

Alignment/Registration Transform Creation Description:

This tool can be used to create a transform which can then be used to align images taken from two different channels/cameras. This is accomplished using reference images of the same object(s) imaged in both channels. Usually these are images of multi-spectral fluorescent beads taken in both the channels or a grid-micrometer. Images of biological objects can also be used to generate a transform, but this is not recommended as there may be other differences between the images in the two channels besides their alignment.

The transformation is generated in two steps. Both steps are optional, but at least one step must be enabled, or there's nothing to do! The first step specifies an initial transform in one of three ways:

1. For bead images with sparse beads, by detecting beads in both channels and determining their misalignment.
2. For dense bead images, or other images, by asking the user to click on the same object in both images several times.
3. An "initial guess" transform can be directly input. See options below.

The second step attempts to refine the initial transformation by minimizing the difference (RMSD) between the two images via non-linear optimization. This step is computationally intensive and may take several minutes to complete - please be patient. Additionally, it is not guaranteed to work, and depending on the quality of the initial transformation and the images, this step may fail in some cases. If this step fails for you, try a better initial transform, different images or pre-processing of the images. As a last resort, you may try using masks generated from the images rather than the images themselves.

Parameter Descriptions:

Images:

This allows you to select which images you want to transform. The base image is also the "reference" image. This is the image that the second channel will be aligned to. The input image is the image which will be aligned with the base image. That is, when the resulting transform is applied to this image, it will align with the base image.

Note: In general, it doesn't really matter which image is the base and which is the input image. What's important is to remember which is which and apply the transform appropriately.

Transformation:

Type of transformation:

This allows you to select the type of transform to use to align the images. The default is projective, which should handle most dual-camera image alignment problems. Polynomial allows higher-order distortion of the image, which can attempt to correct for other effects such as chromatic aberration but is more difficult to refine.

Initial transformation:

This allows you to select one of the three options:

1. Generate the initial transform.
2. Select a transformation from an external file as the initial guess.
3. Do not use an initial transformation. Instead, attempt the refinement directly from the input images. This requires that the misalignment between the images be fairly small (<5 pixels)

Generate initial transformation:

This allows you to select one of the methods for creating the initial transformation methods. Current methods are manual or spot detection. If the spot detection method is selected, an approximate bead radius (in pixels) must be supplied.

Load initial transformation from file:

This allows you to select and load a MAT file containing a transformation for refinement.

Refine transformation:

If this box is checked, the function will attempt to refine the initial transform. This step is highly recommended, only skip it if it is failing!