PHOTONSCORE

PHOTON COUNTING MADE EASY

LINCam

Single Photon Counting System

User Manual

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Contents

Chapter 1

Getting started

The system grants single photon acquisition in a wide-field counting mode. The core of the system is a position sensitive **photomultiplier tube (PMT)** based on **microchannel plates (MPC's)** with a **multi-alkali photocathode**.

The system comprises of the **detector head** and the electronic control module designed to be used in lab conditions. The **detector head** houses MCP-PMT preamplifiers, high-voltage power suppliers and the cooling system. Provided integrated electronic control module includes everything required for robust and reliable single photon counting based imaging. Real-time event selection logic processes registered photons to avoid artifacts like **multi-photon events**, **MCP noise** and **pile-up effect**.

This chapter gives a step-by-step setup guide.

1.1 In the box

Detector head

Acquisition electronics

Chiller

Detector head power

Chiller power

2x cooling tubes

Signal cable

USB 2.0 mini cable

1.2 Before you begin

Please read respective to your competence parts of this manual before operating the system

Always switch the system off performing any manipulations with the detector head.

Avoid overexposure. Extreme caution shall be taken while operating the system to avoid overexposure of the PMT. An irreversible damage of photocathode and/or MCP gain degradation could be caused by exposing the device to high light intensities.

Check liquid circulation. The detector head is cooled by Peltier element. An active secondary liquid cooling device is required to run the system. Stopping or disturbing liquid circulation can result in thermal damage of the detector.

Avoid liquid spoiling electronic components. Before switching the system on please make sure cooling tubes are not damaged and properly connected to the detector and the chiller.

Laser safety. Lifetime or time of flight measurements require pulsed laser light sources. Please consult laser safety manual of your laser.

A **pulsed laser source** is mandatory to perform lifetime and time of flight imaging. The laser shall feature NIM-standard electrical reference output. Using of an **optical constant fraction discriminator (OCFD)** is recommended to form the reference signal for solid-state or gas laser system.

Tunable delay line is highly recommended for flexible and comfortable timing acquisition. If the delay line is not available a corresponding length of the cable with **SMA** end connectors would be sufficient. The delay should roughly correspond to the measuring **time window**. For 50Ω coaxial cable signal propagation speed equals to 5 nanoseconds per meter. The length of the delay shall be optimized for the particular setup geometry.

Recommended **computer configuration**: Intel Xeon or i7 multi-core CPU, 8 Gb of RAM, USB 2.0, Windows 7 64 bits. A large hard drive or network storage facility is highly recommended for data storage.

1.3 Connecting your system

Positioning electronics module

Keep control on high voltage. The electronic module has the switch that controls applying of the high voltage to the photocathode that can be switched on or off any time. It is recommended to keep this switch easily accessible during whole time the system is running as a security measure. This switch shall be used every time when the dramatic change of the light intensity could occur. For example, during the change of the sample, manipulation with objective lenses or change of the position of dichroic cubes carousel.

