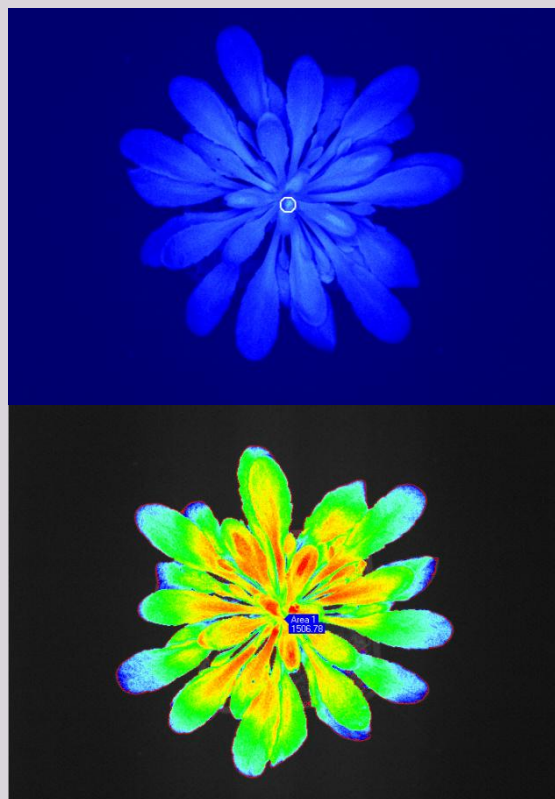


FluorCam PAR- Absorptivity Module & NDVI Measurement

Instruction Manual

Please read this manual before operating this product



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INSTRUCTION MANUAL

GENERAL DESCRIPTION

Normalized Difference Vegetation Index (NDVI) is an important vegetative index based on spectral reflectance of plants in near infrared region ($\lambda = 700-1300$ nm) and in the visible red range ($\lambda = 550-700$ nm) of the electromagnetic spectrum.¹

The photosynthetic chlorophyll pigments in plant leaves strongly absorb light in the blue and red portion of the spectrum and as such have generally low reflectance in this spectrum range. The cell structure of the leaves, on the other hand, strongly reflects near-infrared light (from 0.7 to 1.1 μm) and the amount of this reflectance is determined by the properties of the leaf tissues.

The NDVI is calculated from reflectance measurements in the red and near infrared (NIR) portion of the spectrum:

$$NDVI = \frac{NIR - RED}{(NIR + RED)} \quad (1)$$

where *RED* and *NIR* are the spectral reflectance of the vegetation acquired in the visible (*RED*) and near-infrared regions (*NIR*).

The NDVI vegetative index is successful in predicting photosynthetic activity, because it includes both near infrared and red light. As plant photosynthetic activity is determined by chlorophyll content and activity, NDVI is directly related to the photosynthetic capacity and hence energy absorption of plant canopies.²

Healthy plants absorb most of the visible light and reflect the large amount of the far-red and near-infrared light. In case of the unhealthy plants, the larger amount of the visible light is reflected. The near-infrared light is reflected less than in the healthy plants. These spectral reflectances are themselves ratios of the reflected radiation over the incoming radiation in each spectral band individually, hence they take on values between 0.0 and 1.0. For the healthy plants the NDVI value is in the range between 0.4 – 0.8.³

¹ Kumar, R., L. Silva. 1973. Light ray tracing through a leaf cross- section. Appl. Optics 12: 2950-2954.

² Sellers, P. J. (1985) 'Canopy reflectance, photosynthesis, and transpiration', International Journal of Remote Sensing, 6, 1335-1372.

³ WWW page 'Measuring vegetation (NDVI&EVI) ', available from:
http://earthobservatory.nasa.gov/Features/MeasuringVegetation/measuring_vegetation_2.php, [cit 24-2-2014].

PRINCIPLE OF MEASUREMENT

In the PSI imaging FluorCam systems NDVI index is determined with the use of **PAR-Absorptivity Module**, which is part of available optional accessories.

PAR-Absorptivity Module consists of light panel with precisely defined homogenous distribution of deep-red (660 nm) and far-red (740 nm) LEDs, 7-position filter wheel and appropriate filter set. Light panel is mounted around the camera to ensure homogenous distribution of red and far-red incident light to the imaged object.

Homogenous incident illumination is essential for correct NDVI analysis and correct spatial heterogeneity assessment.

