Nikon Eclipse 2000/C1 Workstation Notes
M.J. Dykstra   August 10, 2003 (Revised March 10, 2005)

Section 1: Most Commonly Used Functions

Start-up Instructions

1. **Determine which light sources are needed for your work and turn them on**

   A **100-W Halogen** light source is used for bright field, phase, and DIC observation. A **Mercury Arc Lamp** light source is available for epifluorescence work and for DIC visualization in the **C-1** program (confocal laser scanning program). Finally, the three **lasers listed below** are available in the **C-1** program.

   a. Turn on the needed high power supplies first, in the order listed, when using laser or mercury vapor lamps. **Leaving the lasers and Mercury Lamp on for the day is preferred to turning them on an off.**

      1. **Mercury Vapor Source** (100 W Mercury Lamp) for epifluorescence
      Hold button on power supply box for 1-2 sec until it fires and the Ready Light comes on. **Always start with both ND filters <u>in</u>.**

      2. **Argon Ion Laser (Laser 1; 488 nm)** blue emission; 40 mW; for GFP, FITC)
      - a. The power supply is the large box with small, keyed remote control
      - b. Make sure that the key on the back of the power supply is in the horizontal position and the switch at the back of the power supply is turned on (I).
      - c. Turn on the small remote control with the attached key
      - d. The first switch on the left should be set at **Current**, the middle switch should be set at **100 mW**, and the switch on the right should be set on **Standby**
      - e. Switch to **Run** position and adjust meter to **6 A**
      - f. Switch back to **Standby** for normal operation (there is usually enough laser emission at this setting)
      - g. The laser is usable almost immediately, but is not totally warmed up and stable for about 20 min.

      3. **Green HeNe Laser (Laser 2; 547 nm)** green emission; 3 mW; for Rhodamine, Cy3)
      Turn on key on labeled power supply. This laser is usable immediately

      4. **Red HeNe Laser (Laser 3; 633 nm** far-red emission; 10 mW; for various nuclear stains other than DAPI (which can only be imaged with the UV cube in the epifluorescence Mercury Vapor Source mode)
      Turn on key on labeled power supply. This laser is usable immediately.

      5. **100W Halogen Light Source**
      After turning on the power supply to the **100W Halogen lamp** (the box on the right side of the table), it can be controlled either by pressing the remote button on the control pad and using the knob on the lower left of the microscope; **or** by turning the remote button off and using the buttons on the control pad. **When the TE2000 control bar is on, the halogen light cannot be controlled from the knob on the lower left of the microscope.**

b. Insert appropriate **Neutral Density (ND) Filters** on laser box. The **ND4 filters** provide ¼ of full laser power, the **ND8 filters** provide 1/8 of full laser power, and if the **ND4 and ND8 filters** are in place, 1% of the laser power is available. **It is usually advisable to start with both filters in to preserve your fluorochromes or cell viability**

The mercury vapor lamp has an expected bulb life of approximately 300 hrs (#103 bulb), while each of the lasers has an expected life of 10,000 hrs.

All lasers go through the single fiber optic cable (single mode fiber), which is polarizer sensitive. Full extinction is provided by the fiber itself (for DIC).

2. Turn on the inverted microscope (Nikon Eclipse 2000E)

The switch is at the rear on the bottom left of the black hub. Turn on the 100W halogen light source, if not already on.

3. Turn on the computers: It is best to turn on the White Box first, followed by the host computer.

a. The **White Box** (Eclipse C-1) is the scan electronics box; turn it on and a **green light (Power)** will come on; when the **green (Ready) light** comes on, it is up and running; this box controls part of the microscope (the rest is in the **host computer**)

b. Turn on the **Black Box** (**Host** computer); this controls the microscope and contains the software for image manipulations (operating in Windows 2000)

4. Open the software

To open the software, click on icon labeled **Nikon EZ-C1 2.01.152**, which controls the Eclipse C1 confocal system and the icon labeled **Nikon TE2000 Control 2.00**, which allows control of the microscope through the computer. **The microscope can be controlled strictly from the control pad without turning on the computers.**

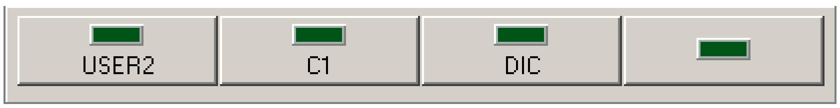
Shut-down Instructions

1. Exit the software and shut off the computer.
2. Switch off the Key Switch for the Argon Laser remote controller. **Wait 15 min and then shut off the Argon Laser power supply switch on the back of the power supply unit.**
3. Switch off the He-Ne power supplies and mercury vapor lamp power supply, if used.
4. Switch off the power switch on the C-1 controller (White Box), the Monitor, the ProScan Box, the Eclipse 2000E controller at the left rear of the microscope base, and the 100W Halogen light source control box to the right of the microscope.

