Microscopy for the Incubator

zenCELL owl

User Manual
zenCELL owl

Software Version 3.4

EN

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Table of Contents

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1 Notes on the user manual

Warning of electrical voltage
This symbol indicates that electrical hazards pose a danger to life and health of persons.

Warning
The signal word indicates a hazard with a medium level of risk which, if not avoided, may result in death or serious injury.

Attention
The signal word indicates a hazard with a low level of risk which, if not avoided, may result in minor or moderate injury.

Note
The signal word indicates important information (such as property damage), but not hazards.

Protective gear
Notes with this symbol indicate that your personal protective equipment is to be worn. Wear safety goggles, gloves, protective clothing and safety shoes.

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2 Legal notice

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2.1 Warranty and liability

The device complies with the essential health and safety requirements of the relevant EU regulations and has been repeatedly tested at the factory for proper function. When claiming the warranty, the device number must be stated (see back of the device). Failure to follow manufacturer's instructions, regulatory requirements, or unauthorized modifications to the equipment, or unauthorized replacement of any part of the equipment, may significantly affect the electrical safety of this product and may void the warranty. Any liability, property damage or personal injury resulting from the device being used contrary to the instructions in this operating manual is excluded. Technical design and design changes due to continuous development and product improvement are reserved at any time without prior notice. For damages that result from improper use, no liability is assumed. Warranty claims then expire as well.

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3 Safety

Please read this manual carefully before putting the device into operation and using it and always keep it in the immediate vicinity of the place of installation or on the device!

3.1 Warning

Read all safety instructions and instructions.
Failure to follow the safety instructions and instructions may result in electric shock, fire and/ or serious injury.

Retain all safety instructions and instructions for the future.
This device may be used by children 14 years old and over, and by persons with reduced physical, sensory or mental capabilities, or lack of experience and knowledge, if they have been supervised or instructed in the safe use of the device and understand the hazards arising therefrom. Children are not allowed to play with the device. Cleaning and user maintenance must not be performed by children without supervision.

- Do not operate the device in potentially explosive areas.
- Do not operate the device in an aggressive atmosphere.
- The device is not a toy. Keep children and animals away.
- Never immerse the device completely in liquid.
- Protect the device from direct sunlight.
- Do not remove safety signs, stickers or labels from the device. Keep all safety signs, stickers and labels in a readable condition.
- Do not open the device with a tool.
- Observe the storage and operating instructions (see chapter Technical data).
- If it can be assumed that the device can no longer be operated without danger, it must be taken out of operation and secured against being restarted.
- The customer/ user may not make any changes or repairs. For maintenance or repair, the unit must be returned to the manufacturer or to a location specified by the manufacturer.
- The safety of the user may be impaired by the device if it has visible damage, is no longer working as prescribed or has been stored in unsuitable conditions. In case of doubt, the device should always be sent to the manufacturer or a body appointed by the manufacturer for inspection, maintenance or repair.

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3.2 Intended use

Only use the device for the microscopy of biological samples and for continuous monitoring of them. Any further use and the utilization of the measurement results of the device are the responsibility of the user. To use the device as intended, use only accessories approved by InnoME or parts tested by InnoME.

3.3 Improper use

Do not use the device in potentially explosive atmospheres, with hazardous substances or in contaminated areas. InnoME assumes no liability for damage resulting from improper use. Warranty claims expire in this case. Unauthorized structural changes and attachments or conversions to the device are prohibited.

3.4 Staff training

The use of the device is reserved for technically trained personnel. Respective persons must also have read and understood the operating instructions, in particular the Safety chapter.

3.5 Residual risks

Protective gear
Wear protective equipment such as gloves, eye protection and protective clothing. Depending on which other products, substances or chemicals you use, further protective measures are necessary. Pay particular attention to the respective safety data sheets for chemicals before using them.

Warning
Do not leave the packaging material careless. It could become a dangerous toy for children.

Warning
The device is not a toy and does not belong in the hands of children.

Warning
This device may pose a hazard if it is used improperly or not as intended by unauthorized persons! Note the personnel qualification.

Note
To avoid damaging the unit, do not expose it to extreme temperatures.

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4 Information about the device

4.1 Device Description

zenCELL owl is a 24 channels microscope for the incubator with automated remote monitoring of cell cultures. 24 independent cameras are used to acquire brightfield or darkfield scans and perform image analysis of up to 24 wells of your cell culture in parallel automatically. Culture quality and data can be comfortably checked from a PC even from outside the lab. Software algorithms evaluate the confluence of each picture and estimate changes in the relative cell number objectively and in real time. Compare mean values and standard deviations of individually selected wells.

zenCELL owl incubator microscope is compatible with a range of standard cell culture vessels like multi-well plates, flasks or individual formats. It leaves enough space in your incubator for other vessels or further zenCELL owl devices. Benefit from easy cleaning and disinfection and a low-maintenance due to absence of mechanical parts. Just plug in the cable, start the intuitive software and get started. Create standardized cell culture conditions and perform long-term measurements. Use zenCELL owl incubator microscope for automatization and parallelization of your routine and specific cell culture applications, e.g.:

- Cell Culture Monitoring
- Scratch Assays or Migration Assays
- Imaging of Spheroids
- Stem cell observation
- Effects of components on cell cultures (e.g. cytotoxicity or biocompatibility)
- Analysis of extracellular conditions (e.g. glucose concentration, pH value or different mediums)

*You can download the zenCELL owl software from https://zencellowl.com/mediasoftware/

