# Kierunki rozwoju optycznych metod obrazowania superrozdzielczego

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**European Young Investigator Award (EURYI)**

CENTER OF QUANTUM OPTICS

# Optyka w Biologii i medycynie - wyzwania

**Biopsja optyczna**

- Obrazowanie na poziomie molekularnym (białkowym)
- Obserwacja dynamiki wirusów i bakterii
- Wysokorozdzielcze obrazowanie przyżyciowe w czasie rzeczywistym oparte na własnym mechaniźmie kontrastującym (rozdzieczość komórkowa dla znacznych objętości)

**Czujniki biologiczne (Biochips)**

- POCT Devices (Optofluidic Lab on a Chip)
- Wyspecjalizowane próbniki nanocząsteczkowe
- Cytometria przepływowa in vivo (Raman)
- Osobisty monitoring stanu zdrowia
- Szybkie czytniki macierzowe do genomiki

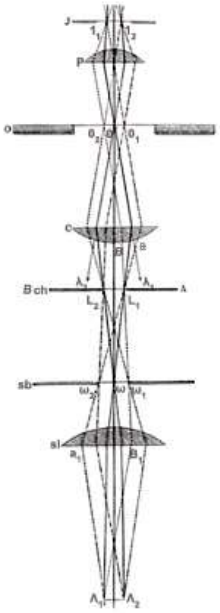
**Diagnostyka kliniczna/ terapie**

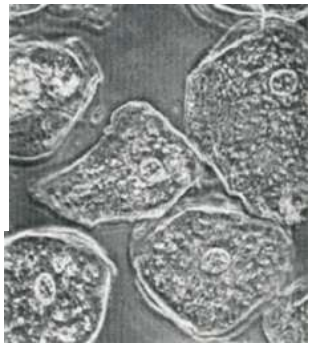
- Farmakokinetyka w czasie rzeczywistym
- Biodozymetry (promieniowanie, ekspozycja na wirusy, bakterie)
- monitoring po terapiach
- diagnostyka POCT
- Obrazowo sterowana mikro/nano chirurgia
- Nieinwazyjne terapie nowotworów
- Monitorowanie i identyfikacja komórek macierzystych
- Zdalne nano-kliniki

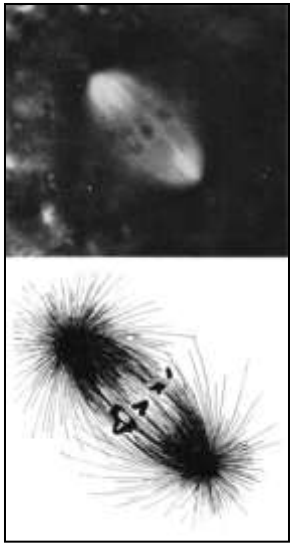
*POCT –point-of-care testing*

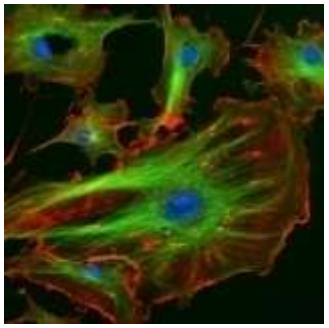
# Rozwój mikroskopii

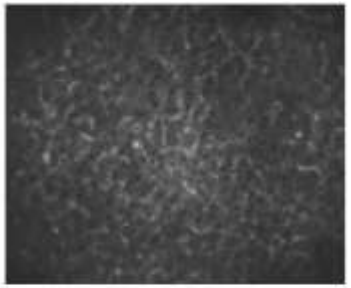
1595 → 1871 → 1873 → 1911 → 1935 → 1939 → 1942 → 1961

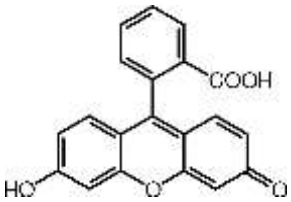












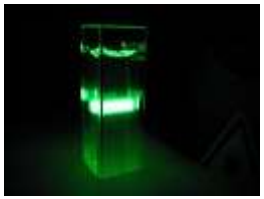
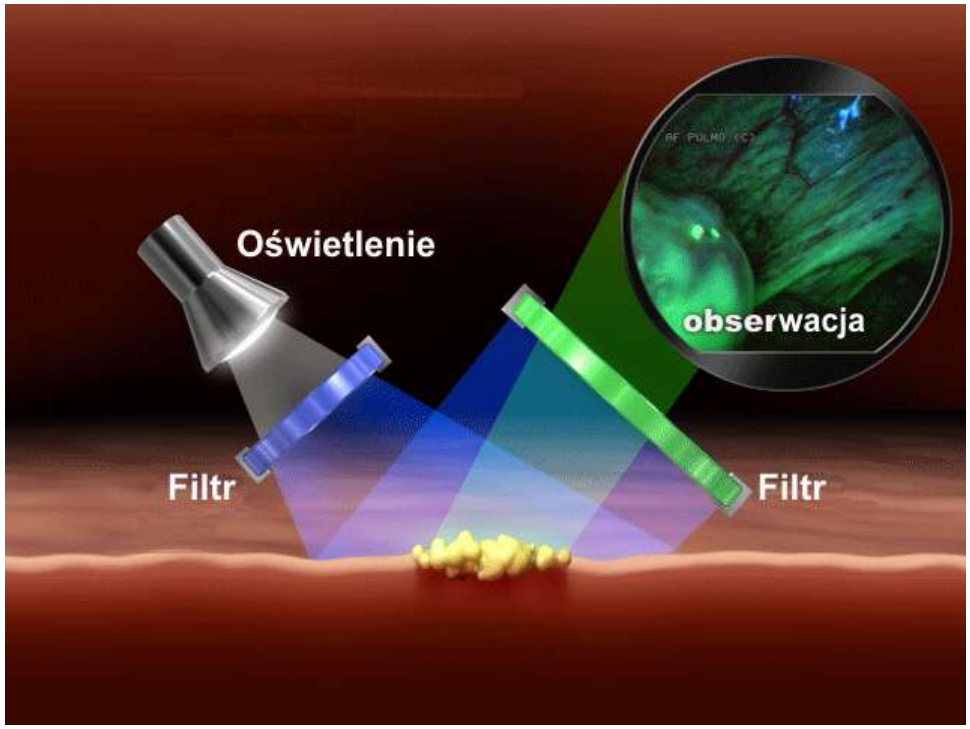



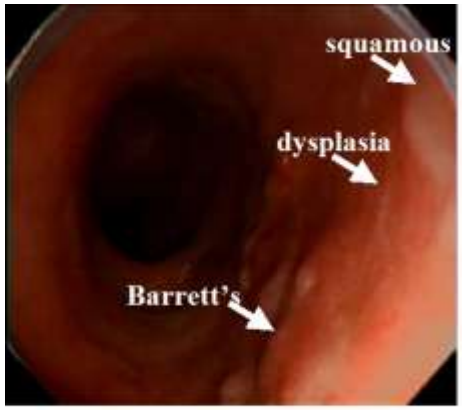
Fig. 1. Photomicrograph through Fluorescein-Microscope of the Liver of a Mouse Mounted with Pneumococcal 3 Injection.

The antigen in the liver stained with fluorescein-carbamido-antipneumococcal 3 rabbit serum. Black areas are blood vessels; grey areas are the blue-grey fluorescence of formalin-fixed hepatic cells; white areas and fine white lines represent the green fluorescence of fluorescein-antibody and indicate the location of pneumococcal 3 antigenic material. 20 × apochromatic Zeiss objective, 10 × ocular; in the ocular Wratten filters ✱2A and ✱8 (K2.). Leitz 1 × microphotographic apparatus. Eastman Ilford Super Ortho Press. Exposure: 20 minutes.

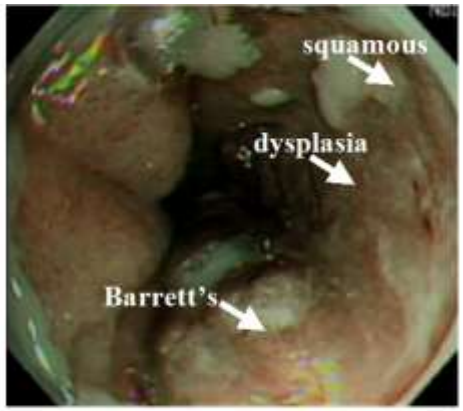
www.nature.com/milestones/light-microscopy

# Poprawa kontrastu w obrazowaniu

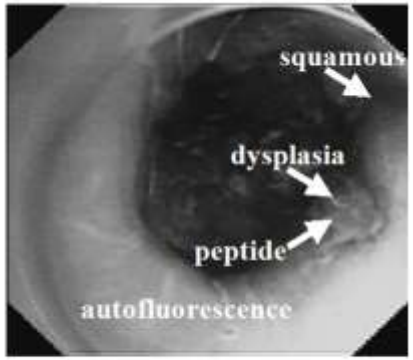



white light



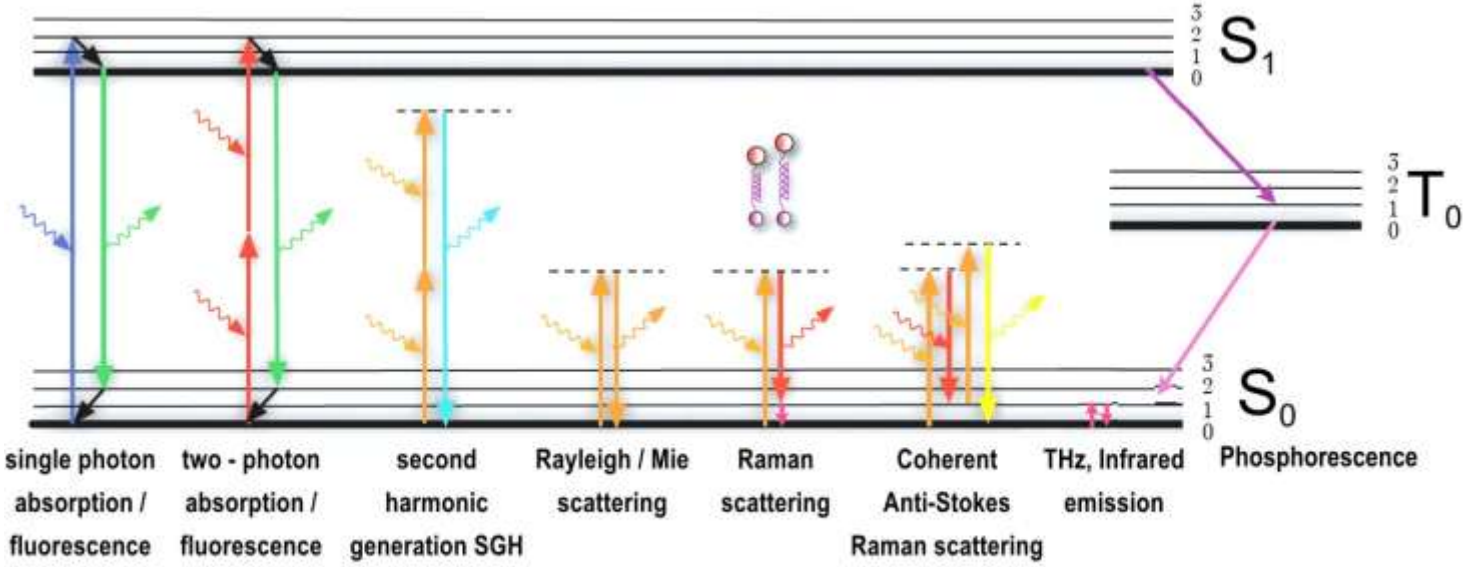

narrow band




fluorescence

# Procesy luminescencji i rozpraszania

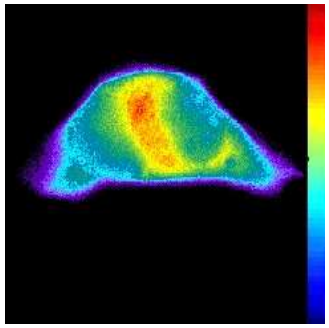
## Diagram Jabłońskiego

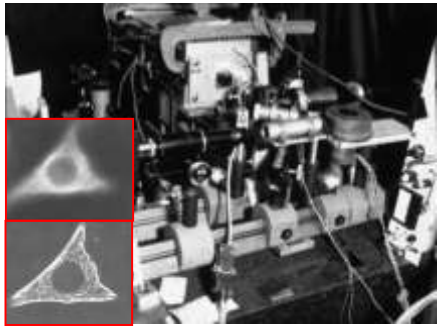


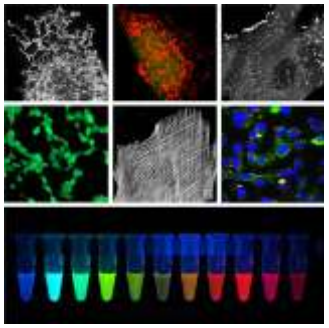

**Aleksander Jabłoński**
**(26 .02.1898 – 09.09.1980 ) , Toruń**
***Efficiency of Anti - Stokes Fluorescence in Dyes***
**(published in 1933, *Nature*)**

