Application of Spectral Cytopathology as a rapid screening method for Barrett’s esophagus

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Abstract
Spectral Cytopathology (SCP), which couples Fourier transform infrared micro-spectroscopy (FTIR) to methods of multivariate analysis, is an accurate and robust method for the detection of subtle biochemical changes within individual cells for the purpose of providing early diagnostic information. Screening for Barrett’s esophagus (BE) could allow for early detection of this premalignant condition, thus allowing a timely diagnosis and intervention for esophageal adenocarcinoma. The aim of this study was to assess the diagnostic performance of SCP for identifying and classifying disease in individual exfoliated cells from the esophagus. Infrared data were collected from 12 patients diagnosed with Barrett’s Esophagus, 10 healthy, 5 dysplastic, and 2 with adenocarcinoma. Infrared data were pre-processed and subsequently analyzed using Principle Component Analysis and Linear Discriminant Analysis (PCA-LDA). Results were then subject to an artificial neural-network (ANN) to measure the diagnostic performance of SCP. The ANN was able to differentiate Barrett’s esophagus from normal squamous with 96.2% specificity, 77% sensitivity and 88% overall accuracy.

Background
Spectral Cytopathology (SCP) is a rapid label-free screening method for the differentiation of human cells at the molecular level. SCP couples Fourier Transform Infrared Micro-Spectroscopy (FTIR-MSP) with unsupervised multivariate statistical analysis (Principal Component Analysis) to detect subtle spectral patterns within individual exfoliated cells. A cellular infrared spectrum provides a superposition of all of the bio-molecules present, including proteins, glycoproteins, nucleic acids, carbohydrates and lipids. This superposition provides a unique fingerprint of the sample’s biochemistry. Using computer algorithms it is possible to detect subtle biochemical changes that occur during the onset and progression of disease.

Esophageal Adenocarcinoma arises from a complex pathway of morphological changes that occur in the cells lining the distal portion of the esophagus and begins with Barrett’s Esophagus. Barrett’s Esophagus is defined as a condition in which the cells lining the distal portion of the esophagus become damaged and repair themselves in such a way that they change to a cell type most commonly found in the intestines. After this transition has occurred the patient is at increased risk to develop esophageal Adenocarcinoma.

Design
Samples from the esophagus were retrieved from 9 healthy patients, 12 patients with Barrett’s Esophagus, 5 with Dysplasia, and 2 with Adenocarcinoma during routine endoscopies. Samples were spun onto low emissivity (low-e) slides via cytocentrifugation (Keyvley, Inc., Chesterland, OH) using a CytoSpin, Thermo Shandon, Pittsburgh, PA.

Infrared data were collected on the Perkin Elmer Spectrum One/Spotlight 400 imaging IR micro-spectrometer with the following parameters: 4 cm⁻¹ spectral resolution, 2 scans/pixel, 6.25 μm pixel size, and 4000-700 cm⁻¹ spectral range. Data were processed using MATLAB based programs. Noise Adjusted Principal Component Analysis (NA-PC) was used to optimize the signal to noise ratio and the PapMap algorithms was used to reconstruct one spectrum per cell. Water vapor corrections and phase corrections were also employed. PCA was performed on 2nd derivative pixel normalized spectra on the 1800-900 cm⁻¹ spectral region.

Results
Infrared spectra from individual cells were analyzed using Principal Component Analysis and Linear Discriminant Analysis (PCA-LDA) and projected onto scores plots shown below. The mean 2nd derivative spectra display small spectral differences, primarily in the fingerprint region, 1350-900cm⁻¹. Infrared data is then used to train an Artificial Neural Network (ANN) to diagnose unknown datasets. The ANN uses a binary classification scheme, in which 59 wave number inputs were passed through 2 nodes in the hidden layer producing a binary output of ‘normal’ or ‘abnormal’. Training and testing spectra were selected randomly and training spectra were not used in testing. The ANN results show high levels of sensitivity, specificity and accuracy for the diagnosis of diseased samples versus healthy squamous.

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