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4.2 Device representation
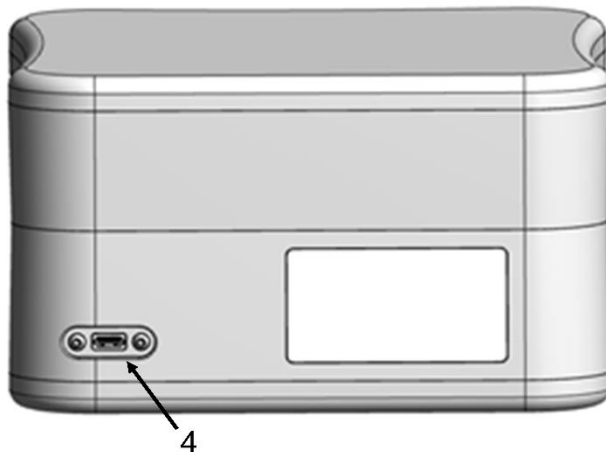
Isometric view
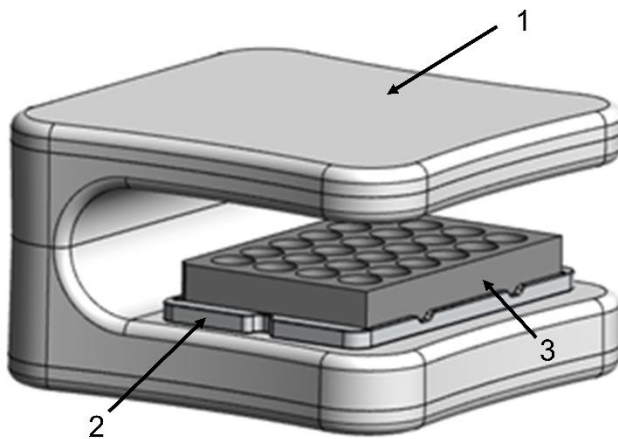
Legend:

1 zenCELL owl

2 Magnetic adapter frame for fixing cell culture vessels

3 Shown: 24-cavity plate, not included

Rear view

4 USB-C socket for data ex-change and power supply

4.3 Connectors & switches

The device can be connected to a computer via the USB-C socket (4) with the supplied USB cable. Only when using a USB cable tested by InnoME, the device is guaranteed to function properly. The device is controlled exclusively via the USB-C interface and a connected computer using the zenCELL owl software.

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5 Technical data

System Requirements	Windows® 8, 10 (32/64 Bit, 2,5 GHz, 4 cores, 8 GB RAM)
Interface (power supply, data exchange)	USB 3.0
Power supply	5 V
Protection	IP62
Operating Environment	20 – 45 °C 20 – 95 % Humidity
Disinfectable	Isopropanol, Ethanol
Dimensions (W x H x D)	10,5 cm x 18 cm x 18 cm
Weight	1050 g
Light Source	LED
Camera	5 MP CMOS
Optical Filters	No Filters
Contrasting methods	Digital Phase contrast
Field of view per microscope	1,2 mm x 0,9 mm
Display resolution per microscope	2592 x 1944
Image Resolution	5 MP
Magnification Camera	10 x (+ digital Zoom)
Image capture	30 s for 24 wells
Adjustable recording interval	10 min – 24 hours
Export Formats	Picture: PNG, JPG, BMP Video: AVI Dataset: CSV
Application	e.g. Cell Culture Monitoring, Scratch Assay, Confluence check, drug treatment

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6 Shipment and Storage

Note

If you store or transport the device improperly, the device may be damaged. Observe the information on transport and storage of the device.

6.1 Shipment

When transporting the device, use a suitable container to protect the device against external influences. The original packaging is a suitable transport container. Make sure that the zenCELL owl is not exposed to improper mechanical loads.

6.2 Storage

- Dry, protected from frost and heat
- Not in contact with hazardous substances
- In a place protected from dust and sunshine
- If necessary, protect it from dust with a cover
- Storage temperature 0 ... +50 ° C

6.3 Delivery

zenCELL owl	x 1
USB-cabel	x 1
Magnetic adapter *	x 1

* among others for Eppendorf 24 cavity plate

Check if the delivery is complete and undamaged. If the shipment shows shipping damage or components are missing, please contact our service (see page 28).

Warning
Do not operate a damaged device as this may cause injury.

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7 Commissioning

The power supply and data exchange take place via the USB-C interface. Only use an InnoME-tested USB cable for commissioning. Only an InnoME-tested USB cable guarantees safe and error-free operation of the device. InnoME assumes no liability for damage resulting from unchecked replacement parts.

Warning
Only connect the device to a terminal that meets the system requirements in the chapter "Technical data". Do not apply overvoltage, otherwise the device may be irreparably damaged.

7.1 First use

Please carefully remove the zenCELL owl from the packaging and check that the device and accessories are intact. Connect the zenCELL owl to your computer with the enclosed USB cable. A first initialization of the 24-channel microscope is performed. After successful initialization, the status LEDs will indicate that the device is ready for use by briefly flashing the status LEDs. On the computer, the zenCELL owl is recognized as a camera in the device manager.

Install the zenCELL owl program on your end device that meets the system requirements from the "Technical Data" chapter. This can be downloaded at https://zencellowl.com/en/software-en/.

7.2 Use

Connect the zenCELL owl to your computer with the enclosed USB cable. After a successful initialization, the green status LED lights to indicate that the device is ready.

When the device is used in an **incubator**, make sure to place it there at least **two hours** before the start of an experiment. This serves to compensate differences in temperatures thus ensures stable processes.

Insert the appropriate magnetic adapter frame with your cell culture vessel and start the software on your computer.

