

Femtosekundumos lézerek, nemlineáris mikroszkópiás módszerek alkalmazása az orvosi diagnosztikában és a gyógyszeriparban

Szipócs Róbert

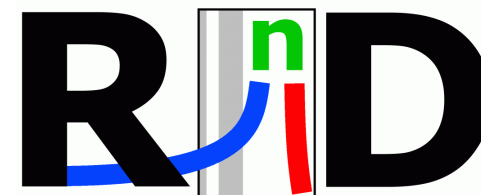
R&D Ultrafast Lasers Kft.

E-mail: r.szipocs@szipocs.com

www.szipocs.com

2014. november 10.





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

BOOTH NUMBER: 8109

R&D ULTRAFast LASERS LTD.



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)

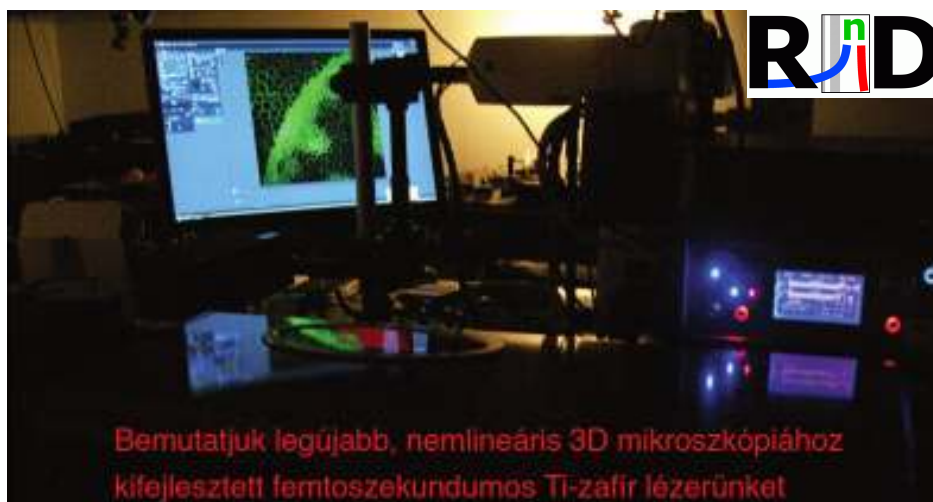
Infrastruktúra: 3 lézeroptikai laboratórium, 1 elektronikus és 1 gépészműhely, irodák, tárgyaló, raktár

Honlap: www.szipocs.com

Miről lesz szó?

1. Fotonika (lézerek + optika)
2. Orvosi diagnosztikai, gyógyszeripari alkalmazások (biológia)

Ezeket szokás együtt **BIOFOTONIKÁ**-nak is nevezni.



FemtoRose 100 TUN Compact/NoTouch (TM)

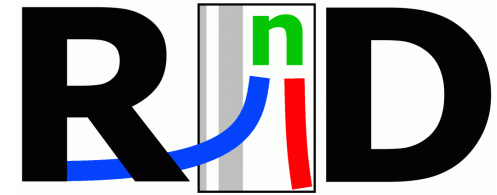
- hargolási tartomány 690–1050 nm
- szobahőmérsékletű ultraszelesávú csőpótló tükrök (UBCM) technológia
- beépített, mikroszkópból vezérelhető fényzár (shutter)
- számítógépről vagy mikroszkópból vezérelhető hűtőrendszer-beállítás
- teljesen automatizált „hands free” működés
- Carl Zeiss mikroszkóp (ZEN szoftver) kompatibilitás
- beépített pumpa lézer
- teljesen zárt dobozolás, környezeti hatásoktól mentes működés

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

Egyéb kapcsolódó termékeink, szolgáltatásaink:

- ionosán porlasztott, kis disperziójú vagy disperziókompenzáló tükrök
- komplett lézertáborok megépítése
- szaktanácsadás, konzultáció
- femtoszekundumos lézerek rendszerének szervizelése, karbantartása

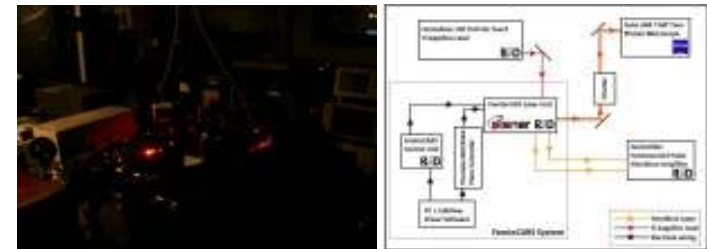




Miről lesz szó?

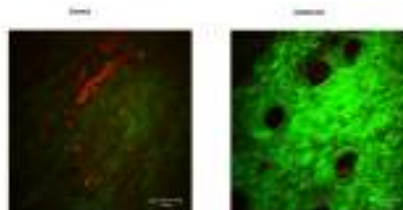
1. Fotonika (lézerek + optika)

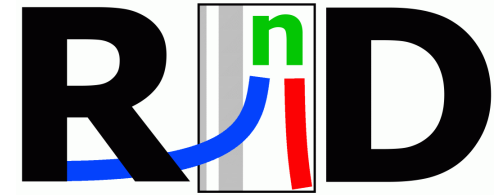
- ps-os, fs-os impulzusüzemű lézerek
- pásztázó nemlineáris mikroszkópia



2. Orvosi diagnosztikai, gyógyszeripari alkalmazások (biológia)

- bőrgyógyászat
- agykutatás
- gyógyszeripar





Miért fontos a Biofotonika, mióta foglalkozunk a szakterülettel?

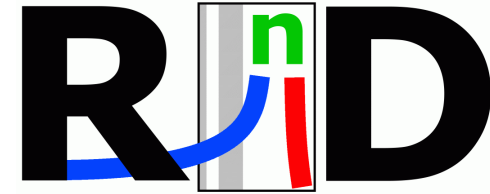


8 OCTOBER 2014



Scientific Background on the Nobel Prize in Chemistry 2014

SUPER-RESOLVED FLUORESCENCE MICROSCOPY



Miért fontos a Biofotonika, mióta foglalkozunk a szakterülettel?

Light microscopy trailblazers win Nobel Prize in Chemistry 2014

10/08/2014

Posted by Lee Dubay

Associate Editor, BioOptics World

Three light microscopy pioneers—Eric Betzig, Stefan W. Hell, and William E. Moerner—have been awarded the Nobel Prize in Chemistry 2014 for two separate achievements in breaking the optical diffraction limit, which enables better resolution ($>0.2 \mu\text{m}$) than half the wavelength of light. With the help of fluorescent molecules, the prizewinners were each able to circumvent this limitation.



SUBSCRIBE TO BIOOPTICS
WORLD

[VIEW MAGAZINE](#)

Miért fontos a Biofotonika, mióta foglalkozunk a szakterülettel?

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.

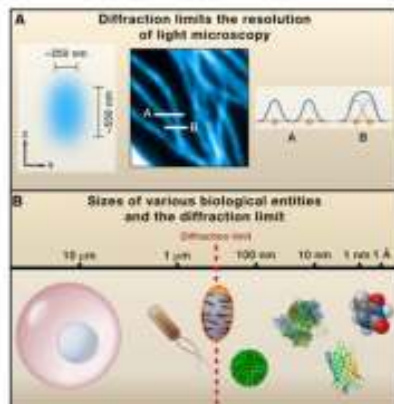
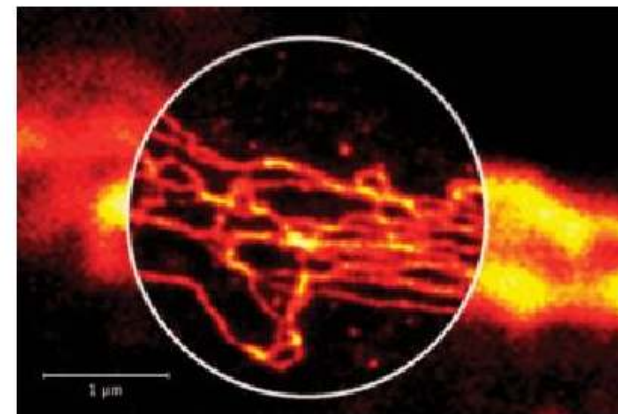
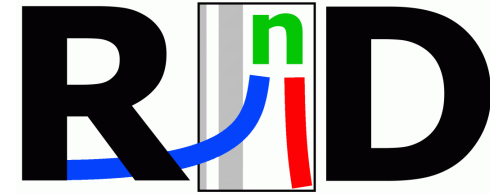


Figure 1: (Huang *et al.*, 2010, *Cell*, 143, 1047 – 57). **A:** (left) Focused laser beam, (middle) structure, (right) resolved (A) and not resolved (B) structural features. **B:** from left to right mammalian cell, *E. coli* cell, mitochondrion, influenza virus, ribosome, GFP, thymine.



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)



Miért fontos a Biofotonika, mióta foglalkozunk a szakterülettel?



ICON NEWSLETTER*

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 (École Normale Supérieure, 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. Martinez (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 Szipócs 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

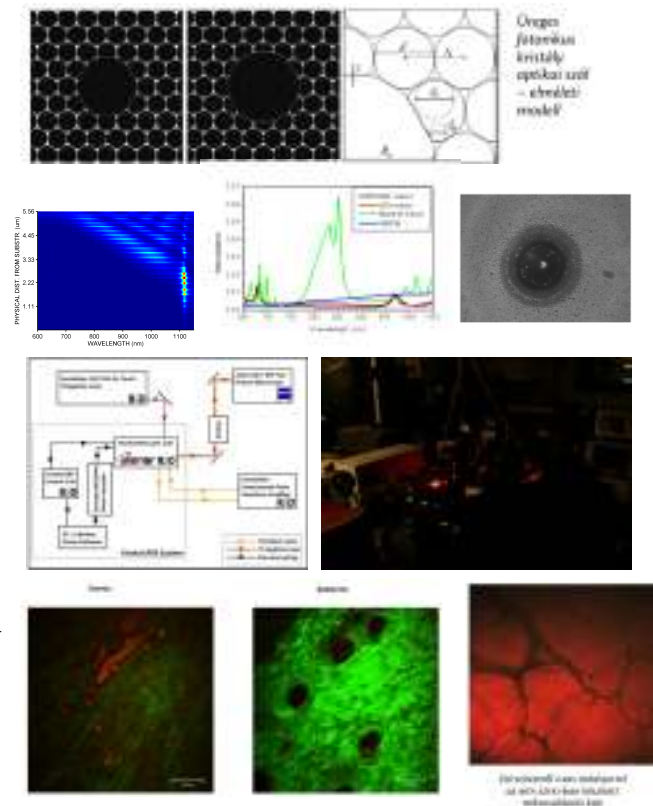
Femtosekundumos lézerfizika, száloptika és nemlineáris mikroszkópia kutatócsoport



Tudományos háttér

Fontosabb kutatási témák:

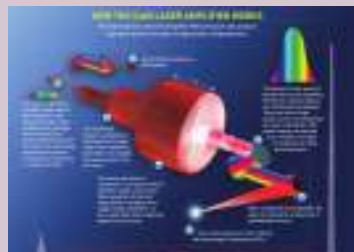
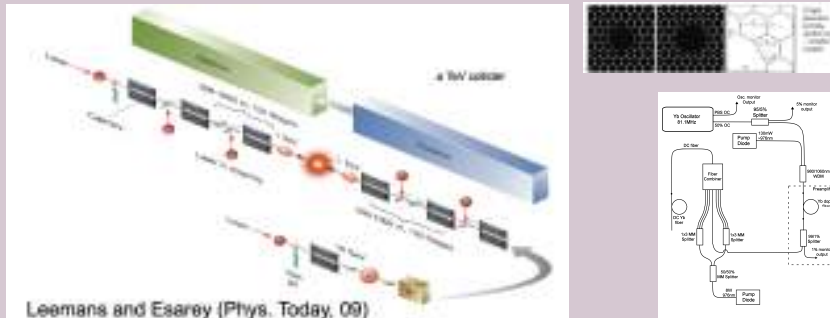
- *Fotonikus kristály optikai szálak elmélete, tervezése, gyártása, minősítése és alkalmazásai optikai szállézekben, erősítőkben és 3D mikroszkópiás orvosi diagnosztikai rendszerekben*
- *Diszperzív lézeroptikai bevonatok roncsolódásának elméleti és kísérleti vizsgálata az 50 ps-nál hosszabb és a femtosekundumos időtartományban*
- *Femtosekundumos szilárdtest- és száloptikai lézerek fejlesztése in vivo, nemlineáris 3D mikroszkópiás alkalmazásokhoz*
- *Két hullámhosszon szinkron működő femtosekundumos lézerrendszer CARS/SRS mikroszkópiához*
- *Nemlineáris mikroszkópia alkalmazásai a bőrgyógyászat, az idegtudományok és a gyógyszeripar területén*
- *Lézeres biztonságtechnikai vizsgálatok*



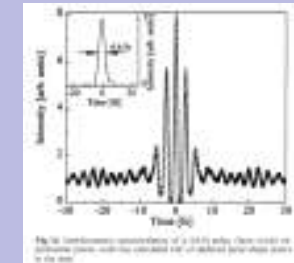
Az SZFI/ANO/femtosekundos lézer kutatócsoport KOMPETENCIÁK



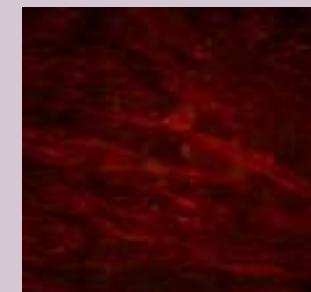
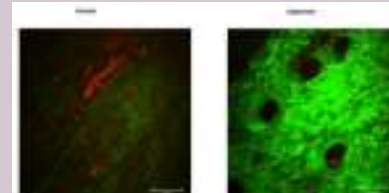
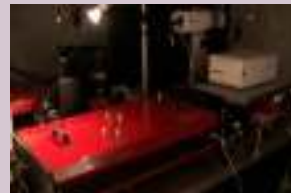
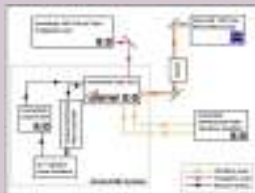
Részecskegyorsítás szállézerekkel (ICAN Projekt)



Femtosekundos szilárdtestlézerek és optika (ELI/Helios Projekt)



LifeScience@Wigner (pl. Nemzeti Agykutatósi Program (NAP))

