OLYMPUS®

User's Manual
[Quick Start]

FLUOVIEW FV1000

LASER SCANNING BIOLOGICAL MICROSCOPE

FV10-ASW [Ver2.0]

Notice

Thank you for your purchase of Olympus microscope at this time.
Hold this manual by your side when using this microscope all the time and keep it with care after reading.

AX7797

Caution

FV1000MPE is a CLASS 4 laser product; FV1000 is a CLASS 3B laser product.

The procedures for using this system are classified as follows:

- Service

  “Service” means any adjustment or repair performed by highly trained and skilled technical personnels who are provided the service training following to the service manual for this system.

  The performance has influence on the feature of this system, and there is a risk which unintended CLASS 3B or CLASS 4 laser light is emitted.

- Maintenance

  “Maintenance” means adjustment or other procedures performed by customers to maintain that this system functions properly.

- Operation

  “Operation” means all performance described in the user’s manuals in this system.

  CLASS 3B or CLASS 4 laser light is only emitted from the objective lens during the actual execution.

The User’s Manuals of this system consist of the following:

In order to maintain the full performance of this system and ensure your safety, be sure to read these user’s manuals and the operating instructions for the laser unit and light source unit before use.

User’s manual constitution of FV1000MPE

- FV1000MPE / FV1000 User’s Manual [Laser Safety Guide]
- FV1000MPE User’s Manual [Safety Manual] or [Safety Guide]
- FV1000 User’s Manual [Safety Guide]
- FV1000MPE User’s Manual [Operation Manual] or [Operation]
- FV1000 User’s Manual [Hardware Manual]
- FV1000 FV10-ASW User’s Manual [Quick Start]

User’s manual constitution of FV1000

- FV1000MPE / FV1000 User’s Manual [Laser Safety Guide]
- FV1000 User’s Manual [Safety Guide]
- FV1000 User’s Manual [Hardware Manual]
- FV1000 FV10-ASW User’s Manual [Quick Start]

Also, we have prepared one service manual for this system as below. Technical personnels who perform the service require to take the service training.

- FV1000MPE / FV1000 Service Manual

In case of purchasing the laser simultaneously, we have prepared the following manual for the laser.

- MaiTai Series User's Manual [Quick Start]

In addition, we have prepared one service manual for the laser as below. Technical personnels who perform the laser service require to take the service training.

- MaiTai Series Service Manual

Registred trademark

CONTENTS

This Quick Start has divided into the volume by the following system configurations.

IX81 (Spectral Type)

IX81 (Filter Type)

BX61 (Spectral Type)

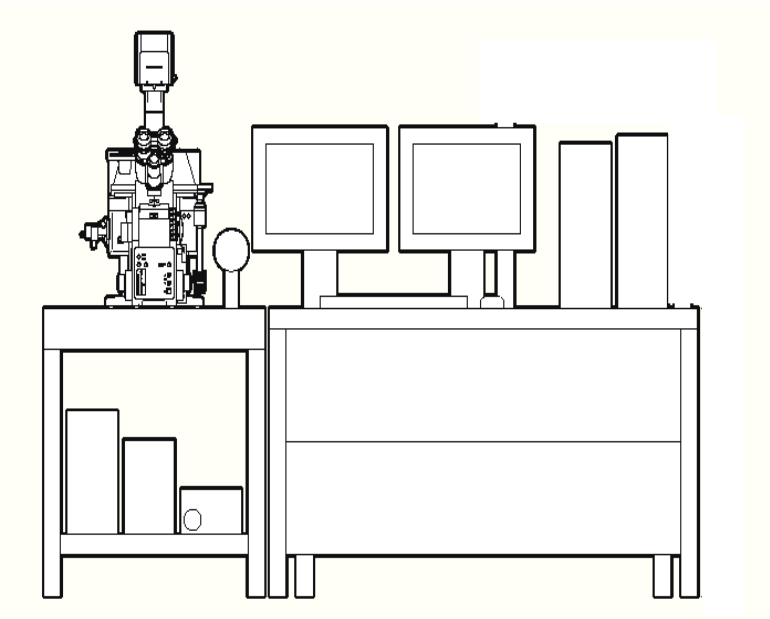
BX61 (Filter Type)

Laser Conforcal Scanning Microscope

FV1000D Spectral Type

(invertedMicroscpeIX81)

Operation Manual



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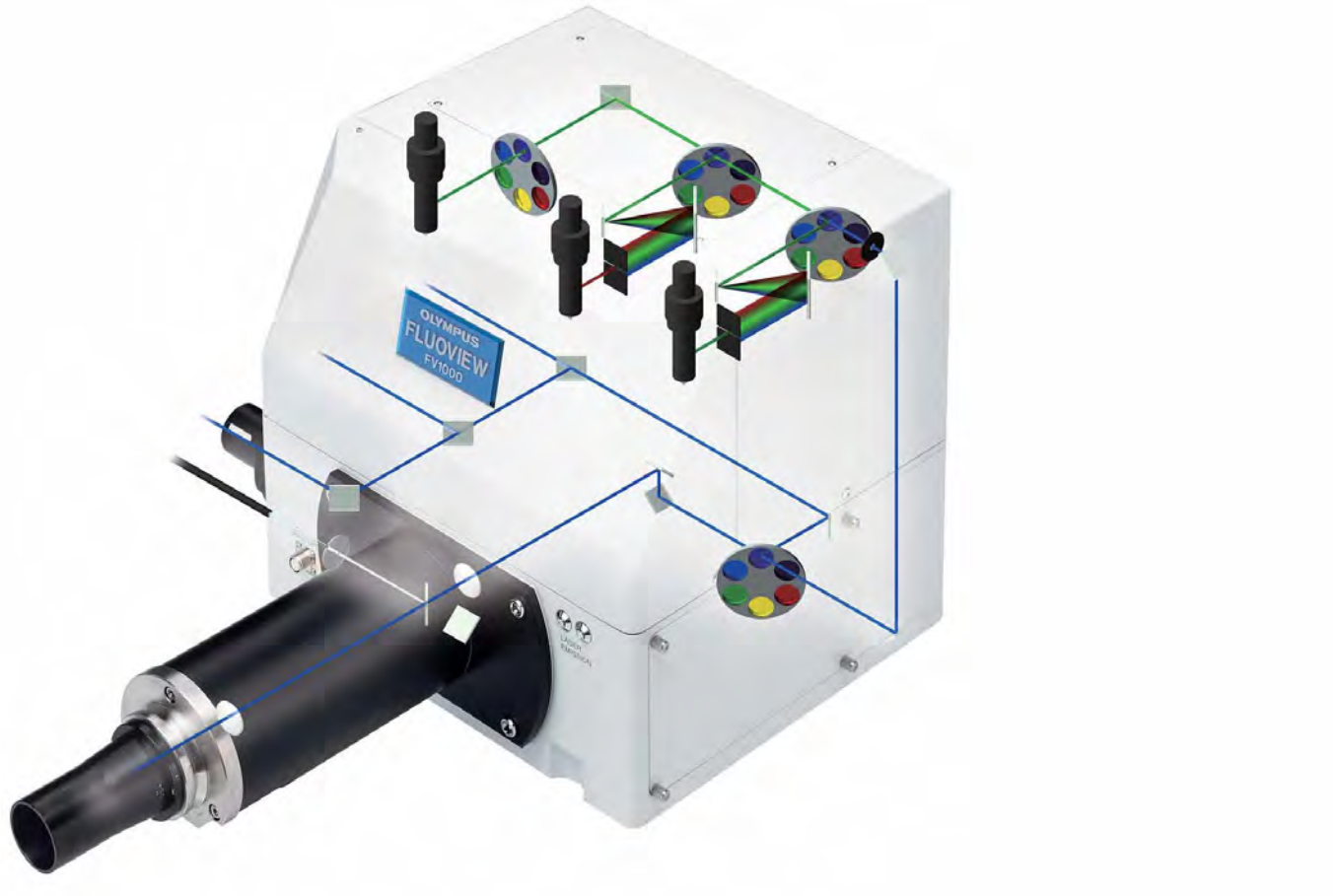
Contents

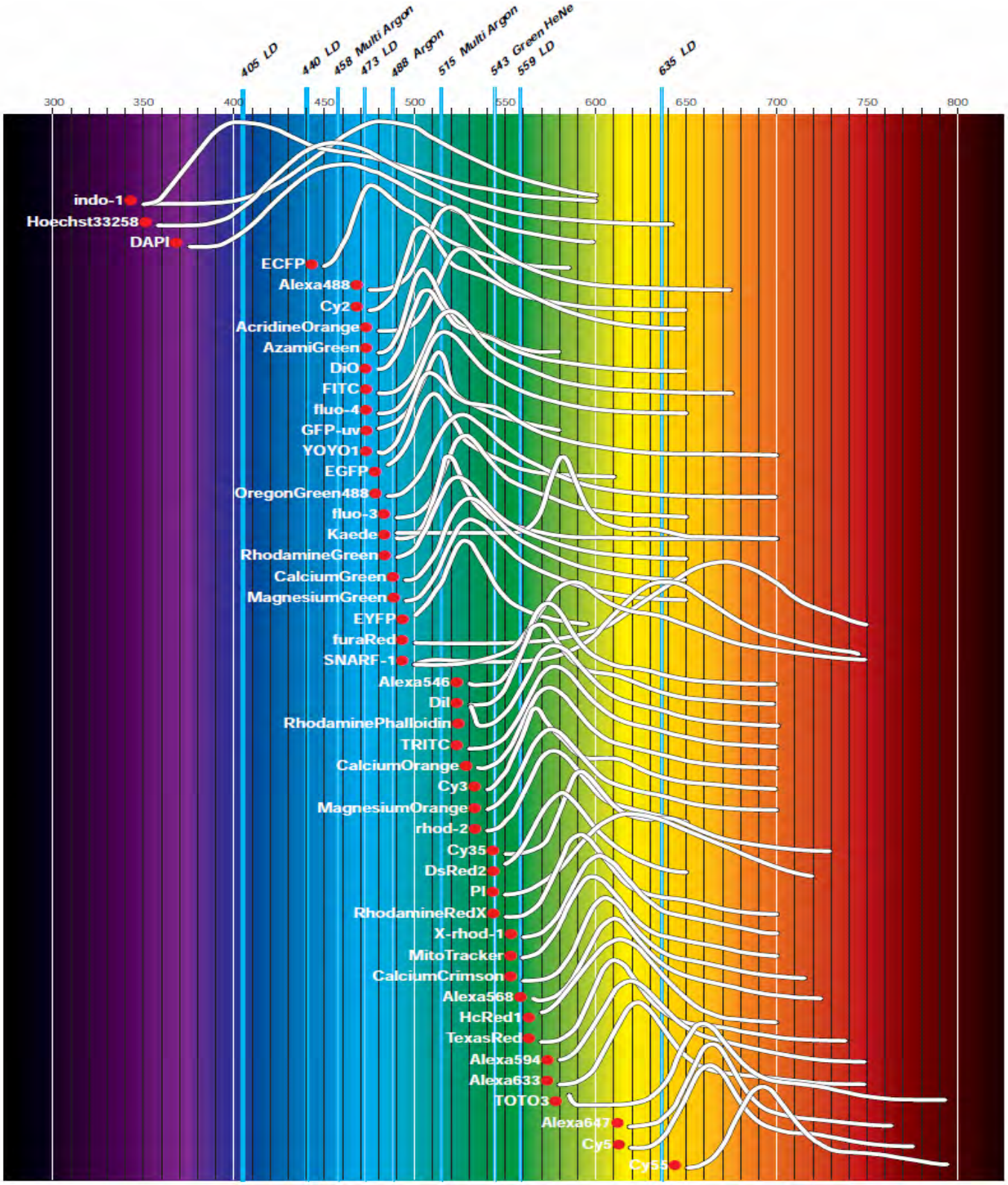
Spectral Type Main Scanner



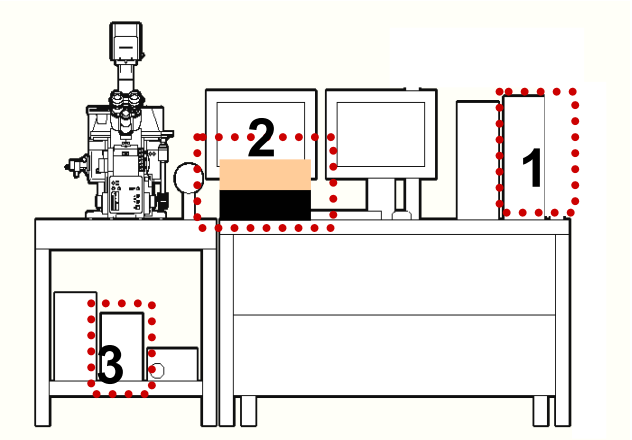
3

Dye List (FV1000D Lasers are available below)

LD405nm LD440nm LD473nm LD559nm LD635nm
Ar458nm Ar488nm Ar515nm
HeNe(G)543nm



System Preparation



1. Turn the computer ON.
   [In case of equipped concentrated power supply, power on it first]

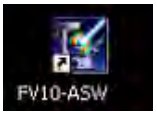
2. Turn the laser ON
   (Turning the key switch)
   2-1. LD559nm ON
   2-2. Multi Ar 458nm 488nm 515nm
   2-2. HeNe(G)(643nm) ON

3. Turn the mercury burner ON for Fluorescence observation.

Wait for a moment until the software is started

4. Log on Windows

Enter Password, Customer name is below
User name: Administrator
Password : fluoview



5. Double click this icon to log on to ASW
User name: Administrator
Password : Administrator

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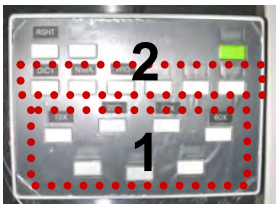
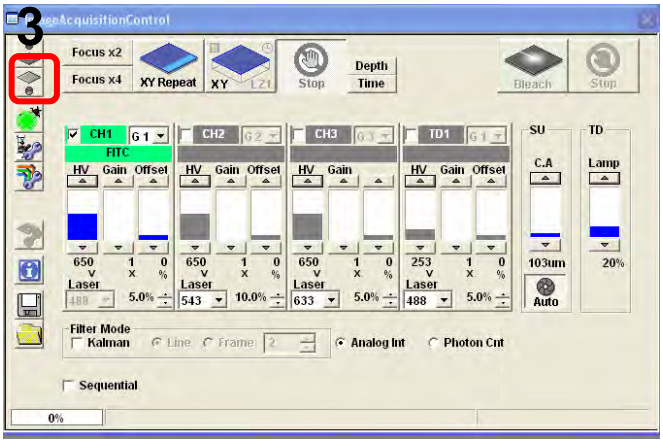
Visual Observation under the Microscope

■■ Observation of Fluorescence Image ■■



Hand switch



1. Select an objective lens
   by using the hand switch

2. Select florescent filter cube

MEMO
Fluorescence filter

NIBA: Blue Excitation / Green Fluorescence
(Ex.:FITC,EGFP)
WIG: Green Excitation / Red Fluorescence
(Ex.:Rhodamine, DsRed)

3.  Click the button
on the Fluoview software

4. Focus to the specimen

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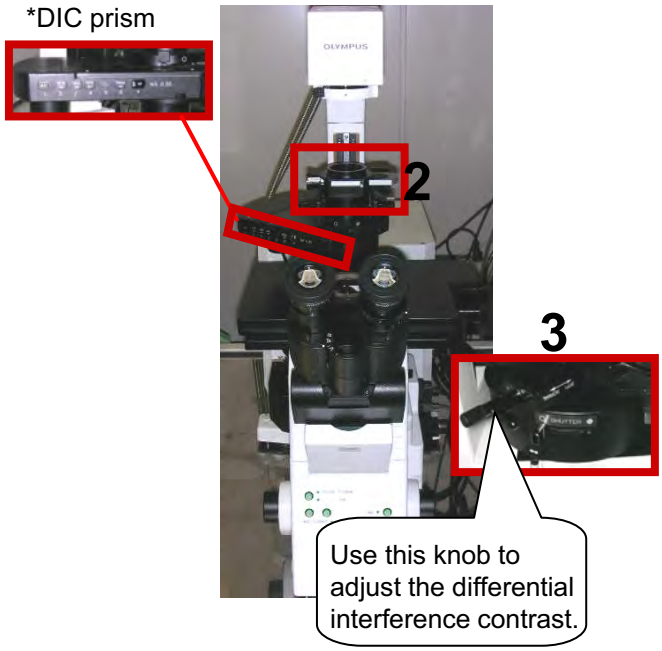
Visual Observation under the Microscope

■■ Observation of Differential Interference Contrast Images ■■

Hand switch

*DIC prism

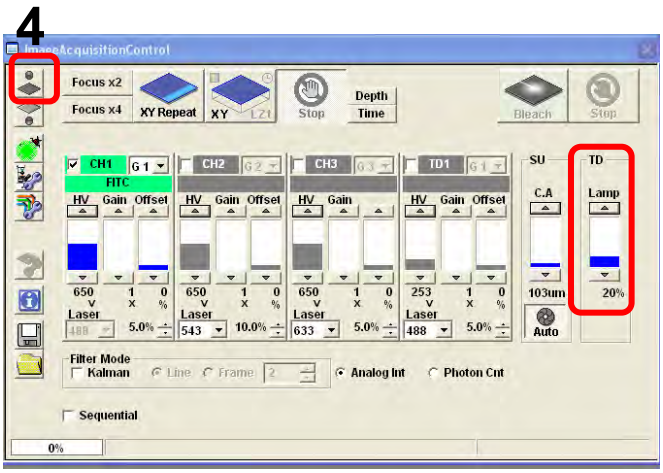


1. Select the Objective Lens

2. Insert the Polarizing Plate in the Light Pass

3. Insert the DIC prism slider in the light pass

4.  Click the button on Fluoview software



5. Focus to the specimen

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Overview of Operation Panel for Image Acquisition

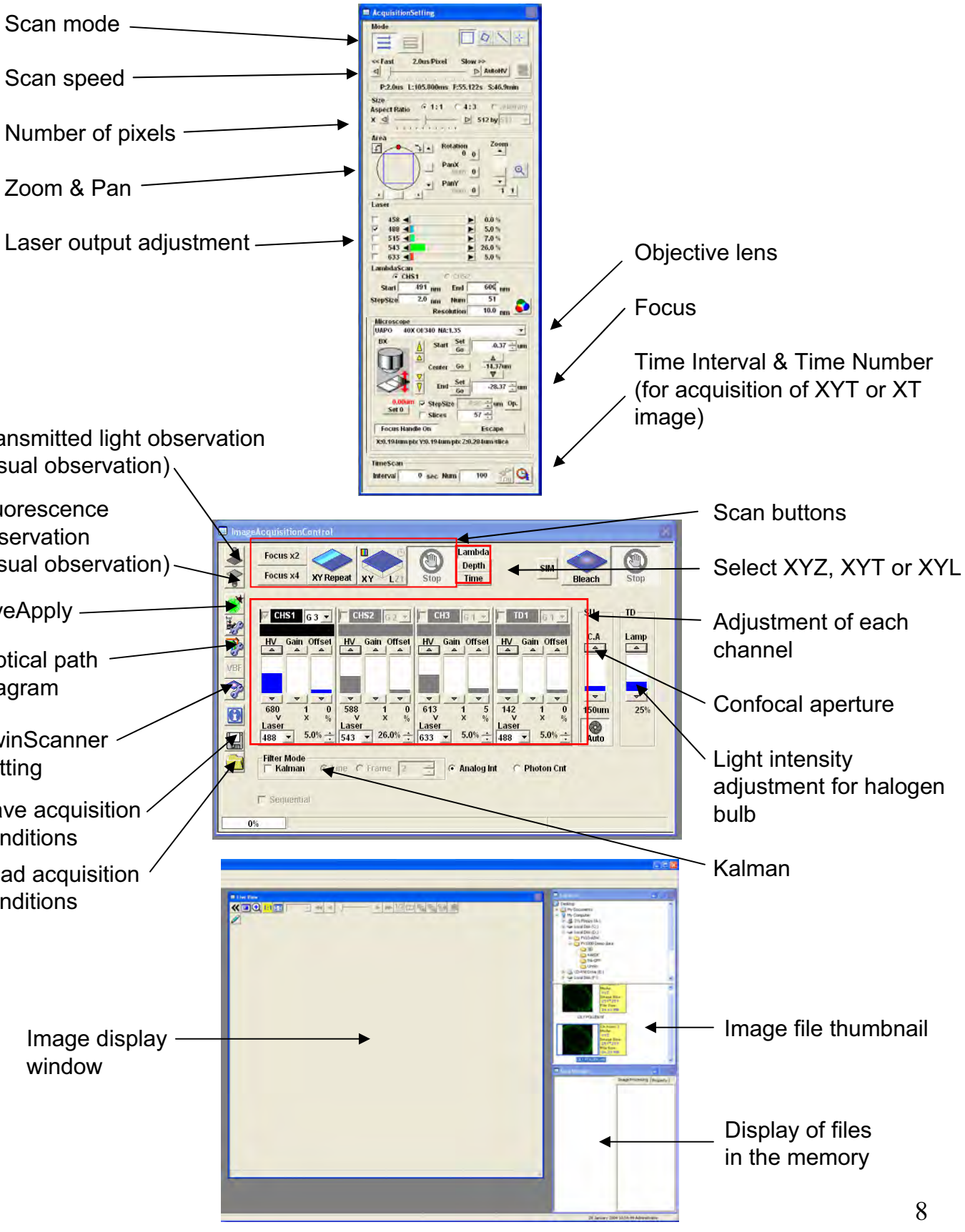

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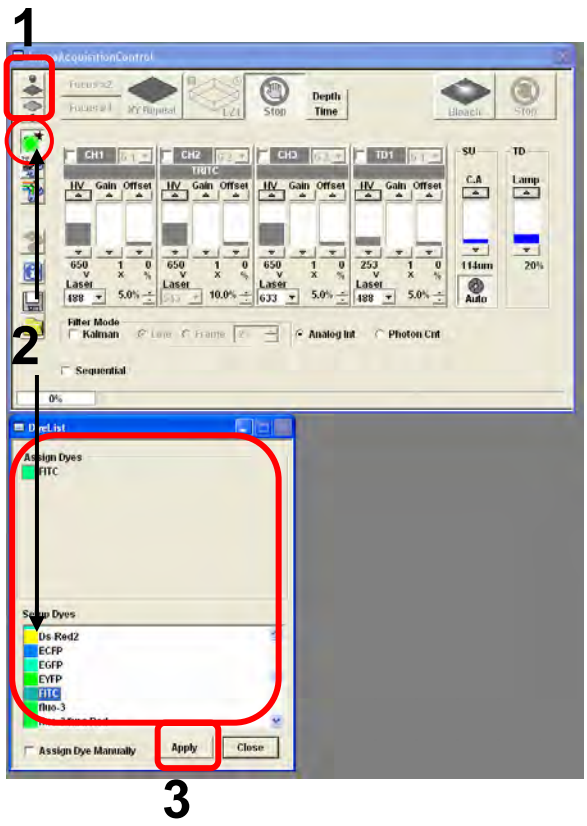
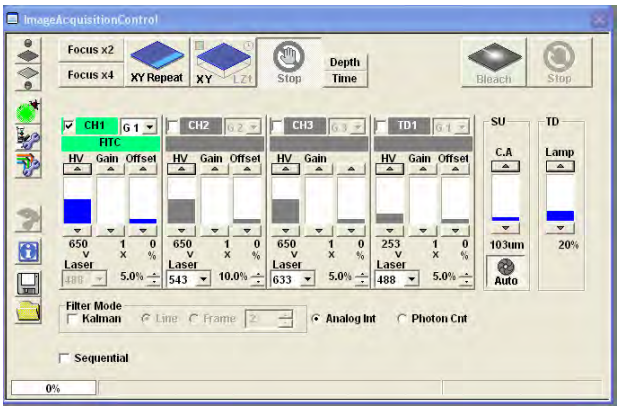
Image Acquisition
(Single Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Single stain of green fluorescence dye (FITC)





1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click on the Apply button.

   (The DyeList panel can be closed by using the Close button.)

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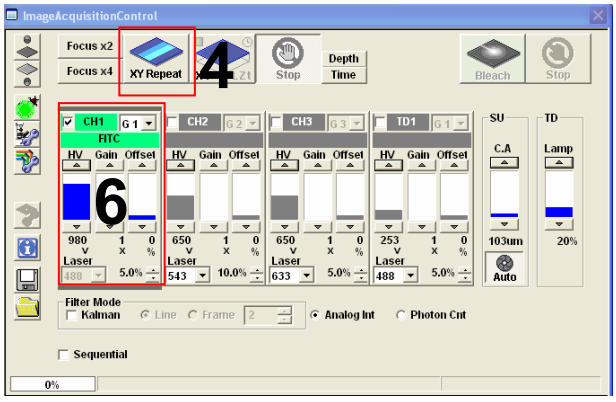
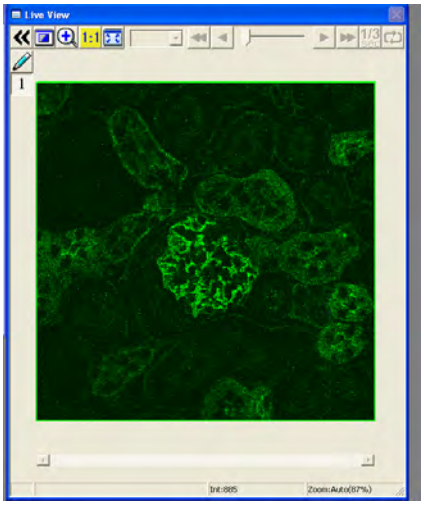
Image Acquisition
(Single Stain on XY Image)





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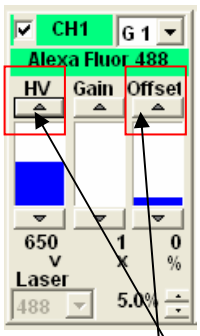
4. Press XY Repeat button click to get image

: Continuous scan mode

5. Focus to the specimen

6. Adjust the green (FITC) image.



· Adjust sensitivity of **HV** and reduce noise by **offset**

7. Press keyboard **Ctrl + H key**
Optimized PMT adjustment brightness intensity 2 color between white and black,
Maximum intensity is 4095(12bit)
if intensity is over 4095, color is changed to red (saturation)

* Basically, **Gain value is 1**

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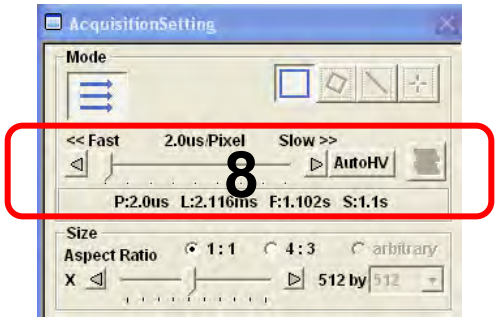
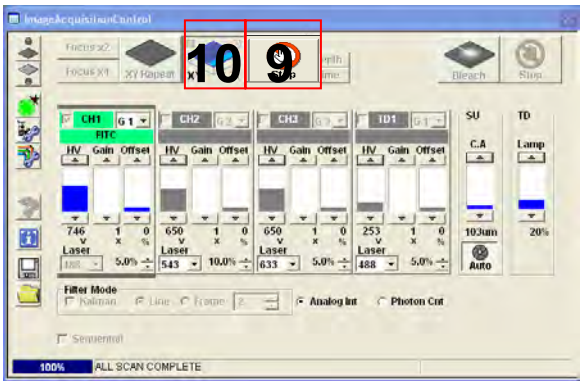
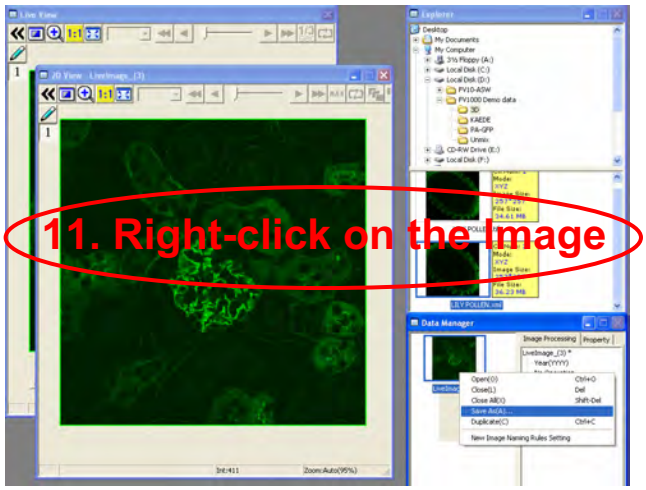
Image Acquisition
(Single Stain on XY Image)







■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

8. Select AutoHV and then select ScanSpeed.
*As the scan speed becomes slower, noise
can be removed while maintaining the current brightness.

9. 

Press the Stop button to stop scanning.

10. 

Click on XY, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

11. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type " oib" or "oif" file format specifically for the FV10-ASW software.)
Save the image as TIFF,BMP,JPEG format Select "Export" and chose the format TIFF, BMP, JPEG.

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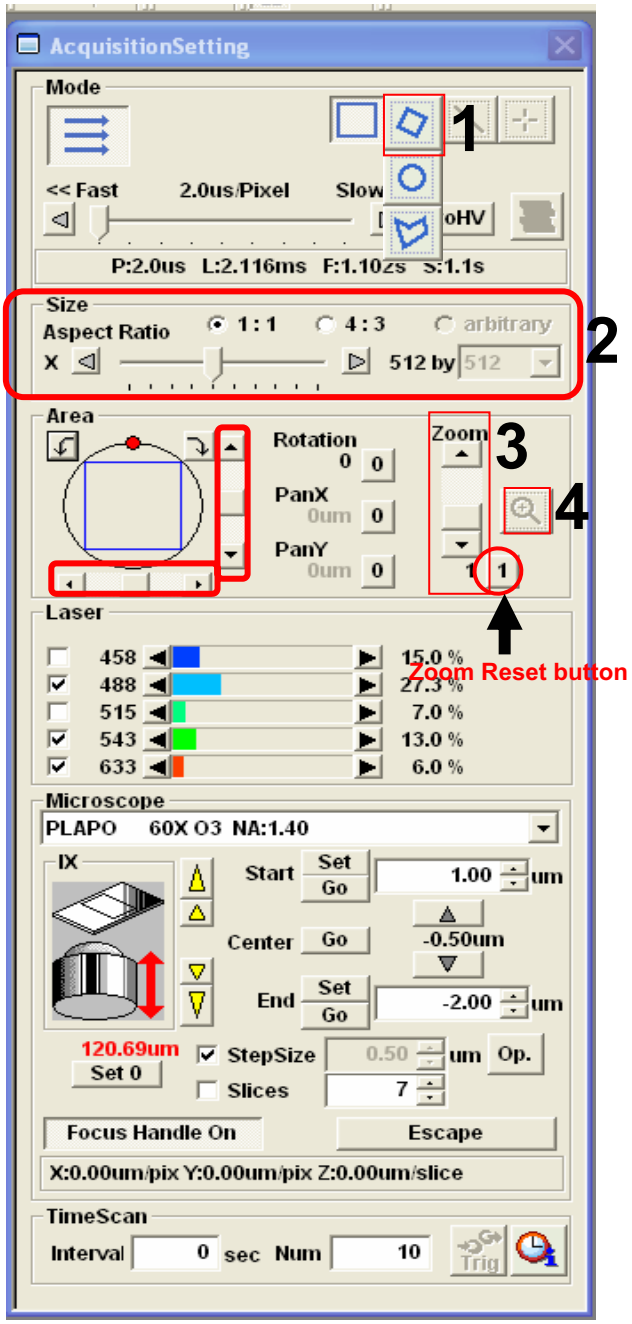
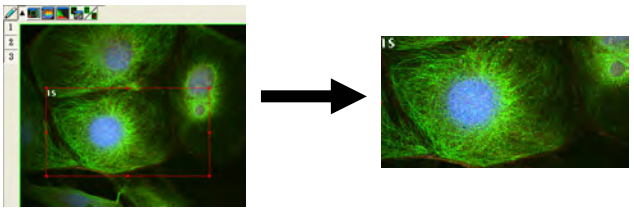
Complement of adjusting the image



1. Click “Clip scan” button , and enclose an interesting region’ s image on the whole image.

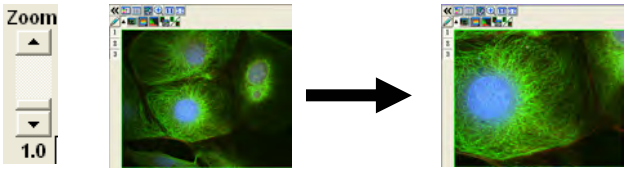


2. pixel setting

* The standard pixel is 512 x 512

3. Zoom Setting

Press “XY Repeat” to scan and set zoom value.

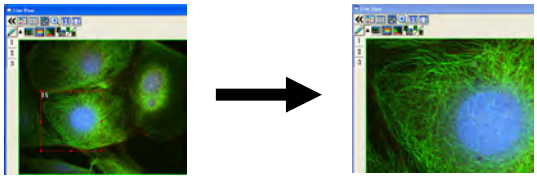


Above image is zoomed From 1 to 2

*** Scan speed and pixel resolution remain even zoom value is changed**

4. Click  Zoom scan, and be able to enclose an interesting region’s on the whole image

Press XYRepeat to scan after enclose the region



*** Scan speed and pixel resolution remain even zoom value is changed**

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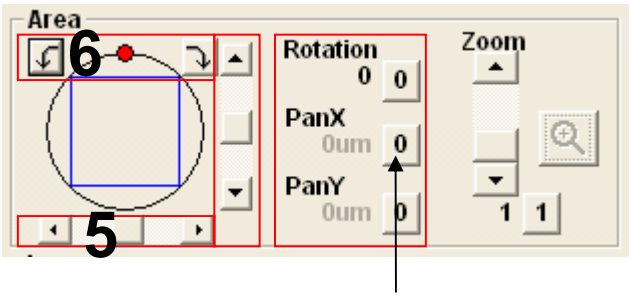
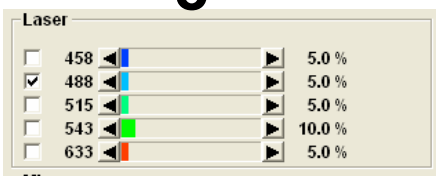
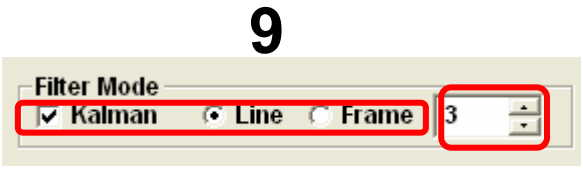
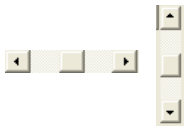
Complement of adjusting the image



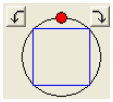
PanX,Y and Rotation reset button





5. Pan X,Y 

Be able to move the field of view to set Pan X,Y without stage action

6. Rotation 

Be able to rotate the whole image.

7. 

Click "Auto" button to acquire Optimized Conforcal aperture
Conforcal aperture · · · change conforcal aperture to larger diameter for dim fluorescence image then, be able to get the more bright image. But Z axis resolution gets worse.

8. Laser Intensity · · · More Laser intensity is increase , more bright image is .

*** More increase laser intensity is , more discoloration image is .**

9. Kalman accumulation · · · Image acquisition is repeated to the specified number of times to provide an averaged image. Consequently, noise is averaged and roughness on the whole image is reduced.

Advantage: The speed of each scan is fast.

Disadvantage: Some blur occurs due to averaging of images.

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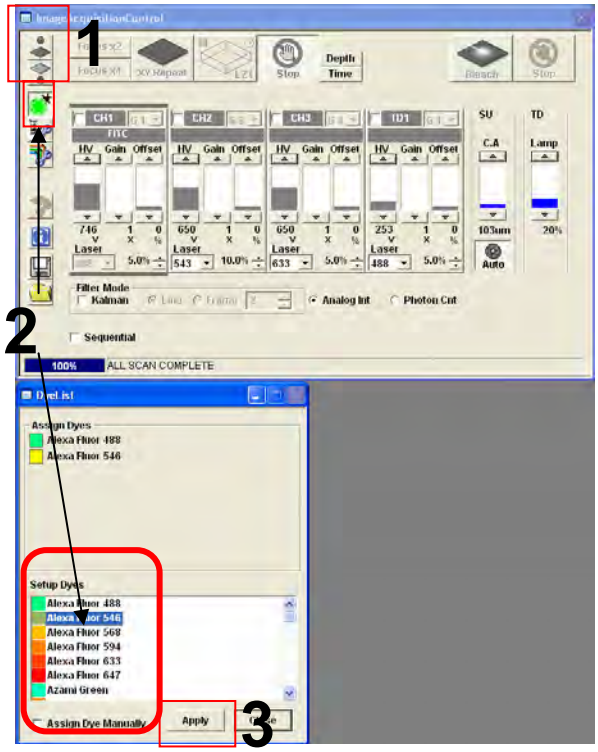
Image Acquisition
(Double Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Simultaneous scan



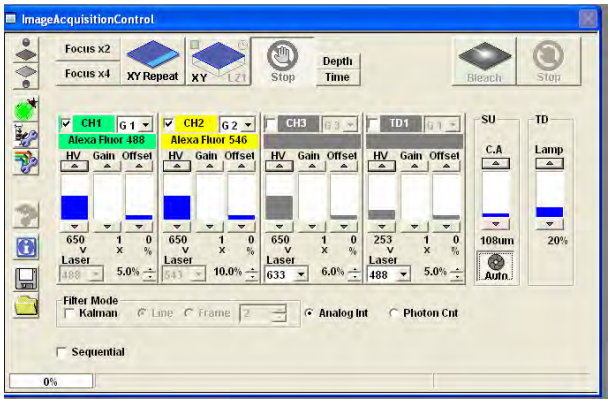
1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click “Apply” button.

   (The DyeList panel can be closed by using the Close button.)



Display after DyeApply is carried out

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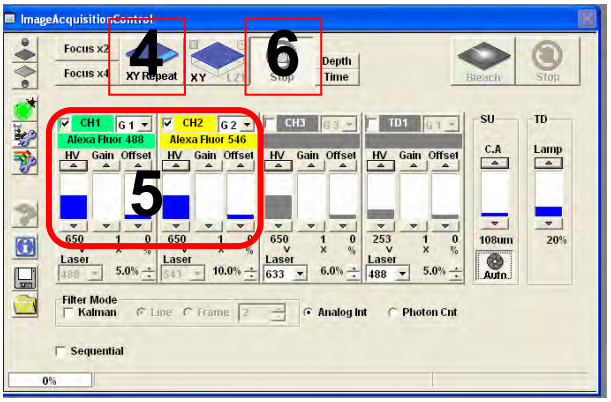
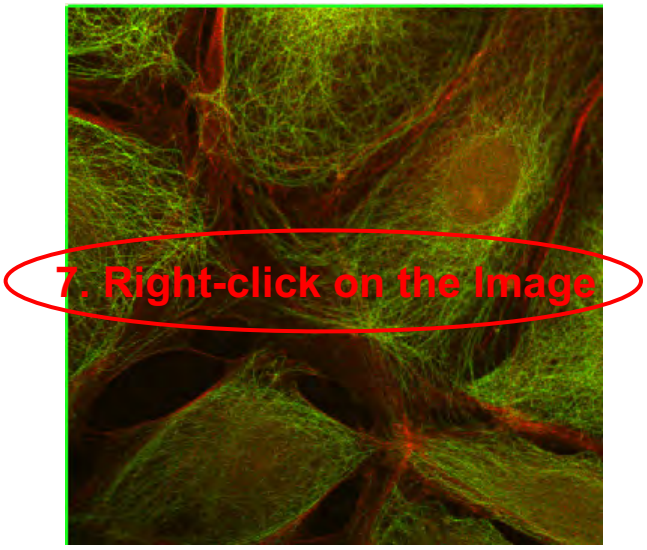
Image Acquisition
(Double Stain on XY Image)





4. Press the XY Repeat button to start scanning.

5. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.
   (The image adjustment is outlined below. For more information, refer to Appendix 1.)

6. Press the Stop button to stop scanning and press XY repeat to acquire the image. (Refer to ■Memo■.)

■Memo■
Scan buttons

: Continuous scan

: Stop scan

: Rough scan (Line skipped)

7. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
   (Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

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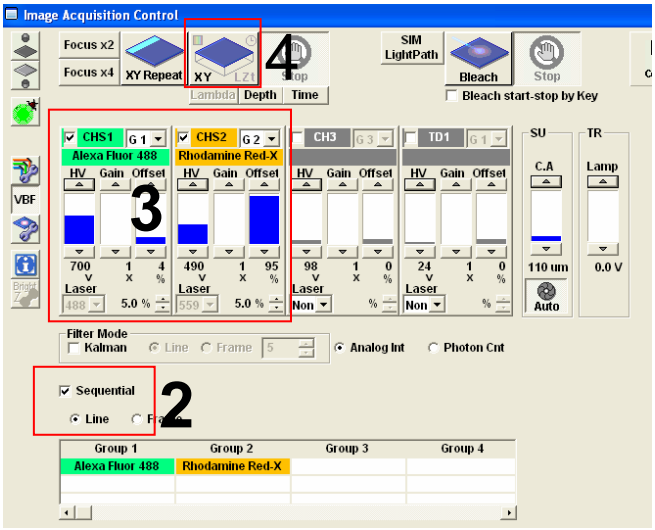
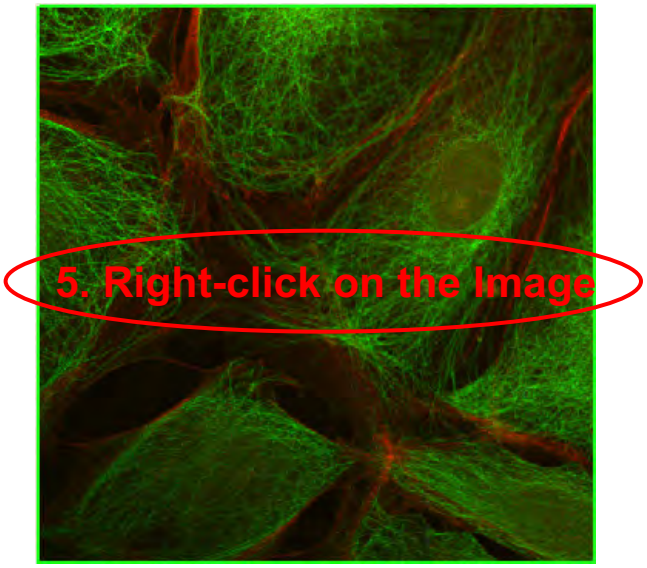
Image Acquisition
(Double Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Sequential scan (Line Sequential is introduced here.)





1. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

2. Check Sequential and select Line.

3. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.

4. Press the XY button to acquire an image.

5. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.) The image is acquired.

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

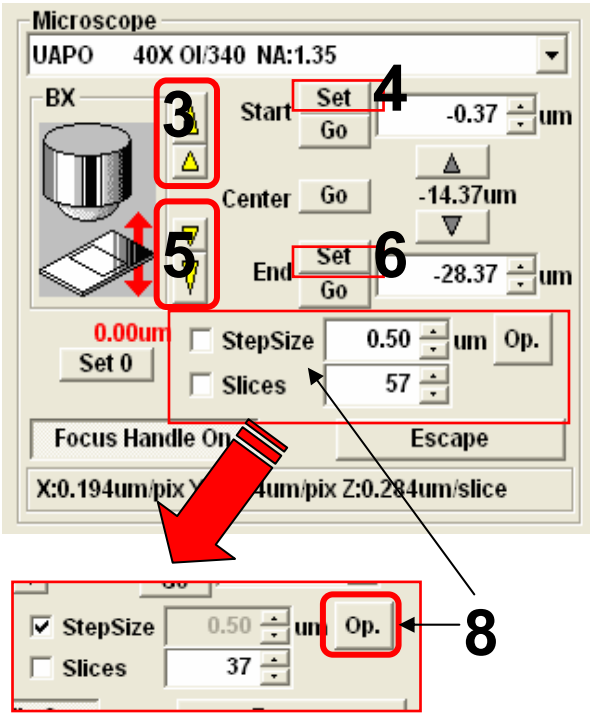
16

Image Acquisition
(Double Stain on XYZ Image)

■■ Acquisition of 3D images (XYZ)
(fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (FITC)
and red fluorescence dye (Rhodamine)

This is the procedure to acquire images
through Line Sequential scanning.



■Memo■

and buttons

: Moves 1.0μm with a single click.

: Moves 0.1μm with a single click.

1. Take steps 1 to 7 described on pages 13 and 14.
2. Press the XY Repeat button to start scanning.
3. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
4. When the sample upper limit is displayed on the image, accept it using the Set button.
5. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
6. When the sample lower limit is displayed on the image, accept it using the Set button.
7. Press the Stop button to stop scanning.
8. Enter StepSize, Slice (the recommended value can be referred to by using the Op button), and check the check box

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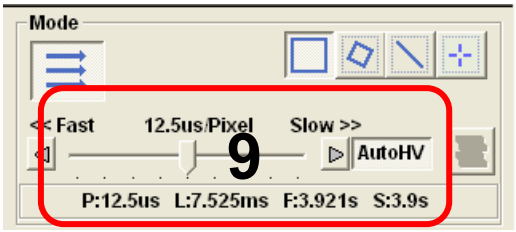
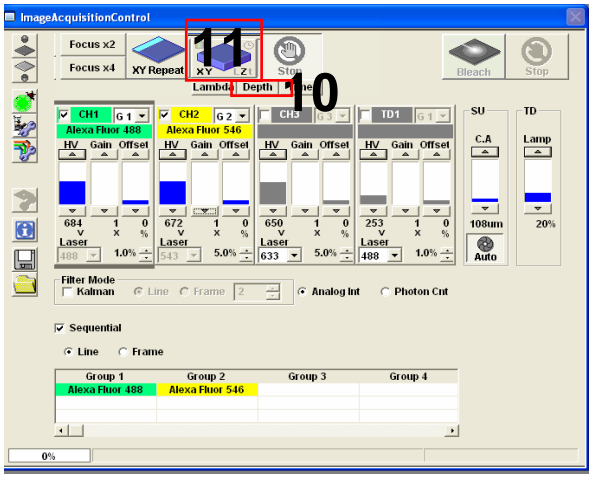
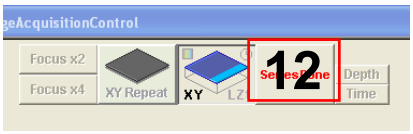
Image Acquisition
(Double Stain on XYZ Image)







9. Select AutoHV and then select ScanSpeed.

10. Select Depth.

11. Press the XYZ button to acquire an image.

12. Click on “SeriesDone”, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

13. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “ oib” or “oif” file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

18

Image Acquisition
(Four Stain on XY Image)

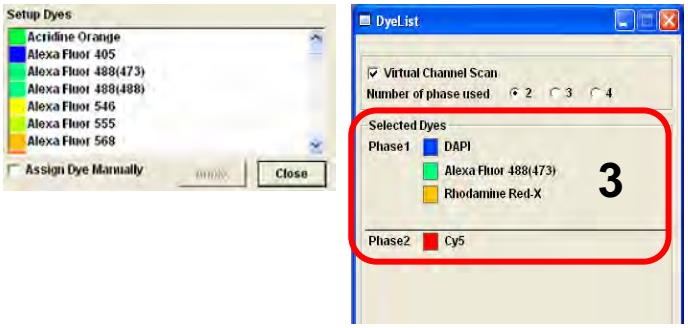
■■ Acquisition of 4 stain images (XY)
(fluorescence image only) ■■

Sample: Four stain of Blue fluorescence dye (DAPI) ,green fluorescence dye (Alexa488) and red fluorescence dye (Rhodamine), far-red fluorescence dye (Cy5)

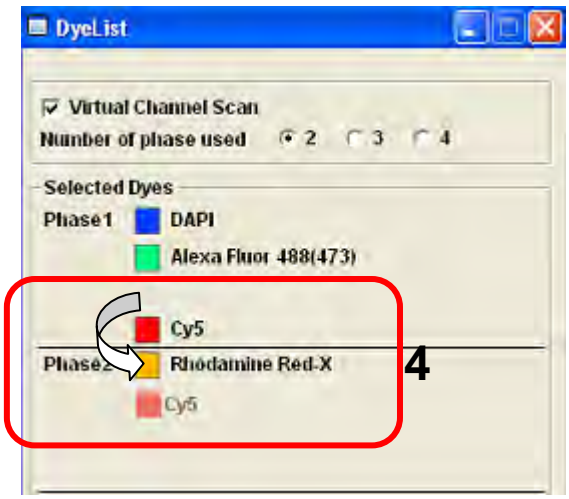
This is the procedure to acquire images
through Virtual Channel scan

1. Virtual Channel Scan Select Virtual channel Scan on the DyeList, and then "**Virtual Channel Controller**" is automatically turned on.

2. Select a number of Virtual Channel from "**Number of phase used**".



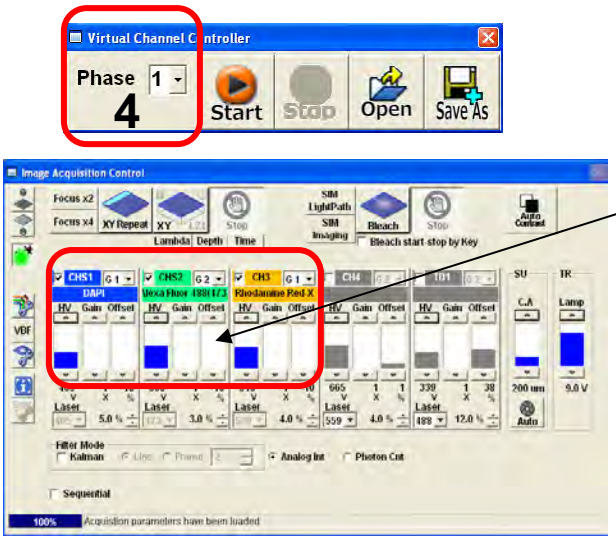
3. Select 4dyes from DyeList 4th dye is registered in "**the Phase 2**".



* **RodaminRed is able to be registered on "Phase2" to drag.**

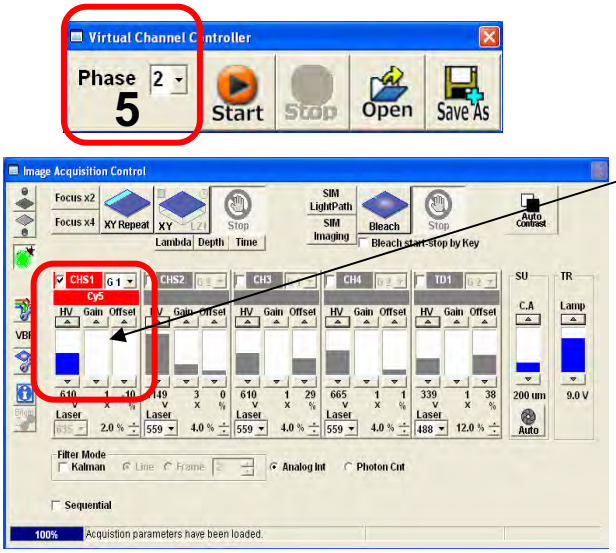
19

Image Acquisition
(Four Stain on XY Image)



4. Select “Phase1”, “DAPI”, Alexa488, RhodaminRed are registered on ImageAquisitionControl.

*** Slit and Filter, DM are automatically set for ”DAPI”, “Alexa488” or ”PhodaminRed”**



5. Select “Phase2”, Cy5 is registered on ImageAquisitionControl

*** Slit and Filter, DM are automatically set for ”Cy5”**

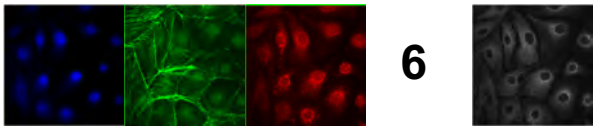
6. Adjust the image to click 

“XY Repeat” at each phases

* If acquire XYZ image, be able to decide upper limit and bottom limit , slices, step size of Z axis at both phases.

Adjust the image at each phases

“Phase1” **“Phase2”**



6

20

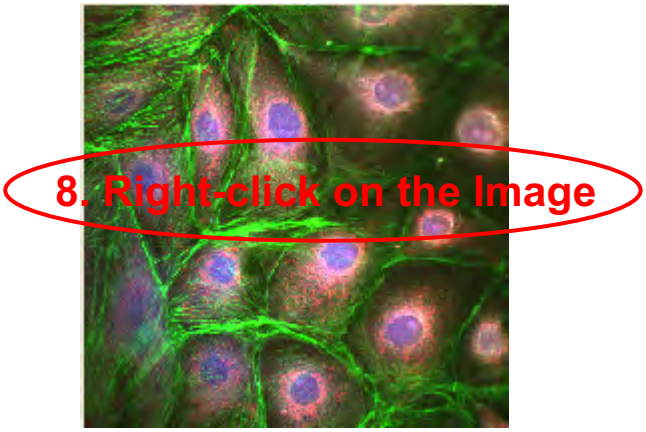
Image Acquisition
(Four Stain on XY Image)

7

7. Click 

on Virtual Channel Controller to acquire the image.

* Be able to start at each Phase.



8. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as Type " oib" or "oif" file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

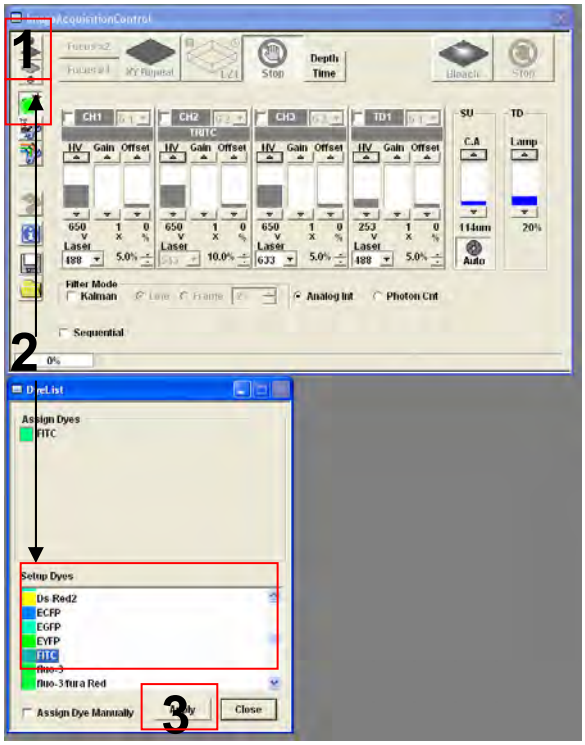
OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

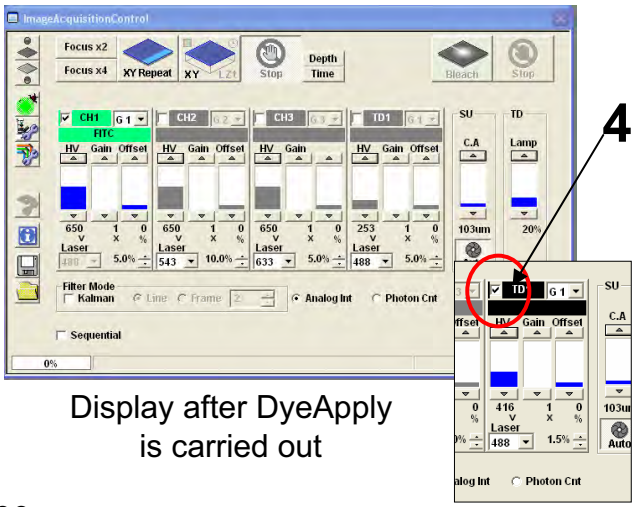
21

Image Acquisition
(Single Stain + DIC on XY Image)

■■ Acquisition of a single image (XY plane)
(fluorescence image and differential interference contrast image) ■■

Sample: Green fluorescence dye (FITC) and differential interference contrast image





Display after DyeApply is carried out

1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

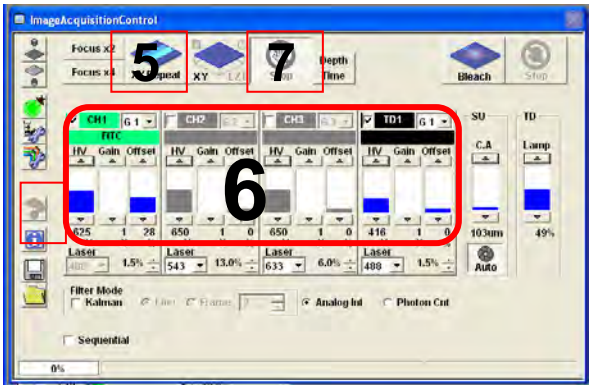
3. Click on the Apply button.

   (The DyeList panel can be closed by using the Close button.)

4. Check TD1.

22

Image Acquisition
(Single Stain + DIC on XY Image)



5. Press the "**XY Repeat**" button to start scanning.

6. Adjust the green (FITC) image and the differential interference contrast image.

7. Press the "**Stop button**" to stop scanning.
8. Press the "**XY button**" to acquire an image.

9. Click on "SeriesDone", and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

10. Right-click on the Image

10. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type " oib" or "oif" file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

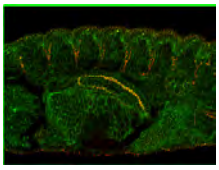
OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

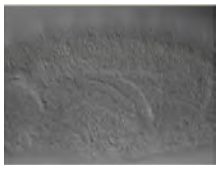
23

Merge the images between fluorescent XY image and DIC image

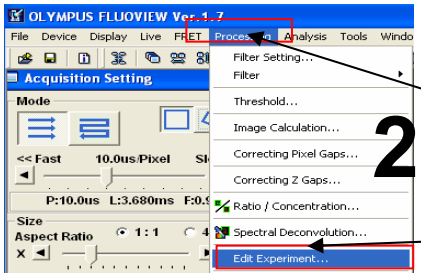
Edit different each files to the same file. This is available for making merge image Between fluorescent image and focused DIC image.



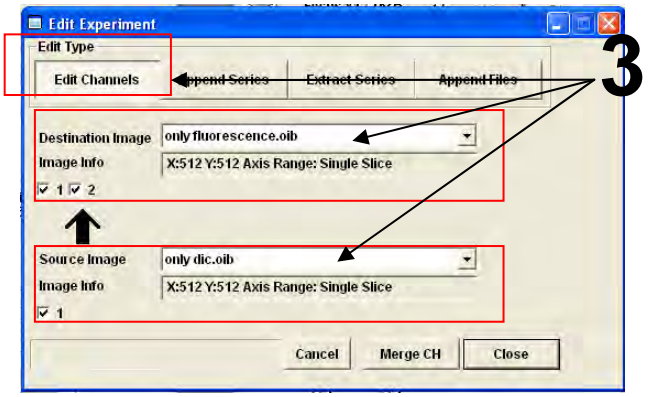
1



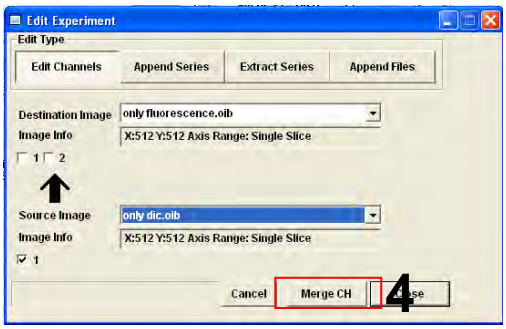
1. Open fluorescent image and DIC image.

