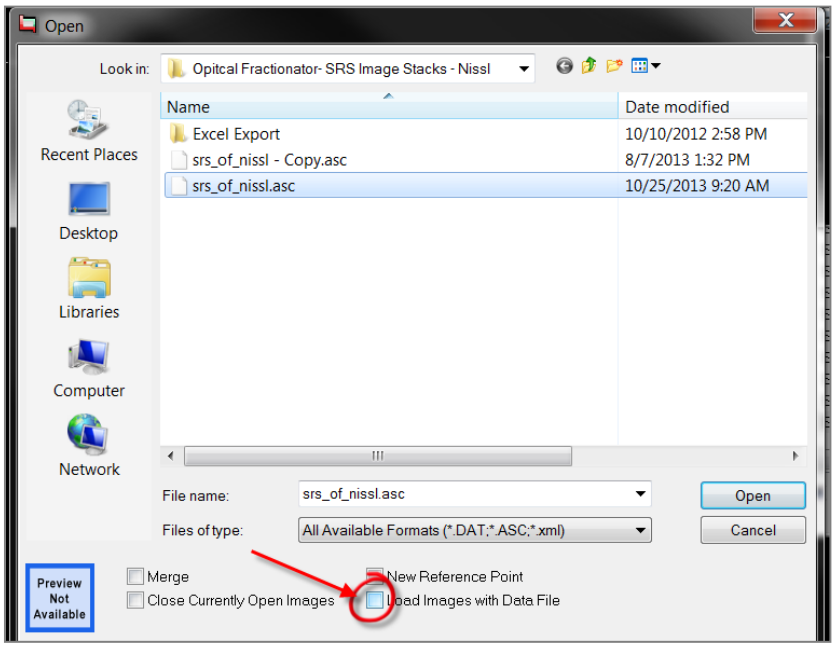
Use the **Optical Fractionator** workflow to perform counting for stacks acquired with the **Acquire SRS Image Stack** workflow.

1. Select **Probes**>**Optical Fractionator Workflow**. The *Optical Fractionator Workflow* dialog box appears.
2. Select **Load subject data from existing file** and click **Browse** to locate the data file.



3. The *Open* dialog box is displayed. Select the desired data file, uncheck **Load Image with Data File** (recommended for faster performance), and click **Open**.
4. Click **OK** to close the *Optical Fractionator Workflow* dialog box. The workflow panel is displayed on the left.
5. Follow the steps in the workflow.

USING THE WORKFLOW

1. Set up the subject

- **Subject Information**: Type your name, the subject of the study, and any notes pertaining to this study or animal.
- **Use Saved Sampling Parameters**: Select **No**.
- **Enter Serial Section information**: The **Section's Cut Thickness** used in the **SRS** workflow is displayed.
  - ☐ To modify the thickness, click the **Edit Serial Sections** button.

2. Measure Mounted Thickness

- **Measure the mounted thickness while counting** is recommended at every sampling site (or at least an evaluation interval) to ensure an unbiased estimation.
- If you select **Manually enter the average mounted thickness**, enter the **Average Mounted Thickness** under **Manual Adjustment**.
- If you select **Measure mounted thickness before counting**, click the **Start Taking Measurements** button below to measure the section thickness at several sites prior to counting.

> If you only collected images for the disector height, enter the section thickness after shrinkage manually.
>
> Keep in mind that, if you enter an incorrect thickness value, you are introducing sampling error and bias to the estimation.

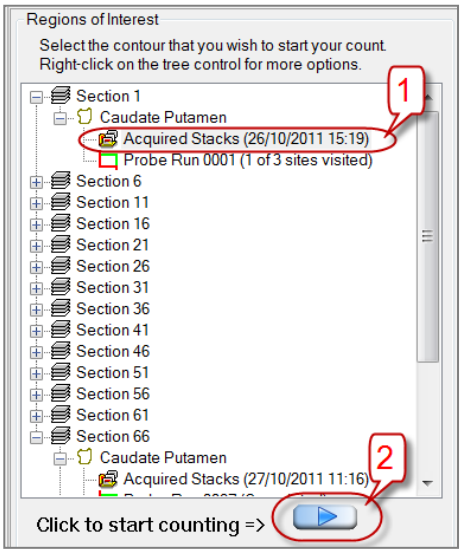
3. Define Disector Options

- If the **Probe Height** and **Guard Zones** were previously defined in the **Acquire SRS Image Stack** workflow, the correct values for the **Top Guard Zone Height** and **Optical Disector Height** are displayed.
- If you didn't enter the **Top Guard Zone Height** and the **Optical Disector Height** values during the **SRS Image Stack** acquisition, enter them now.
- Under **Focus Method**, select **Manual Focus**.

