Working with inverted microscope Nikon T*i* Eclipse

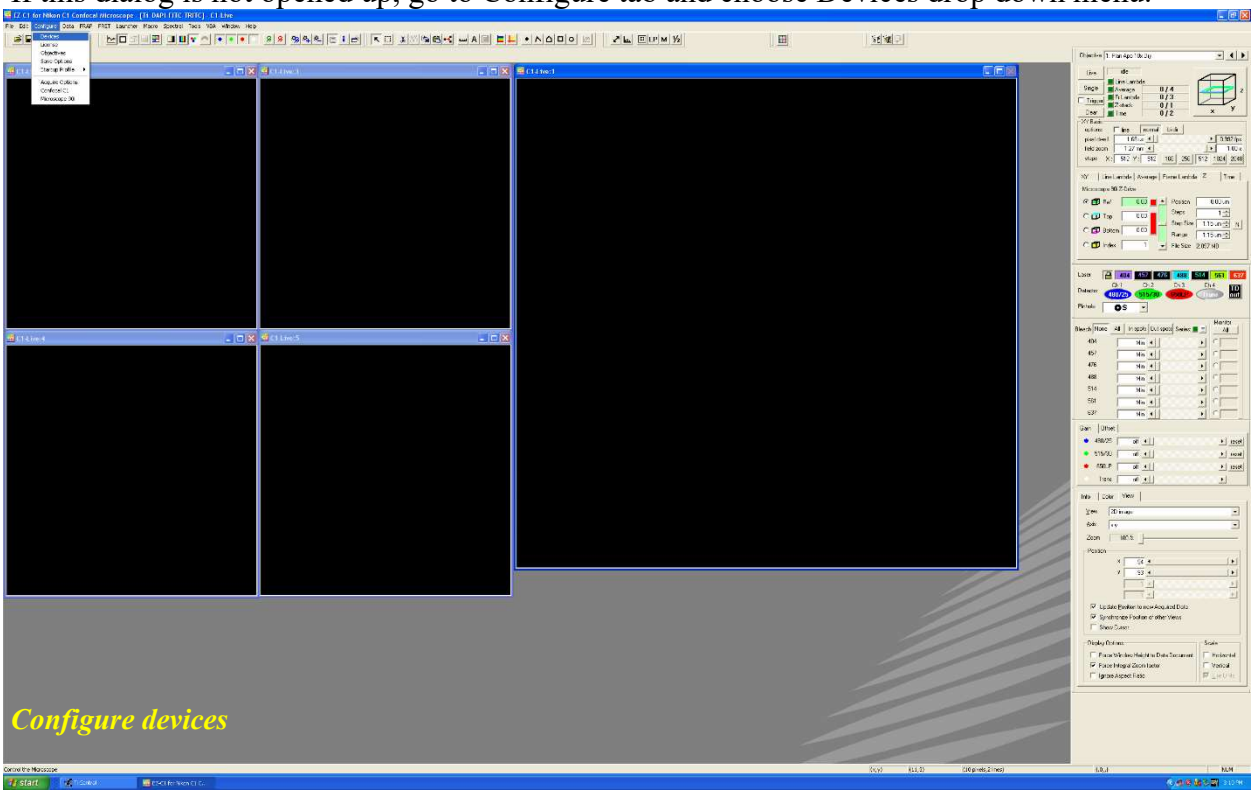
The confocal must be turned on in a sequential manner. The first to be turned on is the key on the lasers (1), turn the key to the right. Next turn on the microscope and the fluorescent light source (2) and (3). Now wait 30 minutes for these items to warm up. After 30 minutes turn on "Proscan II" unit and "D-Eclipse C1" unit (4) and a power light will come on, wait for the ready light underneath the power light to come on before you turn on the computer (5).