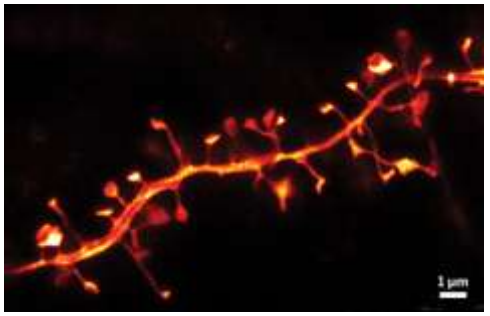
# Od mikroskopii do nanoskopii

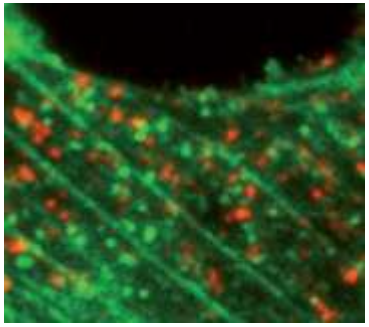
1976 1981 1987 1990 1994 2000 2006

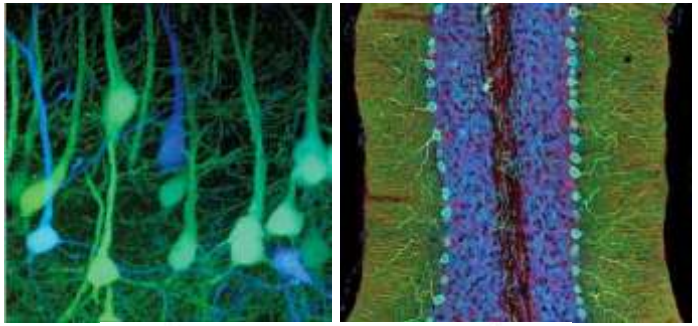


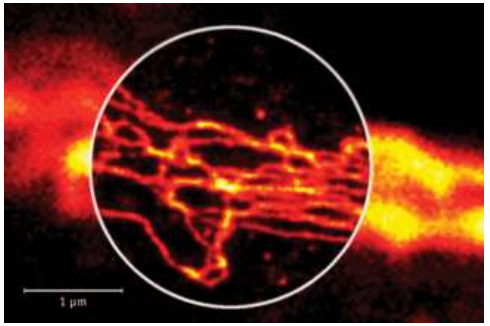


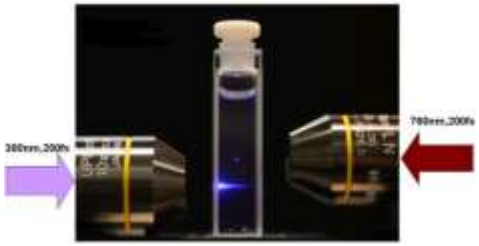


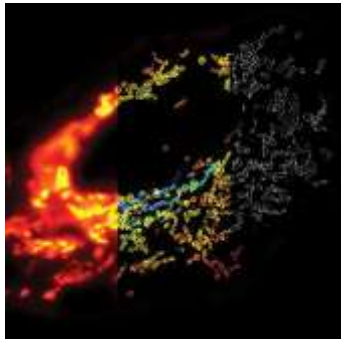






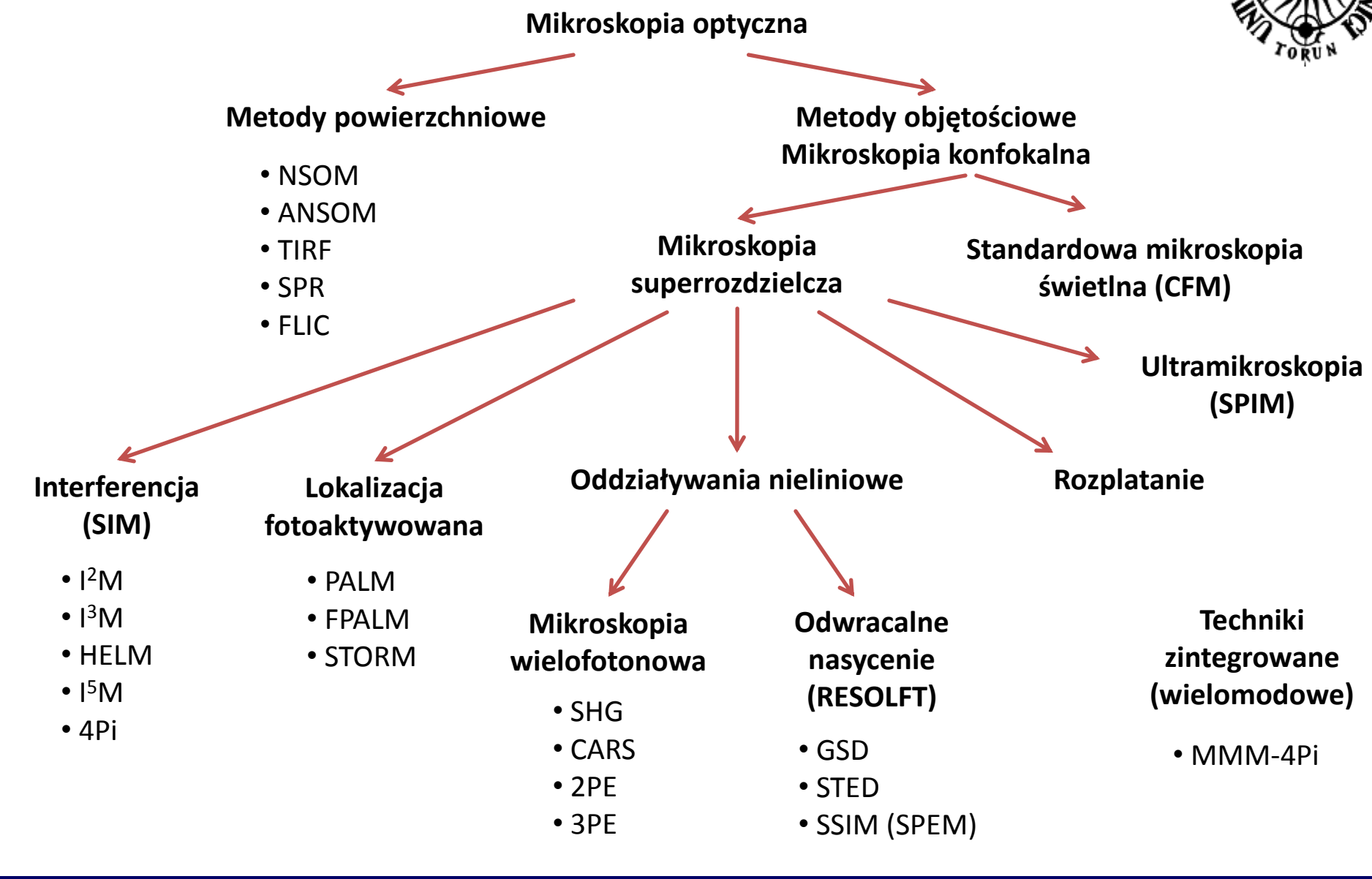






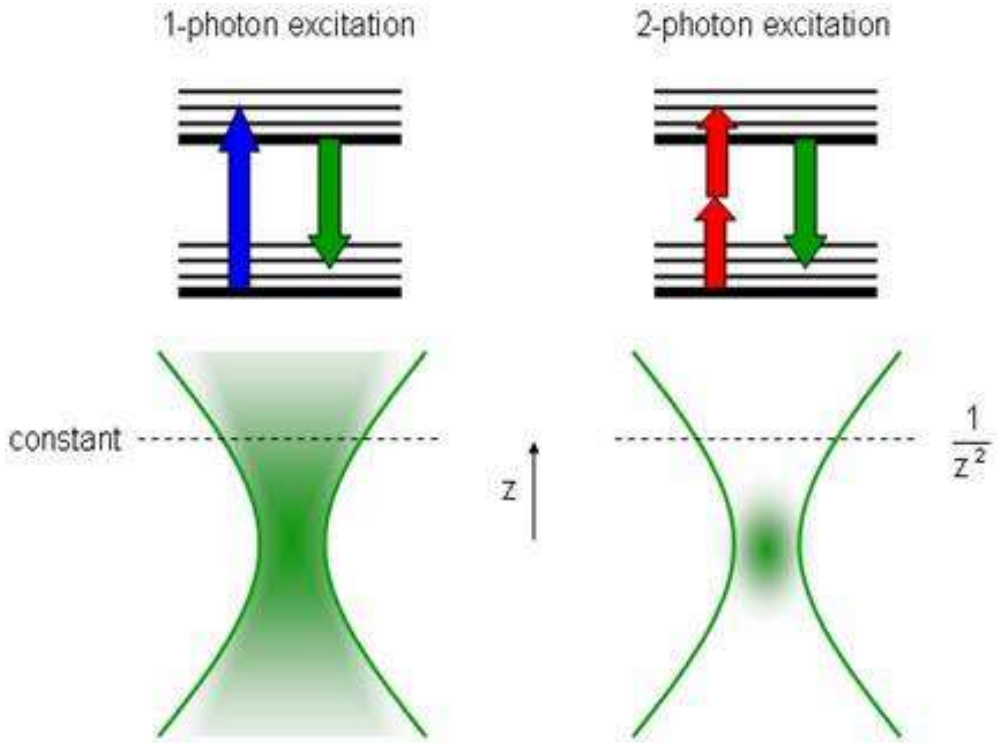
www.nature.com/milestones/light-microscopy

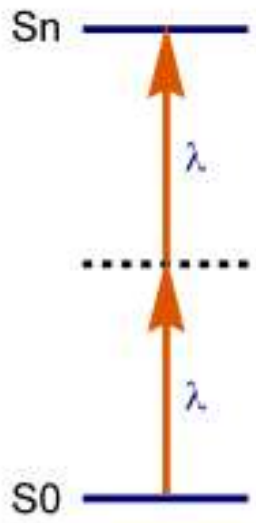
# Współczesne techniki mikroskopowe

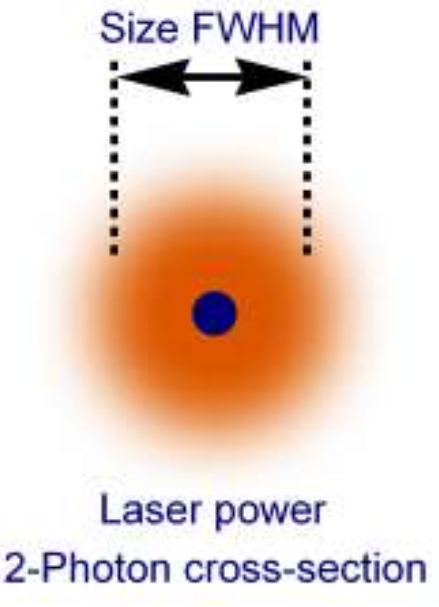


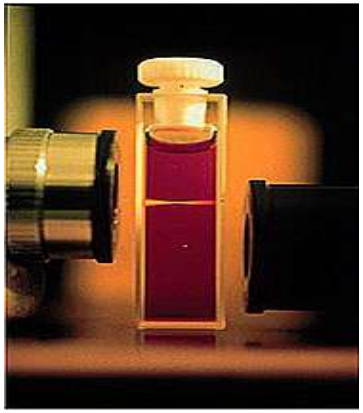
# Wzbudzenie jedno i dwufotonowe

## Automatyczna poprawa rozdzielczości



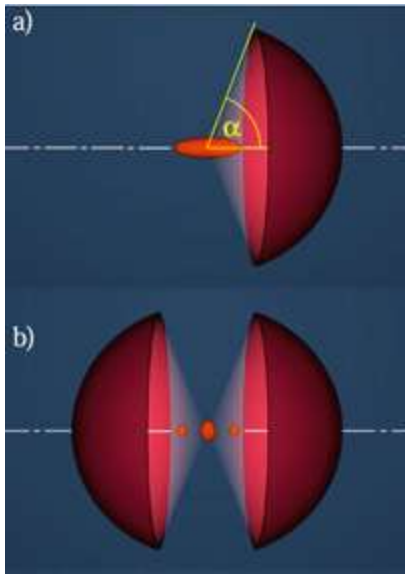



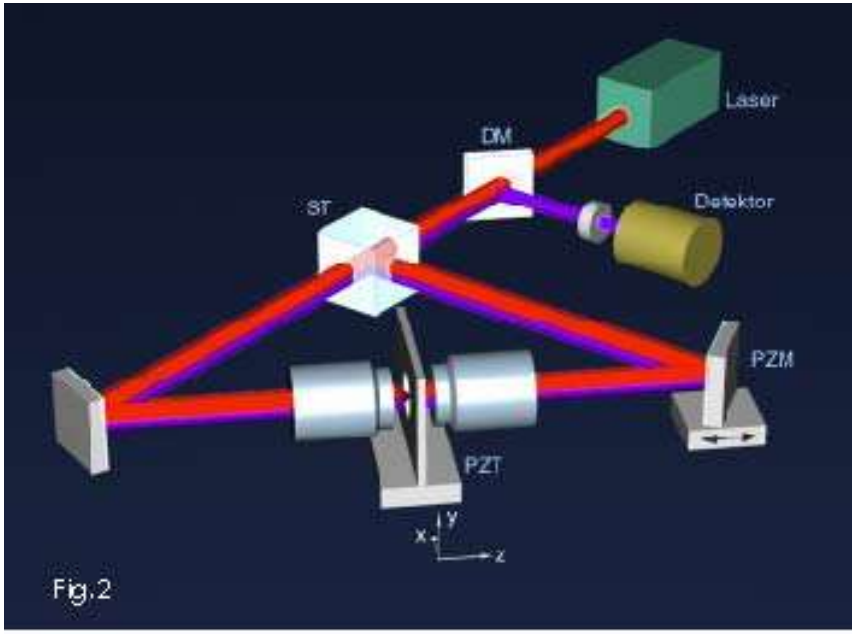
**Wzbudzenie dwufotonowe ~ $I^2$**

# Mikroskopia 4Pi

Objętość wzbudzenia w ognisku



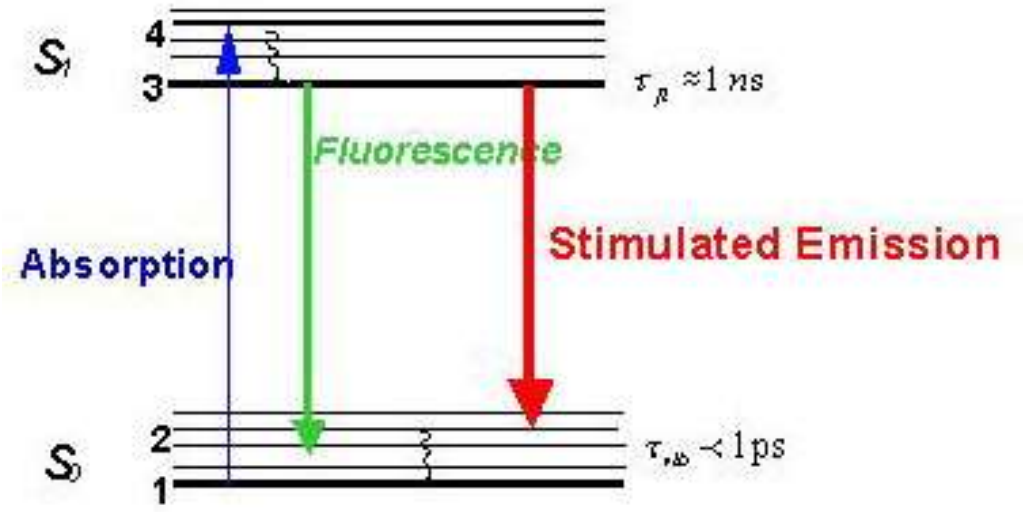

Rozdzielenie światła na dwa obiektywy

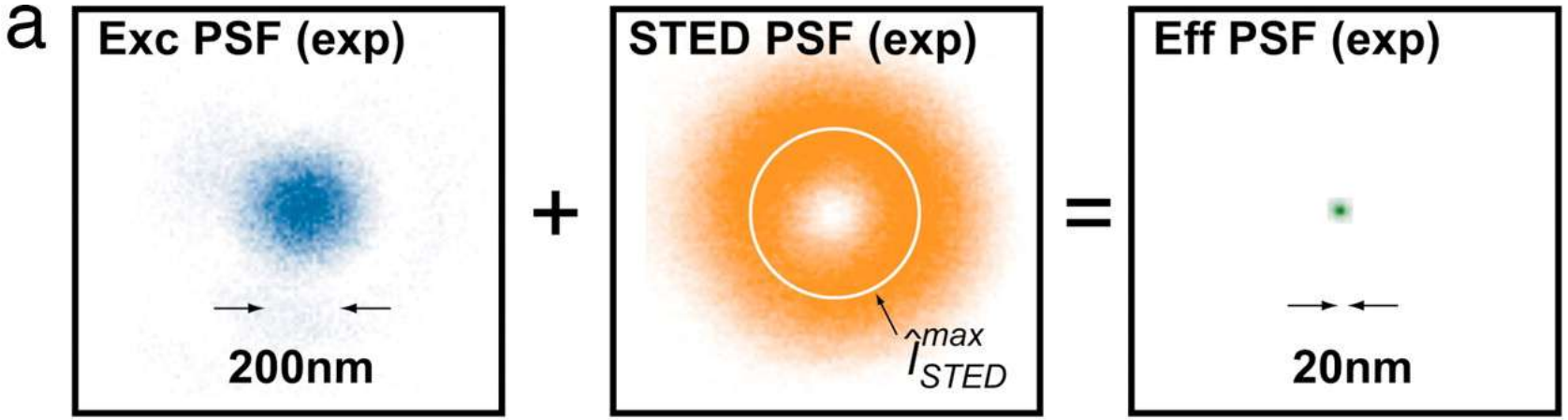



Światło wzbudzające interferuje konstruktywnie w płaszczyźnie ogniskowej tworząc mniejszą plamkę

-Oczekiwana wartość zwiększenia rozdzielczości: 5-7 razy
- Poprawa rozdzielczości w skutek zmniejszenia obszaru wzbudzenia
- Względnie większa apertura numeryczna w układzie zbierającym światło
- optymalnie działa z dwufotonowym wzbudzeniem

