OLYMPUS®

Quick Start

FLUOVIEW FV1000

CONFOCAL LASER SCANNING BIOLOGICAL MICROSCOPE

FV10-ASW【Ver1.4】

Petition

Thank you for your purchase of Olympus microscope at this time.
Prior to using this microscope, read this instruction manual for sure to utilize full-fledged performance of this microscope and ensure customer's safety.
In addition, read section "Safety Guide" and "Hardware" of User's Manual – FLUOVIEW FV1000 as well as instruction manual for microscope to understand how to use the equipment thoroughly.
To use laser system correctly, read instruction manuals that come with each laser equipment and light power system.
Hold this manual by your side when using this microscope all the time and keep it with care after reading.

AX7284

Caution

1. Part or whole of this software as well as manual shall not be used or duplicated without consent.
2. Contents described in this manual are subject to change without notice in future.

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1 About this manual

This manual describes about windows that can be displayed (Section 2) and simple operation procedures (Section 3).

For further details of each window, see Online Help that appears through [Help] – [Help].

2 Windows in this software

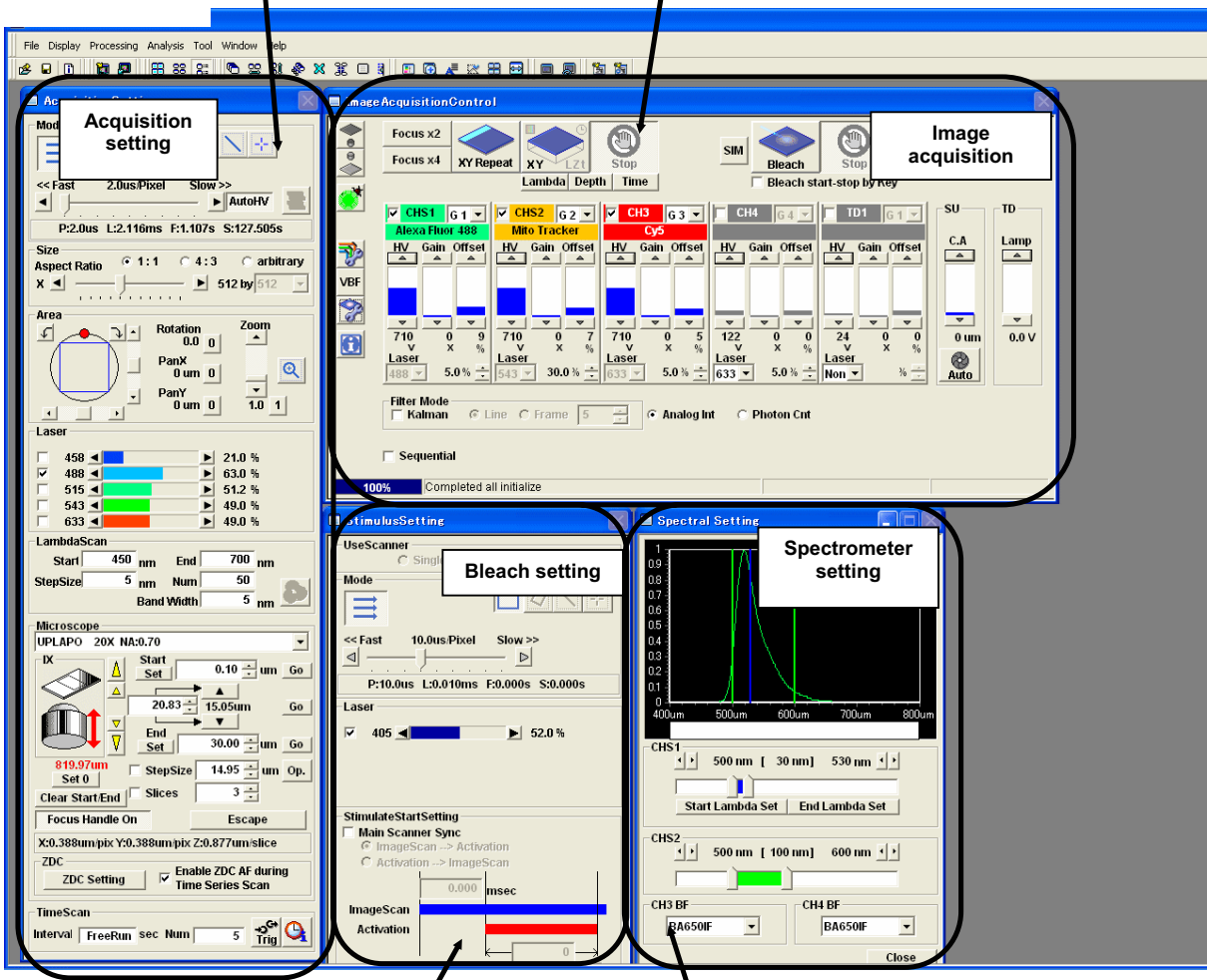
Window used on FV10-ASW (Application Software) is individually introduced.

TIP

When “Acquisition setting” and “Image acquisition” windows are not displayed on start-up of this software, it is not possible to acquire an image (this software starts up with review station). When an image should be acquired with this microscope system, verify conditions of which the above two (2) windows can be displayed.

Window to set image acquisition (See TIP)

Window to acquire image (See TIP)

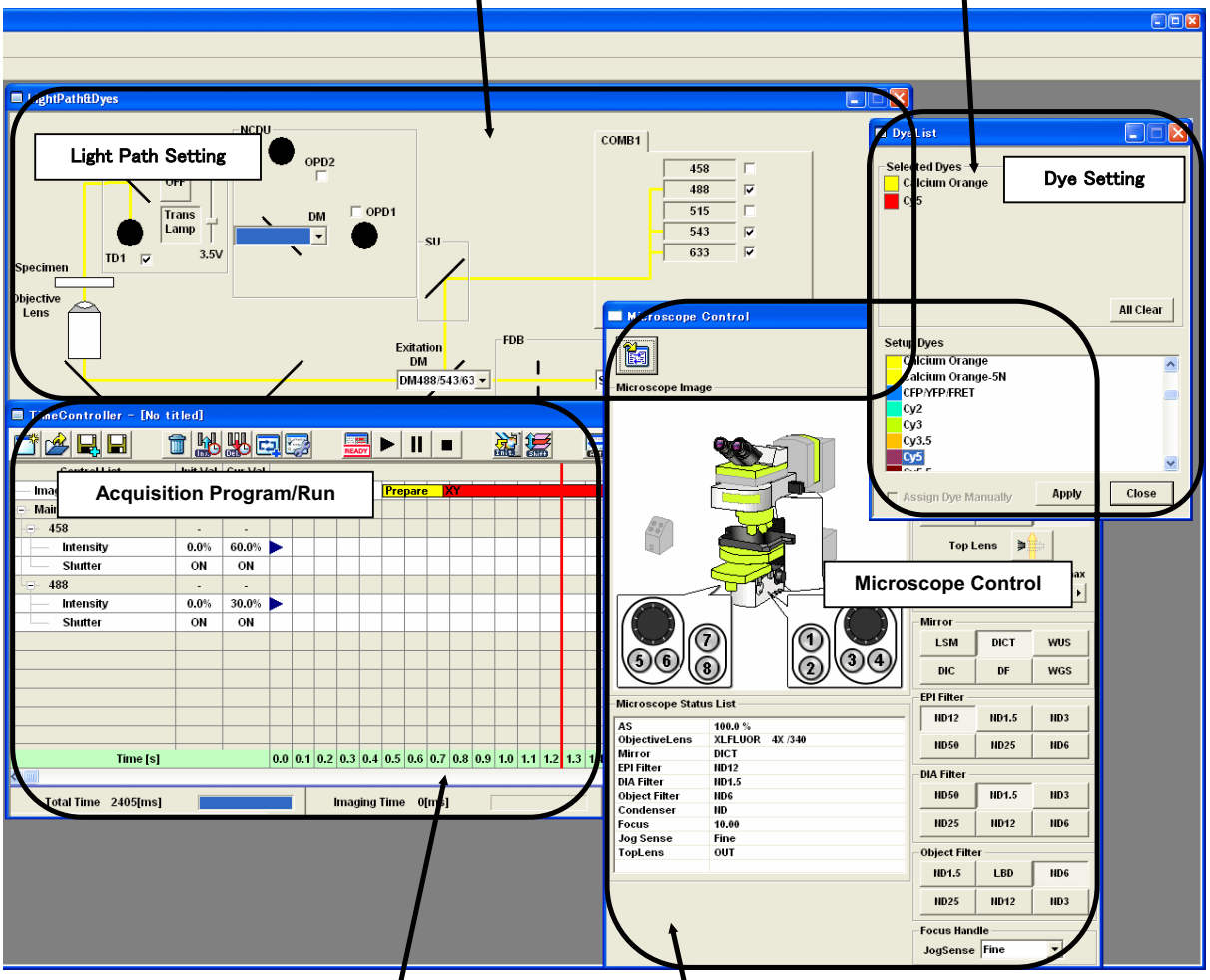


Window that opens when button for “Image Acquisition” is pressed and it is used for bleach setting.
It can be displayed when the SIM scanner exists.

Window that opens when button VBF for “Image Acquisition” is pressed and it is used for spectrometer setting.

Window that opens when button  for "Image Acquisition" is pressed and it is used for light path setting.

Window that opens when button  for "Image Acquisition" is pressed and it is used for Dye setting.



[Device] – [Time Controller]

Window that the acquisition is programmed and the process is executed.

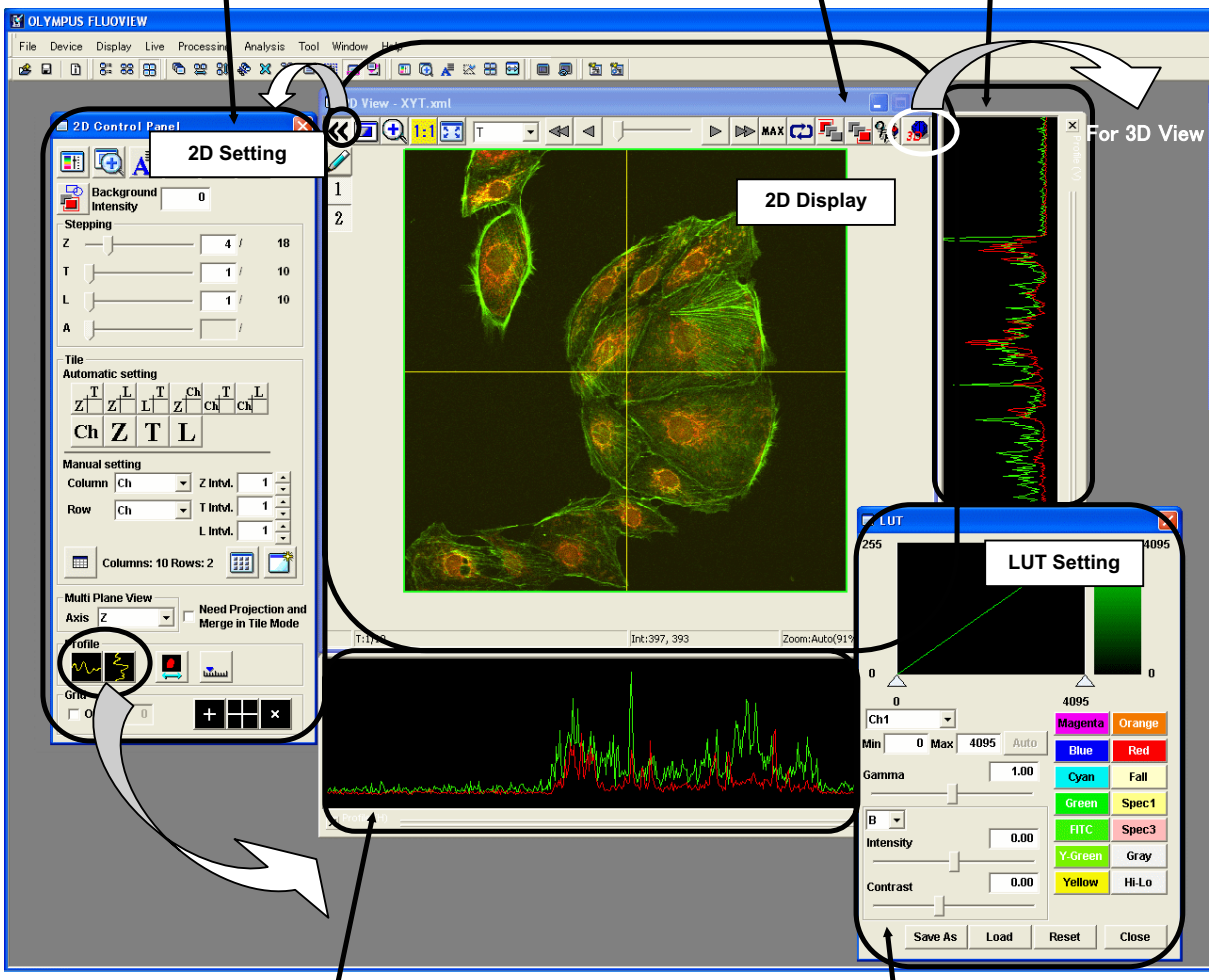
[Device] - [Microscope Controller]

Window to control microscope main unit and motorized section attached to the microscope main unit.

Window that opens when button for "2D Display" is pressed and it is used for 2D display setting.

Window to display 2D. When "Live View" appears, the image being acquired will be displayed.

Window that opens when button for "2D Setting" is pressed and it is used to display intensity profile of 2D image in vertical direction.



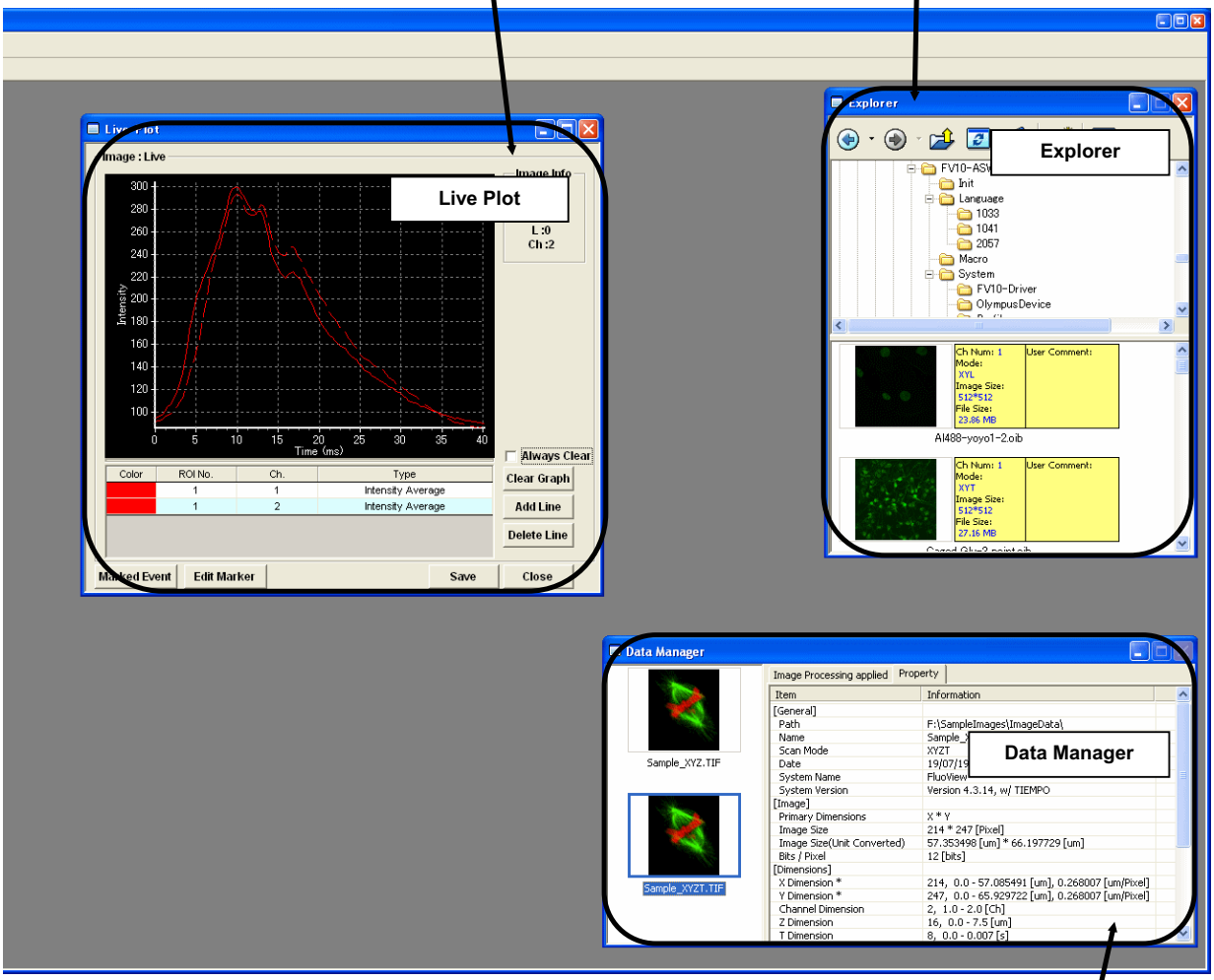
Window that opens when button for "2D Setting" is pressed and it is used to display intensity profile of 2D image in horizontal direction.

Window that opens when button for "2D Setting" is pressed and it is used for LUT setting that changes image display color.

[Live] – [Live Plot]

Window for analysis of an image being acquired and it indicates a graph in real time.

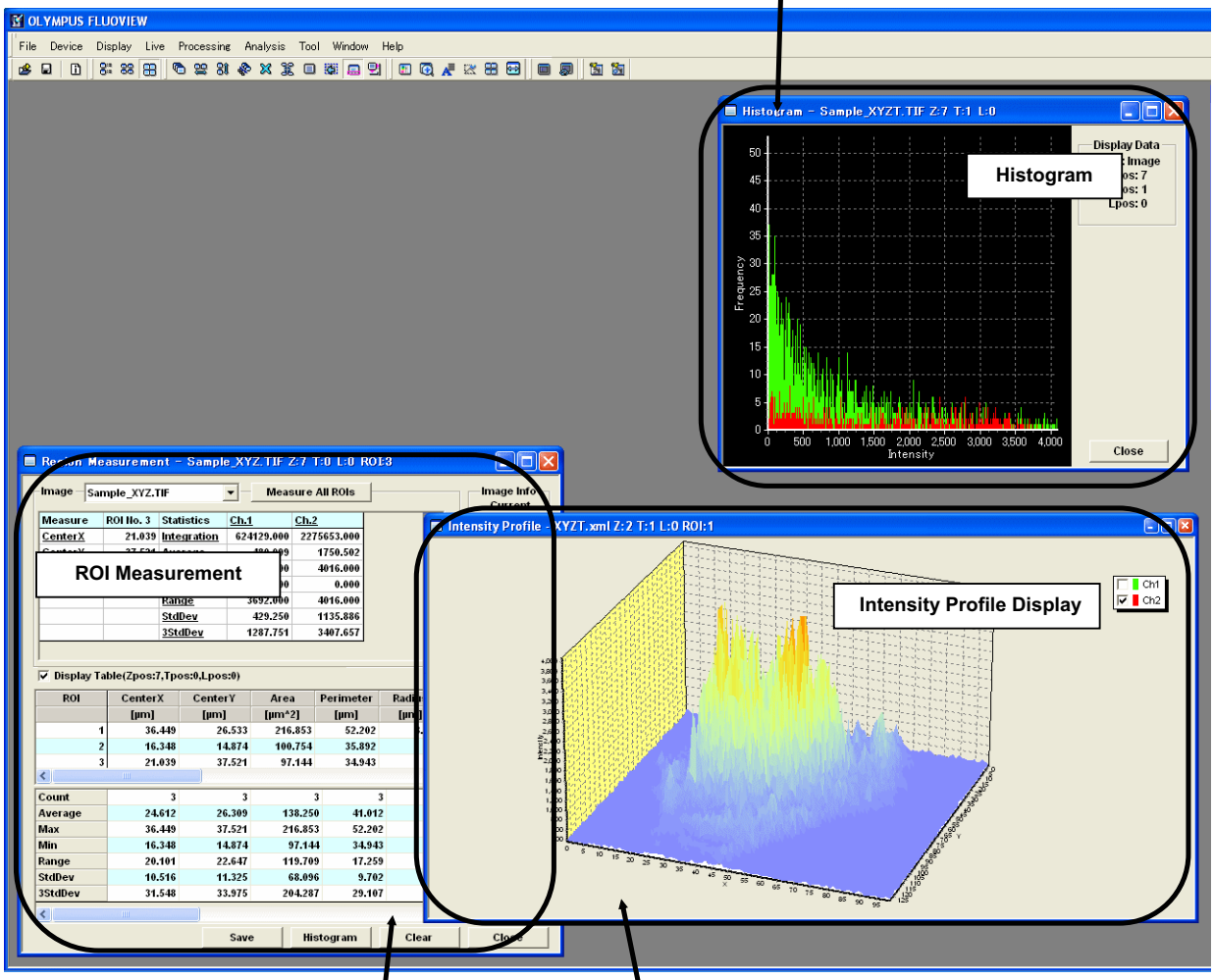
Window to load an image by selecting folder that saves the images. When thumbnail image is double-clicked, the file is called and displayed.



