

## Registration and Alignment of Histopathological Images

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**Background:** Current molecular profiling technologies support three-dimensional correlation between spatial location and molecular profiles. This is especially true of Matrix Associated Laser Desorption Ionization Mass Spectroscopy Imaging (MALDI MS Imaging) in which the resolution across the x-y plane is on the order of 20-50  $\mu\text{m}$ , and successive sections can be imaged at near the same depth (z-axis) resolution. Given the high resolution of the imaging, it would be useful to know the cell types and microscopic structures that correspond to specific molecular profiles.

**Design:** This information can be obtained by registering the spectroscopic profiles to one another in three dimensions together and to histological images taken between the spectroscopic profiles. Three-dimensional registration of the histological images is useful not only to support the forward process of matching the profiles to microscopic structures, but also to support the inverse process of validating that molecular profiles can predict certain cell types or microscopic structure localization. We have investigated registration of serial sections of breast tissue. Serial sectioning is necessary because stained histological images generally cannot be used in a MALDI process. Since breast tissue typically lacks visual features useful for image registration, fiducial marks were created in a tissue sample by inserting a set of parallel needles into the sample prior to sectioning and staining, creating a geometric pattern of holes. The sections are taken from normal breast tissue, fixed in formalin, sectioned at 5  $\mu\text{m}$  in thickness, and stained with hematoxylin and eosin (H&E). Images of sections taken after staining were generated at a magnification of 100X and composited using a Bacus Laboratories (Lombard, IL) BLISS virtual imaging workstation.

**Results and Conclusion:** Sixteen fiducial marks in each sample were manually identified, and the point sets represented by these marks were then registered to one of the samples using a non-rigid quadratic transformation. The tissue samples were deformed during sectioning, staining, and imaging, with the result that a rigid transformation of the fiducial marks (translation and rotation) was found to be inadequate in accurately registering the images. Likewise, registration based on edge detection and alignment was found to yield inferior results to the registration of the image using the fiducial marks. A comparison with fully automatic intensity-based algorithms is ongoing.

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