HIGH-THROUGHPUT RAMAN MICROSCOPY

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With the traditional scanning method of confocal spontaneous Raman imaging, a single pixel has to be exposed for about 1 to 30 seconds in a biological sample. Typically, several hours are required to record a single 100 × 100 pixel image. We hereby present a technique that records a full hyperspectral Raman image consisting of over four million spectra in about 10 minutes.

Confocal Raman imaging is limited by the damage-threshold constrained local light intensity during single point scanning, whilst leaving the rest of the sample unexposed. In contrast, light sheet microscopy \cite{1} exposes the whole field-of-view simultaneously by sideway illuminating. In addition, it archives optical sectioning by not exposing out-of-focus parts.

Light sheet illumination for Raman imaging at a few specific wavelengths was previously reported \cite{2,3}. We have succeeded in combining light sheet illumination with hyperspectral data acquisition to obtain fully resolved hyperspectral information over a large field-of-view within a few minutes by using a Fourier transform spectral imaging approach.

With a total laser power of 2 W at an illumination wavelength of 577 nm, we obtained images (2048 × 2048 pixels) of polystyrene beads at a spectral resolution of 4 cm\textsuperscript{-1} (fig. 1b). We also imaged zebrafish and daisy petal at resolution of 18 cm\textsuperscript{-1} with only few minutes of exposure. The olefinic and aliphatic C-H stretching modes, as well as the fingerprint region are clearly visible along with the broad water peak of the embedding medium (fig. 1a).

Spectrally resolved spontaneous Raman microscopy therefore promises high-throughput imaging for biomedical research and on-the-fly clinical diagnostics.

Figure 1: 2.2 µm Polystyrene beads

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