

Near-field Effects on SHG Imaging

Jarno van der Kolk
Charalambos Kouilos
Lora Ramunno

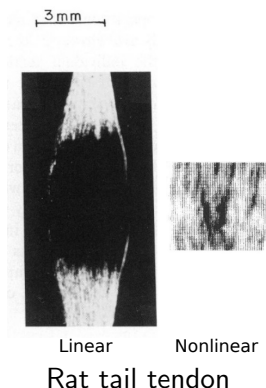
University of Ottawa
Department of Physics
Centre for Research in Photonics

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Imaging collagen fibrils with SHG

- One of the first nonlinear optical microscopes (1986)
- Invisible for linear, visible for nonlinear
- Works especially well for collagen due to high $\chi^{(2)}$

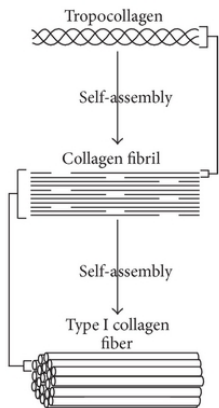


[1] Freund, I., Deutsch, M. & Sprecher, A. Connective tissue polarity. Optical second-harmonic microscopy, crossed-beam summation, and small-angle scattering in rat-tail tendon. *Biophysical journal* 50, 693–712 (1986)

Collagen is everywhere

Why important?

- In almost all biological tissue
- Provides mechanical properties
- Defines structure

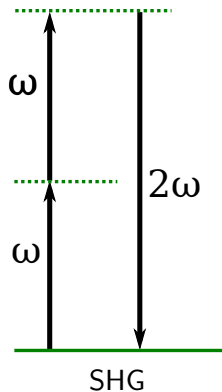


[2]Kruger, T. E., Miller, A. H. & Wang, J. Collagen Scaffolds in Bone Sialoprotein-Mediated Bone Regeneration. The Scientific World Journal 2013, 1-6 (2013).

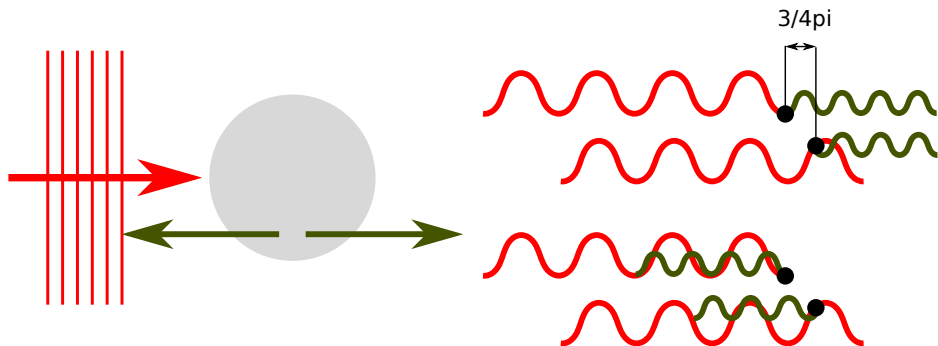
Second Harmonic Generation

Image formation process

- Second Harmonic Generation (SHG)
- Strong signal for collagen due to chiral molecular structure in combination with alignment in fibrils

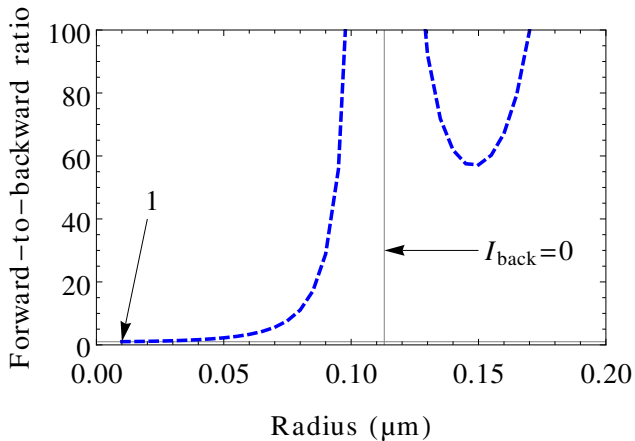


Forward and backward signal

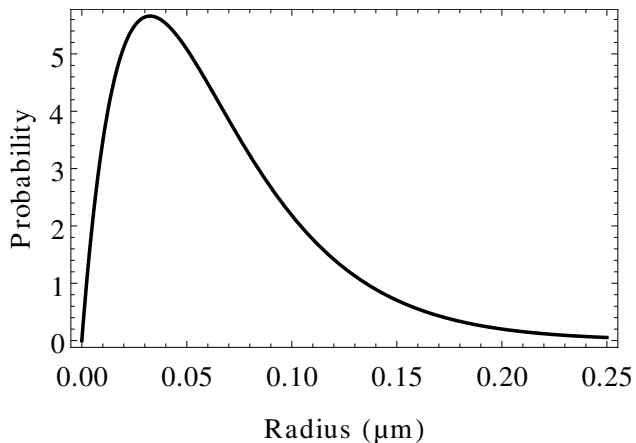


Forward and backward signal

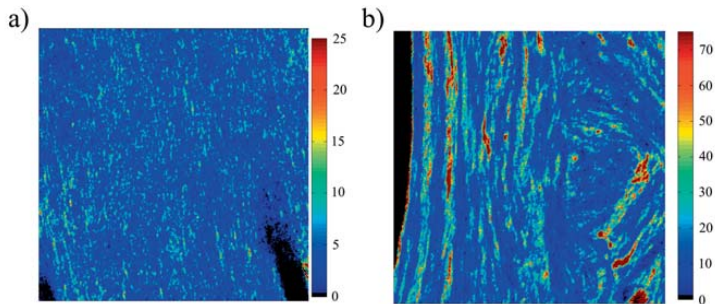
Analytical model for plane wave illumination ($\lambda = 1.0 \mu m$)



