

## Stage Drift correction - Optional

The stage drift correction allows the user to correct for any stage drift during acquisition. It is critical to improve the quality of traction force measurement and we highly recommend it.

1. Setup the stage drift correction step by clicking on its **Settings** button.
2. Select the channels you want to correct (all of them by default). *The first channel of the list of channels to correct must be the channel containing the beads as it will be used to calculate the stage drift.*
3. Click on **Select the reference beads frame** and choose the location of the reference frame to calculate beads displacement.
4. Select a stationary region for stage drift correction by clicking on **Select Stationary region of Interest for registration**. *A pop-up window should open showing the channel and the frame chosen in the setting interface. Adjust the rectangular Region of Interest (ROI) to select a stationary region. Use the frame slider to make sure the ROI is stationary (no cell) during the movie. Select a region where enough beads (~50-200) can be detected for the calculation of the stage drift.*
5. If there is a significant drift (typically greater than 5 pixels) during the experiment, check **Perform pixelwise pereregistration using cross-correlation**.
6. Adjust the template size for calculating stage drift with sub-pixel resolution. *If the selected region of interest is truly stationary, you can use a large template size (~51 pixels) to maximize the precision of the stage drift calculation.*
7. Click on **Apply**.

*The calculated sub-pixel stage drift correction, the registered channels and the registered reference frame are stored in a folder called “stageDriftCorrection”. Clicking on **Result** will open a window showing the registered images padded by a black box. Check that the images have been accurately registered using the frame slider. Note that images are corrected at the pixel level. Sub-pixel component of the stage drift is saved in a MAT file and will be added to the displacement field in the next step.*