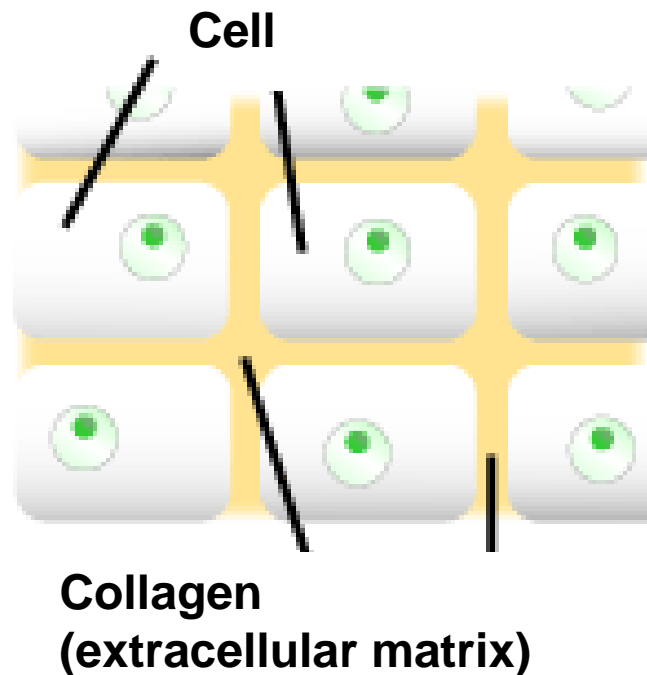


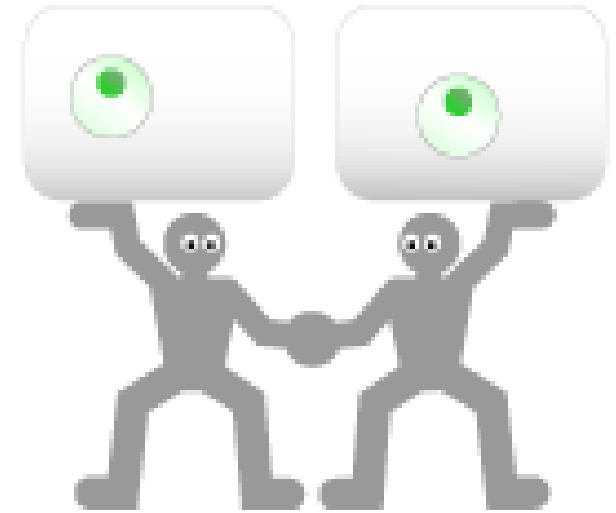
Compact Probe Head of Second-Harmonic-Generation Microscopy for Dermatological Applications

K. Atsuta, E. Hase, and T. Yasui
Tokushima University, Japan

What is collagen?



Collagen plays a role of "glue" to bind cells together or makes partitions between cells to put them in the right and well ordered position

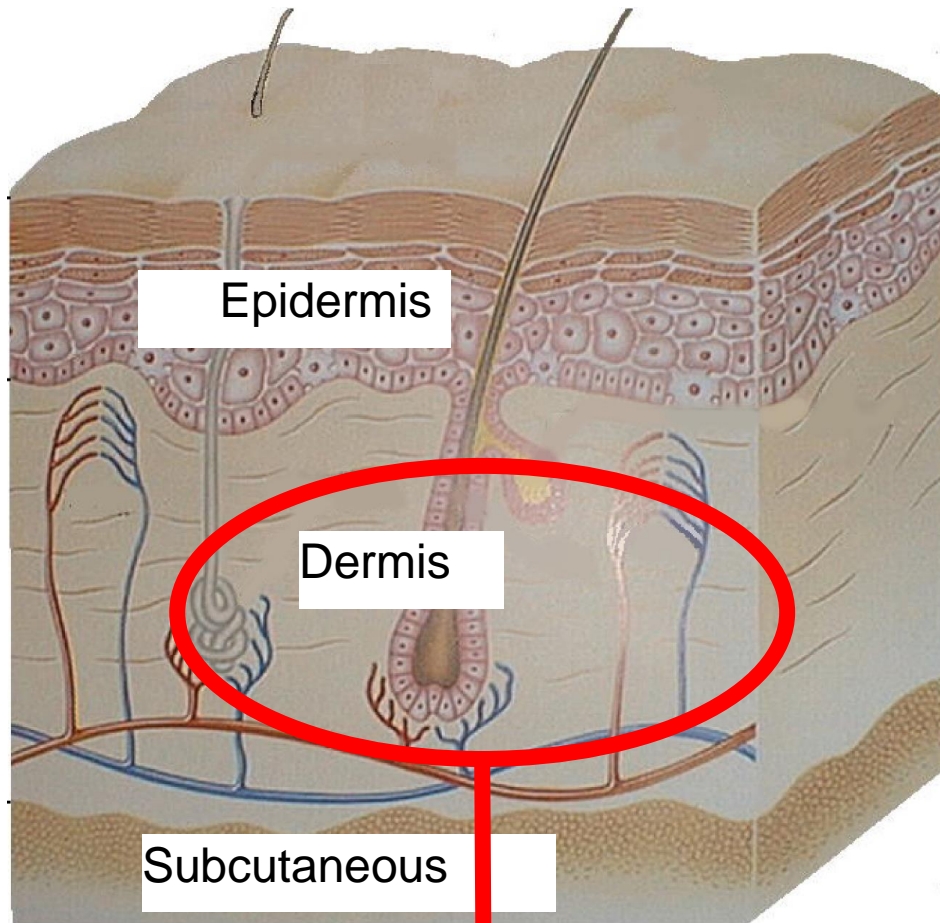


Collagen plays an active role in binding and supporting cells!

