user manual

pco.flim

pco.

PCO asks you to to read this manual carefully before using the pco.flim camera system and follow the instructions.

Contact us for further questions or comments.

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The cover picture shows a typical pco.flim.
The lens is sold separately.

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1. INTRODUCTION

Advantages of the pco.flim

The pco.flim camera system is the first luminescence lifetime imaging camera using a special 2 tap CMOS image sensor. It has all the required generation of frequency domain signals included (5 kHz – 40 MHz) and also allows in a limited frequency range (500 kHz – 40 MHz) the use of external modulation signals. It has an USB 3.0 interface for image data transfer and control of all camera operation modes. In addition it features manifold trigger input / output of trigger input / output signals for integration into any application framework.

Main Features

- high resolution (1008 x 1008 pixel)
- lifetimes from 100 ps to 100 ns
- quantum efficiency up to 39%
- >1000:1 (60dB) dynamic range
- low noise of 45 e- rms
- high frame-rate 90 fps (2-tap readout)
- exposure times from 10 ns to 10 s
- vibration-free water cooling
- modulation frequency range 5 kHz to 40 MHz
- USB3.0 interface
- pixel size 5.6 µm x 5.6 µm

1.1 INTENDED USE

This camera system is designed for use by technicians, engineers and scientists. It is a scientific measuring instrument, providing images. The camera may only be used according to the instructions of this manual. Provisions, limitations and operating conditions stated in this manual must be respected. Unauthorized modifications or alterations of the device are forbidden for safety reasons.

Areas of Application

- fluorescence lifetime imaging
- life science
- scientific imaging
- pressure sensitive paint (PSP)
- FRET
- bioluminescence / chemoluminescence
- imaging of bio markers (e.g. green fluorescent protein, GFP)

2. SAFETY INSTRUCTIONS

Read the safety instructions thoroughly and follow them strictly.

DANGER

DAMAGED POWER CABLE OR POWER PLUG
Danger to life due to electric shock.
➔ Each time the camera is used, check the power cable for damage.

WARNING

ELECTRIC SHOCK WARNING DUE TO VOLTAGE PARTS INSIDE
Risk of injury due to electric shock.
➔ Never slide any items through slits or holes into the camera.

CAUTION

MOISTURE
Risk of injury due to electric shock if moisture enters the camera.
➔ To avoid the risk of water condensation, protect the camera against extreme changes of ambient temperature.

CAUTION

TRIPPING HAZARD
Risk of injury from tripping over loose cables.
➔ Never position the cable in a way that it could become a tripping hazard.

NOTICE

HUMIDTY, DUST OR RADIATION
Humidity, dust or x-rays could damage the camera.
➔ Never operate the camera in humid or dusty environments or in places with high level of x-ray radiation.

NOTICE

JOLT & VIBRATION
To avoid damaging the camera it must be firmly mounted and protected against strong shocks or vibrations.
➔ Use the camera's mounting threads to secure it.

NOTICE

LENS MOUNTING
Screw in the lens gently to avoid thread damage.
➔ To protect the lens connector thread from damage, use minimal force when attaching a lens to the camera.

NOTICE

LIQUIDS DAMAGE CAMERA
If liquids have penetrated the device.
➔ Switch the camera off immediately, detach it from power and contact PCO's customer support.

NOTICE

DAMAGED CAMERA HOUSING
If the camera has been dropped or the housing is damaged.
➔ Switch the camera off immediately, detach it from power and contact PCO's customer support.

NOTICE

IF CAMERA IS NOT WORKING PROPERLY
In case all actions following this manual to get the camera working properly were unsuccessful.
➔ Switch the camera off immediately, detach it from power and contact PCO's customer support.

3. SYSTEM COMPONENTS

The camera system includes the following parts.

Camera

- For standard C-mount lenses and adapters
- Optical connection: the distance between the front edge of the C-mount and the sensor (back focal length) is 17.52 mm.

Rear Panel (see **A1.5**)

- DC Power Jack (connects to power supply)
- USB 3.0 connector
- Trigger connector
- LED indicates camera status

LED green continuous=camera is ready
LED yellow =boot loader
LED orange = camera is armed/blinking while recording
LED red continuous=error

Serial Number Tag

Mounting Thread

3/8''-16 UNC ¼"- 20 UNC M6

Power Supply

AC to 12V/DC power supply

USB 3.0 Cable

USB-A / USB-B cable (5 m)

Digital Camera Tools (USB flash drive content)

- Camware: software for camera control & image acquisition
- Camera driver & tools
- Software development kit (SDK) & demo programs in C / C++

4. INSTALLATION

You will find all necessary files on the accompanying USB flash drive. You may also download the latest versions of our software, camera driver and third party software drivers from our Website (www.pco.de).

Minimum System Requirements

- Clock speed > 2 GHz (Intel i5 Haswell or better)
- RAM > 4 GB
- Windows 7 or higher
- 1280 x 1024 pixel resolution display
- USB 3.0

4.1 CAMERA DRIVER

Start the USB 3.0 driver installation from your USB flash drive.

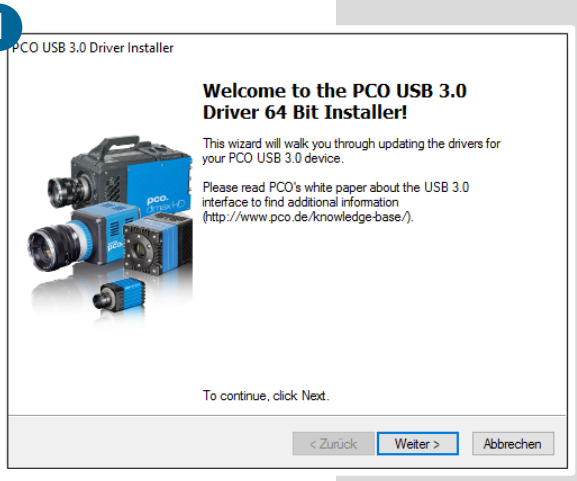
Don't connect the camera to your computer before driver is completely installed.

Run the provided installation file and follow the instructions of the installation wizard. 1

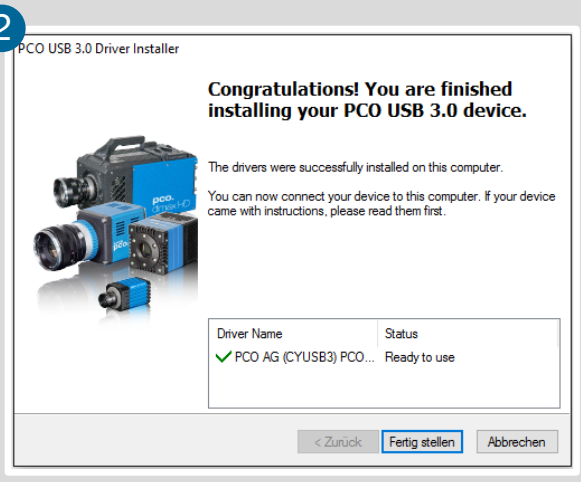
After finishing 2 the driver installation wizard connect your pco.flim camera to its power supply and via enclosed USB 3.0 cable to your computer.

The first time the camera is connected via USB to your computer, Windows will give notice that a new device has been detected.

1



2

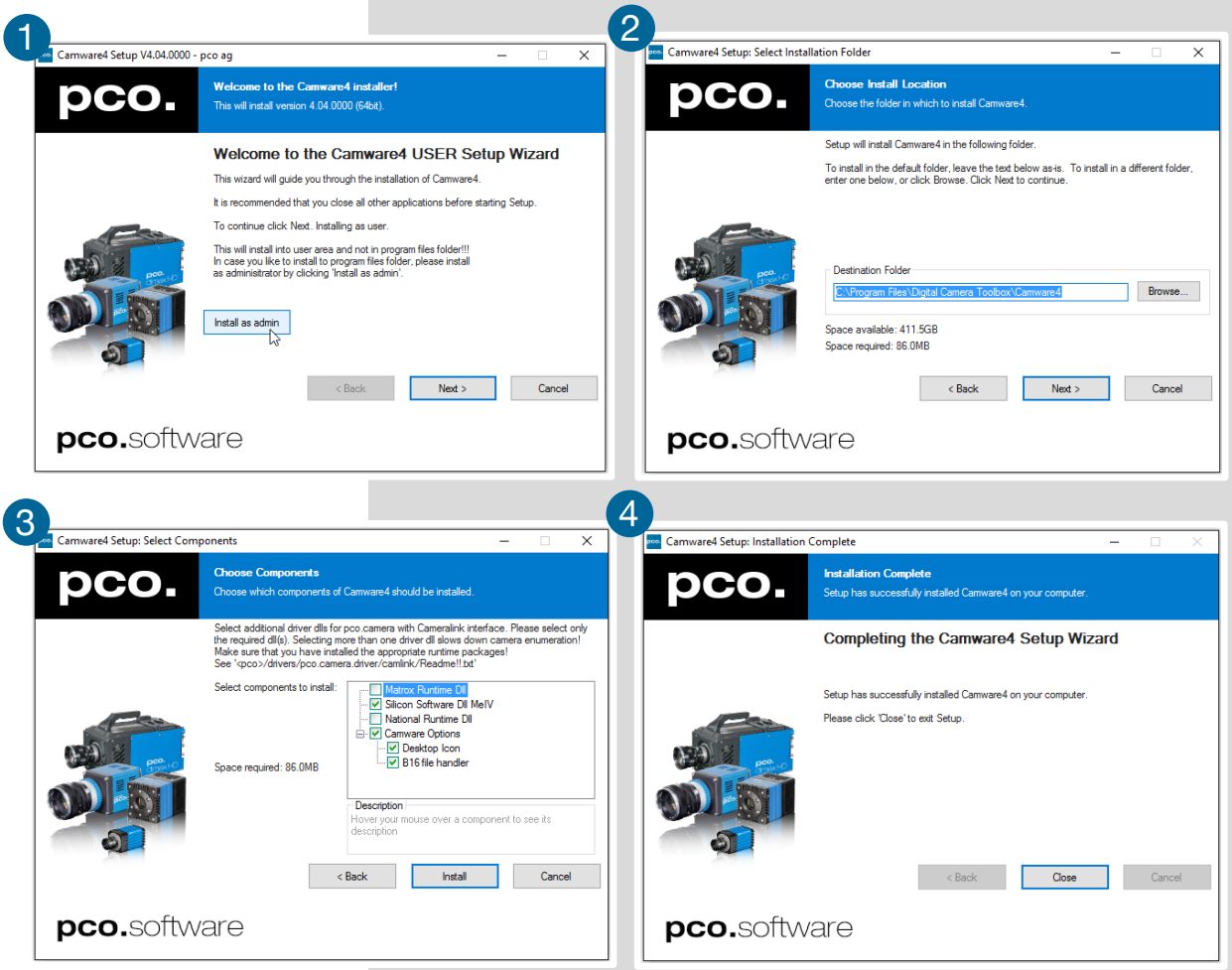


4.2 CAMWARE

The Camware Windows application software enables to control every camera parameter or setting. Images can be displayed on a monitor and may be downloaded and stored. The USB flash drive contains the installation files for the software for latest Windows operating systems in 32 bit and 64 bit.
After a successful installation, you will find the program file Digital Camera Toolbox in your program directory and a Camware 32 / 64 button on your desktop. Other helpful tools are also installed in the same directory.
To uninstall the Camware program, use the software feature under Windows' System Control.

Follow the Installation Wizard

1. ***Install as Admin*** to install to program folder, otherwise it will be installed only to user folder.
2. Choose install directory.
3. Choose components: select additional drivers.
4. After the next two screens installation is complete.



5. QUICK START

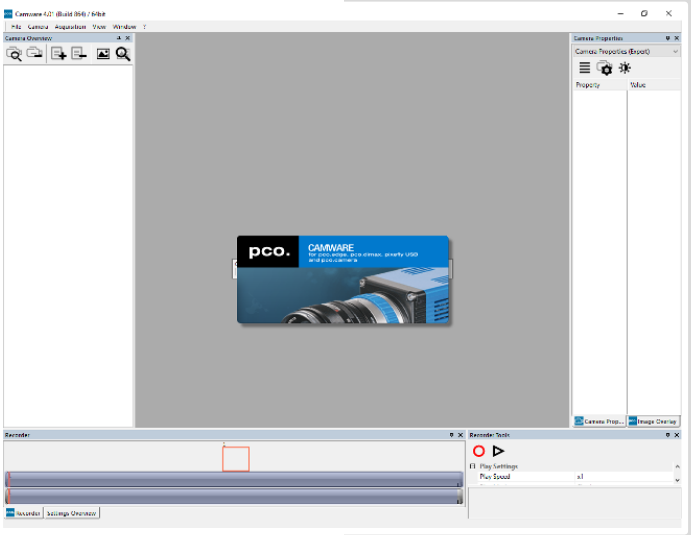
In order to get familiar with your new camera and software it might be helpful, first to aim the camera at an object easy to focus and visible at normal light conditions.

5.1 PREPARATION

- Computer is turned on
- Installation is finished (see chapter **4**)
- An appropriate lens is attached (remove cap) or the camera is attached properly to the microscope, spectrograph or other scientific device
- Camera is connected to your computer
- Camera is connected to the power supply and ready (LED green)

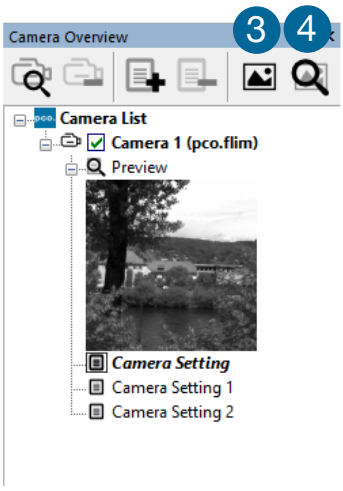
5.2 START

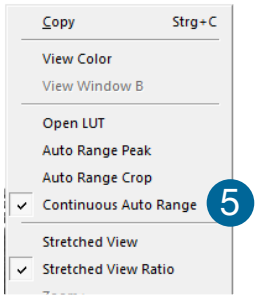
Start Camware and the graphical user interface will start up:



NOTE
Always install latest Camware version to get access to the full functionality of your pco camera (www.pco.de/support).

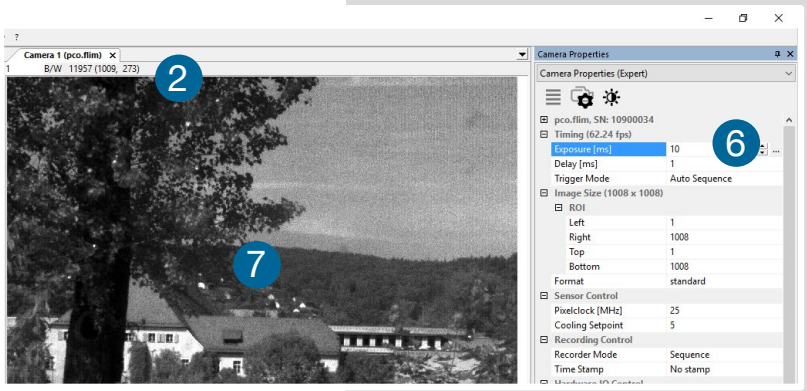
5.3 FIRST IMAGE





Follow the Instructions

- Camware must be started 1
- A ***View Window*** 2 is shown automatically or open a new one 3
- Start ***Live Preview*** 4
- Right-click in the ***View Window*** & apply ***Continuous Auto Range*** 5
- You may have to adjust ***Exposure*** time 6 or aperture and focus of the mounted lens
- Now you should clearly see the object in the window 7



To change ***Exposure*** time (e.g. the image is still either too dark or too bright), see **6.3.1**.

To record and save images, see chapter **6.3.4** and chapter **6.9** for detailed information.

NOTE
Live preview: useful for fast and easy camera adjustment and focusing.

6. CAMWARE 4 SOFTWARE

PCO's Camware an outperforming software for camera control, image acquisition and archiving of images in various file formats. This chapter provides a detailed description of all Camware functions.
Camware works with any kind of PCO camera. Visit PCO **website** for the latest version of this software.

6.1 INTRODUCTION / CONTENT

Chapter 6.2: cameras detected in Camware

6.2 Camera Overview / List	Preview / Connected cameras / Recording profiles

Chapter 6.3: camera settings

6.3.1 Timing	Exposure Time /Trigger Modes
6.3.2 Image Size	ROI / Sensor Format
6.3.3 Sensor Control	Pixelclock / Cooling Setpoint
6.3.4 Recording Control	Recorder Mode / Timestamp
6.3.5 Status	Temperature
6.3.6 Hardware I/O Control	Input and Output Signal Options
6.3.7 Flim Setup	Special Settings for pco.flim
6.3.8 Convert Control Dialog	Contrast / Saturation / Gamma…

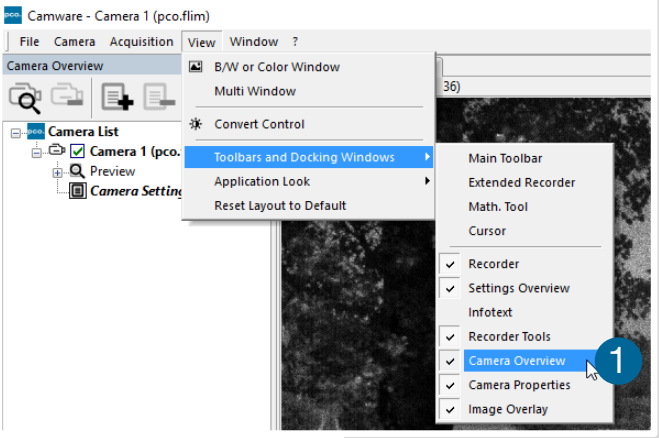
Chapter 6.4 / 6.5 / 6.6 / 6.7 / 6.8: recording

6.4 Image Overlay	Overlay for recorded images
6.5 Recorder Tools	Record / Play and Settings
6.6 View Window	View Window Functions
6.7 Recorder (Images)	Preview Recorded Images
6.8 Settings Overview	Overview of all Parameter Settings / Auto Save

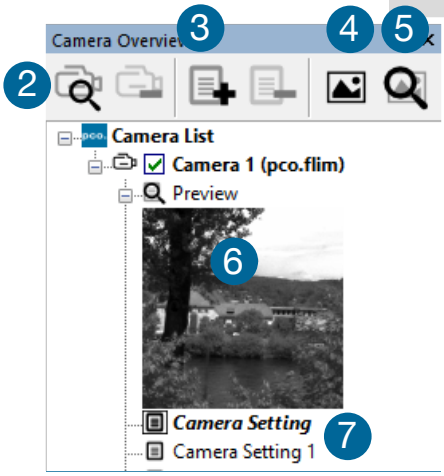
Chapter 6.9: software menus

6.9.1 Demo Mode	No camera connected
6.9.2 File Menu	Open / Save Raw / Export / Options
6.9.3 Camera Menu	Setup / Close / Rescan
6.9.4 Acquisation Menu	Live preview / Acquire Sequence / Rec. Memory Settings
6.9.5 View Menu	New Window / Convert Control / Multi Window / Toolsbars / Application Look
6.9.6 Window Menu	New / Close / Split window
6.9.7 Help Menu	Logfiles / Support File / About
6.9.8 View window Menu	Right-click: Zoom / Flip / Mirror / Rotate / Line Profile / Properties …
6.9.9 Additional Features	Contrast / Short Cut List

6.2 CAMERA OVERVIEW / LIST



If closed, open the ***Camera Overview*** window by selecting the ***View*** tab and ***Toolbars and Docking Windows*** → ***Camera Overview*** 1.



Camera Overview

The ***Camera Overview*** window supports management of more than one PCO cameras and displays a ***Camera List*** of the connected ones. Camware is able to ***Scan Cameras*** 2 or close a connected camera. It allows to define several different ***Camera Settings*** for each camera (max. 30 sets per camera → ***Add Set*** 3).

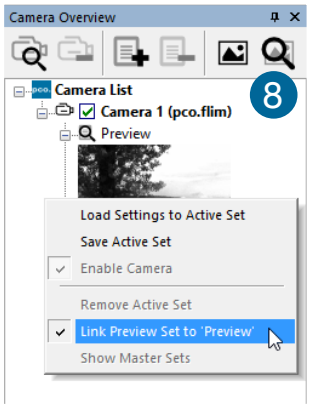
New ***View Windows*** 4 can be opened and the ***Live Preview*** 5 function started. When opened up, the ***Live Preview*** shows a small ***Preview*** window 6 (always monochrome) integrated in the ***Camera List***.

Live Preview 5

Live Preview facilitates the aperture and focus adjustment, allowing a first look at your object. During ***Live Preview Trigger Mode*** is set to ***Auto Sequence***.

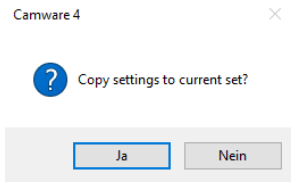
Camera Setting 7

All presettings, such as resolution and frame rate, in the ***Camera Properties*** (see **6.3**) are saved to ***Camera Settings***. Define different ***Camera Settings*** with different ***Preferences*** in ***Camera Properties*** for each of your experiments. ***Camera Settings*** can be switched at any time (not during record) and copied to other cameras.



Link Preview Set to 'Preview'

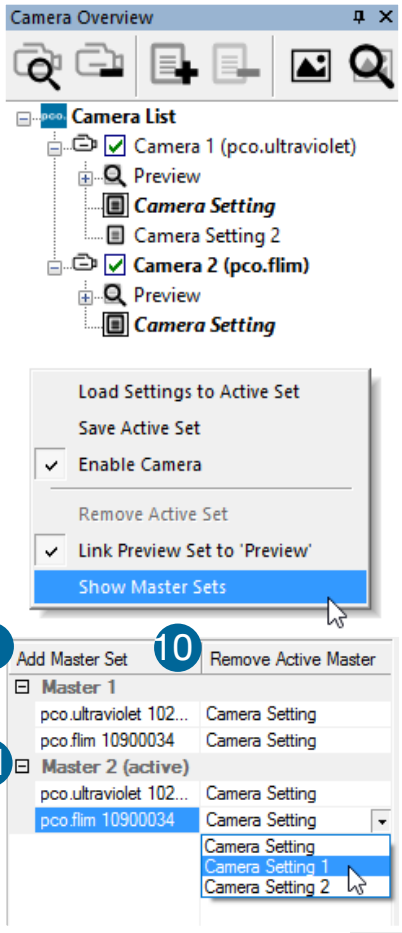
When ***Link Preview Set to 'Preview'*** is ticked the ***Preview*** will always be active with the set parameters when starting a ***Live Preview*** 8.
In case this function is deactivated, the ***Live Preview*** will always show live images with the parameters of your active setting.
Setting a higher exposure time for ***Preview Set*** and linking it to the ***Preview*** function is beneficial if ***Preview*** light conditions are different from those in recording situations.



Copy Settings to Current Set

To copy e.g. ***Camera Setting 1*** to ***Camera Setting 4***, just drag and drop ***Camera Setting 1*** to ***Camera Setting 4*** and Camware will ask to confirm it. It is possible to copy each setting to every camera.

