The zero point is defined as the bottom of the plate (where it contacts the plate carrier).

Focus Height

For **Imaging** you can define a general **focus height** for the whole IMG operation (-200 to +3000 µm). Optionally you can define an offset from the selected focus height for each channel separately (-200 to +200 µm). This can be useful to find the optimum focus plane for the objects to be detected in the respective channel.

The zero point of the focus height is defined as the bottom of the well.

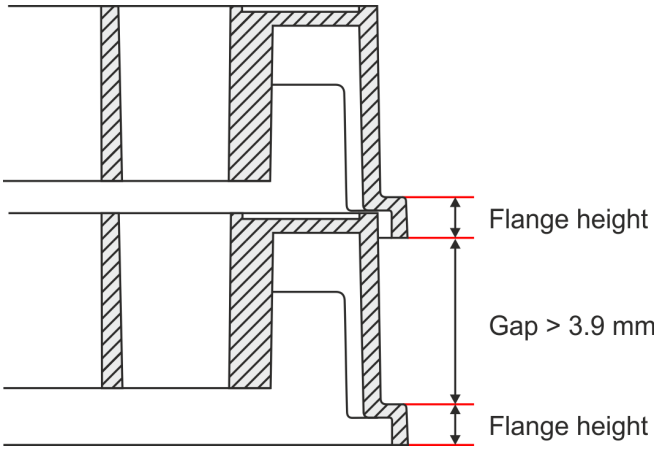
4.11 Plates

4.11.1 General Requirements

- Microplates with 6, 12, 24, 48, 96 or 384 wells
  (for Imaging and Label-free only 96 or 384 wells)
- Maximum outer plate dimensions: 86.0 × 128.2 × 28.0 mm (SBS standard)
- Flexible/deforming plate types are not suited for MMD measurements, because they cannot be gripped properly by the plate carrier and stacker. Some plate types deform at elevated temperatures and hence are unsuitable to be used in combination with the temperature control.

Only when using the **stacker**:

- Lids are not supported when using the stacker.
- The gap between the top of a flange and the bottom of a plate stacked above it has to be > 3.9 mm.



Stacked plates

4.11.2 Technology-specific Requirements

The following chapters contain information and guidelines regarding the compatibility of a plate with the EnSight measurement technologies. Please consider them carefully before setting the compatibility check marks for a new plate type in the Plate Type Wizard (see section 5.10.5.2 “Plate Type”, page 234).

Please also note that certain measurement technologies or operations have additional requirements which are explained in separate chapters:

- 4.11.2.4 “Label-free”, page 55
- 4.11.2.5 “Imaging”, page 56
- 4.11.2.6 “Shake”, page 57

4.11.2.1 Plate Height

- Alpha or Luminescence technology and stacker measurements: 7 – 28 mm
- All other technologies: 3 – 28 mm

4.11.2.2 Plate Color

Plate colors and their recommendations for each EnSight measurement technology:

	White plate	Black plate	Clear plate	Gray plate (AlphaPlate)
ABS Mono	○	●	●	N/A
ABS Filter	○	●	●	N/A
Alpha	○	○	○	●
FI	○	●	○	○
Imaging	○	●	○	N/A
Label-free	N/A	●	N/A	N/A
LUM	●	○	○	●
TRF	●	○	○	○

● Recommended plate color for optimal assay results

○ Does not support optimal assay results, but may work for certain assays

N/A Indicates that the plate color is not available for plates needed for the technology (e.g. AlphaPlates with a clear plate bottom for absorbance).

The color of the plate walls influences the results of an assay. The table shows different plate colors and their recommendations for each EnSight measurement technology. For more details on each of the plate colors please also mind the comments below.

- **White plates** reflect light emerging from the sample and result in high raw signals. Based on that they are generally recommended for **Luminescence** assays that often provide low signal intensities and for **Time-resolved Fluorescence (TRF)** assays. They are badly suited for classical fluorescence as the plate material may cause additional fluorescence background signal.

  TRF is an exception here because fluorophores with large decay times such as Europium chelates are used. A “lag/delay time” after excitation thus diminishes background auto-fluorescence, so white plates are best suited for optimal TRF assay windows.

  Application hint for Luminescence

  A dedicated filter is placed in front of the detector unit to block background phosphorescene from certain microplates. Only in rare cases this phosphorescence can still interfere with luminescence readouts. This can be handled by “dark-adaptation”, i.e. by shielding the plate from light for up to 10 minutes prior to reading the plate.

- **Black plates** are generally recommended for **Fluorescence Intensity** assays as

they minimize the background signal and thus provide highest signal-to-noise values (SNR) and better Z'.

However, compared to white plates their signal level is much lower as no light is reflected from the walls of the wells towards the detector. Thus they are only suitable for luminescence if the assay signal is very high. In that case they may be even better suited than white plate because black plates show no background caused by phosphorescence.

- **Gray plates** (AlphaPlates) are recommended for **Alpha** and **Luminescence** readouts. Gray plates are designed to reduce background caused by auto-fluorescence or phosphorescence while maintaining high signal. AlphaLISA and AlphaScreen assays use strong IR excitation, which in white plates can pass through the walls of the wells and cause cross-excitation. Alpha plates have been optimized to avoid this cross excitation while maintaining a high sample signal.
- **Clear plates** are best suited for **Absorbance** (colorimetric) assays as clear polystyrene plastic does not interfere with measurements in the visible light range (400-900 nm wavelengths). However, assays that measure absorbance in the ultraviolet range (200-400 nm wavelengths) will require plates that are made of a UV-transparent material such as glass or Cyclic Olefin Copolymer (COC), to avoid absorbance of light by the plastic itself.

  However, for higher density plate formats (from 384 wells) light reflected within the plastic material may interfere with the absorbance measurements and can limit the dynamic range. In this case black plates with clear bottom are recommended. For all other measurement technologies clear plates suffer from cross talk and/or auto-fluorescence of the plate material and are not recommended.

4.11.2.3 Plate Characteristics

The following table shows plate characteristics of the plate bottom and top that are incompatible with the measurement of reasonable data for certain technologies or can even damage the instrument.

	Opaque plate bottom	Clear plate bottom	Clear plate bottom + Label-free sensor	Clear U-shaped plate bottom	Protrusions on top of the well** / Lids
ABS Mono	✕	●	●	●	●
ABS Filter	✕	●	●	●	●
Alpha*	●	●	●	●	✕
FI Top	●	●	●	●	●
FI Bottom	✕	●	●	●	●
Imaging	✕	●	●	✕	●
Label-free	✕	✕	●	✕	●
LUM*	●	●	●	●	✕
TRF Top	●	●	●	●	●
TRF Bottom	✕	●	●	●	●

● Compatible with technology

✕ Does not produce reasonable data or even damages the instrument

* Aperture-based technologies. Remove any plate lid before loading the plate or use clear top seals instead.

** Protrusions that exceed the top of the well can interfere with aperture-based technologies, for details see below.

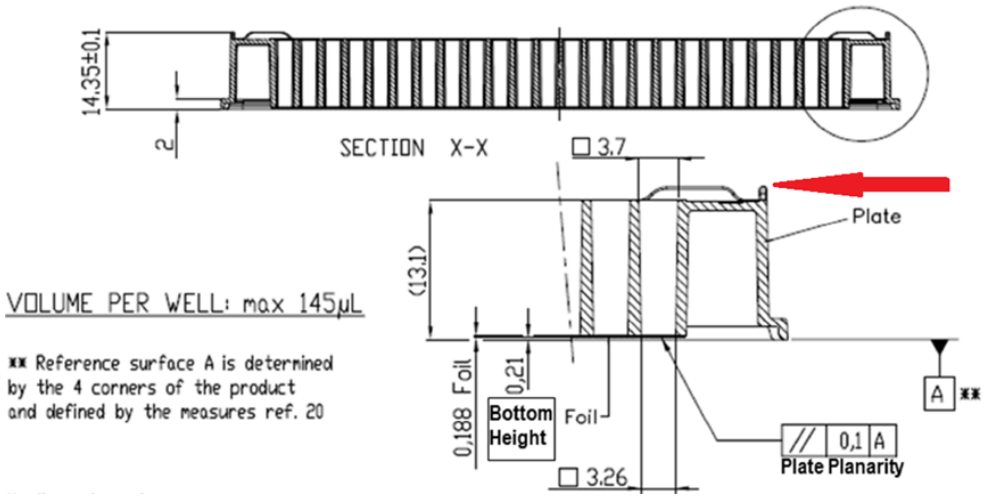
- **Clear plate bottoms** are required for bottom-reading measurement modes such as Absorbance, FI Bottom, TRF Bottom and Imaging.
- **Opaque plate bottoms** are suitable for Alpha, FI Top, Luminescence and TRF.

> **Application hint**
> BackSeals (black or white) can be applied to plates with clear bottoms to convert them functionally to opaque plates.

- Plates with a clear bottom containing a patented **optical biosensor** integrated into each well are mandatory for Label-free, but they are also compatible with all other measurement technologies.

- **Aperture-based technologies** such as Alpha and Luminescence measure with small distances between detector and the plate surface. To prevent collisions, protrusions on top of the well are incompatible with these technologies (see next figure) and plate lids must be removed before loading the plate.

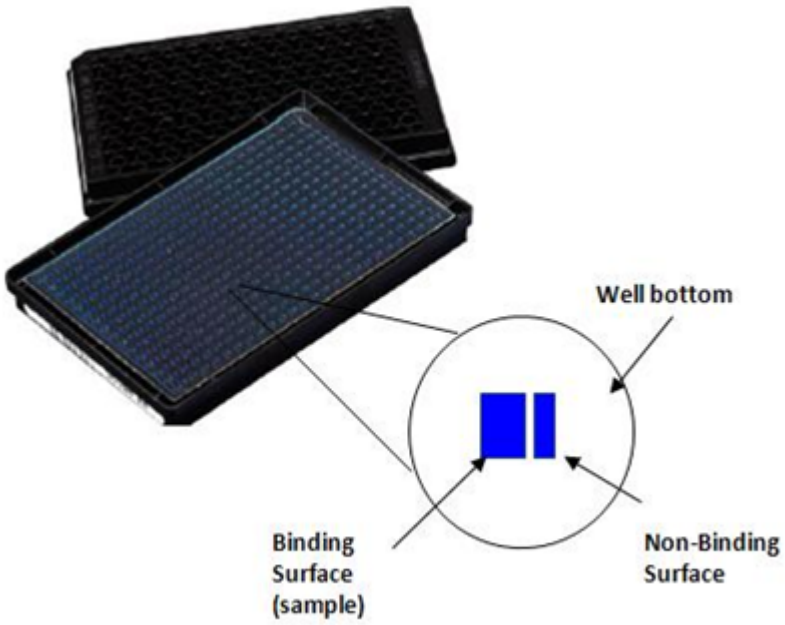
> **Application hint**
> Clear top seals can be used instead of lids.



Example: The technical drawing of a CellCarrier Ultra 384 shows a protrusion on the upper side of the plate (red arrow). This will interfere with aperture-based measurement technologies such as Alpha and Luminescence, if it exceeds 0.1 mm. Such plates are incompatible with Alpha and Luminescence measurements.

4.11.2.4 Label-free

Label-free mode requires PerkinElmer 96- or 384-well label-free enabled microplates, which can be used for a wide range of biochemical and cell-based applications. Highly sensitive optical biosensors are located within the microplate wells.



Biochemical assay label-free microplate with self-referencing dual sensor

Patented dual sensor self-referencing technology used for biochemical assay microplates for protein/ligand assays ensures that only one true analyte binding is reported.

Cell-based assay microplates offer flexibility for many cell types including adherent and suspension cells, mammalian and primary cells. The plates are identical in appearance, but in contrast to biochemical assay plates both sensors are used to generate data and there is no reference subtraction. Coated and uncoated plates are available.

Notice

- Label-free plates should remain sealed until shortly before use, as some of the microplate coatings can degrade when left open and exposed to the atmosphere for an extended period of time.
- For label-free mode, the barcode is essential as the barcode is used to generate the response based on the plate Baseline. All label-free plates will have barcodes affixed in production.

Notice

For dedicated purposes application-oriented documentation is available. Please contact your PerkinElmer specialist for more information.

Application and Technical Notes are published on our web page on a regular basis. Please visit www.perkinelmer.com/ensight.

4.11.2.5 Imaging

Imaging can only be used with 96 and 384 well plates and requires an imaging compatible clear bottom.

Notice

For dedicated purposes application-oriented documentation is available. Please contact your PerkinElmer specialist for more information.

Application and Technical Notes are published on our web page on a regular basis. Please visit www.perkinelmer.com/ensight.

4.11.2.6 Shake

Plates that contain a small number of wells and hold a high **volume per well** are prone to lead to spilling effects during shaking. The shaking intensity has to be adjusted accordingly and should be tested during assay development. The shaking step can be optimized best if using the shake outside function without a stacker. Small volumes in plates with a high number of wells are more difficult to mix efficiently. We recommend shaking of plates with 96 or a lower number of wells.

4.12 Temperature Control

The EnSight is equipped with a temperature control module. The EnSight has a uniquely isolated measurement chamber that allows the plate temperature to be controlled with high precision. The heating system is implemented with 14 resistors, eight above and six below the measurement chamber that provide uniform temperature over the measurement area. In addition, the measurement chamber has its own fans for optimal air flow control.

The temperature control enables for example cellular activity measurements at 37 °C and denaturation of proteins at 42 °C. See section 7.11 “Temperature Control”, page 269 for performance specifications.

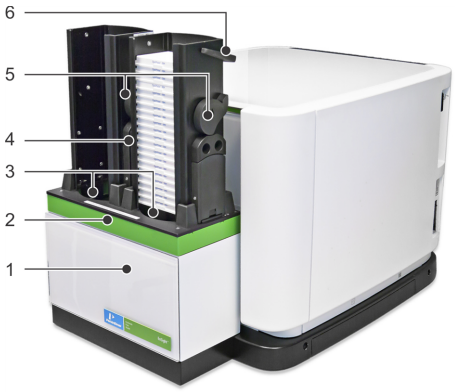
Temperature control can be configured generally in the **Settings** dialog and/or using a **Temperature operation** in the protocol which then overrides the general settings. See the following sections for details:

- 5.10.6 “Temperature (Settings)”, page 235
- “Temperature (TEMP)”, page 106

When the heating is turned off and the **Fast Cooling** option was selected, the plate door is opened and two fans blow the warm air out of the sample chamber. This leads to a faster reduction of the temperature inside the sample chamber.

4.13 Stacker

The EnSight can be equipped with an optional stacker module for automated measurement of multiple plates. It consists of the following components:



1. Stacker main unit
2. Magazine table (removable)
3. Sample loading area
4. Magazines (removable)
   - Right position: input
   - Left position: output
5. Release mechanism
6. Handles

Caution! (For stacker option only)

Moving parts within the sample loading area: The plate lift and the rods in the stacker will move up during operation and initialization and can cause hand injuries.

- *Keep your hands away from the sample loading area.*
- *The magazines have to be removed for loading/unloading plates.*
- *Please note that the stacker mechanics will shortly move up and down during initialization even if the magazine table is not installed and the stacker is not used.*

Before you can use the stacker, you need to place the magazine table on top of the stacker in front of the plate loading door. This determines how plate loading will happen if you start a measurement:

- **Magazine table installed:** Instrument is in stacker mode, stacker is used for automatic plate loading.
- **Magazine table is not installed:** Instrument is in manual mode, plates have to be inserted manually (as with an instrument without stacker).

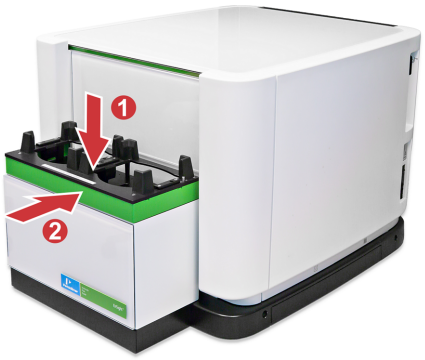
Each stacker comprises two magazines. Plates to be measured have to be loaded into the right magazine (input magazine), the left magazine must be empty. After measurement each plate is moved to the left magazine (output magazine). There are two types of magazines available (capacity of 20 or 50 microplates). These types may not be mixed; you have to use the same type of magazine at the input and output position.

- For technical specifications please see section 7.14 “Stacker”, page 270.
- For instructions how to setup the Kaleido software for a stacker run please see sections 5.3 “Run Protocol”, page 147 and 5.10.7 “Stacker Settings”, page 238.

4.13.1 Preparing Stacker for Measurement

Before you can use the stacker, you need to place the magazine table on top of the stacker in front of the plate loading door.

1. Place the magazine table on top of the stacker main unit.
2. Push the magazine table towards the instrument until it snaps into place.



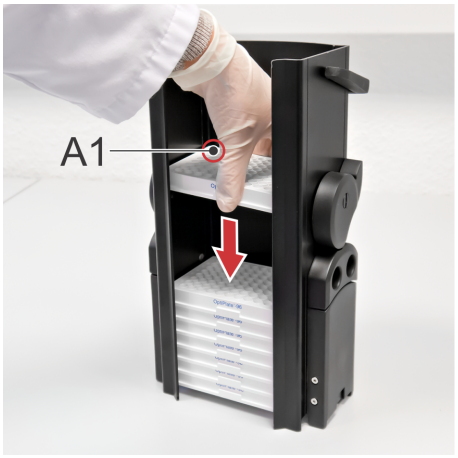
Notice
If the rods of the stacker are still up, press the **Unlock Magazines** button in Kaleido (**Settings – Stacker**) before you install the magazine table.

4.13.2 Preparing Magazines

Magazines are available in two sizes: 20 plate magazine and 50 plate magazine. Plates are usually loaded into the magazine before the magazine is placed on the stacker. All plates used within one protocol must be of the same plate type.

4.13.2.1 Loading Magazines

1. Place the magazine on your lab bench.
2. Load the plates to be measured into the input magazine.



- Plates are inserted into the magazine from the top (see photo above). Load the plates one by one (left image) or in small stacks (right image).

- Make sure the plates are orientated correctly with the A1 position in the left hand corner furthest from you.
- Do not touch the plate bottom with bare fingers to avoid finger prints if the plates are to be used for imaging or other technologies measuring from below.
- The plates should be loaded in the order in which you want them processed by the instrument, i.e. the first plate should be at the bottom of the input magazine.
- When using sealed plates, make sure that all plates are sealed correctly and do not stick together when being stacked on top of each other.
- Do not use plates with lids.

3. When all the plates have been loaded, place the input magazine at the right position of the stacker and place an empty magazine (same capacity) at the left position of the stacker.
4. Check that the handles of both magazines are down during a run so that the plates can move upwards without hindrance.

4.13.2.2 Unloading Magazines

Unloading plates one by one:

1. Lift off the magazine and place it on your lab bench.
2. Unload the plates by lifting them up to the top.

Unloading the entire plate stack:

1. Lift off the magazine and place it on your lab bench.
2. Activate the release mechanism on both sides of the magazine (see A and B in figure below) and hold it in that position until the magazine has been removed.

The plate stack is released and stands on the bench now.

3. Carefully lift off the magazine.

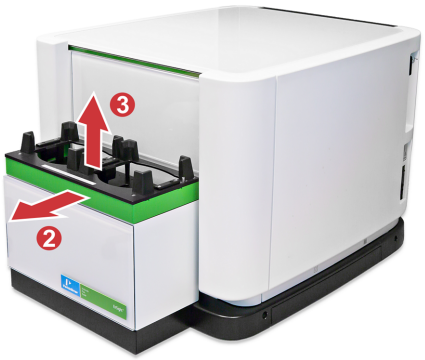
4.13.3 Run Protocol

By installing the magazine table the instrument is automatically in stacker mode. All the plates in the input magazine will be measured using the selected protocol. For detailed instructions and workflows see section 5.3 “Run Protocol”, page 147.

4.13.4 Manual Plate Loading

If you want to load plates manually without using the stacker, you need to remove the magazines and the magazine table.

1. Start by lifting off the magazines.
2. Pull forward the magazine table.
3. Lift it off.



Notice

If you cannot remove the magazines, press the **Unlock Magazines** button in Kaleido (**Settings – Stacker**) to unlock the magazines and the magazine table.

5 Kaleido Software

5.1 Introduction

5.1.1 Start Up

1. Switch on the EnSight.
2. Switch on the user PC and log into Windows®.
3. Double-click the Kaleido icon on the desktop.
4. Log into Kaleido (see section 5.1.3 “Login”, page 63).

   Kaleido is started and the instrument is initialized (status light blinking green). As soon as the status light stops blinking (permanent green), the EnSight is in idle state and can be used. See also section 4.2 “Status Light”, page 31.

Caution!

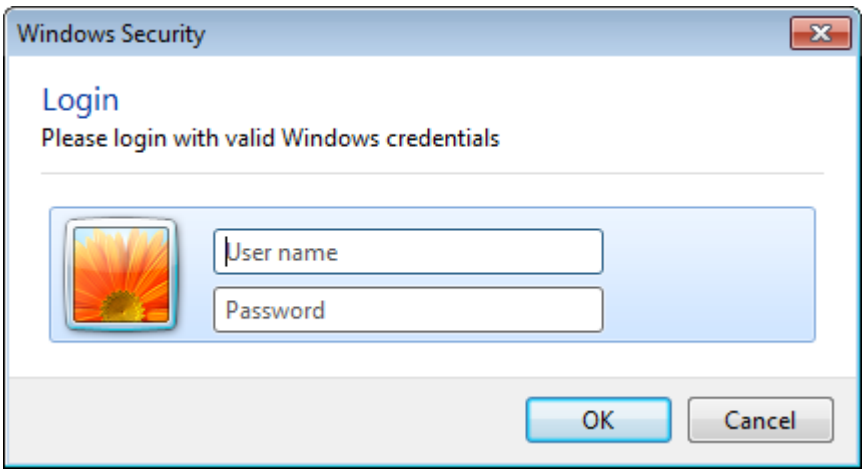
For users with the Stacker option: Keep your hands away from the stacker area when the software is started or restarted. The rods in the stacker will come up during the initialization process.

5.1.2 Shut Down

1. If there is still a plate inside the instrument: Click **Eject** and remove the plate.
2. Click **Load** to move the empty plate carrier back inside and close the plate door.
3. Close the Kaleido software by closing the window in the usual way (X button).
4. Shut down the Kaleido PC.
5. Switch off EnSight using the power switch.

5.1.3 Login

The Kaleido software uses the Windows user management for access control. After starting Kaleido, a Windows **Login** dialog is displayed where you can enter a Windows user name and the corresponding password.



Only user accounts belonging to one of the three Kaleido user groups can login to the software. The user account used for Kaleido login can be different from the currently logged in Windows user. The current user  is also displayed in the **Navigation Bar** (see section "Current User", page 66).

Please see section 5.13 "User Management", page 247 to learn more about user accounts, user groups, restricted actions, and how to add new users.

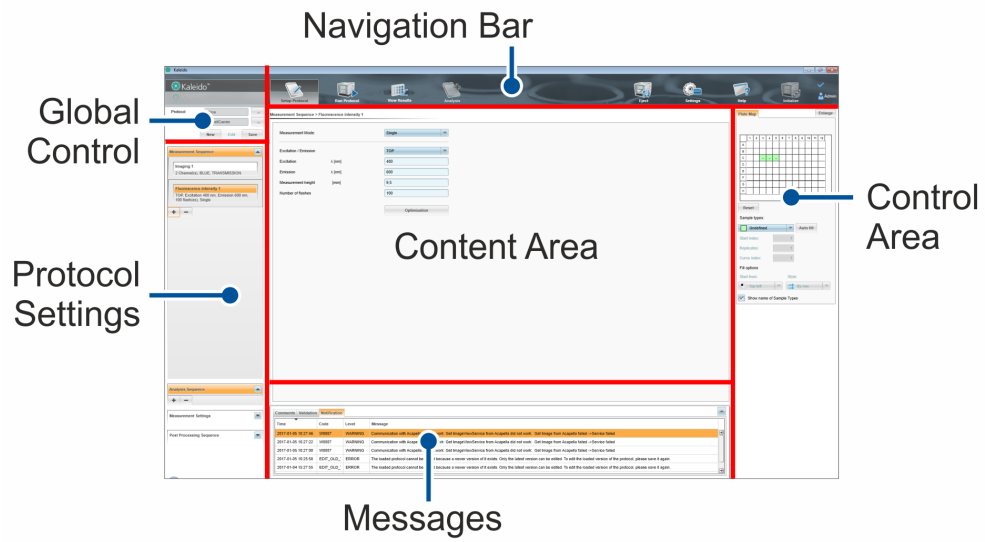
How to switch to a different user

If you are logged in and want to switch to a different user in Kaleido, please shut down and restart the software.

Buttons and Elements

Element	Description
User name	Local Windows user account which has been added to one of the three Kaleido user groups (not case-sensitive).
Password	Password of the selected user account (case-sensitive).
OK	Login information is verified, and Kaleido is started.
Cancel	Cancel the login process.

5.1.4 Software Overview



While certain screen areas in Kaleido are fix (e.g. the upper **Navigation Bar** and the left part of the screen), the following panels can be resized:

- **Control Area** (use **Enlarge/Reduce** button or drag border)
- **Messages** (expand/collapse via toggle button or drag border)

The remaining space in the center of the screen is automatically occupied by the **Content Area**, e.g. for editing parameters or viewing results.

5.1.4.1 Main Screens

Working with Kaleido means working with protocols. Whatever you want to do with your samples and results is defined and configured via protocols. When Kaleido is opened, you see four buttons on the left in the **Navigation Bar** representing the main screens and the Kaleido workflow:

- **Setup Protocol:** Create new protocols and edit existing ones or view settings of a protocol.
- **Run Protocol:** Load and start protocols.
- **View Results:** View measurement and analysis results.
- **Analysis:** Add or change analysis specifications for measurement results of a protocol run and recalculate (enabled when a protocol is already loaded).

All these operations refer to a protocol, which can be loaded via the section in the upper left of the screen, the **Global Control** section. This section always displays the currently loaded protocol. Depending on the selected screen, further elements and buttons are available.

5.1.4.2 Navigation Bar

Instrument Status Symbols

Symbol	Status/Description
	**Idle**
	**Busy** • Preparing hardware • Plate carrier moving • Running protocol • Cancelling
	**Error**
	**Unknown / Uninitialized**
	**Waiting for user action**
	**Service mode**

Further information and error messages are given in the **Messages** area (see section 5.9.3 “Notifications”, page 218).

Current User

Symbol	Status/Description
	**User account** The currently logged in user is displayed next to this icon.

5.1.5 Load/Eject

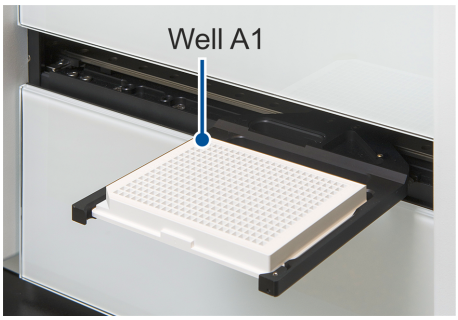
Using this button you can load or eject a plate. The button will toggle its function depending on the current position of the plate carrier (in/out).

How to insert a plate

1. Click **Eject**.

   The plate carrier is moved out.

2. Insert the sample plate and check for correct orientation (see photo).



3. Click **Load**.

   The plate carrier is moved in.

4. Make sure that the correct plate type is selected for your protocol.

Buttons and Elements

Element	Description
	Click **Load** to move the plate carrier into the instrument after inserting a plate. Only available if the plate carrier is outside in handover position.
	Click **Eject** to move the plate carrier out of the instrument to eject a plate and/or insert a different plate. Only available if the plate carrier is inside the instrument.

5.1.6 Initialize

Using this button you can initialize the instrument and all its components. The button is only enabled if the instrument is in error state and requires an initialization.

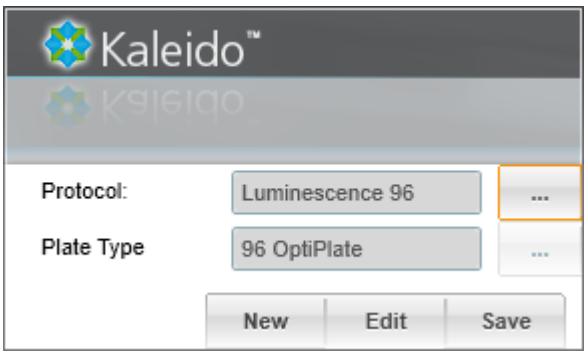
If you want to force an initialization while the instrument is not in error state, please use the corresponding button in the **General Settings** dialog. See section 5.10.2 “General Settings”, page 220.

Buttons and Elements

Element	Description
	If the instrument is in error state or needs to be initialized, the **Initialize** button is enabled. You have to click **Initialize** and initialize the instrument before it can be used again. See also section “Instrument Status Symbols”, page 66.
	If the instrument is not in error state, the button is disabled.

5.2 Setup Protocol

On the **Setup Protocol** screen you can create new protocols, edit or export existing ones or just view your protocol settings. The name of the currently loaded protocol as well as its plate type will always be displayed in the **Global Control** section. The **Measurement Sequence** is displayed on the left in the section beneath, and if you click on one of the operations, its details and settings will be displayed in the **Content Area**.



There are multiple ways to generate new protocols:

- Create a new protocol from scratch
- Load and edit an existing protocol and save it (overwriting)
- Load and edit an existing protocol and save it under a new name (new data object)
- Import a protocol (file format *.kal), e.g. from a different instrument (see section 5.10.3.3 “Import Protocol / Measurement”, page 223)

Buttons and Elements

Element	Description
Protocol [...]	Displays the name of the loaded protocol. You can click ... to open the **Load Protocol** dialog where you can browse and filter all existing protocols to find the desired protocol to be loaded. For details see section 5.6 “Load Dialog”, page 180. **Notice** If the protocol still contains deprecated analysis operations, you will get an error message. Please setup a new analysis sequence. See also section 5.2.2 “Analysis Sequence”, page 120 for details.

Element	Description
Plate Type [...]	Displays the name of the loaded plate type. If you load a protocol, the plate type contained in the protocol will be used. When in edit mode or when a new protocol is generated, you can click ... to open the **Load Plate Type** dialog and load a different plate type. For details see section 5.6 “Load Dialog”, page 180.
[New]	Option to create a new protocol from scratch. Unsaved changes of the current protocol can be saved first or discarded. If you click **New** you will be prompted to enter a name for the new protocol and select an **Execution Type**.
[Edit]	The currently loaded protocol will be set to edit mode so that you can modify it. While in edit mode, the button **Edit** is disabled.
[Save]	Opens dialog **Save Current Protocol**. Perform saving or export with click on **OK** or abort with **Cancel**.

How to load a protocol

1. Click ... next to **Protocol**.

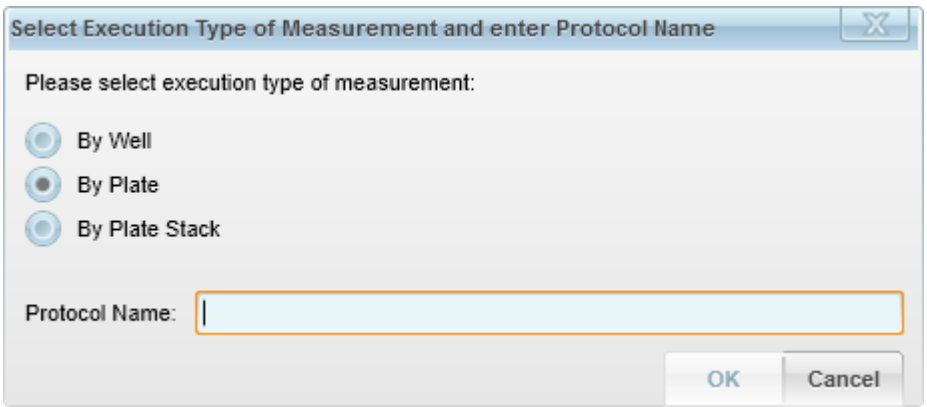
   The **Load Protocol** dialog is opened where you can search for the desired protocol to be loaded. Only the latest version of a protocol is displayed. For details see also section 5.6 “Load Dialog”, page 180.

2. Click **OK**.

   The selected protocol and the contained plate type are loaded.

How to setup a new protocol

1. Click **New**.
2. Select an **Execution Type**.



Notice
The **Execution Type** of a measurement sequence can only be selected when creating a new protocol. It cannot be changed subsequently. For details please see section 5.2.1.1 “Execution Type”, page 75.

3. Enter a **Protocol Name** for the new protocol.

   You will be notified if you enter a protocol name which already exists. To avoid name duplicates you can then change it, but it is also possible to keep the same name. The protocol can also be identified by other properties (owner, date, signature).

4. Click **OK**.

   A new and empty protocol is created and set to edit mode. The protocol name is displayed in the **Global Control** section.

5. Click ... next to **Plate Type** and select the desired plate type using the **Load Plate Type** dialog. For details see section 5.6 “Load Dialog”, page 180.

6. Define the **Plate Map** (see section 5.8.1 “Plate Map”, page 206).

Now you can start to define the **Measurement Sequence** an other elements of the protocol. For details see the following sections.

How to edit a protocol

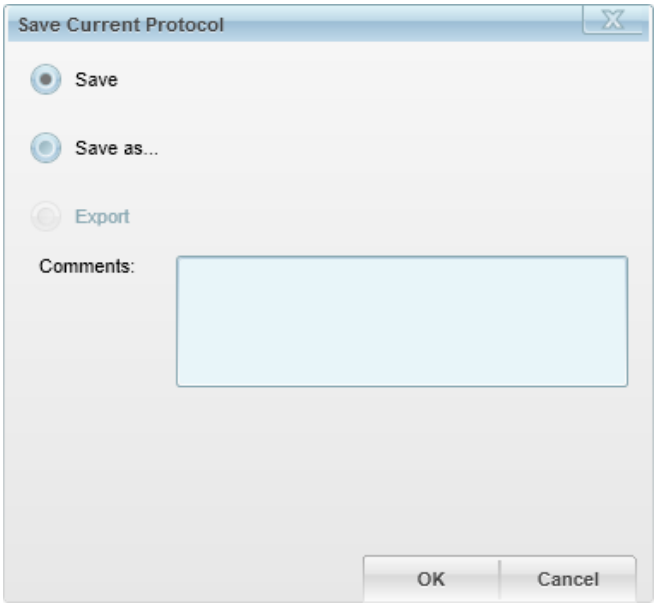
To edit a protocol click the **Edit** button in the **Global Control** section and select an operation you want to edit in the **Protocol Settings** (Measurement, Analysis or Post Processing Sequence). In non-edit mode you can click on an operation to see its details in the **Content Area**.

- If a protocol is already in edit mode, the **Edit** button is disabled.

When switching to a different screen while in edit mode, you will always be asked whether you want to save the current protocol or discard all changes.

How to save a protocol

After completing or changing a protocol it has to be saved. Click on **Save** to open the **Save Current Protocol** dialog. The **Save** button is also enabled in non-edit mode as long as a protocol is loaded.

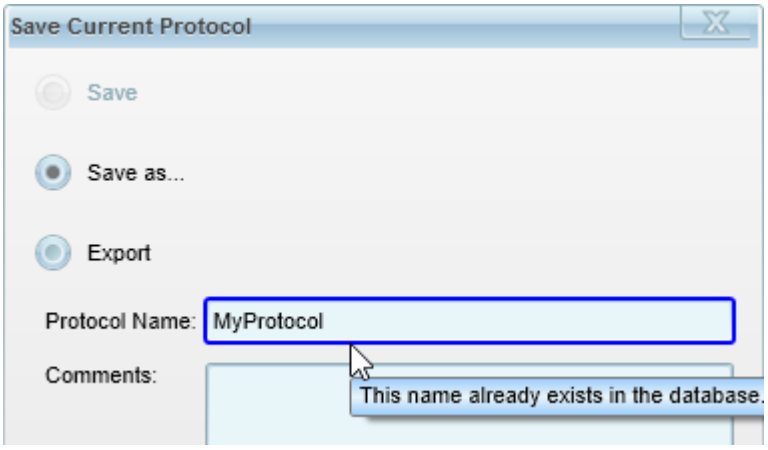


The dialog options are:

- **Save** (only enabled when in edit-mode) will overwrite the currently loaded protocol or save the new protocol under the name shown in the **Global Control** section.

  Notice
  Previous versions of protocols are not available in the **Load Protocol** dialog, only the latest versions will be available. In case you want to check protocol settings of measurement results from former versions of a protocol, you can load the measurement on the **View Results** screen, switch to **Setup Protocol** and click on the operations of interest. The operation parameters will be displayed in the **Content Area**.

- **Save as…** will save the changes as a new and separate data object under the entered name. The currently loaded protocol will not be altered and will still be available in the **Load Protocol** dialog.



  The field **Protocol Name** appears for entering a new name. The current name is displayed by default. A blue outline of the text box indicates if this name already exists. To avoid name duplicates you can then change it, but it is also possible to keep the same name. The protocol can also be identified by other properties (owner, date, signature).

- With **Export** a protocol can be exported as ***.kal** file for use in a different system. Export is only enabled, when the protocol was not in edit mode before, i.e. it has no unsaved changes.

For **Save** and **Save as…** a text can be added with every saving procedure in the **Comments** text box. This comment will be added to the protocol and displayed in the **Messages** section when the protocol is loaded.

To save a protocol, it has to include a name, a plate type, at least one measurement operation and a defined plate map. In case one of these is missing or a value is invalid, you will be notified when trying to save.

When a protocol is changed and saved by a different user (owner), it will be saved under the name of the new user.

5.2.1 Measurement Sequence

For setting up a protocol, the measurement sequence is the most important part, determining the technologies the samples are going to be measured with.

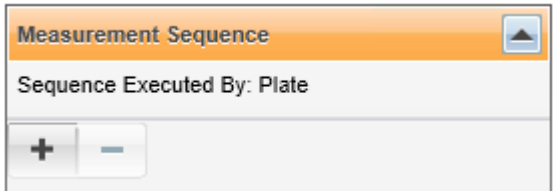
The elements of a measurement sequence are called operations. There are two types:

- **Measurement operations:** They perform the actual measurements using the technologies implemented in the EnSight reader.
- **Non-measurement operations** They are either used for setting up the appropriate conditions for the measurement operations (delay between measurements, shaking or temperature control) or for controlling how the sequence is processed (repeat operations, stop sequence).

You can add multiple operations, which are then processed one after the other until the measurement sequence has been completed.

Execution Type

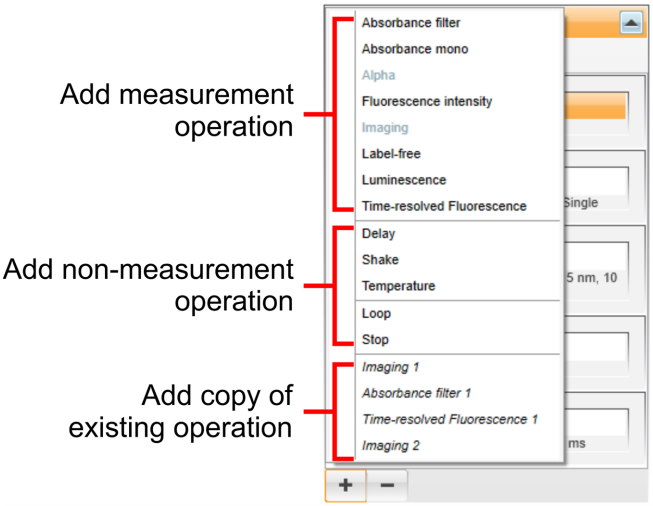
If you create a new protocol, you have to select an **Execution Type**. It defines how the operations of the measurement sequence will be processed (by well, by plate or by plate stack). The selected execution type is displayed in the header of the measurement sequence:



Notice
The **Execution Type** of a measurement sequence can only be selected when creating a new protocol. It cannot be changed subsequently. For details please see section 5.2.1.1 “Execution Type”, page 75.

Add and Configure Operation

If you want to add operations to the measurement sequence, click the + button and select an operation from the pop-up menu.



The order of operations within a measurement sequence cannot be changed. New operations will always be added at the end of the sequence. For each operation added to the sequence further specific settings must be configured. If you click on an operation, its parameters will be displayed in the **Content Area** in the center of the screen.

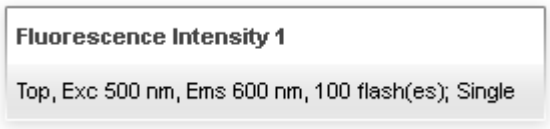
- The first parameter of each measurement operation is the **measurement mode**. It determines the number of actual measuring events in one well. For details see section 5.2.1.3 “Measurement Modes”, page 112.
- For a detailed description of all parameters please refer to the corresponding operation (see section 5.2.1.2 “Protocol Operations”, page 80).

All operations are numbered. If you add the first operation of a type, the number “1” will be added to the name. If you add further operations of the same type, the number will be incremented accordingly. Most operations can be added as often as required to a measurement sequence, but for some operations the max. number is limited:

- There can be only one operation of type **Alpha** or **Label-free** due to the reactions and usage of substances of these technologies during measurement.
- There can be only one or two **Imaging** operations (one per dichroic filter set).
- There can be only 5 **Loop** operations.

If the maximum number of operations is reached, the corresponding option is disabled in the pop-up menu of the + button. For details see also the description of the respective operation.

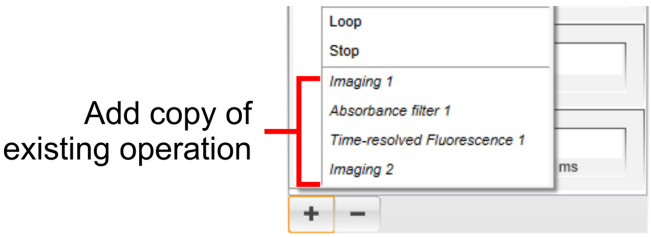
The most important parameter settings of each operation are summarized and displayed below the operation name. Example:



Measurement operation with summary

Copy Operation

After adding an operation to the measurement sequence or to a loop, it will also be offered as a copy of the original operation in the + pop-up menu (at the bottom of the menu, written in *italics*):



If you select such an entry, the existing original operation will be copied and added to the sequence:

- Copied operations have the same name and exactly the same parameter settings as the original operation. They are interpreted as "repeats" of the original operation. The results will be identical to those of an operation which is repeated within a **Loop**.
- You can edit both the original operation and its copy. However, the change will always be synchronized between all operations (original and copy/copies).
- You can add the same copy multiple times.

The following operations cannot be copied:

- Alpha
- Loop
- Stop
- Already existing copies (only the original operations can be copied)

Repeat Operation (Loop)

If you want to repeat one or multiple operations, you can insert a **Loop** operation and add these operations to the loop. You can define the number of loop iterations and the interval between the iterations. For details please see section “Loop”, page 108.

Stop and Continue Measurement

Using a **Stop** operation you can interrupt a measurement, e.g. to dispense a substance to the samples on the plate. Afterwards the measurement can be resumed using the **Continue Measurement** mode. For details please see the following sections:

- “Stop”, page 110
- 5.3.2 “Continue Measurement”, page 153

Remove Operation

A protocol operation can be easily removed from the measurement sequence by

clicking on the − button and selecting the operation to be removed in the pop-up menu.

If you had added one or multiple copies of an operation, these copies will be listed individually in the menu and they also have to be removed individually.

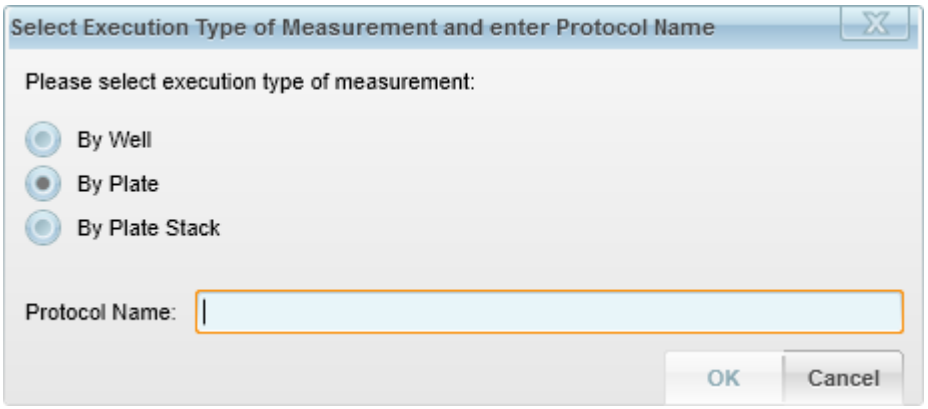
Buttons and Elements

Element	Description
+	Only enabled when protocol is in **Edit** mode. Opens a pop-up menu where you can select a new operation which will be added at the end of the measurement sequence. An entry in the menu may be disabled … • if the max. number of operations of this type has already been inserted. • if an operation is not compatible with the selected execution type (see also section 5.2.1.1 “Execution Type”, page 75).
−	Only enabled when protocol is in **Edit** mode.Opens a pop-up menu where you can select one of the existing operations (original or copy) which will be removed from the measurement sequence. If you remove a **Loop** operation, the operations contained herein will also be removed. To only remove one operation which is contained in a loop, use the − button of the **Loop** operation.

5.2.1.1 Execution Type

If you create a new protocol you will be asked to select the **Execution Type** of the measurement sequence. It determines the order in which the operations are processed.

- **By Well:** The entire measurement sequence is processed in one well (operation by operation), then the next well is processed.
- **By Plate (default):** The first operation is processed in each well of the plate, then the next operation is processed in each well.
- **By Plate Stack (only with optional stacker):** The first operation is processed on each plate in the input magazine (right-hand position). Then the plates are restacked and the next operation is measured on each plate in the input magazine.



Create new protocol dialog

Notice

- The **Execution Type** of a measurement sequence can only be selected when creating a new protocol. It cannot be changed subsequently.
- If you load a protocol, measurement or analysis result, the property **Sequence Executed By** indicates the execution type of the protocol. See also section 5.6 “Load Dialog”, page 180.

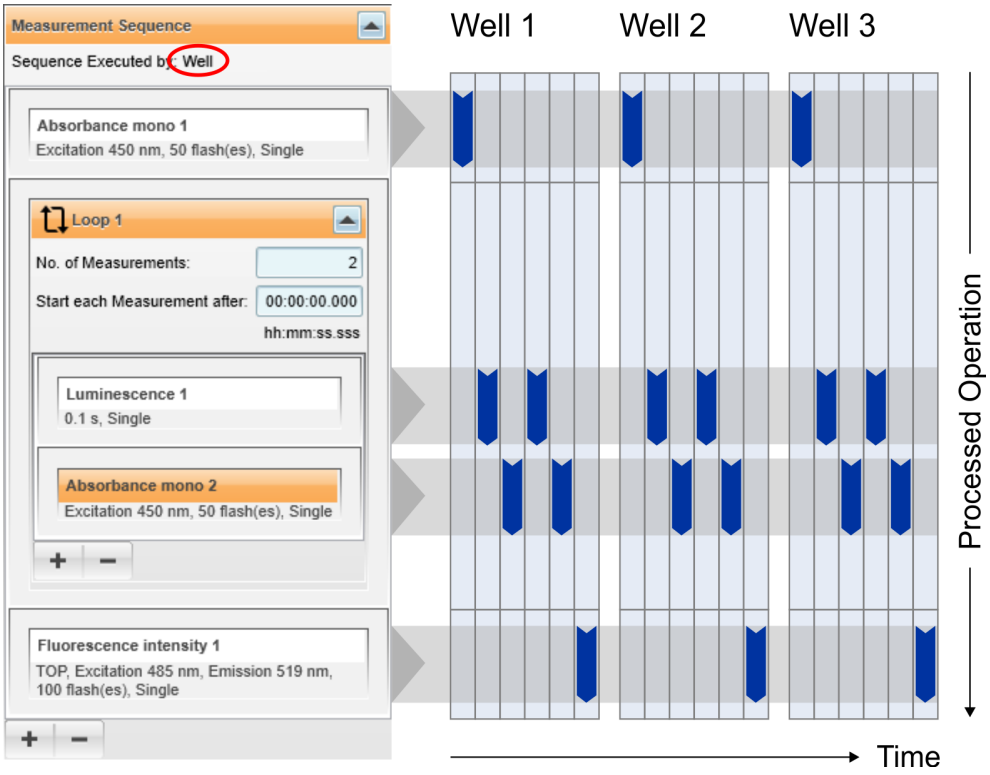
By Well

- The entire measurement sequence is processed in one well (operation by operation). Then the next well is processed until all wells of the plate have been measured.
- *With optional stacker:* When all wells have been processed, the next plate will be loaded and processed according to this pattern until all plates in the input magazine have been measured.

Restrictions

- This execution type cannot be combined with **Stop** operations and the **Continue Measurement** mode.
- It is compatible with the **Automation** mode, but only if the protocol contains no **Stop** operation.
- The following operations are also excluded: **Alpha**, **Label-free**, **Temperature**.
- Only one **Imaging** operation can be added to a **Loop** to avoid frequent changing of the dichroic filter set. **Delay**, **Shake** and **Loop** cannot be added to a **Loop**.
- If you click **Stop** during a measurement, the option "Stop after current Repeat" is disabled. You can only stop a measurement immediately.

Example: By Well

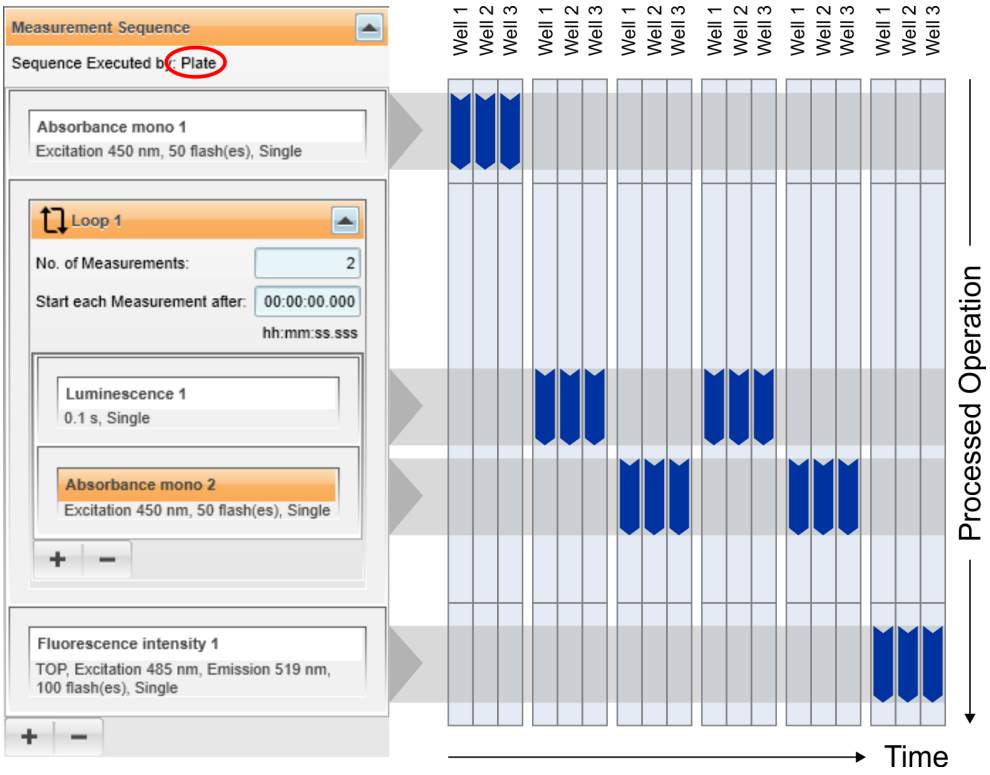


Example for "By Well", plate map with 3 wells

By Plate

- The first operation is processed in each well of the plate. Then the next operation is processed in each well. The measurement is finished if all operations have been processed or a **Stop** operation is reached.
- *With "Stop" operation:* If a **Stop** operation is reached, the measurement will be stopped and saved at this position. When using a stacker, the next plate will be loaded, otherwise the measurement is finished (temporarily).
  The partial measurement can be continued using the **Continue Measurement** mode. See also sections "Stop", page 110 and 5.3.2 "Continue Measurement", page 153.
- *With optional stacker:* When all operations have been processed, the next plate will be processed according to this pattern until all plates in the input magazine have been measured.