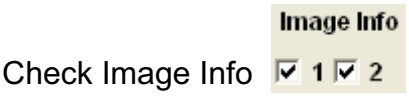


2. Select **Edit experiment** from **Processing**



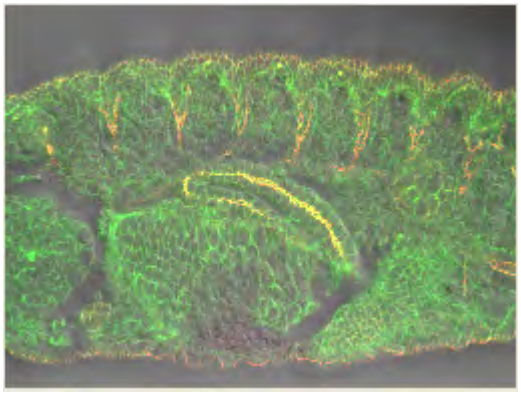
3. Click **Edit Channels**, and select fluorescent image file at **Destination Image**, select DIC image at **Source Image**.





* Check Image Info to make the. merge file. If all channels are checked, all channels are reflected in the new merged file.

4.Click **Merge CH**, and then the fluorescent image and the DIC image are merged as the new file .



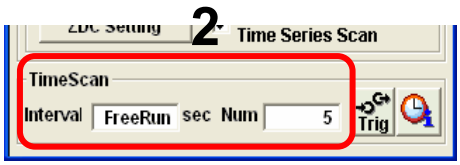
5

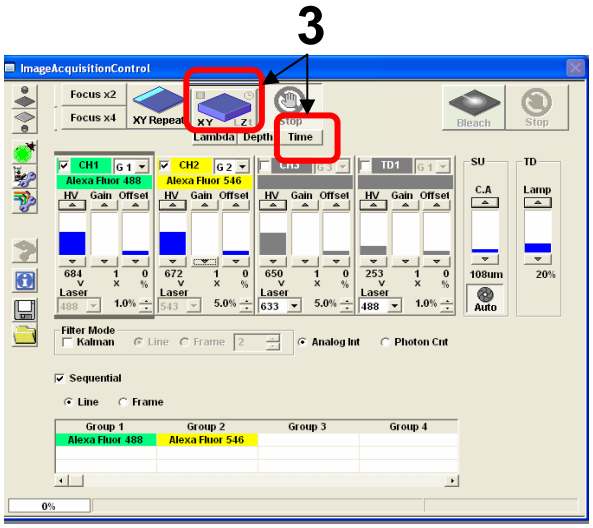
5. Merged image between the fluorescent image and the DIC image.

24

Image Acquisition
(Single Stain on XYZT Image)

This is available for the Time series scan experiment.





1. Adjust the image.
   * Refer P17,18
2. Enter interval time to “Interval”
   Enter interval number to “Num”

   Example: Acquiring time series scan images every 5minutes for 1hour is below,
3. Select “Time” and then click XYTbutton to acquire Time series scan image.
4. Click on “SeriesDone”, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

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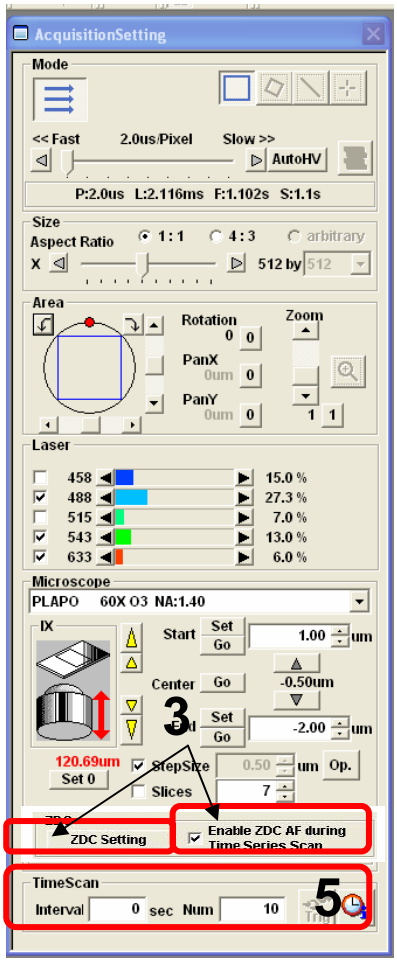
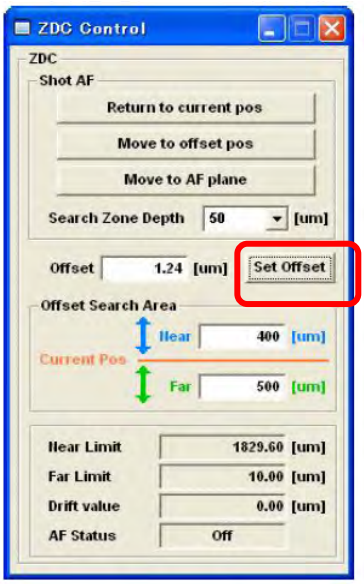
Image Acquisition
(Single Stain on XYZT Image)





1. Adjust the image.
   * Refer P17,18

2. Insert ZDC unit to left side.

3. Check "**EnableZDC AF during Time Series Scan**" ☑ and click "**ZDC setting**".

4. Click "**Set Offset**" to register auto focus position.

   *** Note: Have to use glass bottom dish below, otherwise ZDC doesn't work.**

5. Set "**Interval**" and "**Num**" **and then click "XYZT"** to acquire the time series image.

   *** Note: In case of using ZDC for Time series Scan, follow below limits**
   Interval number is more than 60 sec,
   Rest Time is more than 30 sec,
   otherwise ZDC doesn't work.

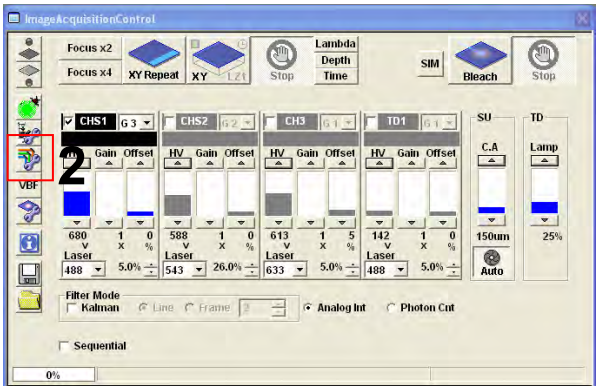
   *** If use "TimeControler", Time Series Scan is able to done even interval number is within 60sec and Rest Time is within 30sec.**

26

Image Acquisition (Spectral Image on XYL Image)

■■ Acquisition of a spectral image (XYL) ■■

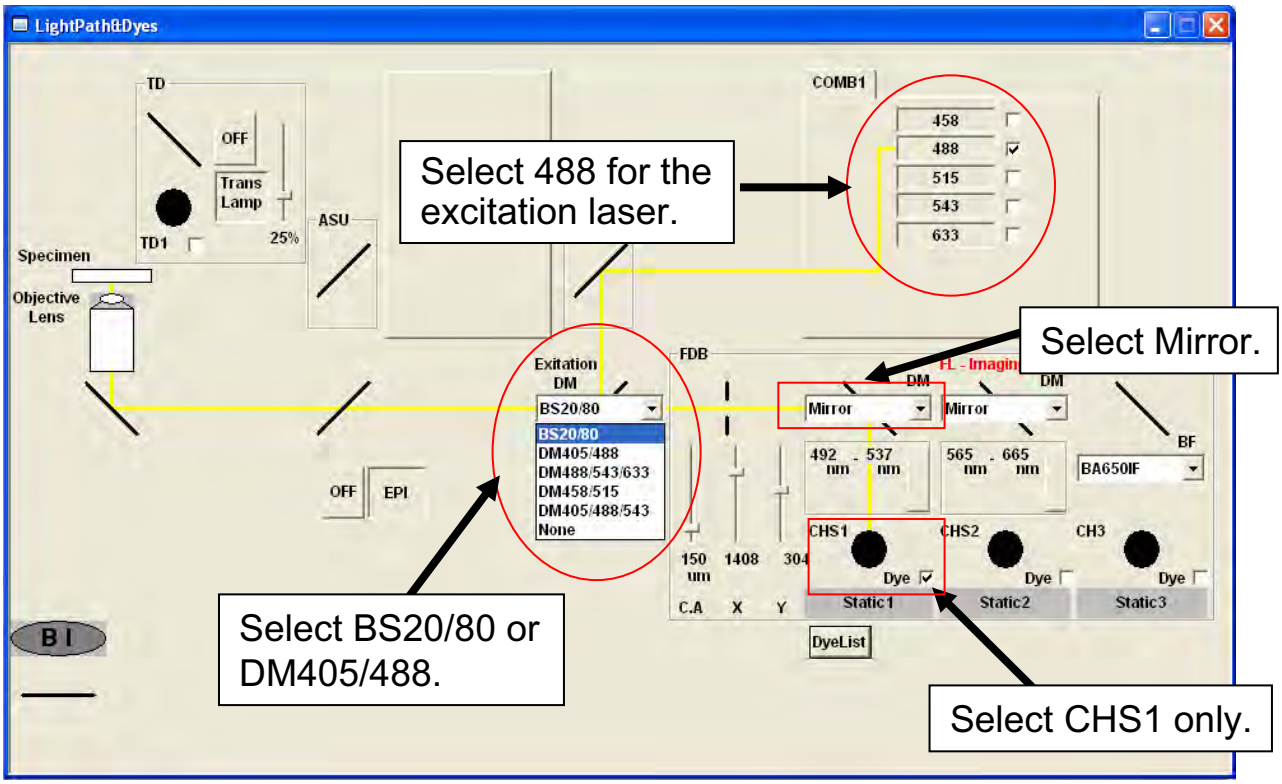
Sample: Double stain of green fluorescence dye (Alexa Fluor 488) and green fluorescence dye (YOYO-1)



1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

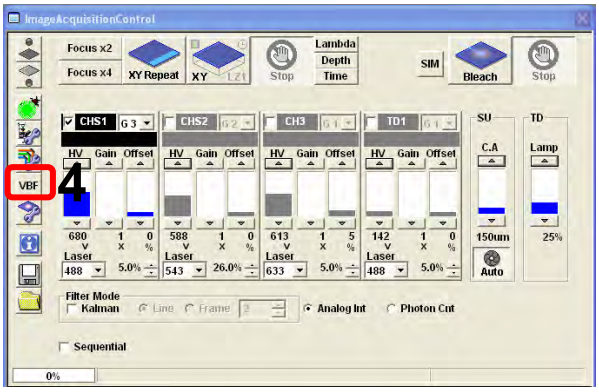
2. Click on the  button to view the optical path diagram.

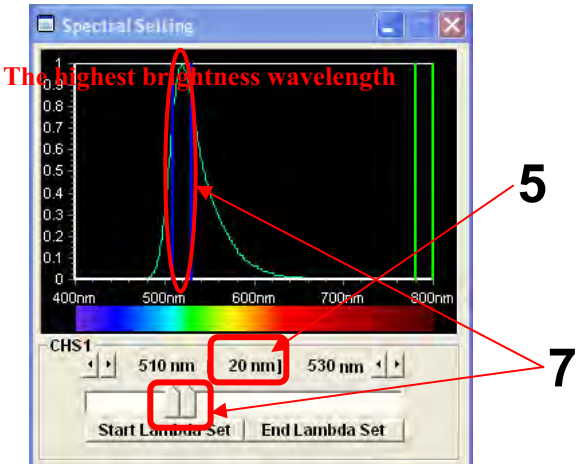
3. Make settings as shown below.

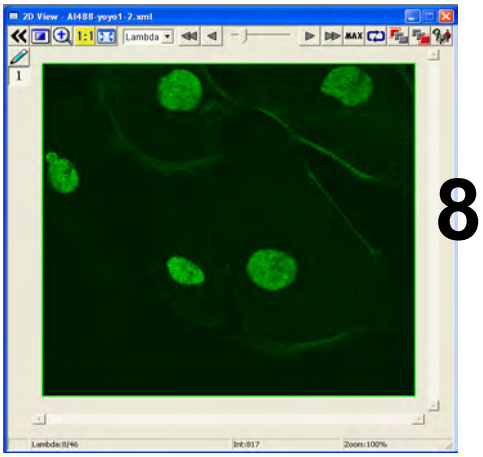


27

Image Acquisition (Spectral Image on XYL Image)







4. Click on th VBF button, and the Spectral Setting window appears.

5. Set the slit width for CHS1 to 20 nm, for example.

6. Press the XY Repeat button to start scanning.

7. While observing the image, Click the left side of slit and drag to the point which the highest brightness is achieved.

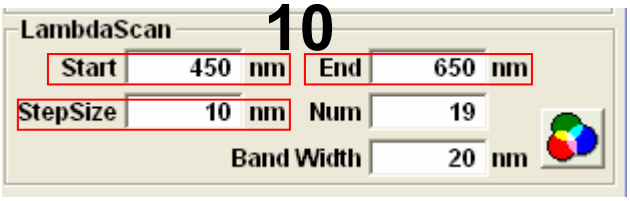
   Note: Move the slit position only while keeping the slit width at 20 nm.

8. Adjust the image on the highest brightness.

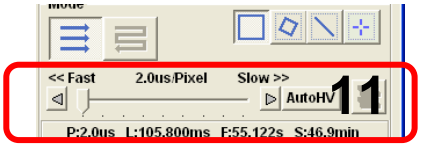
9. Press the Stop button to stop scanning.

28

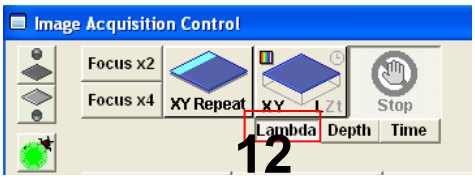
Image Acquisition (Spectral Image on XYL Image)



10. Set the range of wavelength to be acquired, the slit width and the step.
    - Start = Start wavelength
    - End = End wavelength
    - Resolution = Slit width
    - StepSize = Step



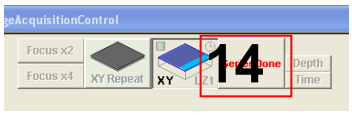
11. Select AutoHV and then select ScanSpeed.
    *As the scan speed becomes slower, noise can be removed while maintaining the current brightness.



12. Select Lambda.

13. 

Press the XYZ button to acquire an image.



14. Click on SeriesDone, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

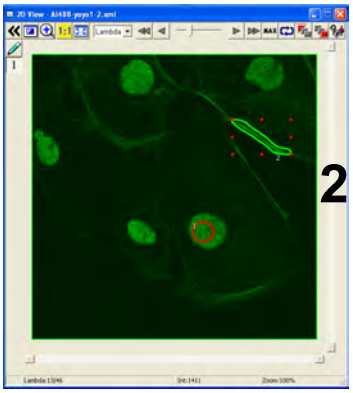
29

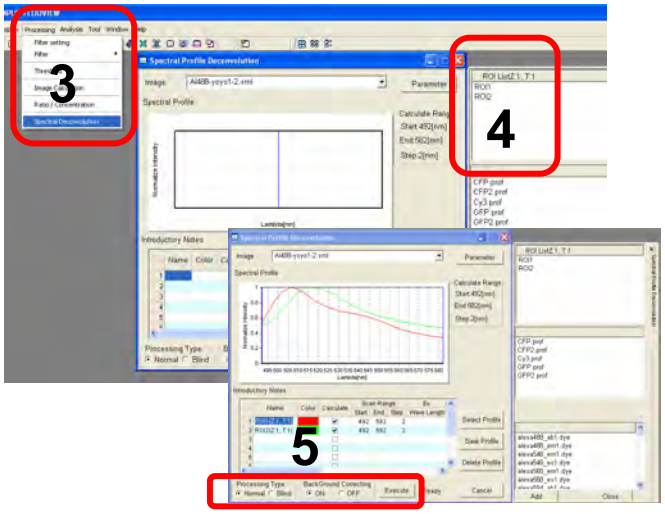
Image Analysis (Unmixing)

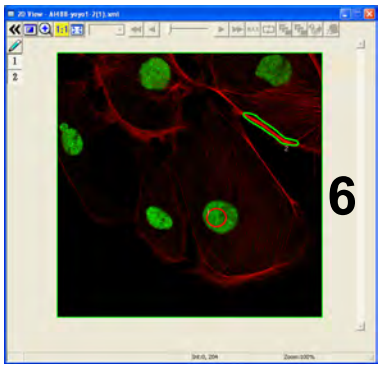
I. When each fluorescence dye point is clear

From an XYL image where fluorescence dyes with similar fluorescence spectrums are present together, derive the fluorescence spectrum for each fluorescence dye and obtain an unmixed image based on the fluorescence spectrums.

Sample: Double stain of green fluorescence dye (Alexa Fluor 488) and green fluorescence dye (YOYO-1)



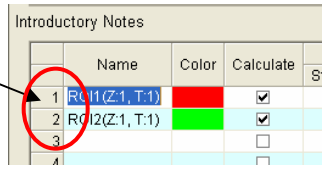




Unmixed image

1. Open an XYL image file with both Alexa Fluor 488 and YOYO1 applied.
2. Enclose a point dyed with Alexa Fluor 488 only and a point dyed with YOYO1 only.
3. From Processing on the menu bar, select Spectral Deconvolution.
4. Double-click on ROI1 and ROI2.
5. Check that the Processing Type is set to "Normal" and click on Execute.
6. An unmixed image is obtained.

Indicates channel assignments of unmixed images

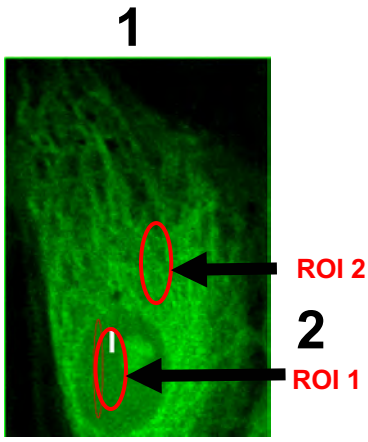


30

Image Analysis (Unmixing)

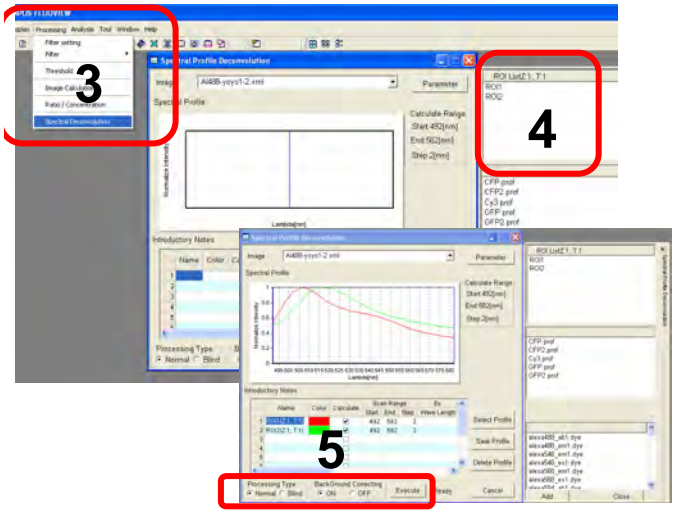
I. When each fluorescence dye point is clear

Sample: single stain of green fluorescence dye (GFP)
and auto fluorescence from cell



1. Open the XYL image (GFP + auto fluorescence).

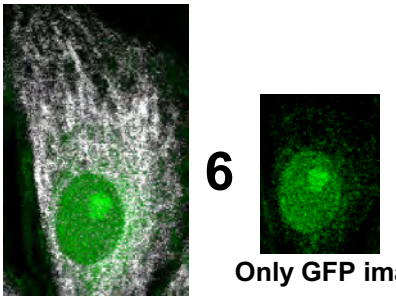
2. Enclose a point dyed with GFP only and a point dyed with auto fluorescence only.



3. From Processing on the menu bar, select Spectral Deconvolution.

4. Double-click on ROI1(GFP) and ROI2(Auto fluorescence).

5. Check that the Processing Type is set to "Normal" and click on Execute .



Only GFP image

Unmixing image between GFP and Auto fluorescence

6. An unmixing image is obtained.

   Green color is GFP.
   Gray color is Auto fluorescence.

31

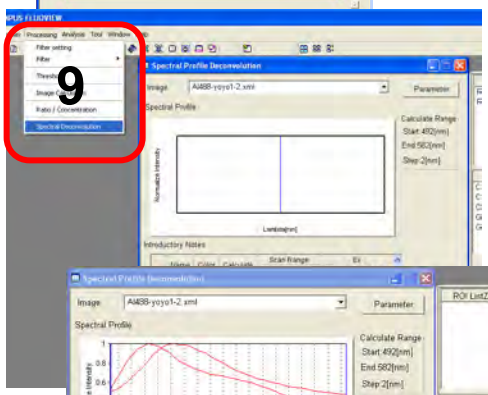
Image Analysis (Unmixing)

II. When a control sample is used

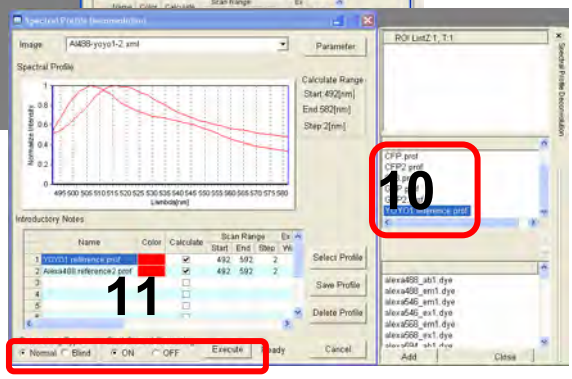
From an XYL image with a single type of fluorescence dye, derive the fluorescence spectrum of the dye and obtain an unmixed image based on the fluorescence spectrum.

Sample: Double stain of green fluorescence dye (Alexa Fluor 488) and green fluorescence dye (YOYO-1)

8. Open an XYL image file with both Alexa Fluor 488 and YOYO1 applied.

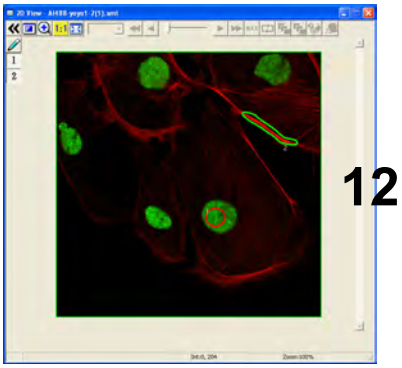


9. From Processing on the menu bar, select Spectral Deconvolution.



10. Double-click on Alexa Fluor 488 and YOYO1 (which have been registered) in the database of fluorescence spectrums.

11. Check that the Processing Type is set to "Normal" and click on Execute.



12. An unmixed image is obtained.

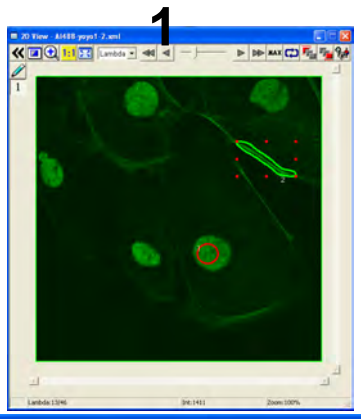
32

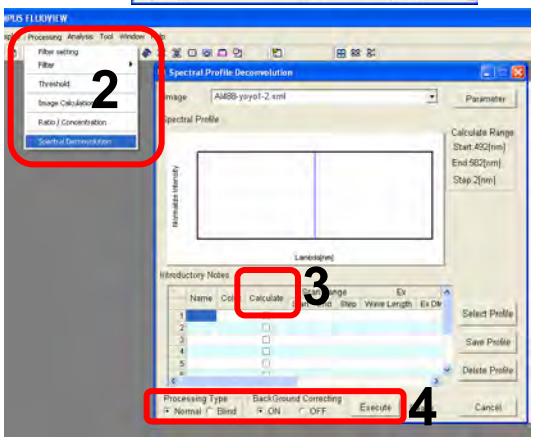
Image Analysis (Unmixing)

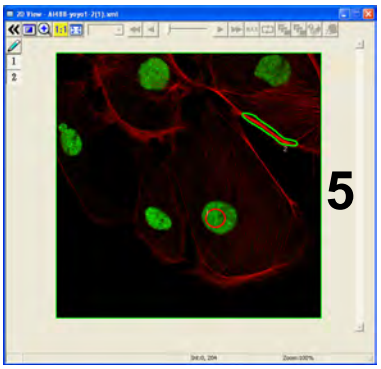
III. When only the number of types of fluorescence dyes is known (Blind Unmixing)

From an XYL image where fluorescence dyes with similar fluorescence spectrums are present together, obtain an unmixed image based on only the number of types of fluorescence dyes.

Sample: Sample with two unknown types of fluorescence dyes







Unmixed image

1. Open an XYL image file for a sample that has two unknown types of fluorescence dyes.

2. From Processing on the menu bar, select Spectral Deconvolution.

3. Click on two Calculate check boxes. (Click on three boxes when three types of fluorescence dyes are used.)

4. Check that Processing Type is set to "Blind" and click on Execute.

5. An unmixed image is obtained.

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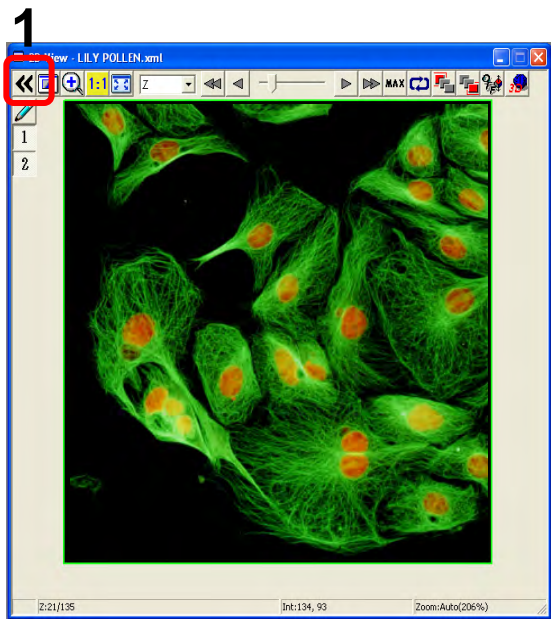
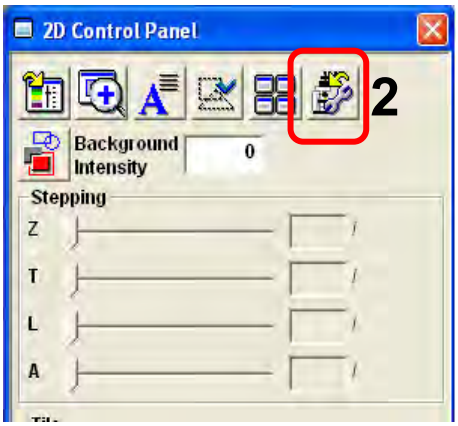
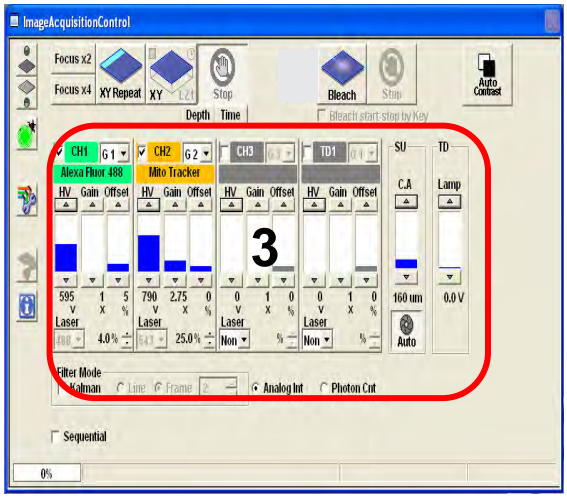
Reload the image conditions



1. Open the file and click 



2. Click 



3. The conditions (HV,Offset, CA and so on) are reloaded .

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Overview of the 2D Operation Panel

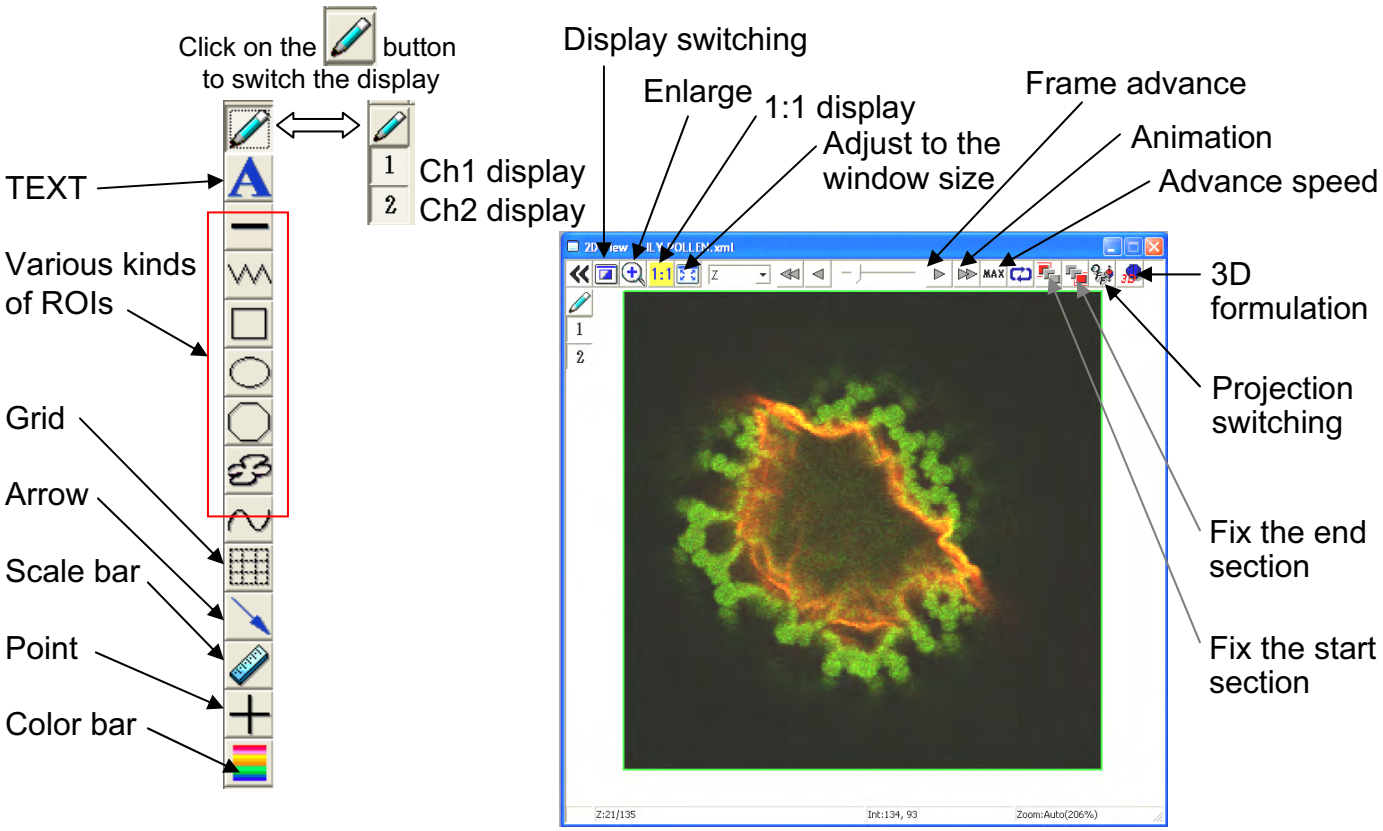
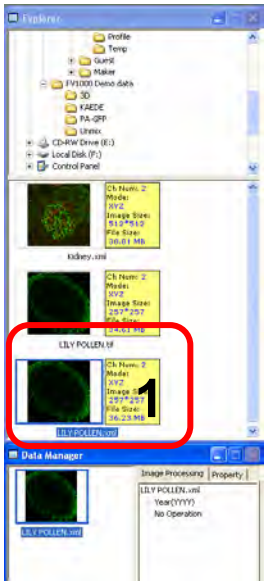


Image Analysis (Opening a File)



1. Double-click on a file to be opened from Explorer.

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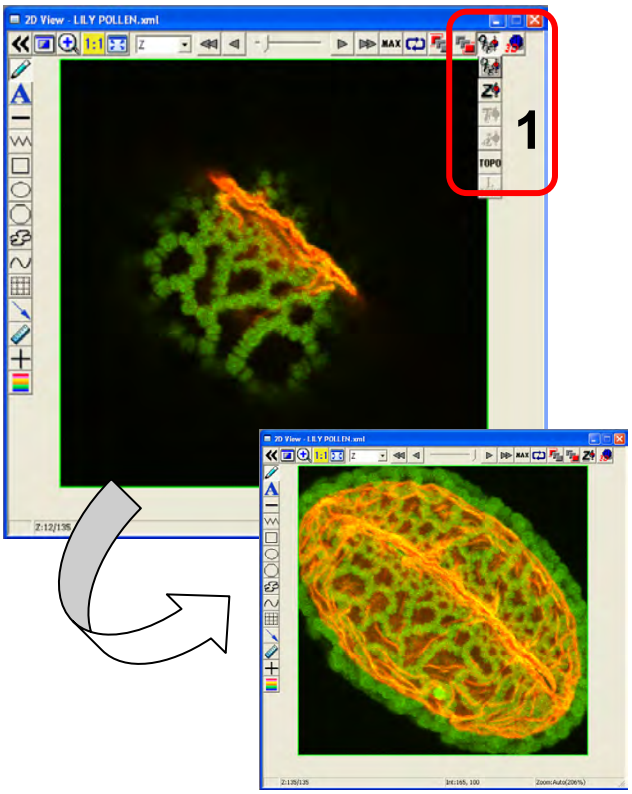
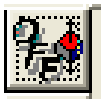
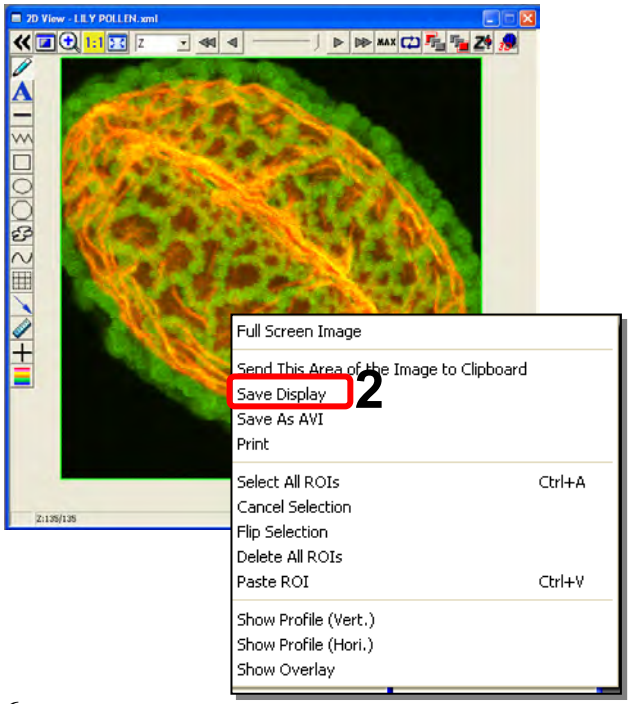
Image Analysis (Acquire a Projection Images)



1. Click on the  button to select  .



2. To save this image, right-click on the image, select Save Display and save the image with a new name.

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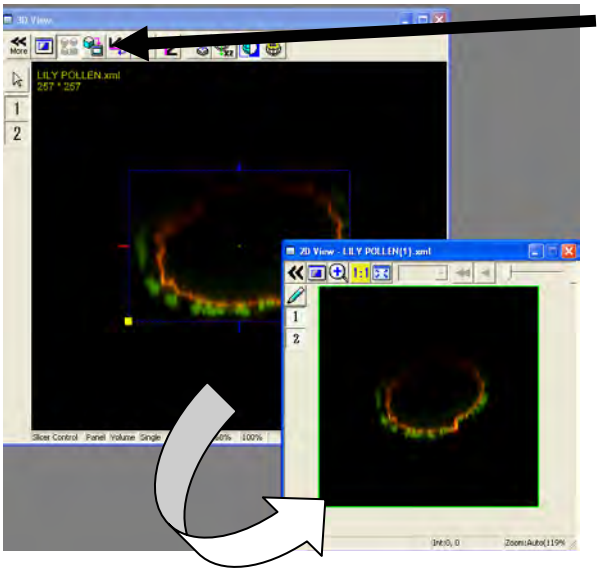
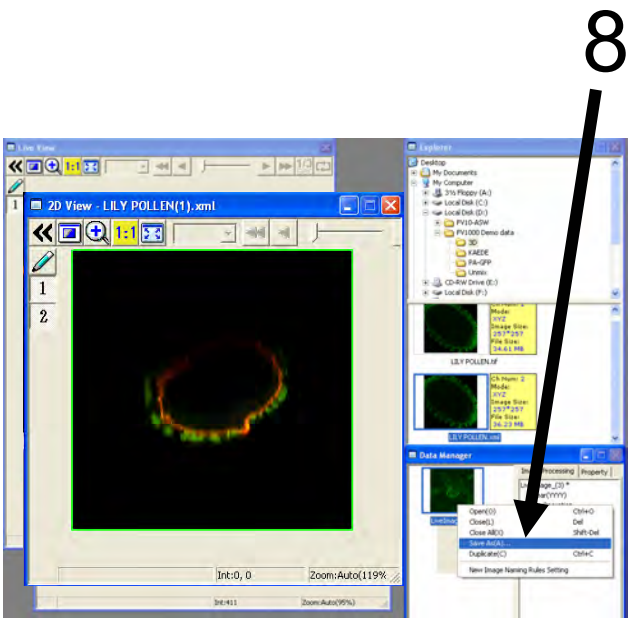
Image Analysis
(Save a Z section Image as 2D file)



Save the image in step 3 or 5

6. Click on the  button.

7. A 2D View-(file name) image is created.



8. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as type "xml" is a file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

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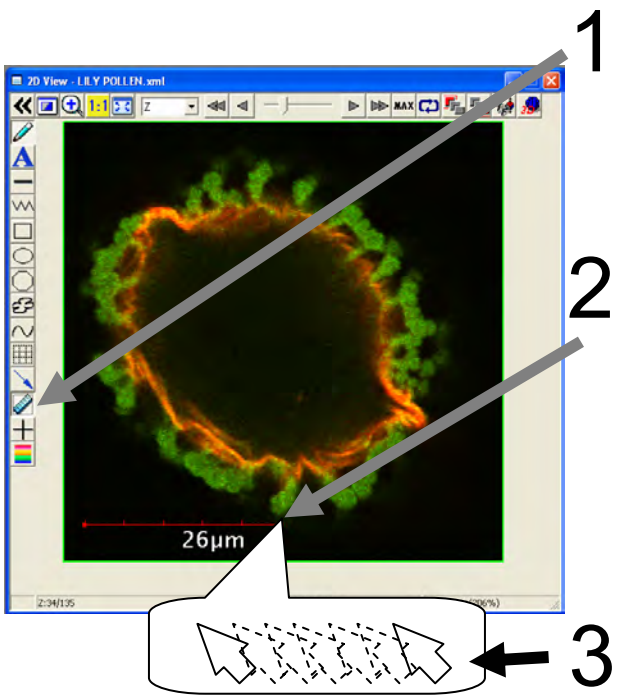
Image Analysis (Inserting the Scale Bar)

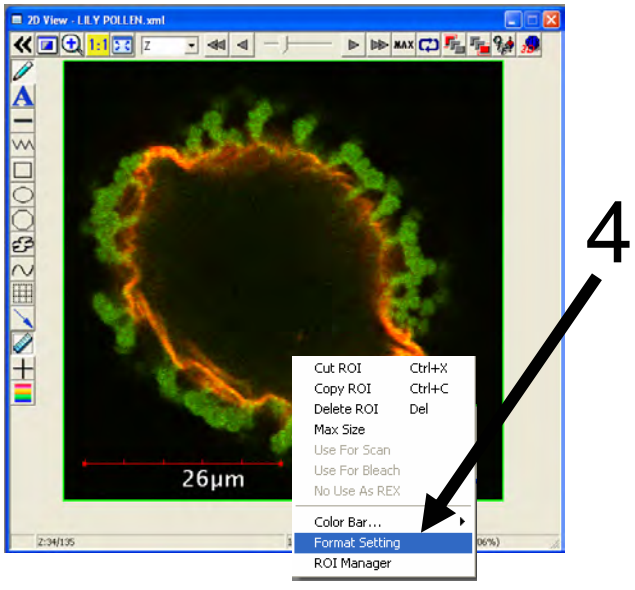


1. Click on the  button.

2. While left-clicking the image, drag and drop it at a certain point.

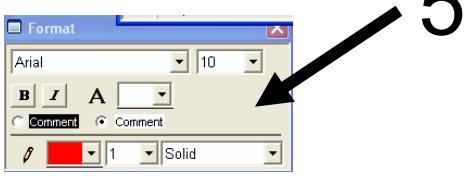
Change the size

3. While clicking the right or left handle, move the mouse from side to side.



Change the text size, color, style, etc.

4. Select Scale Bar and then right-click on Scale Bar to select Format Setting.



5. Change the setting in this window as required.

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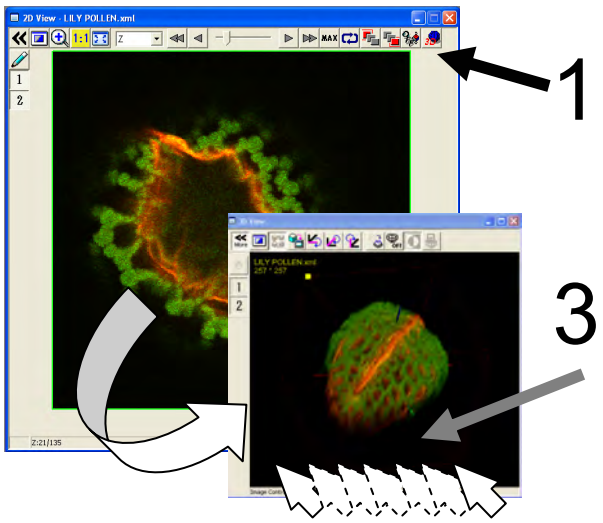
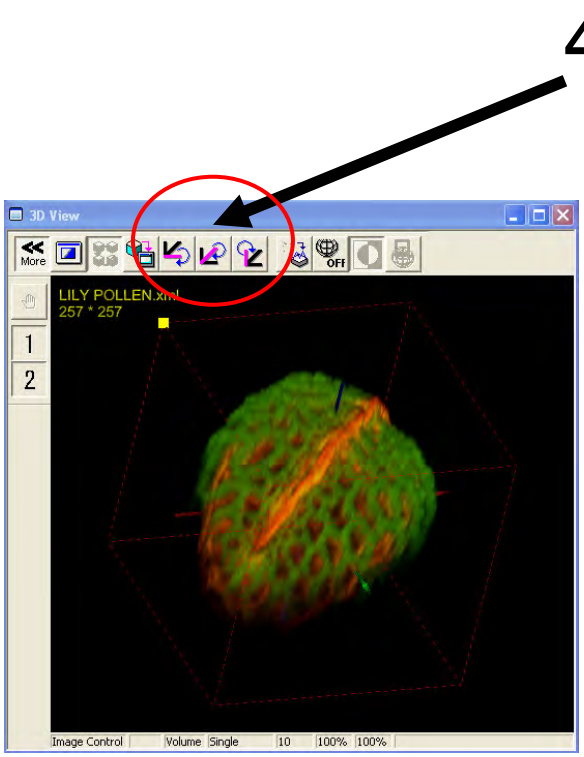
Image Analysis
(Rotating a Three-dimensional Image)



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

2. A 3D view is created.

3. Drag the mouse on the image to observe it at a certain angle.



Simple animation

4. Press and hold the  button to rotate the image around the X-axis. Press it again to stop rotation.

   Press and hold the  button to rotate the image around the Y-axis. Press it again to stop rotation.

   Press and hold the  button to rotate the image around the Z-axis. Press it again to stop rotation.

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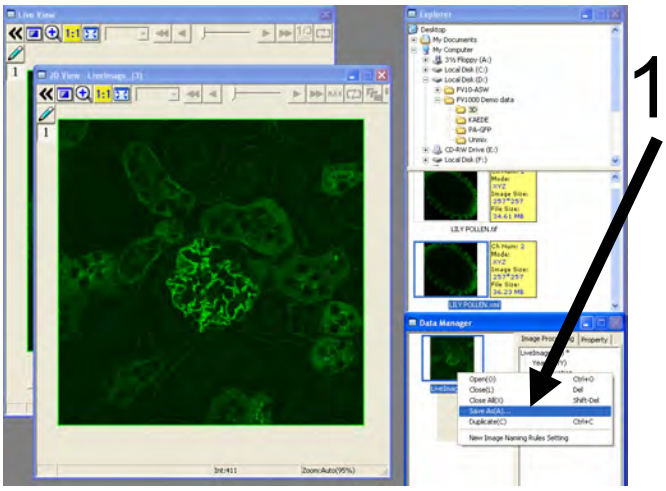
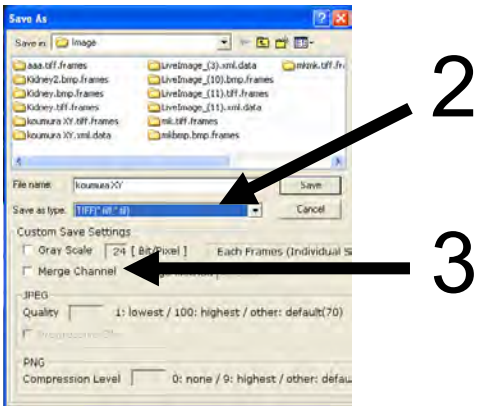
Image Analysis (Saving an Image)



Convert each channel of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to RGB Color.
4. Save the image.

* BMP and JPEG formats are also selectable.

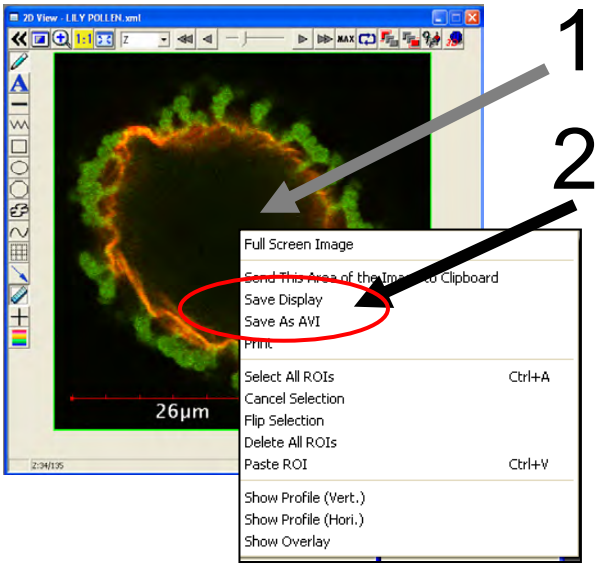


Convert a merge image of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to Merge Channel.
4. Save the image.

* BMP and JPEG formats are also selectable.

****



Convert an image with the scale bar inserted into a BMP format

1. Right-click on the image.
2. Select Save Display and save the image with a new name.

Convert an animated image into an AVI format

1. Right-click on the image.
2. Select Save as AVI and save the image with a new name.

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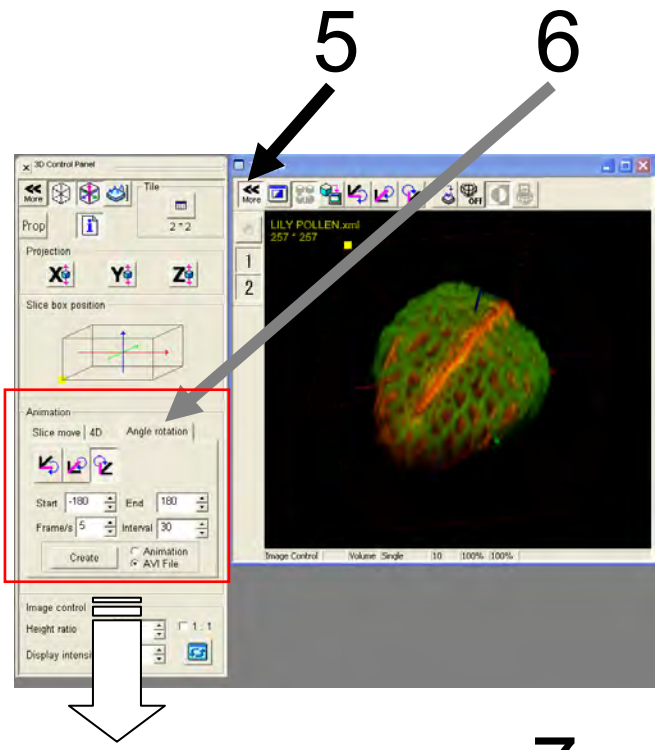
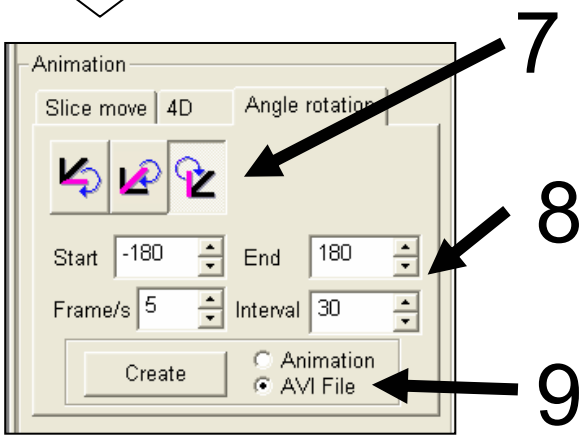
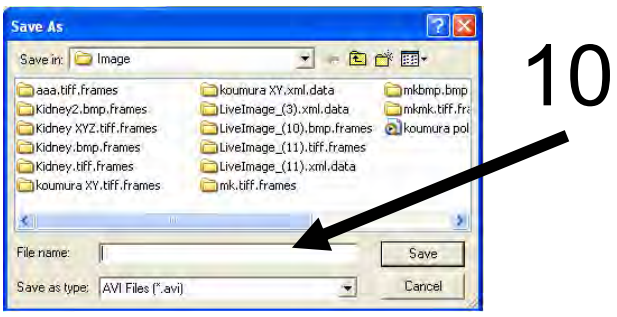
Image Analysis
(Rotating a Three-dimensional animation)

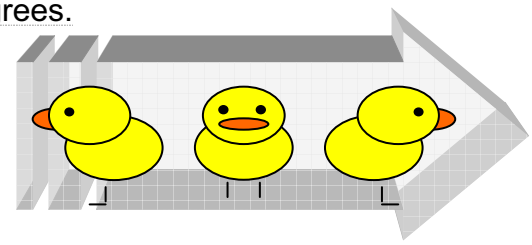






To save a rotation file as an animated image, create three-dimensional images according to the following procedure.

For example, try to rotate an image by 180 degrees.



5. Click on the 

button.

6. Click on the Angle rotation tab.

7. Select the rotation axis.

8. Enter the rotation angle.

Start =	Angle to start rotation
End =	Angle to stop rotation
Frame/s =	Rotation speed
Interval =	Degrees to be rotated at a time

9. Select AVI File and click on Create.

10. Enter a file name and click on Save.

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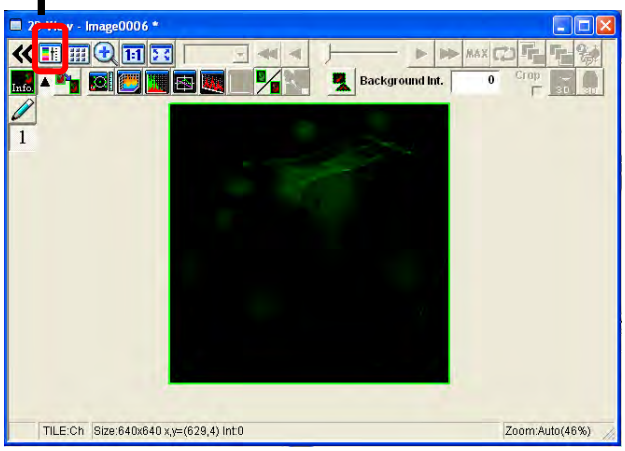
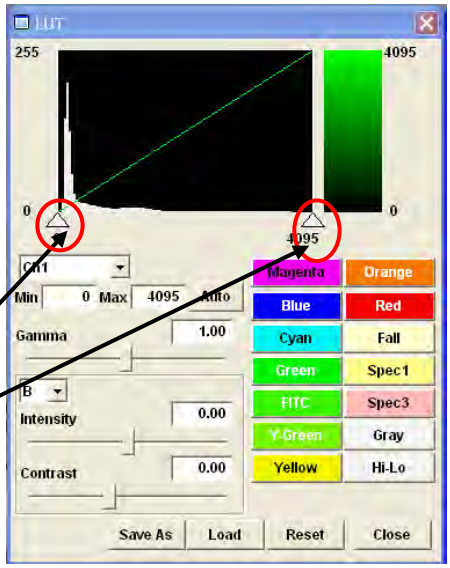
2D Image Analysis (Edit the image color and contrast)



1. Click  "LUT" and then LUT table appears below,

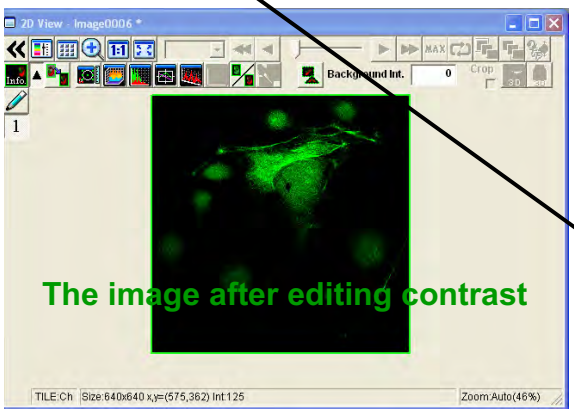


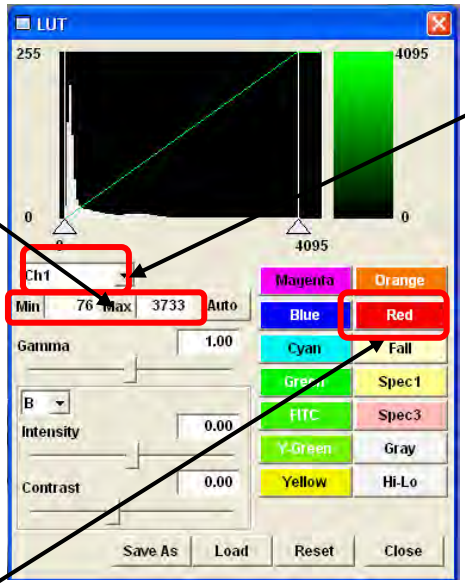
2

2.Edit contrast to drag  to left or right side, and another way to edit contrast is entering value on "Max" and "Min"(Max4095, Min0)

3. Min and Max value are changed and contrast of image is edited.

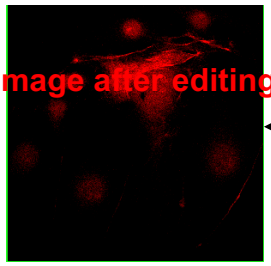
*** According to get Min value up , be able to reduce noise of the image.**





3 **Edit each Ch**

The image after editing color



4

4. To click another color, be able to Edit a color.
   Above example: Change Green to Red to click Red

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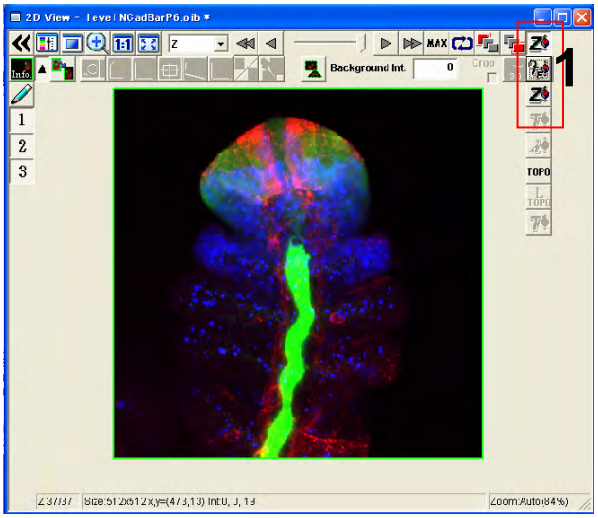
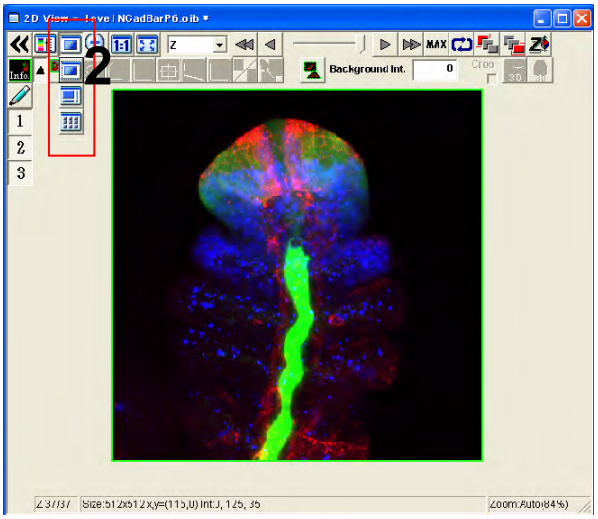
2D Image Analysis (the image of Z section)

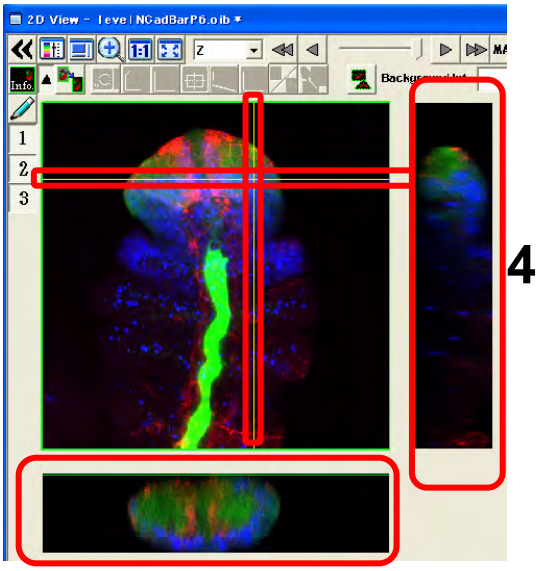


1. Click  and select  again, then Projection image is shown on 2D View after getting XYZ image.



2. Click  and select  .

3. The images of Z section is shown on X axis and Y axis.
   According to Move to left or right side on X axis and to move to ups and down on Y axis, be able to show image of Z section each position.