Master Sets



This function facilitates image acqusition with multiple cameras. Defining two or more ***Master Sets*** allows easy switching between different predefined settings for each camera during an experiment. Each image acquisition or experiment can be recorded with its own ***Master Set***.

To enable ***Master Sets***, right-click in the ***Camera Overview*** window and click ***Show Master Sets***.

Define different ***Master Sets***. Select individual ***Camera Settings*** within each ***Master Set***.

Functions:
Add Master Set 9 or ***Remove Active Master*** 10.
Activate it by clicking on for example ***Master 2*** 11.

6.3 CAMERA PROPERTIES

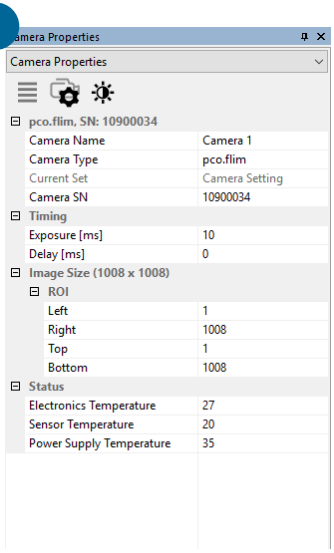
The ***Camera Properties*** window in Camware is the main interface for all camera settings. The active set selected within ***Camera List*** is adjusted here.

Setup: important settings for pco.flim can be made in the ***Setup*** dialog, see chapter **6.3.7**.

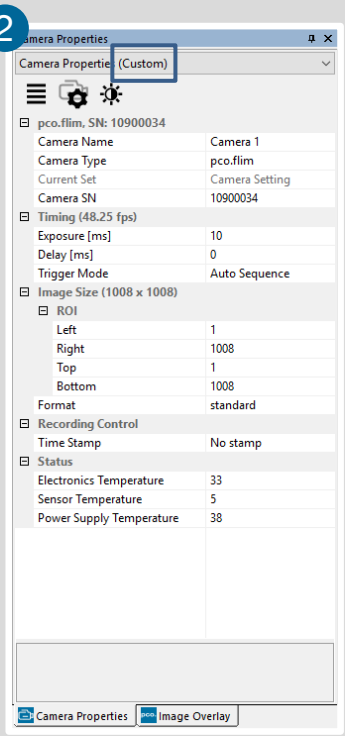
The former main topic ***Camera Control*** (known from Camware 3.x) and the ***Convert Control*** (see **6.3.8**) can be opened additionally.

Three view options with various functions can be selected: ***Basic***, ***Custom*** and ***Expert***.

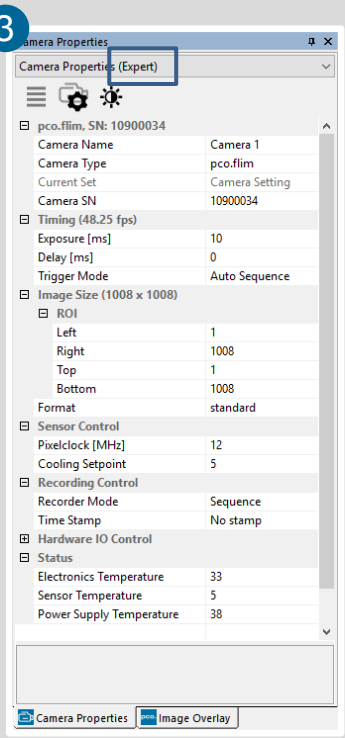
1



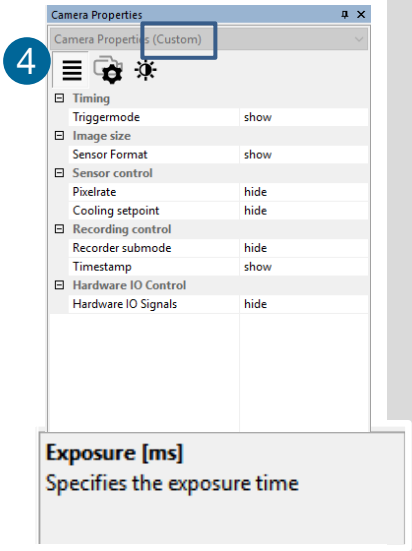
2



3



4



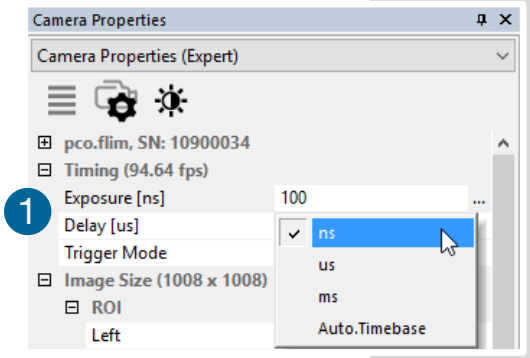
Basic mode 1 only shows camera name, type, set, serial number and exposure time. In ***Basic*** mode the frame rate is always calculated automatically based on the selected exposure time, while exposure time is increased, frame rate decreases. It is recommended for Camware beginners.

Custom mode 2 shows several more setting possibilities and functions are hidden or shown by the ***Custom Properties*** button. 4 Beside the ***Basic*** mode, Trigger Mode, Image Size and Recording Control options are selectable.

Expert mode 3 (for advanced users) shows all possible camera feature settings.

An explanation for every setting is displayed below the ***Camera Properties*** dialog.

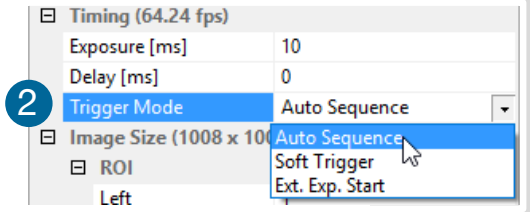
6.3.1 TIMING



Exposure / Delay 1

Time-base from automatic to µs or ms may be changed. If your input is out of the range of the camera, it will be automatically changed to the next possible setting. The current frame rate (fps) is always displayed.
The ***Exposure*** time can be set in steps of 10 ns.

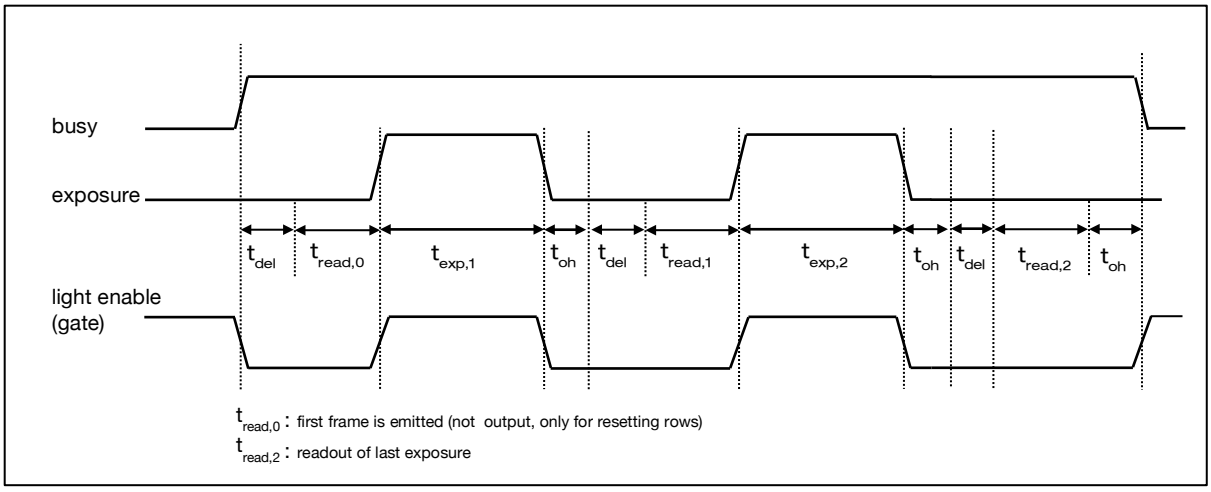
Type	Time range
Exposure time	10 ns – 10 s
Delay time	1 µs – 1 s



Trigger Mode 2

Auto Sequence: the camera optimizes the image recording to achieve the best possible frame-rate.
In the ***Auto Sequence*** exposure control mode the camera determines the highest possible frame rate against the set ***Exposure*** time and the time required for a frame readout. Upon a start command the sequential recording starts and lasts until a stop command.

The following timing diagram shows the exposure sequencing of two exposures times.



t_{td}: trigger delay (incl. jitter)
t_{read}: readout time
t_{del}: delay time
t_{oh}: overhead time
t_{exp}: exposure time

Maximum fps (Auto Sequence mode, i.e. $t_{td} = 0$):

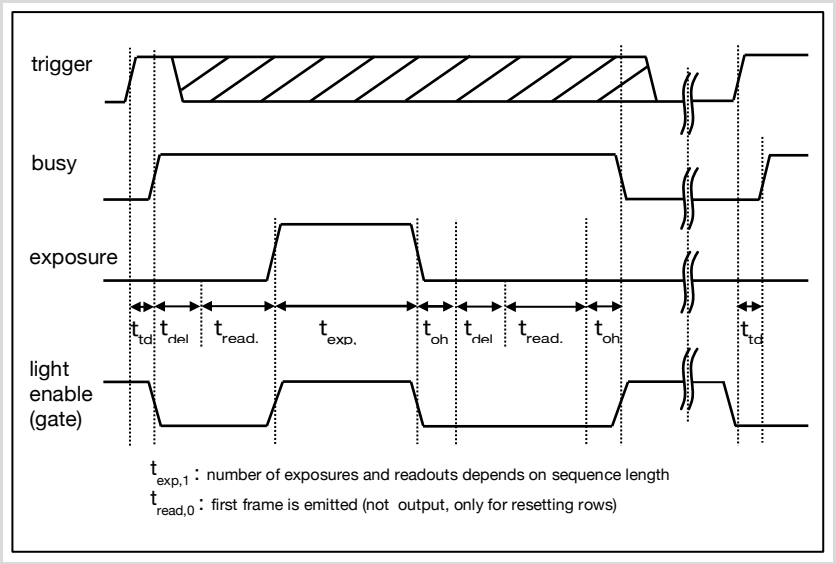
$1/fps_{onetap} = t_{del} + t_{read} + t_{exp} + t_{oh}$ (for one tap)

N_{rows}: number of rows ≙ vertical ROI; $FPS_{bothtaps} = 2 * FPS_{onetap}$

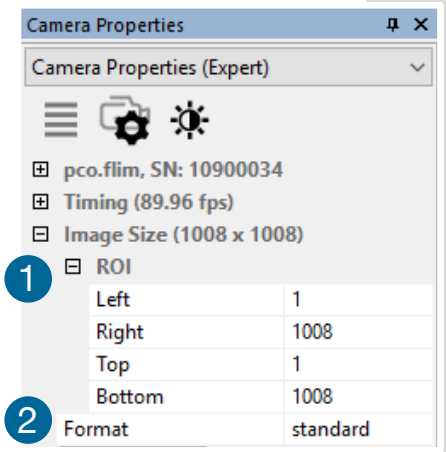
Soft Trigger: single images are recorded by this Camware command. A single image is acquired by pressing the ***Software Trigger*** button. This button appears after pressing the ***Record*** button (see **6.5**). Other signals have no influence on this operating mode.

Timing parameter	Pixel clock 12.5 MHz	Pixel clock 25 MHz
t_{del}	Adjustable 0…1s	Adjustable 0…1s
t_{read}	31.2 µs * N_{rows}	20.96 µs * N_{rows}
t_{exp}	10 ns…10 s	10 ns…10 s
t_{oh}	4.8 µs	4.8 µs
t_{td}	≤ t_{del} + t_{read} + t_{exp} + t_{oh}	

External Exp. Start: image acquisition is triggered by an external signal. The ***Software Trigger*** button acquires a single image for a test.
In the ***External Exp. Start*** exposure control mode, single image acquisition starts by the rising edge of the voltage signal at the trigger input (see **A1.5**).



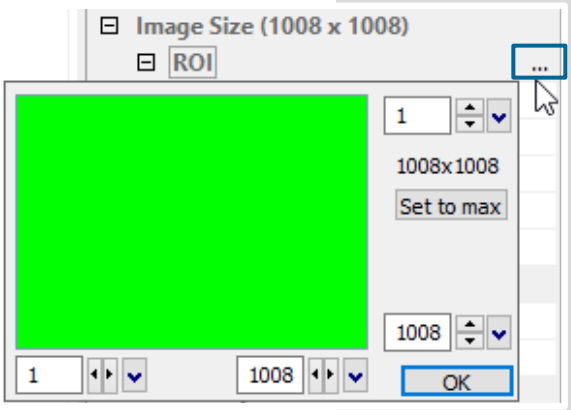
6.3.2 IMAGE SIZE



Region of Interest 1

The ROI (Region of Interest) selects only a part of the sensor to be read out, to speed up the frame-rate and to reduce the amount of image data. The decreased image size you see within Camware is a combination of reduced sensor resolution and software downsizing (Soft-ROI). For ROI by mouse see **6.9.9**. The frame-rate only increases, if the vertical resolution is decreased.

ROI step sizes	
Horizontal steps	16 pixel steps
Vertical steps	1 pixel steps
Minimum ROI	16 x 4 pixels



ROI window

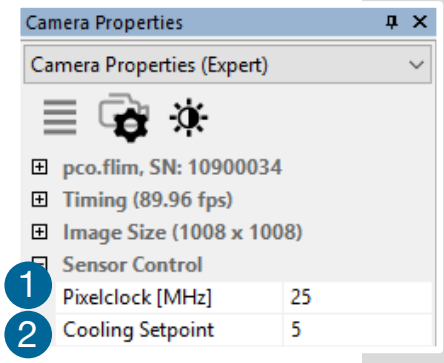
Select the ***ROI*** menu and activate ***ROI window*** by clicking on ***...*** or use the ***...*** right to the ***Left / Right / Top / Bottom*** and click on ***ROI window***.

The ***ROI window*** opens and a new region of interest might be set by dragging a window with the mouse or by typing in the values.

Format 2

This sensor implementation provides the option to either read out the standard size of 1008 x 1008 pixels (effective pixels recommended by the sensor manufacturer) or an extended size of 1024 x 1024 pixel (dark pixels around, the image, which do not contain any information)

6.3.3 SENSOR CONTROL



Pixel Clock 1

The ***Pixel Clock*** sets the clock frequency and therefore the image sensor readout speed.
Possible settings are 12 MHz (real clock frequency is 12.5 MHz) or 25 MHz. At 25 MHz the image sensor is read out with nearly double speed, achieving higher frame-rates.

The following table shows frame-rates for two active taps:

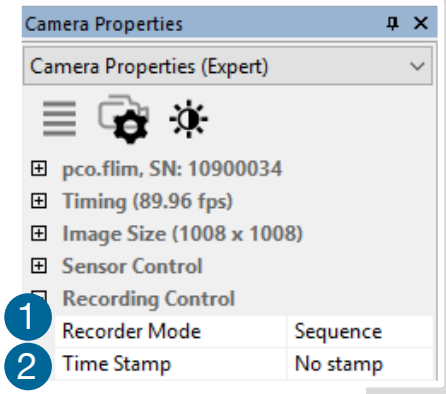
Resolution	12.5 MHz	25 MHz
1008 x 1008	63 fps	94 fps
800 x 600	106 fps	158 fps

Cooling Set Point 2

If a ***pco.flim*** is connected, sensor temperature is adjustable (air- or water-cooling). Recommended sensor temperature is ***5°C***.

Display of sensor temperature: a Peltier cooling unit is used to keep the sensor's dark current to an acceptable minimum and to allow for a continuous operation free of any drift phenomena in image sequences. Either an internal fan or an external water cooling system assures proper heat dissipation from the Peltier element to regulate the temperature of the sensor.

6.3.4 RECORDING CONTROL



Recorder Mode 1

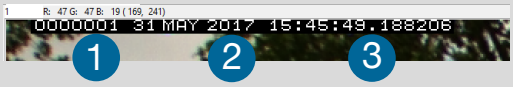
In ***Sequence*** mode the recording stops when (computer) RAM space is full. In ***Ring Buffer*** mode the camera will stop only by a stop command.

Timestamp 2

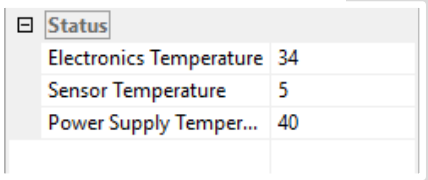
A ***Timestamp*** can be placed into the upper left corner of the image. It can be either set to ***No Stamp***, ***Binary*** or ***Binary + ASCII*** (text). The time resolution is 1µs.

In ***Binary*** mode the first 16 pixels will be filled with the ***Timestamp*** information (binary code). The numbers are coded in BCD with one byte per pixel. Every pixel contains two digits. If the pixels have a resolution of more than 8 bits, the BCD digits are right bound adjusted and the upper bits zero. For further information refer to the SDK.
In ***Binary*** + ***ASCII*** mode text is inserted into the image replacing its content (272 x 8 pixels ASCII).
Timestamp shows the end of exposure time.

Three different information is stamped onto the image: number of the image 1, date 2 and time 3.

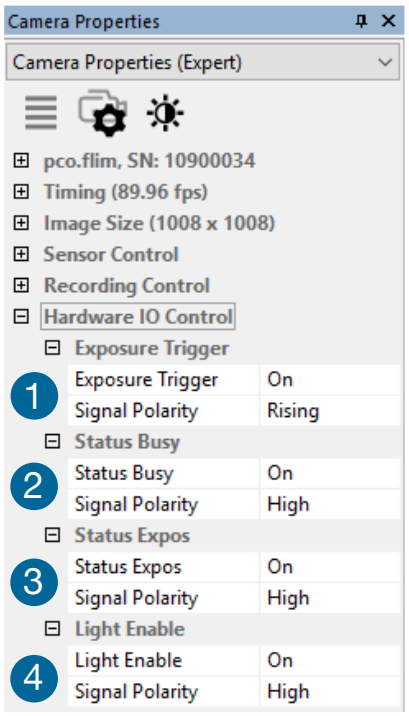


6.3.5 STATUS



Shows the current temperatures of the pco.flim camera.

6.3.6 HARDWARE I/O CONTROL



Exposure Trigger 1

If checked, a signal for ***Ext. Exp. Start*** trigger mode (see chapter **6.3.1**) is accepted at the ***Exposure Trigger*** (see **A1.5**).

Status Busy 2

A signal indicating busy status is available at the status busy output. Once an acceptable trigger edge is received, ***Busy*** will go to status ***High***. As soon as busy goes ***Low*** again, a new trigger edge is accepted (negative logic possible, too!).

Status Expos 3

A signal indicating exposure status is available at the status output. ***Status Expos*** indicates the actual exposure window for one frame.

Light Enable 4

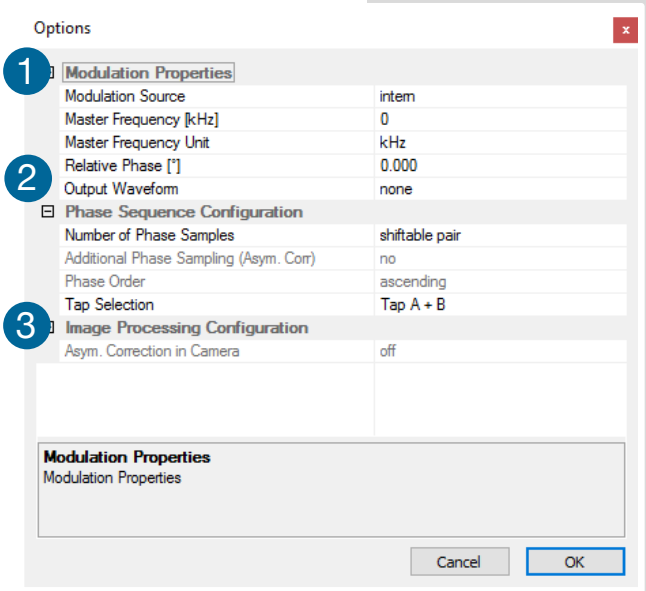
This signal is usually connected to an external light source and enables / disables it synchronously to the image acquisition in order to minimize crosstalk effects in the image sensor.

Enabling and Polarity of I/O Signals

The polarity of the I/O signals indicating their active states is selectable (positive or negative logic).
Level-sensitive signals can be set to ***High*** (positive logic) or ***Low*** (negative logic).
Edge-sensitive signals can be set to ***Rising*** (positive logic) or ***Falling*** (negative logic).

6.3.7 FLIM SETUP

Open ***Camera*** menu (see **6.9.3**) and select ***Setup*** dialog.



Modulation Properties 1

Modulation Source
The source for the modulation signal generation is either ***Internal*** or ***External***. Selecting ***External*** a valid hardware signal must be active at the modulation input.

Master Frequency
Sets the ***Master Frequency*** to the desired value in units of ***kHz*** or ***MHz*** as an integer number if the modulation signal is generated internally.

Master Frequency Unit
Selects the unit of the ***Master Frequency*** value (***kHz*** or ***MHz***).

Relative Phase
Choosing ***Shiftable Pair*** mode in ***Number of Phase Samples*** the ***Relative Phase*** between the image sensor modulation and outgoing / incoming modulation signal can be shifted in the range of 0.0° to 360.0°.

Output Waveform
Sets the ***Output Waveform*** of the outgoing modulation signal to ***None***, ***Sine Wave*** or ***Square Wave***.

Phase Sequence Configuration 2

Number of Phase Samples
Selects the ***Number*** of equidistant ***Phase Samples*** per modulation period of 360° (2, 4, 8 or 16). Additionally, it offers the ***Shiftable Pair*** mode in which taps A and B are manually phase-shifted relative to the modulation signal.

Additional Phase Sampling
If set to ***Yes*** all phase samples are sampled by both ***Taps A*** and ***B***, doubling the total number of phase images. Used to correct asymmetry of the taps.

Phase Order
Determines the arrangement of adjacent phase pairs. Selecting ***Opposite*** all complementary phase pairs are sorted adjacently. Choosing ***Ascending*** the phase pairs cover the modulation period linearly. This selection is available with ***Additional Phase Sampling*** set to ***Yes***.

Tap Selection
Determines which taps are read (***Tap A + B***, ***Tap A*** or ***Tap B***).

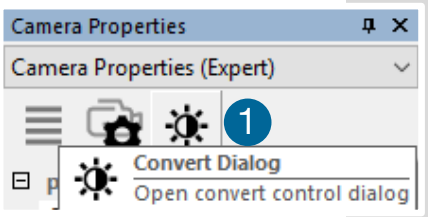
Image Processing Configuration 3

Asym. Correction in Camera

If switched ***On*** the asymmetry correction is performed in the camera by averaging corresponding phase images of ***Taps A*** + ***B***. This mode requires the following settings:

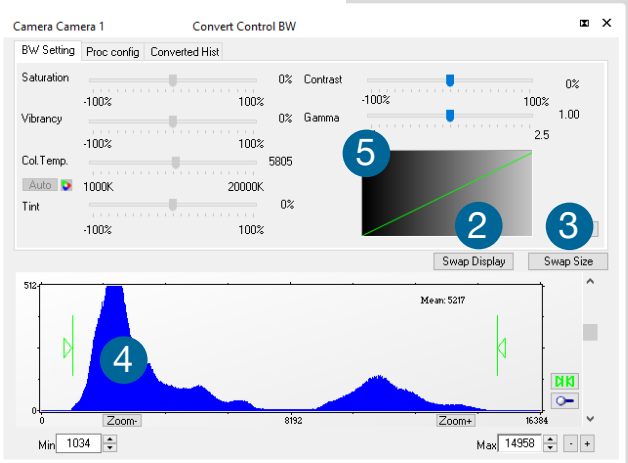
- ***Number of Phase Samples***: all except ***Shiftable Pair***
- ***Additional Phase Sampling***: ***Yes***
- ***Phase Order***: ***Opposite***
- ***Tap Selection***: ***Tap A + B***

6.3.8 CONVERT CONTROL DIALOG



Start the ***Convert Control Dialog*** with the black / white button 1 in ***Camera Properties***.

