



# Ultrafast pulse shaping system for nonlinear imaging

## NANOTECHNOLOGIES

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## Introduction

We develop and test a pulse shaper for application in multiphoton microscopy. Pulse shaping is a technique that reshapes optical pulses into a desirable temporal profile. Here, we present multiphoton imaging results using the SyncRGB-FLIM technique [1], which employs an ultrafast laser coupled to a custom-built inverted microscope. The used ultra-broadband custom-built 7 fs laser system (ENORA) [2] emits in a wavelength range of 630-1080 nm and hosts an integrated d-scan pulse compressor.

## Experimental Details and System Characterization

To compensate for the laser power loss through the spatial light modulator, the total number of reflections on the negatively chirped mirrors was reduced and the remaining negative group delay dispersion (GDD) was added through the active ultrafast pulse shaper (design in Fig.1). Different GDD masks can easily be tested by performing d-scan traces (see Fig. 2). Then, two different phase masks were tested and characterized with d-scan measurements [3]. First, an image was taken with the non-optimized phase mask [see Fig. 4 left]. Then, a second image was taken but with the optical compressor mask applied to the pulse shaper [see Fig. 4 right].

## Results

The pulse shaper allowed us to optimize the two-photon fluorescence signal for a luminescent polymer as well as a triple-stained fixed cell sample ( see Fig. 4 top and bottom.

Without compensation, weak fluorescence is obtained from the cytoplasm region. In the optimal phase mask applied, a significant increase in intensity in the cytoplasm and from signals from the nuclei region is obtained.

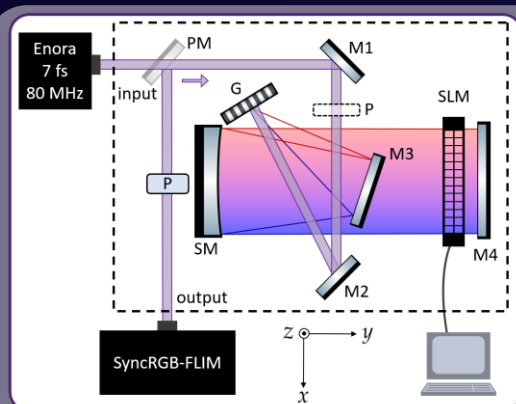


Fig.1: Pulse shaper schematic.

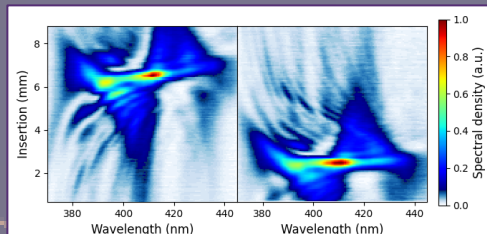


Fig. 2: D-scan traces for two masks.

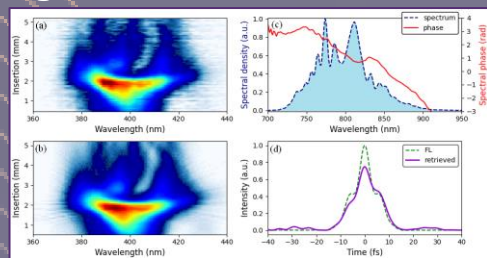


Fig. 3: Measured (a) and retrieved (b) D-scan trace, spectral phase and measured spectrum (c), and retrieved pulse of 11.83 fs.

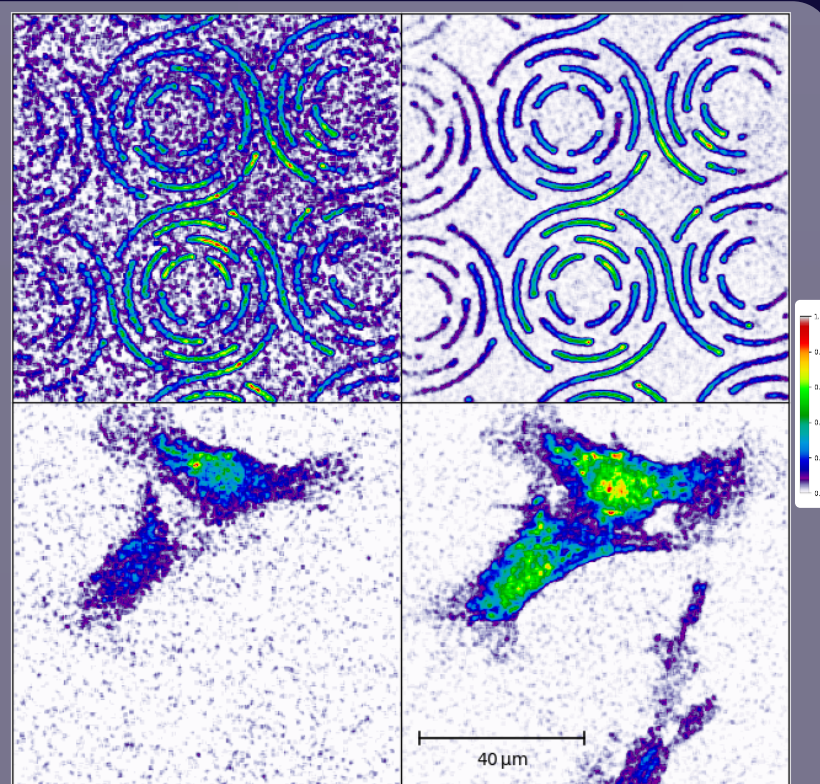


Fig 4: Two-photon fluorescence images, with and without pulse compression using a TPP structure (top) and a multi-color labeled cell sample (FluoCell #1) (bottom).

## Conclusion

An ultrafast pulse shaping system was designed and built together with a Python-based software with a graphical user interface. We demonstrated the enhancement of multiphoton imaging. These results pave the way for automatic pulse compression when the dispersion of the system changes and for selective multiphoton excitation on biological samples to identify distinct cell constituents.

## References

- [1] C. Maibohm et al., Biomed. Opt. Express, 10, 2019.
- [2] R. Weigand et al., Opt. Pura y Apl., 46, 2013.
- [3] M. Miranda et al., Opt. Express, 20, 2012.

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