OPTICAL SENSING OF SINGLE SMALL PROTEINS

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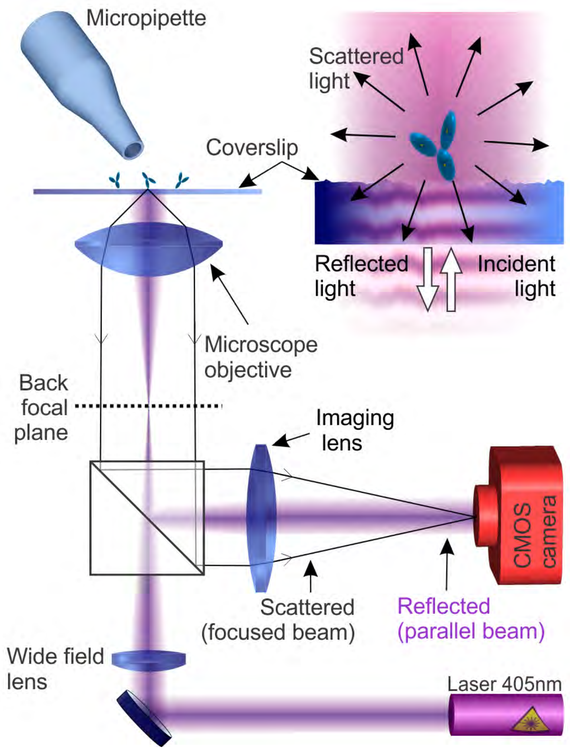
nano-optics -9

INTRODUCTION

We report on the direct label-free detection and imaging of individual proteins via the interference of the light created by Rayleigh scattering and the reflection of the incident laser beam (iSCAT). We demonstrate detection of range of different protein sizes including a specific detection of cancer markers. Furthermore, we introduce super-resolution imaging of protein binding with nanometer localization precision. The ease of iSCAT instrumentation promises a breakthrough for industrial and clinical biosensing as well as fundamental laboratory experiments.

PRINCIPLE OF OPERATION

Interference of the reference beam and scattered beam results in a cross term linearly dependent on E_{sca}, which is proportional to the polarizability of the nano-object. The signal-to-noise ratio (SNR) is intrinsically limited by the shot noise of the illumination [1].



$$I_{det} = |E_{ref}|^2 + |E_{sca}|^2 + 2Re\{E_{ref}{}^* E_{sca}\}$$

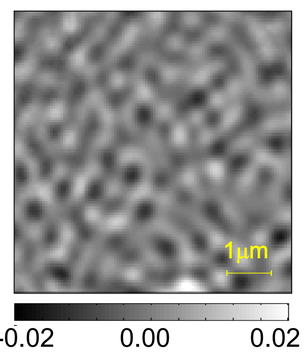
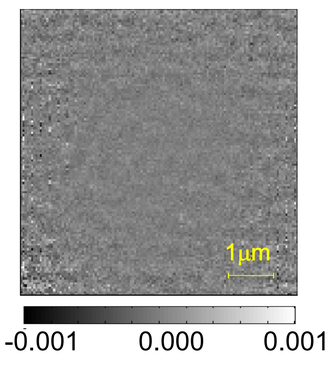


Fig: *iSCAT image of surface roughness of functionalized glass.* ***SNR > 300.*** ***Detection limit contrast ~ 1e-4.***

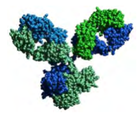
EXPERIMENT

Background fluctuations due to the surface roughness are subtracted revealing the shot-noise-limited nature of background-corrected images (see fig on right). As a result, subtracting consecutive images can reveal the dynamics of analyte molecules arriving at the surface [2].

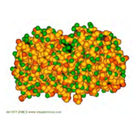


Gold nanoparticle

Fibrinogen 350kDa



Antibody (IgG) 150kDa



Albumin 50kDa

Effective diameter

8 nm

6 nm

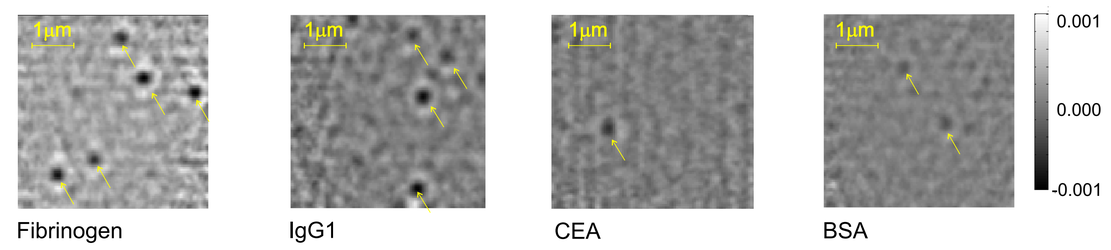
4 nm

Scattering cross section in water

3E-6 µm² **1E-11 µm²** **2E-12 µm²** **2E-13 µm²**

SINGLE PROTEIN BIOSENSING AND IMAGING

A glass substrate functionalized with NHS reactive polymer brush was used to capture protein molecules of different size on the sensor surface.



