3D CARS mikroszkópiás mérőrendszer kifejlesztése és alkalmazása

(MTA Wigner FK SZFI Alkalmazott Kutatási Díj, 2015)

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MTA Wigner SZFI

2016. január 26.

Előadás vázlat

1. Bevezetés a festékjelölésektől mentes 3D mikroszkópiába (2PEF, SHG, CARS)
2. Pásztázó kétfoton (2PEF) mikroszkóp átalakítása SHG és CARS mérésekre (a prototípus)
3. Az ipari kivitelű CARS mikroszkópiás mérőrendszer
4. Alkalmazások a bőrgyógyászatban
5. Alkalmazások az idegtudományban (ld. Ozsvár Attila (SZTE))
6. Összefoglalás, kitekintés (FiberScope/CARS, GRIN lencsék, SRS mérések)

Szipőcs Group

3D képalkotás elve: optikai „szeletelés”

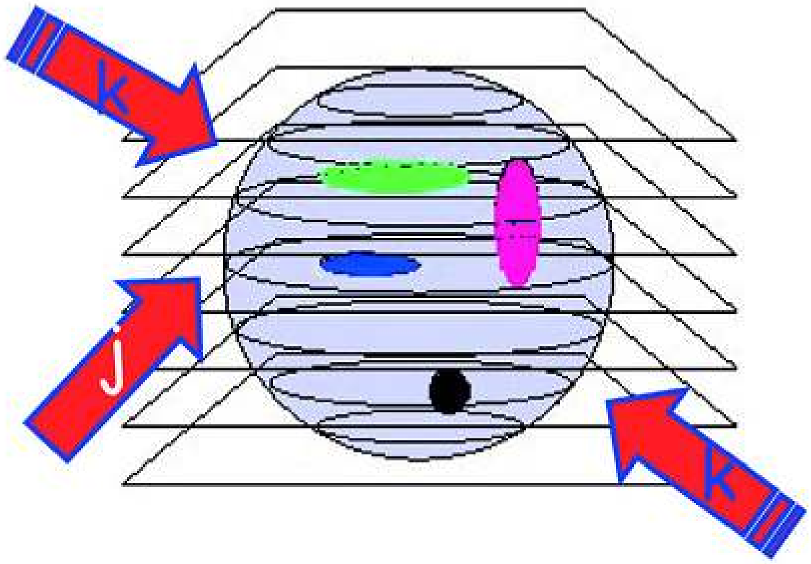
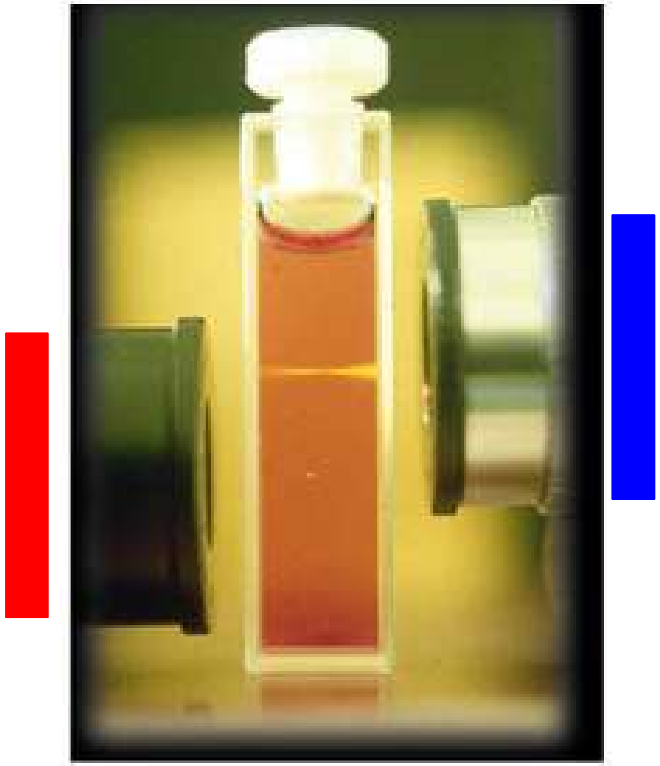
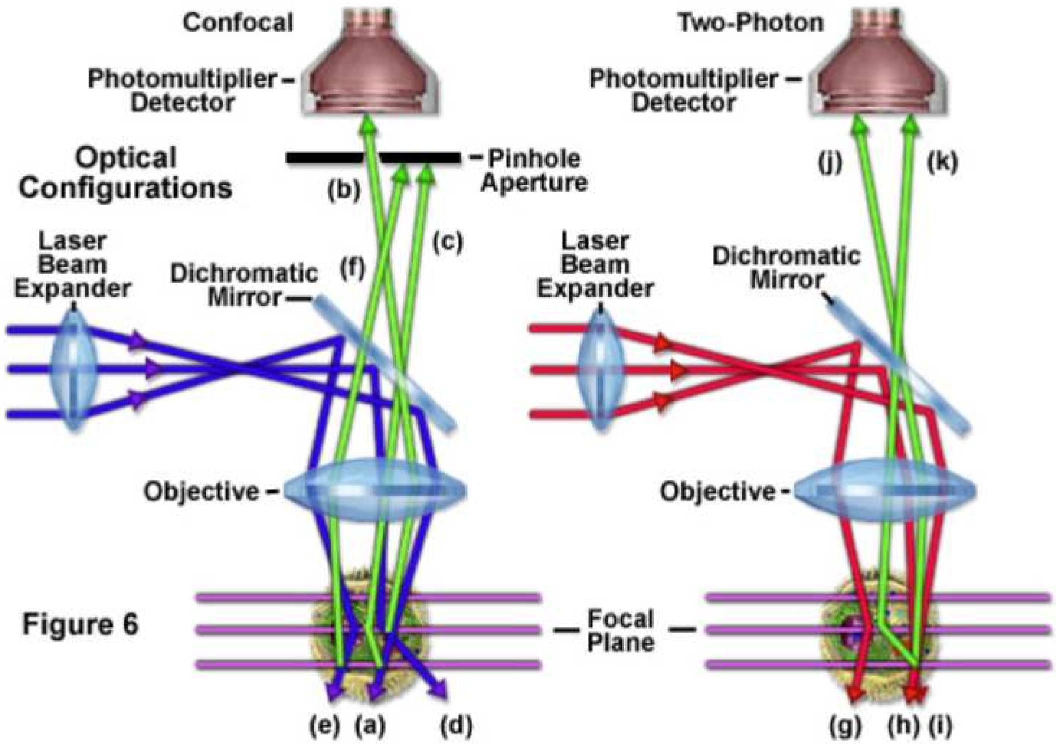


Figure 2
Optical sectioning scheme. A three-dimensional sample can be sketched as a series of optical slices. Let's call slice j the one containing the geometrical focus of the objective and refer to the adjacent planes as k slices. The sample contains a three-dimensional distribution of fluorescently labelled molecules whose intensity distribution is I, slice by slice. The thickness of each optical slice is approximately one half of the axial resolution, say $\approx\lambda/2$.

Optikai „szeletelés” megvalósítása konfokális fluoreszcencia mikroszkópokban és a nemlineáris mikroszkópiában (2PEF, SHG, CARS)

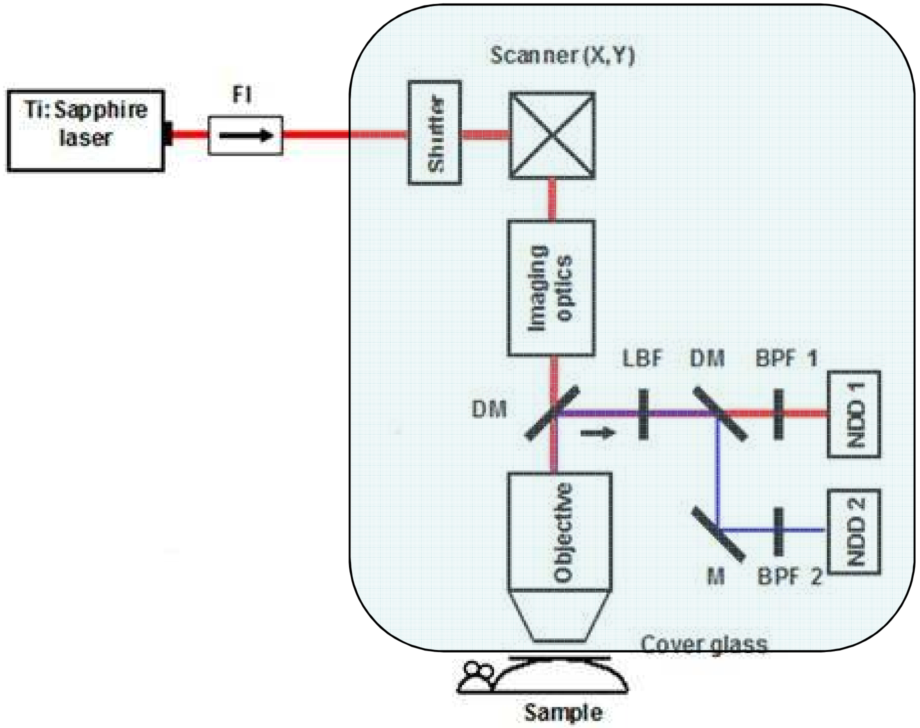




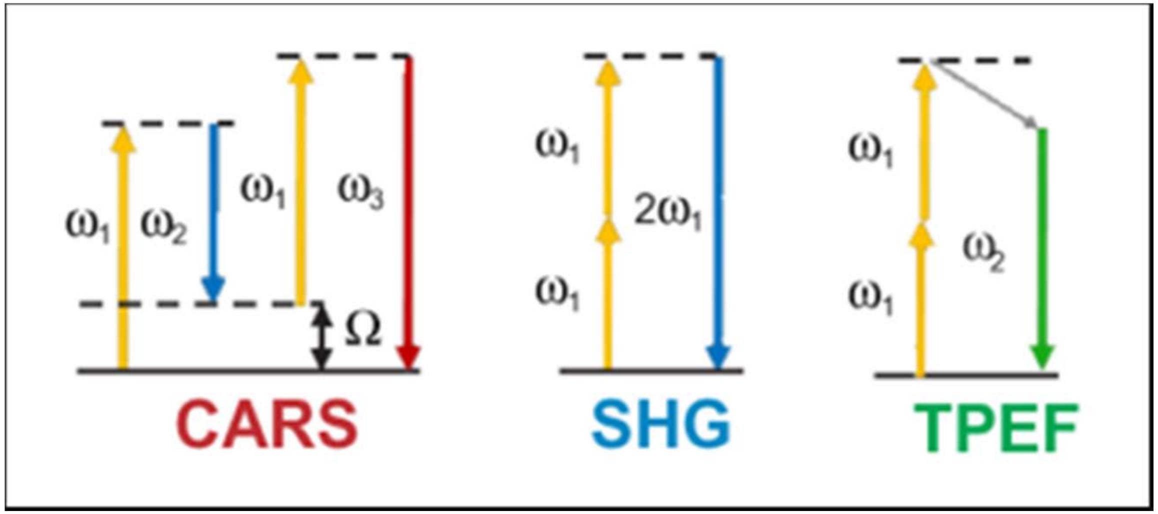
Egy pásztázó 3D 2PEF („kétfoton”) mikroszkóp fontosabb egységei

