SENSIFIC

# ODIN + SOL

# Manual

v1.6


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## Safety and handling

Knowledge of this manual is required for the save operation of the instrument. Please make yourself familiar with the content especially concerning save operation.

The specifications are subject to change. All right reserved in the event of granting of patents or registration of a utility model.

The ODIN device meets the requirements of the RoHS directive and is marked with the CE mark.

This device shall be disposed of in accordance with the WEEE Directive 2021/19/EC.

If it is determined that protective measures are no longer efficient, the instrument must be turned off and secured against inadvertent operation. Please contact Sensific Support in order to repair the system.

Please note that ODIN is a precision opto-electronic device. Incorrect handling may lead to malfunction of the device or even damage it. This will render any warranty claims invalid.

It is mandatory that the system is only used with delivered periphery parts. If new or additional periphery is needed, please contact the Sensific support for the necessary specifications.

# 1 Setup, Interfaces, and Ports

In this section the setup of ODIN, SOL and ODIN SIGNAL HUB is documented.

If all components are connected, the system looks like shown in Figure 1. Depending on the individual configuration changes in the setup might occur.

Optical setups typically follow the schematics in Figure 2. The sensor is able to time-multiplex up to 4 different illumination setups into the same measurement and correlate the data between them. In combination with a very high imaging rate, it is possible to do multichannel fluorescence and combined bright field imaging measurements. A schematic of the time multiplexed “alternated mode” is shown in Figure 3.

ODIN is designed to work with a bright field and epi-fluorescence microscope or similar optical setups.

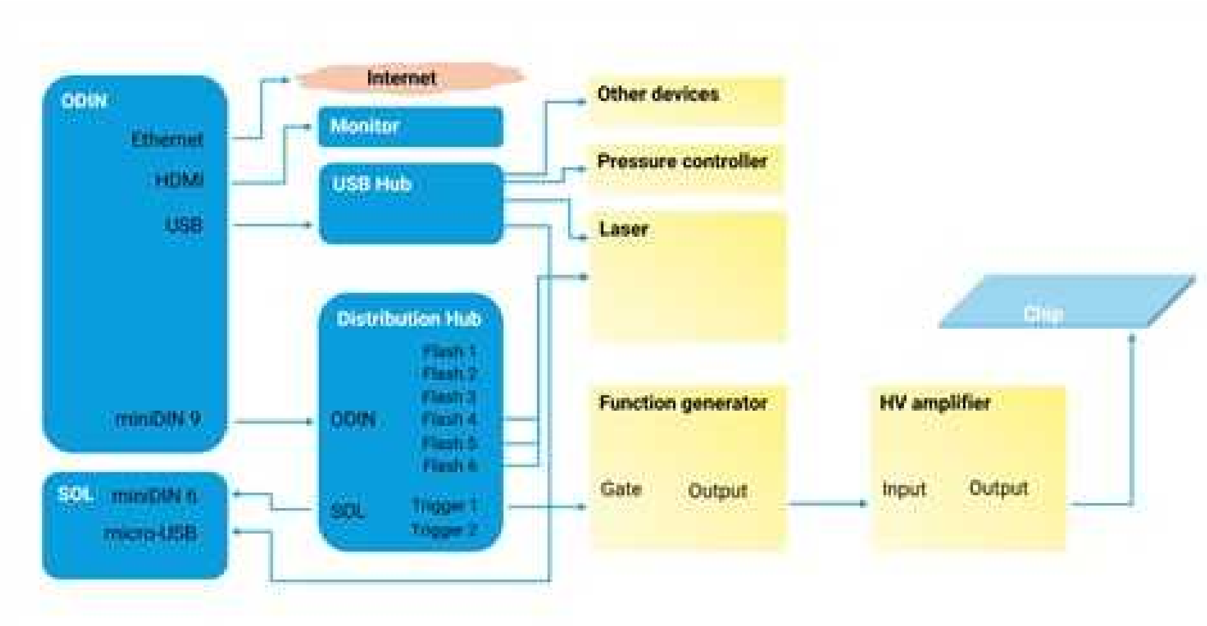



*Figure 1: Electrical connection diagram of a typical setup of ODIN with SOL illumination and sorting. Blue colored devices are part of the standard ODIN system. Yellow colored devices are external components.*

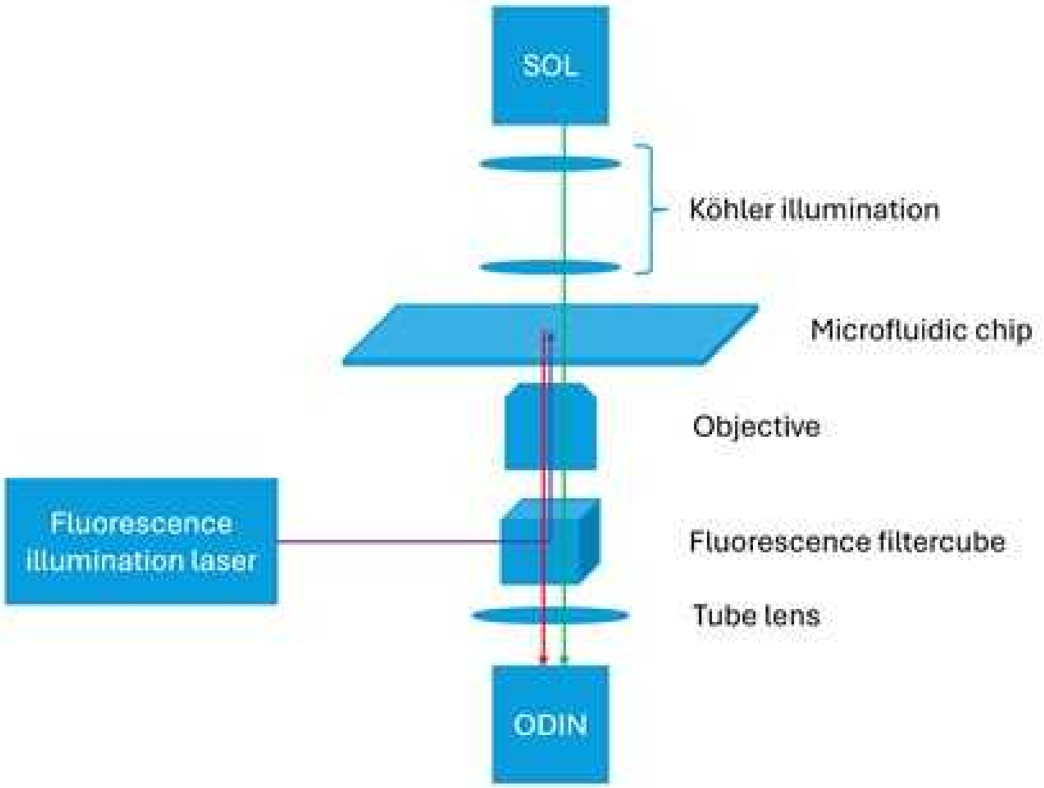



*Figure 2: Optical path for a typical ODIN microfluidic experiment. Alternated time multiplexed illumination is used to image in bright field and fluorescence at the same time. Multiple illumination wavelengths are possible with a multi-bandpass filter cube, which are available with up to 5 different wavelengths.*

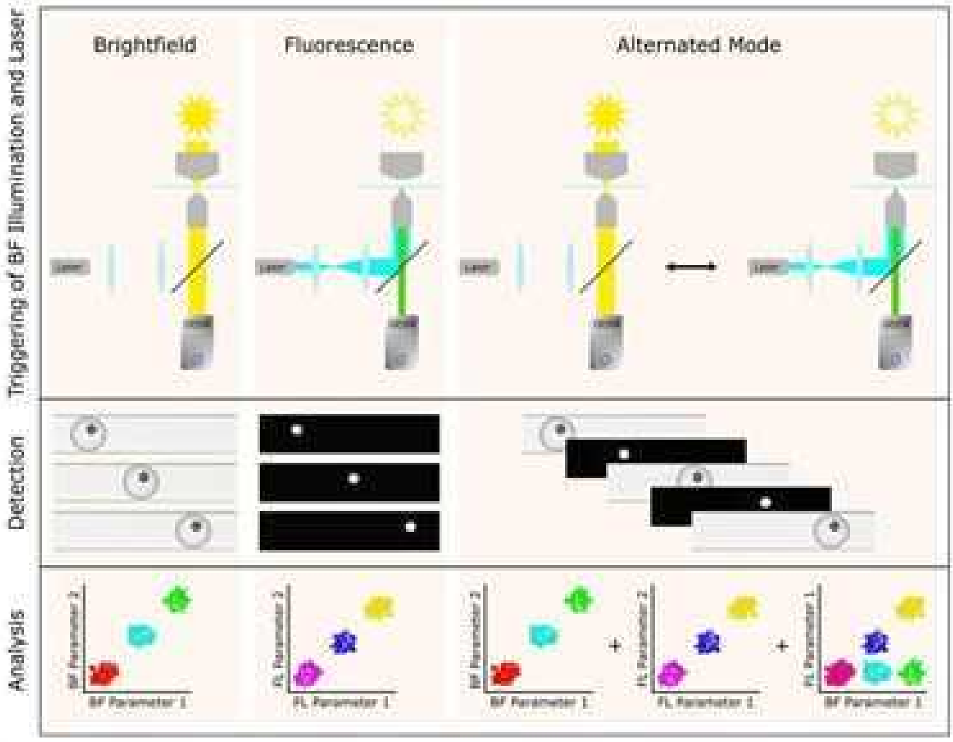



*Figure 3: Time multiplexing as supported by the ODIN sensor. Up to four different modes can be active at the same time. Arbitrary illumination setups like bright field and epi-fluorescence are possible at the same time.*

## 1.1 ODIN

To setup ODIN first connect it with the desired optical system (i.e. to a microscope via the C-Mount port). The mechanical size is shown in Figure 4.

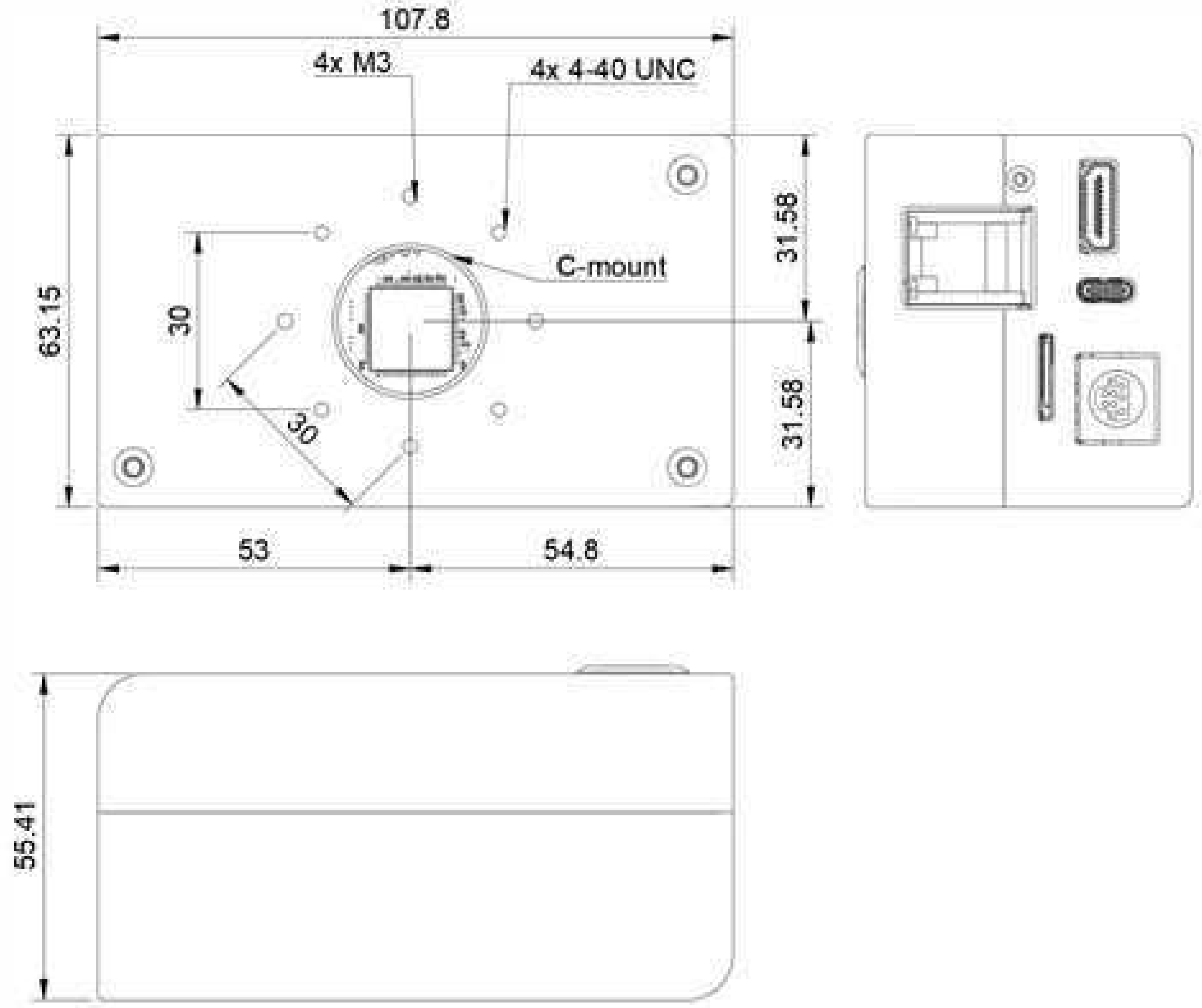



*Figure 4: Mechanical layout of the ODIN sensor. All units are in millimeters.*

Then connect all cables leading to the periphery in the following order

- HDMI 1.3 port (outputs Full-HD video) to Monitor
- Ethernet 1000-BaseT for data transfer via the network (optional)
- USB-2.0 port to the USB Hub where the dongle for mouse and keyboard has to be plugged in
- microSD card (if not already inserted)
- 9 Pin MiniDIN-port to connect with ODIN Hub
- Power supply
    - Old revisions DC 2.1x5.5 barrel jack, center positive, 5 V
    - New revisions USB-C 5 V, 3 A.

The connections are shown in Figure 6 and Figure 5.

⚠ Connecting a power source not supplied with the sensor may destroy the sensor and lead to a loss of any warranty ⚠

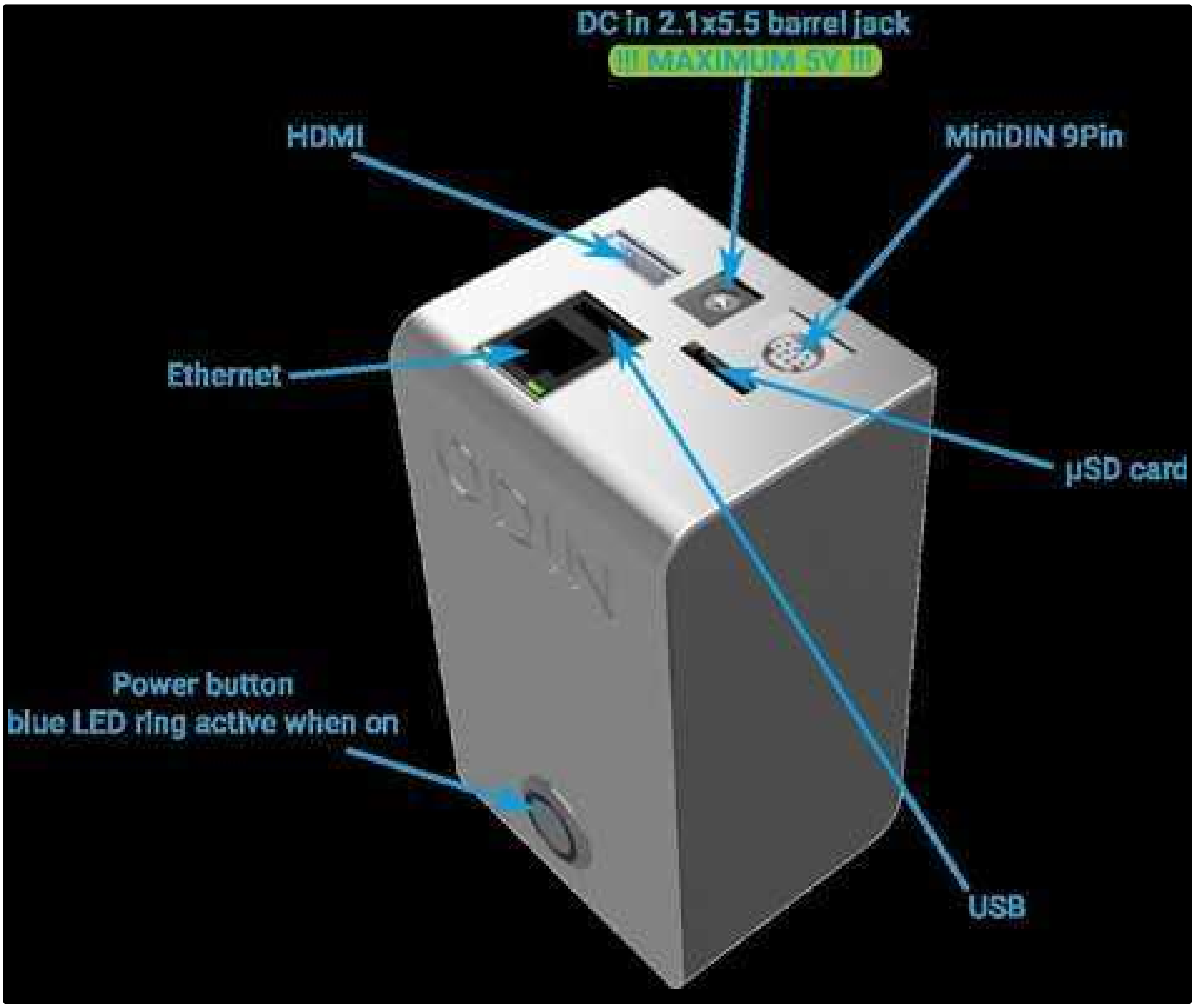



*Figure 6: Electrical connections for ODIN sensors with serial numbers below S3-040.*

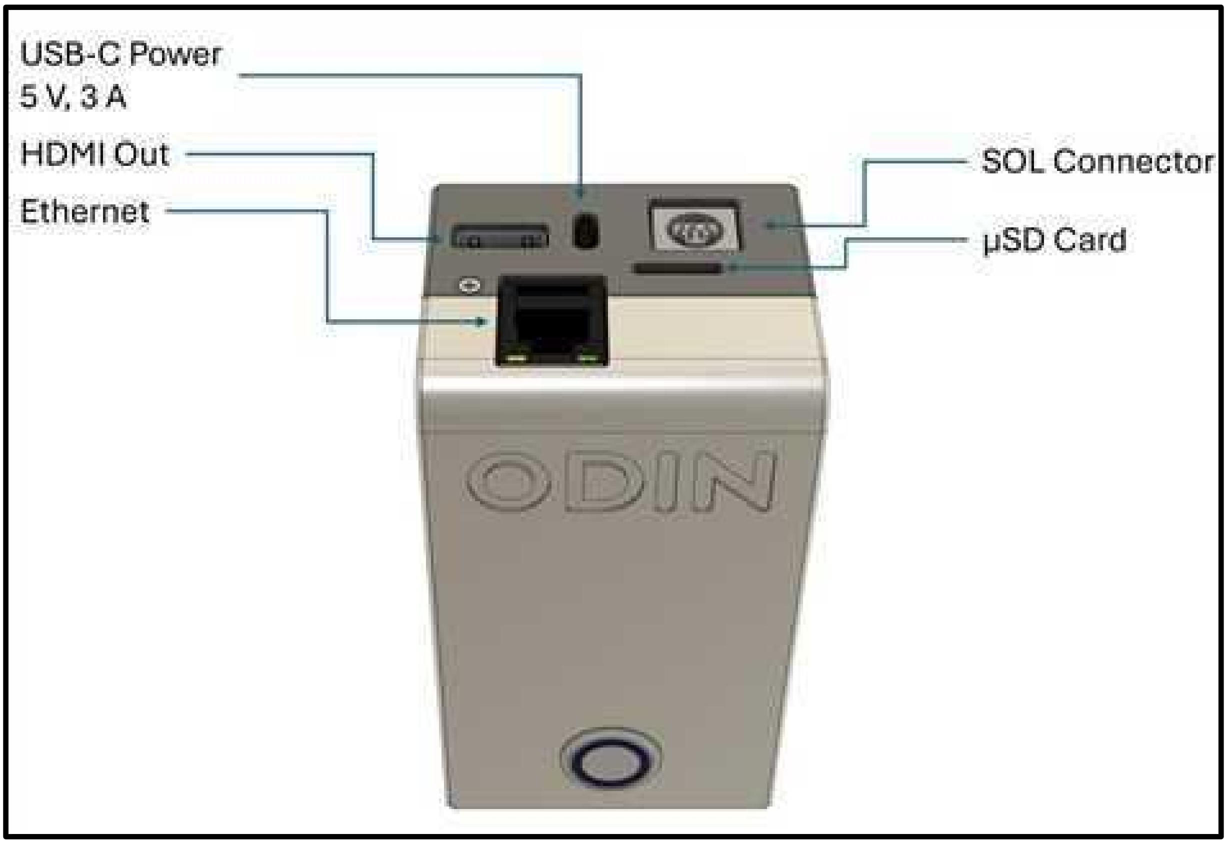



*Figure 7: Electrical connections for ODIN sensors with serial numbers S3-040 and higher (2026 onwards).*

Output of the miniDIN 9 Pin plug is as shown in Table 1. The numbering of the pins on the plug are shown in Figure 8. This port is connected to the Signal-Hub with the provided cable.