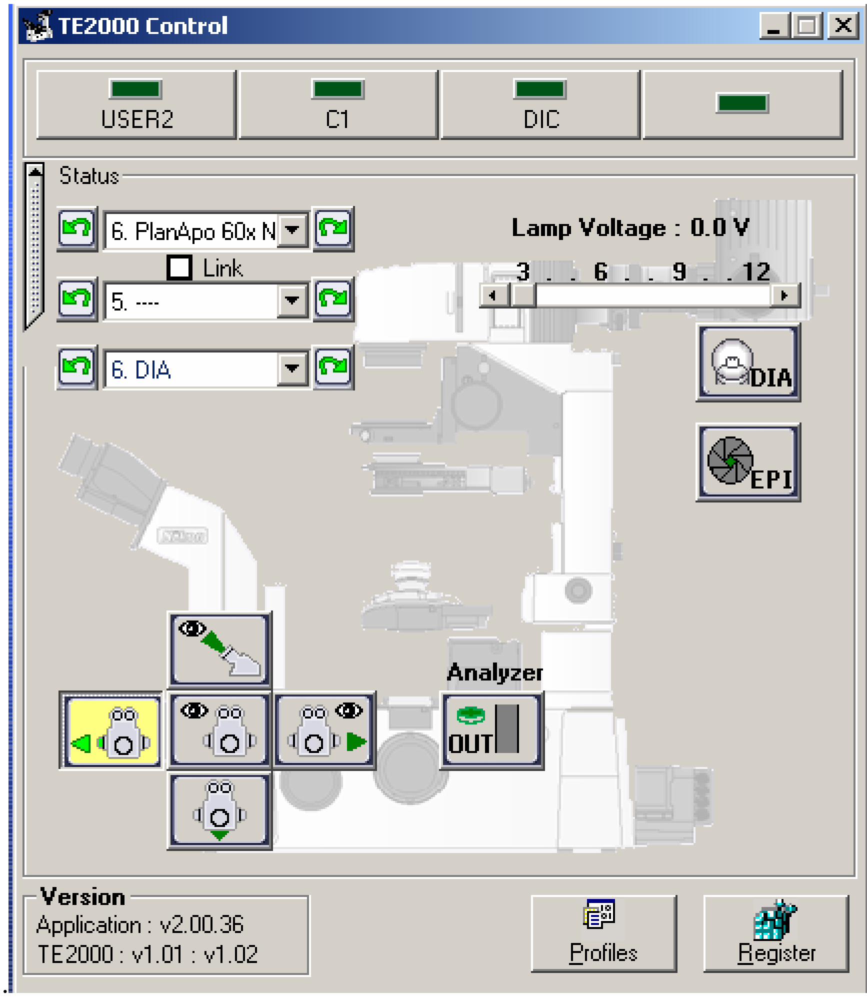
Using the Eclipse 2000E Microscope

Choosing Objective Lenses and Matching Them with Condenser Lens

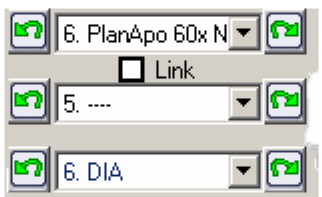
1. Click on the **Blue Objective Button in the C-1 Program** to bring up the microscope control screen. User profiles are set up in the EZ-C1 program
    a. **C-1** is the set-up for confocal work. This must be turned on to get the C-1 program to function fully. The argon laser will still get to the specimen if the C-1 button has not been depressed, but the other lasers will not work and the image from the argon laser will not be good.
    b. **USER 2** is the set-up for the digital camera
    c. **DIC** is the set-up for light microscopy mode
    d. **Unlabeled** has no function ascribed to it



2. When you select an **Objective,** the program makes sure the condenser lens in place is properly matched.
    a. You can move the objective lenses manually to override the matches programmed in.
    b. By selecting an objective either in the C-1 program, or on the keypad, the microscope will put objective/condenser lens back into proper synch if previously overridden manually.
    c. To match objectives and condenser DIC filters, you **must** use the keypad and **must** move the **polarize**r into place at the top of the condenser assembly. Put in the **analyzer** by pressing the right button on the control pad.



3. **Objectives** currently installed can be viewed in the pull down menu in the software program



Objectives Available for the TE-2000E Microscope, with Remarks for their Usage

Description	N.A.	W.D.	Remarks
Plan Apo **4X**	0.20	15.70	
Plan Fluor DL **10X**	0.30	15.20	Ph1
Plan Fluor ELWD DM **20X**	0.45	8.1-7.0	Ph1, DIC; CC 0-2 **Put slides on stage with coverslips <u>up</u>**
Plan Fluor **20X MI (multi-immersion)**	0.75	0.35	Oil/glycerine/water immersion; DIC; CC
Plan Fluor ELWD DM **40X**	0.60	3.7-2.7	Ph2; DIC; CC 0-2
Plan Fluor **40X oil immersion	1.30	0.20	DIC; Spring loaded
Plan Apo **60X water immersion**	1.20	0.22	DIC; Spring loaded; CC 0.15-0.18

*** Correction Collar**
**** Not currently installed on the nosepiece**

<u>Cleaning Objectives:</u> **Please do not <u>rub</u> the lenses (some of which cost almost $8000). This can easily damage the coatings or the glass itself. Gently wick off immersion media with clean lens paper (in upper right drawer of desk). If you have any questions about how to do this, please consult with the LAELOM staff. Isopropyl alcohol (reagent grade) or Sparkle® Glass Cleaner are recommended for cleaning objectives by Chris Cathcart**

The **Plan Fluor 20X Multi-immersion lens** may be used for glycerol, water, or oil immersion. It has a correction collar to adjust for mounts or dishes of different thickness. Vessels such as **WillCo Wells BV; glass bottom Petri dishes** found at WillcoWells.com are best examined with this lens.