# Kształtowanie rozdzielczości - STED




http://www.mpibpc.gwdg.de/groups/hell/

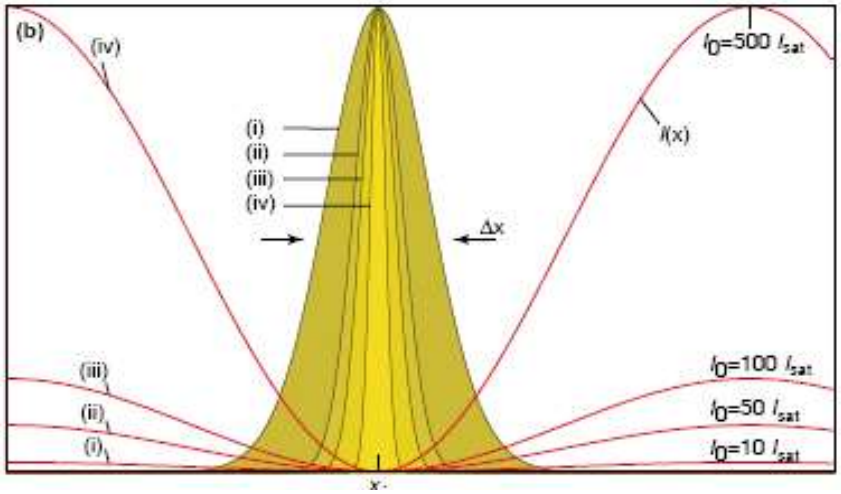
# Mikroskopia STED poza limitem dyfrakcyjnym

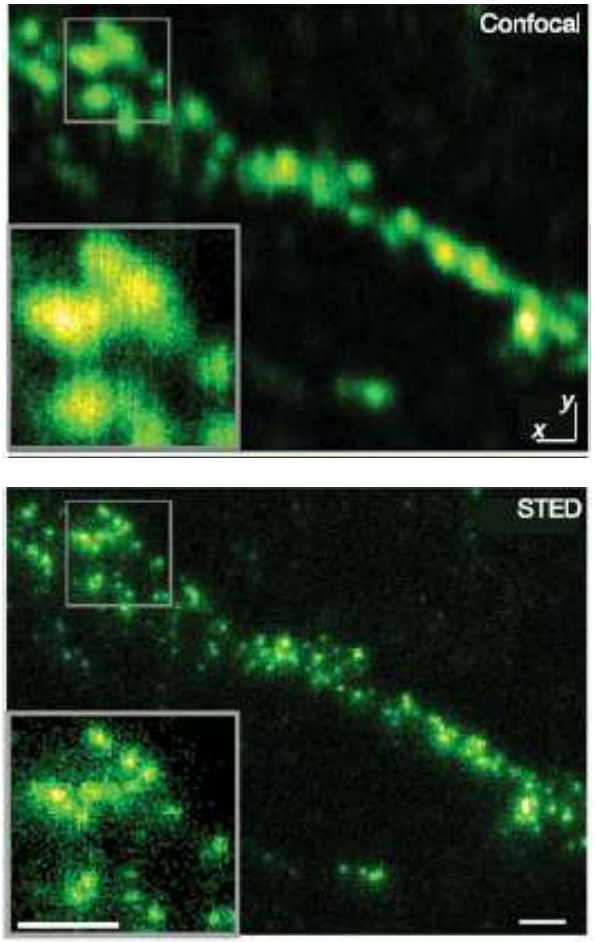
*Confocal*

$$\Delta d \approx \frac{\lambda}{2n\sin\alpha}$$

*STED (Resolft)*

$$\Delta d \approx \frac{\lambda}{2n\sin\alpha\sqrt{1+I/I_{sat}}}$$



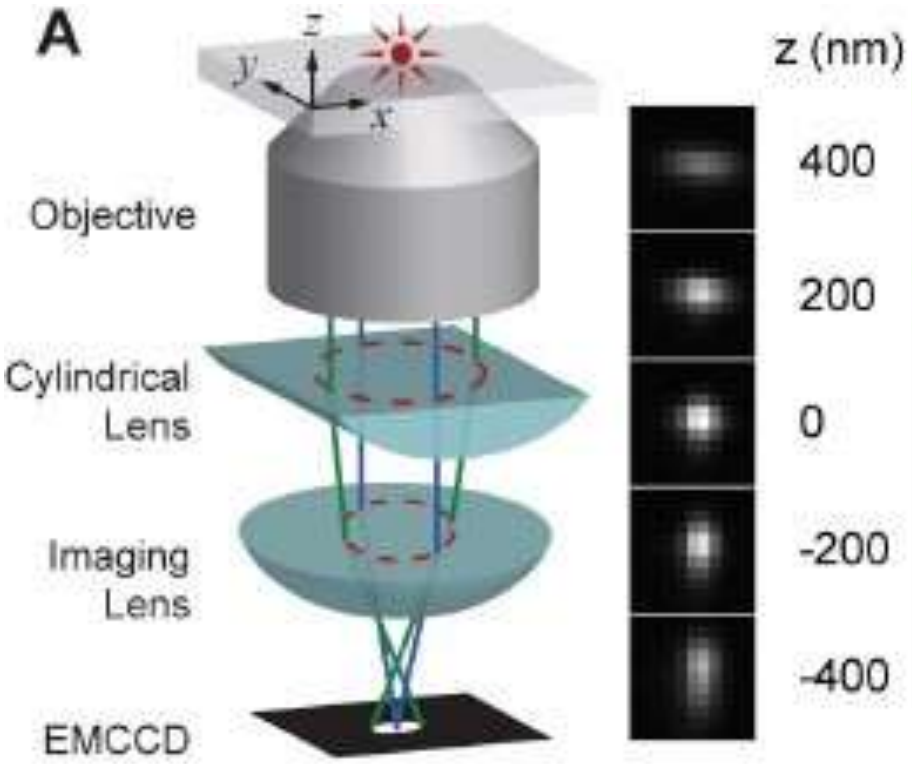



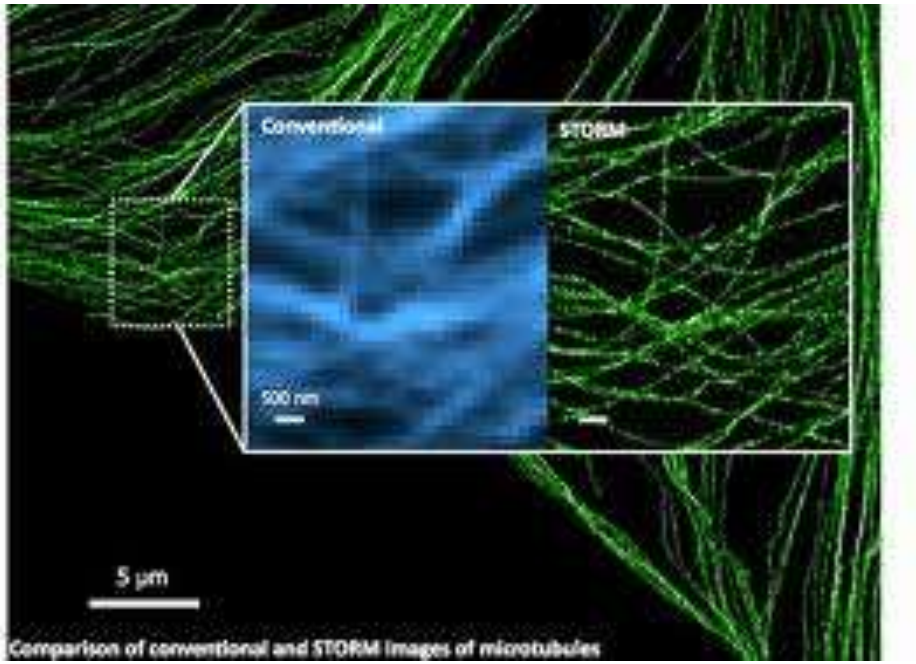

500 nm

Willig, K. I. et al. (2006): Nature 440 (7086): 935-939.

