

## Multicolor Fluorescence Measurement in FluorCam10

UV light (360 or 385 nm) can induce multicolor fluorescence, which provides information about both primary and secondary plant metabolism. FluorCam captures chlorophyll and blue-green fluorescence in the following bands:

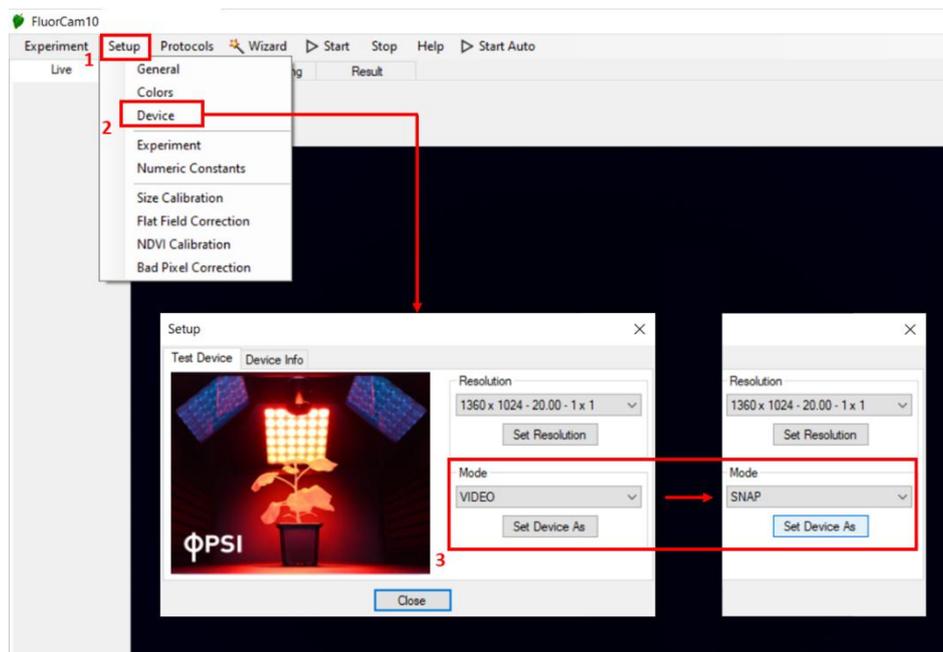
- **440 nm** – Blue
- **520 nm** – Green
- **690 nm** – Red
- **740 nm** – Infrared (IR)

### Quick Start Guide:

#### 1. Switch the measurement mode from Video to Snapshot.

This step is necessary because multicolor fluorescence measurement requires a longer shutter time (100 ms or more), which is not available in Video mode.

- In the upper ribbon menu, select **Setup** (Fig. 1-1), then **Device** (Fig. 1-2).
- When the pop-up window appears, set the **Measuring mode** to **Snapshot** (Fig. 1-3), then press **Set Device As** and **Close**.



**Fig. 1** Setting of the Snapshot mode



**PSI (Photon Systems Instruments), spol. s r.o.**  
Průmyslová 470, 664 24 Drásov  
Czech Republic  
VAT number: CZ60646594  
Trade Register number: C 27560



**Phone:**  
+420 511440012  
+420 511440034  
**Fax:**  
+420 511440901



**E-mail:**  
Inquiries: [info@psi.cz](mailto:info@psi.cz)  
Inquires and orders: [ordering@psi.cz](mailto:ordering@psi.cz)  
Technical support: [support@psi.cz](mailto:support@psi.cz)  
Shipping: [shipping@psi.cz](mailto:shipping@psi.cz)

## 2. Open the Protocol and Wizard Menu.

- In the upper menu, select the **Protocol and Wizard Menu** icon (Fig. 2-1).
- When the **Select Menu Protocol or Wizard Type** window appears, select **Multicolor** from the **Wizard type** section on the right (Fig. 2-2).

