Imaging and characterization of polymer microstructures

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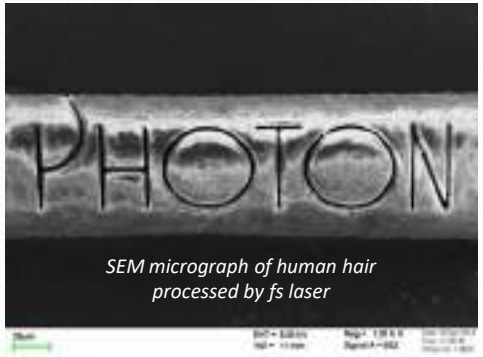
What can light tell us about polymer microstructures

Polymer consists of large amount of similar units bonded together

Artificial polymers (casting, 3D printing..)



SEM micrograph of human hair processed by fs laser

Natural polymers (grown)

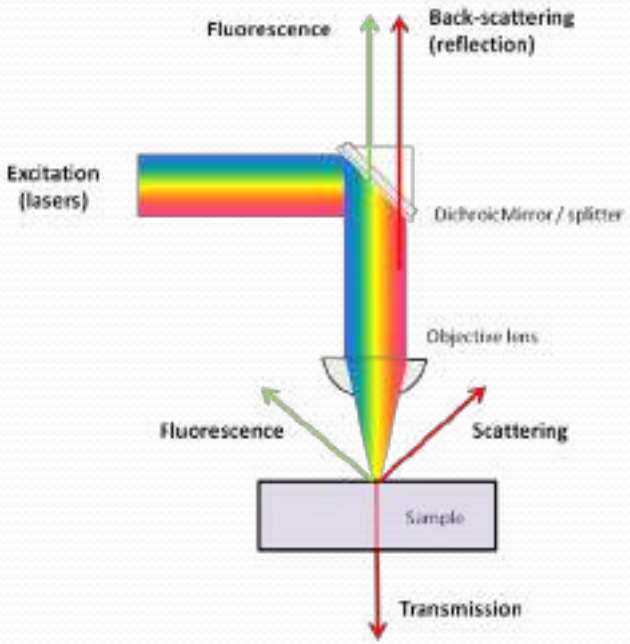
How to see details of polymer structures?

Microscopy (electron, AFM, optical)

Why to use photonics methods such as optical imaging and spectroscopy ?

What can light tell us about polymer microstructures

Interaction of the light with polymers



After impact of the light beam on the polymer surface, different light-matter interactions are possible:

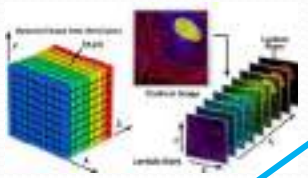
- **Absorption** in the sample,
- Forward-propagating beam - **Transmission**
- Back-scattered beam - **Reflection**
- **Beam scattered arbitrary** around the sample
- **Fluorescence** generated by photochemical excitation of the sample, usually detected in backward direction
- Change of **Polarization** state (e.g. birefringence)

*The **scattered coherent light** could also interfere with another parts of the beam, leading to signal measurement based on **interferometry**.*

In all these cases, we can analyze different properties of light, such as:

- Spatial distribution = **Imaging** (2D, 3D)
- Wavelength properties = **Spectroscopy**
- Time-propagation properties = **Dynamics**

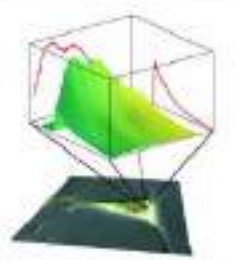
Detection strategies for multi-modal imaging



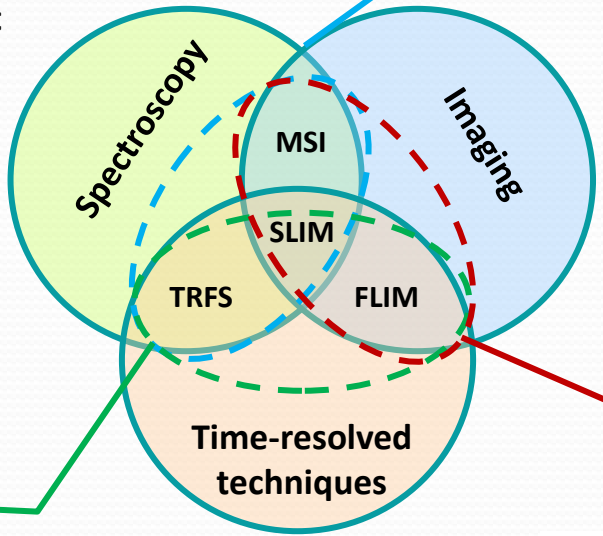
Spectral decomposition

Composition >> Structure

molecular environment classification



Time-series, modulation lifetime analysis



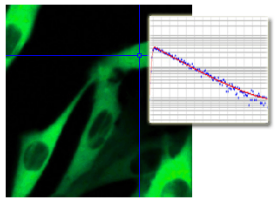
Spatial distribution >> Shape

specificity & labeling segmentation

Image analysis / pattern recognition

Dynamics >> Function

rates, timing, synchrony analysis of behavior

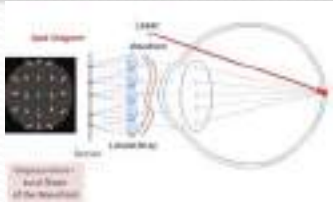


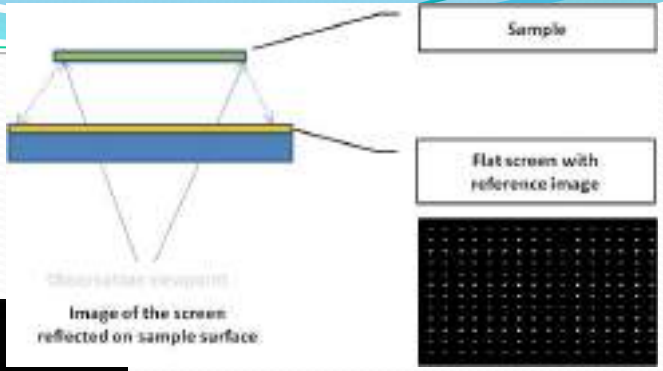
Approaches for visualization and characterization of synthetic polymer structures

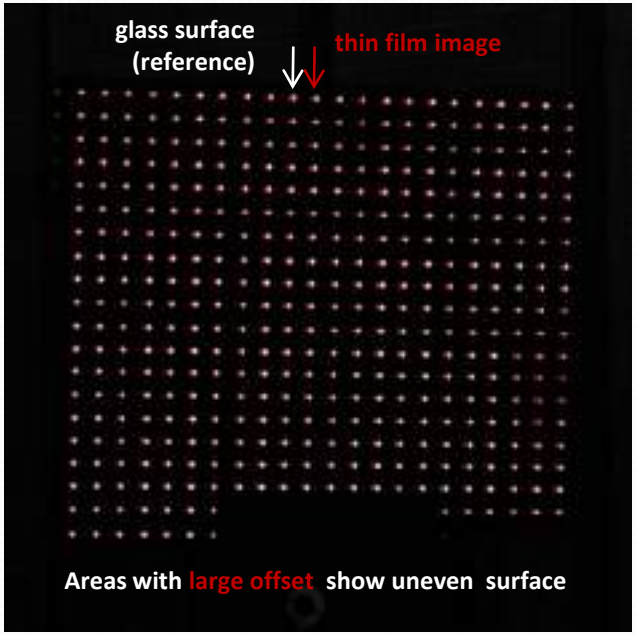
Visualization of large-area thin-films

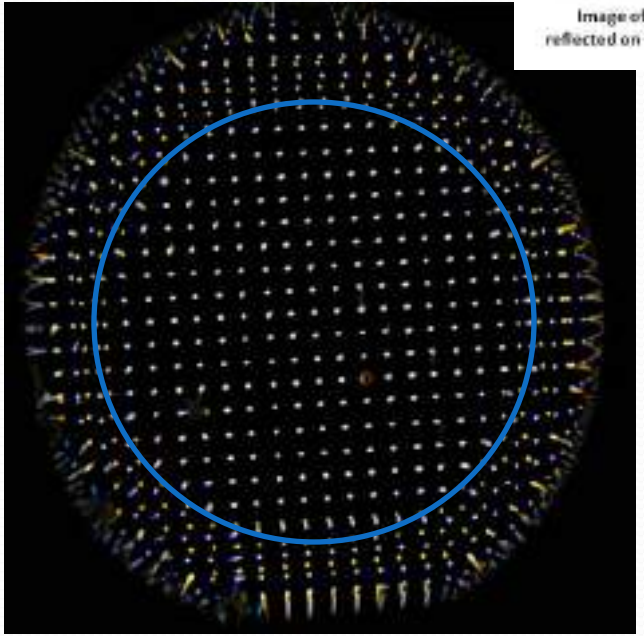
"How good are my deposited resists?"

