DeltaVision™ Elite

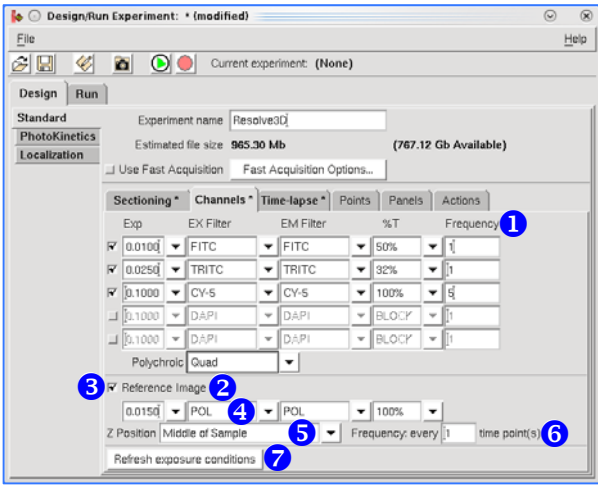
Quick Reference #2
Advanced Acquisition Options

The DeltaVision **Experiment Designer** is a very versatile tool that can accommodate most experiment types a researcher may need to run. The sections below detail some of the less commonly used options in this window.

Sectioning Tab

- **OAI (Optical Axis Integration) Scanning** – Collects a 2D projection of the 3D data contained within the z thickness specified. Ideal for relatively thin and/or light-sensitive biology with distinct signal and low background where z information is important, but z resolution may not be (i.e. yeast, bacteria).
  - In the **Sectioning** tab, define **Sample Thickness** and select the check box next to **Enable OAI Scan**.
  - During acquisition, the system will open the excitation shutter and scan the stage through z, effectively creating a 2D sum projection of the 3D data contained in the z thickness specified.
  - To deconvolve OAI images, select the **Deconvolve Projections** box in the Deconvolution tool.

Channels Tab



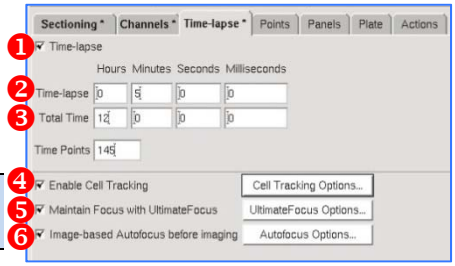
- **Frequency** ❶ **–** Allows a user to collect certain channels at only a subset of defined time points.
- **Reference Image** ❷ **–** Takes a single image in the specified channel and writes that data to a separate file. Often used for DIC or Transmitted light images during time-lapse experiments.
  1. Select the **Reference Image** ❸ check box, load the channel in by the **EX Filter** ❹ and select the **Z Position** ❺.
  2. If DIC is to be used, select **POL** for the **EX** and **EM Filters**.

     Note: Verify that all DIC optics (polarizer and prism in condenser and prism slider under the stage) are in the light path. Use knob on prism slider to adjust image contrast.
  3. To collect a reference image at a subset of time points, enter the interval in the **Frequency every** ❻ field.
- **Refresh Exposure Conditions** ❼ **–** Load in the most recent exposure and %T information for all active channels.

Time-lapse Tab

- Activate the **Time-lapse** ❶ tab and enter the **Time-lapse** ❷ (time between points) and **Total Time** ❸ for the experiment.
- **Enable Cell Tracking** ❹ if observing an isolated cell that is expected to migrate in x and y. Tracking will move the stage to compensate for cellular movement.
- Focus Maintenance options:
  - **UltimateFocus** ❺ will compensate for systematic focus drift, i.e. temperature.
  - **Image-based Autofocus** ❻ can help to compensate for changes in the sample, i.e. dividing cells where cell thickness changes.

Note: If both autofocus options are selected, **UltimateFocus** corrections will be performed first, followed by **Image-based Autofocus**.



Point Visiting Tab

Find and refine points:

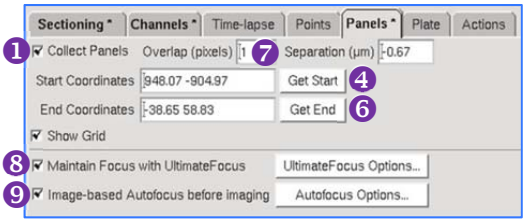
1. Mark points using either the bottom right button on the joystick keypad or the **Mark Point** button in Resolve3D.
2. From the Resolve3D window, open the **Points List**.
3. Without adjusting the microscope focus knob, visit each point using the camera and refine x/y position and focus.
4. Click the **Replace Point** button in the points list to save the updated position.