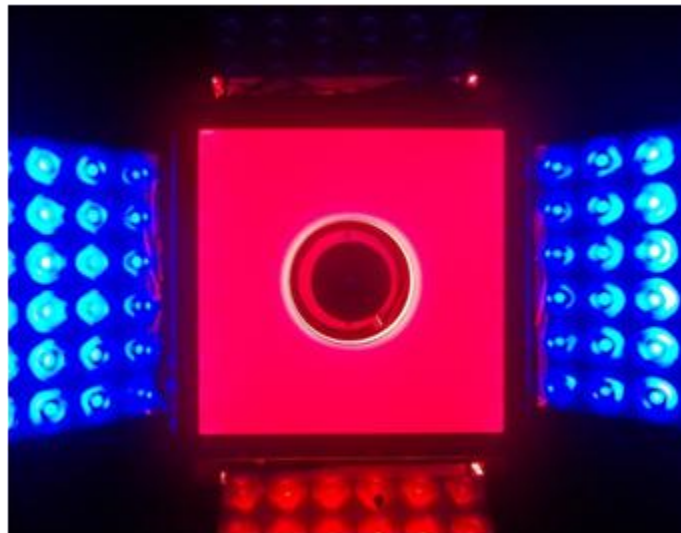


Fig. 1 PAR-Absorptivity panel inside of the Closed FluorCam.

COMPONENTS OF PAR-ABSORPTIVITY MODULE

- Light panel with precisely defined homogenous distribution of deep-red (660 nm) and far-red (740 nm) LEDs. Same number of LEDs is used for both the lights.
- 7-position filter wheel and appropriate filter set (Chl and NDVI filter at least).
- Teflon calibration plate

Light	Wavelength [nm]	Name in FC7
Deep-red	660	ADD1 (RED)
Far-red	740	ADD2 (NIR)

OPERATION INSTRUCTIONS

The next chapter explains how to proceed with the NDVI measurement and how to perform NDVI calibration step, which should be performed prior the NDVI measurement of the plant. For general instruction on the FluorCam hardware and software operation please refer to FluorCam operation manual.

Calibration of the NDVI panel should be always performed prior the initiation of the measurement with the plant samples. During the calibration step both lights used for the NDVI measurement (deep-red and far-red LED lights) are automatically set to equal light intensity. Homogenous incident illumination is essential for correct NDVI analysis of the plants and therefore NDVI calibration step should be performed always prior NDVI measurement.

Important: NDVI calibration should be performed only once at the beginning of the measurement. In case FluorCam Imaging Unit is switched OFF and ON in the meantime of measurement, the calibration step should be repeated. In case FluorCam7 software is closed and launched again, the NDVI calibration does not have to be performed again as the pre-set values are launched again.

NDVI CALIBRATION

Homogenous incident illumination of the light panel with the identical light intensity of both deep-red and far-red lights is essential for correct NDVI analysis and therefore NDVI calibration step should be performed always prior NDVI measurement.

The NDVI Calibration is simple algorithm, which is based on the reflection of the lights (deep-red and far-red) on the calibration standard, the teflon plate. The algorithm sets automatically the intensity of the deep-red (here named as **ADD1**) and far-red LED lights (here named as **ADD2**) to light intensity which is equal for both of the lights. The algorithm is designed to set the final signal intensity to approximately 3000 digits and as such also sets the shutter opening time and the sensitivity for obtaining appropriate quality signal.

Please follow the next steps to perform the NDVI calibration:

1. For the NDVI calibration use the provided teflon plate as calibration standard.

Please note: During the measurement it is important that no outer light effect can influence the calibration step. In case of FluorCam Systems without dark adaptation box, please make sure, that the calibration is performed in the dark without any external light interference.

2. Launch the FluorCam7 software. After launching the FluorCam7 software it is necessary to change the filter type to **NDVI** in the scrolling menu **Filter** – (Fig. 2-1) and (Fig. 2-2). Wait for a moment until the automatic changing is done.