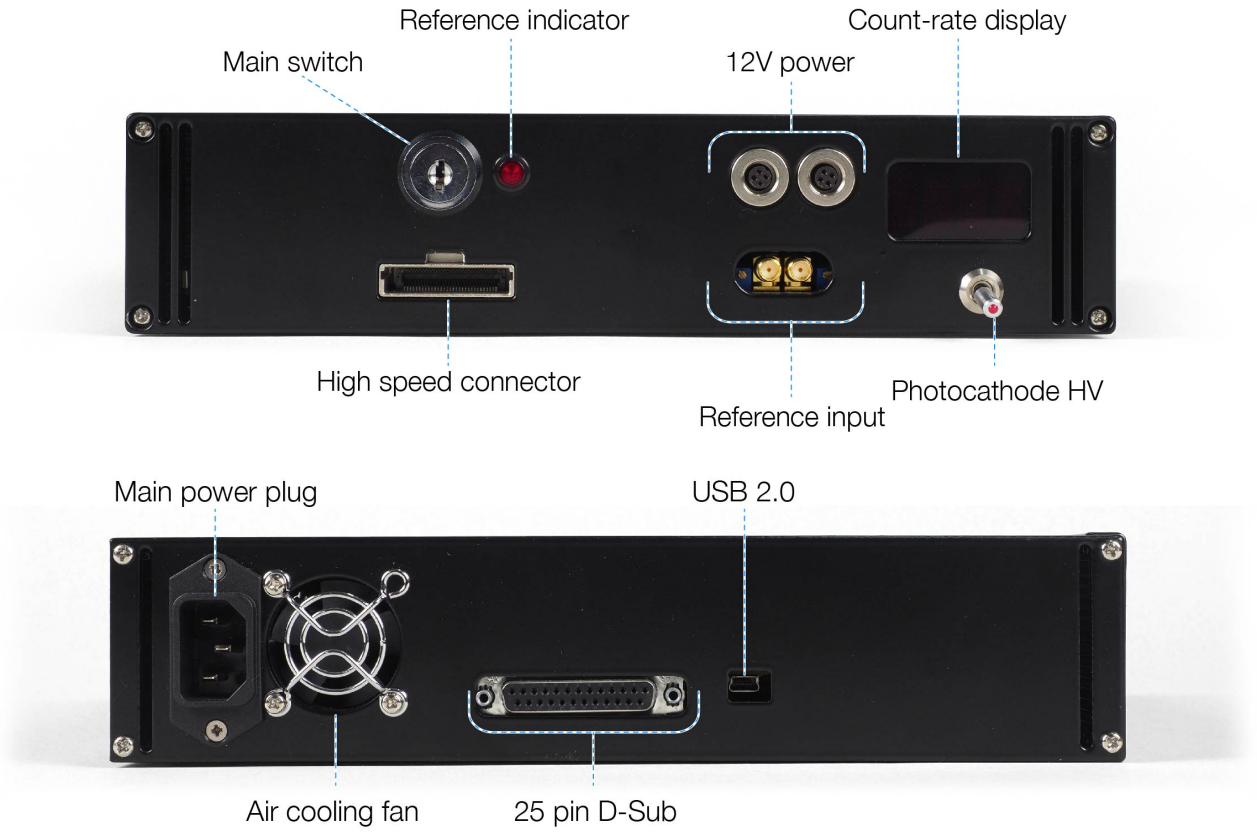
Position the system before connecting the cables. It is recommended to connect the cables and cooling tubes after positioning the camera, electronic module and chiller to avoid accidental damage of the connectors. Furthermore this allows complete freedom of handling the detector head mounting to the imaging device (e.g. microscope).

Keep control on input light flux. The *Count-rate display* should be clearly visible by the user while the system is running. This

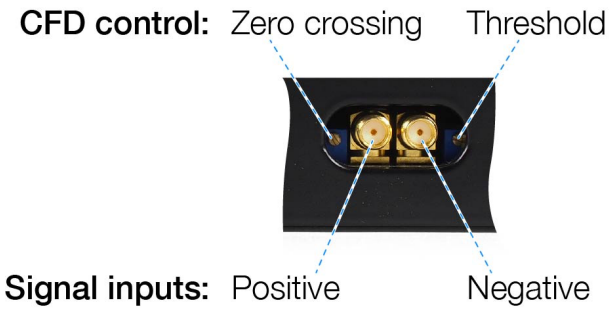
indicator shows the photon rate in thousands per second (KHz). The count-rate value shown by the indicator is measured independently of the two count-rates values sent to the computer. As a security measure it is highly recommended to check this value during the changes of the light intensity.

