

Collagen-sensitive microscopy

Takeshi Yasui

Inst. Tech. Sci., Univ. Tokushima, Japan

Grad. Sch. Engg. Sci., Osaka Univ., Japan

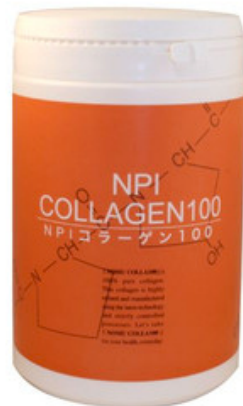
August 2, 2012

To search “collagen” on the internet

Collagen drinks



Collagen supplements



Collagen soup



Collagen cream

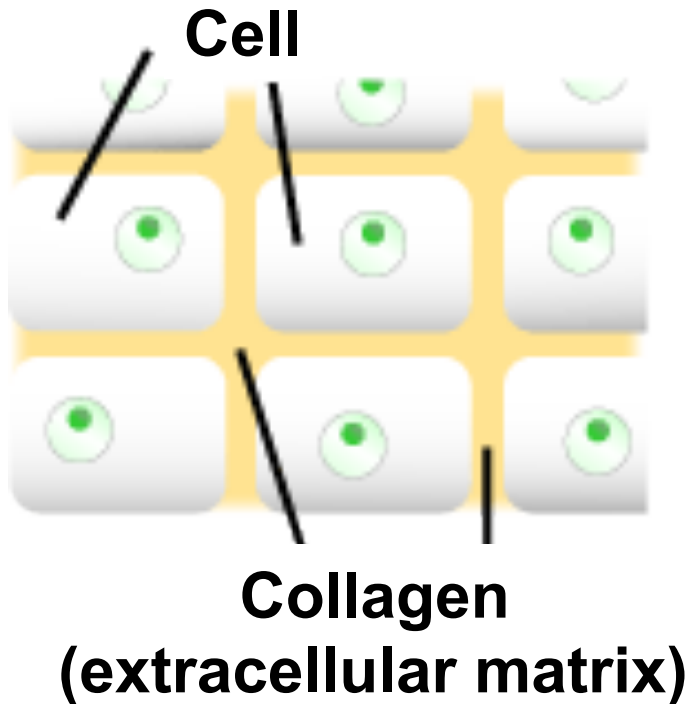


Collagen injections

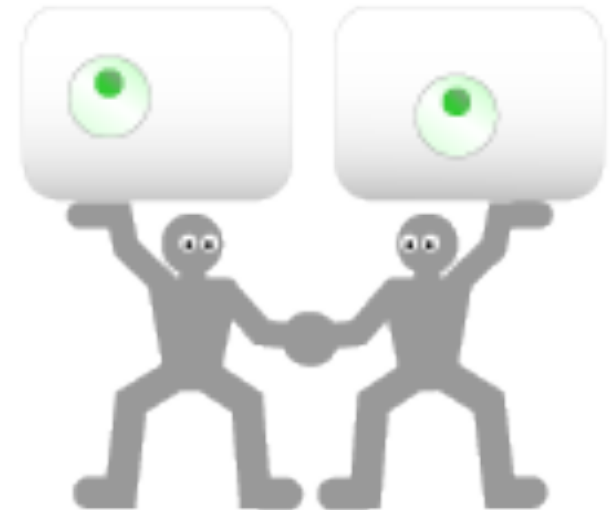


- Important in body
- Huge market in world (¥55 B in Japan@ 2011)
- Questionable ?

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

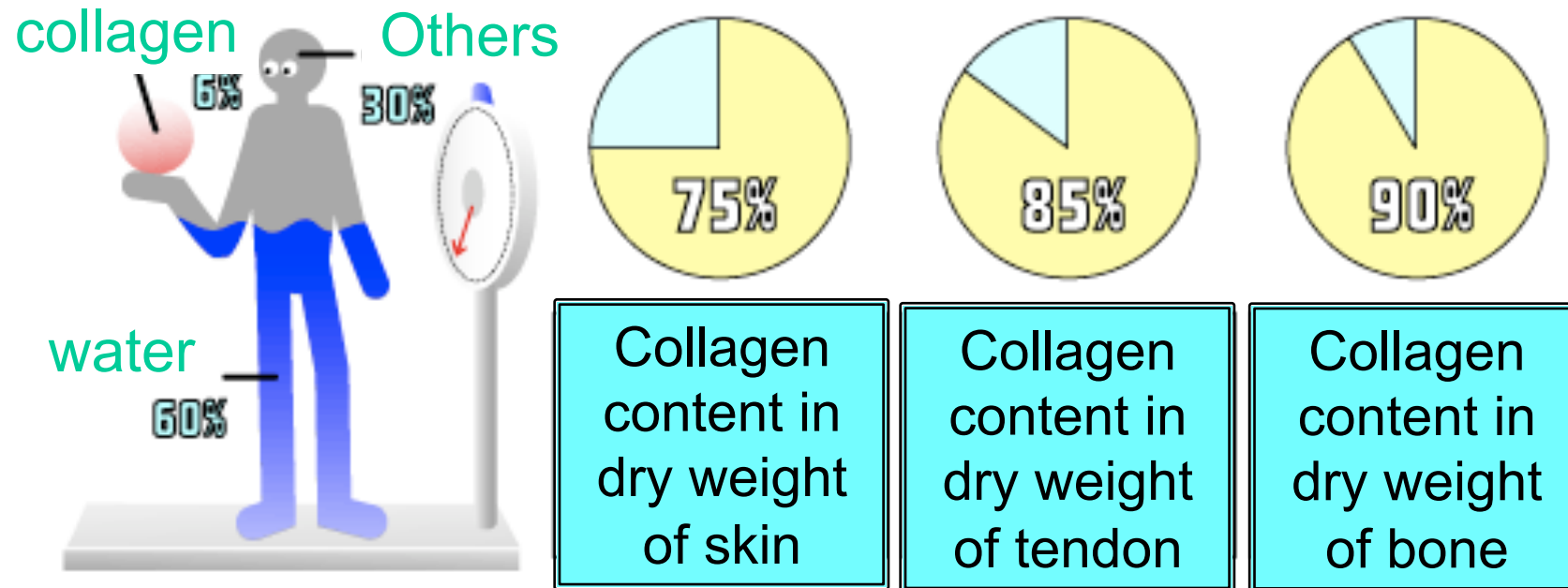


Collagen plays an active role in binding and supporting cells!

- 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

What is collagen?



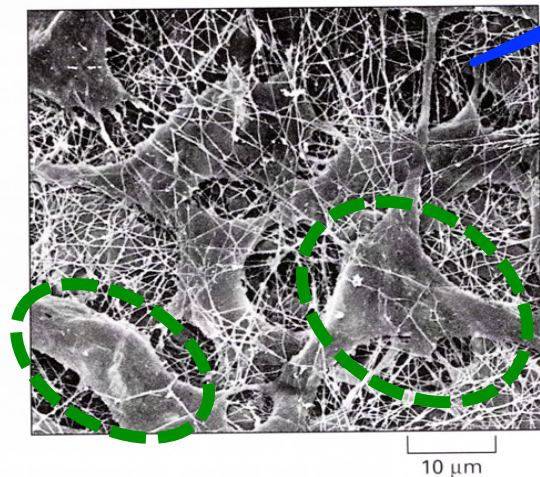
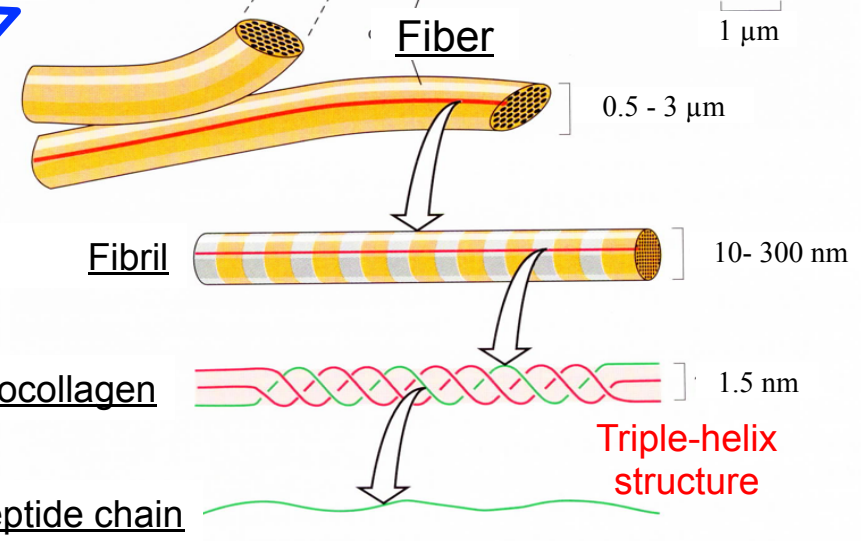
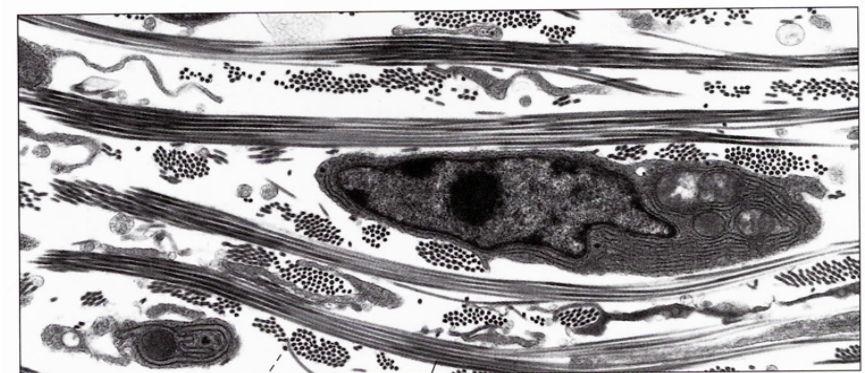
What happens as collagen production decreases?

