

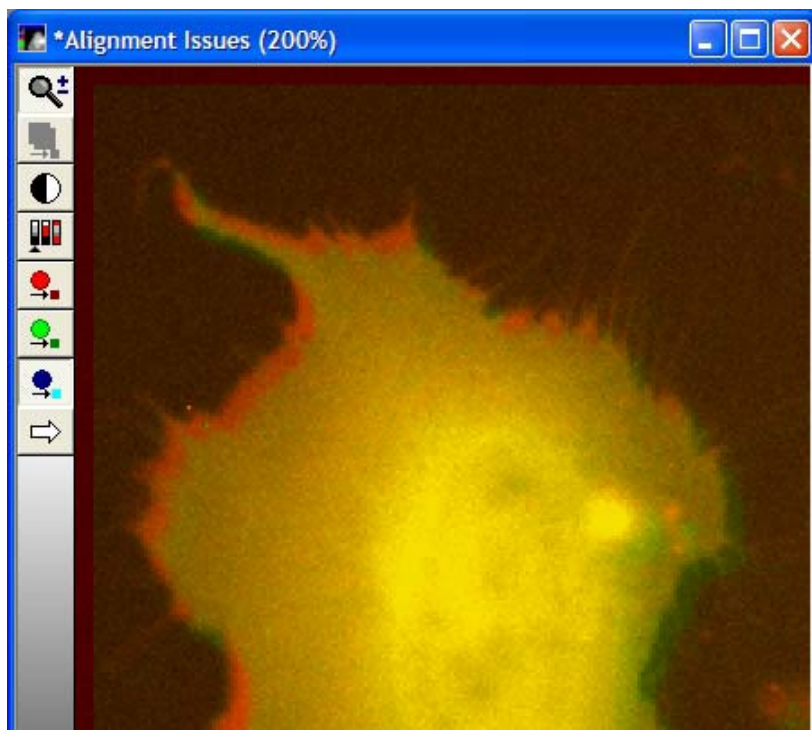
# Registration of Multi-Channel Images

## *Importance of Registering Images*

When using image processing analysis schemes which involve Ratiometric analysis, it is very important that the images are in Register with each other. That is a feature at a particular pixel location in X-Y space for the numerator (I-SO) image must correspond to the identical pixel location for the denominator (EGFP) image. Registration problems, between different fluorescent channels, can be caused by misalignment of the microscope as well as the intrinsic properties of different filters.

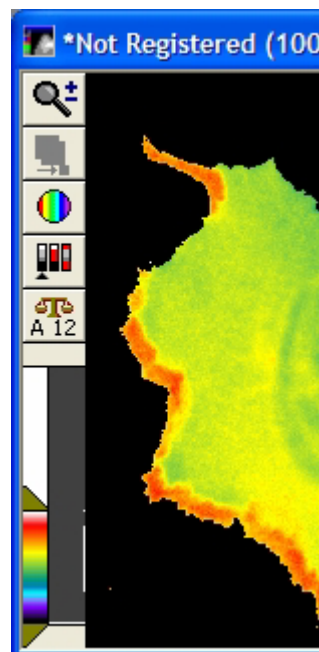
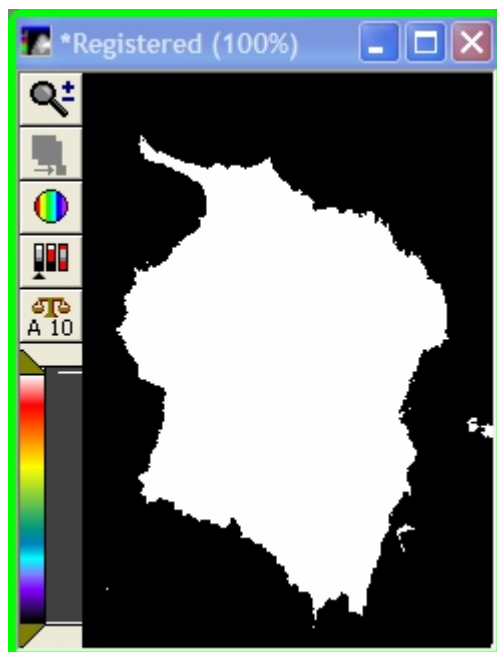
## **Considerations:**

- Standard X-Y offsets should be identical for given set of optics (magnification, fluorescent channels)
- Poor registration is more easily discernable at the cell edges
- Sub-pixel registration is more often than not necessary since Registration offsets are unlikely to be whole numbers
- Rotational corrections may be needed if image sets were collected using a two-camera system
- First correct images for uneven illumination, bad pixels (see instructions for using Metamorph Journals), and subtract backgrounds before putting image sets in Register
- Make sure you've selected the same plane number (time point) for each image stack for identifying the registration offsets



**Left: Misaligned Channels, the I-SO and EGFP Images were mapped into Red and Green Look-up-Tables and overlaid on top of each other. The Red and Green-colored edges indicate the two channels are not aligned properly. Right: In an Aligned Image the Red and Green Overlays should produce a Yellow Colored Pixel.**

**Note: Uneven distributions of the I-SO and EGFP Fluorophores will produce different distributions of Yellow, Red and Green pixels.**



**Left:** A masked I-SO image was divided by itself giving a Ratio Image having values of 1 and 0. **Right:** The Same I-SO image is divided by a copy of itself that has been moved out of Register; this example underscores the importance of Image Registration for quantitative image analysis.