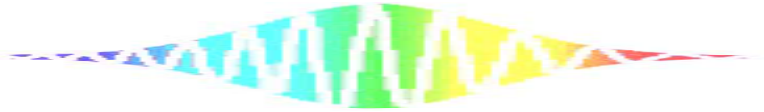

Tomography of collagen fiber orientation in human tissue using depth-resolved second-harmonic-generation polarimetry

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OWLS8 @ MELBOURNE

Introduction

Collagen orientation

Mechanical and functional property of tissue and organ

Non-invasive, non-destructive
non-contact, non-staining

△Conventional optical probe
(scattering, absorption, fluorescence)

Collagen molecule induced

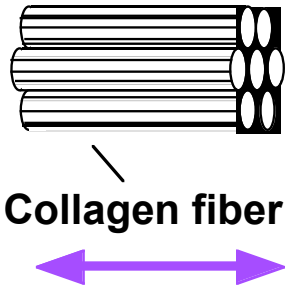
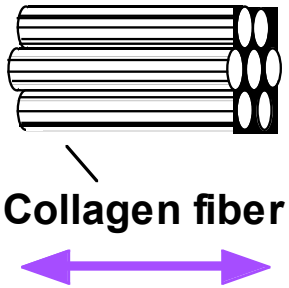
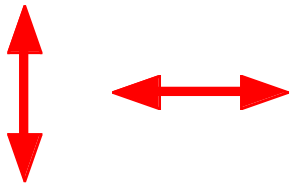
Second-Harmonic-Generation (SHG) light

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

➡ **SHG polarimetry**

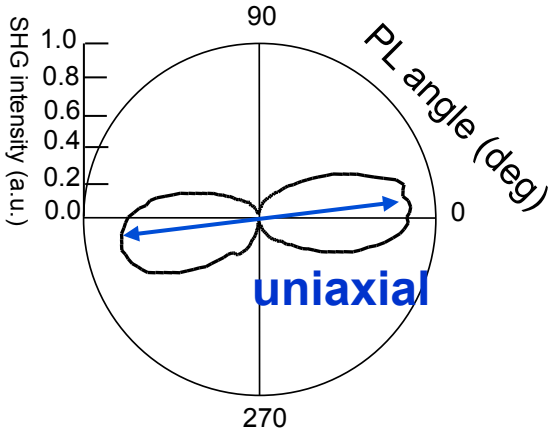
ref) T. Yasui et al, *J. Biomed. Opt.* Vol. 9, pp.259-264 (2004).
T. Yasui et al, *Appl. Opt.*, Vol. 43, pp. 2861-2867 (2004).

Orientation vs. Polarization

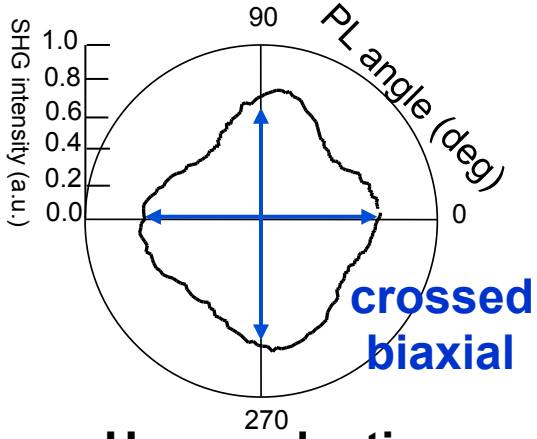
	Axial (Non-centrosymmetric)		Cross-sectional (Centrosymmetric)
	Parallel	Perpendicular	
Collagen orientation			
Laser polarization			
SHG light	Strong	Weak	None

