

# DeltaVision Quick Start Guide – Image Acquisition

## 1 – Mount sample:

- Make sure that the appropriate objective is selected in the Lens Dropdown (3) of the Resolve3D window.
- Select the Info button (7). Enter information under Optical Conditions and note the Recommended Refractive Index.
- Place one drop of oil (w/recommended RI) on Objective lens. Mount slide cover slip down in the repeatable slide holder.

## 2 – Focus on sample through Oculars:

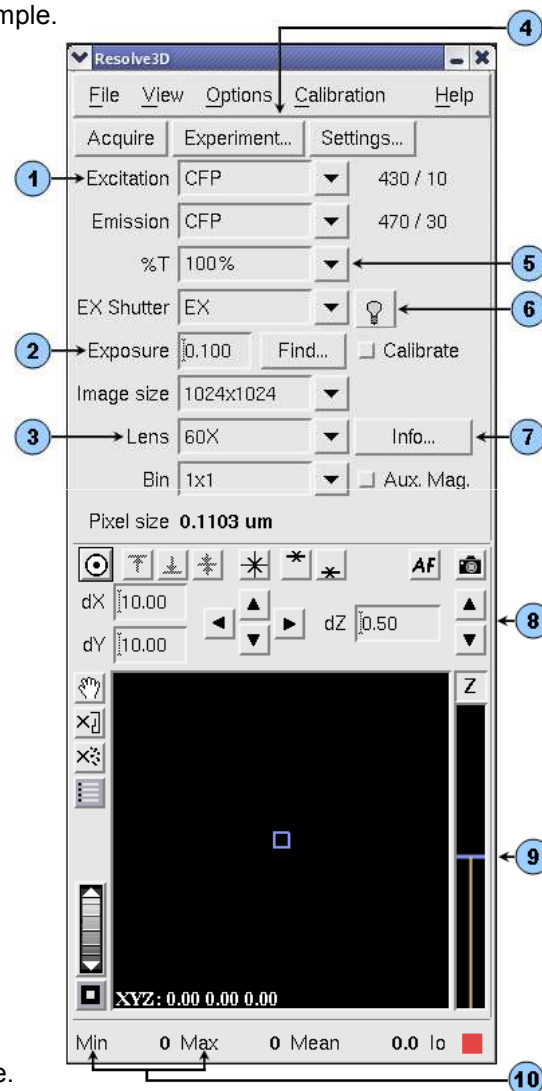
- Bring objective up to the sample by rotating the coarse focus knob towards you just until you see the oil spread.
- Rotate eyepiece filter wheel to your channel of interest.
- Rotate the port selector knob (located on the front of the microscope base) to the eyepiece.
- Open the EX Shutter from the Joystick Keypad (turn on the lamp (6) if necessary).
- Look through the oculars and rotate the fine focus knob to focus on your sample.
- Use joystick to scan your slide in x and y until you find an ROI to image.
- Close the EX Shutter from the Joystick Keypad.

## 3 – Set up experimental conditions:

- Rotate the port selector knob to the camera.
- Acquire an image (8). Make sure the image window is fit to screen (9).
- Use the Centering Tool (10) to center the ROI in the image window.
- Find focal plane (location of highest max intensity (10)) using Z bar (9) or Up and Down arrows (8).
- **NOTE:** Lower exposure time if image is saturated (max intensity of 4095).
- Adjust exposure time (2) and %T (5) (Neutral Density) to achieve a max intensity (10) between 2000-2500 for fixed samples or 3-5 times the background (min intensity) for live samples.
- Switch EX filter (1) to the next channel that will be utilized in your experiment. Find an appropriate exposure time and %T. Repeat for all channels in your experiment.
- Set Z limits for the experiment:
  - Using the Z bar (9), scroll up until you reach the top of your sample. Mark the top (\*).
  - **NOTE:** Scroll up until the structures that are in focus (crisp) at the focal plane appear just slightly out of focus (blurry).
  - Scroll down until you reach the bottom of your sample. Mark the bottom (\*).
- Return to the middle of your sample (†).