Wavefront measurement according to Hartmann principle
(image – Wikipedia)

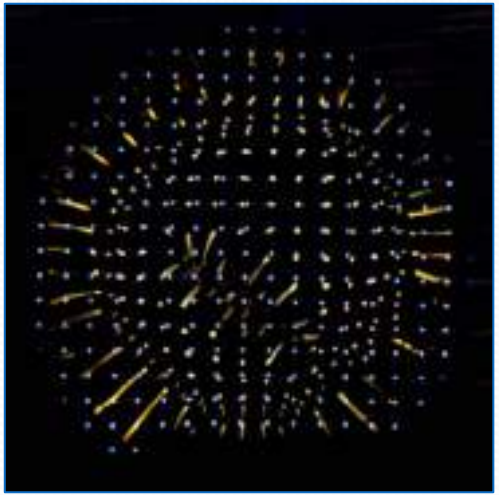








O – area of even resin layer

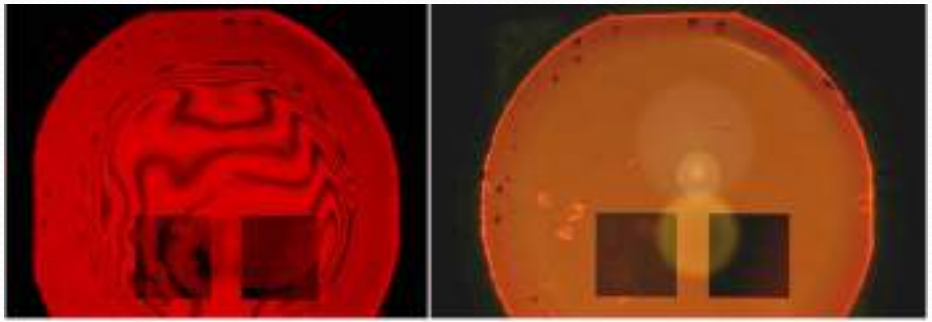


Uneven resin layer

Interferometric imaging of thin structures

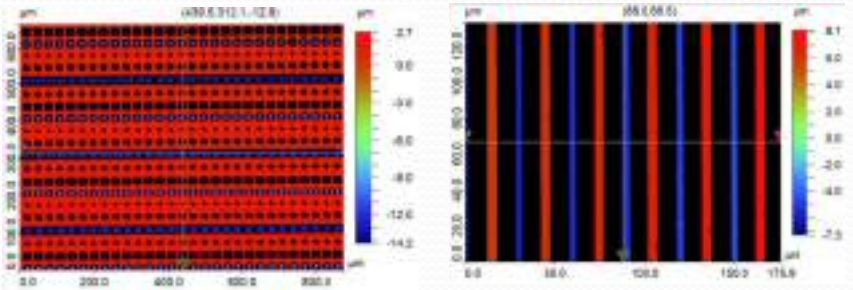
"How to characterize thin films?"

Large-area



Interference imaging can be used for evaluation of substrate / resin homogeneity and surface waviness

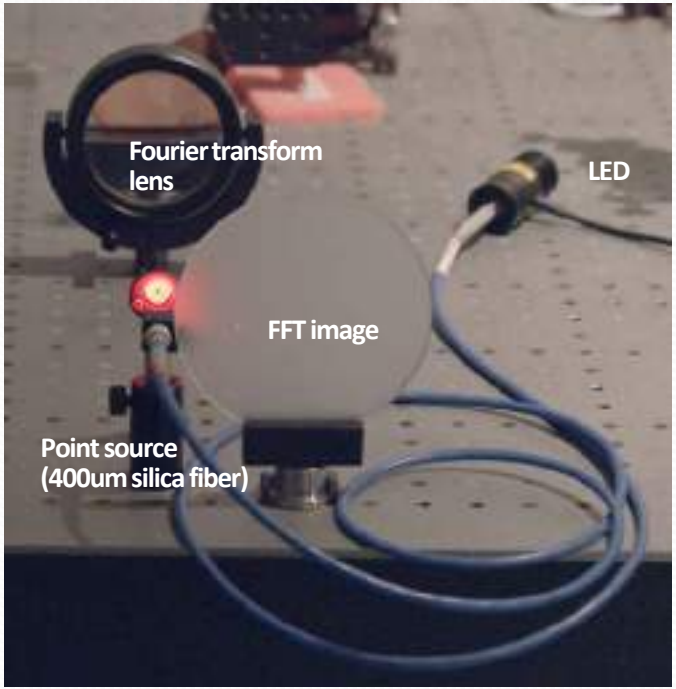
Images by Bruker / Polytec



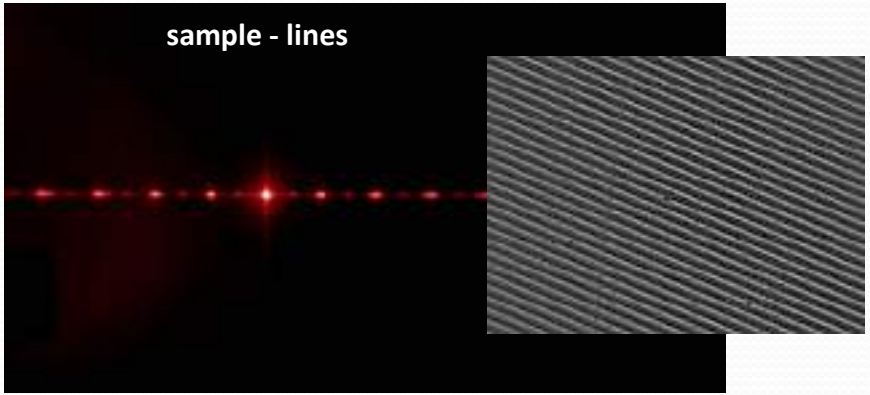
Non-contact profilometry with very high Z-resolution

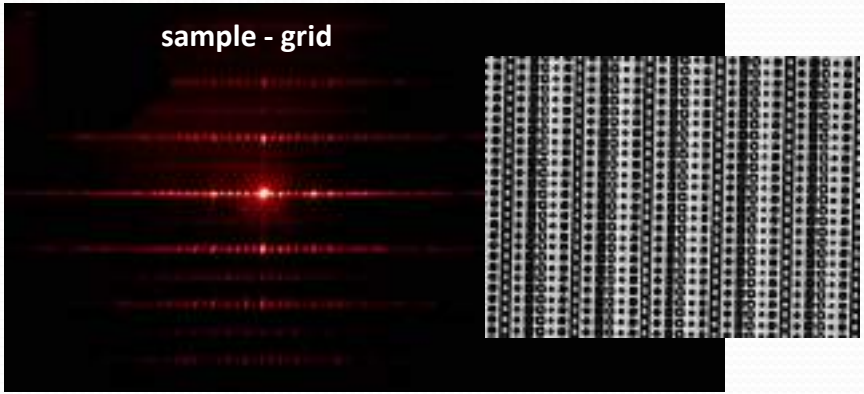
Large-area diffraction imaging of periodic structures

"How to check large patterned areas?"