8 Care and cleaning

- The device is dust and splash-proofed according to the IP62 standard and can be cleaned and sterilized using a spray-wiping process.
- Do not use a brush with hard bristles or other hard objects for cleaning.
- Use a soft cloth or a soft sponge to prevent scratches.
- Do not apply too much pressure and do not use caustic or abrasive cleaners.
- The device is suitable for cleaning with medical cleaning alcohol.
- Apply the disinfectant specifically to the surface to be disinfected.
- Immediately wipe off after applying the disinfectant.
- Pay attention to a complete wetting of the device.
- Alternatively, the disinfectant can be applied directly to a dry cloth to completely wet the device

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9 Operation of zenCELL owl

To properly use the zenCELL owl incubator microscope it is essential to install the zenCELL owl Software downloadable from https://zencellowl.com/mediasoftware/.

Note: Be careful to install the correct Software version corresponding to your device's serial number. This User Manual V3.2 corresponds to Software Version 3.2 and all devices with a Serial Number starting like this: zC01002...

9.1 Start Microscopy with zenCELL owl

After the zenCELL owl Software is started the Main Menu opens.

Quickstart Microscopy

zenCELL owl can be used like a conventional microscope to have a short look on the cells without saving any information (e.g. project setting, pictures or measurement results). For this application use the Menu "Live View".

Main Menu > Views > **Live View** (see page 20)

Start a Project

In order to perform long-term Cell Culture Monitoring or an experiment with automated data recording it is necessary to define distinct Project Settings (e.g. project path). This can be done via using "Project Wizard" to create a new project or adjusting the "Project Settings".

- **Project Wizard** (see page 15)

  Main menu > File > New Project

- **Project Settings** (see page 17)

  Main menu > Settings > Project Settings

Note: While a Project is running, all captured images are saved in the project folder and the selected algorithms for coverage and cell count are executed. All related experiment information and measurement data are saved.

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9.2 Main Menu: File

The File Menu includes actions such as creating or loading projects and templates, or quitting zenCELL owl software.
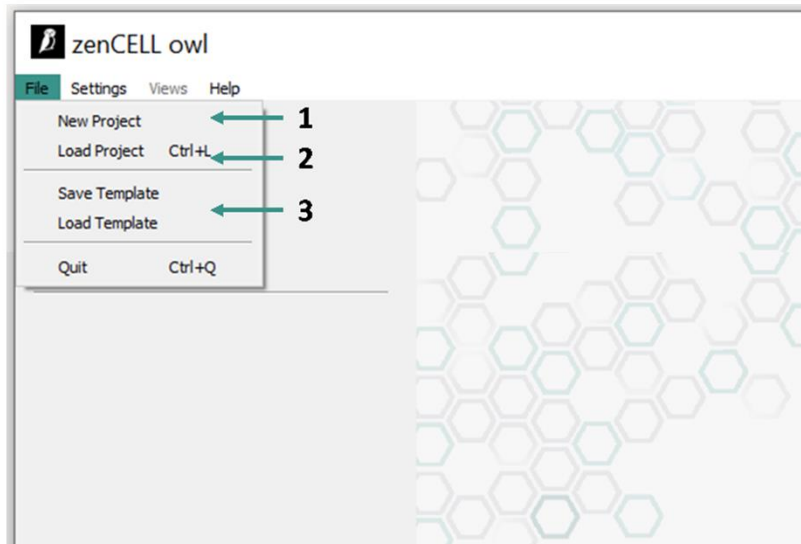
1 Create a new project

In order to perform experiments with automated data recording it is necessary to define distinct project settings such as a project name and project path. Optionally, a description or grouping of wells can be added. **Project Wizard** (see page 15) will guide you while creating a new project.

2 Load project

Experiments already finished can also be viewed retrospectively. For this select the menu item **Load project**. A file dialog will appear, allowing you to navigate to the desired project. Select the **zenCELL owl file** in question and click **Open.**

3 Save template and load

The menu item **Save Template** allows you to save your project settings as a template (*.zct file) and thus makes it easier to repeat experiments under the same conditions. The camera settings last used as well as settings for the experiment are saved (including project settings, algorithms, interval, duration).
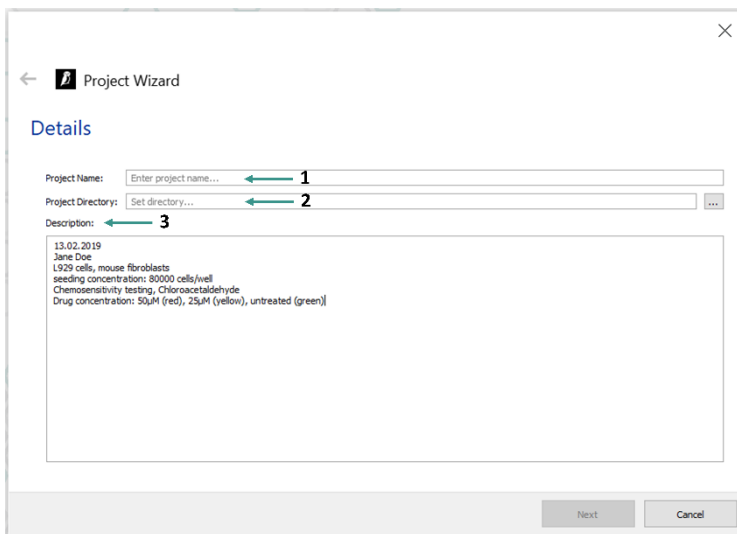
To load the settings, click on **Load Template** and select the desired experiment in the file dialog box. Project Wizard opens for further project settings (see page 15)

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Project Wizard

Main menu > File > **New Project**

Project Wizard will guide you while creating a new project. In order to perform experiments with auto-mated data recording it is necessary to define distinct project settings such as project name and project path. Optionally, a description or grouping of wells can be added.

