MULTIMODE DETECTION

ENSIGHT™

User Manual

PerkinElmer®
For the Better

Release History

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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 use in diagnostic procedures.

The EnSight is an automated multimode plate reader for use in biological-medical 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

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.*

2.5.2 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 EN 60825-1:2007.

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

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 139 for detailed instructions.

2.5.5 Hot Surface

Caution! (For temperature control option only)

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 138 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 140 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.*

Please see section 6.5 “Transport”, page 142 for detailed transport instructions.

2.5.11 Disposal

Caution!

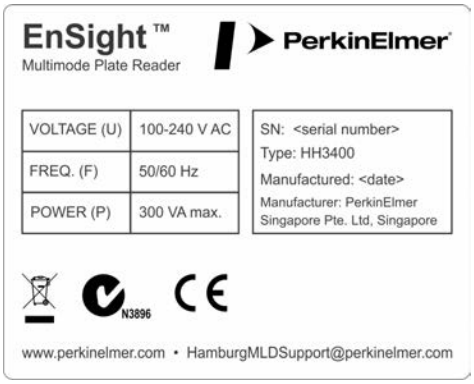
Device may be contaminated by hazardous substances.

- *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 9.3 “WEEE Instructions”, page 158.

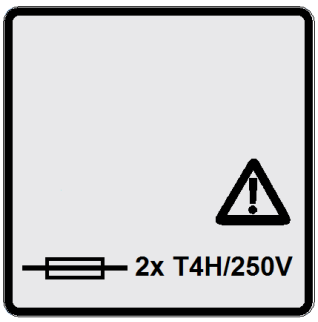
2.6 Location of Warning Signs and Labels

On the back of the instrument:



Classified according to standard
EN 60825-1:2007.

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):

3 Software Installation

This chapter describes how to update Kaleido on the device PC from version 1.0.1 (official release, build 1.0.3028.61) to version 1.2. Older Kaleido versions have to be uninstalled manually before running the setup.

Notice
It is not possible to downgrade from Kaleido 1.2 back to version 1.0.1.

3.1 Backup Database

It is recommended to backup the database before updating Kaleido.

- In Kaleido, open **Settings – Data Management – Backup Database** and follow the instructions of the wizard. See also section 5.9.3.1 “Backup Database”, page 107.

3.2 Update Kaleido

1. Close Kaleido and restart the device PC.
2. Switch on the EnSight instrument.
3. Insert the Kaleido setup DVD.
4. Run the Kaleido setup (\Installation\SetupKaleido.exe). A wizard will guide you through the installation.
   An existing Kaleido 1.0.1 version is uninstalled automatically. Kaleido 1.2 is installed and the instrument’s firmware is updated.

Notice
In Kaleido 1.0.1 the factory preset protocols were not write-protected. If you had modified and saved such a protocol, it has now been automatically renamed to “<OldName>_CUSTOM” by the Kaleido 1.2 setup, so that your changes (and possibly results) are not overwritten by the updated factory preset protocols from PerkinElmer.

3.3 Install WorkOut Plus MMD and Folder Poll

If you want to use the WorkOut data analysis software and the automated result transfer, please install **WorkOut Plus MMD** and **Folder Poll** from the separate **WorkOut DVD**. The applications should be installed in this order:

1. Install/update Kaleido.
2. Install WorkOut Plus MMD.
3. Launch WorkOut Plus MMD and activate the software.
4. Install Folder Poll.

For detailed installation instructions and software documentation please refer to the manuals on the WorkOut DVD.
See also section 5.12 “WorkOut Data Analysis”, page 129.

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 29.

The technologies can be used in different measurement modes (see section 5.2.2.2 “Measurement Modes”, page 71) 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 any of the four sides of the plate.

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.

4.1 Overview

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

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 • 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. • 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.
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.

Signal	Description
White  	**No Connection** The instrument is turned on but not connected to any control software (neither Kaleido nor service software).

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/Init”, page 51). 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.

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! (For temperature control option only)

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.

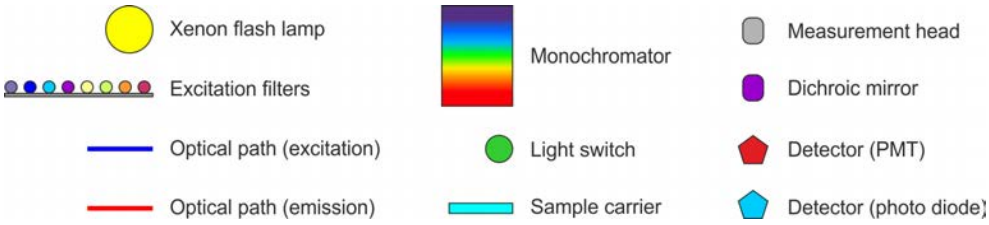
- *Do not remove the glass plate on top of the instrument. User access to this area is not required.*

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.

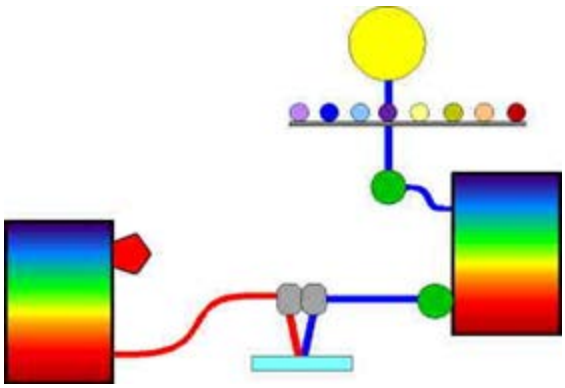
Fluorescence intensity readings can be taken from above or below the plate:

- **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.

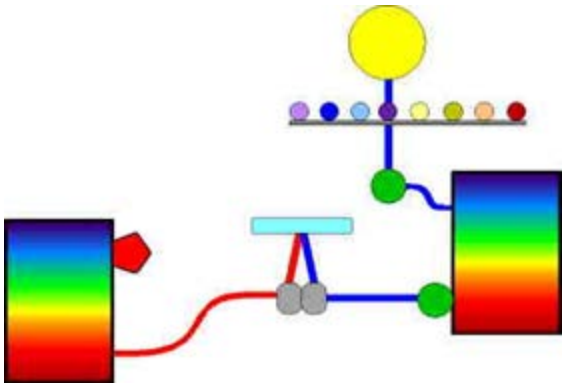
- **FI Bottom:** Excitation and emission from **below** the plate (requires optional detector below plate); for adherent cells and lidded plates, reading from below provides superior efficiency.

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 29.



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

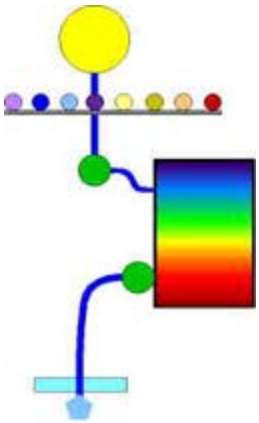
For absorbance measurements (230-1000 nm) the same Xenon light source is used as for FI measurements.

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 29.



Absorbance measurement with the quad-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.

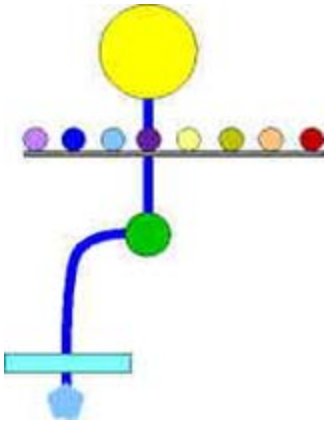
The absorbance value is calculated by the equation

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

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 29.



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.

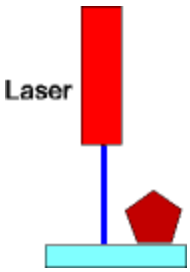
4.5.3 Alpha Technology

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 29.



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 29.

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.

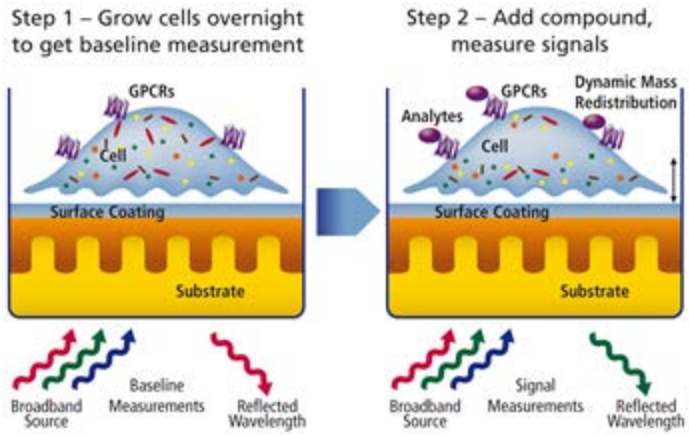
4.5.5 Label-free Technology

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.

Cell-based Assays

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



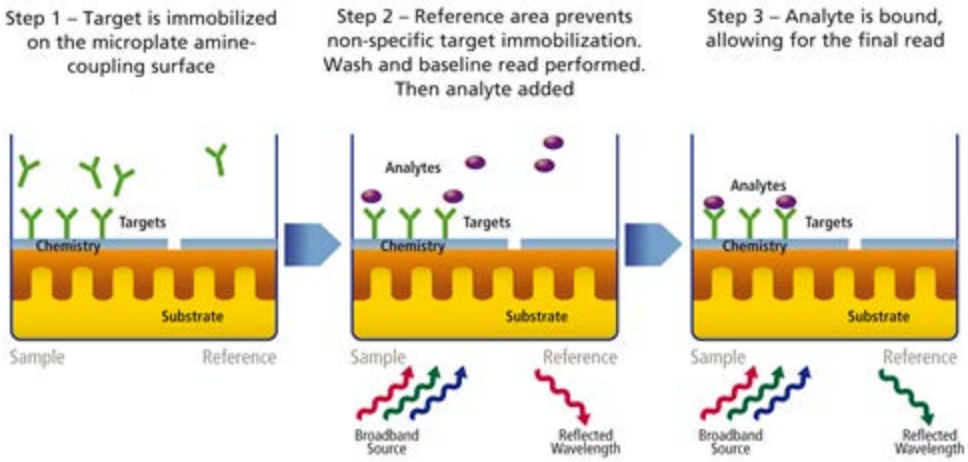
Label-free cell-based assay

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

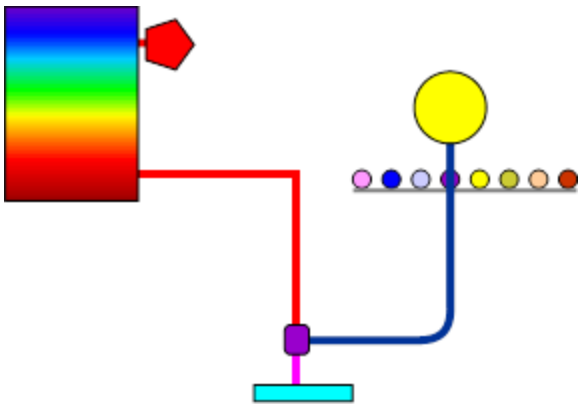
Label-free biochemical assays measure changes in the index of refraction upon a binding event. As in cellular assays, the change is indicated by a shift in wavelength.

4.5.6 Time-resolved Fluorescence (TRF) Technology

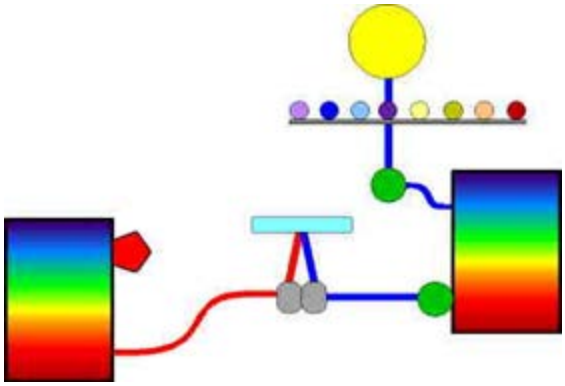
The same excitation light source is used as for FI measurements.

- 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 29.



TRF measurement from the top with excitation filter and emission monochromator

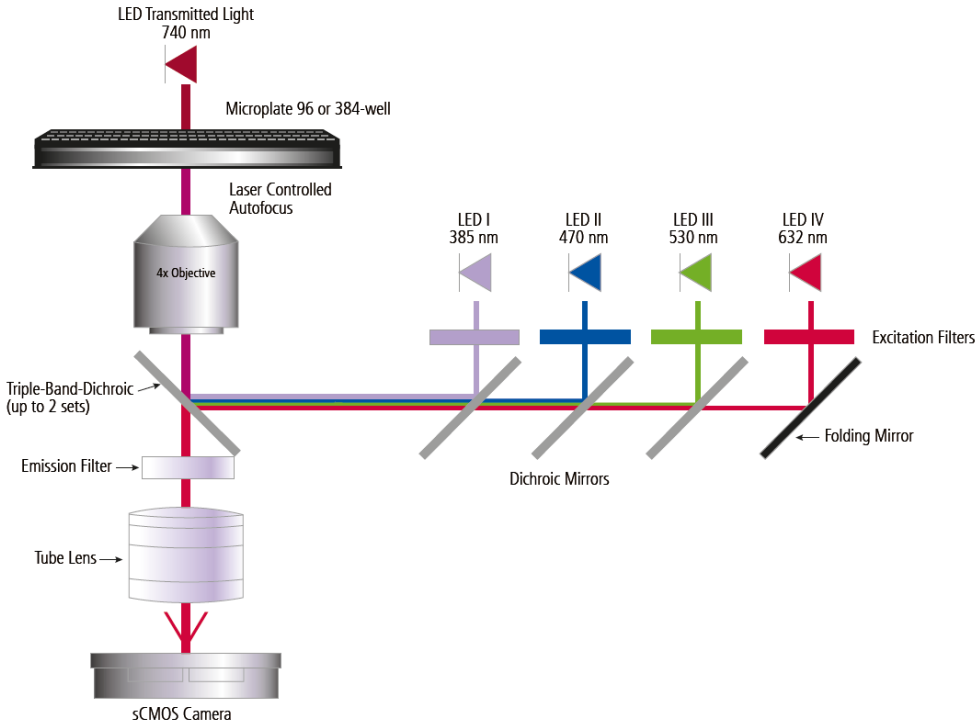


TRF measurement from the bottom with excitation and emission monochromators

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 Well 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.



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.

Well 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 Well 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 well 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 137 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 (supporting points on the plate carrier).

Focus Height

For **Well 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

Microplates with 6, 12, 24, 48, 96 and 384 wells can be measured.