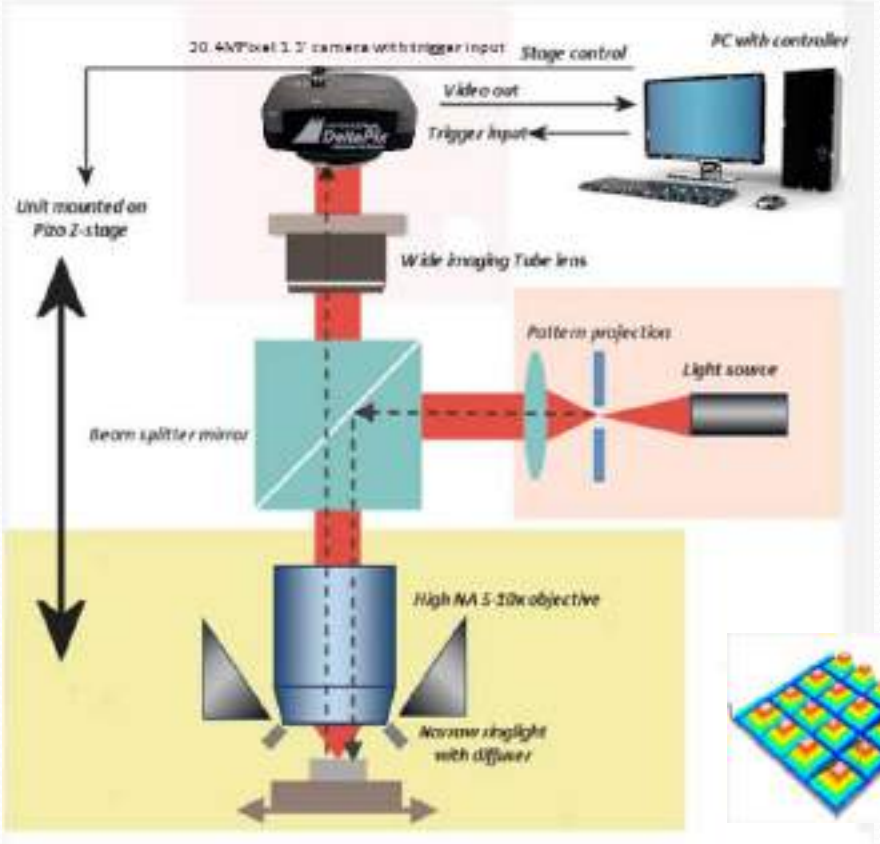
No structure



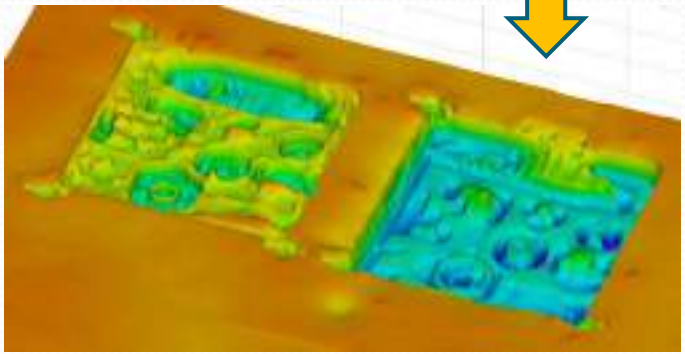


Focus variation microscopy: reconstruction of surface shape

"How can I see my microstructures?"



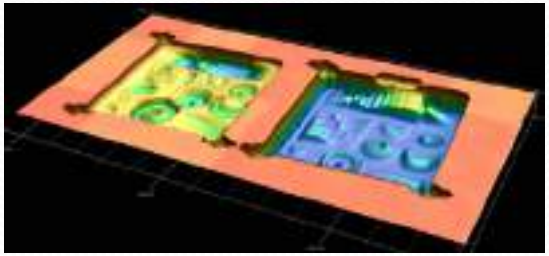
Wide-field microscopy, providing set of Z-stacks



Reconstructed 2.5 D image (surface relief)

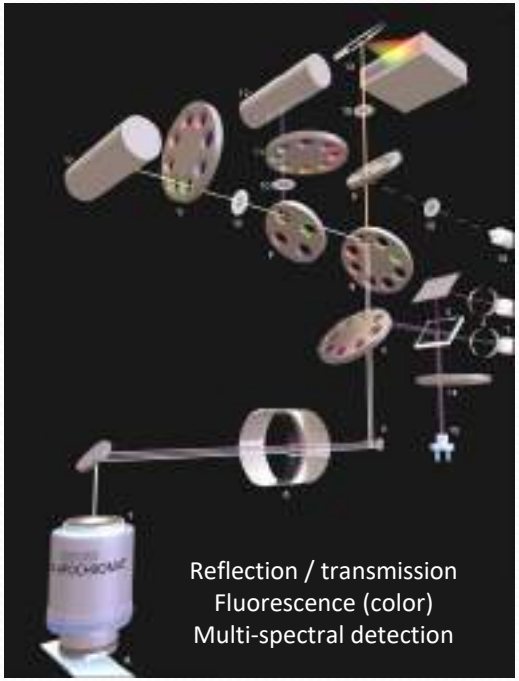
Confocal laser scanning microscopy

"How can I see my microstructures?"

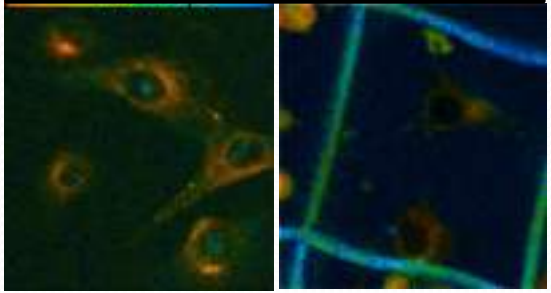


3D scanning and reconstruction by confocal microscope

Scheme of CLSM microscope

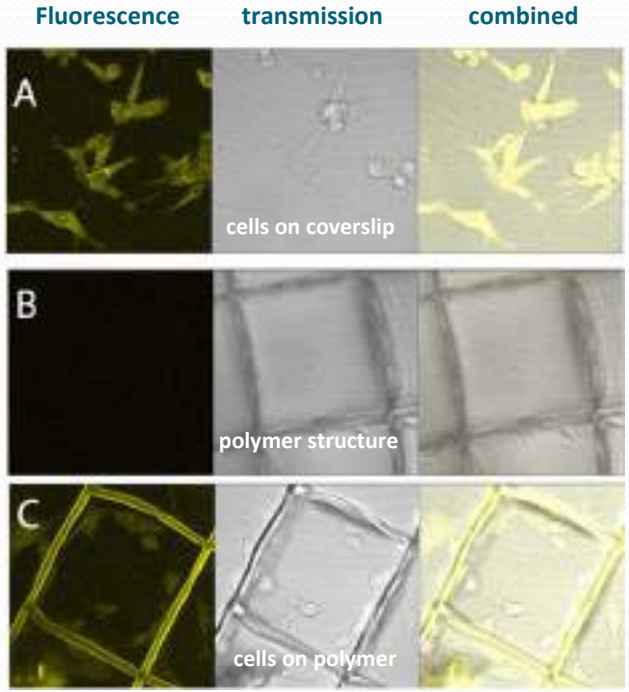


FLIM imaging of U87MG cells on OrmoComp



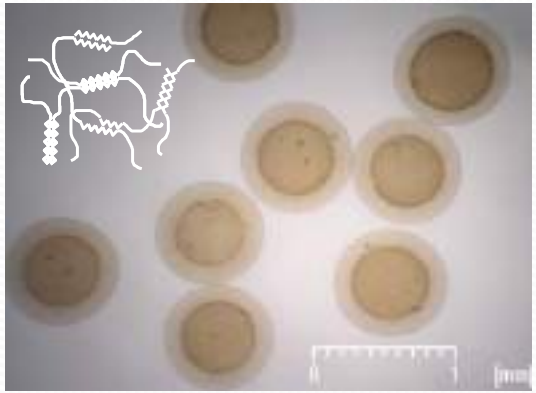
Advanced detection (Fluorescence lifetime imaging)

Interaction of polymers and cells: U87MG cells grown on OrmoComp structures

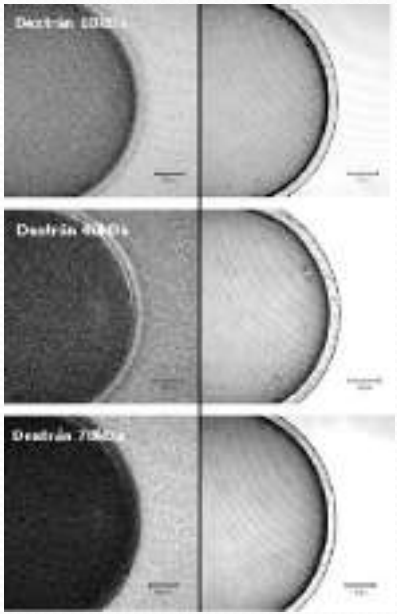


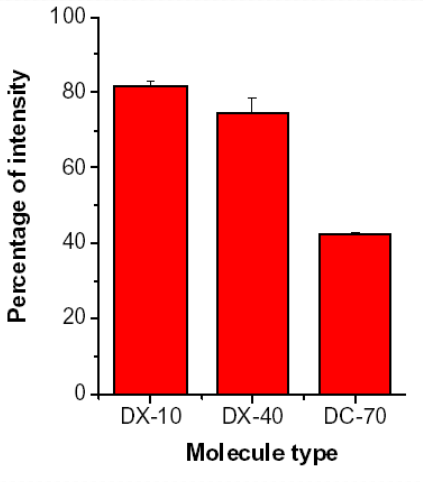
Example 1: Hydrogels and their diffusion properties

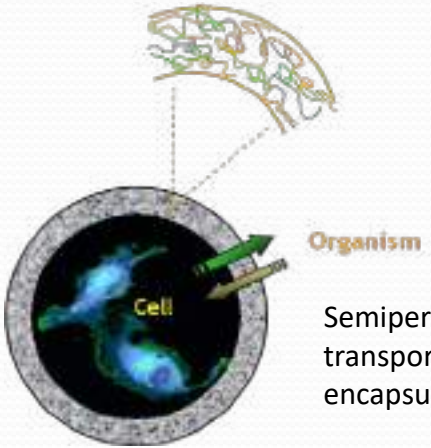
Alginate-based hydrogels for immobilization of living cells and proteins



Measurement of molecular cut-off using probes of different size (dextrans)







Semipermeable membrane for controlled transport of species to and from the encapsulated biological material

➢ **assay with IgG and IgM implemented in Chicago Diabetes project protocols**

www.chicagodiabetesproject.org

Complex polymer matrix and visualization of its structure

CLSM with covalently bound fluorescent labels
Lamprecht A., Schaefer U.F., Lehr C.-M.: Eur.J. Pharm. Biopharm 2000, 49, 1.
Zimmerman H. et al.: Biomaterials 2003, 24, 2083.

