

PHOTOMANIPULATION OF FLUORESCENT PROBES USING A KILOBEAM ARRAY SCANNER IN LIVING CELLS

Peter Lipp, Xin Hui, Ken Bell* and Lars Kaestner
Institute for Molecular Cell Biology, Saarland University
Building 61, Homburg/Saar, Germany
***VisiTech Int. Ltd., Sunderland, UK**
E-mail: peter.lipp@uks.eu

KEYWORDS: fast confocal imaging, living cells, FRAP, FLIP, UV-flash photolysis, multi-beam array scanner, exchangeable pinholes, fluorescent proteins

Today multi-beam scanners have proven as the technique of choice to study the subcellular morphology as well as subcellular function and signal processing in living cells. The major limitation of Nipkow-discs (i.e. non changeable pinholes) was overcome by the introduction of kilobeam array scanners where a single moving mirror serves triple functionality: (i) scanning from a stationary microlens array, (ii) de-scanning onto stationary pinhole arrays and (iii) rescanning onto a CCD-camera for image construction. For the first time such a stationary pinhole plane allowed for exchangeable pinholes with sizes optimised for objectives with a wide range of magnifications. Recently, we have extended this design by including a second optical path and scan mirror into the scanhead (using the same coupling input) that allows fast multi-region photo-manipulation of fluorescent probes in living cells. In addition, the coupling of a UV-flash lamp simultaneously enables the whole-cell manipulation of caged compounds. FRAP and flash photolysis are two optical techniques that have been proven very useful to study diffusion properties of fluorescent dyes or fluorescence fusion proteins as well as organelle characteristics in living cells.

We have applied this technique to a great variety of different studies in living cells:

- (i) in experiments with Lifeact-GFP expressing HeLa cells fast, multi-regional FRAP and FLIP could be demonstrated
- (ii) mitochondria in HEK293 and HeLa cells were labelled with Mito-Tracker Green (Molecular Probes) or mito-eGFP. FRAP experiments in parts of identified individual mitochondria revealed fast fusion and fission between these organelles.
- (iii) cPKC translocation to the plasma membrane was investigated by fast confocal recording (up to 40 frames/s) and UV-flash photolysis of NP-EGTA. We found that the oligomerisation of the fluorescent protein (YFP vs. dsRed2) slowed down translocation.
- (iv) depolymerisation of the actin filaments and/or microtubules did not affect UV-flash induced cPKC translocation to the membrane
- (v) depolymerisation of the actin filaments and/or microtubules did not affect the cytosolic mobility of cPKC as assessed in FRAP experiments

Here we have introduced a novel optical design that in combination with a kilobeam array scanner enables fast confocal multi-beam imaging, very rapid bleaching of arbitrary regions in living cells and global photomanipulation with a UV-flash. We envisage applications similar to those demonstrated here, namely the investigation of diffusion characteristics of fluorescent entities and/or studies of the structure/function relationship of individual organelles in living cells. Studies can also be extended to other photo-activation studies.