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Flourescent Label Free 3D CARS Microscopy for Biomedical Imaging

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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.

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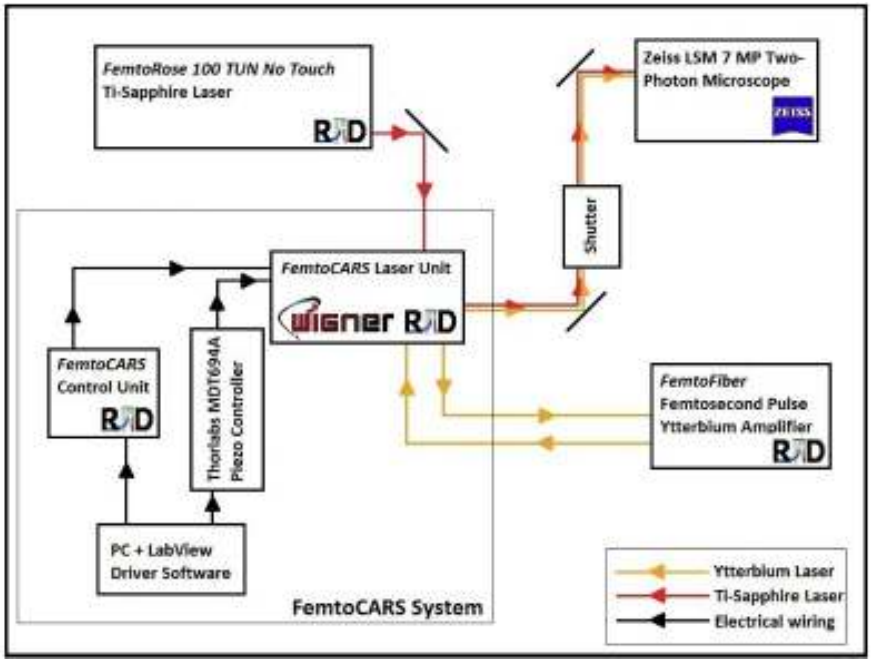
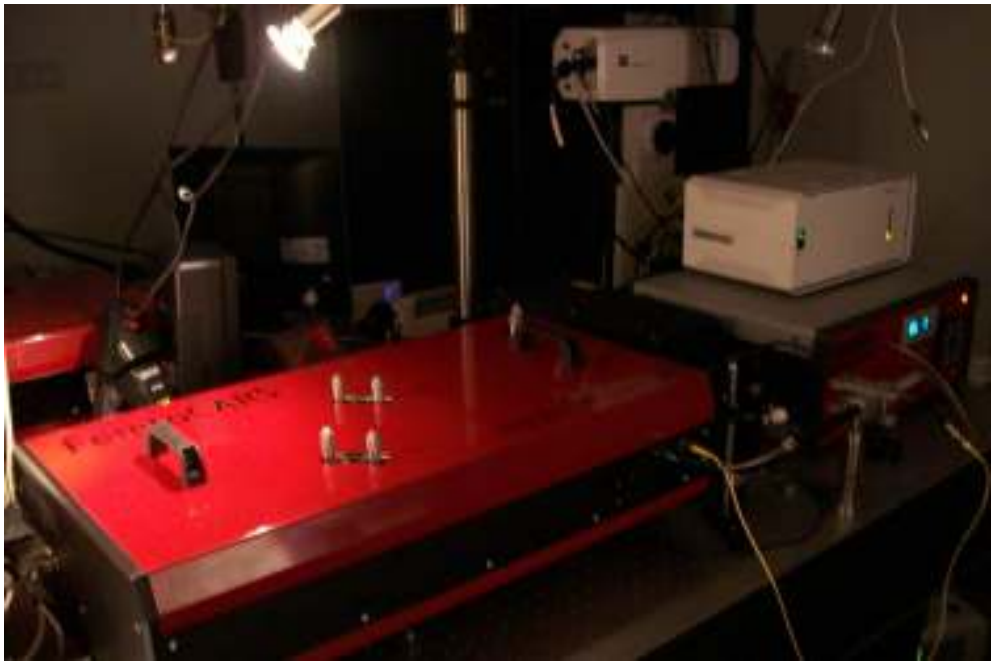
FemtoCARS

the

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





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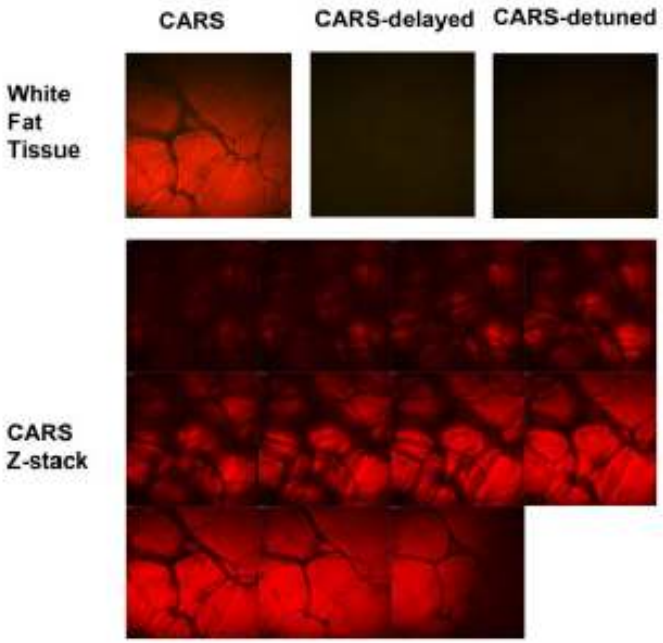
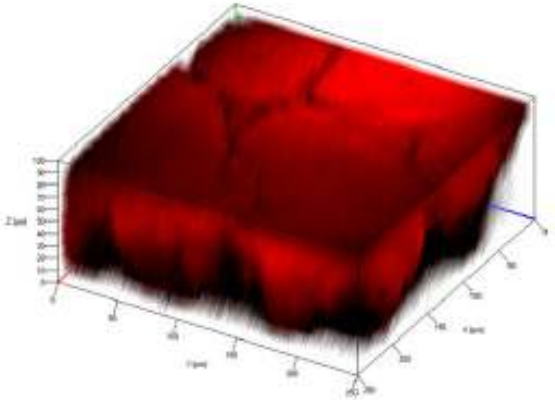


Fig. 3 CARS-images of murine white adipose tissue.



