

was set in flow tube and guided by continuous flow pump to the sample flow pass. The retardation of the sample plasma was calculated from CCD images which was obtained by system at every 5 seconds. After confirming sufficient aggregation of fibrin, heparin was injected to the sample flow pass so as to activate fibrinolytic system of blood. Result: The retardation of plasma increased over time within 5 minutes. In contrast, fibrinolytic system reduced phase differences by melting aggregated fibrin within 6 minutes.

2869-Pos Board B476

Boundary Effects in FRAP Recovery in the Confined Geometries of Animal, Plant and Fungal Cells

James K. Kingsley¹, Jeffrey P. Bibeau², Cem Unsal¹, Iman S. Mousavi¹, Zhilu Chen³, Xinming Huang³, Luis Vidali², **Erkan Tuzel**¹.

¹Department of Physics, Worcester Polytechnic Institute, Worcester, MA, USA, ²Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA, USA, ³Department of Electrical and Computer Engineering, Worcester Polytechnic Institute, Worcester, MA, USA.

Fluorescence Recovery After Photobleaching (FRAP) has been an important tool used by cell biologists in the past few decades to study the diffusion and binding kinetics of proteins, vesicles and other molecules in the cytoplasm, nuclei or the cell membrane. FRAP has been particularly useful in the characterization and development a mechanistic understanding of tip-growth across many cell types over long time (seconds) and length (microns) scales. In FRAP, a high intensity laser beam is applied to a Region of Interest (ROI), photo-bleaching its contents, and the brightness recovery associated with the transport of fluorescent molecules into this area is used to construct a recovery curve, and make quantitative estimates of diffusion coefficient and percentage of bound fraction of molecules. While many FRAP models have been developed over the past decades, the influence of the complex boundaries of three-dimensional geometries on the recovery curves, in conjunction with ROI and optical effects (imaging, bleaching, scanning), has not been well studied in the literature. Here, we developed a three-dimensional computational model of the FRAP process that incorporates particle diffusion, cell boundary effects, and the optical properties of the scanning confocal microscope, and validated this model using the tip-growing cells of *Physcomitrella patens*. This validation also provides an accurate estimation of the diffusion coefficient of 3xmEGFP for moss cells, which allows for the calculation of an effective cytoplasmic viscosity for molecules of this length scale in moss cells for the first time. We then show how the cell boundary and optical effects confound the interpretation of FRAP recovery curves, the bound fraction of fluorescent proteins, and the number of dynamic states of a given fluorescent protein across a wide range of cellular geometries, including the budding yeast, *S. pombe*, tip-growing plant cells, nuclei and lamellipodia of cells. Finally, we illustrate how existing theoretical and computational models perform in each of these scenarios, provide a computational tool and guidelines on how to use FRAP quantitatively in such geometries.

2870-Pos Board B477

First Person Bioimage: An Online Tool for Presentation and Publication of Volumetric Data

Marcus Fantham, Clemens F. Kaminski.

University of Cambridge, Cambridge, United Kingdom.

New microscopy techniques (e.g. SPIM, 3D STORM, OPT, etc.) and many other 3D bioimaging modalities (tomographic EM, MRI, CT, etc.) generate terabytes of volumetric data. Whilst highly specialized software packages are available for the analysis and visualization of such data, their use requires specialized skills and they are often costly. Furthermore the capacity to share data and allow interactive exploration by third parties is highly limited.

Here we present FP BioImage, an easy-to-use and powerful open source visualization tool that permits researchers to share their volumetric image data online and third parties to interact with, and explore, datasets in their entirety. FP BioImage provides the viewer an immersive experience for the exploration of complex 3-dimensional bioimaging data, and makes use of the latest graphics capabilities embedded in all modern web browsers so that no software installation is required.

The tool is fast and user responsive, requires no training for use, and includes advanced rendering and data manipulation capabilities. Data can be intuitively explored from a 'first person perspective', akin to navigating virtual space in modern computer games, allowing users to conceptualize and contextualize details in the data to aid biological interpretation.

From a researcher's perspective the tool makes it now possible to easily share volumetric imaging data globally, providing anyone full and interac-

tive access to the data *via* a web browser. An example website has been set up at <http://fpb.ceb.cam.ac.uk>. In the future, we hope that this changes the way research data are shared and suggest that publishers will use the software for online publication. Data can thus be shared in their entirety, moving on from the current practice of providing selective views or movies shown from a single perspective, which are not capable of providing a full understanding of the data.