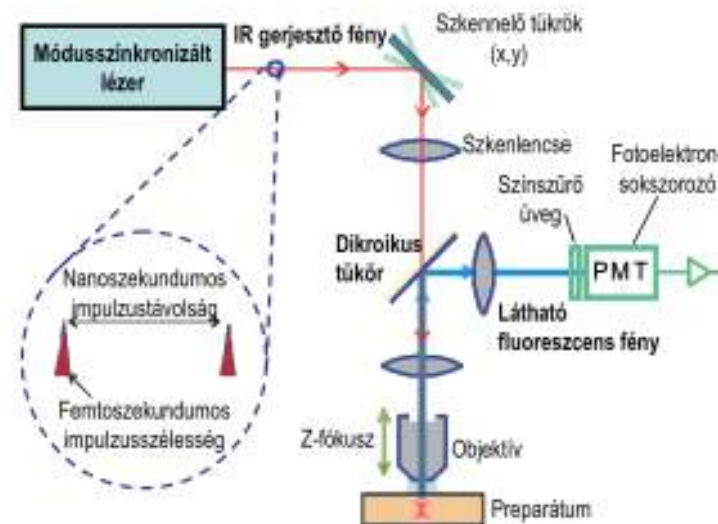


Fontosabb tudományos közlemények (2008-2014)

1. Várallyay Z, Saitoh K, Fekete J, Kakihara K, Koshiha M, Szepcs 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, Szepcs 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, Szepcs R: *All-fiber, all-normal dispersion ytterbium ring oscillator*, *LASER PHYSICS LETTERS* 6, 49-53 (2009)
4. Várallyay Z, Saitoh K, Szabó Á, Szepcs R: *Photonic bandgap fibers with resonant structures for tailoring the dispersion*, *OPTICS EXPRESS* 17, 11869-11883,(2009)
5. Antal P, Szepcs R: *Tunable, low-repetition-rate, cost-efficient femtosecond Ti:sapphire laser for nonlinear microscopy*, *APPL. PHYS. B107*, 17-22 (2012)
6. Antal P, Szepcs 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. Szepcs, S. Kárpáti, *Reduced Inflammatory Threshold Indicates Skin Barrier Defect in Transglutaminase 3 Knockout Mice*, *J. INVESTIGATIVE DERMATOLOGY* 134, 105-111 (2014)
8. Grósz T, Kovács AP, Kiss M, Szepocs 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, Tóke ER, Lőrincz O, Haluszka D, Szepcs 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, Szepcs 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, Szepcs 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)

Mi a pásztázó lézeres 3D fluoreszcencia mikroszkópia, milyen fontosabb típusai vannak?

- ▶ Konfokális mikroszkópia - fototoxicitás
- ▶ Nemlineáris mikroszkópia - nagyobb behatolási mélység
- kicsi gerjesztett térfogat



In vivo pásztázó lézeres nemlineáris 3D mikroszkópia

Nagy intenzitású, impulzusüzemű, többnyire hangolható gerjesztő fényforrás szükséges:

- ps-os és fs-os lézerek (tip. ~ 100 fs)
- ismétlési frekvencia: 1-100 MHz

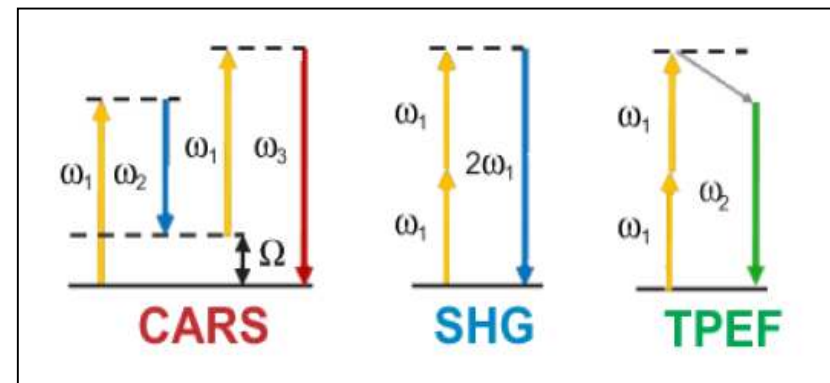
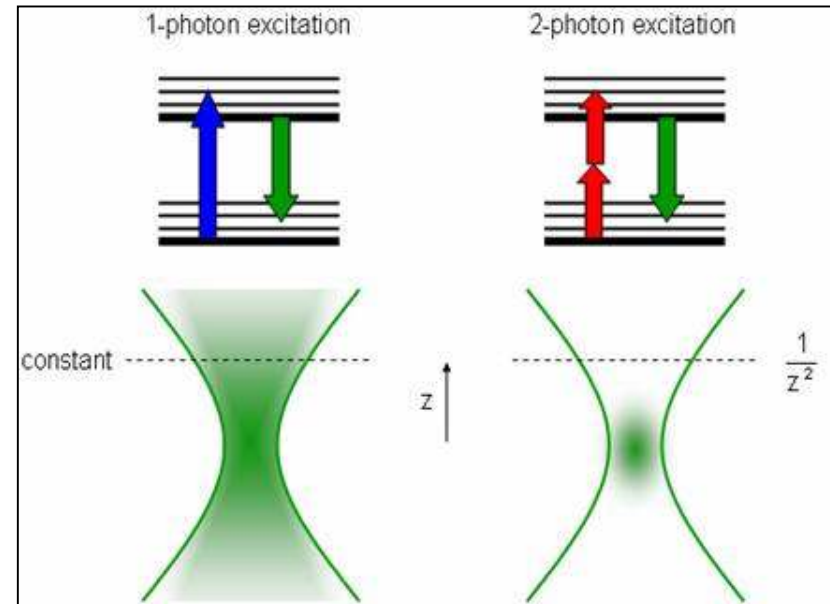
▶ nagy foton sűrűséget képes a fókuszfoltban előállítani, csak ott gerjeszti a mintát

Alkalmazott nemlineáris folyamatok:

- Másodharmonikus keltés (**SHG**) – pl. kollagén
- Két- (**2P**) vagy többfoton abszorpciós fluoreszcencia – pl. elasztin, keratin, melanin, NADH, FAD
- Koherens Anti-Stokes Raman szórás (**CARS**) – pl. lipidek CH₂ kötése

A fluoreszcens indikátorok egyfotonos gerjesztése többnyire 350-550 nm hullámhossz tartományba esik, ezért a kétfoton effektus alkalmazása esetén a lézer hullámhossza ennek kb. a kétszerese

▶ ~ 700-1100 nm tartományban hangolható gerjesztő lézer kell, pl. Ti-zafír (670 -1060 nm)

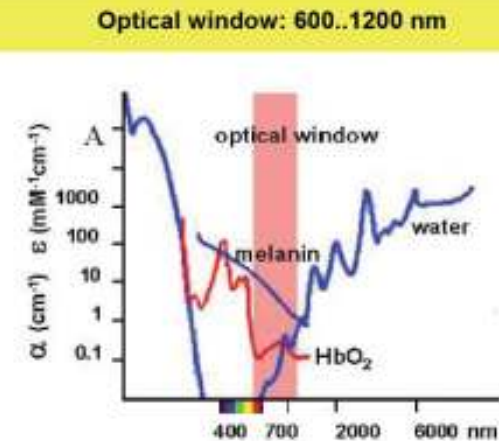


In vivo pásztázó lézeres nemlineáris 3D mikroszkópia előnyei

- Nem használ ionizáló sugárzást
- Nagy térbeli felbontás
- Cél az endogén kromofórok gerjesztése, a szövetek fiziológiai működésének vizsgálata
- Mélyebb penetráció – közel infravörös tartomány 700-1100 nm

Table 1. Endogenous skin chromophores.

Chromophore	Excitation λ_{ex} (nm)	Emission λ (nm)
Fluorescence and SHG		
Retinol (20)	700–830	450
NADH (17,97–100)	340; 690–730	450–470
Vitamin D (20)	< 700	450
Flavins (17)	370; 350; 700–730	430
Melanin (101)	280–450	440; 520; 575
Elastin (101)	300–340; 700–740	420–460
Collagen		
Fluorescence	300–340; 700–740	420–460
SHG (19,101)	720–960 (tunable range of TP laser)	360–480 ($\lambda_{ex}/2$)
CARS		Emission ω (cm^{-1})
C-H stretch	See (23,29)	2845 cm^{-1}
Sebaceous glands		2845 cm^{-1}
Adipocytes		2956 cm^{-1}



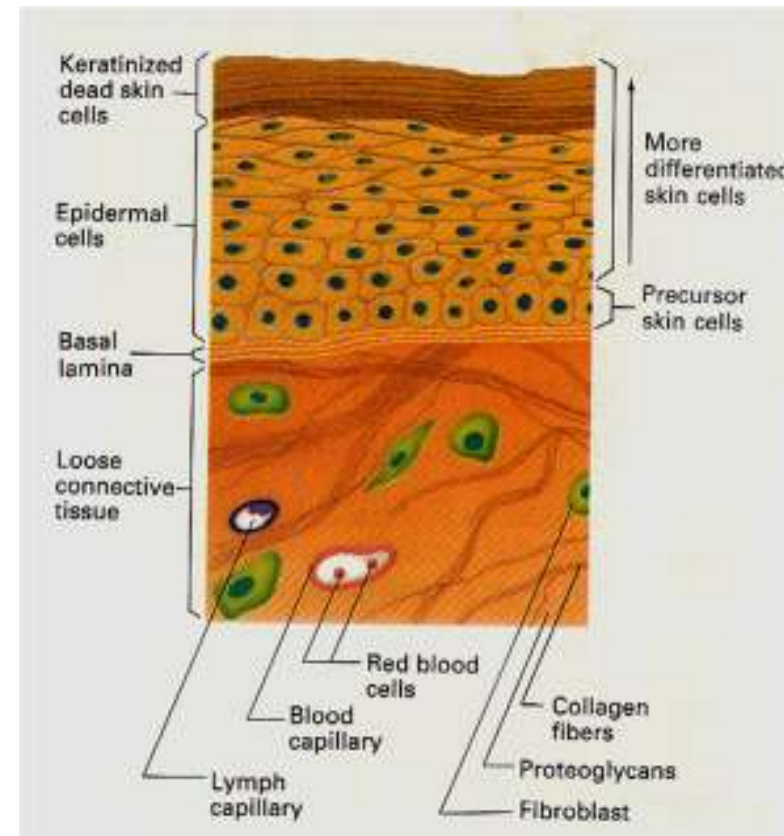
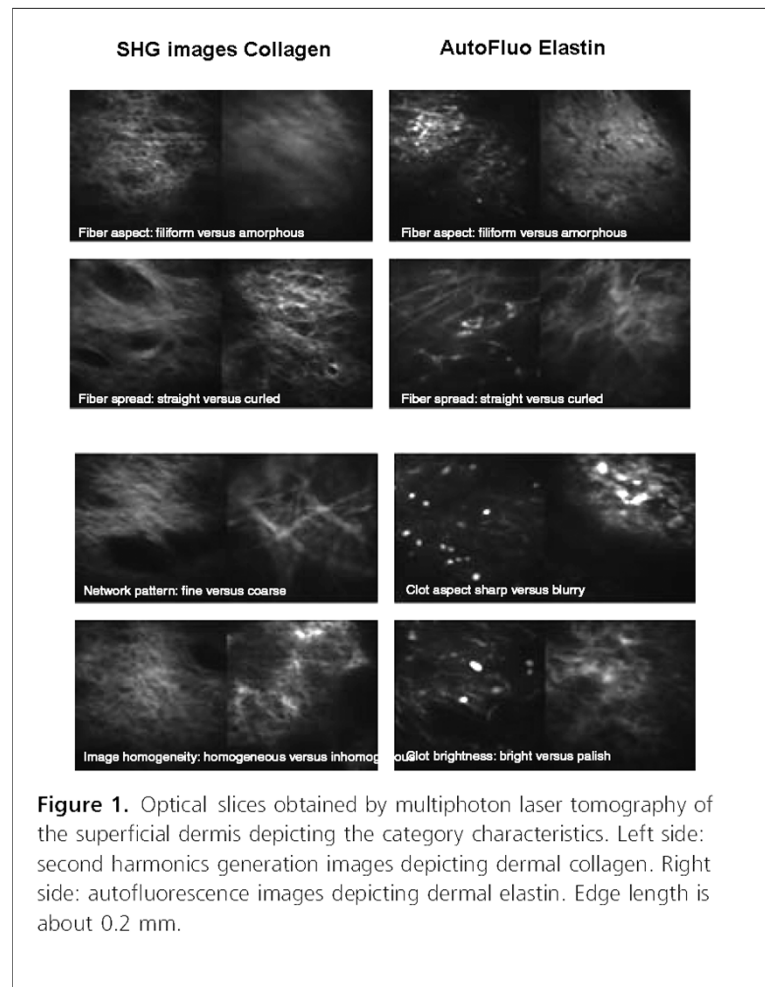
NIR: 700-1100 nm

CÉLUNK:

Olcso, fs-os (vagy ps-os) szállézeret vagy optikai szálba csatolt szilárdtestlézeret fényforrásként használó, *in vivo* diagnosztikai célra alkalmas, kisméretű képalkotó eszköz megalkotása



Természetes fluorofórok a bőrben: kollagén, elasztin, keratin



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

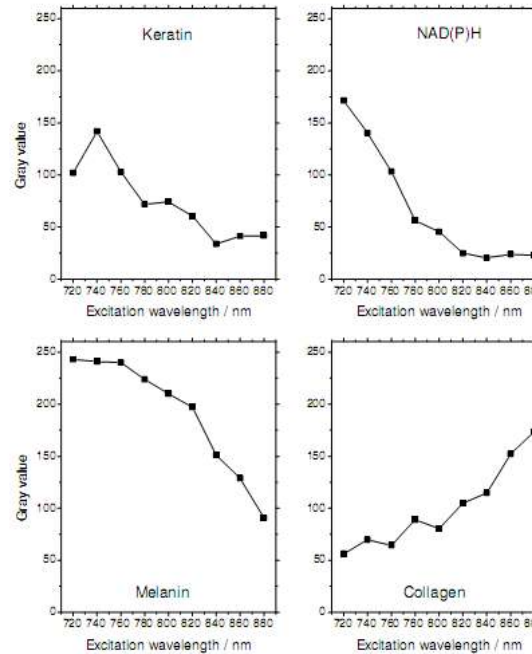


Fig. 8. Signal intensities of main fluorophores of human skin in dependence of excitation wavelength derived from Fig. 3 - Fig. 6.

