



# NONLINEAR OPTICS

## SECOND HARMONIC GENERATION

*Methods for Cell Analysis Course*  
*BioVis – Uppsala, 2014*

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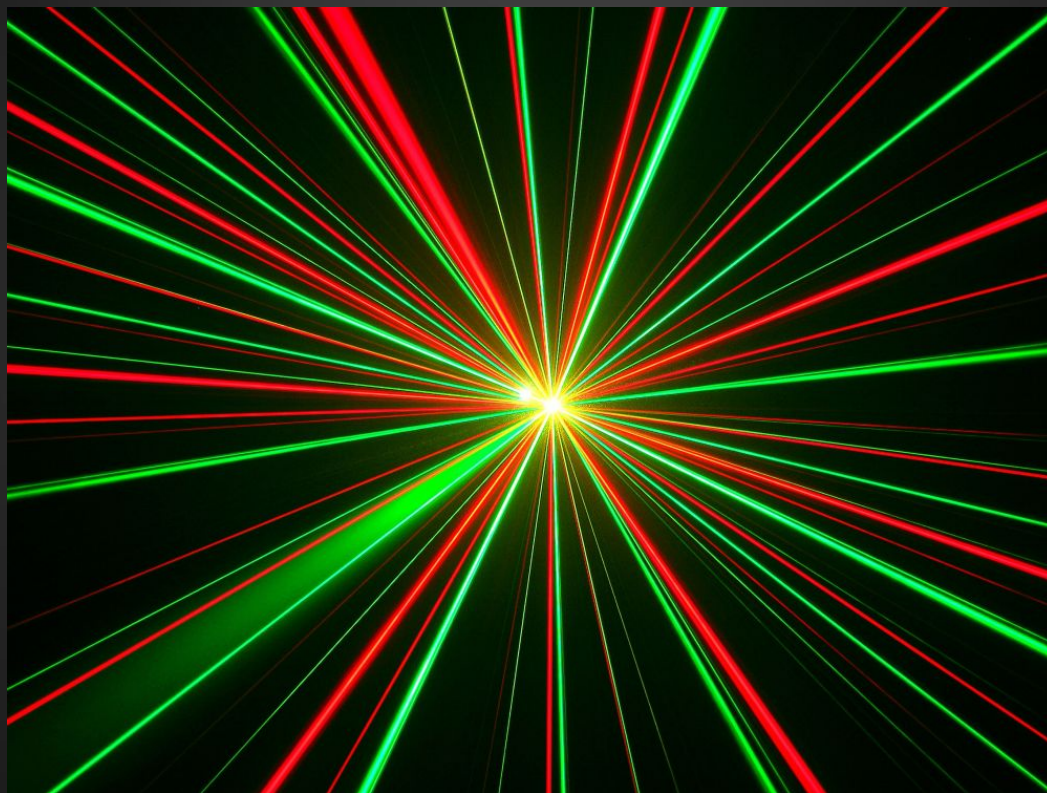
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# Information

- Second harmonic generation microscopy: principles and applications to disease diagnosis, P.J. Campagnola and C.-Y. Dong, *Laser & Photonics Reviews, Volume 5, Issue 1, pages 13–26, January 2011*
- Biological second and third harmonic generation microscopy, Friedl P et al, *Curr Protoc Cell Biol. 2007 Mar;Chapter 4:Unit 4.15*

# BACKGROUND



# Light phenomenon

- Fundamental question:

Is it possible to change the color (frequency/wavelength) of a monochromatic light?



# Light phenomenon

- Answer:

Yes, it is possible.

We need two things for this:


Nonlinear material

Very strong electromagnetic field (femtosecond laser)



# Light phenomenon

## Linear optics:

- Optical properties, such as the refractive index and the absorption coefficient are independent of light intensity.
- The principle of superposition, a fundamental tenet of classical, holds.
  - If input A produces response X and input B produces response Y then input (A + B) produces response (X + Y)  Image ...
- The frequency of light cannot be altered by its passage through the medium.
- Light cannot interact with light; two beams of light in the same region of a linear optical medium can have no effect on each other. Thus light cannot control light.

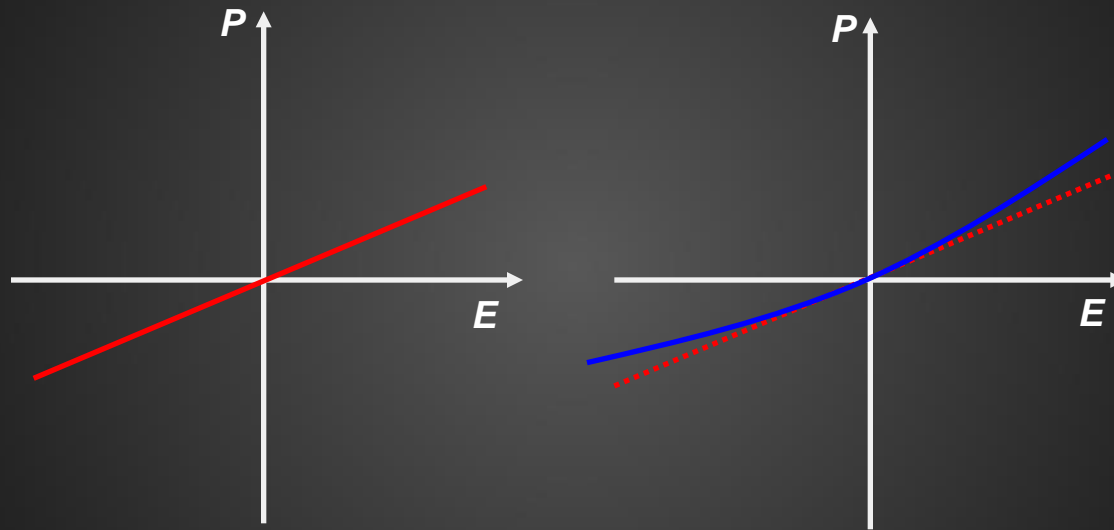
# Nonlinear medium

## Nonlinear optics (When $E$ is very high):

- The refractive index, and consequently the speed of light in an optical medium, does *change* with the light intensity.
- The principle of superposition is violated.
- Light can alter its frequency as it passes through a nonlinear optical material (e.g., from red to blue!).
- Light can control light; photons do interact

**Light interacts with light via the medium. The presence of an optical field modifies the properties of the medium which, in turn, modify nonlinearly another optical field or even the original field itself.**

# Nonlinear medium



**Figure 19.1-1** The  $P$  (polarization density)- $E$  (Electric field) relation for (a) a linear dielectric medium, and (b) a nonlinear medium.



# Nonlinear phenomena

## SECOND ORDER EFFECTS:

- frequency conversion
- Second-harmonic generation (SHG)
- Parametric amplification (OPA)
- Parametric oscillation (OPO)

## THIRD ORDER EFFECTS:

- third-harmonic generation (THG)
- Kerr effect
- self-phase modulation
- self-focusing
- four-wave mixing
- Stimulated Brillouin Scattering
- Stimulated Raman Scattering
  
- Optical solitons
- Optical bistability

# Nonlinear medium

The nonlinearity is usually weak.