The **PlanFluor 40X Oil** can lock the front element down so it can be bypassed to prevent spreading oil to other objectives; **if not in un-sprung condition, poor optical quality will result**

4. The **Condenser lens** currently installed has a numerical aperture of 0.52, which limits the final resolution of the high magnification objectives when doing bright field, phase, or DIC, but has no impact on confocal laser scanning work
5. There are **internal magnification lenses (1X, 1.5X)** for visible light work, selected with the knob on right side of the microscope base
6. The **Knob below the binoculars** has a magnifier and shutter and also has a **Bertrand Lens** position for aligning phase contrast rings
7. The **Epifluorescence Filter Turret** has positions for 6 cubes. The sixth position is left empty for DIC work

Filter Cubes Available for TE-2000E Microscope

Position	Description	Exciter	Barrier	Remarks
1	UV-1A	360-370	420	Narrow exciter minimizes autofluorescence
2	FITC HYQ	460-500	510-560	GFP Green mutant and Humanized S65T, BoDipy,Flo3,Dio
3	Texas Red HYQ	532-587	608-683	
4	CFP (Cyan); 480 bandpass barrier	426-446	460-500	Cyan (CFP) fluorescent protein; designed for dual separate staining with EYFP, will excite EGFP so not recommended for dual stain with EFGP
Not Installed	Endow GFP HYQ; 525 bandpass barrier	450-490	500-550	GFP Green fluorescent protein filters based on Dr. Sharyn Endow, Duke U., with bandpass barrier
5	YFP BP YHQ (10C/Topaz); 535 bandpass barrier	500-517	520-550	Yellow (YEFP) fluorescent protein, excellent for use in separate dual staining with CFP
6	No filter installed			For DIC work

8. The **mercury light source** for epifluorescence has an **HA (heat absorbing) filter**

9. **Filters on the 100W Halogen Source:** (for most work, all but the GIF filter should be in)

   D= diffusion
   ND= neutral density (ND4)
   NCB= blue
   GIF= green interference filter (for black-and-white films)

10. The **Transmitted Light Detector Slider** in front of the **100W halogen** light source must be pushed in for DIC confocal work, and pulled out for regular DIC work utilizing the halogen light source.

11. The **Shutter Mechanism** for the **Binocular** (viewing) and confocal) are set up so that you cannot look into the microscope when in the laser mode; the **confocal position** puts a physical shutter in place in the binocular head; there is also a second interlock that is activated when the **condenser tower** is tipped back

12. There is a **shutter control** on the **control pad** that opens and closes the shutter on the **Mercury lamp** housing

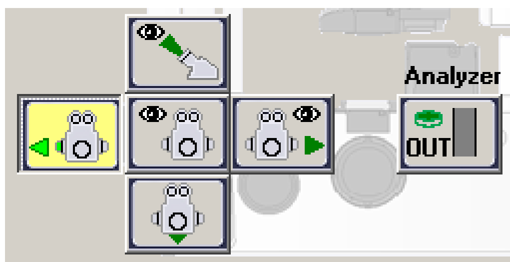
13. After turning on the power supply to the **100W Halogen lamp** (the box on the right side of the table), it can be controlled either by pressing the remote button on the control pad and using the knob on the lower left of the microscope; **or** by turning the remote button off and using the buttons on the control pad. **When the TE2000 control bar is on, the halogen light cannot be controlled from the knob on the lower left of the microscope.**

14. **Stage controls:** After turning on the **ProScan power** supply, the joystick has three speeds (selected with the left button). The small buttons unlock the joystick from being

spring-loaded. **Glass** slides should be installed in the opening provided with the coverslipped side *down* except when using the LWD 20X and LWD 40X lenses

15. **Focusing:** a motorized fine focus knob is on the right side of the microscope, while the coarse focus knob is on the left side of the microscope; the motorized focus control makes long-term viewing accurate (time lapse), with no focus drift. Turning the **locking ring counterclockwise** (the large ring at the base of the focusing knob on the left side of the microscope) will prevent any downward drift of the nosepiece.

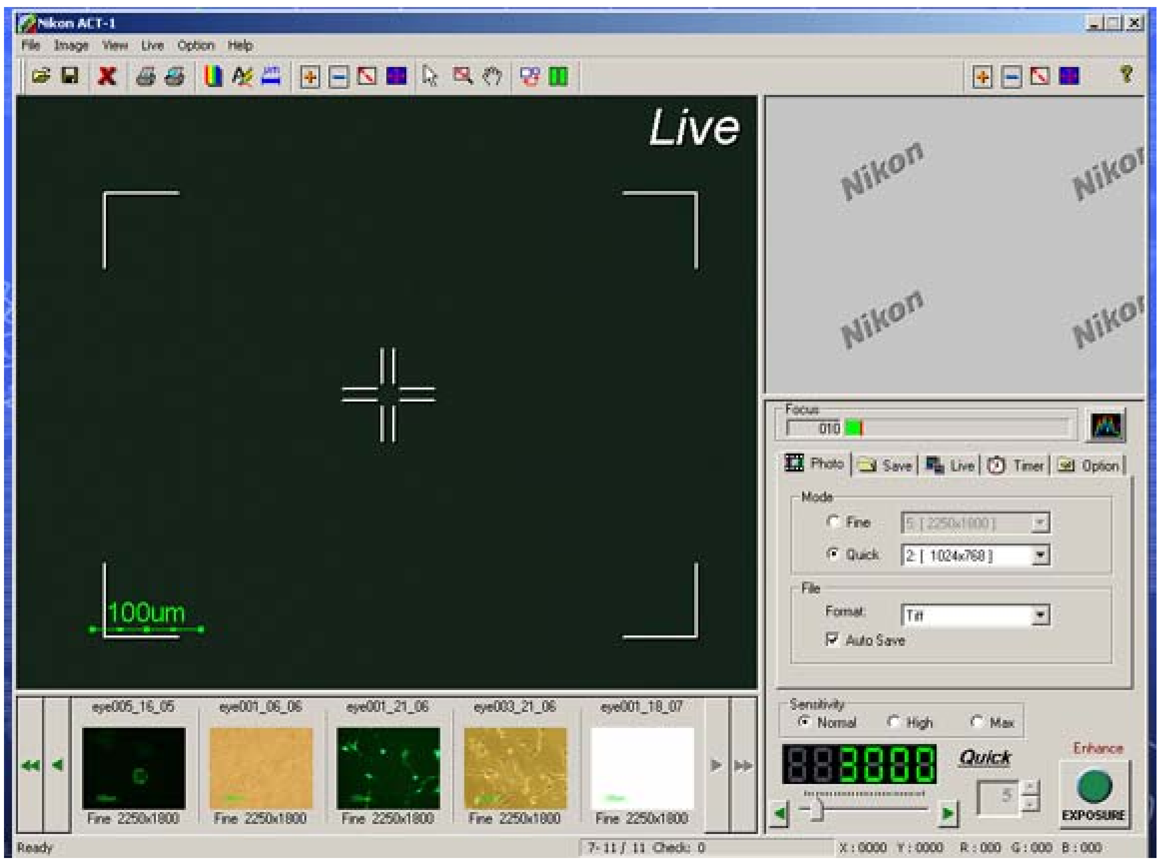
16. **Viewing Ports:** an internal prism allows selection of 5 different ports to which the image can be sent, as shown on the **Control Pad**
    a. Top (viewing by eye)
    b. Bottom (no access to this on the current table)
    c. Right (Nikon DXM1200 digital camera is here)
    d. Front (a 35 mm camera can be installed here)
    e. Left (C-1 system is attached here)