Hangolható (femtosekundumos) lézer kell!

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



Nemlineáris mikroszkópia alkalmazása a bőrgyógyászatban

Haluszka Dóra, PhD hallgató

Semmelweis Egyetem Bőr-, Nemikórtani és Bőronkológiai Klinika,
1085 Budapest, Mária utca 41.
MTA Wigner Fizikai Kutatóközpont Szilárdtest-fizikai és Optikai Intézet,
1525 Budapest, Konkoly-Thege Miklós út 29-33.

Témavezetők: Prof. Dr. Wikonkál Norbert (SE)
Dr. Szipőcs Róbert (MTA Wigner FK SZFI)

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

A bőr szerkezeti képe – multifoton mikroszkópiával

str. cornutum: keratin (TPEF) str. spinosum: NADH (TPEF) str. basale: NADH (TPEF)
 dermis: elastin (TPEF) dermis: kollagén (SHG) subcutis: zsírszövet (CARS)

Biztonságttechnikai vizsgálatok

- Károsító hatások:** hő-, mechanikai és fotofizikai
- Fotokémiai károsító hatások megelőzése intracell. kromofórok két foton excitációja következtében alakul ki, sejtes rendszerekben ez a károsítás teljesen hasonló az UVB besugárzás által okozottakkal
- Cyclobutane pyrimidin dimerek:** közvetlen kereszthatások a DNS azonos láncán lévő szomszédos pirimidin bázisai között - NER rendszer javítja - ritágtalan javítás esetén - mutáció

In: Grigó E. B. and Balazs L., Early events in UV-induced genotoxic DNA damage: Superoxide and strand breaks. *Photochem Photobiol*, 2004, 80(2): 1-10.
 F. Ditzel, S. Vukobrat, S. Ruzsicsák, A. Györfi, M. Borkos, J. Kertész, K. Varga, S. Várkonyi. Mechanism of laser excitation in vivo: photochemical in vivo application. *Biophotonics and Laser Therapy*, Budapest, 2008.
 Hatalmazó, D., Lőrincz, R., Balazs, A., Szegedi, H., Borkos, J., Györfi, A., Balazs, L., Várkonyi, M. The effect of laser excitation on the UV-DNA damage during in vivo application: skin imaging. *J. EP. (WILEY)* DOI: 10.1111/j.1469-7580.2012.01571.x

Atópiás bőrmódel – AF + CARS mérések

in vivo CARS
 keratinociták – „tégla”
 lipid – „habarcs”

Brick-like pattern of the stratum corneum (skin barrier)

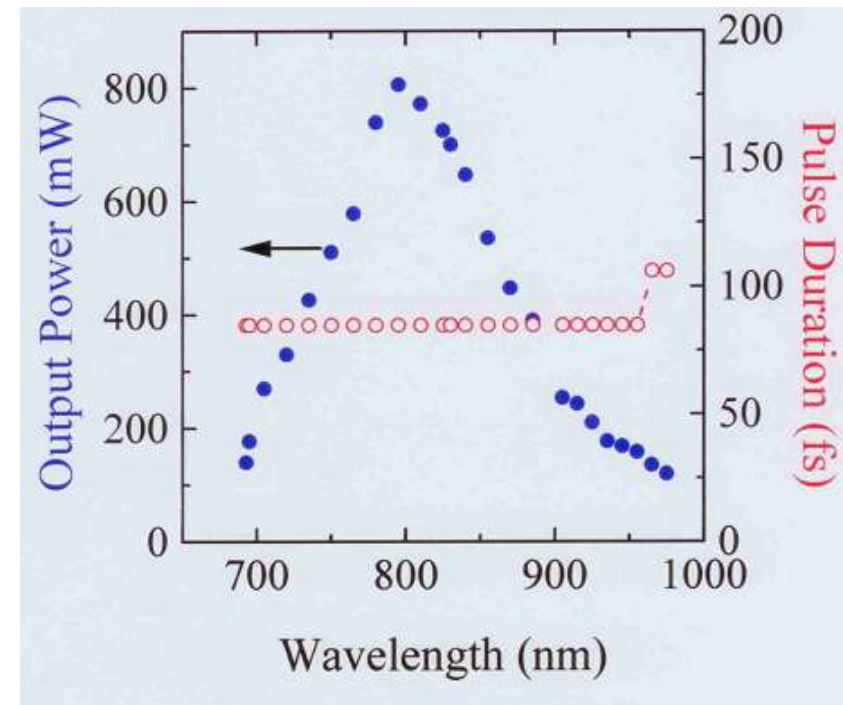
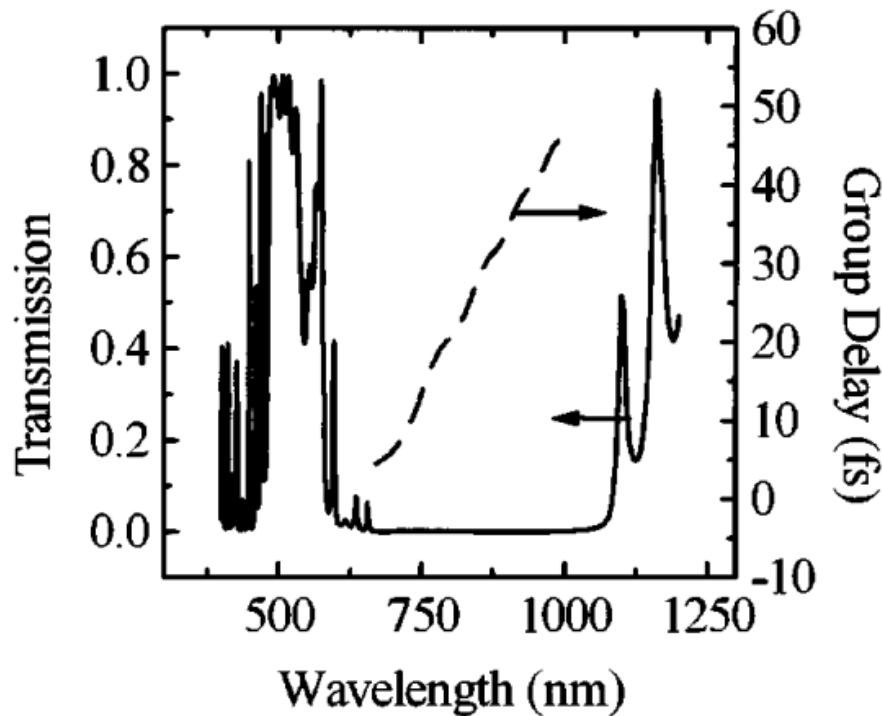
(Keratinocytes)
 (Lipid)

Basaliomák vizsgálata

- 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 excízió, 4-5mm biztonsági zónával
- Ennek ellenére gyakran recidívál
- A lézerek képzőképzés megoldás lehet a pontos metszési sík meghatározásánál
- Kollagén**, mint marker
- Ex vivo minták, műtét után

Bognár P., Nemethi I., Mayer B., Hatalmazó D., Wilczek N., Ostorházi E., Jónás S., Pálinkó M., Bognár N., Pálinkó M., Buzsák D., Szegedi H., Borkos A., Tamasovits E., Koppai S., *Reduced inflammatory threshold activates skin barrier defect in transglutaminase 3 knockout mice. J Invest Dermatol.* 124(1): 105-111 (2014)

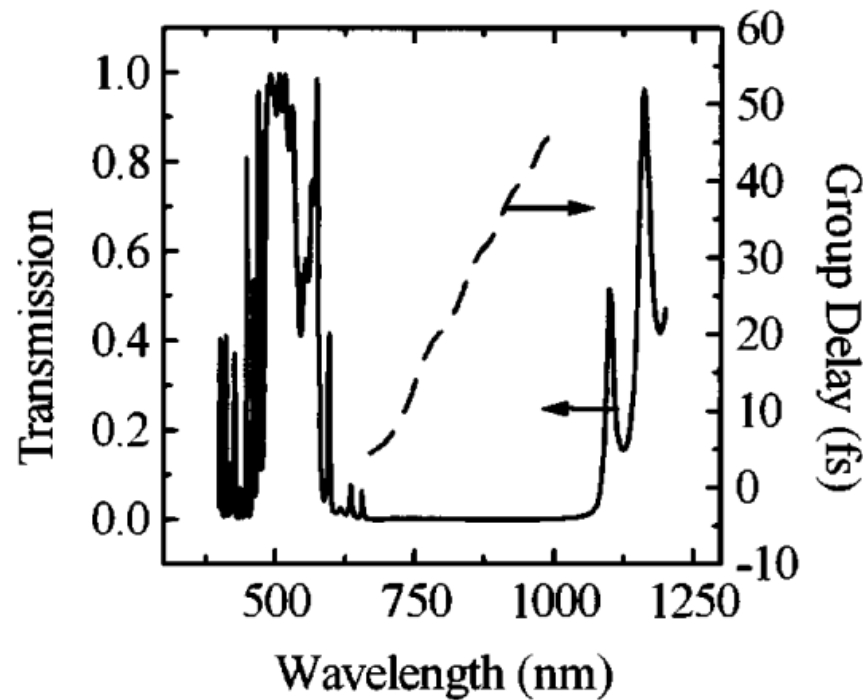
Széles sávban hangolható < 100 fs-os Ti-zafír lézer kifejlesztése



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

Széles sávban hangolható < 100 fs-os Ti-zafír lézer:
alkalmazások a nemlineáris mikroszkópiában

„Broadband Optics with I-track Extend the Reach of Multiphoton Microscopy”



Széles sávban hangolható < 100 fs-os Ti-zafír lézer FemtoRose 100 TUN Compact, NoTouch (10W pump)



NEW! Our latest version of femtosecond pulse Ti:sapphire laser developed for nonlinear 3D microscopy

- tuning range 660 to 1060 nm
- patented ultra-broadband chirped mirror (UBCM) technology
- internal shutter (can be operated directly by the microscope)
- wavelength setting by a computer or a microscope
- compatible with Carl Zeiss microscopes (ZEN software)
- internal pump laser
- fully closed housing, operation is independent of environmental conditions (e.g., humidity)

R&D Ultrafast Lasers Ltd - your partner in nonlinear 3D microscopy

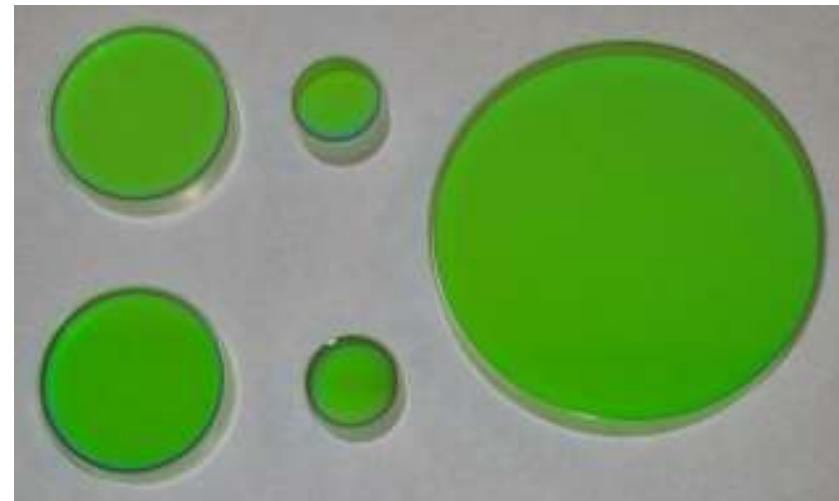
Other related products and services:

- ion beam sputtered, low dispersion or dispersion compensating mirrors
- building complete laser-optical laboratories
- consulting
- service for femtosecond pulse laser system



R&D Ultrafast Lasers Research and Development Ltd.
H-1012 Budapest, Attila st. 73.
R&D Campus: H-1021 Budapest,
Konkoly Thege str. 29-33, Building 6, Floor 1
Phone/Fax: +36 1 932 2392 | E-mail: r.andras@szjpcsi.com
WEB site: www.rslasers.com

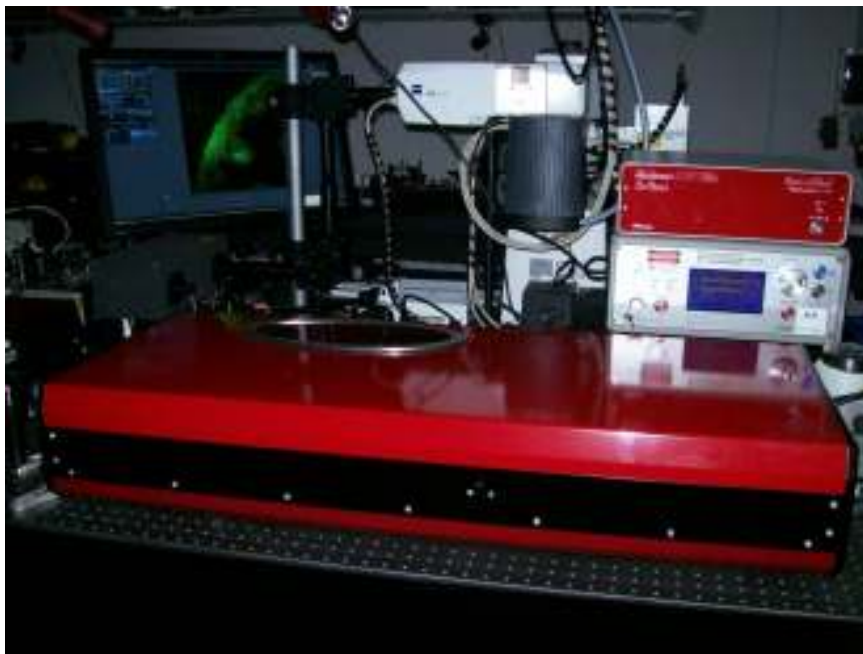
Ultraszélessávú, ionosan porlasztott csörpölt tükrök biztosítják a lézerben a széles sávban való hangolhatóságot



