Capitalizing on the optical absorption of blood hemoglobin, PAM is ideally suited for label-free imaging of the microvasculature, which plays an indispensable role in supplying tissue oxygen and maintaining metabolic activity in vivo. In this talk, I will introduce some of our latest progress on the development of PAM for multi-parametric microvascular imaging and a few representative applications of this enabling technique in brain and cardiovascular research.

Multi-parametric PAM of blood perfusion (concentration of hemoglobin, $C_{\text{Hb}}$), oxygenation (oxygen saturation of hemoglobin, $sO_2$) and flow is based on the statistical, spectroscopic, and correlation analysis we recently implemented (Fig. 1A)$^{1,2}$. At 532 nm, a near-isosbestic point in the absorption spectrum of hemoglobin, PAM is insensitive to $sO_2$. Fluctuation in PAM signals acquired at this wavelength encodes both the Brownian motion and the flow of red blood cells (RBCs). The Brownian motion-induced statistical fluctuation in the amplitudes of successively acquired A-line signals depends on the RBC count within PAM’s detection volume but not the flow speed. Thus, the fluctuation can be used to derive $C_{\text{Hb}}$ in absolute values. In parallel, the blood flow can be quantified using the decorrelation rate of the same set of A-line signals. Combining the readouts at both optical wavelengths (532 and 558 nm), PAM can also quantify $sO_2$. With the aid of vessel segmentation, we can further perform the quantitative analysis at the single-vessel level. Combining the hemodynamic parameters measured in individual microvessels, tissue oxygen extraction fraction and metabolism can be derived.

Using the multi-parametric PAM, we have demonstrated—for the first time—simultaneous high-resolution imaging of $C_{\text{Hb}}$, $sO_2$ and CBF in the live mouse brain (Fig. 1B–D). Observing the strong influence of general anesthesia on the overall brain activity and multiple different forms of hemodynamics, we have developed the first-of-a-kind head-restrained PAM for multi-parametric imaging of the awake mouse brain$^3$. This technique holds great potential to examine the neuroprotective/neurotoxic roles of general anesthetics in a variety of brain disorders and to advance our understanding of neurovascular coupling in the awake behaving brain.

Exploiting the high pulse repetition rate of our newly developed dual-wavelength ns laser and an optical-mechanical hybrid scan scheme$^4$, our latest multi-parametric PAM can acquire the multi-parametric image data with a record-high speed of $1.2 \times 10^6$ A-lines/second, opening new opportunities to study acute hemodynamics and disease processes.