Example: By Plate



Example for "By Plate", plate map with 3 wells

By Plate Stack

This execution type is only available if the instrument has an optional **stacker**.

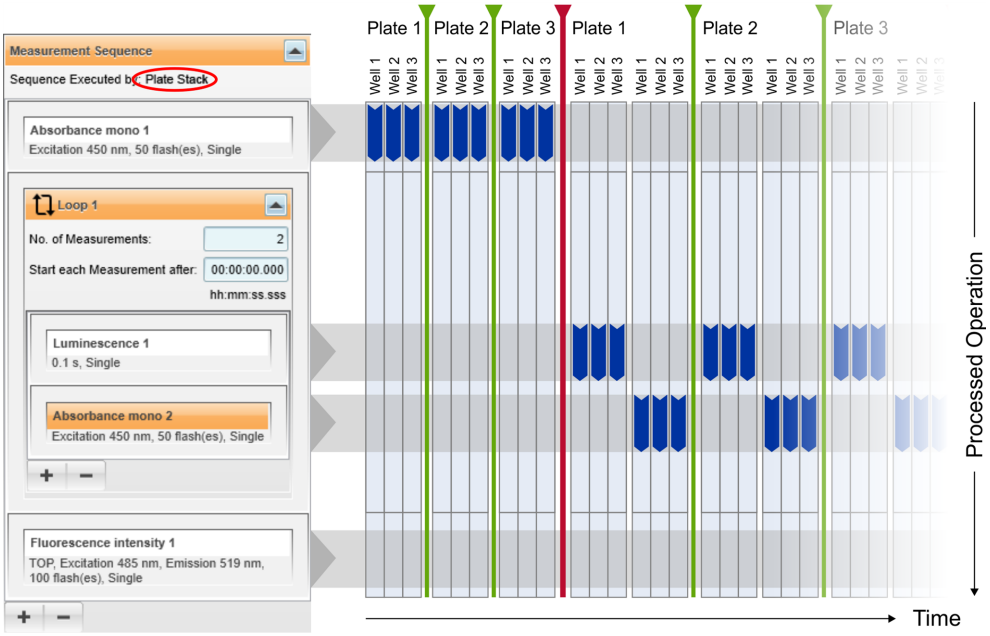
- The first operation is processed in each well of the plate and, one by one, on each plate in the input magazine. Then the plates are automatically restacked from the output magazine back to the input magazine and the next operation is measured on each plate in the input magazine. After the measurement all plates are in the output magazine.
- *With "Stop" operation:* If a **Stop** operation is reached, the measurement will be stopped and saved at this position. The measurement is finished (temporarily). It can be continued using the **Continue Measurement** mode. See also sections “Stop”, page 110 and 5.3.2 “Continue Measurement”, page 153.

Notice

Loops are handled differently by this execution type:

- The operations inside a loop are processed as one group.
- The next plate is only loaded when one entire repeat of the loop has been completed (i.e. all operations processed). See also the example below.

Example: By Plate Stack



Example for "By Plate Stack" (not shown completely):
3 wells selected in plate map, 3 plates in the input magazine of the stacker;
green line = load next plate, red line = restack plates back to input magazine

5.2.1.2 Protocol Operations

In the following sections the protocol operations and their parameters are described in detail.

Absorbance Filter-based (ABS filter)

For filter-based absorbance measurements, light from the Xenon flash lamp is directed through optical filters placed in the filter wheel, selecting for wavelengths between 230 and 1000 nm.

The absorbance measurement head guides the excitation light from the top through the sample, the light is almost parallel. This measurement head can be moved up and down controlled by the software in order to adjust optimum measurement position of the optics. The incident light intensity on the sample (I_0) is measured prior to the actual measurement. The absorbance value is calculated by the equation $A = -log\ (I/I_0)$, where I_0 is the light intensity without any sample and I is the intensity after absorbance.

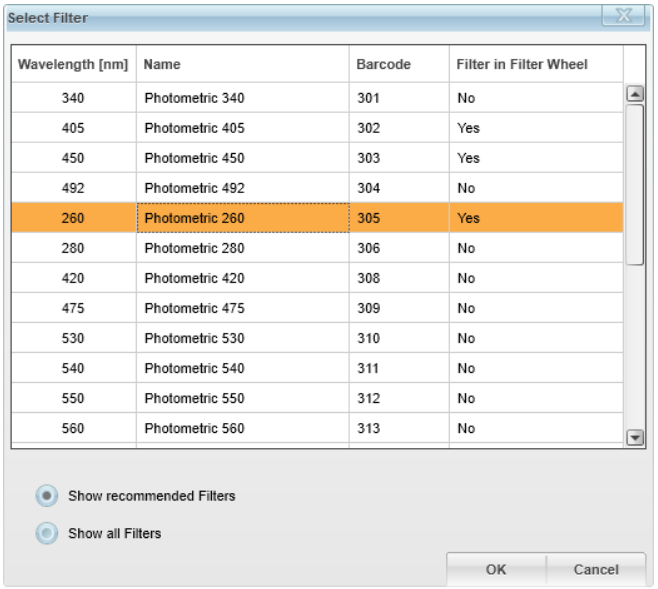
For further details see also sections 4.5.2 “Absorbance Technology”, page 35 and 5.14.4 “Reference value of Xenon flash lamp”, page 252.

Absorbance Filter Parameters

Parameter	Description
Measurement Mode	See section 5.2.1.3 “Measurement Modes”, page 112. Depending on the selected measurement mode, additional parameters are displayed in a separate section below the operation parameters.
Excitation Wavelength / (Barcode)	Click on the ... button to select an excitation filter (**Select Filter** dialog is opened). The wavelength and the barcode of the selected filter will be displayed. See also section “Select Filter Dialog”, page 80.
Measurement Height [mm]	Focus height within the sample, measured from the bottom of the plate (where it contacts the plate carrier). This value can be automatically optimized (see section “Measurement Height”, page 119).
Number of Flashes	Number of flashes for one measurement.
Optimization	This button is only enabled if a plate type was selected. It opens the **Optimization Wizard** (see section 5.2.1.4 “Optimizations”, page 115) which helps you to find the best settings for selected operation parameters. The available optimizations depend on the measurement technology.

Select Filter Dialog

Select the desired filter and click **OK**.
You can sort the table by clicking on a column title.



Parameter	Description
Wavelength [nm]	Excitation wavelength of the filter.
Name	Filter name
Barcode	Filter barcode
Filter in Filter Wheel	Indicates whether the filter is currently available in the instrument or not. To insert the required filter see section 6.1 “Change Excitation Filters”, page 257.
Show recommended Filters	Displays only filters which are suitable for the selected technology.
Show all Filters	Displays all filters regardless of their compatibility.

Absorbance Monochromator-based (ABS mono)

For monochromator-based absorbance measurements, light from the Xenon flash lamp is directed through the excitation double monochromator into the sample. The wavelength can be chosen in a range from 230 and 1000 nm.

The absorbance measurement head guides the excitation light from the top through the sample, the light is almost parallel. This measurement head can be moved up and down controlled by the software in order to adjust optimum measurement position of the optics. The incident light intensity on the sample is measured prior to the actual measurement and the absorbance value is calculated by the equation *A:= -log (I/I0)*, where *I0* is the light intensity without any sample and *I* is the intensity after absorbance.

For further details see also sections 4.5.2 “Absorbance Technology”, page 35 and 5.14.4 “Reference value of Xenon flash lamp”, page 252.

Absorbance Mono Parameters

Parameter	Description
Excitation Wavelength [nm]	Wavelength of light coming from excitation double monochromator.
Measurement Height [mm]	Focus height within the sample, measured from the bottom of the plate (where it contacts the plate carrier). This value can be automatically optimized (see section “Measurement Height”, page 119).
Number of Flashes	Number of flashes for one measurement.
Optimization	This button is only enabled if a plate type was selected. It opens the **Optimization Wizard** (see section 5.2.1.4 “Optimizations”, page 115) which helps you to find the best settings for selected operation parameters. The available optimizations depend on the measurement technology.

Alpha (ALPHA)

The Alpha technology provides a very high sensitivity method of detecting molecular interactions. It is based on the laser excitation of special Alpha technology donor beads and the detection of emission light from bound acceptor beads. In contrast to conventional fluorophores, the emission light has a shorter wavelength than the excitation light. The donor beads are generally coated with molecules allowing capture of the sample. The acceptor beads are coated with appropriate molecular binding partners. The laser illuminates the sample wells at a wavelength of 680 nm, exciting molecules in the donor beads. The excitation time is adjustable within limits of 1 s total measurement time per well. This energy is then transferred to any bound acceptor bead which then emits in the range of 450-645 nm. The emitted light is detected by a very high sensitivity photomultiplier (PMT). The PMT is located right above the sample and reads the well adjacent to the one excited by the laser. Both excitation and detection occur from the top of the sample.

- With Alpha measurements, only execution types **By Plate** and **By Plate Stack** can be used.
- Alpha operations can only run in **Single** measurement mode and cannot be added to a **Loop** operation.
- When working with Alpha technology, bright light and extreme temperature fluctuation should be avoided and only opaque plates should be used.

Notice
The measurement sequence can only contain one Alpha operation.

For further details see also section 4.5.3 “Alpha Technology”, page 37.

Alpha Parameters

Parameter	Description
Distance Between Plate and Detector [mm]	Distance between upper surface of plate and detector, valid range between 0-3 mm, lower distance reduces crosstalk.
Excitation Time [ms]	Length of time the laser is used to excite the sample.
Total Measurement Time [ms]	Comprises excitation and emission times.
Optimization	This button is only enabled if a plate type was selected. It opens the **Optimization Wizard** (see section 5.2.1.4 “Optimizations”, page 115) which helps you to find the best settings for selected operation parameters. The available optimizations depend on the measurement technology.

Fluorescence Intensity (FI)

For fluorescence intensity measurements, light from the Xenon flash lamp is directed through the excitation double monochromator into the sample to excite the fluorochrome. The wavelength can be chosen in a range from 230 up to 835 nm. A signal from a reference photodiode is always read after every flash. The reference signal is then compared to the original reference value taken during the first measurement of a protocol and the results are corrected for the same excitation energy. The fluorescence measurement head can be moved up and down controlled by the software in order to adjust the focal point of the excitation optics.

The fluorescence produced by the excitation light emits at a different (higher) wavelength. This emission light is then directed through the emission monochromator to the detector.

FI measurements are possible from the top or from the bottom of the well. Reading from above is the most efficient way when no seal is used because no plastic surface has to be penetrated. For adherent cells and lidded plates, reading from below provides superior efficiency. Both above and below reading can be used in the same measurement sequence/protocol.

For further details see also sections 4.5.1 “Fluorescence Intensity With Quad-Monochromator”, page 34 and 5.14.4 “Reference value of Xenon flash lamp”, page 252.

Fluorescence Intensity Parameters

Parameter	Description
Measurement Mode	See section 5.2.1.3 “Measurement Modes”, page 112. Depending on the selected measurement mode, additional parameters are displayed in a separate section below the operation parameters. If you select measurement mode **Excitation Scan** or **Emission Scan**, the additional parameters will replace the corresponding operation parameter for excitation or emission wavelength.
Measure from	The measuring direction from above (**Top**) or from below (**Bottom**) can be chosen here.
Excitation Wavelength [nm]	Wavelength (between 230-835 nm) used to excite the fluorochrome in the sample, needs to be at least 15 nm lower than emission wavelength.
Emission Wavelength [nm]	Wavelength (between 245-850 nm) of the resultant fluorescence to be measured; needs to be at least 15 nm higher than excitation wavelength.
Measurement Height [mm]	Focus height within the sample (only editable for **Top**). The height is measured from the bottom of the plate (where it contacts the plate carrier). The value can be automatically optimized (see section “Measurement Height”, page 119).
Number of Flashes	Number of flashes for one measurement.
Optimization	This button is only enabled if a plate type was selected. It opens the **Optimization Wizard** (see section 5.2.1.4 “Optimizations”, page 115) which helps you to find the best settings for selected operation parameters. The available optimizations depend on the measurement technology.

Imaging (IMG)

Notice

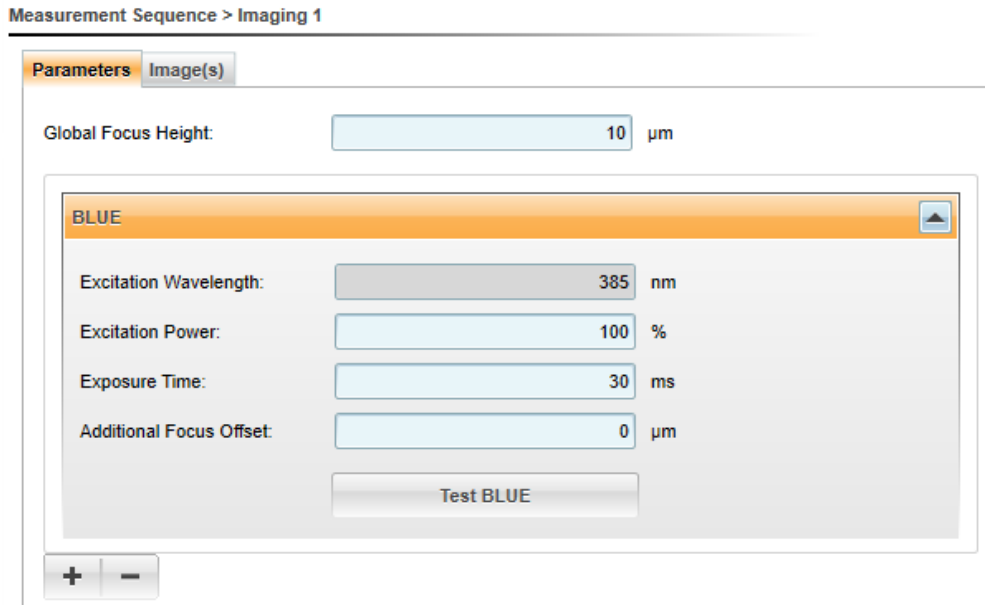
For dedicated purposes application-oriented documentation is available. Please contact your PerkinElmer specialist for more information.

Application and Technical Notes are published on our web page on a regular basis. Please visit www.perkinelmer.com/ensight.

Two different tabs are displayed for an Imaging operation in the **Content Area**:

- **Parameters:** Allows you to add, configure and test imaging channels.
- **Image(s):** Displays test images of a channel or the entire operation.

Parameters



Imaging operation with one channel

For Imaging operations you cannot change the measurement mode (Single). One image will be recorded per well (and per channel).

Measured images are only stored when **Archive Images** is activated in the **Settings** (see section 5.10.3.6 “Image Archive Settings”, page 225). Otherwise only the images of the last measurement are available and will be overwritten by the next imaging measurement.

Notice

- In case of autofocus issues, please see section 5.14.5 “Autofocus Failure (IMG)”, page 253.
- If some of your images are out of focus when using execution type **By Well**, please see section 5.14.7 “Unsharp Images Due to Temperature Changes”, page 254 for troubleshooting.

Imaging Parameters

Parameter	Description
+	Adds a channel to the operation. A new dialog will be opened where you can select the excitation wavelength. In a second step you can confirm or change the default channel name.
−	Allows you to remove one of the existing channels. This channel can be selected from a pop-up list.

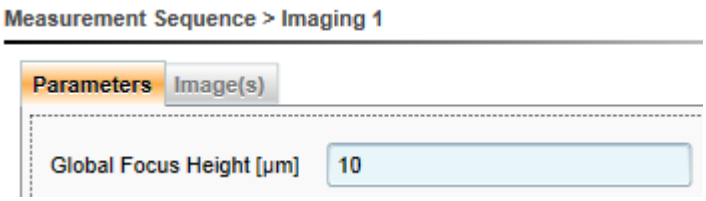
Parameter	Description
Global Focus Height [µm]	Focus height within the sample, measured from the bottom of the well. This value is valid for all channels, but it can be adjusted individually if you enter an **Additional Focus Offset** for a single channel (see below).
Parameters for each Channel	**For Fluorophore and Transmission channels:** • **Excitation Wavelength [nm]** (display only) • **Excitation Power [%]** • **Exposure Time [ms]** • **Additional Focus Offset [µm]:** Allows you to modify the selected **Global Focus Height** by a certain offset (only valid for this channel). **Only for Transmission channels:** Using these parameters you can decide to generate brightfield images, DPC images or both. A separate channel will be created during measurement for each selected option. At least one of the following options must always be selected. To unselect an option, please first select the other option, otherwise the check box is locked. • **Generate Brightfield (BF):** Generates a brightfield image which is available later for display and analysis. If you are only interested in the DPC image, you can uncheck this option. • **Generate DPC:** Computes a Digital Phase Contrast image based on brightfield images. The DPC image can also be generated if the brightfield option (see above) is not checked. In this case only the DPC image will be available later for display and analysis. **Notice** Once the transmission channel has been configured as desired you should make sure that only those images are generated which are really required for later analysis. Thereby you can save disk space which would otherwise be occupied by unused images.

Parameter	Description
Test <CHANNEL>	Each channel has a **Test <CHANNEL>** button. If you select a well (in the plate map on the **Test Imaging** tab) and click this button, a test image of this channel is recorded and displayed on the **Image(s)** tab in the Content Area. You can then optimize the parameters and take new test images. To test all channels of an Imaging operation use the **Test Imaging <1 / 2 >** button(s) below the plate map (see section 5.8.2 “Test Imaging”, page 210). **Notice** Test images cannot be recorded while the magazine table of the optional stacker is installed.

How to add a channel

After adding an Imaging operation to the **Measurement Sequence**, you can add channels and configure their parameters in the **Content Area**.

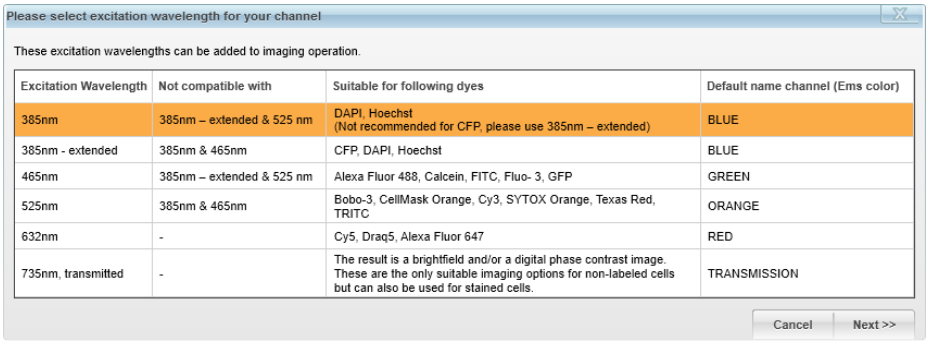
1. Enter the **Global Focus Height** which will be used for all channels (can be corrected later).



2. Click + to add a new channel. New channels will always be inserted at the end of the channel sequence.

   A new window is opened.

3. Select one of the available excitation wavelengths for the new channel.



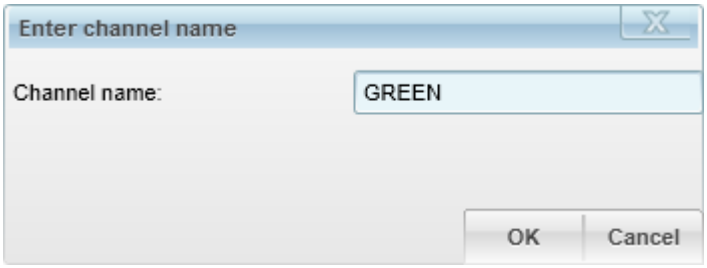
Notice

- The list of available wavelengths depends on the dichroic filter set(s) and on the channels which you have already added to this operation. Certain wavelength combinations may require a second **Imaging** operation. For details see section “Select Excitation Wavelength”, page 91.

4. Click **Next >>**.

   A new dialog is opened.

5. Change or confirm the default channel name.

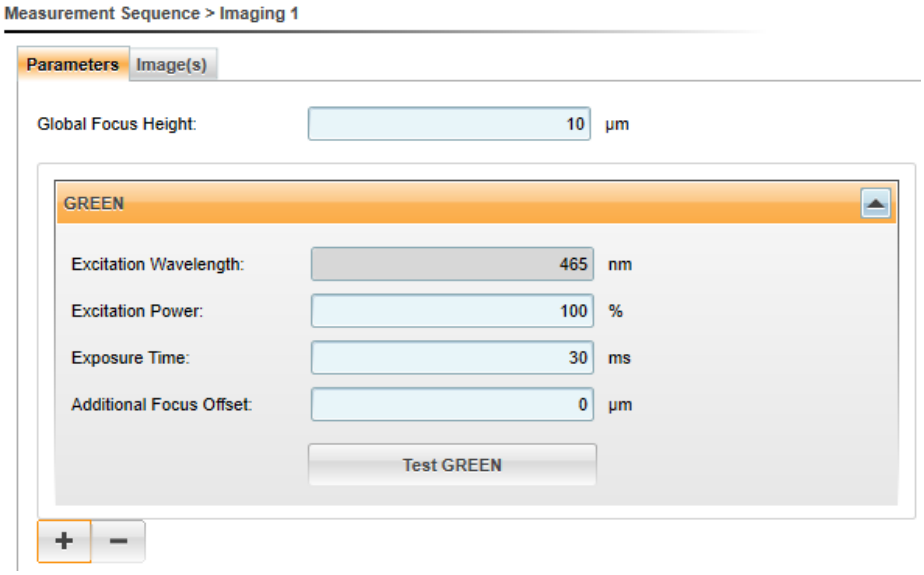


Notice

- It is recommended to use the name of the fluorophore or marker instead. This makes it easier to interpret the analysis results of the channel.
- For the TRANSMISSION channel you can keep the default name. The resulting channel(s) will automatically get a suffix (-BF or -DPC) to indicate the channel type.
- Channel names must be unique within one protocol.

6. Click **OK**.

   The new channel is added to the **Imaging** operation.



How to configure and test a channel

After adding an **Imaging** operation to the **Measurement Sequence**, you can add and configure channels and generate test images. These test images are also required to test an image analysis (see also 5.2.2.1 “Image Analysis”, page 123). Test images are available and displayed until you select a different well or change the parameters of a channel.

1. Configure the parameters of the channel as desired.

   Notice
   The **Global Focus Height** is set for all channels, but you can define an individual **Additional Focus Offset** for each channel.

2. Take test images to find the best settings:
   - Select a well in the plate control (**Test Imaging** tab on the right).
   - Click **Test <CHANNEL>** at the bottom of the channel.

     A test image for this channel will be recorded and displayed on the **Image(s)** tab.

   Notice
   Test images cannot be recorded while the magazine table of the optional stacker is installed.

3. To test all channels of an **Imaging** operation:
   - Select a well in the plate control (**Test Imaging** tab on the right).
   - Click **Test Imaging <1 / 2>** below the plate control.

     Test images for all channels of the operation will be recorded and displayed on the **Image(s)** tab. These images can be used to test the image analysis (see section 5.2.2.1 “Image Analysis”, page 123) and they persist until they are replaced by new test images.

Transmission

If the option **Digital Phase Contrast** (DPC) is used for a **Transmission** channel, the DPC image will be computed based on the measured brightfield images. Using the operation parameters you can decide whether the brightfield shall also be available for display and analysis. If you select to keep both images, they will be displayed In the **Channel** section as two separate channels for brightfield and DPC, e.g. “Channel 1 (BF)” and “Channel 1 (DPC)”, so that you can select and view both images individually. The imaging analysis operations will automatically use the channel which is most suitable for the analysis.

Recommend workflow:
First check the images of the brightfield channel and optimize parameter settings until your cells look fine. Then proceed with DPC or fluorescence channels.

Channels and Filter Sets

The EnSight is equipped with two dichroic filter sets. An **Imaging** operation can only contain channels (i.e. excitation wavelengths) which use the same filter set (see overview below). To add a channel which requires a different filter set, you have to add a second **Imaging** operation (and a second image analysis operation, if applicable).

Notice

- The measurement sequence can only contain one **Imaging** operation per dichroic filter set, i.e. **Imaging 1** or **Imaging 1 and Imaging 2** (each using a different filter set).
- However, you can insert copies of the existing **Imaging** operation(s) (see section "Copy Operation", page 74).
- If using execution type **By Well**, only one **Imaging** operation can be added to a **Loop** to avoid frequent changing of the dichroic filter set.

For further details see also sections 4.5.7 "Imaging Technology", page 42 and 7.15 "Application Wavelengths", page 271.

Overview: Filter Sets and Light Sources

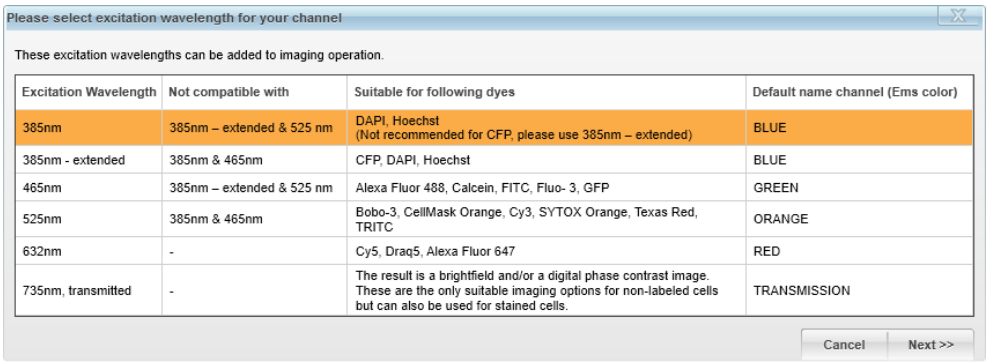
Valid combinations of dichroic filter sets and light source filters/LEDs:

Excitation Wavelength	Emission Color	PRIMARY filter set	SECONDARY filter set (optional)
385 nm	BLUE	●	
385 nm, extended	BLUE		●
465 nm	GREEN	●	
525 nm	ORANGE		●
632 nm	RED	●	●
735 nm, transmitted	TRANSMISSION	●	●

Select Excitation Wavelength

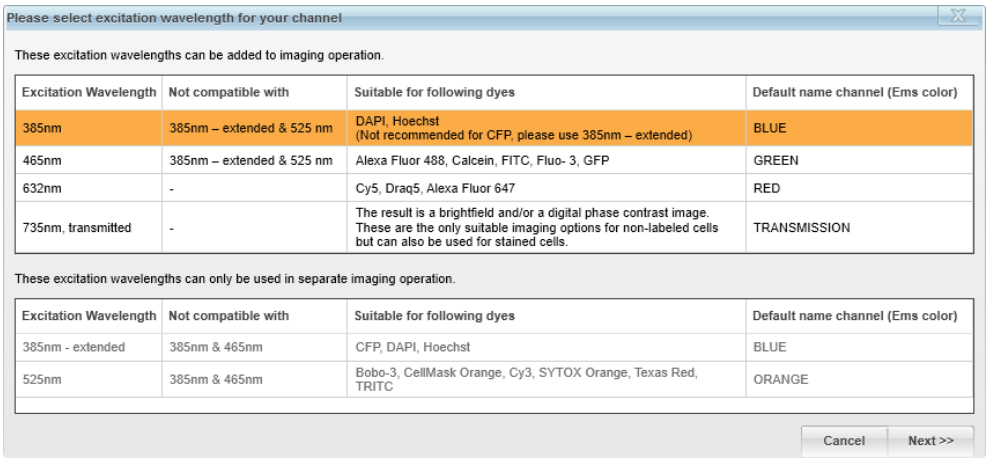
When you add a channel, a separate dialog allows you to select the excitation wavelength of the new channel. The list of available excitation wavelengths depends on the filter set which is used by the **Imaging** operation.

- If you add the first channel to your first **Imaging** operation, there are **no restrictions**. The filter set to be used has not been determined yet.



- As soon as a second operation or further channels have been added, the dialog will also show a second table. It contains excitation wavelengths which are **incompatible** with the filter set assigned to this **Imaging** operation. These

wavelengths can only be used in a separate **Imaging** operation.



Buttons and Elements

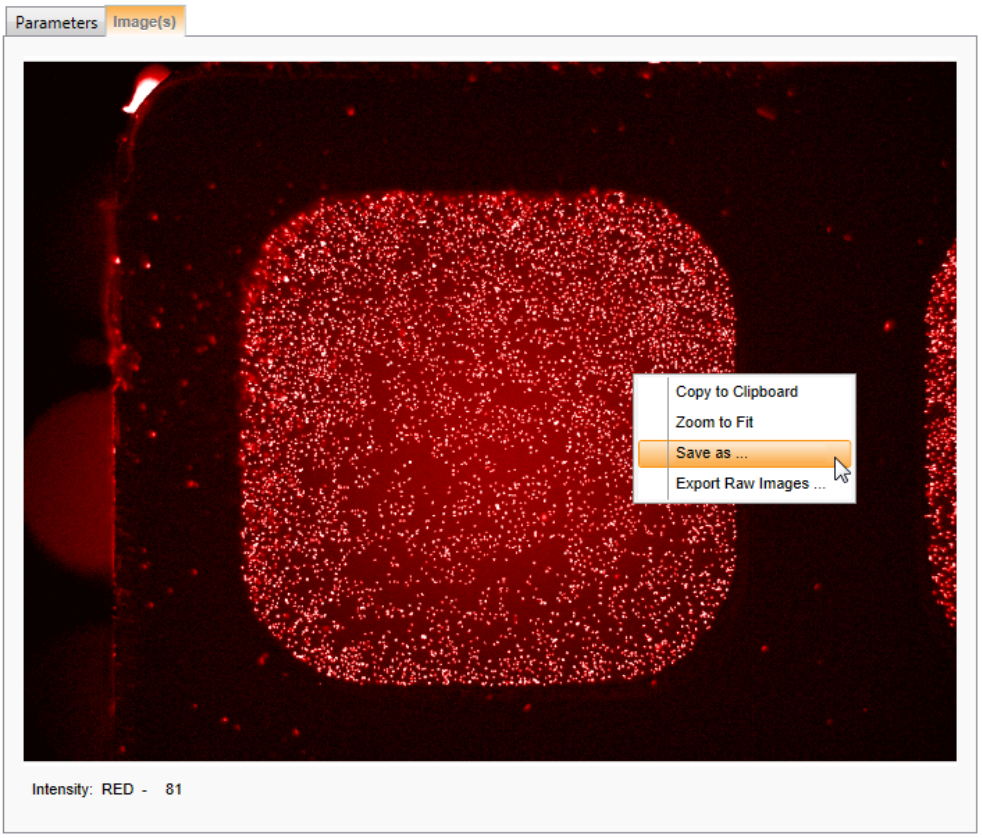
Parameter	Description
Excitation Wavelength	Excitation wavelength / light source
Not Compatible With	List of excitations wavelengths which cannot be combined with this channel, because they require a different filter set. Channels using one of these wavelengths have to be added to a separate **Imaging** operation.
Suitable for Following Dyes	Example dyes which are suitable for this excitation wavelength.
Default Name Channel (Ems Color)	The emission color is used as default name for the new channel. It is recommended to change it and use the name of the fluorophore or marker instead. This makes it easier to interpret the analysis results.

Image(s)

This tab is opened in the **Content Area** as soon as you take test images of a channel.

- If you move the mouse to a spot on the image, the local **intensity** (0-4095) of that spot is displayed below the image (for each channel). The value is updated as soon as you stop moving the mouse.
- You can **zoom** the image using the mouse wheel.
- Drag the image with the mouse to move the field of view.

Using the **Display Options** (beneath the plate map on the right) you can adjust color, brightness and contrast of the image and enable/disable channels and overlays. These settings are only applied to the image display, they do not affect the original images or the image analysis. See also section 5.8.3 “Display Options”, page 211.

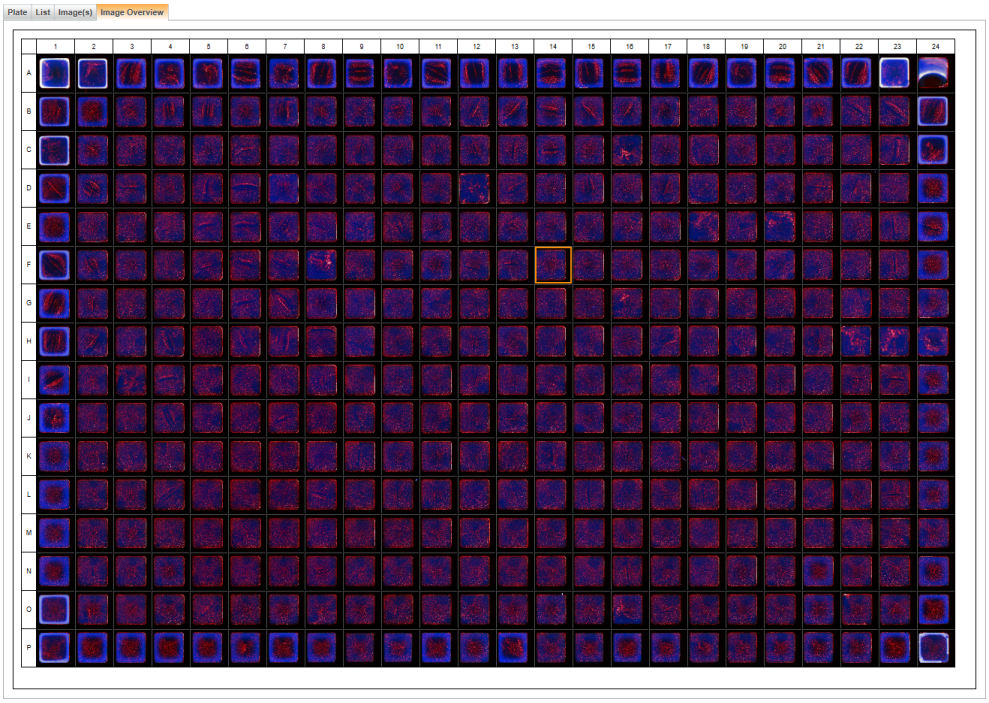


Context Menu

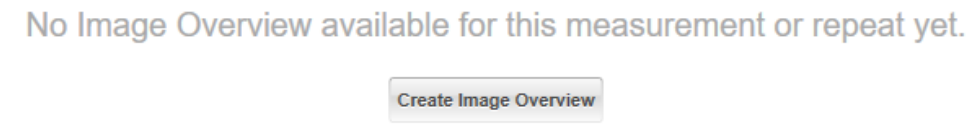
Element	Description
Copy to Clipboard	Copies the image with full resolution (as displayed with colored channels) to the Windows® clipboard to paste it into a different program. This will reduce the color depth from 12 bit grayscale per channel to 8 bit color (no separate channels anymore).

Element	Description
Zoom to Fit	Displays the image so that it fits into the **Content Area**.
Save as ...	Allows you to save the current image with full resolution (as displayed with colored channels). This will reduce the color depth from 12 bit grayscale per channel to 8 bit color (no separate channels anymore). The file format can be selected by appending the corresponding extension to the file name (*.bmp, *.jpg, *.png, *.gif, *.tif or *.tiff).
Export Raw Images ...	Allows you to save the unscaled RAW images with full resolution and original color depth (one 16 bit grayscale TIFF file per channel, first 12 bits are used). To view these images outside of Kaleido you need a third-party image viewer which is capable of displaying 16 bit TIFF images (e.g. IrfanView). Each exported image file will get the same time stamp as the original image so that it indicates the measurement time.

Image Overview



On the **Image Overview** tab you can view thumbnail images of the measured wells directly in the plate layout (reduced resolution). The thumbnail calculation is triggered manually. If no thumbnails are available for the selected measurement (or the selected repeat) yet, the following text will be displayed:



Click **Create Image Overview** to start the calculation. The user interface will be blocked until the calculation has been completed. It cannot be canceled. If the measurement contains repeats, you will have to trigger the calculation individually for each repeat.

Notice
Depending on the number of wells and channels it may take up to a few minutes until the image overview is displayed completely.

Options

- Clicking on a thumbnail selects the corresponding well on the plate map and switches to the **Image(s)** tab to see the full resolution image of the well.
- You can **zoom** the image overview using the mouse wheel.
- The representation of the image overview can be adjusted using the **Display Options** (see section 5.8.3 “Display Options”, page 211) as for regular images. Additionally, there is an option to add a **Label** to each thumbnail (well coordinates, different styles available).

Notice
Please note that any adjustment of brightness or contrast will always be applied to the entire image overview, not to individual thumbnails.

Context Menu

Element	Description
Well Selection	If this mode is activated, clicking on a thumbnail will display the original image on the **Image(s)** tab.
Pan	If this mode is activated, you can pan the view (if view was zoomed in).
Zoom to Fit	Displays the image overview so that it fits into the **Content Area**.
Copy ...	• **Copy Image Overview to Clipboard:** Copies the image overview (as displayed) to the Windows® clipboard to paste it into a different program. • **Save Image Overview as ...:** Allows you to save the current image overview (as displayed) in PNG format.

Label-free (LF)

The Label-free mode utilizes Corning Epic® Label-free technology, which depends on movements and/or binding events taking place upon the sensors embedded in the wells of the microplates. As material moves in the sensing zone and/or binds to the sensor surface, the wavelength of light reflected from the biosensor is changed. The amount of wavelength change is proportional to the amount of material that binds to the sensor surface. The integrated Label-free unit uses a SuperLum diode for excitation light and a spectrometer for detection of reflected light.

- **Cell-based Assays**
  Label-free cell-based assays measure a phenotypic “whole cell” response after ligand addition and stimulation. This response is the result of a series of biochemical signaling interactions within a cell, and includes mass movements of mostly proteins. This mass movement (dynamic mass redistribution, or DMR) changes the refractive index in the “sensing zone”, which in turn changes the wavelength of the light reflected by the biosensor. This change in wavelength is detected by the EnSight.
- **Biochemical Assays**
  Label-free microplates incorporate patented dual-sensor self-referencing technology for protein/ligand assays, ensuring that only true analyte binding is reported. Changes in the index of refraction upon a binding event, indicated by a shift in wavelength, are measured.

For further details see also section 4.5.5 “Label-free Technology”, page 38.

Label-free measurements intend to analyze how the sample properties change over time and thus change the reflected wavelength. Therefore, Label-free measurements usually consist of at least two subsequent runs (Baseline and Final). The initial Baseline run is followed by the addition of compounds that start a biological or chemical reaction inside each well. The Final run contains the kinetic profile as a reaction of that compound addition. It can also consist of multiple measurements.

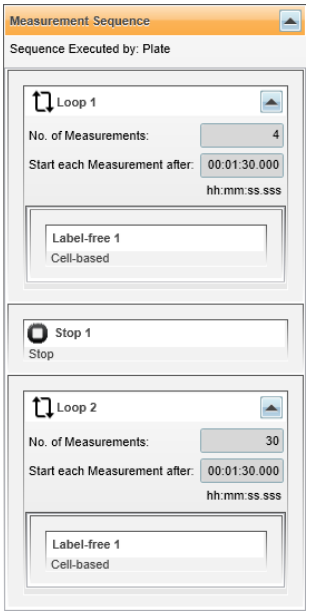
Setting up a Label-free protocol

For setting up a protocol for Label-free measurements, you should be familiar with the following concepts and functions:

- **Continue Measurement** mode (see section 5.3.2 “Continue Measurement”, page 153)
- **Execution Type** (see section 5.2.1.1 “Execution Type”, page 75)
- **Stop** operation (see section “Stop”, page 110)
- **Loop** operation (see section “Loop”, page 108)

Example

Factory-preset protocol *"Label Free 384 Cell-based"*:



- 4 Baseline repeats
- Stop for addition of compounds
- 30 Final repeats

Example: How to set up a Label-free protocol

1. On **Setup Protocol**, create a new protocol and select an **Execution Type**.

   Notice
   Execution type **By Well** is not compatible with Label-free.

2. Select a suitable **Plate Type** for Label-free and define the **Plate Map**. See also section "Plate Map and Measurement Progression", page 99.
3. Add a **Loop** operation to the measurement sequence.
4. Add a **Label-free** operation to this loop. It will be used to measure the Baseline.
5. Select the **Assay Type** for the **Label-free** operation.
6. Configure the loop parameters:
   - **No. of Measurements:** Enter the number of Baseline repeats (here: 4).
   - **Start Each Measurement After:** Enter the time interval for starting a new repeat.

     Notice
     If the specified interval is shorter than it actually takes to measure one repeat, the repeats will be performed as fast as possible in direct succession. See also section 7.16.3 "Maximum Throughput (Time Per Plate)", page 272 for getting an impression of the measurement times.

7. Add a **Stop** operation to the measurement sequence.
8. Add a second **Loop** operation.

9. Add a copy of the **Label-free 1** operation to the loop (see section "Copy Operation", page 74). It will be used to measure the Final repeats. The same **Assay Type** will be used.
10. Configure the parameters of **Loop 2**:
    - **No. of Measurements:** Enter the number of Final repeats (here: 30).

      Notice
      If you are unsure how many repeats are required, select a few more. You can stop the measurement earlier if necessary.

    - **Start Each Measurement After:** Enter the time interval for starting a new repeat.
11. Add a **Post Processing Sequence** to the protocol to automatically export the results for further data analysis.
    - For export to **MyAssays Desktop** select export format **XML**.
    - For details see also section 5.12 "MyAssays Desktop Data Analysis", page 242.
12. Click **Save** to save the protocol.

Performing Label-free measurements

For measuring the first step of the protocol (until the Stop operation) you have to run the protocol **Standard** mode. After the dispensing break you can continue the measurement using the **Continue Measurement** mode. The second step with the Final repeats will be measured.

Example: How to perform a Label-free measurement

1. Switch on the instrument and let it warm up for 45 minutes before starting the measurement.
2. Load the **Label-free** protocol, if necessary.
3. On **Run Protocol**, select the **Standard** mode and click **Start**.

   The first loop with the Baseline repeats is measured (step 1).

   Notice
   Even if you think that no more Baseline repeats are required: Do not stop the measurement, otherwise it cannot be continued anymore!

4. When the **Stop** operation is reached, the partial measurement is stopped and saved. You can now e.g. eject the plate for the addition of compounds.

   Alternatively, restart the same measurement again (step 3) if you are not satisfied with the Baseline results yet.
5. To continue the measurement, switch to the **Continue Measurement** mode on **Run Protocol**. Make sure that your Baseline measurement is loaded (results are displayed on **View Results**).
6. Click **Start**.

The second loop (after the stop) with the Final repeats is processed (step 2).

Notice
You can stop the measurement if no more Final repeats are required (click **Stop** and select option "Stop after current repeat"). Otherwise just wait until the measurement has been completed.

Notice
Protocols containing **Stop** operations cannot be used in automation mode (under remote control via external scheduler). The measurement of such a protocol will be rejected.

Notice
For dedicated purposes application-oriented documentation is available. Please contact your PerkinElmer specialist for more information.

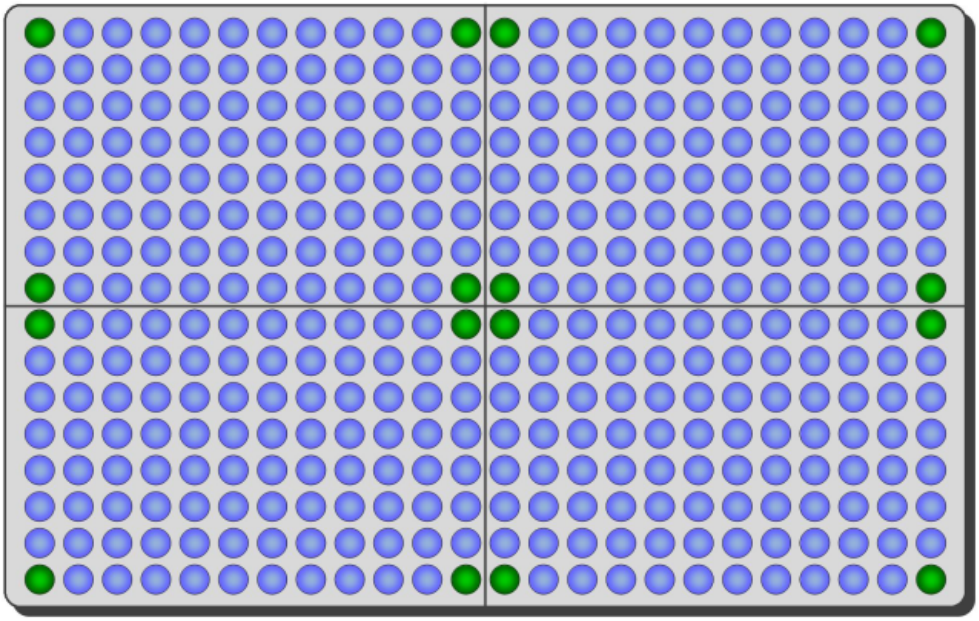
Application and Technical Notes are published on our web page on a regular basis. Please visit www.perkinelmer.com/ensight.

Plate Map and Measurement Progression

The Label-free measurement is carried out quadrant-wise (see figure below).

- As soon as one well of a quadrant is selected in the **Plate Map**, all wells of this quadrant will be scanned completely. However, only the values of the selected wells will be stored.
- By selecting none of the wells of a quadrant, the quadrant will not be measured.

Additionally, all four corner wells of a Label-free plate (see figure below, marked in green) of each quadrant used in the measurement have to be filled with liquid (buffer, assay sample or simply water). If these wells are not filled with liquid or they are accidentally damaged, the corresponding quadrant of the plate will not be recognized by the instrument.



Schematic view of the Label-free quadrant wise plate measurement. A plate is divided into 4 quadrants (separated by thin black lines), which each contain 4 corner wells, marked in green. The corner wells need to be intact and filled with liquid for the corresponding quadrant to be recognized.

Live Response

During a Label-free measurement a **Live Response [pm]** is automatically calculated and displayed on the **Plate** and **Graph** tabs. Raw signals are only displayed on the **List** tab.

The live response helps you to analyze whether further Baseline/Final runs are required. It is defined as follows (individually calculated for each well and for each Baseline/Final repeat x, t = time of repeat x, t_0 = time of repeat 1):

- **Cell-based label-free assay (CBA):**
  Live Response [pm] = (Signal [fm] (t) – Signal [fm] (t_0)) / 1,000
- **Biochemical label-free assay (BCA):**
  Live Response [pm] = (Signal [fm] (t) - Reference [fm] (t)) - (Signal [fm] (t_0) - Reference [fm] (t_0)) / 1,000

The first measured Baseline is subtracted from itself and from each subsequent repeat (Baseline or Final). This leads to a normalization of the live response. If you look at the **Graph** view (or select multiple wells and click **Show Detailed Graph**), all curves start at the zero position and can be compared to each other.

Numbering of Measurements

Repeats are consecutively numbered (irrespective of whether it is a Baseline or Final measurement). On the **List** tab, measurements before or after a **Stop** can be distinguished by means of the **Step** number.

Example with Baseline (step 1: 3 repeats) and Final (step 2: 4 repeats):

Step	Repeat
1	1
1	2
1	3
2	4
2	5
2	6
2	7

Label-free Parameter

Parameter	Description
Assay Type	Biochemical or cell-based assay

Luminescence (LUM)

Detection of biological or chemical induced light emission from samples using a very high sensitivity luminescence photomultiplier (PMT) as detector. It has extremely low background, high dynamic range and spectral response from 450 nm up to 645 nm, measuring the relative amount of the emission light. The detector can be lowered so that it is just above the plate, thus reducing the crosstalk between wells and maximizing detection efficiency. A sensor automatically determines the precise plate height, which prevents the aperture from touching the plate. The aperture is suitable for 96-well as well as for 384-well plates.

For further details see also section 4.5.4 “Luminescence Technology”, page 38.

Luminescence Parameters

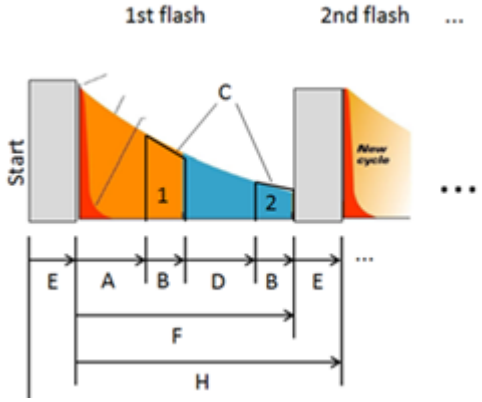
Parameter	Description
Distance Between Plate and Detector [mm]	Distance between upper surface of plate and detector, valid range between 0-3 mm with 0.1 mm steps, lower distance reduces crosstalk.
Measurement Time [s]	Duration of measurement per well.

Time-resolved Fluorescence (TRF)

The technology is based on fluorescence of lanthanide chelates (Europium, Samarium, and Terbium). The fluorescence decay time of these lanthanide chelate labels is much longer than traditional fluorophores, allowing efficient use of temporal resolution for reduction of autofluorescent background. The large Stokes’ shift (difference between excitation and emission wavelengths) and the narrow emission peaks contribute to increasing signal-to-noise ratio.

The excitation light source is a Xenon flash lamp, and the excitation wavelength of the light is selected by an optical filter (excitation filter 320 or 340 nm) placed in the filter wheel for **Top** measurements, and by the excitation monochromator for **Bottom** measurements. The excitation light is directed to the TRF measurement head, employing a dichroic mirror with cut-on wavelength at 400 nm. Excitation light below that wavelength is reflected into to the sample to excite the fluorochrome. This produces fluorescence at a different wavelength.

With both TRF Top and TRF Bottom, the fluorescence from the sample passes the dichroic mirror and enters the emission monochromator where it is detected by the side-on photomultiplier tube detector. In TRF measurements it is operated in digital mode, i.e. every single photon emitted by the sample will be counted. In order to minimize background of the sample, the fluorescence is measured with a delay time for a certain window time. Only those photons after the user-defined time delay and within one or multiple defined time windows contribute to the TRF signal.



Legend

A: Delay

B: Window time

C: Number of sequential windows

D: Time between windows

E: Initialization time of flash unit

F: Max. time frame

H: Time between flashes

At the default flash rate of 500 Hz, the time between two flashes is 2000 µs (H). To calculate the available time for TRF measurements between the flashes, the initialization time of the flash unit (550 µs, E) has to be deducted from this interval. This leads to a maximum time frame of 1450 µs (F). This limitation has to be considered when selecting **Delay**, **Window Time** and **Number of Sequential Windows**. The **Total Time** calculated and displayed by the software may not exceed the maximum time frame (**F**):

$$Total\ Time \leq F$$

Definitions:

$$Total\ Time = A + (C * B) + ((C - 1) * D)$$

$$F = H - E$$

If the allowed time frame is exceeded, you will get an error message as soon as you save the protocol. Reduce one or multiple of these parameters to keep within the allowed range (see example below).

