CGT
CELLGENTEK

Fluorescence Imaging System

FOBI

Fluorescence-labeled Organism Bioimaging Instrument

User manual

Ver 3.3

Fluorescence Imaging System, FOBI User manual.

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Overview

FOBI is a device that imaging and analyzing of fluorescent signals from fluorescent-labeled tissue and organisms. Blue, Green, Red, and NIR channels can be applied to most of the fluorescence using in the biological research. FOBI can distinguish between background and signal without preprocessing by using optimized filter and light source.

The background which the biggest problem of fluorescence imaging can effectively removed by the image analysis.

Diffusion-type LED light shows the precise quantitative value by eliminating a variation in accordance with a position.

FOBI can take high sensitivity images and movie files.

Characteristics of FOBI

1. Subtract background

The biggest problem of fluorescent image is background. Many efforts have been focused for obtain specific emission light and background remove. But completely remove is very difficult in the wide range of wavelengths. NEOimage program of FOBI gives an effective and easy to remove the background.

2. Diffusion type LED Lights: Minimization of variation by sample location

FOBI use the diffuse light source that didn't concentrated in the center. The light will be illuminated evenly in the chamber to remove position variation. It is also very important to obtaining quantitative data.

3. Fast imaging processing

FOBI doesn't need standby time for imaging. The images are shown immediately when the sample was put in the chamber through live window. It is very useful when the signal is not visible at the expected site. Researchers can find an alternative and check the unexpected things right away. FOBI can make video file. It can track the fluorescent material and make a recording of surgery.

4. Accurate analysis

Measure the area and intensity using the result picture. Then, Obtain the volume value of fluorescence signal and save it.

ROI can be defined by user directly or automatically. The obtained quantitative value that uses automatic ROI provide objective result by reduce the error caused from user.

5. Easy to use

Only exposure time and gain value can be defined by user. The other setting values are optimized for Fluorescence *In Vivo* Imaging. Not happen any problems caused by immature user.

Simple and convenient user interface can minimize all the waste of study time about how to use the new equipment. FOBI can help allow the user to focus on animal experiments.