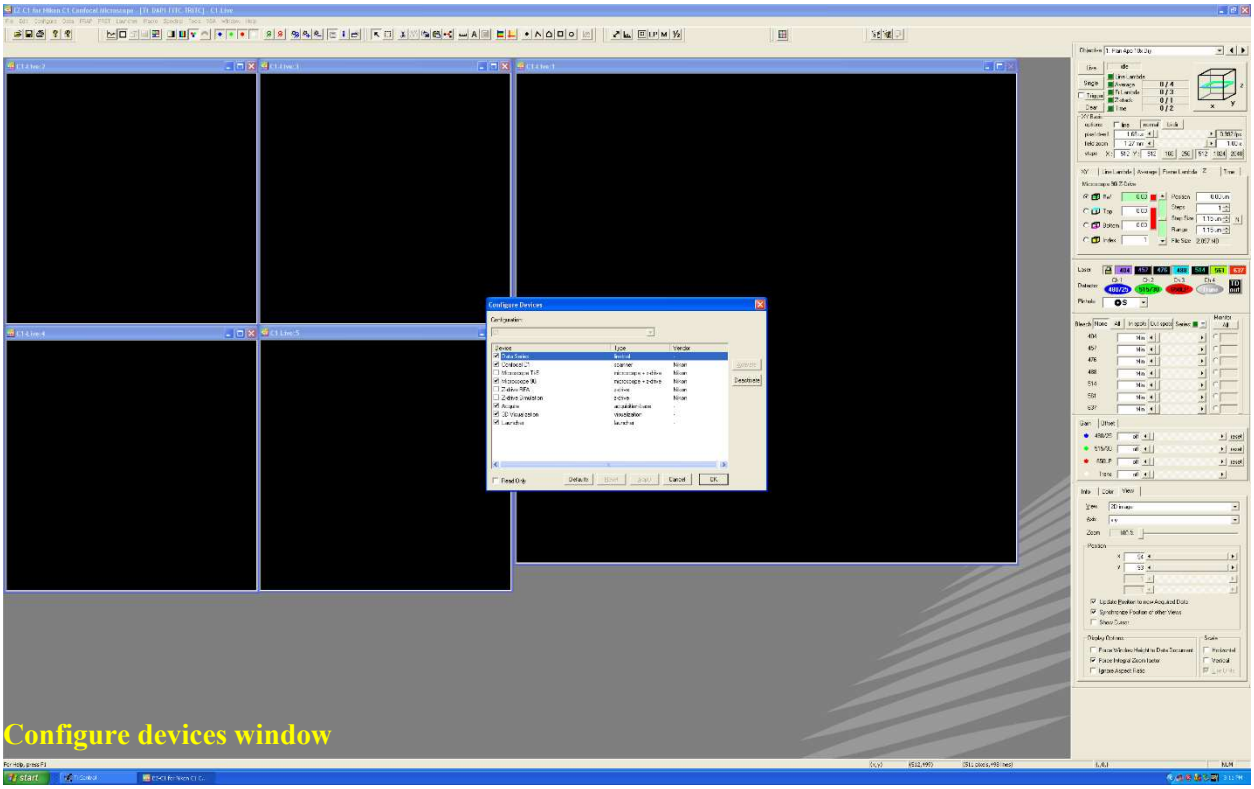
Turning on the Software

On the top left of the screen you will see the icon EZ-C1 3.90, double click to start software.

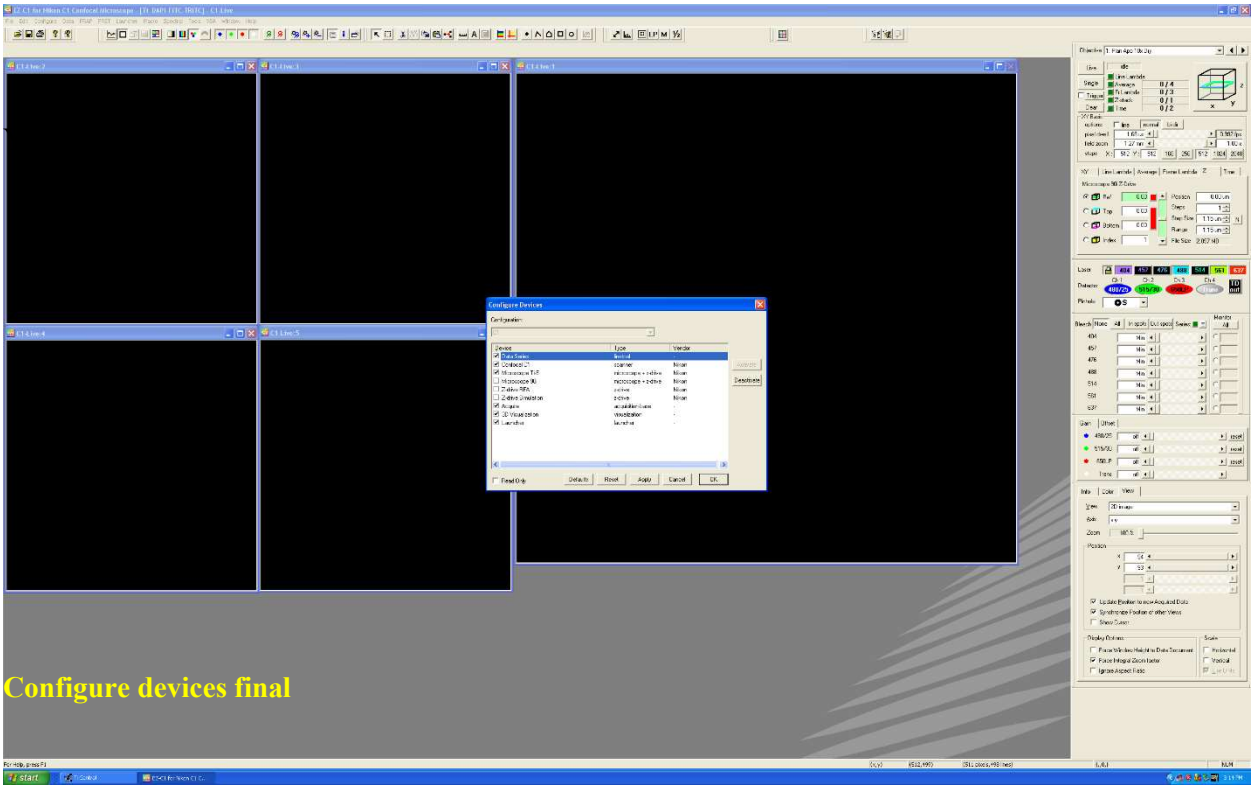
The next dialog box you may encounter is the following and this is the correct configuration for the upright microscope. If you are using the upright 90i microscope this is the proper configuration. If you are using inverted microscope then enable the **MicroscopeTi-E** option and disable **Microscope 90i** option.

