# Light microscopy adapter for laser based microscopy techniques

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## ABSTRACT

Rapid development of micromanipulation and experimental techniques increases the need for easy-to-handle tools, which are suitable for routine operations. Our new concept represents a very suitable platform for incorporating such new experimental techniques into standard laboratory microscopy equipment. Implementing of microscopy methods such as optical trapping, cutting, fluorescence excitation, photopolymerization and most spectroscopic techniques is very expensive and time consuming. In most cases, microscope parts and body must be adapted to insert additional lasers, detectors etc. We overcome these difficulties by inserting source of radiation directly between microscope body and microscope objective. Our concept allows using arbitrary source of radiation (laser diodes, solid-state lasers, gas lasers). Laser diode source can be incorporated inside the adapter. Optical fiber is used to deliver other sources of radiation. Light microscopy adapter (LMA) can be easily mounted on most types of microscopes including special types as confocal microscope.

We demonstrate ability of LMA for stable optical trapping of particle in the range of hundreds of nanometers to tens of micrometers, bleaching (FRAP, FLIP) and microdissection experiments. Mechanical and optical construction of LMA addresses spatial constrains of commercially available microscopes, mainly the fact that optical parts of microscopes are optimized for visible spectrum of light. Our solution preserves all microscope properties unaffected and it is possible to introduce modern microscopy techniques to many types of microscopes.

**Keywords:** laser diode, optical fiber, optical manipulation, photobleaching, microdissection

## 1. INTRODUCTION

Laser assisted microscope techniques are very popular in many areas of science.[1–5] Standard ways how to incorporate this feature in apparatus is quite time consuming and expensive. In most cases guiding of light into the microscope system causes unwanted influencies on correct properties of optical microscope. In systems used for micromanipulation, photopolymerization, photoactivation etc, light is guided into the system by epi-fluorescent port of microscope. For this reason the port is now unused for its purpose and fluorescence experiments must be omitted. Another common problem is with optical coating inside optical path of microscope. Optical path inside microscope is mostly optimized for working in visible part or in some cases in utraviolet region of optical spectra. Due to this fact, standard and nonmodified optical microscope is unsuitable for techniques, which use the light source in near infrared part of optical spectra.

We solve this problem by constructing light microscopy adapters. LMA overcome problems with light guiding into optical path of microscope by inserting light source straightforward between microscope body and microscope objective. For functionality of LMA the microscope body is not necessary and instrument can work standalone, the microscope acts only as the support. LMA does not affect construction of microscope and all features of observing images are well-kept. Presented LMA does not need presence of epi-fluorescencent port in the microscope body, which is mostly omitted in low cost types of microscopes.

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16th Polish-Slovak-Czech Optical Conference on Wave and Quantum Aspects of Contemporary Optics, edited by Agnieszka Popiolek-Masajada, Elzbieta Jankowska, Waclaw Urbanczyk, Proc. of SPIE Vol. 7141, 71410D · © 2008 SPIE · CCC code: 0277-786X/08/$18 · doi: 10.1117/12.822358

