



PerkinElmer UltraVIEW VoX Spinning Disc Confocal Microscope Standard Operating Protocol



Imaging and Flow Cytometry Core

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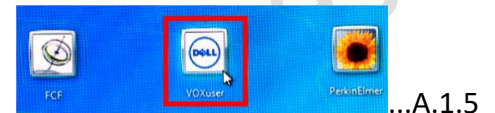
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A. Basic Operation

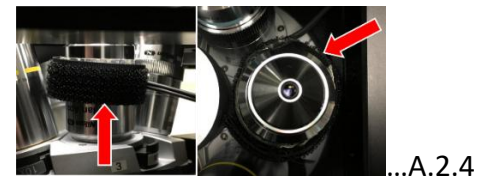
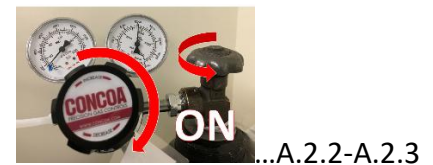
1. Turning ON the system

- 1.1. Switch on the power supply ① and ② mounted on the wall.
- 1.2. Switch ON the modular laser system 2.0 ③.
- 1.3. Switch ON Mercury lamp ④.
- 1.4. Turn ON computer system ⑤.
- 1.5. Log onto “VOXuser” account.



2. ** (Optional) When live cell imaging apply

- 2.1. Turn on temperature and CO₂ control module ⑥.
- 2.2. Turn on CO₂ tank (Anti-clockwise).
- 2.3. Turn the valve of regulator (clockwise), make sure the pressure reach indicated level (blue line).
- 2.4. Install Objective heater on the objective lens (for oil lens ONLY).
- 2.5. Place the incubator on the stage (label towards the top right corner).



3. Volocity Software start up

- 3.1. Launch the Volocity (x64) software on desktop.

