Time-resolved fluorescence spectroscopy facilitates medical research

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Novel fluorescence lifetime-based instrumentation and methodologies enable the development of near-real time clinical diagnostic devices for heart disease and tumors.

Fluorescence is a ubiquitous approach for achieving optical molecular contrast using a wide range of instruments including spectrophotometers, microarrays, microscopes, and endoscopes. Fluorescent molecules emit radiation, which is often visible, in response to an excitation. In biomedical applications, fluorescence measurements have the potential to provide information about biochemical, functional, and structural changes in fluorescent bio-molecular complexes in tissues and cells, such as structural proteins, enzyme metabolic co-factors, lipid components, and porphyrins. Typically, such changes result from pathological transformation or therapeutic intervention.

Fluorescence can be measured using either spectrally-resolved (steady-state or intensity) or time-resolved methods. While fluorescence intensity measurements are straightforward and easy to implement, any quantitative measurement of intensity is hindered by variation in the excitation intensity, the presence of endogenous absorbers (e.g., blood), and variation in the light excitation-collection geometry. Time-resolved techniques are thought to overcome such limitations and to improve the specificity of fluorescence measurements by resolving the fluorescence intensity decay in terms of lifetimes. Conceptually, the fluorescence lifetime is the average time a fluorophore spends in excited states following excitation from its ground energy level. Thus, the lifetime measurement provides additional information about the underlying fluorescence dynamics. Since many of the fluorophores in biological tissues have overlapping spectra, fluorescence lifetime properties can provide a contrast parameter. Yet, since the fluorescence lifetime is independent of the fluorophore concentration and its quantum yield, it is not influenced by any factors that may non-linearly affect the fluorescence intensity.

Despite these recognized inherent advantages, fluorescence lifetime information acquisition has not been broadly imple-
Figure 2. TRFS data correlation with pathological features and classification of the carotid atherosclerotic plaques. IT: intima thickening. N: number of samples. Ca: calcium.

mented in clinical settings due to instrumentation complexity, lengthy data acquisition and analysis, and other barriers. Thus, solutions that enable the development of fluorescence lifetime-based instrumentation appropriate for clinical applications are important. To address these challenges, we have developed time-resolved fluorescence spectroscopy (TRFS) instrumentation and analytical methods that enable fast recording and analysis of fluorescence decays (lifetimes) at multiple wavelengths. This instrumentation (see Figure 1) has been used during surgical interventions to study molecular changes in the body due to a variety of diseases, including primary brain tumors (glioma and meningioma) and atherosclerotic cardiovascular diseases, including high-risk plaques (see Figure 2).

While the most commonly used technique in TRFS is time-correlated single-photon counting, we have chosen an alternative method that takes advantage of fast digitizers and gated photomultipliers: pulse sampling or transient pulse recording. This approach permits detection of many photons per laser pulse, and thus the entire intensity decay profile can be rapidly recorded and digitized. Also, gated detection can be used with low repetition rate lasers, thus allowing for measurements under typical room lighting conditions. These features are particularly important for studies conducted in clinical settings. Moreover, to improve the data acquisition speed of our TRFS devices, we developed a novel combination of optical fibers and bandpass filters that allows near real-time acquisition of spectrally-resolved fluorescence intensity transients. A single detector simultaneously records multiple fluorescence intensity decay profiles in response to a single pulse excitation event. This approach permits recording both fluorescence decay and spectral intensity information from multiple spectral bands within hundreds of nanoseconds.

Each fluorescent biological molecule is characterized by specific fluorescence decay dynamics that carry useful information for the identification of tissue molecular makeup. To analyze these dynamics, we selected a new approach, namely Laguerre expansion of the kernel. This method was found to provide important advantages over more traditional methods such as multi-exponential approximation. For example, Laguerre expansion of the kernel does not require a priori knowledge of the functional or mathematical form of the decay, and it generates unique solutions for decay parameters. In addition, the method can be implemented using fast algorithms, so more than 30 decays can be analyzed in less than 1 second. Thus, it provides an opportunity for near-real time analysis of fluorescence decay information from complex biological systems.

In summary, using autofluorescence-based fluorescence lifetime contrast in clinical and biomedical research is now closer to wide deployment. Our current studies demonstrate the translational research potential of TRFS and show that intrinsic fluorescence signals can be used to provide useful contrast for the diagnosis of high-risk atherosclerotic plaques and primary brain tumors. A combination of new technological advances for fast recording of spectrally-resolved fluorescence lifetime information plus new analytical methods and algorithms for fast processing of the fluorescence intensity decay data will enable further development of compact TRFS diagnostic systems for near-real time diagnoses of tissues.

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