approaches. However, the imaging of whole-mount tissues presents technical problems of its own. One of the major problems using CLSM to image whole organs and embryos is tissue penetration of laser light. High quality morphological images begin by optimizing the sample preparation technique [1, 2]. Additional factors include evaluating CLSM performance by optimizing the acquisition variables (i.e. objective lens, averaging, pinhole size, bleaching, PMT voltage, laser excitation source, and spectral registration.) of the confocal microscope [3, 4].

Confocal microscopy has been used by our laboratory to study cell death and morphology in embryos, ovaries, eyes, ears, kidneys lungs and limbs [1, 2]. The technique has revealed structural morphology and the initiation of cell death by the uptake LysoTracker dye into acidic cells. LysoTracker Red (LT) is fixable by paraformaldehyde and concentrates in acidic compartments of cells. In whole tissues, this accumulation indicates regions of high lysosomal activity and phagocytosis. LT staining is an indicator of apoptotic cell death and correlates with other standard apoptotic assays [1, 2]. LT staining revealed cell death regions in mammalian limbs, neonatal ovaries, fetuses and embryos. The mammalian samples were stained with LT, fixed with paraformaldehyde/glutaraldehyde, dehydrated with methanol (MeOH), and cleared with benzyl alcohol/benzyl benzoate (BABB). The use of BABB matches the refractive index of the tissue within the suspending medium. BABB helps increase the penetration of laser light during CLSM by reducing the amount of light scattering artifacts and allows for the visualization of morphology in thick tissue. Following this treatment, the tissues were nearly transparent. This sample preparation procedure, combined with the optimization of confocal laser scanning microscopy instrument factors, allowed for the detection and visualization of apoptosis in fetal limbs and embryos which were approximately 500 microns thick. Recently, spectroscopic imaging capacity has been incorporated into confocal microscopes. The LT spectra had a maximum peak around 610nm while the fixative, glutaraldehyde (Glut), had a maximum peak around 450nm. Glut was added primarily to preserve the tissue morphology, but also provided molecules emitting in the green fluorescence range that helped to visualize the morphology of the tissue. The understanding of the spectra derived from the tissue was extremely useful in optimizing the staining protocol. We have continued to incrementally improve the tissue staining and preparation techniques to achieve better quality images in 3D.

References

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This abstract of a proposed presentation does not necessarily reflect EPA policy.

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VISUALIZING CELL TRAFFIC OF SINGLE TUMOR CELLS LIVE AND IN VIVO BY A NEW INTRAVITAL MICROSCOPY APPROACH

J.T. Wessels,[1,4] A.C. Busse,[2] G. Seitz ,[3] J. Mahrt,[1] B. Hoffschult,[1] and G. A. Mueller[1]
[1]*Center of Internal Medicine, Dept. Nephrology/Rheumatology, University Hospital Goettingen, Germany*
[2]*Center Radiology, Dept. Diagnostic Radiology, University Hospital Goettingen, Germany*
[3]*Paediatric Surgery, University Hospital Tuebingen, Germany*
[4]*Core Facility Molecular & Optical Live Cell Imaging (MOLCI), University Hospital Goettingen, Germany*

Recent advances in photonics, multi-photon microscopy, intravital microscopy, near infrared fluorescence based imaging and new molecular and genetic tools are empowering scientist from many different fields to answer longstanding unresolved questions in small living animals. To noninvasively image for example cancer cell/blood vessel interaction and drug response at the cellular and subcellular level in live mice new imaging systems and stick objectives now become possible. Sophisticated systems like the OV110 (Olympus) offers a wide range of magnification by the use of parcentred and parfocal lenses enabling both macroimaging the whole animal and microimaging single cells down to resolutions of less than 1μm. Using these approaches we could recently show in vivo cell trafficking of xenotransplanted human tumour cells within the vessels of mice at the single cell level. Animals underwent single injection of transfected tumor cells (approximately 10^6 tumor cells / 100μl) into the tail vein. The mice were anesthetized and an arc-shaped incision was made in the abdominal skin in order to prepare a skin flap. The skin flap was spread and fixed on a flat stand. Cancer cell trafficking was carried out real time within the skin flap down to resolution of single cells. Using this approach we are able to localize single cells within the blood flow, measure size, diameter, motility, nuclear-plasma ratio and fluorescence. Right now this approach can not claim to be a cytometric approach. We have therefore started to optimize and modify the system to additionally readout cellular information like granularity.

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LIVE CELL MICROSCOPY OF GROWTH-FACTOR DEPENDENT SIGNAL TRANSDUCTION PATHWAYS WITH A PROGRAMMABLE ARRAY MICROSCOPE (PAM)

Thomas M. Jovin, Guy M. Hagen, Wouter Caarls, and Donna J. Arndt-Jovin
Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

We have developed an optical sectioning structured illumination microscope denoted *Programmable Array Microscope* (PAM) targeted primarily at live cell studies. The key design feature is the placement of a spatial light modulator (SLM) at an image plane of a conventional fluorescence microscope in order to generate arbitrary patterns of conjugate structured illumination and detection. The major advantages of the PAM are: (1) simple, inexpensive design with no moving parts; (2) 1–2 orders of magnitude speedup

in optical sectioning using pixel illumination duty cycles of up to 50%; (3) optimal detection sensitivity with electron multiplication CCD cameras; (4) arbitrary, continuously programmable, and adaptive optical sectioning modes between and/or within images based on dot, line, or pseudo-random (Sylvester) sequence patterns; (5) efficient and sensitive optical sectioning due to simultaneous detection and processing of both conjugate (in-focus) and non-conjugate (out-of-focus) images; (6) flexible light sources: LEDs, lasers, lamps; (7) generation and detection of arbitrarily patterned polarized states; (8) compatibility with hyperspectral and lifetime-resolved imaging; (9) incorporation of light sources for photochemical activation, destruction, and/or transformation, modes compatible with FRAP, FLIP, and FCS/ICS protocols; (10) minimal photobleaching due to widefield distribution of the excitation energy; and (11) compatibility with schemes for achieving spatial superresolution.

