

Displacement field calculation

This step calculates the displacement field of every detected bead with regard to the reference frame for all frames of the movie. Note this is the longest step of the TFM package as it can easily take ~1-2 days to perform on a single desktop. Intermediate results can be recovered, i.e. when re-running this process on partially analyzed data, the software will ask if previously calculated displacement field should be re-used.

1. Set up the Displacement Field Calculation process by clicking on its **Setting** button.
2. Select the channel containing the beads only.
3. Select the reference frame containing the beads. *If the optional stage drift correction has been performed, the button should be disabled and the software will use the registered reference frame from Step 1 instead.*
4. Adjust the alpha value for detecting beads if necessary.
5. Check High-resolution subsampling of beads if high density displacement field quantification is desired.
6. Check Add non-loc-max points if further addition of displacement vectors is preferred. This will randomly add tracking positions in-between diffraction-limited point sources.
7. Adjust the template size and maximum displacement for calculating displacement field.
8. Check “Use subpixel correlation via image interpolation” if suppression of subpixel uncertainty is desired. Checking this will increase the computation time by about 5 fold for this step.
9. Click on **Apply**.

*The calculated displacement is stored as a MAT file in a folder called “displacementField”. Clicking on **Result** will open a window showing the displacement field as a vector field overlaid onto the images.*