Allowed plate height

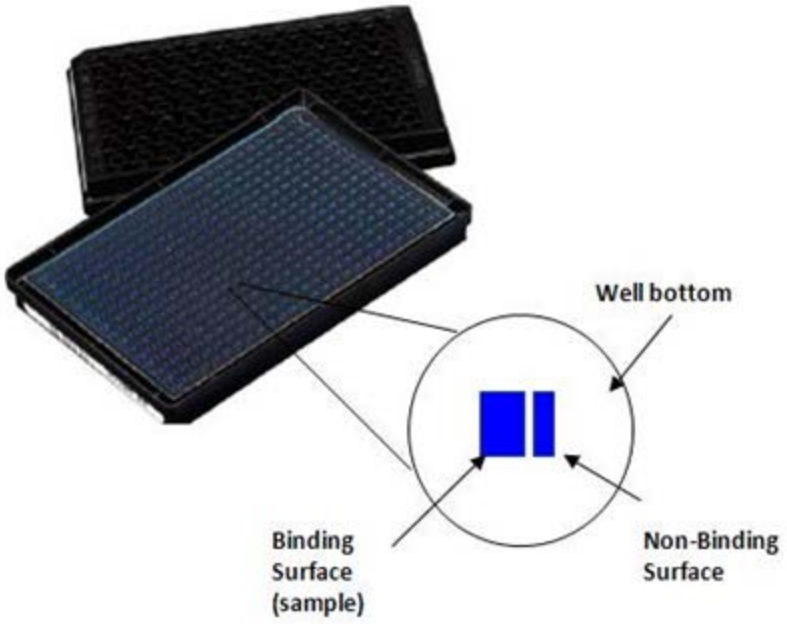
- Alpha or luminescence technology: 7-28 mm
- All other technologies: 3-28 mm

Plate color and type

- Fluorescence intensity: Black
- Absorbance : Black or white
- Time-resolved fluorescence: White
- Luminescence: White preferred
- Alpha: Alpha plate, without lid, without 'top skirt'
- Imaging (fluorescence): Black with clear imaging bottom
- Imaging (brightfield): Black or white with clear imaging bottom

4.11.1 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.

4.11.2 Well Imaging

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

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 147 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:

- 5.9.6 “Temperature (Settings)”, page 113
- “Temperature (TEMP)”, page 68

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.

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 48).

   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 26.

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 51).

Please see section 5.13 “User Management”, page 133 to learn more about user accounts, user groups, restricted actions, and how to add new users.

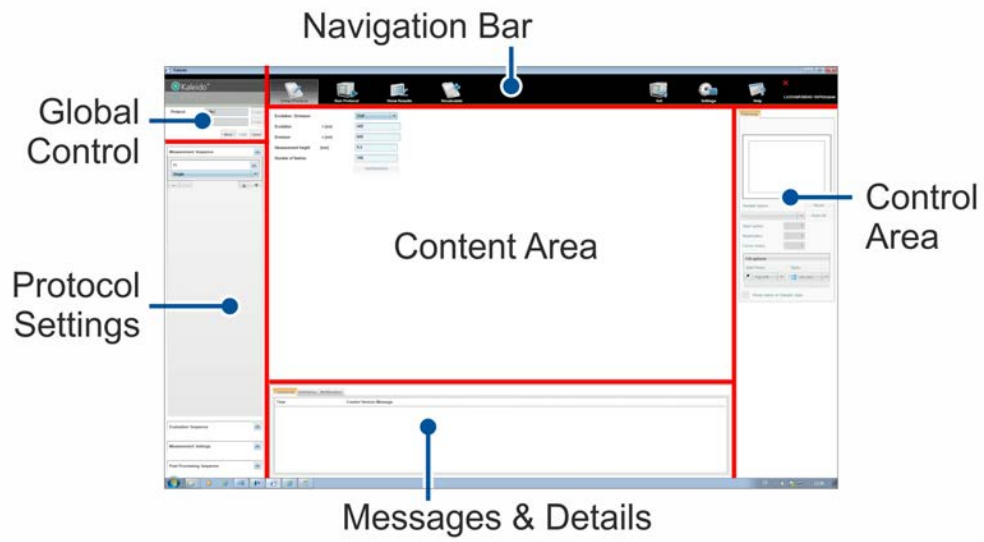
How to switch to a different user

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 & Details** (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.
- **Run Protocol:** Load and start protocols.
- **View Results:** Select protocols and view the results of a run.
- **Recalculate:** 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 has to be loaded via the section in the upper left of the screen, the **Global Control** section. This section always displays the protocol currently loaded and, depending on the selected screen, different elements and buttons are enabled.

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 & Details** area (see section 5.8.3 “Notifications”, page 104).

Current User

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

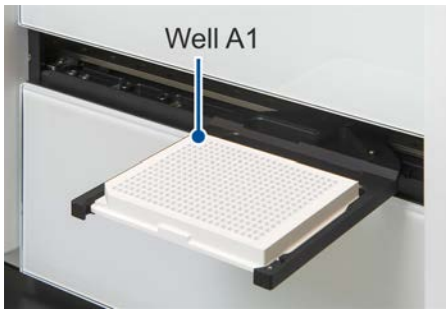
5.1.5 Load/Eject/Init

Using this button you can load and eject a plate and initialize the instrument. The button will toggle its function depending on the current instrument status and the 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.
	If the instrument is in error state or needs to be initialized, the **Init** button is displayed. You have to click **Init** and initialize before the instrument can be used again. See also section “Instrument Status Symbols”, page 51.
	The button is disabled while the instrument is busy.

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
- Save an existing protocol under a new name and edit it
- Edit an existing protocol and save it under a new name
- Import a protocol (file format *.kal), e.g. from a different instrument (see section 5.9.3.3 “Import Protocol / Screen”, page 108)

Buttons and Elements

Element	Description
Protocol [...]	Click ... to open the list with existing protocols in the **Content Area**, and click on a protocol to load it.
Plate Type [...]	Here the plate type of the current protocol is displayed. When in edit mode or when a new protocol is generated, you can click on ... to open the plate type list. To select a plate type for the protocol click **OK** or double click on a plate type, to abort the selection click **Cancel**.
[New]	Option to create new protocol from scratch. Opens a dialog for entering a unique name for the new protocol.
[Edit]	The currently loaded protocol will be set to edit mode. 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**. **Notice** If the protocol still contains analysis operations which have been discontinued, you first have to remove these operations. See also section 5.11 “Analysis Operations”, page 116 for details.

New Protocol

If you want to generate a new protocol [New], you will first be asked to enter a new protocol name. This name has to be unique, otherwise you will be warned by a red frame around the text box and the name is not accepted, i.e. the [OK] button is not enabled. When you entered a valid name and pressed [OK] you will find the new name

in the Global Control and can continue with adding operations to the protocol and select a plate type. The plate type is selected from a list which opens with click on Plate Type […] in the Global Control.

Protocol List

The protocol list is opened in the **Content Area** if you click on … next to **Protocol** in the **Global Control** section. To load a protocol, just click on the desired row in the table.

There are some protocols supplied by the manufacturer which cannot be overwritten (indicated by a icon). However, you can edit such a "factory preset" protocol and save it under a new name to use it as a starting point.

See also section 5.6.1 "Protocol List", page 92 for a detailed description.

Plate Type List

When a protocol is in edit mode or when a new protocol is generated, a click on … next to **Plate Type** in the **Global Control** section will open a new dialog with the list of plate types. To select a plate type for the protocol click **OK** or double click on a plate type.

See also section 5.2.1 "Plate Type List", page 55 for a detailed description.

Edit 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.

Save 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.

Notice
Previous versions of protocols are not available in the protocol list anymore, only the latest versions will be available. In case you want to check protocol settings of screen results from former versions of a protocol, you can load the screen results 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 open a text box "Protocol Name" for entering a new name. Changes will be saved under the new name with click on [OK] and the currently loaded protocol will not be altered.
- 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. was saved before.

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 & Details** 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, 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 Plate Type List

Click on … next to **Plate Type** in the **Global Control** section of the **Setup Protocol** screen to open this dialog. The button is only enabled if a protocol is in edit mode or if a new protocol is created. To select a plate type for the protocol click **OK** or double click on a plate type.

The columns of the plate type list:

- Compatibility of plate type with selected operations (only visible when at least one operation is added to the **Measurement Sequence**):
  - Plate type is **recommended** for the operations in the protocol.
  - Plate type is **unsuitable** (for details see **Comments**).
  - No symbol indicates that the plate type is **applicable**.

  These are only recommendations; measurements can be started even with unsuitable plate types, but this may lead to errors or wrong results.
- When a plate type is **factory preset**, a icon is displayed in the second column.
- Name of the plate type
- Number of rows
- Number of columns

User-defined plate types can be defined or edited in **Settings – Inventory – Define Plate Type**. See also section 5.9.5.2 “Plate Type”, page 111. Factory preset plate types cannot be edited.

- You can sort the plate types if you click on the header of a column.
- If you click a plate type, further details will be displayed in the area below the list.

5.2.2 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 components of a measurement sequence are called operations, comprising **measurement operations** (the technologies implemented in EnSight) and several **non-measurement operations** for setting up appropriate conditions for measurement operations (delay between measurements, shaking or temperature control).The word sequence indicates, that multiple operations can be chosen, which are then run one after the other until the sequence is completed.

With a click on the **[+]** button, you can add operations to the measurement sequence by selecting from a list. For each operation added to the sequence, specific settings must be adjusted. For that, the operation-specific parameters appear in the **Content Area** in the center of the screen, according to the selected operation in the measurement sequence. Most operations can be added as often as required to a measurement sequence; Alpha and Label-free only once due to the reactions and usage of substances of these technologies during measurement.

The order of operations within a measurement sequence can be changed with the **up** and **down** arrow buttons. 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 appearing list.

Buttons and Elements

Element	Description
Plus [+]	Operations can be added to measurement sequence by selecting from opening list. Only enabled when protocol is in **Edit** mode.
Minus [-]	Operations in current measurement sequence can be removed by selecting from opening list. Only enabled when protocol is in **Edit** mode.
Arrow up	Moves up the selected operations within the measurement sequence. Only enabled when protocol is in **Edit** mode.
Arrow down	Moves down the selected operations within the measurement sequence. Only enabled when protocol is in **Edit** mode.

Element	Description
Disclosure buttons 	**Measurement Sequence:** Expand or collapse the measurement sequence. **Protocol Operation:** For several operations (e.g. ABS mono), a measurement mode other than **Single** can be selected (see section 5.2.2.2 “Measurement Modes”, page 71). The disclosure button expands the protocol operation so that a different the measurement mode can be edited.

5.2.2.1 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 is measured prior to the actual measurement and 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 section 4.5.2 “Absorbance Technology”, page 30.

Absorbance Filter Parameters

Parameter	Description
Excitation Filter	Filter with appropriate wavelength can be chosen from a pop-up list.
Measurement Height [mm]	Focus height within the sample, measured from the bottom of the plate (supporting points on the plate carrier). This value can be automatically optimized (see section “Measurement Height”, page 78).
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 5.2.2.3 “Optimizations”, page 74) which helps you to find the best settings for selected operation parameters. The available optimizations depend on the measurement technology.

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 section 4.5.2 “Absorbance Technology”, page 30.

Absorbance Mono Parameters

Parameter	Description
Excitation λ [nm]	Wavelength of light coming from excitation double monochromator.
Measurement Height [mm]	Focus height within the sample, measured from the bottom of the plate (supporting points on the plate carrier). This value can be automatically optimized (see section “Measurement Height”, page 78).
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 5.2.2.3 “Optimizations”, page 74) 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.

Only one measurement per well is recommended because the sample is partially bleached by the excitation light, therefore Alpha technology can only run in **Single** measurement mode. When working with Alpha technology, bright light and extreme temperature fluctuation should be avoided and only opaque plates should be used.

For further details see also section 4.5.3 “Alpha Technology”, page 32.

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.
Total Measurement Time [ms]	Comprises excitation and emission times.
Excitation Time [ms]	Length of time the laser is used to excite the sample. The percentage figure next to the excitation time shows how much of the total measurement time is used for excitation.
Optimization	This button is only enabled if a plate type was selected. It opens the **Optimization Wizard** (see section 5.2.2.3 “Optimizations”, page 74) 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 section 4.5.1 “Fluorescence Intensity With Quad-Monochromator”, page 29.

Fluorescence Intensity Parameters

Parameter	Description
Excitation / Emission	FI reading from above (**TOP**) or from below (**BOTTOM**) can be chosen here.
Excitation λ [nm]	Wavelength (between 230-835 nm) used to excite the fluorochrome in the sample, needs to be at least 15 nm smaller than emission wavelength.
Emission λ [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 available for **TOP**). The height is measured from the bottom of the plate (supporting points on the plate carrier). The value can be automatically optimized (see section "Measurement Height", page 78).
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 5.2.2.3 "Optimizations", page 74) which helps you to find the best settings for selected operation parameters. The available optimizations depend on the measurement technology.

Well Imaging (IMG)

The imaging module acts as an inverted optical (fluorescent) microscope with 4x magnification 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 contrast. The large field of view allows imaging of almost a complete well of a 96-well plate and the resolution of ~3µm per pixel supports analysis of small cell types.

Well imaging can be combined with all other technologies (e.g. Luminescence or Label-free) to allow the analysis of various aspects of cellular assays. For well imaging operations one of two dichroic filter sets (primary or secondary) separating excitation and emission light has to be selected, each allows only certain combinations of light sources of the channels added (see table of parameters below).

If the option **Digital Phase Contrast** (DPC) is used for a **Brightfield** channel, the DPC image will be computed based on the measured brightfield image. In the **Channel** section two separate channels will be displayed 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.

For further details see also section 4.5.7 "Well Imaging Technology", page 37.

Well Imaging Parameters

Parameter	Description
[+]	Adds a channel to the operation. You can enter a channel name or use the default name (Channel x).
[-]	Allows you to remove one of the existing channels. This channel can be selected from a pop-up list.
Parameters for each Channel	• **Light Source:** Click **[...]** to select a light source for the channel. Up to 4 different excitation light sources (LEDs) and brightfield (transmitted light) can be selected from a list with the option **Show compatible light source only** below the list. • **Excitation [%]:** Excitation power. • **Time [ms]:** Exposure time. • **Focus Offset [µm]:** Allows you to modify the selected general **Focus Height** by a certain offset (only for this channel). • **Digital Phase Contrast:** Only enabled when using BRIGHTFIELD as light source.
Filter Set	One of two dichroic filter sets (to separate excitation and emission light) can be chosen, combinations of light sources per filter are specified in the table below.
Focus Height [µm]	Focus height within the sample, measured from the bottom of the well.

Filter Sets and Light Sources

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

Filter Set	BLUE 465 nm	RED 632 nm	BRIGHTFIELD 735 nm, transmitted	True GREEN 525 nm	UV 385 nm
PRIMARY filter set	●	●	●		●
SECONDARY filter set (optional)		●	●	●	●

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.

Label-free measurements intend to analyze changes of the sample properties over time. The average wavelength of the response is of limited relevance. The important information is the kinetic profile of how the contents of each well react over time and thus change the reflected wavelength. Therefore, Label-free measurements usually consist of at least two subsequent runs, an initial Baseline run and one or more Final runs. This 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.

- **Cell-based Assays**
  Label-free cell-based assays measure 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 33.

Notice

- After switching on the instrument the Label-free module needs some time to warm up. Please wait 45 min. before starting measurements.
- Protocols containing Label-free operations cannot be used in automation mode (under remote control via external scheduler). The measurement of such a protocol will be rejected.

Live Response

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

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):

- **Cell-based label-free assay (CBA):**
  Live Response [pm] = (Signal - First Baseline Signal) / 1,000
- **Biochemical label-free assay (BCA):**
  Live Response [pm] = (Signal (Signal Region - Reference Region) - First Baseline (Signal Region - Reference Region)) / 1,000

The first successfully 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

Since Kaleido 1.2, plate repeats are consecutively numbered (irrespective of whether it is a baseline or final measurement). In the **List View**, baseline and final measurements can be distinguished by means of the **Batch Number**.