*Table 1: miniDIN pinout. The outputs are not short tolerant, maximum power drive: 20 mA. All share the same ground reference, which is the ground reference of the sensor.*

| Pin | Name | Voltage level | Purpose |
|---|---|---|---|
| 1 | T1 | 3.3 V TTL out | Triggering output Pin 1 |
| 2 | T2 | 3.3 V TTL out | Triggering output Pin 2 |
| 3 | F1 | 3.3 V TTL out | Flashing output for Flash #1 |
| 4 | F3 | 3.3 V TTL out | Flashing output for Flash #3 |
| 5 | F5 | 3.3 V TTL out | Flashing output for Flash #4 |
| 6 | GND | Signal GND | Ground and ground reference for all signals |
| 7 | F2 | 3.3 V TTL out | Flashing output for Flash #2 |
| 8 | F5 | 3.3 V TTL out | Flashing output for Flash #5 |
| 9 | F4 | 3.3 V TTL out | Flashing output for Flash #4 |

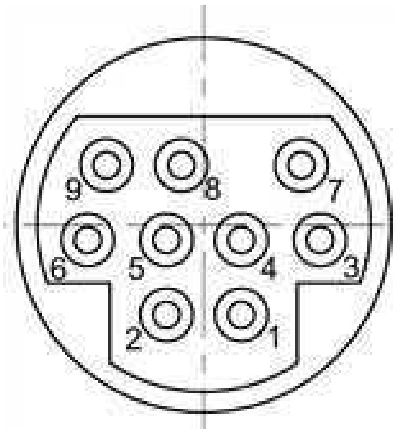



*Figure 8: Pin numbering of the miniDIN 9 plug.*

## 1.2 SOL

SOL is installed at the illumination port of the microscope. It might be necessary to replace the existing illumination of the microscope. Therefor please refer to the handbook of the microscope.

SOL is connected to the rest of the setup by the following cables and plugs

- Voltage supply 5 V DC USB - C
- 6 Pin MiniDIN to connect to SIGNAL HUB
- Micro-USB to connect with the USB Hub that is connected with ODIN

The optical connection of SOL is dependent on customer requirements. The two typical connections are:

- Zeiss dovetail connector
- SM2 internal threaded adapter

A rear side view with all electrical connections is shown in Figure 9.

⚠ Connecting any other power source may destroy SOL or parts of the instrument and leads to a loss of any warranty ⚠

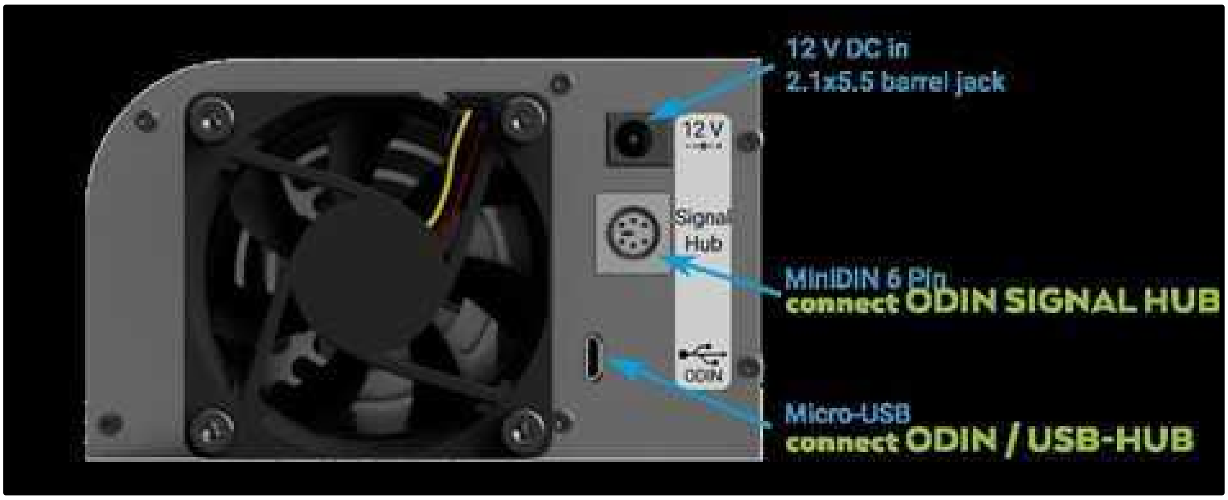



*Figure 9: SOL electrical connections. Depending on the hardware revision, the power can be a USB-C port (5 V, 3 A).*

## 1.3 ODIN SIGNAL HUB

The ODIN SIGNAL HUB can control peripheral instruments such as lasers, function generators and switches.

It is connected to the system by the cables and plugs described below

- 9 PIN MiniDIN cable to connect with ODIN. Supplied with the system.
- 6 PIN MiniDIN cable to connect with SOL. Supplied with the system.
- USB Power source 5V (not included) only connect if the system is run without SOL otherwise SOL will power the ODIN SIGNAL HUB directly.
- Flash-Pins are connected via SMB cables to the designated illumination source the pin numbers are the same as in the software (see section 3.3.1) and by default the flashes 1,2 and 3 control SOL. So, if an additional illumination source should be used together with SOL, it is recommended to first use the flash pins 4, 5 and 6.
- Trigger pins 1 and 2 are also connected via SMB to the designated trigger devices.

A schematic of the connections is shown in figure Figure 10.

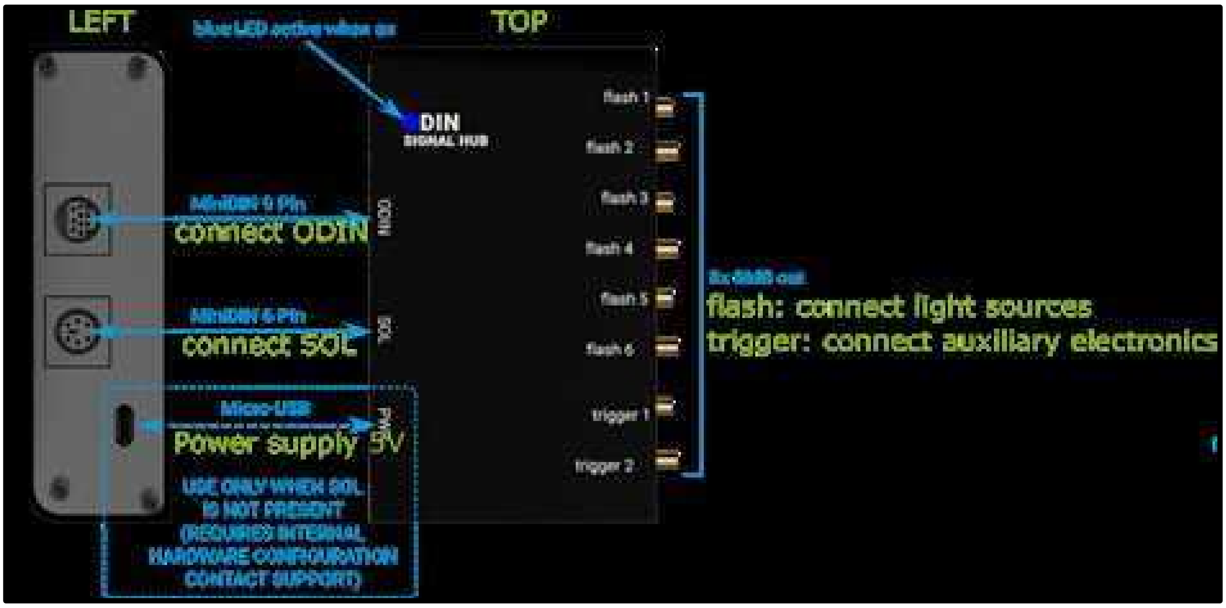



*Figure 10: Signal Hub electrical connections.*

## 1.4 Starting the sensor

- Press the power button of the ODIN device shortly. The ring around the button starts to glow blue.
- ODIN starts booting automatically and the boot sequences are shown on the screen.
- After approximately one minute, the booting process is completed, and the desktop appears with a screen like the one shown in Figure 11.
- To start the measurement tool, double click the ODIN control icon.


*Figure 11: Desktop of the ODIN firmware.*

### 1.5 Shutting down the sensor

To turn off the sensor and to prevent any kind of data loss or hardware corruption it is highly recommended to follow the shutdown sequence as described here.

- Click on the arrow symbol on the lower left corner.
- Select Logoff from the popup menu.
- In the following menu select Shutdown to turn off the sensor.


⚠ It is also possible to force-shutdown the sensor by pressing the power button for 4 seconds. However, this is strongly discouraged as it might lead to corrupted USB-storage devices, a corrupted microSD card or data loss.

⚠ A power loss of the sensor or hard shutdown of the sensor keeps external devices in the state as is. Be aware of potential high voltage or laser safety hazards, especially if safety interlocks are defeated.

## 2 Principle of operation

The ODIN sensor is a fully integrated measurement platform optimized for fast measurements using optical readout methods and integrated analyzing hardware and software for ultra-fast, ultra-low latency image analysis and decision making. The sensor allows real-time, image-based sorting of objects. The sensor is best suited to be applied in flow channel applications e.g. in microfluidic chips.

### 2.1 Image acquisition

The ODIN sensor consists of a fast, sensitive CMOS sensor. The data rate is 210 FPS (frames per second) at full frame and scales to up to 10 000 FPS at reduced readout area. The sensor is a global shutter sensor with a minimal shutter time of approximately 10 µs. The CMOS sensor has an integrated, variable analog gain between 1 and 11 and additionally a digital one up to 32 times. The sensor is capable of recording up to 4 images with different illumination and recording settings by using the external flash outputs and fully configurable exposure times. The framerate is reduced accordingly.

### 2.2 Image correction

The images are analyzed and used for background subtraction, where all non-changing parts of the image are detected and removed without user intervention automatically. The background subtraction is configurable by the user.

### 2.3 Image analysis

The background subtracted images are analyzed to determine a list of features describing the content of the image. A full list of features can be found in section 4.6.

### 2.4 Image filtering

The images can be grouped to avoid double counting and double analyzing and to average over multiple images for noise reduction.

### 2.5 Decision making

The resulting dataset can be analyzed either with a user-built C-Code or with the built-in plotting and sorting tool to control the two sorting pins.

### 2.6 Documentation and visualization

All images and data are handed over to the UI for plotting, analysis, sorting and saving.

The UI provides an intuitive tool to visualize the experiment and control all sensor features.

# 3 Main window, Illumination with SOL and General Settings

After starting the ODIN tool, the *Live Imaging Window* appears as shown in Figure 11. On the left, the *Live Preview* is shown. The *Live View* can be zoomed with **CTRL+MOUSE SCROLL**, scrolled left and right with **SHIFT+MOUSE SCROLL**, and scrolled up and down with **MOUSE SCROLL**.

On the right, the *Image Control Window* with different adjustable parameters is shown. The individual settings and their impact on the measurement are explained below.

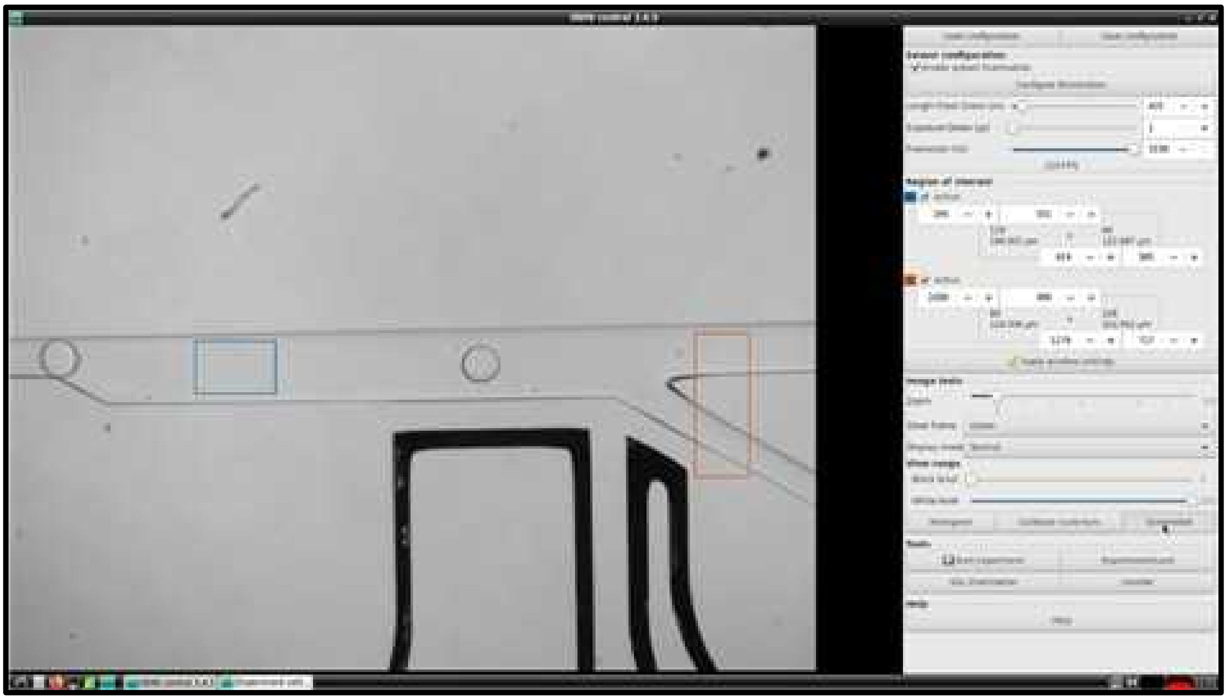


*Figure 12: Typical layout of the main window. A live view of the sensor output is shown on the left. Configuration parameters and buttons to all further windows are shown on the right.*

## 3.1 SOL Illumination

If SOL is used as illumination source, it is activated by clicking in the **SOL Illumination** button in the *Main Window* under **Tools**.

In the *SOL Pulsed Illumination Control Window*, the serial port that SOL is connected over has to be selected in the dropdown. If no port is available, click “Refresh” and check cabling. A typical dialog window is shown in Figure 13.

By activating the **Enable SOL** checkbox the system can be used. Intensity of each color (red, green and blue) can be controlled by the slider or by entering a value between 0 and 1.

To enable the pulsed illumination the **Enable pulsed illumination** checkbox has to be active

**Note:** The pulsing will only start if the checkbox in the main window is also set active.

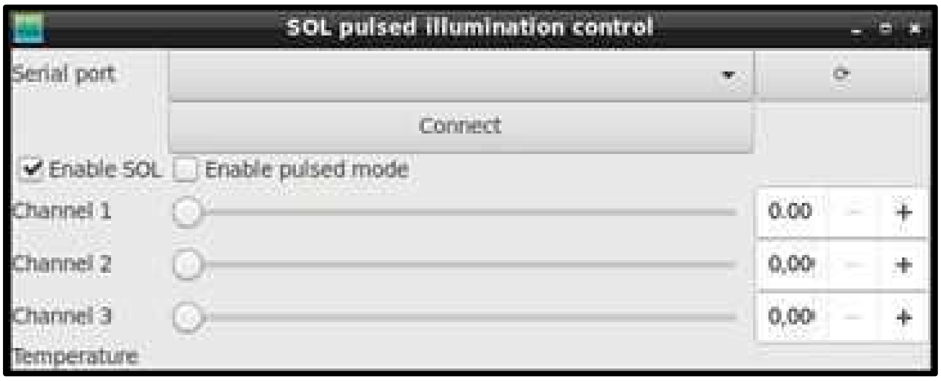
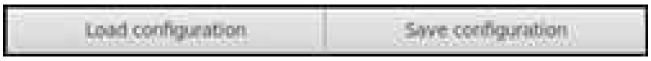



*Figure 13: SOL configuration dialog.*

## 3.2 Configuration loading and saving

All parameters and controls set in the *Image Control Window* can be saved as a *.cfg* file and later be load into the program.




The current configuration is saved automatically to a persistent file on the microSD card and restored when the program is launched again. If the current configuration should be saved, the button **Save configuration** can be used. In the saving dialog the destination and the name of the file can be chosen. The suffix *.cfg* is added to the files name.

If a previously saved configuration should be imported, the **Load configuration** button can be used. In addition to the *.cfg* files also *.exp* files containing the settings from previously saved measurements can be loaded. However, if an *.exp* file is loaded, only the settings for the image window are loaded but not the experiment parameters as explained in the **Experiment Control** section (see section 4).

## 3.3 Sensor Configuration

In the sensor configuration part – shown in Figure 14, all parameters associated with the image acquisition process are set.

If pulsed illumination should be used, it is necessary to enable it by setting the **Enable pulsed illumination** checkbox to active. This activates the Flash Outputs 1-6 on the Signal Hub. Additionally, the flashes 1-3 are routed to the SOL illumination controlling the red, green and blue LED.