Window where thumbnail and property of the image currently loaded is displayed.

Windows in this software

[Analysis] – [Single] – [Histogram]
Window that Histogram is displayed.

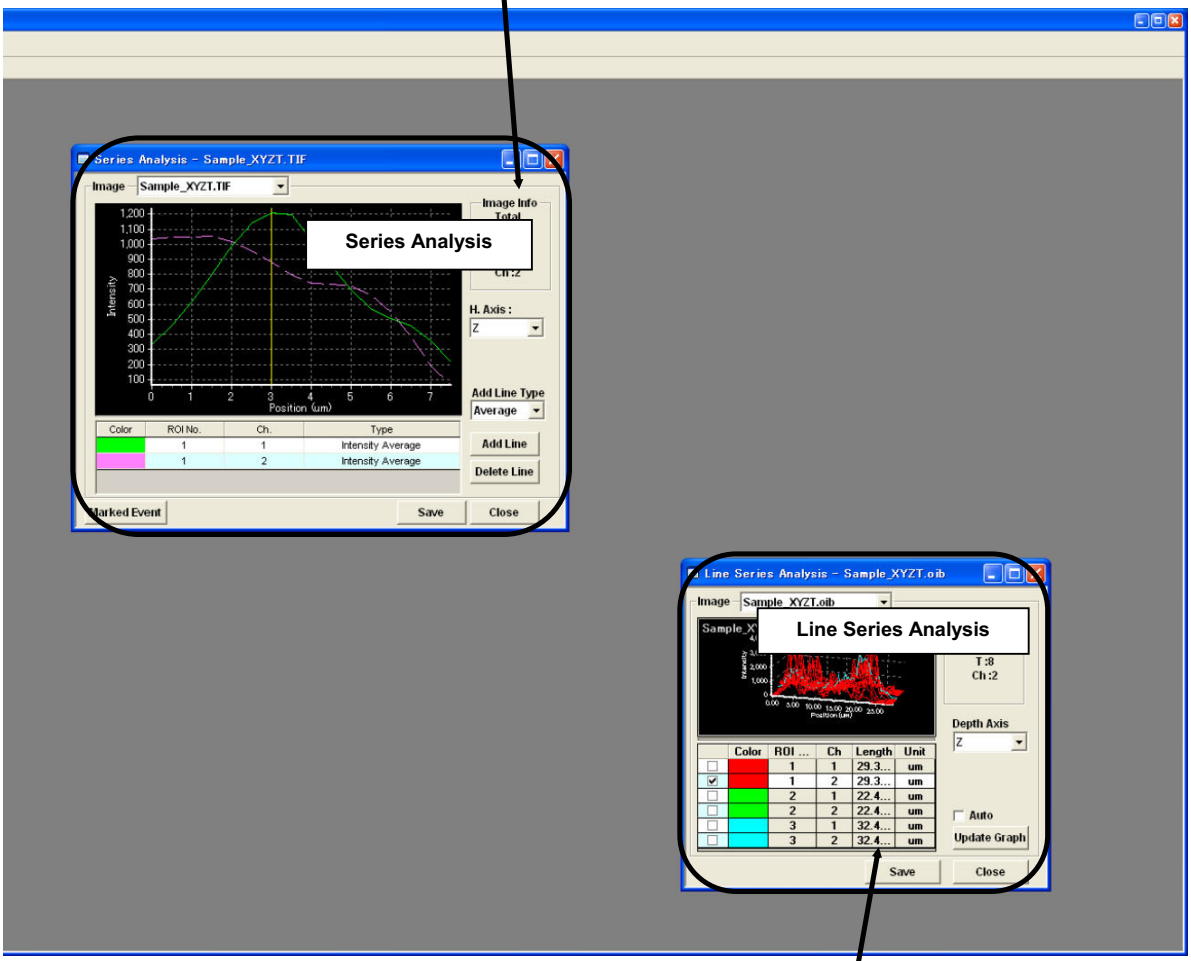


[Analysis] – [Single] – [Measurement]
Window that ROI (Region of Interest) is measured.

[Analysis] – [Single] – [Intensity Profile]
Window that intensity profile inside ROI selected is displayed.

[Analysis] - [Series]

Window that series image analysis is done. Time change for average value of intensity or integrated value can be displayed in graph.



[Analysis] – [Line Series]

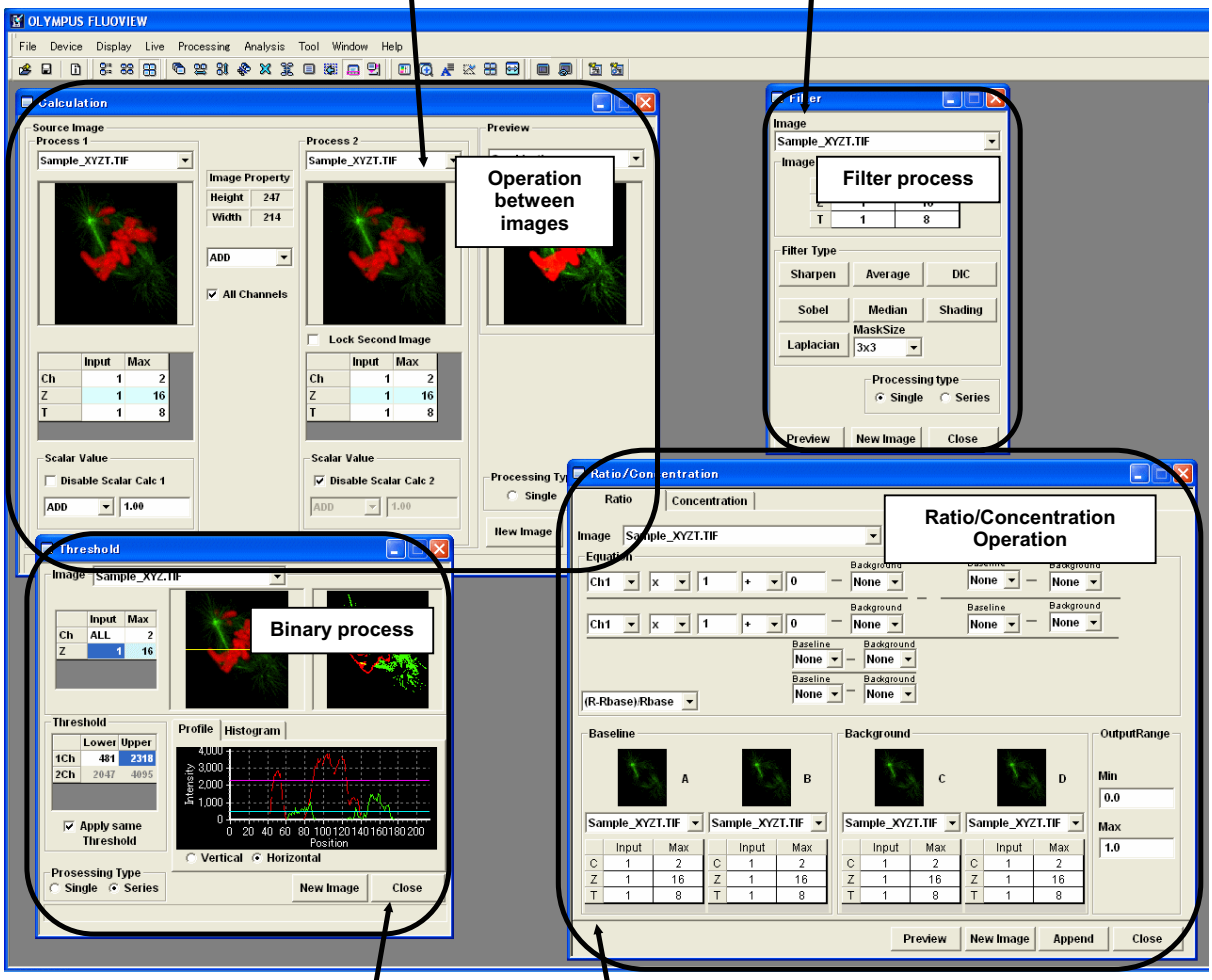
Window used to perform series image analysis on ROI (Region of Interest) of line.

Windows in this software

[Processing] – [Image Calculation]

Window used to perform operations between images. Logic and arithmetic operation for image intensity can be done.

[Processing] - [Filter setting]
Window to enhance the image.



[Processing] – [Threshold]

Window to turn the image to binary data.

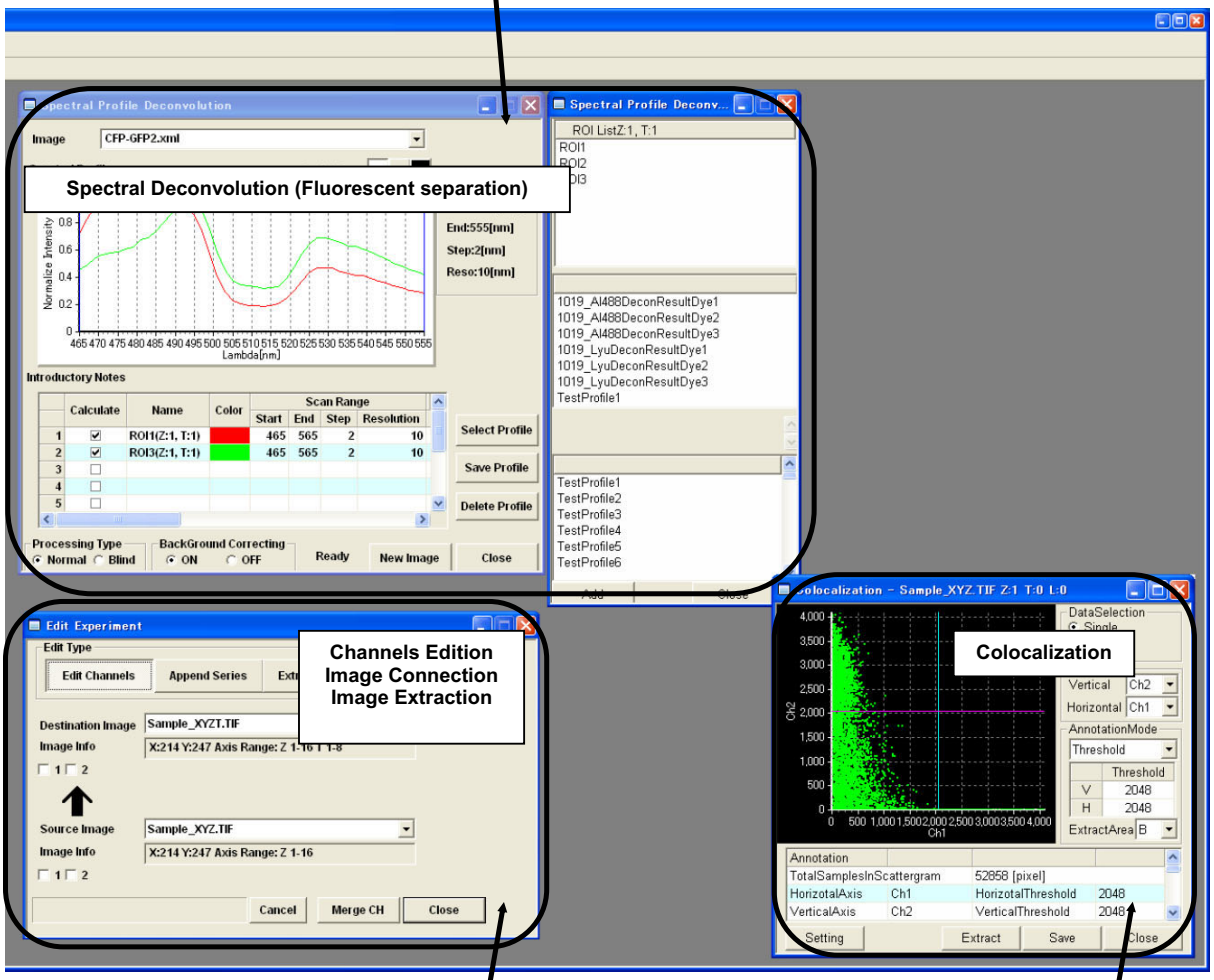
[Processing] - [Ratio/Concentration]

Window where an image of the following value converted to intensity value is created.

- **"Ratio": Intensity ratio between channels**
- **"Concentration": Ion concentration of specimen**

[Processing] – [Spectral Deconvolution]

Window for spectral deconvolution (the process to separate fluorescent from image of multi-dyeing specimen).



[Processing] – [Edit Experiment]

Window that the following image editions are done.

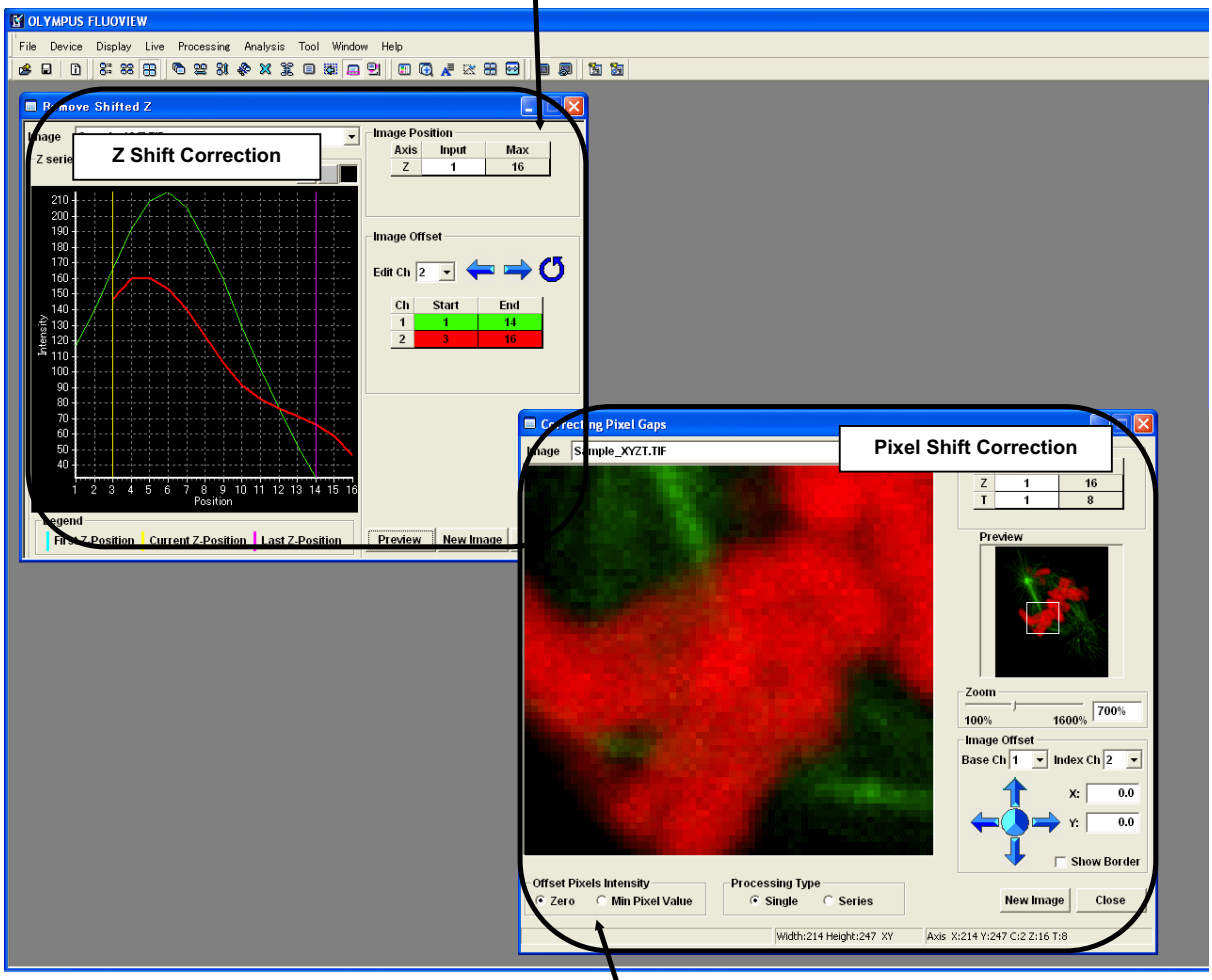
- **"Edit channels": To combine or extract channel or channels.**
- **"Append Series": To append image each other.**
- **"Extract Series": To extract image.**

[Processing] - [Colocalization]

It is the window to see how the intensity is overlapping.

For example, it is possible to see what extent the bright area of Ch1 is overlapping with the bright area of Ch2 quantitatively.

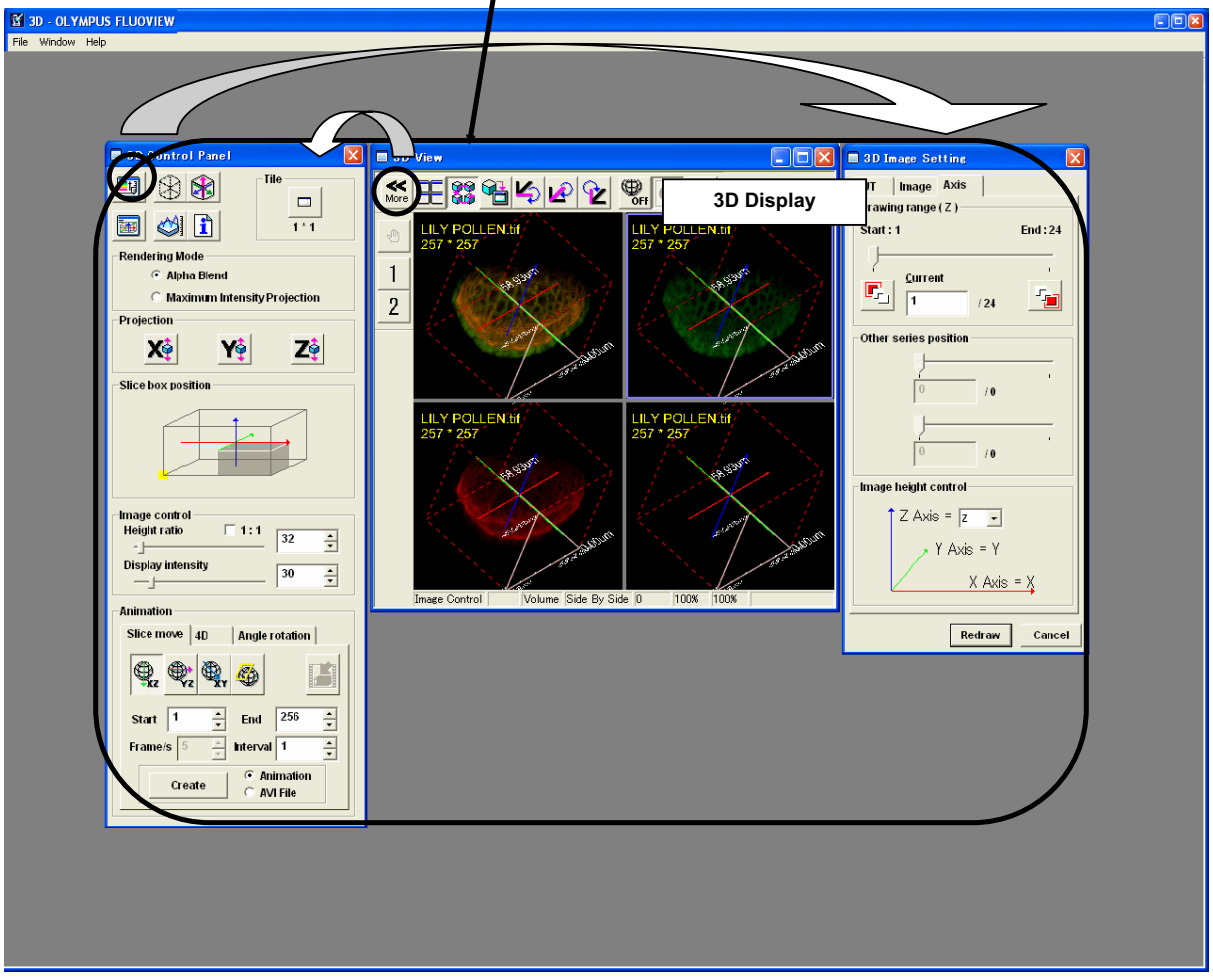
[Processing] – [Correcting Z Gaps]
Window where Z position shift between image channels is corrected.



