Investigating the Biochemical Progression of Liver Disease to Hepatocellular Carcinoma Using Infrared Spectroscopic Imaging

Hari Sreedhar[1], Bennett Davidson[2], Andre Kajdacsy-Balla[2], Grace Guzman[2] and Michael Walsh[2,1]

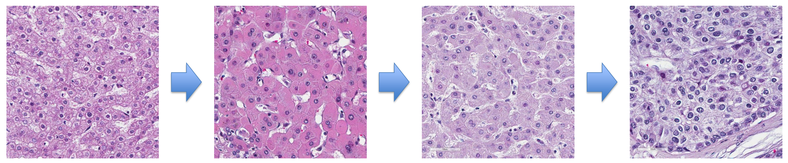
1. Department of Bioengineering, University of Illinois at Chicago
2. Department of Pathology, University of Illinois at Chicago

Introduction

• Hepatocellular carcinoma (HCC) is the most common primary hepatic carcinoma.

• Globally, HCC is the fourth most prevalent malignant tumor and is a leading cause of cancer-related death.

• Carcinogenesis in the liver typically arises through the progression from cirrhosis to dysplasia and ultimately HCC.



Normal | Low-Grade Dysplasia | High-Grade Dysplasia | HCC

• The current clinical gold standard for diagnosis is visual inspection of a stained tissue section by a trained pathologist.

• Fourier-Transform Infrared (FT-IR) imaging and quantum cascade laser (QCL) infrared imaging have shown great potential as label-free chemical imaging approaches for biomedical applications.

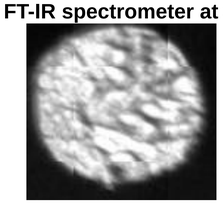
• Recent advances in high resolution IR imaging have opened up new targets of research for histopathology.

• We propose to use IR imaging to provide a new, objective tool for diagnosis of HCC and for tracking the progression of liver disease.

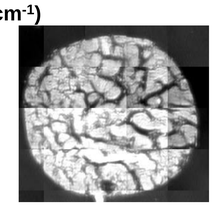
Advances in High Resolution Infrared Imaging

• Conventional IR imaging is limited to spatial resolution around 6.25x6.25 µm, which is insufficient for distinguishing cell types and other structural elements in tissue samples.

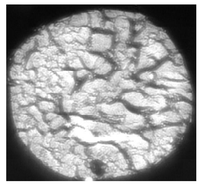
• Novel FT-IR imaging methods have been developed to obtain down to 1.1 µm resolution. (Below, images of the same tissue sample from Agilent Cary 600 Series FT-IR spectrometer at 3275 cm^{-1})



5.5x5.5 µm (15X)

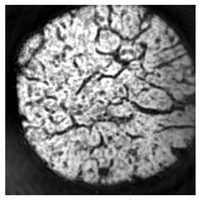


2.2x2.2 µm (36X)



1.1x1.1 µm (74X)

• New IR imaging approaches using QCL allow for high spatial resolution, sparse spectral sampling, and real-time IR imaging of tissue samples. (Right, image of the same tissue sample from Daylight Solutions Spero QCL system at 1640 cm^{-1})



1.4x1.4 µm (Daylight QCL)

• Increased resolution with HD FT-IR imaging and QCL imaging allows for differentiation of features of liver tissue such as hepatocytes and sinusoids which exist at the micron scale.

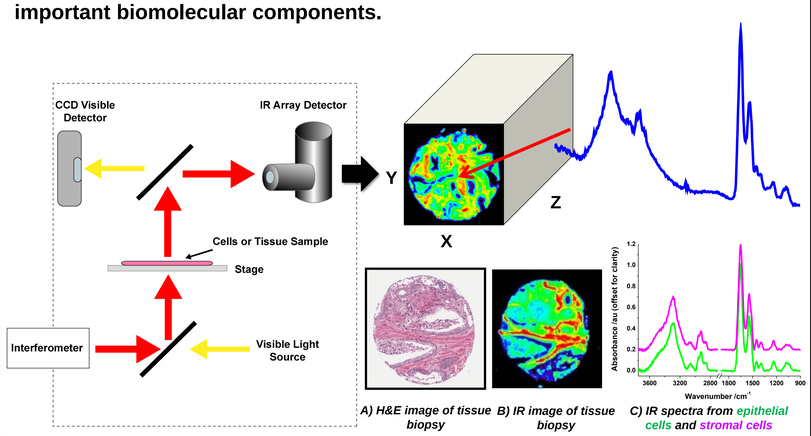
• The biochemical information from the IR data can be correlated with the morphological insights from stained slides.