- Collagen makes up every part of body and supports, binds, detaches cells
- Collagen is a scaffold for cells. Cells divide and proliferate there

Collagen makes significant influence on vital activity and aging

Collagen in skin



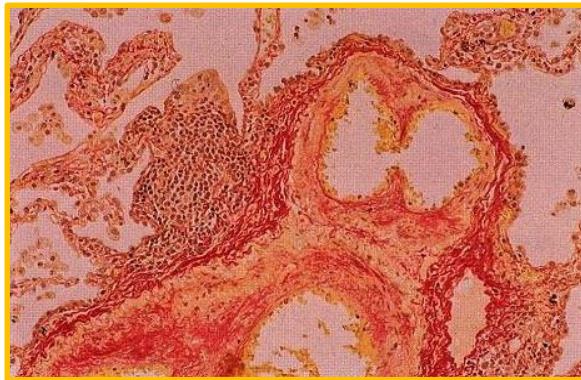
- Collagen comprises 70% of dermis
- Collagen determines mechanical and functional characteristics of skin
- Collagen draws attention in the field of skin cosmetics and anti-aging dermatology

Collagen content = 70wt%

Collagen observation

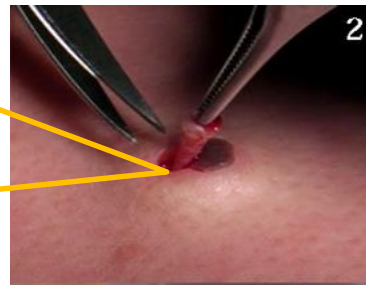
Staining method

e.g. : Van Gieson staining



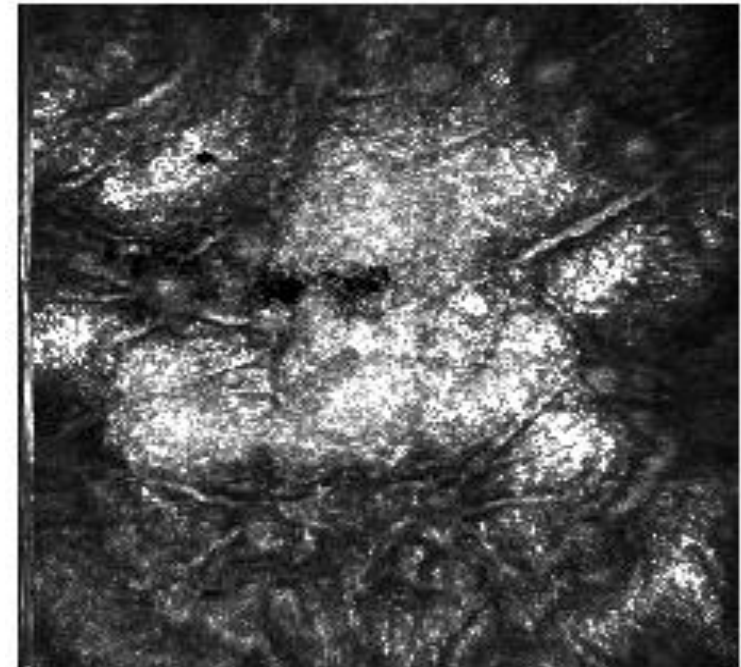
(Collagen= Red)

<http://www.soarer.hoken.med.yamaguchi-u.ac.jp>



Selective visualization
Invasive (skin biopsy)

Confocal microscope



Low-invasive
No molecular selectivity

Difficult to visualize distribution of collagen in living tissue “*in vivo*”

What is SHG light?

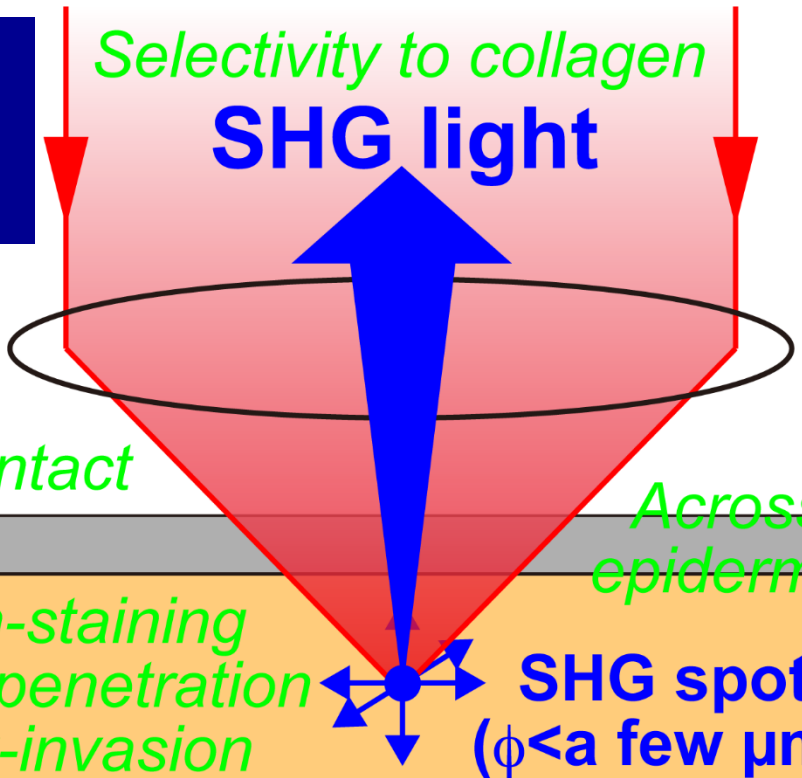
	Linear	Nonlinear
Sound (Keyboard)	<p>Sound (freq. = f)</p> <p>Touch</p>	<p>f $2f$</p> <p>Strong Hit!</p>
Light	<p>CW light ω</p> <p>Reflected and scattered light ω</p> <p>Non centrosymmetric material</p>	<p>Ultraintense pulse light ω</p> <p>SHG light 2ω</p> <p>ω</p> <p>Non centrosymmetric material</p> <p>ex) anisotropic crystal, tissue collagen</p>

