Szipőcs Group

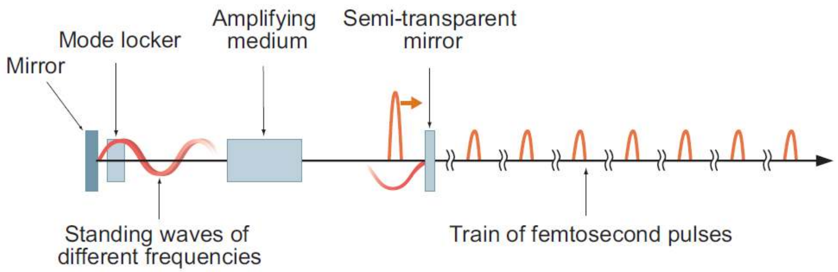
Femtoszekundumos lézerek olcsón, kis méretben, mindenkinek

Szipőcs Róbert, PhD
szipocs.robert@wigner.mta.hu

MTA Wigner Fizikai Kutatóközpont, Budapest
www.wigner.hu

Bevezetés

- **Femtoszekundumos lézerek**



1 fs = 10^{-15} s

1 fs * 300 000 km/s = 0.0003 mm = 0.3 mikron

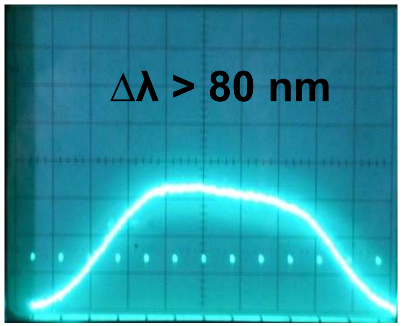
Látható fény hullámhossza: 0.4-0.76 mikron

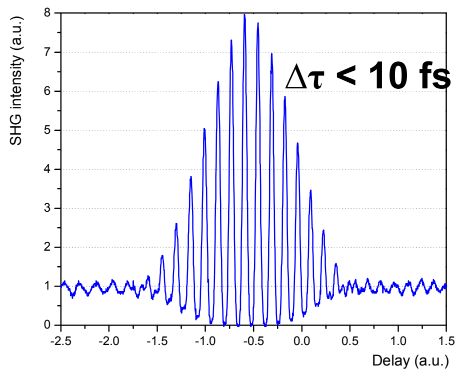
→ Az optikai vékonyréteg rendszerek kiemelkedő szerepe

$\Delta\omega\ \Delta\tau \geq$ állandó

$\Delta\omega$: sávszélesség (kör)frekvenciában

$\Delta\tau$: impulzushossz

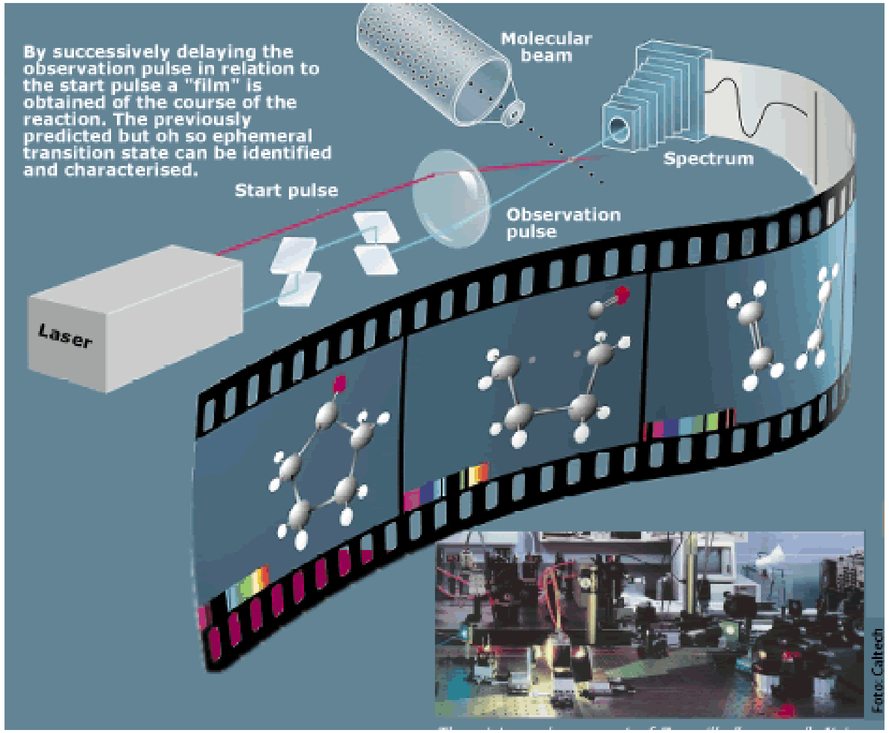


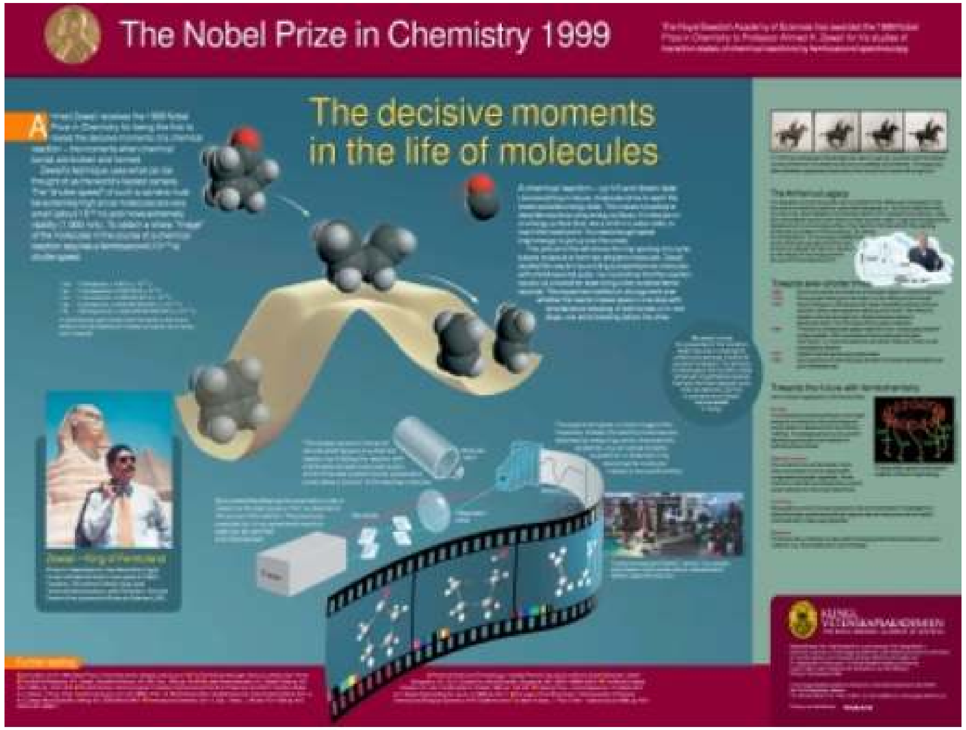


Bevezetés

- **Hol használjuk a femtoszekundomos lézereket?**

- spektroszkópia, időfelbontásos spektroszkópia (ld. még: femtokémia)





Bevezetés

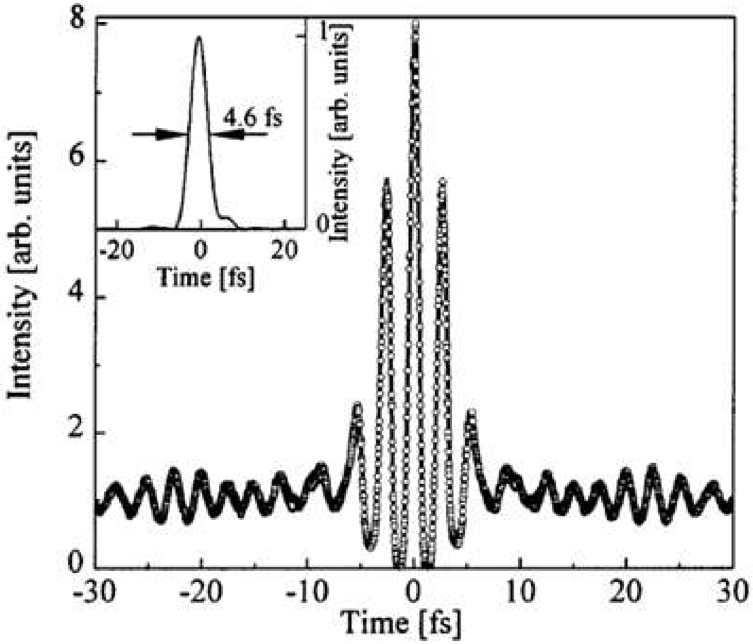


Fig. 14. Interferometric autocorrelation of a 4.6-fs pulse. *Open circles* experimental points, *solid line* calculated IAC of deduced pulse shape shown in the inset

Towards ever-shorter times

1889	Arrhenius predicts the existence of transition states in chemical reactions.
1920s	Mixing experiments give information on the *milli*second time-scale.
1949	Flash photolysis is introduced and makes it possible to study chemical reactions without the reactants needing to be mixed. The method is based on the fact that light can initiate chemical reactions (photochemistry). The *micro*second time-scale is reached.
1960	The laser is invented and rapidly makes the *nano*- and *pico*second time-scales available. Milli- to picosecond time-scales give valuable information on chemical reactions, but direct molecular motion is still impossible to observe.
1987	Zewail's first *femto*chemistry experiments.
1999	Guinness Book of Records gives duration of shortest reported light pulse as 4.5 femtoseconds.

Az 1999-es kémiai Nobel-díj poszterről

A. Baltuška, Z.Wei, M.S. Pshenichnikov, D.A.Wiersma, R. Szipőcs:
"All-solid-state cavity-dumped sub-5-fs laser",
Appl. Phys. B 65, 175–188 (1997)

- **Hol használjuk a femtoszekundomos lézereket?**

- **spektroszkópia (ld. még: frekvencia fésű)**

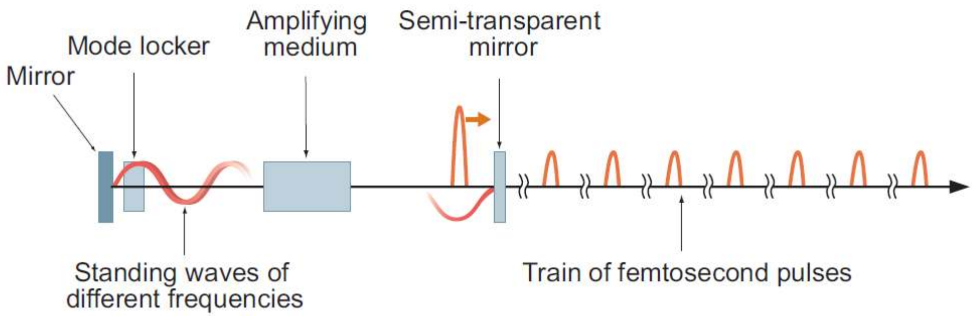
The Nobel Prize in Physics 2005

Photo: J.Reed
Roy J. Glauber
Prize share: 1/2

Photo: Sears.P.Studio
John L. Hall
Prize share: 1/4

Photo: F.M. Schmidt
Theodor W. Hänsch
Prize share: 1/4

The Nobel Prize in Physics 2005 was divided, one half awarded to Roy J. Glauber *"for his contribution to the quantum theory of optical coherence"*, the other half jointly to John L. Hall and Theodor W. Hänsch *"for their contributions to the development of laser-based precision spectroscopy, including the optical frequency comb technique"*.



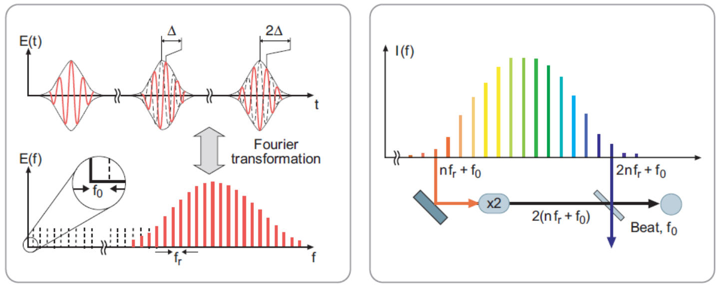


Figure 3. Principles of the frequency comb technique. The upper part of the diagram shows schematically how the laser pulses are created. The lower, left-hand diagram shows both the dependence of the pulses on time, where each pulse contains only a few vibrations, and how the modes are distributed among different frequencies. The spectral distribution consists of a comb of frequencies whose separation is extremely well-defined. The zero point of the spectrum is, however, unknown and represents a displacement f_o. By means of non-linear optical techniques the frequencies of the spectrum can be doubled, as shown in the lower, right-hand diagram. Its lowest frequency can then be compared with its original highest frequency and the displacement f_o can be determined.

MIRROR DISPERSION CONTROLLED Ti:SAPPHIRE LASER

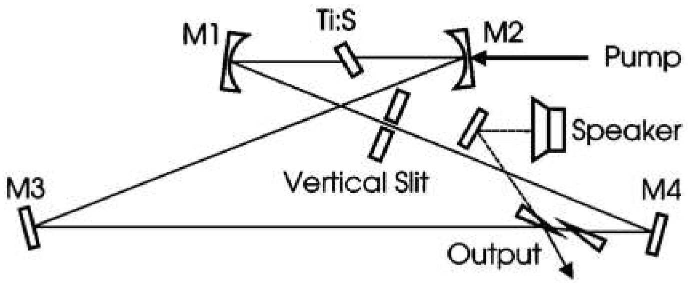


Fig. 1. Schematic of the MDC Ti:sapphire (Ti:S) ring laser. The pump beam is focused with a 40-mm lens onto the Ti:S crystal. M1, M3, M4, chirped mirrors; M2, chirped dichroic mirror. The radius of curvature of M1 and M2 is 50 mm.

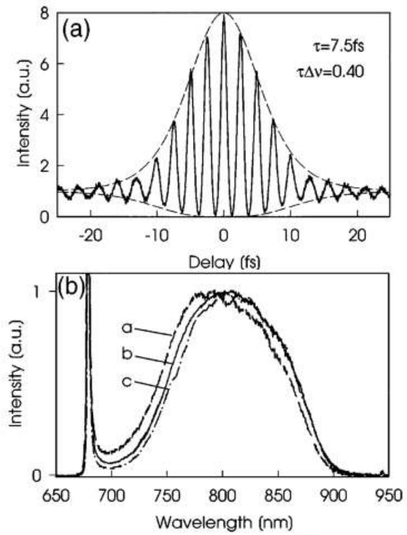


Fig. 2. (a) Single-scan FRAC trace of the output of the MDC Ti:sapphire laser. (b) Spectrum of the mode-locked laser measured through a pinhole positioned at (curve b) the center of the beam and (curves a and c) the half-intensity-maximum points in the horizontal plane. In the vertical plane no spatial chirp was observed.

RING CAVITY

☺ **Highly stable femtosecond pulses with duration of ~7.5 fs**

Lin Xu, Christian Spielmann, Ferenc Krausz and Róbert Szipőcs, *Opt. Lett.* 21, pp. 1259-1261 (1996)

Route to phase control of ultrashort light pulses (TU Wien)

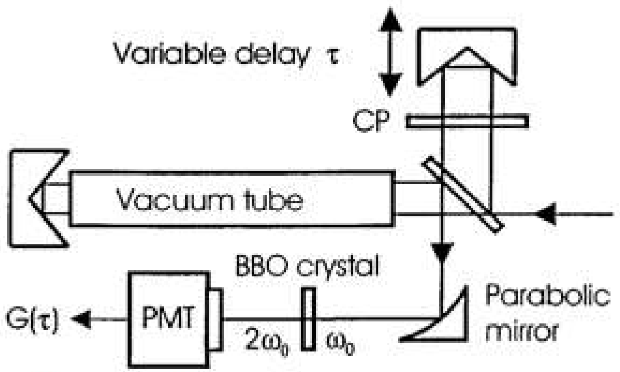


Fig. 1. Schematic diagram of the correlator used to the measure $\Delta\psi$. To balance dispersion in the correlator arms precisely, we evacuate a delay section equal to the resonator round-trip length in the long arm, a compensation plate (CP) is used to introduce the same amount of fused silica into the short arm as the tube windows introduce into the long arm, and pulse splitting and recombination are implemented by identical broadband dielectric coatings on opposite sides of the beam splitter. PMT, photomultiplier tube; BBO, β-barium borate.

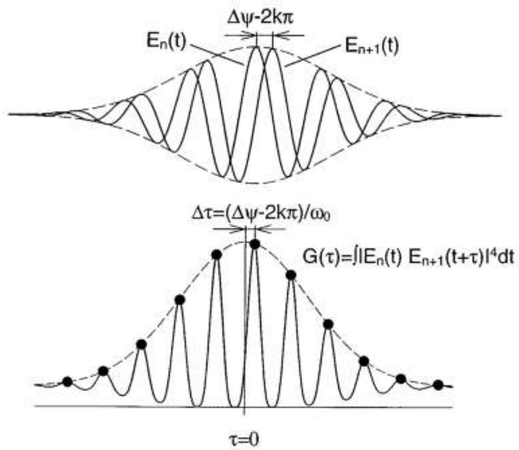


Fig 2. Principle of the measurement of $\Delta\psi$. $E_n(t)$ and $E_{n+1}(t)$ describe successive pulses from the laser. $\Delta\psi - 2k\pi$ can be determined from the position of the fringe peaks on the envelope of $G(\tau)$.

We thank R. Szipöcs and K. Ferencz (Research Institute for Solid State Physics, Budapest, Hungary) for providing the special dispersive mirrors used in the Ti:sapphire laser. This research was supported by Fonds zur Förderung der wissenschaftlichen Forschung grants P-09710 and P-10409, and by Österreichische Nationalbank grants 5335 and 5124.

