One of the primary challenges of biological research in the “post-genomic era” is a comprehensive understanding of the concerted action of multiple genes and their protein products in performing basic life processes. Advanced light microscopy has developed in recent years into a powerful tool for studying molecular and morphological events in cells and tissues. Cell-based screening is an emerging methodology for the exploration of cellular processes and their modulation by multiple chemical or genetic perturbations. Use of the intact live cell as a reporter enables a multi-parametric characterization of responses to such perturbations. These perturbations are screened for effects on a variety of molecular and cellular targets, including sub-cellular localization and dynamic redistribution of proteins, but unlike biochemical screening, they detect the responses within the context of the interconnected structural and functional networks of normal or diseased cells.

The challenges in cell-based screening include preparation of suitable reporter cells, (each presenting a narrow slice of the multidimensional space characterizing cellular systems), automated microscope readouts for high-throughput screening of large libraries, and the development of algorithms for the quantitative analysis and interpretation of terabytes of screened images that is linked to high-level bioinformatics databases. High-resolution microscopy and high-throughput screening are two essential methodologies for detailed characterization of cellular phenotypes, but cell-based platforms typically constitute a compromise between these two rather opposing demands. We developed a screening microscope platform that automatically acquires and interprets high-resolution images at fast rates. The fast acquisition of high-resolution images is made possible by a laser autofocus device [6]. The analysis pipeline is based on the quantification of multiple sub-cellular features and statistical comparisons of their distributions in treated vs. control cells [7]. We applied this platform to screen natural extracts, chemical libraries and siRNA perturbations for their effects on the structure and organization of focal adhesions and the cytoskeleton. Cell based screening opens new opportunities not only in basic cell and systems biology but also in the drug discovery process, for example in a systematic optimization of drug cocktails with cell type specificity.

The platform has been applied to the following projects [7, see also http://www.weizmann.ac.il/mcb/Geiger/Screening.htm]:

### Perturbation of cell adhesions

1. Chemical and natural extracts compounds screens (Y. Paran, B. Geiger).

Examination of effect of chemicals on live cells, including effects on cellular morphology and relocation of structural or signaling proteins, require microscope-based imaging at high resolution. The higher the image resolution, the more sensitive is the readout of drug effects on cellular functions manifested as subtle cell architectural attributes. Here we examined, in collaboration with B. Geiger, the effects of chemical compound library and a library of natural extracts on focal adhesion (FAs) morphology. REF52 cells, stably expressing paxillin-YFP, served as the reporter cells. The cells were cultured in multi-well plates and treated by 2200 extracts and compounds from a library of 10,000 low molecular weight, chemically-defined molecules (ChemDiv Inc., San Diego, CA), using a single compound per well. Following treatment, the cells were fixed and the plates were screened for effects on FAs using the automated microscope at high resolution. Acquired images were subjected to quantitative analysis that pointed to 15 extracts and 100 compounds with interesting cytoskeletal and FA-perturbing effects. The perturbed phenotypes included FA elongation, reduction in FA size, loss of FAs at the cell center, abnormal paxillin localization, and overall cytoskeletal collapse. The automated microscope was also utilized to study the time-and concentration-dependent effects of selected compounds and their mechanism of action, using labeling of cytoskeletal and FA-related proteins.

To explore the mechanism involved in focal adhesion formation, individual signaling and focal adhesion components were knocked-down using a siRNA technology. For that purpose, HeLa JW cells, expressing paxillin-YFP, were transfected with a library of “SMARTpools” RNA duplexes, each consisting of a mixture of four siRNAs, targeting the same gene (Dharmacon, USA). Quantitative multiparametric scores of the effects on cells are being evaluated to identify correlations with groups of genes mediating common mechanisms.

A YFP-tagged cDNA screen for novel structural proteins, based on localization (I. Lavelin, B. Geiger).

Normalized, oriented cDNA library was constructed with poly(A)-primed cDNA, prepared from mixed mRNA pool of rat brain tissue and rat kidney fibroblasts, and randomly fragmented to an average insert size of 500 bp. The fragments were cloned into pLPCX retroviral vector (Clontech) using SfiI sites upstream to the YFP cDNA. REF52 cells were infected with pools of clones generated by subdividing the library. Those clones that contained upstream ORFs that were out of frame with YFP were excluded from the subsequent analysis by FACS sorting. The cells expressing YFP fusion proteins were plated 5 cells per well in 96-well plates. Each well is then “backed up” and frozen in a 96-well plate for future investigation, as well as plated into 384-plates with optical plastic bottoms screening for distinct patterns of YFP fluorescence by the automated microscope. Cells from wells displaying patterns of interest were then subjected to single-cell cloning and re-screened to isolate the clone responsible for the specific pattern. Genes of interest are cloned, sequenced and subjected to further characterization. About 4% of total infected cells display clear cellular localization to nucleus, Golgi complex, mitochondria cytosol, secretory vesicles and others.

Screening for genes affecting cell migration (S. Naffar Abu-Amara, B. Geiger)

Cell migration is essential for the development and maintenance of normal tissues and organs, and is enhanced in malignant cells, causing metastasis. In order to identify genes involved in, or affecting cancer-related cell migration we developed a quantitative high-throughput method for assessing cell motility, based on phagokinetic track formation. Glass-bottom 96 well plates were coated with a monolayer of microbeads, then, cells expressing different genes, derived from metastatic cells, or from libraries of cancer-related genes, were seeded on the bead monolayer and allowed to migrate for 5-9 hours. The migratory tracks, formed by the migrating cells were subjected to computerized analysis of track morphology using multi-scale segmentation methods, leading to extraction of multiple track parameters, including area, maximal length, ratio between long and short axes, perimeter and border roughness which reflect migratory features such as velocity, persistence and lamellar activity.
Selected publications


Cohen, M., Kam, Z., Addadi, L. and Geiger, B.: Dynamic study of the transition from hyaluronan-mediated to integrin-mediated adhesion in chondrocytes. EMBO J. (in print)


Acknowledgements

ZK is the Israel Pollak Professor in Biophysics. Research supported by BSF 2001221, Ministry of Science; The Clore Center for Biological Physics and the Kahn Fund for system biology at the Weizmann Institute of Science.