DeltaVision Deconvolution Microscope Instructions

No Food or Drink Permitted in the Microscope Room
CRITICAL: The microscope can be seriously damaged by improper care of either lenses or the CCD camera. So review the following very carefully before turning on the microscope.

Lens care: Jarring a lens in any way can permanently destroy the alignment of the elements within it. Handle lenses very gingerly. Before examining a sample, make sure there is no foreign material on the sample that could contaminate the lens surface. On this microscope, the 20x objective is dry, and all the other lenses are oil. **Do not try to image an oiled slide with the 20x objective**. After use, clean lenses while in the nosepiece and only with lens paper. Fold the paper several times and rub it back and forth over the lens surface. Use several pieces of lens paper until there is no longer evidence of oil on the paper. Avoid pressing the lens paper with your finger into the transparent part of the lens. If the lens becomes contaminated with any substance, contact the facilities manager for instructions on cleaning. Don't even think of applying any solvents to the lens surface.

CCD camera care: When you turn on or turn off the mercury arc lamp, make sure the CCD camera is off. Voltage spikes caused by turning on or off the arc lamp could damage the CCD chip ($10,000). This means, turn on the mercury arc first in the start up sequence, and turn it off last in the shutdown sequence.

Set up the microscope for standard fluorescence. This microscope uses motor controlled wheels that turn to place different filters in the fluorescence light path. Thus you must enable these filter-wheel motors to even look at a fluorescent specimen through the oculars, and you must control these motors automatically.

1.) Turn on the mercury arc lamp (found on the platform on the floor to the right side of the microscope and labeled Mercury 100W). Turn the black switch to the "on" position.
2.) Turn on the CCD camera and its water circulator. This is done by turning on the flip switch on the lower power strip on the left side of the microscope. You will hear a hum indicating that the pump is on.
3.) Turn on the power for the PC and the microscope motors. This is controlled by the flip switch on the power strip on the table on the left side of the microscope.
4.) Push in the button on the PC box (also on the table) and the button for the monitor.
5.) The motors and PC controller will initialize themselves. During this process, the stage will move automatically, so you should make sure the objectives are lowered before proceeding. (To lower the objectives, turn the focus knob on the left or right side of the microscope base.) Then, watch the PC screen and answer all questions appropriately. Once initialized, the PC screen turns blue, and you are ready to use the microscope.

Now place your specimen on the stage and focus on it. You may focus on the specimen using either bright field light or fluorescence light. (If you do not wish to use bright field light, skip step 1 below). To view your specimen in either bright field or fluorescence, you must send light from the specimen to the oculars (instead of to the camera). To do this, turn the knob on the right side of the scope near the oculars to the "picture of the eye" position. Dial in the appropriate lens manually.

1.) To set up the microscope for **bright field imaging**, turn on the black flip switch on the back, right side of the microscope. You should see light emanating from the lamp housing on the top of the post above the microscope stage. If not, there may be several problems. First, check that the green button on the bottom, front-left panel of the microscope is **not** pushed in. Second, check the PC monitor and look for the transmitted shutter status on the top left corner of the screen. If "TRANS" is "shut", then open the transmitted light shutter by pressing the green button labeled "trans shutter" on the control box with multi-colored buttons on the table on the right side of the microscope. If the microscope condenser (black disk-shaped assembly above the stage) has been rotated out of the light path, rotate it back to be centered above your specimen. You may also adjust the height of this condenser using the black knobs on the right or left sides of the post behind the stage.

2.) To excite your specimen with the correct fluorescence excitation light, you must select the appropriate filters and also open the fluorescence light shutter. Both of these operations are controlled using the buttons on the button control box. The current settings for all operations controlled by the button control box are shown on the PC screen. Observe which filters are currently in the light path under "Filter Wheels" on the right side of the PC screen. The filter wheels can be turned left or right one position at a time by pressing the red buttons labeled "EX" (there are two such buttons for turning the wheels either clockwise or counterclockwise as indicated by the arrows on the buttons). Turn the wheel until you have selected the appropriate filters: DAPI – for uv dyes, FITC – for fluorescein or GFP dyes, RD-TR-PE – for rhodamine or Texas red. Then press the green button labeled "EX SHUT". You should see excitation light of the appropriate color emanating from the objective.
3.) This microscope is also equipped with YFP and CFP filters. Excitation and emission filters for imaging may be selected with the help of button control box. **Attention:** Those two proteins require different dichroic mirror. If you are uncertain, how to change the Dichroic mirror, please, consult Tatiana. The wheel for dichroic mirror is underthe stage, below the handle for manual shutter control. The mirror for CFP and YFP is labeled 'c'. The standard dichroic mirror is labeled PH3. **Always put standard dichroic PH3 mirror back in place before you leave as a courtesy to the next researcher.**
4.) Examine your specimen in fluorescence through the oculars. You must first dial in the appropriate emission filter in front of the oculars. This is controlled by a black wheel under the eyepieces. Small metal dots on this wheel mark different positions: one dot = DAPI, two dots = FITC, three dots = TRITC, four dots = CFP. Once you have the right filters in place, you may need to center your specimen. This is also done by the control box using the yellow buttons with arrows labeled 1-9. There are three speeds for stage movement controlled by the middle white button labeled speed. If after finding a specimen, it appears unusually dim, check that there are no neutral density filters in the light path. These filters are also in a motor-controlled filter wheel, and are ordinarily used to reduce light exposure to sensitive living specimens. The current neutral density filter is also listed on the PC screen under "Filter Wheels" with the heading "ND". 100% indicates complete transmission of the light (i.e. no neutral density filter), and 0% indicates no transmission. You may change the filters using the red buttons labeled "ND" on the button control box. If your specimen is still dim even with 100% transmission, there could be other problems with the fluorescence light path. Check with the facility manager.

