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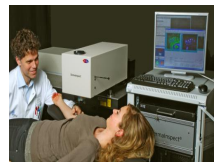
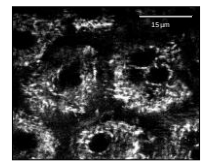
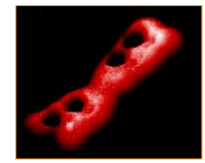
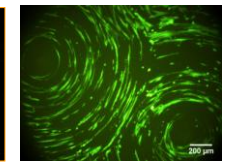
Multiphoton Microscopy

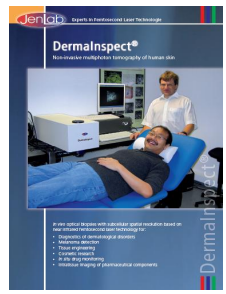
Cellular, tissular and structural imaging











H. G. Breunig

K. König

JenLab

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JenLab GmbH

- Spin-off High-Tech company from the Medical Faculty of the University Jena founded in Sept 1999
- Headquarter (manufacturing, clinical application): Jena

  Jena: birthplace of modern optics (Abbe, Zeiss, Schott), 250 km from Berlin
- Research Center: University Campus Saarbrücken

  on the German-French border (110 min from Paris by TGV / ICE)

Goal

Development of bioinstrumentation
based on femtosecond-laser technology

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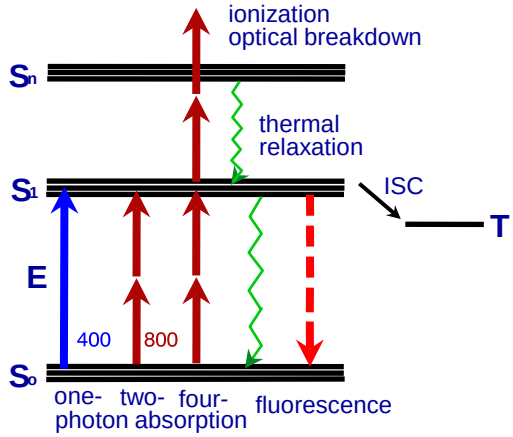
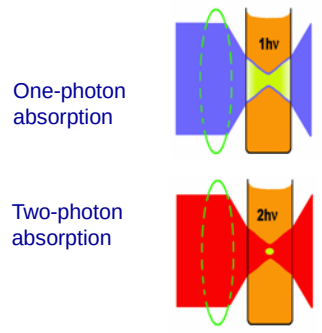
Absorption processes



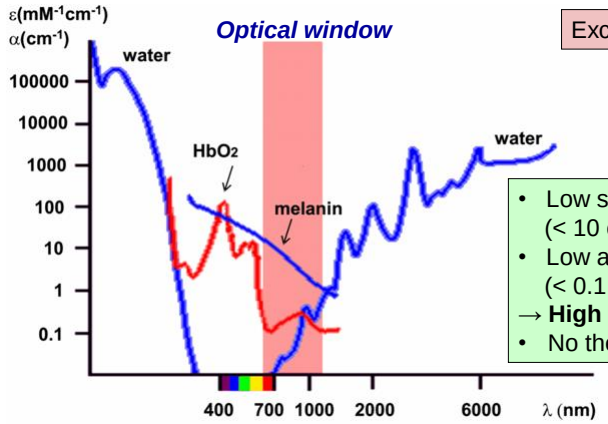
Excitation volumes



Two- and three photon excitation → MW/cm² - GW/cm²

Four- and more photon excitation → TW/cm²

Advantages of *multi*photon tomography



Excitation in NIR range

- Low scattering coefficients (< 10 cm^{-1})
- Low absorption coefficients (< 0.1 cm^{-1})

→ **High light penetration depth**

- No thermal heating

- Highly localized excitation volume
  Fluorescence and SHG based optical sectioning (no pinhole necessary)
- Reduced photostress / photobleaching
  (excitation takes place in the focal volume only)

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Turn-key femtosecond laser sources

compact one-box tunable fs lasers

MaiTai (Spectra Physics): 720 – 980 nm, 80 fs, 80 MHz
Chameleon (Coherent): 680 – 1080 nm, 140 fs, 80 MHz

Integral (Femtolasers): 780±50 fs, 10 fs, 85 MHz
Octavius (MenloSystems) 800 nm, 5 fs, 1 GHz

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Fluorescent biomolecules / SHG-active collagen