Using the Nikon DXM1200 Digital Camera

1. **Click on the ACT-1 icon**

This brings up the camera software and also turns the camera on; the **Camera** is a 3840 X 3072 color mosaic (Bayer mask) image, with 8 bit/color (24 bit total); this provides a 36 MB TIF file; images can be acquired as low as 640 X 480; the **Quick** option gives a 1280 X 1024 (regular video camera resolution image); To crop the image, click the left mouse button.



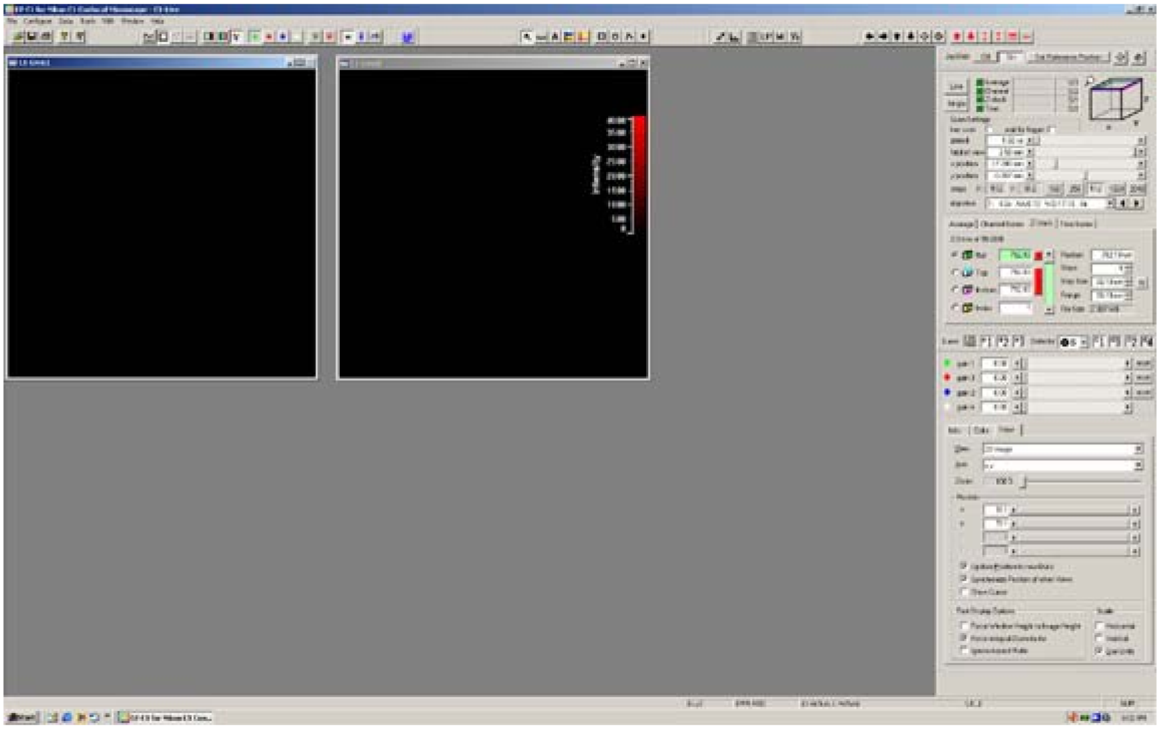
2. Select the **right port**
3. Adjust the **lamp setting** to 100%
4. Click on the button  at the right to get a **histogram**; bring the tops of the RGB lines to below the top of the graph to prevent oversaturation of the camera
5. **White-balancing** should be done monthly (should be done before fluorescence work, too, though no black balance is needed).
    a. Set the **exposure** at 1/300 of a sec (**300**); **set transmitted light at 11 V**, and add ND filters to keep light before overload level for the camera (a Kimwipe can be used to cut down the light if ND filters are not enough)

   b. White balancing can be done with any objective
   c. Click the **Live** button. Go to **point** on the lower right of the screen
   d. Take **Eyedropper** and click on white area in image (doing it on an empty area of a slide may not be in the proper plane). The eyedropper will fill up.
   e. Hit **Start**
   f. Bring the exposure down to **4000-12,000** when finished to get an image
6. **Autosave function:** to use this mode, designate a directory and all images will be saved to that directory; the default is currently **image**, with the thumbnails put under **s**
7. The **image refreshes** at 12 frames/sec
8. **The image can be rotated in the live mode 90 or 180^{o}**
9. Changing the **pixel array size** puts a mask on the screen
10. **Quick Mode** turns off interpixel stepping
11. **Focus**-the highest number gives the best focus (put focus mask on AOI)
12. **Green Button** saves the image and shows the actual image in a second window
13. The **Blue Icon** at the upper right makes a full screen image; press **Escape** to return image to initial size
14. **Timer Function** allows time lapse recording up to 4 frames/sec
15. **DIC:** bring the exposure time up (smaller #), use M prism and objective (20X), push in polarizer at top of condenser tower to adjust contrast
16. **Fluorescence:** choose the field of view, insert appropriate filter cube, turn off transmitted light, and use a very small exposure number
17. **Kohlerize** for DIC (important); the substage condenser diaphragm setting is also important for DIC
18. **Use the epifluorescence mode to see specimen before switching to Confocal Laser Scanning; once in confocal mode, close the digital camera program (it uses a lot of memory)**