Collagen-sensitive SHG microscopy for dermatological applications

Femtosecond laser light

**Selective visualization
of collagen fiber *in vivo***

- Free from BG light
- Reflection light
- Scattering light
- Fluorescence



Epidermis
(no collagen)

Dermis
(70 wt% collagen)

Subcutis
(no collagen)

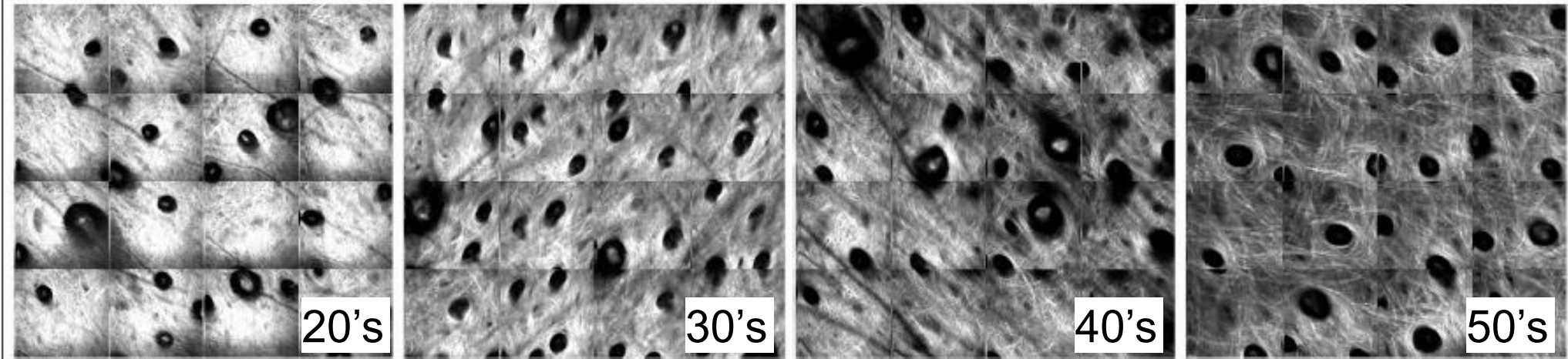
Non-staining
Deep penetration
Low-invasion

in vivo

High spatial resolution
3D imaging

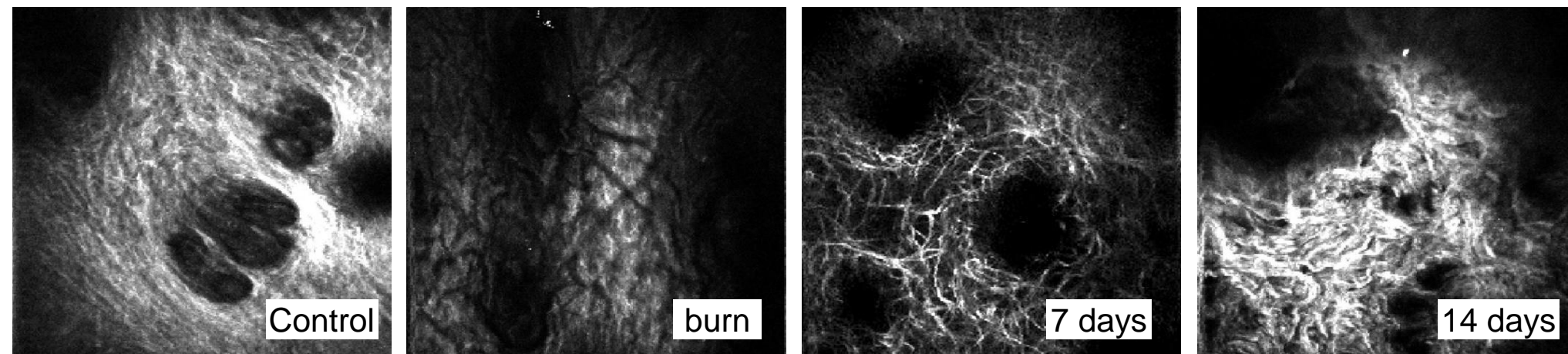
In situ application of SHG microscopy

Structural change of dermal collagen in human cheek by aging



ref) T. Yasui, *J. Biomed. Opt.* **18**, 031108 (2013).