Example with Baseline (3 repeats) and Final (4 repeats):

Batch Number	Plate Repeat	Well Repeat
1	1	1
1	2	1
1	3	1
2	4	1
2	5	1
2	6	1
2	7	1

Label-free Parameters

Parameter	Description
Assay Type	Biochemical or cell-based assay
Baseline	The Baseline run captures the status of each well prior to the experiment. Typically, 4–5 subsequent repeats are run to verify the stability of the sample. These runs are taken with buffer, immobilized proteins only (Biochemical Assays), or equilibrated cells (Cell-Based Assays) for establishing a baseline value for each well. **Measurement Defined by** Multiple baselines can be run to verify that no reaction is taking place, either defined by number of repeats or by total time during which the entire plate is repeatedly counted. **Start Measurement Each** Specifies the amount of time to allow for each repeat of the plate. Since an entire ¼ plate is being read at a time, the specified time needs to be bigger than the minimum amount of time it takes to read the number of quadrants requested. For example, if the minimum time to read a quadrant is 20 seconds and a full plate is to be read, at least 80 seconds have to be specified here for the entire plate. To add a delay between repeats, this time can be increased.
Final	Measurement of reactions taking place. The final run must be preceded by at least one baseline run. **Measurement Defined by** Multiple final runs can be performed based on the chemistry and reaction desired, and are defined either by the number of repeats or by a period of time (total time), for which the entire plate is repeatedly counted. **Start Measurement Each** See above (Baseline).

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 33.

Luminescence Parameters

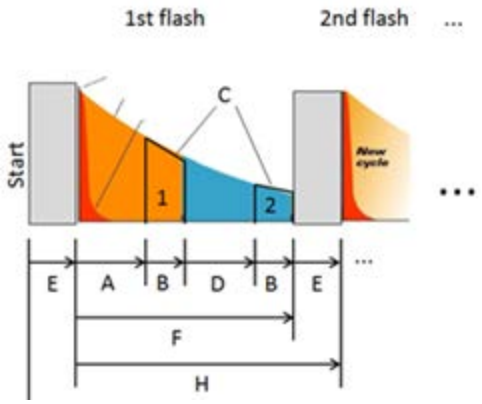
Parameter	Description
Distance between Plate and Detector [mm]	Distance between upper surface of plate and detector, valid range between 0-3 mm with 0.1 mm steps, lower distance reduces crosstalk.
Measurement Time [s]	Duration of measurement per well.
CT2 [%]	Glow (crosstalk 2) correction factor.

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**. If the allowed time frame is exceeded, you will get an error message. Reduce one or multiple of these parameters to keep within the allowed range.

For further details see also section 4.5.6 “Time-resolved Fluorescence (TRF) Technology”, page 35.

Time-resolved Fluorescence Parameters

Parameter	Description
Excitation / Emission	**TOP:** TRF measurement from the top with excitation filter and emission monochromator. **BOTTOM:** TRF measurement from the bottom with excitation and emission monochromators.
Excitation [Filter]/λ [nm]	This parameter depends on the selected measurement direction (TOP/BOTTOM): • **TOP:** Select an appropriate filter from the list to choose the excitation wavelength (230-385 nm). By default the option **Show compatible filters only** is checked. • **BOTTOM:** Enter a wavelength (230-835 nm).
Emission λ [nm]	Wavelength of the resultant fluorescence to be measured, needs to be at least 15 nm higher than excitation wavelength and it depends on the measurement direction (TOP/BOTTOM): • **TOP:** Enter a wavelength (400-850 nm). • **BOTTOM:** Enter a wavelength(245-850 nm).
Measurement Height [mm]	Focus height within the sample (only available for **TOP**). The height is measured from the bottom of the plate (supporting plane). Can be optimized automatically (see section “Measurement Height”, page 78).
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).
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 5.2.2.3 “Optimizations”, page 74) which helps you to find the best settings for selected operation parameters. The available optimizations depend on the measurement technology.

Delay (DELAY)

The **Delay** operation is used to introduce a time gap between measurements. You can use a delay to follow time behavior of assays. This way you can measure a whole plate, wait, and measure again.

Delay Parameters

Parameter	Description
Duration	Length of the delay between the completion of the preceding operation and the start of the next one. The range is 0.1 - 32,000 seconds, different time units are available.
Plate Location	Position of the plate between the measurements, 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.

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 in a range from 0.1 – 6,000 seconds, different time units are available.
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 5.9.6 “Temperature (Settings)”, page 113) are temporarily overridden by the operation.

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
Switch off Temperature Adjustment	This option can be used to reset any temperature adjustment configured by a previous temperature operation. Temperature control will be reset to the configuration defined in the general temperature settings (see section 5.9.6 “Temperature (Settings)”, page 113), i.e. it will only be switched off if it also had been switched off in the general settings before starting the protocol run. To use this function in a protocol you will have to add multiple temperature operations to your protocol: one for setting the desired temperature adjustment and a second one for resetting temperature control later using this option. • **Check box activated:** All other operation parameters will be disabled. Temperature control will be reset. • **Check box disabled:** You can configure desired temperature settings as usual. These settings will override the general temperature settings.
Temperature [°C]	Target temperature in the instrument (15-65 °C, dependent on ambient temperature).
Fast Start	With this option activated, the measurement sequence is continued while the temperature is being regulated – even if the target temperature has not been reached yet. If the fast start is not used, the software waits until the temperature is stabilized to a maximum drift of ±0.3 °C within one minute before proceeding with the next operation.
Fast Cooling	Activates the chamber fan and opens the plate door. 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.
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.

Parameter	Description
Diff. Temperature [°C]	Temperature difference between upper and lower heater (value range: 0- 4 °C, 0.1 °C steps).

5.2.2.2 Measurement Modes

For measurement operations, 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 time (**Kinetics**), 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. Selecting **Kinetic**, **Wavelength Scan** or **Well Scan** for an operation, additional parameters will be displayed in the **Content Area** together with the operation parameters.

Available measurement modes for measurement operations:

Mode	ABS filter	ABS mono	ALPHA	FI	IMG	LF	LUM	TRF
Single	●	●	●	●	●	●	●	●
Kinetic	●	●		●			●	●
Well Scan	●	●		●			●	●
Wavelength Scan (EXC)		●		●				
Wavelength Scan (EMS)				●				●
On-the-Fly	●	●						

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.

Kinetic

In the **Kinetic** measurement mode, one well is measured repeatedly with a definable delay between each measurement, and then the next well is measured with the same settings. Used for fast kinetic assays such as Ca2+ measurements or flash luminescence assays.

Kinetic Parameters

Parameter	Description
Number of Measurements	The desired number of measurement repetitions (up to 1000).

Parameter	Description
Start Measurement each	The time between the end of one repeat and the beginning of the next. Max. 5400 seconds (90 minutes).

Well Scan

In this mode every well is scanned with a definable 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	Distance between measurement points in the well. The maximum distance between points depends on the total number of points and the well size.
Trim to	Rectangular or round wells, depending on plate type.

Wavelength Scan (EXC/EMS)

In the **Wavelength Scan** measurement mode several measurements are performed at different wavelengths per well to get either an excitation (Exc) or an Emisson (Ems) spectrum. These spectra can provide additional information compared to the single point measurement, and 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.

- **Wavelength scan (EXC)**
  For fluorescence intensity and absorbance with monochromator:
  The excitation monochromator scans the samples in definable steps between a minimum and a maximum wavelength. For fluorescence intensity, the resulting fluorescence is measured at the wavelength selected for the emission monochromator.
- **Wavelength Scan (EMS)**
  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 definable steps with the emission monochromator.

Wavelength Scan Parameters

Parameter	Description
Min [nm]	Minimum wavelength to start the scan.
Max [nm]	Maximum wavelength to end the scan.
Step [nm]	Size of the steps 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.

5.2.2.3 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	●	●		●			●	

- For **Well 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 5.7.2 “Optimize IMG Parameter”, page 99). They can be performed for every channel set up in the operation.
- There are no optimizations available for **Label-free** (LF) technology.

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 and continue:** 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 and continue:** 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 maximum absorbance (Abs mono) or emission (FI, TRF BOTTOM) occurs. 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 77).

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 (min. 230 nm).
Exc Max [nm]	Maximum wavelength included in optimization (max. 1000 nm).
Step [nm]	Size of the wavelengths steps to be measured.
Ems [nm] (editable in FI and TRF BOTTOM)	Wavelength (between 245-1000 nm) 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 (min. 245 nm).
Ems Max [nm]	Maximum wavelength included in optimization (max. 1000 nm).

Parameter	Description
Step [nm]	Size of the wavelengths steps to be measured.
Exc [nm]	Wavelength (between 230-1000 nm) 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 121 points are measured in an 11 x 11 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, the X and Y positions defined in the current plate type are displayed.

- If a click on the fields with the highest intensity does not change the offset values (0), the plate was positioned right before.
- If the offset values change, the plate has not been in the right position and can be corrected for the protocol using the offset values.

The results can be saved (with **Save** protocol) so that new plate dimensions will be used for all operations in the protocol.

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 operation.
Size of Scanned Area [µm]	Size of the edges of the area of the wells to be scanned. Default values are 450 for 384 well plates (default value) and 900 for 96 well plates.
Measurement Time (only for Lum)	Duration of measurement of each well, default is the value specified in 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.

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.

5.2.3 Measurement Settings

Depending on the selected technologies and measurement modes, it is possible to repeat the whole measurement sequence on the same plate (**Repeat Plate**). This option is not available when **Label-free** technology is part of the measurement sequence or if the **Kinetic** mode is used by any measurement operation in the sequence.

Parameters

Parameter	Description
Number of Repeats	Defines how often the measurement sequence is repeated on the plate (range: 1-1000).
Start Repeat every	Allows you to define a delay between the plate repeats. Minutes or seconds can be selected with buttons.

5.2.4 Analysis Sequence

In the **Analysis Sequence** you can define analysis operations which will be applied to the images resulting from **Well Imaging (IMG)** measurements. The analyses are performed directly after the exposure. For a detailed description of all analysis operations please see section 5.11 “Analysis Operations”, page 116.

With a click on the **[+]** button, you can add operations to the analysis sequence by selecting from a list. For each analysis operation added to the sequence, specific parameters must be adjusted in the **Content Area** in the center of the screen.

The order of analysis operations within the sequence can be changed with the **up** and **down** arrow buttons. An operation can easily be removed from the sequence by clicking on the **[-]** button and selecting the analysis operation to be removed in the popup menu.

On the **View Results** screen, click an analysis operation in the **Analysis Sequence** to display the results in the **Content Area**. Export and copy functions are the same as for measurement results (see section 5.4 “View Results”, page 87). To change and redo analyses see section 5.5 “Recalculate”, page 90.

Notice

- For analyzing non-imaging measurement data and the results of imaging analysis operations, please use the separate **WorkOut Plus MMD** data analysis software which is now bundled with Kaleido. See section 5.12 “WorkOut Data Analysis”, page 129 for detailed information.
- All non-imaging analysis operations in Kaleido have been discontinued 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 remove the respective operation (s) from such a protocol and save it again.

Buttons and Elements

Element	Description
Plus [+]	Opens a popup menu where you can select the analysis operation to be inserted. Only enabled when protocol is in **Edit** mode.
Minus [-]	Opens a popup menu where you can select the analysis operation to be removed. Only enabled when protocol is in **Edit** mode.
Arrow up	Moves up the selected analysis operation within the analysis sequence. Only enabled when protocol is in **Edit** mode.
Arrow down	Moves down the selected analysis operation within the analysis sequence. Only enabled when protocol is in **Edit** mode.
Disclosure buttons 	Expand or collapse the analysis sequence.

5.2.5 Post Processing Sequence

In the **Post Processing Sequence** you can define an automatic export of results right after the measurements have 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.

The results can be exported either as ***.csv** file (Comma Separated Values) as plate or list, or as ***.xml** file (Extensible Markup Language). Also custom made style sheets (**CUSTOM**) can be defined. Further, **ARCHIVE** offers the export of the result data together with the protocol settings as ***.kal** file. These files can e.g. be imported to Kaleido on a different system.

Notice

For transferring measurement results to **WorkOut** for further analysis, you have to use the **XML** export format. See also section 5.12 “WorkOut Data Analysis”, page 129.

Parameters

Parameter	Description
Export Format	Select an export format (CSV_PLATE, CSV_LIST, XML, CUSTOM, ARCHIVE). Default is CSV_PLATE.
Transformation File	Only enabled when **CUSTOM** is selected as export format. Enter the path of a custom made XSL transformation style sheet or click **[…]** to browse for such a ***.xsl** file.
Export Path	Enter a target path for the export file or click **[…]** to browse for a folder. For exporting XML files to WorkOut it is suggested to use the default Kaleido output folders. See also section 5.12.3.1 “Kaleido Output Folders (Default)”, page 130.
File Name	Enter the desired file name or click **Variables** to select properties of the protocol (e.g. [ProtocolName]) from a list. 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.

5.3 Run Protocol

On the **Run Protocol** screen you can load and run protocols. Measurement results will be displayed on the **Plate View** and **Graph View** tabs in the **Content Area**.

How to run a protocol

1. Click **Run Protocol** in the **Navigation Bar**.
2. If the protocol list is not displayed yet: Click … next to **Protocol** to open the protocol list.
3. Click on a protocol in the protocol list (**Content Area**) to load it.

   Alternatively, you can prepare and save a protocol on the **Setup Protocol** screen. When you switch to **Run Protocol**, the saved 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 on the left)? See also section 5.1.5 “Load/Eject/Init”, page 51.
   - (starting with the first plate if you are running a multi-plate assay)
6. Optional steps:
   - Check the **Continue Screen** option, if applicable (see section “Continue Screen”, page 84).
   - If you are using the **Barcode Reader**, make sure it is activated in the **Settings** (see section 5.9.4 “Barcode Reader”, page 110). For label-free measurements, the barcode is essential as it is used to generate the response based on the plate baseline (all label-free plates have barcodes affixed in production). In case barcode reading fails, the number can be entered manually or a virtual barcode can be generated.
7. Click **Start**.

   The plate is loaded and the measurement is started.

How to prepare automated measurements (remote control)

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 WorkOut has been configured, you should activate the option **Close WorkOut After Analysis** for the corresponding poll operation in the Folder Poll Manager. Otherwise a new instance of WorkOut will be opened after each protocol run. See also section 5.12 “WorkOut Data Analysis”, page 129.

Checklist for automated runs
Selected plate type can be handled by the robot?
Protocol saved in the database?
Protocol does not contain Label-free operations?
Test measurement of one plate successful (to determine protocol duration for plate::works scheduler)?
EnSight is in idle state (green status light and status symbol ✓)?
Plate carrier is empty? (no plate inserted)
“Automation Mode” activated in Kaleido?

Buttons and Elements

Element	Description
Automation Mode	Allows 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 are disabled. Only measurement progress and results are displayed in the **Plate View**. **Notice** Lable-free measurements require the user’s interaction and cannot be used for automated runs.
Protocol [...]	Click [...] to open the list with existing protocols, and click on a protocol to load it. See also section 5.6.1 “Protocol List”, page 92.
Plate Type	Displays the plate type of the currently loaded protocol.

Element	Description
Continue Screen [...]	If screens of the loaded protocol already exist, this box is enabled. When check marked, the last screen will be loaded as default, but can be changed (see **Screen** ... below). **Notice** The option **Continue Screen** cannot be used in automation mode, because the user's interaction is required.
Screen [...]	Enabled when **Continue Screen** is check marked: The last screen of the protocol will be displayed as default, click on ... to choose a different one from a screen list.
[Start]	Starts the protocol, instrument status symbol changes to "Busy" . **Notice** If the protocol still contains analysis operations which have been discontinued, you first have to remove these operations. See also section 5.11 "Analysis Operations", page 116 for details.
[Stop]	Stops measurement after confirmation, if applicable with option to "Stop after current Plate Repeat". If the protocol includes an analysis, all analysis results will be discarded.

