

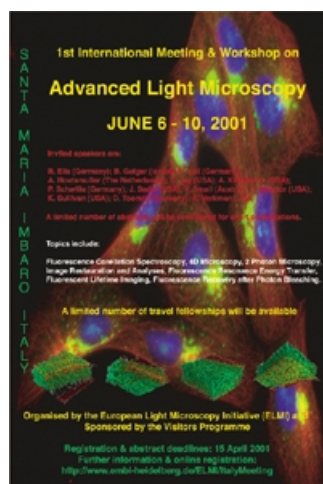
Through the glass brightly

ELMI workshop on advanced light microscopy

Benjamin Geiger[†]

Department of Molecular Cell Biology, Weizmann Institute of Science, IL-76100 Rehovot, Israel

Received July 10, 2001; revised August 20, 2001; accepted August 21, 2001



research community. One avenue towards this goal is the organization of meetings at which advanced approaches in microscopy and their applications are presented. The meeting in Italy was the first of its kind.

The meeting was organized by Christian Boulin (Heidelberg, Germany), Jan Ellenberg (Heidelberg, Germany), Alberto Luini (Chieti, Italy), Agnes de Matteis (Heidelberg, Germany), Tommy Nilsson (Heidelberg, Germany) and Rainer Pepperkok (Heidelberg, Germany), and all members of the European Light Microscopy Initiative (ELMI), a forum initiated by scientists with an interest in the development of novel approaches in light microscopy. ELMI had previously met with leaders of the light microscopy and imaging industry, to discuss new developments and effective means of making advanced light microscopy more accessible to the European

the current 'post-genomic era', with a huge body of structural information available and the obvious next step being to determine how this complex ensemble functions in the live cell. For this mission, one needs the kind of high-resolution approaches that advanced light microscopy is beginning to be able to provide.

The ALM meeting was organized by the European Light Microscopy Initiative (ELMI), a forum initiated by a small group of scientists from the European Molecular Biology Laboratory (EMBL) in Heidelberg and other researchers with a specific interest in the development of novel approaches in light microscopy. The meeting itself was rather unusual in that it focused on a tool rather than addressing a specific scientific topic and as such, brought together a diverse mix of scientists and representatives from industry. In addition to the traditional lectures and poster sessions, the program also included practical workshops where state-of-the-art microscopes and imaging programs were demonstrated by experts. The end result was a unique two-way dialogue both between and amongst the users and the developers of the latest light microscopy techniques.

Introduction

In his 1960 movie *'Through the glass darkly'*, Ingmar Bergman took a focused look into the lives of his sad and complex characters. The first International Meeting & Workshop on Advanced Light Microscopy (ALM), which took place at the Mario Negri Sud Institute in Santa Maria Imbaro, Italy in June 2001, provided a very bright look 'through the glass', giving new insights into the inner lives of cells. As highlighted at the meeting, biological research is currently in great need of new approaches and tools with which to study molecular events in live cells and organisms. Evidently, the cell is highly compartmentalized, maintaining diverse and complex molecular microenvironments that cannot be discerned through standard biochemical approaches, which lack the necessary spatial and temporal resolution. Tools that surpass the current limitations are particularly important in

Higher resolution

One of the most important issues addressed at the meeting was the improvement in the resolution of light microscopy, which is currently ~200 nm in the focal plane and 500 nm in the axial direction. Since small objects spaced by less than these distances cannot be resolved, many subcellular structures (e.g. actin filament meshworks in the leading edges of cells or the internal structures of mitochondria or the nucleus) cannot be distinguished when studied by fluorescence microscopy. John Sedat (San Francisco, CA) described new approaches for the correction of sample-induced aberrations, a common cause of difficulties in resolution. This involves the generation of three-dimensional images through computer-aided deconvolution, using algorithms to correct for out-of-focus data. Summarizing a study conducted in collaboration with Zvi Kam (Rehovot, Israel), Sedat described an approach for removing aberrations specifically

[†]Corresponding author. Tel: +972 8 934 3910 (4069); Fax: +972 8 946 9713; E-mail: benny.geiger@weizmann.ac.il

caused by spatial variations in refractive index within the specimen (either live or fixed). Another common problem, addressed in the talk, is the classical diffraction-limited resolution barrier. Sedat explained that this barrier can now be broken through a combination of structured illumination and interferometry (including the illumination and collection of light from both sides of a sample). This may make it possible to reach a 100 nm or better X,Y,Z resolution in the future. Stefan Jakobs (Göttingen, Germany) approached the same problem using the 'stimulated emission depletion' (STED) technique, where fluorescence emission from the periphery of the focal spot is blocked. These new approaches may increase the effective resolution of the light microscope by a factor of two or more, which may go beyond a threshold of resolution necessary to provide new insight into the internal organization of cells. A conceptually different approach for obtaining high-resolution information is the combination of fluorescence microscopy with subsequent electron microscopy of the same region of the specimen after fixation. Roman Polishchuk (Bethesda, MD) described the use of this strategy, discussing the tracking of highly dynamic transport vesicles at the Golgi-plasma membrane interface by video microscopy, and then examining the same fields by high-resolution electron microscopy.