Fibril radius distribution for rat tail tendon



Fibril diameter from F/B ratio



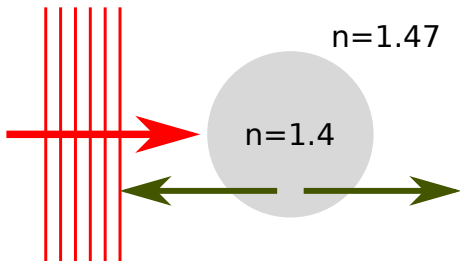
Articular cartilage

Bone

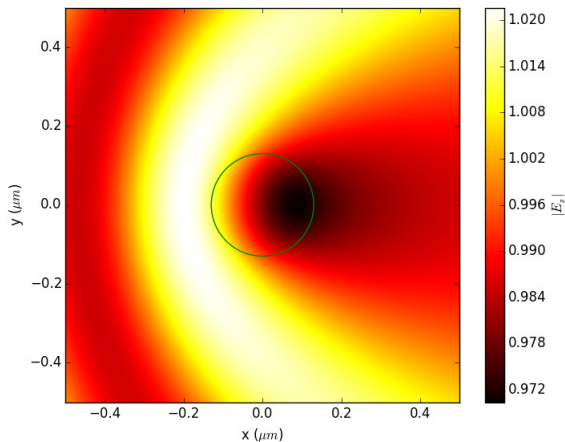
(Images are $100 \times 100 \mu m$)

[3] Houle, M.-A. et al. Analysis of forward and backward Second Harmonic Generation images to probe the nanoscale structure of collagen within bone and cartilage. *Journal of Biophotonics* 8, 993–1001 (2015).

Forward and backward signal

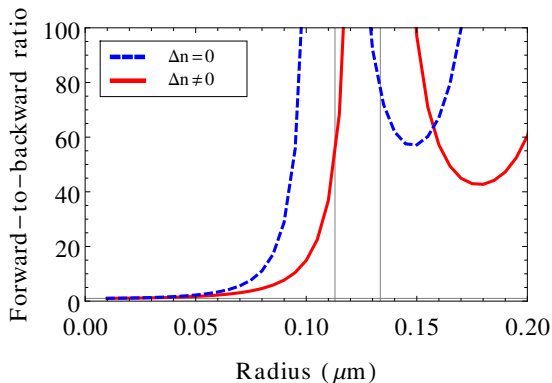


Near-field at pump frequency for index-mismatched cylinder



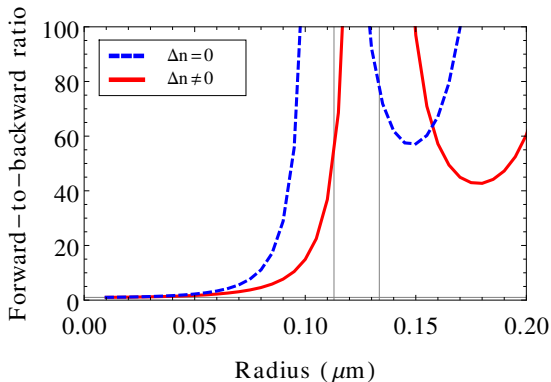
- Only 2% difference in field strength.

Measuring fibril diameter with SHG



- Cylinder appears smaller than it really is.

Measuring fibril diameter with SHG



- Cylinder appears smaller than it really is.
- Caused sensitivity of backward signal to field distribution.

Conclusion




- Difference in the linear index of refraction can alter perceived fibril properties in SHG measurements.
- Reason: Backward signal is very sensitive to near-field distribution.
- Intrinsic effect, cannot be avoided.

Thank you for your attention

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Collagen scaffolds in bone sialoprotein-mediated bone regeneration.
2013:6.
-  Marie-Andrée Houle, Charles-André Couture, Stéphane Bancelin, Jarno Van der Kolk, Etienne Auger, Cameron Brown, Konstantin Popov, Lora Ramunno, and François Légaré.
Analysis of forward and backward second harmonic generation images to probe the nanoscale structure of collagen within bone and cartilage.
Journal of Biophotonics, 8(11-12):993–1001, 2015.

Linear and Nonlinear Microscopes

Wave equation from Maxwell's equations:

$$\nabla \times \nabla \times E(\omega) - \frac{\omega^2}{c^2} E(\omega) = \mu\mu_0 P(\omega)$$

$$P(\omega) = \epsilon_0 \chi^{(1)}(\omega) E(\omega) + \\ \epsilon_0 \chi^{(2)}(\omega = 2\omega_1) E^2(\omega_1) + \\ \dots$$

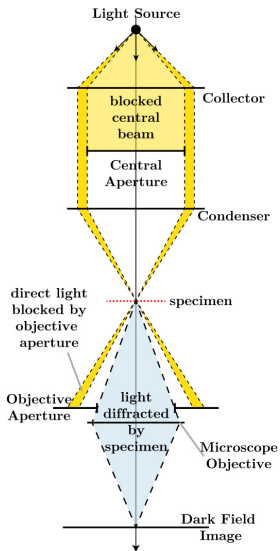
Linear

- Probes $\chi^{(1)}$
- Diffraction limited
- 2D

Nonlinear

- Probes $\chi^{(N)}$, $N > 1$
- Small focal spot
- 3D through scanning

Dark Field Microscope



https://en.wikipedia.org/wiki/Dark_field_microscopy