

2nd Journal review

Clinical application of SHG microscope

2015/10/13 M2 Atsuta

- ① M. Weinigel et al., “Compact clinical high-NA multiphoton endoscopy”, Proc. of SPIE.**8217**, 821706-1(2012).
- ② H. G. Breunig et al. , “Combining multiphoton and CARS microscopy for skin imaging”, Proc. of SPIE.**8588**, 85880N-1(2013).
- ③ M. Weinigel et al., “A novel clinical multimodal multiphoton tomograph for AF, SHG,CARS imaging, and FLIM”, Proc. of SPIE.**8948**, 89481R-1(2014).

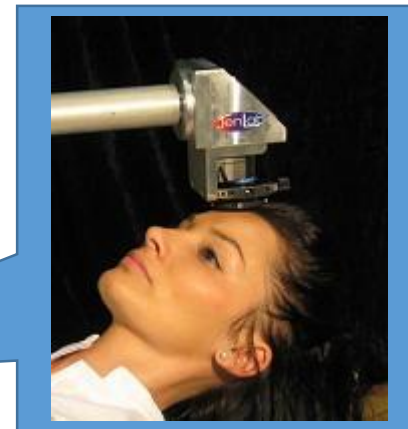
Introduction

SHG microscope : observation of non-centrosymmetric molecules (e. g. collagen)

⇒ **Selective and non-invasive**

To Clinical application...

- Compact & Portable system
- Combination with other microscope



DermalInspect
(Jen Lab.)

① Compact clinical high-NA multiphoton endoscopy

Clinical high-resolution multi-tomography apparatus

Large probe \Rightarrow Restrict external access possibilities
hard-to-reach areas (like wounds)

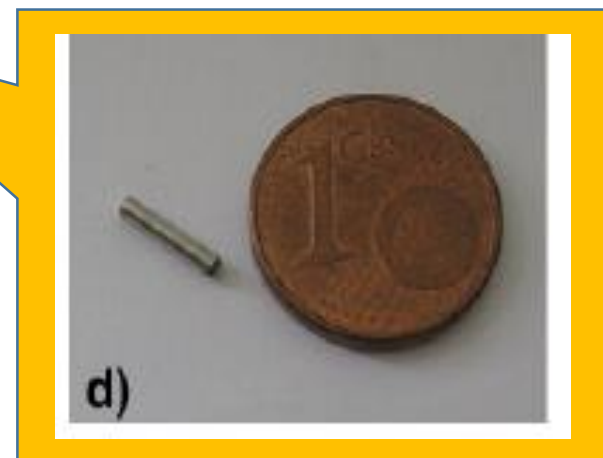
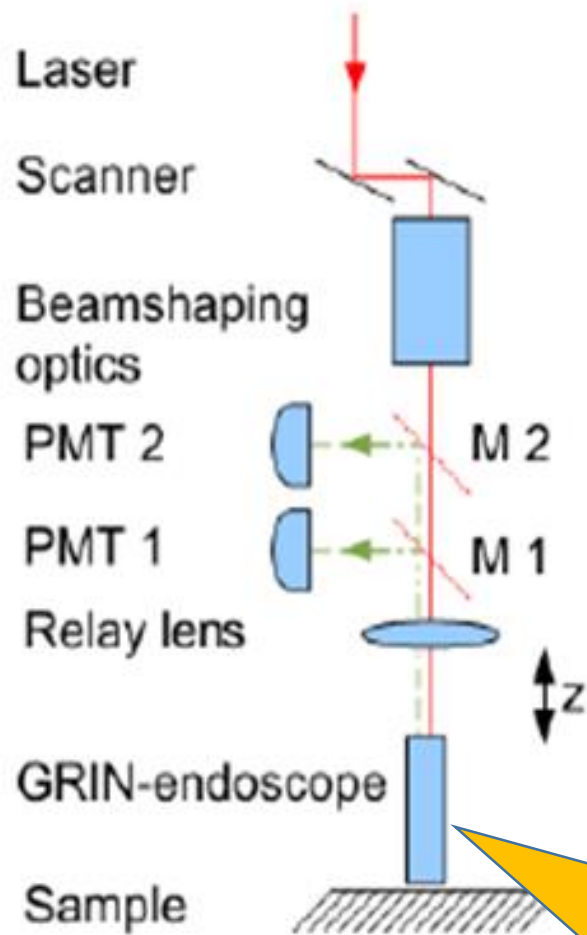
It can be improved by the clinical multiphoton endoscopy.

Using gradient index (GRIN) lenses
& combining multiphoton endoscopy
(Fluorescent & SHG)