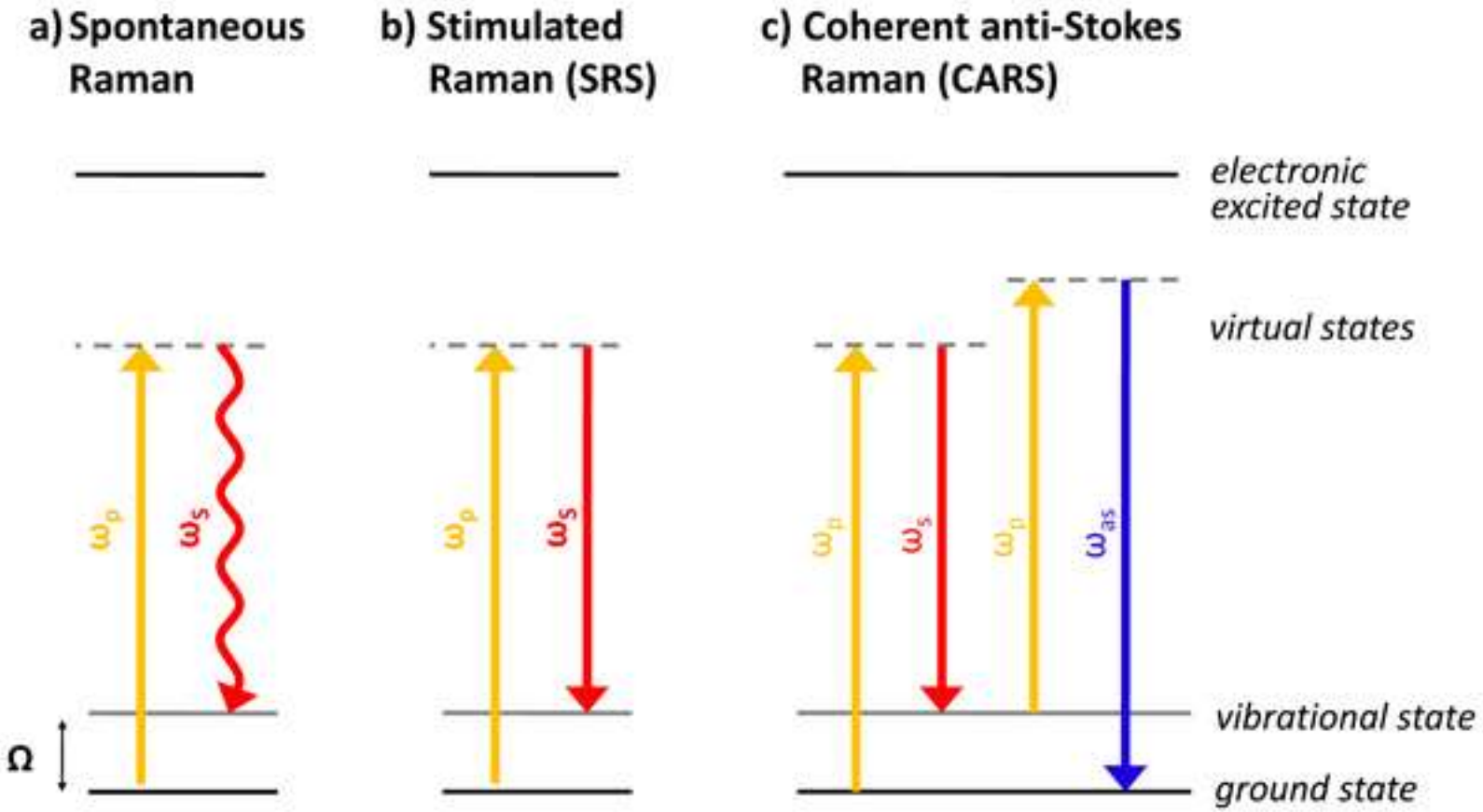
# Mikroskopia PALM / STORM

**Stochastic optical reconstruction microscopy (STORM)**
**photo-activated localization microscopy (PALM)**

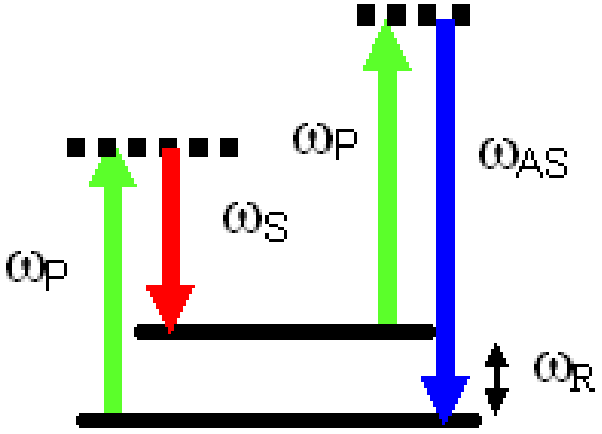


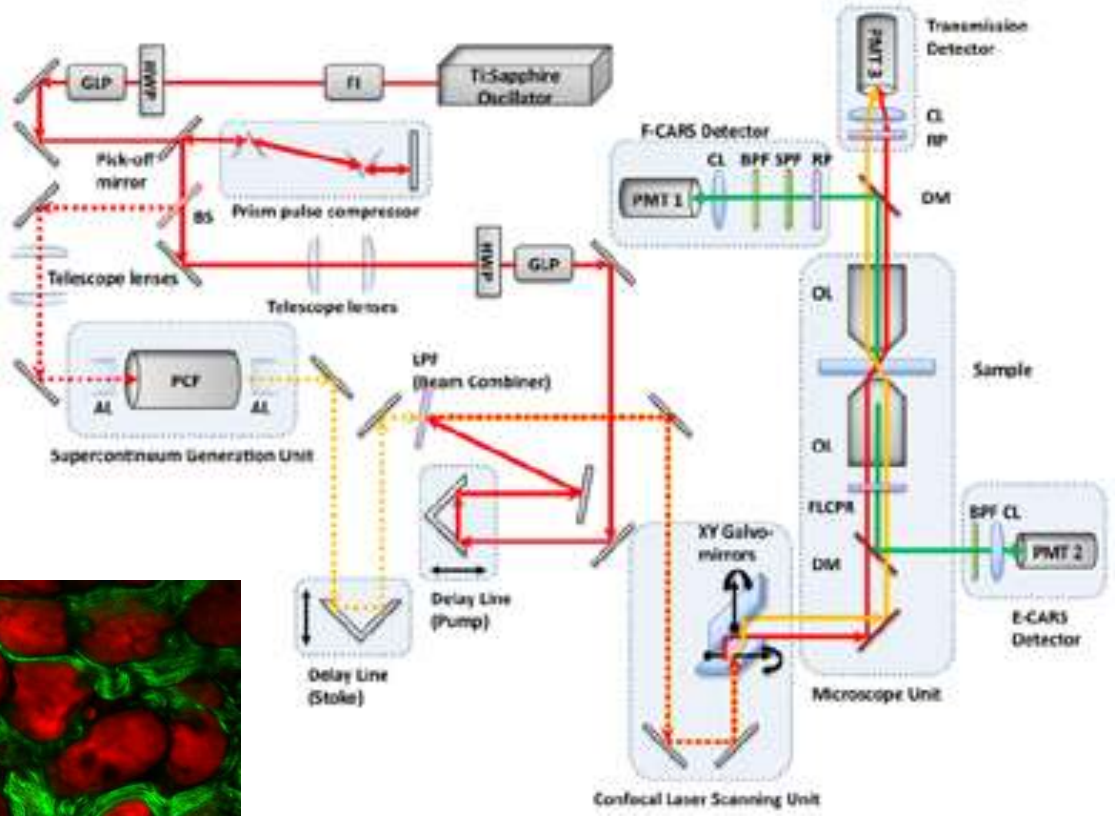
# Intrinsic molecular Contrast -CARS



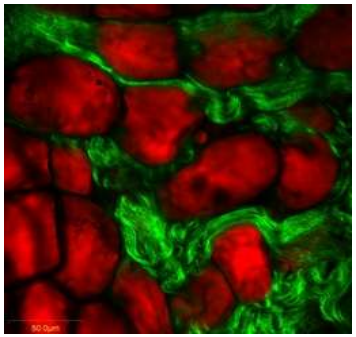
# Intrinsic molecular Contrast -CARS

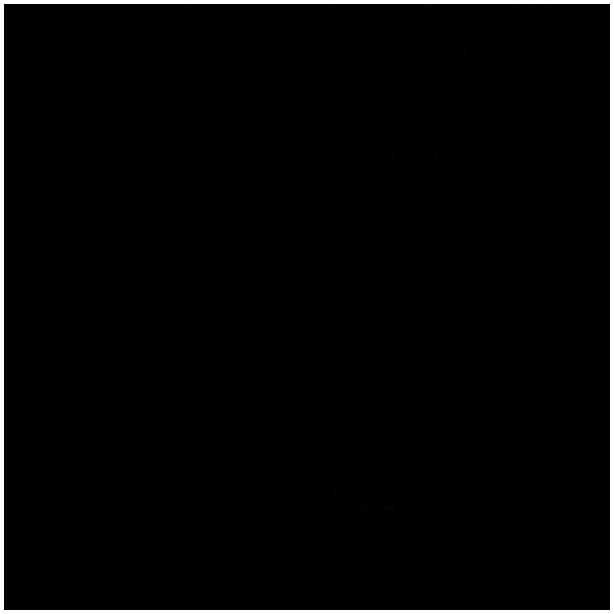



$\omega_R$–vibrational molecular energy




**Komórki tłuszczowe (czerwone )-CARS**
**Włókna kolagenowe (zielone) -SGH**

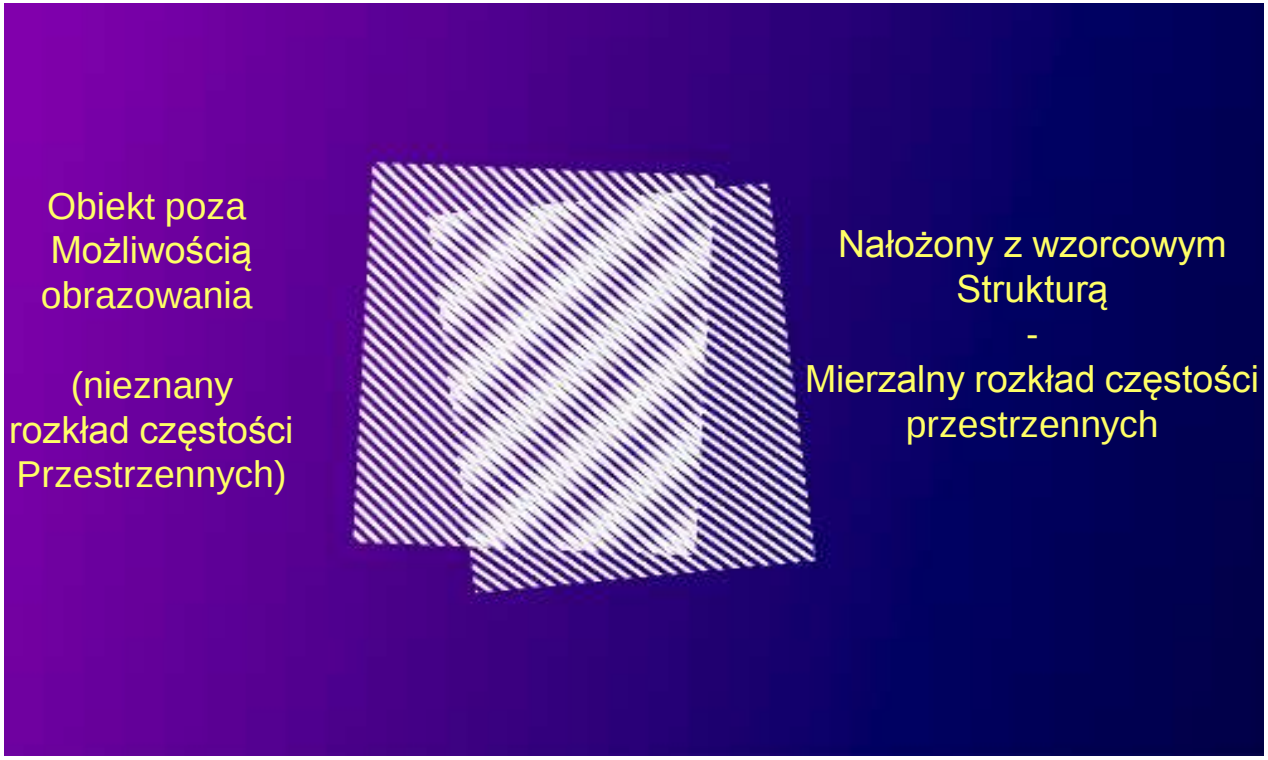




**SRS tissue imaging of fresh mouse skin.** For the acquisition of this image stack in mouse ear, SRL image contrast was tuned into the CH2-stretching vibration. As such, lipid rich structures of the skin were highlighted. From top (beginning of the movie) to bottom (end of the movie):
Polygonal intercellular space of the stratum corneum
Viable epidermis with hair follicle
Sebaceous gland
The image stack was taken on fresh tissue, without any preparation or labeling.

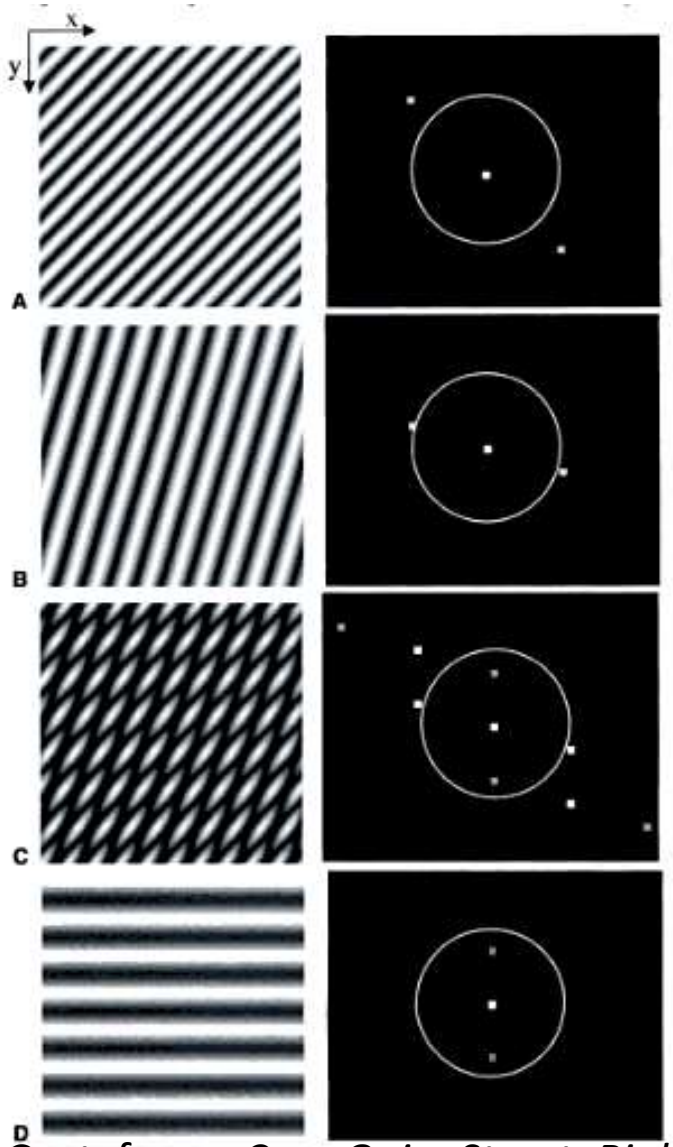
# Mikroskopia z oświetleniem strukturalnym

## Structured illumination microscopy
## idea: prążki Moiré

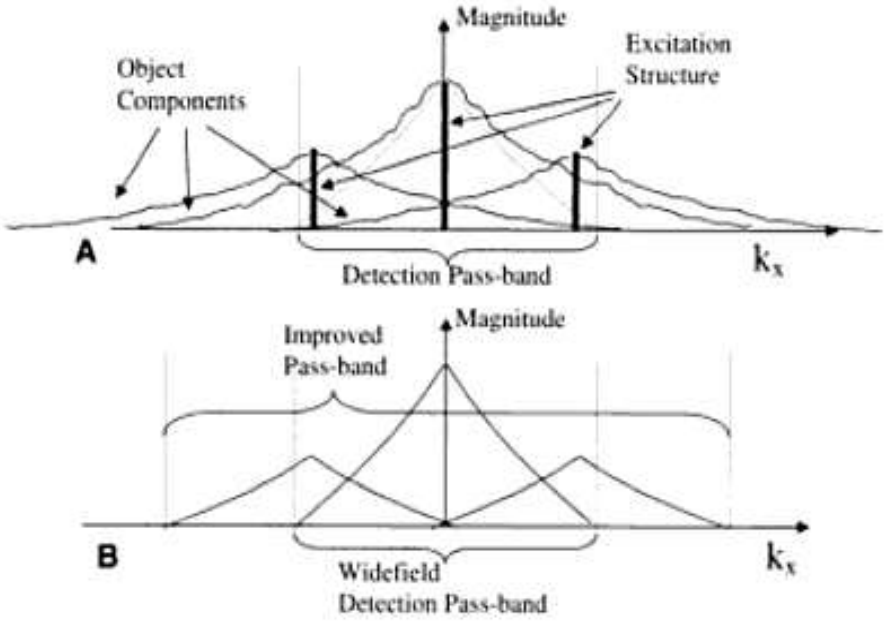



M. Gustafsson, *Curr. Opin. Struct. Biol.* **9**, 627-634 (1999)

# Oświetlenie prążkami



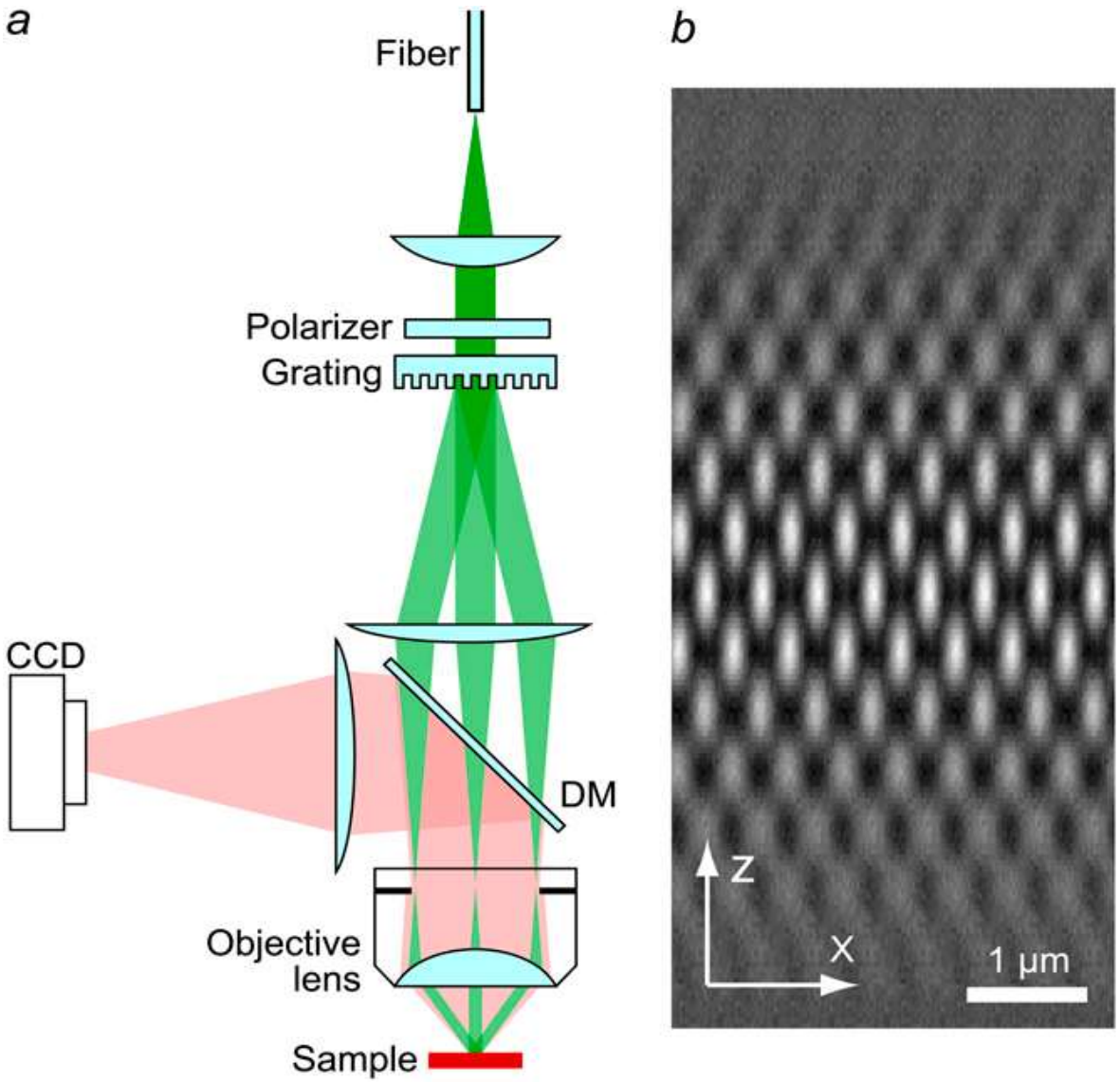

**przesunięcie pasma częstości przestrzennych obiektów względem układu detekcji**





M. Gustafsson, *Curr. Opin. Struct. Biol.* **9**, 627-634 (1999)

# 3-D Sectioning




M. Gustafsson, *Curr. Opin. Struct. Biol.* **9**, 627-634 (1999)

# New ways of light detection

Confocal microscopy with structured illumination


A detailed 3D image of the nucleus of a mouse cell. The picture was captured using a new imaging microscope technique called three-dimensional structured illumination, described in the journal Science.