- Széles sávban hangolható fs-os Ti-zafír lézer

- Lézeres pásztázó mikroszkóp (pl. Carl Zeiss LSM 7MP)



A nemlineáris mikroszkópiában használt folyamatok

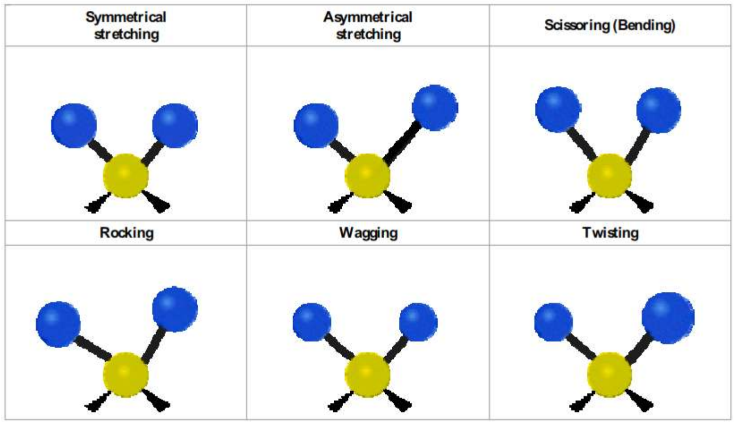


CARS: Coherent anti-Stokes Raman Scattering
SHG: Second-Harmonic Generation
TPEF: Two-photon excitation fluorescence

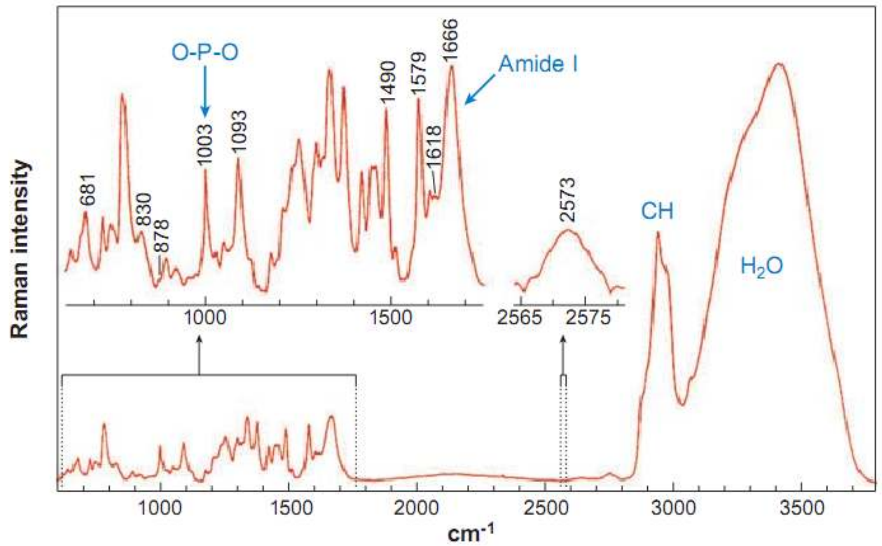
Raman spektroszkópia, infravörös spektroszkópia

Molekuláris rezgési állapotok sajátenergiái

The atoms in a CH_2 group, commonly found in organic compounds, can vibrate in six different ways: **symmetric and asymmetric stretching**, **scissoring**, **rocking**, **wagging** and **twisting** as shown here:

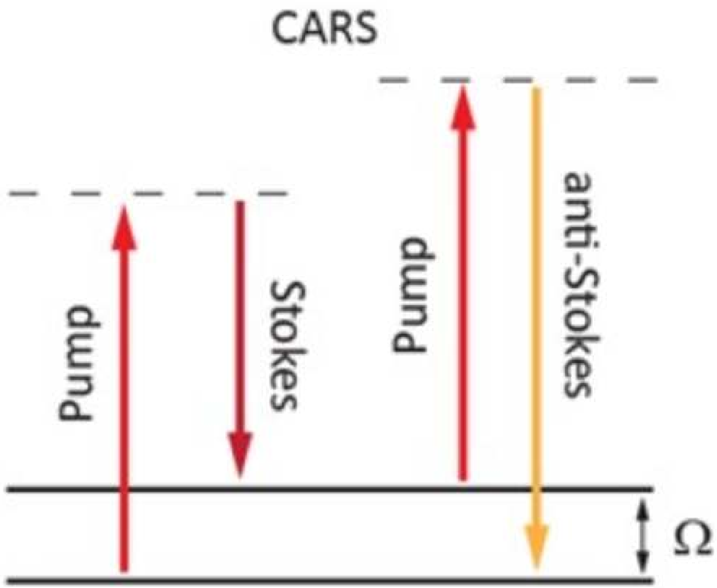


Raman spektrum (pl. vírus)



The Raman spectrum of the P22 virus showing characteristic vibrational frequencies observed in biological samples. Several vibrational modes of particular interest in vibrational microscopy are labeled (*blue*). The O-P-O stretching vibration arises from the vibration of the DNA backbone. The amide-I band is characteristic of proteins and can be used to map out protein density. The CH-stretching band is typically used to image lipids in biological samples. The H_2O-stretching vibrations of water are important for following water flow and density. Adapted with permission from Reference 79.

CARS mikroszkópia, fontosabb összefüggések



$$\Omega_{vib} = \omega_p - \omega_S$$
$$\omega_{as} = 2\omega_p - \omega_S$$

Megjegyzés: két hullámhosszon, azonos ismétlési frekvencián működő impulzusüzemű (fs-os vagy ps-os) lézerrendszer kell!

CARS lézerrendszer, a koncepció

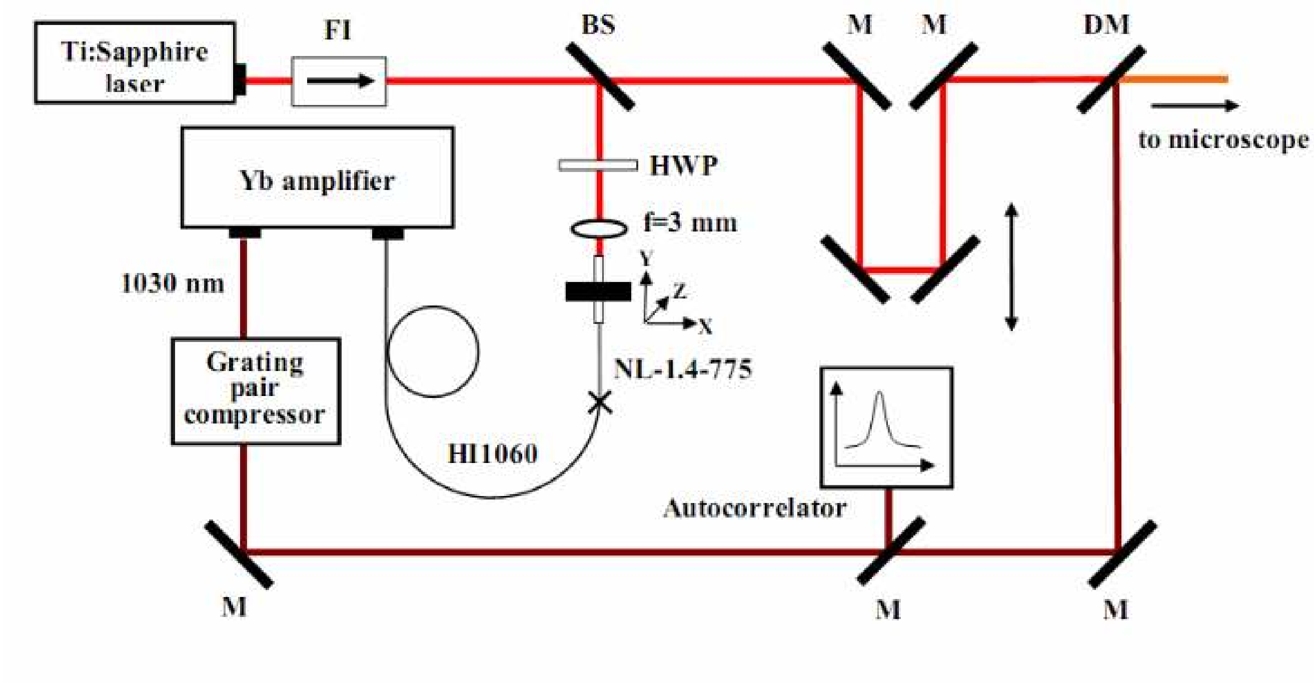
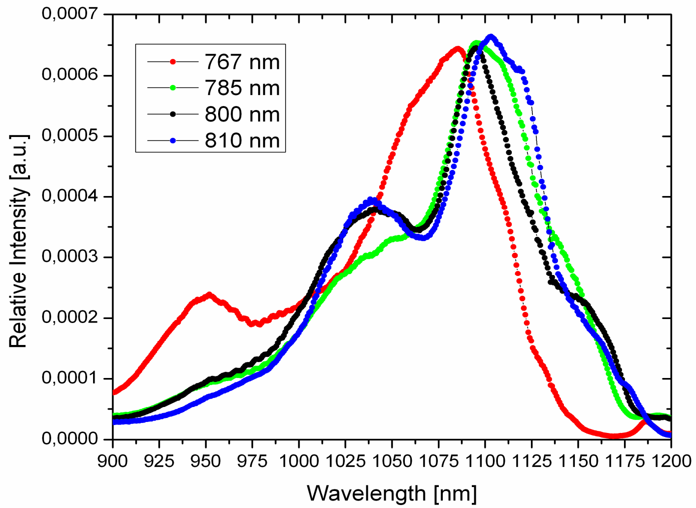
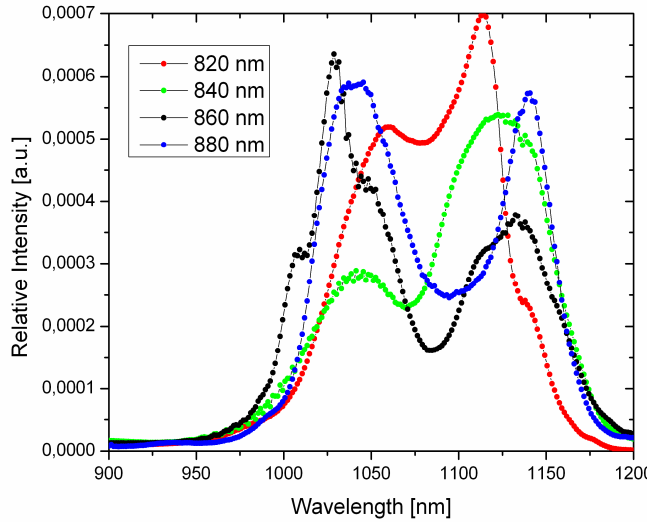