based on with CE-marked clinical tomography



Setup



Results

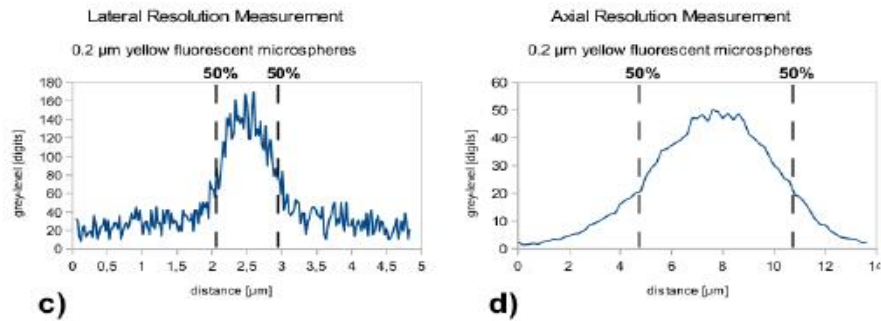
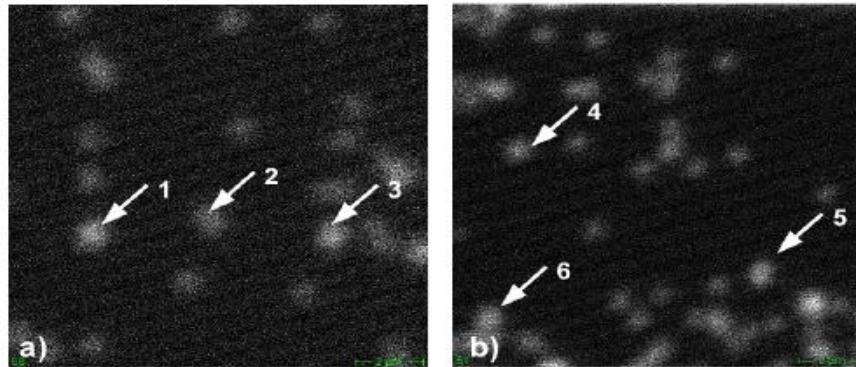


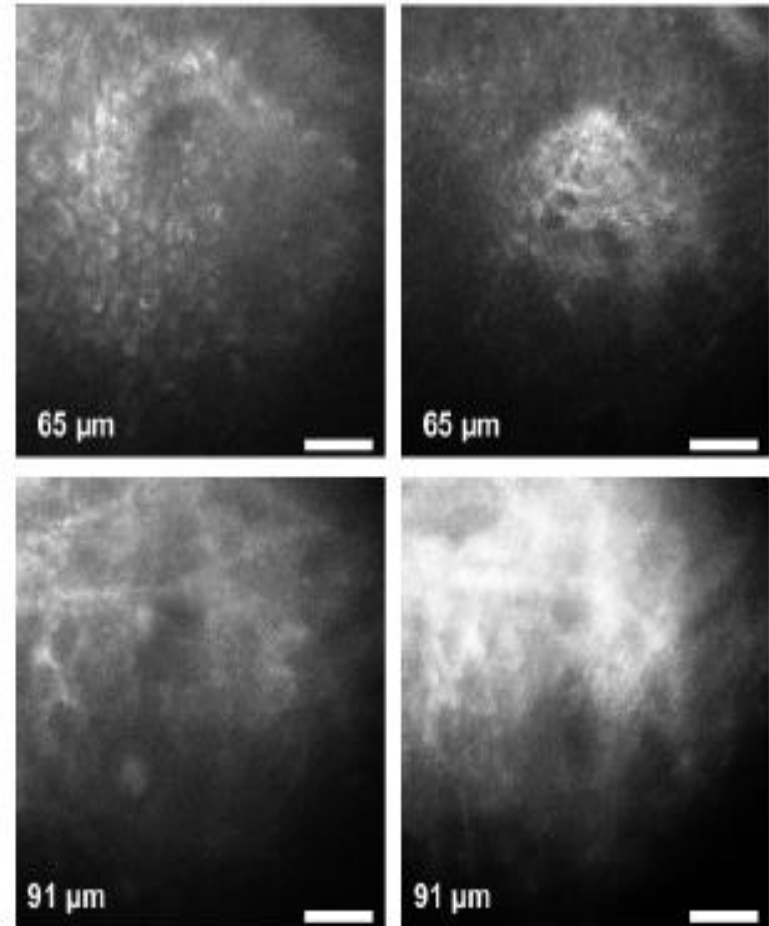
Figure 2: a) and b) Measurement of the point spread function of 0.2 μm spheres; c) Lateral point spread function; d) Axial point spread function.

