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Olympus DeltaVision Microscope Start-Up and Shut-Down Instructions.

The Olympus DeltaVision microscope is controlled by two computers located inside the metal cabinet under the monitor desk:

- a) a LINUX computer runs the DeltaVision softWoRx image acquisition and display software
- b) a PC (running Windows) controls the microscope and camera hardware

Normally, the two computers are always ON.

A single monitor displays feed-back from both computers. A switch box (white switch box to the left of the monitor) toggles the display between the PC (button A) or the LINUX (button B).

1. Start-up procedure.

1. Turn ON Mercury lamp (Power supply on shelf on the right side of microscope table).
2. Activate the monitor by hitting the space bar or moving the mouse. Normally, the monitor switch box (white box to the left of monitor) should be set to **1** (LINUX PC) and the LINUX desktop should be visible on the monitor. Press button **1** if LINUX desktop is not displayed. If LINUX PC is OFF or has crashed, restart the computer (switch in microscope cabinet).
3. Power ON the motors and electronics using the main power strip switch inside the metal cabinet (red monitor light should turn ON). Wait until all the devices are ON (~20 seconds). If the light is already ON, then the system is already running
4. Switch monitor display to PC by using the switch box (button **2**).
5. Lower the objective turret all the way down using focusing knob
6. Start the controller software by double-clicking the icon on the desktop. Follow instructions on screen until hardware is initialized (about a minute). If controller window is already running, you can either proceed with it, or, if there is a problem, you can close it by placing the cursor inside the window and hitting "Esc" twice, and then restart the controller software. If the PC was just turned ON, controller will start automatically. At the end of the Procedure, console will say: "starting normal operations", and a set of fluorescence filters will be displayed.
7. Switch back to LINUX (button **1**)

8. If SoftWoRx is not already running, start it by double-clicking the SoftWoRx icon.

Sometimes, if SoftWoRx is already running, an error message may be displayed; click OK or close the window. (The message appears because SoftWoRx could not communicate with PC controller software that was OFF).

9. From the SoftWoRx menu, start the acquisition software (File>Acquire(Resolve3D)). Click YES on the dialog box to initialize software.

10. Turn ON the microscope tungsten lamp (switch on the back right side of microscope body) if you need transmitted light.

The microscope is now ready for operation.

2. Basic operation.

2.1 set-up

In the Acquire panel, go to Settings>

>Optional: Set "Display" to Auto Color and 3 Waves (to display up to three channels); You can set to Auto Grayscale if you want to display a single monochrome channel. This tab only affects the scratch display window, NOT the files you acquire.

>Files: Under Data Folder, enter /data1/yourname_date_dv

this will create a folder e.g. "jdoe_122507_dv" in the main data folder, where your images will be saved

>Imaging: Make sure the "EX" is checked under "Illumination Shutters" This sets the scope to use fluorescence light whenever you click "Acquire". If you want transmitted light, check "TRANS"

Save the settings and close window.

2.2 running an experiment

Focus your sample; determine the correct exposure times for each channel (max signal around 2,000 works well; camera saturates at 4,095); use ND filter if sample is very bright to cut down illumination intensity. Set your z-stack (be sure to be slightly out of focus on both sides. Go to the middle section.

In the experiment panel, under "Design Experiment":

> Sectioning: click "get thickness" to load your stack parameters. Optimal spacing is 0.2 microns. You can also enter the values manually.

>Channels: click one box for each channel, and select the EX filters. Click "refresh exposure conditions" to load your latest manual exposure settings

> Set other parameters if needed

In the experiment panel, under "Run Experiment":

>Enter an image filename (ex: jdoe_122507_01). SoftWorx will automatically increment after each file is saved.

> add notes if desired.

Save Experiment (use default name Resolve3D.exp), unless you want to reuse your experiment settings in the future.

Click "Start Scan"

Image will be collected and saved automatically. Microscope will be ready for next image.

3. Transferring files using the gFTP application.

Double-click gFTP

In "Bookmarks", select "Decon1" to copy your files to the deconvolution station. Select "fred users" if you want to copy the files straight to your fred/si directory

On the right panel (remote computer),navigate to the folder where you want to copy your files to.

On the left panel (microscope PC), find your folder, and select it

Click the right transfer arrow (>) to copy your files.

When complete, close the gFTP window.

4. Deconvolving Deltavision files.

Typically, this is done on the LINUX "Decon1" station.

If SoftWorx is not running, start the application.

Select "Process" / "Deconvolve"

drag one image file to first line (input). Software should generate an output file in same directory

under "OTF File", software should read and display correct objective. If not, click "OTF File" and find and select correct objective.

Under "Method", select "conservative"

Under "Noise Filtering", select "low" (Also, make sure "Apply Correction: is selected).

Optional: Select "More Options" and check "Save Final Results as Floating-Point"; Close the Deconvolution Options window. This changes the final file format.

> If you have only one image to deconvolve, click "Do It" in the Deconvolve window.

> If you have several images to deconvolve, click "Run Options"

Under Run Options, select "Add to Queue"; Close the Run Options window.

Click "Do It" in the Deconvolve window; this will open the queue window and load the file to deconvolve in it.

Select all the other files to deconvolve and drag them into the queue.

Click "Start Now"

The queue will run and deconvolve all images one after the other, and save them to the original folder.

You may close the "Deconvolve" window, but DO NOT close the Queue manager, since this will terminate the deconvolutions. It is recommended to leave the queue manager maximized on the Desktop, so that other users will be aware that it is running.

