A novel imaging system based on a form of visual-light photon scattering provides real-time views of biological samples.

Sight has long been one of mankind’s most relied-upon senses for attempting to understand the natural world. However, the optical diffraction limit of visible light ordinarily limits the spatial resolution of conventional imaging techniques, such as white-light microscopy, to approximately 250–300nm. This fundamentally restricts our ability to visualize objects at the nanometer scale while retaining spectroscopic (i.e., molecular or atomic) information about the sample. A number of imaging techniques—confocal fluorescence and scanning-probe microscopy, as well as others—have been developed to overcome this limitation. While these methods have been powerful tools for studying the nanoscale world, they have their own limitations. They are generally restricted to imaging fluorescent or fluorescently labeled samples, or they require lengthy imaging times—hours or days—to produce high-quality images of microscopic areas. These long imaging times are driven by the need to move a tiny probe tip over all parts of a microscopic specimen.

We have developed a system that produces dynamic nanometer-scale images with visible light, with no need for fluorescent labeling. It uses a probe that forms images based on Raman scattering. In this process, photons emitted by molecules that have been struck by other photons have different energy and frequency distributions than the original photons. This effect is also known as inelastic scattering, and it is far less common than elastic scattering (also known as Rayleigh scattering), in which the scattered photons all have the same energy as the incident photons.

Imaging with Raman scattering is much slower than with Rayleigh scattering. The reason for making images with Raman scattering is that the wavelength difference between the incident and scattered photons is directly related to the energy—or, more precisely, the vibrational mode—of the bonds in the molecule of interest. Because every molecule contains different bonds, it is...
**A nanoscale probe for dynamic-chemical imaging**

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**Subject terms**

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possible to image only specific molecules (or to identify particular molecules) based on the wavelengths that they scatter.

Raman is a very weak scattering phenomenon. Ordinarily, it would take hours to obtain enough scattered photons to produce a usable image. To overcome this difficulty, we increase the Raman-scattering signal by approximately nine orders of magnitude by placing the tip of our probe in direct contact with the specimen studied. This way, we can obtain good-quality images in less than 1s.

Figure 1 shows our surface-enhanced Raman-scattering (SERS) nanoimaging probe. The SERS probe, using light of 632.8nm wavelength (in the red portion of the visible spectrum) is capable of obtaining 20\(\mu\)m-diameter images of samples with approximately 50nm spatial resolution. These nanoimaging probes combine the molecular specificity and sensitivity of SERS with multispectral imaging to provide the potential for molecular-specific imaging at the macromolecular scale.

The probes are fabricated from coherent fiber-optic imaging bundles containing 30,000 fiber elements. They are tapered on one end in a heated pulling process to provide an array of subwavelength apertures for near-field imaging. Following tapering, the bundles are etched with hydrofluoric acid to produce a uniform array of spikes, six around each fiber element: see Figure 1(C).\(^4\) These cladding spikes then have a SERS-active metal deposited onto them by vacuum evaporation. This process results in fibers with enhancement factors of \(10^9\) to \(10^{11}\), with less than 2% variation in enhancement at any location across the bundle\(^5\) (see Figure 2). Since each of the individual fiber elements in the bundle retains its ability to provide total internal reflection of near-field signals, the resolution is slightly better than twice the diameter of the individual fiber elements.

To obtain nanoscale-resolution chemical images, the tapered active tip of the probe collects SERS signals reflected from the sample it is in contact with and transmits the data to the untapered end. There, an acousto-optic tunable-filter (AOTF)-based multispectral imaging system with 7.5cm\(^{-1}\) spectral resolution provides molecular specificity.\(^6\) Because of the large enhancement factors, it is possible to obtain images with millisecond temporal and sub-100nm spatial resolution.

Figure 3 shows an example of such multispectral chemical images. They show a gelatin sample that has been prepared with the dye brilliant cresyl blue (BCB) dispersed throughout the gelatin, while benzoic acid has been specifically added to the edges. Figure 3(A) corresponds to a Rayleigh-scatter image, revealing the sample’s shape. Figure 3(B) corresponds to a SERS image obtained at the 1002cm\(^{-1}\) ring breathing mode of benzoic acid, revealing its localized presence. Leaving the probe in contact with the sample and simply changing the AOTF-transmitted frequency to a Raman shift of 580cm\(^{-1}\), corresponding to the major band of BCB, it is possible to see the dispersed dye throughout the gelatin: see Figure 3(C). Each image shown in Figure 3 corresponds to a 500ms exposure and provides extremely high signal-to-noise ratios, even at these nanoscale spatial resolutions. By obtaining a series of images, it is possible to monitor the diffusion of the particular chemical species of interest with subsecond temporal resolution.

Our SERS nanoimaging system makes it possible to produce chemical-specific imaging of the surface of biological samples, allowing for unprecedented real-time views of the distribution and interaction of label-free specimens. With the SERS probe, it is possible to both image specific molecules and monitor the breaking and formation of chemical bonds during molecular interactions, because the signals monitored correspond to vibrational energies of the molecule. In the future, we hope to use this ultrahigh-resolution chemical-imaging probe to obtain dynamic images of the chemical composition of samples such as the lipids and integral membrane proteins and receptors in cellular surfaces. Such images of 20\(\mu\)m-diameter areas, with 20 to 50nm spatial resolution and chemical specificity, will provide an unprecedented understanding of many biochemical interactions, such as those involved in the immune-cell receptor response.

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References