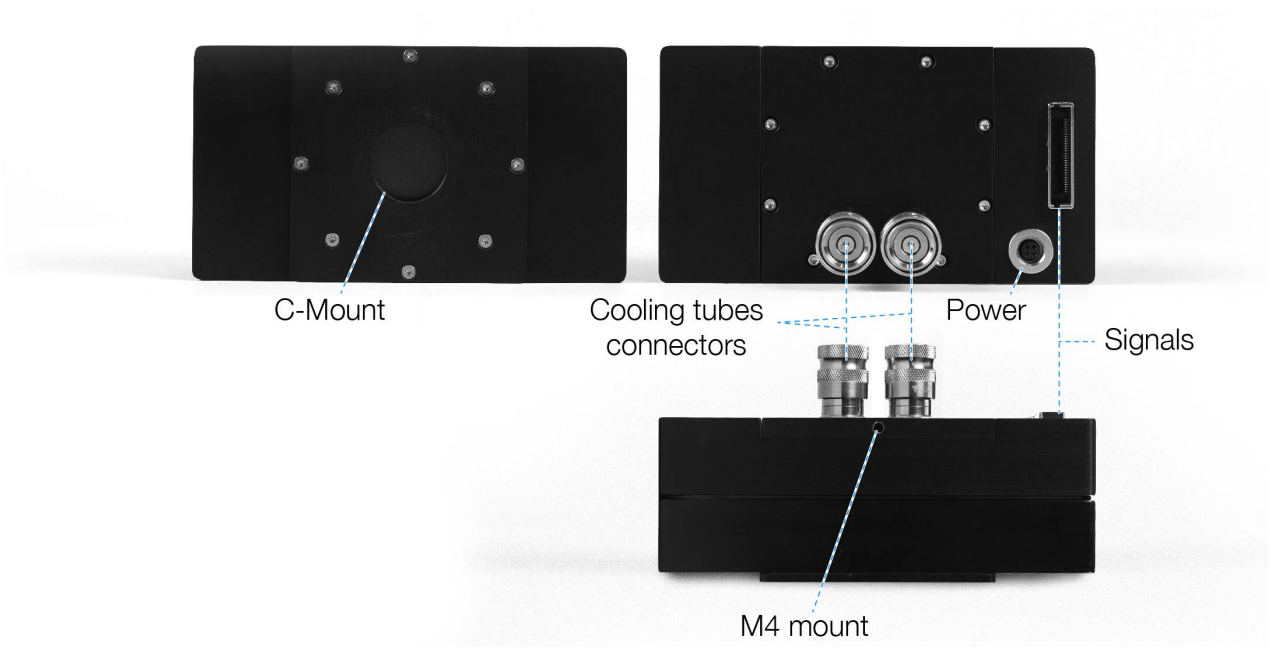
The electronic module contains electronics for position and timing acquisition and real-time preprocessing, temperature control, overexposure protection, count-rate indicator, high voltage control and an USB 2.0 mini connector for communication with the computer. The distance to the acquisition computer can be extended using active USB 2.0 hubs thus it is not limited to the length of the provided cable. The distance between electronics and the detector is limited by the length of the cables thus it should not exceed two meters.

System connectors



The reference input drives embedded constant fraction discriminator (CFD) module accepting positive and negative NIM signals. If **and only if** the reference signal does **not** fully agree with NIM standard, a CFD adjustment might be required. The CFD is controlled by two adjustable resistors setting the threshold level and zero crossing. The presence and the quality of the reference signal is controlled by the control output and the reference indicator.





Attaching the cables

After the detector is mounted, the electronic module and the chiller are placed it's time to connect everything together. Attach the cables as shown on the figure:

The high speed orange cable has two equal ends thus it does not matter which end goes to the acquisition system and with one to the camera body.

Cooling power (12V) connectors are equal on the electronic module and differ at the opposite end: round 4-pin connector shall go to the camera body and the 9-pin D-Sub supplies power to the chiller. The connectors has a position nub and the thread that shall be screwed up to the end.

The reference signal is a standard SMA connector shall be connected to the negative or positive input depending on polarity of the NIM signal your laser system provides. The input circuit can tolerate low current NIM signal of any polarity at any input thus it is not critical if the polarity was not checked before connection. However wrong polarity does not allow the operation of the system, therefore a proper one shall be used.

1.4 Mounting the detector to the microscope

The **input window** is the quartz glass with anti-reflection coating on inner and outer surfaces. This window protects inner volume of the sensor from dust and humidity. The inner volume of the detector is filled by dry air. The o-ring behind the glass grants a vacuum-proven insulation.

The connection to the optical system is a straight-forward operation and per- formed with a standard **C-mount** (25 mm) or **T-mount** **thread** (40 mm) (1-32 UN 2A). Note that C-mount adapters are unique for each microscopic system but flange-photocathode distance is equal to the standard 0.69 inches (17.526 mm).

The detector has two mounting sockets with metric **M4 thread**. These sockets could be used for additional mechanical support of the detector if required.

The controller has two equal plugs for 12V DC supply. Any can be used for the detector connection. All the provided cables (except USB) are symmetric and can be plugged by any end whether to the detector head or to the electronics module.

The mini-USB type A cable on the rear side of the electronic module is used to connect the system to the acquisition computer.

NIM standard negative signal (–0.8V at 50Ω line) of duration more that 5 ns could be used as the **reference laser signal**. The reference signal shall be delayed at the duration of measurement time window.

1.5 Software installation

Download software installation package from our website and follow on-screen instructions. Driver installation requires Administrative privileges on the acquisition computer. Please allow OpalKelly USB device driver installation when asked.

1.6 Switching the system on

After the system is properly connected it is a time to switch it on.