CLSM with electrostatically attracted fluorescent labels
Podskocova J, Chorvat D. et al., Laser Physics, 15, 2005, 545-551

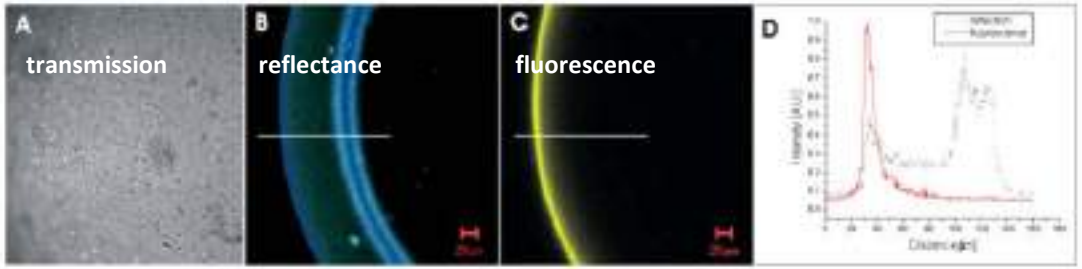
The Bioartificial Pancreas and other Biohybrid Therapies
Hallé J.P., de Vos P., Rosenberg L. (Editors)
Research Signpost (2008)

Chapter 8

Visualisation Techniques in the Characterization of Polymer Microcapsules: Confocal Laser Scanning Microscopy and Atomic Force Microscopy

Igor Lacík[1] and Dušan Chorvát Jr[1,2]

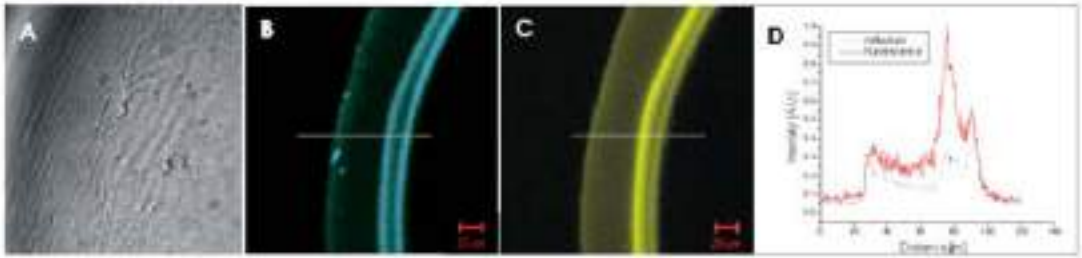
Eosin Y - anionic

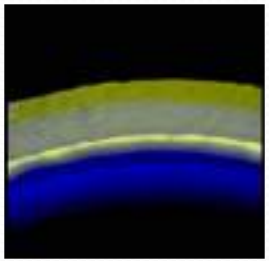


Intensity profile

3D reconstruction

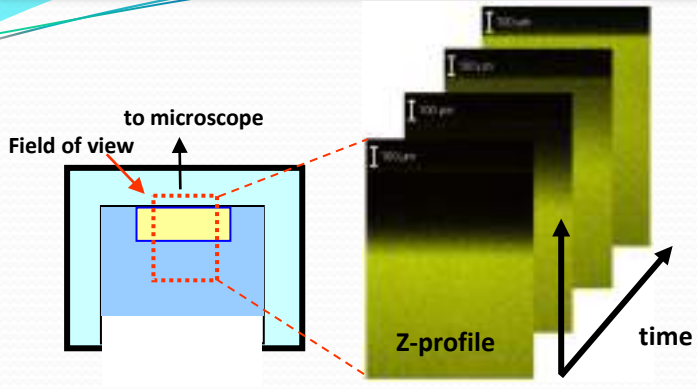
Rhodamin 123 - cationic

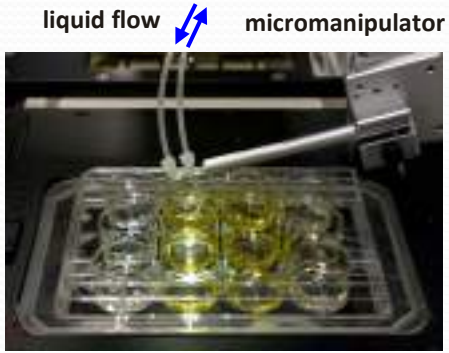




non-covalently bound fluorescence labels are attracted to oppositely charged free polymer groups.

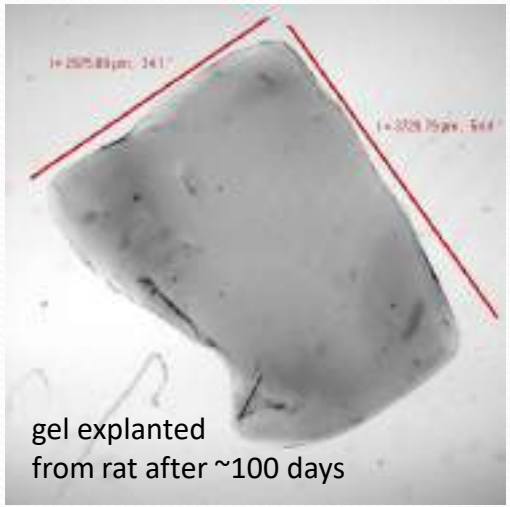
Diffusion properties of internally-gelled hydrogel slabs



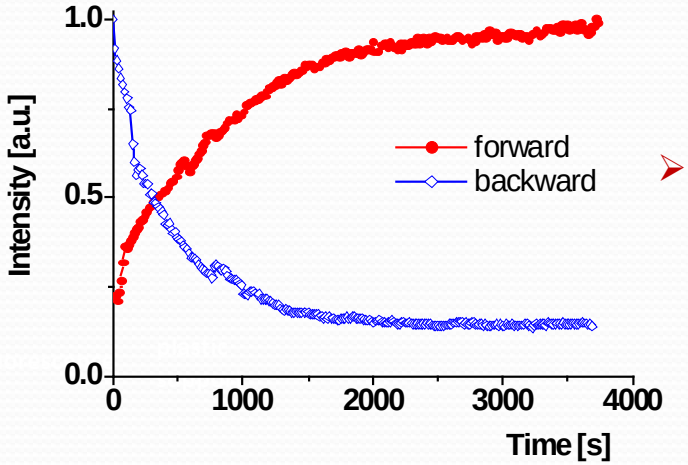


perfusion chamber

Study of the diffusion kinetics of various fluorescent labels in the polyelectrolyte matrix



gel explanted
from rat after ~100 days



- optimization of hydrogel sensor design *(P. Cezanne EC project)*

Nonlinear microscopy

Label-free spectroscopy and imaging of polymeric structures

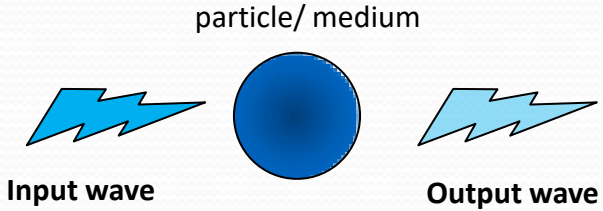
Fundamentals

Linear optics

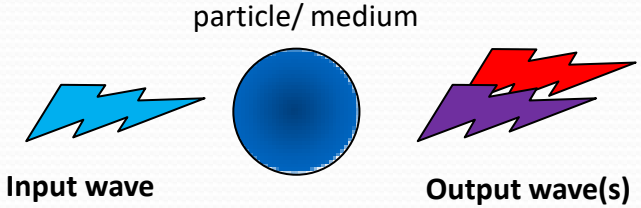
- The optical properties of materials, such as refractive index and absorption coefficient, are **independent of light intensity**
- The frequency of light is never altered by its passage through a medium
- Two beams of light in the same region of a medium have no effect on each other, so that light cannot be used to control light.