The lateral resolution : 0.87 μm

The axial resolution : 5.5 μm

Autofluorescence-
Channel

SHG-
Channel

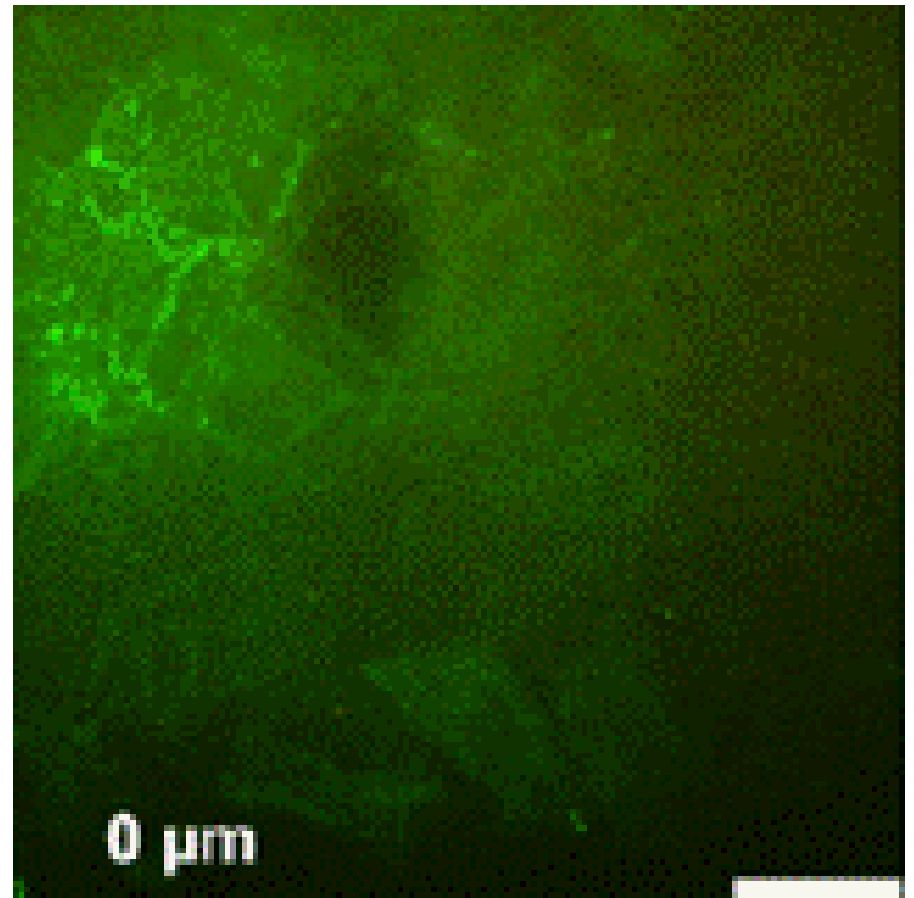


in vivo imaging @ Depth-resolved

12 optical sections
@ steps of 13 μm .

Sample:
Human skin (forearm)

It demonstrates that
high contrast features can be
imaged up to a depth of about
117 μm .



Fluorescence (green)
SHG (yellow)

Summary

- Development of a set-up aimed at a combination of microscopic endoscope and medical certification tomography
- High resolution image has been acquired is in vivo multiphoton image human skin from the above depth 100 μ m

② Combining multiphoton and CARS microscopy for skin imaging

Multiphoton microscope + CARS microscopy

What's CARS microscopy ?

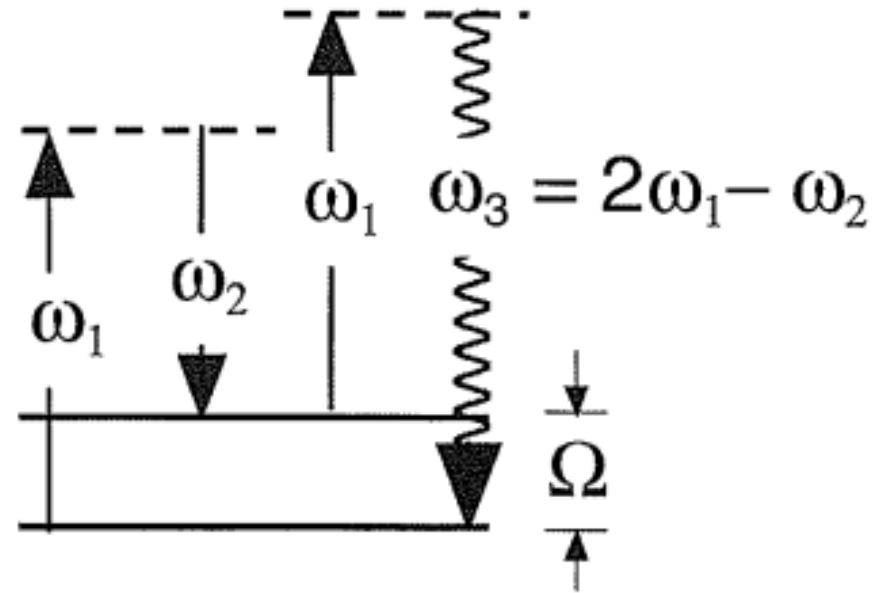
$$\omega_3 = 2\omega_1 - \omega_2 : \text{CARS}$$