4. Count Objects

a. Under **Regions of Interest**, expand **Section 1** by clicking the **+** sign, expand the region of interest by clicking the **+** sign, and highlight **Acquired Stacks**.

b. Click the **Play** button to start counting. The stack is loaded and you are directed to the first sampling site.



> If you changed the sampling parameters between the **Acquire SRS Image Stack** workflow and **Optical Fractionator workflow**, you will see the *Choose Probe Configuration* dialog box.
>
> Select **Use Current Settings** and click **OK**.

c. The *Focus Top of Section* dialog box is displayed. Using the **PageUp** key, focus to the top of the stack, then click **OK** in the dialog box.

d. If applicable, the **Focus Bottom of Section** dialog box is displayed. Using the **PageDown** key, focus to the bottom of the stack, then click **OK**.

e. Select a marker from the **Use Marker** drop-down menu in the workflow panel, or from the marker toolbar.

f. Count the cells following the counting rules.

g. Click the fast forward button to go to the next site.

h. Repeat step 4.a–4.g for all the sections.

a. Click the **I'm Finished Counting** button when done.

i. Click the **Next Step** button to move to Step 5.

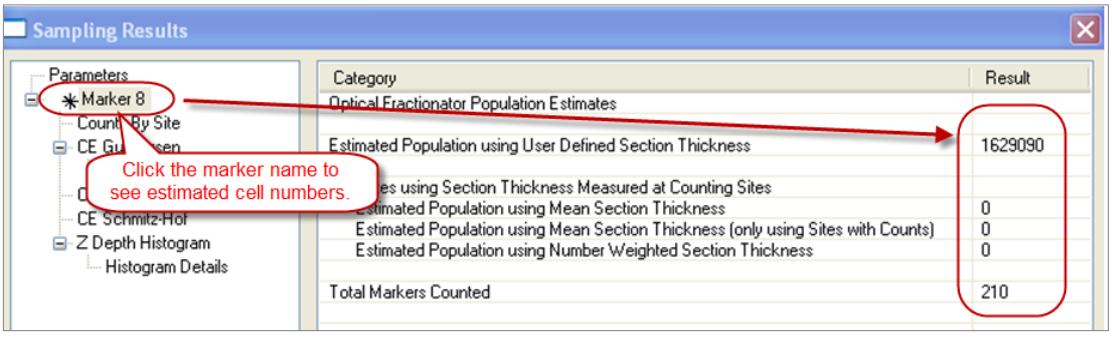
5. View Sampling Results

a. Click the **Display Probe Run List** button.

b. The *Previous Stereological Runs* window is displayed. Select all relevant sections and click the **View Results** button.

c. The *Sampling Results* window is displayed.

- **Parameters** for your study are displayed on the right.
- To see the estimated total cell numbers, highlight the marker name on the left—Do not be alarmed if some of the results are 0.



☐ **Estimated Population using User Defined Section Thickness**—The estimate is calculated using the manual thickness entered prior to counting the cells.

- This is the LEAST accurate estimated value unless you are 100% sure of the thickness of all of your sections.
- Equals zero if you didn't enter the thickness manually.

- ☐ **Estimated Population using Mean Section Thickness**—The estimate is calculated based on the average thickness of the tissue. We refer to the section thickness that you measured within the workflow at every site, whether cells were counted or not.
- ☐ **Estimated Population using Mean Section Thickness (only using sites with counts)**—The estimate is calculated based on the average thickness of the tissue. We refer to the section thickness that you measured within the workflow only for the sites where you marked cells.
- ☐ **Estimated Population using Number Weighted Section Thickness**—The estimate takes into account wavy tissue.
  - Use this number if the section thickness varies between sampling sites.

> To change the section thickness, click the **Edit Mounted Thickness** button in the *Sampling Results* window and enter the correct thickness after shrinkage.

- To display the **CE** results, click their names on the left.
- To view **planimetry** results, click **Planimetry** on the left. The results report the area and volume of the ROI. They are correct only if the contours used to define the ROI were drawn accurately.