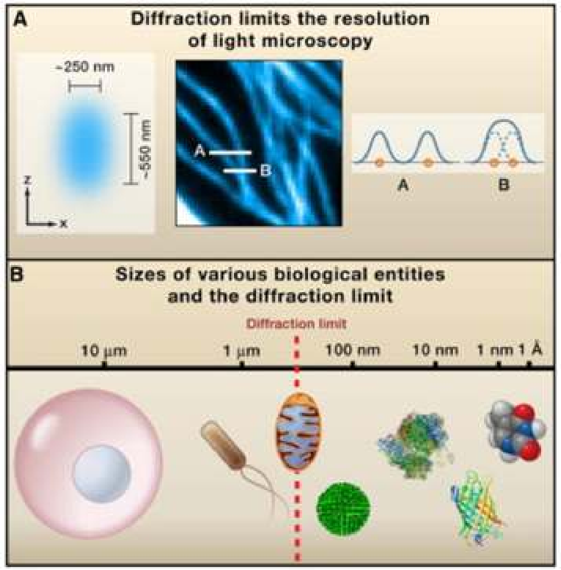
Lin Xu, Christian Spielmann, Ferenc Krausz and Robert Szipőcs, *Opt. Lett.* 21, pp. 1259-1261 (1996)

L. Xu, Ch. Spielmann, A. Poppe, T. Brabec, F. Krausz and T. W. Hansch, *Opt. Lett.* 21, 2008-2011 (1996)

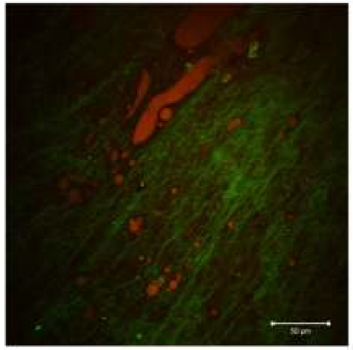
Bevezetés

- **Hol használjuk a femtoszekundomos lézereket?**

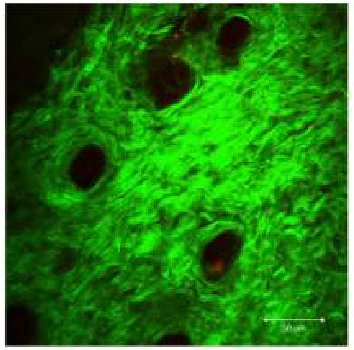
- 3D mikroszkópiás képalkotás (biológia, orvostudomány, gyógyszeripar)

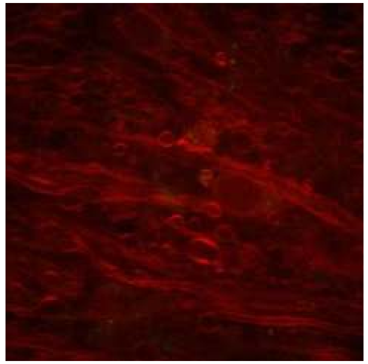


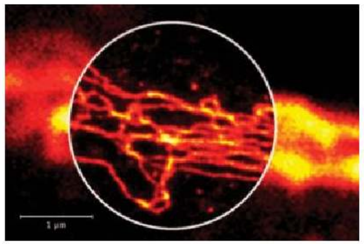
Dermis



Epidermis







The vimentin network of a neuron is revealed by confocal (outer) and nanoscale-resolution STED (inner part) modalities. The STED image shows single filaments that appear in the confocal reference as blurs. (*STED recording described in D.E. Wildanger et al., Opt. Exp., 16, 9614 –9621 [2008]; image courtesy of Stefan W. Hell*)

Scientific Background on the Nobel Prize in Chemistry 2014

SUPER-RESOLVED FLUORESCENCE MICROSCOPY

Szipőcs Group

Hol használjuk a femtoszekundumos lézereket?
- 3D mikroszkópia, 3D nanoszkópia

Hell developed the stimulated emission depletion (STED) microscopy method in 2000, which uses two laser beams—one stimulates fluorescent molecules to glow while another cancels out all fluorescence, except for that in a nanometer-sized volume. Scanning over the sample, nanometer for nanometer, yields an image with a resolution better than the diffraction limit.

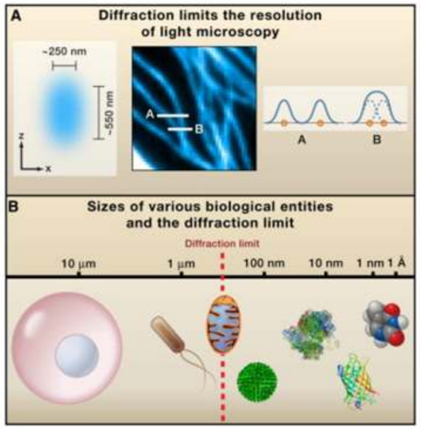
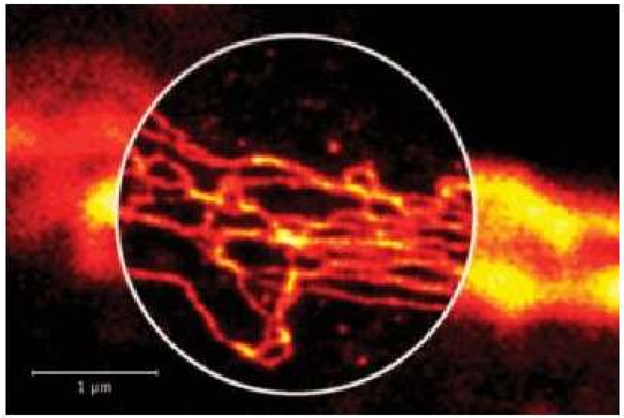


Figure 1: (Huang *et al.*, 2010, Cell, 143, 1047 – 57). **A**: (left) Focused laser beam, (middle) structure, (right) resolved (A) and not resolved (B) structural features. **B**: from left to right mammalian cell, *E. coli* cell, mitochondrion, influenza virus, ribosome, GFP, thymine.



The vimentin network of a neuron is revealed by confocal (outer) and nanoscale-resolution STED (inner part) modalities. The STED image shows single filaments that appear in the confocal reference as blurs. (*STED recording described in D.E. Wildanger et al., Opt. Exp., 16, 9614 –9621 [2008]; image courtesy of Stefan W. Hell*)

Femtoszekundumos lézerek 3D mikroszkópiás rendszerekben

ICONEWSLETTER*

Commission Internationale d'Optique ◆ International Commission for Optics ◆ July 2003

ICTP Winter College on Biophotonics, 10-21 February 2003: report

The Abdus Salam International Center for Theoretical Physics (ICTP) organizes every year the Winter College on subjects relevant to Optics.

A view of the Abdus Salam Center for Theoretical Physics, Miramare, Trieste, Italy (ICTP) where the Winter College 2003 took place

In particular, the following lecturers were delivering specific talks: K. Berg-Sorensen (Niels Bohr Inst., Copenhagen, Denmark); V. Croquette (Ecôle Normale Superieure, Paris, France); C. Depeursinge (EPFL, Lausanne, Switzerland); A. Falaschi (Trieste, Italy); P. French (Imperial College, London, U.K.); S. Hell (Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany); M. S.Z. Kellermayer (Pecs Univ., Hungary); B. Kemper (Univ. Münster, Germany); V. Lakshminarayanan (Univ. of Missouri, St. Louis, USA); O.E. Martínez (Univ. de Buenos Aires, Argentina); R. Marzari (Univ. di Trieste, Italy); A. Oraevsky (Univ. Texas, Houston, USA); U. Osterberg (Thayer School of Eng. Hanover, USA); F.S. Pavone (Univ. di Firenze, Italy); C. Sheppard (Univ. of Sydney, Australia); G. Von Bally (Univ. Münster, Germany).

The lectures covered a broad scope of subjects: Introduction and elements of cell biology, manipulations of biological units, microscopy, optical sources, imaging, metrology, tomography and laser safety.

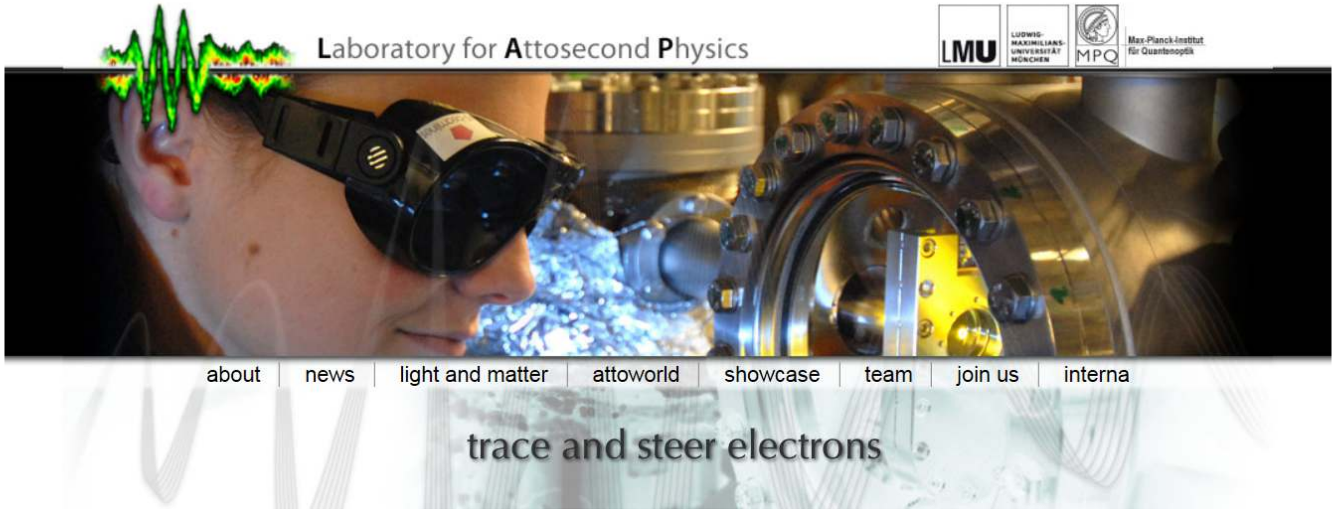
Directors and lecturers observed that the contributions by the participants during the discussions and in the LAMP-Workshops lived up to the high international standard for which ICTP Colleges are known. It was especially apparent that interest and enthusiasm for interdisciplinary research in emerging areas like biophotonics is not restricted to the industrialized countries but it is also evident in the so-called "developing countries" and that this activity can contribute to reducing the technological gap among nations.

The program of the Winter College was extended by the ICO/ICTP Prize ceremony, at which Dr. Róbert Szipőcs from the Research Institute for Solid State Physics and Optics, Budapest, Hungary, was awarded the 2003 prize (see also this ICO Newsletter issue). During this occasion, the winner of the 2002 prize, Dr. Alphan Sennaroglu from Roc University, Department of Physics, Istanbul, Turkey, was also honored for his scientific contributions to the development of solid-state lasers for ultrashort pulse generation and associated power optimization studies. The

Bevezetés

- **Hol használjuk a femtoszekundomos lézereket?**

- **Attoszekundumos lézerfizika (pl. elektronok mozgása az atommag körül)**



No de hol van a Nobel díj?!

Wigner Szipőcs Group

Bevezetés

- **Attoszekundumos lézerfizika**

index

NOBEL | SZÍRIA | MENEKÜLTTÉRKÉP

BELFÖLD KÜLFÖLD GAZDASÁG TECH TUDOMÁNY KULT SPORT VÉLEMÉNY VIDEÓ

TUDOMÁNY | KRAUSZ FERENC | NOBEL-DÍJ | FIZIKA | ATTOFIZIKA

Magyaré lehet a fizikai Nobel-díj?

A Science Watch nevű oldal évről évre összeállítja a Nobel-díj-esélyes kutatók listáját a különböző témák felkapottsága, és a vele foglalkozó kutatók idézettségi indexe alapján. A fizikai Nobel-díjra számításaik szerint 2015-ben egy Németországban élő magyar kutató, Krausz Ferenc, a müncheni Ludwig Maximilians Egyetem kísérleti fizikai tanszékének vezetője és a Max Planck Kvantumfizikai Intézet igazgatója is esélyes. Holtversenyben elsőnek szerepel a listán Paul B. Corkum kanadai kutatóval közösen. Az oldal szerint az attofizika kifejlesztéséért érdemelnének díjat.

Compression of high-energy laser pulses below 5 fs

522 OPTICS LETTERS / Vol. 22, No. 8 / April 15, 1997

Compression of high-energy laser pulses below 5 fs

M. Nisoli, S. De Silvestri, and O. Svelto

Centro di Elettronica Quantistica e Strumentazione Elettronica—Consiglio Nazionale delle Ricerche, Dipartimento di Fisica, Politecnico, Piazza L. da Vinci 32, 20133 Milano, Italy

R. Szipöcs and K. Ferencz

Szilárdtestfizikai Kutatóintézet, Pf. 49, H-1525 Budapest, Hungary

Ch. Spielmann, S. Sartania, and F. Krausz

Abteilung Quantenelektronik und Lasertechnik, Technische Universität Wien, Gusshausstrasse 27, A-1040 Wien, Austria

Received October 25, 1996

High-energy 20-fs pulses generated by a Ti:sapphire laser system were spectrally broadened to more than 250 nm by self-phase modulation in a hollow fiber filled with noble gases and subsequently compressed in a broadband high-throughput dispersive system. Pulses as short as 4.5 fs with energy up to 20-μJ were obtained with krypton, while pulses as short as 5 fs with energy up to 70 μJ were obtained with argon. These pulses are, to our knowledge, the shortest generated to date at multigigawatt peak powers. © 1997 Optical Society of America

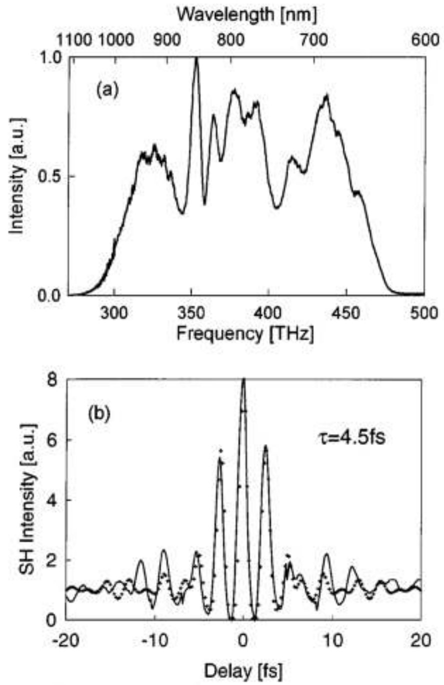
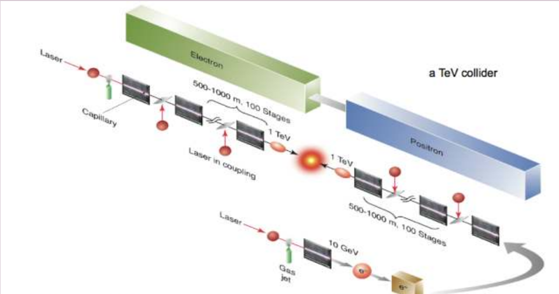


Fig. 2. (a) Spectral broadening in krypton at $p = 2.1$ bars and $P_0 = 2$ GW. A low-intensity pedestal (~1% of the peak) extends below 600 nm. (b) Measured (solid curve) and calculated (crosses) autocorrelation trace; an evaluation of the pulse duration (FWHM) is also given.

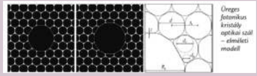
Az SZFI/ANO/femtoszekundumos lézer kutatócsoport KOMPETENCIÁK

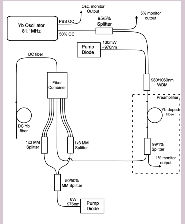
Wigner Szipőcs Group

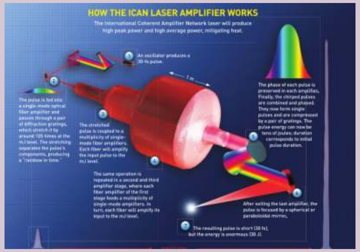
Részecskegyorsítás szállézerekkel (ICAN Projekt)

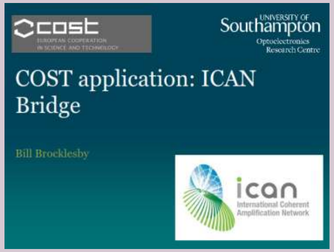


Leemans and Esarey (Phys. Today, 09)

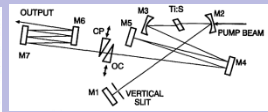


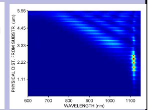


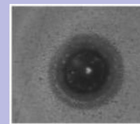


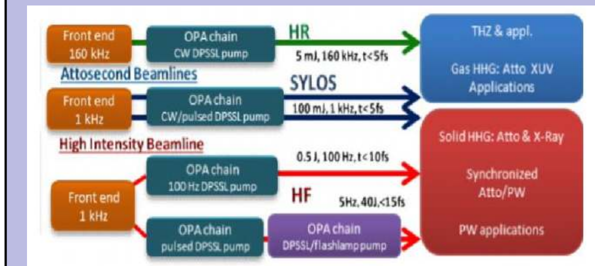


Femtoszekundumos szilárdtestlézerek és optika (ELI/Helios Projekt)









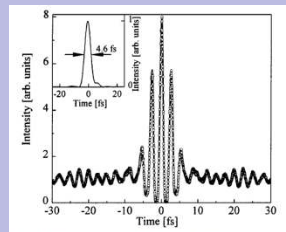
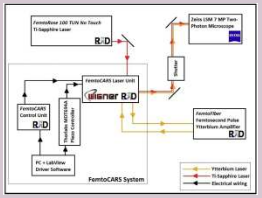
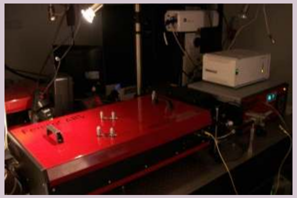
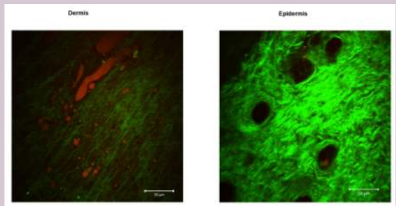


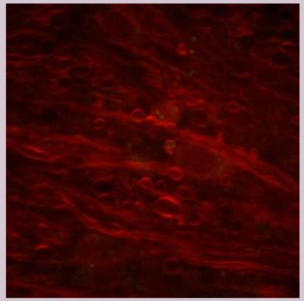
Fig. 14. Interferometric autocorrelation of a 4.6-fs pulse. *Open circles* experimental points, *solid line* calculated IAC of deduced pulse shape shown in the inset

LifeScience@Wigner (pl. Nemzeti AgykutatásiProgram (NAP))









Femtoszekundumos lézerfizika, száloptika és nemlineáris mikroszkópia kutatócsoport

Szipőcs Group

Tudományos háttér

Fontosabb kutatási témák:

● *Fotonikus kristály optikai szálak elmélete, tervezése, gyártatása, minősítése és alkalmazásai optikai szállézerekben, erősítőkben és 3D mikroszkópiás orvosi diagnosztikai rendszerekben*

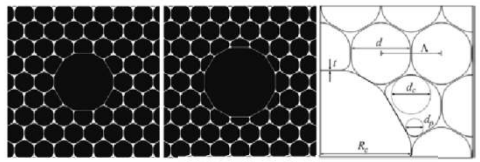
● ***Diszperzív lézeroptikai bevonatok roncsolódásának elméleti és kisérleti vizsgálata az 50 ps-nál hosszabb és a femtoszekundumos időtartományban***

● ***Femtoszekundumos szilárdtest- és száloptikai lézerek fejlesztése in vivo, nemlineáris 3D mikroszkópiás alkalmazásokhoz***

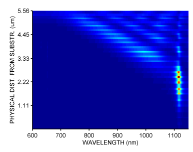
● ***Két hullámhosszon szinkron működő femtoszekundumos lézerrendszer CARS/SRS mikroszkópiához***

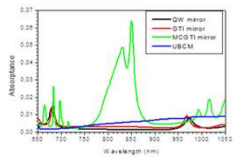
● ***Nemlineáris mikroszkópia alkalmazásai a bőrgyógyászat, az idegtudományok és a gyógyszeripar területén***

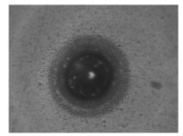
● ***Lézeres*** *biztonságtechnikai vizsgálatok*

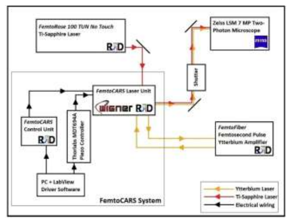


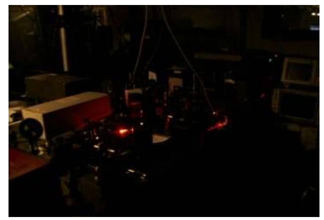
Üreges fotonikus kristály optikai szál – elméleti modell