Fig. 1 Setup of the CARS extension unit

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)

CARS lézerrendszer, a koncepció

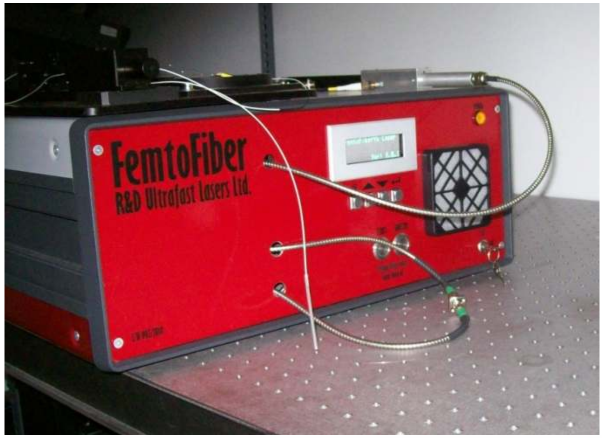


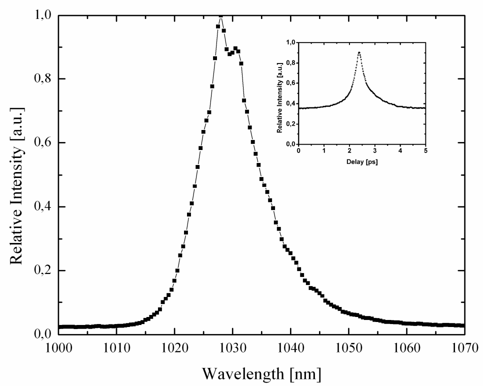


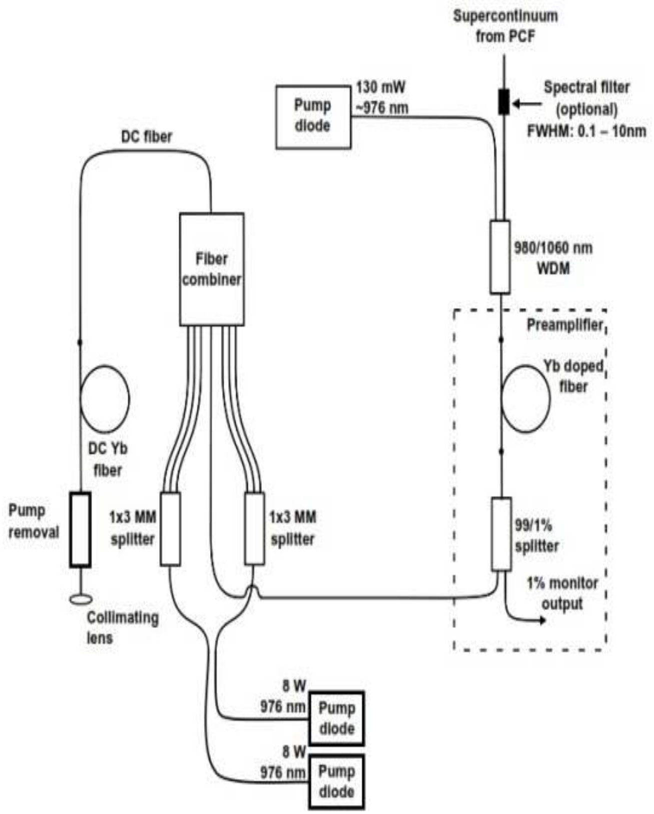
Magimpuzusok keltése Yb-erősítőhöz fotonikus kristály szálban

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)

Az Yb-szálerősítő felépítése

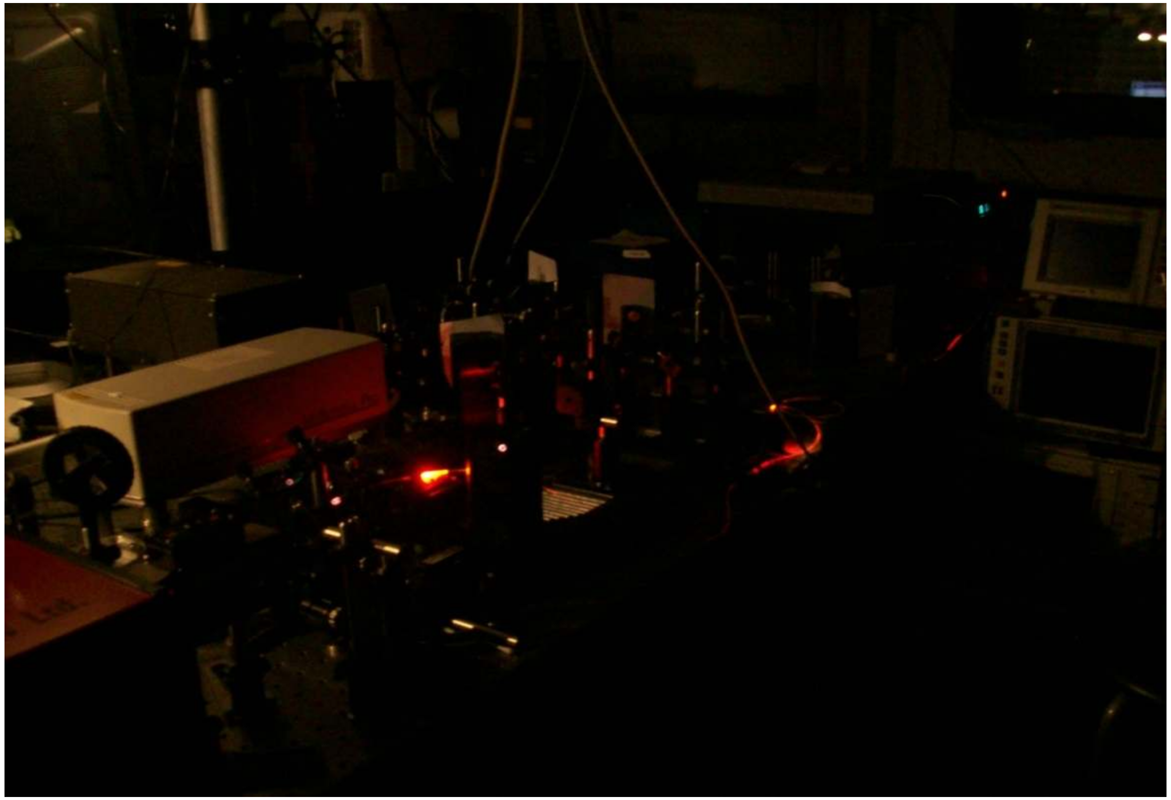






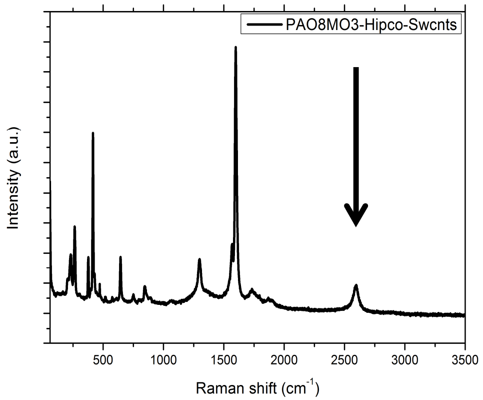
Kolonics A, Csáti D, Antal P, Szipőcs R; ***A simple, cost efficient fiber amplifier wavelength extension unit for broadly tunable, femtosecond pulse Ti-sapphire lasers for CARS microscopy****; In: Proc. BIOMED Biomedical Optics and Digital Holography and Three Dimensional Imaging (Miami, Florida, United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

A Wigner FK-ban elkészített prototípus CARS rendszer



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)

Tesztmérések szén nanocsöveken

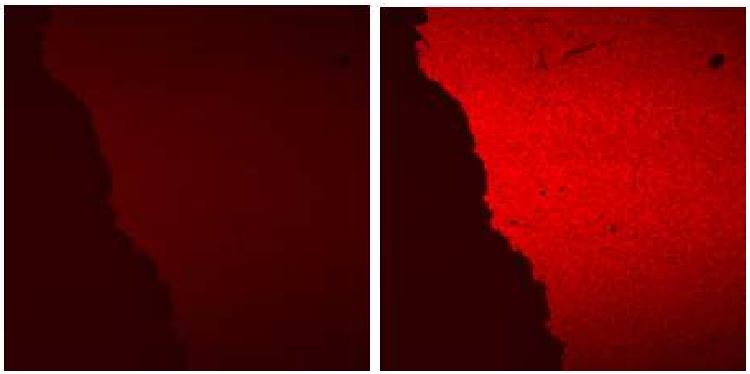


Raman csúcs: Ω = 2577 cm^{-1}

Yb lézer hullámhossza:1030 nm (9708 cm^{-1})