SHG radar graph

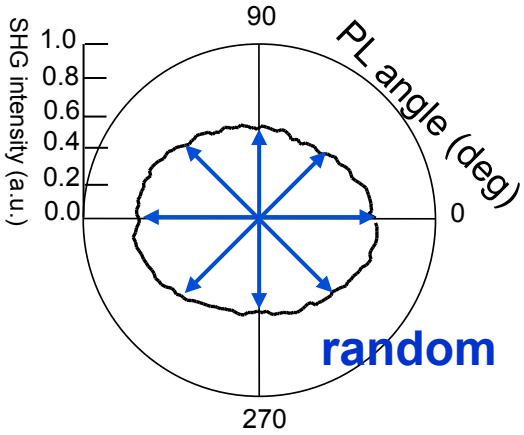
Expected orientation



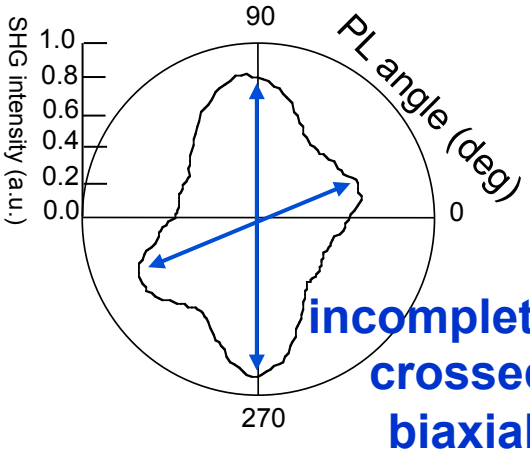
Human Achilles tendon



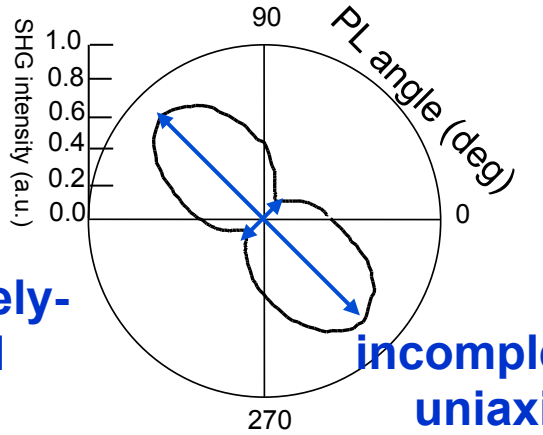
Human dentin



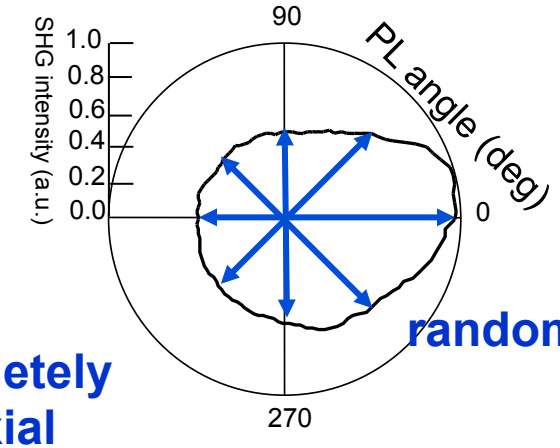
Collagen sponge



Human anklebone



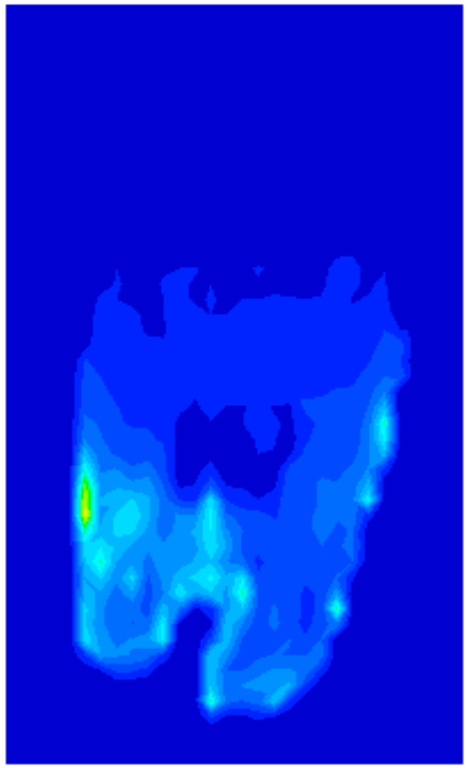
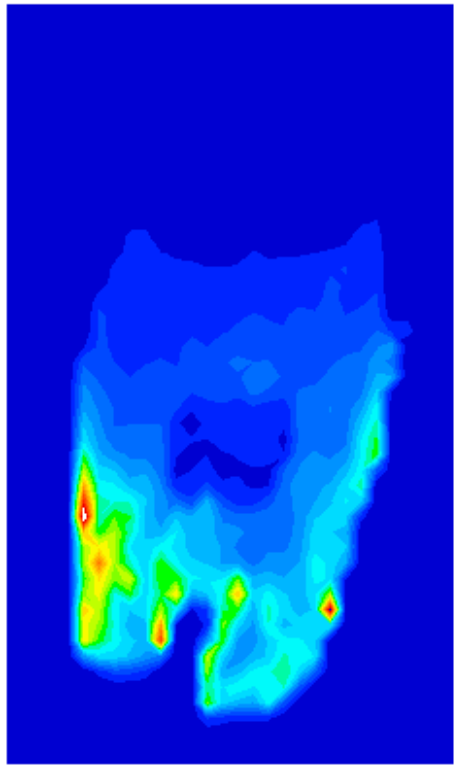
Human reticular dermis



Human papillary dermis
(across epidermis layer)

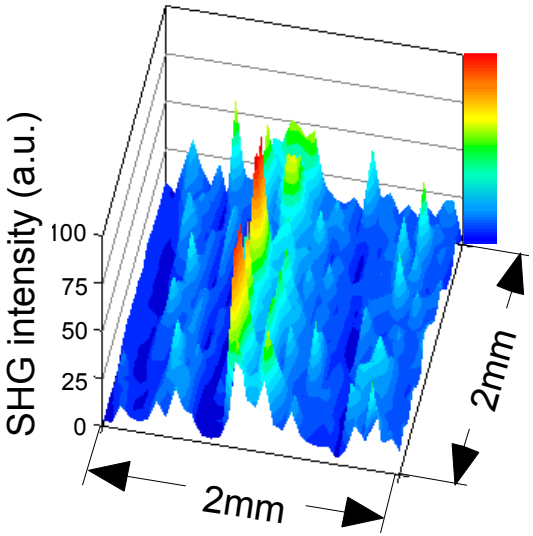
Tissue dependence of collagen orientation

Polarization-resolved SHG imaging @ human dentin

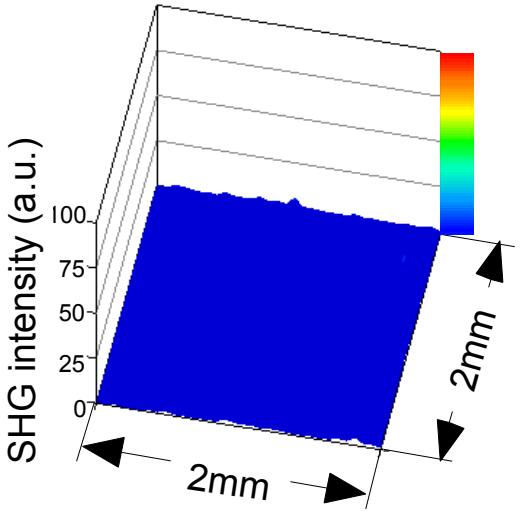
Polarization angle	Vertical ↕	Horizontal ↔
Enamel / Dentin		

Localization of SHG light

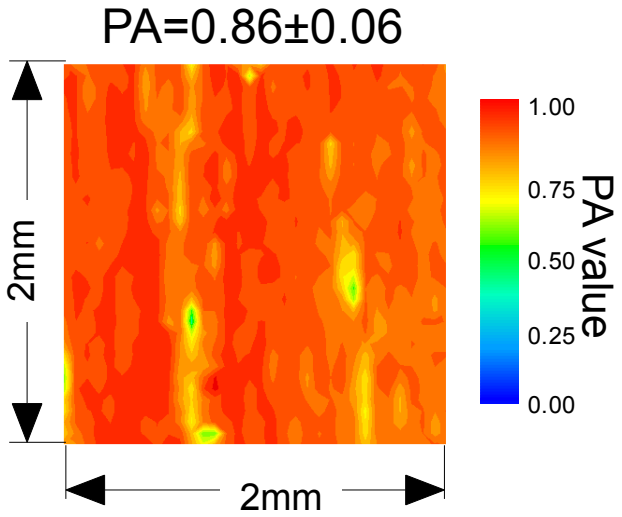
Polarization-resolved SHG imaging @ human Achilles tendon