Continue Screen

In case a protocol has been run before, the option **Continue Screen** is available in the **Global Control** area. A screen is one run of a protocol, and screens (with the results) of the same protocol are numbered consecutively, starting again with 1 for every new version of a protocol.

Example
Screen number *51-2-3* means:

- Protocol number 51
- Second version of protocol
- Third screen

Data of a new measurement can be added to an existing screen instead of generating a new screen. By check marking the option **Continue Screen** the last screen will be loaded as default, but can be changed after opening the screen list (click on ... next to **Screen**).

Notice

- The option **Continue Screen** cannot be used in automation mode, because the user's interaction is required.
- When protocol includes an analysis (not IMG) and **Continue Screen** is selected, the new analysis results will be based on all screen results belonging to the screen. For IMG the previous results are copied and added to the new analysis results.

The **Continue Screen** option has also to be used when running the Baseline and Final measurements of a protocol which includes the **Label-free** technology. If you activate **Continue Screen**, you will be asked if you want to make a Baseline or Final measurement. See also section "Label-free (LF)", page 62.

Load Protocol

Click on a protocol in the protocol list (**Content Area**) to load it.

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

Start

Click **Start** to run the protocol. If a setting of the protocol is invalid, e.g. a filter is not in the filter wheel of the instrument, a message with respective information would appear now.

View Measurement Progress and Results

During measurement, you can follow its progress in the content area either as **Plate View** or **Graph View**, 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 View** is only enabled for repeated measurements or scans in the operation (measurement mode). With each new operation in the protocol, the displayed results are overwritten. The currently measured plate, plate repeat and well repeat is displayed in the boxes below the plate map.

For details please see the following sections:

- 5.6.2 "Plate View", page 93
- 5.6.3 "Graph View", page 93

Stop

To abort a running protocol, click on **Stop**. You will be asked to confirm before the measurement is actually stopped. If you confirm with **Yes**, the measurement will be terminated and the data measured so far will be saved in the database. If the option **Stop After Current Plate Repeat** is check marked, the current plate repeat will be completed before the measurement is stopped. You may use this option when the protocol includes plate repeats or a Label-free operation.

If an analysis is part of the protocol, all analysis results will be discarded.

If you stop a measurement and start a new one with **Continue Screen**, the protocol will start from the beginning regardless at which point the previous screen was stopped.

5.4 View Results

On the **View Results** screen, **screen results** and **analysis results** of every protocol can be viewed.

A screen is the run of a protocol, and screens (including protocol, results and meta data) of the same protocol are numbered consecutively, starting again with 1 for every new revision of a protocol.

Example
Screen number *51-2-3* means:

- Protocol number 51
- Second version of protocol
- Third screen

The list of analysis results comprises their name as well as the time and date when they were produced. Analyses of measurement results can be included in the protocol and directly performed during **Run Protocol**. In this case the analyzed results are given a default name (date and time). If an analysis has been done using **Recalculate**, a name has to be defined by the user.

After a running a protocol, Kaleido automatically switches to **View Result** with the just created screen loaded. Click on the desired measurement operation or analysis on the left to view the corresponding results.

How to open a different screen result

1. Load a protocol (click … next to **Protocol** in the **Global Control** section and click on a protocol in the list).

   Hint
   If you **double-click** a protocol, the list of existing results is displayed directly.

2. Click … next to **Result** to open a list with all existing screen results of the currently loaded protocol, including time and date of the run. The list will be displayed in the **Content Area**. The … button next to **Result** is disabled when no screen has been done for the loaded protocol yet.
3. To actually display screen results into the **Content Area**, select an operation within the **Measurement Sequence**. The screen results of that selected operation are displayed in **Plate View** as default, but they can be also viewed in **List View** or **Graph View** (**Graph View** is only enabled when appropriate screen results are loaded). See also section 5.6 “Content Area”, page 92.

How to open an analysis result

1. Load a protocol and a screen result.

2. Click … next to **Analysis Result** to open the list with existing analysis results of the selected screen. If no Analysis Result has been generated yet for the loaded screen, the button is disabled.

Buttons and Elements

Element	Description
Protocol […]	Click … next to **Protocol** to open the list with existing protocols, and click on a protocol to load it. See also section 5.6.1 “Protocol List”, page 92.
Result […]	Click … next to **Result** and click on the desired screen results in the list in the **Content Area**. Click on an operation in the **Measurement Sequence** to view the results.
Analysis Result […]	Click … next to **Analysis Result** to open a list with existing analyses of the currently loaded screen results. Click on an analysis in the **Content Area** to load the analysis results.
Plate Type	Displays the plate type of the current protocol.
[Export]	Opens dialog for manual export of results. After recalculation, first save the **Analysis Results** since only saved data can be exported. For automated export of results see section 5.2.5 “Post Processing Sequence”, page 81.
[Add Com.]	Allows you to add a comment to the current screen or to the loaded analysis result <name>.
[Save]	Opens a dialog for saving analysis results or/and saving analysis to protocol. Only enabled when viewing unsaved analysis results.
[Edit Anly.]	Opens the **Recalculate** screen where analyses can be added, edited and applied to operation results.

List View

In List View screen results of the currently loaded operation are displayed. See section 5.6.4 “List View”, page 94 for details.

Graph View and Plate View

Graph View and Plate View are not available for analysis results. Please see the following sections for details:

- 5.6.2 “Plate View”, page 93
- 5.6.3 “Graph View”, page 93

Edit Analysis

A click on **Edit Anly.** will open the **Recalculate** screen. You can edit current analyses or, if no analysis exists yet, create a new one and recalculate based on currently loaded screen results. For further details see section 5.5 “Recalculate”, page 90.

The button **Edit Anly.** and the **Recalculate** button in the **Navigation Bar** are only enabled when a screen result has been loaded. After a recalculation, the new and unsaved **Analysis Result** can be viewed. An asterisk is displayed instead of a name in the **Global Control** section to indicate that the results have not been saved yet. The **Save** button is enabled now.

Save

The **Save** button is only enabled when the recalculated analysis result is unsaved. Click on **Save** to open the dialog with two options:

- **Save Analysis Result:** The analysis results will be saved under the entered name, and the protocol will not be changed. A comment can be added which appears 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 Analysis Sequence to Protocol:** With this option selected 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 & Details** area when the respective protocol in loaded.

Add Comment

The **Add Comment** button is enabled as soon as a screen result has been loaded. In the dialog you can chose between two options where to add the comment:

- **Add comment to current screen <name of current screen>:** This option is always enabled and selected as default. The comment will be displayed in the **Messages & Details** area when the respective screen result is loaded.
- **Add comment to current analysis result <name of current analysis result>:** This option is only enabled if the currently loaded analysis result has been already saved. The comment will be displayed in the **Messages & Details** area when the respective analysis result is loaded.

Export

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

- **CSV_PLATE, CSV_LIST:** Comma separated values formatted as plate or list
- **XML:** Format e.g. for data transfer to WorkOut, see also section 5.12 “WorkOut Data Analysis”, page 129.
- **CUSTOM:** Custom-made style sheets
- **ARCHIVE:** Offers the export of the result data together with the protocol settings as *.kal file. These files can then be imported to a different system.

For automated export of results see section 5.2.5 “Post Processing Sequence”, page 81.

5.5 Recalculate

On the **Recalculate** screen you can change a protocol's analysis sequence or define a new one if the currently loaded protocol does not include one, and recalculate. You can also change the plate map in case you only want to analyze results of some of the measured wells. On the **View Result** screen you can switch to **Recalculate** either by click on **Edit Anly.** or directly via the **Navigation Bar**.

The **Recalculate** screen is only enabled if a screen has been loaded and its name is displayed next to **Results** in the **Global Control** section.

- If analysis results have already been loaded when opening **Recalculate**, these analysis results will be displayed directly and are ready to be changed.
- If no analysis results have been loaded, a new **Analysis Sequence** can be created.

Buttons and Elements

Element	Description
Protocol	Name of the currently loaded protocol.
Result	Name of the currently loaded measurement result.
Analysis Result	Name of the currently loaded analysis result.
Plate Type	Plate type of the current protocol.
Recalc	Start recalculation. **Notice** If the protocol still contains analysis operations which have been discontinued, you first have to remove these operations. See also section 5.11 "Analysis Operations", page 116 for details.

Create a new analysis sequence

Click on the **[+]** button in the **Analysis Sequence** and select the desired analysis operations to be added. With **[–]** you can select an analysis operation to be removed from the sequence. In the **Content Area** you can define the parameters of the selected analysis operation.

Recalculation of Well Imaging analyses

You can test **Imaging** analysis results beforehand and thereby optimize the analysis parameters. Click **[+]** in the **Analysis Sequence** and select an analysis operation from the **Well Imaging** menu or select one existing analysis operation in the **Analysis Sequence**. Next to the **Plate Map** tab in the **Control Area** another tab **IMG** is opened

and you can select one of the wells on this plate to load the respective image taken during screen. Enter the appropriate parameter values and click on **Test**. Optimize the parameters until you are satisfied with the results.

Start Recalculation

A click on **Recalc** will start the analysis. Since in **Recalculate** a new analysis result is generated, the **Analysis Results** box will display an asterisk as place holder name. After execution of the recalculation the **View Results** screen is opened and the new analysis results can be saved, exported or edited again. See also section 5.4 “View Results”, page 87.

5.6 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:

- List of protocols (see section 5.6.1 “Protocol List”, page 92)
- Operation parameters (see section 5.2.2.1 “Protocol Operations”, page 58)
- Measurement results
- Analysis results

5.6.1 Protocol List

After starting Kaleido, the **Run Protocol** screen is displayed and shows the **Protocol List** with existing protocols for a quick start of measurements. If an instrument is connected, only compatible protocols will be displayed (depending on the technologies the instrument is equipped with). You can also open the list from the **Setup Protocol** and **View Results** screen (click on **Protocol […]** in **Global Control** area).

- If you click on a protocol in the list, it will be loaded and its name appears in the **Protocol** text box.
- Only on **View Results**: If you double-click a protocol, the list of existing results is displayed directly. You can then click on a screen result to load it.

Existing protocols are displayed in the protocol list including the following columns:

- In the first column **factory preset protocols** are indicated by a lock icon . Such protocols are “read-only”. However, you can edit them and then save with a different name to use them as a starting point for your protocols.

  Notice
  In Kaleido 1.0.1 the factory preset protocols were not write-protected. If you had modified and saved such a protocol, it has now been automatically renamed to “<OldName>_CUSTOM” by the Kaleido 1.2 setup, so that your changes (and possibly results) are not overwritten by the updated factory preset protocols from PerkinElmer.

- Protocol name
- Name of owner (user) who created/last edited the protocol
- Date and time of the last modification

Only the last revision of a protocol (head revision) is displayed in the protocol list. The protocol list remains visible until you select another function which uses the **Content Area** (e.g. if you select an operation).

- You can **sort the protocols** in ascending/descending order if you click the header of a column.
- You can **filter the protocols** by technology and owner in the section above the list.

5.6.2 Plate View

In the **Plate View** tab in the **Content Area** you can view the screen results (during a measurement or afterwards). The result values are displayed in the wells, and the well color is determined by these values so that you see a heatmap of your results. This way you can see immediately which samples were below, above, or within a certain range. You can configure the heatmap in the **Control Area** (see section 5.7.4 “Heatmap”, page 100). The results are already converted into technology specific units (e.g. OD, [CPS], ..) and are replaced by new results as soon as the well has been measured again in the same protocol run.

Especially looking at 384-well plate, you might want to zoom into the plate view. By scrolling with the mouse you can **zoom in and out**, the area where the cursor is located will be centered when zooming in. Further options are available via context menu (right click plate).

- **Well Imaging** analysis operations have multiple analysis results per well which cannot be displayed simultaneously in the **Plate View**. Only the values of the **primary result** parameter are displayed (specific for each analysis operation). Result values for the other parameters can only be seen in the **List View**. See also section 5.11 “Analysis Operations”, page 116.
  Notice: For imaging data no heat map is available.
- For **Label-free** measurements, an automatically calculated **Live Response** is displayed (see also section “Live Response”, page 63). Raw signal values can only be seen in the **List 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 will be copied to the clipboard as tab separated data. • **Copy all Results to Clipboard:** All (so far measured) results of the current plate will be copied to the clipboard as tab separated data. • **Copy Heatmap to Clipboard:** The whole current plate with so far measured results will be copied to clipboard as picture. • **Save Heatmap as:** A dialog opens and the whole current plate with measured results can be saved as *.png image file.

5.6.3 Graph View

In the **Plate View** tab in the **Content Area** you can view the screen results (during a measurement or afterwards). The **Graph View** tab is only enabled when the protocol includes several measurements of the same point in a well, e.g. when using Plate Repeat or measurement modes like Kinetic and Wavelength Scan. In these cases you will see a miniature plot being created for each well. For well scan, 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 zooming, copying and saving functions are the same as described in section 5.6.2 “Plate View”, page 93. Again, the well scan is an exception, since the miniature heat maps can be copied as picture, but not as tab delimited result tables. The heat map functions, as described for **Plate View** are enabled for the well scan heat maps.

For the miniature graphs you can check mark the option **Normalize to Well** to adjust the y-axis to the minimum and maximum y-values within the well. Besides the overview of all wells, you can also view one or several selected wells in detail with a click on **Show Detailed Graph** in the **Control Area**. A large diagram with the graphs of all selected wells is opened. A click in **Show points** will add points of measurement to the lines. For **Well Scan** no detailed view is available.

- Imaging analysis operations have multiple analysis results per well which cannot be displayed simultaneously in the **Graph View**. Only the values of the **primary result** parameter are displayed (specific for each analysis operation). Result values for the other parameters can only be seen in the **List View**. See also section 5.11 “Analysis Operations”, page 116.
- For Label-free measurements, the automatically calculated **Live Response** is displayed (see also section “Live Response”, page 63). Raw signal values can only be seen in the **List View**.

5.6.4 List View

On the **List View** tab in the **Content Area**, screen results (loaded on **View Results**) of currently loaded operation are displayed in a list. It shows the following properties:

- Plate Number
- Barcode
- Batch Number (is incremented when using “Continue Screen”)
- Plate Repeat (consecutively numbered, is not reset by Batch number)
- Well Repeat
- Well ID
- Row
- Column
- Further details depending on the operation and the technology and measurement mode

Results are given in units depending on technology:

- Alpha in counts
- Absorbance in OD (optical density)
- Luminescence and time-resolved fluorescence in CPS (counts per second)
- Fluorescence intensity in RFU (relative fluorescence units)
- Label-free signal and reference in [fm] of wavelength, live response in [pm] of wavelength
  See also section “Label-free (LF)”, page 62.

Analysis Result sheets vary depending on the analyses performed.

5.7 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 **Well Imaging** parameters is controlled from here.

5.7.1 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**.

5.7.1.1 Plate Map on Setup Protocol

During set up of a protocol you can select the wells to be measured with the selected technologies (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. Analyses require specific sample types which you can chose from a drop down list:

Sample Type		Abbreviation	Function
	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.
	EC50 sample	**E**	For curve fitting calculation where the results is the IC50/ED50 concentration (the half maximal concentration).
	Z Low	**ZL**	At least two replicate negative samples or samples with a low concentration for Z’ measure to determine the assay quality.