- Skin loses firmness and elasticity, and sags and wrinkles appear
- Bone density decreases causing osteoporosis
- The gums deteriorate and periodontal disease occurs
- Blood vessels lose elasticity causing hypertension
- Nails become liable to split or chip

What is collagen?

- Most abundant fibrous protein; principal component of ECM
- Produced and remodeled by fibroblast
- Scaffold to maintain tissue structure
- Control production, specialization, and morphogenesis of cells
- Play an important role as a scaffold for cells in engineering tissues

Hierarchical structure of collagen

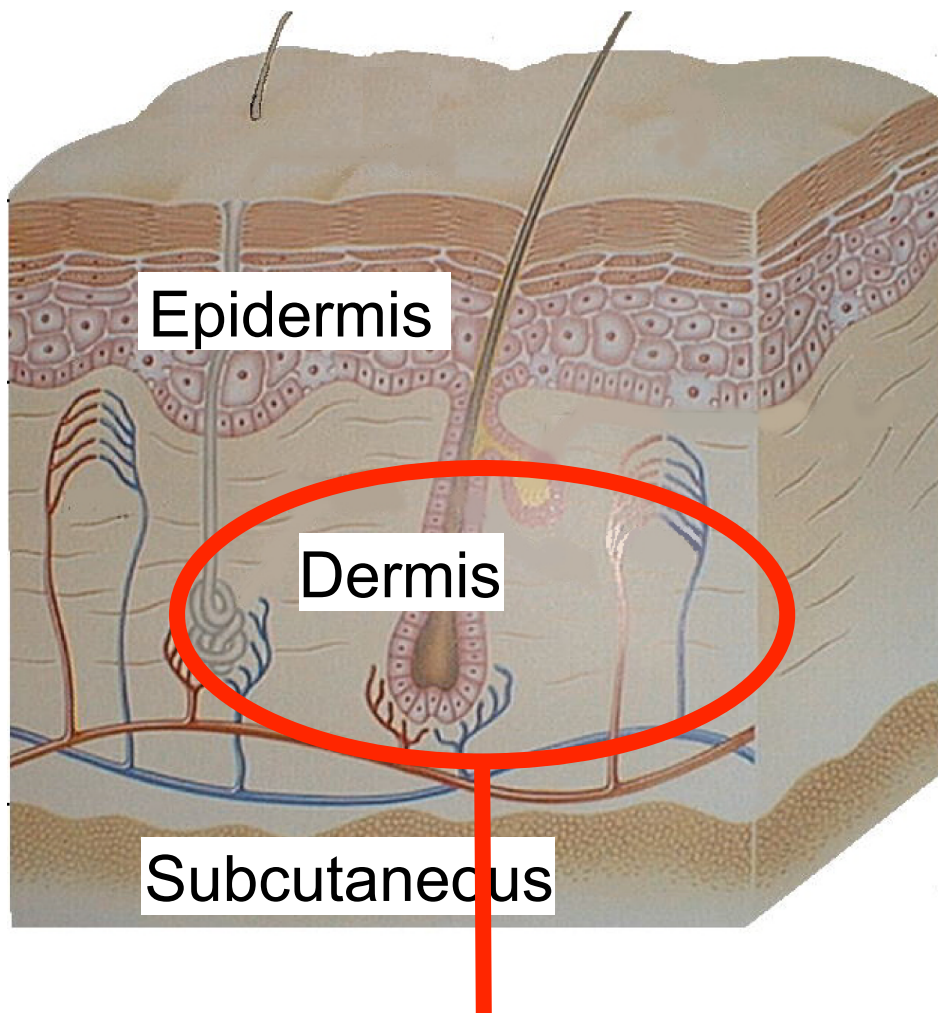


Collagen

Fibroblast

10 μm

Collagen in skin

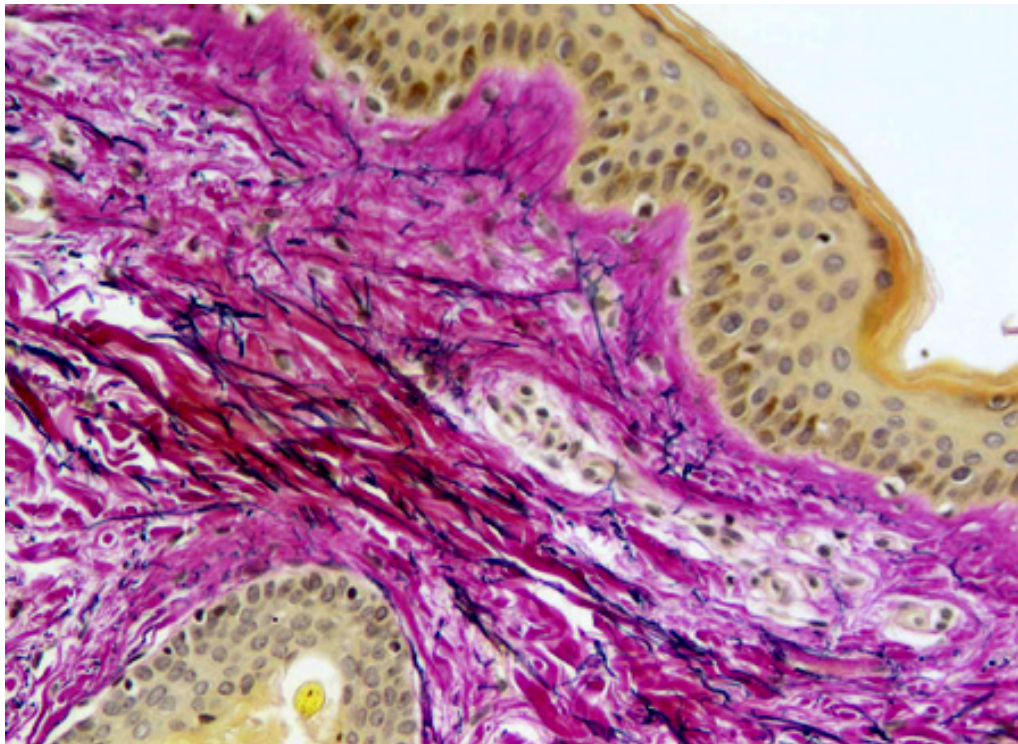


Collagen content = 70wt%

- 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

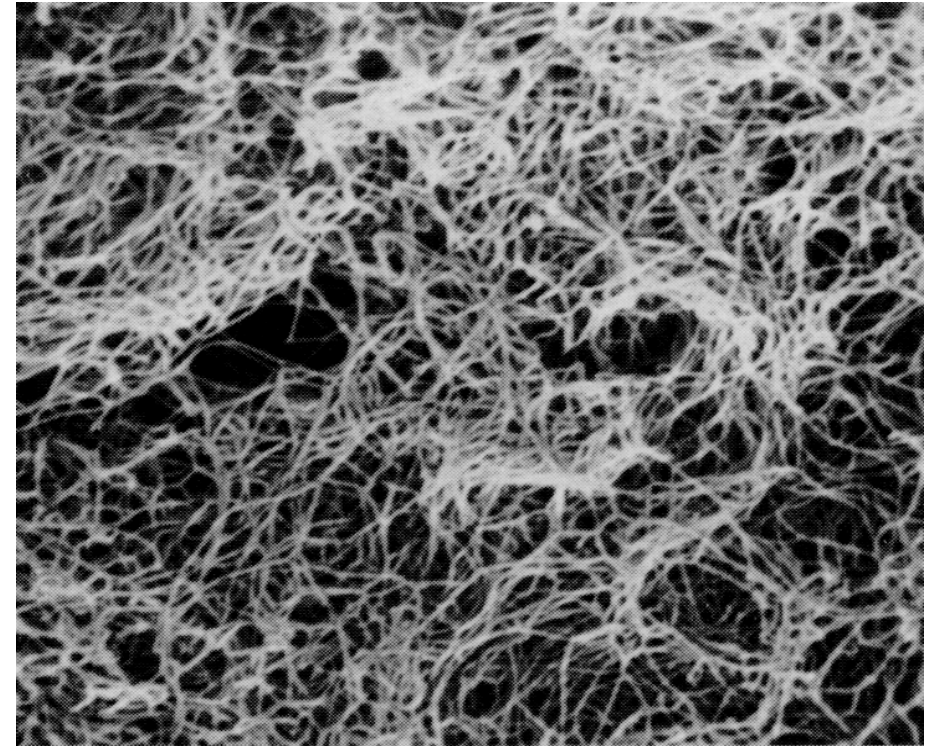
Conventional method to observe collagen