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The Label-free,

3D Microscopic Imaging System

for Brain Research

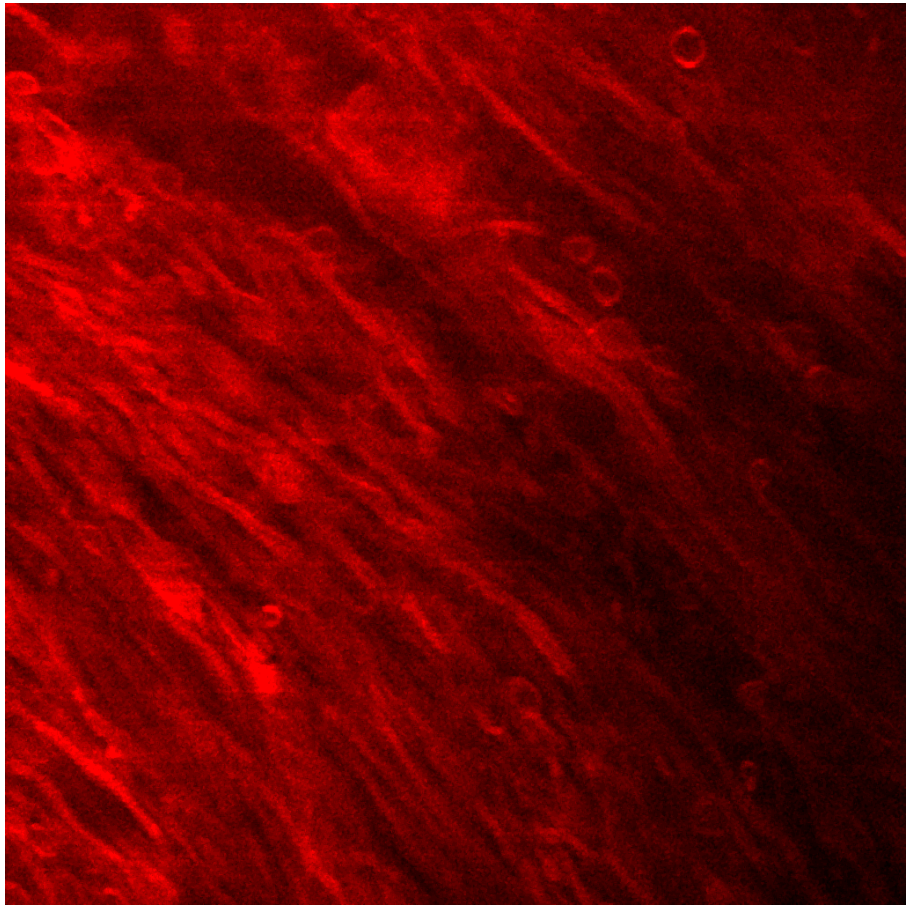

Figure 1. CARS image of myelin fibers in the white matter of rat. In vivo preparation.

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The concept

BSuJA.28.pdf Biomedical Optics and 3D Imaging OSA 2012

A simple, cost efficient fiber amplifier wavelength extension unit for broadly tunable, femtosecond pulse Ti-sapphire lasers for CARS microscopy

A. Kolonics[1,2], D. Csáti[3], P. Antal[1], R. Szipőcs[1,2,*]
[1] Research Institute for Solid State Physics and Optics, P.O. Box 49, H-1525 Budapest, Hungary
[2] R&D Ultrafast Lasers Ltd, P.O. Box 622, H-1539 Budapest, Hungary
[*] r.szipocs@szipocs.com

Abstract: An inherently synchronized Yb-fiber amplifier based extension unit for femtosecond pulse, broadly tunable Ti-sapphire lasers is introduced, which is well suited for coherent anti-Stokes Raman scattering microscopy.
OCIS codes: (140.7090) Ultrafast lasers; (180.4315) Nonlinear microscopy

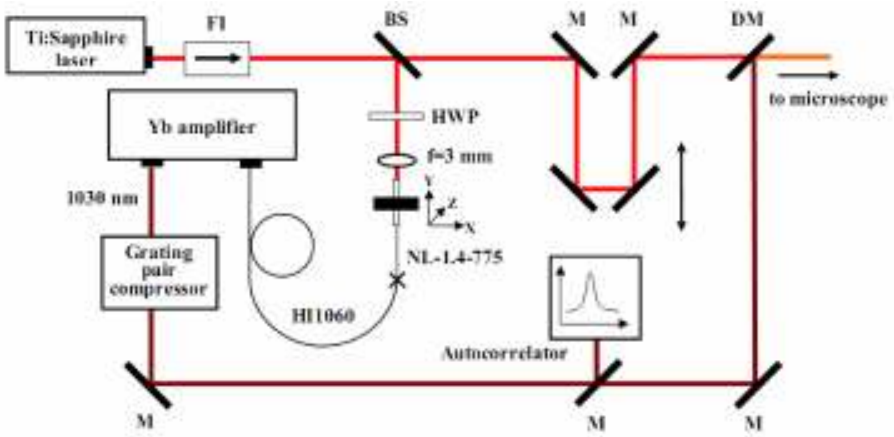

Fig. 1 Setup of the CARS extension unit

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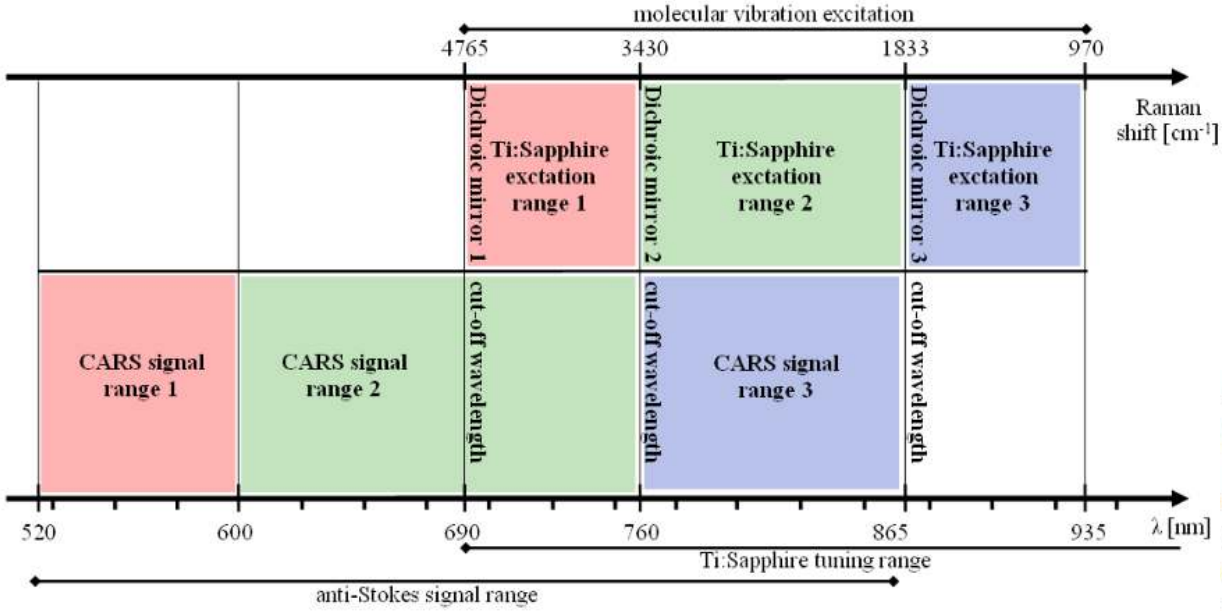

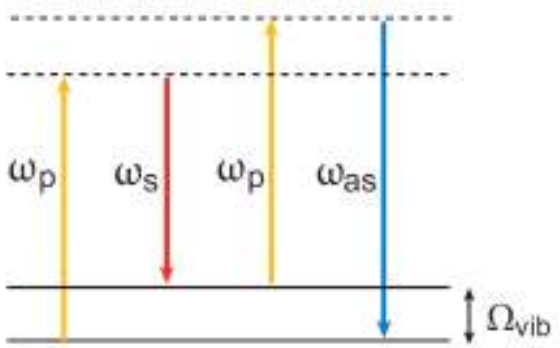

Fig. 1 The energy diagram of the CARS process: ω_p – the frequency of Ti-sapphire laser (pump), ω_S – the frequency of Yb-amplifier (Stokes), ω_{as} – the frequency of light generated during the CARS process, Ω_{vib} – vibration frequency of the investigated molecule

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How to make a CARS upgrade for an existing two-photon microscope?

- Add a CARS wavelength extension unit to your femtosecond, tunable Ti:Sapphire laser
- Replace dichroic beam splitter according to excitation and signal wavelengths
- Select laser blocking filter according to excitation wavelength in order to protect detector unit

What will you need?