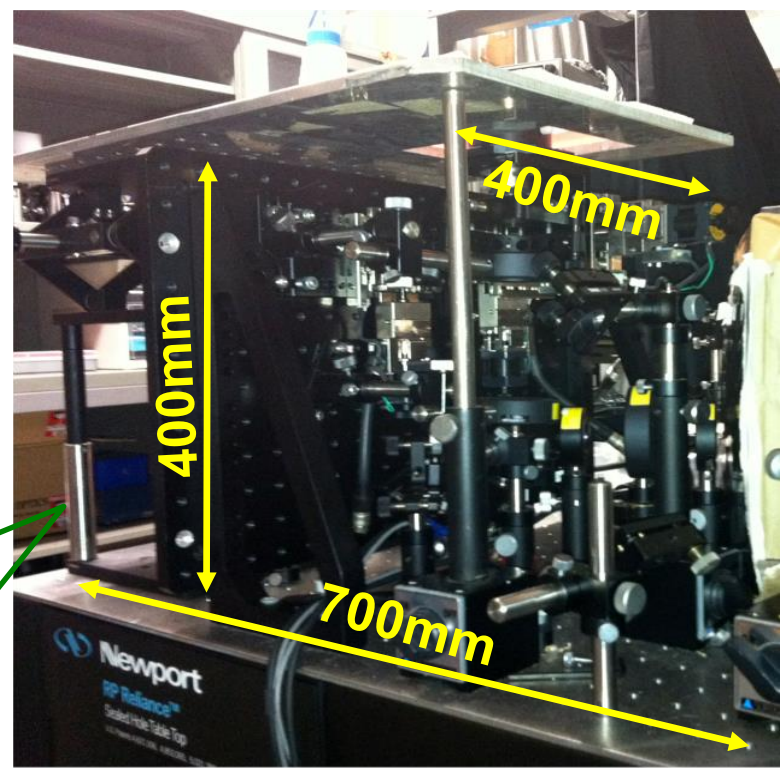
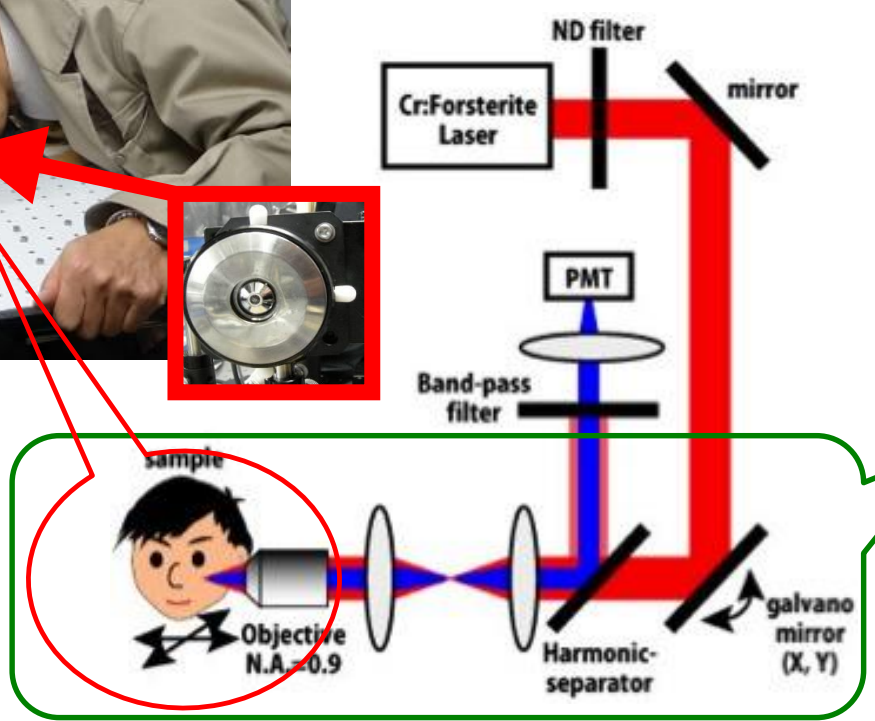
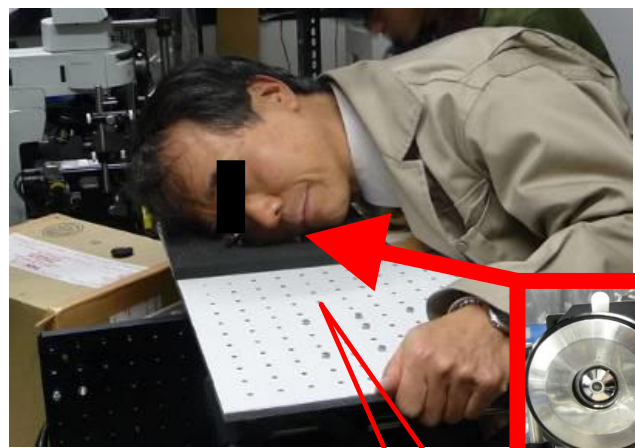
Healing process of skin burn in animal model



ref) T. Yasui, *Proc. SPIE.* **8948**, 89480B (2014).