The relation between  $\mathbf{P}$  and  $\mathbf{E}$  is approximately linear for small  $\mathbf{E}$ , deviating only slightly from linearity as  $\mathbf{E}$  increases.

$$\mathbf{P} = \varepsilon_0 \chi \mathbf{E}$$

But for very high  $\mathbf{E}$ ,  $\mathbf{P}$  is nonlinear :

$$\mathbf{P}(t) = \varepsilon_0 (\chi^{(1)} \mathbf{E}(t) + \chi^{(2)} \mathbf{E}^2(t) + \chi^{(3)} \mathbf{E}^3(t) + \dots) .$$

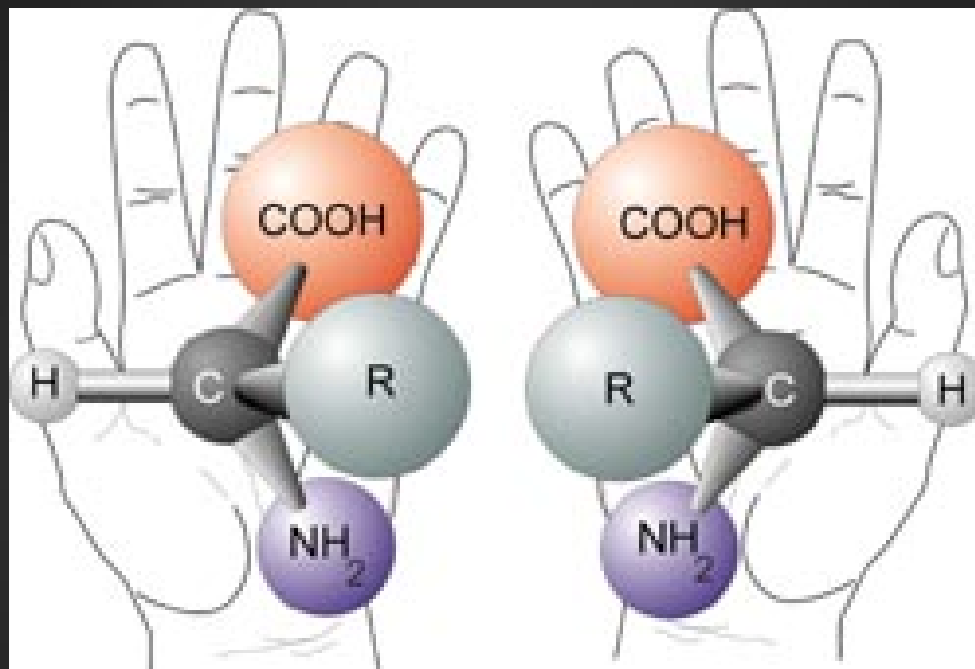
basic description for a nonlinear optical medium

- **In centrosymmetric media,  $\chi^{(2)}$  vanish (=0), and the lowest order nonlinearity is the third order.**
- SHG has strong symmetry requirement (non-centrosymmetric materials)

# Centrosymmetry

- The term **centrosymmetric**, is generally used in crystallography, refers to a point group which contains an inversion center as one of its symmetry elements. In such a point group, for every point  $(x, y, z)$  in the unit cell there is an indistinguishable point  $(-x, -y, -z)$ . Crystals with an inversion center cannot display certain properties, such as the piezoelectric effect.
- Point groups lacking an inversion center (**non-centrosymmetric**) are further divided into *polar* and *chiral* types. A chiral point group is one without any rotoinversion symmetry elements. *Rotoinversion* (also called an 'inversion axis') is rotation followed by inversion; for example, a mirror reflection corresponds to a twofold rotoinversion. Chiral point groups must therefore only contain (purely) rotational symmetry. These arise from the crystal point groups 1, 2, 3, 4, 6, 222, 422, 622, 32, 23, and 432. Chiral molecules such as proteins crystallize in chiral point groups. (Wikipedia)

# An example for non-centrosymmetry (Chirality)



An object or a system is chiral if isn't identical to its mirror image, it cannot be superposed onto it. A chiral object and its mirror image are called **enantiomorphs** (Greek *opposite forms*) or, when referring to molecules, **enantiomers**. A non-chiral object is called **achiral** (sometimes also **amphichiral**) and can be superposed on its mirror image.

# Second harmonic generation

In centrosymmetric media:  $\chi^{(2)} = 0$



The lowest order nonlinearity is of third order

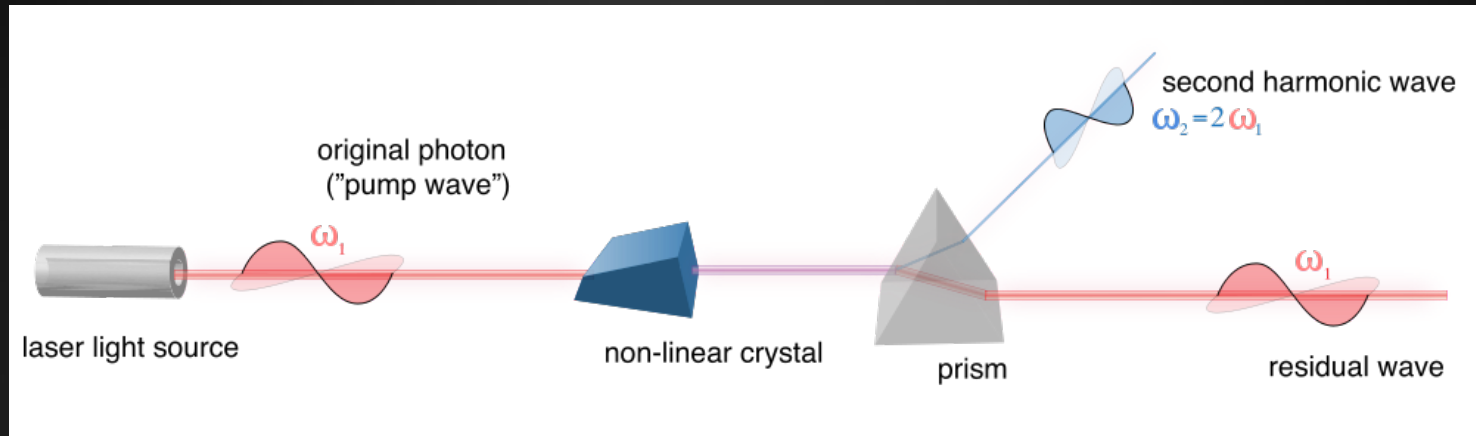


Typical values:

$$\chi^{(2)} = 2 * 10^{-11} \text{ m/V}$$

$$\chi^{(3)} = 4 * 10^{-23} \text{ m/V}$$

# SHG in a simple way

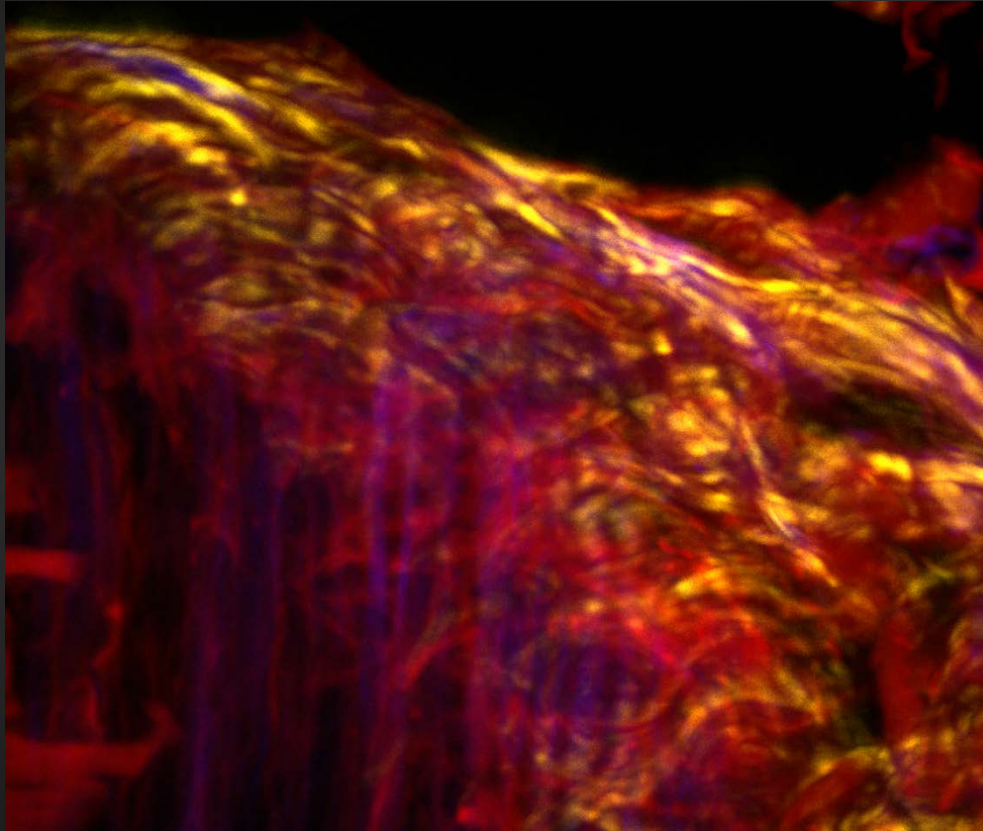


- Due to the  $\chi^{(2)}$  nonlinearity, the fundamental (pump) wave generates a nonlinear polarization wave which oscillates with **twice the fundamental frequency**. According to Maxwell's equations, this nonlinear polarization wave radiates an electromagnetic field with this **doubled frequency** (half the wavelength).
  - 800 nm laser input and 400 nm SHG signal
- For THG -  $\chi^{(3)}$  : output frequency three times higher than the input frequency