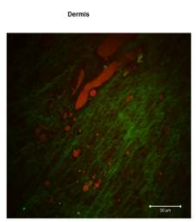


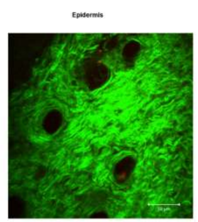


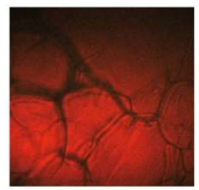












Zsírszövetről CARS módszerrel az MTA SZFKI-ban készített mikroszkópiás kép

Femtoszekundumos lézerfizika, száloptika és nemlineáris mikroszkópia kutatócsoport

Szipőcs Group

Fontosabb tudományos közlemények (2008-2014)

1. Várallyay Z, Saitoh K, Fekete J, Kakihara K, Koshiba M, Szipőcs R: *Reversed dispersion slope photonic bandgap fibers for broadband dispersion control in femtosecond fiber lasers*, *OPTICS EXPRESS* 16, 15603-15616 (2008)

2. Fekete J, Várallyay Z, Szipőcs R: *Design of high bandwidth one- and two-dimensional photonic bandgap dielectric structures at grazing incidence of light*. APPLIED OPTICS 47, 5330-5336 (2008)

3. Fekete J, Cserteg A, Szipőcs R: *All-fiber, all-normal dispersion ytterbium ring oscillator*, *LASER PHYSICS LETTERS* 6, 49-53 (2009)

4. Várallyay Z, Saitoh K, Szabó Á, Szipőcs R: *Photonic bandgap fibers with resonant structures for tailoring the dispersion*, *OPTICS EXPRESS* 17, 11869-11883,(2009)

5. Antal P, Szipőcs R: *Tunable, low-repetition-rate, cost-efficient femtosecond Ti:sapphire laser for nonlinear microscopy*, *APPL. PHYS. B107*, 17–22 (2012)

6. Antal P, Szipőcs R: *Relation between group delay, energy storage and loss in dispersive dielectric mirrors*, *CHINESE OPTICS LETTERS* 10, 053101/1-4 (2012)

7. P. Bognár, D. Haluszka, N. Wikonkál, A. Kolonics, R. Szipőcs, S. Kárpáti, *Reduced Inflammatory Threshold Indicates Skin Barrier Defect in Transglutaminase 3 Knockout Mice, J. INVESTIGATIVE DERMATOLGY* 134, *105-111* (2014)

8. Grósz T, Kovács AP, Kiss M, Szipocs R, Measurement of higher order chromatic dispersion in a photonic bandgap fiber: Comparative study of spectral interferometric methods, *APPLIED OPTICS* 53, *1929-1937* (2014)

9. Kolonics A, Csiszovszki Zs, Tőke ER, Lőrincz O, Haluszka D, Szipőcs R, *In vivo study of targeted nanomedicine delivery into Langerhans cells by multiphoton laser scanning microscopy, EXPERIMENTAL DERMATOLOGY* 23, *596-605* (2014)

10. Toke ER, Lorincz O, Csiszovszki Z, Somogyi E, Felföldi G, Molnár L, Szipőcs R, Kolonics A, Malissen B, Lori F, Trocio J, Bakare N, Horkay F, Romani N, Tripp CH, Stoitzner P, Lisziewicz J, *Exploitation of Langerhans cells for in vivo DNA vaccine delivery into the lymph nodes*, *GENE THERAPY* 21, *566-574.*(2014)

11. Várallyay Z, Szipőcs R, *Stored Energy, Transmission Group Delay and Mode Field Distortion in Optical Fibers, IEEE JOURNAL OF SELECTED TOPICS IN QUANTUM ELECTRONICS* 20, 0904206/1-6 (2014)

SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

Bemutatkozás:
Az R&D Ultrafast Lasers Kutatási és Fejlesztési Kft.

BOOTH NUMBER: 8109

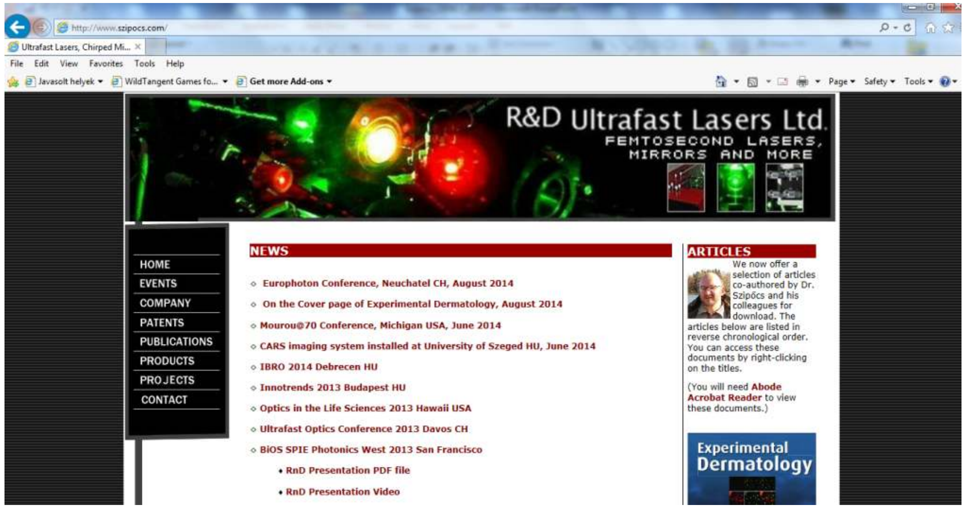
R&D ULTRAFAST LASERS LTD.

BiOS SPIE Photonics West

Company Description

Featured Product: Dual wavelength fs laser system for 3D CARS imaging including tunable Ti.sapphire and Yb fiber laser

Manufacturer of single or double wavelength ultrafast laser systems including ultrashort (ps or fs) pulse, ultrabroadband or broadly tunable Ti:sapphire lasers, Yb-doped fiber lasers, amplifiers and optical parametric oscillators. Their typical applications include time resolved or CARS spectroscopy or nonlinear (2P, SHG or SRS/CARS) microscopy. Manufacturer of ultrafast laser optical coatings including different dispersive mirrors such as chirped mirrors. Complete laser laboratory construction.



Alapítva: 1997-ben

Telephely: 1121 Budapest, Konkoly Thege út 29-33. 6. ép. I. em. (KFKI Campus)

Infrastruktúra: 3 lézeroptikai laboratórium, 1 elektronikus és 1 gépészműhely, irodák, tárgyaló, raktár

Honlap: www.szipocs.com

LASERS

BiOS SPIE Photonics West

Company Description

Featured Product: Dual wavelength fs laser system for 3D CARS imaging including tunable Ti.sapphire and Yb fiber laser

Manufacturer of single or double wavelength ultrafast laser systems including ultrashort (ps or fs) pulse, ultrabroadband or broadly tunable Ti:sapphire lasers, Yb-doped fiber lasers, amplifiers and optical parametric oscillators. Their typical applications include time resolved or CARS spectroscopy or nonlinear (2P, SHG or SRS/CARS) microscopy. Manufacturer of ultrafast laser optical coatings including different dispersive mirrors such as chirped mirrors. Complete laser laboratory construction.

H-1012 Budapest, Attila str. 73, HUNGARY
www.fslasers.com

Phone/Fax: +36 (1) 392 2582
r.szipocs@szipocs.com

SPIE Corporate Member

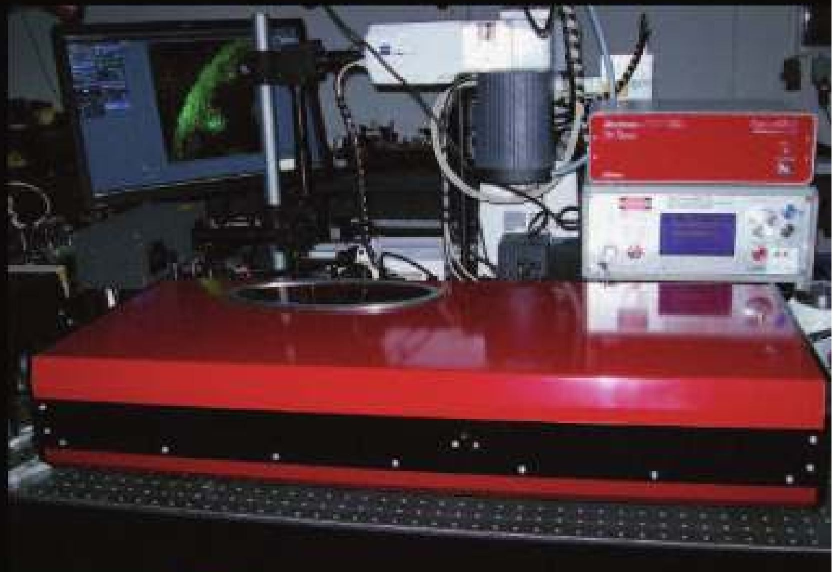
R&D ULTRAFAST LASERS LTD.

SPIE Corporate Member

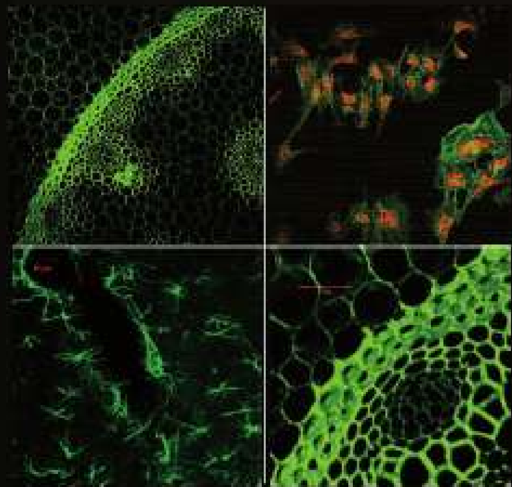
R&D ULTRAFAST LASERS LTD.

FemtoRose 100 TUN Compact/NoTouch (TM)

– hangolási tartomány 690–1050 nm
– szabadalmaztatott ultraszélessávú csörpölt tükör (UBCM) technológia
– beépített, mikroszkópból vezérelhető fényzár (shutter)
– számítógépről vagy mikroszkópból vezérelhető hullámhossz-beállítás
– teljesen automatizált, „hands free" működés
– Carl Zeiss mikroszkóp (ZEN szoftver) kompatibilitás
– beépített pumpa lézer
– teljesen zárt dobozolás, környezeti hatásoktól mentes működés



Az R&D Ultrafast Lasers Kft. – az Ön partnere a nemlineáris 3D mikroszkópiában



Egyéb kapcsolódó termékeink, szolgáltatásaink:

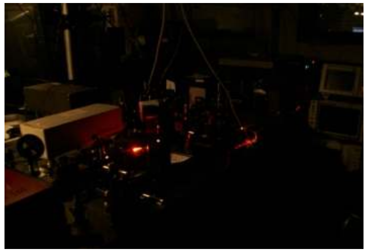
– ionosan porlasztott, kis diszperziójú vagy diszperziókompenzáló tükrök
– komplett lézerlaboratóriumok kiépítése
– szaktanácsadás, konzultáció
– femtoszekundumos lézerrendszerek szervizelése, karbantartása

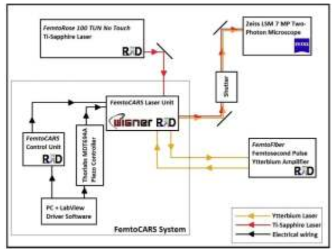
Szipőcs Group

Miről lesz szó?

1. **Fotonika (lézerek + optika)**

- **ps-os, fs-os impulzusüzemű lézerek**
- **pásztázó nemlineáris mikroszkópia**

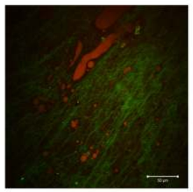




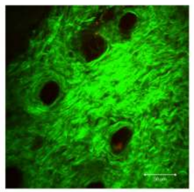
2. Orvosi diagnosztikai, gyógyszeripari alkalmazások (biológia)

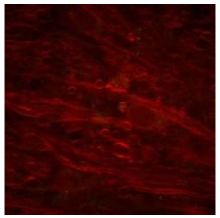
- **bőrgyógyászat**
- **agykutatás**
- **gyógyszeripar**

Dermis



Epidermis





Femtoszekundumos lézerek működésének alapjai

1 fs = 10^{-15} s

1 fs * 300 000 km/s = 0.3 mikron = 0.0003 mm

Látható fény hullámhossza: 0.4-0.76 mikron

→ Az optikai vékonyréteg rendszerek kiemelkedő szerepe

$\Delta\omega\ \Delta\tau \geq$ állandó

$\Delta\omega$: sávszélesség (kör)frekvenciában, $\Delta\tau$: impulzushossz

Femtoszekundumos lézerműködés alapvető feltételei:

- ❑ **Nagy sávszélességgel rendelkező erősítő közeg**
- ❑ **Módusszinkronizálás (pl. Kerr-lencsés)**
- ❑ **Diszperziókompenzálás (szolitonszerű impulzusformálódás)**

Femtoszekundumos lézerek

Szipőcs Group

A Ti-zafír kristály, mint nagy sávszélességgel rendelkező erősítő közeg

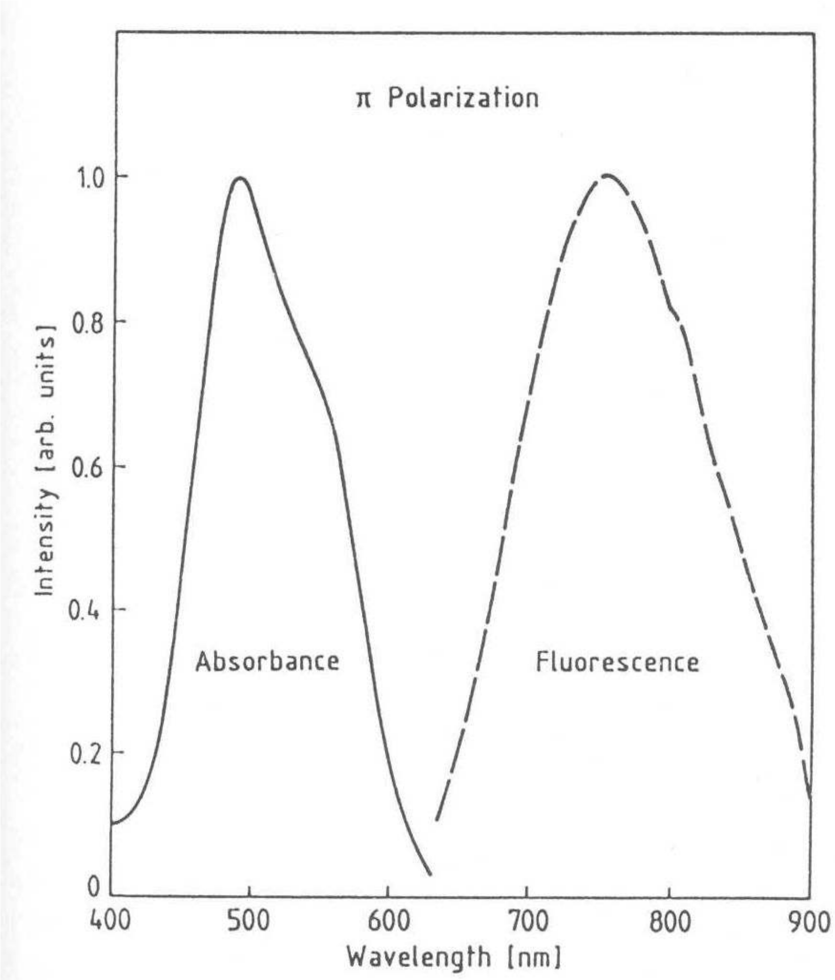


Fig. 2.24. Absorption and fluorescence spectra of the Ti^{3+} ion in Al_2O_3 (sapphire) [2.165]

Table 2.12. Laser parameters of $Ti:Al_2O_3$

Index of refraction	$n = 1.76$
Fluorescent lifetime	$\tau = 3.2\,\mu s$
Fluorescent linewidth (FWHM)	$\Delta\lambda \sim 180\,nm$
Peak emission wavelength	$\lambda_p \sim 790\,nm$
Peak stimulated emission cross section	
parallel to c axis	$\sigma_{p\parallel} \sim 4.1 \times 10^{-19}\,cm^2$
perpendicular to c axis	$\sigma_{p\perp} \sim 2.0 \times 10^{-19}\,cm^2$
Stimulated emission cross section	
at $0.795\,\mu m$ ($\parallel$ c axis)	$\sigma_{\parallel} = 2.8 \times 10^{-19}\,cm^2$
Quantum efficiency of converting a $0.53\,\mu m$ pump photon into an inverted site	$\eta_Q \approx 1$
Saturation fluence at $0.795\,\mu m$	$E_{sat} = 0.9\,J/cm^2$

A Cr:LISAF kristály, mint nagy sávszélességgel rendelkező erősítő közeg

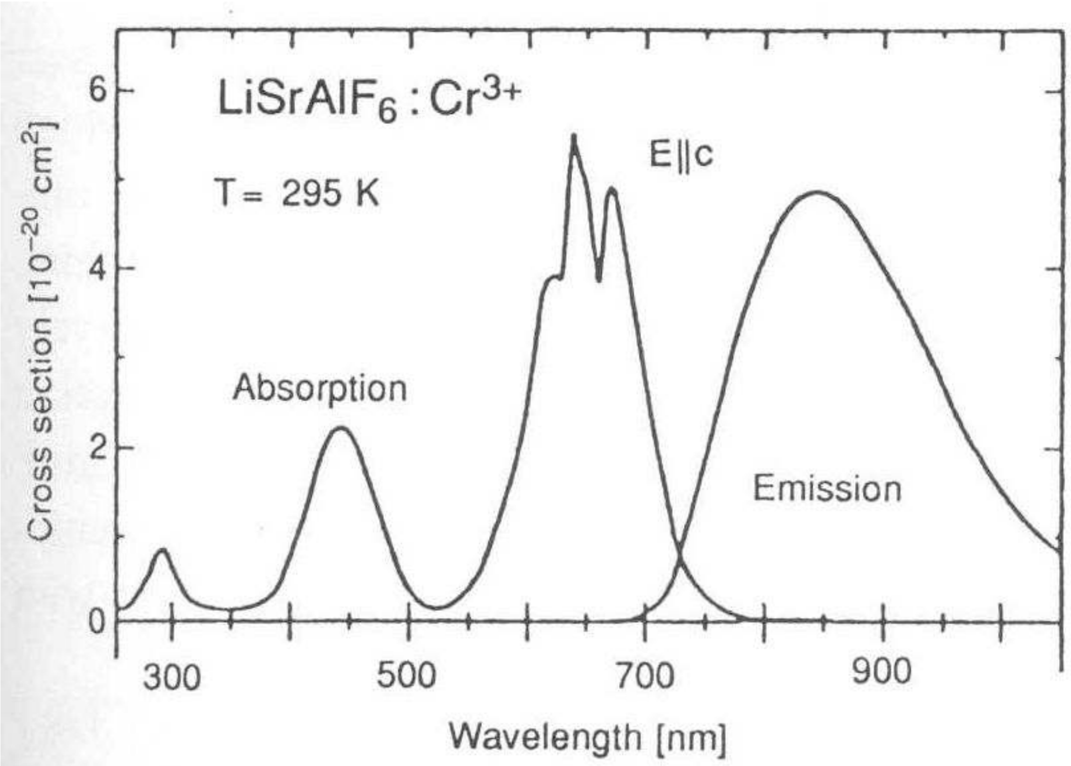


Fig. 2.26. Absorption and emission spectra of Cr : LiSAF

Table 2.13. Comparison of relevant laser parameters for Cr:LiSAF and Ti:Sapphire

	Cr:LiSAF	Ti:Sapphire
Peak wavelength [nm]	850	790
Linewidth [nm]	180	230
Emission cross section [10^{-19} cm^2]	0.5	4.1
Fluorescence lifetime [μs]	67	3.2
Refractive Index	1.41	1.76
Scattering loss [cm^{-1}]	0.002	0