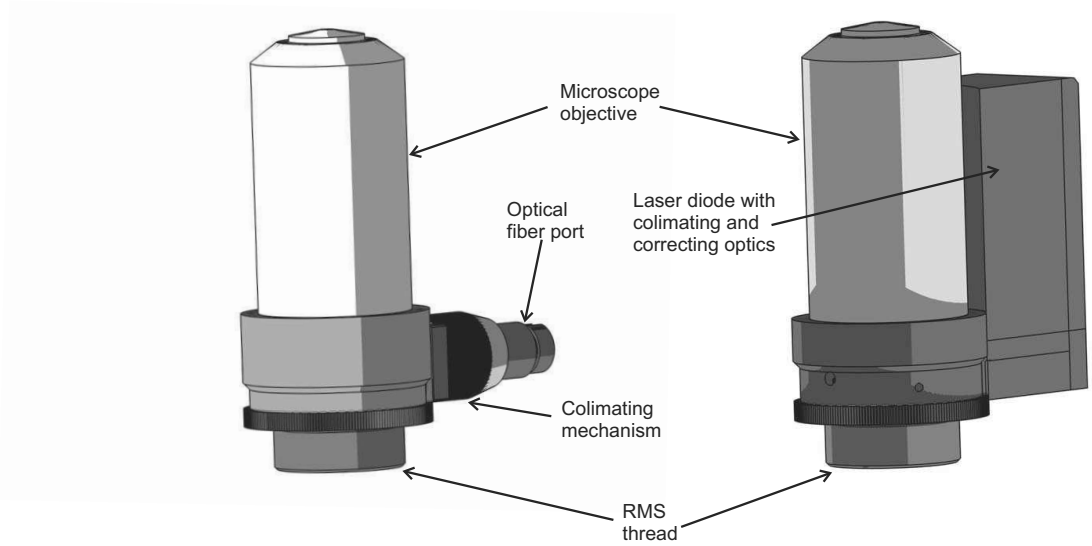


Figure 1. Mechanical construction of LMA with optical fiber; LMA2 (left) and LMA with laser diode; LMA1 (right).

## 2. CONSTRUCTION

We constructed two types of LMAs. The first type is with source of radiation incorporated directly in LMA. We selected laser diode Sanyo DL-8031-031A as a laser radiation source of emitted light with wavelength 808 nm and base transversal profile $TEM_{00}$, maximal output power 200 mW. This wavelength is suitable for biological objects[6] and could be used for optical trap generation. Emanated beam is then collimated by aspherical lens with focal length 8 mm (Geltech 352240-B) and elliptical beam profile was corrected by anamorphic prism pair (Thorlabs PS871-B).[7] The improved beam was then retroreflected by a pair of mirrors into infinity-corrected microscope objective. The first mirror was coated with multilayer for maximum reflectance for wavelength of laser diode. The second mirror has multilayer with maximum reflectance for laser diode in one direction and maximum transmittance for visible light in opposite direction. This optical system was entered to Zemax optical program and optimization was performed. After optimizition we obtained diffraction limited laser beam. Stability of power in the focal point was achieved by temperature stabilization of laser diode by Peltier module (Supercool PE-017-06-11) and appropriate selection of construction materials. Stability of current to the laser diode was done by homemade current controller.[8] If ultraviolet laser diode is used as laser light source, LMA can by used as a optical scissors[9–12] or for photopolymerization techniques. LMA can also act as source for excitation of spectral lines with another types of laser diodes.

The second type of LMA is very flexible due to using optical fibers as the source of radiation. Fibers could deliver radiation from various sources (solid state, gas, spectroscopy and others). Beam emanating from fiber, single mode or multimode with FC/PC connector, was collimated by achromatic lens (Thorlabs AC080-20 with appropriate coating). Divergention of the beam could be fine tuned by on axis movement of this lens. This improoved beam was then retroreflected by dichroic mirror, with maximal reflectance for used laser sources, into the infinity-corrected microscope objective. Optimization of optical system was performed and we obtained diffraction limited spot in the focal plain.

## 3. EXPERIMENTS

We made many types of experiments with various types of speciments, techniques and microscope types to prove functionality of LMA. The first type of the experiments was made for verification function of LMA1 as optical tweezers. Trapping power of LMA1 was checked in combination with inverted microscope Olympus IX-70. Microscope objective Olympus Ach 100x was used as a focusing element (see Fig. 2). Three-dimensional manipulations with polymer particles with diferent sizes (Duke Scientific) freely diffused in deionized water were performed. Power in the focal point was kept for inverted microscope at level 55 mW. Images were aquired with b/w CCD camera (Mintron, 63W1C) and digitized by framegrabber card (Picasso PCI-2SQ, Arvoo). Position of the image plane is changed by lateral movement of microscope stage and longitudinal movement of microscope objective. Image of trapped particle is still focused (see Figs. 3, 4)