4. The image of Z section on Y axis.

5. The image of Z section on X axis.

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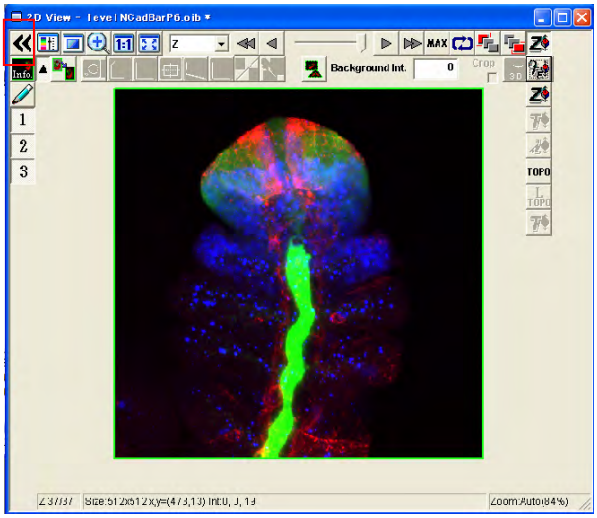
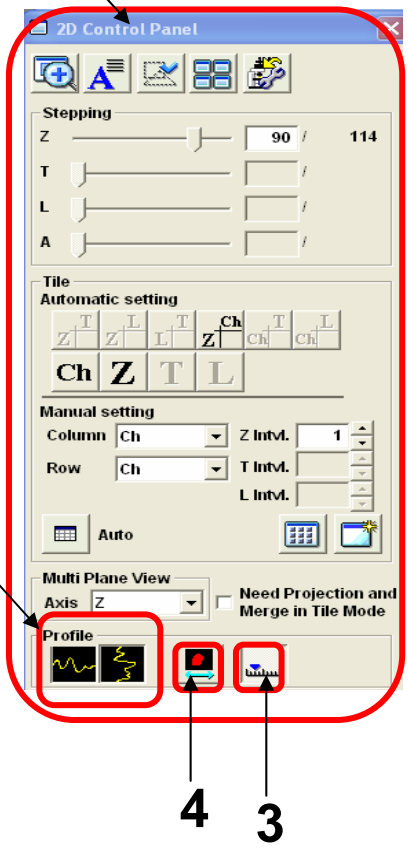
2D Image Analysis (Intensity Profile of each Z sections)

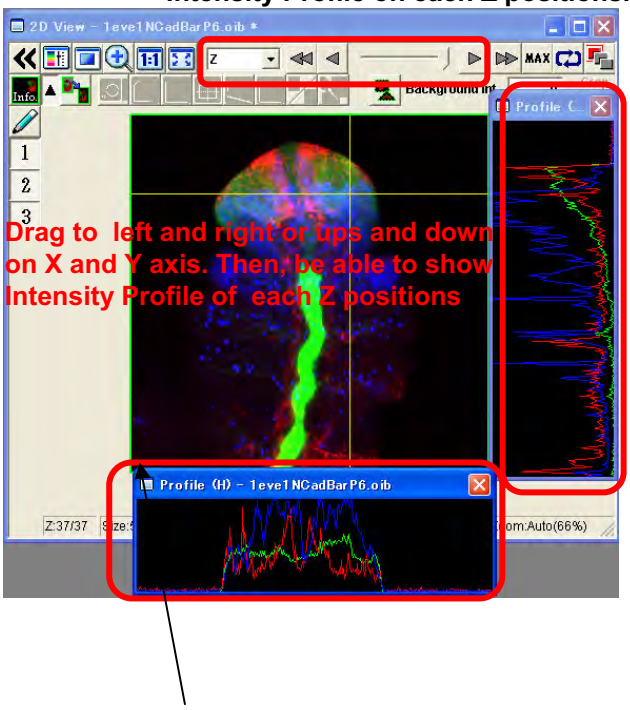


1. Click  and then 2D Control Panel is shown below,



2. Click  "Profile" and then Intensity Profile of each Z sections is shown on the X and Y axis.

To move to Z position ,be able to show Intensity Profile on each Z positions.



3. Click  to show Scale on Intensity Profile

4. According to click  , be able to show as equal scale of Profile window as 2Dimage.

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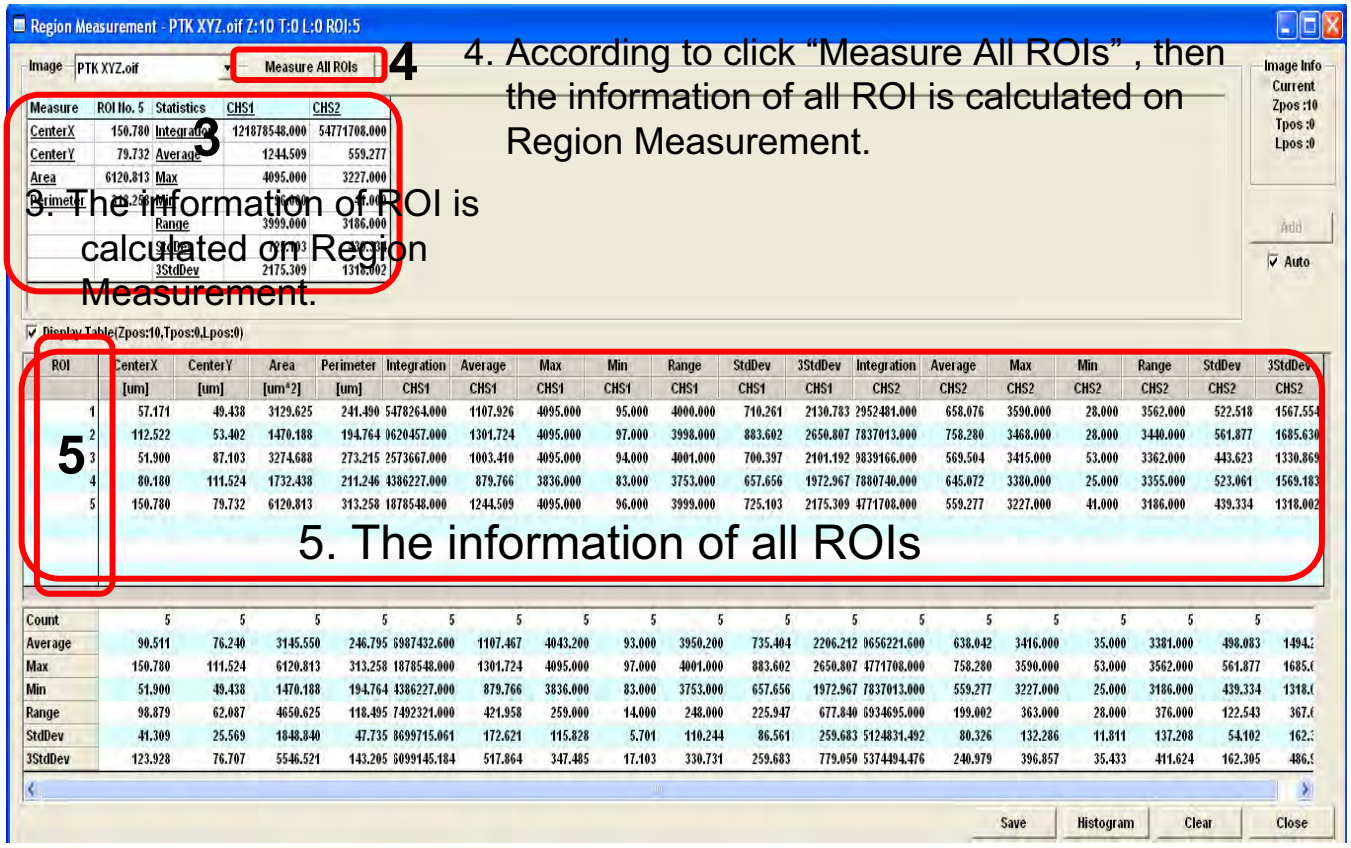
2D Image Analysis (Measure)

1. Enclose interesting regions by ROI

   Line on interesting positions by ROI —

2. Click  “measure”.



4. According to click “Measure All ROIs” , then the information of all ROI is calculated on Region Measurement.

3. The information of ROI is calculated on Region Measurement.

5. The information of all ROIs

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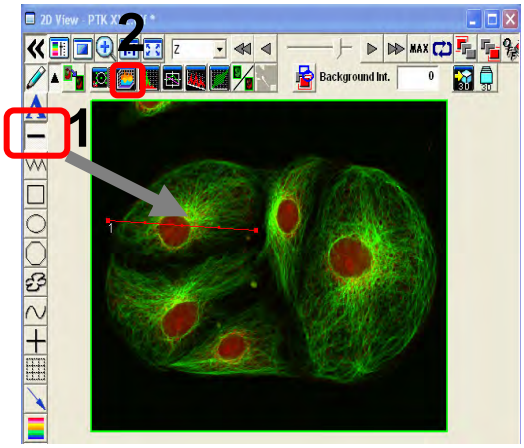
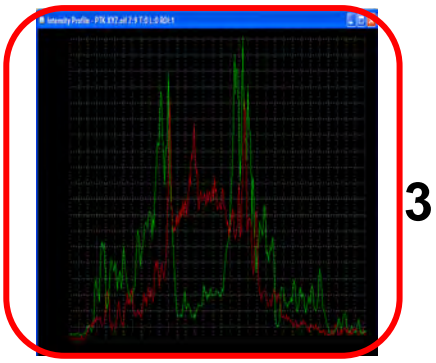
2D Image Analysis (Line Intensity Profile on the 2D image)



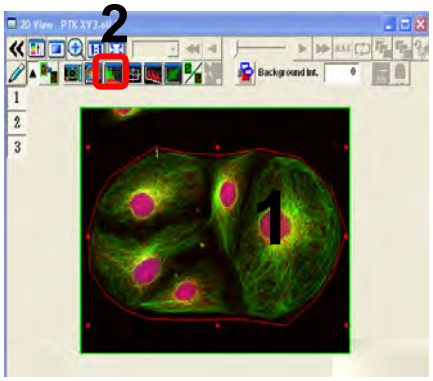


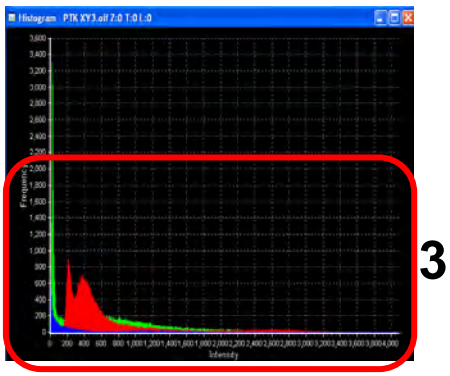
1. Line on the 2D image by ROI 

2. Click  “Intensity Profile”

3. “Intensity Profile” on the line is shown as intensity graph .

2D Image Analysis (Histogram)





1. Enclose interesting regions by ROI.

2. Click  **“Histogram”**

3. “Histogram” window is shown as a graph, frequency of intensity of each pixels is plotted on the area enclosed by ROI.

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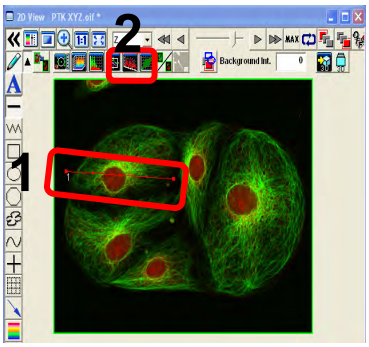
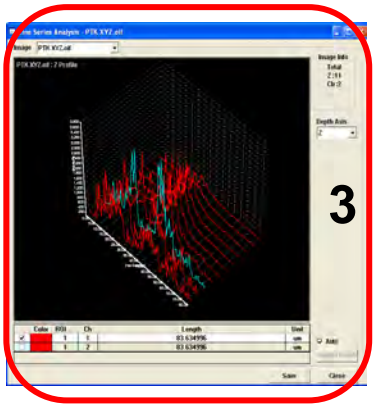
2D Image Analysis (Line Series Analysis)



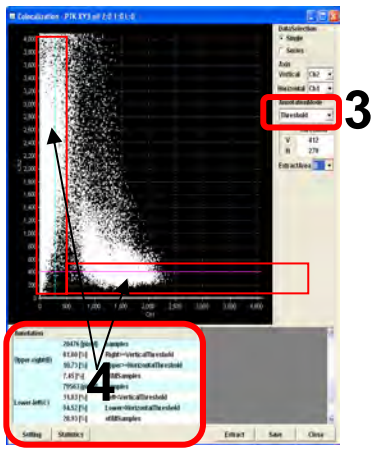


1. Line on the 2D image.

2. Click  “Line Series Analysis”

3. Intensity of Z position/ time on the line is shown as a graph .

2D Image Analysis (Co-localization)



1. Enclose an interesting regions by ROI.
2. Click 
3. Select 

Threshold from Annotation Mode.
4. According to move Thresholds of X,Y axis to right and left ,ups and down (Enclose red color X,Y axis), Co-localization result between 2ch is changed .

   Information of Co-localization is listed under the scatter plot.

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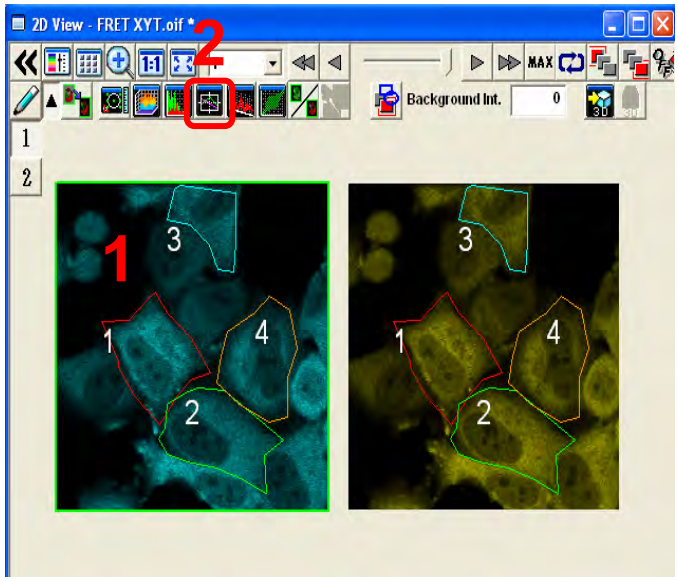
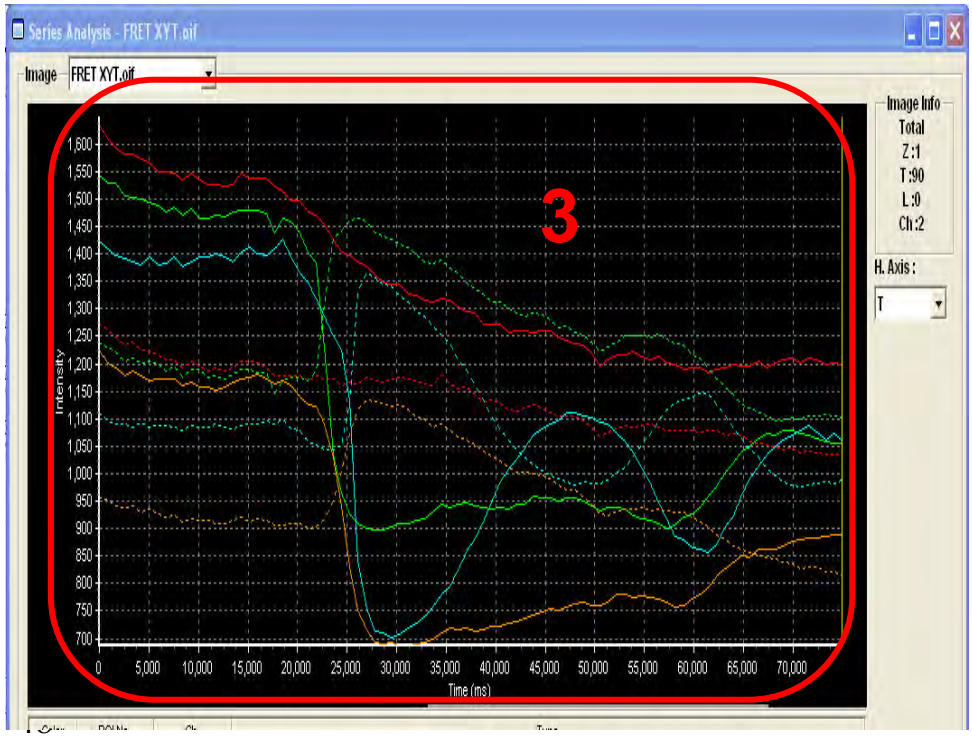
2D image Analysis (Series Analysis TimeLapse)



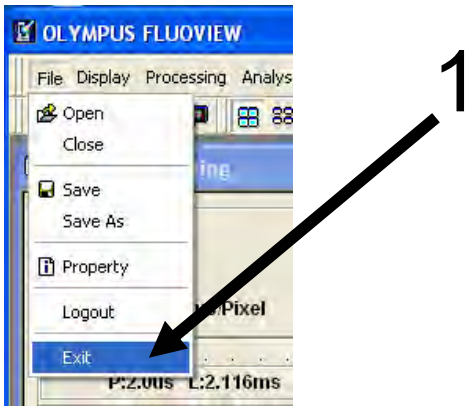
1. Enclose interesting regions by ROI

2. Click  "Series Analysis"

3. "**Series Analysis**" graph is shown below, Y axis shows intensity, X axis shows time and then be able to see time series reaction each ROIs.



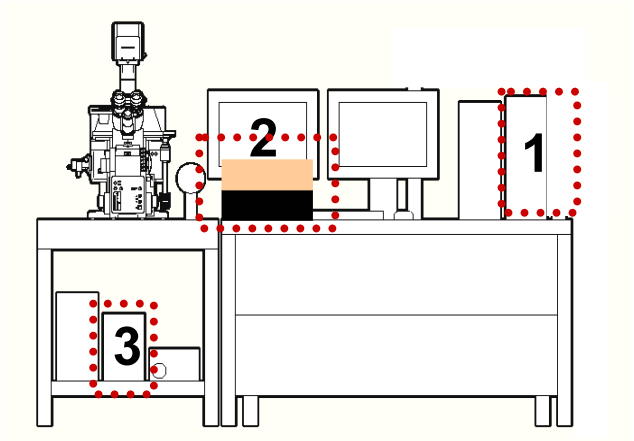
Closing the System



1. Exit the FV10-ASW software by selecting File/Exit.

2. Exit the Windows.

(1) Select Start/Shut Down.
(2) On the Shut Down Window, select Shut Down and click on OK.



3. Turn the laser OFF.
(Turn the key switch to the OFF position.)

3-1. LD559nm OFF

3-2. Multi Ar (458 nm, 488 nm, 514 nm) OFF

3-3. HeNe (G) (543 nm) OFF

4. Turn the mercury burner power OFF.

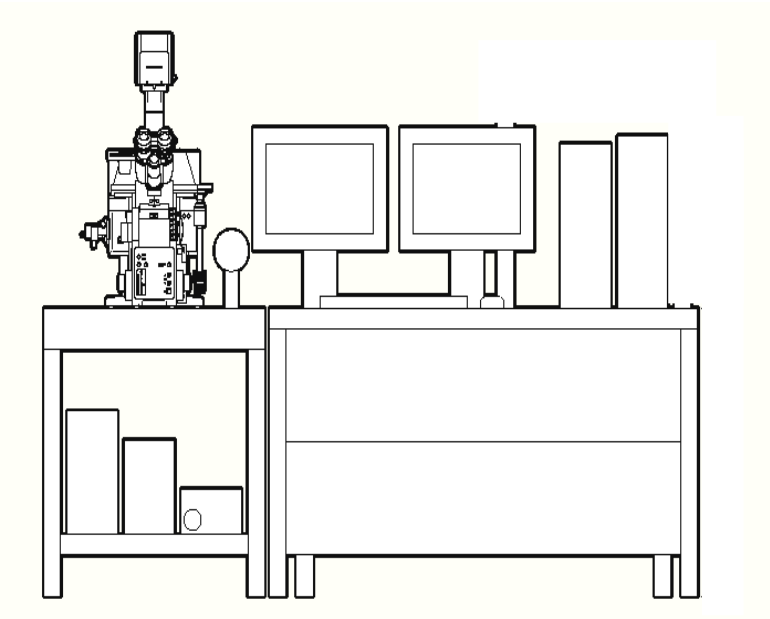
49

Laser Conforcal Scanning Microscope

FV1000D Filter Type

(invertedMicroscpeIX81)

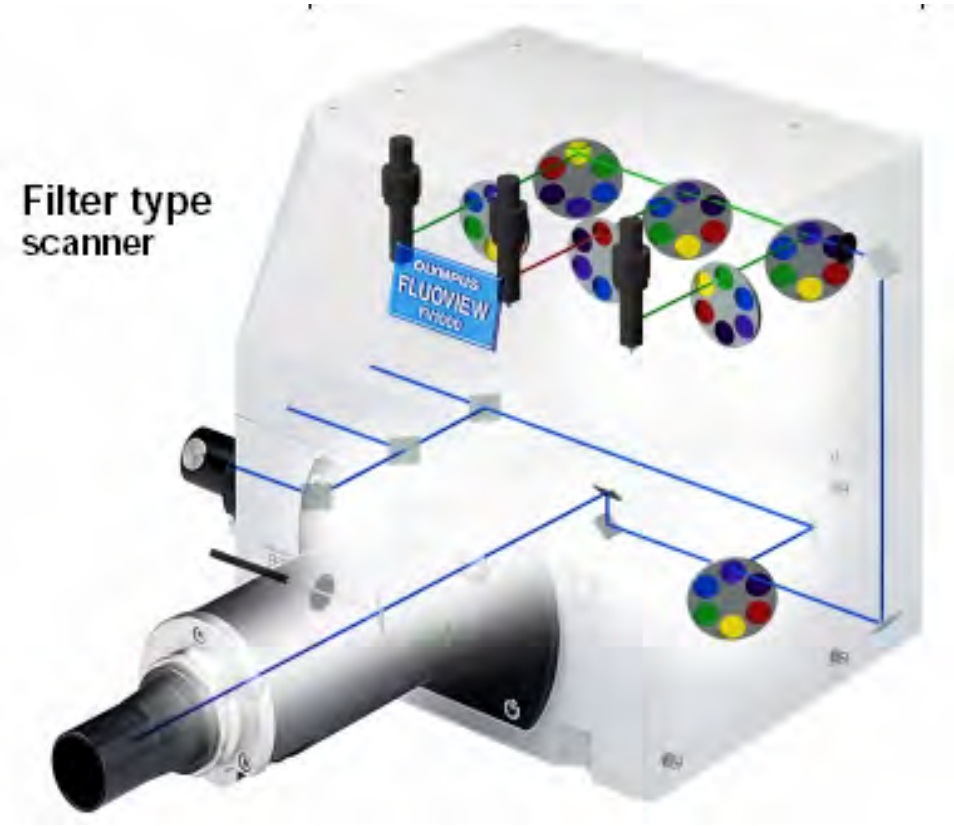
Operation Manual



1

Contents

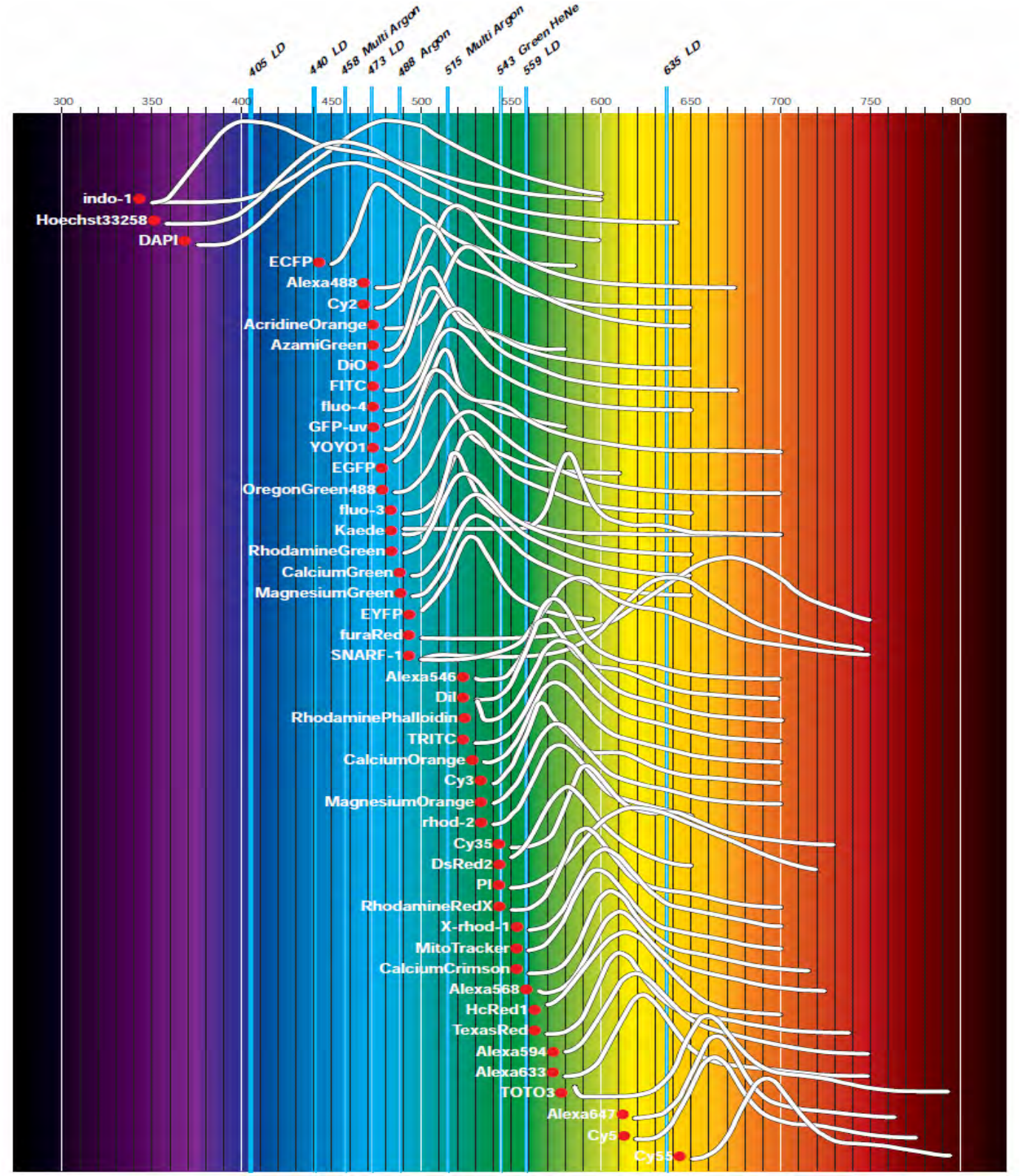
Filter Type Main Scanner



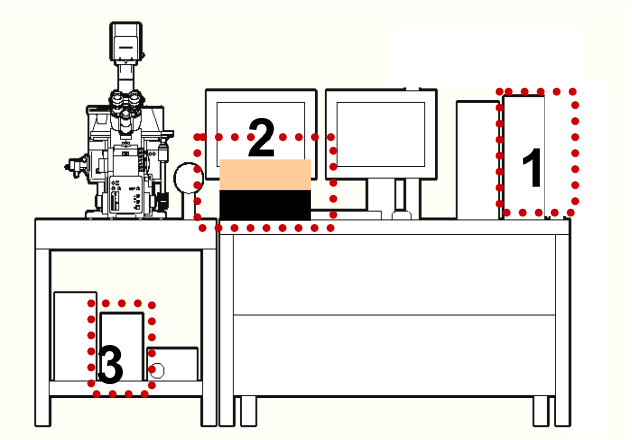
3

Dye List (FV1000D Lasers are available below)

LD405nm LD440nm LD473nm LD559nm LD635nm
Ar458nm Ar488nm Ar515nm
HeNe (G) 543nm



System Preparation



1. Turn the computer ON.
   [In case of equipped concentrated power supply, power on it first]

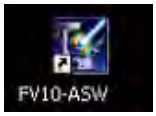
2. Turn the laser ON
   (Turning the key switch)
   2-1. LD559nm ON
   2-2. Multi Ar 458nm 488nm 515nm
   2-2. HeNe(G) (643nm) ON

3. Turn the mercury burner ON for Fluorescence observation.

Wait for a moment until the software is started

4. Log on Windows

   Enter Password ,Customer name is below
   User name: Administrator
   Password : fluoview

5. 

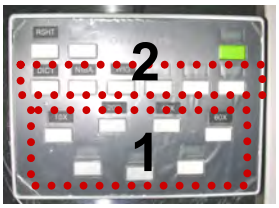
Double click this icon to log on to ASW

   User name: Administrator
   Password : Administrator

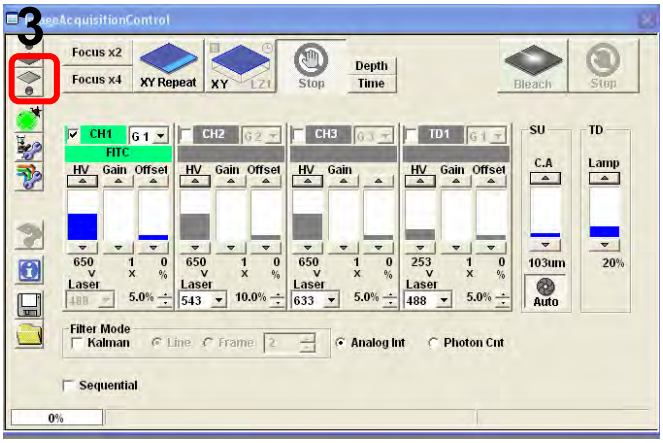
5

Visual Observation under the Microscope

■■ Observation of Fluorescence Image ■■



Hand switch



1. Select an objective lens
   by using the hand switch

2. Select florescent filter cube

MEMO
Fluorescence filter

NIBA: Blue Excitation / Green Fluorescence
(Ex.:FITC,EGFP)
WIG: Green Excitation / Red Fluorescence
(Ex.:Rhodamine, DsRed)

3.  Click the button
on the Fluoview software

4. Focus to the specimen

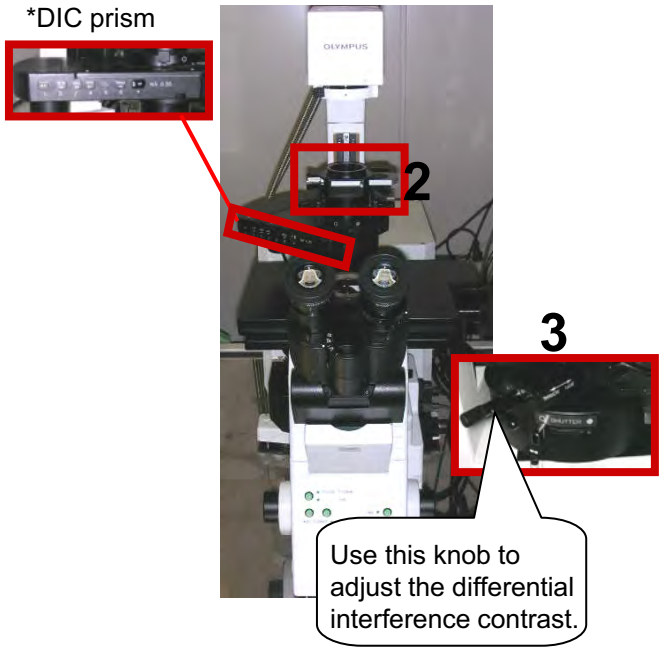
6

Visual Observation under the Microscope

■■ Observation of Differential Interference Contrast Images ■■

Hand switch

*DIC prism

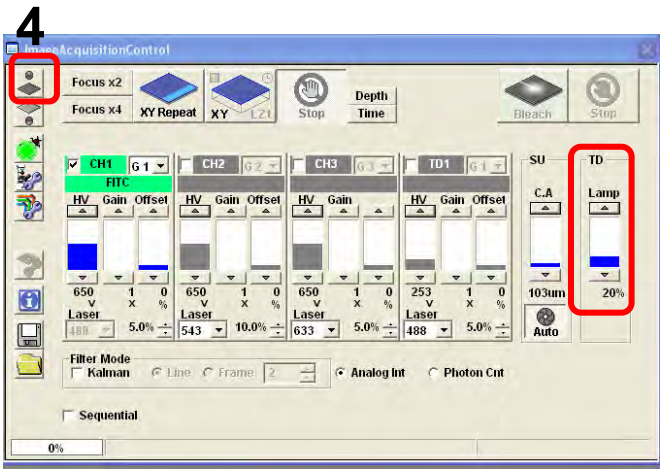


1. Select the Objective Lens

2. Insert the Polarizing Plate in the Light Pass

3. Insert the DIC prism slider in the light pass

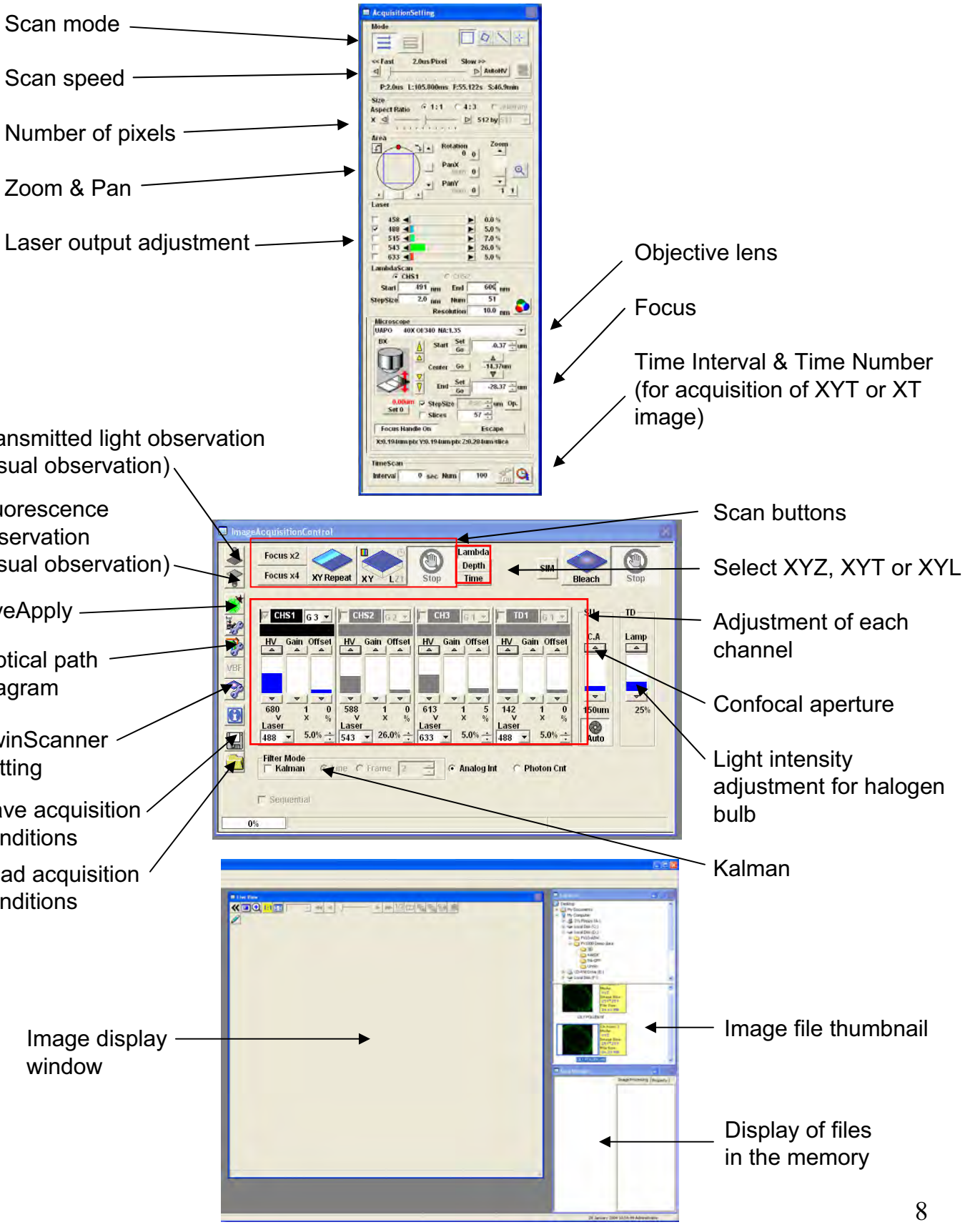
4.  Click the button on Fluoview software



5. Focus to the specimen

7

Overview of Operation Panel for Image Acquisition

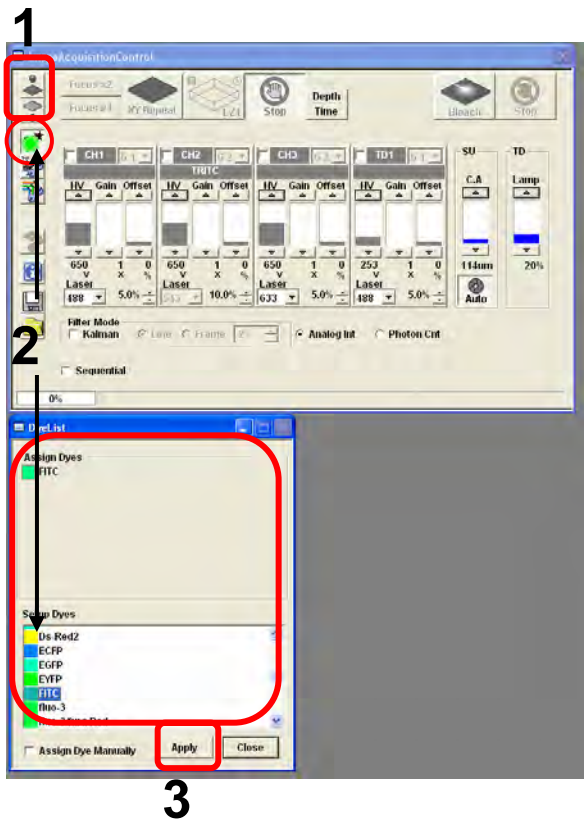


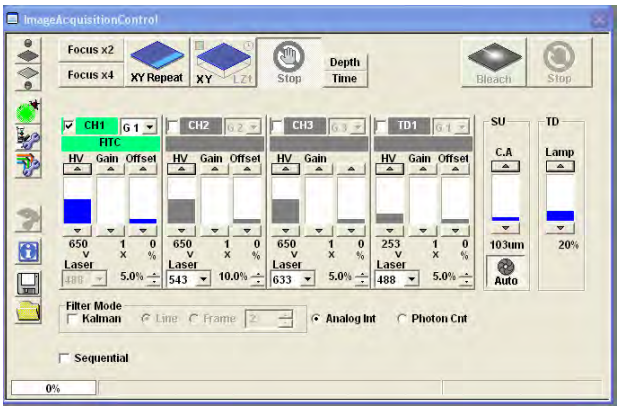
8

Image Acquisition
(Single Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Single stain of green fluorescence dye (FITC)





1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

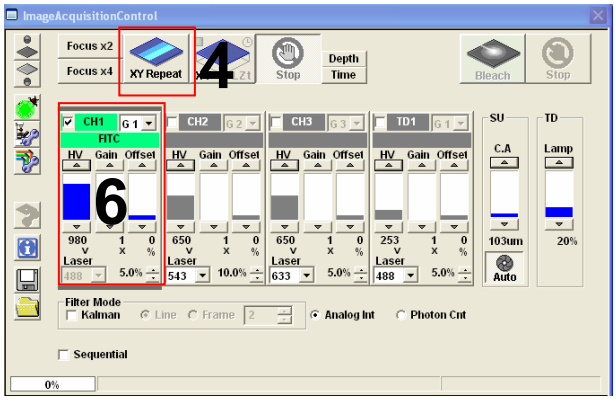
   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

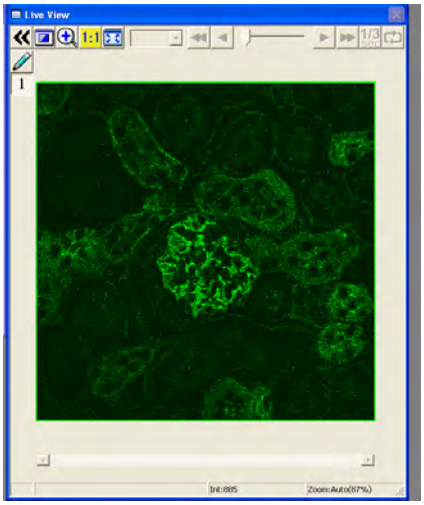
3. Click on the Apply button.

   (The DyeList panel can be closed by using the Close button.)

9

Image Acquisition
(Single Stain on XY Image)





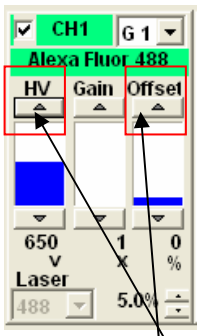
7

4.Press XY Repeat button click to get image

: Continuous scan mode

5. Focus to the specimen

6. Adjust the green (FITC) image.



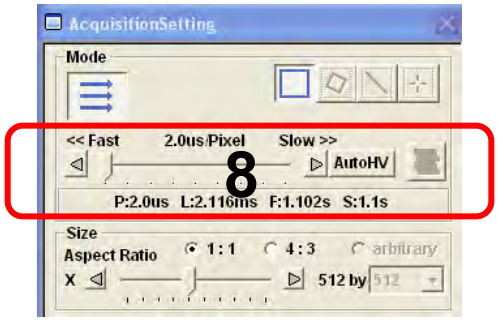
· Adjust sensitivity of **HV** and reduce noise by **offset**

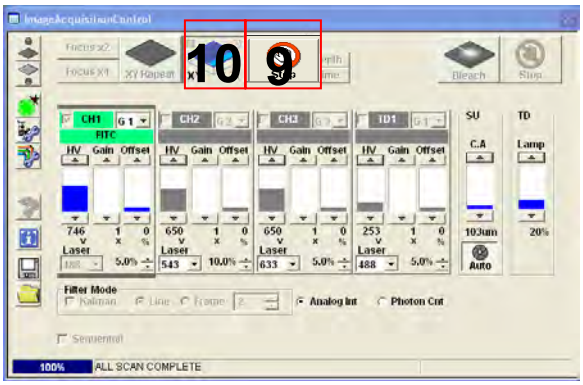
7. Press keyboard **Ctrl + H key**
Optimized PMT adjustment brightness intensity 2 color between white and black,
Maximum intensity is 4095 (12bit)
if intensity is over4095, color is changed to red (saturation)

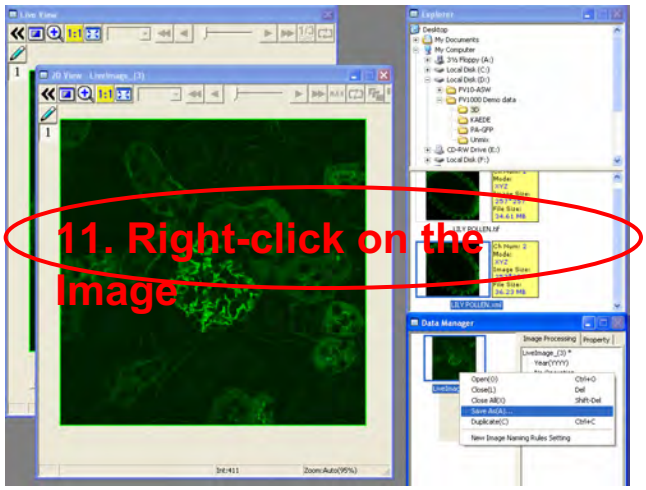
* Basically, **Gain value is 1**

10

Image Acquisition
(Single Stain on XY Image)







8. Select AutoHV and then select ScanSpeed.
   *As the scan speed becomes slower, noise
   can be removed while maintaining the current brightness.

9. 

Press the Stop button to stop scanning.

10. 

Click on XY, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

11. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type " oib" or "oif" file format specifically for the FV10-ASW software.)
Save the image as TIFF, BMP, JPEG format Select "Export " and chose the format TIFF, BMP, JPEG.

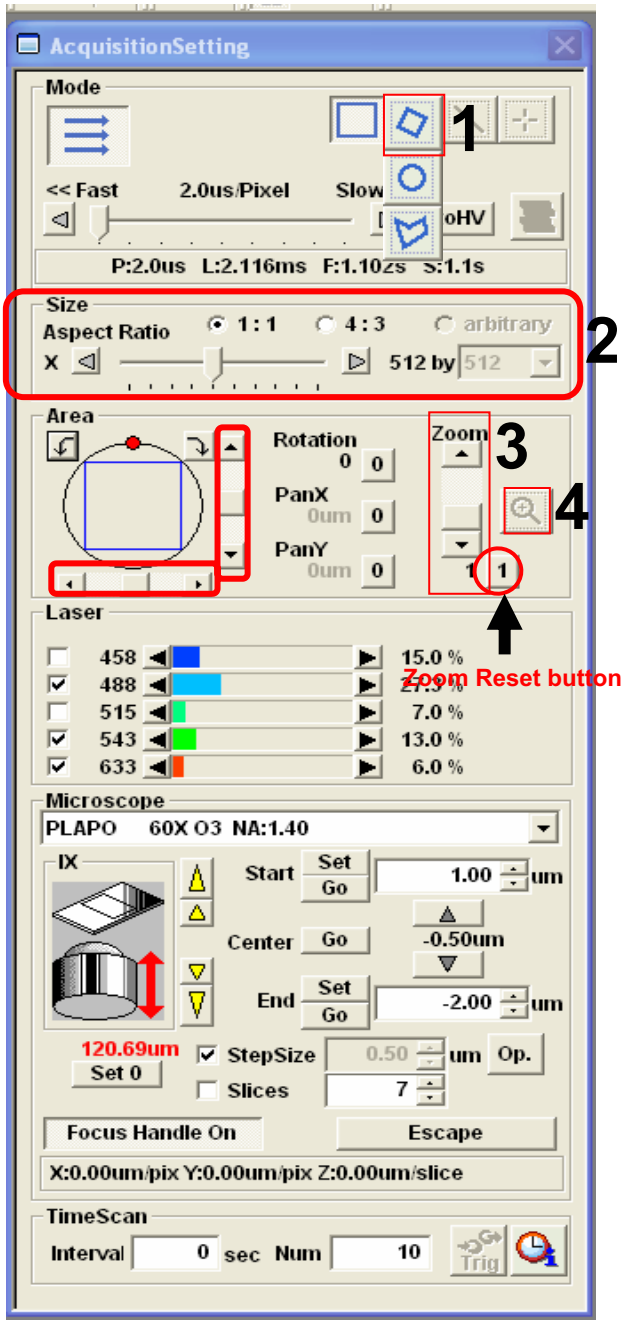
■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

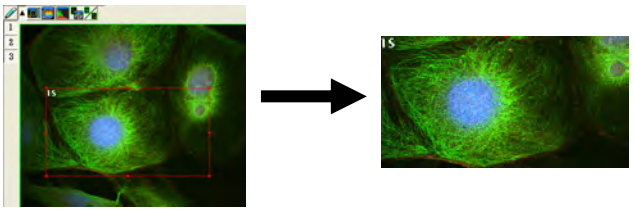
OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

11

Complement of adjusting the image



1. Click “Clip scan” button , and enclose an interesting region’s image on the whole image.

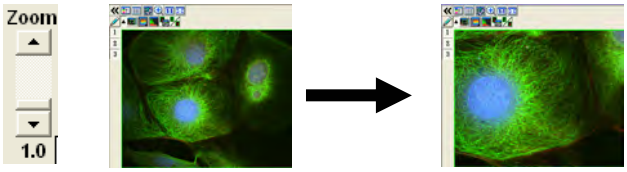


2. pixel setting

* The standard pixel is 512 x 512

3. Zoom Setting

Press “XY Repeat” to scan and set zoom value.

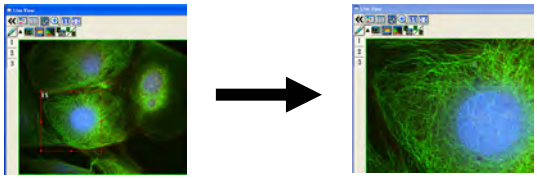


Above image is zoomed From 1 x to 2

*** Scan speed and pixel resolution remain even zoom value is changed**

4. Click  Zoom scan, and be able to enclose an interesting region’s on the whole image

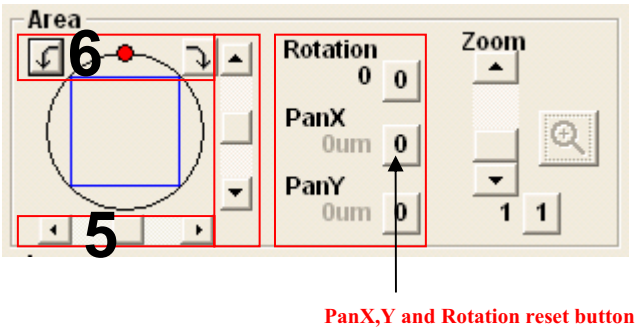
Press XYRepeat to scan after enclosing the region



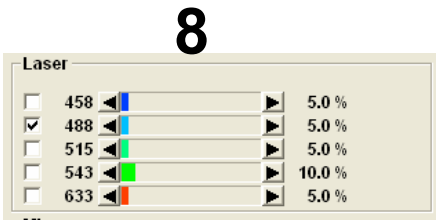
*** Scan speed and pixel resolution remain even zoom value is changed**

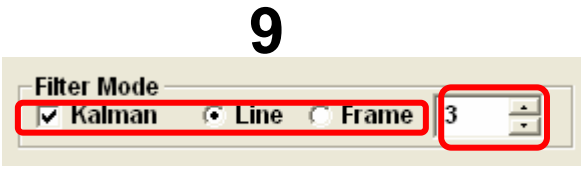
12

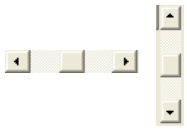
Complement of adjusting the image



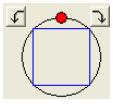
PanX,Y and Rotation reset button





5. Pan X,Y 

   Be able to move the field of view to set Pan X,Y without stage action

6. Rotation 

   Be able to rotate the whole image.

7. 

   Click "Auto" button to acquire Optimized Conforcal aperture
   Conforcal aperture · · · change confocal aperture to larger diameter for dim fluorescence image then, be able to get the more bright image. But Z axis resolution gets worse.

8. Laser Intensity · · · More Laser intensity is increase , more bright image is .

   *** More increase laser intensity is , more discoloration image is .**

9. Kalman accumulation · · · Image acquisition is repeated to the specified number of times to provide an averaged image. Consequently, noise is averaged and roughness on the whole image is reduced.

   Advantage: The speed of each scan is fast.

   Disadvantage: Some blur occurs due to averaging of images.

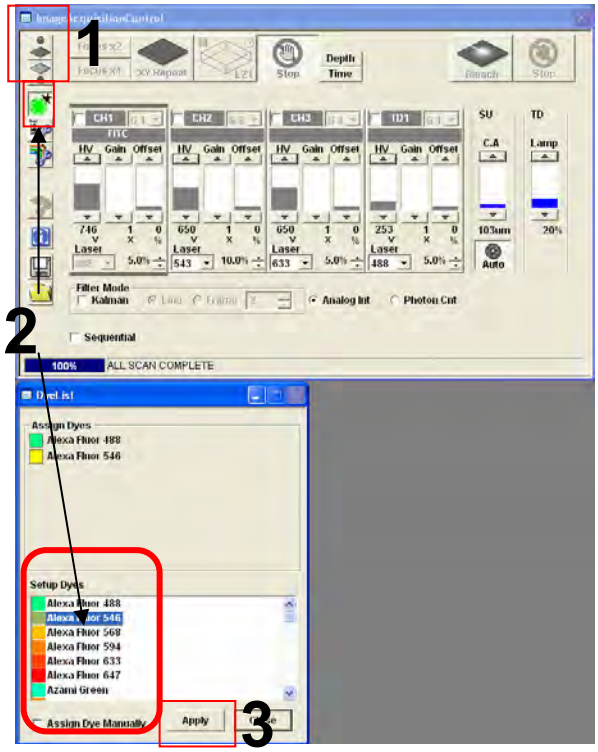
13

Image Acquisition
(Double Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Simultaneous scan



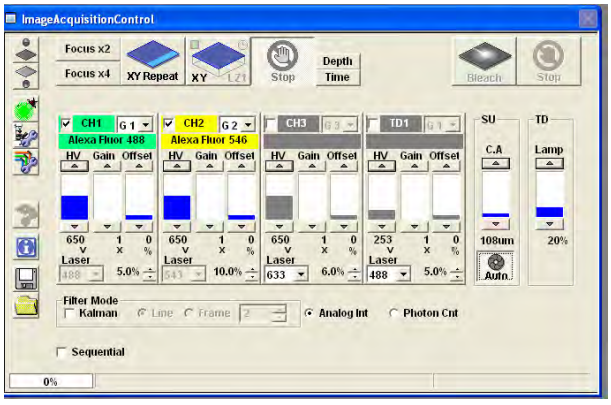
1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click “Apply” button.

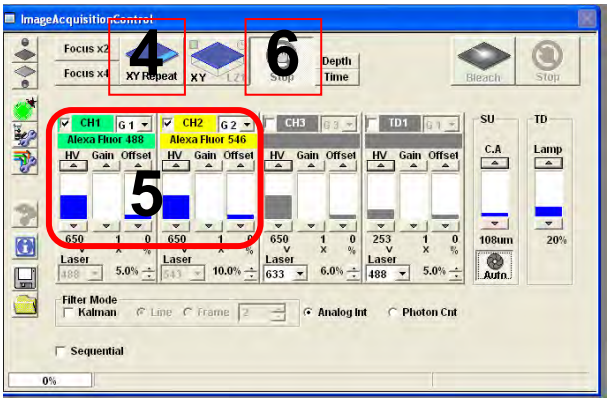
   (The DyeList panel can be closed by using the Close button.)

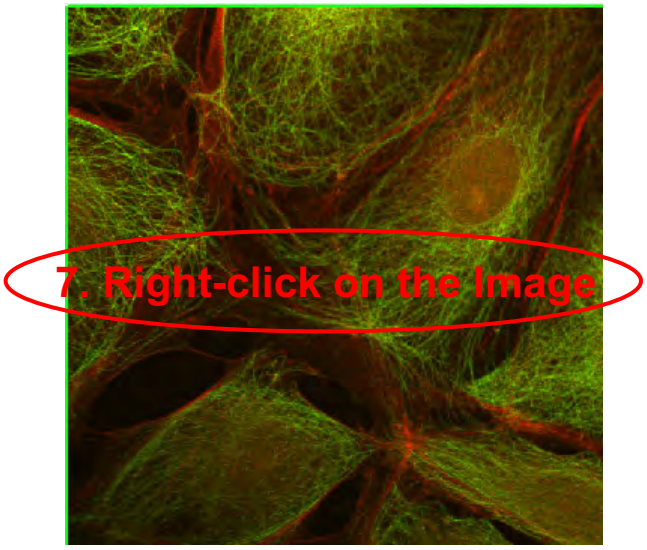


Display after DyeApply is carried out

14

Image Acquisition
(Double Stain on XY Image)





4. Press the XY Repeat button to start scanning.

5. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.
   (The image adjustment is outlined below. For more information, refer to Appendix 1.)

6. Press the Stop button to stop scanning and press XY repeat to acquire the image. (Refer to ■Memo■.)

■Memo■
Scan buttons

: Continuous scan

: Stop scan

: Rough scan (Line skipped)

7. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
   (Save as Type “oib” or “oif “file format specifically for the FV10-ASW software.)

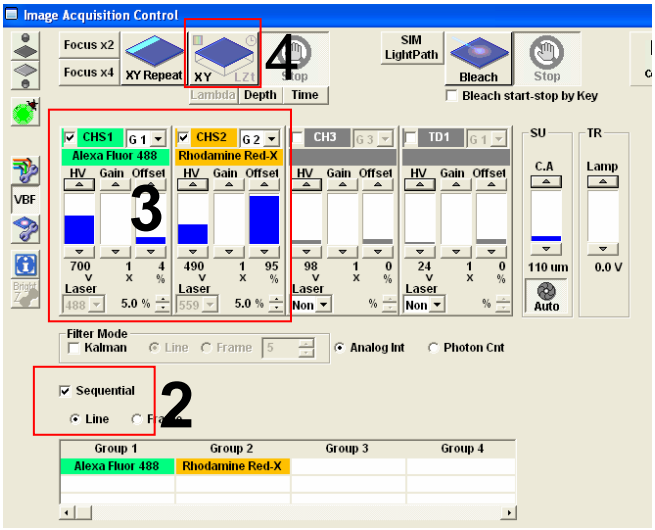
15

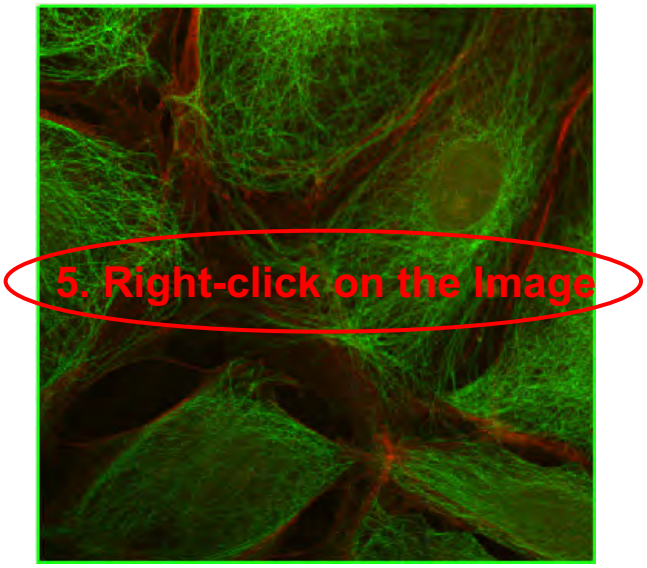
Image Acquisition
(Double Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Sequential scan (Line Sequential is introduced here.)





1. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

2. Check Sequential and select Line.

3. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.

4. Press the XY button to acquire an image.

5. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.) The image is acquired.

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

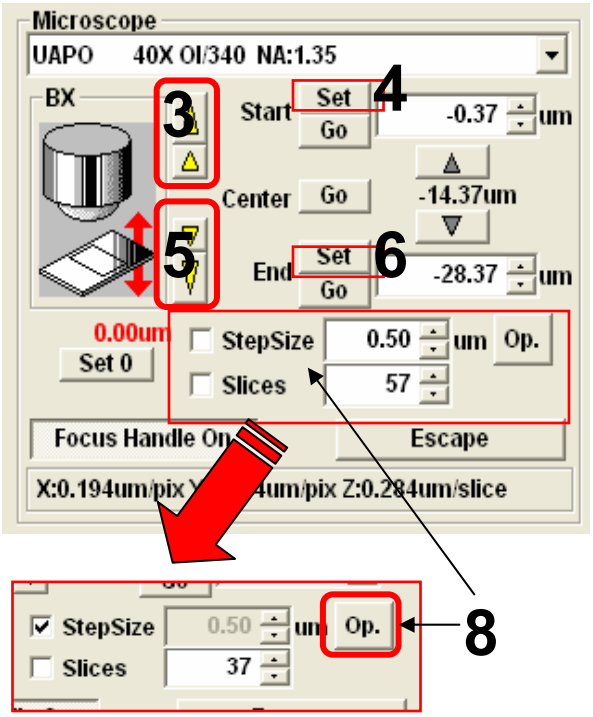
16

Image Acquisition
(Double Stain on XYZ Image)

■■ Acquisition of 3D images (XYZ)
(fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (FITC)
and red fluorescence dye (Rhodamine)

This is the procedure to acquire images
through Line Sequential scanning.



■Memo■

and buttons

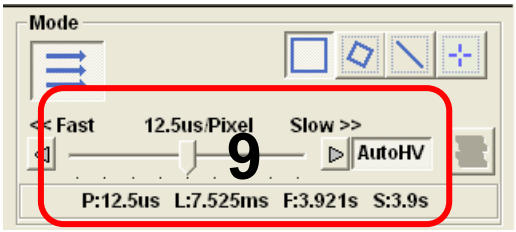
: Moves 1.0μm with a single click.

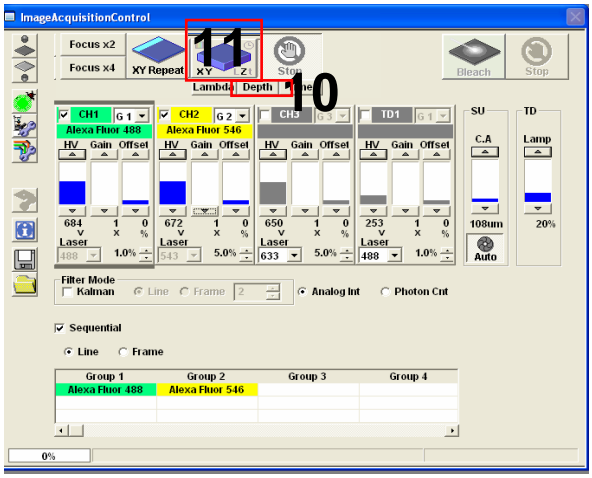
: Moves 0.1μm with a single click.

