DeltaVision™ Localization Microscopy

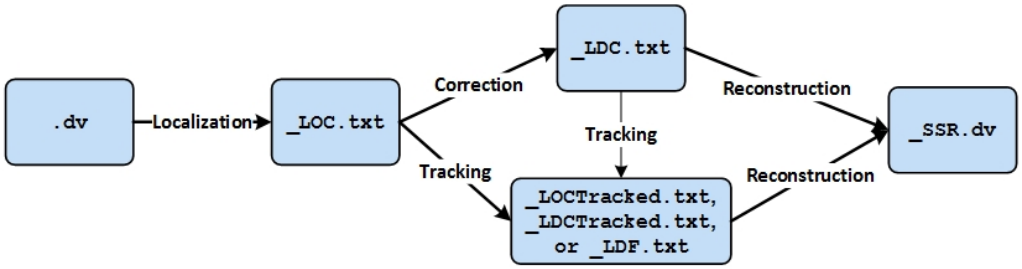
Quick Reference #2
Analysis

Localization Image Analysis Workflow

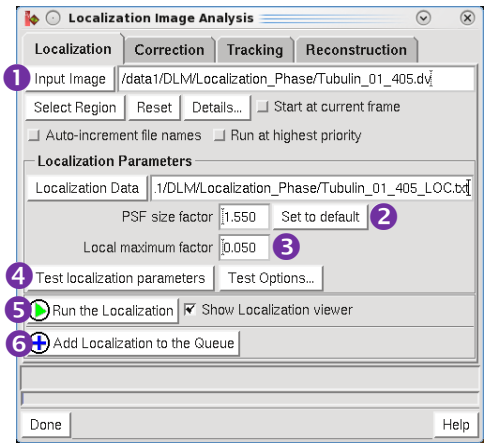
In softWoRx™, click **Measure | Localization Image Analysis** to open the **Localization Image Analysis** tool. There are multiple tabs in the **Localization Image Analysis** tool and they are designed to be used from left to right. Using this order will simplify workflow by auto-populating many fields.

- **Localization** – Identifies all blinking events in the data set.
- **Correction** – Applies an image correlation algorithm to account for drift over time.
- **Tracking** – Uses an algorithm to identify persistent fluorophores and groups their appearances together into a unique track identifier.
- **Reconstruction** – Builds an image file from the identified localizations.

A .dv image file is the input for the first **Localization** tab, but the output from the **Localization**, **Correction** and **Tracking** tabs will be text files. These text files are used as input data in the **Reconstruction** tab, where output is a reconstructed image file.



Define Localization Tab Options



1. Open a localization image file and drag the window ID number into the **Input Image** ❶ field of the **Localization** tab.

   Note: An image file (.dv) must be open to run many localization processes.

2. Next to the **PSF size factor** field, click **Set to Default** ❷.
3. Use the default value of 0.05 for the **Local maximum factor** ❸.
4. Scroll to a point in the data where individual blinking events can be detected.
5. Click **Test Localization Parameters** ❹.
6. Scroll through image data and assess the localization results. Adjust image contrast if required.
7. Adjust **PSF Size factor** ❷ and **Local maximum factor** ❸ (LMF) using the guidelines in the table below.

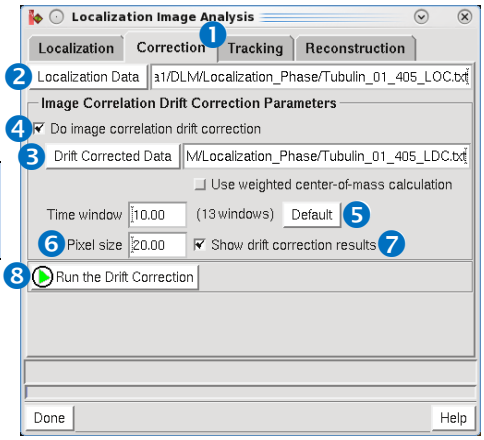
   Note: Testing at several points in the data set (beginning, middle, and end) is recommended.

8. Once parameters are optimized, run the localization in one of the following ways:
   - Click **Run the Localization** ❺ to run immediately,

   ...or
   - Click **Add Localization to the Queue** ❻ to process later.

Problem	Cause	Action
False positives	LMF too lenient	Lower LMF (i.e. if 0.05 was used, try 0.01)
Events not identified where there is real signal	LMF too strict	Raise LMF (i.e. if 0.05 was used, try 0.1)
Multiple events localized as too few peaks	PSF size factor too large	Check default PSF size factor, was data collected with non-standard configuration?
Multiple events localized as too many peaks	PSF size factor too small	Check default PSF size factor, was data collected with non-standard configuration?

Define Correction Tab Options



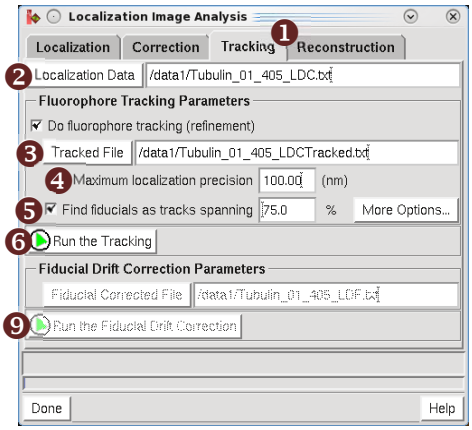
1. Once Localization is finished, click on the **Correction** ❶ tab. The **Localization Data** ❷ and **Drift Corrected Data** ❸ fields will automatically populate.
2. Select the **Do image correlation drift correction** ❹ check box.
3. Click the **Default** ❺ button next to the **Time window** field. This automatically divides the data set into 12-15 windows.
4. Set **Pixel size** ❻ to 10-20nm (20 is default).