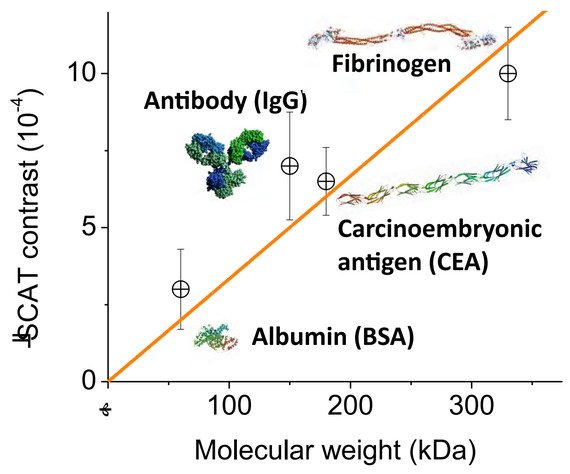


Fig: *Relationship between the measured iSCAT contrast and the molecular masses of different proteins.*

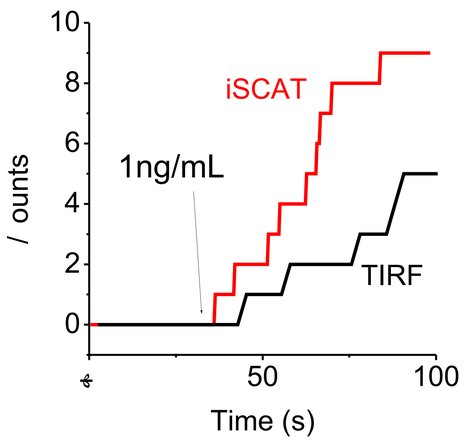


Fig: *Comparison of IgG1 detection kinetics obtained with iSCAT and fluorescence measurement.*

SUPER-RESOLUTION MICROSCOPY OF PROTEIN BINDING SITES

Direct imaging biosensor registers the spatial coordinates of each molecule with nanometer precision.

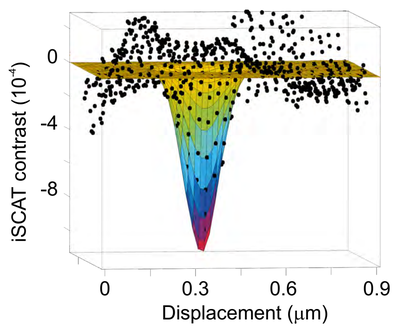
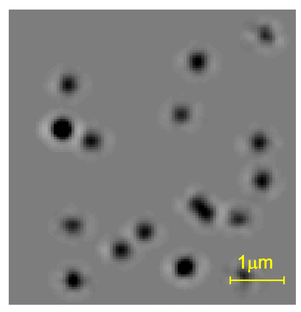
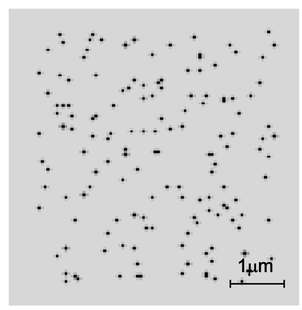


Fig: *An iSCAT image of a molecule fitted with a two-dimensional Gaussian, yielding a localization precision of 5 nm.*



Recorded bindings after 15 seconds

Fig: *Images of individual molecules accumulated in 15 s*



150 localized bindings after 150 second

Fig: Superresolution image, showing the localized positions of the binding events accumulated in 150 s.

SUMMARY

It is possible to detect the Rayleigh scattering of a single unlabeled biomolecule in direct optical measurements. This approach can count proteins, is compatible with a wide range of functionalization methods, provides nanoscopic spatial information, can be easily parallelized, and is not limited to confined optical fields [4].

REFERENCES

[1] K. Lindfors, T. Kalkbrenner, P. Stoller, V. Sandoghdar, Phys. Rev. Lett. 93, 037401-1 (2004)
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[4] M. Piliarik V. Sandoghdar, arXiv:1310.7460 (2013).