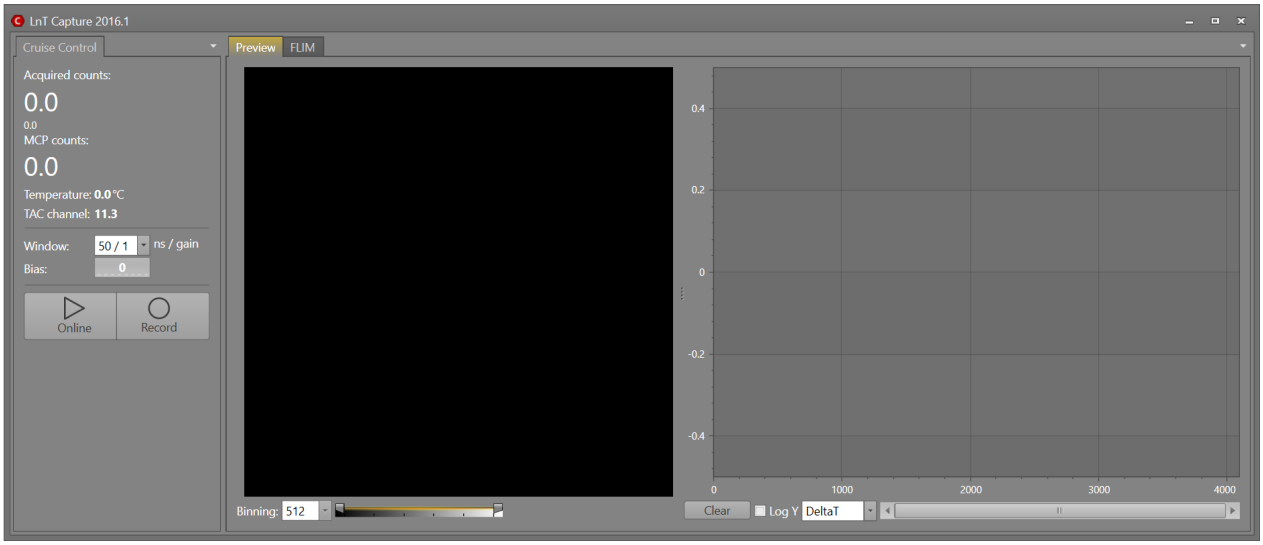
Plug the power cable to the wall socket and switch the electronics on using the **key switch** on the front side of the module. A control LED glows.

Initially an **alarm** ring will be on. Start **LnTCapture** program and after a short initialization the ring will go off. In case of the communication problem

Chapter 2
Knowing your system

2.1 Software controls

After the software is installed and the system is initialized a home screen of the acquisition software should look like shown on the figure below.