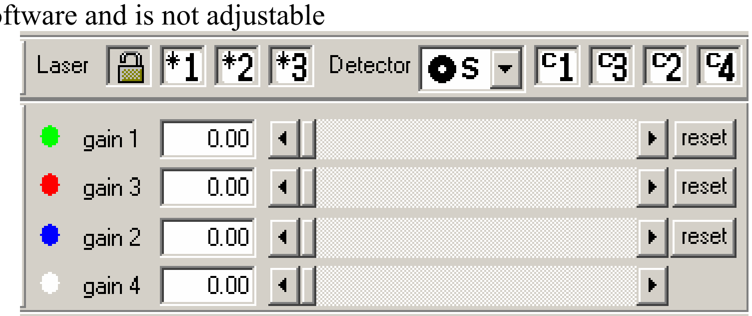
Using the C1 Confocal Laser Scanning Program

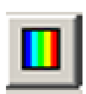
1. Click on **EZ-C1 2.01-152** icon: 

the program uses an 8-10 year old program developed by cytomorphology folks (IDS format); can reopen IDS files and resave them as TIF or jpeg files for other applications; this program runs both the confocal microscope and the lasers; the **C1** button located in the window is the preset to use the confocal; click here to set the microscope to the proper objective and condenser; this does not turn on the lasers. Make sure that the C1 button is depressed. If it is left off, the C-1 program will still deliver one laser source to the scanning head and produce an extremely dim, granular image, but good images and multiple lasers will not function.

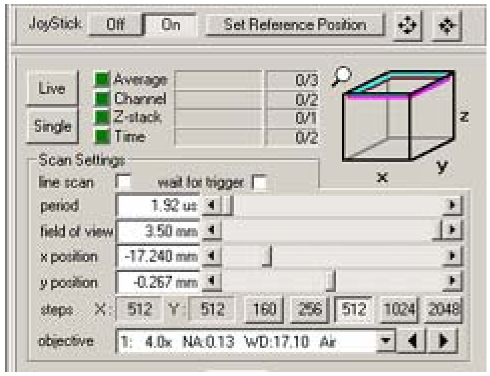


a. The **Photomultiplier** converts a few photons into many electrons (signal). Increasing the gain increases the number of electrons and also the noise. **Set Gain at about 6**. The gain can be increased until you see noise. Stop just below the lower threshold of acceptable noise. The offset is incorporated into the software and is not adjustable



b. **Pseudocolor**  adds false color to a grayscale image; 0= black and 4096= white. Use the 500-3000 range to avoid extremes. While in pseudocolor, aim for a balance of high gain with no noise (only a very small amount of pink/red)
c. 12 bit **data files** are produced, rather than an image file
d. Lots of header information is collected along with image. Your own information can be added manually
e. Pull up an image, bring up pseudocolor image and adjust grayscale to **not** oversaturate photomultiplier. Gain should be set between 0 and 3500 out of 4096 gray levels (leaving 10-5% headspace). Go to **configure** and then **grabber**. There are 4 **Gain** settings, one for each photomultiplier They are set at the default value now. Increasing gain increases noise. You want to see some noise. A histogram of the image can be viewed by clicking on the histogram icon.

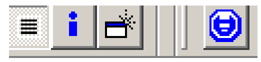
2. **Using the C-1 Software**

Upper right on screen: 

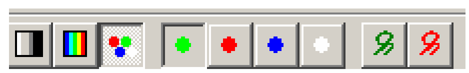
1. **Live** turns on the scanner; constantly refreshing
2. **Single** scans the sample 1X;or 3X if set up to average 3 frames
3. **Period** is the dwell time of the scan; 512 X 512 pixels is 1 sec/scan; can slow the scan down to excite fluorochrome
4. **Field of View** slider can be moved to change optical magnification; if downsized, more energy is put into smaller area of specimen
5. **Region of interest** scans a smaller area with a smaller number of pixels and reduces the scan time and consequent photobleaching; a scan area of 2048 takes 8 sec
6. **Averaging**
   a. Sum a number of images (3-4); more will cause more photobleaching
   b. **Quality** looks at the SNR; determines if there is change in the SNR on the image; useful for very thick specimens; going deeper into a specimen degrades image quality; 200 µm is a thick specimen
7. **Channel Series:** single laser to single detector switching to 2^{nd} laser, 2^{nd} detector
8. **Z-stack**: go through focus to find top and bottom; calculates range (volume); calculates number of steps, based on objective being used; calculates appropriate step size; click on **z-stack**; can manually override to decrease number in stack to reduce photobleaching; select objective that magnifies AOI as much as possible; microscope will determine how many z-steps should be collected; 2.3 X the sampling rate according to Nyquist theory; selecting the **refresh** button will replace the preset values
9. **Time Series**
10. **Laser Controls:** shutters to turn on and off
11. **Detector Controls:** 

smallest pinhole typically set up by computer. The **largest pinhole** that does not produce excessive noise is the best
   a. **Three detectors in box:** remote from laser heads; preselect emission filters; emission filters cost about $100 each
   b. **4^{th} detector:** at top of light column for bright field/DIC/phase images; these images can be merged with laser images
   c. **85-110 Gain** gives best SNR; if you go above that, use averaging to collect image

