

Instruction Guide



Flat Handy FluorCam FC 1300-H

Please read the Guide before operating this product



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The contents of this manual have been verified to correspond to the specifications of the device. However, deviations cannot be ruled out. Therefore, a complete correspondence between the manual and the real device cannot be guaranteed. The information in this manual is regularly checked, and corrections may be made in subsequent versions.

The visualizations shown in this manual are only illustrative.

This manual is an integral part of the purchase and delivery of equipment and its accessories and both Parties must abide by it.

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1 WARNINGS AND SAFETY PRECAUTIONS

PLEASE READ THE FOLLOWING INSTRUCTIONS CAREFULLY BEFORE TURNING THE FLUORCAM ON!

GENERAL ELECTRICAL SAFETY GUIDELINES:

- Perform a routine check of the devices and their wiring.
- Replace worn or damaged cords immediately.
- Use appropriate electrical extension cords/power bars and do not overload them.
- Place the devices on a flat and firm surface. Keep them away from wet floors and counters.
- Avoid touching the device, socket outlet or switch if your hands are wet.
- Do not perform any alterations to the electrical part of the devices or their components.

The following table presents basic highlight symbols used in this manual:

Symbol	Description
	Important information, read carefully.
	Complementary and additional information.

WARNING:

The FluorCam device is considered Class 1M* LED Product. LED radiation may be harmful to eye. Avoid direct and strongly reflected exposure. It is recommended to use protective glasses.

**Class 1M: Laser and LED equipment that is safe under reasonable conditions of operation for use with the naked eye. Looking directly into the source of radiation by employing optics within the beam such as magnifying glass, telescope or microscope can be potentially hazardous.*



2 INTRODUCTION

Each quantum of light absorbed by a chlorophyll molecule raises an electron from the ground state to the excited state. The light energy absorbed by chlorophyll molecules may undergo one of three competing fates:

- it may be used to drive photosynthesis (83 % of energy in healthy plants),
- it may be dissipated as heat (up to 15 % of the energy) or
- it may be re-emitted as a red **chlorophyll fluorescence** (3–5%).

The three fates are complementary, and therefore changes in fluorescence yield reflect changes in photochemical efficiency and heat dissipation or non-photochemical quenching. Chlorophyll fluorescence imaging has so become one of the most powerful and popular tools to monitor changes in the photosynthetic performance of plants in response to biotic and abiotic stimuli or environmental changes. Changes in chlorophyll fluorescence kinetic parameters often occur before other effects of stress become apparent. The Detection of chlorophyll fluorescence is fast, non-invasive, and the spread of inhibition can be observed and quantified over time. Heterogeneity in the location of inhibition can be easily shown and quantified by chlorophyll fluorescence imaging systems.

FluorCam devices are used to monitor fluorescence kinetics in pulse-amplitude modulated mode and saturation pulse method, which provides a wealth of information about a plant's photosynthetic performance, physiological and metabolic condition, as well as its susceptibility to various stress conditions. Chlorophyll fluorescence yield is estimated after the application of a short saturating flash (saturation pulse) in dark-adapted plants or when plants are light-adapted or illuminated with photosynthetically active actinic light. The changes in chlorophyll fluorescence are used to describe plants' performances for photochemical and non-photochemical quenching of light energy supplied to the plant's surface.

2.1 CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence emission competes with photosynthesis for excitation energy (Fig. 1). The more effective is photosynthetic energy conversion, the lower the chlorophyll fluorescence yield, and *vice versa*. In a healthy, dark-adapted plant, photosynthetic capacity is available at its maximum and, thus, chlorophyll fluorescence yield is minimal (F_0). The photosynthetic performance can be reduced to zero by herbicide or by a pulse of strong light that transiently congests the photosynthetic electron transport pathway. With photosynthetic yield at zero, fluorescence emission reaches a maximum (F_M). An exhaustive review of all aspects of chlorophyll fluorescence emission is provided by Govindjee and Papageorgiou (2004). Several models of photosynthetic apparatus and of fluorescence emission dynamics are available at www.e-photosynthesis.org.

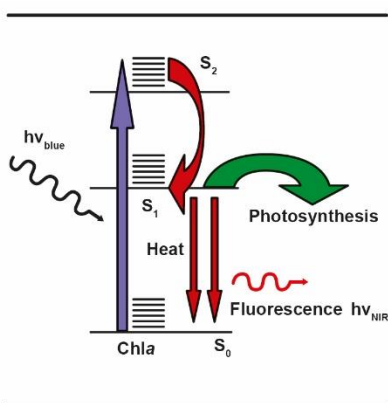


Fig. 1 Chlorophyll fluorescence

The change from the absorbed blue light to the emitted red fluorescence is explained by the Jablonski scheme. The energy of the blue absorbed photon brings the chlorophyll molecule into the upper excited singlet state, here S₂. Part of the energy is rapidly lost to heat changing the energetic state to the lowest excited singlet state, here S₁. The S₁ energy is, in photosynthetic organisms, largely used for photosynthetic energy conversion in the reaction centers. Only a fraction of the S₁ energy is lost to either heat dissipation or to fluorescence emission.

2.2 OXYGENIC PHOTOSYNTHESIS

Oxygenic photosynthesis starts with light absorption in photosynthetic antenna complexes (Fig. 2). The antenna complexes deliver captured energy to Photosystem II and Photosystem I reaction centers. The effectiveness of the energy capture, and of its transfer to the reaction centers, is determined by the effective antenna cross section σ_{PSII} and σ_{PSI} ¹.

The excitonic energy delivered to the reaction centers is largely used for primary charge separation. A fraction of the energy is lost to fluorescence and heat dissipation. The fluorescence yield is low (F_0) when the photochemical yield is maximal, and it is high (F_M) when photosynthesis is blocked, e.g.: by congestion of the plastoquinone pool that mediates electron transport from Photosystem II to the Cyt b/f complex (Fig. 2). The variability of chlorophyll fluorescence yield originates from Photosystem II as the fluorescence yield of Photosystem I does not depend on the photochemical state of its reaction centers.

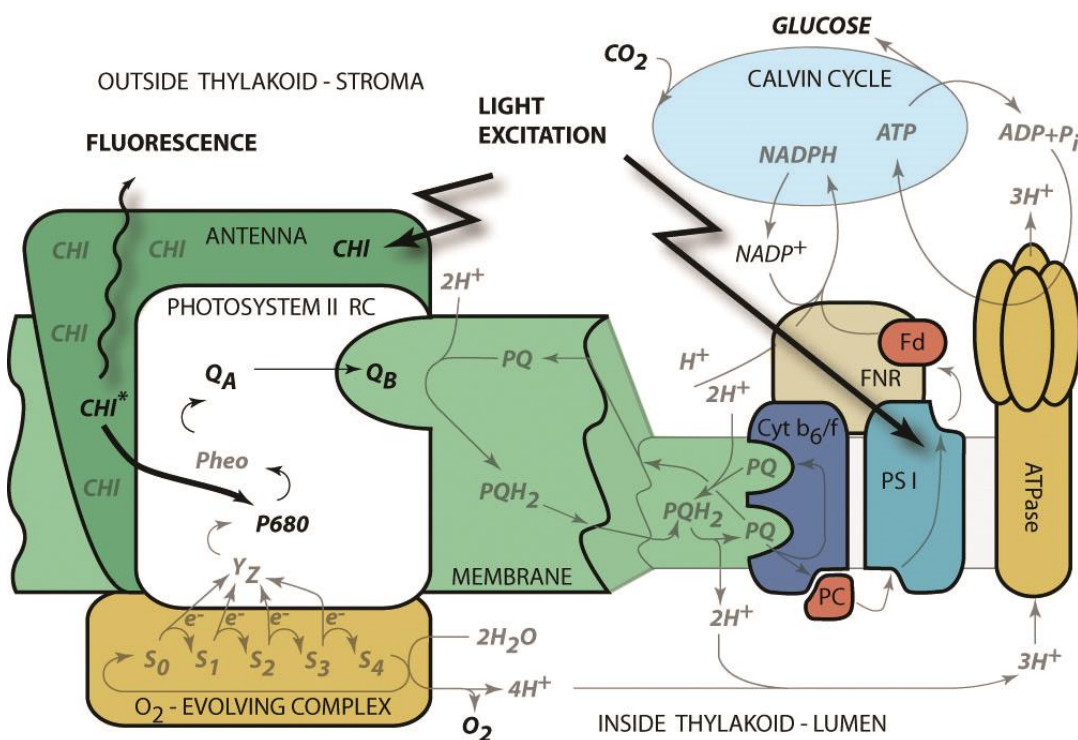


Fig. 2 Schematic presentation of the principal photosynthetic modules in plants and green algae

Light is absorbed by antenna pigments of Photosystem II (in front) and of Photosystem I (in back). The excitons generated in the antennae are rapidly transferred to the reaction centers where their energy serves to drive the primary charge separation. In PSII, the primary charge separation to P680⁺-Pheo⁻ is followed by secondary charge transfer processes: the electrons are extracted by the oxidized primary donor P680⁺ from water by the O₂-evolving complex and by the YZ donor. On the acceptor side, the electron is rapidly stabilized by a transfer from pheophytin (Pheo) to the primary quinone acceptor QA. A mobile plastoquinone pool shuttles two electrons sequentially taken from QA⁻ and two protons taken from the stromal side of the membrane to the luminal side of cytochrome b₆/f complex where the protons are released and electrons are sent to PSI. PSI uses the excitonic energy to generate reducing NADPH.H⁺. The charge transfer reactions in the thylakoid membrane result also in accumulation of protons on the luminal side, and depletion on the stromal side of the thylakoid. The difference in electrochemical potentials is used by ATPase to generate ATP that is used together with NADPH.H⁺ in the Calvin-Benson cycle to assimilate inorganic CO₂ into sugars.

¹ Irradiance of 2,000 $\mu\text{mol}(\text{photons})\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, typical of a sunny summer day at noon in Central Europe, results in ca.1,200 excitons arriving to the reaction center per second when the effective antenna cross section of 100 \AA^2 .

3 FLAT HANDY FLUORCAM

Handy FluorCams are lightweight, portable devices designed for time-resolved chlorophyll fluorescence imaging. Handy FluorCams monitor fluorescence kinetics using pulse-amplitude modulated mode and saturation pulse method, offering detailed insights into a plant's photosynthetic performance and stress response. Beside a CCD camera, these compact devices include four fixed LED panels, two for measuring pulses, and two for actinic illumination and saturating flashes. An optional leaf clip can be applied for handling larger specimens. Handy FluorCams work well with a typical sample size of up to 40 × 40 mm, and are especially convenient for experiments with plant leaves, small plants, leaf segments, mosses, lichens, seeds, roots, tissues or algal colonies on plates. Powered *via* mains or batteries, Handy FluorCam devices can be utilized both in the laboratory and in the field.

Available models:

- **Flat Handy FluorCam FC 1300-H:** A model with orange-red and white light panels, and a chlorophyll filter for **chlorophyll fluorescence imaging**
- **Flat Handy GFP Cam FC 1300-H/GFP:** An alternative model with orange-red and royal blue light panels, and interchangeable chlorophyll and GFP filters allowing for both **chlorophyll fluorescence and GFP imaging**

Each Handy FluorCam comes with a power source, a stand and a laptop preinstalled with comprehensive control software FluorCam10 (Fig. 3). FluorCam10 includes common experimental protocols, and tools for data acquisition and image processing. For more information about additional equipment, please follow chapter 5 HW Installation and Assembly Instructions starting on page 14.

