Personal Deltavision Quick Reference Sheet
ACRF Cancer Biology Imaging Facility
Institute for Molecular Bioscience
University of Queensland
13 March 2012

First and foremost: be mindful of what you are doing at all times. The Deltavision microscope is delicate and must be treated as such.

SLIDE CLEANING AND PREPARATION

- Make sure your slide is *scrupulously* clean. All dust, old oil and mounting medium should be removed with a little 70% EtOH
- If you can use a mounting medium that sets hard, please do so.
- Be sure to give your slides and sealants at least eight hours to set.

STARTUP

- Inspect the microscope to be sure the objectives are clean, that there is no oil where it should not be, no medium spilled on the microscope, etc.
- Ensure that the objective nosepiece has been fully lowered using the coarse focus wheel.
- Turn on the computer on the left and allow it to start fully.
- Turn on the computer on the right and log in.
- Start *softWoRx* by double-clicking the *softWoRx* desktop icon
- When *softWoRx* starts, go to *Menu > File > Acquire (Resolve 3D)*.
- Select the **Lens** you wish to use in the software and rotate it into place on the microscope.
- Consult the **Deltavision Filter Chart** (on the computer table) and decide which set of filters you would like to use. The set 'Filter Turret #1 "Standard"' is the same as the original filter set.
- Ensure that the filter turret below the nosepiece is set to the appropriate position.
- In the **Resolve 3D** window, click on the settings button to bring up the **Resolve 3D Settings** window*.*
- Select the **Misc.** tab and inspect the **Filter Wheel Sets** section. Ensure that the filter wheel sets correspond to the set you wish to use.
- If the displayed sets do not correspond to the set you wish to use, select the appropriate set from the drop-down menus for each filter set.
- When you have ensured that the proper filter sets have been selected, click the **Activate Filter Sets** button, click the **Save Settings** button to save your settings and the click the **Done** button.
- Press the **Info…** button in the user interface next to the objective you've selected.
    - Set the **Coverslip Thickness** – 0.17 is default, but if you're using #1 coverslips set this to 0.15.
    - Set the **Approximate Distance to Specimen.**
        - 0 is the correct setting for adherent cells.
        - Use a larger distance if you wish to image deeper into thick tissues, *e.g.* half the tissue thickness
    - Set the **Temperature** of the specimen - 25°C is the default, but check the room temperature at the thermometer above the monitor, and use 37°C for live cell imaging.
    - Set the **Mounting Medium** – glycerol is the default, but you can select from several others.

Figure 1 - Location of Settings Button

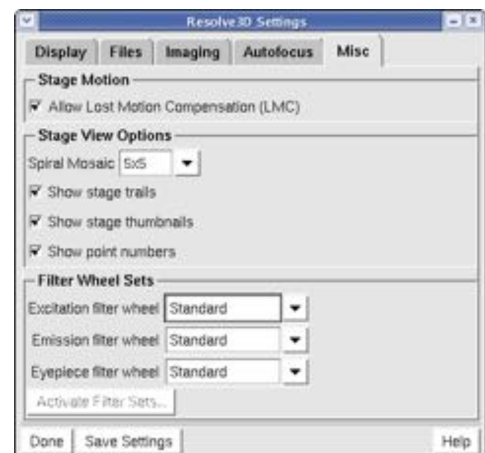

Figure 2 - Resolve 3D Settings Window

- Take note of the **Recommended Oil Refractive Index** – 1.515 is the default
- Select the indicated immersion oil from the box of oils on the table.
- Raise the objective a bit and use the black knob on the stage, as well as the joystick, to position the stage so that your coverslip will be approximately centred over the objective.
- Carefully place a drop of oil on the objective.
- Mount your slide with the coverslip facing down towards the objective.
- Set the *xy* stage speed to **Fast** on the keypad and centre your coverslip over the objective.
- Bring the objective up using the microscope coarse focus knob until the oil touches the coverslip
- Set the eyepieces for your comfort, being sure that the Dioptre adjustment is set to zero, and focus on your sample.
    - Turn the selector knob on the front of the microscope to **Eyepiece**.
    - Rotate the wheel under eyepiece tube until the **Filter Monitor** window in the software has either **DAPI, FITC, TRITC** or **POL** in three of the boxes
    - Press the **Trans** button on the keypad for brightfield (use the POL eyepiece setting) or
    - Press the **EX** button on the keypad for fluorescence (use DAPI, FITC or TRITC).
    - Focus on the sample using the microscope focus knobs.
- Set the *xy* stage speed to **Slow** on the keypad and centre an object of interest.
- Press the Trans or Ex button on the keypad to deactivate the light source.
- Turn the selector knob on the front of the microscope to **Camera**.
- Choose an **Excitation Dye** (*e.g.* TRITC) in the **Acquire** menu.
- Set **%T** (the percent transmission) of the xenon lamp to a reasonable value: lower for less photobleaching/phototoxicity, higher for faster image acquisition.
- Select the **Image Size** you would like. 512 × 512 and 1,024 × 1,024 are common settings.
- Se the **Binning** to **1 × 1**. Only use higher values if your sample is **very** dim or you are willing to trade resolution for speed.
- Set the **Exposure**
    - Use manual exposure to set the time to a reasonable value (less than one second) and capture an image either by pressing the **Acquire** button at the top of the screen or the small camera button or…
    - Use the **Find** button to perform an automatic exposure, and accept the default **Target Intensity Value**. N.B.: this may bleach your specimen.
    - Either way, be sure that the image is not over-exposed. Generally this means making sure that the highest grey-level in your image is no greater than 3,600.
    - For thick samples you may need to check the exposure at several focal planes.
- You can acquire continuously by selecting *Menu > File > Continuous Acquire* in the *Resolve 3D* window.
- You can use the continuous acquire mode with the joystick to find objects of interest.
- You can centre an object of interest by clicking on the Circle button and then clicking in the image frame.
- Focus on an object of interest
    - Adjust the focus automatically by pressing the **AF** button, OR
    - Adjust the focus manually by dragging the **z position indicator** up or down, OR
    - Adjust the focus manually by pressing the up down arrow keys in the software or on the keypad, OR
    - Adjust the focus manually by using continuous acquire and using the microscope fine focus knob.
- Once the sample is in focus, adjust the Exposure settings either manually or using the Auto-Expose button. Ensure that the captured image is not over-exposed, *i.e.*: the Intensity is less than 3600.
- ***N.B.:* Saturated pixels will not deconvolve, so it's much better to have a weak image than a bright one.**

