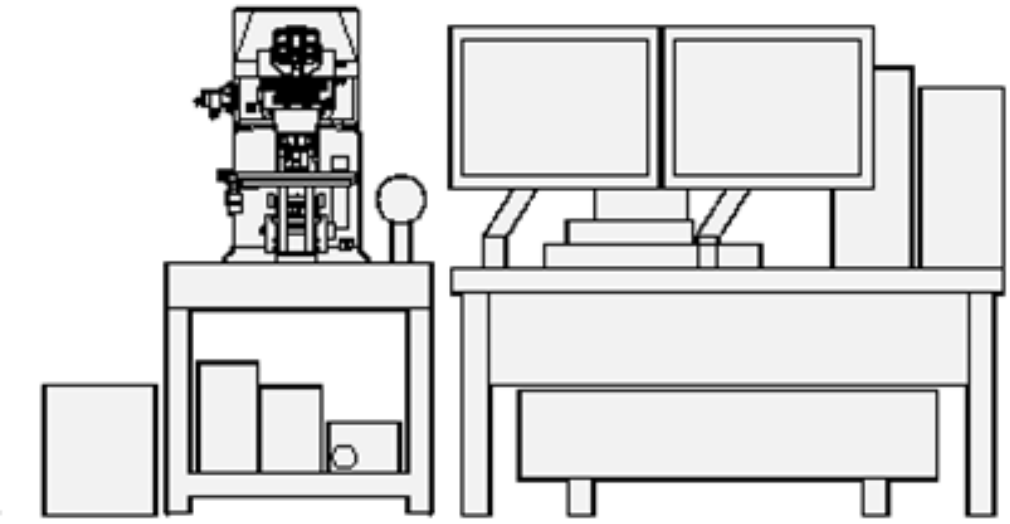
OLYMPUS

Confocal Laser Scanning Microscopy

FV1000-D

BX61（Filter Type）

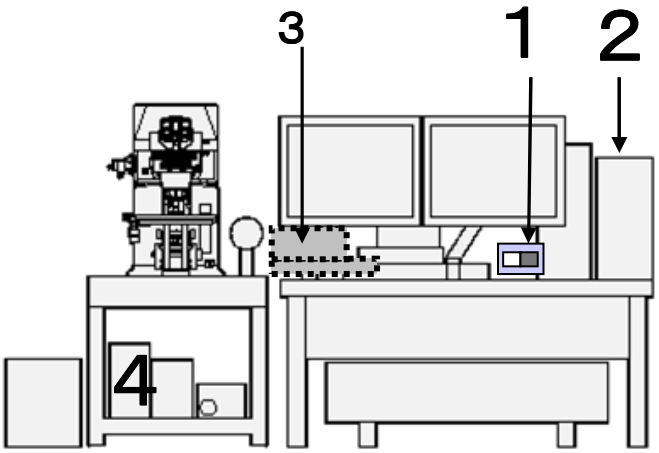
Operation Manual



1

Contents

Starting the System



1. Turn the Concentrated Power Supply ON.

2. Turn the computer ON.

3. Turn the laser ON.
   HeNe-G(543nm) : Black power supply.
   Turn the key switch ON.
   (473nm is connected to concentrated power supply)

3

4. Turn the mercury burner ON for Fluorescence observation.

5. Log on Windows

User ID: **fluoview**
Password: **fluoview**

6.  Double click this icon to log on to FV10-ASW

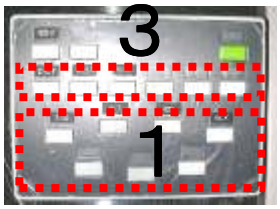
6

User ID: **Administrator**
Password: **Administrator**

3

Visual Observation under the Microscope

■ ■ Observation of Fluorescence Image ■ ■

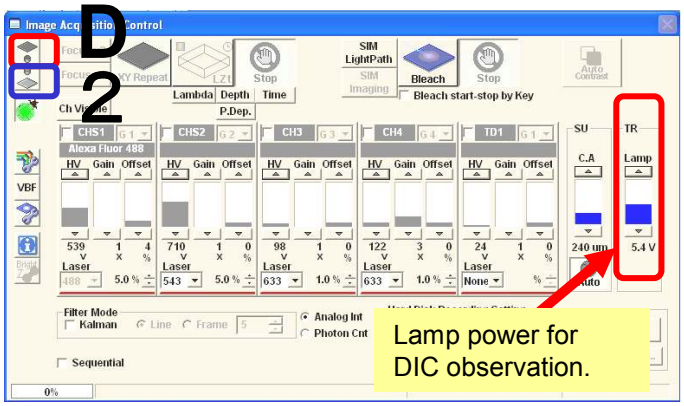


Hand Switch

■ Memo ■
Fluorescence filter

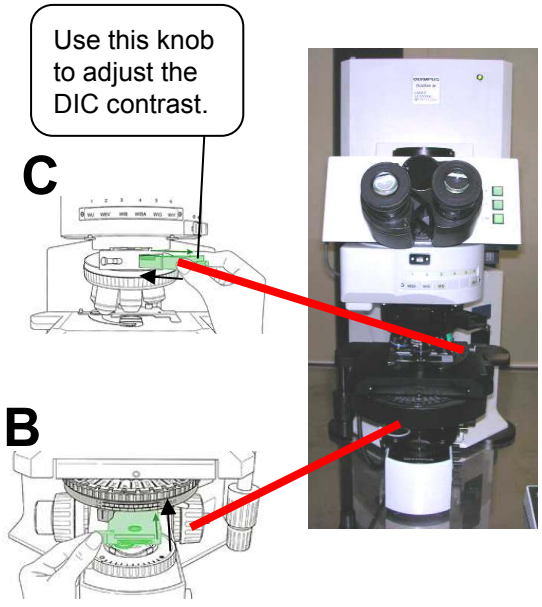
NIBA: Blue Excitation /
Green Fluorescence
(Ex : FITC、EGFP)

WIG : Green Excitation /
Red Fluorescence
(Ex : Rhodamine、DsRed)



1. Select an objective lens by using the hand switch.
2.  Click the button on the Fluoview software.
3. Select fluorescent filter cube by using the hand switch.
4. Focus to the specimen.
5.  After observation, click the button to close the shutter.

■ ■ Observation of Differetial Interference Contrast Images ■ ■

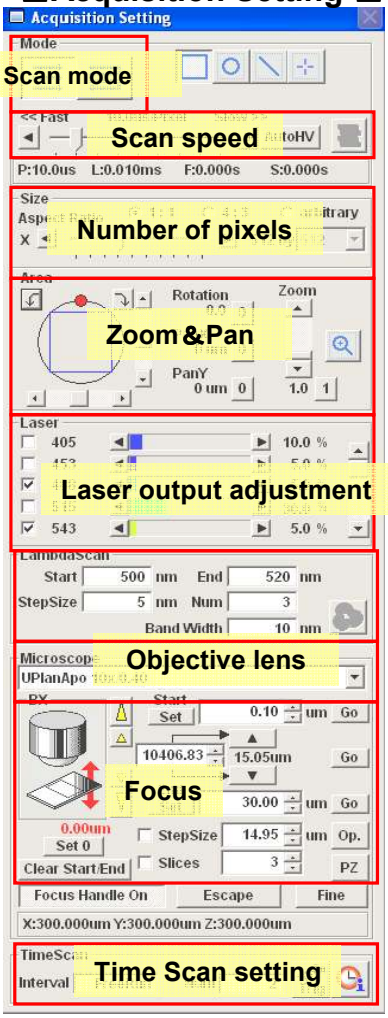


A. Select the Objective Lens.
B. Insert the Polarizing Plate in the Light Pass.
C. Insert the DIC prism slider in the Light Pass.
D.  Click the button on Fluoview software.
E. Focus to the specimen.
F.  After observation, click the button to close the shutter.