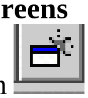
12. **Adjusting Data Set:** information"i" in top row of buttons shows data set page



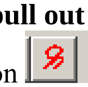
13. **Selecting C-1 button** moves all controls to confocal mode; puts filters (epifluorescent) to empty hole. **Manually turn off halogen light source.** Turn up **gain** to find image
14. **Color Mapping of C-1**
    a. **Gain 1=** Green
    b. **Gain 2=** Blue
    c. **Gain 3=** Red
    d. **Can select what color each channel is**



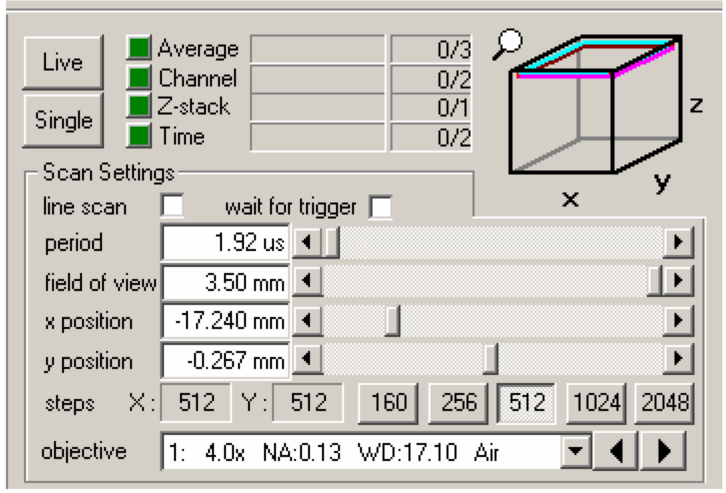
15. **Setting up multiple data screens**

    Click on the data screen icon  to bring up more data screens. Up to 6 can be displayed simultaneously. Select a screen and click on the color channel buttons above to ascribe that color channel to the live screen.

16. **Recording DIC Images in Confocal Mode**
    a. Find image with laser (red He-Ne will give best signal, but any of the lasers will work
    b. Push the Polarizer filter in
    c. Push the button on the microscope control pad to remove the Analyzer
    d. Push condenser buttons until DIC "H" position is at the front of the condenser assembly
    e. Adjust contrast with the Polarizer filter
    f. Adjust brightness of the DIC image with the substage condenser aperture

17. **Capturing an image**
    a. Set the gain to 1300, 1 scan
    b. Left click in 3-D cube icon to get a crop box
    c. Set process to single scan
    d. To eliminate noise, use averaging or slow down scan with period slider
    e. **Lower laser power and lower scan times make prettier pictures; oversaturated conditions give less information; best to start low and pull out as you increase**
    e. Can use **best** button  with 12 bit data files (only), not with an image file. This control takes a 4096 (12 bit) color value and converts it to 256 colors (8 bit) on the monitor
    f. **Z-stack acquisition:**

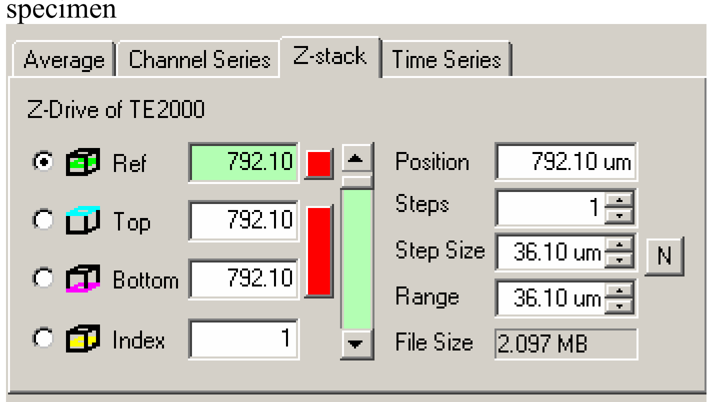
i. click on **live**



ii. increase the **gain** by dragging the slider to the right (about 6 usually is good)

iii. adjust the fine focus and then decrease the gain so that there are no white pixels in the pseudocolor window

iv. Go to the **z-stack tab** button and fine focus to the bottom of the specimen



v. Click **bottom** when below the focal point (counterclockwise)

vi. Turn the fine focus in the opposite direction and click **top** (clockwise)

vii. Turn off **live**

viii. Click **z-stack green**, click **no** to live dialog box, and open new window, which will give z-dimensions*

ix. Click **single** (pass), click **color**, and **best**

x. To rotate, tilt, and wobble the final image, click **data**, **volume, render, and best fit** in the top menu bar

xi. **Put 3^o for step size**

xii. **Rotation at –30 to 30**

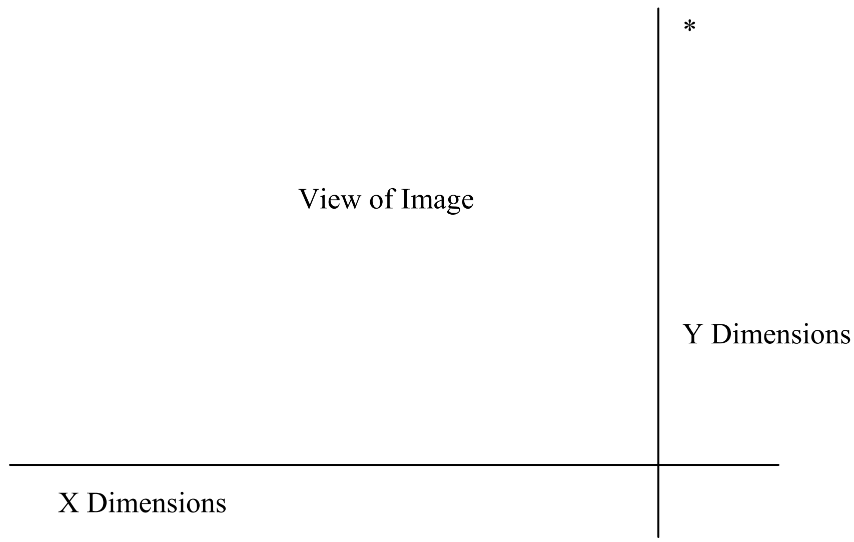
xiii. **Select 21 steps (60^o divided by 3=20, plus 1)**