# Why SHG is good for us?

- We can separate SHG from the input laser signal with filters
- Powerful tool in *in situ* bioimaging: we don't have to label the specimen to get specific signal from the tissue
- SHG can be combined with other label-free imaging methods such as: CARS, THG, two-photon autofluorescence

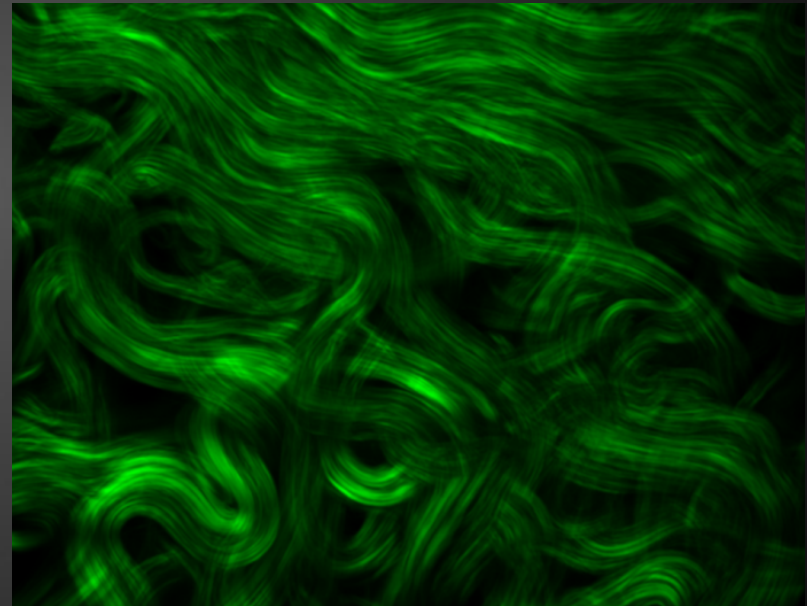
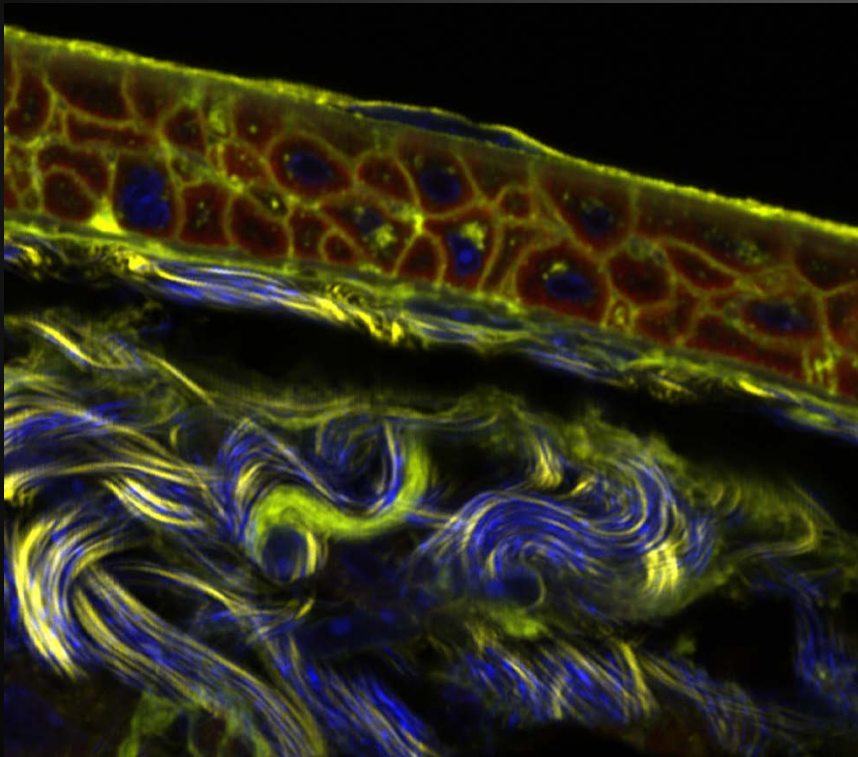
# APPLICATION OF SHG IN BIOIMAGING





# Collagen

Collagen: noncentrosymmetric, gives SHG signal with around 800 nm femtosecond laser

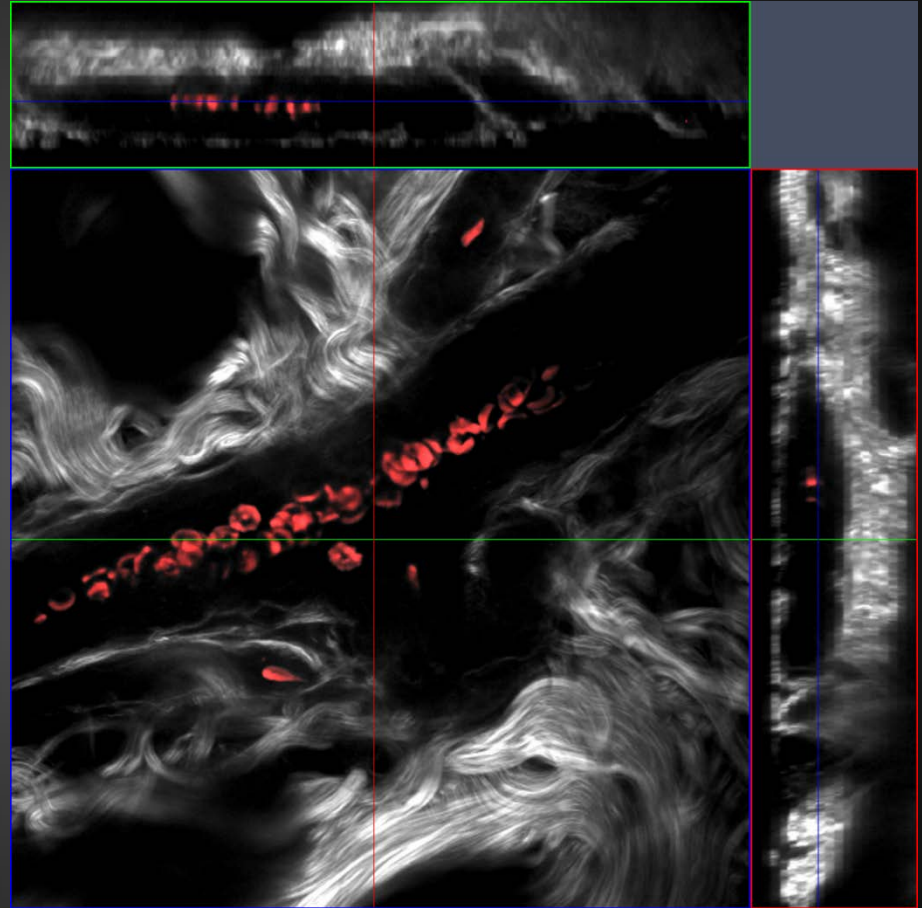


## More info about collagen SHG:

- Second Harmonic Generation Confocal Microscopy of Collagen Type I from Rat Tendon Cryosections, Theodossis A. Theodossiou et al. *Biophys J.* 2006 December 15; 91(12): 4665–4677