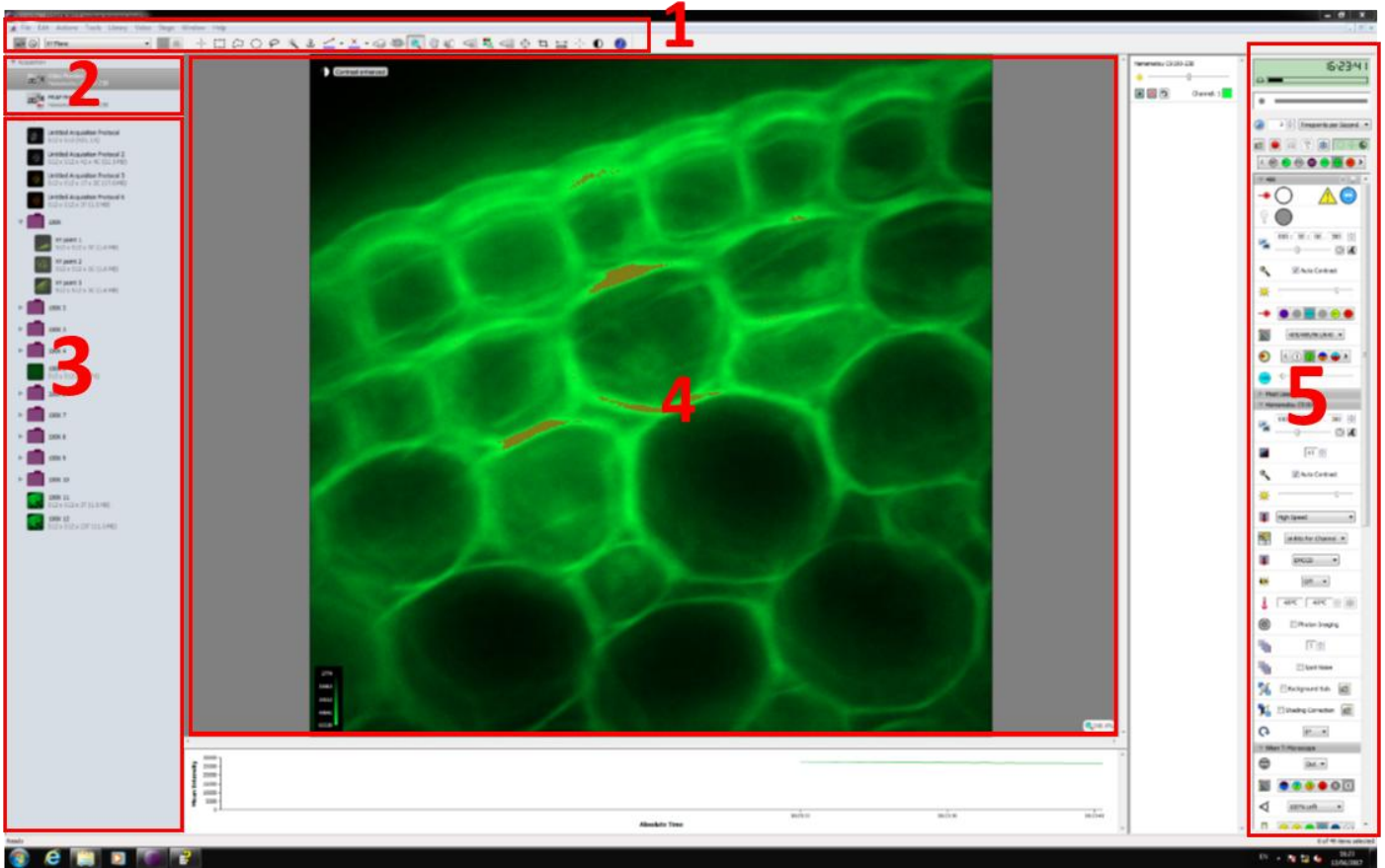


- 3.2. **Create a new library** or choose **Open an existing library** if you want to append to existing image set.
- 3.3. Create your folder under:
E:\Data\PI\your name



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B. Overview of the Volocity User Interface



- 1 Menu bar
- 2 Acquisition
- 3 Library
- 4 Video Preview window
- 5 Device Controls

C. Microscope setup and locating your specimen

1. Select suitable lens for your sample.
2. (Optional) apply immersion oil if 60X or 100X lens is selected.
3. Mount your sample onto the stage with suitable sample holder (select between slide or Petri dish holder)

4. Click on 'Video Preview'  button. And select 'BF' light path on the 'Device Control' to view your specimen under bright field via eyepiece.

5. And select 'FL' light path to view your specimen under fluorescence.
5.1. You can select suitable filter set on the TI-RCP controller.



...C.4



...C.5

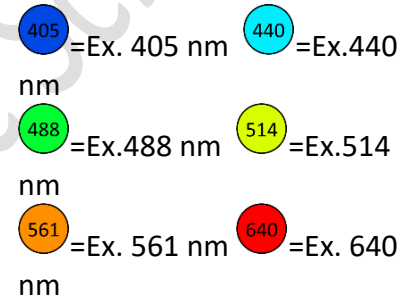
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...C.5.



...C.6



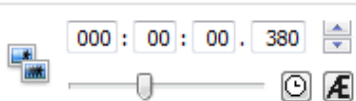
6. Select 'SD' on light path selector to prepare for image acquisition.

7. Change your light path to suitable wavelength then click 'unfreeze'  and ensure the laser emission is 'ON'.

8. You should now be able to view fluorescent image in the video preview window.

9. Toggle between different light paths according to the excitation wavelengths of labelling dye used on your specimen. Adjust the following parameters for each channel:

9.1. Exposure time (ms):




9.2. Sensor sensitivity: (maximum value = 200)



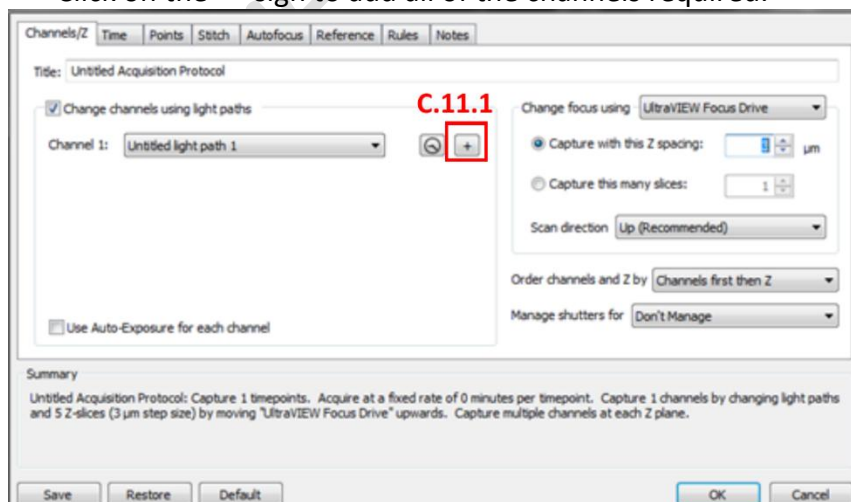
9.3. Laser power: (typical range 3-10%)



10. Click 'Save'  after you have adjusted your imaging parameters for each channel.

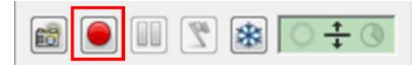
11. Right click on the acquisition control panel to view the acquisition setup window.