⚠ Pulsed illumination can be hazardous to eyes even if the brightness seems to be low. Take necessary precautions. ⚠

The **Configure Illumination** button opens a window where the setup of the illumination can be set. This is explained in detail together with the parameters in section 3.3.1.

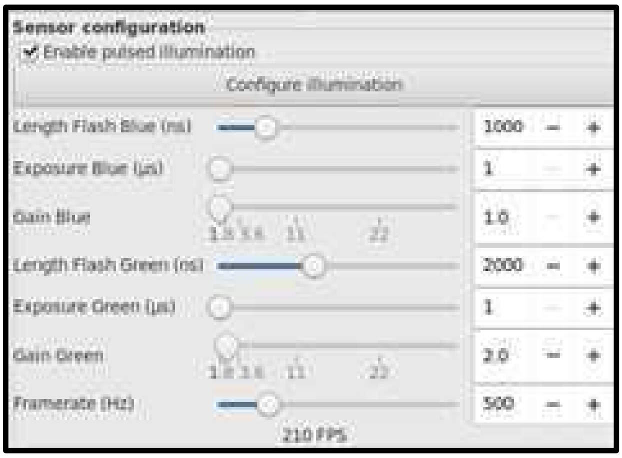



*Figure 14: Sensor configuration pane. All parameters shown here can also be adjusted and hidden/shown using the „Configure illumination“ button.*

**Framerate:** This parameter is relevant for all illumination modes. The framerate can be set to a minimal value of 60 Hz. The maximal available framerate is dependent on the size and shape of the selected region of interest and the selected exposure times.

When the sensor is read out in fullscreen mode, the maximum achievable framerate is 210 FPS. The less pixels the sensor has to read, the faster it can read out a complete frame. The slider and the numeric entry field will always clamp the framerate one can select to the currently maximum achievable framerate. Things which might block higher framerates are:

- Exposure: An exposure > 1 might limit the framerate. This applies to all exposures when having multiple illumination modes active. The highest exposure defines the maximum framerate.
- Region of interests: The more area the sensor needs to read out, the more time it takes, reducing the framerate. The sensor also needs a fixed amount of time per row of pixels. Therefore the height of the ROIs normally influences the maximum framerate stronger than the width. See section 3.4 for more information.

### 3.3.1 Illumination Configuration

The **Configure Illumination** button opens the window for all settings associated with the illumination process. This is shown in Figure 15.

First the number of illumination channels must be chosen in the ***Number of configurations* dropdown** menu. ODIN can handle up to 4 different configurations at once. This means that by time multiplexing images are taken with different illumination sources consecutively. For the sake of simplicity here we assume a 2-channel measurement, but settings are the same for one, three or four channels.

Since ODIN uses time multiplexing the effective framerate is reduced. For two configurations it is only the half, for three one third and for four one fourth of the individual framerate.

First the name of the channel can be entered, for example related to the color of illumination or the wavelength of the laser.

The color of the plotting is important to choose. In a later step all parameters related to this channel will be plotted with the chosen color.

To control illumination the **flash to use** for this channel must be selected. In a conventional setup the SOL LEDs are activated with channels 1 to 3 (red with 1, green with 2 and blue with 3). The ODIN SIGNAL HUB also breaks out the flash channels 1-3. These are shared with SOL and can be used with custom illuminations. It is recommended to use the flash signals 4-6 on the SIGNAL HUB for external illumination sources (e.g. diode lasers). The flashes 1-3 are limited to 5 μs illumination time as this is the maximum SOL can do. Flashes 4-6 are not limited by that amount and can illuminate to a maximum duration of 200 ms.

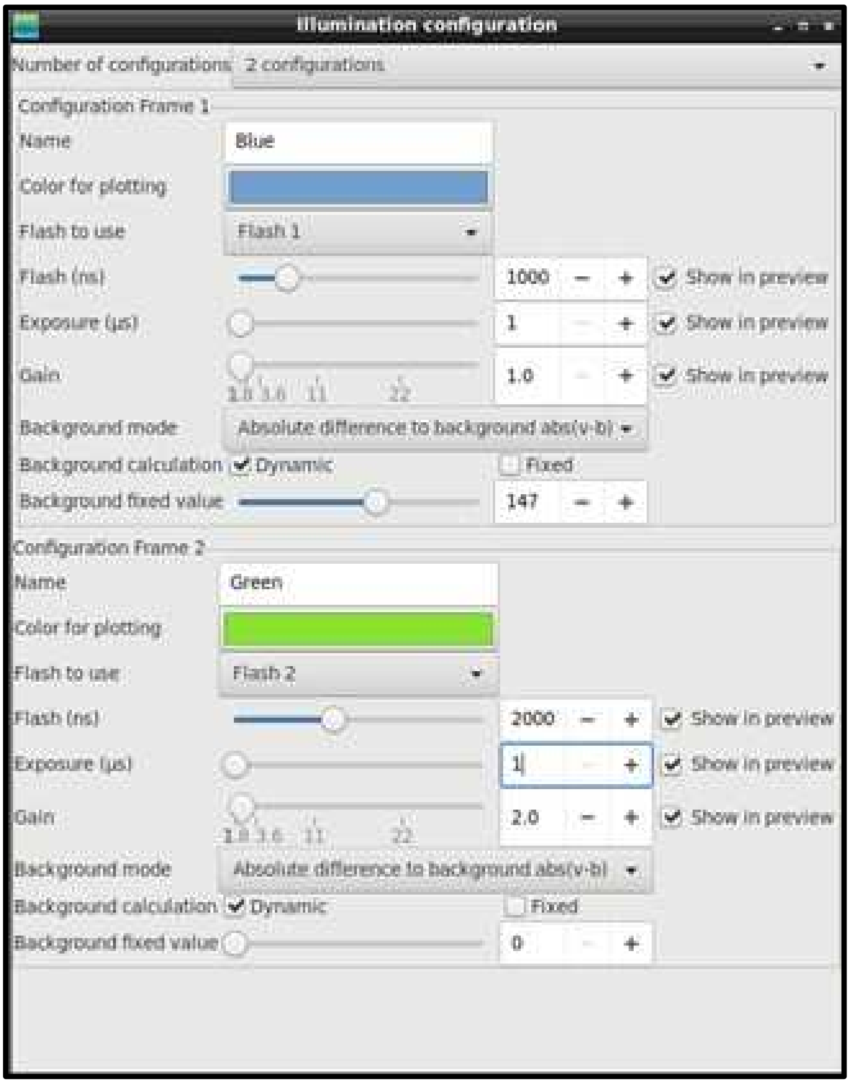



*Figure 15: The „Illumination configuration“ dialog. The Configuration Frame <n> is shown when the number of configurations is changed.*

ODIN can apply different settings for the channels by reconfiguring the sensor before each image. Therefor the flash (i.e. the on time of the light source), the exposure time and the gain must be given. The checkbox **Show in preview** determines if this setting will be available for adjustments in the main window or if it is only accessible via the *Illumination Configuration Window*.

**Flash:** The flash length determines the on-time of the light source and can be set between 10 and 5000 ns for channels 1 to 3 and between 10 and 150 000 ns for channels 4 to6.

**Exposure:** The exposure length is relevant if an illumination time longer than approximately 10 µs is chosen, either by using continuous illumination or using external pulsed light sources with flash lengths greater than 10 µs. The minimal exposure setting is 1 µs, although the hardware only achieves roughly 10 µs and the maximal exposure is determined by the framerate. Longer exposure times lead to brighter images but also to an increased motion blur. For very long exposure times it is possible that the framerate and the exposure time influence each other. Longer exposures reduce the maximum framerate and higher framerate limit the maximum exposure times. The software limits either one to the maximum value currently possible, so one might need to be lowered before the other can increased to the desired level.

**Gain:** The apparent brightness of the image can be increased by choosing a higher gain value. If the gain is set to values larger than 1, the signal is increased digitally.

At gain values of 1.8 and 11 an analog signal amplification is carried out additionally. Analog gain increases the signal before the conversion to digital values at the expense of higher noise. The analog gain has only 3 fixed values. By the digital multiplication of the sensor values, a smooth gain scaling can be achieved but does not increase the information content of the image.

The maximal gain value is 32. In general, higher gain values come at the cost of a higher image noise. However, it can still be beneficial to use higher gains to reduce the necessary illumination time and hence to decrease motion blur.

**Background mode**: One core aspect of the sensor and the high image quality is the built-in automatic background subtraction. This field allows the user to control the background subtraction. In the dropdown the user can choose 3 different modes of background subtraction. *Absolute difference to background* calculates the background subtracted image with the absolute function meaning that brighter and darker (as the background) areas will be shown in the residual image. The mode *Brighter than background clamped* will only keep features brighter than the background while *Darker than background clamped* conserves only the darker ones. This setting should be chosen carefully with respect to the expected sample and its brightness distribution. When in doubt, use *Absolute difference to background* for the start. The background subtraction can be directly observed in real time in the main window by selecting the **Display mode** to *Background subtracted*.

**Dynamic**: If this checkbox is activated, the sensor automatically detects the background of the image and isolates objects in the foreground. This mode reaches its limits if there are more foreground objects than images of the background. In general, this is the case if the frequency of passing objects is in the same order of magnitude as the framerate.

Unchecking this feature freezes the background in time. This can be used before an experiment is started to set the background a priori and prevent problems in case of high object rates

**Fixed:** If this feature is active, a fixed background value is used. It is adjustable by the *background fixed value* slider. This is only recommended if the background is very uniform in brightness.

These settings are available for each illumination channel and can be set individually.

## 3.4 ROI

This field shows the placement and size of the two regions of interest (ROI). An example configuration is shown in Figure 16. The sensor only evaluates the images in these regions. To enable a ROI, check the **Active** box. The position and shape of the ROI is either adjusted by entering the numbers in the controls or by dragging the ROI with the mouse in the live preview. To start an experiment or to get a preview of the current ROI, press the button **Apply window settings** below. This button will stay in a clicked visual state until the ROIs are disabled by clicking again. ROI settings can only be changed while the ROI is disabled. However, illumination settings can be changed while a ROI is activated.

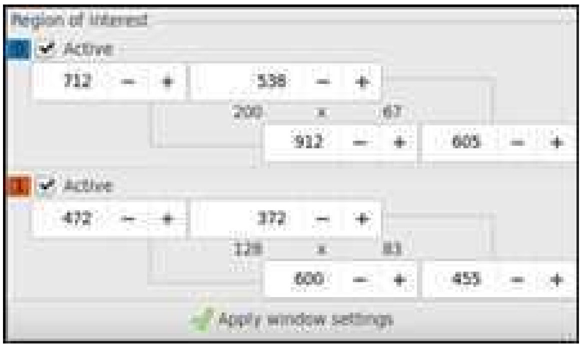



*Figure 16: ROI pane. Used to control the regions of interest the sensor is recording and analyzing.*

The smaller the chosen ROIs, the higher the maximum framerate will be. The height and the width of a ROI have a different impact on the maximum achievable framerate. Every row takes a fixed minimum amount of readout time equivalent to 256 pixels. Therefore, there is no speed advantage by reducing the width of a frame to less than 256 pixels. If the framerate is too low, first consider changing the rotational alignment of the setup and sensor to reduce the number of lines (the vertical height) which needs to be read out, then try to minimize the width of the read-out region.

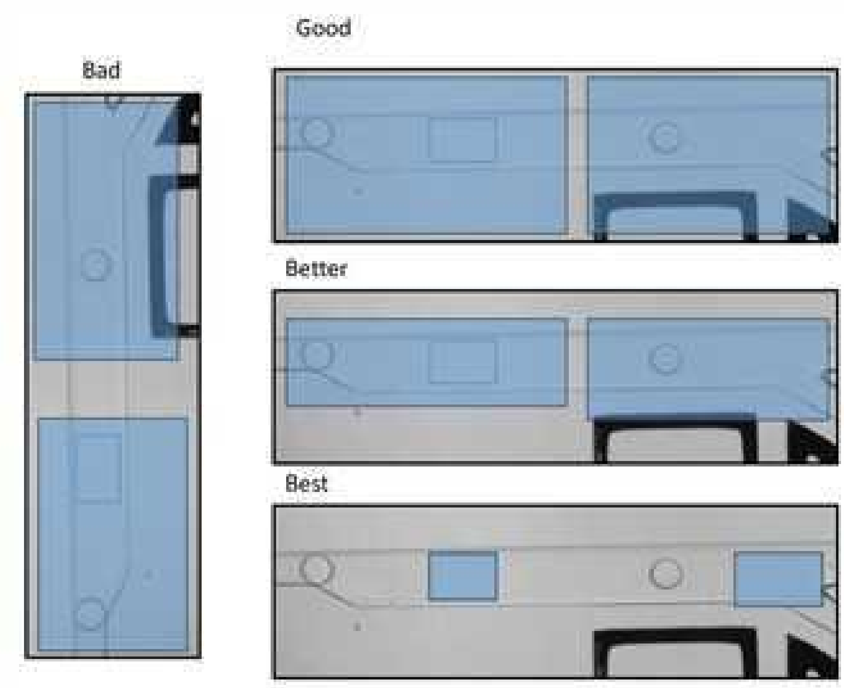
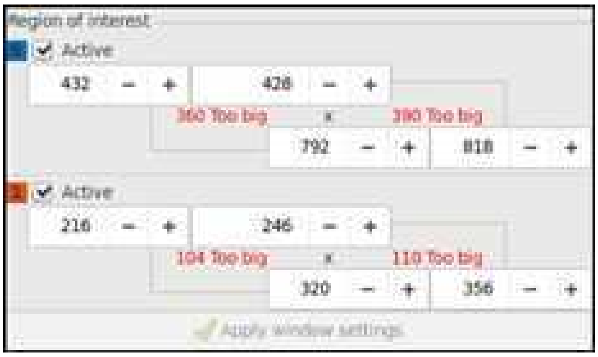



*Figure 17: ROI size and alignment guide. Minimize the height of the readout region and the total area for the fastest possible framerate.*

Some ROI combinations are invalid due to inefficient readout conditions. If such a combination is chosen, the **Apply window settings** button will be grey and a note is shown in the ROI selection field. There is also an upper limit on how big the ROIs can be chosen. Larger values will also disable the **Apply window settings** button. The maximum ROI size is determined by the width of the ROI rounded up to a multiple of 32 pixels and the height. The maximum per ROI pixel count is 262144 px ($2^{18}$ px). Two possible invalid configurations are shown in Figure 18.

It is also possible to measure with one ROI by unticking the second.


*Invalid combination* *ROI too large*

*Figure 18: Invalid ROI configurations.*

## 3.5 Image tools

These tools help to find the best image settings for the specific application and facilitates adjusting the different parameters. The tools are shown in Figure 19.


*Figure 19: Image tools. Allows zooming and showing the different frames for different recording modes as well as viewing what the sensor sees and the computed background.*

The live view can be zoomed with the **Zoom** slider (also possible with **CTRL+MOUSE SCROLL** in the live view).

⚠️ Note: **Zoom** only applies to the preview image, not to the analyzed or saved images.

In the **Show frame** dropdown menu, the user can select the imaging channel that is shown in the live preview. The names correspond to the settings done in the Illumination Configuration window described in 3.3.1.

Choosing the **Display mode** helps to check what happens after background subtraction or to see what the actual background image looks like.

**Show background**: Checking this shows the background instead of the image in the live preview.

**Show background subtracted image**: Checking this shows the background subtracted image instead of the real image in the live preview. The dynamic range is adjusted and often less than the bright field image, so that the appearance might be different than expected

**Show binarized image**: The image is background subtracted and binarized according to the thresholds set in the Experiment Control Section 4.

## 3.6 View range

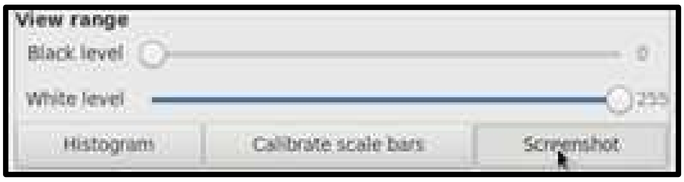



*Figure 20: View range. Can be used to enhance hard to see features.*

Grey values can be compressed for improved visibility by moving the **View range** sliders as shown in Figure 20. Values below **Black level** will be drawn in dark red, values above **White level** will be drawn in a bright red. This helps to adjust illumination condition in order to adjust the setup by the operator.

⚠️ Note: **View range** only applies to the preview image, not to the analyzed or saved images. It is only a change in visualization!

### 3.6.1 Histogram

The histogram button opens a histogram view of the current ROI. It is a versatile feature to adjust the illumination settings and to ensure reproducibility among different illumination setups and experiments. The histogram window as shown in Figure 21 always shows the histogram for the currently shown preview alternating configuration.

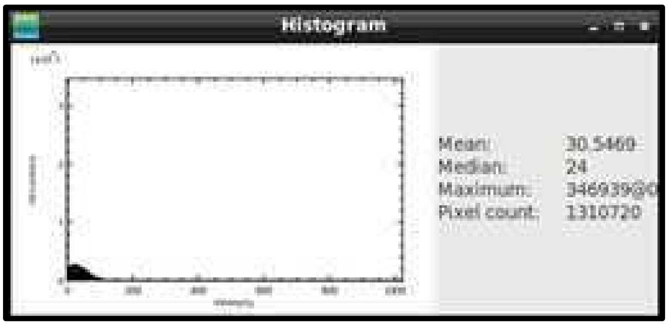



*Figure 21: Histogram window*

### 3.6.2 Calibrate scale bars

This function controls the visibility and enables the calibration of the scale bar in the image. The window looks like shown in Figure 22.


*Figure 22: Image scale calibration window. The two radio buttons define which method is used for the scale bare and calibration. Mark in image allows to select two points in the image to determine their distance, which is then correlated with the entered known distance to calculate the magnification. Here, the optical total magnification is used as calibration.*

The scale bar can be inserted into the preview image by checking the **Show scale bar** box. There are two possible ways to set the scale bar:

**Optical total magnification** uses the total optical magnification of the microscope. It is important that not only the objective magnification but also the additional magnification (e.g. tube lens and post magnification) is considered.

**Mark in image** allows the user to mark a region in the image and enter its known dimensions. Click the button, then click in the preview window on the first and then on the second point, the distance in pixels will then be automatically computed and inserted in the “px” input field.

Alternatively, the conversion factor can be entered directly if known from previous experiments. Check the radio button in front of the computation variant you want to use.

This scale is used to scale all length scales and parameters containing length in the measurements if “Use scale” is active (see Experiment Control).

### 3.6.3 Screenshot

By clicking the **Screenshot** button, a picture of the preview window is taken and the destination for saving can be selected in the opening dialog.

A normal ***Print screen*** screenshot from the operating system will only capture a green field where the live preview is displayed.

## 3.7 Tools


*Figure 23: The Tools.*

After adjusting all image and sensor relevant parameters, these buttons are the most important ones for starting the experiment. The buttons look like shown in Figure 23 and might be hidden by the window size if lots of controls are shown in the image control section. Therefore, one might need to scroll down to see the buttons.

**Start experiment** opens the main window to analyze passing objects. Here, all settings related to the measurement are made. Also capturing video sequences, adjusting sorting parameters and recording results are controlled in the **Experiment control window** as described in section 4.

**ExperimentGuard** opens a tool to visualize and observe various properties of passing objects in real time. This feature helps to adjust the experiment (e.g. object rate or droplet size). See section 5 **Experiment Guard** for further information.