To use the CCD camera to acquire an image, you must use the SoftWoRx/Resolve3D program running on the SGI. This enables control of the camera, filter wheels and microscope x,y,z position.

1.) Log onto the Silicon Graphics computer.
2.) To start up the data collection program, double click on the "SW" icon. The SW control panel menu should appear.
3.) On the menu under the "File" option, select "Acquire/Resolve3D." Resolve3D is the program controlling all aspects of data collection on the Deltavision microscope. The Resolve3D control panels should now appear along the right hand side of the Silicon Graphics computer screen.
4.) First, select the lens that you are using under the Lens button. This will insure proper xy stage movement if you use these features to center your specimen. In addition, the lens information will be stored with all images you collect, permitting appropriate image processing of your data.
5.) To direct light to the camera, turn the dial under the oculars from the picture of the eye to the SPL position.
6.) To take a picture, click on the acquire button at the top of the Resolve3D window. An image window will appear with a picture of your specimen in it.

To fine tune your image there are a number of variables that can be adjusted.

1.) Exposure to the specimen can be adjusted. Your image may either be too dim or too bright, but in either case the displayed image is automatically scaled such that it will **appear** bright. To determine if you are using the appropriate exposure, it is therefore critical to check the actual maximum and mean intensity values in your image. These are shown above the blue graph on the Resolve3D window. The

CCD camera has a maximum intensity of 4095. If your maximum intensity equals this, then you should reduce your exposure because you may be missing features in the bright region of your image. For a specimen in which bleaching is not a problem, the maximum intensity should be close to 4095. For light sensitive specimens, maximum intensity as low as a few hundred is often adequate.

To adjust exposure, you may change the exposure time (Exp) on the menu (this value defaults to 1 second). You may also change neutral density filters in the light path (note this can be done with the button control box as described above, or directly through the Resolve3D interface by clicking adjacent to "ND filter". Higher numbers for ND filters on the Silicon Graphics screen correspond to less light).

2.) Resolution of the specimen can be adjusted. You may think you would always want higher resolution, but there is a trade-off. More resolution requires more excitation light, which could bleach or damage your specimen. The camera's resolution is controlled by "binning". The Resolve3D panel shows the binning factor in use (default setting is 1) and the current pixel size in the image. Binning by two results in a pixel that is twice as big, and therefore yields half the resolution. When you bin by a factor of two however, each pixel in your image becomes four times brighter. Experiment with different binning factors to see for yourself the trade-off between light intensity and resolution, but also note point #3 below regarding image size and permissible binning factors.

To adjust resolution, change the binning factor in the Resolve3D window. Higher binning yields lower resolution.

3.) The size of the image can be adjusted (e.g. 1024x1024, 512x512,…). Smaller image sizes are better because they take up less space and are faster to process and display. Choose the smallest size possible so that the important features of your image are included. Note that if you bin by more than 1, then larger dimensions are not possible, and you will receive an error message if you try. The basic rule is that the binning factor multiplied by the "Image Size" should not exceed 1024.

To adjust dimensions, click on the window in Resolve3D and select the appropriate image size.

4.) Any feature in your image can be centered by choosing the center object button in Resolve3D. Next click on the feature in your image to be centered, and a new image will be acquired with that feature at its center. If you use these controls, and find that centering fails, check to be sure you have entered the correct lens in the Resolve3D parameter list.

5.) Fine focus can be adjusted from the Resolve3D menu. The step size for z focus change can be adjusted by entering a value of your choice. Then click on the arrow buttons to move up or down by the step size entered. The up button is the equivalent of having the lens move up closer to your specimen. Note that x,y position of the stage can also be adjusted through Resolve3D.

Time-lapse, 3D or multi-color images, and saving your images.