Note: If camera settings have been manually modified before, a pop-up window asks you whether to continue with these settings or set camera settings to default.
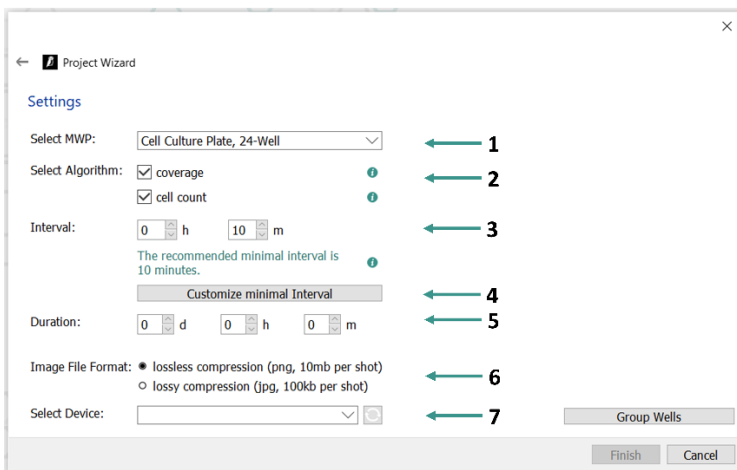

1 Project Name

Use a unique name for the project.

2 Project Directory

Set the path where project and measurement data are saved.

3 Description

A description allows further clarification for the project to be created.

1 Select MWP and cameras

Set the type of cell culture vessel used for the experiment. This defines the cameras which are activated during experiment.

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2 Select Algorithm

Algorithms are selected by default. Deselect the Algorithms you would not like to run during the experiment.

Note: Coverage and cell count can not be calculated retrospectively!

Coverage: Percentage of the substrate's surface covered by cells. Coverage corresponds to confluence of the cell culture.

Cell Count: Total number of all visible cells. Cell number is added from "Adherent Cells" and "Detached Cells". "Adherent Cells" determines the number of cells attached to the surface of the substrate and have a spread shape. "Detached Cells" however show a round shape and are not attached to the surface.

Note: There is no differentiation possible between dead or living cells!

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3 Set Measurement Interval

Set the time interval for recording measurement data (i.e. pictures and the selected algorithms). Choose a time interval between 10 min and 24 hours.

Note: The minimal interval depends on the number of activated algorithms and wells selected.

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4 Customize minimal Interval

To have an interval smaller then 10 min you can customize the minimum interval.
Let the Software calculate your individual minimal possible time interval. Calculating a new interval may take several minutes depending on your hardware.

---

5 Set Duration

Set the duration of the measurement. Choose time periods between 1 min and 10 days. Experiment will stop after the selected duration. The experiment can also be stopped manually at any time.

---

6 Select Image File Format

Select *lossless compression* to save images as *.png file format (10 mb per shot) or *lossy compression* for *.jpg file format (100 kb per shot).

---

7 Select device

The **device manager** lists all zenCELL owl devices connected to the computer. Click on a zenCELL owl to select it and set it active.

Group Wells: It is possible to color wells and group them together. Grouped wells are used for calculating mean and standard deviation, displayed in "Custom Plot" (see page 25). Grouping of wells is shown in "Settings" (page 17 f.) in detail.

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9.3 Main Menu: Settings

Main Menu > **Settings**

The Settings Menu gives an overview of details of an experiment. A couple of settings or information can be changed or complemented even after an experiment has already finished.

Import the data of an already finished experiment via File > Load Project and select experiment path.

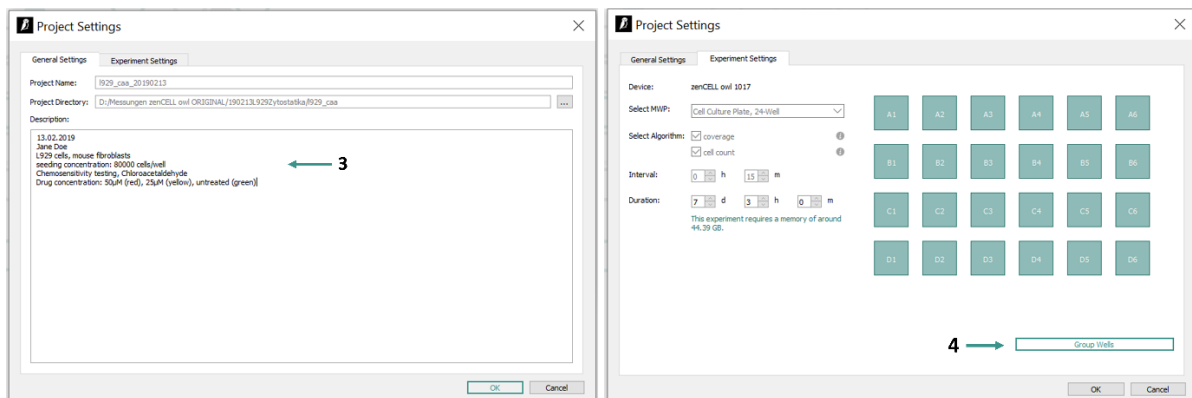**Note:** Experiment data is saved as a *.zenCellOwl file.
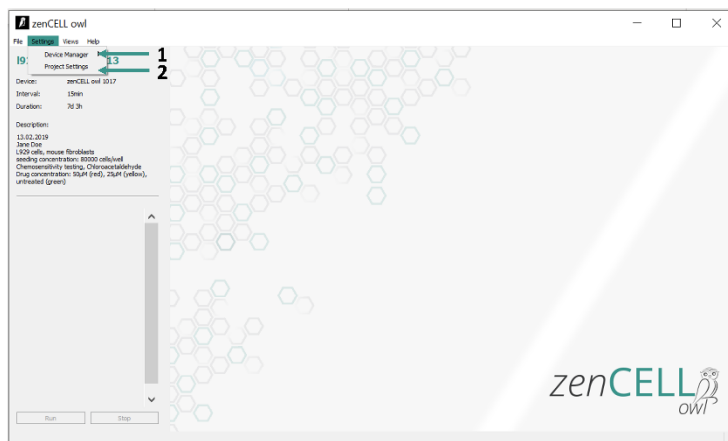
1 Device manager

The **device manager** lists all zenCELL owl devices connected to the computer. Click on a zenCELL owl to select it and set it to active.