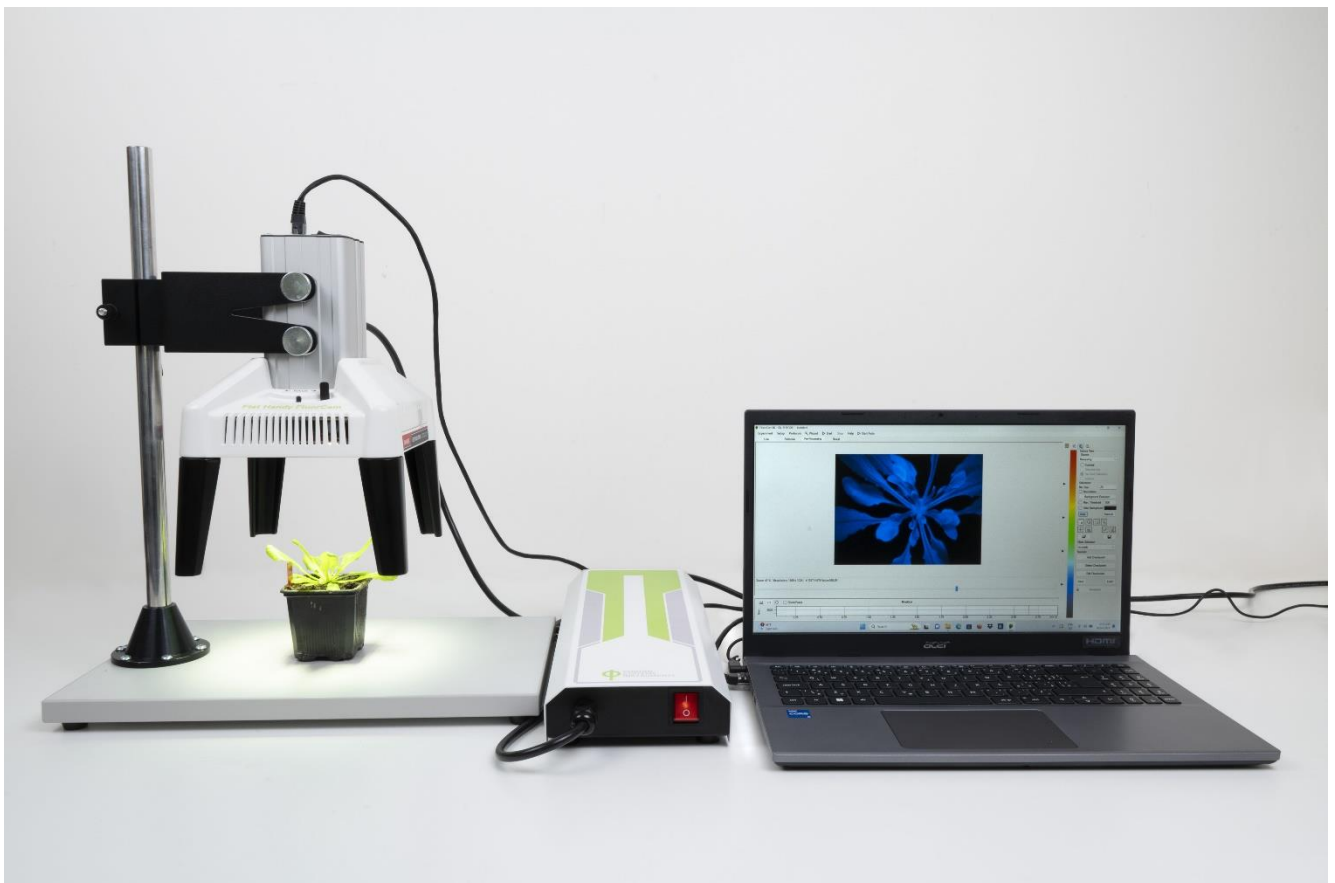


Fig. 3 Handy FluorCam with a stand and a PC

4 TECHNICAL SPECIFICATIONS

CCD Detector		
Camera	High resolution CCD camera TOMI-2	
Resolution	1 360 × 1 024 pixels	
A/D Converter Resolution	16 bit (65 536 grey levels)	
Pixel Size	6.45 μm × 6.45 μm	
Frame Rate	20 frames per second for full resolution	
CCD Detector Wavelength Range	400 – 1 000 nm	
Spectral Response	QE max at 540 nm (~72%), 50% roll-off at 350 nm and 800 nm	
Read-Out Noise	< 8 electrons RMS	
Full-Well Capacity	> 22 000 electrons	
Dynamic Range	65 dB	
Connectivity	Control and data: Gigabit Ethernet	
Operating Modes	Video mode (ChIF), optionally Snapshot mode (long integration times for FPs detection)	
Lights		
Light Sources	FC 1300-H Red-Orange 610 - 630 nm White 5 300 K Far-Red 710 - 740 nm	FC 1300-H/GFP Red-Orange 610 - 630 nm Royal Blue 440 - 450 nm Far-Red 710 - 740 nm
Super Pulse Intensity	> 4 500 μmol.m ⁻² .s ⁻¹	
Actinic Light Intensity	Up to 1 200 μmol.m ⁻² .s ⁻¹	
Detection channels		
Filters	FC 1300-H Chlorophyll filter: 695 - 770 nm	FC 1300-H/GFP Chlorophyll filter: 695 - 770 nm GFP filter: 510 - 541 nm
Lens		
Lens Type	Ricoh FL-CC1614-2M 2/3"	
Focal Length	16 mm	
Brightness	F1.4	
Field of View	40 × 40 mm (homogenous illumination)	
Technical Data		
Dimensions (W × D × H)	194 × 182 × 280 mm	
Weight	2 kg	
Electrical	100 – 240 V AC	
Power Consumption	210 W	
Operating Temperature	5 – 40°C	
Operating Humidity	0 to 90% (non-condensing)	
Software		
FluorCam10	<ul style="list-style-type: none"> fully automated control of FC device image acquisition <i>via</i> automated experimental protocols numerous image manipulation tools automated data analysis and parameters computation (F0, FM, FV, F0', FM', FV', FT, FV/FM, FV'/FM', ΦPSII, NPQ, qN, qP, Rfd, ...) 	

5 HW INSTALLATION AND ASSEMBLY INSTRUCTIONS

The Flat Handy FluorCam FC 1300-H and Flat Handy GFP Cam FC1300-H/GFP are compact devices which are delivered assembled. Below are the basic installation tips for both devices.

The set-up (Fig. 4) consists of:

- a **Flat Handy FluorCam device**
- a stand with a holder
- a power source
- a PC with **FluorCam10** software and a battery charger
- 3 thumb screws
- a power cable
- an Ethernet cable
- a lens cover
- deployable chlorophyll and GFP filters (in GFP version only)



Fig. 4 Flat Handy FluorCam parts

A) A Flat Handy FluorCam device. B) A stand with a holder. C) A power supply with a connection cable. D) A laptop with software FluorCam10. E) Three metal thumb screws for installation into the device stand. F) A power cable. G) An Ethernet cable. H) Two interchangeable filters (for Flat Handy GFP Cam only). I) A lens cover for the lens of the Flat Handy FluorCam.

Additional Accessories for Flat Handy FluorCam:

- Leaf Clip: Facilitates handling larger leaves such as tobacco, with a gentle lock/release mechanism to prevent leaf damage.
- Tripod Stand: Supports outdoor measurements or measuring larger plants when standard holders are inadequate.
- Battery Pack: Enables device operation during outdoor measurements where power access is restricted.



Please note that these additional accessories are not standard and must be ordered separately.

5.1 ASSEMBLY INSTRUCTIONS FOR THE FLAT HANDY FLUORCAM



Recommended tools: Allen key (provided).
Assembly Ease: Can be easily installed by one person.

Step 1: Setup

- Carefully unpack the device.
- Place all components on a dry, stable surface.
- Verify all parts against the enclosed packing list.
- Ensure the Flat Handy FluorCam and the power supply are switched off.

Step 2: Stand Assembly

- Prepare the stand with the metal rod and the holder.
- Attach the holder to the stand with the provided Allen key if needed.
- Adjust the holder to the correct height and secure the holder by tightening the lever (Fig. 5-B).

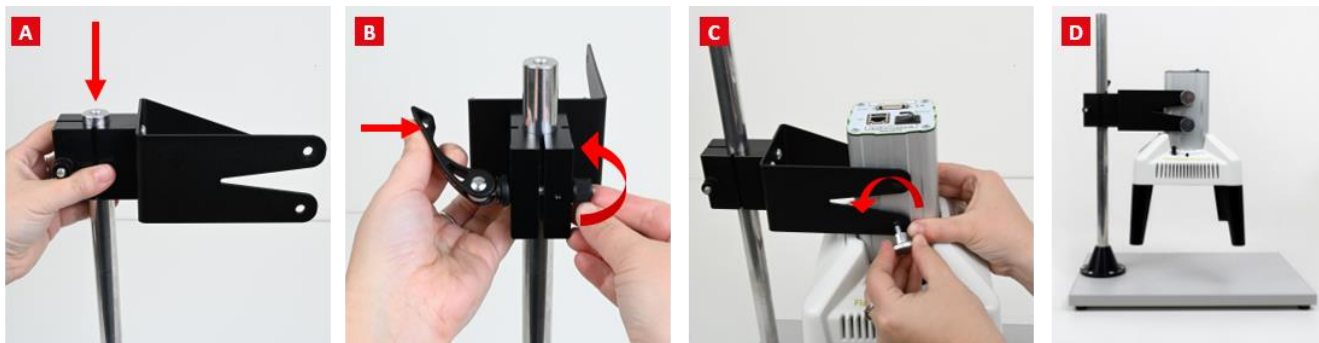


Fig. 5 Flat Handy FluorCam stand assembly.

Step 3: Lens preparation

- Carefully remove a lens cover from the Flat Handy FluorCam device
- For the GFP version only: Secure the appropriate emission filter without touching the glass. Handle the filter by the rim to avoid damage. Tighten the filter by turning it clockwise.



WARNING! Do not touch the filter glass.

Step 4: Final Assembly

- Slide the camera into the stand's holder using the three metal thumb screws (Fig. 5-C, D).
- Connect the Flat Handy FluorCam to the computer using the provided Ethernet cable (Fig. 6) and to the power source using the provided thick power cord. The socket, located on the side of the FluorCam device body, is marked as DC 15.6 V (Fig. 7-A).
- Plug the power source into a 110/230V outlet (Fig. 7-B).
- Switch on the power source, the Flat Handy FluorCam, and the computer (Fig. 8).

Before Operating the Flat Handy FluorCam:

- Ensure the device is turned off before connecting the power supply.
- Properly align male and female connectors to prevent damage during connection.
- Verify that the voltage ratings of the FluorCam, power supply, and battery pack are compatible.
- Avoid operating the device in wet conditions (such as rain) and allow sufficient time for temperature adjustment when transitioning between different environments.
- Do not look directly at the LED panels when the Handy FluorCam is active.



Fig. 6 Flat Handy FluorCam connection with PC through Ethernet cable



Fig. 7 Flat Handy FluorCam power source connection

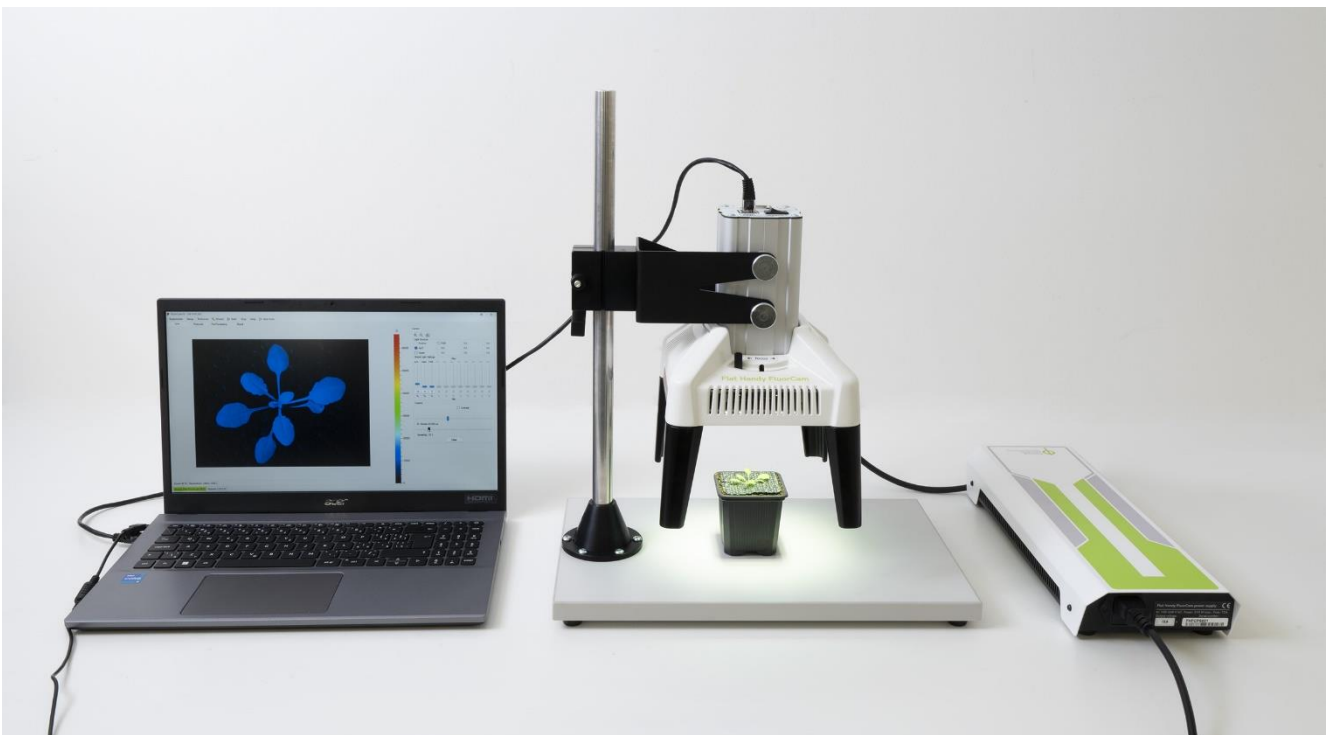


Fig. 8 Assembled Handy FluorCam

5.2 BATTERY PACK BOX

Unless specified by a user or restricted by transport conditions of the carrier, the battery pack box is equipped with a high-capacity Li-Ion battery pack which requires regular charging to maintain optimal performance.

5.2.1 LI-ION BATTERY PACK

The battery pack box is embedded with the high quality 24 V Li-Ion rechargeable battery pack sealed with industrial PVC (Fig. 9, Tab. 1). The battery pack is made of 21 Brand-new A grade cylindrical 18650 cells with PCB for full protection. This battery pack is light weight and possesses higher energy density than any rechargeable battery and longer storage life than NiMH battery. The battery is recharged by Li-ion 29.4 V Charging Voltage Battery Charger without any memory effect. Built-in IC chip prevents battery pack from over charge, over discharge and prolongs battery pack's life.



Fig. 9 Example of a Li-Ion battery pack.