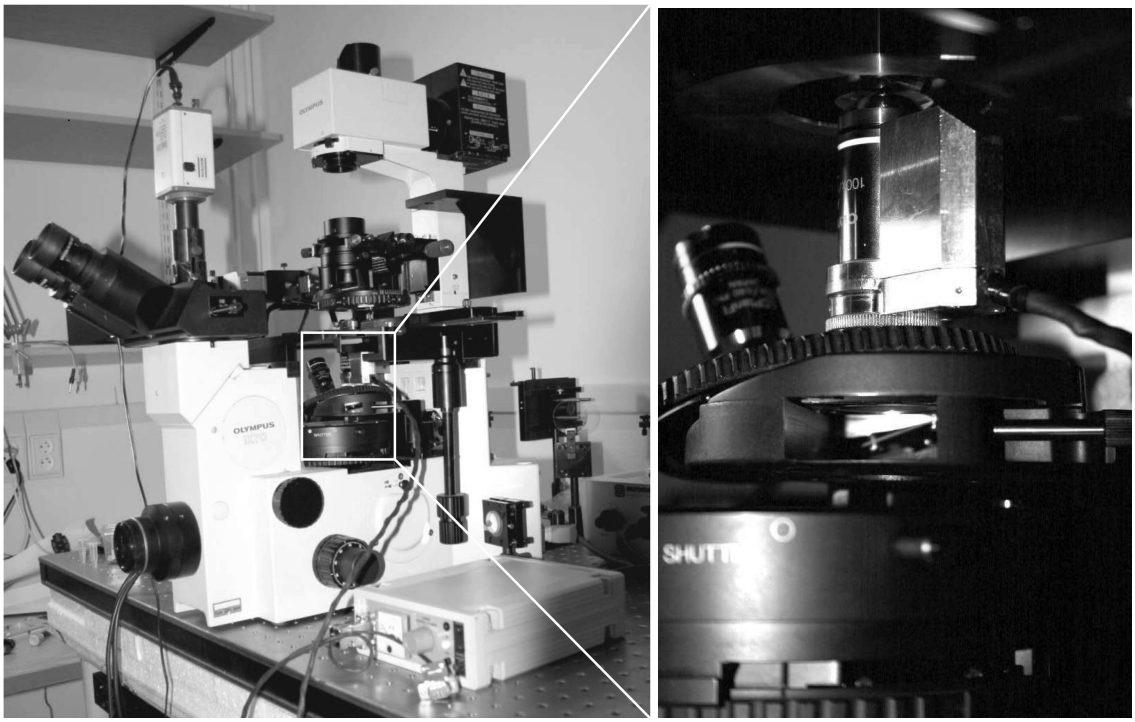


Figure 2. LMA with laser diode mounted on inverted microscope Olympus IX-70. View of the apparatus (left) and detailed view of the LMA1 (right).

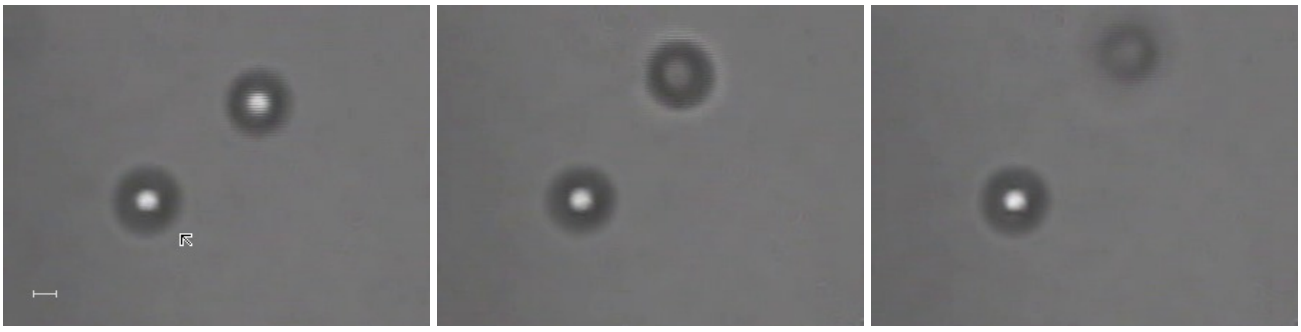

Figure 3. Arrow signed polymer microsphere with diameter 2 $\mu$m (4k-2 Duke Scientific) in water solution is trapped by LMA1. Object in right part of left image laying on the microscope slide is focused. The position of the optical trap is changed by vertical movement of the microscope objective and image of the right particle blurs. Trapped particle stays focused. Power in the focal point is 55 mW. Vector on the first picture has length 1 $\mu$m.