TiS lézer hullámhossza: 814 nm (12285 cm^{-1})

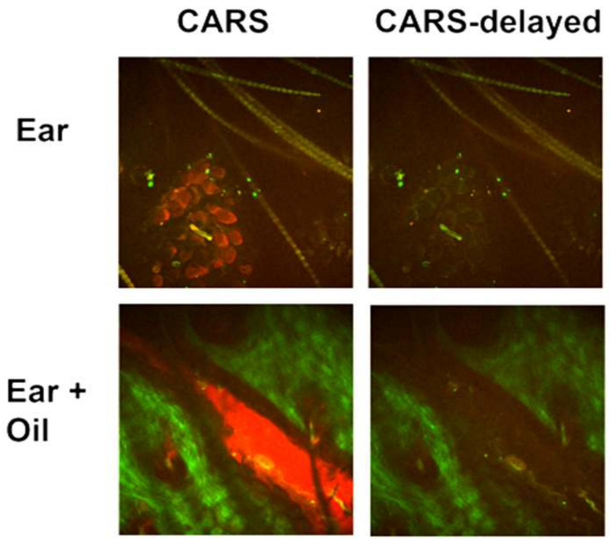
anti-Stokes jel: 673 nm-en



The 2P autofluorescence (left) and the CARS signal (right) detected on the "red" channel of the nonlinear microscope for non-zero (left) and zero (right) delay difference between the pump and the Stokes pulses

Csáti D, Antal P, Szipőcs R: „An inherently synchronized Yb fiber laser extension unit for broadly tunable, femtosecond pulse Ti-sapphire lasers for CARS microscopy," In: *Proc.* ***FILAS Fiber Lasers and Applications****, (San Diego, California, February 1-3, 2012)*; OSA Technical Digest Series; Paper JTh2A.29/1-3 (2012)

Tesztmérések olajjal kezelt egérfülön



Raman csúcs: Ω = 2845 cm^{-1}

Yb lézer hullámhossza:1030 nm (9708 cm^{-1})

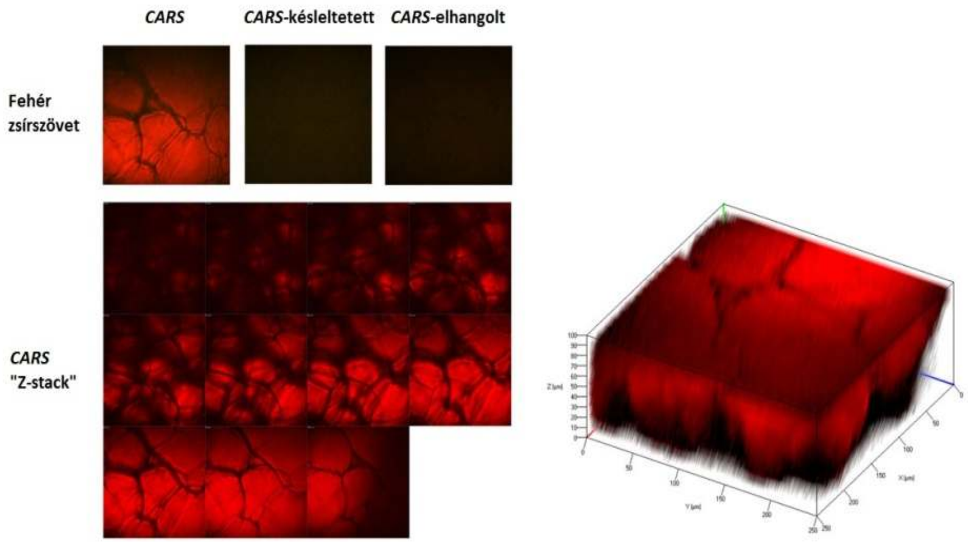
TiS lézer hullámhossza: 796 nm (12563 cm^{-1})

anti-Stokes jel: 651 nm-en

Olajjal kezelt és nem kezelt egérfülről készített CARS mikroszkópiás képek

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)

Tesztmérések in vitro zsírszöveten



Raman csúcs: Ω = 2845 cm^{-1}

Yb lézer hullámhossza:1030 nm (9708 cm^{-1})

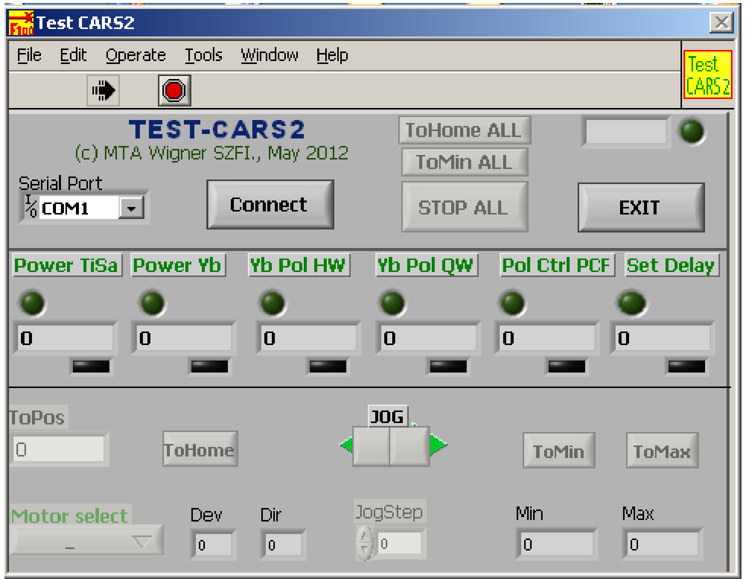
TiS lézer hullámhossza: 796 nm (12563 cm^{-1})

anti-Stokes jel: 651 nm-en

Egér zsírszövetről készített 3D CARS mikroszkópiás képek

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)

Ipari kivitelű CARS mikroszkópiás mérőrendszer



Gépészeti tervezés, elektronikus, léptetőmotoros vezérlés, LabView kontrol program

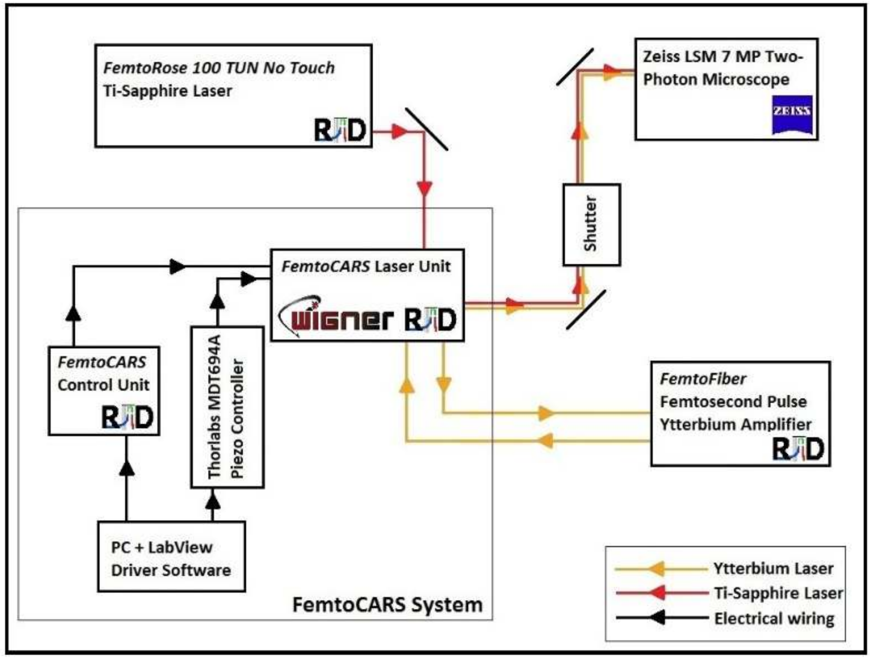
SPIE Corporate Member

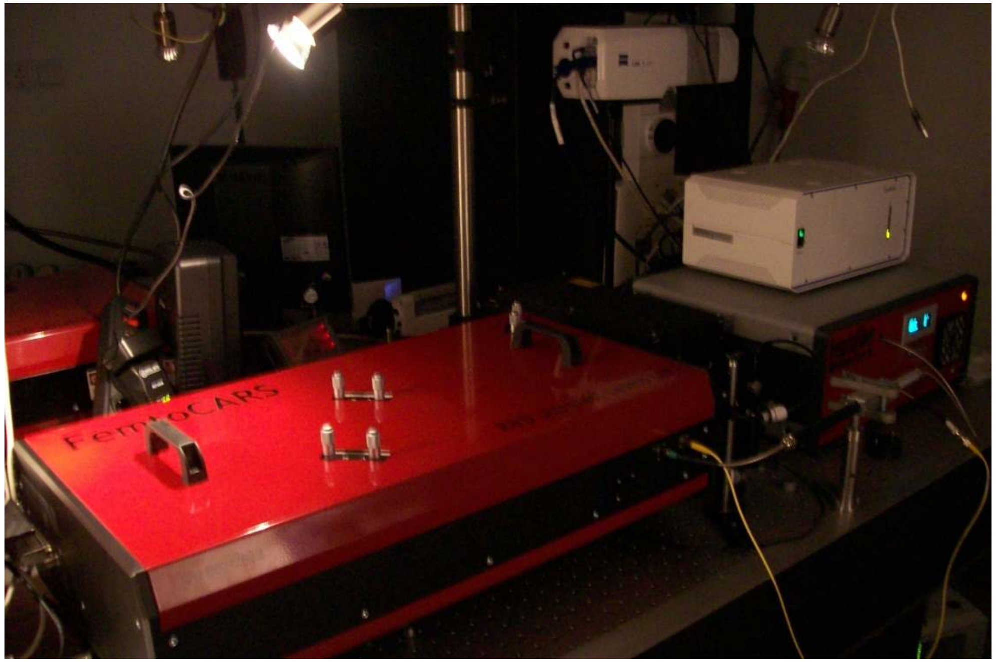
R&D ULTRAFAST LASERS LTD.