Click on the '+' sign to add all of the channels required.



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12. Click on 'Record button'



D. Setting up z-stack confocal scan

Prerequisite:

Follow Part C (p.2) to have the specimen in focus and visible in the video preview window.

1. Click on the z adjust button under 'UltraVIEW' to view z control panel
2. Use the slider on right side of the panel to adjust focal plane position (z)
3. 'Set Top' and 'Set Bottom' z-positions as desired.

3.1. You can preview the stack by clicking:

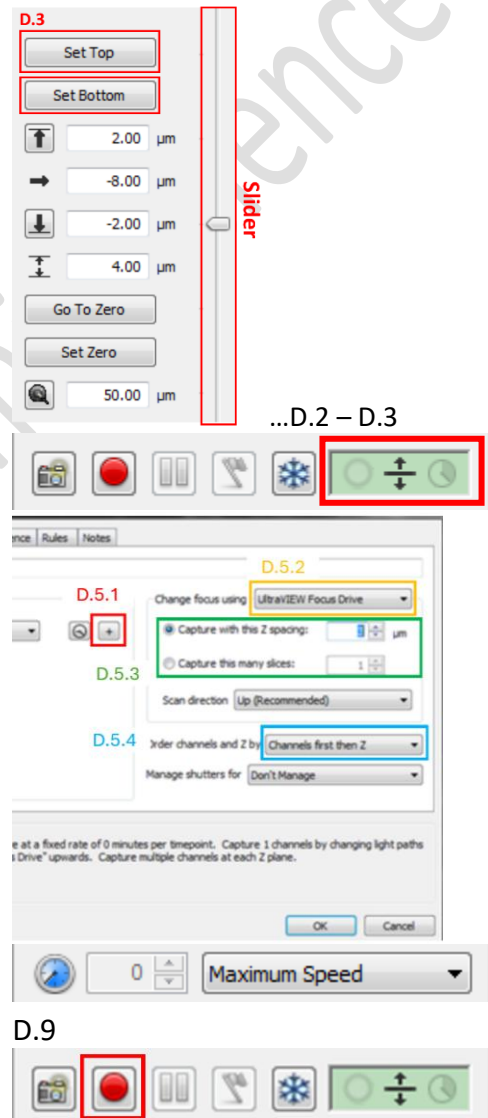
Go to top:

Go to bottom:

4. Right click on the acquisition control panel to view the acquisition setup window.
5. Adjust the following parameters in the 'acquisition setup' menu:
 - 5.1. Click on the '+' sign to add all of the channels required.
 - 5.2. Select 'UltraVIEW focus Drive' for changing focus
 - 5.3. Adjust axial increment per optical slice
 - 5.4. Choose z-scan priority:
Channels / z-direction
 - 5.5. Click 'OK'

6. Set the timepoint option to 'Maximum speed'

7. Click on 'Record button'



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E. Setting up multi-timepoint experiments

Prerequisite:

- Follow Part C (p.2) to have the specimen in focus and visible in the video preview window.
 - Follow Part D step 5-7 to view 'acquisition setup' window.
1. After you have your specimen in focus press 'ON' on the microscope to activate the Perfect Focusing System (PFS). This option will enable the microscope to lock onto your sample over long imaging sessions.



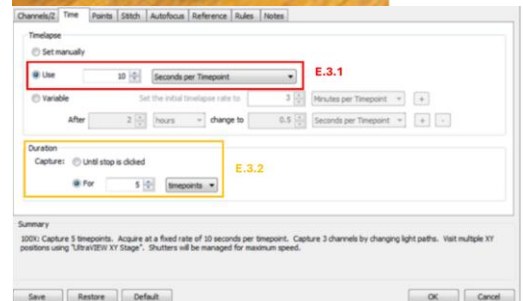
...E.1

2. **Fine-tune the focus with PFS Offset controller**

Note: This will work best for medium filled coverslip-bottom Petri dishes or chamber slides.