The stretching vibration energy of CH_2

$$\Omega = 2845 \text{ cm}^{-1}$$

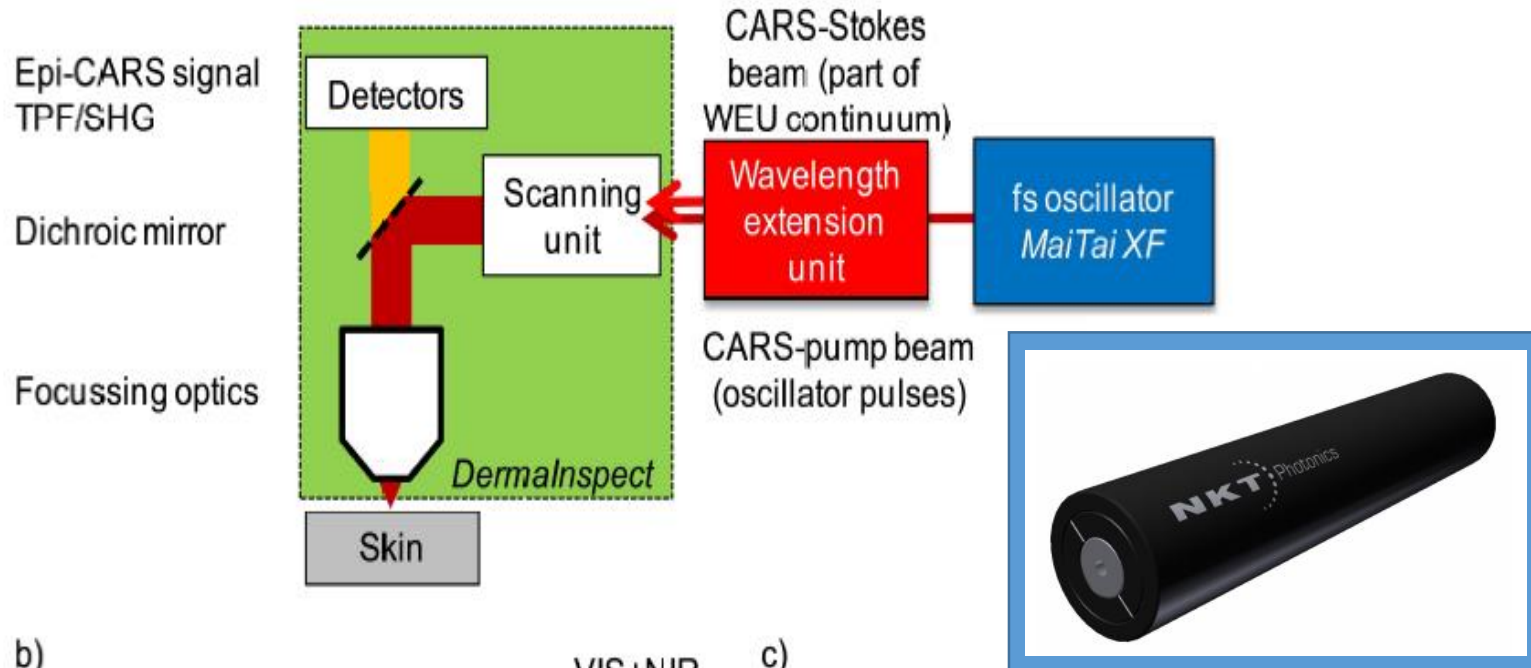
Biological measurement...

known for **lipids**

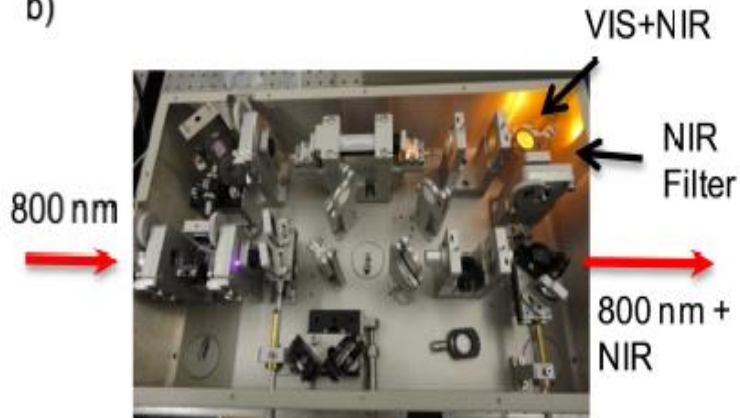


Setup

a)



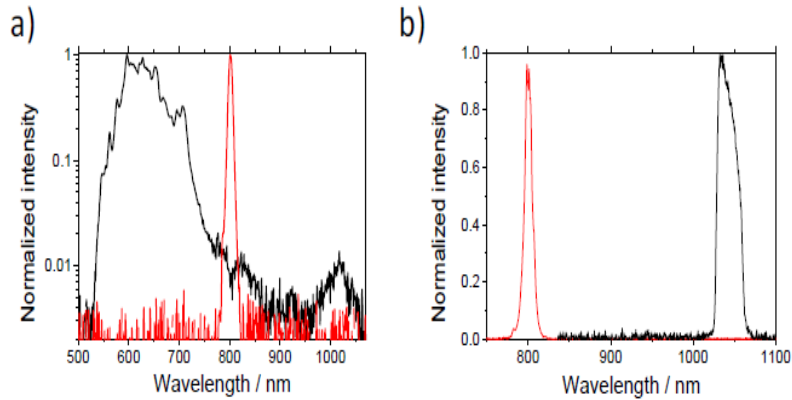
b)



c)



