OPTICAL COHERENCE TOMOGRAPHY

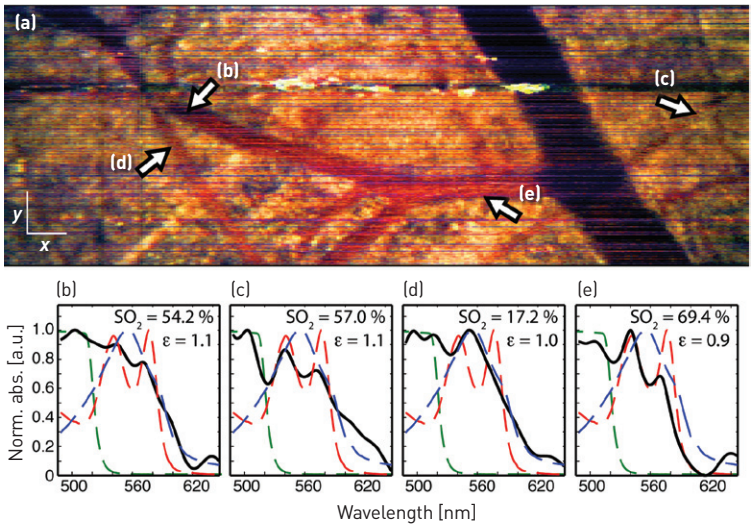
True Color Molecular Imaging with METRiCS OCT

Being able to assess, structure and quantify the molecular content of biological samples *in vivo* is paramount to improving our understanding of many diseases. To this end, we have developed molecular imaging true-color spectroscopic optical coherence tomography (METRiCS OCT), which derives functional information from the spatially resolved spectral content of scattered and absorbed light.[1]

The main working principle behind METRiCS OCT is spectroscopic OCT (SOCT), which provides the same noninvasive, high-resolution tomographic imaging capabilities as OCT with the addition of spectral information at each voxel of the sampled volume.[2] We use a wide spectral bandwidth laser source centered in the visible spectrum, thus allowing quantification of hemoglobin oxygenation (SO_2),[3] providing contrast from other readily available absorbers and enabling a true-color tomographic representation of samples.

In addition, METRiCS OCT uses a novel method for assessing spatially resolved spectral information. This method, termed dual window (DW), uses two short-time Fourier transforms with carefully chosen windows to reconstruct a time-frequency distribution and achieve high spatial and spectral resolution.[4] DW has also been shown to avoid the artifacts associated with other commonly used time-frequency methods, thereby providing spectra with high fidelity.

To demonstrate the capabilities of METRiCS OCT, we acquired images from an *in vivo* CD1 nu/nu normal mouse dorsal skinfold window chamber model. Our results demonstrate that both endogenous and exogenous chromophores—from Hb and sodium fluorescein (NaFS), respectively—provide unique colorimetric contrast. The figure illustrates the full potential of the method. As it shows, the rich spectral content allows quantification of each species—specifically, SO_2 and the ratio between Hb and NaFS ($\varepsilon \equiv$ NaFS/Hb).



(a) En-face (*x*-*y*) METRiCS OCT image using exogenous contrast and spectral profiles. Arrows indicate points where spectra were extracted. White *x* and *y* scale bars are 100 µm. (b-e) Spectral profiles from points as noted by arrows in (a). Measured spectral profiles (black) are superposed with the theoretical oxy (dashed red) and deoxy (dashed blue) Hb normalized extinction coefficients and normalized absorption of NaFS (dashed green). Also shown are the SO_2 levels and the relative absorption of NaFS with respect to total Hb ($\varepsilon \equiv$ NaFS/Hb). All spectra were selected from depths immediately beneath each corresponding vessel.

Adapted from F. E. Robles et al. Nature Photon. **5**, 744–7 (2011).

We believe that METRiCS OCT has the potential to become an incisive tool for many applications, including basic research into tumor development (e.g., angiogenesis and hypoxia); the diagnosis of ophthalmologic pathologies (retinal microvasculature perfusion and oxygenation) and cancer; and the delivery and monitoring of therapeutic agents. A recent continuation of this work has shown that plasmonic nanoparticles also provide useful colorimetric contrast.[5] This is an important step forward, particularly for the implementation of this method to deliver and monitor therapeutic agents. OPN

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