FemtoCARS

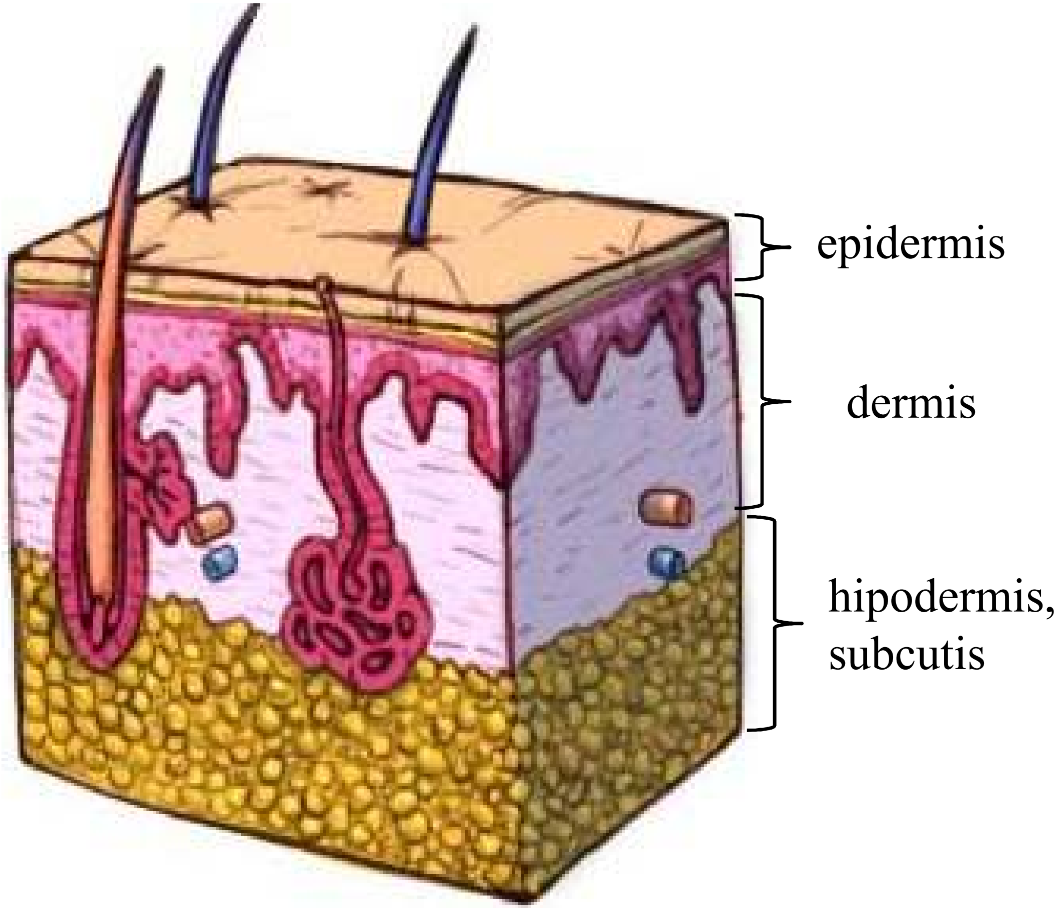
the

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

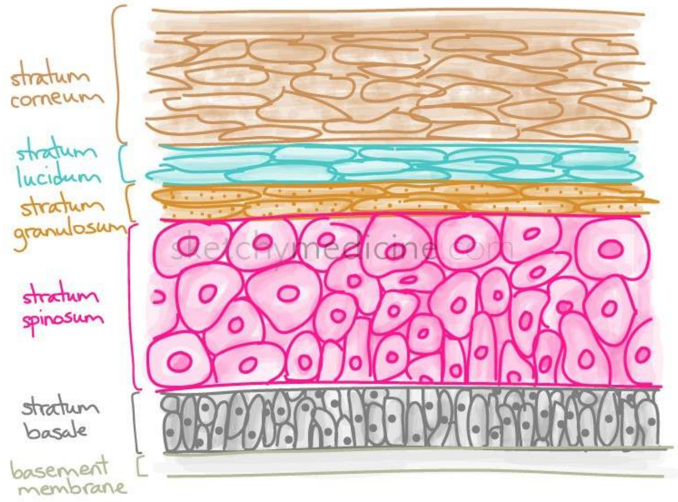




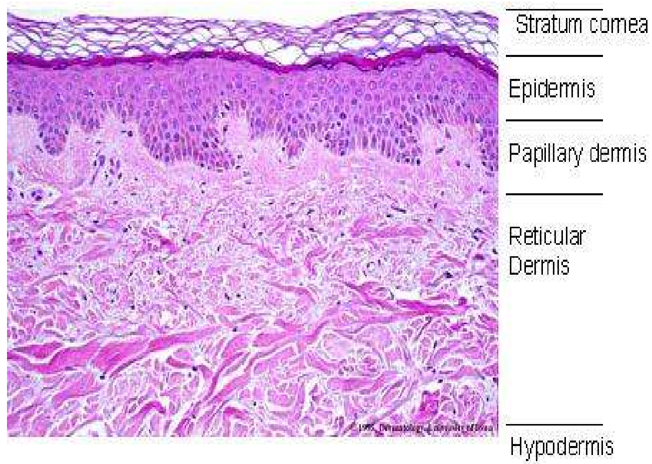
Nemlineáris mikroszkópia a bőrgyógyászatban



epidermis rétegei



dermis szerkezete



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A bőr szerkezeti képe – multifoton mikroszkópiával

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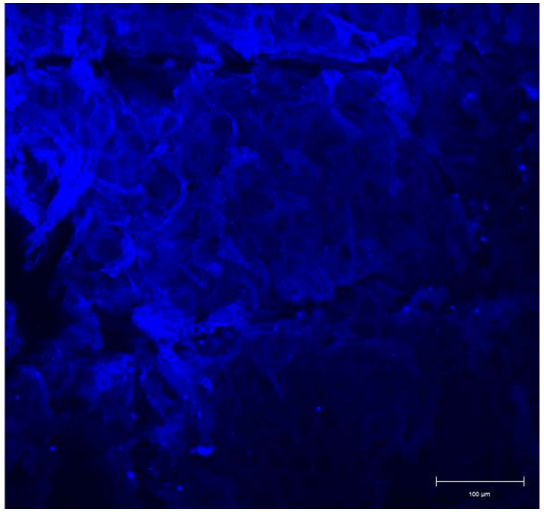
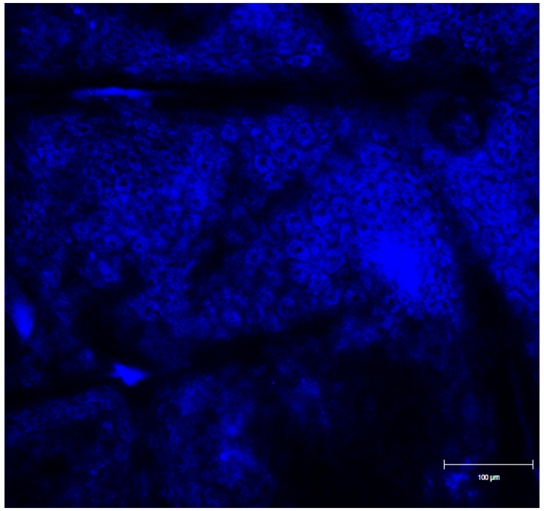
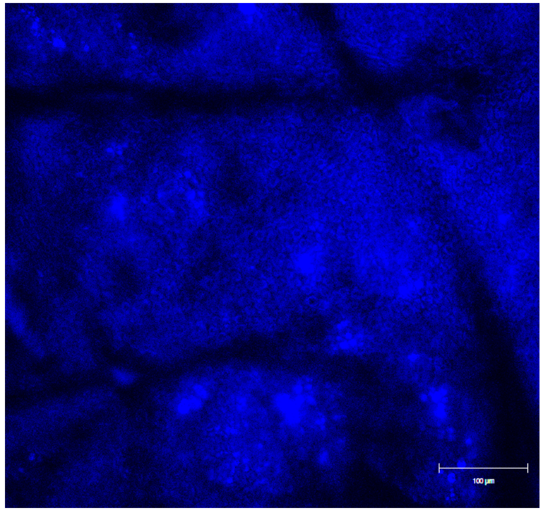
str. corneum: keratin (TPEF)



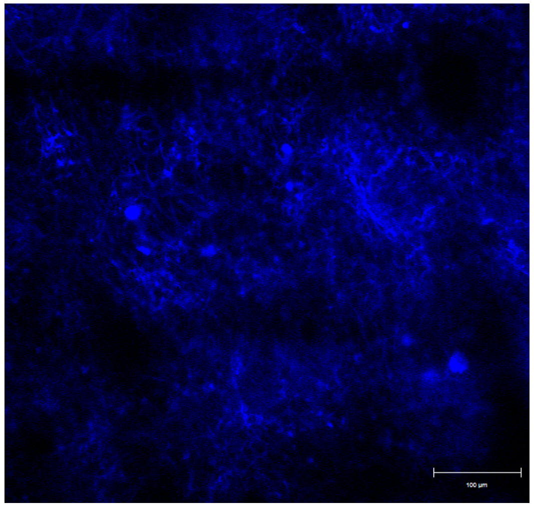
str. spinosum: NADH (TPEF)



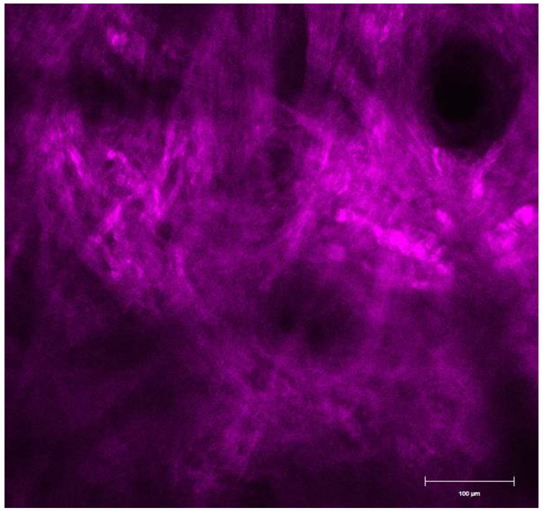
str. basale: NADH (TPEF)