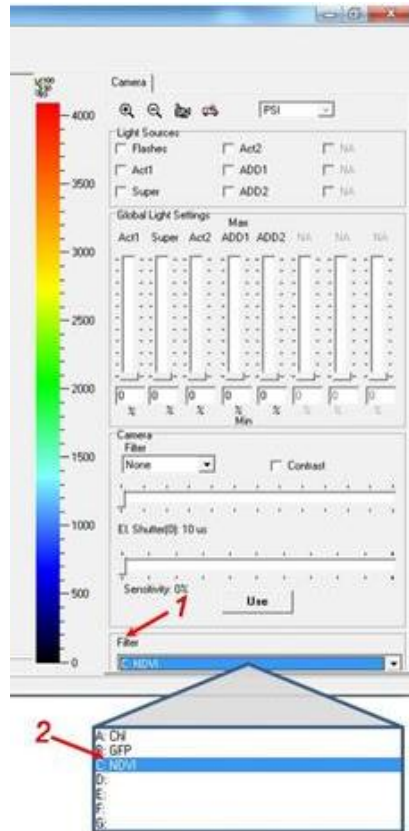


Fig. 2 NDVI calibration – Filter selection

3. Place the fluorescence size calibration standard on the top shelf, set the far-red light (here named as **ADD2**) ON and check that the imaged object is in focus in the **Live** window. In case the object is OUT of focus follow the next instructions:
 - First, turn on one of the lights (**WARNING!** Set only low light intensity and do not look directly to the light panels during the focusing/zooming!)
 - For focus gently loosen the bottom screw (do not unscrew it out) (Fig. 3-1) and carefully rotate the bottom part of the objective (Fig. 3-2) until the image of the object under the camera (as can be observed in **Live** window) is sharp.
 - For zoom gently loosen the top screw (do not unscrew it out) (Fig. 3-3) and carefully rotate the top part of the objective (Fig. 3-4) until the object under the camera is zoomed.
 - The result of zoom or focus is possible to observe in the **Live** window in the FluorCam7 software. FOCUS and ZOOM should be kept during the whole experiment otherwise the level of the absolute fluorescence signal could be changed.

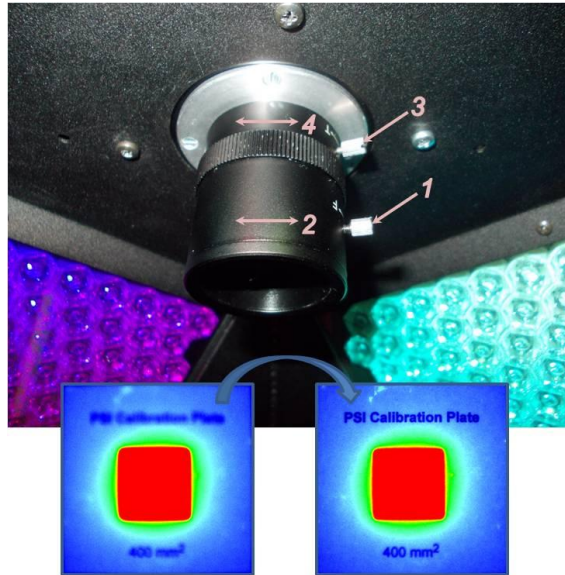


Fig.3 Focus and zoom of the objective

- When the object is in focus, you can proceed with the calibration step. In the menu **Setup** select **NDVI calibration** (Fig. 4-1 and 4-2). When the pop-up window **Information** shows up, put the teflon calibration plate on the top shelf in the FluorCam device and press **OK** (Fig. 4-3). The plate should be in field of view of the camera.

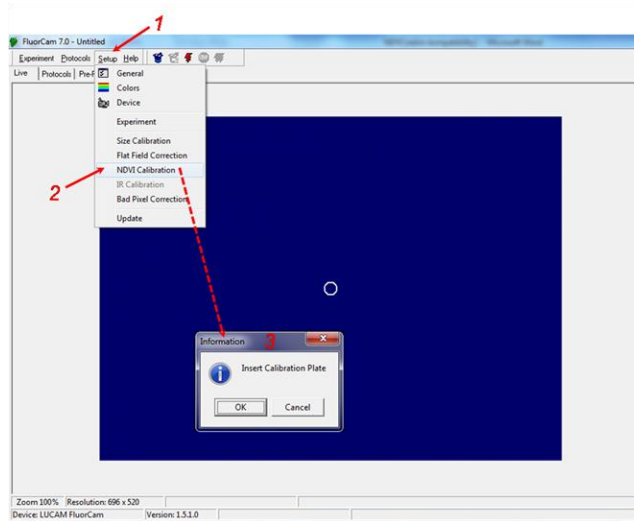


Fig. 4 Launching of the NDVI calibration

- After the pressing button **OK** in the window **Information**, the software starts automatically calibrate the shutter opening time, camera sensitivity and far-red light intensity – **ADD2** (Fig. 5-1, 5-2 and 5-3). The intensity of the deep-red light – **ADD1** is always set to 50% (which approximately corresponds to 3000 digits) and the other parameters are calibrated according to this setting. The NDVI calibration takes less than one minute. After calibration is finished, correct light intensity, shutter and sensitivity is set for proper NDVI measurement. Do not change the light intensity, shutter and sensitivity settings! Leave the teflon plate in the same position in the FluorCam device and in the top panel menu select **Wizard** icon (Fig. 5-4) for selecting the NDVI protocol. When the pop-up window **Protocol menu**