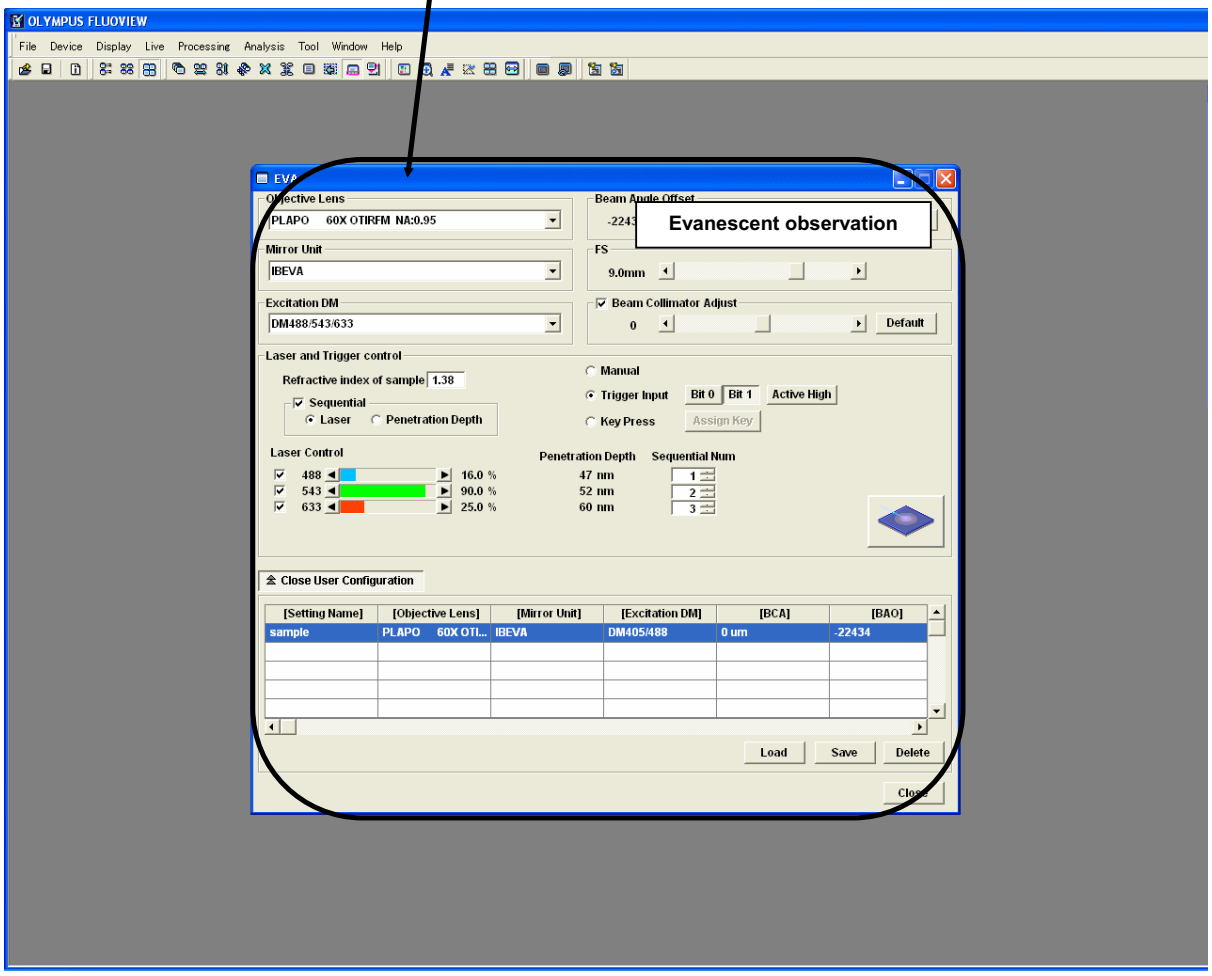
[Processing] – [Correcting Pixel Gaps]
Window where pixel position is corrected in XY direction, if it is shifted, between light receiving channels while image is being acquired.

Window for 3D display that opens

when 2D Display  **button is pressed.**



This is a window that is opened with EVA button on “Image acquisition” window (Page 2) and it is used for evanescent observation. It can be displayed when EVA exists.

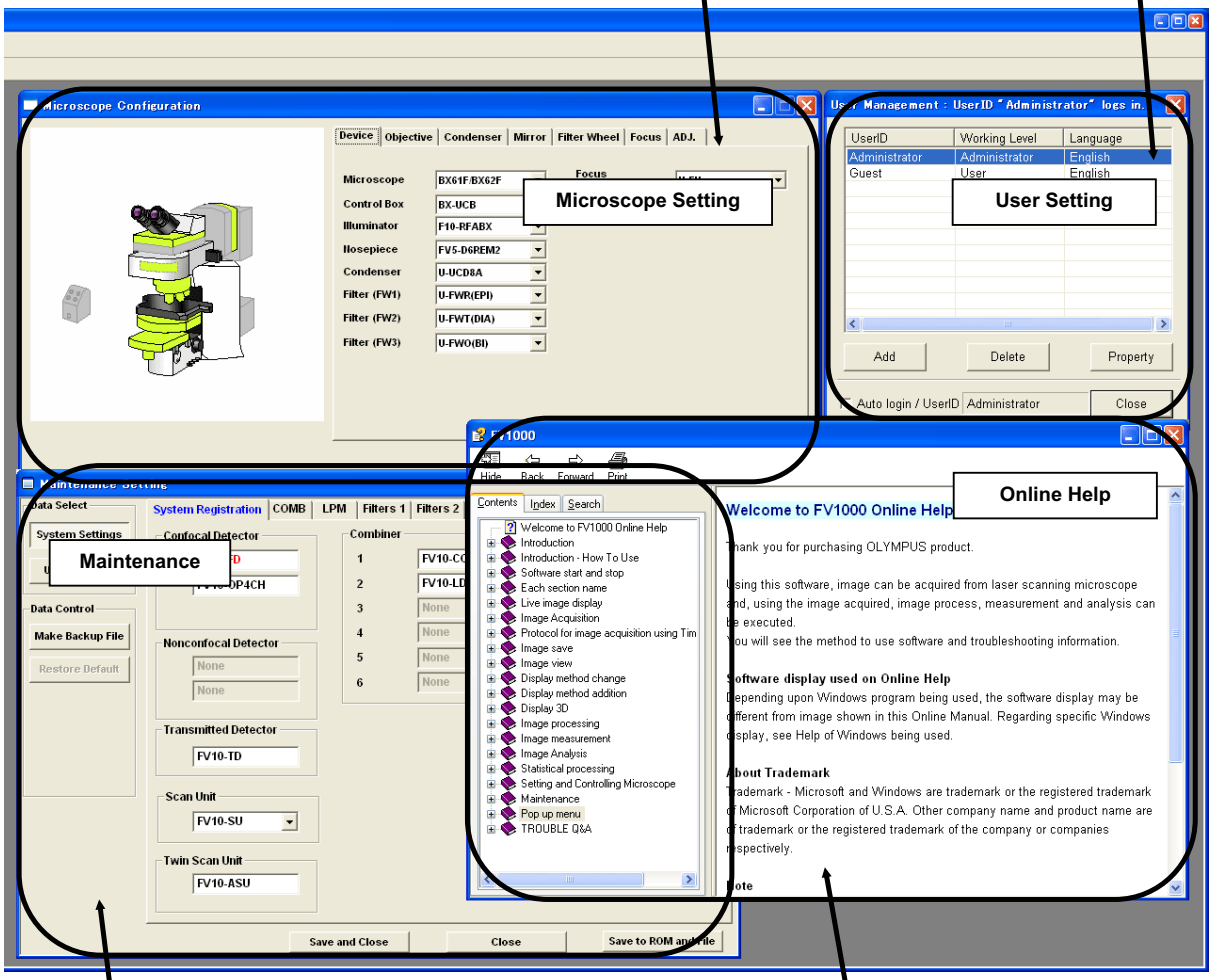


[Tool] - [Microscope Configuration]

Window for setting microscope main unit and motorized section attached to microscope main unit.

[Tool] - [User Setting]

Window for user information setting.
New addition, deletion or change of user can be set.



[Tool] – [Maintenance]

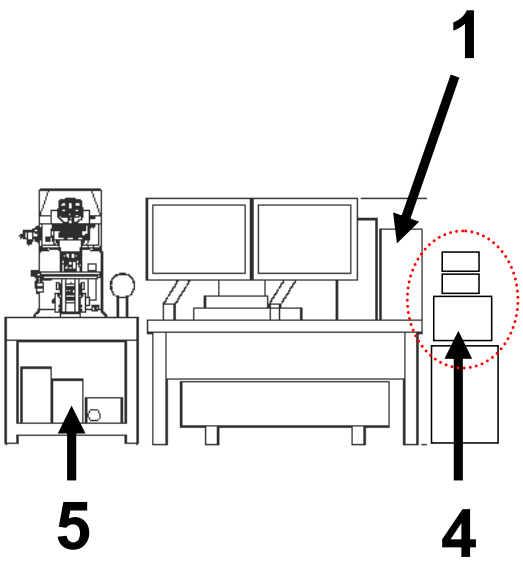
Maintenance of system can be done.

[Help] – [Help]

Online Help. See here for the details of operation methods.

3 Basic operation procedures

3.1 System Start Up



1. Turn computer to ON.
2. Turn FV10-PSU to ON.
3. Turn BX-UCB or IX2-UCB to ON.
4. Turn laser to ON.
   (Turn key switch.)
   4-1. Multi Ar
   （458nm･488nm･514nm）ON
   4-2. HeNe（G）(543nm） ON
   4-3. HeNe（R）(633nm） ON

5. Turn mercury lamp power to ON.

6. Enter user name/password and logon to WindowsXP.

   User name: _____
   Password: _____

7. Double-click 

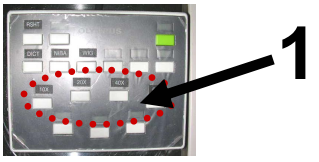
and this software starts up.

   User name: _____
   Password: _____

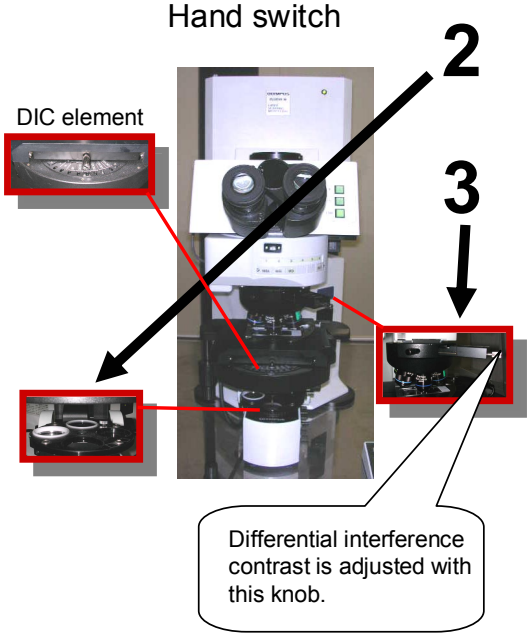
＊It takes a certain period of time for start up.

3.2 Visual observation with microsocpe (Differential Interference Image Observation)

3.2.1 Erecting type BX



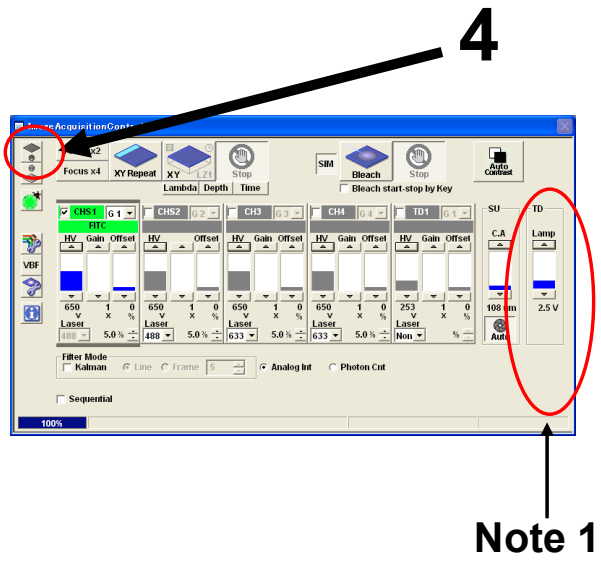
1. Select objective lens with hand switch.
(Ref: - Memo -)



2. Insert polarizer.

3. Insert differential interference prism slider.

4. Click  of “Image Acquisition”.



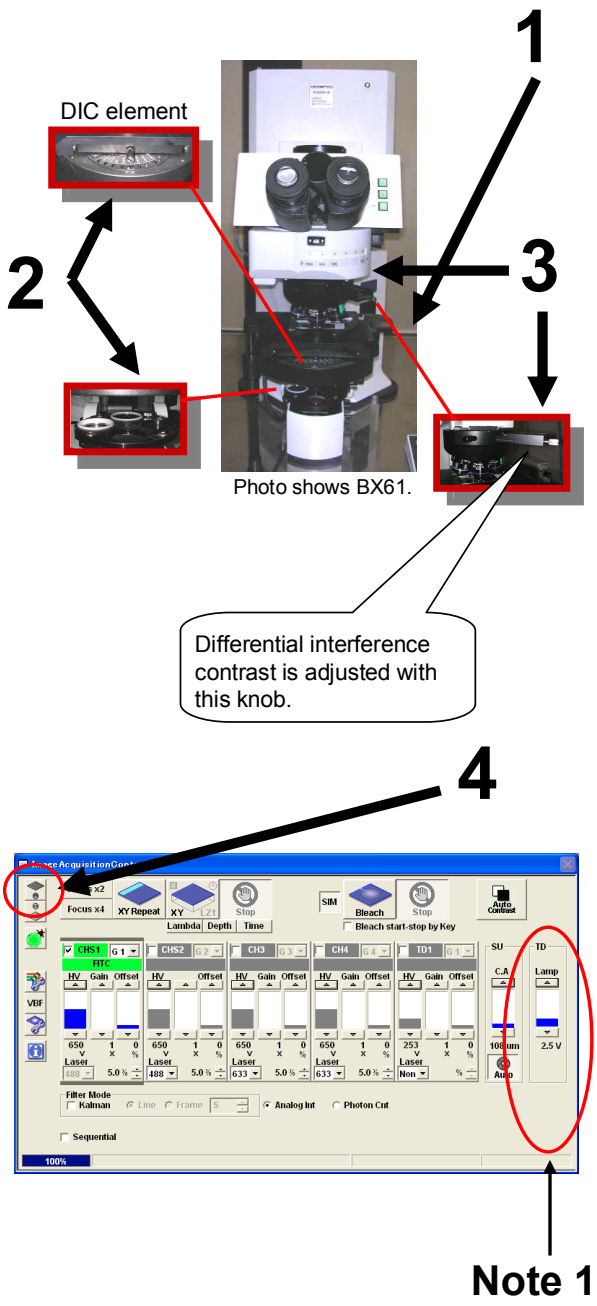
Note 1: Light of halogen lamp is adjusted, using TD Lamp slider on “Image Acquisition”.

Note 2: Check if the filter turret is set to 6. DIC. If not, press DICT button of hand switch.

5. Adjust focus.

- Memo -
When magnification change is required hereafter, do procedure 1 only and the followings will be changed accordingly:;
- Objective lens
- Optimum DIC element to each objective lens

3.2.2 Erecting type BXWI



1. Select objective lens.

2. Insert polarizer and select DIC element.

3. Insert differential interference prism slider and select differential interference analyzer (DICT).

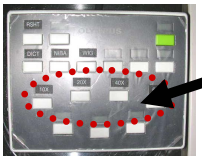
4. Click  of "Image Acquisition".

Note 1: Light of halogen lamp is adjusted, using TD Lamp slider on "Image Acquisition".

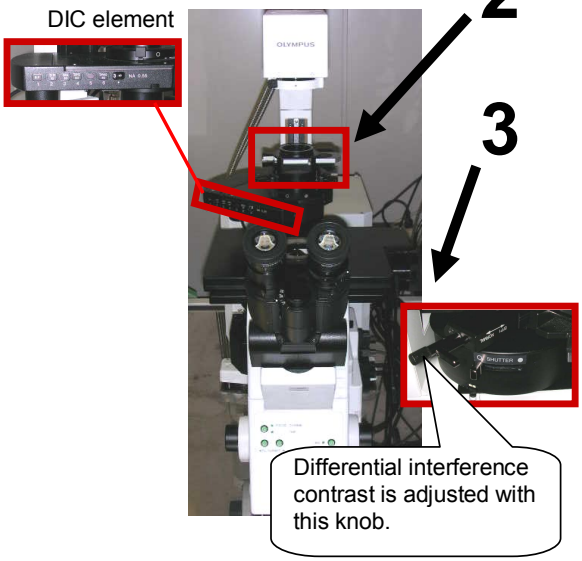
Note 2: Check if the filter turret is set to 6. DICT. If not, press DICT button of hand switch.

5. Adjust focus.

3.2.3 Inverted type IX



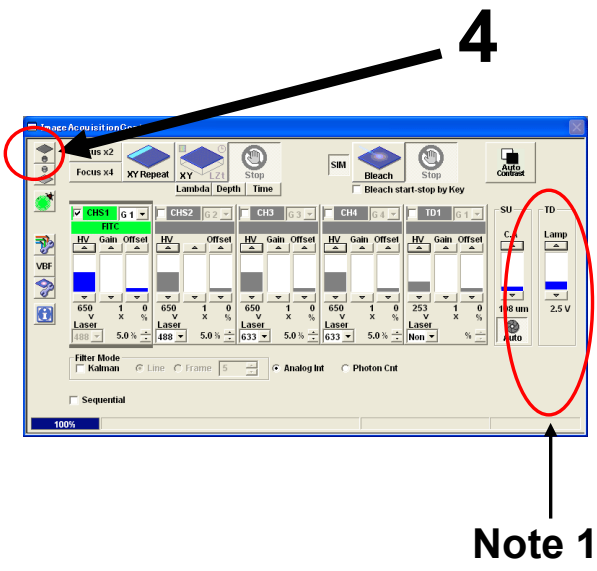
1. Select objective lens with hand switch.
   (Ref: -Memo-)



2. Insert polarizer.

3. Insert differential interference prism slider.

4. Click  of “Image Acquisition”.



Note 1: Light of halogen lamp is adjusted, using TD Lamp slider on “Image Acquisition”.

Note 2: Check if the filter turret is set to 6. DIC. If not, press DICT button of hand switch.

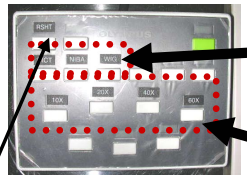
5. Adjust focus.

- Memo -

When magnification change is required hereafter, do procedure 1 only and the followings will be changed accordingly;
- Objective lens
- Optimum DIC element for each objective lens

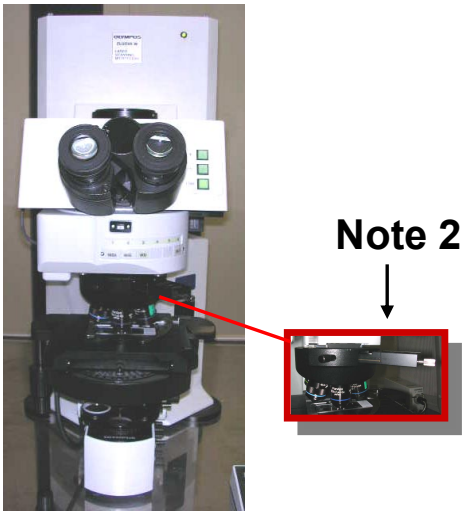
3.3 Visual observation with microscope (Fluorescent image observation)

3.3.1 Erecting type BX, BXWI



1. Select objective lens with hand switch.

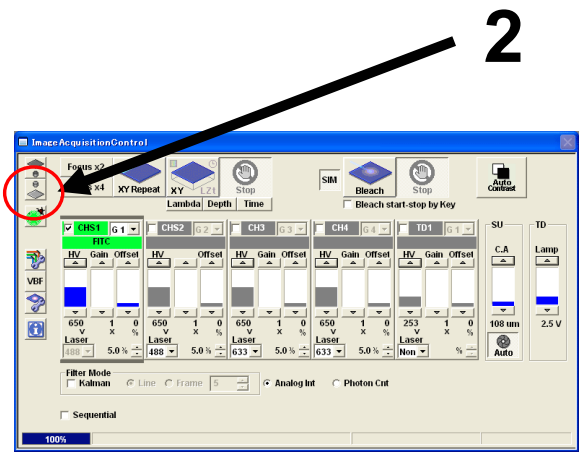
2. Click  of “Image Acquisition”.



Note 1: Operation in procedure 2 turns the mode to fluorescent visual mode. At this moment, mechanical shutter of mercury lamp will open. Be careful. (Mercury lamp shutter – Close is done from hand switch.)

Note 2: Verify that the differential interference slider is pulled out.

3. Select fluorescent filter with hand switch. (Ref: - Memo -)

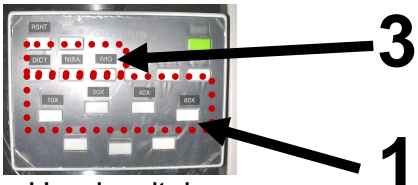


- Memo -
About fluorescent filter

NIBA: Blue excitation/Green fluorescent
(Example: FITC, EGFP etc.)
WIG: Green excitation/Red fluorescent
(Example: Rhodamine, DsRed. etc.)

