NANOBIOTECHNOLOGY

Imaging without labels

Researchers report an optical method to detect and image single proteins without using any labels.

Labels are indispensable molecular visualization tools, but ultimately they are decoys, not direct signals, and they can interfere with molecule function. Some scientists have therefore been interested in developing technologies for directly detecting and imaging single molecules.

Marek Piliarik and Vahid Sandoghdar of the Max Planck Institute for the Science of Light in Germany recently reported a direct optical sensing technique that can detect and image individual proteins without any labeling. This feat is made possible by a method called iSCAT, or interferometric detection of scattering.

In iSCAT, a laser beam is used to illuminate a glass substrate. The light is partially reflected by the surface, and any individual molecules on the substrate cause the light to be scattered. The reference light and the scattered light are detected by a camera as coherent, interfering waves. Sandoghdar's group established the iSCAT principle in previous work, and they showed in *Nature Methods* in 2009 that it could be used to track single virus particles with a diameter of ~45 nanometers. However, it was previously thought that proteins were just too small to scatter light to a measurable degree.

In new work reported in Nature Communications, Piliarik and Sandoghdar made a small but crucial tweak to the method: they added a step to subtract background noise generated by the surface roughness of the substrate. This provided the needed sensitivity to detect individual proteins including fibrinogen, mouse immunoglobulin IgG1, carcinoembryonic antigen and bovine serum albumin—average-sized proteins that are about 1,000 times smaller than an average-sized virus.

To demonstrate the method's potential as a practical biosensing tool, the researchers decorated the substrate with anti-IgG1 antibodies and thus detected varying concentrations of IgG1. They also were able to specifically detect carcinoembryonic antigen in the presence of a high concentration of IgG1.

Perhaps most interestingly, iSCAT has the ability to localize individual molecules with nanometer precision. Piliarik and Sandoghdar obtained super-resolution images of fibrinogen association, demonstrating iSCAT's capability as a label-free super-localization technique—a potential that could be quite powerful indeed.

Allison Doerr

RESEARCH PAPERS

Piliarik, M. & Sandoghdar, V. Direct optical sensing of single unlabelled proteins and super-resolution imaging of their binding sites. *Nat. Commun.* **5**, 4495 (2014).