**Sol Configuration** allows activating the SOL device if connected as described in section 3.1.

**Counter** allows to count events in real time to e.g. see droplet rates while adjusting an experiment. See section 6 for details.

**Pump control** allows controlling of microfluidic pumps. The list of available vendors is expanded over time. See section 7 for details.

**Laser control** allows to control selected laser drivers. See section 8 for details.

## 3.8 Help

The **Help** button shows detailed information about the program version and Copyright data. Some options which should not be changed without consultation by a support agent can be changed here. The MQTT and HTTP server are experimental and therefore disabled per default.

**Max # of points** in line plot defaults to 5000. Max # of points in scatter plot to 1000. **Plot frames vertically** is default disabled. **Advanced settings** is default enabled and allows to adjust and use all functionality of the software and sensor.

The combobox with languages enables translations of the UI to other languages. Support for them is experimental and not complete.

**About** shows some legal data, the software version and build date. This information is important when communicating with Sensific support.

# 4 Experiment Control

The **Experiment Control** window is the heart of der ODIN Software and is opened by clicking on **Start experiment** in the main window. Here, all parameters for particle detection, sorting, analysis and saving are set. A sample screen is shown in Figure 24. It is split into four parts. On the left the settings for particle detection, analysis and sorting are set. In the upper middle part images of the last recorded sequence are shown while directly below the corresponding graph can be seen. In the lower part the settings for capturing and saving of measurements can be made. The right-hand side shows the detected parameters of the corresponding preview image. The individual settings are described below.

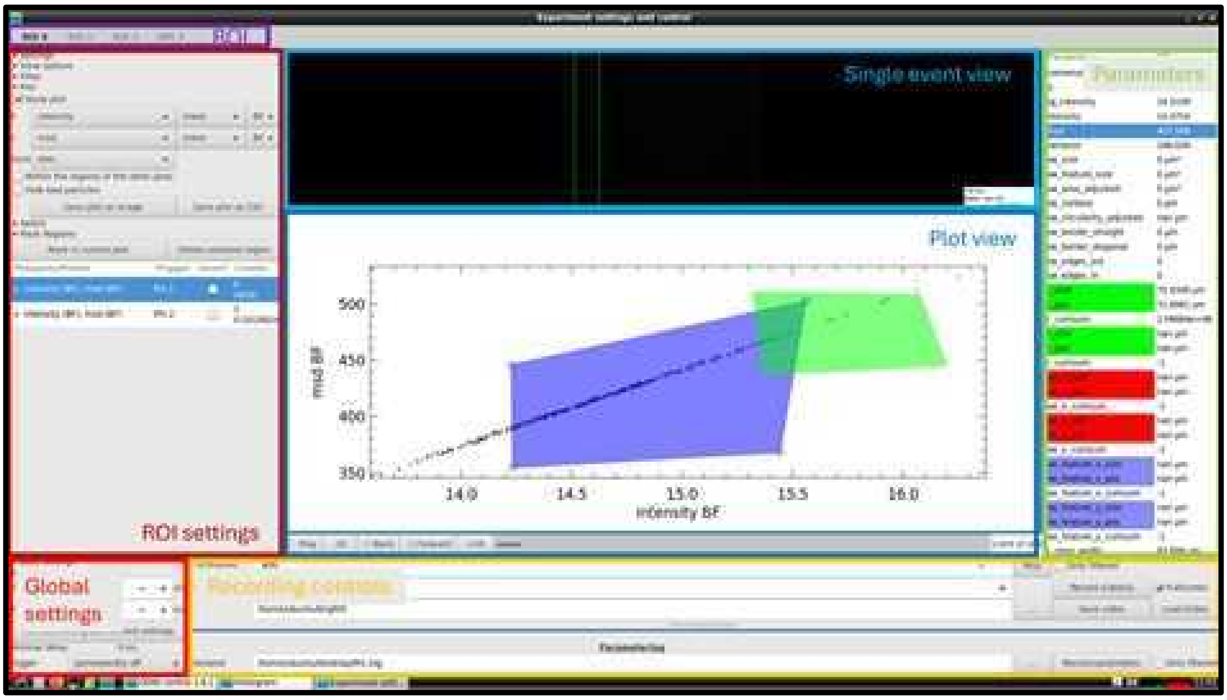



*Figure 24: The Experiment settings and control window. Marked are the main areas "Recording controls", "ROI", "ROI settings", "Single event view", "Plot view", "Parameters", and "Global settings".*

## 4.1 Record control

In this field the viewing, recording and saving of videos and parameter files is controlled.

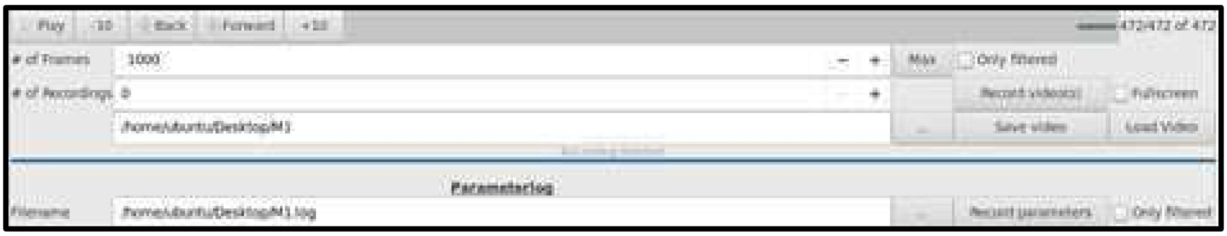



*Figure 25: Recording controls.*

### 4.1.1 Video controls

The individual frames of a recording can be assessed by moving the slider. The current frame and the maximum frame count is displayed to the right.
The buttons **-10**, **Back**, **Forward** and **+10** can be used to move the slider by 10 or 1 frames back and 1 or 10 frames forward. The **Play** button plays the recording at a speed of 2 frames per second.

A blue circle in the plot (if a plot is active) shows the values of the current particle. If too many frames for plotting are in the recording (visible by the text *Showing xxx out of yyyy* in the plot), then not every frame selectable by the slider will have a blue circle in the plot, because it is not plotted.

The text **xxx/yyy of zzz** is the currently selected frame **xxx** in the recording, out of the number of frames **yyy** passing the filter currently of the total number of all frames **zzz** in the recording, regardless of passing the filter.

### 4.1.2 Settings

After activating the ROIs in the main window, the first step to record a video is to enter the number of images that should be recorded in the **# of Frames** field. By clicking the **Max** button, the maximum number of frames possible with the set configurations can be selected.

If a series of recordings should be captured, the **# of Recordings** can be set to a value higher than 0. After one recording is finished, the file is saved and a new recording starts immediately. This feature is useful if many objects should be recorded without changing the parameters or the system. Hence, improved statistics can be achieved without increasing the workload.

The name entered in the **Save dialog**, will be the name of the folder containing all files of the recording. It contains two folders named ROI1 and ROI0 (if both ROI are active, otherwise only the active ROI is saved) and two files called *realtime.c* containing the settings for the trigger and sorting control, and *settings.exp* where all settings of the experiment such as ROI, exposure, time are saved (See section 12.1). Within the ROI folders, the image data is saved. Either as *.avi* or as *.rec* file, depending on the settings of the corresponding dropdown menu. The determined parameters are saved in the *.log* file as a tab separated text file. The filename and location can be set by clicking **"..."** and selecting the path.

Checking the **Only filtered** box allows for longer recordings by applying the filter settings (see *section 4.3.3*) directly when capturing the data. This means that only images and the corresponding parameters matching the filter settings will be saved. This is useful in case of rare events since this feature enables a longer recording time. Thus, more objects can be characterized within the same amount of data. The drawback is, that if one wants to widen the filter after recording the data, the images and the data has already been discarded, so the data is lost.

**Record Video** starts recording the videos and parameters. While a recording is running, the button description changes to "**Stop recording**" and clicking it will stop the recording as soon as possible.

**Save Video** saves the current captured data to the previously selected location. Multiple recordings made with the **# of Recordings** field are saved automatically.
⚠ If **Auto file numbering** (see section ) is unchecked and the file already exists in the folder designated for saving, this file will be overwritten.

⚠ The video saved is the one currently shown in the data plot and the preview. Hence, if the filter option (See Section 4.3.3) is active, only these data points and images are saved. If you want to save all frames, you need to uncheck the checkbox **Enable filter** in the Settings pane of the ROI (see Section 4.3.3) first before pressing **Save video**.

⚠ If more than one ROI is active, all ROIs are saved with their respective parameter and filter settings.

**Fullscreen** is a convenience button for the **Apply window settings** button in the main window and mirrors its functionality and state.

**Load video** allows to load a previously recorded video and the corresponding parameters. This is useful if the setting of new trigger parameters should be compared with older measurements. It is only possible to load AVI or REC videos. Therefore, the *.avi* or *.rec* file has to be used. Also, only ROI0 of the video will be loaded.

### 4.1.3 Parameterlog

The parameterlog directly saves the detected parameters without saving the corresponding images. This enables quasi-infinitely long measurements only limited by the available storage capacity.

If filtering is active and the **Only filtered** checkbox is active, only the filtered parameters are recorded. The filename and location can be set by clicking **...** and selecting the path. The logging format is CSV with "," as a separator and "." as decimal point.

Recording is started by clicking the **Record parameters** button and stopped the same way. While the recording log is running, the **Record parameters** button appears to be stuck in the pressed-in state.

⚠ If filter criteria are changed during measurement, this will not be reflected by the parameter log. To account for the changes, the parameter logging must be restarted.

## 4.2 ROI

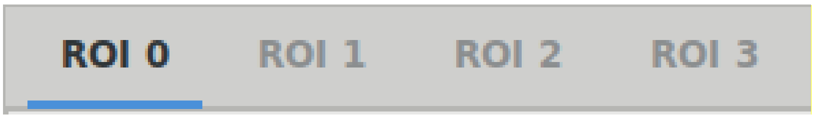



*Figure 26: ROI tab selector*

On the left-hand side of the Experiment Control Window, the settings for particle detection, filtering and triggering are placed.

First thing to select is the ROI selector. ODIN can capture and evaluate up to two ROIs at the same time. Also, two additional ROIs can be used for imaging, even though they are not analyzed by the sensor. For a better overview, the experiment window only shows the images and data of one ROI. With the ROI selector the user can switch between the different ROIs.

⚠ During experiments the ROI not shown will also contribute to triggering, saving, filtering etc. if it is set active in the main window (see section 3.4) even if it is not currently the active tab in the *Experiment Control Window*. Make sure to check the settings and results of all active ROIs to avoid misleading results. It is highly recommended to deactivate the ROIs if they are not needed.

## 4.3 ROI settings

This pane configures the various settings for a single ROI. They can be set independently for all ROIs. In the next section each subgroup will be explained.

### 4.3.1 Settings

To get the best out of the experiment, the parameters for the ODIN algorithms have to be set before the experiment. This is done in the upper part of the setting area. Settings from previously saved measurements can be loaded by clicking the **Load from .*exp*-file** button and selecting an .*exp* file.

For a successful experiment, the settings have to be adjusted to the imaging condition. This varies primarily with contrast, brightness and signal to noise ratio of the image but also with the magnification, imaging method (e.g. brightfield, phase contrast, darkfield, fluorescence, ...), and sample type. In the following the settings and their impact on the experiment are explained and recommendations on how to adjust them are given.

Depending on the number of imaging channels used (see section 3.3.1) more than one column for adjusting parameters is shown. For each channel the parameters can be set individually. A sample settings field is shown in Figure 27.

In general, two classes of parameters can be adjusted. The *Threshold* values are applied directly on the image that is summed in a row and column like manner. If the summed intensities exceed a certain threshold value, a particle is detected. The *Bw* (Black-white) labeled values correspond to the binarized image processing.

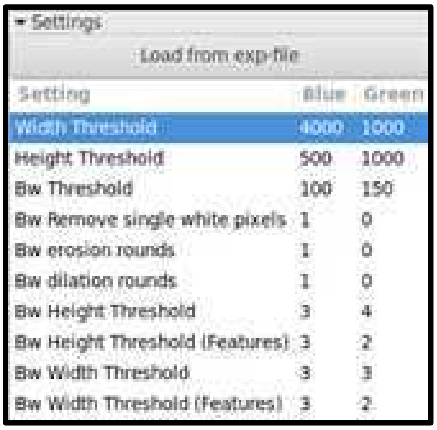



*Figure 27: Settings for analyzing images. They can be adjusted by clicking on them.*

#### *4.3.1.1 Width/Height Threshold*

The threshold should be chosen high enough to reliably detect the particles relevant for the experiment while not reacting on dirt or noise. Values of several thousands are a good start when a high contrast image is analyzed. For low contrast images, a starting value of 1000 is recommended.

**Adjustment guide:** If no particle is detected, the threshold should be lowered. If too many particles, dirt or noise is detected as a particle, the threshold should be raised.

#### *4.3.1.2 Bw Values*

In most experiments the black-white detection method is the better choice due to its robustness. The most important value here is the *Bw Threshold*, influencing all following settings. The value of these parameters must be between 0 and 1024. It is defined as the threshold for each pixel. If the pixel brightness value has a lager difference from the background image (see section 3.3.1) i.e., it is significantly brighter or darker than the background it is labeled as “bright”, otherwise it is labeled “dark”. This results in a binary image of bright and dark pixels. This image is then further processes by the following parameters and algorithms. The number in brackets in the *Value range* column indicates typical values from where the user can start to customize them to the specific conditions in the experiment. See section 9 for further explanations. A detailed explanation of all settings is shown in Table 2.

*Table 2: Setting parameters. The typical value range should not be exceeded. The description tries to aid in determining the effect of changing the parameter.*

| Name | Value range (typ.) | Description |
|---|---|---|
| *Bw Remove single white pixels* | 0-1 (1) | Removes single isolated white pixels from the binarized images. This helps reducing artifacts from noise. |
| *Bw erosion* | 0-16 (0) | The binarized image can be eroded to remove halos or sparks in images. Use when necessary and begin with small values. Often it can be set to |

| | | |
|---|---|---|
| | | 0. Using this feature changes the apparent size of the object so use with care. |
| *Bw dilation* | 0-16 (0) | The binarized image can be grown to obtain smoother reliefs. Use when necessary and begin with small values. Often it can be set to 0. Using this feature changes the apparent size of the object so use with care. |
| *Bw Height Threshold* | 0-1024 (3) | Determines how many pixels in a single row need to be above the threshold to indicate the beginning or end of particle. Value should be significantly smaller than expected particle height. The larger the ROI and the higher the noise, the larger the value should be. This applies for all parameters where all black pixels within closed white areas are "flooded" i.e., set to white. |
| *Bw Height Threshold (Features)* | 0-1024 (3) | See "Bw Height Threshold". But with the binarized but otherwise unaltered, non-flooded image. |
| *Bw Width Threshold* | 0-1280 (4) | Determines how many pixels in a single column need to be above the threshold to indicate the beginning or end of particle. Value should be significantly smaller than expected particle width. |
| *Bw Width Threshold (Features)* | 0-1280 (4) | See "BW Width Threshold" and "BW Height Threshold (Features)". |

### 4.3.2 View options


*Figure 28: View options in the experiment control window.*

The **View options** change the visualization of the captured images. It works like the menu in the main window. This helps while setting up and adjusting the parameters. The upper and lower limits of the displayed brightness values can be adjusted here. **Black Level** controls the value for black, **White Level** for white. When the pixel values exceed these limits, the pixel is colored in red. Bright red if the upper limit is exceeded, dark red if the lower limit is exceeded.

Checking **Show Bw** approximates what the binarization module of the sensor calculates. It binarizes the contrast between the current image and the surrounding images and thresholds it by the value set in *Bw Threshold* in the *Settings* menu.

**Show background subtracted** shows the image as it is seen by the sensor by approximating the background and calculating the average image from the neighboring 10 frames.

**Show neighboring images** shows the directly neighboring images to the side in the image viewing area, if saved in memory. Directly neighboring can also mean from another alternated frame, e.g. bright field and fluorescence combination. When recorded in filtered mode, most often the direct neighbor is not stored in memory and therefore the image is not displayed.

⚠ These setting have nothing to do with the parameter detection or the saved images, they only serve to adjust the dynamic range of the preview image.

### 4.3.3 Filter

Filtering the result of a measurement is very useful to focus only on important events or to enable long term measurements without producing huge amounts of irrelevant background data. Only data points meeting the filter criteria will be shown and displayed in the data plot. All filter parameters are enabled with the **Enable filter** checkbox. The Filter settings are shown in Figure 29.

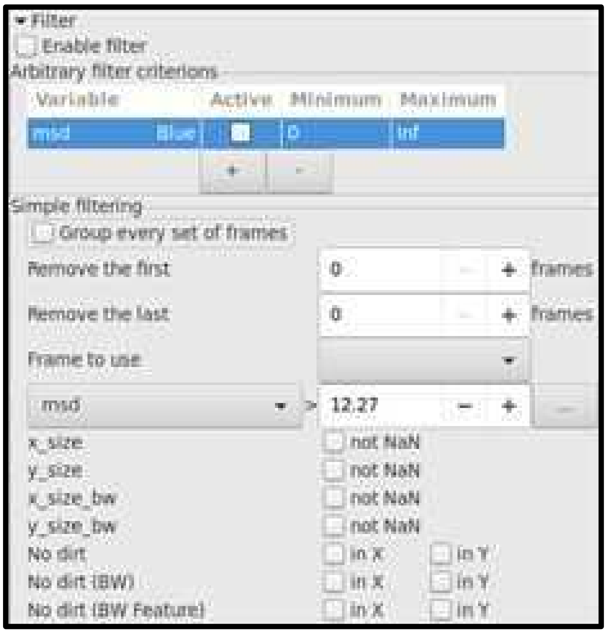



*Figure 29: Filter settings.*

The **Arbitrary filter criterions** are very powerful. In the *Variable* section a parameter from the parameter list (see section 4.6) is chosen. A *Minimum* and *Maximum* value can be set for each parameter and for each imaging channel. With the **+** button more rows i.e., parameters can be added and removed by the **–** button. The variable selected needs to be between *Minimum* and *Maximum* to keep the frame. The rows are only applied if *Active* is checked. This allows fast cycling between different filter criteria, if wanted. This section is only visible, if the **Enable advanced settings** mode is active (see Section 3.8). If no filter criterion is possible to be expressed by the

**Simple filtering** mode, then select a dummy variable in the simple filter mode (e.g. intensity>0, which is basically impossible to not pass).

The **Simple filtering** section is suited for quick filtering of the frames. Normally this is sufficient and can be used without using the **Advanced settings** mode.

If **Group every set of frames** is active, the sensor groups together all parameters and images belonging to one event. The beginning and end of the event is classified by the filter criterion active. This means that all consecutive images are grouped together and when the filter criterion is not met for at least one frame, ODIN senses a new particle and starts grouping the next one. As residual image the median image (i.e. the middle image of the sequence) of the grouped series is taken and for this the parameters are used. If particles enter and leave the region of interest during several frames (i.e. are relatively slow) it can be of advantage to use the **Remove the first** and **Remove the last … frames**. As the name suggests, this drops the selected number of frames at the beginning and ending of a group sequence.

