Ex vivo evaluation of a relation between wrinkling direction and collagen orientation in photoaged rat skin

in collaboration with Osaka Univ.

Optical probe of cutaneous photoaging

Conventional study

Combined SHG and two-photon autofluorescence microscope has been applied as an indicator of changes in dermal collagen and elastin content.
ref) Lin, OL **30**, 2275 (2005)   Koehler, OL **31**, 2879 (2006)
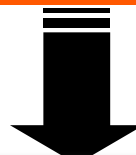
Change viewpoint!

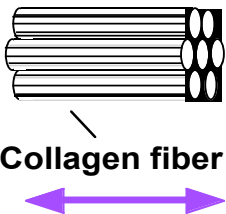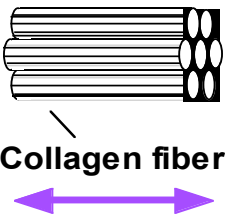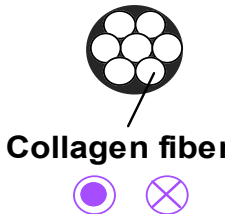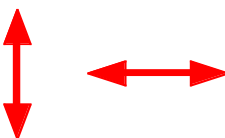*Deep wrinkles characteristic of photoaged skin*

stretch

Wrinkle formation is closely related to directional property of collagen fiber and repeated mechanical stretch to the skin.

Relation between collagen orientation and wrinkle direction using polarization-resolved SHG microscope
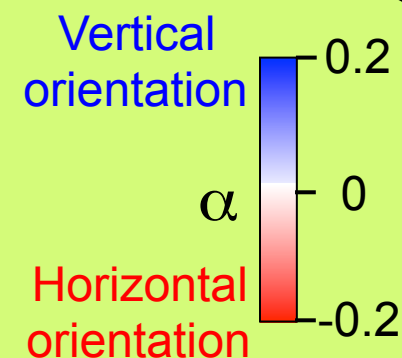
Collagen orientation *vs.* Laser polarization

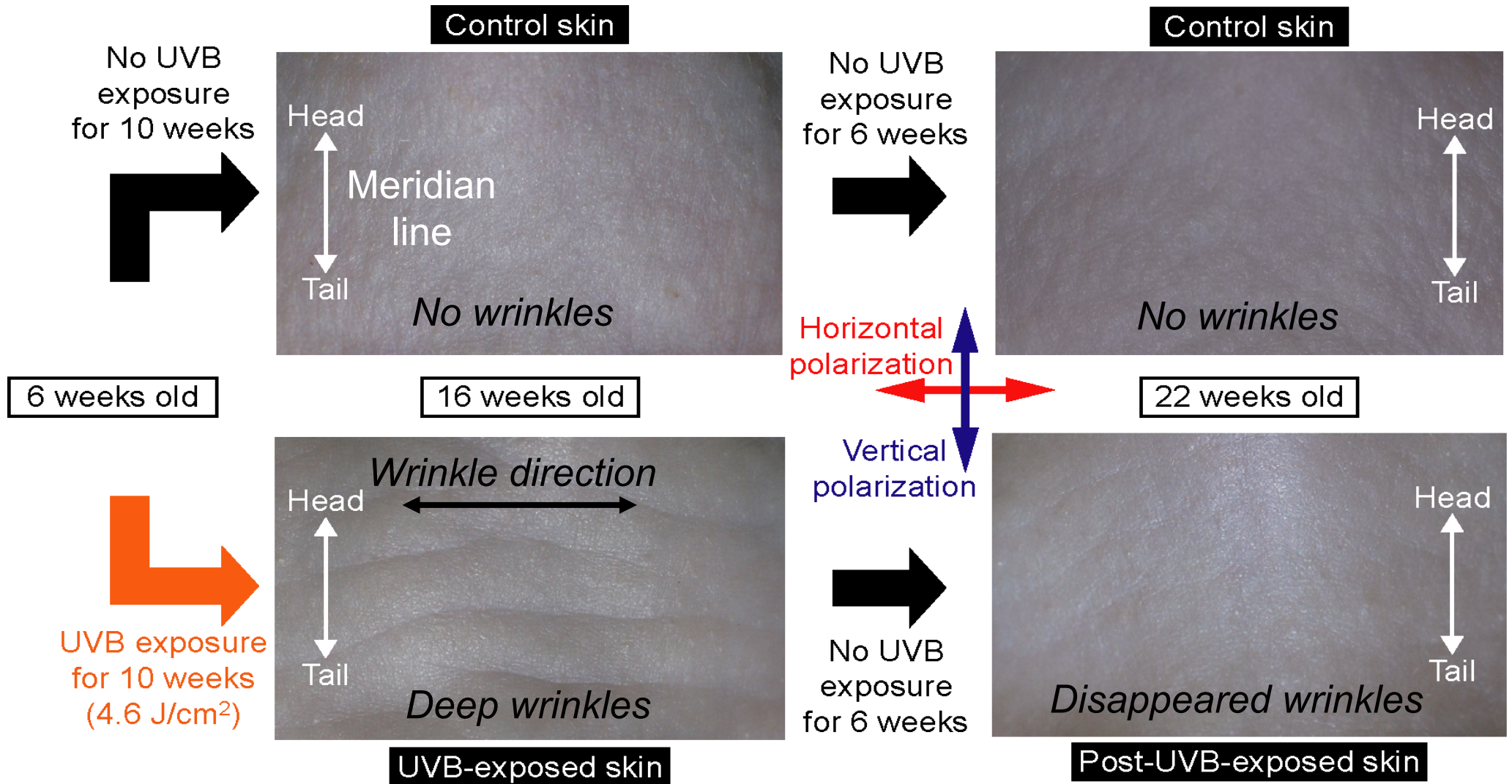|	Axial (Non-centrosymmetric)		Cross-sectional (Centrosymmetric)
	Parallel	Perpendicular	
Collagen orientation	Collagen fiber	Collagen fiber	Collagen fiber
Laser polarization			
SHG light	Strong	Weak	None