For further details see also sections 4.5.6 “Time-resolved Fluorescence (TRF) Technology”, page 40 and 5.14.4 “Reference value of Xenon flash lamp”, page 252.

Example for Parameter Validation

- Delay (A): 400 µs
- Window Time (B): 300 µs
- Number of Sequential Windows (C): 3
- Time Between Windows (D): 5 µs (fix)
- Time Between Flashes (H): 2000 µs (flash rate = 500 Hz)

$$Total\ Time = 400 + (3 * 300) + ((3 - 1) * 5) = 1310\ \mu s$$

$$F = 2000 - 550 = 1450\ \mu s$$

$$1310\ \mu s \leq 1450\ \mu s$$

The validation is successful, these operation parameters will work.

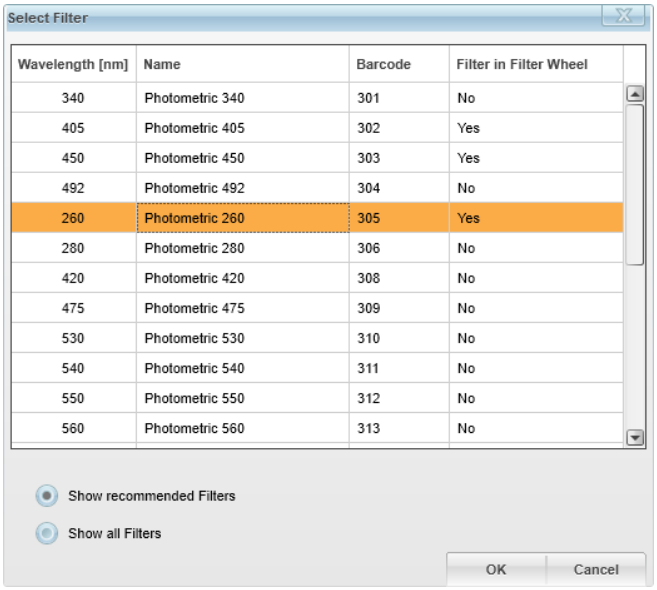
Time-resolved Fluorescence Parameters

Parameter	Description
Measure from	**Top:** TRF measurement from the top with excitation filter and emission monochromator. **Bottom:** TRF measurement from the bottom with excitation and emission monochromators.
Excitation Wavelength [nm] / Barcode	This parameter depends on the selected measurement direction (Top/Bottom): • **Top:** Click on the ... button to select an excitation filter (**Select Filter** dialog is opened). The wavelength and the barcode of the selected filter will be displayed. See also section "Select Filter Dialog", page 104. • **Bottom:** Enter an excitation wavelength.
Emission Wavelength [nm]	Wavelength of the resultant fluorescence to be measured, needs to be at least 15 nm higher than the excitation wavelength.
Measurement Height [mm]	Focus height within the sample (only editable for **Top**). The height is measured from the bottom of the plate (supporting plane). Can be optimized automatically (see section "Measurement Height", page 119).
Number of Flashes	Number of flashes for one measurement.
Delay [µs]	Time between flash and measurement of first window.
Window Time [µs]	Period of time during which photons are counted/measurement.
Number of Sequential Windows	Number of windows measured per flash, separated by 5 µs from each other (fixed value).

Parameter	Description
Total Time [µs]	Automatically calculated total time per flash according to the former three entries, max. 1450 µs.
Optimization	This button is only enabled if a plate type was selected. It opens the **Optimization Wizard** (see section 5.2.1.4 “Optimizations”, page 115) which helps you to find the best settings for selected operation parameters. The available optimizations depend on the measurement technology.

Select Filter Dialog

Select the desired filter and click **OK**.
You can sort the table by clicking on a column title.



Parameter	Description
Wavelength [nm]	Excitation wavelength of the filter.
Name	Filter name
Barcode	Filter barcode
Filter in Filter Wheel	Indicates whether the filter is currently available in the instrument or not. To insert the required filter see section 6.1 “Change Excitation Filters”, page 257.
Show recommended Filters	Displays only filters which are suitable for the selected technology.
Show all Filters	Displays all filters regardless of their compatibility.

Delay (DELAY)

The **Delay** operation is used to introduce a waiting time between protocol operations. You can specify whether the plate will be inside or outside the instrument during this time.

Delay Parameters

Parameter	Description
Duration	Length of the delay between the completion of the preceding operation and the start of the next one. **Notice** • The input field is pre-formated in **hh:mm:ss.sss** pattern *(hh = hours, mm = minutes, ss = seconds, sss = milliseconds)*. • Editing these values works in "overwrite" mode: Place the cursor in front of the digits which you want to edit and start typing. The colons and the decimal point can be ignored. • If you delete single digits or the entire time definition, the respective digits are reset to "00". • You can only enter numbers. • Pasting content from the clipboard is disabled.
Plate Location	Position of the plate during the waiting time, either at measuring position (**Inside**) or **Outside** the instrument.

Shake (SHAKE)

The **Shake** operation can be used to mix the solutions in the wells between measurements.

Notice

The **Shake** operation with parameter **Plate Location** set to **“Outside”** cannot be used in the following combinations:

- **Using the optional stacker**
  Remove the stacker magazines and take off the magazine table before running such a protocol or change the parameter **Plate Location**. See also section 4.13 “Stacker”, page 58.
- **Protocol with execution type "By Plate Stack"**
  See also section 5.2.1.1 “Execution Type”, page 75.

Shake Parameters

Parameter	Description
Shake Mode	Type of shaker motion: straight line (**Linear**), circular (**Orbital**) or figure of eight (**Double Orbital**). The setting for **Shake Mode** affects the range for the **Speed** parameter.
Duration	Duration of shaking operation. **Notice** • The input field is pre-formated in **hh:mm:ss.sss** pattern *(hh = hours, mm = minutes, ss = seconds, sss = milliseconds)*. • Editing these values works in "overwrite" mode: Place the cursor in front of the digits which you want to edit and start typing. The colons and the decimal point can be ignored. • If you delete single digits or the entire time definition, the respective digits are reset to "00". • You can only enter numbers. • Pasting content from the clipboard is disabled.
Speed [rpm]	Sets the speed as number of revolutions per minute. The range for this depends on the **Shake Mode** and the **Diameter**, the minimum is 30.
Diameter [mm]	Distance between the extremes of the movement of the center of a well in the plate. The default 0.1 mm means that the shaking moves the center of the plate ± 0.05 mm. The setting for **Diameter** affects the range for the **Speed** parameter, the maximum is 10 mm.
Plate Location	Position of the plate during shaking, either at measuring position (**Inside**) or **Outside** the instrument.

Temperature (TEMP)

With this operation the instrument starts to heat or cool until it reaches the selected temperature. It will keep the target temperature until a new temperature operation is started or until the protocol has been completed.

Notice
If a protocol contains a **Temperature** operation, the general temperature settings for the instrument (see section 5.10.6 “Temperature (Settings)”, page 235) are temporarily overridden by the operation. After the measurement, the general temperature settings are applied again.

The instrument cannot reach temperatures lower than the ambient temperature. The approximate minimum temperature is ambient temperature +2 °C. The lowest allowed ambient operating temperature for the instrument is +15 °C.

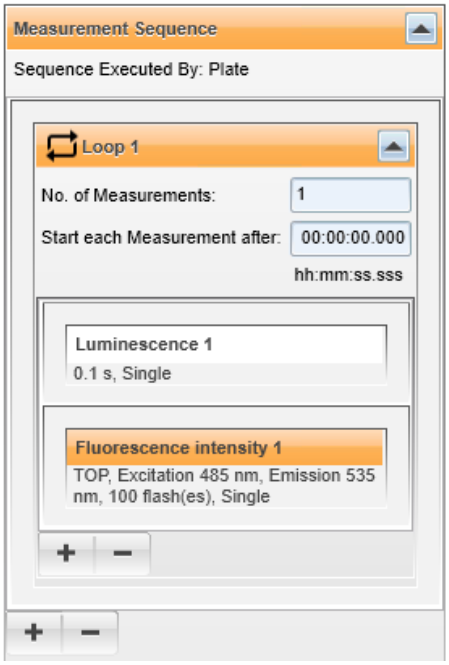
Condensation droplets forming on covers of sealed plates can be avoided by setting the temperature of the heater above the assay plate higher than the temperature of the heater below the plate. The amount of this difference can be maximum 4 °C.

Temperature Parameters

Parameter	Description
Temperature [°C]	Target temperature in the instrument (15-65 °C, dependent on ambient temperature).
Set Target and Continue Immediately	**Activated:** The measurement sequence is continued while the temperature is being regulated – even if the target temperature has not been reached yet. **Not activated:** The temperature is adjusted to the target temperature until the **Ready** state (see below) is reached. When this temperature is stable for at least 60 sec., the next operation will be started. Definition of "Ready" state: • Target = 15 – 50 °C → ± 1 °C • Target = 51 – 60 °C → ± 2 °C • Target = 61 – 65 °C → ± 3 °C
Condensation Prevention for Sealed Plates	Turns condensation prevention **ON** or **OFF**. The following options in this section are only available if the function is ON.
Upper Heater is ... Than Lower Heater	• **Warmer:** Increases the temperature of the upper heater by the offset entered below. • **Colder:** Reduces the temperature of the upper heater by the offset entered below.
Diff. Between Upper and Lower Heater [°C]	Temperature difference between upper and lower heater (value range: 0–4 °C, 1 °C steps).
Switch off Temperature Adjustment	This option can be used to switch off temperature control. Any temperature adjustment configured by a previous temperature operation or by the general temperature settings will be reset until another temperature operation is reached or the measurement is finished. After the measurement, the general temperature settings will be applied again (see section 5.10.6 “Temperature (Settings)”, page 235). • **Check box activated:** All other operation parameters are disabled. Temperature control will be switched off. • **Check box disabled:** You can configure desired temperature settings as usual. These settings will override the general temperature settings.

Loop

The **Loop** operation can be used to repeat operations which have been added to the loop. You can define the number of repetitions for the loop and the time span between starting the repeats. After the loop has been processed completely, the next operation outside the loop will be processed. It also depends on the selected **Execution Type** how the measurement sequence is processed exactly (see section 5.2.1.1 “Execution Type”, page 75).



How to define a loop

1. Click the + button of the **Measurement Sequence** and add a **Loop** operation.
2. Click the + button of the **Loop** operation and add the operation(s) to be repeated.
3. Enter the number of repeats in **No. of Measurements**.
4. Define the time span between starting two repeats using the **Start each Measurement after** parameter.

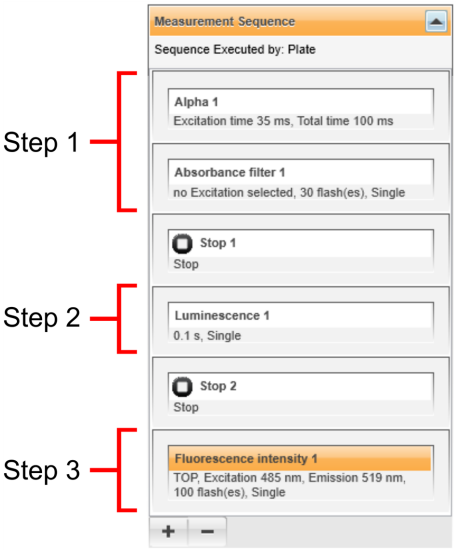
Loop Parameters and Buttons

Element	Description
	Opens a pop-up menu where you can select a new operation which will be added to the loop (last position). An entry in the menu may be disabled ... • if an operation is generelly not allowed inside a loop (e.g. Alpha, Temperature, Loop, Stop). • if the max. number of operations of this type has already been inserted. • if an operation cannot be used with the selected **Execution Type** of the measurement sequence. See also section 5.2.1.1 “Execution Type”, page 75. Only enabled when protocol is in **Edit** mode.
	Opens a pop-up menu where you can select one of the existing operations which will be removed from the loop. Only enabled if the protocol is in **Edit** mode and the loop is not empty. To remove the entire **Loop** operation, use the  button of the **Measurement Sequence**.
No. of Measurements	Defines the number of repitions for the loop and its operations.
Start Each Measurement after	Defines the **minimum** time span between two repeats (starting times). The real time span may be longer than the selected interval, if processing the loop takes longer. • If you enter *"00:00:00.000"*, the loop repeats will be processed as fast as possible in direct succession. • For an estimation of measurement times for different technologies please see section 7.16.3 “Maximum Throughput (Time Per Plate)”, page 272. **Notice** • The input field is pre-formated in **hh:mm:ss.sss** pattern *(hh = hours, mm = minutes, ss = seconds, sss = milliseconds)*. • Editing these values works in "overwrite" mode: Place the cursor in front of the digits which you want to edit and start typing. The colons and the decimal point can be ignored. • If you delete single digits or the entire time definition, the respective digits are reset to "00". • You can only enter numbers. • Pasting content from the clipboard is disabled.

Stop

Using one or multiple **Stop** operations you can interrupt a measurement, e.g. to dispense a substance to the samples on the plate. This operation has no parameters. Afterwards the measurement can be resumed at this position using the **Continue Measurement** mode (see section 5.3.2 “Continue Measurement”, page 153).

Stop operations split up the measurement sequence into multiple **steps** (see below). If you run such a protocol, it will only be processed until the next **Stop** operation is reached (e.g. step 1 completed). Then the measurement is interrupted and the partial measurement is saved.



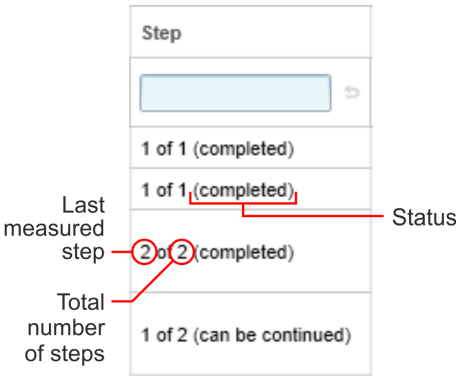
You can continue this partial measurement using the **Continue Measurement** mode. The next unmeasured step will then be processed until another **Stop** operation is reached or the measurement sequence is completed. For details see also section 5.3.2 “Continue Measurement”, page 153.

In the **Load Measurement(s)** dialog, the property **Step** indicates how many steps of the measurement sequence have already been processed. See also section 5.6.5 “Load Measurement(s)”, page 187.

- **Example:** *"2 of 3 (can be continued)"*

The text in the brackets shows the measurement status. Only measurements with status **"can be continued"** will be available when loading a measurement in **Continue Measurements** mode.

- **"can be continued":** Still one or multiple steps to be processed, can be continued.
- **"completed":** All steps measured, cannot be continued.
- **"canceled":** Aborted by user or due to error, cannot be continued.
- **"unknown":** Measured with previous Kaleido version, cannot be continued.



Notes

- The **Stop** operation may not be the first or last operation in the **Measurement Sequence**. A **Stop** operation may not be positioned directly after another **Stop** operation, there must be other operations in between.
- A **Stop** operation can only be added to a measurement sequence with execution type **By Plate** or **By Plate Stack**. Type **By Well** is not compatible with the **Stop** operation (and thus also not with the **Continue Measurement** mode).
- Protocols to be measured in **Automation** mode may not contain a **Stop** operation. For a workaround see section 5.14.6 “Replacing Stops in Automation Mode”, page 254.

5.2.1.3 Measurement Modes

The first parameter of each measurement operation is the measurement mode. Different measurement modes are available (see table), determining the number of actual measuring events in one well. These events can differ from each other by position in the well (**Well Scan**) and wavelengths (**Wavelength Scan**). A further measurement mode, **On-the-Fly**, is available for Absorbance technology for higher throughput.

For the **Single** measurement mode, no further parameters have to be set and the wells are measured once according to the settings in the measurement operation. If you select **Wavelength Scan** or **Well Scan** for an operation, additional parameters will be displayed in the **Content Area** in a separate section below the regular operation parameters.

Available measurement modes for measurement operations:

Mode	ABS filter	ABS mono	ALPHA	FI	IMG	LF	LUM	TRF
Single	●	●	●*	●	●*	●*	●	●
Well Scan	●	●		●			●	●
Excitation Scan		●		●				
Emission Scan				●				●
On-the-Fly	●	●						

** Measurement mode is implicit, no selection possible*

Notice

The measurement mode **Kinetic** has been replaced by the execution type **By Well** and the **Loop** operation. Please see the following sections:

- 5.2.1.1 “Execution Type”, page 75
- “Loop”, page 108

Single

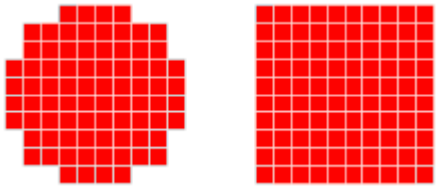
The **Single** measurement mode is the default mode for all operations added to the measurement sequence. Single point measurements are performed in one well after the other using the selected technology and measurement parameters.

Well Scan

In this mode every well is scanned with a selectable number of measurement points (up to 100). The measurement points are arranged in a grid of columns and rows, and the distance between these measurement points can be set, defining the scanning area. Well scanning is used especially for assays using adherent cells, for example Green Fluorescent Protein (GFP) assays.

Well Scan Parameters

Parameter	Description
Number of Columns (points X)	Number of points in the X-direction (1-10).
Number of Rows (points Y)	Number of points in the Y-direction (1-10).
Distance Between Points [mm]	Distance between measurement points in the well. The maximum distance between points depends on the number of points and the well size. The following formula is used for validation when the protocol is saved: $(Points_{max} - 1) * Distance < WellDiameter * 1.1$ • $Points_{max}$: Max. number of points in X- or Y-direction • Distance: Distance between points • WellDiameter: Well size of current plate type If the validation fails, you will be notified and you have to reduce the number of points or the distance between the points. **Example** • Number of Columns (points X): 10 • Number of Rows (points Y): 10 • Distance between Points: 1.0 mm • Well Diameter: 7.13 mm (Plate Type: 96 Optiplate) $(10 - 1) * 1.0 < 7.13 * 1.1$ $9 < 7.843$ (= validation fails when you save the protocol) Reduce the number of points or the distance, e.g.: • Distance between Points: 0.8 mm $(10 - 1) * 0.8 < 7.13 * 1.1$ $7.2 < 7.843$ (= validation successful)

Parameter	Description
Well Scan Pattern	Allows you to adapt the well scan pattern to the well shape of the used plate type (round or rectangular). 

Excitation/Emission Scan

In the **Excitation Scan** and **Emission Scan** measurement modes several measurements are performed at different wavelengths per well to get either an excitation or an emission spectrum. These spectra can provide additional information compared to the single point measurement. They are used e.g. when spectral distributions are more important than single wavelength signal strengths (e.g. of pigments). Spectra can be used to characterize the fluorescence behavior of the samples and find the optimal excitation or emission wavelengths of unknown dyes or substances.

- **Excitation Scan**
  For fluorescence intensity and absorbance with monochromator:
  The excitation monochromator scans the samples in selectable steps between a minimum and a maximum wavelength. For fluorescence intensity, the resulting fluorescence is measured at the wavelength selected for the emission monochromator.
- **Emission Scan**
  For fluorescence intensity and TRF:
  A single excitation wavelength is selected with the excitation monochromator and the resultant fluorescence intensities are measured over a range of wavelengths scanned in selectable steps with the emission monochromator.

Excitation/Emission Scan Parameters

Parameter	Description
Excitation/Emission Wavelength – Start [nm]	Lower limit of the scan range.
Excitation/Emission Wavelength – End [nm]	Upper limit of the scan range.
Step Size [nm]	Step width to be used during the scan.

On-the-fly

In this measurement mode the plate does not stop at the measuring position but is measured as it is moving past. Only one flash is used. This speeds up the measuring process but requires adequate signals from the sample. There are no parameters in addition to the operation parameters.

5.2.1.4 Optimizations

For most measurement operations, several optimizations are available for better measurement performances, corrections and hence results (see table below). Optimizations can correct or refine:

- parameters of single operations (e.g. wavelengths, measurement height)
- the dimension of the plate for exact reading points for the whole measurement sequence

For one operation, you can select and configure several optimizations in one go using a wizard. The optimizations are processed one after the other. The measurements for optimizations are done on selected wells which have to be filled with appropriate solutions or samples. After every single optimization process, the results are presented and can be edited, discarded or saved for the respective operation in the protocol. You can repeat an optimization at any time.

Optimization	ABS filter	ABS mono	ALPHA	FI TOP	FI BOTTOM	LUM	TRF TOP	TRF BOTTOM
Wavelength Excitation		●		●	●			●
Wavelength Emission				●	●		●	●
Plate Dimension	●	●	●	●	●	●	●	●
Baseline	●	●						
Measurement Height	●	●		●			●	

Notice

- For **Imaging** (IMG) the optimizations are not done with the optimization wizard, but in the plate map area on the right of the content area during setting up protocol (see section 5.8.2 “Test Imaging”, page 210). They can be performed for every channel set up in the operation.
- There are no optimizations available for **Label-free** (LF) technology.
- Optimizations are not compatible with the optional **stacker**. They are not available while the magazine table is installed.

Optimization Wizard

For optimizations, the protocol has to be in **Edit** mode and an operation has to be selected. The **Optimization** button is shown below the parameters of the operation in the **Content Area**. Clicking this button will open the optimization wizard. The plate carrier will be moved out so that you can insert the required sample plate. The plate will be loaded automatically as soon as you start the optimization process.

First Step: Configure Optimization

The first window of the wizard consists of three sections.

- In the section **Select Optimization** on the left all optimizations are listed and only those applicable for the current operation can be selected. Several optimizations can be selected at the same time.
- The **Plate Map** in the middle indicates in which well(s) the optimization measurements will be performed. The well colors are specific for each optimization. Most optimizations allow changing the preset well(s). If you want to change the preset well, select the optimization type in the drop-down menu below the plate map and click on a well in the plate map.
- In the section on the right – **Set Parameter for Optimization** – you can edit all parameters required for the selected optimizations or accept the default values. The parameters for each optimization are explained in the following sections.

Click the **Start** button to initiate the optimization process. The plate carrier will be moved in.

Second Step: Optimization Process

In this step, a progress bar is displayed for the currently processed optimization.

Third Step: Results

After every optimization process, the results are shown in a window where you can edit them manually if required. You have the following options:

- **Save + Continue/End Optm.:** Accept the optimized values and use them in the protocol.
  Depending on whether a further optimization is queued or not, the next one will be initiated or the wizard will be closed.
- **Don't save + Continue/End:** Discard the results (protocol is not modified).
  Depending on whether a further optimization is queued or not, the next one will be initiated or the wizard will be closed.
- **Cancel:** Stop the optimization process and close the wizard.
  Any queued optimizations will not be processed.

All accepted optimization results will also be listed in the **Comments** tab of the **Messages** area.

Notice
After running optimizations you have to **save** the protocol to apply the changes and use them for future measurements.

Wavelength Excitation

This optimization determines the excitation wavelength at which the maximum absorbance (Abs mono) or emission (FI, TRF Bottom) is measured. The wavelength range to be tested can be specified in the wizard. For FI and TRF Bottom also the emission wavelength can be changed for optimization tests. In the results window a plot of excitation wavelength (x-axis) against counts (y-axis) is displayed and the wavelength of the highest counts value is set automatically and displayed in the text box.

To change this value manually you can either move the cursor in the graph area and select a wavelength by mouse-click or directly enter a value in the text box. Saving the result will change the value for the excitation wavelength of the respective operation in the protocol.

Notice
Wavelength optimization for **Absorbance mono** requires a result from the **Baseline** optimization. Therefore Baseline optimization is selected automatically and is run first (see section "Baseline", page 119).

Wavelength (Excitation) Optimization Parameters

Parameter	Description
Number of Flashes	Number of flashes for one measurement, by default same value as defined in operation.
Exc Min [nm]	Minimum wavelength included in optimization.
Exc Max [nm]	Maximum wavelength included in optimization.
Step [nm]	Size of the wavelengths steps to be measured.
Ems [nm] (editable in FI and TRF Bottom)	Wavelength of the resultant fluorescence to be measured; needs to be at least 15 nm higher than maximum excitation wavelength.

Wavelength Emission

This optimization determines the wavelength at which maximum emission occurs. The wavelength range to be tested can be specified in the wizard. In the same dialog you can also change the excitation wavelength (only monochromator) for optimization tests. In the results window a plot of emission wavelength (x-axis) against counts (y-axis) is displayed and the wavelength of the highest counts value is set automatically and shown in the text box.

To change this value manually you can either move the cursor in the graph area and select a wavelength by mouse-click or directly enter a value in the text box. Saving the result will change the value for the emission wavelength of the respective operation in the protocol.

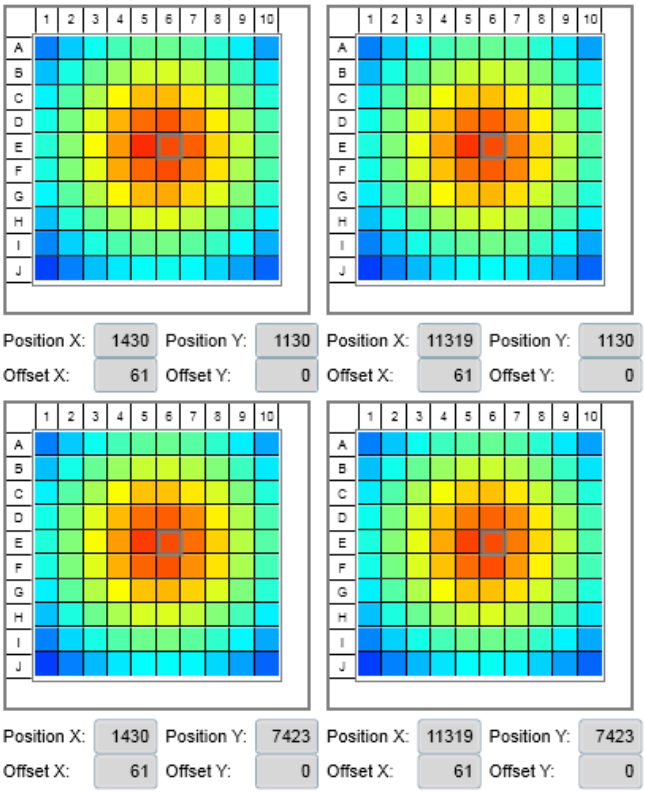
Wavelength (Emission) Optimization Parameters

Parameter	Description
Number of Flashes	Number of flashes for one measurement, by default same value as defined in operation.
Ems Min [nm]	Minimum wavelength included in optimization.

Parameter	Description
Ems Max [nm]	Maximum wavelength included in optimization.
Step [nm]	Size of the wavelengths steps to be measured.
Exc [nm]	Wavelength used to excite the fluorochrome in the sample, needs to be at least 15 nm lower than minimum emission wavelength.

Plate Dimension

This optimization determines the exact position of the wells leading to a centered measurement point adjustment. For this process always the four corner wells of the plate are used. During the scan 100 points are measured in a 10 x 10 array. These four arrays (one array for each corner well) are displayed in the results window showing the signal intensity in heat maps, with red being the most intense.



Below the heat map of each corner well, the X and Y positions defined in the current plate type are displayed. In each well, click on the field with the highest intensity.

- If the offset values do not change (all offset values = "0"), the plate was positioned right before.
- If the offset values change after clicking on the fields with the highest intensity, the plate has not been in the right position and the required offset values are displayed.

Click **Save + Continue** / **Save + End Optm.** to correct the plate dimensions using the displayed offset values. The new plate dimensions will be used for all operations in the protocol. **Save** the protocol to apply the changes.

Plate Dimension Optimization Parameters

Parameter	Description
Number of Flashes (only for Abs, FI and TRF)	Number of flashes for one measurement, by default same value as defined in the operation.
Size of Scanned Area [mm]	Displays the edge length of the well area which will be scanned: • 384-well plates: 4.5 mm • 96-well plates: 9 mm
Measurement Time	Displays the measurement time (specified in the operation).

Baseline

This optimization is only available for **Abs mono**.

- It is required and automatically selected if a **Wavelength (Exc)** optimization is selected for Abs mono, but can also be performed alone.
- It is also required whenever measurement mode **Wavelength Scan (EXC/EMS)** is used.

In contrast to a single absorbance measurement, a **Wavelength (Exc)** optimization for absorbance will determine the absorbance at every wavelength step of the absorbance range. Therefore, the initial light intensities at every wavelength need to be known. These are determined and recorded before during the **Baseline** optimization and include sample and plate specific absorbance properties. This step is not necessary in single-wavelength measurements, since a background measurement is taken routinely before each protocol starts.

The preset well for the blank sample on the plate map (in green) can be changed. The Baseline is recorded against the blank sample for the full absorbance range (230 - 1000 nm) in 1 nm steps. A graph in the results window shows the signal intensity (y-axis) over wavelengths (x-axis). These results of the optimization can be saved for the protocol, and the Baseline values are automatically compensated for the measurement signals in the results.

Baseline Optimization Parameters

Parameter	Description
Number of Flashes	Number of flashes for one measurement, by default same value as defined in operation.

Measurement Height

With this function the optimal distance between optics and samples can be determined. You can change the preset (pink) well in the plate map of the optimization wizard. The well will be measured between 0 and 20 mm from the bottom of the plate in

1 mm steps. The resulting graph shows the signal intensity (y-axis) over measurement height (x-axis) and the wavelength of the highest counts value is set automatically and shown in the text box.

To change this value manually you can either move the cursor in the graph area and select a wavelength by mouse-click or directly enter a value in the text box. By moving the cursor in the graph area and selecting a height by mouse click, you can change the proposed optimal measurement height, shown in the box on the left. Saving the result will change the value for the measurement height in the respective operation of the protocol.

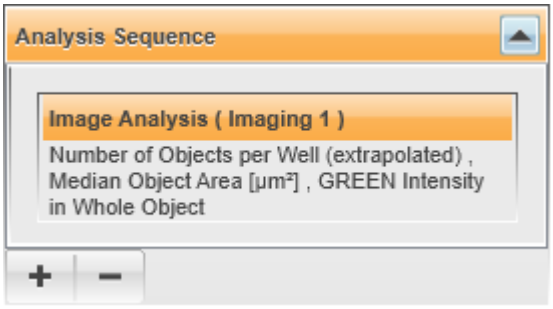
Measurement Height Optimization Parameters

Parameter	Description
Number of Flashes (for Abs, FI, TRF)	Number of flashes for one measurement, by default same value as defined in operation.

5.2.2 Analysis Sequence

In the **Analysis Sequence** (**Setup Protocol** screen) you can define analysis operations which will be applied to the images resulting from **Imaging (IMG)** measurements. What the image analysis does is defined in the interactive image analysis configuration in the **Content Area**. The analysis will be started as soon as the first well has been measured completely. It will run in parallel to the continuing image acquisition.

For a detailed description of all analysis parameters please see section 5.2.2.3 “Image Analysis Parameters”, page 129.



Analysis Sequence with one Image Analysis operation (for measurement operation "Imaging 1", the first three result parameters of the image analysis are displayed as a summary

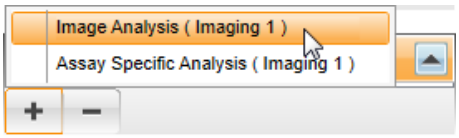
Using the + button you can add **one** analysis operation for each **Imaging** operation defined in the **Measurement Sequence**.

- There can be one or two **Imaging** operations at most. See also section “Imaging (IMG)”, page 84.
- An analysis operation has only access to the images of the assigned **Imaging** operation (the name is indicated in brackets).

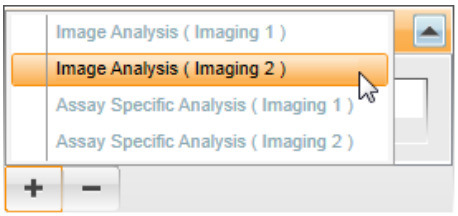
There are two types of analysis operations. All operations in the analysis sequence must have the same type. Incompatible options will be disabled in the + menu after you have added the first operation.

- **Image Analysis:** Standard image analysis
  See also section 5.2.2.1 “Image Analysis”, page 123.
- **Assay Specific Analysis:** Custom analysis script, requires a *.KALA file (Kaleido Assay Specific Analysis).
  See section 5.2.2.2 “Assay Specific Analysis”, page 127.

Examples for available menu options



One Imaging operation in Measurement Sequence ("Imaging 1"), no analysis operation added yet (both types available)



Two Imaging operations in Measurement Sequence ("Imaging 1" and "Imaging 2"), Image Analysis operation already added for Imaging 1

How to add an analysis operation

1. Click the + button.
2. Select an operation to be added from the flyout menu.

   Depending on the number of **Imaging** operations in the **Measurement Sequence** and of previously added analysis operations, certain options may be disabled (see examples above).
3. *Only for type "Assay Specific Analysis":* A new dialog will be opened where you can select an analysis script file (*.KALA).
4. After adding an analysis operation to the sequence, you have to configure the image analysis in the **Content Area** in the center of the screen. See section 5.2.2.1 “Image Analysis”, page 123.

Buttons and Elements

Element	Description
Disclosure buttons 	Expand or collapse the analysis sequence.

Element	Description
	Opens a flyout menu where you can select the analysis operation to be inserted. Only enabled when protocol is in **Edit** mode and if at least one **Imaging** operation has been defined in the **Measurement Sequence**. Depending on the number of Imaging operations defined in the Measurement Sequence and on previously added operations, some options in the menu may be disabled. See also above for details.
	Opens a flyout menu where you can select the analysis operation to be removed. Only enabled when protocol is in **Edit** mode and if there is an operation which can be removed.

Notice

- All analysis operations created with Kaleido version 1.2 or older are **deprecated** and cannot be used any more. You can still load and view old results containing such analysis operations, but it is not possible to edit, run or recalculate a protocol which makes use of these operations. Please set up a new analysis sequence, if required.
- For analyzing non-imaging measurement data and the results of image analysis operations, please use the separate **MyAssays Desktop** data analysis software which is bundled with Kaleido. See section 5.12 “MyAssays Desktop Data Analysis”, page 242 for detailed information.

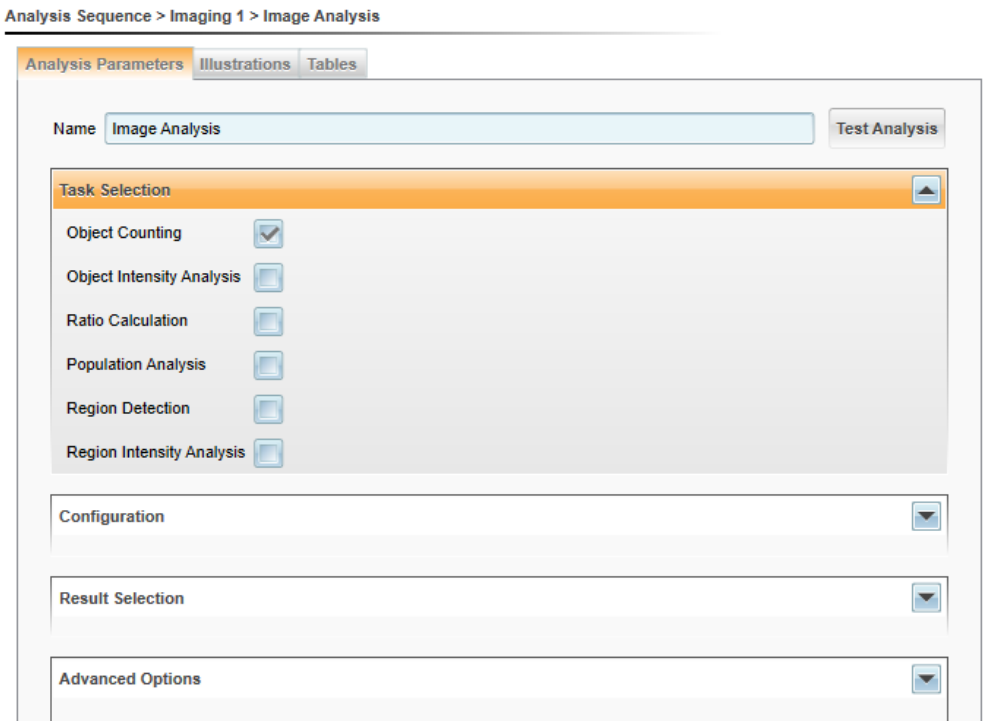
5.2.2.1 Image Analysis

Image analysis operations take the acquired images as an input and calculate numerical results, e.g. the number of cells per well or a fluorescent marker intensity.

Three different tabs are displayed for an analysis operation in the **Content Area**:

- **Analysis Parameters:** Allows you to setup and test an image analysis.
- **Illustrations:** Displays visual results (test images with an overlay of detected objects and regions) when testing an analysis.
- **Tables:** Displays numerical results for the selected result parameters when testing an analysis.

Analysis Parameters



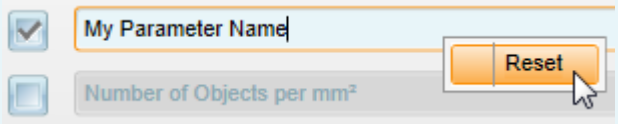
What the image analysis does is defined interactively on the **Analysis Parameters** tab. There are four groups of parameters:

- **Task Selection:** Select one or multiple analysis tasks.
  See section "Task Selection", page 129 for a description of the tasks.
- **Configuration:** Define mandatory input parameters.
- **Result Parameters:** Select result parameters.
- **Advanced Options:** Define optional parameters.

For a detailed description of all parameters please see section 5.2.2.3 "Image Analysis Parameters", page 129.

Notice

You can reset a numerical parameter or the name of a result parameter to its default value by selecting **Reset** from the context menu.



How to configure and test an image analysis

Necessary preparations:

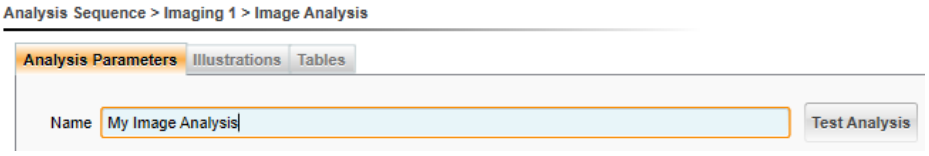
- Setup **Measurement Sequence** (incl. **Imaging** operation).
  See section 5.2.1 “Measurement Sequence”, page 72.
- Add **Image Analysis** operation to **Analysis Sequence**.
  See section 5.2.2 “Analysis Sequence”, page 120.

Workflow:

1. Click on the title of the analysis operation in the **Analysis Sequence**.

   The parameters of the image analysis are displayed in the **Content Area**.

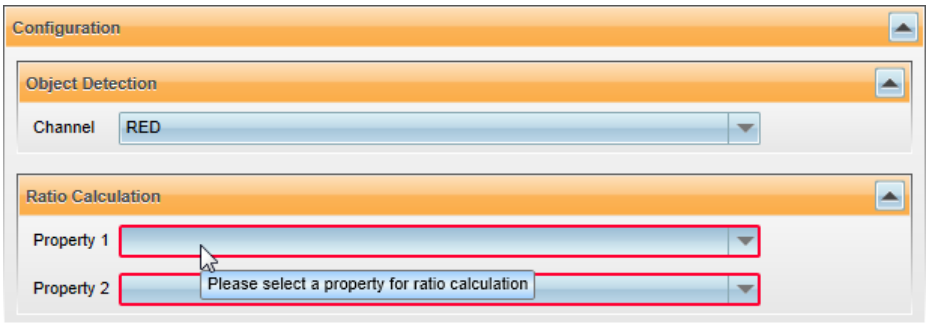
2. Enter a **Name** for the image analysis. This name will be displayed in the header of the analysis operation.



3. Select one or multiple tasks in the **Task Selection** pane.

4. Review and adjust the **mandatory** inputs in the **Configuration** pane.

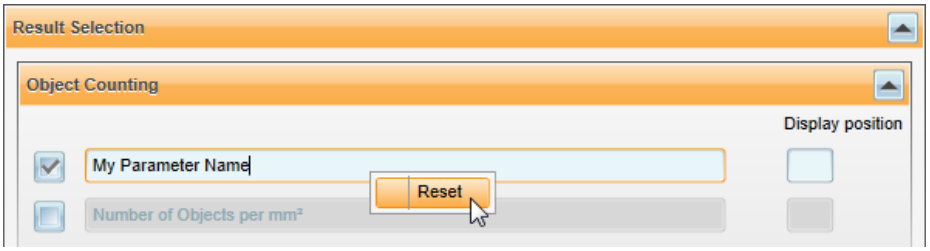
   Depending on the selected tasks different mandatory inputs are listed here. The parameters are displayed with a red outline and an explanatory tooltip (see below) until all required inputs have been configured.



All other parameters in the following sections are optional, so you could already test the analysis now (see step 10).

5. *Optional:* Select the desired result parameters in the **Result Selection** pane.

   All available result parameters for the selected tasks are listed, the default parameters have been checked.



   - Check a parameter to add it to the analysis results. The default name can be edited as required. If the name is already used within the analysis sequence, it will automatically get a suffix (1).
   - To restore the default name, select **Reset** from the context menu.
   - Uncheck a parameter to remove it from the analysis results.
   - *Optional:* Enter a display position to determine the result order and list most important results first.

6. *Optional:* Adjust optional parameters in the **Advanced Options** pane.

   This section gives access to rarely used options which usually have good default values and need no user attention for most applications.

7. *If not done yet:* Generate test images for the respective **Imaging** operation:

   - Select a well in the **Plate Map**.
   - Click **Test Imaging 1/2** (below the plate map).

     A set of test images of all channels in the operation will be acquired.

8. On the **Analysis Parameters** tab, click **Test Analysis**.

   The analysis is validated and tested using the acquired test images. The **Illustrations** tab is opened automatically to show the graphical results. Numerical results can be viewed on the **Tables** tab.

9. Optimize the input parameters and test the analysis again until you are satisfied with the results.

Illustrations

This tab is opened automatically in the **Content Area** as soon as you test an image analysis. It displays a color overlay of selected channels and a set of overlays returned by the analysis, e.g. the outlines of the detected well and cells. This allows visual inspection how analysis results match with different channels and combinations of channels.

- If you move the mouse to a spot on the image, the local **intensity** (0-4095) of that spot is displayed below the image (for each channel). The value is updated as soon as you stop moving the mouse.
- You can **zoom** the image using the mouse wheel.
- Drag the image with the mouse to move the field of view.

Using the **Display Options** (beneath the plate map on the right) you can adjust color, brightness and contrast of the image and enable/disable channels and overlays. These settings are only applied to the image display, they do not affect the original images or the image analysis. See also section 5.8.3 “Display Options”, page 211.

Context Menu

Element	Description
Copy to Clipboard	Copies the image with full resolution (as displayed with colored channels and overlays) to the Windows® clipboard to paste it into a different program. This will reduce the color depth from 12 bit grayscale per channel to 8 bit color (no separate channels anymore).
Zoom to Fit	Displays the image so that it fits into the **Content Area**.
Save as ...	Allows you to save the current image with full resolution (as displayed with colored channels and overlays). This will reduce the color depth from 12 bit grayscale per channel to 8 bit color (no separate channels anymore). The file format can be selected by appending the corresponding extension to the file name (*.bmp, *.jpg, *.png, *.gif, *.tif or *.tiff).
Export Raw Images ...	Allows you to save the unscaled RAW images with full resolution and original color depth (one 16 bit grayscale TIFF file per channel, first 12 bits are used). To view these images outside of Kaleido you need a third-party image viewer which is capable of displaying 16 bit TIFF images (e.g. IrfanView). Each exported image file will get the same time stamp as the original image so that it indicates the measurement time.

Tables

The table shows all numerical results returned by the current analysis. All parameters selected in the **Result Selection** are shown. The order can be modified by defining a **Display Position** for a result parameter (see section “Result Selection”, page 132).

You can copy selected values or the entire table to the clipboard using the context menu.

In addition to the selected result parameters, the **Analysis Quality** is listed in the result table. This parameter warns you about potential issues with the image analysis.

Analysis Quality Parameter

Analysis Quality	Description
Good	No problems found.
Well Detection: estimated	**Well Detection** is considered not reliable. Well outlines may be unprecise.
Overexposed <channel name> Channel	The channel used for **Object Detection** or **Region Detection** contains more than 400 overexposed pixels inside the analyzed well ROI (region of interest). Note that channels which are only used for intensity measurement are not taken into account (might be valid for some applications).
Empty <channel name> Channel	No intensity change found inside the analyzed well ROI of the **Object or Region Detection** channel. Criterion: *Max intensity < 3 * Min intensity*
Combination of above warnings	List of warnings seperated by a comma. Example: „Well Detection: estimated, Empty UV Channel“

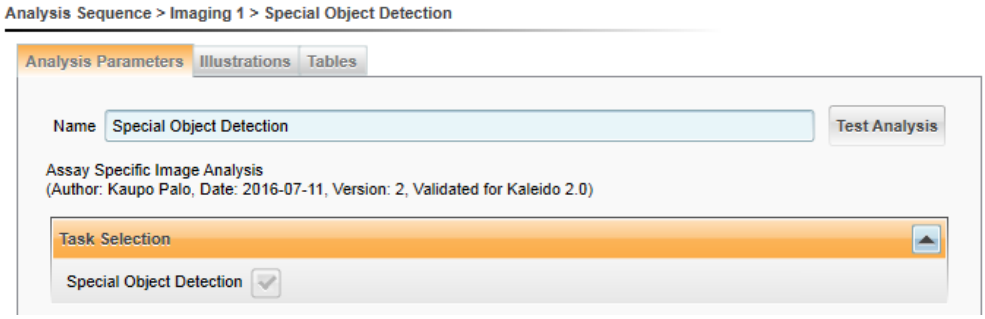
Context Menu

Element	Description
Select All (Ctrl+A)	The entire table is selected.
Copy (Ctrl+C)	Copies the selected table rows to the clipboard.

5.2.2.2 Assay Specific Analysis

Assay specific analyses are customized analysis scripts created by PerkinElmer. Using such scripts special applications can be realized which are not possible using a standard **Image Analysis** operation. If you are interested in this service please contact your PerkinElmer sales representative.

The assay specific analysis is contained in a single file (*.KALA, "Kaleido Assay Specific Analysis”). If you add an **Assay Specific Analysis** operation to the **Analysis Sequence**, you will be asked to choose a *.KALA file. Version details are displayed under the analysis name (see below).



You cannot select/unselect any tasks, because **Assay Specific Analyses** are by design only written for one specific task. Input and output parameters depend on the customized analysis script. Otherwise the general handling is the same as described for standard **Image Analysis** operations (see section 5.2.2.1 “Image Analysis”, page 123).

A copy of the script is stored in the image analysis configuration, i.e. it will be included if you save the protocol. This guarantees that the protocol is self-contained and will always run.

Notice

Assay Specific Analyses cannot be combined with standard **Image Analyses** in case of two **Imaging** operations. E.g. if for one of the **Imaging** operations an **Assays Specific Analysis** is selected, the second operation can only be an **Assay Specific Analysis** as well.

5.2.2.3 *Image Analysis Parameters*

Please see the following sections for a detailed description of all image analysis parameters.

For instructions how to set up an image analysis please see section 5.2.2.1 “Image Analysis”, page 123.

Task Selection

All available tasks are listed and can be checked/unchecked. Unavailable tasks are grayed out in the list (e.g. tasks operating on fluorescent images if only brightfield images are available).

Object Counting

This analysis is designed to detect and count any fluorescently stained objects in the image. Best results can be obtained for well separated objects, e.g. stained nuclei of a cell. Good results can also be achieved for cells stained with a whole cell stain or digital phase (DPC) images of unstained cells. The objects of interest must be brighter than their surrounding background.

Object Intensity Analysis

Add this task to measure fluorescence intensities inside and around the detected objects. The desired results can be selected in the **Result Selection** section.

- Use this analysis e.g. to measure specific fluorescent label intensities in the nucleus or cytoplasm region of a cell indicating e.g. cell health.

Ratio Calculation

Add this task to calculate ratios of the measured object properties, e.g. fluorescent marker intensities in different compartments and channels.

- Use this analysis e.g. to quantify the translocation of a fluorescent marker from the cytoplasm to the nucleus of a cell by the ratio "Marker Intensity in Inner Region" / "Marker Intensity in Outer Region".

Population Analysis

Add this task to identify different sub-populations (phenotypes) of the detected objects, e.g. mitotic cells, apoptotic cells or cells with low mitochondrial potential. Any number of sub-populations can be defined. For each sub-population any number of

conditions can be combined to define it (e.g. object size thresholds, thresholds for different markers, thresholds for marker intensities in different compartments). The desired outputs for each sub-population can be defined in the **Result Selection** section.

Region Detection

This analysis is designed to reliably identify regions covered with cells or tissue in brightfield or fluorescence images. Use this analysis e.g. to determine the confluency of a cell layer, measure colony growth, scratch wound healing or properties of micro tissues or zebra fish.

- The main readout is the area of the detected region. The region can be further analyzed using the **Region Intensity Analysis** task.
- Note that the region detection is based on texture analysis. Objects do not need to be small and brighter than the surrounding background like in the **Object Counting** task.

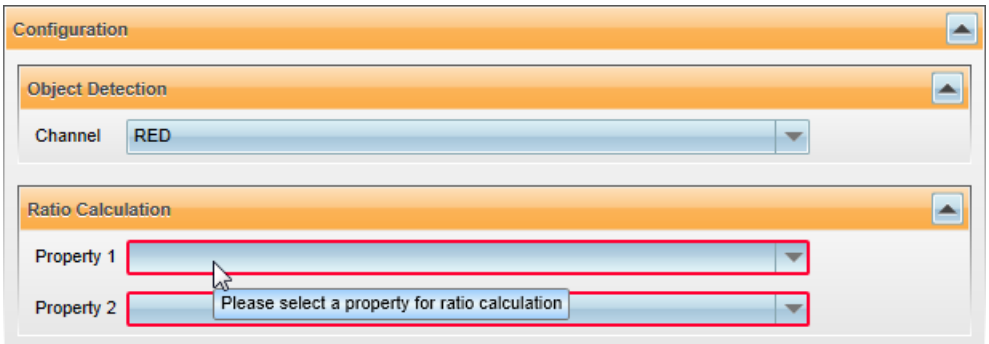
Region Intensity Analysis

Add this task to further characterize regions identified by the **Region Detection** task. The region is divided into parts that are positive and negative for a fluorescent marker. In the "Positive" region the marker intensity is above a given threshold.

- Use this analysis e.g. to determine sub-regions with transfected cells or to measure fluorescent markers in micro tissues or zebra fish.

Configuration

In the **Configuration** pane, all mandatory input parameters are listed which are required for the selected tasks. The parameters are grouped by the selected tasks. You have to review and adjust these inputs before the configuration can be used. A red outline and an explanatory tooltip are displayed for parameters which have not been configured correctly yet. Other parts of the user interface may be blocked until valid options have been selected for all parameters.



If you want to restore the default value of a parameter, right-click and select **Reset** from the context menu (available for numerical values in text boxes only).

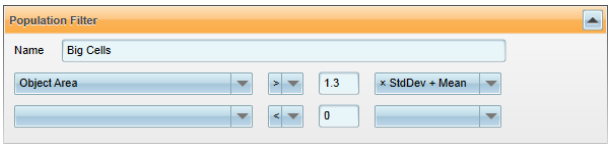
Configuration Parameters – Object Detection

Parameter	Description
Channel	Imaging channel to be used for object detection.