2871-Pos Board B478

The Extra Microscope

Alberto Diaspro^{1,2}, Luca Lanzani¹, Paolo Bianchini¹, Giuseppe Vicidomini¹, Marti Duocastella¹, Francesca Cella Zanacchi¹, Colin J.R. Sheppard¹.

¹Nanophysics, Istituto Italiano di Tecnologia, Genoa, Italy, ²Department of Physics, University of Genoa, Genova, Italy.

In the last 40 years, optical fluorescence microscopy, due to its inherent ability of imaging living systems during their temporal evolution, had a continuous update on three main tracks, namely: three-dimensional (3D) imaging, penetration depth at low perturbation and resolution improvements. However, computational optical sectioning, confocal laser scanning, two-photon excitation and super-resolved methods can be considered as milestones in optical microscopy. With the recent Nobel Prize in Chemistry in 2014 for the development of super-resolved fluorescence microscopy, it has been revealed how the optical microscope can offer unimaginable performances in terms of spatial resolution. [Diaspro A. 2014. *Il Nuovo Saggiatore*]. Starting from this point, considering important revolutions like the ones of confocal and two-photon excitation microscopy coupled to the advent of green fluorescent proteins [Diaspro A. and van Zandvoort M.A.M.J. (eds) 2016. *Super-resolution Imaging in Biomedicine*. CRC press], I will discuss about some converging and correlative techniques that can be used for what I like to name "the extra microscope". The meaning is related to series of advances and new methods that allow getting information at the nanoscale or preferable at the molecular scale referring to both spatial resolution or structural information. Three examples are related to resolution improvement by image subtraction microscopy using the STED donut, volumetric imaging fast-inertia approach and fluorescence lifetime and correlation methods (SPLIT, PLICS) [Scipioni et al. 2016. *BJ*]. Within this framework second harmonic generation, phase methods and Mueller matrix [Sheppard C.J.R, Castello M. and Diaspro A., 2016. *JOSA A* 33 (4), 741-751] signatures based on polarization are opening a new window for multimodal imaging including label free approaches. The Extra Microscope has tunable and flexible performances depending on the biological question and reflects the integration in a flexible way of several approaches [Teodori L. et al. 2016. *J.Biophotonics*]. It is worth noting that new correlative approaches coupling optical super resolved methods with scanning probe microscopes are providing interesting developments that will be outlined [Chacko, J.V. et al. 2013. *Cytoskeleton*]. Considering all these aspects we can re-phrase the sentence related to the Galilei's "occhialino" into "microscopium extraordinarium nominare libuit".

2872-Pos Board B479

Efficient Parametric Imaging with GPU Computing

Dianwen Zhang¹, Xiang Zhu², Angelo Bifone³, Alessandro Gozzi³, Silvia Capuani⁴, Marco Palombo^{4,5}.

¹Microscopy Suite, Beckman Institute for Advanced Science & Technology, UIUC, Urbana, IL, USA, ²China Agricultural University, Beijing, China, ³IIT, Center for Neuroscience and Cognitive Systems UniTn, Rovereto, Italy, ⁴IPCF-UOS Roma, Physics Department, "Sapienza" University of Rome, Rome, Italy, ⁵CEA/DSV/I2BM/MIRcen & CEA-CNRS URA 2210, Fontenay-aux-Roses, France.

Parametric imaging plays a critical role in modern biophysical, biomedical research and clinical diagnosis. It can provide useful visual representations of a sample with respect to the parameters underlying the mathematical models associated with sample data. For this technique, nonlinear model fitting optimization is a commonly used approach to estimate the parameters on a pixel-by-pixel basis to create parametric maps. With the increased sophistication of modern imaging systems, the amount of data processed with parametric imaging techniques is exploding and the processing time is often limiting the advancement of these technologies for real-time and automotive applications. However, in many applications, the computation for each image pixel can be carried out independently of any others, and such sort of computations can profit tremendously from parallel processing. Nowadays, graphics processing unit (GPU) has become a standard tool in high-performance parallel computing. To realize real-time automated image reconstruction for parametric imaging techniques, such as multi-parametric microscopy and magnetic resonance imaging (MRI), we have developed a