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User Guide for Multi-Fluorescence tool of PROGRES GRYPHAX® software

The "Multi-Fluorescence tool" of PROGRES GRYPHAX® software enables user to create single or multi-color fluorescence images. Also you can merge RGB and colored fluorescence images easily.

Note: The *Multi-Fluorescence* tool is available for both monochrome and color cameras. Color cameras will be automatically set to the monochrome mode when this function is activated.

Preparations general:

Important note: All camera image parameter (e. g. capture resolution and storage format) should be set in advance before you start with *Multi-Fluorescence* image tool.

We are recommend to use "16 bit" image format to reach best image results with *Fluorescence tool*.
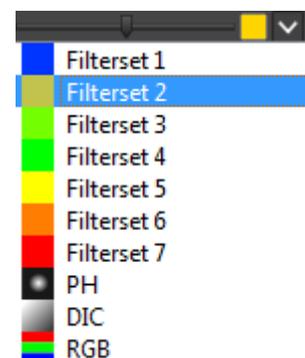
In case of scanning camera use, please set the capture resolution to a non-scanning mode under "Preferences/Device Configuration". Otherwise the image capturing time can be up to 36 minutes depending on used Exposure Time and Gain value!

To reach to best results with *Fluorescence tool* you have to activate the option *"Minimize Gain during Record"*.

To enhance the image results of long exposed image, we strongly recommend to create a so called: *"black shading"* correction to reduce the noise level <u>before</u> starting fluorescence image capture.

The *Multi-Fluorescence* tool is designed to save up to <u>10 different</u> filter parameter settings sets.

- Filter set 1 to 7 for different Fluorescence filter
- PH settings for Phase Contrast
- DIC settings for Differential Interference Contrast
- And RGB settings for colored images from color cameras

Filterset 1
Filterset 2
Filterset 3
Filterset 4
Filterset 5
Filterset 6
Filterset 7
PH
DIC
RGB

Note: All user interaction and changes of filter set parameter will be saved after change accordingly.

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Overview:

Multi-Fluorescence tool contains two separate windows:

- **Filter list** with active filter sets
- **Settings widget** which contains all camera parameter

Fluorescence filter set (1) of 7 different available set-ups. Can be changed by filter menu.

Dropdown filter menu ⌄ to change filter set number, order and position at *Fluorescence* tool filter list.

Checkbox ☑ to activate or deactivate filter set to combine at "merge" image.

Intensity slider ▭ to adjust the filter intensity after image record. To correct brightness on merge image.

Color selector ▢ - by click on the color icon the "*Wavelengths Selector*" will be opened and you can enter the exact emission wavelengths in **nm** for the appropriate filter set.
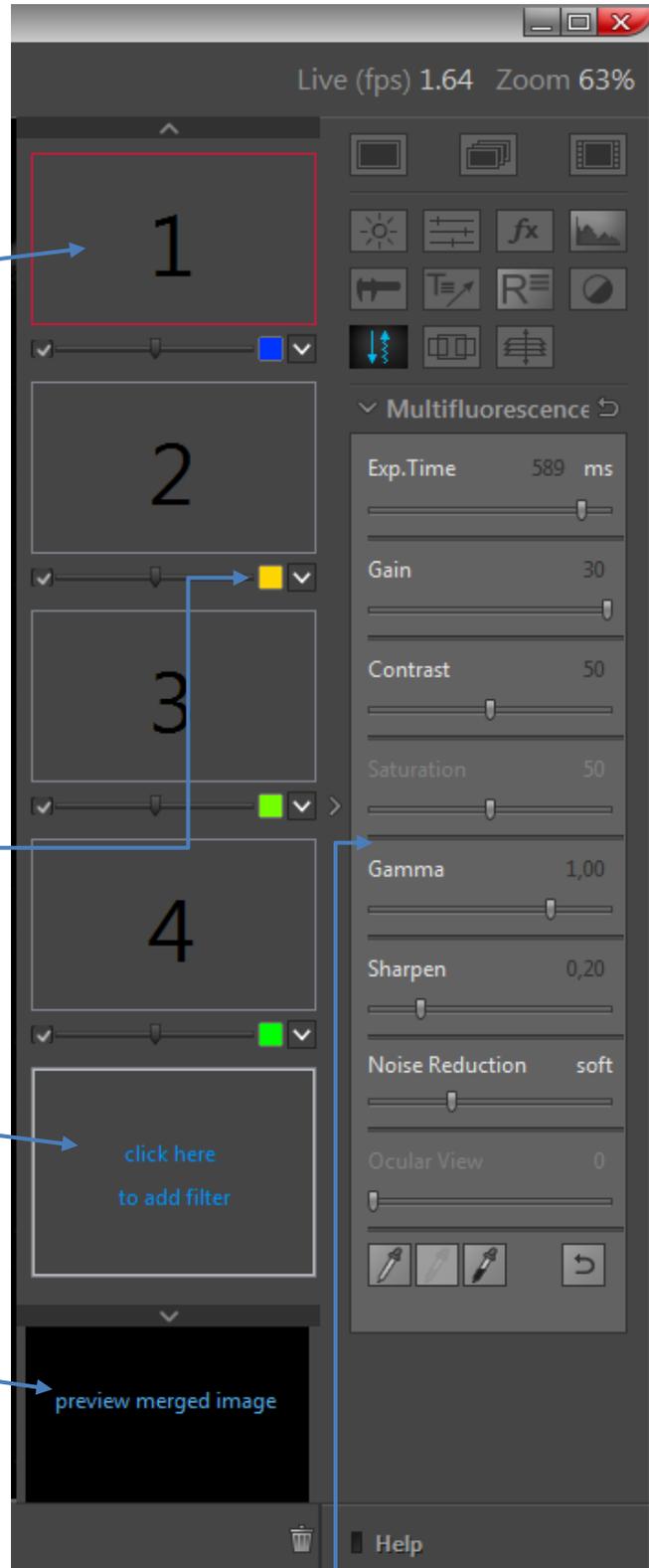
Add new filter - click on empty filter set to add new filter to *Fluorescence tool* filter list.