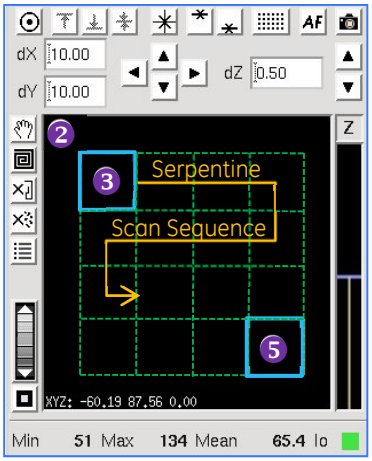
Set up an experiment to visit points:

- Activate the **Points** tab, list the points you would like to use in your experiment (i.e. all or 1-3, 5,10-14).
- The experiment will apply all **Sectioning**, **Channels** and **Time-lapse** settings to all points selected.
- **UltimateFocus** and **Image-based Autofocus** options can be defined.

Panels Tab

A **Panels** experiment collects a series of overlapping images that can be stitched together to form a large FOV. To collect **Panels**:





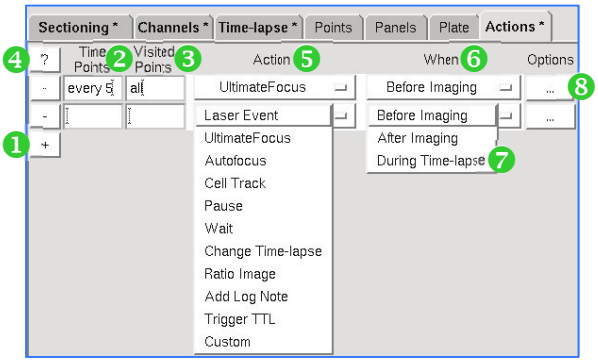
1. Click the **Collect Panels** ❶ check box.
2. In the **Resolve3D Trails** ❷ window, move the stage to the top left-hand corner ❸ of the area to be imaged ❸. Click **Get Start** ❹.
3. Move stage to the bottom right-hand corner ❺, click **Get End** ❻.
4. If panels will be deconvolved, set **Overlap** ❼ to at least twice the border roll-off value.

Note: Border roll-off can be found in **More Options** menu of Deconvolution tool.

5. Activate either **UltimateFocus** ❽ (only for samples in aqueous medium) and/or **Image-based Autofocus** ❾ if desired. Autofocus events will be performed prior to each panel to account for focus variations across the panel area.
6. Before starting experiment, return to first panel position and refine focus.

Actions Tab

During a **Time-lapse** experiment, the user has the flexibility of adding **Actions** to customize the experiment. Actions can be applied at every time point and visited point or at a subset of each/both. To use **Actions**:



1. Set up **Sectioning**, **Channels**, **Time-lapse** and/or **Points** tabs.
2. Click the **+** ❶ button to add an action.
3. Specify at which **Time Points** ❷ and/or **Visited Points** ❸ you would like the action to occur. Click the **?** ❹ button to view acceptable syntax.
4. Select the **Action** ❺ from the drop-down menu.
5. Select **When** ❻ the action should be applied. If **During Time-lapse** ❼ is selected, a pop-up window will appear to specify when during the time-lapse wait interval to apply the action.
6. If required, click on the **Options** button ❽ to define Action options. Repeat Steps 2-6 for each additional Action.

Fast Acquisition

Fast Acquisition is an imaging mode in which speed takes precedence over all other processes and should be used for dynamic experiments. Activate **Fast Acquisition** from the **Design** tab of the Experiment Designer. **Fast Acquisition Options** include:

- **Image Scan Sequence:**
  - **Wavelength then Z** – System will image each channel before moving in z. Use when channel registration is very important and/or when sample is very dynamic.
  - **Z then Wavelength** – System will image the entire z stack in a single channel before switching filters. Faster scan sequence due to reduction of filter wheel moves, but can lead to mis-registration between channels.
- **Open Shutter For Each** – Exposure should always be used except for those applications that require ultra-fast frame rates.
- **Camera Readout Mode** – Normal or Fast/Aggressive modes are recommended. Very Fast/Aggressive should not be used.

Note: Not all cameras will show noticeable frame rate differences between modes.

- **Use Conventional Time-lapse** – This check box allows for use of the **Actions** tab when **Fast Acquisition** is selected.

Run Options

- **Deconvolve during experiment** ❶ will deconvolve the last time point as the system is waiting to acquire the next time point. Use during long time-lapse experiments to utilize idle system time.
- Click the **Enable Post-Acquisition Processing** ❷ check box to automatically process all image files after acquisition is completed. Click the **Processing Tasks...** ❸ button to set up specific processing tasks. These tasks can be run immediately or at a later time/date through the Queue Manager.