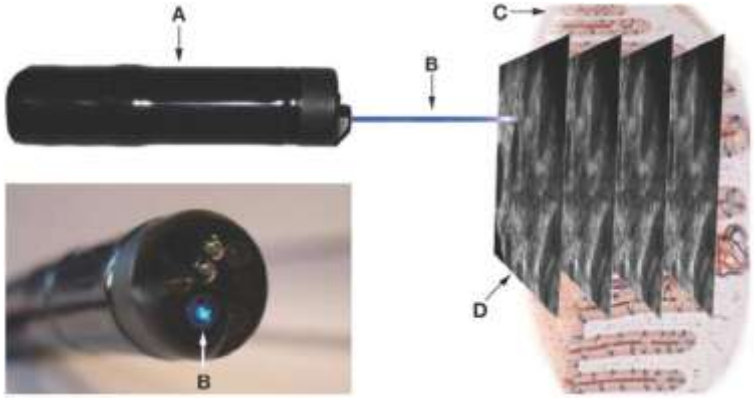
**Nucleus of Mouse cell**

# Obrazowanie przyżyciowe - *in vivo*

| | Mikroskop optyczny | Mikroskop konfokalny (CFM) | Mikroskop Fluo-rescencyjny | Oświetlenie strukturalne | Mikroskop Ramanowski (CARS, SARS) |
|---|---|---|---|---|---|
| Zdolność wyr. pojedynczej warstwy | ✗ ----- | ✓ średnio | ✓ b. dobrze (STED) | ✓ b. dobrze | ✓ średnio |
| Bezpieczeństwo (moc prom. na próbkę) | ✓ b. dobrze | ✓ w normach | ✗ ----- | ✓ słabo | ✗ ----- |
| Czas pomiaru | ✓ b. dobrze | ✓ b. dobrze | ✗ ----- | ✗ ----- | ✗ ----- |
| Mechanizm kontrastu | rozpraszanie | rozpraszanie | fluorescencja | fluo/rozpr. | Autofluoresc. |

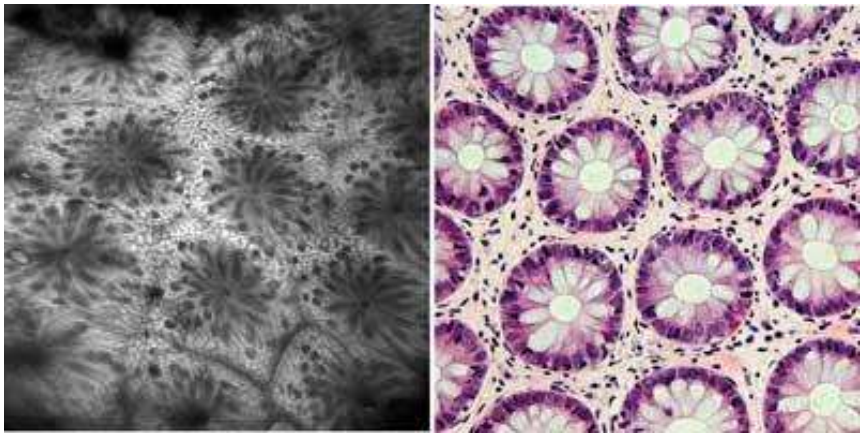
# Examples of confocal imaging

## Scanning laser microendoscopy



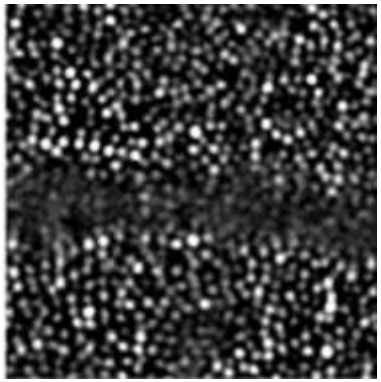

In vivo

Histology



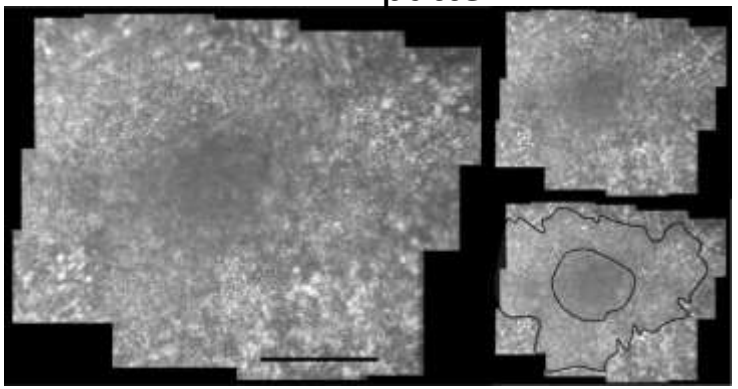
**Technology Insight: confocal laser endoscopy for *in vivo* diagnosis of colorectal cancer** R. Kiesslich, M. Goetz, M. Vieth, P.R. Galle and M.F. Neurath *Nature Clinical Practice Oncology* (2007) **4**, 480-490 / **Optiscan, Australia**

**Adaptive optics Scanning Laser Ophthalmoscopy**



Photoreceptor mosaics

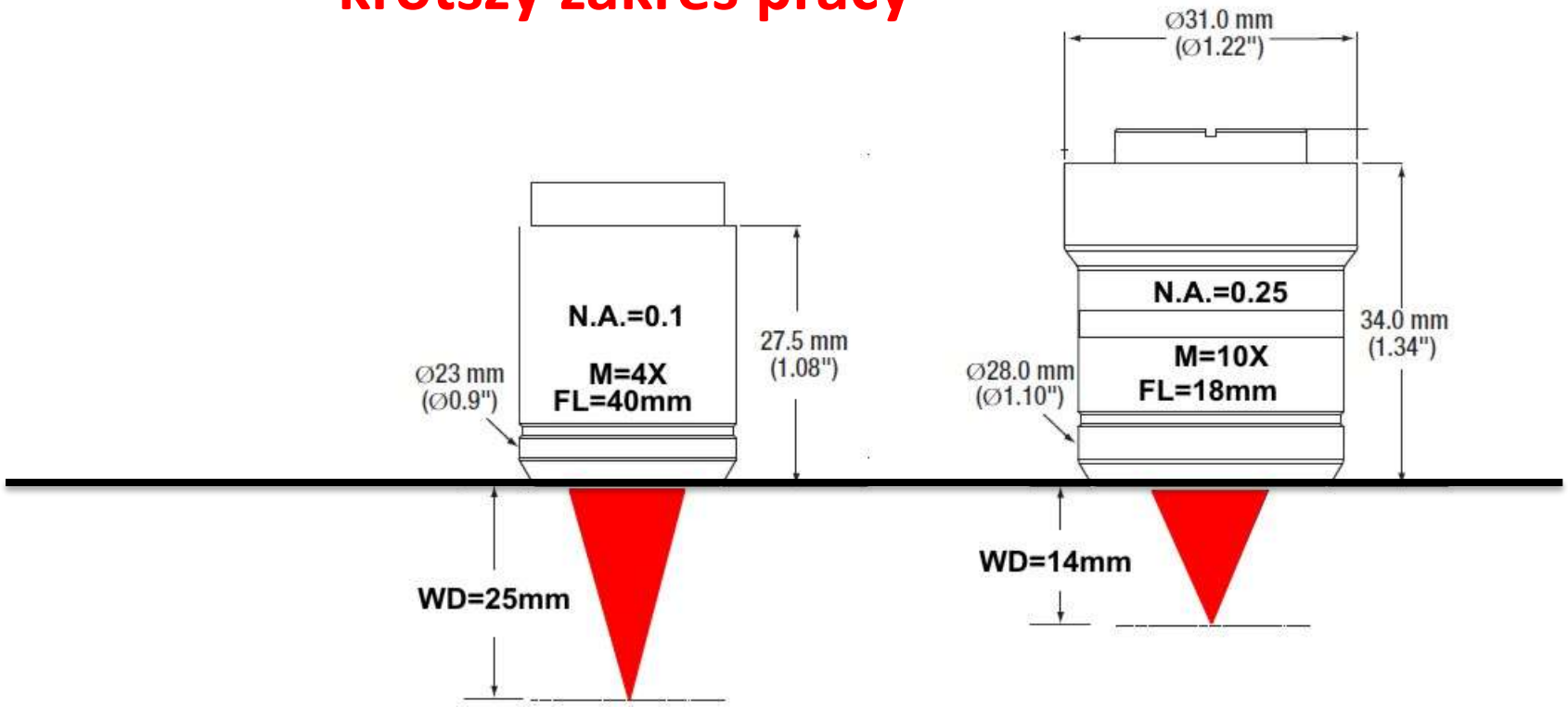
RPE pattern



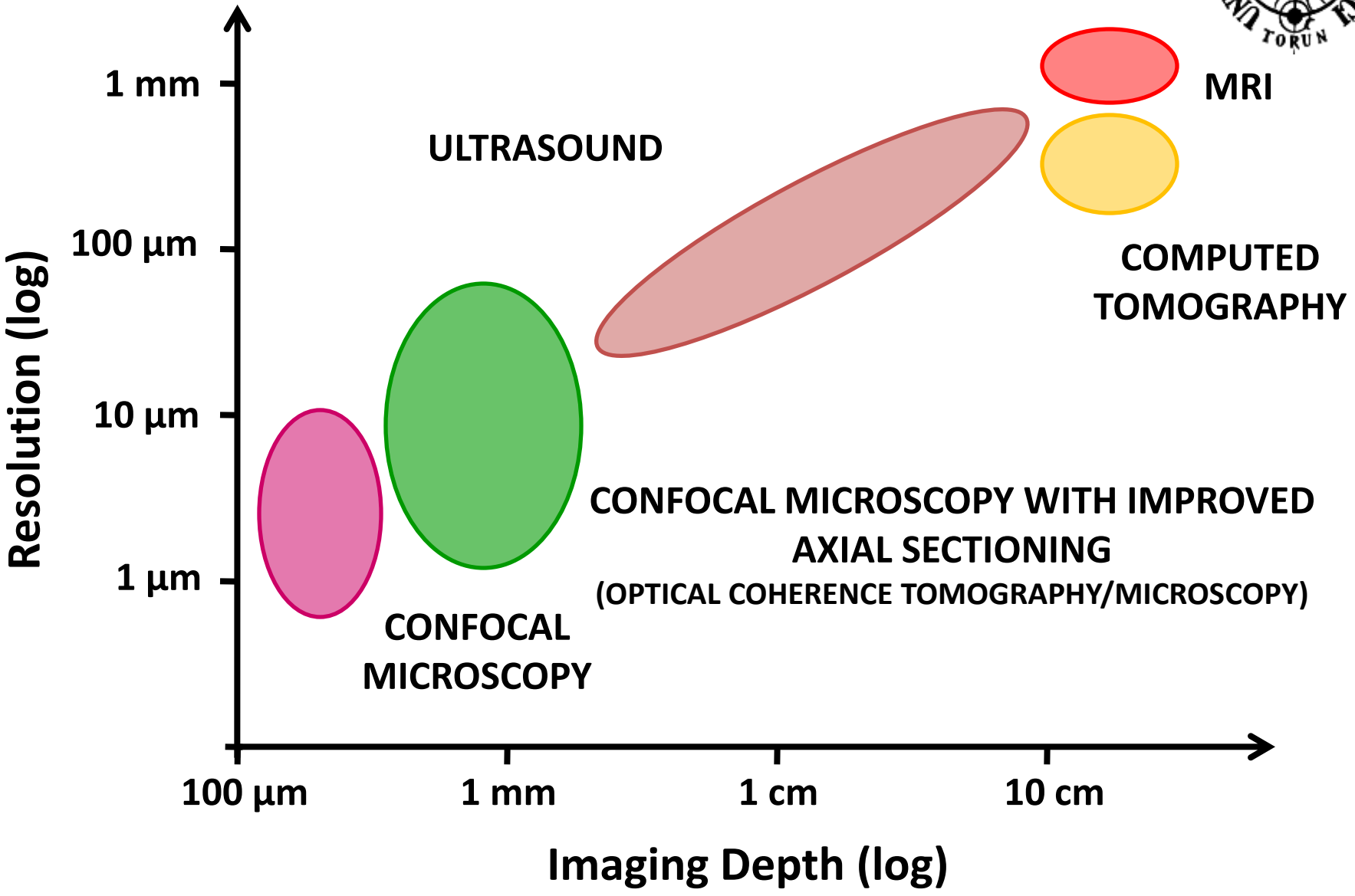
*Roorda et al.,IOVS, May 2007, Vol. 48, No. 5*

# Fundamentalne ograniczenie

**W mikroskopii konfokalnej**
**Większa apertura – lepsza rozdzielczość**
**- krótszy zakres pracy**

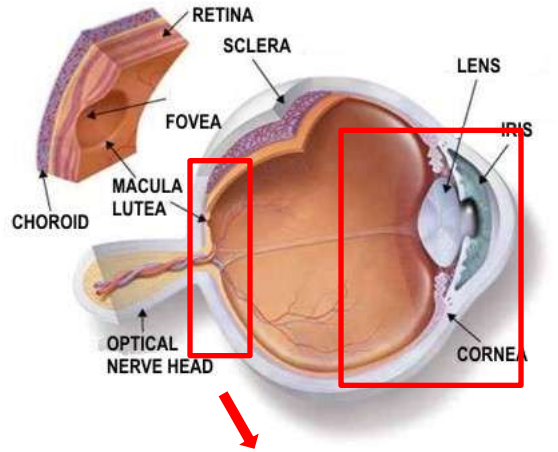


# In vivo 3-D Imaging

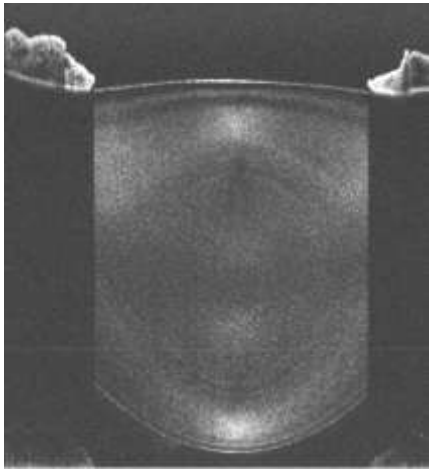


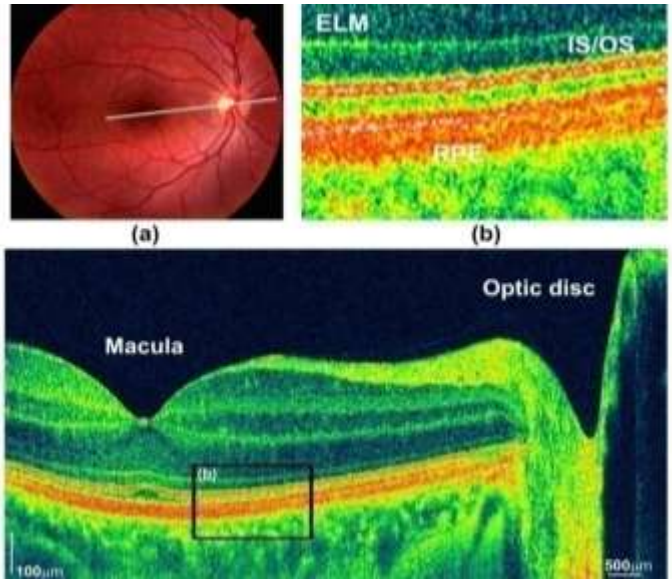
# Obrazowanie z wykorzystaniem spójności światła

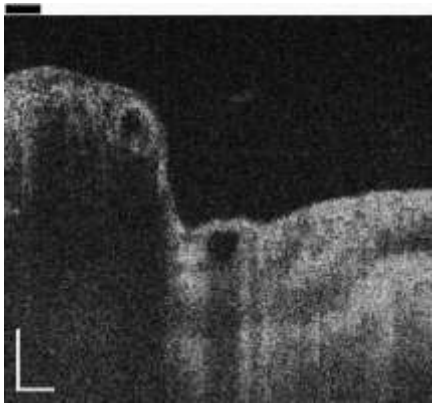
Tomografia OCT





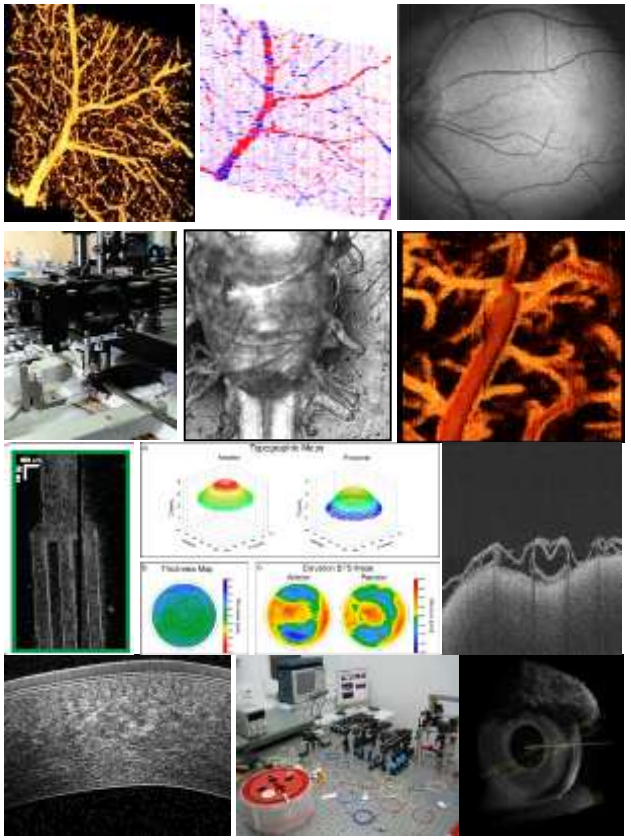




# Cel aktywności badawczej OBIGu

**Prowadzenie wysokiej jakości badań naukowych i stosowanych oraz prac wdrożeniowych w dziedzinie obrazowania biomedycznego**