Infrared Spectroscopic Imaging

• IR spectroscopic imaging, through FT-IR or QCL, derives chemical images from tissues based on their inherent biochemistry.

• IR imaging yields data cubes which comprise of spatial (X,Y) and spectral (Z) information, associating each image pixel with a wealth of chemical information.

• IR is absorbed by chemical bonds that form the chemical basis of key cellular biomolecules, giving a measure of levels and conformations of proteins, lipids, collagen, glycogen, carbohydrates, DNA, RNA, and other important biomolecular components.

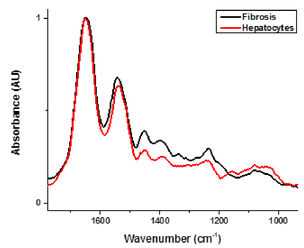


A) H&E image of tissue biopsy *B) IR image of tissue biopsy* *C) IR spectra from epithelial cells and stromal cells*

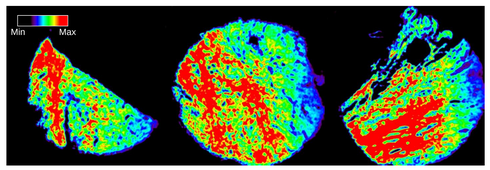
Chemical Characterization of Liver Tissue

• Tissues from liver biopsies can be sectioned onto IR-appropriate substrates and scanned in IR, and their absorbance spectra can be used to distinguish between different chemical states, such as in the case of fibrosis, or scarring.

Average spectrum for regions of hepatocytes compared to the average spectrum for regions with fibrosis, where collagen and lymphocytes are present.



• Images of absorbance at individual wavelengths can be used to detect features of tissue, such as the presence of fibrosis.



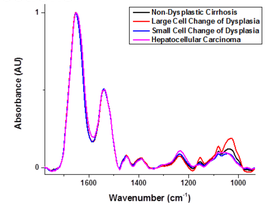
General regions of fibrosis visible in red, in rainbow mapped image of absorbance at 1640.5 cm^{-1}.

•These images can be collected extremely quickly using QCL-based sparse sampling and even displayed in real time at 30 frames per second.

Classification of Disease State on the Basis of Spectral Data

•Representative spectra for four disease states were extracted from regions from two tissue microarrays of liver biopsies from patients with cirrhotic, dysplastic, and cancerous tissues, identified by pathologists' diagnoses of hemotoxylin and eosin (H&E) stained serial sections.

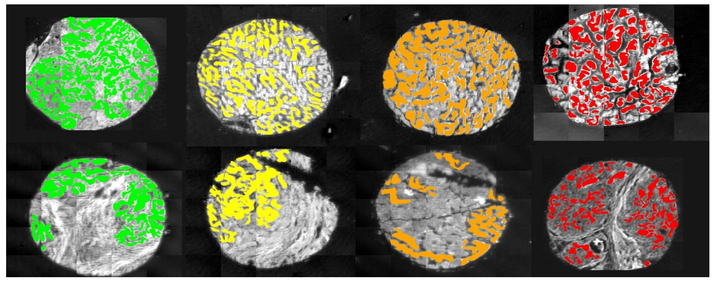
Average spectra for pixels of each disease state (class), baseline corrected and normalized. Classes cannot be distinguished by human observers alone.



•The processes of liver disease progression and hepatocarcinogenesis occur in a highly variable and chemically complex context, so machine learning was selected as a tool for distinguishing between these disease states.

• A Random Forest classifier was built to distinguish between the different disease states on the basis of the spectral data.

Non-Dysplastic Cirrhosis	Large Cell Change	Small Cell Change	Hepatocellular Carcinoma



•Classification of pixels by disease state on a validation set (n=8) from each class was achieved using a small training array (n=30), with AUCs greater than ___ for all classes.

•Black-box approaches like this are extremely powerful, but should be used with caution, and much larger data sets, as they may overtrain or train to noise in the data rather than to the inherent biochemical differences that are present.

Conclusion

• IR imaging can provide a powerful tool to aid with the diagnosis of HCC, and the study of hepatocarcinogenesis.

• Future work will focus on studying different aspects of liver disease, such as the identification of different liver pigments, the diagnosis of grades of HCC, and the incorporation of morphological information into classification algorithms.

•Faster QCL-based IR imaging approaches open the potential for applications such as real-time imaging and a role in intraoperative detection of tumor margins.

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