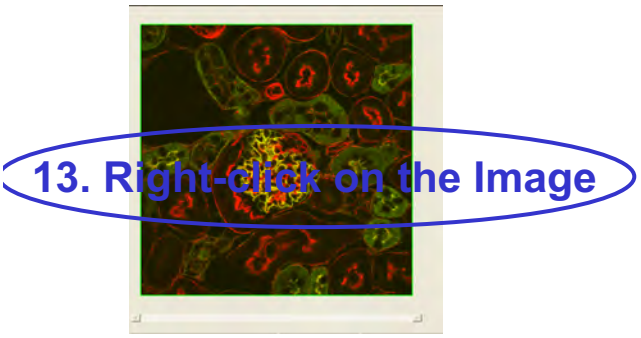
1. Take steps 1 to 7 described on pages 13 and 14.
2. Press the XY Repeat button to start scanning.
3. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
4. When the sample upper limit is displayed on the image, accept it using the Set button.
5. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
6. When the sample lower limit is displayed on the image, accept it using the Set button.
7. Press the Stop button to stop scanning.
8. Enter StepSize, Slice (the recommended value can be referred to by using the Op button), and check the check box

17

Image Acquisition
(Double Stain on XYZ Image)







9. Select AutoHV and then select ScanSpeed.

10. Select Depth.

11. Press the XYZ button to acquire an image.

12. Click on "SeriesDone", and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

13. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

18

Image Acquisition
(Four Stain on XY Image)

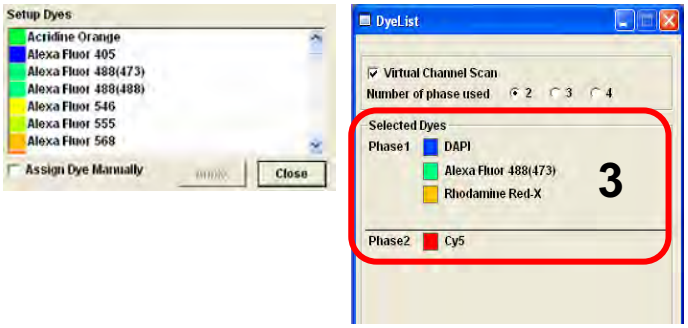
■■ Acquisition of 4 stain images (XY)
(fluorescence image only) ■■

Sample: Four stain of Blue fluorescence dye (DAPI) ,green fluorescence dye (Alexa488) and red fluorescence dye (Rhodamine), far-red fluorescence dye (Cy5)

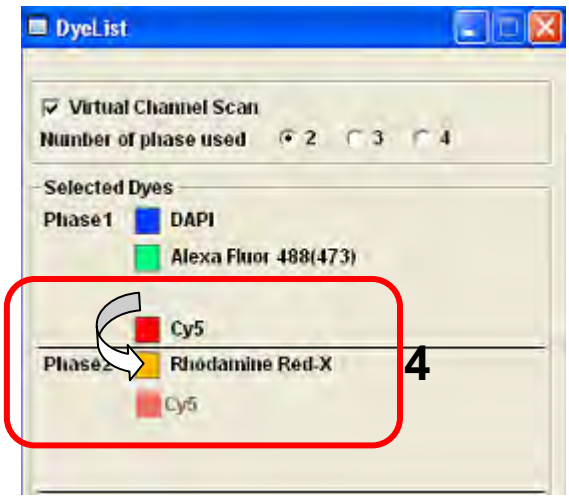
This is the procedure to acquire images
through Virtual Channel scan

1. Virtual Channel Scan Select Virtual channel Scan on the DyeList, and then "**Virtual Channel Controller"** is automatically turned on.

2. Select a number of Virtual Channel from "**Number of phase used**".



3. Select 4dyes from DyeList 4th dye is registered in "**the Phase 2**".



* **RodaminRed is able to be registered on "Phase2" to drag .**

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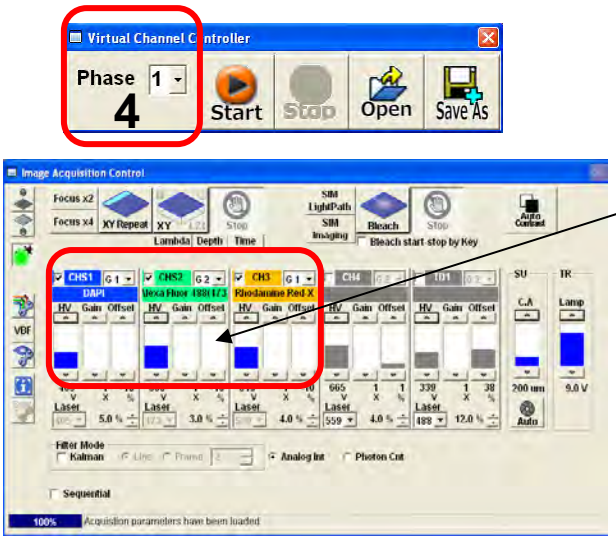
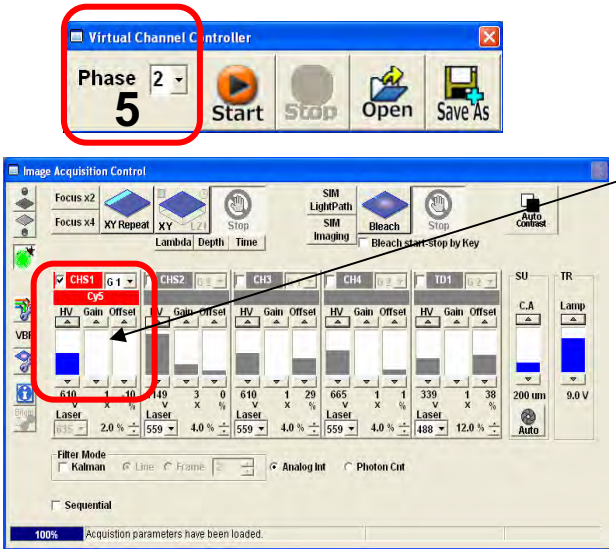
Image Acquisition
(Four Stain on XY Image)



4. Select “Phase1”, “DAPI”, Alexa488, RhodaminRed are registered on ImageAquisitionControl.

*** Slit and Filter, DM are automatically set for “DAPI”, “Alexa488” or “PhodaminRed”**

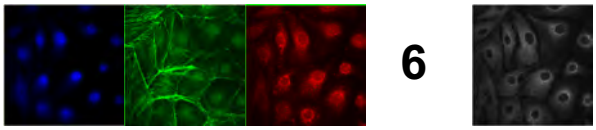


5. Select “Phase2”, Cy5 is registered on ImageAquisitionControl

*** Slit and Filter, DM are automatically set for “Cy5”**

Adjust the image at each phases

“Phase1” **“Phase2”**



6

6. Adjust the image to click 

“XY Repeat” at each phases

* If acquire XYZ image, be able to decide upper limit and bottom limit , slices, step size of Z axis at both phases.

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Image Acquisition
(Four Stain on XY Image)

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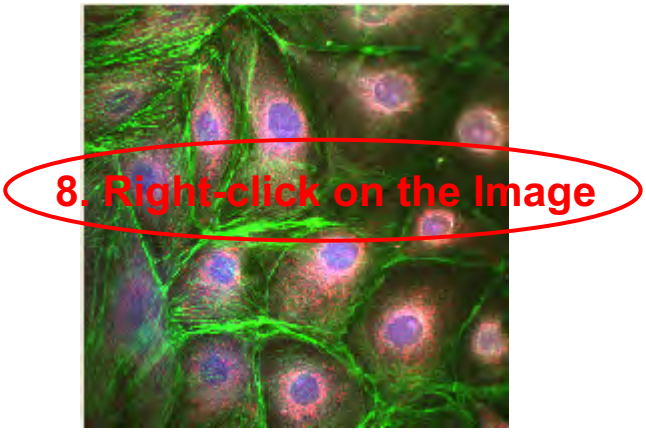
7. Click 

on Virtual Channel Controller to acquire the image.

* Be able to start at each Phase.



8. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

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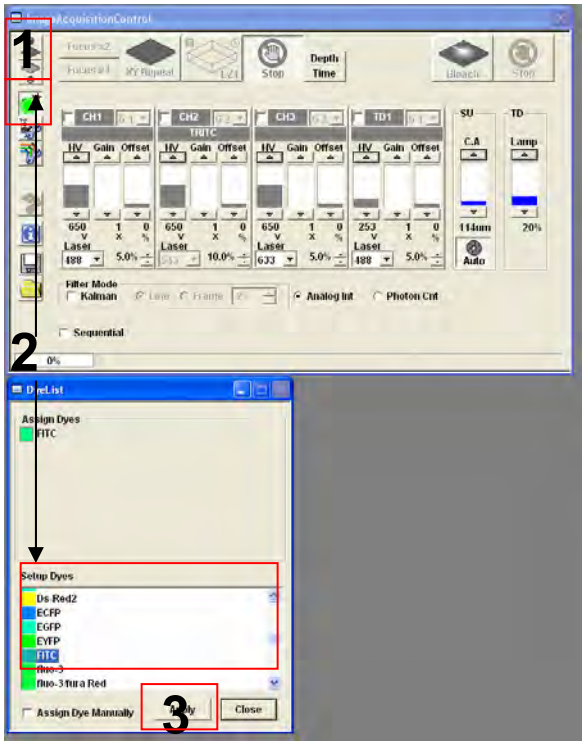
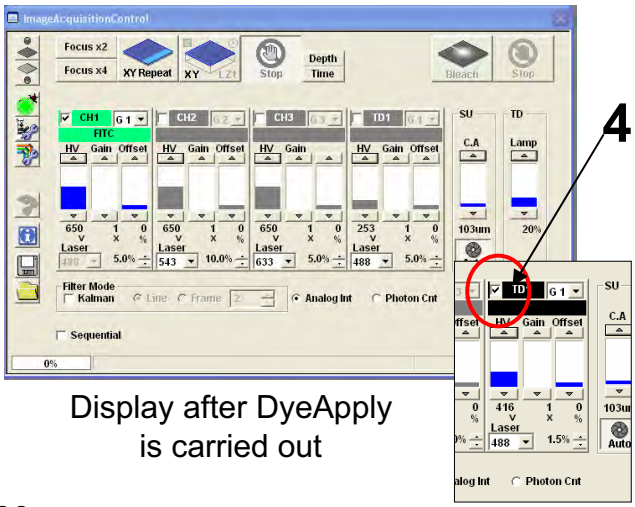
Image Acquisition
(Single Stain + DIC on XY Image)

■■ Acquisition of a single image (XY plane)
(fluorescence image and differential interference contrast image) ■■

Sample: Green fluorescence dye (FITC) and differential interference contrast image





Display after DyeApply is carried out

1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click on the Apply button.

   (The DyeList panel can be closed by using the Close button.)

4. Check TD1.

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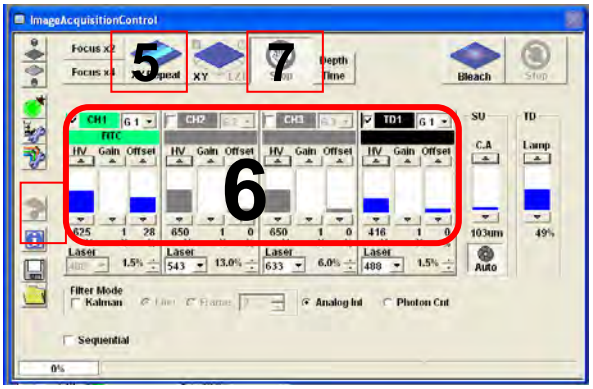
Image Acquisition
(Single Stain + DIC on XY Image)



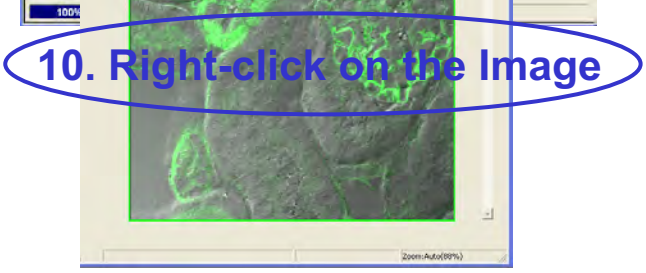
5. Press the "**XY Repeat**" button to start scanning.

6. Adjust the green (FITC) image and the differential interference contrast image.

7. Press the "**Stop button**" to stop scanning.

8. Press the "**XY button**" to acquire an image.

9. Click on "SeriesDone", and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.



10. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type " oib" or "oif" file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

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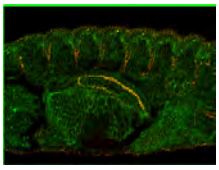
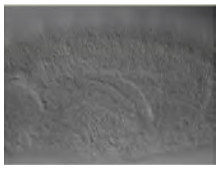
Merge the images between fluorescent XY image and DIC image

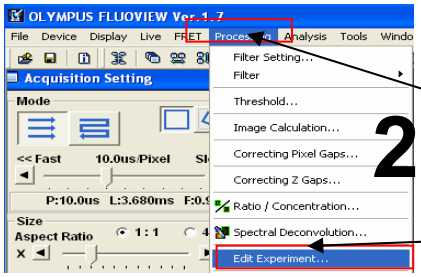
Edit different each files to the same file. This is available for making merge image Between fluorescent image and focused DIC image.



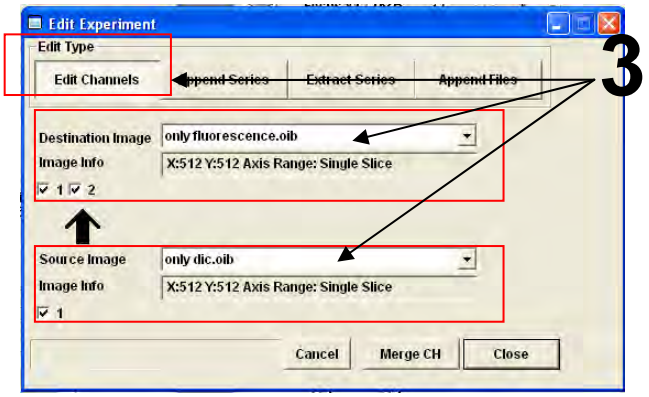
1



1. Open fluorescent image and DIC image.

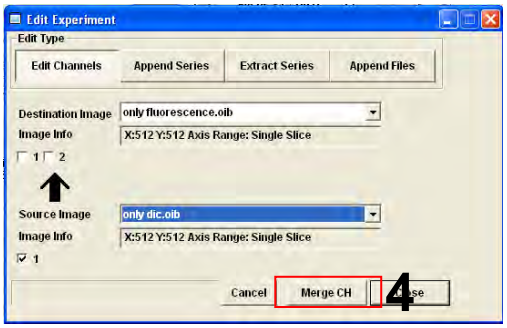


2. Select **Edit experiment** from **Processing**

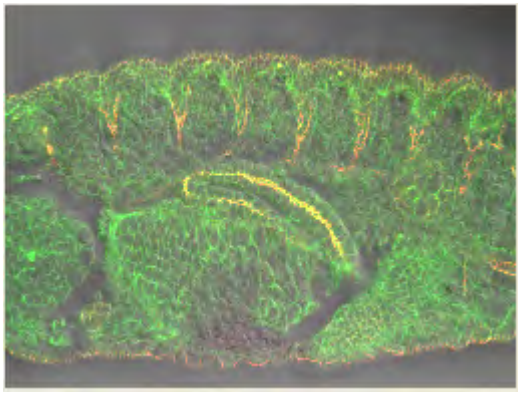


3. Click **Edit Channels**, and select fluorescent image file at **Destination Image**, select DIC image at **Source Image**.

* Check Image Info to make the. merge file. If all channels are checked ,all channels are reflected in the new merged file.



4.Click **Merge CH**, and then the fluorescent image and the DIC image are merged as the new file .



5

5. Merged image between the fluorescent image and the DIC image.

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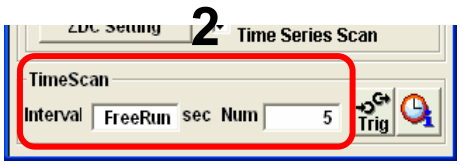
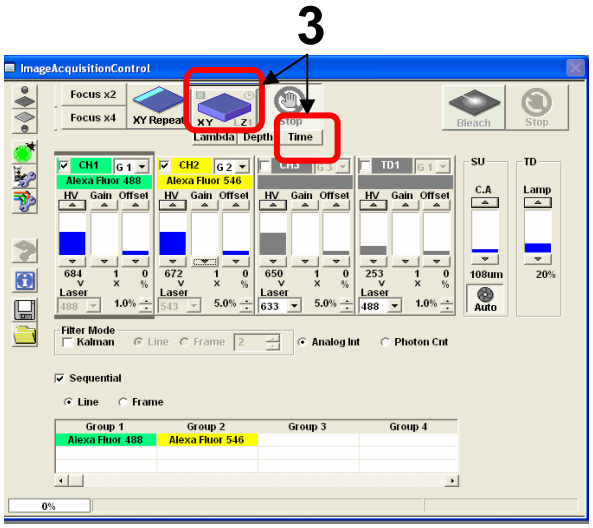
Image Acquisition
(Single Stain on XYZT Image)

This is available for the Time series scan experiment.





1. Adjust the image.
   * Refer P17,18
2. Enter interval time to “Interval”
   Enter interval number to “Num”

   Example: Acquiring time series scan images every 5minutes for 1hour is below,
3. Select “Time” and then click XYTbutton to acquire Time series scan image.
4. Click on “SeriesDone”, and “2D View-LiveImage(x)” is displayed on the window bar for the image that has been acquired.

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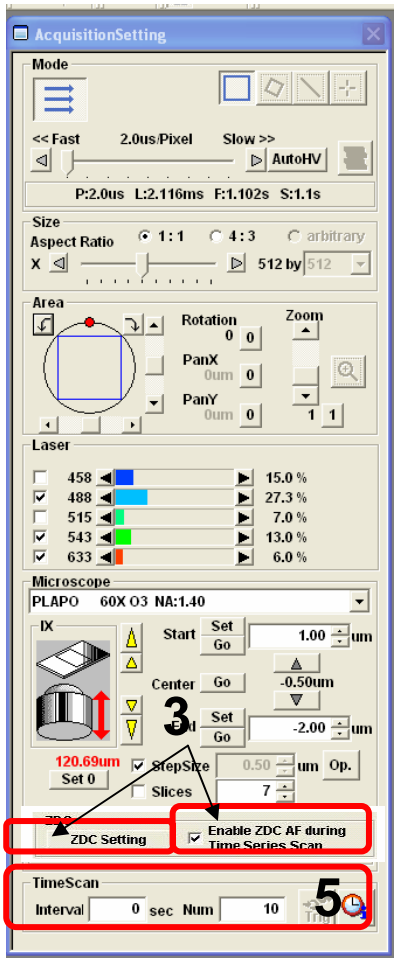
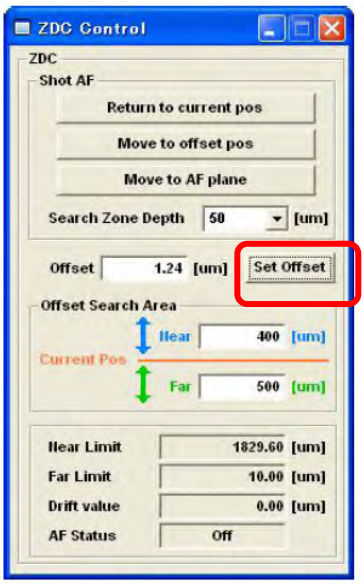
Image Acquisition
(Single Stain on XYZT Image)





4

1. Adjust the image.
   * Refer P17,18

2. Insert ZDC unit to left side.

3. Check "**EnableZDC AF during Time Series Scan**" and click "**ZDC setting**" .

4. Click "**Set Offset**" to register auto focus position.

   *** Note: Have to use glass bottom dish below, otherwise ZDC doesn't work.**

5. Set "**Interval**" and "**Num**" **and then click "XYZT"** to acquire the time series image.

   *** Note: In case of using ZDC for Time series Scan, follow below limits**
   Interval number is more than 60 sec,
   Rest Time is more than 30 sec,
   otherwise ZDC doesn't work.

   *** If use "TimeControler", Time Series Scan is able to done even interval number is within 60sec and Rest Time is within 30sec.**

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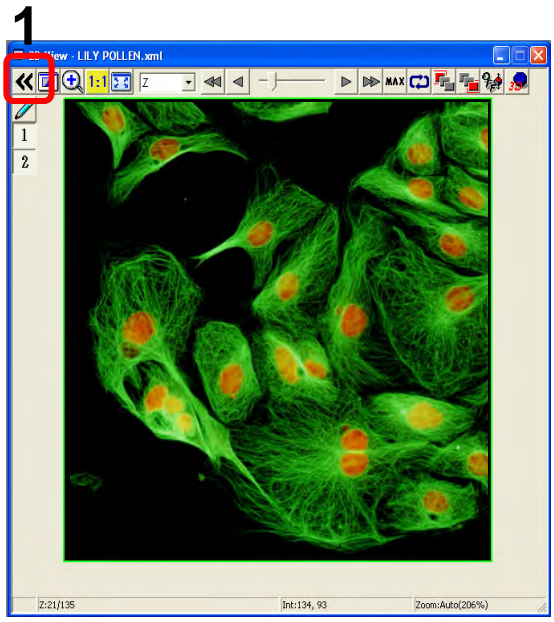
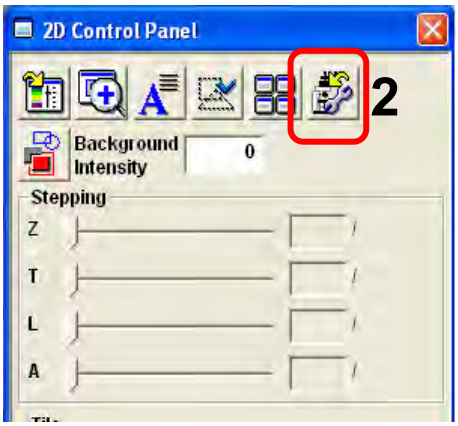
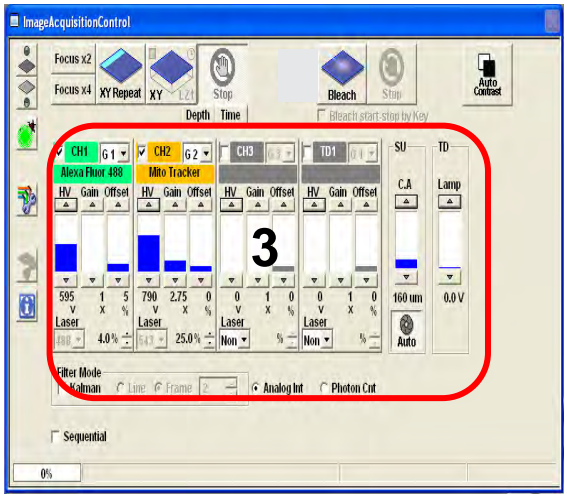
Reload the image conditions



1. Open the file and click 



2. Click 



3. The conditions (HV,Offset, CA and so on) are reloaded .

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Overview of the 2D Operation Panel

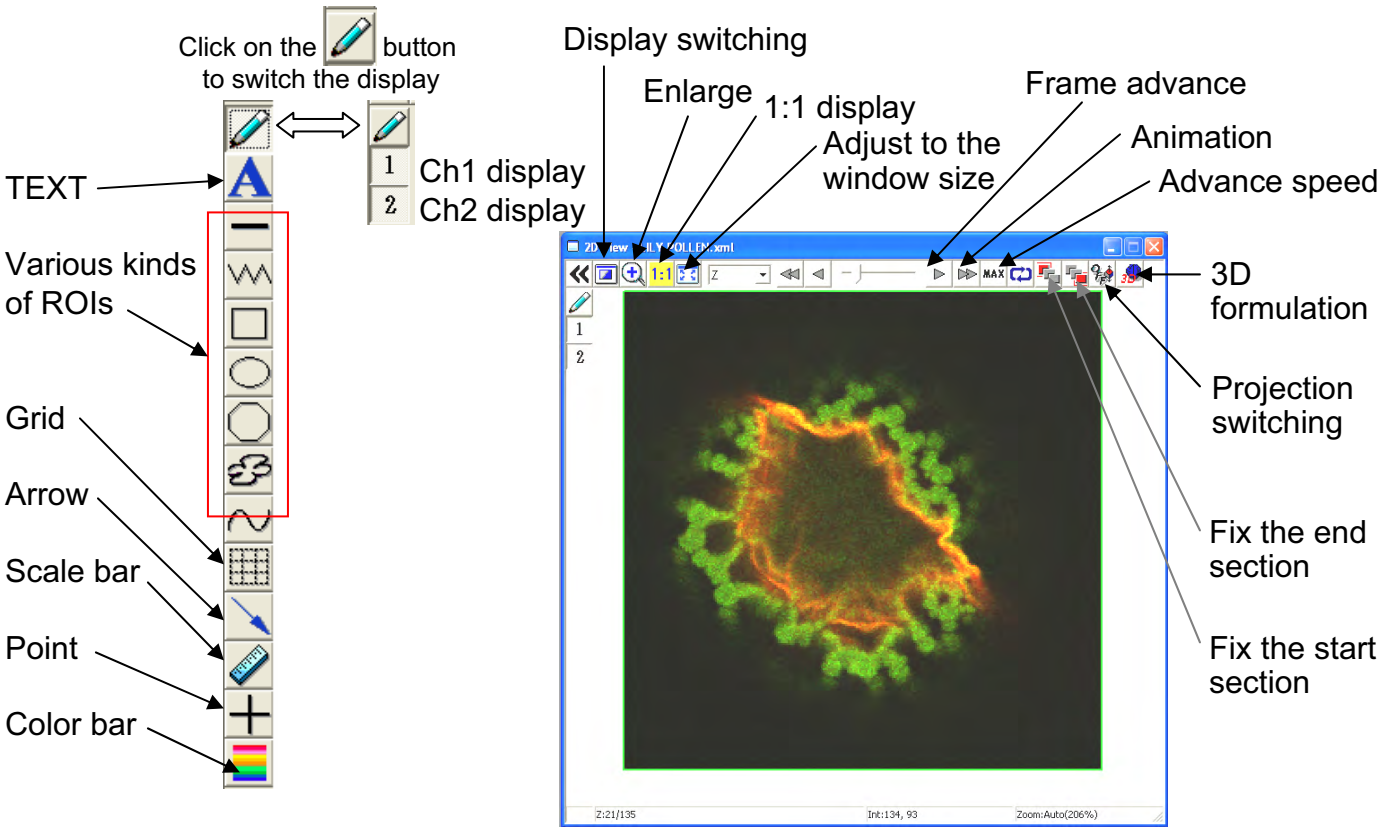
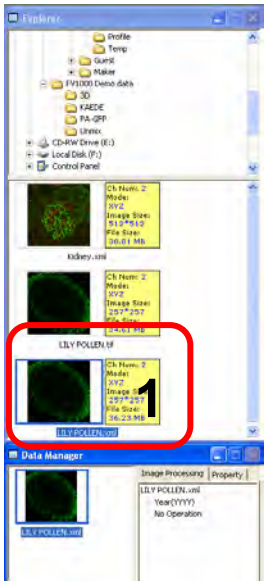


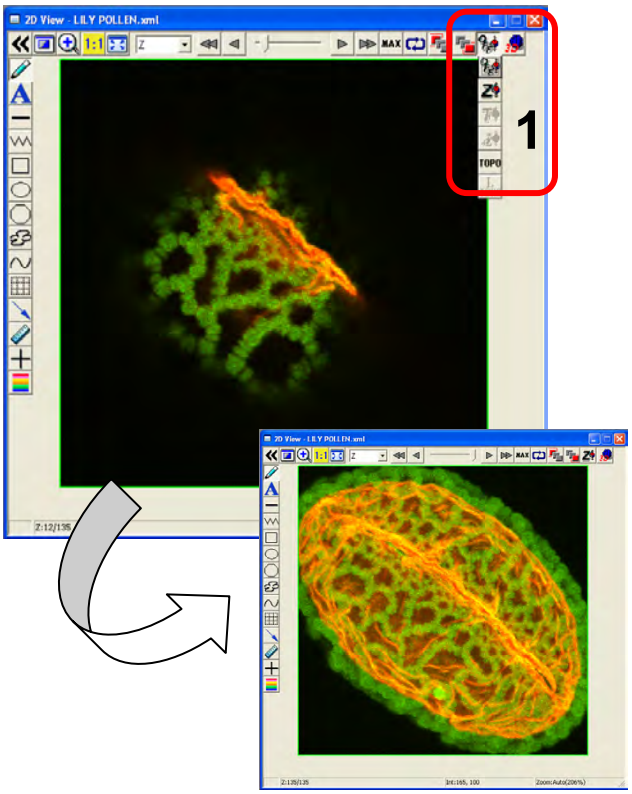
Image Analysis (Opening a File)

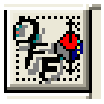


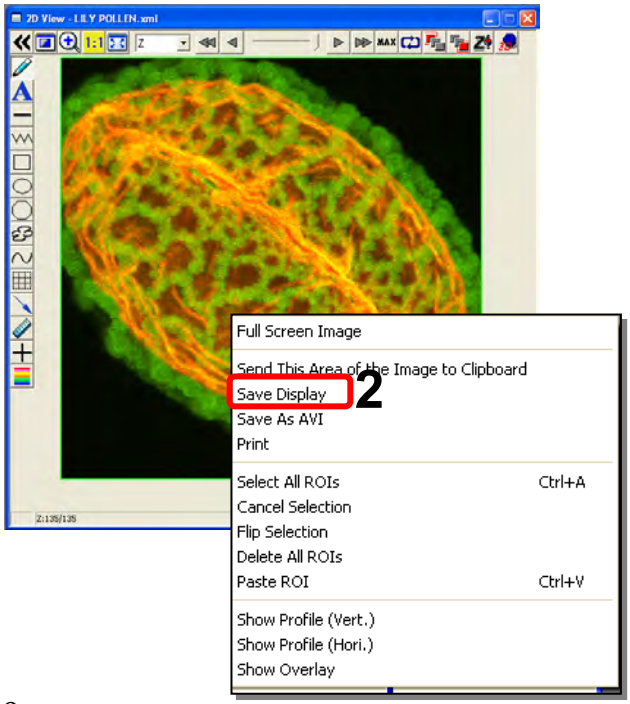
1. Double-click on a file to be opened from Explorer.

28

Image Analysis (Acquire a Projection Images)



1. Click on the  button to select  .



2. To save this image, right-click on the image, select Save Display and save the image with a new name.

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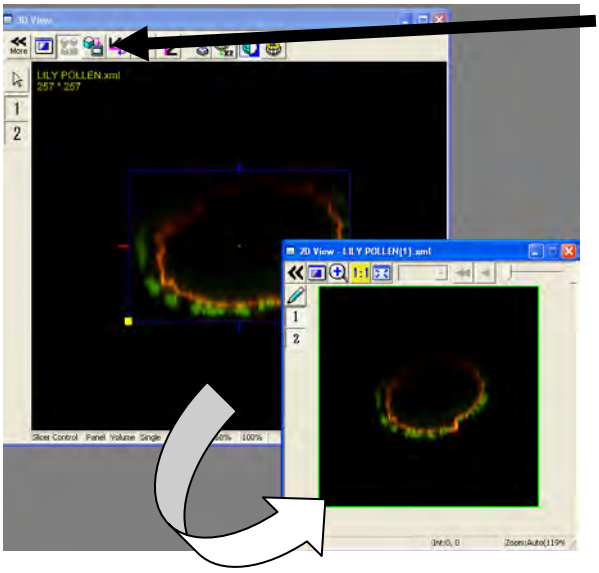
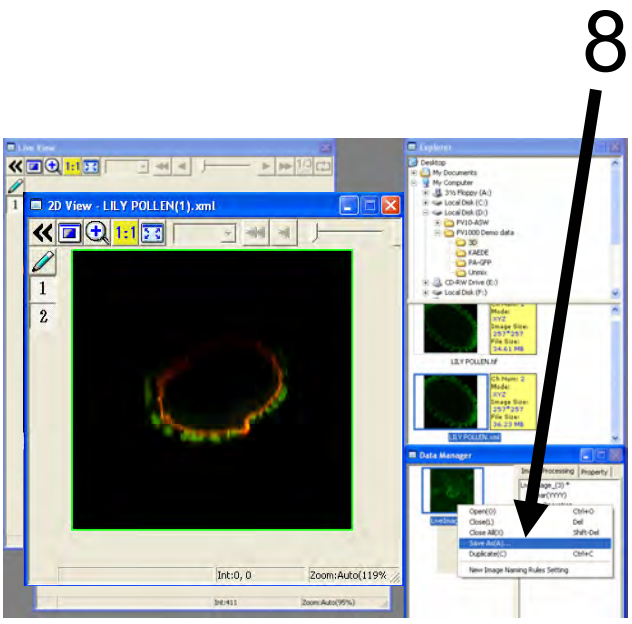
Image Analysis
(Save a Z section Image as 2D file)



Save the image in step 3 or 5

6. Click on the  button.

7. A 2D View-(file name) image is created.



8. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as type "xml" is a file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

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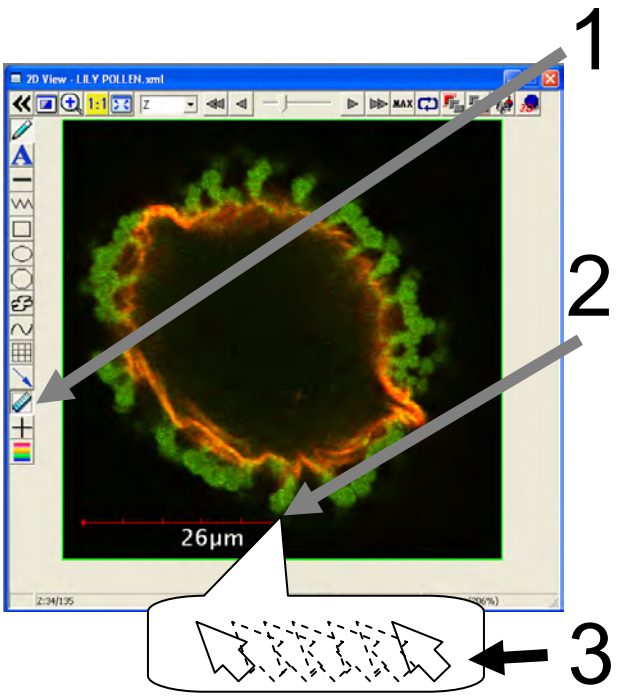
Image Analysis (Inserting the Scale Bar)

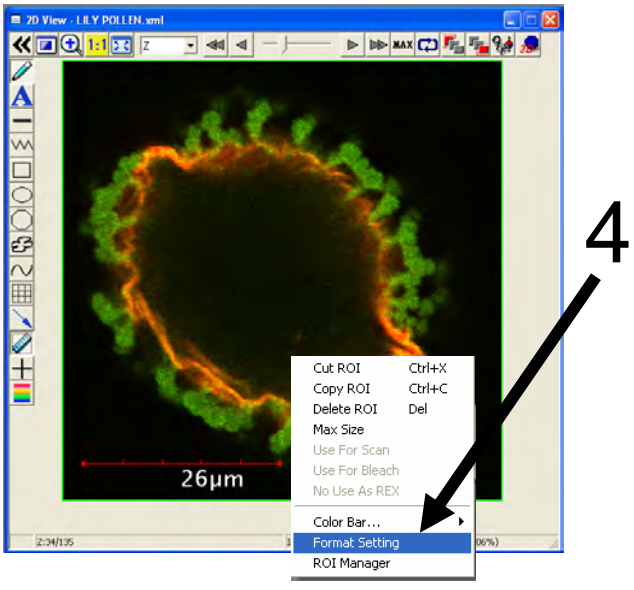


1. Click on the  button.

2. While left-clicking the image, drag and drop it at a certain point.

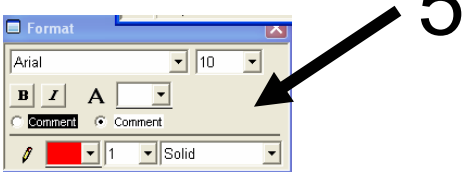
Change the size

3. While clicking the right or left handle, move the mouse from side to side.



Change the text size, color, style, etc.

4. Select Scale Bar and then right-click on Scale Bar to select Format Setting.



5. Change the setting in this window as required.

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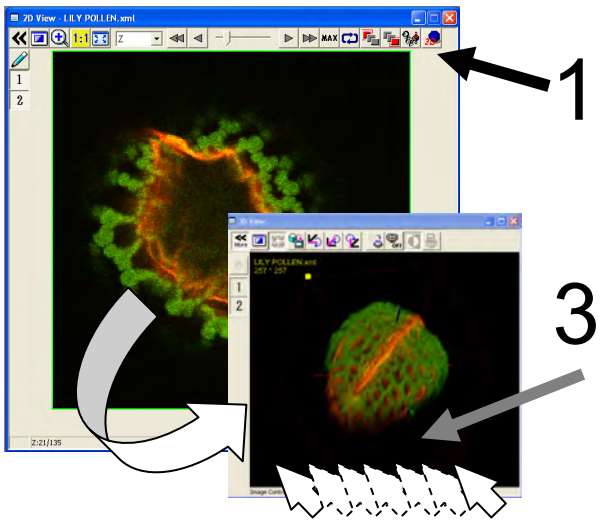
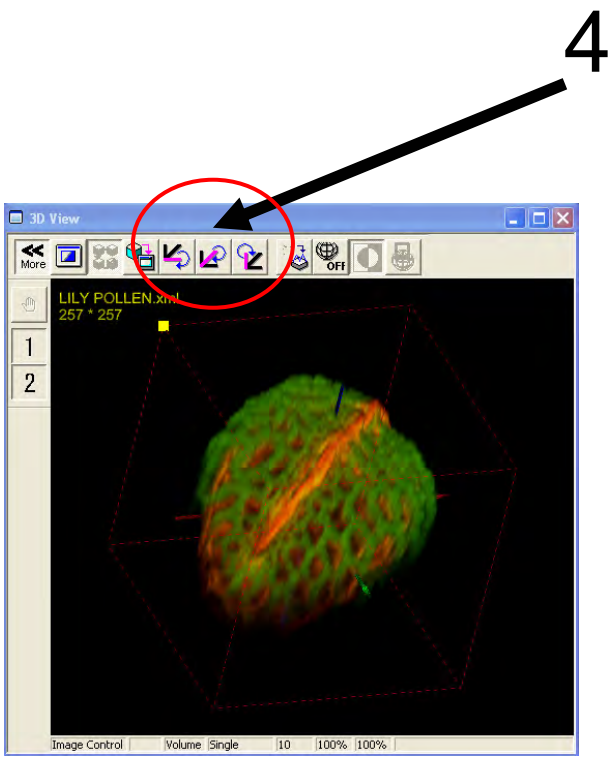
Image Analysis
(Rotating a Three-dimensional Image)



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

2. A 3D view is created.

3. Drag the mouse on the image to observe it at a certain angle.



Simple animation

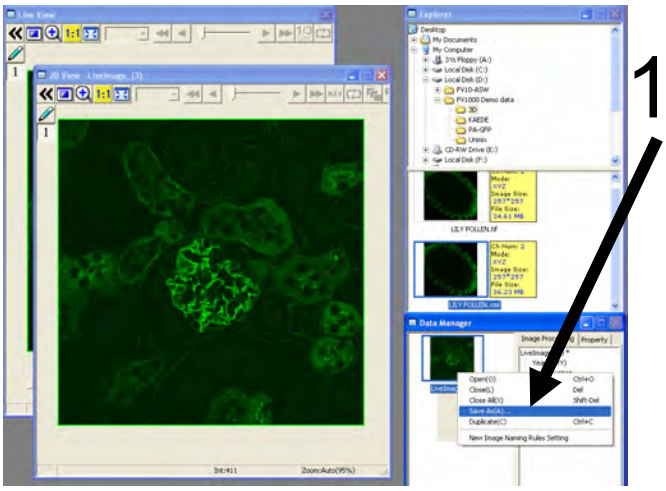
4. Press and hold the  button to rotate the image around the X-axis. Press it again to stop rotation.

   Press and hold the  button to rotate the image around the Y-axis. Press it again to stop rotation.

   Press and hold the  button to rotate the image around the Z-axis. Press it again to stop rotation.

32

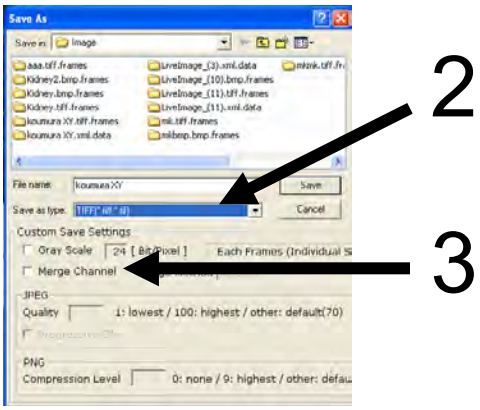
Image Analysis (Saving an Image)



Convert each channel of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to RGB Color.
4. Save the image.

* BMP and JPEG formats are also selectable.

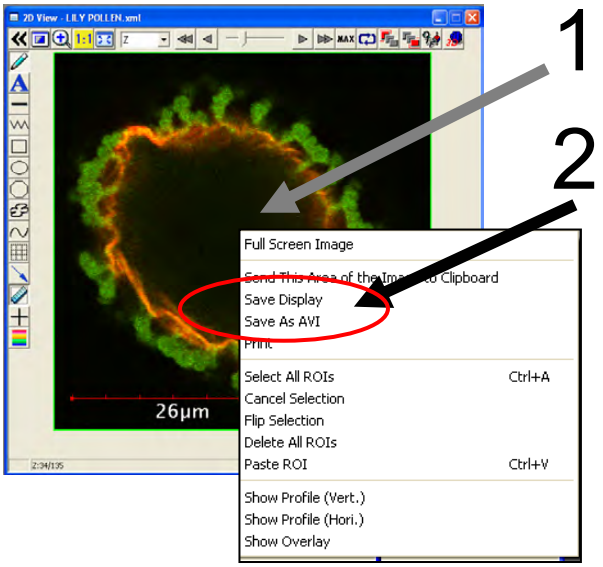


Convert a merge image of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to Merge Channel.
4. Save the image.

* BMP and JPEG formats are also selectable.

****



Convert an image with the scale bar inserted into a BMP format

1. Right-click on the image.
2. Select Save Display and save the image with a new name.

Convert an animated image into an AVI format

1. Right-click on the image.
2. Select Save as AVI and save the image with a new name.

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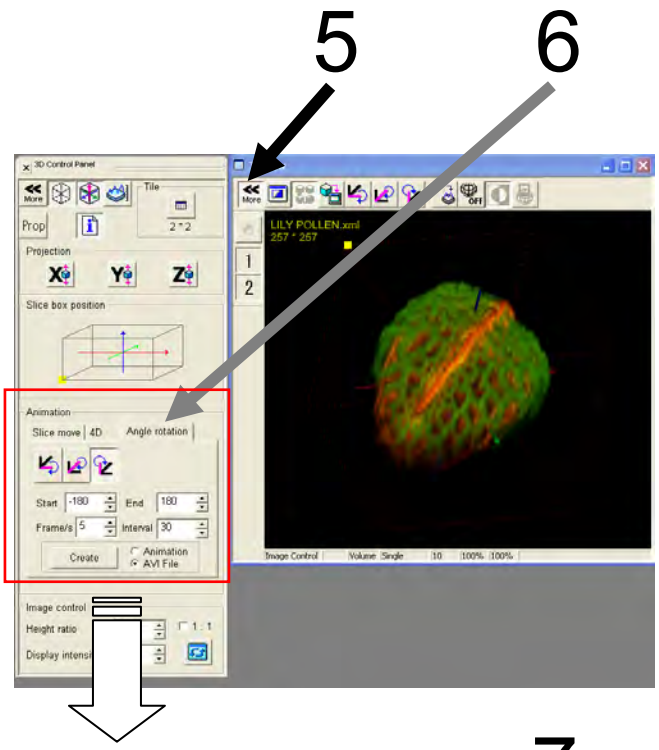
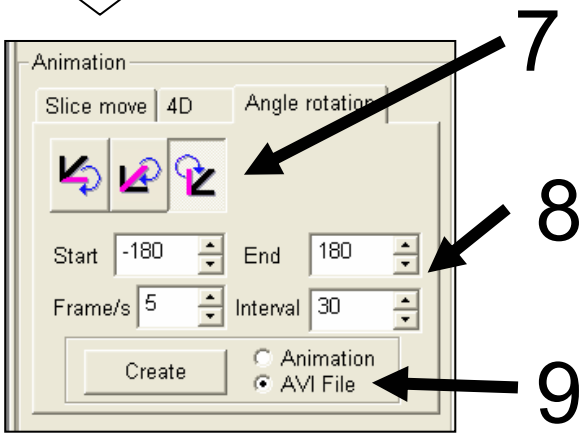
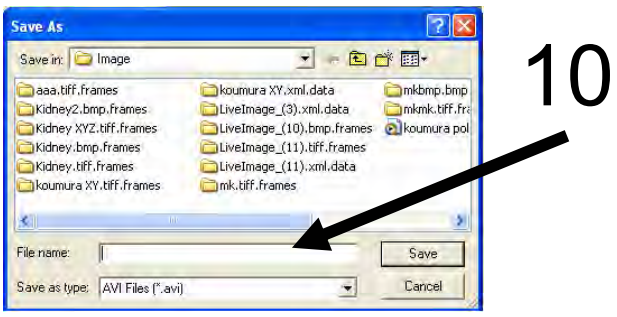
Image Analysis
(Rotating a Three-dimensional animation)

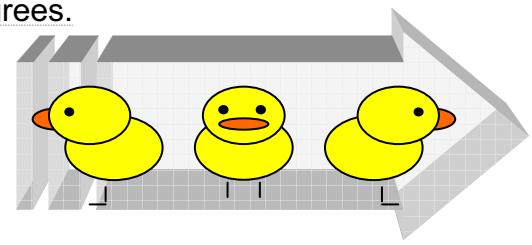






To save a rotation file as an animated image, create three-dimensional images according to the following procedure.

For example, try to rotate an image by 180 degrees.



5. Click on the 

button.

6. Click on the Angle rotation tab.

7. Select the rotation axis.

8. Enter the rotation angle.

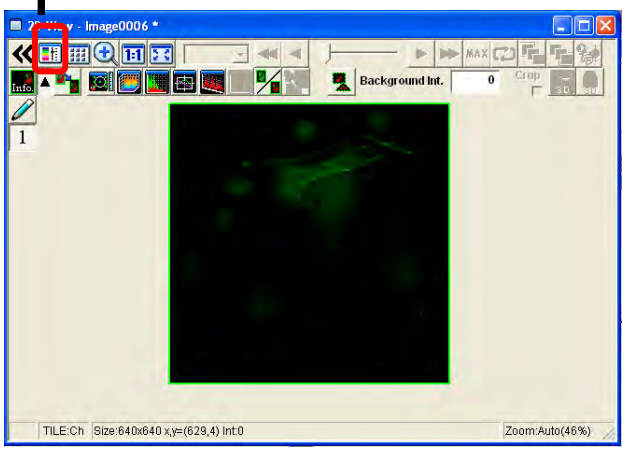
Start =	Angle to start rotation
End =	Angle to stop rotation
Frame/s =	Rotation speed
Interval =	Degrees to be rotated at a time

9. Select AVI File and click on Create.

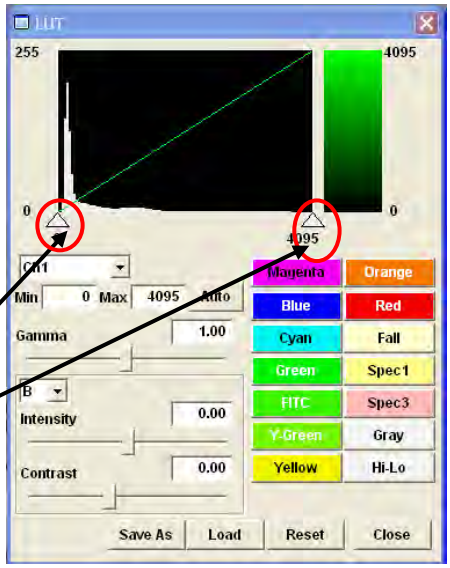
10. Enter a file name and click on Save.

34

2D Image Analysis (Edit the image color and contrast)



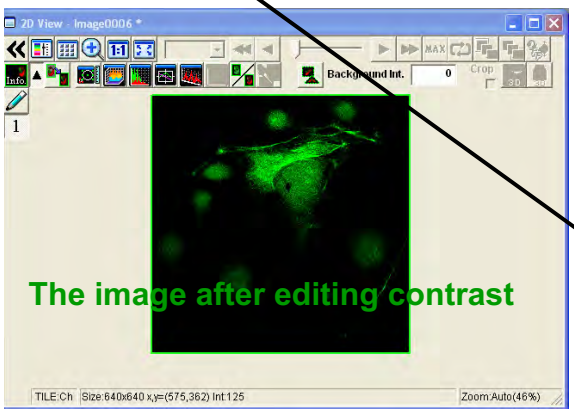
1. Click  "LUT" and then LUT table appears below,

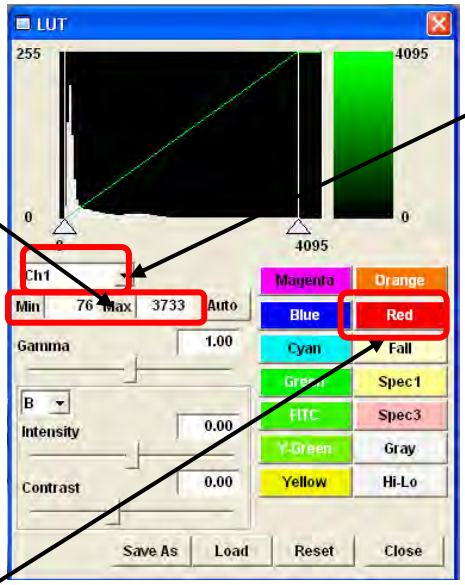


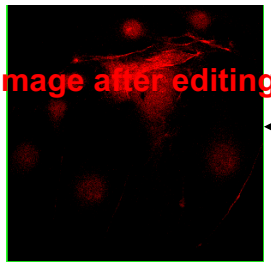
2. Edit contrast to drag  to left or right side, and another way to edit contrast is entering value on "Max" and "Min"(Max4095, Min0)

3. Min and Max value are changed and contrast of image is edited.

*** According to get Min value up , be able to reduce noise of the image.**



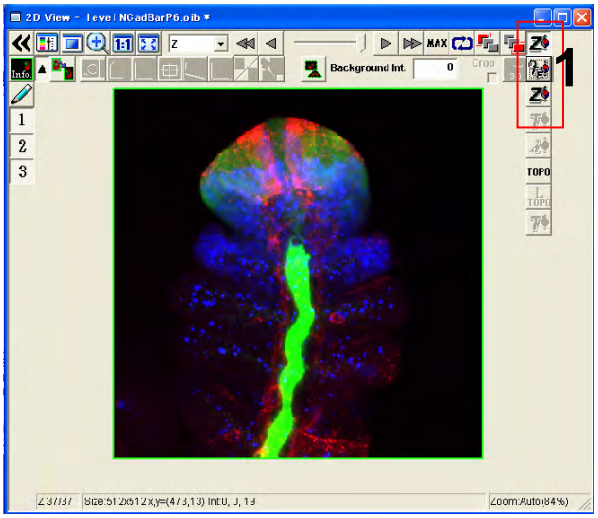




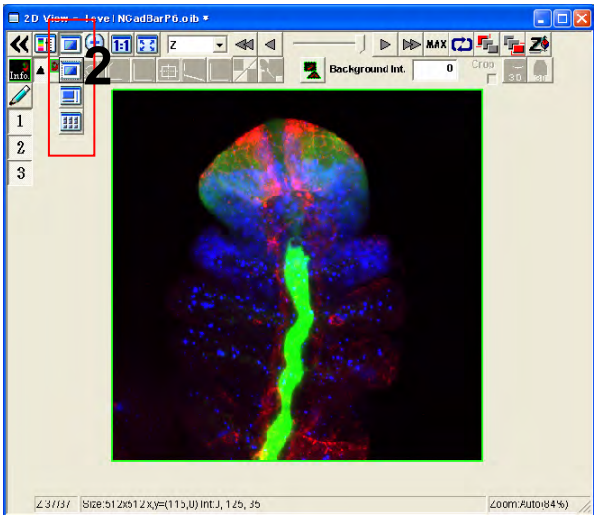
4. To click another color, be able to Edit a color.
   Above example: Change Green to Red to click Red

35

2D Image Analysis (the image of Z section)

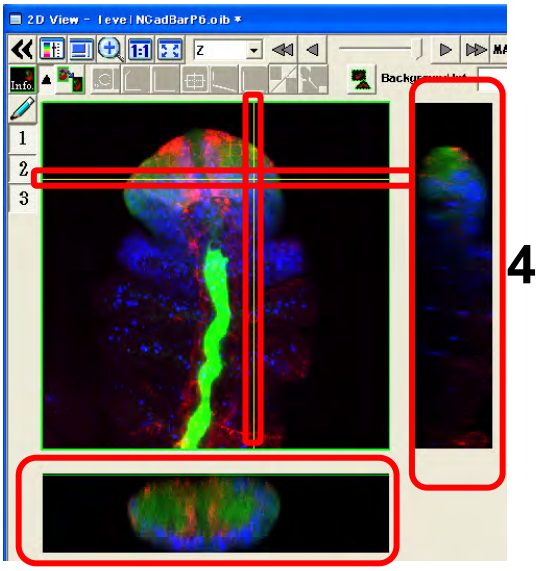


1. Click  and select  again, then Projection image is shown on 2D View after getting XYZ image.



2. Click  and select  .

3. The images of Z section is shown on X axis and Y axis.
   According to Move to left or right side on X axis and to move to ups and down on Y axis, be able to show image of Z section each position.



4. The image of Z section on Y axis.

5. The image of Z section on X axis.

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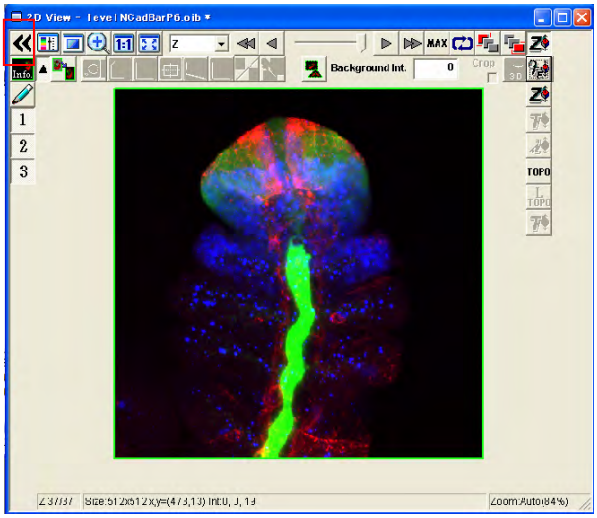
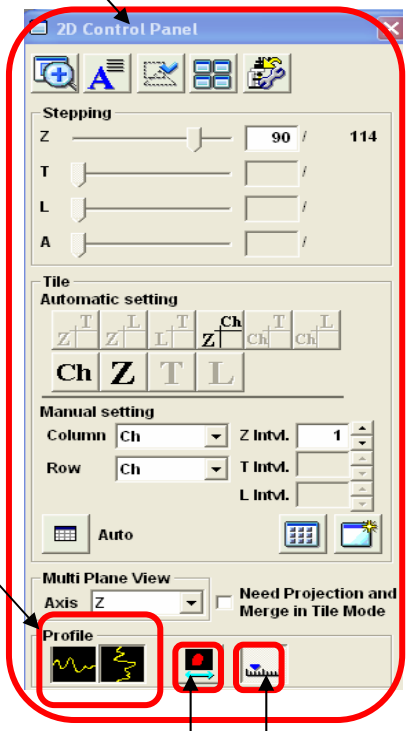
2D Image Analysis (Intensity Profile of each Z sections)

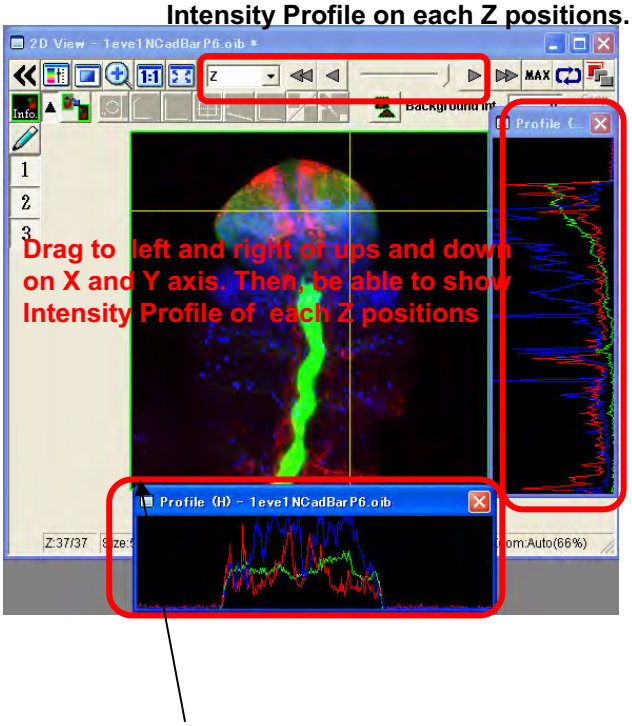


1. Click  and then 2D Control Panel is shown below,



2. Click  "Profile" and then Intensity Profile of each Z sections is shown on the X and Y axis.

To move to Z position ,be able to show Intensity Profile on each Z positions.



