Microscopy TODAY

Coming Events

2011

TMS 2011 Annual Meeting
February 27–March 3, 2011
San Diego, CA
www.tms.org

PITTCON
March 13–18, 2011
Atlanta, GA
www.pittcon.org

Histochemistry 2011
March 30–April 1, 2011
Woods Hole, MA
www.histochemistry2011.org

FASEB Experimental Biology
April 9–13, 2011
Washington, DC
eb@faseb.org

MRS Spring Meeting
April 25–29, 2011
San Francisco, CA
www.mrs.org/spring2011

EMAS 2011
May 15–19, 2011
Angers, France
www.emas-web.net

IUMAS-V
May 22–27, 2011
Inchon, South Korea
www.iumas5.org

Inter/Micro: 62nd Conference
June 11–15, 2011
Chicago, IL
www.mcri.org/home/section/101/intermicro

Microscopy & Microanalysis 2011
August 7–11, 2011
Nashville, TN

FEMMS 2011
September 18–23, 2011
Sonoma County, CA
www.femms2011.llnl.gov

CIASEM 2011
September 25–30, 2011
Mérida, Mexico
www.ciasem.com

2012

Microscopy & Microanalysis 2012
July 29–August 2, 2012
Phoenix, AZ

2013

Microscopy & Microanalysis 2013
August 4–8, 2013
Indianapolis, IN

2014

Microscopy & Microanalysis 2014
August 3–7, 2014
Hartford, CT

More Meetings and Courses
Check the complete calendar near the back of this magazine and in the MSA journal *Microscopy and Microanalysis*.

Carmichael's Concise Review

A (Relatively) Simple Method to Visualize Single Molecules

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Fluorescence microscopy can be used to study certain single molecules in solution or attached to a surface. Two conflicting challenges to overcome are: (1) to image freely moving molecules for long times and (2) to image immobilized single molecules when there is a highly fluorescent background. The fact that these two goals are inversely related is illustrated by epifluorescence, which is good for observing freely diffusing molecules but poor for detecting single molecules, whereas the reverse is true for zero-mode waveguides. Plus, these and other techniques require elaborate (read: expensive) equipment with computerized controls. Sabrina Leslie, Alexander Fields, and Adam Cohen have developed an ingenious (relatively) simple technique that can image freely moving single molecules [1].

Their technique of convex lens-induced confinement (CLIC) restricts molecules to a tapered gap of nanoscale depth, formed between a plano-convex lens and a flat coverslip. The shallow depth of the imaging volume is perpendicular to the imaging plane. The confinement reduces the vertical dimension of the imaging volume and thereby provides a 20-fold greater rejection of background fluorescence than is achieved with total internal reflection fluorescence imaging. By preventing the molecules from diffusing out of the focal plane, the device yields an approximately 10,000-fold greater diffusion-limited observation time per molecule than is achieved with confocal fluorescence correlation spectroscopy.

The design of the microscope is theoretically simple. A convex lens, curved side down, rests on the surface of a nearly perfectly flat coverslip. The gap between the lens and the coverslip is zero at the point of contact and increases with the radial distance from this point. The gap is dependent on the radius of curvature of the lens that can be varied with the lens used. The lens is mounted at the end of a lever, and a micrometer on the other end of the lever is used to gently lower the lens to the coverslip. The lens is placed in the optical path of an inverted fluorescence microscope.

To demonstrate some of the capabilities of CLIC, Leslie et al. measured single immobilized molecules in the presence of freely diffusing fluorescent molecules, counted transmembrane proteins in freely diffusing lipid vesicles, and directly measured the size and compressibility of double-stranded DNA molecules. They were able to observe DNA oligonucleotides labeled with a single fluorophore and immobilized on a coverslip in the presence of up to 2 μM concentration of the same dye species freely diffusing in solution. As for observing freely diffusing fluorescent molecules, the observation times were limited either by photobleaching or lateral diffusion out of the field of view. Vesicles could be observed for up to 25 seconds, and the results suggested that the protein probe inserts into vesicles as a monomer. The third feasibility test took advantage of the fact that molecules are physically excluded from regions in which the gap height is less than the molecular diameter. This principle was applied to determine the diameter of plasmid DNA and protein molecules on the nanometer scale. In contrast to "hard" spheres, these DNA-protein complexes appeared to be flexible, and some could "squeeze" into the narrow spaces under the lens, although these conformations were entropically disfavored. This demonstrated that CLIC could not only estimate molecular size but also estimate how some interacting molecules could change their configuration and therefore their size.

This novel adaptation of basic optical physics demonstrates how a (relatively) simple microscopic setup can accomplish sophisticated measurements that heretofore could only be determined with methods that are much more complicated and correspondingly expensive. This new technology developed by Leslie et al. promises to be extremely useful for single-molecule microscopy, particularly for tracking the diffusion of single molecules. A challenge that remains is the interpretation of what is observed during diffusion of single molecules in a restricted space of varying geometry. The exact form of the geometrical constraint and its effect on the diffusion of a particle may be difficult to obtain. CLIC will become a very practical tool if it is able to provide quantitative data for observed processes of diffusion [2].

References

[1] SR Leslie, AP Fields, and AE Cohen, *Anal Chem* 82 (2010) 6224–29.
[2] The authors gratefully acknowledge Dr. Adam Cohen for reviewing this article.

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