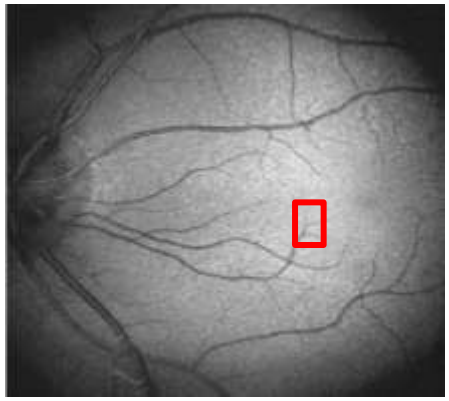


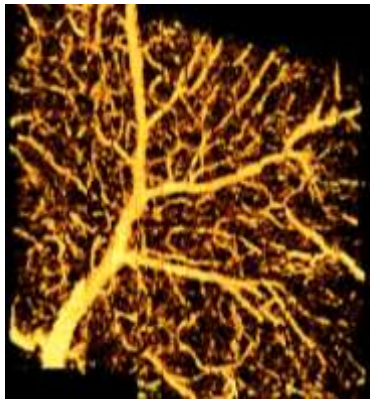
Tematyka prac:

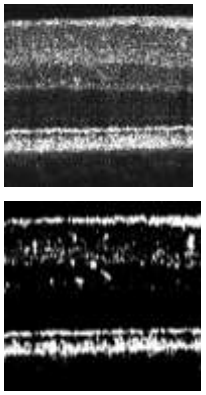
- Strukturalne i czynnościowe obrazowanie oka
- Rozwój nowych optycznych technik mikroskopowych
- Optyczne czynnościowe badania neuronalne
- Propagacja światła w ośrodkach rozpraszających
- Rozwój nowych metod analizy danych

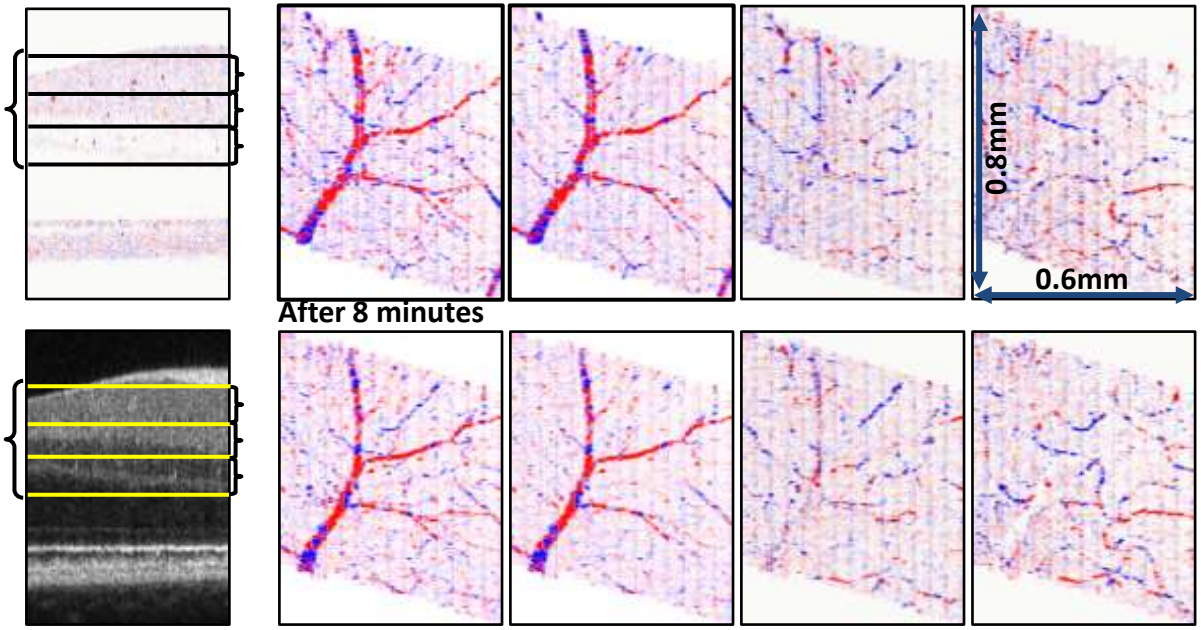
Wsparcie dla prowadzenia dydaktyki na wysokim poziomie dla różnych specjalności włączając: fizykę doświadczalną, fizykę medyczną, fizykę techniczną oraz zastosowania informatyki.

# Obrazowanie układu mikrokapilarnego siatkówki



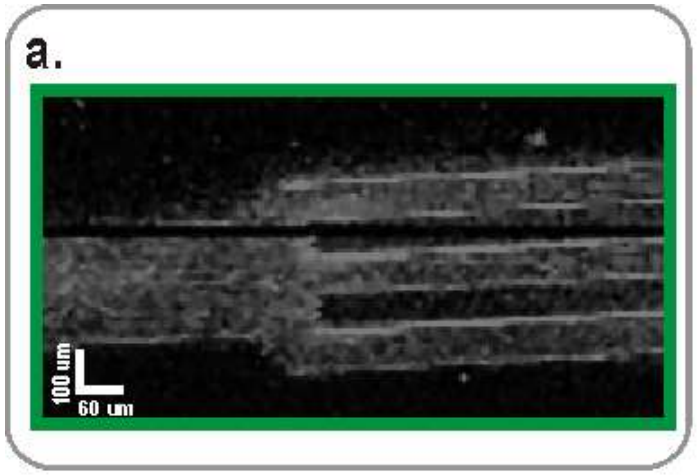


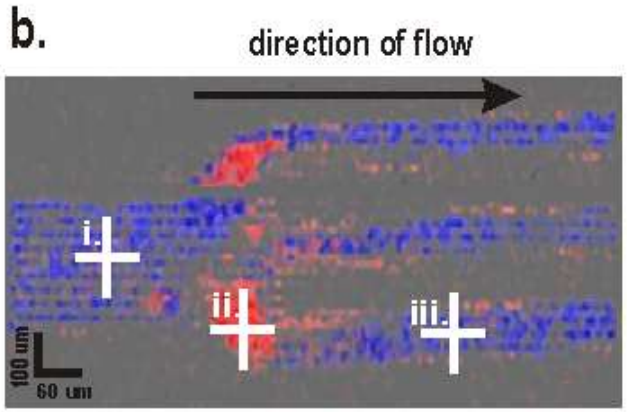


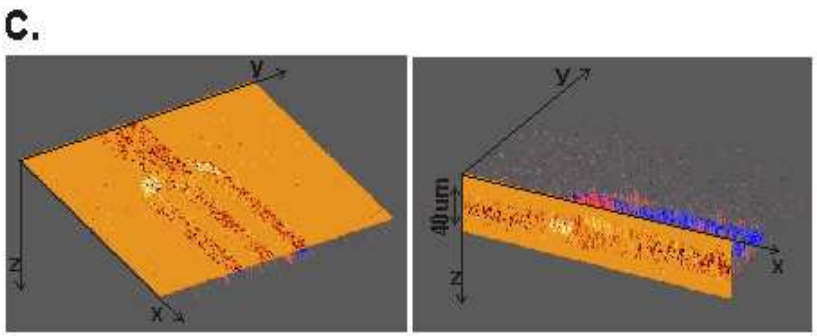


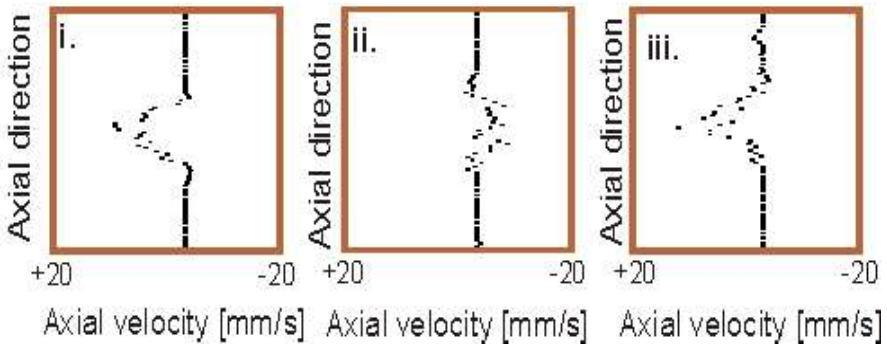
# Opto-microfluidics of blood

**Mikroskopia OCM**



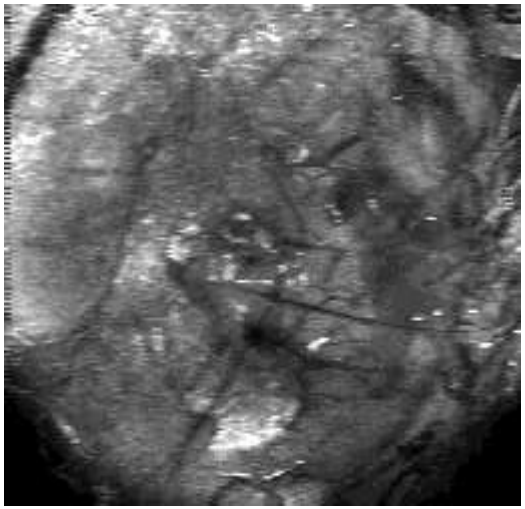



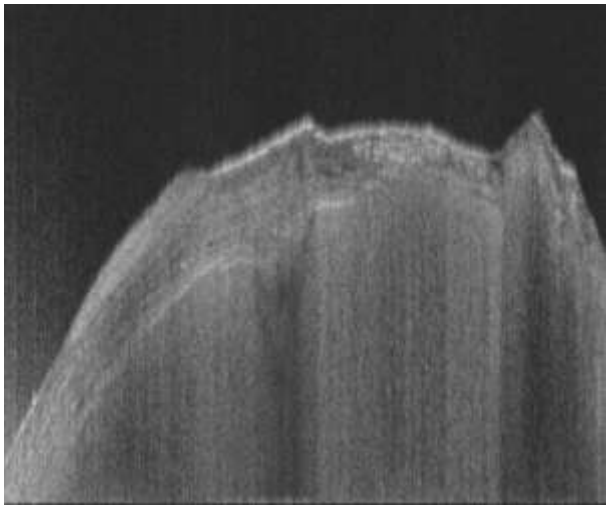



# Neuro Imaging – mouse brain

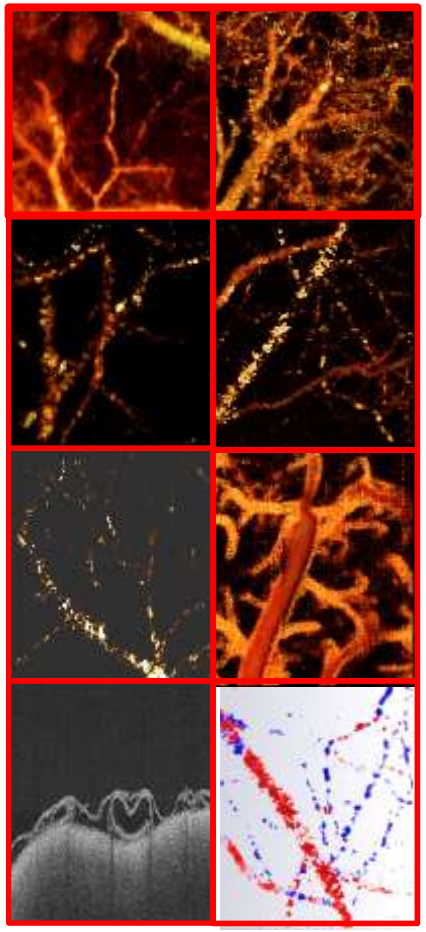
**En –face OCT image**



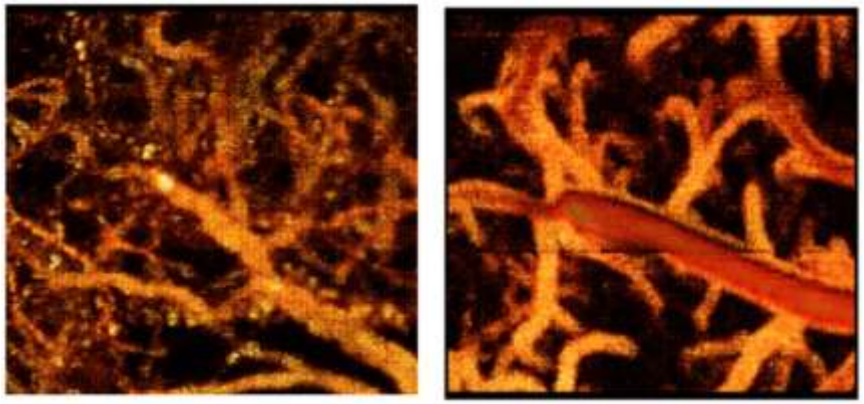
**Cross-srctional OCT image**



instytut biologii doświadczalnej im. M. Nenckiego PAN

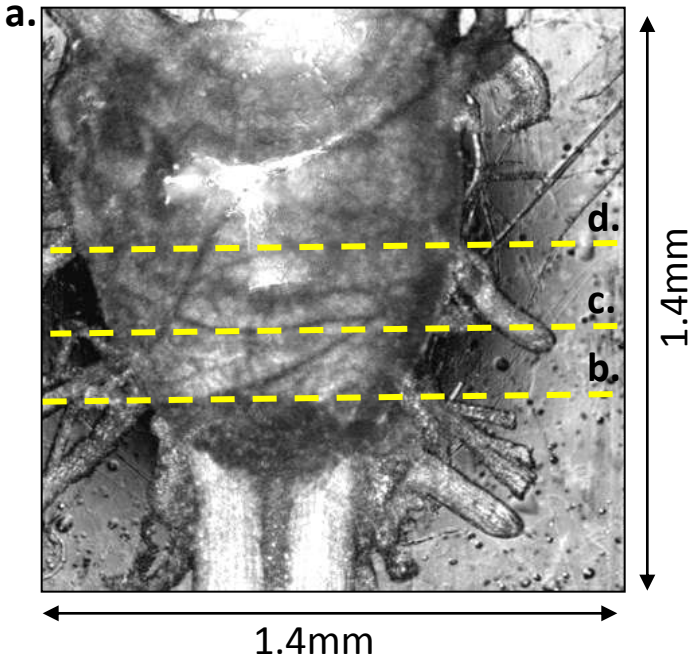


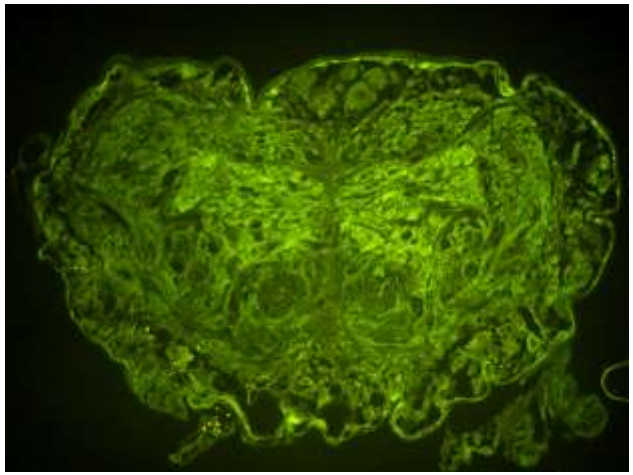
**Functional OCT –visualization of mouse brain vasculature**



