7.1 Guide

super-resolution correlator usage guide

Open and display data

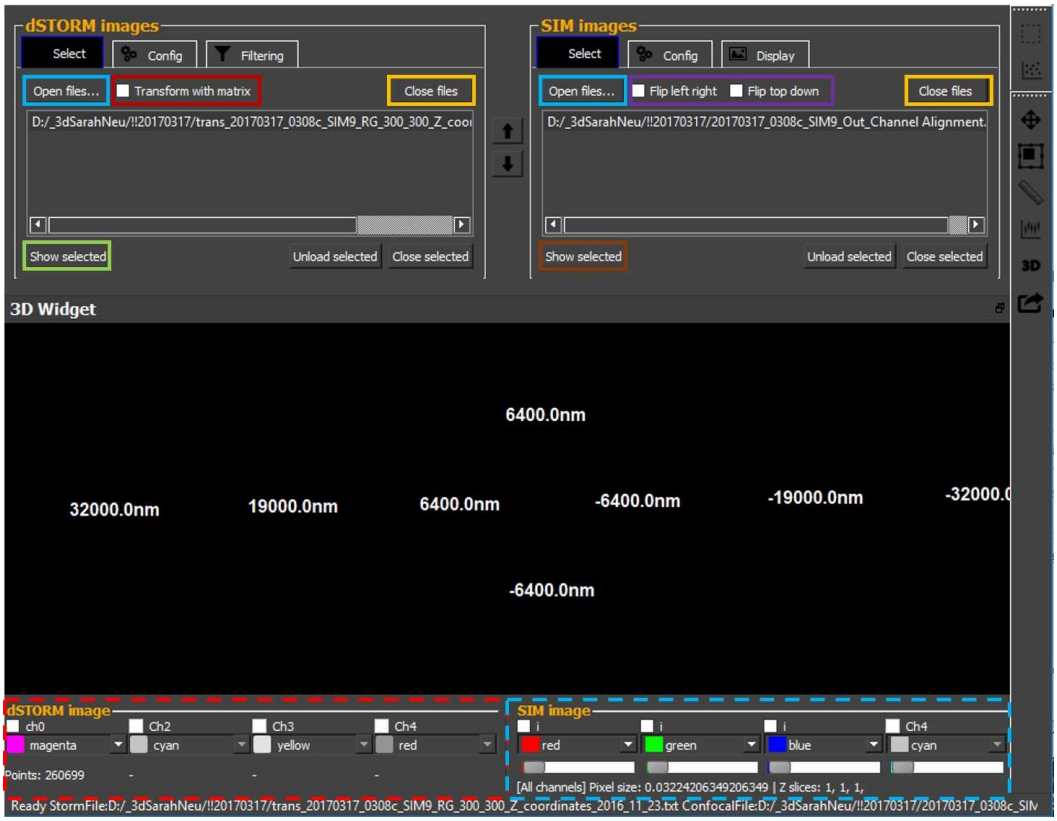
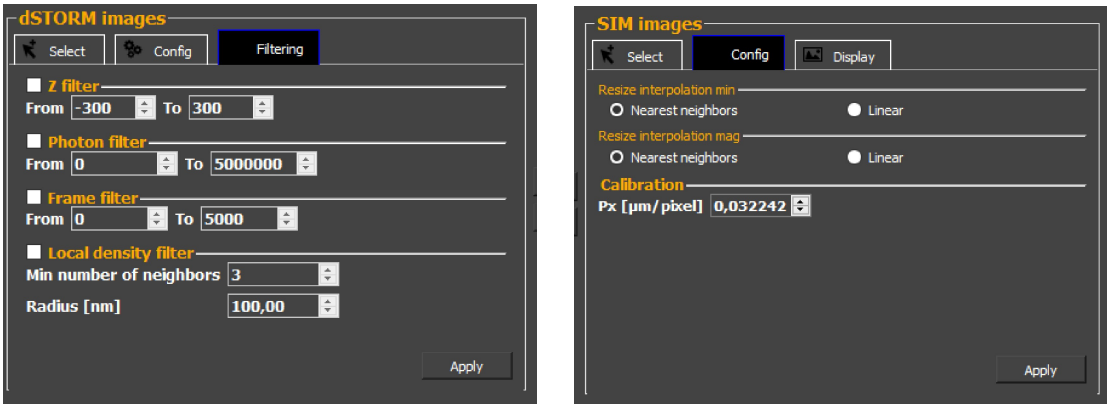