Note: if you have collected images with different objectives, you should do as above for all the images taken with one objective; then, you should reload a sample image taken with the other objective into the "Deconvolve window", make sure the new objective OTF is listed, and click "Do it" to load the new image in the queue. Then, select all other images with the SAME objective, and add them to the queue. The reason for this is that when you add a file directly to the queue, the objective info is not updated; you need to go through the "Deconvolve" window for that.

5. File conversion.

There are several ways to view your DeltaVision files: you can purchase a DeltaVision viewer software from Applied Precision... probably not worth it

You can open DeltaVision files with public domain software imageJ. You need to download a recent version of the software and also download the DeltaVision opener plugin. Once you open your files with imageJ, you can convert them to TIFF, and do various types of processing and analysis.

Finally, you can convert your files directly from our deconvolution station.

To do that, you need to

1. open your images with SoftWoRx.
2. Adjust image brightness and contrast, if necessary
3. From the image window, choose: "Save as TIFF..."
4. If desired, select a region, or a range of sections, to save
5. Choose the final format, and click "Save"

SoftWorx will save each section as a separate TIFF file. files will be numbered with channel and section number information.

Notes about converting your files to TIFF:

DeltaVision files are acquired in 16 bit per channel format. Therefore, each channel is a separate 16-bit greyscale image. 16-bit means that the intensities can range from 0 (zero) to about 64,000 (2 to the power 16). There are three standard image file formats: 8-bit tiff (monochrome, 256 levels of grey), 16-bit tiff (monochrome, 64,000 levels of grey), and 24-bit RGB (standard color images with 3 channels, Red, Green, Blue, and 8-bit per channel; these are generally referred to as "millions of colors, because they have 256x256x256 possible colors).

When you convert DeltaVision files to 16-bit TIFF, you save the original data for each channel, separately, in 16-bit format. These files can be viewed with imageJ, or Photoshop (although the contrast needs to be readjusted, otherwise they tend to look dark).

The other option is to convert to 24-bit RGB and save the color image. This is convenient. However, when you do this, the original intensities are recalculated from a 16-bit to an 8-bit scale for each channel.

By default, the lowest intensity value in the original 16-bit image is converted to 0 (zero) or black, and the highest value, for instance 7,046, is converted to 255, or white. Therefore, each image will have a different scale conversion factor, depending on its own range of intensities.

By adjusting the brightness and contrast of your DeltaVision image, you can change the min and max (and gamma) values for the conversion.

If you want to be able to compare intensities between different images, you need to (a) collect them with the same settings (such as exposure time), and (b) use the same conversion factor, by copying the scale of one image to another, before converting.

All this can appear rather complicated, so please don't hesitate to ask Scientific Imaging staff to show you the various methods of conversion, and how to use them properly.

6. Shut-down procedure.

(Shut system down ONLY after hours)

1. Quit Resolve 3D; you may leave SoftWoRX running
2. Switch monitor to PC (button A on white switch box)
3. Quit the controller software from the controller window menu (put cursor inside window and click Esc twice (Esc, Esc).
4. Turn OFF microscope transmitted light (rear right switch on microscope body)
5. Turn OFF Mercury lamp (power supply on floor under microscope table).
6. Turn OFF main power switch in cabinet (indicated by the red arrow)
7. Gently wipe oil from objectives with lens tissue ONLY. Lower objective turret all the way down.
8. Cover microscope (leave uncovered if lamp housing is hot).

7. Some simple rules.

1. Always use properly mounted samples that are clean, and have been sealed. If your samples are not sealed, be extra careful to avoid transferring salt solutions or fluorescent media to the microscope optics. These will ruin your, and everybody else's images, and will require time-consuming cleaning. For best resolution, use #1.5 coverslips. Objectives have generally been corrected for #1.5 coverslip thickness.

Oil objectives have a working distance of about 100-150 microns. Therefore the sample to be imaged has to be within that distance of the coverslip. If you have cells on the slide, and have a thick layer of mounting medium between the cells and the coverslip, you may not be able to focus on your cells. Typically, 20 microliters of mounting medium are enough for a 24x24 mm coverslip.

2. Be careful with immersion oil. Only use the immersion oil provided by Scientific imaging. Do not mix oils (or you and the people after you will get poor images). Apply a SMALL amount of oil to your sample (~20 microliters is enough), not to the objective. Transfer a small drop to the coverslip, but avoid contact between the glass rod and coverslip. This will prevent transfer of dirt or fluorescent substances to the oil. Avoid using too much oil, or it will end up on the stage or condenser. Cleaning oil from optical components is difficult and time-consuming. Be sure to use immersion oil ONLY with immersion lenses (check objective or chart on the wall). Always close the bottle of immersion oil and leave it on its absorbing pad. An open bottle will soon end up in a messy spill.

3. Be considerate and clean your objectives when you are done. Use lens tissue ONLY. take one or two sheets, fold them along their length, and gently apply the tissue to the objective front element. gently wipe the metal cover to remove excess oil. If oil has been spilled on the stage or optical components, please inform staff immediately. Place the unused lens tissues back in their protective envelope. DO NOT use tissues that have been sitting uncovered on the desk. They may have dust particles or grease that may damage the objective lens.

4. If you are working after hours or during the week-end, please follow the shut-down procedure. Generally, it is a good idea to cover the microscope at the end of the day, but make sure you have turned OFF all lamps, and that the housings are not HOT. If in doubt, leave uncovered.

5. When working with fluorescence, always minimize unnecessary exposure of the sample to reduce photobleaching. Some samples can fade away in a matter of seconds. Use illumination field diaphragm, density filters, or close down the shutter whenever possible.

6. If you are unfamiliar with the operation of the instrument, or if you think there is a problem, talk to the staff, or leave a note. In case of emergency after hours, contact Julio Vazquez at 206-789-0369.

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