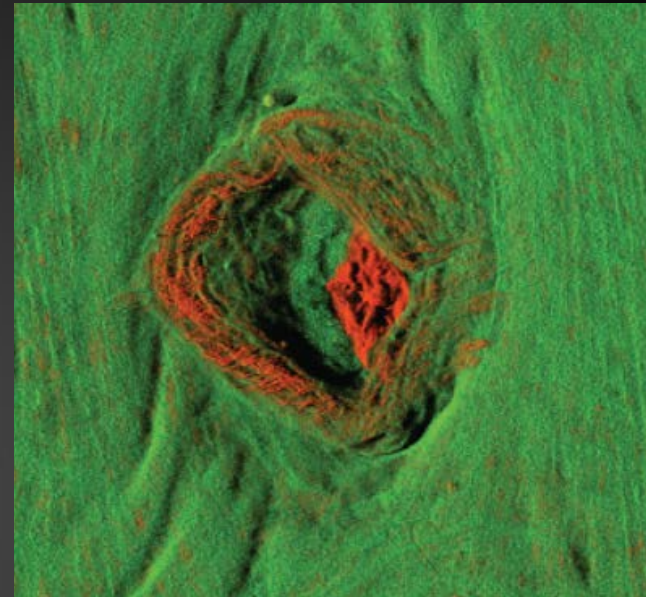
# Collagen

- Collagen (white, SHG): surrounding of a blood vessel
- Red blood cells (red): gives nice two-photon autofluorescence



# Collagen

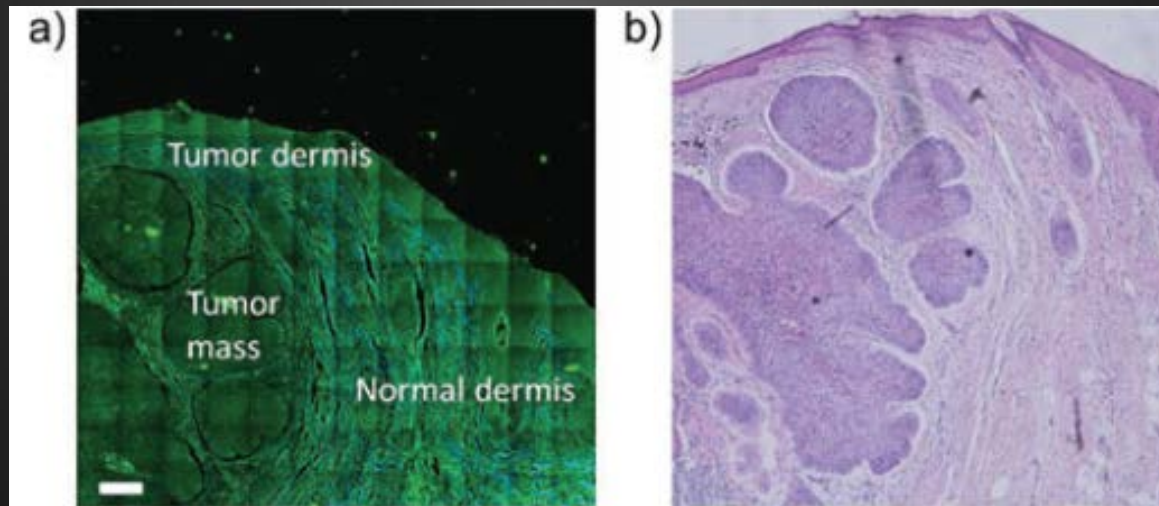
- **Skin**
  - Dermal photoaging diagnostics with SHG and 2PH AF (two-photon autofluorescence)
- **Cornea stroma** – cornea is invisibly with single photon techniques but can be visualize with 2PH AF and SHG
  - Bacterial infections
  - Keratoconus: abnormal reorganization of corneal collagen
  - Scars from damage or surgery
- **Blood vessel - atherosclerosis**
  - Monitor the changes in the arterial wall structure and composition
- **Musculo-skeletal disorders**



3D rendering of atherosclerosis-susceptible intervertebral branch point with a ring of exposed collagen around the ostia. Red elastin autofluorescence and Green collagen SHG. *P.J. Campagnola and C.-Y. Dong: Second harmonic generation microscopy*

# Collagen - cancer

- Skin cancer imaging:
  - With SHG and 2PH AF the tumor mass can be detected without any labeling
  - allows real time monitoring of the tumor without tissue removal

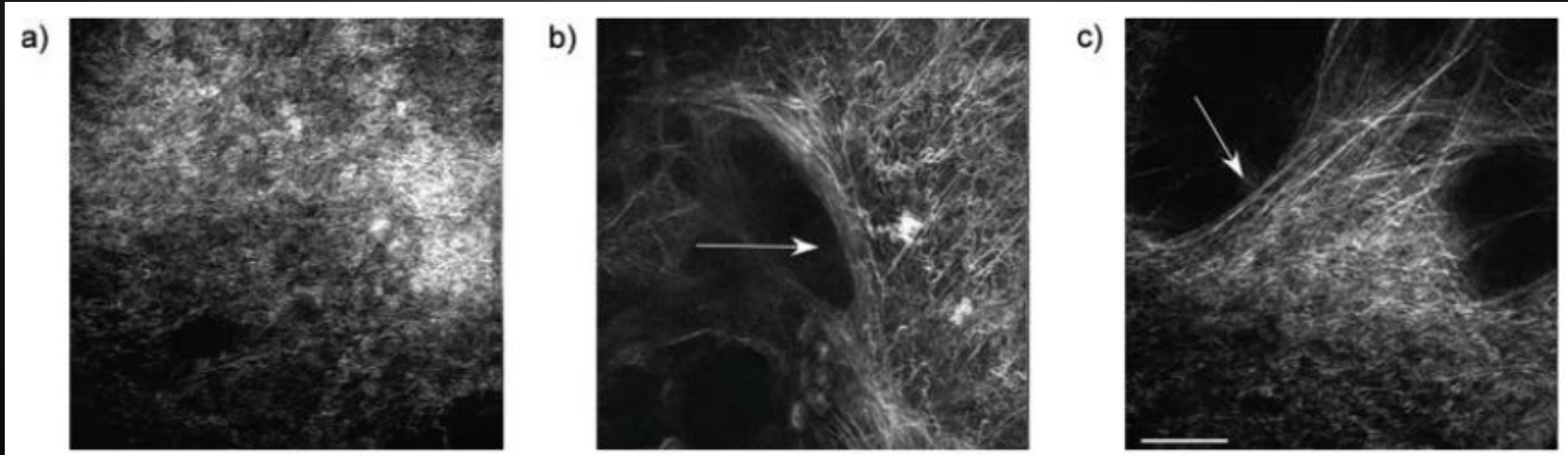


Optical diagnosis of ex-vivo human basal cell carcinoma using a) SHG and multiphoton autofluorescence imaging, where blue SHG, green autofluorescence and b) adjacent H&E histological section.