Staining method



Selective visualization
Invasive (skin biopsy)

Electron microscopy

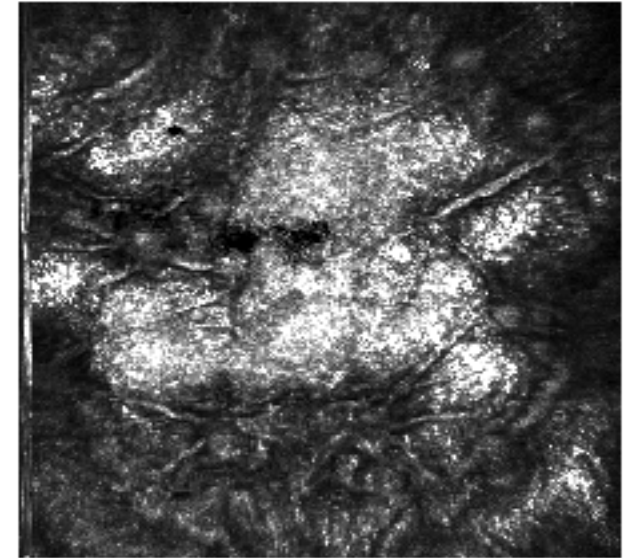


Highly spatial resolution
Invasive (skin biopsy)

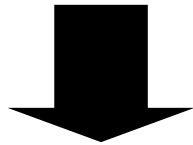
Difficult to visualize distribution of collagen in living tissue “*in vivo*”

Optical probe methods

Laser confocal microscopy



- Simple and rapid
- Non-contact and non-invasive
- Directly applied to the skin



in vivo measurement
No selectivity to collagen

Collagen molecule induced

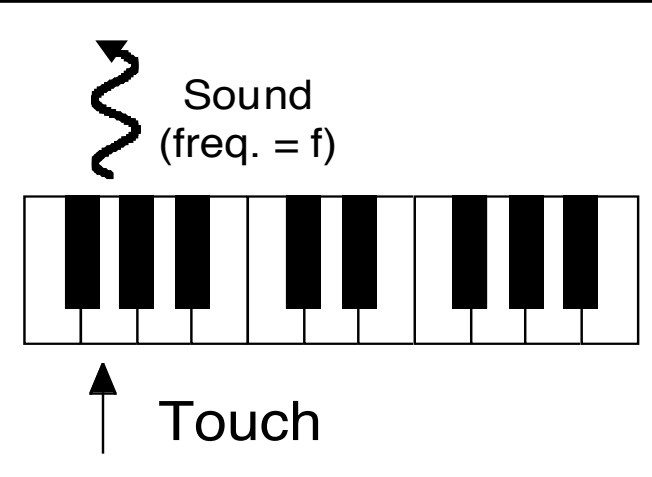
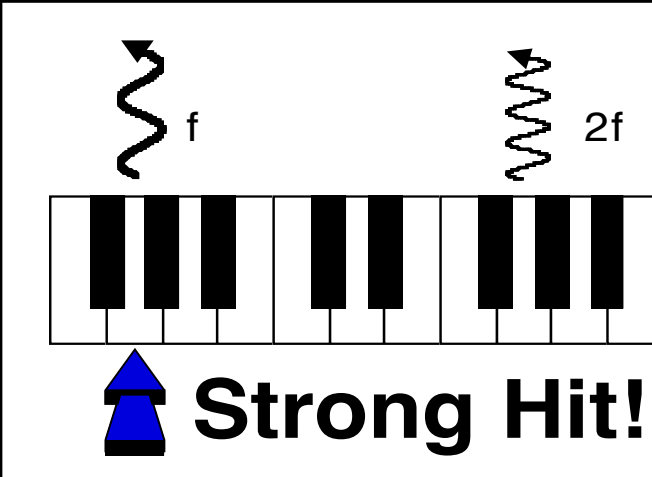
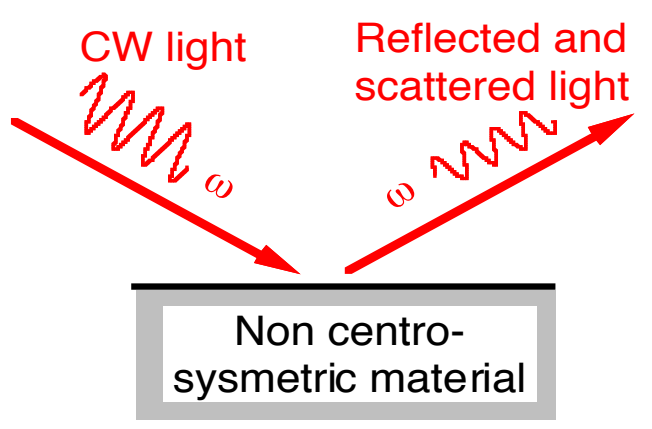
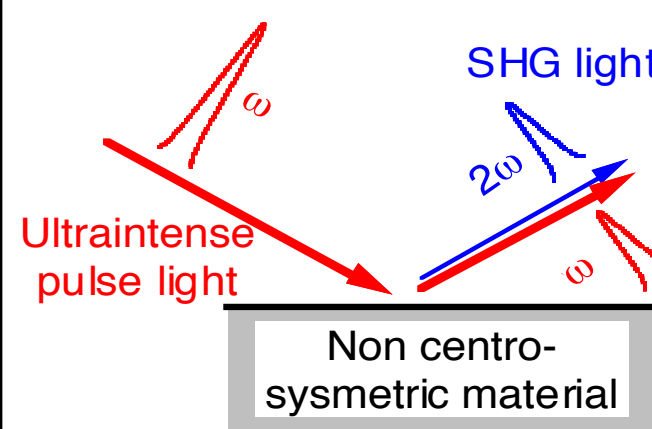
Second-Harmonic-Generation (SHG) light

- Nonlinear optical interaction with ultrashort pulse light
- Specifically generated from collagen molecule in tissue components
- Sensitive to collagen structure and orientation