Results

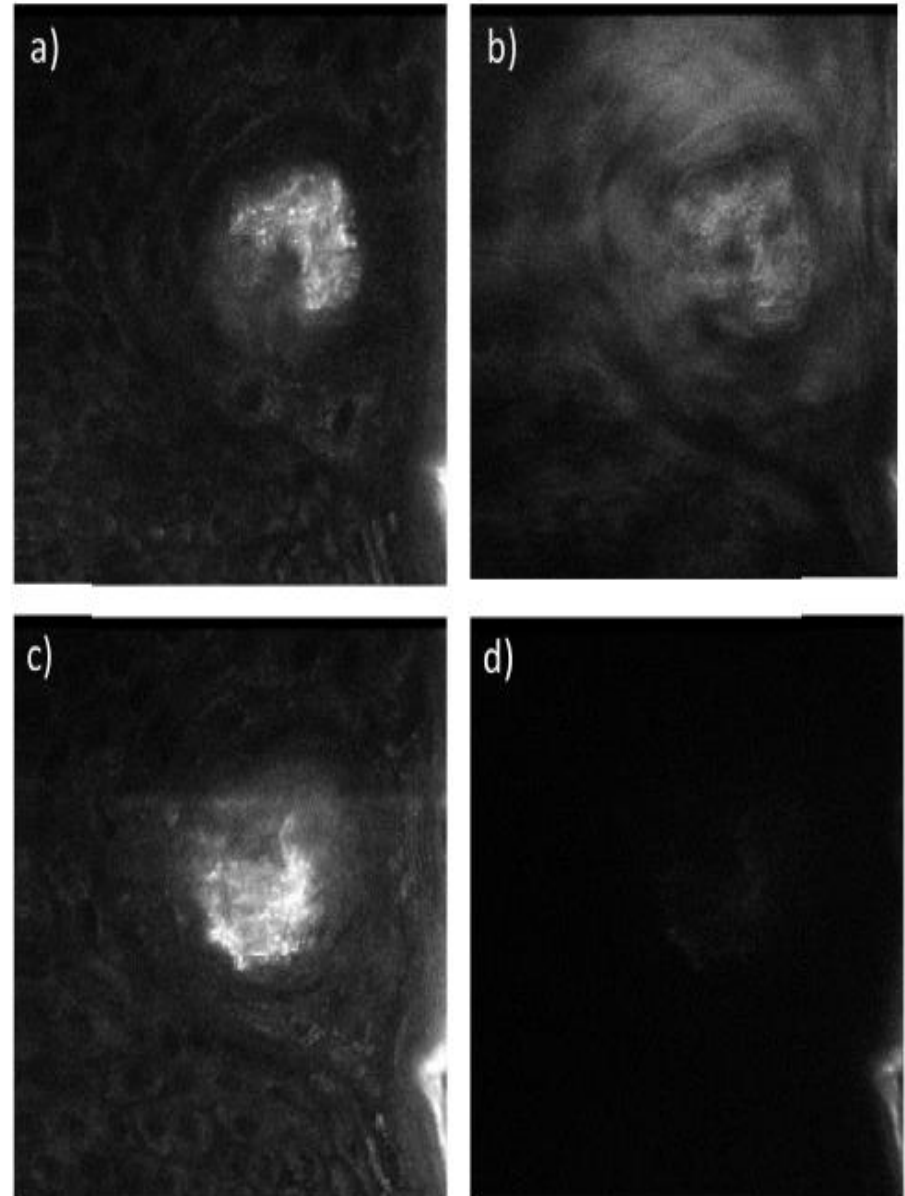


Pump : 800nm

Stokes : 1035nm

CARS : 652nm

7.4 s/frame for images
with 512 x 512 pixels
(FOV : 200 x 200 μm)