Configuration Parameters – Ratio Calculation

Parameter	Description
Property 1	Two object properties have to be selected.
Property 2	$Ratio = \frac{Property\ 1}{Property\ 2}$

Configuration Parameters – Population Filter

Parameter	Description
Name	Enter a name for the resulting population. • As soon as you enter a name, a new box for an additional population will be added below. • If you delete the name, this box will be removed (except for the first box).
Parameter	Parameter to be used as filter criterion. • As soon as you select one parameter, a new line for an additional filter criterion will be added. If multiple filter criteria are defined, they will be combined by a logical AND operation. • If you select the empty entry from the list, this line will be removed (except for the first line).
Relational Operator	Select a relational operator (<, ≤, >, ≥, =).
Value	Enter a value which is compared to the object parameters or used as a factor for the statistical value.
Statistical Method	Optionally, you can select a statistical value to be calculated for the whole population e.g. the mean value or mean + standard deviation. See example below. Statistical values include mean, median, standard deviation and median absolute deviation (MAD). Use median and MAD instead of mean and standard deviation to minimize the influence of outliers. **Example:** Select all cells that are bigger than the mean cell area + 1.3 times the standard deviation. 

Configuration Parameters – Region Detection

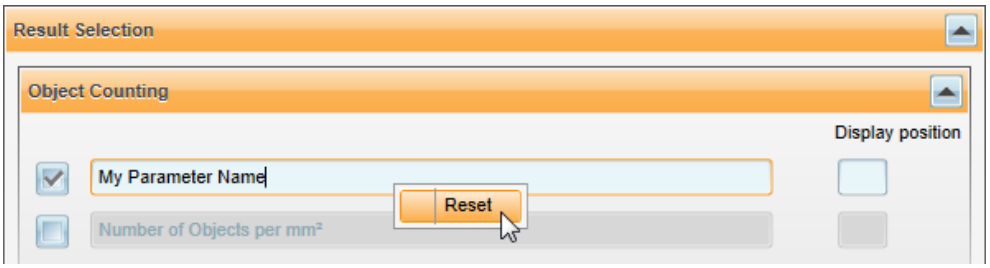
Parameter	Description
Channel	Imaging channel to be used for region detection.

Configuration Parameters – Region Intensity Analysis

Parameter	Description
Marker Channel	Channel to be used for sub-region detection.
Marker Threshold	Intensity threshold for the sub-regions. Areas with intensity above this threshold are included in the "Positive" sub-region, all other parts are in the "Negative" sub-region.

Result Selection

In the **Result Selection** pane you can select (and rename) the desired output parameters of the image analysis. The result parameters are grouped by the selected tasks.



To add a result parameter to the output, activate the corresponding **check box**. The name of the result parameter can be edited as desired. If you want to restore the default name of a parameter, right-click the text box and select **Reset** from the context menu.

Per default, the result parameter columns will appear in the same order as listed in the **Result Selection** section (pre-defined order). If you prefer a different order, you can enter a **Display Position** for the most important or for all parameters. Parameters without a position value will be listed after all other values.

Result Parameters – Object Counting

Parameter	Description
Number of Objects per Well (extrapolated)	Number of counted objects extrapolated to the full nominal well area.
Number of Counted Objects	Objects counted in the region of interest.

Parameter	Description
Number of Objects per mm²	Number of objects counted in the region of interest devided by the area of the region of interest.
Median Object Area [µm²]	Median object area of all detected objects.

Result Parameters – Object Intensity Analysis

The following three parameters are available for each measured fluorescence channel. I.e. if two channels were measured, six results are generated.

Parameter	Description
[Channel Name] Intensity in Whole Object	Intensity in the "Whole Object" region. Median of all selected objects. See section “Object Detection”, page 137 for the definition of the regions.
[Channel Name] Intensity in Inner Region	Intensity in the inner region. Median of all selected objects.
[Channel Name] Intensity in Outer Region	Intensity in the outer region. Median of all selected objects.

Result Parameters – Ratio Calculation

Parameter	Description
Intensity Ratio	Intensity ratio between the two selected properties: $Intensity\ Ratio = \frac{Inner\ Intensity}{Outer\ Intensity}$ Median of all selected objects.

Result Parameters – Population Analysis

The three intensity properties are available for each measured channel, i.e. if two channels were measured , six results are generated.

Parameter	Description
[Population Name] Count	Number of objects in the sub-population.
[Population Name] Percentage	Percentage of detected objects that are contained in the sub-population.
[Population Name] Object Area	Median area of the objects in the sup-population [µm²].

Parameter	Description
[Population Name] [Channel Name] Intensity in Whole Object	Intensity in the "Whole Object" region. Median of all objects in the sub-population. See section "Object Detection", page 137 for the definition of the regions.
[Population Name] [Channel Name] Intensity in Inner Region	Intensity in the inner region. Median of all objects in the sub-population.
[Population Name] [Channel Name] Intensity in Outer Region	Intensity in the outer region. Median of all objects in the sub-population.
[Population Name] Intensity Ratio	Intensity ratio between the two selected properties: $Intensity\ Ratio = \frac{Inner\ Intensity}{Outer\ Intensity}$ Median of all objects in the sub-population.

Result Parameters – Region Detection

Parameter	Description
Confluency [%]	Percentage of the well area covered by the detected region, i.e. the confluency in case a cell layer is analyzed.
Region Area [µm²]	Area of the detected region.
Number of Fragments	Number of separate parts of the detected region. E.g. if the total region consists of two cell colonies which are separated by background region, the number of fragments is two.
Foreground Roughness Mean	Mean roughness value in the detected region, e.g. the area covered by cells (foreground). The roughness is a measure for the texture of the region. Lower values mean less intensity variations like in empty regions of the well, higher values correspond to stronger intensity variations like in tissue or cell layers.
Foreground Roughness SD	Standard deviation of the roughness value in the detected region (foreground).
Background Roughness Mean	Mean roughness value in the background area.
Background Roughness SD	Standard deviation of the roughness value in the background area.

Parameter	Description
Total Roughness Mean	Mean roughness value over the whole analyzed area (ROI).
Roughness Range Factor	Ratio between highest and lowest roughness value in the analyzed area. This value is useful for quality control. The value is low if the contrast between foreground and background areas is low, e.g. due to bacterial contamination of the well.

Result Parameters – Region Intensity Analysis

Parameter	Description
Positive Region [%]	Percentage of the total region with a marker intensity above the specified threshold ("Positive Region"). E.g. the percentage of a cell layer that contains transfected cells.
Mean Intensity in Positive Region	Mean marker intensity in the positive region.
Mean Intensity in Negative Region	Mean marker intensity in the negative region.
StdDev of Intensity in Positive Region	Standard deviation of marker intensity in the positive region.

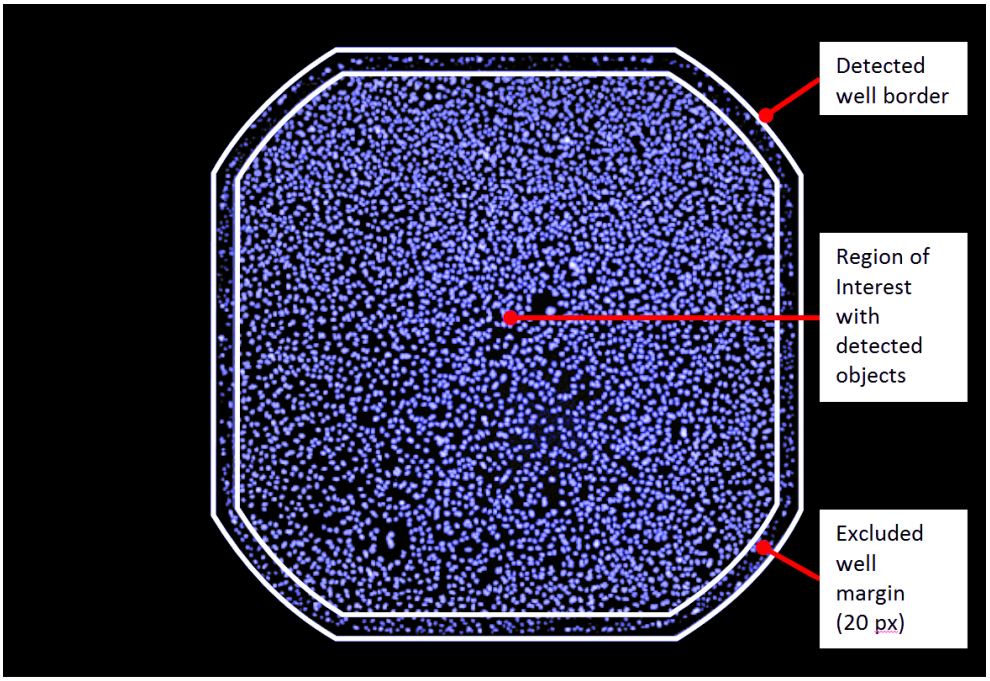
Advanced Options

This section gives access to rarely used options which usually have good default values and need no user attention for most applications. They are needed to enable some applications, which are otherwise impossible to do. Examples are well detection options for non-standard plates or specific selection of an object detection method for some assays. Parameters are grouped into different logical sections, depending on the algorithm they refer to, e.g. “Well Detection” or “Object Detection”. A copy of the mandatory parameters contained in the **Configuration** pane is also listed here.

If you want to restore the default value of a parameter, right-click the text box and select **Reset** from the context menu.

Well Detection

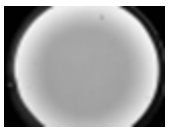
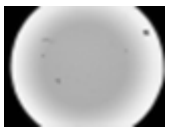
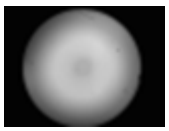
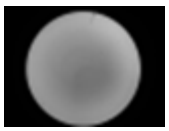
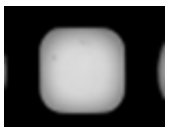
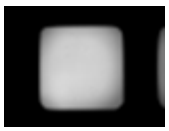
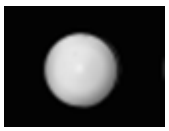
The first step of the analysis is to detect the well outlines to define the region of interest (ROI) for object or region detection. The well detection takes the well shape and diameter specified in the plate definition as a starting point and adapts it to the actual position and size of the well in the image.



Advanced Options – Well Detection

Input Parameter	Description
Excluded Well Margin [px]	Width of the excluded outer rim of the well. Can be used to exclude artifacts visible at the well border (e.g. glue rim in the well or cells aggregating in the corner). See illustration above. Default value: 20 px.
Well Detection Method	"Standard" (default) or "Fast". "Standard" is more reliable but slower.
Well Dimensions	• **Automatic** (default): Well dimensions of selected plate type definition are used. • **Manual:** You can manually enter values for **Well Shape** and **Well Diameter**.
Well Shape	*Only enabled if **Well Dimensions: Manual** was selected.* • “Round" or "Rectangle"
Well Diameter [mm]	*Only enabled if **Well Dimensions: Manual** was selected.* • Please enter a manually specified diameter (in case of "Rectangle": side length of the square well).
Channel	Channel used for well detection. Use "Automatic" to automatically select the best suited channel in each well. If automatic fails in your experimental setup, select a brightfield or fluorescence channel that works well in your setup.

Examples of pre-defined well dimensions in the Kaleido database

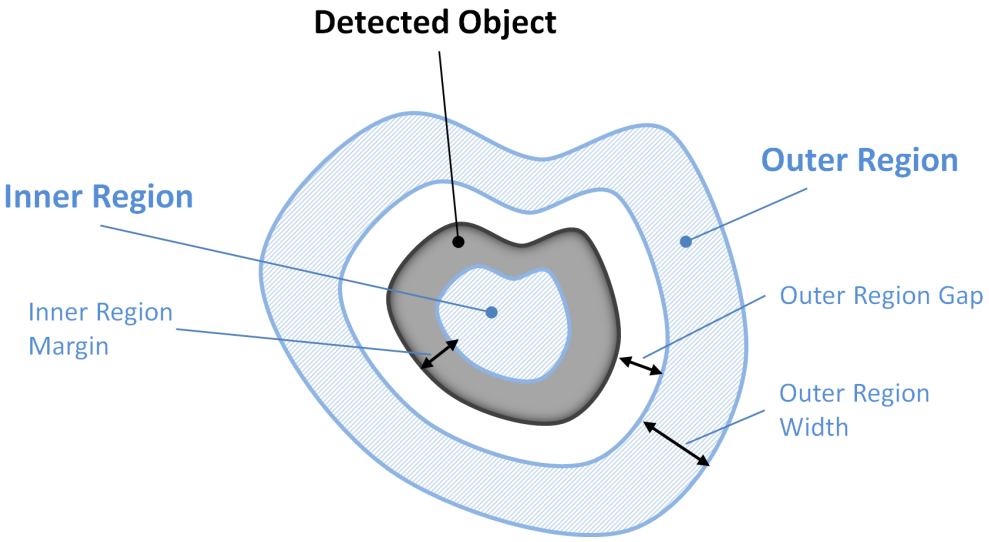
Plate Type	Well Shape	Well Diameter [mm]	Example Image (Brightfield)
ViewPlate-96, Glass bottom	Round	6.00	
ViewPlate-96	Round	6.10	
Cell Carrier-96	Round	6.58	
Label-free-96	Round	4.50	
ViewPlate-96, 1/2 Area	Round	4.38	
ViewPlate-384	Rectangular	3.30	
CellCarrier-384 Ultra	Rectangular	3.26	
Label-free-384	Round	2.82	

Object Detection

The next step is to detect objects inside the detected region of interest (ROI). There are three different detection methods available:

- **Method A:** Lower sensitivity. Low contrast objects may be missed, but higher robustness against false positive detected objects.
- **Method B (default):** Higher sensitivity. More low contrast objects are detected, but higher risk of false positive objects.
- **Method C:** Optimized for high cell densities and small objects, e.g. suspension cells. Still works well at cell densities when A and B already fail, but may pick up more false positive objects at very low cell densities.

For most assays method B (default) or C work best. To identify the best settings for your specific assay, visually test all methods with a couple of representative images. Make sure that you cover various cases, e.g. treated cells may look differently or there may be huge cell density or staining variations across different plates or wells.

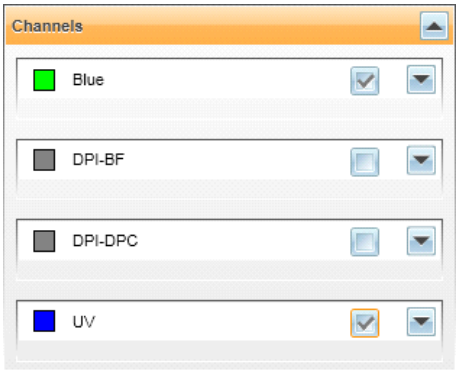
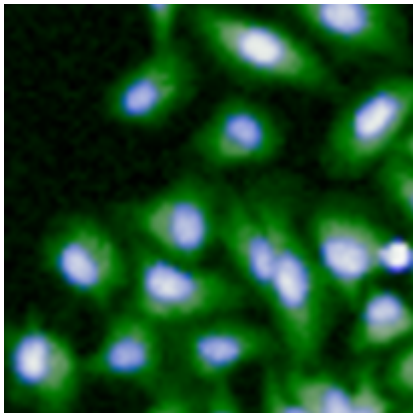


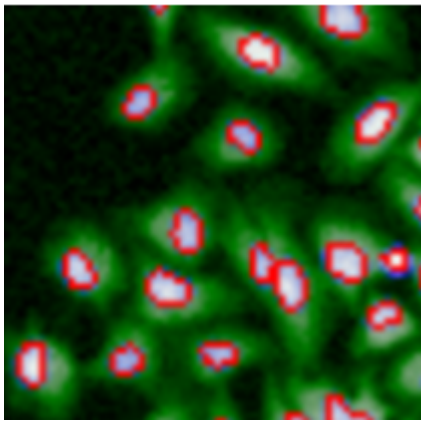
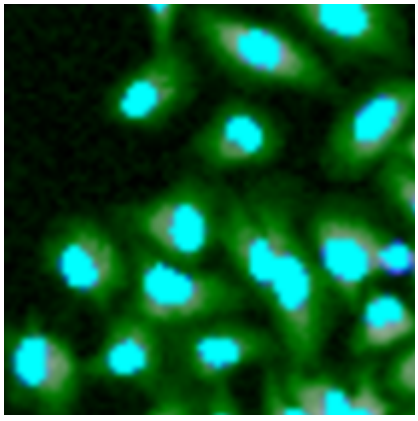
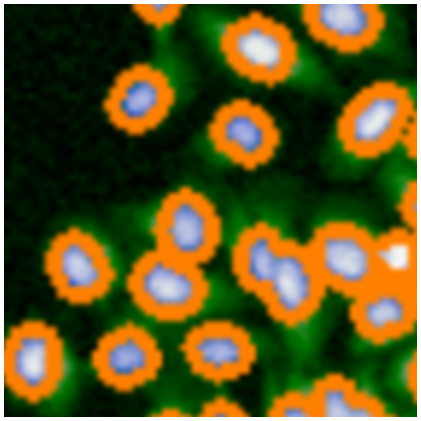
To adjust the **inner and outer region**, use the image view display:

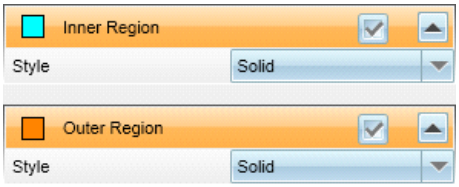
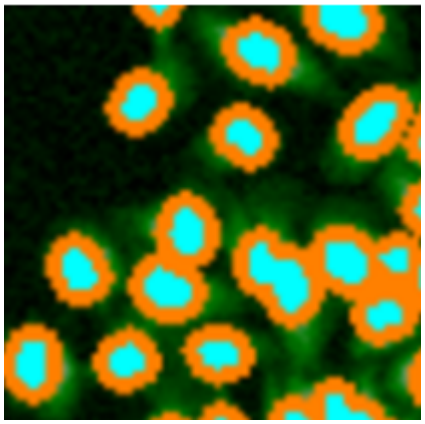
1. Zoom in to see a representative object.
2. Switch on and off the overlays for the **Object Region**, **Inner Region** and **Outer Region**.
3. Adjust color and style to best see the region outlines.

See also the following example and section 5.8.3 “Display Options”, page 211.

Example: Using different image view overlays to adjust and check the Inner and Outer Region parameters

Display Options	Overlay
 Overlay of two channels of the imaging operation. Adjust channels as needed. Also consider using black/white coloring.	

Display Options	Overlay
 Outline (border) of detected objects added as red line. The UV channel was used for object detection here.	
 „Inner Region“ area colored solid cyan.	
 „Outer Region“ area colored solid orange.	

Display Options	Overlay
 „Inner“ and „Outer Region“ overlays both switched on.	

Advanced Options – Object Detection

Input Parameter	Description
Channel	Fluorescence imaging channel for object detection. Alternatively, a digital phase imaging channel can be used.
Method	• Method **A** – lower object detection sensitivity but stricter filtering for false positive objects. • Method **B** – method with higher object detection sensitivity (default). • Method **C** – optimized for high cell densities and small objects, e.g. suspension cells. Still works well at cell densities when A and B already fail, but may pick up more false positive objects at very low cell densities.
Minimum Object Area [µm²]	Minimum area of objects to count. Objects smaller than this are discarded. Typical values are in the range of 30 to 300 µm². Default is 50 µm².
Inner Region Margin [px]	Margin from detected object border. Default: 0. See figure above. Negative values may be used to extend the region **outside** the detected object.
Outer Region Gap [px]	Distance of the outer region from the object border. Default: 0 px. See figure above.
Outer Region Width [px]	Width of the outer region around the object. Default: 3 px. See figure above.

Intensity Analysis

For the intensity measurements in the different regions of detected objects (whole object, inner region, outer region) it can be specified if a background intensity correction is used or not.

The intensity signal visible in the fluorescence channel image usually consists of an unspecific background signal originating e.g. from fluorescence of the supernatant of the sample, and the desired signal from the sample itself. The background signal can be neglected if it is low compared to the sample signal (e.g. in case of careful washing of the sample and low unspecific staining of the sample). If the background signal is higher and varying between or inside wells it may significantly bias or even falsify intensity measurements. In this case a background correction can be applied to improve results.

The background correction estimates the background fluorescence by analyzing the neighborhood intensity of each object and subtracting it from the measured intensity.

The correction assumes small objects like single cells. It gets inaccurate for larger closely packed agglomerates of objects without significant intensity drop between the objects.

Background correction is especially useful when selecting object populations based on intensity thresholds.

Advanced Options – Intensity Analysis

Input Parameter	Description
Background Correction	Switch on background correction of measured intensity values (default: off).

Ratio Calculation

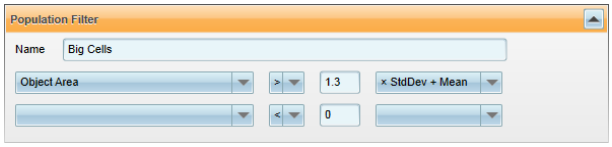
Advanced Options – Ratio Calculation

Parameter	Description
Property 1	Two object properties have to be selected. $Ratio = \frac{Property\ 1}{Property\ 2}$
Property 2	

Population Filter

Advanced Option – Population Filter

Parameter	Description
Name	Enter a name for the resulting population. • As soon as you enter a name, a new box for an additional population will be added below. • If you delete the name, this box will be removed (except for the first box).

Parameter	Description
Parameter	Parameter to be used as filter criterion. • As soon as you select one parameter, a new line for an additional filter criterion will be added. If multiple filter criteria are defined, they will be combined by a logical AND operation. • If you select the empty entry from the list, this line will be removed (except for the first line).
Relational Operator	Select a relational operator (<, ≤, >, ≥, =).
Value	Enter a value which is compared to the object parameters or used as a factor for the statistical value.
Statistical Method	Optionally, you can select a statistical value to be calculated for the whole population e.g. the mean value or mean + standard deviation. See example below. Statistical values include mean, median, standard deviation and median absolute deviation (MAD). Use median and MAD instead of mean and standard deviation to minimize the influence of outliers. **Example:** Select all cells that are bigger than the mean cell area + 1.3 times the standard deviation. 

Region Detection

Region Detection is designed to reliably discriminate regions containing a sample (cells, tissue, small animals) from empty background regions. This is done based on the texture of the region: Sample regions (cells, tissue) usually have a stronger texture than the empty background of the well.

In contrast to the object detection this analysis does not require the sample to be brighter than the background. It works for a wide range of samples and is very robust with respect to absolute intensity changes.

The drawback is, that the region detection does not allow to detect individual small objects like single cells. The whole region covered with objects is detected as a single entity.

Advanced Options – Region Detection

Input Parameter	Description
Channel	Fluorescence or brightfield imaging channel for region detection.

Input Parameter	Description
Minimum Area	Region parts smaller than this are discarded if they are not connected to a larger region. Typical values are in the range 100 to 1000 µm². The default value is 400 µm².
Contrast Threshold	Areas with roughness higher than this threshold are considered as cell area. Areas with roughness smaller than this are considered as background. Typical values are in the range of 20 to 150. Default is 30.

Region Intensity Analysis

Configuration Parameters – Region Intensity Analysis

Parameter	Description
Marker Channel	Fluorescence imaging channel for sub-region detection.
Marker Threshold	Threshold for sub-region detection: Regions with intensity above the threshold are assigned to the "Positive" region.
Minimum Area	Minimum area of positive region fragments in square pixels. Areas smaller (or equal) than this value are discarded if they are not connected to a larger region. The default value is 1 (single pixel areas are discarded). Can be used to remove obvious artifacts.

5.2.3 Post Processing Sequence

In the **Post Processing Sequence** you can define an automatic export of results right after the measurement (and the analysis, if defined) has been finished.

Click the + button and select **Export to file** from the pop-up menu to add an **EXPORT** operation. If you click the operation, its parameters will be displayed in the **Content Area** where you can configure format and target location of the export. You can add multiple operations to export results in multiple formats or to different locations. Click − to remove an operation.

Export Format	Extension	Description
CSV_ PLATE	*.csv	Comma-separated values, formatted as plate layout
CSV_ LIST	*.csv	Comma-separated values, list of results
XML	*.xml	Transfer to e.g. MyAssays Desktop data analysis software
ARCHIVE	*.kal	Export results together with protocol settings. These ***.kal** files can e.g. be imported to Kaleido on a different system.

Notice

- For transferring measurement results to **MyAssays Desktop** for further analysis, you have to use the **XML** export format. See also section 5.12 “MyAssays Desktop Data Analysis”, page 242.
- When opening ***.csv** result files in Microsoft Excel®, the number format of the **Time** column will not be set correctly. Please define a custom format for this column using the following pattern: *[h]:mm:ss.000*

Parameters

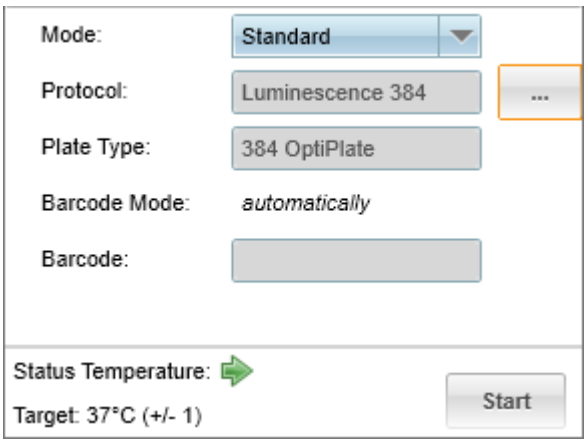
Parameter	Description
Export Format	Select an export format (CSV_PLATE, CSV_LIST, XML, ARCHIVE). Default is CSV_PLATE.

Parameter	Description
Format Options	Only enabled if **CSV_PLATE** or **CSV_LIST** is selected as export format. • **Standard:** Default format incl. file header, footer and column headers. • **DataOnly (with CSV_LIST):** Recommended for Spotfire® users without SciStream (no file header and footer, but with column headers). • **DataOnly (with CSV_PLATE):** Recommended for LIMS users (very simple format, only result values). **Notice** When using the **DataOnly** format, the export file will have no file header or footer. The content can only be identified by choosing a suitable file name.
Export Path	Enter a target path for the export file or click **[…]** to browse for a folder. For exporting XML files to MyAssays Desktop it is suggested to use the default Kaleido output folders. See also section 5.12.5.1 “Kaleido Output Folders (Default)”, page 243.

Parameter	Description
File Name	Enter the desired file name or click **Variables** to select properties of the protocol (e.g. [ProtocolName]) from a list. **Using Variables:** The selected variables will appear in the text box and will be replaced by the corresponding property during export. • More than one variable can be used and combined with manually entered text. • Changing the text between the square brackets [] or removing these will disable the automated conversion of the variable. • Existing export files will be overwritten if the same file name is used again. **Notice** • When exporting multiple measurements (e.g. during a stacker run or in **Automation** mode), the data of each measurement will be exported to a separate file. Please make sure to insert variables which generate unique file names (e.g. date variables). Otherwise the export file will be overwritten again and again. • The [MeasurementDate] variable uses the start date of the measurement. Please note that this date is identical for continued measurements, i.e. using this variable does not always generate unique file names. • To ensure that no files are overwritten, add the [ExportDate] variable. **Resulting File Names** Date and signature variables get an additional letter at the beginning to indicate the type of variable. See the following examples: • [ExportDate]: **E_**20160921-162853 • [MeasurementDate]: **M_**20160720-104340 • [MeasurementSignature]: **M_**469ffbf3-6f94-4d06-82f8-f00c0c17d6b5 • [ProtocolSignature]: **P_**2440c5aa-269b-4bee-9f96-6b3e32870ab5 Dates are stated in this format: *YYYYMMDD-hhmmss* (24-hour notation)

5.3 Run Protocol

On the **Run Protocol** screen you can load and run protocols. The measurement progress and results will be displayed on the **Plate**, **Well** and **Graph** tabs in the **Content Area**.



Global control section in Standard mode

In the global control section you first select a **Mode** for the measurement (see descriptions below):

- **Standard:** Normal measurement. Local control, plate loading manually or via stacker.
- **Continue Measurement:** Continue an existing partial measurement. Local control, plate loading manually or via stacker.
- **Automation:** Automated measurement controlled by external scheduler (remote control), not compatible with stacker.

Stacker (optional)

Plate loading can be done manually (using Load/Eject) or using a stacker. If the instrument has a stacker, the magazine table determines whether automatic plate loading is used or not:

- Magazine table installed: Automatic plate loading (stacker)
- Magazine table not installed: Manual plate loading (Load/Eject)

See also section 4.13 “Stacker”, page 58 for detailed instructions.

Compatibility of the stacker with other functions

- Operation "Shake (outside)" cannot be used (to avoid crash with magazine table). Please use "Shake (inside)" instead.
- Barcode settings:
  - Option "Enter barcode manually" cannot be used. Please select "Generate virtual barcode" for plates without barcode.
  - Barcode reading must be activated for any measurement in **Continue Measurement** mode because a real barcode is required.
- Automated runs via external scheduler (**Automation** mode) cannot be combined with the stacker. The software will immediately switch to **Standard** mode as soon as the magazine table is installed.

Buttons and Elements

Element	Description
Mode	**Standard:** Default mode for normal measurements (local control). See also section 5.3.1 “Standard”, page 150. **Continue Measurement:** Allows you to select and continue an existing measurement. See also section 5.3.2 “Continue Measurement”, page 153. **Automation:** Allows an external scheduling software to control the instrument. As long as the automation mode is active, most functions are disabled. See also section 5.3.3 “Automation”, page 158. **Notice** • Measurements with **Stop** operations cannot be used in **Automation** mode. • *For optional stacker only:* The stacker cannot be used in **Automation** mode. If the magazine table is installed, this mode cannot be selected.
Protocol [...]	Displays the name of the currently loaded protocol. Click ... to load a protocol from the database. See also section 5.6 “Load Dialog”, page 180. **Notice** If the protocol still contains deprecated analysis operations, you will get an error message. Please setup a new analysis sequence. See also section 5.2.2 “Analysis Sequence”, page 120 for details.
Plate Type	Displays the plate type of the currently loaded protocol.

Element	Description
Measurement [...]	Only visible in **Continue Measurement** mode: Allows you to select the measurement(s) to be continued. Click ... to load a measurement from the database. See also section 5.6 “Load Dialog”, page 180. After the selection, the measurement name will be displayed. If multiple measurements have been selected (only possible with stacker), "Multiple" will be displayed. **Note:** If a measurement was already loaded on the **View Results** screen before you activated **Continue Measurement** mode on **Run Protocol**, the current measurement will automatically be selected (measurement name will be displayed).
Barcode Mode	Displays a summary of the barcode reader configuration. To modify the settings and to view all details, use the **Settings – Barcode Reader** dialog. See also section 5.10.4 “Barcode Reader”, page 232. • **Automatically:** Barcode reader activated. • **Virtually:** Barcode reader deactivated, virtual barcode will be generated (time stamp). • **Manually:** Barcode reader deactivated, user will be prompted to enter barcode manually before measurement.
Barcode	Displays the plate barcode of the current measurement.
Temperature	Displays status of the temperature control and target temperature (if activated): : **Start-up (heating)**, current temperature > 4 °C below Ready state : **Start-up (cooling)**, current temperature > 4 °C above Ready state : **Regulating**, current temperature < 4 °C below/above Ready state, not stabilized yet : **Ready**, target value reached and stable since > 60 sec. (see definition below) : **Operating Error** **Off**: Temperature control deactivated Definition of **Ready** state is variable and depends on target temperature: • Target = 15 – 50 °C → ± 1 °C • Target = 51 – 60 °C → ± 2 °C • Target = 61 – 65 °C → ± 3 °C Max. temperature = 65 °C

Element	Description
[Start]	Starts the protocol, instrument status symbol changes to "Busy" . *For stacker only:* If the magazine table is attached, automatic plate loading via stacker will be used. For details see section 4.13 "Stacker", page 58. If the database if more than 90 % full, you will get a warning if you try to start a measurement. For troubleshooting see also section 5.14.3 "Database or disk is full", page 251.
[Stop]	Opens a dialog which allows you to stop the measurement (further confirmation required). See also section "How to stop a measurement", page 152.

5.3.1 Standard

The **Standard** mode is used for regular measurements (local control). Plate loading can be done manually or automatically by means of the optional stacker.

How to run a protocol (manual plate loading)

For stacker only: If you want to load plates manually, please first remove the magazines and the magazine table.
See also section 4.13 "Stacker", page 58.

1. Click **Run Protocol** in the **Navigation Bar**.
2. Select the **Standard** mode.
3. Click … next to **Protocol** and load a protocol.
   See also section 5.6 "Load Dialog", page 180.

   Alternatively, you can prepare and save a protocol on the **Setup Protocol** screen or use a protocol loaded on **View Results** or **Analysis**. When you switch to **Run Protocol**, the protocol will still be loaded and ready to be run.

   The respective plate layout will be displayed in the **Plate Map** section on the right.
4. If the plate carrier is in the instrument, click the **Eject** button in the **Navigation Bar** and the plate carrier will come out.
5. Put a suitable sample plate onto the plate carrier. Check the following:
   - Correct plate type used?
   - Plate filled as defined in the plate map?
   - Plate orientation correct (well A1 in the upper left corner)? See also section 5.1.5 "Load/Eject", page 66.
6. *Optional:* If you want to use the **Barcode Reader**, make sure it is activated in the **Settings** (see section 5.10.4 "Barcode Reader", page 232).
7. Click **Start**.

   The protocol settings are validated, the plate is loaded and the measurement is started.

How to prepare the stacker

See also section 4.13 “Stacker”, page 58 for detailed instructions how to handle the stacker equipment.

1. Make sure that no plate is inserted (plate carrier must be empty).

   Notice

   If a plate was forgotten inside the instrument the measurement will not be started until you remove the plate and initialize the instrument.

2. Install the magazine table. This will activate automated plate loading via stacker.
3. Prepare the magazines:
   - Load the plates to be measured into the input magazine and place it at the right position of the magazine table.
   - Place an empty magazine (same capacity) at the left position.

How to run a protocol (automatic plate loading with optional stacker)

1. Prepare the stacker as described above (see section “How to prepare the stacker”, page 151).
2. Click **Run Protocol** in the **Navigation Bar**.
3. Select the **Standard** mode.
4. Click … next to **Protocol** to select and load a protocol.
   See also section 5.6 “Load Dialog”, page 180.

   Alternatively, you can prepare and save a protocol on the **Setup Protocol** screen or use a protocol loaded on **View Results** or **Analysis**. When you switch to **Run Protocol**, the protocol will still be loaded and ready to be run.
5. Open the **Settings – Stacker** dialog and enter the **Flange Height** of the used plate type.

   Notice

   See section 5.10.7 “Stacker Settings”, page 238 for background information and instructions.

6. In the global control section of **Run Protocol**, click **Start**.
   - The magazines and the magazine table are locked.
   - The first plate is loaded from the input magazine (right) and the plate is measured. The measurement is not finished until the analysis (if applicable) and possible plate repeats have also been completed.
   - The measured plate is unloaded into the output magazine (left) and the next plate is loaded.
   - The run is finished if there is no plate left in the right magazine.
   - The magazines and the magazine table are unlocked.

How to view measurement progress and results

During measurement, you can view the progress in the **Content Area** on the **Plate**, **Well** or **Graph** tab, showing which well is being measured and the results for each well in live view. Since a graph requires at least two measurement points, the **Graph** tab is only enabled for repeated measurements or wavelength scans in the operation (measurement mode). The **Well** tab is only available for well scans.

For details please see the following sections:

- 5.7.1 “Plate”, page 194
- 5.7.3 “Well”, page 199
- 5.7.2 “Graph”, page 196

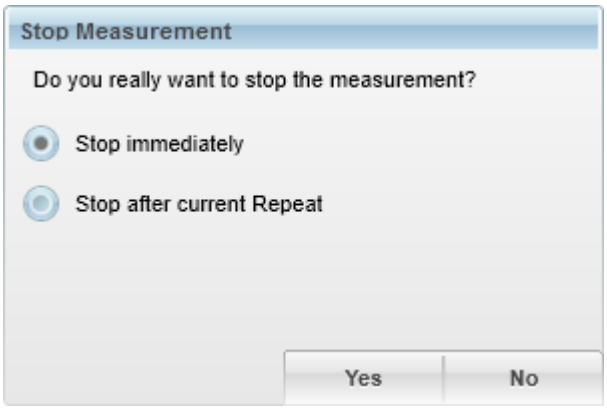
The active operation of the protocol is highlighted in orange. With each new operation, the displayed results are refreshed in the **Content Area**. You can only see progress and results of the current operation and plate or plate repeat. The barcode of the currently measured plate is displayed in the global control section, and the current plate repeat is displayed on the right in the **Navigation** pane.

After the measurement the **View Results** screen is opened automatically. Using the controls below the **Plate Map** you can select and view the measurement results of all repeats.

How to stop a measurement

1. To abort a running protocol, click on **Stop**.

   The following dialog is opened, but the measurement is not aborted yet.



2. Select the desired option:
   - **Stop immediately:** The measurement will be terminated and the data measured so far will be saved in the database.
   - **Stop After Current Repeat** (not available for execution type **By Well**): the current repeat will be completed before the measurement is stopped. You may use this option to finish a current repeat.
3. Click **Yes** to confirm and stop the measurement or **No** to cancel.

Consequences of stopping a measurement:

- If an analysis is part of the protocol, all wells measured so far will be analyzed.
- If the measurement was part of a stacker run, the current plate will be unloaded to the left magazine (output). All remaining plates will not be measured.
- If you abort a measurement manually using the **Stop** button, this measurement can *never* be continued. See also section 5.3.2 “Continue Measurement”, page 153.

5.3.2 Continue Measurement

Using this mode you can continue an existing partial measurement (e.g. for time series measurements with breaks).

The measurement to be continued must contain **one or multiple Stop operations**. These stops split up the measurement sequence into multiple steps (see section “Stop”, page 110).

- The **first step** of the protocol (until the first **Stop** operation is reached) has to be measured in **Standard** mode.
- The **next step(s)** have to be measured in **Continue Measurement** mode (see detailed instructions below).

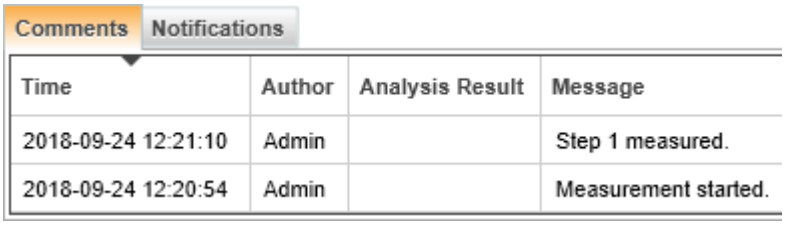
Notice

- There must be **steps** left which have **not yet been measured**. When all steps of a measurement have been completed, it cannot be continued anymore.
- Only measurements with execution type **By Plate** or **By Plate Stack** can be continued. See also section 5.2.1.1 “Execution Type”, page 75.

How to measure the first step of a measurement

1. Prepare a protocol which contains at least one **Stop** operation.
2. On **Run Protocol**, select **Standard** mode and **Start** the protocol.

   The first step of the protocol is measured, i.e. all operations are processed until the (first) **Stop** operation is reached and the measurement is stopped. You will be notified in the **Comments** area:



How to continue a measurement (manual plate loading)

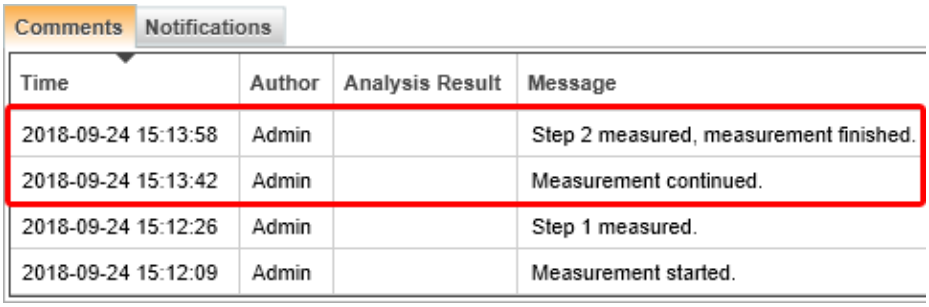
1. On **Run Protocol**, select the **Continue Measurement** mode.

   An additional field **Measurement** is displayed. If a measurement had already been loaded on the **View Results** screen, this measurement will be selected and its name will be displayed in this field.

2. Click ... next to **Measurement** and select the measurement to be continued. See also section 5.6.5 “Load Measurement(s)”, page 187.

   Protocol, plate type and plate barcode of the selected measurement are displayed.

3. If the plate carrier is in the instrument, click the **Eject** button in the **Navigation Bar** and the plate carrier will come out.
4. Put the sample plate with the displayed barcode onto the plate carrier. Check if the plate orientation is correct (well A1 in the upper left corner). See also section 5.1.5 “Load/Eject”, page 66.
5. Click **Start**.
   - The protocol settings are validated, the plate is loaded and the measurement is started.
   - The next unprocessed step of the measurement sequence will be measured according to the selected execution type. The partial measurement is stopped when a **Stop** operation or the end of the sequence is reached. See also **Comments** area:



   - The new measurement results will be added to the existing measurement.

Continue Multiple Measurements/Plates (Stacker)

The **Continue Measurement** mode can also be used in combination with a **stacker**. The workflow for preparing the stacker and activating automatic plate loading is the same as described for the **Standard** mode (see before).

- Instead of a single measurement you can also select **multiple measurements/plates** to be continued. These measurements must be compatible to each other:
  - They must have been measured with the identical **protocol version**.
  - They must have the same **Step** value (e.g. 2 of 3 steps processed).

> **Notice**
> The progress of a partial measurement is displayed by the **Step** property in the **Load Measurement(s)** dialog. The **Show Compatible** option can be used to filter the list for measurements which match regarding the protocol version and the **Step** value. See also section 5.6.5 “Load Measurement (s)”, page 187.

- A plate in the stacker will be measured again if its barcode matches one of the selected measurements and if its measurement sequence is not completed yet. Otherwise the plate will not be processed and unloaded to the output magazine.
- Also, if you select measurements which have a manually entered or virtual barcode, these measurements will not be processed, because the corresponding plate cannot be identified. Only measurements with real barcodes can be used.
- It depends on the selected **execution type** how the measurement will be performed. See also section 5.2.1.1 “Execution Type”, page 75 for examples.
  - **By Plate:** The next unprocessed step of the measurement sequence is executed completely, i.e. all operations are measured until the next **Stop** operation or the end of the sequence is reached. Then the next plate is loaded.
  - **By Plate Stack:** Only the first operation of the next unprocessed step of the measurement sequence is executed. Then the next plate is loaded and measured the same way. This is repeated until this operation has been applied to all plates in the stacker. Then the plates are automatically restacked back into the input magazine and the next operation is applied to all plates in the stacker. The measurement is finished if all plates have completed this step (i.e. reached Stop operation or end of sequence).

Notice

- *For stacker only:* Barcode reader must be activated and only plates with barcode can be measured and continued. See also section 5.10.4 “Barcode Reader”, page 232.
- You cannot continue measurements which had been performed with an older software version (e.g. Kaleido 2.0).
- Measurements cannot be continued in **Automation** mode.
- Aborted measurements (after an error or manually canceled by the user) cannot be continued.

How to continue multiple measurements (automatic plate loading via stacker)

1. Prepare the stacker and activate automatic plate loading as described for the **Standard** mode (see section “How to prepare the stacker”, page 151).

   Notice
   If you want to measure the same plates again and restore the initial order of plates, you can use the **Restack Plates** function to transfer the plates from the output magazine (left) back to the input magazine (right). See also section “Restack Plates”, page 240. If the order is not important, you can just switch the magazines.

2. Click **Run Protocol** in the **Navigation Bar**.
3. Select the **Continue Measurement** mode.

An additional field **Measurement** is displayed. The ... button for loading protocols is disabled, the protocol contained in the respective measurement will be used automatically.

4. Click ... next to **Measurement** and select the measurements to be continued (multiple selection is possible with stacker only).

   The load dialog is opened. For details see also section 5.6 “Load Dialog”, page 180.

   - Select one of the measurements to be continued. You can add or remove columns and set filters as required to find the desired object.
   - Activate the filter **Show compatible** in the first column.

     Now multiple selection is enabled and only those measurements are displayed which have been measured with the same protocol version (identical protocol signature) and which have the same **Step** value (processing state) as the selected measurement.

     Notice
     Make sure to select only measurements with **real barcodes** (no manual or virtual barcodes). Otherwise these measurements will not be processed, because the corresponding plate cannot be identified.

   - Select the desired measurements and click **OK**.

   In the Global Control section, the protocol name is displayed and "Multiple" is displayed as measurement name and barcode.

5. Open the **Settings – Stacker** dialog and enter the **Flange Height** of the used plate type.
   See section 5.10.7 “Stacker Settings”, page 238 for background information and instructions.

6. Final checks:
   - No plate inserted? Plate carrier must be empty.
   - Barcode reader activated? Barcode mode "Automatically" must be displayed, see also section 5.10.4 “Barcode Reader”, page 232.

7. Click **Start**.
   - The magazines and the magazine table are locked.
   - The first plate is loaded from the input magazine (right) and the barcode is read. If one of the selected measurements contains this barcode, the plate will be measured. Otherwise it will be skipped.
   - The new measurement results will be added to the existing measurement as additional plate repeat.
   - The measured plate is unloaded into the output magazine (left) and the next plate is loaded.
   - The run is finished if there is no plate left in the right magazine.
   - The magazines and the magazine table are unlocked.

Troubleshooting: Continue multiple measurements (stacker)

If multiple measurements and plates were selected for continuation, but some of them were not continued, the possible reasons are:

Possible reason	Solution
Respective plate was not in stacker magazine.	Make sure to load the plates of all selected measurements into the input magazine. Compare the barcodes on the plates with those in the selected measurements.
Respective plate (with intact barcode) was in the stacker magazine, but no measurement with this barcode was selected to be continued.	
Barcode of respective plate could not be read (see notifications).	Check if the plate barcode is missing, soiled or damaged.
Measurements with a manual/virtual barcode were selected. Real barcodes are required for plate identification.	Select only measurements with real barcodes.

5.3.3 Automation

Activate this mode to allow an external scheduling software to control the instrument (for using EnSight in an automated environment). As long as the automation mode is active, most functions in Kaleido are disabled. Only measurement progress and results are displayed on the **Plate** tab (**Run Protocol** screen).

Notice

- Protocols to be measured in **Automation** mode must not contain **Stop** operations. For a workaround see section 5.14.6 “Replacing Stops in Automation Mode”, page 254.
- *For optional stacker only:* The stacker cannot be used in **Automation** mode (magazine table must be detached). Protocols with execution type **By Plate Stack** will be rejected.

How to prepare automated measurements (Automation mode)

Notice

- Eject and remove any sample plate before starting an automated run. Such a plate could lead to a crash of the robot and damage the instrument.
- Check prior to your run if the protocol(s) can be executed and no error message regarding the plate type is shown. If you load a protocol, the plate type definition will be read directly from the protocol, not from the current plate type definition in the database (which may have been changed in the meantime). To prevent any crashes, please check prior to your run if the plate type used in the protocol can *really* be handled by the robot. We recommend checking this under supervision. If you want to make sure to use the current plate type definition from the database, you can edit the protocol and select the plate type again. Any old plate type version will be overwritten.
- If an automated result transfer to MyAssays Desktop has been configured, you should activate the option **Close MyAssays Desktop After Analysis** for the corresponding poll operation in the Folder Poll Manager. Otherwise a new instance of MyAssays Desktop will be opened after each protocol run. See also section 5.12 “MyAssays Desktop Data Analysis”, page 242.

Checklist for automated runs
Selected plate type can be handled by the robot?
Filter wheel contains all required excitation filters?
Execution type of the protocol is "By Well" or "By Plate"? ("By Plate Stack" will be rejected)
Protocol does not contain "Stop" operations?
Protocol saved in the database?

Checklist for automated runs
Test measurement of one plate successful (to determine protocol duration for scheduler)?
EnSight is in idle state (green status light and status symbol ✓)?
Plate carrier is empty? (no plate inserted)
“Automation” mode activated in Kaleido (Run Protocol)?

Kaleido functions behaving differently in Automation mode

- Barcode handling
  See section 5.10.4 “Barcode Reader”, page 232 for details.
- Temperature control
  “Turn OFF after next run of protocol” will be ignored, no automatic shutdown of the temperature control.
- General Settings
  "Load plate into instrument automatically" (disabled)
- Stop operations
  Not allowed in Automation mode (measurement will be rejected)

How to specify the protocol to be measured in the external scheduler

In your external scheduling software you need to define the exact Kaleido protocol which is to be measured. The easiest way to do this is to select the protocol from a **list of all protocols** which has been requested from the instrument – if your scheduler supports this feature (e.g. PerkinElmer's plate::works scheduler).

Otherwise the protocol has to be specified manually using the following parameters. It is not sufficient to specify only the protocol name, because protocol names in Kaleido are not unique anymore. One of the following options can be used to uniquely identify a protocol:

- *Signature*
- *User$ProtocolName* (latest protocol version is used)
- *User$ProtocolName$Date*

Notice

- The exact way how to enter the required parameters and which of the above options are supported will depend on the used scheduling software. Please refer to the manufacturer's documentation.
- It is recommended to copy and paste the required parameters directly from Kaleido to avoid typos. The “Load …” dialogs in Kaleido allow you to copy properties via context menu.

Legend:

- **Signature:** Database signature of the protocol (displayed in the "Load ..." dialogs)

- **User:** Owner of the protocol

  Notice
  The full user name is required, i.e. including the computer name of the EnSight PC.
  Example: *"EnSight-PC\UserXY"*

- **ProtocolName:** Name of the protocol
- **Date:** Timestamp of the protocol in the database, if not present the latest version will be used.

  Supported date formats:

  - YYYY-MM-DD HH:MM:SS
  - MM/DD/YYYY HH:MM:SS a
  - YYYY-MM-DD HH:MM:SS a

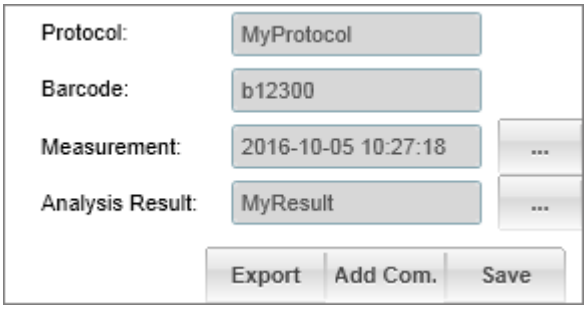
How to stop an automated run

Ideally, an automated run should be stopped via the external scheduler. If it does not offer a corresponding function, you can stop the run locally:

1. On **Run Protocol**, switch to back **Standard** mode.
2. Click **Stop**.
3. Select the desired option:
   - **Stop immediately:** The measurement will be terminated and the data measured so far will be saved in the database.
   - **Stop After Current Plate Repeat**: The current plate repeat will be completed before the measurement is stopped.
4. Click **Yes** to confirm and stop the measurement or **No** to cancel.