# OCT imaging of american cockroach nervous system

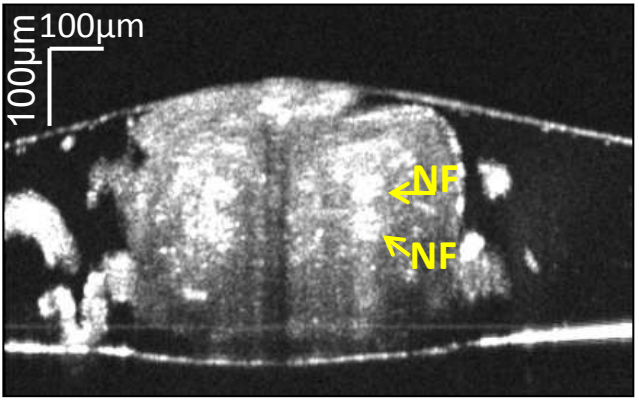
Mikroskopia OCM



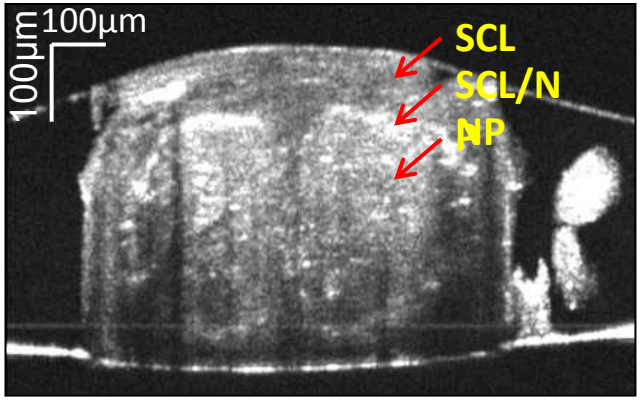



Histology section - formaldehyde induced fluorescence of a cockoach terminal abdominal ganglion slice.

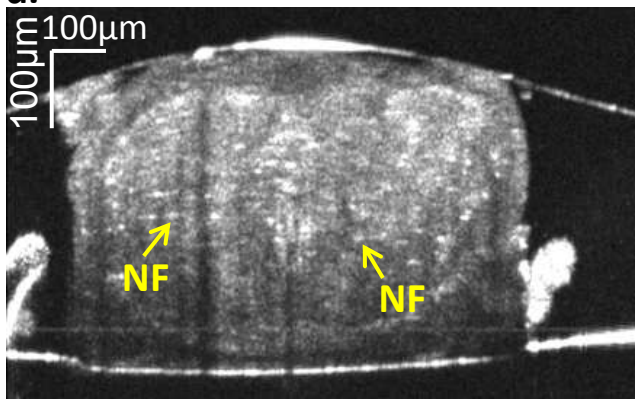
b.




c.




d.



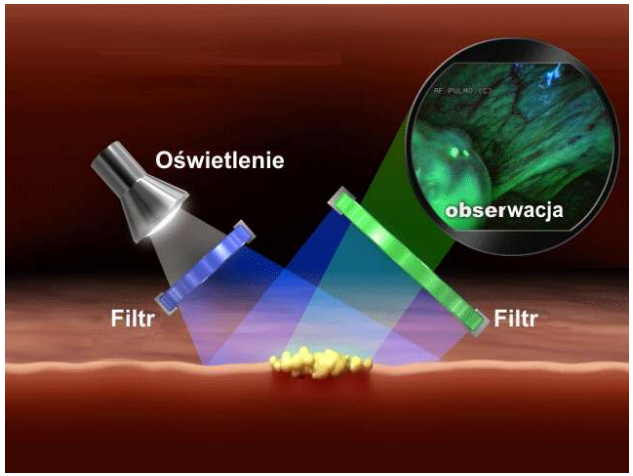
# Plany badawcze OBIGu

**mechanizmy kontrastu** + **metody detekcji**

**badania podstawowe grant NCN** **badania stosowane TEAM**

1. Kontynuacja prac stosowanych nad rozwojem interferometrycznych technik obrazowania:
   a. Metody obrazowania czynnościowego
   b. Tomografia Optyczna OCT i Mikroskopia OCM
   c. Elastografia optyczna
   d. 3-D Mikroskopia fazowa w badaniach morfologii krwi
2. Obrazowanie z wykorzystaniem fotoidukowanej autofluorescencji
3. Badania nad obrazowaniem w ośrodkach silnie rozpraszających z użyciem interferometrii światła częściowo spójnego oraz optyki adaptywnej.
4. Zwiększanie rozdzielczości poprzecznej przez wykorzystanie przestrzennej modulacji fazy światła (metody superrozdzielcze w obrazowaniu in-vivo)
5. Optyczna tomografia z wykorzystaniem światła polichromatycznego oraz oświetlenia strukturalnego (k-microscopy)
6. Rozwinięcie detekcji interferometrycznej w technikach CARS i SRS

# Zadanie 1 – Autofluorescencja siatkówki



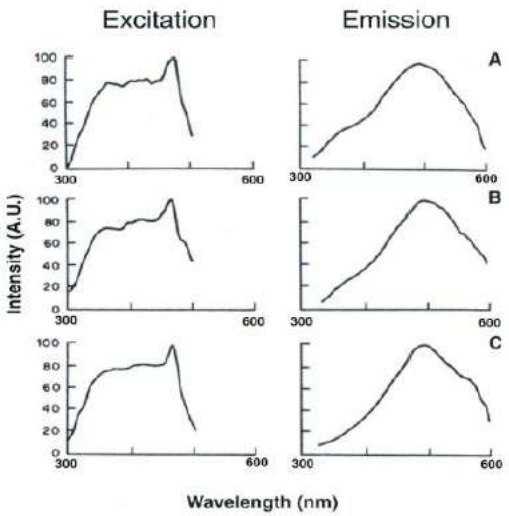
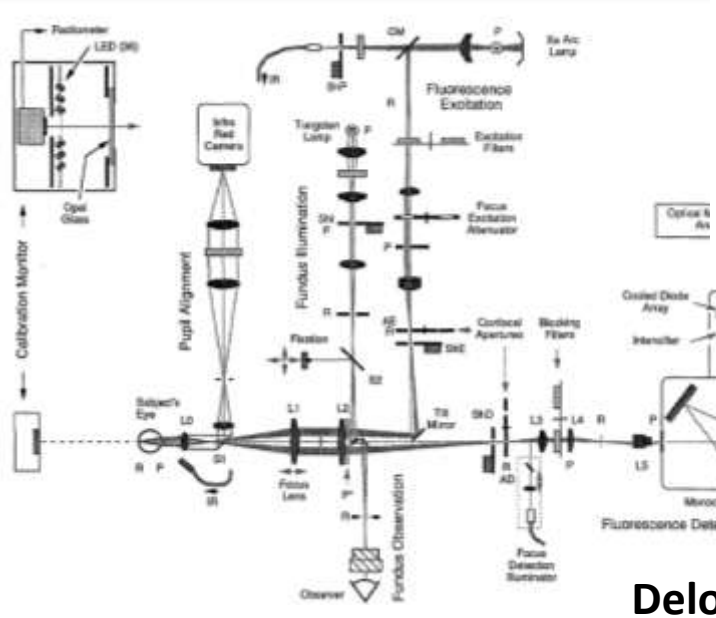
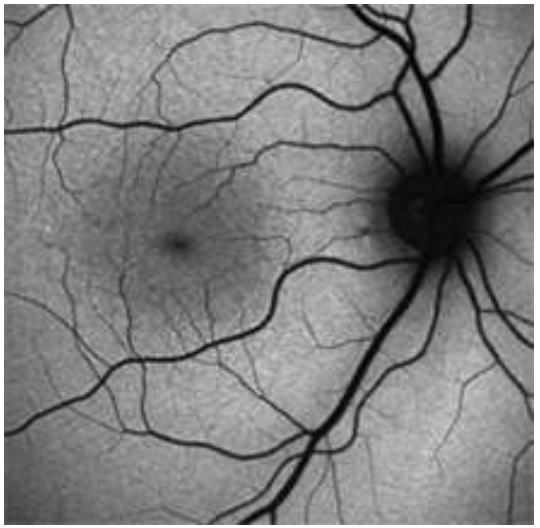
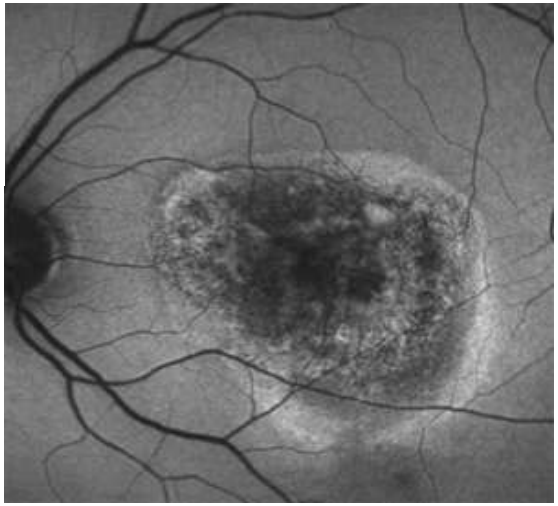




Fig. 1. Excitation and emission spectra of intact lipofuscin granules isolated from donors of different ages. Excitation were monitored with emission performed at 570 nm and emission spectra were monitored with excitation performed at 364 nm. Lipofuscin granules were prepared from different age groups:



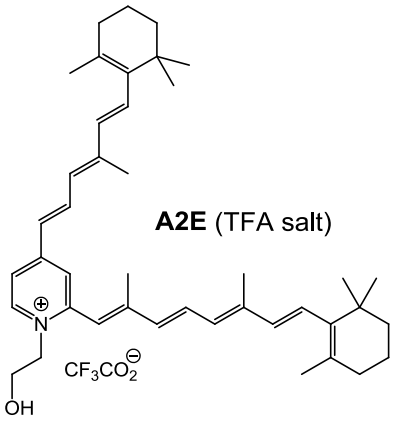



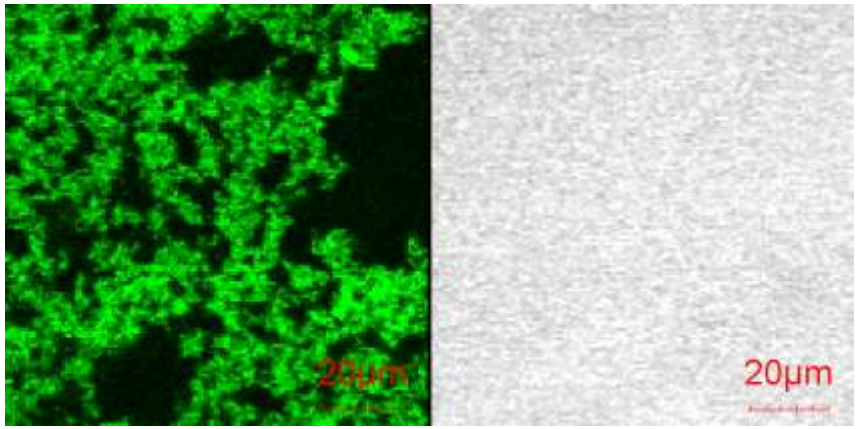


**Delori et al. 80-90**

# Optical Modulation of Fluorescence Emission from A2E/xlinked-BSA (Sample_c)

JM-2-58_c (fluor./brightfield)

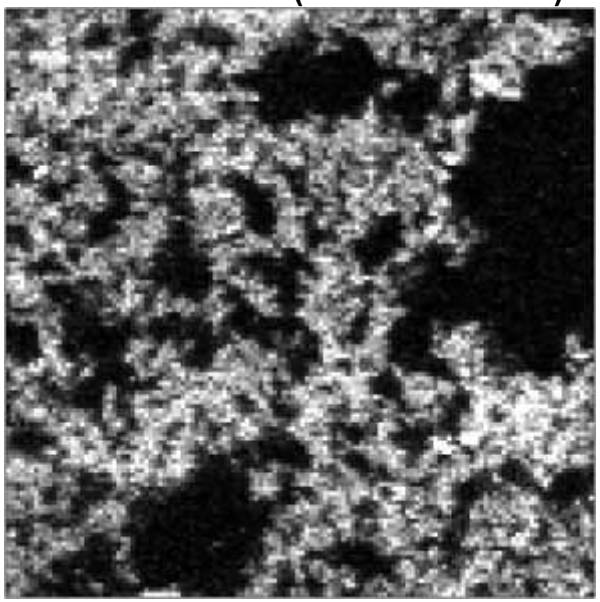


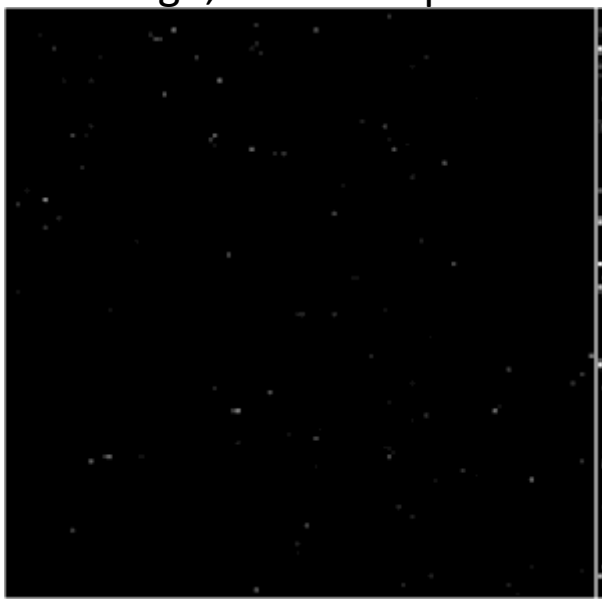

Fourier-domain images at different modulation powers:

Mod. laser: λ= 808 nm
mod freq. ~1 Hz
max. power: 10 W/cm$^2$ ?
Recording: 5.3 fps

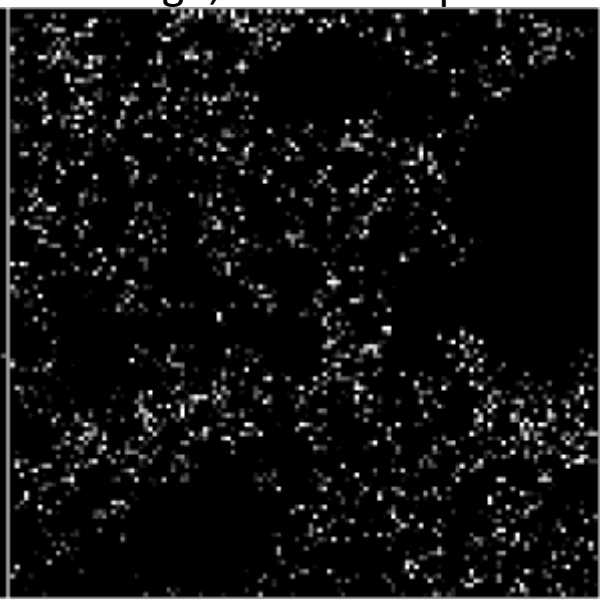
Fluorescence (FITC channel)



FD image; 12% mod.power



FD image; 50% mod. power



# Zwiększenie czułości - modulacja fluorescencji

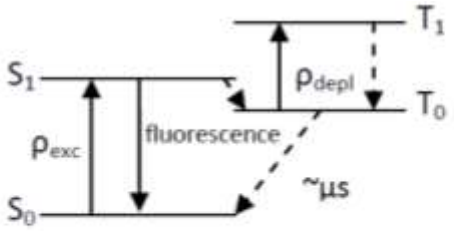



Fig. 3. Model of energy levels and main transitions involved in modulated luminescence depletion.