Stratum spinosum

SHG & CARS イメージ

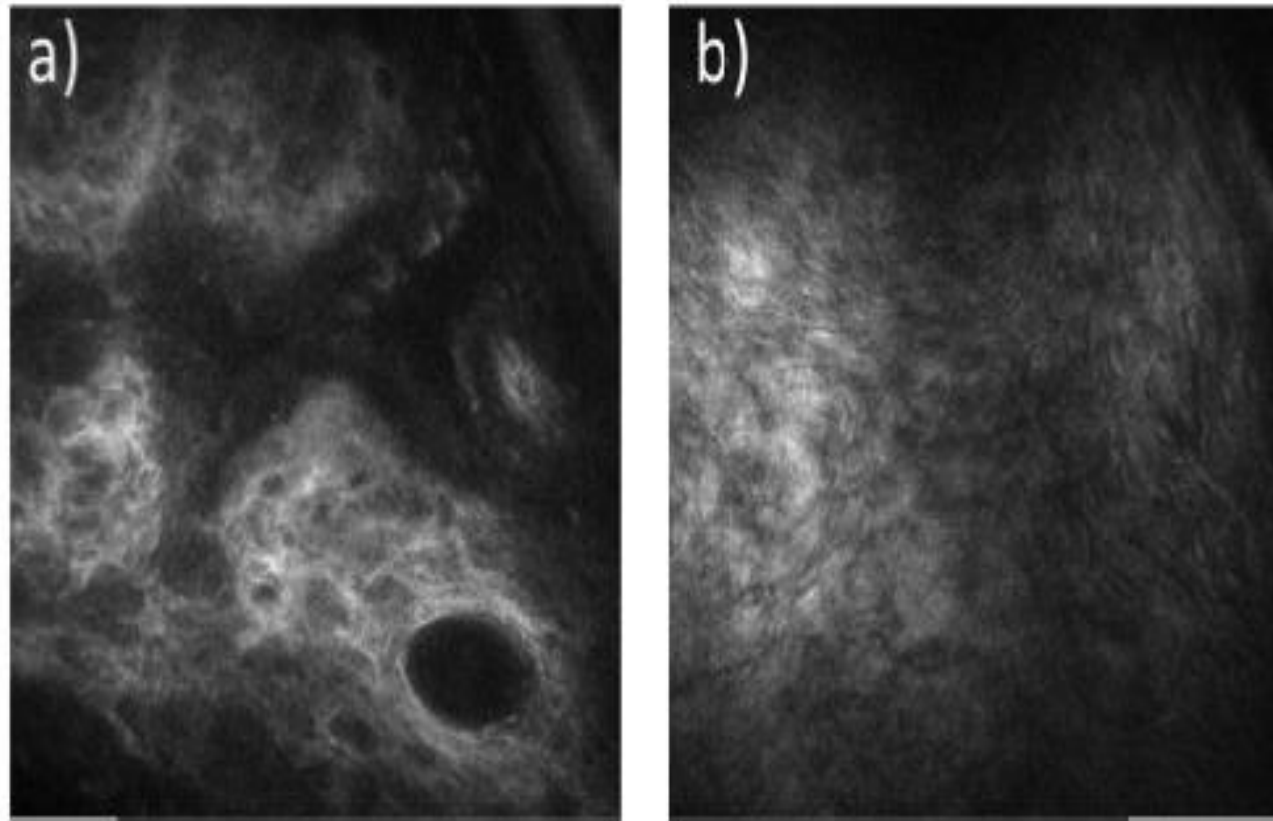


Fig. 6 In vivo multiphoton imaging of the human skin about 40 μm below the skin surface (stratum papillare). a) TPF/SHG-channel signal. b) CARS-channel signal. Each image depicts an area of $153 \times 153 \mu\text{m}^2$.

Summary

- Clinical CARS / TPF / SHG multi-photon human skin in vivo imaging of the imaging system has been shown.
- Different fluorophores and CH2 molecular distribution was observed inside the deeper layers of the skin cell structure and elastin or collagen are present.

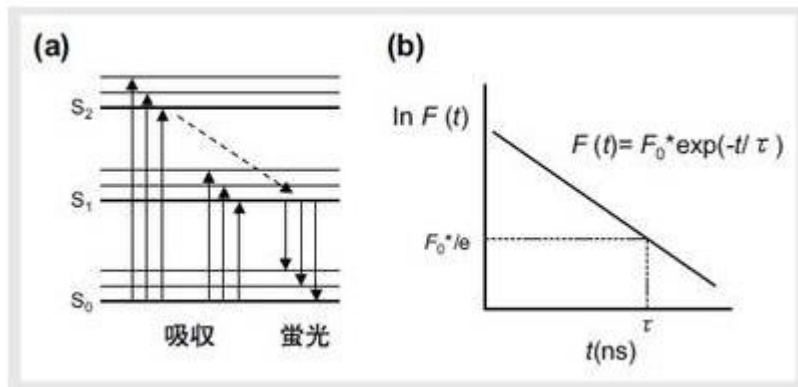
③ A novel clinical multimodal multiphoton tomograph for AF, SHG, CARS imaging, and FLIM

medical certification tomography
combination **AF** / SHG / CARS microscope

elastin, melanin, and NAD(P)H

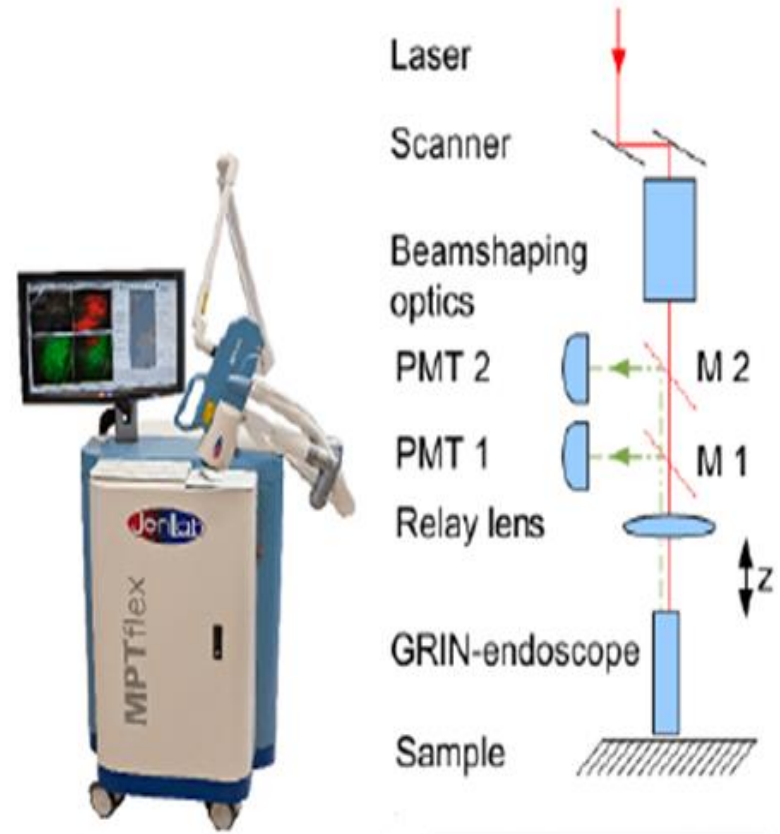
⇒ fluorescence lifetime imaging (FLIM)

