Review of Scientific Instruments

Vol. 84, no. 5

Wide-area scanner for high-speed atomic force microscopy

Hiroki Watanabe¹, Takayuki Uchihashi¹,², Toshihide Kobashi¹, Mikihiro Shibata³, Jun Nishiyama³, Ryoei Yasuda³,⁴, and Toshio Ando¹,²
¹Department of Physics, College of Science and Engineering, Kanazawa University, Kanazawa 920-1192, Japan
²Bio-AFM Frontier Research Center, College of Science and Engineering, Kanazawa University, Kanazawa 920-1192, Japan
³Department of Neurobiology, Duke University Medical Center, Durham, North Carolina 27710, USA
⁴Max Planck Florida Institute, Jupiter, Florida 33458, USA

High-speed atomic force microscopy (HS-AFM) has recently been established. The dynamic processes and structural dynamics of protein molecules in action have been successfully visualized using HS-AFM. However, its maximum scan ranges in the X- and Y-directions have been limited to ∼1 µm and ∼4 µm, respectively, making it infeasible to observe the dynamics of much larger samples, including live cells. Here, we develop a wide-area scanner with a maximum XY scan range of ∼46 × 46 µm² by magnifying the displacements of stack piezoelectric actuators using a leverage mechanism. Mechanical vibrations produced by fast displacement of the X-scanner are suppressed by a combination of feed-forward inverse compensation and the use of triangular scan signals with rounded vertices. As a result, the scan speed in the X-direction reaches 6.3 mm/s even for a scan size as large as ∼40 µm. The nonlinearity of the X- and Y-piezoelectric actuators’ displacements that arises from their hysteresis is eliminated by polynomial-approximation-based open-loop control. The interference between the X- and Y-scanners is also eliminated by the same technique. The usefulness of this wide-area scanner is demonstrated by video imaging of dynamic processes in live bacterial and eukaryotic cells.

Sensorless enhancement of an atomic force microscope micro-cantilever quality factor using piezoelectric shunt control

M. Fairbairn and S. O. R. Moheimani
School of Electrical Engineering and Computer Science, The University of Newcastle, Callaghan, NSW 2308, Australia

The image quality and resolution of the Atomic Force Microscope (AFM) operating in tapping mode is dependent on the quality (Q) factor of the sensing micro-cantilever. Increasing the cantilever Q factor improves image resolution and reduces the risk of sample and cantilever damage. Active piezoelectric shunt control is introduced in this work as a new technique for modifying the Q factor of a piezoelectric self-actuating AFM micro-cantilever. An active impedance is placed in series with the tip oscillation voltage source to modify the mechanical dynamics of the cantilever. The benefit of using this control technique is that it removes the optical displacement sensor from the Q control feedback loop to reduce measurement noise in the loop and allows for a reduction in instrument size.

Vol. 84, no. 6
Nothing of interest.
Proceedings of the National Academy of Sciences of the USA
Vol. 110, no. 23,24,25,26,27,28
Nothing of interest.
Modeling Stochastic Kinetics of Molecular Machines at Multiple Levels: From Molecules to Modules

Debashish Chowdhury

Department of Physics, Indian Institute of Technology, Kanpur, India

A molecular machine is either a single macromolecule or a macromolecular complex. In spite of the striking superficial similarities between these natural nanomachines and their man-made macroscopic counterparts, there are crucial differences. Molecular machines in a living cell operate stochastically in an isothermal environment far from thermodynamic equilibrium. In this mini-review we present a catalog of the molecular machines and an inventory of the essential toolbox for theoretically modeling these machines. The tool kits include 1), nonequilibrium statistical-physics techniques for modeling machines and machine-driven processes; and 2), statistical-inference methods for reverse engineering a functional machine from the empirical data. The cell is often likened to a microfactory in which the machineries are organized in modular fashion; each module consists of strongly coupled multiple machines, but different modules interact weakly with each other. This microfactory has its own automated supply chain and delivery system. Buoyed by the success achieved in modeling individual molecular machines, we advocate integration of these models in the near future to develop models of functional modules. A system-level description of the cell from the perspective of molecular machinery (the mechanome) is likely to emerge from further integrations that we envisage here.

Tracking Image Correlation: Combining Single-Particle Tracking and Image Correlation

A. Dupont†, K. Stirnnagel‡, §, D. Lindemann‡, § and D.C. Lamb†, ¶

† Department of Chemistry, Center for NanoScience and Center for Integrated Protein Science Munich, Ludwig Maximilians Universität, Munich, Germany
‡ Institute of Virology, Medizinische Fakultät “Carl Gustav Carus”, Technische Universität Dresden, Dresden, Germany
§ CRTD/DFG-Center for Regenerative Therapies Dresden-Cluster of Excellence, Technische Universität Dresden, Dresden, Germany
¶ Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL

The interactions and coordination of biomolecules are crucial for most cellular functions. The observation of protein interactions in live cells may provide a better understanding of the underlying mechanisms. After fluorescent labeling of the interacting partners and live-cell microscopy, the colocalization is generally analyzed by quantitative global methods. Recent studies have addressed questions regarding the individual colocalization of moving biomolecules, usually by using single-particle tracking (SPT) and comparing the fluorescent intensities in both color channels. Here, we introduce a new method that combines SPT and correlation methods to obtain a dynamical 3D colocalization analysis along single trajectories of dual-colored particles. After 3D tracking, the colocalization is computed at each particle’s position via the local 3D image cross correlation of the two detection channels. For every particle analyzed, the output consists of the 3D trajectory, the time-resolved 3D colocalization information, and the fluorescence intensity in both channels. In addition, the cross-correlation analysis shows the 3D relative movement of the two fluorescent labels with an accuracy of 30 nm. We apply this method to the tracking of viral fusion events in live cells and demonstrate its capacity to obtain the time-resolved colocalization status of single particles in dense and noisy environments.
Single-Molecule Motility: Statistical Analysis and the Effects of Track Length on Quantification of Processive Motion

