SECOND-HARMONIC GENERATION IMAGING OF COLLAGEN-BASED SYSTEMS

presented by

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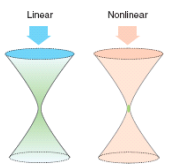
LECTURE OUTLINE

- Second-harmonic generation review
- Application of SHG imaging to qualitative studies
- Application of SHG imaging to quantitative studies
- Fourier Transform-SHG microscopy

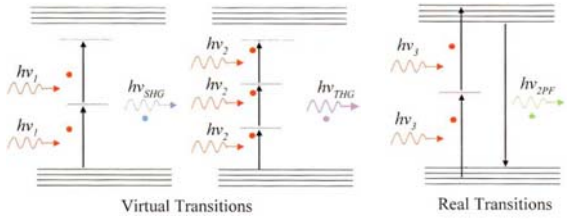
REVIEW: NONLINEAR MICROSCOPY

- Nonlinear methods – approaches whereby output intensity is proportional to I^n, where I is the input intensity and n is the number of photons involved in the interaction
- Permits "optical histology"
- Deeper penetration depths (~600 μm compared to 50 μm)
- Reduced photodamage
- Reduced photobleaching



Denk et al, *Nature Methods* **2**, 932 - 940 (2005)

REVIEW: BASIC CONCEPT



- SHG, THG → "no energy" is absorbed
- 2PF → energy is absorbed

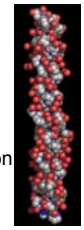
Image Source: Handbook of Biological Confocal Microscopy

REVIEW: SHG IN BIOLOGY

- Applies to noncentrosymmetric systems, i.e., those that are highly ordered (spatially organized)
- Examples in biology include proteins: collagen, myosin, and tubulin

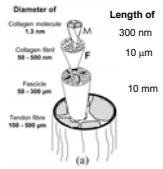
COLLAGEN

- Accounts for 25% of total protein mass in mammals
- Molecule is 1.5-nm width, 300-nm length
- Fibrillar collagen found in connective tissues
- Displays high degree of (supramolecular) organization
- Provides tensile strength in bones, transparency in cornea, elasticity in skin

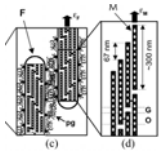


http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mcb.section.6542

COLLAGEN (TENDON EXAMPLE)



Tendon consists of mostly type I



- Gap (G) contains 4 collagen molecules

- 3D arrangement is not well understood

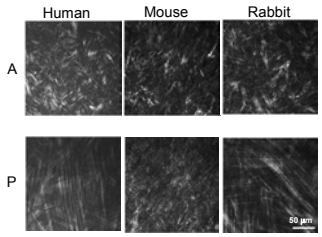
Fratzl, P., *Current Opinion in Colloid and Interface Science* **8**, 32-39 (2003)

COLLAGEN AND SHG

- SHG has definitely been observed for Type I, II, III (w/ I the most crystalline)
- Form 90% of types found in body
- Used to study (normal and abnormal) structure in tendon, skin, cornea, bone, etc.
- Occasionally SHG imaging combined with other modalities to increase sensitivity

QUALITATIVE APPLICATION OF SHG MICROSCOPY

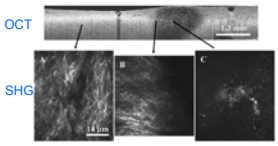
- Studied collagen fiber organization in corneal stroma between human, mice, and rabbits
- Observation of bands of fibers (lamellae) in forward SHG
- Observed 2 types of lamellar organization common in all species in anterior (interwoven) and posterior (orthogonal)
- Only human showed transverse lamellae that may serve anchoring role



