

# Quantitative multidimensional light microscopy

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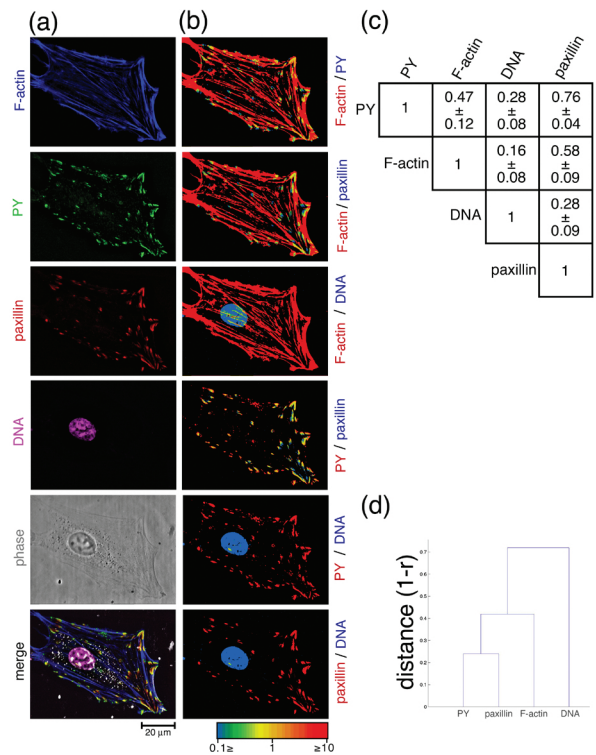
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Modern light microscopy has become a most powerful analytical tool for studying molecular processes in cells. Recent advances combining sample preparation, microscope design and image processing allow the generation of 'multidimensional' image data, simultaneously reporting the three-dimensional distribution and concentrations of several different molecules within cells and tissues at multiple time points with sub-micron spatial resolution and sub-second temporal resolution. Molecular interactions and processes that were approached by biochemical analyses *in vitro* can now be monitored in live cells. Our research focuses on the development of novel methodologies for light microscopy, and on its application to the molecular organization of cell adhesions and the cytoskeleton.

In Fig. 1a we present a simple example of multicolor image, showing the distributions of four fluorescently labeled cellular components: F-actin (TRITC-conjugated phalloidin), phosphotyrosine (PY; polyclonal anti-phosphotyrosine + FITC-conjugated goat-anti-rabbit antibodies), paxillin (monoclonal anti-paxillin + Cy5-conjugated goat-anti-mouse antibodies) and DNA (DAPI), as well as the phase-contrast image of the same cell, depicting the nucleus, various phase-dense organelles, and the cell outline. The 'merged' image combine visually the information from all five images. Pixel-by-pixel ratio between images in Fig. 1b visualize the local variations in molecular composition: a uniform ratio image represents similarity, while variations represents heterogeneity. Cross-correlation coefficients quantify the similarity between distributions of pairs of components (see Table 1c), and provide image-averaged scores for molecular interactions. The dendrograms using these correlations (Fig. 1d) yields a hierarchical tree of similarity between cellular distribution patterns of the above molecules, picking the two colocalized focal adhesion components PY and paxillin, and the partial overlap with actin.

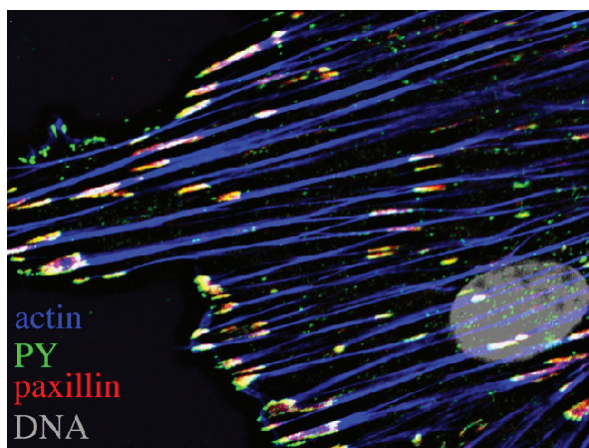
Another kind of quantitative image analysis method relies on recognition of structures by defining the corresponding regions in space (segmentation). Binary decision to define structures when the labeling intensity of a constituting molecule is above a threshold is typically impossible due to non-uniform and high



**Fig. 1** (a) Multicolor imaging of four cellular components and phase (separately and merged). (b) Ratio imaging (blue: lowest red: highest ratio). (c) Cross-correlation,  $r$  ( $r=1$  overlapping,  $r=-1$  complementary non-overlapping). (d) Dendrogram, distances are  $(1-r)$ .

background. We have developed a range of three-dimensional image segmentation algorithms and background estimates for various cellular sub-structures, such as nuclei, focal adhesions, cytoskeletal fibers, vesicles and mitochondria. Such analyses generates statistical data on the morphological properties (e.g. size, orientation, elongation) and molecular composition of the segmented objects. For example, scattergraphs depicting the total amounts of two adhesion proteins reveal distinct subpopulations with different protein composition, information that cannot be revealed by biochemical determination of the average structure composition.

In Fig. 2 a different approach to segmentation of cellular structures is displayed. It is based on similarity of the pattern of molecular composition. As compared to cross-correlation, this analysis can deal simultaneously with more components (practically limited to 4-5 independent fluorescent channels), yet the number is still much smaller than the number of molecular components assembled and interacting within typical cellular structures. It is our goal to devise methods that will assemble the global information about complex biological networks of interacting molecules from the sub-spaces accessible to experimental measurements.



**Fig. 2** Cluster analysis of color component patterns in multi-labeled cell images. Clustered pixels (painted with distinct colors) share stoichiometry. The algorithm was written by U. Alon and N. Barkai.

Dynamical studies performed with live cells expressing chimeras containing inherently fluorescent proteins are experimentally demanding, and usually limited to small number of examined cells. Nevertheless, they have been a source of new and exciting information. When compared to statistical analysis of cell populations that are fixed, live cell analysis avoids the variability between individual cells. The resulting high sensitivity to changes allow to evaluate effects induced by drugs and by mutations. We are developing a platform that will allow fast, systematic and sensitive reporting of such changes employing high-resolution microscopy.

In collaboration with B. Geiger and A. Bershadsky's groups.

#### **Selected Publications**

- Zamir, E., Katz, B-Z., Yamada, K.M., Geiger, B. and Kam Z. (1999) Molecular diversity of cell-matrix adhesions. *J. Cell Sci.* 112, 1655-1669
- Zamir, E., Katz, M, Posa, Y., Erez, N., Yamada, K.M., Katz, B-Z, Lin, S., Lin, D.C., Bershadsky, A., Kam, Z. and

Geiger, B. (2000) Dynamics and segregation of cell-matrix adhesions in cultured fibroblasts. *Nature Cell Biol.* 2, 191-196.

Katz, B-Z, Zamir, E., Bershadsky, A., Kam, Z., Yamada, K.M. and Geiger, B. (2000) Physical state of the extracellular matrix regulates the structure and molecular composition of cell-matrix adhesions. *Mol.Biol. Cell* 11, 1047-1060.

Kam, Z, Zamir, E. and Geiger, B. (2001) Probing molecular processes in live cells by quantitative multidimensional microscopy. *Trends Cell Biol.* 11, 328-333.

Kam, Z. (2001) Generalized analysis of experimental data for interrelated biological measurements. *Bull. Math. Biol.* (in print)

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