Tab. 1 Li-Ion Battery Pack Technical Specifications

Li-Ion Battery Pack Technical Specifications	
Full Capacity (standard/high capacity)	9 000 mAh
Voltage	24 V (peak at 29.4 V)
Dimensions (Height × Length × Thickness)	70 × 130 × 65 mm
Weight	1.5 kg
Max. Discharge Current	15 A
Charging Current	2 A
Over-Charge Protection	29.4 V
Over-Discharge Protection	19.25 V



The battery pack box is designed for 24V 9Ah 7S3P 18650 Battery Lithium Battery 29.4 V Electric Bicycle Moped /Electric/Lithium-ion Battery Pack charged by the 29.4V 2A charger produced by ShenZhen Battis Batteries Company.

5.2.2 BATTERY INSTALLATION

For the successful battery installation, please follow next steps:

1. Unscrew two screws on each side of the battery box and open it (Fig. 10-A). Loosen and unscrew the screws of the battery holder (Fig. 10-B). Use an Allen key (size 2 mm) and a side key (size 5.5 mm).



Fig. 30 Unscrew the two screws on each side of the battery box and open it, then remove the four screws securing the battery holder at the bottom of the battery pack.

2. Place the Li-Ion battery pack to the battery holder and insert whole bundle to the battery pack box (Fig. 11). Note that holes of the holder must be positioned above the holes of the battery pack box. Fix the battery holder using screws and nuts.

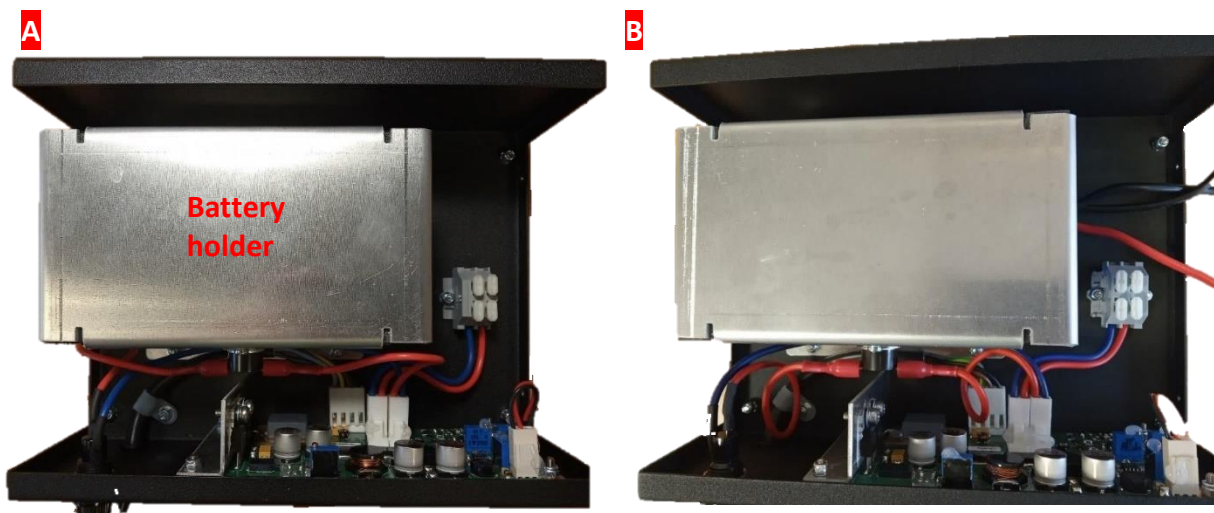


Fig. 4 Place the Li-Ion battery below the battery holder (A) and whole compound insert to the battery pack box (B)

3. Remove 8 mm of the isolation from the end of black wire of the Li-Ion battery pack and twist its fibers into the rope. Plug the black wire to the terminal block opposite to the dark blue wire from PCB. Do the same operation with the red wire (Fig. 13). Plug the red wire to the terminal block opposite to the red wire from PCB. During plugging of wires is necessary to press the white knob on the terminal block by finger or by flat screwdriver (Fig. 12-B)

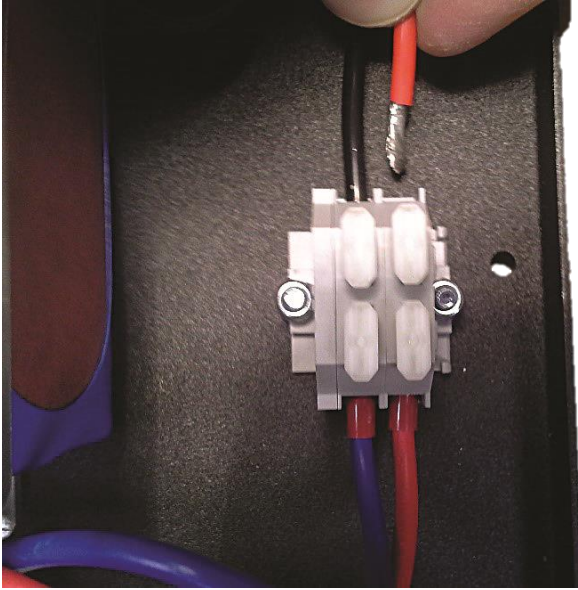
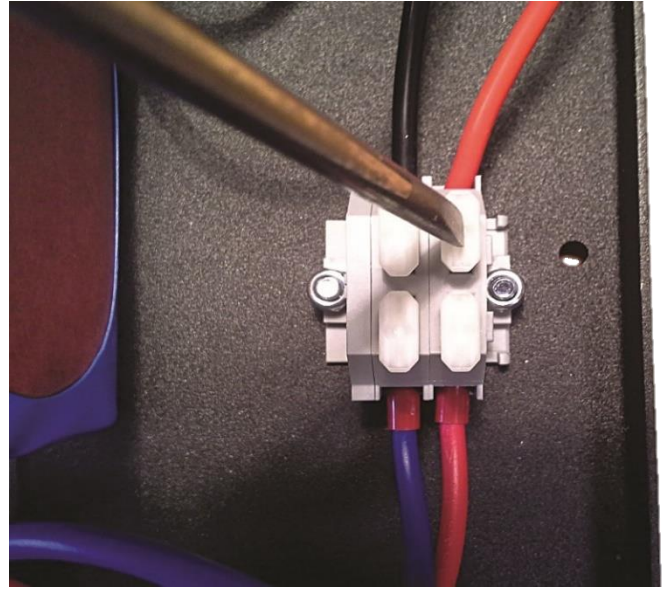
A**B**

Fig. 52 Plug the black and red wires of the Li-Ion battery pack to the terminal block (A). Press white knob on terminal block (B).



WARNING! Do not remove isolation from second wire before first wire without isolation is plugged to terminal block. Accidental short connection of naked wires can damage battery. Check, if any released fiber of rope not stay in battery pack box.

4. Close the battery pack box with the cover and tighten it with screws (Fig. 13).

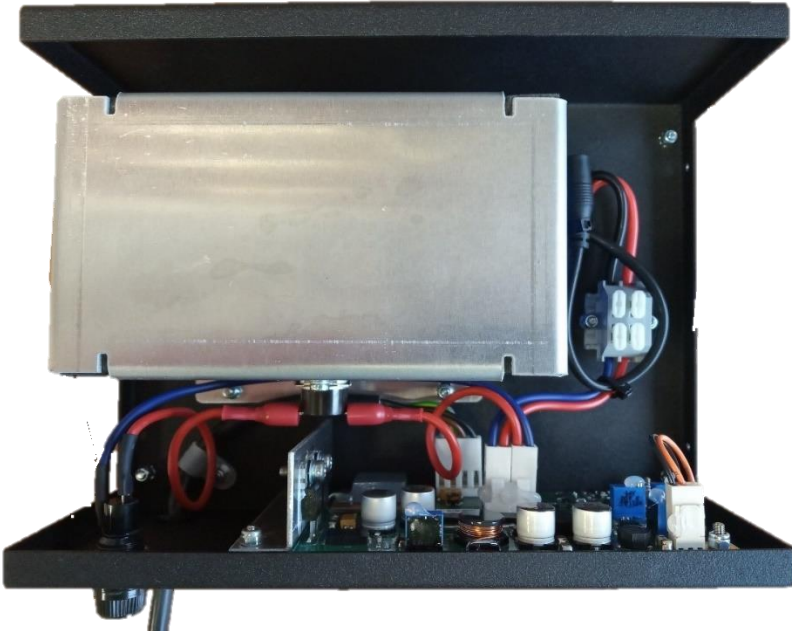


Fig. 6 Close the battery pack box and tighten the cover with screws.

5.3 GUIDE FOR USING ULTRA-LIGHT BATTERY PACK



WARNING! Lithium-ion batteries must be charged regularly to prevent capacity loss. Always store batteries charged and follow safety instructions to avoid hazards.

5.3.1 SAFETY INFORMATION

- Store the battery pack securely and out of reach of unauthorized users.
- Keep the battery pack dry and wait for temperature adjustment when moving between environments.
- Use only the designated battery pack for the Flat Handy FluorCam.
- Charge the battery pack in a safe location away from flammable materials and monitor it during charging.
- Lithium is a hypergolic material; always charge the battery pack outside of a bag.
- Never store the battery pack in a car or any container that may become hot.
- Avoid short-circuiting the batteries.
- Always replace the whole battery pack.
- If a lithium battery becomes damaged, there is a risk of fire. Store it outside and monitor its status regularly.
- Dispose of batteries properly and never in fire; do not attempt to open, solder, or weld batteries (Fig. 14).
- Never puncture a lithium cell or touch its contents.



Fig. 14 Safety symbols on the Li-Ion battery pack.

5.3.2 USER GUIDE

1. Connect the battery pack with Flat Handy FluorCam
2. Turn power switch ON.
3. The battery's condition is indicated by five LEDs:
 - **Green LED:**
 - 25%: Battery charge is between 0 to 25%.
 - 50%: Battery charge is between 25 to 50%.
 - 75%: Battery charge is between 50 to 75%.
 - 100%: Battery charge is between 75 to 100%.

- **Red LED:**

Indicates that the battery is empty. If the red LED turns on during use (voltage falls below the usable range), the battery pack will automatically shut off. Restarting the power switch after three seconds can reactivate the green LEDs, indicating a higher no-load voltage. If the intensity of the Saturating pulse decreases, the battery pack can be used for a longer duration. Charge the battery pack as soon as it is empty. If not used for more than four weeks, test and recharge the battery pack.

5.3.3 BATTERY PACK CHARGING

1. Connect battery pack with charging power supply adapter
2. Charging phase is indicated by **orange LED** and typically takes about 11 hours
3. To avoid overcharging, do not leave the charger connected for more than 15 hours.



Do not leave charging adapter connected to battery pack for significantly longer time than 15 hours for saving the battery lifetime.
Never charge battery pack, when its temperature is below 0 °C.

5.4 LEAF CLIP

The additional leaf clip serves for the measurement of the leaves of bigger plants, it easier the manipulation with the leaf, provides the dark adaptation of the selected leaf segment before the chlorophyll fluorescence measurement or dark environment for fluorescence proteins assessment and holds this leaf segment for repetitive measurements, which ensures the measurement of the same area of interest. Leaf clip has gentle lock/release mechanism for avoiding leaf damage.



The Phillips screwdriver is necessary for the leaf clip installation.

Please follow next steps for successful leaf clip installation:

1. Remove the Flat Handy FluorCam from holder and place it on the solid surface. Remove the Flat Handy FluorCam's legs (Fig. 15). The legs are hold by screws in the middle of each piece, for their releasing use the Phillips screwdriver.
2. Prepare the leaf clip on the solid surface.
3. Place the Flat Handy FluorCam device on the leaf clip frame.
4. Using the same screws, fasten the leaf clip to the Handy FluorCam by Phillips screwdriver.



Fig. 7 Removal of the Flat Handy FluorCam's legs and installation of the Leaf clip

The use of the leaf clip is very easy:

1. Fasten the Flat Handy FluorCam to the table holder or the tripod.
2. Press the black lever on the side of the clip.
3. Insert a leaf carefully.

4. Release the black lever.

The leaf clip can be used either in the laboratory, the greenhouse or outdoors conditions (Fig. 16).



Fig. 86 The leaf clip can be used either in the laboratory, the greenhouse or outdoors conditions

5.5 TRIPOD ASSEMBLY

Depending on the measured plant material, the Flat Handy FluorCam can be mounted in the table holder (standard equipment) or in the tripod (additional equipment). The tripod is suitable for measurement of larger plants or measurement in outdoor conditions.



Tripod package consists of three parts – tripod legs section, center column and device holder.

For the tripod assembly, please follow these steps:

1. Prepare the tripod stand to the desired height (Fig. 97-A).
2. Prepare the Flat Handy FluorCam device holder (Fig. 9-B).
3. Tighten the Handy FluorCam device holder to the tripod stand by rotating the metal gear on the holder (Fig. 9-C).
4. Move the device holder to desired position and fix it by two black screws on the side of the holder (Fig. 9-D).
5. Move the Flat Handy FluorCam device between the holder arms (Fig. 9-E).
6. Fasten the Flat Handy FluorCam device to the holder using three metal thumb screws. (Fig. 9-F).

5.6 EMISSION LENS FILTER CHANGING

The Flat Handy FluorCam FC 1300-H comes standardly with built-in emission filter for detection of chlorophyll fluorescence. However, Flat Handy GFPCam FC 1300-H/GFP is able to measure not only the chlorophyll fluorescence, but also can detect the green fluorescence protein GFP using the correct emission filter. The two replaceable emission filters are embedded in a special rim, which can be fastened to the device's lens.



When manipulating with the filter, do not touch the filter glass, hold it by the black plastic rim only!

