Novel clinical technology for rapid detection of tissue fluorescence wavelength-time matrices

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Abstract: Clinically-compatible technology was developed to measure wavelength- and time-resolved fluorescence intensities from biological tissues. Validation studies were conducted on tissue-simulating phantoms and the results were consistent with theoretical predictions with < 4% deviation.

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1. Introduction

Histology, the gold standard for clinical tissue disease diagnosis and pre-clinical monitoring of tissue-engineered devices, is qualitative, invasive, subjective, and destructive. Fluorescence sensing shows promise for a wide variety of applications in tissue diagnostics because it is quantitative, non-invasive, objective, and non-destructive [1]. Here we report novel, clinically-compatible technology for the rapid detection of wavelength-time matrices (WTMs) of fluorescence intensity. The technology is validated on tissue-simulating phantoms by employing Monte Carlo (MC) simulations of photon migration [2, 3] to quantitatively account for effects of scattering and absorption.

2. Novel fluorescence detection technology for wavelength-time matrix measurements in tissues

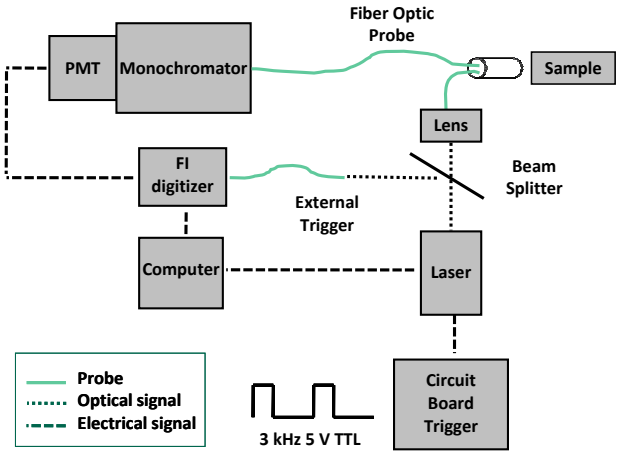

Fig. 1. Experimental setup developed to collect WTMs from the tissue-simulating phantoms for the study.

The instrumentation (Fig. l) consisted of a 473 nm microchip laser (3 kHz pulse repetition, 1-3 ns pulse width), 600 μm diameter optical fibers for remote sensing in tissue-simulating phantoms, and a specialized transient FI digitizer (Fluorescence Innovations, Inc.) for fluorescence WTM detection. Fiber-optic probe holders were attached to micrometer stages to enable variable center-center source-detector spacing.

3. Fluorescence wavelength-time matrices (WTMs)

Wavelength-time matrices (WTMs) of fluorescence intensity temporal decay at different wavelengths (illustrated in Fig. 2) can be acquired with the specialized digitizer described above [4-6]. The WTM contains two dimensions of fluorescence intensity information: wavelength-resolved and time-resolved. Integrating the WTM over time would yield the fluorescence spectrum traditionally measured in fluorescence spectroscopy experiments. Integrating the WTM over wavelength would yield the fluorescence temporal decay traditionally measured in time-resolved fluorescence lifetime spectroscopy experiments.

Fluorescence lifetimes can be extracted from the WTM by using a least-squares fitting algorithm and the corresponding instrument response. The WTM acquired can be employed for global fitting to further extract the number of components and the lifetime of each component when multiple fluorophores are present in a sample.

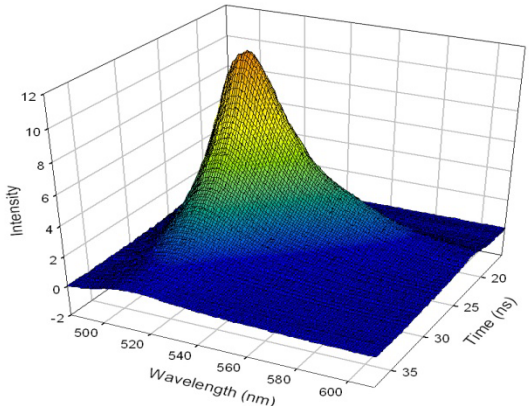

Fig. 2. WTM of 0.1 μM fluorescein in 0.1 N NaOH: fluorescence intensity was recorded as a function of both wavelength of emission and the time following sample excitation.

