

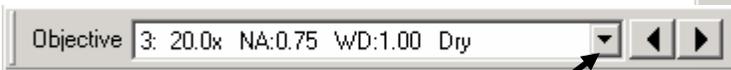
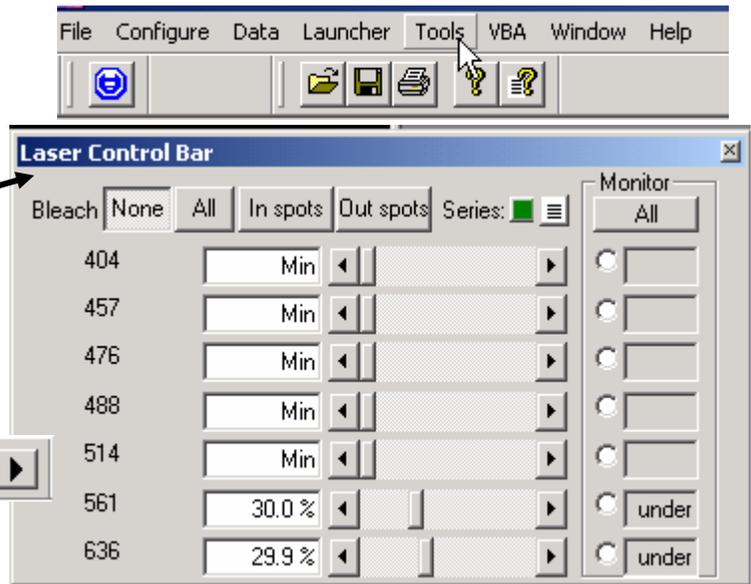
Confocal EZ-C1 Instructions

Setting Up:

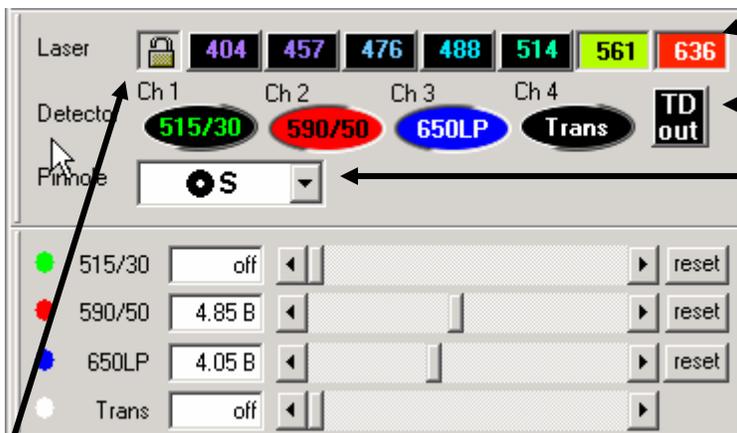


Follow startup procedure and then open EZ-C1 software.

Under the Tools Menu, open the Laser Control Bar.
Set the lasers you are using to about 30% power.
Close the Control Bar.



Select an objective.



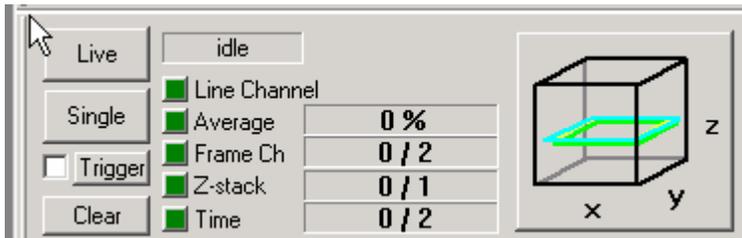
Select the laser shutters and Detectors you will be using. Black button = OFF; Colored button = ON

For transmitted light, select Trans and TD in.

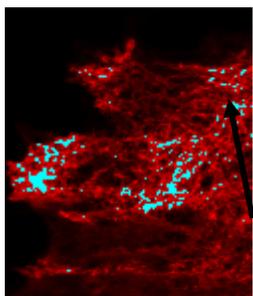
Set the pinhole to S unless you know you need a larger pinhole

Set the active **detector gains** to about half way.