*P.J. Campagnola and C.-Y. Dong: Second harmonic generation microscopy*

# Collagen - cancer

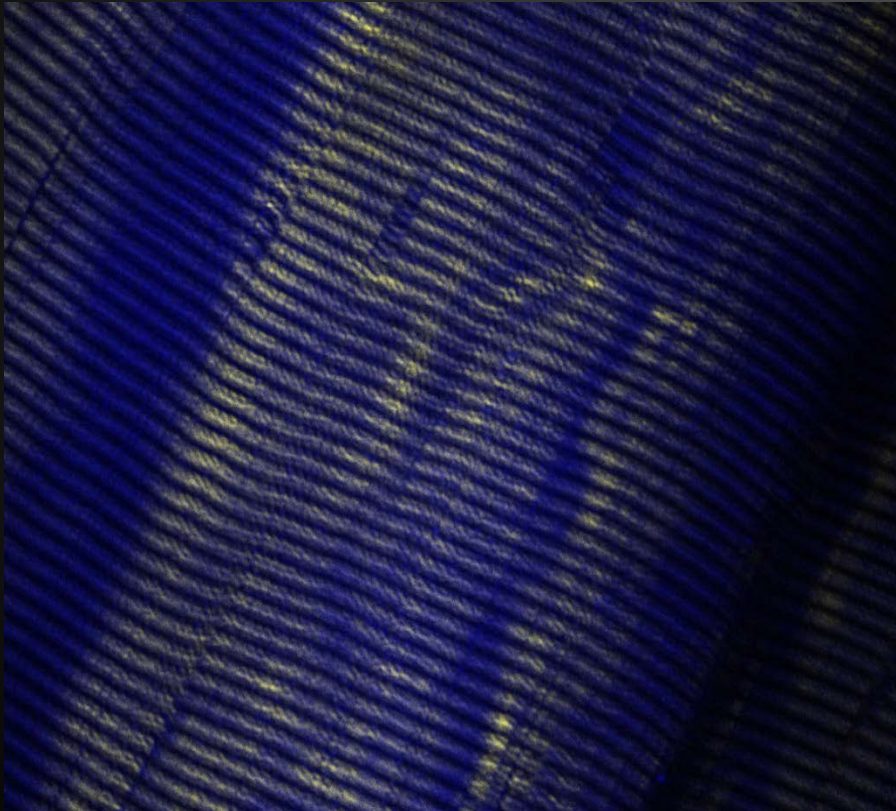
- Breast cancer imaging:
  - Collagen structure changes near the tumor, Tumor Associated Collagen Signatures (TACS)
  - TACS: defined stages of tumor progression
  - The signatures:
    - Dense collagen localized around small tumors during early stages
    - Collagen fibers that are parallel to the tumor boundary
    - Collagen fibers that are normal ( $90^\circ$ ) to the tumor boundary for invasive disease
  - Invasion, metastasis: tumor cells migrate through and along collagen fibers – SHG is a very useful tool to visualize



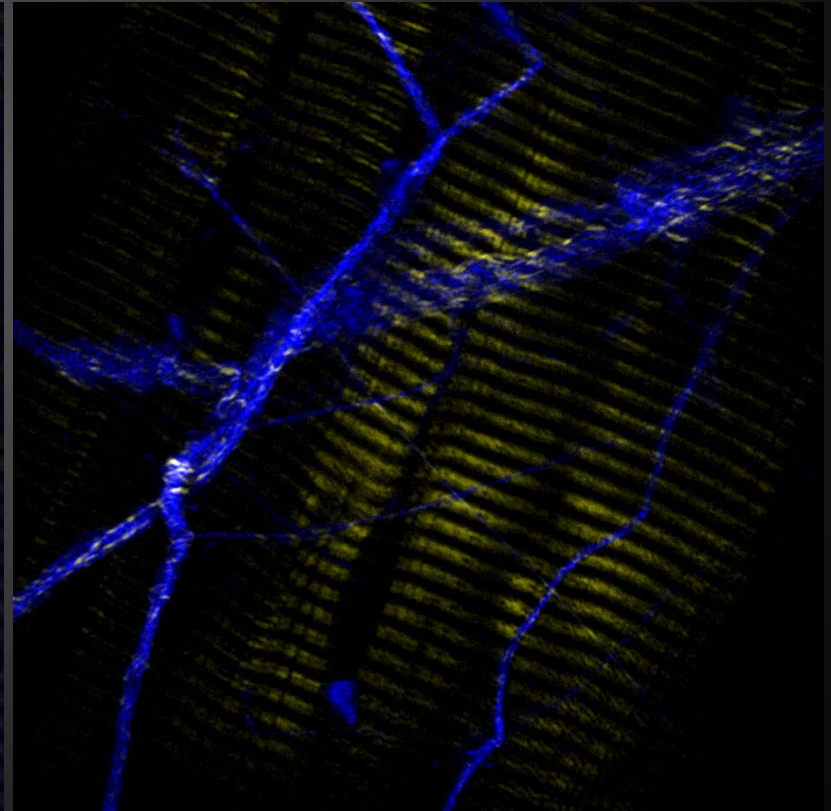
*P.J. Campagnola and C.-Y. Dong: Second harmonic generation microscopy*

# Skeletal muscle

Myosin: gives SHG signals at around 800 nm with femtosecond laser



Skeletal muscle SHG



Collagen and skeletal muscle SHG

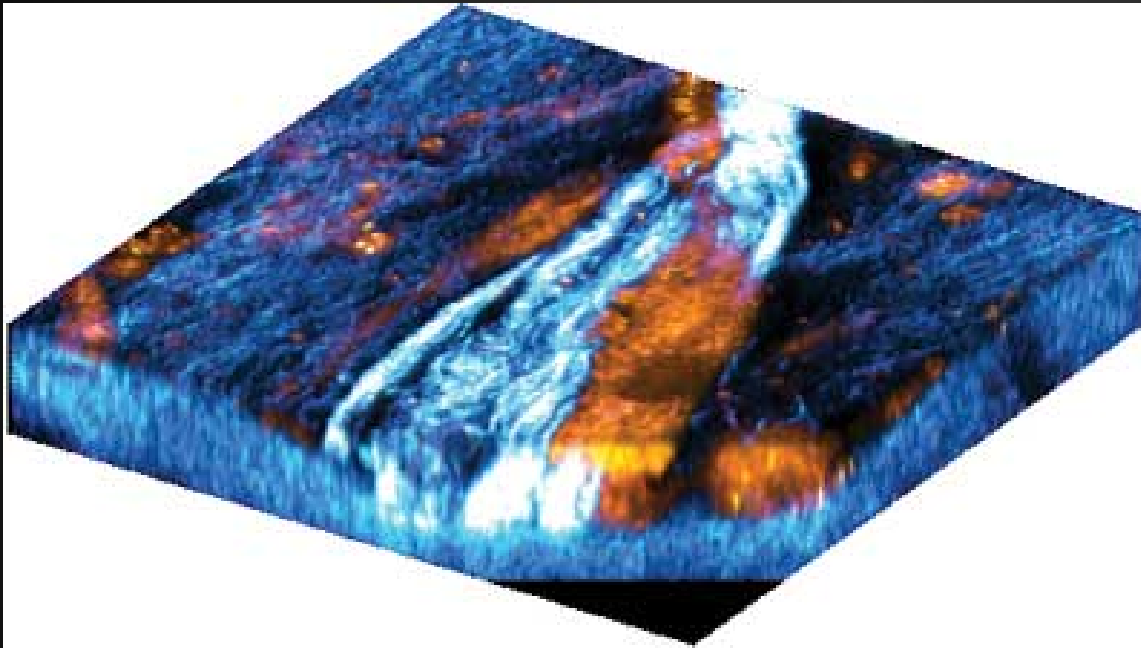
# Skeletal muscle

- With SHG the structure of skeletal muscle can be visualized, bands of sarcomers can be measured
- Disease models that can be visualized with SHG:
  - Disuse-induced atrophy
  - Hereditary muscular dystrophy (mild (mdx) and severe (mdx/UTR) forms)
  - Sarcopenia of aging

## More info:

- Characterization of the Myosin-Based Source for Second-Harmonic Generation from Muscle Sarcomeres, Sergey V. Plotnikov et al., *Biophysical Journal*, Volume 90, Issue 2, 15 January 2006, Pages 693–703