3. Click  to show Scale on Intensity Profile

4. According to click  , be able to show as equal scale of Profile window as 2Dimage.

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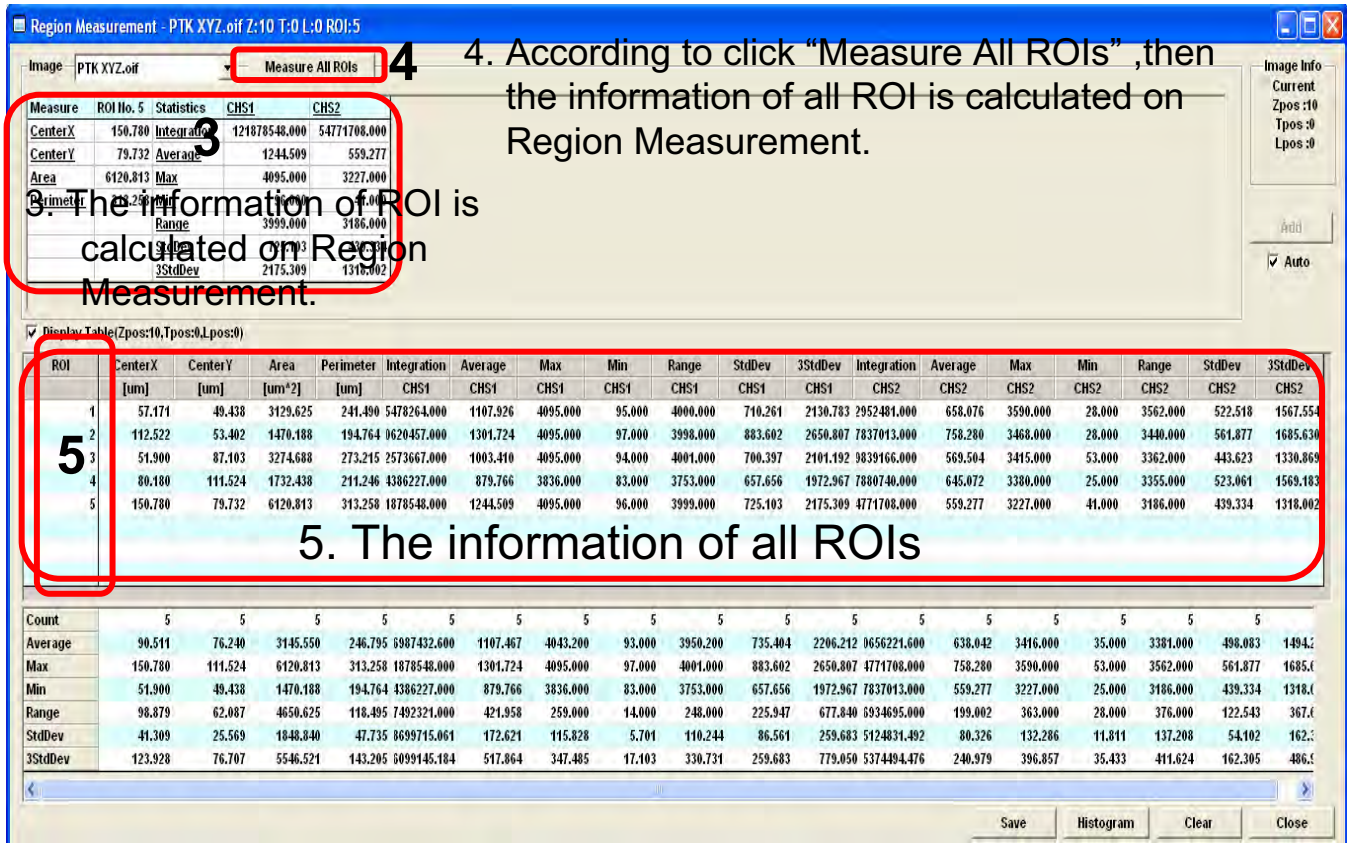
2D Image Analysis (Measure)

1. Enclose interesting regions by ROI

   Line on interesting positions by ROI —

2. Click  “measure”.



4. According to click “Measure All ROIs” ,then the information of all ROI is calculated on Region Measurement.

3. The information of ROI is calculated on Region Measurement.

5. The information of all ROIs

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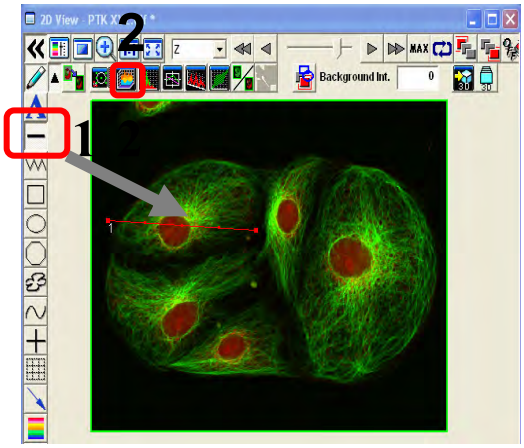
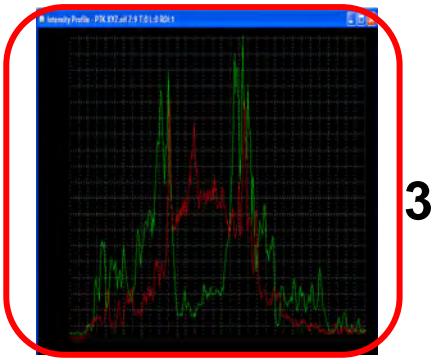
2D Image Analysis (Line Intensity Profile on the 2D image)





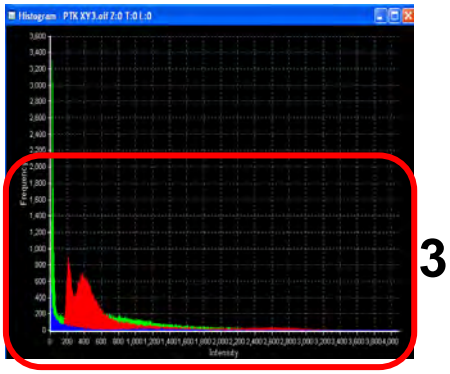
1. Line on the 2D image by ROI 

2. Click  “Intensity Profile”

3. “Intensity Profile” on the line is shown as intensity graph .

* State of colocalization between each Chs is figured out apart from intensity .

2D Image Analysis (Histogram)



1. Enclose the region by ROI.

2. Click  “**Histogram”**

3. “Histogram” window is shown as a graph, frequency of intensity of each pixels is plotted on the region enclosed by ROI.

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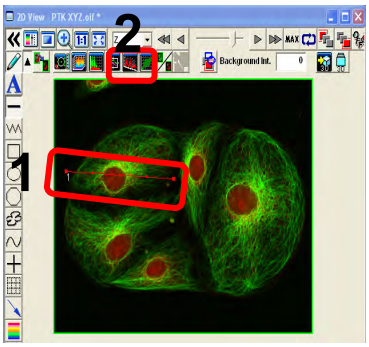
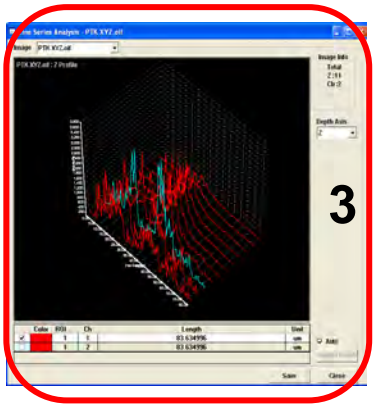
2D Image Analysis (Line Series Analysis)



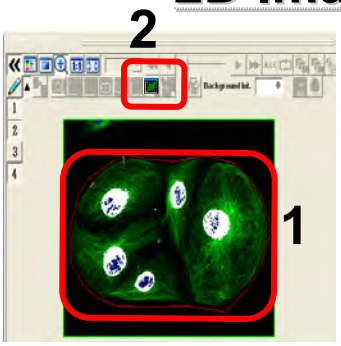


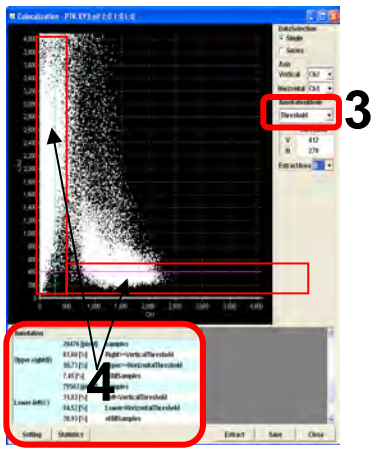
1. Line on the 2D image.

2. Click  “Line Series Analysis”

3. Intensity of Z position/ time on the line is shown as a graph .

2D Image Analysis (Co-localization)





1. Enclose an interesting region by ROI.
2. Click 
3. Select 

Threshold from Annotation Mode.
4. According to move Thresholds of X,Y axis to right and left ,ups and down (Enclose red color X,Y axis), Co-localization result between 2ch is changed .

   Information of Co-localization is listed under the scatter plot.

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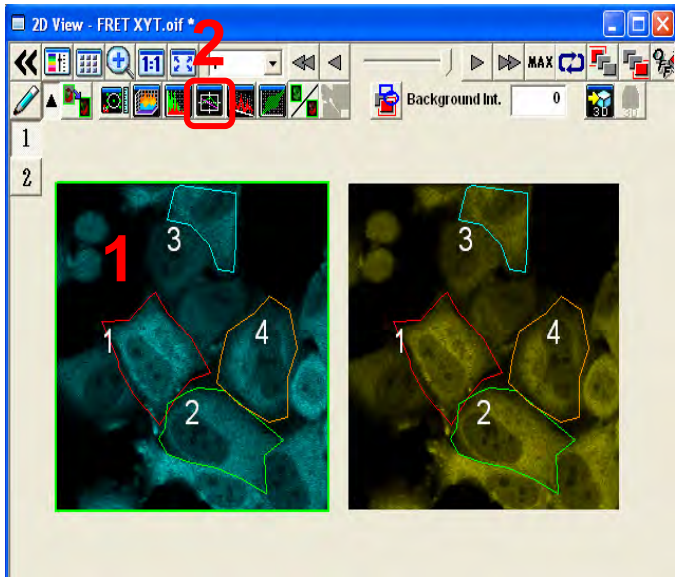
2D image Analysis(Series Analysis TimeLapse)

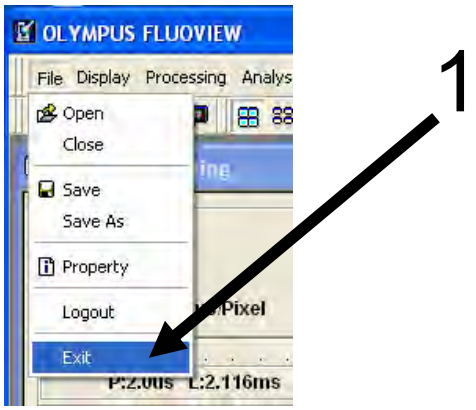


1. Enclose interesting regions by ROI

2. Click  “Series Analysis”

3. “Series Analysis” graph is shown below, Y axis shows intensity, X axis shows time and then be able to see time series reaction each ROIs.

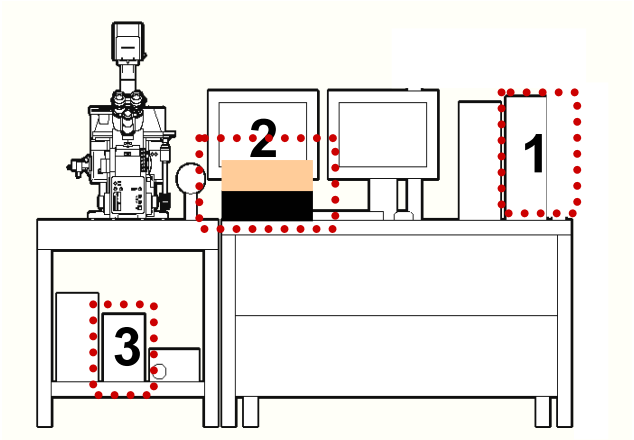
Closing the System



1. Exit the FV10-ASW software by selecting File/Exit.

2. Exit the Windows.

(1) Select Start/Shut Down.
(2) On the Shut Down Window, select Shut Down and click on OK.



3. Turn the laser OFF. (Turn the key switch to the OFF position.)

3-1. LD559nm OFF

3-2. Multi Ar (458 nm, 488 nm, 514 nm) OFF

3-3. HeNe (G) (543 nm) OFF

4. Turn the mercury burner power OFF.

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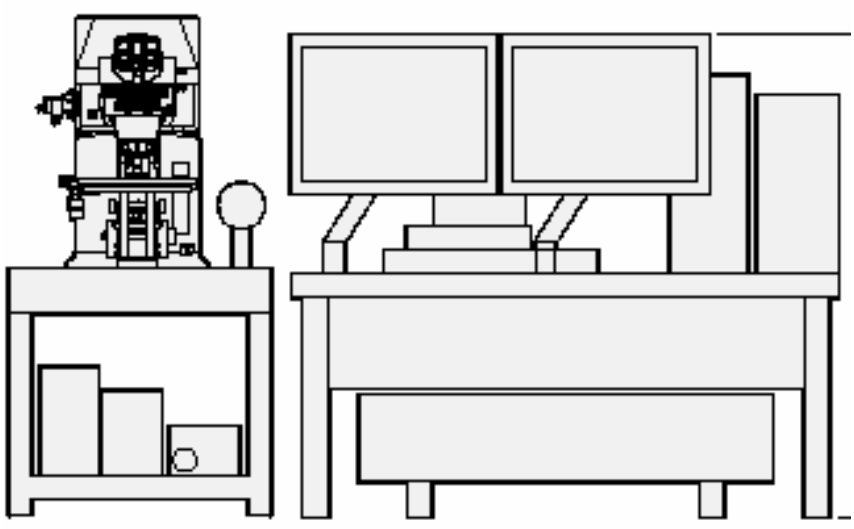
Laser Conforcal Scanning Microscope
FV1000D Spectral Type
(Upright Microscope BX61)

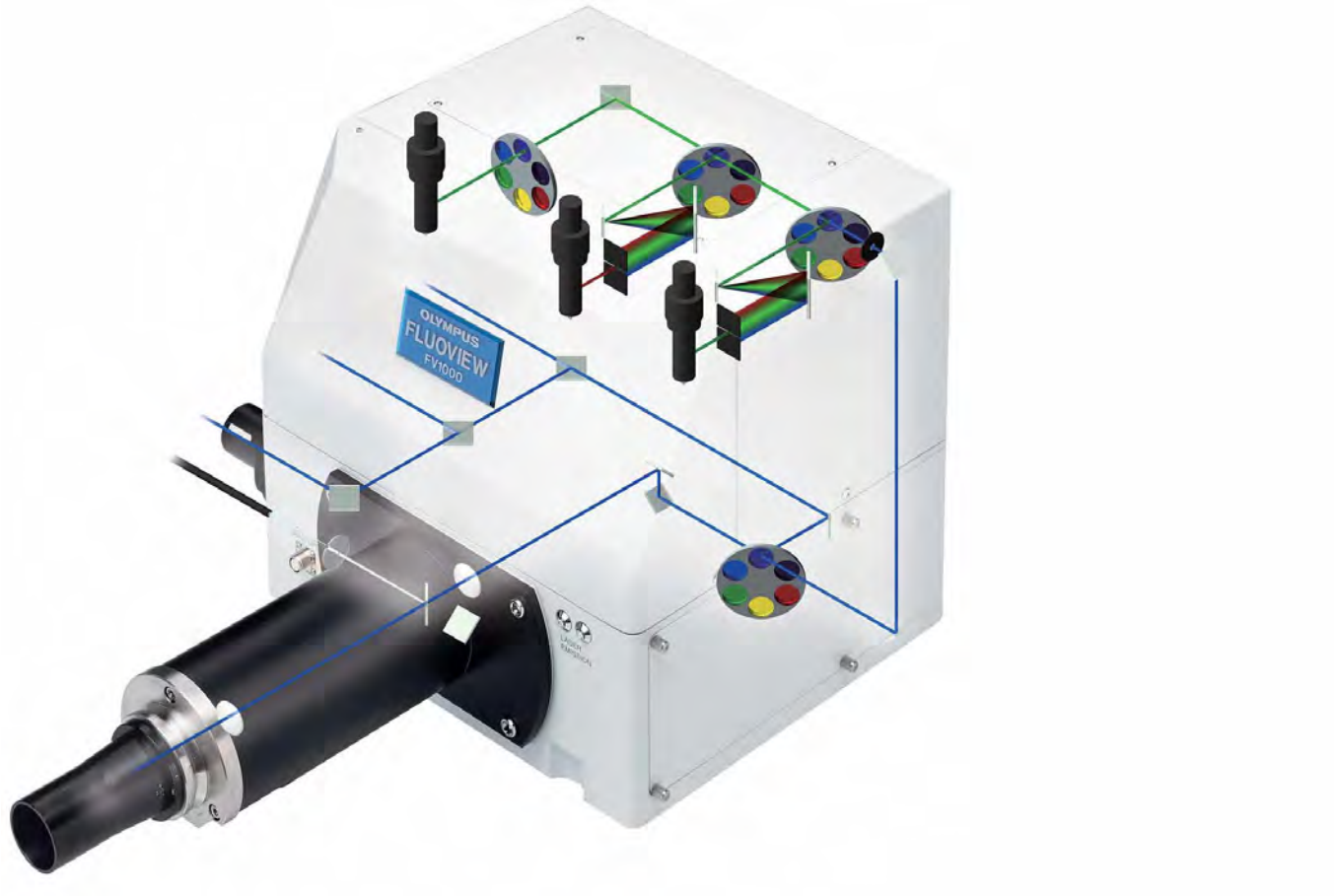
Operation Manual



1

Contents

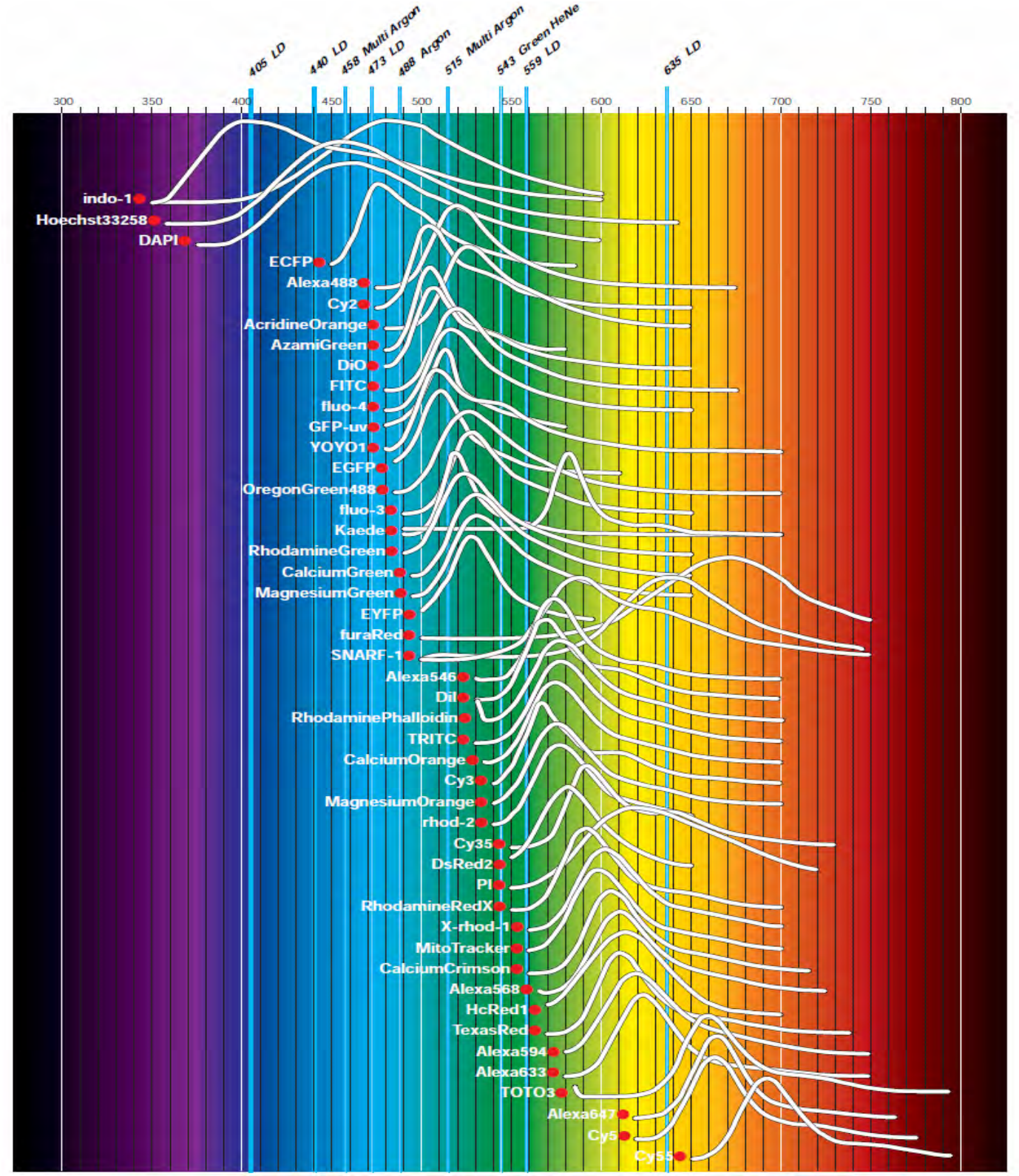
Spectral Type Main Scanner



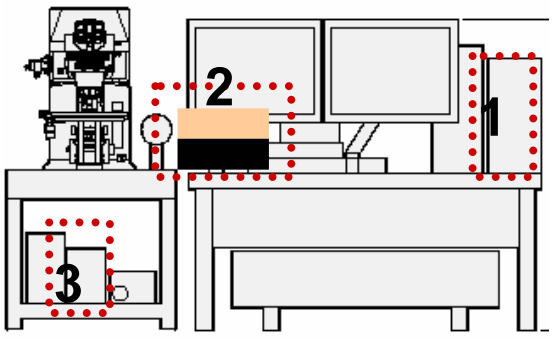
3

Dye List (FV1000D Lasers are available below)

LD405nm LD440nm LD473nm LD559nm LD635nm
Ar458nm Ar488nm Ar515nm
HeNe (G) 543nm



System Preparation



1. Turn the computer ON.
   [In case of equipped concentrated power supply, power on it first]

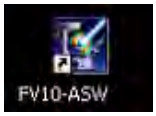
2. Turn the laser ON
   (Turning the key switch)
   2-1. LD559nm ON
   2-2. Multi Ar 458nm 488nm 515nm
   2-2. HeNe(G) (643nm) ON

3. Turn the mercury burner ON for Fluorescence observation.

Wait for a moment until the software is started

4. Log on Windows

   Enter Password ,Customer name is below
   User name: Administrator
   Password : fluoview

5. 

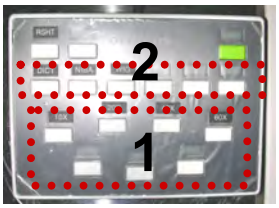
Double click this icon to log on to ASW

   User name: Administrator
   Password : Administrator

5

Visual Observation under the Microscope

■■ Observation of Fluorescence Image ■■



Hand switch

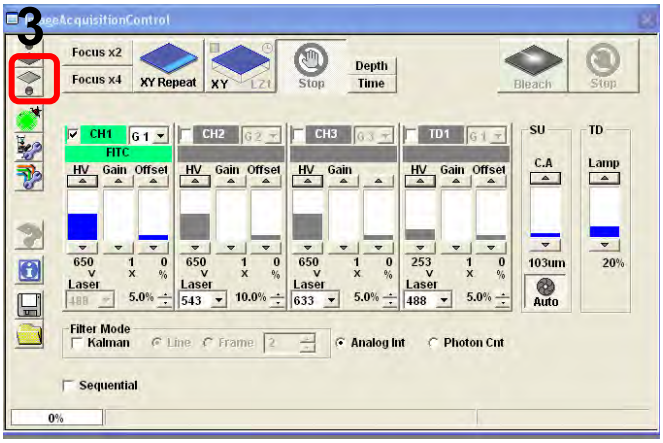
1. Select an objective lens
   by using the hand switch

2. Select florescent filter cube

MEMO
Fluorescence filter

NIBA: Blue Excitation / Green Fluorescence
(Ex.:FITC,EGFP)
WIG: Green Excitation / Red Fluorescence
(Ex.:Rhodamine, DsRed)

3.  Click the button
on the Fluoview software



4. Focus to the specimen

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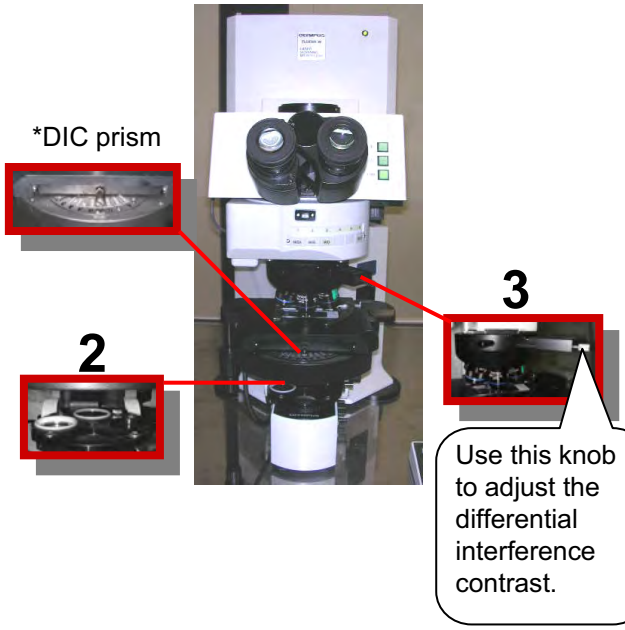
Visual Observation under the Microscope

■■ Observation of Differential Interference Contrast Images ■■

Hand switch

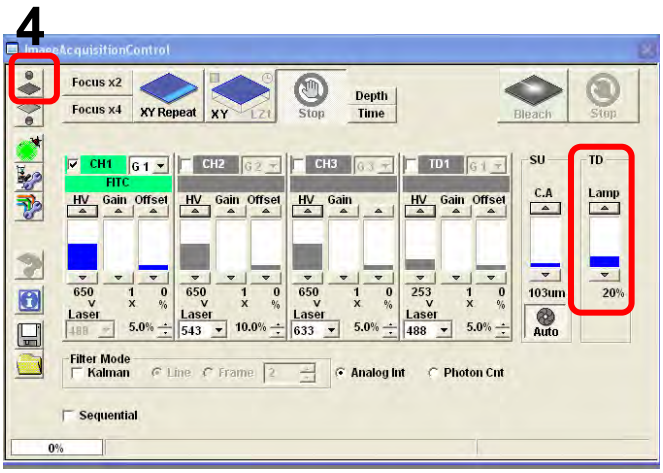


1. Select the Objective Lens

2. Insert the Polarizing Plate in the Light Pass

3. Insert the DIC prism slider in the light pass

4.  Click the button on Fluoview software



5. Focus to the specimen

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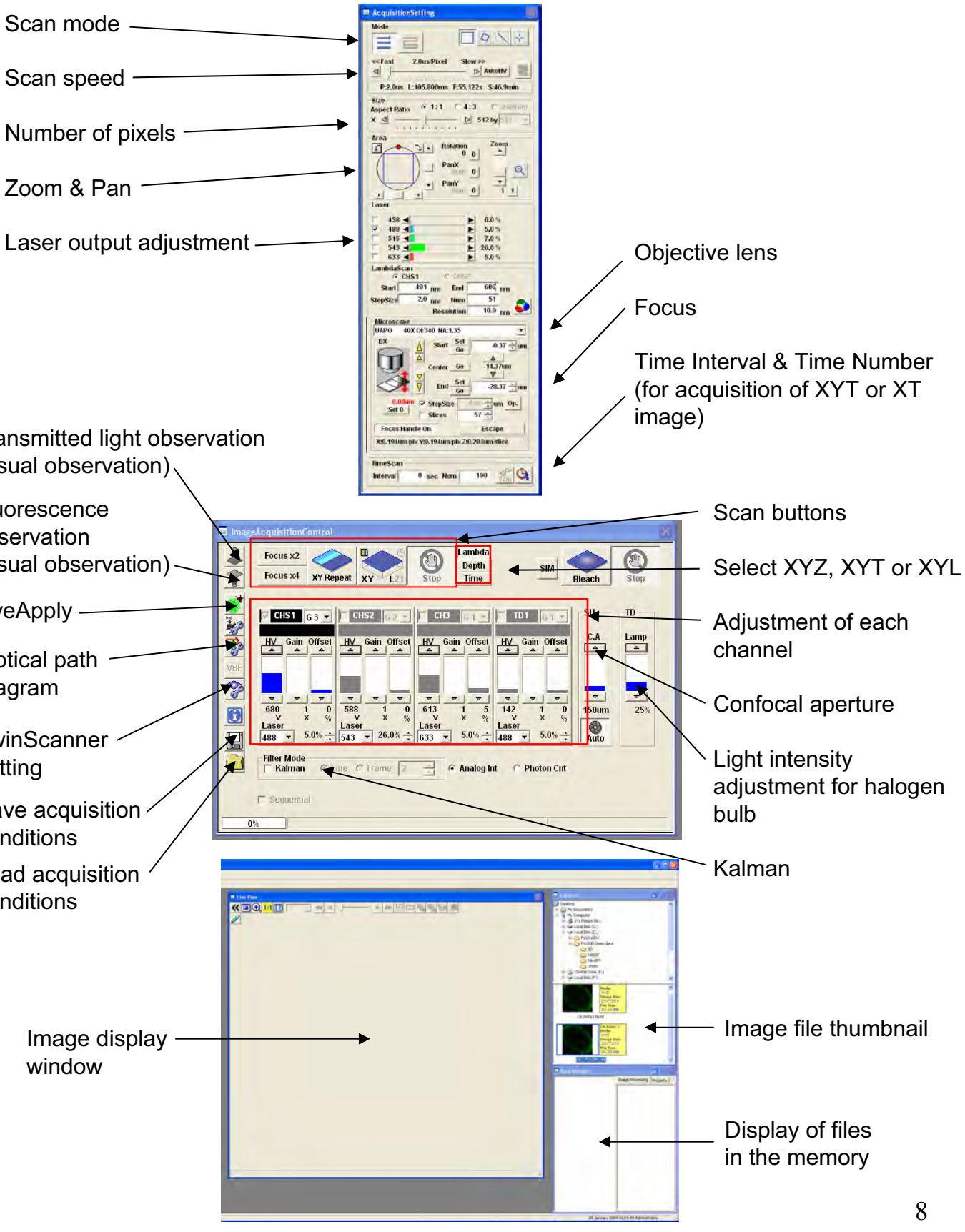
Overview of Operation Panel for Image Acquisition

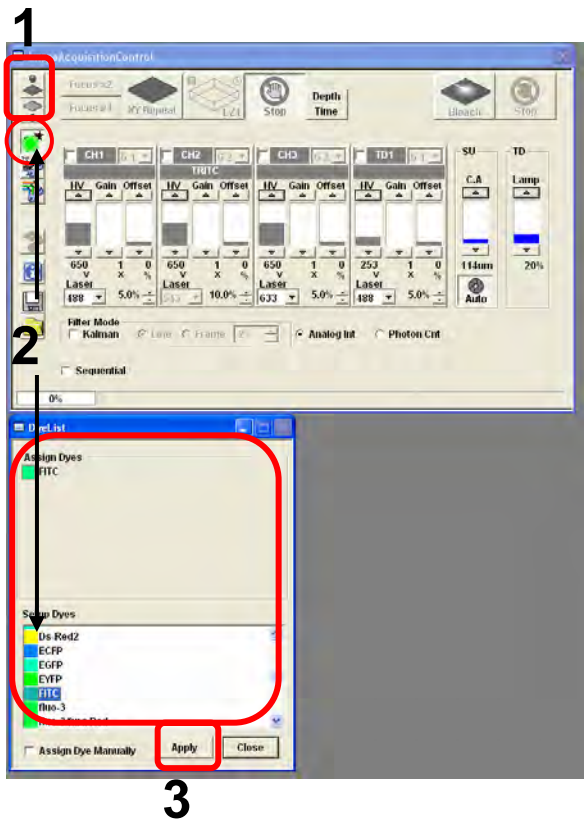


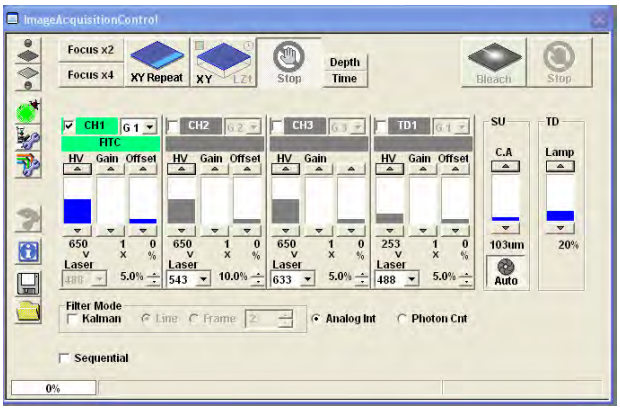
8

Image Acquisition
(Single Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Single stain of green fluorescence dye (FITC)





1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click on the Apply button.

   (The DyeList panel can be closed by using the Close button.)

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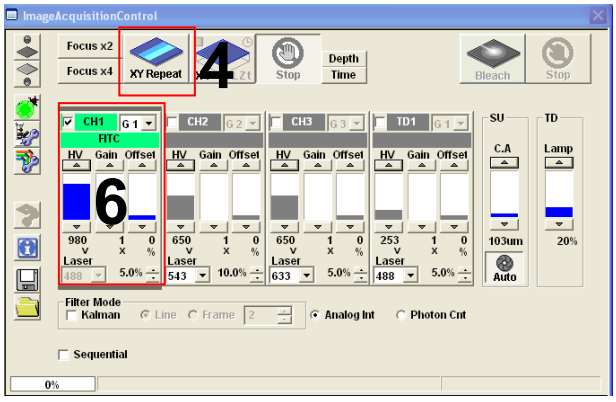
Image Acquisition
(Single Stain on XY Image)

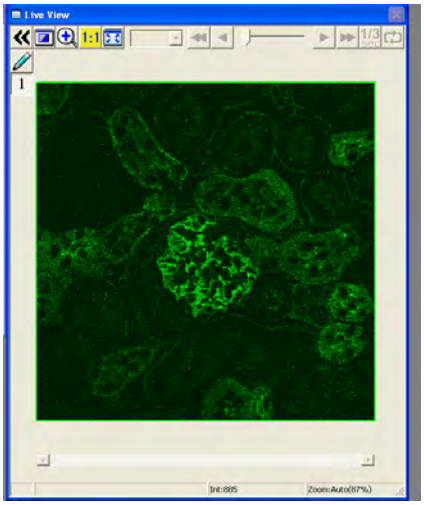


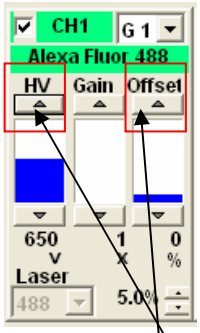
4. Press XY Repeat button click to get image

: Continuous scan mode

5. Focus to the specimen

6. Adjust the green (FITC) image.





· Adjust sensitivity of **HV** and reduce noise by **offset**

7. Press keyboard **Ctrl + H key**
Optimized PMT adjustment brightness intensity 2 color between white and black,
Maximum intensity is 4095 (12bit)
if intensity is over4095, color is changed to red (saturation)

7

* Basically, **Gain value is 1**

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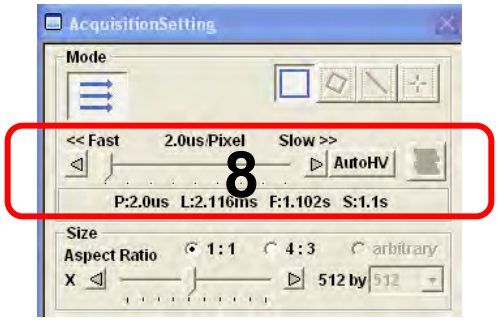
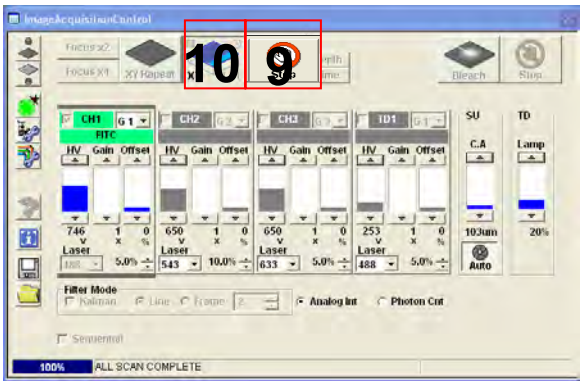
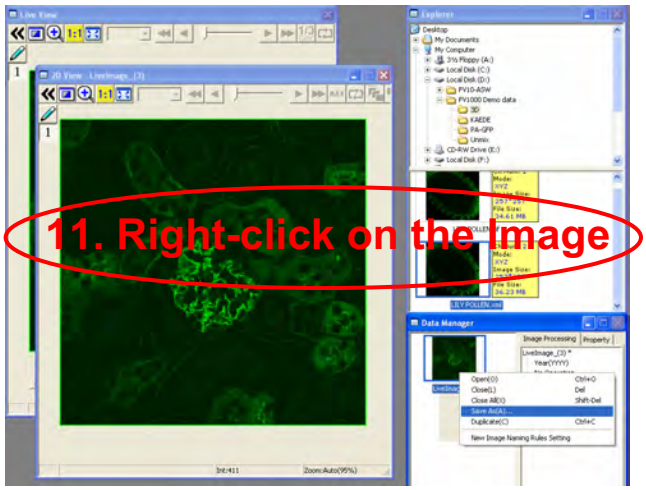
Image Acquisition
(Single Stain on XY Image)







■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

8. Select AutoHV and then select ScanSpeed.
*As the scan speed becomes slower, noise
can be removed while maintaining the current brightness.

9. 

Press the Stop button to stop scanning.

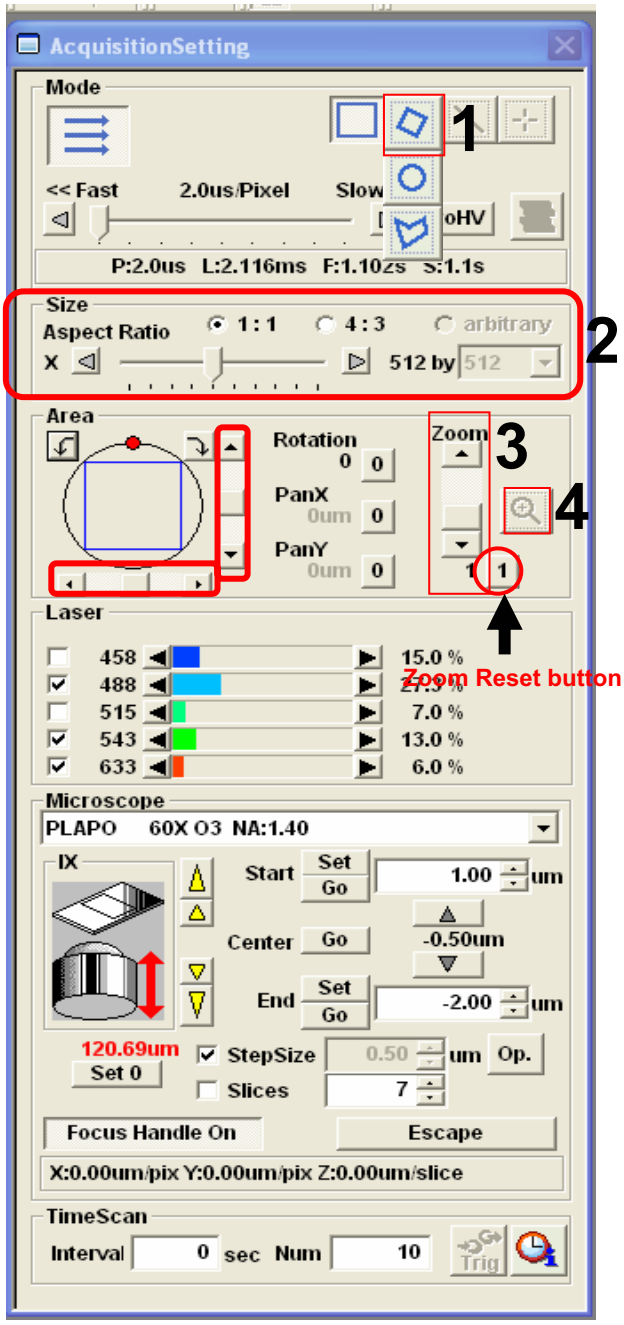
10. 

Click on XY, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

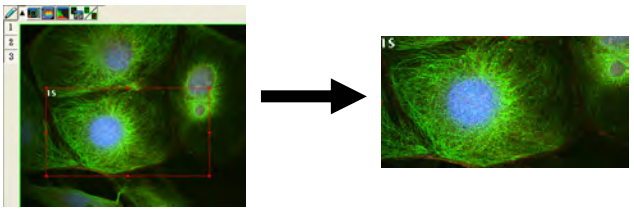
11. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)
Save the image as TIFF,BMP,JPEG format Select "Export" and chose the format TIFF, BMP, JPEG.

11

Complement of adjusting the image



1. Click “Clip scan” button , and enclose an interesting region’s image on the whole image.

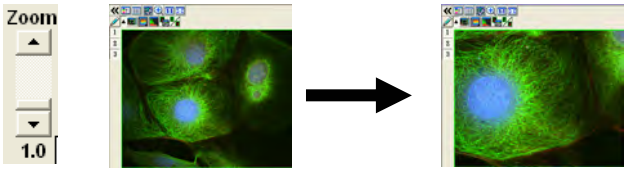


2. pixel setting

*The standard pixel is 512 x 512

3. Zoom Setting

Press “XY Repeat” to scan and set zoom value.

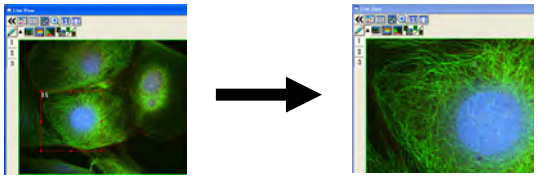


Above image is zoomed From 1x to 2

*** Scan speed and pixel resolution remain even zoom value is changed**

4. Click  Zoom scan, and be able to enclose an interesting region on the whole image

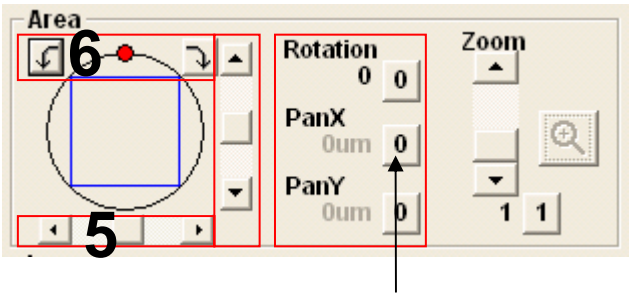
Press XYRepeat to scan after enclosing the area



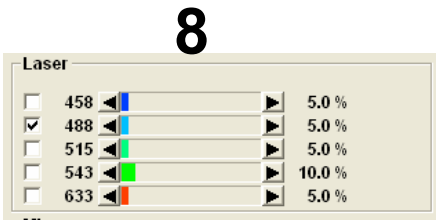
*** Scan speed and pixel resolution remain even zoom value is changed**

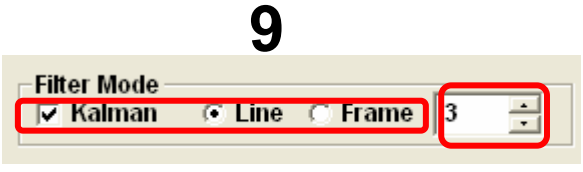
12

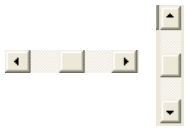
Complement of adjusting the image



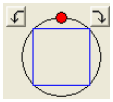
PanX,Y and Rotation reset button





5. Pan X,Y 

   Be able to move the field of view to set Pan X,Y without stage action

6. Rotation 

   Be able to rotate the whole image.

7. 

   Click "Auto" button to acquire Optimized Conforcal aperture
   Conforcal aperture · · · change conforcal aperture to larger diameter for dim fluorescence image then, be able to get the more bright image. But Z axis resolution gets worse.

8. Laser Intensity · · · More Laser intensity is increase , more bright image is .

   *** More increase laser intensity is , more discoloration image is .**

9. Kalman accumulation · · · Image acquisition is repeated to the specified number of times to provide an averaged image. Consequently, noise is averaged and roughness on the whole image is reduced.

   Advantage: The speed of each scan is fast.

   Disadvantage: Some blur occurs due to averaging of images.

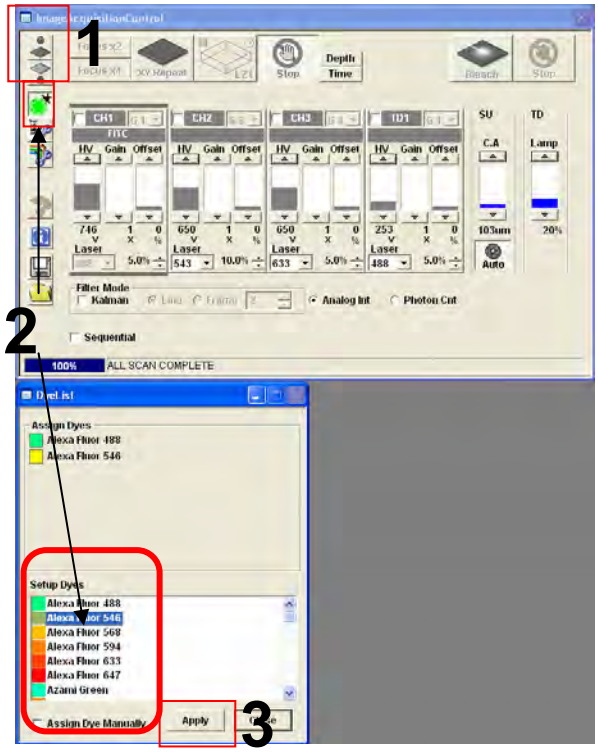
13

Image Acquisition
(Double Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Simultaneous scan



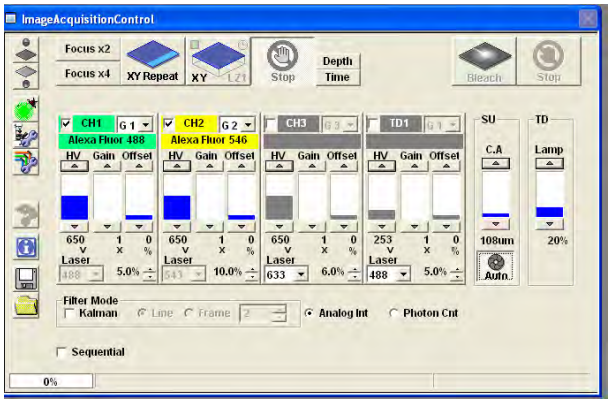
1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click “Apply” button.

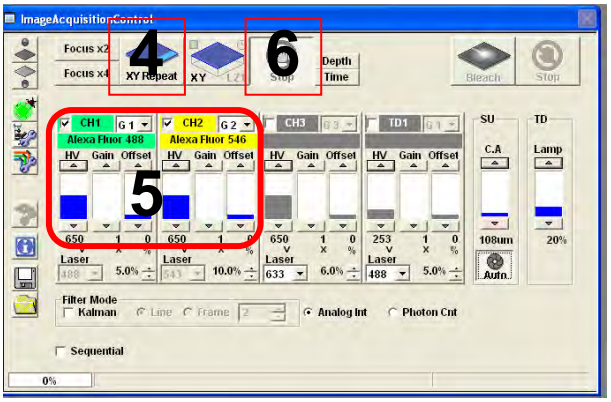
   (The DyeList panel can be closed by using the Close button.)

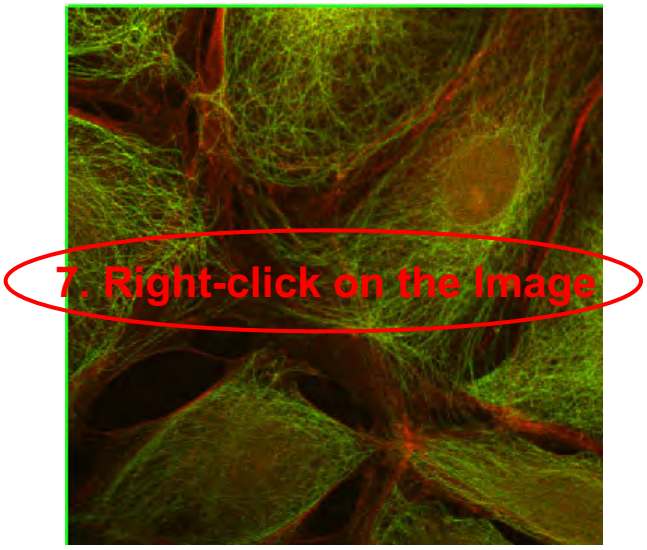


Display after DyeApply is carried out

14

Image Acquisition
(Double Stain on XY Image)





4. Press the XY Repeat button to start scanning.

5. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.
   (The image adjustment is outlined below. For more information, refer to Appendix 1.)

6. Press the Stop button to stop scanning and press XY repeat to acquire the image. (Refer to ■Memo■.)

■Memo■
Scan buttons

: Continuous scan

: Stop scan

: Rough scan (Line skipped)

7. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
   (Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

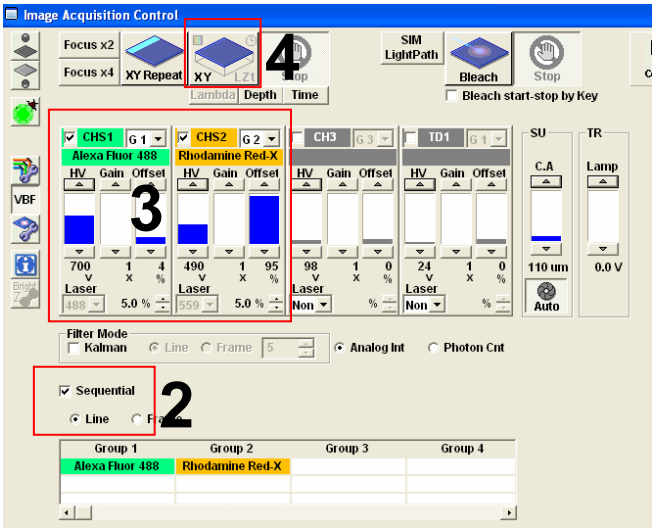
15

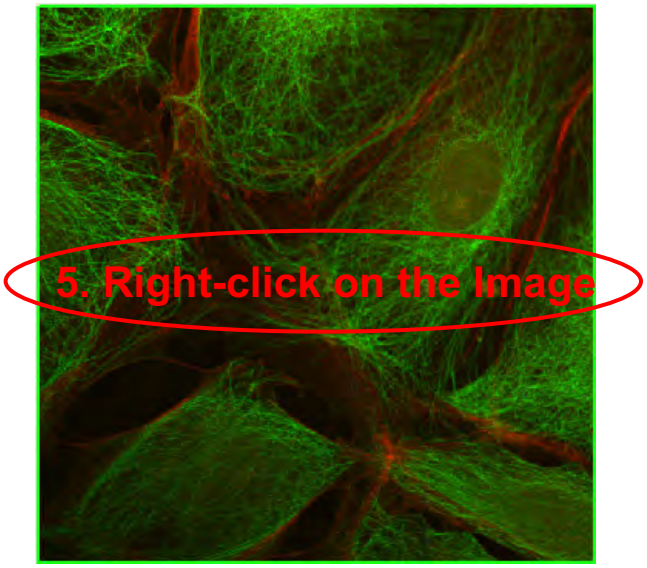
Image Acquisition
(Double Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Sequential scan (Line Sequential is introduced here.)





1. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

2. Check Sequential and select Line.

3. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.

4. Press the XY button to acquire an image.

5. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.) The image is acquired.

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

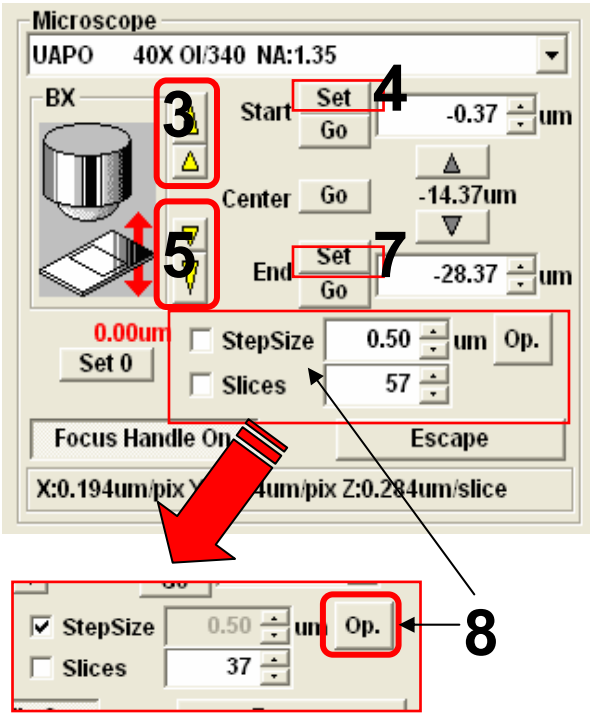
16

Image Acquisition
(Double Stain on XYZ Image)

■■ Acquisition of 3D images (XYZ)
(fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (FITC)
and red fluorescence dye (Rhodamine)

This is the procedure to acquire images
through Line Sequential scanning.



■Memo■

and buttons

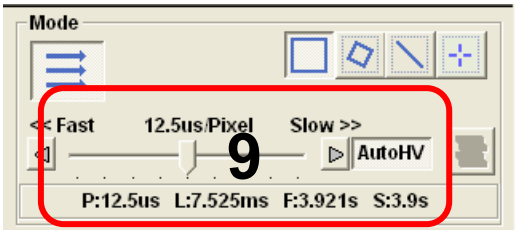
: Moves 1.0μm with a single click.

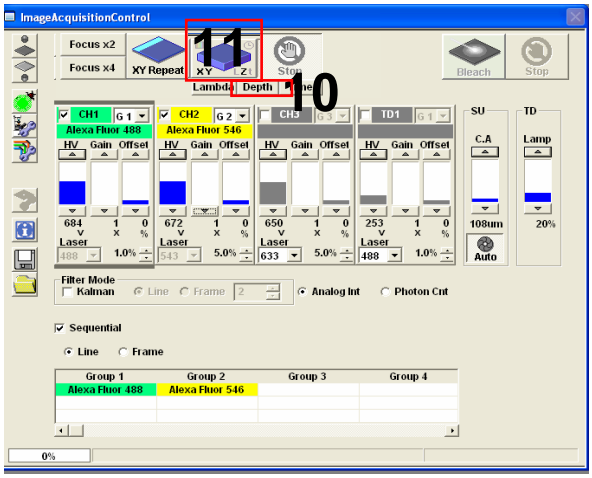
: Moves 0.1μm with a single click.

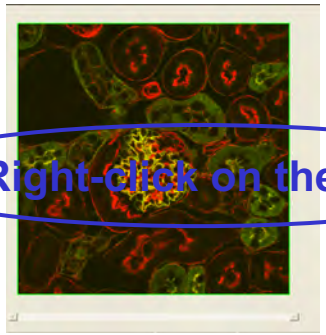
1. Take steps 1 to 7 described on pages 13 and 14.
2. Press the XY Repeat button to start scanning.
3. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
4. When the sample upper limit is displayed on the image, accept it using the Set button.
5. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
6. When the sample lower limit is displayed on the image, accept it using the Set button.
7. Press the Stop button to stop scanning.
8. Enter StepSize, Slice (the recommended value can be referred to by using the Op button), and check the check box

17

Image Acquisition
(Double Stain on XYZ Image)







9. Select AutoHV and then select ScanSpeed.

10. Select Depth.

11. Press the XYZ button to acquire an image.

12. Click on “SeriesDone”, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

13. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

18

Image Acquisition
(Four Stain on XY Image)

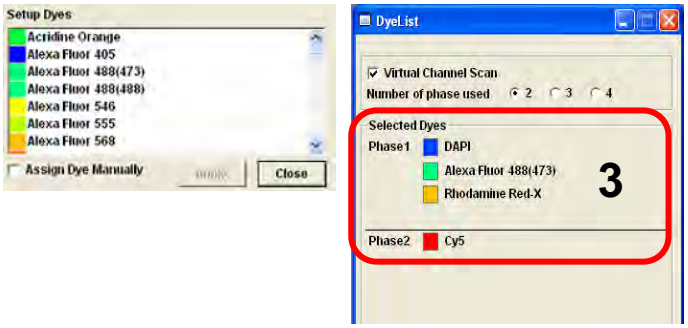
■■ Acquisition of 4 stain images (XY)
(fluorescence image only) ■■

Sample: Four stain of Blue fluorescence dye (DAPI) ,green fluorescence dye (Alexa488) and red fluorescence dye (Rhodamine), far-red fluorescence dye (Cy5)

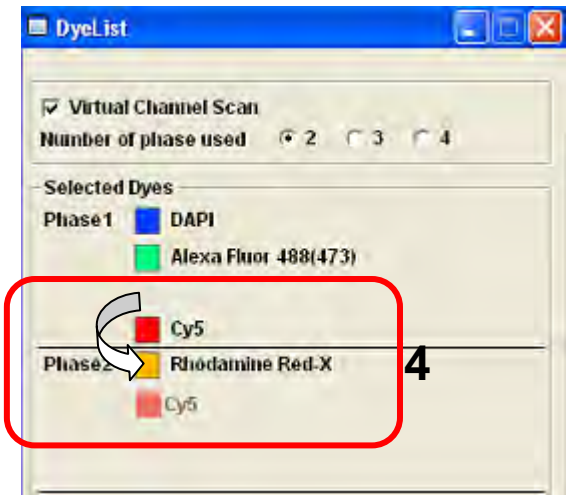
This is the procedure to acquire images
through Virtual Channel scan

1. Virtual Channel Scan Select Virtual channel Scan on the DyeList, and then "**Virtual Channel Controller**" is automatically turned on.

2. Select a number of Virtual Channel from "**Number of phase used**".



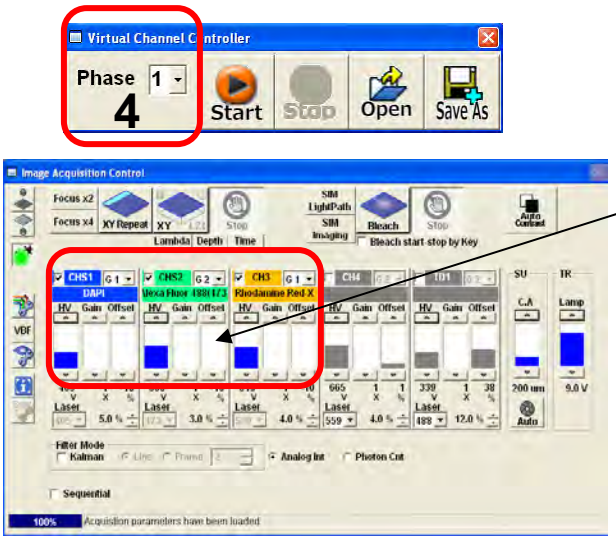
3. Select 4dyes from DyeList 4th dye is registered in "**the Phase 2**".

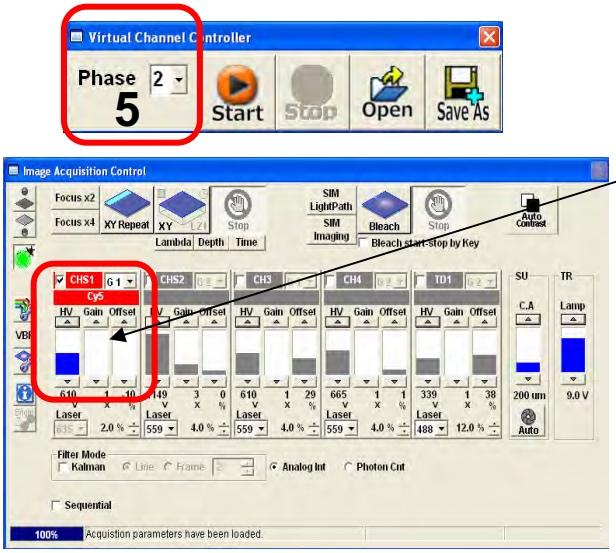


* **RodaminRed is able to be registered on "Phase2" to drag.**

19

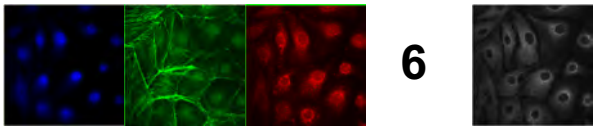
Image Acquisition
(Four Stain on XY Image)





Adjust the image at each phases

“Phase1” **“Phase2”**



6

4. Select “Phase1”, “DAPI”, “Alexa488”, “RhodaminRed” are registered on ImageAquisitionControl.

*** Slit and Filter, DM are automatically set for “DAPI” “Alexa488” “PhodaminRed”**

5. Select “Phase2”, Cy5 is registered on ImageAquisitionControl

*** Slit and Filter, DM are automatically set for “Cy5”**

6. Adjust the image to click 

“XY Repeat” at each phases

* If acquire XYZ image, be able to decide upper limit and bottom limit , slices, step size of Z axis at both phases.

20

Image Acquisition
(Four Stain on XY Image)

7

7. Click 

on Virtual Channel Controller to acquire the image.

* Be able to start at each Phase.

8. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

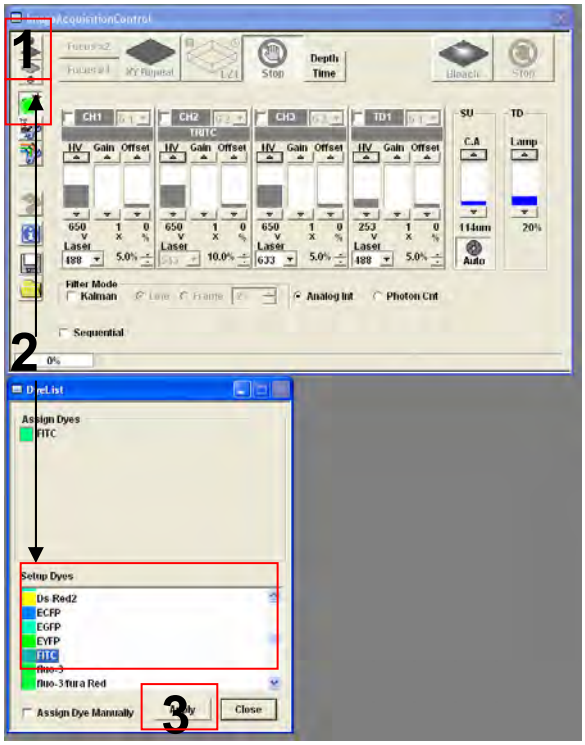
OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

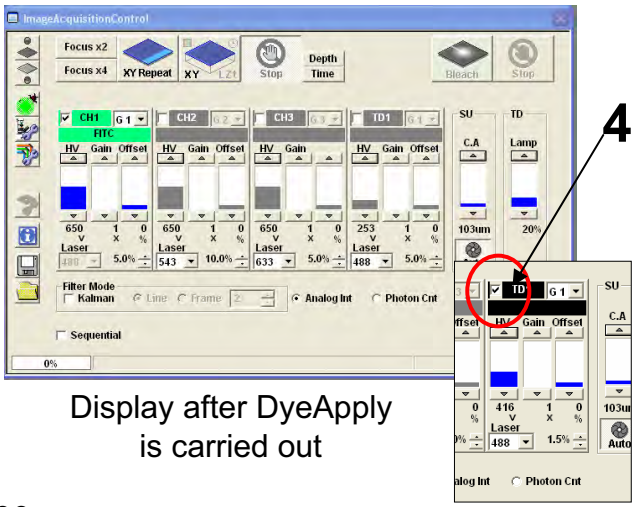
21

Image Acquisition
(Single Stain + DIC on XY Image)

■■ Acquisition of a single image (XY plane)
(fluorescence image and differential interference contrast image) ■■

Sample: Green fluorescence dye (FITC) and differential interference contrast image





Display after DyeApply is carried out

1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

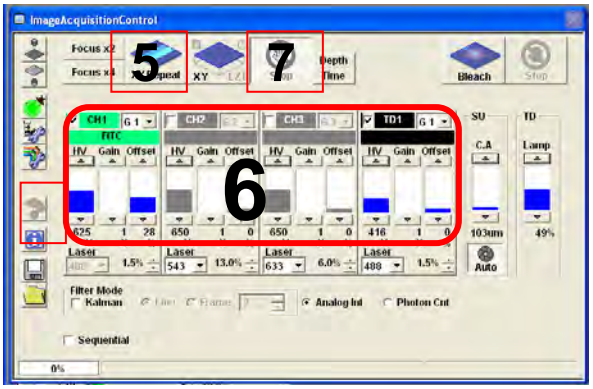
3. Click on the Apply button.

   (The DyeList panel can be closed by using the Close button.)

4. Check TD1.

22

Image Acquisition
(Single Stain + DIC on XY Image)



5. Press the "**XY Repeat**" button to start scanning.

6. Adjust the green (FITC) image and the differential interference contrast image.

7. Press the "**Stop button**" to stop scanning.

8. Press the "**XY button**" to acquire an image.

9. Click on "SeriesDone", and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

10. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

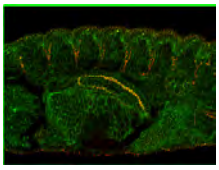
OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

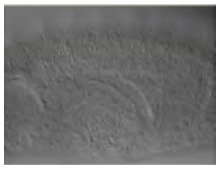
23

Merge the images between fluorescent XY image and DIC image

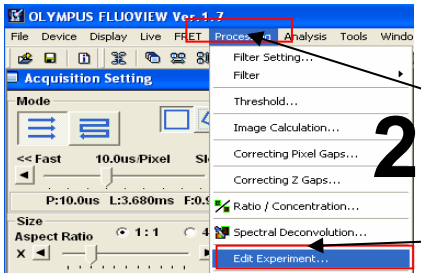
Edit different each files to the same file. This is available for making merge image Between fluorescent image and focused DIC image.



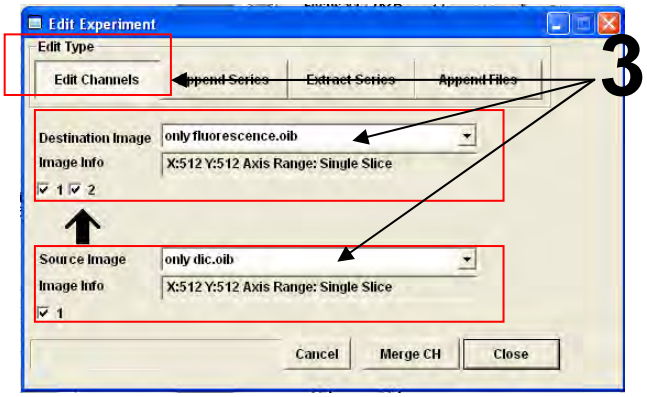
1



1. Open fluorescent image and DIC image.

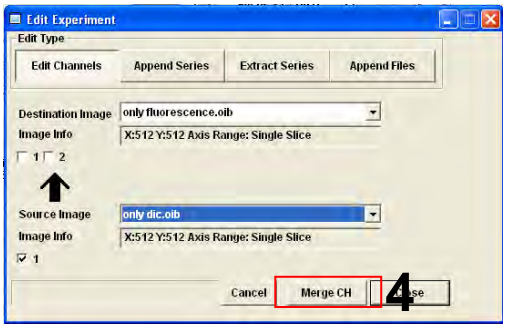


2. Select **Edit experiment** from **Processing**

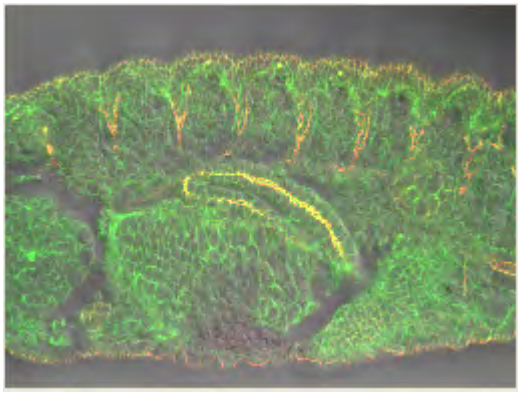


3. Click **Edit Channels,** and select fluorescent image file at **Destination Image** , select DIC image at **Source Image** .

* Check Image Info to make the. merge file. If all channels are checked, all channels are reflected in the new merged file.



4.Click **Merge CH** ,and then the fluorescent image and the DIC image are merged as the new file .



5

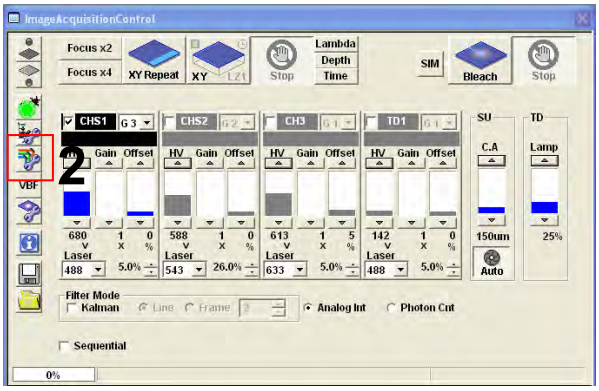
5. Merged image between the fluorescent image and the DIC image.

24

Image Acquisition (Spectral Image on XYL Image)

■■ Acquisition of a spectral image (XYL) ■■

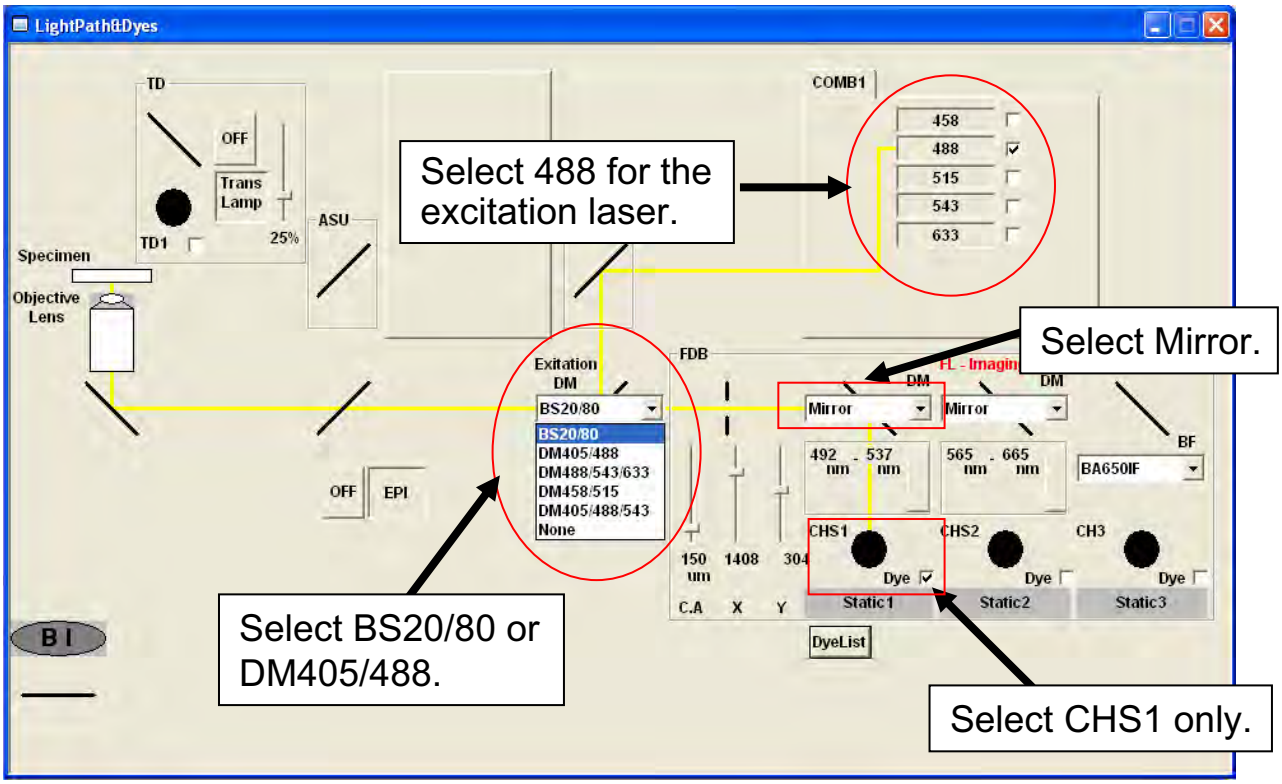
Sample: Double stain of green fluorescence dye (Alexa Fluor 488) and green fluorescence dye (YOYO-1)



1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

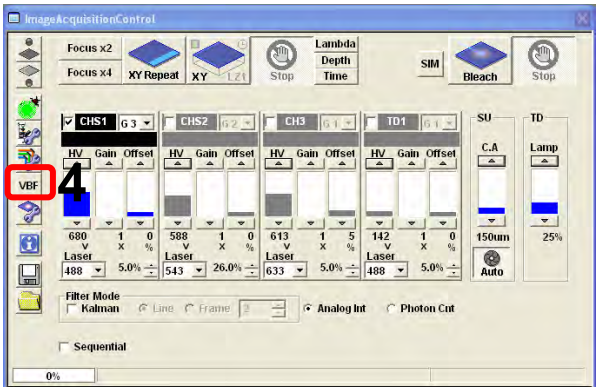
2. Click on the  button to view the optical path diagram.

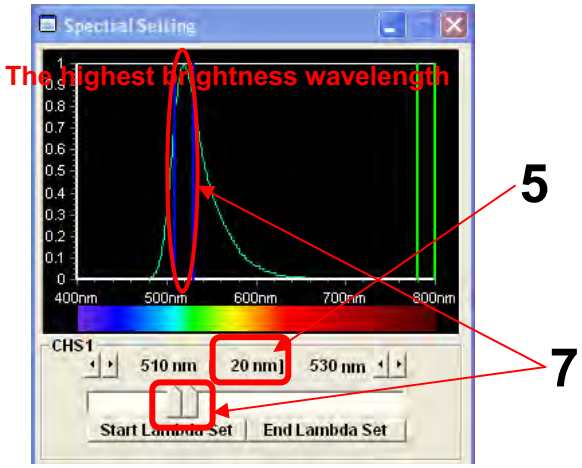
3. Make settings as shown below.

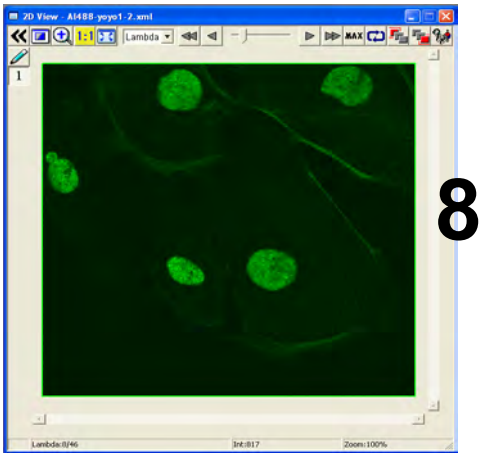


25

Image Acquisition (Spectral Image on XYL Image)







4. Click on the VBF button, and the Spectral Setting window appears.

5. Set the slit width for CHS1 to 20 nm, for example.

6. Press the XY Repeat button to start scanning.

7. While observing the image, Click the left side of slit and drag to the point which the highest brightness is achieved.

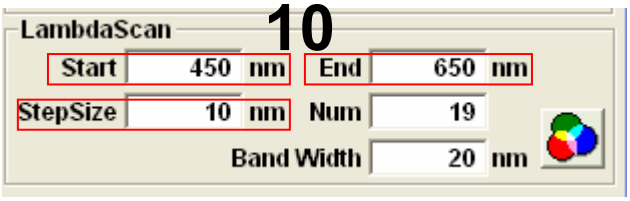
   Note: Move the slit position only while keeping the slit width at 20 nm.

8. Adjust the image on the highest brightness.

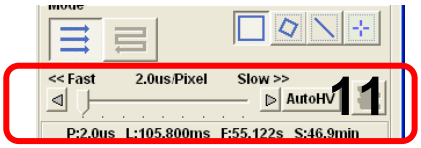
9. Press the Stop button to stop scanning.

26

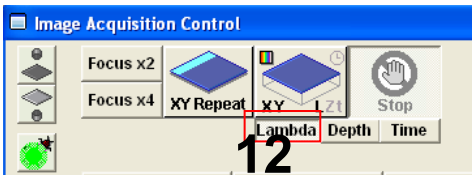
Image Acquisition (Spectral Image on XYL Image)



10. Set the range of wavelength to be acquired, the slit width and the step.
    - Start = Start wavelength
    - End = End wavelength
    - Resolution = Slit width
    - StepSize = Step



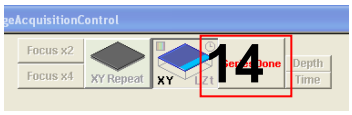
11. Select AutoHV and then select ScanSpeed.
    *As the scan speed becomes slower, noise can be removed while maintaining the current brightness.



12. Select Lambda.

13. 

Press the XYZ button to acquire an image.



14. Click on SeriesDone, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

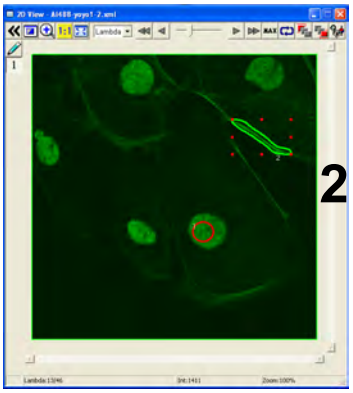
27

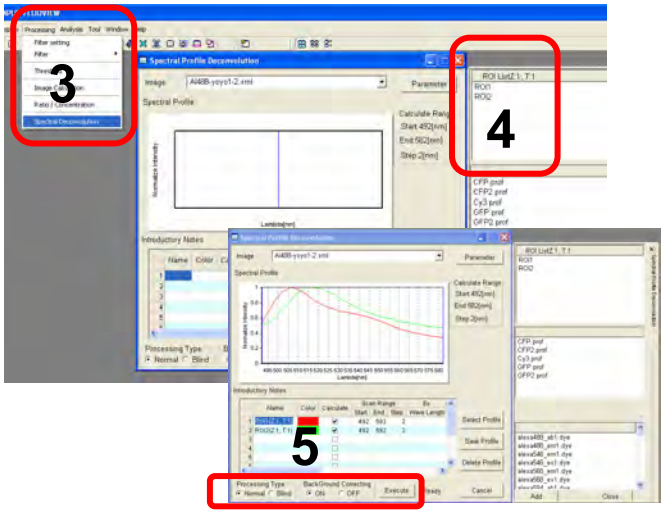
Image Analysis (Unmixing)

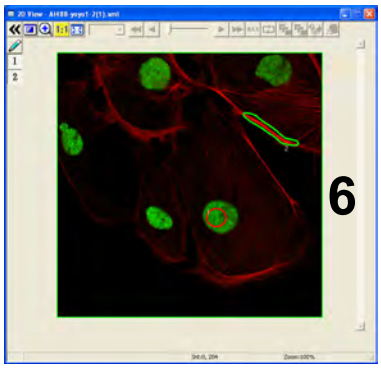
I. When each fluorescence dye point is clear

From an XYL image where fluorescence dyes with similar fluorescence spectrums are present together, derive the fluorescence spectrum for each fluorescence dye and obtain an unmixed image based on the fluorescence spectrums.

Sample: Double stain of green fluorescence dye (Alexa Fluor 488) and green fluorescence dye (YOYO-1)







Unmixed image

1. Open an XYL image file with both Alexa Fluor 488 and YOYO1 applied.
2. Enclose a point dyed with Alexa Fluor 488 only and a point dyed with YOYO1 only.
3. From Processing on the menu bar, select Spectral Deconvolution.
4. Double-click on ROI1 and ROI2.
5. Check that the Processing Type is set to "Normal" and click on Execute.
6. An unmixed image is obtained.

Indicates channel assignments of unmixed images

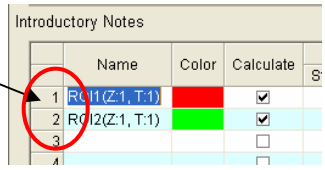


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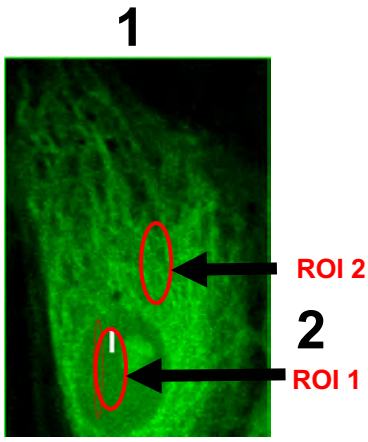
Image Analysis (Unmixing)

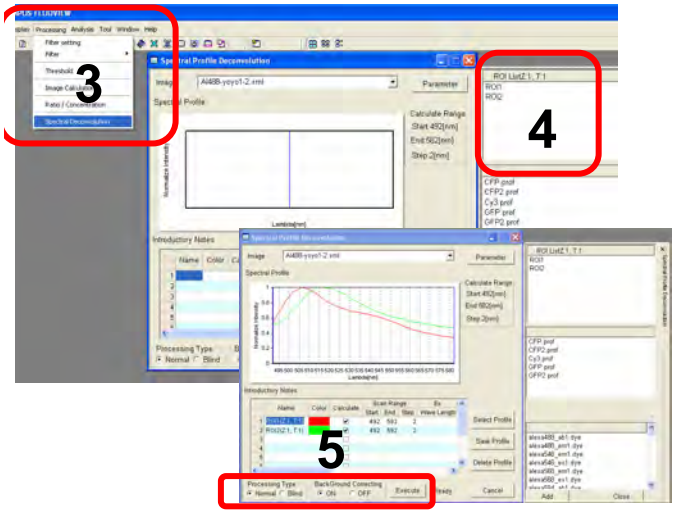
I. When each fluorescence dye point is clear

Sample: single stain of green fluorescence dye (GFP)
and auto fluorescence from cell



1. Open the XYL image (GFP + auto fluorescence).

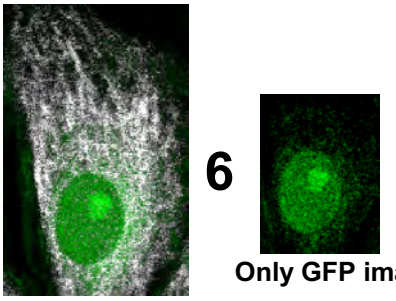
2. Enclose a point dyed with GFP only and a point dyed with auto fluorescence only.



3. From Processing on the menu bar, select Spectral Deconvolution.

4. Double-click on ROI1(GFP) and ROI2(Auto fluorescence).

5. Check that the Processing Type is set to "Normal" and click on Execute.



Only GFP image

6. An unmixing image is obtained.

   Green color is GFP.
   Gray color is Auto fluorescence.

Unmixing image between GFP and Auto fluorescence

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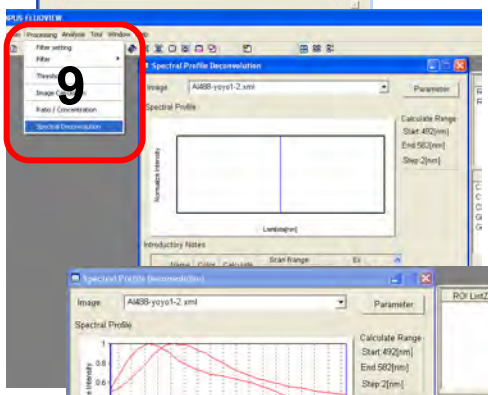
Image Analysis (Unmixing)

II. When a control sample is used

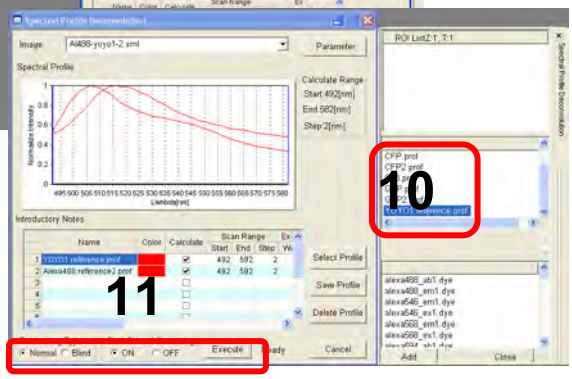
From an XYL image with a single type of fluorescence dye, derive the fluorescence spectrum of the dye and obtain an unmixed image based on the fluorescence spectrum.

Sample: Double stain of green fluorescence dye (Alexa Fluor 488) and green fluorescence dye (YOYO-1)

8. Open an XYL image file with both Alexa Fluor 488 and YOYO1 applied.

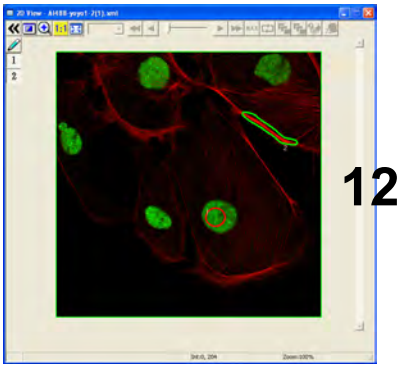


9. From Processing on the menu bar, select Spectral Deconvolution.



10. Double-click on Alexa Fluor 488 and YOYO1 (which have been registered) in the database of fluorescence spectrums.

11. Check that the Processing Type is set to "Normal" and click on Execute.



12. An unmixed image is obtained.

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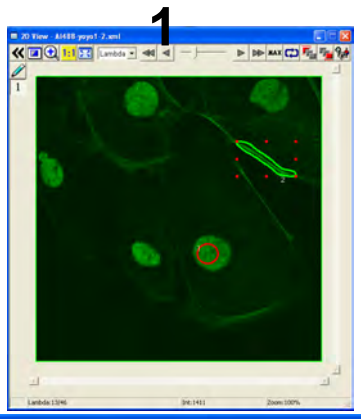
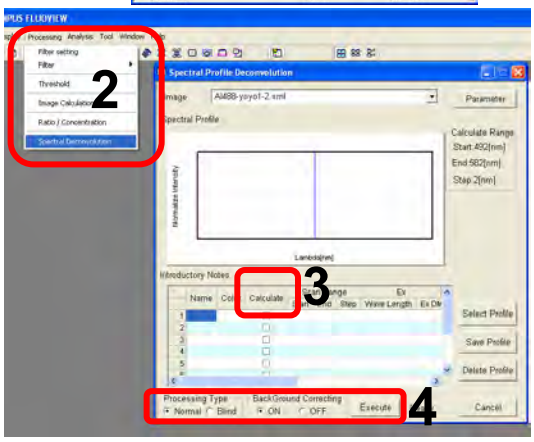
Image Analysis (Unmixing)

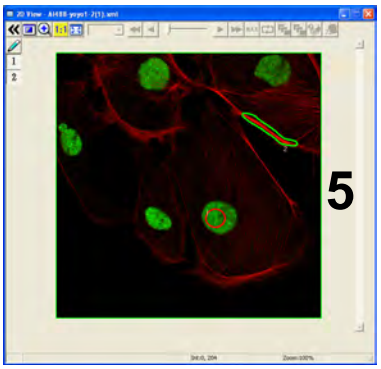
III. When only the number of types of fluorescence dyes is known (Blind Unmixing)

From an XYL image where fluorescence dyes with similar fluorescence spectrums are present together, obtain an unmixed image based on only the number of types of fluorescence dyes.

Sample: Sample with two unknown types of fluorescence dyes







Unmixed image

1. Open an XYL image file for a sample that has two unknown types of fluorescence dyes.
2. From Processing on the menu bar, select Spectral Deconvolution.
3. Click on two Calculate check boxes. (Click on three boxes when three types of fluorescence dyes are used.)
4. Check that Processing Type is set to "Blind" and click on Execute.
5. An unmixed image is obtained.

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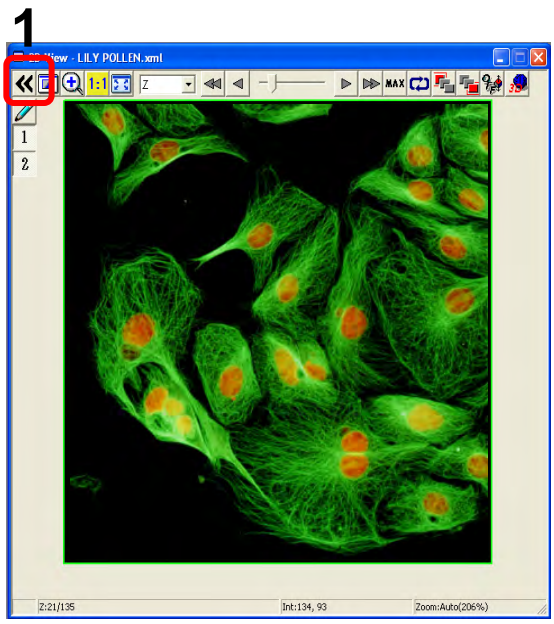
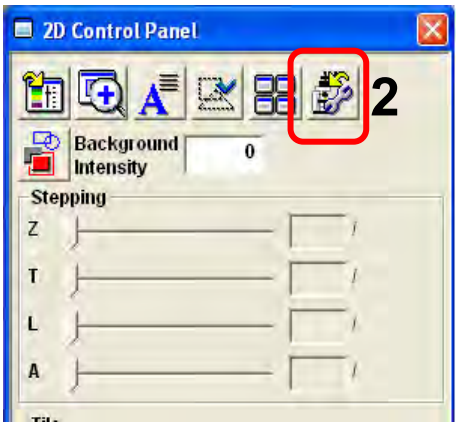
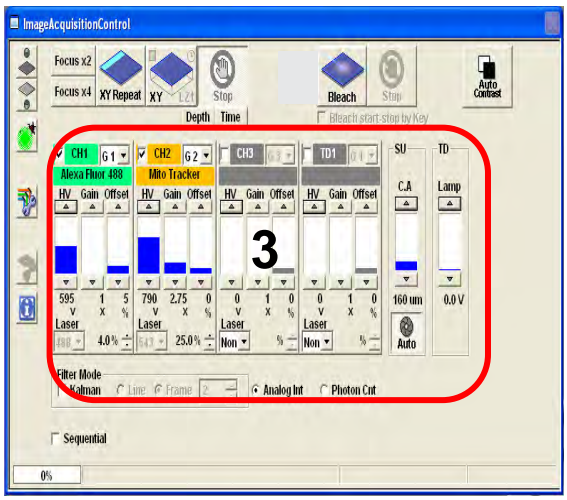
Reload the image conditions



1. Open the file and click 



2. Click 



3. The conditions (HV,Offset, CA and so on) are reloaded .

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Overview of the 2D Operation Panel

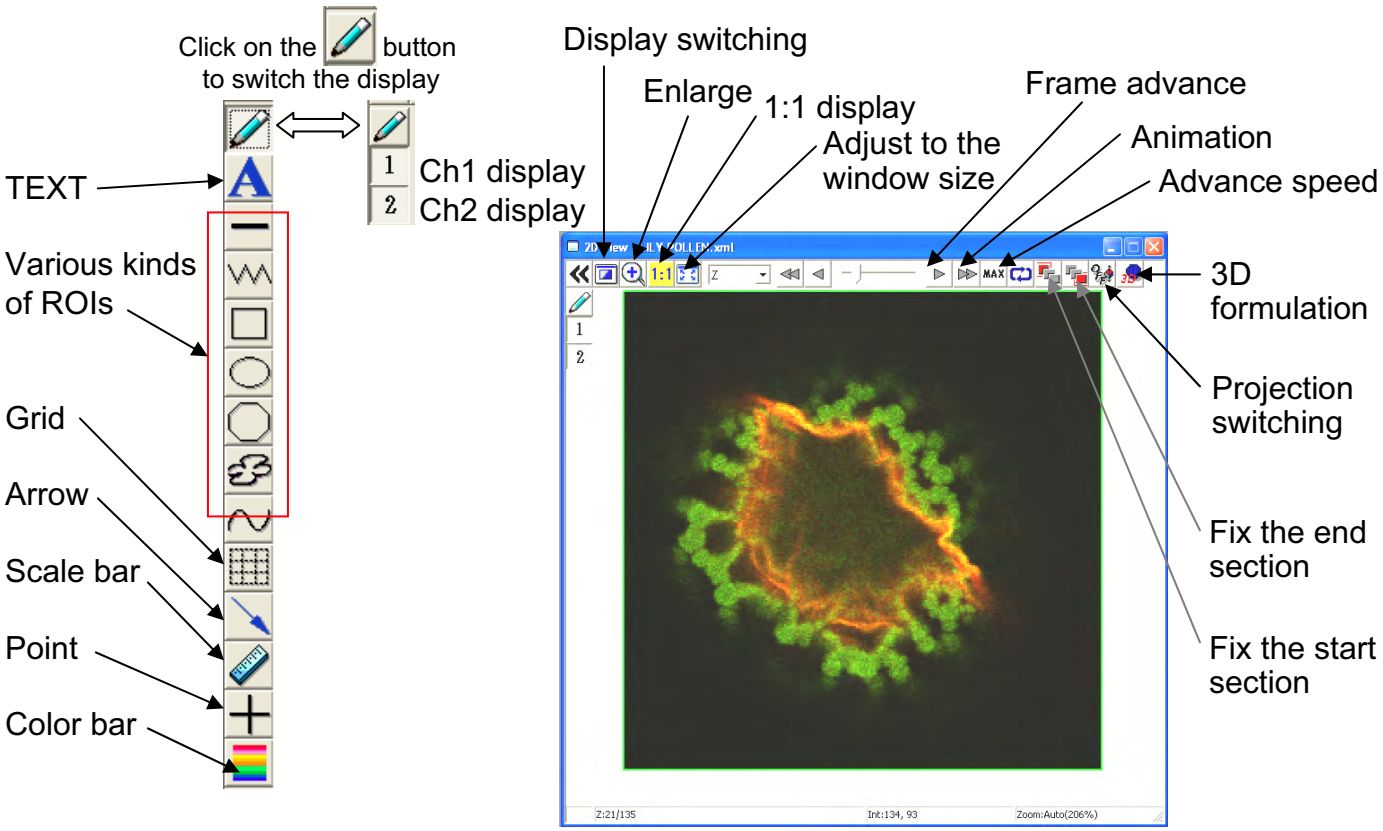
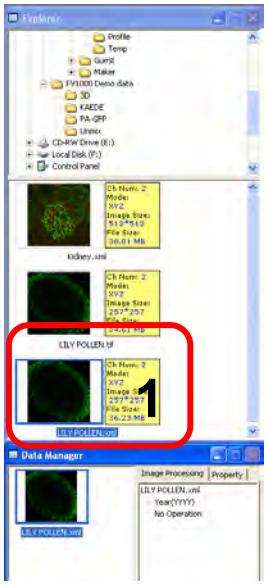


Image Analysis (Opening a File)



1. Double-click on a file to be opened from Explorer.

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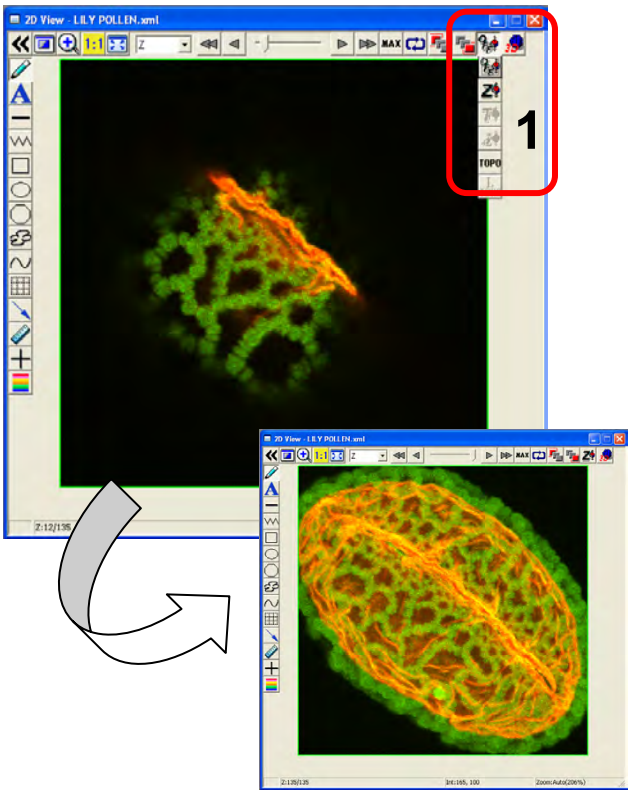
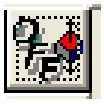
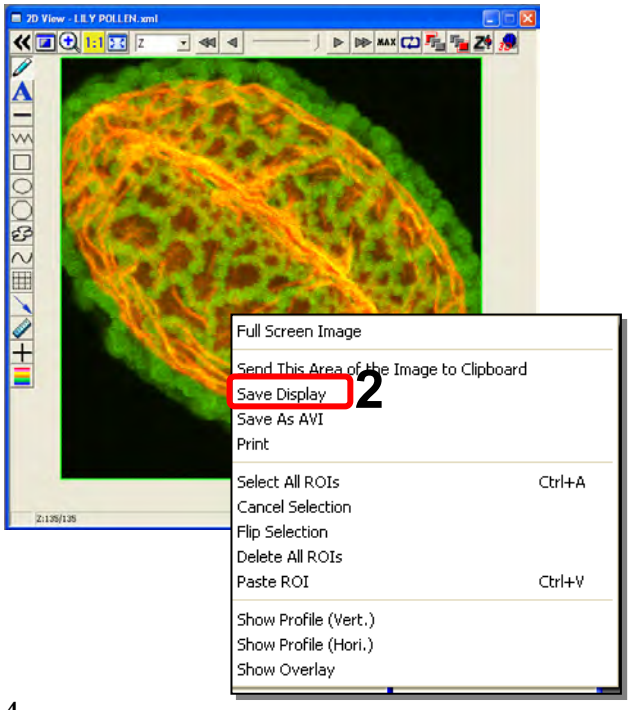
Image Analysis (Acquire a Projection Images)



1. Click on the  button to select  .



2. To save this image, right-click on the image, select Save Display and save the image with a new name.

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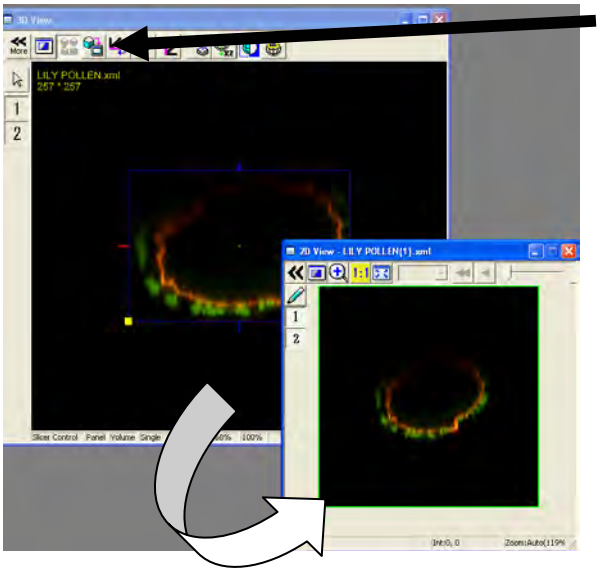
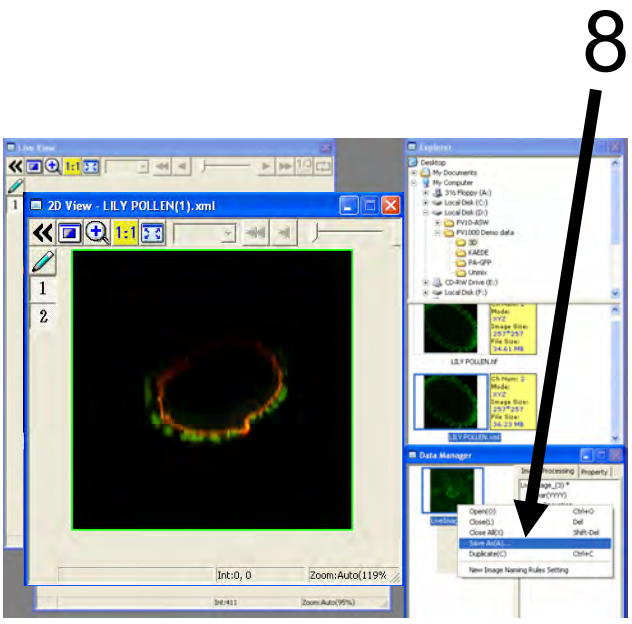
Image Analysis
(Save a Z section Image as 2D file)



Save the image in step 3 or 5

6. Click on the  button.

7. A 2D View-(file name) image is created.



8. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as type "xml" is a file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

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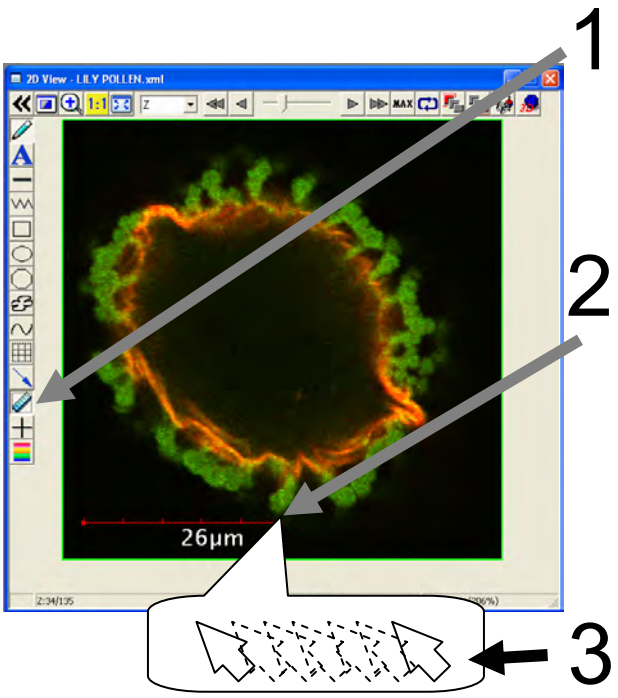
Image Analysis (Inserting the Scale Bar)

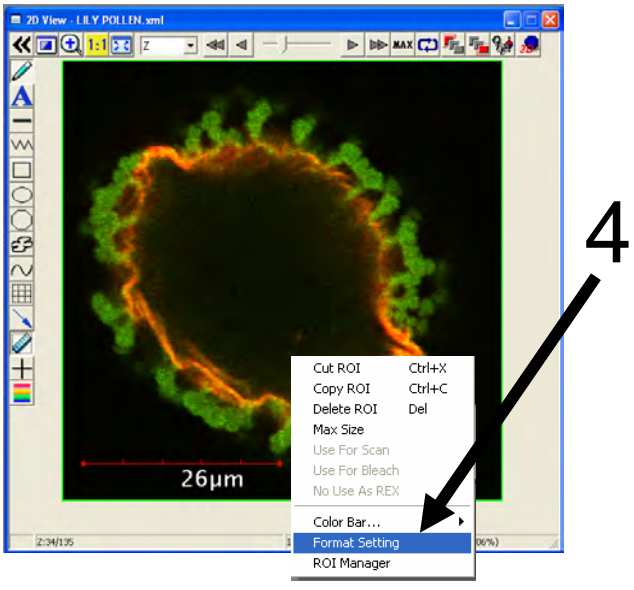


1. Click on the  button.

2. While left-clicking the image, drag and drop it at a certain point.

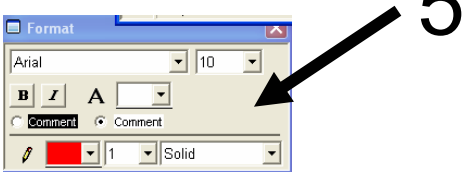
Change the size

3. While clicking the right or left handle, move the mouse from side to side.



Change the text size, color, style, etc.

4. Select Scale Bar and then right-click on Scale Bar to select Format Setting.



5. Change the setting in this window as required.

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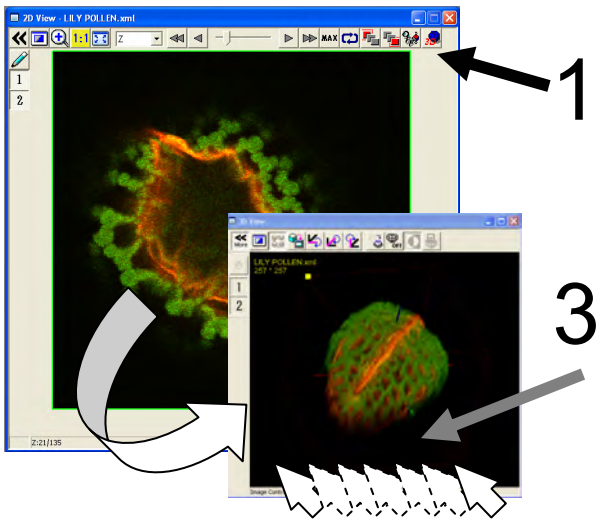
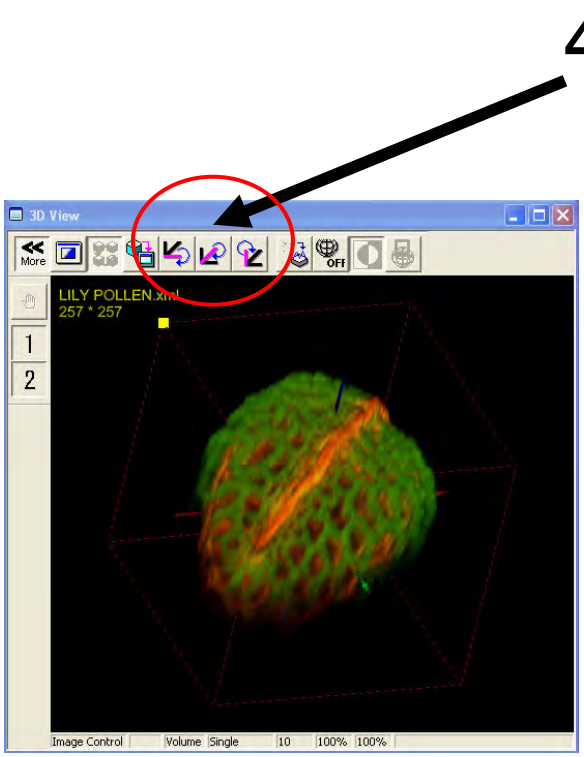
Image Analysis
(Rotating a Three-dimensional Image)



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

2. A 3D view is created.

3. Drag the mouse on the image to observe it at a certain angle.



Simple animation

4. Press and hold the  button to rotate the image around the X-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Y-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Z-axis. Press it again to stop rotation.

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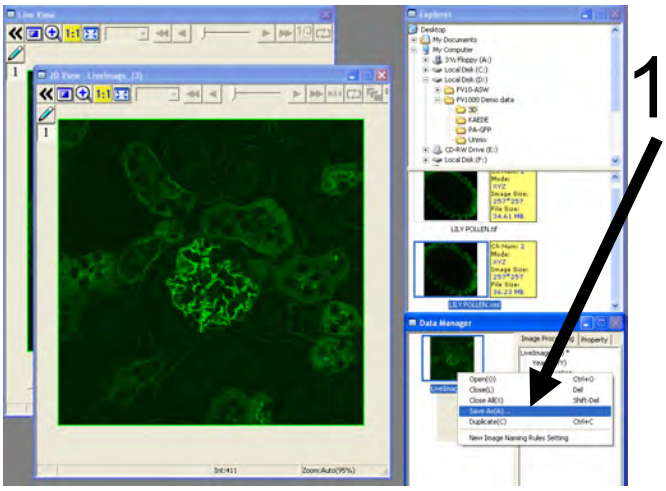
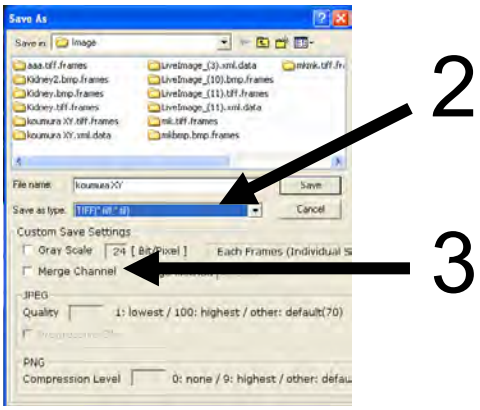
Image Analysis (Saving an Image)



Convert each channel of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to RGB color.
4. Save the image.

* BMP and JPEG formats are also selectable.

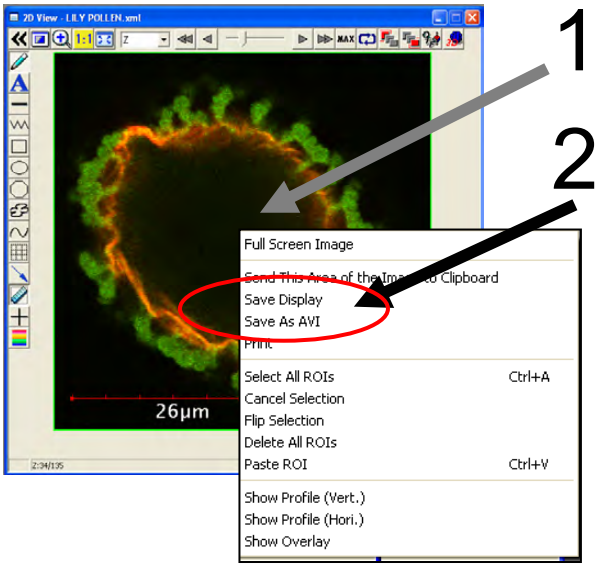


Convert a merge image of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to Merge Channel.
4. Save the image.

* BMP and JPEG formats are also selectable.

****



Convert an image with the scale bar inserted into a BMP format

1. Right-click on the image.
2. Select Save Display and save the image with a new name.

Convert an animated image into an AVI format

1. Right-click on the image.
2. Select Save as AVI and save the image with a new name.

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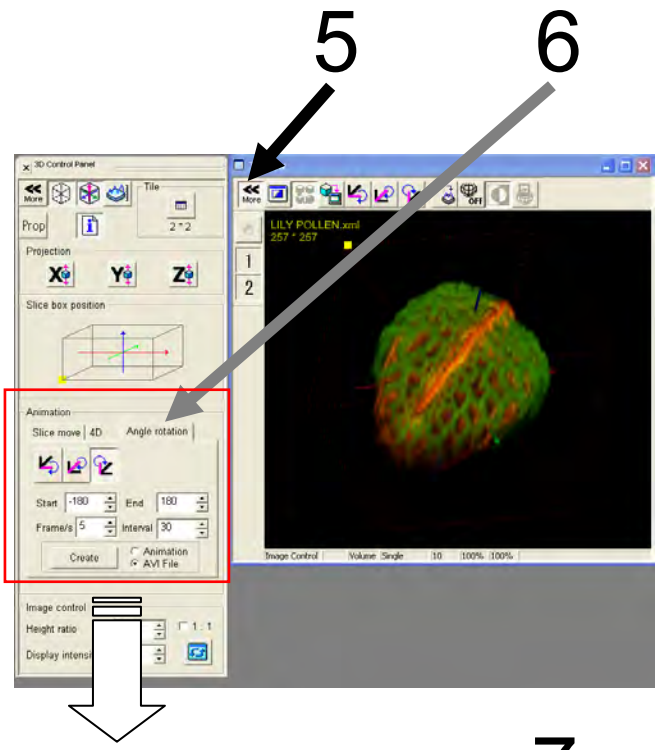
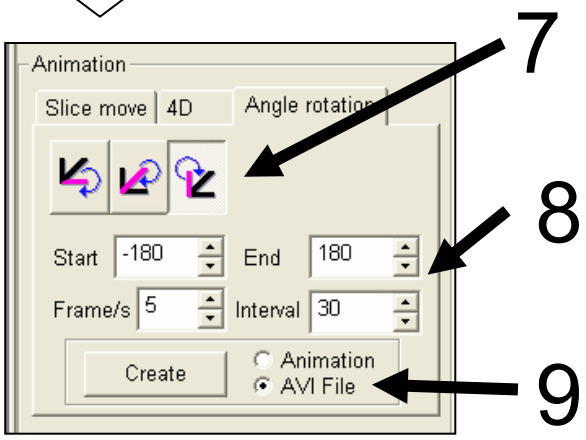
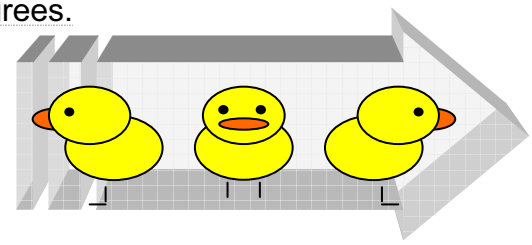
Image Analysis
(Rotating a Three-dimensional animation)





To save a rotation file as an animated image, create three-dimensional images according to the following procedure.

For example, try to rotate an image by 180 degrees.



5. Click on the 

button.

6. Click on the Angle rotation tab.

7. Select the rotation axis.

8. Enter the rotation angle.

Start =	Angle to start rotation
End =	Angle to stop rotation
Frame/s =	Rotation speed
Interval =	Degrees to be rotated at a time

9. Select AVI File and click on Create.

10. Enter a file name and click on Save.

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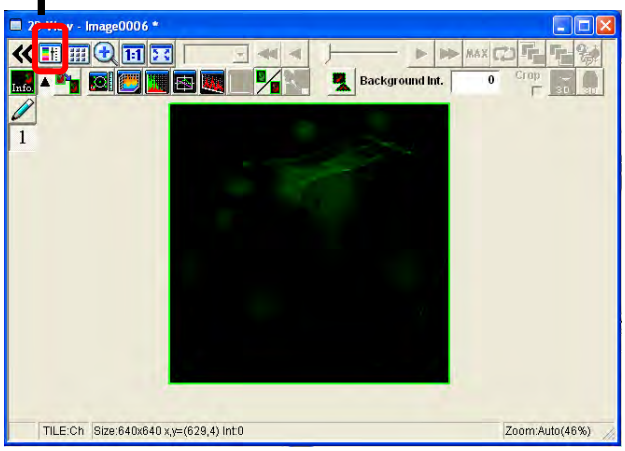
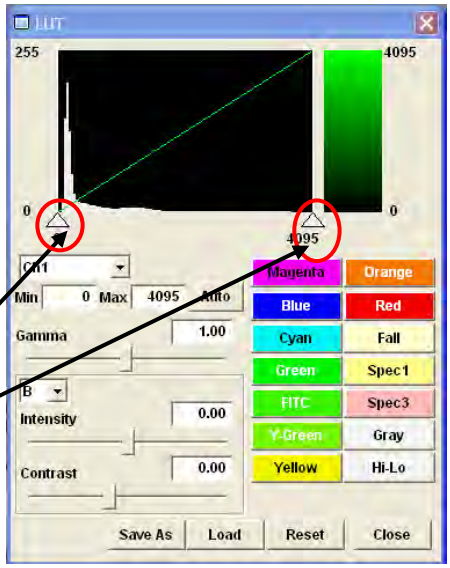
2D Image Analysis (Edit the image color and contrast)



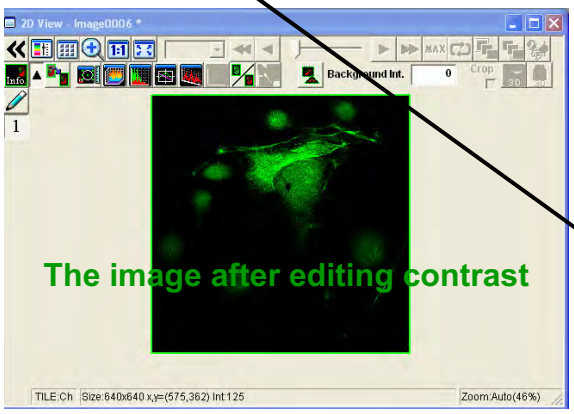
1. Click  "LUT" and then LUT table appears below,

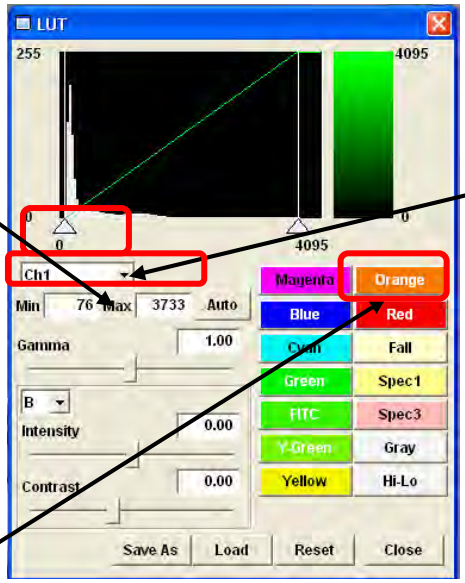


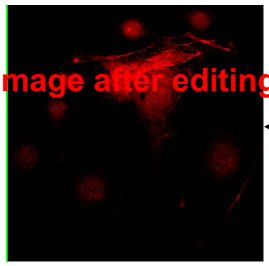
2.Edit contrast to drag  to left or right side, and another way to edit contrast is entering value on "Max" and "Min" (Max4095, Min0)

3. Min and Max value are changed and contrast of image is edited.

*** According to get Min value up , be able to reduce noise of the image.**







4. To click another color, be able to Edit a color.
   Above example: Change Green to Red to click Red

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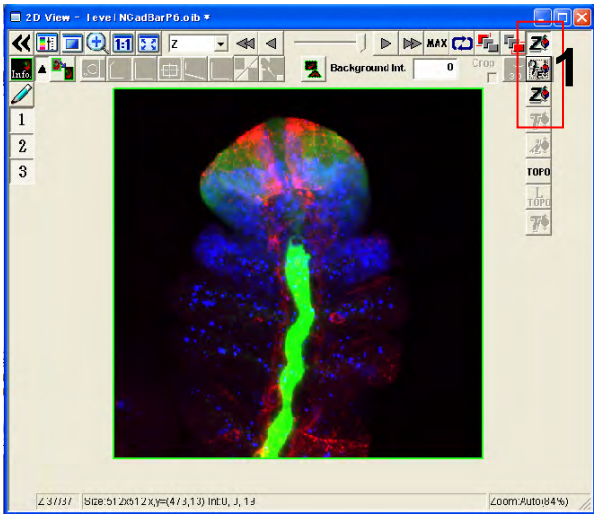
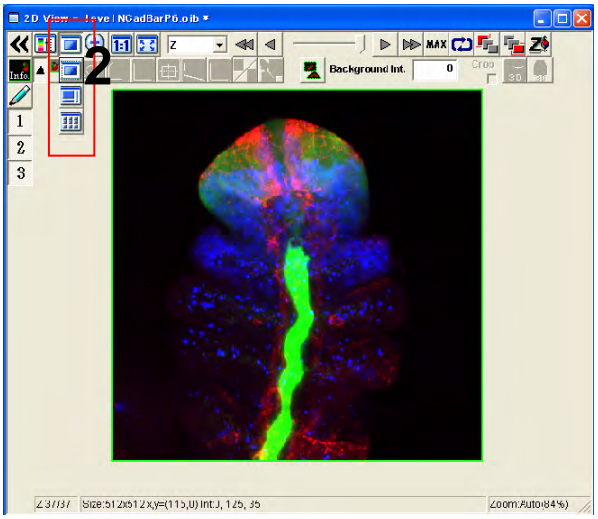
2D Image Analysis (the image of Z section)

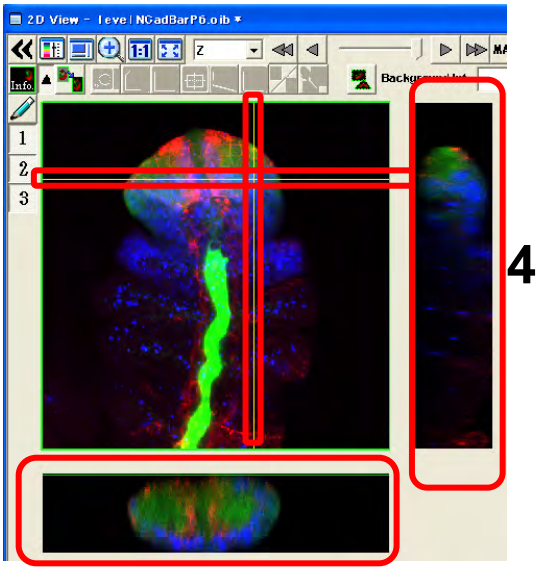


1. Click  and select  again, then Projection image is shown on 2D View after getting XYZ image.



2. Click  and select  .

3. The images of Z section is shown on X axis and Y axis.
   According to Move to left or right side on X axis and to move to ups and down on Y axis, be able to show image of Z section each position.