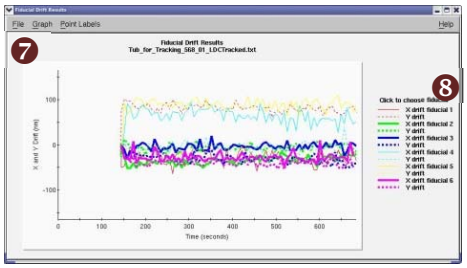
   Note: This pixel size does *not* correlate to the resolution of the drift correction. Using pixel sizes < 10nm only increases calculation time, *without* improving image correlation drift correction results.

5. Select the **Show drift correction results** ❼ check box.
6. Click **Run the Drift Correction** ❽.
7. The output is an `LDC.txt` (localization drift corrected) file, with updated x and y coordinates for all identified localizations.

Define Tracking Tab Options

1. Click on the **Tracking** ❶ tab. The **Localization Data** ❷ and **Tracked File** ❸ fields will automatically populate.
2. Use the default value of 100 for the **Maximum localization precision** ❹ field.
3. If fiducial markers are present, select the check box next to **Find fiducials as tracks spanning** ❺. Adjust percentage or use default value (75%).
4. Click **Run the Tracking** ❻.
   - If fiducial markers are not present, proceed to the **Create a Reconstructed Image** section below.
   - If fiducial markers are present, complete Steps 5 - 7 below.
5. In the **Fiducial Drift Results** ❼ window, select the tracks to use for fiducial drift correction by clicking on the bold line in the legend ❽ for each fiducial you want to use.
6. Close the **Fiducial Drift Results** window.
7. Click **Run the Fiducial Drift Correction**❾.

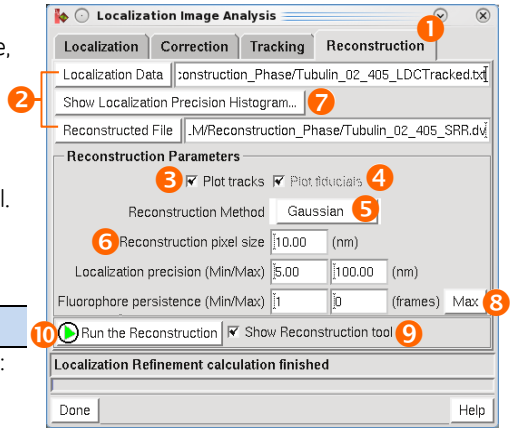


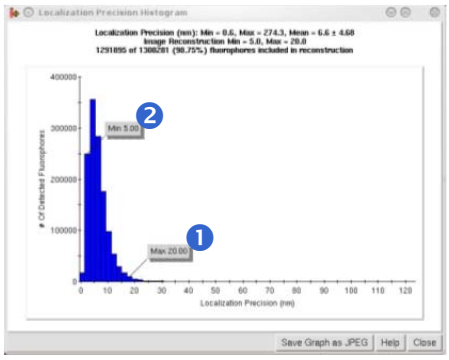


Create a Reconstructed Image

1. Click on the **Reconstruction** ❶ tab. The **Localization Data** ❷ and **Reconstructed File** ❷ fields will automatically populate.
2. Select **Plot Tracks** ❸.
3. If fiducial markers are present and you would like to visualize them in the reconstructed image, select **Plot Fiducials** ❹.
4. Select a **Reconstruction Method** ❺
   - **Gaussian** – each event is plotted as a 2D Gaussian with width determined by its localization precision.
   - **Histogram** – each event, regardless of its localization precision, is plotted as a single pixel.
5. Input an appropriate **Reconstruction pixel size** ❻
   - use 10nm for 256x256 or larger image sizes
   - use 5-10nm for 128x128 or smaller image sizes

   Note: This pixel size does *not* correlate to the resolution of the structures in the final image.
6. Examine Localization Precision Histogram to determine Max/Min Localization Precision values:
   a) Click **Show Localization Precision Histogram** ❼ button.
   b) Drag the **Max Localization Precision** ❶ marker to the left. All events with a localization precision larger than the selected max will be excluded as outliers.
   c) Drag the **Min Localization Precision** ❷ marker to the right. All events with a measured localization precision smaller than the selected minimum will be plotted with the minimum localization precision.
7. In the **Reconstruction** ❶tab, use the minimum **Fluorophore persistence** value of 1. Click the **Max** ❽ button to set the **Fluorophore persistence** max to the maximum track length in the data set.
8. Select **Show Reconstruction tool** ❾ check box to display the Localization Image Reconstruction window after reconstruction is complete. This tool allows you to zoom in on an ROI and create a new reconstruction with different parameters (i.e. smaller pixel size).
9. Click **Run the Reconstruction** ❿.





Creating ROI images with Localization Image Reconstruction Tool

1. After reconstruction, the **Localization Image Reconstruction** window will open. **Input** and **Localization Data** fields will automatically populate.
   ...or
   Click **Measure | Localization Image Reconstruction** to open the **Localization Image Reconstruction** tool. Drag the window ID number of an open `_SSR.dv` file into the **Input** field of the **Localization Image Reconstruction** window and the corresponding localization file (`LOC`, `LDC`, `LOCTracked`, `LDCTracked`, or `LDF` text file) into the **Localization Data** field.
2. Use the **Select Region** tool or **Details** button to select an ROI.
3. Click the **Show Localization Precision Histogram** button. Use the min/max markers to set min/max localization precision for reconstruction.
4. Select either **Gaussian** or **Histogram** for the **Reconstruction Method**.
5. Input an appropriate pixel size for the **Reconstruction method** to be used.
6. Click **Do It**.

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