The **Frame to use** selects which image to take the parameter from. In the dropdown menu the parameter for filtering is selected and a value can be entered in the field as lower boundary. By clicking the **"…"** button, the plot will automatically change the y-axis of the plot to the selected parameter and a click in the plot will use the height as the new threshold value.

If only the data of detectable particles should be saved, the **not NaN** option is very handy. If checked, only those datasets where the respective parameter (width (**x_size**), height (**y_size**), width in the binarized image (**x_size_bw**) or height in the binarized image (**y_size_bw**)) has a valid value are kept.

The same applies for the **No dirt** part. A particle is characterized as dirt if it is not completely in the ROI. This means that if the rim of the particle is not visible within the image, it is labeled as dirt. Therefore, three different calculation modes (normal, Bw and Bw Feature) can be selected. A image passes the filter if the following is true:

- x_size not NaN: x_size is not NaN (not a number), x_size is valid
- y_size not NaN: y_size is not NaN (not a number), y_size is valid
- x_size_bw not NaN: x_size_bw is not NaN (not a number), x_size_bw is valid
- y_size_bw not NaN: y_size_bw is not NaN (not a number), y_size_bw is valid
- No dirt: x: left_dirt=0 and right_dirt=0, y: top_dirt=0 and bottom_dirt=0
- No dirt (BW): x: bw_left_dirt=0 and bw_right_dirt=0, y: bw_top_dirt=0 and bw_bottom_dirt=0
- No dirt (BW Feature): x: bw_feature_left_dirt=0 and bw_feature _right_dirt=0, y: bw_feature _top_dirt=0 and bw_feature _bottom_dirt=0

### 4.3.4 Plot

Plotting the recorded parameters for a sequence is a very important feature especially when parameters for sorting or filtering have to be determined. The plot panel is shown in Figure 30.


*Figure 30: Plot configuration options. The parameter to plot and the channel to take the parameter from can also be chosen by right-clicking on the parameter in the parameter table on the right side of the Experiment window.*

To visualize the data, the **Plot** checkbox must be activated and two parameters must be selected from the dropdown menus representing the x- and y-axis. In the **Style** dropdown the options are **lines** to connect the values in a chronological order, this is best used to connect datasets especially when the x-axis is associated with time. In all other cases the **dots** options for creating a scatter plot is recommended. In the dropdown menu behind the parameter, the plotting style for each axis can be chosen to be *linear* or *logarithmic*. If *logarithmic* is chosen as plot tool, be aware, that the lines drawn by polygons might be misleading, as they are straight lines in the linear space and not straight lines in the logarithmic space, even if the are plotted as straight lines. In the last menu the frame respectively the imaging channel can be chosen. The names refer to the settings in the illumination control menu (see section 3.3.1). If *All* is selected, all channels are plotted at the same time. Choosing different colors for the frames in the Illumination control menu allows you to differentiate between the different channels. The parameter and channel can also be chosen from a right click context menu of the parameter table (see Section 4.6).

If a polygon for sorting in other projections of the dataset (so other combinations of the x- and y-axes) is already selected (see section 4.3.6), checking the **Within the regions of the other plots** box shows only points which are in these already selected polygons. The points are either black dots, for unevaluated measurement points, a green plus (**+**) for good evaluated points, and a red cross (**x**) for bad evaluated points (see section 4.3.5).

**Hide bad particles** excludes points, which have been marked **Bad** by the user.

Clicking on a point in the plot the values and the corresponding image nearest to that point is selected and displayed. The plot can be zoomed by clicking and dragging the mouse. The original zoom is restored by a double click.

**Save plot as image**: Save the plot as a *.png* file to the designated path.

**Save plot as CSV:** Saves the parameter displayed in the plot as a .csv file to the designated path. Note: only the plotted parameters will be saved, nothing else.

### 4.3.5 Select

To facilitate sorting of complex and unknown samples the **Select panel** can be used. The panel is shown in Figure 31.

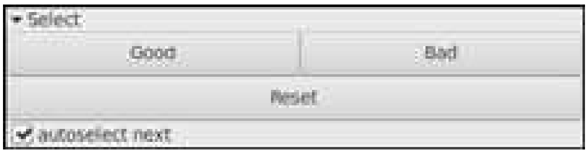



*Figure 31: Select buttons.*

To visualize events that should be sorted or not, the currently displayed image can be marked by clicking the **Good** (F1 key) or **Bad** (F2 key) buttons. If the **autoselect next** box is checked, the next image of the sample is displayed automatically. In the data plot the marked points are shown as green **+** (Good) and red **x** (Bad). This helps to identify regions of the parameter space where the different populations are clustered or separated.

### 4.3.6 Mark regions

Marking different regions in scatterplots to select clusters of events for sorting is the most important function for reliable and precise sorting. A sample selection area is shown in Figure 32.

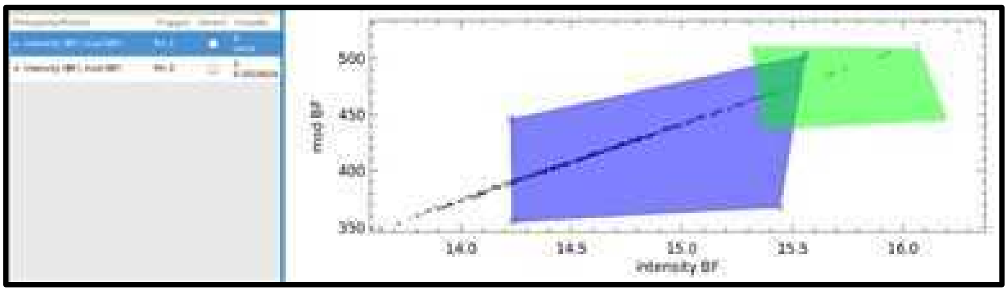



*Figure 32: Mark regions pane and a sample selected areas in a measurement. A blue shade is used for Trigger Pin 1 and a green shade is used for Trigger Pin 2. A trigger event is generated, if the data point falls into any shaded region, the shade intensity has no deeper meaning.*

The button **Mark in current plot** enables the user to draw an arbitrarily shaped polygon in the scatterplot region by clicking on the desired corner points. These points are then interconnected by a straight line. If the polygon should be closed, the **ENTER** key can be used directly or a click near the start point will close it. Data points within the polygon are then selected for setting the trigger pin. If more than one combination of parameters should be considered, a second polygon in a different scatterplot can be drawn. Now only data points serving both conditions will be sorted. The **Delete selected region** button removes the active polygon. The straight lines are always meant to be in the linear space.

The selected points can be seen in the dropdown list below the names of the parameters.

The regions define the **Trigger** pin used for sorting when activated. Click on the Pin name and select “Pin 1” or “Pin 2” to define the pin. All polygons with the same trigger pin in the ROI form a group. If the current frame passes the polygons in the group as defined in Section 4.3.7, the trigger pin is activated (see Section 4.7.2)

The region trigger mode can also be **inverted** to trigger when a data point falls outside of the polygon. Note that this inversion is not indicated in the plot.

A counter information is shown for the polygon, listing how many particles have fallen into the polygon region and at which rate over the counter integration time (see Counter section 6 for more information).

### 4.3.7 Multiple regions combining rules

If multiple regions are defined for a single trigger pin, the regions are combined as follows:

- Regions within the same attribute (parameters) area are combined with logical OR, meaning a data point must lie in at least one of the regions to be considered “in” and trigger the sorting pin.
- Regions with different attribute (parameter) pairs are combined with logical AND, meaning for all attribute combinations, the data point must lie within at least one of the defined regions for that combination to be considered “in” and trigger the sorting pin.

⚠ It is recommended not to use more than 50 points to define the polygon in order to guarantee smooth runtimes.

## 4.4 Single event view

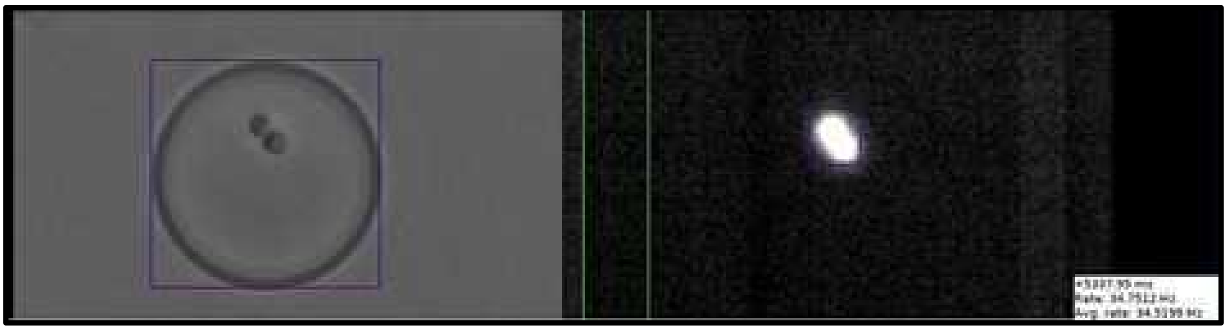


*Figure 33: Single event view. Showing the alternated illumination captures and some minimal statistical information*


*Figure 34: Single event video selector*

The single event view shows the current data point, as it can be selected in the classical video controls below the plot as shown in Figure 34 and looks like shown in Figure 33. The event to display can also selected by clicking onto a data point in the plot view.

## 4.5 Plot view

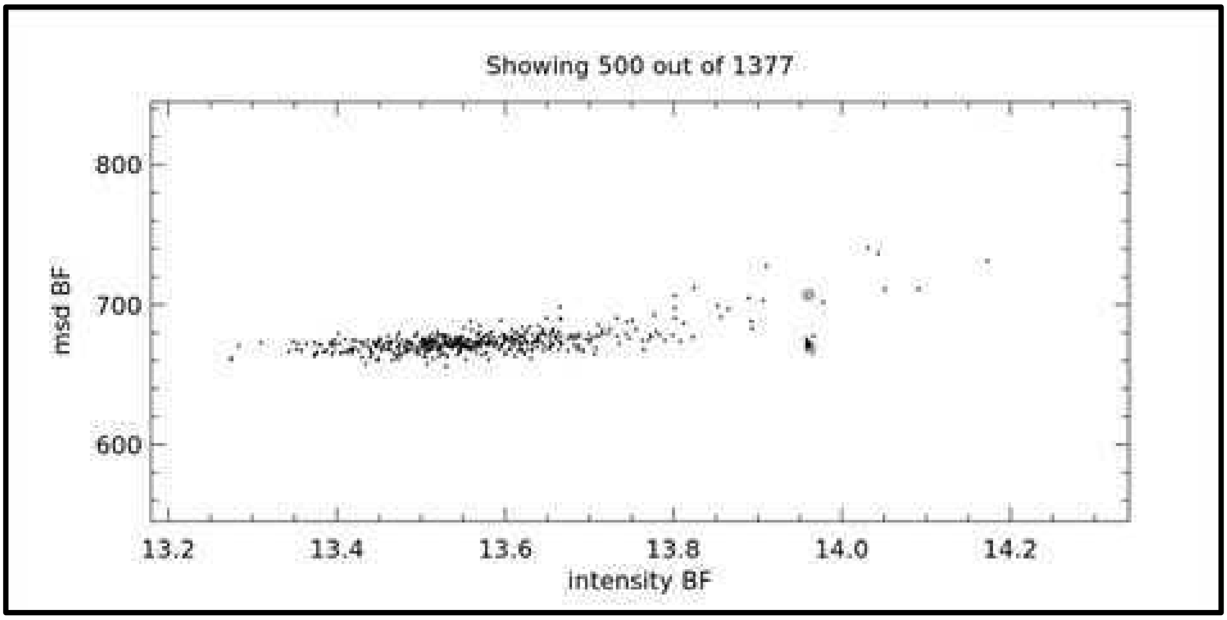



*Figure 35: Plot window. Shown is a scatter plot with msd over intensity*

The plot view shows the configured plot (see section 4.3.4). The maximum number of points plotted can be limited by the settings in the Help dialog as described in section 3.8. Clicking onto a data point shows it in the Single event view. Clicking and dragging creates a zoom rectangle. Double clicking resets the zoom to show all data points. The color of the dots and lines is set using the color for the illumination mode (see section 3.3.1).

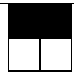
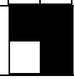
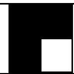
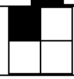
## 4.6 Parameters

On the right-hand side, the determined parameters of the active image or images/channels mode is used are given. This is the list of parameters the sensor is computing always and in real time while it is running. These parameters qualify and quantify the content of the image. Some parameters are computed without any input from the user, some need settings adapted to the content the user expects to see (see Section 4.3).

The **Scaled** checkbox indicates if the parameters are given in pixels (default) or if the scaling set in the *Calibrate Scalebar* menu (see section 3.6.2) is applied.

In red, green and blue parameters that are also displayed as lines in the preview image are marked. In the following table a brief description of every parameter is given. All parameters starting with *Bw* are calculated from the thresholded black-white image.

*Table 3: List of parameters with description*

| Name | Value range | NaN? | Description |
|---|---|---|---|
| framenumber | 0-2^32-1 | No | Framenumber, increases from 0 at the point where the sensor is started |
| Ts | 0-2^32-1 | No | Timestamp of the image from a 10 ns step freerunning 32 bit counter |
| bg_intensity | 0-130 000 000 | No | The sum of all pixel brightness values of the background image |
| intensity | 0-1.3E9 | No | The sum of all pixel brightness values after background subtraction |
| msd | 0-1.3E12 | No | Sum of the squared brightness values of each pixel after background subtraction |
| variance | 0-4.3e11 | No | Variance of the background subtracted image |
| bw_size | 0-1310720 | No | Number of white pixels in the binarized image (including filling black seas white) |
| bw_feature_size | 0-1310720 | No | Number of white pixels in the binarized image |
| bw_area_adjusted | 0-1310720 | No | Area (bw_size) adjusted by the border pixels (0.5 pixel size) |
| bw_contour | 0-1310720 | No | Contour sum of all particles in the image (circumfence) |
| bw_circularity_adjusted | 0-1 | Yes | Circularity 2 sqrt(pi)sqrt(bw_area_adjusted)/bw_contour |
| bw_border_straight | 0-130 000 | No | Number of straight border elements with a length of 1 px  |
| bw_border_diagonal | 0-130 000 | No | Number of diagonal border elements with a length of $\sqrt{2}$ px  |
| bw_edges_out | 0-130 000 | No | Number of “out” edges. Kernel element:  |
| bw_edges_in | 0-130 000 | No | Number of “in” edges. Kernel element:  |
| x/y_size | 0-1280 | Yes | Size of the particle in x/y (greyscale method) |
| x/y_pos | 0-1280 | Yes | Position of the particle in x/y (greyscale method) |
| x/y_cumsum | 0-1.3E9 | Yes | Intensity of the area from the start of the particle to the end of the particle in x/y |
| bw_x/y_size | 0-1280 | Yes | Size of the particle along the dimension (x or y) extracted from the binarized and flooded image |
| bw_x/y_pos | 0-1280 | Yes | The position as the center between the beginning and the end of a particle along the indicated direction (x or y) extracted from the binarized and flooded image |
| bw_x/y_cumsum | 0-130 000 | Yes | Number of white pixels within the area marked by *Bw_x/y_size* and *Bw_x/y_pos* |
| bw_feature_x/y_size | 0-1280 | Yes | Size of the particle along the dimension (x or y) extracted from the binarized image. |

| | | | |
|---|---|---|---|
| bw_feature_x/y_pos | 0-1280 | Yes | The position as the center between the beginning and the end of a particle along the indicated direction (x or y) extracted from the binarized image |
| bw_feature_x/y_cumsum | 0-130 000 | Yes | Number of white pixels or sum of the intensity within the corresponding area marked by *Bw_feature_x/y_size* and *Bw_feature_x/y_pos* |
| x/y_chan_width | 0-1280 | Yes | Size of a channel if both borders are bright and the middle in between is dark in x/y (greyscale method) |
| bw_x/y_chan_width | 0-1280 | Yes | Size of a channel if both borders are bright and the middle in between is dark in x/y (binarize method with filling) |
| bw_feature_x/y_chan_width | 0-1280 | Yes | Size of a channel if both borders are bright and the middle in between is dark in x/y (binarize method) |
| left/right/top/bottom_dirt | 0, 1 | No | 1 if a particle is on the left/right/top/bottom side border, 0 otherwise |
| bw_left/right/top/bottom_dirt | 0, 1 | No | 1 if a particle is on the left/right/top/bottom side border, 0 otherwise (binarize method with filling) |
| bw_feature_left/right/top/bottom_dirt | 0, 1 | No | 1 if a particle is on the left/right/top/bottom side border, 0 otherwise (binarize method) |
| x/y_move | 0-1280 | Yes | Movement of the particle in x/y (greyscale method) |
| bw_x/y_move | 0-1280 | Yes | Movement of the particle in x/y (binarize method with filling) |
| bw_feature_x/y_move | 0-1280 | Yes | Movement of the particle in x/y (binarize method) |
| bw_perimeter | 0-240 000 | No | $\sqrt{2}$ ·Bw_border_diagonal+bw_border_straight |
| bw_particle_count | 0-327680 | No | Number of particles (computed by the edges_out and edges_in parameter) |
| bw_circularity | 0-Inf | Yes | Circularity is defined as $4\pi A/C^2$ with A the size (*bw_size*) and C the circumfence (*bw_perimenter*) |
| elongation | 0-Inf | Yes | $\frac{x\ size}{y\ size}$ |
| bw_elongation | 0-Inf | Yes | $\frac{Bw\ x\ size}{Bw\ y\ size}$ |
| intensity_difference | -1310720 – 1310720 | No | intensity-bg_intensity |
| intensity_quotient | 0-Inf | Yes | intensity/bg_intensity |
| NCA | 0-Inf | Yes | Normalized Contrast area (bw_size-bw_feature_size)/bw_size |
| count_all | 0-2^32-1 | No | Counts all particles, works only if sorting code has been generated |
| count_sorted | 0-2^32-1 | No | Counts all particles which satisfied the trigger criterions |
| count_unsorted | 0-2^32-1 | No | Counts all particles which do not satisfied all trigger conditions |
| count_averaged | 0-2^32-1 | No | Number of frames used for the computation of this particle |
| is_good | 0-1 | Yes | NaN when the user has not marked the image, 0 if *bad* and 1 of *good* marked. Cannot be used as sorting criterion |

Before any computation step takes place, an automatically computed background image is defined and gets subtracted from the image.

The parameter for *Width* and *Height* depend on the setting of *Width Threshold* and *Height Threshold*. The higher the threshold, the bigger the particle needs to be to get detected. These values have sub-pixel precision. See Section 4.3.1.1 for more information.

The *non-Bw* values are calculated by summing the grey values column and row wise. These curves are then thresholded to determine the parameters.

The computation of all *BW* values starts with thresholding and binarization of the image as described in the *Experiment guard* (section 5) and *Settings* (see 4) section. After binarization and the optional removal of single white pixels, every white area gets reduced by the outer row of pixels as often as *Bw erosion rounds* are set. Then, every remaining white pixel island grows by one row of pixels for *Bw dilation rounds* times. Then, the border detection and summing of pixels along each dimension (x and y) takes place. Finally, these curves are thresholded to determine the particle size.

