DeltaVision™ Elite

Quick Reference #1
Startup & Basic Acquisition

System Startup

1. If system is equipped with an electronics cabinet, turn on the power strip.
2. Turn on the IC/MIC and workstation. The order in which they are turned on does not matter.
3. When prompted, log into the Linux workstation:

   Username: <worx>

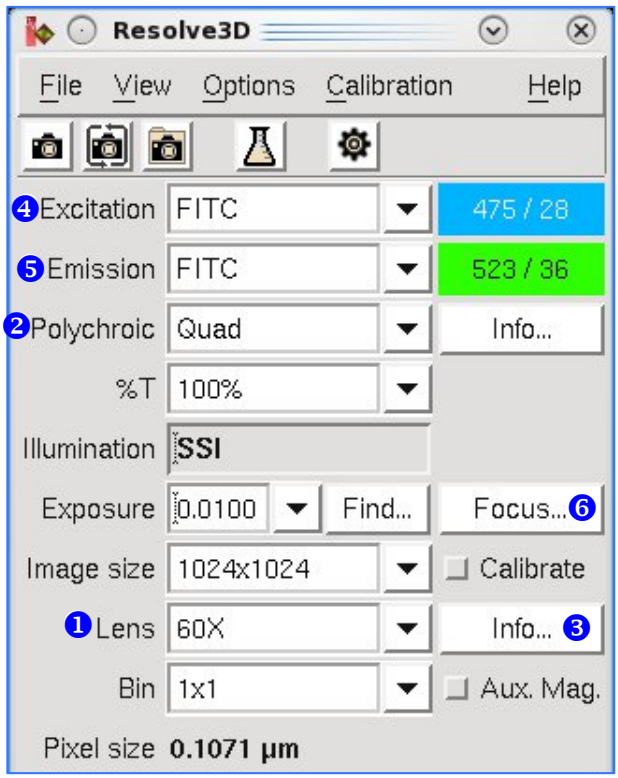
   Password: <system serial number> (i.e. cn807XX)
4. Click on the **Start softWoRx™**  icon.
5. From the main softWoRx menu, click on the microscope  icon or **File | Acquire (Resolve3D)** to initialize the system.

Using the Oil Calculator:

1. Click the **Info...** ❸ button next to the **Lens** drop-down menu.
2. Enter information in the **Optical Conditions** section:
   - **Distance from Coverslip to Specimen** – Enter a value in um equal to half the thickness of your cell type. Can also be thought of as the point within the sample where you would like to focus.
   - **Temperature** – Enter room/chamber temperature in °C.
   - **Specimen Refractive Index** – Use drop-down menu to select the medium that is closest to what the sample is mounted in. Live cells in media/PBS should use the Water entry.
   - **Coverslip Thickness** – Enter the coverslip thickness in um. A #1.5 coverslip (recommended) is 170µm and a #1 is 150µm.
3. Note the **Recommended RI** and choose the appropriate oil.

Mount Sample

1. Rotate objective turret until appropriate objective is in the active position. Update selection in the **Lens** ❶ drop-down menu in Resolve3D.
2. Select the appropriate dichroic for the experiment:
   - If system has a motorized turret, use the **Polychroic** ❷ drop-down menu.
   - If a manual turret is installed, rotate the turret until the appropriate dichroic is in place. If **Polychroic** ❷ drop-down menu is also present, update selection.
3. Click the **Info...** ❸ button and use oil calculator to select the appropriate oil for the sample. See **Using the Oil Calculator** (above) for more details.
4. Place one or two drops of oil with recommended RI on the objective or coverslip. Mount slide coverslip facing downward in the repeatable slide holder.



Focus on Sample and Find a Region of Interest

1. Turn the coarse focus knob toward you, just until the lens makes contact with the slide and the oil spreads.
2. Rotate the eyepiece filter wheel to an appropriate channel.
3. Check **Filter Monitor** window to ensure EX and EM filters match. If not, adjust **Excitation** ❹ and **Emission** ❺ filters accordingly.
4. Rotate the port selector knob (on the front of the microscope base) to the eyepiece.
5. Open the **EX Shutter** from the joystick keypad.
6. Look through the oculars and rotate the fine focus knob to focus on your sample.
7. Use the joystick to scan your slide in x and y until you find a region of interest.
8. Close the **EX Shutter** from the joystick keypad.

