Detailled Summary.

Optogenetics relies on the genetically targeted expression of light sensitive proteins in specific cell populations. This novel field has had a large impact in neuroscience, allowing both monitoring and stimulating the activity of specific neuronal populations, in intact brain preparations. Optogenetic tools have been used to record functional signals, such as changes in membrane potential or intracellular calcium concentration, as well as to modulate the excitability of neurons. To fully exploit the potentiality of optogenetics, new microscopy techniques have been developed to optimize illumination of photo-active compounds *in situ*. In particular, an important effort has been directed towards improving the spatial and temporal resolution of light stimulation, in order to match the dynamics of physiological processes. In this frame, the use of two-photon excitation becomes necessary to ensure penetration of light in scattering biological tissues, as well as confining the excitation volume and improve the specificity of illumination. My thesis was dedicated to the development and use of advanced optical methods for two-photon excitation of optogenetic tools. In a first project, we combined optical approaches (generalized phase contrast and temporal focusing) to perform two-photon activation of neurons expressing the light-sensitive cationic channel channelrhodopsin-2 (ChR2). Our work demonstrated for the first time the simultaneous generation of action potentials in multiple neurons, while maintaining a micrometric axial and lateral resolution. These results pointed out the advantages of light sculpting to increase both the specificity and the flexibility of photo-stimulation.

In order to investigate the potential of this technique for efficient in-depth stimulation, we therefore studied the propagation through scattering biological media of laser beams generated by two different light patterning techniques, generalized phase contrast and digital holography in combination with temporal focusing. We demonstrated that temporal focusing enabled the excitation volumes to maintain micrometric axial confinement, as well as to maintain well defined patterns deep inside tissues. We also demonstrated for the first time the activation of ChR2 at depth over 200 µm.

Finally, the last part of my PhD was focused on testing light patterning methods for the activation of two other photosensitive proteins, the excitatory channel C1V1 and the inhibitory pump, halorhodopsin.