Fluorophore	Emission (nm)	Lifetime τ (ns)
NADH(P)H free	460	0,3
NADH(P)H-protein	440	2,0-2,3
Flavines	530	5,2
Elastin	420-460	0,3/ 2
Collagen	420-460	0,3/ 2
SHG	λ/2	0
Melanin	440, 520, 575	0,1/1,9/8
PPIX	635, 710	10-12

Review article: K. König (2008): *Clinical multiphoton tomography*, J. Biophotonics **1**,13–23

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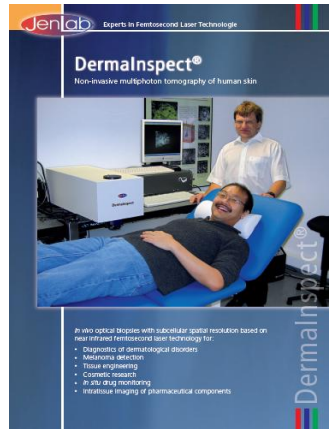
Manufacturer of the world's first clinical Multiphoton Tomograph

DermaInspect®



CE marked medical device

High resolution *in vivo* imaging: optical biopsies

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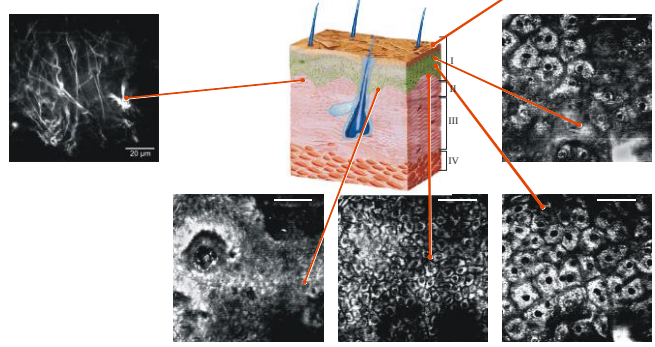
View inside the skin with DermaInspect

I Epidermis

A Stratum corneum
B Stratum granulosum
C Stratum spinosum
D Stratum basale

II-IV Dermis

II Corium: Stratum papillare
III Corium: Stratum reticulare
IV Corium: Subcutaneous layer



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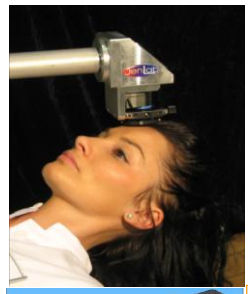
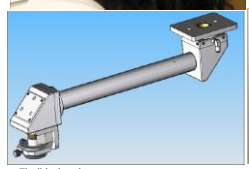
In vivo Multiphoton Tomography of Human Skin

no biopsy required, no stain required
5D tissue imaging tool (5D-IVT)
with submicron spatial resolution
and 270 ps temporal resolution (FLIM)
and 10 nm spectral resolution



Costumers

Beiersdorf AG Hamburg, L'Oreal, Fraunhofer Society, companies at Berlin, Tokyo …
hospitals in Paris, Jena, Lübeck, Modena, London, Brisbane, Magdeburg, Münster, Saarbrücken, Risoe, Berlin



Flexible head

80 MHz/90MHz femtosecond laser
(MaiTai, Chameleon)
in situ pJ pulse energies

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MPT*flex*

second generation mobile multiphoton tomograph with flexible arm

The new CE-marked multiphoton tomograph MPT*flex*

Released at Photonics West 2010, San Fransisco

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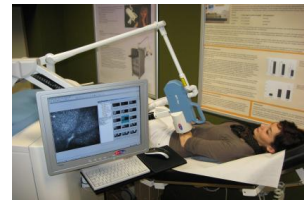
Novel flexible clinical multiphoton tomograph MPT*flex*

Setup:

- **Ti:Sapphire laser:**
- MaiTai (Spectra Physics, CA)
- Pulse duration ≤ 100 fs
- 710 – 920 nm tuneable wavelength
- 80 MHz repetition rate

- **optical unit:**
- optical power attenuator
- beam stabilization device
- safety unit

- **articulated mirror arm:**
- optimized for IR-radiation



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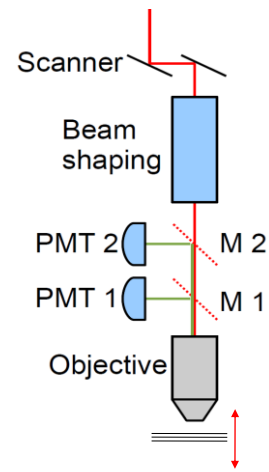
Novel flexible clinical multiphoton tomograph MPT*flex*

Setup:

- **detector head:**
- scanner (xy-scanning)
- beam shaping optics
- piezo-driven high NA focusing optics
- dual-channel detectors
- z-scan (optical sectioning)