Unlock the laser lock

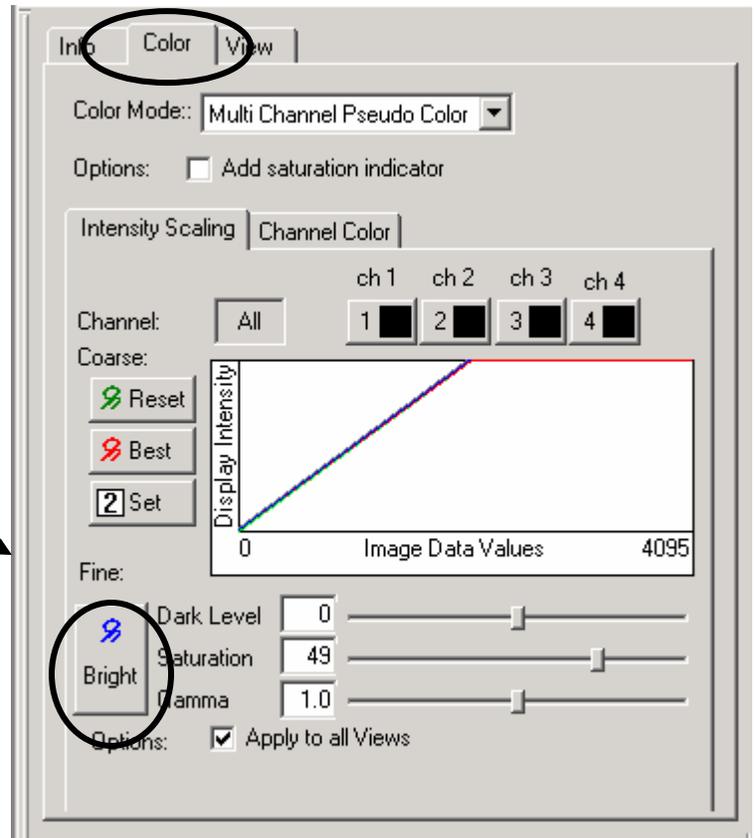


Hit **Single**. If you don't see anything in one or more channels, hit "**Bright**" under the **Color** tab.

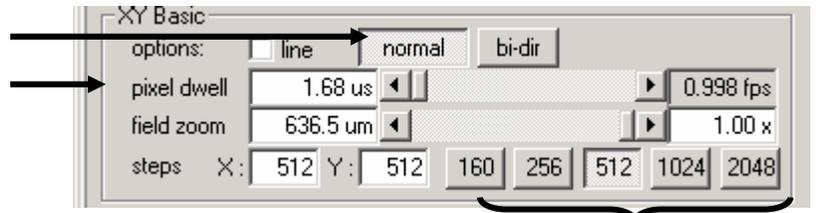


Under Color, choose multi channel pseudo color and check the add saturation indicator box. This will display saturated pixels in a contrasting color.

Adjust the detector gains until just a few saturated pixels are visible.

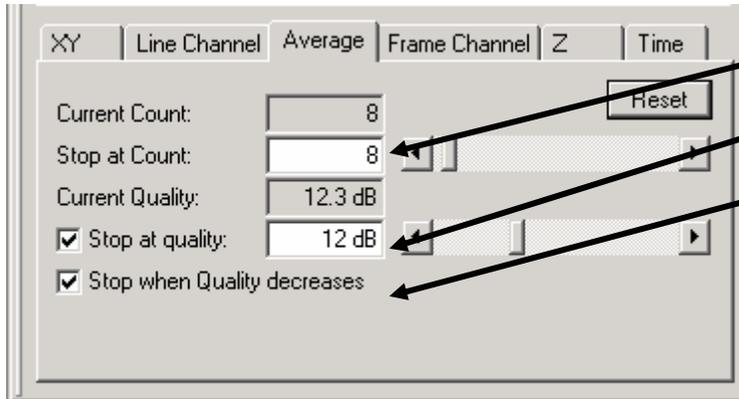


Leave as normal
Pixel dwell: the longer the dwell, the slower the laser scans = brighter image, faster bleaching. To start with, leave it at minimum



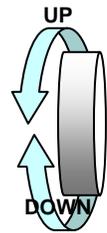
Default image size is **512x512 pixels**. Larger = higher resolution, larger file, slower scan. Smaller = faster scan, smaller file, lower resolution.