N. Morishige *et al*, *J. Cataract Refract Surgery* **32**, 1784 (2006)

QUALITATIVE APPLICATION OF SHG MICROSCOPY

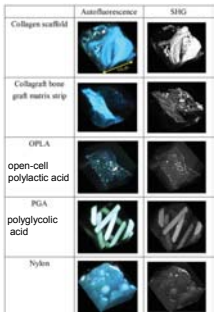
- SHG combined with OCT and histopathology to study wound healing in skin-equivalent tissue models (composed of type I collagen)
- Tissue exposed to thermal (laser-induced) injury
- OCT used to monitor reduction in (linear) light scattering; SHG used to monitor reduction in collagen organization



A. Yeh *et al*, *J. Biomedical Optics* **9**, 248 (2004)

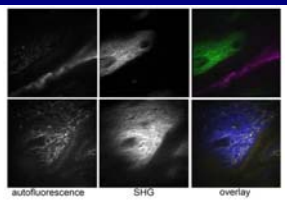
QUALITATIVE APPLICATION OF SHG MICROSCOPY

- SHG combined with 2PF to distinguish between various scaffold materials used in tissue engineering
- Monitored difference in emission spectrum and SHG intensity
- Alternative to using histology (and staining) for examination



Y. Sun *et al*, *Microscopy Research and Technique* **71**, 140 (2008)

QUALITATIVE APPLICATION OF SHG MICROSCOPY: CLINICAL USE



JenLab GmbH

- Potential for clinical use
- Development of high-NA GRIN optics permits high-resolution imaging
-Combined with 2PF microscopy

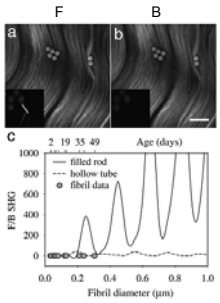
Konig *et al*, *Journal of Biophotonics* **1**, 506 (2008)

WHAT ABOUT QUANTITATIVE ANALYSIS?

- Why do we care?
- Attempt to model/quantify changes in collagen fibril organization due to physical injury or disease (lupus, Marfan syndrome, assess collagen content in tumors)
- Need to develop metrics to use as markers (correlate with morphology/physiological function)

APPLICATION OF SHG MICROSCOPY: QUANTITATIVE

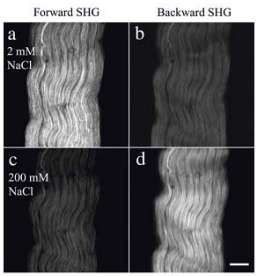
- Used the ratio of forward-to-backward SHG (F/B) to study tendon collagen
- Deduced that SHG emanates from fibril shell rather than from its bulk
- F/B ratio sensitive to ionic strength of solution; results in change in shell thickness



R. Williams *et al*, *Biophysical Journal* **88,** 1377 (2005)

APPLICATION OF SHG MICROSCOPY: QUANTITATIVE

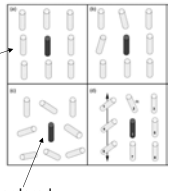
- Morphological changes are not observable from images
- Obvious changes in contrast is indicative of sensitivity to ionic concentration
- Fibril shell thickness is believed to "thin" with increasing ionic concentration



R. Williams *et al*, *Biophysical Journal* **88,** 1377 (2005)

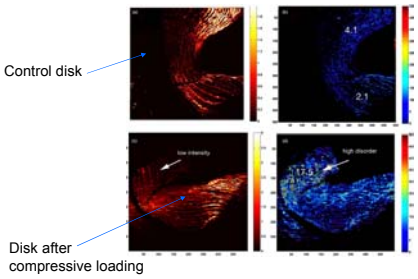
APPLICATION OF SHG MICROSCOPY: QUANTITATIVE

- Developed metrics for disorder in colllagenous tissue using polarization-modulated SHG
- Disorder index
- Looked at mouse model of intervertebral disk injury
- Orientation of fibers is estimated by modulating input polarization