Andrew R. Thompson, Gregory J. Hoeprich and Christopher L. Berger

Department of Molecular Physiology and Biophysics, University of Vermont, Burlington, Vermont

In vitro, single-molecule motility assays allow for the direct characterization of molecular motor properties including stepping velocity and characteristic run length. Although application of these techniques in vivo is feasible, the challenges involved in sample preparation, as well as the added complexity of the cell and its systems, result in a reduced ability to collect large datasets, as well as difficulty in simultaneous observation of the components of the motility system, namely motor and track. To address these challenges, we have developed simulations to characterize motility datasets as a function of sample size, processive run length of the motor, and distribution of track lengths. We introduce the use of a simple bootstrapping technique that allows for the quantification of measurement uncertainty and a Monte Carlo permutation resampling scheme for the measurement of statistical significance and the estimation of required sample size. In addition, we have found that, despite conventional wisdom, the measured characteristic run length is directly coupled to the characteristic track length that describes the microtubule length distribution. To be able to make comparisons between motility experiments performed on different track populations as well as make measurements of motility when motors and tracks cannot be simultaneously resolved, we have developed a theoretical framework for the determination of the effect that track length has on observed characteristic run lengths. This shows good agreement with in vitro motility experiments on two kinesin constructs walking on microtubule populations of different characteristic track lengths.

Quantitative Analysis of Three-Dimensional Fluorescence Localization Microscopy Data

Dylan M. Owen, David J. Williamson, Lies Boelen, Astrid Magenau, Jérémie Rossy and Katharina Gaus

† Centre for Vascular Research and Australian Centre for Nanomedicine, University of New South Wales, Sydney, Australia
‡ Department of Medicine, Imperial College London, London, United Kingdom

Identifying the three-dimensional molecular organization of subcellular organelles in intact cells has been challenging to date. Here we present an analysis approach for three-dimensional localization microscopy that can not only identify subcellular objects below the diffraction limit but also quantify their shape and volume. This approach is particularly useful to map the topography of the plasma membrane and measure protein distribution within an undulating membrane.

Quantifying the Diffusion of Membrane Proteins and Peptides in Black Lipid Membranes with 2-Focus Fluorescence Correlation Spectroscopy

Kerstin Weiß, Andreas Neef, Qui Van, Stefanie Kramer, Ingo Gregor and Jörg Enderlein

III. Institute of Physics, Georg-August-University Göttingen, Göttingen, Germany
Protein diffusion in lipid membranes is a key aspect of many cellular signaling processes. To quantitatively describe protein diffusion in membranes, several competing theoretical models have been proposed. Among these, the Saffman-Delbrück model is the most famous. This model predicts a logarithmic dependence of a protein's diffusion coefficient on its inverse hydrodynamic radius \(D \propto \ln \frac{1}{R}\) for small radius values. For large radius values, it converges toward a \(D \propto \frac{1}{R}\) scaling. Recently, however, experimental data indicate a Stokes-Einstein-like behavior \(D \propto \frac{1}{R}\) of membrane protein diffusion at small protein radii. In this study, we investigate protein diffusion in black lipid membranes using dual-focus fluorescence correlation spectroscopy. This technique yields highly accurate diffusion coefficients for lipid and protein diffusion in membranes. We find that despite its simplicity, the Saffman-Delbrück model is able to describe protein diffusion extremely well and a Stokes-Einstein-like behavior can be ruled out.
Visualization of Plasma Membrane Compartmentalization by High-Speed Quantum Dot Tracking

Mathias P. Clausen and B. Christoffer Lagerholm *
MEMPHYS – Center for Biomembrane Physics and DaMBIC – Danish Molecular Biomedical Imaging Center, University of Southern Denmark, DK-5230 Odense M, Denmark

In this study, we have imaged plasma membrane molecules labeled with quantum dots in live cells using a conventional wide-field microscope with high spatial precision at sampling frequencies of 1.75 kHz. Many of the resulting single molecule trajectories are sufficiently long (up to several thousand steps) to allow for robust single trajectory analysis. This analysis indicates that a majority of the investigated molecules are transiently confined in nanoscopic compartments with a mean size of (100-150 nm)$^2$ for a mean duration of 50-100 ms.

Vol. 13, no. 7
Nothing of interest
Multiple Hypothesis Tracking for Cluttered Biological Image Sequences

Chenouard, N.
New York University School of Medicine, New York
Bloch, I.; Olivo-Marin, J.

In this paper, we present a method for simultaneously tracking thousands of targets in biological images sequences, which is of major importance in modern biology. The complexity and inherent randomness of the problem lead us to propose a unified probabilistic framework for tracking biological particles in microscope images. The framework includes realistic models of particle motion and existence, and of fluorescence image features. For the track extraction process per se, the very cluttered conditions motivate the adoption of a multiframe approach which enforces tracking decision robustness to poor imaging conditions and to random target movements. We tackle the large-scale nature of the problem by adapting the Multiple Hypothesis Tracking algorithm to the proposed framework, resulting in a method with a favorable trade-off between the model complexity and the computational cost of the tracking procedure. When compared to the state-of-the-art tracking techniques for bioimaging, the proposed algorithm is shown to be the only method providing high quality results despite the critically poor imaging conditions and the dense target presence. We thus demonstrate the benefits of advanced Bayesian tracking techniques for the accurate computational modeling of dynamical biological processes, which is promising for further developments in this domain.