4. Adjust focus.

3.3.2 Inverted type IX



Hand switch

Note 2

Note 1 & 3

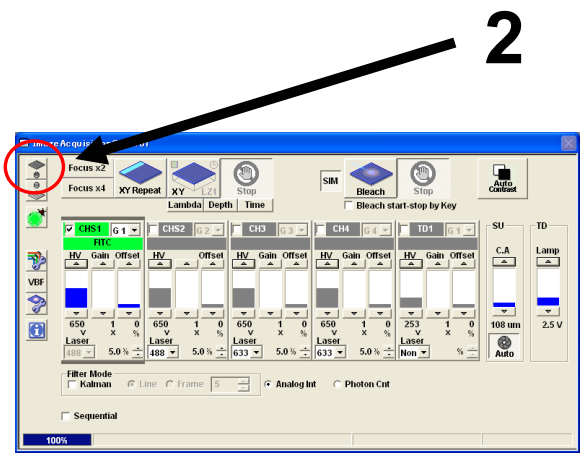
1. Select objective lens with hand switch.

2. Click  of “Image Acquisition”.

Note 1: Operation in procedure 3 turns the mode to fluorescent visual mode. At this moment, mercury lamp mechanical shutter will open. Be careful. (It is recommended that the mercury lamp manual shutter is set to Close ● beforehand.)

Note 2: Verify that the differential interference slider is pulled out.

3. Select fluorescent filter with hand switch. (Ref: - Memo -)



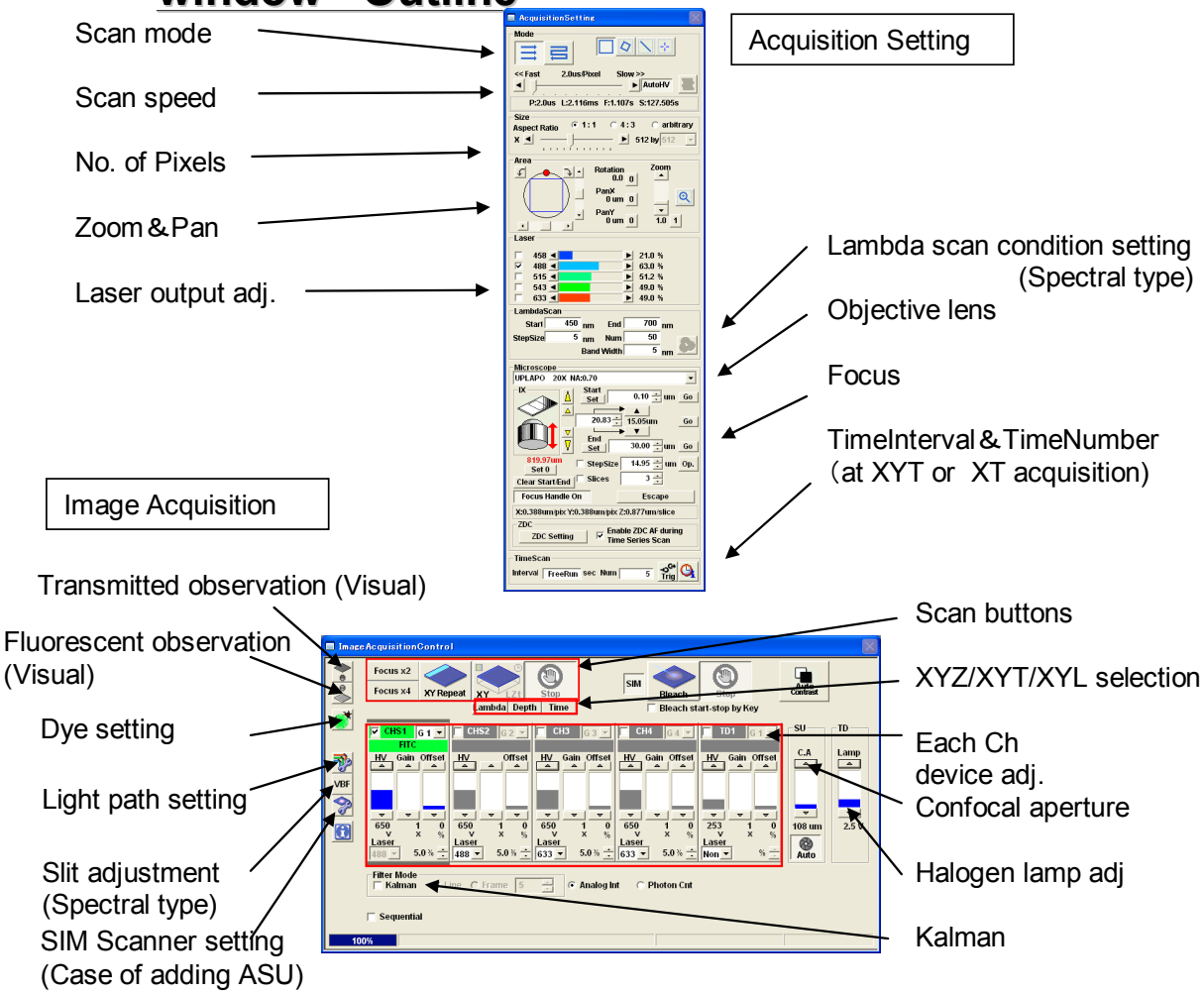
Note 3: When viewing through microscope, verify whether or not the mercury lamp mechanical shutter is set to Open ○.

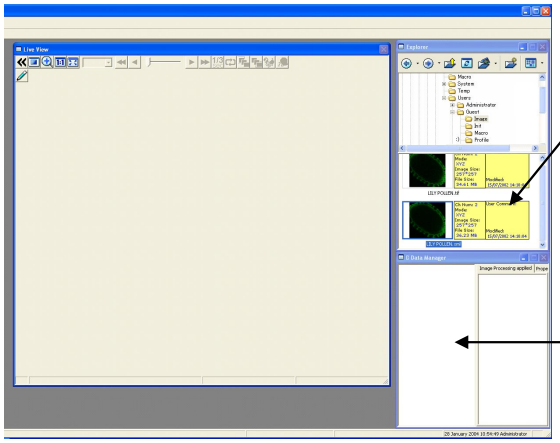
4. Adjust focus.

- Memo -
About fluorescent filter

NIBA: Blue excitation/Green fluorescent
(Example: FITC, EGFP, etc.)
WIG : Green excitation/Red fluorescent
(Example: Rhodamine, DsRed, etc.)

3.4 “Acquisition Setting”, “Image acquisition” window - Outline



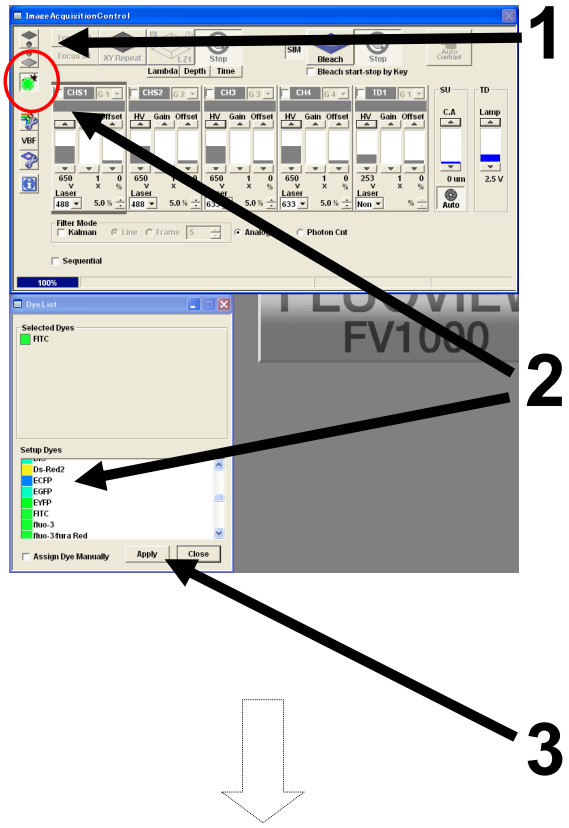


3.5 Image acquisition

3.5.1 Single dyeing color XY

-- 1 slice of image acquisition (XY plane) (Fluorescent image only) --

Example: Single dyeing with green fluorescence (FITC)



1. Click  of “Image Acquisition” and set it to “not pressed” state and close shutter of fluorescent lamp.

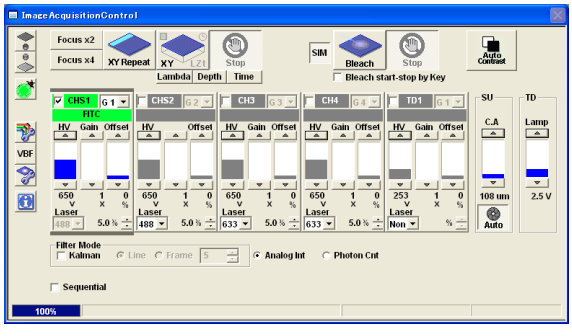
   Click  and set it to “not pressed” state and close shutter of halogen lamp.

2. Click <DyeList> button and double-click fluorescent reagent (FITC) that should be observed from “DyeList” window.

   * When reselecting, double-click the fluorescent dye in [AssignDyes] one time to clear it, and then, perform operation in procedure 2.

3. Click <Apply> button.

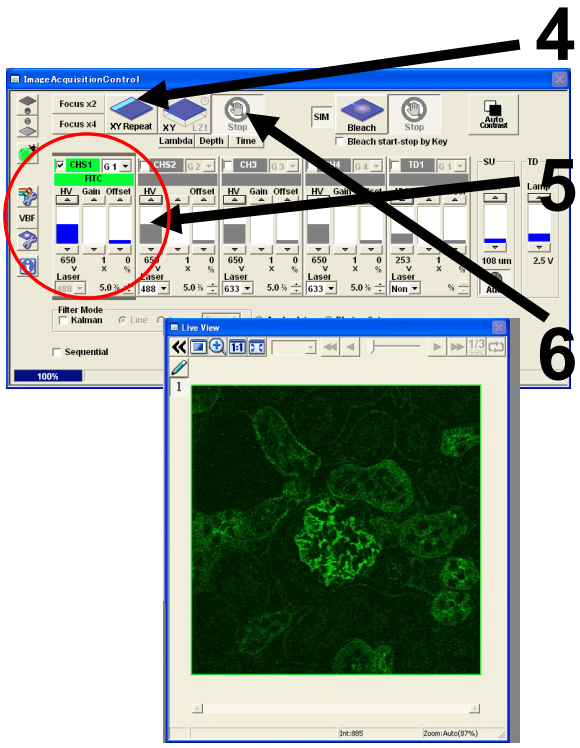
   (“DyeList” window can be closed with <Close> button.)



Window after DyeApply is clicked

- In case of Spectral type -
When this operation is done, the fluorescent wavelength suit to Fluorescent Dye selected is defined.

Moreover, the fluorescent wavelength is manually changeable.
For further details, see Appendix C.



4. Click <XY Repeat> button to perform scanning.

5. Adjust green (FITC) image. (See below for outline of image adjustment. For further details, see Appendix A.)

6. Click <STOP> button to stop scanning. (Ref: - Memo -)

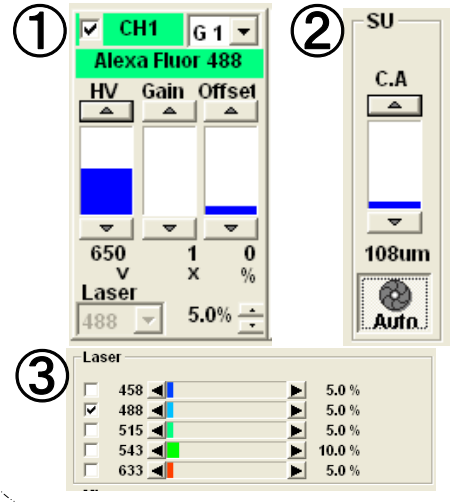
- Memo -
About scan button

: Consecutive scan

: Scan stop

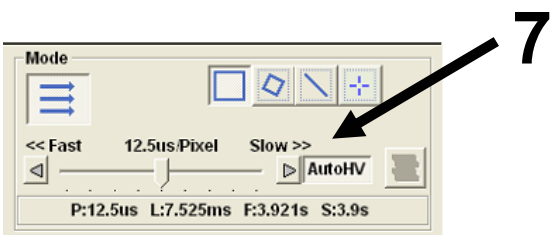
: Rough scan
(Lines skipped and scanned)

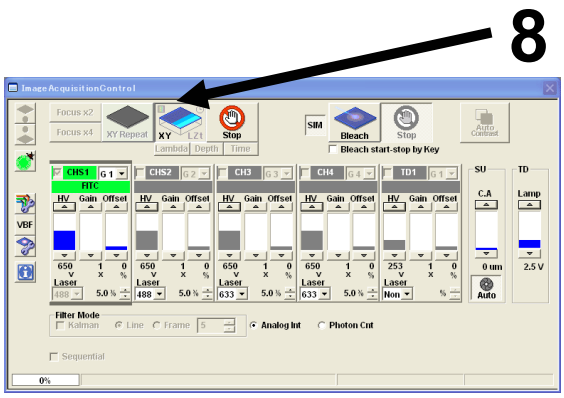
Outline of image adjustment

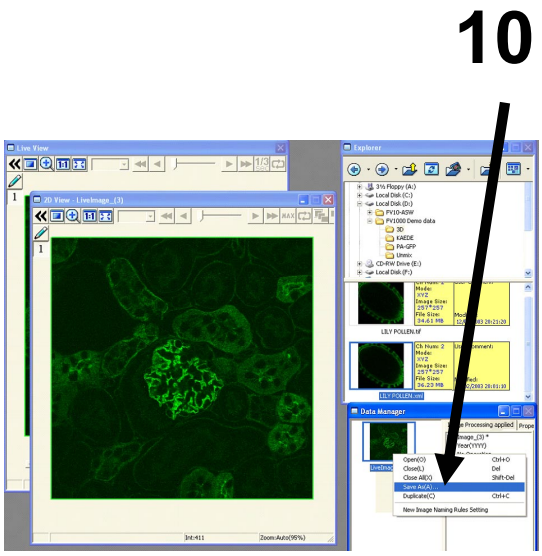


① Sensitivity of detector adjustment (HV)
② Confocal aperture adjustment (CA)
③ Laser output adjustment (Laser)

Adjustment method (Example: HV)
When any place on slider is clicked, the HV can be jumped UP or DOWN to the point where it is clicked. For fine tuning, click  or use mouse wheel.







7. Select <AutoHV> button and select [ScanSpeed].

   * The slower the speed set, the more the noise only can be reduced by keeping current brightness.
   (In addition, Kalman integration is available as a separate method to remove noise.
   For further details, see Appendix B.)

8. Click <XY> button to acquire an image.
9. When acquisition is completed, “2D View-(File Name)” will appear on title bar of the image acquired.
10. Image save:
    Click mouse right button over Image displayed on “DataManager” and then, select [SaveAs].

    (Save as Type “oib” or “oif” is the dedicated file format for this software.)

- Memo -
Dedicated file format for this software

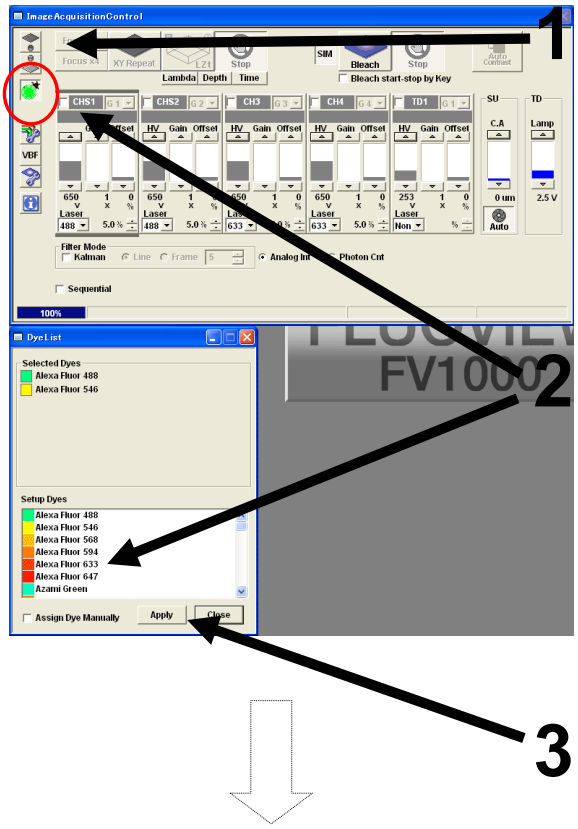
OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

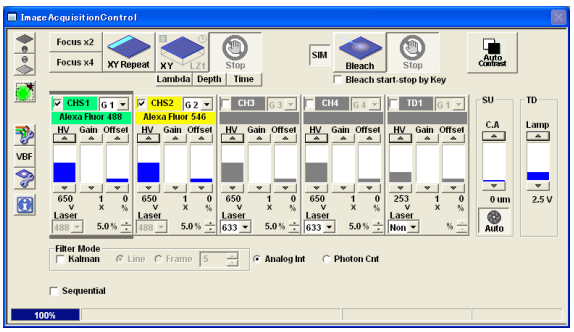
3.5.2 Double dyeing color XY (Simultaneous scan version)

-- 1 slice of image acquisition (XY plane) (Fluorescent image only) --

Example: Green fluorescence (Alexa488) + Red fluorescence (Alexa546)

Double dyeing





Window after DyeApply clicked

1. Click  of “Image Acquisition” and set it to “not pressed” state and close shutter of fluorescent lamp.
   Click  and set it to “not pressed” state and close shutter of halogen lamp.

2. Click <DyeList> button and select fluorescent reagent (Alexa488, Alexa546) from “DyeList” window and double-click it.

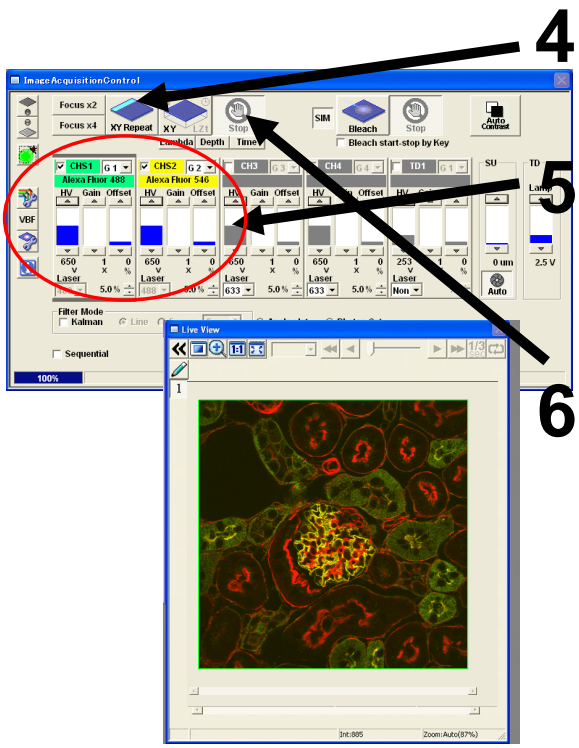
   * When reselecting, double-click the fluorescent reagent in [AssignDyes] one time to delete it and then, do procedure 2.

3. Click <Apply> button.

   (“DyeList” window can be closed with <Close> button.)

- In case of Spectral type -
When this operation is done, the fluorescent wavelength suit to Fluorescent Dye selected is defined.

Moreover, the fluorescent wavelength is manually changeable.
For further details, see Appendix C.



4. Click <XY Repeat> button to perform scanning.

5. Adjust image of Green (AlexaFluor488) and Red (AlexaFluor546). (See below regarding outline of image adjustment. For further details, see Appendix A.)

6. Click <Stop> button to stop scanning. (Ref: - Memo -)

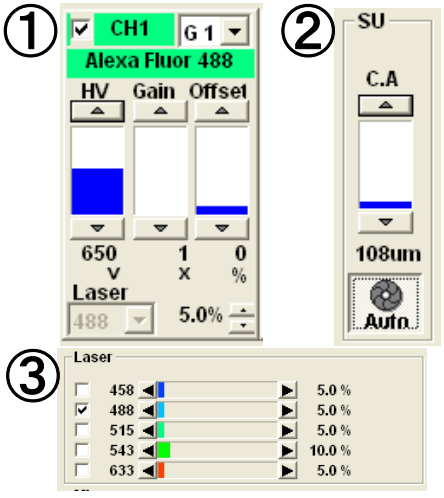
- Memo -
Scan buttons

: Consecutive scan

: Scan stop

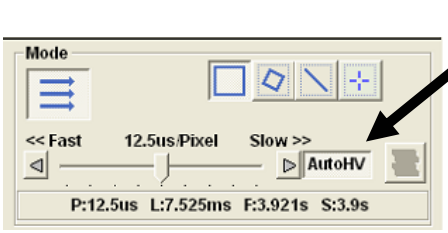
: Rough scan
(Lines skipped and scanned)

Outline of image adjustment

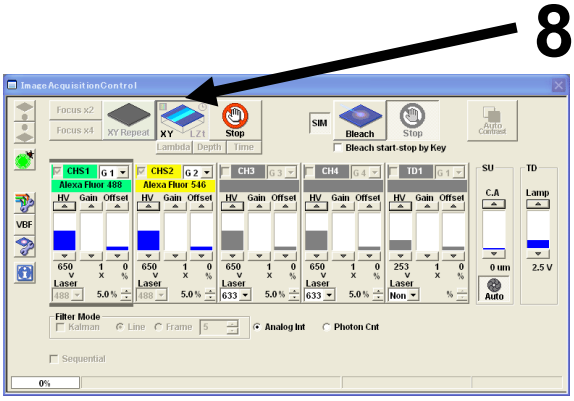


① Sensitivity adjustment of detector (HV)
② Confocal aperture adjustment (CA)
③ Laser output adjustment (Laser)

Adjustment method (Example: HV)
When any place on slider is clicked, the HV can be jumped UP or DOWN to the point where it is clicked. For fine tuning, click ▲ or use mouse wheel.