2 Project settings

Project settings can manually be changed, even though a measurement is already finished.

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3 General Settings

The description of the project can be changed. It is not possible to change the Project Name or Project Directory.

4 Experiment Settings

It is possible to change the grouping of wells retrospectively. Device, MWP or cameras and Algorithms can not be changed in retrospect.

5 Grouping of wells

Mark the wells, select the color and press Add to Group or Ungroup. For calculation of Median and Standard Deviation the grouping is decisive. Resulting graphs are plotted in "Custom Plot" (see page 25).

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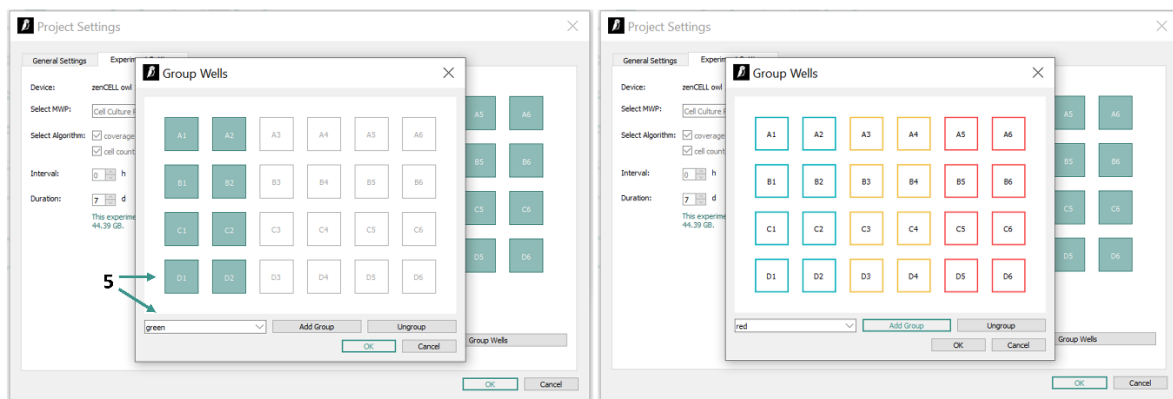
9.4 Main Menu: Views

Overview

Main menu > Views > **Overview**

The Overview window shows the current pictures of all available cameras of the selected zenCELL owl device. If groupings of wells have been made via the project settings (see page 17 f.), the wells are marked in the selected color.
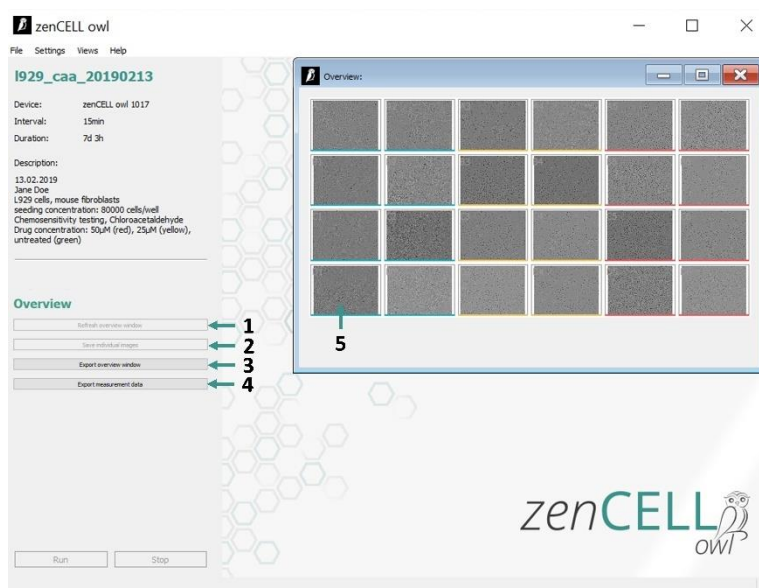

1 Refresh overview window

Take current pictures of all enabled cameras and refresh the overview.

2 Save images

Save individual images to a selected folder as a *.png file. A file dialog box sets the location and name.

3 Export overview window

Overview window can be exported to a selected folder as *.png file. A file dialog box sets the location and name.

4 Export measurement data

Measurement data can be exported to a selected folder as *.csv file. A file dialog box sets the location and name.

5 Single-click and double-click

A **single-click** on a well opens the **Live View** (see page 20).
A **double-click** on a well opens the **Well History** window (see page 22).

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Live View

Main menu > Views > **Live View**

Live View allows live microscopy of the selected well. Only one Well View window can be displayed at once. Fine adjustments such as lighting, exposure, gain and focus level can be set for each well. These settings can be used for a subsequent measurement.