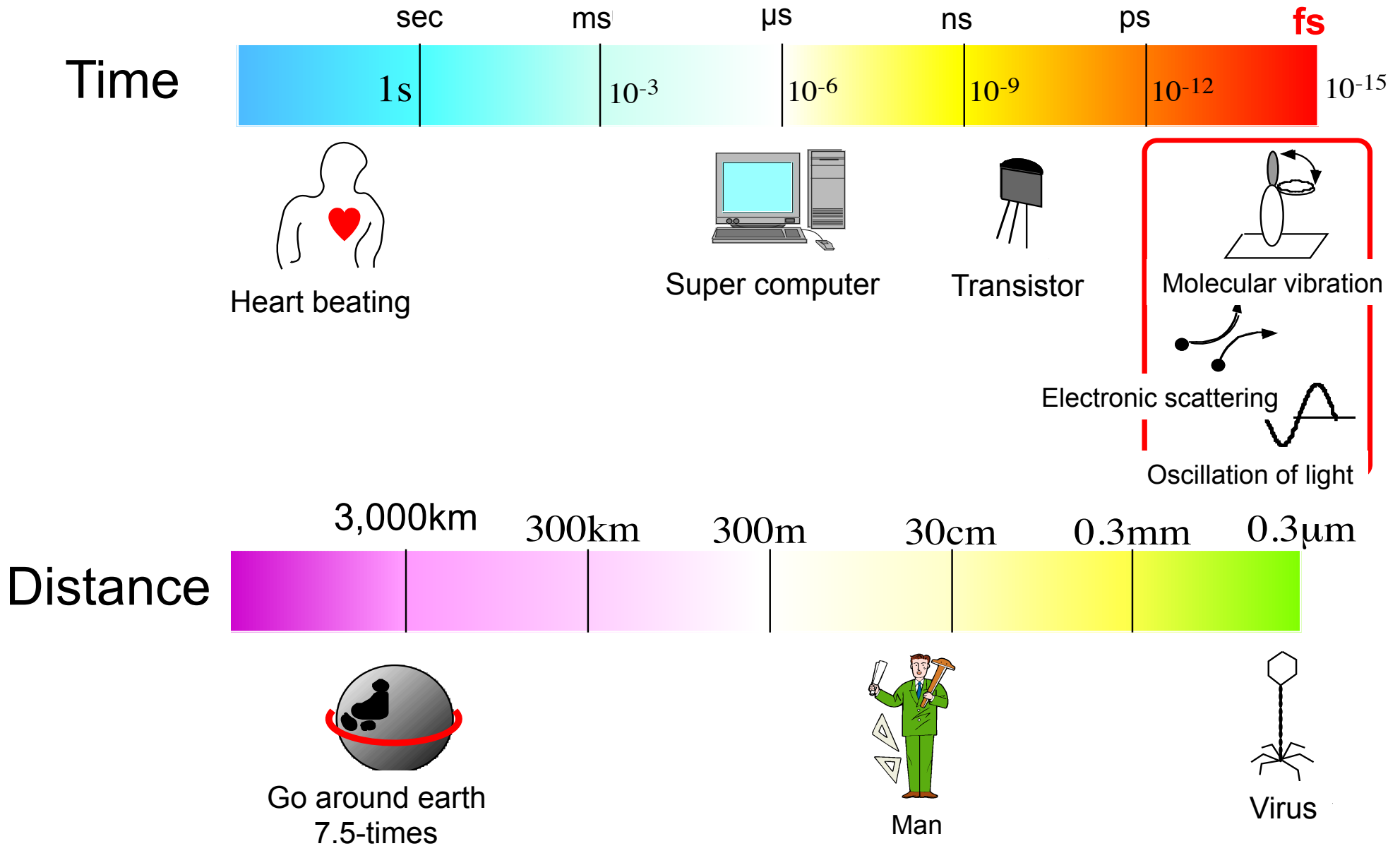
ref) T. Yasui et al, *J. Biomed. Opt.*, Vol. 9, pp. 256-264 (2004).

T. Yasui et al, *Appl. Opt.*, Vol. 43, pp. 2861-2867 (2004).

What is SHG light?

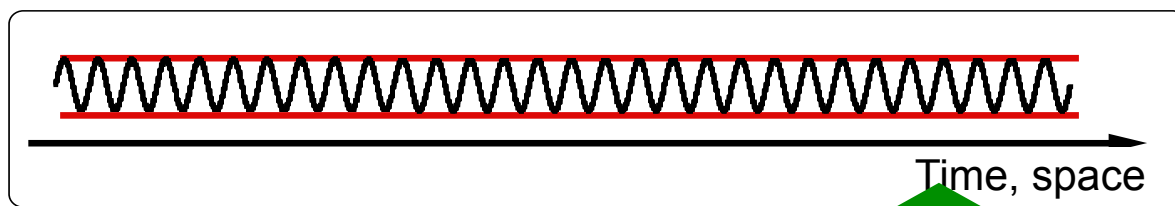
	Linear	Nonlinear
Sound (Keyboard)	 <p>Sound (freq. = f)</p> <p>Touch</p>	 <p>f $2f$</p> <p>Strong Hit!</p>
Light	 <p>CW light Reflected and scattered light</p> <p>ω ω</p> <p>Non centrosymmetric material</p>	 <p>SHG light</p> <p>Ultrainense pulse light</p> <p>ω 2ω ω</p> <p>Non centrosymmetric material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

What is femtosecond (10^{-15} sec) ?

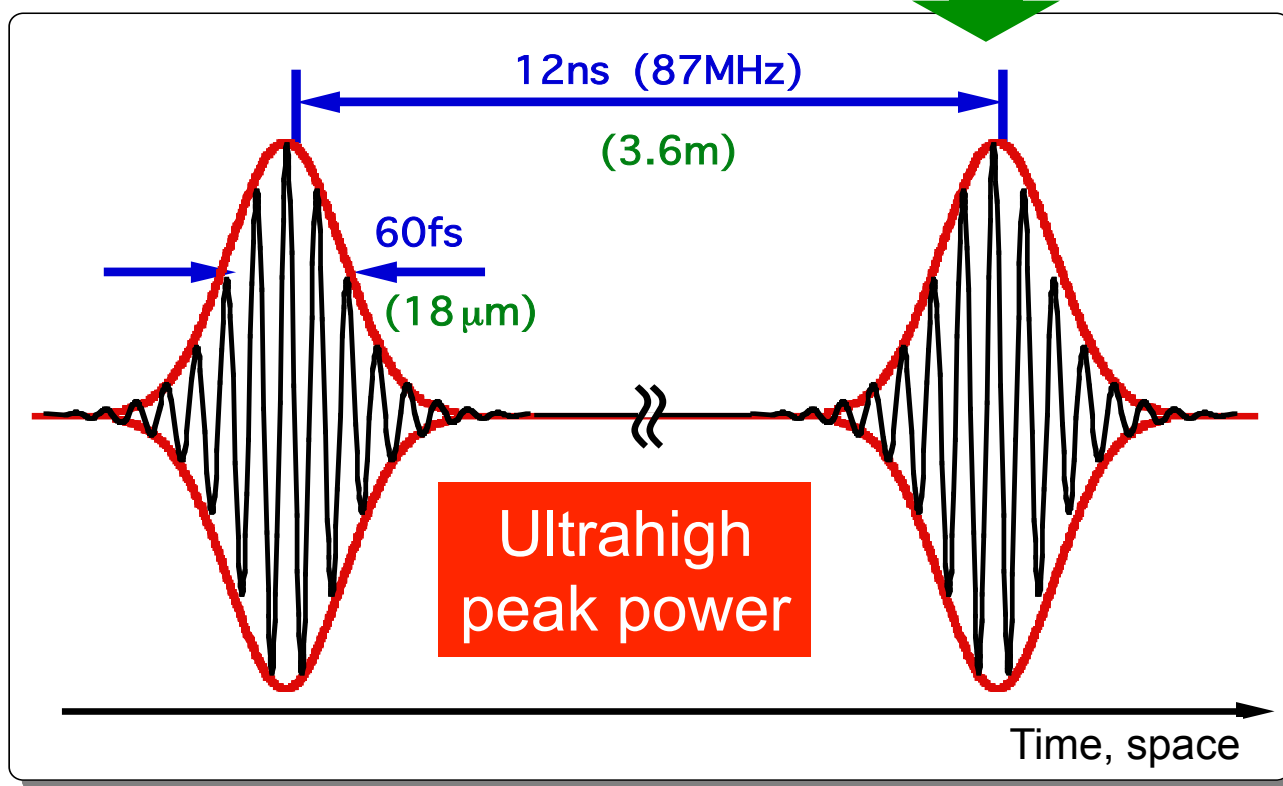


CW (continuous-wave) light vs. fs pulse light

CW (continuous-wave) laser



fs pulse laser



Average power is equal to each other.

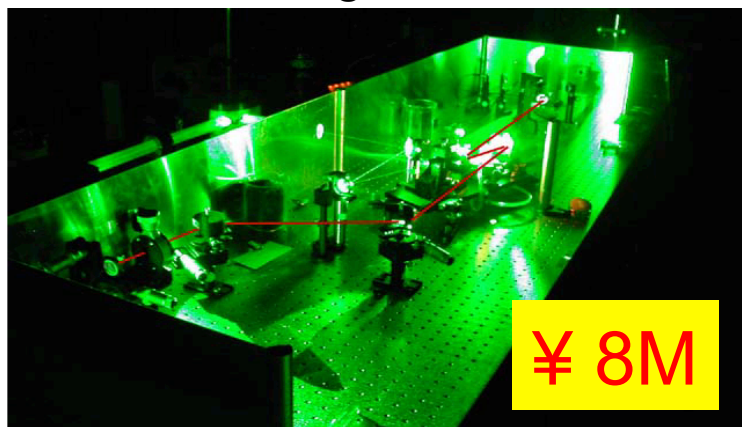
Electric field of light is temporally localized within region of fs order.



