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A Dark-field Scanning *In Situ* Spectroscopy Platform for Broadband Imaging of Resected Tissue

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Abstract

A dark-field geometry spectral imaging system is presented to raster-scan thick tissue samples *in situ* in 1.5cm square sections, recovering full spectra from each 100 microns diameter pixel. This spot size provides adequate resolution for wide field scanning, while also facilitating scatter imaging without requiring sophisticated light-tissue transport modeling. The system is demonstrated showing accurate estimation of localized scatter parameters and the potential to recover absorption-based contrast from broadband reflectance data measured from 480nm up to 750nm in tissue phantoms. Results obtained from xenograft pancreas tumors show the ability to quantitatively image changes in localized scatter response in this fast imaging geometry. The polychromatic raster scan design allows the rapid scanning necessary for use in surgical/clinical applications where timely decisions are required about tissue pathology.

The lack of visual contrast between normal (or benign) and malignant tissue often leads to incomplete resection of tumors during many cancer surgeries. For example, in the case of breast lumpectomy, studies indicate that a significant percentage (as high as 70% [1]) of patients are called back for re-excision because of positive (for cancer) margins resulting from the initial surgery. Thus, intraoperative imaging systems capable of reliably delineating involved tumor margins could have significant potential in more effectively guiding surgery in procedures such as lumpectomy. This report presents a dark-field, telecentric scanning system prototype, which rapidly scans resected tissue, recovering broadband spectral remission at each pixel in order to estimate the spectral constituents of tissue for immediate classification.

Several fiber-probe methods have been demonstrated to acquire localized measures of broadband reflectance in bulk tissue [2-4], but they involve individual fiber bundles, which are not able to scan large fields of view at a resolution sufficient to account for variations within a resected specimen. The extension of probe techniques to large field-of-view imaging has been recently reported where either the sample [5] or the probe is mechanically scanned [6,7] to generate the image. These systems allow accurate co-registration of optical measures to histopathology, but in most cases are too slow to be suitable for scanning a large number of samples or lack enough resolution for margin delineation. In this report, a fast raster-scanning design is demonstrated as a platform technology for multimodal imaging of tissue with dense spectral sampling at each pixel.

Localized measures of broadband reflectance are known to be useful for extracting optical scattering and absorption parameters that are predictive of the underlying tissue morphology

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and biochemical composition. However, high variability often exists in these measures within a given pathology and across patients, requiring sophisticated classification techniques for automated detection, the utility of which directly depends on the quality of the datasets used to “train” the algorithms. Imaging the entire tissue field, in contrast to probing at discrete locations, allows accurate co-registration with histopathology and helps characterize intra-specimen variations, which are critical to optimal training of the classification algorithm needed to complete the functionality of the system for clinical use.

The high-throughput scanning spectroscopy platform described here has been designed to operate over large fields-of-view. It uniquely combines a broadband telecentric scanning design with a dark-field illumination/detection optical path to allow efficient rejection of specular light while maintaining consistent sampling geometry across the entire imaging field. Figure 1 (a) shows a schematic of the imaging system. A broadband supercontinuum laser (SuperK Blue, NKT Photonics, Denmark) is used as the source. The current configuration generates sufficient light signal across the 480 nm to 750 nm wavelength range; however, the usable span depends upon integration time and overall tissue albedo. In the illumination path, light from the source is coupled into a bank of multimode fibers terminating in a 50-micron core diameter multimode source fiber (NA=0.22, Thorlabs Inc., NJ). Light exiting from the distal end of this fiber is collimated using an achromatic lens ($f = 80\text{mm}$; Newport Corp, Irvine, CA), which is bonded to a 45° micro-rod mirror (Diameter = 6mm; Edmund Optics Inc., Barrington, NJ) at its center. An aperture stop is used along with this assembly to produce a hollow cylindrical beam of light of 10 mm outer diameter and 6 mm inner diameter, which is steered using two orthogonally-mounted galvanometer-based scan mirrors (Cambridge Tech., Cambridge MA) that are optimally placed at the entrance pupil of a custom-designed broadband (400 nm – 750 nm), telecentric, f-theta scan lens (Special Optics, Wharton, NJ). A manual XY translation stage with a glass slide insert is used to hold the sample (Semprex Corporation, Campbell, CA) and is mounted to locate the optimal focus plane on the top surface of the glass plate.

In the detection path, the light scattered from the sample is collimated back by the telecentric lens, de-scanned by the same scan mirrors and is reflected by the mirrorized side of the micro-rod mirror into the focusing achromatic lens L3 ($f=40\text{mm}$, Newport Corp., Irvine, CA). This unique dark-field geometry rejects almost all specularly reflected light from the glass-tissue interface and from other optical surfaces along the illumination path. An additional aperture stop is placed immediately in front of the focusing lens to further eliminate trace specular light that can couple into the detection path due to slight losses in telecentricity caused by optical aberrations. For a field size of 10 mm x 10 mm, this aperture stop is typically set to 4 mm in diameter, yielding an effective collection NA of ~ 0.05 . A detection optical fiber of 50- micron core diameter is placed at the focal point of achromat L3 with its distal end coupled to a CCD-based spectrometer (Princeton Instruments, NJ). The focal length of this achromatic lens was chosen to have the lateral magnification in the detection path be approximately 0.5. Thus, the 50-micron core diameter fiber detects light scattered from $\sim 100\text{-micron}$ diameter spot size on the sample plane, overlapping the illumination spot. This low NA, low magnification design offers extended depth of focus at the sample plane, which minimizes the effects of slight variations in the flatness of the tissue surface imaged. A customized LabVIEW software program allows the sample plane to be raster-scanned with full spectral data acquisition at each pixel position. A scan field of up to 1.5 cm x 1.5 cm can be acquired, typically at a lateral resolution of 100 microns.