Non-Linear optics

- The refractive index / speed of light in a nonlinear optical medium **does depend on light intensity**, optical output intensities does NOT correspond linearly to the input intensities.
- The frequency of light is altered as it passes through a nonlinear optical medium;
- Photons do interact within the confines of a nonlinear optical medium so that light can be used to control light = light induces changes to itself as it propagates through the medium



For low optical intensities, nonlinear effects become very weak. However, ultra-high fields provided by pulsed (femtosecond) lasers allow to utilize a broad range of nonlinear optical phenomena.



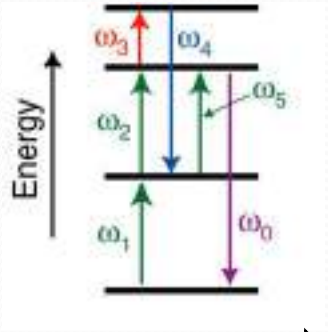
Nonlinear optics - techniques

NLO describes the behavior of light in nonlinear media, when the dielectric polarization P responds nonlinearly to the electric field E of the light. This nonlinearity is typically only observed at very high light intensities provided by pulsed lasers.

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

The spectrum of NLO techniques comprise of e.g.:

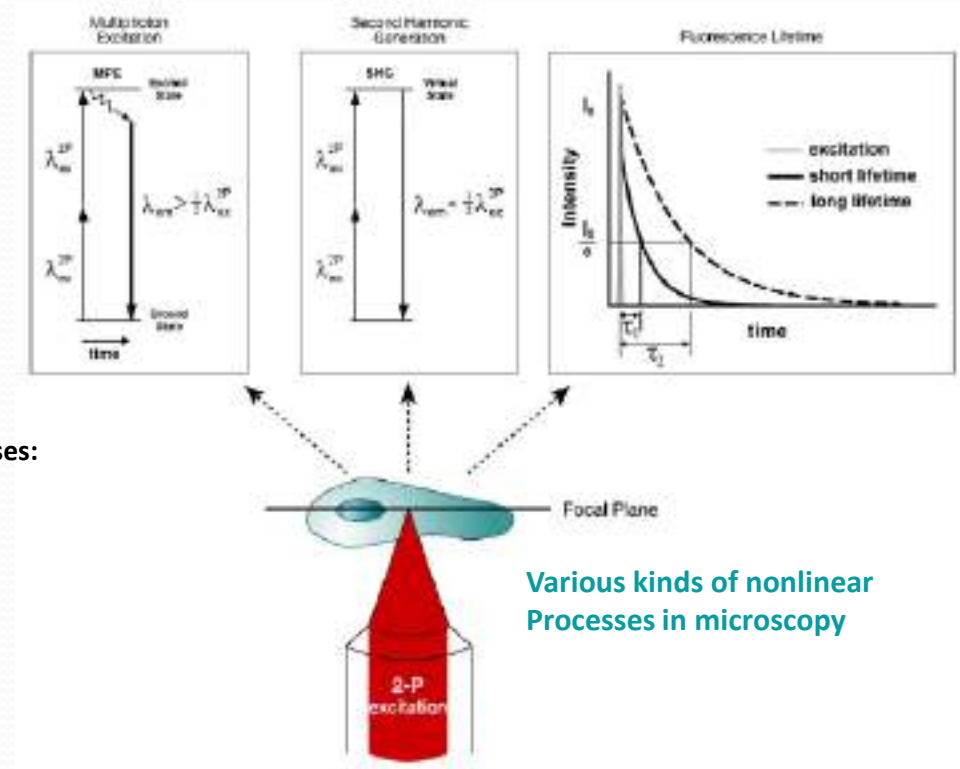
- Frequency mixing processes (FWM, SFG, SHG, HHG, OPA, ..)
- Optical Kerr effect - intensity dependent refractive index
- multi-photon absorption/ionization ...



Various kinds of three-wave mixing processes:

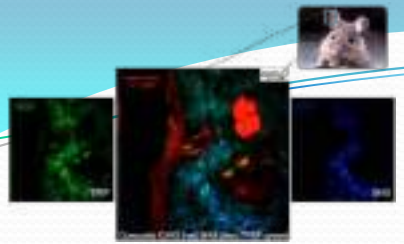
- second-harmonic generation,
- sum-frequency generation,
- difference-frequency generation,
- optical rectification.

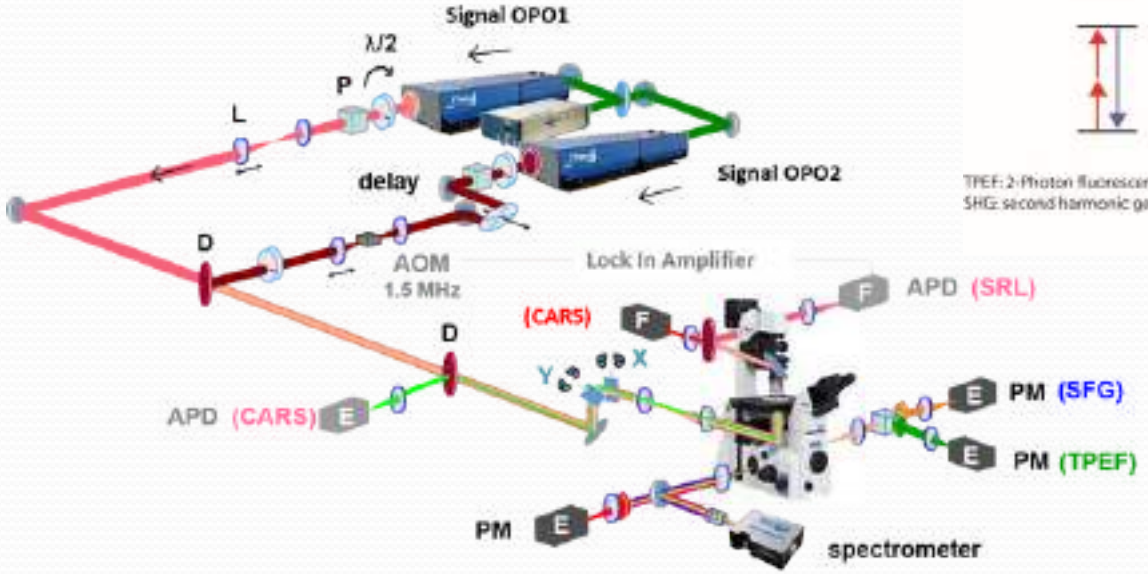
$2\omega_1$, $2\omega_2$, $\omega_1 + \omega_2$, $\omega_1 - \omega_2$, and 0