Conventional SHG microscope

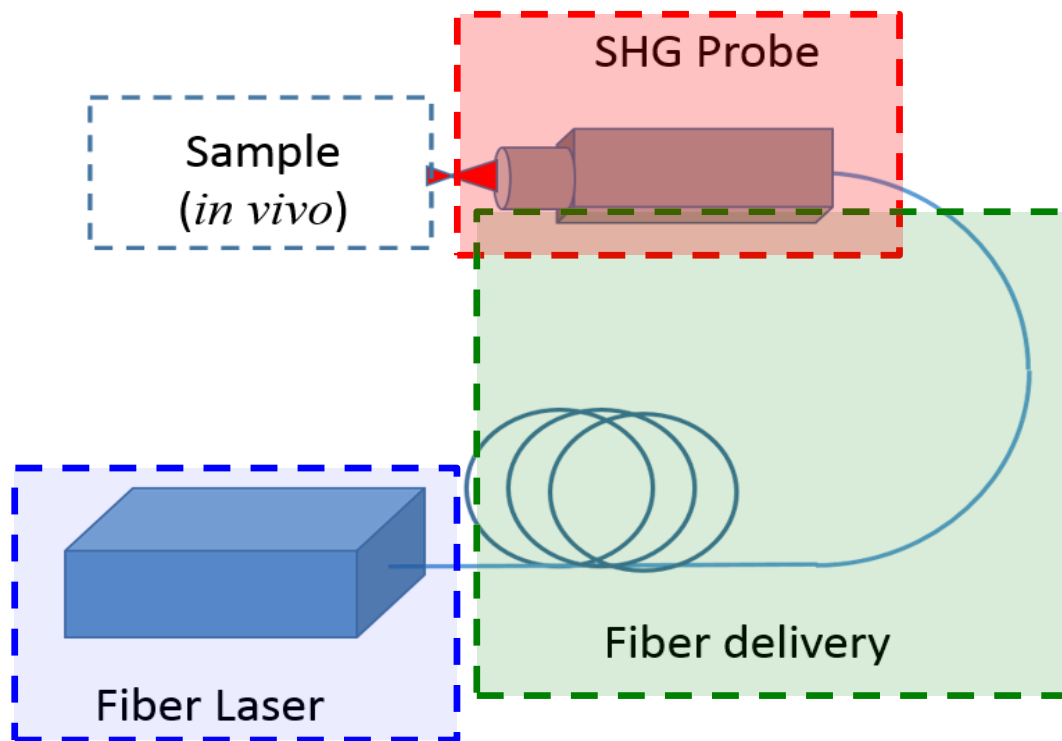
Large, bulky, complicated, daily alignment



Difficult to apply clinical applications

SHG fiberscope

Present talk



Key technologies

- (1) Compact fiber laser source
- (2) Fiber delivery of ultra-short pulsed light with dispersion compensation
- (3) Compact SHG probe

Compact, flexible, robust, alignment-free

SHG probe equipped with external galvano mirror

Pulse width : 100 fs
Center Wavelength : 1250 nm
Repetition frequency : 73 MHz

Cr:Forsterite laser

M

Galvano mirror (external)



