Femtosecond lasers for nonlinear microscopy

Róbert Szipőcs, Gergely Szipőcs

Dispersive dielectric mirrors for ultrafast lasers. — In December of 2023, Ferenc Krausz received the Nobel Prize in Physics for generation of the first isolated attosecond (as) pulses suitable for time resolved spectroscopy of electrons in atoms on the as time scale. In January, I published a paper on this topic in the Hungarian journal of physics (Fizikai Szemle) [1], in which I described the road to this outstanding result at the Technical University of Vienna including our contribution to his work in Budapest, at the optical coating laboratory of our institute. In a second paper, I presented the story of the first chirped mirror designed and manufactured in Budapest [2], which was a milestone result for the generation of high energy, nearly one-cycle (~5 fs) optical pulses at 800 nm (see Fig. 1). Note that such pulses are required for high-harmonic generation in experiments generating isolated as pulses [1].

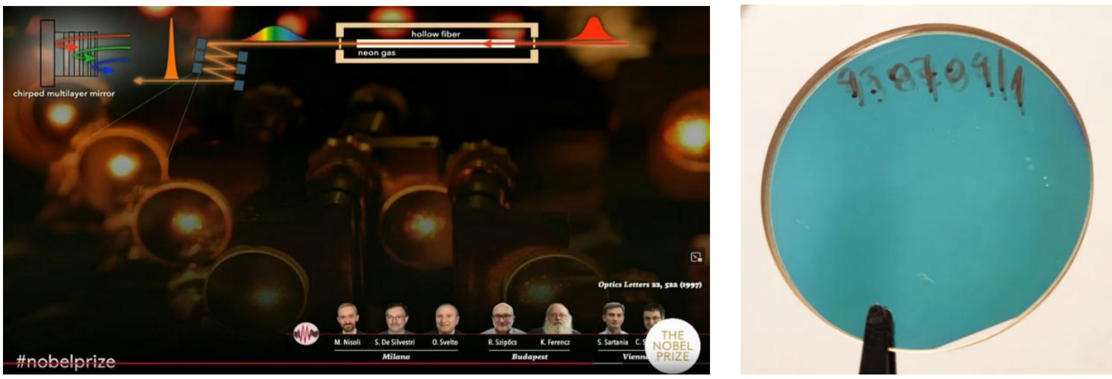

Figure 1. Snapshot from the lecture of Ferenc Krausz presented during the Nobel Prize for Physics, 2023 ceremony in Stockholm. In this slide, he explained how high energy, single cycle optical pulses were generated by spectral broadening in neon gas filled hollow fiber (contribution of Politechnico Milano, Italy), the dispersion of which was compensated by chirped mirrors designed and manufactured at our institute (left) [1]. Photo of the first chirped mirror designed and manufactured in Budapest (right) [2].