K. Reiser *et al*, *Journal of Biomedical Optics* **12**, 064019 (2007)

APPLICATION OF SHG MICROSCOPY: QUANTITATIVE



K. Reiser *et al*, *Journal of Biomedical Optics* **12**, 064019 (2007)

POLARIZATION

- Often polarization microscopy is used on the same samples as SHG microscopy
- Contrasts are not the same
- Polarization microscopy is based on linear birefringence
- SHG microscopy depends on nonlinear dependence on input power

POLARIZATION IN SHG IMAGING

- Used to determine matrix elements
- Provides info on degree of organization of the molecular dipoles via anisotropy parameter
- Extract orientation information

SHG AND ANISOTROPIC MATERIALS

$$P = \chi^{(1)}E + \chi^{(2)}E^2 + \chi^{(3)}E^3 + ...$$

Induced polarization

$$\begin{bmatrix} P_1(2\omega) \\ P_2(2\omega) \\ P_3(2\omega) \end{bmatrix} = \begin{bmatrix} \chi_{11} & \chi_{12} & \chi_{13} & \chi_{14} & \chi_{15} & \chi_{16} \\ \chi_{21} & \chi_{22} & \chi_{23} & \chi_{24} & \chi_{25} & \chi_{26} \\ \chi_{31} & \chi_{32} & \chi_{33} & \chi_{34} & \chi_{35} & \chi_{36} \end{bmatrix} \begin{bmatrix} E_x(\omega)^2 \\ E_y(\omega)^2 \\ E_z(\omega)^2 \\ 2E_z(\omega)E_y(\omega) \\ 2E_x(\omega)E_z(\omega) \\ 2E_y(\omega)E_x(\omega) \end{bmatrix}$$

NONCENTROSYMMETRIC CRYSTAL CLASSES

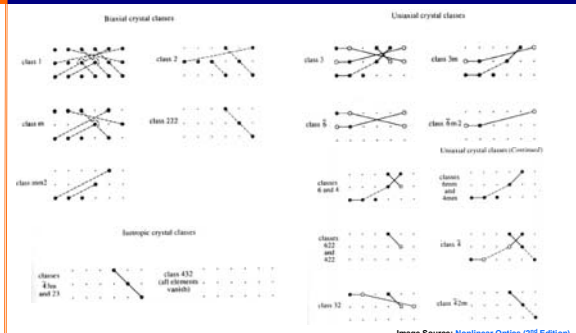
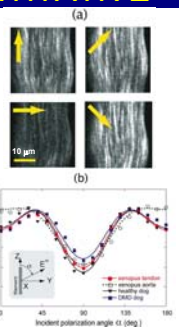


Image Source: Nonlinear Optics (2nd Edition)

APPLICATION OF SHG MICROSCOPY: QUANTITATIVE

- Polarization degree of freedom was used
- Ratio of $\chi^{(2)}$ elements used as a metric
- Different tissues had different collagen types (I and III)

Frog tendon



F. Tiaho *et al*, *Optics Express* **15**, 12286 (2007)

APPLICATION OF SHG MICROSCOPY: QUANTITATIVE

$$\begin{bmatrix} 0 & 0 & 0 & 0 & d_{15} & 0 \\ 0 & 0 & 0 & d_{15} & 0 & 0 \\ d_{31} & d_{31} & d_{33} & 0 & 0 & 0 \end{bmatrix}$$

$$I^{2\omega} \sim [\sin^2 2\alpha + (\frac{\chi_{31}}{\chi_{15}} \sin^2 \alpha + \frac{\chi_{33}}{\chi_{15}} \cos^2 \alpha)^2].$$

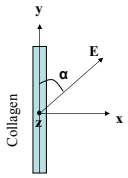


Table 1. Ratio of coefficients χ_{31}/χ_{15} and χ_{33}/χ_{15} for myosin-rich and collagen-rich tissues obtained from fit of Eq. 2 with the experimental data of Fig. 2(b) and Fig. 3(b). The orientation parameter D and the effective orientation angle θ_e are defined by Eq. 4.