Table 2.14. Comparison of thermal and physical properties of LiSAF and glass

	Cr:LiSAF	Glass
Thermal shock resistance [W/m$^{1/2}$]	~ 0.4	~ 0.4
Fracture strength [kg/mm^2	3.9	5
Thermal expansion coefficient ($\times 10^{-6}$/°C	22	11.4
Young's modulus [Gpa]	100	50
Microhardness [kg/mm^2]	197	~ 500
Fracture toughness [MPam$^{1/2}$]	0.4	0.45
Thermal conductivity [Wm^{-1}K^{-1}]	3.09	0.62

A módusszinkronizálás

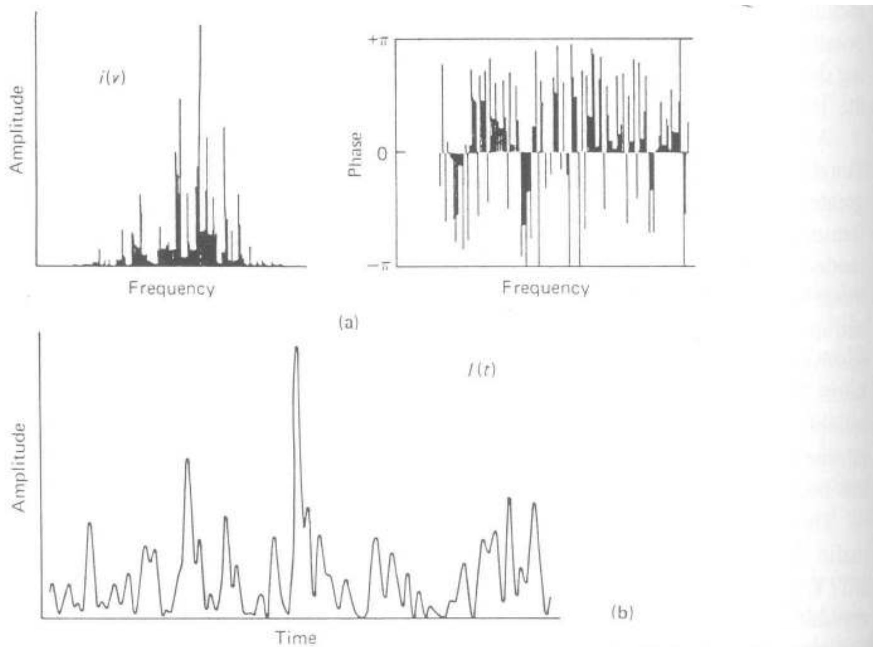


Fig. 9.1a,b. Signal of a non-mode-locked laser. In the frequency domain **(a)** the intensities $i(\nu)$ of the modes have a Rayleigh distribution about the Gaussian mean and the phases are randomly distributed. In the time domain **(b)** the intensity has the characteristic of thermal noise

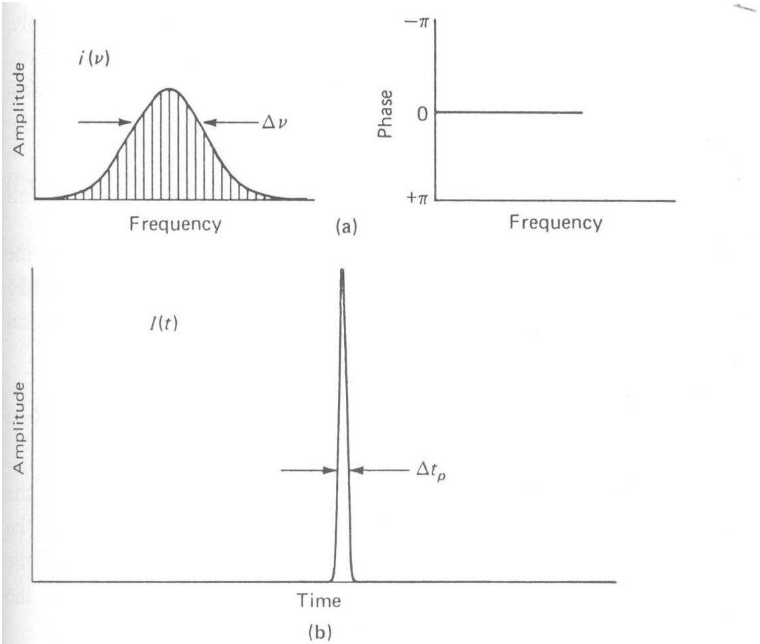


Fig. 9.2a,b. Signal structure of an ideally mode-locked laser. The spectral intensities **(a)** have a Gaussian distribution, while the spectral phases are identically zero. In the time domain **(b)** the signal is a transform-limited Gaussian pulse

A diszperzió hatása az impulzusokra

$$\phi(\omega) = \phi_0 + \phi'(\omega-\omega_0) + {}^1\!/_2\phi''(\omega-\omega_0)^2 + {}^1\!/_6\phi'''(\omega-\omega_0)^3 + \ldots \quad (1)$$

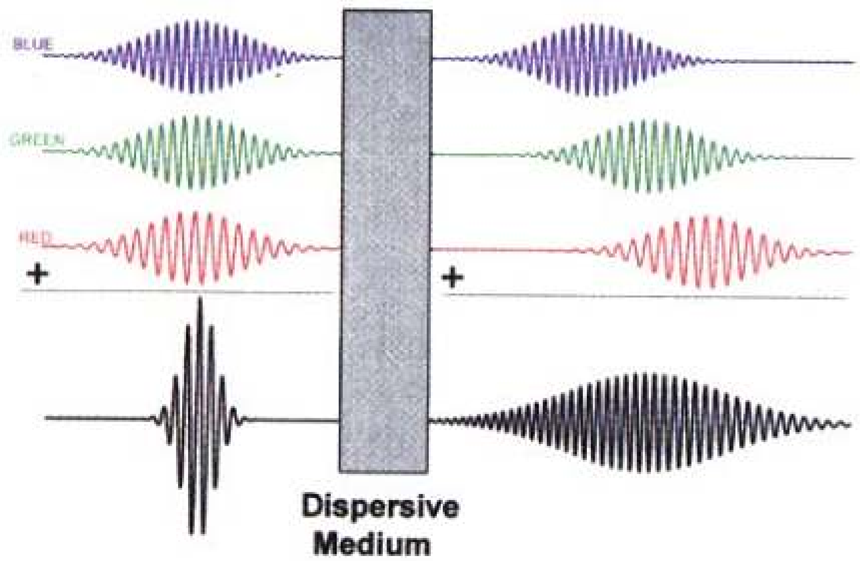
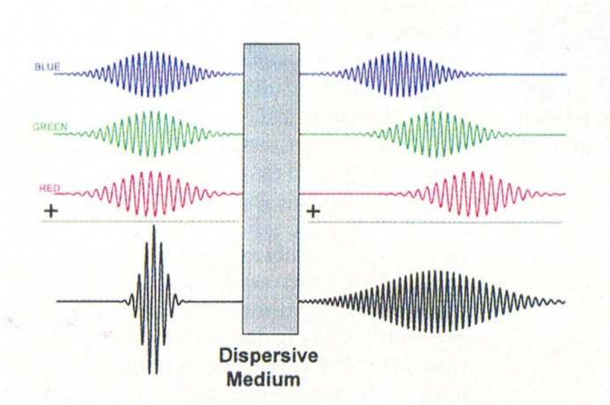
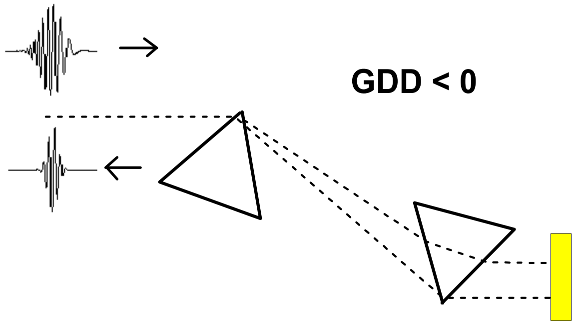


Figure 1. Optical pulse propagation through a dispersive medium: a frequency-dependent group delay leads to a pulse broadening and to a carrier frequency sweep.

Szipőcs Group

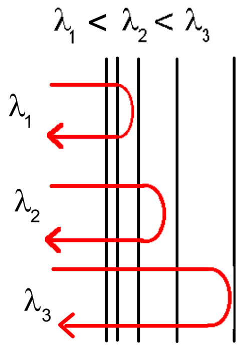
The effect of dispersion

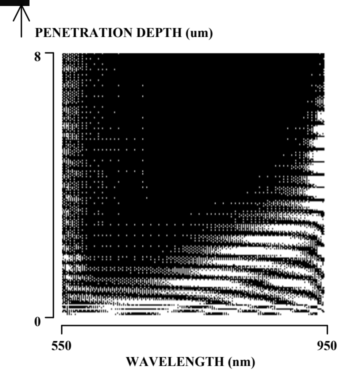




Zs. Bor, B. Rácz, Opt. Comm., 54, 165 (1985)

Chirped mirrors for dispersion control





R. Szipőcs, K. Ferencz, Ch. Spielmann, F. Krausz, *Opt. Lett*. 19(3), 201-203 (1994)

R. Szipőcs, A. Kőházi-Kis, Appl. Phys. B65, 115 (1997)

R. Szipőcs, F. Krausz U. S. Pat. No.: 5, 734, 503

A csörpölt tükrök előnyös tulajdonságai:

1. Közel állandó másodrendű illetve tervezhető harmadrendű diszperzió

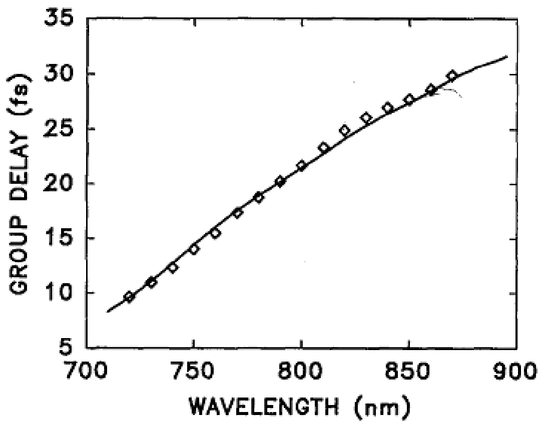


Fig. 3. Computed group delay as a function of wavelength (solid curve) together with experimental data (squares) for the multilayer design of Fig. 1. Note that the absolute delay could not be measured; therefore a wavelength-independent constant delay was added to the measured relative data.

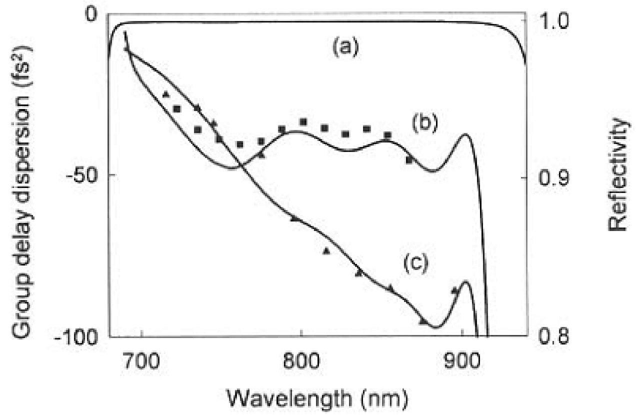


Figure 3. Curve (a): calculated reflectivity of chirped mirrors described in the text. The reflectivity is somewhat less (≈ 99.5%) in reality due to scattering losses not included in the calculations. Curves (b) and (c): calculated GDD of chirped mirrors designed for GDD and TOD compensation, respectively. The squares and triangles represent measured data points obtained with spectrally-resolved[15] and conventional[16] white-light interferometry, respectively.

R. Szipőcs, K. Ferencz, Ch. Spielmann, F. Krausz, *Opt. Lett*. 19(3), 201-203 (1994)

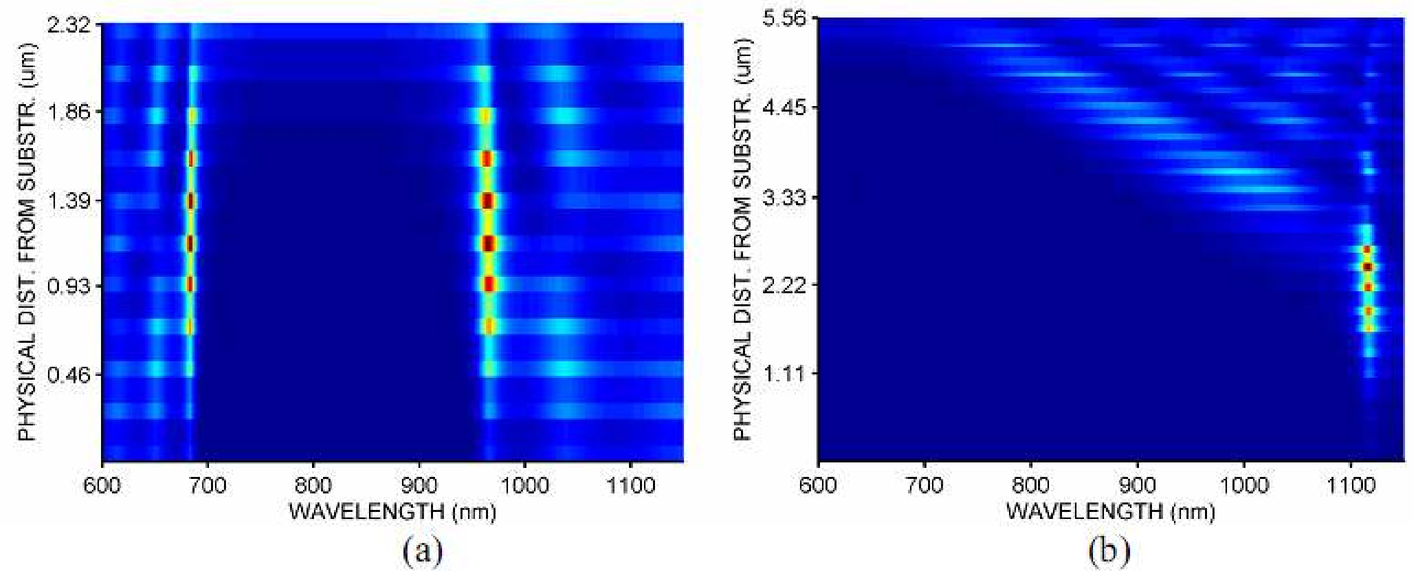
R. Szipőcs, A. Kőházi-Kis, Appl. Phys. B65, 115 (1997)

R. Szipőcs, F. Krausz U. S. Pat. No.: 5, 734, 503

Szipőcs Group

A csörpölt tükrök előnyös tulajdonságai:

2. A csörpölt tükrök megnövekedett spektrális sávszélessége



(a) Kisdiszperziójú negyedhullámú tükör (HR tartomány :~ 700-900 nm)

(b) Ultraszélessávú csörpölt dielektrikumtükör (HR tartomány: ~ 660-1060 nm)

OPTICS

BiOS
SPIE Photonics West

Femtosecond Dispersive and Broadband Optics by IBS technology

- Chirped mirrors (CM)
- Low dispersion ripple, highly dispersive negative dispersion mirrors (MCGTI)
- Ultrabroadband chirped mirrors (UBCM)

H-1012 Budapest, Attila str. 73, HUNGARY
www.fslasers.com

Phone/Fax: +36 (1) 392 2582
r.szipocs@szipocs.com

BOOTH NUMBER: 8109

R&D ULTRAFAST LASERS LTD.

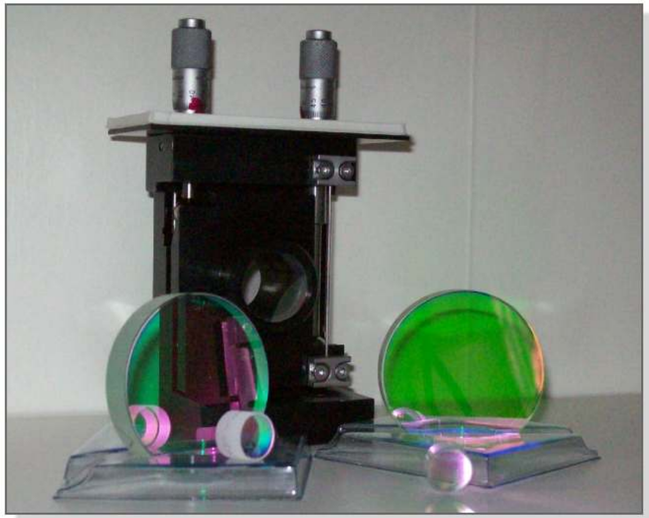
BiOS
SPIE Photonics West

Pioneering Ultrafast LaserTechnology by R&D

INVENTING CHIRPED MIRRORS

Femtosecond Dispersive
and Broadband Optics by IBS technology

Patents

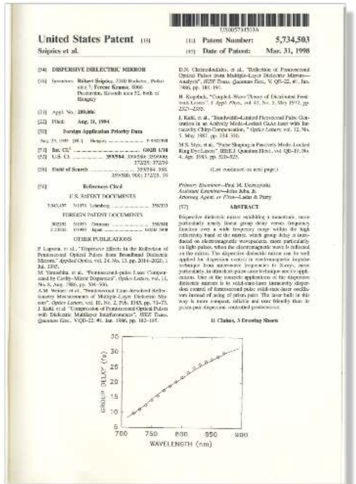



SZABADALMI OKIRAT


The United States of America

The Commissioner of Patents and Trademarks

United States Patent


United States Patent

H-1012 Budapest, Attila str. 73, HUNGARY
www.fslasers.com

Phone/Fax: +36 (1) 392 2582
r.szipocs@szipocs.com

DISPERSIVE MIRRORS, CHARACTERIZATION: WHITE LIGHT INTERFEROMETRY

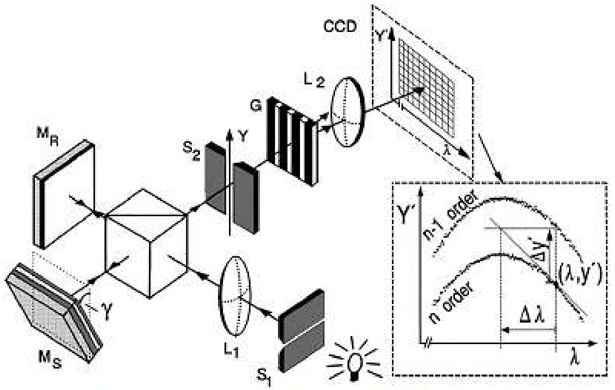
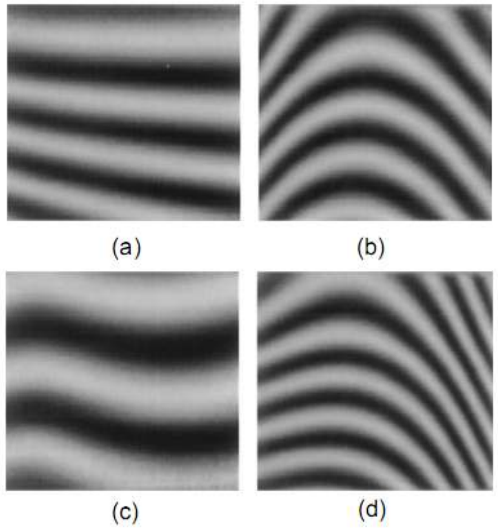


Fig. 1. Spectrally resolved white-light interferometer for group-delay measurement of dielectric mirrors. L_1, L_2, achromatic lenses; S_1, S_2, slits; M_S, sample mirror; M_R, reference mirror; G, transmission grating.