FLIM microscopy for life science and quantum microscopy — Last year we applied Fluorescence Lifetime Imaging Microscopy (FLIM) on NAD(P)H and Lipofuscin fluorophores in the liver of epigenetically altered rats for investigation of the development of metabolic syndrome in low running capacity rats [3]. For our FLIM studies, a Carl Zeiss LSM 7MP two-photon microscope was used, which was upgraded by a Becker&Hickle FLIM data acquisition and image processing system four years ago. In collaboration with the Gali group of Wigner RCP and R&D Ultrafast Lasers Ltd, we started a Hungarian-German bilateral Eureka project on "quantum microscopy", in which our research target is to apply a fluorescence lifetime measurement technique on single- or multiple NV color centers in nano- or bulk diamond samples in order to determine their quantum states under different microwave excitation conditions. Like in case of Opticaly Detected Magnetic Resonance (ODMR) measurements, this novel time resolved technique (FLMR) could be also used for high accuracy, high spatial resolution magnetic field measurements. For our first FLIM measurements on NV color centers in diamond, a Ti-sapphire laser with an "industry standard" repetition rate frequency of 76 MHz was used, with an operating wavelength adjusted within the two-photon excitation wavelength of diamond containing NV color centers. Based on our FLIM measurements on NV color centers in nanoscale diamond (ND) samples, we found that the measured lifetime varies significantly with sample size and impurity, and thus in our opinion they are not suitable for magnetic field characterization in this form. In case of bulk diamond samples, we found that the contamination of the samples and the inhomogeneity of the color centers within the sample can also be a significant problem, making it difficult (or impossible in the case of contamination) to obtain bi-exponential lifetime data fitting required for quantum state characterization. The only reproducible data were obtained for an electron irradiated bulk diamond sample (product of Element 6), which was provided by our German consortium partner (University of Ulm). We note that the detection system of our two-photon microscope is currently not suitable for detection above 750 nm, which is of our interest for the efficient separation of the emission of NV- and NV0 color centers. Another important finding was that to measure the lifetime of NV0 and NV- color centers (~8-25 ns), the repetition rate of our two-photon laser needs to be significantly reduced. Accordingly, we are currently working on extending the ~800 nm operation wavelength of our fiber-coupled, ~20 MHz repetition rate, sub-ps Ti-sapphire [4] to wavelengths of ~920 nm and ~1000 nm, which are required for efficient two-photon excitation of NV color centers. To this end, we apply our novel low reflection loss dispersion compensation scheme developed for broadly tunable sub-ps solid state lasers [5], which is a Gires-Tournois interferometer (GTI) comprising an ion-beam sputtered ultrabroadband chirped mirror [1,2] as a high reflector and a slightly wedged fused silica plate as a partial reflector. Beside this, two additional saturable absorber mirrors (SAM 920 and SAM 1000) are mounted next to the original SAM device (SAM 800) on the same copper heat sink. Control software and electronics of the GTI device (piezo-controller) and that of the laser cavity (step motor controller) were developed and manufactured by our industrial partner R&D Ultrafast Lasers Ltd. Note that the same software, dispersive optics, SAM devices and electronics have been succesfully tested recently in a fiber-coupled, ~69 MHz repetition rate laser system developed for a head-mounted two-photon imaging system at Nanyang Technological University, NOBIC Imaging Laboratory, Singapore [5]. In their imaging system developed for Alzheimer research, ~800 nm, ~920 nm and ~1000 nm pulses are used for two-photon excitation of different fluorophores labelling different tissue components of the brain.

Fiber lasers for nonlinear microscopy. — Continuing on research on ultrashort pulse fiber lasers, an Er-fiber oscillator-amplifier system (Fig. 2) operating at around 1560 nm was developed in our laboratory, the output of which is frequency doubled for generation of ~780 nm optical pulses being suitable of two-photon excitation of NADH [3] for FLIM studies.

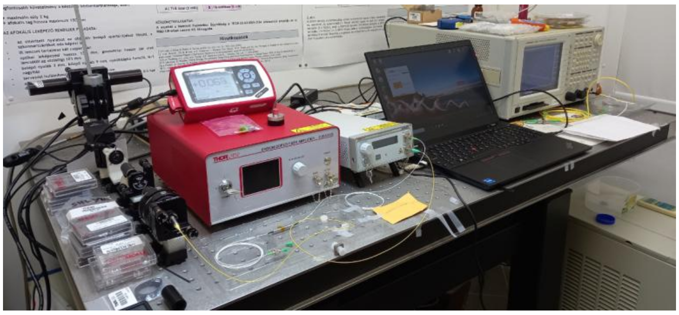

Figure 2. Er-fiber oscillator-amplifier system with an SHG unit for generation of ~780 nm optical pulses.

References:

[1] https://www.szipocs.com/picks/Fizikai_Szemle_2024_jan_Szipocs_R.pdf

[2] https://www.szipocs.com/picks/Fizikai_Szemle_2024_jul_aug_Szipocs_R.pdf

[3] https://doi.org/10.1117/12.3005632

[4] https://doi.org/10.3390/life14020231

[5] https://doi.org/10.1364/CLEO_AT.2023.JTh2A.121