FemtoCARS Unit

FemtoFiber Yb-fiber amplifier

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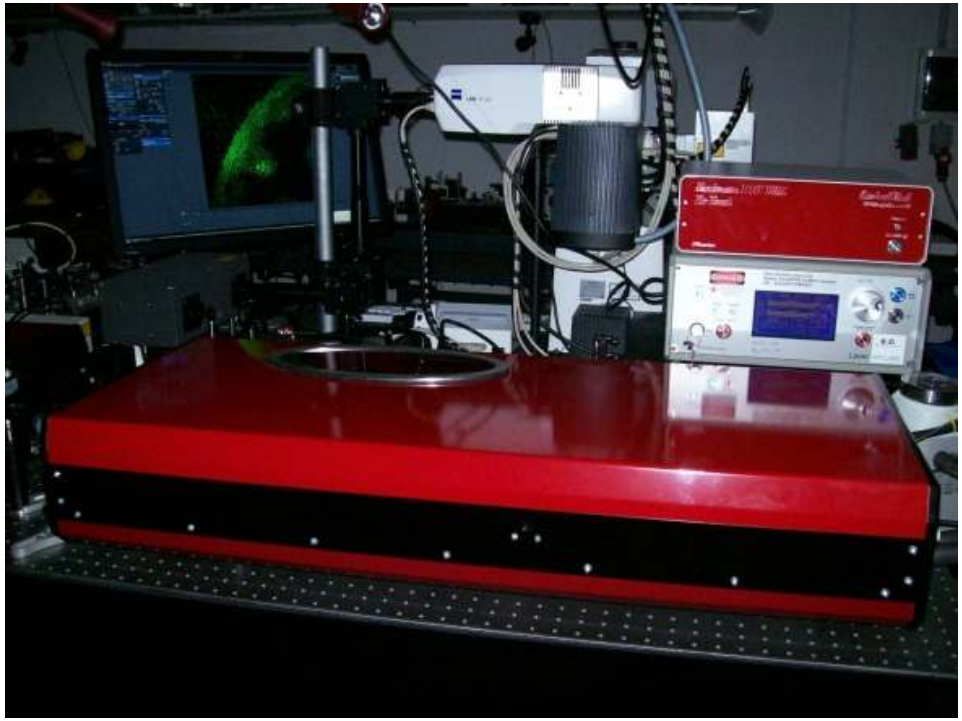
How to make a CARS upgrade for an existing two-photon microscope?



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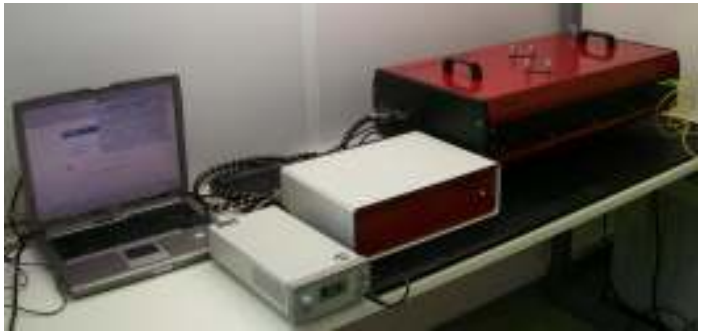

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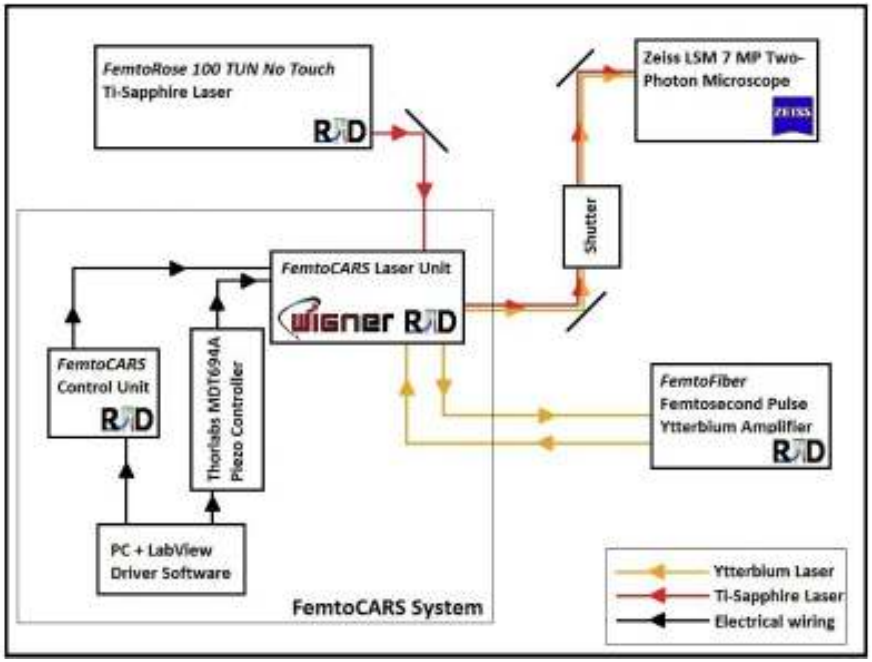

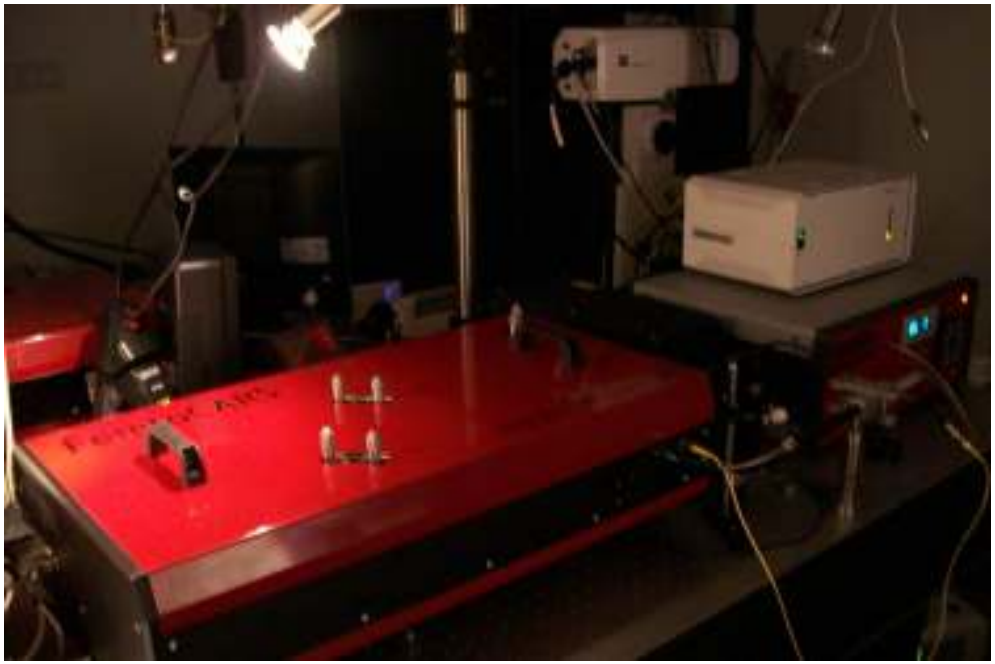

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Multicolored Stain-free Histopathology with Coherent Raman Imaging

