Training Guide – Olympus FV1000

Updated on September 16, 2014

The Olympus FV1000 is designed for timelapse imaging of living cells, tissues, or organ explants. If you have any problems, please contact David Pfaff @ extension 8044 or 540-463-6685. In case of emergency contact Dr. Robert Peterson with Olympus America at 919-619-9902.

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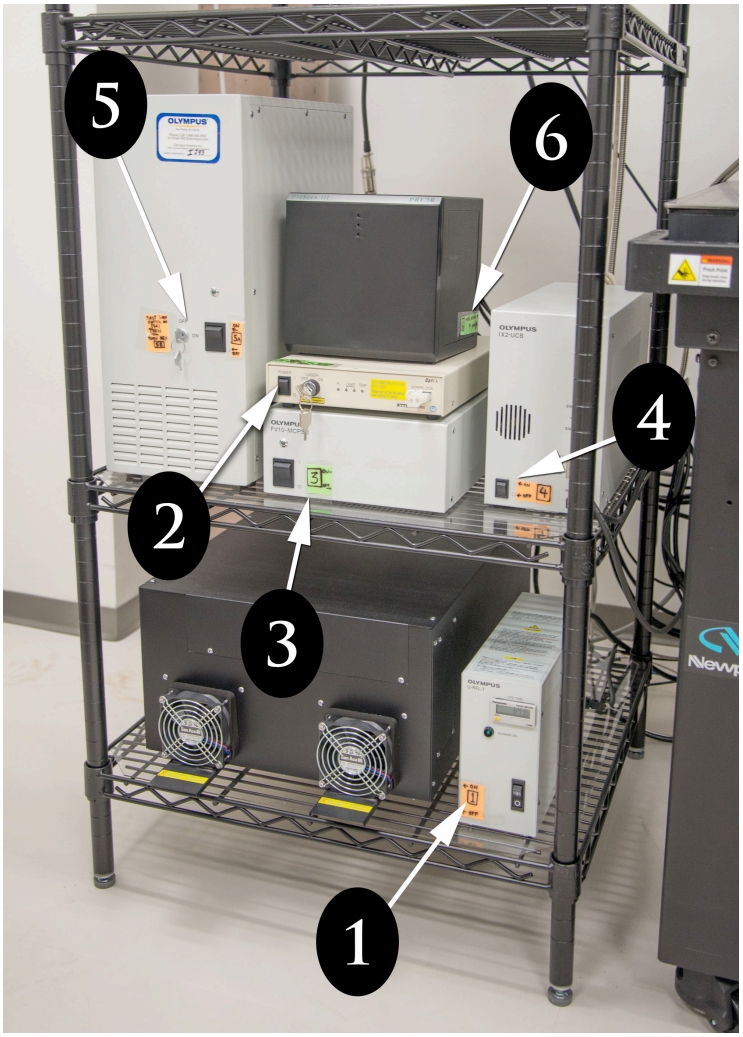
Starting the System

No.	Equipment Description
1	Mercury, flip the switch to activate the mercury lamp
2	559 Laser. Turn on button, wait for light to stop flashing, turn key.
3	Multi-combiner power switch.
4	UCB. Push the button to activate.
5	Main PSU. Push the button and turn the key to activate the scanner power supply.
6	SIM PSU. Push the button and turn the key to activate the SIM scanner power supply.
7	Turn on computer (not shown)

Turn-on procedure.

1. To log into the computer using your W&L login and pwd.
2. The program you need to use is **FV10-ASW,** once you open it use the account created for you.
3. The first thing you should do is to initialize the stage, go to “Device” and choose Multi Area Time Lapse. When the pop up window appears, click “OK” for the stage to initialize.



Placing Specimens in the Chamber

1. Open front of environmental chamber by turning knobs until white dot is face down.
2. The condenser can be tilted back to allow access to the adjustable stage, then place the dish tightly into the adjustable stage insert.
3. Tilt the condenser back into place and close the front of the environmental chamber.