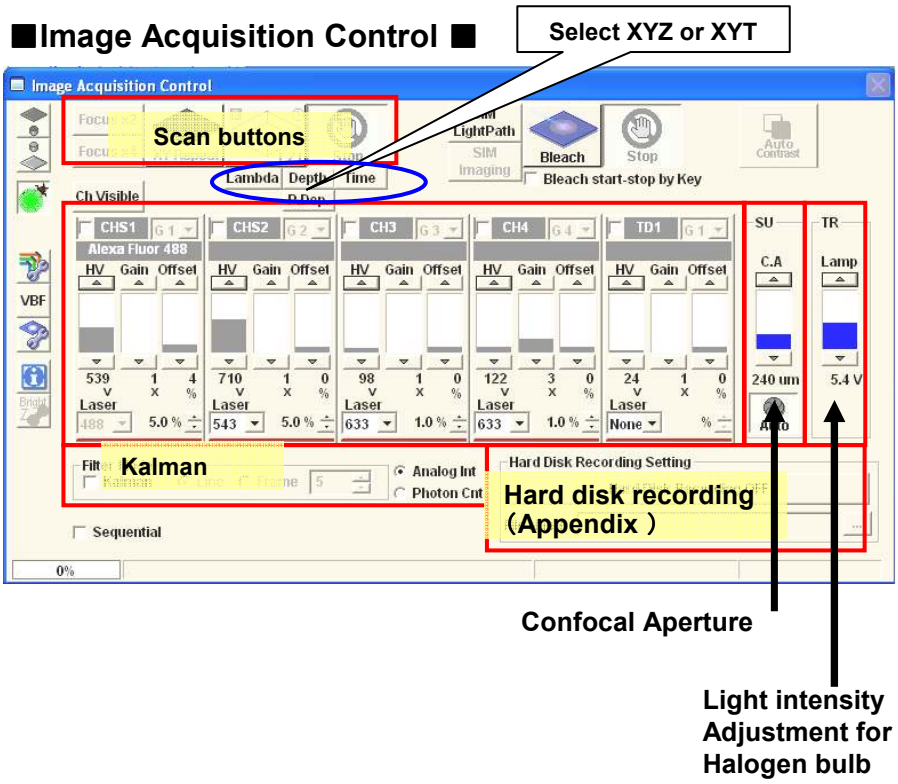
4

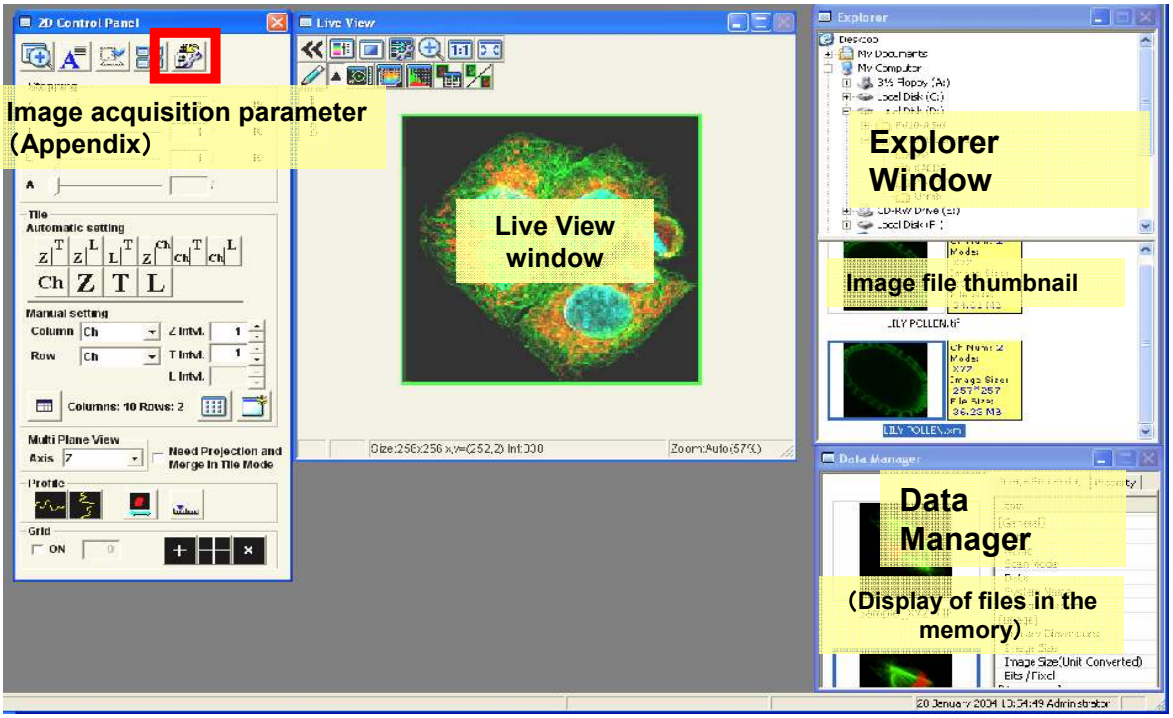
Overview of Operation Panel for Image Acquisition

■Acquisition Setting ■



■Image Acquisition Control ■

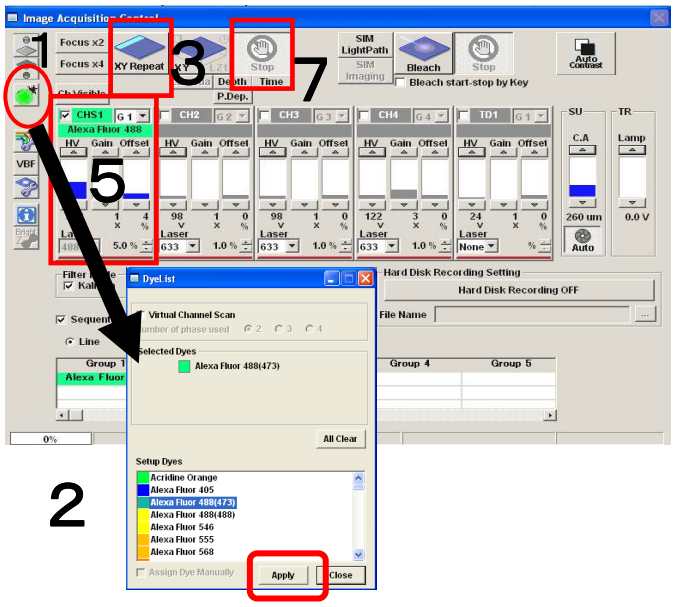




5

Image Acquisition
(Single Stain on XY Image)

Sample : Single stain of green fluorescence dye (Alexa fluor 488)



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

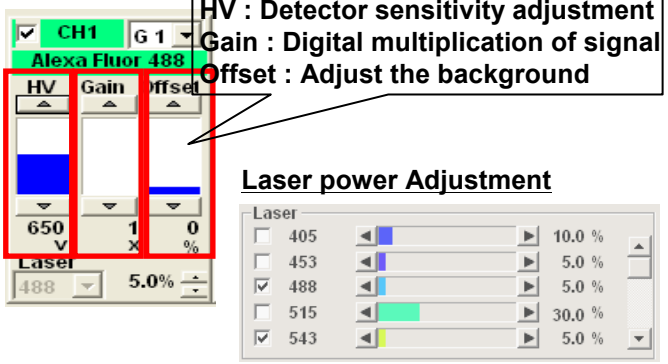
2. Click on the Apply button.

3. Click XY Repeat button 

to get live image.

4. Focus to the specimen.

5



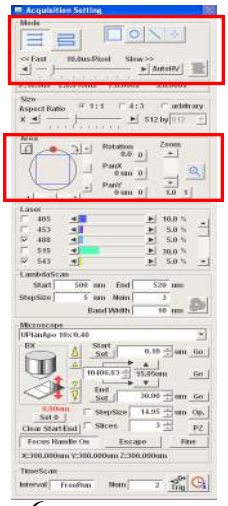
Laser power Adjustment

5. Adjust the green (Alexa488) image.
(Laser power, HV, Offset, etc)

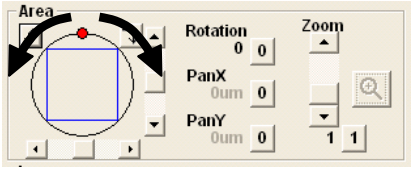
6. Use zoom and rotation if it is needed.

7. After the image adjustments, click Stop button 

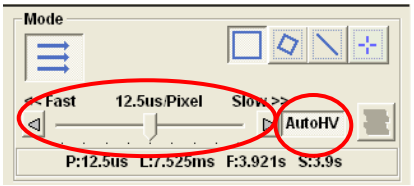
to stop scanning.