Sample Type		Abbreviation	Function
	Z High	**ZH**	At least two replicate positive samples or samples with a high concentration for Z' measure to determine the assay quality.
	LANCE Blank	**LB**	For LANCE normalization: buffer wells, measured in the acceptor channel
	LANCE Crosstalk	**LC**	For LANCE normalization: crosstalk control wells
	LANCE High	**LH**	For LANCE normalization: samples with maximum signal, measured in the acceptor channel

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 from selection** and click on the respective wells or headers.

The sample types **Control, Standard, Unknown** and **EC50** 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 after confirming a warning message, but then the plate map will be reset.

Buttons and Elements

Element	Description
Enlarge/Reduce	Will enlarge / reduce display of plate map.
Reset	Clears complete plate map from sample types.
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.

Element	Description
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, …).
Curve index	For defining more than one EC50 curve in one plate map. The curve index will be set before the sample index, e.g. first curve: E1.1, E1.2, E1.2, … second curve: E2.1, E2.2, E2.3, …. By default the curve index is 1 and changing the curve index will reset the Start index to 1.
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 names of sample types and indices in plate map.

5.7.1.2 Plate Map in Run Protocol

On the **Run Protocol** screen the plate map of the currently loaded protocol is shown but cannot be edited. The **Navigation** box displays which plate or repeat is currently being measured. Measurement progress and values are displayed in the **Content Area**.

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	Indicates which **Plate** or **Repeat** is being measured.

5.7.1.3 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, the controls beneath the plate map can be used to navigate back and forth through the results displayed in the **Content Area** (**Plate View, Graph View, List View**).

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.
Record selection boxes	For navigation through results of different plates, (well) repeats, well positions or wavelengths.

5.7.1.4 Plate Map on Recalculate

On the Recalculate screen, the plate map of the current protocol is displayed and can be edited and redefined for the recalculation, e.g. if you do not want to analyze all samples that have been measured or apply different analyses requiring different samples types. The functions are the same as for the plate map displayed on the **Setup Protocol** screen (see section 5.7.1.1 “Plate Map on Setup Protocol”, page 95).

Buttons and Elements

Element	Description
Enlarge/Reduce	Will enlarge / reduce display of plate map.
Reset	Clears complete plate map from sample types.
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, …).
Curve index	For defining more than one EC50 curve in one plate map. The curve index will be set before the sample index, e.g. first curve: E1.1, E1.2, E1.2, … second curve: E2.1, E2.2, E2.3, …. By default the curve index is 1 and changing the curve index will reset the Start index to 1.
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.

Element	Description
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 names of sample types and indices in plate map.

5.7.2 Optimize IMG Parameter

During setup of a **Well Imaging** operation, its parameters can be optimized using the **Optimize IMG Parameter** tab in the **Control Area**. After selecting in which well you want to perform the optimization, click on **Test [name of active channel]** or **Test all Channels** below the plate map. 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 **Images** tab in the **Messages & Details** section. For further details see also section “Well Imaging (IMG)”, page 61.

Buttons and Elements

Element	Description
Enlarge/Reduce	Will enlarge / reduce display of plate map.
Optimize IMG Parameter	Plate map indicating in orange which well is being measured for optimizations. Well can be chosen freely by click on different well.
Test [channel x]	Only the channel currently shown in the content area will be tested.
Test All Channels	All channels set so far will be tested.

5.7.3 Image Control

Setup Protocol

When in edit mode of **Setup Protocol** with an **IMG** operation selected, the **Image Control** below the **Plate Map** section offers several options to inspect the images generated by imaging optimization. Here the test well is specified again (**Actual Well**) and the coloring modes can be selected from a drop down list. Using a check box a fixed scale bar (500 µm) can be added or removed. For every channel tested an expander is opened below the **Display** options showing the color and the maximum intensity of the respective image. Depending on the filters selected the coloring of the images is defined (see **Buttons & Elements** section below).

By default all channels are activated and the images are overlain, but single images can be removed from the display using the respective check boxes. You can change the parameter in the **Well Imaging** operation and generate new test images until you

find the best results. Since you are still in edit mode on **Setup Protocol**, you can switch to the **Plate Map** tab anytime for editing. Also analyses can be included in the optimization of the operation parameters.

View Results

The same **Image Control** options are available in **View Results**:

- In **List View** select one result and the image will open together with the image controls.
- In **Plate View** select one well, and all images done in this well for the currently selected operation are displayed as well as the image controls.

Buttons and Elements

Element	Description
Actual Well	Coordinates of tested well.
Coloring mode	Select **Standard** (default), **Enhanced** or **Highlight** from a drop down list. Image will update immediately.
Show scale bar	Using this check box a fixed scale bar in µm can be added to or removed from the image.
Disclosure buttons of channel box	opens info box for respective channel. closes info box for respective channel.
Channel activation check box	By default all channels are activated and their images overlain. Unchecking will remove respective channels from the image display.
Color	Depending on the filters selected the coloring of the images is as follows: • UV filter: blue • Blue and green filter: green • Red filter: red • Brightfield: gray
Intensity	The maximum intensity measured in the image is specified here.

5.7.4 Heatmap

In **Plate View** and **Graph View** (only well scan), 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 5.9.2 “General Settings”, page 106).

Value	Color (Monochrome)	Color (Rainbow)
Low value	Light blue	Blue

Value	Color (Monochrome)	Color (Rainbow)
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 either by using the sliders or by changing values in the text box. Both will update each other automatically, and clicking the **Reset** button will change the values back to original values. 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. Bars can be shifted with pressed left mouse button, and Min/Max values will be updated.
Linear	Set as default scale.
Logarithmic	Check mark to see results in logarithmic scale.
Min	Editable minimum value, corresponds to left slider in color scale.
Max	Editable maximum value, corresponds to right slider in color scale.
Reset	Resets **Min** and **Max** values to automatically set values.

5.7.5 Detailed Graph

On **Run Protocol** and **View Results**, the tab **Graph View** is only enabled if more than one measurement is being performed on single wells. If you select one or multiple miniature graphs in the **Content Area** and click **Show Detailed Graph**, you can view these graphs in detail (not available for heatmaps of well scans). A detailed chart is opened in the **Content Area**. Graph colors are explained in a legend in the **Control Area**.

The y-axis corresponds to the signal measured; the x-axis corresponds with repeats, wavelengths or windows. Selecting wells in the legend will highlight respective line and vice versa (hold down [**Strg**] to select multiple wells). Activating **Show points** will add point markers to the graphs. Hovering with the cursor over the graph area shows you the exact positions on the x- and y- axis. Again, you can zoom into the graph area with the mouse wheel, whereby the position of the cursor is centered, and you can pan the area with a pressed left mouse button. The context menu offers:

- **Fit to view:** Will bring the graph area back to its original size fitting the content area.
- **Copy screenshot:** Copies the graph area to the clipboard.
- **Save screenshot:** Saves the graph area as *.png file.

Buttons and Elements

Element	Description
Show Detailed Graph/ Back to Overview	Pressing this button will show detailed and expanded version of selected miniature graphs or vice versa.
Normalize to Well	Enabled only in overview. Use this check box to adjust y-axis to the minimum and maximum values within each well.
Show points	Adds all points of measurement to the graphs.
Legend:	Displays colors of graphs with corresponding well coordinates. Graphs can be highlighted by click on respective well(s) with pressed [Strg] key.

5.8 Messages & Details

The area at the bottom of the screen is called **Messages & Details** section. Here you can find for example system notifications and comments. Images recorded by a **Well Imaging** operation are also displayed here.

5.8.1 Comments

Comments are short messages that are added to a protocol, a screen or an analysis result by Kaleido or manually by the user. Comments are listed chronologically with timestamps in the Comments tab which is always available. Once created, comments cannot be edited or removed.

How to add a comment to a protocol

When a protocol is loaded on **Setup Protocol**, the **Save…** button is enabled. In the **Save current protocol** dialog select **Save** or **Save as…** and enter your comment in the text box. Proceed with saving and the comment will appear in the **Comments** tab including a time stamp, the name of the user logged in, the version number of the protocol 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 in Kaleido in **Setup Protocol**. Further are comments listed in export files of screens.

How to add a comment to a screen or analysis result

- As soon as a screen or analysis result is loaded in **View Results**, the **Add Com.** button is enabled. A click will open the **Add Comment** dialog. With the radio buttons you select whether the comment is to be added to the screen (Add comment to current screen <number of current screen>) or to the analysis result (Add comment to the current analysis result <name of analysis result>). If no analysis result is available for a screen, 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 **Recalculate**, the comments of loaded screens and analyses are always displayed in the **Comment** tab. Comments of analysis results are displayed together with the respective screen name, i.e. the entry in the column **Calculation** indicates to which analysis result of the screen the entry in **Messages** refers. This way you have a good overview of all comments created for the screen.

Examples for automatically added comments of screens

- Screen was started
- Screen was stopped
- Screen was continued
- Analyses: count cells: D02 Image is empty or overexposed

- Autofocus failed for G3

5.8.2 Validation

This tab only appears on **Setup Protocol** and reports inconsistencies between the components in the protocol (e.g. plate type not applicable for the chosen operation or Label-free cannot be combined with a plate repeat). As soon as the inconsistency is removed, the validation message will disappear.

5.8.3 Notifications

Like comments, this property sheet is always displayed and shows notifications coming from the instrument while running a protocol or during test analysis. These notifications from the instrument are available on **View Results** later on but not saved to the screen result. Like comments, notifications are not editable.

Examples for notifications

- Analysis was completed
- Label Free set time couldn't be done
- Warning: CytoNuc failed

5.8.4 Images

The **Images** tab is used when working with **Well Imaging** operations on the **Setup Protocol** or **View Results** screen.

- **Setup Protocol:** Test images recorded for optimization will be displayed on the **Images** tab.
- **View Results:** If you select an image from the **List View** or a well in the **Plate View**, the respective images are displayed on the **Images** tab. In case of well or plate repeats you can navigate through the repeats (see also section 5.7.1.3 "Plate Map on View Results", page 97) and the image will be updated.

For both test images and results images, there is the **Image Control** section on the right side of the screen, offering information about the images and options for modifying the display of the images (show/hide channels, select colors etc.). See also section 5.7.3 "Image Control", page 99.

By scrolling with the mouse wheel you can zoom into the image where the cursor is located (cursor area will be centered). Hover with the mouse over the image, and the text box on the bottom right shows the intensity of the area where the cursor is located for each channel. Further options like copying and saving images are available in the context menu (right-click).

Context Menu

Element	Description
Copy to Clipboard	Image as displayed in tab (single channel or overlay of channels) is copied to the clipboard.

Element	Description
Zoom to Fit	Image view is reset (total view).
Save as…	A dialog opens and the image as displayed (single channel or overlay of channels) can be saved in different formats.
Export Raw Image	Each of the displayed channel images is saved as a separate raw file (*.tiff images). The same format is used when images are archived (see section 5.9.3.6 “Archive Images”, page 109).

5.8.5 Results

The **Results** tab is created as soon as a test of a **Well Imaging** analysis is performed. All output parameters of the respective analysis are listed here with the result values. The values will be overwritten with every new analysis test. Test results are not saved and will be discarded when you e.g. switch to another operation and back during setup.

5.9 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.

Please note that certain functions are not available for all users. For details see section 5.13.3 “Restricted Actions”, page 134.

5.9.1 Instrument Options

This dialog lists various properties of the connected instrument (read-only).

- Serial number
- Kaleido software version
- Available reading positions of the barcode reader
- Installed instrument options and technologies

5.9.2 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 5.7.4 “Heatmap”, page 100).

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

5.9.3 Data Management

5.9.3.1 Backup Database

You can use this function to make a backup of all protocols and screen 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 5.9.3.2 “Restore Database”, page 107).

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 your database and click **Next >>**.
3. 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.
4. When the backup is finished, click **Finish** to close the wizard.

5.9.3.2 Restore Database

Using this function you can restore protocols and results from a previously created database backup file (see section 5.9.3.1 “Backup Database”, page 107). The backup will overwrite your current database.

Notice
If a backup is restored, all existing protocols and screen 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 your database and click **Next >>**.
3. Select the backup file. If you click the "**...**" button, you can browse for the file. Click **Next >>**.
4. 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.
5. Click **Yes** to confirm the selection and restore the database.
6. When the process is finished, click **Finish**.

   Kaleido is closed and restarted automatically. After logging in you can work with the restored database.

5.9.3.3 Import Protocol / Screen

Using this function you can import a protocol or screen 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 screen (then it contains protocol + results). See also the following sections:

- Export protocol: 5.2 "Setup Protocol", page 53 (save dialog)
- Export protocol + results (manually): 5.4 "View Results", page 87
- Export protocol + results (automatically): 5.2.5 "Post Processing Sequence", page 81

How to import a protocol/screen

1. Open **Settings > Data Management** and click the **Import Protocol / Screen** 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 necessary, you can edit the name. The protocol name has to be unique and must not exist in the database yet.
4. If the import file also contains screen results, the **Screen Start Time** is displayed and you have the following options for the import:
   - **Import Screen** checked: Import protocol and screen
   - **Import Screen** unchecked: Import protocol only
5. Click **OK** to start the import.

5.9.3.4 Database Info

After opening the dialog you have to select your database and click **OK**. The following status information will be displayed:

- Used space in database
- Free space in database
- Time stamp of last backup

5.9.3.5 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.

5.9.3.6 Archive Images

This dialog is only visible if the connected instrument is equipped with the Imaging technology. The user can define a path where all recorded images will be saved permanently. This option can be switched on (default) and off.

Notice
Images fill up disk space very rapidly. Check the available free disk space before starting an imaging run.

Buttons and Elements

Element	Description
Save Images	**On:** The images will be saved into the cache folder AND into the **Image archive folder** (see below). If the next screen starts they will be deleted from the cache folder but not from the Image archive folder. **Off:** The images will be saved only temporarily and deleted again if the next screen is started.
Image archive folder	Allows you to specify the path of the Image archive folder. Enter a UNC path or click **"..."** to browse for a folder.
Cancel	Close the dialog discarding any changes.
OK	Close the dialog and apply changes.

5.9.4 Barcode Reader

In this dialog you can switch barcode reading on or off and define where the barcode is read.

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.

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	The barcode can be read on all four sides of the plate. • Select **one** of the four sides (options 1-4). 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. • **Enter barcode manually:** You will be prompted to enter a barcode manually.

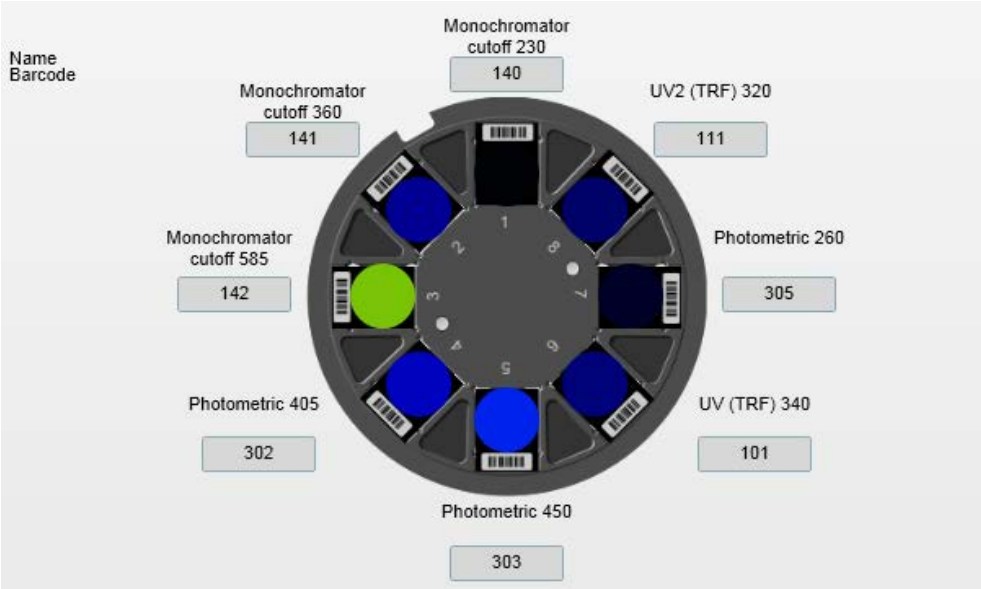
5.9.5 Inventory

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.

