Advancing Digital Pathology with CytoViva Hyperspectral Microscopy

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Digital Pathology is the fastest growing area of research within pathology and focuses on data analysis of digitized specimen slides. By digitizing the optical image of a pathology slide, machine learning software can be utilized to improve diagnosis, prognosis and prediction of a wide range of diseases.

Today, the digitization of pathology slides primarily focuses on capturing simple optical microscopy images of traditional H&E stained slides. This allows the software to stitch these images together so they can be analyzed based on RGB (red, green, blue) color channels or shape of the tissue. While this process can serve to advance the accuracy of disease diagnosis vs traditional qualitative observations of tissue, it is a limited dataset relative to other imaging modalities.

Hyperspectral Microscopy of specimen slides represents a potential quantum leap in the advancement of digital pathology. Hyperspectral images are digital images that look very similar to a traditional digitized optical image with a very significant difference. Each pixel of a hyperspectral image contains the full visible near-infrared (VNIR) spectrum from 400nm-1,000nm. This spectral data can be produced at a very high spectral resolution of ~2nm and pixel sizes can be as small as 100nm depending on the camera utilized for the image capture. This incredibly data-rich hyperspectral image provides an opportunity for significantly enhanced digital characterization of a wide range of disease tissue states versus simple digital optical images.

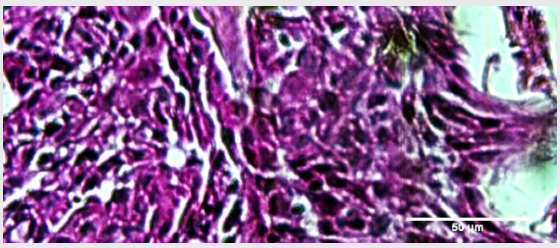

Figure 1: H&E stained squamous cell carcinoma tissue

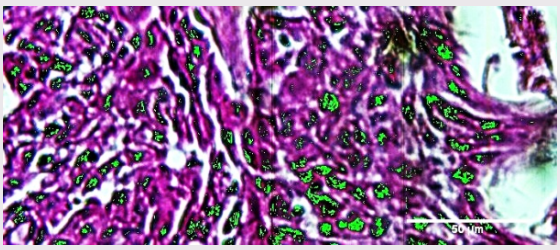

Figure 3: Spectral mapping in green of unique cancerous elements in the tissue

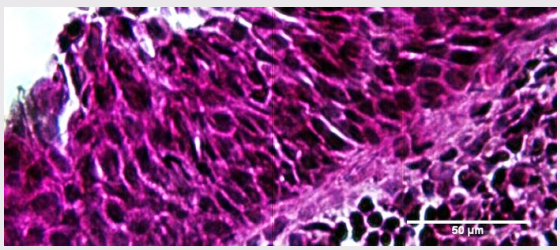

Figure 2: H&E stained negative control tissue

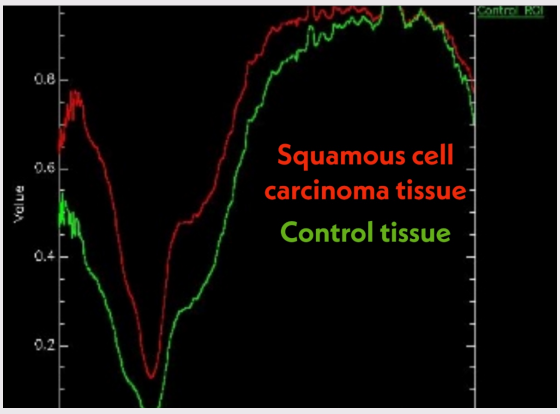

Figure 4: Mean spectral differences between cancerous and non-cancerous tissue

CytoViva, Inc. is the world-wide leader in the field of hyperspectral microscopy and a leading developer of hyperspectral imaging-related digital pathology applications. CytoViva provides turnkey hyperspectral microscope systems that include custom microscopy configurations, including its patented enhanced darkfield and traditional brightfield imaging. CytoViva utilizes high-resolution, line scan-based hyperspectral imaging integrated onto the microscope, which provides 2nm of spectral resolution across the entire VNIR 4000nm-1,000nm spectral range. These systems also come equipped with a full suite of custom hyperspectral image analysis functionality, which provides significant utility in pathology-related applications.