Polarization // Orientation



Polarization ⊥ Orientation

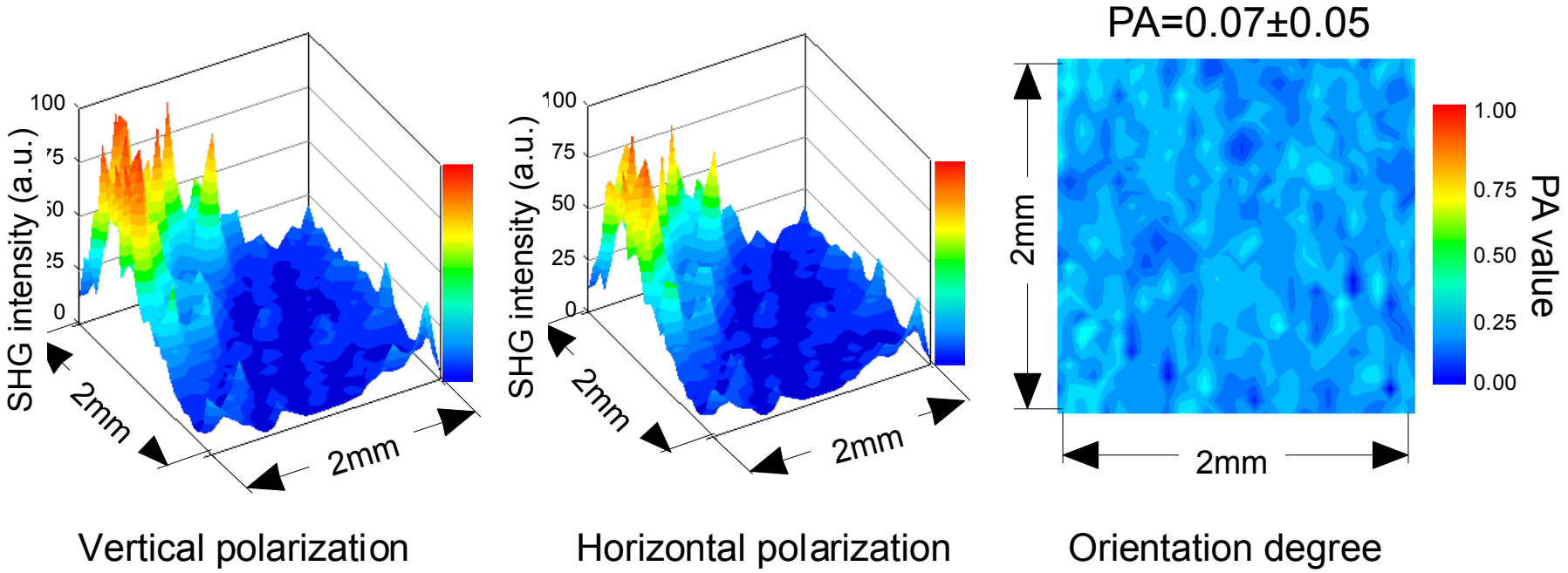


Orientation degree

Uniform distribution of high PA values

Collagen orientation arranged with high organization

Polarization-resolved SHG imaging @ collagen sponge

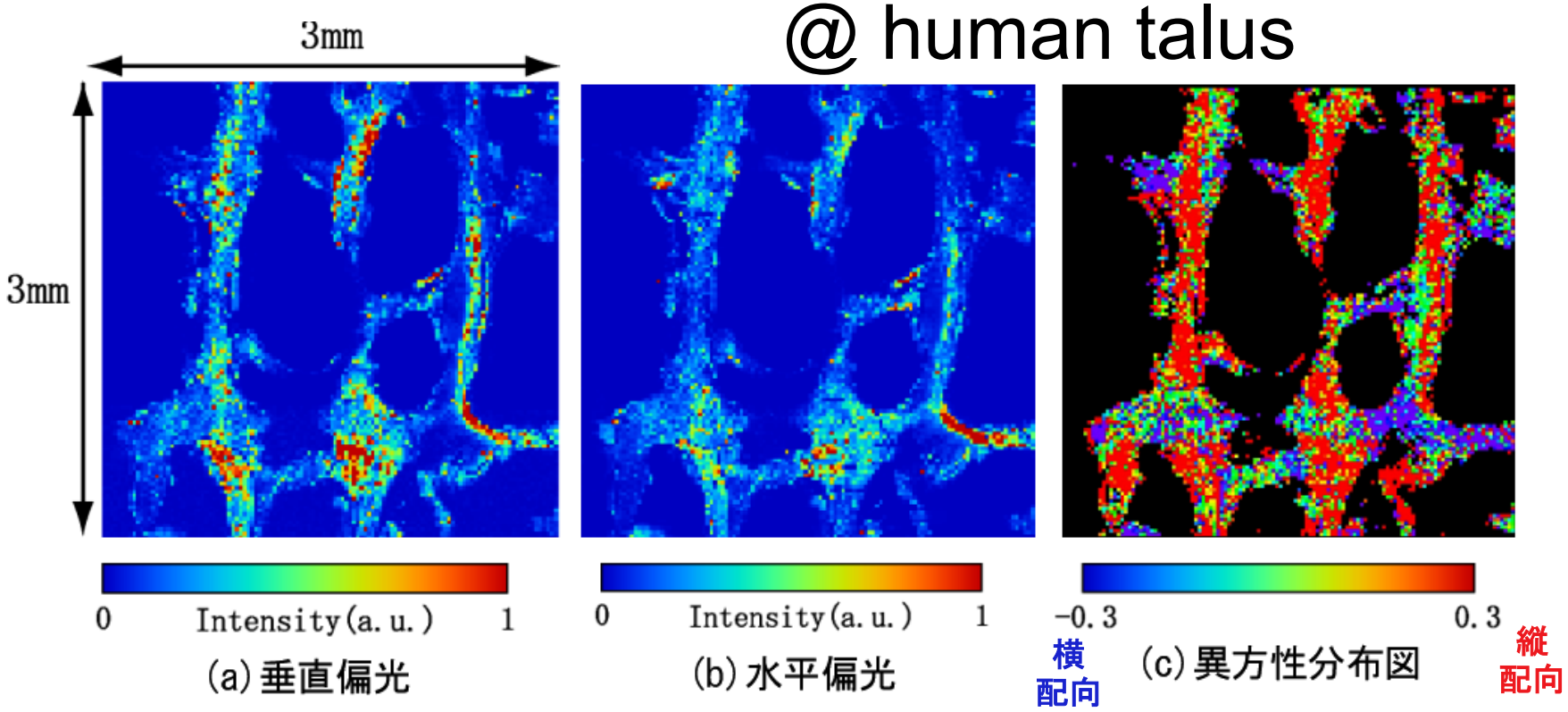


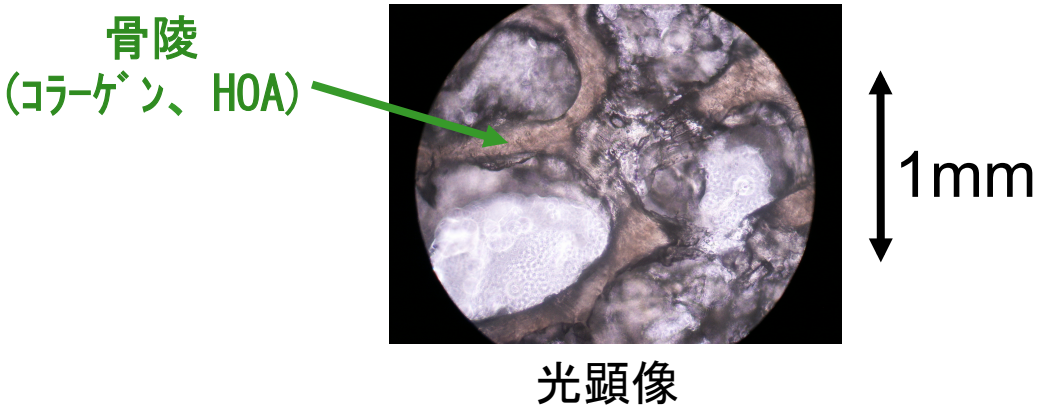
Vertical polarization | Horizontal polarization | Orientation degree