Spectroscopy and quantitative analysis

Another important development described at the meeting was the application of novel spectroscopic approaches and quantitative analyses to light microscopy. Biochemical methodologies have been most powerful in characterizing molecular events and interactions. However, classical biochemical approaches lack spatial resolution and usually fail to provide information about the 'real' biological processes that take place in the three-dimensional and highly compartmentalized cellular context. Microscope-based spectroscopic approaches are designed to address this issue, not only in terms of the morphological, but also the molecular properties of different subcellular structures. Petra Schwille (Göttingen, Germany) reported on the use of fluorescence correlation spectroscopy (FCS), a method that measures interactions between molecules in a defined environment based on their diffusion patterns. She described its use in probing a variety of molecular parameters such as local concentrations of, mobility of, and interactions between specific proteins in a variety of systems. Paul French (London, UK) described the application of fluorescence lifetime imaging (FLIM) for probing the local microenvironments in terms of parameters such as pH and calcium concentration. The system described can record spectral and lifetime information at high spectral, spatial and temporal resolutions, functioning as a 'five-dimensional microscope'. These and additional studies presented at the ALM meeting reflect the growing awareness of new methods that can be used for studying molecular processes *in vivo*.

Quantitative microscopy studies of cell adhesions were described by Benny Geiger (Rehovot, Israel). These studies are based on accurate measurements of local intensities of green fluorescent protein (GFP)-tagged molecules in live cells, as well as of fixed cells, multiple-labelled for a variety of focal adhesion components. Analysis of the relative local intensities of the

different molecules pointed to a remarkable molecular heterogeneity of adhesion sites, and time-lapse recording revealed the mechanisms responsible for the generation of this diversity. In addition, segmentation procedures that allow an automatic identification of adhesion sites and determination of their morphological parameters, including size, intensity, orientation, axial ratio, etc., were discussed. These studies made possible new classification of cell-matrix adhesions, based on fine molecular composition and architecture, as well as on their dynamic properties (for additional information see Kam *et al.*, 2001).

Visualizing cellular dynamics

An important feature of modern light microscopy is the capacity to study dynamic processes, both cellular and molecular. Many of the studies presented in the talks, as well as in the poster session, made use of GFP and its variants for tracing dynamic molecular processes in live cells. The impressive progress in these applications can clearly be attributed to recent major developments including the availability of different variants of intrinsically fluorescent proteins that can be introduced into cells for multiple labelling. For example, this has resulted in the possibility of using properly selected pairs of such fluorescent proteins, e.g. the cyan and yellow derivatives of GFP, to measure nanometer-scale intermolecular distances by fluorescence resonance energy transfer (FRET) in which the photo-excitation of the donor fluorophore (CFP, in this case) leads to the selective excitation of nearby acceptor molecules (YFP), thus providing information on the inter-molecular proximity of the two molecules.

Cell motility is a common dynamic process that is involved in diverse events such as embryonic development, wound healing, inflammation and cancer metastasis. Daniel Zicha (London, UK) described the visualization of the movements of metastatic sarcoma cells in a specialized chemotactic chamber that provides a chemotactic gradient. Vic Small (Salzburg, Austria) showed movies of cells expressing fluorescent components of adhesion sites or of the cytoskeleton. Using these studies, he established the molecular relationships between the different molecules examined and highlighted the role of microtubules in directing motility and cell-matrix adhesion. The power of four-dimensional microscopy (i.e. three dimensions over time) was also demonstrated by studies on the dynamic relationships between the mitotic spindle and the cell cortex, as presented by Jim Dompierre (Orsay, France).

Several talks focused on dynamic events in the nucleus, and their relationships to major nuclear processes. David Spector (Cold Spring Harbor, NY) showed the spatio-temporal relationships between a gene and its product in live cells, as well as visualizing DNA decondensation, using a CFP/YFP combination of fluorophores and the lac activator/repressor system. He further showed different sub-nuclear speckles as well as the PML body and followed their dynamics. The shuttling of transcription factors of the STAT family into the nucleus and out again using GFP fusion proteins was presented by Mario Koester (Braunschweig, Germany), while Adriaan Houtsmuller (Rotterdam, The Netherlands) used fluorescence recovery or loss after photobleaching (FRAP and FLIP assays, respectively) to study, *in situ*, DNA repair and gene transcription. It was shown that the diffusion coefficient of different molecules is inversely related to their molecular weight,

and that DNA repair factors become immobilized following DNA damage.