Collagen orientation parameter (α)

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

I_V: SHG intensity with *vertical polarization*
I_H: SHG intensity with *horizontal polarization*

Vertical orientation —0.2

α — 0

Horizontal orientation —-0.2

Sample : back skin of hairless mice
*(sample size = 30mm*30mm)*

Control skin

No UVB exposure for 10 weeks

Head
↕
Tail

Meridian line

No wrinkles

Control skin

No UVB exposure for 6 weeks

Head
↕
Tail

No wrinkles

Horizontal polarization

Vertical polarization

6 weeks old

16 weeks old

22 weeks old

UVB exposure for 10 weeks (4.6 J/cm²)

Head
↕
Tail

Wrinkle direction
↔

Deep wrinkles

UVB-exposed skin

No UVB exposure for 6 weeks

Head
↕
Tail

Disappeared wrinkles

Post-UVB-exposed skin

*ref) Klingman, J. Am. Acad. Dermatol. **21**, 623-631 (1989).*

UVB-exposed vs. age-matched control

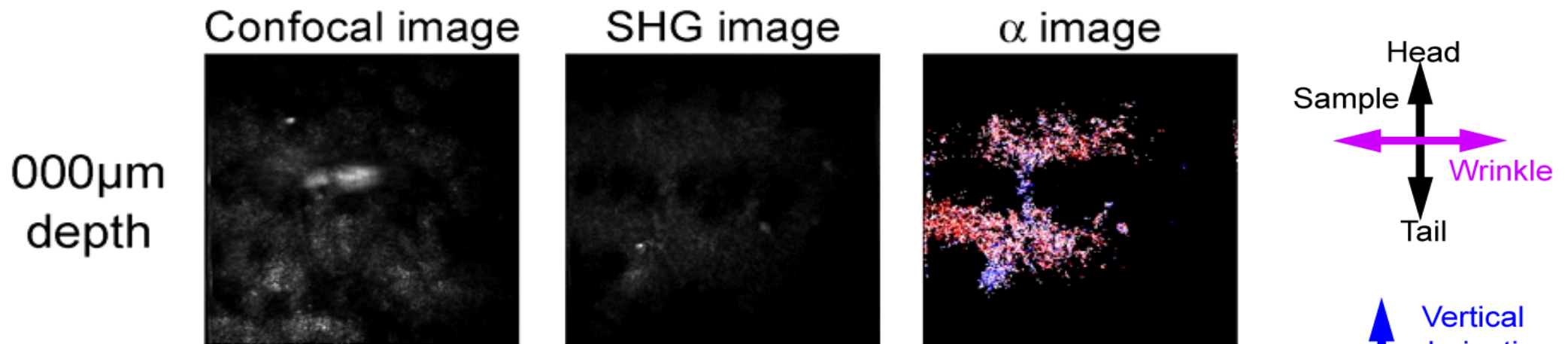*(image size = 800μm*800μm)*
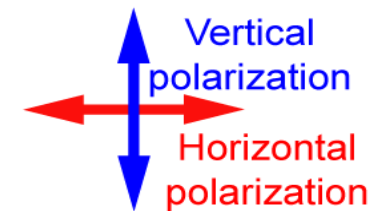

