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Fluorescence Fluctuation Microscopy Techniques to Study mRNA Synthesis and Dynamics

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Probing Short-Range Protein Brownian Motion in the Cytoplasm of Living Cells

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The translational motion of small molecules in cells appears to be suppressed compared to what is observed in dilute solutions. Although, the rotation of small proteins is almost unhindered, pointing out a local aqueous environment. Different theoretical models provide explanations for this apparent discrepancy but with predictions that drastically depend on the nanoscale organization assumed for macromolecular crowding agents. A conclusive experimental test of the nature of the translational motion in cells is still missing owing to the lack of techniques capable of probing protein motion with the required temporal and spatial resolution. We show that fluorescence-fluctuation analysis of raster scans at variable time scales can provide this information. By using GFP, we measure protein translational motion at the unprecedented time-scale of 1 microsecond, unveiling unobstructed Brownian motion from 25 to 100 nanometers, and partially-suppressed diffusion above 100 nm. Experiments on in vitro model systems attribute this effect to the presence of relatively immobile structures rather than to diffusing crowding agents. In this regard, internal membranes (e.g. the ER sheets, vesicles, Golgi apparatus, etc.) appear to be the more likely candidates as selective disruption of the microtubules network by treatment with Nocodazole did not significantly alter GFP behavior in the cytoplasm. Also, the same measurement in a structurally-different (e.g devoid of membranes) intracellular environment, such as the nucleoplasm, yields a different behavior, in which GFP motion is never coincident with that in a dilute solution. Finally, we believe the present findings coupled with use of genetically-encoded fluorescent markers pave the way to novel studies of biomolecular processes in live cells at the physiologically-relevant spatio-temporal scale. Supported by grants NIH P41-GM103540 and NIH P50-GM076516 (grants to EG), MIUR under FIRB-RBAP11X42L and Fondazione Monte dei Paschi di Siena (grants to FB).
The availability of a system such as the MS2-GFP fusion protein, which directly labels the mRNA, has allowed obtaining an estimation of the mRNA pol- 
ymerase elongation rate in vivo. This is due to the high large heterogene-
ity observed in RNA Polymerase II (PolII) elongation rates measured by 
fluorescence assays.

To shed further light on the source of this heterogeneity we introduce and 
discuss here a novel method based on the phasor analysis of steady state 
MS2-mRNA fluorescence trajectories. When applied to the study of PolII 
kinetics, we demonstrate that this approach allows resolving PolII elongation 
rates in a range from a few to hundreds of basepairs per seconds.

In order to couple this information to what happens to mRNA molecules once 
they leave the active transcription site, we combine 3D orbital particle tracking 
with Pair Correlation Analysis to investigate the diffusive routes taken by 
mRNA molecules within the nucleoplasm. With this approach we observe 
that the mean mRNA molecule takes to leave the transcription site is highly 
variable, ranging from a few to tens of minutes. Work supported in part by 
Grants NIH P41-GM103540 and NIH P50-GM076516

1630-Pos Board B581
Nanoscale Protein Diffusion by STED-Based Pair Correlation Analysis 
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We describe for the first time the combination between cross-pair correlation 
function analysis (pair correlation analysis or pCF) and stimulated emission 
depletion (SEDE) 3D spatial diffusion maps at spatial resolution below the 
optical diffraction limit (super-resolution). Our approach was tested in systems 
characterized by high and low signal to noise ratio, i.e. Capsid Like Particles 
(CLPs) bearing several (>100) active fluorescent proteins and monomeric 
fluorescent proteins transiently expressed in living Chinese Hamster Ovary 
cells, respectively. The latter system represents the usual condition encountered 
in living cell studies on fluorescent protein chimeras. Spatial resolution of 
STED-pCF was found to be about 110 nm, with a more than twofold improve-
ment over conventional confocal acquisition. We successfully applied our 
method to highlight how the proximity to nuclear envelope affects the mobility 
features of proteins actively imported into the nucleus in living cells. Remark-
ably, STED-pCF unveiled the existence of local barriers to diffusion as well as 
the presence of a slow component at distances up to 500-700 nm from either 
sides of nuclear envelope. The mobility of this component is similar to that 
previously described for transport complexes. Remarkably, all these features 
were invisible in conventional confocal mode.