HR tartomány: 660 - 1060 nm

FemtoRose 100 TUN/NoTouch

the
Broadly Tunable, femtosecond pulse Ti:sapphire laser



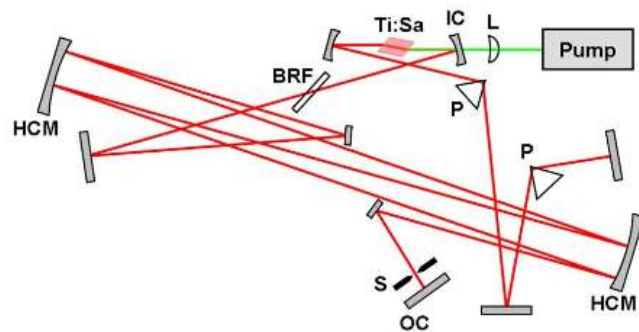
- Stable, easy mode-locking (with starter electronics)
- Soliton-like, nearly transform-limited pulses
- Patented Ultrabroadband Chirped Mirror™ optics
 - single optics set from 680 to 1040 nm
- Built in Millennia™ / Verdi™ / Finesse™ pumping (6W, 8W, 10W) – diode-pumped stability
- Sealed, purgeable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

**ÚJ FEJLESZTÉSI EREDMÉNYÜNK:
Hangolható, femtoszekundumos, hosszúrezonátoros Ti-zafír lézer**

FemtoRose 300 TUN/NoTouch

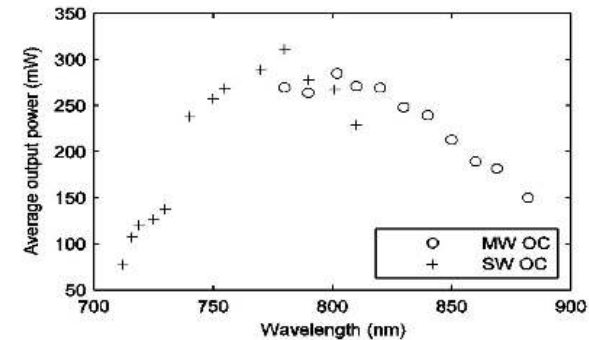
The Concept

Schematic of the oscillator



L: pump focusing lens, IC: input coupler mirror, Ti:Sa: titanium-sapphire crystal, BRF: birefringent filter for tuning, P: prisms, HCM: Herriott-cell mirrors, OC: output coupler, S: slit for hard-aperture KLM

Typical measured output power vs. wavelength (at 2.6 W pump)



Two different output couplers were used for short wavelengths (SW OC, crosses) and for longer wavelengths (MW OC, circles).

FemtoRose 300 TUN/NoTouch

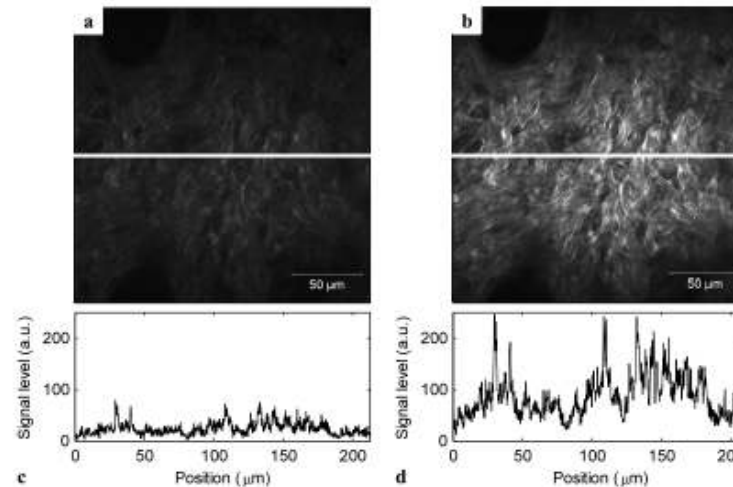
Low Average Power, High Quality Imaging in Two-Photon Microscopy

Előnyök:

- Olcsóbb pumpáló lézer (532 nm, max. 4 W) kell !
- Jobb képminőség (jel/zaj arány) ugyanannál az átlagteljesítménynél

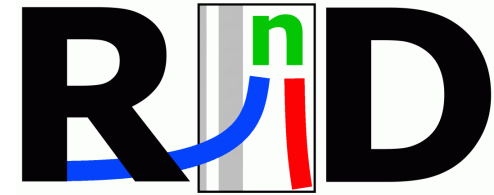
Tunable, low-repetition-rate, cost-efficient femtosecond Ti:sapphire laser for nonlinear microscopy

Fig. 6 Two-photon absorption fluorescence raw images of mouse dorsal skin using (a) the 76 MHz laser and (b) the 22 MHz laser, at nearly the same excitation power (3.081 mW for the 76 MHz laser and 3.015 mW for the 22 MHz laser). (c) and (d) show the corresponding intensity profiles along the white horizontal line in the middle of the images



BOOTH NUMBER: 8

R&D ULTRAFAST LASERS LTD.



FemtoRose 300 TUN/NoTouch

The Cost Efficient

Long-Cavity, Broadly Tunable, femtosecond pulse Ti:sapphire laser



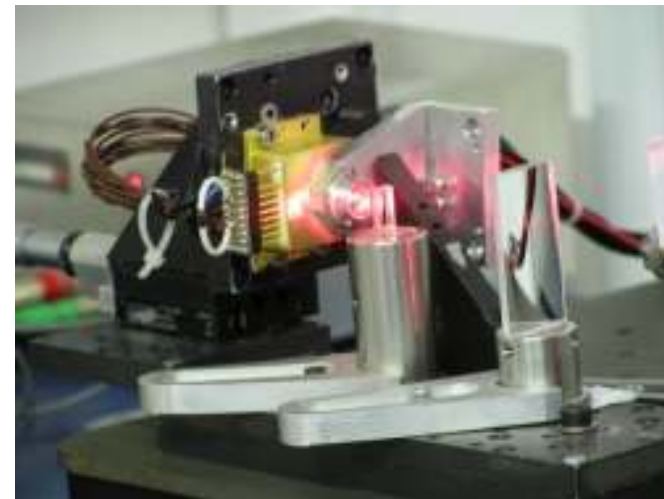
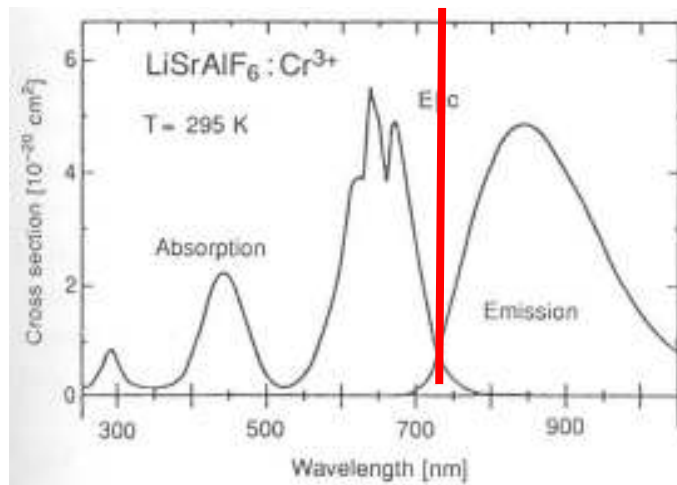
Key features

- Low pump laser cost (~ 2.6 W pumping)
- Low, 22 MHz repetition rate
- Higher fluorescence signal
- Lower thermal damage in sample
- No extra-cavity chirp control is required
- Wavelength control by a Zeiss 2P microscope

Applications

- Multiphoton microscopy
- Ultrafast spectroscopy

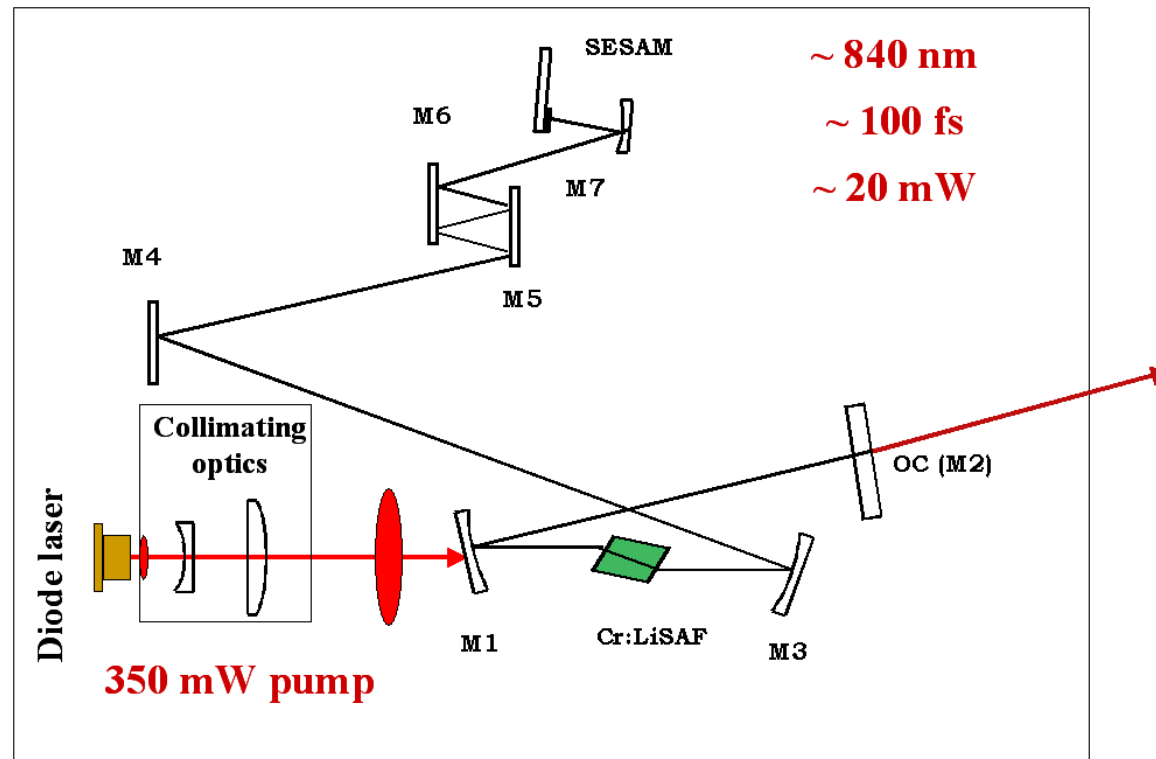
További árcsökkentési lehetőség:
Diódapumpált, tükrökompenzált fs-os Cr:LISAF lézer



- Optikai pumpálás 670 nm-es lézerdiódával, $P = 350$ mW teljesítménnyel (!)
- 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

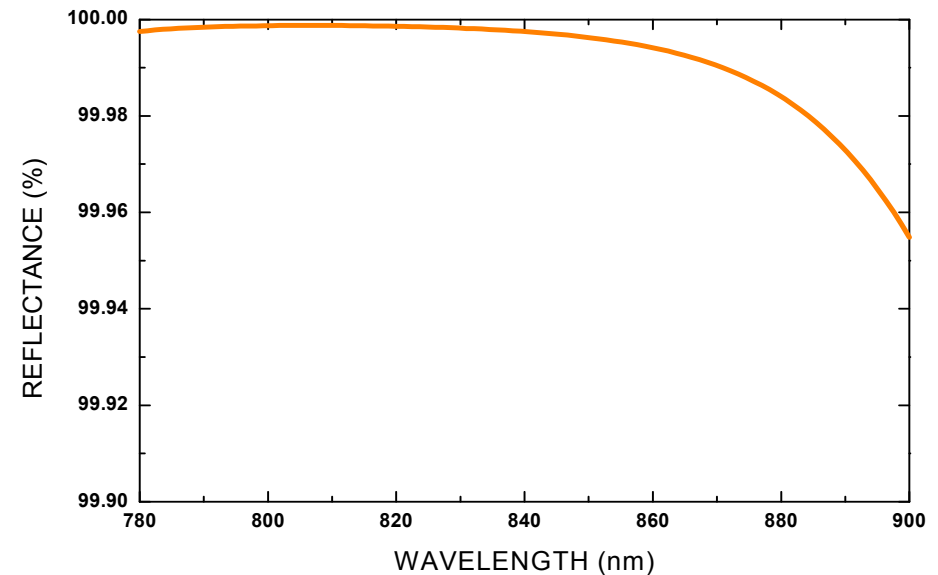
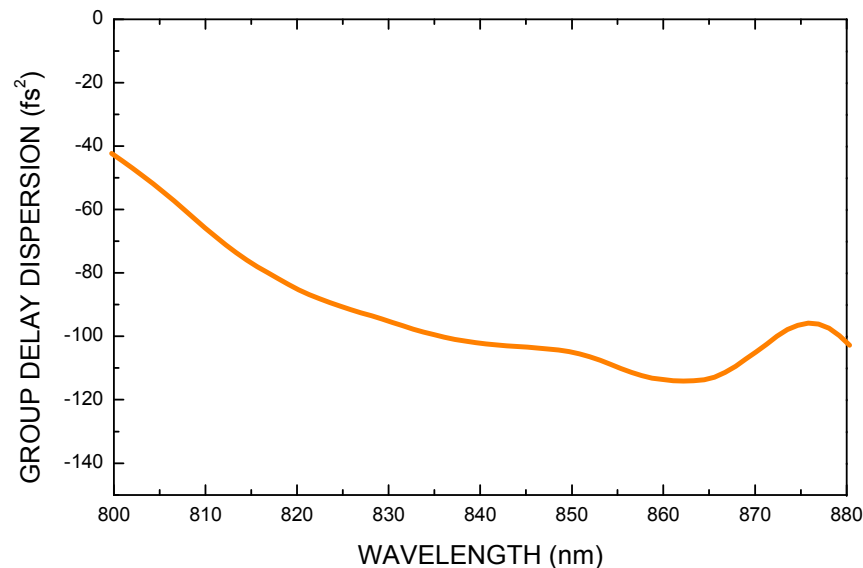
A diódapumpált Cr:LiSAF lézer felépítése



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

Diódapumpált, tükrökompenzált fs-os Cr:LISAF lézer

Diszperzió kompenzálás: extrém kis veszteségű, ionosan porlasztott MCGTI tükrökkel