Changing the emission filters, please follow next steps (Fig. 18):

1. Turn OFF the Flat Handy FluorCam device and lay it to one side.
2. Gently take away the emission filter by anticlockwise turning from the lens.
3. Place the removed emission filter to plastic bag immediately to avoid the scratches on the filter surface.
4. Fasten the selected emission filter by clockwise turning to the lens.
5. Turn ON the Flat Handy FluorCam device.

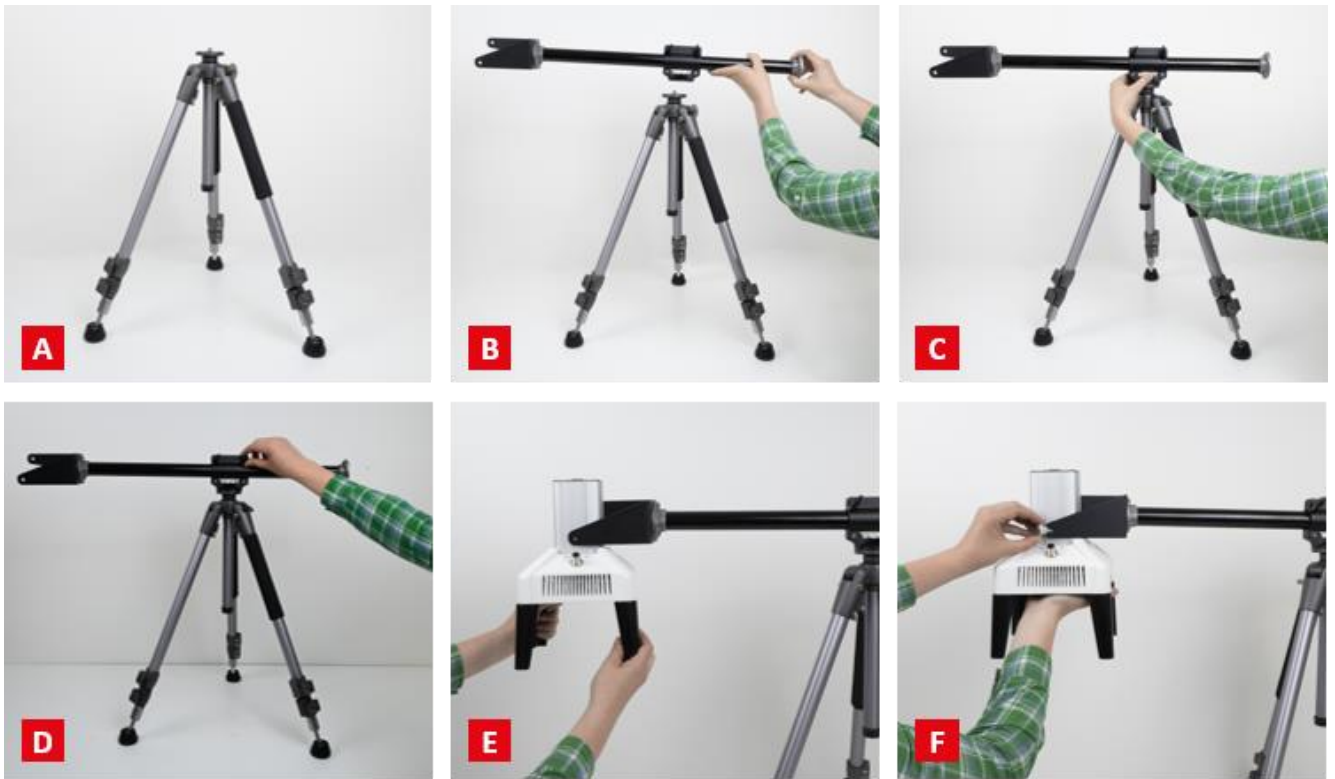


Fig. 9 Tripod assembly



Fig. 18 Emission filters changing

6 FLUORCAM 10 SOFTWARE

The Flat Handy FluorCams are compatible with the FluorCam10 software. Unlike FluorCam7, FluorCam10 is a 64-bit application. This change enables FluorCam10 to access more memory than 32-bit applications and so run complex protocols more smoothly. The setting of FluorCam10 resembles FluorCam7 and is quite intuitive. The useful functions of FluorCam7 have been preserved so the details can be found in the FluorCam7 instruction guide if necessary.

6.1 DEVICE CONNECTION

First of all, the proper connection of the switched-on Flat Handy FluorCam with the computer through the Ethernet cable should be checked. If the device is properly connected, the “Live” tab of FluorCam10 contains information about the device in the bottom left corner (highlighted by the green color) as shown in the Figure 19. If the device is not connected, check Network & Internet Settings and set proper IP address via Internet Protocol version 4 (TCP/IPv4: IP address: 172.23.0.1, Subnet mask: 255.255.240.0).

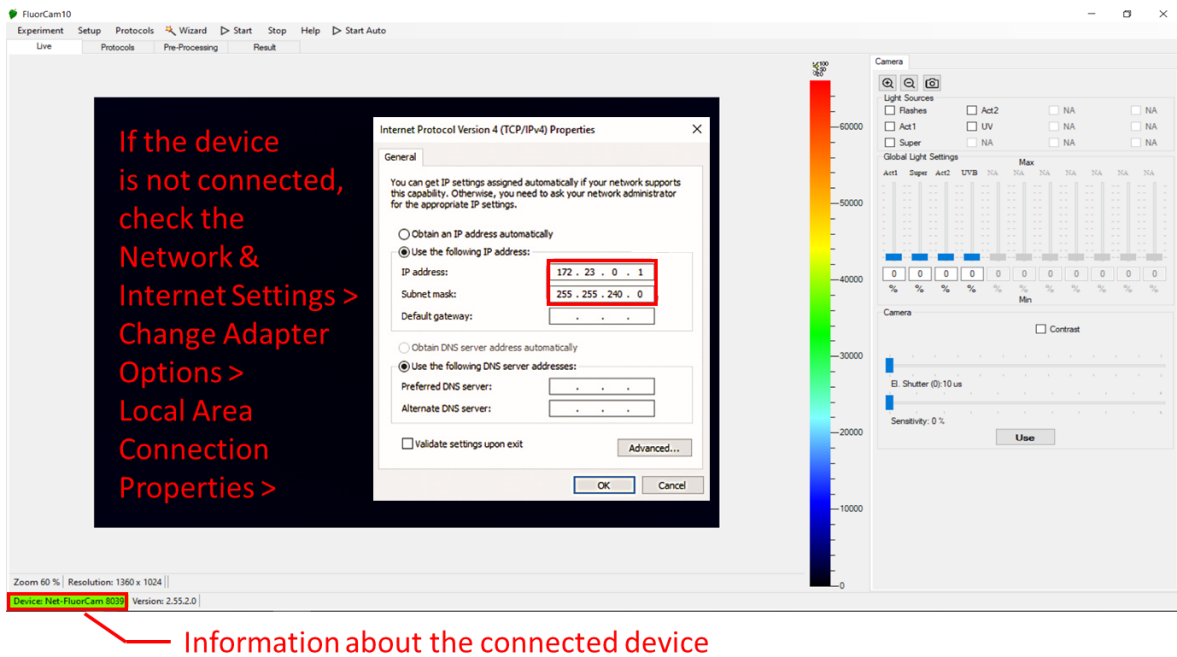


Fig. 19 Indication of properly connected FluorCam 1300/2020-C in FluorCam10 and Ethernet settings

6.2 SOFTWARE LICENSE AND AUTOMATION LICENSE

Unlike FluorCam7, the FluorCam10 is a licensed software. It is important to insert **FC10 License Code** into the software to activate most of functionalities of FluorCam10. The unique FC10 License Code is put onto the delivered USB flash drive in the folder “User Keys”. The FluorCam10 software will be licensed (Fig. 20) after pasting FC10 License Code in the following way: Help > About > Not Licensed > License Code > OK.

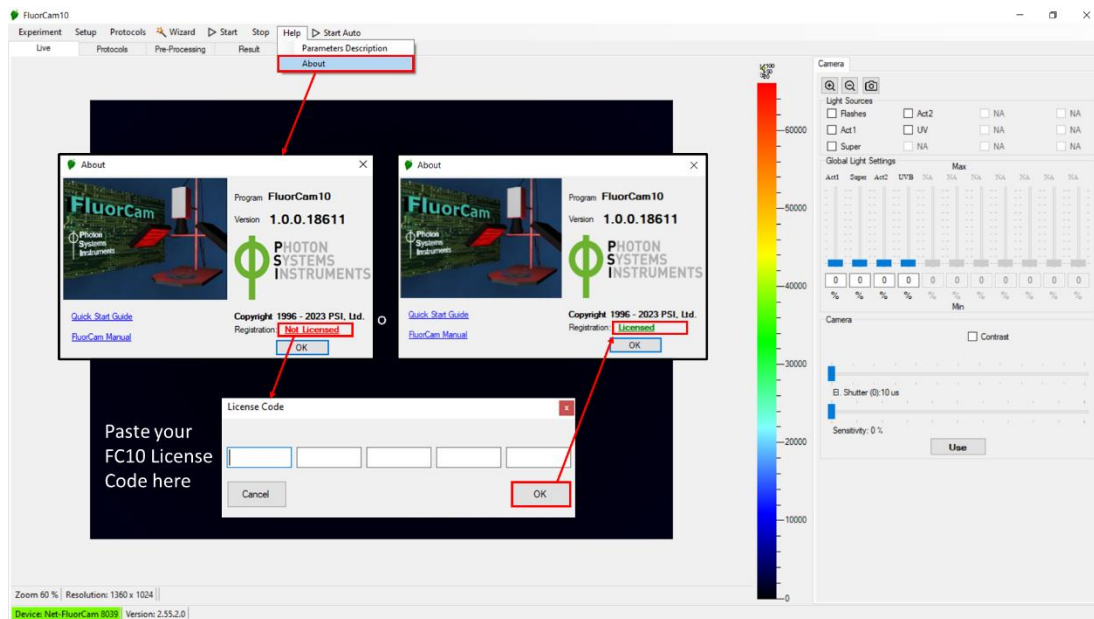


Fig. 20 Activation of FluorCam10

To activate the purchased **Advanced multiple function/Automation license**, the file with FC10 Automation License key has to be inserted into the software in the following way: Setup > General > Advanced > Select file > Close as described in the Figure 21. Then, the multiple protocols can be triggered by a click on “▶ Start Auto”.

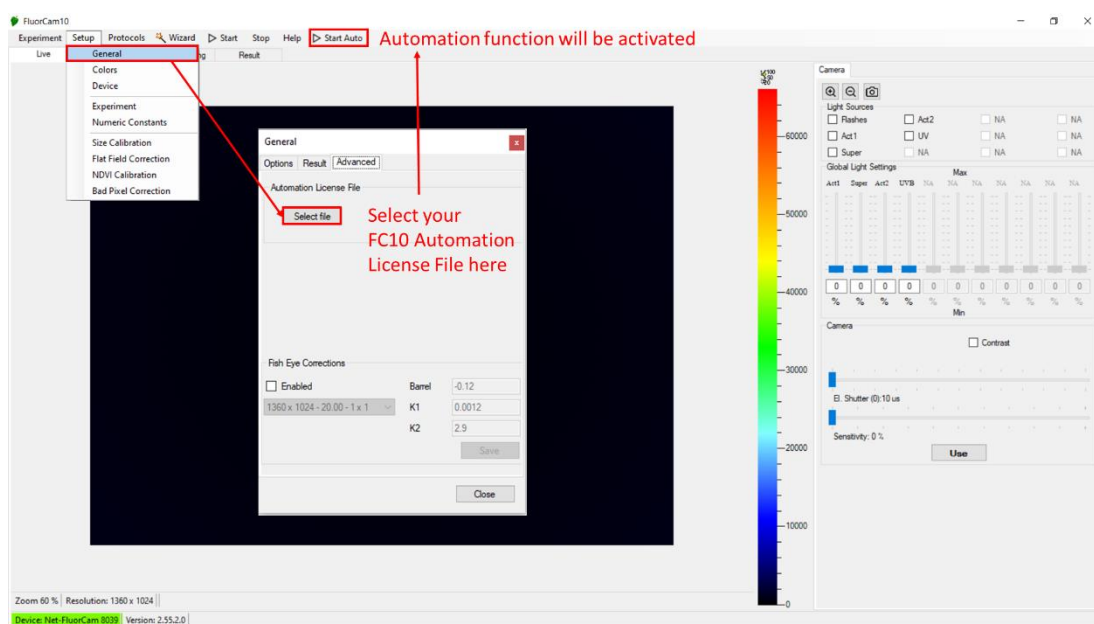


Fig. 21 Activation of the purchased Automation license

6.3 THE LIVE TAB

Then, the “**Live**” tab can be used for focusing the sample of interest (this is done manually) and selection of right parameters, such as light intensities, shutter, sensitivity and zoom, for the experiment. The predefined protocols are offered after a click on the “Wizard” button (Fig. 22). Please note that no automatic filter selection is possible in the Flat Handy FluorCam.