Arrows ▭ to scroll manually at Fluorescence filter list.

Preview thumbnail of "merged" image, depending on selected filter set.

Trash 🗑 to delete selected filter set from Fluorescence filter list. All previously settings will be saved and reused after add filter set number again.

Filter specific camera settings are connected to each separate filter set.

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Status of filter set:

To support different interactions, the filter set thumbnails indicates the following status of filter set.
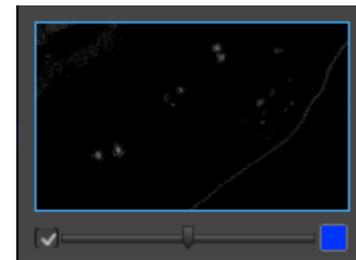
Red frame rectangle indicates ready for record. All image parameter of Fluorescence tool are active to adjust all parameter. The corresponding live image is activated and displayed on the image main window of GRYPHAX software.

Press "REC" button to save filter set image into the temporary image list of *Fluorescence tool*. The Filter set preview thumbnail will be updated accordingly.
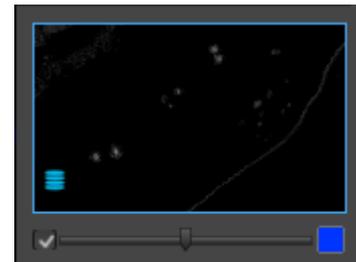

Blue frame rectangle indicates displaying of already recorded filter set image. The recorded image is activated and displayed on the image main window of GRYPHAX software. The image parameter of Fluorescence tool are deactivated.
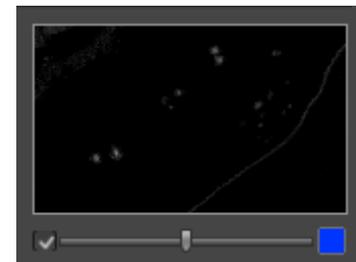
Press "REC" button to save filter set image into the Gallery.

After successful save of filter set image the preview thumbnail will be marked with a save icon .

Grey frame rectangle indicates that no interaction can be performed.

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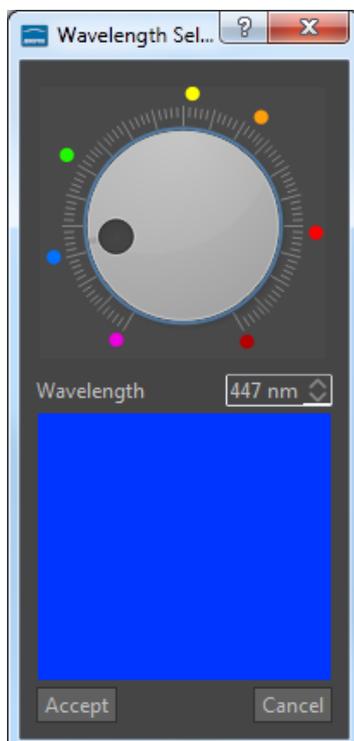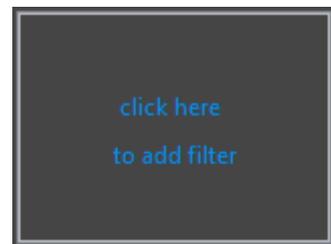
Start & initial set-up:

After successfully preparation you can open *"Multi-Fluorescence tool"* by click on Fluorescence icon at *Tool bar*.