Mirror: $\Phi 3\text{mm}$
Fast mirror: 1kHz

150mm
PMT

Photon-counting type
Max. count rate = 1.5MHz

Pass band = 625nm

BPF

sample

Oil immersion
N.A. = 0.9
W.D. = 350 μm

DM

objective

50mm

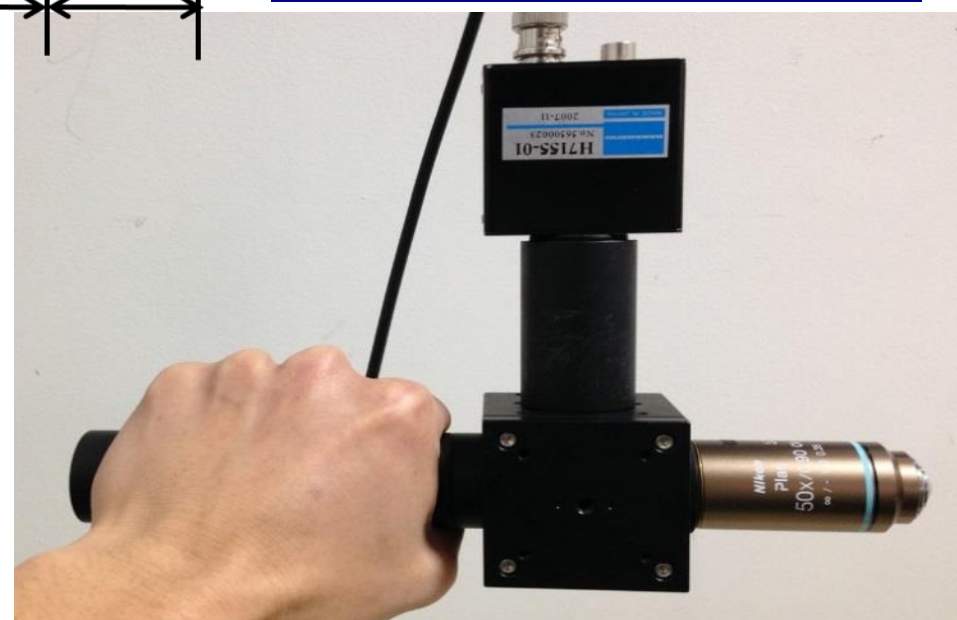
165mm

60mm

Hand-held probe

Transmittance: 1250nm
Reflection: 625nm

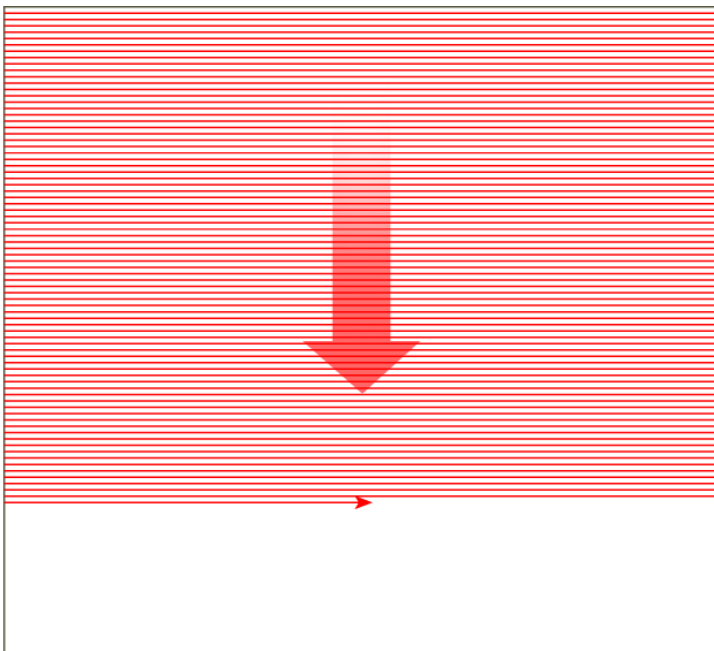
All-in-one SHG microscope



2D scanning of focus spots

Raster scan

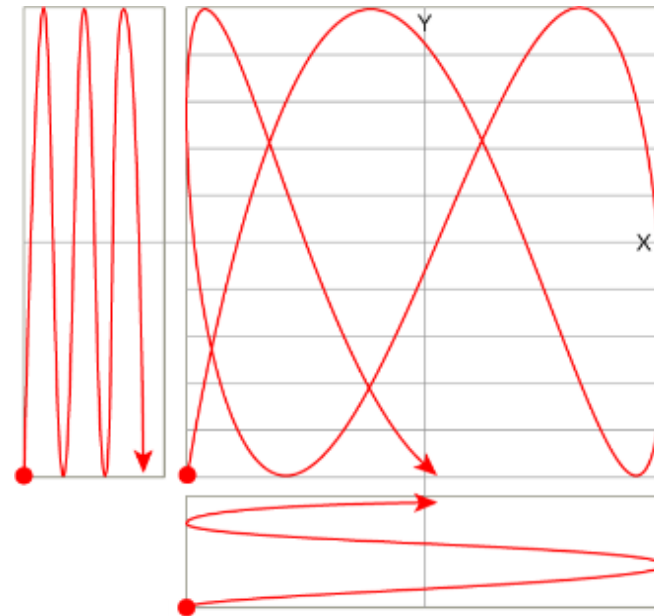
Normal galvano mirror, polygon mirror, etc



- ✓ Simple image reconstruction
- ✓ Limited scanning speed due to ramp waveform

Lissajous scan

Resonant galvano mirror, MEMS mirror, etc



- ✓ Faster scanning speed due to sinusoid waveform
- ✓ Complicated image construction

ref) <http://www.signal.co.jp/vbc/mems/sp/ecoscan/>

SHG image of human bone artery (400 μ m*400 μ m)

Raster scan

Ramp wave
(fast: 140Hz, slow: 0.5Hz)

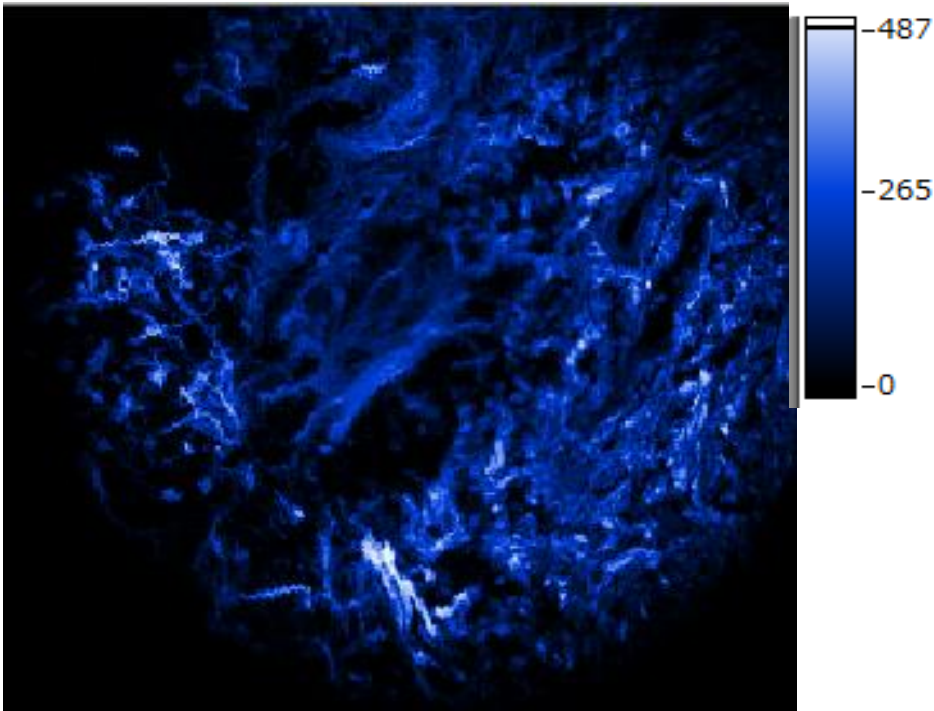
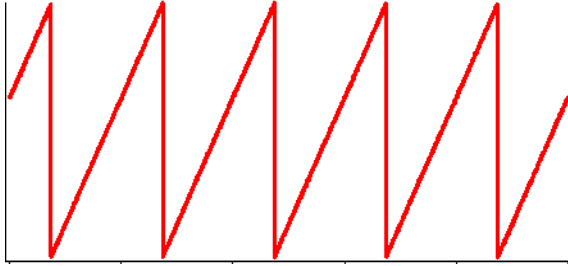


