

**MicroSeer: Software for Monte Carlo Evaluation of Colocalization Statistics Using Shifts in Channel Registration****Greiling, Dunrie<sup>\*1</sup>, Bement, William<sup>2</sup>, Rommel, Robert<sup>1</sup>****<sup>1</sup>BioMedware, Inc., Ann Arbor, MI, USA; <sup>2</sup>University of Wisconsin, Madison, WI, USA**

Currently, researchers can obtain remarkably detailed images of living cells and their constituent proteins using molecular genetic and microscopy-based approaches in conjunction with sophisticated microscopy hardware. Available image analysis techniques and software, however, lag behind the power of this new imaging equipment to visualize the microscopic world. This phase I SBIR project applies existing technology in spatial analysis of satellite image data to the analysis of micrographs, creates new statistical techniques specific to the study of spatial association of proteins in cells, and creates software that implements these statistics for use in the analysis of spatial association in time-lapse *in vivo* biomedical imagery.

The analysis of colocalization is limited by the subjectivity of visual interpretation, the quantification of channel overlap as the number of yellow pixels, and by a lack of a standard of comparison. The software under development, MicroSeer, will provide objective quantification of coincidence of pattern in double-label confocal fluorescence micrographs. The MicroSeer project is developing new statistical methods for colocalization analysis: comparing the minimum distances between edges detected on in each channel separately. Then, we obtain the significance of those distances by comparison to two standards: complete spatial randomness and toroidal registration shifts of a single channel of the image. We evaluate these methods using several example micrographs. For comparison to current techniques, we also count the number of yellow pixels in each of the images. Alignment of the two channels of the original images was significant when compared to randomizations or registration shifts of the green channel. The shift method can be considered a restricted randomization that preserves the geometry of each channel yet randomizes the registration of the two channels. It is a more conservative point of comparison than complete spatial randomness. Yet, it robustly illustrates the colocalization/alignment in the example images. This research has the potential of automating colocalization analysis using objective metrics, thereby improving the interpretation of colocalization experiments and spatial associations and biological interactions among proteins in cells.

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