Components
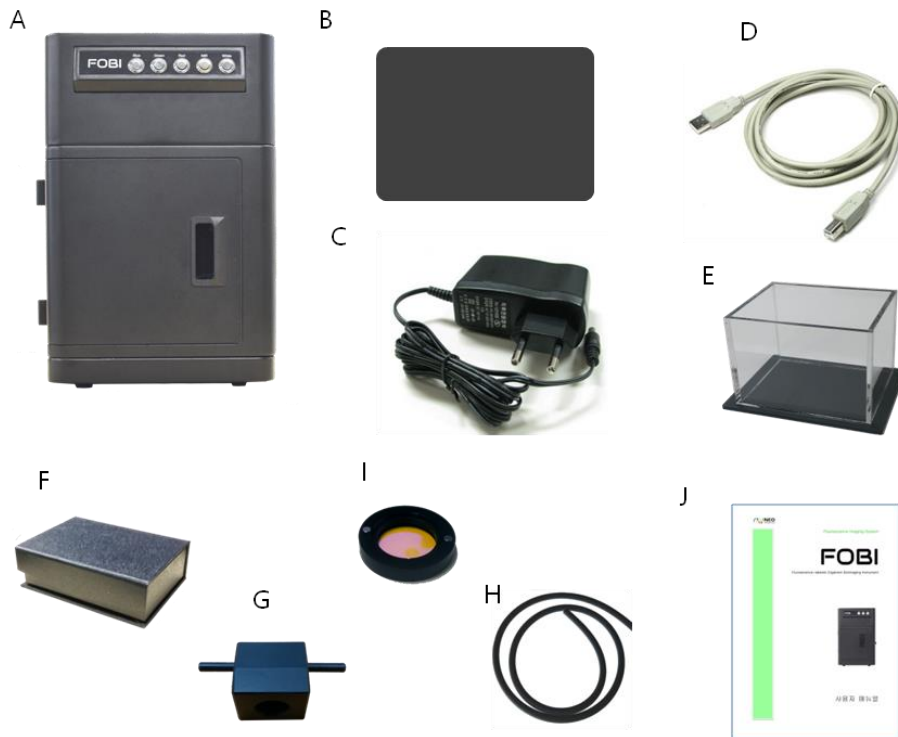
A: FOBI

B: Black plate

C: DC adaptor

D: USB connect cable

E: Mouse trap

F: Program USB memory card

G: Anesthesia module
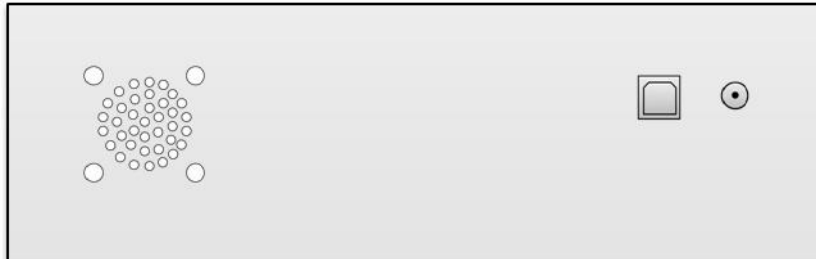
H: Anesthesia module connecting tube

I: Em filter

J: User manual

How to install and use

Please install the program in the following manner for use.

1. Connect the USB cable to the back of FOBI and computer.

2. Connect the DC adaptor to DC jack at back of the FOBI.

Channel selection

Select a channel according to the type of using fluorescence.
Install the emission filter you want and turn on the suitable lights for excitation.

1. Blue channel: GFP, Cy2, FITC, DyLight 488 etc.
2. Green channel: RFP, Cy3, RITC, Alexa Fluor 546 etc.
3. Red channel: Cy5.5, DiD, Alexa Fluor 680, DyLight 680, XenoLight CF680 etc.
4. NIR channel: ICG, Cy7, DiR etc.

Driver connection and program installation

1. Driver Connection

1) Connect the USB cable to the FOBI and the computer.

2) Connect "NEOimage for FOBI" USB memory card to the computer.

3) Double-click "Driver for FOBI" in "Dirver" folder of USB card and install the driver

2. Program Installation

1) Double-click "NEOimage FOBI setup.msi" in the USB card to start installation

2) Follow the instruction and click "Next" to proceed installation.

3) Click "Yes" on the pop up message allowing the publisher to continue installation.

4) When the Installation complete message pops up, installation is completed.

5) Double-click "vc_redist.x86" in the USB card to installation.

6) Double-click "NEOimage.exe icon on desktop or start menu to activate the program

Quick start

1. Click the NEOimage to activate program. (Desktop or start menu)

2. Click on the "LIVE" will pop up the live window.

3. Turn on the white light.

4. Remove the filter in front of the lens, and adjust focus watching the Live window.

5. Adjust brightness using adjust time of Live Exposure.

6. Click the "CAPTURE" and Take General picture.

 (Can use with the Signal picture for Merge)

7. Put the required emission filter to adaptor in front of the lens.

8. Can watch the target signal through Live window after turn on the same channel light with emission filter.

9. Adjust brightness using Live Exposure and Gain.

10. Click the "CAPTURE" to take signal picture (If necessary, press the VIDEO gets a movie. Video is a good to limit to 1 min).

11. Use the "Analysis" for removes the background of signal.

12. Select the signal to be measured using the Edit Tool Bar area (square, circle, polygon, automatic). Click the "Measure" confirms the results of the measurement (Background must be removed for accurate quantiation).

13 If necessary of the merge of the General Photos and signal pictures.

14. Select the required picture and save it under a different name for results backup.

NEOimage

1. Dialog Bar

1) User: You can manage to register a user name. You can press "Add" to add a user to choose from. If you add a user, a subfolder is created in C / FOBI data folder can be divided manage data.

2) Project: As a "User", you can add and choose project. If you add the "Project", a subfolder is created in the User folder.

3) Channel: According to the type of the fluorescent material you can enter the channel information. Channel information is essential when analyze. Wrong input channel can be modified in the Info tab for each photo.