The *non-feature bw* values are calculated with an additional computing step where every black region that is not connected to the outside with a black line is filled with white. E.g. a white ring gets filled with white, because the black inside does not have a connection to the black outside. This allows an easier measurement of the size in terms of pixel count. The number of black pixels converted into white ones can be determined by *Bw_size - Bw_feature_size*. This measure can be inexact when *Bw erosion rounds* and *Bw dilation rounds* are used.

Some typical operations with these parameters are given in a context menu as shown in figure Figure 36 when **Right clicking** on a given parameter.


*Figure 36: Right click menu of a parameter.*

The **Plot on X-axis** and **Plot on Y-axis** sets the currently marked parameter as the plotting parameter of the plot in the center of the window. This is equivalent to selecting the parameter in the X and Y dropdown menu in the plot configuration. **Hide** hides the parameter from showing up in the menus, but it is still saved and exported. This allows to hide parameters which for a given experiment are of reduced meaning. To show all hidden parameters again, click **Show all** on a random parameter.

## 4.7 Global settings

The global settings are region independent and therefore set at a global level. The different settings are described below.

### 4.7.1 Save settings

In this section the setting for saving the data can be adjusted. If an additional file format in addition to the .log file should be saved this can be activated and selected here.

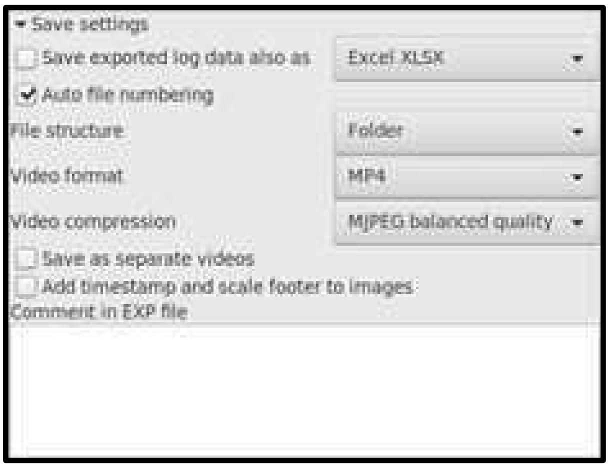



*Figure 37: Save settings. Define the export format, the folder structure, the video compression and add a comment.*

The parameters are saved as a large CSV file (separator is "," line separator is "\n", no quotes, no special characters, float separator is ".", scientific notation uses "e" for the exponent, "nan" is used for Not a Number, which occurs if a calculation run into an error), containing a header row delineating the names of each row, followed by n rows, n being the number of frames in the recording. Each row corresponds to the frame with the same number (without counting the header row).

**Save exported log data also as** allows the log data directly to be saved as XLSX, XLS, ODS, and a bad compressed version of XSLX (Excel XLSX-fast).

⚠ Saving additional file types will significantly increase the time needed for saving.

If **Auto file numbering** is checked and the folder/file already exists, a number at the end of the filename is added or increased by 1. Otherwise, if the name of the file is not changed (see section 4.1.1) for each new file, the old one will be overwritten.

In the **File Structure** menu the structure of the final dataset can be chosen. We recommend using the *Folder* structure, so each measurement is assigned to a new folder allowing for a clearer structure. Otherwise, the files will be prefixed with the filename provided.

For the folder structure, the following is created:

- Folder name as set in the recording Filepath
    - settings.exp: The settings for the experiment. Can be reloaded by the software. INI-style sectioned entries.
    - realtime.c: The code executed on the realtime processor for sorting. Mostly for debugging and diagnostic purposes.
    - ROI0
        - ROI0[_frameX]_background.png: The background at the start of the measurement, [_frameX] is inserted when storing separate videos and having more than one active imaging mode.
        - ROI0.log: The log file
        - ROI0.xlsx: The log file as excel (optional)
        - ROI0[_frameX].[rec|avi|mp4]. The video files. Extension depends on the selected type, [frameX] is inserted when storing separate and having more than one active imaging mode.
        - ROI0[_frameX].00000012.[bmp|jpg]. The image files, if selected an image format for storing.
    - ROI1: The equivalent to ROI0.

If **File prefix** is selected as storage variant, all what would have been a folder separator in paths is replaced by an underscore ("_"). Yielding

- <prefix>_realtime.c
- <prefix>_settings.exp
- <prefix>_ROI0.log
- <prefix>_ROI0.xlsx (optional)
- <prefix>_ROI0.[rec|avi|mp4] or <prefix>_ROI0.12345678.[jpg|bmp]
- <prefix>_ROI0[_frameX]_background.png

The **Video Format** can be chosen for different containers and different compression settings. Containers supported are:

- REC: Easy to parse format. See 12.2 Description of the REC file format for a description.

- AVI: Standard AVI container format.
- MP4: MP4 container format.
- JPEG: Numbered Jpeg compressed images. Note: Lossy compression, even with "Best quality" selected.
- BMP: Numbered bitmaps. Lossless.

For containers which can contain different formats (AVI, MP4) or support compression (AVI, MP4, JPEG), the **video compression** dropdown contains the options for compression:

- Uncompressed: Uncompressed bitmap single channel 8 bit Grayscale.
- MJPEG high quality: Compressed JPEG images, JPEG quality setting 100. Note: This is still a lossy compression.
- MJPEG balanced quality: Compressed JPEG images, JPEG quality setting 90.
- MJPEG fast speed: Compressed JPEG images, JPEG quality setting 50.

With activating the **Save as separate videos** checkbox the channels can be split into separate video files if more than one imaging channel is active. If unchecked, the images of the different channels will be saved in one video file beside each other.

If one wants to have additional information directly in the images, the option **Add timestamp and scale footer to images** adds a small timestamp and a scale to the images, making them slightly larger in height.

Additional comments can be added to a separate field of the .exp file (see section 12.1) for example to describe the experimental conditions in more detail. A sample configuration is shown in Figure 37.

### 4.7.2 Realtime filter

The **Realtime filter** control is crucial for setting the trigger pins correctly. A sample configuration is shown in Figure 38.

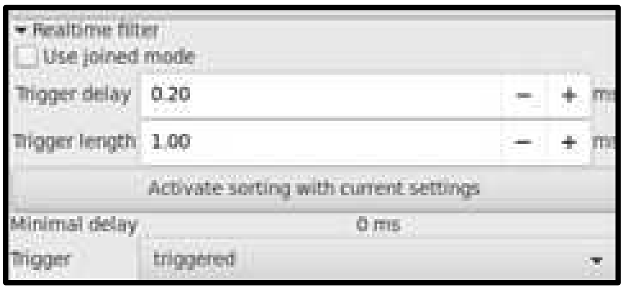



*Figure 38: Realtime filter pane. In this configuration the trigger pins are active and will rise 200 µs after a frame is within the polygons and be logical HIGH for 1 ms before going to logical LOW again.*

The **Trigger delay** determines the time in ms between the particle is detected by ODIN and the rising edge of the trigger pin. This parameter must be adjusted correctly for each experiment, especially if the distance of the detection and the position of the actual sorting have been changed. The flow speed of the particles and the distance has to be calculated carefully. For each setting there is a minimal delay shown in the menu, when a sorting program has been loaded. This is the time ODIN needs to check if the detected particle is within the marked polygon or not. The selected trigger delay must be larger than this value, otherwise the trigger signal might silently be dropped.

The **Minimal delay** will show the current delay setting the sensor achieved for the last image the sorting switch was activated. It also checks the delay against the set trigger delay and if that time is undercut, it prints a warning text in red. The minimal delay is influenced by the size of the ROIs read out, the settings for analyzing, especially the *Bw erosion rounds* and *Bw dilation rounds*. It is not influenced by the framerate itself.

The **Trigger length** determines how long the trigger pin is active. This needs to be adjusted for the respective sorting device. Care should be taken so that the active-time is shorter than the expected inter-particle-time because otherwise the pin might appear to be permanently on.

By clicking **Activate Sorting with current settings** the settings are transferred to the processor and the code is active. This must be executed every time if one of the following points is changed

- Settings in the filter menu
- A region in the mark regions menu
- Trigger delay or length

Otherwise, the old configuration will still be active and running. The configured sorting settings run until new ones are loaded, this is even true if the software is restarted. The sorting configuration is lost on a restart of the system, or by overwriting it with a new configuration. An empty configuration is also a valid configuration, doing nothing.

In the dropdown menu, **triggered** must be selected if the generated code should generate output signals on the trigger pins. There is also the option for **permanently off** if no trigger signal is needed or **permanently on** if the trigger pin should be high for all times e.g. for testing the signals and the periphery.

# 5 Experiment Guard

The **Experiment Guard** is activated in the *Main Window* (see section 3) and shows the detected parameters in a graphical way. The parameters can be added to the graph by checking the respecting box in the list of parameters. A sample configuration is shown in Figure 39.

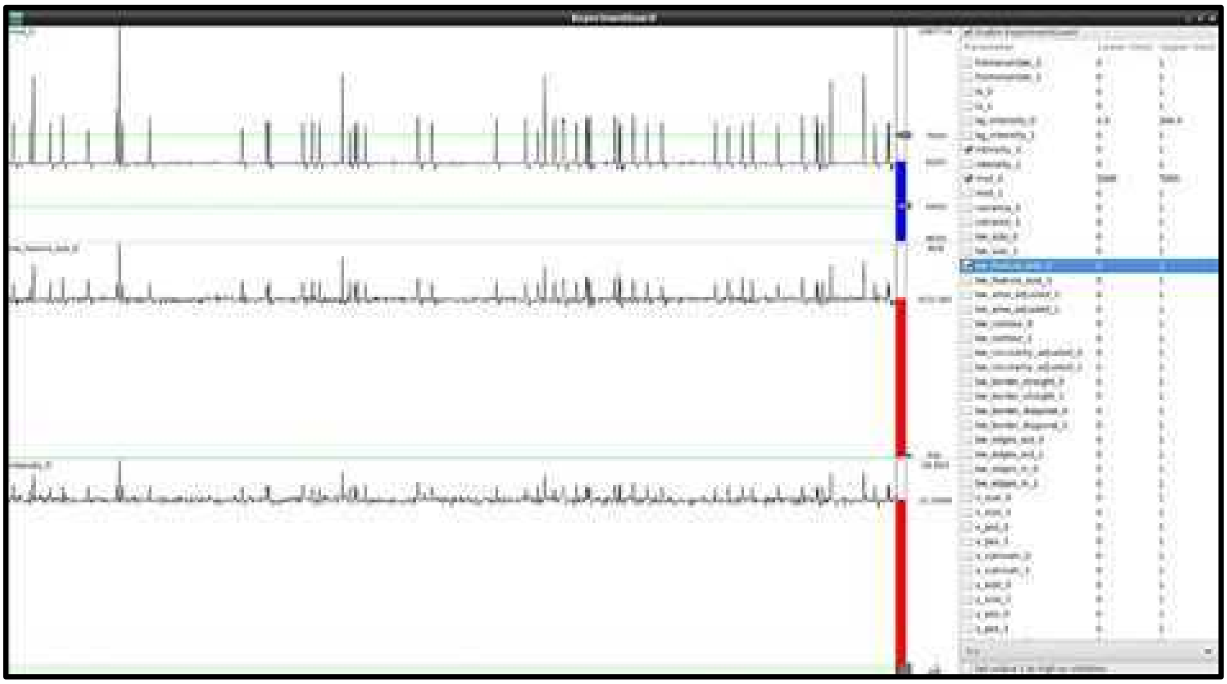


*Figure 39: Experiment guard window with 3 activated parameters, of which 2 are out of range.*

To enable the plotting and guarding, the **Enable ExperimentGuard** checkbox has to be checked. As this, especially at high sensor frame rates, needs a lot of computation power, it is encouraged if the ExperimentGuard is not used, to disable it.

The left part of the window shows plots of the current sensor data over time. This is helpful to determine good values for sorting and see changes in the experiment. Limits for the surveillance of a parameter can be set in the right part of the window.

Setting **Set Trigger 2 to high on violation** forces the Trigger Pin 2 to high when the set limits are surpassed and reset the pin when all watched parameters are within the limit again. This disables the sorting pin functionality of the Trigger Pin 2.

To avoid spurious alarms for short term irregularities, a smoothing interval for computing the values can be set in the dropdown menu. The smoothing is visualized in the bars on the right side of the plots. As soon as the bars cross the thresholds, the output 1 will be set high. The settings in the dropdown menu correspond to the smoothing duration applied.

# 6 Counter

The counter counts image frames passing through the filter per ROI and passing through the polygon per polygon.

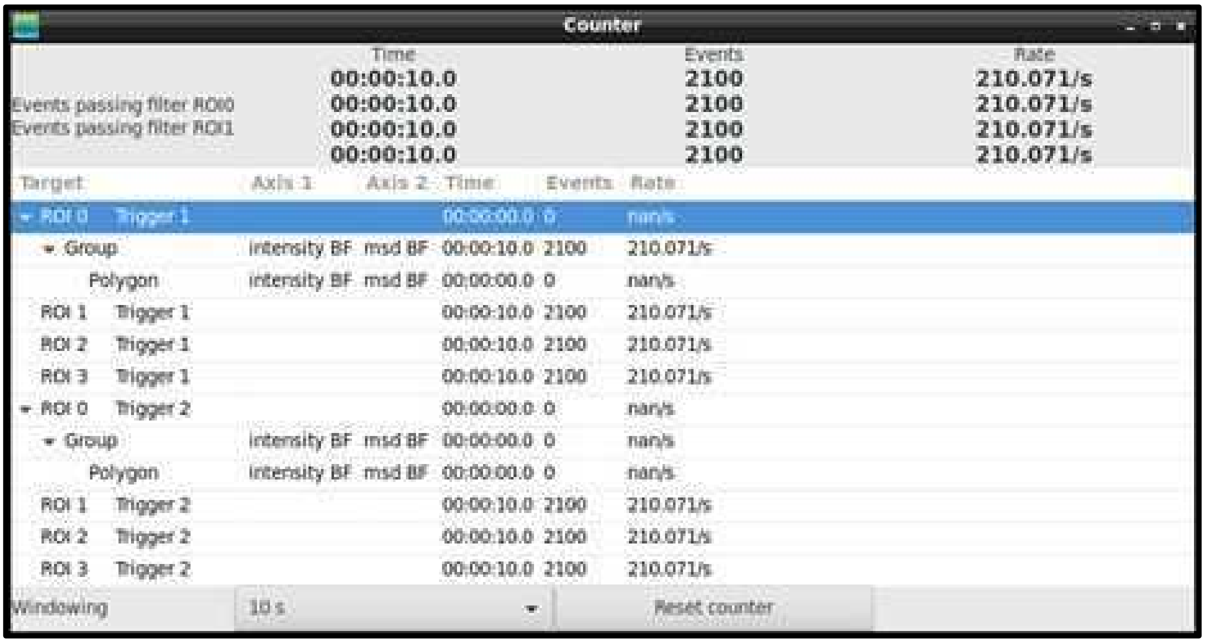



*Figure 40: Counter window. The polygons are listed here and also the trigger events generated per trigger.*

**Time** is the time used to compute the average; **Events** is the number of events happening in **time**; and rate is “(events-1)/time”, as the rate of events.

The event count and the rate are also displayed in the Experiment window for the corresponding polygon and update live there.

The table with the triggers and the polygons tries to visualize the hierarchical filtering and decision making to the individual trigger pins from the polygons and ROIs.

**Windowing** can be set to infinitely long, 1 s, 10 s, 1 minute and 5 minutes moving window averaging.

**Reset counter** will reset everything to start from the beginning. This also happens if you update the polygons.

# 7 Pump control

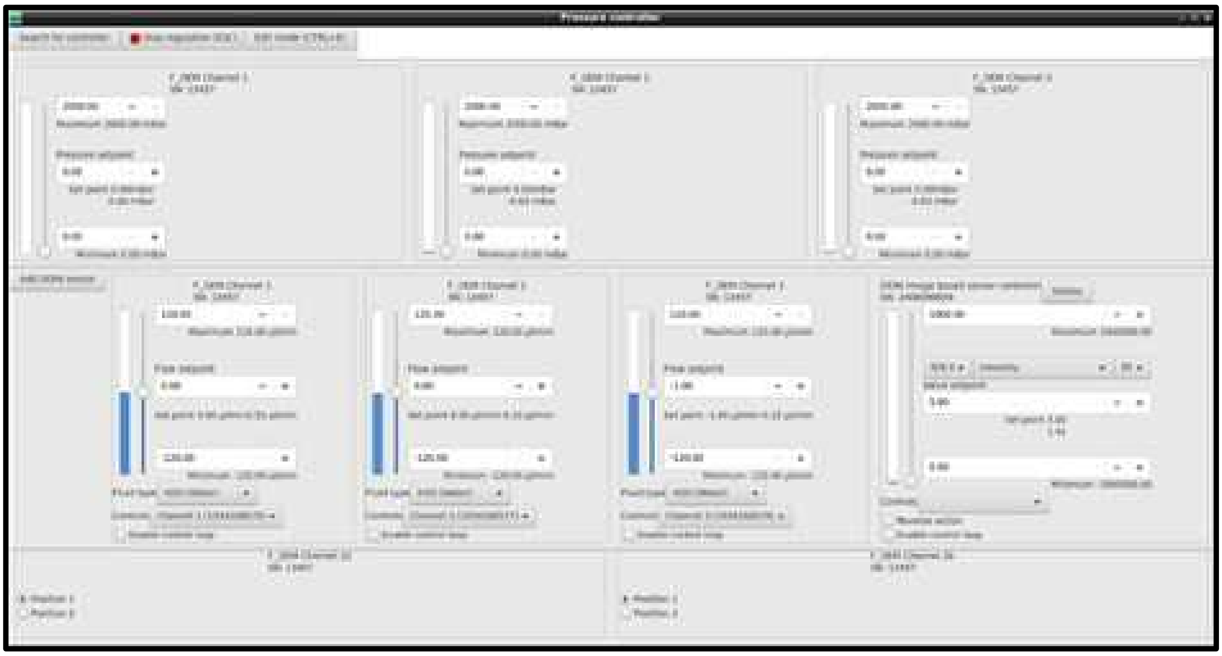


*Figure 41: Pump control window.*

*On the very top the user can rescan the connected devices for pump controllers, can stop the regulation (also via <ESC>) and enable the edit mode, which will hold the pressures until the mode is disabled. On the top three connected channels are visible. In the middle 3 flow sensors configured for H2O and a ODIN sensor configured for intensity are configured. All sensors are not controlling pressure channels. On the bottm two switches are configured, both set to the default position "1".*

The pressure pumps are controlled via the **Pump control** window which can be shown with the Button **Pump control** in the main window. An example view of the window is shown in Figure 41.

All pressure control entries can also be changed in 1 mBar steps by using the scrollwheel on the mouse while hovering the mouse pointer above the entry itself.

**Search controller** rescans the system for supported pressure pump controllers.

**Stop regulation (ESC)** will stop the regulation of the pumps and set the pressure for all pumps to "0". Note, that the hotkey <ESC> only works when the "Pump control" window has focus.

The **Edit mode (CTRL+E)** is active when starting the software. When active, it freezes the pressure targets for the pumps. It allows setting up multiple pressure settings and when deselecting the edit mode to update all channels simultaneously.

On **Software start** the **Edit Mode** will always be active, as the last pressure settings are prefilled for each pump. This avoids surprises when starting the software, as the user needs to explicitly release the edit mode to apply pressure through the pumps.