$$F(t) = F_0 \cdot \exp(-t/\tau)$$



Ref.) <http://www.horiba.com/jp/>

Conventional system



Setup

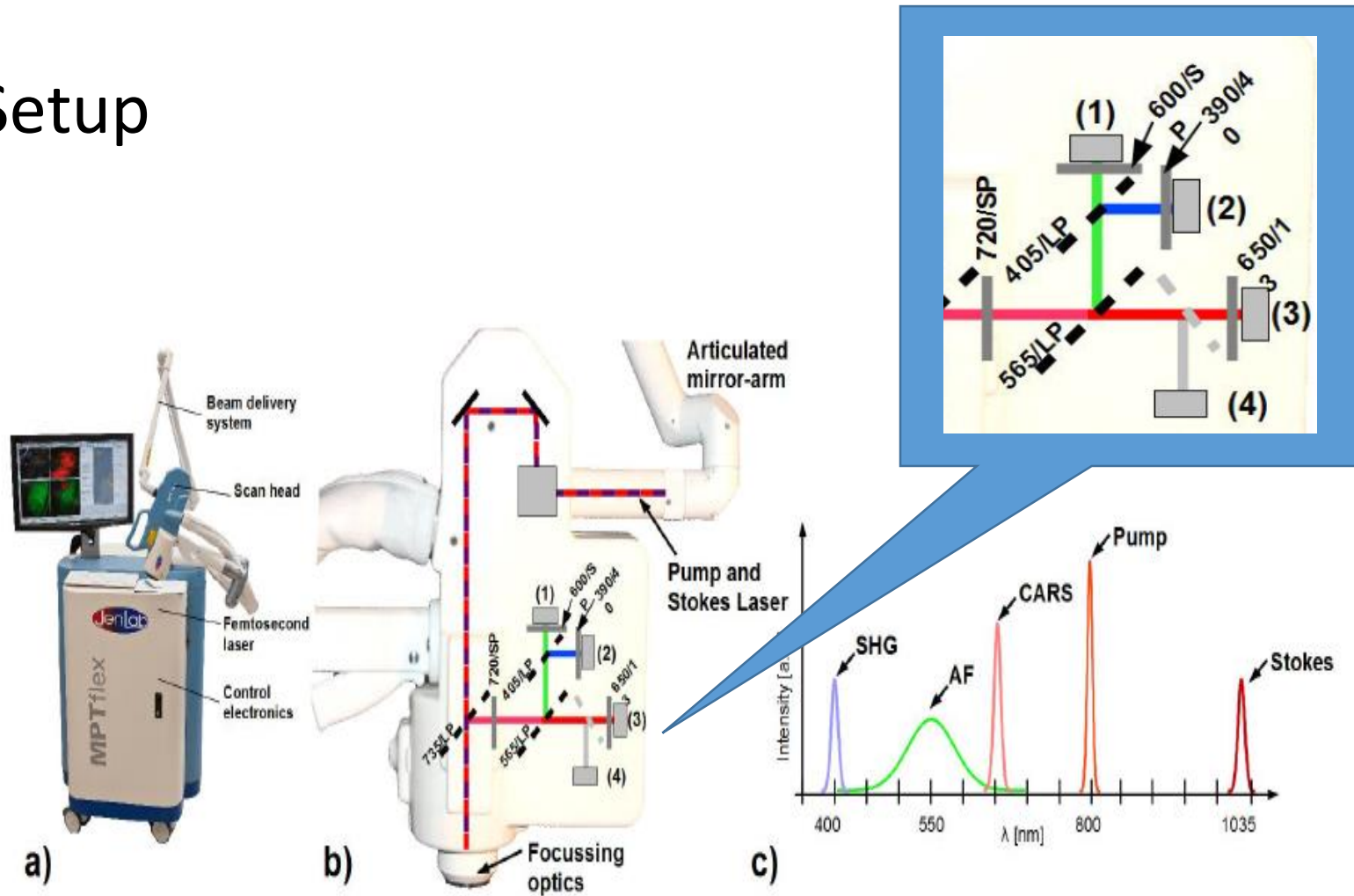


Figure 1: a) Flexible multimodal tomograph MPTflex CARS; b) Scan head with four-detector arrangement; c) Schematic spectrum of excitation and detection signals.

