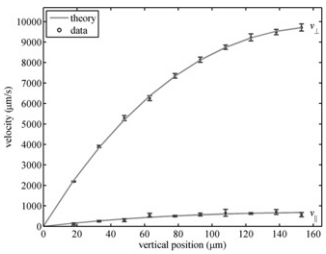
of molecules in the sample. Current fluorescence lifetime technology is too slow by **a factor of at least 1000** to detect this decay accurately within the millisecond timescale of a typical biochemical transient. Our system, based on a direct acquisition data collection approach, records the entire fluorescence decay with S/N > 100 from a single pulse form a 10 kHz microlaser. Using this approach, we can resolve individual fluorescence lifetime components comprising as little as 10% of a complex multi-exponential fluorescence decay. When used to monitor structural transitions by time resolved FRET, we are capable of isolating individual structural states within complex structural ensembles. Using this approach we were able to simultaneously monitor the binding of myosin to different classes of binding sites on actin filaments. This type of analysis is not possible with conventional fluorescence based stopped flow measurements.

3031-Pos

Dual-Focus Confocal Microscopy for Flow and Brightness Measurements

Tyler J. Arbour[1,2], Anastasia Loman[3], Ingo Gregor[3], Jörg Enderlein[3].
[1]Molecular Foundry, Lawrence Berkeley National Lab, Berkeley, CA, USA, [2]Department of Earth and Planetary Science, UC Berkeley, Berkeley, CA, USA, [3]Georg-August-Universität Göttingen, Göttingen, Germany.

Fluorescence correlation spectroscopy (FCS) is a confocal microscopy method mostly used to measure the dynamic properties of molecules in solution and in biological membranes. In 2007, Dertinger *et al.* introduced a new two-beam confocal method called *dual-focus fluorescence correlation spectroscopy* (2f-FCS). [1] This development has been extremely important to the field because it overcomes major artifacts that limit conventional FCS as a quantitative measurement technique. In this poster we present two applications of 2f-FCS. One is measuring the velocity profile in a microfluidic channel with high accuracy and sub-micrometer spatial resolution. Second, we present a scheme to measure the absolute brightness of single fluorescent molecules, and thereby directly observe the heterogeneity intrinsic to biological systems. Measured velocity profile in a 300-by-300 micrometer-squared capillary.



1. Dertinger, T., et al., *ChemPhysChem*, 2007. **8**(3): p. 433-443.

3032-Pos

Dual-Focus Fluorescence Correlation Spectroscopy: Measuring Translational and Rotational Diffusion of Biomolecules

Joerg Enderlein[1], Anastasia Loman[1], Iris von der Hocht[2], Claus Bernhard Mueller[1], Qui Van[1], Ingo Gregor[1], Sebastian Springer[3].
[1]3rd Institute of Physics, Goettingen, Germany, [2]Max-Planck Institute of Biophysics, Frankfurt, Germany, [3]Jacobs University Bremen, Bremen, Germany.

We present numerous applications of the recently developed dual-focus fluorescence correlation spectroscopy (2fFCS) to the measurement of translational diffusion of proteins and protein complexes. The method is applied to measuring Ca^{2+}-binding curves of wild-type and mutant of the proteins calmodulin and recoverin. 2fFCS is also capable of quantifying the conformational flexibility of macromolecular complexes, which is exemplified on the peptide binding of the major histocompatibility complex I (MHC I). Furthermore, we extended 2fFCS to measure fluorescence correlation at the nanosecond time scale, allowing also for measuring rotational diffusion. We performed a comparative study of translational and rotational diffusion (and related hydrodynamics size) of proteins, and present results for several globular proteins (BSA, human serum albumin, aldolase, ovalbumin). In all cases, measurements are performed at pico- to nanomolar sample concentrations, and with an accuracy of determining hydrodynamic size of a few percent. For performing 2fFCS measurements, a protein or protein complex needs only unspecific fluorescent labeling which is easily accomplished with commercially available dyes. Thus it is hoped that 2fFCS becomes a widely used and easy to handle technique in biophysical research.

References

1. Dertinger T.; Loman A.; Ewers B.; Müller C.B.; Krämer B.; Enderlein J., *Opt. Expr.* **16**, 2008, 14353-14368.
2. Müller C.B.; Loman A.; Pacheco V.; Koberling F.; Willbold D.; Richtering W.; Enderlein J., *Eur. Phys. Lett.* **83**, 2008, 46001.
3. Dertinger T.; Pacheco V.; von der Hocht I.; Hartmann R.; Gregor I.; Enderlein J., *ChemPhysChem* **8**, 2007, 433-443.