## 7.1 Pressure controllers

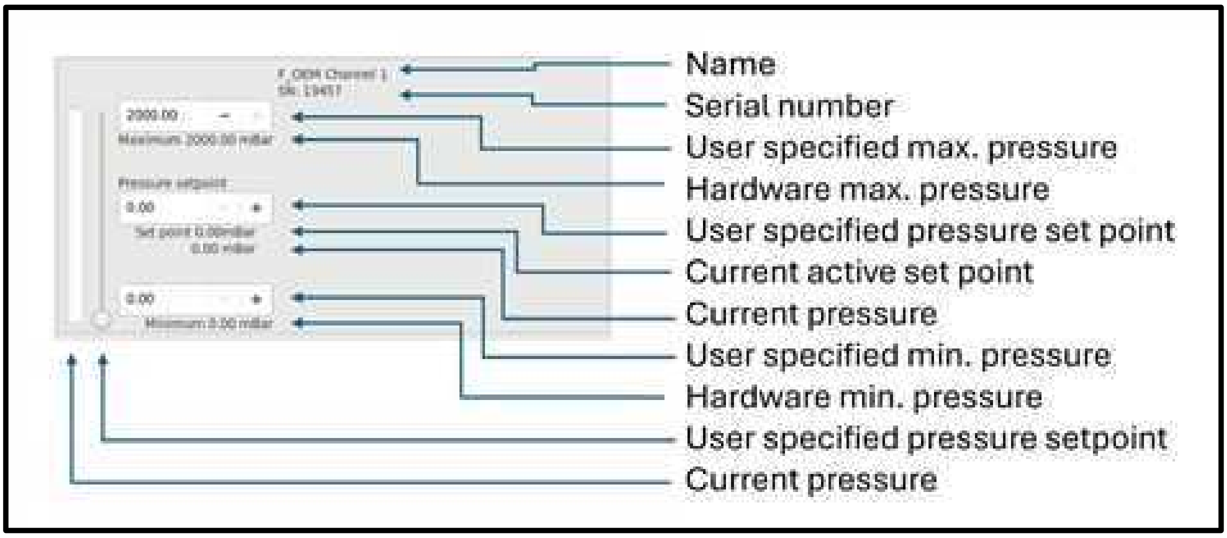



*Figure 42: Pressure controls*

The first row of controls is a control for the pressure channels. See Figure 42 for an example view. The user can limit the maximum and the minimum value anyone can enter as setpoint by updating the **maximum** and **minimum**. This is limited by the hardware reported maximum and minimum pressures. This is a failsafe to avoid fat-finger accidents when entering pressures very fast.

**Pressure setpoint** sets the pressure value the regulator tries to achieve. If there is leakage in the system, this might not be reached. The status information will show the actual measured pressure. Also, the slider on the left can be used to change the setpoint. The bar will show the pressure achieved. In edit mode it shows what setpoint was previously selected and is maintained currently as well as which setpoint will be set when the edit mode will be ended.

## 7.2 Flow sensors

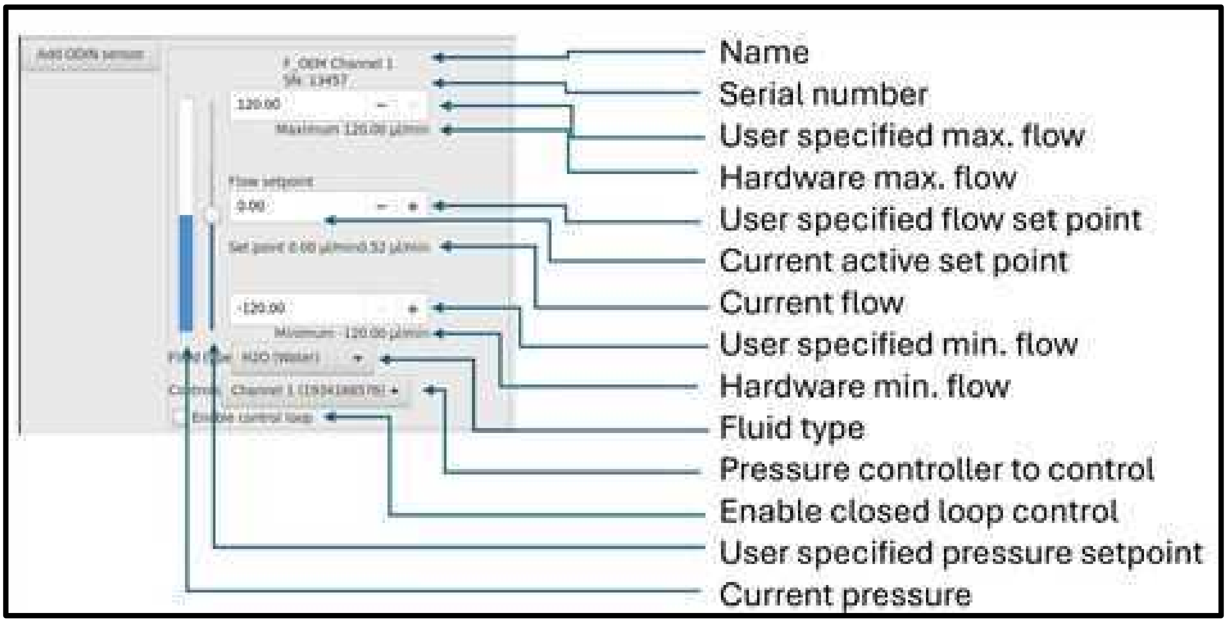



*Figure 43: Flow controller status and setting block.*

The second row shows sensors which can be coupled to the pressure regulators.

Sensors can come in a variety of forms. Typical hardware sensors are flow sensors measuring the flow of liquids. Sensors can also be formed using ODIN image analysis. By clicking **Add ODIN sensor** a virtual sensor can be added, which will monitor an ODIN parameter. Hardware flow sensors and ODIN virtual sensors have very similar capabilities.

All sensors can be used to form a feedback loop to the pressure controllers to regulate the pressures to achieve a certain goal, e.g. fixing a system parameter to a certain value.

Figure 43 shows an example flow controller which measures the flow rate of liquids. The controller is primed to set the flow rate to 0, and currently measured is a rate of 0.52 µl/min. The control loop is not active, so the user needs to set the pressures on the configured channel “Channel 1”.

Depending on the sensor type, different calibration curves are available. The user needs to select the proper fluid type to get reliable sensor measurements for the flow sensors.

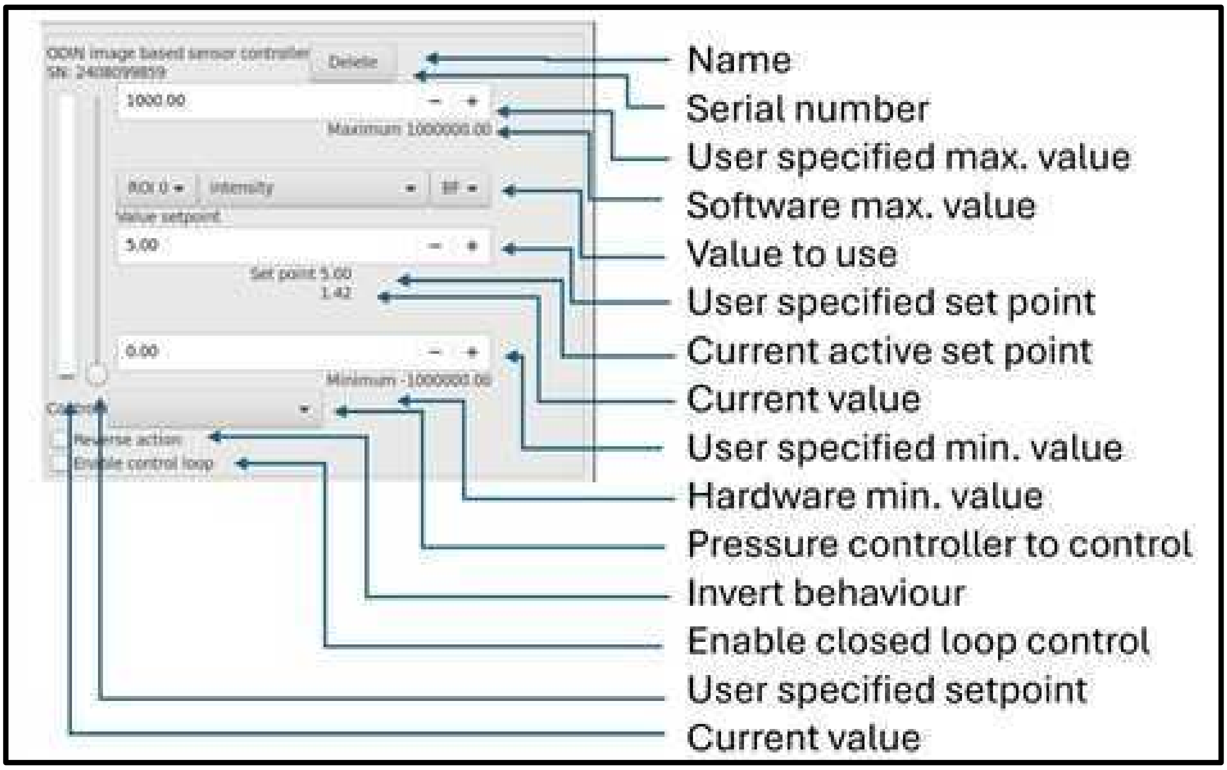



*Figure 44: ODIN parameter pressure controller example. The minimum and maximum virtual sensor value can and must be adjusted to the expected extreme values.*

As shown in Figure 44 the ODIN parameter virtual sensor uses a very similar structure. The user selects which parameter should be controlled to a set point, sets the set point and specifies which channel should be controlled. For the ODIN sensor, also the user needs to specify the action direction. Normal action expects a rise of the value if the pressure is increased. If that is not the case, **reverse action** should be selected to achieve proper regulation.

- Reverse action: Use if it is expected that the sensor value increases, when the pressure is increased.
- Standard action: Use if it is expected that the sensor value decreases, when the pressure is increased.

⚠ The ODIN sensor control loop scales its sensitivity based on the distance between the **maximum** and **minimum** values entered by the user. The narrower the values, the stronger it changes the pressures to achieve the target values. Setting these values too far apart will lead to a very slow regulation performance or no pressure regulation at all. Setting these values too tight will lead to a very fast and possibly oscillating pressure regulation.

There is no limit on the number of ODIN sensors. As only one sensor can control a pressure regulator, the number of useful ODIN sensors in use is limited by the number of pressure regulators.

All sensors show the current value, the target point and the range of the measurement values possible. ODIN sensor values can use all parameters from ODIN and of any ROI which the sensor and the pressure controllers then try to keep constant. All sensors allow the coupling between the sensor and a pressure controller.

## 7.3 Switch control

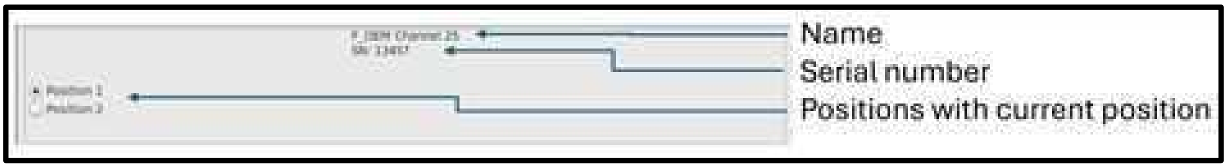



*Figure 45: Switch valve control*

The last row contains fluid switch valves. See Figure 45 for an example. The valves normally start up in “Position 1”. The action behind switching a valve is defined by the connections outside of the control of ODIN itself.

# 8 Laser control

The sensor supports controlling some laser drivers. Currently supported is the Oxxius Laser system L4Cc and L6Cc as well as the standalone laser heads. See Figure 46 for an example view.

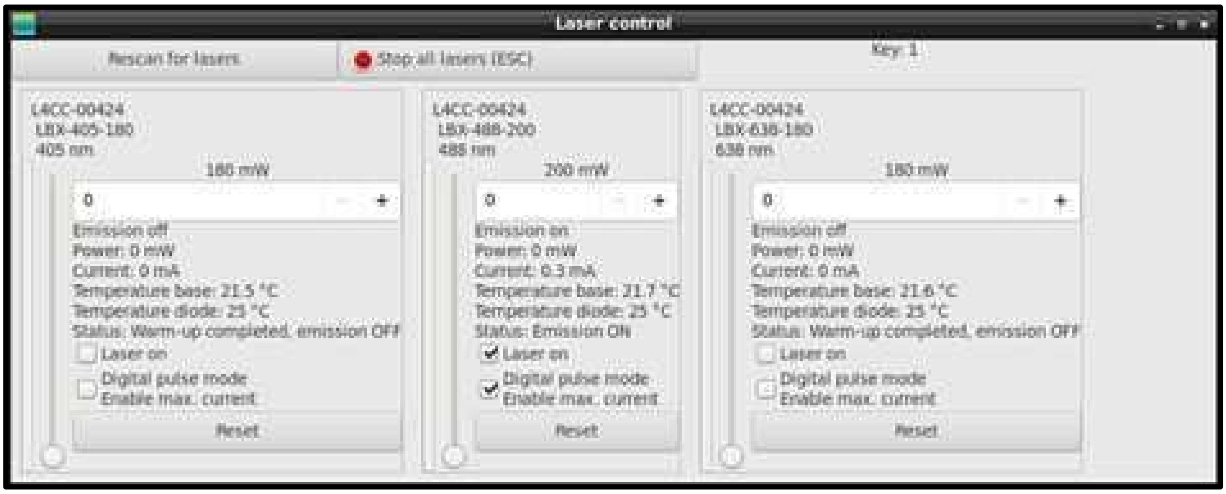



*Figure 46: Laser control*

The system allows setting the Laser power, mode of operation and provides diagnostic information. The Laser system itself is provided with a control unit with Key, and interlock for CDRH standard conformance. The interlock needs to be closed and the key needs to be turned on (not kept on, it needs to go from off to on after power up) to allow for lasing, otherwise the system will be in emission inhibition mode.

As visible in Figure 46, the UI shows the status of the key turning point as “0”: Key in off position. “1”: Key in on position. It also shows the laser modules detected, their emission status (“on” or “off”), their current average power, their average current, their temperatures and their status.

The controls include “Laser on” to enable a laser module; and “Digital pulse mode”, which sets the laser to digital modulation, current control and maximum allowed current. This is the mode normally used with the alternating illumination for high-speed fluorescence microscopy. A “Reset” button provides a reset if the laser gets overheated, or other protection mechanisms stopped the laser module.

In CW mode, the power can be set with the vertical slider or the numeric input box.

⚠ Laser radiation is harmful to eye and skin, wear adequate protection (suitable laser safety goggles), follow proper procedures and do not defeat the interlock protection mechanism. ⚠

# 9 Sensor values

In this section some calculated parameters are explained in more detail to assess their importance and significance as markers for real physical values.

## 9.1 Intensity values

### 9.1.1 MSD

The mean squared difference or SSD (sum of squared difference) value is computed by summing up all squared intensity values of the image.

### 9.1.2 Intensity

This value is the sum of the individual pixel values of the image and is linear proportional to the visible feature size in the sensor image.

### 9.1.3 Comparing MSD and Intensity

MSD and intensity signals have different meanings for data interpretation. The MSD is a better marker if small, but very strong visible features should be analyzed or detected. However, the intensity is better suited to characterize larger objects with a weaker optical signal.

## 9.2 Proportions

The determination of the proportions of objects by the computed parameters is probably the most important task of the ODIN sensor. Therefore, the different methods and metrics are described here briefly

### 9.2.1 Circumference

The black and white images have a clear border and can be used to calculate the circumference of the objects. This is realized by drawing horizontal, vertical and diagonal lines around objects. These lines are then added together to form the

*BwCirc* and *BwFeatureCirc* parameters. As they work on the level of pixels and are very discretized, they normally overestimate the true circumference. This gets more pronounced the smaller the object is.

#### 9.2.2 Derived values

Some values can be derived from the previously mentioned parameters

- Width = WidthEnd-WidthBegin
- Height = HeightEnd-HeightBegin
- Position in x = (WidthEnd+WidthBegin)/2
- Position in y = (HeightEnd+HeightBegin)/2
- Elongation = Width/Height
- Circularity = $c = \frac{1-\sqrt{Area}}{circumfence}$

In addition, the speed of particles can be derived by position and time. This can be done for the grey value position, the *Bw position* and the *Bw feature position*, yielding the 3 move parameters *MoveX/Y*, *BwMoveX/Y*, und *BwFeatureMoveX/Y*.

# 10 Troubleshooting/FAQ

**Q: The sensor is showing only a black image**

A: Please check if the optical path is clear of any blocking elements and the illumination is turned on (check SOL configuration 3.1, and in the main window **Enable pulsed illumination**). If in doubt, increasing the gain to the maximum value should make the noise clearly visible. Also check if you are previewing with background subtraction enabled. (See *View mode* in 3.5).

**Q: My recording is always empty**

A: Did you enable a filter and the image data no longer passes it? Disable the filter by unchecking **Enable filter** (see 4.3.3). Also check if the selected ROI active.

**Q: I cannot change the ROI positions**

A: Disable the **Enable ROI configuration** button by clicking it. If it is greyed out, you need to stop the current recording by clicking **Stop recording** in the Experiment window.

**Q: I do not get a trigger signal, but the sensor is showing that it triggered**

A: Did you activate the current configuration for sorting? (See 4.7.2). Please also check if the trigger output is set to *always off*. Furthermore, is your *Trigger delay* long enough? Too short delays will be dropped silently.

**Q: My image looks very badly, and all particles seem to have a shadow in one direction.**

A: This is the result of an unoptimized illumination. Please follow the manual of your microscope manufacturer to align your microscope. Best results can be achieved using the so-called Köhler illumination. The steps are as follows:

1. Focus on your sample/channel
2. Close the field aperture of your illumination until you see the borders.
3. Move the field aperture into the center of your view.
4. Focus the field aperture until it is sharply imaged
5. Open the field aperture that wide that you do not get vignetting (seeing parts of the aperture in the image).

Also closing the illumination aperture to increase the depth of field can have a huge influence on image quality.

**Q: My image is looking bad and small particles are cross-shaped**

A: Most likely you have a cylindrical element in your optical path. This can happen if you image a PDMS channel through the PDMS and the PDMS is not completely flat. This can be caused by uneven pouring, edge effects when curing and by holders squeezing the PDMS.

**Q: The sensor grouping feature is not working, I often/always get the particles at the border of the ROI.**

A: If your particles are spaced apart less than the size of the ROI, the sensor will have a hard time separating them. In that case, make the ROI smaller until only one particle is imaged.

If your particle rate is fast, it is also possible that the sensor is not recording enough images per particle to select a nice, uncropped image. In that case increase the framerate or increase the ROI. The parameter *count_averaged* contains the number of frames averaged for the group. If that value is smaller than 3, cropped particles are to be expected.

**Q: I have a spinning disk confocal microscope and I do not get any nice images out of ODIN. The microscope was very expensive, why is it not working.**

A: ODIN does not support confocal microscopes. Please use a standard microscope for bright field and epi-fluorescence imaging.