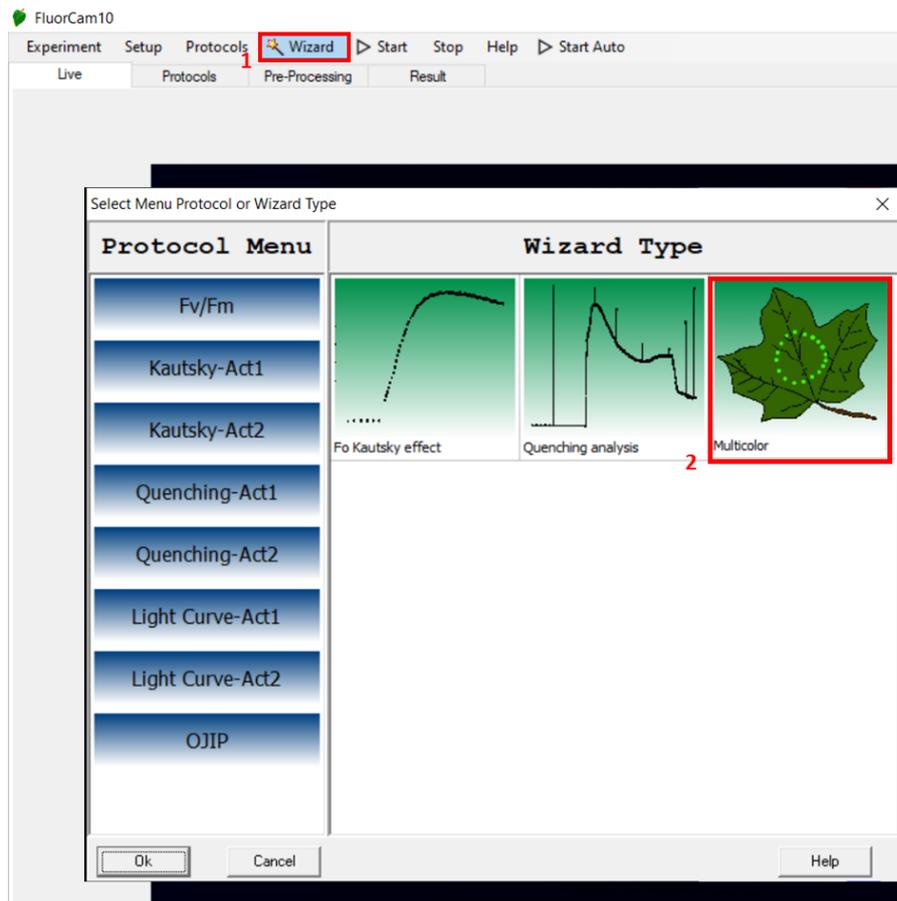


Fig. 2 Wizard Menu

## 3. The Multicolor window is divided into two sections:

- **Light** (Fig. 3-1 and 3-2)
- **Parameters** (Fig. 3-3, 3-4 and 3-5)



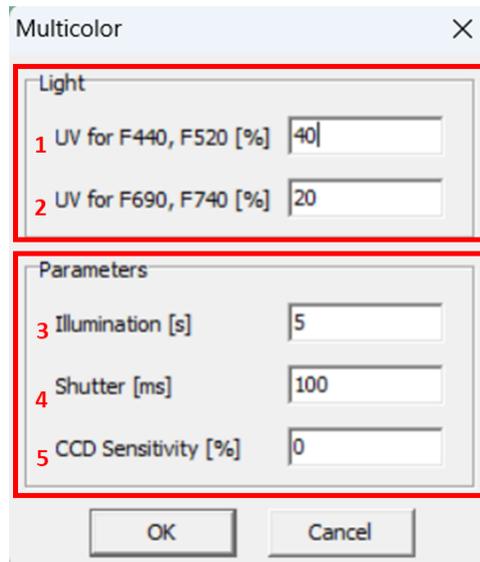
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Inquires and orders: [ordering@psi.cz](mailto:ordering@psi.cz)  
Technical support: [support@psi.cz](mailto:support@psi.cz)  
Shipping: [shipping@psi.cz](mailto:shipping@psi.cz)