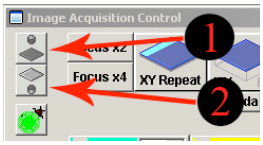
Setting up Imaging Using DIC

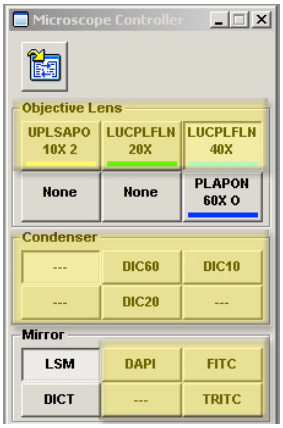
1. Rotate the condenser turret to engage the suitable optical element for the objective in use in the light path.
2. Move the polarizer detaching lever 1 on the polarizer unit to engage the polarizer in the light path.
3. Attach the objective to be used to the revolving nosepiece and rotate it to engage the objective in the light path.
4. Move the polarizer rotation/clamping knob 2 on the polarizer unit horizontally in the counterclockwise direction around the axis until the position with which the field of view is darkest.
5. Clamp the polarizer rotation/clamping knob so that the polarizer will not rotate.
6. Place a specimen on the stage and bring the specimen into focus.
7. Adjust the field iris diaphragm so that its image circumscribes the field of view.
8. Adjust the aperture iris diaphragm to enhance the contrast.
9. Move the prism movement knob of the DIC slider to select the interference color that can provide the optimum contrast in accordance with the specimen.

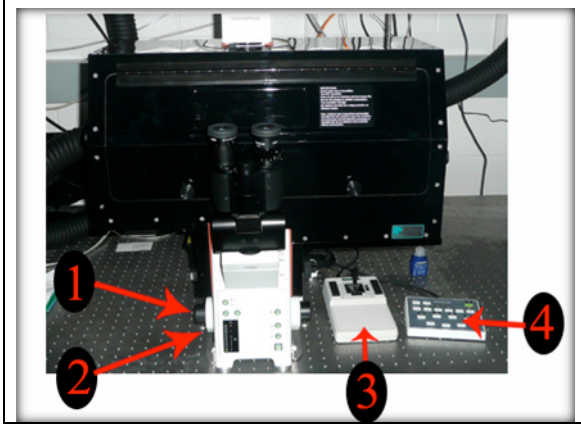
Capturing Images

Finding your specimens

1) To see your samples, click on the button for transmitted light (1) or epifluorescence (2).
2) Adjust the "Microscope Controller" or the handpad that is located next to the microscope.
   a) **Objective** – chose the objective you want, usually starting with 10X objective to find the specimen and then moving to higher magnification.
   b) **Condenser** – this will adjust itself automatically.
   c) **Mirror** – changes the cubes for which fluorophores you will view through the eye pieces.
3) To find your samples you will need to use several of the microscope controls.







No.	Equipment
1	Focal Knob.
2	Course/Fine adjustment. The front button, below the focus knob, toggles between Coarse and Fine focus.
3	Stage Control. The joystick controls X/Y axis movement of the motorized stage.
4	Objective/Turret Control. This pad controls which objective is chosen and which fluorescent cube is in position. However, this can also be controlled through the software.

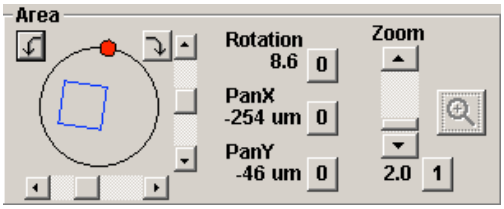
4) Once the specimen is in focus and the correction collar is adjusted, switch off the epifluorescence or transmitted light.

5) Choose which dyes you wish to image by clicking on the Dye List button and then 

choosing your desired fluorophores from the provide list (image to right) and hitting Apply.