The display of the original 14 bit image intensity values (x-axis) in the shown 8 bit values (y-axis) can be arranged in following ways.



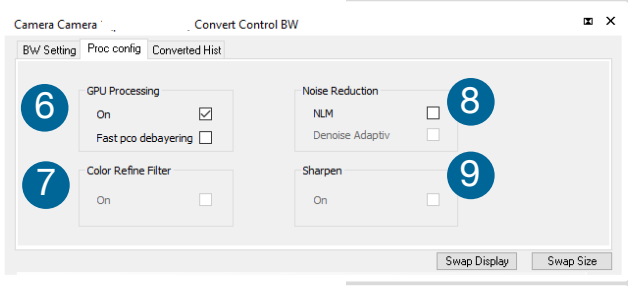
BW Settings

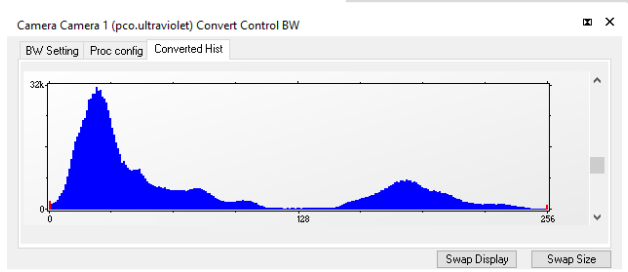
It is possible to hide the histogram of original data 2 and to switch tab / histogram 3.

Green sliders in histogram 4
Left slider: min controller (corresponds to value 0 of the 8 bit display). Values below that mark are set to 0, i.e. displayed as black.
Right slider: max controller (corresponds to value 255). Values above that mark are set to 255, i.e. displayed as white.

The values in-between are converted into a value between 0 and 255 depending on ***Contrast*** and ***Gamma*** settings, the small graph 5 , reflects the calculation.

Other functions (Saturation, Vibrancy, Col. Temp, Tint) are inactive for this camera.





Proc. Config (Process configuration)
Due to proprietary high-end algorithms used for these image processing features, no detailed description is given here.

GPU Processing 6
On: switch on in order to significantly reduce processing time (increases refresh rate of the live image.
Fast pco debayering: not available

Color Refine Filter 7
On: not available

Noise Reduction 8
NLM: non local means algorithm
Denoise Adaptive: not available

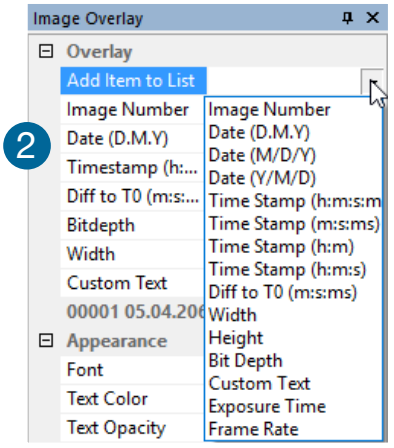
Sharpen 9
On: not available

Converted Hist

This tab shows the histogram of converted data.

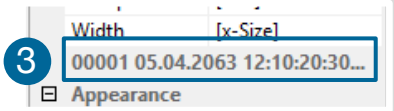
6.4 IMAGE OVERLAY

Open ***Image Overlay***: these buttons allow easy switching between ***Camera Properties*** and ***Image Overlay*** windows.1
If not available, see **6.9.5 *View Menu*** to activate this menu.

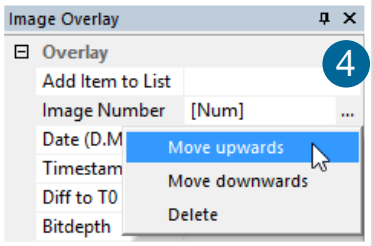


This function enables an individually configurable image overlay, to display information within the images.
Many options are available by clicking ***Add Item to List*** 2

Also the ***Appearance*** is configurable:
Font, ***Text Color***, ***Text Opacity***, ***Background Color***, ***Background Opacity*** and ***X Position*** or ***Y Position***.

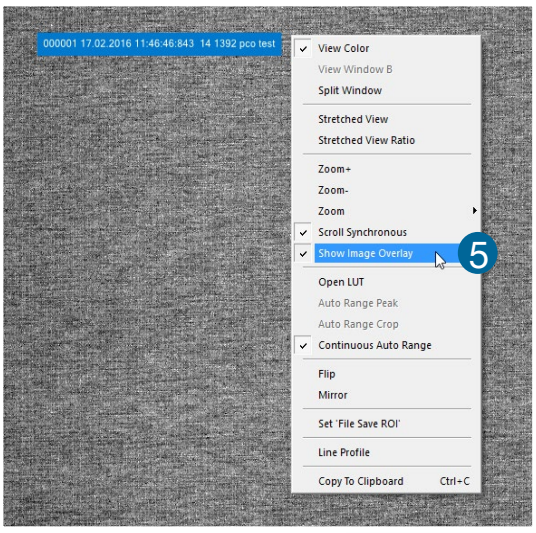


Preview of the image overlay. 3



Each item can be moved or deleted: ***Move Upwards***, ***Move Downwards*** or ***Delete*** by clicking on ***...*** 4

Move the ***Image Overlay*** to your favorite position in the image by drag & drop.



Right-click in the image window to activate ***Show Image Overlay.***5

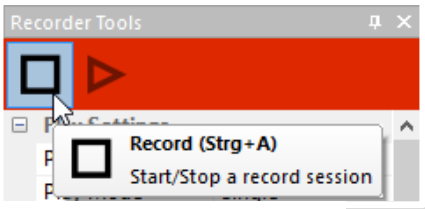
NOTE
This function does not overwrite image data.

6.5 RECORDER TOOLS

Recorder Tools provides ***Record*** and ***Play*** function, ***Play Settings*** and ***Record Settings***.
Located on the right lower side of Camware or, if closed, activated by ***View*** menu (see chapter **6.9.5**)

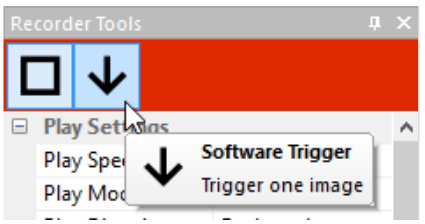
Record

Start/Stop record: with ***Record*** button
Second option: press enter key or Strg + A to Start / Stop recording.



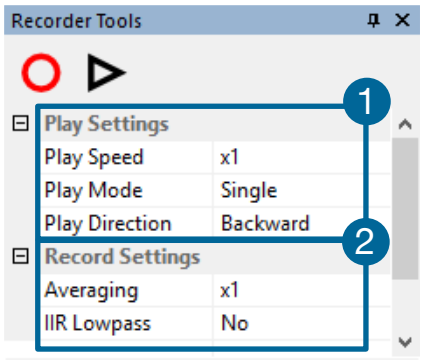
Record: in record state Camware software is highlighted in red.

Exposure time may be changed during recording. See **6.3** ***Camera Properties***.



Software Trigger: after record is started an arrow pointing downwards appears. Clicking on it triggers a single image (see **6.3.1*)***.

Play Settings ❶



Play Speed: selectable ***Play Speed*** from ***x1*** to ***x256*** or from ***1 fps*** to ***16 fps***.
E.g. in mode x1 a recording with 1000 fps is played with 25 fps.
1 fps means that only one frame per second is played.

Play Mode: selectable play mode of the recorder (***Single*** or ***Continuous*** (re)play).

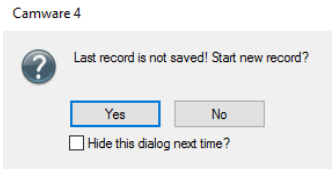
Play Direction: selectable direction of record play (***Forward*** or ***Backward***)

Record Settings ❷

Averaging: averaging images in the buffer reduces statistically independent (image) noise. Set a value higher than ***x1*** in the drop-down list and this number of images will be averaged.

IIR Lowpass: another option to reduce noise is the activation of the infinite impulse response ***IIR Lowpass*** filter. It takes 90% of the previous image and 10% of the new image to create images with clearly reduced noise.

Image (actual) = Image (act - 1) * 0.9 + Image (new) * 0.1



Reminder Dialog

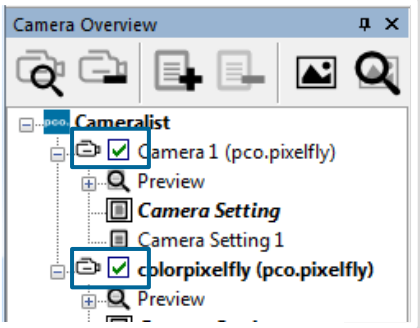
If you made a recording but did not save it yet, Camware will remind you to save the record before starting a new one.

Extended Recorder

Extended Recorder can be activated (see **6.9.5**)

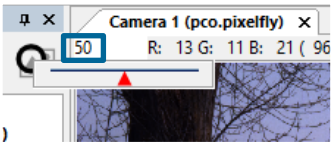
1. ***Record*** / ***Stop*** record / ***Play***
2. ***First image*** (jump to first image) / ***Back fast*** (jump backward) / ***Back*** (jump one image backward)
3. ***Forward*** (jump one image forward) / ***Forward fast*** (jump forward) / ***Last image*** (jump to last image in record)

Recording with Multiple Cameras



With all cameras activated recording is started simultaneously for all cameras.
Recorder will use ***Recorder Mode*** settings (Sequence or Ring Buffer) of the active camera for all cameras (see **6.3.4**)
For single camera recording, deactivate cameras by removing the check mark from the box.

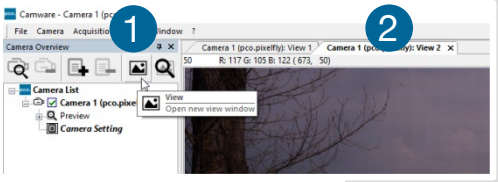
6.6 VIEW WINDOW



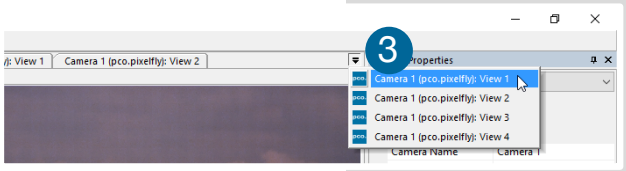
Quick Scrolling

Having recorded at least 50 images, you may scroll through them quickly by holding down the left mouse button on the image number. Or enter the desired image number directly into the number field.

More View Windows

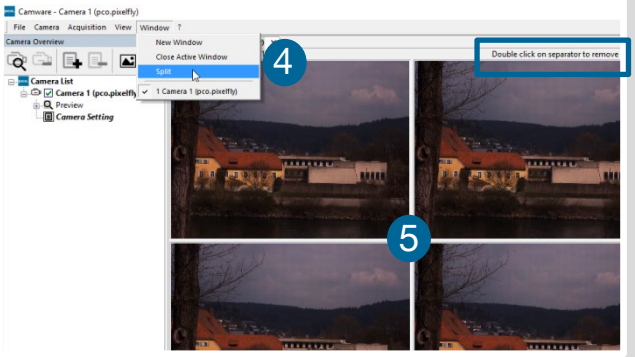


To open more ***View Windows*** from one camera: click ***View Window*** button 1 and Camware will create a new ***View window*** 2 .
Even with multiple ***View Windows*** (or from multiple cameras) open, the same image number is always shown in all of the ***View Windows***.

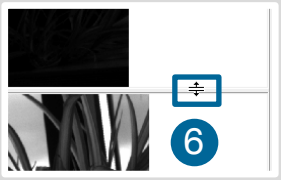


A dropdown menu 3 helps to select a ***View Window***. If there are more view windows than can be displayed on the desktop, you may select individual ***View Windows***.

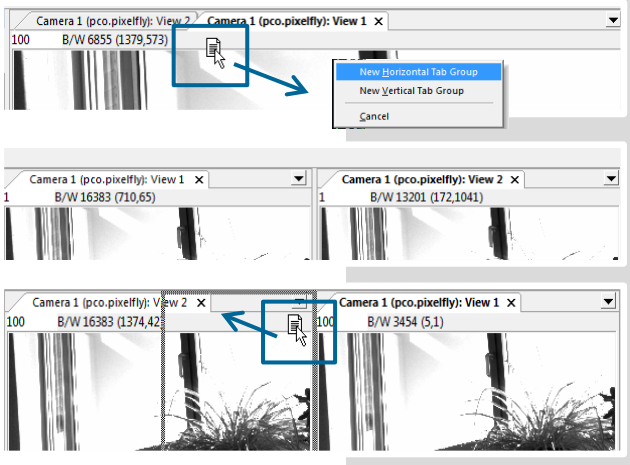
Split View Window



The ***View Window*** can be ***Split***. Click ***Window*** → ***Split*** 4 and a split cross is shown. Adjust the size of the splitted window elements by grabbing and dragging the dividing lines 5.
The main reason for this function is to view four sections of the image in one view. Choose the ***Zoom±*** function to zoom in the image (first turn off ***Stretched View***, see **6.9.8**)
To undo the ***Split***, double-click on the dividing line (after symbol 6 is visible).



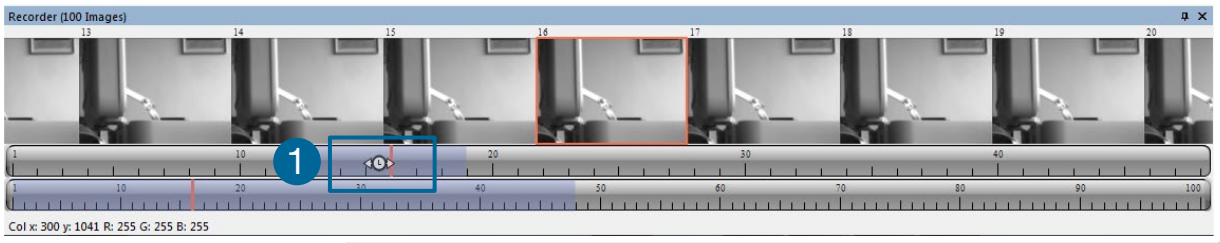
New Horizontal / Vertical Tab Group



To view two tabs side by side or arranged one above the other just drag a tab and Camware will ask you whether you want to create a ***New Horizontal / Vertical Tab Group***. Undo this by draging the tab back to its former position.
This also applies for ***View Windows*** of several cameras.

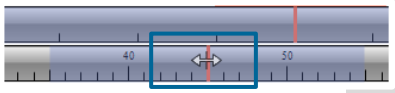
6.7 RECORDER (IMAGES)

Once recording is done, small preview images (thumbnails) are built and displayed automatically in the ***Recorder (Images)*** docking window. It takes some time depending on the performance of your computer system and the interface used.

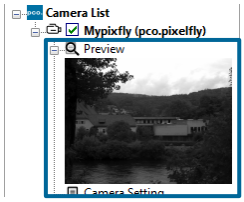


Clicking (left mouse button) within the upper scale bar 1, adjusts the number of images shown by moving the mouse left or right.
In this scale the minimum is 20 images and the maximum is half of the recorded images

Quick Scrolling



Scroll through the thumbnails by dragging the orange bar with the mouse or by mouse wheel while the cursor is over the image number bar.

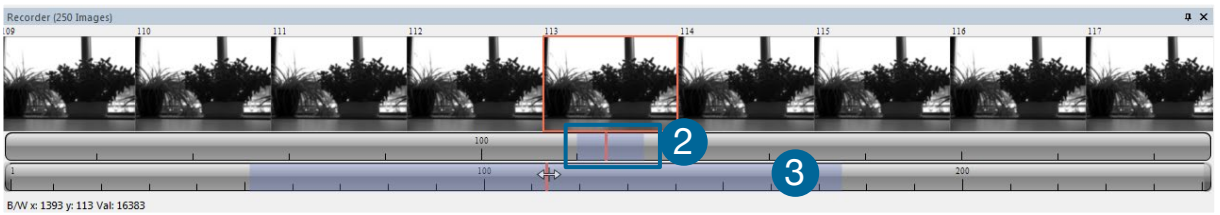


While quick scrolling, the ***Preview Window*** displays the active image sequence, allowing to scroll through the image sequence and showing the images in the ***Preview Window*** forwards or backwards. The ***View Window*** will not actively show live images during quick scrolling (only in normal scrolling speed by mouse-wheel).

Thumbnail Image

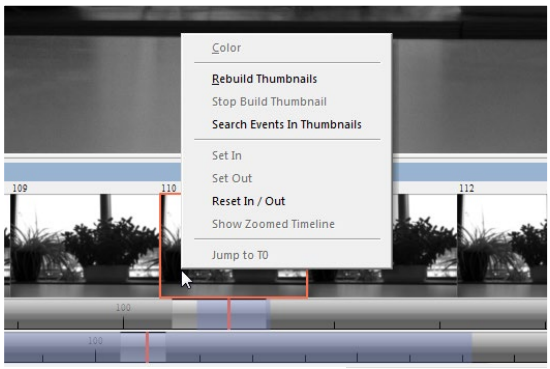
Clicking on a thumbnail image it will be shown in the ***View Window***. Scroll via mouse wheel through the thumbnails.

The upper blue bar 2 matches with the number of displayed thumbnails. The lower blue bar shows the range of the upper scale in relation to the whole record.



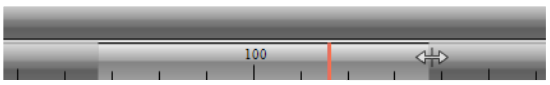
The second scale shows the total number of recorded images. It allows fast scrolling through the recorded images 3.

Right–Click Menu

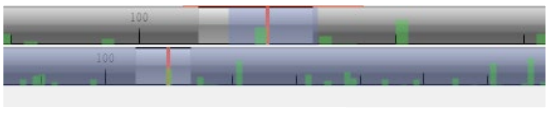


Right-click on thumbnails.
Allows to rebuild all thumbnails and to search for events.

Furthermore, the ***Set In / Out*** enables to set values for a sequence, which can be played via play button. ***Reset In / Out*** discards these settings.
Set In / Out is active: if you save / export your images, only the selected ones are saved / exported (see **6.9.2**).

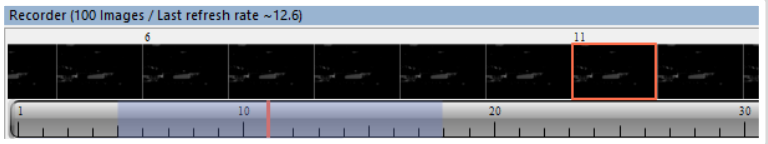


The light gray area in the upper scale shows an ***In-Out*** example area. To define a new area: just right-click on the start and end frame in one of the scales.
The ***In*** image must be left of the red bar, the ***Out*** image to the right of the red bar.
Adjust the ***In / Out*** area by holding down the left mouse button and slide the borders to increase / decrease.



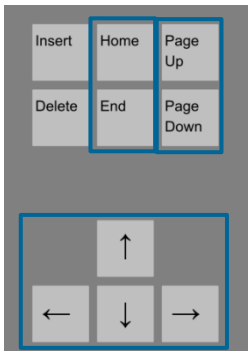
Search Events in Thumbnails: detected events are shown as green bars.

Too Dark or Bright Thumbnails



If thumbnails are too dark or too bright, right-click in ***View Window*** (see **6.9.8**) and select ***Auto Range Peak*** or ***Auto Range Crop***. Then right-click on a thumbnail image and select ***Rebuild Thumbnails***. Now the thumbnail images should conform the ***View Window***.

Keyboard Scrolling



Use your Keyboard to scroll through the Images

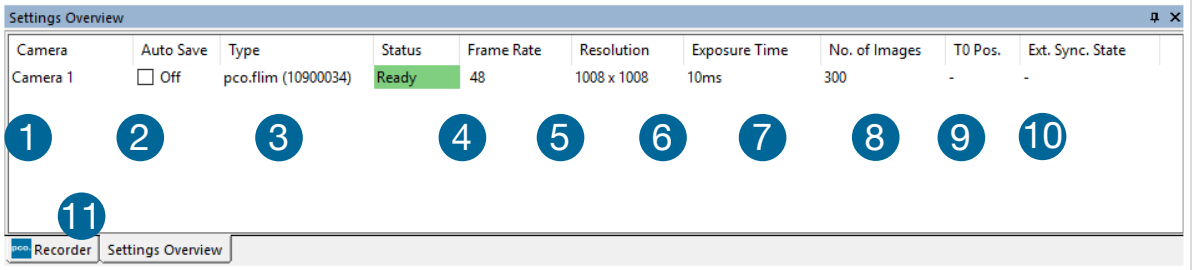
Page up / down keys: 10 images up or down.

Arrow keys: quick scrolling through the images.
Advantage: fluent video playback in the ***View window*** (forwards or backwards).

Home / Pos1 key: first image.

End key: last image.

6.8 SETTINGS OVERVIEW



Settings Overview shows the most important parameters of your camera(s) at a glance. For more than one camera connected, each camera and its parameters are listed.

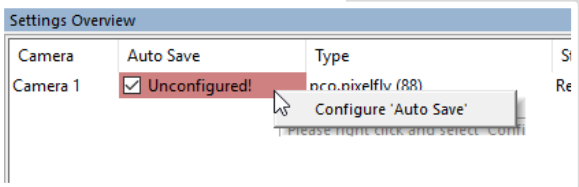
The parameters can only be changed using **6.3 *Camera Properties***.

Switch easily between the ***Recorder (Images)*** section and the ***Settings Overview*** 11.

No.	Function	Description
1	Camera name	Name
2	Auto Save	Off , Unconfigured (red), OK (green)
3	Type	Camera type and serial number
4	Status	Ready or Recording Green background: Images are in memory
5	Frame rate	Currently selected frame rate
6	Resolution	Resolution in pixels
7	Exposure time	Selected exposure time
8	Number of images	Number of images to be recorded
9	T0 Position	Not available
10	Ext. Sync. State	Not available

6.8.1 AUTO SAVE

Auto Save helps to save recorded images or sequences in an easy way. There is no need to save each image / sequence separately from each connected camera. Therefore this function is very useful if you use more than one camera. Once configured ***Auto Save*** allows acquiring and saving as many images / sequences as needed during your experiment. This function stores ***RAW*** (e.g. b16, TIFF) and ***Export*** (compressed e.g. AVI, JPG) files.
Standard file save see ***File*** menu **6.9.2**.
Explanations are shown in the info text window at the bottom of the menu.