Abbildung 19: **Display data**

The 'Open files...' (blue rectangle) button in the dSTORM group box (top left) opens a standard file dialog to select the desired dSTORM text file. If the check box 'Transform with matrix' (red rectangle) is checked, another file dialog opens after dSTORM data selecting. A bUnwarpJ raw matrix can be selected to transform the dSTORM coordinate points. The 'close files' button (yellow rectangle) unloads all files. The 'Show selected' button (green rectangle) loads the data into memory. The avaible channels and meta data are now displayed in the group box dSTORM image (red dashed rectangle). By checking the check box of the corresponding channel data is displayed in the 3D Widget (mid). Opening a SIM image works in a similar way. The SIM group box (top right) additionally contains two check boxes for image flipping. By checking them and subsequently reloading the data with the button show selected, the image in the 3D Widget ist flipped. Channel selection and Meta data can be found in the 'SIM image' group box (blue dashed rectangle). The sliders under the corresponding channel allow a slice selection (in 2D mode only).

Filter and render options



(a) (b)

Abbildung 20: **Data settings**

There are various filter options for dSTORM data which can be found in the 'Filtering' tab of the dSTORM group box. To use a filter check the corresponding check box, enter the limits and press the 'Apply' button. Z-filter, Photonfilter and Frame filter select localizations in the defined ranges. The 'Local density filter' checks each localization for neighbouring localizations in a certain radius. In the displayed settings for example every localization having less than 3 neighbours within 100 nm is excluded from the visualization. The SIM configuration, which can be found in the SIM group box allows the manual setting of a pixel size (should happen automatically) and a choice of interpolation for zooming in and out.