**Q: I have a nice infrared fluorophore; can I use ODIN with that?**

A: The sensor sensitivity of ODIN is as in image Figure 47. Normally the NIR sensor is used to increase the sensitivity for standard yellow-red emitting fluorophores. Please check the diagram in Figure 47 to see if the sensor has the required sensitivity.

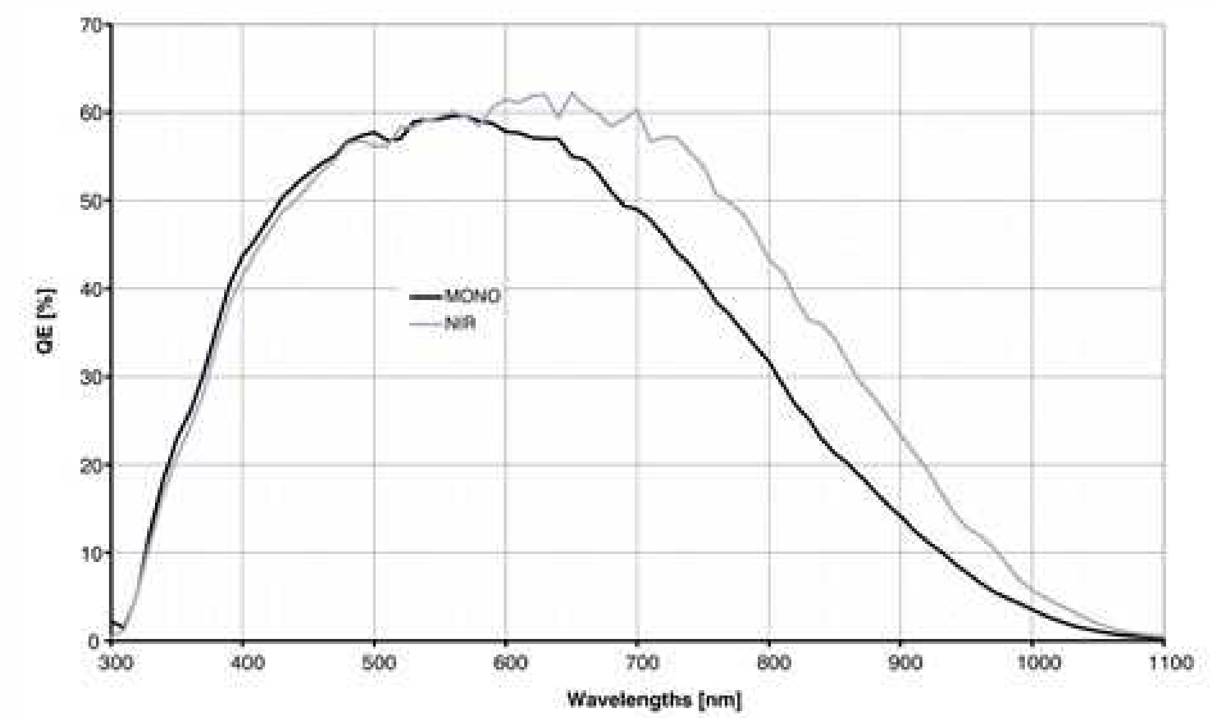



*Figure 47: Sensitivity and Qantum efficiency of the ODIN sensor. Normally the NIR sensor is used.*

**Q: I have a nice FPGA idea and want to use the FPGA for my own algorithm. Is that possible?**

The FPGA is fully utilized with the ODIN software. If you want to use custom algorithms, we can provide on request the API so you can use external components (e.g. computer, GPUs, FPGAs) to analyze the parameters or the images and generate sorting signals.

**Q: I found a bug!**

A: Please describe the bug and how to reproduce it as good as possible and written an email to info@sensific.de . Any help is welcome and we always try to improve the sensor, software, and hardware.

**Q: I have a nice idea, I want to use device xyz with ODIN!**

A: That’s great, please describe your idea or your device you want to use the best you can and write us an email at info@sensific.de we will see what is possible to be realized.

**Q: My laser safety officer is asking about interlocks.**

A: ODIN does not provide any interlocking mechanism, please work together with your safety officer to make your workplace up to standard and safe to use. SOL does not require interlocks as long as no optical device is used to look at the lamp output port. Safest is to remove the eyepieces and permanently seal all non-used ports of the microscope. Some HV generator support interlocks via the IO connector, please connect proper switches to it.

# 11 Advanced options

This section is for power users only or when advised to do so by the Sensific support. It is possible to permanently disable or destroy the sensor.

## 11.1 Updating the sensor

Sensor updates can be performed by using a Sensific provided ODIN_OS.img file on the root folder of an USB stick. Upon boot, the sensor asks to flash the new software version. Answer “y” or “yes” to flash it.

⚠ Note: You will lose all data on the sensor. Make sure you have it backed up safely.

## 11.2 Mounting network storage

Network storage can directly be accessed using the sensor.

- Connect the sensor to the network (Ethernet)
- If the network has a DHCP server, the sensor will configure itself
- If the network has no DHCP server, the user has to configure the network
  - Click on the network symbol on the lower right
  - Click Edit connections...
  - Click on “Wired connection 1”
  - Click on the gear
  - Edit your network settings (e.g. IPv4 Settings)
  - Click save
  - Click again on the network icon
  - Click on “Wired connection 1”
- Open the file manager
- Enter the network address you want to connect in the bar on top
  - Windows network smb://<server name>/<share name>/
  - NFS nfs://<server name>/
  - FTP ftp://<server name>/
  - SFTP sftp://<server name>/
- Press enter
- A dialog asks for connection credentials, enter them
- Now you can see and access the files
- If you want to get the path of this network drive, click Tools->”Open current folder in terminal”, the prompt will tell you the path

## 11.3 System user credentials

To gain root privileges, use the “sudo” command. The user “ubuntu” is part of the sudoers group and can therefore elevate itself to root.

Default user name: ubuntu

Default user password: ubuntu

## 11.4 Reflashing the µSD card

Reflashing can be necessary on µSD card loss or other system faults.

If the µSD card is lost or otherwise unusable, a new µSD card can be reflashed with the newest firmware.

- Got to https://www.sensific.de/odin-firmware and download the firmware image.
- Download Etcher (https://www.balena.io/etcher/) for your operating system
- Start etcher
- Select the downloaded firmware image as image
- Select the µSD card as the target
- Flash
- Plug in the µSD card to the ODIN sensor
- Start the sensor
- The sensor automatically extracts the system image and installs it, this takes approximately 10 minutes on an average µSD card
- The sensor reboots and boots the new sensor version

If the sensor needs only recovered from an invalid configuration or other system errors, a soft reset can be executed.

For newer firmwares with ODIN 1.5.6 and higher, a USB stick or similar USB device is needed. Copy a ODIN_OS.img from the firmware download site (see above) onto root folder of the drive, plug it into the sensor and make sure you can see the file. Then reboot, now the sensor should find the ODIN_OS.img and asks to flash the firmware. Answer "y" or "yes" to flash then the new firmware. Also, firmware upgrades can be performed in that manner.

For older versions, the µSd card has a FAT32 partition with the files EXTRACT_HERE_FOR_RECOVERY.zip and ODIN_OS.img. The partition should be readable with any computer (Windows, MacOS, Linux, Android). For a system refresh extract the zip file content (a file called BOOT.bin) in the root directory of this partition, overwriting the existing BOOT.bin. Then start the sensor with this SD card plugged in. It should extract the OS image and flash this firmware to the sensor and then boot.

# 12 Appendix

## 12.1 Description EXP file of recording

The exp file (short for experiment) saves all relevant parameters of the sensor state at the time of the recording. Its basic structure is that of a INI-file, meaning, the name is followed by a equal sign ("=") and then followed by the value followed by a newline (New line, "\n"). For values which might have an equal sign or a new line, they are quoted with double quotes, the quotes in the quotes are escaped with a double quote (""""). The file should not have any excess spaces or newlines. Entries without "=" are treated as having an empty value.

An example entry is

filename=/home/ubuntu/Documents/M1/M1.exp

comment="This is a
multiline comment, containing ""quotes"" terminated by a single quote and newline"

The exp file is arranged in sections, sections are grouped by square brackets of the header name (e.g. "[settings]". Note however, that these sections are merely informal. The values named roi_idx and alt_idx should preface the actual values of the corresponding ROI or multiplexed alternate mode.

Entries in the files are listed in Table 4.

*Table 4: Description of the fields in the EXP file*

| Name | Description |
|---|---|
| [[GENERAL]] | General section |
| Filename | The filename or folder it was initially saved to |
| Framerate | The framerate of the recording |
| Timestamp | The timestamp at the saving point |
| timestamp_start | The time when the recording was started |
| timestamp_end | The time when the recording was finished |
| trigger_delay | The set delay of the trigger in ms |
| trigger_duration | The set duration of the trigger in ms |
| illumination_enable | Was the pulsed illumination active |
| alternating_count | The number of alternating frame configurations |
| view_min | Minimal brightness in preview before clipping |
| view_max | Maximal brightness in preview before clipping |
| scale_um_per_px | The scale in µm/px for this recording (if the user did not calibrate or adjust the settings, it might be wrong) |
| scale_active | The scaling was used when saving the recording |
| software_version | The software version |
| save_mode | "folder": In a single folder<br>"separate": In files prefixed with the name |

| | |
|---|---|
| save_separate | Separate videos per alternating mode, not merged to a single video file |
| video_format | The extension of the video file, either AVI or REC |
| | |
| *Mainwindow numbers* | |
| alt_exposure0 | Exposure length in µs for alternate configuration 1 |
| alt_exposure1 | Exposure length in µs for alternate configuration 2 |
| alt_exposure2 | Exposure length in µs for alternate configuration 3 |
| alt_exposure3 | Exposure length in µs for alternate configuration 4 |
| alt_gain0 | Gain for alternate configuration 1 |
| alt_gain1 | Gain for alternate configuration 2 |
| alt_gain2 | Gain for alternate configuration 3 |
| alt_gain3 | Gain for alternate configuration 4 |
| alt_bg_val0 | Background value for alternate configuration 1 |
| alt_bg_val1 | Background value for alternate configuration 2 |
| alt_bg_val2 | Background value for alternate configuration 3 |
| alt_bg_val3 | Background value for alternate configuration 4 |
| frame_length | Frame rate |
| illumination_length0 | Pulsed illumination length in ns for flash 1 |
| illumination_length1 | Pulsed illumination length in ns for flash 2 |
| illumination_length2 | Pulsed illumination length in ns for flash 3 |
| illumination_length3 | Pulsed illumination length in ns for flash 4 |
| illumination_length4 | Pulsed illumination length in ns for flash 5 |
| illumination_length5 | Pulsed illumination length in ns for flash 6 |
| view_min | Dark view level for preview |
| view_max | Bright view level for preview |
| scale_objective_multiplier | The objetive magnification of the scaling dialog |
| scale_px | The pixel value of the scaling dialog |
| scale_um | The µm value of the scaling dialog |
| xbegin0 | lower x for ROI 0 |
| xbegin1 | lower x for ROI 1 |
| ybegin0 | lower y for ROI 0 |
| ybegin1 | lower y for ROI 1 |
| xend0 | upper x for ROI 0 |
| xend1 | upper x for ROI 1 |
| yend0 | upper y for ROI 0 |
| yend1 | upper y for ROI 1 |
| *Mainwindow booleans (0,1)* | |
| scale_bar_show | Show scale bar in preview |
| scale_use_mag | Scale uses the magnification |
| scale_use_cal | scale uses the calibration |
| check_roi0 | Enable ROI 0 |
| check_roi1 | Enable ROI 1 |
| alt_flash_show0 | Show flash in mainwindow for alternate configuration 1 |
| alt_flash_show1 | Show flash in mainwindow for alternate configuration 2 |

| | |
|---|---|
| alt_flash_show2 | Show flash in mainwindow for alternate configuration 3 |
| alt_flash_show3 | Show flash in mainwindow for alternate configuration 4 |
| alt_exposure_show0 | Show exposure in mainwindow for alternate configuration 1 |
| alt_exposure_show1 | Show exposure in mainwindow for alternate configuration 2 |
| alt_exposure_show2 | Show exposure in mainwindow for alternate configuration 3 |
| alt_exposure_show3 | Show exposure in mainwindow for alternate configuration 4 |
| alt_gain_show0 | Show gain in mainwindow for alternate configuration 1 |
| alt_gain_show1 | Show gain in mainwindow for alternate configuration 2 |
| alt_gain_show2 | Show gain in mainwindow for alternate configuration 3 |
| alt_gain_show3 | Show gain in mainwindow for alternate configuration 4 |
| alt_bg_set0 | Set the background image to a fixed value for alternate configuration 1 |
| alt_bg_set1 | Set the background image to a fixed value for alternate configuration 2 |
| alt_bg_set2 | Set the background image to a fixed value for alternate configuration 3 |
| alt_bg_set3 | Set the background image to a fixed value for alternate configuration 4 |
| alt_bg_dyn0 | Dynamically calculate background for alternate configuration 1 |
| alt_bg_dyn1 | Dynamically calculate background for alternate configuration 2 |
| alt_bg_dyn2 | Dynamically calculate background for alternate configuration 3 |
| alt_bg_dyn3 | Dynamically calculate background for alternate configuration 4 |
| *Mainwindow text entries* | |
| alt_name0 | Name of alternate configuration 1 |
| alt_name1 | Name of alternate configuration 2 |
| alt_name2 | Name of alternate configuration 3 |
| alt_name3 | Name of alternate configuration 4 |
| *Mainwindow combo boxes* | |
| preview_frame | Which frame to preview, can be 0,1,2,3 |
| preview_mode | The mode of preview, can be normal, background, background_s, and bw |
| alt_count | number of alternate configurations (1,2,3,4) |
| alt_flash_mode0 | Mode of flashing (-1,0,1,2,3,4,5,6) of flash for alternate configuration 1 |

| | |
|---|---|
| alt_flash_mode1 | Mode of flashing (-1,0,1,2,3,4,5,6) of flash for alternate configuration 2 |
| alt_flash_mode2 | Mode of flashing (-1,0,1,2,3,4,5,6) of flash for alternate configuration 3 |
| alt_flash_mode3 | Mode of flashing (-1,0,1,2,3,4,5,6) of flash for alternate configuration 4 |
| alt_bg_mode0 | Background mode (abs,dark,bright) of alternate configuration 1 |
| alt_bg_mode1 | Background mode (abs,dark,bright) of alternate configuration 2 |
| alt_bg_mode2 | Background mode (abs,dark,bright) of alternate configuration 3 |
| alt_bg_mode3 | Background mode (abs,dark,bright) of alternate configuration 4 |
| Colors | |
| alt_color0 | Color for plotting of alternate configuration 1 |
| alt_color1 | Color for plotting of alternate configuration 2 |
| alt_color2 | Color for plotting of alternate configuration 3 |
| alt_color3 | Color for plotting of alternate configuration 4 |
| *Comments* | |
| Comment | User comment on the measurement |
| *ROI specific information* | |
| [[ROI]] | Marks the beginning of a ROI confugration |
| roi_idx | Index of the ROI (0,1): Note that must be placed directly after [[ROI]] |
| [settings] | beginning of the settings |
| [alt] | Setting for alternate configuration |
| alt_idx | index of the alternate configuration. Note that must be placed directly after [alt] |
| width_threshold | Width threshold |
| height_threshold | Height threshold |
| bw_threshold | Binarizing threshold |
| bw_remove_singles | Remove single pixels |
| bw_shrink | Erosion rounds |
| bw_grow | Dilation rounds |
| bw_height_threshold | Bw Height threshold |
| bw_width_threshold | bw width threshold |
| bw_height_feature_threshold | Bw height feature threshold |
| bw_width_feature_threshold | Bw width feature threshold |
| flashing_idx | Flashing pin used |
| [position] | The position of the ROI |
| Enabled | Is it enabled? |
| Xbegin | lower x value |
| Ybegin | lower y value |
| Xend | higher x value |
| Yend | higher y value |
| [trigger] | Trigger and filter settings |

| | |
|---|---|
| filter_enable | Filtering active |
| filter_name | Which name is used for thresholding for filtering |
| filter_value | Thresholding value |
| filter_alt_idx | Thresholding alternate configuration |
| filter_x | Filter dirt in x |
| filter_y | filter dirt in y |
| filter_bw_x | filter dirt in x binarized |
| filter_bw_y | filter dirt in y binarized |
| filter_group | Group the frames after filtering |
| filter_variable_count | How many custom filters are installed |
| filter_variable_<i>_name | The filtered variable name |
| filter_variable_<i>_alt_idx | The alternate configuration the variable is from |
| filter_variable_<i>_active | Is this filter active |
| filter_variable_<i>_lower | The lower level for filtering |
| filter_variable_<i>_upper | The upper level for filtering |
| [select region] | Regions of arbitrary polygons for sorting (can be present multiple times) |
| name_x | Name of the x axis variable (in the form <alt_idx>_<name>) |
| name_y | Name of the y axis variable (in the form <alt_idx>_<name>) |
| coords_x | The x coordinates of the polygon (closed, comma separated) |
| coords_y | The y coordinates of the polygon (closed, comma separated) |
| [autosolver] | The autosolver feature |
| Enabled | Is it active |
| Mask | The mask of the parameters, comma separated booelans |
| Subs | The subtraction values |
| Dims | The dimensionality |
| mults_<i> | The multiplication values (one per dimension) |
| [pca] | The calculated PCA |
| Enabled | Is the PCA calculated? |
| Mask | The mask of paramters, comma separated Booleans |
| Subs | The subtraction values |
| Dims | The dimensionality |
| mults_<i> | The multiplication values (one per dimension) |

## 12.2 Description of the REC file format

The rec file format is an easy to parse and easy to read format to store binary frame data. It is adapted from the REC format of Mikrotron cameras, but not 100% compatible. Most likely a parser for this format can read the Mikrotron format, but not vice versa.

The file consists of a header, and at a specified offset, of the raw image data. No compression or additional framing is applied.

The header consists of a INI file style section, finished by the String “End of header\n\n”, line termination is “\n”.

The header looks like

[RAWHEADER]
ColorMode=0
BayerFilterStart=0
BitsPerPixel=8
NumberOfFrames_00=1234
OffsetToFirstFrame=4096
FrameWidthInPixel=128
FrameHeightInPixel=64
FrameRate=5678
End of header

Description of the header values are shown in Table 5.

*Table 5: Description of the header of the REC file format*

| **Name** | **Description** |
|---|---|
| [RAWHEADER] | Magic cookie, required |
| ColorMode | 0=binary, others are not specified |
| BayerFilterStart | For Bayer raw images, the matrix shape |
| BitsPerPixel | Fixed for the sensor to 8 |
| NumberOfFrames_00 | Number of frames in the file |
| OffsetToFirstFrame | The byte offset from the beginning of the file to the raw data |
| FrameWidthInPixel | Frame width |
| FrameHeightInPixel | Frame height |
| FrameRate | The framerate of the recording |

The data then follows at an offset “OffsetToFirstFrame” (e.g. 4096) from the start of the file, no padding is applied, in this case (and the only supported mode by the sensor) every pixel has one Byte in size. Therefore, ColorMode must be fixed to 0, BayerFilterStart is ignored, BitsPerPixel must be 8. As the sensor is working on a granulary on the x axis of 8, the data is always QWORD aligned. The sensor software expects it to be aligned on DWORD.

# 13 List of tables



# 14 List of figures