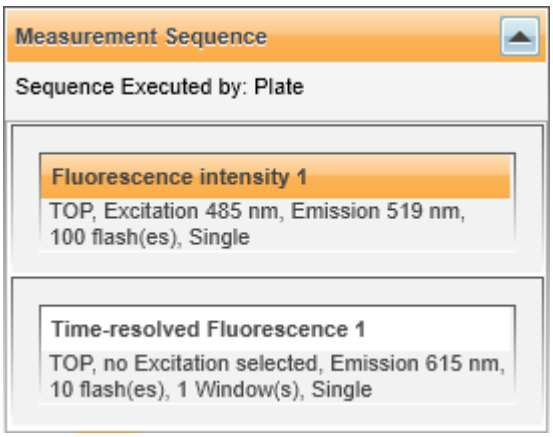
5.4 View Results

On the **View Results** screen, you can view **measurement** and **analysis results**.

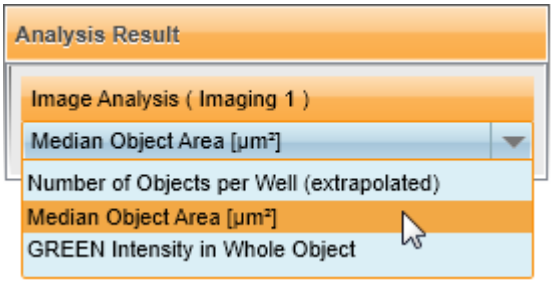


A measurement is the result of a protocol run. After running a protocol, Kaleido automatically switches to the **View Results** screen and displays the just finished measurement.

- To view measurement results, click on the desired operation in the **Measurement** section on the left. In case of an **Imaging** operation, the corresponding image will be displayed automatically on the **Image(s)** tab if you click a well on the **Plate** tab or **Plate Map**.



- To view analysis results, click on the desired operation in the **Analysis Result** section on the left. You can choose one result to be displayed as heatmap on the **Plate** tab (via drop-down menu below operation title).



Measurement or analysis results of the selected operation will be displayed in the **Content Area**. There are multiple tabs (depending on selected operation), see the following sections for details:

- 5.7.1 “Plate”, page 194
- 5.7.2 “Graph”, page 196
- 5.7.3 “Well”, page 199
- 5.7.4 “List”, page 201
- 5.7.5 “Image(s)”, page 203
- “Image Overview”, page 94

Buttons and Elements

Element	Description
Protocol	Displays the protocol name of the loaded measurement.
Barcode	Displays the plate barcode of the loaded measurement.
Measurement	Displays the name of the currently loaded measurement. Click … next to **Measurement** to open the **Load Measurement(s)** dialog and load a measurement.
Analysis Result	Displays the name of the loaded analysis result. Click … next to **Analysis Result** to open the **Load Analysis Result(s)** dialog and load an analysis result. Note that the corresponding measurement will be automatically loaded as well.
Export	Opens dialog for manual export of results (see also section 5.4.3 “Export Results”, page 167). After re-calculation, first save the **Analysis Results** since only saved data can be exported. For automated export of results see section 5.2.3 “Post Processing Sequence”, page 144.
Add Com.	Allows you to add a comment to the currently loaded measurement or to the analysis result. See also section 5.4.4 “Add Comment”, page 172.
Save	Opens a dialog for saving analysis results or/and saving analysis to protocol. Only enabled when analysis results have been loaded. See also section 5.4.5 “Save”, page 172.

5.4.1 Load Measurement

Normally you can only load a single measurement. Using the **Show compatible** filter in the load dialog you can also pre-select multiple measurements generated with exactly the same protocol (identical signature). Then you can browse the different measurement results using the navigation tools below the plate map. For detailed instructions see the following sections.

How to load and view a single measurement

1. Click … next to **Measurement**.

   The **Load Measurement(s)** dialog is opened where you can search for the desired measurement to be loaded. For details see also section 5.6 “Load Dialog”, page 180.

2. Select the desired measurement and click **OK**. You can also double-click a

measurement.

The dialog is closed and the measurement is loaded.

- The corresponding protocol used for this measurement is contained in the measurement. It is also loaded and displayed on **Setup Protocol** and **Run Protocol**.
- Any previously loaded analysis result will be removed from the user interface.
- For details see also section 5.6.2 “Inheritance of Objects and Properties”, page 184.

3. The measurement results of the first measurement operation are displayed on the **Plate** tab. To view results of a different measurement operation, select an operation from the **Measurement** section on the left. Results are displayed on the **Plate** tab as default, but they can be also viewed on the **List**, **Graph** or **Well** tab (**Graph** and **Well** are only available when appropriate measurement results are loaded). See also section 5.7 “Content Area”, page 194.

How to select and view measurements of a batch of plates (e.g. stacker run)

1. Click … next to **Measurement**.

   The **Load Measurement(s)** dialog is opened where you can search for the desired measurement to be loaded. For details see also section 5.6 “Load Dialog”, page 180.

2. Select the desired measurements:
   - Select one of the measurements to be loaded. You can add or remove columns and set filters as required to find the desired object.
   - Activate the filter **Show compatible** in the first column.

     Now multiple selection is enabled and only those measurements are displayed which have been measured with the same protocol version (identical protocol signature) as the selected measurement.
   - Select multiple measurements:
     - Multiple single objects: Hold down **Ctrl** key and click objects.
     - Group of objects: Click first object, hold down **Shift** key, click last object.
     - Using the mouse: Click on an object, hold down the mouse button and drag the mouse.
3. Click **OK**.

   The dialog is closed and the measurements are loaded.
   - The corresponding protocol used for the measurements is also loaded and displayed on the **Setup Protocol** screen.
   - Any previously loaded analysis result will be removed from the user interface.
   - For details see also section 5.6.2 “Inheritance of Objects and Properties”, page 184.
4. Select the actual measurement results to be displayed in the **Content Area**:

- Select an operation from the **Measurement Sequence**.
  The measurement results of that operation are displayed on the **Plate** tab as default, but they can be also viewed on the **List**, **Graph** or **Well** tab (**Graph** and **Well** are only available when appropriate results are loaded). See also section 5.7 “Content Area”, page 194.
- Use the navigation controls below the plate map to select the desired measurement, plate repeat or well repeat. See also section 5.8.1.3 “Plate Map on View Results”, page 209.

5.4.2 Load Analysis Result

Normally you can only load a single analysis result. Using the **Show compatible** filter in the load dialog you can also pre-select multiple analysis results of different plates, e.g. for inspecting analysis results of a batch of plates. You can only select analysis results which ...

- belong to measurements which were generated using exactly the **same protocol** (identical signature)

  AND

- were created using the same **analysis sequence**. The analysis sequence can be created in two different places or situations:
  - **Protocol** (if an analysis sequence is defined before the measurement)
  - **Analysis result** (subsequent recalculation on **Analysis** screen, based on existing imaging measurement or existing analysis result)

Then you can browse the measurement results using the navigation tools below the plate map. The corresponding analysis results will be loaded automatically. For detailed instructions see the following sections.

How to load and view a single analysis result

1. Click … next to **Analysis Result**.

   The **Load Analysis Result(s)** dialog is opened where you can search for the desired analysis result to be loaded. For details see also section 5.6 “Load Dialog”, page 180.
2. Select the desired analysis result and click **OK**. You can also double-click an

object.

The dialog is closed and the analysis result is loaded.

- The corresponding protocol and measurement are contained in the analysis result. They are also loaded and displayed on the corresponding Kaleido screens.
- Any previously loaded data object will be replaced.
- For details see also section 5.6.2 “Inheritance of Objects and Properties”, page 184.

3. To actually view analysis results in the **Content Area**, select an operation from the **Analysis Sequence**. The analysis results of that operation are displayed on the **List** tab. See also section 5.7 “Content Area”, page 194.

How to view analysis results of a batch of plates (e.g. stacker run)

1. Click … next to **Analysis Result**.

   The **Load Analysis Result(s)** dialog is opened where you can search for the desired analysis results to be loaded. For details see also section 5.6 “Load Dialog”, page 180.

2. Select the desired analysis results:
   - Select one of the analysis results to be loaded. You can add or remove columns and set filters as required to find the desired object.
   - Activate the filter **Show compatible** in the first column.

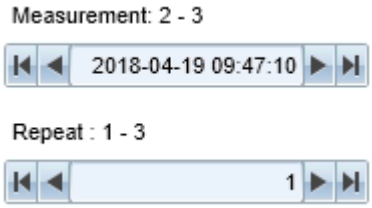
     Now multiple selection is enabled and only "compatible" analysis results are displayed (generated with the same protocol version and using the same analysis sequence).
   - Select multiple analysis results:
     - Multiple single objects: Hold down **Ctrl** key and click objects.
     - Group of objects: Click first object, hold down **Shift** key, click last object.
     - Using the mouse: Click on an object, hold down the mouse button and drag the mouse.

3. Click **OK**.

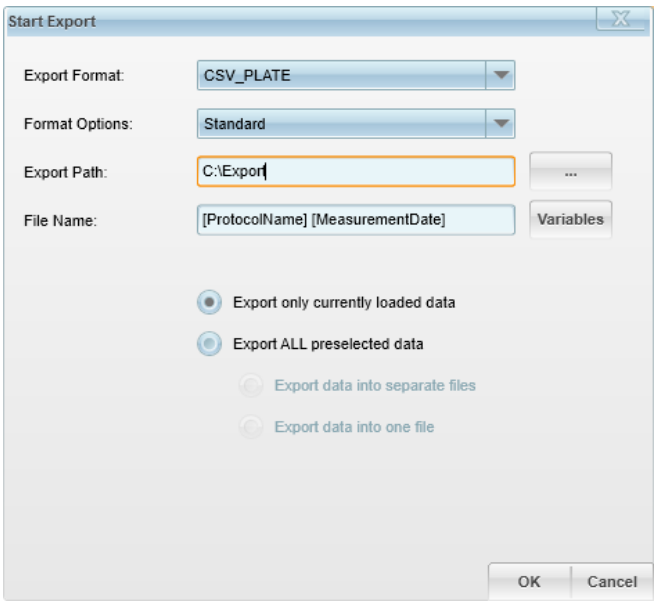
   The dialog is closed and the first analysis result is loaded.
   - The corresponding protocol and measurement are contained in the analysis result. They are also loaded and displayed on the corresponding Kaleido screens.
   - Any previously loaded data object will be replaced.
   - For details see also section 5.6.2 “Inheritance of Objects and Properties”, page 184.

4. Select the actual measurement results to be displayed in the **Content Area**:
   - Select an operation from the **Analysis Sequence**.
     The analysis results of that operation are displayed on the **Plate** tab. See also section 5.7 “Content Area”, page 194.

- Use the measurement selector below the plate map to select and view a different measurement. The corresponding analysis result is loaded automatically. See also section 5.8.1.3 “Plate Map on View Results”, page 209.



5.4.3 Export Results



Click on **Export** to open the dialog with manual export options. Measurement or analysis results can be exported in the following formats:

Export Format	Extension	Description	Multi-export to one file*
CSV_ PLATE	*.csv	Comma-separated values, formatted as plate layout	Yes
CSV_ LIST	*.csv	Comma-separated values, list of results	Yes
XML	*.xml	Transfer to e.g. MyAssays Desktop data analysis software	Yes
ARCHIVE	*.kal	Export results together with protocol settings. These ***.kal** files can e.g. be imported to Kaleido on a different system.	No

** Some export formats also allow you to export multiple preselected datasets into one file while others can only generate separate files for each dataset. For details see below.*

Notice

- For transferring measurement results to **MyAssays Desktop** for further analysis, you have to use the **XML** export format. See also section 5.12 “MyAssays Desktop Data Analysis”, page 242.
- When opening ***.csv** result files in Microsoft Excel®, the number format of the **Time** column will not be set correctly. Please define a custom format for this column using the following pattern: *[h]:mm:ss.000*

For automated export of results see section 5.2.3 “Post Processing Sequence”, page 144.

Parameters

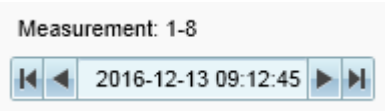
Parameter	Description
Export Format	Select an export format (CSV_PLATE, CSV_LIST, XML, ARCHIVE). Default is CSV_PLATE.
Format Options	Only enabled if **CSV_PLATE** or **CSV_LIST** is selected as export format. • **Standard:** Default format incl. file header, footer and column headers. • **DataOnly (with CSV_LIST):** Recommended for Spotfire® users without SciStream (no file header and footer, but with column headers). • **DataOnly (with CSV_PLATE):** Recommended for LIMS users (very simple format, only result values). **Notice** When using the **DataOnly** format, the export file will have no file header or footer. The content can only be identified by choosing a suitable file name.
Export Path	Enter a target path for the export file or click **[…]** to browse for a folder. For exporting XML files to MyAssays Desktop it is suggested to use the default Kaleido output folders. See also section 5.12.5.1 “Kaleido Output Folders (Default)”, page 243.

Parameter	Description
File Name	Enter the desired file name or click **Variables** to select properties of the protocol (e.g. [ProtocolName]) from a list. **Using Variables:** The selected variables will appear in the text box and will be replaced by the corresponding property during export. • More than one variable can be used and combined with manually entered text. • Changing the text between the square brackets [] or removing these will disable the automated conversion of the variable. • Existing export files will be overwritten if the same file name is used again. **Notice** • When exporting multiple datasets into separate files, please make sure to insert variables which generate unique file names (e.g. date variables). Otherwise the export file will be overwritten again and again. • When exporting multiple datasets into one file, the properties of the first measurement (displayed on top in the load dialog) will be used for replacing variables in the file name. • The [MeasurementDate] variable uses the start date of the measurement. Please note that this date is identical for continued measurements, i.e. using this variable does not always generate unique file names. • To ensure that no files are overwritten, add the [ExportDate] variable. **Resulting File Names** Date and signature variables get an additional letter at the beginning to indicate the type of variable. See the following examples: • [ExportDate]: **E_**20160921-162853 • [MeasurementDate]: **M_**20160720-104340 • [MeasurementSignature]: **M_**469ffbf3-6f94-4d06-82f8-f00c0c17d6b5 • [ProtocolSignature]: **P_**2440c5aa-269b-4bee-9f96-6b3e32870ab5 Dates are stated in this format: *YYYYMMDD-hhmmss* (24-hour notation)

Parameter	Description
Export options	*Only available if multiple measurements or analysis results have been selected:* • **Export only currently loaded data:** Exports only results of the currently loaded measurement (and analysis if applicable) displayed in the **Global Control** section of the **View Results** screen. • **Export ALL preselected data:** Exports results of all selected measurements (and analyses, if applicable). ◦ **Export data into separate files:** Useful if measurements/analysis results belonging to one screening campaign/assay are exported at different time points. Please note that in this case you have to choose export variables that are different from file to file (e.g. containing the measurement date), otherwise they will overwrite each other (see above for details). We recommend to collect the separate files in one folder. ◦ **Export data into one file (not available for ARCHIVE format):** Useful if subsequent evaluation software cannot handle batch import of separate files or if the batch of files has an inherent connection like e.g. in a multi plate assay (Standard on one plate, Samples on all other plates). The data sets in the export file will be sorted chronologically by measurement date.

How to export the current measurement/analysis result

1. Open the **View Results** screen.
2. Load the measurement or analysis result to be exported.
   - Alternatively you can also directly use the currently loaded measurement/analysis result (displayed in the **Global Control** section).
   - If multiple measurements/analysis results have been pre-selected, you can select the active measurement/analysis result using the navigation tools below the plate map.



3. Click **Export**.

   The export dialog is opened.
4. Select an **Export Format** (and **Export Options**, if applicable).
5. Enter an **Export Path** or browse for a folder.
6. Enter a **File Name**. You can also insert variables using the **Variables** button.
7. Click **OK**.

   The current measurement/analysis result is exported.

How to export multiple measurements/analysis results

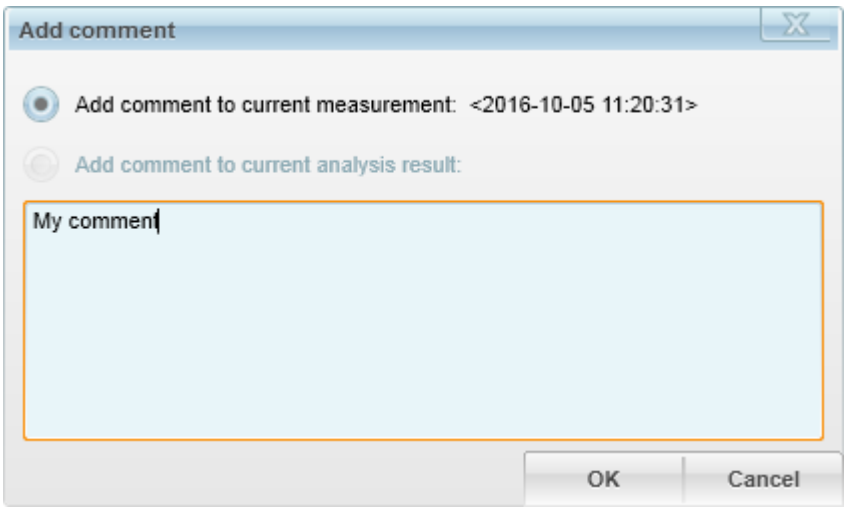
1. Open the **View Results** screen.
2. Load the measurements or analysis results to be exported.
   - Click ... next to **Measurement** or **Analysis Result** and select multiple measurements/analysis results.
   - Multi-selection in the load dialog is only enabled if you select a data object and activate the **Show Compatible** filter. Only objects with the same protocol signature will be displayed and can be selected for export.
   - For details see section 5.6 “Load Dialog”, page 180.
3. Click **Export**.

   The export dialog is opened.
4. Select an **Export Format** (and **Export Options**, if applicable).
5. Enter an **Export Path** or browse for a folder.
6. Enter a **File Name**.

   Notice
   Especially if exporting into separate files, make sure to insert **Variables** which generate unique files names (e.g. date variables). Otherwise the export file will be overwritten again and again.
7. Select the option **Export ALL preselected data**.
8. Select one of these options:
   - **Export data into separate files** (default)
   - **Export data into one file** (not available for ARCHIVE format)
9. Click **OK**.

   All preselected measurements/analysis results are exported (into one or multiple files, depending on your selection).

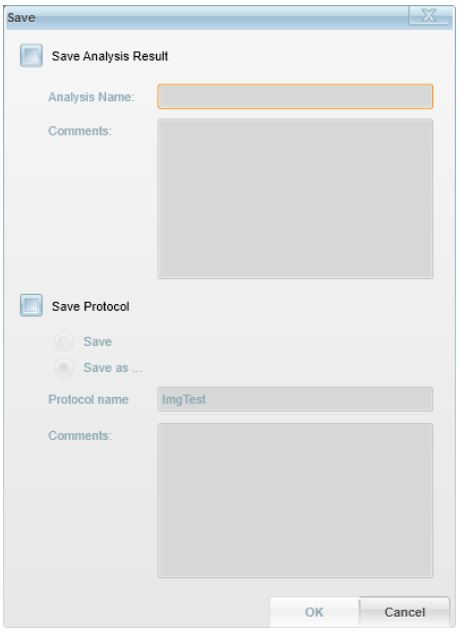
5.4.4 Add Comment



The **Add Comment** button is enabled as soon as a measurement or analysis result has been loaded. In the dialog you can chose between two options where to add the comment:

- **Add comment to current measurement:** This option is always enabled and selected as default. The comment will be displayed in the **Messages** area when the respective measurement is loaded.
- **Add comment to current analysis result:** This option is only enabled if the currently loaded analysis result has already been saved. The comment will be displayed in the **Messages** area when the respective analysis result is loaded.

5.4.5 Save



Click on **Save** to open the dialog with two options:

- **Save Analysis Result:** The analysis result will be saved under the entered name, and the protocol will not be changed. A comment can be added which is displayed when the respective analysis result is loaded. The name of the analysis result will appear in the **Global Control** section, replacing the preliminary asterisk.
- **Save Protocol:** With this option the current analysis sequence will be saved to the protocol, either by changing the existing protocol with **Save** or by creating a new protocol with **Save as ...** and entering a protocol name. Also a comment can be added here, which will be displayed in the **Messages** area when the respective protocol in loaded.

5.5 Analysis

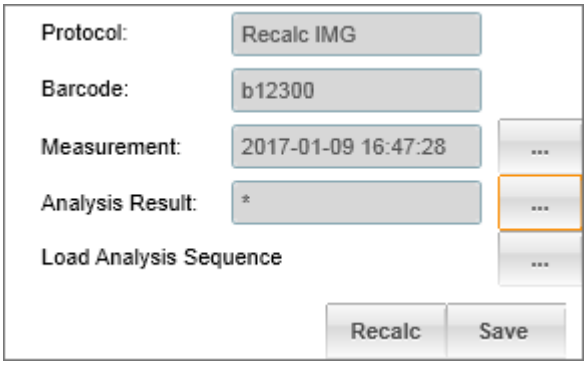
The **Analysis** screen provides tools to analyze or re-analyze existing measurements.

Notice
The functions for setup, test and modification of an image analysis are identical to those on the **Setup Protocol** screen. See the following sections for details:

- Setting up an **Analysis Sequence**: 5.2.2 “Analysis Sequence”, page 120
- Setting up and testing an **Image Analysis**: 5.2.2.1 “Image Analysis”, page 123
- Reference of all **Image Analysis Parameters**: 5.2.2.3 “Image Analysis Parameters”, page 129

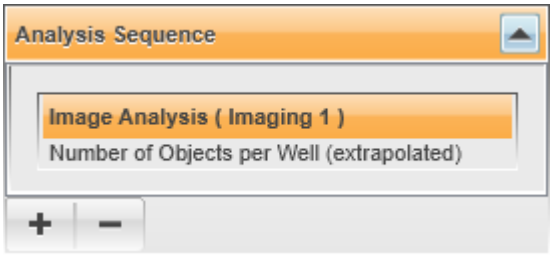
5.5.1 Overview

Overview of loaded and displayed data.



- The **Measurement** part of the loaded protocol and the **Plate Map** are displayed for reference but cannot be edited.

- The **Analysis Sequence** part of the currently loaded protocol can be viewed and modified. The modified analysis can be tested and optimized on single wells just like on **Setup Protocol** (see section 5.2.2 “Analysis Sequence”, page 120). The only difference is that test images do not have to be acquired. The images of the loaded measurement are used instead. You can also load and re-use the analysis sequence from a different protocol.

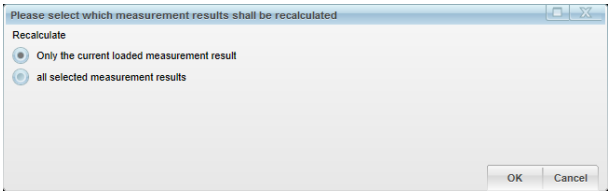


- The measurement (or a batch of multiple measurements) can be re-analyzed with the new analysis (**Recalc** button).
  - After the recalculation the analysis results are automatically displayed on the **View Results** screen.
  - The new **analysis results** are only transient. If you want to keep them, you have to use the **Save** button on **View Results**.
  - The modified **protocol** can be saved using the **Save** button on the **View Results** or **Analysis** screen.

Notice
A measurement can only be re-analyzed if the **Archive Images** option was activated during measurement and if the acquired images are still available. For details see section 5.10.3.6 “Image Archive Settings”, page 225.

Buttons and Elements

Element	Description
Protocol	Displays the protocol name of the loaded measurement.
Barcode	Displays the plate barcode of the loaded measurement.
Measurement	Displays the name of the currently loaded measurement. Click … next to **Measurement** to open the **Load Measurement(s)** dialog and load a measurement or pre-select multiple measurements.
Analysis Result	Displays the name of the loaded analysis result. Click … next to **Analysis Result** to open the **Load Analysis Result(s)** dialog and load an analysis result or pre-select multiple analysis results. Note that the corresponding measurement and analysis sequence will be automatically loaded as well. **Notice** If the protocol still contains deprecated analysis operations, the analysis sequence is removed automatically. Please setup a new analysis sequence. See also section 5.2.2 “Analysis Sequence”, page 120 for details.

Element	Description
Load Analysis Sequence	Click ... next to **Load Analysis Sequence** to open the **Load Protocol** dialog and load a protocol of which you want to re-use the analysis sequence. Only the **Analysis Sequence** part of the protocol will be loaded. It will replace the current analysis sequence, if applicable.
Recalc	Starts a recalcution of the loaded measurement (whole plate) using the current analysis sequence. If multiple measurements have been pre-selected, you will be asked if you want to analyze all pre-selected measurements or only the currently loaded one:  • The new analysis result of a single measurement will not be saved automatically. You can inspect the result first on the **View Results** screen and decide to discard or save the analysis result manually on the **View Results** screen. • Analysis results of multiple pre-selected measurements are saved automatically using the following naming pattern: *Recalc <measurement date>*
Save	Opens a dialog for saving the modified protocol.

5.5.2 Modify Analysis Sequence

The currently loaded analysis sequence can be modified, e.g. to optimize it or to extract more or different information out of the images.

How to add a new analysis

1. Click ... next to **Measurement** to load the measurement that should be analyzed on the **Analysis** screen.

   For details see also section 5.6 “Load Dialog”, page 180.
2. Create a new **Analysis Sequence** (see also section 5.2.2 “Analysis Sequence”, page 120).
3. Select a well on the **Plate Map** and click **Test Analysis** in the **Content Area** to test the new analysis.
4. Select different wells on the **Plate Map** to check the analysis on different samples. The analysis results are automatically updated when changing to a new well.
5. Run the analysis (see next chapter).

How to modify an existing analysis

1. Click ... next to **Analysis Result** to load the measurement that should be re-analyzed on the **Analysis** screen.

   For details see also section 5.6 “Load Dialog”, page 180.

2. Modify the **Analysis Sequence** as desired (see also section 5.2.2.1 “Image Analysis”, page 123).
3. Select a well on the **Plate Map** and click **Test Analysis** in the **Content Area** to test the new analysis.
4. Select different wells on the **Plate Map** to check the analysis on different samples. The analysis results are automatically updated when changing to a new well.
5. Run the analysis (see next chapter).

How to use the analysis sequence of a different protocol

Example: You have a protocol which includes a well working analysis sequence. Now you want to re-use this analysis for another slightly different measurement without re-typing the whole analysis sequence.

1. Click ... next to **Measurement** to load the measurement that should be re-analyzed on the **Analysis** screen.
2. Click ... next to **Load Analysis Sequence** to open the **Load Protocol** dialog showing all available protocols.
3. Find and select the protocol containing your desired analysis (see also section 5.6 “Load Dialog”, page 180. Click **OK**.

   Only the **Analysis Sequence** part of the selected protocol is loaded now, the **Measurement** part is not changed.

4. Select a well on the **Plate Map** and click **Test Analysis** in the **Content Area** to test the new analysis.

   If the analysis does not fully match with the measurement it returns an error describing the mismatch, e.g. missing channels (via red boxes + tooltip).

5. Correct the analysis, e.g. by correcting the channel selection.
6. Run the analysis (see next chapter).

5.5.3 Run Analysis

The currently loaded measurement(s) can be analyzed with the currently loaded analysis sequence to create new results.

How to re-analyze a single plate

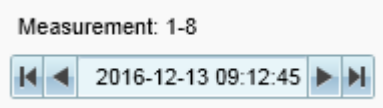
1. Click **Recalc** to run the analysis (whole plate).

   When the analysis is completed it switches to the **View Results** screen for inspection.

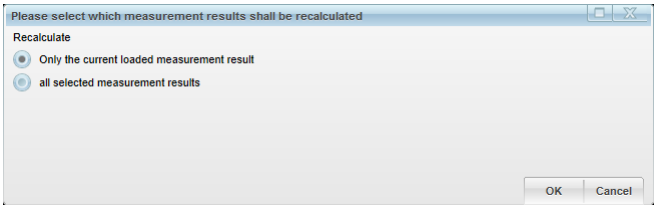
2. Save the results and/or the modified protocol if wanted (**Save** button on **View Results**).

How to analyze a batch of plates (e.g. stacker run)

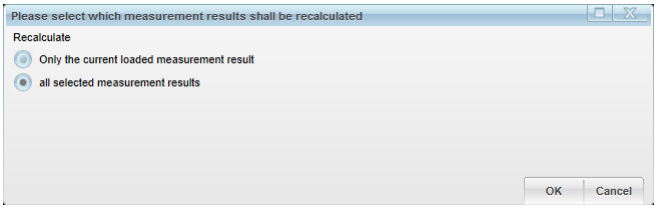
1. Either on **Analysis** or **View Results**, click ... next to **Measurement** and select the desired measurements:
   - In the **Load Measurement(s)** dialog, select one measurement and activate the **Show compatible** filter. Now multiple selection is enabled and only those measurements are displayed which have been measured with the same protocol version (identical protocol signature) as the selected measurement.
   - For detailed instructions see sections "How to select and view measurements of a batch of plates (e.g. stacker run)", page 163and 5.6 "Load Dialog", page 180.
2. Click **OK**.
3. On the **View Results** screen, select a well in the **Plate Map** and navigate to a good example measurement using the measurement selector.



4. Go to the **Analysis** screen and set up the analysis.
   - Create a new **Analysis Sequence** from scratch. See also section 5.2.2 "Analysis Sequence", page 120.
     or
   - Load the **Analysis Sequence** of a different protocol. See also section "How to use the analysis sequence of a different protocol", page 177.
5. Modify the analysis until it works fine (check with **Test Analysis** on single wells).
6. Click **Recalc** to recalculate the full plate.



7. Select **Only the current loaded measurement result** and click **OK** to test the analysis only with one plate.
8. Check the analysis results on the **View Results** screen.
9. Repeat steps 5-8 until the analysis is fine.
10. Got to the **Analysis** screen and click **Recalc**.



Select **All selected measurement results** and click **OK** to start the analysis of the full batch of plates.

All plates are analyzed with the modified analysis sequence now.

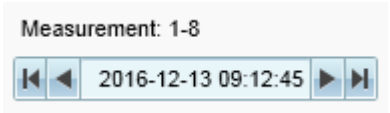
Notice

This may take considerable time and cannot be aborted before all processing of all plates is completed.

How to save the new analysis results

- Analysis results of a **single measurement** will not be saved automatically. You can inspect the results on the **View Results** screen first. If you are satisfied with the analysis, you can save the results and/or the protocol (**Save** button). Otherwise you can just go back to the **Analysis** screen and further modify and re-run the analysis. Unsaved analysis results are indicated by an asterisk * in the **Analysis Result** field on **View Results**.
- Analysis results of a **batch of multiple measurements** are saved automatically in the database using the following naming pattern: *Recalc <measurement date>*

  After the recalculation the measurements remain pre-selected. You can use the measurement selector on **View Results** to navigate between different measurements and view their results.



How to save a modified protocol

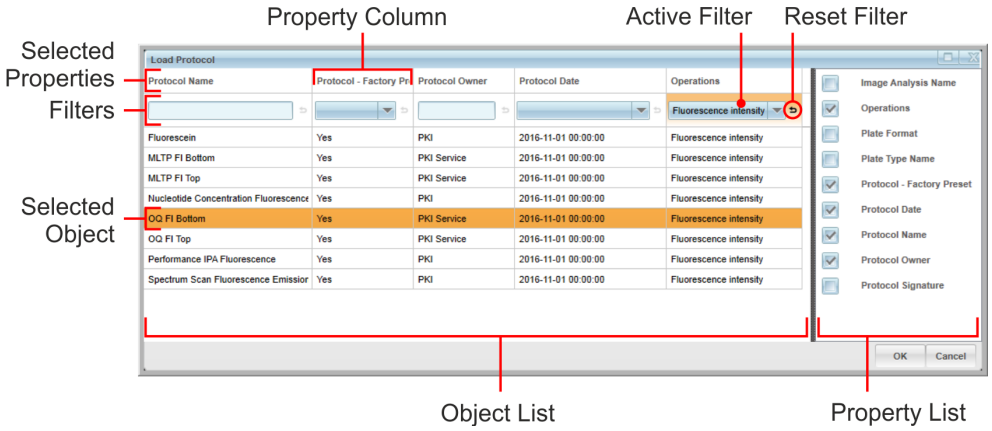
If you want to save the modified protocol for later use, click the **Save** button on the **View Results** or the **Analysis** screen.

- On the **Analysis** screen, **Save** opens the **Save Current Protocol** dialog.
- On the **View Results** screen, the **Save** dialog allows saving the current analysis results and/or the current protocol, if you check the corresponding options.

5.6 Load Dialog

This dialog is used in various situations to load or select data objects from the database. You can **filter** all objects by selected **properties** to quickly find the desired object. The current filter configuration is saved and restored individually for each user.

5.6.1 Overview



If you open the dialog for the first time, the default properties are selected and no filter is active, i.e. all objects are listed in the **Object List**. In most load dialogs a certain object type is pre-selected, e.g. the "Load Protocol" dialog will only show protocols in the object list. The different variations of this dialog are described in the following chapters.

You can now add or remove property columns by selecting/unselecting properties in the **Property List** on the right. Furthermore you can apply **filters** to narrow down the search for the desired object (see instructions below). If a filter is active, the corresponding cell in the table header will be highlighted with orange background color. You can remove an existing filter by clicking on its black reset icon (see figure above).

The current configuration of filters and the selected properties will be saved (per user) and restored if you use this dialog the next time. The most recently loaded object (if loaded via this dialog) will still be selected. To restore the default configuration of selected properties and to remove all filters, use the context menu of the **Property List** and select **Reset to Default View**.

Once you have found and selected the desired object, click **OK** or double-click the object to load it.

Notice

It is recommended to first set the desired filter(s) before selecting objects, because the selection will be lost after adding or modifying a filter.

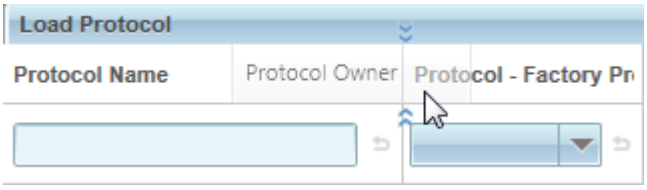
How to add/remove property columns

- **Add:** Check the desired property in the **Property List**.
  A new column will be added to the **Object List** (as last column on the right).
- **Remove:** Uncheck the desired property in the **Property List**.
  The corresponding column will be removed from the **Object List**.

How to sort columns

The columns of the **Object List** can be sorted via drag & drop:

1. Click a column title and hold down the mouse button.
2. Move the column (left/right) to the desired position.



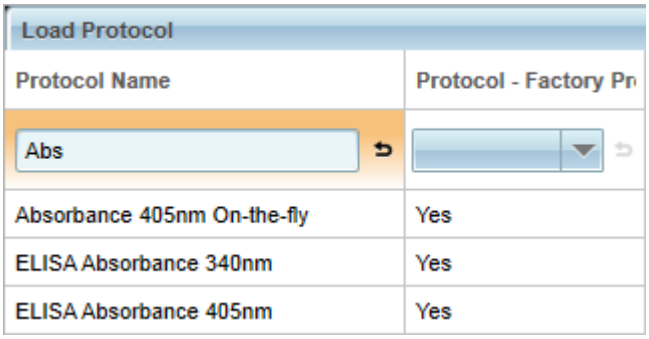
   The position where the column will be inserted is indicated by blue arrows.
3. Release the mouse button to move the column to this position.

How to filter the Object List

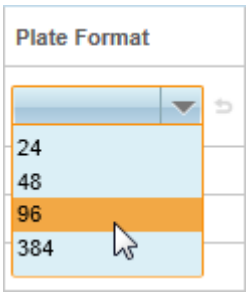
Filters can be configured in the second row of the **Object List** (below the column title). An active filter is indicated by an orange cell background. To deactivate a filter, click its small black reset button.

There are different types of filters:

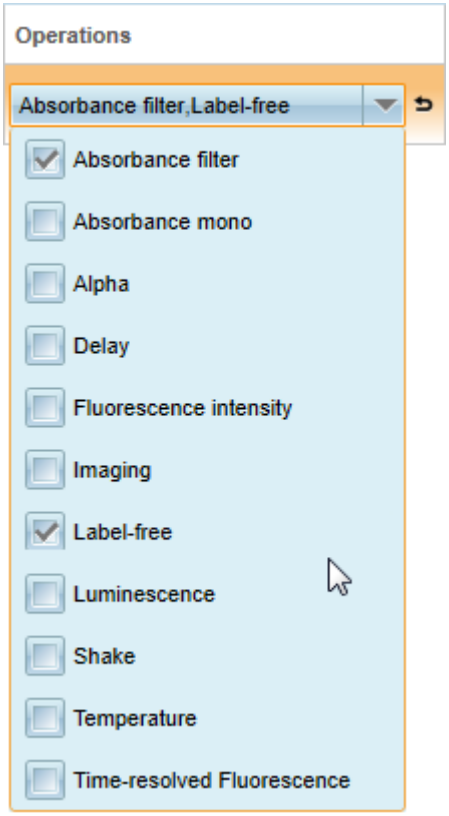
- **Free text:** You can enter a search term. Only objects which **contain** this expression anywhere in their property value will be listed. As soon as you start typing the filter is activated and the list is updated.



- **Single value from dropdown list:** You can select one value from a list of pre-defined values. Only objects with this property value will be listed.



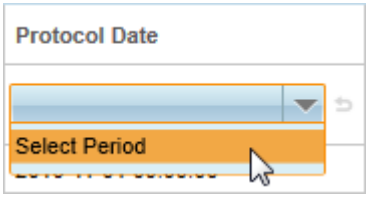
- **Multiple values from dropdown list:** You can select multiple values from a list of pre-defined values. An object is only displayed if all selected property values match.



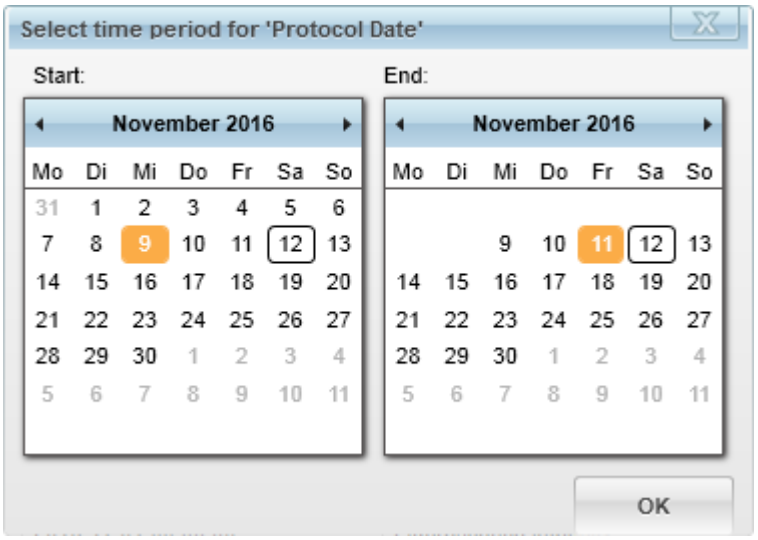
Example: Operations (Load Protocol)
All protocols containing (at least) the selected operations "Absorbance filter" AND "Label-free" will be displayed. The protocols may contain further operations.

- **Date/time period:** You can select a period of time (start date and end date) to filter objects by date.

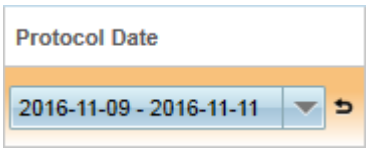
1. Click on the dropdown element of a date property and click **Select Period**.



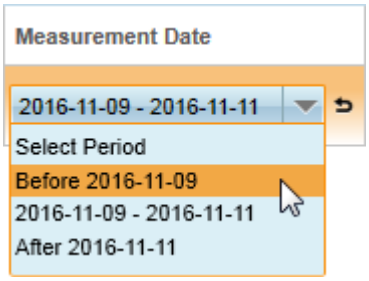
2. Select start and end date of the desired period. To select only one day, select the same date for start and end date. The current date is indicated by a black frame.



3. Click **OK**. The selected period will be displayed and the **Object List** will be filtered.



4. As soon as a period has been selected, there will be additional options in the dropdown menu:



   - **Before [...]:** Select all dates before start date of selected period.
   - **After [...]:** Select all dates after end date of selected period.

- **Show compatible:** This option is only available in the **Load Measurement(s)** and **Load Analysis Result** dialogs.
  For detailed instructions and use cases please see sections 5.6.5 “Load Measurement(s)”, page 187 and 5.6.6 “Load Analysis Result(s)”, page 190.

  1. Select one object (one row).
  2. Activate the **Show compatible** filter.

  Only objects which are compatible with the selected object will be listed (i.e. measurements generated with the same protocol).

3. Now you can also select **multiple** objects:
   - Multiple single objects: Hold down **Ctrl** key and click objects.
   - Group of objects: Click first object, hold down **Shift** key, click last object.
   - Using the mouse: Click on an object, hold down the mouse button and drag the mouse.

How to sort the listed objects

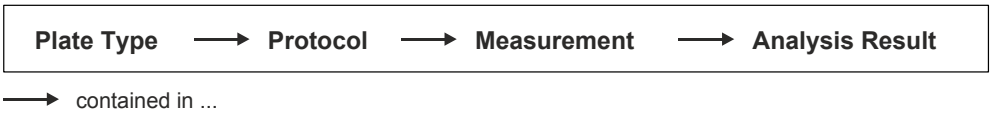
- The **Object List** can be sorted by one property if you click on the corresponding column title.
- Click again to reverse the sorting direction (indicated by a small triangle).

How to copy values to the clipboard

- **All displayed property values of an object:**
  1. Select an object in the **Object List** (one row is highlighted).
  2. Press CTRL+C to copy all displayed properties to the clipboard (tab-separated values).
- **Single property value of an object:**
  1. Select an object in the **Object List** (one row is highlighted).
  2. Right-click on the selected object to open the context menu.
  3. Click **Copy Selected** and then select the desired property name from the fly-out menu.

     The value of the selected property is copied to the clipboard.

5.6.2 Inheritance of Objects and Properties



If an object is saved, all other objects which were used by this object will also be embedded. Thereby the objects are self-contained.

Example
If a measurement is saved in the database, it will also contain the protocol and plate type used for that measurement.

The same applies to the object properties which are displayed in the load dialog. The properties are passed on in the hierarchy of objects. These properties help you to sort and filter the object list and to find the desired objects in the database. However, not all properties are visible, only the most relevant ones.

What happens if you load an object

- The contained object(s) will also be loaded and displayed on the corresponding screens.
- All objects which rank higher in the hierarchy of objects (towards the right in the illustration above) will be unloaded and removed from the user interface.

Example: Loading a measurement

- The measurement will be loaded and displayed on **View Results** and **Analysis**. Any previously loaded measurement will be replaced.
- The contained protocol will be displayed on **Setup Protocol**, but also on the other screens.
- Any previously loaded analysis result will be removed. The corresponding fields on **View Results** and **Analysis** are empty.

5.6.3 Load Protocol

- Only protocols are displayed.
- Only the latest version of a protocol is displayed.
- A protocol is only displayed if all operations used in the measurement sequence are supported by the instrument. However, if a required excitation filter is currently not inserted in the instrument, such a protocol is displayed nevertheless.

Protocol Properties

The default properties are highlighted with gray background in the following table.

Property	Description
Protocol Name	Name of the protocol (user-defined). Protocol names are not unique. If two protocols have the same name, they can be distinguished by date and signature (see below).
Protocol – Factory Preset	**Yes:** Factory preset **No:** User-defined
Protocol Owner	The owner is the Kaleido user who created and saved the protocol. In case of "Factory Preset" protocols the owner is "PKI" or "PKI-Service".
Protocol Date	Time stamp when the protocol was saved (using **New** or **Edit**). Format: *YYYY-MM-DD hh:mm:ss*.
Operations	List of all operations included in the measurement sequence of the protocol. Each type of operation is listed only once, even if multiple operations of this type are used.

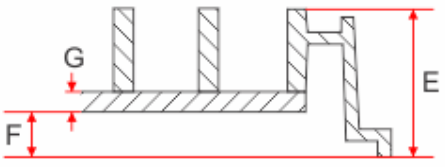
Property	Description
Image Analysis Name	Name(s) of the image analysis operation(s) (if applicable).
Plate Format	Plate format of the plate type (number of wells).
Plate Type Name	Name of the plate type used in the protocol.
Protocol Signature	The signature is a unique identifier of an object, e.g. "4sdii-4hs89mn-ddi39-ll". While protocol names are *not* unique, two protocols will never have the same signature. If you edit and save a protocol, it will get a new signature. Note: You can copy this value to the clipboard for further use (context menu: **Copy Selected...**).
Sequence Executed By	Execution type of the measurement sequence. See also section 5.2.1.1 “Execution Type”, page 75.

5.6.4 Load Plate Type

- Only plate types are displayed.

Plate Type Properties

The default properties are highlighted with gray background in the following table.

Property	Description
Plate Type Name	Name of the plate type.
Plate Format	Plate format of the plate type (number of wells).
Plate Type – Factory Preset	**Yes:** Factory preset **No:** User-defined
Plate Type Owner	The owner is the Kaleido user who created and saved the plate type. In case of "Factory Preset" plate types the owner is "PKI".
Plate Parameter – Bottom Height [mm]	Plate parameter **"F"** from the **Plate Type Wizard**, see illustration below. 

Property	Description
Plate Parameter – Bottom Thickness [mm]	Plate parameter **"G"** from the **Plate Type Wizard**, see illustration before.
Plate Parameter – Well Volume [µl]	Well volume [µl] of the plate type.
Plate Type - Compatible with	List of all operations and other options like stacker, shake, temperature etc. which can be used with this plate type. This information is available for all factory preset plate types.
Plate Type Date	Time stamp created when the plate type was saved. Format: *YYYY-MM-DD hh:mm:ss*.
Plate Type - Recommended by PKI for …	List of operations for which this plate type is particularly suitable (recommendation by PerkinElmer). Only available for factory preset plate types.
Plate Type Signature	The signature is a unique identifier of an object, e.g. *"4sdii-4hs89mn-ddi39-ll"*. Two plate types will never have the same signature. Note: You can copy this value to the clipboard for further use (context menu: **Copy Selected...**).

5.6.5 Load Measurement(s)

- Only measurements are displayed.
- All measurements for a plate barcode are displayed (each with a different start date).
  - When opening this dialog from the **Run Protocol** screen, only measurements will be displayed which could be started or continued on the instrument (instrument is equipped with required technologies).
  - When opening this dialog from the **View Results** screen, all measurements will be displayed regardless of the technologies installed.
- If you load a measurement, the corresponding protocol is contained and will also be loaded (and displayed on **Setup Protocol** and **Run Protocol**).
- Normally, you can only load one **single** measurement. If you select a measurement and activate the **Show compatible** filter, only measurements will be displayed which are compatible with the selected measurement (i.e. measured with exactly the same protocol version and which have the same **Step** value). In this mode you can also select **multiple** measurements for the following purposes:
  - **Run Protocol – Continue Measurement mode (stacker):** Selecting multiple measurements (and thereby plate barcodes) to be continued within a stacker run (see also section "How to continue multiple measurements (automatic plate loading via stacker)", page 155). **Show compatible** is only available if the instrument has an optional stacker (stacker main unit

detected).

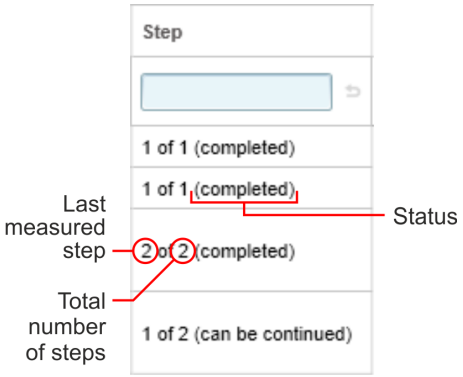
- **View Results:** Pre-selecting multiple measurements for viewing, comparing and exporting results (see also section "How to select and view measurements of a batch of plates (e.g. stacker run)", page 163).
- **Export multiple measurement results:** See section 5.4.3 "Export Results", page 167.

Measurement Properties

The default properties are highlighted with gray background in the following table.

Property	Description
Protocol Name	Name of the protocol (user-defined). Protocol names are not unique. If two protocols have the same name, they can be distinguished by date and signature (see below).
Measurement Owner	The owner is the Kaleido user who performed the measurement.
Measurement Date	Measurement start date. Format: *YYYY-MM-DD hh:mm:ss*. If using **Continue Measurement** mode, the measurement date will not change (start date of initial measurement will be displayed for all appended measurements).
Barcode	Barcode of the measured plate. This can be a ... • real barcode (scanned by barcode reader) • manual barcode (entered by user) • virtual barcode (e.g. if barcode reading failed), naming pattern: "V-{date} {time}"
Operations	List of all operations included in the measurement sequence of the protocol. Each type of operation is listed only once, even if multiple operations of this type are used.
Instrument Serial No.	Seven-digit serial number of the instrument (as displayed in **Settings – Instrument Options**).
Measurement Name	The measurement name is set automatically (start date of the measurement). Format: *YYYY-MM-DD hh:mm:ss*.
Measurement Signature	The signature is a unique identifier of an object, e.g. "4sdii-4hs89mn-ddi39-ll". Two measurements will never have the same signature. Note: You can copy this value to the clipboard for further use (context menu: **Copy Selected...**).
Plate Format	Plate format of the plate type (number of wells).
Plate Type Name	Name of the plate type used in the protocol.
Protocol – Factory Preset	**Yes:** Factory preset **No:** User-defined

Property	Description
Protocol Signature	The signature is a unique identifier of an object, e.g. *"4sdii-4hs89mn-ddi39-ll"*. While protocol names are *not* unique, two protocols will never have the same signature. If you edit and save a protocol, it will get a new signature. Note: You can copy this value to the clipboard for further use (context menu: **Copy Selected...**).
Protocol Date	Time stamp created when the protocol was saved (using **New** or **Edit**). Format: *YYYY-MM-DD hh:mm:ss*.
Protocol Owner	The owner is the Kaleido user who created and saved the protocol. In case of "Factory Preset" protocols the owner is "PKI" or "PKI-Service".
Sequence Executed By	Execution type of the measurement sequence. See also section 5.2.1.1 “Execution Type”, page 75.
Stacker Run – Start Date	Time point when a stacker run was started. All plates of a stacker run can be identified by their identical **Stacker Run – Start Date**. This property is empty if the instrument has no stacker or if the stacker has not been used.

Property	Description
Step	The group of operations in a measurement sequence before and after a **Stop** operation is called "step". See also section "Stop", page 110. This property is helpful if you are looking for a measurement which can be continued.  It indicates the last processed step number and the total number of steps of the measurement sequence. The text in the brackets indicates if the measurement can be continued using the **Continue Measurement** mode. • **"can be continued":** Still one or multiple steps to be processed, can be continued. • **"completed":** All steps measured, cannot be continued. • **"canceled":** Aborted by user or due to error, cannot be continued. • **"unknown":** Measured with previous Kaleido version, cannot be continued.

5.6.6 Load Analysis Result(s)

- Only analysis results are displayed.
- If you load an analysis result, the corresponding measurement and protocol are contained and will also be loaded (and displayed on the different screens).
- Normally, you can only load one **single** analysis result. If you select an analysis result and activate the **Show compatible** filter, only analysis results will be displayed which are compatible with the selected analysis result (i.e. done with the same protocol version and the same analysis sequence). In this mode you can also select **multiple** analysis results for the following purposes:
  - **View Results:** Pre-selecting multiple analysis results for viewing, comparing and exporting results (see also section "How to view analysis results of a batch of plates (e.g. stacker run)", page 165).
  - **Analysis:** Batch recalculation of the selected analysis results (see section "How to analyze a batch of plates (e.g. stacker run)", page 178).