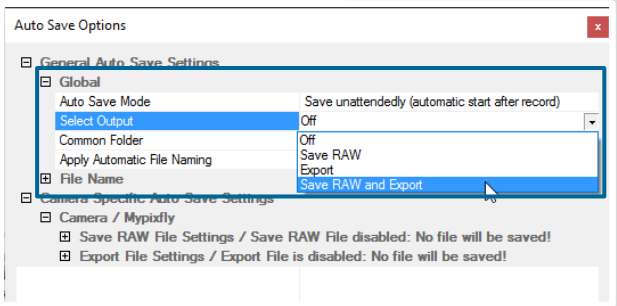
Enable ***Auto Save*** by clicking on the check box. The text changes to ***Unconfigured!*** (red background).

Right-click on the ***Unconfigured!*** field and click on ***Configure 'Auto Save'***. The ***Auto Save Options*** dialog is displayed.

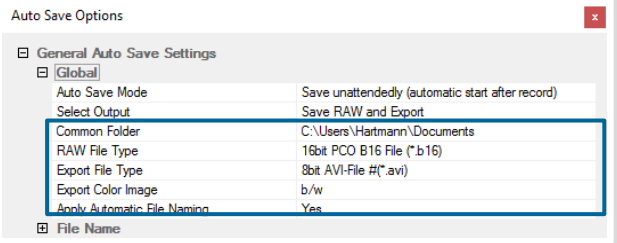
General Auto Save Settings

Global

Auto Save Mode: two different modes are available, ***Save manually*** and ***Save unattendedly***.
The ***Save manually*** mode allows to store RAW images and export images after a recording session, when hitting the ***ALT and D*** keys. This allows to cut the image sequence in the ***Recorder Toolbar*** before saving.
The ***Save unattendedly*** mode enables to download all RAW images and to export the complete image sequences of all cameras immediately after an active recording is stopped.

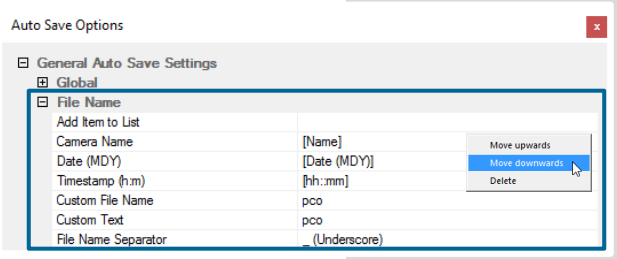


Select Output:
Off: Auto Save is deactivated
Save RAW: only 16 bit RAW files are stored (b16, PCORAaw-File, MultiTif-File, tiff)
Export: only compressed files are stored (bmp, jpg, tiff, avi, mpeg, wmv)
Save RAW and Export: RAW and compressed files are stored simultaneously



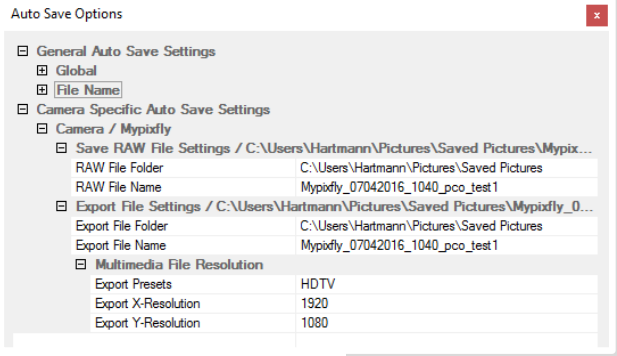
Common Folder: select main folder for stored files
RAW and ***Export File Type:*** select the type of RAW and compressed file
Export Color Image: select to export color images (only for color cameras)
Apply Automatic File Naming: if set to ***Yes***, stored files are automatically named by Camware according to your automatic file name settings.

File Name



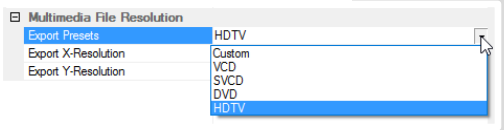
Set file name individually by adding or deleting items. Position these elements as needed.

Camera Specific Auto Save Settings



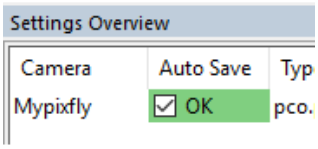
Configure camera specific settings for each connected camera.

Save RAW File Settings: set ***RAW File Folder*** and ***RAW File Name*** (if not set to automatic file naming).
Export File Settings: set ***Export File Folder*** and ***Export File Name*** (if not set to automatic file naming).



Multimedia File Resolution: set predefined video export resolution or enter a ***Custom*** x- and y-resolution.
Most likely you may have to set the configured resolution of your camera here.

Finish the configuration by clicking ***OK***.

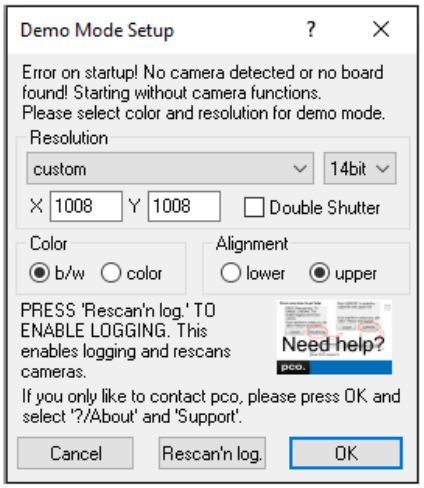


After configuration is finished, ***Auto Save*** status turns ***OK*** (green background).

6.9 CAMWARE MENU TAB & FEATURES

This chapter describes in detail the Camware Demo Mode and the Camware Tabs: File, Camera, Acquisition, View and Window. Furthermore the right-click menu and some additional features are listed.

6.9.1 DEMO MODE



Upon start Camware automatically recognizes the type of the connected and running cameras.

Camware starts in ***Demo Mode***, if your camera is off or no camera is connected.
In this mode all image processing features are available, but all camera settings are deactivated. State in Camware the image type and the ***Demo Mode Setup*** window opens, requesting the corresponding input.

Need Help? Having troubles to run the camera this window will pop up. Follow instructions of chapter **6.9.7**.

Settings to view the b16 files of the pco.flim:

Resolution	Bit	Double Shutter	Color	Alignment
1008x1008	14	no	b/w	upper

Resolution

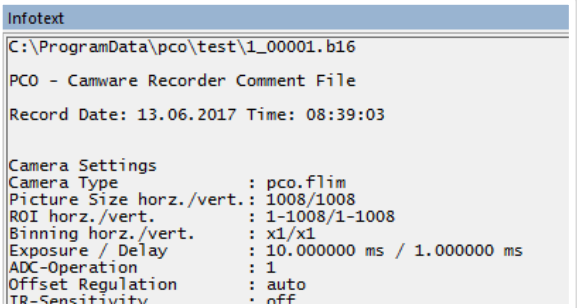
The drop down list shows the PCO camera image sensor resolutions. Select the specific resolution and bit depth of the images to be opened.

Color

Select color mode. (pco.flim b/w only)

Alignment

Adjust whether MSB (most significant bit) aligned (upper) or LSB (least significant bit) aligned (lower) images have been stored.

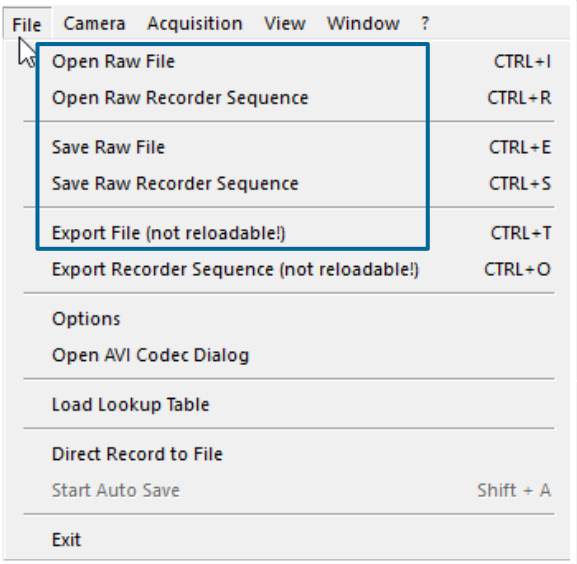


Infotext

The ***Infotext*** is automatically shown in Camware when a stored image sequence is opened.
The ***Camera Settings***, storing location and ***Record Date*** are listed in this file.

Infotext can be activated in the ***View*** menu **6.9.5** at any time.

6.9.2 FILE MENU



NOTE
Be aware of the different storage characteristics of the formats, for example *.bmp - the bitmap format stores 8 bit values only and therefore the image content of a 16 bit image is reduced, if stored as bitmap.

Open Raw File

This command imports a single image into the active image window. Only files with the extension and format ***.b16 (=PCO proprietary binary image format)*** and *.tif (16 bit TIFF image format) can be imported. If the recorder is enabled, each imported image is transferred to the buffer shown in the picture number. The image itself is fitted to the current image size. If the recorder is disabled, the current image sizes is set to the parameters of the imported image.

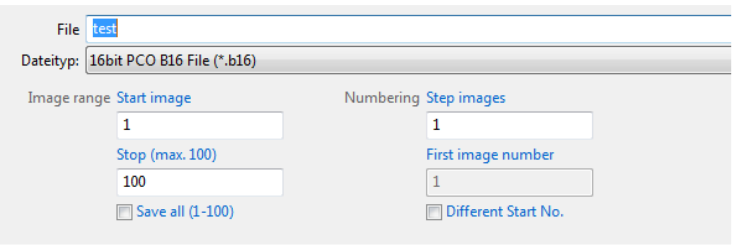
Open Raw Recorder Sequence

Imports a sequence of images. If more than one camera is connected and an image window is open, the sequence is loaded to the active window. If no image window is open, the images are loaded to camera #1. This command opens the ***Open RAW File Recorder*** dialog box. Only files with the extension and the format *.b16, *.pcoraw, *.tif and multi tif can be imported.

Save Raw File

Saves the image displayed in the active window and opens the Save file dialog. The image file can be saved in ***16bit *.b16*** and ****.tif*** format. If more than one camera is connected, it is possible to save all current images by selecting Export all images in the ***Save RAW File*** dialog box. This feature saves one image of each active camera within one step (it is not necessary to repeat the save process for each camera). The Save command is not available, if no image window is open. For ***Auto File Save*** see **6.8.1**

Save Raw Recorder Sequence

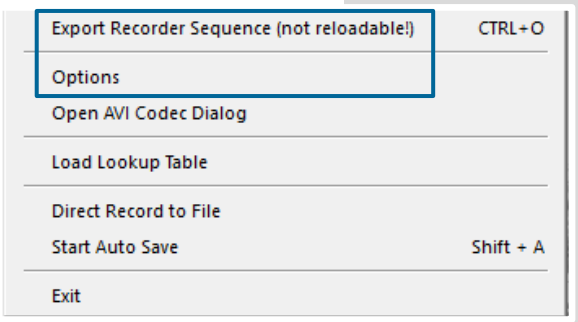


This command should be used to save or export image sequences. If more than one camera is connected and an image window is open, the record of the active window is saved. The command opens the ***Save Recorder*** file dialog box. It is possible to select the number of saved images, to step images and to choose the first image number. For ***Auto File Save*** see **6.8.1**

Export File

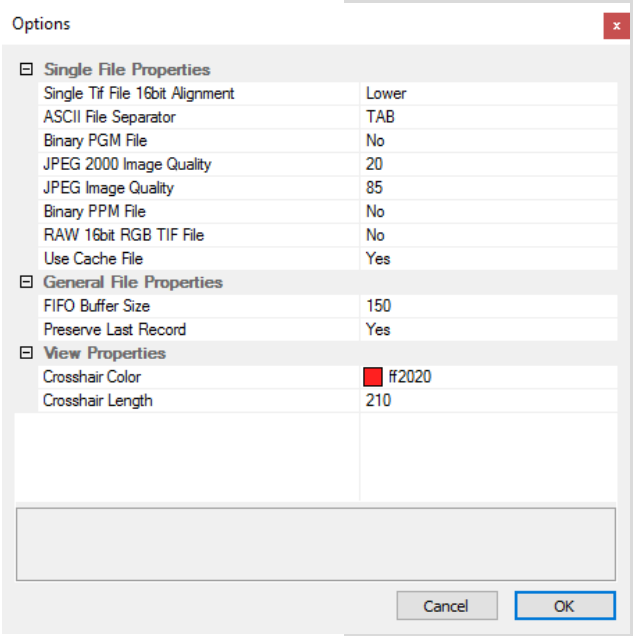
This is not reloadable! Exports the image of the active image window. This command opens the ***Export Image*** dialog box. Files with the extensions fts, tif, bmp, asc, jpg, and jp2 can be exported. This topic is not visible, if no image window is open. For ***Auto File Save*** see **6.8.1**

Export Recorder Sequence



This is not reloadable! Exports a sequence of images. If more than one camera is connected the image record of the currently open window will be saved. If no image window is open the ***Export Recorder Sequence*** menu does not appear. This command opens the ***Export Recorder*** box. Files with the extensions fts, tif, bmp, asc, avi, mpg, jpg, jp2, and wmv can be exported (see Appendix **A4**). For ***Auto File Save*** see **6.8.1**

Options



Single File Properties

Single Tif file 16 bit Alignment: Upper / Lower
ASCII File Separator: select a separator for the values in the ASCII file. Select: TAB, SPACE, SEMICOLON, COLON, COMMA, HYPHEN, SLASH, BACKSLASH.
Binary PGM file: set the format of the PGM (portable gray map) file. Select: Yes, No.
JPEG 2000 Image Quality: set compression from 20 to 100%.
JPEG Image Quality: set compression from 20 to 100%.
Binary PPM File: set format of the ppm (portable pixmap) file. Select: Yes, No
RAW 16bit RGB TIF File: save RAW TIF without color balance. Select: Yes, No.
Use Cache File: caches image data on disc for a camera with camera internal memory. Select: Yes, No (n/a for pco.flim)

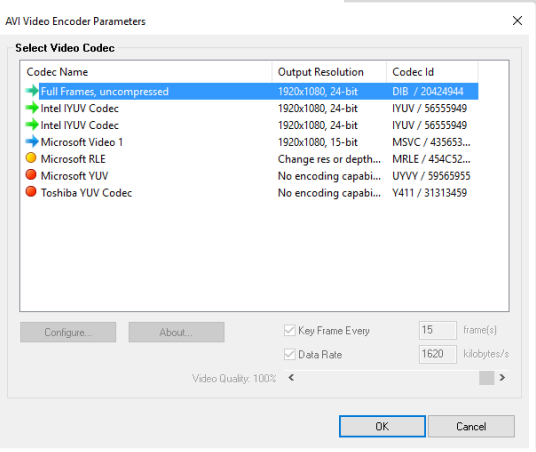
General File Properties

FIFO Buffer Size: set the FIFO buffer size in number of images. This avoids gaps during file write delays. Usually it is set to 150.
Preserve Last Record: preserves current recorded images. When set, the user will be asked whether to really start a new record or to close.

View Properties

Crosshair Color: set the crosshairs color for Save ROI and Line Profile.
Crosshair Length: set the crosshairs length in pixel.
Activate crosshairs: see chapter **6.9.8**

Open AVI Codec Dialog



Using Auto File Save and selecting AVI for video output affects stored video sequences.
You only need to set this option, if you use ***Auto File Save*** see **6.8.1**

Select the (compression) codec you want to use for stored sequences. All installed codecs are listed here.

Load Lookup Table

This feature assigns pseudo colors (LUT) to a monochrome image. Either select one of the four predefined or create your own. The result is shown in the color view window.

Direct Record to File

Presets a certain number of images to be stored. If the camera captures images faster than the computer can save to disk, you lose images. Images display doesn't interfere with the record process.

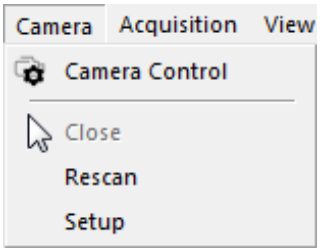
Start Auto Save

Only available if ***Auto Save*** is activated (see **6.8.1**)

Exit

Exits the program and closes all channel dialog windows. Window positions, settings and sizes are stored in the Microsoft Windows registry and will be loaded again at next start-up.

6.9.3 CAMERA MENU



Camera Control

Opens the camera control window (see **6.2**).

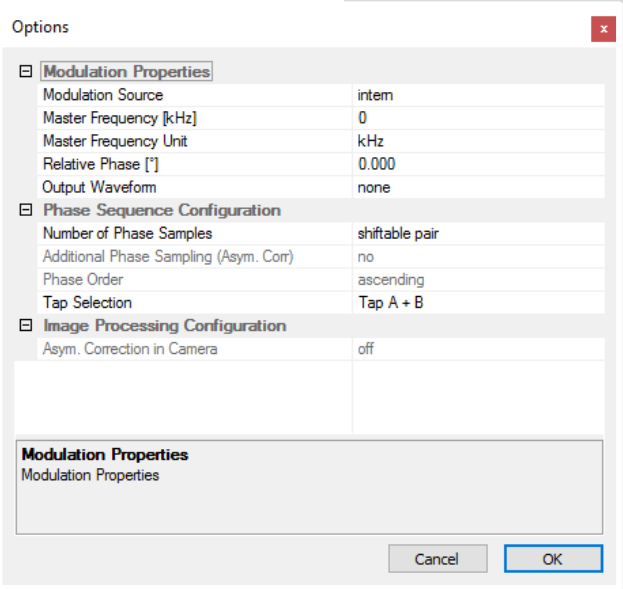
Close

Disconnects camera and switches Camware to Demo Mode. In case of multiple cameras, all cameras must be closed for Camware to switch to Demo Mode.

Rescan

Disconnects and reconnects all cameras.

Setup



Specific settings for recording with the pco.flim (explanation see **6.3.7**).

Modulation Properties

Modulation Source: intern / extern
Master Frequency (kHz): 0 – 50 MHz
Master Frequency Unit: kHz / MHz
Relative Phase: 0 -360°
Output Waveform: none / sine wave / square wave

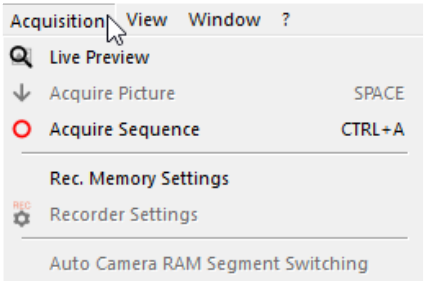
Phase Sequence Configuration

Number of Phase Samples: shiftable pair / 2 / 4 / 8 / 16
Additional Phase Sampling (Asym. Corr): no / yes
Phase order: ascending / opposite
Tap selection: Tap A+B / Tap A / Tap B

Image Processing Configuration

Asym. Correction in Camera: off / on

6.9.4 ACQUISITION MENU



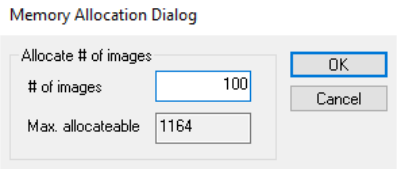
Live Preview

Live Preview for quick and easy adjusting and focusing of the camera. The active window will be refreshed. To see another window, simply click on the window.

Acquire Picture (not available)

Acquire Sequence

Starts recording images into the system memory according to Trigger Mode selection (see **6.3.1**). During recording, all camera controls are locked.



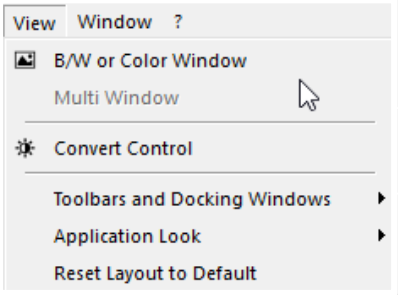
Rec. Memory Settings

This sets the number of images recorded in one sequence. The maximum is defined by approved RAM size.

Recorder Settings (not available)

Auto Camera RAM Segment Switching (not available)

6.9.5 VIEW MENU



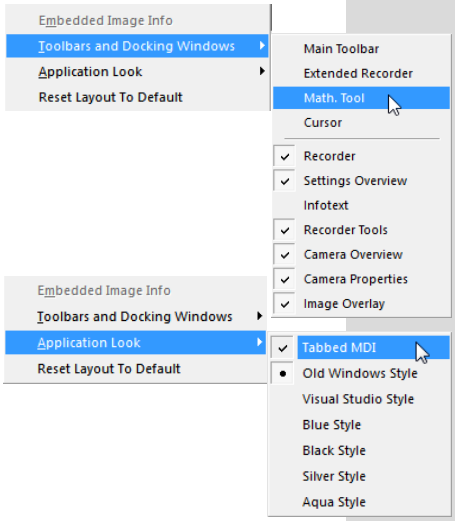
B/W or Color Window

Opens a new ***View window***.

Multi window (not available)

Convert Control

See 6.3.8

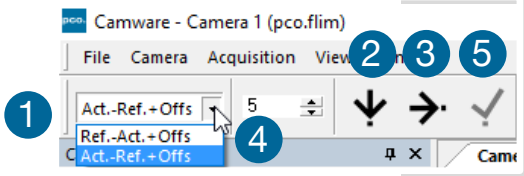


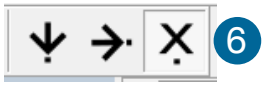
Toolbars and Docking Windows

Standard toolbars of Camware 4 are Recorder/ Recorder Tools / Camera Overview / Camera Properties and Image Overlay.
Additional Toolbars are displayable, but not essential: Main Toolbar / Extended Recorder / Math. Tool / Cursor. See below. For function ***Infotext*** see **6.9.1**

Application Look

Style and look of Camware can be customized; many different style sheets are selectable. The Tabbed MDI function (un)docks the view windows.

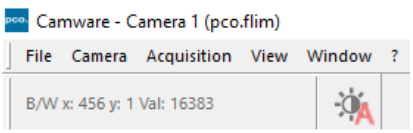




Math. Tool

Calculate the difference between a reference image and the actual image. Activating ***Math. Tool*** every new acquired image is subtracted from the reference image or vice versa:

1. Reference (image) – Actual (image) + Offset or
   Actual (image) – Reference (image) +Offset
2. A reference picture is acquired and copied to reference buffer
3. Last acquired image is copied to reference buffer
4. Add offset to avoid negative values, which would not be visible
5. Enable math function
6. Disable math function



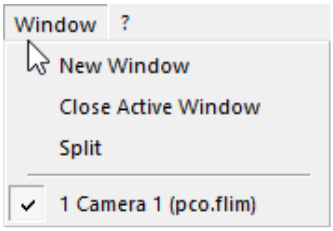
Cursor

Shows position of mouse cursor.
B/W (black/white camera) x-axis: 456; y-axis: 1; Value: 16383 counts

Reset Layout to Default

This resets all your customized changes and restores the default layout.

6.9.6 WINDOW MENU



New Window

A new view window appears.

Close Active Window

Active window closes.

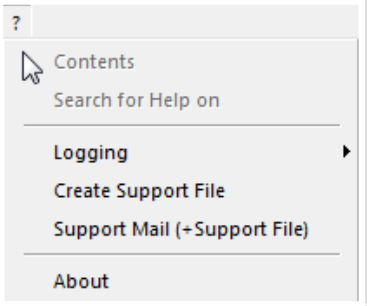
Split

The view window is split in four quadrants.

