A Dark-field Scanning Spectroscopy Platform for Localized Scatter and Fluorescence Imaging of Tissue

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ABSTRACT

Tissue ultra-structure and molecular composition provide native contrast mechanisms for discriminating across pathologically distinct tissue-types. Multi-modality optical probe designs combined with spatially confined sampling techniques have been shown to be sensitive to this type of contrast but their extension to imaging has only been realized recently. A modular scanning spectroscopy platform has been developed to allow imaging localized morphology and molecular contrast measures in breast cancer surgical specimens. A custom designed dark-field telecentric scanning spectroscopy system forms the core of this imaging platform. The system allows imaging localized elastic scatter and fluorescence measures over fields of up to 15 mm x 15 mm at 100 microns resolution in tissue. Results from intralipid and blood phantom measurements demonstrate the ability of the system to quantify localized scatter parameters despite significant changes in local absorption. A co-registered fluorescence spectroscopy mode is also demonstrated in a protophorphyrin-IX phantom.

Keywords: scatter spectroscopy, fluorescence spectroscopy, multimodal spectroscopy, scanning spectroscopy, dark-field spectroscopy

1. INTRODUCTION

Localized tissue spectroscopy offers sensitivity to morphological and molecular level contrast in tissue, which could be used to distinguish various types of tissue pathologies. In particular, parameters related to elastic scattering [1-2] and fluorescence [3] have shown significant promise in imaging this contrast. However, high variability in tissue makes interpreting these measures a challenging problem. A new dark-field scanning spectroscopy platform has been developed to acquire localized broadband reflectance spectra across large tissue fields. The scanning beam spectroscopy design is optimized for dense spectral recovery at each 100-micron diameter pixel over 1.5 cm x 1.5 cm fields, allowing accurate co-registration with standard histopathology. The instrument also allows co-registered sampling of localized fluorescence emission spectra with simple a modification to the optical subsystem.

2. MATERIALS AND METHODS

2.1 Dark-field Scanning Spectroscopy

The design of the dark field localized scanning spectroscopy (DLSS) platform is described in [4]. Figure 1a illustrates the illumination and collection geometry employed. In broadband scatter spectroscopy mode, a tungsten halogen source is...
coupled to the source path, which produces a focused illumination spot of ~100 microns diameter at the sample plane. A fiber coupled CCD-based spectrometer allows measuring the broadband spectral remission (467-830 nm) from the ~100 micron diameter detection spot overlapping the illumination spot.

In fluorescence mode, the broadband source is replaced with an appropriate excitation laser source. For protophorphyrin IX (PPIX) imaging a blue excitation source (405 nm) was used. The fluorescence emission for this fluorophore is in the red band (~610 – 800 nm). The detection spectrometer grating was centered to cover this region, while blocking the strong 405 nm excitation laser peak, which is mixed with the fluorescence remission in the detection path. This strategy could potentially increase the amount of stray light reaching the CCD detector. However the spectrometer used here has effective baffling mechanisms to minimize the effects of stray light.

Figure 1: a) A schematic illustrating the dark-field spectroscopy geometry b) illumination and detection scheme for fluorescence imaging. For PPIX imaging, the source fiber is coupled to a 405 nm excitation laser source. The detection fiber detects the fluorescence emission in the red band mixed with the scattered excitation light, which are decoupled at the spectrometer.

2.2 Phantom Experiments

For characterizing broadband scatter spectroscopy capabilities a matrix of intralipid phantoms were measured using the scanning spectroscopy system. In each batch of intralipid phantoms the concentration of intralipid (% volume fraction of lipids) ranged from 0.5% to 1%. The hemoglobin (Hb) concentration was set to remain same within a given batch of intralipid phantoms, but varied in the range of 0 µM - 30 µM across the batches. Figure 2 illustrates the phantom setup showing the actual concentrations used. The concentration values were chosen cover the range typically measured in breast tissue.
Figure 2: An illustration of a matrix of IntraLipid/Blood phantoms used to characterize the spectroscopy system.

A PPIX based fluorescence phantom was also measured. 1 µg/ml of PPIX was dissolved in 2% intralipid stock solution containing 20% tween to prevent aggregation of PPIX in the phantom.

Figure 3: Measured spectra from the intralipid/blood phantoms. The concentration of hemoglobin varied from 0-30 µM within each group (identified by the concentration of intralipid) of intralipid phantoms.
3. RESULTS

Figure 3a shows the spectra acquired from intralipid/blood phantom matrix shown in Figure 2. All measurements were referenced to a 10% intralipid reference phantom to remove instrument spectral response. In the region outside the hemoglobin absorption peaks (> 610 nm), the measured spectra are shown to be less sensitive to changes in local hemoglobin concentration, which is expected in this highly localized measurement geometry. The wavelength-integrated reflectance in this waveband (not shown here) was found to increase linearly with concentration of intralipid, despite large changes in the local hemoglobin concentration.

Figure 4 shows the fluorescence spectra measured from the PPIX phantom. It should be noted that co-registered reflectance spectra could be acquired for this sample by switching to the broadband source, which will allow computing the normalized Born ratio of fluorescence for the sample [5]. This is particularly useful in tissue measurements where extreme changes in local optical properties make fluorescence quantification hard.

4. DISCUSSION

A scanning spectroscopy platform for characterizing morphology and molecular contrast in breast pathologies has been developed. The platform allows rapid scanning of surgical specimens to generate pathology-correlated datasets, which are critical in accounting for large variability observed within a given pathology and...
across patients. Results from the intralipid/blood phantom measurements indicate the ability of the system to quantify scatter measures independent of changes in local absorption. Capability to acquire fluorescence spectra is also demonstrated in a PPIX phantom.

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REFERENCES