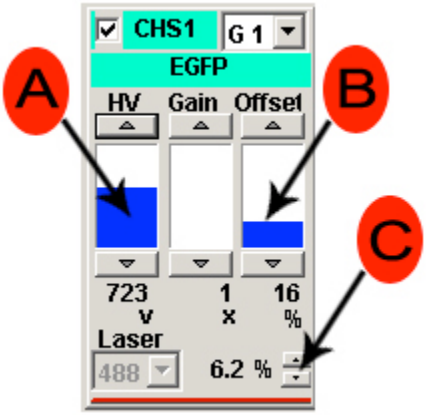
6) If you are using multiple fluorophores then you will need to click on the sequential button located below channel 1. This will set single fluorophores into different channels by default.
7) If you are going to be scanning sequentially then you can obtain better pictures by opening the VBF tab (pictured to the right) and adjusting each slider to its maximum of 100nm.

YOU ARE FINALLY READY TO SCAN!



1) Make sure the AutoHV button is depressed and then perform basic functions using the FastX2 or FastX4 setting.
2) You will want to focus using the microscope knobs set to "Fine Focus", zoom in to your sample, rotate the picture and Pan to center your sample using the set of controls pictured to the right.
3) At this point you should also determine what pinhole width you want using the controls to the right. The "SU" sets the pinhole diameter, where "Auto" sets it to 1 Airy Unit. As you increase the diameter of the pinhole you increase the amount of light that passes through and the thickness of the sample.

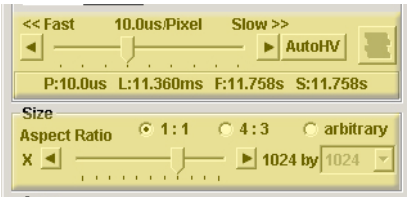
4) After you center and zoom your sample to the right size you will need to adjust the image using the HV, Offset, and laser power (see the image to the right).
   a) Before starting press Ctrl-H to switch the image to "High-Low", which gives you a range of brightness and darkness in two colors.
   b) In the figure, (A) points to the HV. This is equivalent to the "Gain" in other systems. All of these terms refer to the voltage (HV) that passes through the PMT detector. The more voltage you have, the brighter your sample looks. Go too high and you get non-specific background. For quantification, adjust the HV until you just see red and then back of one notch.
   c) In the figure, (B) points to the Offset. Offset is used to decrease the background. For quantification, adjust the offset until you see blue background and then back off one notch.
   d) In the figure, (C) points to the laser power. If you find that your HV is below ~500 then your laser power is too high. Adjust the laser power down until your gain is somewhere between 500-700 in a best case scenario.



5) At this point you will do the final touches using XY Repeat 

. There are a number of settings you should adjust (highlighted to the right in yellow):
   a) Speed, set it 2 or 3 notches from the right for final images.

b) Aspect Ratio, you can do standard 512x512 up to 1024x1024 or you can do arbitrary settings such as 256x1024 for long, narrow specimens.

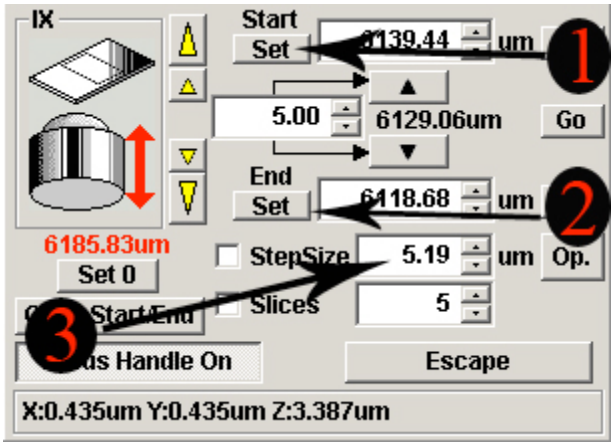


c) Kalman averaging is found under the Gain/Offset box for Channel 1, see figure below.