Scalebar

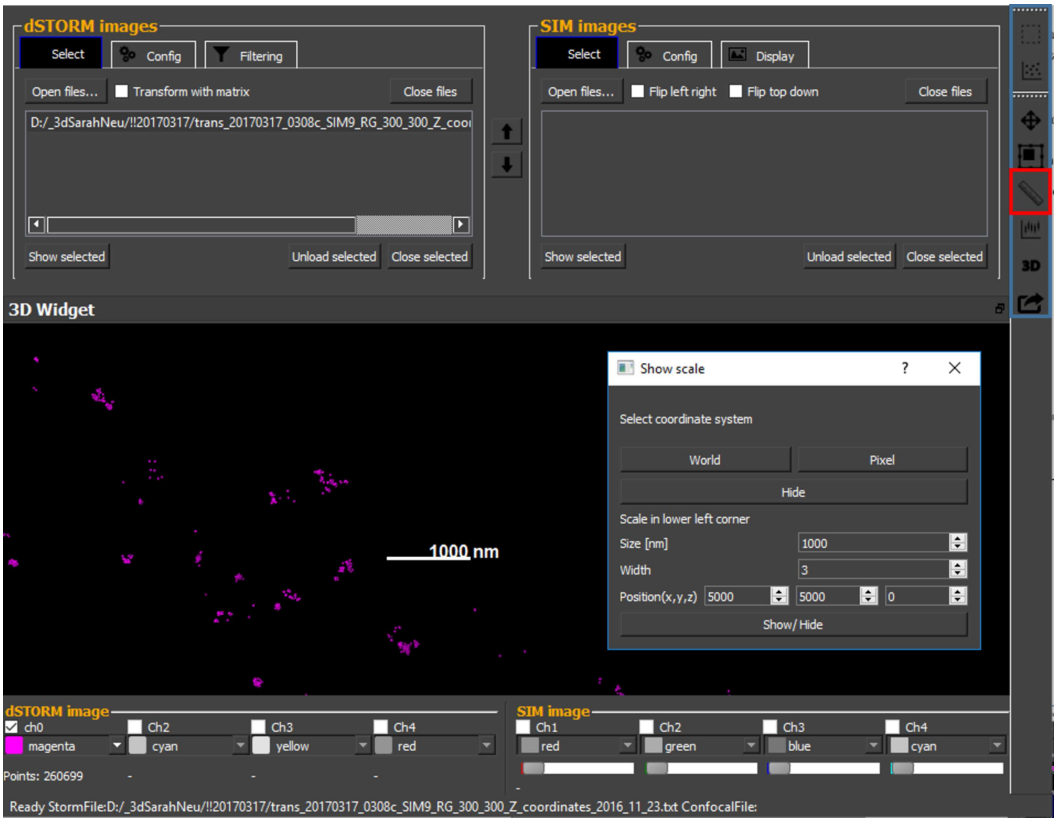


Abbildung 21: **Scalebar options**

Different scales can be rendered in the image by opening the 'scale dialog' by clicking on the scale symbol (red rectangle) in the toolbox (blue rectangle). The buttons 'World' or 'Pixel' (only if SIM image is opened) render a coordinate system displaying the corresponding scales. Additionally a custom scalebar can be rendered, by entering size width and position and subsequently clicking the 'Show/Hide' button