# Bacterial and plant cellulose



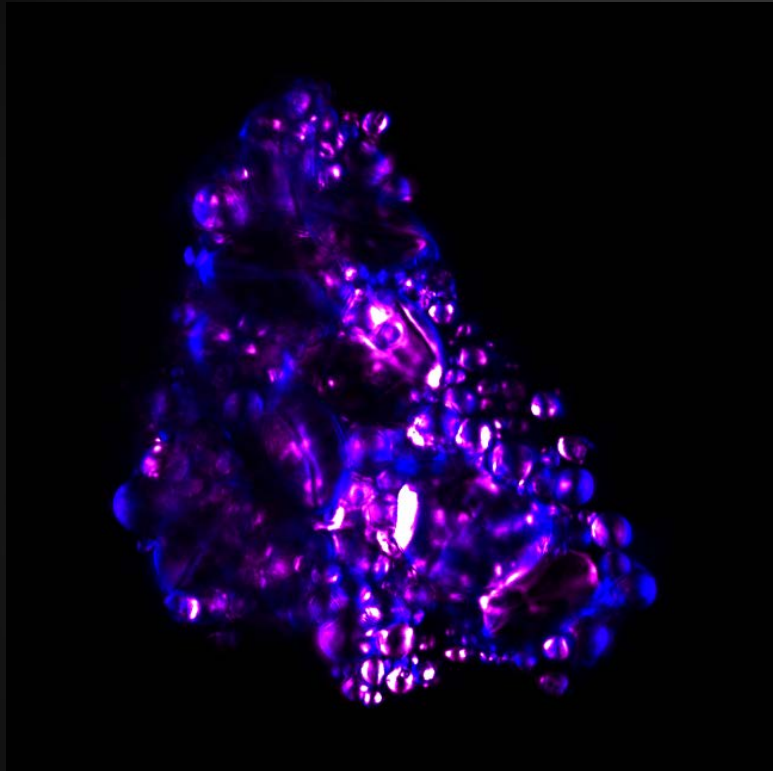
Coherent anti-Stokes Raman scattering microscopy of human smooth muscle cells in bioengineered tissue scaffolds, Brackmann C et al., *J Biomed Opt.* 2011 Feb;16(2):021115

Bacterial cellulose for artificial tissue matrix in regenerative medicine

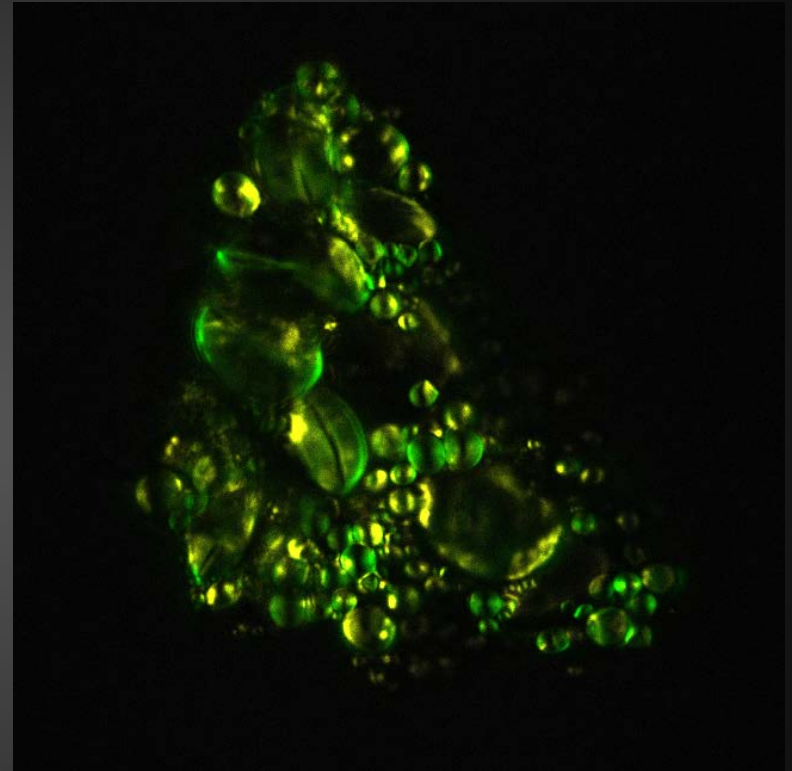
- Blue: cellulose SHG
- Yellow: smooth muscle cell CARS
- No labeling were used, 817 nm laser source



# Starch



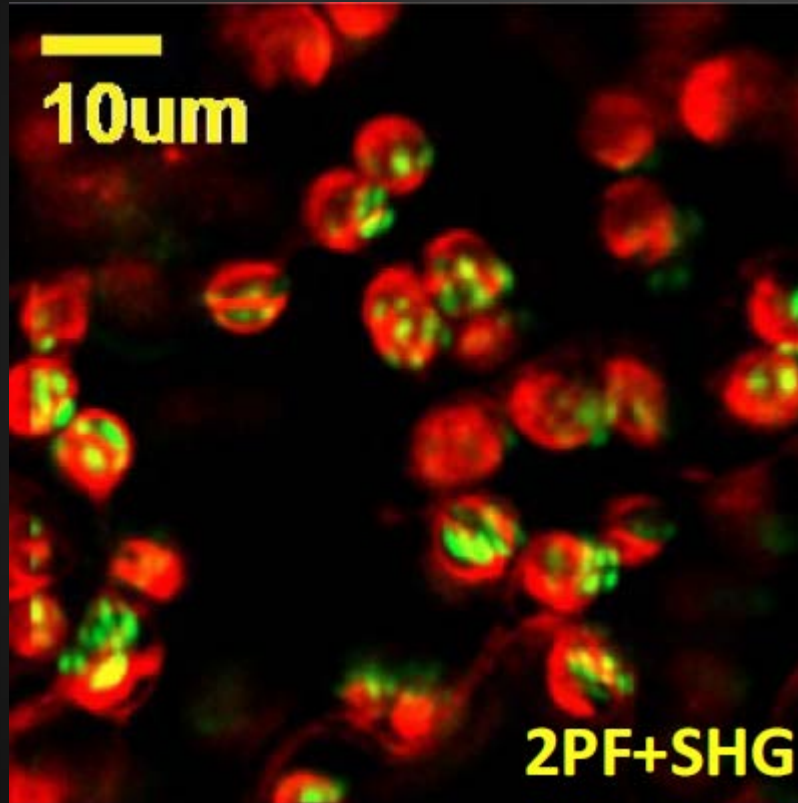
Wheat starch SHG at 800 nm  
Magenta: transmitted  
Blue: back-scattered



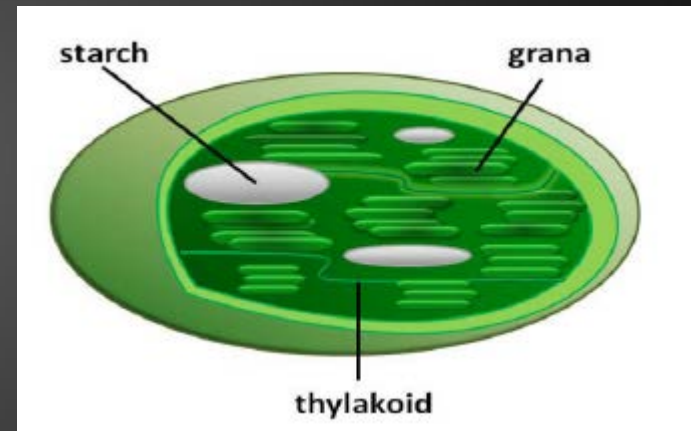
Wheat starch SHG at 1030 nm  
Yellow: transmitted  
Green: back-scattered