UVB-exposed sample (16 weeks old)
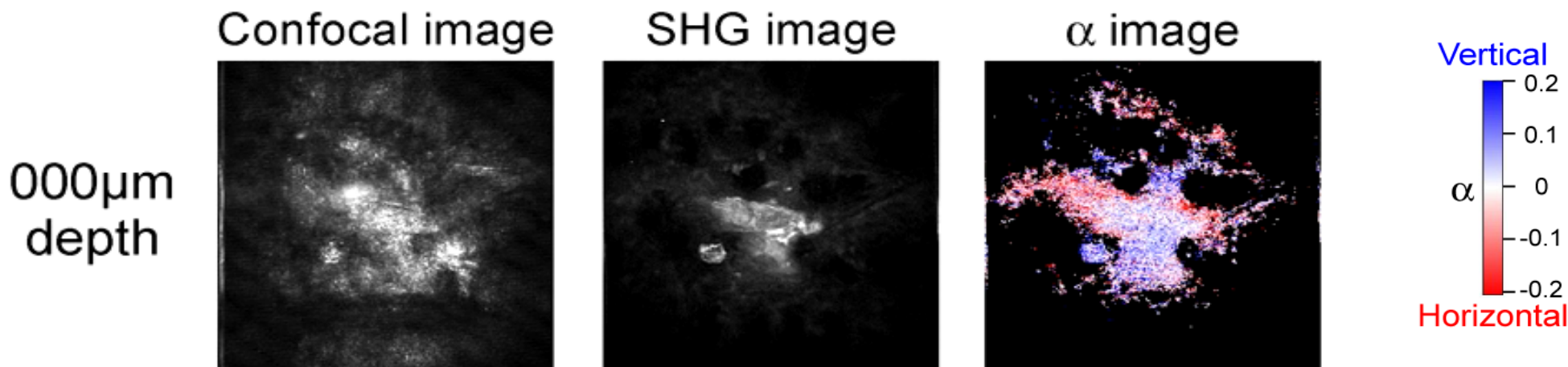
Confocal image SHG image α image

