# Implantable Micro-optics for label-free non-linear imaging

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**Abstract.** Non-linear excitation microscopy offers superior in-vivo imaging but faces challenges in deep tissue. High numerical aperture beams suffer spherical aberrations, while tissue scattering impacts image quality. To address this, we propose implantable microlenses for precise focusing below the skin in lab animals. By using low numerical aperture lasers, we avoid spherical aberrations induced by high NA objectives. Our study presents various microlens designs differing in size, shape, and fabrication methods, all on glass or organo-hybrid ceramic substrates. This approach shows promise for enhancing deep tissue imaging, facilitating better understanding of biological processes in vivo.

## 1 Introduction

Non-linear excitation microscopy offers several advantages for in-vivo imaging compared to conventional confocal techniques. However, two major issues must be overcome to obtain high resolution optical images in deep tissue. Firstly, high numerical aperture beams suffer from high spherical aberrations when propagating through the in-homogenous tissue. Secondly, the tissue scatters the excitation and emitted light. For non-linear excitation, the scattering effect on the excitation beam has most prominent effect on the image signal/noise ratio when reaching deep layers (below approximately 100 $\mu m$). Still, the quality of the images acquired under non-linear excitation benefits from the reduction of the tissue induced spherical aberrations. In fact, the use of high numerical aperture objectives required for the non-linear excitation microscopy, works to increase the spherical aberrations. We approach these issues by developing implantable microlenses of an optical quality that is sufficient to induce non-linear excitation of the tissue and be implanted in deep layers below the skin of lab animals. In this way, one can use low numerical aperture laser beam to enter the animal tissue, reach the microlenses and focus the laser right at the target layer below the implanted microlenses.
We present here our results on the development and test of various types of microlenses. They differ in size (from 0.3 to 0.6 mm in diameter), shape (plano-convex or aspheric-parabolic) and in the production methodology (2 Photon Polymerization, 2PP, or Nano Imprinting Lithography, NIL). In all cases, the microlenses are fabricated on a planar slab of glass or hybrid organo-ceramic material (SZ2080 or Ormocomp).

## 2 Results

Since we want to focus at about 100 $\mu m$ below the surface of the micro-optics, plano-convex lenses cannot be larger than about 160 $\mu m$, limiting their numerical aperture (NA). We then resort to use aspheric shapes. In particular we tested the parabolic sag shape. In this case, the parametric law for the outer shell of the lens is given by [1]:

$$s(r) = \sqrt{f^2 + r^2} - f \qquad (1)$$

This shape has been developed for thin diffractive metasurfaces. In our refractive case, the focal parameter is related to the actual focal length of the microlens once we know the refractive index of the lens material and that of the surrounding medium. We developed such a relation and tested on the experimental realization of these lenses. The hybrid material SZ2080™ was employed in the production of the microlenses. The outer layer of the

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microlenses was fabricated using 2-photon polymerization lithography, followed by the bulk UV polymerization of the inner volume (Figure 1).We tested plano-convex microlenses with NA =0.2 in the form of hexagonal symmetry microarray. Higher values of NA can be reached with aspheric-parabolic shapes for which NA $\simeq$ 0.5 in air and NA $\simeq$ 0.7 in the tissue.

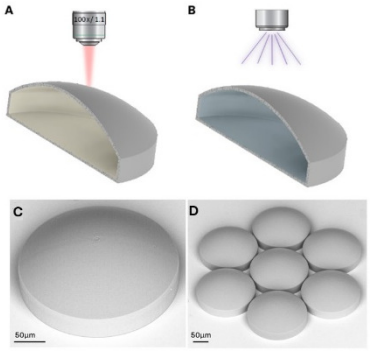


**Fig. 1.** Scheme of the microlens fabrication protocol. A: 2PP of the lens outer surface focusing the laser beam with a 100x water immersion objective (NA = 1.1). B: UV exposure to crosslink the unpolymerized core of the lens. C: SEM image of one microlens obtained from the optimization of the 2PP fabrication process and UV exposure. D: SEM image of one array of seven microlenses symmetrically set in a hexagonal configuration covering an area 800 µm in diameter.

The lenses were characterized optically by measuring the wavefront with a Shack-Hartman sensor. In addition, the MTF of the fabricated microlenses was derived by inserting a sharp edge in the sample plane, and by collecting and digitizing an image to retrieve the Edge Spread Function (ESF). We computed the derivative of the ESF to obtain the Line Spread Function (LSF), whose Fourier transform is the Modulation Transfer Function. MTF (Figure 2). A 10% modulation is reached at a cutoff frequency of about 490 lines pairs/mm [2], which is comparable to the cutoff frequency of more sophisticated microfabricated optics in the recent literature.

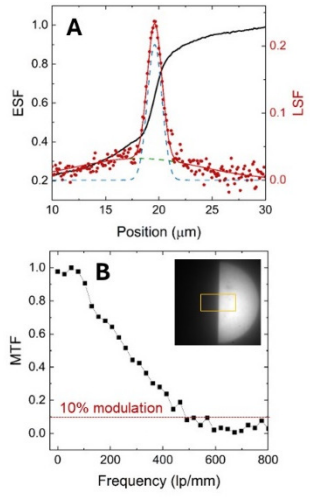


**Fig.2** Optical characterization of the microlenses. Measurement of the MTF of the microlenses by the knife edge method. A: Edge Spread Function (ESF, black solid line) and Line Spread Function (LSF, red circles) of a single microlens. To eliminate the background contribution, the LSF is fitted to a two-component Gaussian function (dashed lines), the background wider Gaussian component is subtracted, and the resulting LSF is Fourier transformed to retrieve the MTF. B: MTF of the microlens derived as the Fourier transform of the background-subtracted LSF in panel A. Inset: Image of a sharp razor blade produced by the microlens and exploited for the ESF quantification. The ESF is extracted as the average intensity profile along the horizontal axis within the yellow Region of Interest; the LSF is computed as the first derivative of the ESF.

Through the arrays of plano-convex lenses coupled to commercial confocal and two-photon excitation scanning microscopes, we could retrieve magnified fluorescence images of fibroblasts. The signal-to-noise ratio of the images is not substantially affected by the use of the microlenses, and the magnification can be adjusted by changing the relative position of the microlens array to the microscope objective and the immersion medium. These results are promising for the application of implanted micro-optics for optical in-vivo inspection of biological processes. We test this possibility on chicken embryos[2,3] and mice.

As an example on chicken embryo data, we discuss the possibility to build a cell atlas entirely reliant on label-free, in vivo non-linear excitation imaging (second-harmonic generation and autofluorescence signals) performed through implanted microlenses [3]. This atlas is instrumental in quantifying the number of recruited cells in the tissue and identifying granulocytes, collagen, and microvessels and can be coupled to machine learning clusterization algorithms of the various types of cells in tissues. A careful comparison with conventional H&E histological image analysis suggests that the label free approach can substitute to a large extent the histological approach and indicates the absence of a significant foreign body reaction to the implant of the micro-structured optics. This finding paves the way for the potential use of similar microstructures, coupled with biomaterials, for in vivo imaging of the interface between tissue and biomaterial using label-free non-linear excitation microscopy. The approach presented here promises to be a valuable alternative to conventional histopathology, for the validation of biomaterials in in vivo longitudinal studies.

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