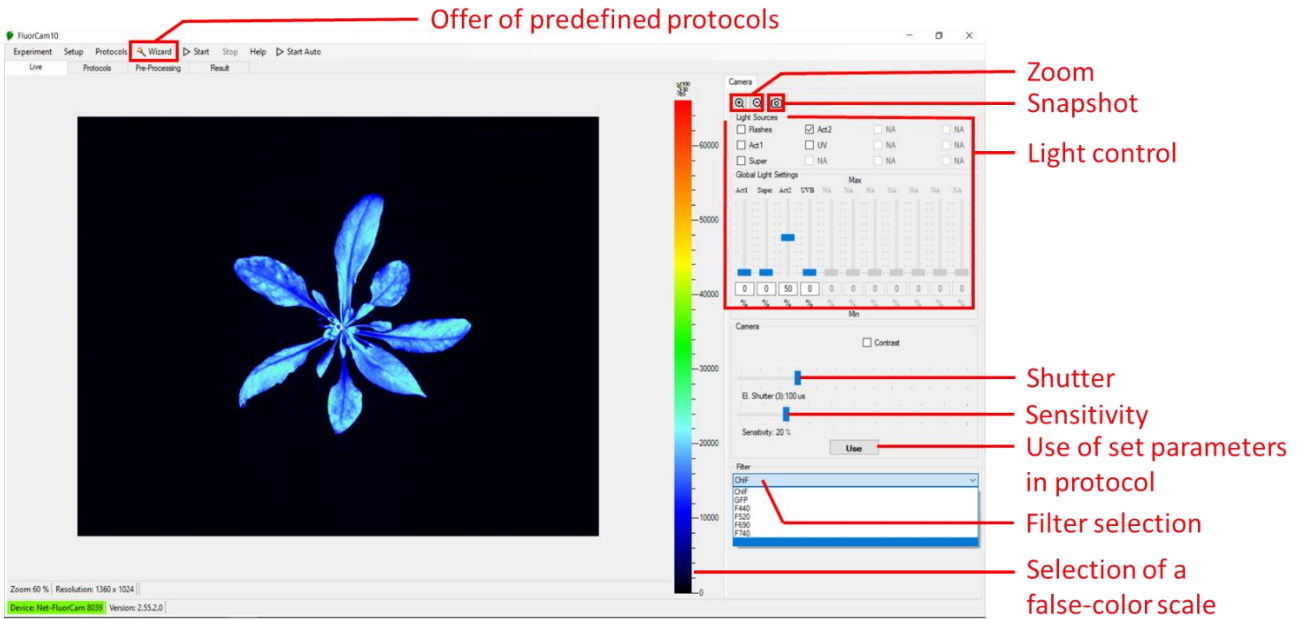


Fig. 22 The “Live” tab of the FluorCam10

The GFP protocol should be used after the manual change of the GFP filter and selection of the “**Snapshot mode**”. The “Live” tab can be switched from the “Video mode” into the “Snapshot mode” in the following way: Setup > Device > Mode SNAP > Set Device As > Close (Fig. 23). After setting parameters, the “**Get Frame**” button should be used to see the image.

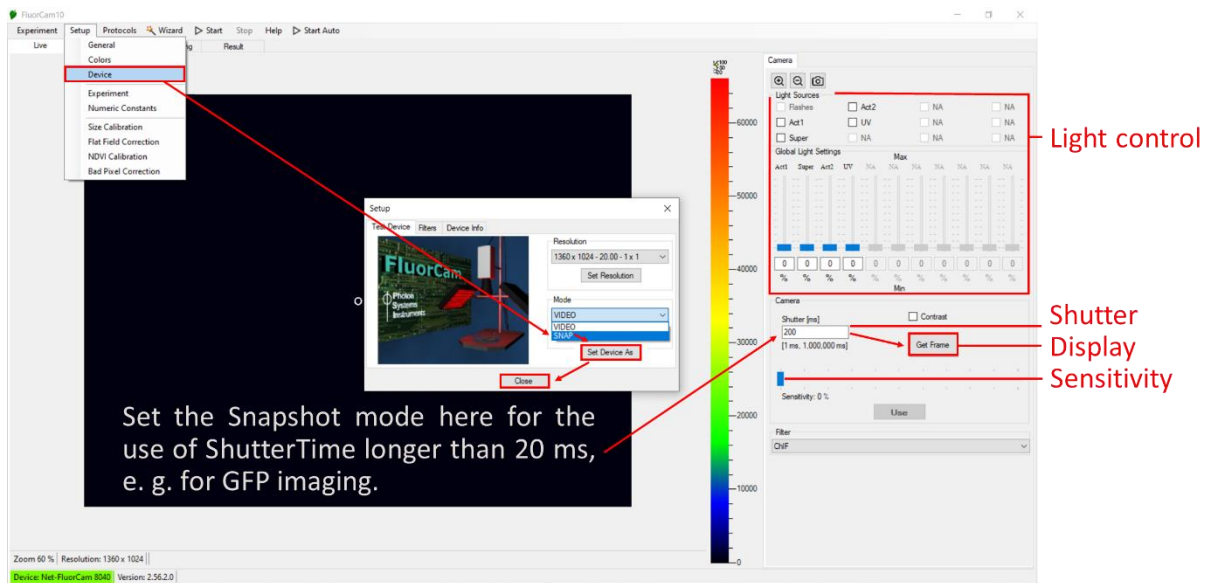


Fig. 23 The selection of the Snapshot mode in the FluorCam10

6.4 THE PROTOCOL TAB

The “Use” button can be used for import of desired parameters into the selected protocol in the “**Protocols**” tab. The protocol is triggered by the “Start” button as shown in the Figure 24.

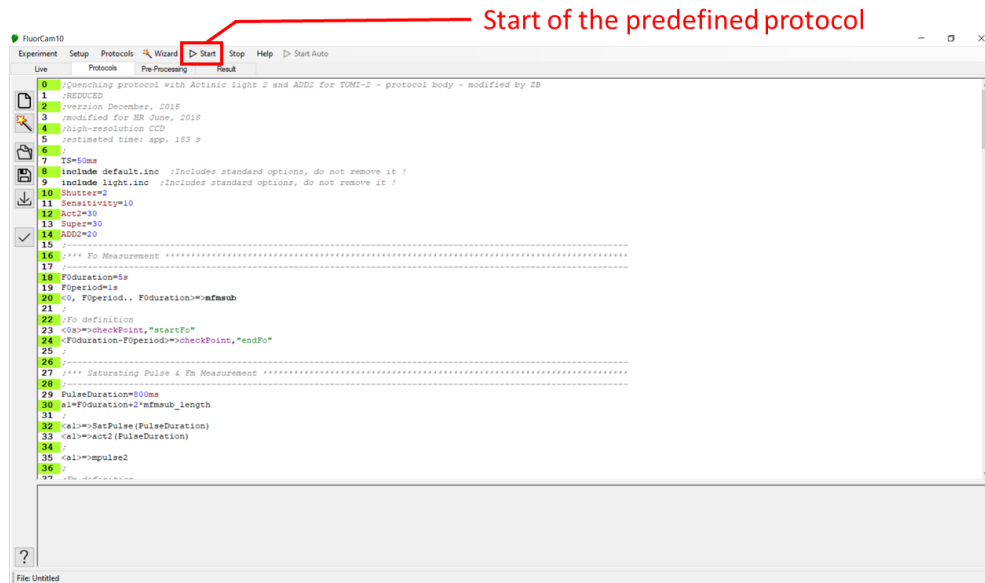


Fig. 24 The "Protocol" tab of FluorCam10

6.5 THE PRE-PROCESSING TAB

The desired mask can be selected in the "Pre-processing" tab (Fig. 25). The automatic mask is applied after choosing the "Background Exclusion" button. Small objects will be excluded from the analysis when the minimal object size is given. The mask can be defined manually as well. All masks can be saved and open in future experiments. After calibration of the FluorCam10 with the size calibration standard the predefined masks for Petri dishes and microtiter plates can also be applied.

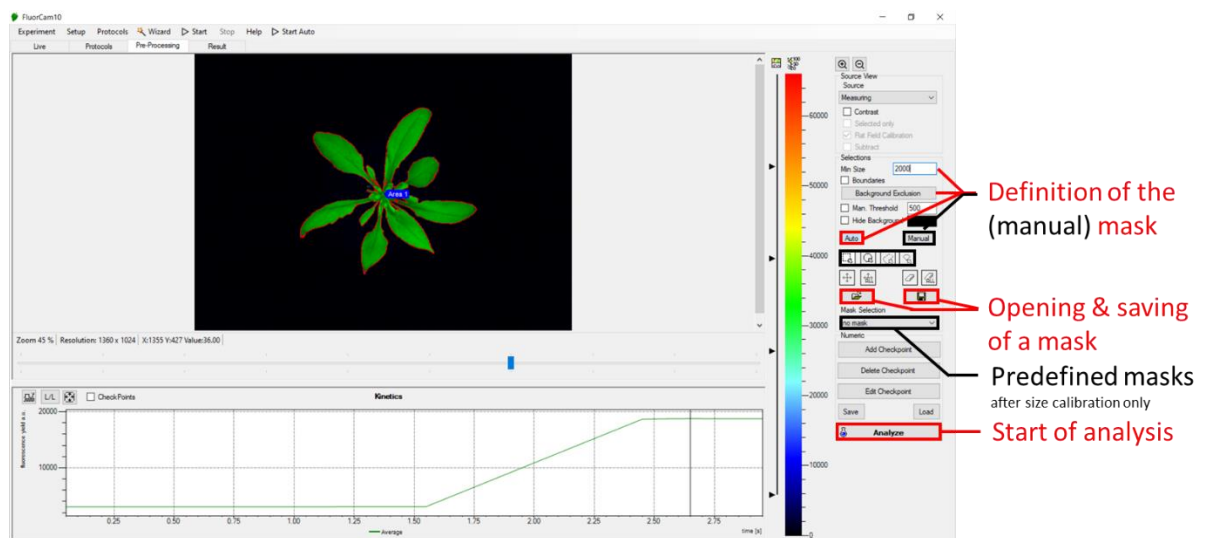


Fig. 25 The "Pre-Processing" tab of FluorCam10

6.6 THE RESULT TAB

The results (curves, parameters and images) can be visualized in the “Result” tab and saved or exported in Experiment menu (Fig. 26).

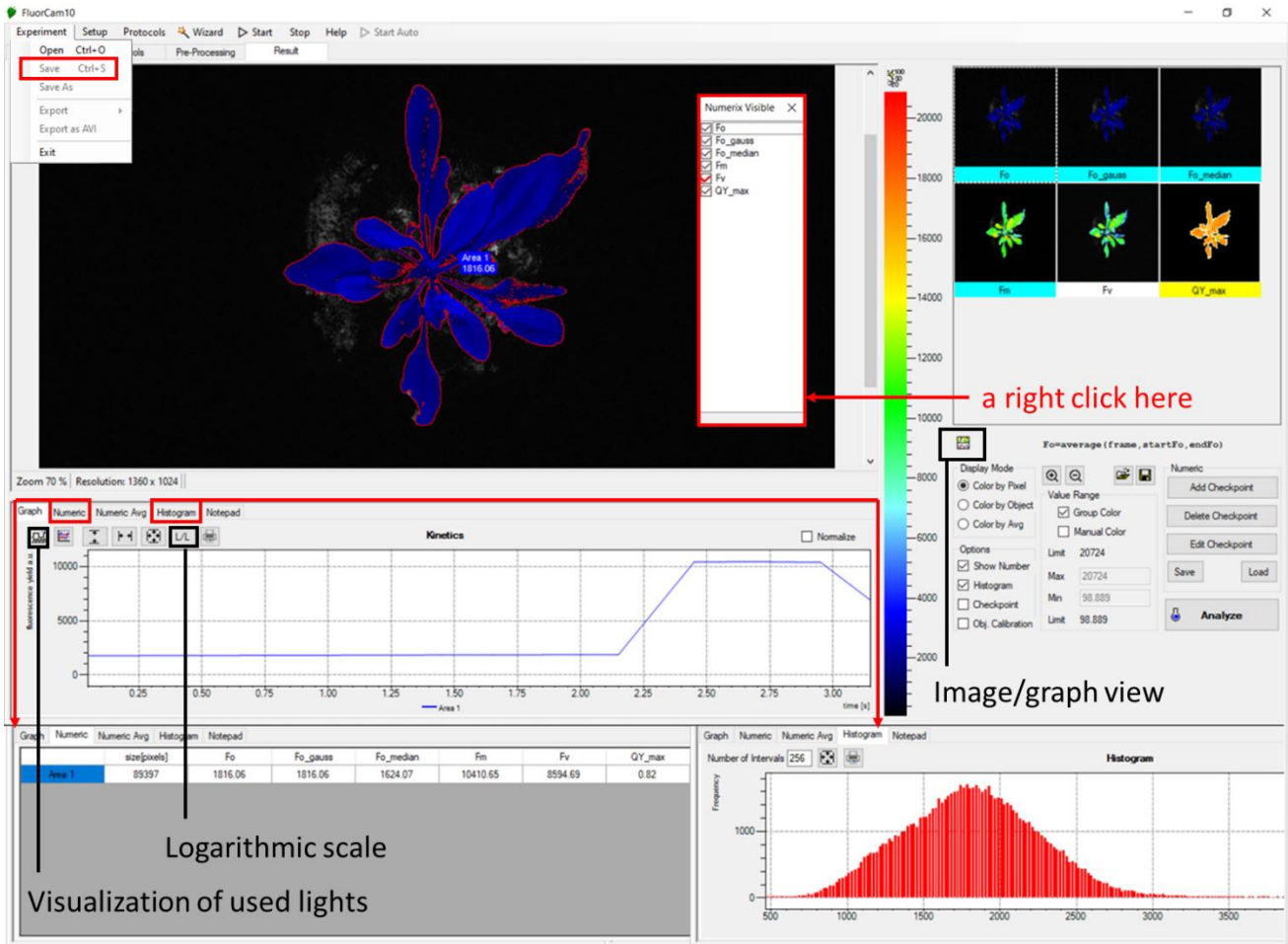


Fig. 26 The “Result” tab of FluorCam10

7 OPERATION

7.1 PRE-DEFINED PROTOCOLS

Various protocols (Tab. 2-7, Figs. 27-31) can be used for the chlorophyll fluorescence analysis. Based on the type of protocol different parameters can be measured and calculated.