Look Up Table

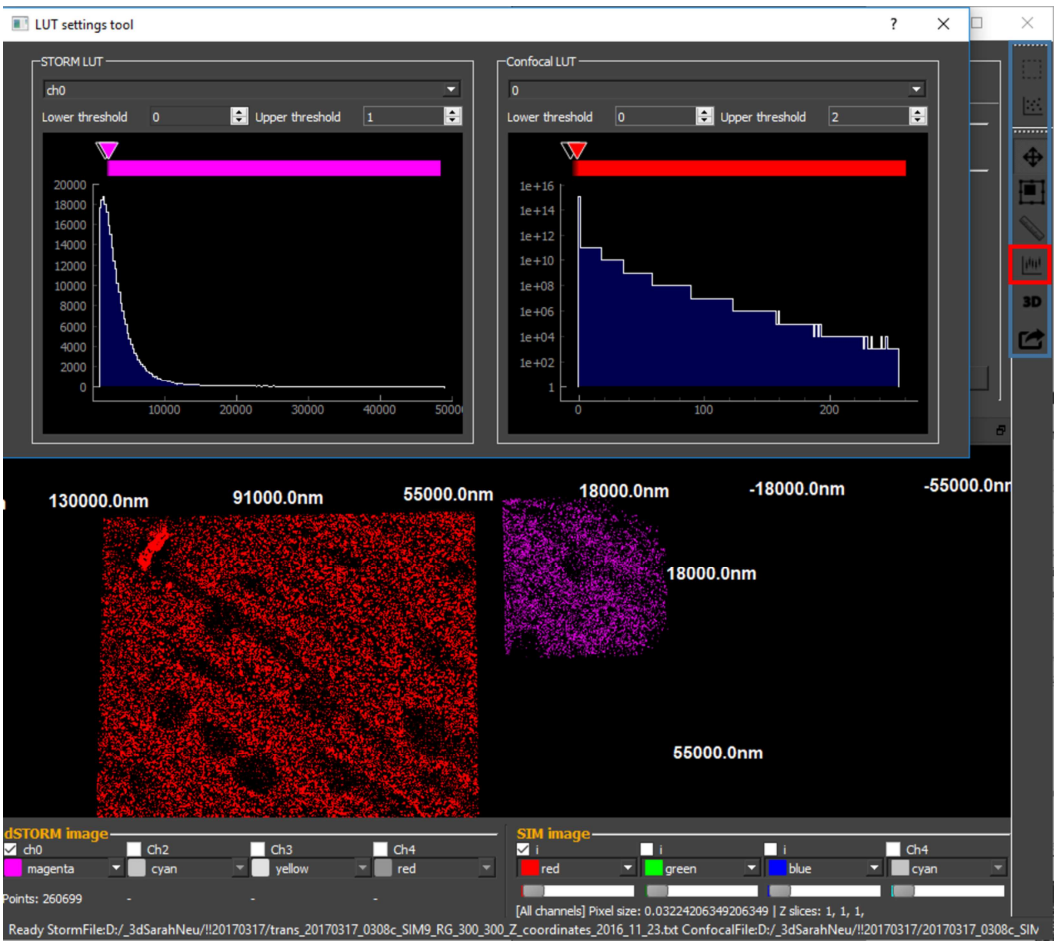


Abbildung 22: **Change intensity via LUT**

The intensity of the displayed data can be changed by editing the lower and upper threshold in the 'LUT settings tool' dialog (red rectangle), which can be found in the tool box. dSTORM data points are rendered as spheres with different alpha values, depending on their emission strength. Every data point with emission strength higher than the upper threshold is displayed in full intensity. Every data point under the lower threshold isn't displayed at all. Intensity between the thresholds decays exponentially. SIM LUT works similar using pixel intensity instead of emission strength.

