as Y. pestis are able to circumvent the innate immune system. Finally, we will exploit the dual-color STORM capabilities to simultaneously image LPS and TLR4 receptors in the cellular membrane at resolutions at or below 40nm.

1924-Plat
High Speed Resolution Enhancement by Optical Pupil Function Modulating System
Tomonobu M. Watanabe[1], Yoshikazu Tsukasaki[2], Tatsuya Saitoh[1], Shizuo Akira[1], Toshio Yanagida[1].
[1]Osaka University, Osaka, Japan, [2]RIKEN Center for Developmental Biology, Kobe, Japan.
Superresolution fluorescent microscopy (nanoscopy) offers unprecedented resolution of biological structures by overcoming the fundamental diffraction limit of light. There are several superresolution technologies like stimulated emission depletion (STED), structured illumination microscopy (SIM), stochastic optical reconstruction microscopy (STORM) and fluorescence photoactivation localization (FPALM) all show promise as they offer subdiffraction limit optical resolution. The further challenge in superresolution is the improvements of the temporal resolution, because the temporal resolutions of the above four methods are too low to pursue the movements of proteins at ms timescales. However, it looks so difficult because FPALM and STORM are based on single molecular detection and stochastic photoswitching while STED and SIM require laser illumination scanning. Here, we constructed a microscopic system that can voluntarily modulate optical transmission of the pupil function by placing a reflective liquid crystal micro mirror array (LCMM) onto the Fourier plane that alters the point spread function (PSF) shape. It has been reported that the diffraction limit was overcome by subtracting the normal PSF from the low-pass filtered PSF (*). Our present system can obtain a subtracted image of superresolve-masked image and the low-pass filtered image within one frame of a camera. We achieved 2 ms temporal resolution at 120 nm spatial resolution without any special probes and scanning systems. Furthermore, because the present technique is compatible with fluorescent probes that lack polarization or coherency, we can apply it to various biological investigations. In this meeting, we show basic principles and constructions of our present nanoscopy, and demonstrate the potentials of it for live cell imaging both on the membrane and intracellular, and for single particle tracking.
(*) Heintzmann R, Sarafis V, Munroe P, Nailon J, Hanley QS, Jovin TM., Micron. 34,293-300 (2003).

1925-Plat
Correlative Microscopy of Living Cells between Fluorescence and Quantitative Phase Imaging with a High Resolution Wavefront Sensor
Julien Savatier[1], Pierre Bon[1,2], Didier Marguet[3], Serge Monneret[1].
[1]Institut Fresnel - Université Aix-Marseille - Ecole Centrale - CNRS UMR 6133, Marseille, France, [2]Phasics, Palaiseau, France, [3]Centre d'Immunologie de Marseille Luminy, Université de la Méditerranée, INSERM U631, CNRS UMR 6102, Marseille, France.
Phase visualization of cells is used for decades, with setups such as Zernike phase contrast or Nomarski-DIC equipments. Those techniques use the fact that light passing through a sample accumulates phase shift. Nevertheless, they are quite impossible to give information on the quantitative phase shift of each pixel of the image and are commonly used only to increase contrast. We describe here the use of quadri-wave lateral shearing interferometry [1] for wavefront sensing, in order to measure quantitatively the local phase shift within a sample, with a high sensitivity.
We use a high resolution wavefront sensor and we get a 300x400 sampling points on the sample, with both phase and intensity information. The method is simple and can be implemented on a conventional microscope: its native bright-field illumination system is used as the light source, and the wavefront sensor has only to be mounted on a video port, with the possibility to combine phase and fluorescence imaging.
Correlative microscopy between wide-field fluorescence and optical phase on COS-7 living cells is considered, either on regular substrate or fibronectin-coated micro-patterns. The phase visualization of intracellular organelles is clearly possible with a high contrast, and targeted fluorescence allows a precise and simultaneous identification of each component. A multidimensional image is then obtained by combining those two images. Many organelles, such as mitochondria and endocytosis vesicles, can be specifically determined by their relative phase shift and shape, with a high degree of confidence. Refractive index anisotropy is also used to visualize phase shift of anisotropic components, such as cytoskeleton, with different polarizations of transmitted light.
[1] Bon, Maucort, Wattellier and Monneret, "Quadriwave lateral shearing interferometry for quantitative phase microscopy of living cells," Opt. Express 17, 13080-13094 (2009).