Camera Overview

Shows all connected cameras, e.g. ***1 Camera (pco.flim)***

6.9.7 HELP MENU



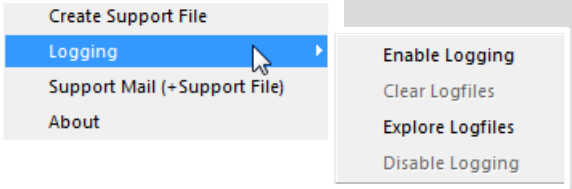
Contents (not available)

Opens the main page of the program help.

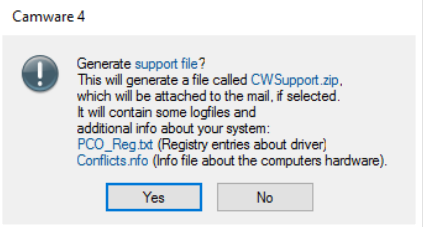
Search for Help on (not available)

Opens an index list for help.

Logging



Enable Logging: activates Camware log files (this cuts down performance)
Clear Logfiles (only visible if logging is enabled): erases all actual log files
Explore Logfiles: opens windows explorer. Log files are formatted as e.g. SC2_Cam.log
Disable Logging (only visible if logging is enabled): disables logging



Create Support File

This will activate the Camware log files. Click ***Yes*** to activate log files. Reboot Camware and your pco.flim.
After log files are activated it is possible to create a support file. Send this file to the PCO support (see **A5.4**).

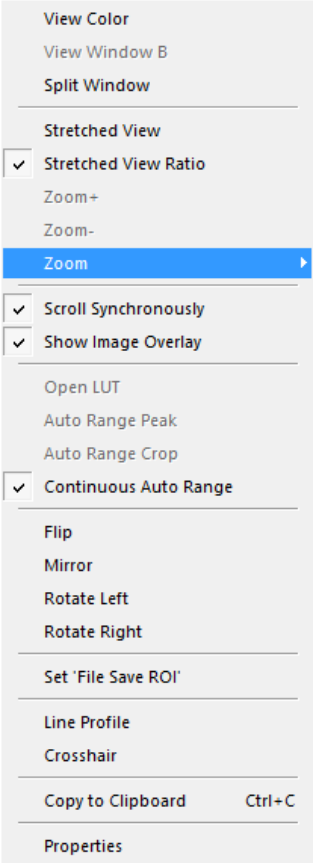
Support Mail

Opens your email-program and the created support file is added automatically as attachment.

About

Shows program version information.

6.9.8 VIEW WINDOW MENU



Right-click in the ***View Window*** to open this menu.

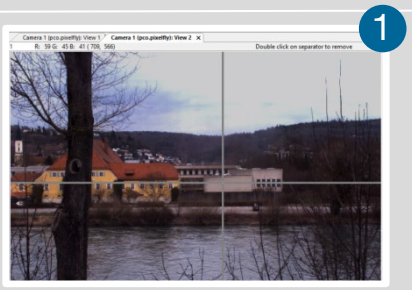
View Color

Color window.

View Window B (not available)

Split Window 1

Splits the ***View Window*** in four quadrants. Double-click on separator to undo.



Stretched View

Image is fitted into the ***View Window***.

Stretched View Ratio

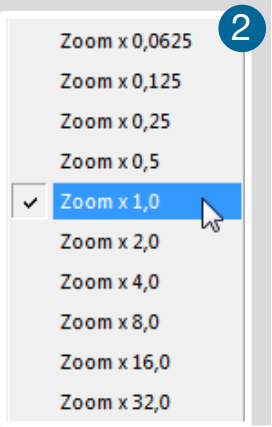
Aspect ratio is maintained.

Zoom +/– 2

Image zoom (only available if ***Stretched View*** is deactivated).

Zoom

Sets the factor of the zoom (from 0.0625 to 32).



Scroll Synchronously

If more than one ***View Window*** is open, you may scroll through all images synchronously (only available if ***Stretched View*** is deactivated on all images).

Show Image Overlay

Activates the overlay see **6.4**

Open LUT

Opens ***look-up table*** file for false-color representation.

Auto Range Peak

Searches for the minimum and maximum 14 bit intensity values of the image. Given these numbers the converter scales the 8 bit display (256) within these two values.

Auto Range Crop

Sets the converter to ignore the extreme intensity values of the image and scales the display in a smaller range. Thus dark or bright light spots, reflections, etc. are cut off.

Continuous Auto Range

(Crop) Enables the automatic min / max function (Auto Range Crop) during record and replay.

Flip / Mirror

Image will be flipped or mirrored.

Rotate Left / Right

Rotates the image in steps of 90°.

Set 'File Save ROI'

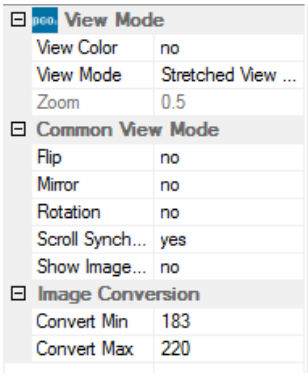
To save just a part of the recorded image (region of interest), draw a rectangle with the mouse. This rectangle is valid for all recorded images and can be dragged at its edges.

Line Profile

Point the mouse where the line should start and left-click. Move the mouse to the desired line end and left-click again. The line may be stretched, shrunk or moved by grasping its end point. A ***Line Diagram*** opens. The graph in the length of the line (units: pixel) is displayed showing the intensity values of the pixels along the red line.

Crosshairs

Activates centered crosshairs. Size and color are selectable see chapter **6.9.2** → ***Options***.
To move the crosshairs drag it by mouse. Reset it to center position by double-click into center of the crosshairs.



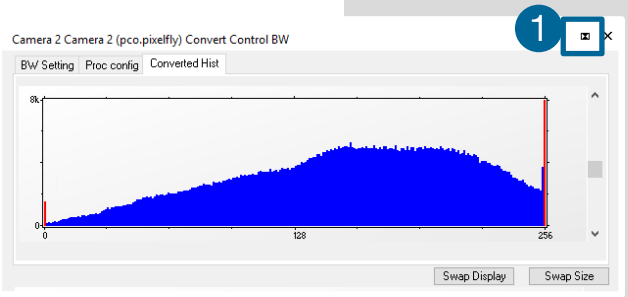
Copy to Clipboard

Copies the actual image to clipboard.

Properties

Displays the current settings for ***View Mode*** / ***Common View Mode*** and ***Image Conversion.***

6.9.9 ADDITIONAL FEATURES



Fold Up Window

The ***Convert Control*** window can be minimized / folded 1 . Move the pointer over the bar and the window will unfold again 2.

Setting Contrast Area by Mouse

Control the minimum and maximum values used for the conversion from 14 bit to 8 bit with the mouse. Move the mouse cursor into a region which should be shown with maximum contrast. Press the shift and the left mouse button. Hold down the mouse button while increasing the size of the rubber band window with mouse moves. After releasing the mouse button the coordinates of the rubber band window act as a border for calculating the minimum and maximum values.

Setting new ROI by Mouse

In the same manner you can setup a new region of interest (see ***ROI* 6.3.2)** for the camera. Press the CTRL (STRG) button and drag an area with the left mouse button instead of the shift button. The coordinates of the rubber band window are used for calculating a new region of interest, which will be adapted to the camera capabilities automatically. Reset the ROI to maximum by pressing the CTRL (STRG) button and the right mouse button.

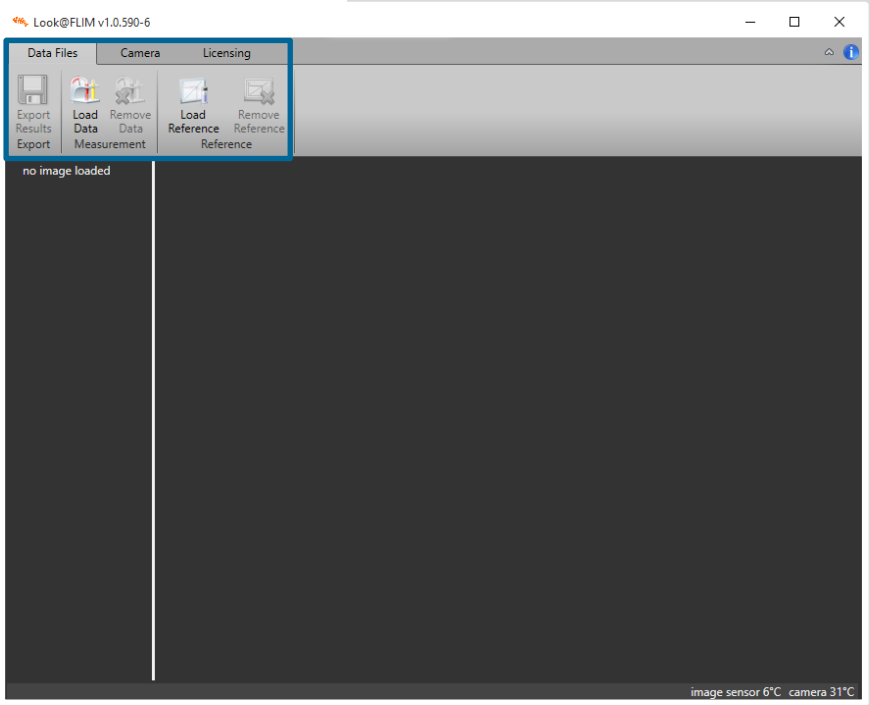
Short Cut List

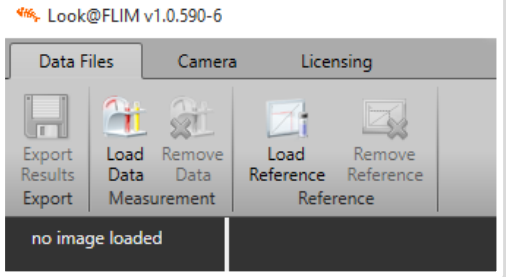
- Start / Stop record: ENTER
- Acquire Picture: SPACE (Soft Trigger mode)
- Acquire Sequence: STRG + A
- Auto Save: ALT + D
- Export File: STRG + T
- Export Recorder Sequence: STRG + O
- Open Raw Image File: STRG + I
- Open Raw Recorder Sequence: STRG + R
- Save Raw Image File: STRG + E
- Save Raw Recorder Sequence: STRG + S

7. LOOK@FLIM SOFTWARE

The ***Look@FLIM*** software is a tool for image recording and analysis with a pco.flim camera system.
Install the ***Look@FLIM*** software from the PCO usb flash drive or download the latest version from this website.

7.1 START





Double-click on the icon starts the program, the start window appears with following topics.

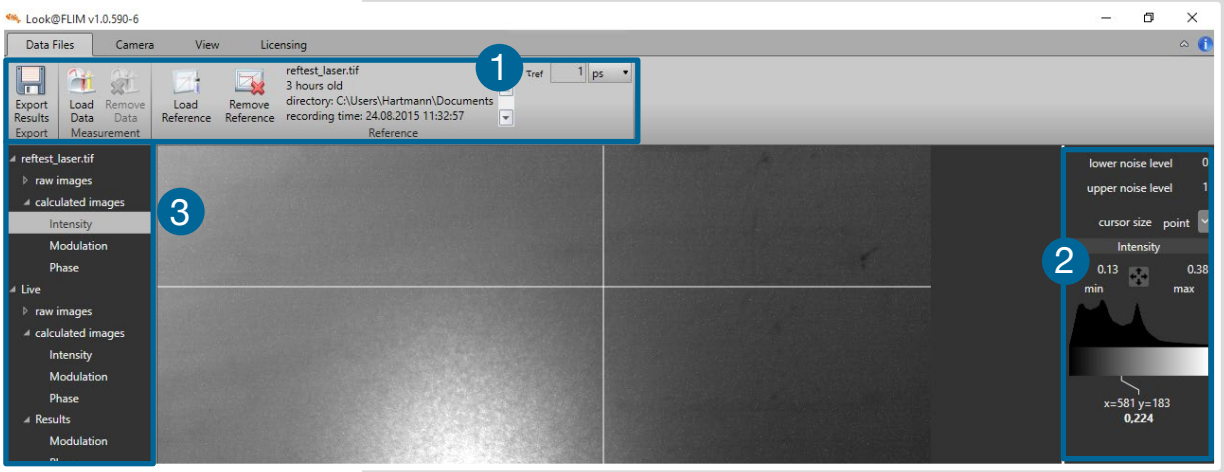
The menu tabs are: ***Data Files*** (see **7.2**) – ***Camera*** (see **7.3**) and ***Licensing*** (see **7.5**).

7.2 DATA FILES MENU

The ***Data Files*** menu processes stored image data sequences. It distinguishes between reference data, which can be loaded with the ***Load Reference*** button and measured data, which can be loaded with the ***Load Data*** button. Depending on the selection, additional information is required. Usually, before measured data can be evaluated, a reference data set should be loaded.

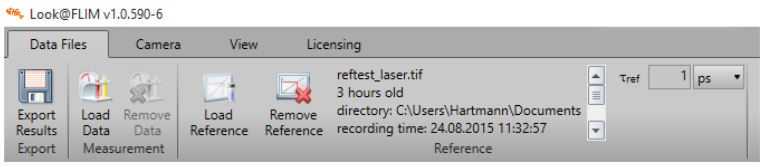
7.2.1 LOAD REFERENCE

The main window has three operation areas: menu bar 1, control window 2 and file / data tree window 3.

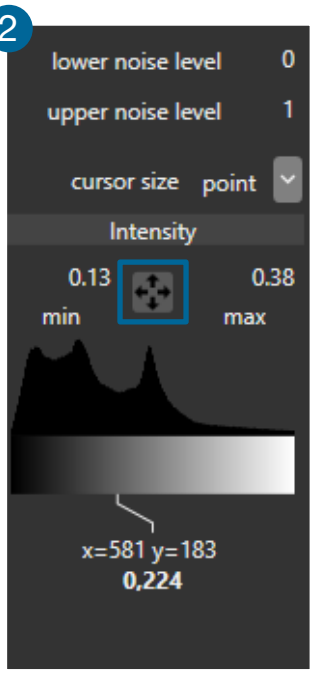


The main window shows the calculated intensity image in a normalized way, such that the value range is 0.0 – 1.0. Within the menu bar some new fields appear after loading the reference data file.

Menu Bar 1



Next to the ***Load Reference*** button, a ***Remove Reference*** button appears. It removes the reference data from the program memory. The filename of the currently loaded reference file is also shown. Below the filename is a checkbox for asymmetry correction. Here, the user has input whether the reference image sequence contains a second sequence for asymmetry correction. The default value of the checkbox is checked, so the software expects sequences containing additional images for asymmetry correction.
Further, for the conversion of phase angle and modulation index values into valid fluorescence lifetime distributions, it is necessary to instruct the software which modulation frequency [f_{mod}] has been used and which is the known lifetime [τ_{ref}] of the reference luminophore. The values can be typed in and the drop-down list beside the number fields allows to select the appropriate unit.



Control Window 2

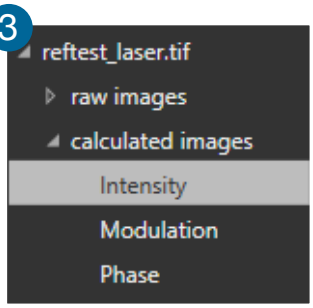
The control window (column) allows for the adjustment of the processing and delivers results and information of the active image.

Lower / Upper Noise Level: on top there are two number fields which define so-called noise levels. Before the images are processed, the intensity image is pixel-wise compared with these levels, and pixels which are out of the given range (value is smaller than the ***Lower Noise Level*** or larger than the ***Upper Noise Level***) are ignored and not used for calculation and processing. In the resulting images this is indicated by setting the pixel values to black or zero. Since the levels refer to the normalized intensity image, the range is always from 0.0 – 1.0.

Cursor Size: the drop-down list right to the cursor size designator allows the selection of single pixel readout or the average value of a field of (3 x 3), (9 x 9) or (21 x 21) pixels.

Min / Max: below the noise levels there are two number fields which are named ***Min*** and ***Max*** with a push button with four arrows between them. These values scale the display of the image pixel values according to the value range of the active image (for intensity image from 0.0 – 1.0, for raw data from 0 – 16383, for lifetime distribution from 0.0 – x in [s] etc.). The settings change from active image to active image. The push button performs a sophisticated auto scaling which can lead to undesired behavior in case the noise levels were not properly chosen.

Histogram with scaled grey or color wedge and cursor readout: the histogram below the minimum / maximum values shows the distribution of the values of the active image within the defined minimum / maximum range. Below the histogram the corresponding grey or color wedge is displayed, which is like a horizontal grey or color bar. The line, which points down to further values, is the readout of the cursor, located in the active image. The values given below the histogram correspond to the x–y–coordinates of the cursor in the active image, and the bold value is either the cursor readout or the average of the selected area around the cursor, which is defined by the cursor size selection. It can be a single pixel value or the average of a field of (3 x 3), (9 x 9) or (21 x 21) pixels. The standard deviation is given as σ value.



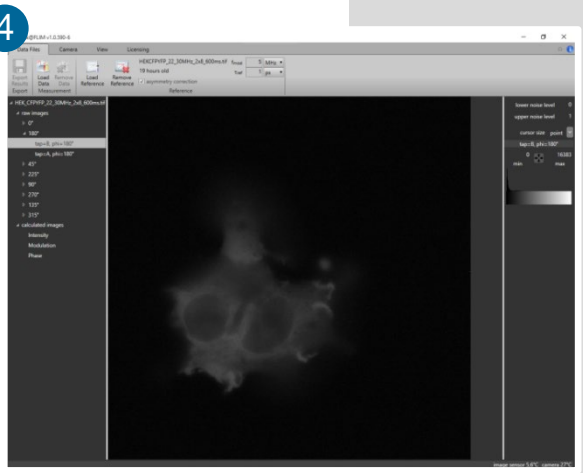
File Tree Window 3

In this window (column) all loaded image files, processed images and live images are listed and can be selected. The selected image is displayed as active image in the central window, and the control and readout values from the control column relate to this active image. Each of the raw images can be selected as well as the calculated and referenced images, if exist, and can be activated for display.
First the filename is shown or live if it is a live image. Then all the ***Raw Images*** of the sequence can be selected if the list is unfolded by clicking on the arrow.

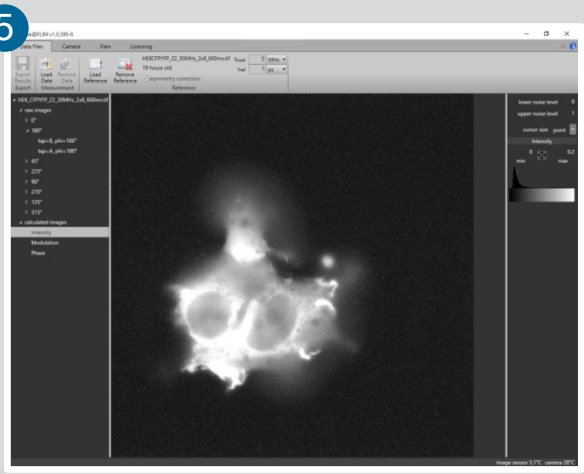
Below the raw images the relevant ***Calculated Images*** are listed: ***Intensity*** – ***Modulation*** (index) distribution – ***Phase*** (angle) distribution.

4 ***Raw Images***
5 ***Intensity***
6 ***Modulation*** index
7 ***Phase*** angle

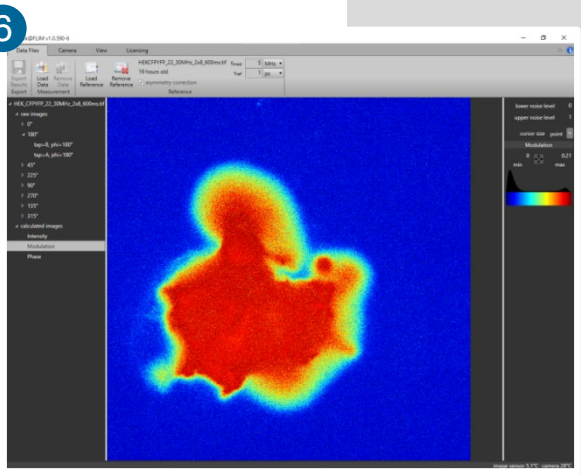
4



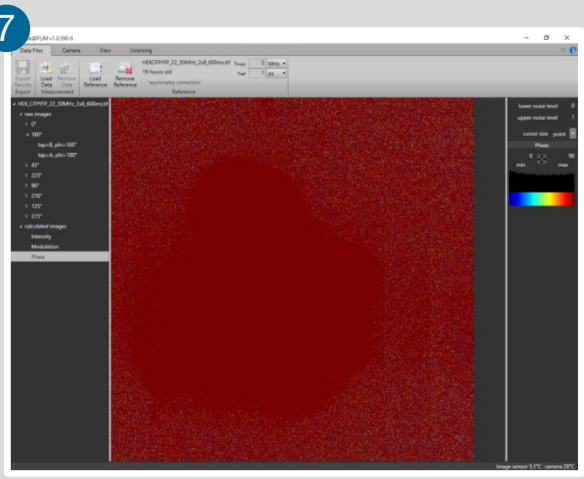
5



6



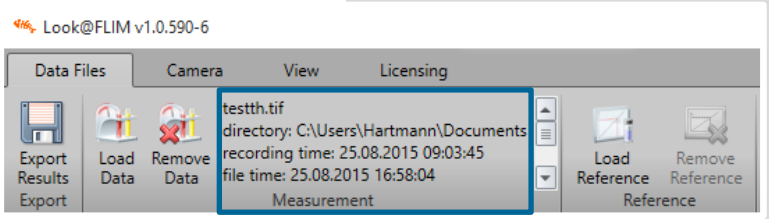
7



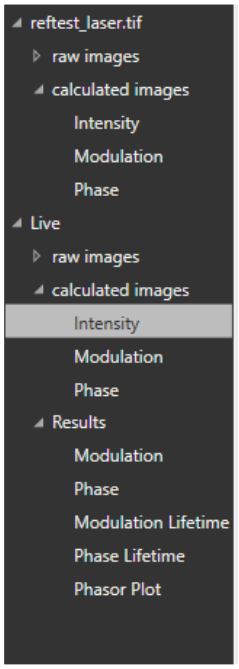
7.2.2 LOAD DATA

Loading a data file adds some elements to the menu bar.