If this dialog is not opened up, go to Configure tab and choose Devices drop down menu.



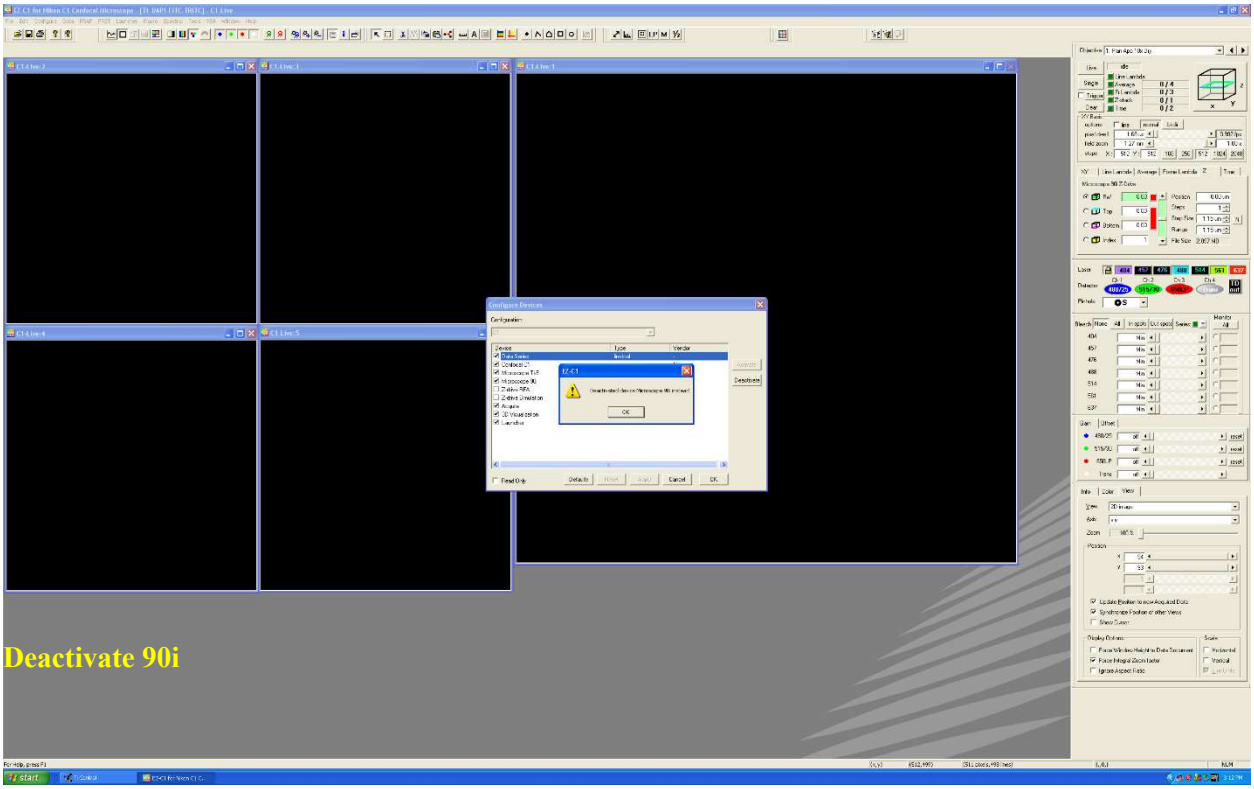


Configure devices window

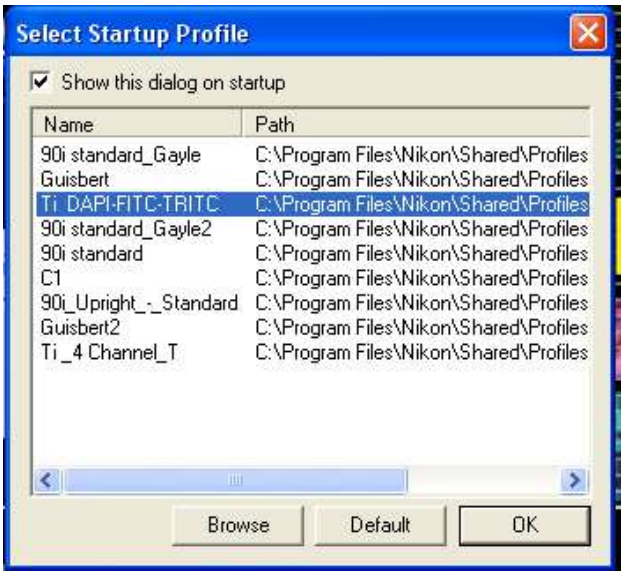


Configure devices final

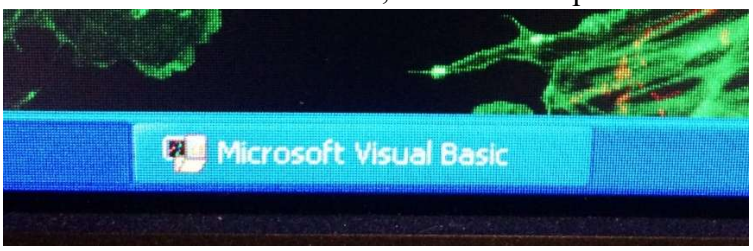
.