4. The image of Z section on Y axis.

5. The image of Z section on X axis.

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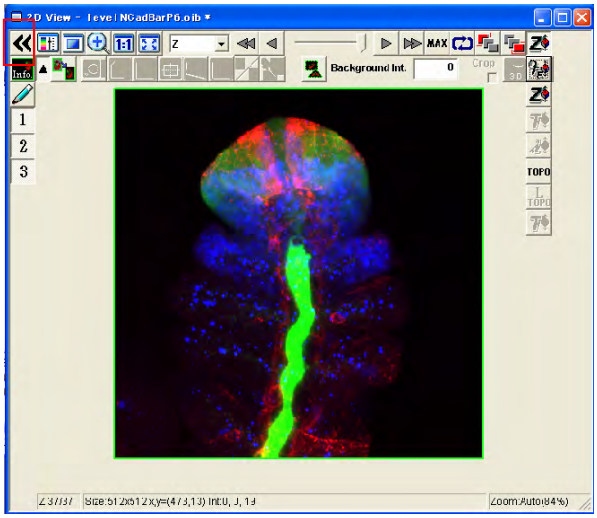
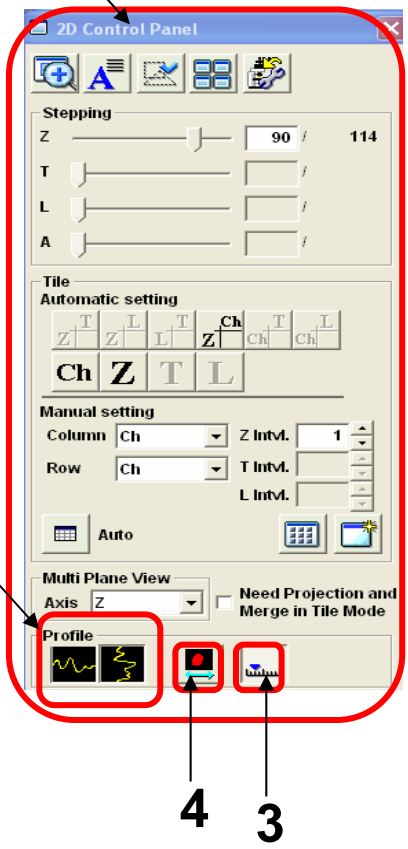
2D Image Analysis (Intensity Profile of each Z sections)

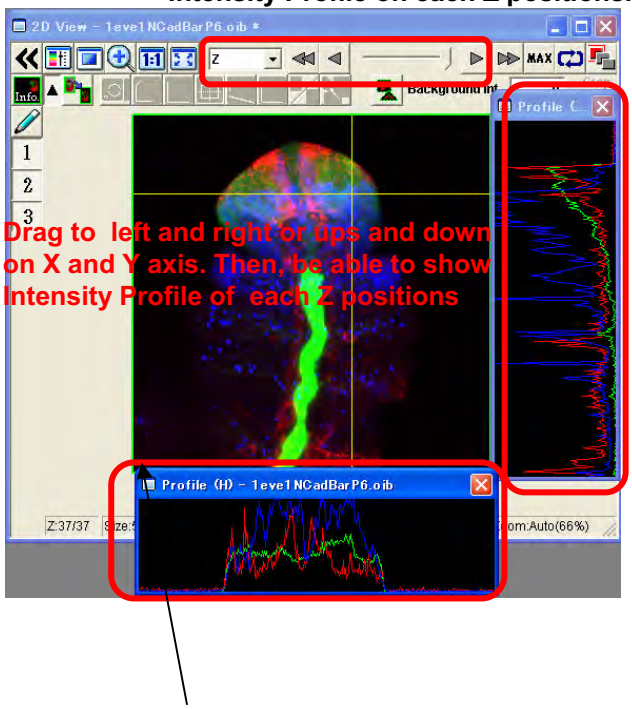


1. Click  and then 2D Control Panel is shown below,



2. Click  "Profile" and then Intensity Profile of each Z sections is shown on the X and Y axis.

To move to Z position ,be able to show Intensity Profile on each Z positions.



3. Click  to show Scale on Intensity Profile

4. According to click  , be able to show as equal scale of Profile window as 2Dimage.

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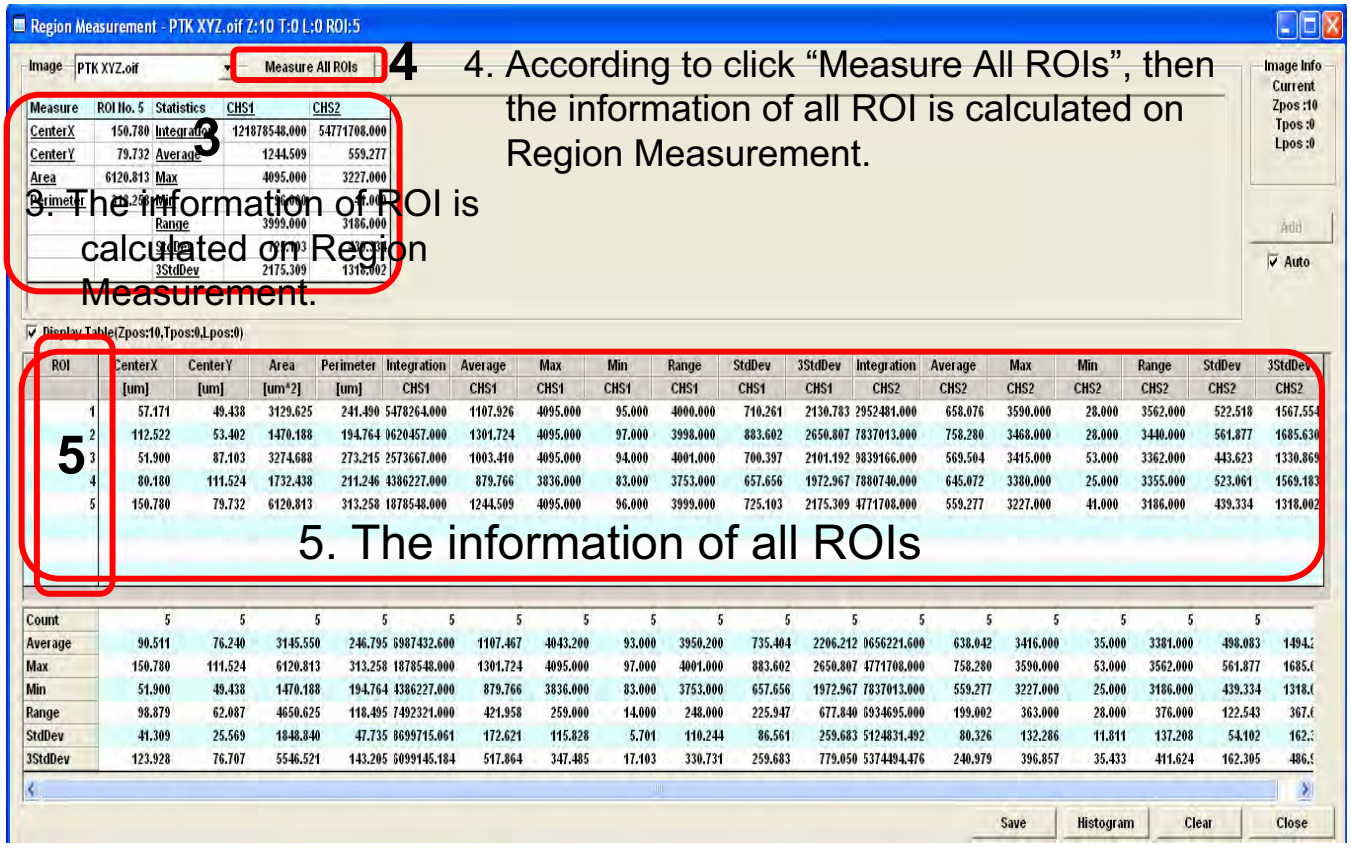
2D Image Analysis (Measure)

1. Enclose interesting regions by ROI

   Line on interesting positions by ROI —

2. Click  “measure”.



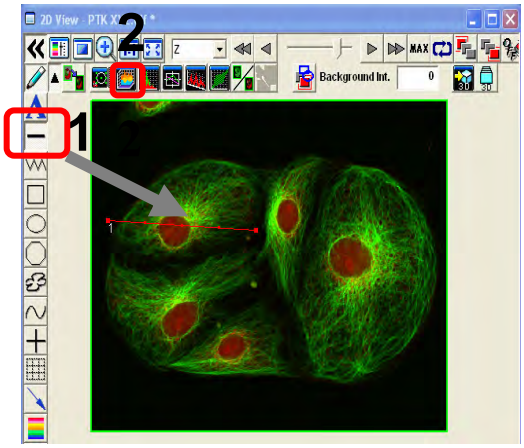
4. According to click “Measure All ROIs”, then the information of all ROI is calculated on Region Measurement.

3. The information of ROI is calculated on Region Measurement.

5. The information of all ROIs

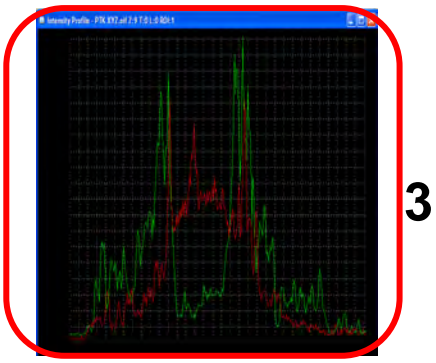
43

2D Image Analysis (Line Intensity Profile on the 2D image)



1. Line on the 2D image by ROI 

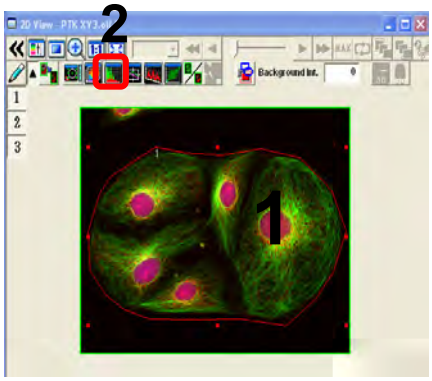
2. Click  "Intensity Profile"



3. "Intensity Profile" on the line is shown as intensity graph .

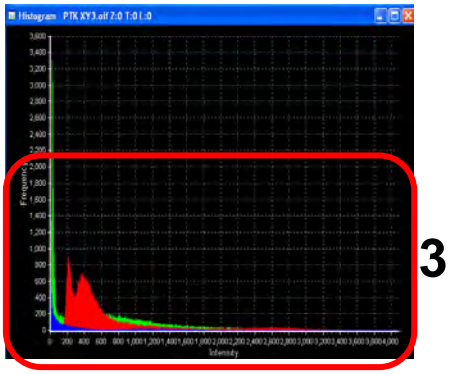
* State of colocalization between each Chs is figured out apart from intensity .

2D Image Analysis (Histogram)



1. Enclose the interested region by ROI.

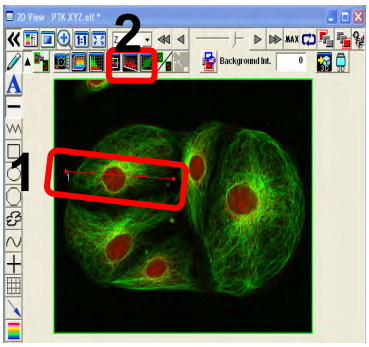
2. Click  **"Histogram"**

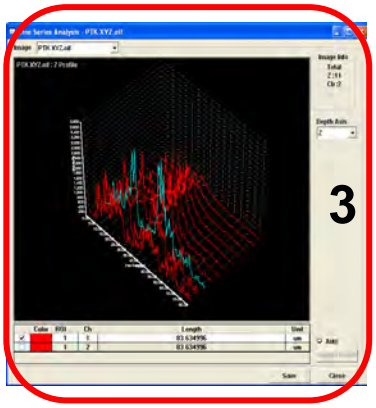


3. "Histogram" window is shown as a graph, frequency of intensity of each pixels is plotted on the region enclosed by ROI.

44

2D Image Analysis (Line Series Analysis)



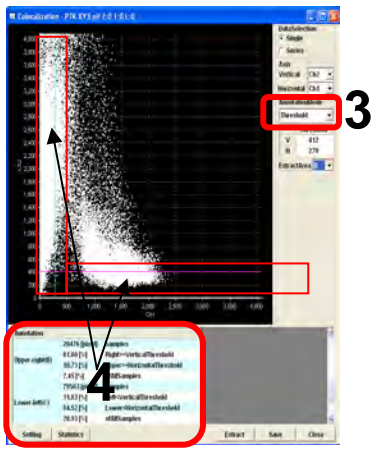


1. Line on the interesting region.

2. Click  “Line Series Analysis”

3. Intensity of Z position/ time on the line is shown as a graph .

2D Image Analysis (Co-localization)



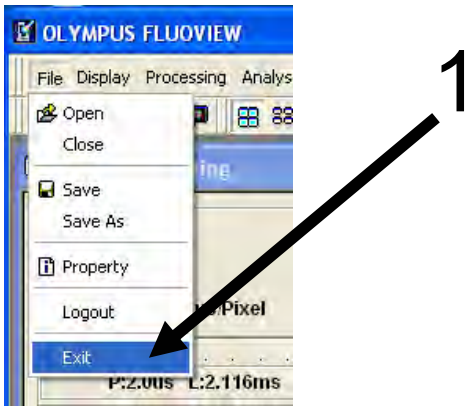
1. Enclose an interesting area by ROI.
2. Click 
3. Select 

Threshold from Annotation Mode.
4. According to move Thresholds of X,Y axis to right and left ,ups and down (Enclose red color X,Y axis), Co-localization result between 2ch is changed .

   Information of Co-localization is listed under the scatter plot.

45

Closing the System



1. Exit the FV10-ASW software by selecting File/Exit.

2. Exit the Windows.

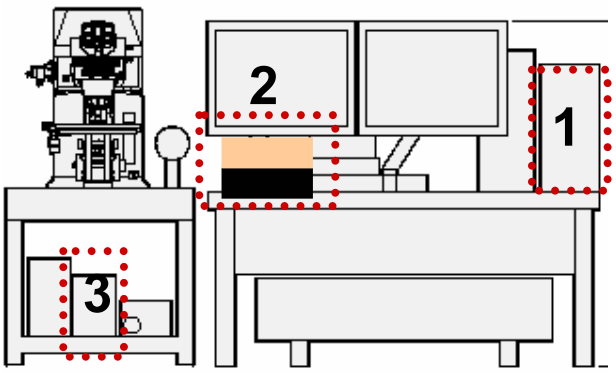
(1) Select Start/Shut Down.
(2) On the Shut Down Window, select Shut Down and click on OK.

3. Turn the laser OFF. (Turn the key switch to the OFF position.)

3-1. LD559nm OFF

3-2. Multi Ar (458 nm, 488 nm, 514 nm) OFF

3-3. HeNe (G) (543 nm) OFF



4. Turn the mercury burner power OFF.

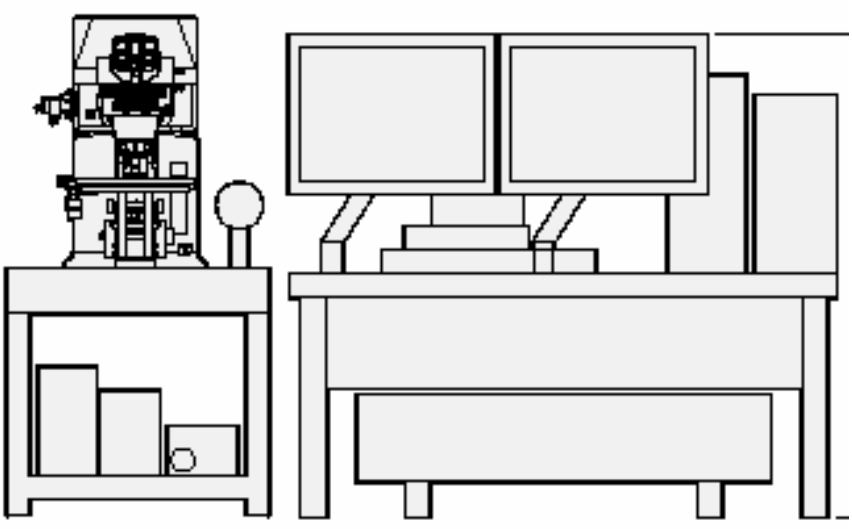
46

Laser Conforcal Scanning Microscope

FV1000D Filter Type

(Upright Microscope BX61)

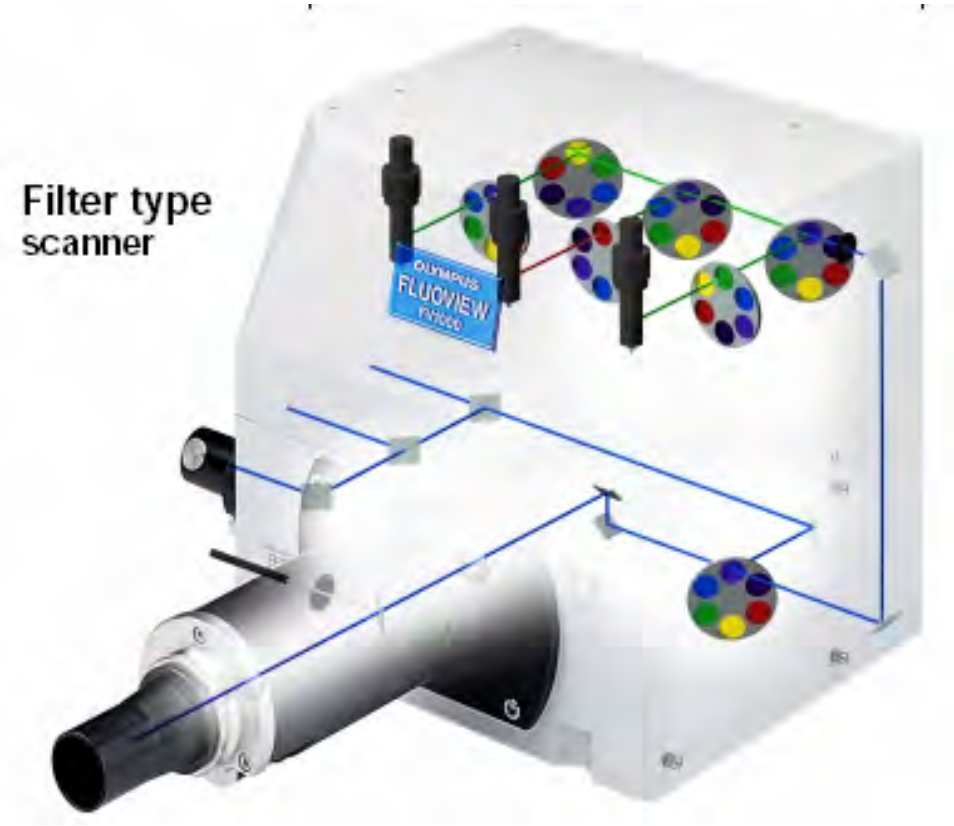
Operation Manual



1

Contents

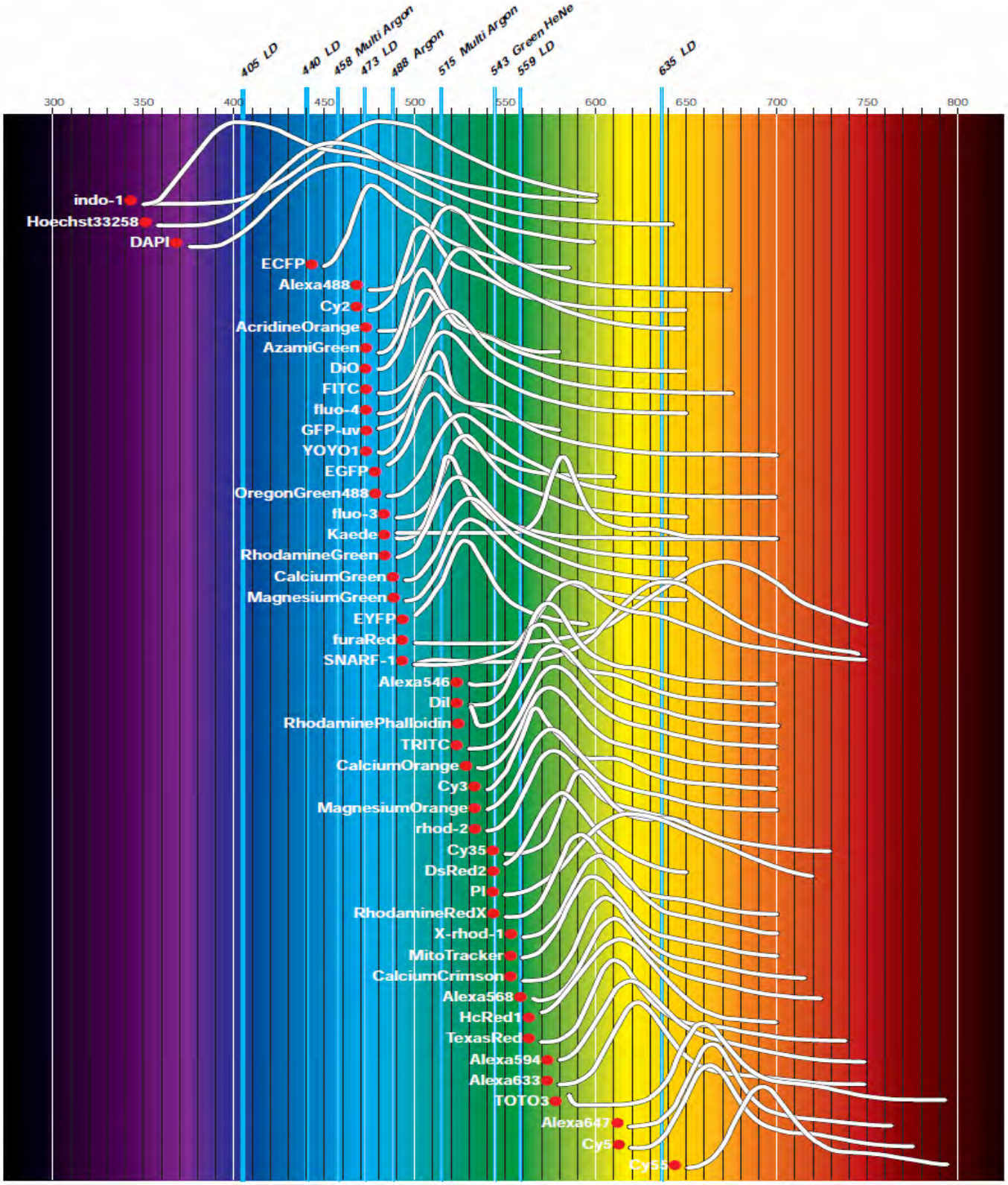
Filter Type Main Scanner



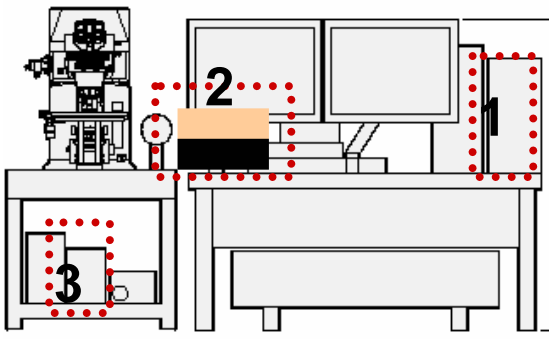
3

Dye List (FV1000D Lasers are available below)

LD405nm LD440nm LD473nm LD559nm LD635nm
Ar458nm Ar488nm Ar515nm
HeNe(G)543nm



System Preparation



1. Turn the computer ON.
   [In case of equipped concentrated power supply, power on it first]

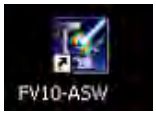
2. Turn the laser ON
   (Turning the key switch)
   2-1. LD559nm ON
   2-2. Multi Ar 458nm 488nm 515nm
   2-2. HeNe(G)(643nm) ON

3. Turn the mercury burner ON for Fluorescence observation.

Wait for a moment until the software is started

4. Log on Windows

Enter Password,Customer name is below
User name: Administrator
Password : fluoview

5. 

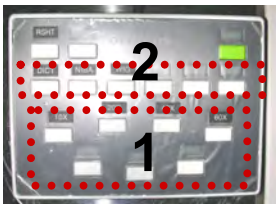
Double click this icon to log on to ASW

User name: Administrator
Password : Administrator

5

Visual Observation under the Microscope

■■ Observation of Fluorescence Image ■■



Hand switch

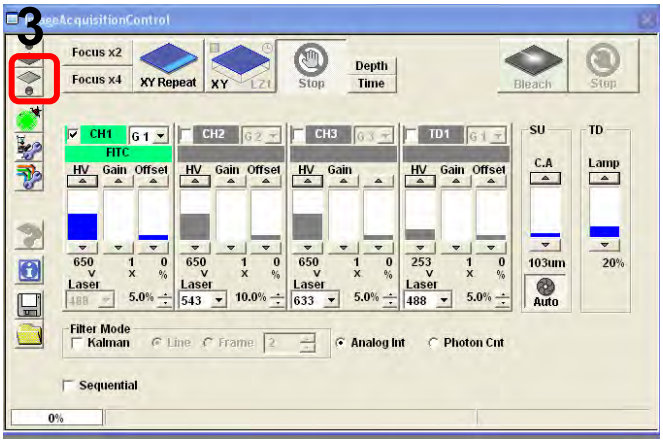
1. Select an objective lens
   by using the hand switch

2. Select florescent filter cube

MEMO
Fluorescence filter

NIBA: Blue Excitation / Green Fluorescence
(Ex.:FITC,EGFP)
WIG: Green Excitation / Red Fluorescence
(Ex.:Rhodamine, DsRed)

3.  Click the button
on the Fluoview software



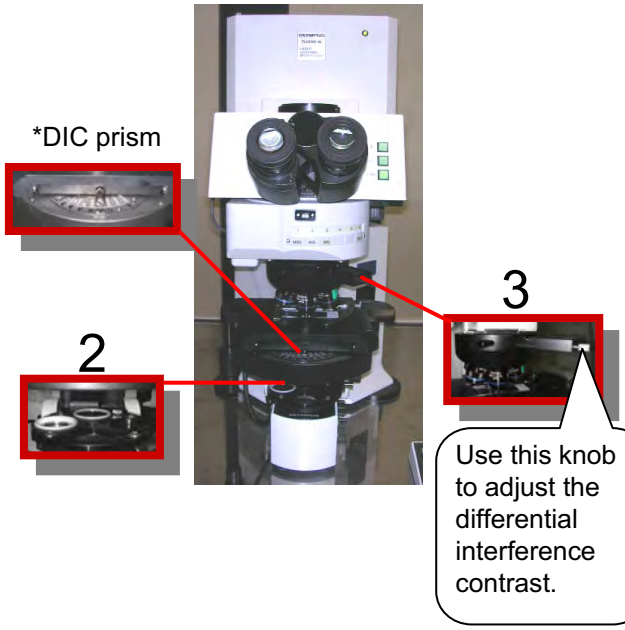
4. Focus to the specimen

6

Visual Observation under the Microscope

■■ Observation of Differential Interference Contrast Images ■■

Hand switch

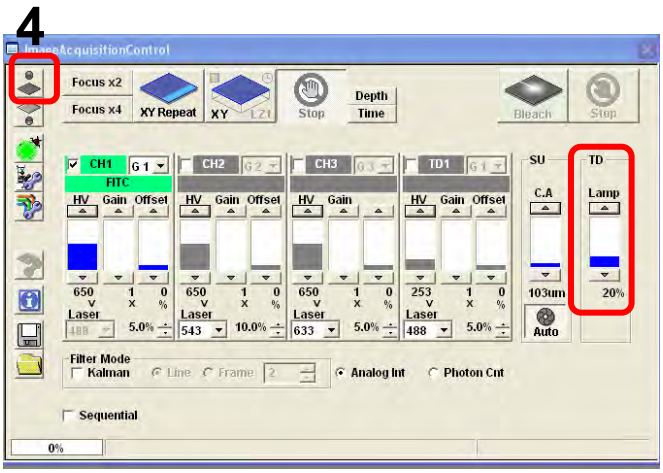


1. Select the Objective Lens

2. Insert the Polarizing Plate in the Light Pass

3. Insert the DIC prism slider in the light pass

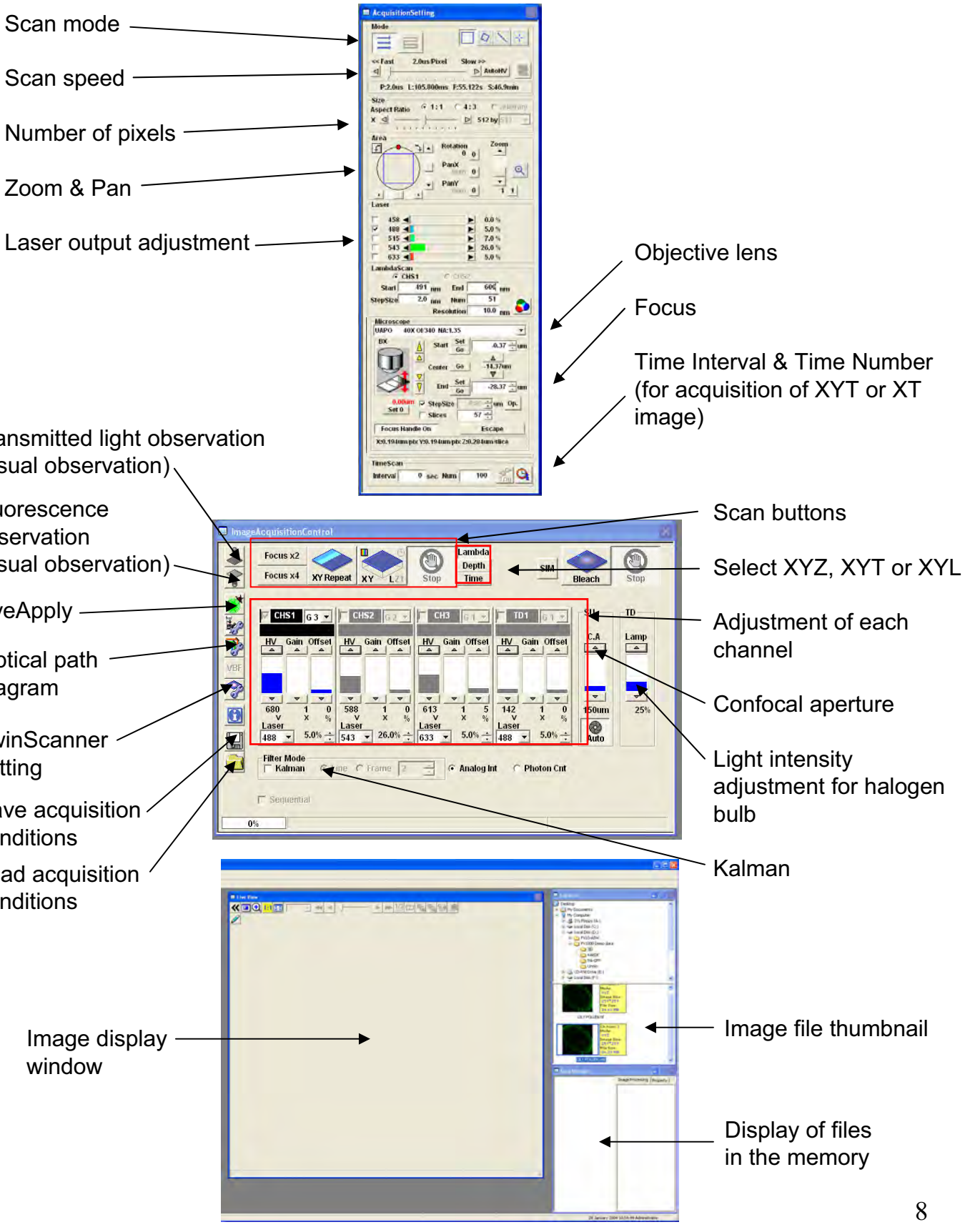
4.  Click the button on Fluoview software



5. Focus to the specimen

7

Overview of Operation Panel for Image Acquisition

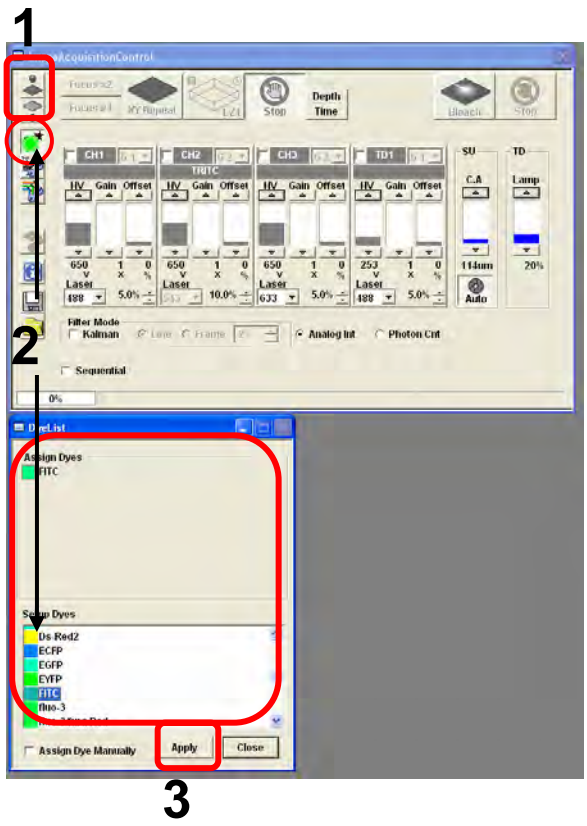


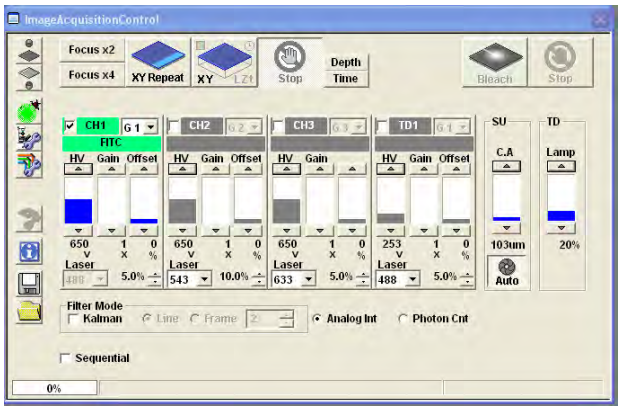
8

Image Acquisition
(Single Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Single stain of green fluorescence dye (FITC)





1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

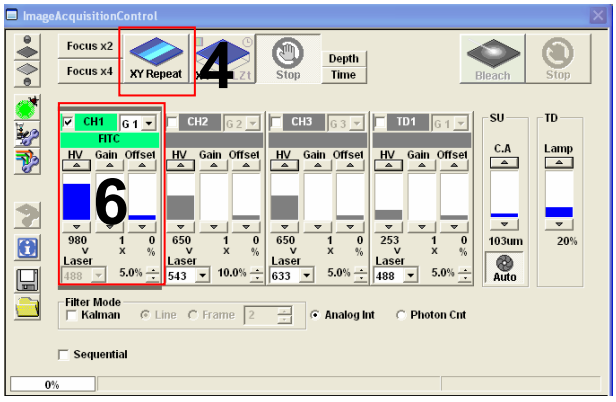
   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

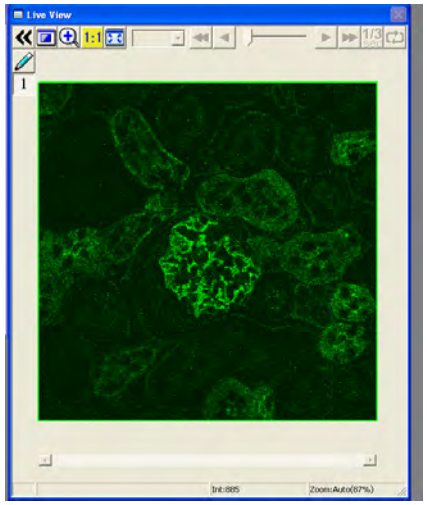
3. Click on the Apply button.

   (The DyeList panel can be closed by using the Close button.)

9

Image Acquisition
(Single Stain on XY Image)





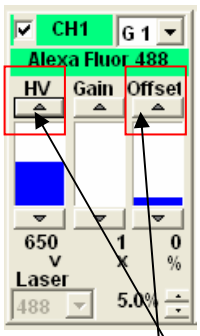
7

4. Press XY Repeat button click to get image

: Continuous scan mode

5. Focus to the specimen

6. Adjust the green (FITC) image.



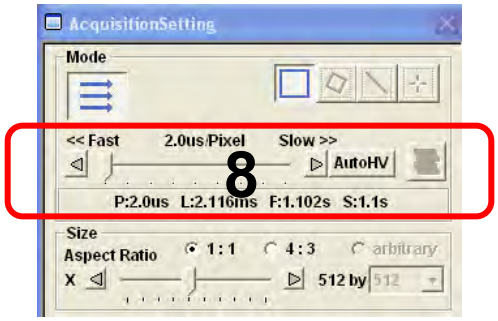
· Adjust sensitivity of **HV** and reduce noise by **offset**

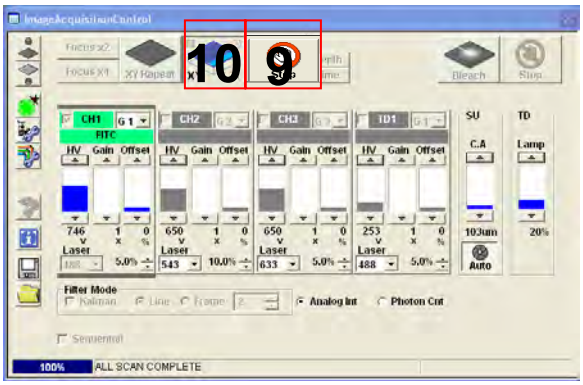
7. Press keyboard **Ctrl + H key**
Optimized PMT adjustment brightness intensity 2 color between white and black,
Maximum intensity is 4095(12bit)
if intensity is over4095, color is changed to red (saturation)

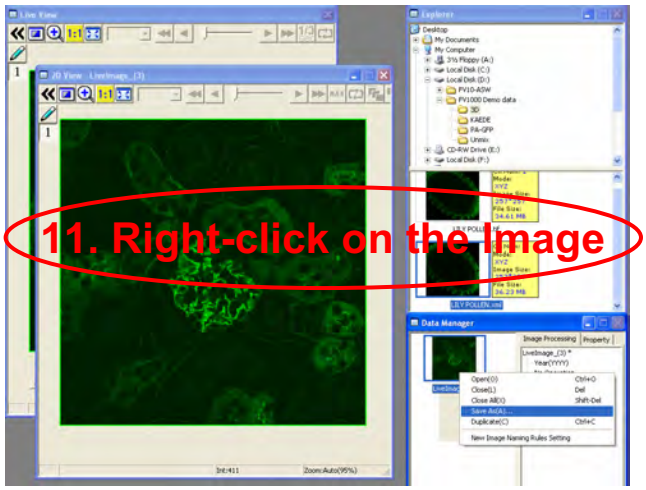
* Basically, **Gain value is 1**

10

Image Acquisition
(Single Stain on XY Image)







■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates “a folder that contains an image (16-bit TIFF)” and “an accessory file,” which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

8. Select AutoHV and then select ScanSpeed.
*As the scan speed becomes slower, noise
can be removed while maintaining the current brightness.

9. 

Press the Stop button to stop scanning.

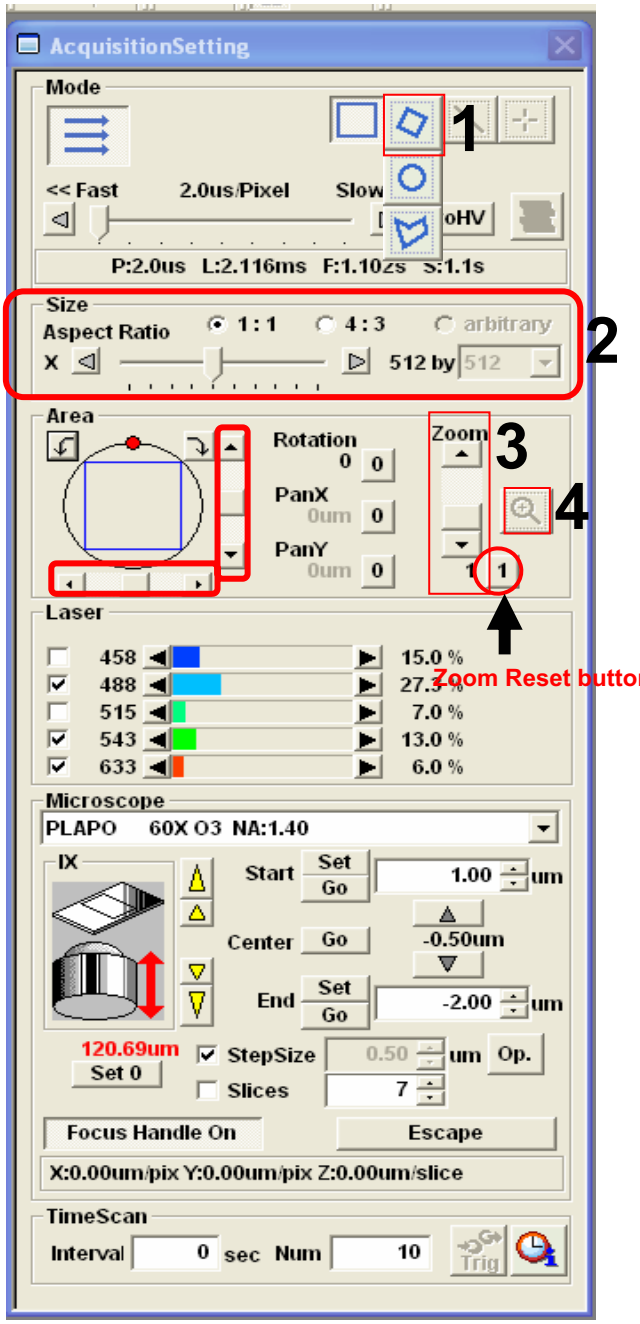
10. 

Click on XY, and “2D View-LiveImage(x)” is displayed on the window bar for the image that has been acquired.

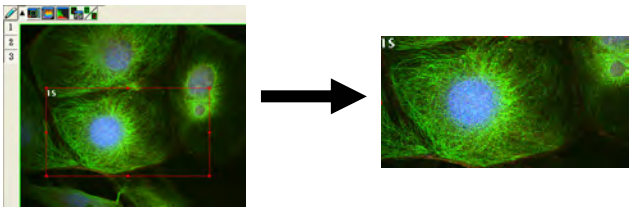
11. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “ oib” or “oif” file format specifically for the FV10-ASW software.)
Save the image as TIFF, BMP, JPEG format Select “Export” and chose the format TIFF, BMP, JPEG.

11

Complement of adjusting the image



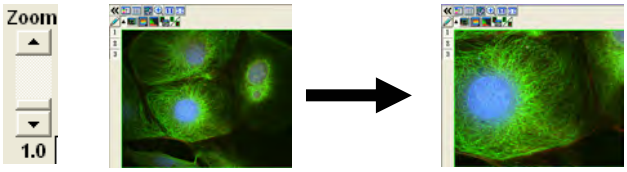
1. Click “Clip scan” button , and enclose a interesting region’s image on the whole image.



2. pixel setting
   * The standard pixel is 512 x 512

3. Zoom Setting

Press “XY Repeat” to scan and set zoom value.

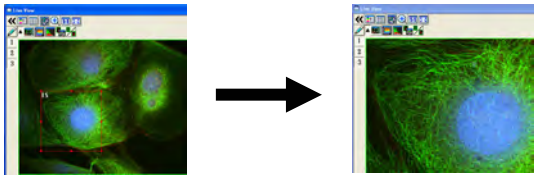


Above image is zoomed From 1x to 2

*** Scan speed and pixel resolution remain even zoom value is changed**

4. Click  Zoom scan, and be able to enclose an interesting region’s on the whole image

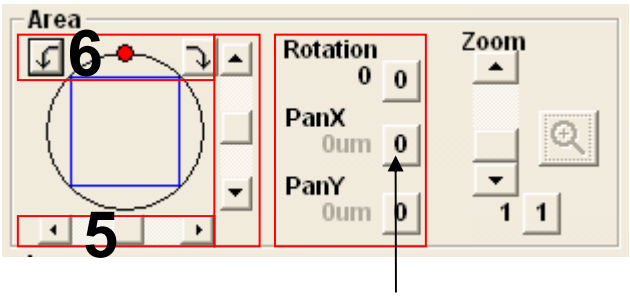
Press XYRepeat to scan after enclosing the area



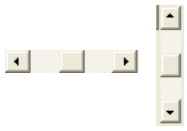
*** Scan speed and pixel resolution remain even zoom value is changed**

12

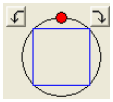
Complement of adjusting the image



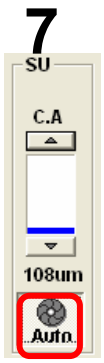
PanX,Y and Rotation reset button

5. Pan X,Y 

Be able to move the field of view to set Pan X,Y without stage action

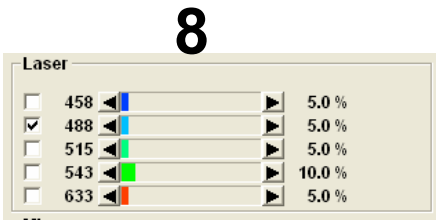
6. Rotation 

Be able to rotate the whole image.



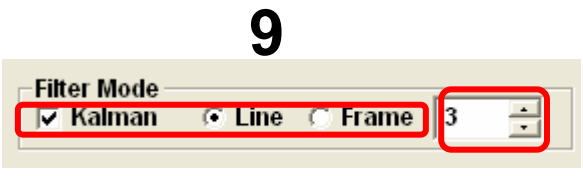
7. 

Click "Auto" button to acquire Optimized Conforcal aperture
Conforcal aperture · · · change conforcal aperture to larger diameter for dim fluorescence image then, be able to get the more bright image. But Z axis resolution gets worse.



8. Laser Intensity · · · More Laser intensity is increase , more bright image is .

*** More increase laser intensity is , more discoloration image is .**



9. Kalman accumulation · · · Image acquisition is repeated to the specified number of times to provide an averaged image. Consequently, noise is averaged and roughness on the whole image is reduced.

Advantage: The speed of each scan is fast.

Disadvantage: Some blur occurs due to averaging of images.

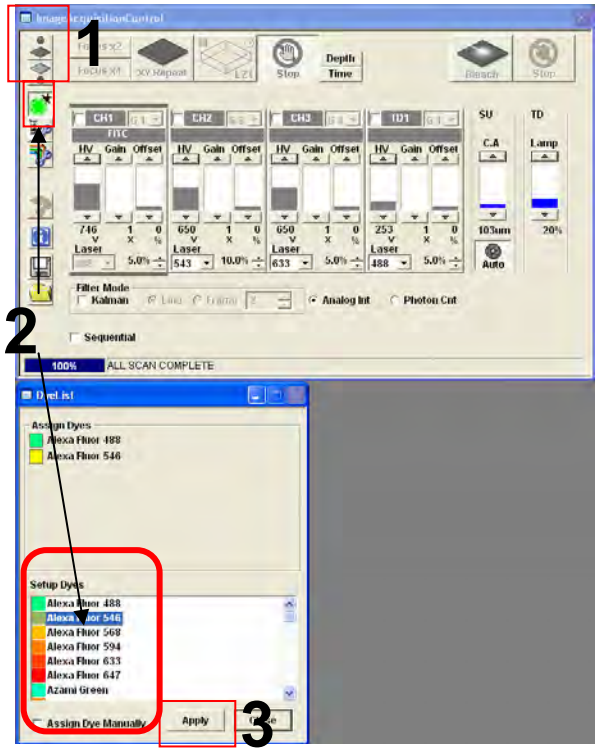
13

Image Acquisition
(Double Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Simultaneous scan



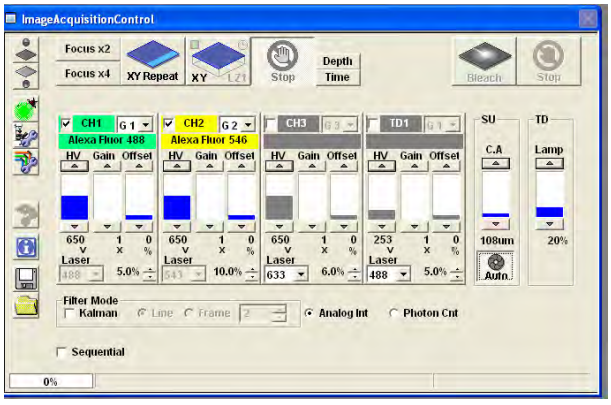
1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click “Apply” button.

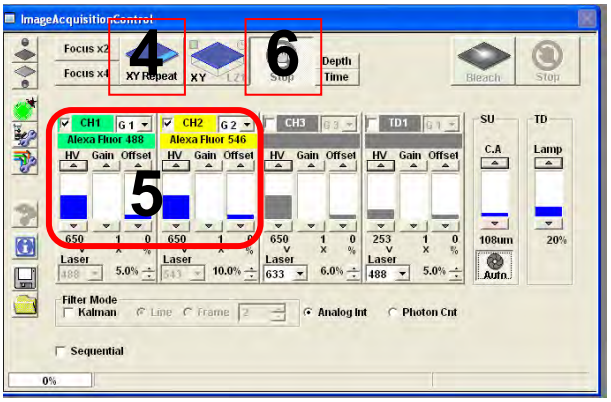
   (The DyeList panel can be closed by using the Close button.)

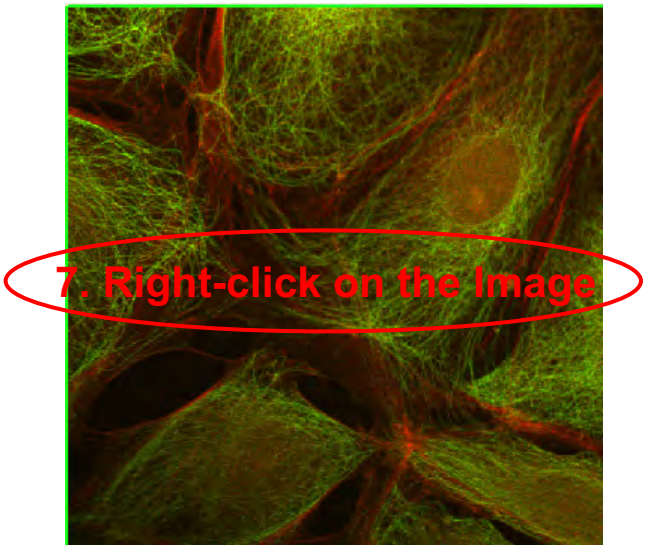


Display after DyeApply is carried out

14

Image Acquisition
(Double Stain on XY Image)





4. Press the XY Repeat button to start scanning.

5. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.
   (The image adjustment is outlined below. For more information, refer to Appendix 1.)

6. Press the Stop button to stop scanning and press XY repeat to acquire the image. (Refer to ■Memo■.)

■Memo■
Scan buttons

: Continuous scan

: Stop scan

: Rough scan (Line skipped)

7. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
   (Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

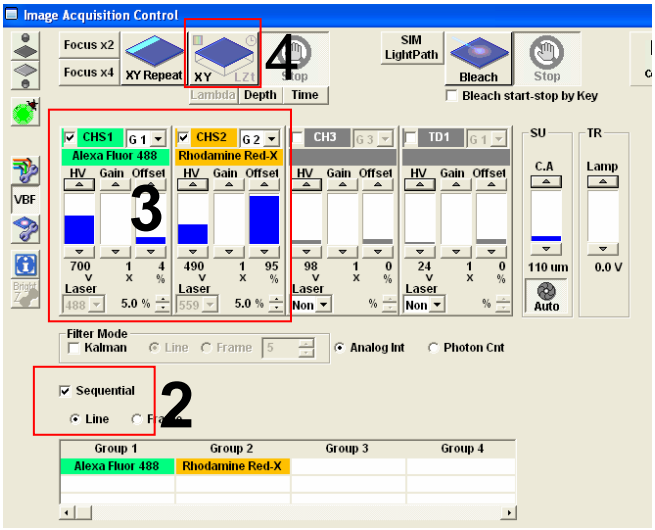
15

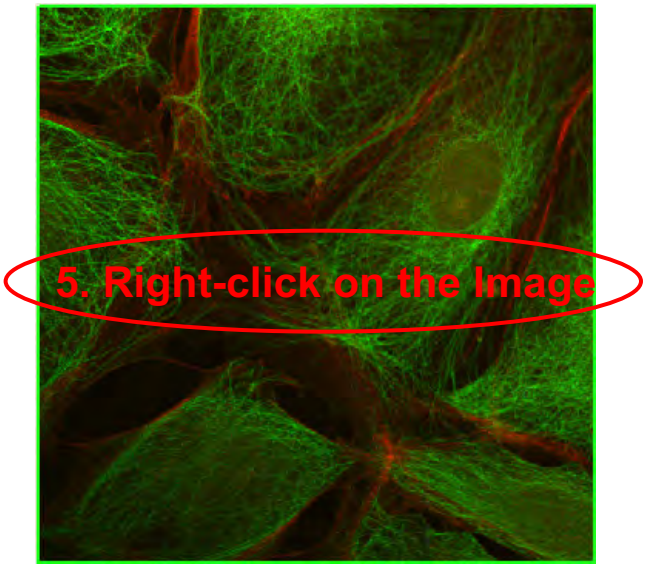
Image Acquisition
(Double Stain on XY Image)

■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Sequential scan (Line Sequential is introduced here.)





1. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

2. Check Sequential and select Line.

3. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.

4. Press the XY button to acquire an image.

5. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.) The image is acquired.

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates “a folder that contains an image (16-bit TIFF)” and “an accessory file,” which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

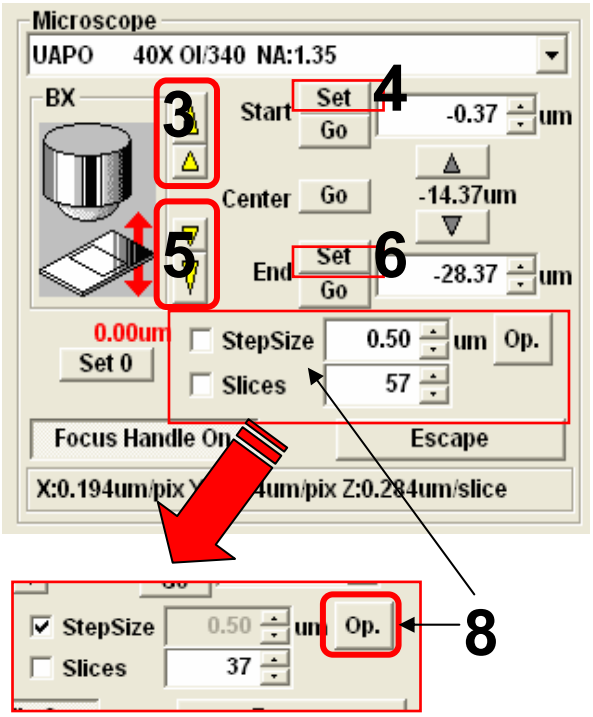
16

Image Acquisition
(Double Stain on XYZ Image)

■■ Acquisition of 3D images (XYZ)
(fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (FITC)
and red fluorescence dye (Rhodamine)

This is the procedure to acquire images
through Line Sequential scanning.



■Memo■

and buttons

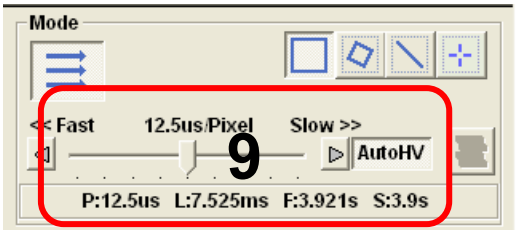
: Moves 1.0μm with a single click.

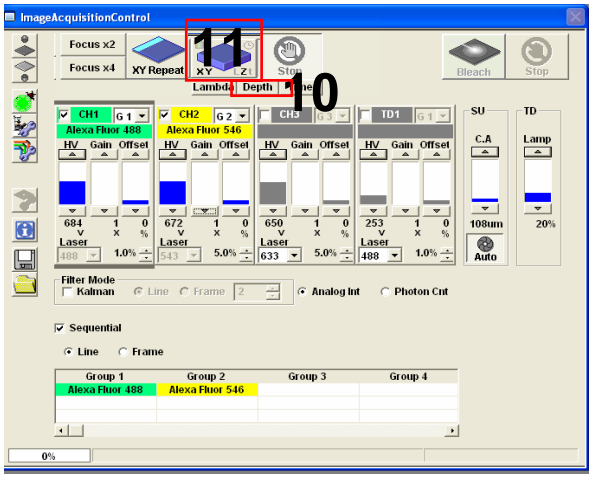
: Moves 0.1μm with a single click.

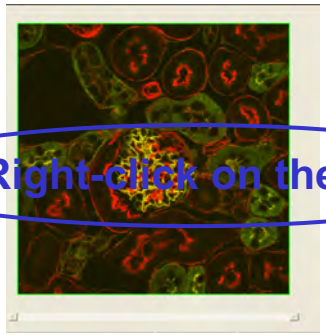
1. Take steps 1 to 7 described on pages 13 and 14.
2. Press the XY Repeat button to start scanning.
3. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
4. When the sample upper limit is displayed on the image, accept it using the Set button.
5. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
6. When the sample lower limit is displayed on the image, accept it using the Set button.
7. Press the Stop button to stop scanning.
8. Enter StepSize, Slice (the recommended value can be referred to by using the Op button), and check the check box

17

Image Acquisition
(Double Stain on XYZ Image)







9. Select AutoHV and then select ScanSpeed.

10. Select Depth.

11. Press the XYZ button to acquire an image.

12. Click on "SeriesDone", and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

13. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

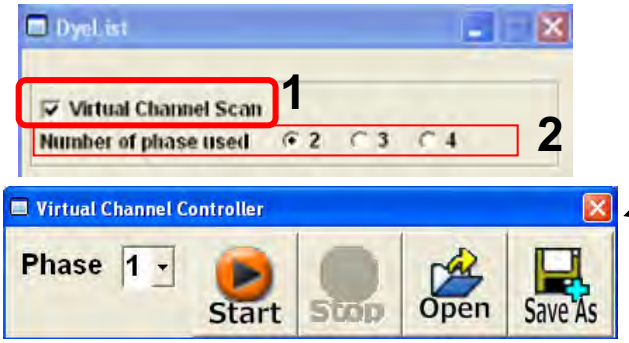
18

Image Acquisition
(Four Stain on XY Image)

■■ Acquisition of 4 stain images (XY)
(fluorescence image only) ■■

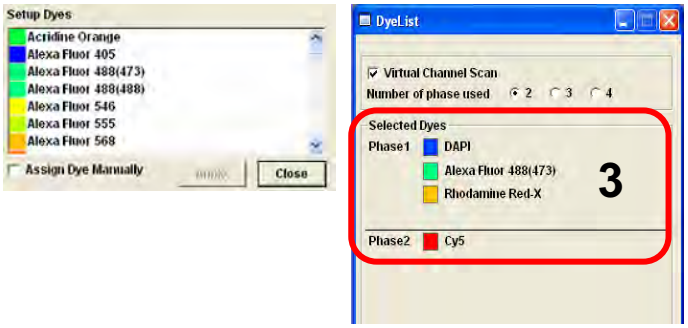
Sample: Four stain of Blue fluorescence dye (DAPI) ,green fluorescence dye (Alexa488) and red fluorescence dye (Rhodamine), far-red fluorescence dye (Cy5)

This is the procedure to acquire images
through Virtual Channel scan

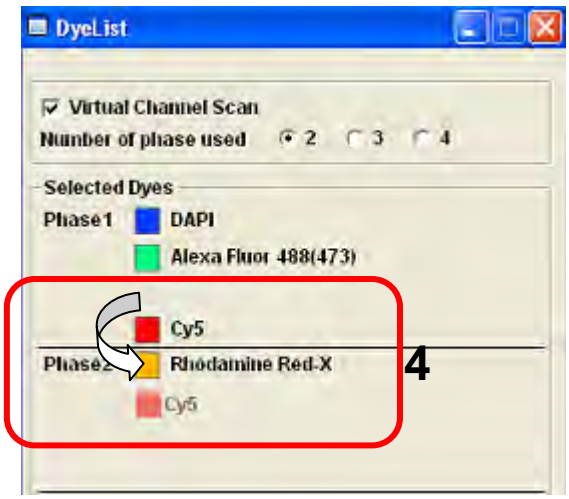


1. Virtual Channel Scan Select Virtual channel Scan on the DyeList, and then "**Virtual Channel Controller**" is automatically turned on.