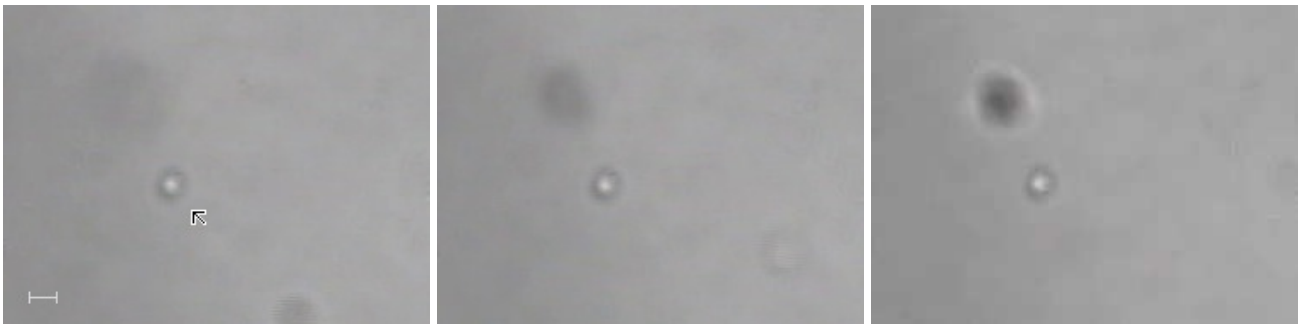

Figure 4. Arrow signed polymer microsphere with diameter 520 nm (3k-500 Duke Scientific) in water solution is trapped by LMA1. The position of the optical trap is changed by vertical movement of the microscope objective and image on CCD camera focuses. Power in the focal point is 55 mW. Vector on the first picture has length 1 $\mu$m.

We also used upright microscope Olympus BX-50 in combination with microscope objective Olympus UplanApo 100x for verification of LMA1 function in revert configuration. As a specimen we used yeast cells *Saccharomyces cerevisiae*. Yeasts were diluted in water solution and enclosed by cover slip and microscope slide. Power in the focal point was kept at level 30 mW. Images were aquired by b/w CCD camera (Mintron, 63W1C) and digitized by framegrabber card (Picasso PCI-2SQ, Arvoo). Position of image plane is changed by lateral and longitudinal movement of the microscope stage. Image of trapped yeast is still focused (see Figs. 5, 6).

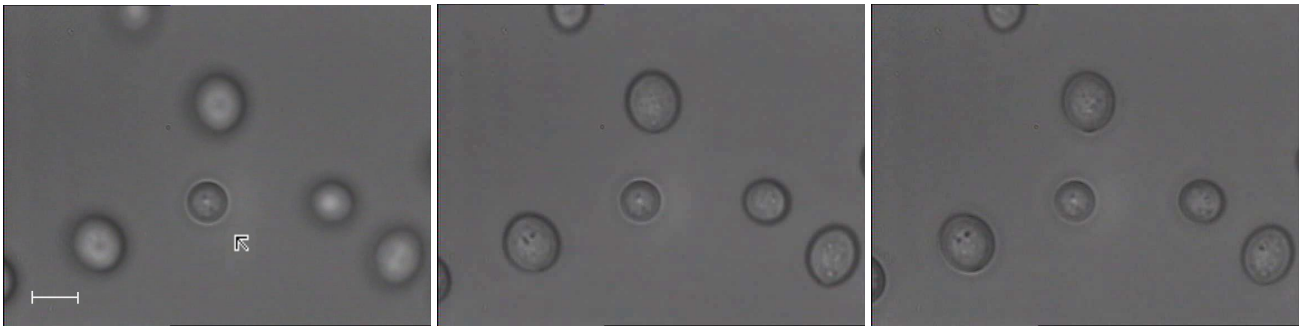

Figure 5. Manipulation with yeast cells Saccharomyces cerevisiae (arrow signed), trapping power 30 mW. Yeast cell freely levitated in water solution is trapped by LMA1 and speciment is moving longitudinal. Image of yeast cell is stil focused, yeast cells laying on microscope slide are focusing.Vector on the first picture has length 5 $\mu$m.