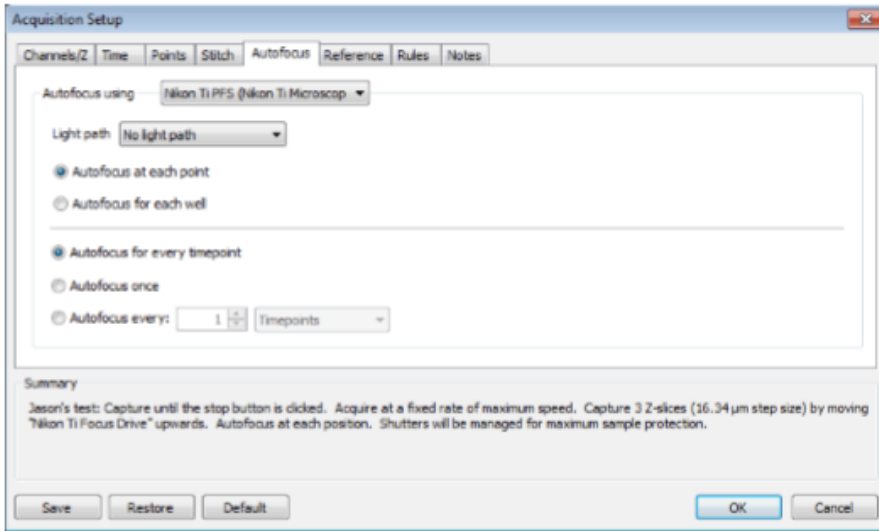
3. In the Acquisition setup dialog, go to 'Time' tab
 - 3.1. Adjust duration for each timepoint
 - 3.2. Adjust how many timepoints to be imaged.
4. Click 'OK'



...E.2

5. In the Acquisition setup dialog, go to 'Autofocus' tab
6. set "Auto-focus using" to "Nikon Ti PFS".

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7. Click on 'Record button'

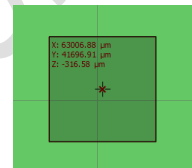


F. Setting up multi-point experiments

To setup multipoint scans position have your specimen in focus.

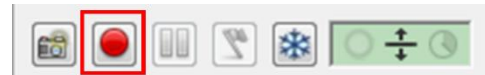
1. At your desired location, add point by:
2. Stage\Add point (or press Ctrl+Shift+A)
3. To view the coordinates of different points saved, right click the preview image and choose 'XY stage'

Under this option, the image area and coordinates (x,y,z) is displayed.



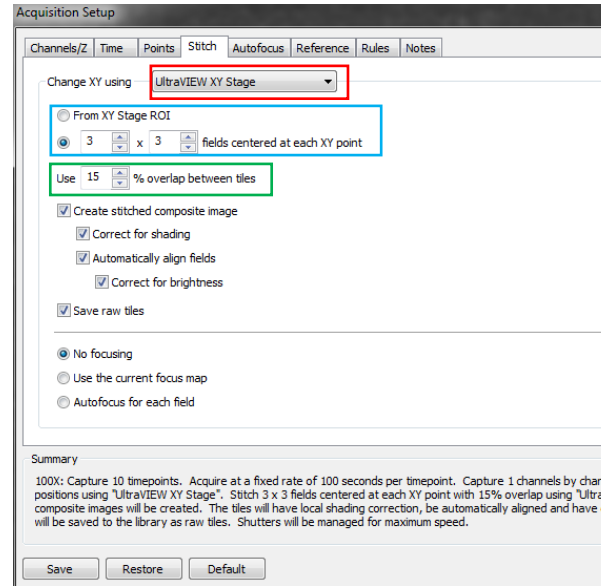
...F.3

4. Review point
5. Go to acquisition setup to verify imaging parameters then click on 'Record button'
6. Multi-point scans is compatible with z-stack (Part D), multiple time-point experiments (Part E) and tile scan function.



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7. Tile scan function can be accessed in the 'Stitch' tab in acquisition window.
 - 7.1. **Select** UltraVIEW XY Stage as control.
 - 7.2. **Choose** number of fields to be imaged per coordinate.
 - 7.3. **Determine** the % of overlap between tiles.
 - 7.4. Click 'OK' and then start recording.