6 **Rotation** **Zoom**



8 **Rotation**



8. Select AutoHV and then select Scan speed.

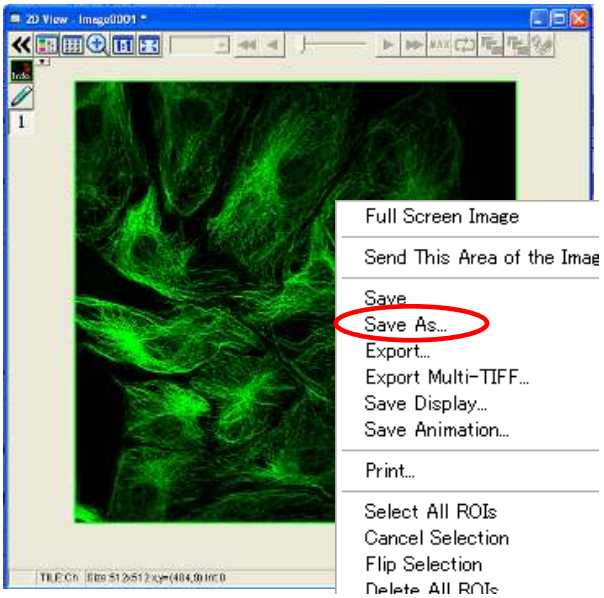
※As the scan speed becomes slower, noise can be removed while maintaining the current brightness.
You can also use Kalman, to remove the noise. Look at P11.

6

Image Acquisition
(Single Stain on XY Image)

Sample : Single stain of green fluorescence dye (Alexa fluor 488)

9



9. Click XY button to Acquire the image.

10. Saving the image : Right-click on the 2D view and select Save As to save the image.

■ **Memo** ■
File formats specifically for the FV10-ASW

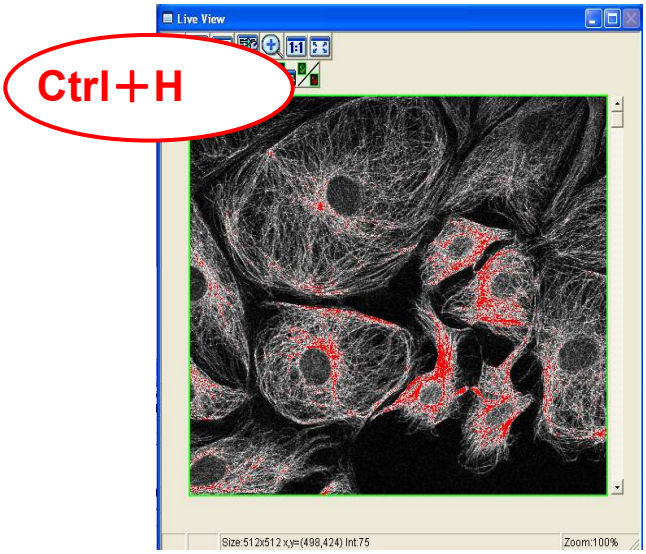
OIF format : Creates “a folder that contains an image (16-bit TIFF)” and “an accessory file,” which cannot be opened separately from each other.
OIB format : Creates the OIF format files in a single file, which is convenient for migration and other operations.

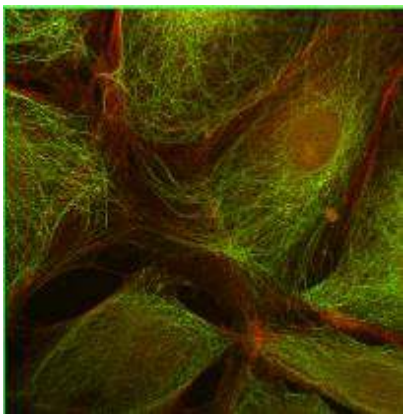
Look at Appendix1 to export the images to TIFF, BMP, or JPEG.

■Adjustment brightness intensity■

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).
If intensity is under 0, color is changed to blue (no signal).



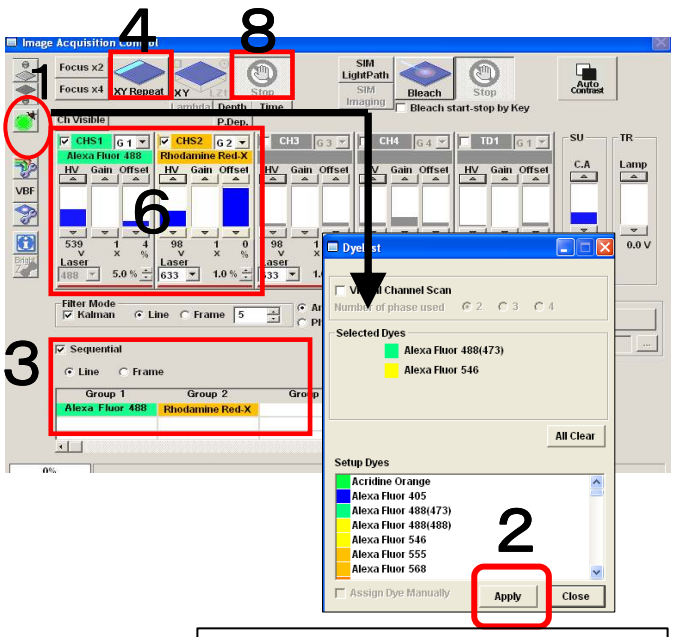


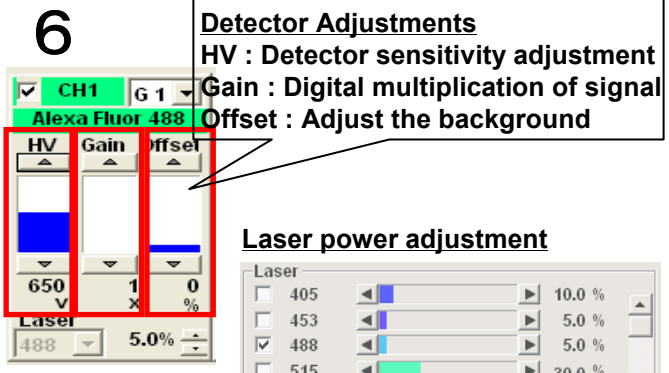
7

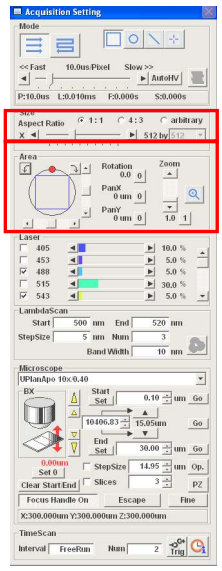
Image Acquisition（Double stain on XY）

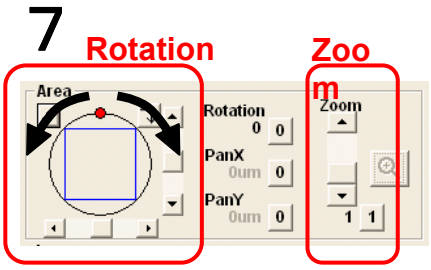
Sequential scan

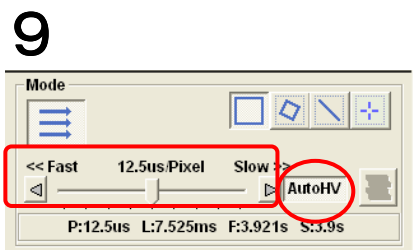
※Sequential scan is a one way to remove the fluorescence cross talk.











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

2. Click on the Apply button.

3. Check ‘Sequential’, and select ‘Line’.

4Click XY Repeat button 

to get live image.

5. Focus to the specimen.

6. Adjust the green（Alexa488）image.
（ Laser power, HV, Offset, etc ）

7. Use zoom and rotation if it is needed.

8. After the image adjustments, click Stop button 

to stop scanning.

9. Select AutoHV and then select Scan speed.

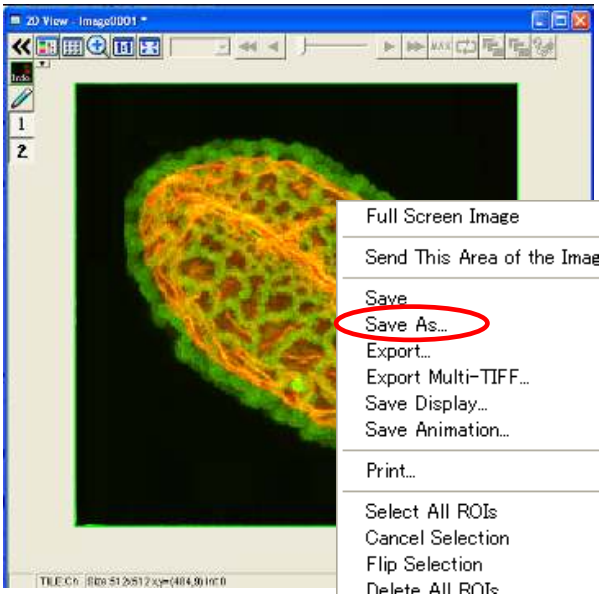
※As the scan speed becomes slower, noise can be removed while maintaining the current brightness.
You can also use Kalman, to remove the noise. Look at P11.