Tips for Live Cell Imaging:

- For samples mounted in aqueous medium (live cells), use the **Focus Assist** tool to find focus:
  - Click on the **Focus...**❻ button.
  - Follow instructions in the **Focus Assist** window. Click **Start Focus Assist**.
  - Rotate the focus knobs toward you, until the purple bar reaches a maximum position.
  - Click **Stop Focus Assist** and close **Focus Assist** window. Your sample should be in focus.
- If you must use eyepieces to focus on your cells, use DIC/Transmitted Light instead of fluorescence.
- To find rare events or dual-labeled cells:
  - Scan slide with eyepieces using DIC to mark areas that are densely populated.
  - Switch to software and do a **Spiral Mosaic** in fluorescence at each marked point to find rare events or dual-labeled cells.

Determine the Acquisition Parameters

1. Rotate the port selector knob to the camera.
2. **Acquire** an image. In the image window, select **Fit to Screen**.
3. Use the **Centering Tool** to center the specimen in the image window.
4. Find focal plane (z section with highest **Maximum Intensity** ❶) using **Z bar** ❷ or **Up and Down arrows** ❸ in Resolve3D.

> **Note:** It may be necessary to lower **Exposure** ❹ time and/or **%T** ❺ (percent transmission) if image is saturated (max. intensity of 4095 for HQ² or 32767 for sCMOS).

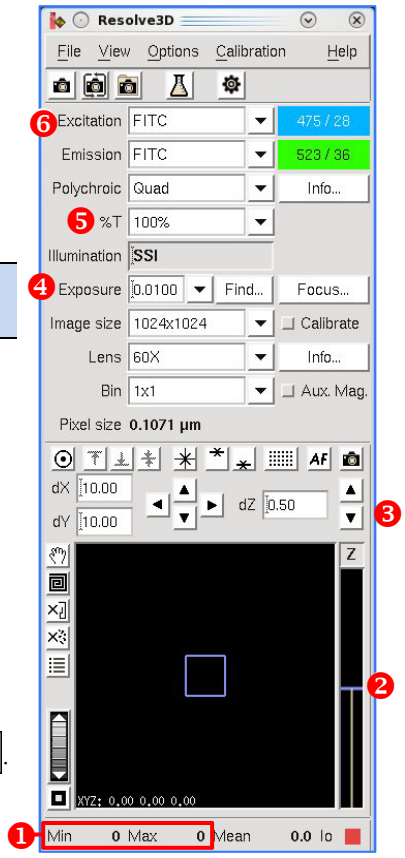
5. Adjust **Exposure** ❹ time and **%T** ❺ to target a **Max** ❶ intensity according to the guidelines in the chart below:

	sCMOS	HQ²
Fixed Cells	5000-7000 counts	2000-2500 counts
Live Cells	5-7x background	3-5x background

6. Switch **Excitation** ❻ to the next channel and find appropriate **Exposure** ❹ time and **%T** ❺. Repeat for all channels that will be utilized in your experiment.
7. Use the **Z bar** ❷ to scroll up until the top of the sample is reached. **Mark the top**.

> **Note:** Scroll up until the structures of interest are just slightly out of focus.

8. Use **Z bar** ❷ to scroll down until bottom of the sample is reached. **Mark the bottom**.
9. Return to the **middle of the sample**.



Design and Run the Experiment

1. Select the **Experiment** button in the Resolve3D window.
2. In the **Sectioning** ❶ tab, select the **Get Thickness** ❷ button. This will load the Z thickness set previously. Verify that you have elected to start from the **Middle of the Sample** ❸.
3. In the **Channels** ❹ tab, activate the number of channels ❺ to be used in your experiment. Load the channels using the **EX Filter** ❻ drop-down menu.
4. If required, select the appropriate **Polychroic** ❼.
5. Click the **Save** icon to save the experiment macro as the default filename (Resolve3D.exp).

> **Note:** Macro information is written to the image log file so unique macro names are not required.

6. Click on the **Run** ❽ tab.
7. Select the **Settings...** ❾ button to create a folder or change the directory where your image file will be saved.

> **Note:** Image files should always be saved within the /data1 directory. Use subfolders within that directory to organize files.

8. Enter an **Image File Name** ❿. Do NOT use spaces or special characters in the file name.
9. Select the **Play** button.