Various kinds of nonlinear Processes in microscopy

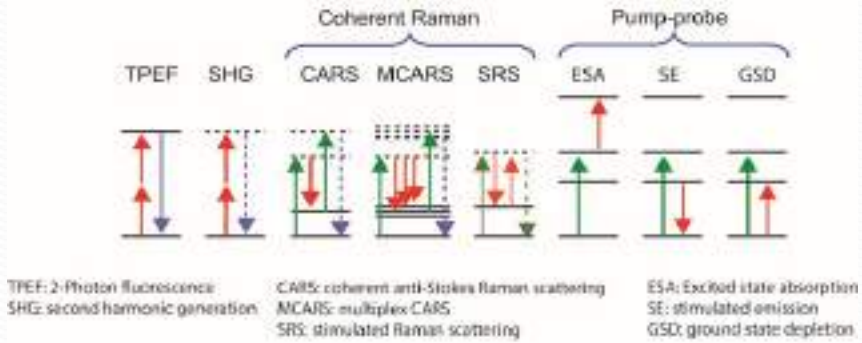
Advanced optical microscopy today

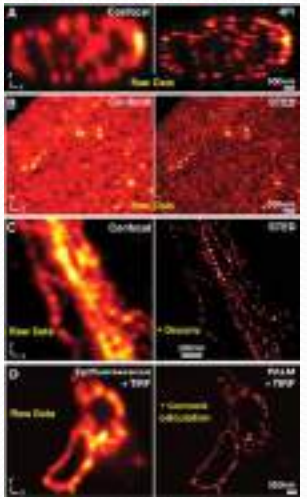




TPEF, SHG, CARS, SRS multimodal microscope

H. Rigneault et al., www.fresnel.fr/spip/spip.php?article1691

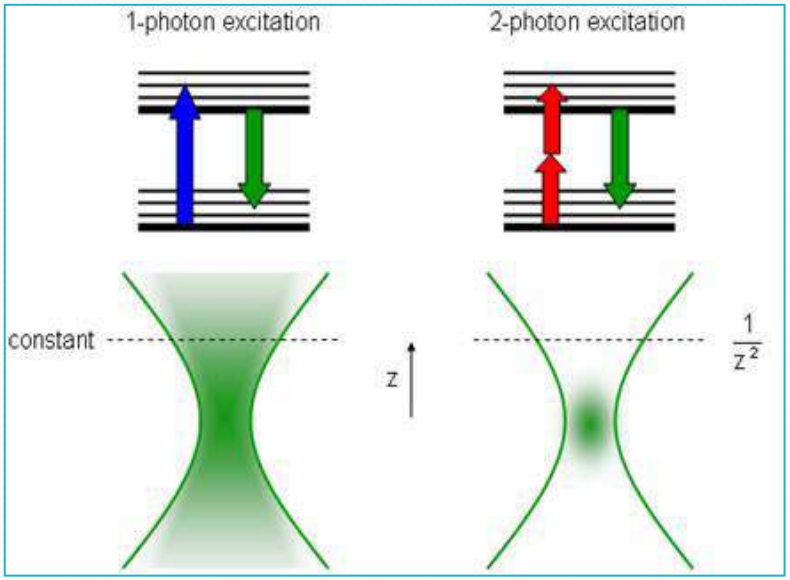




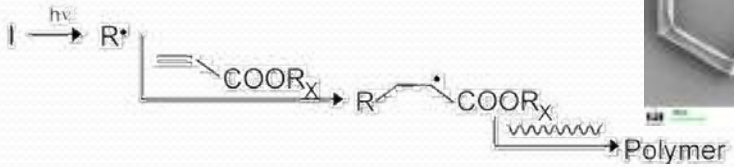
Nobel prize for chemistry 2014
Erik Betzig, Stefan W. Hell, William E. Morner
Nobel Prize for Physics 2018
G.Mourou, D.Strickland, A.Ashkin

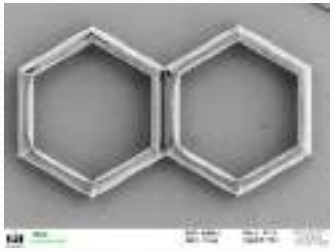
Multi-photon excitation as a tool for fabrication and imaging

Same process, different applications

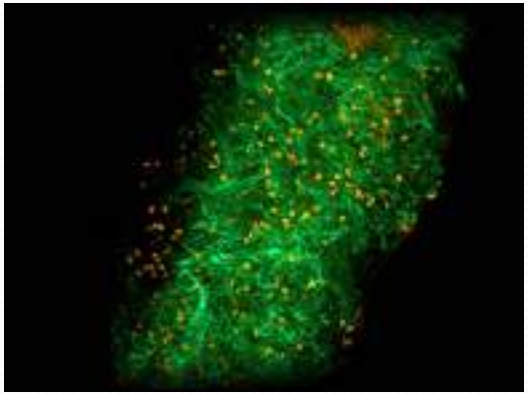


Two photon polymerization





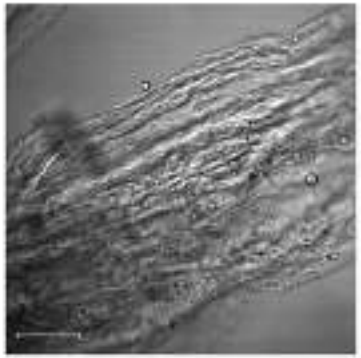
Two photon imaging



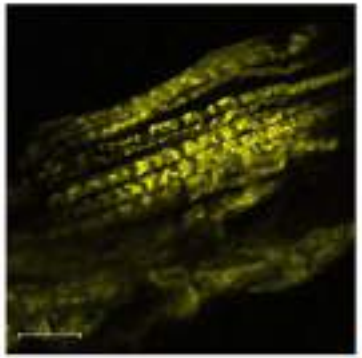
Example 2 :Nonlinear optical imaging of collagen

Analysis of collagen orientation in tissues

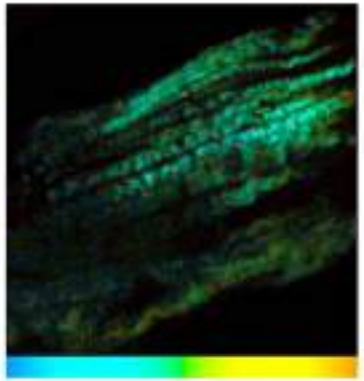
SHG microscopy is a well-established technique for characterizing collagen morphology and its organization.



Transmission



SHG



FLIM / TPE

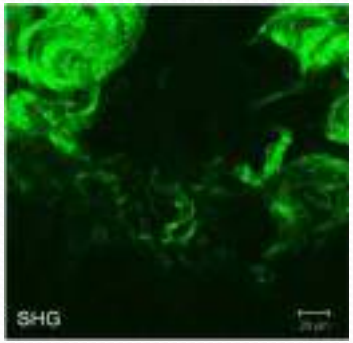
J Biophotonics. 2024 Aug;17(8):e202400090. doi: 10.1002/jbio.202400090. Epub 2024 Jun 27.

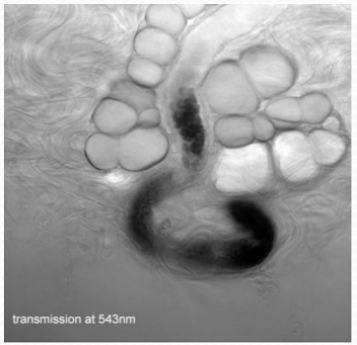
Extraction of collagen morphological features from second-harmonic generation microscopy images via GLCM and CT analyses: A cross-laboratory study

R Cicchi [1,2], E Baria [2,3], M Mari [4], G Filippidis [4], D Chorvat [5]

Affiliations + expand

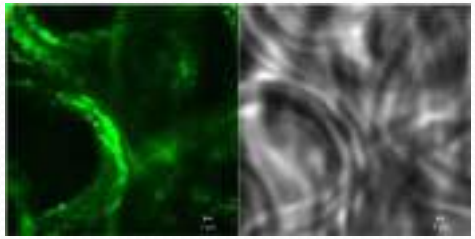
PMID: 38937995 DOI: 10.1002/jbio.202400090





The aim is to obtain good contrast in the image (using many modalities)

Optimal signal detection in SHG microscopy



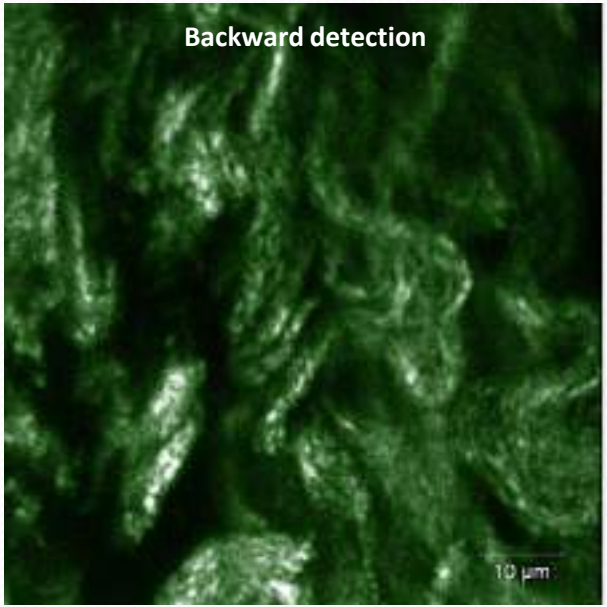
SHG image has good intrinsic contrast and sub-micron resolution

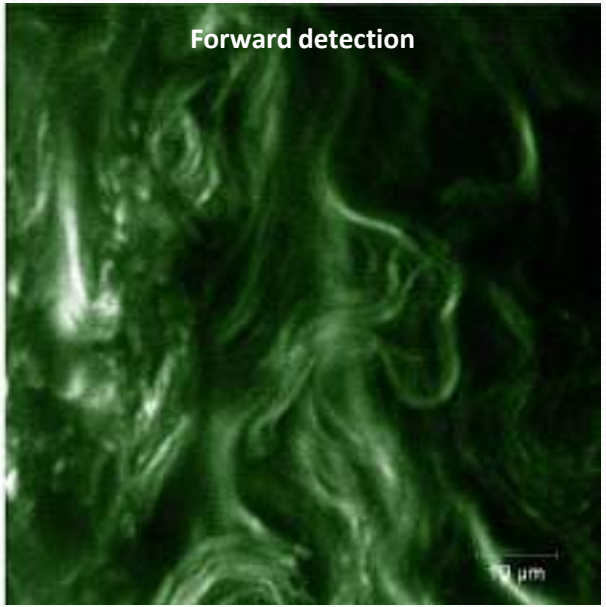
However, in **backward direction** SHG shows selectively structures that are both **generating SHG and scattering**.