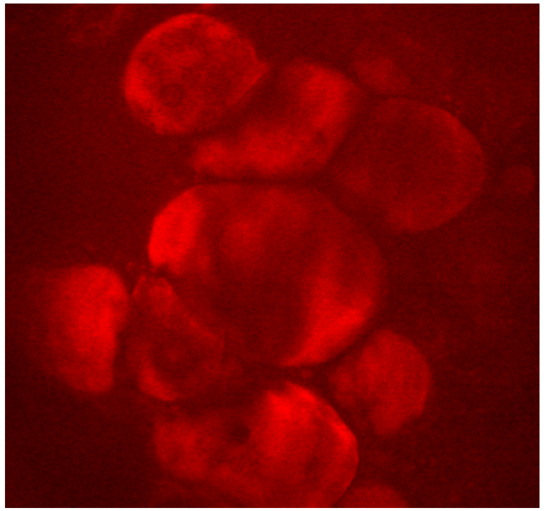
dermis: elasztin (TPEF)



dermis: kollagén (SHG)



subcutis: zsírsejtek (CARS)



In Vivo Second-Harmonic Generation and Ex Vivo Coherent Anti-Stokes Raman Scattering Microscopy to Study the Effect of Obesity to Fibroblast Cell Function

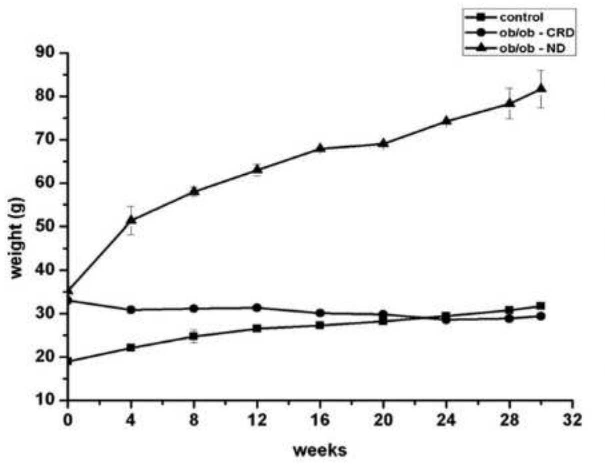


Fig. 2. Results of body weight changes and SHG intensity of dermal collagen measured and followed-up for 30 weeks. **A**: Increase of body weight in groups of mice during 30 weeks. At the beginning of the experiments (week 0) animals in various diet groups were already different; animals with leptin deficiency had higher body weight compared to controls. At week 8 the body weight increase of ob/ob—ND group was approximately twofold higher compared to other groups. The body weight of ob/ob—CRD group stayed relatively constant from week 8. Error bars represent standard deviation (SD) **B**: SHG

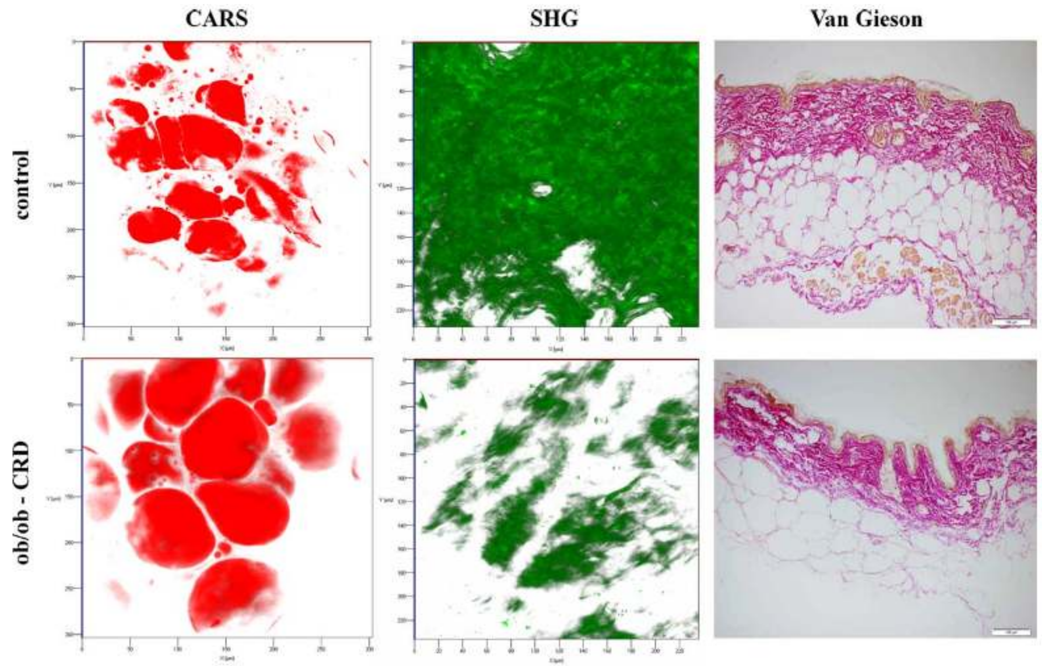


Fig. 4. Comparison of adipocyte sizes by CARS measurement and the collagen morphology, determined by SHG, and *ex vivo* van Gieson staining of control and ob/ob—ND groups. The 3D CARS and SHG images are projected in *z*-direction and imaging area is ~0.6 × 0.6 mm^2. The enlarged adipocytes, damaged collagen fibers and thinner dermis in case of leptin deficient mice are clearly visible, in contrast to control group. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Haluszka, D., Lőrincz, K., Molnár, G., Tamás, G., Kolonics, A., Szipőcs, R., Kárpáti, S. and Wikonkál, N. M: **„In vivo second-harmonic generation and ex vivo coherent anti-stokes Raman scattering microscopy to study the effect of obesity to fibroblast cell function using an Yb-fiber laser-based CARS extension unit,”** Microsc. Res. Tech. **78,** 823–830 (2015)

A 2. típusú diabétesz és a bőr kollagén szerkezetének vizsgálata ob/ob knockout egereken, in vivo

Semmelweis Egyetem
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Ob/Ob egerek

- a 2-es típusú diabétesz (csökkent inzulin érzékenység) modellállatai
- ob gén – leptin – étvágy szabályozása
- leptint kódoló gén mindkét allélja hibás – falánk, elhízott
- **2-es típusú diabétesz – bőr kollagén szerkezetének változásai**
- **3 egércsoport**
- AGEs – (advanced glycation endproducts) glükóz eredetű glikált végtermékek
- bőr autofluoreszcencia és SHG intenzitás értékei változnak
- megnövekedett zsírsejtek-szabad zsírsavak – csökken a fibroblast sejtek proliferációja + növelik a MMP13 szintjét – további szabad-gyök termelés – metabolikus betegségek + a bőr korai öregedése

Ezure T. and Amano S., *Negative regulation of dermal fibroblasts by enlarged adipocytes through release of free fatty acids.* J Invest Dermatol, 2011. **131**(10): p. 2004-9.

D. Haluszka, R. Szipőcs, N. Wikonkál, A. Kolonics: *Characterization of Obesity in Murine Skin in vivo by CARS and SHG Microscopy Using a Cost Efficient, Fiber Laser Based Wavelength Extension Unit*, paper WeP-T1-P-13 (2014)

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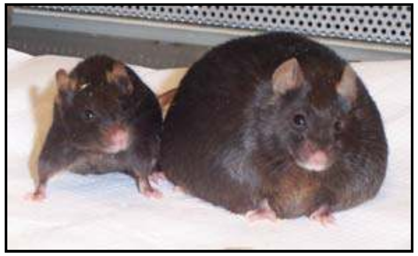
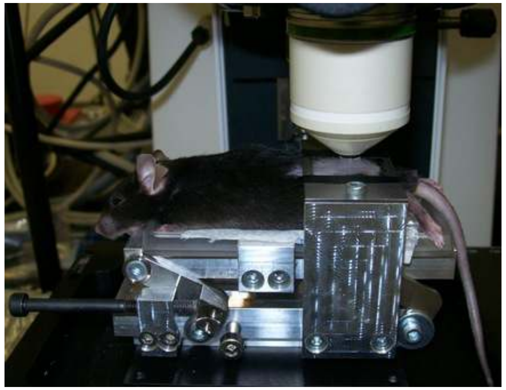
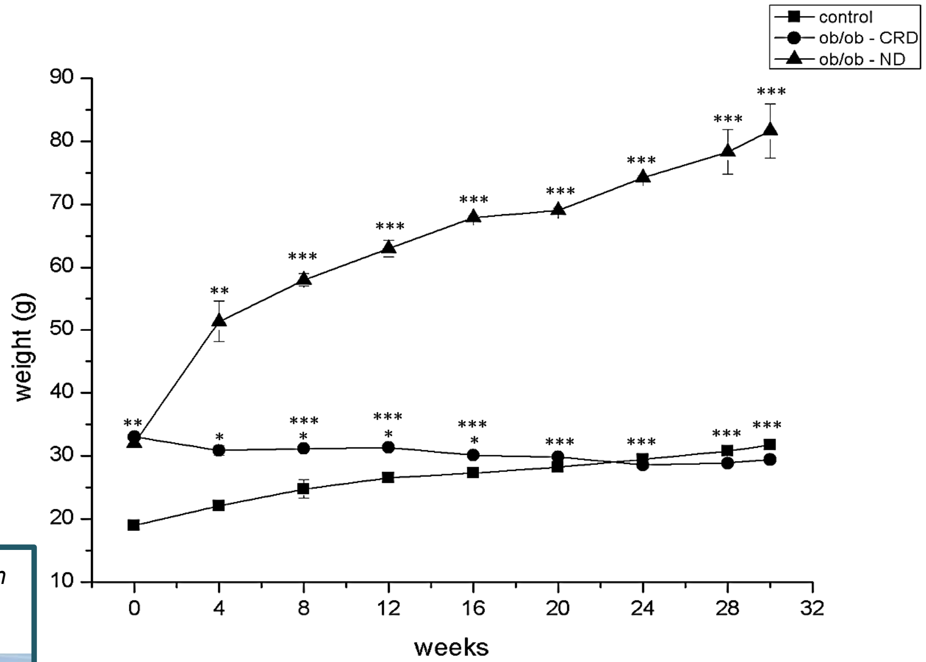
24-29 August 2014
Neuchâtel, Switzerland