appears (Fig. 5-5), choose protocol **NDVI** and confirm your choice by **OK** (Fig. 5-6 and 5-7). Return back to the **Live** window and press button **Use** in the bottom of **Live** window to import camera and light settings to the protocol and confirming the calibrated settings (Fig. 5-8). After that, launch the experiment by button **Start experiment** (Fig. 5-9) in the top panel menu.

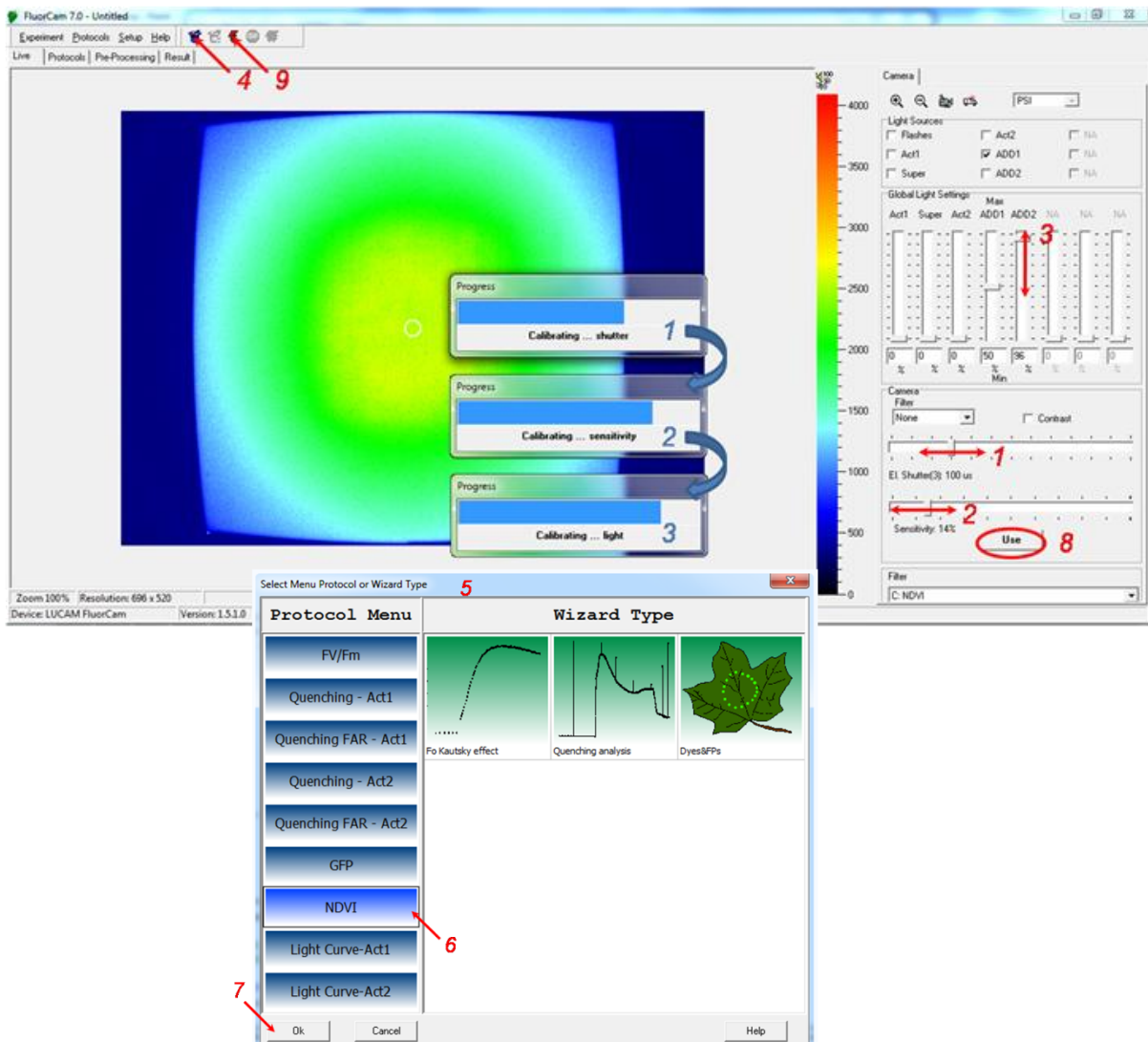


Fig. 5 NDVI calibration process

6. When NDVI experiment ends, select the area of interest in the **Pre-Processing** window. There are two ways, how to do it:
 Automatically: press the button **Auto** (Fig. 6-1) and then the button **Background exclusion** (Fig. 6-2). This option selects whole plate (Fig. 6-5).
 Manually: press the button **Manual** (Fig. 6-3) and select some part of the plate as shown in the window (Fig 6-4).