Solution: detection of **forward-directed photons with circular polarization**

Backward detection

Forward detection



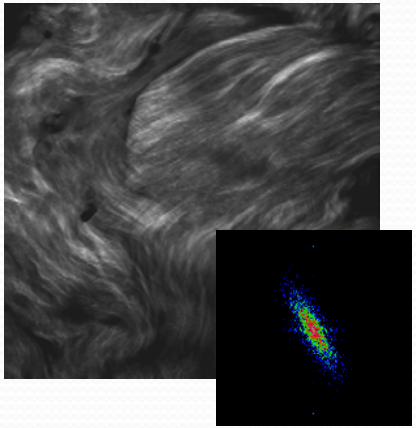


Fast-Fourier Transform (FFT) and Curvelet transform (CT) analysis of SHG images

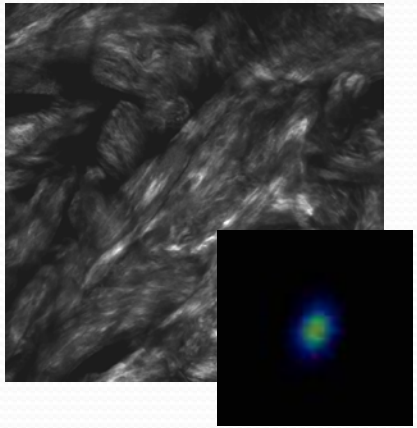
FFT approach is useful to perform an analysis of the spatial frequency components of an image. The distribution of spatial frequencies in the FFT can be used to infer information on the pattern of the original image, hence to characterize the geometry of the image texture.

Curvelets represent a generalization of Fourier Transform (FT) that can be used for denoising images and enhancing fiber-edge features. Such property can be exploited by fiber extraction (FIRE) algorithms.

Healthy dermis



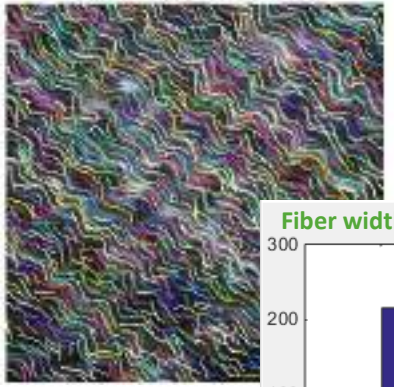
Keloid



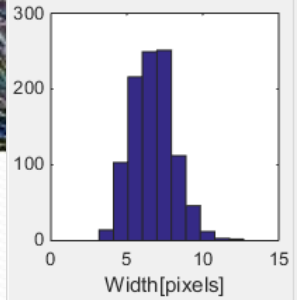
Cicchi et al, J Biophoton (2010)

Equine pericardium

Fiber extraction



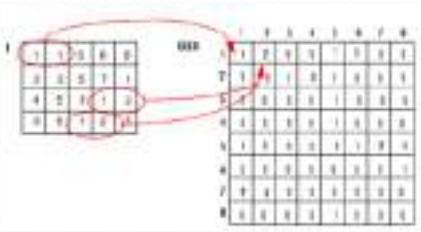
Fiber width distribution



monitoring of the fiber anisotropy, length and orientation

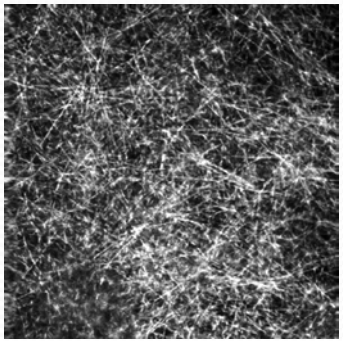
Grey-level Co-occurrence Matrix (GLCM) analysis

The **GLCM** represents the relationships between neighbors of an intensity matrix (e.g. an image). GLCM-derived functions describe image properties such as contrast and **correlation between adjacent pixels.**



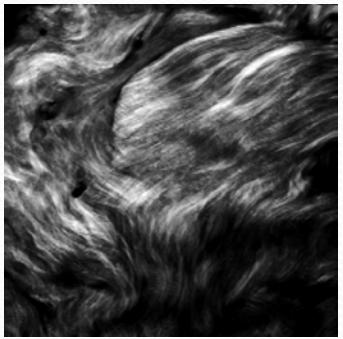
The analysis of the GLCM-correlation function can be used to evaluate the typical size of supra-molecular formations (e.g. the diameter of collagen fibers observed in Second-Harmonic Generation images).