Note: It is recommended to use Live View function only as long as necessary. Because Live View requires a continuous activation of lighting that can result in heating of the well. For the savety of your experiment, Live View stops automatically. You can manually pause warning for 1, 3 or 10 minutes in the pop-up window.
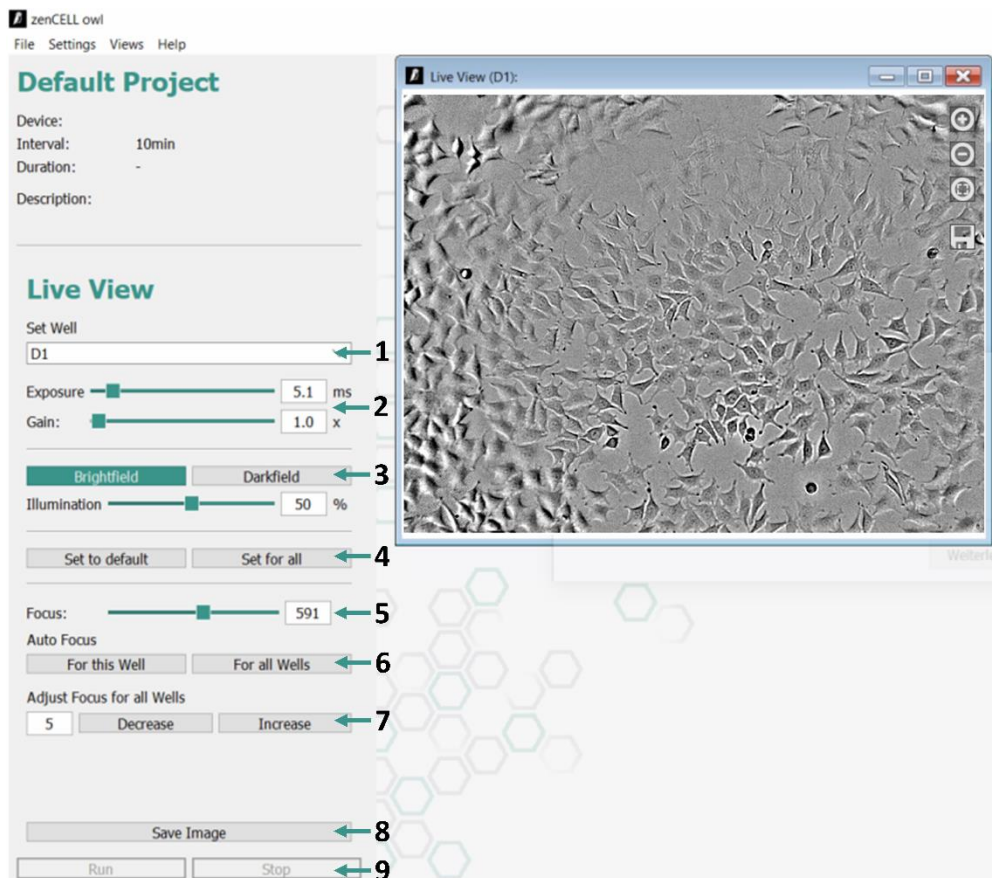
1 Select a well

Select or change the displayed well.

2 Exposure and Gain

Make your required adjustments for Exposure and Gain.

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3 Select Brightfield or Darkfield imaging and adjust illumination.

4 Set for all

The camera adjustments can be transfered to the other wells. The focus settings will not be applied to the other cameras.

Set to default button resets the configuration to the default.

5 Focus

Adjust the focus level. The maximum focus range is 280 µm, adjustable in 1050 steps.

6 Auto Focus

Focus can be set automatically whether for the selected well or for all Wells at the same time. To determine the best focus qualitity, an algorithm scans pictures taken with different focus levels.

7 Adjust Focus for all Wells

It is possible to change focus level simultaneous for all wells for the same value.

8 Save Image

Export the image as a *.png file to a selected folder. A file dialog box sets the location and name

9 Run or stop

Run or Stop experiment can be used to start or stop a long-term measurement.
Clicking the **run** button opens Project Wizard to define necessary project settings such as project name and project path (see page 15). Then experiment can be started.

**Note:** To continue with **camera settings** adjusted in **Live View** make sure to confirm the camera settings in the pop-up window.

Note: While the experiment is running, all captured images are saved in the project folder and the selected algorithms for cell coverage and the cell count are executed and results saved as a *.csv file. While the experiment is running, this file must remain closed.

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Well History

Main menu > Views > **Well History**

Well History gives detailed Information about all measurement data that has been recorded since the start of the experiment for a selected well.
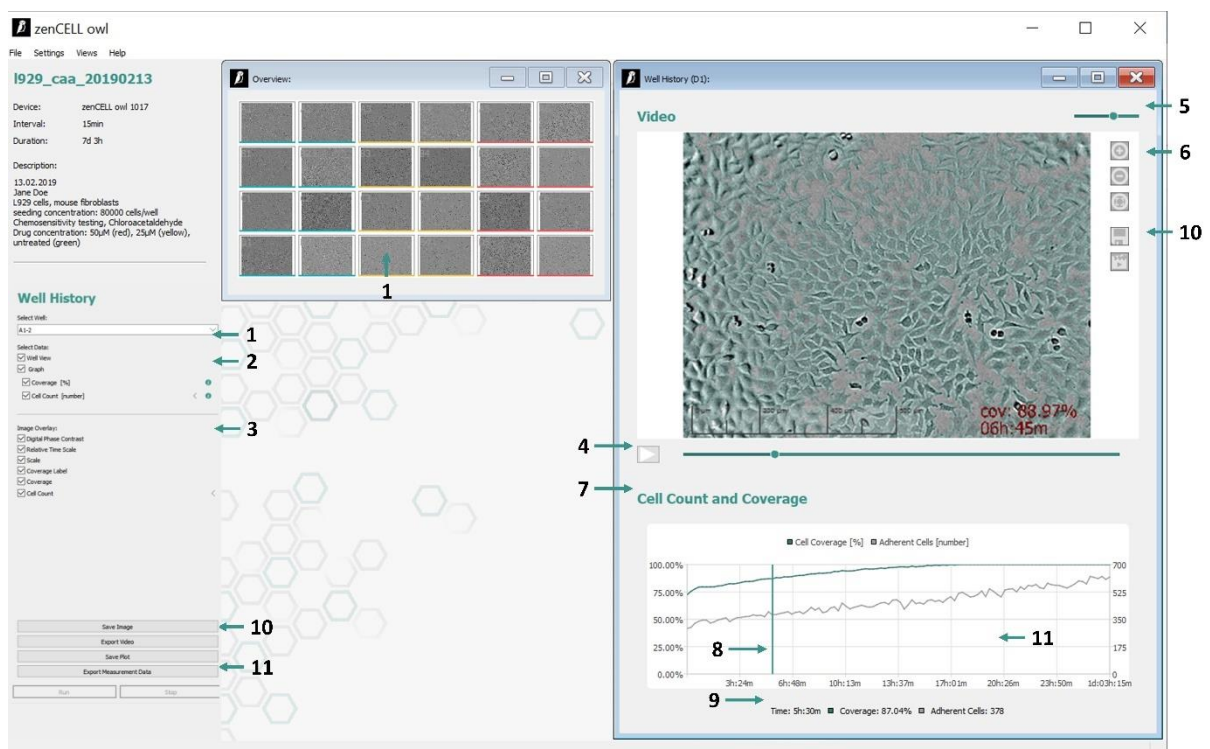
1 Select a well