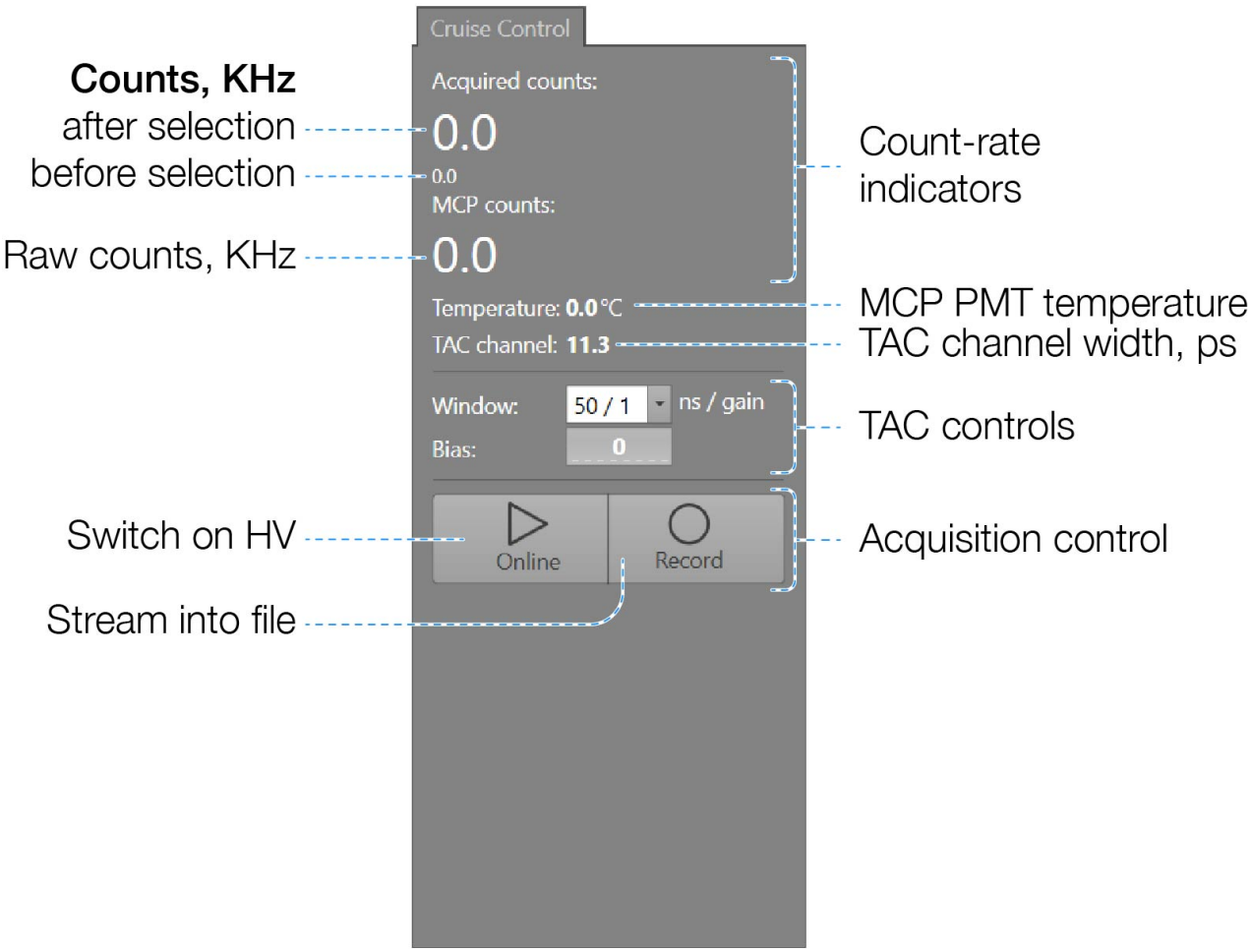
Cruise control

All the acquisition module control and feedback are gathered in the **Cruise Control** window.

There are three count-rate indicators shown. The first is a large one in acquired counts section. This indicator shows the rate of the photons that are actually displayed and stored to the disk (e.g. useful photons). The second smaller one displays count-rate of the events that include all the photons transferred to the computer. Normally this number is a bit higher

than useful photons because of the software selection logic. For example, if the observed decay is too long the photons falling outside the TAC window are included in “before selection” counts and do not contribute into “after selection” counts. A large discrepancy between after/before selection counts might be an indication of low quality of the reference signal or inadequate TAC settings.

MCP counts correspond to number of photons that yield an electron avalanche. This counts include all the events including those that are rejected due to the electronics dead-time.



The embedded TAC features selection of two **windows** of 50 and 100 ns optionally divided by 2, 4 or 8. For example 50/4 roughly corresponds to the measurement window of 12.5 ns. **Bias** control allows to induce additional delay to place time zero point into desired place.

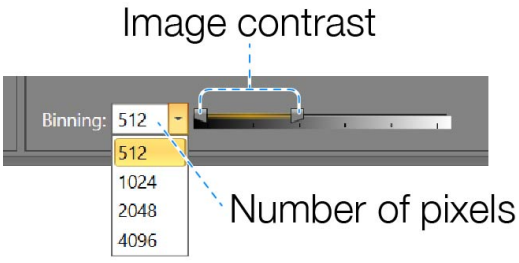
Starting acquisition is pretty straight forward. The **Online** toggle button applies HV to the photocathode allowing the photons to be detected. In addition to the software controlled PC-HV a hardware switch on the electronic module has to be used. For adjusting one can change TAC Window and bias.

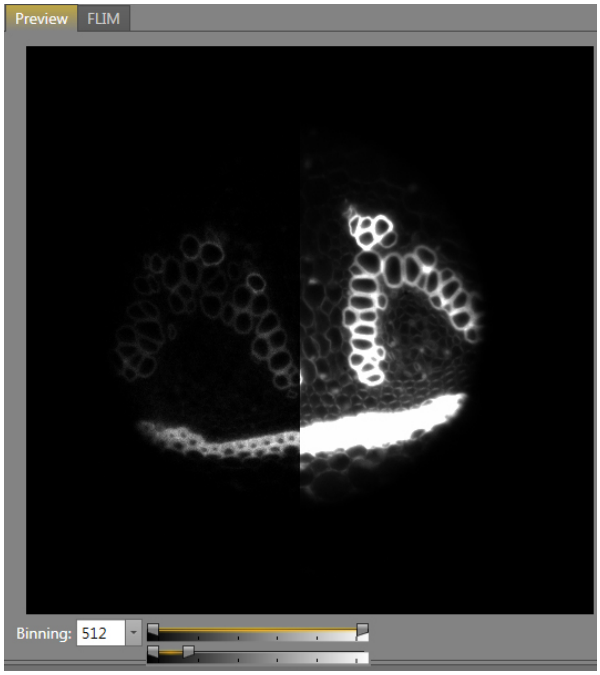
Please note that hardware switch has a priority over the software control, e.g. when the PC-HV is off it will remain off independently to the software change.