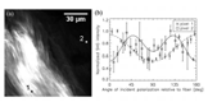
	χ_{31}/χ_{15}	χ_{33}/χ_{15}	D	θ_e (deg)
myosin of stage 32 xenopus	0.89	0.55	0.21	62.3
myosin of stage 37 xenopus	0.95	0.64	0.24	60.5
myosin of stage 46 xenopus	0.95	0.38	0.16	66.4
myosin of adult xenopus	1.12	0.70	0.26	59.4
myosin of DMD Golden retriever dog	0.87	0.54	0.21	62.5
myosin of 71 years-old human female	0.97	0.69	0.25	59.6
collagen of adult xenopus tendon	1.02	1.44	0.41	50.3
collagen of adult xenopus aorta	1.41	2.15	0.52	43.9
collagen of DMD Golden retriever dog	1.21	1.56	0.44	48.5
collagen of healthy Beagle dog	0.94	1.43	0.41	49.7

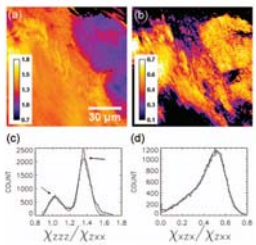
Determined "effective" orientation of collagen harmonophores

F. Tiaho *et al*, *Optics Express* **15**, 12286 (2007)

APPLICATION OF SHG MICROSCOPY: QUANTITATIVE

- Again, ratio of $\chi^{(2)}$ elements used as a metric
- This time, $\chi^{(2)}$ determined as a function of space
- Looked at human dermis and tendon-muscle junction of chicken wing

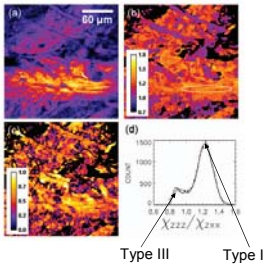




W. Chen *et al*, *Applied Physics Letters* **94**, 183902 (2009)

APPLICATION OF SHG MICROSCOPY: QUANTITATIVE

- Histogram distribution for $\chi^{(2)}$ for human dermis suggests detection of type III collagen in addition to type I



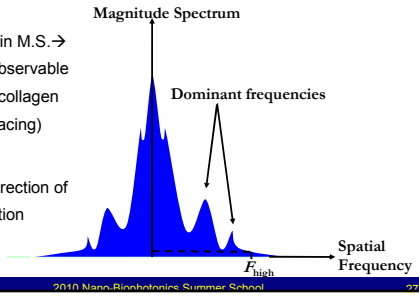
W. Chen *et al*, *Applied Physics Letters* **94**, 183902 (2009)

APPLICATION OF SHG MICROSCOPY: QUANTITATIVE (FOURIER ANALYSIS)

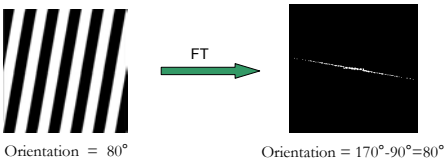
- SHG is excellent for imaging fibrous structures
- Fourier transforms is a useful tool for analyzing fibers/fiber arrays
- Some information about spatial organization can be obtained without the use of complex algorithms or experimental setup

FT-SHG: SPATIAL FREQUENCY

- F_{high} and peaks in M.S.→ info about min. observable feature size and collagen fiber packing (spacing)
- Evaluated for direction of preferred orientation

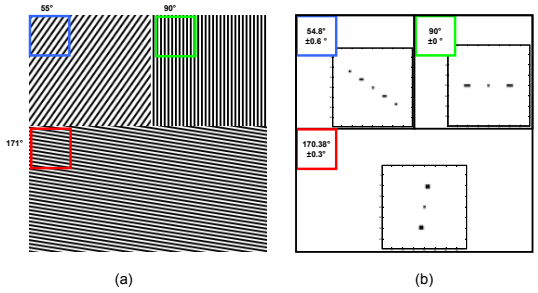


FT-SHG: ORIENTATION

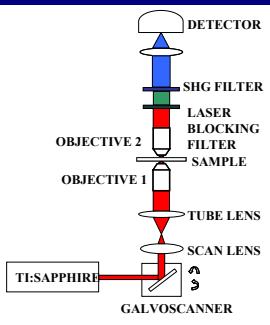


- FT picks up preferred direction of variation in image
- Creation of binary image of dominant spatial frequencies → apply best-fit line