Rendering options

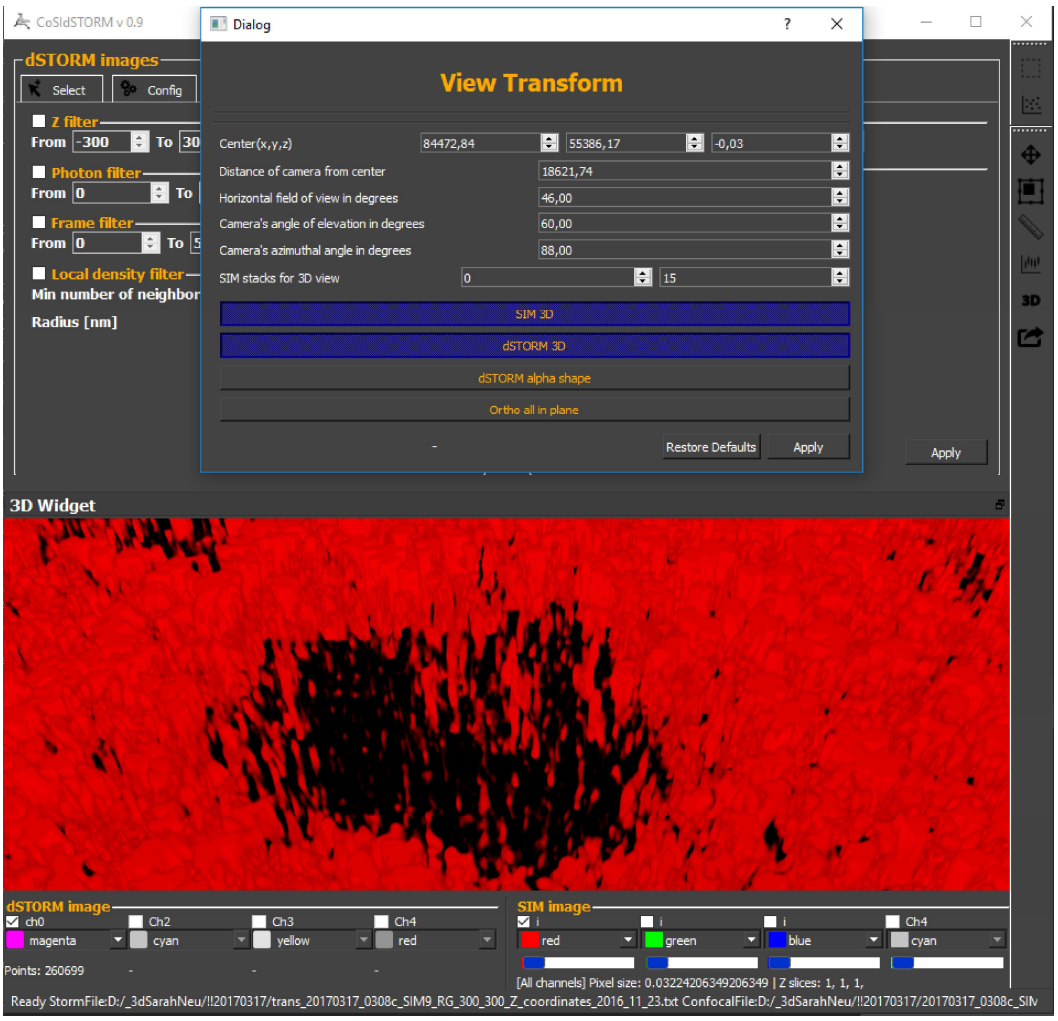


Abbildung 23: **Switch easily between 2D and 3D and adjust the view manually**

The 'View Transform' dialog can be opened by clicking the 3D symbol in the tool box. The center of the coordinate system can be adjusted as well as camera distance from plane, horizontal field of view, angle of elevation and azimuthal angle. The push buttons 'SIM3D' and 'dSTORM3D' allow switching between 2D and 3D visualization. In the fields 'SIM stacks for 3D view' the SIM slices for 3D rendering can be defined. The button dSTORM alpha shape renders an alpha shape with alpha 130 of the underlying data points. The button 'Ortho in plane' sets the view orthogonal and display to 2D. Clicking the 'Apply' button updates the view.