4) LIVE: It shows the status within the chamber in real time. When the white light is on, the general picture of the sample can be seen and the fluorescent signal can be observed in real time when the excitation light is turned on. The live image is 50% of the real image. Where FOBI is not connected, the live feature does not work.

5) CAPTURE: The image shown on the live screen is captured in the picture form. The newly-created picture is shown in a separate window (50%) on the top left corner of the computer screen and is automatically backed up. The image is stored in the "C₩FOBI data" folder. When a program is shut down without storing a file by accident, the file can be restored in this folder. The obtained file is stored in the YearMonthDay_HourMinuteSecond.tif format indicating the time when the picture is taken
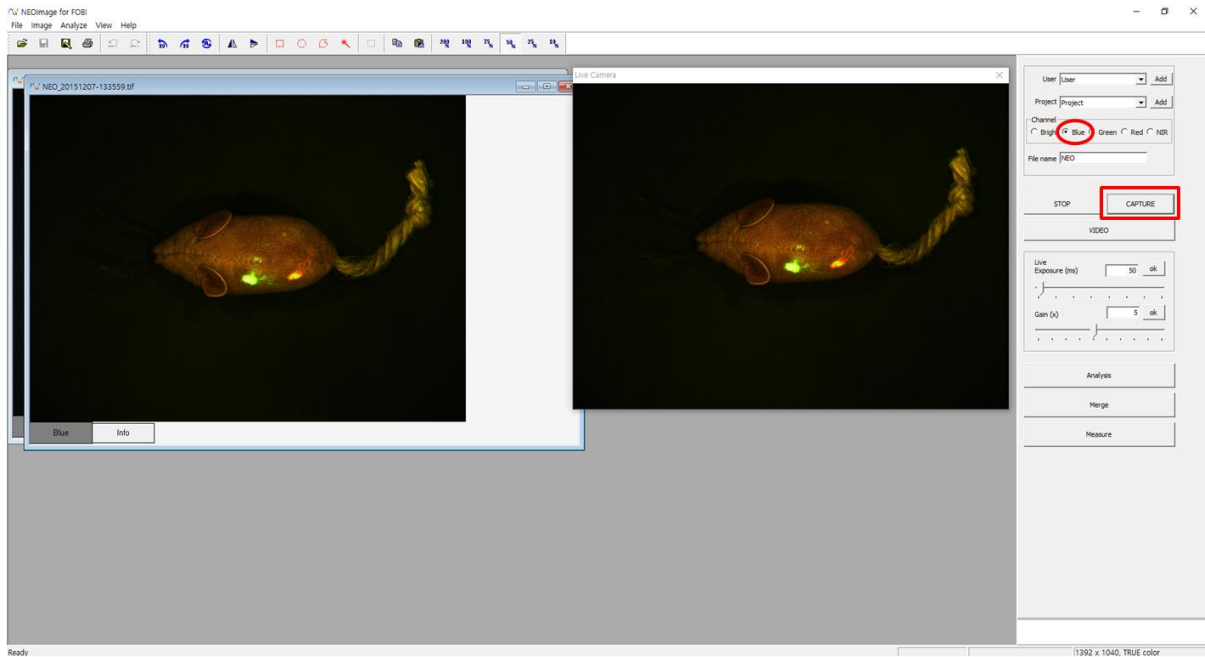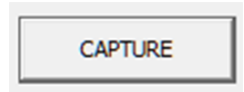