Image acquisition = 2sec

Lissajous scan

Sinusoidal wave
(fast: 221Hz, slow: 13.5Hz)

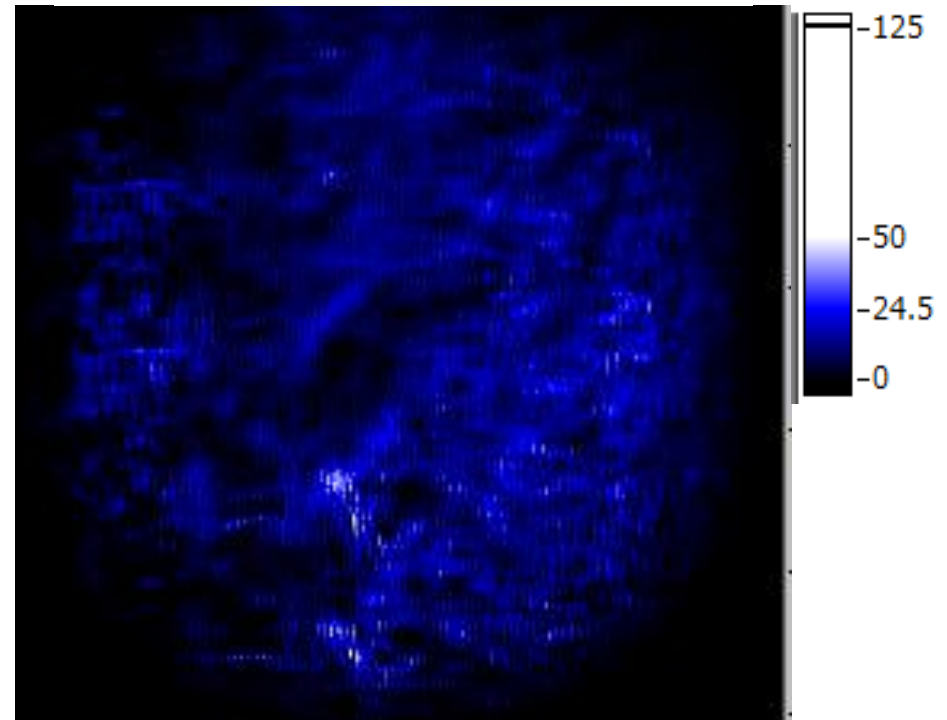
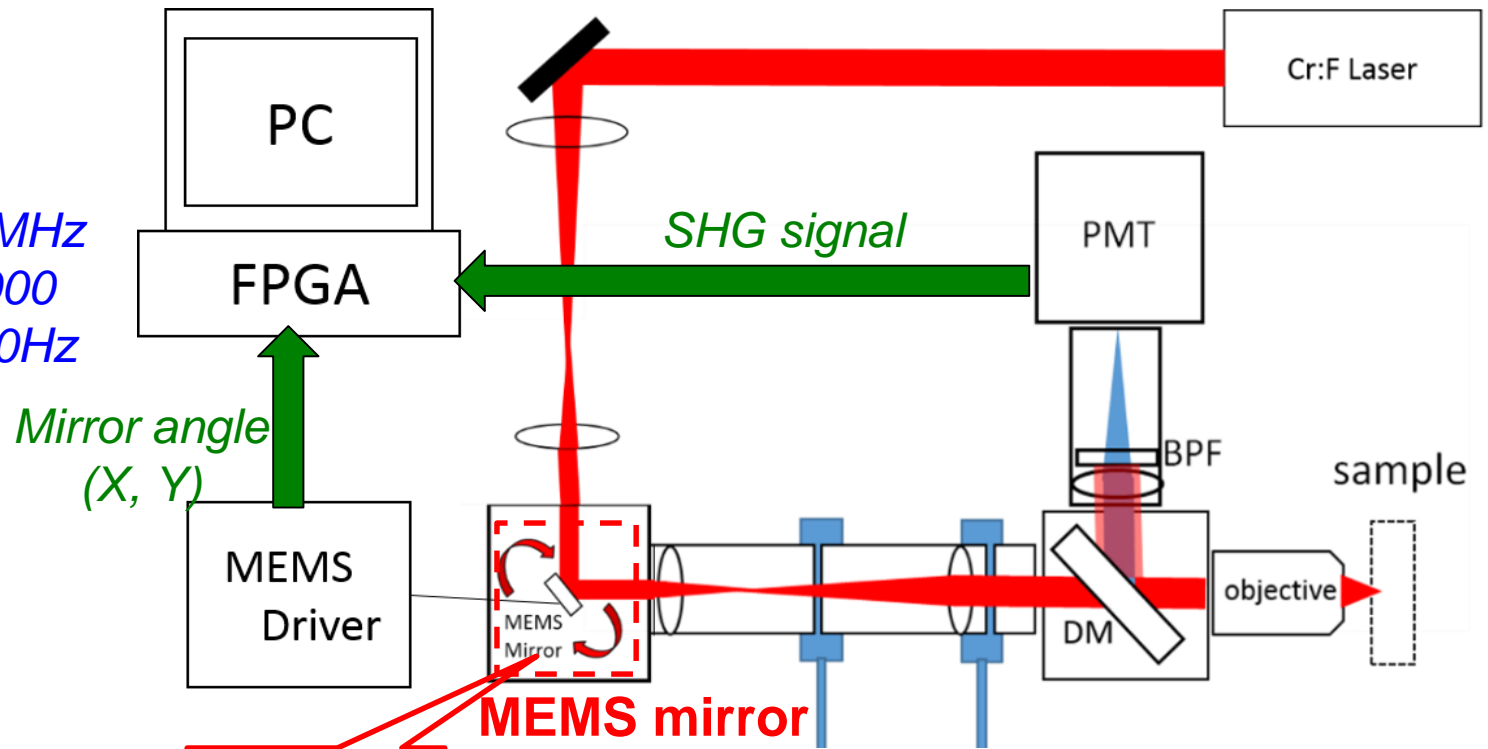