Christian W. Freudiger[1], **Rolf Pfannl**[2], **Daniel A. Orringer**[3,4,5], **Brian G. Saar**[1,†], **Minbiao Ji**[1], **Qing Zeng**[6,7], **Linda Ottoboni**[8], **Wei Ying**[9], **Christian Waeber**[9], **John. R. Sims**[9], **Philip L. De Jager**[8,10,11], **Oren Sagher**[5], **Martin A. Philbert**[12], **Xiaoyin Xu**[6,7], **Santosh Kesari**[13,14], **X. Sunney Xie**[1], and **Geoffrey S. Young**[6,7,*]

NIH-PA Author Manuscript

Abstract

Conventional histopathology with hematoxylin & eosin (H&E) has been the gold standard for histopathological diagnosis of a wide range of diseases. However, it is not performed *in vivo* and requires thin tissue sections obtained after tissue biopsy, which carries risk, particularly in the central nervous system. Here we describe the development of an alternative, multicolored way to visualize tissue in real time through the use of coherent Raman imaging (CRI), without the use of dyes. CRI relies on intrinsic chemical contrast based on vibrational properties of molecules and intrinsic optical sectioning by nonlinear excitation. We demonstrate that multi-color images originating from CH_2 and CH_3 vibrations of lipids and protein, as well as two-photon absorption of hemoglobin, can be obtained with subcellular resolution from fresh tissue. These stain-free histopathological images show resolutions similar to those obtained by conventional techniques, but do not require tissue fixation, sectioning or staining of the tissue analyzed.

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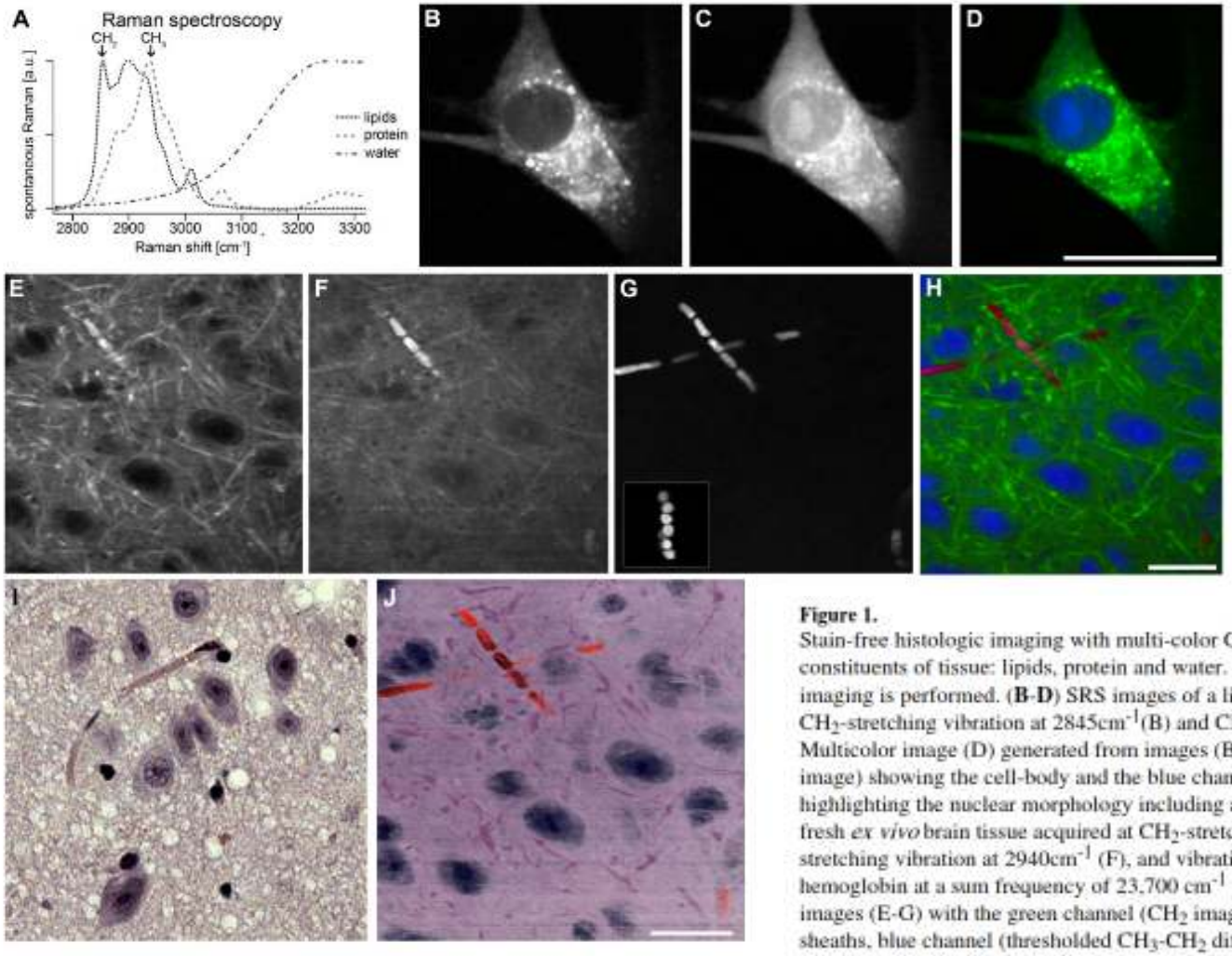

Figure 1.
Stain-free histologic imaging with multi-color CRI. (**A**) Vibrational spectra of the major constituents of tissue: lipids, protein and water. Arrows indicate Raman shifts at which imaging is performed. (**B-D**) SRS images of a live C2C12 mammalian cell acquired at the CH_2-stretching vibration at 2845cm^{-1}(B) and CH_3-stretching vibration at 2940cm^{-1} (C). Multicolor image (D) generated from images (B) and (C) with the green channel (CH_2 image) showing the cell-body and the blue channel (thresholded CH_3-CH_2 difference image) highlighting the nuclear morphology including a bright nucleolus. (**E-H**) SRS images of fresh *ex vivo* brain tissue acquired at CH_2-stretching vibration at 2845cm^{-1} (E), CH_3-stretching vibration at 2940cm^{-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 (CH_2 image) highlighting cytoplasm and myelin sheaths, blue channel (thresholded CH_3-CH_2 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**) Same 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.

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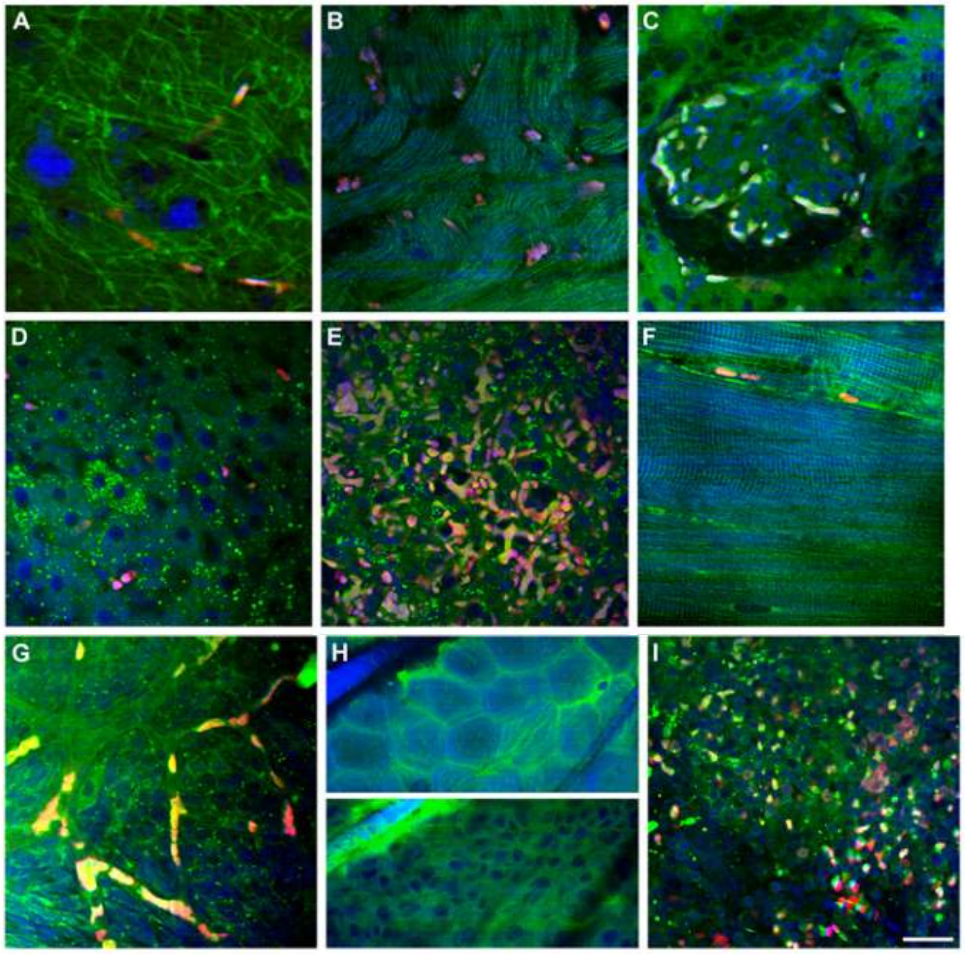