1.) After identifying the basic parameters for a single focal plane image, you can save the image or acquire a series of images by clicking on the "Experiment" button in Resolve3D. A new window appears. In this window, click on Design. This produces another window that provides five different options for data collection.

a.) Sectioning (3D images). You must enter three parameters: starting position in z, plane spacing and number of optical sections (i.e. focal planes). Note if you want just one optical section, then enter 1 in the box labeled "number of optical sections".

b.) Wavelength (multicolor images). Check off the number of wavelengths and choose the appropriate filters. If nothing is checked, you will take an image with the current position of the filter wheels.

c.) Time Lapse (Repeated images over time). Enter the number of time points and the interval between time points. If you need to adjust the focus while the system waits for the next time point, find purple 'control mode' button on the control panel next to joystick, click on it. You should see on the PC screen 'Control: local'. After that focus through the eyepieces. Note that the z coordinate on PC screen will not change - computer does not know that you changed the focus.

Click on the same purple button and check that control will change back to 'remote' (i.e. computer-operated).

Final note: If you specified z-scan plus time lapse in multiple channels, be realistic, calculate, how long will it take, and input appropriate long time interval between time points.

d.) Point Visiting. (For parallel time lapse studies from different locations on your slide). First, define the points. Click on 'Stage' button and select 'Mark and Visit'. Find the cell through the eyepieces, using a joystick, and click on the button 'Mark point'. Continue, until you will find all points you want. Second, include point visiting into your 'Design Experiment' routine. Specify all the parameters in the order they are written in the window. Specify the number of sections and step size. Specify the imaging channels. Check off the 'Visit list' and specify the numbers of the points to visit. For instance, if points 1, 2, 3 and 5 of your list need to be visited, type in 1,2,3,5. 1-3,5 is supposed to work also, but sometimes it does not. Finally, go to 'File'/'Save and Exit" and run the experiment.

Unfortunately, you cannot change the point coordinates while the program is running. Therefore, if you need to correct focus or cell position between time points, do not specify any time lapse in Define Experiment window. Collect all images for the first time point. Then, before the next time point, visit all the points (cells), correct the focus, 'replace' points (cells) and run experiment for the second time point. Continue in the same way with the rest of time points.

e.) Panel Collection. (For creating a larger image by acquiring multiple adjacent images). See the Facility Manager.

2.) Once you've selected the appropriate parameters, click on "File" and select "Save & Exit". Then return to the Resolve3D Experiment window and click on "Run". This produces a new window where you must click on "Do It". You will then be prompted for a file name to save your data. Enter this and then click on "OK" to start data collection. Your files will automatically be saved in a standard directory under your name. Once you begin to generate a lot of data, you may want to make different directories to organize your data. For this, you must learn some basic UNIX commands. Ask the Facility Manager for help.

Viewing files

1.) Choose "View" under File in the original SoftWoRx window. This produces a new window where you must click on "Input". This yields (of course) another window with a list of files. Now select the name of the file you want to view. You may scroll through the list of files in your current directory or change to a new directory by clicking on the new directory name. To move up one directory, move to the top of your file list and click on the ".." entry. After clicking on the file name, it is entered into the Input line in the View File window. Now click on "Do It" to bring up the image.

2.) Experiment with the different scroll bars at the edge of your image. These can shift the image laterally, or step in z, or change time points (depending on the type of data you have collected). The rotating dial bar on the bottom left of the image window will zoom your image. Image contrast can be adjusted using the icon on the upper left depicting a line and graph.

Shutdown

Move your files to the SC-B41-SSA_SSA_41_G1_SERVER/SSA_G1 /LRBGEIMAGE/YourFolder.This server will be automatically mounted on all the computers of the facility. If you will not see this server mapped on your personal computers, map it yourself. Find red N in the lower right corner of the computer screen, right-click on it. Select Novell Map Network Drive and for the "network path to resource" field, use the following syntax \\lrbgeimage.nci.nih.gov\ssa_41_g1\lrbgeimage.

1.) This can be done by FTP using xdir program, please, consult staff, if you are uncertain how to do it. Also, there is a description hanging on the bulletin board. Files left on SGI img_decon after imaging session will be deleted from this computer by facility staff.

2.) Exit the SoftWoRx program by clicking on Exit under File in the Main menu.

3.) Logout of the Silicon Graphics by moving the cursor outside of a window, and then pressing the right mouse button. A new window appears. Choose logout. PLEASE, DO NOT SWITCH OFF THE SILICON GRAPHICS COMPUTER.
4.) Shutdown the PC by first hitting the esc key on the PC keyboard. Answer yes to "Are you sure…?" The Program Manager window appears. Click on File and select "Exit Windows". Then turn off the PC monitor button on the lower right corner of the monitor. Also turn off the PC using the button on the PC box, and turn off the power strip on the table.
5.) Turn off the camera by turning off the lower power strip.
6.) Turn off the mercury arc lamp.
7.) Turn off the bright field light (right back side of the microscope).
8.) Lower and clean lenses with lens paper.
9.) Wipe down the stage and table with window cleaning detergent sprayed into a Kimwipe.
10.) Sign the log book and note any problems.