Uniform distribution of low PA values

Collagen orientation distributed equally in all directions

@J@Polarization-resolved SHG imaging @ human talus



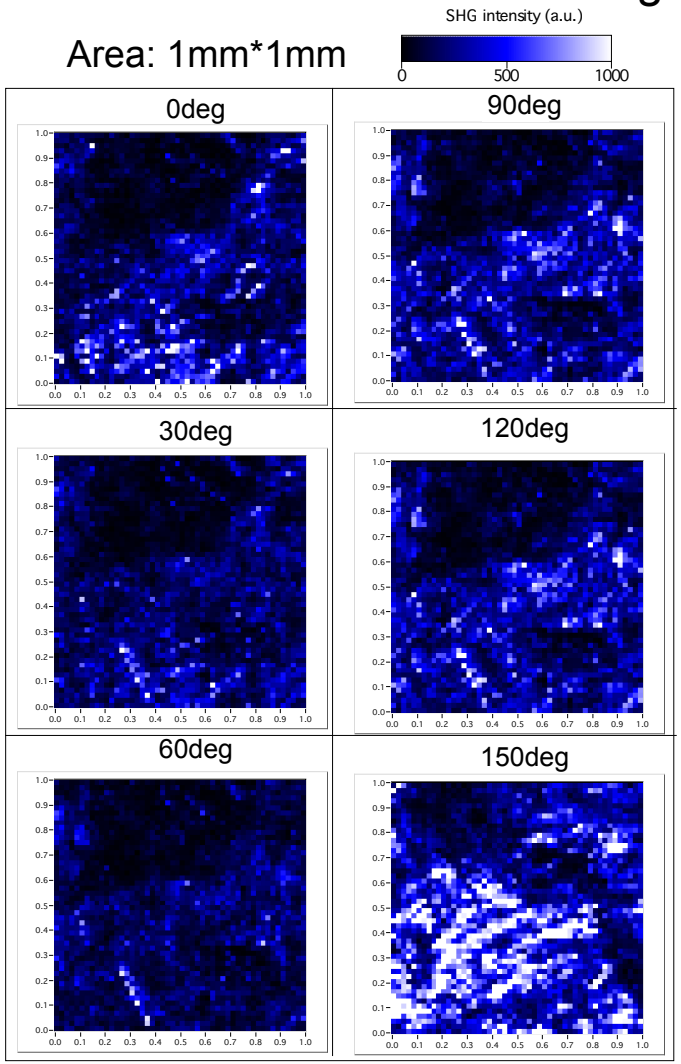


骨陵に沿った
コラーゲン配向

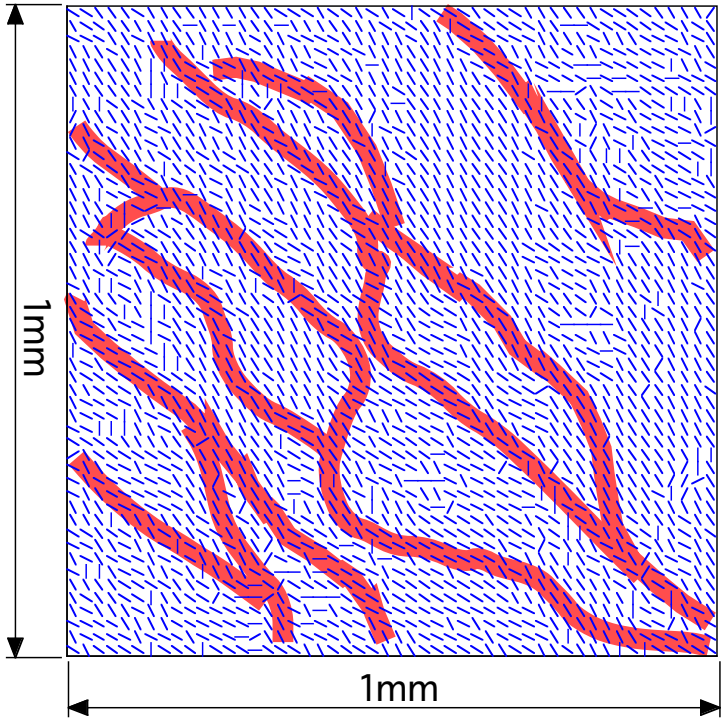
Microscopic 2D distribution of collagen orientation angle @reticular dermis

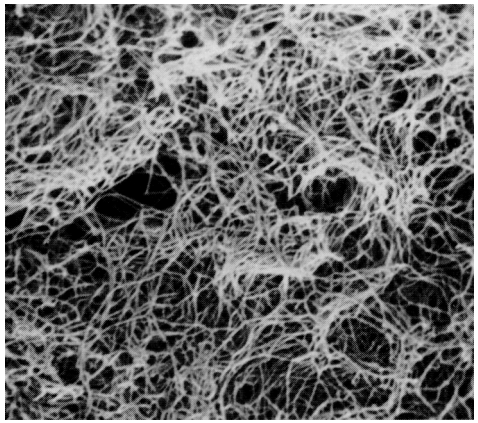
Polarization-resolved SHG image

Area: 1mm*1mm



2D mapping of orientation angle





Microphotograph of bovine dermis

Entangled structure of collagen fiber

Introduction

Collagen orientation

Mechanical and functional property of tissue and organ

Non-invasive, non-destructive
non-contact, non-staining

△Conventional optical probe
(scattering, absorption, fluorescence)

Collagen molecule induced

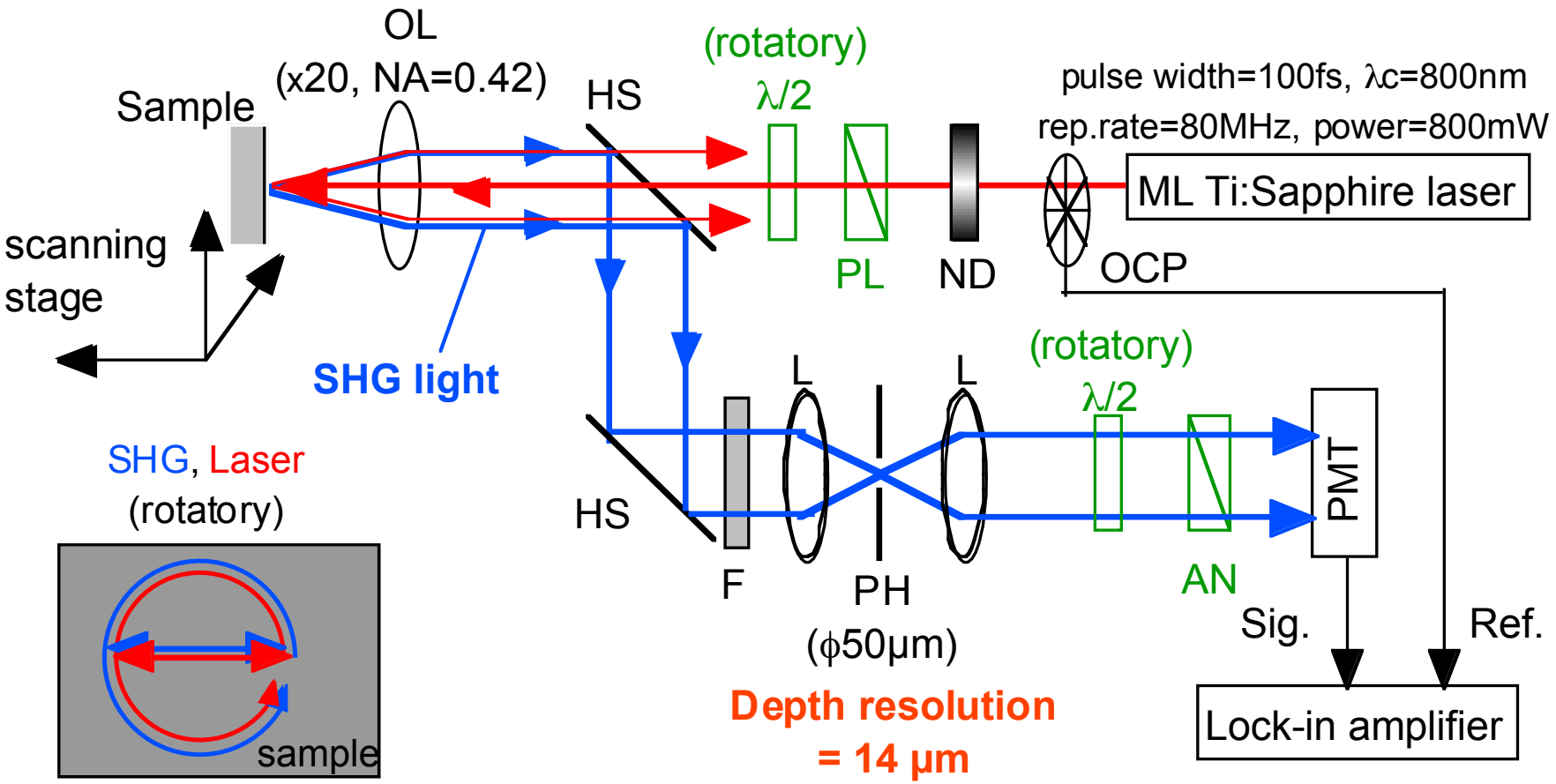
Second-Harmonic-Generation (SHG) light

- **SHG polarimetry**
- **Polarization-resolved SHG imaging**

Present talk

Tomography of collagen fiber orientation in human tissues based on depth-resolved SHG polarimetry