CONFIGURE EXPERIMENT

- Select the first channel you wish to capture under the Excitation drop-down, for example: TRITC.
- Capture an image.
- Optimise the excitation settings for the *z* stack as shown above, *i.e.:* Hit AF button then set exposure so that the captured image intensity is less than 3600
- Mark Z Stack Range
    - Using the software z controls move to the top of the sample, and click the **Mark Top** button
    - Move to the bottom of the sample and click the **Mark Bottom** button
    - Move back to the middle of sample using the **Move to Center** button

- Select the next dye Channel (*e.g.*: FITC) from the Excitation list and adjust the exposure settings as required, do not adjust the Z position.
- Repeat last step for other dye Channels, *e.g.* DAPI, Cy5 etc.
- Press the **Experiment** button.
- Adjust the **Fast Acquire** settings if necessary (use the help menu).
  - Lamp off after acquisition will shut down the xenon lamp after a long run (good for long time-lapse experiments running during the night).
- Tick the following checkboxes and configure their settings to perform the following types of experiments.
  - **Sectioning Tab**
    - Activate **Z Sectioning**
    - Set the **Focus point when scan starts** to the current position of your focus in the sample.
    - Set the **Optical section spacing** to either 0.10 or what is recommended in the Lens Info Window.
    - Press the **Get Thickness** button.
    - Adjust **Total Thickness** or Spacing if the stack is too large.
    - Enabling **OAI Scan** produces a 2D *xy* image of the *z*-stack (useful for fast imaging).
  - **Channels Tab**
    - Activate **Channels**
    - Select the channels you wish to image, then press **Refresh exposure conditions** to ensure your exposure times are correct.
    - **Reference Image** is used for creating a DIC reference image to overlay with your fluorescence images.
  - **Time-Lapse**
    - Activate **Time-lapse**
    - Adjust any two of the three settings below to setup your time-lapse experiment.
      - **Time-Lapse** sets the interval time between images.
      - **Total Time** sets the total time of the experiment.
      - **Time Points** sets the number of images.
    - Enabling **Cell Tracking** will move the *xy* stage to keep cells centered in the camera field of view.
    - Enabling **Autofocus before Imaging** will allow you to autofocus before capturing an image.
  - **Points**
    - Activate **Points**
    - Mark the points using the **Points List Menu**.
    - Type the points into the **Visit Point List** data entry panel, *i.e.*: 1,2,3 or 5,1,7,6 etc.
    - Enabling Autofocus will allow you to autofocus before capturing an image.
  - **Panels**
    - Activate **Collect Panels**.
    - Enter a suitable **Overlap**, *i.e.*: 12 pixels for a 512x512 image, 24 pixels for a 1024x1024 image; then hit *Enter* or *Tab*.
    - Hit the **Get Start** button when your current image is your required start image.
    - Move the stage via joystick or software to the required end image or your Panel/Mosaic, then hit the **Get End** button.
  - **Actions**
    - Actions are used to create events that occur during experiments, such as autofocussing, pauses, ratio imaging, etc.
- Run Experiment
  - Press the **Settings** button in the *Resolve 3D* window to choose or create a folder for your data within the *Data1* folder, *i.e.*: *Data1\j.bloggs*.
  - Press the **Run Experiment** tab.
  - Press **Yes** to overwrite the *Resolve3D* macro.
  - Enter *Image Name*, etc.
  - Press the green **Run** button.
  - Wait for the *Experiment* to finish acquiring.
- Deconvolution
  - In the *softWoRx* window, select *Menu > Process > Deconvolve.*
  - Drag the **1** from the captured image window to *Input* of the *Deconvolve* window.
  - Select **wavelengths** to deconvolve.

- Ensure correct OTF file is selected for your imaging objective.
    - Adjust *deconvolution options* as required.
    - Press **Do It**.
    - Wait for the deconvolution to finish, or you can start acquiring anew during a deconvolution.
- Stitching Panels
    - If you have performed a *Panel* experiment, you can stitch the panels together to form one large "Mosaic" image. Stitching should be performed *after* deconvolution.
    - In the *softWoRx* window, select *Menu > View > Stitch.*
    - Drag the collected data file number, *e.g.* "1", to the *Input* section of the *Stitch* window.
    - Adjust the parameters as required.
    - Press **Do It** to produce a *Mosaic* image.
- Shutdown Procedure
    - When finished remember to turn off the xenon lamp via the small *lamp button*.
    - Clean the objective then wind it down fully.
    - *Quit* the software.
    - Copy the files you created in the */Data1* directory to *userhome* or a USB stick.
    - Shut the PC down.
    - When the PC has fully shut down, press the controller PC button for a count of 3, then let go. The system should shut down after a few seconds.
    - If you forgot to turn off the xenon lamp, you may have to hold down the Controller button for 10 seconds before the system will shutdown.