This report will describe a 2nd generation commercially available PAM created by the combined efforts of our Department (MPIbpc) and Cairn Research Ltd. (UK). The PAM module, including the light source(s) and detector(s), is incorporated in a stand-alone assembly featuring a ferroelectric liquid-crystal-on-silicon (LCoS) SLM. It can be attached to an exit port of a conventional, unmodified fluorescence microscope. The current MPI-Cairn prototype system incorporates an Andor iXon emCCD camera and is mounted on an Olympus IX71 fluorescence microscope equipped with 60–150x objectives, dual excitation/emission filter wheels, and a Prior Scientific ProScan *x/y/NanoScanZ-Piezo* stage/focusing system. Further enhancements include hyperspectral detection and lifetime imaging (FLIM) based on Lambert Instruments phase-modulation modules. Optical sectioning and display can be implemented with single 16 ms exposure cycles and at overall 20 Hz full-field frame rates.

The PAM is currently being applied to studies of signal transduction, e.g. based on the use of quantum dot-conjugated ligands. Tracking of individual diffusing and /or trafficking nanoparticles on and within cells is readily achieved. The combined use of a 405 nm diode laser and LED/ion laser sources permits arbitrary patterns and cycles of photoconversion (e.g. DRONPA) and thus high-speed multiloci determinations of molecular 3D movement.

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CAUSES AND CONSEQUENCES OF CHROMOSOMAL ANEUPLOIDY IN CANCER CELLS

Thomas Ried, Kundan Sengupta, Jordi Camps, Amanda B. Hummon, Hesed M. Padilla-Nash, Marian Grade, B. Michael Ghadimi, and Michael J. Difilippantonio

Genetics Branch, CCR, NCI/National Cancer Institute, University Medical Center, Goettingen, Germany

Chromosomal aneuploidies are observed in essentially all sporadic carcinomas. Aneuploidy results in tumor specific patterns of genomic imbalances that are acquired early during tumorigenesis, continuously selected for and faithfully maintained in cancer cells. In order to characterize patterns of global transcriptional deregulation in primary colon carcinomas, we performed gene expression profiling of 73 tumors (UICC stage II, n=33 and UICC stage III, n=40) using oligonucleotide microarrays. For 30 of the tumors, expression profiles were compared to those from matched normal mucosa samples. We identified a set of 1,950 genes with highly significant deregulation between tumors and mucosa samples ($P < 1e\text{-}7$). A significant proportion of these genes mapped to chromosome 20 ($P=0.01$). Finally, we established a relationship between specific genomic imbalances, which were mapped for 32 of the analyzed colon tumors by comparative genomic hybridization, and alterations of global transcriptional activity. Previously, we had conducted a similar analysis of primary rectal carcinomas. The systematic comparison of colon and rectal carcinomas revealed a significant overlap of genomic imbalances and transcriptional deregulation, including activation of the Wnt/-catenin signaling cascade, suggesting similar pathogenic pathways.

These results were confirmed experimentally: we generated artificial trisomies in a karyotypically stable diploid, yet mismatch-repair deficient, colorectal cancer cell line using microcell mediated chromosome transfer. We then used global gene expression levels to determine what effect chromosome copy number increases have on the average expression levels of genes residing on the trisomic chromosomes. Our results show that, regardless of chromosome or cell type, chromosomal trisomies result in a significant increase in the average transcriptional activity of the trisomic chromosome. We therefore postulate that the genomic imbalances observed in cancer cells exert their effect through a complex pattern of transcriptional deregulation. Finally, we could show that aneuploid chromosome assume a nuclear position that is conserved and similar to the one in diploid cells. This might indicate that active transcription requires conservation of the 3D position of chromosome territories.

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PROTEOME-WIDE DETERMINATION OF SUBCELLULAR LOCATION BY AUTOMATED MICROSCOPY

Robert F. Murphy

Departments of Biological Sciences, Biomedical Engineering and Machine Learning, Center for Bioimage Informatics, Molecular Biosensor and Imaging Center, Carnegie Mellon University, Pittsburgh, USA

Systems Biology requires comprehensive, systematic data on all aspects and levels of biological organization and function. In addition to information on the sequence, structure, activities, and binding interactions of all biological macromolecules, the creation of accurate, predictive models of cell behavior will require detailed information on the distributions of those molecules within cells and the ways in which those distributions change over the cell cycle and in response to mutations or external stimuli. Current information on subcellular location in protein databases is limited to unstructured text descriptions or sets of terms assigned by human curators. These entries do not permit basic oper-