5.9.5.1 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 5.9.5.3 “Define Filter”, page 112.

If Kaleido is not connected to the instrument (simulation mode), a pre-defined virtual filter configuration will be displayed.



Filters available in simulation mode

5.9.5.2 Plate Type

The Plate Type Wizard can be used to create new plate types or to edit existing user-defined plate types. The factory preset plate types cannot be edited.

How to use the Plate Type Wizard

1. Open **Settings > Inventory** and click the **Plate Type** icon.
2. Select **New** and click **Next >>**.

   Alternative options:

   - If you choose **New based on**, you can select an existing plate type and use its parameters as a starting point.
   - If you choose **Edit**, you can select and modify an existing user-defined 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. Mark all operations which can be used with the new plate type. Click **Next >>**.
11. Enter a unique name for the plate type.

    If you edit an existing plate type, the current plate type name will be displayed (read-only).
12. Click **Finish** to complete the wizard.

    The plate type will be saved in the inventory database.

How to update protocols after editing a plate type

If you edit a plate type, protocols using this plate type will not be updated automatically. To apply the changes, perform the following steps:

1. Load the corresponding protocol and click **Edit**.
2. Reload the plate type.
3. **Save** the protocol.

5.9.5.3 Define Filter

A wizard helps you to create new filters or edit existing user-defined filters to be used in the excitation filter wheel. The factory preset filters cannot be edited.

How to use the Filter Wizard

1. Open **Settings > Inventory** and click the **Filter** icon.
2. Select **New** and click **Next >>**.

   Alternative option: If you choose **Edit**, you can select and modify an existing user-defined filter.
3. Enter the barcode of the new filter (three digit number) and click **Next >>**. This barcode must not exist in the database yet.

   If you edit a filter, the barcode is only displayed and cannot be changed.
4. Enter the filter properties.
5. Mark the operation(s) for which the filter is suitable. Click **Next >>**.
6. Enter a unique name for the filter.

   If you edit a filter, the existing filter name is displayed.
7. Click **Finish** to complete the wizard.

   The filter will be saved in the inventory database.

How to update protocols after editing a filter

If you edit a filter, protocols using this filter will not be updated automatically. To apply the changes, perform the following steps:

1. Load the corresponding protocol and click **Edit**.
2. Reload the filter.
3. **Save** the protocol.

5.9.6 Temperature (Settings)

This dialog allows you adjust the general temperature control.

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 68.

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 Adjustment

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 affect 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.

Element	Description
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, the instrument will be cooled down (by normal ventilation).
Fast Cooling	Enables faster cooling by opening the plate door and activating additional fans inside the chamber. It is recommend to remove the plate from the plate carrier before the fast cooling is activated in order to avoid unnecessary evaporation, contamination or light exposure of the samples. Fast cooling is automatically deactivated ... • if the target temperature is reached. • if the temperature stabilizes at its minimum temperature (i.e. ambient temperature +2 °C). • if you uncheck this option.
Turn Temperature Adjustment 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).

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. Temperature [°C]	Temperature difference between upper and lower heater (value range: 0- 4 °C, 0.1 °C steps).

5.10 Help

Click the help icon in the **Navigation Bar** to open the online help for EnSight and Kaleido (welcome page).

5.11 Analysis Operations

Well Imaging analysis operations take the acquired images as an input and calculate numerical results, e.g. the cell number in the image or a fluorescent marker intensity.

Unlike all other technologies, imaging returns multiple results per well, e.g. the cell count, the mean cell area and the mean cell intensity at the same time. Since Kaleido is only capable of displaying one result per well in the **Plate View** display (**View Results** screen), only the first imaging result is shown (primary result). In the **List View** representation all results per well are shown.

While setting up the parameters of an imaging analysis operation it should be tested on test images using the **Test Analysis** button (see section "Well Imaging (IMG)", page 61). As a result the detected objects are shown on the test image and the numerical results are shown on the **Results** tab. Input parameters can be adjusted until the desired result is achieved.

As a last step the **Well Imaging** analysis operation can be saved to the protocol or immediately applied to an existing screen result on the **Recalculate** screen (provided that the images of the screen result have been saved).

Screen images are only stored when **Archive Images** is activated in the **Settings** (see section 5.9.3.6 "Archive Images", page 109). Otherwise the images of previous screens will be overwritten.

Currently there are three analysis operations available for **Well Imaging**. Please see the following sections for a detailed description.

5.11.1 Brightfield Confluency

This analysis operation is designed to work on a brightfield image, i.e. no fluorescent cell staining is required. If the analyzed **IMG** operation also includes channels with fluorescence light sources, these do not disturb the analysis operation but are automatically excluded. The analysis uses the intensity "roughness" to discriminate between empty image areas (background) and areas covered by cells. Areas with low roughness are considered as background. Areas with roughness exceeding an adjustable threshold are considered as cell area.

Brightfield Confluency Parameters

Input Parameter	Description
Source	Select the image source for the analysis. All **Well Imaging** operations in the current protocol are listed in a drop down menu.
Minimum Cell Area [µm²]	Minimum cell area in square micrometers. Foreground areas smaller than this are discarded. Typical values are in the range 100 to 1000 µm². The default value is 400 µm².

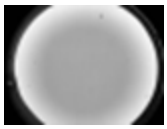
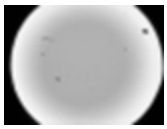
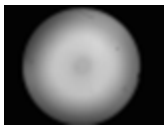
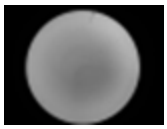
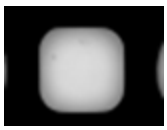
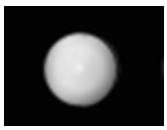
Input Parameter	Description
Contrast Threshold	Areas with roughness higher than this threshold are considered as cell area. Areas with roughness smaller than this are considered as background. Typical values are in the range of 20 to 150. Default is 30.

Output Parameter	Description
Confluency [%] (primary result)	Confluency of cells, i.e. percentage of the analyzed area which is covered by cells.
Foreground Roughness Mean	Mean roughness value in the area covered by cells (foreground).
Foreground Roughness SD	Standard deviation of the roughness value in the area covered by cells (foreground).
Background Roughness Mean	Mean roughness value in the background area.
Background Roughness SD	Standard deviation of the roughness value in the background area.
Total Roughness Mean	Mean roughness value over the whole analyzed area.
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.
Used Well Area [px²]	Area that has been used for the analysis.

5.11.2 Count Cells – Version 1.3

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 first step of the analysis is to detect the well outlines to define the region of interest (ROI) for object 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. Here are 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	2.50	

The second step is to detect objects inside the detected well region (ROI). There are two different detection methods available:

- **Method a:** Lower sensitivity. Low contrast objects may be missed, but higher robustness against false positive detected objects.
- **Method b:** Higher sensitivity. More low contrast objects are detected, but higher risk of false positive objects.

For most assays method b (default) works best. To identify the best settings for your specific assay, visually test both 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.

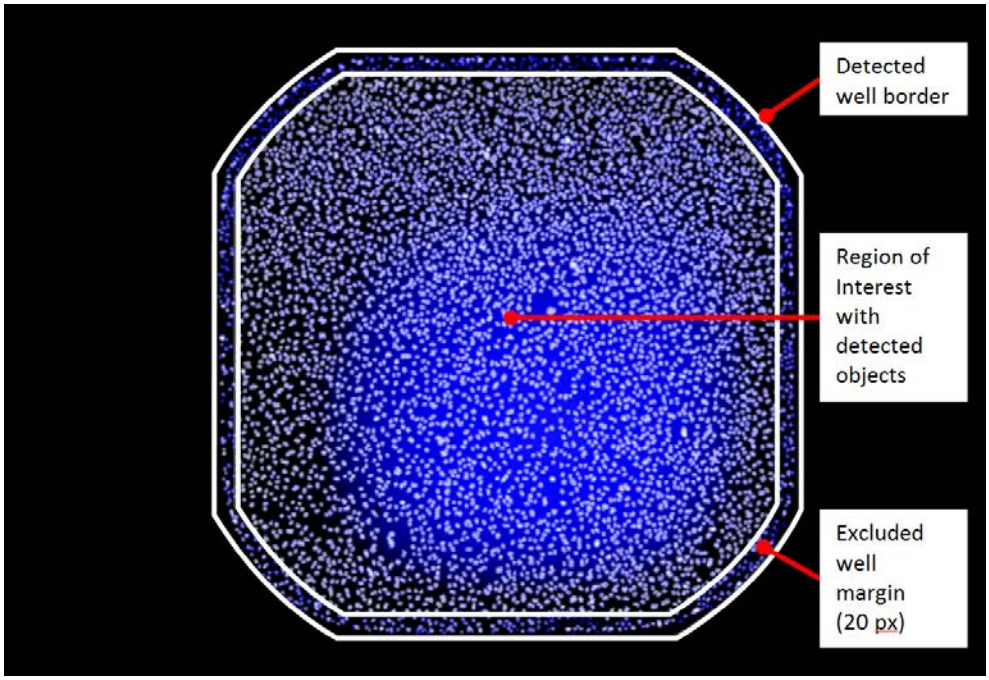
The third step is the calculation of object properties like intensity and size, removal of objects that do not meet specific criteria (e.g. minimum size), and reporting of the results (e.g. median area of the detected objects).

Count Cells – Version 1.3 Parameters

Input Parameter	Description
Source	Select the image source for the analysis. All **Well Imaging** operations in the current protocol are listed in a drop down menu.
Channel	Fluorescence imaging channel for object detection. Alternatively, a digital phase imaging channel can be used.
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.
Object Detection Method	• Method “a” – lower object detection sensitivity but stricter filtering for false positive objects. • Method “b” – method with higher object detection sensitivity (default).
Well Detection Method	Standard or Fast. Standard is more reliable but slower.
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 below. Default value: 20 px.
Parameters only needed to process legacy data:	

Input Parameter	Description
Well Shape	• "Use Plate Definition" Uses the value stored in the image during acquisition (default). • "Round – Manually Specified" Please enter a manually specified diameter. • "Square – Manually Specified" Please enter a manually specified diameter (= side length of the square well).
Manually Specified Diameter [mm]	Needed to evaluate legacy data without well diameter available in the images (acquired with Kaleido versions <1.2). In this case the diameter and well shape can be manually specified in the analysis. Default value is 0.0. If the specified value is bigger than 8 mm the whole image is used as well area. In this case the "Excluded Well Margin [px]" value is subtracted from the whole image area to determine the region of interest for object detection.

Output Parameter	Description
Number of Objects per Well (primary result)	Number of counted objects extrapolated to the full nominal well area.
Number of Counted Objects	Objects counted in the region of interest.
Number of Objects per Area [1/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.
Median Object Intensity	Median object intensity of all detected objects.
Error Code	• 0 – No error • 1 – Well detection quality check failed, detected well outlines might be incorrect. • 2 – Object detection failed, see **Notification** pane for further information and instructions.



5.11.3 CytoNuc – Version 1.3

This analysis is designed to detect objects and measure intensities inside and in the surrounding of each object. This allows e.g. to quantify the translocation of a fluorescently labeled protein from the cytoplasm of a cell into the nucleus of the cell.

Object detection and intensity measurement can be done in two separate channels, e.g. HOECHST 33342 for object detection and Alexa 488 for intensity measurement of a fluorescently labeled protein.

The location and size of the regions inside and outside the object can be adapted by a number of input parameters.

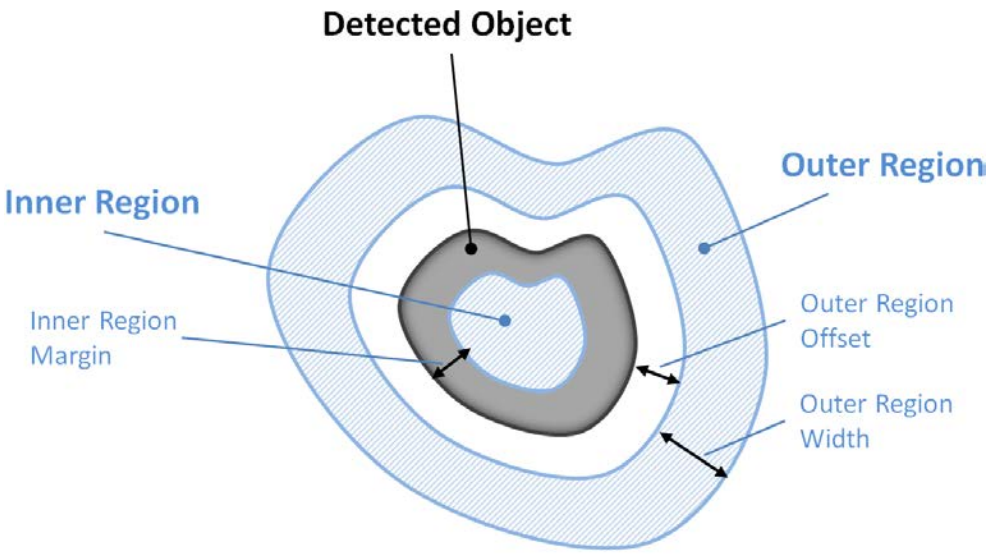
CytoNuc – Version 1.3 Parameters

Input Parameter	Description
Source	Select the image source for the analysis. All **Well Imaging** operations in the current protocol are listed in a drop down menu.
First Channel (Object Detection)	Fluorescence or digital phase imaging channel used for object detection.
Second Channel (Signal)	Fluorescence imaging channel used for intensity measurement in inner and outer region.
Minimum Object Area [µm²]	Objects with an area smaller than this are discarded. Typical values are in the range of 30 to 300 µm². Default value is 50 µm².

Input Parameter	Description
Object Detection Method	• "a" – lower object detection sensitivity but stricter filtering for false positive objects. • "b" – method with higher object detection sensitivity.
Inner Region Margin [px]	Margin from detected object border. Default: 0. See figure below.
Outer Region Offset [px]	Distance of the outer region from the object border. Default: 1 px. See figure below.
Outer Region Width [px]	Width of the outer region around the object. Default: 2 px. See figure below.
Well Detection Method	Standard or Fast. Standard is more reliable but slower.
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 in section 5.11.3 "CytoNuc – Version 1.3", page 121. Default value: 20 px.
Options only needed to process legacy data:	
Well Shape	• "Use Plate Definition" Uses the value stored in the image during acquisition (default). • "Round – Manually Specified" Please enter a manually specified diameter. • "Square – Manually Specified" Please enter a manually specified diameter (= side length of the square well).
Manually Specified Diameter [mm]	Needed to evaluate legacy data without well diameter available in the images (acquired with Kaleido versions <1.2). In this case the diameter and well shape can be manually specified in the analysis. Default value is 0.0. If the specified value is bigger than 8 mm the whole image is used as well area. In this case the "Excluded Well Margin [px]" value is subtracted from the whole image area to determine the region of interest for object detection.