In the dark- adapted state a “measuring light- flashes” are used to measure minimum value of chlorophyll fluorescence, termed F_0 . Measuring light is of intensity too low to induce electron transport through PSII but high enough to result in minimal level of fluorescence emitted. Application of saturating pulse to a dark-adapted leaf induces maximum level of fluorescence, termed F_M , by driving the closure of all reaction centers. At this phase in healthy plant no non-photochemical is occurring. The difference between F_M and F_0 is variable fluorescence, termed F_V . From this F_V/F_M parameter can be calculated, which is used as robust indicator of maximum quantum yield of PSII photochemistry (in healthy plants is 0.83). Most healthy plants have a very conservative value of F_V/F_M of about 0.8. With many abiotic stresses, this value declines markedly, and well before any visible indicators of stress are apparent visually. Thus, measurements of F_V/F_M can identify the onset of stress much more effectively than measuring the change in slope of a growth curve. Changes in F_V/F_M can also be used as alarms to trigger rescue protocols for stressed plants, such as irrigation of plants undergoing drought conditions.

To address the proportion of energy used for photochemical reaction and amount of energy used for non-photochemical heat dissipation, quenching protocols can be used. During quenching protocol dark adapted sample is illuminated is first illuminated with saturating pulse (for F_V/F_M calculation) and subsequently with actinic light. Initial rise in fluorescence is observed after actinic light application, which is subsequently quenched as a result of the increasing competition between photochemical and non-photochemical events. Number of saturating pulses can be applied during the actinic light exposure, which allows determine operating efficiency of PSII photochemistry (F_q'/F_M'), the level of photochemical quenching of PSII (F_q'/F_V') and the level of non-photochemical quenching (NPQ). The light curve protocol is used to quantify the rate of photosynthesis at gradually increasing photon irradiances using. The light curve protocol was proven to provide detailed information on ChlF under stress (Brestic and Zivcak, 2013).

Measured and calculated parameters depend on the used protocol that can be either measured in video mode with max shutter time of 20 ms or in shapshot mode with integration time up to several seconds. Measuring time depends on the used protocol. The protocols are pre-defined in the protocol menu specifically for the given actinic light used.

Tab. 2 List of protocols

List of protocols		
Protocol name	Description	Mode
Fv/Fm	Investigation of dark-adapted plant's maximum quantum yield of PSII.	VIDEO
Kautsky-Act1	Protocol for investigation of the dynamics of photosynthetic reactions during a transition from dark to light.	VIDEO
Quenching-Act1	Protocol for investigation of the transitions from the dark-adapted state to light-adapted state and back.	VIDEO
Quenching FAR-Act1	Protocol for investigation of the transitions from the dark-adapted state to light-adapted state and back. Includes FAR illumination for measurement of F_0' .	VIDEO
Light Curve-Act1	Light Curve protocol in FC is designed to measure quenching analysis in light adapted state at different light irradiances.	VIDEO
GFP (for Flat Handy GFPCam only)	Green fluorescence protein detection protocol	SNAP



Please note for the measurement of the dark-adapted F_m and F_0 values, or the fluorescent signal of proteins, the imaging must be performed in complete darkness without any ambient light interference.

7.1.1 FV/FM PROTOCOL

FV/FM protocol enables determination of QY_{max} (F_v/F_m parameter, Fig. 27, Tab. 3). This robust indicator of maximum quantum yield of PSII photochemistry reaches in healthy plants 0.83.

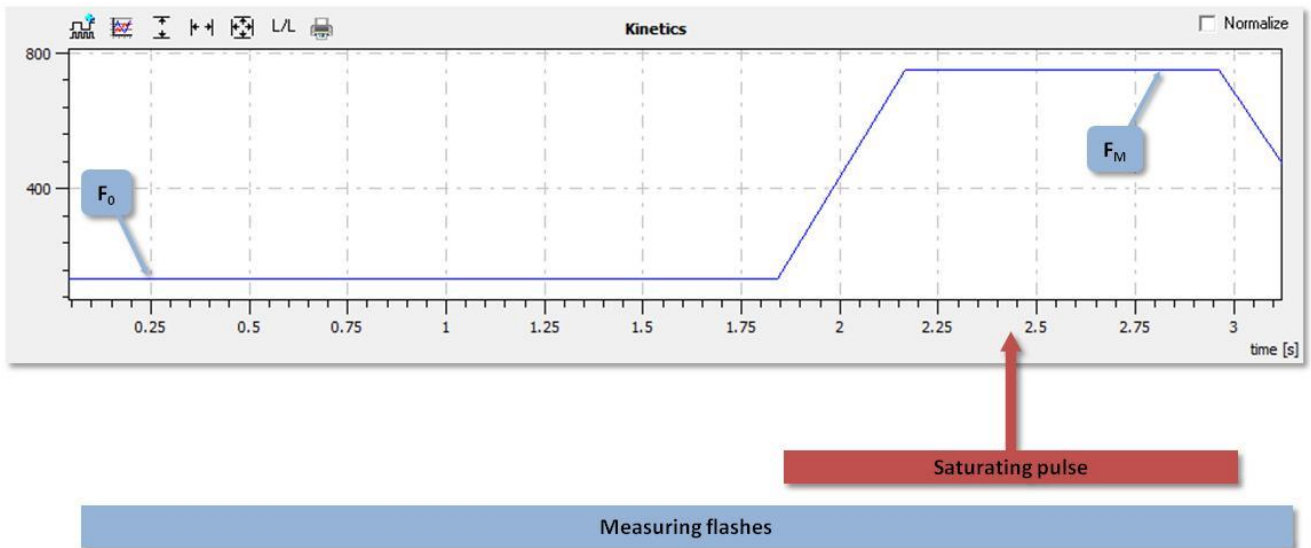


Fig. 27 Schematics of Fv/Fm protocol for dark-adapted plant samples.

Protocol duration 3-6 seconds.

Tab. 3 List of parameters in the Fv/Fm protocol

F _v /F _m protocol		
F ₀	Measured	Minimum fluorescence in dark-adapted state
F _m	Measured	Maximum fluorescence in dark-adapted state
F _v	F _m – F ₀	Variable fluorescence in dark-adapted state
F _v /F _m (QY _{max})	F _v / F _m	Maximum PSII quantum yield

7.1.2 KAUTSKY CURVE PROTOCOL

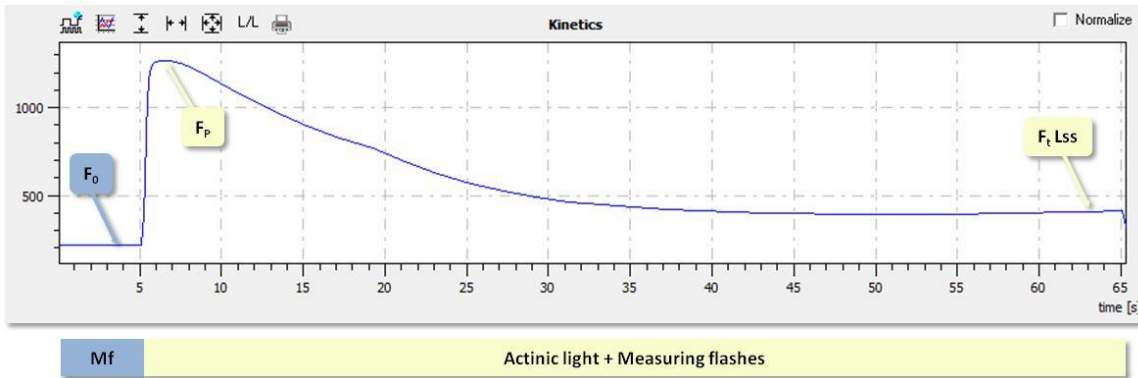


Fig. 28 Schematics of Kautsky curve protocol. Requires dark-adaptation of plant samples.

Protocol duration of 60 seconds.

Tab. 4 List of parameters in the Kautsky protocol

Kautsky protocol		
F_0	Measured	Minimum fluorescence in dark-adapted state
F_p	Measured	Peak fluorescence during the initial phase of the Kautsky effect
F_t_Lss	Measured	Steady-state fluorescence in light
F_v/F_m (QY_max)	F_v / F_m	Maximum PSII quantum yield
Rfd_Lss	$(F_p - F_t_Lss) / F_t_Lss$	Fluorescence decline ratio in steady-state

7.1.3 QUENCHING PROTOCOL

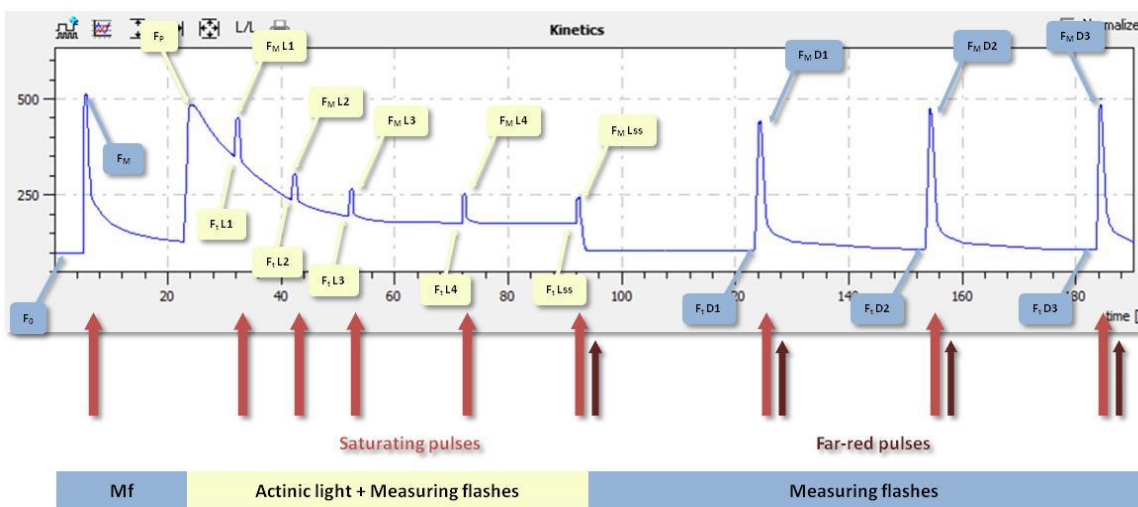


Fig. 29 Schematics of quenching analysis protocol (Quenching Act1 or 2)

It is used to quantify photochemical and non-photochemical quenching capacity of the plants. Requires dark-adaptation of plant samples. Protocol duration of approx. 180 seconds.

Tab. 5 List of parameters in the Quenching protocol

Quenching protocol			
F_0	Measured	Minimum fluorescence in dark-adapted state	
F_{0_gauss}			
F_{0_median}			
F_M	Measured	Maximum fluorescence in dark-adapted state	
F_V	$F_M - F_0$	Variable fluorescence in dark-adapted state	
F_P	Measured	Peak fluorescence during the initial phase of the Kautsky effect	
$F_P - median$	Measured		
$F_P - gauss$	Measured		
$F_P - average$	Measured		
F_{M_Ln}	Measured	Maximum fluorescence during light adaptation	
F_{M_Lss}	Measured	Steady-state maximum fluorescence in light	
F_{M_Dn}	Measured	Instantaneous maximum fluorescence during dark relaxation	
F_{t_Ln}	Measured	Instantaneous fluorescence during light adaptation	
F_{t_Lss}	Measured	Steady-state fluorescence in light	
F_{t_Dn}	Measured	Instantaneous fluorescence during dark relaxation	
F_{0_Ln}	$(F_0 / ((F_V / F_M) + (F_0 / F_{M_Ln})))$	Minimum fluorescence during light adaptation	
F_{0_Lss}	Quenching with FAR lights	Quenching without FAR	Steady-state minimum fluorescence in light
F_{0_Dn}	Quenching with FAR lights	Quenching without FAR	Minimum fluorescence during dark relaxation
F_{V_Ln}	$F_{M_Ln} - F_{0_Ln}$	Variable fluorescence in light	
F_{V_Lss}	$F_{M_Lss} - F_{0_Lss}$	Variable fluorescence in light-adapted state	
F_{V_Dn}	$F_{M_Dn} - F_{0_Dn}$	Variable fluorescence during dark relaxation	
F_{q_Ln}	$F_{M_Ln} - F_{t_Ln}$	Difference in fluorescence between F_{M_Ln} and F_{t_Ln} in light	
F_{q_Lss}	$F_{M_Lss} - F_{t_Lss}$	Difference in fluorescence between F_{M_Dn} and F_{t_Dn} in light adapted state	
F_{q_Dn}	$F_{M_Dn} - F_{t_Dn}$	Difference in fluorescence between F_{M_Dn} and F_{t_Dn} during dark relaxation	
F_V / F_M (QY_max)	F_V / F_M	Maximum quantum efficiency of PSII photochemistry	
F_V / F_{M_Ln}	F_{V_Ln} / F_{M_Ln}	PSII maximum efficiency of light adapted sample	
F_V / F_{M_Lss}	F_{V_Lss} / F_{M_Lss}	PSII maximum efficiency of light adapted sample in steady-state	
F_V / F_{M_Dn}	F_{V_Dn} / F_{M_Dn}	PSII maximum efficiency during dark relaxation	
QY_Ln	F_{q_Ln} / F_{M_Ln}	Instantaneous PSII quantum yield during light adaptation	
QY_Lss	F_{q_Lss} / F_{M_Lss}	Steady-state PSII quantum yield	
QY_Dn	F_{q_Dn} / F_{M_Dn}	Instantaneous PSII quantum yield during dark relaxation	
NPQ_Ln	$(F_M - F_{M_Ln}) / F_{M_Ln}$	Instantaneous non-photochemical quenching during light adaptation	
NPQ_Lss	$(F_M - F_{M_Lss}) / F_{M_Lss}$	Steady-state non-photochemical quenching	
NPQ_Dn	$(F_M - F_{M_Dn}) / F_{M_Dn}$	Instantaneous non-photochemical quenching during dark relaxation	
qN_Ln	$(F_M - F_{M_Ln}) / (F_{M_Ln} - F_{0_Ln})$	Coefficient of non-photochemical quenching during light adaptation	
qN_Lss	$(F_M - F_{M_Lss}) / (F_{M_Lss} - F_{0_Lss})$	Coefficient of non-photochemical quenching in steady state	
qN_Dn	$(F_M - F_{M_Dn}) / (F_{M_Dn} - F_{0_Dn})$	Coefficient of non-photochemical quenching during dark relaxation	
qP_Ln	F_{q_Ln} / F_{V_Ln}	Coefficient of photochemical quenching during light adaptation	
qP_Lss	F_{q_Lss} / F_{V_Lss}	Coefficient of photochemical quenching in steady-state	
qP_Dn	F_{q_Dn} / F_{V_Dn}	Coefficient of photochemical quenching during dark relaxation	
qL_Ln	$(F_{q_Ln} / F_{V_Ln}) * (F_0 / F_{t_Ln})$	Fraction of PSII centers that are 'open' during light adaptation	
qL_Lss	$(F_{q_Lss} / F_{V_Lss}) * (F_0 / F_{t_Lss})$	Fraction of PSII centers that are 'open' in steady state	
qL_Dn	$(F_{q_Dn} / F_{V_Dn}) / (F_{0_Dn} / F_{t_Dn})$	Fraction of PSII centers that are 'open' during dark relaxation	
Rfd_Ln	$(F_P - F_{t_Ln}) / F_{t_Ln}$	Instantaneous fluorescence decline ratio in light	
Rfd_Lss	$(F_P - F_{t_Lss}) / F_{t_Lss}$	Fluorescence decline ratio in steady-state	