- **control hardware:**
- controls dual-channel detectors
- 3D- scanning (optical sectioning)
- detection mode: current-mode
  SPC-mode



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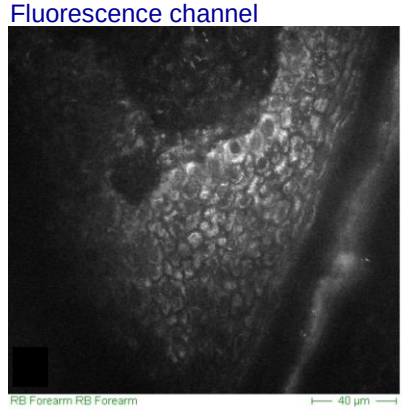
Dual-Channel Signal Detection with MPT*flex*

NAD(P)H/elastin | **collagen (SHG)**

Fluorescence channel



SHG channel

z=80 µm

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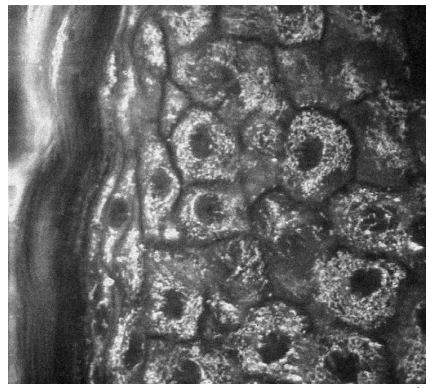
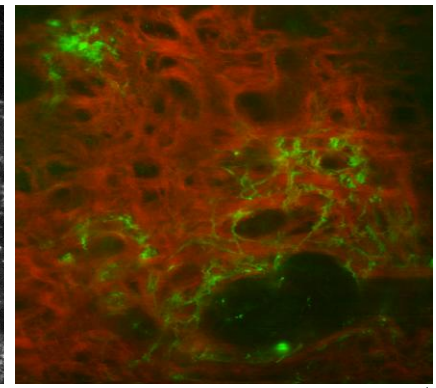
Examples:

Cells of stratum granulosum



Stratum Granulosum; z= 15 µm

Collagen structures/ Elastin fibers



Dermis; z=100 µm

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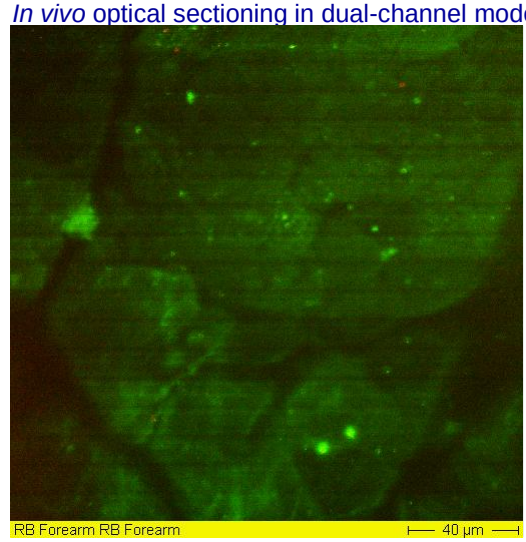
In vivo optical sectioning in dual-channel mode:

Examples:

z = 5 µm

Δz = 5 µm



512x515 pixels; dual-channel (merged)

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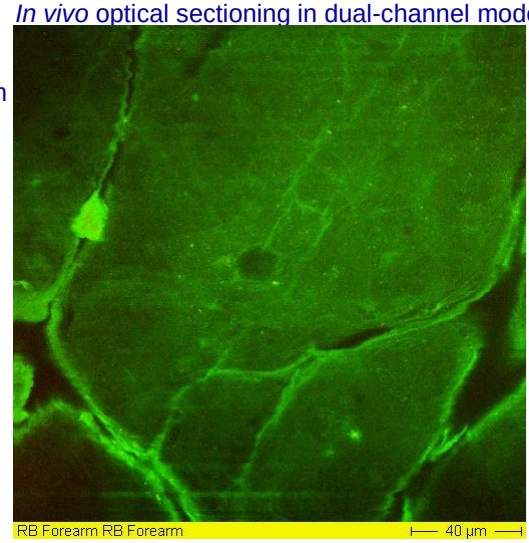
In vivo optical sectioning in dual-channel mode:

Examples:

z = 10 µm

Δz = 5 µm



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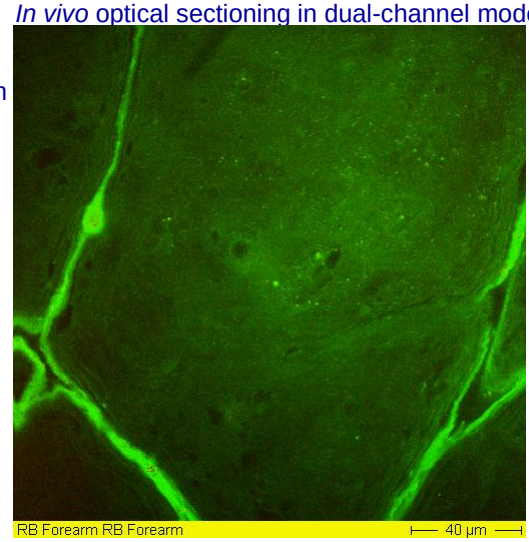
In vivo optical sectioning in dual-channel mode:

Examples:

z = 15 µm

Δz = 5 µm



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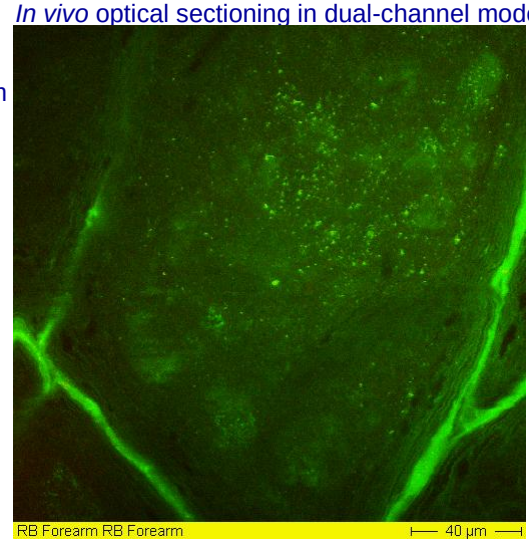
In vivo optical sectioning in dual-channel mode:

Examples:

z = 20 µm

Δz = 5 µm



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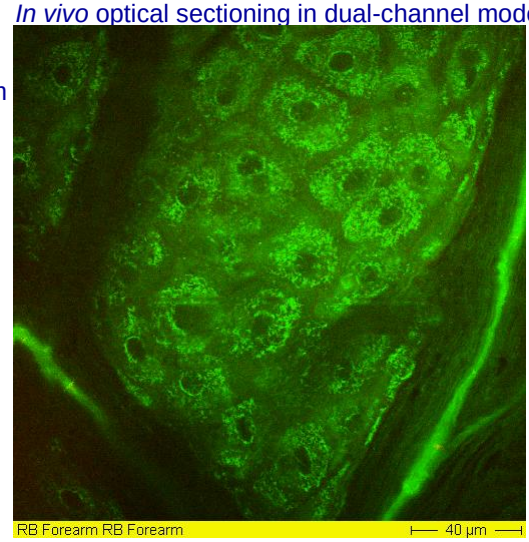
In vivo optical sectioning in dual-channel mode:

Examples:

z = 25 µm

Δz = 5 µm



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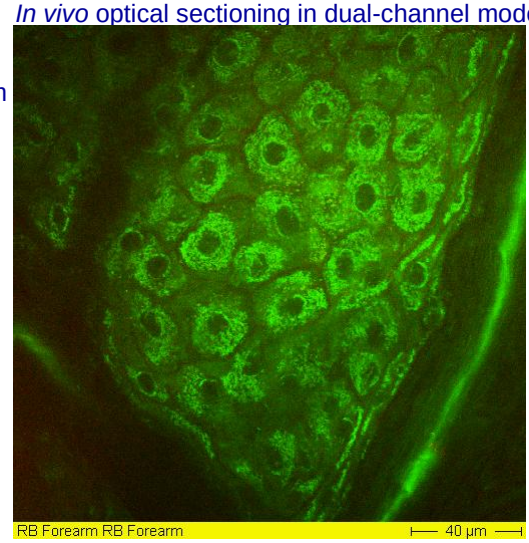
In vivo optical sectioning in dual-channel mode:

Examples:

z = 30 µm

Δz = 5 µm



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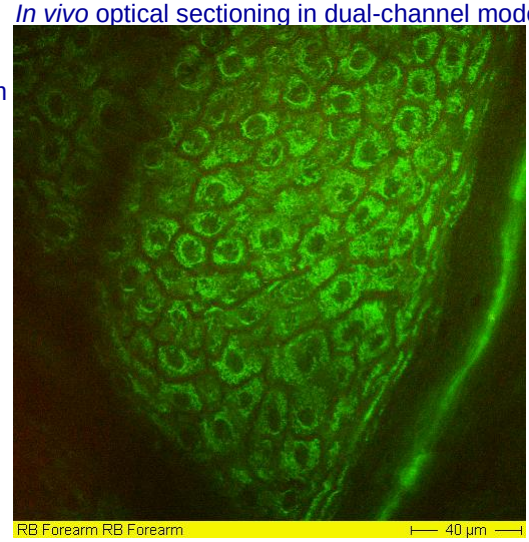
In vivo optical sectioning in dual-channel mode:

Examples:

z = 35 µm

Δz = 5 µm



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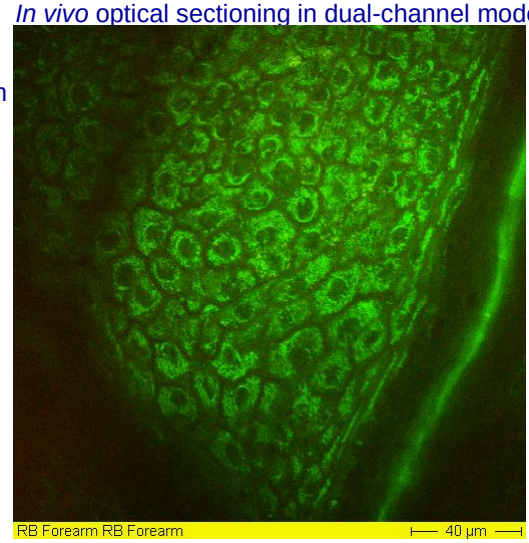
In vivo optical sectioning in dual-channel mode:

Examples:

z = 40 µm

Δz = 5 µm



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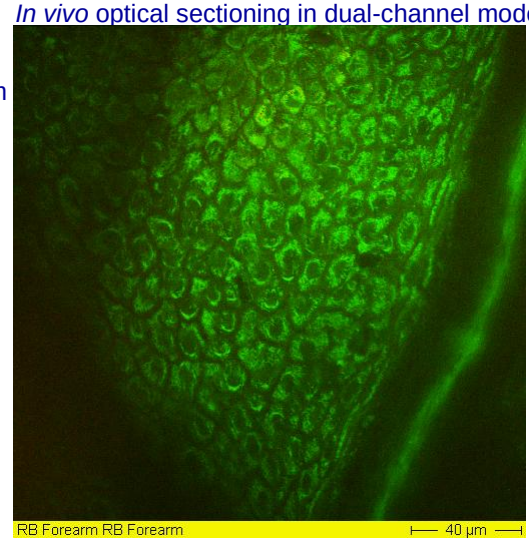
In vivo optical sectioning in dual-channel mode:

Examples:

z = 45 µm

Δz = 5 µm



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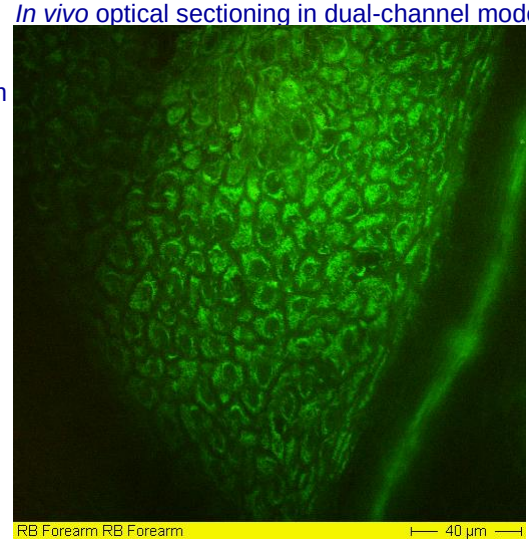
In vivo optical sectioning in dual-channel mode:

Examples:

z = 50 µm

Δz = 5 µm



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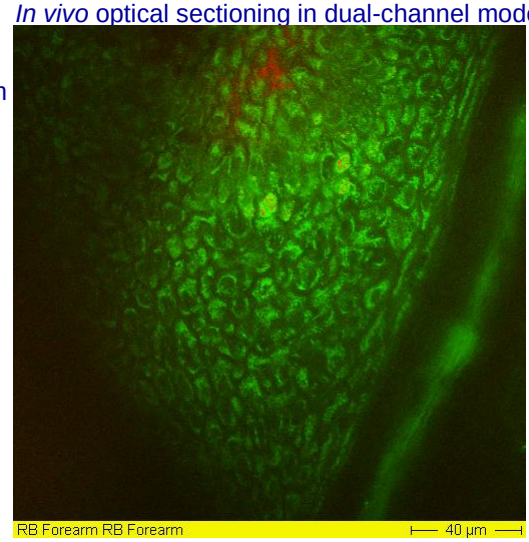
In vivo optical sectioning in dual-channel mode:

