Zvi Kam
Benjamin Geiger

Yael Paran
Irina Lavelin
Suha Naffar Abu-Amara
Yuvalal Liron
Tal Shay

Cell-based high-resolution and high-throughput microscope screening

Department of Molecular Cell Biology

Tel. 972 8 934 **3473** **Fax.** 972 8 934 **4125**
E-mail: zvi.kam@weizmann.ac.il
Web page: www.weizmann.ac.il

The conceptual framework of our studies is an image of a cell as an integrated network of interacting molecules, forming a large variety of sub-cellular structures and mediating signaling events in response to diverse external stimuli. Only some of the components of such networks are known, and just a fraction of their interactions is characterized in detail. Molecular components can often display an apparent redundancy or may participate in different cellular processes. Such networks must, therefore, be analyzed and characterized in the context of the live cell, that contains all the (known and unknown) components, and allows multiple molecular and functional perturbations. The molecular interactions within the particular cellular networks can be deduced from the temporal and spatial responses to these perturbations. When analyzed by high magnification microscopy, resolving sub-cellular structures such as the cytoskeleton, cell adhesions, mitochondria, ER, Golgi apparatus, cytoplasmic vesicles etc., these readouts allow to probe with extreme sensitivity both major responses to perturbations, that dramatically affect cell shape and behavior, and subtle, transient responses to stimuli. Such responses are best analyzed at single cell level, which highlights the variability that is ignored in biochemical studies, where only population averages are recorded. Unlike gene knock-out approaches, perturbation by drugs, environmental conditions (temperature, ions, radiation), and siRNA-mediated suppression of specific gene expression can be introduced within short time frames, and dynamically monitored. Modern microscopy allows for such high-sensitivity and high-specificity analyses, using inherently fluorescent protein constructs and other labeling techniques. This is extremely important for the modeling of complex processes by reverse-engineering approaches.

Led by such considerations we developed a screening microscope system that can image, at high resolution, cells grown in multiwell plates. This system can acquire 40Gbytes of high-magnification images per day in a continuous, computer-controlled operation, scanning thousands of individual cells exposed to multiple conditions. On-line, automated quantitative analysis procedures, are currently being developed, including novel segmentation and quantification algorithms, to turn the Terra-Bytes of

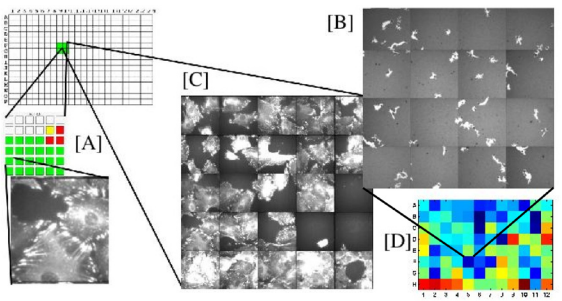

Fig.1 *Typical images acquired during a screen for drugs affecting cell motility and adhesion. Cells are cultured in multiwell plates, treated with the drugs and microscopically scanned and imaged inside these plates at high magnification. Multiple fields in each well are imaged [A]. All images in each well can be combined into a montage-picture for easy visualization [B,C]. The assay for cell motility is based on phagokinetic tracks: Cells are plated over bead-covered surface, and migrating cells clear beads from the area they migrate through [B]. Parameters characterizing the motility are extracted from tracks area and shape. Cell adhesion structures are analyzed from high-resolution fluorescent images of reporter cells stably expressing YFP-tagged Paxillin, a cell adhesion protein that localizes to cell-substrate adhesion sites [C]. The drug-induced effects are quantified by evaluating the number, size, density, intensity and morphology of the adhesion sites. A color-coded score for each well summarizes the results of mobility for each plate [D].*

image data for each experiment into compact and meaningful functional results.

Among the presently pursued projects we screen for natural extracts that affect cell motility and adhesion, and for new proteins that interact with cell adhesions and with the actin cytoskeleton or modulate their integrity [Fig. 1].

In order to optimize our microscopic imaging we develop and improve high-resolution imaging technologies in live cells, including fast autofocusing device, aberration corrections for thick samples in buffer media using adaptive optical elements, and fast method for imaging thick 3D samples by "sweeping focus" acquisition [Fig 2]

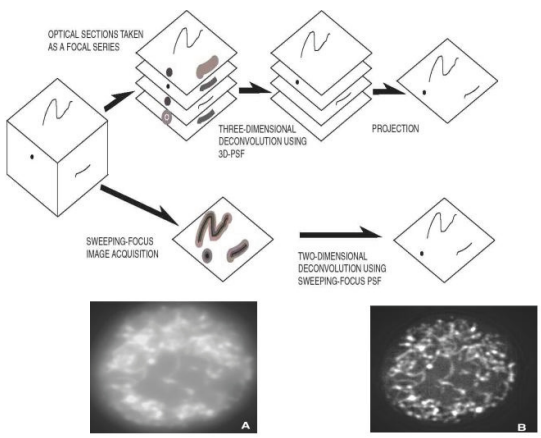

Fig.2 *A fast method for imaging thick specimens, replacing the conventional acquisition of three-dimensional focal series. The camera integrates the signal while the focus is sweeps across the cell height, yielding the blurred projected image of the mitochondria labeled by transfecting SV80 cells with a mitochondrial localization vector [EYFP-Mito, Clontech] [A]. 2D deconvolution using the projection of the microscope point spread function (PSF) shown in [B] is equivalent to the projection of the 3D de-blurred data [see flow diagram], includes sharp features throughout the thickness of the cell, yet requires a small fraction of both acquisition and deconvolution time. This method is therefore practical for high-throughput screening applications, for example studying the affects of drugs on cell metabolism.*

Selected Publications

Lichtenstein, N., Geiger, B. and Kam, Z. (2003) Quantitative analysis of cytoskeletal organization by digital fluorescent microscopy. Cytometry. 54A: 8-18.

Kirchner, J., Kam, Z., Tzur, G., Bershadsky, A.D. and Geiger, B. (2003) Live-cell monitoring of tyrosine phosphorylation in focal adhesions following microtubule disruption. J Cell Sci. 116:975-86. Erratum in: J Cell Sci. 2003 116:1389.

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Kam, Z., Zamir, E., Geiger, B. (2001) Probing molecular processes in live cells by quantitative multidimensional microscopy. Trends Cell Biol. 11: 329-34.

Kam, Z., Hanser B, Gustafsson MG, Agard DA, Sedat JW. (2001) Computational adaptive optics for live three-dimensional biological imaging. Proc Natl Acad Sci U S A. 98:3790-5.

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