 Then check the graph below (Fig. 6-6). The average reflection kinetics of the both lights in this area should have the same values during the measuring time and create straight line if the lights are properly set to the same intensity. For analyzing the selected part of the teflon plate (or whole) press button **Analyze** (Fig. 6-7).

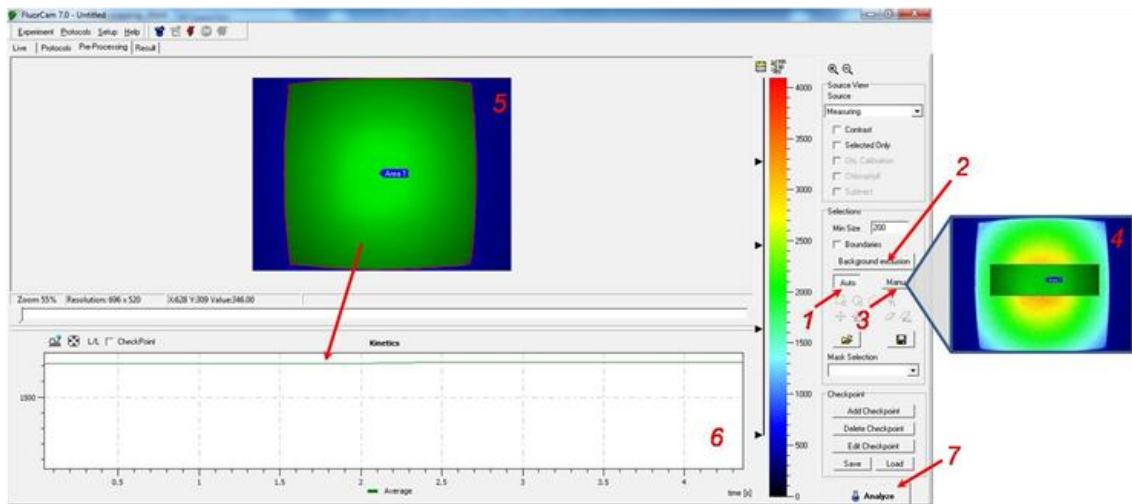


Fig. 6 NDVI calibration - Pre-processing window

- In the **Result** window in the table **Numeric** (Fig. 7-1) is shown calculated value for NDVI parameter. This value should be $NDVI = 0.00$ or very close (acceptable is ± 0.02) (Fig. 7-2). It means that there is no difference in the reflection between deep-red and far-red light and the lights are correctly set to the same intensity.