Examples:

z = 55 µm

Δz = 5 µm



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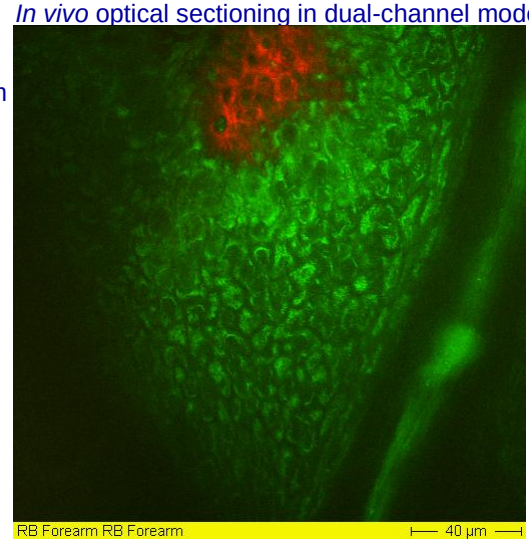
In vivo optical sectioning in dual-channel mode:

Examples:

z = 60 µm

Δz = 5 µm



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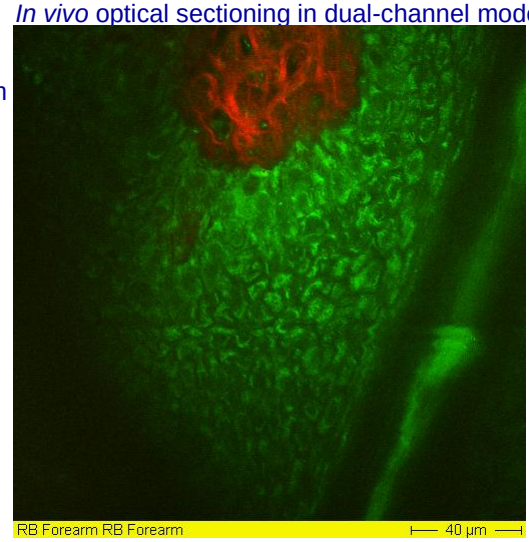
In vivo optical sectioning in dual-channel mode:

Examples:

z = 65 µm

Δz = 5 µm



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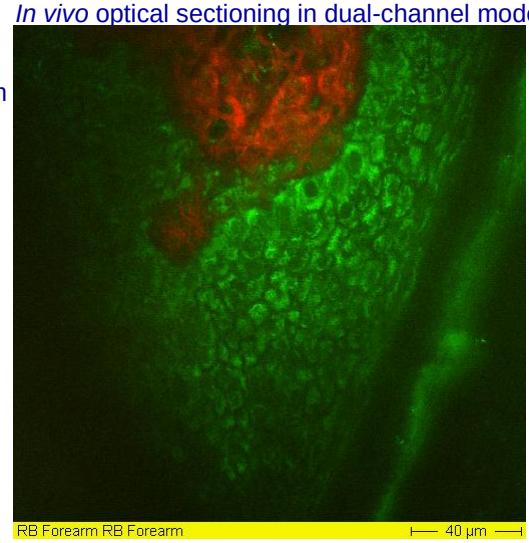
In vivo optical sectioning in dual-channel mode:

Examples:

z = 70 µm

Δz = 5 µm



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Skin aging index SAAID

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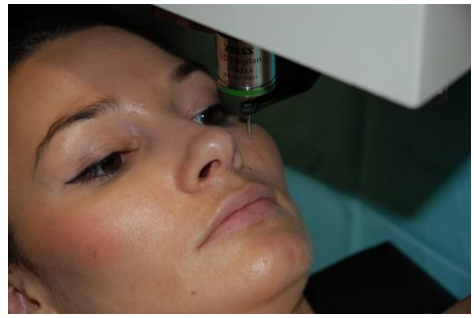

$$SAAID=(I_{SHG}-I_{AF})/(I_{SHG}+I_{AF})$$

Köhler, König, Elsner, Bückle, Kaatz.
In vivo assessment of human skin aging by multiphoton laser scanning tomography.
Optics Letters **31** (2006)

Endoscopy: K. König, A. Ehlers, I. Riemann, S. Schenk-L, R. Bückle, M. Kaatz.
Clinical Two-Photon Microendoscopy.
Microscopy Research and Technique **70** (2007)