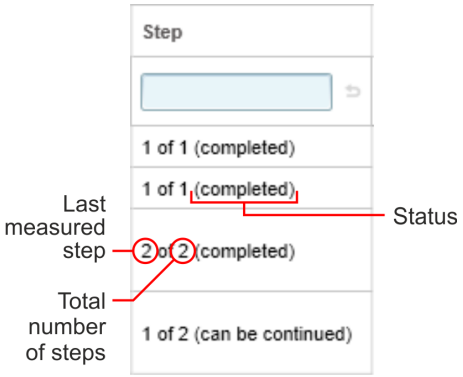
- **Export multiple analysis results:** See section 5.4.3 “Export Results”, page 167.

Analysis Result Properties

The default properties are highlighted with gray background in the following table.

Property	Description
Analysis Result Name	• **Online Analysis:** Analysis result is saved automatically and named with a time stamp. Format: *default YYYY-MM-DD hh:mm:ss* • **Recalculation...** ◦ of one measurement: user-defined name ◦ of multiple measurements (batch recalculation): "Recalc" + user-defined name
Analysis Result Date	Time stamp when the analysis result was saved – manually (using the **Save** button on the **Analysis** screen) or automatically (online analysis during measurement). Format: *YYYY-MM-DD hh:mm:ss*
Analysis Result Owner	The owner is the Kaleido user who performed the measurement (with online analysis) or the recalculation.
Barcode	Barcode of the measured plate. This can be a ... • real barcode (scanned by barcode reader) • manual barcode (entered by user) • virtual barcode (e.g. if barcode reading failed), naming pattern: "V-{date} {time}"
Protocol Name	Name of the protocol (user-defined). Protocol names are not unique. If two protocols have the same name, they can be distinguished by date and signature (see below).
Operations	List of all operations included in the measurement sequence of the protocol. The order of this enumeration corresponds to the order of operations in the measurement sequence.
Analysis Result Signature	The signature is a unique identifier of an object, e.g. "4sdii-4hs89mn-ddi39-ll". While analysis result names are *not* unique, two analysis results will never have the same signature. Note: You can copy this value to the clipboard for further use (context menu: **Copy Selected...**).
Image Analysis Names	Name(s) of the image analysis operation(s). This is always the original name from the protocol which was used to perform the measurement. Even if you recalculate a measurement and edit the name of the image analysis, this will not affect this property.
Instrument Serial No.	Seven-digit serial number of the instrument (as displayed in **Settings – Instrument Options**).

Property	Description
Measurement Date	Measurement start date. Format: *YYYY-MM-DD hh:mm:ss*. If using **Continue Measurement** mode, the measurement date will not change (start date of initial measurement will be displayed for all appended measurements).
Measurement Name	The measurement name is set automatically (start date of the measurement). Format: *YYYY-MM-DD hh:mm:ss*.
Measurement Owner	The owner is the Kaleido user who performed the measurement.
Measurement Signature	The signature is a unique identifier of an object, e.g. "4sdii-4hs89mn-ddi39-ll". Two measurements will never have the same signature. Note: You can copy this value to the clipboard for further use (context menu: **Copy Selected...**).
Protocol – Factory Preset	**Yes:** Factory preset **No:** User-defined
Protocol Date	Time stamp created when the protocol was saved (using **New** or **Edit**). Format: *YYYY-MM-DD hh:mm:ss*.
Protocol Owner	The owner is the Kaleido user who created and saved the protocol. In case of "Factory Preset" protocols the owner is "PKI" or "PKI-Service".
Protocol Signature	The signature is a unique identifier of an object, e.g. "4sdii-4hs89mn-ddi39-ll". While protocol names are *not* unique, two protocols will never have the same signature. If you edit and save a protocol, it will get a new signature. Note: You can copy this value to the clipboard for further use (context menu: **Copy Selected...**).
Sequence Executed By	Execution type of the measurement sequence. See also section 5.2.1.1 “Execution Type”, page 75.
Stacker Run – Start Date	Time point when a stacker run was started. All plates of a stacker run can be identified by their identical **Stacker Run – Start Date**. This property is empty if the instrument has no stacker or if the stacker has not been used.

Property	Description
Step	The group of operations in a measurement sequence before and after a **Stop** operation is called "step". See also section "Stop", page 110. This property is helpful if you are looking for a measurement which can be continued.  It indicates the last processed step number and the total number of steps of the measurement sequence. The text in the brackets indicates if the measurement can be continued using the **Continue Measurement** mode. • **"can be continued":** Still one or multiple steps to be processed, can be continued. • **"completed":** All steps measured, cannot be continued. • **"canceled":** Aborted by user or due to error, cannot be continued. • **"unknown":** Measured with previous Kaleido version, cannot be continued.

5.7 Content Area

The **Content Area** is the section in the center of the screen. It is used to display various types of information depending on the context. Some examples:

- Parameter configuration of measurement and analysis operations
- Measurement progress and results
- Images
- Analysis results

5.7.1 Plate

In the **Plate** tab in the **Content Area** you can view measurement and analysis results (during a measurement or afterwards).

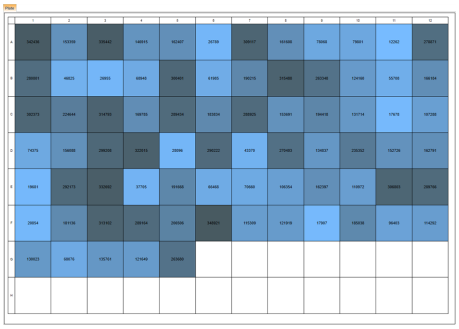
Especially when looking at 384-well plate, you might want to zoom into the plate view. Using the mouse wheel you can **zoom in and out**. Further options are available via context menu (right click plate view).

Context Menu

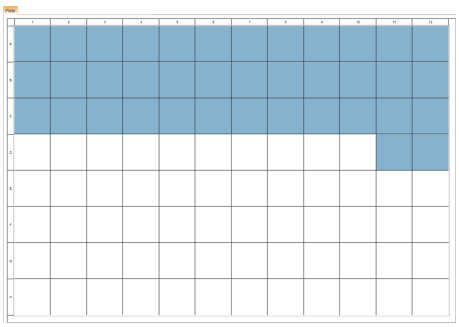
Element	Description
Select Wells	Mode to select and copy wells (default setting). If this option is selected, several copy functions are enabled (see **Copy**).
Pan	Allows you to pan the plate.
Copy	• **Copy Selected Wells to Clipboard:** Only the results of selected wells (current repeat) will be copied to the clipboard as tab separated data. • **Copy all Results to Clipboard:** All (so far measured) results of the current plate (current repeat) will be copied to the clipboard as tab separated data. • **Copy Heatmap to Clipboard:** The whole current plate with so far measured results (current repeat) will be copied to clipboard as picture. • **Save Heatmap as:** A dialog opens and the whole current plate with measured results (current repeat) can be saved as *.png image file.

5.7.1.1 Measurement Progress

During a measurement on the **Run Protocol** screen the **Plate** tab shows the measurement progress of the current operation. For non-imaging measurements the numerical result values are displayed directly in the wells. Analysis results are not displayed during the measurement.



Progress and results of non-imaging measurement (heatmap)



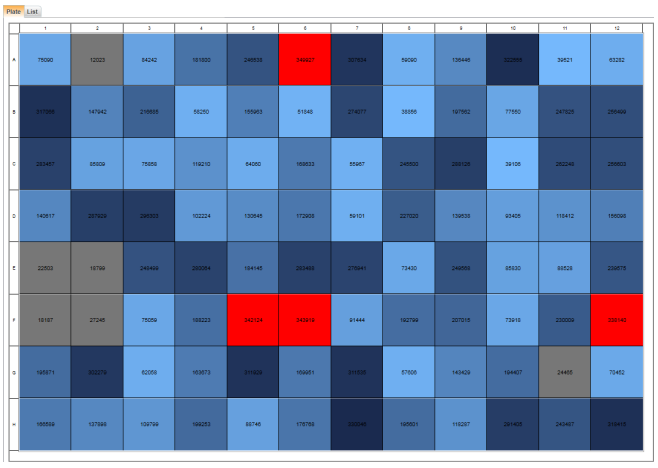
Progress of imaging measurement

Notice

If execution type **By Well** is combined with very fast kinetics, the heatmap of the live view is updated very often (for each repeat). This can produce an unpleasantly flickering display. In this case, switch to the **Graph** tab instead.

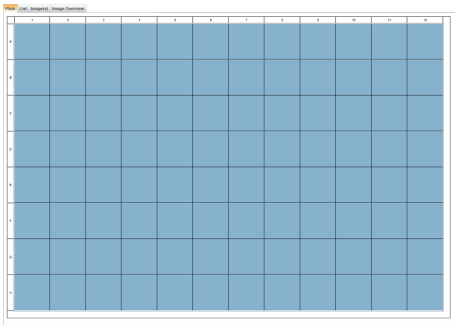
5.7.1.2 *Viewing Results*

Directly after a measurement, the **View Results** screen is opened and the measurement results (and analysis results) are displayed on the **Plate** tab as a heatmap. You can also load an existing measurement or analysis result from the database. Select a measurement or analysis operation to view the corresponding results. You can configure the heatmap in the **Control Area** on the right (see section 5.8.4 “Color Range”, page 215).

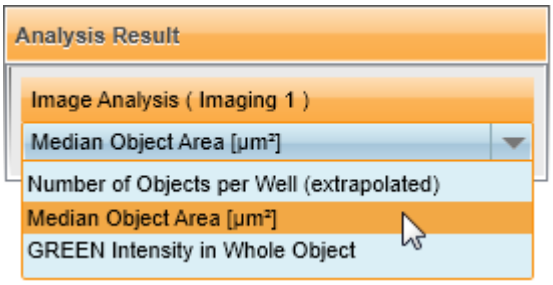


- **Imaging measurements** generate images instead of numerical results. Therefore, there are no values on the **Plate** tab and no heatmap if you select an **Imaging** operation in the **Measurement** section (**View Results**). Measured wells are displayed with blue background. If you click such a well, the corresponding image(s) of this well are automatically displayed on the **Image(s)** tab. You can also calculate an **image overview** with thumbnails of the measured images (see

section “Image Overview”, page 94).



- **Image Analysis** operations have multiple analysis results per well which cannot be displayed simultaneously on the **Plate** tab. You have to select the parameter to be displayed in the **Analysis Result** section (see below). On the **List** tab all result parameters are shown.



Selecting the result parameter to be displayed in the Plate view

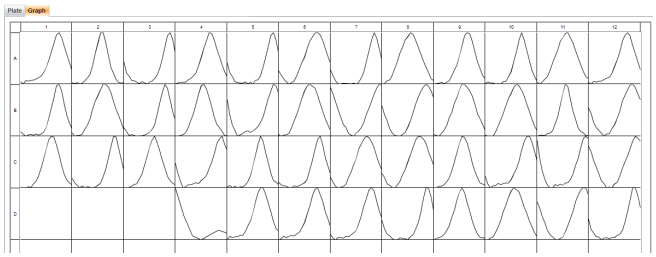
- For **Label-free** measurements, an automatically calculated **Live Response** is displayed (see also section “Live Response”, page 100). Raw signal values can only be seen on the **List** tab.

5.7.2 Graph

The **Graph** tab is only displayed on **Run Protocol** and **View Results** if the protocol includes several measurements of the same point in a well, e.g. when using **Repeats** or measurement modes like **Wavelength Scan**.

5.7.2.1 Measurement Progress

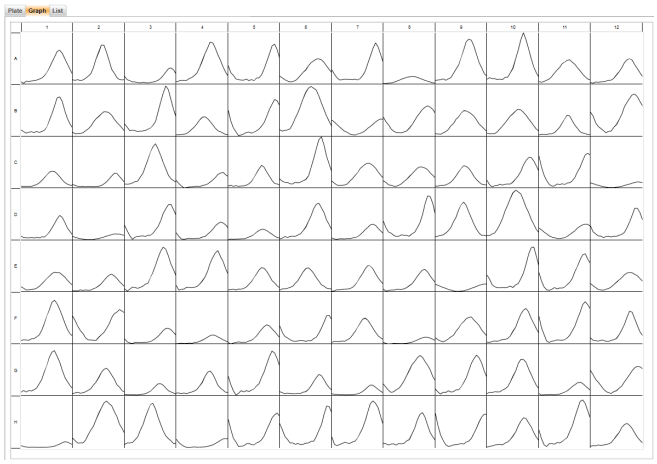
During measurement on **Run Protocol** you will see a miniature plot being created for each well. The y-axis is **normalized per well** to the minimum and maximum y-values.



Miniature plots (normalized per well)

5.7.2.2 Viewing Results

On the **View Results** screen the miniature plots of the loaded measurement are normalized to the min/max values of the whole plate, not separately for each well. For well scans, where different points in a well are measured, heat maps are generated for every well in the resolution defined for the operation (maximum 10 x 10 points per well). The heat map can be configured as usual (see section 5.8.4 “Color Range”, page 215).

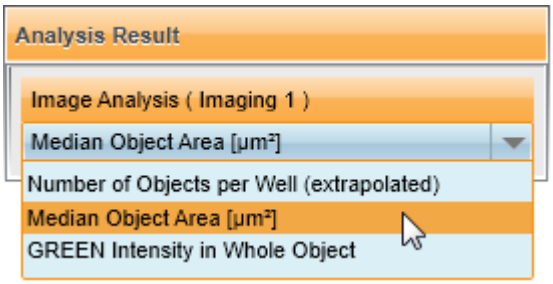


Miniature plots for each well (normalized per plate)

Using the **Selected X-Axis** combo box you can define the x-axis of the displayed graphs (Repeat, Time or Wavelength, depending on measured data).

You can also view one or several selected wells in detail if you click on **Show Detailed Graph** in the **Plate Map** pane on the right (see instructions below). A large diagram with the graphs of all selected wells will be opened.

- **Image Analysis** operations have multiple analysis results per well which cannot be displayed simultaneously on the **Graph** tab. You have to select the parameter to be displayed in the **Analysis Result** section (see below). On the **List** tab all result parameters are shown.

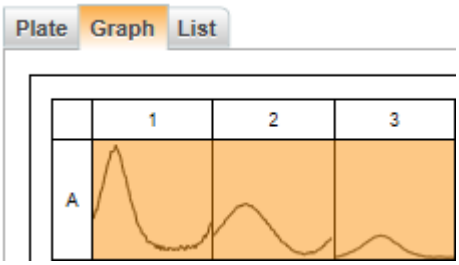


Selecting the result parameter to be displayed on the Graph tab

- For **Label-free** measurements, an automatically calculated **Live Response** is displayed (see also section “Live Response”, page 100). Raw signal values can only be seen on the **List** tab.

How to view a detailed graph

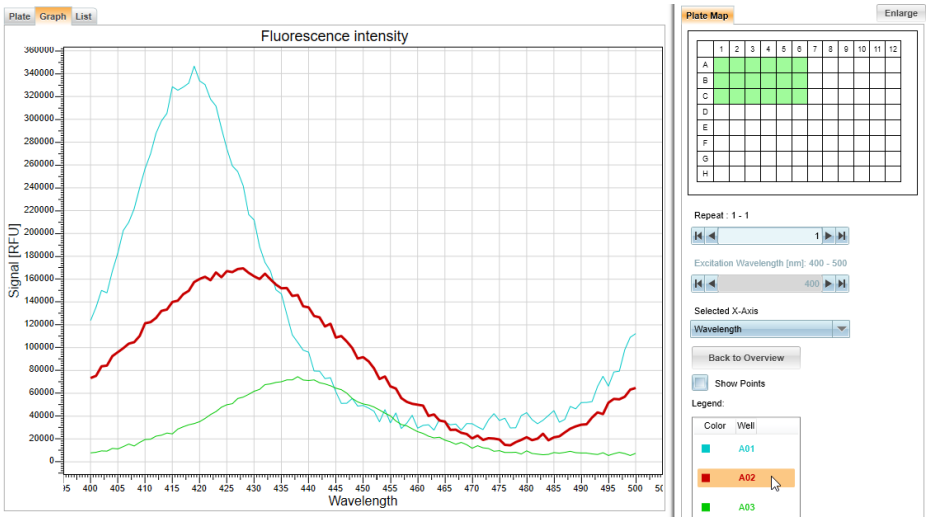
1. Select one or multiple wells on the **Graph** tab.



2. Click **Show Detailed Graph** (below **Plate Map** on the right).

   A large diagram is displayed instead of the plate overview. If multiple wells have been selected, the graph for each well is plotted with a different color (see legend below the **Plate Map**).

   - You can zoom the view using the mouse wheel.The position of the cursor will be centered. Further options are available via context menu.
   - If you select a well in the legend, the corresponding curve is highlighted in the diagram (and vice versa).
   - You can define the x-axis of the graphs using the **Selected X-Axis** combo box.
   - You can switch between different well positions, repeats or wavelengths using the **Record Selection Boxes** below the plate map (if applicable).



3. Click **Back to Overview** to switch back to the **Graph** view for the whole plate.

Context Menu – Graph

Element	Description
Select Wells	Mode to select and copy wells (default setting). If this option is selected, several copy functions are enabled (see **Copy**).

Element	Description
Pan	Allows you to pan the plate.
Copy	• **Copy Graph View to Clipboard:** The whole current plate with so far measured results will be copied to clipboard as picture. • **Save Graph View as:** A dialog opens and the whole current plate with measured results can be saved as *.png image file.

Context Menu – Detailed Graph

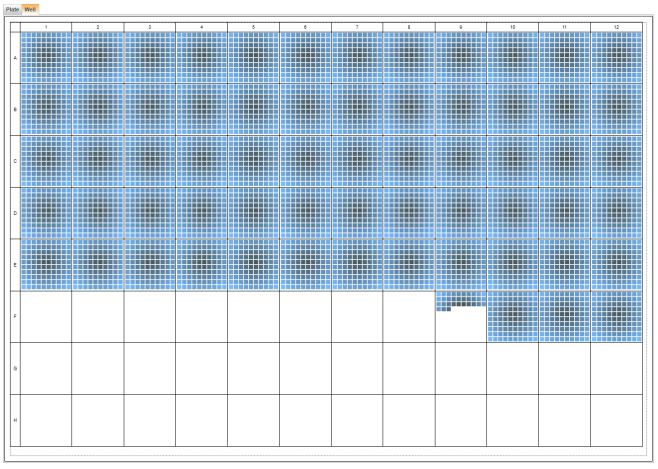
Element	Description
Fit to View	Brings the entire graph (or all graphs, if applicable) back into view.
Copy Screenshot	Copies a screenshot of the displayed diagram to the clipboard.
Save Screenshot	Allows you to save a screenshot of the displayed diagram as *.png file.

5.7.3 Well

The **Well** tab is only displayed on **Run Protocol** and **View Results** if measurement mode **Well Scan** was used for the selected operation (i.e. a grid of data points was measured in each well). See also section "Well Scan", page 112.

5.7.3.1 Viewing Progress

During measurement on **Run Protocol** you will see a miniature heat map being created for each well.



5.7.3.2 Viewing Results

On the **View Results** screen, a heat map is generated for every well which shows the data points in this well. The heat maps can be configured as usual (see section 5.8.4 "Color Range", page 215).

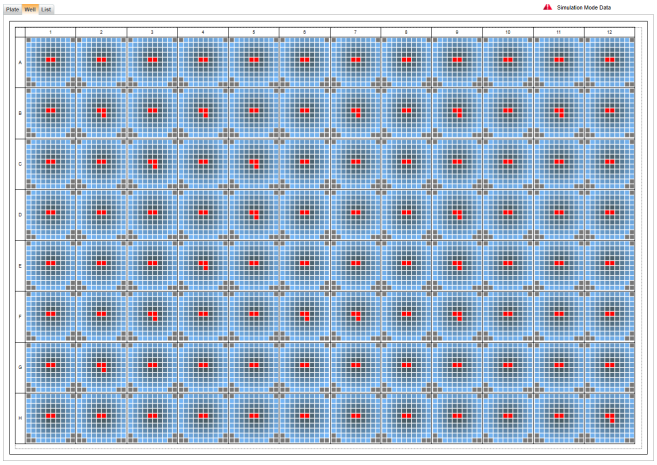


Plate
Well
List
Simulation Mode Data
1
2
3
4
5
6
7
8
9
10
11
12
A
B
C
D
E
F
G
H

5.7.4 List

The **List** tab on **View Results** displays all results of the currently loaded measurement or analysis result. Only results of the selected operation (and of its copies and repeats) are displayed.

There are a few default properties and further parameters (i.e. columns) depending on the measurement operation, technology, measurement mode and analysis.

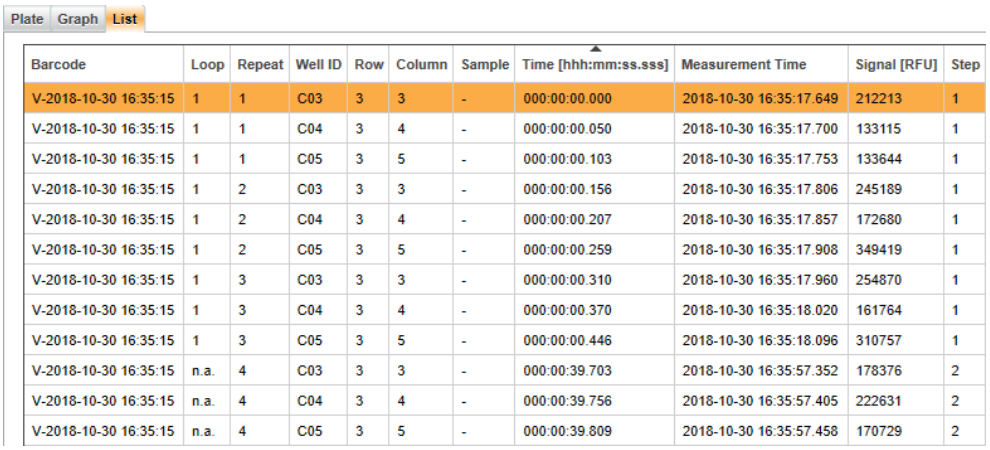


Plate Graph List

Barcode	Loop	Repeat	Well ID	Row	Column	Sample	Time [hhh:mm:ss.sss]	Measurement Time	Signal [RFU]	Step
V-2018-10-30 16:35:15	1	1	C03	3	3	-	000:00:00.000	2018-10-30 16:35:17.649	212213	1
V-2018-10-30 16:35:15	1	1	C04	3	4	-	000:00:00.050	2018-10-30 16:35:17.700	133115	1
V-2018-10-30 16:35:15	1	1	C05	3	5	-	000:00:00.103	2018-10-30 16:35:17.753	133644	1
V-2018-10-30 16:35:15	1	2	C03	3	3	-	000:00:00.156	2018-10-30 16:35:17.806	245189	1
V-2018-10-30 16:35:15	1	2	C04	3	4	-	000:00:00.207	2018-10-30 16:35:17.857	172680	1
V-2018-10-30 16:35:15	1	2	C05	3	5	-	000:00:00.259	2018-10-30 16:35:17.908	349419	1
V-2018-10-30 16:35:15	1	3	C03	3	3	-	000:00:00.310	2018-10-30 16:35:17.960	254870	1
V-2018-10-30 16:35:15	1	3	C04	3	4	-	000:00:00.370	2018-10-30 16:35:18.020	161764	1
V-2018-10-30 16:35:15	1	3	C05	3	5	-	000:00:00.446	2018-10-30 16:35:18.096	310757	1
V-2018-10-30 16:35:15	n.a.	4	C03	3	3	-	000:00:39.703	2018-10-30 16:35:57.352	178376	2
V-2018-10-30 16:35:15	n.a.	4	C04	3	4	-	000:00:39.756	2018-10-30 16:35:57.405	222631	2
V-2018-10-30 16:35:15	n.a.	4	C05	3	5	-	000:00:39.809	2018-10-30 16:35:57.458	170729	2

Overview of result parameters

Result	Description
Barcode	Scanned barcode, manual barcode or virtual barcode
Loop	*Only displayed if protocol contained Loop operation* Number of the Loop operation
Repeat	Number of repeat (consecutively numbered). The repeat number is incremented for each loop iteration and each copy of an operation.
Well ID	Well ID, e.g. A01
Row	Row number of the well
Column	Column number of the well
Sample	Sample type abbreviation of the well
Time [hhh:mm:ss.sss]	Relative time since first measured data point
Measurement Time	Absolute time
Wavelength [nm]	*Only displayed for Exc/Ems Scan:* Wavelength of the single data point

Result	Description
Window	*Only displayed for TRF:* Window number
Window Start [µs]	*Only displayed for TRF:* Calculated for each data point (see "Total Time" in section "Time-resolved Fluorescence (TRF)", page 101).
Point X	*Only displayed for Well Scan:* X position in the well
Point Y	*Only displayed for Well Scan:* Y position in the well
Channel	*Only displayed for Imaging:* Channel name (defined in the protocol)
File name	*Only displayed for Imaging:* Name of the image file
<Name of Analysis Result>	*Only displayed for image analysis results:* Numerical result value of image analysis (one column per result parameter)
Analysis Quality	*Only displayed for image analysis results:* Quality rating of image analysis result. Examples: • Good • Well Detection: estimated • Empty <channel name>
Signal [<unit>]	Unit depends on technology: • Alpha: [Counts] • ABS: [RU] • LUM: [Counts] • FI: [RFU] (relative fluorescence units) • LF: [fm] • TRF: [Counts]
Result [<unit>]	*Only displayed if signal is converted:* • ABS: Result [OD] (optical density) • LUM: [CPS] (counts per second) • TRF: [CPS] (counts per second)
Reference [fm]	*Only displayed for Label-free:* Reference value (always "0" for cell-based assays)
Sign-Ref [fm]	*Only displayed for Label-free:* Calculated value = Signal – Reference
Live Response [pm]	*Only displayed for Label-free:* Calculated value (see section "Live Response", page 100)

Result	Description
Step	*Only displayed if protocol contains Stop operation:* Stop operations split up the measurement sequence into multiple "steps". The step number indicates to which step of the measurement sequence the results belong. See also section “Stop”, page 110.

Gray background = default property, always displayed

How to sort the result table

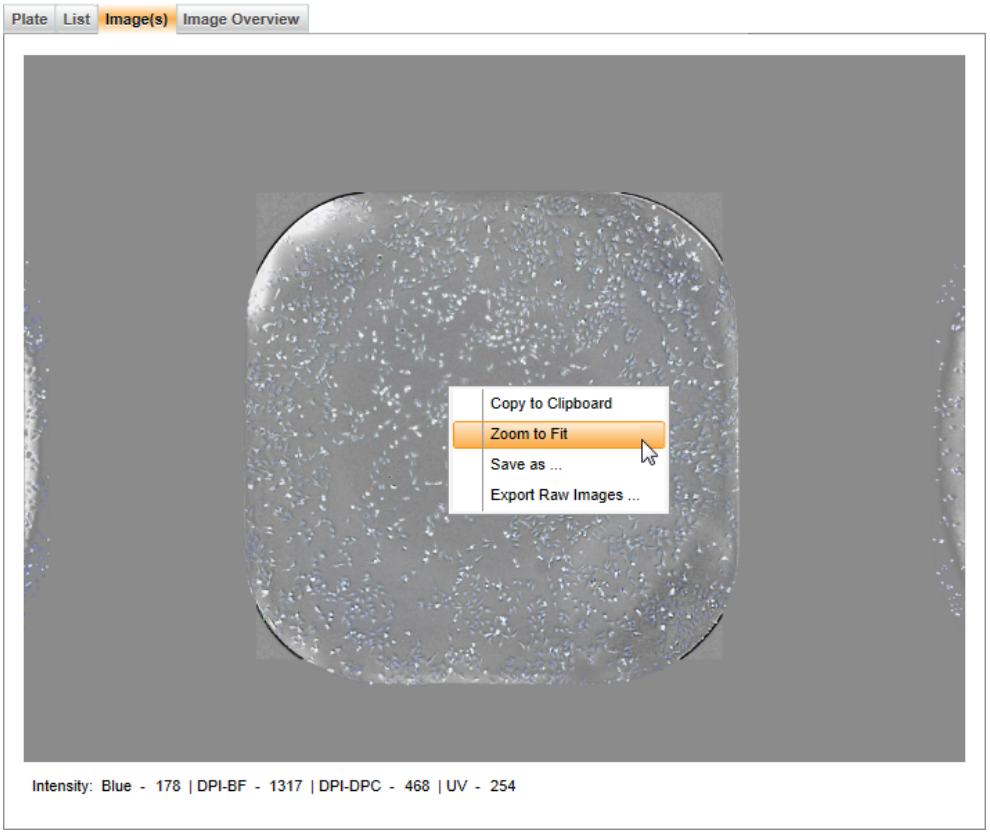
- The result table can be sorted by one parameter if you click on the corresponding column title.
- Click again to reverse the sorting direction (indicated by a small triangle).

How to copy results to the clipboard

- **Entire result table:**
  1. Press **CTRL+A** to select all table rows.
  2. Press **CTRL+C** to copy all datasets to the clipboard (tab-separated values). Alternatively, you can also use the **Copy** command in the context menu.
- **Single dataset:**
  1. Select one dataset in the result table (one row is highlighted).
  2. Press **CTRL+C** to copy the selected dataset to the clipboard (tab-separated values). Alternatively, you can also use the **Copy** command in the context menu.

5.7.5 Image(s)

This tab is only available on **View Results** if an **Imaging** measurement operation has been selected in the **Measurement** section on the left. If you click one well in the **Plate Map** or **Plate** tab, the corresponding image will be displayed automatically (overlay of channels).



- If you move the mouse to a spot on the image, the local **intensity** (0-4095) of that spot is displayed below the image (for each channel). The value is updated as soon as you stop moving the mouse.
- You can **zoom** the image using the mouse wheel.
- Drag the image with the mouse to move the field of view.

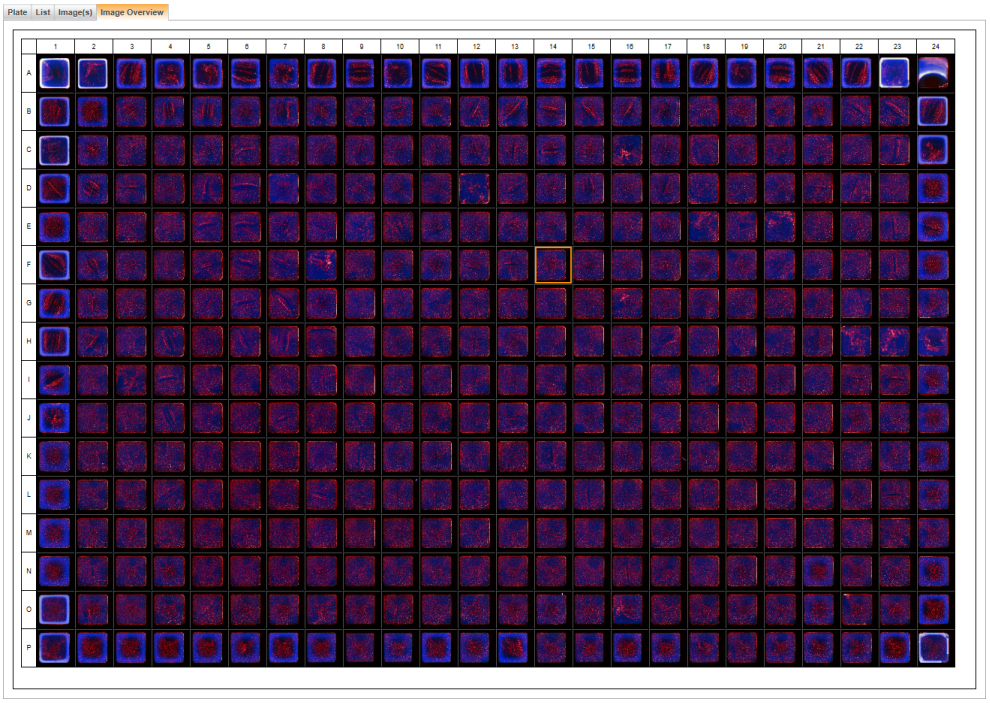
Using the **Display Options** (beneath the plate map on the right) you can adjust color, brightness and contrast of the image and enable/disable channels and overlays. These settings are only applied to the image display, they do not affect the original images or the image analysis. See also section 5.8.3 “Display Options”, page 211.

Context Menu

Element	Description
Copy to Clipboard	Copies the image with full resolution (as displayed with colored channels) to the Windows® clipboard to paste it into a different program. This will reduce the color depth from 12 bit grayscale per channel to 8 bit color (no separate channels anymore).
Zoom to Fit	Displays the image so that it fits into the **Content Area**.

Element	Description
Save as ...	Allows you to save the current image with full resolution (as displayed with colored channels). This will reduce the color depth from 12 bit grayscale per channel to 8 bit color (no separate channels anymore). The file format can be selected by appending the corresponding extension to the file name (*.bmp, *.jpg, *.png, *.gif, *.tif or *.tiff).
Export Raw Images ...	Allows you to save the unscaled RAW images with full resolution and original color depth (one 16 bit grayscale TIFF file per channel, first 12 bits are used). To view these images outside of Kaleido you need a third-party image viewer which is capable of displaying 16 bit TIFF images (e.g. IrfanView). Each exported image file will get the same time stamp as the original image so that it indicates the measurement time.

5.7.6 Image Overview



This tab is only available on **View Results** if an **Imaging** measurement operation has been selected in the **Measurement** section on the left. On this tab you can view thumbnail images of the measured wells directly in the plate layout (reduced resolution). The thumbnail calculation is triggered manually. For details please see section “Image Overview”, page 94.

5.8 Control Area

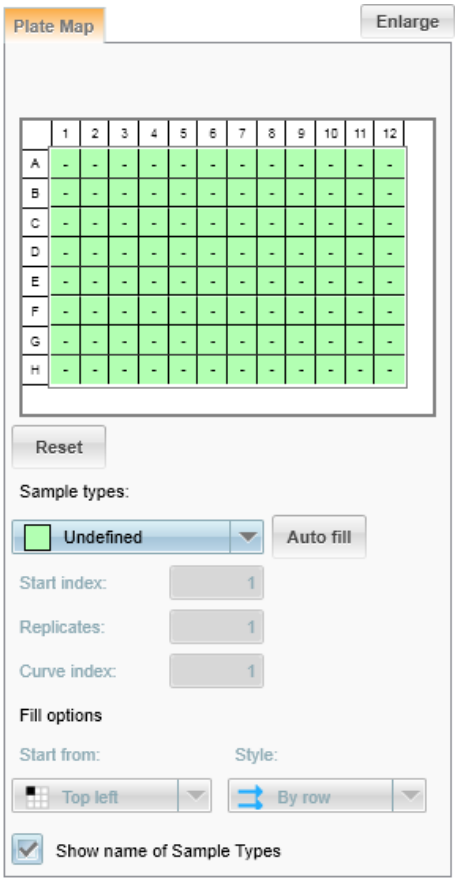
The right section of the screen is called **Control Area**. It contains the **Plate Map** and, depending on the context, various sections with control elements for modifying the information displayed in the **Content Area**. Furthermore, the optimization of **Imaging** parameters is controlled from here.

5.8.1 Plate Map

The plate map is always displayed in the upper right section of Kaleido. However, it has different functions and controls on the different screens. These are described in the following sections.

At any time you can use the **Enlarge/Reduce** button to magnify the representation of the plate map. You can also resize this area manually by dragging the inner border of the **Control Area**.

5.8.1.1 Plate Map on Setup Protocol



During set up of a protocol you can select the wells to be measured with the selected technologies (measurement operations) and define the type of the samples, e.g. required for an analysis. A protocol cannot be saved and run unless at least one well is

selected in the plate map. According to the plate format selected in the **Global Control** area, a plate layout is displayed in the **Plate Map** section. The sample type "Undefined" is selected for all wells by default.

When running a measurement sequence, wells to which a sample type has been assigned will be measured; white wells will be omitted. Specific sample types can be selected from a drop down list:

Sample Type		Abbreviation	Function
	Clear Well(s)		Clear sample type of selected wells.
	Blank	**B**	Samples without concentrations for blank corrected curve fitting calculations.
	Undefined	**–**	Default sample type.
	Control	**C**	Samples with known concentrations for curve fitting calculations.
	Standard	**S**	Samples with known concentrations for standard curves.
	Unknown	**U**	Samples with unknown concentrations for curve fitting calculations.
	Z Low	**ZL**	At least two replicate negative samples or samples with a low concentration for Z' measure to determine the assay quality.
	Z High	**ZH**	At least two replicate positive samples or samples with a high concentration for Z' measure to determine the assay quality.

There are multiple ways to assign the selected sample type to the wells:

- **Select single wells:** Click on a well in the plate map.
- **Select a group of wells:** Draw a rectangle with the pressed left mouse button. The selected wells will be colored immediately.
- **Select complete rows or columns:** Click on the header of the row or column.
- **Select the whole map:** Click on the top left corner of the plate map.
- **Clear sample type assigned to a well:** Select **Clear Well(s)** (in drop-down list of sample types) and click on the respective wells or headers.

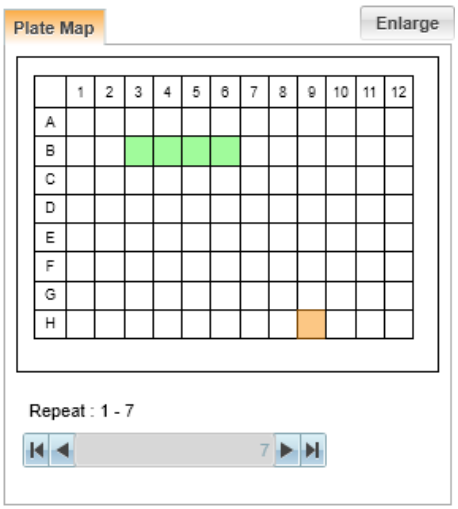
The sample types **Control, Standard** and **Unknown** have an indexing function. The wells that are defined with these sample types will be consecutively numbered according to the indexing option(s) chosen (see table below).

The plate map is saved in the protocol, you can change it anytime in the **Edit** mode on **Setup Protocol**. You can also change the plate type. If the new plate type has a different format (e.g. 96 instead of 384 wells), the plate map will be reset after you have confirmed a warning message.

Buttons and Elements

Element	Description
Enlarge/Reduce	Will enlarge / reduce display of plate map.
Reset	Clears the entire plate map, no wells and sample types selected.
Sample types	Choose a sample type from drop down list (see table above).
Auto Fill	The selected sample type will be assigned to all wells that have no sample type yet.
Start index	For sample types with indexing function. The indexing will start with the number defined in the text box and wells selected with the same sample type will be numbered consecutively, e.g. S1, S2, S3, ... while the start index is updated. The default start index after changing sample types is 1, even if indexed samples of this kind already exist in the plate map.
Replicates	For sample types with indexing function. Replicate samples are given the same number (e.g. start index = 3, replicates = 2: S3, S3, S4, S4, S5, S5, …).
Fill options - Start from	With the Fill options you can define in which way you want to index the samples if you are selecting more than one well at a time. Indexing can start from top left, bottom left, top right or bottom right independent from which direction you start to mark the wells.
Fill options – Style	Wells can be indexed by row or columns (indexing will start every new line from the same direction as defined in “Start from”) or bi-directional by row or column (indexing initially starts from the same direction as defined before, and returns in the next line).
Show Name of Sample Type	Unchecking this box will hide the abbreviations of sample types and indices in plate map.

5.8.1.2 Plate Map on Run Protocol



On the **Run Protocol** screen the plate map of the currently loaded protocol is shown but cannot be edited. The **Repeat** box displays the current repeat number and the total number of repeats. Depending on the measurement further parameters can be displayed for orientation (e.g. wavelength, well position etc.). Measurement progress and result values (of the current repeat) are displayed in the **Content Area**. Switching between differents repeats (or other parameters) is only possible after the measurement on the **View Results** tab.

Buttons and Elements

Element	Description
Enlarge/Reduce	Will enlarge / reduce display of plate map.
Plate Map	Shows the plate map as defined in the current protocol.
Repeat	Indicates which repeat is being measured and the total number of repeats.

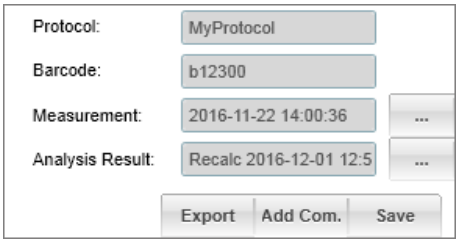
5.8.1.3 Plate Map on View Results

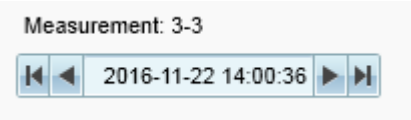
On the **View Results** screen the plate map of the currently loaded results is shown but cannot be edited.

If multiple measurements have been performed per well or if multiple measurements or analysis results are pre-selected, the **navigation controls** beneath the plate map can be used to navigate back and forth through the results displayed in the **Content Area**. Example:

There are controls for selecting different measurements, repeats, well positions, wavelengths or TRF windows. Which controls are available depends on the context (**Plate**, **Graph**, **Well** or **List** tab selected) and the loaded measurement(s) or analysis results.

If multiple measurements have been pre-selected and you switch to a different measurement, the corresponding measurement will be loaded and its name is displayed in the **Global Control** section:





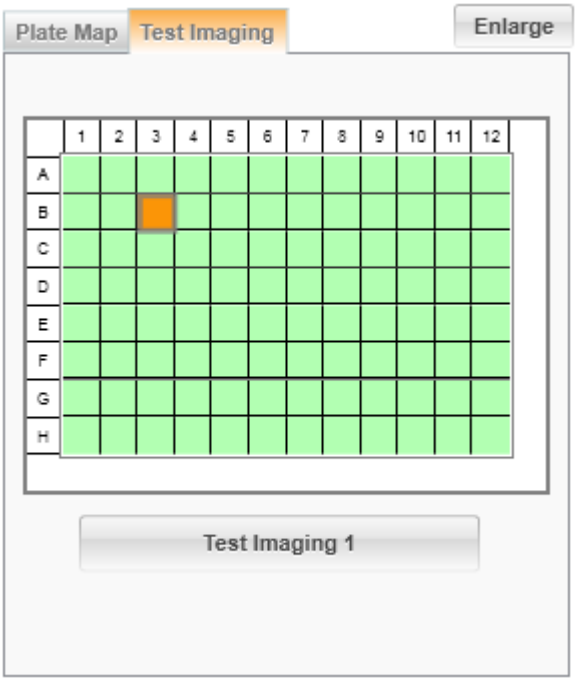
Buttons and Elements

Element	Description
Enlarge/Reduce	Will enlarge / reduce display of plate map.
Plate Map	Shows the plate as defined in the current protocol.
Navigation Controls	For browsing through results of different measurements, plate repeats, well repeats, well positions, wavelengths or TRF windows. Label above the control: For example, *"Measurement: 1-3"* means: first measurement of three pre-selected measurements in total.

5.8.1.4 Plate Map on Analysis

On the **Analysis** screen, the plate map of the current protocol is displayed but cannot be edited for the recalculation.

5.8.2 Test Imaging



During setup of an **Imaging** operation, its parameters can be tested and optimized using the **Test Imaging** tab in the **Control Area** (on the right). The generated test images are also required to test the image analysis (see section "How to configure and test an image analysis", page 124) and they persist until they are replaced by new test images, the imaging parameters are changed or a different well is selected.

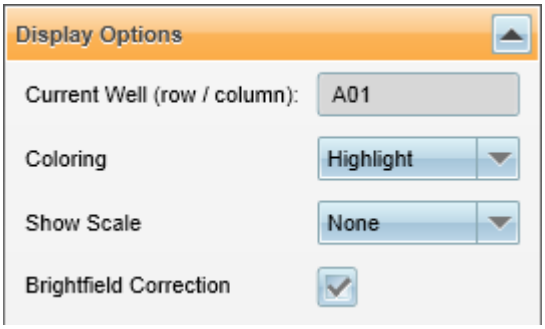
After selecting a well in the plate map, click on **Test Imaging [1 or 2]** to generate test images for all channels of the selected **Imaging** operation. The buttons are disabled as long as parameters are not set yet or set incorrectly (e.g. incompatible filter, no plate type defined etc.). The generated images are displayed on the **Image(s)** tab of the selected **Imaging** operation in the **Content Area**.

- Setting up an Imaging operation: "Imaging (IMG)", page 84
- Setting up an Image Analysis operation: 5.2.2.1 "Image Analysis", page 123
- You can use the **Display Options** pane (below **Test Imaging** tab) to configure how the images and channels are displayed. See section 5.8.3 "Display Options", page 211.

Buttons and Elements

Element	Description
Enlarge/Reduce	Will enlarge / reduce display of plate map.
Plate Map	Allows you to select one well where the test image(s) will be recorded.
Test Imaging 1 / 2	Test images will be recorded for all channels of the desired **Imaging** operation. • If there is only one **Imaging** operation, only **Test Imaging 1** will be available. • If there are two **Imaging** operations, **Test Imaging 1** and **Test Imaging 2** will be available.

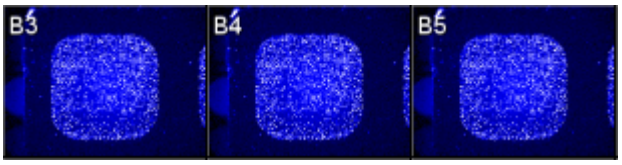
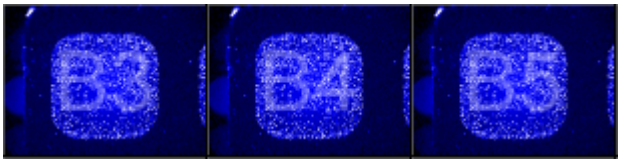
5.8.3 Display Options



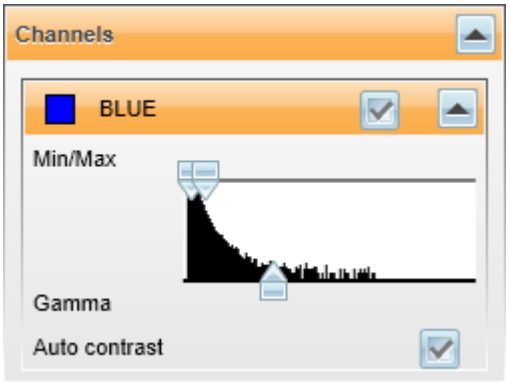
The **Display Options** can be used to modify the appearance of the image or illustration currently displayed in the **Content Area**. All changes are transient and only affect the display of the images. The original images are not modified.

Display Options (for all channels) – Buttons and Elements

Element	Description
Disclosure button	 expand pane,  collapse pane
Current Well	Coordinates of selected well (row/column).
Coloring	The images recorded by the EnSight have a much higher dynamic range than the computer screen (12 bit instead of 8 bit). There is no way to display all information even of a single image channel in a single image on a computer screen without loss of information. In Kaleido, the user can select one of three display modes to view the image as required. • In the **Highlight** mode (default), the color channels are intelligently adapted to show as many details as possible within the dynamic range of the display. Colors are more pure and saturated. *Drawback:* Very bright objects are all white, independent from which color they have been assigned to. This is a good tradeoff to get a better representation of “middle intensity” regions in the image. In many applications it is clear from the image context to which channel the “white” areas belong. • The **Enhanced** mode is mostly identical to the **Highlight** mode, except for the fact that very bright regions are colored in the assigned channel color (or a mixture of colors for co-localized regions). *Drawback:* Gives a less clear view for the middle intensity range (which mostly is the largest part of the image). • In the **Standard** mode, image intensities are not modified to enhance image details. Intensities can be directly judged on the displayed image. **Notice** The **Coloring** mode has no influence on the intensity values displayed in the **Content Area** (see section 5.7.5 “Image(s)”, page 203). It also has no influence on any analysis results, only the display is affected.

Element	Description
Label	*Only available for image overview:* Displays well coordinates on the thumbnail images of an image overview. • **None** (deactivated, default) • **Small:** Well coordinates as small text  • **Watermarked:** Well coordinates as large watermark 
Show Scale	Allows you to select a corner of the image where a fixed scale bar in µm will be displayed. Select "None" to hide the scale.
Brightfield Correction	The local background intensity of brightfield images is estimated and subtracted. Structures like cells are much clearer visible, the intensity drop towards the edges of the well is compensated for. Default is ON. **Note:** Only the display is affected, analysis results are not changed by the setting.

5.8.3.1 Channels



By default all channels are activated and displayed as a colored overlay.

- Click the colored square in front of the channel name to select a different **color** for a channel.
- Single channels can be removed from the display by deactivating the check box in the channel's header.
- Brightness and contrast of the image can be adjusted using the **Min/Max** and **Gamma** sliders of the histogram. Intensity values will be displayed as a tooltip if

you move a slider.

- Check **Auto Contrast** to determine the best settings automatically.

Notice

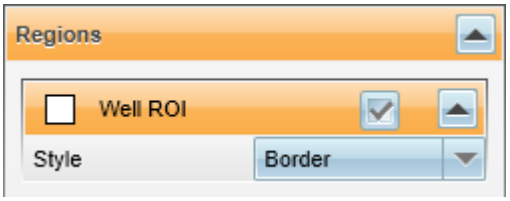
- All settings only affect the image display – neither the image itself nor any analysis results are affected by the display settings.
- In case of an **image overview**, brightness and contrast adjustments are applied to the entire image overview, not to individual thumbnails. Thereby you can compare individual wells.

Channels – Buttons and Elements

Element	Description
Disclosure button of channel	 expand channel,  collapse channel
Select Color	The little square in front of the channel name shows the channel color. Depending on the excitation wavelength the default coloring of the images is as follows: • 385 nm / 385 nm extended: blue • 465 nm: green • 525 nm: orange • 632 nm: red • 735 nm, transmitted: gray If you click the colored square you can select a different color for this channel.
Channel activation check box	• **Activate** the check box to display this channel in the image. If multiple channels are activated, they will be displayed as an overlay. • **Deactivate** the check box to ignore this channel for image display. This does not modify or delete the channel, it just controls the appearance of the image.
Min/Max/Gamma	The sliders of the histogram can be used to adjust brightness and contrast of the channel in the display. The **Auto Contrast** checkbox is cleared when moving any slider. The manual slider settings persist until the **Auto Contrast** checkbox is enabled again. • **Min:** Adjust the **minimum intensity** value of the image. • **Max:** Adjust the **maximum intensity** value of the image. • **Gamma:** Adjust the **Gamma** value. Gamma correction is a nonlinear intensity enhancement of dark image areas in order to make the structure inside these areas visible.
Auto Contrast	Activates or deactivates automatic contrast adjustment. This does not modify the image, it just controls the appearance of the image.

5.8.3.2 Regions

This section is only available when image analysis results are displayed in the **Content Area**, e.g. when setting up and testing an image analysis.



The list of available regions (e.g. overlay masks of detected cells) depends on the tasks selected in the image analysis.