Optical nonlinear effect can be easily induced without thermal damage!

Various femtosecond lasers in our lab

“Lab-made” femtosecond Ti:Sapphire laser
at wavelength of 800 nm



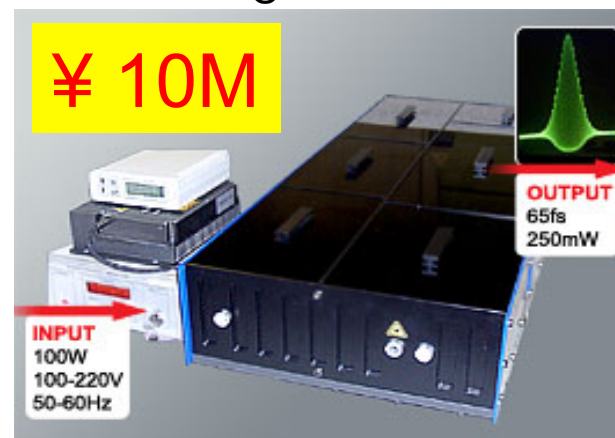
“Amplified” femtosecond Ti:Sapphire
laser at wavelength of 800 nm



“Compact” femtosecond fiber laser
at wavelength of 1550 nm

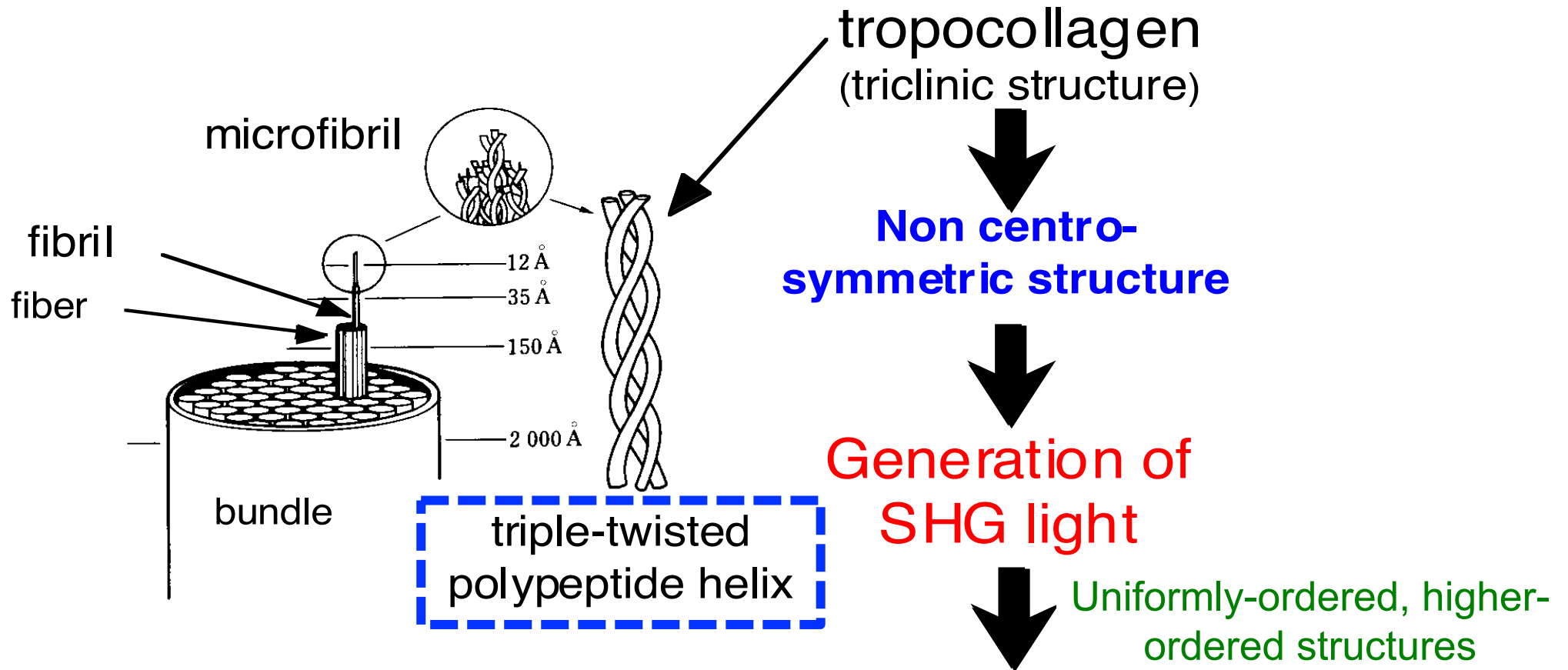


“Penetrative” femtosecond
Cr:Forsterite laser
at wavelength of 1250 nm



Good for
biomed. opt.

Structure of tissue collagen



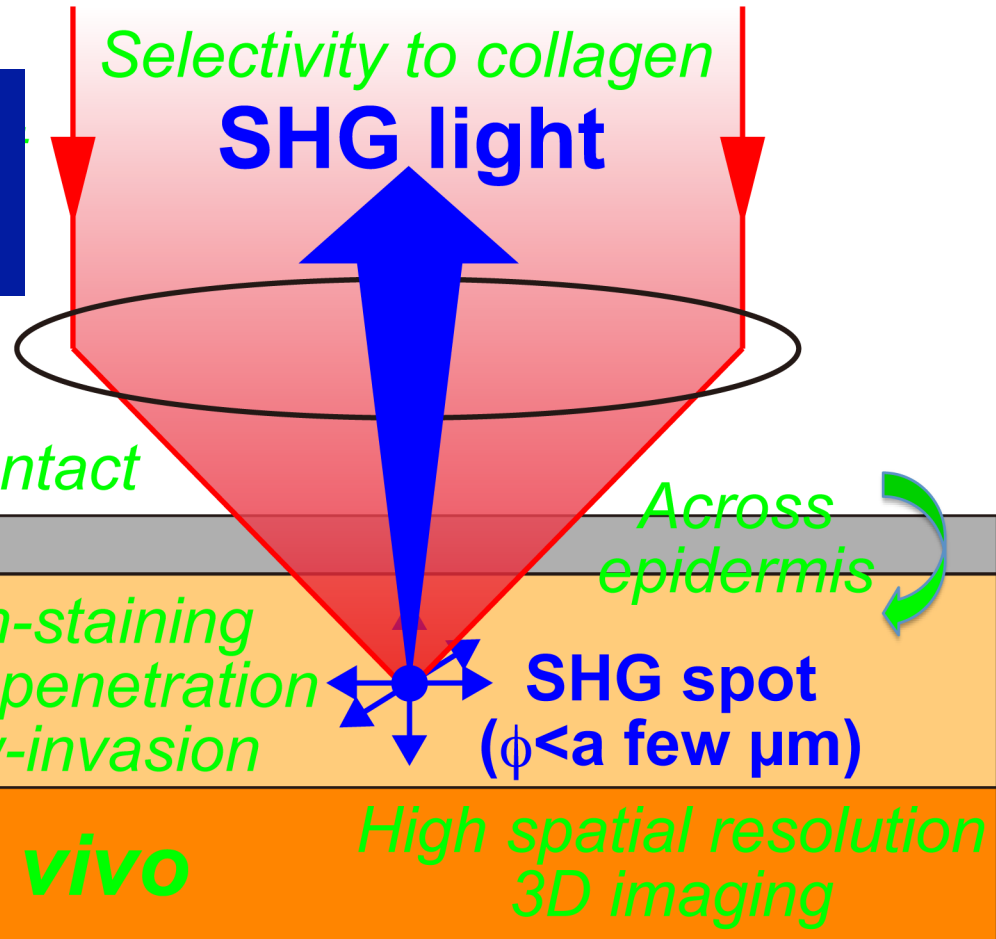
Enhanced SHG light due to constructive interference

Collagen-sensitive SHG microscopy for dermatological applications

Femtosecond laser light

Selective visualization of collagen fiber *in vivo*

- Fluorescence



Dermis (70 wt% collagen)

Epidermis (no collagen)

Subcutis (no collagen)