We also check suitability of LMA1 for fluorescent experiments. From Fig. 7 is clearly seen that influence of LMA1 on fluorescence signal is very low.

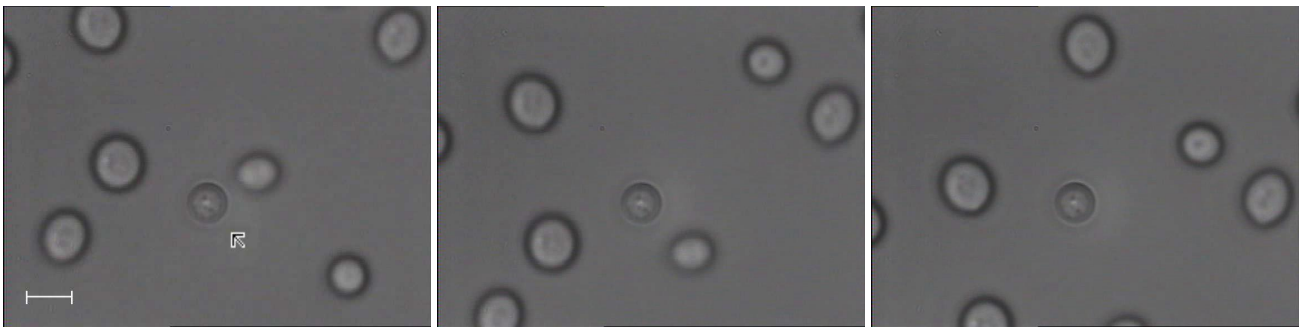

Figure 6. Manipulation with yeasts Saccharomyces cerevisiae (arrow signed), trapping power 30 mW. Yeast cell is trapped by LMA1 and speciment is moving transversal, yeasts laying on microscope slide are moving to bottom of image, trapped yeast cell has same position. Vector on the first picture has length 5 $\mu$m.

Figure 7. Manipulation with fluorescently labelled polystyrene particles (denoted by arrow) trapped by LMA1, trapping power 30 mW. Vector on the first picture has length 5 $\mu$m.

Finally we measured optical trap stiffneses for various types of microscope objectives see Tab. 1. We conclude from measurements that best trapping power was reached with two types of microscope objectives. First type was basic and realtively non-expensive achromatic microscope objective. Second type of microscope objective was expensive top company model. Reason for this fact is that middle class microscope objectives has lower transmission in near-infrared region, and also optical abberation has significant influence on optical trap quality. Good results for entry class objectives is due smaller number of optical elements, because its influence on transmition is lower.

Table 1. Optical trap stiffness for various types of microscope objectives, current of laser diode I=180 mA. The second rows for corresponding objectives are for repeated measurements of different microparticle in the same place of the speciment.

| Microscope objective | $\kappa_{Bx}$ [$10^{-5}$ Nm$^{-1}$] | $\kappa_{By}$ [$10^{-5}$ Nm$^{-1}$] | $\kappa_{Bz}$ [$10^{-5}$ Nm$^{-1}$] |
|---|---|---|---|
| Olympus Ach 100x/1,25 Oil Iris | $7,0 \pm 0,4$ | $4,1 \pm 0,3$ | $1,5 \pm 0,1$ |
| | $7,0 \pm 0,3$ | $3,8 \pm 0,2$ | $2,0 \pm 0,1$ |
| Olympus Ach 100x/1,25 Oil Ph3 | $6,3 \pm 0,3$ | $1,9 \pm 0,2$ | $1,0 \pm 0,1$ |
| | $6,1 \pm 0,5$ | $2,3 \pm 0,4$ | $1,0 \pm 0,1$ |
| Olympus UPlanFl 100x/1,3 Oil Iris | $1,3 \pm 0,2$ | $2,3 \pm 0,5$ | $1,01 \pm 0,03$ |
| | $1,7 \pm 0,3$ | $2,7 \pm 0,4$ | $1,1 \pm 0,04$ |
| Olympus UPlanApo 100x/1,35 Oil Iris Ph3 | $2,6 \pm 0,1$ | $7,3 \pm 0,2$ | $1,40 \pm 0,04$ |
| | $3,1 \pm 0,2$ | $7,5 \pm 0,2$ | $1,5 \pm 0,1$ |