Output Parameter	Description
Median Inner Intensity (primary result)	Signal intensity in the inner region.
Median Outer Intensity	Signal intensity in the outer region.
Median Intensity Ratio	Intensity ratio between background corrected signal in inner and outer region: (Inner Intensity - Background Intensity) / (Outer Intensity - Background Intensity).

Output Parameter	Description
Number of Objects per Well	Number of counted objects extrapolated to the full nominal well area.
Number of Counted Objects	Objects counted in the region of interest.
Number of Objects per Area [1/mm²]	Number of objects counted in the region of interest devided by the area of the region of interest.
Median Object Area [µm²]	Median area of all detected objects.
Median Object Intensity	Median intensity of all detected objects (in object detection channel).
Median Background Intensity	Median background intensity of the signal channel over all objects. The individual background intensity for an object is determined in a fixed ring region around the object starting at **Outer Region Offset** + 5 px and a width of **Outer Region Width**. In case any region has zero area (because the object is completely surrounded by neighbor objects) the object intensity is not taken into account in any intensity calculations.
Error Code	• 0 – No error • 1 – Well detection quality check failed, detected well outlines might be incorrect. • 2 – Object detection failed, see **Notification** pane for further information and instructions.



5.11.4 Deprecated Versions

5.11.4.1 Count Cells – Version 1.2 (Deprecated)

This analysis is included for backward compatibility only. This allows running existing protocols which use this analysis. Version 1.2 will be removed in future versions of Kaleido. For new protocols use 5.11.2 “Count Cells – Version 1.3”, page 118.

Compared to the old “Count Cells” analysis (see section 5.11.4.2 “Count Cells (Deprecated)”, page 125) the detection algorithm has been modified to provide better sensitivity for object detection. Objects which were missed before can now be detected. A new parameter “Detection Method” allows to select between two variants: method “a” (lower sensitivity) and “b” higher sensitivity (default). In case too many false positive objects are detected, e.g. due to very high background fluorescence areas, switch to method “a” for a stricter filtering of objects. Method “a” gives similar (but not identical) results as the old Count Cells analysis.

Count Cells – Version 1.2 Parameters

Input Parameter	Description
Source	Select the image source for the analysis. All Well Imaging operations in the current protocol are listed in a drop down menu.
Channel	Fluorescence imaging channel used for cell detection. It is assumed that the nuclei have been stained. Alternatively, a brightfield channel with digital phase imaging.
Minimum Object Area [µm²]	Minimum area of object to count in square micrometers. Bright areas smaller than this are not considered an object. Typical values are in the range of 30 to 300 µm².
Detection Method	• “a” – lower object detection sensitivity but stricter filtering for false positive objects • “b” – method with higher object detection sensitivity

Output Parameter	Description
Number of Objects (primary result)	Number of counted objects in analyzed well area (typically between 0 and 6000).
Number of Solitary Objects	Objects that are well separated from adjacent objects.
Median Area [µm²]	Typical size of an object. Typical values are in the range of 30 to 400 µm².
Median Intensity	Typical intensity of the objects counted. Typical values are in the range of 10 to 3000.
Confluency [%]	Fraction of well covered by stained objects.
Number of objects per mm²	Number of objects normalized to the analyzed area.

5.11.4.2 Count Cells (Deprecated)

This analysis is included for backward compatibility only. This allows running existing protocols which use this analysis. This analysis will be removed in future versions of Kaleido. For new protocols use 5.11.2 “Count Cells – Version 1.3”, page 118.

This analysis was designed for stained nuclei but works for stained cells as long as they are split. Therefore all validly counted stains in this analysis are referred to as objects. The size limit of what is to be counted can be set in the parameters.

Count Cells Parameters

Input Parameter	Description
Source	Select the image source for the analysis. All Well Imaging operations in the current protocol are listed in a drop down menu.
Channel	Fluorescence imaging channel used for cell detection. It is assumed that the nuclei have been stained. Alternatively, a brightfield channel with digital phase imaging.
Minimum Object Area [µm²]	Minimum area of object to count in square micrometers. Bright areas smaller than this are not considered an object. Typical values are in the range of 30 to 300 µm2.

Output Parameter	Description
Number of Objects (primary result)	Number of counted objects in analyzed well area (typically between 0 and 6000).
Number of Solitary Objects	Objects that are well separated from adjacent objects.
Median Area [µm²]	Typical size of an object. Typical values are in the range of 30 to 400 µm².
Median Intensity	Typical intensity of the objects counted. Typical values are in the range of 10 to 3000.
Confluency [%]	Fraction of well covered by stained objects.

5.11.4.3 CytoNuc – Version 1.2 (Deprecated)

This analysis is included for backward compatibility only. This allows running existing protocols using this analysis. Version 1.2 will be removed in future versions of Kaleido. For new protocols use 5.11.3 “CytoNuc – Version 1.3”, page 121.

Compared to the old “CytoNuc” analysis (see section 5.11.4.4 “CytoNuc (Deprecated)”, page 127) the detection algorithm has been modified to provide better sensitivity for object detection. Objects which were missed before can now be detected. A new parameter “Detection Method” allows to select between two variants: method “a” (lower sensitivity) and “b” higher sensitivity (default). In case too many false positive objects are detected, e.g. due to very high background fluorescence areas, switch to method “a” for a stricter filtering of objects. Method “a” gives similar (but not identical) results as the old CytoNuc analysis.

CytoNuc – Version 1.2 Parameters

Input Parameter	Description
Source	Select the image source for the analysis. All Well Imaging operations in the current protocol are listed in a drop down menu.
First Channel (nucleus)	Fluorescence imaging channel used for nuclei detection. It is assumed that the nuclei have been stained. Alternatively, a brightfield channel with digital phase imaging can be used.
Second Channel (signal)	Fluorescence imaging channel used for signal detection, the intensity of which is calculated both inside the nucleus and in a ring area around it, separated by a gap.
Minimum Nucleus Area [µm²]	Minimum nucleus area in square micrometers. Bright areas smaller than this are not considered a nuclei. Typical values are in the range of 30 to 300 µm².
Detection Method	"a" – lower object detection sensitivity but stricter filtering for false positive objects. "b" – method with higher object detection sensitivity
Margin [px]	Width of a ring around the nucleus that is used for intensity comparison; typical values are in the range of 1 to 10 pixels.
Inner Gap [px]	Width of the nucleus inner border that is excluded from the analysis. Positive values shrink the nucleus, negative expand it. Typical values are in the range of -3 to 3 pixels.
Outer Gap [px]	Distance of the margin ring from the original nucleus border. Positive values move the ring further out, negative further in. Typical values are in the range of -3 to 3 pixels.

Output Parameter	Description
Median Inner Intensity (primary result)	Marker intensity inside the nuclear area. Typical values are in the range of 100 to 3000.
Number of Objects	Number of counted nuclei in analyzed well area. Typical values are in the range of 0 to 6000.
Number of Solitary Objects	Nuclei that are well separated from adjacent nuclei.
Number of Objects per mm²	Number of objects normalized to the analyzed area.
Median Area [µm²]	Typical size of a nucleus, normally in the range of 30 to 400 µm².

Output Parameter	Description
Median Reference Intensity	Typical intensity of the objects counted. Typical values are in the range of 10 to 3000.
Median Outer Intensity	Marker intensity inside the ring area, typically between 100 and 3000.
Median Background Intensity	Marker intensity the background. Typical values are in the range of 0 to 1000.
Median Intensity Ratio	Ratio between inner (nuclear) and outer (cytoplasmic) intensity.

5.11.4.4 CytoNuc (Deprecated)

This analysis is included for backward compatibility only. This allows running existing protocols using this analysis. “CytoNuc” will be removed in future versions of Kaleido. For new protocols use 5.11.3 “CytoNuc – Version 1.3”, page 121.

Statistics of intensities discriminating between nuclear (inner) and cytoplasmic (outer) compartments. Cell segmentation is based on stained nuclei and a ring around the nuclear region which can be adjusted using input the parameter. The ring covers the outer cytoplasmic region. For each cell, the local background is subtracted from inner and outer intensities before calculating the intensity ratio.

CytoNuc Parameters

Input Parameter	Description
Source	Select the image source for the analysis. All Well Imaging operations in the current protocol are listed in a drop down menu.
First Channel (nucleus)	Fluorescence imaging channel used for nuclei detection. It is assumed that the nuclei have been stained. Alternatively, a brightfield channel with digital phase imaging can be used.
Second Channel (signal)	Fluorescence imaging channel used for signal detection, the intensity of which is calculated both inside the nucleus and in a ring area around it, separated by a gap.
Minimum Nucleus Area [µm²]	Minimum nucleus area in square micrometers. Bright areas smaller than this are not considered a nuclei. Typical values are in the range of 30 to 300 µm².
Margin [px]	Width of a ring around the nucleus that is used for intensity comparison; typical values are in the range of 1 to 10 pixels.
Inner Gap [px]	Width of the nucleus inner border that is excluded from the analysis. Positive values shrink the nucleus, negative expand it. Typical values are in the range of -3 to 3 pixels.

Input Parameter	Description
Outer Gap [px]	Distance of the margin ring from the original nucleus border. Positive values move the ring further out, negative further in. Typical values are in the range of -3 to 3 pixels.

Output Parameter	Description
Number of Nuclei (primary result)	Number of counted nuclei in analyzed well area. Typical values are in the range of 0 to 6000.
Number of Solitary Nuclei	Nuclei that are well separated from adjacent nuclei.
Median Area [µm³]	Typical size of a nucleus, normally in the range of 30 to 400 µm².
Median Nucleus Intensity	Typical intensity of the objects counted. Typical values are in the range of 10 to 3000.
Median Inner Intensity	Marker intensity inside the nuclear area. Typical values are in the range of 100 to 3000.
Median Outer Intensity	Marker intensity inside the ring area, typically between 100 and 3000.
Median Background	Marker intensity the background. Typical values are in the range of 0 to 1000.
Median Intensity Ratio	Ratio between inner (nuclear)and outer (cytoplasmic) intensity.

5.12 WorkOut Data Analysis

The **WorkOut Plus MMD** data analysis software is now bundled with Kaleido. The software is able to import Kaleido XML files for further data analysis. WorkOut Plus MMD can be used to analyze data exported from Kaleido. This includes:

- numerical measurement results (resulting from MMD operations)
- numerical analysis results (resulting from imaging analysis operations)

There are multiple ways to transfer the data to WorkOut, from manual import to fully automated import including data analysis and report using pre-defined WorkOut protocols.

Notice

The video tutorials on this web page help you getting started with **WorkOut Plus MMD** and explain how to set up the **Folder Poll** tool for fully automated import of Kaleido XML files (see section “Working with EnSight”):

- http://www.myassays.com/workout-plus-mmd

See also the user manuals on the **WorkOut DVD** for detailed information.

5.12.1 Manual Export of Kaldeido XML Files

1. On the **View Results** screen, load the screen results which you want to analyze in WorkOut.
2. Click **Export** and save the results in XML format.
3. Import the Kaleido XML file in WorkOut:
   - Create a new WorkOut protocol (*.apr) for importing the results.
   - Or use one of the pre-defined WorkOut protocols (EnSight *.apr) which is suitable for the used measurement operation(s).

5.12.2 Automated Export after Measurement

If you add a **Post Processing Sequence** to your protocol, you can automatically export the screen 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 screen results will automatically be exported to the specified folder in XML format. You can then import the files manually in WorkOut. Alternatively, you can configure an automated import using the **Folder Poll Manager** (see next section).

5.12.3 Automated Import in WorkOut

Folder Poll is a separately installed software tool (see WorkOut Setup DVD) 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 WorkOut protocol (*.apr) which has been assigned for this purpose. Depending on the WorkOut protocol, the measurement can be imported and displayed, but you could also let WorkOut automatically analyze the results and generate a report.

For a demonstration and further details see also the video tutorials linked above.

5.12.3.1 Kaleido Output Folders (Default)

There are two default output folders for Kaleido XML exports:

- *C:\ProgramData\PerkinElmer\Kaleido\Workout**Assay development***
  This folder is intended for measurements which shall only be imported and displayed (WorkOut protocol without analysis). You can then start to analyze the data in WorkOut manually.
- *C:\ProgramData\PerkinElmer\Kaleido\Workout**Screening***
  This folder is intended for measurements which shall be imported and analyzed automatically (WorkOut 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**.

5.12.3.2 Example Protocols in Kaleido

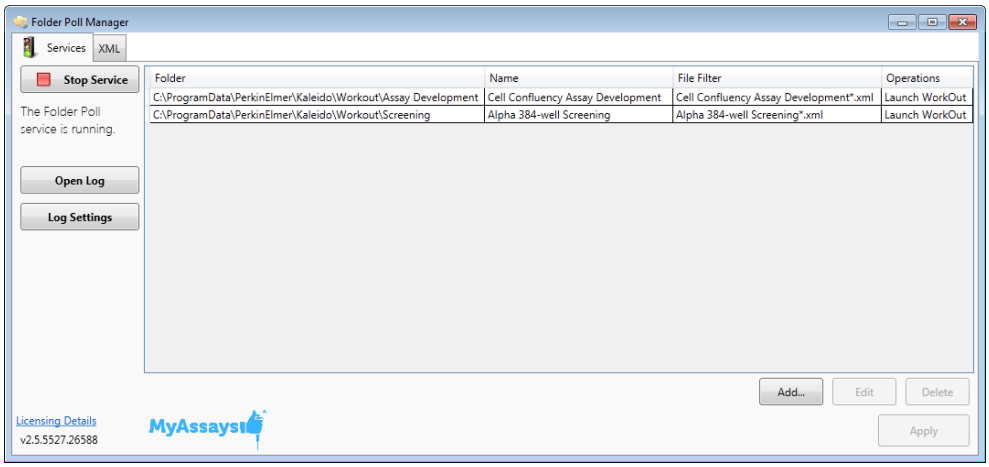
Kaleido comes with two factory-preset protocols which demonstrate the automated data transfer to WorkOut. If WorkOut and Folder Poll have been installed and you run one of these protocols, the results will automatically be opened in WorkOut.

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 WorkOut protocol.

- *Cell Confluency Assay Development* (without analysis)
- *Alpha 384 well Screening* (with analysis)

5.12.3.3 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 WorkOut.



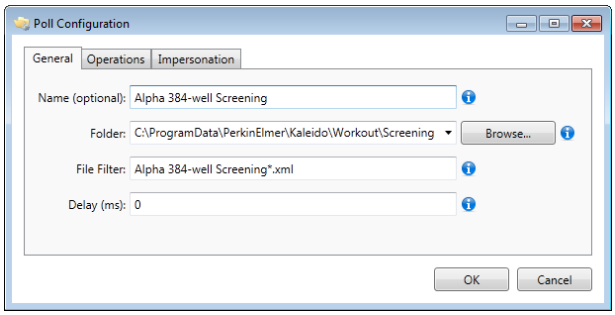
You can add and configure your own folder polls to configure the automated transfer for other protocols.

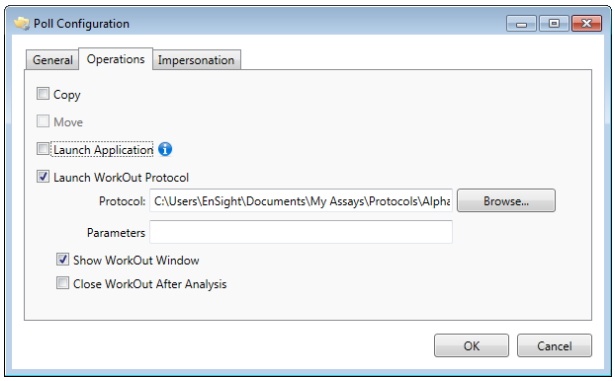
How to setup your own automated transfer to WorkOut

1. Add a **Post Processing Sequence** to your protocol so that an XML file is automatically exported after measurement (see section 5.12.2 “Automated Export after Measurement”, page 129).
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 WorkOut, depending on the selected naming pattern for your XML file.
4. Select a WorkOut protocol (*.apr) which shall be used to import the data. This can be one of the provided default protocols (EnSight *.apr) or a protocol which you have already prepared and saved in WorkOut.

