For a solution, our Kalman-filter-inspired approach, Structure-from-FRET, generates an atomistic protein trajectory most likely responsible for the observed smFRET distance constraint: a statistical inference inverse problem.
As a general analysis and visualization tool, we apply the method to the model hinge protein adenylate kinase. Millisecond-resolution FRET efficiencies indicate approximate Lid/Core distance traces, which constrain inference of global protein geometry. Resultant trajectories span extended temporal (seconds) and conformational (tens of Angstroms) scales, without imposed potentials or perturbations between reference states. Here, the inter-residue distances constrain a state-space estimation with backbone atom coordinates as variables. Experimental (constraint) uncertainty is explicitly considered and each update step maximizes spatial information from previous 'frames'. We compare generated trajectories against those from interpolative or elastic network approaches, but also with known PDB structures and long run MD simulations. Results suggest Structure-from-FRET is reliable for modeling novel biocatalysts, inhibitor targets, or other proteins for which multiple structures or atomistic simulations are unavailable.

2582-Pos Board B568
Single Particle Tracking of QD-IgE in Hyperspectral Fluorescent Images
Patrick J. Cutler, Michael Malik, Sheng Liu, Fang Huang, Jason Byars, Diane Lidke, Keith Lidke.
In recent years, single particle tracking of quantum dot (QD) labeled membrane proteins has yielded significant biological insights. In conventional single particle tracking, the experimentalist is limited to 2-3 colors of quantum dots and sparse labeling density. In order to circumvent these limitations, we have developed a hyperspectral line scanning microscope with the ability to acquire hyperspectral images (128 spectral channels over 500 to 800 nm) at a rate of 30 frames per second. Hyperspectral images possess a wealth of information that can be used to improve multicolor single particle localization and tracking. Here we describe new algorithms for the analysis of hyperspectral images for single particle localization and trajectory building.
In conventional single particle localization, a two dimensional Gaussian is often used to estimate the point spread function of the microscope. In hyperspectral images, the spectral characteristics of single QDs can be estimated using a Gaussian distribution in the wavelength dimension. Combining this with a two dimensional Gaussian estimate for the microscope point spread function gives a three dimensional Gaussian estimate for the position of a single quantum dot in both spatial dimensions and the spectral dimension. We extend our high speed two dimensional [1] fitting to three dimensions and implement the routine on GPU architecture giving more than an order of magnitude increase in analysis speed over an equivalent CPU based implementation.
Analysis of hyperspectral data allows the relatively unique spectral signature of each QD to be used to help resolve indeterminacy during connection of points into trajectories, allowing for single particle tracking in relatively densely labeled samples. The hyperspectral line scanning microscope and single particle tracking algorithms are demonstrated with experiments of QD- IgE bound to Fc (epsilon)RI on live cells.
Smith, Nat. Methods 7, 373-375 2010.

2583-Pos Board B569
Optimal Estimation of Location and Orientation of Myosin V Lever Arm from Focused Diffraction-Limited Images of Single, Double-Bound Fluorophore
Jongmin Sung, Kim I. Mortensen, Henrik Flyvbjerg, James A. Spudich.
In "... the most complete theoretical description of localization microscopy to date ..." (1,2), we showed how to estimate location and orientation of fixed, fluorescent probes from focused images. We demonstrated the superiority of the theoretical point spread function over a 2D Gaussian and of maximum likelihood estimation over least squares fitting. Here we compare our approach to popular methods that use de-focused imaging and/or 2D Gaussians (3). Experimentally, we verify theoretical expectations using internally labeled dsDNA as benchmark. Then we show from motility assays that our method provides high-resolution angular and positional information about myosin V dynamics. Specifically, bifunctional rhodamine-labeled calmodulin is exchanged to the lever arm of myosin V. The orientation of the dye shows two angular states during stepping, which very stably are separated by ~70 deg. This result is consistent with the 70 deg rotation of the lever arm between pre- and post-stroke states.
References:
1. Kim I. Mortensen, L. Stirling Churchman, James A. Spudich, and Henrik Flyvbjerg: Optimized localization-analysis for single-molecule tracking and super-resolution microscopy. Nature Methods 7, 377-381 (2010).
2. Characterization by Daniel R. Larson: The economy of photons. News and Views, Nature Methods 7, 357-359 (2010).
3. Erdal Toprak, Joerg Enderlein, Sheyum Syed, Sean A. McKinney, Rolfe G. Petschek, Taekjip Ha, Yale E. Goldman, and Paul R. Selvin: Defocused orientation and position imaging (DOPI) of myosin V. PNAS 103, 6495-6499 (2006).