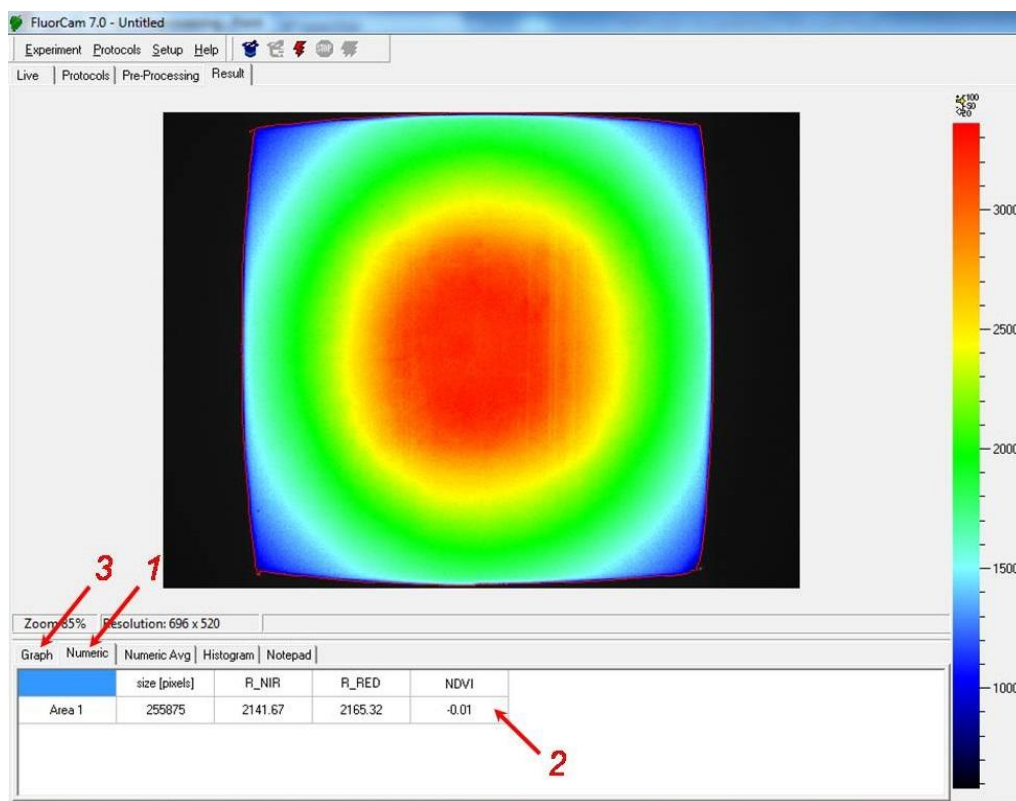


Fig. 7 NDVI calibration - Result window

NDVI MEASUREMENT

For NDVI measurement in FC systems is used NDVI protocol which first turns on the far-red lights and measures reflection of the desired object and then turns on the deep-red light and again measures the reflection of the object under deep-red light. From these reflection values, NDVI of the plant is calculated (according to the formula (1)), which should be in the range 0.4 – 0.8 if the plant is healthy.

Please note: it is not necessary to provide dark adaptation to the plant prior NDVI measurement, but it is important to perform the measurement in dark without any external light interference.

For successful NDVI measuring please follow next steps:

1. Take out the teflon calibration plate and move the shelf to the position where the imaged object, e.g. rosette of the plant is in the same plane level as the top shelf. Place the plant on the shelf in FluorCam device. Go to the **Live** window (Fig. 8-1) in the FluorCam7 software. **Important:** Please, do not change the settings of the light intensity in **Global Light Settings** menu (Fig. 8-2)! In case the light settings are changed, please perform again the NDVI calibration step. Switch on **ADD1** light ON → mark check-box **ADD1** in **Live** window. Change False – color scale to Extended spectrum or Extended spectrum 3_0_3 (the most sensitive color scales for human eye). Adjust the settings of **El. Shutter** (Fig. 8-3) and **Sensitivity** (Fig. 8-4) by trucking the bar to get a signal in the range around 1000 digital units (blue or light blue color of the object). The shutter controls, how long is the signal integrated. Generally, the higher value, the better is resulting signal quality. Sensitivity controls signal amplification, but should be used as second, for the final fine tuning, because it also decreases quality of the signal and decreases signal to noise ratio. It is recommended to keep the sensitivity below 50%.
2. If there are any changes in settings of the shutter and the sensitivity, confirm them by **Use** (Fig. 8-5). Because the NDVI protocol is already load and prepared from the previous calibration, it is not necessary to prepare it again. Launch the experiment by clicking the icon **Start experiment** (Fig. 8-6) in the top panel menu.