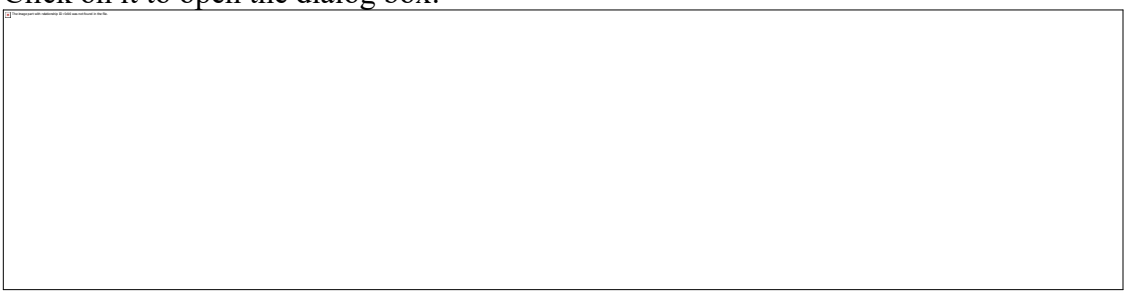
You most times will encounter the pop-up on the left, chose the appropriate startup profile and click OK.



Click OK and the software will begin to launch. After a short time you will encounter a Microsoft Visual Basics error, it will show up in the task bar at the bottom of your screen.

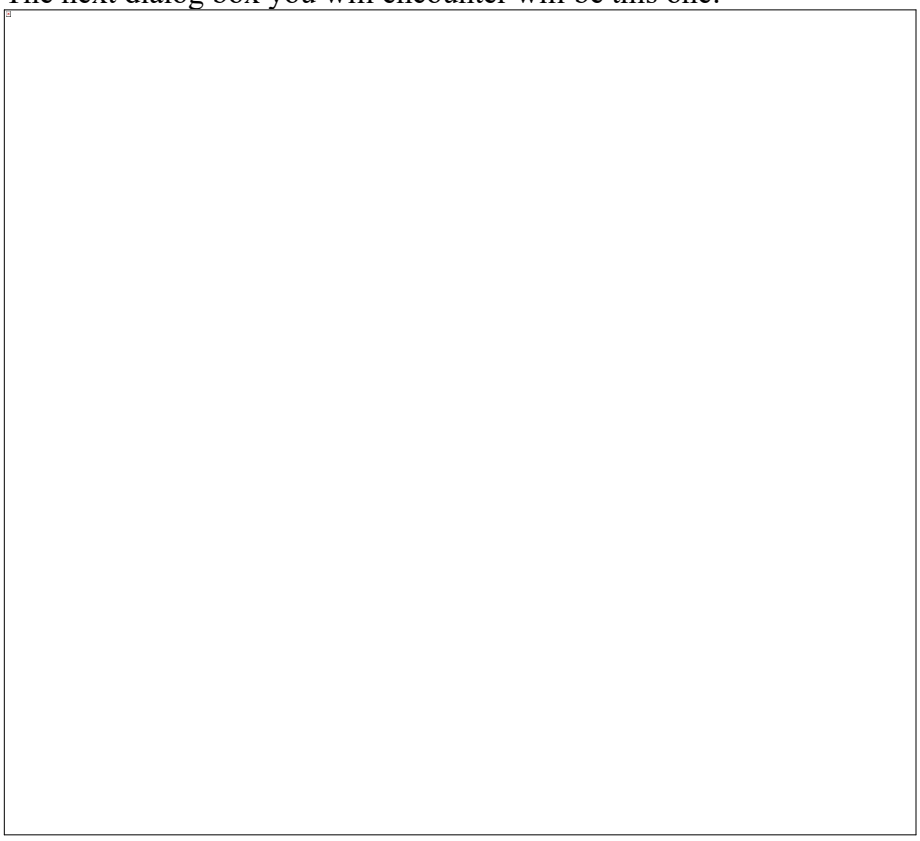


Click on it to open the dialog box:

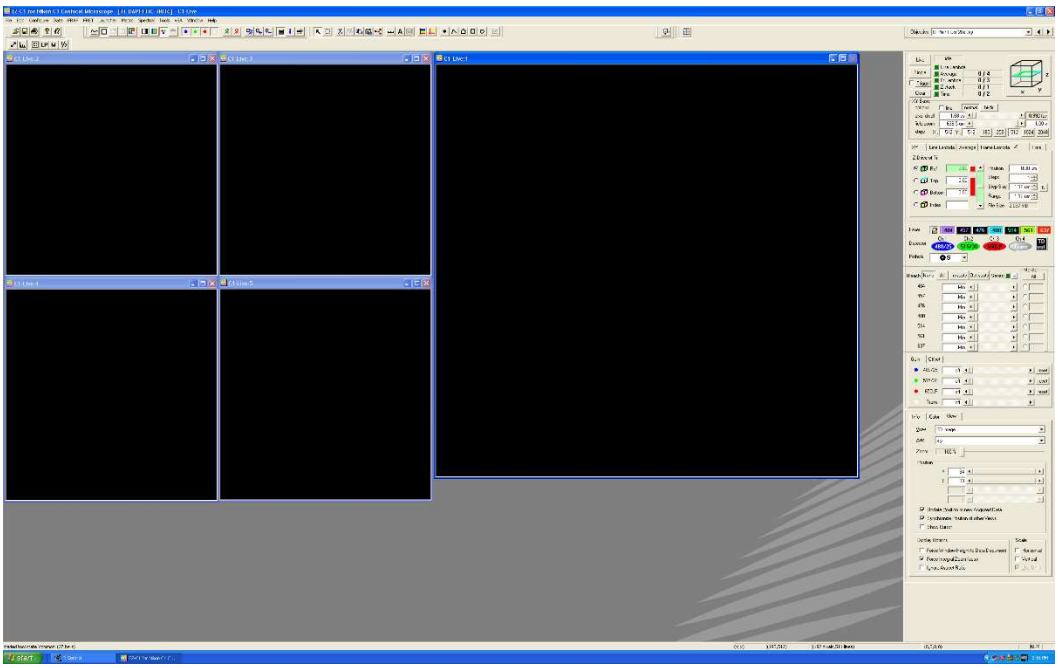


Click on OK, you will get another Microsoft Visual Basic dialog box to which you will also click OK.

The next dialog box you will encounter will be this one:



Click on the red X in the right hand corner to close out the box and you will get the Microsoft Visual Basic box in the middle of the screen. Click on OK and the box will close. You will encounter one more Microsoft Visual Basic error, click on the OK and the EZ-C1 will launch. This is how the screen should look:

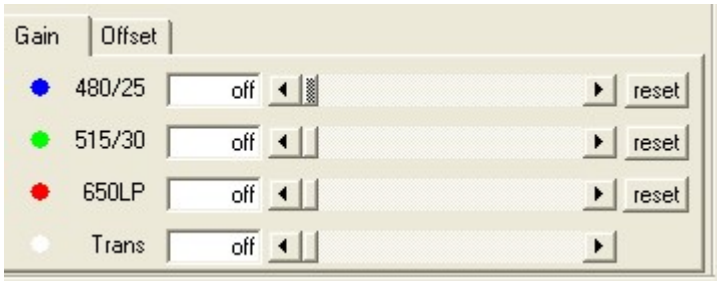


On the Gain menu choose the gain

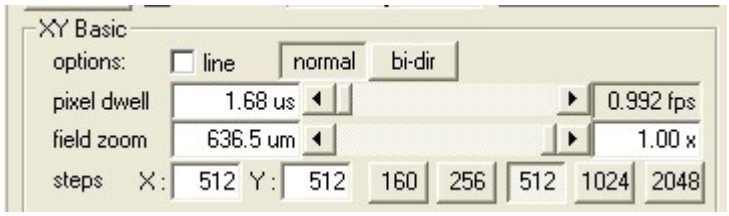
6.30 for all the channels or for green only

(for example if you are imaging falloidin

staining) as the initial point.