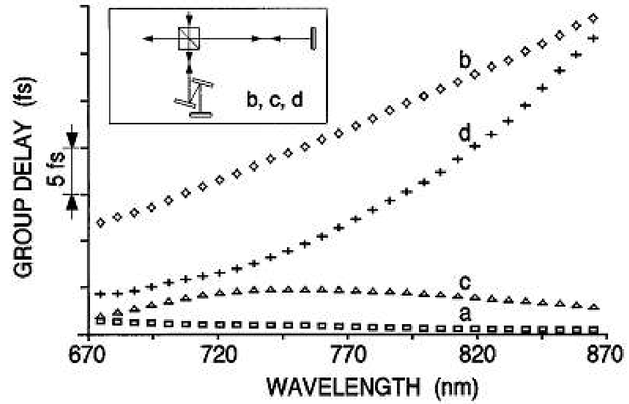


Fig. 4. Measured group-delay functions obtained by computer processing of the images shown in Fig. 3 (every fifth point is plotted). The curves correspond to a single reflection. Inset: four-reflection arrangement used for measuring curves b–d.

(a) Low dispersion sample (linear phase shift)

(b) Chirped mirror sample (quatratic phase shift)

(c) Gires-Tournois Intereferometer mirror (cubic phase shift)

(d) (c)+(d)

A.P. Kovács, K. Osvay, Zs. Bor, R. Szipőcs, Opt. Lett. 20, 788-790 (1995)

Ultrabroadband chirped mirrors for ultrafast lasers

528 OPTICS LETTERS / Vol. 22, No. 8 / April 15, 1997

Ultrabroadband chirped mirrors for femtosecond lasers

E. J. Mayer, J. Möbius, A. Euteneuer, and W. W. Rühle

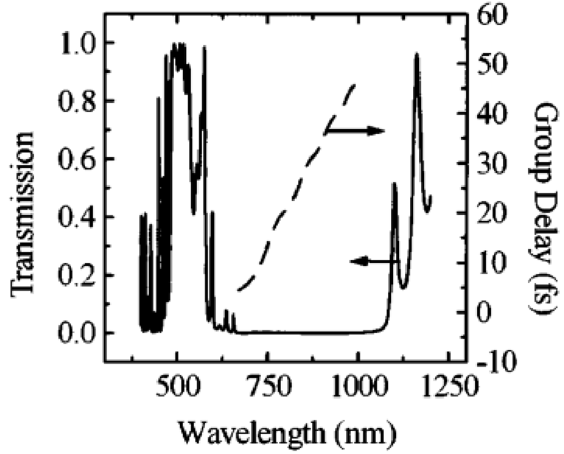
Department of Physics, Philipps University, Renthof 5, D-35032 Marburg, Germany

R. Szipőcs

R&D Lézer-Optika Bt., P.O. Box 622, H-1539 Budapest, Hungary

Received November 25, 1996

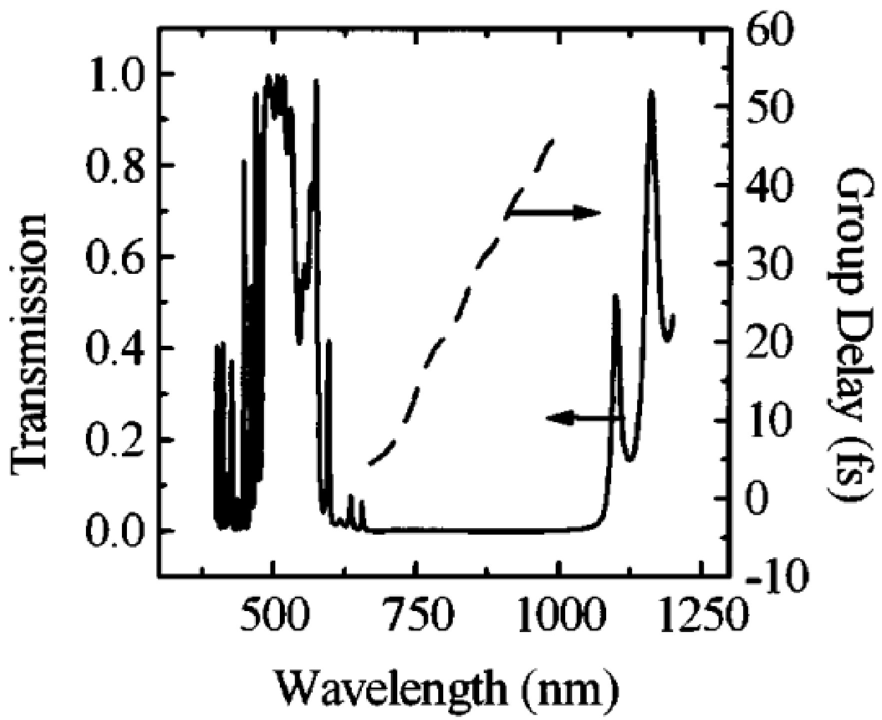
We report on the performance of widely tunable femtosecond and continuous-wave Ti:sapphire lasers that use a newly developed ultrabroadband mirror set. The mirrors exhibit high reflectivity ($R > 99\%$) and smooth variation of group delay versus frequency over a wavelength range from 660 to 1060 nm. Mode-locked operation with pulse durations of 85 fs was achieved from 693 to 978 nm with only one set of ultrabroadband mirrors. © 1997 Optical Society of America

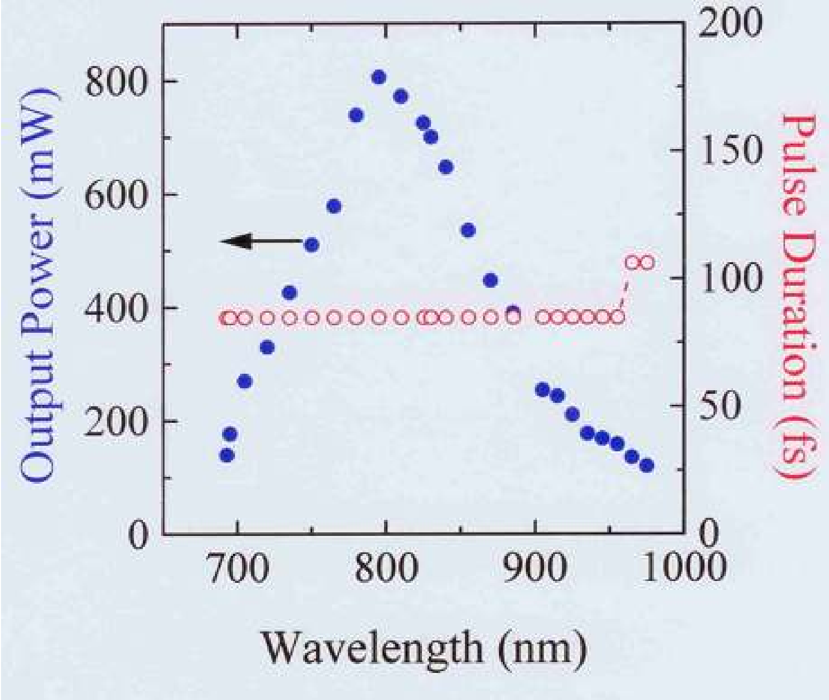


- The first widely tunable femtosecond pulse Ti:sapphire laser
- High reflectivity (*R . 99%) and* smooth variation of group delay over a wavelength range from 660 to 1060 nm
- Mode-locked operation from 693 to 978 nm using one set of mirrors

E.J. Mayer, J. Möbius, A. Euteneuer, W. Rühle, R. Szipőcs: Opt. Lett. 22, 528-530 (1997)

Széles sávban hangolható < 100 fs-os Ti-zafír lézer kifejlesztése





- **Ultraszélessávú csörpölt tükrök (HR tartomány: 660-1060 nm)**
- **Széles hangolási tartomány tükörkészlet cseréje nélkül (693-978 nm)**

E.J. Mayer, J. Möbius, A. Euteneuer, W. Rühle, R. Szipőcs: Opt. Lett. 22, 528-530 (1997).

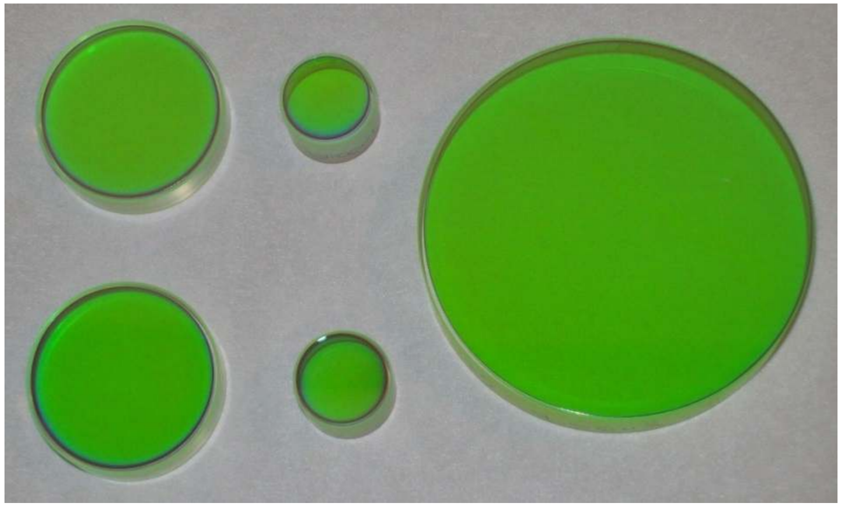
SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

Széles sávban hangolható < 100 fs-os Ti-zafír lézer

FemtoRose 100 TUN Compact, NoTouch (10W pump)

Ultraszélessávú, ionosan porlasztott csörpölt tükrök biztosítják a lézerben a széles sávban való hangolhatóságot



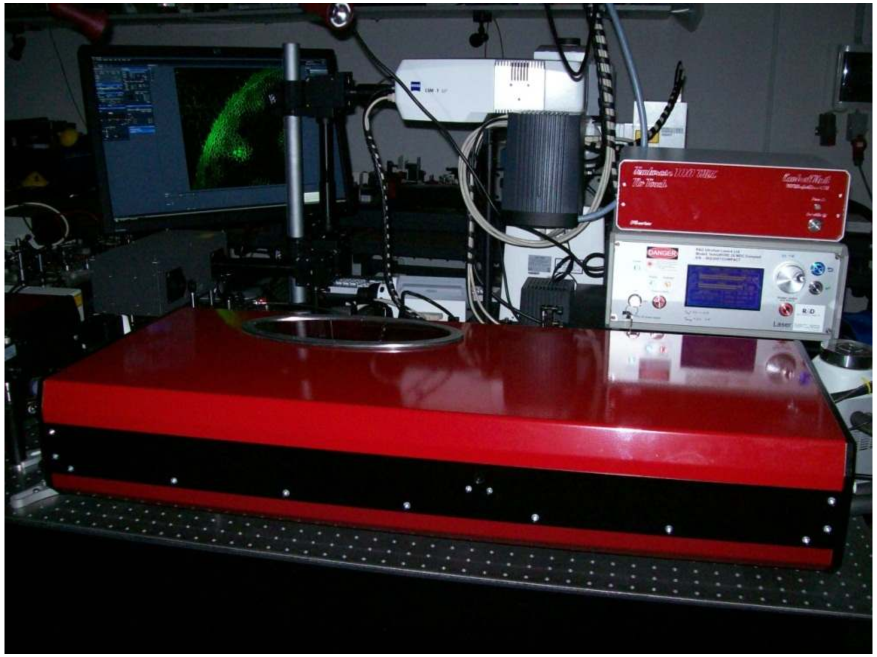
HR tartomány: 660 - 1060 nm

SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

FemtoRose 100 TUN/NoTouch

the
Broadly Tunable, femtosecond pulse Ti:sapphire laser



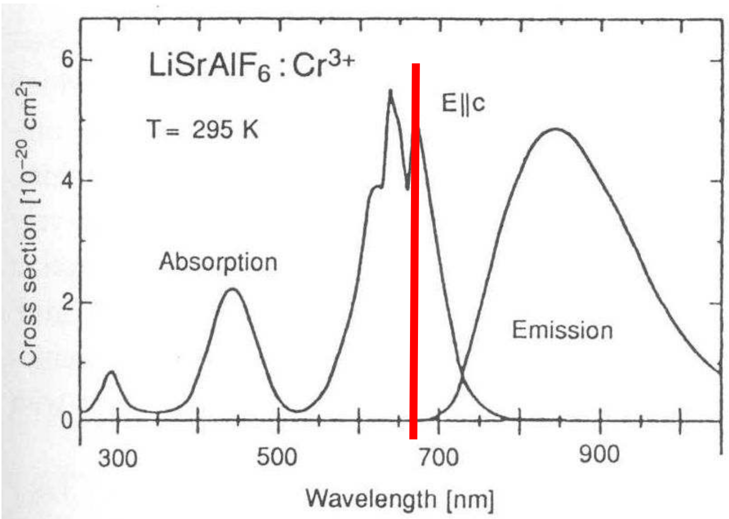
- Stable, easy mode-locking (with starter electronics)
- Soliton-like, nearly transform-limited pulses
- Patented Ultrabroadband Chirped Mirror™ optics
  – single optics set from 680 to 1040 nm
- Built in Millennia™ / Verdi™ / Finesse™ pumping (6W, 8W, 10W) – diode-pumped stability
- Sealed, purgeable enclosure
  – reliability, full wavelength coverage
- 15 years of experience
- Labview interface program
- Turn-key, truly hands-off operation (automatic cavity control)

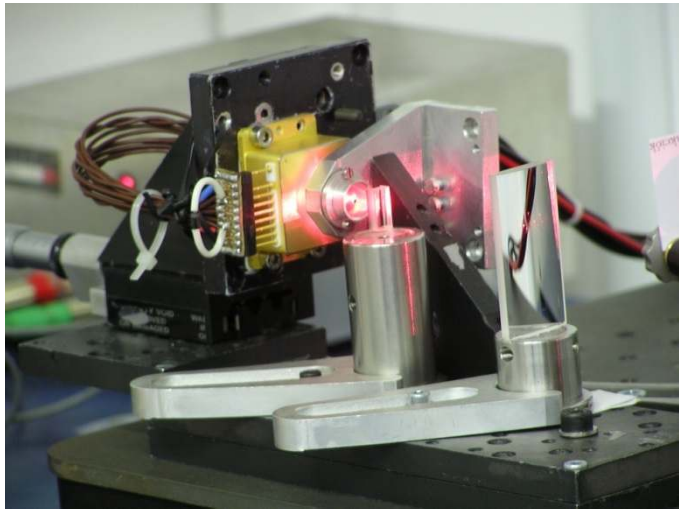
SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

Diódapumpált, tükörkompenzált fs-os Cr:LISAF lézer

Erősítő közeg: Cr:LISAF kristály



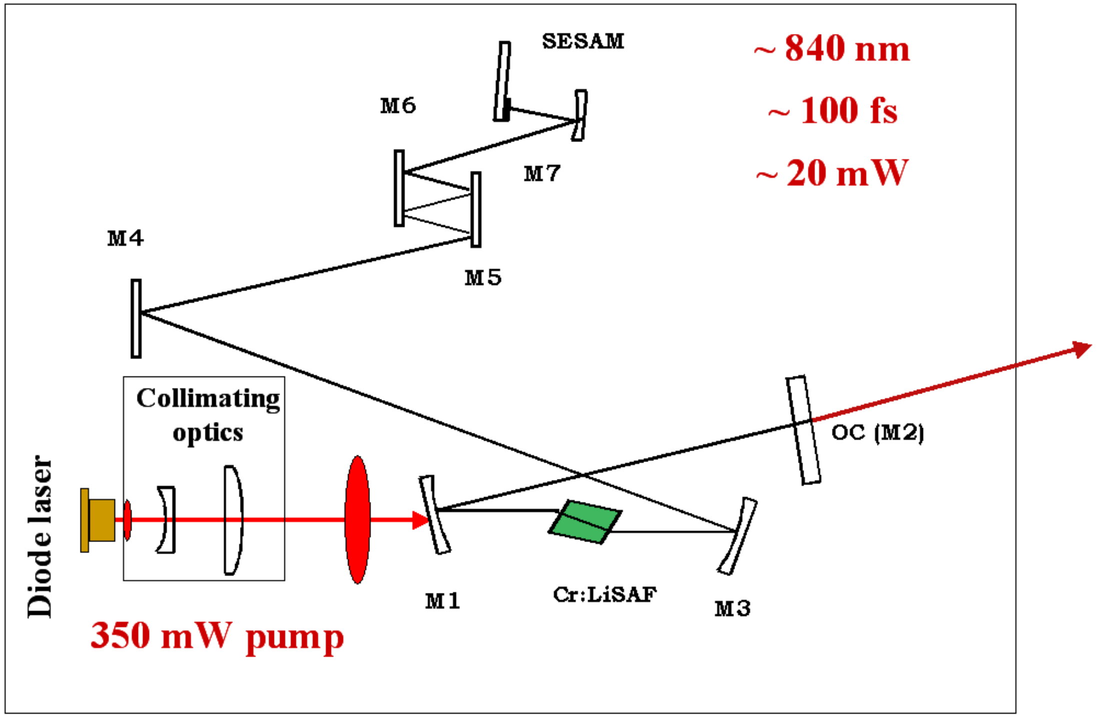


•Optikai pumpálás 670 nm-es lézerdiódával, P = 350 mW teljsítménnyel (!)