Select or change the displayed well. Further Well History windows can be opened by double-clicking on the desired well in the Overview window.

2 Select data

Choose the data to be shown in the detailed view of the Well History window. By activating the check-box the display of images or graphs can be switched on and off. Select well view and graphs of Coverage and Cell Count.

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Note:

- The cell count algorithm was completely revised and will allow users to individually define models in the future. Feel free to contact our support if you are interested to test this new cell count functions as a beta user: info@zencellowl.com
- **Total cell number**: the cell number corresponds to the sum of the counted adherent and detached cells. Note: as soon as one of the counting algorithms is no longer correct, the total cell count also deviates.
- **Coverage**: The algorithm for cell coverage is defined by a percentage calculation of the "overgrown" area. This area is defined by the empty background and contrast via cells. The covered areas are marked green in the image of the Well History. It is specified that there is no coverage higher than 100%, since the overlapping of cells is not identified by the algorithm. Note: small gaps between cells and areas beyond the cell membranes are partly calculated in addition to the covered area.

---

3 Image Overlay

Select whether the Digital Phase Contrast, Relative Time Scale, Coverage, a scale bar, Coverage Value and/or Cell Count Value should be shown in the image.

Note: The Relative Time Scale and the corresponding Coverage Values and Cell Count Values can be displayed in the picture resp. video and are also shown in the exported data.

---

4 Video

All pictures stored up to this time are put together into a time-lapse video. The experiment can be played in fast motion using the **Play** button.

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5 Playback speed

The playback speed can be adjusted via the slider.

---

6 Zoom buttons

Using the buttons, the image can be zoomed in and zoomed out with the digital zoom.

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7 Graph with the algorithm results

In the window "Cell Count and Coverage" the results of the selected algorithms are graphically displayed.

---

8 Data cursor

The marker in the graph indicates the value of the algorithm associated with the displayed image.

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9 Data values

The values corresponding to the data cursor are listed below the graph. The point in time is also stated. When the mouse pointer is moved over the graph the values change correspondingly.

10 Export image and video

- Export the shown image as a *.png File. A file dialog box sets the location and name. The image is saved with the respectively selected image overlays.
- Export a video (*.avi) of the shown well. A file dialog box sets the location and name.

**11 Export Graph and Measurement Data**

- Export the active Grapgh as *.png File. A file dialog box sets the location and name. The image is saved with the respectively selected graphs.
- Export the Measurement Data of the active Well as *.csv File.

By right-clicking into the graph both Export options also can be chosen. Both export options only contain the data shown in the graph.

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Custom Plot

Main Menu > Views > **Custom Plot**

Custom Plot allows an individualized display of measurement curves and calculated results. Use Custom Plot for presentation and export of measurement data for selected single wells or means of grouped wells.
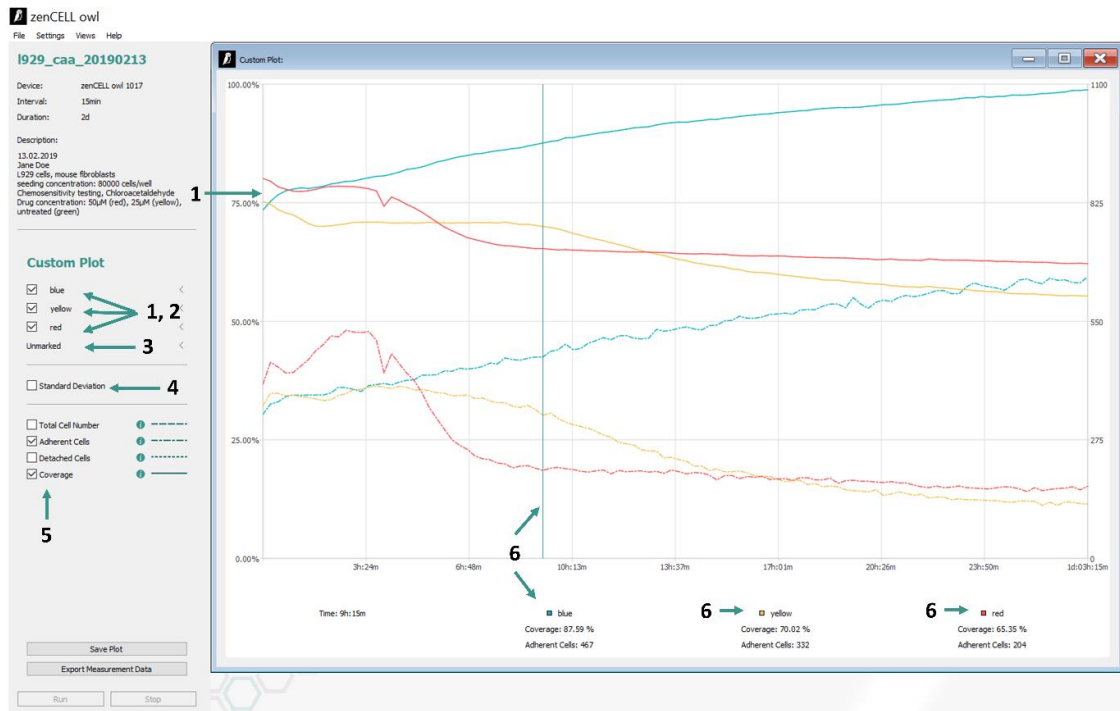
1 Mean graphs