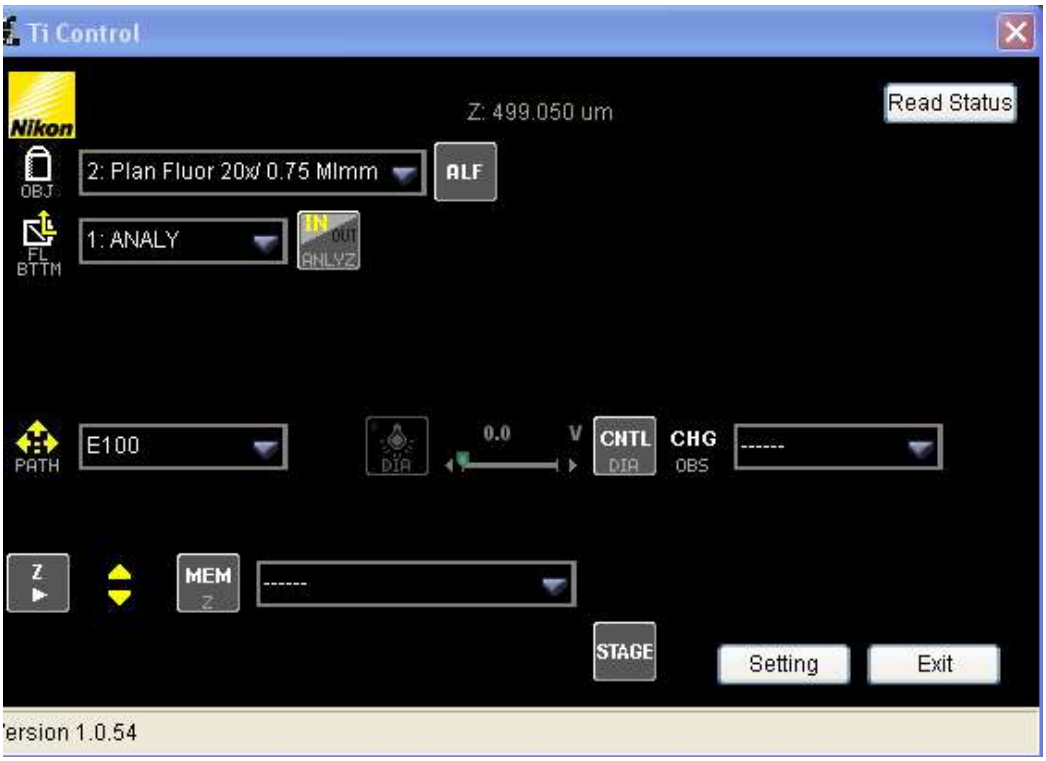


To start out the scan you will want a quick response from to get your sample set up. Set the step to 512. You will do your final scan at 1024 or 2048 resolution.



Place the slide on the microscope.

Bring up the T*i* controls by clicking on iControl next to the EZ-C1 3.90 software.

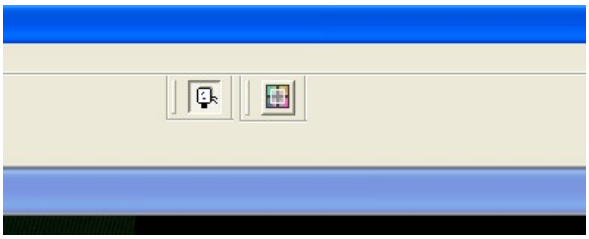


Above: Notice the first line is the objectives selection, the second one is the filter cube, line three is the path of the laser: E100 will be the eyepiece while L100 will be the path for the scan head. If using white light on the microscope to find and focus your sample, the CNTL box must be selected. On the inverted microscope there is not an option to switch between white light and fluorescent. To open the path for the fluorescent light on the microscope slide this bar (below), located on the lower right of the objectives on the microscope, away from you.

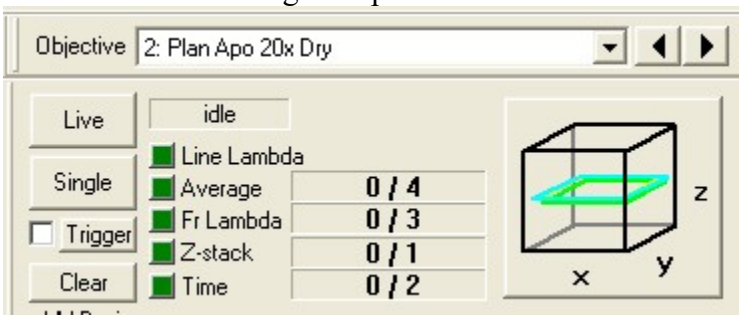
Imaging Your Slide

Place your sample under the microscope and open the shutter for the fluorescent light source. Turn the filter cube to FITC (as an example for imaging falloidin stained sample). Once you find the spot that you wish to image:

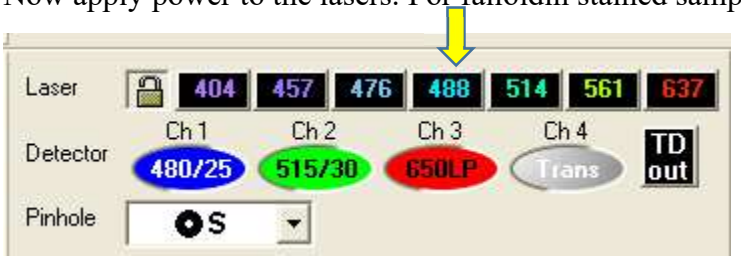
- close the fluorescence light shutter
- on the T*i* control menu change path to L100
- On the right side of the menu bar you will see two icons together in the menu bar (inverted microscope only). The Laser Control icon is to switch the image from the eyepieces to the scan head for confocal microscopy.