8

Image Acquisition（Double stain on XY）

Sequential scan

10



9. Click XY button to Acquire the image.

10. Saving the image : Right-click on the 2D view and select Save As to save the image.

■ **Memo** ■
File formats specifically for the FV10-ASW

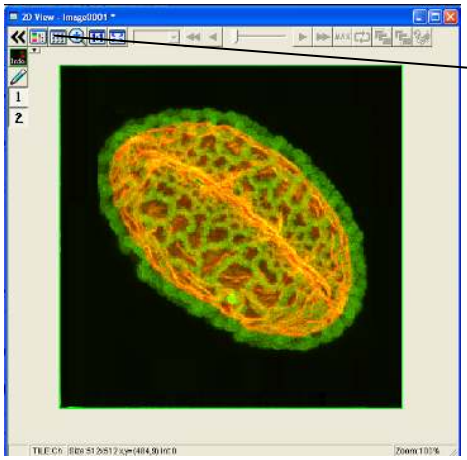
OIF format : Creates “a folder that contains an image (16-bit TIFF)” and “an accessory file,” which cannot be opened separately from each other.
OIB format : Creates the OIF format files in a single file, which is convenient for migration and other operations.

Look at Appendix1 to export the images to TIFF, BMP, or JPEG.

■Change View mode■

In Live View and 2DView, there are three pattern for multicolor sample.
1. 1 panel of merge image.
2. Tile view of each channel image.
3. Tile view of each channel and merge image.

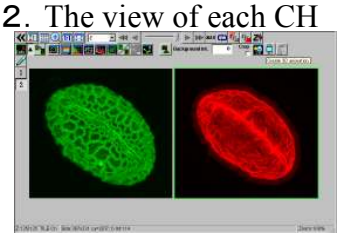
Tile view and 1 panel view is changed on the icons and if you need each channel and merge channel, select ‘Add a View’ on the right click menu.



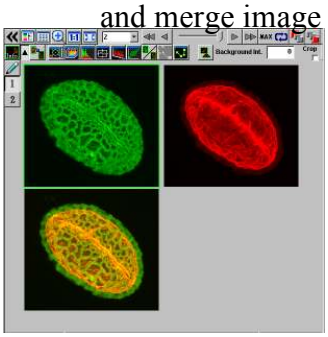
: 1panel view

: tile view

2. The view of each CH

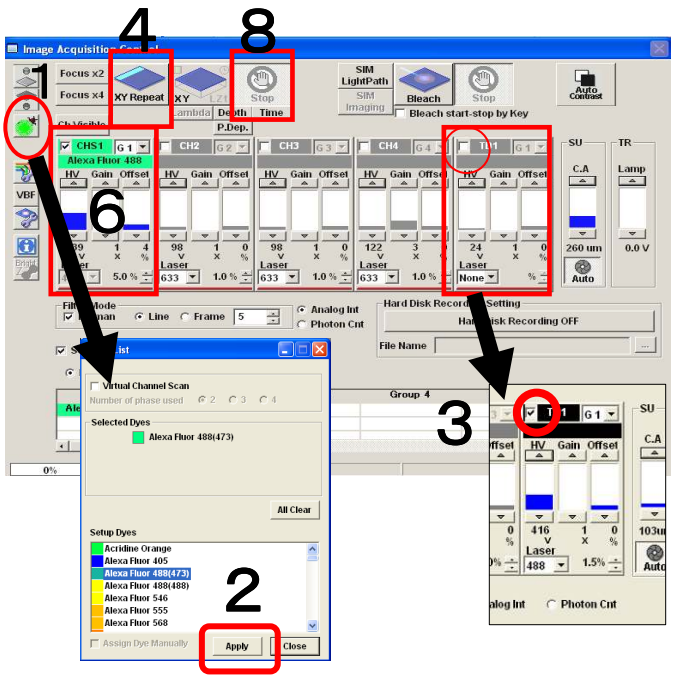


3. The view of each CH and merge image



9

Image Acquisition
（DIC with fluorescence on XY）



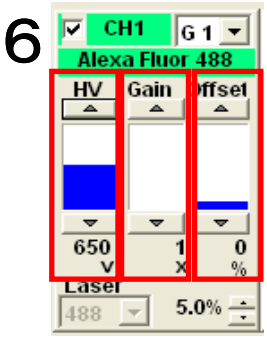
1. Click on the ‘Dye List’ button. On the Dye List panel, double-click on a fluorescence reagent to be used for observation.

2. Click on the Apply button.

3. Check ‘TD1’.

4. Click XY Repeat button 

to get live image.



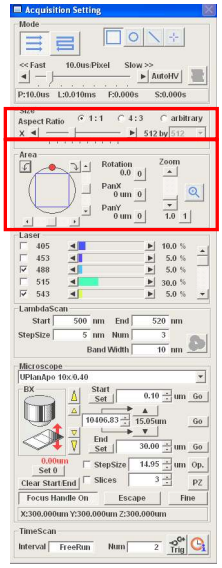
HV:検出器の感度調整をします

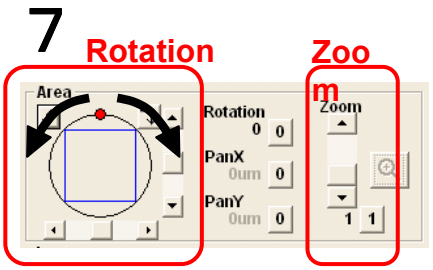
Gain:設定倍率で画像を明るくします。

Offset:背景を暗くします。

5. Focus to the specimen.

6. Adjust the green（Alexa488）image.
（ Laser power, HV, Offset, etc ）

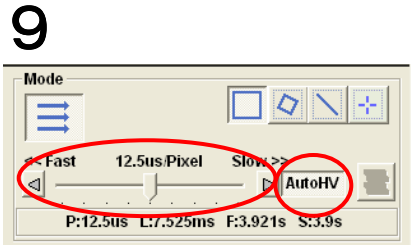




7. Use zoom and rotation if it is needed.

8. After the image adjustments, click Stop button 

to stop scanning.



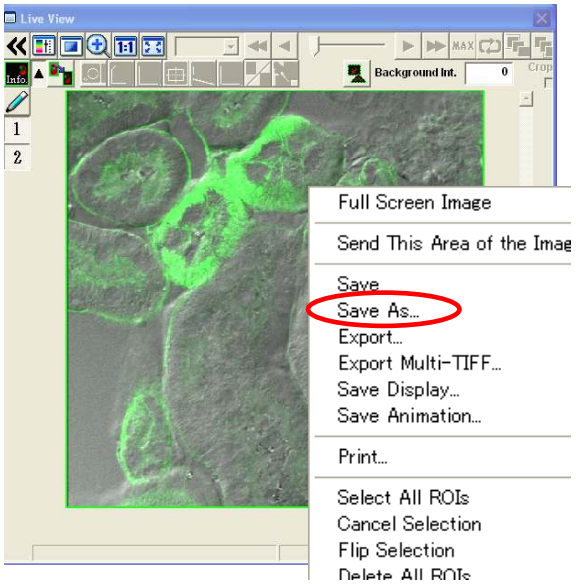
9. Select AutoHV and then select Scan speed.

※As the scan speed becomes slower, noise can be removed while maintaining the current brightness.
You can also use Kalman, to remove the noise. Look at P11.

10

Image Acquisition
（DIC with fluorescence on XY）

9



9. Click XY button to Acquire the image.

10. Saving the image : Right-click on the 2D view and select Save As to save the image.

■ **Memo** ■
File formats specifically for the FV10-ASW

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【Points to get a good images】

Signal is too dark

Turn up HV: Turn up sensitivity of detector. If you cannot get a good brightness in over 700V, take another methods.

Turn up Laser power: Fluorescence brightness will raise by laser power. But it is too much power, bleach occurs.

Changing CA: In normally, 

button is on to set the perfect size of pinhole by automatically. If you want to change it, undo the 

button and change it.

Turn up Gain: Digital multiplication of signal.