4. Tissue phantom preparation and experimental methods

Tissue phantoms consisting of rat-tail collagen (#354236, BD Biosciences; 3.68 mg/ml) and fluorescent beads (A7303, Invitrogen) were developed to mimic tissue engineered constructs under study in our laboratory. The materials, including the solutions of the beads, collagen, 10X PBS and 1N NaOH, and a tube of sufficient capacity to contain the final volume (1.072 ml) of the gel mixture were first put on ice. Then the materials were added into the tube in the following order: 100 μl ice cold 10X PBS, 27.7 μl ice cold 1N NaOH, 175.3 μl bead solution, and then 769 μl collagen solution. The contents were mixed with air bubbles avoided. The mixture was poured into one 35 mm Petri dish, and then incubated at 37^{O} C for 30 minutes for solidification. The final gel mixture has 2.64 mg/ml collagen and 1.67 x 10^5 beads per 60 μl gel. For these phantoms, fluorescence lifetimes were extracted from the decay curves measured at 550 nm. A stock solution of the fluorescent beads was also measured and the standard deviation of fluorescence intensity from the 550 nm time-resolved decay curve was calculated by averaging standard deviations of intensity around the emission peak (36–38 ns).

Wavelength parameters for WTM collection were selected prior to acquisition by defining start and end wavelengths, as well as wavelength spacing. The temporal range was fixed from 0 – 127.8 ns with resolution of 0.2 ns. Phantoms were measured from 500-700 nm, spaced by 1 nm. WTMs were collected from the collagen and beads phantom with no probe separation and with 1.25 mm separation to analyze the accuracy in detected lifetime and average fluorescence intensity ratio described in Section 6.

5. Monte Carlo simulations for quantitative lifetime sensing in tissues

Monte Carlo (MC) simulations were run to extract fluorophore lifetimes by matching simulated time-resolved fluorescence decay curves with experimentally-measured time-resolved fluorescence decay curves. The MC simulation approximated the tissue phantom as a homogeneous, single-layered, semi-infinite turbid medium [7]. An integrating sphere setup was employed to measure the diffuse reflectance and transmittance of the sample, and the inverse adding-doubling method was used to obtain the reduced scattering coefficient (μ_s') and absorption coefficient (μ_a) from the integrating sphere measurements [7]. These parameters were used in the MC simulations, where photon trajectories were constructed by stochastic sampling of scattering angles (specified by the anisotropy g) and path lengths (specified by the scattering coefficient $\mu_s = \mu_s'(1 - g)$). For our initial simulations, the value of g was approximated to be 0.9, which is commonly used for biological tissue. This approximation allowed us to obtain μ_s and g without performing collimated transmittance measurements, which can be highly prone to experimental errors. The simulations also accounted for the refractive index mismatch caused by the glass Petri dish (n = 1.5) beneath the phantom. The simulation inputs for the phantom included μ_s = 10 cm^{-1}, μ_a = 0 cm^{-1} (no absorption), refractive index = 1.4 and thickness = 4 mm, while the simulation inputs for the probe included fiber radius = 300 μm, center-to-center distance between source and detector = 660 μm, numerical aperture = 0.22, and refractive index = 1.53. MC simulated decays had three different input lifetime values ($<\tau>$ = 1 ns, 2 ns, 3 ns) and were compared to the decay

curves measured on collagen-bead phantoms by calculating the percent deviation between simulation and experiment from 1-2.5 ns.