Artificial collagen



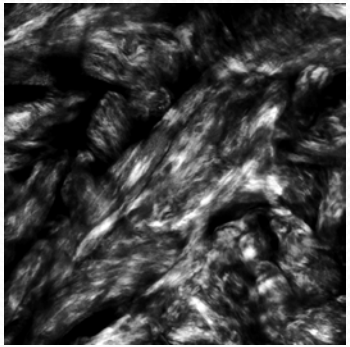
Corr. Length:
1.0 ± 0.1 mm

Healthy dermis

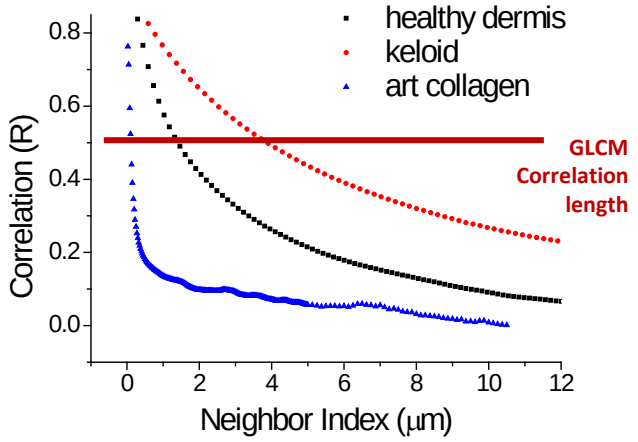


Corr. Length:
3.7 ± 0.1 mm

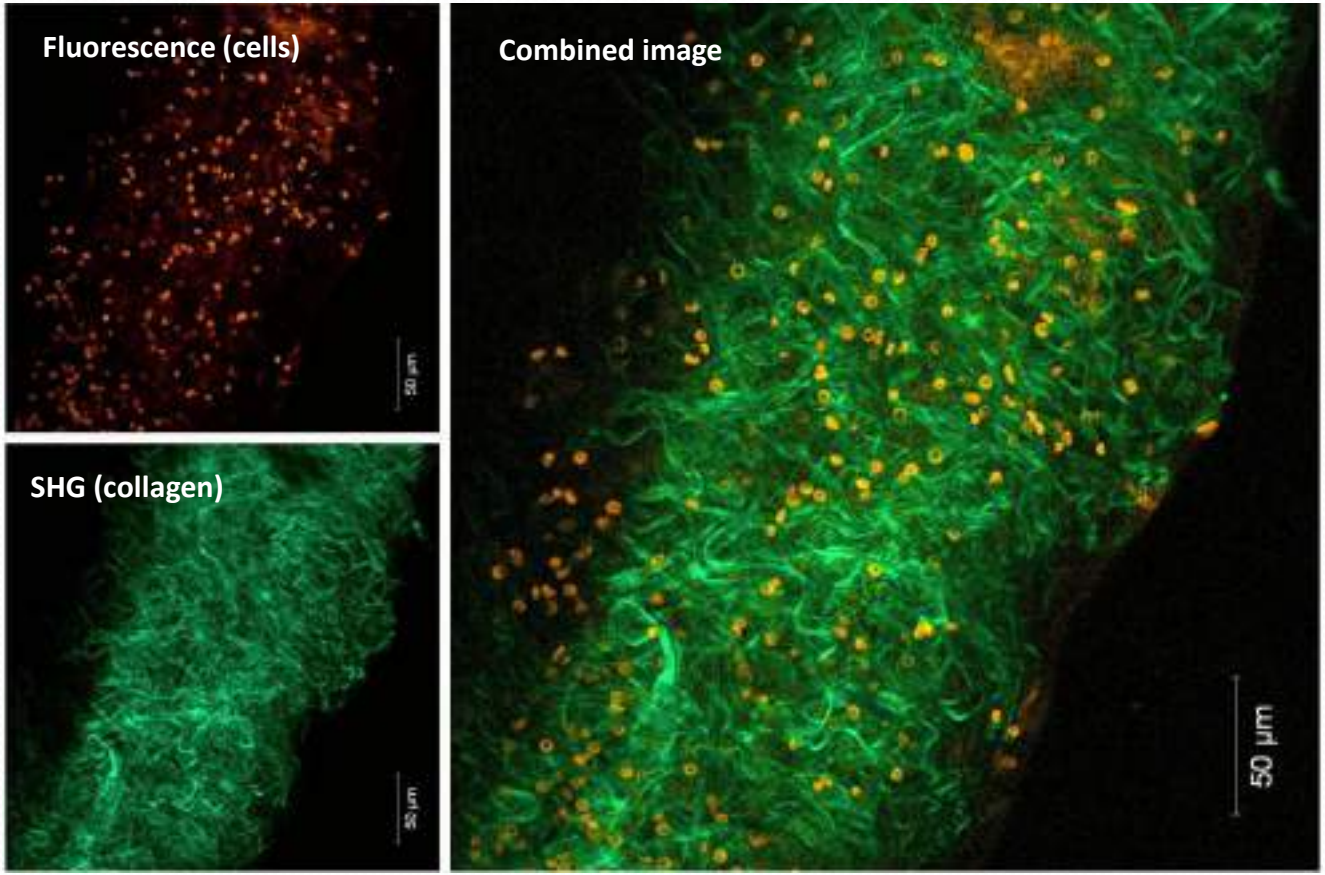
Keloid



Corr. Length:
6.8 ± 0.1 mm



Simultaneous SHG and fluorescence imaging



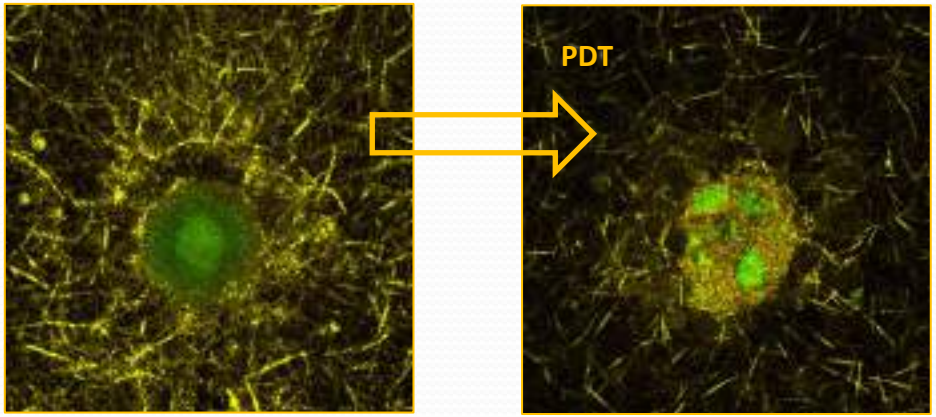
Spontaneous formation of collagen network by hydrogel synthesis

"What it is used for?"

Collagen-1 gel matrix allows to keep the cells alive in conditions closer to their natural state (not spreading over the coverslip).

Structure of the collagen network can be visualized by CLSM using reflection or SHG contrast

KYSE 450 cells stained by FOSCAN,
before and after photodynamic therapy (PDT):



before

after

- **How is the 3D network affected by the cell?**
- **Is the physiology of entrapped cell different compared to coverslip cultures?**

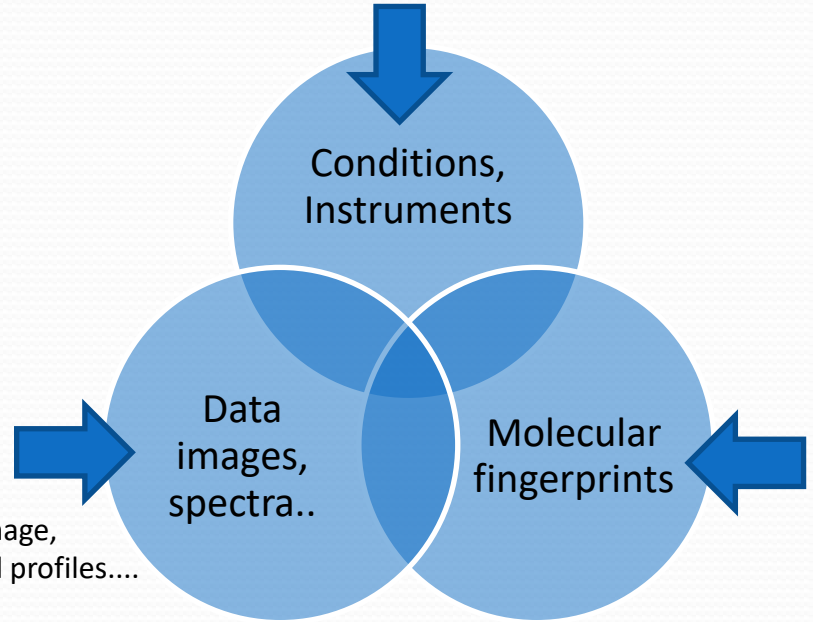
Data management in advanced microscopy

Multi-modal image databases

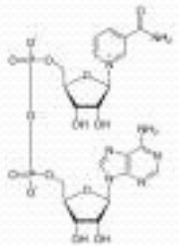
The Open Microscopy Environment

"How to archive and access large scale data?"

OME

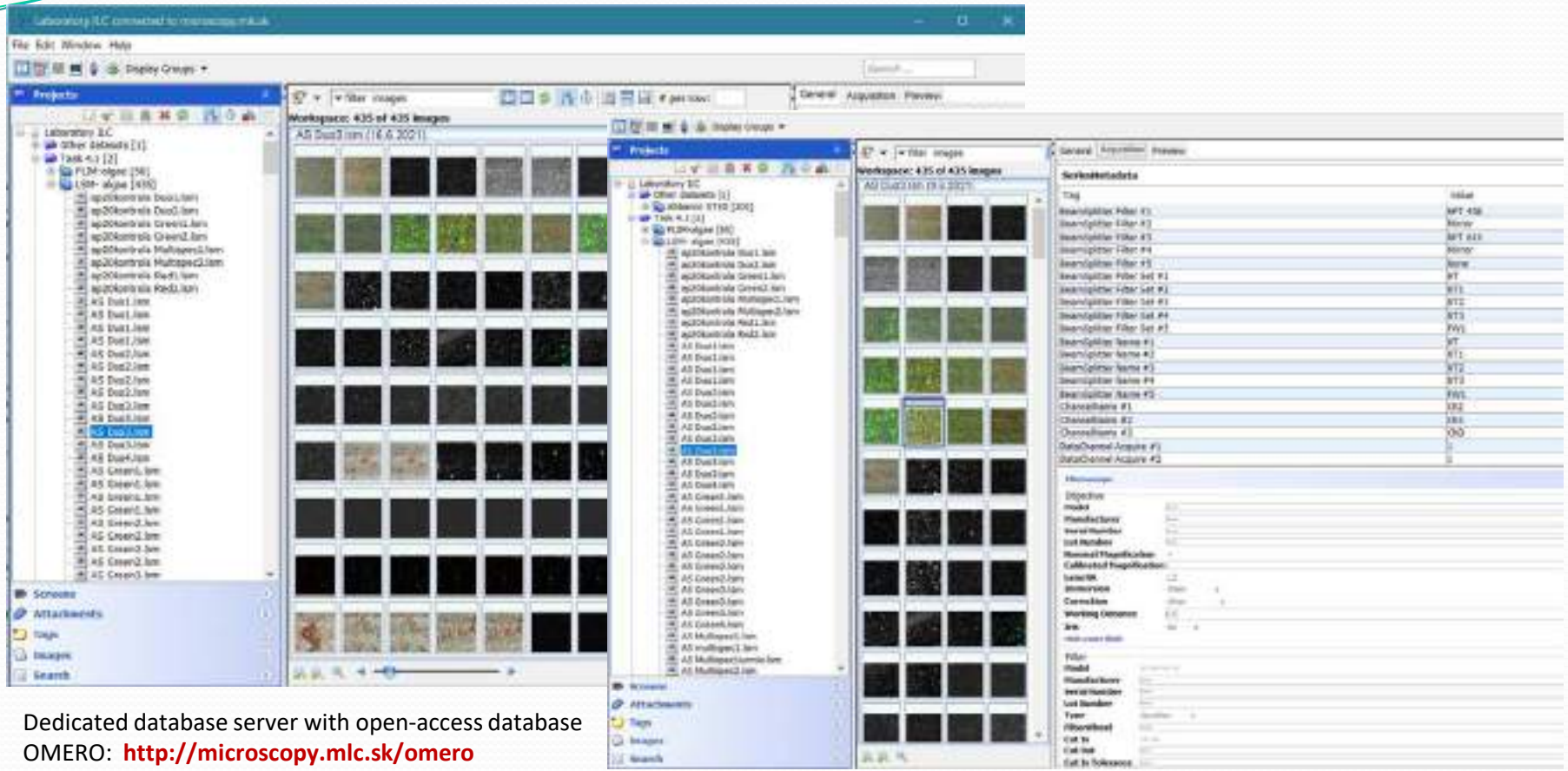


FLIM image,
spectral profiles....



Identified targets
(collagen, NADH...)

Multi-modal image databases



Dedicated database server with open-access database OMERO: **http://microscopy.mlc.sk/omero**

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