## 4 – Design and Run Experiment:

- Select the Experiment button (4) in the Resolve3D window.
- Z Sectioning tab:
  - Make sure that you have elected to start from the Middle of the Sample.
  - Select the **Get Thickness** button. This loads the Z thickness set in Section 3.
- Channels tab:
  - Activate the number of channels you wish to use in your experiment.
  - Load the channels using the EX Filter drop-down menu. Verify that exposure times and filters are correct.
  - If desired, add and set exposure conditions for a Reference Image at the bottom of the Channels Tab.
- Select the **Run Experiment** tab. When prompted, click “OK” to save the experiment macro and then “Yes” to save the macro as the default filename (Resolve3D.exp).
- Select the **Settings** button to enter the name of the folder you would like the image file saved in.
- Enter an Image File Name. Do NOT use spaces or special characters in the file name.
- Select Play (10).




# DeltaVision Quick Start Guide – Image Processing

## 1 – Experiment File Location:

- All experiment files are saved in subfolders within the main Data Folder (Data1). This folder can be opened from the Main softWoRx window by selecting the **Data Folder** button or by double clicking on the Data1 folder on the Desktop.



## 2 – Image Processing:

- Deconvolution: Select **Process > Deconvolve**. All other information is loaded from the raw image file. Default settings are adequate for the majority of applications. Select **Do It**.
- Quick Projection: Select **View > Quick Project**. This will project your 3D data (X, Y and Z) into a 2D format (X and Y). There are three methods for projecting data; Max Intensity, Average and Sum. Select a method then select **Do It**.
- Volume View: Select **View > Volume Viewer**. Select the Rotation you prefer then select **Do It**.
- General Processing Tips:
  - To load files into processing tools, first open the tool. Then, drag and drop the window ID number of the file into the Input line of the tool. The window ID number is found on the upper left-hand side of the image window.
  - Many tools have a **Details** button. This is where you can choose to process only a subset of your data by selecting only certain Z sections, Time Points, etc.
  - Many tools also have a **Select Region** button. This is where you can select only a particular x-y area to process. Select the **Select Region** button then draw a box in your image window. The **Reset** button will erase a selected region.
  - Not all tools automatically save their output. You will be prompted to save unsaved files before closing.
  - Select the  button to open the image histogram (or use the small histograms located on the top of the image window). This will adjust display scaling but will not alter your experimental data.

## 3 – Save As:

- Movie: In the image window, select **File > Save as Movie**. Select the preferred file format (MPEG, AVI or QuickTime), and Frame Rate then select **Preview**. When the speed of the movie looks acceptable, select **Do It**.
- Tiff: In the image window, select **File > Save as Tiff**.
  - Decide whether you would like to scale the Tiff images by selecting or deselecting the **Scale Using Min/Max/Exp Values Below** option.
  - Select the appropriate Output Size:
    - 8-bit Grayscale – Saves each channel as a separate Tiff file. Compresses 16-bit data.
    - 16-bit Grayscale – Saves each channel as a separate Tiff file. Preserves all intensity data, therefore, unscaled 16-bit Tiffs are the recommended format for quantification purposes.
    - 24-bit RGB – Saves 3 channels in one Tiff file (8-bit per channel). Compresses 16-bit data.
  - Select **Do It**.
- JPEG: In the image window, select **File > Save as JPEG**. Select **Do It**. JPEGs should NOT be used for quantification.

## 4 – Advanced Experimental Design:

- OAI (Optical Axis Integration) Scanning: In the Sectioning Tab, select the box next to OAI scan to run an experiment that will collect a 2D projection of the 3D data contained within the Z thickness specified. Before deconvolving these files, be sure to select the **Deconvolve Projections** box in the Deconvolution tool.
- Time Lapse Tab:
  - Activate the time lapse tab and enter the time between points and total time you wish to collect.
  - To run deconvolution during time lapse, select the **Deconvolve during experiment** option in the Run Experiment tab. This is not recommended during extremely taxing time lapse experiments, as it may interfere with acquisition.
- Point Visiting Tab:
  - Mark points using either the bottom right button on the Joystick keypad or the button  in Resolve3D.
  - From the Resolve3D window, open the Points List . Visit each point and scroll through Z to find the focal plane. Select the **Replace Point** button in the points list to save this Z position.
  - In the Design Experiment window, activate the Points tab. List the points you would like to use (i.e. 1-3, 5,10-14).
  - The experiment will apply all Z sectioning, Channels and Time Lapse settings to all points selected.
- Panels Tab: Define paneling area by defining opposite corners of a rectangular area. To stitch select **View > Stitch**.
- Actions Tab: Define actions (Autofocus, Pause, Wait, etc.) to be performed during a time lapse experiment.