Non-contact

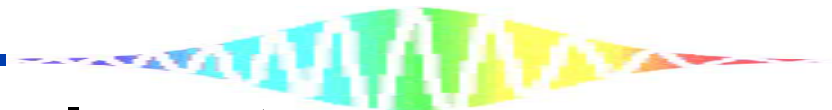
Across epidermis

*Non-staining
Deep penetration
Low-invasion*

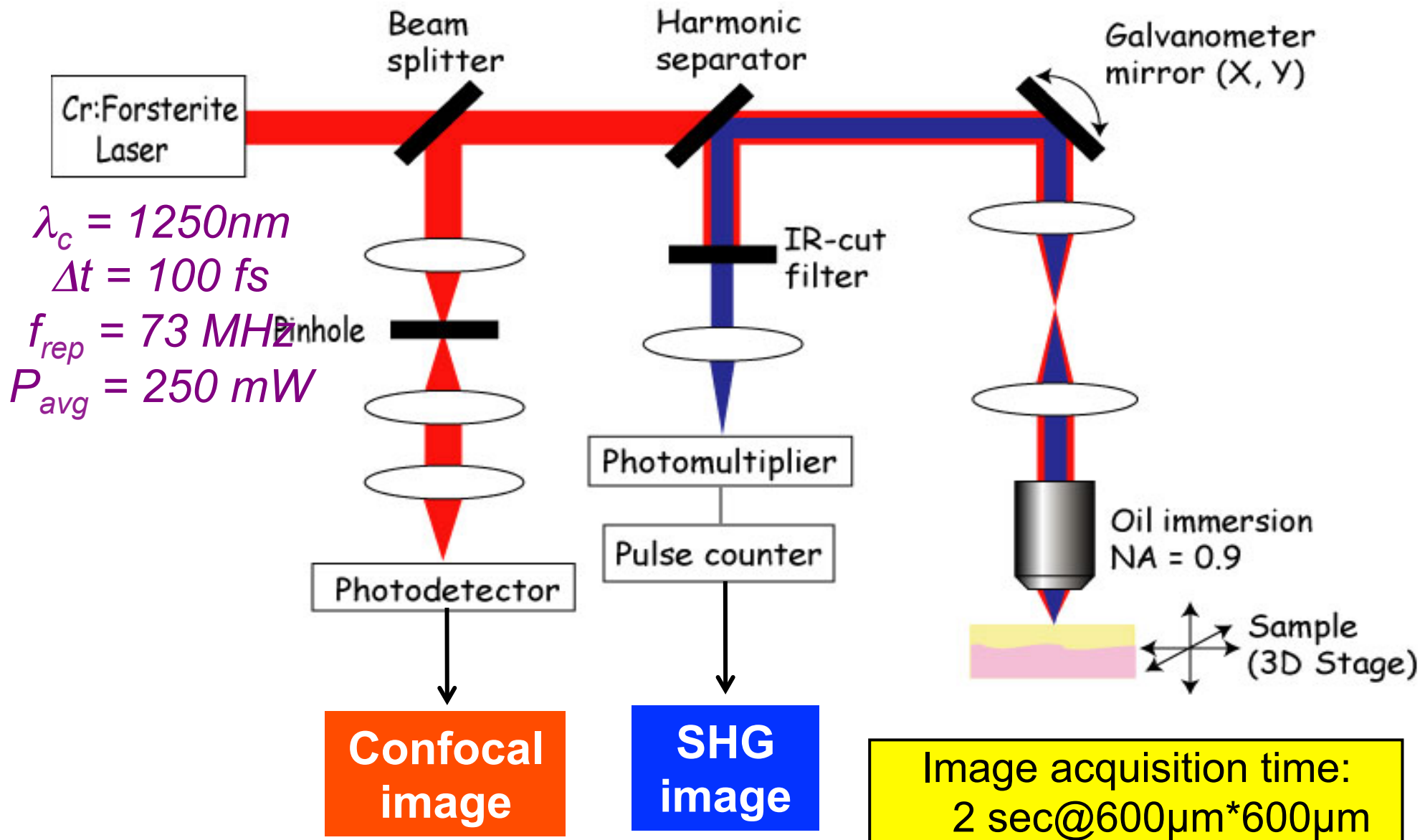
SHG spot
($\phi < \text{a few } \mu\text{m}$)

in vivo

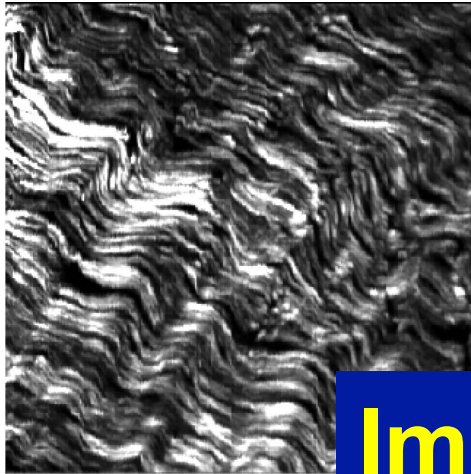
*High spatial resolution
3D imaging*



Experimental setup



SHG imaging of sliced specimen



Uniform orientation of thick collagen fiber

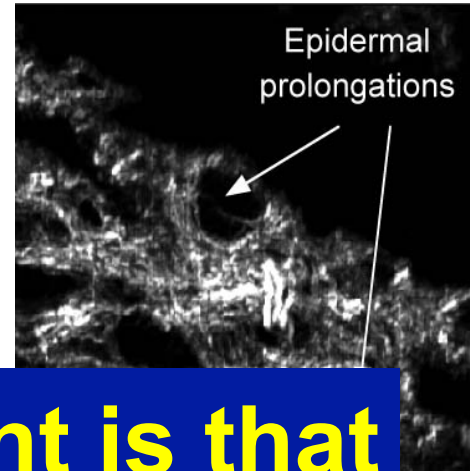
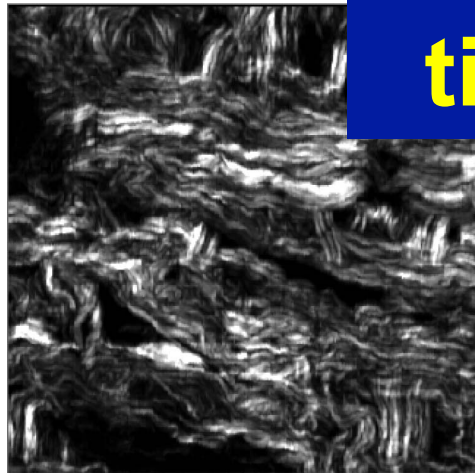


Image size 400μm*400μm

Dense distribution of thin collagen fiber

Important point is that only collagen fiber in tissue is visualized!



Entangles structure of thick collagen fiber

Upper reticular dermis

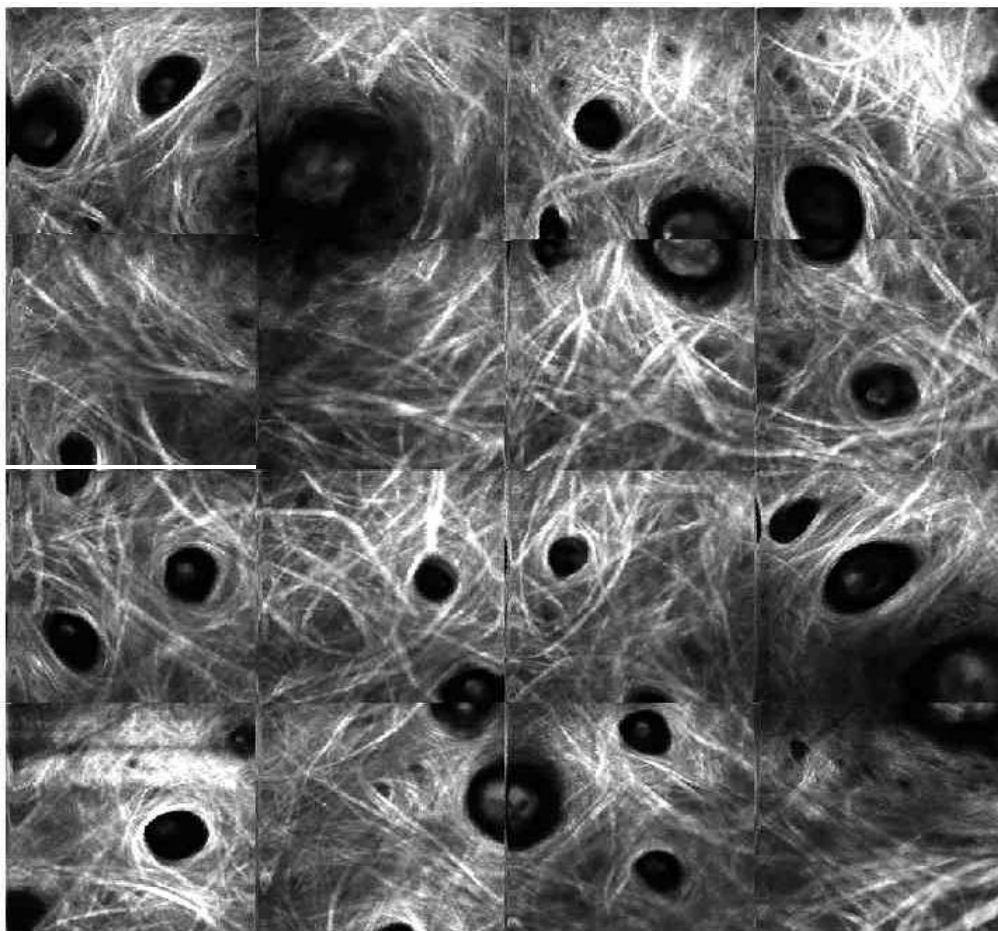


Entangles structure of thick collagen fiber

Lower reticular dermis

Large-area SHG imaging

(Image area: 2.4 mm×2.4 mm)