The **Record** toggle button will popup a file save dialog and start streaming the data into existing or newly created file. Changing TAC settings is not allowed during the acquisition.

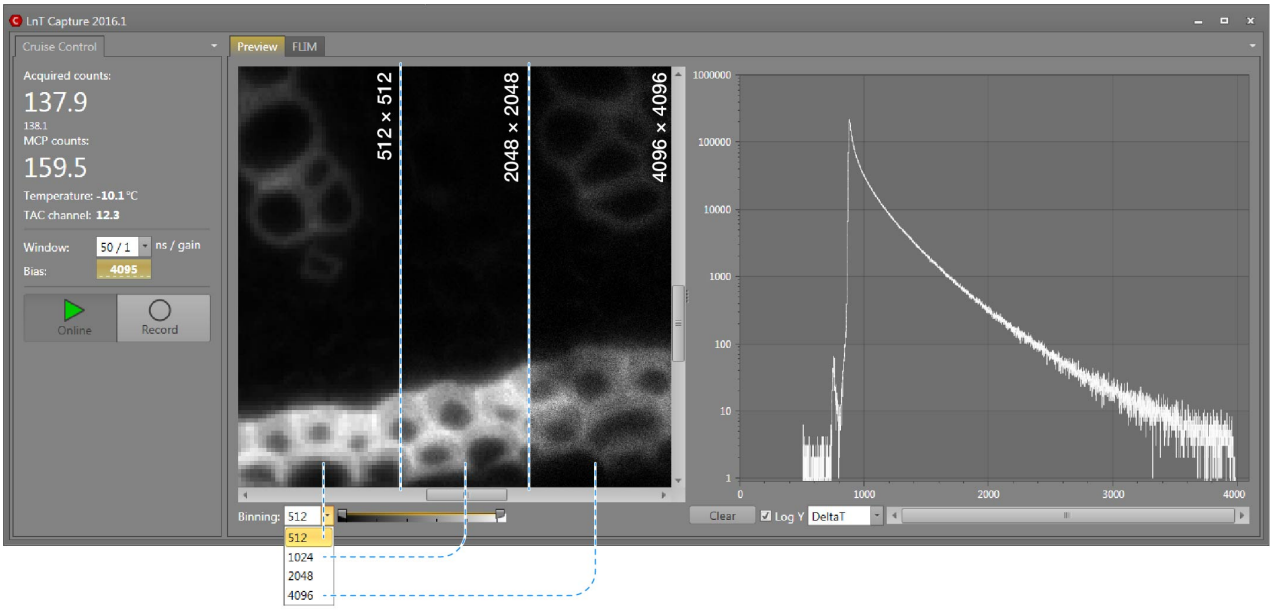
Image preview

The acquired counts are put into a set of two-dimensional histograms. The binning can be set dynamically to one of the following values: 512, 1024, 2048 or 4096. An optimal binning depends on intensity distribution and photon flux. The image can be zoomed in and out with a mouse wheel.

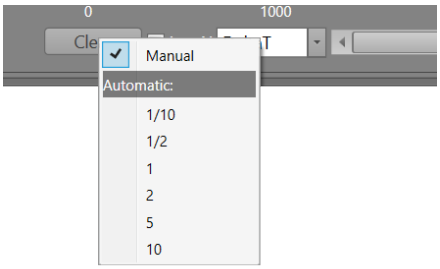




Effect of different binning settings on the image:

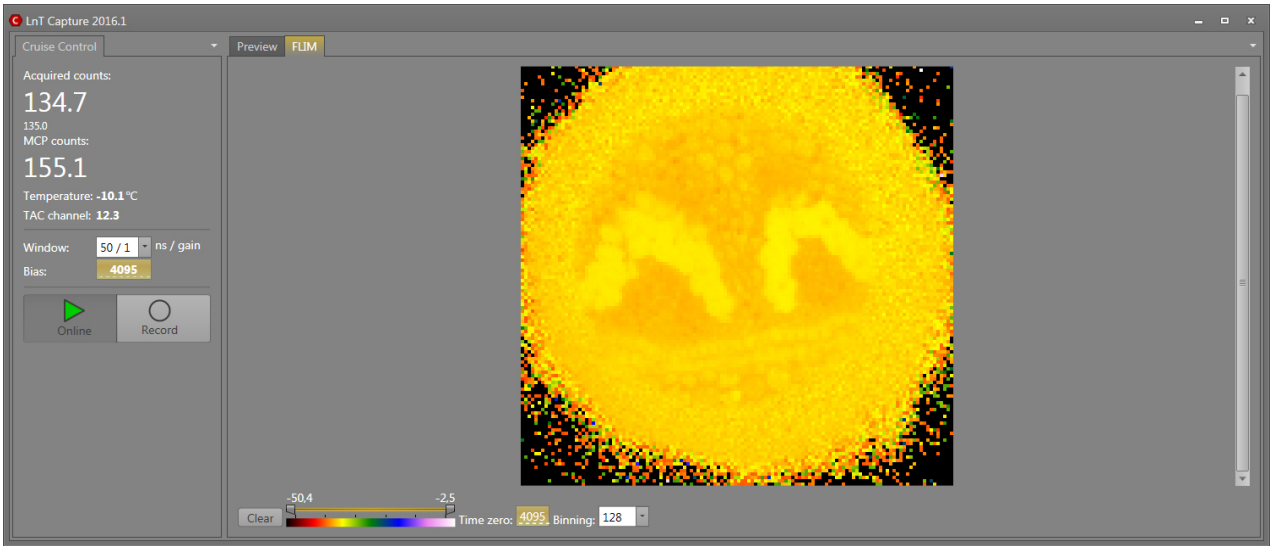


By default all the photons are accumulated into the histograms. Clicking **Clear** button erases all 1D and 2D histograms. For the adjusting it's handy to use automatic refresh feature that is available with a right mouse button click on clear button. The refresh rate defines the period of time the photons are accumulated in memory and displayed. That is if 2 seconds interval is selected the program will display in image preview and 1D spectra all the photons detected within the last 2 seconds.



FLIM preview

A fast calculated mean lifetime image is provided by mouse click on **FLIM** next to **Preview** on top of the preview image.