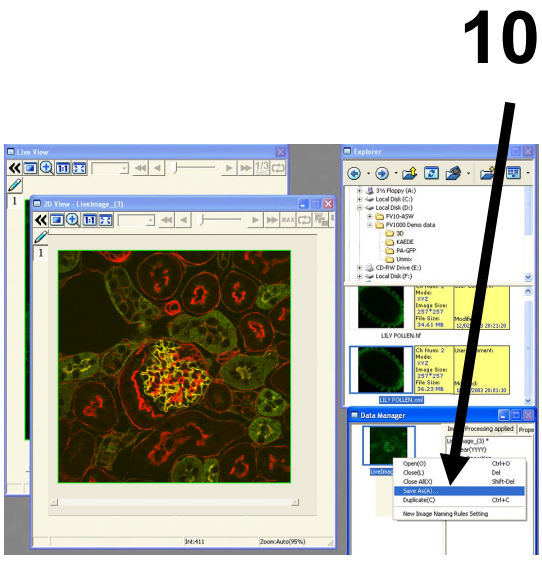
7. Select <AutoHV> button and select [ScanSpeed].
   * The slower the speed set, the more the noise only can be reduced by keeping current brightness.
   (In addition, Kalman integration is available as a separate method to remove noise. For further details, see Appendix B.)



8. Click XY button to acquire an image.

9. When acquisition is completed, "2D View-(File Name)" will appear on title bar of the image acquired.

10. Image save:
    Click mouse right button over Image displayed on "DataManager" and then, select [SaveAs].
    (Save as Type "oib" or "oif" is the dedicated file format for this software.)



- Memo -
Dedicated file format for this software

OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.

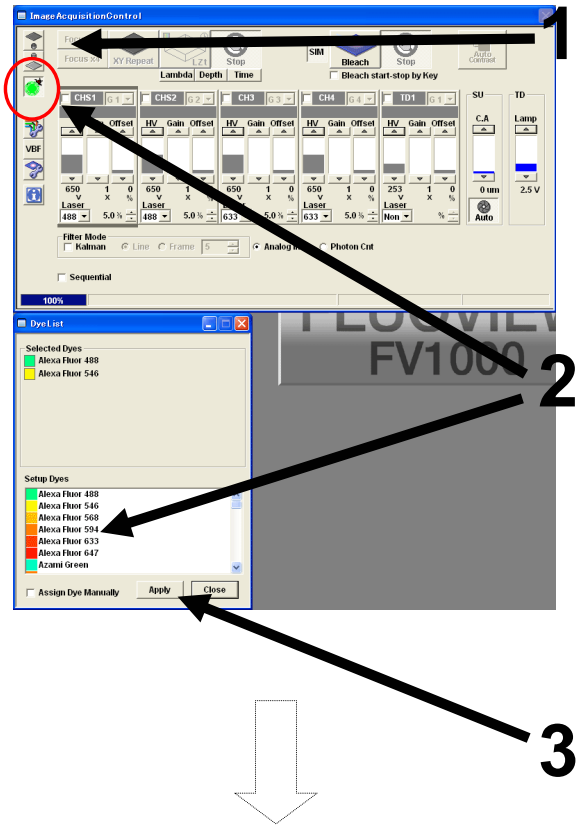
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

3.5.3 Double dyeing color XY (Line sequential scan version)

-- 1 slice of image acquisition (XY plane) (Fluorescent image only) --

Example: Green fluorescence (Alexa488) + Red fluorescence (Alexa546)

Double Dyeing



1. Click  of “Image Acquisition” and set it to “not pressed” state and close shutter of fluorescent lamp.

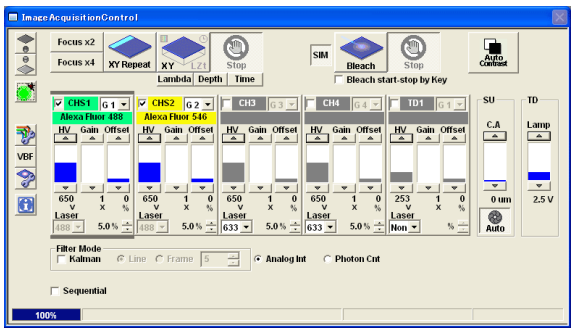
   Click  and set it to “not pressed” state and close shutter of halogen lamp.

2. Click <DyeList> button and select fluorescent reagent (Alexa488, Alexa546) from “DyeList” window and double-click it.

   * When reselecting, double-click the fluorescent reagent in [AssignDyes] one time to delete it and then, do procedure 2.

3. Click <Apply> button.

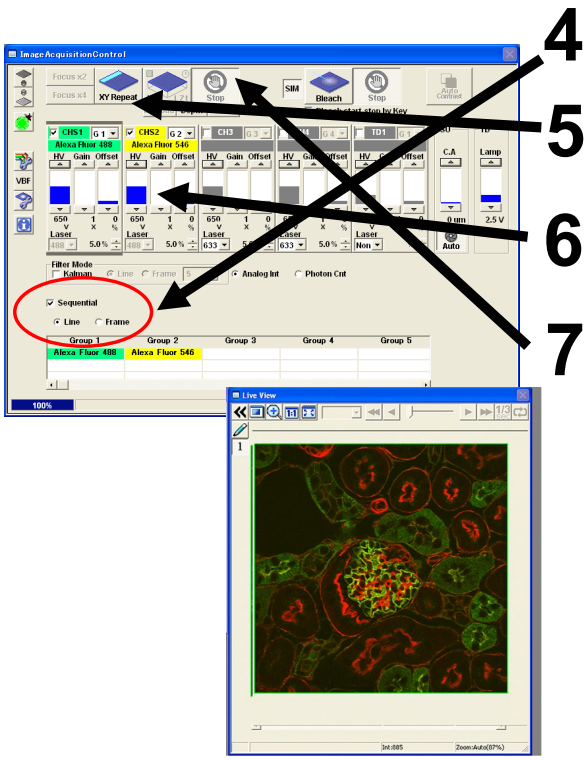
   (“DyeList” window can be closed with <Close> button.)



Window after DyeApply clicked

- In case of Spectral type -
When this operation is done, the fluorescent wavelength suit to Fluorescent Dye selected is defined.

Moreover, the fluorescent wavelength is manually changeable.
For further details, see Appendix C.



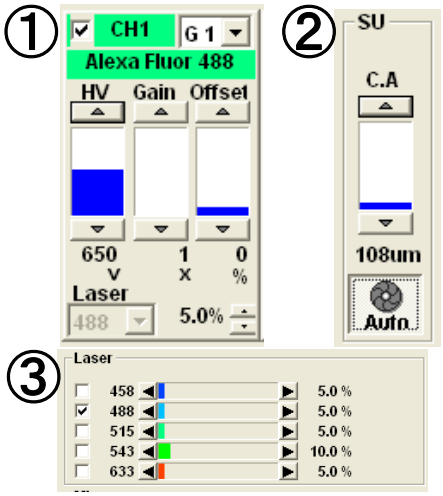
4. Check [Sequential] and select [Line].

5 Click <XY Repeat> button to perform scanning.

6. Adjust image of Green (AlexaFluor488) and Red (AlexaFluor546). (See below regarding image adjustment outline. For further details, see Appendix A.)

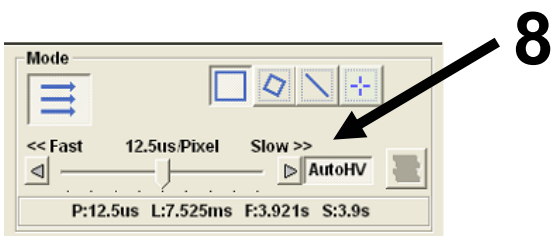
7. Click <Stop> button to stop scanning.

Outline of image adjustment

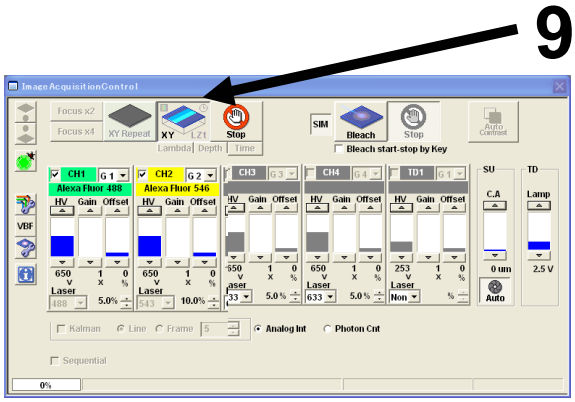


① Sensitivity adjustment of detector (HV)
② Confocal aperture adjustment (CA)
③ Laser output adjustment (Laser)

Adjustment method (Example: HV)
When any place on slider is clicked, the HV can be jumped UP or DOWN to the point where it is clicked. For fine tuning, click ▲ or use mouse wheel.



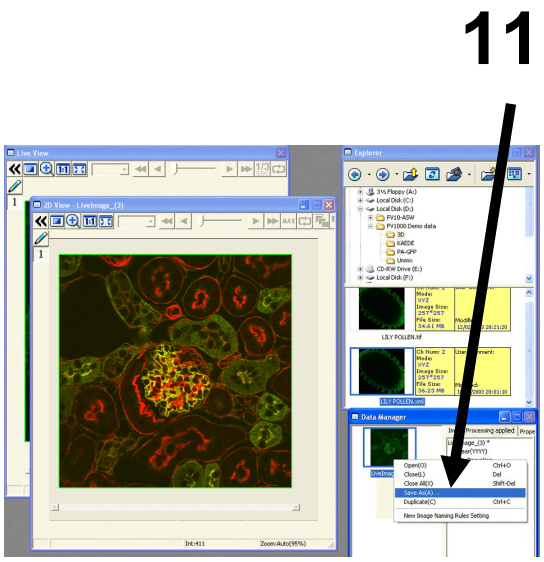
8. Select <AutoHV> button and select [ScanSpeed].
   * The slower the speed set, the more the noise only can be reduced by keeping current brightness.
   (In addition, Kalman integration is available as a separate method to remove noise. For further details, see Appendix B.)



9. Click <XY> button to acquire an image.

10. When acquisition is completed, "2D View-(File Name)" will appear on title bar of the image acquired.

11. Image save:
    Click mouse right button over Image displayed on "DataManager" and then, select [SaveAs].
    (Save as Type "oib" or "oif" is the dedicated file format for this software.)



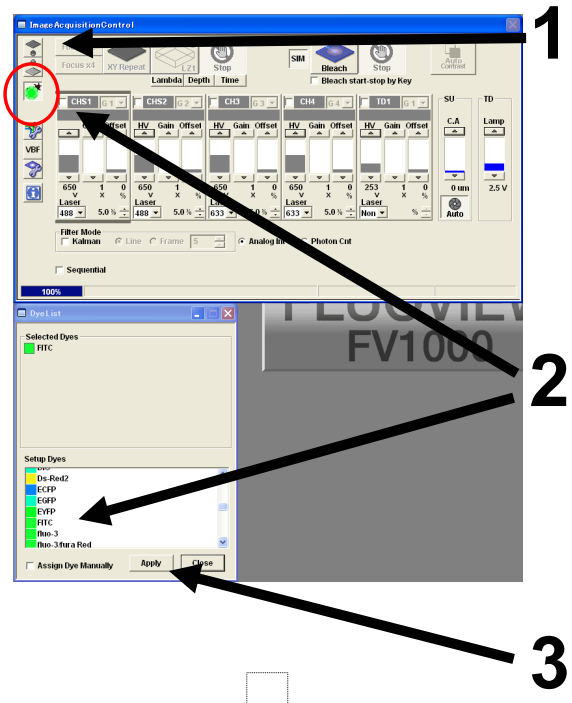
- Memo -
Dedicated file format for this software

OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

3.5.4 Single dyeing color + DIC XY

-- 1-slice Image (XY plane) acquisition
(Fluorescent Image + Differential Interference) --

Example: Green fluorescence (FITC) + Differential Interference



1. Click  of “Image Acquisition” and set it to “not pressed” state and close shutter of fluorescent lamp.

   Click  and set it to “not pressed” state and close shutter of halogen lamp.

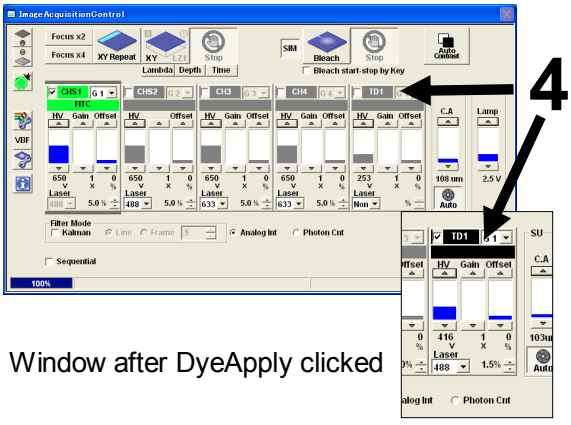
2. Click <DyeList> button and select fluorescent reagent (FITC) to be observed from “DyeList” window and double-click it.

   * When reselecting, double-click the fluorescent dye in [AssignDyes] one time to clear it and then, perform procedure 2.

3. Click <Apply> button.

   (“DyeList” window can be closed with <Close> button.)

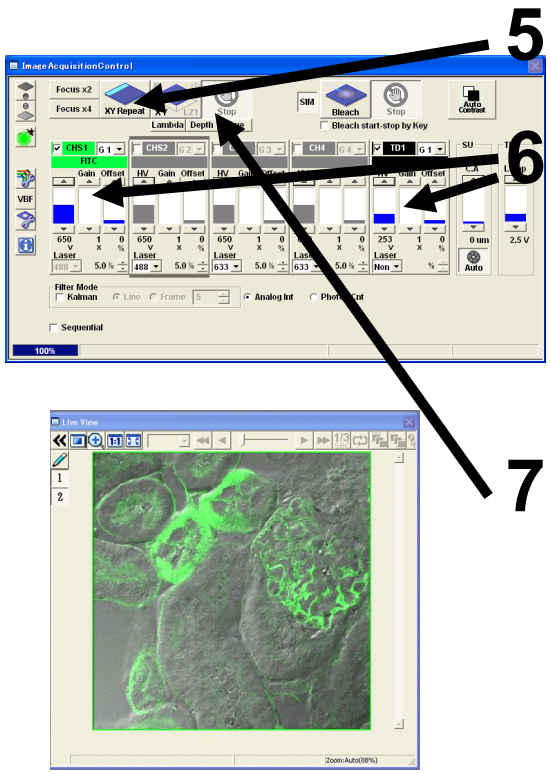
4. Check [TD1].



Window after DyeApply clicked

- In case of Spectral type -
When this operation is done, the fluorescent wavelength suit to Fluorescent Dye selected is defined.

Moreover, the fluorescent wavelength is manually changeable.
For further details, see Appendix C.



5. Click <XY Repeat> button to perform scanning.

6. Adjust Green (FITC) image and differential interference image. (See below regarding image adjustment outline. For further details, see Appendix A.)

7. Click <Stop> button to stop scanning. (Ref: - Memo -)

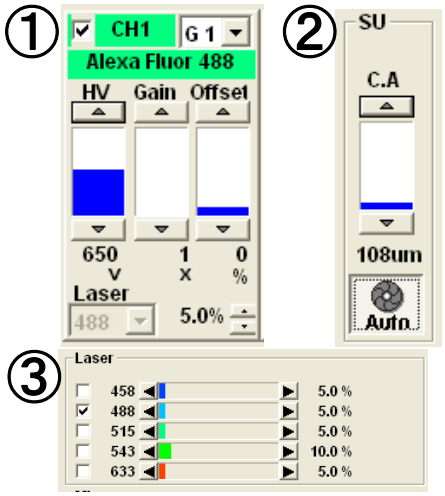
- Memo -
Scan buttons

: Consecutive scan

: Scan stop

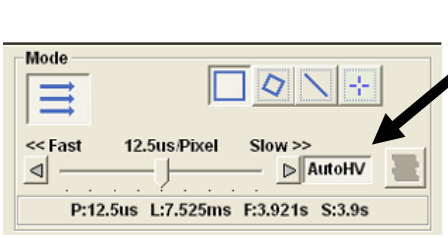
: Rough scan
(Line skipped and scanned)

Outline of image adjustment

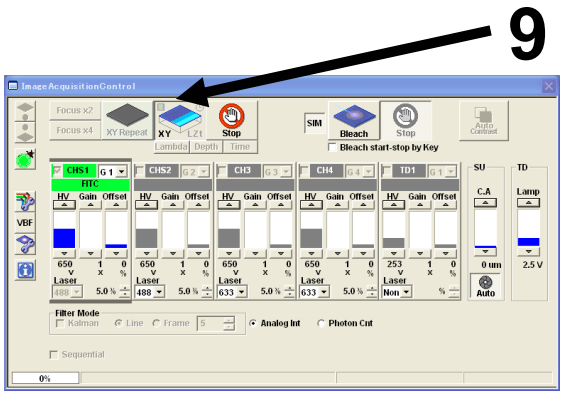


① Sensitivity adjustment of detector (HV)
② Confocal aperture adjustment (CA)
③ Laser output adjustment (Laser)

Adjustment method (Example: HV)
When any place on slider is clicked, the HV can be jumped UP or DOWN to the point where it is clicked. For fine tuning, click  or use mouse wheel.



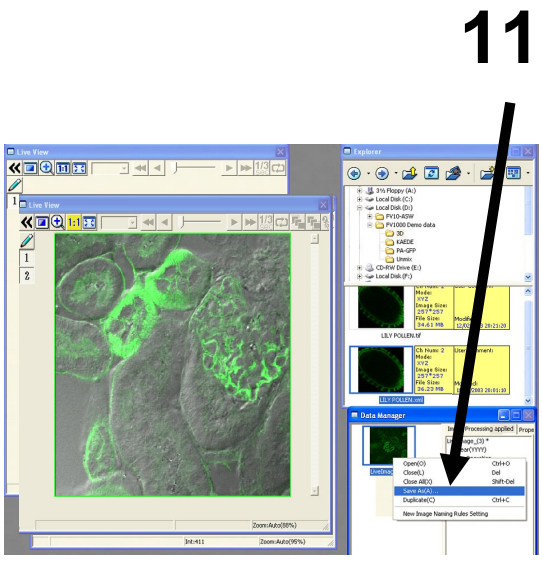
8. Select <AutoHV> button and select [ScanSpeed].
   * The slower the speed set, the more the noise only can be reduced by keeping current brightness.
   (In addition, Kalman integration is available as a separate method to remove noise. For further details, see Appendix C.)



9. Click <XY> button to acquire an image.

10. When acquisition is completed, "2D View-(File Name)" will appear on title bar of the image acquired.

11. Image save:
    Click mouse right button over Image displayed on "DataManager" and then, select [SaveAs].
    (Save as Type "oib" or "oif" is the dedicated file format for this software.)



- Memo -
Dedicated file format for this software

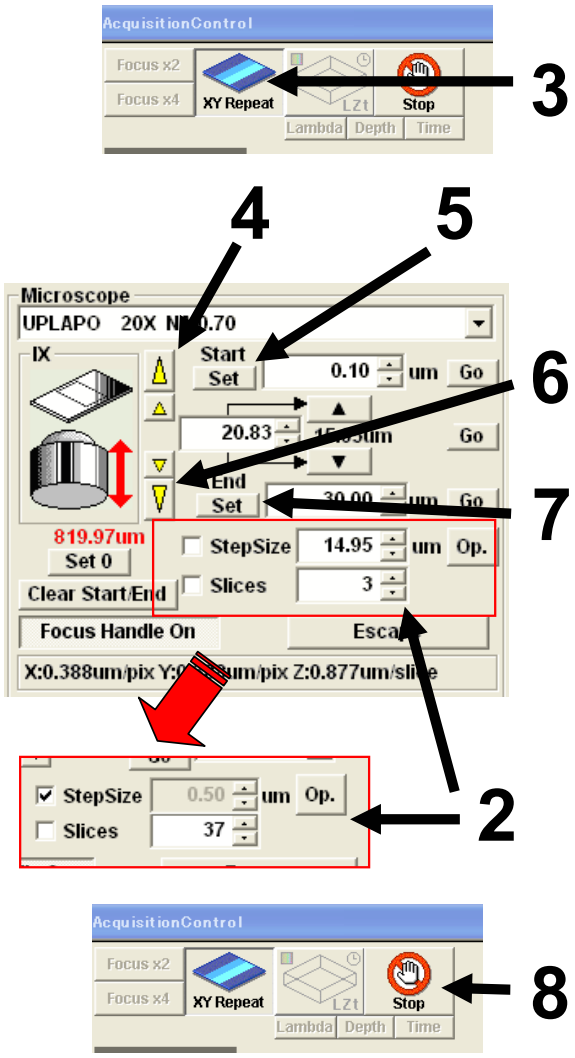
OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.

OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

3.5.5 Double dyeing color XYZ (Line sequential scan version)

-- Consecutive Cross-section Image (XYZ) Acquisition (Fluorescent Image only) --

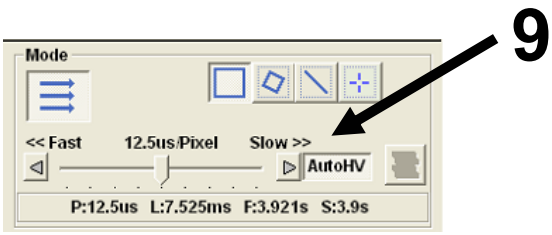
Example:Green fluorescence (Alexa488)+Red fluorescence (Alexa546) Double Dyeing



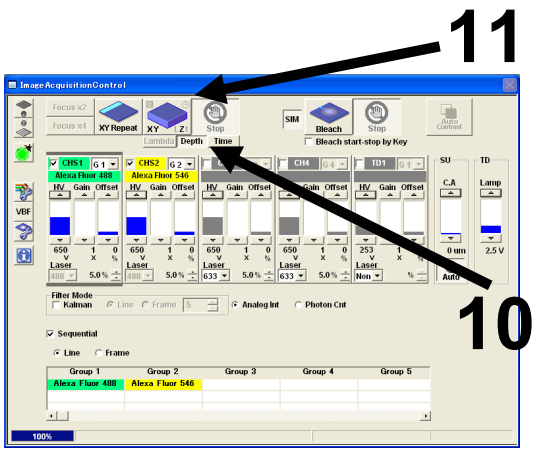
1. Perform procedures 1 through 7 described in page 26 and 27.

Upper and lower limit of consecutive cross-section image is determined here.

2. Enter [StepSize] and check  .
3. Click <XY Repeat> button to perform scanning.
4. Click  or  to bring the object in out of focus.
5. When sample upper limit is displayed on the sample, click <Set> button.
6. Click  or  to bring the object in out of focus.
7. When sampler lower limit is displayed on the sample, click <Set> button.
8. Click <Stop> button and stop scanning.

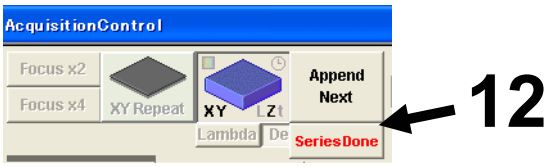


9. Select <AutoHV> button and select [ScanSpeed].
   * The slower the speed set, the more the noise only can be reduced by keeping current brightness.
   (In addition, Kalman integration is available as a separate method to remove noise.
   For further details, see Appendix B.)

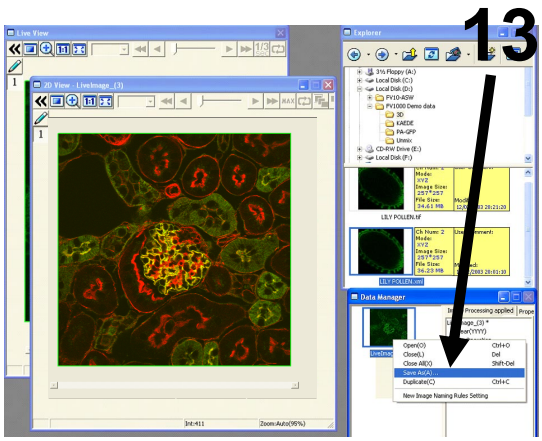


10. Select <Depth> button.

11. Click <XYZ> button to acquire an image.



12. Click <SeriesDone> button so that, on Title bar of the image acquired, "2D View-(file name)" will appear.



13. Image save:
    Click mouse right button over thumbnail displayed on "DataManager" and then, select [SaveAs].
    (Save as Type "oib" or "oif" is the dedicated file format for this software.)

- Memo -
Dedicated file format for this software

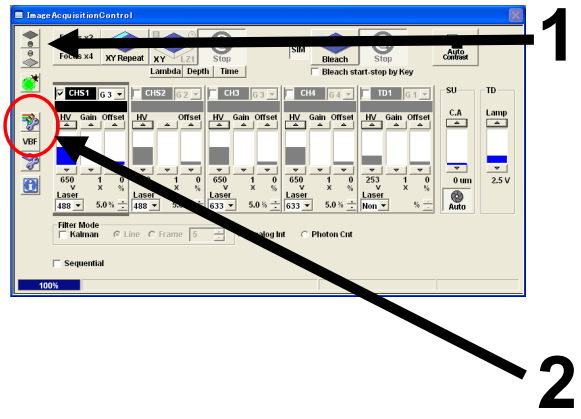
OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

3.5.6 Spectral XYL - Spectral type -

-- Spectral Image (XYL) Acquisition --

Example: Green fluorescence (AlexaFluor488) + Green fluorescence (YOYO-1)

Double dyeing



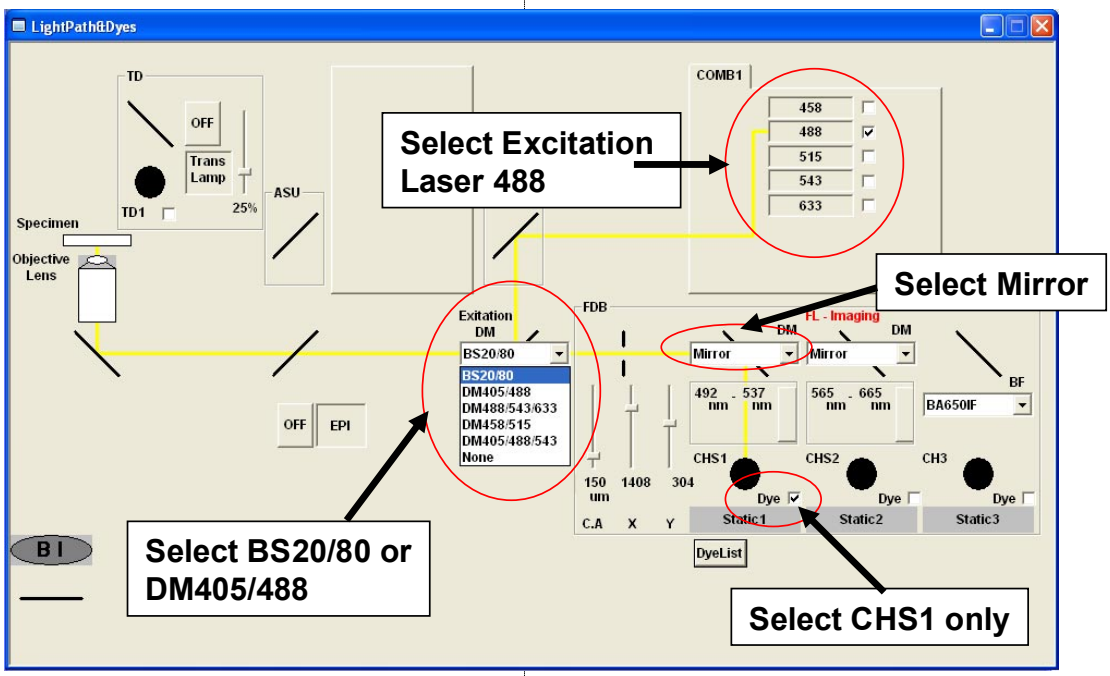
1. Click  of “Image Acquisition” and set it to “not pressed” state and close shutter of fluorescent lamp.

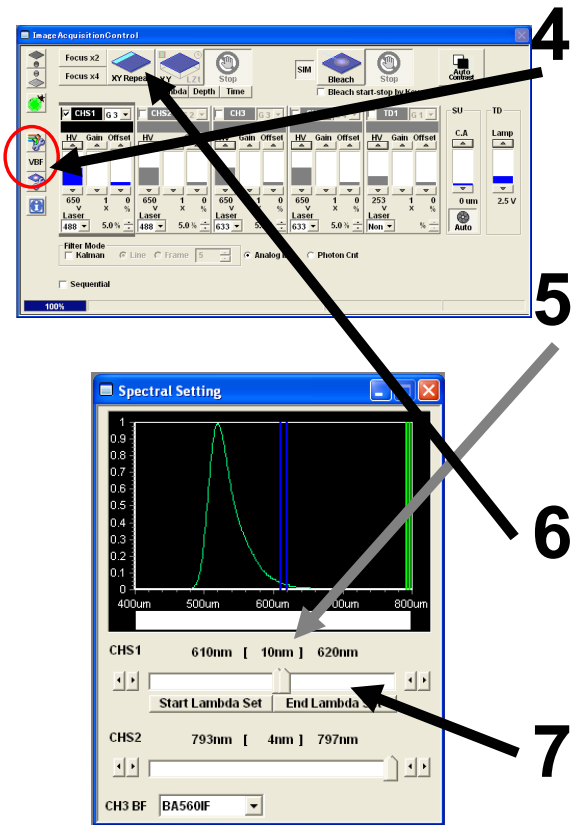
   Click  and set it to “not pressed” state and close shutter of halogen lamp.

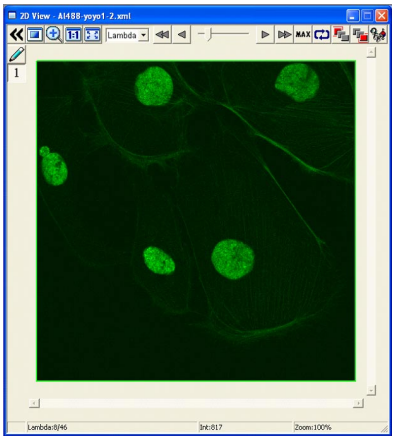
2. Click  and display light path diagram.

3. Set as shown below.

3







4. Click  button and display [SpectralSetting] window.

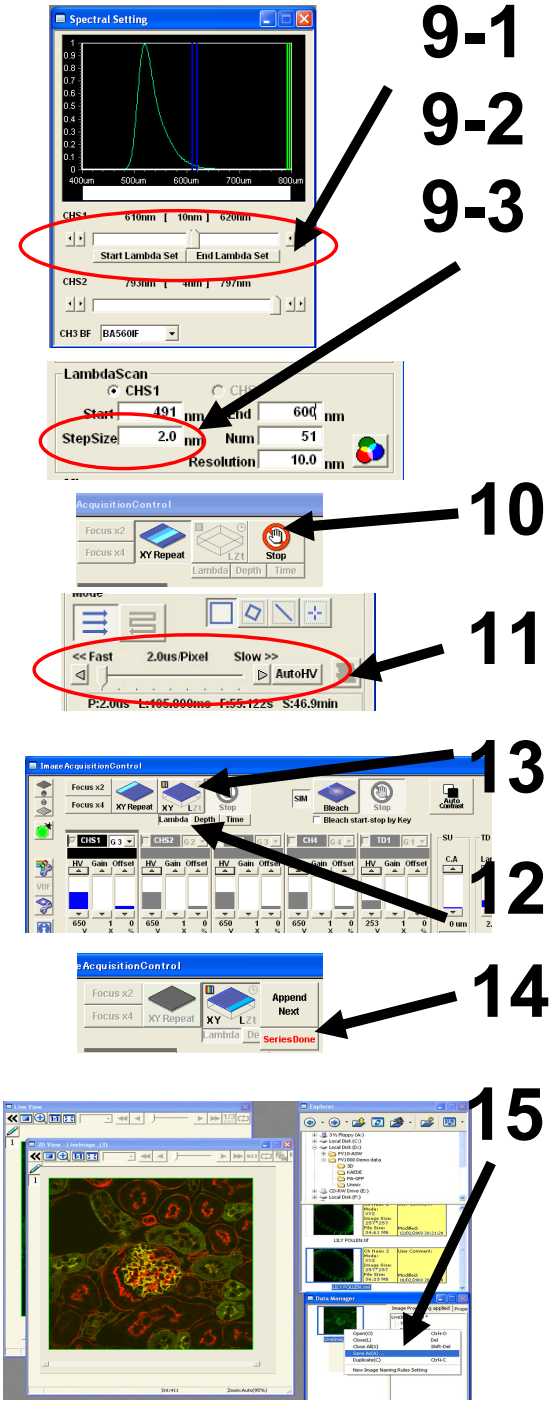
5. Set slit width of CHS1 to 10nm (as example).
(To change slit width, see Appendix C.)

6. Click <XY Repeat> button to perform scanning.

7. Viewing the image, move slit position to the brightest position.
(To change slit position, see Appendix C.)

Note: Leave slit width to 10nm “as is” and move position only.

8. Adjust the image.
(Regarding image adjustment, see Appendix A.)



9. Setting range/step of wavelength to be acquired.

9-1 : Move slit to wavelength start and click <Start LambdaSet> button.

9-2 : Move slit to wavelength end and click <End LambdaSet> button.

9-3 : Enter [Step Size].

10. Stop scanning.

11. Select <AutoHV> button and select [ScanSpeed].

∗ The slower the speed set, the more the noise only can be reduced by keeping current brightness.
(In addition, Kalman integration is available as a separate method to remove noise.
For further details, see Appendix B.)

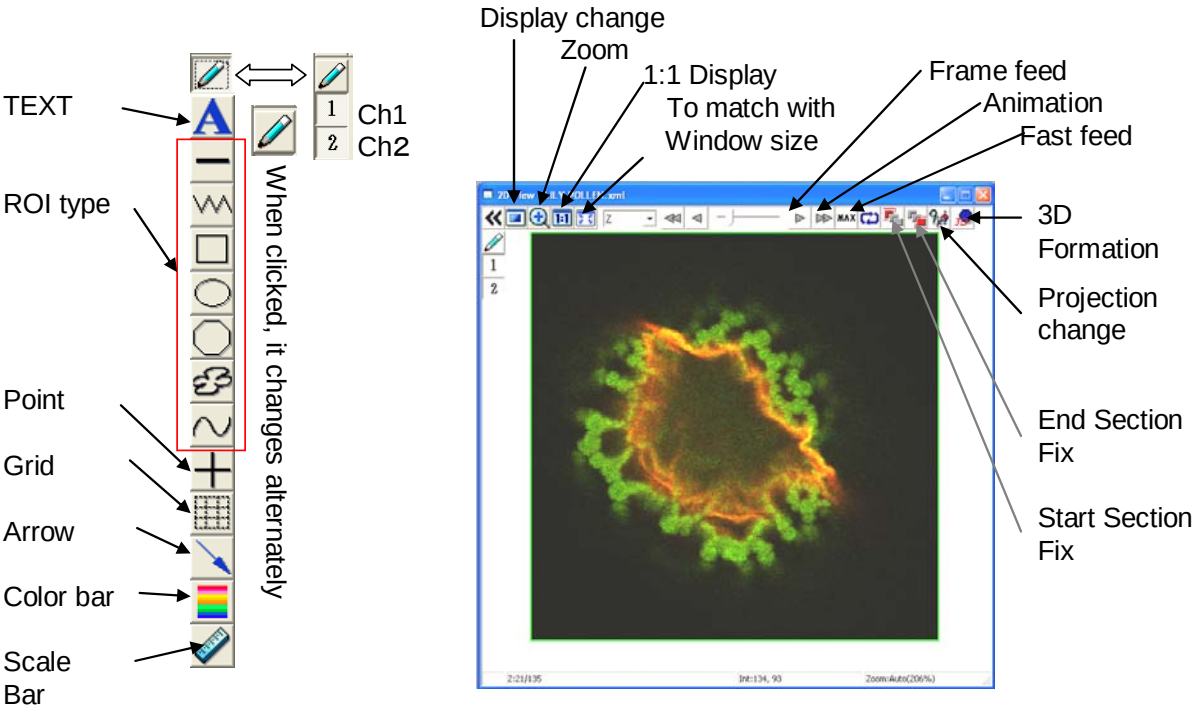
12. Select <Lambda> button.

13. Click <XYL> button to acquire an image.

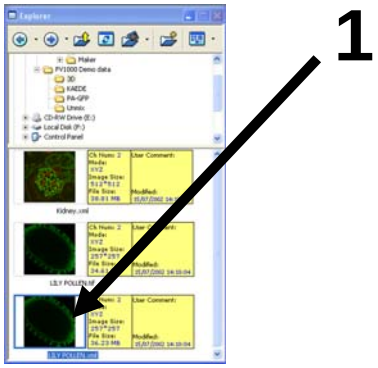
14. Click <SeriesDone> button so that, on Title bar of the image acquired, “2DView-(file name)” will appear.

15. Image save:
Click mouse right button over thumbnail displayed on “DataManager” and then, select [SaveAs].
(Save as Type “oib” or “oif” is the dedicated file format for this software.)

3.6 “2D Display” window - Outline

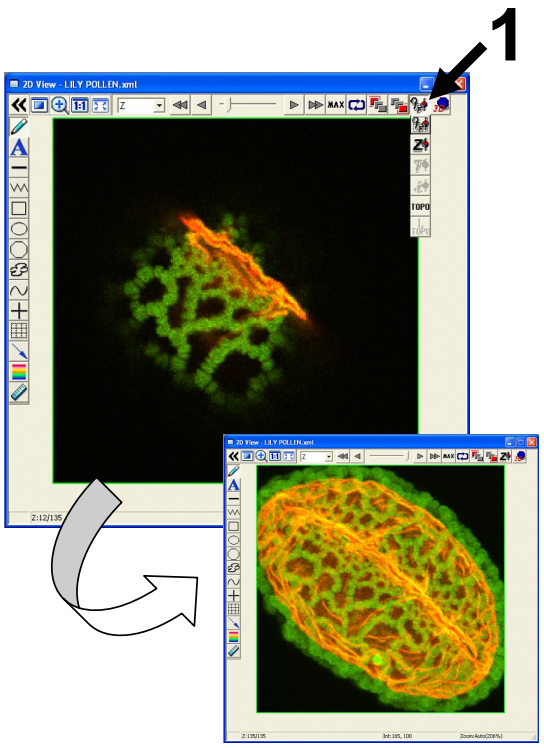


3.7 File open

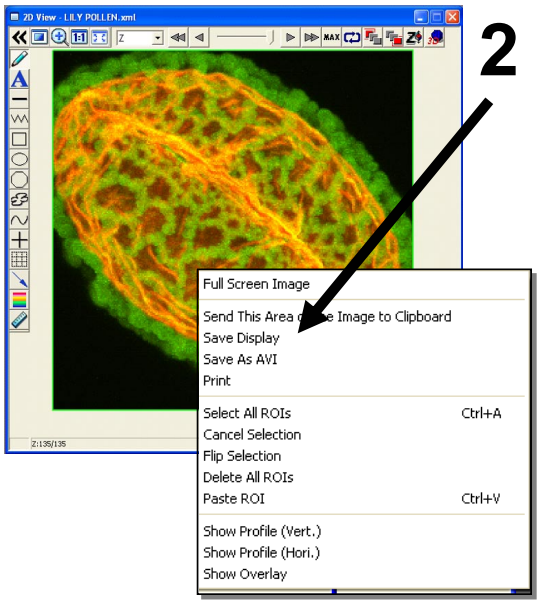


1. Double-click a file that should be opened from Explorer.

3.8 Display of XYZ Image (Cross-section image overlap)

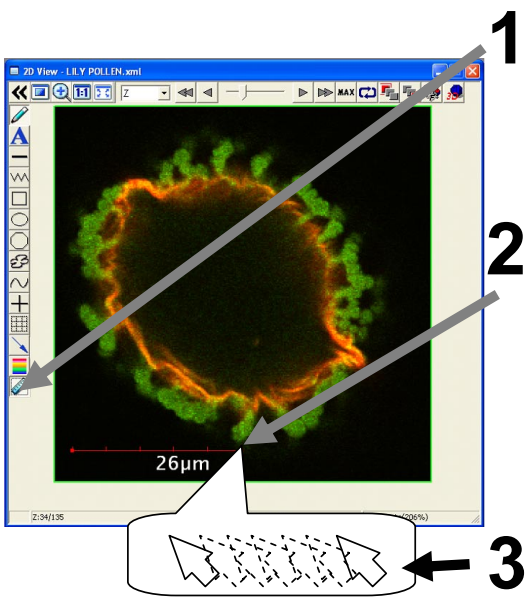


1. Click  and select  .



2. In case that this image is saved, click mouse right button over the image and select [Save Display] and save it with a name assigned.