Experimental setup



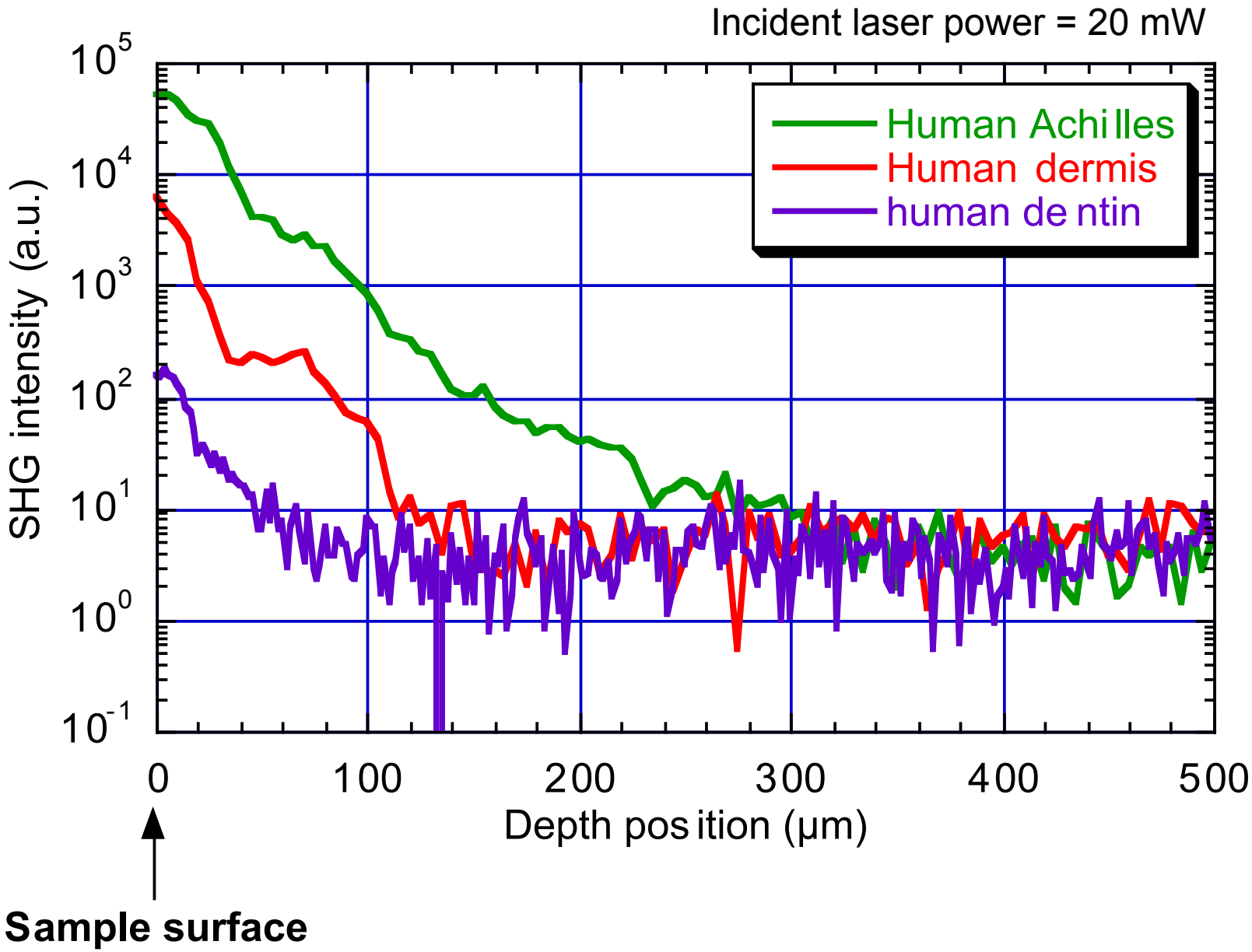
Polarization condition

OCP; optical chopper, ND; neutral density filter, P; polarizer, λ/2; half waveplate, HS; harmonic separator, OL; objective lens, PH; pinhole, F; blue pass filter, A; analyzer, PMT; photomultiplier

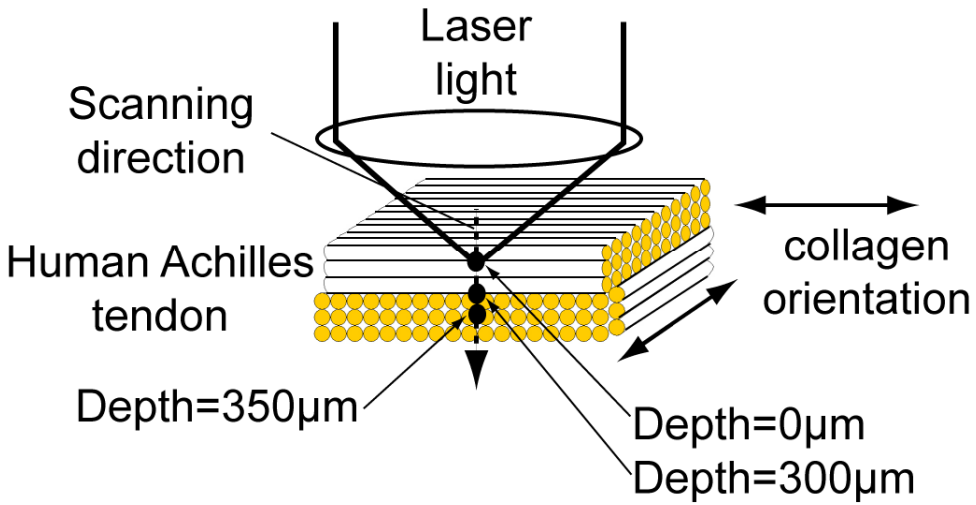
Human tissue sample

Soft tissue	Hard tissue
Back dermis • formalin fixation, washing and drying • reticular layer • 1mm thickness **Achilles tendon** • formalin fixation, washing and drying • sliced along axial direction • 2mm thickness	**Dentin** • sliced along tooth axis • 1mm thickness **Anklebone** • sliced across section • 1mm thickness