Notice

- Please note that the selected WorkOut protocol has to be compatible to the measurement in terms of plate format and number of measurements.
- WorkOut 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 WorkOut (Options – Data), e.g. a common folder for multiple users. See WorkOut Plus MMD User’s Guide for details.
- If the EnSight is used in automation mode (controlled by an external scheduler) and an automated transfer to WorkOut has been configured, you should activate the option **Close WorkOut After Analysis** for the corresponding poll operation in the Folder Poll Manager. Otherwise a new instance of WorkOut 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 screen results will be transferred automatically to WorkOut.

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

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

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 Users

Creating new 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 the user group he belongs to:

Restricted Actions	Kaleido_ Administrators	Kaleido_ Editors	Kaleido_ Operators
Create Protocol. This includes: • Option ”Save as ...“ of protocols in non-edit-mode • Import protocol/screen	Yes	Yes	No
Run Protocol	Yes	Yes	Yes
Edit Protocol. This includes: • Optimizations • Add comments to protocol (save dialog) • Within Recalculation: save edited analysis sequences to protocol	Yes	Yes	No
Recalculate	Yes	Yes	Yes
View and edit basic settings. This includes: • View database information • View instrument options • View Filter Wheel configuration • Edit General Settings • Edit Barcode Settings • Edit Temperature Settings	Yes	Yes	Yes
Backup Database	Yes	Yes	Yes
Restore Database	Yes	No	No
Edit Data-Management Settings: • Change settings of a scheduled task regarding Backup Database • Change settings of Archive Images	Yes	No	No

Restricted Actions	Kaleido_ Administrators	Kaleido_ Editors	Kaleido_ Operators
Plate Type Wizard: • Create new plate types • Edit existing plate types	Yes	Yes	No
Filter Wizard: • Create new filter • Edit existing filter	Yes	Yes	No

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 or logical error detected). A tooltip explains how to enter the data correctly. Please correct the entry before proceeding.

All other errors will be reported on the **Notifications** tab (**Messages & Details** area). For details see section 5.8.3 “Notifications”, page 104.

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 5.9.5.1 “Filter Wheel”, page 111.

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.
- You can remove dust by means of very clean and dry compressed air or special canned air for optics cleaning.

Notice

Do not direct the air flow towards the opened plate loading door to prevent dust from getting into the instrument.

- 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

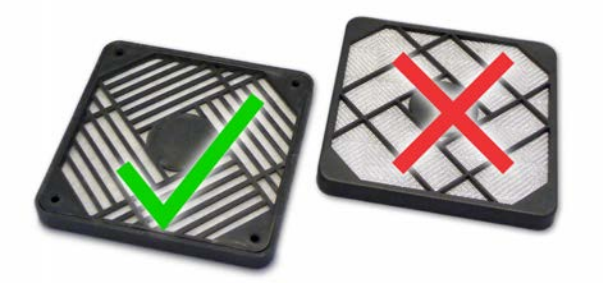
Cleaning/changing the central air filters

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.*

1. Prepare the instrument for transport using the corresponding software tool (*Windows® Start Menu – PerkinElmer – Kaleido 1.2 – Prepare for Transport*). Follow the instructions on screen and load the transport plate.

2. Remove the magnetic cover of the instrument's base plate (right-hand side).

3. Pull the handles out of the storage.
4. Remove the caps of the threaded holes on both sides of the base plate.
5. Screw the four handles completely into the threaded holes (see right figure).
6. Lift the instrument carefully using the four handles (at least 2 persons required).
7. After moving the instrument, unscrew the handles, put them back into the storage and mount all caps and the magnetic cover.
8. When you start the instrument for the first time after the transport, the plate carrier will be ejected automatically. 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

- Height:
  - Closed: 460 mm
  - Opened: 610 mm
  - For Service: 716 mm
- Width: 564 mm
- Depth: 632 mm
- Weight: about 75 kg (fully loaded)

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® 7, SP1, 64bit, English (Windows® 8/10 is not supported)

Component	Description
CPU	≥ Intel® Core™ i5-4670
RAM	≥ 4 GB RAM
Harddisk	≥ 1 TB HDD
Expansion slot	PCIe x16 (full height)
Monitor	≥ 24“

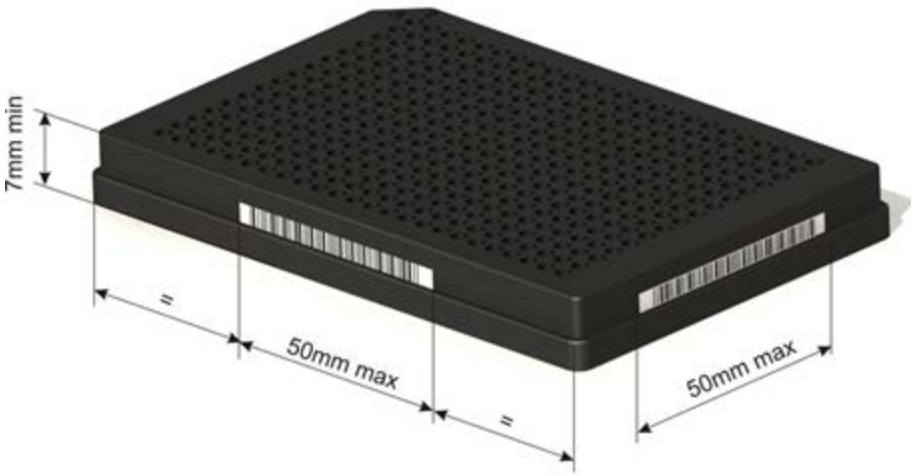
7.6 Plates

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. Both opaque and clear plates are suitable.

- For photometric measurements a clear bottom is required.
- Lable-free technology requires special Label-free plates (see also section 4.11.1 “Label-free”, page 45).
- Imaging can only be used with 96 and 384 well plates and requires an imaging compatible clear bottom.

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 all four sides of a microplate



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
Well Imaging	For fluorescence excitation: • 3 or 4 different high power LEDs • Wavelengths: 375 / 470 / (530) / 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
Well 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
Well Imaging (fluorescence excitation)	Bottom	Bottom
Well Imaging (transmission/brightfield)	Top	Bottom

7.11 Temperature Control

- Maximum temperature: 65 °C
- Minimum temperature: ambient temperature + 2 °C (heating only)
- Temperature accuracy: ± 1 °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 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	
Well Imaging		PIMARY dichroic filter set	SECONDARY dichroic filter set (opt.)
	385	413-443	413-498
	470	499-604	–
	530	–	555-604
	632	664-750	664-750
	735 (Brightfield)		

7.15 Performance

7.15.1 Photometric Performance With Monochromators

- Wavelength range: 230-1000 nm
- Wavelength selection: monochromator, tunable in 0.5 nm increments
- Photometric resolution: 0.001 OD

7.15.2 Fluorescence Intensity Performance With Monochromators

- Wavelength selection: monochromators, tunable in 0.5 nm increments

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® 7, SP1, 64bit, English (Windows® 8/10 is not supported)
CPU	≥ Intel® Core™ i5-4670
RAM	≥ 4 GB RAM
Harddisk	≥ 1 TB HDD
Expansion slot	PCIe x16 (full height)
Monitor	≥ 24“

8.2 Computer Systems

8.2.1 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.

- The usage of any additional network adapter of any computer of the “EnSight System” is not supported.

8.2.2 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.

8.2.3 Operating System, Software & Software Applications

Problems with software should be reported to the PerkinElmer Service.

1. Supported operating system: Windows® 7, SP1, 64 bit, English
   - 32 bit operating systems are generally not supported.
   - The 64 bit versions of Windows® 10, Windows® 8, Windows Vista® and Windows® XP are not supported.
   - Using any virtual PC (e.g. VMWare, Virtual-PC) is not supported.
2. Set “Automatic Microsoft Updates” to “Turn Off Automatic Updates” or “Notify me but don´t automatically download and install them.” because they may disturb the “EnSight System”.
3. Deactivating the virtual memory for Microsoft Windows® is not allowed.

8.2.4 Microsoft Windows Security Updates / Service Packs

The Kaleido software was tested and released with:

- Microsoft Windows® 7, Service Pack 1, 64 bit

This includes all security patches until the Kaleido release date (see Release Notes).

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” it is recommended to disable Windows updates.

8.2.5 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 1.0\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 to be provided.

8.2.6 Data

1. PerkinElmer is not liable for any data loss due to data management processes like backups, etc.
2. When harddisk space on the EnSight PC is exhausted we recommend to set the “Archive folder” to a different location (server etc.) or to disable the archiving option for images in Kaleido.
3. Images should only be archived to reliable servers which are integrated into the customer's backup system.
4. PerkinElmer recommends a Gigabit network connection.

8.2.7 Back Up

1. PerkinElmer is not responsible for the implementation of an effective backup strategy.
2. PerkinElmer is not responsible for the backup of any files from the “EnSight System”.
3. PerkinElmer recommends to backup all data on the EnSight PC on a regular basis using third-party backup software.

8.3 Default Users and User Groups

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

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.

8.4 Remote Support

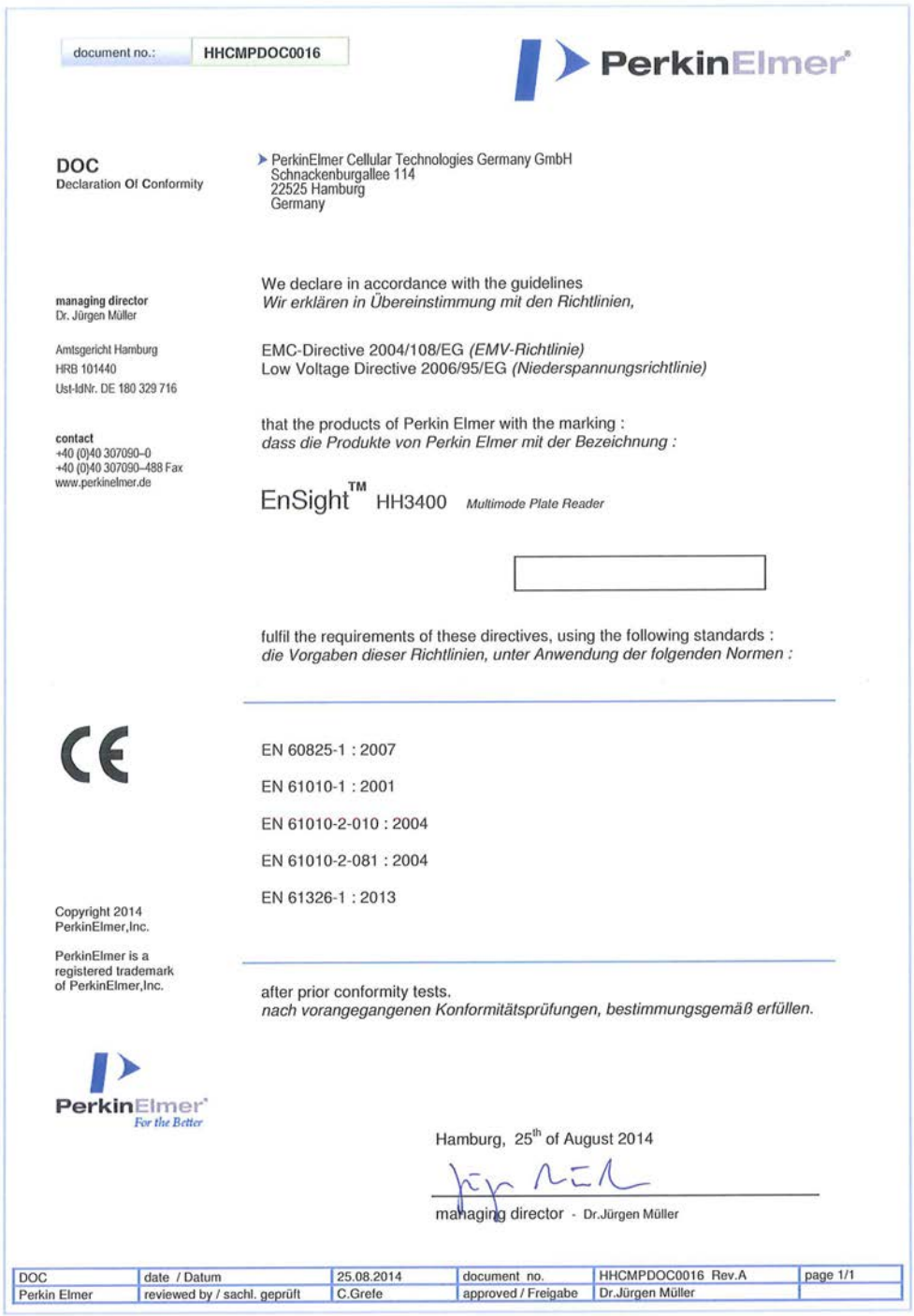
PerkinElmer uses the service of LogMeIn (https://secure.logmeinrescue.com/) to solve issues of “EnSight Systems” by remote support. If LogMeIn support is not allowed please contact PerkinElmer Service and ask for other options. Please note: Other options may increase costs for the service contract.

Minimum system requirement for LogMeIn remote support:

- Broadband connectivity to the internet (i.e. T1, cable modem, ISDN, or DSL)

9 Compliance

9.1 Declaration of Conformity



document no.: HHCMPDOC0016

PerkinElmer

DOC
Declaration Of Conformity

PerkinElmer Cellular Technologies Germany GmbH
Schnackenburgallee 114
22525 Hamburg
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managing director
Dr. Jürgen Müller

Amtsgericht Hamburg
HRB 101440
Ust-IdNr. DE 180 329 716

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We declare in accordance with the guidelines
Wir erklären in Übereinstimmung mit den Richtlinien,

EMC-Directive 2004/108/EG *(EMV-Richtlinie)*
Low Voltage Directive 2006/95/EG *(Niederspannungsrichtlinie)*

that the products of Perkin Elmer with the marking :
dass die Produkte von Perkin Elmer mit der Bezeichnung :

EnSight™ HH3400 *Multimode Plate Reader*

fulfil the requirements of these directives, using the following standards :
die Vorgaben dieser Richtlinien, unter Anwendung der folgenden Normen :

CE

EN 60825-1 : 2007

EN 61010-1 : 2001

EN 61010-2-010 : 2004

EN 61010-2-081 : 2004

EN 61326-1 : 2013

after prior conformity tests.
nach vorangegangenen Konformitätsprüfungen, bestimmungsgemäß erfüllen.

PerkinElmer
For the Better

Hamburg, 25th of August 2014

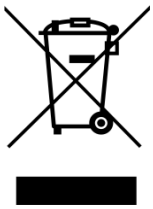
managing director - Dr.Jürgen Müller

DOC	date / Datum	25.08.2014	document no.	HHCMPDOC0016 Rev.A	page 1/1
Perkin Elmer	reviewed by / sachl. geprüft	C.Grefe	approved / Freigabe	Dr.Jürgen Müller	

9.2 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 residual area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

9.3 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/pages/010/onesource/environmental-health-and-safety/environmental-directives-compliance.xhtml

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.

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