1631-Pos Board B582
Analysis of Trabecular Bone Architecture using Two Photon Fluorescence 
Microscopy 
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Biomechanical competence of trabecular bone is dependent on the makeup of 
its architecture. Alterations in the trabecular architecture can lead to fractures 
in metabolic bone diseases like osteoporosis. Therefore, it is paramount to un-
derstand the signaling mechanisms that dictate these changes in bone growth 
and fracture repair. Two photon fluorescence microscopy revolutionized the 
imaging of biological specimens utilizing its unique capabilities. The three-
dimensional (3D) imaging based on nonlinear excitation of the fluorophores 
brings multiple advantages for imaging skeletal tissue. However, noise gener-
atated by the subsurface signal and auto-fluorescence of the local tissue make 
imageing of trabecular bone problematic. Imaging of calcified tissue presents 
a unique challenge to address the aberrations produced through the noise gener-
atated. Also a general practice of immunolabeling of the plasticized bone for 
antigen stability are to be optimized. We demonstrate here for the first time using 
two-photon fluorescence imaging of trabecular bone and its architecture iden-
tifying the structural differences and cell populations lining the trabecular cav-
ity and also the cells embedded in it. Furthermore, we developed a shortened 
method of immunohistochemistry for plastic embedded bone tissue providing 
antigen stability for antibody labeling. Two photon fluorescence imaging 
greatly reduces photo damage and helps image of specimens of uneven planes 
to submicrometer resolution making this an ideal source for imaging in vivo 
signaling of trabecular bone. We demonstrate here labelling of multi colored 
fluorophores measuring Smad and ERK activity in trabecular bone growth in 
mice that are systemically injected with Bone Morphogenetic Protein 2 
(BMP2). We optimized the conditions for in vivo imaging of bone tissue that 
is calcified and plasticized. We demonstrate here two photon fluorescence mi-
croscopy of the trabecular bone can be used for understanding the molecular 
mechanisms which control bone growth and development in vivo.

1632-Pos Board B583
Non-Linear Microscopy of Mitochondrial Damage and Abnormal Lipid 
Metabolism in Beta-Amyloid Expressing Yeast 
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One of the earliest pathological hallmarks of Alzheimer’s disease is the forma-
tion of soluble β-amyloid (Aβ) oligomers, also believed to be the primary 
novel neurotoxic agents long before the accumulation of amyloid plaques. However, 
the mechanisms by which the Aβ oligomers cause cell dysfunction and eventu-
tially cell death are poorly understood. The yeast Saccharomyces cerevisiae 
has here emerged as a valuable model for systemic studies of the intracellular 
cytotoxicity of Aβ species, revealing that Aβ transits through the different 
dendritic compartments and disrupts cell-, mitochondrial-, lysosomal- 
and ER membranes (for a review of the different aspects of amyloid-membrane in-
teractions) finally activating the mitochondrial apoptotic pathway. In order to 
form a better understanding of the cause and consequences of mitochondrial 
damage, seemingly one of the central cytotoxic mechanisms, we have done 
a multi-parametric study on living GFP-Aβ42 expressing yeast using non-
linear microscopy. The intracellular distribution of GFP-labelled Aβ42 was 
correlated with the corresponding distribution and morphology of mitotracker-labelled mitochondria by means of 2-photon fluorescence micro-
scopy. Furthermore, the consequences of the dysfunctional mitochondria and 
the resulting oxidative stress were visualized by the monitoring of the general 
NADH levels based on their 2-photon-excited intrinsic fluorescence and the 
content and distribution/morphology of lipid stores by means of CARS micro-
scopy (probing natural carbon-hydrogen vibrations). We could observe how 
Aβ-expressing yeast accumulates significant amounts of lipid stores and follow 
their coalescence to larger store units, which can be recognized as a general stress 
response, in this case most likely due to oxidative stress.

1633-Pos Board B584
Using Surface Plasmon Resonance to Study Species Transport across Lipid 
Membranes 
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Studying species transport across lipid membranes by membrane transport 
proteins is important for various biological applications. Although patch-clamp 
technique is well developed for recording the ion transport across lipid mem-
branes, the technique requires well trained personals for the challenging and 
delicate operation. In this study, we demonstrated using the surface plasmon 
resonance (SPR) based platform to detect the concentration change of the target 
species across the lipid membrane. We created sub-micron sized pore structure 
on the platform, in which the bottom surface is gold and the top surface is silica, 
and spanned lipid membranes over the pore. The process created a space inside 
the pore separated from the outside environment by the free-standing lipid 
membrane for further studying the species transport across the membrane. The 
platform geometry allowed us to combine plasmon-waveguide resonance 
(PWR) to the system to simultaneously monitor the refractive index change in 
the pore space, which is correlated to the target species concentration, and 
the refractive index change on the membrane above the top silica surface, which 
is correlated to the binding events occurring on the membrane surface. We expect 
to use this platform to monitor how various inhibitors or ligands could influence 
the transport dynamic of interested membrane transport proteins.

1634-Pos Board B585
Applications of High Resolution Surface Plasmon Resonance Imaging to 
Adherent Cells: Single Mammalian Cells to Bacterial Biofilms 
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High resolution surface plasmon resonance imaging (SPRI) allows label-free 
imaging of subcellular features when performed using a high numerical aper-
ture objective lens with a digital light projector to precisely position incident 
angle excitation. The SPRI signal is a result of the mass of material within