The "Info" tab of Photos record information about your photos. Experimenter's name, project name, files storage location, channel, exposure time and gain. You can check whether the analysis. Other information can not be changed, but you can change channels.
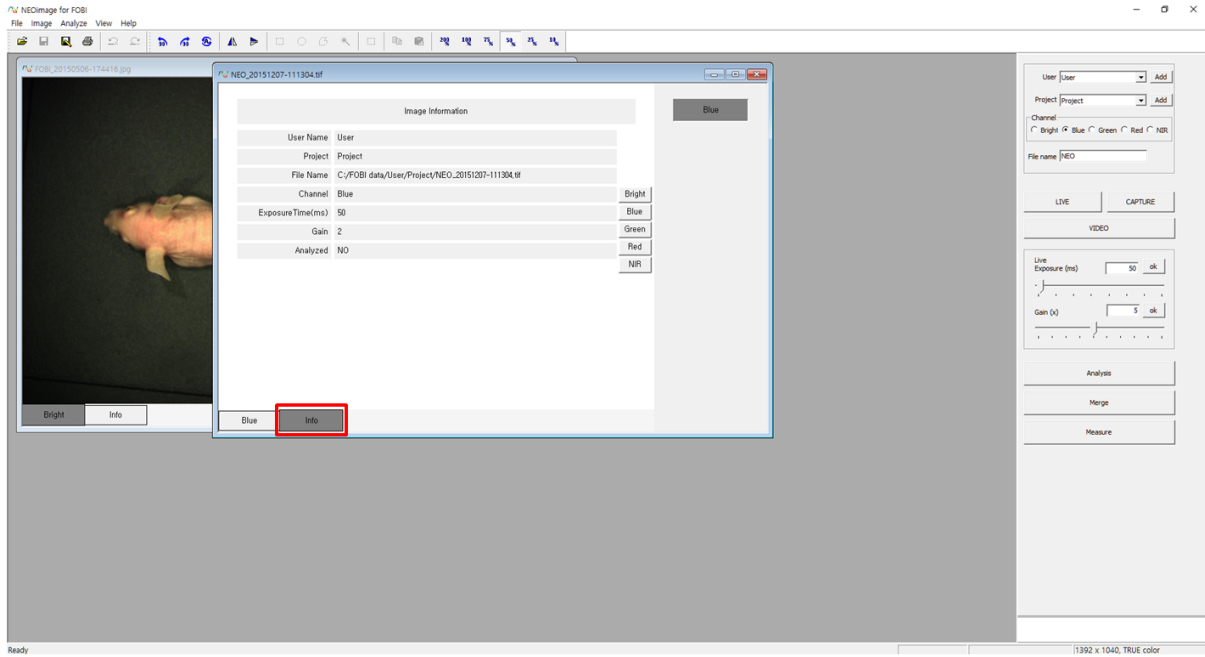
6) VIDEO: Can save video file literally the image shown in the LIVE window. Like CAPTURE, the video file is stored in the "C₩FOBI data₩video" folder. The obtained file is stored in the YearMonthDay_HourMinuteSecond.avi