Menu Bar Add Ons



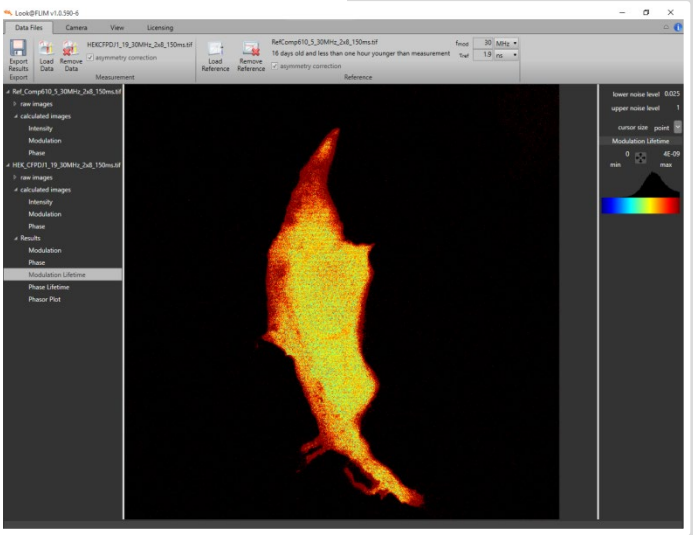
Loading reference data, the filename of the measured data appears and a checkbox is added to tell the software whether the data images have been recorded with a second sequence for asymmetry correction or not. As default the software expects software expects sequences with images for asymmetry correction. An additional icon appears for the removal of the data from the program memory.



File Tree Window

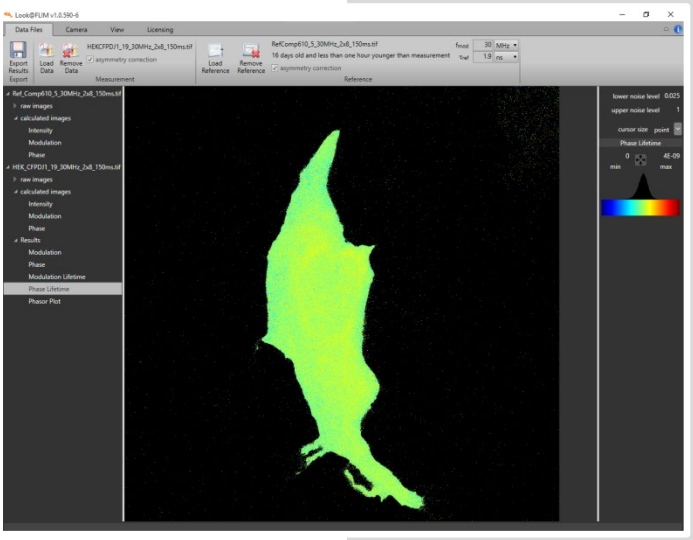
The files or images from the ***Load Data*** event are shown in the file tree window. The raw images can be selected, as well as the calculated images, as for the reference data, and a new section appears called ***Results***.

In the ***Results*** the calculated and referenced images are presented. The modulation index and the phase angle distribution images have now referenced values, which can be converted into luminescence lifetime distributions each. Further, the modulation index as the magnitude of a vector or phasor and the phase angle as its angle can be displayed in one plot, the phasor plot, which is a type of Nyquist plot.



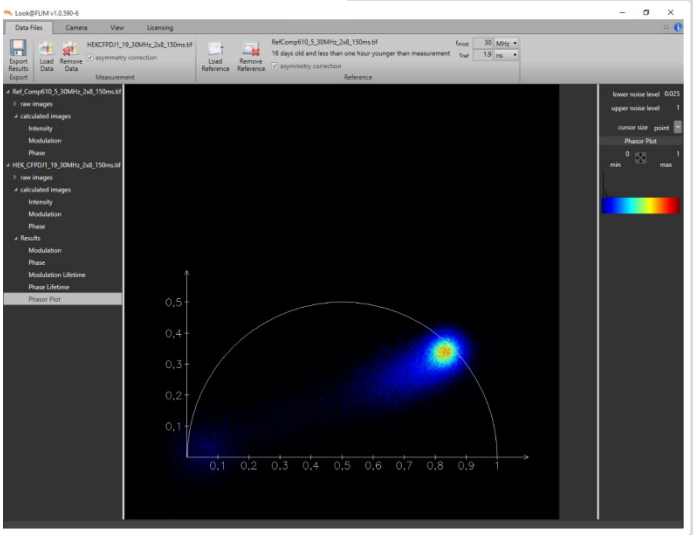
Modulation Lifetime

This is the luminescence lifetime distribution derived from the referenced distribution of the modulation indices per pixel.



Phase Lifetime

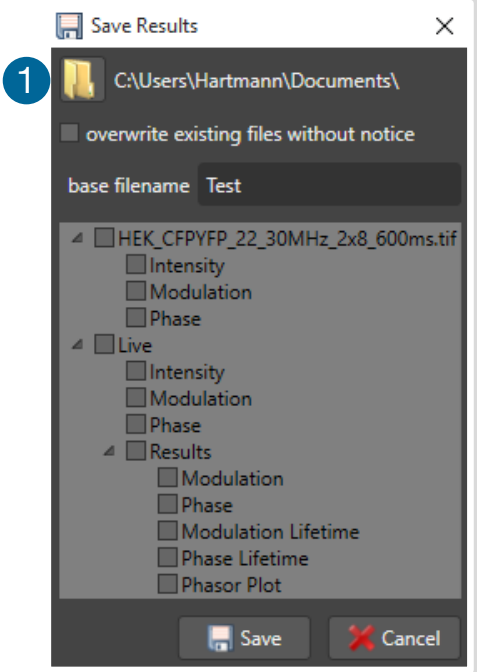
This is the luminescence lifetime distribution derived from the referenced distribution of the phase angle per pixel.



Phasor Plot

This is a Nyquist plot where each pixel represents a vector with a magnitude given by the referenced modulation index and an angle towards the x-axis given by the referenced phase angle.

7.2.3 EXPORT RESULTS



When the ***Export Results*** button is pressed, the following dialog window pops up:

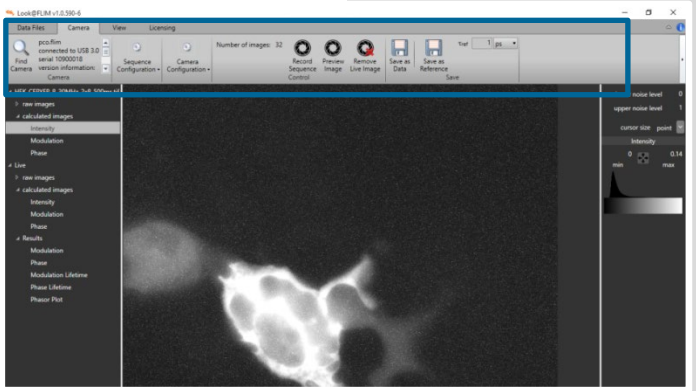
The folder button 1 selects a different directory for storing the results with an additional file manager dialog. In case the calculations have been repeated with another reference file, and the old results are no longer required, the checkbox ***Overwrite Existing Files Without Notice*** may be ticked. Old files with the same filename are overwritten.
The suggested base filename for the result files is the original data filename without extension. It can be edited and changed by an input into the text window beside ***Base Filename***.

The file tree window shows all calculated images:

Reference:	***Intensity – Modulation – Phase***
Data:	***Intensity – Modulation – Phase***
Results:	***Modulation – Phase – Modulation Lifetime – Phase Lifetime – Phasor Plot***

If the higher level checkbox is ticked, the lower level files are all ticked automatically. All ticked files / images will be stored as TIFF files containing floating numbers, so they become images with non-typical image content. Software like Matlab and ImageJ can process these value distributions. The filenames are created as the combination of the base filename plus the corresponding name in the file tree window.

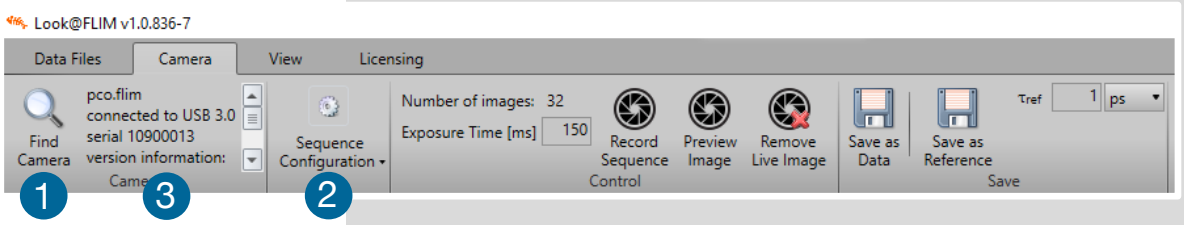
7.3 CAMERA MENU



The ***Look@FLIM*** program opens with the ***Camera*** menu window if an active camera is connected.

7.3.1 MENU BAR

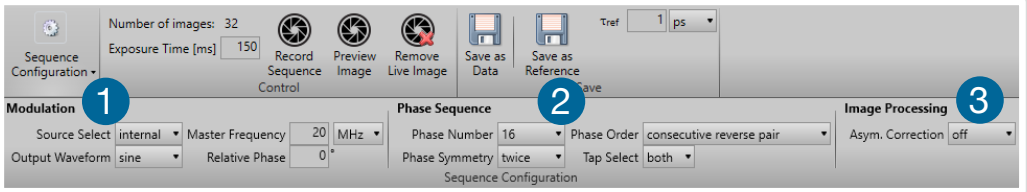
The menu bar contains:



7.3.2 FIND CAMERA

The ***Find Camera*** button ❶ is helpful, in case the camera is switched on after the program has been started or if for some reason the camera has been switched off or disconnected while the program is running. If pressed, the program scans for connected and available cameras.
In case the program found a camera, a text beside the ***Find Camera*** button describes which camera is connected via which interface and the serial number (which is relevant for the licensing).
In this case: a ***pco.flim connected to USB 3.0 serial 1090013*** ❷.
Next to the right, the ***Sequence Configuration*** button ❸ follows, which serves to unfold different configuration tabs.

7.3.3 SEQUENCE CONFIGURATION TAB



All settings for frequency domain FLIM measurement control are made in this dialog box.

NOTE
Relative Phase:
This feature is only available in the special case the phase number (see below) has been set to manual and the camera is in recording state.

Modulation 1

The relevant settings for the internal direct digital synthesizer, the modulation signal generation, are made here.
Source Select: the dropdown list proposes an ***Internal*** modulation signal generation or an ***External*** modulation signal input.
Output Waveform: the dropdown list proposes ***None*** for no signal output, ***sinusoidal*** for a sinusoidal output signal or ***Rectangular*** for a rectangular output signal shape.
Master Frequency: the input window allows for the numerical input (as integer) of the desired modulation frequency in the range from 5 kHz to 40 MHz, while the dropdown list allows to choose the corresponding frequency unit: Hz, kHz and MHz.
Relative Phase: this allows the manual control of the phase angle shift between image sensor modulation signal and the modulation output signal to an external device. The values should be in degrees.

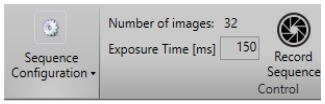
Phase Sequence 2

Phase Number: select in the dropdown list the number of phase angles per modulation period (360°), which shall be measured in a data sequence for the reconstruction of the sinusoidal or higher harmonic signal, which is detected: 2 – 4 – 8 – 16.
Phase Symmetry: select in the dropdown list ***Singular*** or ***Twice***. This is the main selection if a single sequence (singular) shall be recorded or a second sequence (twice) is appended with opposite phase angles in the taps A and B, if the asymmetry correction should be performed by either the camera or the PC.
Phase Order: this selection is only relevant if the ***Phase Symmetry*** is set to ***Twice***. It defines whether the inverse (opposite) measurements are made as ***Consecutive Reverse Sequence***, such that a first normal sequence is recorded and then the reverse (appended) sequence, or as ***Consecutive Reverse Pair***, which means that the reverse measurement is made directly after the corresponding dual tap read out image.
Tap Select: this dropdown list states whether ***Tap A***, ***Tap B*** or ***Both*** taps are readout.

Image Processing 3

Asymmetry Correction: choosing ***On***, the camera performs the asymmetry correction internally. If ***Off*** is selected from the dropdown list, the camera does not perform the asymmetry correction internally. Make sure the asymmetry correction checkbox in the **LOAD DATA** section of the Look@FLIM program is unchecked, when loading data recorded and saved with the internal correction option set to ***On***, since the correction has already been made by the camera.

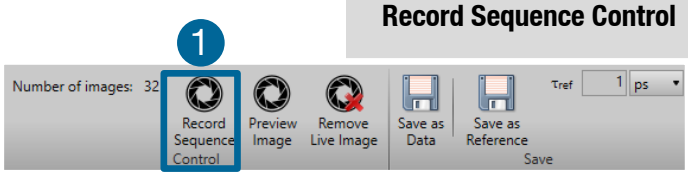
7.3.4 EXPOSURE TIME / NUMBER OF IMAGES

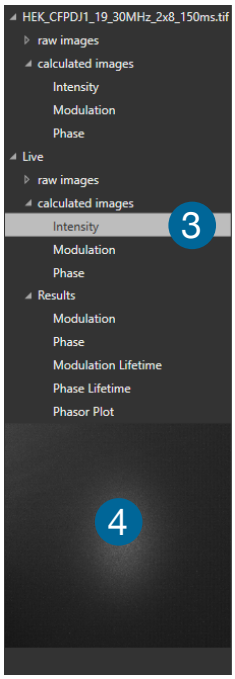


Exposure Time [ms]: with the numerical input into this window, the exposure time for a double (both taps) or a single (tap A or tap B) image is set in milliseconds.

Number of Images: displays the number auf recorded images; can bet set by the SEQUENCE CONFIGURATION TAB **7.3.3**

7.3.5 FUNCTION BUTTONS





Record Sequence Control

If pressed 1 one complete data sequence is recorded by the camera using the corresponding settings from the configuration tabs. The image data are directly displayed, and if a reference data set has been loaded or recorded prior to that, the result images are calculated as well. Additionally the total number of recorded images is displayed left of this button.

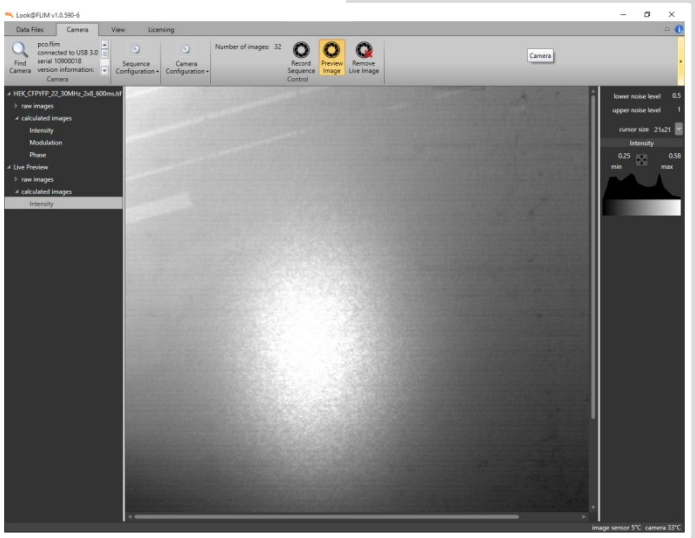
All recorded and calculated images are listed in the file tree window on the left.

Not yet saved recorded data are named as ***Live*** data with the normal names of calculated images and result images. By selecting the desired image 3 in the file tree, this image is active and displayed in the main window.

While recording a small preview window 4 is shown in the left column.

Preview Image

Sets the camera back to a continuous dual tap but single image recording at the configured exposure time. The button is highlighted when on. During operation the live intensity image is continuously updated to make adjustments of the optical setup easy or to figure out the optimum exposure time.



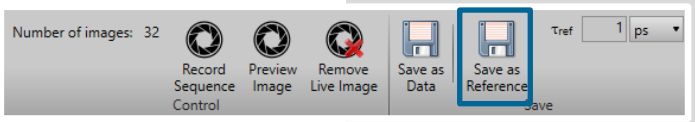
Since the shortest possible sequence length is used here, this mode is well suited for focusing purposes.

When pushed again, it is deactivated and the original sequence configuration settings are loaded back to the camera.

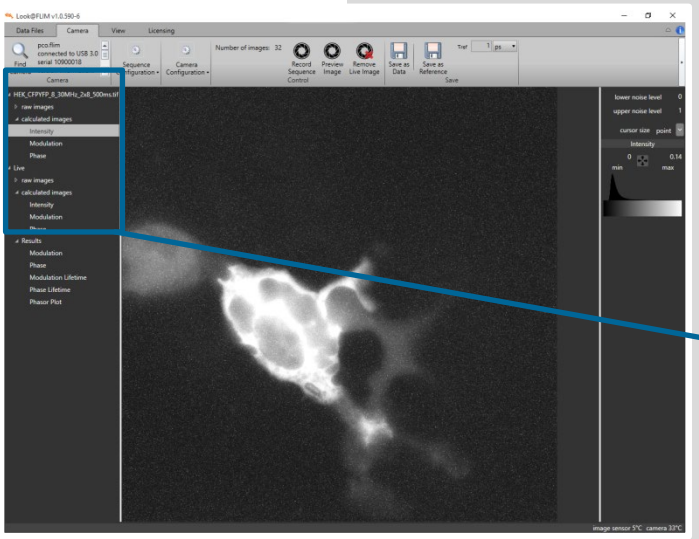
Save as Data

When pressed, a save file dialog pops up, and the user can store the recorded image sequence as data.

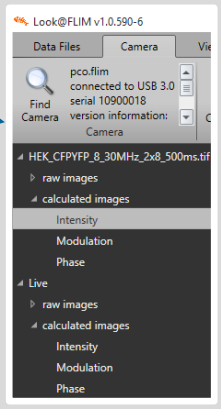
Save as Reference



If pressed, a save file dialog pops up, and the user can store the recorded image sequence as reference data.

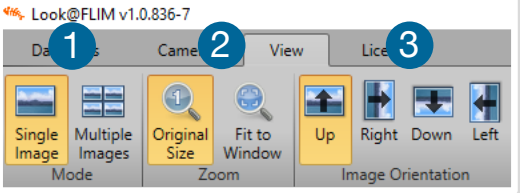


Thereupon, the reference image sequence appears in the file tree window on the left side with the defined filename. It is organized using the listing described above.



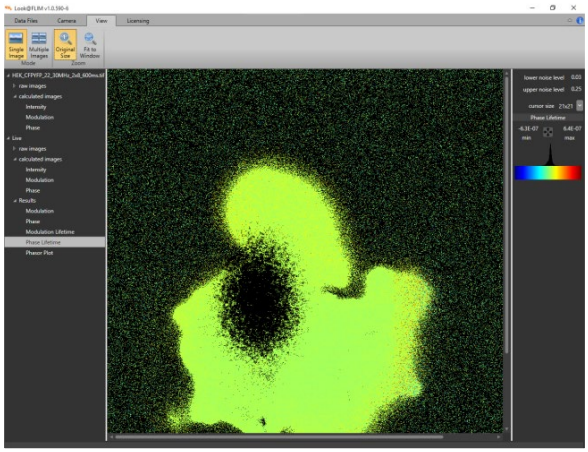
NOTE
Now the reference data set is used for referencing the following measurements and calculating the results. In order to obtain valid lifetime information the user has to define the known lifetime of the reference by switching to the ***Data Files*** menu and typing in the lifetime with the appropriate time unit.

7.4 VIEW MENU



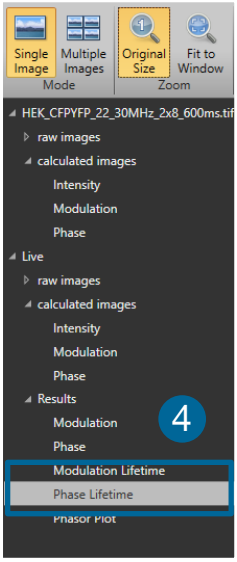
The ***View*** menu enables to control and select the displayed images and corresponding information windows ranging from ***Single*** to ***Multiple Images*** display. The zoom level can be set to ***Original Size*** or the whole image can be fitted to the display window (***Fit to Window***). The menu bar has the following options.

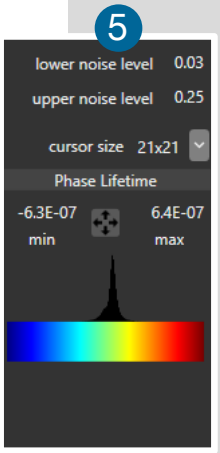
Image Orientation: 3 selectable orientation - ***Up*** (standard) – ***Right*** – ***Down*** - ***Left***



Modes

There are two display modes available, ***Single Image*** and ***Multiple Images*** 1.
If ***Single Image*** is selected, one image is displayed at a time (either in ***Original Size*** or ***Fit to Window*** zoom stage 2).

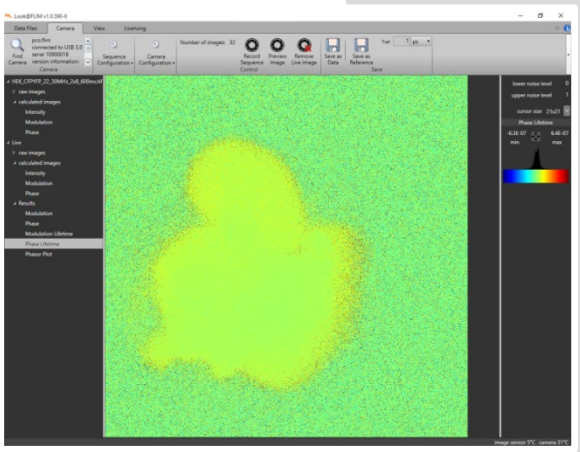


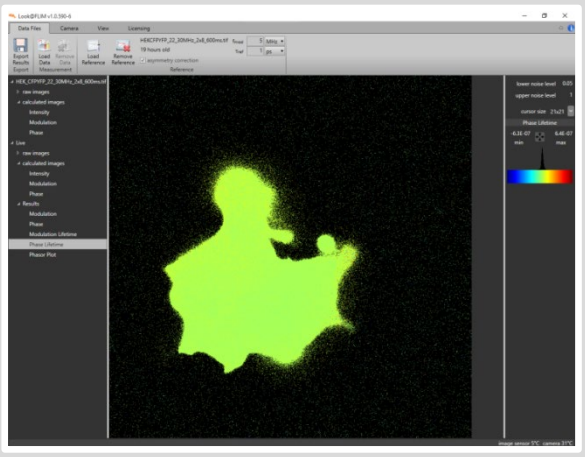


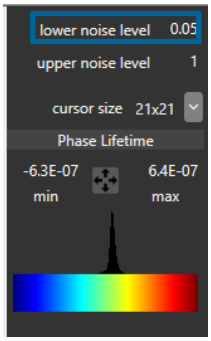
The image selection is activated by clicking on the appropriate image name in the file tree window on the left side. 4

The selected image is highlighted by a light grey background behind the name. On the right side the control column 5 adapts the scaling of the image, control cursor size and read values at the current cursor position.