*Obtained by arranging 16
SHG images
(600 μ m*600 μ m) as a matrix
of four rows and four lines.
Image acquisition time:1min*

Optically-sectioning, depth-resolved SHG imaging

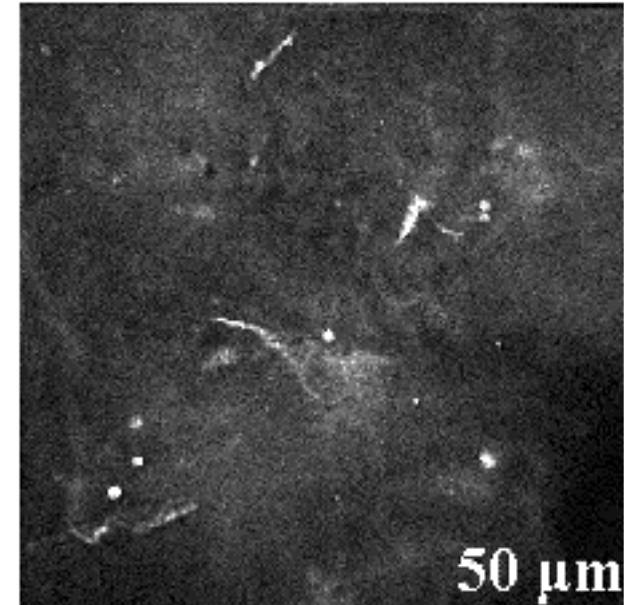
Sample: frozen porcine skin

Area: $400\ \mu\text{m} \times 400\ \mu\text{m}$

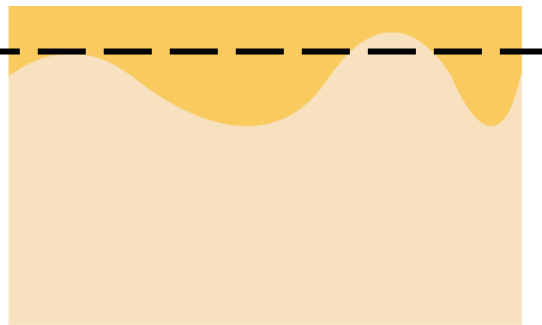
Pixel number: 256×256

Acquisition time: 10 sec/ image

laser power = 10mW



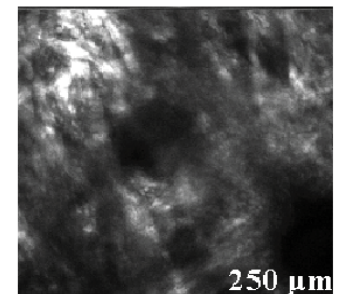
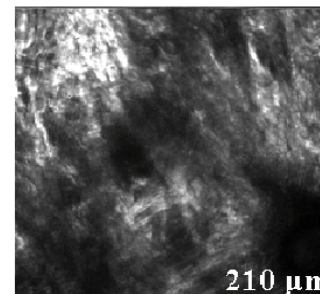
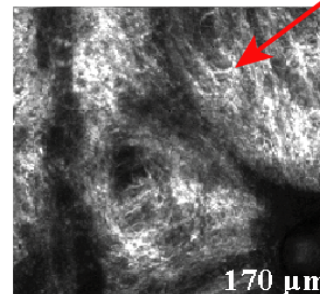
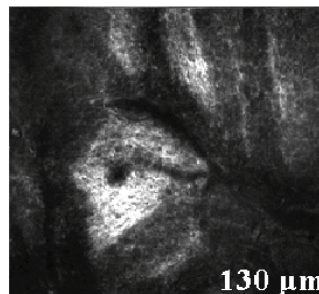
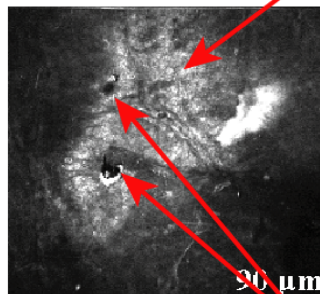
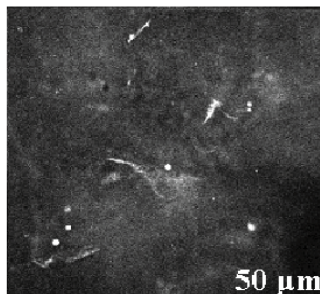
Probing plane



Epidermis
Dermis

Dense distribution of fine collagen fiber

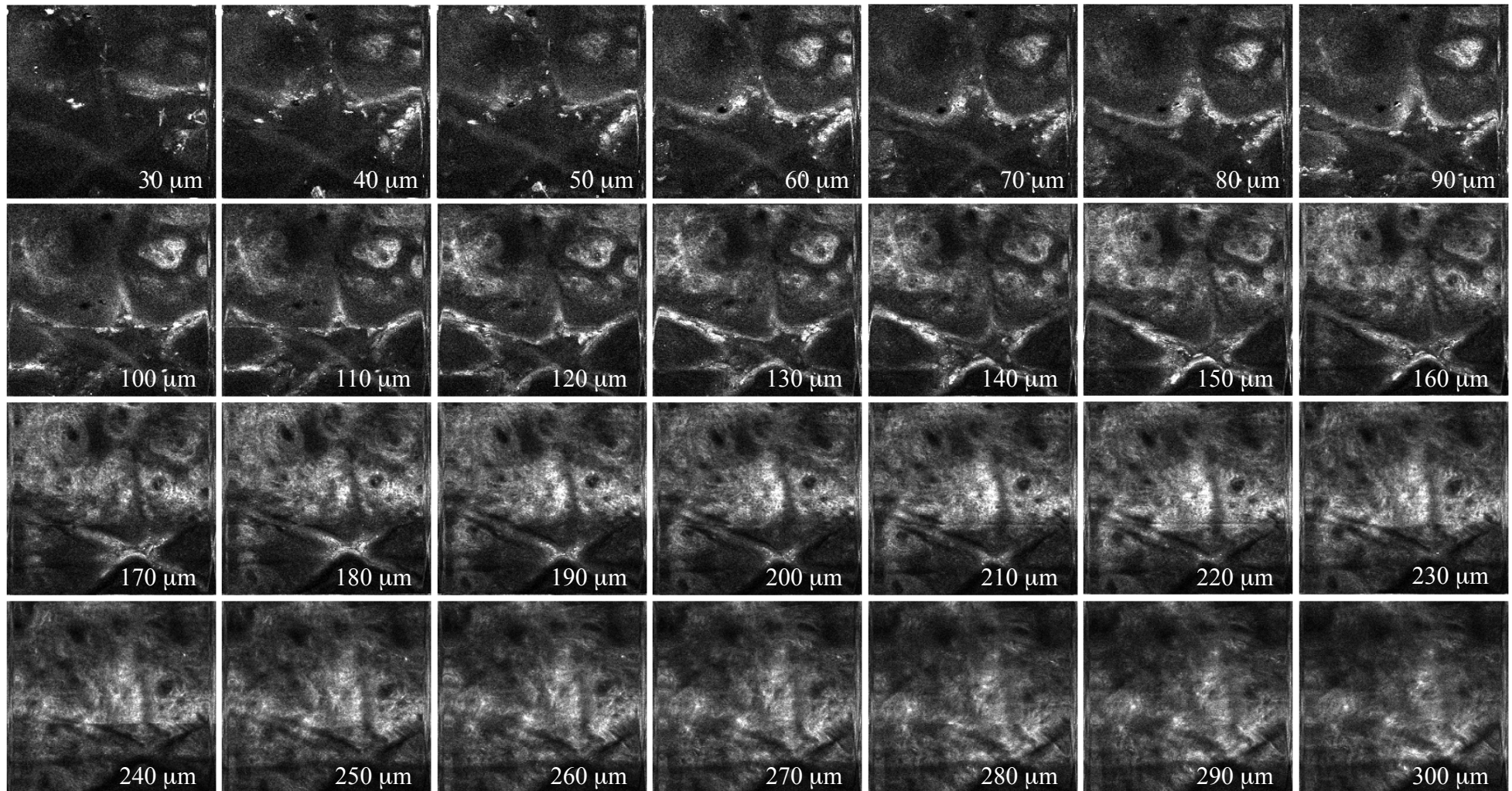
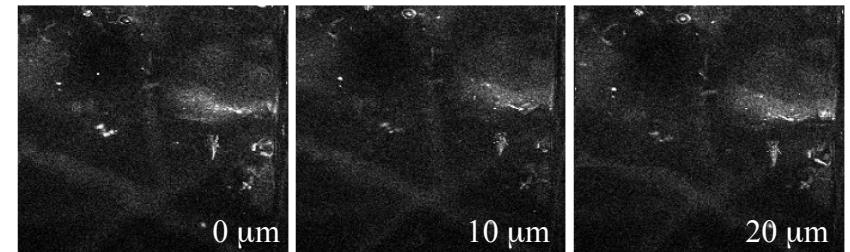
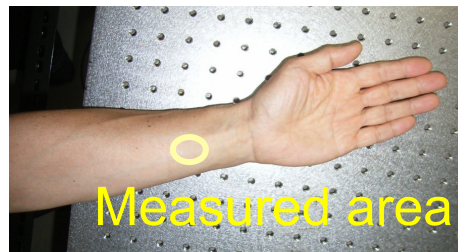
Thickly growing collagen fiber