Starch SHG can be obtained with laser source from 800 – 1050 nm

# Starch in chloroplasts

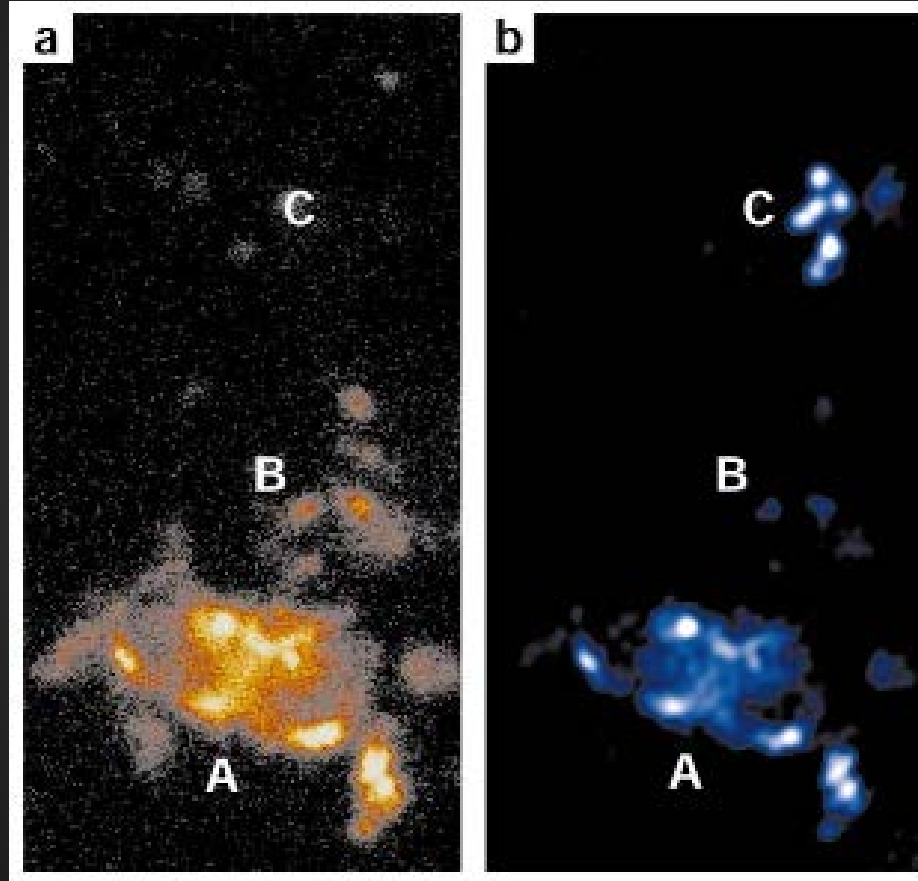


Red: 2 photon autofluorescence of thylakoid (chloroplast)  
Green: starch SHG  
(Laser source: 1030 nm)



Multiphoton Imaging to Distinguish Grana and Starch inside an Intact Leaf, Mei-Yu Chen et al., *Proc. of SPIE Vol. 8588 858822-1*

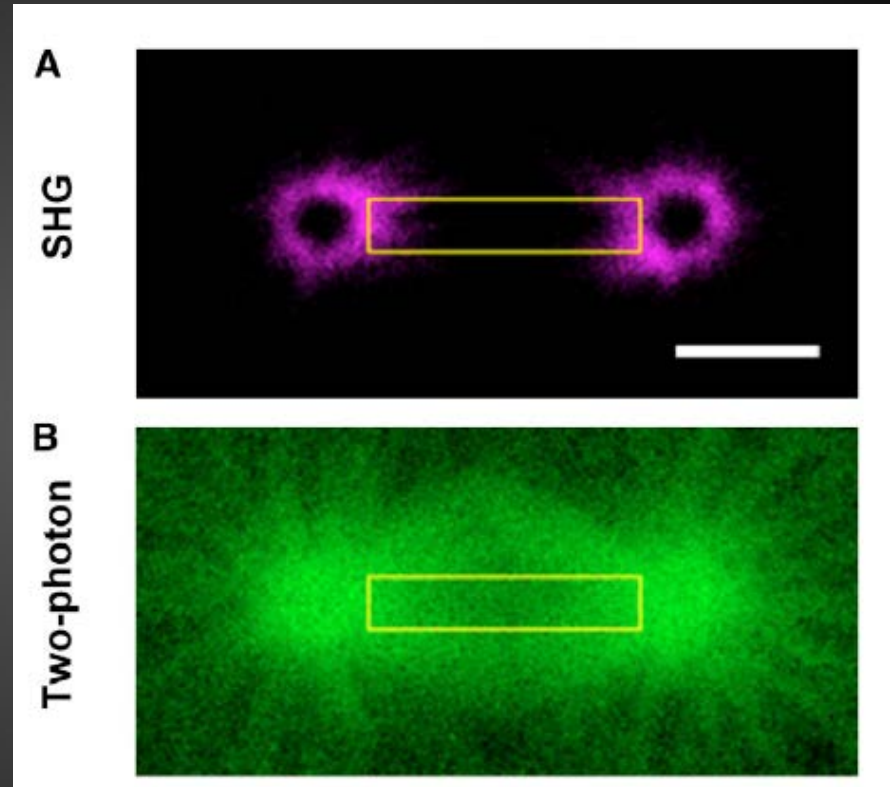
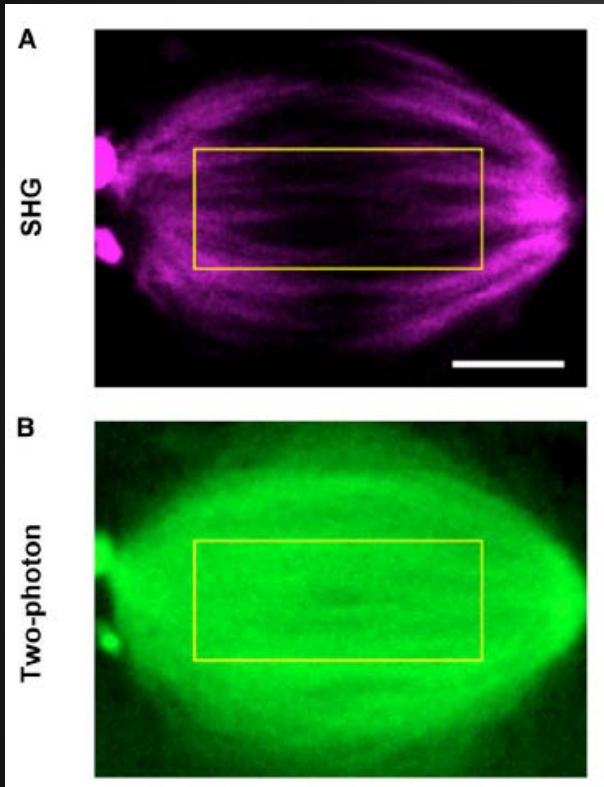
# DNA



a) TPF image and (b) SHG image of the same region of a specimen containing *B. Tyroni* fruit fly polytene chromosomes (800 nm excitation)

Simultaneous multichannel nonlinear imaging: combined two-photon excited fluorescence and second-harmonic generation microscopy, R. Gauderon et al., *Micron* 32 (2001) 685-689