• Módusszinkronizálás félvezető telítődő abszorbens (SESAM) alkalmazásával

B. Császár, A. Kőházi-Kis, R. Szipőcs: **Low reflection loss ion-beam sputtered negative dispersion mirrors with MCGTI structure for low pump threshold, compact femtosecond pulse lasers**
In *Proc. Advanced Solid State Photonics, February 6-9, 2005, Vienna, Austria* (2005), Paper WB17

A diódapumpált Cr:LISAF lézer felépítése



B. Császár, A. Kőházi-Kis, R. Szipőcs: **Low reflection loss ion-beam sputtered negative dispersion mirrors with MCGTI structure for low pump threshold, compact femtosecond pulse lasers**
In *Proc. Advanced Solid State Photonics, February 6-9, 2005, Vienna, Austria* (2005), Paper WB17

SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

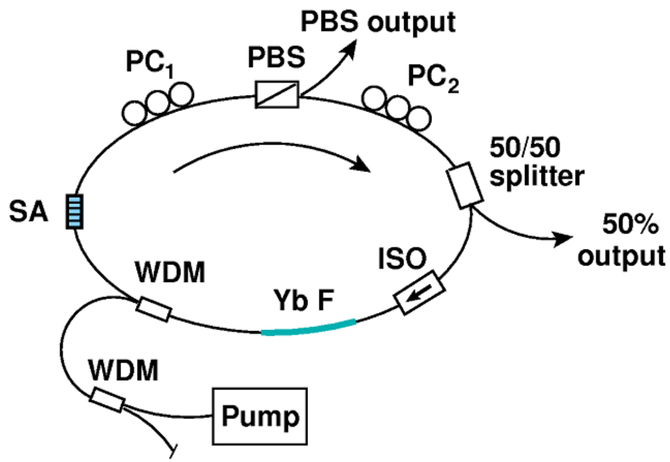
Szálintegrált, femtoszekundumos AND Yb-szállézer

Femtobiológia projekt: R&D Ultrafast Lasers Kft, Furukawa Electric (FETI) közös laboratórium

J. Fekete, A. Cserteg, Szipőcs; **All-fiber, all-normal dispersion ytterbium ring oscillator,** Laser Physics Letters 6, 49-53, 2009

All-fiber, all-normal dispersion ytterbium ring oscillator

- Operation determined by interplay between **gain**, **self-phase modulation**, **dispersion** and **filtering** effects
- Pulse shaping is based on **nonlinear polarization rotation** in the fiber together with **spectral and temporal filtering** by a polarizing element



PC: polarization controller

PBS: polarizing beam splitter

ISO: isolator

Yb F: Ytterbium doped fiber

WDM: wavelength division multiplexer

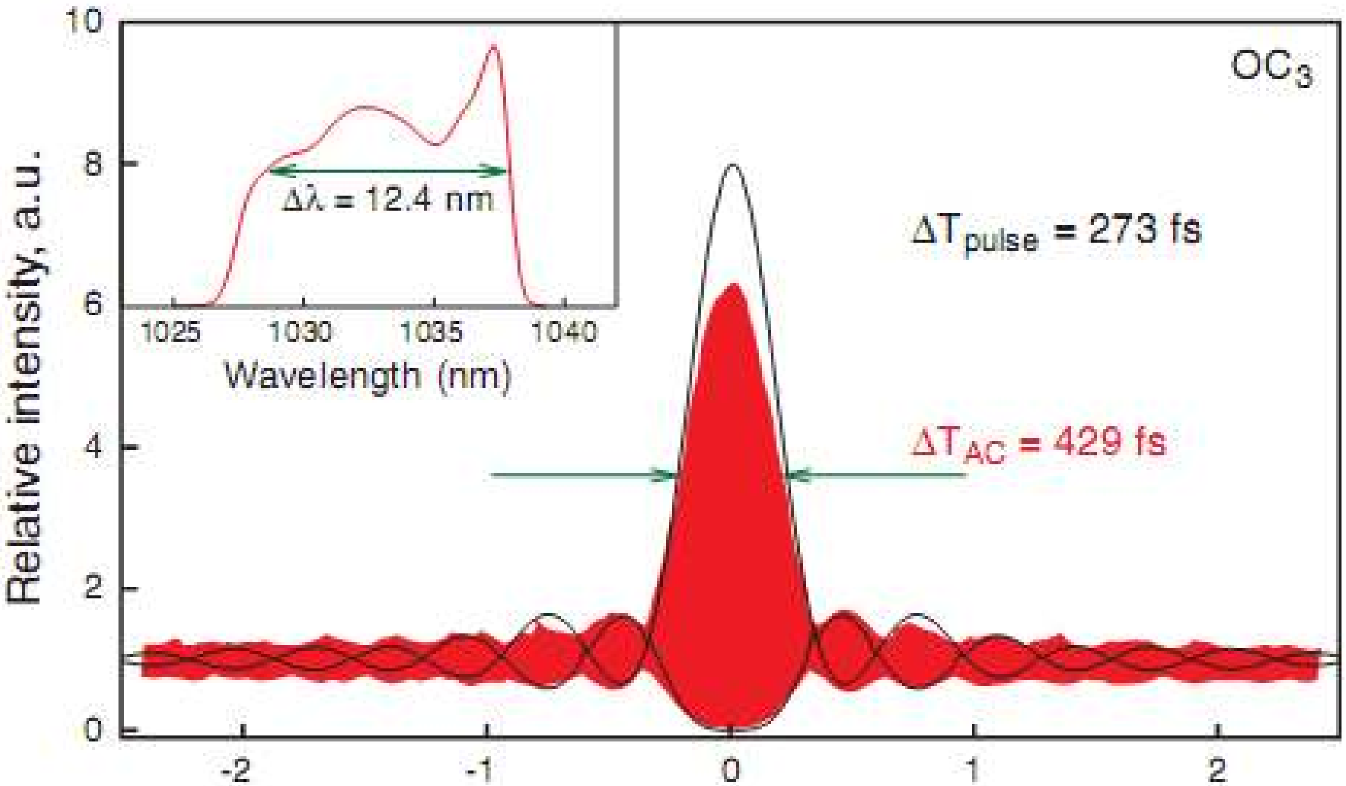
SA: saturable absorber

J. Fekete, A. Cserteg, Szipőcs; All-fiber, all-normal dispersion ytterbium ring oscillator, Laser Physics Letters 6, 49-53, 2009

Copyright Wigner RCP and R&D Ltd (2012)

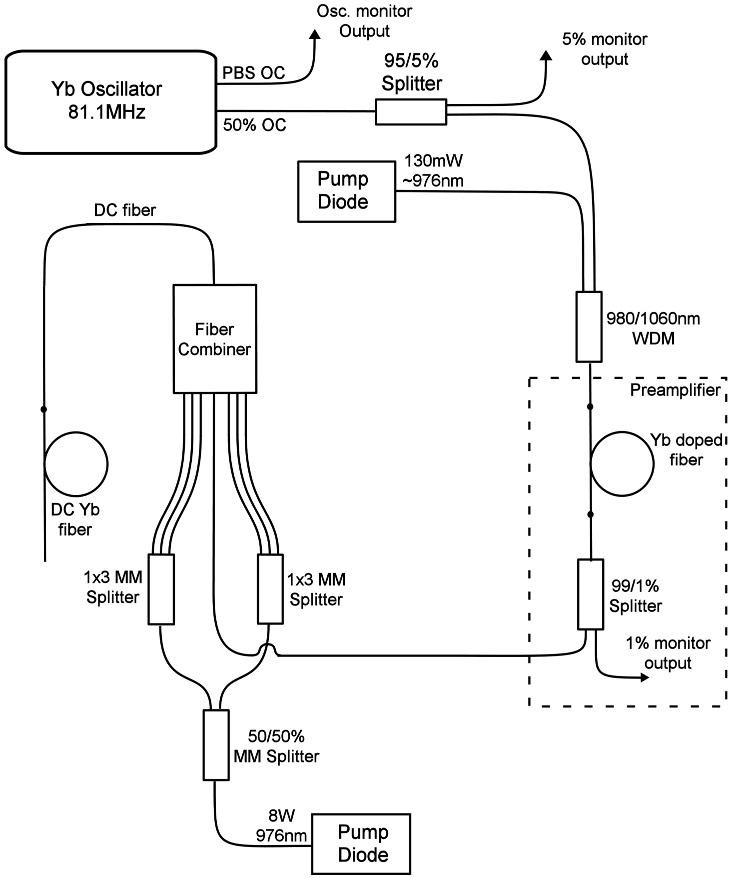
Szálintegrált, femtoszekundumos AND Yb-szállézer

Mért jellemzők: spektrális sávszélesség és impulzushossz

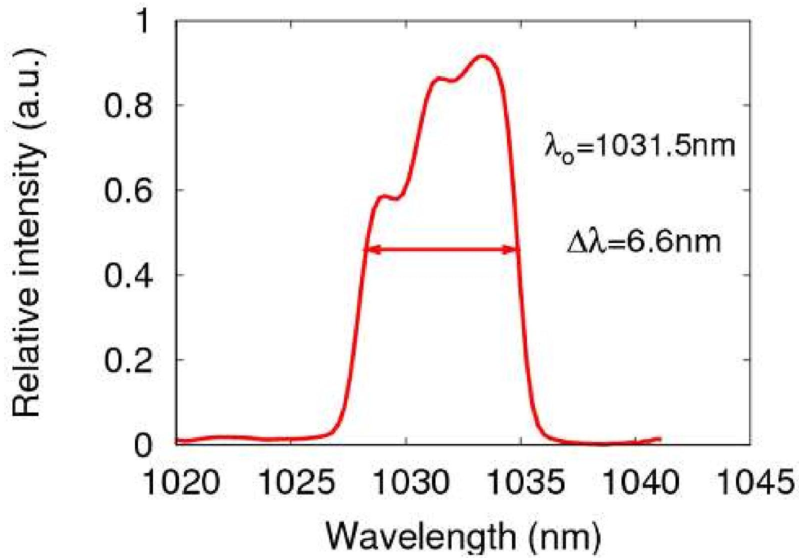


J. Fekete, A. Cserteg, Szipőcs; **All-fiber, all-normal dispersion ytterbium ring oscillator**
Laser Physics Letters 6, 49-53, 2009

Yb szálerősítő felépítése



Mért bemeneti spektrum



P_{osc} ~ 2-5 mW

P_{preamp} ~ 40-60 mW

P_{amp} ~ 600 - 900 mW

τ ~ 300-400 fs (kompresszált)

FemtoFiber: fs pulse yb-fiber oscillator/amplifier system

- **Polarization is controlled by a built in PolaRITE III polarization controller**
- **Control voltages of the PolCont are set by a computer through an RS232 interface**
- **Pump powers of the diodes are set by a built in microcontroller unit**

Copyright Wigner RCP and R&D Ltd (2012)

SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

FemtoCARS

the

Label-free, 3D Microscopic Imaging System for Real-time in vivo Diagnostics

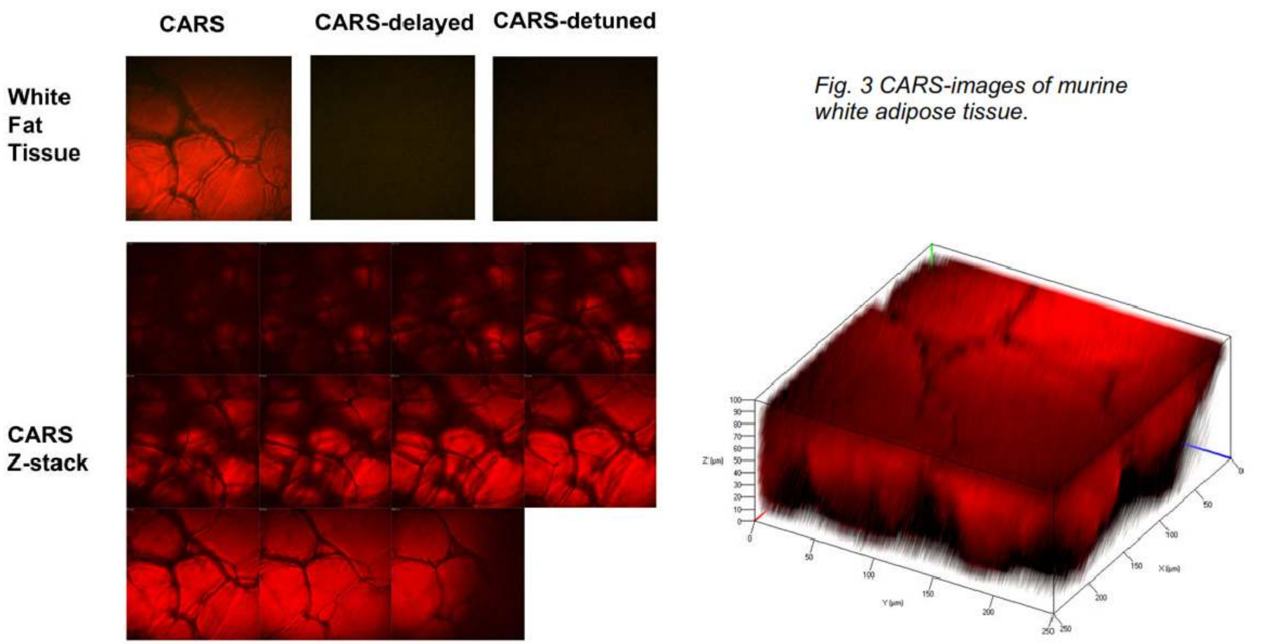


Fig. 3 CARS-images of murine white adipose tissue.

Kolonics A, Csáti D, Antal P, Szipőcs R; A simple, cost efficient fiber amplifier wavelength extension unit for broadly tunable, femtosecond pulse Ti-sapphire lasers for CARS microscopy; In: Proc. BIOMED Biomedical Optics and Digital Holography and Three Dimensional Imaging (Miami, Florida, United States, April 28-May 2 2012); OSA Technical Digest Series; BSu3A.28 /1-3 (2012)

SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

FemtoCARS
the
Label-free, 3D Microscopic Imaging System for Real-time in vivo Diagnostics

Dermis

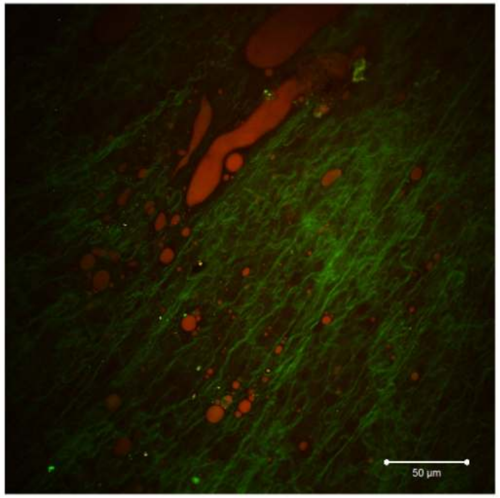


Photo: Kolonics/Szipőcs

Epidermis

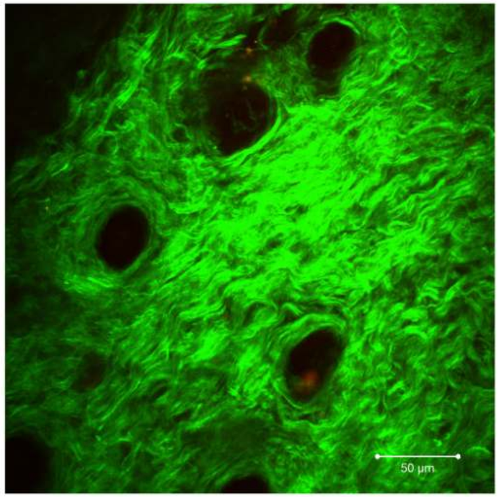
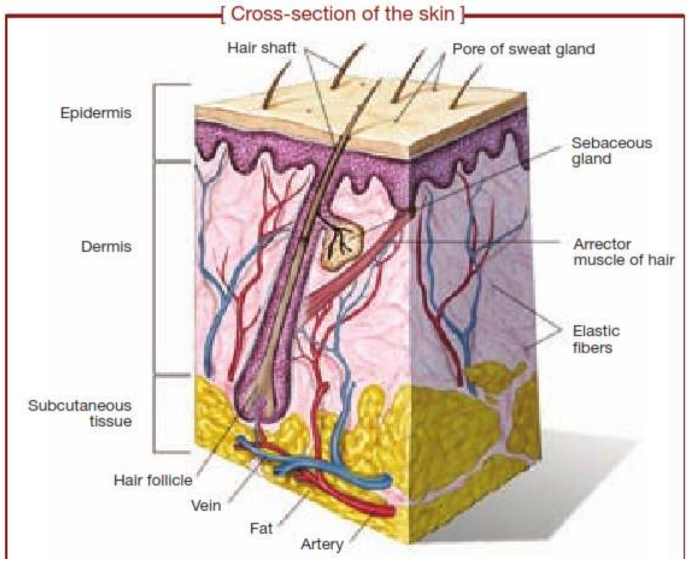


Photo: Kolonics/Szipőcs



red: CARS signal from lipids, green: SHG signal of collagene

SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

FemtoCARS

the

Label-free, 3D Microscopic Imaging System for Neurology

In 2012, a novel, cost efficient CARS imaging setup was developed at Wigner RCP of HAS (Csati, 2012), which was used for label-free, 3D microscopic imaging of different biological samples, such as white adipose tissue (Kolonics, 2012). A commercial version of the prototype CARS system at Wigner RCP has been constructed and built for the University of Szeged (USZ) by the end of 2012 by the R&D Ultrafast Laser Ltd in collaboration with Carl Zeiss Jena. The CARS imaging system comprises a femtosecond pulse, tunable Ti:sapphire laser (R&D), an inherently synchronized two-stage Yb-fiber amplifier unit (R&D), a CARS Unit (R&D) and a CARS-upgraded version (Wigner) of Axio Examiner LSM 7 MP microscope (Carl Zeiss). Using this commercial setup, researchers at USZ (Molnár G) and Wigner RCP (Szipőcs R) have demonstrated that this novel CARS-imaging setup allows for label free imaging of the brain (Figure 1.). For instance, tuning their CARS setup to CH_2 vibration of myelin lipids, they could record high quality 3D CARS images of the myelin. Since myelin loss and axonal degeneration are the pathological hallmarks of several inherited and acquired neurological disorders, a method that allows simultaneous visualization of the two inter-related processes in live tissues may have great research utility. Such method had not been available prior to the introduction of the CARS system.

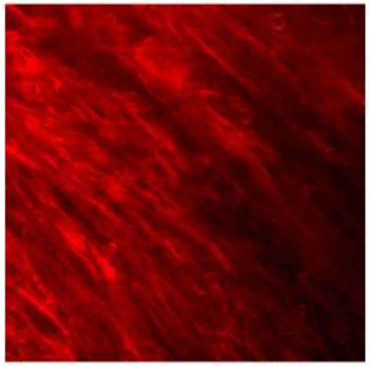
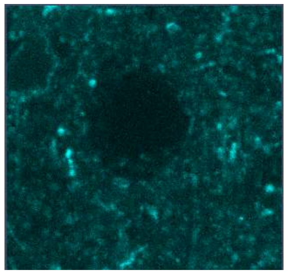


Figure 1. CARS image of myelin fibers in the white matter of rat. In vivo preparation.

HUNGARIAN BRAIN RESEARCH PROGRAM
NEMZETI AGYKUTATÁSI PROGRAM



3D mikroszkópia már létező klinikai alkalmazásai

The confocal microscope illuminates a portion of skin with the point source of light and detects the reflected light through a pinhole.