Image acquisition = 8sec

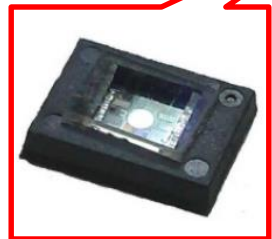
SHG probe equipped with internal MEMS mirror

Use of MEMS mirror reduces the size of SHG image and increase the image acquisition speed!

Sampling rate = 20MHz
Data length = 500,000
Acquisition rate = 40Hz



MEMS mirror (internal)



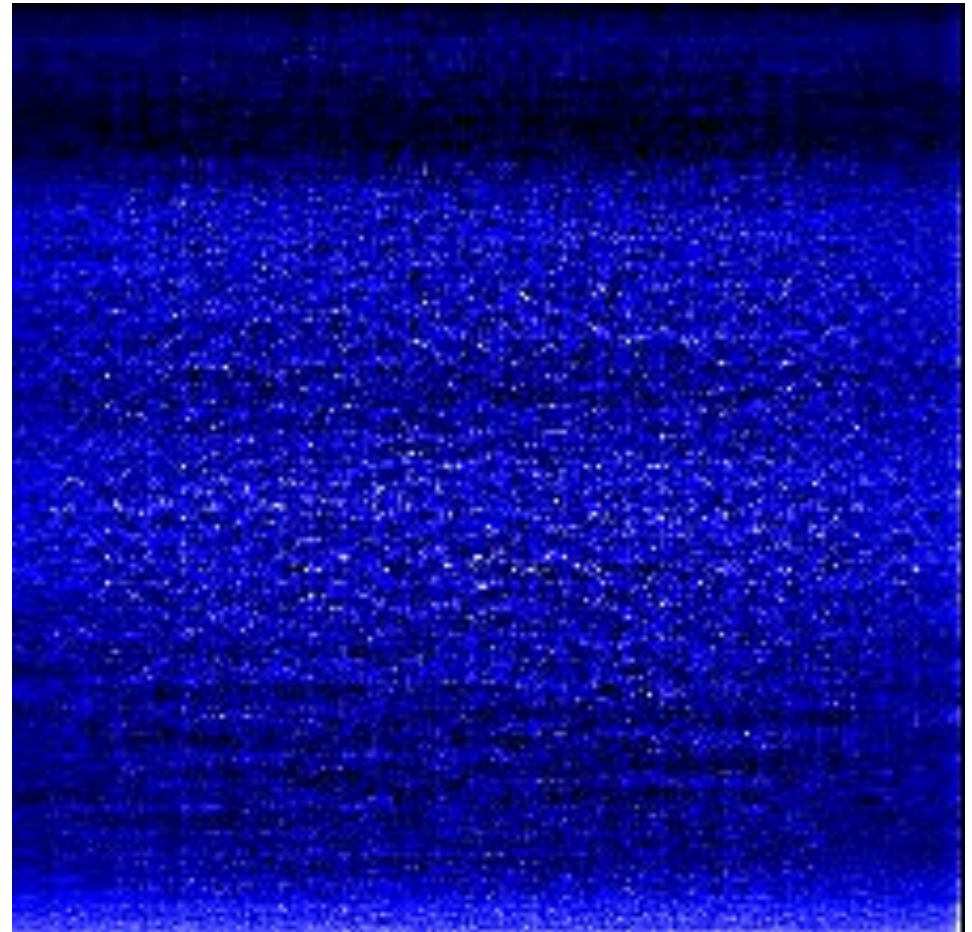
2D MEMS mirror
Fast : $\pm 25^\circ$ max @22.1kHz
Slow : $\pm 18^\circ$ max @1.35kHz

SHG image of LiNbO_3 powder

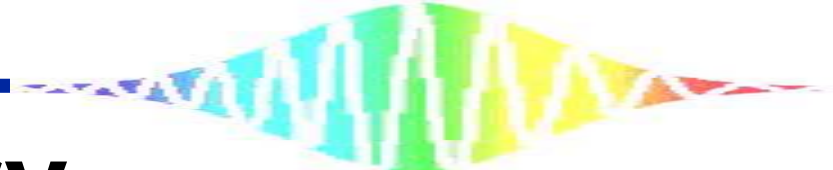
Optical photograph



SHG image (200 μm *200 μm)



The SHG image shows the unevenness of the sample



Summary

- ✓ Development of compact, all-in-one SHG probe
- ✓ SHG imaging with external galvano mirror
- ✓ SHG imaging with internal MEMS mirror

Future Plans

- Fiber delivery of the ultrashort pulse light from the laser source to the SHG probe