3.9 Putting Scale bar

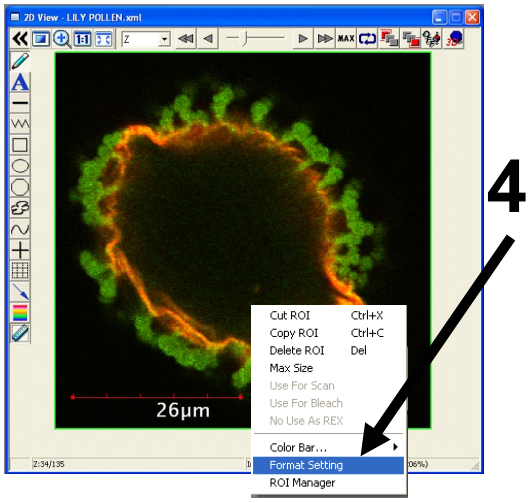


1. Click  button.

2. While left mouse button is clicked over the image, drag the mouse and release the button at proper point.

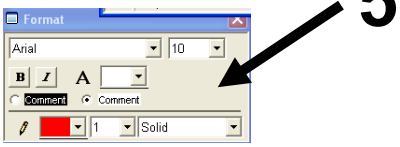
Size change

3. While right or left handle is clicked, move the mouse, left or right.



Change of character size, color and style, etc.

4. After selecting [ScaleBar], click mouse right button over [ScaleBar] and select [FormatSetting].



5. In this Window, change portions that must be changed.

3.10 3D Display

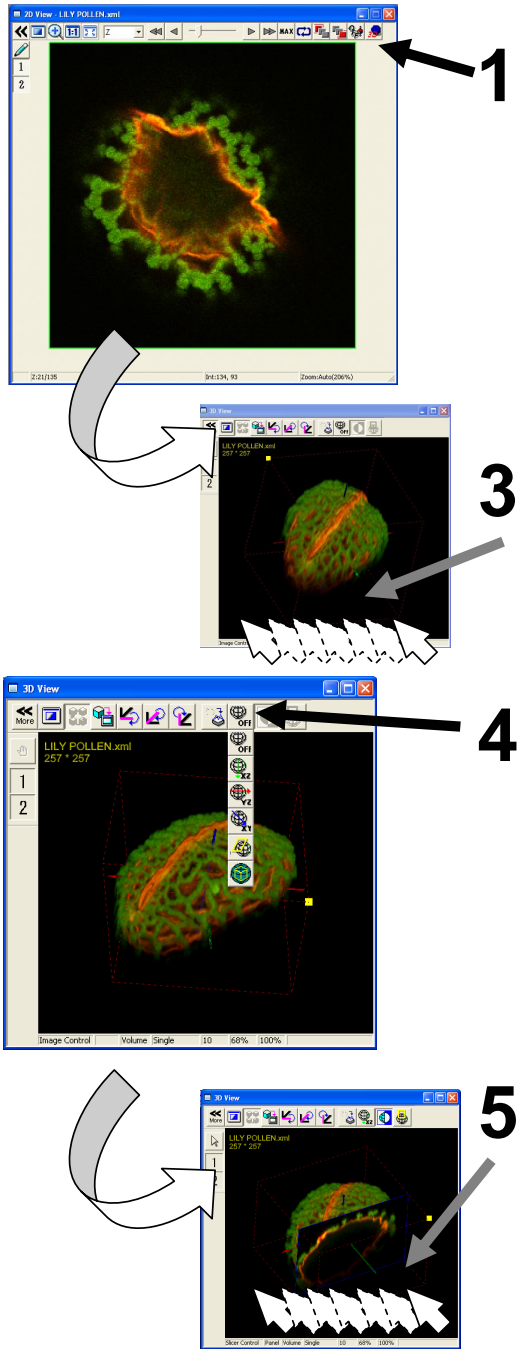


Image is observed from arbitrarily selected angle.

1. Click  button on 2D View-(file name) image.

2. [3D-OLYMPUS FLUOVIEW] window starts up and “3D View” will be built up.

3. Dragging mouse over the image, observe the image from arbitrarily selected angle.

→ To save this image, see procedure 6 in next page.

Cross-section image at optional angle is observed.

4. Click  and select  .

5. Dragging mouse over the image, move, left or right, to see cross-section at optional angle.

→ To save this image, see procedure 6 in next page.

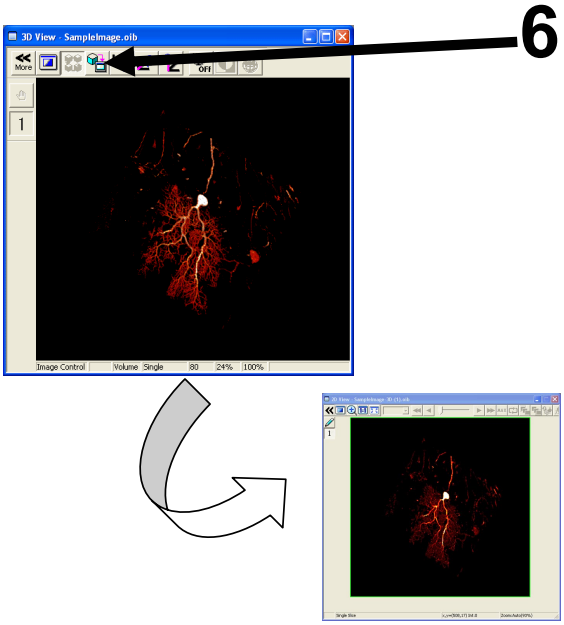


Image in procedure 3 or 5 is saved.

6. Click  button.

7. 2D View-(file name) image appears.

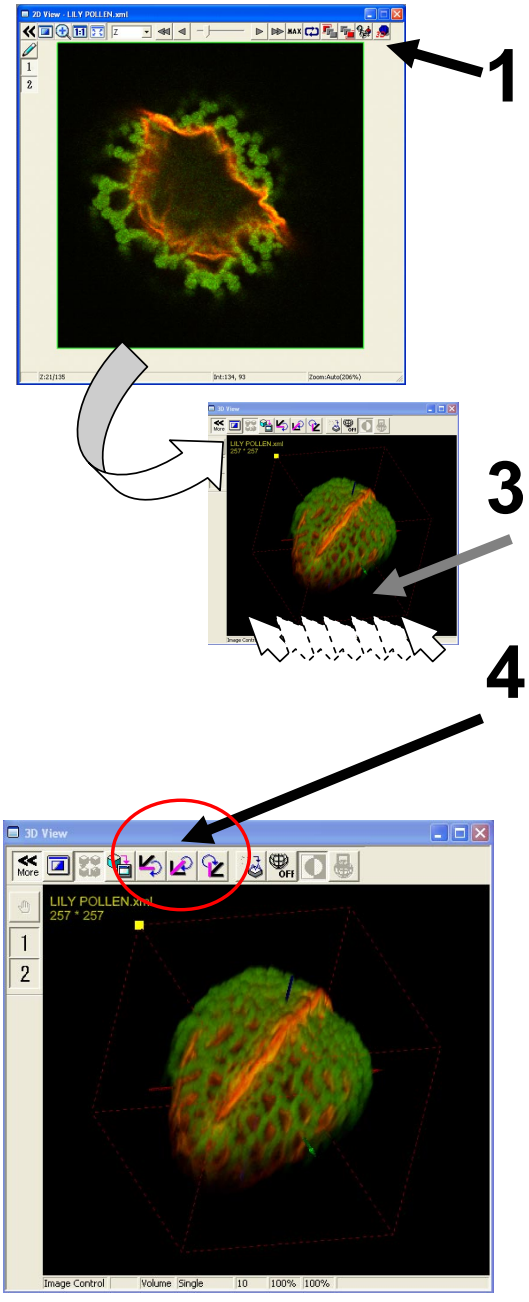
8. Image save:
   Click mouse right button over thumbnail displayed on "DataManager" and then, select [SaveAs].

   (Save as Type "oib" or "oif" is the dedicated file format for this software.)

- Memo -
Dedicated file format for this software

OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

3.11 Rotation of cubic image



1. Click  button on 2D View-(file name) image.

2. [3D-OLYMPUS FLUOVIEW] window starts up and “3D View” will be built up.

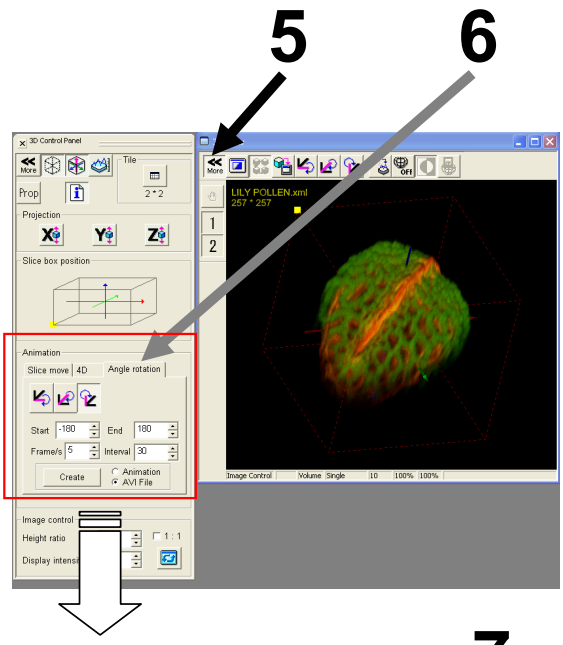
3. Dragging mouse over the image, observe the image from arbitrarily selected angle.

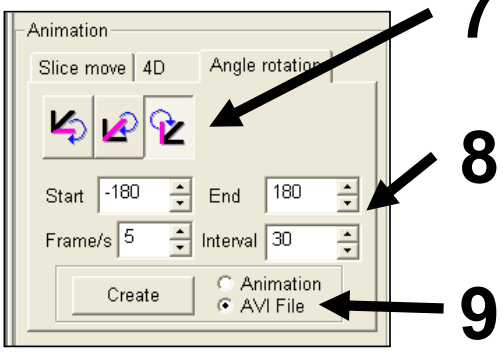
Simplified animation

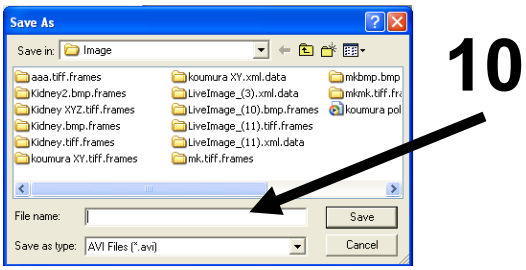
4. When  button is clicked with long press mode, the image turns with X-axis as rotation center. When clicked again, the turn would stop.

When  button is clicked with long press mode, the image turns with Y-axis as rotation center. When clicked again, the turn would stop.

When  button is clicked with long press mode, the image turns with Z-axis as rotation center. When clicked again, the turn would stop.

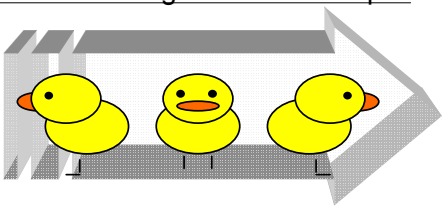






When a rotation file is saved as movie, 3D formation must be executed as follows.

Let's turn an image 180° as example.



5. Click 

button.

6. Double-click [Angle Rotation] tab.

7. Select rotation angle axis.

8. Enter rotation angle.

[Start]=From what degree /
[End]=To what degree/
[Frame/s]=Rotation speed /
[Interval]=per what degree

9. Select [AVI File] and click <Create> button.

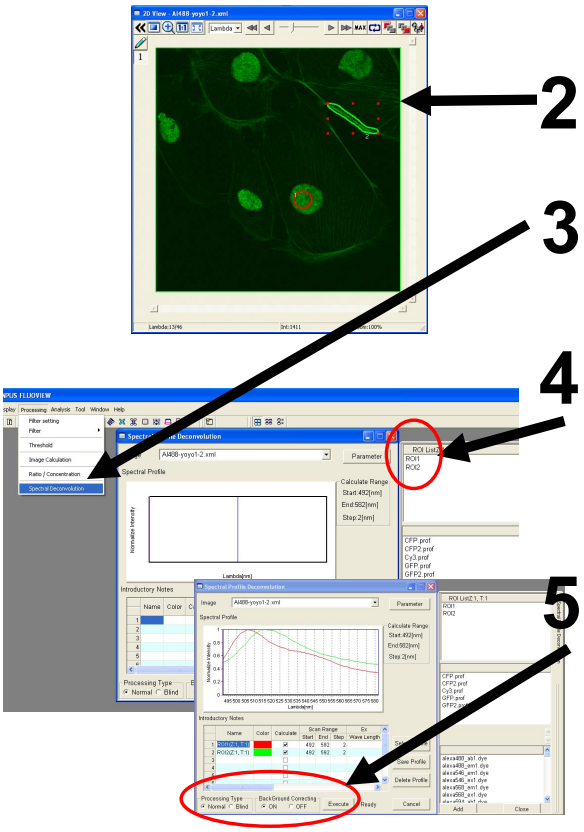
10. Enter file name and click <Save> button.

3.12 Fluorescence separation - Unmixing (Spectral type)

3.12.1 In case that Dye place for each fluorescence is known

Fluorescent spectrum of each fluorescent dye are extracted from XYL image where a plural number of fluorescent dyes having fluorescent spectrum similar to each other exist and; with the said fluorescent spectrum as reference, a method is presented here to acquire a fluorescence separated image.

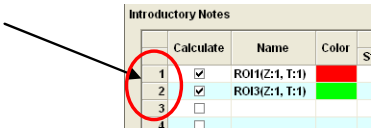
Example: Green fluorescence (AlexaFluor488) + Green fluorescence (YOYO-1)



1. Open XYL image file of AlexaFluor488 + YOYO1 of double dyeing.
2. Surround AlexaFluor488 and YOYO1 region only with ROI.
3. Select [SpectralDeconvolution] from [Processing] on menu bar.
4. Double-click ROI1 and ROI2 respectively.
5. Verify that [ProcessingType] is set to “Normal” and [BackGround Correcting] is turned “ON” and then, click <NewImage> button.
6. Image of fluorescent separation can be acquired.

Image after fluorescence separating (Unmixing)

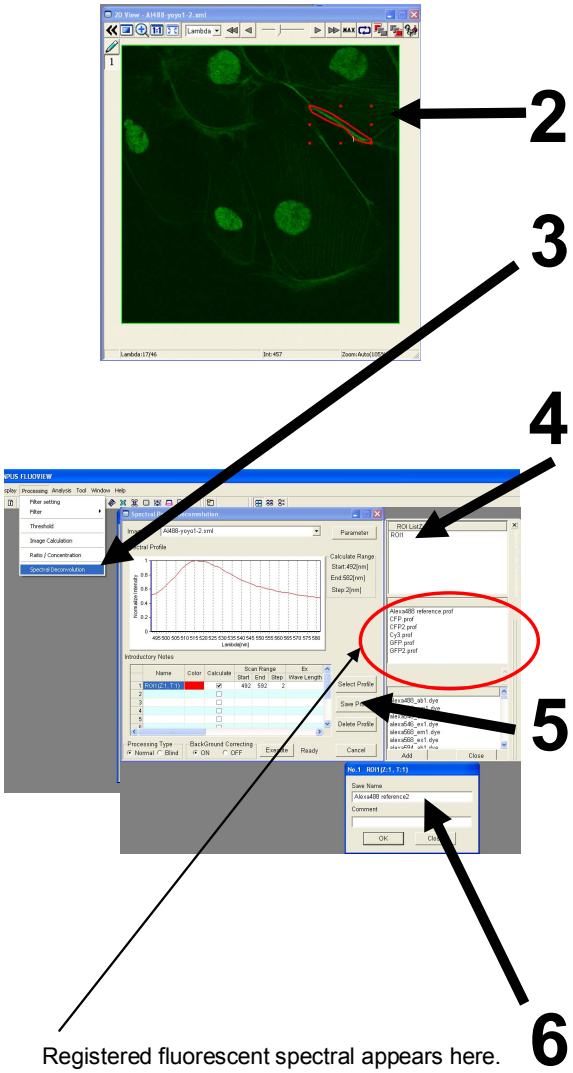
After fluorescence separation (Unmixing), the assignment of image to Ch is indicated.



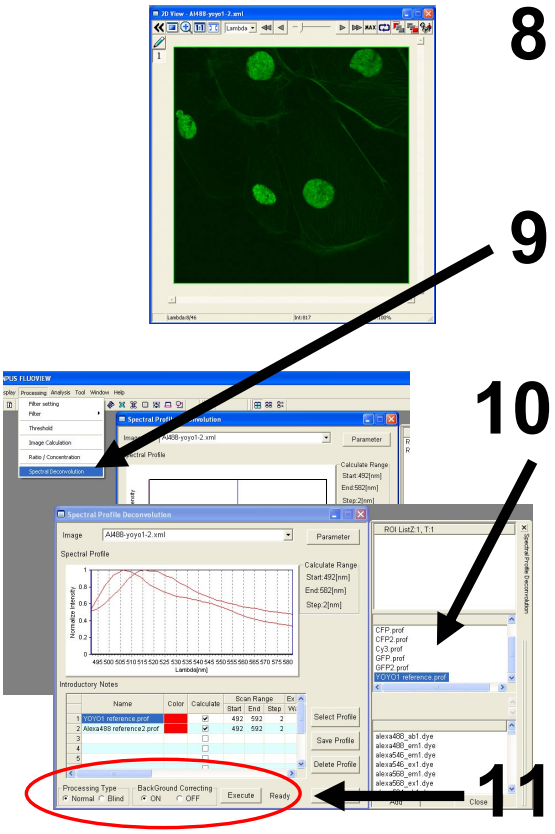
3.12.2 In case that control sample is used

Method to acquire a fluorescence separated image with reference to fluorescent spectrum. The said spectrum are extracted from one kind of fluorescent dye on XYL image.

Example: Green fluorescence (AlexaFluor488) + Green fluorescence (YOYO-1) Double Dyeing



1. Open XYL image file of control sample (the sample dyed with AlexaFluor488 only).
2. Surround AlexaFluor488 region with ROI.
3. Select [SpectralDeconvolution] from [Processing] on menu bar.
4. Double-click ROI1.
5. Click <SaveProfile> button.
6. Enter name to be saved and click <OK> button and the fluorescent spectral of AlexaFluor488 is registered to data base.
7. Perform the same operation, 1 to 6, for YOYO1.



8. Open XYL image file of AlexaFluor488 + YOYO1 of double dyeing.

9. Select [SpectralDeconvolution] from [Processing] on menu bar.

10. Double-click AlexaFluor488 and YOYO1 (previously registered) from data base of fluorescent spectrum.

11. Verify that [ProcessingType] is set to “Normal” and [BackGround Correcting] is turned “ON” and then, click <NewImage> button.

12. Image of fluorescence separated can be acquired.

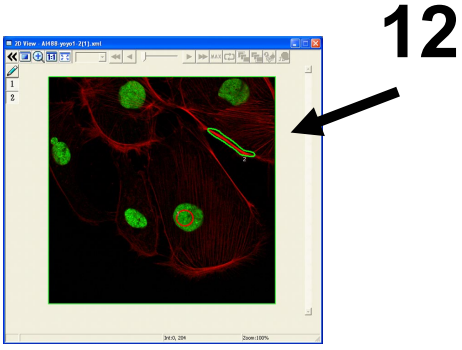
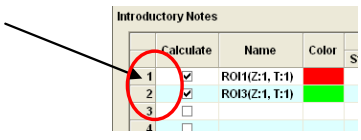


Image after fluorescence separating (Unmixing)

After fluorescence separation (Unmixing), the assignment of image to Ch is indicated.



3.12.3 In case that number of fluorescent dye kinds only is known (Blind Unmixing)

Method to acquire a fluorescence separated image with a little information such as number of fluorescent dye kinds only known as a hint from XYL image where a plural number of fluorescent dyes having fluorescent spectrum similar to each other exist.

Example: Sample having 2 kinds of unknown fluorescent dyes

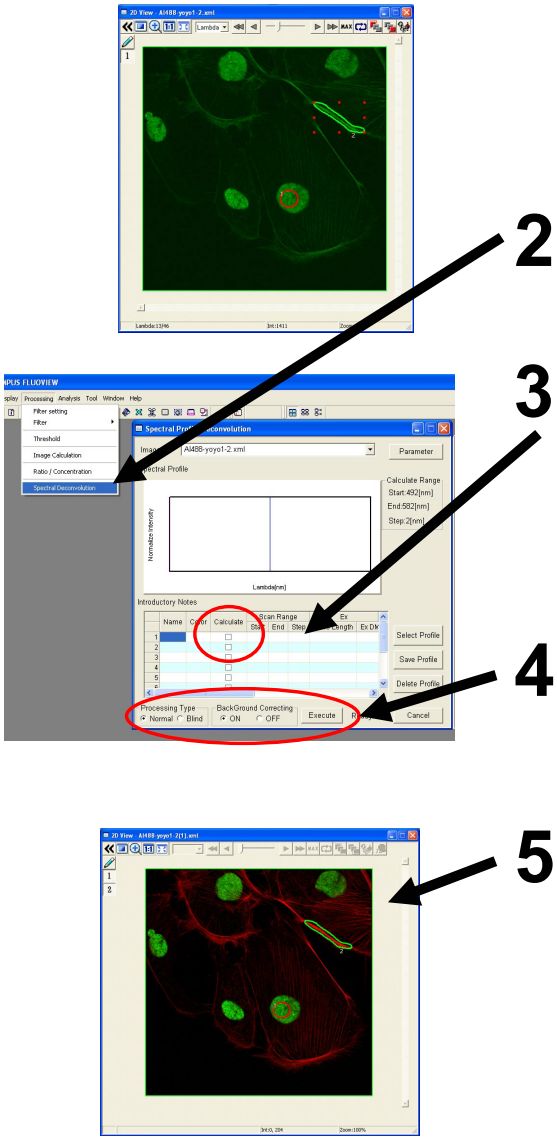
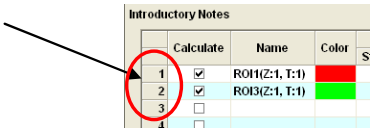


Image after fluorescence separating (Unmixing)

1. Open XYL image file of sample that has 2 kinds of unknown fluorescent dyes.
2. Select [SpectralDeconvolution] from [Processing] on menu bar.
3. Put check marks at 2 places for [Calculate] check box. (When 3 kinds of fluorescent Dyes exist, click 3 places to put check marks.)
4. Verify that [ProcessingType] is set to “Blind”, [BackGroundCorrecting] is turned “ON” and then, click <NewImage> button.
5. Image of fluorescence separated can be acquired.