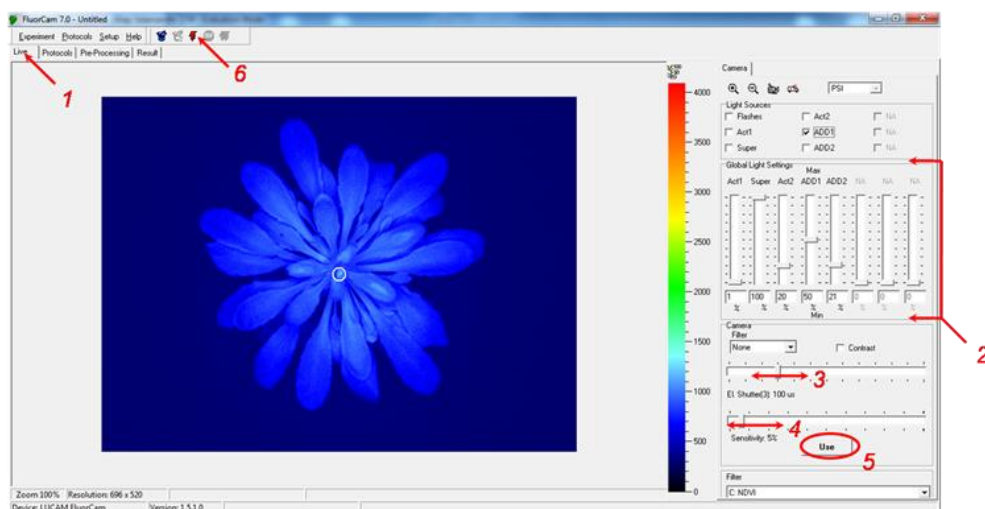


Fig. 8 NDVI measuring on the plants

- When the NDVI protocol ends, choose the area of interest in the **Pre-processing** window. There are two ways, how to do it:

Automatically: press the button **Auto** (Fig. 9-1) and then the button **Background exclusion** (Fig.9-2) for excluding the objects (mold, algae, ...), which also reflects the light, from analyzed area. Other way, how to exclude them, is setting the intensity interval by pulling the black arrow (Fig. 9-3) and selecting the area of interest more precisely.

Manually: if the image is too noisy, use **Manual** (Fig. 9-4) choosing of area, select the type of segments (Fig. 9-5) and draw the borderline around the object (Fig. 9-6). The graph of the kinetics of the measurement (Fig. 9-7) is shown on the bottom of the **Pre-processing** window. In the kinetics graph, first part refers to values of the far-red reflection; the lower part is represented by the reflection of the deep-red light.

After that, click the button **Analyze** (Fig. 9-8) for obtaining the results for the selected area of interest. Results of the fluorescence transient measurement for the defined regions of interest are shown shortly in the **Result** window.

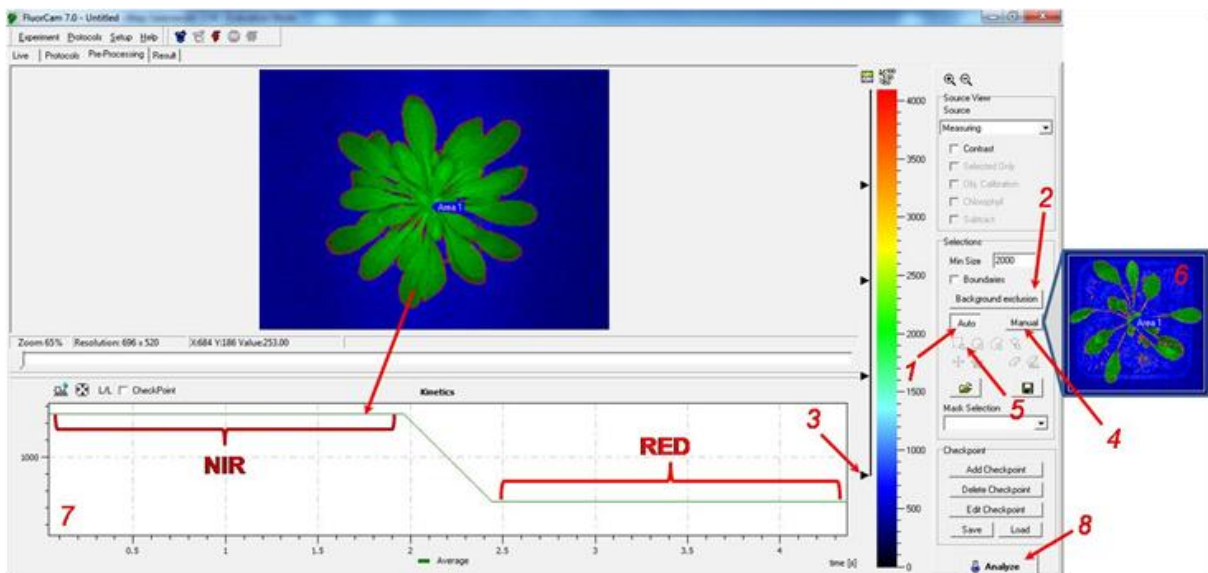


Fig. 9 NDVI measurement - Pre-processing window

- In the **Result** window kinetic graph of the measurement, calculated numeric values and the kinetic images of the analyzed plant are shown. The calculated values of the NDVI parameter are shown in the table **Numeric** (Fig. 10-1) and **Numeric Avg**. The NDVI value (here named as parameter **NDVI**), should be in the range $NDVI = 0.4 - 0.8$, if the plant is healthy and with sufficient chlorophyll content (Fig. 10-2). In the table **Graph** (Fig 10-3) the kinetics curve is shown for the NDVI measurement.