**Fig. 3 The Multicolor Window**

These sections allow you to configure the illumination and camera settings for the experiment:

- **UV for F440, F520 [%]:** UV light intensity for measurements using blue and green filters (Fig. 3-1).
- **UV for F690, F740 [%]:** UV light intensity for measurements using red and IR filters (Fig. 3-2).
- **Illumination [s]:** Duration of UV exposure, in seconds (Fig. 3-3).
- **Shutter [ms]:** Camera integration time, in milliseconds (Fig. 3-4).
- **CCD Sensitivity [%]:** Sensitivity of the camera's CCD sensor (Fig. 3-5).

#### **Restrictions:**

The minimum UV light intensity that can be set is **5%**.

The maximum shutter value is **1 000 ms**.

Recommended settings are preconfigured in the **Multicolor** window. Once all parameters are set, click the **OK** button and start the experiment by clicking the Start icon ►.



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Inquires and orders: [ordering@psi.cz](mailto:ordering@psi.cz)  
Technical support: [support@psi.cz](mailto:support@psi.cz)  
Shipping: [shipping@psi.cz](mailto:shipping@psi.cz)

### Multicolor Wizard Description:

1. Camera and light intensity settings
2. Timing for each fluorescence color measurement
3. Automatic filters, UV light exposure, and measurement settings
4. Definition of checkpoints

```
;protocol body - Generated by wizard
;Multicolor Plant Fluorescence
;version ZB December 13, 2017
;
;run in Snapshot mode, min timing 1s!!!
;
include default.inc ;Includes standard options, do not remove it !
include light.inc ;Includes standard options, do not remove it !
include FW.inc ;Includes standard options, do not remove it !
;
UV = 100
Sensitivity=0
Shutter=100
__Irradiance1 = 40
__Irradiance2 = 20
;
FWDelay = 5s
UVMeasure = 1s
;
BF = 0s ;Blue Fluorescence
GF = BF + (FWDelay + 5s) ;Green Fluorescence
RF = GF + (FWDelay + 5s) ;Red Fluorescence
IrF = RF +(FWDelay + 5s) ;IR Fluorescence
;
; Multicolor fluorescence *****
BF + <0s>=> SET_FILTER(F440)
BF + <1s>=> SI_UV(__Irradiance1)
BF + <FWDelay>=>UV(5s)
BF + FWDelay + <0s, UVMeasure ..5s> =>m
;
```

1

2

3



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Shipping: [shipping@psi.cz](mailto:shipping@psi.cz)

```
GF + <0s>=> SET_FILTER(F520)
GF + <FWDelay>=>UV(5s)
GF + FWDelay + <0s, UVMeasure ..5s> =>m
;
RF + <0s>=> SET_FILTER(F690)
RF + <1s>=> SI_UV(__Irradiance2); WRITE UV INTENSITY FOR RED AND IRF
RF + <FWDelay>=>UV(5s)
RF + FWDelay + <0s, UVMeasure ..5s> =>m
;
IrF + <0s>=> SET_FILTER(F740)
IrF + <FWDelay>=>UV(5s)
IrF + FWDelay + <0s, UVMeasure ..5s> =>m
;
;***** CheckPoints *****
<BF + FWDelay>=>checkPoint,"startF440"
<BF + FWDelay + 5s>=>checkPoint,"endF440"
;
<GF + FWDelay>=>checkPoint,"startF520"
<GF + FWDelay + 5s>=>checkPoint,"endF520"
;
<RF + FWDelay>=>checkPoint,"startF690"
<RF + FWDelay + 5s>=>checkPoint,"endF690"
;
<IrF + FWDelay>=>checkPoint,"startF740"
<IrF + FWDelay + 5s>=>checkPoint,"endF740"
;
<IrF + FWDelay+ 5s - UVMeasure>=>checkPoint,"timeVisual"
;
;end
```

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