xiv. Save files as **IDS** file

xv. Go to **render**

xvi. Select **View** tab

xvii. Click on **movie play** button



If the communications between the various components gets corrupted and re-booting doesn't fix the problem:

1. Turn on the microscope
2. Turn on the computer (black box)
3. Turn on the controller (white box) and make sure both green lights are on
4. Click on Nikon EZ-C1 icon and follow the instructions

Adjusting Evenness of Laser

1. Use solid fluorochrome slide
2. Adjust allen screws in top of laser head
3. Edge fall-off is normal (view in pseudocolor)

Supplementary Instructions for Specific Functions of the C-1 System
January 6, 2005 Information from Chris Cathcart

Basic Setup for Image Capture

1. Select **C-1 Live 1** screen
2. Check **Force Window Height to Image Height**, then uncheck
3. Check **Force Integral Zoom Factor**
4. Set up channels:
   #1: Live green/red
   #2: Pseudocolor
   #3: Live green
   #4: Live red

5. Gains are color coded and in order of photo multipliers shown in diagram of laser filter box elsewhere in this manual
6. Go to pseudo color screen and reduce whites and yellows to point where image is almost entirely blue; at this point, the live colored images will be very faint
7. Click on **Color** and increase saturation on **Intensity Scaling** screen, which only changes intensity of display
8. Increase **Pixel Dwell** near top of screen at right and then reduce saturation further on **Intensity Scaling** screen
9. **To open Navigation Window:**
   a. go to upper right of screen and click on **Scan Area** box; move box to area of interest on screen
   b. go to **Navigation Tab** and click on **Crop,** then check **"Try to use best pixel size"** (this optimizes pixel size for the field of view and gives the best resolution)

Creation of a Z-Stack

1. Click on gray **Z-stack** box, click on red boxes to zero reference
2. Go to **Navigation Window** (top right box on screen)
3. Click **Live**, monitor on **Pseudocolor** screen
4. Turn focus knob **Clockwise** to find **bottom, counterclockwise** to find **top**
5. Find approximate middle of image
6. Click on bottom, find bottom
7. Click on top, find top
8. Click **live** off
9. Click on green **Z-stack** button in **Navigation Window** (bars next to **ref., bottom, top** bullseyes turn yellow); focus position moves to top. Click on **live**.
10. **Optional:**
    Go to **Crop**, choose **"Try to use best pixel size"**
    Go to **View** and select **3D orthogonal** to see process

Volume Rendering

1. Go to **Data**
2. Select **Volume Render**
   Accumulative works best for small features (filopodia)
   Maximum works best for most things
3. Click on **Enable**
4. Click on red best fit symbol in tool bar
5. **Three standard projections are typically used:**

   a. **Full 360°:** select 5°, set steps at 72
   b. **60°:** set **Start** at -30° (for ± 30°), set at 3° and 21 steps
   c. **90°:** set **Start** at -45° (for ± 45°), set at 3° and 31 steps

6. Click **once**, then **enable**
7. Click **View**, then **Play tab**; turns in one direction
8. Go to **Render** and turn off **Bounding box visible** at lower right
9. Go to **View**
10. Click on ↔ to get image rocking back and forth
11. Speed may be adjusted by moving slider below **Play tab** (default is slowest)
12. To decrease image saturation, go to **Color tab** and move **Saturation slider**

Selecting Subset of Z-stack

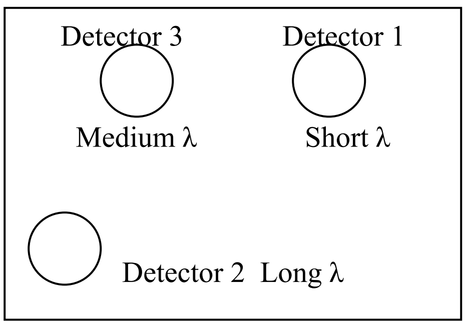
1. Go to **3D Orthogonal view** (3D box); edit from here
2. Go to **Data, Reduce, Crop**
   a. Crop xy view (click on image first)
   b. Crop Z volume (click on **Cropped volume** screen on right to see number of Z steps left
3. Go to **Data, volume, render** to view reduced stack
4. Click on **Enable** to get 2D image of reduced data set
5. To see 3D rendering, click **Enable;** select -30 as start, 3°, 21 steps, click on **Once**
6. Turn off **Bounding box**, go to **View Tab,** click on **player**

Reflected Light Imaging with the C1 System
November 1, 2004 M.J. Dykstra (from Chris Cathcart)

1. **Purpose:** To produce reflected light images of a specimen. The typical use is to produce a series of z-stack images that can be assembled in a volume to give 3-D images of topographic details of a specimen.