Roland Eils (Heidelberg, Germany) presented advanced approaches for simultaneous visualization of several components at high spatial and temporal resolution, combined with powerful object detection and tracking methods. These approaches were applied to the study of (i) the dynamics of nuclear envelope breakdown and re-assembly during mitosis, and (ii) the relationships between the centrosome and site of chromatin de-condensation. This system was also used extensively for studying chromosome organization and positioning in live cells.

Alan Verkman (San Francisco, CA) addressed the rate and anisotropy of the diffusion of small molecules as well as macromolecules in different cellular compartments. Using FRAP and other methods, he showed that diffusion in the cytoplasm was considerably slower than in saline. For small molecules the reduction in diffusion rate was inversely related to the molecular radius, suggesting that local viscosity plays an important role. Larger, interacting macromolecules such as DNA fragments and different enzymes were slowed down further. These approaches were applied to the study of fluid dynamics in cells, for example the diffusion of the water channel aquaporin within the membrane of the endoplasmic reticulum.

Applications in genomics

Advanced light microscopy brings some exciting possibilities for functional genomic studies. Jeremy Simpson (Heidelberg, Germany) described a microscope-assisted cDNA screen for new proteins, based on their association with specific subcellular structures. This strategy offers exciting possibilities for the isolation of new genes that encode components of a wide variety of sub-cellular structures. In principle, one may prepare cDNA libraries encoding fusion proteins consisting of full-length, or segments of, diverse proteins fused to GFP. Large-scale screening for novel structural proteins is thus possible. Its wide application depends, to a large extent, on the development of an automated microscope system capable of multiple sample screening, autofocusing and the appropriate image processing.

Cellular manipulations

Finally, light microscopy can also be used to perturb specific structures at high spatial precision, and to study the molecular and cellular consequences of this manipulation. Alexey Khodjakov (Albany, NY) applied laser microsurgery to the ablation of centrosomes in live cells. Centrosomes were visualized using GFP- γ -tubulin (for which the cDNA had been transfected into the cells), and destroyed with a focused laser beam. It was shown that interphase microtubules that were associated with the ablated centrosomes disassembled. Interestingly, destruction of

both centrosomes during prophase did not block the formation of a bipolar spindle. Another approach for inactivating specific proteins *in situ* is the micro-scale chromophore-assisted laser inactivation (micro-CALI), which was described by Daniel Jay (Boston, MA). In this method, non-perturbing antibodies specific for particular cellular proteins, labelled with Malachite Green, are introduced into cells and illuminated by a laser beam at 620 nm. The excitation of the Malachite Green produces short-lived hydroxyl radicals that inactivate the antibody's nearest neighbours (namely the cellular protein with which it interacts). In the study described at the meeting, the roles of different myosin isoforms (Ib, II and V) in processes such as the extension of filopodia from the growth cones of neuronal cells were studied using micro-CALI.

Conclusion

The work presented at the ALM meeting, part of which was outlined here, provided an excellent opportunity to learn about current developments in advanced microscopy, and to gain an appreciation for the great potential of these rapidly developing technologies for modern biological research. Many of the participants felt that some courses of action besides the usual sharing of ideas and information should be taken to ensure that the momentum created at the meeting will be used to promote the development of light microscopy and increase its accessibility to researchers throughout Europe and beyond. In an open discussion conducted at the end of the meeting, it was agreed that some concrete steps would be taken. These include the organization of an annual ALM meeting with lectures, posters, workshops and tutorial sessions, the promotion of dialogue between researchers and the microscopy industry, and the sharing of procedures and imaging software between researchers in the field. One on-going avenue of activity is the establishment of 'microscopy centres' at different research institutes in Europe, for example at the European Advanced Light Microscopy Facility (EurALMF, <http://www.EMBL-Heidelberg.DE/ExternalInfo/EurALMF/>) in Heidelberg. The aim of such centres is to enable visiting researchers to use unique equipment which is not available to them at their home institutions. Most importantly, it was felt that the power of modern light microscopy should be presented to the broader community of cell and molecular biologists, particularly those whose training is primarily based on biochemical and molecular genetic approaches.

References

Kam, Z., Zamir, E. and Geiger, B. (2001) Probing molecular processes in live cells by quantitative multidimensional microscopy. *Trends Cell. Biol.*, **11**, 329–334.

DOI: 10.1093/embo-reports/kve205