2584-Pos Board B570
Probing Diffusion in Live E. coli using Single-Molecule Tracking
Benjamin P. Bratton, Somenath Bakshi, James C. Weisshaar.
Measurement of protein diffusion *in vivo* probes the effects of bacterial cytoplasm on protein mobility. We used single-particle tracking (SPT) and fluorescence recovery after photobleaching (FRAP) to study diffusion of the photoactivatable protein Kaede inside live *E. coli* cells. Photo-activation of the molecule Kaede enables us to study only one or two fluorescent molecules at a time. The non-spherical shape of *E. coli* led us to separate the tracking data into displacements along the long axis and short axis of the cell. Kaede molecules diffuse rapidly, exploring the entire cell on a timescale of 100 ms. This containment effect resulted in sub-linear increase of the mean-square displacement (MSD) vs time. We used Monte Carlo simulations and the analytical expression for the long-time MSD in a spherocylinder to fit the SPT data.
Within a single cell, the tracking data were consistent with homogeneous diffusion. Across a population of cells, the tracking data had a mean diffusion constant of $\langle D_{SPT} \rangle = 7.2 \pm 1.9\ \mu m^2/s$. The value of the MSD at long times as measured by SPT was consistent with the predicted long time MSD based on the cell shape and size. This implies that the molecules explore the entire volume of the cell. The sub-linear increase of MSD with time can be explained completely by the containment of diffusing molecules by the cell. For a few cells, we also performed FRAP on the unactivated state of Kaede, yielding $\langle D_{FRAP} \rangle = 8.5 \pm 2.0\ \mu m^2/s$ in agreement with the SPT result for the activated state. Among single cells, D_{SPT} and D_{FRAP} were correlated (r = 0.32).

2585-Pos Board B571
Dye-Conjugated Dendrimers as Bright and Photostable Fluorescent Labels for Single Molecule Microscopy
Younghoon Kim, Sung Hoon Kim, Melikhan Tanyeri, John A. Katzenellenbogen, **Charles M. Schroeder**.
In this work, we report a new class of bright and photostable fluorescent labels for single molecule fluorescence microscopy based on chemically functionalized dendrimers. We synthesized polymeric dendrimers containing multiple fluorophores and versatile functional groups, thereby enabling facile fluorescent labeling of biomolecules (proteins, DNA, RNA) via specific chemical conjugation. We characterized the photophysical properties of single dye-conjugated dendrimer molecules immobilized to chemically modified surfaces using total internal reflection fluorescence microscopy (TIRF-M). Fluorescence photobleaching lifetimes of dye-conjugated dendrimers were observed to far exceed the lifetimes of single conventional organic dye molecules, both in the presence or absence of oxygen scavengers. In addition, single dendrimer molecules are significantly brighter than single conventional organic dye molecules. Furthermore, we determined the precision of centroid position determination for single molecule localization by fitting the fluorescence emission from single dendrimers to a 2-dimensional Gaussian function. Dye-conjugated dendrimers exhibit several advantages as polymeric fluorophores for labeling biological molecules. First, single dendrimer molecules exhibit a relatively small size which is comparable to common GFP-family fluorescent proteins and smaller than inorganic quantum dots. Second, fluorescently-labeled dendrimers are synthesized to contain multiple fluorophores, thereby overcoming blinking in single dyes and short fluorescence "on" times before photobleaching, which are common limitations of single organic dyes for applications in single molecule microscopy. Third, a wide variety of commonly used fluorophores can be conjugated to polymeric dendrimers, thereby increasing their utility for labeling. Overall, we anticipate that dye-conjugated dendritic polymers will be a versatile tool for single molecule microscopy applications, including fine-scale characterization of protein structural dynamics and super-resolution microscopy.

2586-Pos Board B572
Tracking Processive DNA Synthesis with Single-Molecule Fluorescence
Charles E. Wickersham, **Everett A. Lipman**.
We have recently shown that double-stranded DNA labeled with a periodic series of fluorescent dyes can be used to track a single helicase. Here we demonstrate that this technique can be adapted to follow processive DNA synthesis. By monitoring strand displacement, we measure the motion of a single phi29 DNA polymerase without labeling or altering the enzyme or the template strand, and without applying any force. We observe a wide range of speeds, with the highest exceeding by several times that observed in other single-molecule experiments. Because this method enables repeated observations of the same polymerase traversing identical segments of DNA, it should prove useful for determining the effects of sequence on DNA replication and transcription.