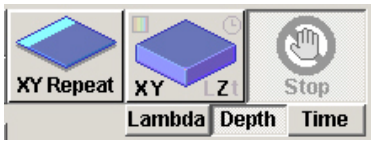
i) Use Analog Int, either Line or Frame, and set the averaging at 2, 3, or 4.

Capturing a Z-stack

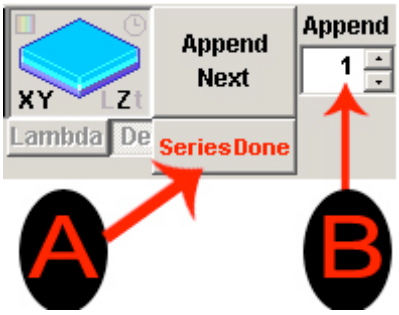
1) Focus to the top of your sample using the arrows. The large arrows move the objective the distance listed in the Step Size window (#3), while the small arrows move half this distance.
2) Click the Set button when you reach the top of your sample (#1).
3) Repeat this for the bottom of your sample (#2).
4) If the Step Size is too large, I recommend hitting the Op. (optimize) button, which gives you an "optimal" z-stack step size.



5) Once you have set the top and the bottom of your stack you are ready to collect the image. In the picture below, you will need to depress the "Depth" tab (shown depressed). This turns the image in the button above into a cube representing X/Y/Z collection. Hitting this button will collect your image.

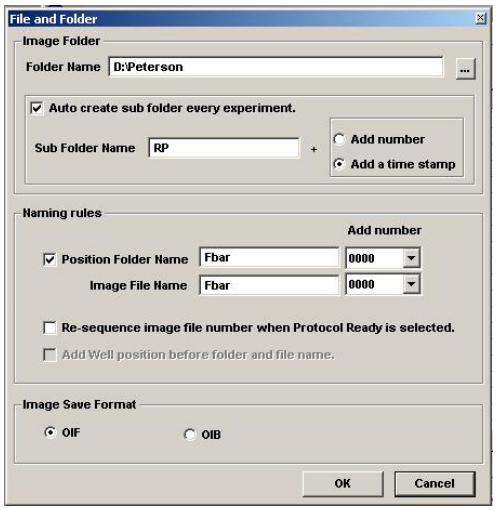


6) When your series is finished you will need to click on "Series Done" for the individual images to be Appended. If you would like the series to go longer, you can add by clicking on Append and then hit Appened Next.

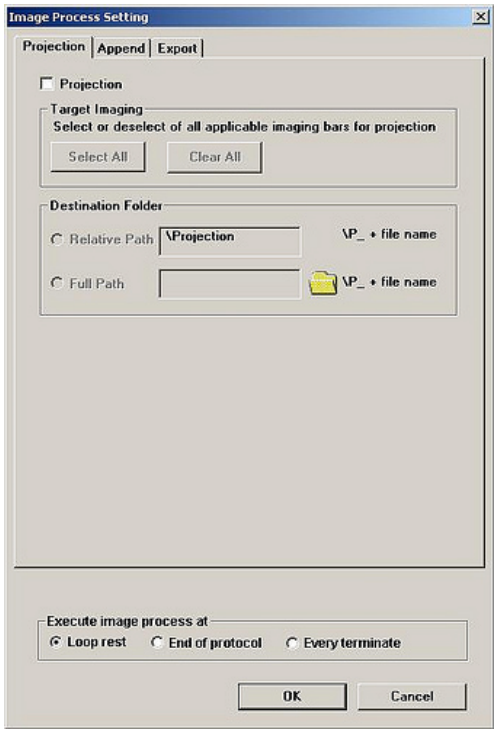


Scanning Multiple Positions

1) Set all your parameters the way you want your final image collected, this includes speed, frame size, rotation, Kalman averaging, and z-sectioning.
2) Open the Multi Area Time Lapse Controller. If the Registered Point List does not open, click on this button below  to see it.
3) Adding data points to the Registered Point List is fairly straight-forward, you simply click this button () every time you have a new position set (with all parameters set the way you want them in the final image.
4) Before you scan your points, in the Registered Point List panel above there are several things you will need to do.