Figure 2.
Multicolor stain-free images of various mouse organs (green: CH_2 image; blue: CH_3-CH_2 difference image; red: hemoglobin image) of (A) brain, (B) heart, (C) kidney, (D) liver, (E) lung, (F) muscle, (G) ovary, (H) skin with *stratum corneum* (top) and *stratum basale* (bottom) and (I) spleen. Scale bar, 25 μm.

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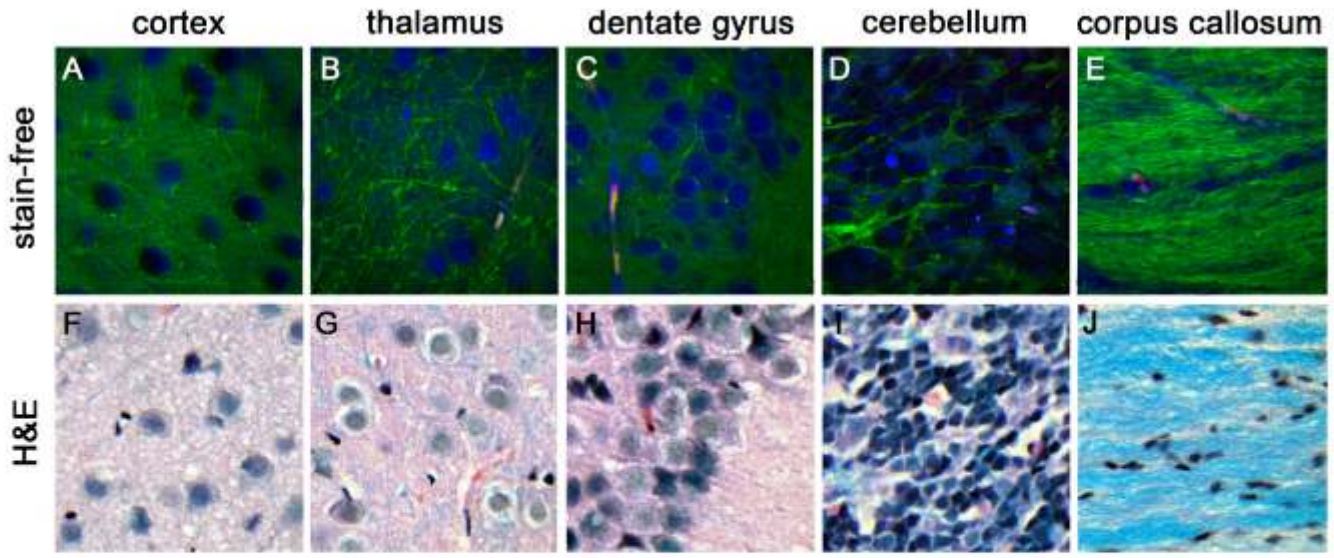

Figure 3.
Multicolor stain-free images of various brain regions in a wild-type mouse in comparison with paraffin-embeded, H&E and Luxol-stained sections.(green: CH_2 image; blue: CH_3-CH_2 difference image; red: hemoglobin image) of (**A**) cortex, (**B**) thalamus, (**C**) dentate gyrus, (**D**) cerebellum, and (**E**) corpus callosum. (**F**-**J**) show H&E/luxol stained section of corresponding regions.

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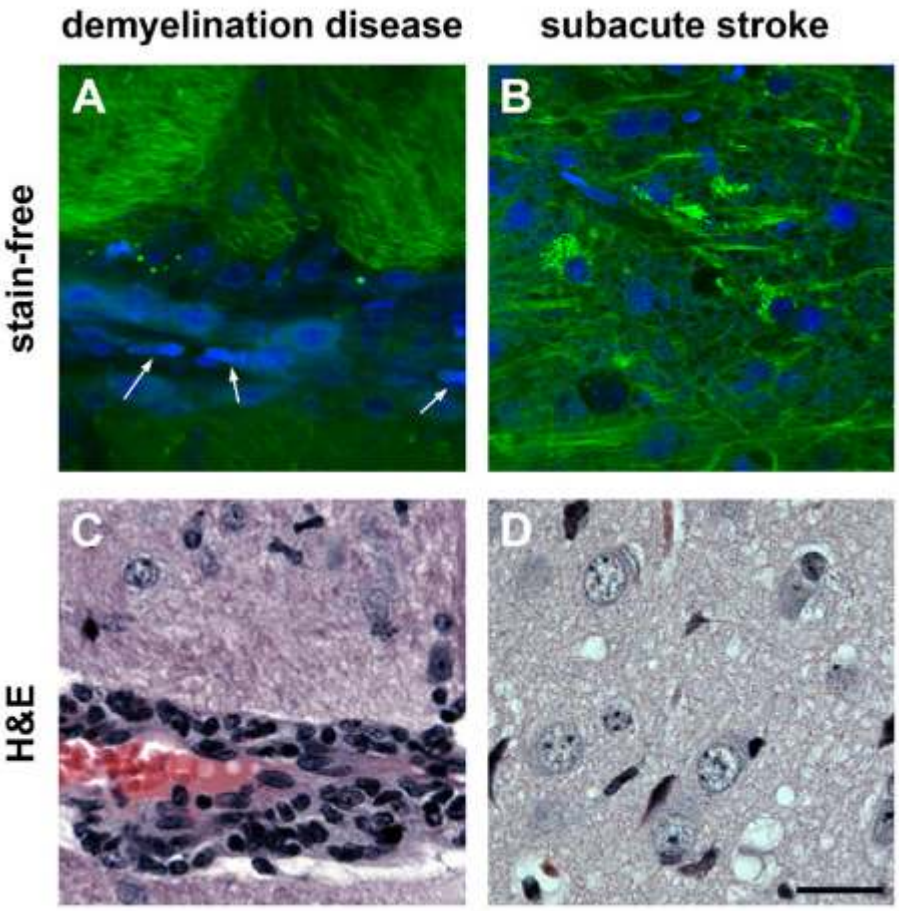

Figure 5.
SRS label-free images of demyelination and stroke mouse models. (**A**) Two-color SRS image (green: CH_2 image; blue: CH_3-CH_2 difference image) of a demyelinating lesion in a mouse with experimental allergic encephalomyelitis (EAE). (A) Perivascular inflammatory cells and histiocytes with large lipid droplets from phagocytized myelin are visible. Red blood cells in a capillary are highlighted by arrows (Hemoglobin image, red, not used in this picture). (**B**) Two-color SRS image of brain tissue in a mouse stroke model three days post-stroke. Macrophages with many small lipid droplets are dispersed throughout the tissue. Dark areas represent edema fluid. (**C,D**) H&E stained micrographs of (C) EAE and (D) stroke model from the same region. Scale bar, 25 μm.