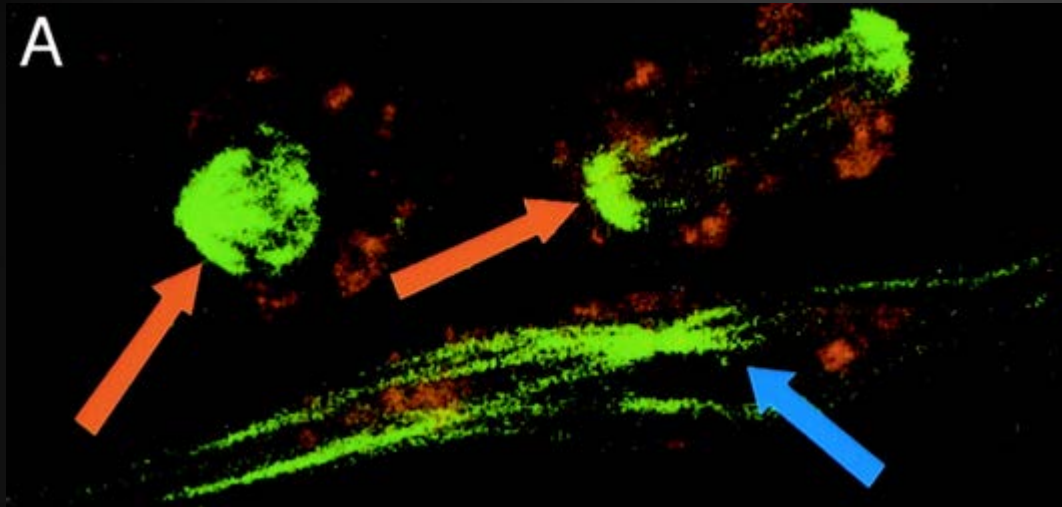
# Tubulin - microtubule



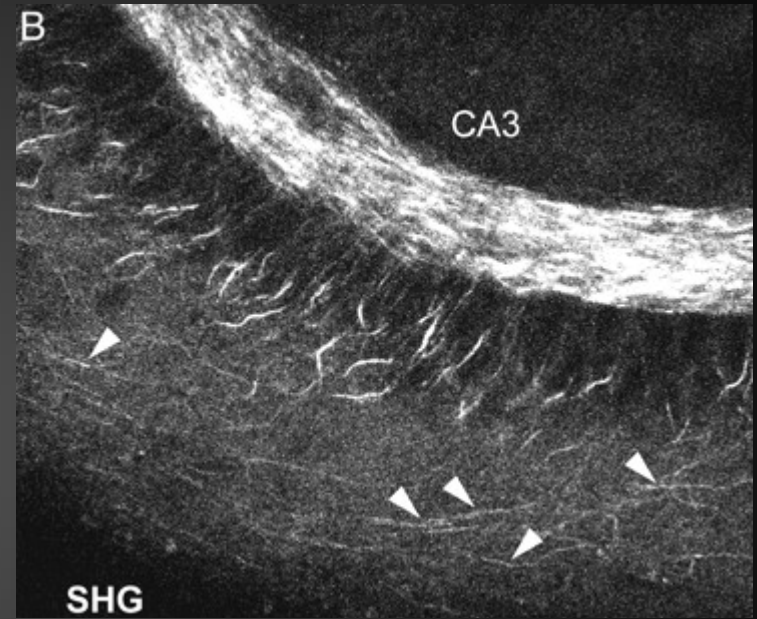
- Mitotic spindle, laser source: 850 nm. Labeled with GFP for 2 PH fluorescence
- Only the centromere region is visible with SHG, here the microtubules enter in radial direction, the orientation is random. However this effect is not yet clear.

Che-Hang Yu et al: Measuring Microtubule Polarity in Spindles with Second-Harmonic Generation; Volume 106, Issue 8, 15 April 2014, Pages 1578–1587

# Tubulin – brain tissue, axons



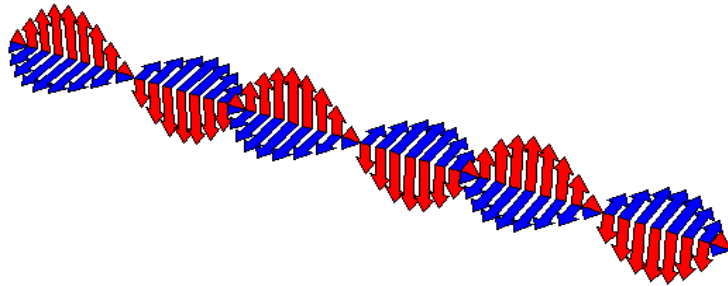
SHG (green) is seen from mitotic spindles (orange arrows) and from interphase MT ensembles (blue arrow). Red is autofluorescence. Horizontally polarized laser source at 880 nm.



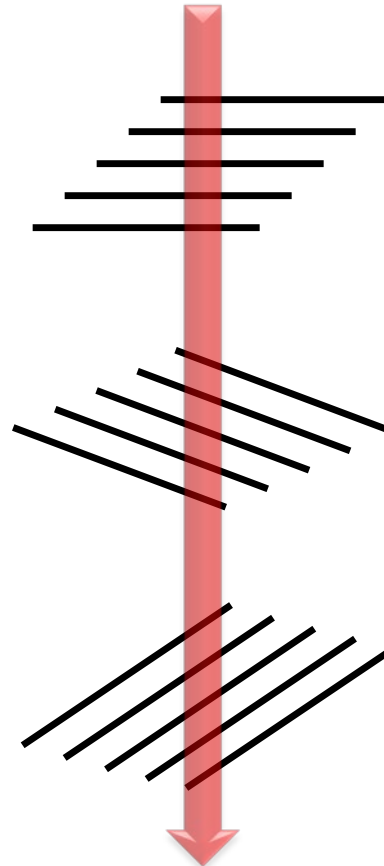
SHG image shows individual axons emanating from the pyramidal neurons (arrowheads). Circularly polarized laser source at 880 nm.

Uniform polarity microtubule assemblies imaged in native brain tissue by second-harmonic generation microscopy, Daniel A. Dombeck et al., *PNAS*, June 10, 2003, vol. 100, no. 12, 7081–7086

# Anisotropy – polarization angle with SHG



Laser beam is **polarized *per se*** due to the characteristic of the laser resonator, cavity, Brewster window etc..



Periodic and non-centrosymmetric sample in different orientation

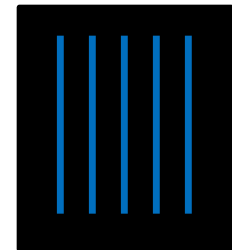
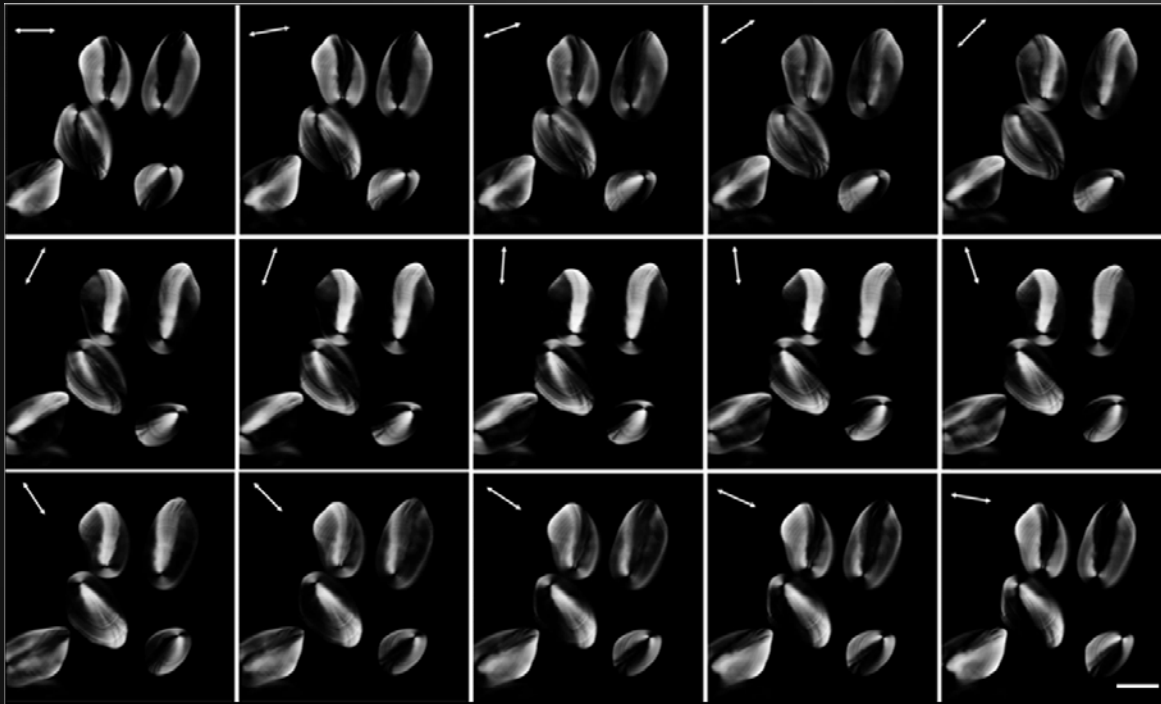


Image (SHG) intensity changes

# Anisotropy – polarization angle with SHG



Starch SHG in different orientation

With  $\chi^{(2)}$  tensor analysis one can calculate back the structural organization and molecular orientation information of the sample

$$\chi^{(2)} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & \chi_{16}^{(2)} \\ \chi_{21}^{(2)} & \chi_{22}^{(2)} & \chi_{21}^{(2)} & 0 & 0 & 0 \\ 0 & 0 & 0 & \chi_{16}^{(2)} & 0 & 0 \end{bmatrix}$$
$$= \chi_{16}^{(2)} \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 1 \\ 0.54 \pm 0.38 & 2.04 \pm 0.28 & 0.54 \pm 0.38 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \end{bmatrix}$$

Zhuo ZY et al: Second harmonic generation imaging - a new method for unraveling molecular information of starch; J Struct Biol. 2010 Jul;171(1):88-94

**THANKS FOR YOUR ATTENTION!**