7) Exposure: It adjusts the exposure time when obtaining the image and can be adjusted from 50ms up to 3500ms. Numeric numbers can be entered directly as the exposure time or the length of time can be adjusted by moving the bar with the mouse. Live window and save video are adjustable from 0 to 500ms.
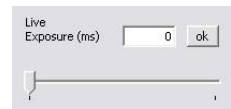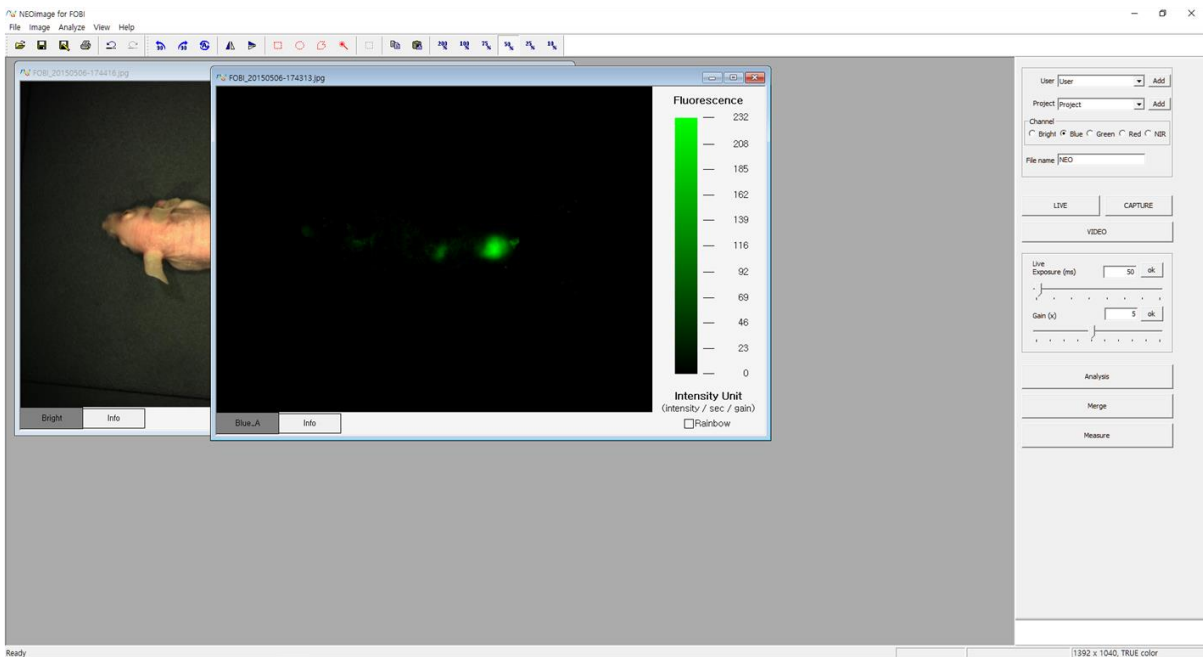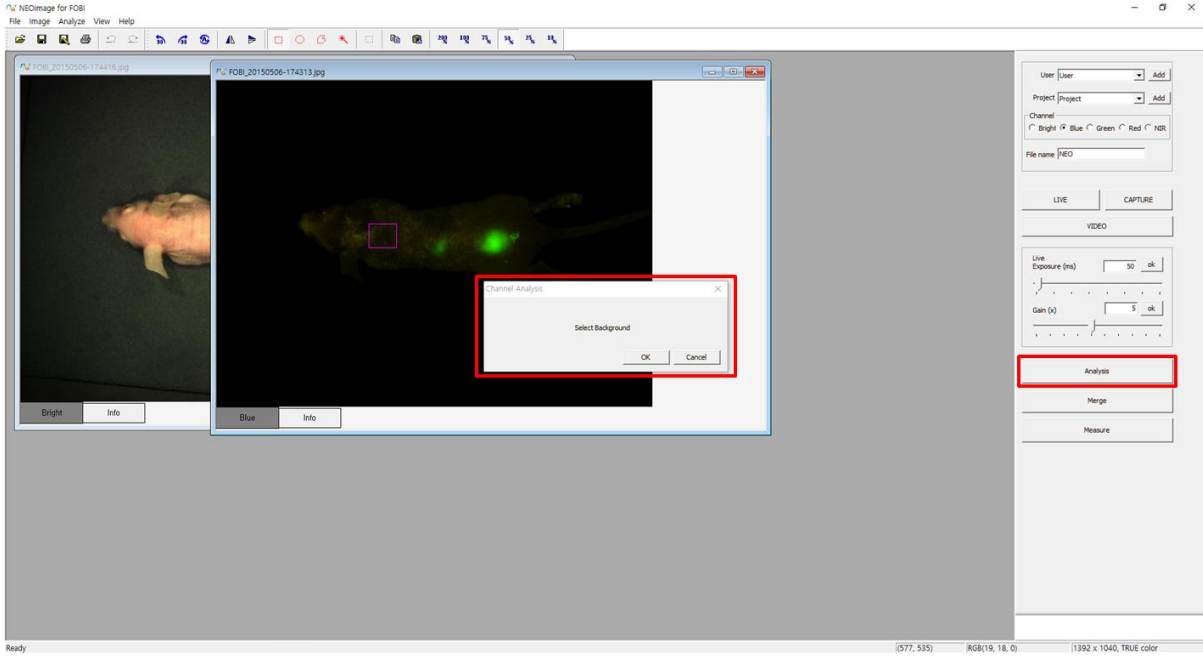
8) Gain: It increases the intensity value on the obtained image and the value can be adjusted up to 10.