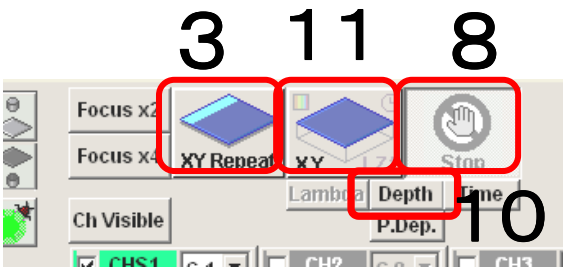
Too many noise

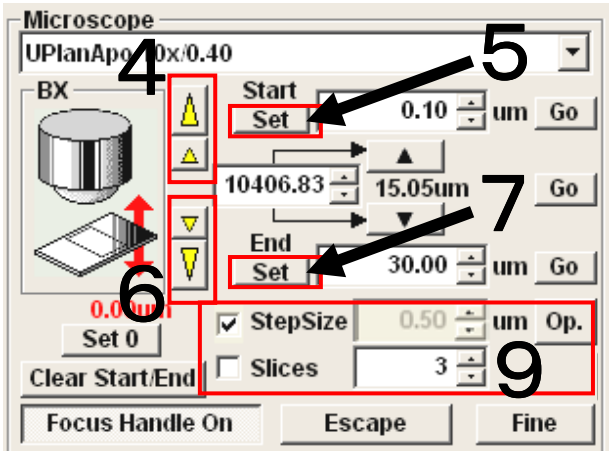
Kalman･･･Average the images to remove the random noise.
Click Kalman, and select Line and input the number of images for average.

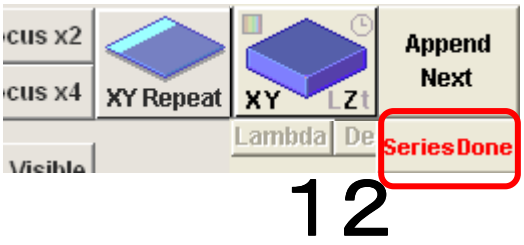
11

Image Acquisition（XYZ）

Acquisition of 3D image







1. Adjust XY image.
   Look at from page 6 to page 9

2. Click ‘Clear Start/End’ to reset the upper and lower position. And also, click ‘Set 0’ to change the base point.

3. Click XY Repeat button to get live image.

4. Click this to change the Z position and decide upper position.

5. Click Start ‘Set’ button to set the upper position.

6. Click this to change the Z position and decide lower position.

7. Click End ‘Set’ button to set the lower position.

8. Click Stop button to stop live view.

9. Put in a value to StepSize or Slices.
   （You can see the recommendation by Op. button）

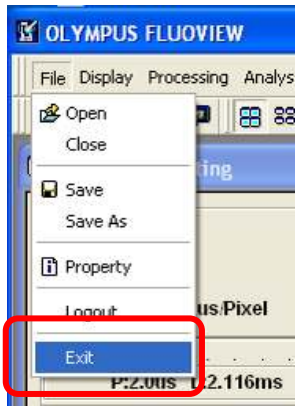
10. Click on ‘Depth’.

11. Click XYZ button to acquire the XYZ image series.

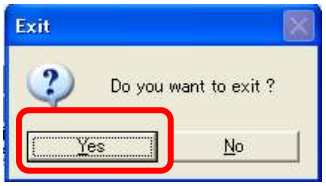
12. Click ‘Series Done’ and save the image.

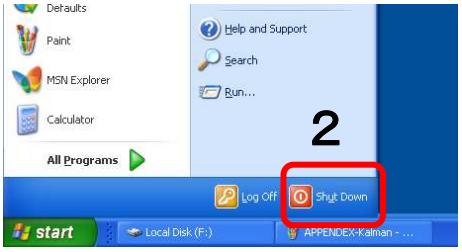
12

Closing the System



1

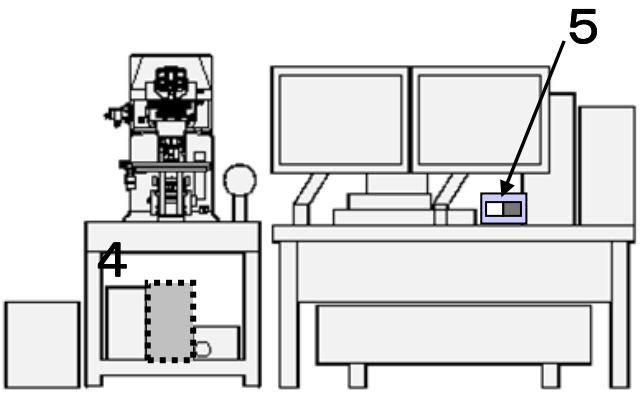




2

3

Key switch off



1. File/Exit to shut down the software.

2. Shutdown Windows.

3. Turn laser off.
   HeNe-Green laser (543nm)

4. Turn the mercury burner Off.

5. Turn the Concentrated Power Supply Off.

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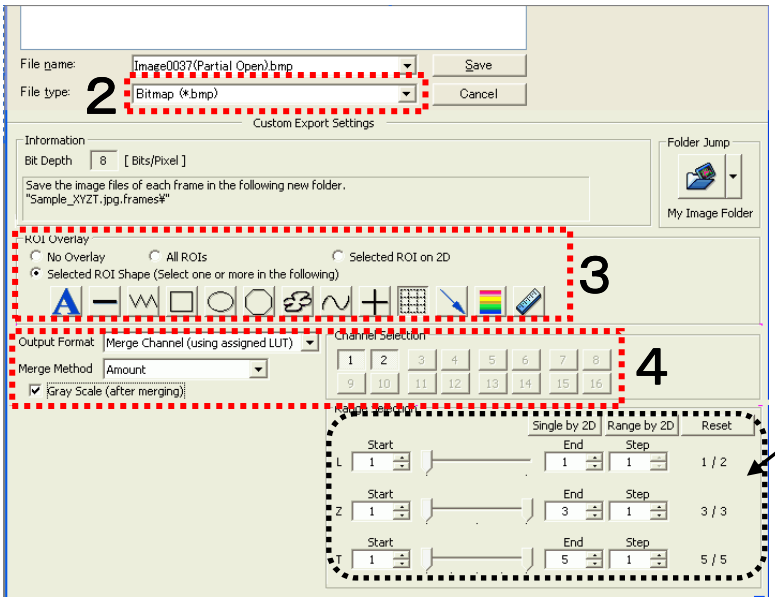
Appendix 1

Export Images

Export to Bitmap (*.bmp)・JPEG (*.jpg, *.jpeg)・TIFF (*.tif, *.tiff)・PNG (*.png)

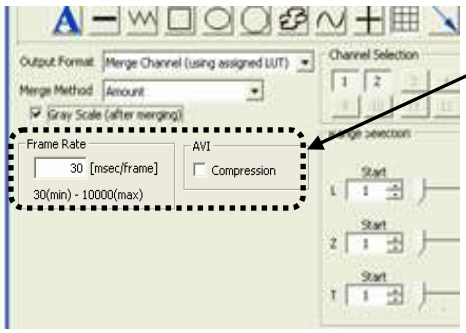
1. Choose 'Export' in the right click menu on the 2D View window of data to export.
2. Select file directory, name and format.
3. Select ROI shape if you needed. (ex scale)
4. Choose format in Output Format, channels in Channel Selection and range in Range Selection (Z/T).
5. Click 'Save' to export.



Range Selection
Select start frame and end frame of series.

Export to Movie Files (AVI、MOV形式は、XYZ・XYT・XYλ・XZT・XλT・XλZ画像で可能です)

1. Choose 'Export' in the right click menu on the 2D View window of data to export.
2. Select file directory , name, format, ROI shape, Output format, Channels and range.
3. Input the Frame Rate.
4. Click 'Save' to export.