Press live on the Navigation panel:



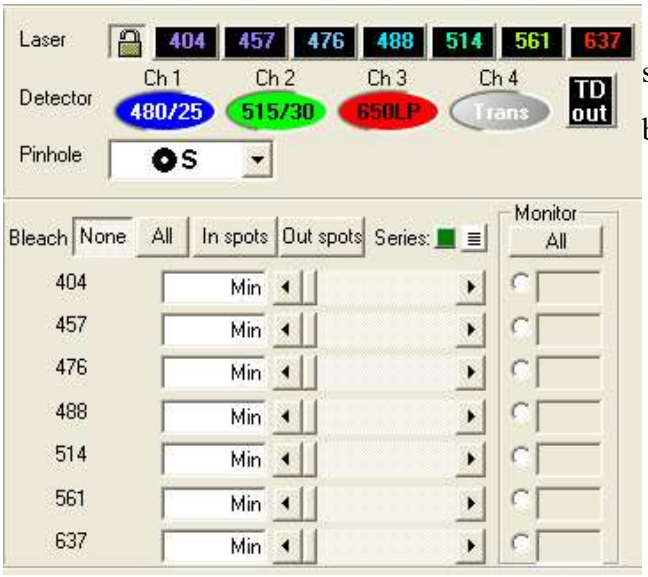
Now apply power to the lasers. For falloidin stained sample use 488 laser.



If you do not know which laser to use, then apply power to the lasers starting with the highest numbered laser and working your way down. You do this because the lower the laser the more damage to your sample so as you add the lower powered lasers you minimize damaging your sample.

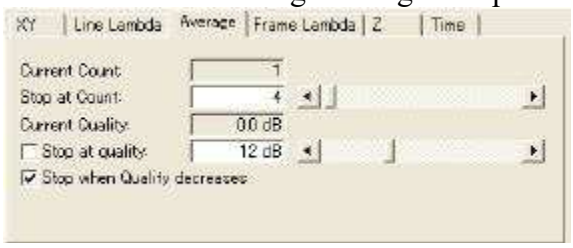
Please note you may not use the 514 or the 457 laser without changing some filters. Please see microscopy center manager or approved representative before use of those two lasers.

When the background of the Laser and Detector is blacked out (above) the power to that laser and/or detector is off. Click on the laser(s)/detector(s) you would like to use to turn it on. Even though the laser is turned on it is not emitting until you move the slide bars for that laser (next).

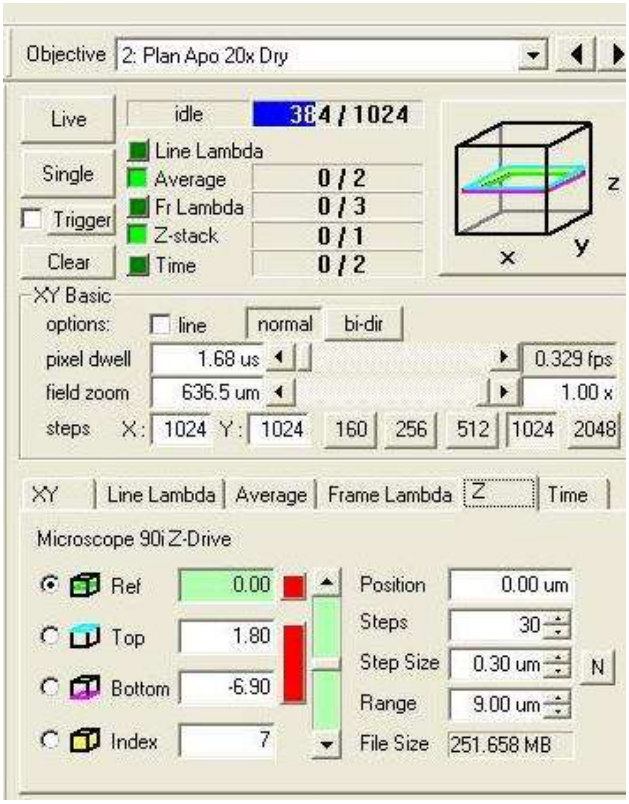


Start moving the laser power levers until you start getting a signal. If you don't get a signal by 50% power your sample may not fluoresce at that laser power or the focus is not ideal.

On the XY menu change average "Stop at count" 2.



Then Z stack menu.



Check ref. circle and then click on the red bars to get all the positions to "0".

Check the bottom circle. Then move the microscope stage away from you (=focus nobe) until the image is no longer visible. Position numbers will decrease.

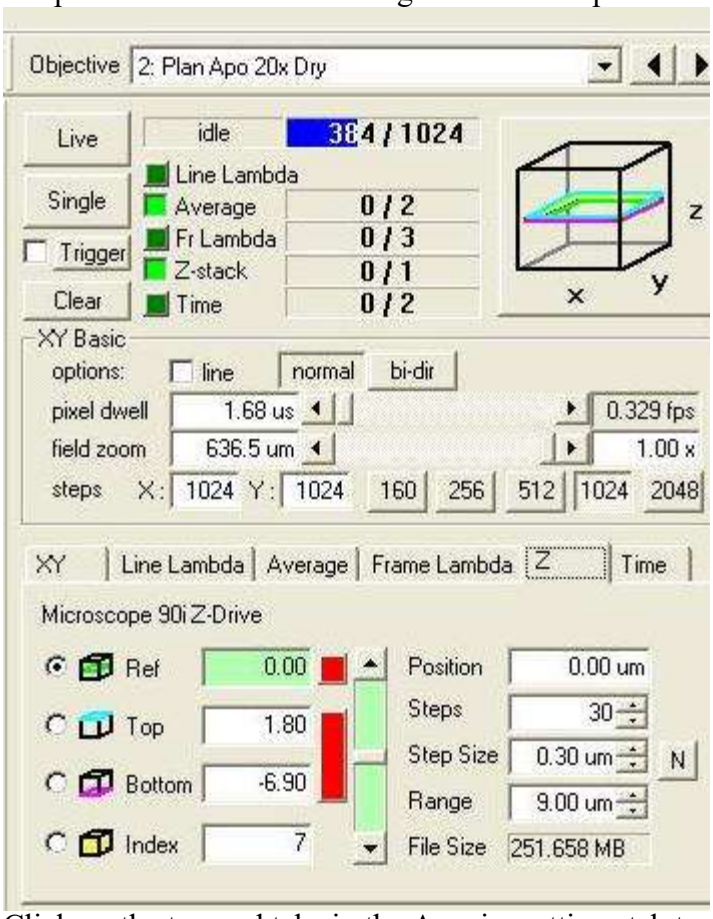
Next check top circle and move the focus nobe towards you.