We tested LMAs, especially LMA2 in routine biological techniques such as bleaching and microdissection on uppright fluorescence microscope (see Fig. 8) equipped with sensitive CCD camera. The first experiment shows performance of LMA2, bleached region in the fluorescently labeled slices is very nice localized horizontally. Vertical localization is presented by nearby slices with distance 500 nm acquired in the volume of speciment (see Fig. 9). The second experiment introduce LMA2 in very popular and frequently used biological technique microdissection. Membranes of two cucumber cell (see Fig. 10), which are close together, were exposed by single ns shot of UV laser puls. Membranes were broken and intra volumes of cell were mixed together.

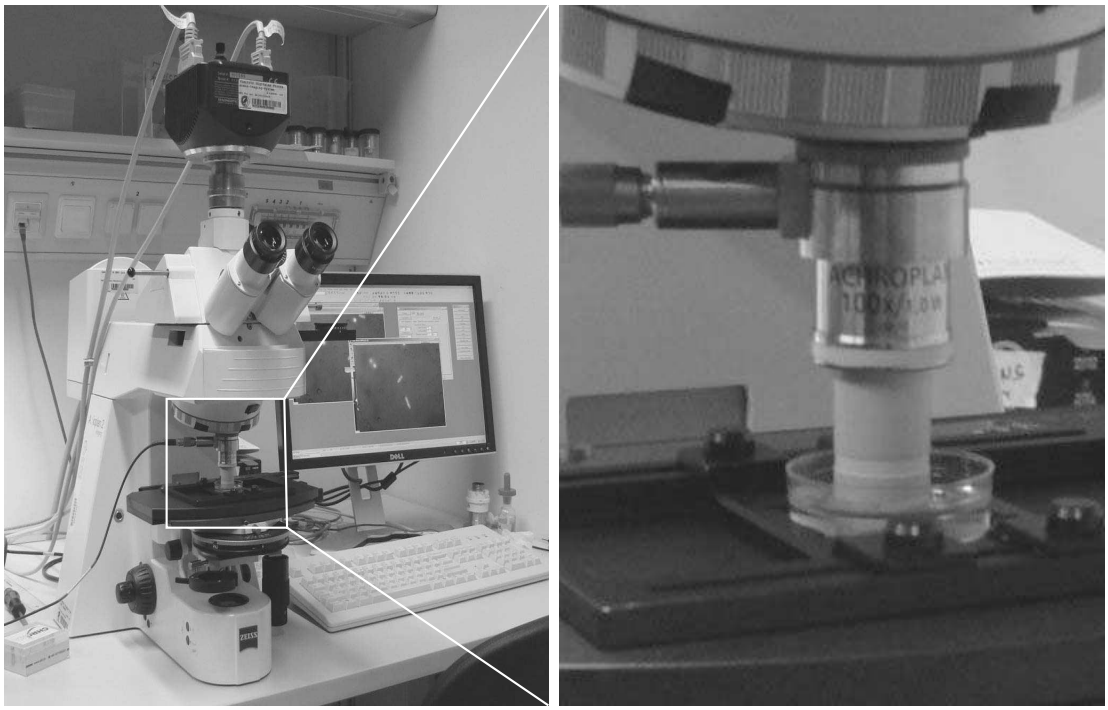

Figure 8. LMA with optical fiber mounted on uppright microscope Zeiss Axioplan2. View of the apparatus (left) and detailed view of the LMA2 (right).