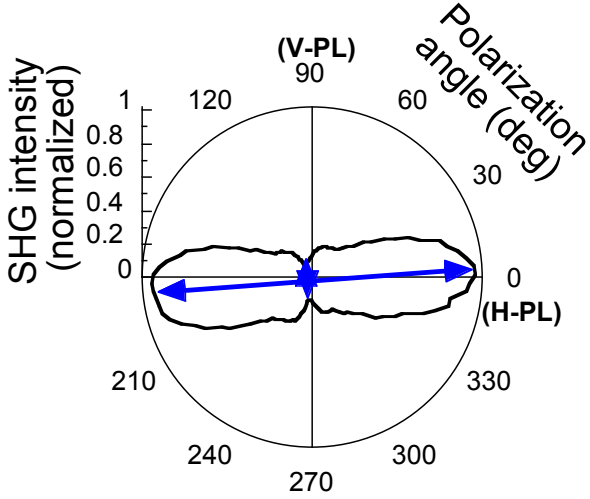
Penetration power of depth-resolved SHG measurement



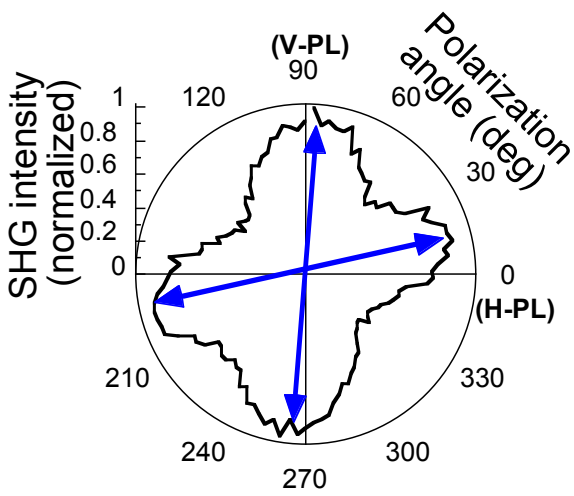
Basic performance of depth-resolved SHG polarimetry



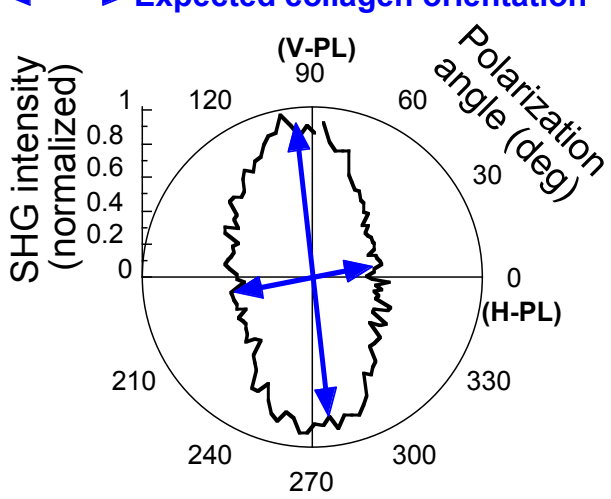
Expected collagen orientation



Depth=0μm
(suface of 1st layer)



Depth=300μm
(boundary between 1st and 2nd layers)



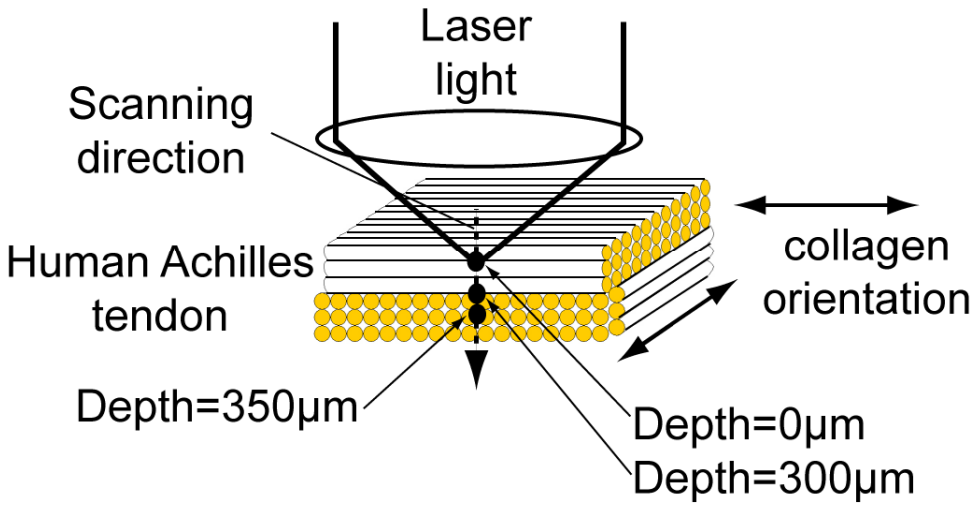
Depth=350μm
(2nd layer)

Horizontal Uniaxial

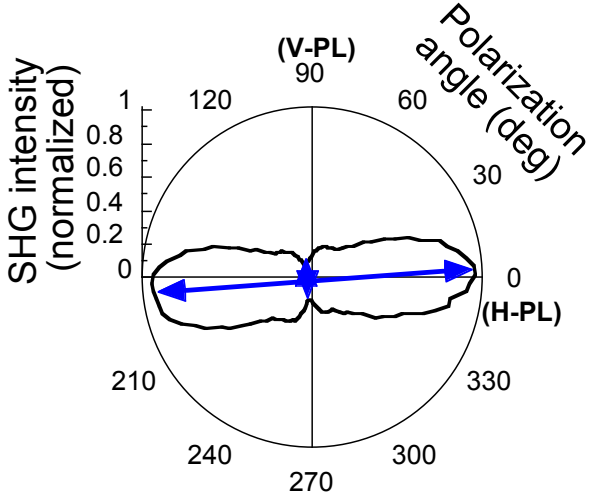
Mixture of horizontal and vertical

Vertical

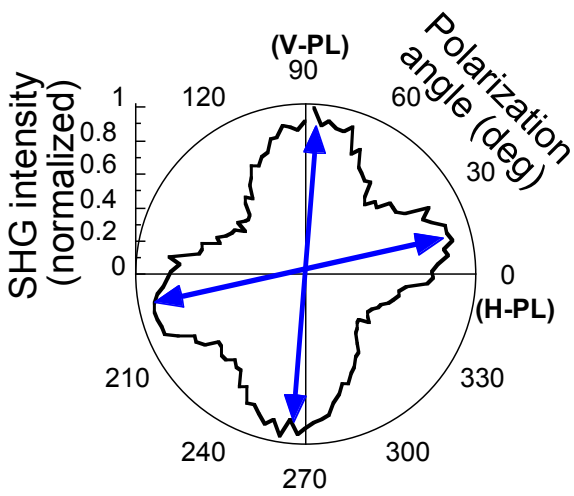
Basic performance of depth-resolved SHG polarimetry



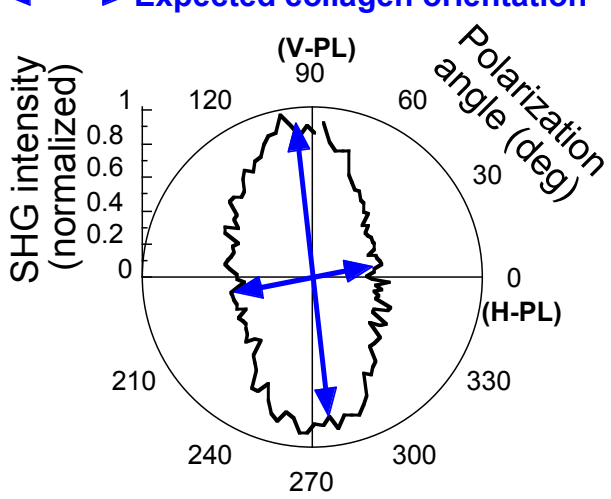
Expected collagen orientation



Depth=0μm
(suface of 1st layer)