By clicking the small images in the right side of the **Result** window, the reflectance image of the plant in deep-red light (Fig. 10-5) and far-red light (Fig. 10-4) can be visualized. In addition to the inspection of the spatial heterogeneity of the far-red and deep-red light induced reflectance of the imaged plant, the NDVI (Fig. 10-6) value distribution over the area of the plant can be visualized.

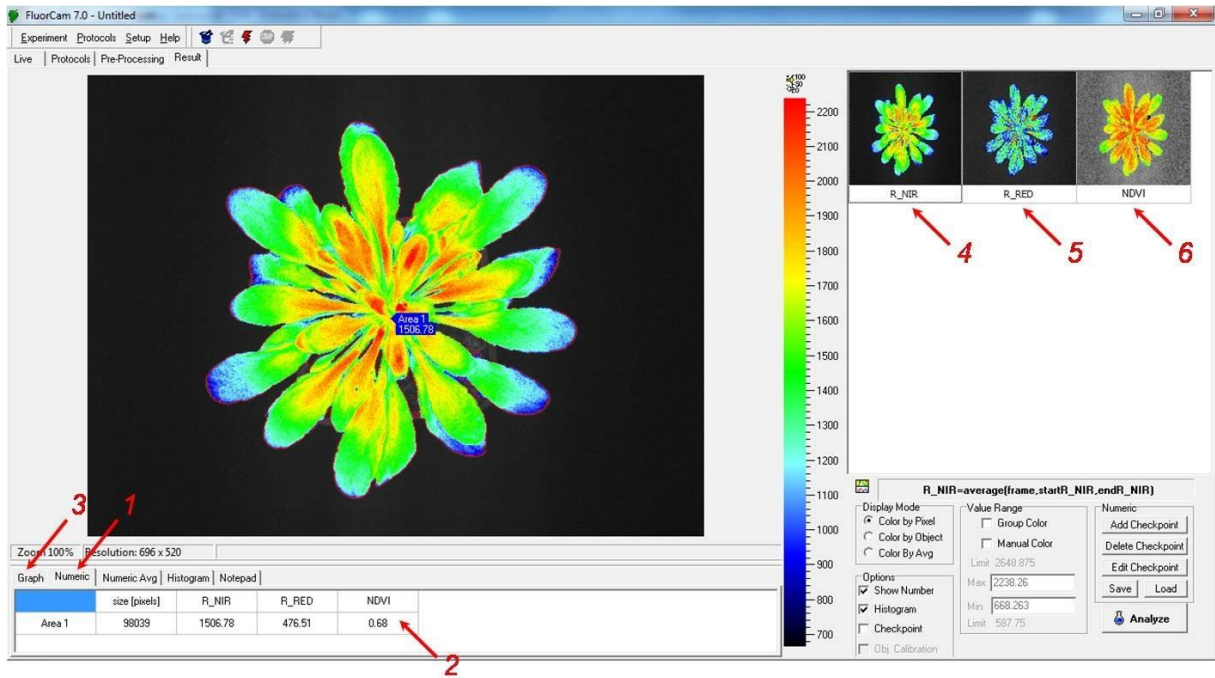


Fig. 10 NDVI measuring - Results

WARRANTY TERMS AND CONDITIONS

- This Limited Warranty applies only to the FluorCam series devices. It is valid for one year from the date of shipment.
- If at any time within this warranty period the instrument does not function as warranted, return it and the manufacturer will repair or replace it at no charge. The customer is responsible for shipping and insurance charges (for the full product value) to PSI. The manufacturer is responsible for shipping and insurance on return of the instrument to the customer.
- No warranty will apply to any instrument that has been (i) modified, altered, or repaired by persons unauthorized by the manufacturer; (ii) subjected to misuse, negligence, or accident; (iii) connected, installed, adjusted, or used otherwise than in accordance with the instructions supplied by the manufacturer.
- 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.
- The manufacturer repairs or replaces faulty instruments as quickly as possible; the maximum time is one month.
- The manufacturer will keep spare parts or their adequate substitutes for a period of at least five years.
- 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.
- PSI also offers out-of-warranty repairs. These are usually returned to the customer on a cash-on-delivery basis.
- **WEAR & TEAR ITEMS** (such as sealing, tubing, padding, etc.) 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.

TROUBLESHOOTING AND CUSTOMER SUPPORT

In case of troubles and for customer support, please, write to support@psi.cz or contact your local distributor.

Manual Version: 2014/04

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