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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*

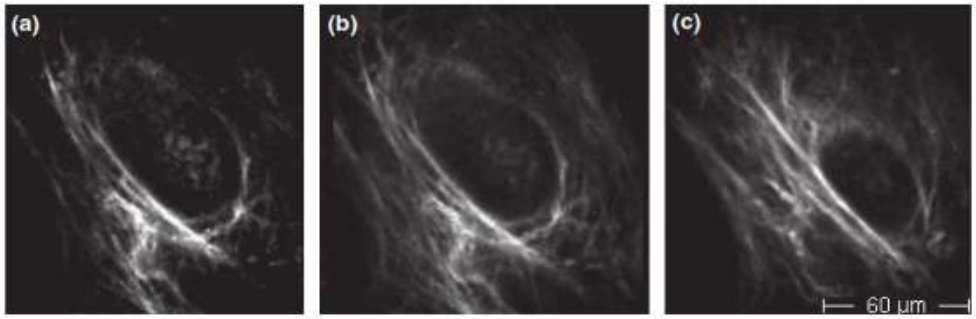


Fig. 4. Basal cell carcinoma. (a) 100 µm depth, excitation wavelength 800 nm. Shifting the wavelength to 800 nm, basaloid cells become less visible; employing an excitation wavelength of 820 nm basaloid cells disappear and it is possible to observe empty spaces surrounded by collagen fibres (phantom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

2P mikroszkópiás mérések, BCC vizsgálata

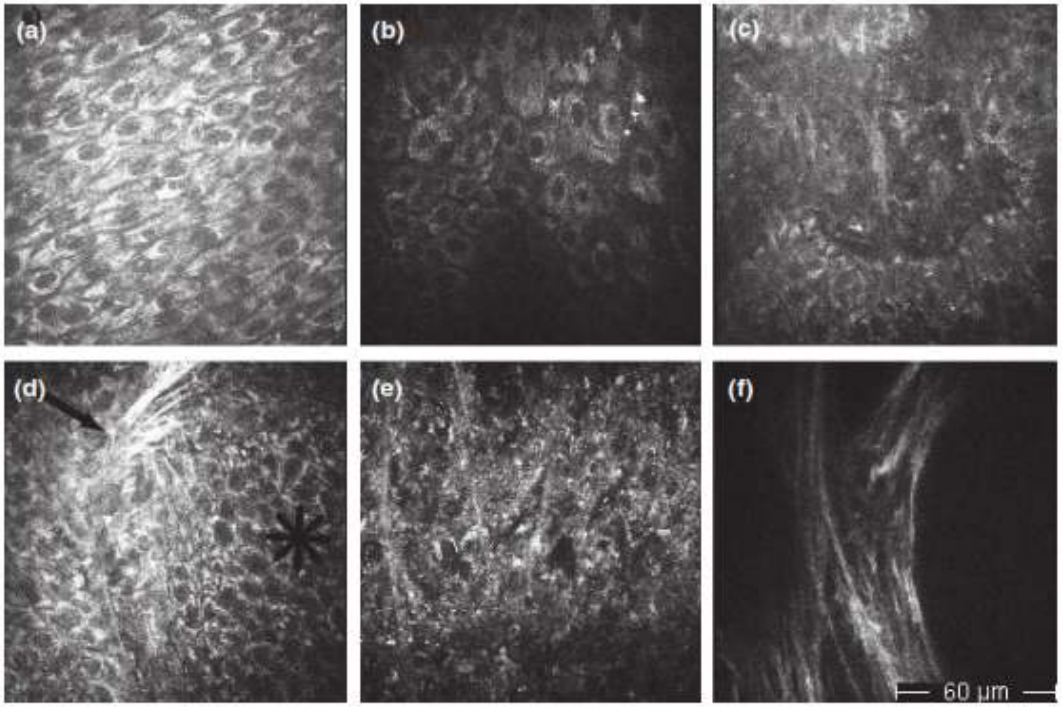
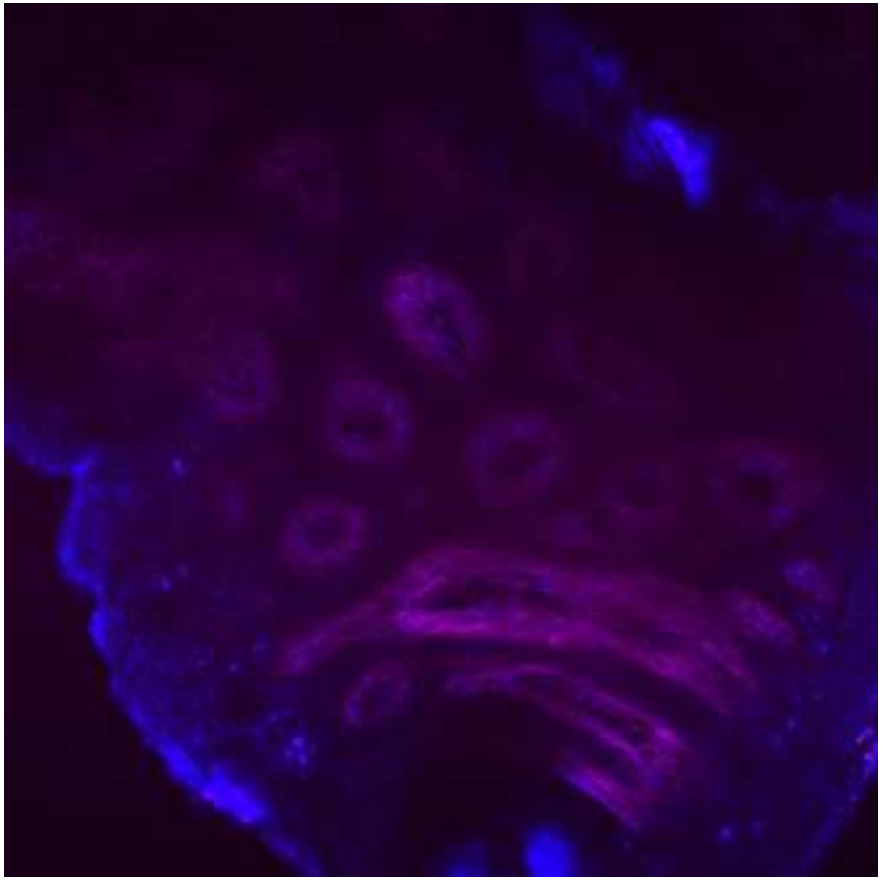


Fig. 3. Descriptors of basal cell carcinoma. (a) 50 µm depth with an excitation wavelength of 760 nm. Basaloid cells are disposed in a regular order; they are aligned and elongated in the same direction, showing elongated shape and nuclei, and tightly packed together; (b) 50 µm depth with an excitation wavelength of 760 nm. Sheets of basaloid cells are elongated and aligned in two different directions; (c) 80 µm depth employing an excitation wavelength of 760 nm. At the periphery of basaloid nests, the cells are oriented perpendicularly to the surrounding extracellular matrix (palisading); (d) 90 µm depth with an excitation wavelength of 760 nm. A basaloid nodule (asterisk) is observable as a cell aggregate surrounded by fibres (arrow); (e) 30 µm depth employing an excitation wavelength of 760 nm: sheets of cells intermingled with fibres; (f) 75 µm depth with an excitation wavelength of 800 nm. Increasing the wavelength to 800 nm basaloid cells disappear and only the fibres of the extracellular matrix are visible (phantom island).