2. **Set-up**

a. Turn on C-1 system
b. Get fluorescent image
c. Turn off laser scan and pull **left cube** out of the laser cube housing
   1. The position 1 cube has no upper lens and goes into the right slot
   2. The position 2 cube has an upper lens and goes into the left slot
d. Close laser cube housing



e. To collect reflected light image:
   1. Use long wavelength laser (Red HeNe) to reduce toxicity; can use lots of power
   2. Go to color tab and pull down gray scale for #2 photomultiplier
   3. Use large pinhole and adjust photomultiplier #2 to get image
   4. Build Z-stack in reflected light mode for topography

Dichroic cubes are about a 90:10 split

In reflected light mode, all of laser light is impinging on specimen and all of reflected light is being collected.

Instructions for Installation and Use of Hoffman Modulation Contrast Optics on Nikon Eclipse 2000E Inverted Light Microscope
M. J. Dykstra April 21, 2004

1. Description

This optical package consists of:

a. A **condenser assembly**, a 0.6 NA, 40 mm working distance condenser lens that attaches to the bottom of the condenser assembly and a polarizing filter holder that attaches to the top of the condenser assembly.

b. Three **objective lenses**:

1. A 20 X Extra Long Working Distance (ELWD) Plan Fluor 0.45 NA lens with a 7.4 mm working distance and a focusing collar to adjust for 0 to 2 mm-thick coverglasses or culture vessel wall thicknesses
2. A 40 X ELWD Plan Fluor 0.60 NA lens with a 2.7 to 3.7 mm working distance and a focusing collar to adjust for 0-2 mm-thick coverglasses or culture vessel wall thicknesses
3. A 60 X ELWD Plan Fluor 0.70 NA lens with a 1.5 to 2.1 mm working distance and a focusing collar to adjust for for 0.5-1.5 mm-thick coverglasses or culture vessel wall thicknesses

All of these lenses are DRY lenses and are not to be used with any immersion media.

2. Purpose: This optical package produces images with enhanced three-dimensionality that are similar to images from Differential Interference Contrast (DIC) optics, though through utilization of another optical method. Hoffman Modulation Contrast (HMC) can be used with objects of greater thickness than work well with DIC and can be used to look through plastic vessel walls, which is difficult with DIC, since DIC will show the stress lines in plastic materials. A potential downside is that more of the Z-axis of a specimen is brought into the viewing plane than with DIC optics, which means that objects slightly above or below the plane of interest may be seen as well as those objects specifically in the plane of interest.

3. Set-up:

a. Remove the standard condenser assembly by using the red-handled hex wrench in the wrench rack on the left of the Eclipse 2000E stand to loosen the topmost hex screw on the left side of the condenser assembly.
b. Next, look on the right side of the condenser assembly for a hex screw of the same size at the same level. It may be necessary to slightly turn the condenser assembly to see the screw in the recessed hole on the right side. This recessed screw is actually the screw holding the condenser assembly in place.
c. Gently remove the entire condenser assembly by pulling it forward and lay it down on the white box to the left rear of the microscope.
d. Cover with a piece of lens paper so that it will not get overly dusty.
e. Slide the HMC condenser assembly into the place where the standard condenser assembly was located. Tighten the recessed hex screw on the right side of the condenser assembly. Tighten the chrome hex screw on the left side of the condenser assembly.

f. Loosen the recessed hex screw at the top right side of the condenser assembly track **while holding the condenser assembly** and raise the condenser assembly to the top of the track.
g. Gently screw the condenser lens into the bottom of the condenser assembly. **The screw threads are very delicate, so if it does not go on smoothly, STOP and ask for assistance. It is expensive to repair cross-threaded condenser assemblies.**
h. Gently screw the polarizing lens assembly into the top of the condenser assembly.
i. Adjust the condenser assembly so that the condenser lens is approximately 40 mm from the specimen.
j. Remove three objectives from the objective turret and install the three HMC objective lenses.
k. Using the 10 X objective, Kohlerize the microscope. When done, open the field diaphragm completely.

4. **Using the HMC Optics:**
a. Select either BF (bright field), HMC 20, HMC 40 or HMC 60 positions on the condenser assembly to match the objective you are using. Bright Field viewing can be done with any of the objectives (HMC or not) on the objective turret. The HMC condenser settings will only work with the HMC objectives. The slit modulators are already aligned, so you do not need to adjust them. If, for some reason, they do not seem to be working, consult with the LAELOM staff.
b. Adjust the focusing collar on the adjustable HMC objectives for the thickness of the medium through which you are looking. A setting of "1" is a good starting point for plastic vessels. This is an empirical adjustment that requires changing the objective setting and refocusing on the specimen until the sharpest image is observed.
c. Rotate the polarizing lens at the top of the condenser assembly to enhance the image to get the image that is most pleasing to you.

5. **The HMC objectives can be used with the laser light sources.** Thus, a fluorescent image can be acquired at the same time an HMC image is captured so that both can be displayed in the same window, showing excellent fluorescence localization at the same time excellent cytological detail can be seen.

Control Pad Programming

Programming Cube Positions

1. Press **Mode**
2. Select **Edit**
3. Select "**Other 4**" (for programming information for filter cube 4)
4. Enter letters on name line (e.g., CYAN) and then **enter**; after each letter is entered, move over 1 with right arrow before adding next letter
5. Press **enter**
6. Select **Ex** (excitation), use adjust buttons (arrows above) to find present values or enter numbers with the key pad
7. Press **enter**
8. In this example, select DM and then press **enter**
9. Select **BA** and then 460/500 (BA480), then **enter**
10. Press **Mode**
11. Search menu for **CYAN**

Programming Objectives

1. Find **Objective** line after pressing **Mode** and proceed as above.
2. If the microscope is seeking the entire z-stack range, make sure the objectives can't hit the stage. This situation exists when the control pad has lost its program and is indicated when the stage takes over 30 sec to quit moving.

Section 2: Nikon C-1 Hands-on Instructions (Beta version from June 2003)