Note: To start a new Fluorescence session you have to toggle the icon , all settings of filter set will be saved and the previously recorded images are removed and cannot be re-loaded.

At the first use of *Multi-Fluorescence* tool you have to set-up your specific filter set-up of Microscope. No initial filter set is displayed.
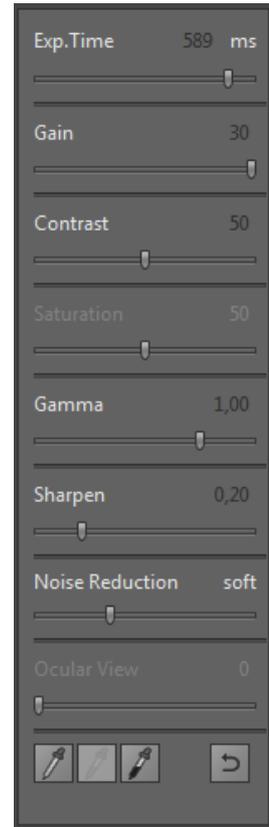
1) **Add new filter set** with click on empty filter thumbnail a new filter set will be **added** to the filter list *Fluorescence tool*. Add the number of necessary filter sets according to your number of fluorescence filter.

2) Change Emission wavelength by click on the Color selector the *"Wavelengths Selector"* will be opened and you can enter the exact emission wavelengths in *"nm"* for the filter set / fluorochromes.

With *"Accept"* of the selected emission wavelengths, the wavelengths is saved to the filter set and the recorded monochrome image will be colored with this color after press *"REC"* button.
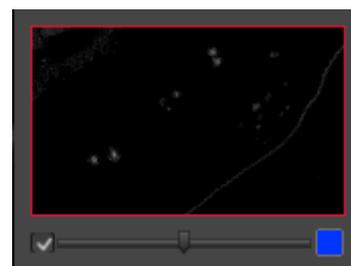
3) **Adjust camera specific parameter** separately for each created filter set.

4) Press "REC" button to **start record** filter set image into the filter set. The already recorded filter set images will be displayed at the merge preview thumbnail and updated after change or add of each filter set image.

5) *Fluorescence tool* will **jump to the next** "empty" filter set automatically. In order from top to button of the filter list.

6) **Repeat** the points 2. to 4. to fill-up all filter set.

7) After record of each filter set image the software jump to the merge preview thumbnail and will display the merge image at the image window of GRYPHAX software.

8) Press "REC" button to save merge image to Gallery. The save icon will display that the currently displayed "merge" image is saved to the Gallery.

9) With the intensity slider of each filter set you can adjust the filter image brightness afterwards according to your needs.

10) To save the adjusted "merge" image, press "REC" button again to save new "merge" image into the GRYPHAX Gallery.

Re-record images:

Re-record filter set images with click into the filter set thumbnail at any time. **Red frame** is displayed to the filters set. The live image is started with previously used parameters on image windows again. All image parameter can be adjusted.

Press "REC" button to start record filter set image into the filter set.

The preview thumbnail and the "merge" image will be <u>updated</u> according to the new filter set image.

Save to Gallery:

Save "Merge" image - click to the "Merged" image thumbnail. The blue frame rectangle will be visible at "merge" thumbnail.

Press the "REC" button to save the composed merge image to the GRYPHAX Gallery, at any time if you are satisfied with the result image. After successful save of "merge" image the thumbnail will be marked with an save icon .

Save "Filter set" image – <u>double click</u> to the filter set thumbnail at any time during *Fluorescence* tool is active. The blue frame rectangle will be visible at "filter set" thumbnail.

Press the "REC" button to save the filter set image to the GRYPHAX Gallery. After successful save of "filter set" image the thumbnail will be marked with an save icon .

In case of large image size the remaining time to save the images will be displayed. The image record can be aborted by press "Cancel" button.

Additional hints:

Show emission wavelengths of filter set via mouse over. Hold the mouse pointer over according color box. The tool tip will display the currently selected emission wave lengths.

Limitations:

a) Measurement and Annotation are not allowed during *"Fluorescence tool"*.
b) Preferences and Gallery are not reachable during *Fluorescence tool*.
c) The number of images and filter set for Fluorescence tool is limited by approx.: 2 GB of memory.

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We looking forward to hearing from you and remain sincerely,

Your PROGRES GRYPHAX® Team

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