SHG (kollagén) + CARS (zsírsejtek)

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Eredmények – kollagén szerkezet (SHG)

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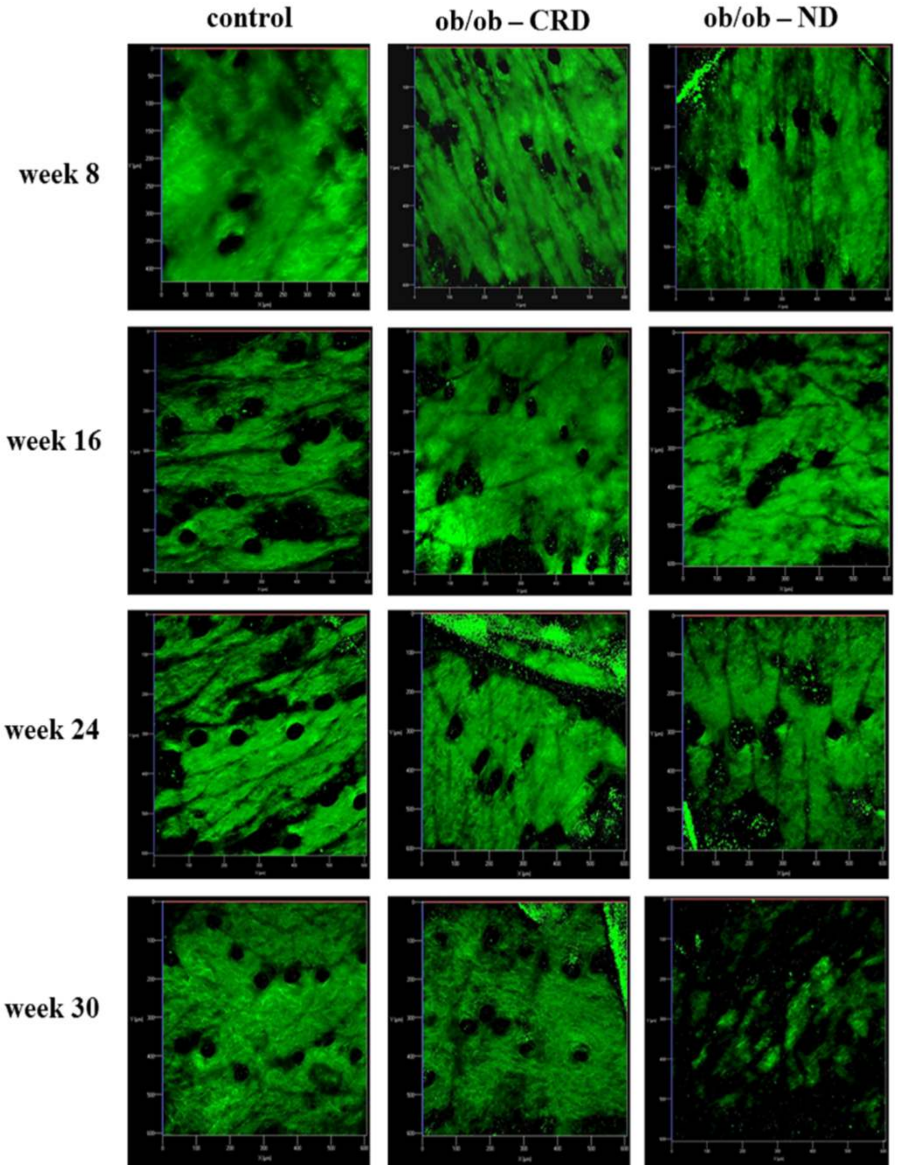

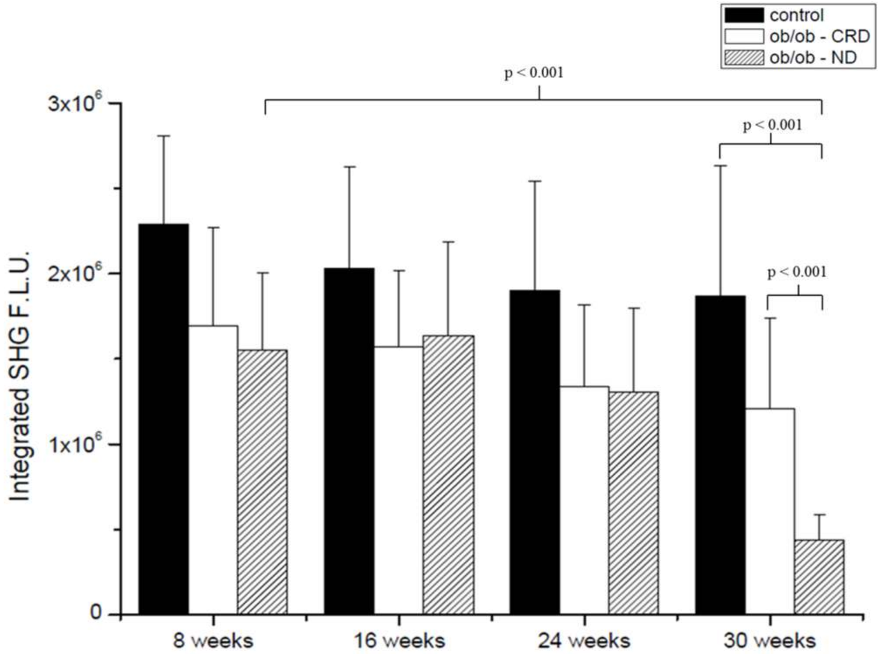

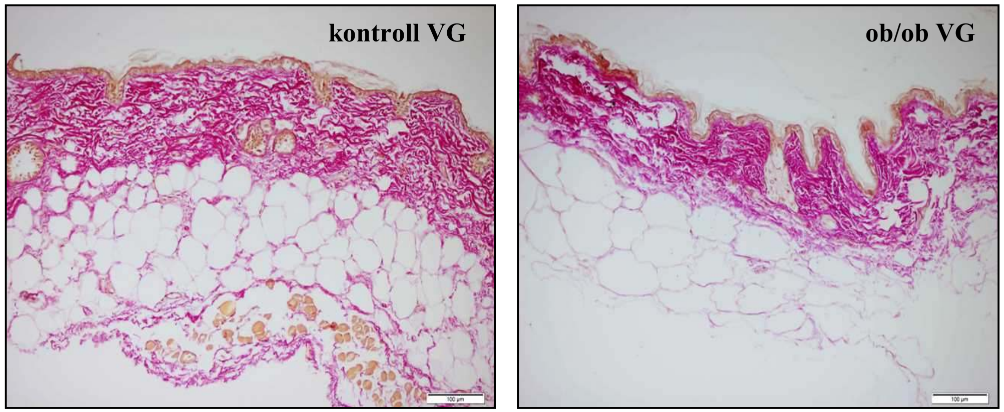

rutin szövettani festés

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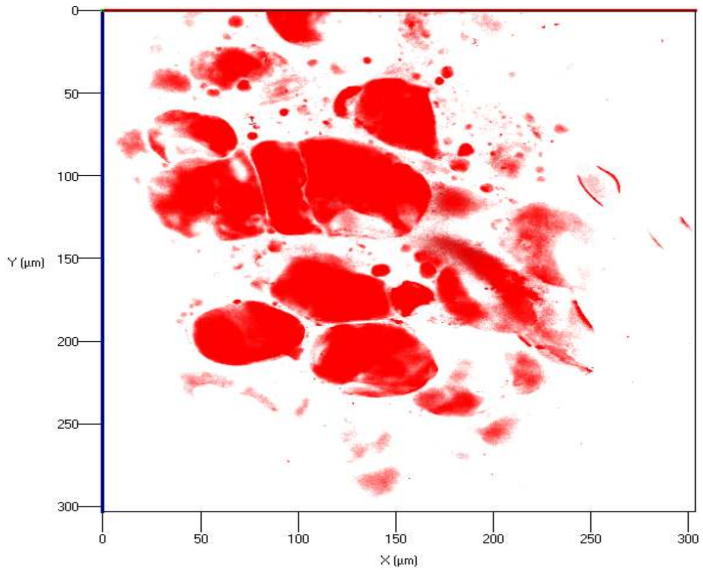
Eredmények – zsírsejtek (CARS)