Capillaries

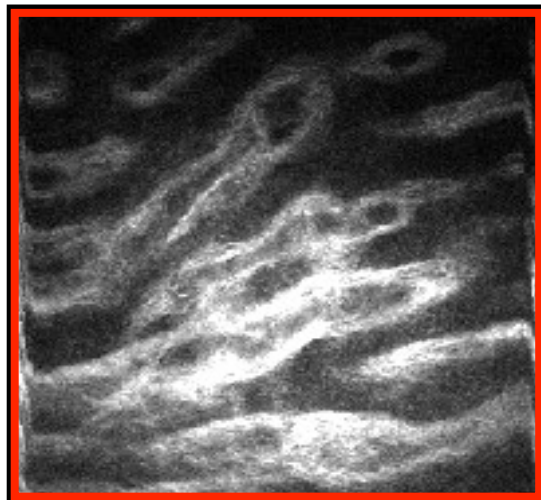
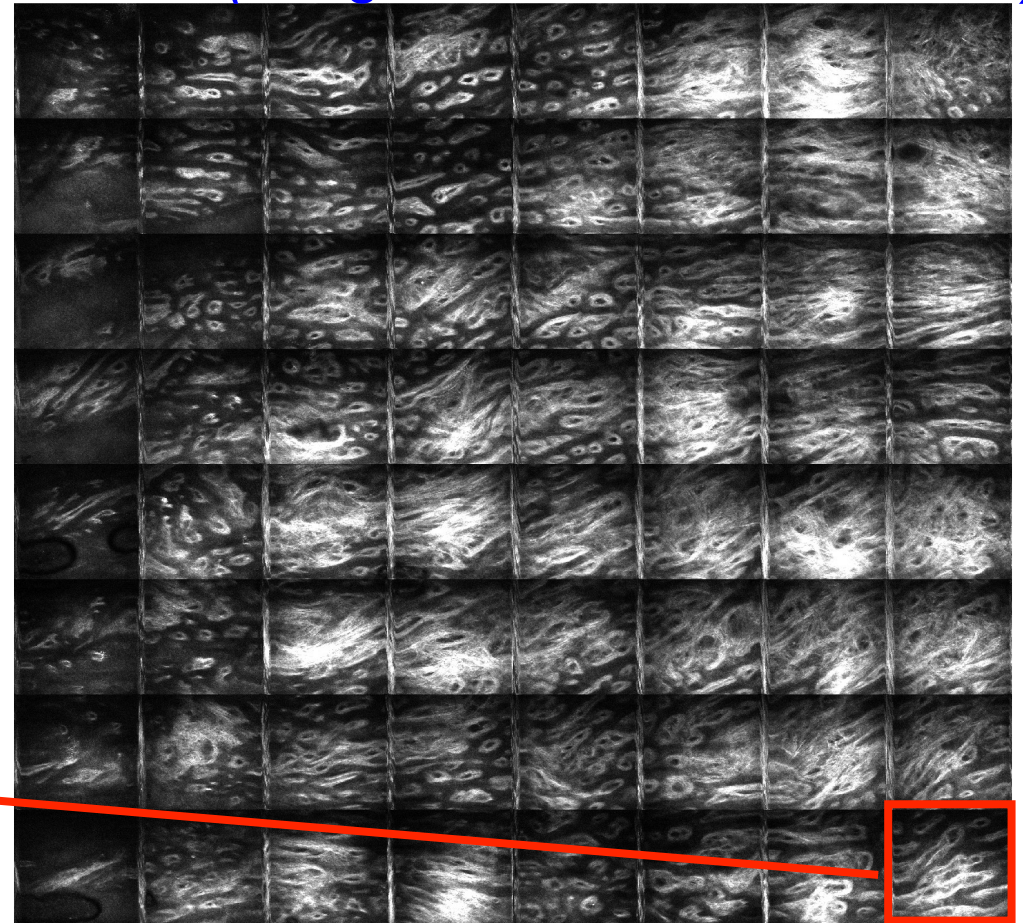
in vivo depth-resolved SHG imaging of human forearm

58-years male
area: 600×600mm
time: 2 sec/image



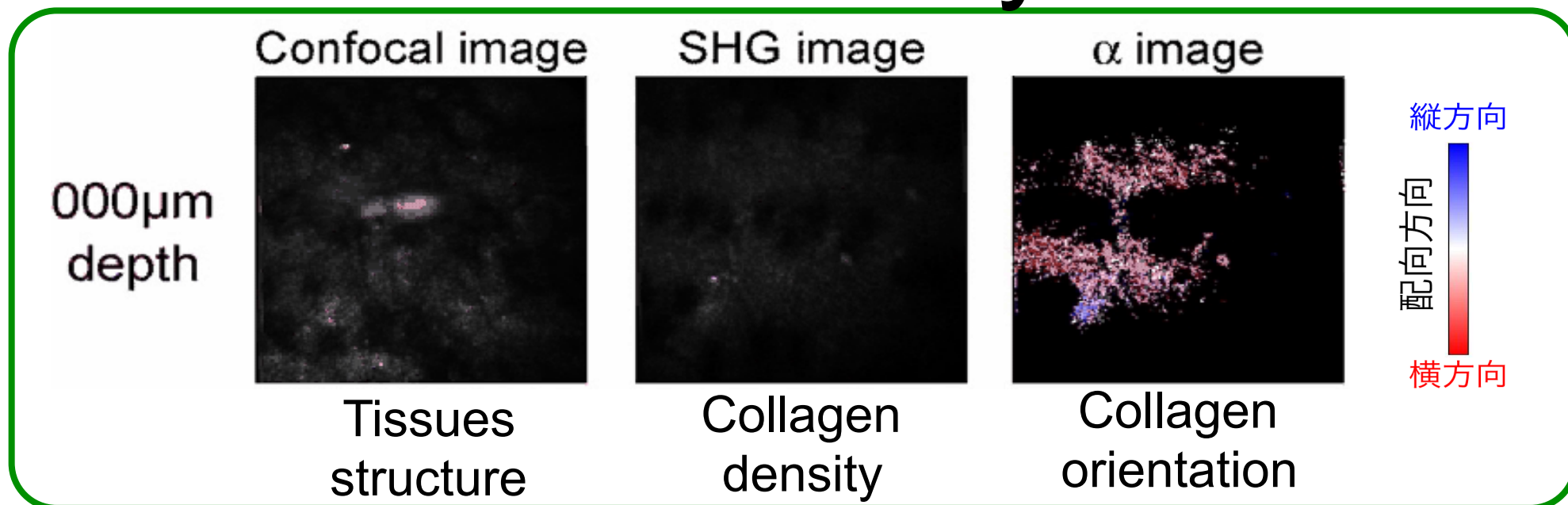
in vivo large-area SHG imaging of hypertrophic scar in human forearm

(image size = 4.4mm*4.4mm)



Abnormal structure of collagen fiber caused by imperfect healing process of injury

Summary



in vivo visualization of “**living**” collagen