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

FiberScope, a kézben tartott nemlineáris mikroszkóp

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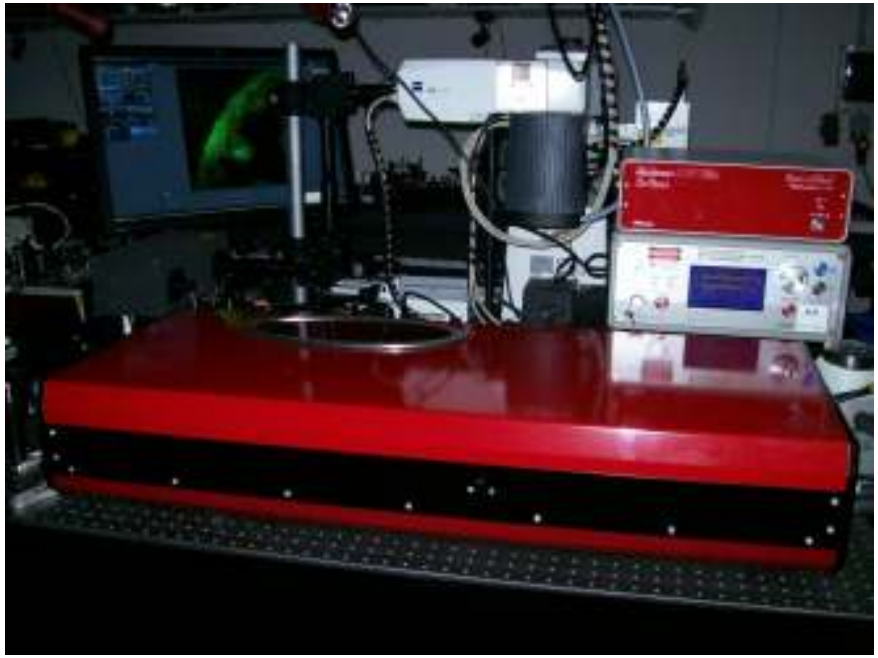
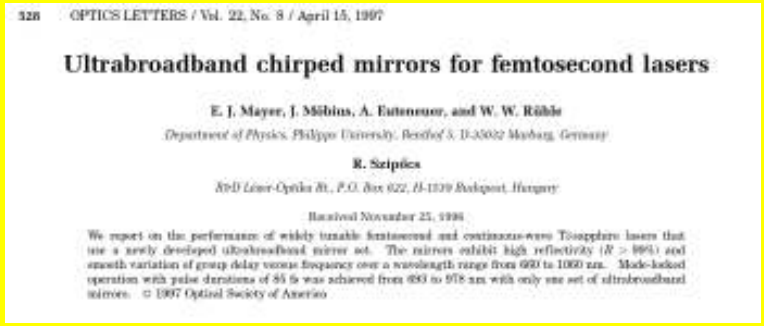
The First
Broadly Tunable, femtosecond pulse Ti:sapphire laser



Pioneering Ultrafast LaserTechnology by R&D

INTRODUCING THE FIRST ULTRABROADBAND CHIRPED MIRRORS FOR BROADLY TUNABLE FEMTOSECOND LASERS



328 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 Laser-Optika Rt., 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 680 to 978 nm with only one set of ultrabroadband mirrors. © 1997 Optical Society of America

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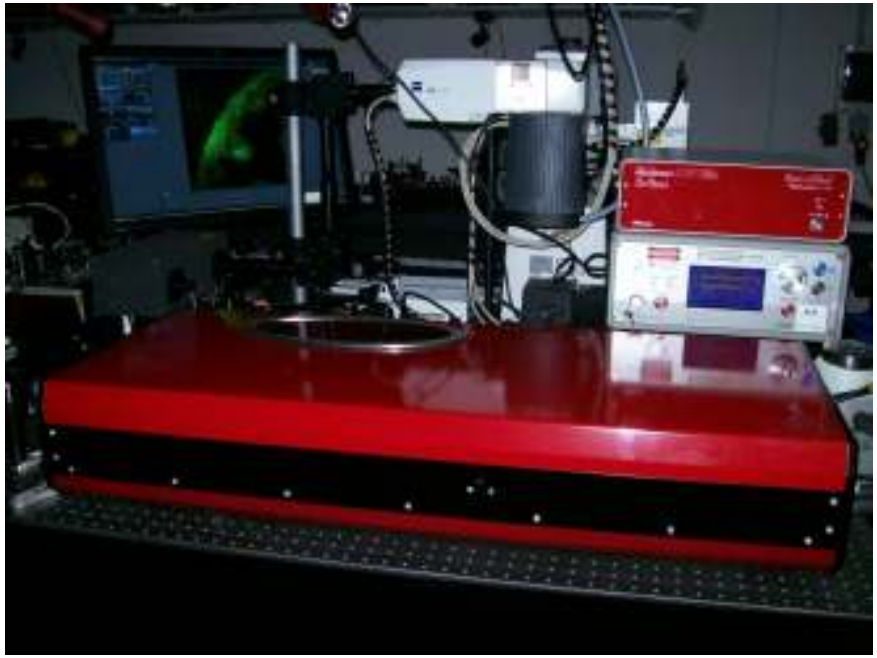

- Stable, easy mode-locking (with starter electronics)
- Soliton-like, nearly transform-limited pulses
- Patented Ultrabroadband Chirped Mirror™ optics
  – single optics set from 680 to 1040 nm
- Built in Millennia™ / Verdi™ / Finesse™ pumping (6W, 8W, 10W) – diode-pumped stability
- Sealed, purgeable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

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Long-Cavity, Broadly Tunable, Femtosecond Pulse Ti:sapphire Laser

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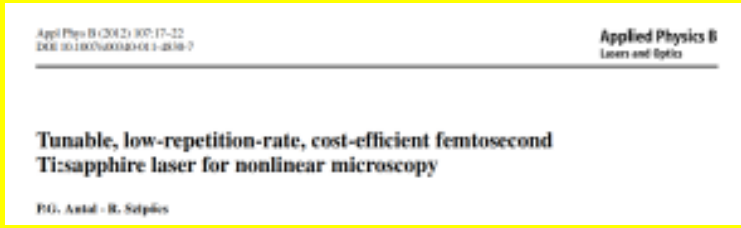
Appl Phys B (2012) 107:17–22
DOI 10.1007/s00340-011-4838-7

Applied Physics B
Lasers and Optics

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

P.G. Antal · R. Szipőcs

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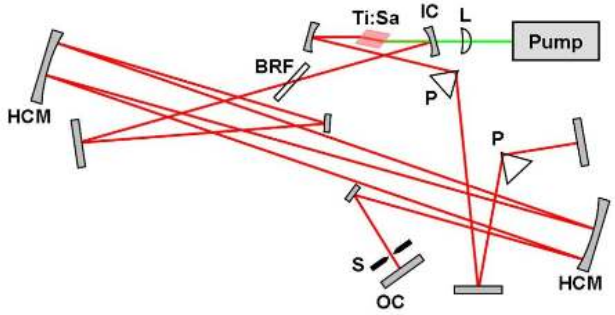
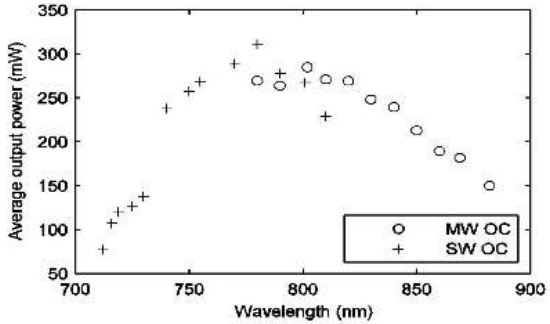
The Concept

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).