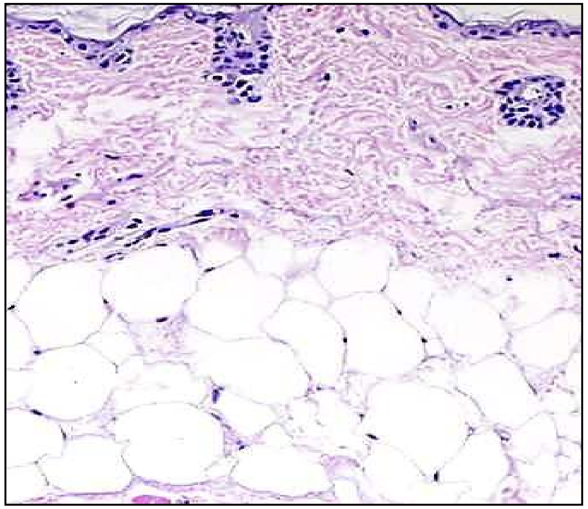
Wigner
Szipőcs Group

Koherens anti-Stokes Raman szórás mikroszkópia

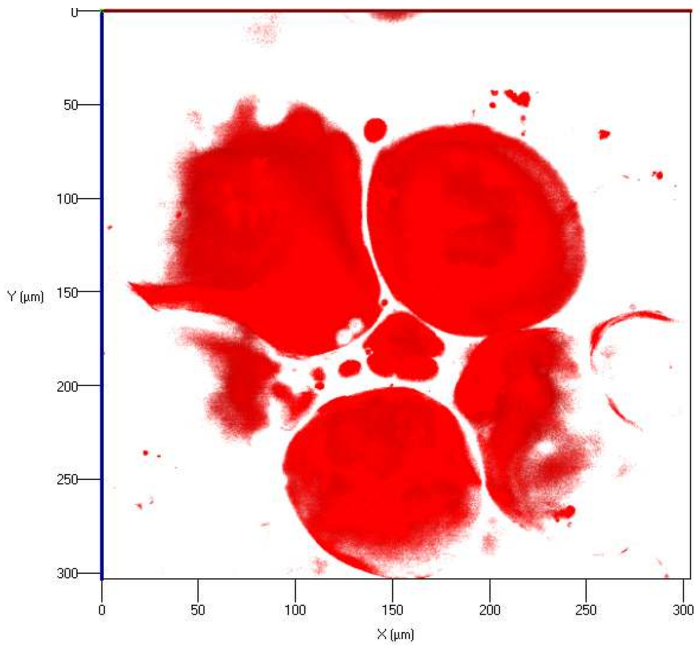
B6 kontroll CARS



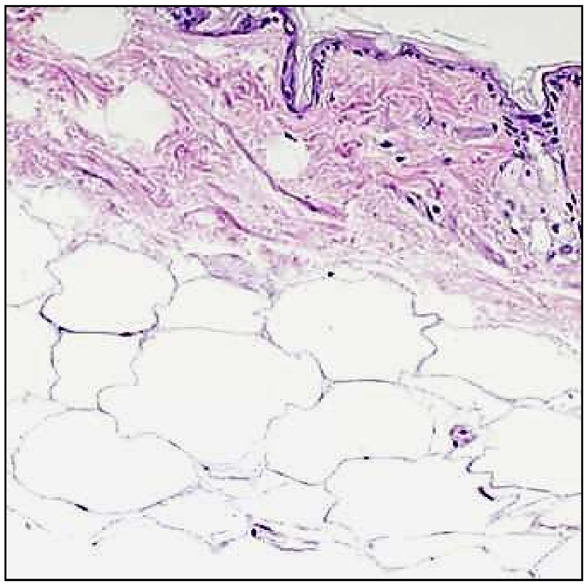
B6 kontroll HE



Ob/Ob CARS

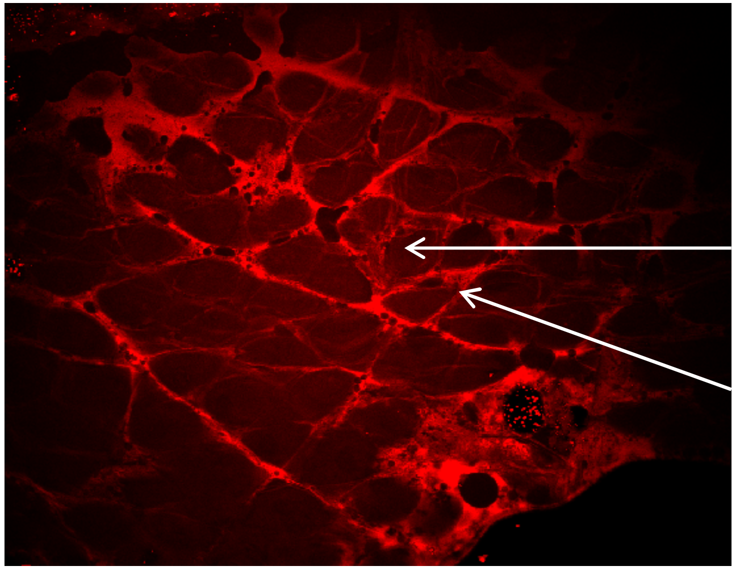


Ob/Ob HE



rutin szövettani festés

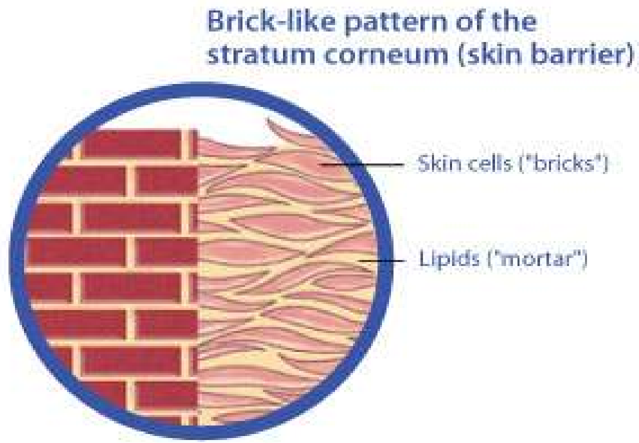
Atópiás bőrmodell – AF + CARS mérések

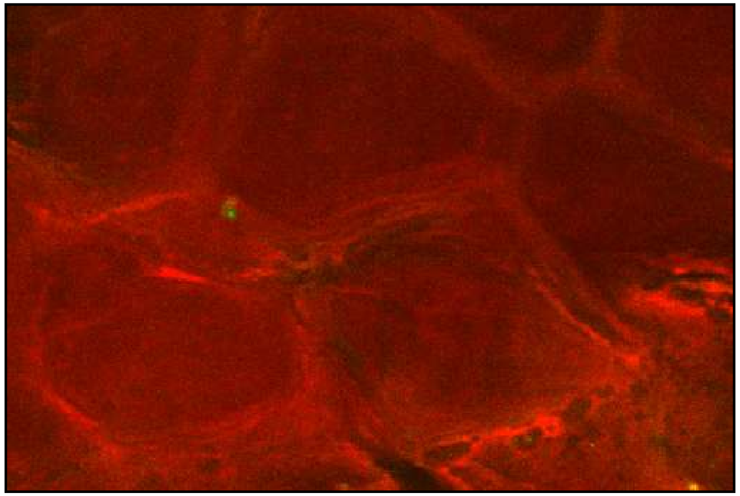


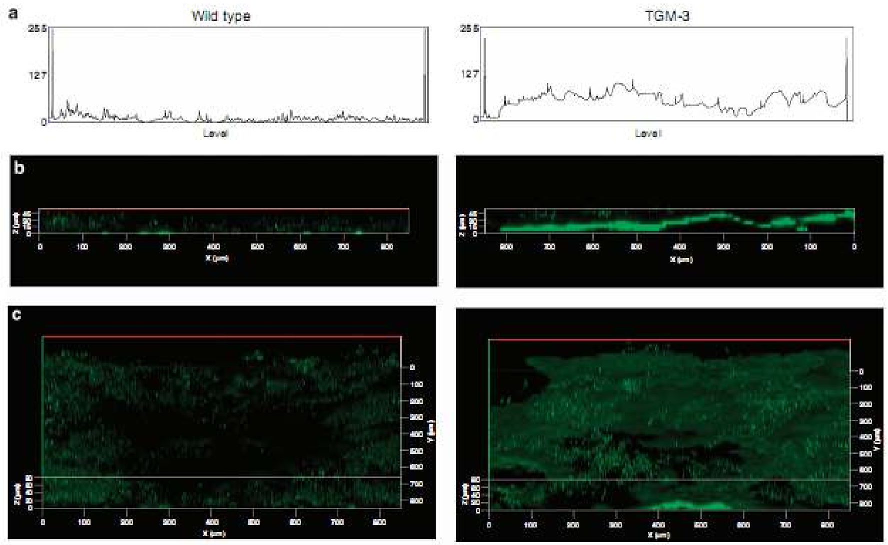
in vivo CARS

keratinociták – „tégla”

lipid – „habarcs”







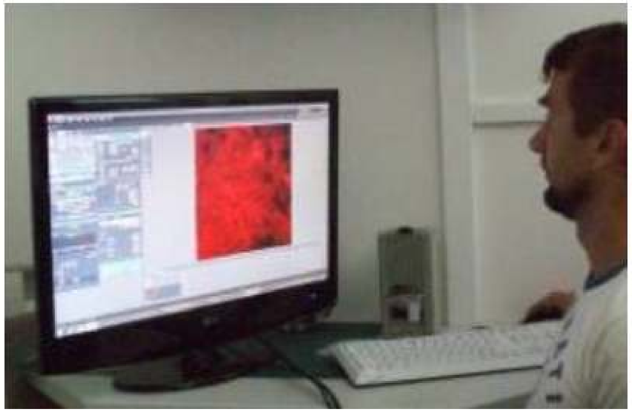
Bognar P, Nemeth I, Mayer B, Haluszka D, Wikonkal N, Ostorhazi E, John S, Paulsson M, Smyth N, Pasztoi M, Buzas EI, Szipocs R, Kolonics A, Temesvari E, Karpati S., *Reduced inflammatory threshold indicates skin barrier defect in transglutaminase 3 knockout mice.* J Invest Dermatol, **134**(1) 105-111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

CARS SYSTEM INSTALLED AT UNIVERSITY OF SZEGED

Related articles

CARS imaging system installed at the University of Szeged, Department of Neurology (Prof. Gábor Tamás lab), June 2014

Photo Gallery



HUNGARIAN BRAIN RESEARCH PROGRAM
NEMZETI AGYKUTATÁSI PROGRAM

Wigner
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CARS mikroszkópia alkalmazása az idegtudományokban (ld. Ozsvár Attila (SZTE) előadását)

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.

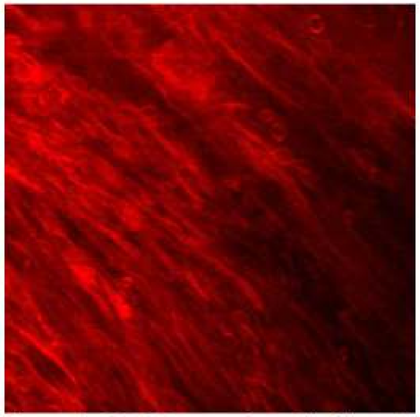

Figure 1. CARS image of myelin fibers in the white matter of rat. In vivo preparation.