Depth=300μm
(boundary between 1st and 2nd layers)



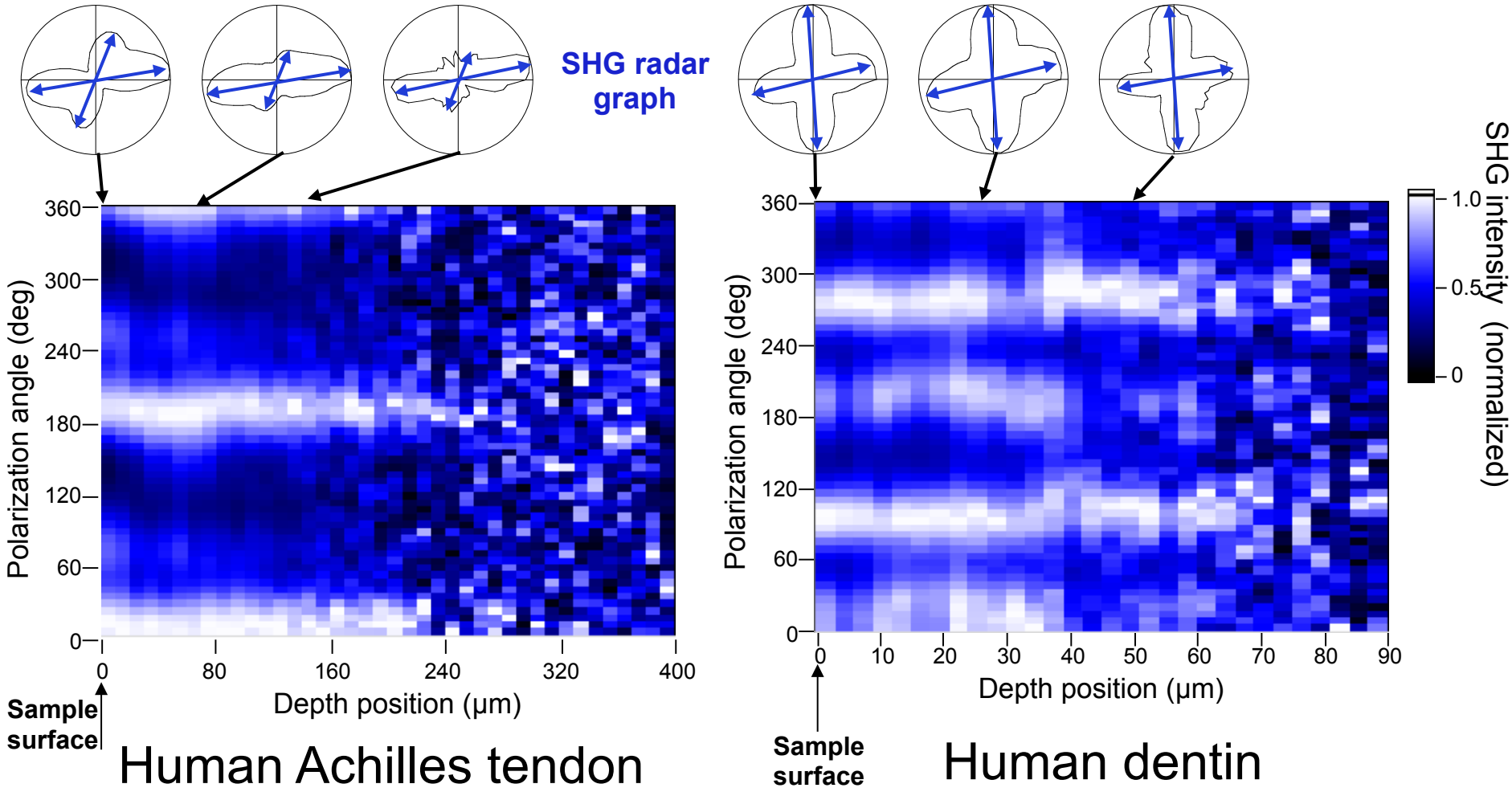
Depth=350μm
(2nd layer)

Horizontal Uniaxial

Mixture of horizontal and vertical

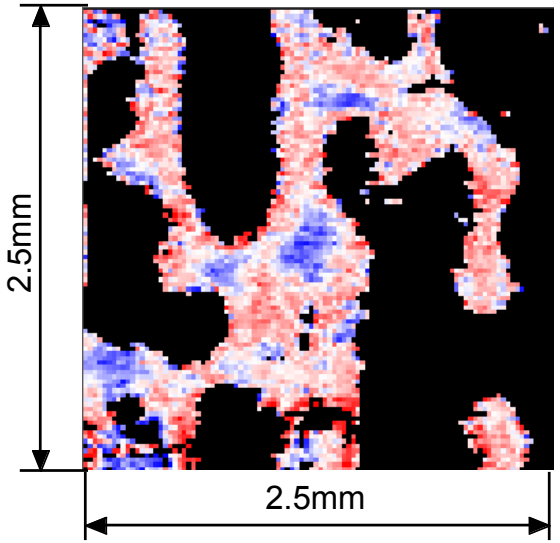
Vertical

Depth-resolved SHG radar graph of human tissue

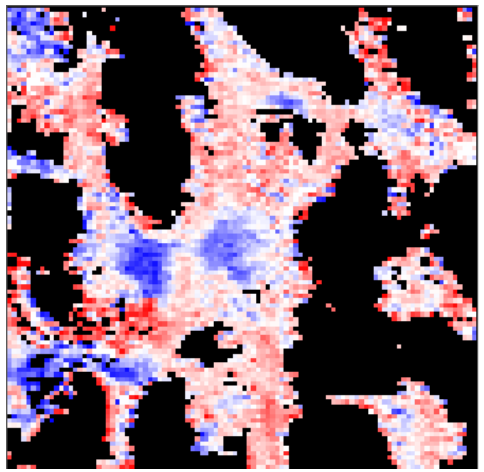


Uniform distribution of collagen orientation along depth direction

Depth-resolved imaging of 2D lateral distribution of collagen orientation in human anklebone



Depth = 0μm
(sample surface)



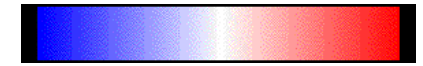
Depth = 50μm

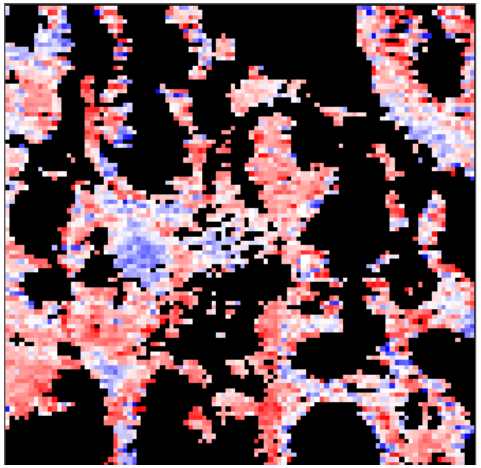
Orientaion degree

$$ORD = \frac{I_V - I_H}{I_V + I_H}$$

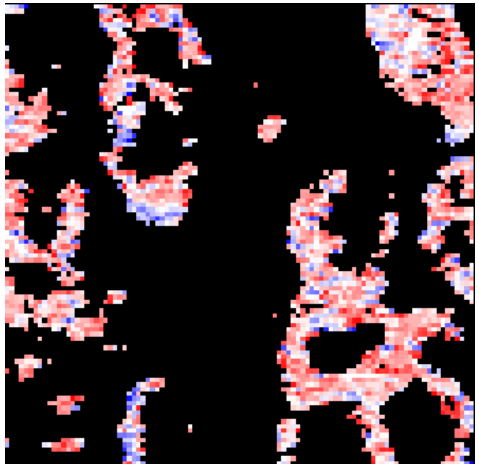
I_V; vertically-polarized SHG intensity
I_H; horizontally-polarized SHG intensity