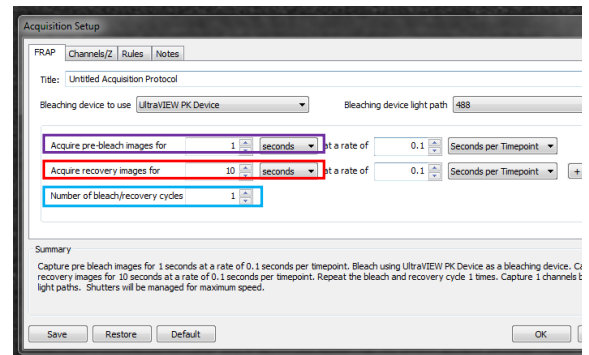
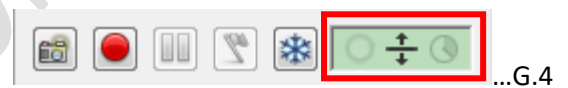
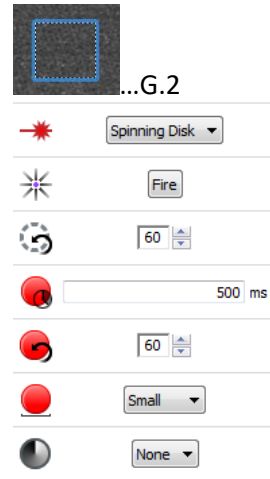


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G. FRAP function

FRAP function can be accessed under the **UltraVIEW** device panel.

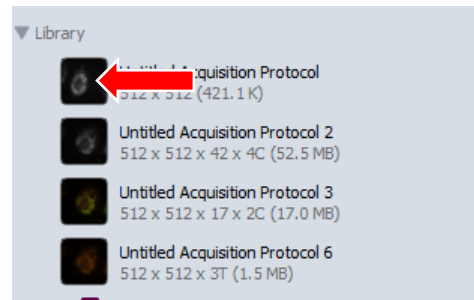
1. Select 'FRAP Preview'
2. Draw an ROI around the area you want to administer the treatment.
3. Adjust FRAP parameters:
 - 3.1. Double click on the  to choose laser wavelength
 - 3.2.  UltraVIEW PK cycles (determine the number of rounds the laser will go through the ROI)
 - 3.3.  Spot period
 - 3.4.  Spot cycle
 - 3.5.  Spot size
 - 3.6.  Attenuation (decrease FRAP strength)
4. Right click on the acquisition control panel to view the acquisition setup window.
5. Set the number of
 - 5.1. images to be acquired BEFORE bleaching.
 - 5.2. images to be acquired AFTER bleaching.
 - 5.3. cycles of treatment to be performed.



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H. Viewing and exporting acquired Images

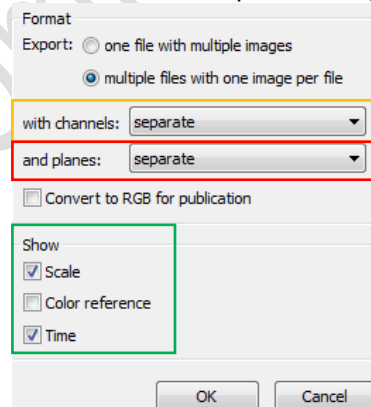
1. Double click the icon in library panel to open up a new window containing the images.
2. Choose between different viewing modes on menu bar. On the right listed a few useful ones of the viewing modes.
3. To export the images, right click on the image and select 'Export'
Alternatively, go to File\Export\
4. Choose from different file types.
5. Export:
 - 5.1. Separate channel
 - 5.2. Separate z-planes
 - 5.3. Label images with scalebar, color reference and time stamp.
6. Save to C:\Data\PI\your name"
7. Upload to your own server as soon as possible.



...H.1

XYZ = 2D view with orthogonal slices
3D Plane = manually rotate the z-stack
3D Opacity = render images into 3D objects
Extended Focus = combine the z-stack into a deeper depth-of-view image.

'Item as': more options available
'View as': direct export of image view



...H.5

I. Turning OFF the microscope

1. Exit Volocity software
2. Turn off computer ⑤.
3. Switch OFF Mercury lamp ④.
4. Switch OFF the modular laser system 2.0 ③. Wait until light and fan off
5. Clean oil objective (with lens cleaning tissue only but NOT Kimwipe)
6. Remove residue oil from the objective lens with a dry lens cleaning tissue
7. Clean the lens with a new lens cleaning tissue with 100% absolute ethanol
8. Dry the lens with another new lens cleaning tissue
9. Switch OFF power supply mounted on the wall ① and ②.
10. Replace microscope cover.