2. Select a number of Virtual Channel from "**Number of phase used**".



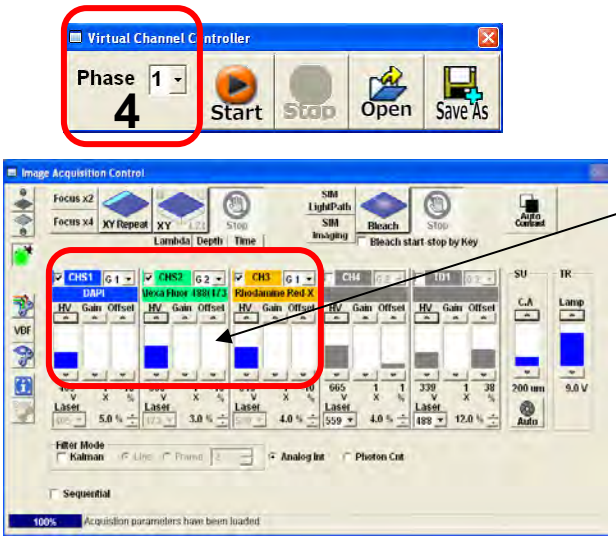
3. Select 4dyes from DyeList 4th dye is registered in "**the Phase 2**".



* **RodaminRed is able to be registered on "Phase2" to drag .**

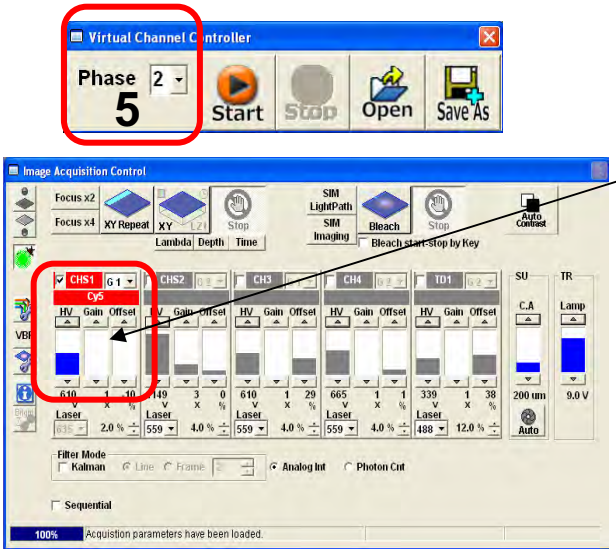
19

Image Acquisition
(Four Stain on XY Image)



4. Select “Phase1”, “DAPI”, Alexa488, RhodaminRed are registered on ImageAquisitionControl.

* **Slit and Filter, DM are automatically set for “DAPI”, “Alexa488”, “PhodaminRed”**



5. Select “Phase2”, Cy5 is registered on ImageAquisitionControl

* **Slit and Filter, DM are automatically set for “Cy5”**

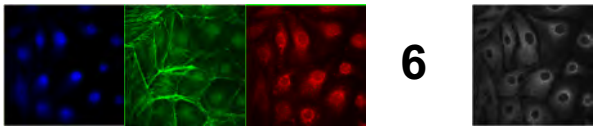
6. Adjust the image to click 

“XY Repeat” at each phases

* If acquire XYZ image, be able to decide upper limit and bottom limit , slices, step size of Z axis at both phases.

Adjust the image at each phases

“Phase1” **“Phase2”**



6

20

Image Acquisition
(Four Stain on XY Image)

7

7. Click 

on Virtual Channel Controller to acquire the image.

* Be able to start at each Phase.

8. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.

■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates “a folder that contains an image (16-bit TIFF)” and “an accessory file,” which cannot be opened separately from each other.

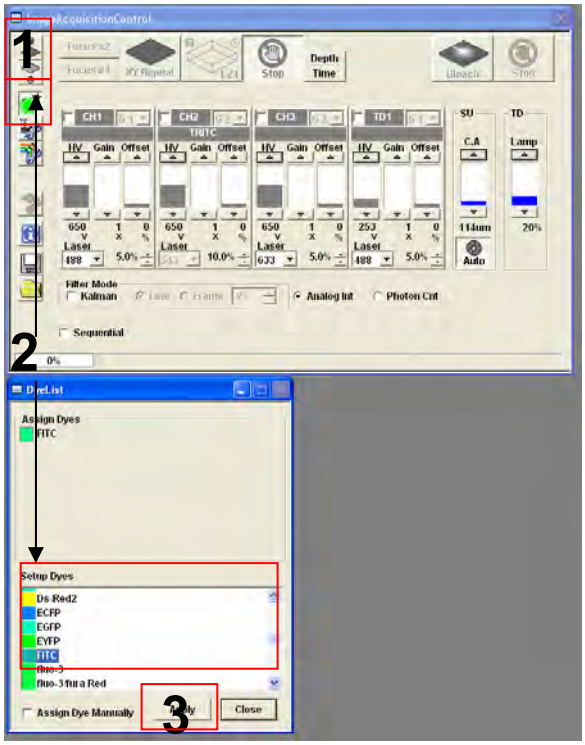
OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

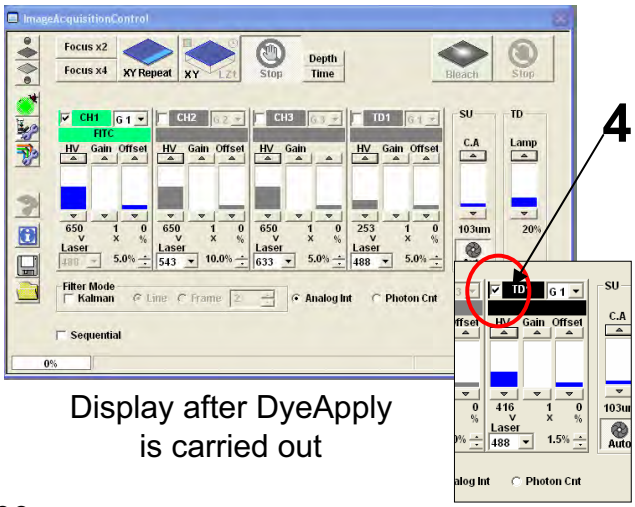
21

Image Acquisition
(Single Stain + DIC on XY Image)

■■ Acquisition of a single image (XY plane)
(fluorescence image and differential interference contrast image) ■■

Sample: Green fluorescence dye (FITC) and differential interference contrast image





Display after DyeApply is carried out

1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
   Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

   * To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

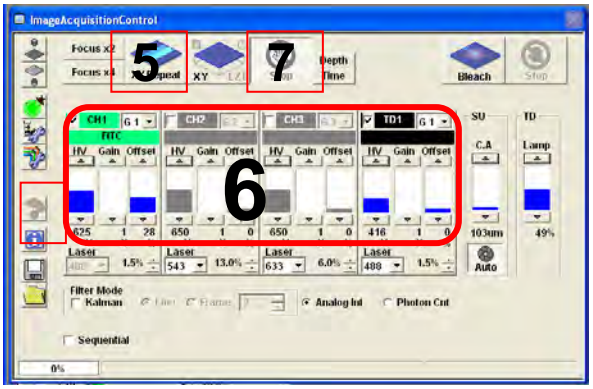
3. Click on the Apply button.

   (The DyeList panel can be closed by using the Close button.)

4. Check TD1.

22

Image Acquisition
(Single Stain + DIC on XY Image)



5. Press the "**XY Repeat**" button to start scanning.

6. Adjust the green (FITC) image and the differential interference contrast image.

7. Press the "**Stop button**" to stop scanning.

8. Press the "**XY button**" to acquire an image.

9. Click on "SeriesDone", and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

10. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type "oib" or "oif" file format specifically for the FV10-ASW

■Memo■
File formats specifically for the FV10-ASW

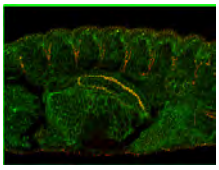
OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

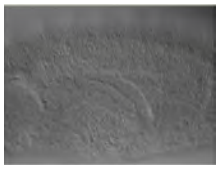
23

Merge the images between fluorescent XY image and DIC image

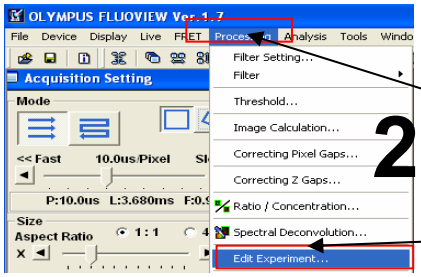
Edit different each files to the same file. This is available for making merge image Between fluorescent image and focused DIC image.



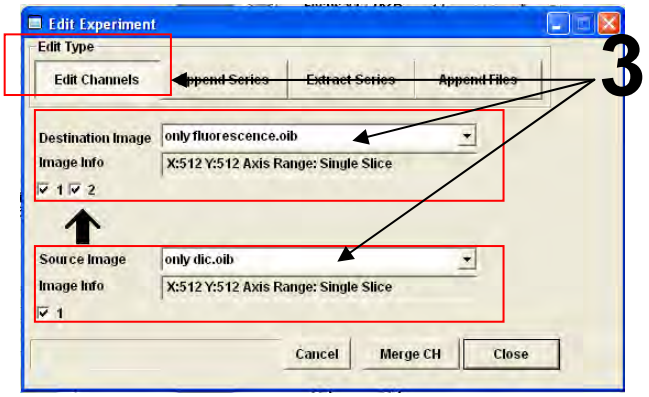
1



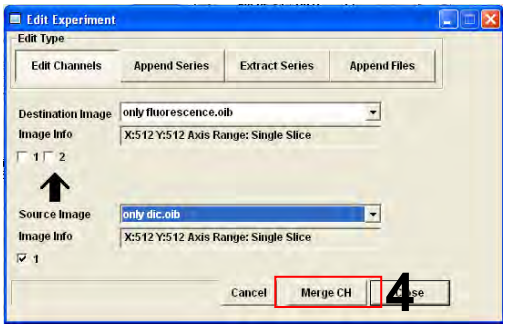
1. Open fluorescent image and DIC image.



2. Select **Edit experiment** from **Processing**



3. Click **Edit Channels**, and select fluorescent image file at **Destination Image**, select DIC image at **Source Image**.

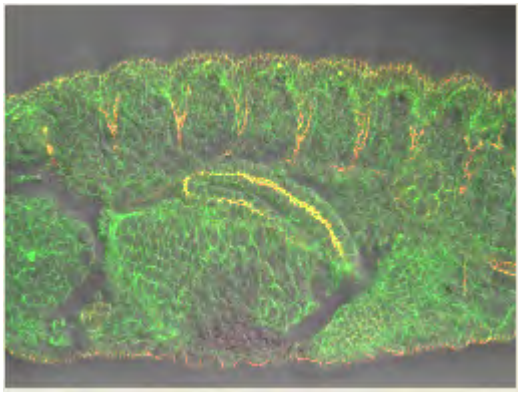


4

* Check Image Info 

to make the. merge file. If all channels are checked ,all channels are reflected in the new merged file.

4.Click **Merge CH**, and then the fluorescent image and the DIC image are merged as the new file .

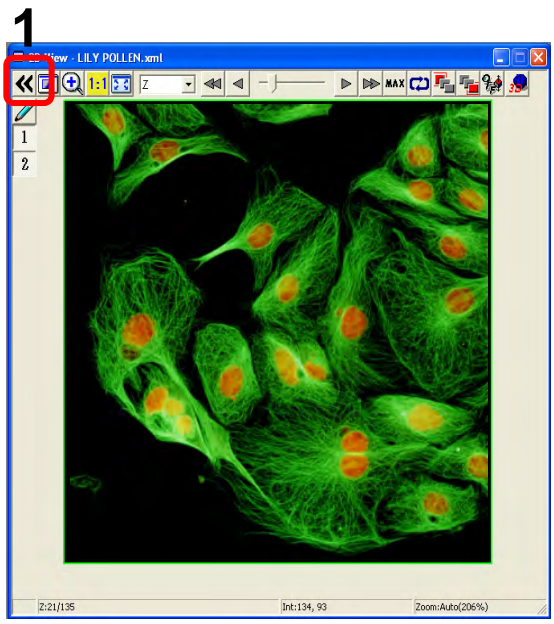


5

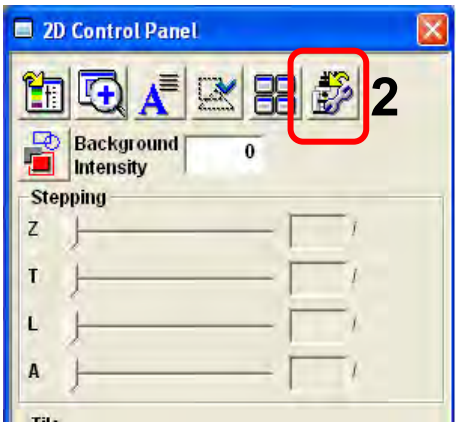
5. Merged image between the fluorescent image and the DIC image.

24

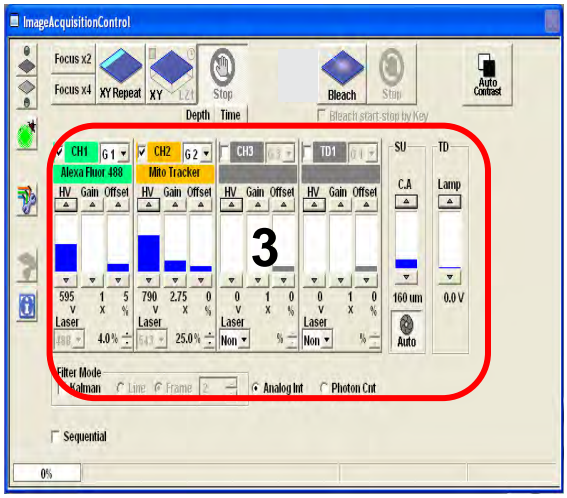
Reload the image conditions



1. Open the file and click 



2. Click 



3. The conditions (HV,Offset, CA and so on) are reloaded .

25

Overview of the 2D Operation Panel

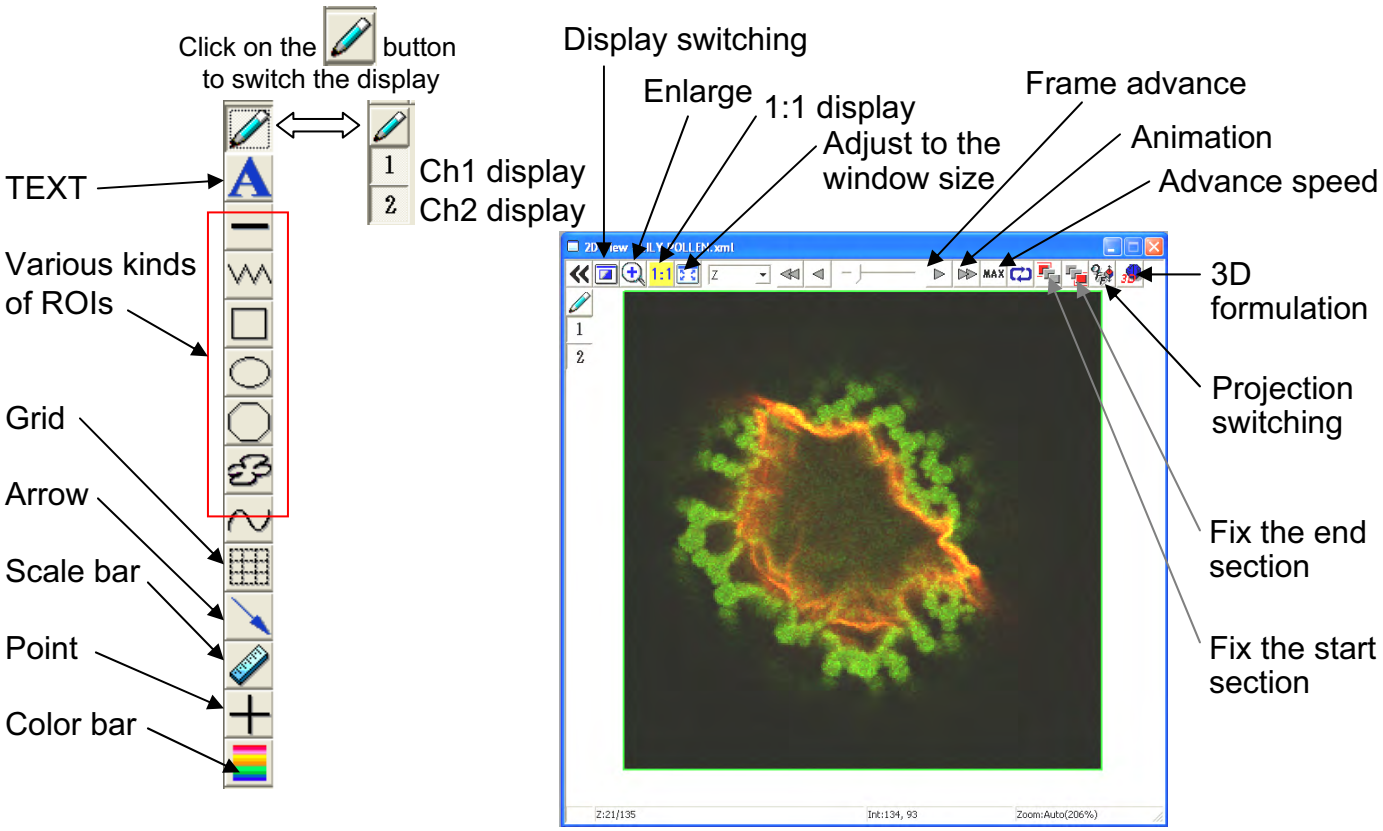
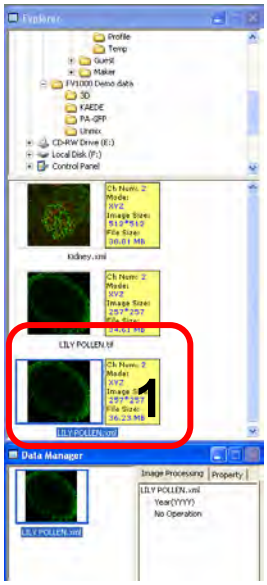


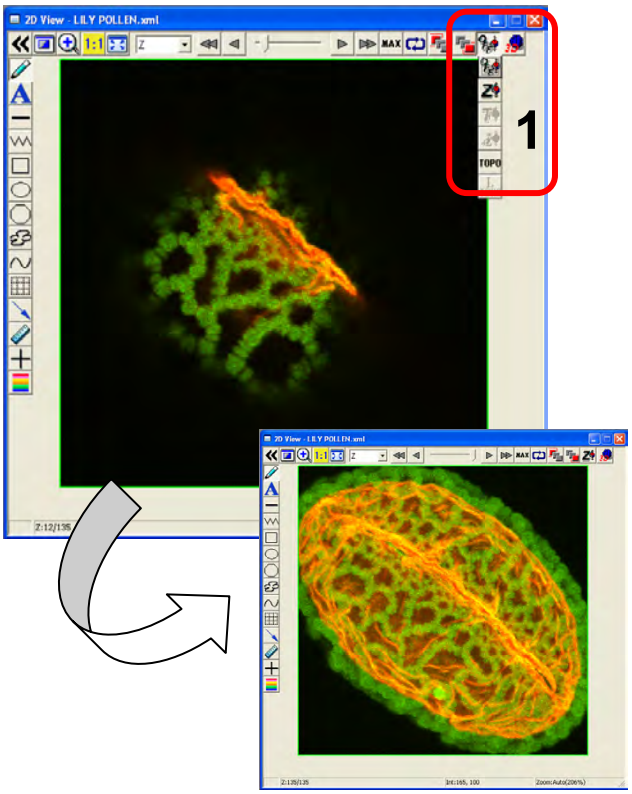
Image Analysis (Opening a File)

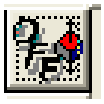


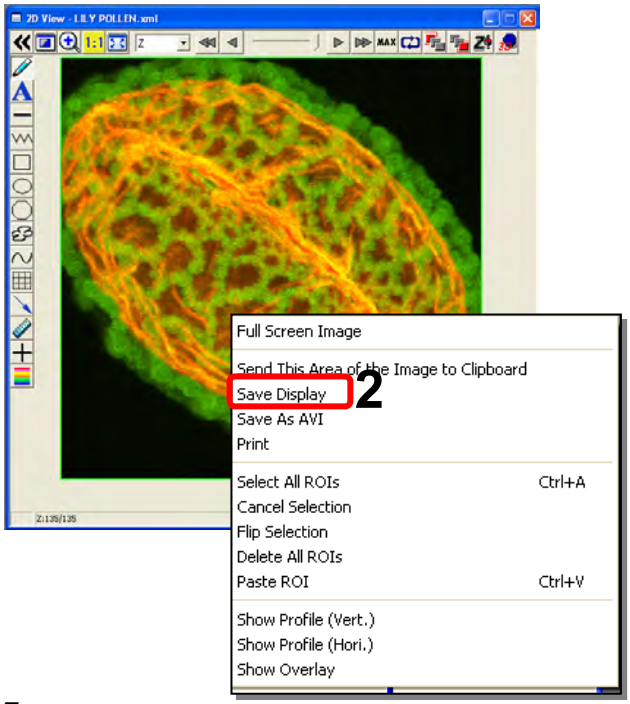
1. Double-click on a file to be opened from Explorer.

26

Image Analysis (Acquire a Projection Images)



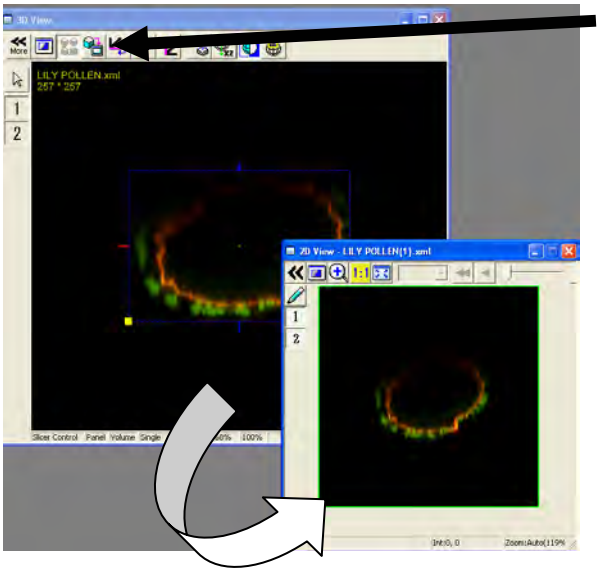
1. Click on the  button to select  .



2. To save this image, right-click on the image, select Save Display and save the image with a new name.

27

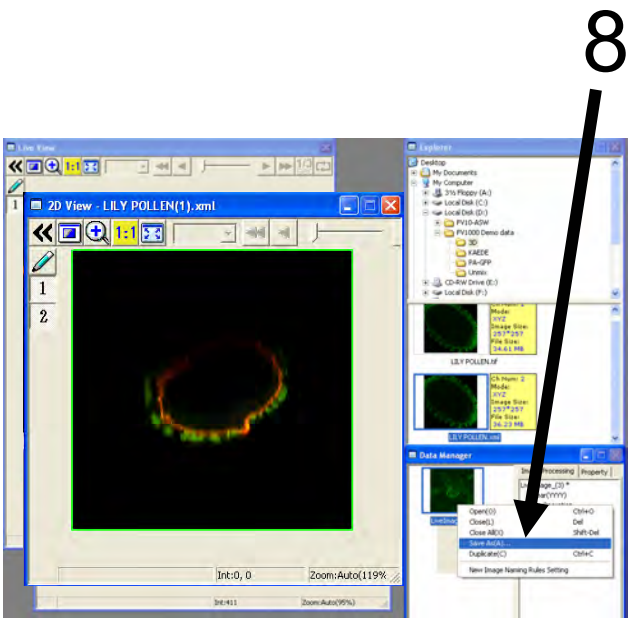
Image Analysis
(Save a Z section Image as 2D file)



Save the image in step 3 or 5

6. Click on the  button.

7. A 2D View-(file name) image is created.



8. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as type “xml” is a file format specifically for the FV10-ASW software.)

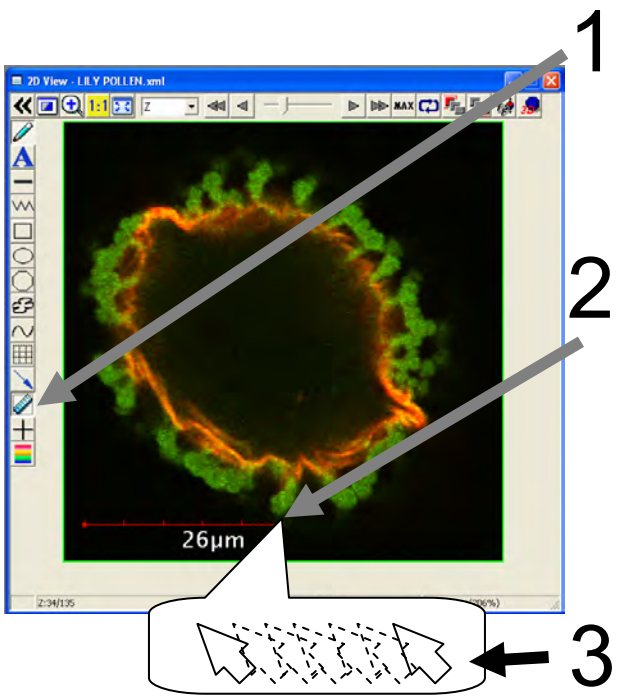
■Memo■
File formats specifically for the FV10-ASW

OIF format:
Creates “a folder that contains an image (16-bit TIFF)” and “an accessory file,” which cannot be opened separately from each other.

OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

28

Image Analysis (Inserting the Scale Bar)

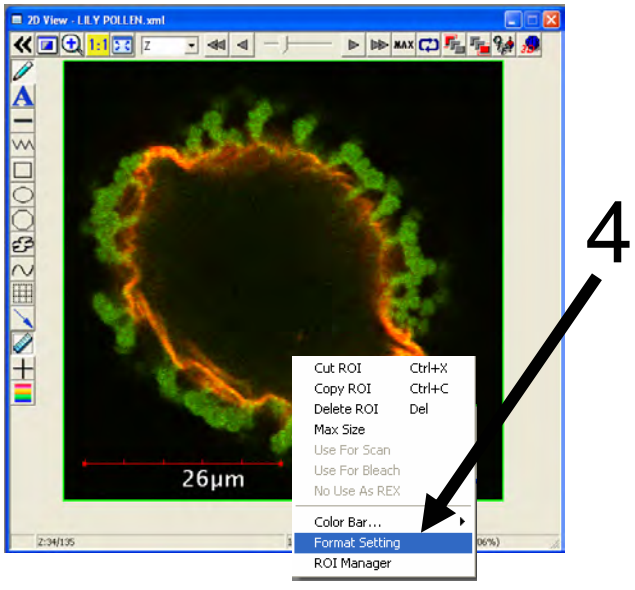


1. Click on the  button.

2. While left-clicking the image, drag and drop it at a certain point.

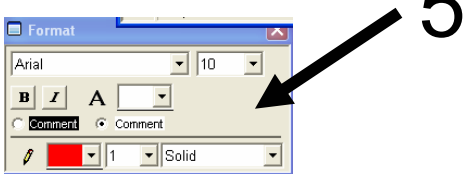
Change the size

3. While clicking the right or left handle, move the mouse from side to side.



Change the text size, color, style, etc.

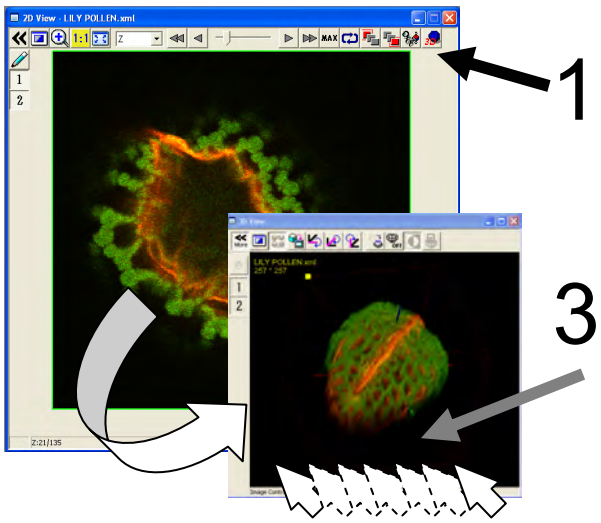
4. Select Scale Bar and then right-click on Scale Bar to select Format Setting.



5. Change the setting in this window as required.

29

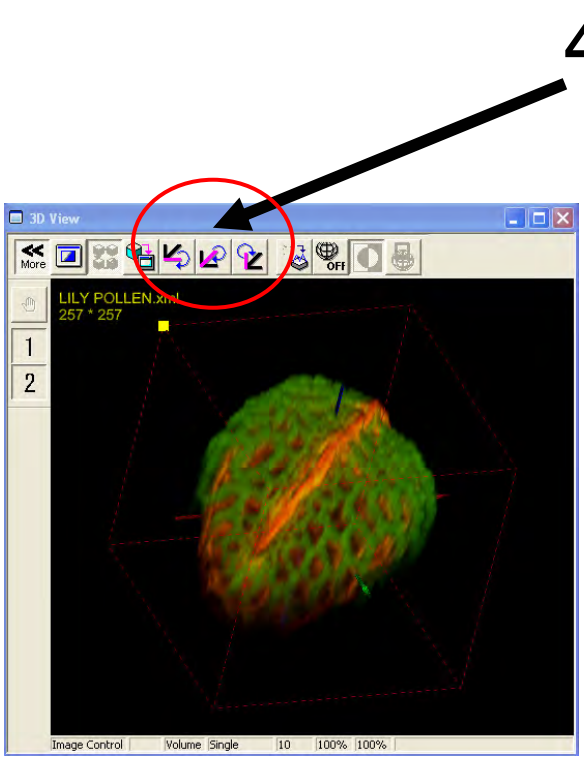
Image Analysis
(Rotating a Three-dimensional Image)



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

2. A 3D view is created.

3. Drag the mouse on the image to observe it at a certain angle.



Simple animation

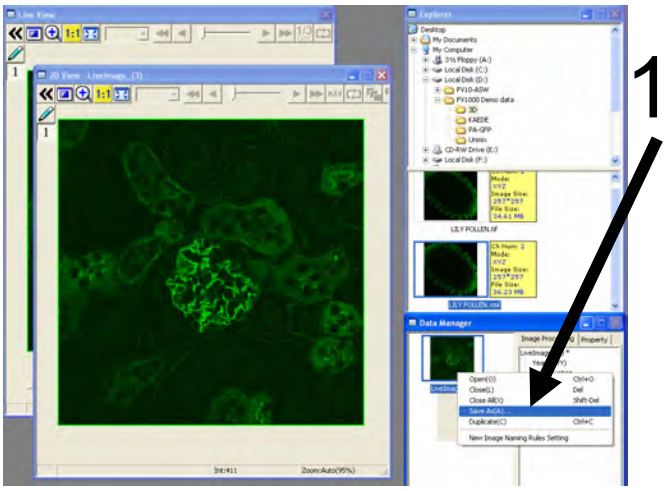
4. Press and hold the  button to rotate the image around the X-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Y-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Z-axis. Press it again to stop rotation.

30

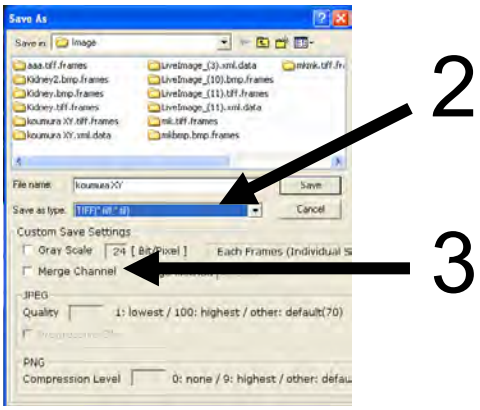
Image Analysis (Saving an Image)



Convert each channel of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to RGB Color.
4. Save the image.

* BMP and JPEG formats are also selectable.

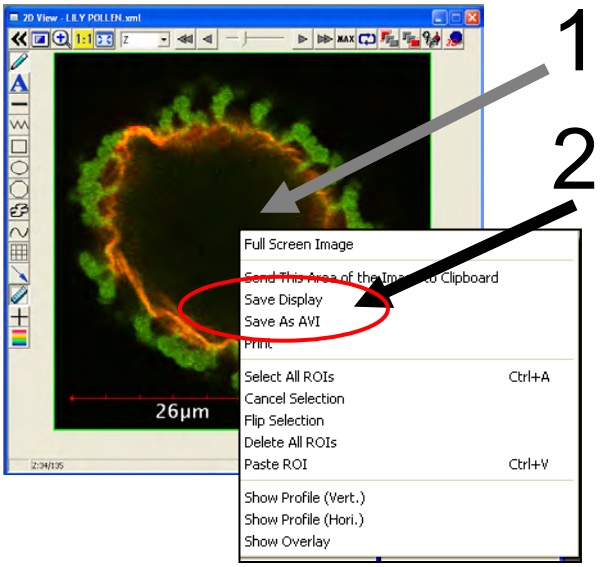


Convert a merge image of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to Merge Channel.
4. Save the image.

* BMP and JPEG formats are also selectable.

****



Convert an image with the scale bar inserted into a BMP format

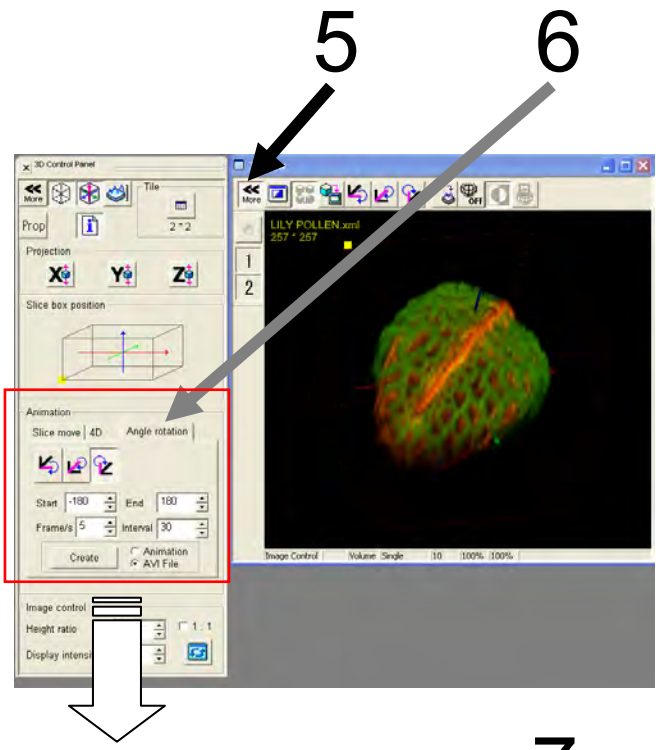
1. Right-click on the image.
2. Select Save Display and save the image with a new name.

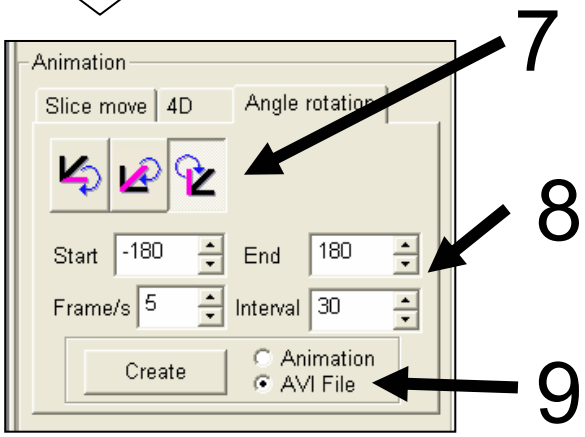
Convert an animated image into an AVI format

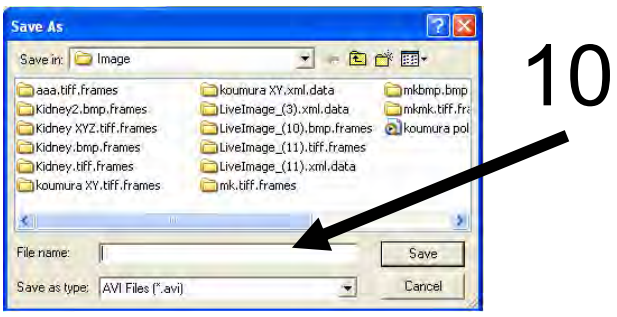
1. Right-click on the image.
2. Select Save as AVI and save the image with a new name.

31

Image Analysis
(Rotating a Three-dimensional animation)

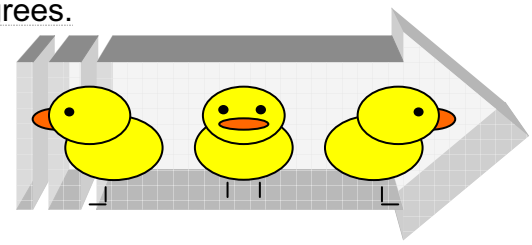






To save a rotation file as an animated image, create three-dimensional images according to the following procedure.

For example, try to rotate an image by 180 degrees.



5. Click on the 

button.

6. Click on the Angle rotation tab.

7. Select the rotation axis.

8. Enter the rotation angle.

Start =	Angle to start rotation
End =	Angle to stop rotation
Frame/s =	Rotation speed
Interval =	Degrees to be rotated at a time

9. Select AVI File and click on Create.

10. Enter a file name and click on Save.

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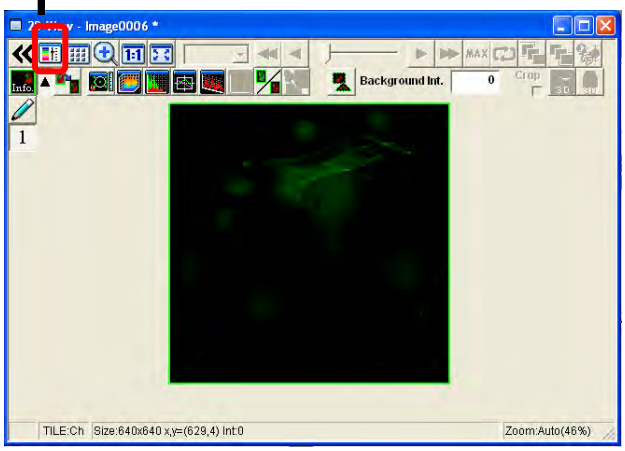
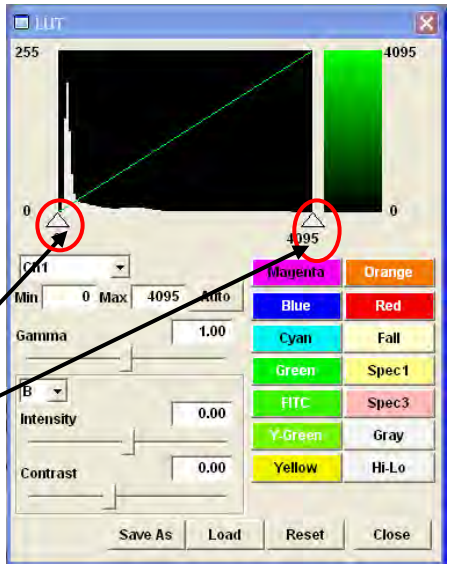
2D Image Analysis (Edit the image color and contrast)



1. Click  "LUT" and then LUT table appears below,

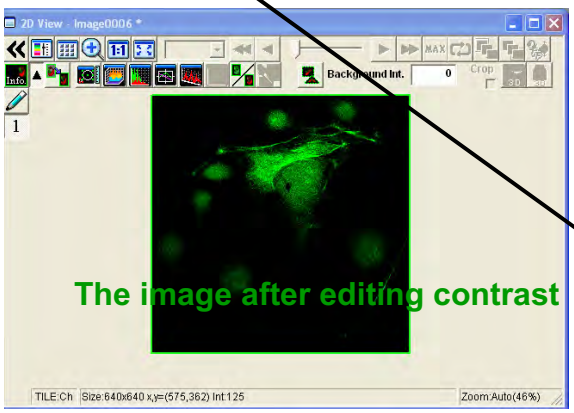


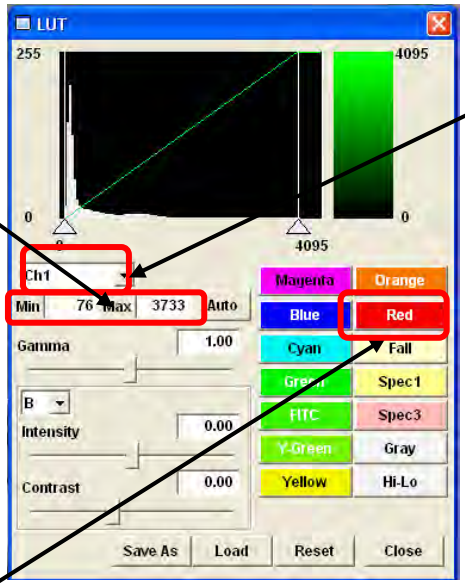
2

2.Edit contrast to drag  to left or right side, and another way to edit contrast is entering value on "Max" and "Min" (Max4095, Min0)

3. Min and Max value are changed and contrast of image is edited.

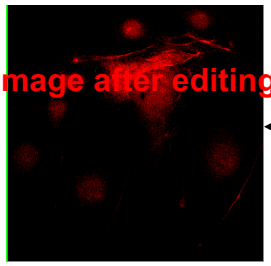
***According to get Min value up, be able to reduce noise of the image.**





3 **Edit each Ch**

The image after editing color



4

4. To click another color, be able to Edit a color.
   Above example: Change Green to Red to click Red

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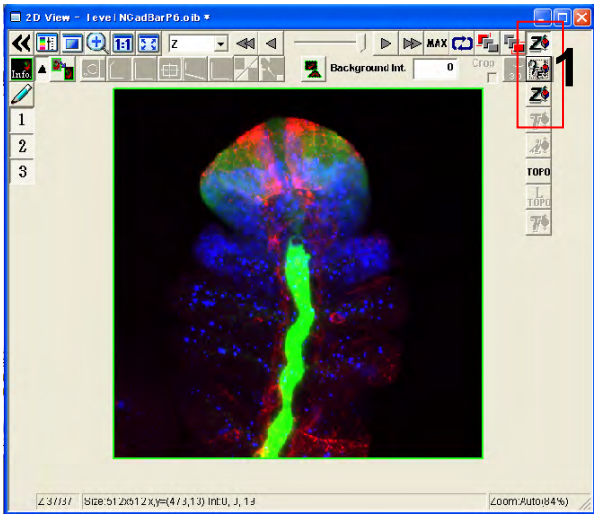
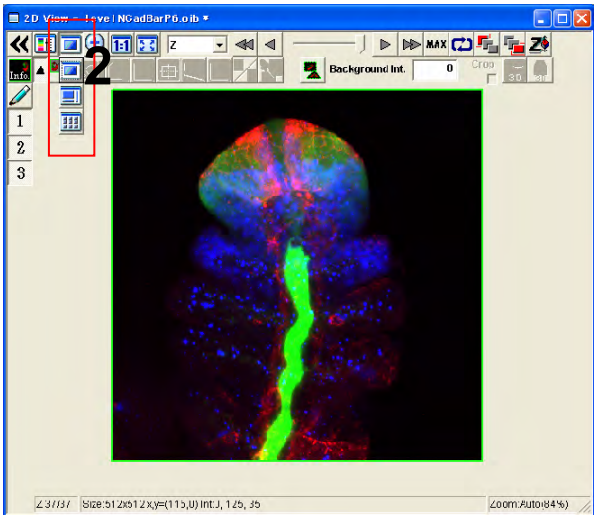
2D Image Analysis (the image of Z section)

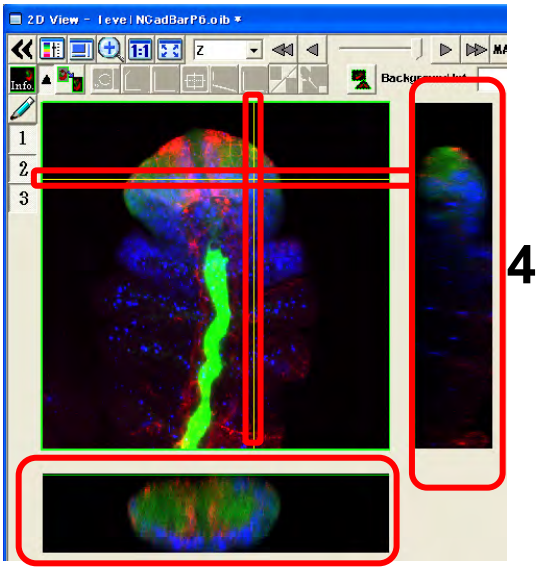


1. Click  and select  again, then Projection image is shown on 2D View after getting XYZ image.



2. Click  and select  .

3. The images of Z section is shown on X axis and Y axis.
   According to Move to left or right side on X axis and to move to ups and down on Y axis, be able to show image of Z section each position.



4. The image of Z section on Y axis.

5. The image of Z section on X axis.

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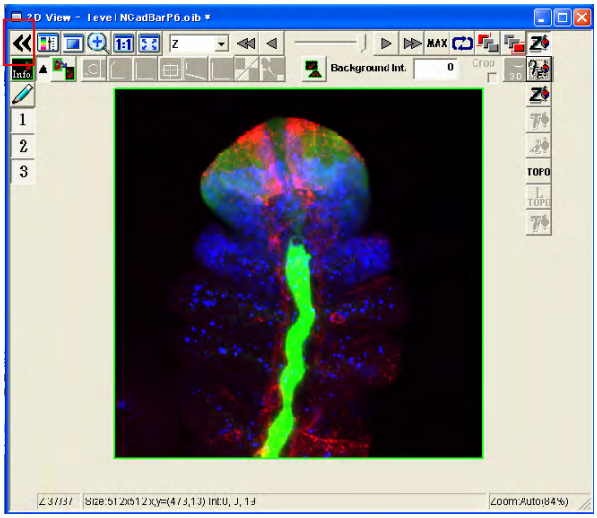
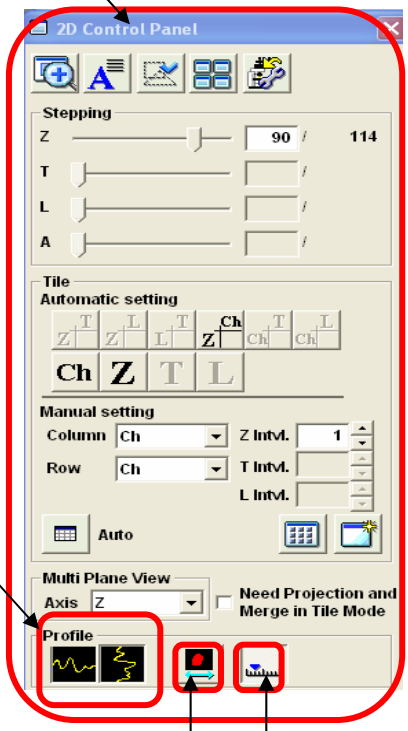
2D Image Analysis (Intensity Profile of each Z sections)

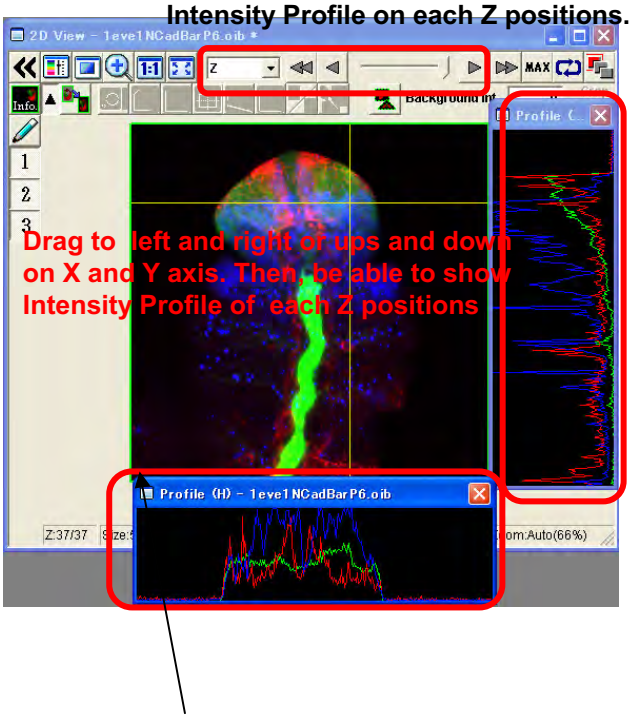


1. Click  and then 2D Control Panel is shown below,



2. Click  "Profile" and then Intensity Profile of each Z sections is shown on the X and Y axis.

To move to Z position ,be able to show Intensity Profile on each Z positions.



3. Click  to show Scale on Intensity Profile

4. According to click  , be able to show as equal scale of Profile window as 2Dimage.

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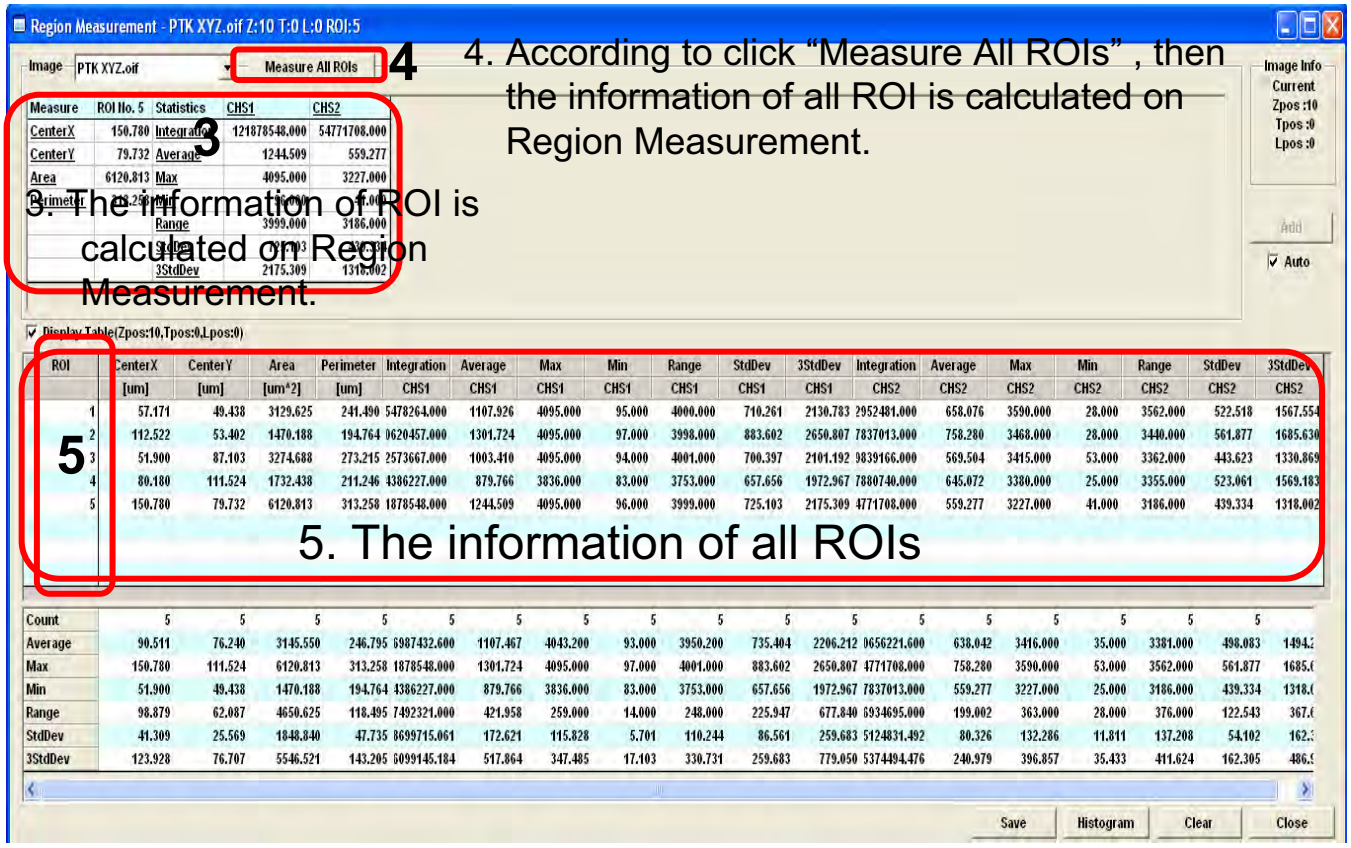
2D Image Analysis (Measure)

1. Enclose interesting regions by ROI

   Line on interesting positions by ROI —

2. Click  “measure”.



4. According to click “Measure All ROIs” , then the information of all ROI is calculated on Region Measurement.

3. The information of ROI is calculated on Region Measurement.

5. The information of all ROIs

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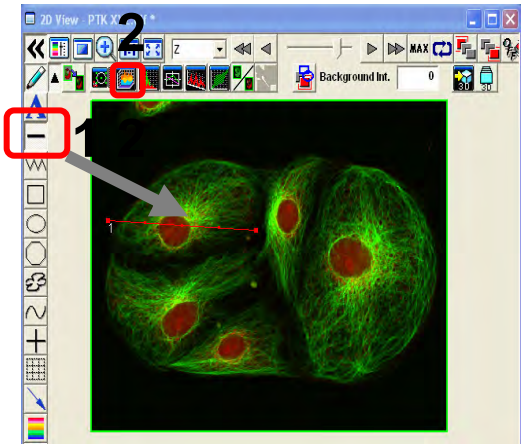
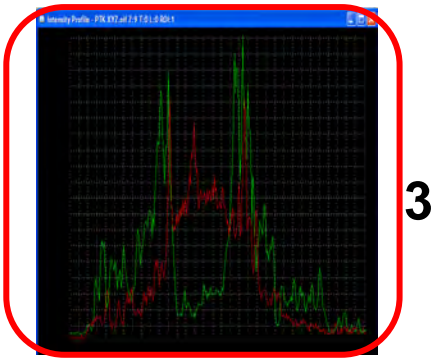
2D Image Analysis (Line Intensity Profile on the 2D image)



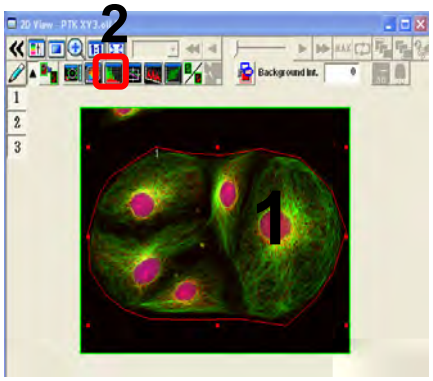


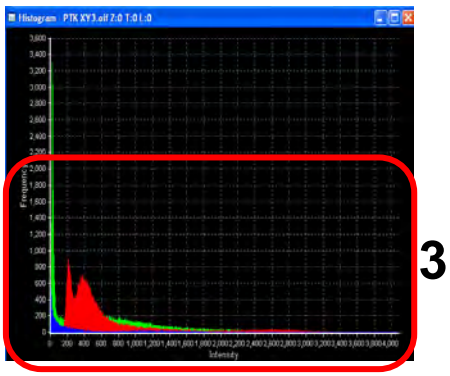
1. Line on the 2D image by ROI 

2. Click  “Intensity Profile”

3. “Intensity Profile” on the line is shown as intensity graph .

2D Image Analysis (Histogram)





1. Enclose an interested area by ROI.

2. Click  **“Histogram”**

3. “Histogram” window is shown as a graph, frequency of intensity of each pixels is plotted on the area enclosed by ROI.

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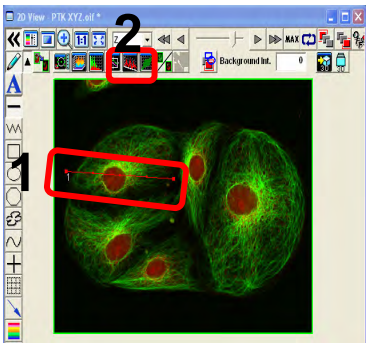
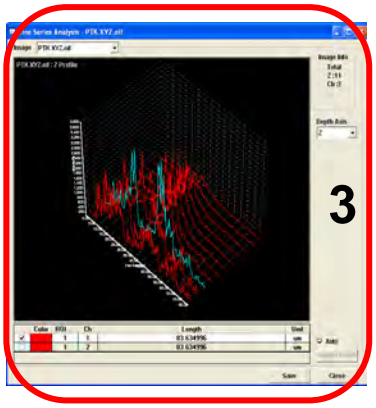
2D Image Analysis (Line Series Analysis)



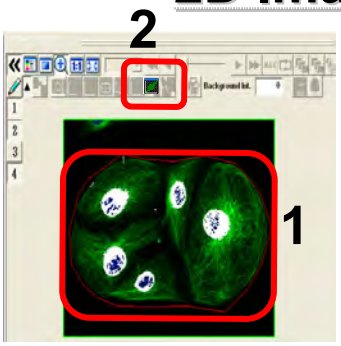


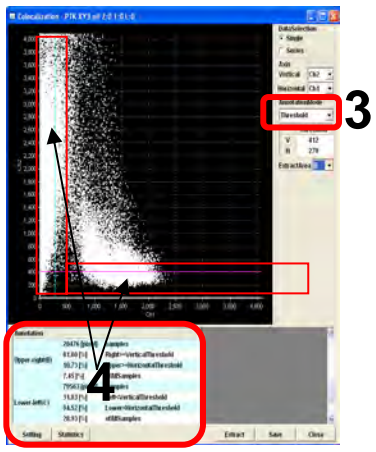
1. Line on the interesting region.

2. Click  “Line Series Analysis”

3. Intensity of Z position/ time on the line is shown as a graph .

2D Image Analysis (Co-localization)





1. Enclose an interesting region by ROI.
2. Click 
3. Select 

Threshold from Annotation Mode.
4. According to move Thresholds of X,Y axis to right and left ,ups and down (Enclose red color X,Y axis), Co-localization result between 2ch is changed .

   Information of Co-localization is listed under the scatter plot.

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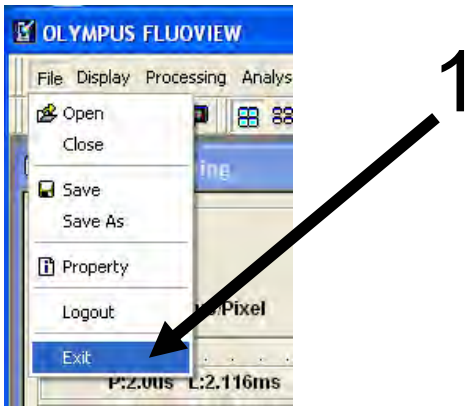
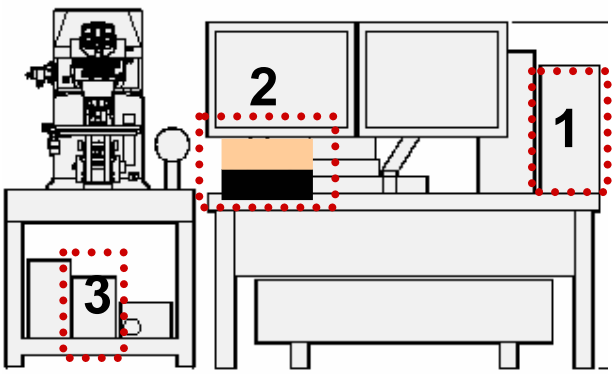
Closing the System



1. Exit the FV10-ASW software by selecting File/Exit.

2. Exit the Windows.

(1) Select Start/Shut Down.
(2) On the Shut Down Window, select Shut Down and click on OK.



3. Turn the laser OFF. (Turn the key switch to the OFF position.)

3-1. LD559nm OFF

3-2. Multi Ar (458 nm, 488 nm, 514 nm) OFF

3-3. HeNe (G) (543 nm) OFF

4. Turn the mercury burner power OFF.

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