000μm depth
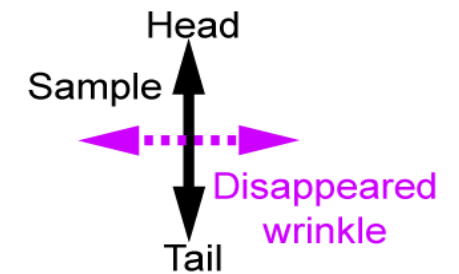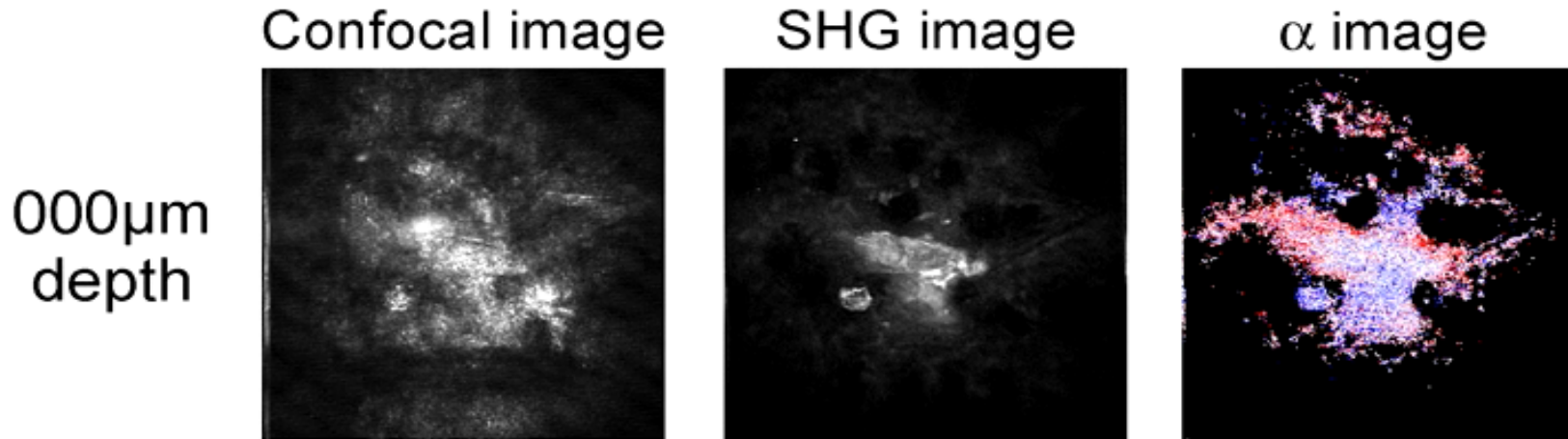
Age-matched control sample (16 weeks old)