7.1.4 LIGHT CURVE PROTOCOL

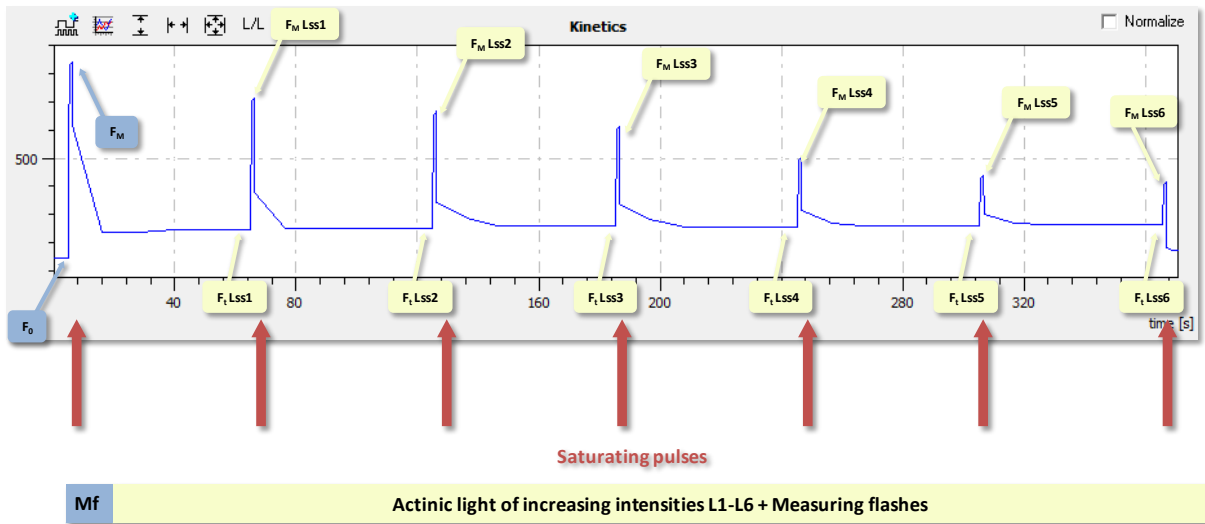


Fig. 30 Schematics of rapid light curve protocol (Light curve Act1 or 2).

Requires dark-adaptation of plant samples. Protocol duration of approx. 360 seconds.

Tab. 6 List of parameters for the light curve protocol

Light curve protocol		
F ₀	Measured	Minimum fluorescence in dark-adapted state
F _M	Measured	Maximum fluorescence in dark-adapted state
F _V	F _M -F ₀	Variable fluorescence in dark-adapted state
F _{M_LSS}	Measured	Steady-state maximum fluorescence in light
F _{t_LSS}	Measured	Steady-state fluorescence in light
F _{0_LSS}	$F_0 / ((F_V/F_M) + (F_0/F_{M_LSS}))$	Steady-state minimum fluorescence in light
F _{V_LSS}	F _{M_LSS} -F _{0_LSS}	Variable fluorescence in light-adapted state
F _{q_LSS}	F _{M_LSS} -F _{t_LSS}	Difference in fluorescence between F _{M_Dn} and F _{t_Dn} in light adapted state
F _{V/F_M} (QY_max)	F _V / F _M	Maximum PSII quantum yield
F _{V/F_M_LSS}	F _{V_LSS} /F _{M_LSS}	PSII quantum yield of light adapted sample in steady-state
QY_LSS	F _{q_LSS} / F _{M_LSS}	Steady-state PSII quantum yield
NPQ_LSS	(F _M - F _{M_LSS})/F _{M_LSS}	steady-state non-photochemical quenching
qN_LSS	(F _M -F _{M_LSS})/(F _M -F _{0_LSS})	Coefficient of non-photochemical quenching in steady state
qP_LSS	F _{q_LSS} /F _{V_LSS}	Coefficient of photochemical quenching in steady-state
qL_LSS	(F _{q_LSS} /F _{V_LSS}) / (F _{0_LSS} /F _{t_LSS})	Fraction of PSII centers that are 'open' in steady state
PAR*	LightA * LightIntensity + LightB	Photosynthetically active radiation calculated from light calibration curves
ETR_LSS	0.8 * 0.5 * QY_LSS * PAR	Calculated electron transport rate in steady-state

* For PAR calculation, please add the values for Coefficient A and Coefficient B from Light Intensity Calibrations sheet into the protocol body row __LightA and __LightB. Use the coefficient for the given actinic light used in the light curve protocol.

7.1.5 GFP PROTOCOL

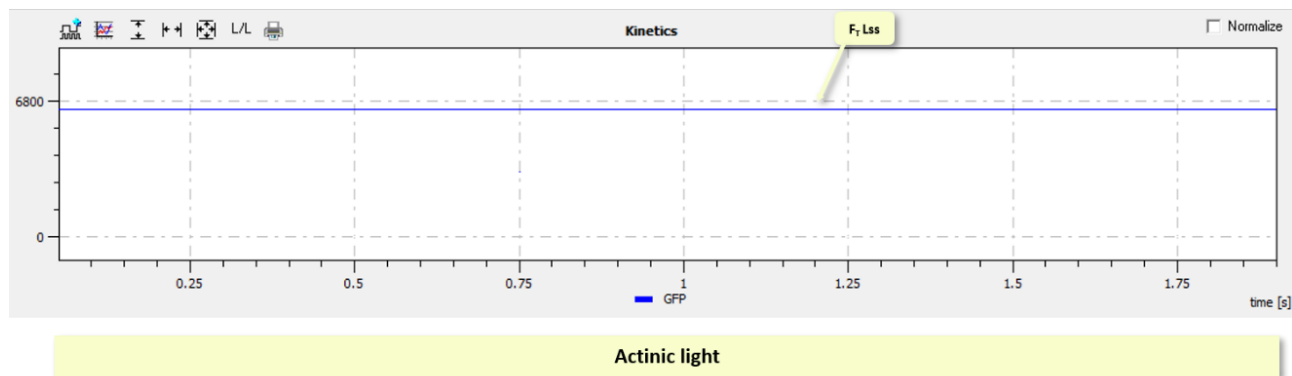


Fig. 31 Schematics of protocol for GFP detection

Tab. 7 List of parameters for GFP detection protocol

FPs protocols		
F _t _Lss	Measured	Steady-state fluorescence in light

7.2 HOW TO START

There are few simple steps the user should follow before starting fluorescence measurement with FluorCam. The steps are briefly described in the following section (a detailed description of the individual steps is in the next sections).

1. Define the FluorCam protocol or Wizard used for the measurement. Select the protocols from the Wizard Protocol Menu or open previously used and/or modified protocol
2. Optimize the measurement settings. Adjust the camera settings, such as electronic shutter and sensitivity and set the light intensity used for the measurement (actinic light intensity or intensity of some of the additional lights).
3. Perform the test measurement with the optimized protocol and defined settings.
4. Start the protocol with optimized settings. Analyze and store obtained results.

7.2.1 PROTOCOL SELECTION AND INITIAL SET-UP

Before starting a measurement, the user needs to decide on the type of measuring protocol to be used. After selecting the protocol, a number of settings needs to be optimized, such as light intensity, sensitivity and shutter duration. In the steps below, it is described how to find correct settings for light and camera before starting a real experiment.

CAMERA MODE (ONLY for imaging of fluorescent proteins): Switch the camera from the video mode to the snapshot mode, since during measurement of the fluorescence proteins it is necessary to use longer integration time. Go to the upper ribbon menu Setup/Device and select Snapshot mode.

PROTOCOL: Open the protocol Menu and choose the measuring protocol. There is a set of predefined protocols or user alone can modify the protocols (e.g., duration of the light phase) by using Wizard function.

The predefined protocol F_V/F_M is a simple protocol determining F₀, F_M and F_V/F_M. This can be used to check if Saturating pulse is strong enough before running any other more complicated protocol.

CAMERA SETTINGS: Let measuring flashes switched on and adjust EI. Shutter and Sensitivity in LIVE window. Change false-color scale to Extended spectrum or Extended spectrum 3_0_3 (the most sensitive color scales for human eye). Keep EI. Shutter as low as possible (between 1-2), otherwise measuring pulses would be too strong causing actinic effect. Adjust Sensitivity by trucking the bar to get a signal in the range of 2000-2500 digits for (dark blue or blue color). For getting signal with less noise, keep the Sensitivity at lower level if possible (up to 20 – 30 %, 40 % max).

LIGHT SETTINGS – ACTINIC LIGHT OR ADDITIONAL LIGHT: Choose the intensity of Actinic light (Act1): (a) either desired absolute light intensity can be chosen with respect to cultivation conditions, or (b) it can be adjusted according to the fluorescence transient.

Light calibration curves for all lights available in the FC panel are part of the documentation provided and allow the conversion of the light intensity recorded in % to $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Let measuring flashes switched on and adjust Actinic light intensity by trucking the bar in Global light settings table in LIVE window. An intensity between 20 – 50 % is usually strong enough for plants grown in chambers.

After choosing the Actinic light intensity, test the image quality: turn on the measuring pulses and the desired actinic light and check if the signal is not too low or saturating. If the fluorescence signal is too low, increase the Sensitivity to use a best adynamic scale. TOMI-2 CCD camera A/D converter is 16-bit, therefore the signal can be acquired on the scale 0 – 65 536. If the signal is saturated, an error message will appear. In this case, it is necessary to decrease Sensitivity or both Sensitivity + El. Shutter.

LIGHT SETTINGS – SATURATING PULSE INTENSITY: Truck the Super bar in Global light settings table in LIVE window to set the proper intensity of saturating pulse and then mark check box Super in Light Sources panel. The saturating pulse is only switched for limited time (800 ms), so it must be switched on each time the intensity is changed. Finally, test image quality with all 3 lights (Flashes + Actinic light Act1 + Super) switched on as described above.

IMPORTING SETTINGS TO PROTOCOL: Click button Use in the bottom of LIVE window to import camera and light settings to the protocol.

TEST THE SETTINGS: Start Experiment in the top panel to launch the measurement. In the PRE-PROCESSING window select areas of interest, press button Analyze and observe the results (kinetics and parameters) in RESULT window for estimation of FC settings correctness. If the settings are correct (e.g., healthy green plant has QY_{max} value of 0.83), proceed with next step, if not, repeat the entire procedure.

SAVING RESULTS: Apply the protocol with correct settings. Analyze the results using the manual or automatic mask and save the experiment.



For a more detailed description of the FC measurement protocol set-up, please also refer to FluorCam SW Manual.

7.2.2 SIZE CALIBRATION

The image recorded by a FluorCam system can be calibrated from pixels to real size units such as millimeters (mm^2) or inches. One of the applications, inevitably coupled with this function, is an employment of pre-defined masks for Petri dishes or micro-titrate plates for automated image analysis in Pre-processing window of FluorCam10 application.