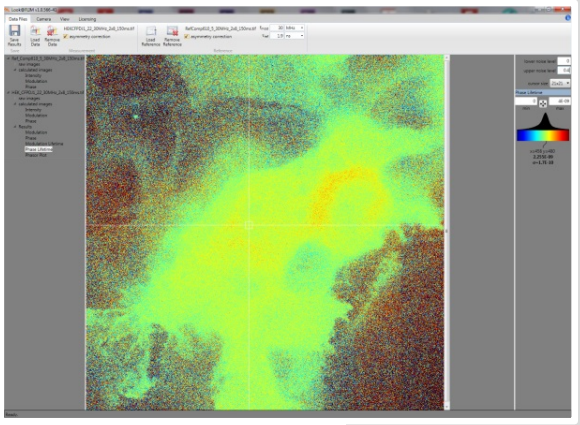
Effect of the Noise Levels in the Images

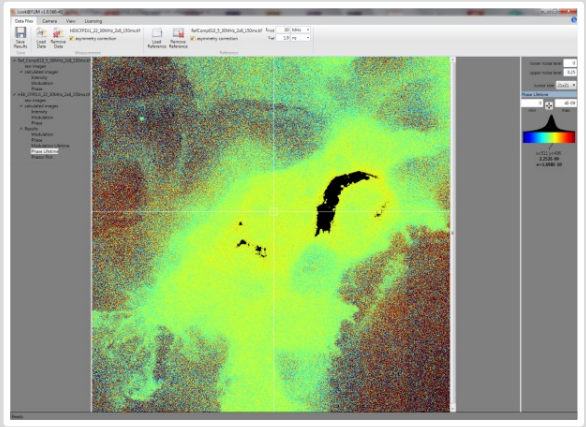


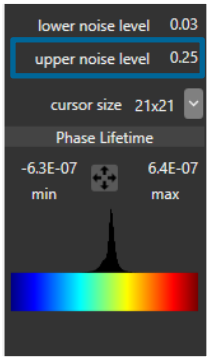




The left image shows the phase angle distribution of a bright data image with the ***Lower Noise Level*** set to 0 and the ***Upper Noise Level*** set to 1, thus everything is visible. The right image shows the effect of setting the ***Lower Noise Level*** to 0.05 (in units of the normalized intensity image: 0.0 – 1.0). Most of the surrounding noise of the fluorescing cell is suppressed.

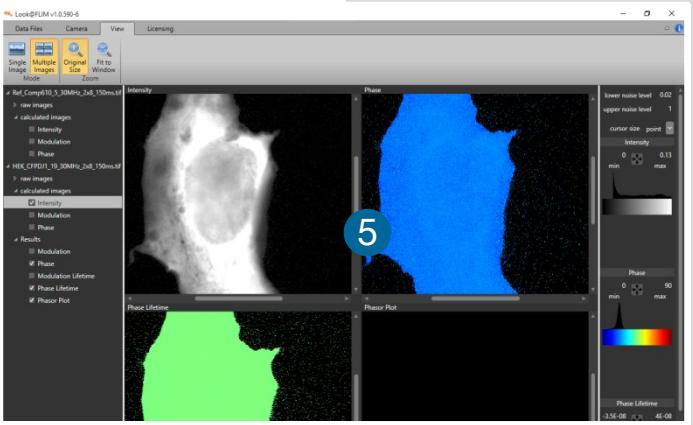




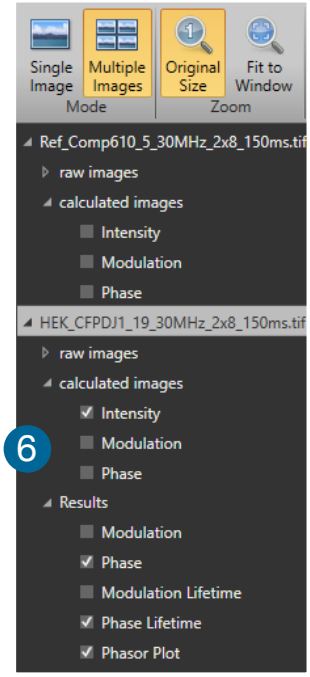


If the signal is too bright, i.e. the mean intensity becomes more than approximately 0.5, clipping appears. Although the image itself is not overexposed, derived values from bright parts might already be clipped, such that modulation index and phase angle start to distort. If now the ***Upper Noise Level*** is set to 0.5, the parts of the image exhibiting a too large signal are suppressed. This is shown by means of the black area within the sample, indicating that the significantly different phase angle in this area, which is reddish in the left image, is an artefact.

Multiple Images

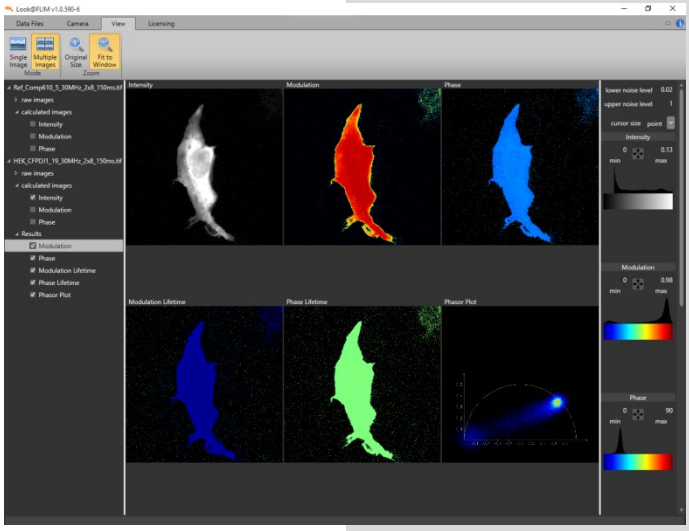


Selecting ***Multiple Images***, several images are displayed in the image area at once (in the example with the ***Original Size*** zoom factor 5).

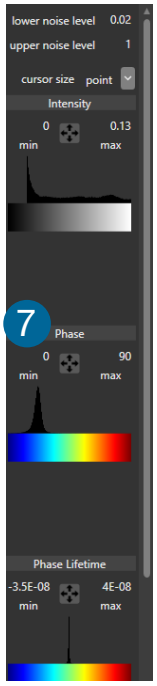


Now in the file tree column checkboxes appear to the left of each image, such that the user can select those images to be displayed 6 .

Depending on the total number of selected images the available space in the image area is subdivided to display all the images (maximum of eight images), such that the maximum space is used for each image.

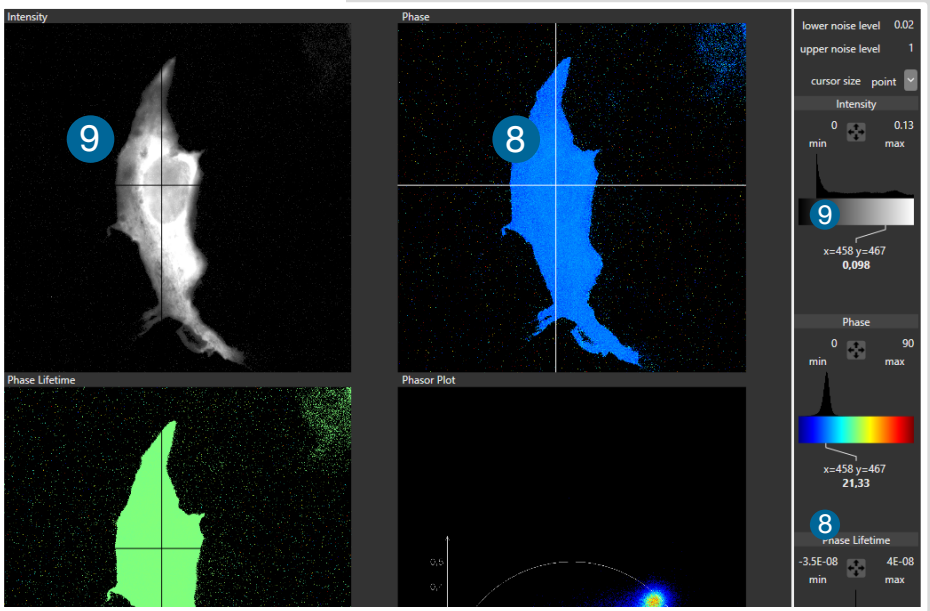


The following image shows an example with six images displayed simultaneously.



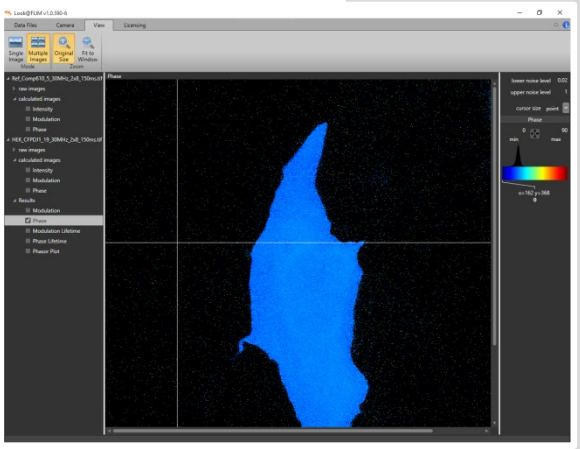
Using the ***Multiple Windows*** selection, each of the selected images has a corresponding histogram with a scale bar for scale adjustments in the control column on the right side, where all corresponding information can be identified by the titles of the images and the histograms 7 .

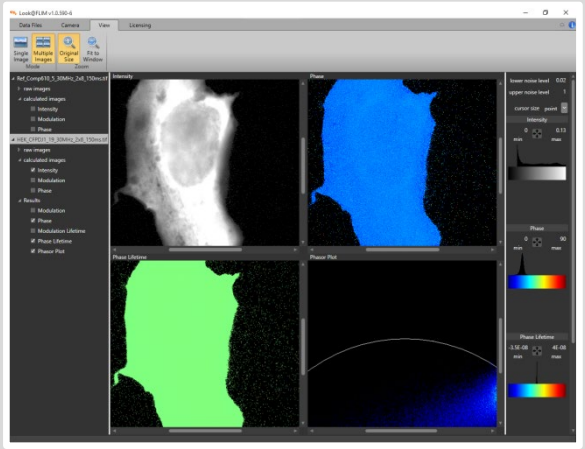
The crosshairs of the cursor in the current image is displayed in white 8 while the corresponding position in all other images is displayed in black 9 . Nevertheless in all images (except the phasor plot) the values at the same cursor positions are read and displayed below the corresponding histograms.



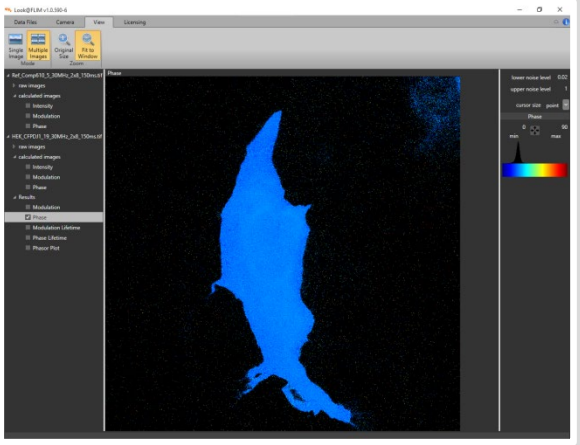
Zoom

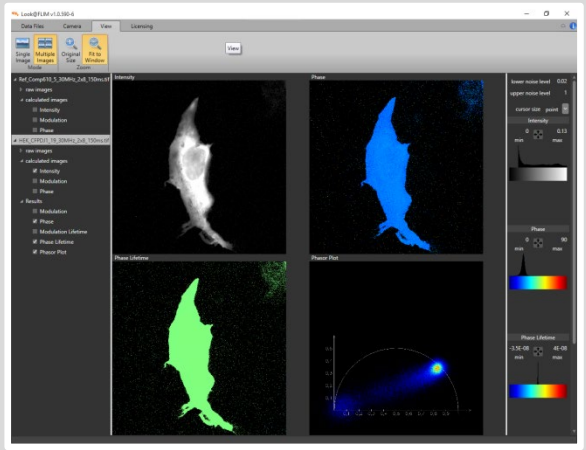
Two different zoom factors are available: the ***Original Size*** which corresponds to a pixel by pixel representation of the measured image as shown for ***Single*** and ***Multiple Images***:





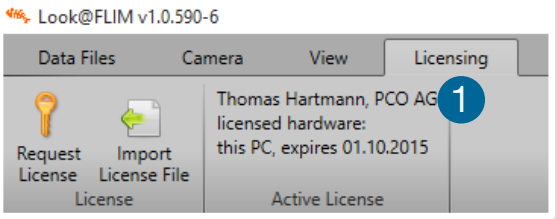
Or the images are fitted to the available image space, when ***Fit to Window*** has been selected, as it is shown for ***Single*** and ***Multiple Images***:





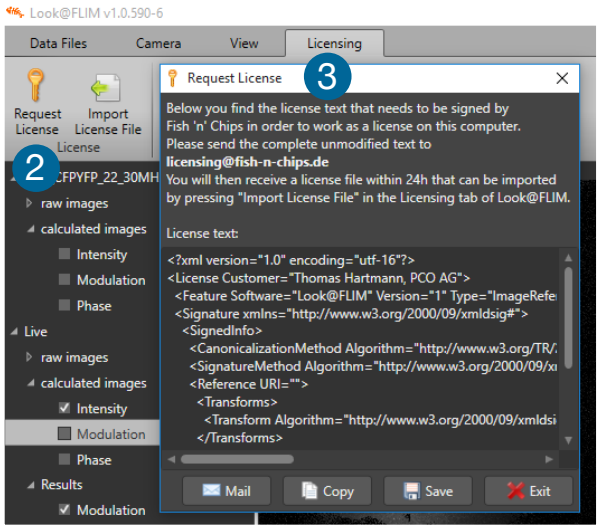
7.5 LICENSING MENU

The last menu is the ***Licensing*** menu informing the user which license for ***Look@FLIM*** is active and allows to request or to import a license file in case the computer has no web access.



Active License

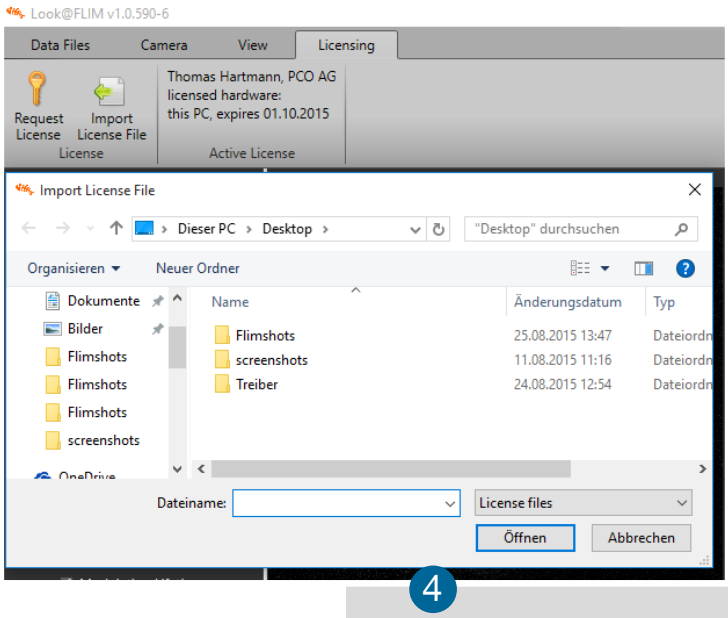
The text field advises the name of the user and his affiliation, type of license (per PC or per camera) and expiry date, if existing (1).



Request License

Pressing the ***Request License Button*** (2), the following window pops up (3).

The program gathers required information about the computer and sends it to the company ***Fish'n-Chips***, the originator of the ***Look@FLIM*** software. With this information a license file is created and uploaded to the computer.
In case there is no access to the internet a text file with the required computer or camera information is created and the user is asked to send it to a provided e-mail address to receive the license file via e-mail.



Import License File

Once a license file is received, it shall be installed. a license file, it has to be imported. Pressing the ***Import License File*** button a file manager dialog opens. Here the user has to switch to the directory where the license file has been stored. By selecting the license file and pressing ***open*** (öffnen) (4) the license will be directly processed by the program.

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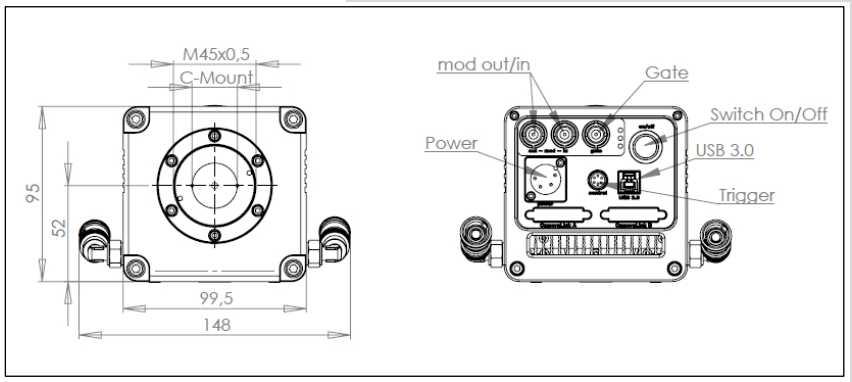
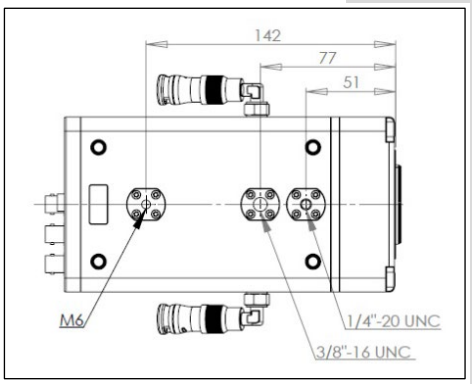
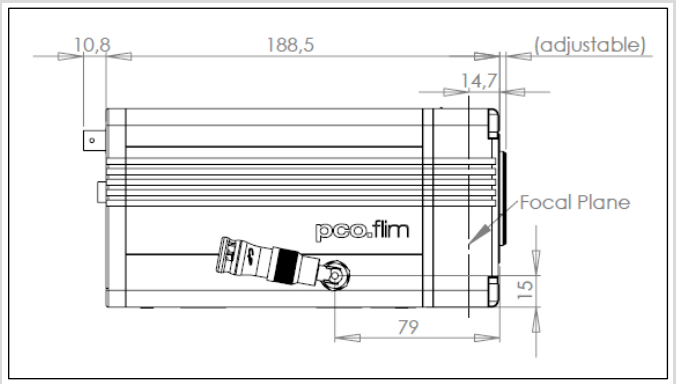
A1 TECHNICAL DATA

A1.1 MECHANICAL DIMENSIONS

All dimensions given in millimeter.







A1.2 DATA SHEET

Image sensor	
Type of sensor	CMOS
Image sensor	Proprietary
Resolution (h x v)	1008 x 1008
Pixel size (h x v)	5.6 µm x 5.6 µm
Sensor format / diagonal	5.7 mm x 5.7 mm / 8.1 mm
Shutter mode	Rolling reset / global exposure
Fullwell capacity	52 000 e^- (typical)
Readout noise	48 e^- rms (typical)
Dynamic range	> 1000:1 (>60 dB)
Quantum efficiency	Appr. 39 % @ 520 nm (peak)
Spectral range	370 nm – 780 nm (FWHM)
Dark current	1220 e^- / (s*pixel)
DSNU	56 e^- rms
PRNU	0.7 %

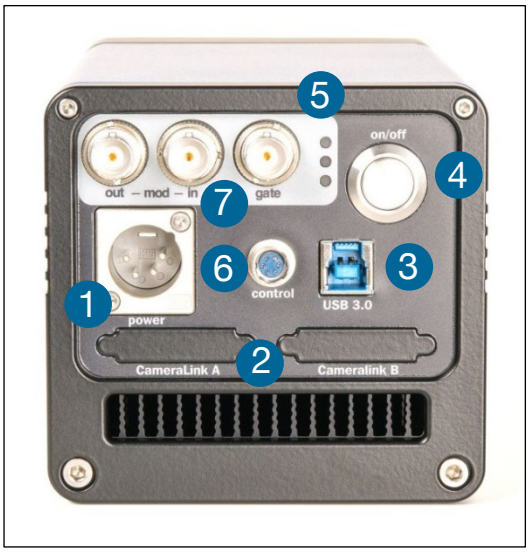
Camera	
Max. frame rate (full frame, full resolution)	90 fps (2 tap readout)
Modulation frequency	Internal 5 kHz – 40 MHz External 500 kHz – 40 MHz
Modulation signal shape	Sinusoidal / rectangular
Exposure / shutter time	10 ns – 42 s
Dynamic range A/D	14 bit
A/D conversion factor	3.4 e^- / count
Region of interest (ROI)	Steps of 16 x 1 pixel
Rhermoelectrical cooled	+5 °C
Nonlinearity	< 1 %
Trigger input signals	Exposure start (phase sequence trigger)
Trigger output signals	Exposure, busy, gate (light enable)
Modulation signal output	1 $V_{peak-peak}$ in 50 Ω, AC coupled
Modulation signal input	Max. ±5 V in > 1 kΩ
Data interface	USB 3.0

General	
Power supply	90 – 260 VAC (12 VDC optional)
Power consumption	40 W max.
Weight	2.4 kg
Ambient temperature	+5 °C to +40 °C
Operation humidity range	10 % - 90 % (non-condensing)
Storage temperature range	-20 °C to +70 °C
Optical interface	C-mount
CE / FCC certified	Yes

A1.3 INPUT WINDOW

The input window of the pco.flim camera has a standard coating (providing better light transmission).

A1.4 REAR PANEL



Power Input 12 V 1

Camera Link A & B connectors (not available) 2

USB 3.0 connector 3

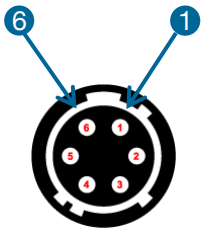
Power Switch (on / off) 4

Status LEDs 5

LED	Color	Function
1st	Green	Normal operation
	Red	Error
2nd	Orange	Camera is armed
		Blinks continuously
3rd	Yellow	Lights up during boot-up (< 10s)

Control Connector 6

Hirose HR10-7R-6S (73)



Pin	Description
1	Not used
2	Trigger
3	Not used
4	Exposure
5	Busy
6	Ground

Electrical Specification	Inputs (Trigger)	Outputs (Exposure, Busy)
Type	Digital	Digital
Level	3.3 V LVTTL (5 V tolerant)	3.3 V LVTTL
Coupling	DC	DC
Input impedance / load current	1 kΩ	Max. 100 mA (short-term)

BNC Connectors 7

Mod out (Modulation Output)	
Type	Analog
Frequency	5 kHz-40 MHz
Amplitude	1 $V_{peak-peak}$ (in 50 Ω)
Coupling	AC
Impedance	50 Ω
Waveform	Sinusoidal, rectangular, off

Mod in (Modulation Input)	
Type	Analog / digital (Schmitt trigger)
Frequency	500 kHz-40 MHz
Amplitude	Max. ±5 V
Coupling	AC
Impedance	> 1 kΩ

Gate (Light Source Enable)	
type	Digital
level	3.3 V LVTTL
coupling	DC
current	Max. 100 mA (short-term)

Timing & Signals

Busy = TRUE:	Camera is recording or sensor readout in progress
Busy = FALSE:	External trigger is ready to accept
Exposure = TRUE:	Exposure of image sensor

In ***Auto Sequence*** trigger mode the busy signal is always set to TRUE, while the exposure signal indicates the real exposure of the image sensor.
In ***External Exp. Start*** trigger mode the camera is ready to accept a new trigger signal on PIN #2 (trigger) when the busy signal is set to FALSE. After a successful trigger event the busy signal stays TRUE until the exposure is finished and the image is completely read out.