Confocal image SHG image α image

000μm depth

Head
Sample
Tail
Wrinkle

Vertical polarization
Horizontal polarization

Vertical
0.2
0.1
0
-0.1
-0.2
α
Horizontal

UVB-exposed vs. Post-UVB-exposed
*(image size = 800μm*800μm)*

Post-UVB-exposed vs. age-matched control
*(image size = 800μm*800μm)*
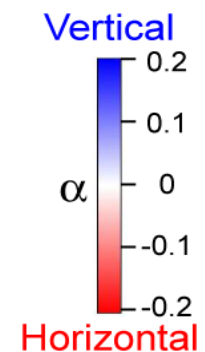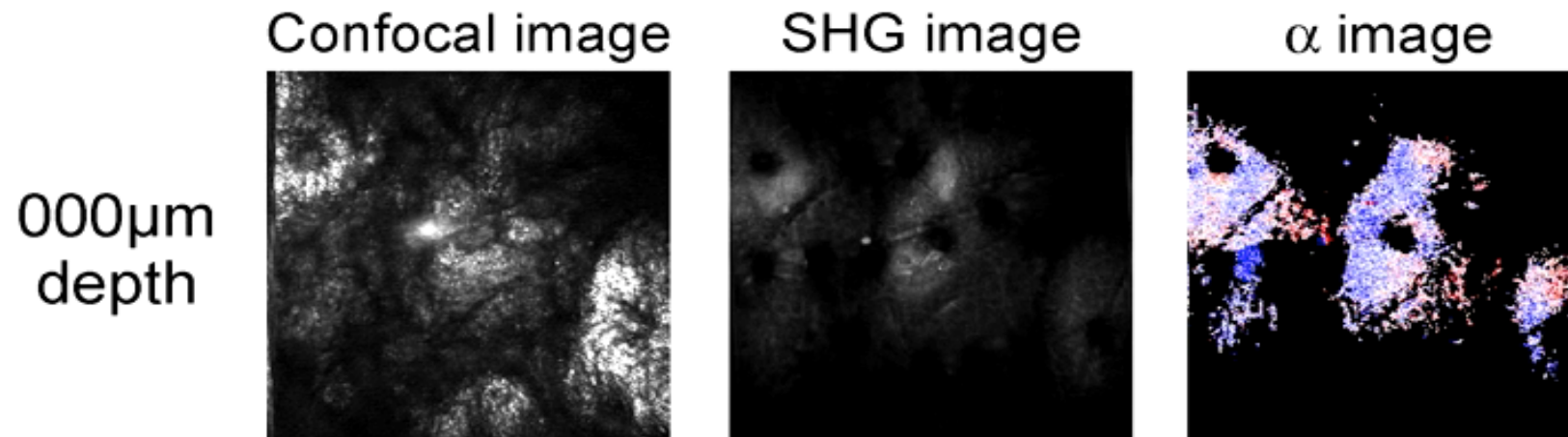
Statistics of α parameters for 3D volume
*(800µm*800µm*300µm-depth)*
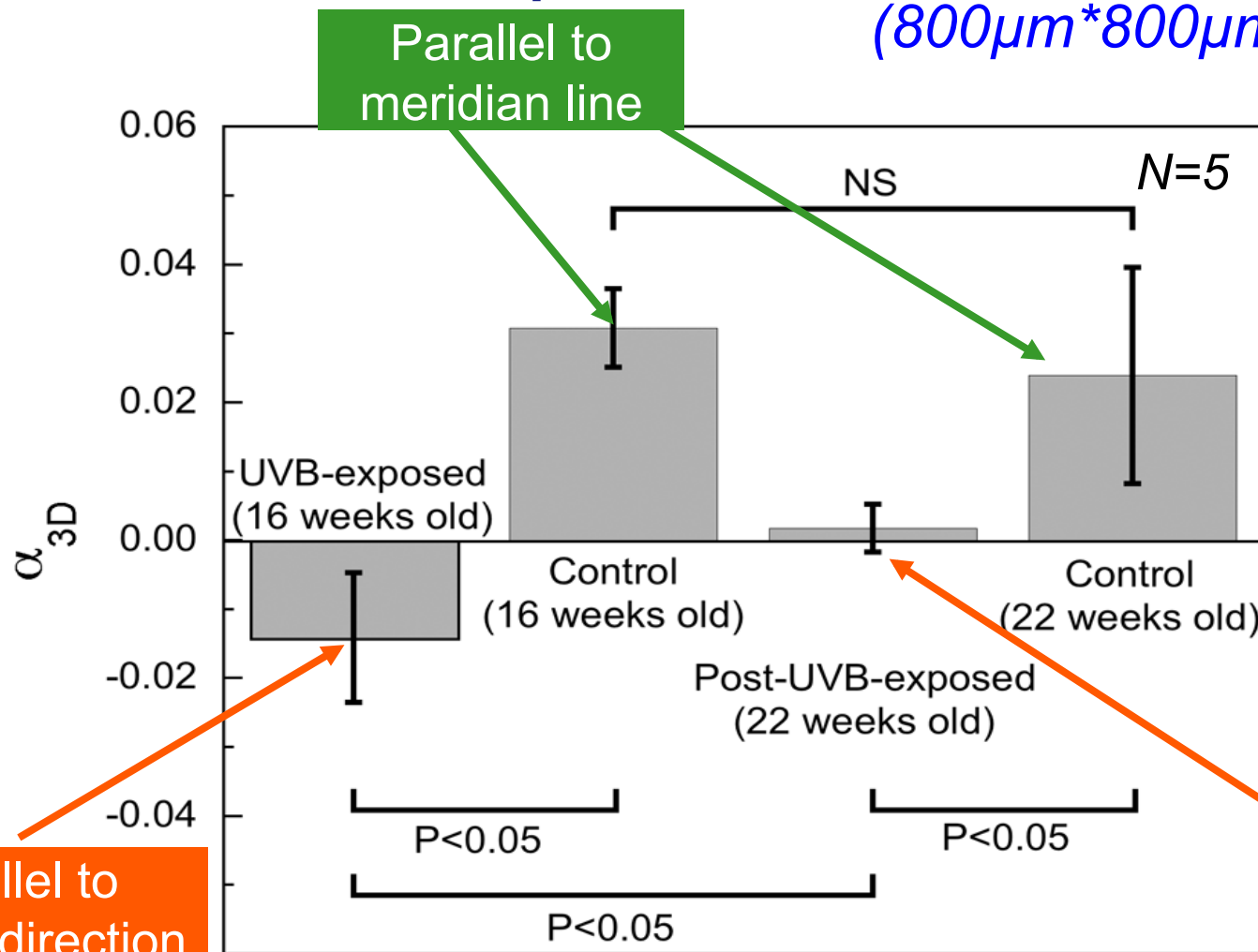
UVB-induced wrinkles are closely related to the change of collagen orientation!