Noninvasive Functional Microscopy Using Coherent Raman Methods

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Over the past decade many microscopy approaches have been developed using coherent Raman scattering as a contrast mechanism. Most of these have used coherent anti-Stokes Raman scattering (CARS) or stimulated Raman scattering (SRS). Each of these methods feature label-free contrast and aim to provide noninvasive, functional imaging. Depending on the chemical and morphological complexity of objects being imaged, “functional” imaging may be achieved using a single vibrational mode or may require many vibrational modes for image contrast. In this talk I will give a brief overview of CARS and SRS methods, including issues remaining to be resolved for both approaches. I will then focus my remarks on development efforts in broadband CARS microscopy.

Biological materials imaging is often performed on spatio-chemical environments that are extremely complex, or discriminating subtly different species. In these applications it is desirable to have as much chemical specificity as possible, and broadband coherent Raman is a natural choice. We and others have developed broadband CARS methods that provide as much chemical specificity as spontaneous Raman, but acquire signal in a few milliseconds - as much as 100 times faster than spontaneous Raman methods. (1-4)

Figure 1 exemplifies the chemical resolving power of broadband CARS microscopy. From the Raman spectrum obtained at each pixel we are able to track signaling vesicles related to wound healing response in white blood cells (1a), and can observe changes in chemistry as well as the morphology of stem cells as they differentiate into bone or fat cells (1b).

Although broadband CARS is much faster than spontaneous Raman, imaging speed remains a major drawback, hindering wide-spread adoption of this method. I will also describe a signal acquisition approach that we believe will allow us to acquire high-quality full spectra in 100s or even 10s of microseconds.