1926-Plat
Nanoscale Imaging of Fish Probe Binding to Metaphase Chromosomes
Linda J. Johnston[1], Roderick Chisholm[1], Peter K. Rogan[2], Wahab Khan[3], Joan H. Knoll[4], Seyed M. Tadayon[3], Peter R. Norton[3].
[1]National Research Council Canada, Ottawa, ON, Canada, [2]Cytognomix Inc, London, ON, Canada, [3]University of Western Ontario, London, ON, Canada, [4]University of Western Ontario, Londond, ON, Canada.
Fluorescence in situ hybridization (FISH) is widely used for diagnosis of genetic abnormalities. We have developed multimodal, nanoscale imaging methods using a combination of fluorescence and atomic force microscopy to determine the precise binding location of FISH probes. The use of high resolution imaging methods is important to validate and improve the binding efficiency for small single copy FISH probes that are being developed for cytogenetic analysis at higher genomic resolution than is possible with current commercial FISH probes. Chromosome topography is imaged by AFM and superimposed on FISH probe location determined by confocal or epifluorescence microscopy. Image processing procedures are used to quantify probe context, relative to neighboring 30 nm chromatin fiber bundles imaged by AFM, and to quantify probe binding to homologs within the same cell. We have correlated optical and topographic images of multicopy centromeric probe for chromosome 17 and have examined the spatial relationship between centromeric DNA and the formation of kinetochore structures and sister chromatid segregation. Interestingly, segregation of sister chromatids is not coordinated with the formation of these structures within individual homologous chromosomes, occurring after the formation of these structures. We hypothesize that asynchronous segregation of previously replicated sister chromosomes may account for differences in the hybridization patterns for probes in different cells from the same individual.

Platform AN: Physical Chemistry of Proteins & Nucleic Acids

1927-Plat
Effect of Pressure on the Stability of Short DNA Hairpins
Robert Macgregor, Amir R. Amiri.
University of Toronto, Toronto, ON, Canada.
DNA hairpins are models for more complex, naturally occurring loop-containing nucleic acid structures. They consist of two distinct structural domains: a double stranded stem and a single-stranded loop that connect the two strands of the stem. Previous studies of short DNA hairpins have revealed that the base sequence of the loop and stem significantly affect the relative thermodynamic stability of the hairpin and coil conformations. We have studied the effect of hydrostatic pressure on the heat-induced helix-coil transition of short DNA hairpins in order to gain knowledge about the role of hydration in the stability of the secondary structure adopted by these self-complementary DNA molecules. We report the effect of hydrostatic pressure on the helix-coil transition temperature (T_M), monitored by absorption, for eleven 16-base DNA hairpins at different sodium chloride concentrations. The studied hairpins form by intramolecular folding of 16-base self-complementary DNA oligodeoxyribonucleotides. Each of the hairpins structures has a six-base pair duplex-forming stem linked by a four-base loop. We have varied only loop sequence and identity of the duplex base pair adjacent to the loop; the first four base pairs in the stem are the same for every molecule. Transition parameters based on a two-state denaturation process, such as ΔH_{vH} and transition volume (ΔV), were calculated from the optical melting transitions. Experiments revealed the ΔV for denaturation of these molecules range from -2.35 to $+6.74$ cm^3mol^{-1}. We propose that the sequence dependence of the molar volume changes is attributable to differences in the hydration of these molecules.

1928-Plat
Contributions of Ordered Solvent to Long-Range DNA-Dendrimer Interactions
Maria Mills[1], Bradford G. Orr[2], Mark M. Banaszak Holl[2], Ioan Andricioaei[1].
[1]University of California Irvine, Irvine, CA, USA, [2]University of Michigan, Ann Arbor, MI, USA.
Water is essential to nearly all biological reactions, and yet the role of solvent is often overlooked in studying such interactions. In particular, the ability of highly charged molecules to orient the dipole moments of water molecules has not been thoroughly explored. While short-range solvent ordering effects have been previously investigated, we report evidence of the existence of ordered waters between charged molecules at large distances and characterize the free energy contributions of this solvent ordering to the interaction. We present evidence from molecular dynamics simulations that the hydrogen bonding network in ordered waters between a strand of DNA and a highly charged nanoparticle, a generation 3 polyamidoamine (PAMAM) dendrimer, contribute