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IT-3-P-5941 Development of the pump-probe nanoscopy architecture.

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Modern super-resolution microscopy techniques can provide high quality images with sub-diffraction resolution. However, a significant limitation for most of the methods is the use of fluorescence as readout, which results in photo-bleaching, and reduction of penetration depth, and significantly complicates the deep tissue imaging process due to strong scattering inside the sample. These issues can be avoided by using infra-red (IR) vibrational spectroscopy, but in that case, the image resolution is very low, due to the diffraction limit. As a new solution, the infrared absorption microscopy method was recently proposed [1, 2].

Strictly speaking the IR absorption microscopy method combines two well known techniques – transient absorption spectroscopy and stimulated emission depletion microscopy (STED) [3, 4]. In particular, it is based on a saturation effect, where the first laser pulse creates contrast within the sample, and the second pulse, at different frequency, detects the change. This allows label free transient images to be obtained. By introducing an additional doughnut-shaped depletion pulse, the excited area can be transiently saturated in the periphery of the focal spot, allowing collection of a signal from the central sub-diffraction area. By choosing the pumping and probing wavelengths, we can image different non-fluorescent species. The possibility to use IR light for sub-diffraction imaging is especially vital for deep tissue imaging, because the IR spectrum lies in the transparency window of biological tissues.

To verify the concept, we designed and assembled prototype of such a system presented in this work. The microscope combines two home-built setups – a pump-probe spectroscope and STED nanoscope. This configuration allows achieving high temporal and spatial resolution in the very same instrument. The setup is based on a femtosecond laser coupled with an OPA, which can generate laser pulses in a broad spectral range from visible to near IR, according to experimental needs. Therefore, explicit spatial and dynamical information about the sample can be obtained. This is very useful for cell biophysics and nanochemistry applications. We also present the possibility of implementing a 3D super-resolution capability.

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