Improvement of Stitching Results using a Correction for Camera to Stage Misalignment

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Microscopes with motorized stages and the capability to visualize large fields of view by sequential acquisition of smaller tiles are common. An important calibration step required for efficient acquisition of tiles for stitching is the precise alignment of the camera to the stage. Nevertheless, based on usage data*, we found that camera to stage misalignment is a routine problem. Although many systems are aligned to within 0.1 degrees of rotation between the camera and stage axes it can exceed 0.5 degrees in some microscopes. Even a 0.1 degree misalignment corresponds to a systematic shift of 3.5 pixels for every stage translation of 2000 pixels (roughly one field of view).

Hence, to correct for this systematic error, we propose integrating a global (affine) camera to stage alignment transformation which is beneficial for the overall stitching results in two ways:

- ensures improved positioning of “difficult to align” tiles (i.e. with insufficient image content),
- provides reduced long range positioning errors.

The alignment transformation can be robustly estimated from a subset of overlapping tiles that provide a reliable cross correlation. The transformation can then be applied to all tiles, even those that do not have a reliable cross correlation with neighbors, significantly improving the positioning of “difficult to align” tiles. In addition, a global alignment transformation is not prone to error propagation across regions of unreliable cross correlation, which can improve the precision of long range morphological measurements.

We observed that the correction for camera to stage axes misalignment within the stitching software greatly improves stitching results on any system with imperfect alignment without any negative impact on systems with perfect calibration. We show examples of our approach on images where some tiles are “difficult to align” and on images where error propagation across long ranges is improved.

*Thanks to users who agreed to collection of usage data