Texture analysis applied to second harmonic generation image data for disease classification& development of multi-view second harmonic generation imaging platform

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Many diseases, e.g. ovarian cancer, breast cancer and pulmonary fibrosis, are commonly associated with drastic alterations in surrounding connective tissue, and changes in the extracellular matrix (ECM) are associated with the vast majority of cellular processes in disease progression and carcinogenesis: cell differentiation, proliferation, biosynthetic ability, polarity, and motility. We use second harmonic generation imaging for visualization of the ECM because it is a non-invasive, non-linear laser scanning technique ideal for visualizing collagen.

In this thesis, we are interested in developing imaging techniques to understand how the extracellular matrix is remodeled in diseases. We developed imaging techniques to acquire 3D extracellular matrix imaging by directing laser focus from four different directions for reconstruction. To quantitate remodeling, we implement a 3D texture analysis to delineate the collagen fibrillar morphology observed in second harmonic generation microscopy images of human normal and high grade malignant ovarian tissues. In the learning stage, a dictionary of “textons”—frequently occurring texture features that are identified by measuring the image response to a filter bank of various shapes, sizes, and orientations—is created. By calculating a representative model based on the texton distribution for each tissue type using a training set of respective second harmonic generation images, we then perform classification between images of normal and high grade malignant ovarian tissues. For 3D texture, we did classification based on the area under receiver operating characteristic curves (true positives versus false positives). The local analysis algorithm is a more general method to probe rapidly changing fibrillar

morphologies than global analyses such as FFT. It is also more versatile than other texture approaches as the filter bank can be highly tailored to specific applications (e.g., different disease states) by creating customized libraries based on common image features.

Further, we discussed the development of multi-view 3D second harmonic generation imaging platform. Unlike fluorescence microscopy, SHG excites intrinsic characteristics of collagen, bypassing the need for additional primary and secondary imaging labels. However, single view image collection from endogenous SHG contrast of collagen molecules is not “a true 3D technique,” because collagen fibers oriented along the plane of the lasers used to excite them are invisible to the detectors. The loss of information means that researchers can’t resolve the 3D structure of the ECM using this technique. We are developing a new, multi-view approach that involves rotation of agarose embedded sample in FEP tubing, so that the excitation beam path travels to from multiple angles, to reveal new insight in understanding the 3D collagen structure and its role in normal and diseased tissue.