

DeltaVision|OMX Acquisition

Acquisition

1. Ensure needed lasers and fiber shaker are on and system has been started according to startup instructions
2. Start omxN.bat on omxmaster if not already running
3. Activate the desired cameras by clicking on the necessary **Channels**
 - a. Camera labels will appear in the image viewing area
 - b. Change the **View Mode** to 4 cameras if necessary
4. Select **Camera Mode** for each active channel, and **EM Gain** if desired
 - a. EM = electron multiplication
 - b. Conv = no electron multiplication
 - c. 10MHz, 5MHz, or 1MHz readout speeds
5. Move stage into position to image, using **DV Points** or the stage controls
6. Mount sample onto OMX stage
 - a. If using **DV Points**, the sample must be oriented the same way it was on the pDV
7. Set the imaging conditions to desired state
 - a. Set the **Light Path**
 - i. Conventional if doing time-lapse or widefield imaging
 - ii. SI if doing structured illumination imaging
 - b. Set the **Imaging Mode**
 - i. Simultaneous if high-speed is necessary and/or crosstalk is not an issue
 - ii. Sequential if crosstalk is an issue and/or high-speed is not necessary
 - c. Set the **Image Size** to control the portion of the CCD chip to collect data from
 - i. 512x512 is the maximum chip size for the EMCCD cameras
 - d. Set the **Bin** size
 - i. 1x1 for any SI imaging
 - ii. Higher values for lower signal or faster imaging of the full field of view
8. Focus on sample using the stage controls and **Z touchdown** if applicable
9. Adjust the field of view using the stage controls if necessary
10. Adjust **Exposure** time and **%T** settings
 - a. Minimum, maximum, and mean intensity values are displayed at the bottom of every image area
 - b. If in Simultaneous **Imaging Mode**, the **Exposure** time for all channels must be the same
 - i. Use the **%T** settings to control relative signal levels, or the **EM Gain** (if applicable)
11. Set the camera background(s) if necessary
 - a. Open the **Dark Image** tool (File | Dark Image)
 - b. **Acquire** a dark image

- c. Look at the **Min/Max/Mean** info at the bottom of the image areas
 - i. Mean values should be around 50-100 counts
 - d. If background value(s) need to be changed, open the **Settings** information (File | Settings)
 - e. Adjust the background values for the current **Camera Mode** and cameras and **Save Settings**
 - f. **Acquire** another dark image to test the new settings
 - g. When acceptable background levels have been found **Close** the **Dark Image** tool and **Close** the **Settings** information
12. Determine the thickness of scan to perform
- a. Use the z-slider to move to the top of the image stack
 - b. Mark this position by clicking the Mark Top button
 - c. Use the z-slider to move to the bottom of the image stack
 - d. Mark this position by clicking on the Mark Bottom button
 - e. The **Sample thickness** is displayed
 - i. Stack height is limited to 30um (range of the z-piezo)
13. Move to the **Experiment** tab
- a. Select **Conv** to run a non-structured illumination scan, or a time-lapse experiment
 - i. Ensure the **Light Path** is set to Conventional
 - ii. Ensure the current position in z (z-slider) matches the **Focus point when scan starts**
 - iii. Set the **Optical section spacing** to desired thickness
 - iv. Set sample thickness by
 1. Typing in the **Number of optical sections**
or
 2. Typing in the **Sample thickness**
or
 3. Clicking the **Get Thickness** button if the top and bottom were previously marked
 - v. Set the **Time-lapse** parameters if desired
 - vi. Select a **Flatfield file** if flatfield correction is desired
 - b. Select **SI** to run a structured illumination experiment
 - i. Ensure the **Light Path** is set to SI
 - ii. Ensure the current position in z (z-slider) matches the **Focus point when scan starts**
 - iii. Set the **Optical section spacing** to desired thickness
 - iv. Set sample thickness by
 1. Typing in the **Number of optical sections**
or
 2. Typing in the **Sample thickness**
or
 3. Clicking the **Get Thickness** button if the top and bottom were previously marked
 - c. Update the **Filename** and **Path** if desired
 - d. Click on the **Run** button

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- e. The experiment progress will be shown and the stage controls will re-activate when the experiment is done