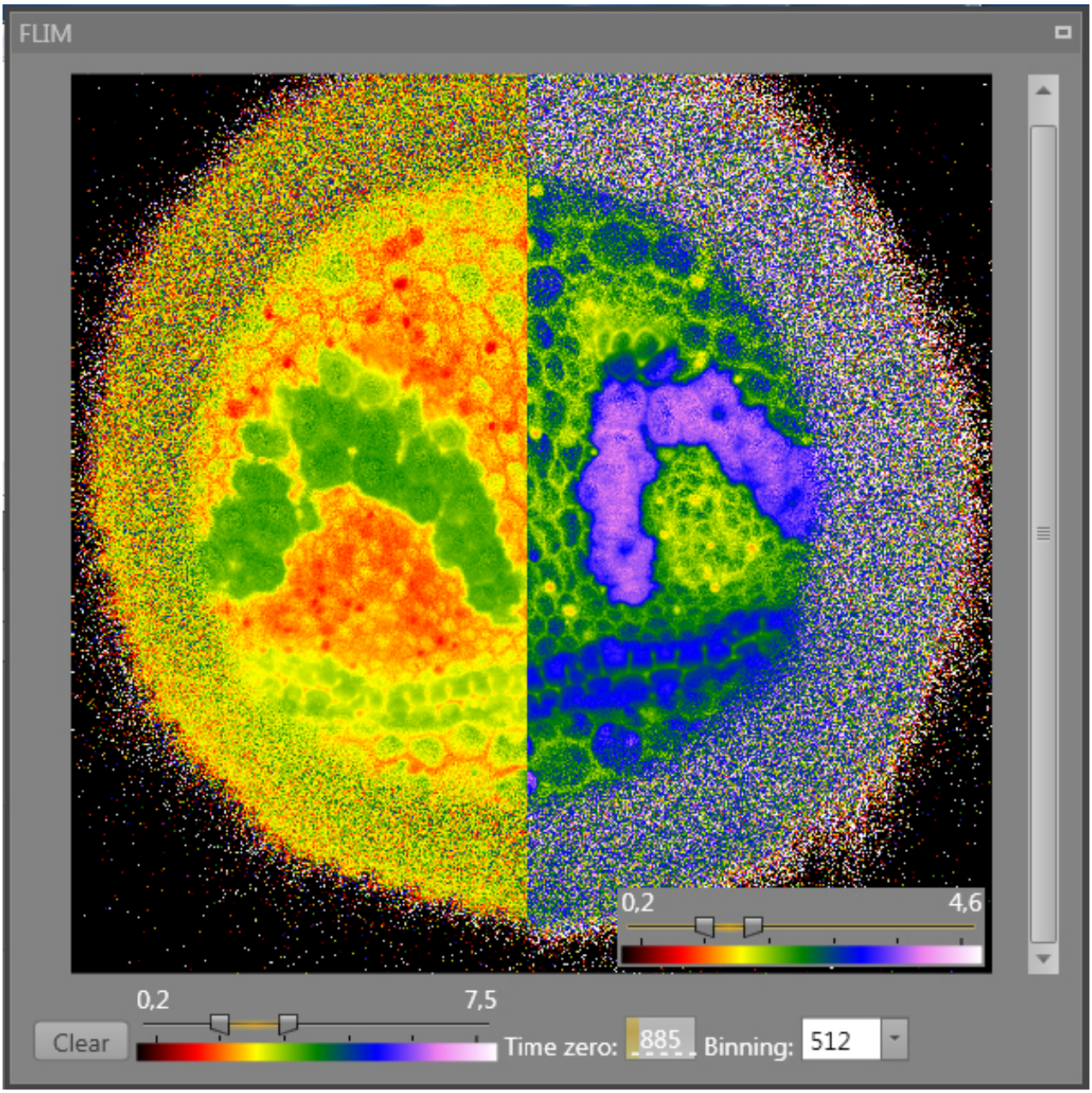


All pixels containing a photon are color coded by its mean lifetime according to the colormap below the image. Changes of the image parameters are comparable to the **Image preview** (see previous section).

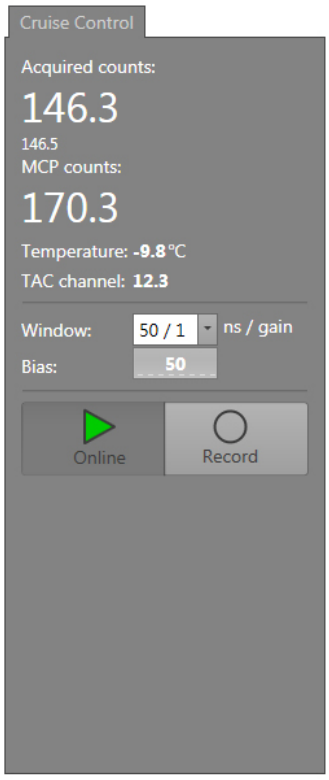
To adjust the timing of FLIM image **Time zero** corresponds to the starting limit of lifetime calculation. Time points below this limit get negative values. Usually **Time zero** marks the time, when the excitation LASER pulse hits the sample. For a good contrast the maximum of the fluorescence decay can be used.

The lifetime values are given in nanoseconds and take the width of a single **TAC channel** into account. The image is calculated continuously. The **Clear** button next to the colormap resets the time point of incident photons for calculation. The **Binning** menu gives the opportunity to change the image resolution from a 128x128, 256x256 to 512x512 pixels binning. Note, to resize the image on the screen use the mouse wheel. The slider on top of the colormap changes the minimum and maximum lifetime limits to the adjust the contrast of the FLIM image. Slider buttons for limits are

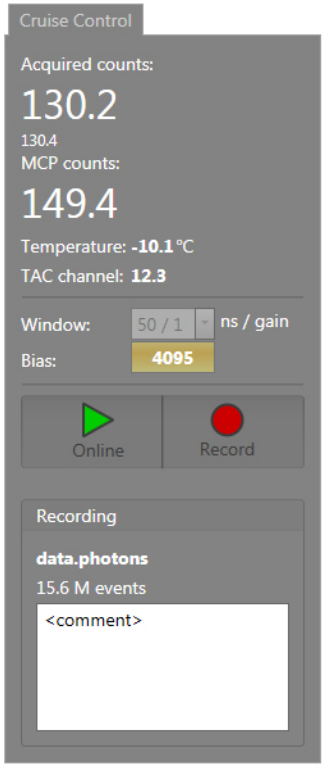
accessible by mouse „click and pull“ or using the mouse wheel to shift the selected range.



2.2 Recording data



Clicking **Online** toggle button will apply the high voltage on the photocathode. When the hardware key is also on, the detected photons shall be displayed on the screen. In this mode one can adjust TAC settings and find desirable image area. After that one can start recording.



Clicking **Record** toggle button will popup standard OS “Save As…” dialog box where the location of the output file and name has to be specified. After clicking save button actual data recording starts. During the recording TAC controls are disabled preventing accidental data acquisition conditions change.

It is possible to write the comment that is accessible via `/comment` attribute in the datafile.

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