Frame Rate
Frame rate of the movie and with or without compression selection

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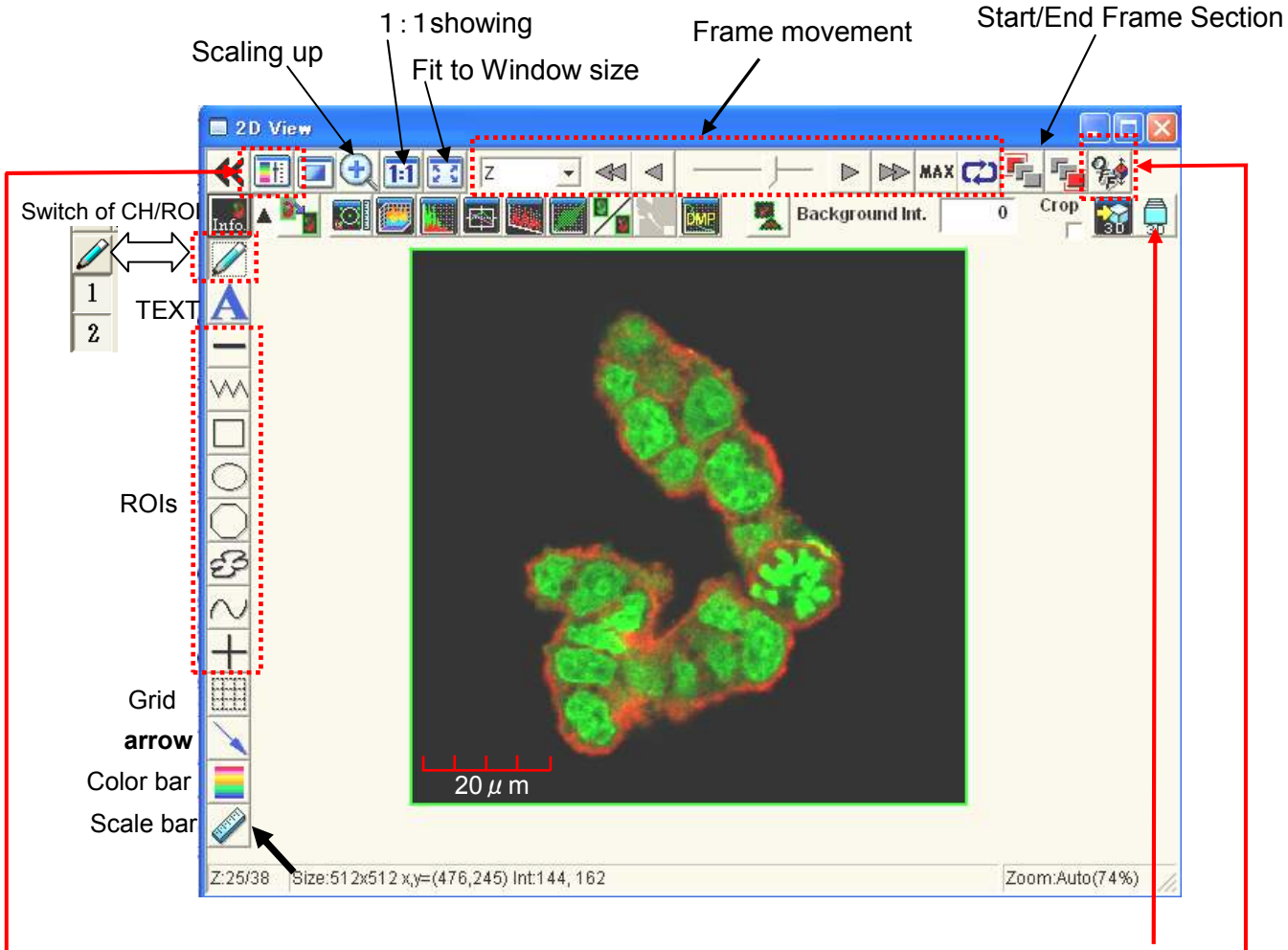
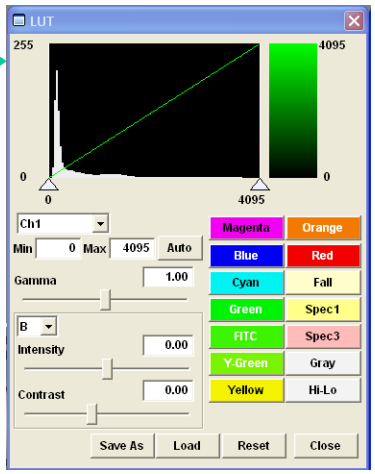
Appendix2

2D View Window



Make rotating 3D animation

LUT settings



Projection switching

(Projection Off) button: no projection.

(Z intensity projection) button: Z intensity projection is displayed.

(T intensity projection) button: T intensity projection is displayed.

(Lambda intensity projection) button: Lambda intensity projection is displayed.

(Z topographic projection) button: Z topographic projection is displayed.

(Lambda topographic projection) button: Lambda topographic projection is displayed.

(T series average) button: The average of T series images is displayed.

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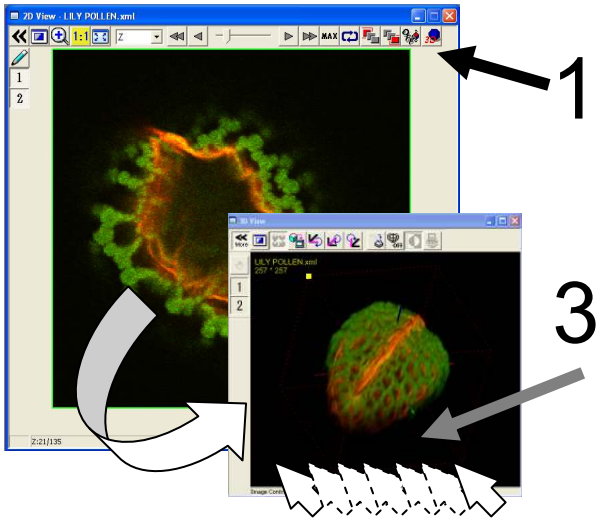
Appendix3

Image Analysis (Creating a Three-dimensional Image)



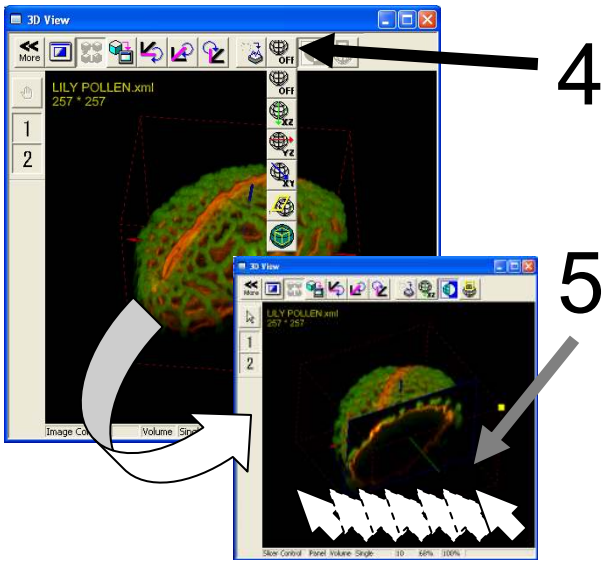
Observe the image at a certain angle

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.

→ To save this image, proceed to step 6 on the next page.



Observe a certain cross-section surface of the image

4. Click on the  button and select  .

5. Drag the mouse from side to side on the image to observe a certain vertical section.

→ To save this image, proceed to step 6 on the next page.

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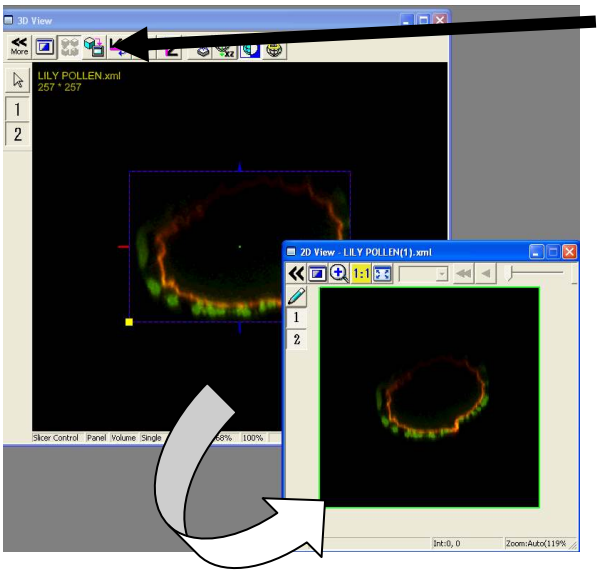
Appendix3

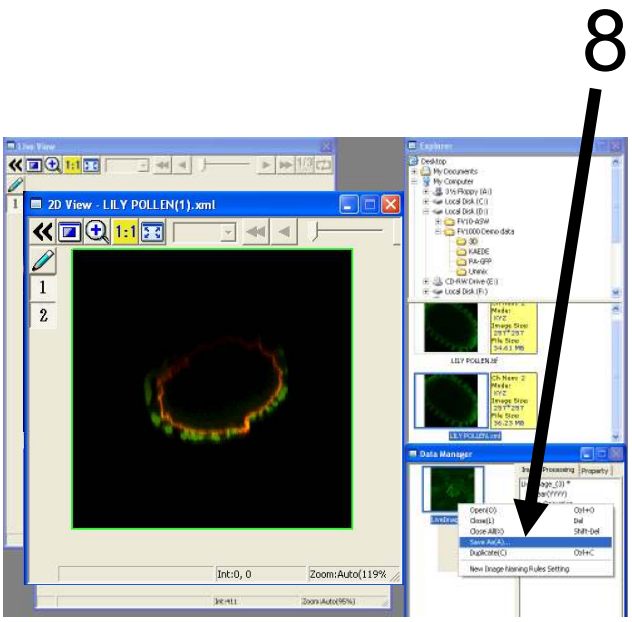
Image Analysis (Creating a Three-dimensional Image)