Then set step size to 0.5 um.

Check icons for Average and Z-stack, then Click Single and the software will scan multiple plane in Z .

Creating a Z-stack

When your sample is not a single plane of focus a Z-stack helps to give you a better image by taking multiple images throughout the focal range of the sample. Set up your sample as above to get the focus, laser power, and gain set up, then click on the Z Tab. You should be scanning your sample under Live while setting the Z-Stack up.



Scan control

XY basics

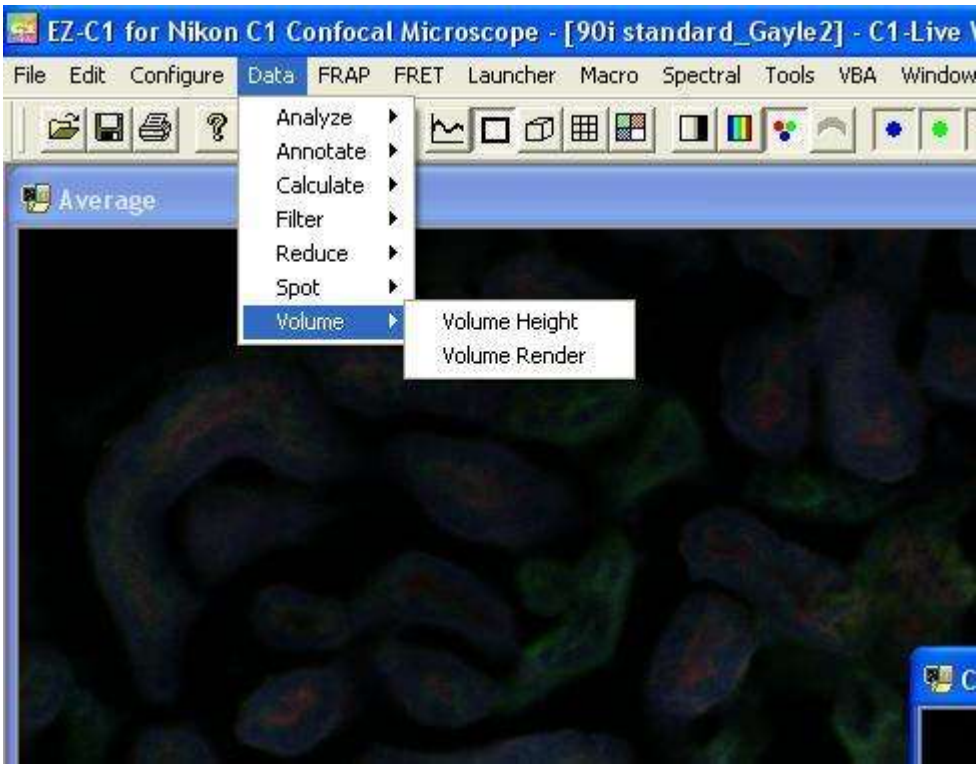
Acquire settings

Click on the two red tabs in the Acquire settings tab to clear the Ref, Top, and Bottom to zero. Next click on the circle to the left of the Bottom icon. On the Upright microscope reach over and start turning the focus knob towards you just before you stop getting any signal from your sample, on the Inverted you will turn the focus knob away from you. Next, click on the circle to the left of the Top icon. This time turn the focus knob away from you on the Upright and towards you on the Inverted until you almost lose your signal. Turn off Live.

Set your number of steps or Step Size, what you choose is entirely up to you but the larger number of steps the longer it takes to scan and the higher the chance of photo bleaching of your sample. For very thick samples you can easily make the Step Size 2.5 – 5.0µm. For thinner samples 0.5µm or maybe less will be okay.

Click on the Average Tab and change the count to 2 for thick samples. On the XY Basics tab change the steps to 1024.

On the Scan Control tab click on click on Z-stack and Average, and then click Single. Depending on the number of steps times the number of Averages you selected this can take several minutes to hours. Once you click on Single two windows will open, Average and C1-Live Volume 1.



You have several choices on this screen, if you want a 3D image you can press the Enable, if you want a flattened/stacked image you change the zoom to 1 and click on Once.