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In vivo ZnO nanoparticles penetration into human skin (forearm)

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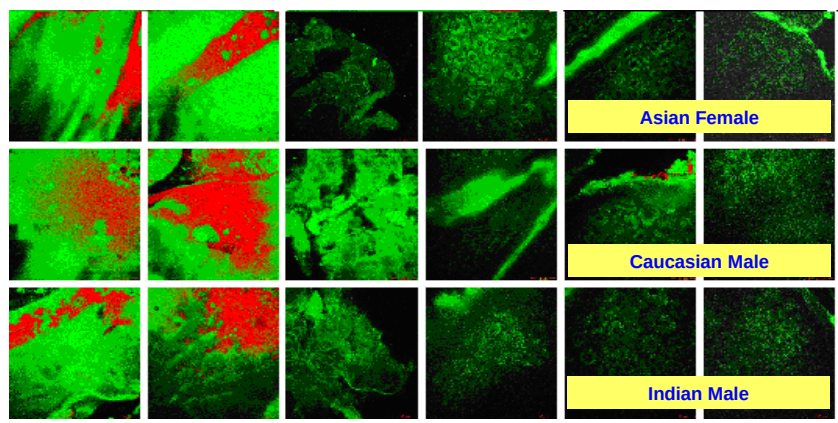
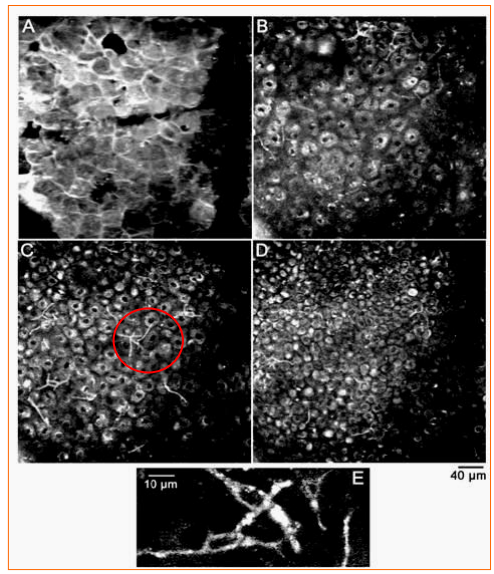


Australia's Therapeutic Goods Agency (TGA's) perspective:
"The weight of current evidence is that they (ZnO and TiO_2) remain on the surface of the skin and in the outer dead layer (stratum corneum) of the skin."

Michael S. Roberts *et al.*, *In vitro and in vivo imaging of xenobiotic transport in human skin and in the rat liver.* J. Biophoton. **1**(6) (2008)

Autofluorescence image of human malignant melanoma *in vivo*



3D imaging:
Dendritic cells (melanocytes) of patients with malignant melanoma

Diagnostic classification:
- Sensitivity value range: **71–95%**
- Specificity value range: **69–97%**
- Accuracy values up to **97%** (by logistic regression analysis)

Dimitrow, Kaatz, Ziemer, Köhler, Norhauer, König.
Sensitivity and specifity of multiphton laser tomography for in vivo and ex vivo diagnosis of malignant melanma.
J. Invest. Dermatol. **129**(7), 2009

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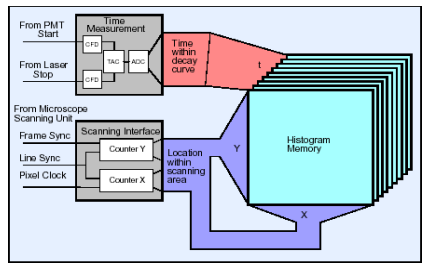
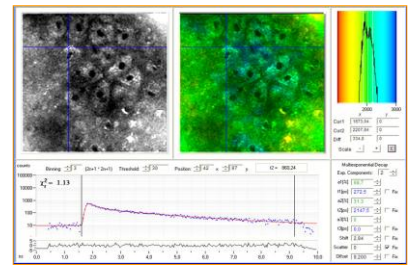
Fluorescence Lifetime Imaging (FLIM) of human skin

time-correlated single photon counting (SPC 830, Becker&Hickl GmbH)



(λ: 800 nm, z: 15 µm).
left : fluorescence intensity image
middle: FLIM;
right: histogram (lifetime distribution);
lower part: fluorescence decay in one pixel
multiexponential decay
t_1: 0.2 ns (69%), t_2: 2.1 ns (31%)