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 types "oib" and "oif" are file formats 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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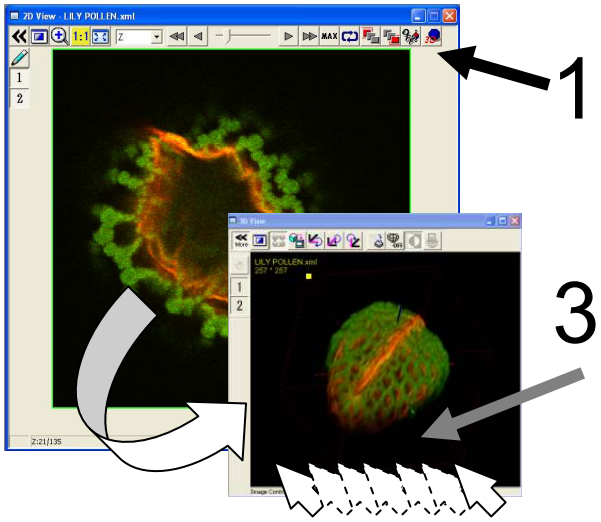
Appendix4

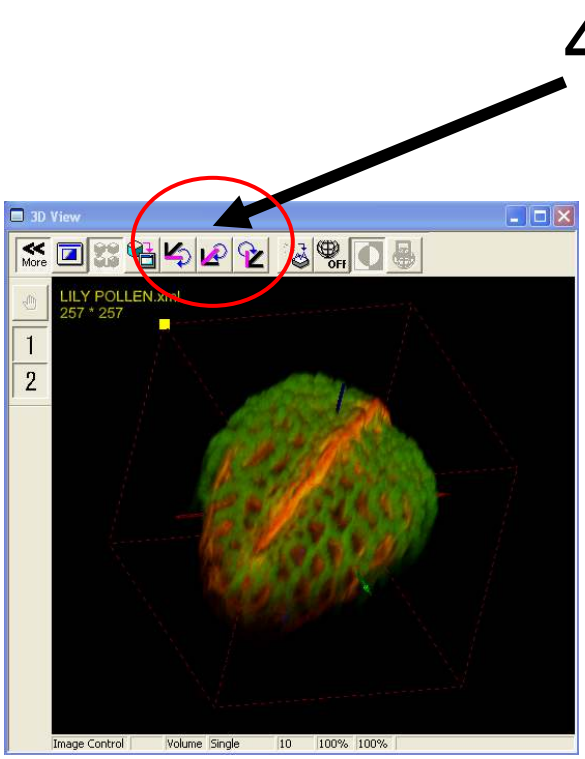
Image Analysis (Rotating a Three-dimensional Image)



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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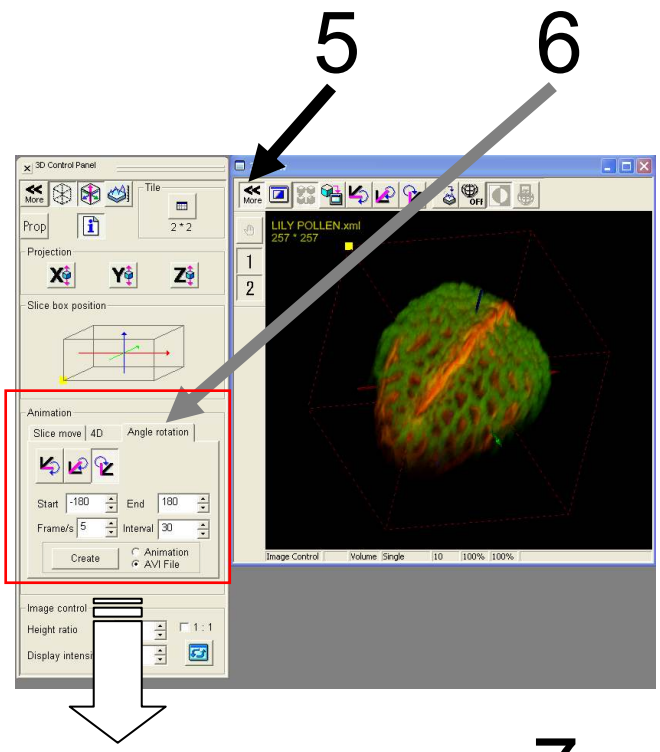
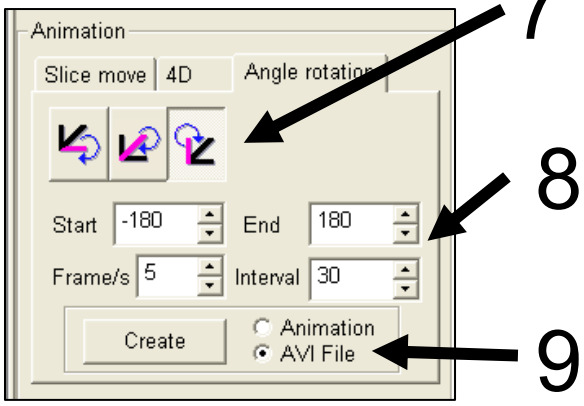
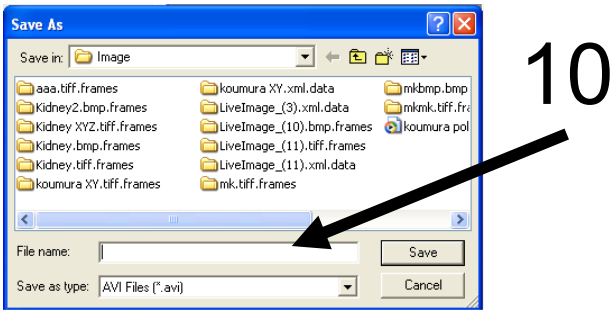
Appendix4

Image Analysis (Rotating a Three-dimensional Image)

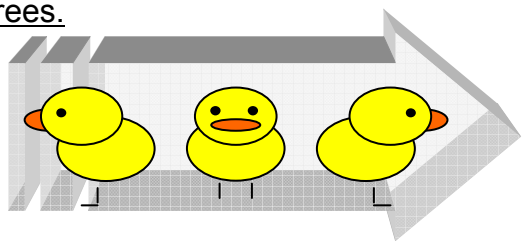






To save a rotation file as an animated image, create three-dimensional images according to the following procedure.

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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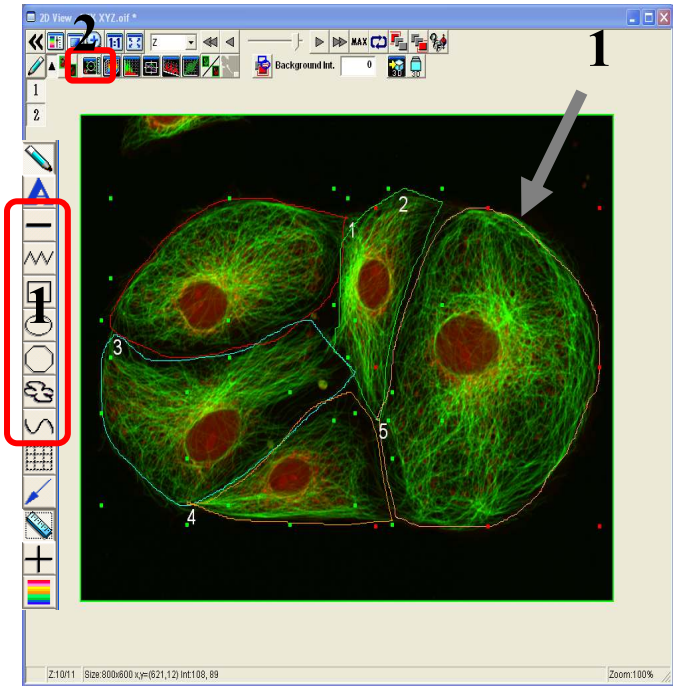
Appendix5