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

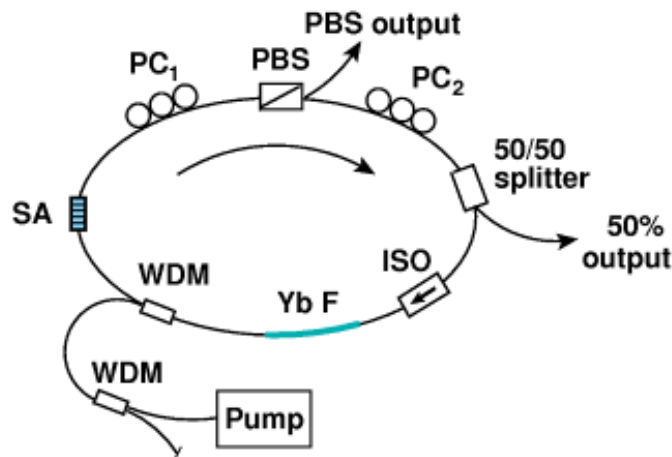
Szálintegrált, femtoszekundumos AND Yb-szállézer



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

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

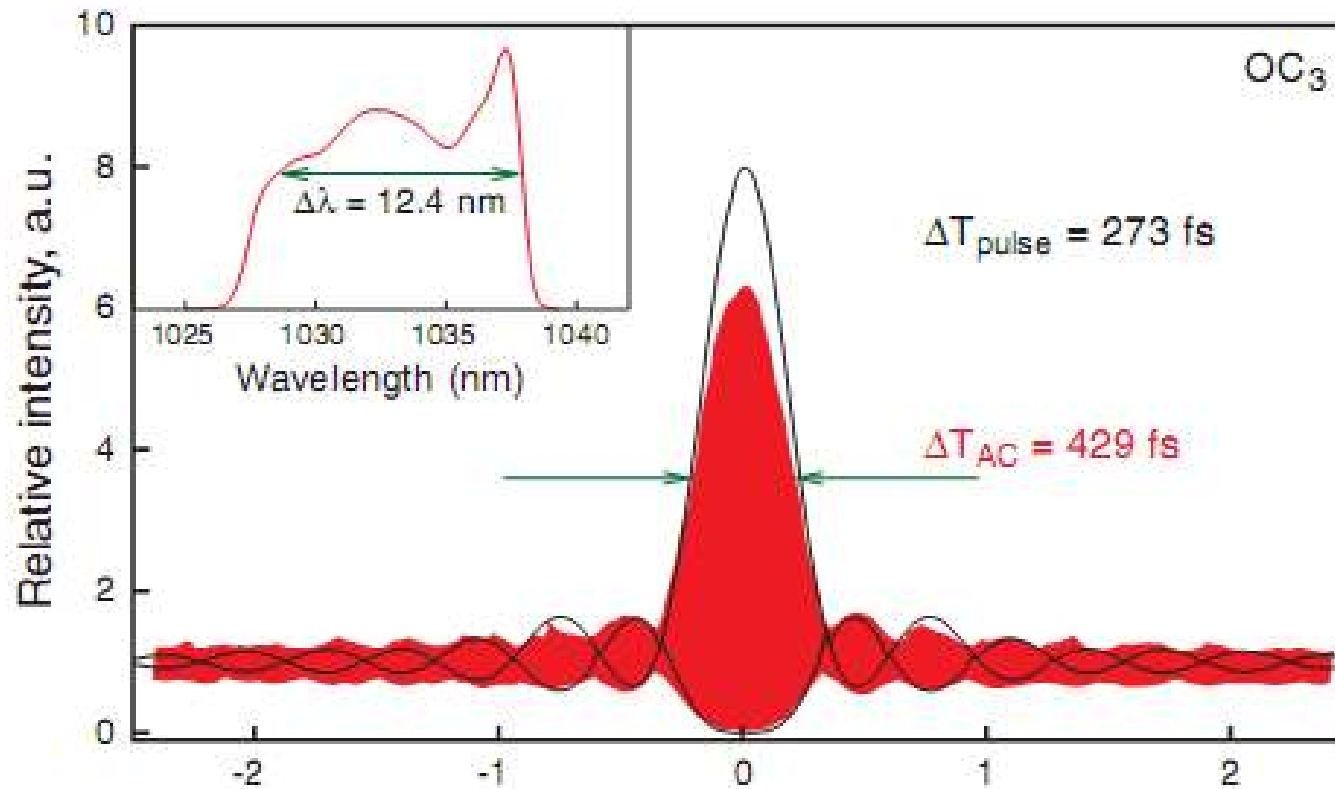
WDM: wavelength division multiplexer

SA: saturable absorber

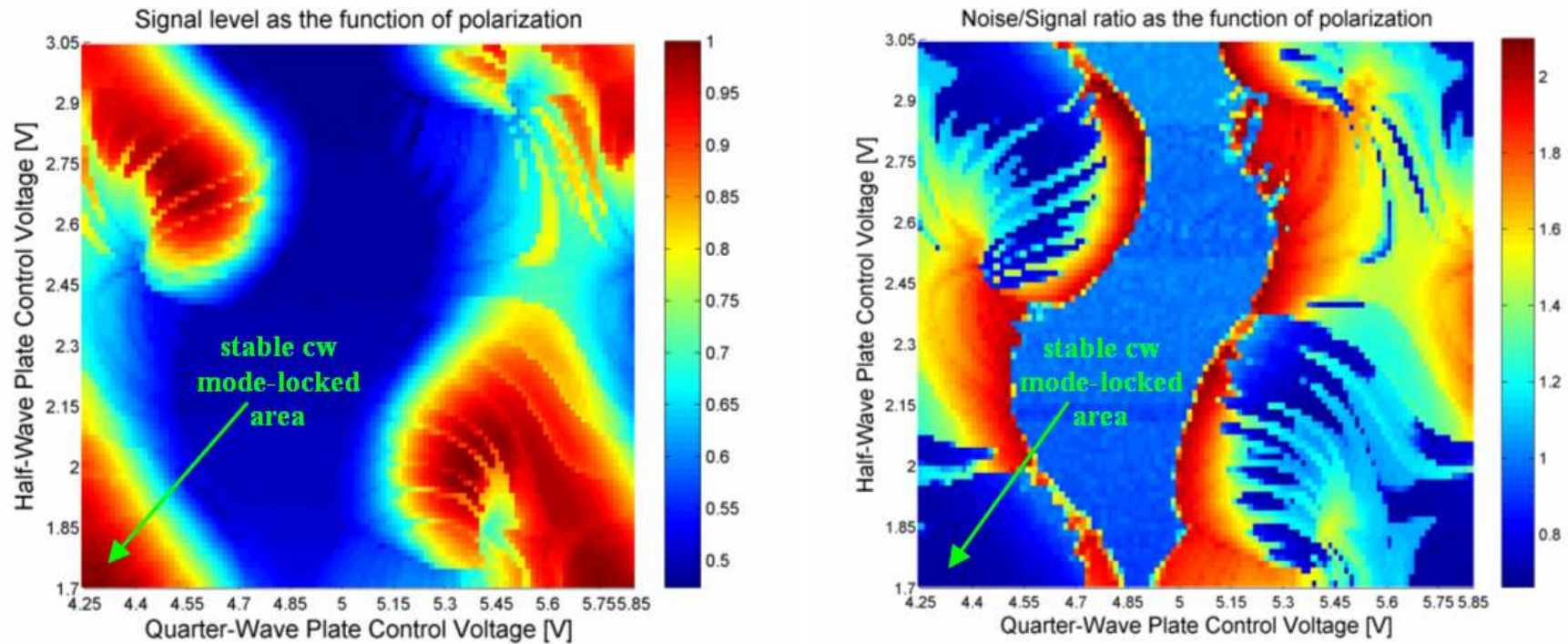
J. Fekete, A. Cserteg, Szipőcs; All-fiber, all-normal dispersion ytterbium ring oscillator, *Laser Physics Letters* 6, 49-53, 2009

Szálintegrált, femtoszekundumos AND Yb-szállézer

Mért jellemzők: spektrális sáv szélesség és impulzushossz

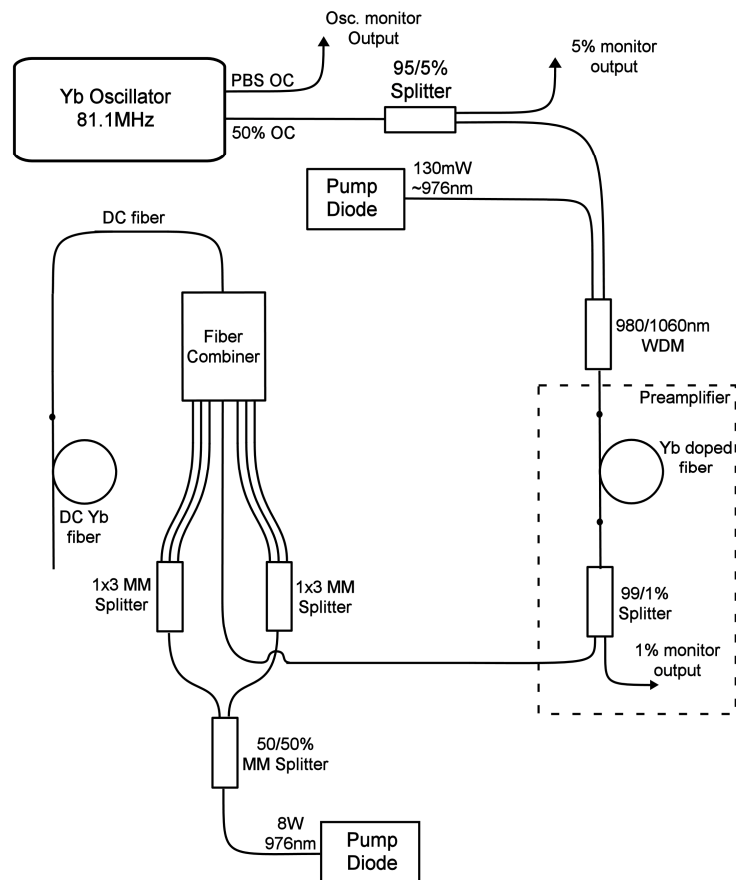


Recording of „stability maps” using SLH circuits

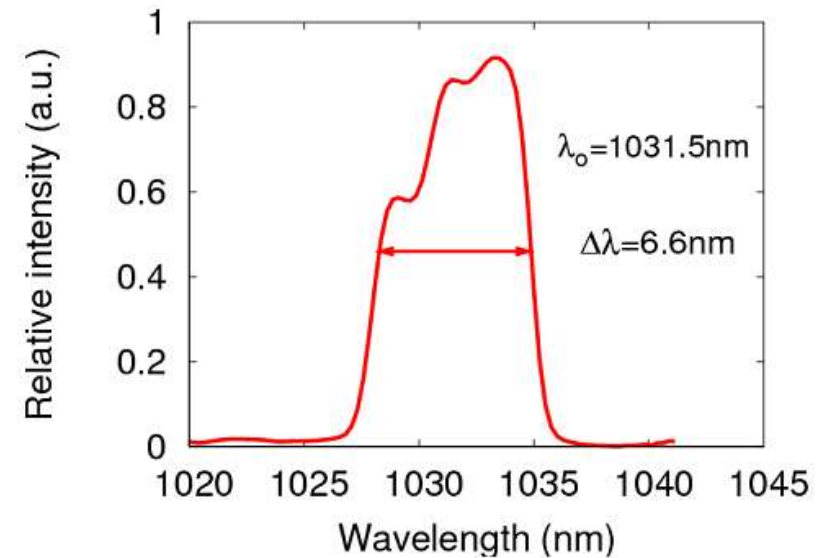


Measured signal power (left) and normalized noise power (right) as the function of control voltage on the polarization controllers.

Yb szálerősítő felépítése



Mért bemeneti spektrum



$P_{\text{osc}} \sim 2\text{-}5\text{ mW}$

$P_{\text{preamp}} \sim 40\text{-}60\text{ mW}$

$P_{\text{amp}} \sim 600\text{ - }900\text{ mW}$

$\tau \sim 300\text{-}400\text{ fs (kompresszált)}$

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

FemtoCARS

The concept

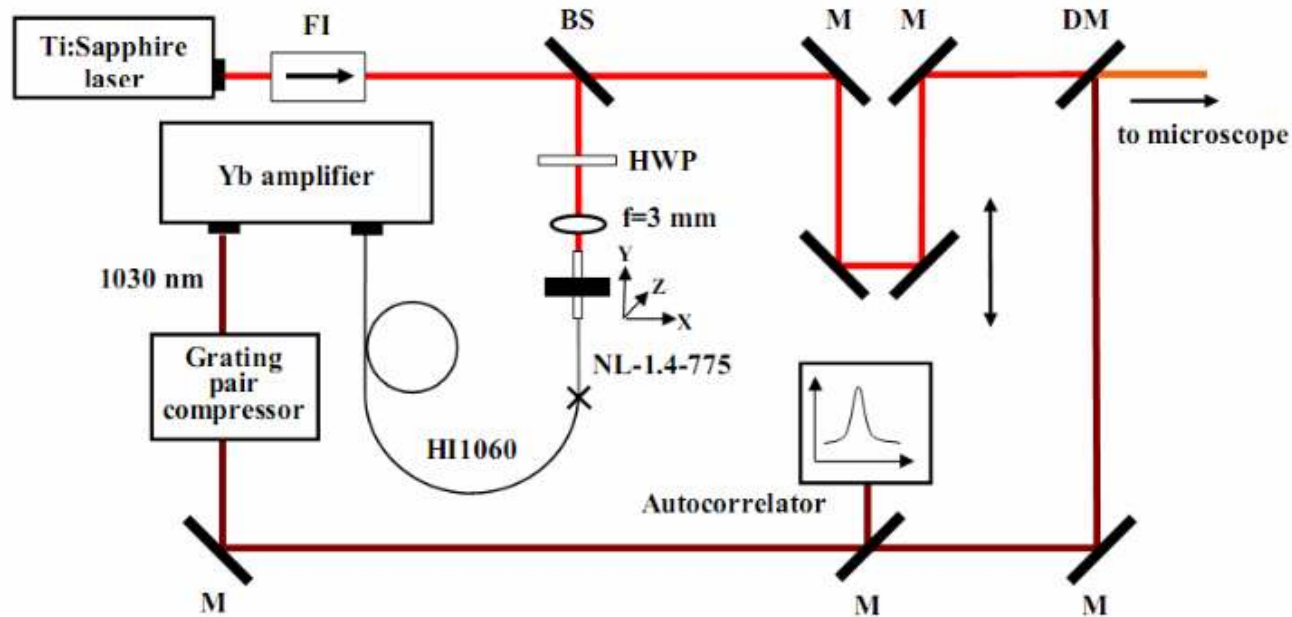


Fig. 1 Setup of the CARS extension unit

Kolonics A, Csáti D, Antal P, Szipócs 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)