5) First, click on the File and Folder button  .
6) In the File and Folder window, set the Folder Name where you want to save, the SubFolder you want to create, and the name for each Folder and File that will be produced (Fbar below). Leave the rest of the settings as they are.
7) After clicking OK, go back to the Registered Point List panel and click on this button  to open the Image Process Settings Panel.
8) In this panel, you will need to click on two tabs to make changes. The boxes you will need to check include:
   a) Projection (under Projection Tab)
   b) Append (under Append Tab)
   c) Normal (under Append Tab)
   d) With Projection (under Append Tab)
   e) End of Protocol, on the bottom of the window, you can do this under any tab.
9) At this point, when you hit Okay, you will have the settings for all your images ready.



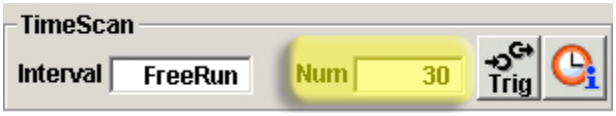
10) Now, set the interval in the bottom of the Registered Point List for how often you collect your images and the number of intervals you want to perform.

11) **YOU ARE READY**, so click Ready (

). At this point the Scan arrow should be available and you can start a scan.

Time Scanning

1. To perform time scanning of a single spot, set the speed, pixel size, zoom, rotation, HV, offset, etc.
2. In the TimeScan box located in the bottom left hand corner you can set the time interval (FreeRun will collect one image after the other with no lapse) and the number of images you want to collect.



3. When the prescribed number of images has been captured you will be given the choice of adding more images through “Append” or to finish the series (the same as is shown in **Capturing a Z-stack** above).

SIM Scanning and Bleaching

1. There are two types of bleaching and activation.

    A. **Main Scanner** stimulus allows for the use of any laser line. However, it cannot be performed simultaneously with image capture. It can be used to stimulate and then capture images or it can be used to capture images, pause, stimulate, then continue capturing images. This is very useful for things like Kaeda transformation where changes are going to be observed overnight.

    B. **SIM Scanner** stimulus can be used with any laser. However, it can be synced with the image scanning so that stimulation and image capture can occur simultaneously. This is exceptionally useful for experiments that measure changes in seconds or minutes.

2. When the SIM LightPath button 

is depressed, the light path is changed for SIM scanner use. The acquired image will shift a little by changing the light path. If this button is not pressed, only the main scanner is available.

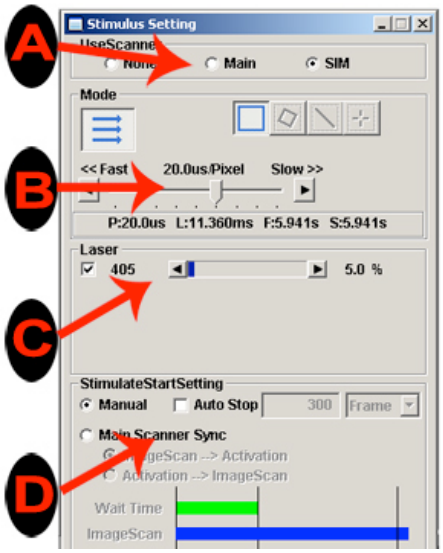
3. When the SIM Imaging button 

is clicked, scanning by the SIM and its main scanners is synchronized. This enables the image of the laser irradiation points obtained by the SIM scanner to be acquired on the main scanner.