A2 USB 3.0

A2.1 HARDWARE RECOMMENDATIONS

The pco USB 3.0 interface is based on the Cypress EZ-USB FX31 device and compatible to pco software such as Camware and SDK. To run a pco USB 3.0 camera successfully the user should consider a number of important issues that are discussed in the following chapters.

Motherboard and Chipset Configuration

PCO recommends a motherboard with a state of the art USB 3.0 host controller for the onboard USB 3.0 ports. The latest generation of USB 3.0 xHCI host controllers manufactured by Intel, Renesas (NEC), Fresco, Via Labs, ASMedia and Texas Instruments Inc. (TI) are approved by Cypress Semiconductor Corporation. (see Cypress EZ-USB® FX3TM SDK Release Notes, Version 1.2.3, chapter 1.3). Currently pco recommends only motherboards with the following onboard xHCI host controllers:

- Renesas/NEC µPD720202 Host Controller
- Intel® USB 3.0 eXtensible Host Controller

If the motherboard does not have an onboard USB 3.0 port or the onboard USB 3.0 port does not work properly with a pco USB 3.0 camera, use an independent PCIe USB 3.0 extension card. In this case, pco recommends the following:

- DELOCK 89348 (U3-PCIE1XG202-10)
  PCIe 1x (µPD720202 host controller)
  (This board comes along with your pco.flim USB 3.0 camera system)
- DELOCK 89325 (U3X4-PCIE4XE101)
  PCIe 4x (µPD720202 host controller)

USB 3.0 Extension Card with xHCI4 Host Controller

The following companies manufacture USB 3.0 xHCI host controller:

- Renesas Electronics America Inc. (earlier NEC)
- Texas Instrument Inc.
- VIA Labs Inc (Diamond)
- Fresco Logic Inc.
- Intel Inc.
- AMD Inc.
- Etron Technology Inc.

NOTE
To find manufacturers of suitable xHCI controller, use the Windows Device Manager. For example, in Windows 7 drop down the USB Controller entry and find the USB 3.0 host xHCI controller of the USB 3.0 device in the sub-tree. Right-click to open the properties dialog, click the ***details*** tab, and choose ***Vendor*** in the properties pull-down box.

The following table gives an overview about tested and recommended system configurations or hardware components.

Component	Recommendation
Motherboard	There is no recommendation for a manufacturer. But if an onboard USB 3.0 port is used, the Renesas´s xHCI host controller µPD720202 is suggested.
USB 3.0 extension card	• DELOCK 89348 (U3-PCIE1XG202-10) PCIe 1x (µPD720202 host controller), • DELOCK 89325 (U3X4-PCIE4XE101) PCIe 4x (µPD720202 host controller)
PCIe Slot	A PCIe 4x, 8x or 16x slot is recommended.
USB 3.0 Cable	USB 3.0 cable included in the packaging of your pco USB 3.0 camera
USB 3.0 Hub	Not recommended
Operating System	Windows XP 32 Bit, Win7 32 Bit / 64 Bit, Win8 32 Bit / 64 Bit
Multiple pco USB 3.0 cameras connected to a PC	A separate DELOCK 89348 (U3-PCIE1XG202-10) extension board for each camera should be used to guarantee maximum data throughput for each camera.

Recommended Software Components

The pco USB 3.0 driver supports Windows 7/8 (x86 or x64) and Windows XP x86 operating systems. Microsoft does not support USB 3.0 natively for Win 7 or older Windows versions. Therefore use the manufacturers' supplied xHCI host controller driver. By contrast, in Windows 8 Microsoft does provide a native generic xHCI Driver but pco recommends using the driver from the corresponding manufacturer of the xHCI host controller. Currently there are seven different vendors producing USB 3.0 host controllers:

- Renesas Electronics America Inc. (earlier NEC)
- Texas Instrument Inc.
- Intel Inc.
- VIA Labs Inc (Diamond),
- Fresco Logic Inc.
- AMD Inc.
- Etron Technology, Inc.

Make sure the most recent driver from the manufacturer is installed before a pco USB 3.0 camera is connected to a USB 3.0 port of your computer. The appropriate driver for the USB 3.0 port could be loaded from the website of the manufacturer of the USB 3.0 host xHCI controller; or from the website of the USB 3.0 extension card vendor. The current driver of the extension card included in the packaging of your pco USB 3.0 camera is added to the PCO flash drive delivered with each pco.flim.

NOTE
To find out which driver is currently used for the xHCI controller, consult the Windows Device Manager. For example in Win 7, drop down the USB-Controller entry and find the USB 3.0 host xHCI controller of the USB 3.0 device in the sub-tree. Then right-click to open the Properties dialog, click the driver tab and choose driver details

The pco USB 3.0 driver must be installed before connecting the camera pco.flim USB 3.0 to the computer (see **4.1**).
Get the latest USB 3.0 driver from www.pco.de/support.

A2.2 INSTALLATION OF THE USB 3.0 BOARD

WARNING

An external USB 3.0 host controller board comes along with the camera.

Hardware Installation

First shut down your computer and install the USB 3.0 Host Controller. Hardware Installation must be performed by a technician, because high voltages can occur on the device.

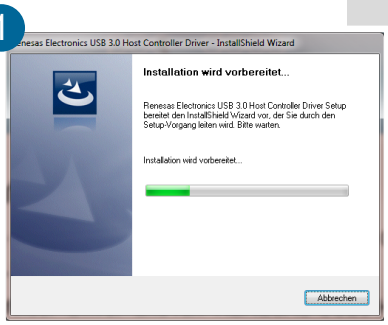
ELECTRIC SHOCK WARNING DUE TO VOLTAGE PARTS INSIDE
Risk of injury due to electrical shock.
➔ Always pull the mains plug before opening the computer.

Driver Installation Instructions

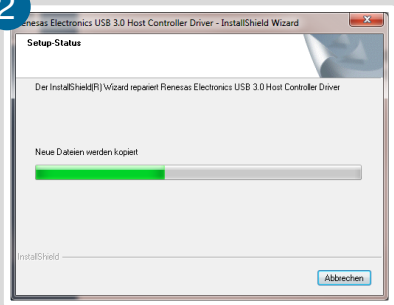
- Within the provided installation files USB_HBA, open the folder U3X4-PCIE4XE101, U3X4-PCIE1XE101, U3-PCIE1XG202.
- Open the subfolder Driver and run RENESAS-USB3-Host-Driver-30230-setup.exe.
- On Win 7/8 with User Account Control enabled the installation wizard prompts to start the setup: accept with ***Yes***.

1. Installation is prepared.
2. Software components are copied automatically.
3. Installation is completed and the Delock USB 3.0 extension card can be used.

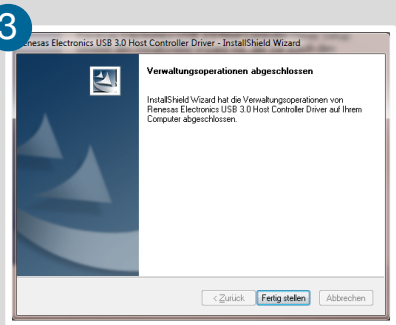
1



2



3



A3 WATER COOLING PCO.AQUAMATIC II

A3.1 SYSTEM COMPONENTS

This is the re-cooling system for pco cameras with water-cooling.

	Material No.	Description
1	30108000211	pco.aquamatic II
2	30108000212	Connection Tube 5m PVC 3541-01 PCO (with Colder NS212 fittings)
3	50402000055	Power supply 1.2m
4	20307500024	Power cable
5	10307000130	WAT camera cable 5m FGG-RG58- NC3MX
6	10305000190	Innovatec Protect IP 1L

pco.aquamatic handling is easy. Normally no maintenance and nearly no attention are needed. Only the liquid level of the reservoir (water tank) should be controlled from time to time.

Only use Innovatec Protect IP for the pco.aquamatic!

Do not use or add any other cooling liquid or normal water! If you need to add cooling liquid to maintain the tank level, contact PCO for additional supply.

The cooling liquid will turn yellow after some hours of operation. This is normal and no sign of wear or malfunction. The optimum pH-value is between 8 and 9 (check this value if you are concerned about the cooling liquid's quality).

NOTE
The recommended service interval for the change of the cooling liquid is four years.

A3.2 FIRST TIME INSTALLATION

Take care to place the unit on a flat and firm surface. Do not cover the cooling vents of the unit. Ensure free airflow around the pco.aquamatic for maximum cooling performance. All tubes and power cords need to run kink-free.

Before installation of the unit carefully read the Innovatek Protect IP safety datasheet (see Innovatek Website).

outlet flow

inlet flow

Follow steps 1 – 6

1. Connect tubes to cooling unit and camera. The two arrows on the housing of the cooling unit only indicate flow direction. Either connection of the camera can be used for inlet or outlet
2. Connect to power
3. Open tank cover
4. Turn power switch on
5. Slowly fill in the cooling liquid while the unit is running. Refill as needed to keep the level
6. While the cooling liquid flows back to the tank make sure all air gets out of the system – this takes a few minutes (move hoses if necessary)

The cooling liquid tank is filled when liquid level is approximately 1-2 cm from the top of the tank. The integrated pump only works when the pump chamber is completely filled. To ensure this move hoses or remove air by evacuating. Tank capacity is approximately 500 ml.

After steps 1 – 6 are completed successfully the system is ready for operation.

NOTE
The hose connectors are waterproof in not connected state. Maybe they lose one drop of cooling liquid from time to time. You don't have to empty the hoses while storing the camera system.

First connect the ***Power out*** of the cooling unit with the ***Power in*** of the pco.flim camera by using the PCO WAT camera cable. Or use separate power supplies for camera and aquamatic.

The cooling unit provides two operation modes.

Operation Mode ***on***: the cooling unit is on and provides permanently power to the camera. The camera can be switchted on and off as needed.

Operation mode ***follow***: the cooling unit turns on when the camera is switched on and vice versa.

Error Codes

The coolant temperature sensor is located in the water tank.

Temperature	Action
27°C	Fan turns off
36°C	Fan turns on
55°C	Warning message
60°C	Error message

If a warning level is passed, the power LED blinks slowly and the error LED displays the error code. If a failure level is passed, the power LED blinks fast and the error LED shows the error code.

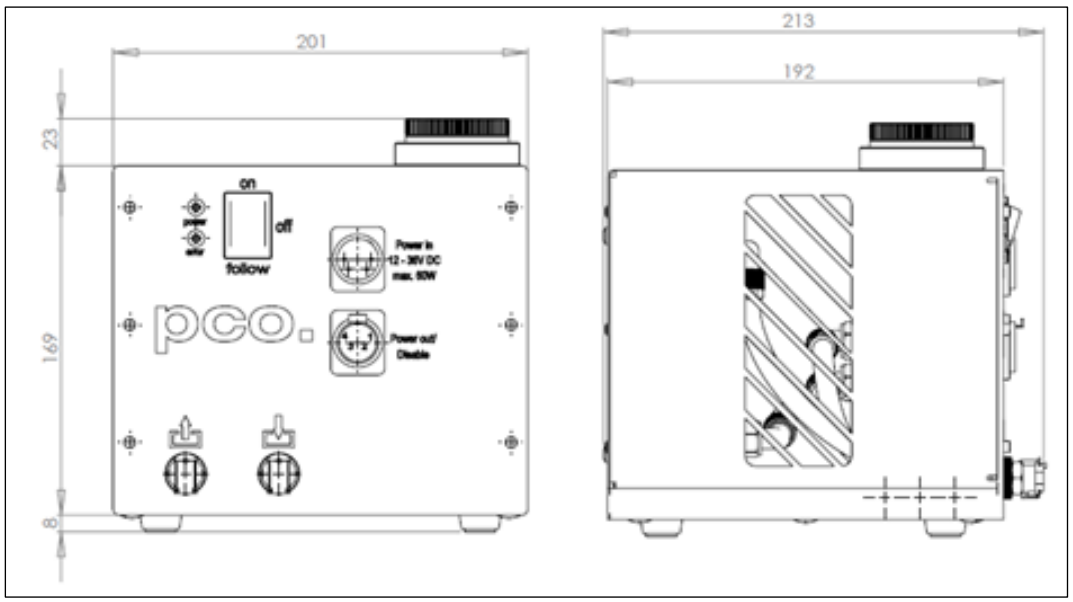
power	error	Error / Failure
On	Off	None
1Hz flash	One short flash	Warning when temperature at 55 °C (also if sensor is defect or missing)
2Hz flash	One short flash	Failure when temperature at 60 °C
1Hz flash	Two short flashes	Fan speed (also if a high deviation of the standard value is reached)

NOTE
The camera has its own protection circuit and will shut down automatically when the electronics temperature exceeds safety level.

A3.4 DIMENSIONS

All dimensions are given in millimeter.

Weight: 4kg (completely filled cooling liquid tank)



General Information

You are not obliged to purchase the pco.aquamatic system - it is possible to use your own water cooling device. A separate power supply will be provided to every pco.flim camera with water connection. The hardware of the pco.flim cameras with USB 3.0 interface is designed to work with or without a water cooling system. A fan inside the camera provides adequate cooling anyway.

In case you use an own water cooling system, make sure the liquid you use to cool your camera is always ABOVE the dew point of the ambient temperature! To avoid any occurence of condensation, use a cooling liquid at room temperature. A liquid flow rate of 1–2 litres per minute is sufficient.

A4 IMAGE FILE FORMATS

There are different file formats available for saving camera images with Camware.

b16

The b16 16 bit format is similar to the bmp format. However, 16 bit pixel values are used instead of 8 bit pixel values.

The file format consists either of a Basic Header (6 Long-parameter) or of an Extended Header (32 Long-parameter), the latter of which is optional for additional information. It may follow a variable comment field (ASCII code). Finally, there is the actual data set that is saved linearly (as in the case of BMP files).

With the exception of the first value, all parameters are Long Integers (4 Byte). The first 6 parameters must always exist. The rest of the parameters, as well as the comment field, are optional.

	Parameter	Function
1	PCO-	The first 4 byte are the characters PCO-
2	File size	File size in byte
3	Header length	Header size + comment field in byte
4	Image width	Image width in pixel
5	Image height	Image height in pixel
6	Extended header	-1 (true), extended header follows
7	Color mode	0 = black/with camera, 1 = color camera
8	B/w min	Black/white LUT-setting, minimum value
9	B/w max	Black/white LUT-setting, maximum value
10	B/w linlog	Black/white LUT-setting, 0 = linear, 1 = logarithmic
11	Red min	Red LUT-setting, minimum value
12	Red max	Red LUT-setting, maximum value
13	Green min	Green LUT-setting, minimum value
14	Green max	Green LUT-setting, maximum value
15	Blue min	Blue LUT-setting, minimum value
16	Blue max	Blue LUT-setting, maximum value
17	Color linlog	Color LUT-setting, 0 = linear, 1 = logarithmic
18 ... 266	Internal use	
Comment file in ASCII characters with variable length of 0…XX. The length of the comment filed must be documented in the header length field.		
	16 bit pixel data	
Line 1, pixel 1	value of the first pixel	
Line 1, pixel 2	value of the second pixel	
…	…	

PCO recommends that all images should be saved first in the b16 or TIFF format. The advantage is to have the b16 or tiff images available all the time, having the maximum 16 bit information. Note that not all image analysis programs can accommodate 16 bit data. The 8 bit format saves only the information displayed on the monitor screen. The 16 bit information will be lost and cannot be recovered.

pcoraw

This 16 bit PCO file format is based on the new BigTIFF format, thus allowing for file size > 4GB. A new PCO proprietary compression scheme is added if necessary.

Standard File Formats

TIFF

Tag Image File Format, version 6.0 and lower. There is a 16bit monochrome and color image format.

BMP

Windows Bitmap Format, b/w or color 8 bit format-images, which have been saved in BMP format can be loaded later only as 8 bit images, i.e. part of the original information (16 bit) is lost.

FTS

Flexible Image Transport System, Version 3.1. It is a 16 bit image format defined by NASA / Science Office of Standards and Technology (NOST). Some programs use the FIT extension for this format.

ASCII

16 bit format, some mathematical programs prefer ASCII data.

JPG

JPEG (named after the Joint Photographic Experts Group who created the standard) is a commonly used method of lossy compression for photographic images. The degree of compression can be adjusted, allowing a selectable tradeoff between storage size and image quality.

JP2

JPEG 2000 is a wavelet-based image compression standard and coding system. It was created by the Joint Photographic Experts Group committee in the year 2000 with the intention of superseding their original discrete cosine transform-based JPEG standard (created 1992).

AVI

Audio Video Interleave is a multimedia container format introduced by Microsoft in November 1992 as part of its Video for Windows technology.

MPG

MPEG-1, similar to JPEG, is a standard for lossy compression of video and audio developed by the Moving Picture Experts Group (MPEG).

WMV

Windows Media Video (WMV) is a compressed video format for several proprietary codecs developed by Microsoft. The original video format, known as WMV, was originally designed for Internet streaming applications, as a competitor to RealVideo.

A5 CUSTOMER SERVICE

A5.1 SERVICE

The camera is designed to operate with no need of special adjustments or periodic inspections.

A5.2 MAINTENANCE

CAUTION

UNPLUG CAMERA BEFORE CLEANING
Risk of injury due to electric shock!
- ➔ Unplug the camera from any power supply before cleaning it.

NOTICE

CLEANING
- ➔ Use a soft, dry cloth for cleaning the camera.
- ➔ Do not clean the input window unless it is absolutely necessary.
- ➔ Be careful and avoid scratches and damage to the input window surface.
- ➔ Do not use liquid cleaners or sprays.

NOTICE

LENS CLEANING
- ➔ The lens is best cleaned with pressurized air or with liquid cleaners such as pure alcohol or with special optical cleaners are available at premium photo stores
- ➔ Use a cotton swab dipped in pure alcohol or optical cleaning liquid and wipe only on the glass surface.
- ➔ Do not get any cleaning liquid on the metallic parts such as the lens thread, because tiny detached particles may scratch the surface.

NOTICE

CLEANING LIQUIDS
Aggressive cleaning liquids can damage your camera.
- ➔ Never use aggressive cleaning liquids such as gasoline, acetone, spirits or nitro cleanser.
- ➔ Every time the input window is cleaned, there is the possibility of surface damage.

NOTICE

PROTECTIVE CAP
Always store the camera with the protective cap or with a lens mounted to avoid dust and dirt on the input window.

A5.3 RECYCLING

To dispose your camera, send it to PCO or take it to a local recycling center.

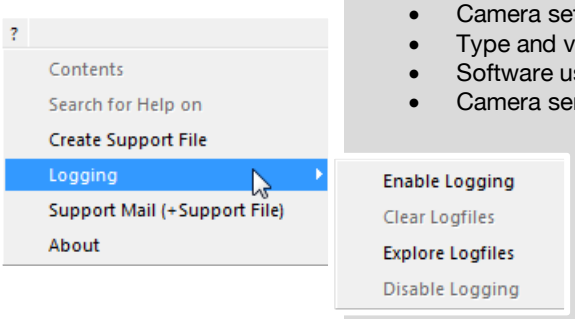
The camera includes electronic devices, which can contain materials harmful to the environment. These electronic devices must be recycled.

A5.4 TROUBLESHOOTING

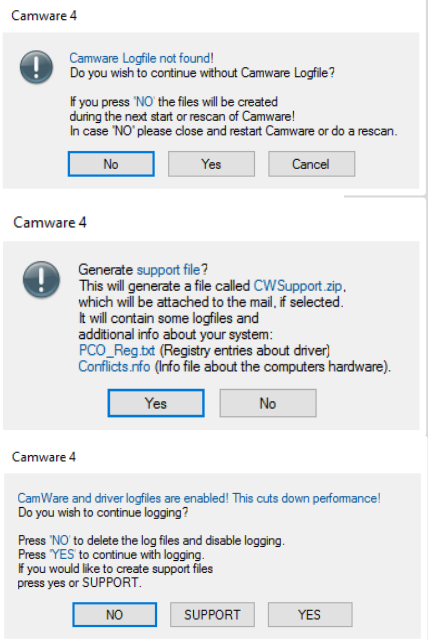
If you have a question, which is not adequately addressed in this manual, contact PCO or your local dealer.

To Speed up Your Request

- Short description of the problem
- Description of your application
- Camera settings
- Type and version of camera
- Software used
- Camera serial number
- Operating system (PC)
- Processor type (PC)
- Memory
- Graphic card
- Graphic card setup



How to Create Logfiles



Steps	Explanation
Enable ***Logfiles***	Open ***? Help*** menu → Logging → Enable log file →Camware will ask you to press ***NO*** to activate ***Logfiles*** after restart of Camware
Repeat	The workflow which produces the faults
Open ***? Help*** menu	Click Support Mail (+ Support File) → Camware will ask you: ***Generate support file?***
Save this file	(CWSupport.zip – don't rename it) and send it to PCO Support (support@pco.de)
Or visit website:	http://www.pco.de/support/ and upload the support file with our support form

Repair

Before sending the camera for repair, first contact your local dealer or PCO respectively.

When shipping the camera for repair, be certain to carefully pack the camera with proper shipping materials. If possible use the original packaging. Use the protection cap to protect the camera on the lens thread.

Firmware, Software and Driver Update

You will find all necessary software and drivers on the accompanying USB flash drive.

For the latest versions check PCO website.

A6 INDEX

NOTE:
The mentioned page is always the starting page of a chapter!

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Chapter 7: Look@FLIM

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ABOUT PCO

pco.history

"PCO" stands for what we are: a Pioneer in Cameras and Optoelectronics. With 30 years of expert knowledge and experience PCO has forged ahead to becoming a leading specialist and innovator in digital imaging used in scientific and industrial applications such as life and physical science, high-speed imaging and machine vision. However, the beginning of PCO's story of success dates back to the 1980s and a research project of the founder, Dr. Emil Ott, who was working at the Technical University Munich for the Chair of Technical Electrophysics. While performing measurements with intensified slow scan cameras, Dr. Ott realized that the existing standard did not meet the sophisticated requirements of scientific applications – and so PCO came to life in 1987. With a small team of engineers Dr. Ott began to develop his first image intensified camera followed by several variations on the original model, geared to overcoming all the existing flaws and surpassing standards of the day. During these early years PCO developed a now well established core of advance technologies used as the foundation to develop cutting edge products.

In the early 1990s PCO expanded its business activities to the global market by successfully establishing an international network of highly trained sales partners and customers. We entered additional fields beyond traditional scientific research expanding the potential for our cameras' applications in life science, automotive testing and even broadcasting. This step paved the way for a wide range of innovative highlights:

As of 2017, PCO has three decades of technical know-how and expert knowledge in the development and manufacturing of high-performing camera systems. In-house competence of all significant technical disciplines and partnering with leading image sensors manufactures ensures cutting edge sCMOS, CMOS and CCD technology for all PCO cameras.

pco.prospect

"If you want to do something special, particularly in the high end fields, you have to develop your own image sensors. So we work with partner companies who develop tailored sensors made especially for us. This is something we are doing continuously, so we're already working on the next generation of cameras that we will introduce in the coming years" – Dr. Emil Ott.

In PCO's first 30 years, Dr. Emil Ott took a company that he started right after finishing university and has built it into a major player in scientific and industrial cameras – and there's plenty more to come.

.