Uniaxial for ORD=±1
Random or Biaxial for ORD=0





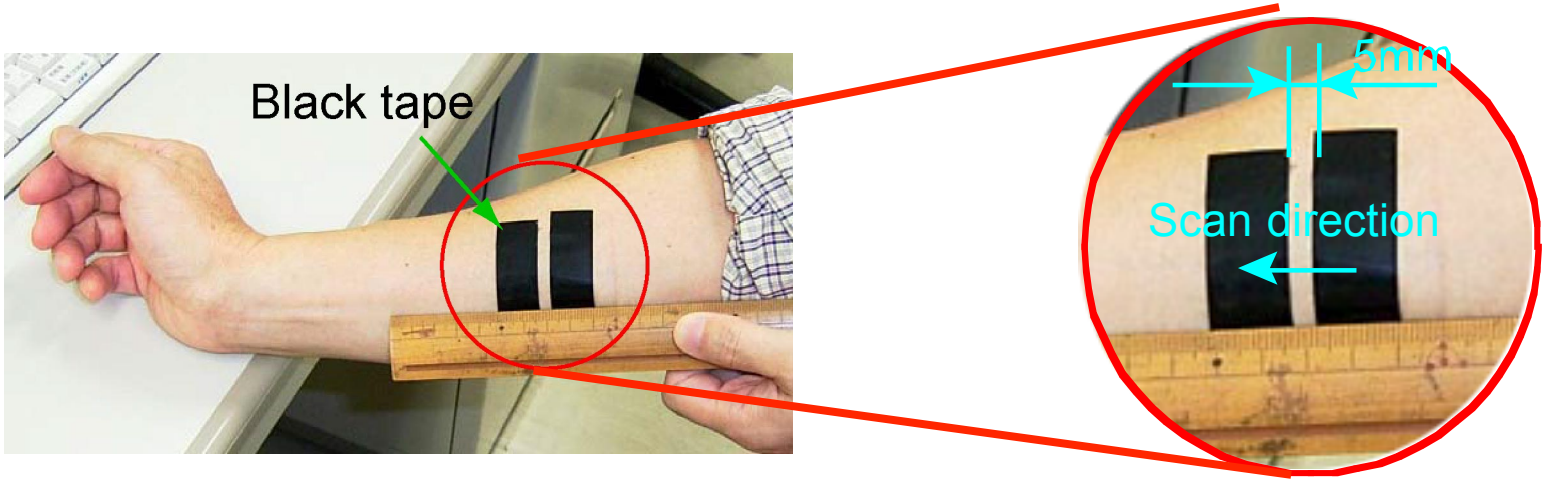
Depth = 100μm



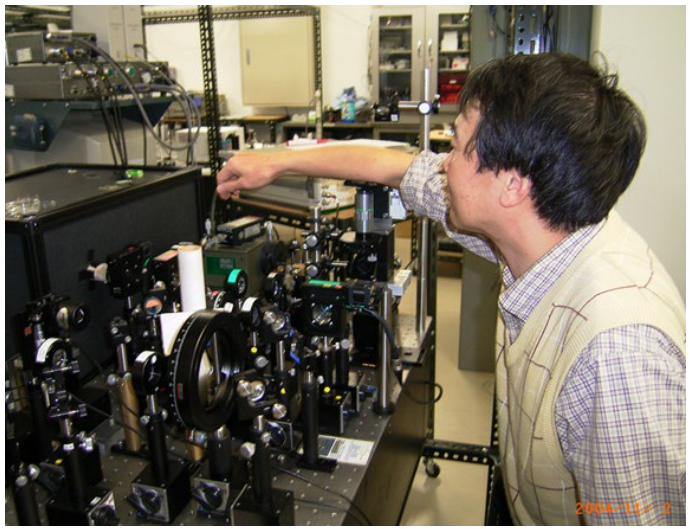
Depth = 150μm

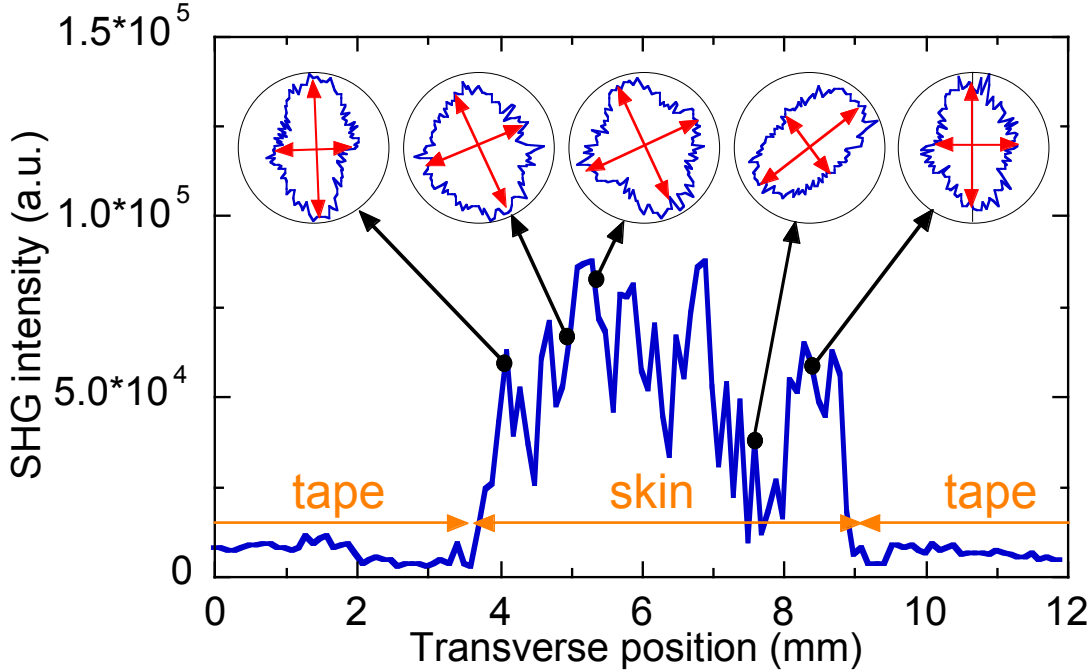
Collagen orientation parallel to bone crest direction developing along depth direction

In vivo measurement of human skin



Volunteer's forehand





Summary

(1) Depth-resolved SHG polariemtry

- Depth resolution = 14 μm
- Penetration power = 100μm@human hard tissue
  150~300μm@human soft tissue

(2) Tomography of collagen orientation in human tissue

- Depth-resolved SHG radar graph
- Depth-resolved imaging of 2D lateral distribution of collagen orientation

(3) *In vivo* measurement of human skin