The examples in this application note serve to illustrate a simple utilization of hyperspectral microscopy for pathology-based initiatives. The first example in Figure 1 above shows a hyperspectral image of H&E stained squamous cell carcinoma tissue under traditional brightfield microscopy illumination. Figure 2, is a negative control or non-cancerous hyperspectral image of the same tissue. Figure 3 is a hyperspectral image of the cancerous tissue, with mapping in green of all pixels containing optical spectrum unique to the cancerous elements of the tissue. The spectrum used for spectral mapping was identified by comparing thousands of pixels in the positive control sample versus all pixels in the negative control sample using a proprietary supervised machine learning algorithm known as Filter Spectral Library. This algorithm identified spectrum unique to the cancerous tissue, which was created as a spectral library. Then, a mapping algorithm known as Spectral Angle Mapper was utilized to map the cancerous elements based on the created spectral library. In figure 4, an example mean spectrum is shown to illustrate nuanced spectral differences between the control tissue and areas mapping for cancerous tissue.

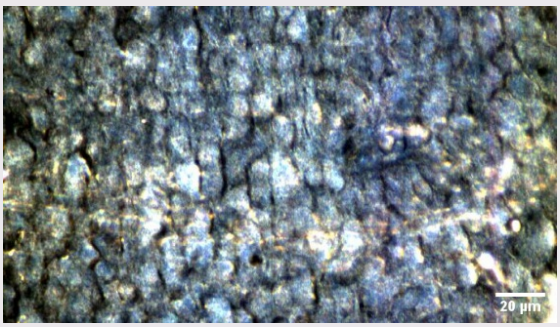

Figure 5: Unstained Pancreatic Cancer Tissue

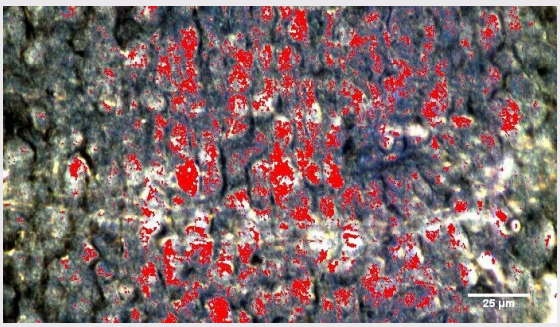

Figure 7: Spectral mapping in red of unique cancerous elements in tissue

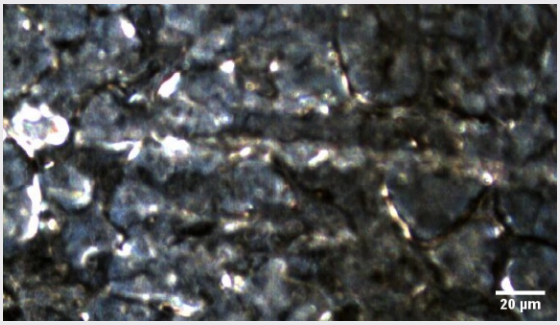

Figure 6: Unstained negative control tissue

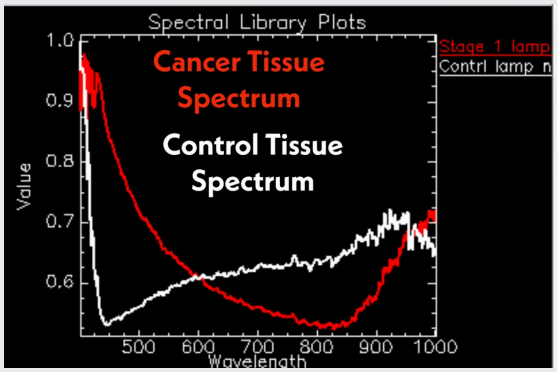

Figure 8: Mean spectral differences between cancerous and non-cancerous tissue

With CytoViva's hyperspectral microscopy, there is also an opportunity to characterize disease versus healthy tissue based on the tissue's endogenous optical spectrum, without any traditional staining or other sample preparation. This is typically done using CytoViva's enhanced darkfield microscope optics, which serves to create a high contrast scattering effect from tissue, without interference from the source illumination. In the second example above, Figure 5 illustrates an enhanced darkfield hyperspectral image of stage 1 cancerous pancreatic tissue. Figure 6 is a negative control pancreatic tissue sample (no cancer). Just like in the stained tissue example, a supervised machine learning algorithm was utilized to identify the spectral characteristics that are unique to cancerous elements of the unstained pancreatic tissue. This resulted in the mapping of cancerous tissue based on the unique spectral characteristics as shown in figure 7. Figure 8 shows the mean spectral differences between pancreatic negative control

tissue versus areas mapping for cancerous pancreatic tissue.

These two examples illustrate how hyperspectral microscopy can be easily applied to quantitatively identify cancerous tissue in both traditional stained and unstained environments. This system and these analysis principles can be applied across many different tissue samples to identify a wide range of disease states. To learn more about hyperspectral microscopy for pathology-related applications, please contact CytoViva at info@cytoviva.com.