9) Analysis: Click it, and a message of "Select Background" pops up. If a user designates a square as the background, the "OK" button on the window is activated. Click "OK", then the result of analysis of the image is shown in the window.
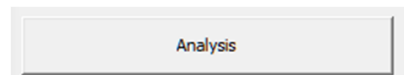
10) Merge: It overlaps the general picture and the fluorescent picture of the sample. This function is paste the fluorescence signals to general image.
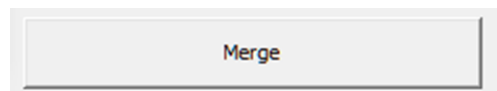

When the Merge button is activated, a message requiring a user to select the ordinary picture and fluorescent picture opens up. "OK" button is clicked after choosing the corresponding pictures, then the two pictures are merged and a new window is created. To aid in the understanding of the image, general picture used only gray color
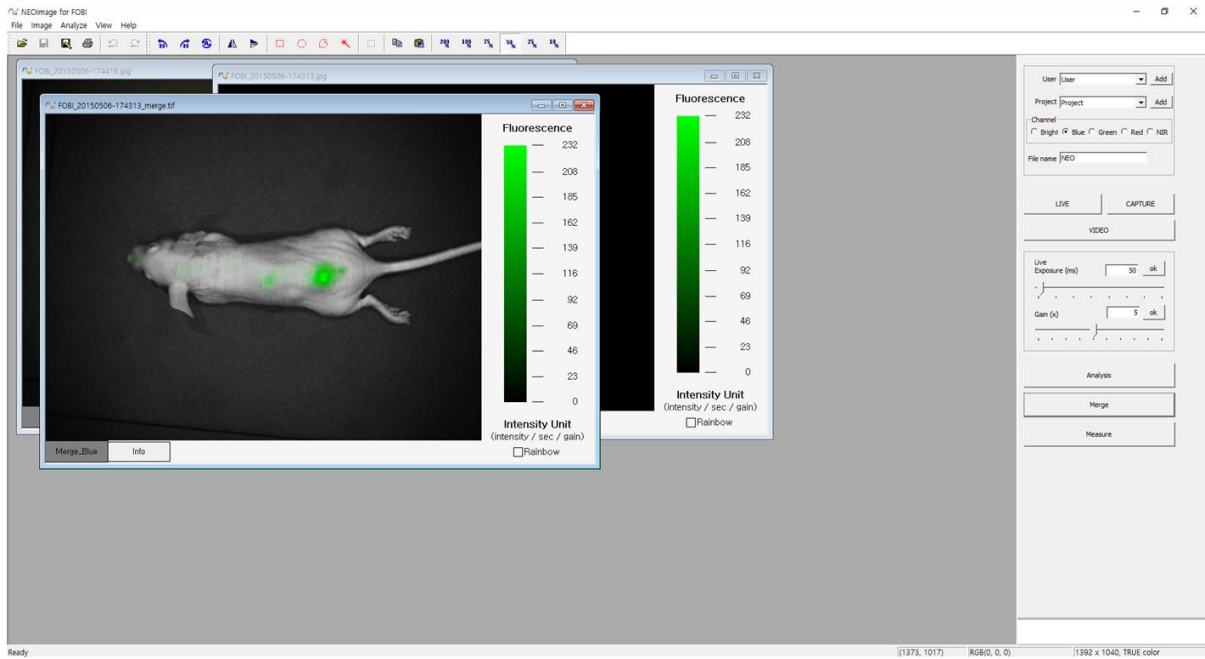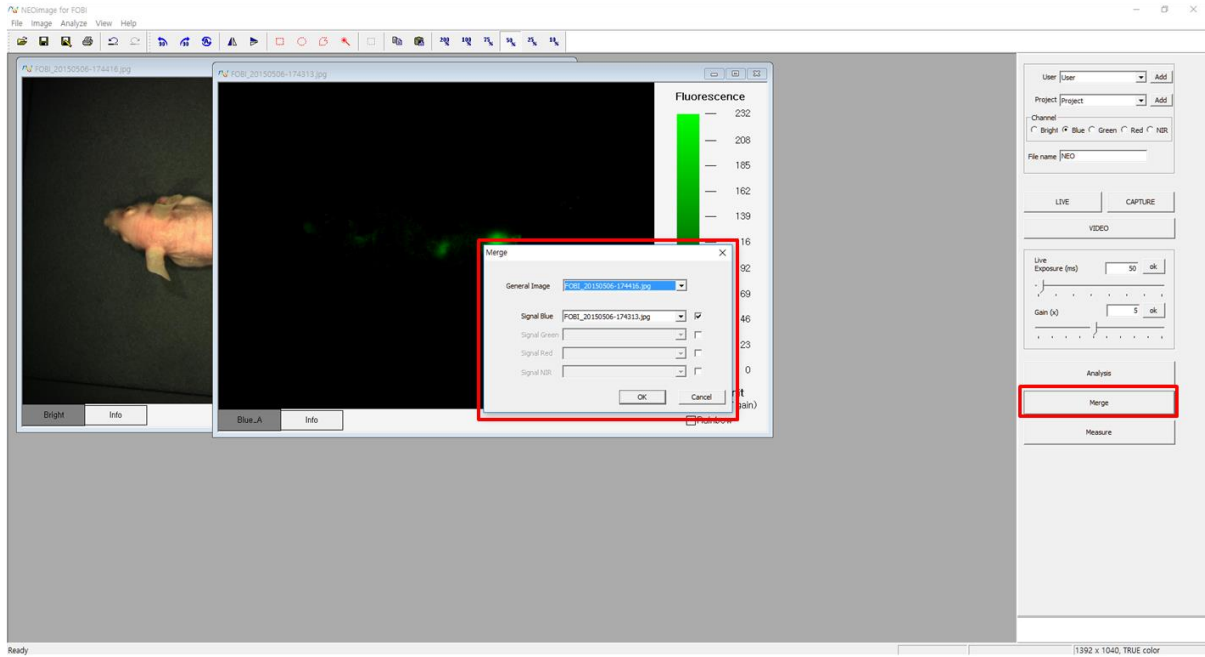
11) Measure: It designates ROI (Region of interest) on the obtained fluorescent image and secures the number of pixels, intensity value and volume value

