**Non-Linear Excitation Fluorescence Imaging through**

**Two-Photon Laser Polymerized Microlenses**

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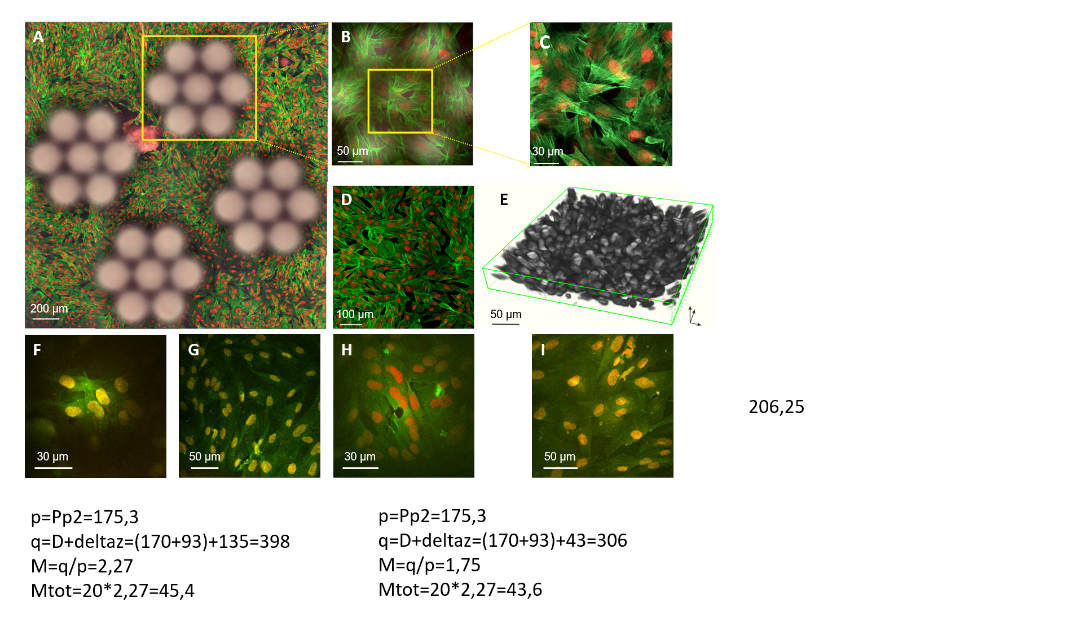
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# We report on methods for laser fabrication of microlenses by 2 Photon Polymerization (2PP) and their use for non-linear excitation microscopy. These lenses can help, once implanted, to reduce the spherical aberrations induced by the tissue in *in vivo* imaging.

Microlenses have already been fabricated by 2PP [1] but with too low numerical aperture to allow non-linear excitation microscopy. Therefore, up to now, no attempt has been made to use laser fabricated microlenses for non-linear excitation fluorescence microscopy of biological samples.

We show that microlenses with high numerical aperture (0.4) and large diameter (280), can be fabricated with a medium throughput, about 8 minutes/lens, by limiting the 2PP fabrication to a ≃ 5 thick outer crust, followed by a post-development volume polymerization. This procedure leads to sufficient optical quality (roughness ≃ 80nm) to use the lenses for fluorescence confocal and two-photon excitation microscopy. We report on the use of the microlenses for fluorescence non-linear excitation microscopy of cells cultures (Figure 1) and thick specimens [2].



**Figure 1. A:** Confocal image of human fibroblasts (FITC-phallodin, cytoskeleton and DRAQ5/Hoechst, nuclei staining). **B**: fluorescence confocal image through a microlenses array. **C**: Cropped scan area through a single microlens. **D**: Full field-of-view confocal microscope image of cells at the glass coverslip focal plane (20x dry objective for **A-D**). **E**: 3D reconstruction of cells through an array of microlenses. **F-I:** Fluorescence images of the cells under two-photon excitation (. **F**: Fluorescence image through the microlenses coupled to a 20x dry objective. **G**: Control image through the 20x dry objective. **H**: Image of the cells through the microlenses coupled to a 25x water immersion objective. **I:** Control image through the 25x water immersion objective.

This work opens the possibility to use implanted micro-lenses for non-linear excitation of tissues, allowing the direct and continuous optical inspection of biological dynamics in vivo.

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