FemtoCARS

The concept

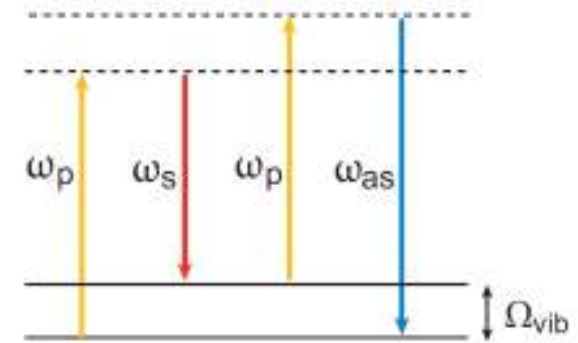
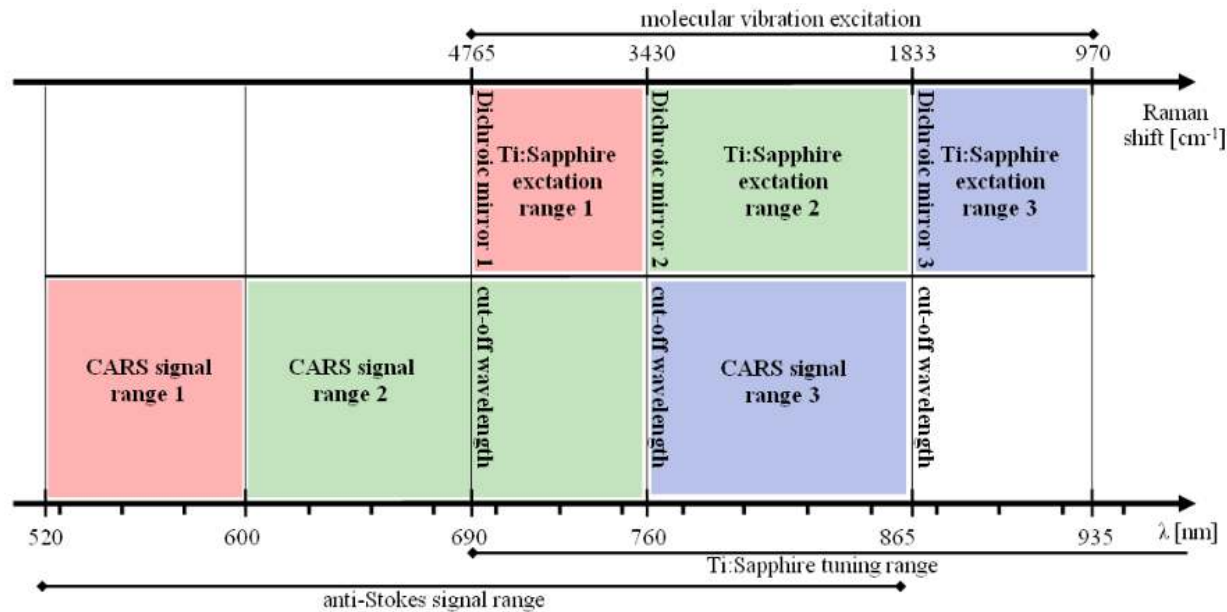
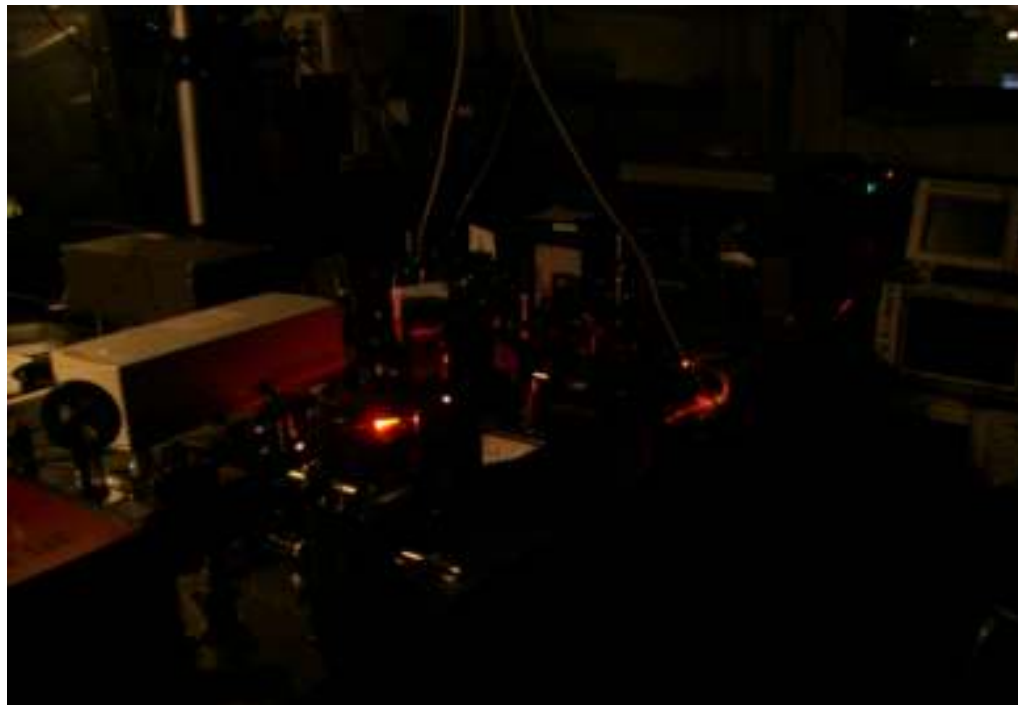


Fig. 1 The energy diagram of the CARS process: ω_p – the frequency of Ti-sapphire laser (pump), ω_s – the frequency of Yb-amplifier (Stokes), ω_{as} – the frequency of light generated during the CARS process, Ω_{vib} – vibration frequency of the investigated molecule

Kolonics A, Csáti D, Antal P, Szipócs 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)

FemtoCARS

Prototype at Wigner RCP

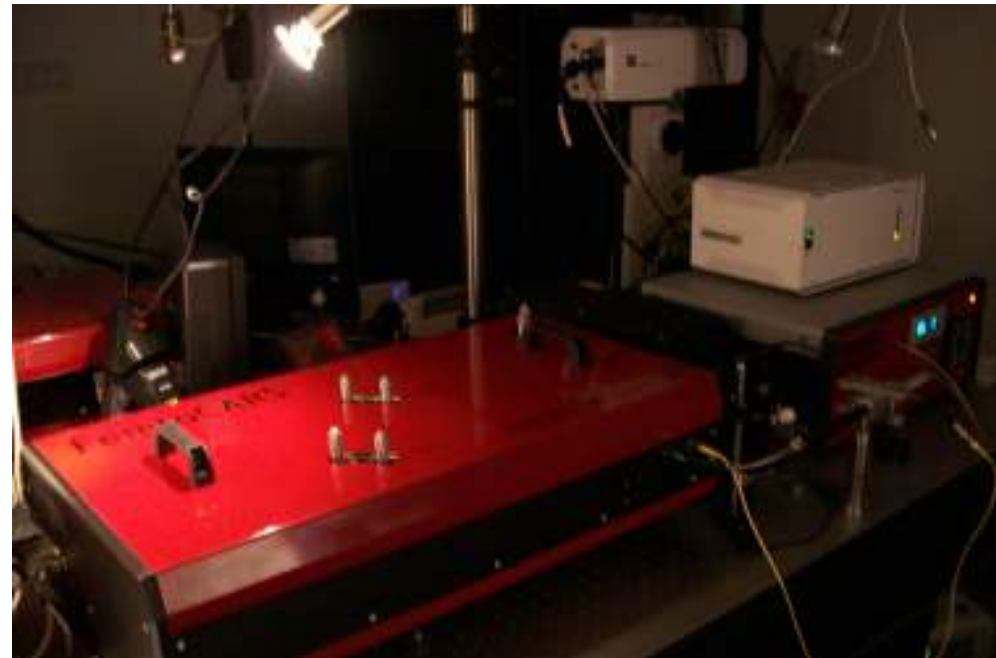
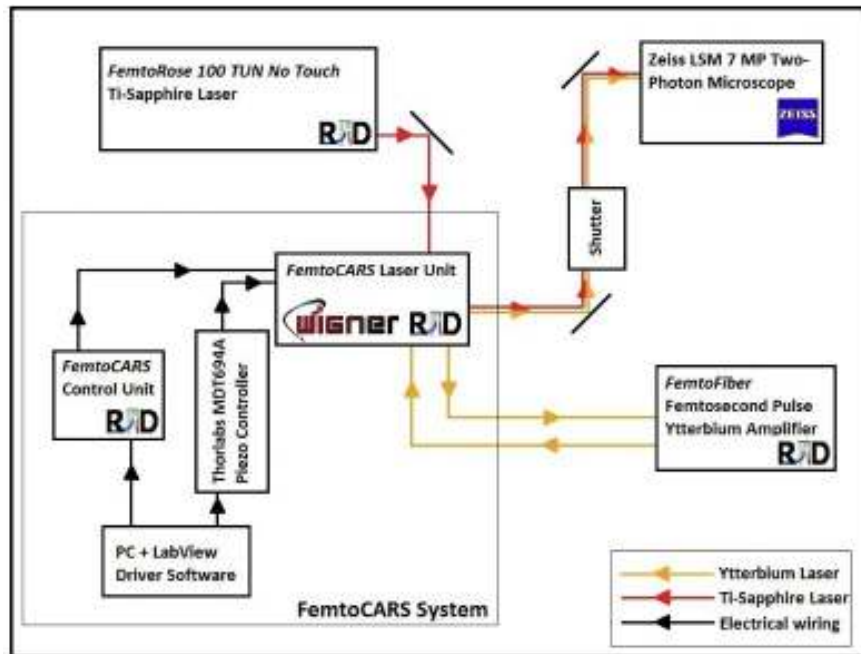


Kolonics A, Csáti D, Antal P, Szipócs R; A simple, cost efficient fiber amplifier wavelength extension unit for broadly tunable, femtosecond pulse Ti-sapphire lasers for CARS microscopy; In: Proc. BIOMED Biomedical Optics and Digital Holography and Three Dimensional Imaging (Miami, Florida, United States,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

FemtoCARS

the

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



FemtoCARS

the

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

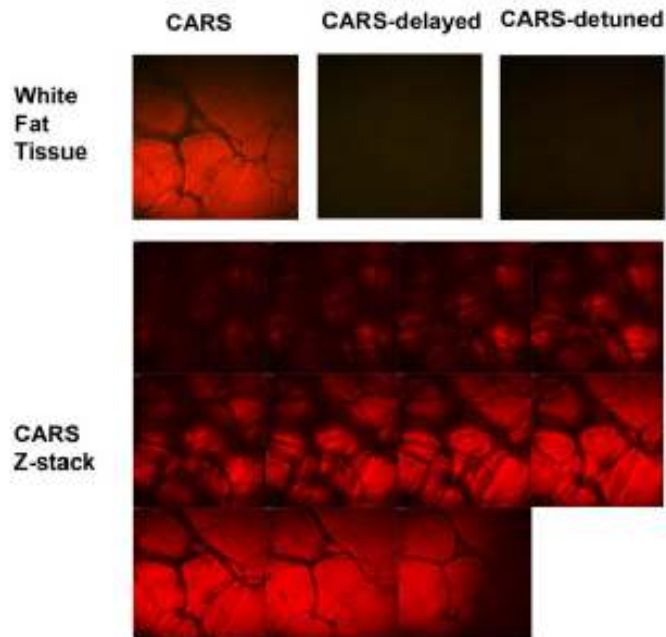
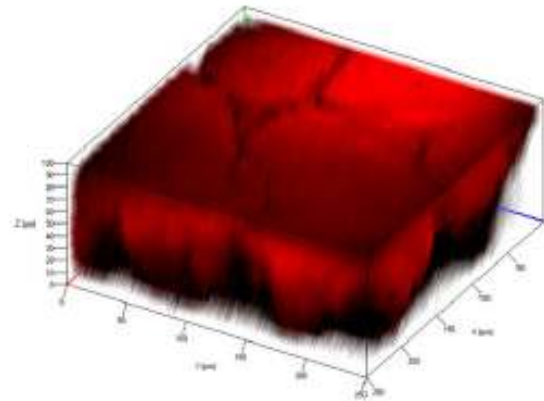


Fig. 3 CARS-images of murine white adipose tissue.



Kolonics A, Csáti D, Antal P, Szipócs 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)

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

Dermis

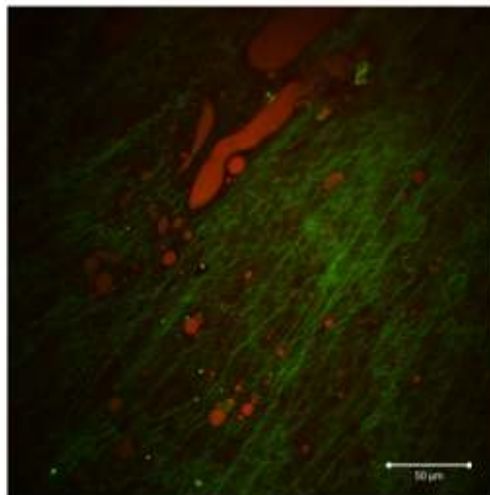


Photo: Kolonics/Szipöcs

Epidermis

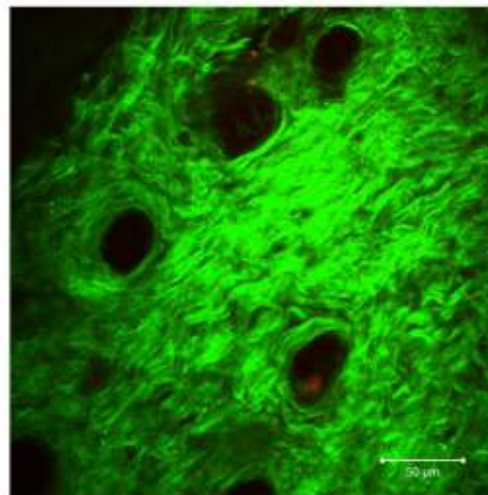
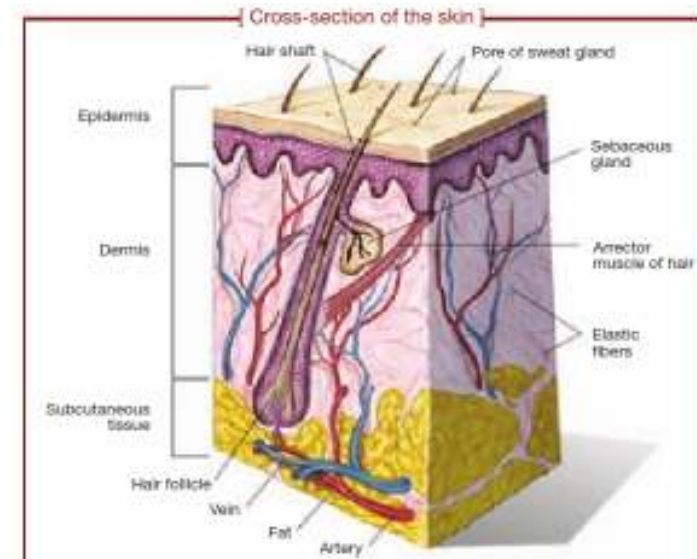


Photo: Kolonics/Szipöcs



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

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 (Kolonic, 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 (Szipócs 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 CH₂ 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.