Alignment

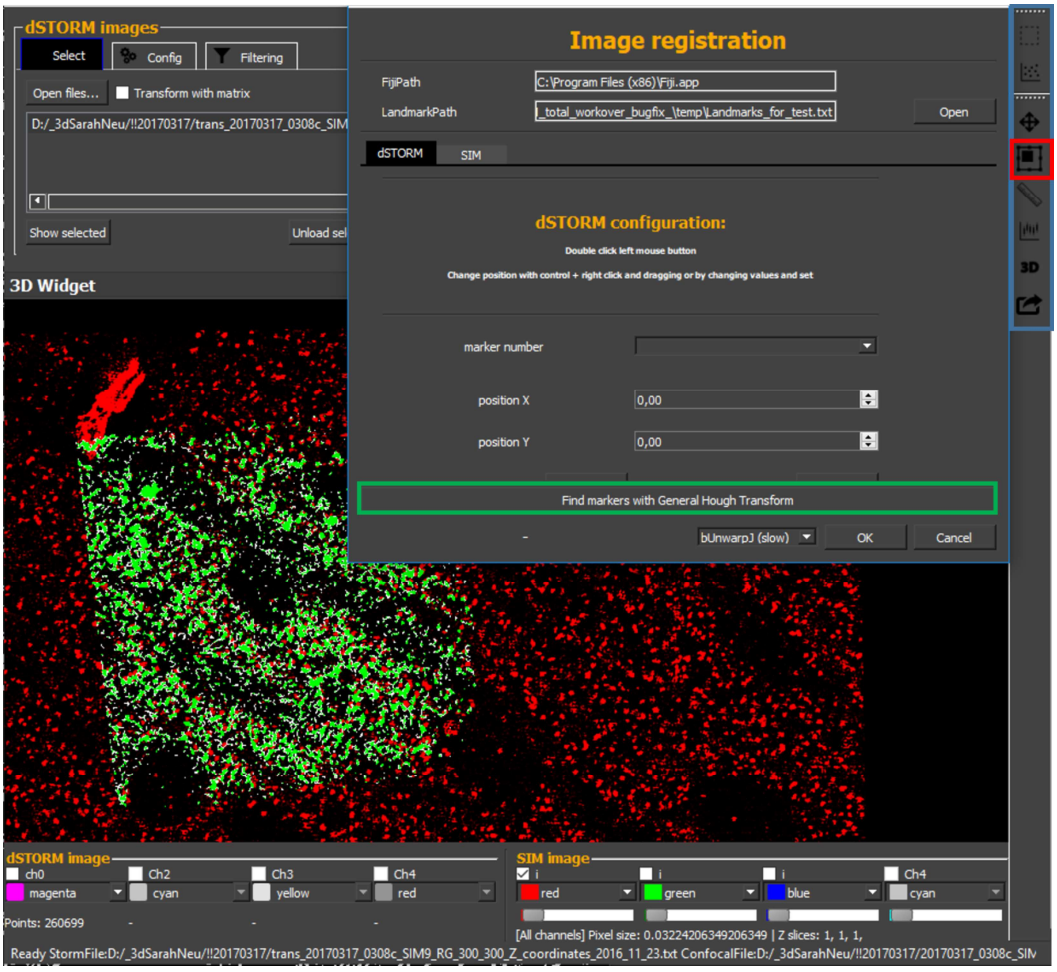


Abbildung 24: **Automated alignment via General Hough Transform**

The 'Image registration' dialog can be opened by clicking the registration symbol (red rectangle) in the tool box. The easiest way to align is, to press the button "Find markers with General Hough Transform". It requires, that the alpha shape for dSTORM data was already created and suitable LUT settings of the SIM image. I.e. the underlying structure should be clearly visible. (Image for reference). After successful computation dSTORM data points are aligned. The offset has to be adjusted manually. To display the corresponding alpha shape it has to be recreated by double clicking the 'alpha shape' button in the 'View Transform' dialog. Should the automated alignment fail, you can try to change settings in the dSTORM local density filter or the SIM LUT. If you want to align data manually markers can be set in dSTORM space (double click left) and corresponding in SIM space (double click right). It can be chosen between Affine transformation and elastic transformation with bUnwarpJ (not much difference for correlation).

Roi and correlation

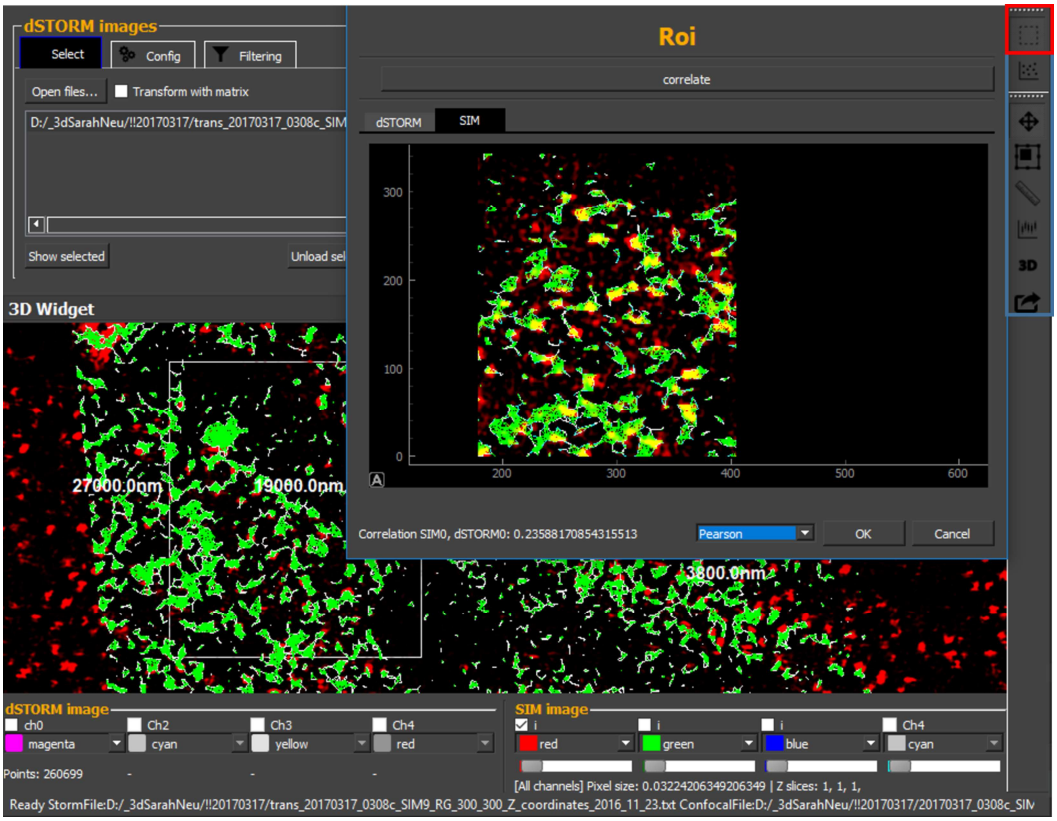


Abbildung 25: **Correlating regions of interest**

The 'Roi' dialog can be opened by clicking the ROI symbol (red rectangle) in the tool box. By dragging a rectangle in the main window a ROI can be selected. The correlation index of the chosen structure appears automatically, at the bottom left of the dialog. The dSTORM tab shows the pure alpha shape. The SIM tab shows an overlay of both. By clicking cancel the ROI mode is closed.