The size calibration is a simple algorithm based on automated recognition of fluorescent object of known dimension/area. The Size calibration standard (Fig. 20) is supplied with each FluorCam system for optimal calibration. This procedure is optimized for chlorophyll filter. Please note, that calibration standards would have different dimensions according to the type of your device. The size of the standard is always listed directly on it – on the back or front side (see size of calibration standard on Fig. – 400 mm^2).

5. Put calibration standard under FluorCam camera approximately to the same position (distance) as your experimental material (leaf or plant) would be placed – usually on the top shelf. Adjust focus (Fig. 33-A)
6. In top panel menu in FluorCam10 application, select Setup (Fig. 33-B) and then Size Calibration. Once the message INSERT CALIBRATION PLATE appears, the excitation light is turned on and software starts to adjust optimal exposure settings automatically.
7. Place fluorescent area of the calibration standard to the center of the field of view, wait until the image is stable and confirm it by button OK.
8. The new pop-up window invites you to enter the size of identified area in mm^2 . The correct value is typically written on the bottom of the calibration standard (Fig. 32).



Fig. 32 Fluorescence standard

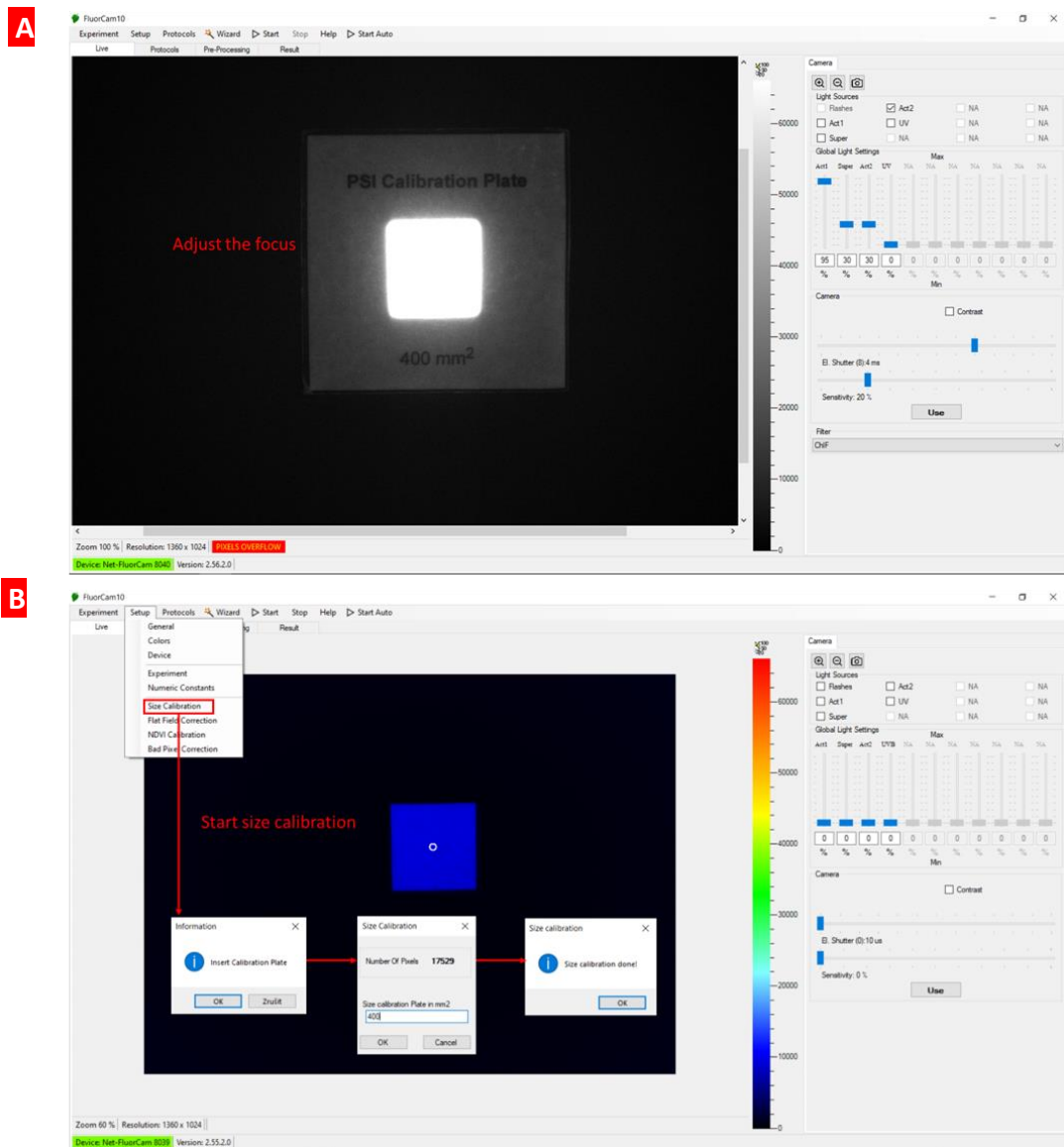


Fig. 33 Size calibration setup



The system must be re-calibrated whenever the distance between experimental object and camera is changed. Once the system is calibrated, this information is saved together with measured experiment. Thus, the Size calibration must be done in advance, before the experiment is measured.

After successful Size calibration, the Area of analyzed object in Result window is displayed in mm² or inches instead of number of pixels.

7.2.3 BAD PIXEL CORRECTION

The appearance of bad pixels in the image can be fixed by the Bad Pixel Correction:

1. Cover the lens with a lens cap.
2. In top panel menu in FluorCam10 application, select Setup (Fig. 34) and then Bad Pixel Correction. Once the message COVER OBJECTIVE appears, the software starts to detect bad pixels automatically.
3. Wait until the calibration is done and confirm it by button OK.
4. The application of the Bad pixel correction is enabled in the Setup/Experiment menu after selection of the Bad Pixel Correction.

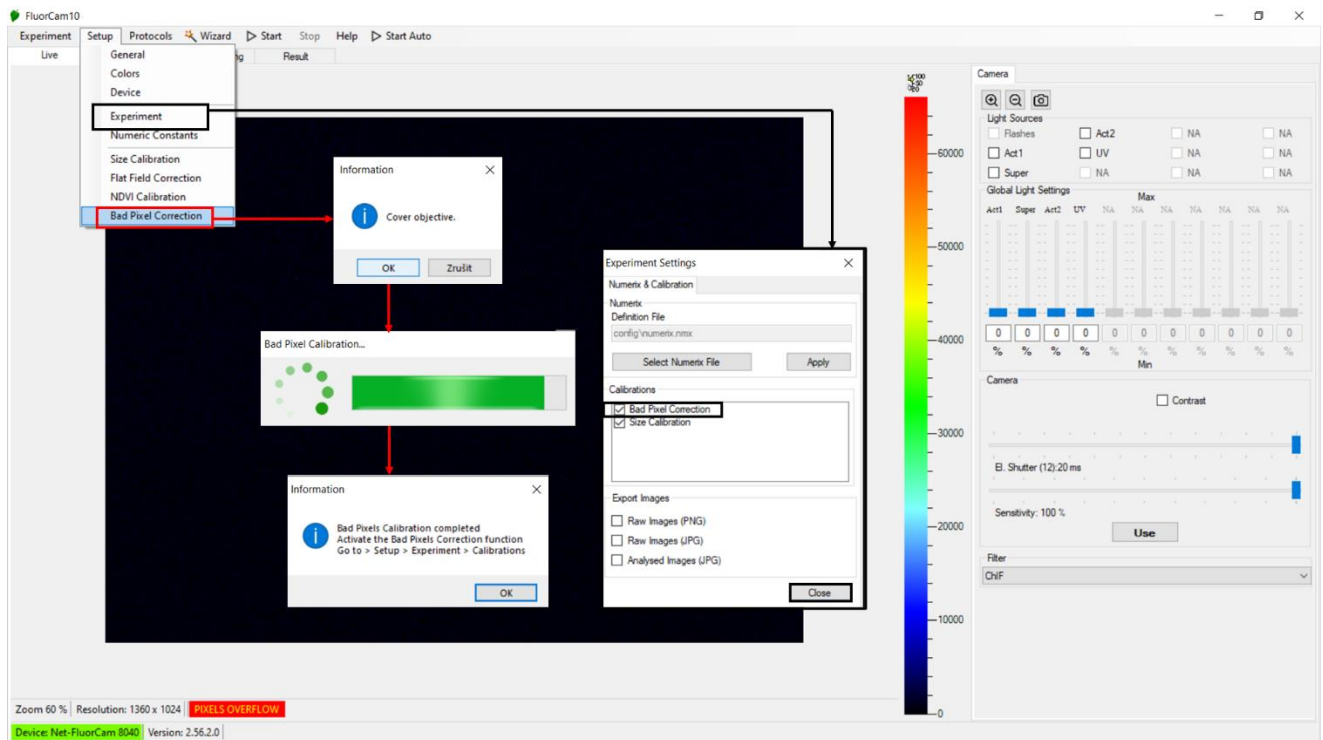


Fig. 34 Bad pixel correction setup

7.3 SMALL ACCESSORIES

Safety goggles are always a part of the Flat Handy FluorCam package. Safety goggles must be worn every time, where is possibility of the eye contact with the turned-on LED panels. All the FluorCam LED panels are class 1M LED Product. As LED radiation may be harmful to eyes, avoid direct and strongly reflected exposure and use protective glasses when necessary.

A flash USB driver is added to each Flat Handy FluorCam package, it contains:

- FluorCam10 application,
- video tutorial for FluorCam10 application operation,
- FluorCam operational and technical manuals,
- light calibration curves especially assessed for each device,
- demo data (measured example experiments with ideal camera and light settings),
- additional applications,
- user key for activation of the FluorCam10 software and optionally a key for unlocking of the Automated function.

8 FLUORCAM KEYWORDS

Flashes – for accurate measurement of minimal fluorescence (F_0) determination. The duration of flashes is controlled by shutter time. Try to keep shutter as low as possible (low resolution CCD between 0-1, high resolution CCD between 1 – 2), otherwise measuring pulses would be too strong causing actinic effect.

Actinic light 1 – (here usually white or royal blue) corresponds to the ambient light in which the plants are growing

Saturating pulse – Short and strong pulse of light used for maximal fluorescence (F_M value) determination

FAR – far-red light for determination of parameter F_0

TOMI-2 – High resolution CCD camera

Binning - the procedure of combining a cluster of pixels into a single pixel

Shutter (time) – controls the exposure time. It is the length of time during which light is admitted by the image sensor of the camera. Controls also duration of the measuring flashes. User can usually choose shutters 0 – 12 representing values in range 0 μ s – 20 ms.

Sensitivity – The sensitivity of the camera sensor to light

Protocol – predefined set of instructions for the Fluorcam

Video mode – automated measurement defined by the protocol. Video mode is used for measurement of chlorophyll fluorescence protocols. If the flashes are not used in the measurement, maximal shutter time is set to 20 ms.

Snap mode – capturing the single images with longer shutter times (up to 1 000 seconds). This mode is useful for measurement of fluorescent proteins, where long integration time is necessary. Measuring in snap mode can be also automated using the protocol.

Size calibration – for image calibration from pixels to real size units such as millimeters (mm^2) or inches.

Background exclusion – button used for excluding background and the objects (mold, algae, ...), which also reflects the light, from analyzed area.

Emission filters - transmit a wavelength range that corresponds to the respective fluorophore emission spectrum, while blocking out unwanted wavelengths

Fluorescence standard – is made of pink fluorescence material, which reacts very strongly to excitation illumination and is used for different system calibrations (size calibration, focusing etc.)

Wizard menu – contains Protocol menu with the list of predefined protocols and Wizard type window where user can modify settings of the given protocols.

Fluorescence protein measurement - detection and imaging of different fluorescent proteins, mostly of green fluorescent protein (GFP), red-shifted GFP (EGFP). The FluorCam device must be then equipped with LED panels with appropriate wavelength for selected fluorescent protein excitation and a emission filter for correct fluorescence detection.

9 WARRANTY CONDITIONS

1. Photon Systems Instruments, Ltd. (PSI) warrants all its instruments to be free from defects in materials or workmanship for a period of **one year** from the date of invoice/shipment from PSI.
2. If at any time within this warranty period the instrument does not function as warranted, return it and PSI will repair or replace it **at no charge**. The customer is responsible for shipping and insurance charges (for the full product value) to PSI. PSI is responsible for shipping and insurance on return of the instrument to the customer.
3. No warranty will apply to any instrument that has been (i) modified, altered, or repaired by persons unauthorized by PSI; (ii) subjected to misuse, negligence, or accident; (iii) connected, installed, adjusted, or used otherwise than in accordance with the instructions supplied by PSI.
4. The warranty is return-to-base only, and does not include on-site repair charges such as labor, travel, or other expenses associated with the repair or installation of replacement parts at the customer's site.
5. PSI repairs or replaces the faulty instruments as quickly as possible; maximum time is one month.
6. PSI will keep spare parts or their adequate substitutes for a period of at least five years.
7. Returned instruments must be packaged sufficiently so as not to assume any transit damage. If damage is caused due to insufficient packaging, the instrument will be treated as an out-of-warranty repair and charged as such.
8. PSI also offers out-of-warranty repairs. These are usually returned to the customer on a cash-on-delivery basis.
9. **Wear & Tear Items** are excluded from this warranty. The term **Wear & Tear** denotes the damage that naturally and inevitably occurs as a result of normal use or aging even when an item is used competently and with care and proper maintenance.
10. Some PSI instruments use accessories made by other manufacturers. In such case, these accessories may be covered by a different warranty period.
11. Contact us at support@psi.cz in case of any support with the assembly and installation of the device is needed.