Averaging



Averaging will stop after set number of scans, after the image reaches a set quality, or when additional iterations are no longer improving the quality of the image (whichever happens first)

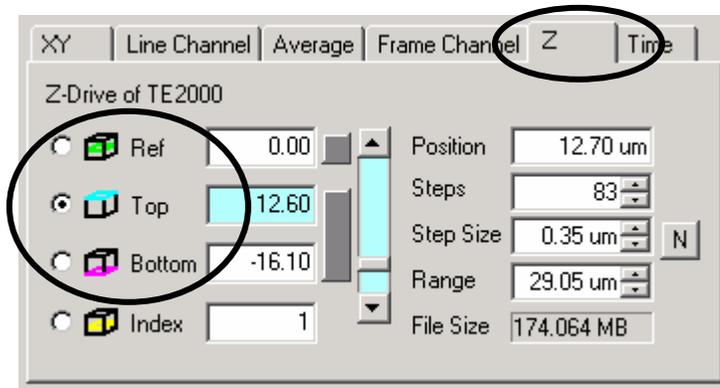
To start with, set quality to 12dB



Setting up Z-stacks

While scanning Live:

1. focus to mid -plane of sample, hit the red box next to "Ref" (sets zero point)
2. hit bottom, **then** focus to the bottom of the sample
3. hit top (it will take you back to 0.00), **then** focus to the top of your sample.
4. Stop Live

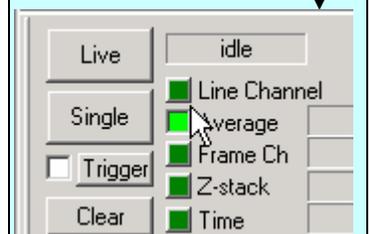


Note:

The software selects the optimal step size. Step size can be changed to reduce total number of steps and total file size. To keep small steps but reduce step number, range (top-bottom) can be reduced.

To scan with averaging:

1. select Average
2. hit Single



To make a z-stack:

1. Select Z-stack
2. Select Average (if desired)
3. Hit Single

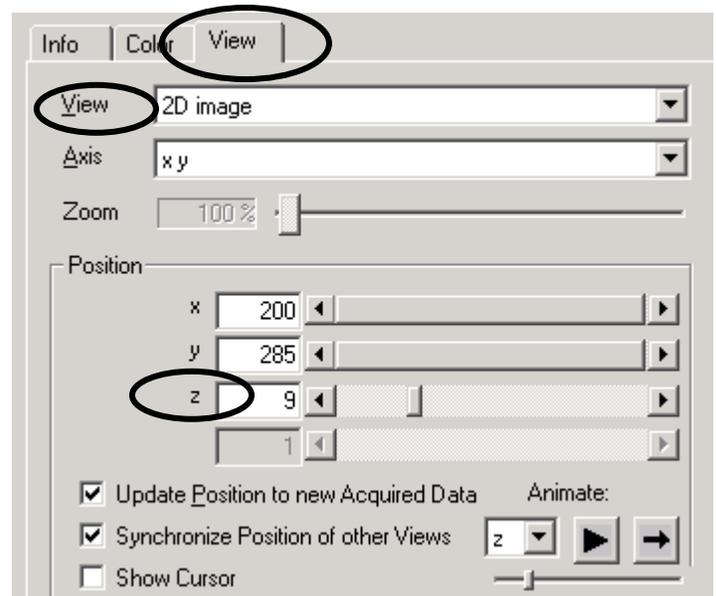
To view the stack:

Select the View tab.

Choose a view

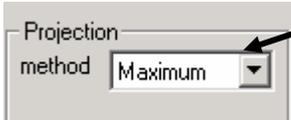
In 2D image view, you can scroll through the steps using the z slider bar, or hit the play button to animate

In 3D orthogonal view, you can move the cross hairs to see data from all three dimensions



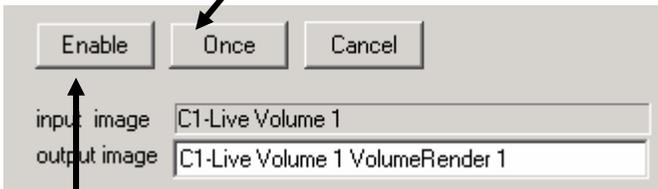
To make a projection:

Under Data in the main toolbar, choose Volume > Volume Render
 This opens a new tab in the Info/Color/View window