Measure

within the ROI. First, designate the area to be measured as the ROI and click "Measure" to create a new window containing the measured value in the table form. In the table,

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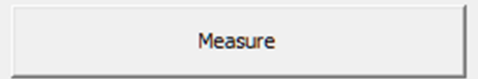
"ROI" indicates the ID number of the designated ROI. "Area" means the number of pixels within the designated ROI. "IU" is the average of "intensity value / sec / gain" of the from each pixel within the ROI. "Integrated Density" is the value of the "Area" value multiplied with the "IU" value. When the sizes of the fluorescent signals within the ROI are compared, "Integrated Density" values shall be compared. The obtained quantitative value can be stored as the CSV file by clicking "Export" (It can be opened on the Excel program).
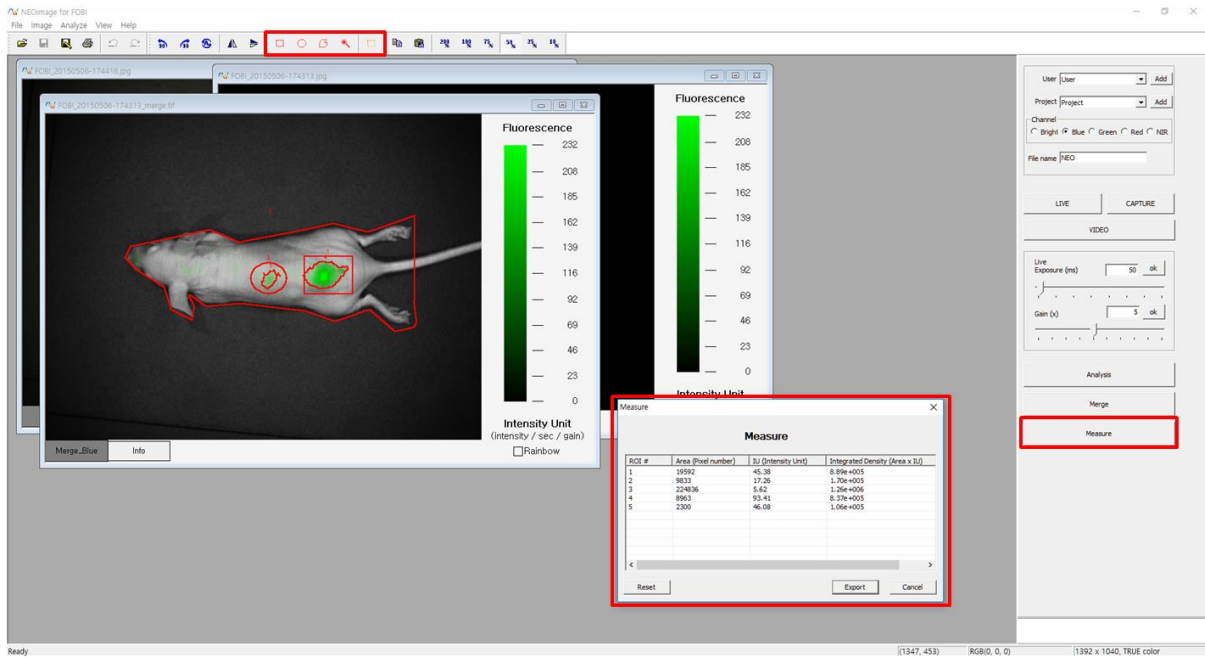
2. Menu Bar
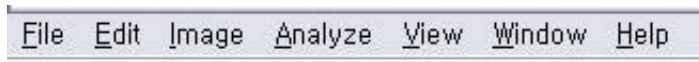
1) File: It opens, closes, saves, saves as, opens Rainbow Standard and closes the obtained picture. Also, it allows a user to open up to nine recent photos without browsing.