4. To open the Stimulus Settings Panel, click on this button  , which is located below the VBF button.

5. To use the SIM scanner, depress both SIM LightPath and SIM Imaging buttons.

6. The SIM scanning panel has four panels that need to be adjusted.



    A. Use Scanner should be set to Main or SIM depending on how you want to bleach or stimulate.

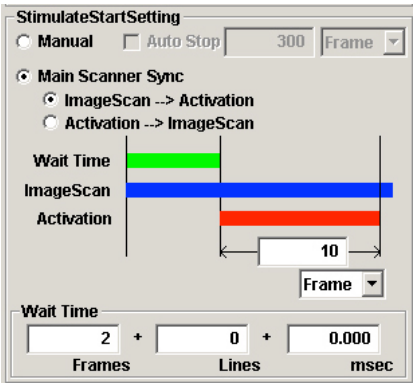
    B. The Mode can be used to choose a ROI and to adjust the speed. A slow speed is usually better for bleaching.

    C. Laser power should be adjusted on control samples to determine the correct levels before proceeding with the experiment.

    D. The Stimulate settings can be set in a

multitude of ways. Manual will allow you to use the Bleach button (see below) to lase the entire sample or an ROI.

7. Using the SIM with “Main Scanner Sync” will allow you to adjust several options.

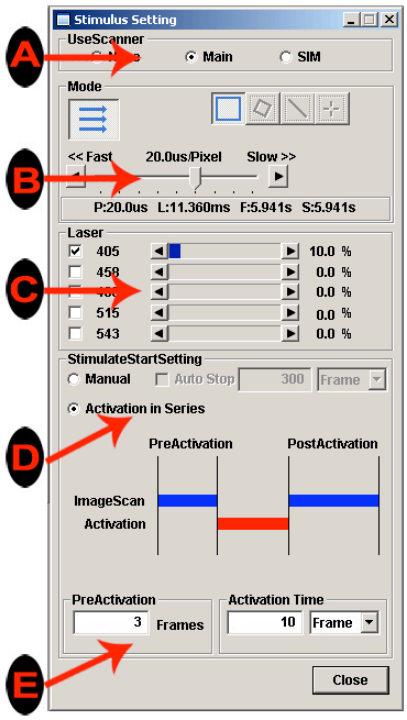


   A. You can choose to either scan the image for a period of time and then activate (followed by more image scanning) or you can choose to activate for a period of time and then start collecting images.

   B. The wait periods can be in frames, lines, or msec.

   C. This option should be used in combination with a time scan as discussed above in Time Scanning. Set your Main Scanner Sync the way you prefer it and then adjust your time settings to perform a single-spot timelapse as needed. Then, click the time box under the XY Scan. Now, starting your XY scan makes Image Scanning and Activating synchronized.

8. When using the Main Scanner there will be some new choices.



   A. Depress the Main button.

   B. Choose your speed based on desired effect.

   C. Any of the lasers can be used for bleaching/activation.

   D. Generally, you will be using the Main Scanner for “Activation in Series”.

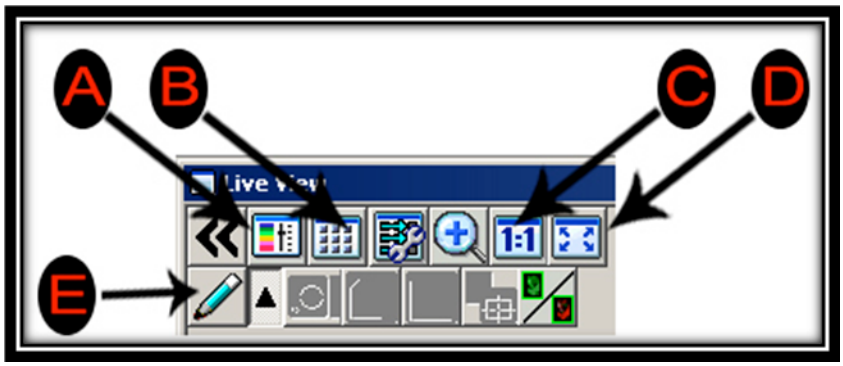
   E. This setup allows you to capture a number of frames, then to activate for a number of frames using the speed and lasers chosen above, then to continue capturing images afterwards.