3033-Pos

Spatio-Temporal Control in Multiphoton Fluorescence Laser-Scanning Microscopy

Debabrata Goswami, Arijit Kumar De.
Indian Institute of Technology Kanpur, Kanpur, India.

Confocal microscopy, one of the modern day fluorescence laser-scanning microscopic techniques, is suffered from having sufficient out-of-focus signal. On the other hand, in multiphoton microscopy ultrafast laser pulses are commonly used to circumvent low multiphoton absorption cross-sections of common fluorophores; due to broad overlapping two-photon absorption (TPA) spectra of fluorophores and large spectral bandwidth of a short pulse, simultaneous excitation of many fluorophores is common demanding selective excitation of individual fluorophores if required. Addressing the first issue, our recent work has shown that ultrafast one-photon pulsed illumination leads to increased signal-to-noise ratio by controlling the fluorophore photo-physics.[1-2] Considering TPA, we have demonstrated that photo-thermal corruption due to pulse pile-up effect is largely solvent-mediated and a rather slow process which can be taken care of by simple intensity modulation of a pulse-train.[3-4] We have recently shown how precise delay between pair of ultrafast pulses can lead to possible selective excitation in microscopy.[5] We have also demonstrated how gigantic peak-power of a femto-second laser pulse (with rather low average power) leads to stable optical trapping of latex nano-particles which is otherwise impossible with continuous-wave excitation (at the same average power).[6] All these cutting-edge topics will be discussed in the presentation.

1. A K De and D Goswami, J. Fluorescence, **19**, 931 (2009).
2. A K De and D Goswami, J Microscopy, **233**, 320 (2009).
3. A K De and D Goswami, J. Fluorescence, **19**, 381 (2009).
4. A K De and D Goswami, J. Microscopy, **235**, 119 (2009).
5. A K De and D Goswami, J. Biomed. Opt., in press.
6. A K De, D Roy, A Dutta and D Goswami, Appd. Opt., **48**, G33 (2009).

3034-Pos

Lifetime Resolved Fluorescence Fluctuation Spectroscopy

Peng Guo, Neil Anthony, Keith M. Berland.
Emory University, Atlanta, GA, USA.

Fluorescence correlation spectroscopy (FCS) has been widely used investigate molecular dynamics and interactions in biological systems. FCS typically resolves the component species of a sample either through differences in diffusion coefficient or molecular brightness. Diffusion based assays currently have a major limitation which requires that the diffusion coefficients of component species in a sample must be substantially different in order to be resolved. This criterion is not met in many important cases, such as when molecules of similar molecular weight bind to each other. This limitation can be overcome, and resolution of FCS measurements enhanced, by combining FCS measurements with measurements of fluorescence lifetimes. By using of global analysis on simultaneously acquired FCS and lifetime data we show that we can dramatically enhance resolution in FCS measurements, and accurately resolve the concentration and diffusion coefficients of multiple sample components even when their diffusion coefficients are identical provided there is a difference in the lifetime of the component species. We show examples of this technique using both simulations and experiments. It is expected that this method will be of significance for binding assays studying molecular interactions.

3035-Pos

Measuring Molecular Mobility with Fluorescence Anisotropy Macro Imaging

Claudia Y. Lee[1], Andrew Zepfel[2], LeeAnn Allen[2], Sean Gallagher[1].
[1]UVP, LLC, Upland, CA, USA, [2]The Vaccine Development Institute, Pitzer College, Claremont, CA, USA.

Macro imaging systems with fields of view of tens of centimeters across are required for the whole body imaging of plants and small animals, but rarely have such systems been able to deliver molecular level information. We present a novel advancement to camera and lamp based macro imaging systems which introduces the capability of steady state anisotropy imaging. Our new fluorescence anisotropy macro imaging (FAMI) system provides the capability of rapidly and easily measuring molecular mobility both in-vitro and in-vivo. We show that the read-out on in-vitro assays can be highly quantitative. We also used FAMI to non-invasively examine the effects of temperature on live plants carrying fluorescent probes. The results indicate that low temperatures increase the internal viscosity of plants, and that the degree of increase is related to the anatomy of the plants. Our finding on plants verifies the results of others which showed that temperature and the water consumption of plant tissue are positively correlated and dependent on the tissue type. We also discuss further applications of FAMI to Förster Resonance Energy Transfer (FRET)-based sensors in assays and live animals.