2) Edit: A menu that allows you to edit your photos.   Can be used for color conversion, rotation and mirroring. Can be used for Reverse, full reverse and run again. You can also copy and paste of the specified ROI.

3) Image: It undoes, repeats the operation and uses Color, Merge, RGB Split, RGB Merge, Rotate, Flip and Zoom features.

4) Analyze: Image Calculate, GFP Analyze, Subtract Background, Set ROI, Measure features

5) View: It selects Standard Tool Bar, Edit Tool Bar, Status Bar, Dialog Bar visibly or invisibly.

6) Window: Can determine how to align the opened pictures.

7) Help: It checks the information of NEOimage.

3. Standard Tool Bar

The icons of Open, Save, ROI Copy, ROI Paste, Undo, Repeat, etc. can be easily used.

4. Edit Tool Bar

1) Turn: It turns the picture 90 degrees left or right, or by a custom angle. When the custom angle is a positive number, the picture is rotated to the right and when the custom angle is a negative number, the picture is turned to the left. Also, the picture can be flipped by 180 degrees horizontally or vertically.

2) ROI Designation: The ROI can be designated by selecting the feature of Square, Circle, Polygon or Auto form. The ROI shape is chosen on the selected picture and saves the image by dragging the shape on the picture. The designated ROI can be moved or corrected. However, the designation of the Auto feature cannot be undone.

3) Zoom: The size of the image can be in 10%, 25%, 50%, 75%, 100%, and 200%.

5. Status Bar

(733, 627)   RGB(27, 127, 57)   1392 x 1040, TRUE color

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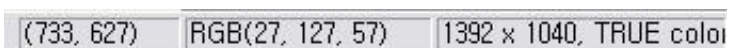
It confirms the mouse coordinate, individual RGB intensity, picture size, picture types. The coordinate and the size of the picture are shown in pixels.

Sepcifications

	FOBI
Image Sensor	½″ Interline Sony ICX205 1.4 megapixel colorCCD sensor
Effective Pixels	1392x1040, 4.65μm square pixels
Frame Rate	15 fps at 1392 x 1040
Digital Output	24-bit
Interface	Standard USB 2.0 high-speed interface
Light source	Diffuse type LED
Channel	Blue (460nm) - GFP, Cy2, FITC, DyLight 488 Green (520nm) – RFP, Cy3, RITC, Alexa Fluor 546 Red (630nm) - Cy5.5, DiD, Alexa Fluor 680, DyLight 680 NIR (730nm) – ICG, Cy7, DiR
Weight	9kg
Size (WxDxH)	260 x 260 x 400 mm

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