   F. Again, this is used in combination with the Time Scan settings discussed above.

Glossary of Controls and Buttons

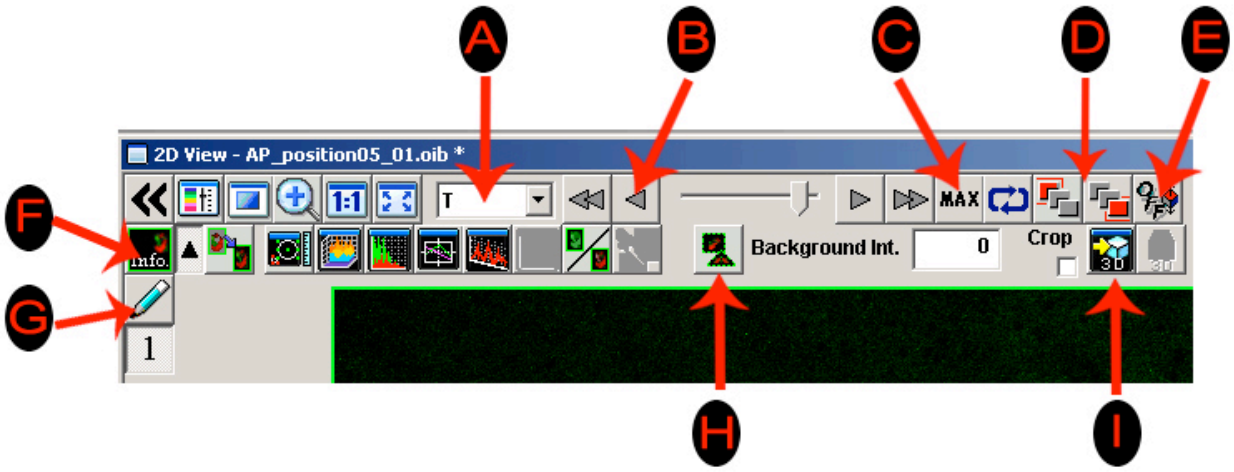
Live View Window Controls

These buttons can be found on the Live View window where images appear while being captured.



A. The LUT Setting (Look Up Table) button can be used to change the color of the image or to set up the “High-Low” that is used for setting HV and Offset. (A shortcut to the High-Low is Ctrl-H).
B. The view mode button can be used to switch between overlay view and gallery view (which gives you the ability to see all channels simultaneously).
C. The original scale button will switch to a view that is the actual pixel dimension of the image. In the case of 1024x1024 that will result in not being able to see the entire image. Using 512x512 will give you a small picture which looks good.
D. The fit to screen button will fit the image to the size of the screen. This will shrink the 1024x1024 to fit inside the field of view and it will zoom the 512x512 to fill the field of view.
E. The ROI toolbar button will open a palette of tools for drawing and measuring on the picture.

2D View



A. The AXIS pulldown menu controls whether you are viewing a single z-series (Z) or a single plane over time (T).
B. The single arrow buttons move you along the chosen axis one-step at a time. The double arrows animate the series.
C. This button adjusts the speed of the animation.
D. These buttons set the current position as the lower or upper limit for the animation.
E. This projection/topography button can be used to create a projection of single z-planes, all z-planes within a time series, or of a time series.
F. The Active Overlay button adds a timestamp to your timeseries.
G. The ROI Toolbar is the same as in the above.
H. The Crop Button can be used to create a new image from an ROI defined using the ROI Toolbar. This includes creating a movie of an ROI from a Z or T stack.
I. Clicking this button will open the 3D viewer software that can be used to create rotations of 3D projections. The crop button next to it can be used to create a crop that will open in the 3D viewer software.