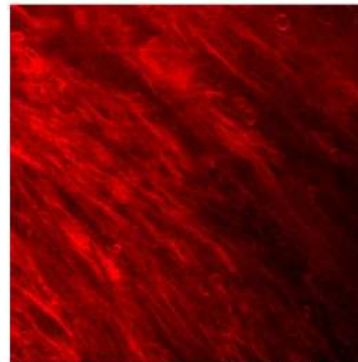
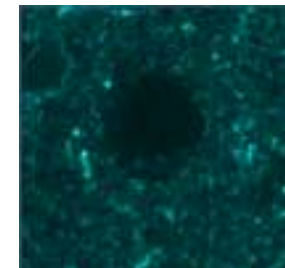


Figure 1. CARS image of myelin fibers in the white matter of rat. In vivo preparation.



NATIONAL INSTITUTES OF HEALTH
NIH-PA Author Manuscript

NIH Public Access
Author Manuscript
Lab Invest. Author manuscript; available in PMC 2013 April 10.

Published in final edited form as:
Lab Invest. 2012 October ; 92(10): 1492–1502. doi:10.1038/labinvest.2012.109.

Multicolored Stain-free Histopathology with Coherent Raman Imaging

Christian W. Freudiger¹, Rolf Pfanni², Daniel A. Orringer^{3,4,5}, Brian G. Saar^{1,T}, Minbiao Ji⁶, Qing Zeng^{6,7}, Linda Ottoboni⁸, Wei Ying⁹, Christian Waeber⁹, John R. Sims⁹, Philip L. De Jager^{8,10,11}, Oren Sagher⁵, Martin A. Philbert¹², Xiaoyin Xu^{6,7}, Santosh Kesari^{13,14}, X. Sunney Xie¹, and Geoffrey S. Young^{6,7,*}

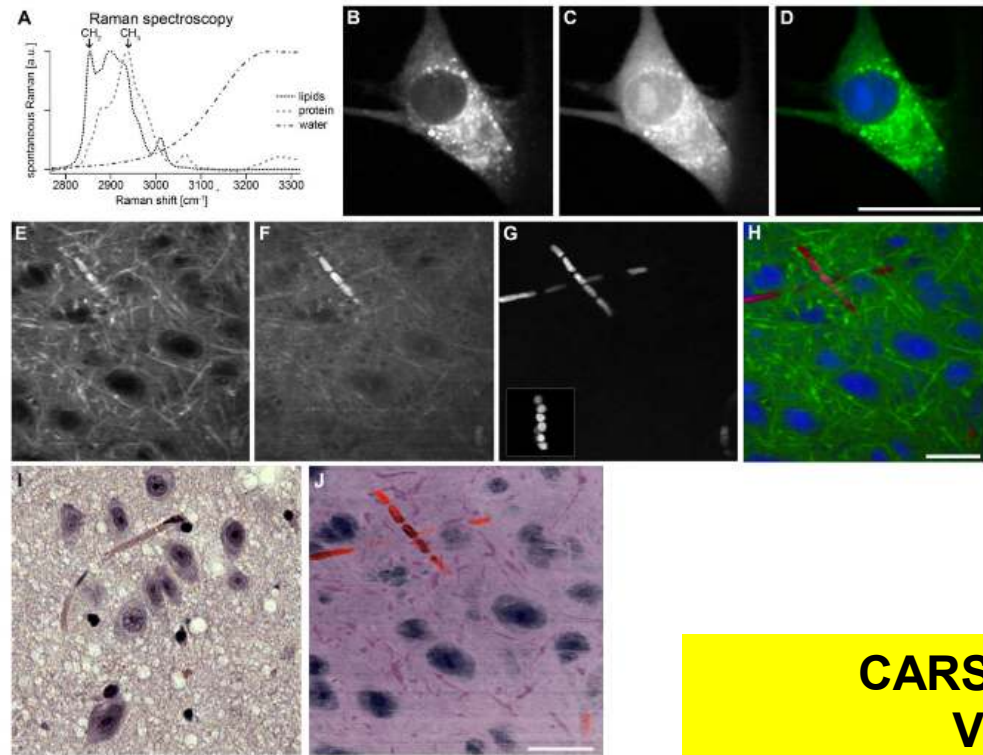


Figure 1. Stain-free histologic imaging with multi-color CRI. (A) Vibrational spectra of the major constituents of tissue: lipids, protein and water. Arrows indicate Raman shifts at which imaging is performed. (B-D) SRS images of a live C2C12 mammalian cell acquired at the CH₂-stretching vibration at 2845 cm⁻¹ (B) and CH₂-stretching vibration at 2940 cm⁻¹ (C). Multicolor image (D) generated from images (B) and (C) with the green channel (CH₂ image) showing the cell-body and the blue channel (thresholded CH₂-CH₂ difference image) highlighting the nuclear morphology including a bright nucleolus. (E-H) SRS images of fresh ex vivo brain tissue acquired at CH₂-stretching vibration at 2845 cm⁻¹ (E), CH₂-stretching vibration at 2940 cm⁻¹ (F), and vibrationally off-resonant showing TPA of hemoglobin at a sum frequency of 23,700 cm⁻¹ (G). Multicolor image (H) generated from images (E-G) with the green channel (CH₂ image) highlighting cytoplasm and myelin sheaths, blue channel (thresholded CH₂-CH₂ difference image) showing the nuclear morphology, and the red channel (hemoglobin image) highlighting red blood cells. (I) H&E-stained micrograph from the same region in the brain. (J) Some multicolor image as (H) with a different pseudo-color scheme, chosen to mimic the appearance of an H&E-stained micrograph, illustrates the similar image content and appearance of stain-free images and H&E stained sections. Scale bar, 25 μm.

**CARS IN VIVO PATOLÓGIAI
VIZSGÁLATOKHOZ**

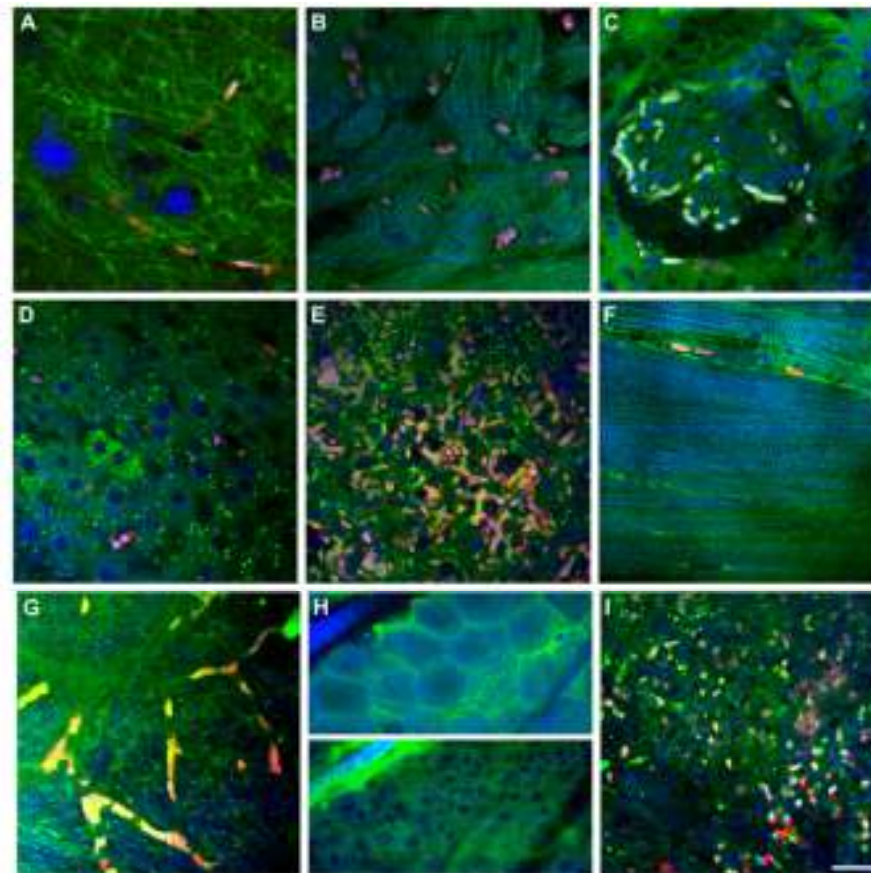


Figure 2. Multicolor stain-free images of various mouse organs (green: CH_2 image; blue: $\text{CH}_3\text{-CH}_2$ difference image; red: hemoglobin image) of (A) brain, (B) heart, (C) kidney, (D) liver, (E) lung, (F) muscle, (G) ovary, (H) skin with *stratum corneum* (top) and *stratum basale* (bottom) and (I) spleen. Scale bar, 25 μm .

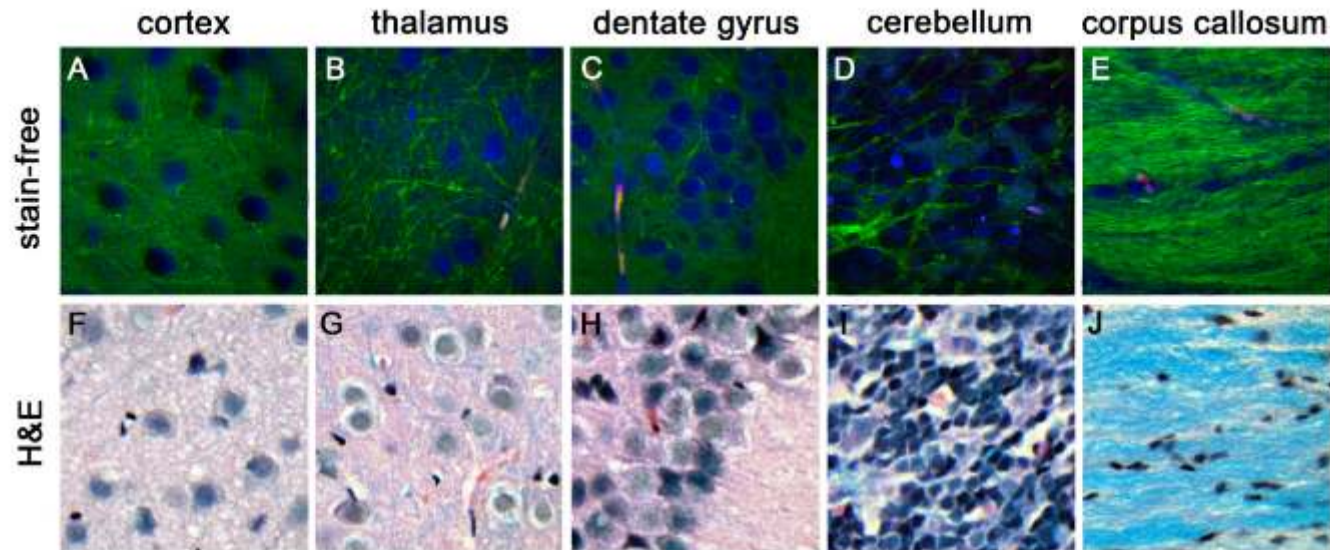


Figure 3.

Multicolor stain-free images of various brain regions in a wild-type mouse in comparison with paraffin-embedded, H&E and Luxol-stained sections. (green: CH_2 image; blue: $\text{CH}_3\text{-CH}_2$ difference image; red: hemoglobin image) of (A) cortex, (B) thalamus, (C) dentate gyrus, (D) cerebellum, and (E) corpus callosum. (F-J) show H&E/luxol stained section of corresponding regions.

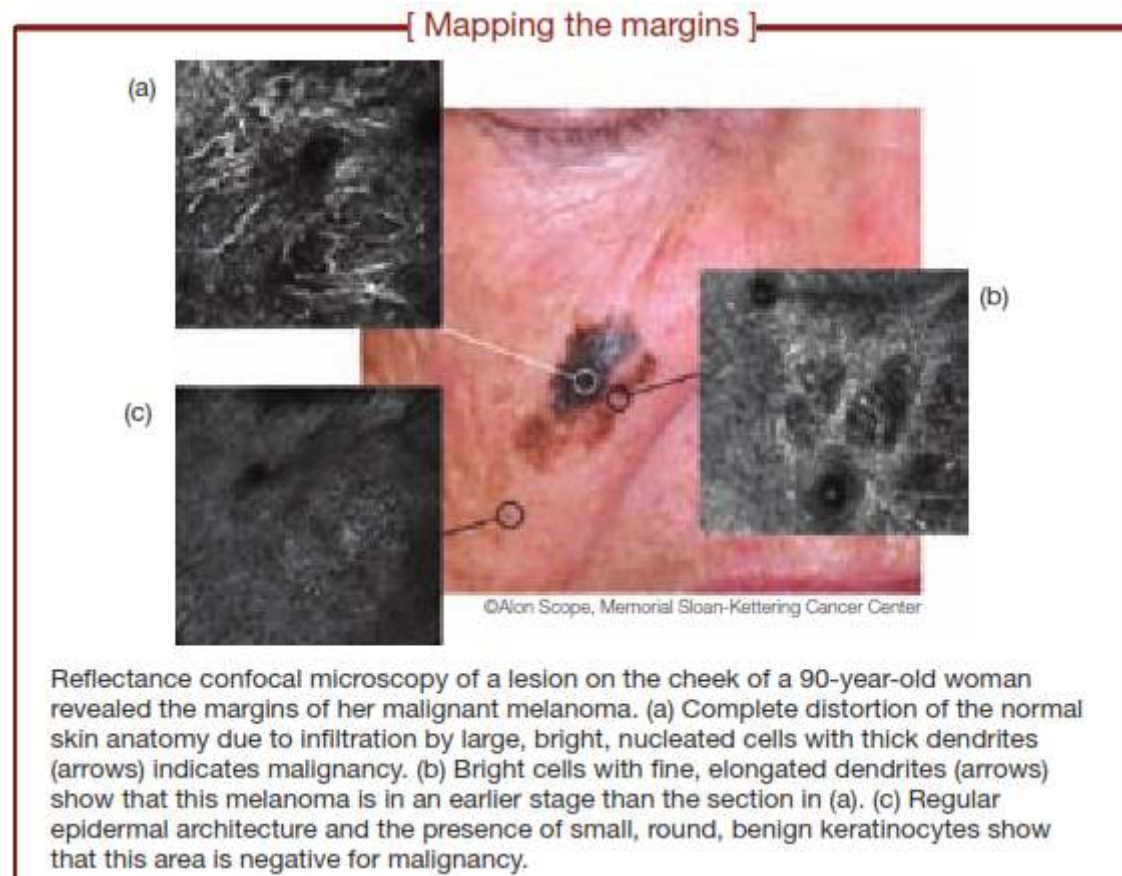
3D mikroszkópia már létező klinikai alkalmazásai



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.