6. Results and discussion

In Fig. 3(a), the experimental measured standard deviation compared well to the theoretical prediction from Poisson noise distribution and the propagation of uncertainty. The time-resolved fluorescence decay curves in Fig. 3(b) were similar in shape even though the red decay curve included more noise due to the lower number of shots averaged (5 vs. 1250 shots). To collect a WTM with 201 wavelengths and with a standard deviation of 1% relative peak intensity at 550 nm, the data acquisition time would be ~8.4 seconds (arrow in Fig. 3(a)). If the WTM included fewer wavelengths with 10 nm spacing between measurements, the collection time would be reduced to ~0.88 seconds (roughly a factor of 10), which is a rapid detection time desirable in a clinical setting.

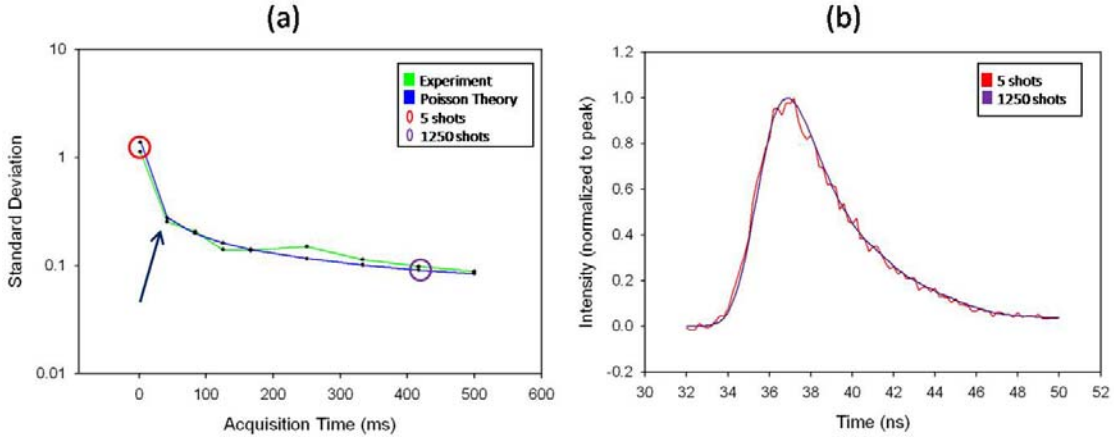

Fig. 3. (a) Measured fluorescence emission intensity standard deviation (green curve) of stock solution of fluorescent beads. Theoretical curve (blue) is square root of mean intensity divided by square root of shots averaged (equal to number of decay curves averaged), multiplied by a scaling factor of 1.3 to match the scale of experimental results. Acquisition times correspond to 5, 25, 50, 75, 100, 150, 200, 250, and 300 shots averaged, respectively. (b) Normalized fluorescence decay curves for 5 (red), corresponding to red circle in (a) and 1250 shots averaged (blue), corresponding to purple circle in (a). The arrow in (a) denotes data acquisition parameter for 25 shots averaged is ~0.25 (a.u.) with a peak signal intensity of 25 (a.u.).

For collagen-bead tissue phantoms, the MC simulation with a mean lifetime value of $<\tau>$ = 2 ns provided the most accurate reconstruction of the measured fluorescence decay curve. There was less than 4% deviation between the measurement (extracted lifetime of 2.18 ns) and the simulation. In addition, when probe spacing was 1.25 mm, the average fluorescence intensity from 550-650 nm was measured to be half that obtained with no probe spacing and this result matched the MC prediction to better than 10%. These preliminary results confirm the highly accurate fluorescence detection capability afforded by this technology. Additional validation studies are underway.

7. Potential for clinical use

The accuracy, speed of data acquisition, and portability of the novel instrumentation reported here, coupled with the information-rich nature of WTM fluorescence data, indicate that this technology is highly suitable for eventual translation to a clinical biomedical setting and shows promise for a variety of biomedical optics applications.

8. References

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