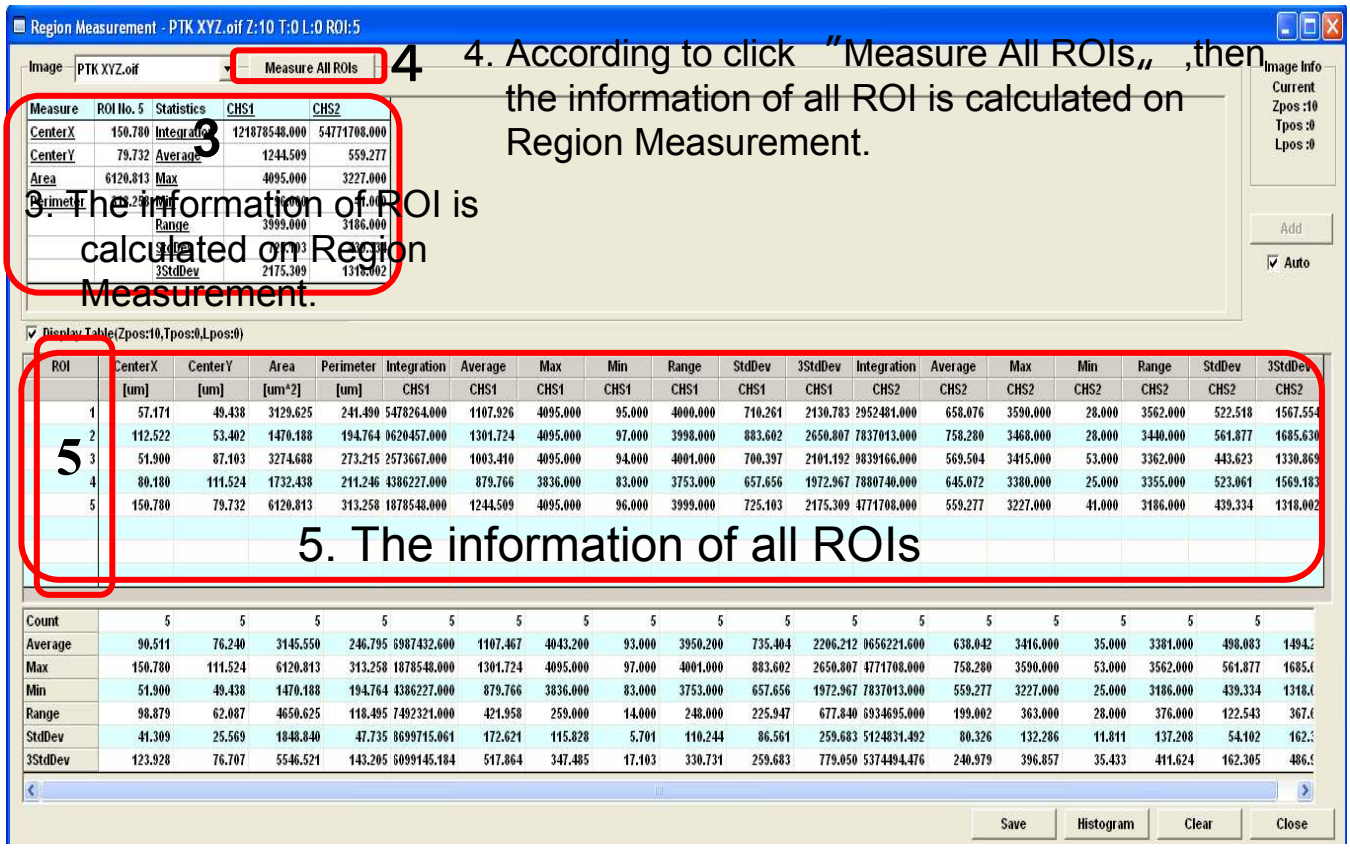
2D Image Analysis (Measure)



1. Enclose interesting regions by ROI

   Line on interesting positions by ROI —

2. Click ″measure„ .



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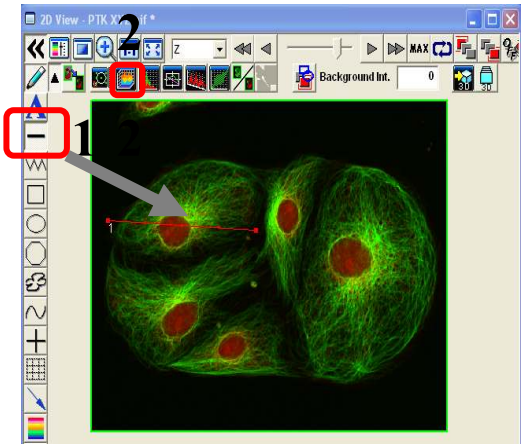
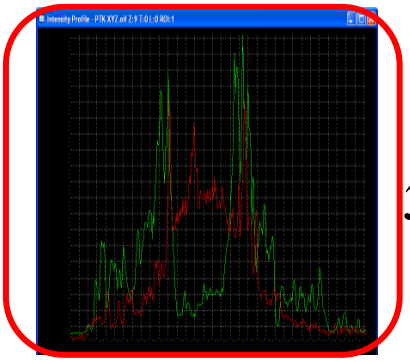
2D Image Analysis (Line Intensity Profile on the 2D image)



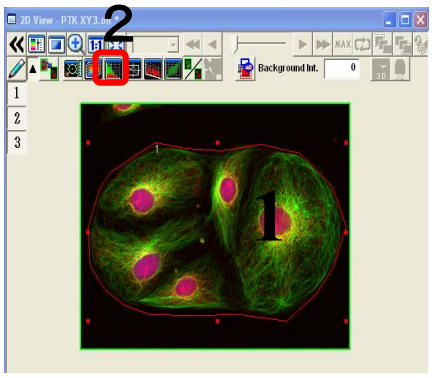


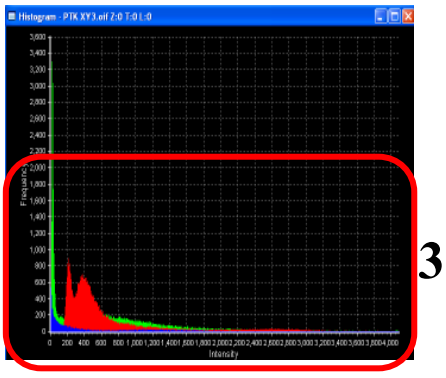
1. Line on the 2D image by ROI 

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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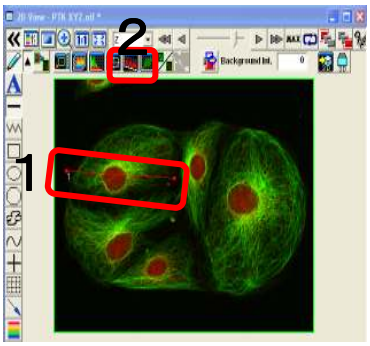
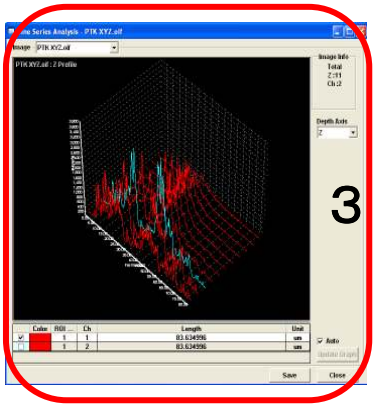
2D Image Analysis (Line Series Analysis)



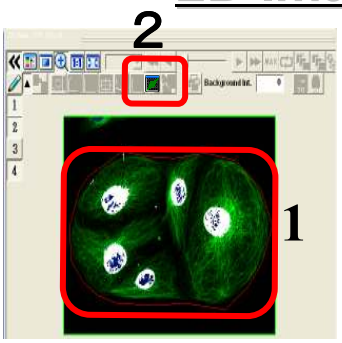


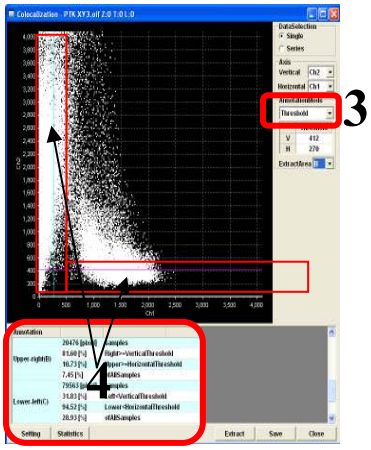
1. Line on the interesting region.

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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