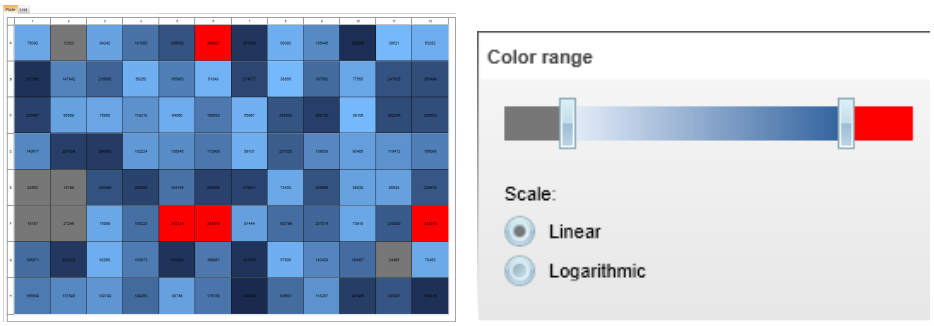
- Click the colored square in front of the region name to select a different **color** for a region.
- Regions can be removed from the display by deactivating the check box in the region's header.
- You can select a **Style** for each region to determine which information is displayed in the overlay mask.

Regions – Buttons and Elements

Element	Description
Disclosure button of region	expand region, collapse region
Select Color	The little square in front of the region name shows the color of the overlay mask. If you click the colored square you can select a different color for this region.
Region activation check box	• **Activate** the check box to display this overlay mask in the image. • **Deactivate** the check box to hide this region.
Style	• **Border:** Displays a border around the detected region. • **Body:** Displays the whole area of the region (semi-transparent). • **Solid:** Displays the whole area of the region (no transparency). • **Center:** Indicates only the center of the region with a small circle. • **Numbers:** Displays only the region number. • **Circle:** Draws a circle around the detected region. • **Borderless:** Same as "Body" but without border.

5.8.4 Color Range

On the **View Results** screen, on the **Plate** and **Well** tabs (only for well scans), the intensities of the measured results are visualized as a heat map of colored wells. The colors can be configured in this section.



You can generally choose between a **Monochrome** style (shades of blue) or a **Rainbow** style. The style can be selected in the **Settings** dialog (see section 5.10.2 “General Settings”, page 220).

Value	Color (Monochrome)	Color (Rainbow)
Low value	Light blue	Blue
High value	Dark blue	Red
Lower than selected minimum	Gray	Light gray
Higher than selected maximum	Red	Dark gray

Minimum and maximum values are set automatically and can be changed by using the sliders. You can change the scale for the signal intensity from linear to logarithmic by using the check boxes.

Buttons and Elements

Element	Description
Slider (color)	Shows the color settings for the results in the wells. Left slider defines the minimum value, the right slider the maximum value.
Linear	Set as default scale.
Logarithmic	Check mark to see results in logarithmic scale.

5.9 Messages

The area at the bottom of the screen is called **Messages** section. Here you can find for example system notifications and comments.

- The tables can be sorted by clicking on the desired column title. Click again to reverse the sorting direction.
- Selected rows can be copied to the clipboard using the context menu command **Copy** or by pressing **Ctrl+C**. There are multiple ways to select rows:
  - Single row: Click one row with the mouse.
  - Multiple rows: Hold down **Strg** and click multiple rows or hold down **Shift** and click to select all rows inbetween. You can also hold down the mouse button and just drag the mouse.
  - All rows: Press **Ctrl+A**.

Create Report

In case of technical issues you can use the **Create Report** button to generate a zipped file with all relevant log files and system information for sending them to PerkinElmer Service. For detailed instructions please see section 5.14.2 “Reporting Technical Issues”, page 251.

5.9.1 Comments

Comments are short messages that are added to a protocol, a measurement or an analysis result by Kaleido or manually by the user. Comments are listed chronologically with timestamps in the **Comments** tab by default. Once created, comments cannot be edited or removed.

How to add a comment to a protocol

Precondition: Protocol loaded or created on **Setup Protocol**.

1. On **Setup Protocol**, click **Save…**.
2. In the **Save Current protocol** dialog select **Save** or **Save as…** and enter your comment in the **Comments** text box.
3. Click **OK**.

   The comment will appear in the **Comments** tab including a time stamp, the name of the user logged in and the actual message.

Comments of all earlier versions of the protocol remain and comments are also included when exporting a protocol in ***.kal** format keeping their original timestamps. Comments of protocols can only be viewed on **Setup Protocol**. Comments are also listed in export files of measurements.

How to add a comment to a measurement or analysis result

- As soon as a measurement or analysis result is loaded on **View Results**, the **Add Com.** button is enabled. Click this button to open the **Add Comment** dialog. Select where the comment is to be added:
  - **Add comment to current measurement <measurement date>** or
  - **Add comment to the current analysis result <name of analysis result>** If no analysis result is available for a measurement, the respective radio button is disabled.

  Enter your comment in the text box and click **OK**.
- After a **Recalculation** you can save the new analysis results and/or save the new analysis sequence to the protocol and thereby add a comment.
- On **Run Protocol**, **View Results** and **Analysis**, the comments of the loaded measurement and analys result are always displayed on the **Comments** tab. For comments of analysis results the corresponding name is displayed in the column **Analysis Result**. If this column is empty, the comment belongs to the measurement.

Examples for automatically added comments of measurements

- Measurement was started
- Measurement was stopped
- Measurement was continued
- Analyses: count cells: D02 Image is empty or overexposed
- Autofocus failed for G03
- Imaging 1 – Archive Images was activated during measurement and images have been archived to folder […]
- Temperature control activated by settings. Target: 37 °C
  Condensation prevention for sealed plates is deactivated.

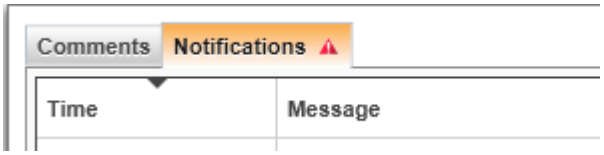
5.9.2 Validation

This tab only appears on **Setup Protocol** and reports inconsistencies between the components in the protocol (e.g. plate type not suitable for the chosen operation). As soon as the inconsistency is removed, the validation message will disappear.

5.9.3 Notifications

Like **Comments**, this tab is always displayed and shows notifications and error messages coming from the instrument during initialization and during/after a measurement. These notifications are available on **View Results** after the measurement but they are not saved with the measurement result.

If new notifications are available, the header of the **Notifications** tab flashes orange and a red warning symbol is displayed:



Examples for notifications

- One or more instrument components did not initialize properly. [...]
- An error occurred during your measurement, please note that this may have distorted your data. [...]

5.10 Settings

The **Settings** dialog allows you to view and edit the instrument's configuration and general Kaleido settings. The settings are grouped by category. Click the desired icon to open the corresponding dialog or the next navigation level.

Notice
Certain functions are not available for all users. For details see section 5.13.3 “Restricted Actions”, page 248.

The settings in Kaleido can be **global** (affect all users) or **user-specific** (affect only the currently logged in user). The following overview contains only functions where settings can be displayed or modified:

Category	Sub Window / Function	Scope
Instrument Options	View options	Global
General Settings	All settings	User-specific
Data Management	Backup Database (backup path)	Global
	Scheduled Task	Global
	Archive Images (on/off, archive path)	Global
Barcode Reader	All settings	User-specific
Temperature	All settings	User-specific
Stacker	All settings	User-specific

5.10.1 Instrument Options

This dialog lists various properties of the connected instrument (read-only).

- Serial number
- Kaleido software version
- Acapella software version (image analysis)
- Installed instrument options and technologies
- Available reading positions of the barcode reader

5.10.2 General Settings

This dialog includes various general settings grouped in three categories.

Plate Carrier Settings

Element	Description
Soft Plate Moving	If the plate wells are very full, this option allows you to select that the plates will be moved more slowly than normal in order to avoid spillage. **Notice** Using this option will lead to longer measurement times.
Load Plate into instrument automatically [min]	If you activate this option, you can give a time in minutes. The plate carrier will automatically be moved into the instrument when this time has elapsed (after the instrument switched from ”busy“ to ”idle“ state). **Notice** This option is not applicable in automation mode and will be ignored.

Color Scale of Heat Map

Option to select a color style which will be used to visualize result values in Kaleido (see also section 5.8.4 “Color Range”, page 215).

Element	Description
Monochrome (default)	Different shades of blue will be used for coloring the heat map. Gray or red are used for values out of the selected range. • **Light blue:** low value • **Dark blue:** high value • **Gray:** lower than selected min. value • **Red:** higher than selected max. value
Rainbow	Multiple colors will be used. • Color scale (from lowest to highest value): blue, green, yellow, orange, red

Instrument Initialization

Element	Description
Initialize Instrument	The **Initialize Instrument** function re-initializes all instrument components and the hardware control software. This may help in case of undefined error states and is an alternative to manually closing and restarting the device and the hardware control software.

5.10.3 Data Management

5.10.3.1 Backup Database

You can use this function to make a backup of all protocols and results in the database. The content of the database will be saved as a compressed file (*.bak). To restore a database from such a backup file, please use the **Restore Database** function (see section 5.10.3.2 “Restore Database”, page 222).

How to backup a database

1. Open **Settings > Data Management** and click the **Backup Database** icon.

   A wizard is opened which will guide you through the backup procedure.

2. Select a destination path for the backup file. If you click the ”**...**“ button, you can browse for a folder. The backup file will be named automatically using current date and time (naming pattern: *YYYYMMDDhhmmss.bak*). Click **Next >>**.

   The backup is started.

3. When the backup is finished, click **Close** to close the wizard.

5.10.3.2 Restore Database

Using this function you can restore protocols and results from a previously created database backup file (see section 5.10.3.1 “Backup Database”, page 222). The backup will overwrite your current database.

Notice
If a backup is restored, all existing protocols and results in the current database will be lost and replaced by those in the backup. Before restoring a backup, make sure to either create a backup or export single protocols and results which are still needed. After restoring the database you can easily re-import them.

How to restore a database

1. Open **Settings > Data Management** and click the **Restore Database** icon.

   A wizard is opened which will guide you through the restoration procedure.

2. Select the backup file. If you click the ”**...**“ button, you can browse for the file. Click **Next >>**.

3. You have now the option to backup the current database before restoring the data from the backup file. Select the option as desired and click **Next >>**.

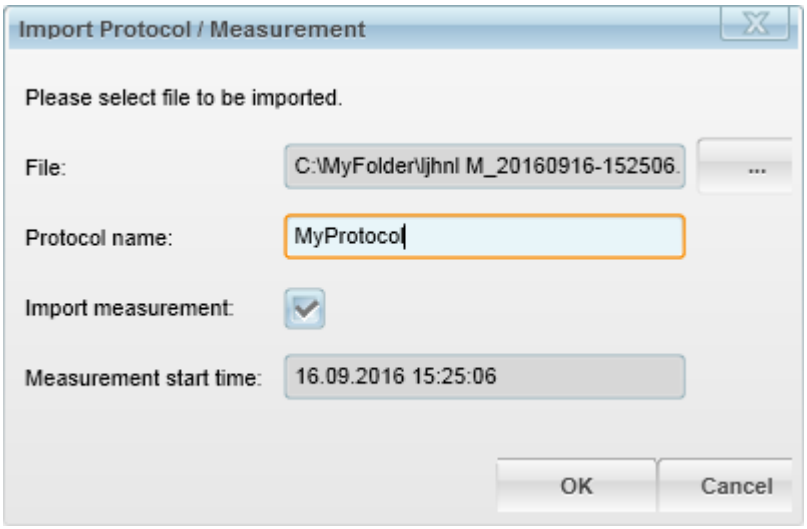
   - If you checked this option, you will be asked for a destination folder and then the backup will be created.

4. Click **Yes** to confirm the selection and restore the database.

5. When the process is finished, click **Close**.

   Kaleido is closed and restarted automatically. After logging in you can work with the restored database.

5.10.3.3 Import Protocol / Measurement



Using this function you can import a protocol or measurement from a Kaleido export file (*.kal). Such a file can be created by exporting a protocol (then it includes only the protocol) or by exporting a measurement (then it contains protocol + results). See also the following sections:

- Export protocol: 5.2 “Setup Protocol”, page 68 (save dialog)
- Export protocol + results (manually): 5.4 “View Results”, page 161
- Export protocol + results (automatically): 5.2.3 “Post Processing Sequence”, page 144

How to import a protocol/measurement

1. Open **Settings > Data Management** and click the **Import Protocol / Measurement** icon.

   The import dialog is opened.

2. Select the **File** to be imported (*.kal). If you click the ... button, you can browse for the file.

3. The **Protocol Name** of the selected file is displayed in the text box.
   - If only a protocol is imported (see step 4), you can edit the name. The protocol name may exist in the database already. Identification is also easily possible via other properties like owner, date or signature.
   - If measurement + protocol are imported, the protocol name cannot be modified.

4. If the import file also contains measurement results, the **Measurement Start Time** is displayed and you have the following options for the import:
   - **Import Measurement checked:** Import protocol and measurement.
   - **Import Measurement unchecked:** Import protocol only.

5. Click **OK** to start the import.

Buttons and Elements

Element	Description
File	Displays the path and file name of the *.kal file to be imported. Click ... to select the desired *.kal file.
Protocol Name	Displays the protocol name of the selected import file (*.kal). If only a protocol is imported, you can edit the protocol name. You will be notified if the protocol name exists already in the database. In this case you can change the protocol name if you want to avoid name duplicates. However identification is also easily possible via other attributes like owner, date, etc. Please do not use following special characters \ / : * ? „ < > |.
Import Measurement	*Only available if the import file contains measurement results:* • Checked: Protocol + measurement results are imported. • Unchecked: Only protocol is imported.
Measurement Start Time	*Only if the import file contains measurement results:* Displays the start date of the measurement.
OK	Imports the selected file and closes the dialog.
Cancel	Closes the dialog without importing anything.

5.10.3.4 Database Info

The following status information will be displayed:

Database Size

- **Used space** in database
- **Free disk/database space**
  - Database using *Microsoft SQL Server **Express*** (Kaleido standard installation):
    In this configuration the size of the database is limited. The remaining **free database space** is displayed.
  - Database using *Microsoft SQL Server **Standard*** (optional):
    The size of the database is only limited by the available **free disk space**, hence it is displayed in this case.

For troubleshooting see also section 5.14.3 “Database or disk is full”, page 251.

Last Backup

- **Time stamp** of last database backup

5.10.3.5 Scheduled Task

Using this function you can activate a regular notification which reminds you of creating a database backup. If the next backup is due, a corresponding message will pop up each time when Kaleido is started until you create a new backup. Per default,

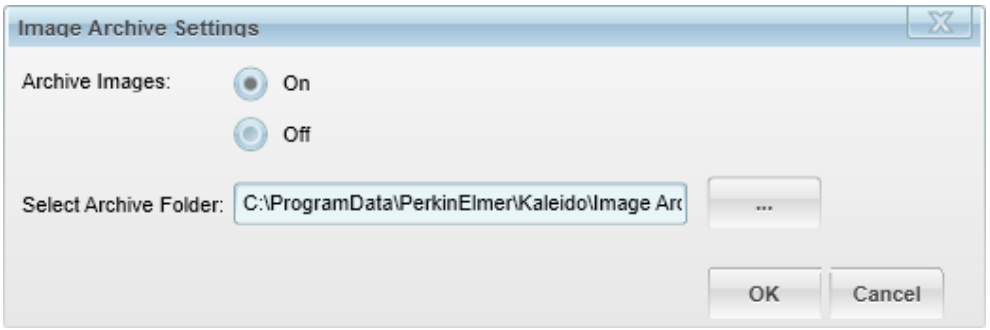
this function is activated with a daily notification.

How to define a scheduled task

1. Click the **Scheduled Task** icon to open the dialog.
2. Click **Yes**.
3. Select the desired interval for the reminder:
   - daily (default)
   - every week
   - every 2 weeks
4. Click **OK** to apply the settings and close the dialog.

5.10.3.6 Image Archive Settings

In this dialog you can define a path where all recorded images will be saved permanently. This option can be switched on (default) and off.



The specified archive folder is also the location where Kaleido will search for images to be displayed if you load an imaging measurement on the **View Results** or **Analysis** screen (irrespective of whether **Archive Images** is on or off). If the images have been moved, you have to adapt the path to view the images.

Notice

- Images fill up disk space very quickly. Check the available free disk space before starting an imaging run.
- In case of large numbers of imaging measurements it is recommended to set the archive path to an external storage or network server. Otherwise the local disk may fill up quickly. In case of insufficient space the system may become unstable and runs may be canceled.
- Each imaging measurement will automatically get a comment indicating whether **Archive Images** was turned on or off during measurement. If it was turned on, also the path of the archive folder configured at that time will be stated.
- If you move the images of a measurement, it is recommended to add a comment to the measurement stating the new location. This will help you to find the images if you need them at a later time.
- Do not change the archive folder while using the **Continue Measurement** mode (i.e. before continuing a partial measurement). The resulting images would be saved to different folders so that some images will not be accessible and no image overview can be created.

Buttons and Elements

Element	Description
Archive Images	**On:** The images will be saved into the cache folder AND into the **Archive Folder** (see below). If the next measurement starts they will be deleted from the cache folder but not from the archive folder. **Off:** The images will be saved only temporarily in the cache folder and deleted again if the next measurement is started.
Select Archive Folder	Allows you to specify the path of the image archive folder. Enter a UNC path or click ... to browse for a folder. Kaleido will also use this folder to search for images to be displayed (even if **Archive Images** is off).
OK	Close the dialog and apply changes.
Cancel	Close the dialog discarding any changes.

Archive Folder Structure

If **Archive Images** is on, for each imaging measurement one subfolder will be created in the specified archive folder. This folder with all its data objects is self-contained, i.e. it contains all information to import a measurement in a different database and view the images.

Subfolder name: <*MeasurementDate*>_<*MeasurementSignature*>

Example: *2010-10-05_153345_ 01c31c6c-bf85-4350-a311-e5448da7e2cf*

- <MeasurementDate>: Start time of the measurement, format "*YYYY-MM-DD_ hhmmss*"

- <MeasurementSignature>: Unique identifier of the measurement

Notice
Do not rename the measurement subfolders. Otherwise the link to the measurement in the database is lost.

Data objects contained in each subfolder

- *<MeasurementDate>_<MeasurementSignature>**.kal***:

  Contains the protocol, measurement result (and analysis result, if applicable). This file is generated automatically as soon as the measurement is finished . It can be used to transfer measurements to a different instrument or to restore measurements which had been removed from the database. See also section 5.10.3.3 “Import Protocol / Measurement”, page 223.

- *<Timestamp>_c<Channel-ID>_<Well-ID>-<ChannelType>**.tiff:***

  Example: *20161005_153345.21_ c4_0403-BF.tiff*

  The measured images, one file per channel and well (only if **Archive Images** was on).

  - Timestamp format: *YYYYMMDD_hhmmss.msms*
  - Channel-ID:
    Channel 0 = LED 1 = UV
    Channel 1 = LED 2 = Blue
    Channel 2 = LED 3 = Green
    Channel 3 = LED 4 = Red
    Channel 4 = BF / DPC
  - Well-ID = <Row><Col>
    Row: Row number (A=01, B=02, ...)
    Col: Column number
  - Channel Type:
    BF: Brightfield
    DPC: Digital Phase Contrast
    No suffix: Fluorescence

- *<MeasurementDate>_<MeasurementSignature>**.index.idx.xml***:

  Contains metadata of the measurement (only existing if **Archive Images** was on).

Troubleshooting

Selected archiving folder is not accessible

Possible Reason	Solution
User has no access rights for the selected folder.	Check the access rights for this folder.
The selected folder is located on a server and there is currently no network connection.	Check the network connection.

Images of the loaded imaging measurement are not displayed

Possible Reason	Solution
Archive Images was **off** during the measurement. The images have not been archived.	
Archive Images was **on**, but the images have been moved manually (e.g. to a server).	• Update the path of the archive folder in the settings to the current location of the images. Or: • Move the archive folder back to its former location.
Archive Images was **on**, but a different archive folder has been specified in the settings.	• Update the path of the archive folder in the settings to the current location of the images. Or: • Move the images to the currently selected archive folder.

5.10.3.7 Delete Data

This dialog allows you to delete the following objects from the database:

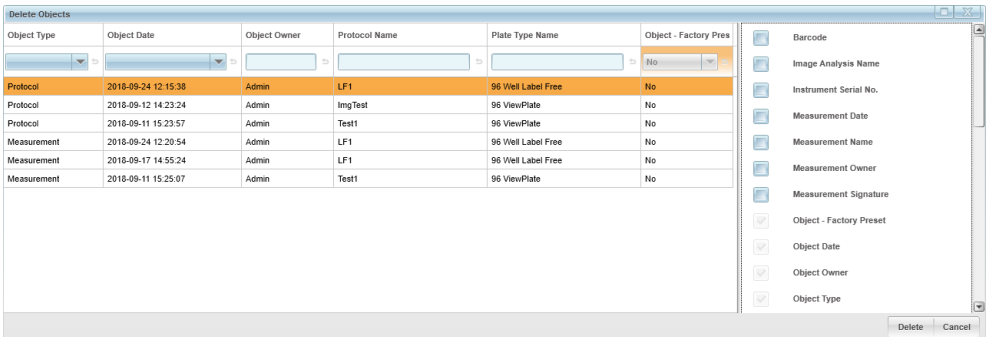
- Protocols (only user-defined, not factory-preset)
- Measurements
- Plate types (only user-defined, not factory-preset)

Notice

Access to this function depends on the user's user group:

- **Operators** have no access to this dialog.
- **Editors** can only delete their own objects.
- **Administrators** can delete all objects.

For details see section 5.13.3 “Restricted Actions”, page 248.



The list of objects in this dialog is restricted to objects which actually can be deleted by the current user (due to object type and user group). The **Delete Data** dialog is very similar to the **Load ...** dialogs. For a general description and for instructions how to filter and sort the listed objects, please see section 5.6 “Load Dialog”, page 180.

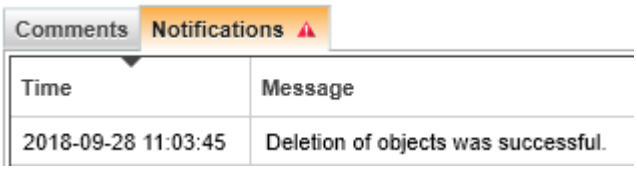
Effects of the deletion process:

- If you delete a **plate type** or **protocol**, this will not affect other database objects (and vice versa), e.g. measurements performed with this protocol. They will remain unchanged.
- If you delete a **measurement** which contains an **Imaging** operation, a few things have to be considered:
  - After clicking **Delete**, you will be asked if you also want to delete associated **images**. See section “How to delete an Imaging measurement and associated images”, page 229.
  - If **analysis results** exist for this measurement, these results will also be deleted.

How to delete a database object

1. Open **Settings > Data Management > Delete Data**.
2. Select the desired object(s) (protocol, plate type or measurement) in the object list.
   - You can sort and filter the list to find the desired object. For detailed instructions see section 5.6 “Load Dialog”, page 180.
   - To select multiple objects:
     - Hold down the **Ctrl** key and click multiple single objects.
     - Or click the first object to be deleted, hold down the **Shift** key and click the last object.
3. Click **Delete**.

   The selected object(s) are deleted and a notification is displayed:



How to delete an Imaging measurement and associated images

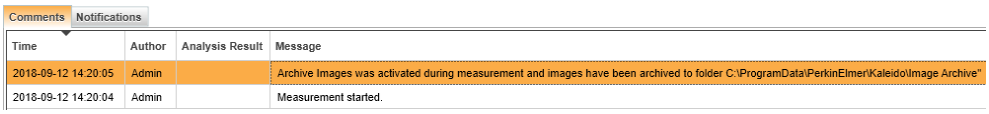
If you delete a measurement containing an **Imaging** operation, you will be asked if you want to delete associated images. Please note:

- The system cannot detect whether images for this measurement still exist. The corresponding dialog will appear in any case.
- The images of an **Imaging** measurement are only saved permanently if **Archive Images** was activated during the measurement. See also section 5.10.3.6 “Image Archive Settings”, page 225.

- The **comments** of a measurement indicate whether **Archive Images** was active during the measurement and which **archive path** was used. This path is required for deleting associated images.

Recommended workflow:

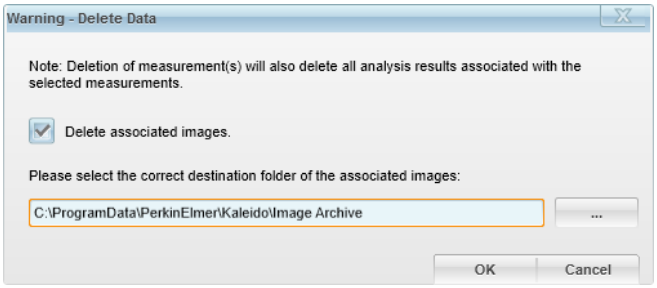
1. Load the measurement to be deleted and check the **Comments**:



   If **Archive Images** was activated, make a note of the archive path. Otherwise no images have been saved and there are no images which could be deleted.

2. Open **Settings > Data Management > Delete Data**.
3. Select the measurement and click **Delete**.

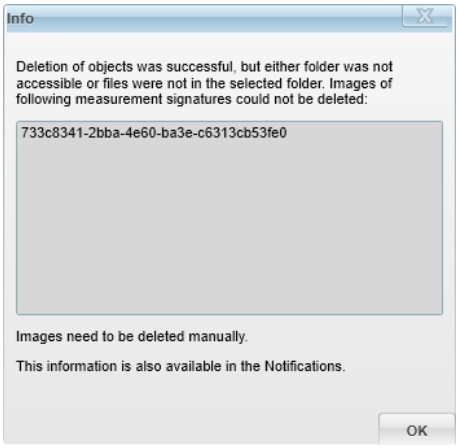
   The following dialog will be displayed:



4. If images exist for your measurement and if you want to delete them:
   - Check mark **Delete associated images**.
   - Enter the archive path (or the current folder if the images have been moved in the meantime) where the images are located. The images will only be deleted if they are found in the specified folder.
5. Click **OK**.

   The measurement including associated images and analysis results (if applicable) will be deleted.

Troubleshooting: Images could not be deleted

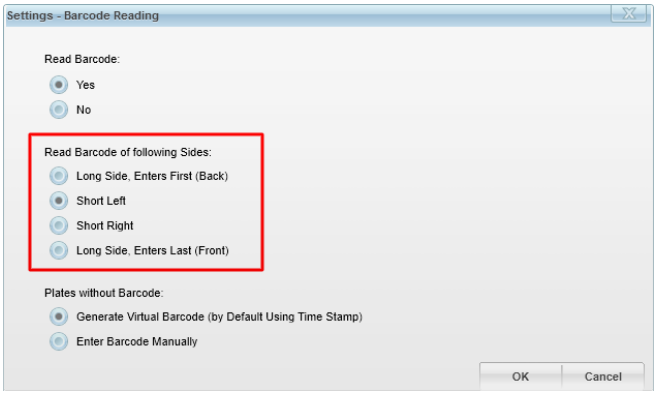


Possible reason	How to fix it
Folder with images was not accessible.	Delete the images manually. To avoid this issue in the future: • Check the network connection (if the folder is located on a different computer or server). • Check the access rights of the folder. • If using a removable drive, make sure that it has been connected.
Image files were not in the selected folder.	Find out the actual location of the images and delete them manually. The signature(s) of the measurement(s) concerned is stated in the info dialog and also in the **Notifications** area. This can help you to find the images, because the subfolder(s) containing the images is automatically named after the measurement signature.

5.10.4 Barcode Reader

In this dialog you can switch barcode reading on or off and define where the barcode is read (red box).

- If **one** barcode reader is installed (default), the left side of the plate is automatically set as reading position.
- If **four** barcode readers are installed (optional), you can select a reading position.



If barcode reading is switched off, the reading position is not displayed anymore:

The selected reading position will also be used in **Automation** mode (under remote control by an external scheduler). All other barcode settings will be ignored.

Barcode handling in Automation mode

- **Case 1: Barcode provided by scheduler**

  If the scheduler provides a barcode with the RUN command, Kaleido will use this barcode and will not look for a barcode on the plate (regardless of whether barcode reading is enabled or disabled in Kaleido).

- **Case 2: No barcode provided by scheduler**

  If no barcode is provided by the scheduler, Kaleido will look for a barcode only if barcode reading is switched on in the Kaleido settings. The selected reading position (see red box above) will also be applied if the instrument is controlled via external scheduler. This parameter can only be configured directly in Kaleido, not via scheduler.

  A virtual barcode is automatically created if barcode reading is switched off or if no barcode could be detected. The last group of parameters in “Settings –

Barcode Reader" (Plates Without Barcode) will be ignored, i.e. you cannot enter a barcode manually in **Automation** mode.

Buttons and Elements

Element	Description
Read Barcode	**Yes:** Barcode of every plate is read automatically. **No:** Barcode reading is switched off.
Read Barcode of Following Sides	*Selection is only possible if four barcode readers are installed (optional):* Select **one** of the four sides (see screenshot above). The barcode will be read exclusively on this side.
Plates Without Barcode	A barcode is required for each measured plate. If a plate does not have a barcode, or if barcode reading is switched off, you can decide how to handle such plates using the following options: • **Generate Virtual Barcode:** A virtual barcode consisting of a time stamp is created automatically. The virtual barcode follows the pattern: ◦ V-{date} {time} ◦ V-2015-02-28 16:01:13 • **Enter Barcode Manually:** You will be prompted to enter a barcode manually.

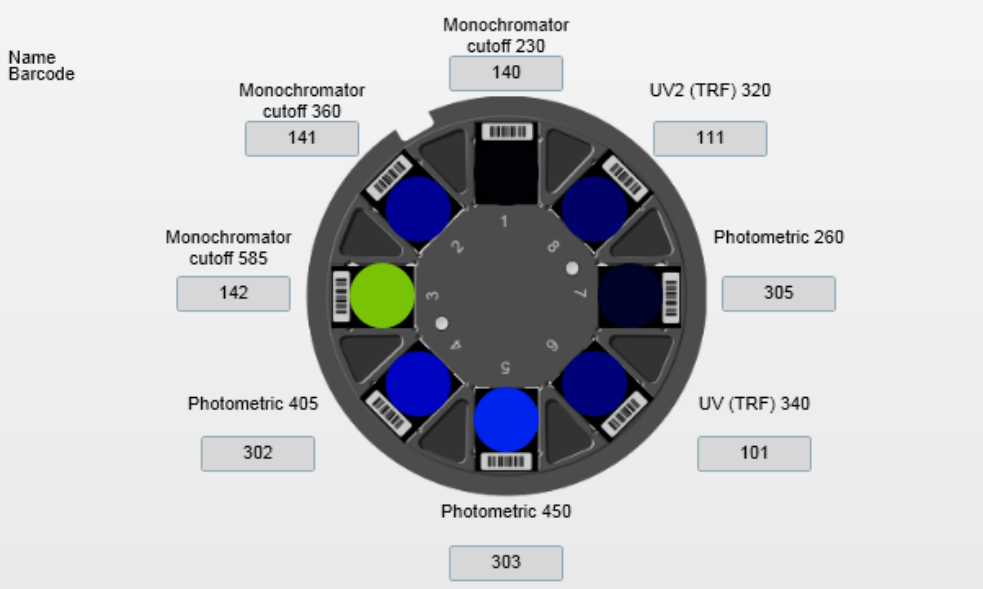
5.10.5 Inventory

The functions grouped in this sub window allow you to view and edit the instrument's filter configuration and the list of available plate types.

5.10.5.1 Filter Wheel

This dialog shows a picture of the filter wheel with the currently installed excitation filters, their barcodes (in text box) and their position in the wheel. In the **Define Filter** dialog you can modify existing filters or create new filters. See also section 5.10.5.3 “Define Filter”, page 235.

If Kaleido is not connected to the instrument (simulation mode), a pre-defined virtual filter configuration will be displayed.



Filters available in simulation mode

5.10.5.2 Plate Type

The Plate Type Wizard can be used to create a new plate type from scratch or based on an existing plate type. It is not possible to edit a plate type. Alternatively, you can create a new plate and delete the old plate type (see section 5.10.3.7 “Delete Data”, page 228).

How to use the Plate Type Wizard

1. Open **Settings > Inventory** and click the **Plate Type** icon.
2. Options:
   - If you choose **New**, you can create a new plate type from scratch.
   - If you choose **New based on**, you can select an existing plate type from the **Plate Types** combo box and use its parameters as a starting point. The parameters of the selected plate type will be displayed as default settings in

the following steps of the wizard so that you only have to edit the parameters where the value differs compared to your new plate type.

3. Select a **Plate Format** and click **Next >>**.
4. Enter the plate dimensions (**A** to **D**) according to the figure and click **Next >>**.
5. Enter further plate properties (**E** to **H**) according to the shown figures.
6. Select a **Well Shape** (round/rectangle).
7. Enter the **Well Volume**.
8. Define the **Optical Index** of the plate bottom material. You can select one of the pre-defined materials or **Custom** to enter a value directly.
9. Click **Next >>**.
10. Check all operations which can be used with the new plate type. Click **Next >>**.

> **Notice**
> Before setting the compatibility check marks for a new plate type, please check the plate properties carefully. For detailed information regarding compatibility of a plate with the EnSight measurement technologies see also section 4.11 “Plates”, page 51.

11. Enter a unique name for the plate type.
12. Click **Finish** to complete the wizard.

    The plate type will be saved in the inventory database.

5.10.5.3 Define Filter

A wizard helps you to create new filters to be used in the excitation filter wheel.

How to use the Filter Wizard

1. Open **Settings > Inventory** and click the **Filter** icon.
2. Select **New** and click **Next >>**.
3. Enter the barcode of the new filter (three digit number) and click **Next >>**. This barcode must not exist in the database yet.
4. Enter the filter properties.
5. Check the operation(s) for which the filter is suitable. Click **Next >>**.
6. Enter a unique name for the filter.
7. Click **Finish** to complete the wizard.

   The filter will be saved in the inventory database.

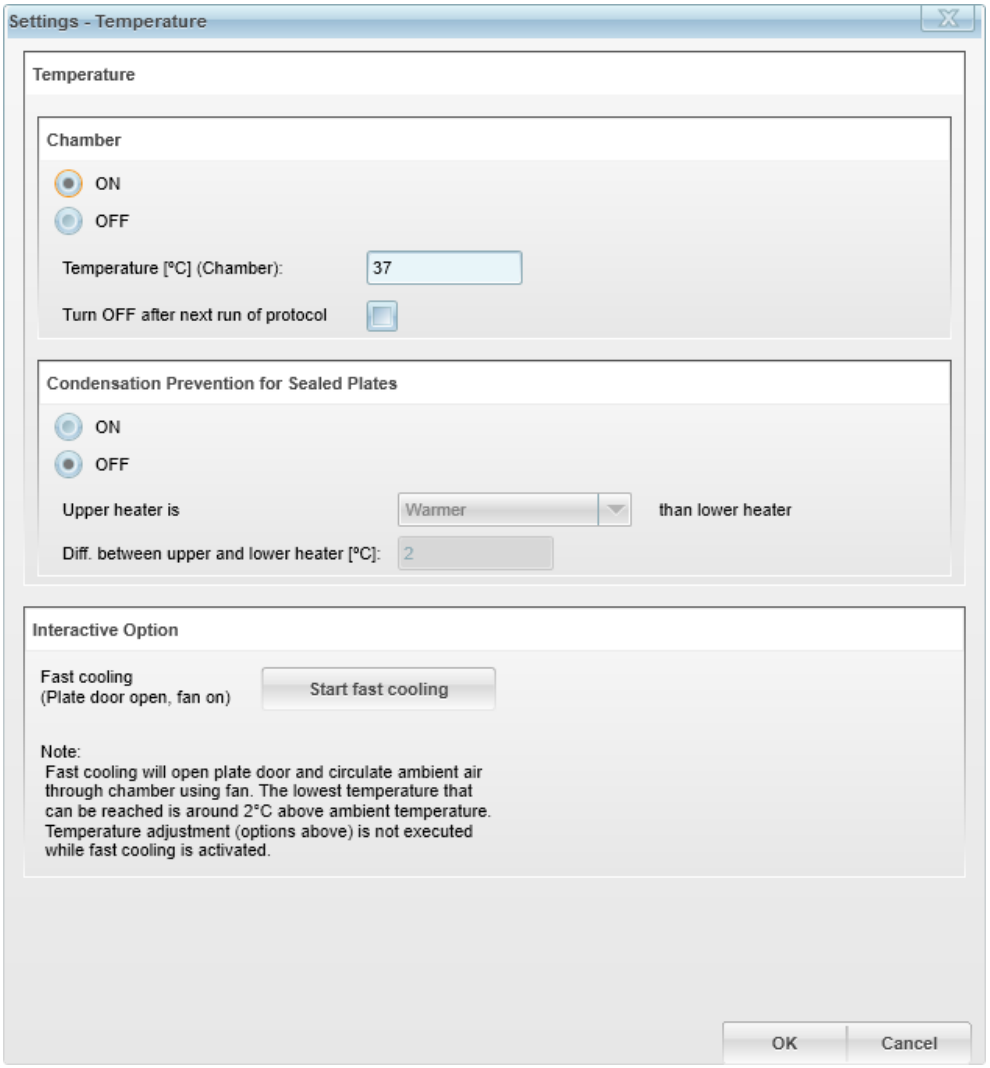
5.10.6 Temperature (Settings)

This dialog allows you adjust the general temperature control. The current status of the temperature control is displayed on the **Run Protocol** screen (see section 5.3 “Run Protocol”, page 147).

If you set up a protocol, you can add a **Temperature** operation and override these general settings temporarily, if necessary. See also section “Temperature (TEMP)”, page 106.

Example

You could use this dialog to define a general temperature and warm up the instrument. The final temperature during the measurement could be configured in the **Temperature** operation of the protocol.



Temperature – Chamber

Element	Description
ON/OFF	Turns temperature control ON or OFF. The other options in this section are only available if temperature control is ON. **Notice** When the temperature adjustment is off, the instrument does not actively control the temperature of the instrument. For example, if the instrument was heated to an elevated temperature before the temperature adjustment was turned off, the instrument will slowly start to cool down near ambient temperature.
Temperature [°C] (Chamber)	Target temperature inside the measurement chamber (value range: 15-65 °C). **Notice** The instrument cannot reach temperatures lower than the ambient temperature. • If the current chamber temperature is lower than the target temperature, the instrument will be heated up (using heating elements below and above the sample plate). • If the target temperature is lower, heating will be switched off and the instrument will cool down slowly (by normal ventilation, no additional fans).
Turn OFF after next run of protocol	Turns the temperature control off after the next protocol run has been finished. This parameter will be ignored if the instrument is in automation mode (remote control via external scheduler). In case of a stacker run, temperature control will be switched off after the last plate has been processed.

Temperature – Condensation Prevention for Sealed Plates

This function allows you to define an offset by which the temperature of the heater above the assay plate is warmer or colder than the temperature of the heater below the plate.

Keeping the upper heater at a higher temperature than the lower heater avoids the formation of condensation droplets at the underside of the seal when using a sealed plate.

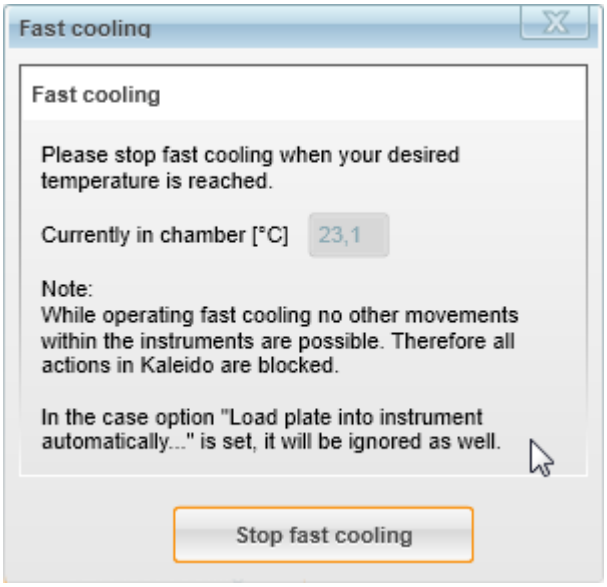
Element	Description
ON/OFF	Turns condensation prevention ON or OFF. The other options in this section are only available if the function is ON.

Element	Description
Upper heater is ... than lower heater	• **Warmer:** Increases the temperature of the upper heater by the offset entered below. • **Colder:** Reduces the temperature of the upper heater by the offset entered below.
Diff. between upper and lower heater [°C]	Temperature difference between upper and lower heater (value range: 0- 4 °C, 0.1 °C steps).

Interactive Option (Fast Cooling)

Enables faster cooling by opening the plate door and activating additional fans inside the chamber.

It is recommended to remove the plate from the plate carrier before fast cooling is activated in order to avoid unnecessary evaporation, contamination or light exposure of the samples.

Element	Description
Start Fast Cooling	If you click this button, the plate door is opened and additional fans inside the chamber are activated to cool down the chamber. Temperature control will be disabled as long as fast cooling is running. A small window is opened where the current chamber temperature is displayed:  If the desired chamber temperature has been reached, click **Stop Fast Cooling** to close the window and stop cooling. **Note:** Fast cooling does not stop automatically.

5.10.7 Stacker Settings

This dialog is only available if your instrument is equipped with an optional stacker. For further details see also section 4.13 “Stacker”, page 58.

Caution! (For stacker option only)

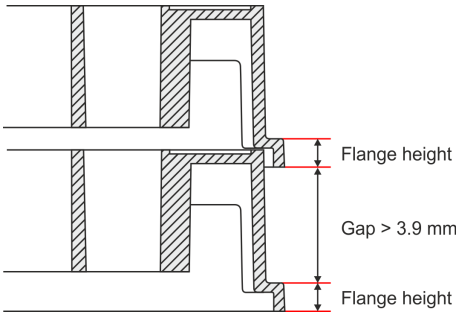
Moving parts within the sample loading area: The plate lift and the rods in the stacker will move up during operation and initialization and can cause hand injuries.

- *Keep your hands away from the sample loading area.*
- *The magazines have to be removed for loading/unloading plates.*
- *Please note that the stacker mechanics will shortly move up and down during initialization even if the magazine table is not installed and the stacker is not used.*

Flange Height

Before starting a stacker measurement or using the **Restack Plates** function you have to determine and enter the **Flange Height** of the plate type used in the protocol (see figure below).

Using this parameter and the data from the plate type definition the software can then verify if the plate type can be handled by the stacker. The size of the gap between the flanges of the stacked plates is calculated. This space is required by the holders that support the stack of plates in the magazines.



Flange height of factory-preset plate types (compatible with stacker)

Plate Name	Flange Height [mm]
96 CellCarrier	2.50
96 CellCarrier Ultra	2.00
96 GravityTRAP Plate	2.70
96 HalfAreaPlate	2.50
96 IsoPlate	6.00
96 OptiPlate	2.89
96 ProxiPlate	2.87
96 ViewPlate	6.10

Plate Name	Flange Height [mm]
96 ViewPlate (Glass)	3.20
96 Well Label Free	6.10
384 AlphaPlate	2.93
384 AlphaProxiPlate	2.76
384 CellCarrier	2.00
384 CellCarrier Ultra	2.00
384 OptiPlate	2.92
384 ProxiPlatePlus	2.75
384 ViewPlate	2.50
384 Well Label Free	6.17

Unlock Magazines

Initializes the stacker and unlocks the magazines and the magazine table.

Please use this function only if the magazines erroneously stay locked. This function is not needed for routine operation, the magazines are always unlocked automatically when a stacker run is finished or if an error occurred that requires user intervention.

Restack Plates

Restacks all plates from the left magazine (output) back to the right magazine (input). The button is only enabled if the magazine table has been attached.

This function can be useful e.g. after a measurement to restore the initial order of plates in the input magazine or to start another run processing the plates in the same order.

Notice

- Before using **Restack Plates** make sure that a protocol with the used plate type is selected and that the correct **Flange Height** has been entered (see above).
- If you run a protocol with execution type **By Plate Stack**, the plates will automatically be restacked to the loading magazine when required (see also section 5.2.1.1 “Execution Type”, page 75). This ensures that the order of the plates in the repeat measurements is the same as that during the first measurement.

How to use Restack Plates

If you use **Restack Plates** directly after a stacker run has been completed, you can just click the button and the plates will be restacked.

In all other situations (e.g. stacker not used before or different protocol loaded) you should first check the following requirements:

- Magazine table attached?
- Both magazines placed on the stacker?
- Active protocol uses the correct plate type?
  If necessary, load any protocol which uses the current plate type. This is required to determine the correct plate height.
- Flange height of used plate type entered/verified?

Click **Restack Plates**. The plates will be restacked from the left to the right magazine.

5.11 Help

Click the help icon in the **Navigation Bar** to open the online help for EnSight and Kaleido (welcome page).

5.12 MyAssays Desktop Data Analysis

5.12.1 WorkOut Plus MMD Replaced by MyAssays Desktop

The data analysis software **WorkOut Plus MMD** used to be bundled with Kaleido. It has now been replaced by **MyAssays Desktop** (Pro license).

5.12.2 Download and Installation

Please download **MyAssays Desktop** and **Folder Poll** from the MyAssays website and install it according to the manufacturer's instructions:

- https://www.myassays.com/perkinelmer-mmd (see section for Kaleido 3.0)

On this website you will find:

- Download links for software, release notes and user's guides
- Installation instructions for MyAssays Desktop and Folder Poll (including upgrade scenarios if you have been using WorkOut or a previous version of MyAssays Desktop and Folder Poll)
- Video tutorials for using MyAssays Desktop and Folder Poll with Kaleido 3.0

5.12.3 Manual Export of Kaldeido XML Files

1. On the **View Results** screen, load the measurement results which you want to analyze in MyAssays Desktop.
2. Click **Export** and save the results in XML format.
3. Import the Kaleido XML file in MyAssays Desktop:
   - Create a new MyAssays protocol (*.assay-protocol) for importing the results. In the Protocol Wizard you can optionally activate "Automatically launch this protocol when a new measurement file is detected". This will allow you to directly configure a corresponding rule for the Folder Poll Manager (see section 5.12.5 “Automated Import in MyAssays Desktop”, page 243).
   - Or use one an existing protocol which is suitable for the used measurement operation(s) and plate format.

5.12.4 Automated Export after Measurement

If you add a **Post Processing Sequence** to your protocol in Kaleido, you can automatically export the results after measurement.

1. On the **Setup Protocol** screen, create a new protocol or edit an existing one. Configure the protocol as desired.
2. Expand the **Post Processing Sequence** section.
3. Click the **[+]** button and select **Export to file** from the popup menu.
4. Configure the export settings in the **Content Area**:
   - Select export format **XML**.
   - Enter/select the desired export path.

- Enter a name for the export file or create a naming pattern using pre-defined variables.

5. **Save** the protocol.

If you run this protocol, the measurement results will automatically be exported to the specified folder in XML format. You can then import the files manually in MyAssays Desktop. Alternatively, you can configure an automated import using the **Folder Poll Manager** (see next section).

5.12.5 Automated Import in MyAssays Desktop

Folder Poll is a separately installed software tool which allows you to monitor one or multiple Kaleido output folders. As soon as a new Kaleido XML file (matching a certain naming pattern) is saved to such a folder, the result file will be opened using a pre-defined MyAssays protocol (*.assay-protocol) which has been assigned for this purpose. Depending on the MyAssays protocol, the measurement can be imported and displayed, but you could also let MyAssays Desktop automatically analyze the results and generate a report.

For a demonstration and further details see also the video tutorials linked in chapter 5.12.2 “Download and Installation”, page 242.

5.12.5.1 Kaleido Output Folders (Default)

There are two default output folders for Kaleido XML exports:

- *C:\ProgramData\PerkinElmer\Kaleido\Data Transfer**Assay development***
  This folder is intended for measurements which shall only be imported and displayed (MyAssays protocol without analysis). You can then start to analyze the data in MyAssays Desktop manually.
- *C:\ProgramData\PerkinElmer\Kaleido\Data Transfer**Screening***
  This folder is intended for measurements which shall be imported and analyzed automatically (MyAssays protocol incl. analysis).

It is suggested to use these output folders, but you can also create other folders and define corresponding rules in the **Folder Poll Manager**.

5.12.5.2 Example Protocols in Kaleido

Kaleido comes with two factory-preset protocols which demonstrate the automated data transfer to MyAssays Desktop. If MyAssays Desktop and Folder Poll have been installed and you run one of these protocols, the results will automatically be opened in MyAssays Desktop.

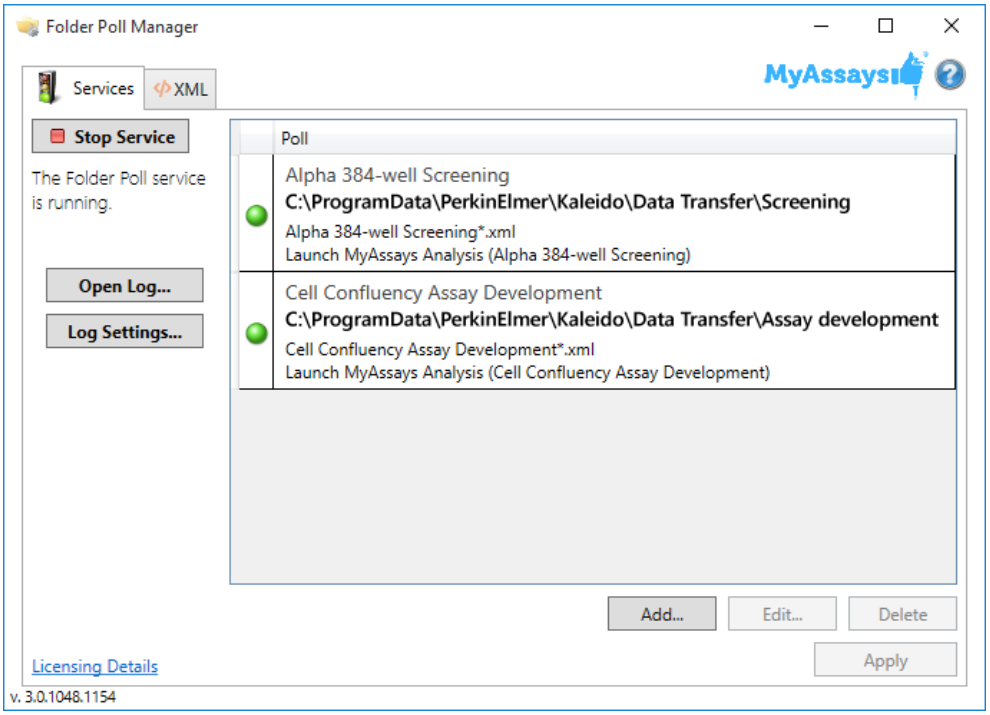
Examples

The following factory-preset protocols contain a **Post Processing Sequence** which will export the results to an export folder which is monitored by the **Folder Poll** tool. The XML files will then be imported using a pre-defined MyAssays protocol.

- *Cell Confluency Assay Development* (without analysis)
- *Alpha 384 well Screening* (with analysis)

5.12.5.3 *Folder Poll Manager*

In the default configuration of the **Folder Poll Manager**, there are two rules to monitor the Kaleido default output folders. File filters have been applied so that only export files resulting from the two factory-preset protocols (see above) will trigger the transfer to MyAssays Desktop.