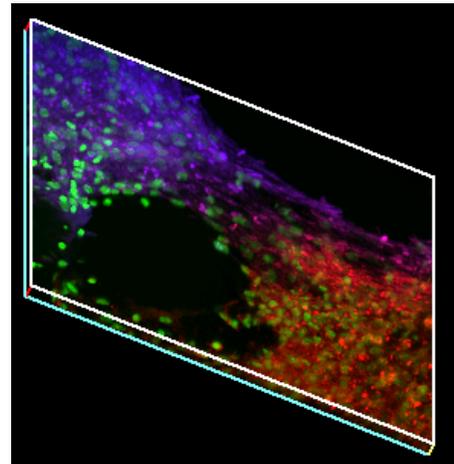


Under Method, choose Maximum or Sum (usually you will want Maximum)

Click Once to construct the projection
 If nothing is visible, open the color tab and click Bright

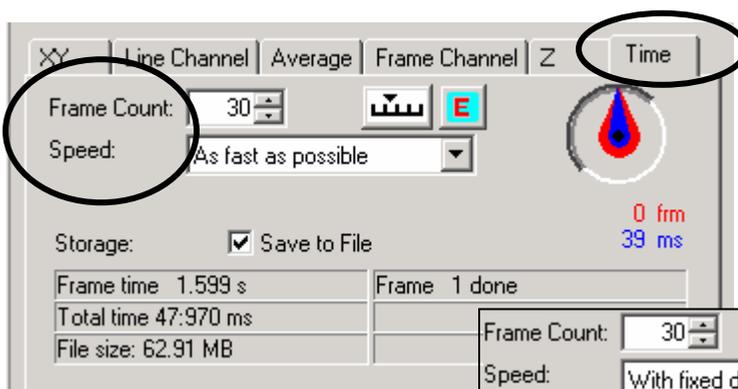


The projection can be rotated by grabbing with the mouse.
 Choose Enable to continuously update the image as it is rotated.



Time Series

You can choose number of images taken (Frame count) and time between images (speed). Together these make up the total duration of the time series.



To make a time series:

1. Select Time
2. Select a location and file name for saving the series
3. Select Average (if desired)
4. Hit Single

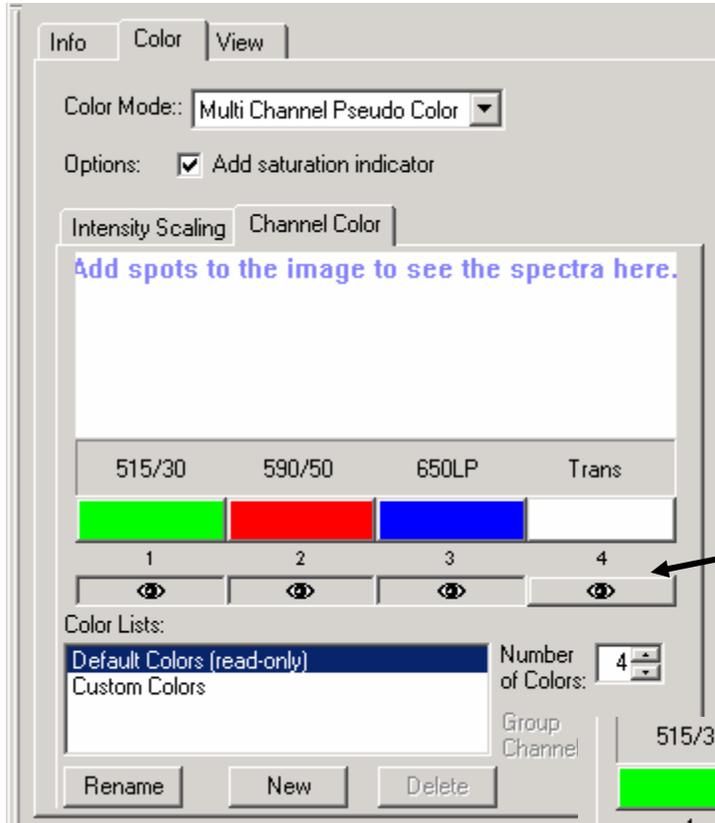
30 frames at max speed gives less than 1 min footage and uses 63MB



Note the trade-off between frames/sec and file size:

30 frames at 1 frame/min gives 30 min footage for the same file size.

Other functions and Trouble shooting



You can display each channel in a different window (get new windows from the main toolbar: Window > New) or as an overlay in one window.

To choose which channels are visible in a window, click on the window, then go to the color tab, select the Channel Color tab, and click on the eye symbols to turn channels on and off in that window.

Red green and blue channels all visible as an overlay

Only red channel is visible