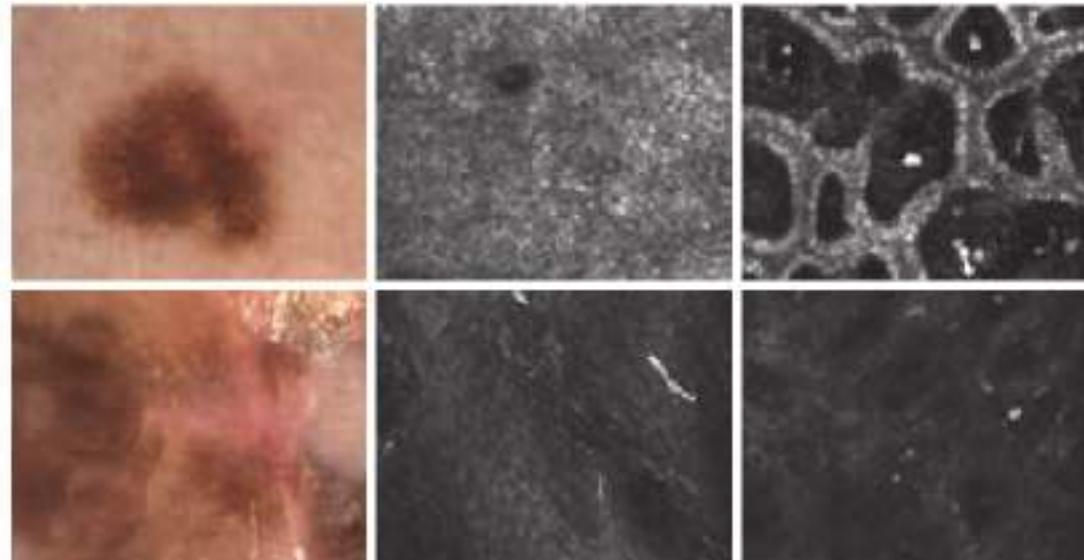
Miben segíthetnek a 3D mikroszkópiás módszerek?



Miért előnyös az in vivo 3D mikroszkópia?

- ☺ Tumor határának pontos meghatározásának lehetősége
- ☺ Nem kell várni a patológiai vizsgálatok eredményére!

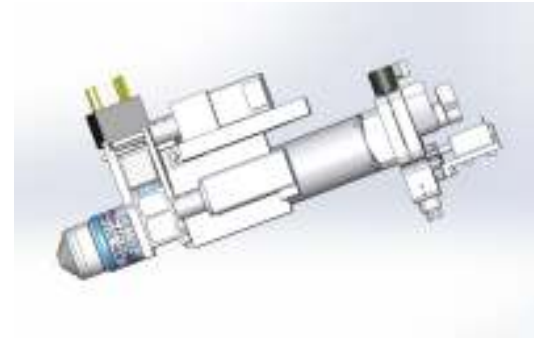
Given that this imaging form lets physicians **look into tissue in vivo** without any of the processing that is done in the pathology lab, confocal imaging may well reveal new skin features that cannot be directly correlated to the pathology.



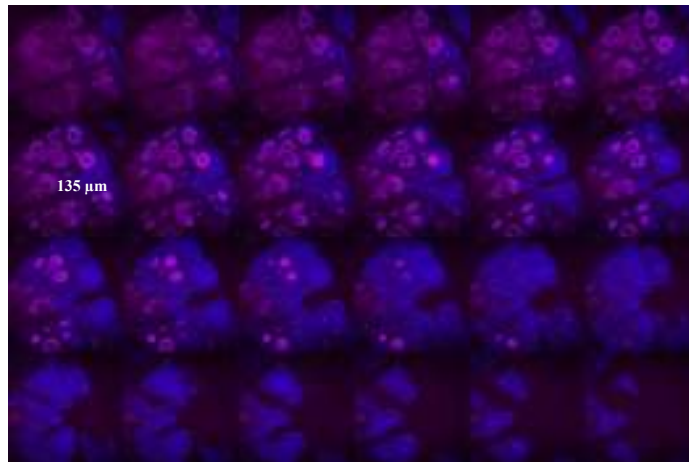
(Top) A benign mole viewed from (left) the skin's surface at visible wavelengths, (center) at a depth of 10 μm and (right) at 161 μm with infrared confocal microscopy.
(Bottom) Malignant melanoma at (left) surface level in visible light, (center) at a depth of 35 μm and (right) at 95 μm with confocal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



Basaliomák 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)



Semmelweis Egyetem
Bőr-, Nemifertani
és Bőronkológiai Klinika

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 Seidenari¹, Federica Arginelli¹, Sara Bassoli¹, Jennifer Cautela¹, Anna Maria Cesinaro², Mario Guanti¹, Davide Guardoli¹, Cristina Magnoni¹, Marco Manfredini¹, Giovanni Ponti¹ and Karsten König^{3,4}

¹Department of Dermatology, University of Modena and Reggio Emilia, Modena, Italy,

²Department of Pathology, University of Modena and Reggio Emilia, Modena, Italy,

³Department of Biophotonics and Lasertechnology, Saarland University, Saarbrücken, Germany and ⁴JenLab GmbH, Schillerstrasse 1, 0745, Jena, Germany

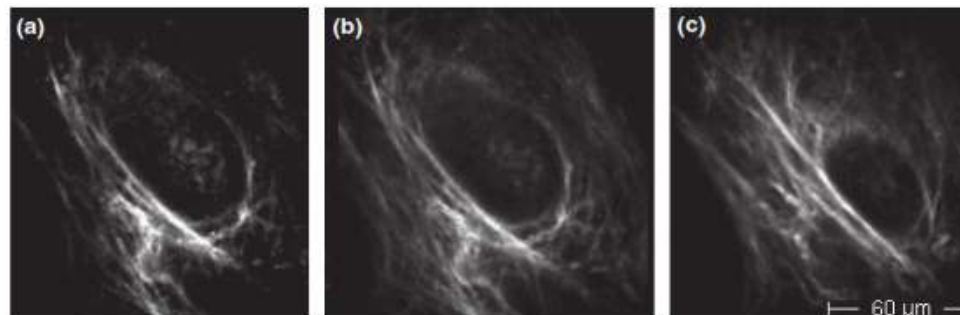
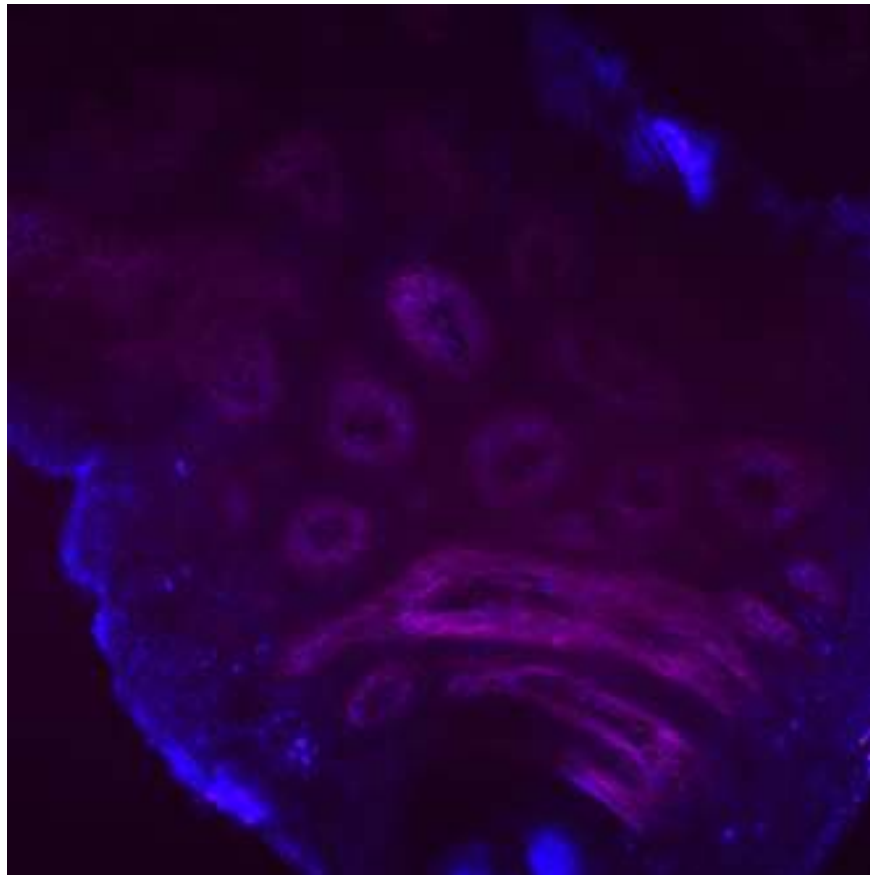


Fig. 4. Basal cell carcinoma. (a) 100 μm depth, excitation wavelength 800 nm. Shifting the wavelength to 800 nm, basaloid cells become less visible; employing an excitation wavelength of 820 nm basaloid cells disappear and it is possible to observe empty spaces surrounded by collagen fibres (phantom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



Basalioma vizsgálata – 3D FiberScope fejlesztése

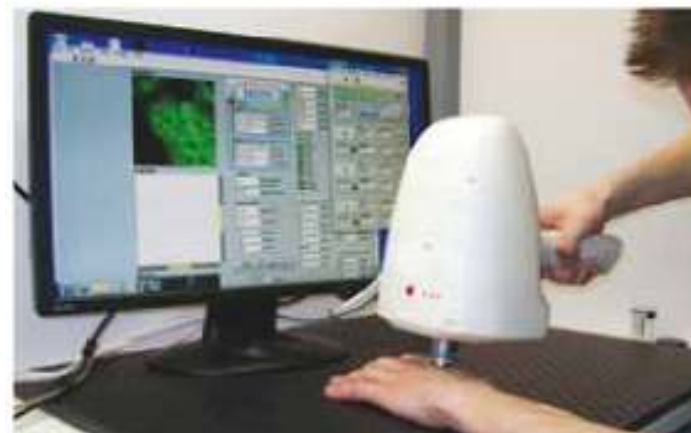


Kollagén szerkezet – SHG
Sejtek - autofluoreszcencia

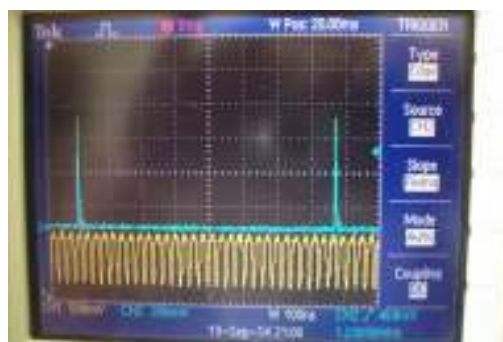
- Lézer hullámhossza
- Színszűrők kiválasztása

Szállézer specifikálása
Optikai szál specifikálása

Biztonságtechnikai vizsgálatok



FiberScope, a kézben tartott nemlineáris mikroszkóp



Lézeres fényforrás: 2-36 MHz-es ismétlési frekvenciájú, 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



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}, Enikő R. Tőke^{3,5}, Orsolya Lőrincz^{3,5}, Dóra Haluszka^{1,4} and Róbert Szípcős^{1,2}

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

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

⁵Present 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–moisty-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.

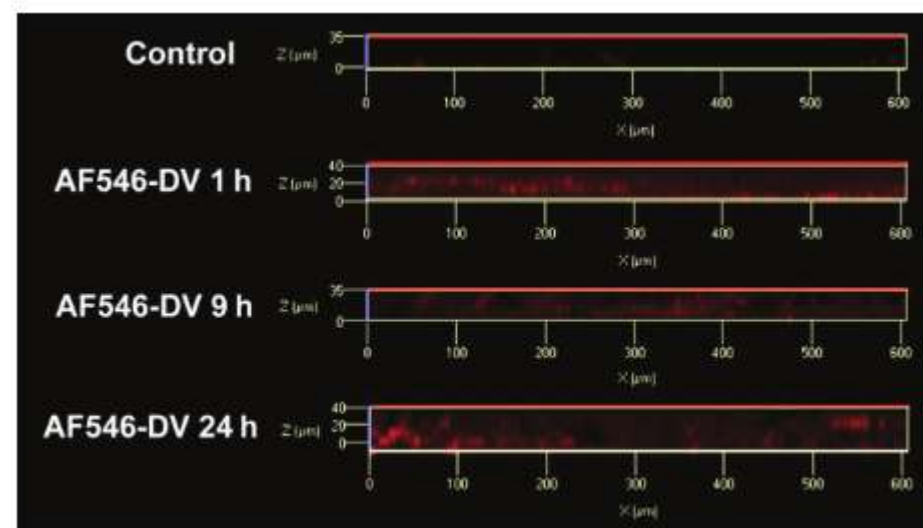




Figure 2. Kinetics of AF546-DV uptake by Langerhans cells (LCs) in eGFP-Langerin knock-in mouse ear *in vivo*. Nearly all LCs had incorporated AF546-DV after 1 h of topical treatment: strong colocalization was detected in both channels [NDD 2 – green/eGFP (middle column) versus NDD 1 – red/AF546-DV (left column)] as presented on the merged pictures (right column). Images of red light emission also revealed that the nanoparticles were distributed homogeneously 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 .