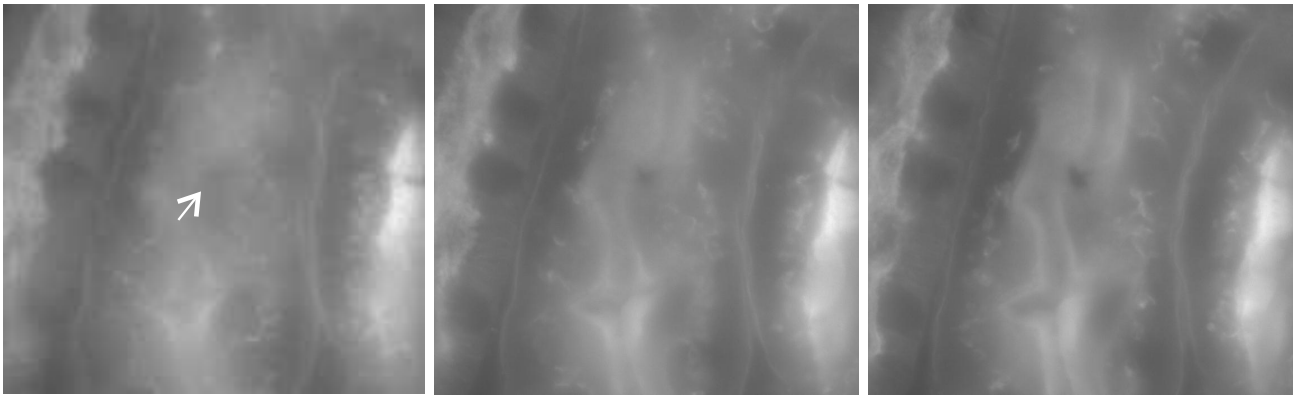

Figure 9. Bleaching of mouse kidney tissue slices stained with Alexa 488. Vertical stack 5 $\mu$m, LMA2, PicoQuant laser, driver PDL 808, laser head LDH-405, water immersion objective Zeiss Neofluar 100x, microscope Zeiss Axioplan 2 (in cooperation with MPI-CBG).

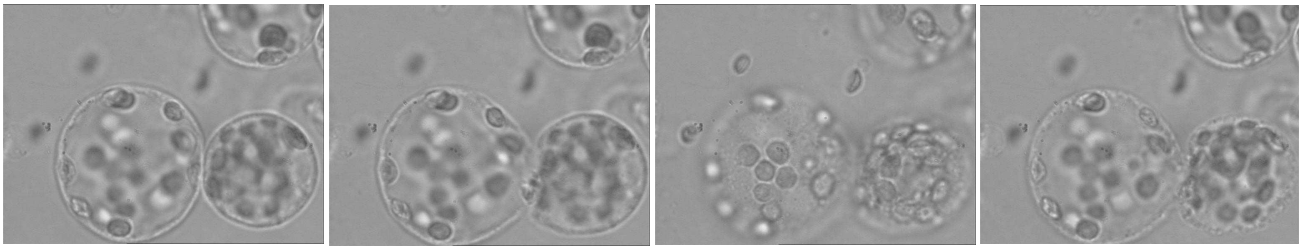

Figure 10. Microdissection of protoplast of cucumber. Membranes at the cell contact are exposed to a single shot which leads to their perforation. LMA2, laser Continuum Minilite II, $\lambda$=355 nm, 6 ns pulse, oil immersion objective Olympus UPLANFl 100x/1,25 Oil, microscope Olympus IX-70 (in cooperation with UP Olomouc).

## 4. CONCLUSION

We have proved the ability of LMA to trap optically the particles in the range of hundreds of nanometers to tens of micrometers. Using LMA we have also bleached fluorescently labeled samples (FRAP, FLIP methods) and performed microdissection experiments. We currently develop various stable fiber coupled laser sources, pulsed and continuous, from UV to IR, which are fully compatible with LMA's. Our solution preserves all the microscope properties unaffected and, therefore, it paves the way for easy and fast utilization of the modern microscopy techniques in various types of microscopes.

## ACKNOWLEDGMENTS

This work was supported by the Ministry of Industry and Trade of the Czech Republic (project No. FT-TA2/059), GAAS (project No. KJB100650601), ISI IRP (project No. AV0Z20650511) and MEYS CR (project No. OC08034).

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