Before construction, the entire optical system was modeled in a double pass configuration in the ZEMAX optical design software (ZEMAX Corp., Bellevue, WA). Figure 1(b) shows the 3-d shaded model of the entire optical system depicting the illumination and detection paths for on-axis and extreme-field positions, which span the full 1.5 cm square sampling area.

The illumination path traces rays emitted from the optical fiber source to the sample plane, which is mirrorized to guide the specularly reflected light from the illumination point back through the detection optics. The illumination optical fiber, was modeled as a circular extended source represented by a collection of point sources at the center and extremities of a 50-micron diameter disc and the actual lens specification data from the manufacturers were used to model all other optical components in the ZEMAX layout. The design was optimized to achieve a polychromatic spot size of approximately 100 microns in diameter in the sample plane. Figure 1 (c) shows the polychromatic spot diagrams at the center and extremes of the full field, with different spot colors representing different wavelengths used to span the design waveband of 400-750 nm. The patterns observed within each spot diagram are depictions of the discretely sampled geometric point spread functions, shown for both on-axis and off-axis cases.

Preliminary phantom measurements were obtained with this system to test linearity and reduced sensitivity to local absorption in wavelengths above 610nm. Serial dilutions of intralipid solution, covering a range of 0.5% - 4% of lipids by volume, were measured. All spectral measurements were background-subtracted and referenced to the spectrum of Spectralon (Labsphere, North Sutton, NH) acquired on the same system. The dwell time per pixel was 10 msec and the total scan time for a 10 mm x 10 mm field at 100 micron sampling resolution was approximately 4 min. Figure 2 (a) shows the linear response of average scattered irradiance as a function of intralipid concentration. Figure 2 (b) shows results from another set of phantom measurements where the concentration of animal blood was varied between 0.5%-4% by volume in a 1% intralipid stock solution. For this large range of concentrations, the measured reflectance spectra above 610 nm are mostly independent of absorption due to blood in the spectral band outside the main hemoglobin peaks, allowing direct imaging of changes in local scatter response.

A murine pancreatic tumor sample was also scanned. The scan field was set to 1 cm square with a scanning resolution of 100 microns. The data from this experiment was processed using techniques outlined previously [5]. Figure 2 (f) shows the white light image of the tumor sample. Images of scatter amplitude, scatter power and average scattered irradiance, all relative to the spectralon standard, are shown in Figures 2(c) – 2(e), respectively. Representative spectra and corresponding fits to the reflectance model are shown in Figure 2 (g). The range of variations in average scattered irradiance and scatter power appear to be consistent with results obtained earlier for pancreas xenograft tissue samples [5]. In addition to imaging scatter, the platform also offers the potential to image absolute chromophore concentrations from these spectra using sophisticated Monte-Carlo based approaches to model the effective path length [8].

The system performed as expected in scanning resected tissue samples and demonstrates the ability to quantify spectral parameters rapidly, which is vital for translation of this technology into surgical applications. The key features reported in this letter are the design of the dark field, telecentric illumination and detection scheme for rapid point spectroscopy in raster scanning mode using an optical train, which allows broadband polychromatic illumination. The dark field design allows effective specular light rejection and the simultaneous detection of all wavelengths makes the imaging fast. The telecentric scanning architecture maintains a consistent normal illumination and collection geometry across the entire field, which is crucial to sampling scatter related parameters that have inherent angular dependence. For most pancreatic tumor samples that have significantly higher albedo, the dwell time per pixel can be reduced to ~1 ms which will allow significant speed up in acquisition time in combination with hardware-synchronized raster scanning schemes. Future work will involve complete scanning of breast resection specimens to assess the accuracy of the system in classifying tissues during lumpectomy surgery. Addition of

polarization and fluorescence spectroscopy to the platform is also readily accomplished. Thus, the technology described here serves as a novel platform, which could have significant clinical impact once fully evaluated for its diagnostic potential.

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Figure 1.

(color online) (a) A schematic of the prototype imaging system. The inset illustrates the dark-field illumination and collection geometry. (b) A photograph of the assembled prototype. (c) 3-d shaded model of the full optical train modeled in ZEMAX optical design software. Different ray colors represent different field positions. (d) Full field spot diagrams shown at the center and extreme positions of a 1.5 cm^2 scan field at the sample plane. The solid circle measures $100 \mu\text{m}$ in diameter.

Figure 2.

(color online) (a) A plot of average reflectance versus IntraLipid concentration showing a linear response. (b) Plots of spectral reflectance shown for different volume fractions of porcine blood (0.5% - 4%) in 1% IntraLipid stock solution. Note that the spectral response remains approximately same in the > 610 nm spectral region, despite a large change in blood concentration. (c-e) Average reflectance, scatter amplitude and scatter power images of a murine pancreatic tumor specimen [5]. The scan field measures 1cm x 1cm. (f) A photographic image of the tumor specimen. (g) Representative remission spectra from two different pixel locations. The solid line shows fits to the empirical reflectance model [5].