1313 ULTISTACKER™: EASY TO USE MULTIMODAL IMAGE CO-REGISTRATION WITH SUB-CELLULAR ACCURACY

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Background Ultivue InSituPlex technology (ISP) enables whole-slide multiplex immune-fluorescence (mIF) staining of multiple protein targets on an autostainer and for conducting multiple rounds of ISP imaging and a terminal or initial H&E stain of the same slide. But to accurately phenotype each cell, one must co-register or 'stack' the images of each round with sub-cellular accuracy. This can be a demanding task due to scanner artifacts (*e.g.*, field selection, stitching deformations, focus errors), staining variations and possible tissue damage during the staining process. Here we describe Ultivue's UltiStacker™, an easy-to-use Windows OS application that performs whole slide image co-registration from a variety of scanners with high accuracy and high throughput.

Methods The DAPI channel of each ISP round is used to perform the co-registration. For H&E images, a 'synthetic DAPI' image is produced by defining the color of the nuclear stain in Hue Saturation Value color space. H&E images may also need to be corrected for pixel size differences and rotation with respect to the IF images. The base (1st round) image is divided into a regular grid of overlapping tiles and the offset of each base tile to its location in each round image is found using Normalized Cross-Correlation. The offsets are then regularized and used to restitch each round image to match the base image. The accuracy and precision of the co-registration of the final stack is then independently validated.

Results We show evidence that the algorithm can routinely co-register IF rounds with an accuracy of 1µm (~3 pixels at 20X) over the whole slide. Similar results are also seen for H&E matching for good quality samples with high nuclear density. The algorithm is also tolerant to regions of scanning or staining artifacts, tissue damage or loss, etc. With built-in parallel processing, most samples can be co-registered in a few minutes to an hour, depending on tissue area, staining quality, scanner type, hardware performance, etc.

Conclusions UltiStacker provides a convenient means to co-register and therefore phenotype all the cells of whole slide images across multiple rounds of fluorescent and brightfield scanning. It reads several common image formats and can combine IF, RNA-ISH UltiStacker can routinely obtain 1µm co-registration needed to accurately phenotype cells across multiple rounds of mIF imaging and it enables visualization and analysis of those cells in multiple modalities for a greater understanding of the Tumor Microenvironment.

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