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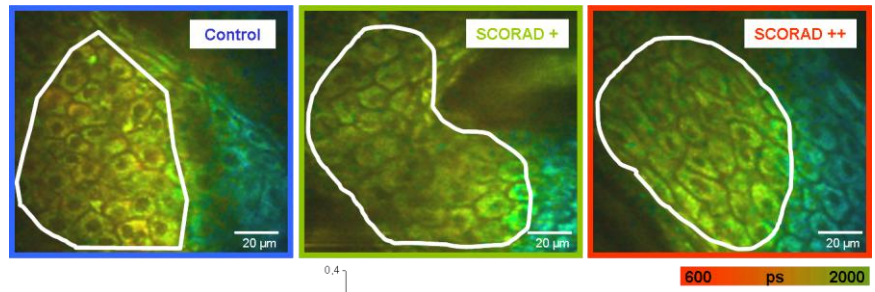
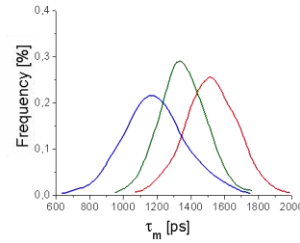
FLIM of Atopic Dermatitis





1.15 ns to 1.55 ns

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τ-histogram. The SCORAD index correlates with the τ-value.

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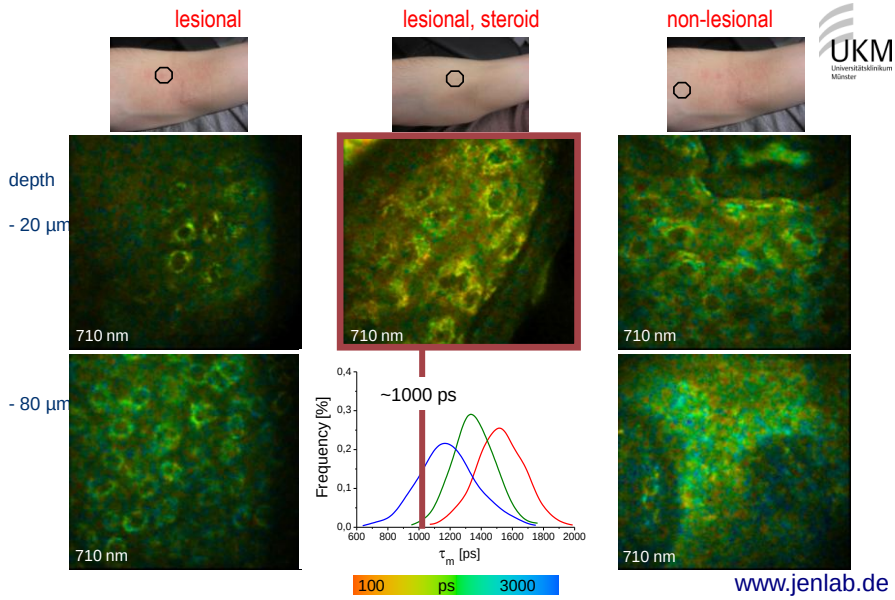
FLIM: effect of treatment



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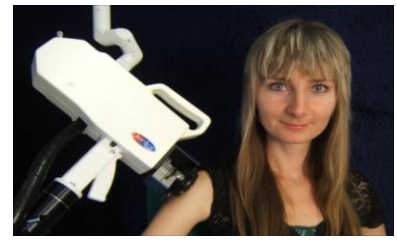
Conclusions

Multiphoton imaging technology is now in clinical use
in Australia, Japan, UK, France, Italy, Demark, Germany
based on two-photon excited autofluorescence and SHG

CE-marked multiphoton tomographs *DermaInspect* and *MPTflex*



more than 1,000 patients and volunteers

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Targeted transfection by sub-20 femtosecond laser pulses

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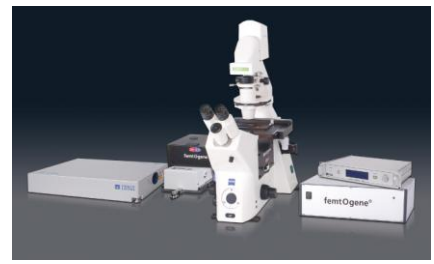

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How safe is multiphoton tomography?

F. Fischer, B. Volkmer, S. Puschmann, R. Greinert, W. Breitbart, J. Kiefer, R. Wepf.
Risk estimation of skin damage due to ultrashort pulsed focused near infrared laser radiation at 800 nm.
J Biomed Optics 13(2008)041320-1

Comparison UV lamps versus DermaInspect
DNA damage in biopsies of volunteers taken after exposure
UV lamps have higher damage potential
DermaInspect exposure = sun exposure of 15 min walk