FT-SHG: ORIENTATION



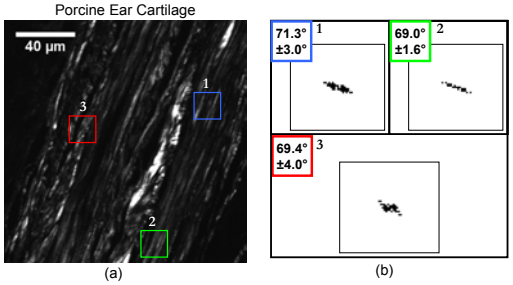
EXPERIMENTAL SETUP



R. Ambekar *et al*, *Optics Express* **17**, 14534 (2009).

FT-SHG: ORIENTATION

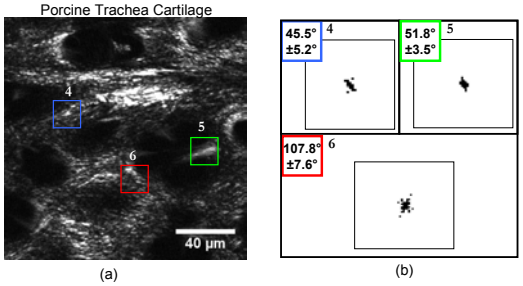
Porcine Ear Cartilage



R. Ambekar *et al*, *Optics Express* **17**, 14534 (2009).

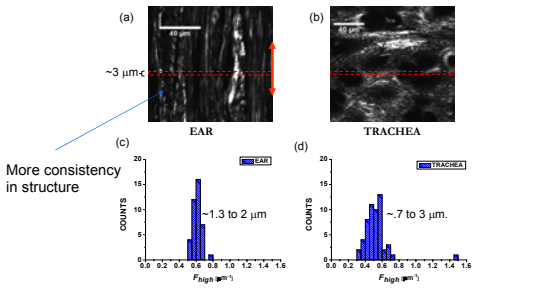
FT-SHG: ORIENTATION

Porcine Trachea Cartilage



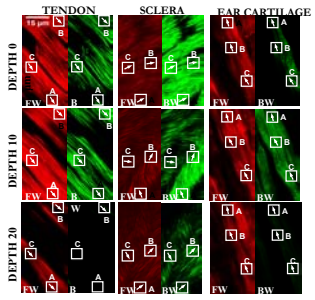
R. Ambekar *et al*, *Optics Express* **17**, 14534 (2009).

FT-SHG: SPATIAL FREQUENCY



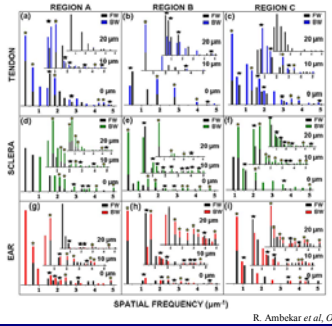
R. Ambekar *et al*, *Optics Express* **17**, 14534 (2009).

FT-SHG: FORWARD VS. BACKAWARD



R. Ambekar *et al*, *Optics Letters* **34**, 3779 (2009).

FT-SHG: FORWARD VS. BACKAWARD



R. Ambekar *et al*, *Optics Letters* **34**, 3779 (2009).

SUMMARY

- SHG is a nonlinear optical technique (coherent scattering)
- Useful in microscopy for non-invasive 3D imaging
- Recent work has focused on combining with other nonlinear imaging modes
- Development of quantitative metrics could help with assessment of tissue morphology

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http://probe.mechse.illinois.edu