

## ARBITRARY MULTI-SITE TWO-PHOTON EXCITATION

Vincent Ricardo Daria<sup>1,2</sup>, Christian Stricker<sup>2</sup>, Hans-A Bachor<sup>1</sup> and Stephen Redman<sup>2</sup>

<sup>1</sup>Physics Department, Australian National University, 0200 Canberra, Australia

<sup>2</sup>Division of Neuroscience, John Curtin School of Medical Research, Australian National University, 0200 Canberra, Australia

e-mail: [vincent.daria@anu.edu.au](mailto:vincent.daria@anu.edu.au)

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The applications of nonlinear two-photon excitation points to cross-disciplinary research ventures starting from photonics, micro-fluidics, semiconductor manufacturing, molecular biology and neuroscience. In a two-photon absorption process, an incident beam with two low energy photons is used to stimulate stable molecules. The requirement of two photons to be simultaneously absorbed by a molecule compels temporal and spatial constraints on the excitation source. A femtosecond ( $1 \text{ fs} = 10^{-15} \text{ s}$ ) pulsed laser source provides high photon densities in a very short time interval while focusing the same laser using a high numerical aperture objective lens results in high spatial photon density within the focus.

Rather than performing two-photon excitation on a single focus from an incident laser, we use holographic methods to split the laser into multiple beams in order to project arbitrary optical field patterns at the output or at the focal region of a lens. Here, we make use of programmable phase-only spatial light modulator (SLM), which is encoded with a computer-generated phase-hologram. The optical field patterns are projected at output following an optical lens transformation of the hologram at the input. Holographic splitting of a collimated  $\text{TEM}_{00}$  laser is transformed into optical field patterns characterized with several intensity maxima or multiple spots. We show that the various intensity maxima has sufficient energy and spatiotemporal photon density for localized two-photon absorption. The multiple spots, which can be arbitrarily positioned by generating an appropriate phase-only hologram, allow for user-defined multi-site non-linear two-photon excitation within the focal volume of a lens.

Fig. 1 shows multi-site two-photon excitation using  $2\text{-}\mu\text{m}$ -diameter fluorescent microspheres. The yellow-green two-photon fluorescence signal from the micro-spheres correspond to user-defined positions of the multiple-spot array.

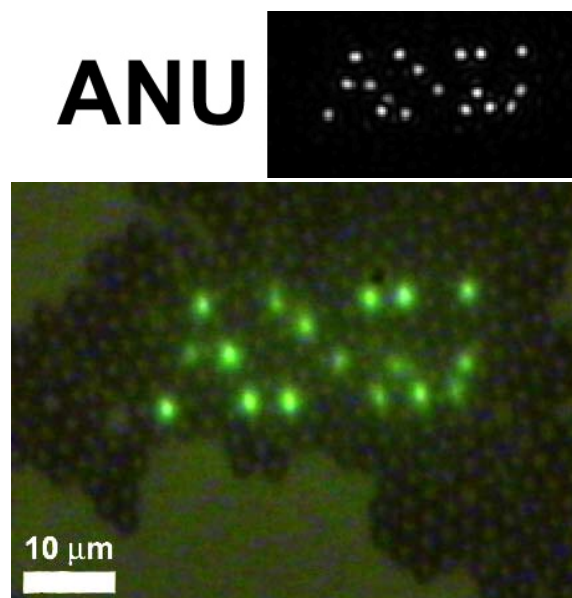


Figure 1. Optical field pattern at the focal region of a lens (top-right) and corresponding two-photon fluorescence (bottom) showing points of the letters, “ANU.”