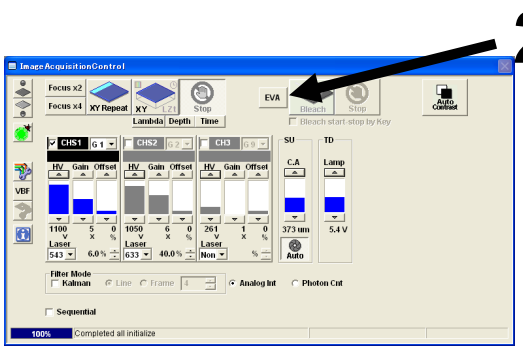
After fluorescence separation (Unmixing), the assignment of image to Ch is indicated.

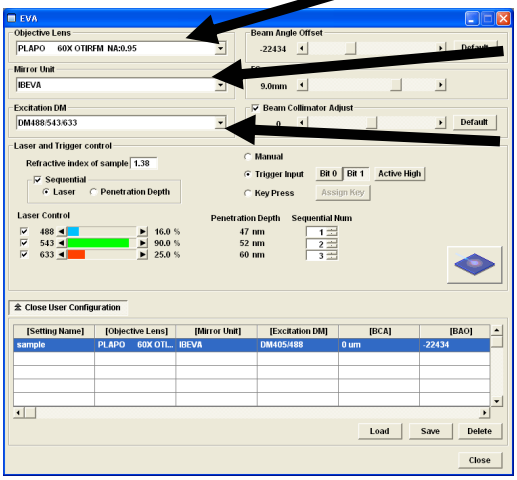


3.13 Evanescent light observation

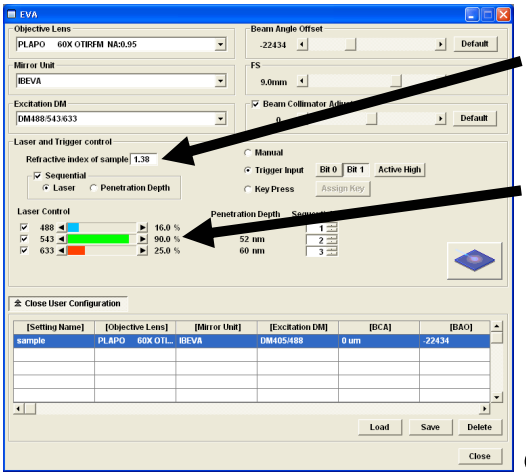
When EVA is used

It is a method to excite fluorescent particles only that exist near surface of cover glass by using "evanescent light" that comes out in submicron order at total reflection side of the cover glass (specimen side) as excitation beam.





1. Remove the Nomarski prism.
2. Click EVA button. [EVA] window appears.
3. With [Objective Lens], select the objective lens indicated as "TIRFM".
4. With [Mirror Unit], select "IBEVA".
5. With [Excitation DM], select the excitation DM indicated as "488".



6. Enter refractive index of the specimen in [Refractive index of sample].

7. Check [Laser Control] check box and set the output with slider.

8. Set [Beam Angle Offset] to “0”.

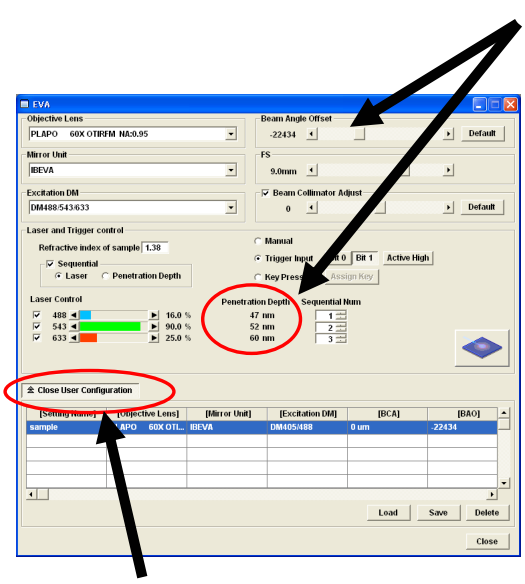
9. Set [FS].
   ✳ Recommended value - 9.0mm.

10. Press [Laser Emission] button.
    Laser emission will start.

11. Align focus.

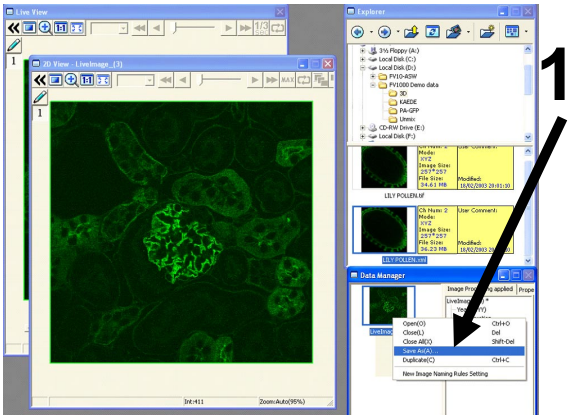
12. Move [Beam Angle Offset] slider and adjust it so as to set [Penetration Depth] to the value other than “0”.

13. Make a fine adjustment of focus.



Using [User Configuration], the setting condition can be saved or called out.

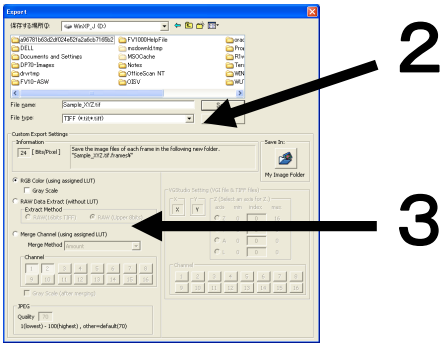
3.14 Image save



Each channel for XY or XYZ Image is converted to TIFF.

1. Click mouse right button over thumbnail displayed on "DataManager" and select [Export].
2. Set [Save as Type] to "TIFF" and <Save> button.

* Other type, BMP/JPEG/PNG can be selected.



Merge image of XY or XYZ Image is converted to TIFF.

1. Click mouse right button over thumbnail displayed on "DataManager" and select [Export].
2. Set [Save as Type] to "TIFF".
3. Check [MergeChannel] and <Save> button.

* Other type, BMP/JPEG/PNG can be selected.

****

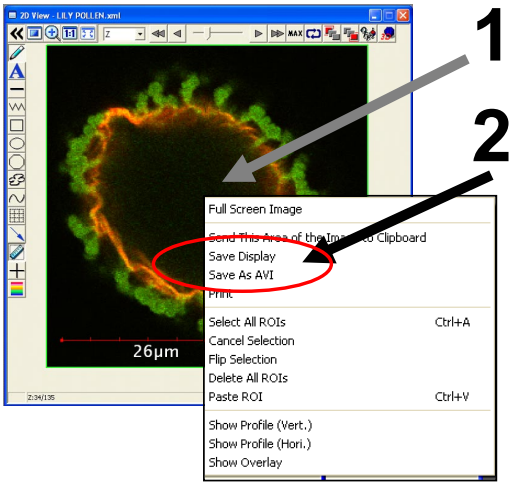


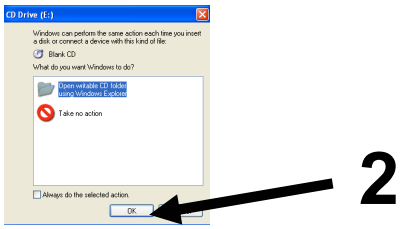
Image with Scale bar is converted to BMP.

1. Click mouse right button over image.
2. Select [Save Display] and save the image with a name assigned.

Movie is converted to AVI.

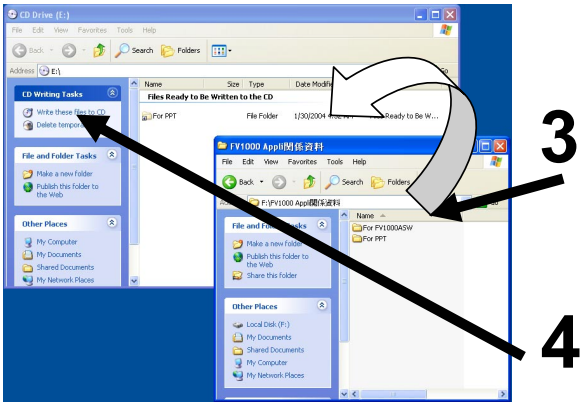
1. Click mouse right button over image.
2. Select [Save as AVI] and save the image with a name assigned.

3.15 Save to CD-R



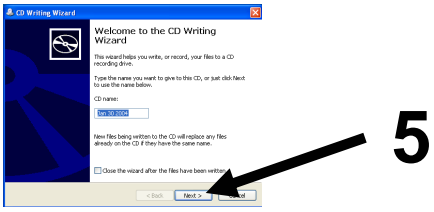
1. Insert CD-R media.

2. Click <OK> button.

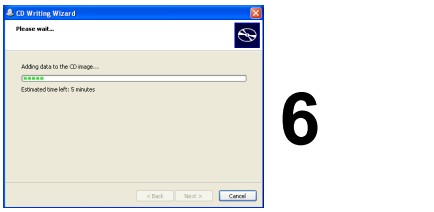


3. Select a file and move it to Window for CD-R with drag-and-drop technique.

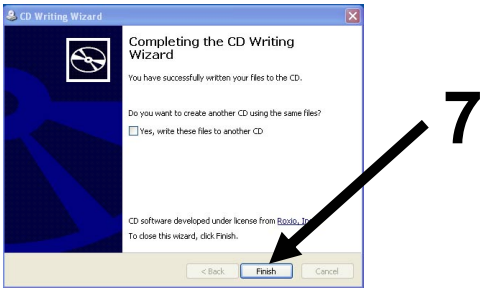
4. Click [Write these files to CD].



5. Click <Next> button.

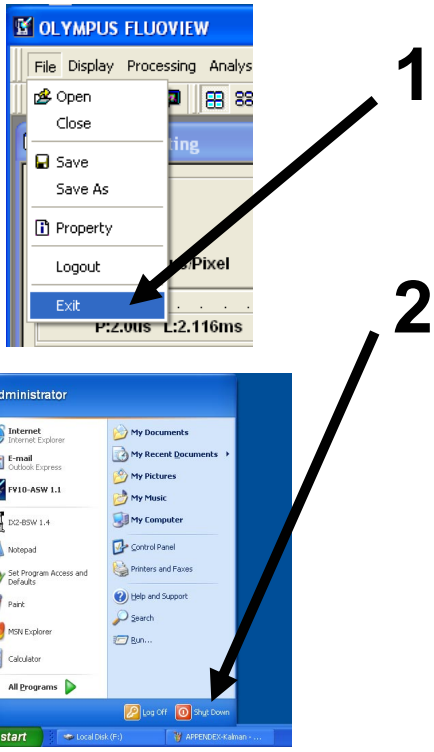


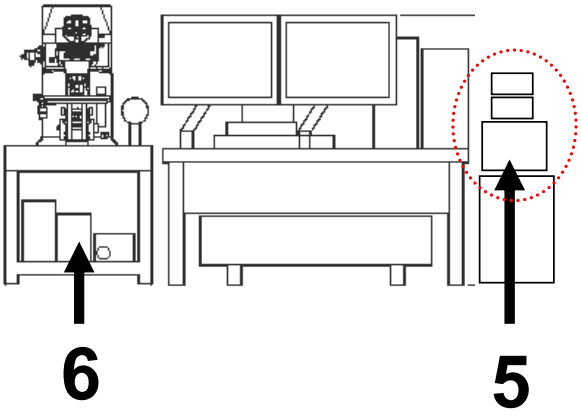
6. Write will start.



7. Click <Finish> button so that the save to CD-R is completed.

3.16 System shut down





1. Shut dwon this software with [File]-[Exit].

2. Shut down WindowsXP.

① Select [Start] – [Shut Down].

② Select “Shutdonwn” on [ShutDown] window and click <OK> button.

3. Turn FV10-PSU to OFF.

4. Turn BX-UCB or IX2-UCB to OFF.

5. Turn laser to OFF.
(Return key switch to OFF position)

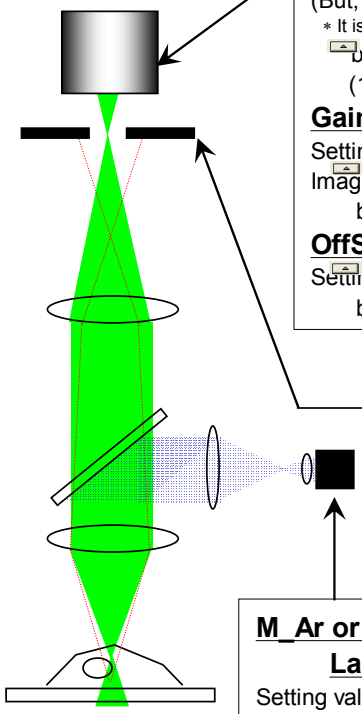
5-1. Multi Ar
（458nm･488nm･514nm） OFF

5-2. HeNe（G）（543nm） OFF

5-3. HeNe（R）（633nm） OFF

6. Turn mercury lamp power to OFF.

Appendix A Relationship between confocal principle and tuning mechanism



HV: Sensitivity adjustment of detector (Voltage)

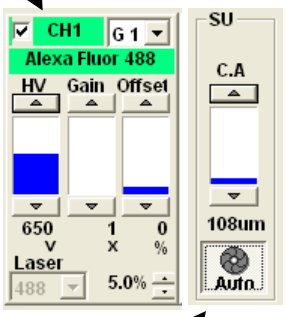
Setting value ↑ > Sensitivity ↑ > Image Brightness ↑
(But, noises are getting noticeable.)
* It is recommended that the voltage is set at about 700V or less.
button: changes by 1V step.
(1V in case of transmitted image)

Gain adjustment (x)

Setting value ↑ > Image Brightness ↑
Image gets brighter with setting value.
button: changes by x0.001

OffSet adjustment (%)

Setting value ↑ > Image Brightness ↓
button: changes by 1%

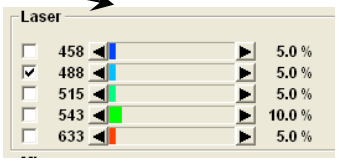


C.A.: Confocal aperture adjustment (μm)

Setting value ↑ > C.A. Size ↑ > Image Brightness ↑
(But, optical cross-section is getting thicker).
* It is recommended that C.A. Is used with Auto.)
When C.A. should return to default setting after move,
s Auto again.
button: changes by 1μm.

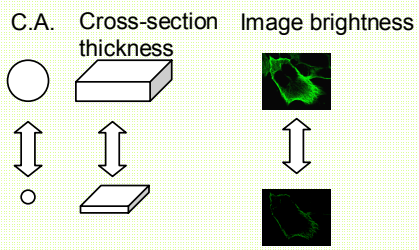
M_Ar or HeNeG、HeNeR: Laser output adjustment (%)

Setting value ↑ > Output strength ↑ > Brightness ↑
(But, fluorescent discoloring is getting larger.)
* Use with a value as small as possible recommended.
button; changes by 0.1%

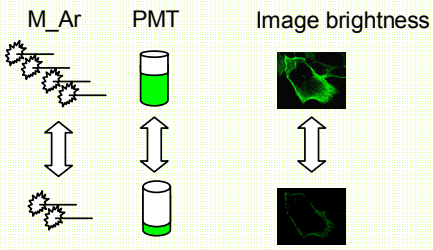


■ Memo ■

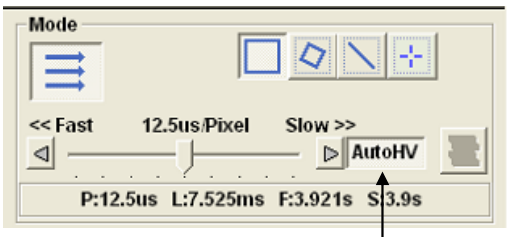
Relationship between confocal aperture and optical cross-section, image brightness



Relationship between laser output/ detector sensitivity and image brightness



Appendix B Image acquisition of less noises



AutoON: The slower it is set, the more noise can be removed by keeping current image brightness.
AutoOFF: The slower it is set, the more it is getting brighter and noise is also removed.

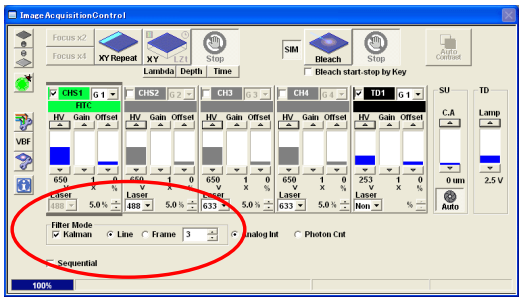
Method to make scanning speed slower

Set scanning speed slower so that an image can be acquired without detecting noises from the beginning.

Merit:
● With Kalman integration, comparatively sharp image can be acquired.
Demerit:
● Speed of scanning at one time is slow.

1. Select [ScanSpeed].

****



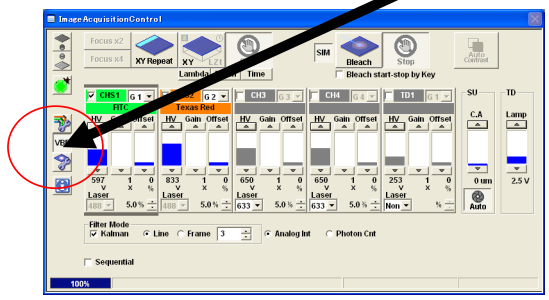
Method to use Kalman Integration

Images are averaged while image is being acquired for number of times specified. As the results, noises are also averaged so that roughness of whole image can be suppressed.
Merit:
● Speed of scanning at one time is fast.
Demerit:
● Images are averaged so that an image may get dim, more or less.

1. Click [Kalman] and select [Line] or [Frame].
2. Enter number of integration times for the image (number of scanning cycles).

Appendix C Method to change width or positiron of slit in manual mode

1

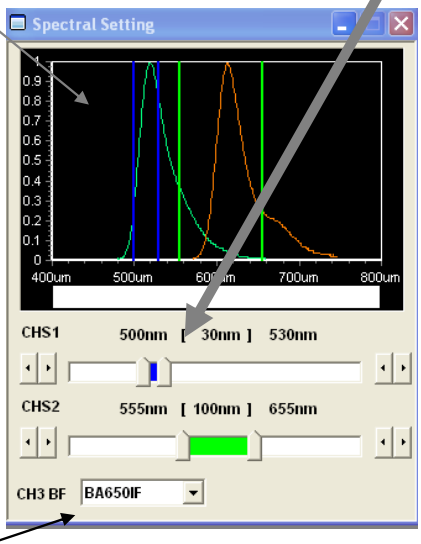


1. Click VBF button and display SpectralSetting window.

2. Using  or  , move left or right.

2

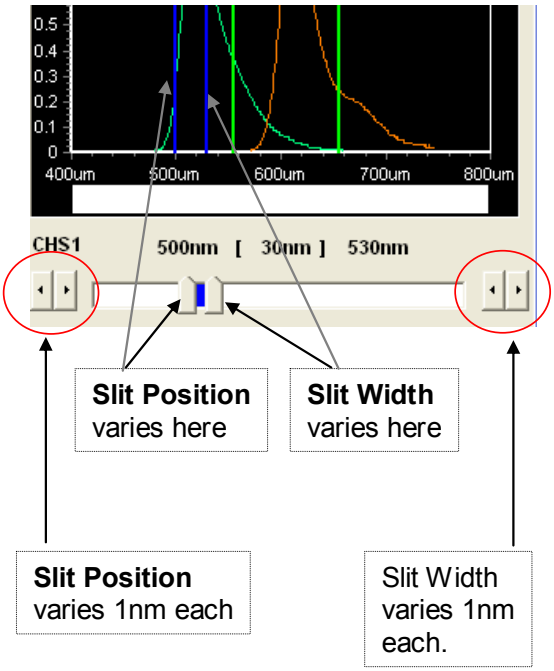
Spectral of fluorescent dye selected and Slit width and position are displayed.



CH1 Slit Adj

Ch2 Slit Adj

Ch3 Fluorescent filter selection



* Numeric can be entered.

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