Results

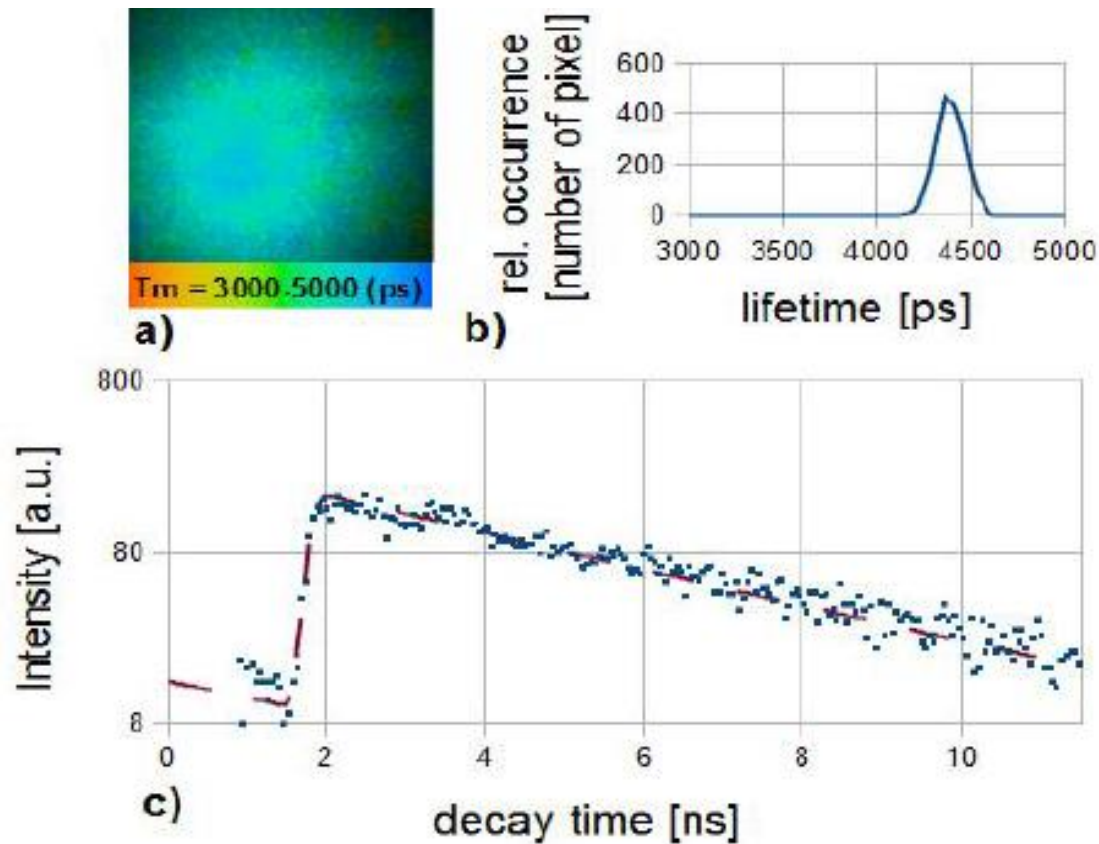


Figure 2: a) 2D lifetime distribution of fluorescein solution, image size: $100 \times 100 \mu\text{m}^2$; b) lifetime distribution histogram; c) representative decay function acquired in the center of a) (measurement data (dotted values), mono-exponential fit (dashed)).

Results

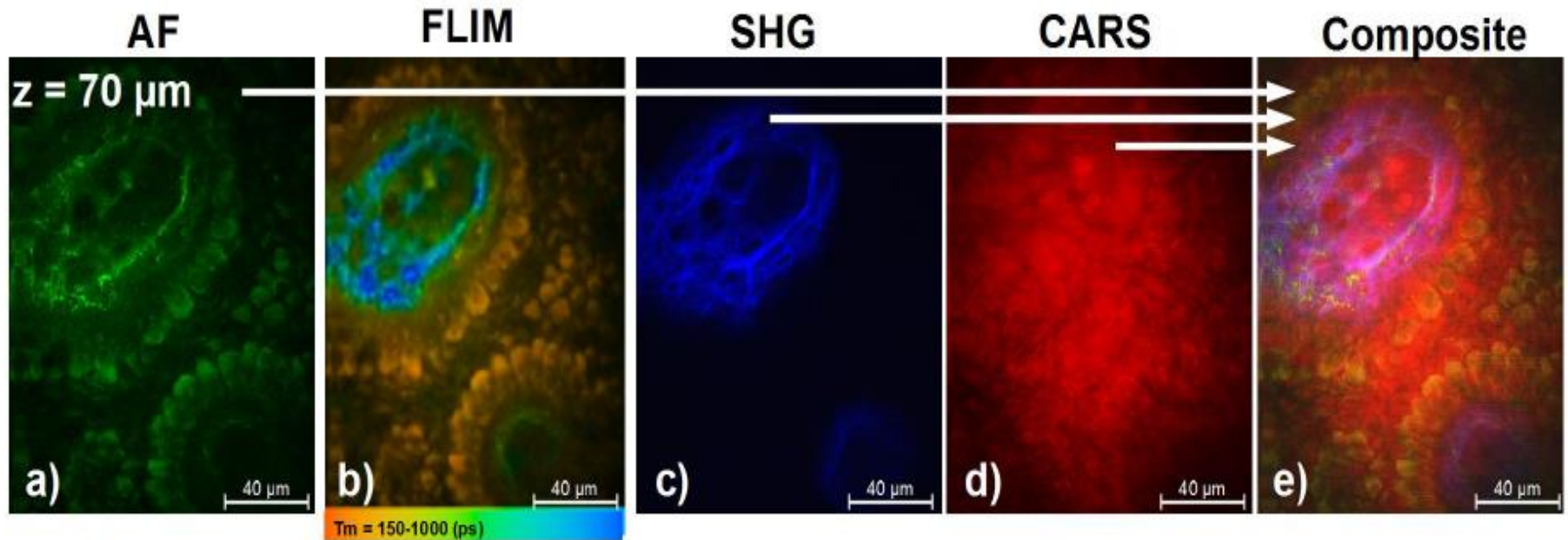


Figure 3: Multichannel imaging of human skin (forearm) *in vivo* at dermal papillae; bar: 40 μm .

healthy human skin @ a depth of 70 μm (dermal papillae)

FLIM : Melanin has a strong short lifetime component (orange color coded) and elastin longer lifetimes (blue-green color coded)

Summary

- Multiphoton tomography, based on autofluorescence and SHG, to allow tissue imaging of high resolution.
- Additional information about the non-fluorescent and non-SHG-active tissue component can be obtained by CARS.
- New and it is compact, multi-channel simultaneous detection, AF · FLIM, separation of the SHG and CARS signal has become possible.

Summary

Multiphoton microscope is compact, and built into clinical high-resolution multi-tomograph, and the observation field is spread by combining with other systems.

These are expected to be a very powerful tool for the skin test in the future of the medical field and cosmetic field.

That's all.