You can add and configure your own folder polls to configure the automated transfer for other protocols.

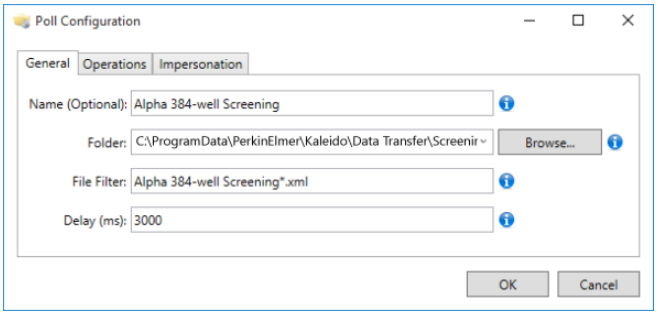
How to setup your own automated transfer to MyAssays Desktop

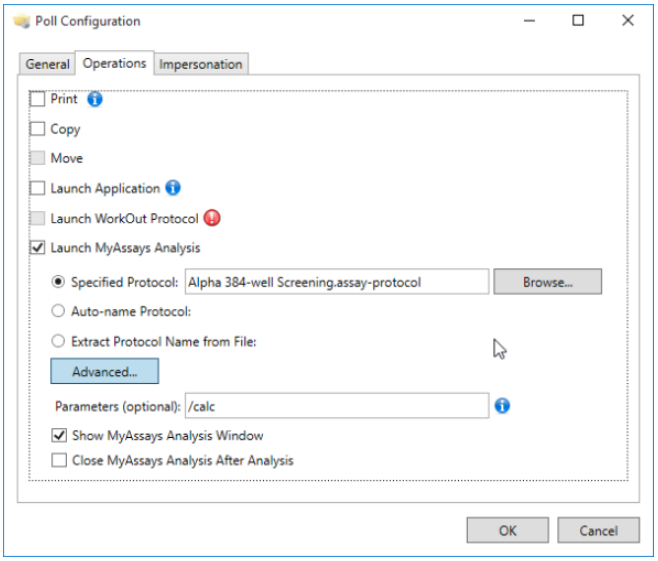
1. Add a **Post Processing Sequence** to your protocol so that an XML file is automatically exported after measurement (see section 5.12.4 “Automated Export after Measurement”, page 242).
2. Open the **Folder Poll Manager** and add a new rule for the output folder which you have specified in the **Post Processing Sequence**.

3. Configure a file filter so that only XML files resulting from suitable protocols can trigger the transfer to MyAssays Desktop, depending on the selected naming pattern for your XML file.
4. Select a MyAssays protocol (*.assay-protocol) which shall be used to import the data. This can be one of the provided default protocols or a protocol which you have already prepared and saved in MyAssays Desktop.

Notice

- Please note that the selected MyAssays protocol has to be compatible to the measurement in terms of plate format and number of measurements.
- MyAssays protocols are stored separately for each Windows® user (default configuration). If you cannot find the desired protocol, make sure to look in the correct user folder. Alternatively, you can configure a different parent directory for user files in MyAssays Desktop (File > Data), e.g. a common folder for multiple users. See MyAssays Desktop User's Guide for details.
- If the EnSight is used in automation mode (controlled by an external scheduler) and an automated transfer to MyAssays Desktop has been configured, you should activate the option **Close MyAssays Desktop After Analysis** for the corresponding poll operation in the Folder Poll Manager. Otherwise a new instance of MyAssays Desktop will be opened after each protocol run.





5. Click **OK** to add the new poll operation.

6. Click **Apply** to apply the current configuration.
7. Run your protocol in Kaleido. The measurement results will automatically be processed by MyAssays Desktop.

5.13 User Management

The Kaleido software uses the Windows user management for access control. Each Kaleido user corresponds to a local Windows user account on the Kaleido PC. Creating new users and changing their access rights is done using the corresponding Windows functions.

5.13.1 Default Users and User Groups

PC Login

For Windows® login at the device PC please use only the default user account (has admin rights):

- Windows 10: "MMD-User" (default password: "qwerty")
- Windows 7: "EnSight" (default password: "qwerty")

The Windows feature "Fast User Switching" is not supported and must remain deactivated.

Kaleido Login

The Kaleido software uses the Windows user management for access control. Each Kaleido user corresponds to a local Windows user account on the Kaleido PC. Creating new users and changing their access rights is done using the corresponding Windows functions.

During installation of Kaleido, three user groups will be created on the Windows system. Each user must be a member of one these three user groups to be able to login to Kaleido. The following default users will be available after installation:

User Name	User Group	Password (default)
Operator	Kaleido_Operators	Operator
Editor	Kaleido_Editors	Editor
Admin	Kaleido_ Administrators	Admin
PerkinElmer (for service only, do not change or delete)	*Kaleido_ Administrators*	

The default users are intended for initial login only. Please change the default passwords as soon as possible. Create user accounts for each Kaleido user and assign each user to one of the three Kaleido user groups. The default users can also be deleted, if not required anymore.

5.13.2 Creating and Modifying Kaleido Users

Creating new Kaleido users and changing their access rights is done using the corresponding Windows functions. After creating a new local Windows user, it is important to add this user to one of the three Kaleido user groups. Otherwise access to Kaleido will be denied.

How to create a new user account

1. Open **Computer Management** by clicking the **Start** button, typing **computer management** into the search box, and then pressing **Enter**.

   If you're prompted for an administrator password or confirmation, type the password or provide confirmation.
2. In the left pane of **Computer Management**, click **Local Users and Groups**.
3. Right-click the **Users** folder, and then click **New User...**.
4. Type the appropriate information in the dialog box, and then click **Create**.
5. When you are finished creating user accounts, click **Close**.

How to add a user account to a user group

1. Open **Computer Management** by clicking the **Start** button, typing **computer management** into the search box, and then pressing **Enter**.

   If you're prompted for an administrator password or confirmation, type the password or provide confirmation.
2. In the left pane of **Computer Management**, click **Local Users and Groups**.
3. Double-click the **Groups** folder.
4. Right-click one of the three Kaleido groups you want to add the user account to, and then click **Add to Group...**
5. Click **Add...**, and then type the name of the user account.
6. Click **Check Names**, click **OK**, and then click **OK** again.

5.13.3 Restricted Actions

The restricted actions of a user are defined by his user group:

Menu	Sub Menu / Action	Admin	Editor	Operator
Setup Protocol	Create New Protocol	Yes	Yes	No
	Edit + Save Protocol	Yes	Yes[1]	No
Run Protocol	Run + Continue Measurement	Yes	Yes	Yes
View Results	Load Measurement, Load Analysis Result, Export, Add Comment	Yes	Yes	Yes
	Save Analysis Result	Yes	Yes	No
	Save Protocol	Yes	Yes[1]	No

Menu	Sub Menu / Action	Admin	Editor	Operator
Analysis	Load Measurement, Load Analysis Result	Yes	Yes	Yes
	Recalc	Yes	Yes	No
	Save Protocol (changed Analysis Sequence)	Yes	Yes[1]	No
	Load Analysis Sequence	Yes	Yes	No
Settings				
Instrument Options	View options	Yes	Yes	Yes
General Settings	All settings	Yes	Yes	Yes
Data Management	Backup Database	Yes	Yes	Yes
	Restore	Yes	No	No
	Import Protocol/Meas.	Yes	Yes	No
	Database Info	Yes	Yes	Yes
	Scheduled Task	Yes	No	No
	Archive Images	Yes	No	No
	Delete Data (user-defined protocols, plate types and measurements)	Yes	Yes[2]	No
Barcode Reader	All settings	Yes	Yes	Yes
Inventory	Filter Wheel	Yes	Yes	Yes
	PlateType (Create)	Yes	Yes	No
	Filter (Create)	Yes	Yes	No
Temperature	All settings	Yes	Yes	Yes
Stacker	All settings	Yes	Yes	Yes

[1] Only allowed for the user's own protocols. Protocols from other users can be edited, but they can only be saved using the option "Save as ..." (i.e. copied).

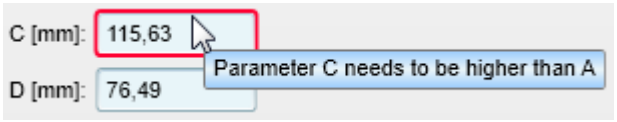
[2] Only allowed for the user's own protocols, measurements or plate types. Database objects from other users cannot be deleted.

5.14 Troubleshooting

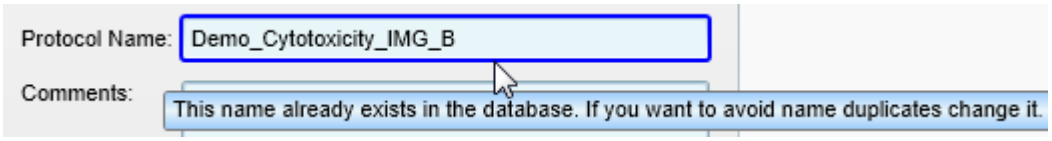
5.14.1 Feedback via Tooltips

Data entered into the input boxes of the user interface is checked for plausibility (if possible) and errors or instructions are indicated by a colored border:

- **Red outline:** The entered data is incorrect (e.g. parameter value out of range, not allowed characters or logical error detected). A tooltip explains how to enter the data correctly. Please correct the entry before proceeding.



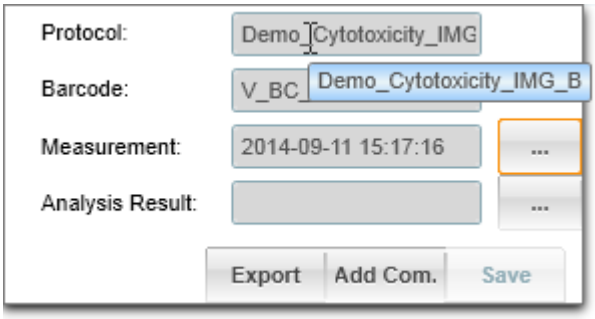
- **Blue outline:** A tooltip is available explaining the behavior of the user interface and giving helpful instructions.



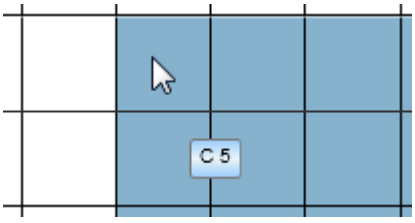
All other errors will be reported on the **Notifications** tab (**Messages** area). For details see section 5.9.3 “Notifications”, page 218.

Tooltips are also displayed in the following situations:

- Display of full object names (e.g. of loaded protocol, measurement etc.) in all **Global Control** sections.
  This is useful if the name is too long to be displayed completely in the corresponding text box.



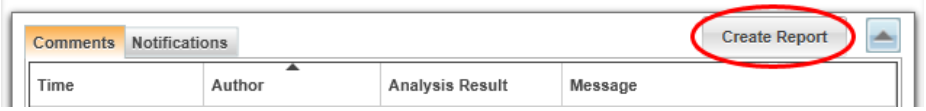
- Display of well coordinates on the **Plate** tab.



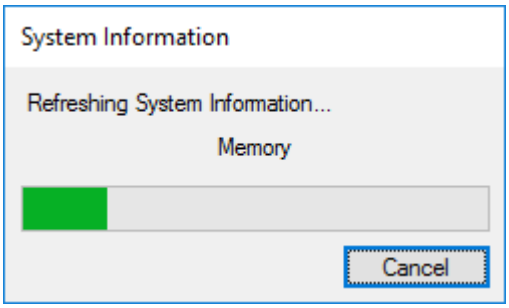
5.14.2 Reporting Technical Issues

If you experience technical issues, please use the **Create Report** function to generate a report file containing all relevant log files and system information. Send this file to PerkinElmer Service, together with a description of the issue.

1. In the **Messages** section click **Create Report**.



The log files and system information are collected.



All data is automatically compressed to a ZIP file and saved on the Windows® Desktop. Naming pattern of the report file:

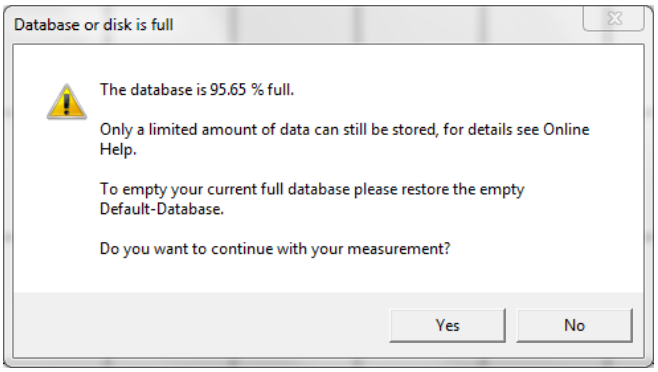
- *Report_[Serialnumber]_[Timestamp].zip*

2. Send the report file and a description of the issue via email to PerkinElmer Service.

Notice
The log files may contain sensitive data (e.g. user names and text entered as object name or comment). PerkinElmer will, of course, treat your data confidentially and only use it for solving your issue. If you do not want to share certain pieces of information, please remove them manually before sending the report file to PerkinElmer.

5.14.3 Database or disk is full

If you try to start a measurement and the database is > 90 % full, the following warning will be displayed:



With a database fill level of 90 % you can still store a few normal measurements. However, it is not recommended to start e.g. a stacker run with 50 plates. In this case, data may be lost if the space is not sufficient.

- Click **Yes** to continue the measurement.
- Click **No** to cancel the measurement.

To empty your current full database, you can restore the empty default database which is provided on the Kaleido USB drive:

- *<USB drive>:\Installation\FactoryDefaultDatabase.bak*

See also section 5.10.3.2 “Restore Database”, page 222.

Notice
If you restore the default database, it will replace your current database, i.e. all data will be lost!

5.14.4 Reference value of Xenon flash lamp

What is the "initial reference value"?

All technologies which make use of the Xenon flash lamp (FI, TRF, ABS) have to compensate the ageing of the flash lamp. This is done by correcting the results with the signal of a reference photodiode after each flash.

- When a protocol is measured for the first time, the **"initial reference value"** is measured and saved with the protocol.
- For all subsequent measurements of this protocol, the reference signal is then compared to the initial reference value taken during the first measurement of the protocol. The results are corrected for the same excitation energy.
- The initial reference value is reset as soon as you edit the protocol (excitation/emission wavelength or number of flashes) or just save it under a different name (**Save as ...**).

Notice

If the **initial reference value** of a protocol is not measured correctly, this will falsify the results of all subsequent measurements of this protocol.

If you get an error message (see below) during the first measurement of such a protocol, reset the initial reference value of the protocol. This is triggered by saving a copy of the protocol with **Save as ...** and using the new copy. It is recommended to delete the old protocol to avoid confusion.

Related error messages

- *"The reference value of the flash unit for operation <XYZ> , well <XY> is out of range."*
- *"Well <XY>. Operation <XYZ>. FlashUnit failure, data not valid."*
- *"An error occurred during your measurement, please note that this may have distorted your data."*

Possible Reason	Solution
Xenon flash lamp is not functioning properly.	Repeat the measurement as the issue may be transient. If it persists, please see below.
Initial reference value of the protocol was not measured correctly.	Reset the initial reference value of the protocol. This is triggered by saving a copy of the protocol with **Save as ...**and using the new copy. It is recommended to delete the old protocol to avoid confusion.

If the problem still persists, please contact PerkinElmer Service.

5.14.5 Autofocus Failure (IMG)

Error message

"Cannot find autofocus for several wells [...]." (Imaging measurement)

Possible Reason	Solution
No plate in the instrument.	Load the sample plate and start the measurement again.
Plate type defined in the protocol and actual plate type of the sample plate do not match.	Edit the protocol and change the **Plate Type** to the actual plate type of your sample plate. Alternatively, use a different sample plate which has the same plate type as selected in the protocol.

Possible Reason	Solution
Plate type definition is wrong.	Check the properties of the selected plate type in the Plate Type wizard (see section 5.10.5.2 “Plate Type”, page 234), especially the values of these parameters: • (F) Bottom height • (G) Bottom thickness • Optical index You cannot edit a plate type. If the values need to be corrected, please create a new plate type and delete the old one.

If the problem still persists, please contact PerkinElmer Service.

5.14.6 Replacing Stops in Automation Mode

Protocols to be measured in **Automation** mode may not contain **Stop** operations. If you need to interrupt the measurement at certain positions in the protocol, you can use the following workaround:

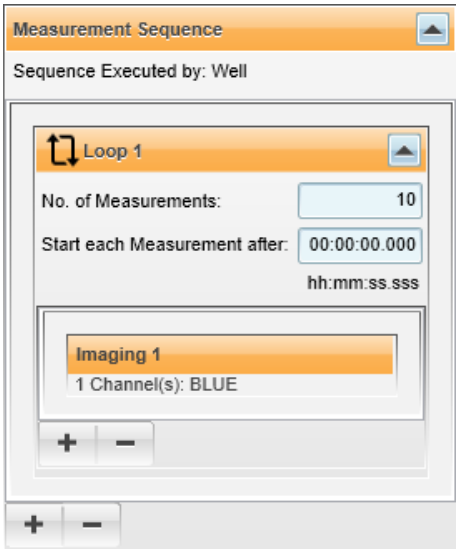
1. Split up your protocol into multiple steps and create a separate protocol for each step.
2. Let your scheduler send these separate protocols as required, e.g. combined with other processes or interruptions.

The only drawback is that you will get individual results for each separate measurement. You will have to use third-party software to bring the results together. This cannot be done in Kaleido.

5.14.7 Unsharp Images Due to Temperature Changes

Issue

The following combination is prone to unsharp images due to a drift of the focus position:



- Execution type: **By Well**
- **Loop** with **Imaging** operation
  or
  multiple **Imaging** operations (copies) in direct succession
- Temperature control activated or temperature difference between plate and instrument

With execution type **By Well** the focus position is only detected once per well at the first **Imaging** measurement. All further repeats of the **Imaging** operation will be measured without new focus scan, because there is no plate movement until the next well is processed.

If the temperature is not constant during this time, the first image in the well will be sharp, but then the focus position will start to drift due to the plate deformation so that the images get more and more unsharp.

How to avoid this

- Make sure that instrument and plate have the same constant temperature.
- Let the instrument temperature stabilize before starting measurements.
- If necessary, let the plate warm up inside the instrument until both have the same temperature.

6 Retooling and Maintenance

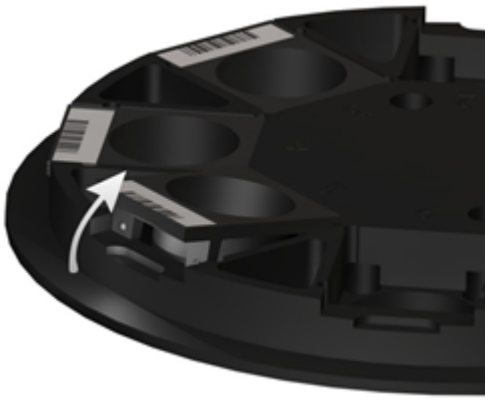
Retooling and maintenance procedures described in this chapter may be carried out by the user. Repair and service may only be performed by the PerkinElmer Service.

6.1 Change Excitation Filters

The excitation filters in the filter wheel can easily be changed.

1. Pull down the handle of the filter wheel holder on the right side of the instrument.

2. Take out the filter wheel.
3. Remove any filter from the wheel that is not needed.



4. Insert the required filters into the wheel.
5. Insert the filter wheel into the holder.
6. Lift the handle to move the filter wheel into operating position.

   The instrument will read the filter barcodes and identify the filters. The new filter configuration is displayed in the **Settings – Inventory – Filter Wheel** window in Kaleido. See also section 5.10.5.1 “Filter Wheel”, page 234.

6.2 Cleaning

Warning!

Ethanol or other cleaning solvents may cause fire or injury if used in great amounts.

- *Observe the cleaning instructions.*
- *Keep the concentration of dangerous solvents as low as possible.*
- *Do not use organic solvents.*
- *Ensure the area is well ventilated.*

Cleaning the instrument (outside)

- For cleaning the outer surface of the EnSight use only a slightly moistened, damp cloth. Do not use any aggressive detergents or alcohol.

Cleaning the plate carrier

- The plate carrier should be kept clean to avoid dust and dirt entering into the optics at the measuring position. This could increase the measurement background of the system especially if wavelengths in the ultraviolet region are used.
- At least once a week the plate carrier should be cleaned using a soft cloth or tissue paper soaked in a mild detergent solution or alcohol.

Cleaning optical filters

- Filters should be free of finger prints. Finger prints on filters should be removed with ethanol or iso-propanol on microfiber cloth.

For cleaning the workstation inside, the operator must specify suitable precautions, especially with regard to biological contaminated and infectious materials. The instrument has to be decontaminated before any service visit.

6.3 Replacing a Fuse

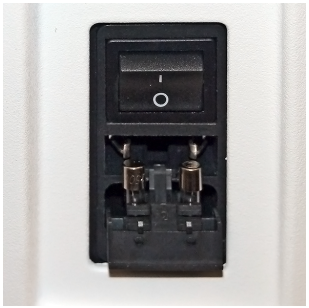
If one of the two fuses of the EnSight has blown, it can be exchanged by the user.

Danger!

Direct electrical contact – Electrical shock.

- *Unplug from mains before replacing fuses.*
- *For continued protection against risk of fire, replace only with certified fuse of same type and rating: T4.0 H / 250 V (T = time delay; 4.0 = 4 Ampere; H = high breaking capacity).*

1. Switch off the EnSight and unplug the power cable.
2. Pull out the fuse holder (below power switch).



3. Replace the blown fuse(s).
4. Close the fuse holder and push it into its former position.
5. Reconnect the power cable.

6.4 Changing Air Filters

Caution!

Covered ventilation openings may cause malfunctions or damage to the equipment.

- *Make sure that none of the ventilation openings of the instrument (in the back and bottom) are obstructed.*
- *Keep a distance of at least 5 cm (2 in) to walls and other objects.*

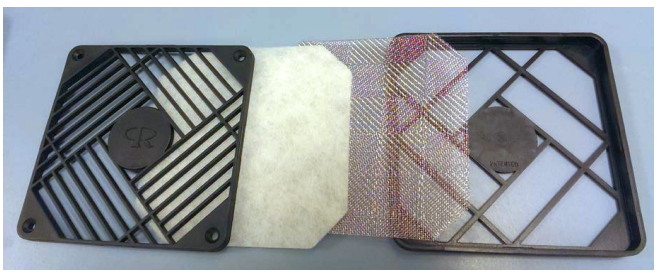
There are two types of air filters in the EnSight:

- **Rear air filters** (exhaust air)
  These filters are not accessible for the user. Please let PerkinElmer Service check the filters once a year.
- **Central air filters** (fresh air)
  These filters can be cleaned or changed by the user (see below). Please contact PerkinElmer Service to request new filters.
  Recommended interval: once a year

Cleaning/changing the central air filters

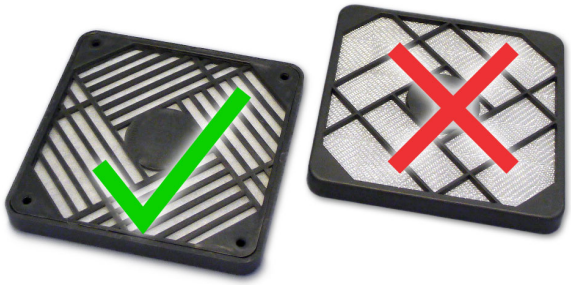
1. Remove the magnetic cover on the right-hand side of the instrument's base plate.
2. Pull out the two filter cartridges.

3. Take each filter cartridge apart.



4. Carefully wash out the dust from the white filter material with water (or replace the filter fleece if necessary).
5. Dry it thoroughly, e.g. between paper towels.

6. Put the cartridges back together and slide them back into place.
   Make sure that the correct side is facing up (as shown below).



7. Mount the magnetic cover of the base plate.

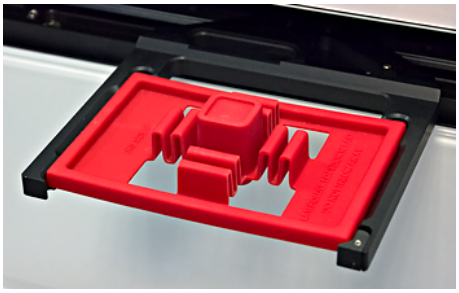
6.5 Transport

Warning!

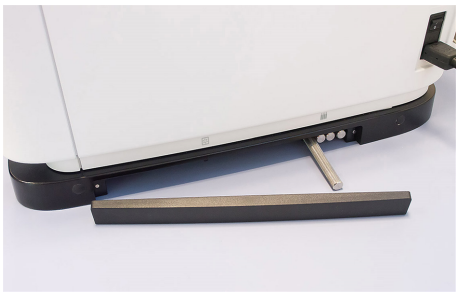
The instrument is heavy (75 kg / 165 lb) and the glass plate at the top is not fixed.

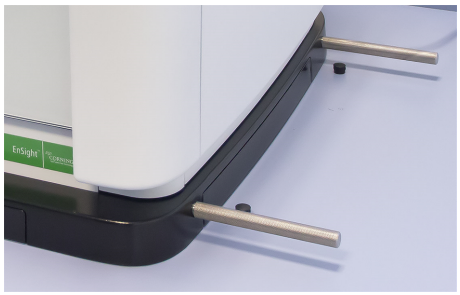
- *At least two persons are required to transport the instrument.*
- *Do not try to lift or transport the instrument without all transport handles mounted correctly.*
- *All four transport handles have to be screwed in completely. Do not use inadequate force or tools for this manual process, otherwise the handle threads may be damaged.*
- *Keep the instrument in upright position, otherwise the glass plate could fall down.*
- *An instrument with optional stacker is heavier (+9 kg, without magazine table and magazines) and the instrument's center of gravity is closer towards the front.*

1. *With optional stacker only:* Lift off the magazines and remove the magazine table. See also section 4.13.4 "Manual Plate Loading", page 61.
2. Prepare the instrument for transport using the corresponding software tool (*Windows® Start Menu – PerkinElmer – Kaleido 3.0 – Prepare for Transport*). Follow the instructions on screen and load the transport plate.



3. Remove the magnetic cover of the instrument's base plate (right-hand side).





4. Pull the handles out of the storage.
5. Remove the caps of the threaded holes on both sides of the base plate.
6. Screw the four handles completely into the threaded holes (see right figure).
7. Lift the instrument carefully using the four handles (at least 2 persons required) and bear in mind the decentralized center of gravity.

8. After moving the instrument, unscrew the handles, put them back into the storage and mount all caps and the magnetic cover.
9. When you start the instrument for the first time after the transport, click **Eject** in the Kaleido software and remove the transport plate.

7 Specifications

7.1 Environmental Conditions

- Indoor use only
- Altitude: up to 2000 m
- Operating conditions:
  +15 °C to +30 °C, relative humidity 10 - 80%
- Operating conditions for Alpha technology:
  +20°C to +25°C, relative humidity < 80%
- Operating conditions for Label-free technology:
  23 °C ± 3 °C, relative humidity < 70 %, non-condensing

7.2 Power Requirements

- Mains voltage: 100-240 V, 50/60 Hz
- Power consumption: Max. 300 VA

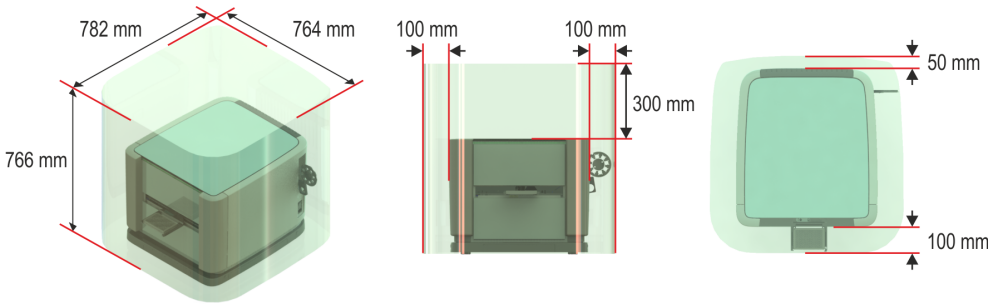
7.3 Physical Dimensions

Component	Width	Depth	Height	Weight
EnSight	564 mm (22.2 in)	632 mm (24.9 in)	Height: 466 mm (18.3 in) Clear height above: min. 300 mm (11.8 in)	75 kg (165 lb)
EnSight with optional stacker	564 mm (22.2 in)	770 mm (30.3 in)	Total height incl. magazines for: • 20 plates: 649 mm (25.6 in) • 50 plates: 1047 mm (41.3 in) Clear height above: min. 120 mm (4.7 in)	85 kg (188 lb) + magazines (empty): 2.2 kg (for 20 plates) or 4.3 kg (for 50 plates)
PC Mini Tower	154 mm (6.1 in)	274 mm (10.8 in)	350 mm (13.8 in)	10 kg (22 lb)
Monitor 24”	550 mm (21.7 in)	180 mm (7.1 in)	up to 492 mm (19.4 in)	8 kg (18 lb)

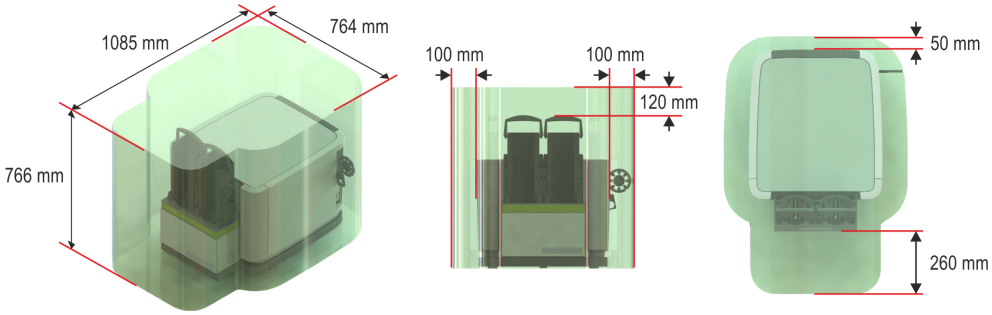
During normal operation and especially during service visits additional space is required (see illustrations below).

Required space overall and minimum distances to other objects

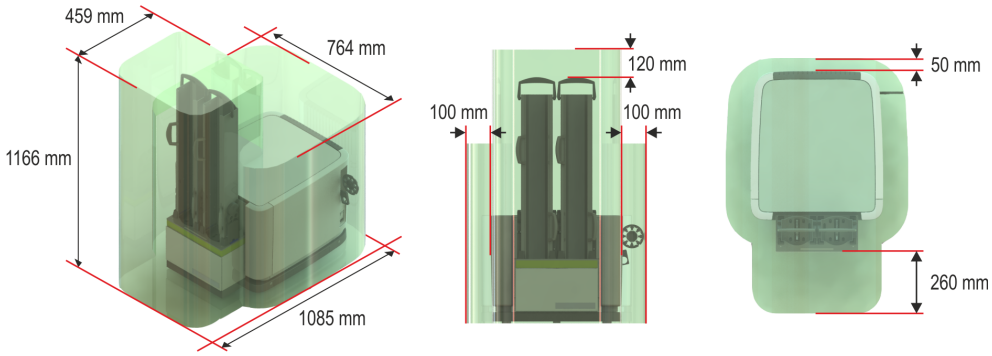
The minimum distance between the back of the instrument (ventilation outlets) and other objects (e.g. walls) should be 50 mm (2 in).



EnSight without stacker



EnSight with stacker (magazines for 20 plates)



EnSight with stacker (magazines for 50 plates)

7.4 Input and Output Connections

- USB: Universal Serial Bus Type B (connection to control PC, cable length 3 m max.)
- CAN: 9-pin D-sub, male (CAN Out)
- FireWire: 2x cables with FireWire 800 (1394b); for instrument with Imaging option only

7.5 Control PC

The PC running the Kaleido software and controlling the instrument is equipped as follows (minimum configuration):

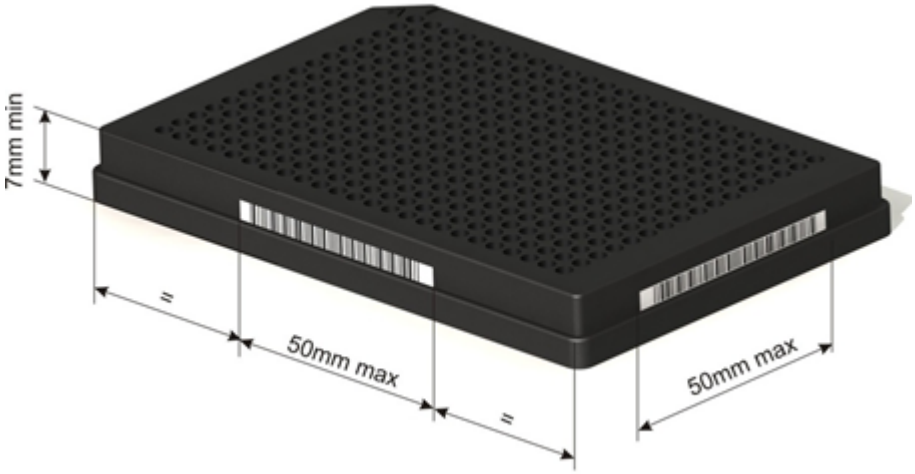
Component	Description
Operating system	Windows® 10 IoT Enterprise 2015/2019 LTSB, 64 bit, English or Windows® 7, SP1, 64 bit, English (other operation systems are not supported)
CPU	≥ Intel® Core™ i5
RAM	≥ 8 GB RAM
Harddisk	≥ 1 TB HDD
Expansion slot	PCIe x16 (full height)
Monitor	≥ 24“

7.6 Plates

6, 12, 24, 48, 96 and 384-well plates are compatible with the instrument. The maximum outer dimensions are 86.0 x 128.2 x 28.0 mm (SBS standard). For detailed requirements and plate compatibilities for each EnSight technology please see section 4.11 “Plates”, page 51.

7.7 Plate Barcode

Property	Value
Barcode length	Max. 50 mm, 6-20 characters
Barcode height	Min. 5 mm
Empty space at the ends of barcode label	Min. 10 mm
Minimum bar width	Min. 0.25 mm
Bar-space ratio	1/3
Label material	Non-fluorescent
Code types (variable number of digits, no check digit)	• CODE39 • INTERLEAVED 2/5 • CODABAR • CODE128
Barcode reading	From the left side of a microplate (with one barcode reader) Optional: From any of the four sides of a microplate (with four barcode readers)



Correct barcode positioning

7.8 Light Sources

The flash light source used for most measurement technologies is a UV Xenon flash tube, spectral range 230-1000 nm. Further light sources are available if your instrument is equipped with one of the following optional technologies.

Technology	Light source
Alpha	• Semiconductor laser diode • Wavelength: 680 nm • Nominal output power: 400 mW • Laser class 3B
Label-free	• Superluminescent diode • Wavelength: 832 nm • Nominal output power: 0.6 mW • Laser class 3R
Imaging	For fluorescence excitation: • 3 or 4 different high power LEDs • Wavelengths: 385 / 465 / (525) / 632 nm
	For transmission (brightfield) and digital phase imaging: • LED • Wavelength: 735 nm
	Autofocus laser: • Diode laser • Wavelength: 850 nm • Nominal output power: 10 mW • Laser class 3B

7.9 Detection Units

Technology	Detector
Absorbance	• Photodiode • Detection range: 230-1000 nm
Fluorescence Intensity (Top/Bottom)	• Photomultiplier tube (in accordance of the monochromator) • Detection range: 230-850 nm
Alpha, Luminescence	• Photomultiplier tube • Detection range: 400-650 nm • Very high sensitivity
Time-resolved Fluorescence	• Photomultiplier tube (in accordance of the monochromator) • Detection range: 230-850 nm
Label-free	• Optical biosensor (waveguide resonant grating) • System variability: 5 pm
Imaging	• sCMOS camera • Image size: 1920 x 1440 px • Resolution: ~3.3 µm per pixel • 4x objective lens

7.10 Measurement Directions

Technology	Excitation	Detection
Fluorescence Intensity (FI Top)	Top	Top
Fluorescence Intensity (FI Bottom)	Bottom	Bottom
Absorbance	Top	Bottom
Alpha	Top	Top
Luminescence	---	Top
Time-resolved Fluorescence (TRF Top)	Top	Top
Time-resolved Fluorescence (TRF Bottom)	Bottom	Bottom
Label-free	Bottom	Bottom
Imaging (fluorescence excitation)	Bottom	Bottom
Imaging (transmission/brightfield)	Top	Bottom

7.11 Temperature Control

- Maximum temperature: 65 °C
- Minimum temperature: ambient temperature + 2 °C (heating only)
- Temperature accuracy:

- Target = 15 – 50 °C → ± 1 °C
- Target = 51 – 60 °C → ± 2 °C
- Target = 61 – 65 °C → ± 3 °C

7.12 Plate Shaking

- Plate shaking modes: linear, orbital, double orbital
- Three speed levels
- Adjustable amplitude
- Available for all technologies

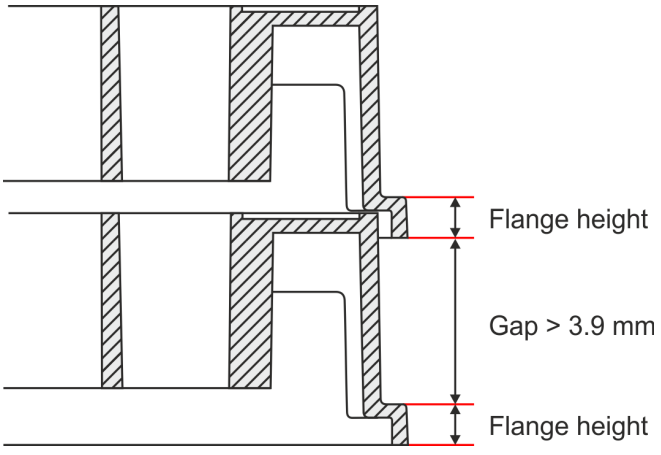
7.13 Scanning

Scanning of wells (several measuring points within a well) are available for most technologies.

7.14 Stacker

The stacker can hold magazines for 20 or for 50 plates. All plates used within one protocol must be of the same plate type. They have to fulfill the following additional requirements:

- Plate height: 7 – 28 mm
- No lids
- Maximum load: 5.2 kg
- Gap between the top of a flange and the bottom of a plate stacked above it: > 3.9 mm



Stacked plates

Before starting a measurement using the stacker, the flange height of the used plate type has to be entered in the **Settings** dialog in Kaleido (see section 5.10.7 “Stacker Settings”, page 238).

For detailed plate requirements see section 4.11 “Plates”, page 51.

7.15 Application Wavelengths

Technology	Excitation [nm]	Emission [nm]	
Absorbance	230-1000	–	
Alpha	680	450-645	
Fluorescence Intensity	230-835	245-850	
Label-free	832	–	
Luminescence	–	450-645	
TRF Top TRF Bottom	230-385 230-835	400-850 245-850	
Imaging		PIMARY dichroic filter set	SECONDARY dichroic filter set (opt.)
	385	413-443	–
	385 – extended	–	413-498
	465	499-604	–
	525	–	555-604
	632	664-750	664-750
	735 (Brightfield)		

7.16 Performance

7.16.1 Photometric Performance With Monochromators

- Wavelength range: 230-1000 nm
- Wavelength selection: monochromator, tunable in 0.5 nm increments
- Photometric resolution: 0.001 OD

7.16.2 Fluorescence Intensity Performance With Monochromators

- Wavelength selection: monochromators, tunable in 0.5 nm increments

7.16.3 Maximum Throughput (Time Per Plate)

These values represent the maximum throughput under ideal conditions. Before starting real assay measurements, perform a test measurement to determine the realistic measurement time for your assay.

Technology Overview

Technology	96-well	384-well
Well Imaging	1 min 30 sec	4 min
Label-free	55 sec	1 min 30 sec
Alpha	27 sec	1 min 35 sec
Fluorescence	23 sec	1 min 14 sec
Time-resolved fluorescence	25 sec	1 min 25 sec
Absorbance standard mode	22 sec	1 min 15 sec
Absorbance on-the-fly	7 sec	14 sec
Luminescence	35 sec	1 min 40 sec

Label-free

Plate format	Quadrants	BCA	CBA
384-well	4	≥ 160 sec	≥ 88sec
	3	≥ 120 sec	≥ 66 sec
	2	≥ 80 sec	≥ 44 sec
	1	≥ 40 sec	≥ 22 sec
96-well	4	≥ 80 sec	≥ 48 sec
	3	≥ 60 sec	≥ 36 sec
	2	≥ 40 sec	≥ 24 sec
	1	≥ 20	≥ 12 sec

8 IT Policy

This chapter contains policies and general information about the IT infrastructure and rules for integration of the EnSight System into any network environment.

Notice

The purpose of the EnSight IT Policy is to ensure the effective protection and proper usage of the computer systems belonging to the “EnSight System”. The IT Policy will assist in maintaining systems at operational level. Contraventions of the IT Policy could seriously disrupt the operation of the “EnSight System” and could involve PerkinElmer support billable at the current Service rate.

Notice

PerkinElmer is not responsible for problems caused by violating the following policies. Any effort required to verify this type of problem is billable at the current service rate and is not covered by guarantee and/or service contract.

8.1 EnSight PC Configuration

Notice

Due to numerous differences in PC hardware, PerkinElmer cannot guarantee that our software will run on a computer supplied by the customer, even if the system meets the minimum specifications described below. PerkinElmer installation of a computer supplied by the customer is available for an additional fee. PerkinElmer is not responsible for problems caused by unspecified system components, software, and/or accessories.
Any effort required to verify this type of problem is billable at the current service rate. PerkinElmer may not provide maintenance service on the computers supplied by the customer.

The PC running the Kaleido software and controlling the instrument is equipped as follows (minimum configuration):

Component	Description
Operating system	Windows® 10 IoT Enterprise 2015/2019 LTSB, 64 bit, English or Windows® 7, SP1, 64 bit, English (other operation systems are not supported)
CPU	≥ Intel® Core™ i5
RAM	≥ 8 GB RAM
Harddisk	≥ 1 TB HDD
Expansion slot	PCIe x16 (full height)
Monitor	≥ 24“

8.2 Network

The EnSight PC can receive the network address from a DHCP server. The general configuration is to obtain the IP address automatically from a DHCP.

1. The usage of any additional network adapter of any computer of the “EnSight System” is not supported.
2. PerkinElmer recommends a Gigabit network connection.
3. If you add the EnSight PC to a domain, there could be side effects to the Kaleido software. Since domain policies are complex and can have very far-reaching implications, we cannot guarantee that the system will work properly in every case.

8.3 Hardware

Requirements for new PC hardware should be discussed in advance with PerkinElmer Service to assess the detailed specification. Problems with hardware should also be reported to PerkinElmer Service.

- Modification of any hardware of the EnSight PC is not supported.

8.4 Operating System, Software & Software Applications

Problems with software should be reported to the PerkinElmer Service.

1. Supported operating system:
   - Microsoft Windows® 10 IoT Enterprise 2015/2019 LTSB, 64 bit, English or Microsoft Windows® 7, SP1, 64 bit, English
   - 32 bit operating systems are generally not supported.
   - The 64 bit versions of Windows® 8, Windows Vista® and Windows® XP are not supported.
   - Using any virtual PC (e.g. VMWare, Virtual-PC) is not supported.
2. Required region and language settings:
   - Windows® 10:
     - *Control Panel – "Language":*
       Windows Display Language (enabled): English (United States)
     - *Control Panel – "Region":*
       Format: English (United States) or select "Match Windows display language"
   - Windows® 7: *Control Panel – "Region and Language"*
     - Format: English (United States)
     - Display Language (if multiple languages installed): English (United States)
3. Deactivating the virtual memory for Microsoft Windows® is not allowed.

8.5 Security Updates / Servicing Channels

8.5.1 Microsoft Windows

1. The Kaleido software was tested and released with:
   - Microsoft Windows® 10 IoT Enterprise 2015/2019 LTSB, 64 bit
   - Microsoft Windows® 7, Service Pack 1, 64 bit

   This includes all security patches until the Kaleido release date (see Release Notes).
2. Set Windows Update settings to “Never check for updates” or “Check for updates but let me choose whether to download and install them” because they may disturb the “EnSight System”.
3. Install only "important updates", not "optional" updates or "preview" updates.

Notice

PerkinElmer cannot guarantee that future Windows updates provided by Microsoft will not compromise the stability of the “EnSight System”. We assume that the impact is low but to ensure to always have a stable “EnSight System” we recommend that you …

- disable Windows Server Update Services (WSUS), if applicable,

  or

- disable internet access temporarily during screening campaigns to avoid unattended installation of updates during a screening run.

8.5.2 Microsoft Windows 10 IoT Enterprise 2015/2019 LTSB

The operating system of the provided device PC is **Microsoft Windows® 10 IoT Enterprise 2015/2019 LTSB**.

- This version will receive regular **security updates** (supported until October 2025/January 2029).
- It will skip any **feature updates** to ensure stability.
- Do not change the preset Servicing Channel (LTSC/LTSB). This will not be supported by PerkinElmer.

For further details see the FAQ below.

What is Windows 10 IoT Enterprise?

As a part of the entire EnSight system the device PC uses a Windows® 10 IoT Enterprise license. This is a full version of Windows® 10 that delivers enterprise manageability and security to IoT (internet of things) solutions. Windows® 10 IoT Enterprise is a binary equivalent to Windows® 10 Enterprise, so you can use the same familiar development and management tools as client PCs and laptops.

- https://docs.microsoft.com/en-us/windows/iot-core/windows-iot-enterprise

What is Long-term Servicing Channel (LTSC)?

For an MMD device it makes more sense that it is kept stable and secure than kept up to date with user interface changes.

- The LTSC servicing model prevents Windows® 10 Enterprise LTSB* devices from receiving the usual feature updates and provides only quality updates to ensure that device security stays up to date.

  * LTSB = Long-term Servicing Branch (recently renamed to LTSC, but still present in the name of previous releases)

- https://docs.microsoft.com/en-us/windows/deployment/update/waas-overview

How to get security updates?

- If the device PC is connected to the internet, you will be notified when new security updates are ready to be downloaded. After going to Windows Update, you can download and install the update.
- Updates which require a restart will not take effect until the device PC is restarted manually. Please check regularly for corresponding notifications and restart the PC.
- If the update behavior is not as described above, please open the Group Policy Editor (run "gpedit.msc"), verify the update settings and reset to the configuration below, if necessary. Administrator rights are required for this step. The "Windows Update" section can be found in this branch: "Computer Configuration – Administrative Templates – Windows Components – Windows Update".
  - "Configure Automatic Updates": Enabled + option 2 selected ("2 – Notify for download and notify for install")
  - "No auto-restart with logged on users for scheduled automatic updates installations": Enabled

Can I upgrade to a new LTSC release to get new features?

No, the license is only valid exactly for Microsoft Windows® 10 IoT Enterprise 2015/2019 LTSB.

8.6 Security Settings & Anti-Virus Protection

The system is delivered without anti-virus software installed. The Windows® Firewall is activated. The Kaleido software is tested with Symantec Endpoint Protection.

1. PerkinElmer is not responsible for the implementation of an effective virus security strategy. It is suggested to exclude the following folders (incl. subfolders) from any scan:
   - C:\Program Files\PerkinElmer
   - C:\Program Files\Microsoft SQL Server
   - C:\ProgramData\PerkinElmer
2. Please ensure that the “InstrumentHost.exe” is not blocked by any firewall

(*C:\Program Files\PerkinElmer\Kaleido 3.0\Bin*). For this program you have to enable:

- Protocols TCP & UDP
- Access to any local address
- Access to any port

3. In the unexpected case of returning a PC back to PerkinElmer for trouble shooting any security software like virus scanner or firewall has to be removed before shipping and the current Windows® admin password has to be provided.

8.7 Data

1. PerkinElmer is not liable for any data loss due to data management processes like backups, etc.
2. To avoid that the hard disk space on the EnSight PC is quickly exhausted, we recommend to set the “Archive folder” to a different location (server, external USB hard disk etc.) or to disable the archiving option for images in Kaleido.
3. Images should only be archived to reliable servers or external hard disks which are integrated into the customer's backup system.

8.8 Backup

1. PerkinElmer is not responsible for the implementation of an effective backup strategy or for the backup of any files from the “EnSight System”.
2. PerkinElmer recommends to back up all data on the EnSight PC on a regular basis using third-party backup software.

8.9 Default Users and User Groups

PC Login

For Windows® login at the device PC please use only the default user account (has admin rights):

- Windows 10: "MMD-User" (default password: "qwerty")
- Windows 7: "EnSight" (default password: "qwerty")

The Windows feature "Fast User Switching" is not supported and must remain deactivated.

Kaleido Login

The Kaleido software uses the Windows user management for access control. Each Kaleido user corresponds to a local Windows user account on the Kaleido PC. Creating new users and changing their access rights is done using the corresponding Windows functions.

During installation of Kaleido, three user groups will be created on the Windows system. Each user must be a member of one these three user groups to be able to login to Kaleido. The following default users will be available after installation:

User Name	User Group	Password (default)
Operator	Kaleido_Operators	Operator
Editor	Kaleido_Editors	Editor
Admin	Kaleido_ Administrators	Admin
PerkinElmer (for service only, do not change or delete)	*Kaleido_ Administrators*	

The default users are intended for initial login only. Please change the default passwords as soon as possible. Create user accounts for each Kaleido user and assign each user to one of the three Kaleido user groups. The default users can also be deleted, if not required anymore.

8.10 Remote Support

PerkinElmer uses the service of LogMeIn (https://secure.logmeinrescue.com/) to solve issues of “EnSight Systems” by remote support. If LogMeIn support is not allowed please contact PerkinElmer Service and ask for other options. Please note: Other options may increase costs for the service contract.

Minimum system requirement for LogMeIn remote support:

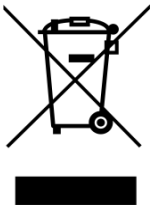
- Broadband connectivity to the internet (i.e. T1 or DSL)

9 Compliance

9.1 Protection Against Harmful Interference

This equipment has been tested and found to comply with the limits for a **Class A** digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate frequency energy and, if not installed and used in accordance with the provided manuals, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

9.2 WEEE Instructions



A label with a crossed-out wheeled bin symbol and a rectangular bar indicates that the product is covered by the Waste Electrical and Electronic Equipment (WEEE) Directive and is not to be disposed of as unsorted municipal waste. Any products marked with this symbol must be collected separately, according to the regulatory guidelines in your area.

The objectives of this program are to preserve, protect and improve the quality of the environment, protect human health, and utilize natural resources prudently and rationally. Specific treatment of WEEE is indispensable in order to avoid the dispersion of pollutants into the recycled material or waste stream. Such treatment is the most effective means of protecting the customer's environment.

Requirements for waste collection, reuse, recycling, and recovery programs vary by regulatory authority at your location. Instructions to both PerkinElmer customers and recyclers/treatment facilities wishing to obtain disassembly information are provided on the PerkinElmer website:

http://www.perkinelmer.com/lab-products-and-services/environmental-health-and-safety/environmental-directives-compliance.html

Products from other manufacturers may also form a part of your PerkinElmer system. These other producers are directly responsible for the collection and processing of their own waste products under the terms of the WEEE Directive. Please contact these producers directly before discarding any of their products.

10 Legal Information

10.1 Kaleido™ Software License Agreement

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11 Index

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