Reference

Antal P, Szigligeti A, Kolonics A, Szipőcs R; Tunable, Low Repetition Rate, Femtosecond Pulse Ti:Sapphire Laser for In Vivo Imaging by Nonlinear Microscopy; In: Optics int he Life Sciences Congress (OSA, 4-6 April 2011, Monterey, CA) Paper JTuA12, 2011

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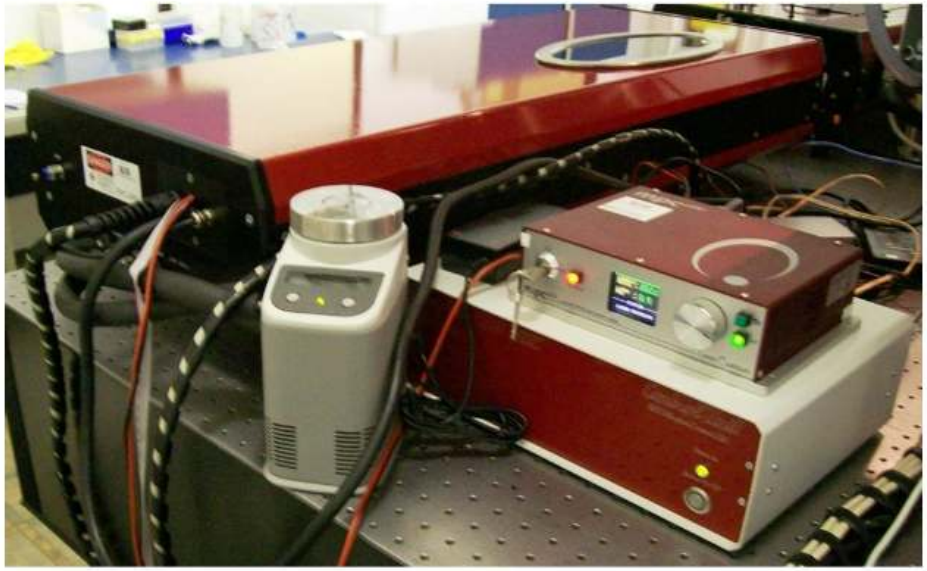
FemtoRose 300 TUN/NoTouch

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

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- Ultrafast spectroscopy

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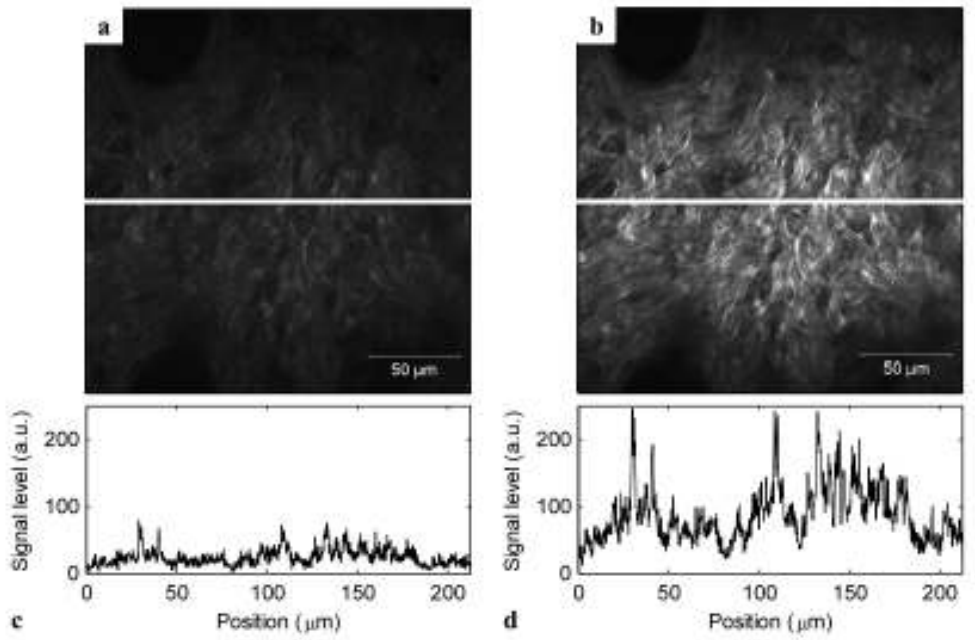
Low Average Power, High Quality Imaging in Two-Photon Microscopy

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*



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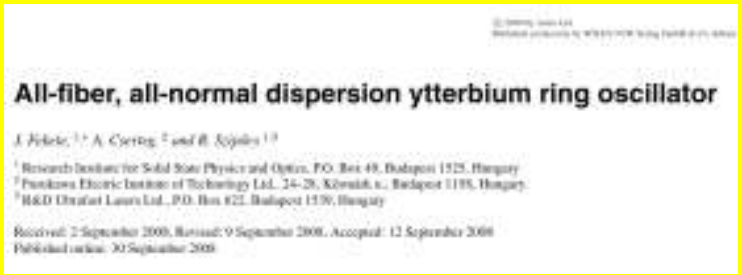
INTRODUCING THE FIRST ALL-FIBER, ALL NORMAL DISPERSION, FEMTOSECOND Yb-FIBER LASER



All-fiber, all-normal dispersion ytterbium ring oscillator

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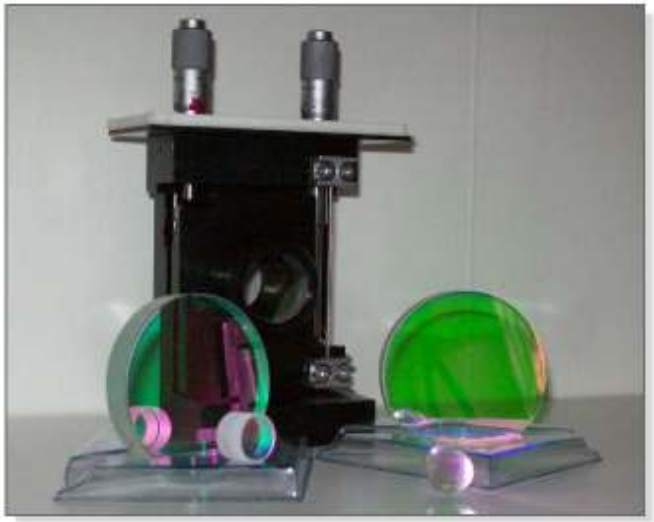

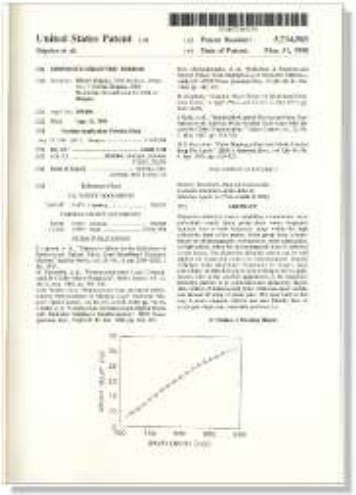

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