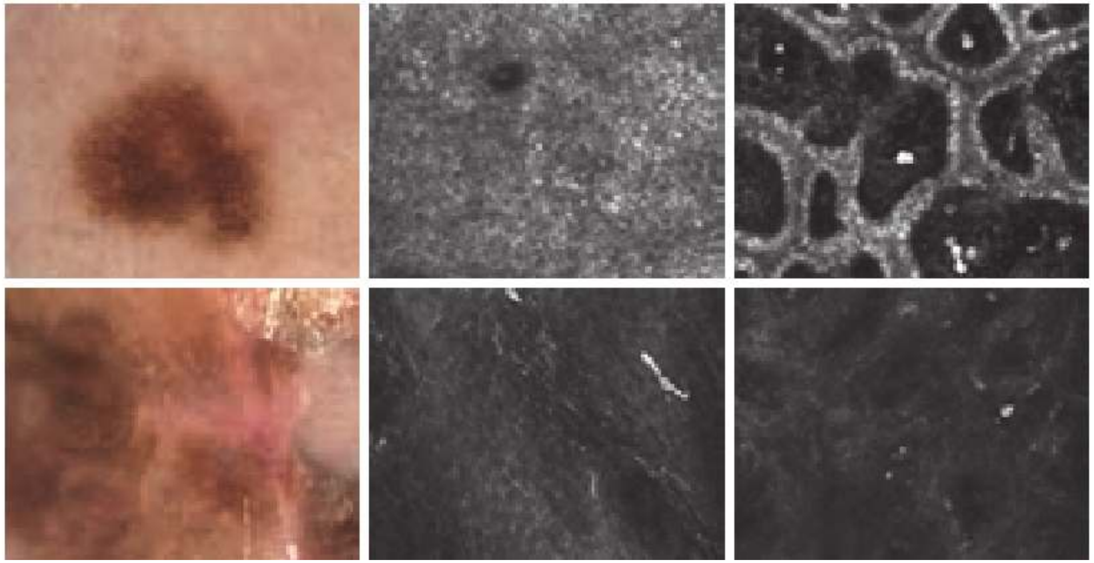
Lucid's non-invasive VivaScope confocal microscopes provide cellular resolution images to help identify various skin conditions.

Miért előnyös az in vivo 3D mikroszkópia?

☺ **Tumor határának pontos meghatározásának lehetősége**

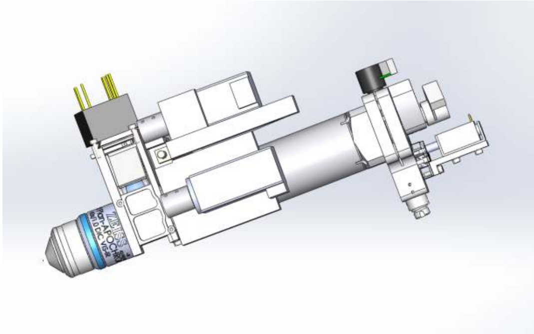
☺ **Nem kell várni a patológiai vizsgálatok eredményére!**

Given that this imaging form lets physicians look into tissue *in vivo* without any of the processing that is done in the pathology lab, confocal imaging may well reveal new skin features that cannot be directly correlated to the pathology.

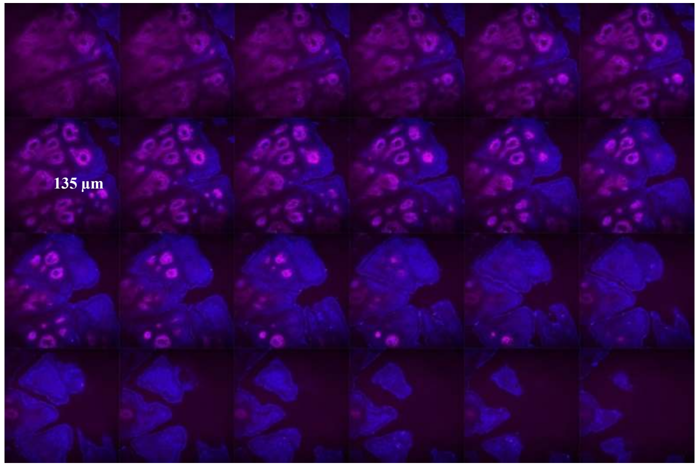


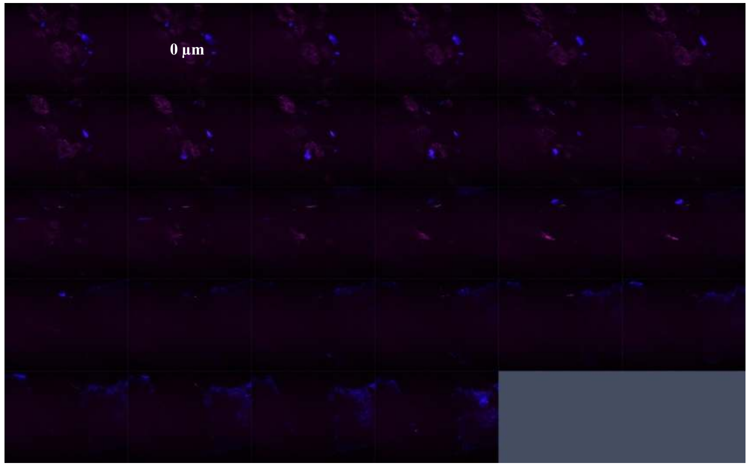
(Top) A benign mole viewed from (left) the skin's surface at visible wavelengths, (center) at a depth of 10 μm and (right) at 161 μm with infrared confocal microscopy. (Bottom) Malignant melanoma at (left) surface level in visible light, (center) at a depth of 35 μm and (right) at 95 μm with confocal microscopy.

Basaliomák in vivo vizsgálata nemlineáris mikroszkópia módszerekkel




FemtoFiber + scanning head for confocal/2PF imaging = FiberScope





31 éves nőbeteg ép terület/tumor terület z-stack sorozat felvétel (AF+SHG)

SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

Természetes fluorofórok a bőrben: kollagén, elasztin, keratin

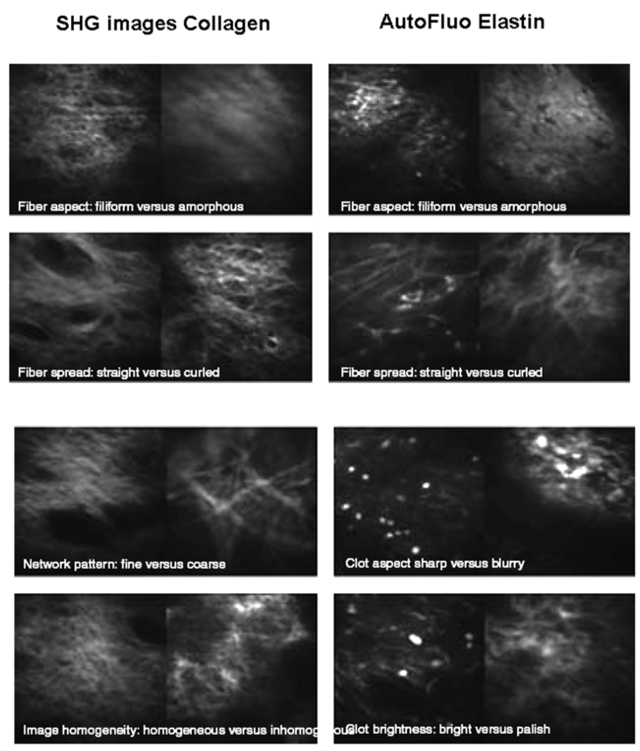
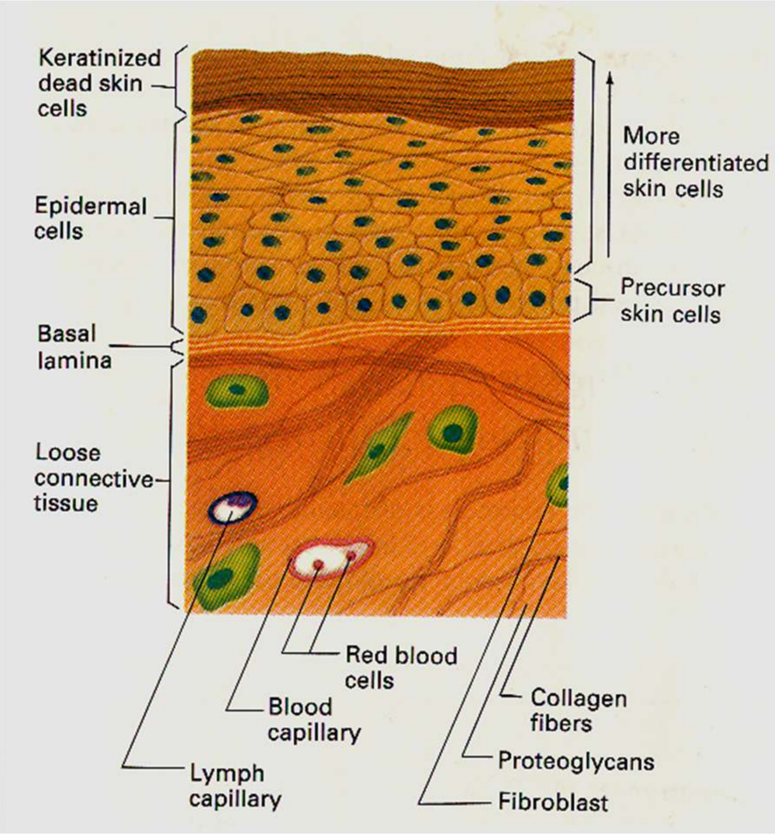


Figure 1. Optical slices obtained by multiphoton laser tomography of the superficial dermis depicting the category characteristics. Left side: second harmonics generation images depicting dermal collagen. Right side: autofluorescence images depicting dermal elastin. Edge length is about 0.2 mm.



Hans Georg Breunig, Hauke Studier and Karsten König, Opt. Exp. 18, 7857 (2010)

SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

Természetes fluorofórok a bőrben: kollagén, keratin, NADH, melanin

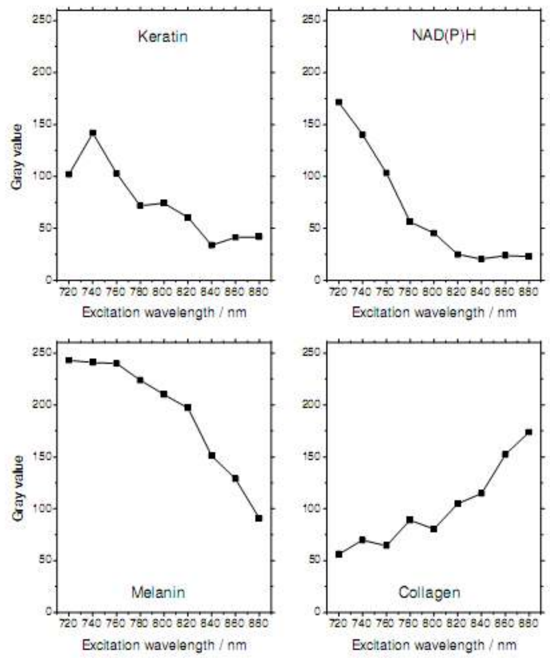


Fig. 8. Signal intensities of main fluorophores of human skin in dependence of excitation wavelength derived from Fig. 3 - Fig. 6.

Hangolható (femtoszekundumos) lézer kell!

Hans Georg Breunig, Hauke Studier and Karsten König, Opt. Exp. 18, 7857 (2010)

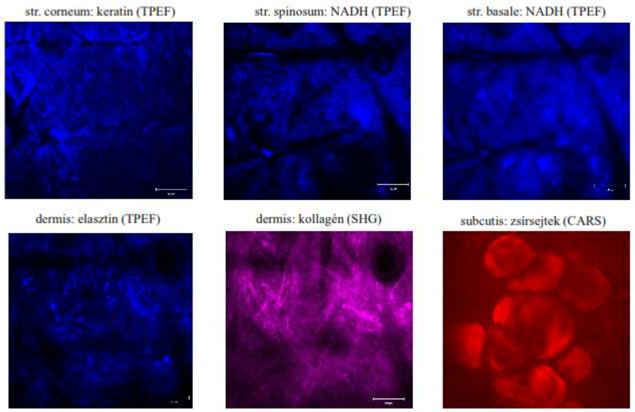
SPIE Corporate Member

R&D ULTRAFAST LASERS LTD.

Mit, hogyan mérünk in vivo a bőrgyógyászatban?
(a lézerekkel és a leképező optikákkal kapcsolatos elvárások)

A bőr szerkezeti képe – multifoton mikroszkópiával

Semmelweis Egyetem Bőr-, Nemikórtani és Bőronkológiai Klinika — Wigner Szipőcs Group

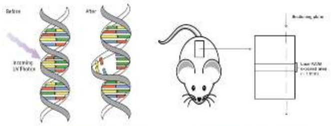


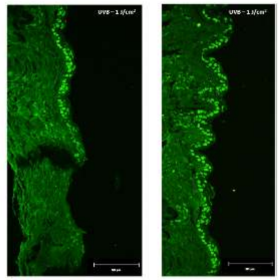
4

Biztonságtechnikai vizsgálatok

Semmelweis Egyetem Bőr-, Nemikórtani és Bőronkológiai Klinika — Wigner Szipőcs Group

- **Károsító hatások**: hő-, mechanikai és fotokémiai
- Fotokémiai károsító hatások detektálása: intracell. kromoförok két foton excitációja következtében alakul ki, **sejtes rendszerekben ez a károsodás teljesen hasonló az UVB besugárzás által okozottakkal**
- **Cyclobutane pyrimidin dimerek**: kovalens keresztkötések a DNS azonos láncán lévő szomszédos pirimidin bázisai között - NER rendszer javítja – elégtelen javítás esetén – mutáció





de Gruijl F.R. and Rebel H., *Early events in UV carcinogenesis-DNA damage, target cells and mutant p53 foci*. Photochem Photobiol, 2008. **84**(2): p. 382-7.

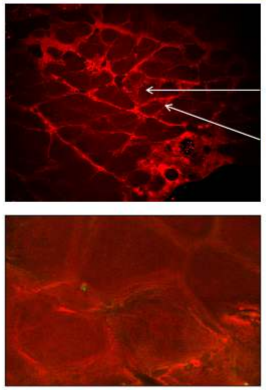
F.Fischer, B.Volkmer., S. Puschmann, R. Greinert., W. Breitbart, J. Kiefer, R. Wepf, *Skin imaged by femtosecond laser irradiation: a risk assessment for in vivo applications*. Biophotonics and New Therapy Frontiers, 2006.

Haluszka D, Lorincz K, Banvolgyi A, Gyongyosi N, Kolonics A, Szipocs R, Karpati S, Wikonkal N: *The effect of repair mechanisms on risk of DNA damage during in vivo two-photon skin imaging*, J. OF INVESTIGATIVE DERMATOLOGY 133:(1) p. S219. (2013)

5

Atópiás bőrmodell – AF + CARS mérések

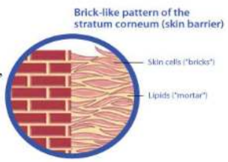
Semmelweis Egyetem Bőr-, Nemikórtani és Bőronkológiai Klinika — Wigner Szipőcs Group

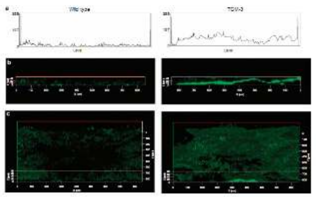


in vivo CARS

keratinociták – „tégla"

lipid – „habarcs"





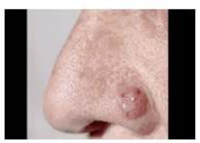
Bognar P, Nemeth I, Mayer B, Haluszka D, Wikonkal N, Ostorhazi E, John S, Paulsson M, Smyth N, Pasztoi M, Buzas EI, Szipocs R, Kolonics A, Temesvari E, Karpati S., *Reduced inflammatory threshold indicates skin barrier defect in transglutaminase 3 knockout mice*. J Invest Dermatol, **134**(1) 105-111 (2014)

17

Basaliomák vizsgálata

Semmelweis Egyetem Bőr-, Nemikórtani és Bőronkológiai Klinika — Wigner

- A hám bazális sejtrétegéből kiinduló rosszindulatú daganat, de áttétet nem képez
- Általában napfénynek kitett bőrfelületen alakul ki: arc, fül, nyak, vállak
- Több formája létezik
- Kezelés: sebészi excisió, 4-5mm biztonsági zónával
- Ennek ellenére gyakran recidívál
- A lézeres képalkotás megoldás lehet a pontos metszési sík meghatározásánál
- **Kollagén,** mint marker
- Ex vivo minták, műtét után



13

Basaliomák vizsgálata
nemlineáris mikroszkópia módszerekkel

Skin Research and Technology 2013; **19**: e297–e304
Printed in Singapore All rights reserved
doi: 10.1111/j.1600-0846.2012.00643.x

© 2012 John Wiley & Sons A/S
Skin Research and Technology

Diagnosis of BCC by multiphoton laser tomography

Stefania Seidenari[1], Federica Arginelli[1], Sara Bassoli[1], Jennifer Cautela[1], Anna Maria Cesinaro[2], Mario Guanti[1], Davide Guardoli[1], Cristina Magnoni[1], Marco Manfredini[1], Giovanni Ponti[1] and Karsten König[3,4]

[1]*Department of Dermatology, University of Modena and Reggio Emilia, Modena, Italy,*
[2]*Department of Pathology, University of Modena and Reggio Emilia, Modena, Italy,*
[3]*Department of Biophotonics and Lasertechnology, Saarland University, Saarbrücken, Germany and* [4]*JenLab GmbH, Schillerstrasse 1, 0745, Jena, Germany*

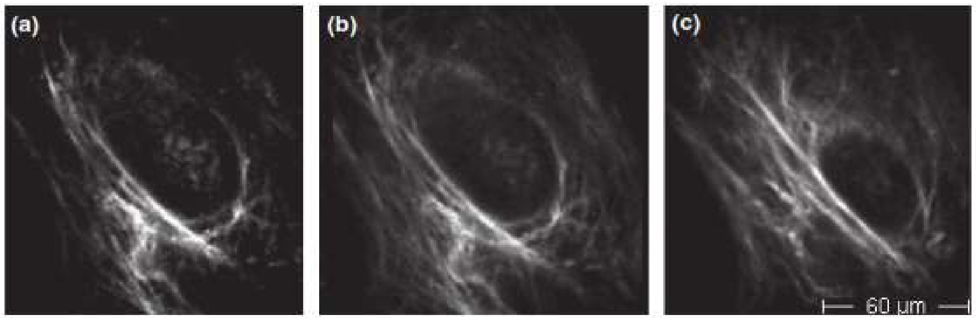
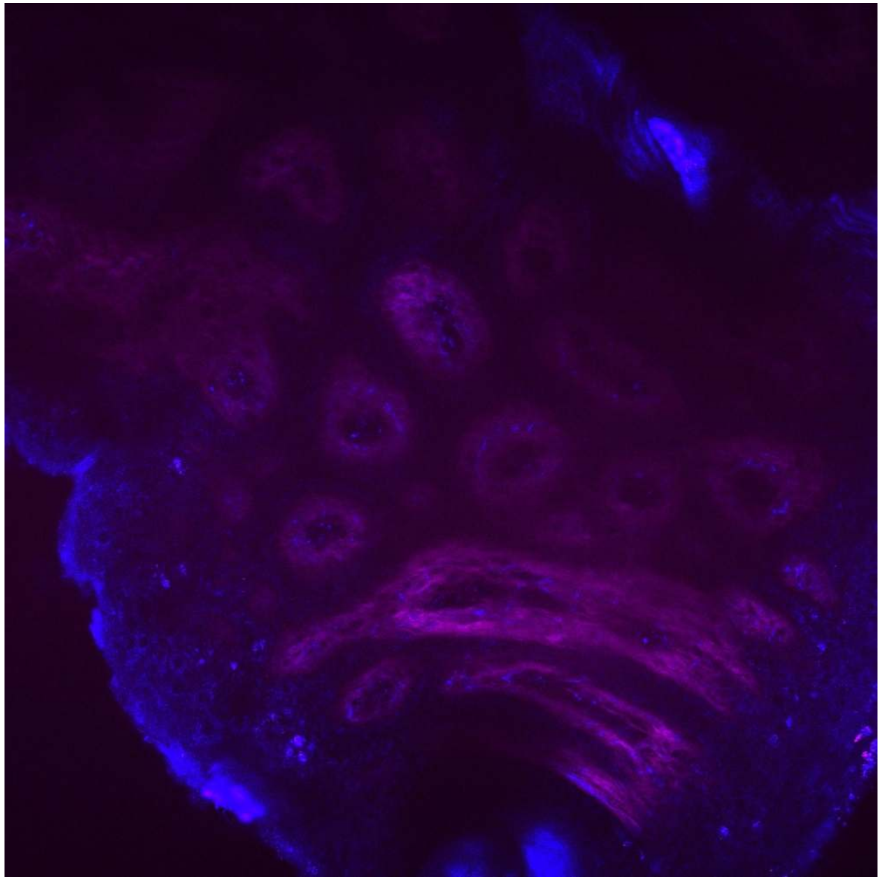


Fig. 4. Basal cell carcinoma. (a) 100 µm depth, excitation wavelength 800 nm. Shifting the wavelength to 800 nm, basaloid cells become less visible; employing an excitation wavelength of 820 nm basaloid cells disappear and it is possible to observe empty spaces surrounded by collagen fibres (phantom island); (b) 100 µm depth; (c) 120 µm depth.