If groupings of wells (see page 17) were made before, means of the marked wells are calculated and visualized. Select or deselect the plotted mean graphs.

2 Renaming the groupings

By double clicking the groupings' name, you can choose an individual name for this grouping.

3 Ungrouped wells

Wells, which do not belong to any grouping, can be selected under **unmarked**.

4 Standard deviation

You can select or deselect the **standard deviation** by setting a checkmark.

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5 Selection of graphs

By setting a corresponding checkmark you can choose to visualize the following calculations: cell coverage, cell count, detached and/or attached cells.

---

6 Data cursor

The marker in the graph indicates the value of the algorithm associated with the displayed image. Measurement data are shown for each graph at the selected time point.

---
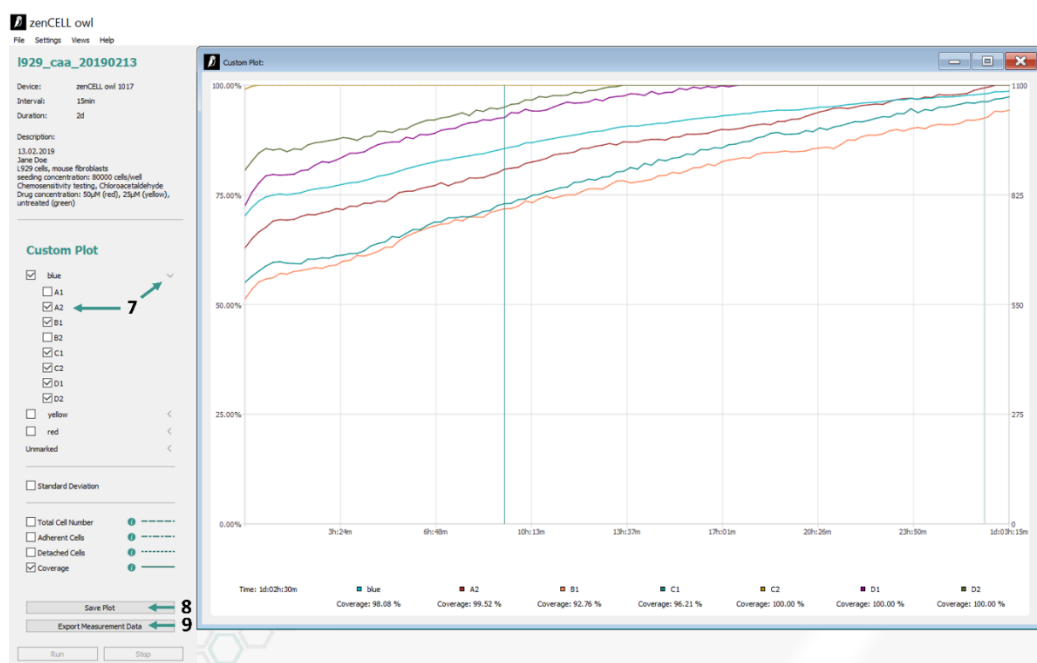
7 Selection and deselection of plotted wells

The data of individual wells can be select or deselect from groupings, to include or exclude their data from mean calculation.

---

8 Save Plot Button

Use the **Save Plot button** to save the custom plot as an image (*.png).

---

9 Export Measurement Data Button

Data of selected wells can be exported as *.csv file. This data can be opened with any calculation programme of choice.

Groupings of the wells can be changed under the menu item **Settings > Project Settings > Experiment Settings** (see page 17)**.**

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9.5 Main Menu: Help

Supporting Material

Here you find the latest versions of the operating instructions (pdf), Adapter adjustment (pdf) and adapter installation (mp4).

Check for updates

Here a **software update** can be carried out. You will be automatically redirected to the associated link.

About

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10 Maintenance and repair

In case of a defect, please contact info@zencellowl.com.

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11 Disposal

The crossed-out wheeled bin symbol on a WEEE indicates that it should not be disposed of with household waste at the end of its life. For your free return collection points for WEEE are available near you. The addresses can be obtained from your city or local government. Separate collection of WEEE is designed to enable the reuse and recycling of WEEE, and to avoid adverse effects on the environment and human health from the disposal of potentially hazardous substances contained in the appliances.

The WEEE registration number of InnoME is: DE 43441295.

You are responsible for the deletion of personal data that may be present on the waste equipment to be disposed of.

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12 License information

Pillow Library:

The Python Imaging Library (PIL) is

Copyright © 1997-2011 by Secret Labs AB

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Pillow is the friendly PIL fork. It is

Copyright © 2010-2018 by Alex Clark and contributors

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