Basalioma vizsgálata – 3D FiberScope fejlesztése



Kollagén szerkezet – SHG
Sejtek - autofluoreszcencia

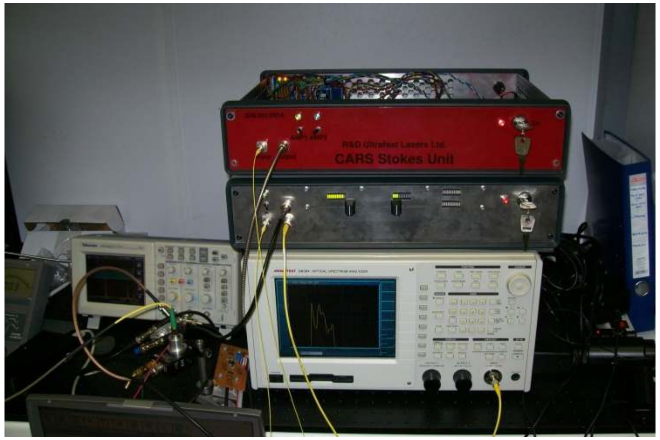
- Lézer hullámhossza
- Színszűrők kiválasztása

Szállézer specifikálása
Optikai szál specifikálása

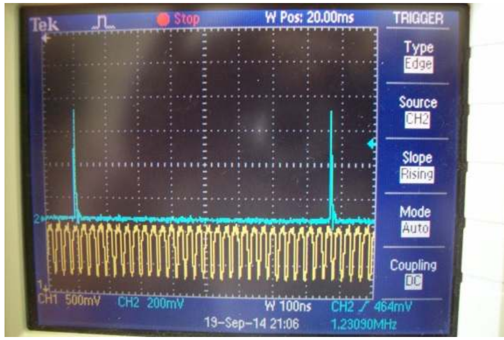
Biztonságtechnikai vizsgálatok

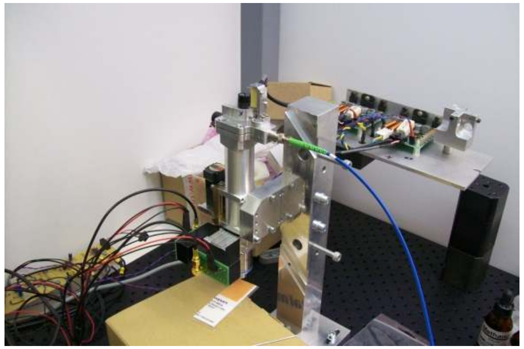
2P mikroszkópiás mérések, BCC vizsgálata

Szipőcs Group



FiberScope, a kézben tartott nemlineáris mikroszkóp





Lézeres fényforrás: 2-36 MHz-es ismétlési frekveniájú, impulzusüzemű Yb-szállézer, erősítő rendszer

Leképező optika: kisméretű pásztászó mikroszkóp

Mind a lézerforrás, mind a mikroszkóp optimalizált az adott orvosi diagnosztikai feladathoz: alacsony ár!

Biztonságtechnikai vizsgálatok

2P mikroszkópiás mérések, BCC vizsgálata

Szipőcs Group

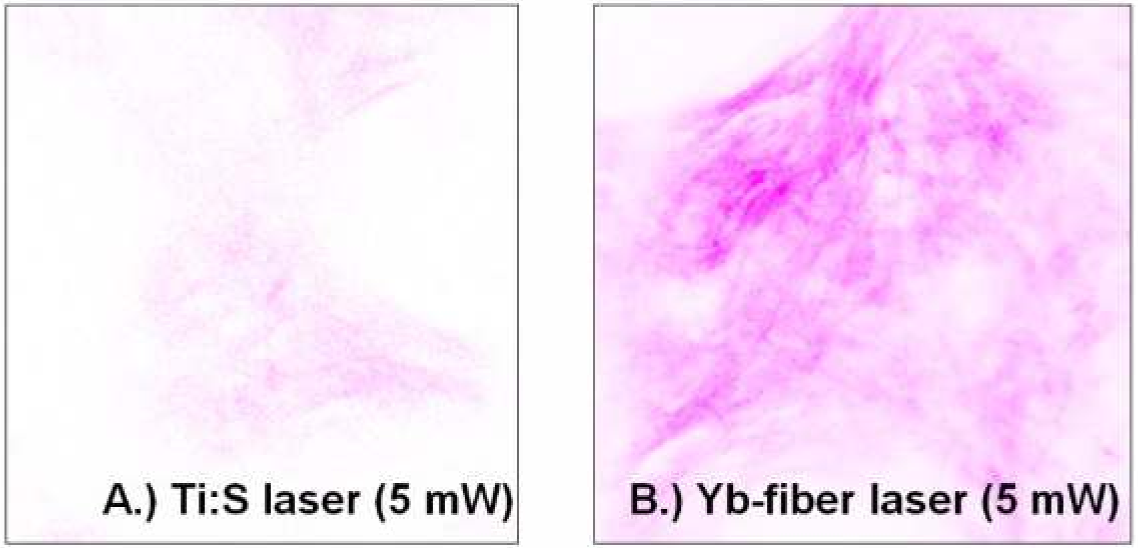


Figure 1. *Comparison of SHG imaging performance of different mode-locked lasers having the same average power of 5 mW (on the sample) for nonlinear microscopy. Ex-vivo murine skin sample, imaging depth: z = 30 µm, same microscope settings. Collagen distribution measured by A) an industry standard, 80 MHz Ti:sapphire laser, and by B) our newly developed Yb-fiber oscillator and amplifier system (with a variable repetition rate).*

2P mikroszkópiás mérések
Gyógyszeripai alkalmazások

Szipőcs Group

DOI: 10.1111/exd.12464
www.wileyonlinelibrary.com/journal/EXD

Letter to the Editor

In vivo study of targeted nanomedicine delivery into Langerhans cells by multiphoton laser scanning microscopy

Attila Kolonics[1,2], Zsolt Csiszovszki[3,5], Enikö R. Töke[3,5], Orsolya Lörincz[3,5], Dóra Haluszka[1,4] and Róbert Szipöcs[1,2]

[1]Institute for Solid State Physics and Optics of Wigner RCP, Budapest, Hungary; [2]R&D Ultrafast Lasers Ltd, Budapest, Hungary; [3]Genetic Immunity Kft, Budapest, Hungary; [4]Department of Dermatology, Venereology and Dermatooncology, Semmelweis University Hungary, Budapest, Hungary

Correspondence: Róbert Szipöcs, Institute for Solid State Physics and Optics of Wigner RCP, PO Box 49, H-1525 Budapest, Hungary, Tel./Fax: +36 1 3922582, e-mail: szipocs.robert@wigner.mta.hu

[5]*Present address: eMMUNITY Inc., 4400 East West Hwy, Bethesda, MD 20814, USA*

Abstract: Epidermal Langerhans cells (LCs) function as professional antigen-presenting cells of the skin. We investigated the LC-targeting properties of a special mannose–moiety-coated pathogen-like synthetic nanomedicine DermaVir (DV), which is capable to express antigens to induce immune responses and kill HIV-infected cells. Our aim was to use multiphoton laser microscopy (MLM) *in vivo* in order to visualize the uptake of Alexa-labelled DV (AF546-DV) by LCs. Knock-in mice expressing enhanced green fluorescent protein (eGFP) under the control of the langerin gene (CD207) were used to visualize LCs. After 1 h, AF546-DV penetrated the epidermis and entered the eGFP-LCs. The AF546-DV signal was equally distributed inside the LCs. After 9 h, we observed AF546-DV signal accumulation that occurred mainly at the cell body. We demonstrated in live animals that LCs picked up and accumulated the nanoparticles in the cell body.

Key words: eGFP-Langerin knock-in mice – *in vivo* – Langerhans cells – multiphoton laser microscopy – nanomedicine formulation

Accepted for publication 3 June 2014

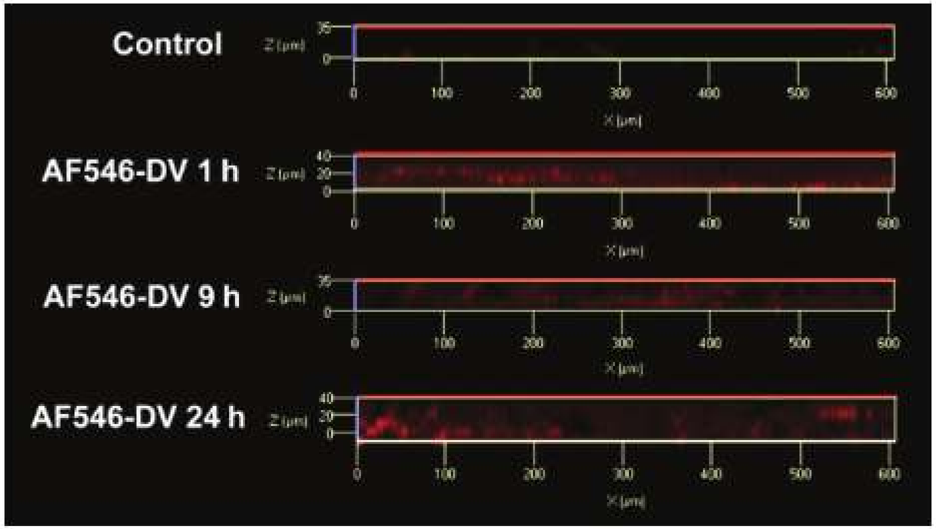


Figure 1. Penetration kinetics of AF546-DV through the stratum corneum in enhanced green fluorescent protein (eGFP)-Langerin knock-in mouse ear *in vivo*. *xz*-Multitracking sections were composed from a stack of *xy*-optical sections with 5 μm distances between the sections. The sections were recorded from the stratum corneum ($Z = 0$ μm) to the epidermis ($Z = 35$–40 μm). These representations reveal the penetration profiles of AF546-DV into eGFP-Langerin knock-in mouse skin reaching an average of 20 μm penetration depth underneath the honeycomb-shaped corneocyte layer after 1 h of topical treatment. AF546-DV diffused in the whole depth of the skin after 9 or 24 h despite of the fact that a part of the AF546-DV formula dried on the stratum corneum. Control: intact skin without AF546-DV.

2P mikroszkópiás mérések
Gyógyszeripai alkalmazások

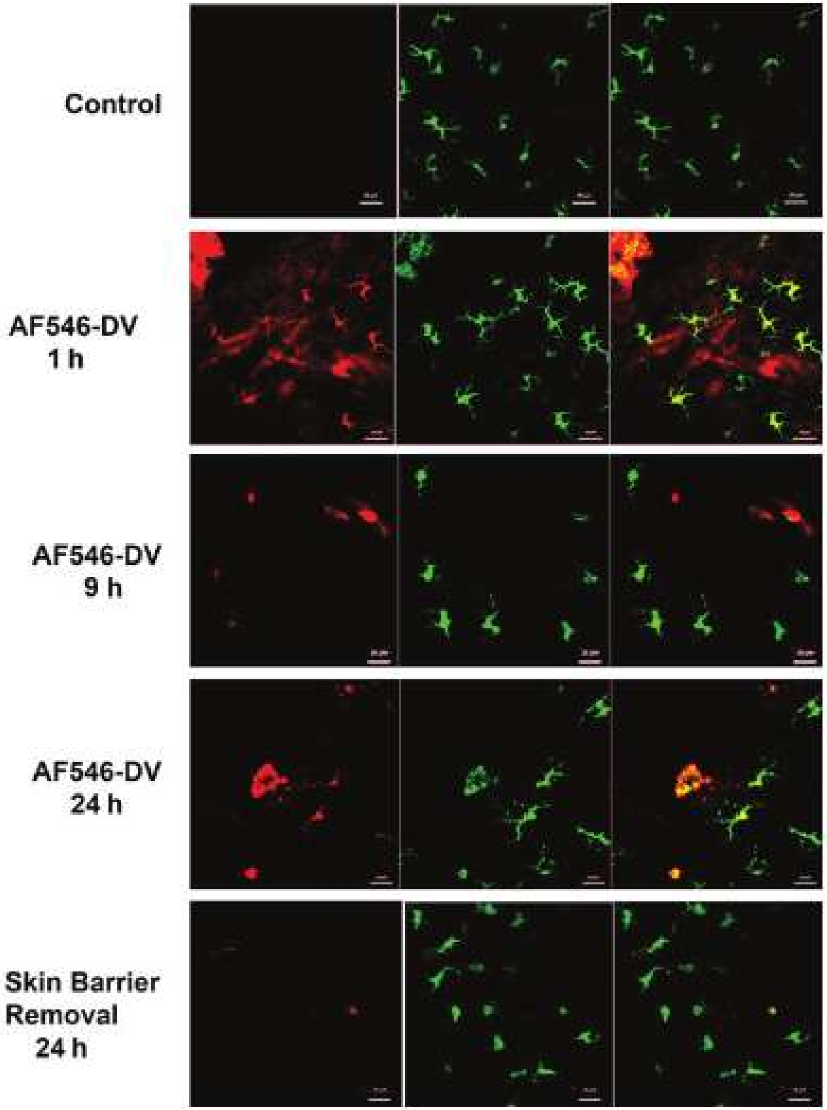
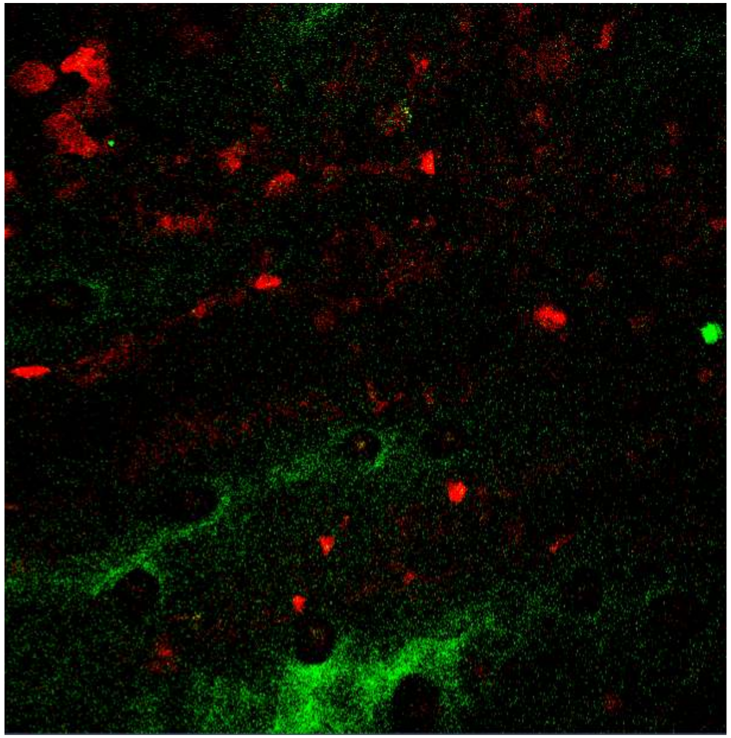
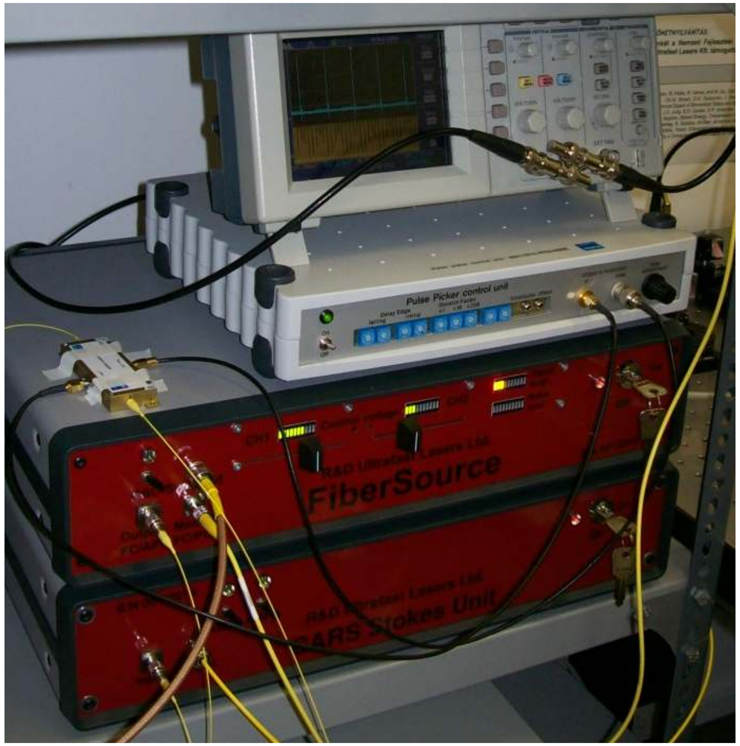


Figure 2. Kinetics of AF546-DV uptake by Langerhans cells (LCs) in eGFP-Langerin knock-in mouse ear *in vivo*. Nearly all LCs had incorporated AF546-DV after 1 h of topical treatment: strong colocalization was detected in both channels [NDD 2 – green/eGFP (middle column) versus NDD 1 – red/AF546-DV (left column)] as presented on the merged pictures (right column). Images of red light emission also revealed that the nanoparticles were distributed homogenously in all parts of the LCs. After 9 h, the intensity of red light emission by AF546-DV decreased significantly and disappeared from the dendrites and concentrated around the nucleus. Intriguingly, after 24 h, the nuclear location as well as a weak signal of AF546 in the dendrites could still be observed. The removal of the stratum corneum resulted in the activation of the vast majority of the LCs characterized by a rounded potato-like shape. The scale bar represents 20 μm.

FiberScope 2P/SHG mikroszkópiás mérések
Gyógyszeripai alkalmazások



Zöld: kollagén SHG jele
Piros: Alexa-546 jelölt nanomedicina (DermaVir)



A mérésekhez használt 2 MHz ismétlési
frekvenciájú Yb-szállézeres erősítő rendszer