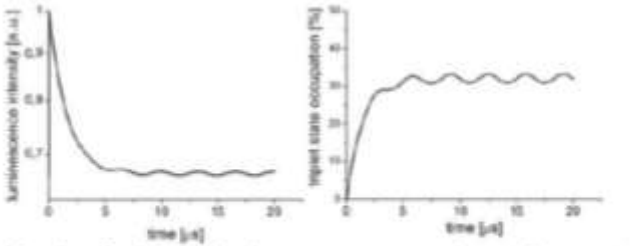
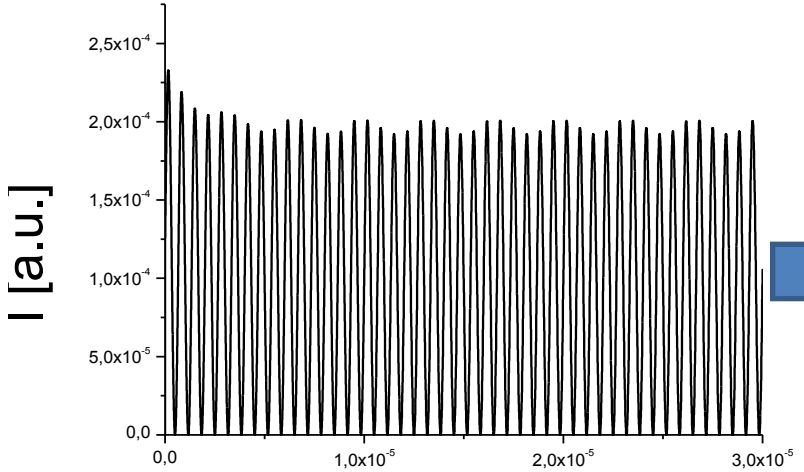
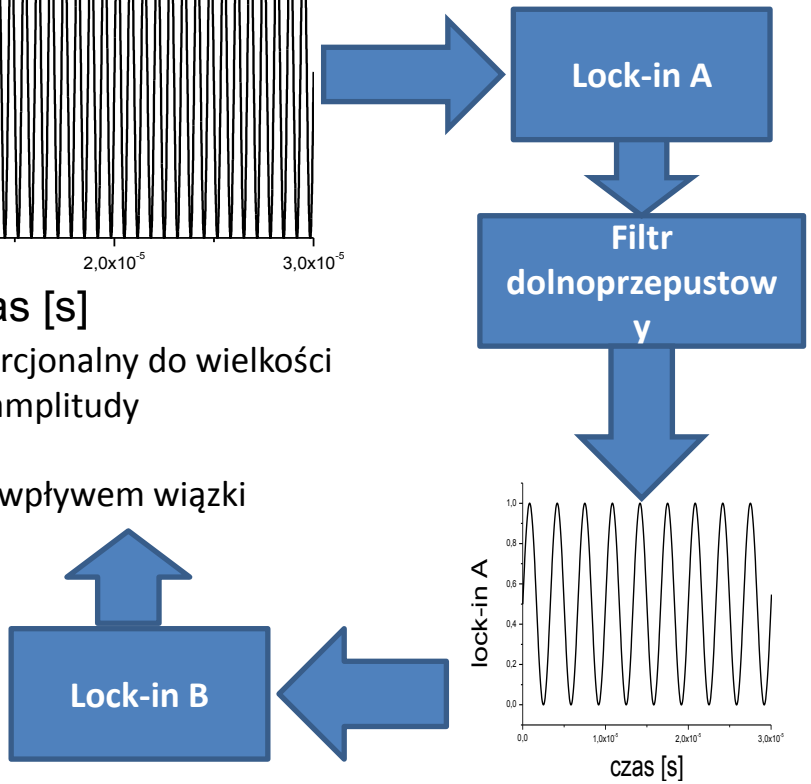


Fig.4. Calculated luminescence and triplet state modulation




Stały sygnał, proporcjonalny do wielkości powolnych zmian amplitudy luminescencji
zachodzących pod wpływem wiązki modulującej.



# Motywacja

Chemical Science

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www.rsc.org/chemicalscience

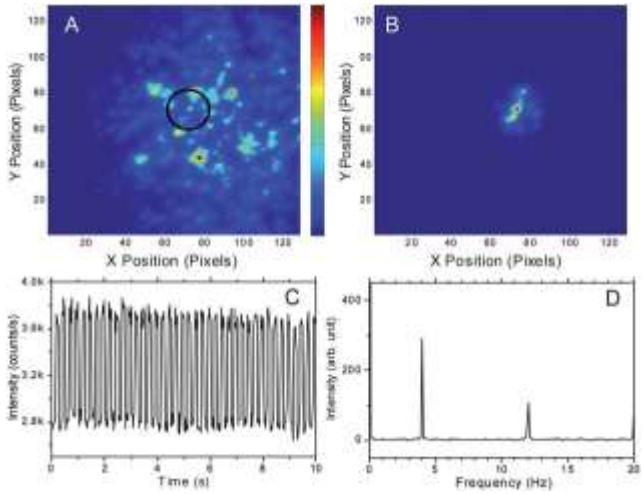
**EDGE ARTICLE**

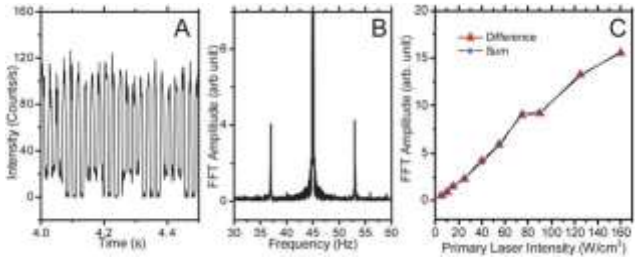
## All-optical fluorescence image recovery using modulated stimulated emission depletion

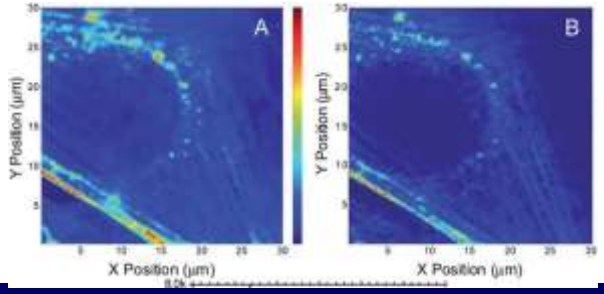
**Chaoyang Fan, Jung-Cheng Hsiang, Amy E. Jablonski and Robert M. Dickson***

***Received 20th December 2010, Accepted 14th March 2011***
**DOI: 10.1039/c0sc00637h**

Fluorescence modulation for selective recovery of desired fluorescence signals has to date required careful fluorophore selection combined with repeated optical recovery from long-lived photoinduced dark states. Adapting an all-optical scheme, modulated stimulated emission depletion (STED) generalizes such modulation schemes by eliminating the need for dark state residence by directly optically depopulating the emissive state at any externally applied frequency. Using two overlapped Gaussian laser spots with the depletion beam being intensity-modulated, fluorescence modulation is readily achieved with a depletion ratio governed by the intensity of the depleting laser. Selective image recovery of otherwise unmodulatable fluorophore signals is directly achieved through this all-optical modulation, and common STED-degrading multiphoton-excited background is readily discriminated against. Both beads and dyes in solution, as well as fluorophores bound within fixed cells are readily imaged in this manner.



# Dalsze perspektywy

NANO LETTERS

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## Far-Field Autofluorescence Nanoscopy

Jakob Bierwagen,[†,§] Ilaria Testa,[†,§] Jonas Fölling,[†,§] Dirk Wenzel,[†] Stefan Jakobs,[†] Christian Eggeling,[†] and Stefan W. Hell*[,†]

[†]Max Planck Institute for Biophysical Chemistry, Department of NanoBiophotonics and [†]Electron Microscopy Facility, Am Fassberg 11, 37077 Göttingen, Germany

**ABSTRACT** We demonstrate far-field optical imaging at the nanoscale with unlabeled samples. Subdiffraction resolution images of autofluorescent samples are obtained by depleting the ground state of natural fluorophores by transferring them to a metastable dark state and simultaneously localizing those fluorophores that are transiently returning. Our approach is based on the insight that nanoscopy methods relying on stochastic single-molecule switching require only a single fluorescence on−off cycle to yield an image, a condition fulfilled by various biomolecules. The method is exemplified by recording label-free nanoscopy images of thylakoid membranes of spinach chloroplasts.

**KEYWORDS** Microscopy, resolution, chlorophyll, ground state depletion, nanoscopy, GSDIM

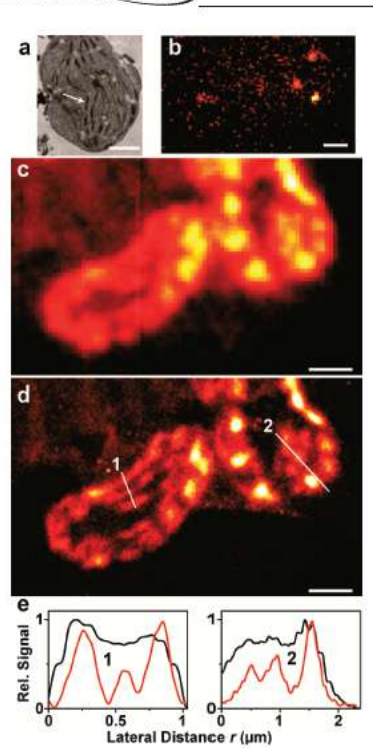



FIGURE 2. Subdiffraction resolution imaging of autofluorescent chloroplasts. (a) Electron micrograph pinpointing (arrow) the thylakoid membranes (darker areas) in the chloroplasts. (b) Typical single camera frame image showing single chlorophyll molecules. (c, d) Autofluorescence wide-field (c) and GSDIM (d) images of thylakoid membranes in the chloroplasts and selected (white line) line profiles (e). The wide-field images were created by summing up all camera frame images after background subtraction. Optical images recorded with a 488 nm wavelength continuous wave beam of 28 kW/cm$^2$ focal intensity. Scale bar 1 μm.

# Obrazowaniem w ośrodkach silnie rozpraszających

LETTERS
PUBLISHED ONLINE: 14 FEBRUARY 2010 | DOI: 10.1038/NPHOTON.2010.3
nature photonics

## Exploiting disorder for perfect focusing

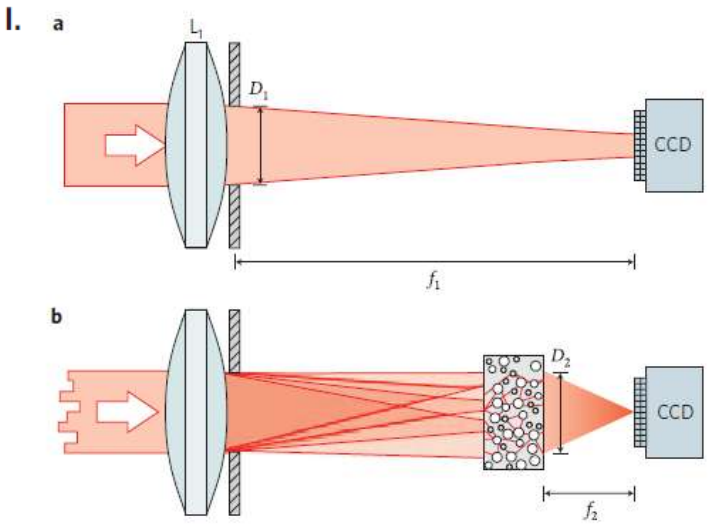



**Figure 1 | Schematic of the experiment.** Lens $L_1$ with focal distance $f_1$ focuses light onto a camera. A pinhole controls the aperture $D_1$ of the lens. **a**, 'Clean' system without disorder. The focus is, at best, as sharp as the diffraction limit of the lens. **b**, System with disorder. A disordered sample at distance $f_2$ from the camera randomly scatters the light. After shaping the incident wavefront with a spatial light modulator (modulator and imaging telescope not shown), the light focuses through the sample to a spot that is narrower than the diffraction limit. $D_2$, diameter of the diffuse spot at the back of the sample.

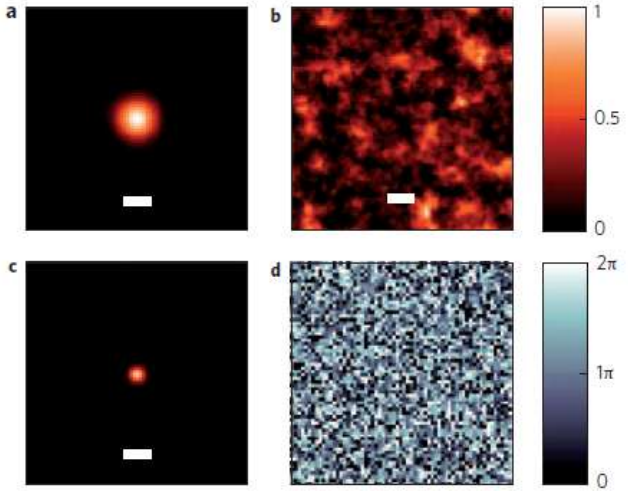



**Figure 2 | Measured intensity distribution in the focal plane at 200±3 mm from the glass lens. a**, Clean system with an unmodified incident wavefront. The focal width is of the order of the diffraction limit (62 μm, white bar). **b**, Intensity transmission of a 6-μm layer of airbrush paint for the unmodified incident wavefront. No focus is discernible. **c**, System with the sample present, and the wave shaped to achieve constructive interference in the target. A high-contrast, extremely sharp focus is visible. **d**, Pattern on the spatial phase modulator for the set-up in **c**. The intensity plots are normalized to the brightest point in the image.

# Obrazowaniem w ośrodkach silnie rozpraszających

ARTICLES
PUBLISHED ONLINE: 22 MAY 2011 | DOI: 10.1038/NPHOTON.2011.72
nature photonics

## Focusing and compression of ultrashort pulses through scattering media

Ori Katz, Eran Small, Yaron Bromberg and Yaron Silberberg*

**Light scattering in inhomogeneous media induces wavefront distortions that pose an inherent limitation in many optical applications. Examples where this occurs include microscopy, nanosurgery and astronomy. In recent years, ongoing efforts have made the correction of spatial distortions possible using wavefront-shaping techniques. However, when ultrashort pulses are used, scattering also induces temporal distortions, which hinder the use of such pulses in nonlinear processes such as multiphoton microscopy and quantum control experiments. Here, we show that correction of both spatial and temporal distortions can be achieved by manipulating only the spatial degrees of freedom of the incident wavefront. By optimizing a nonlinear signal, we demonstrate spatiotemporal focusing and compression of chirped ultrashort pulses through scattering media, and refocusing in both space and time of 100 fs pulses through thick brain and bone samples. Our results open up new possibilities for optical manipulation and nonlinear imaging in scattering media.**

# Obrazowaniem w ośrodkach silnie rozpraszających

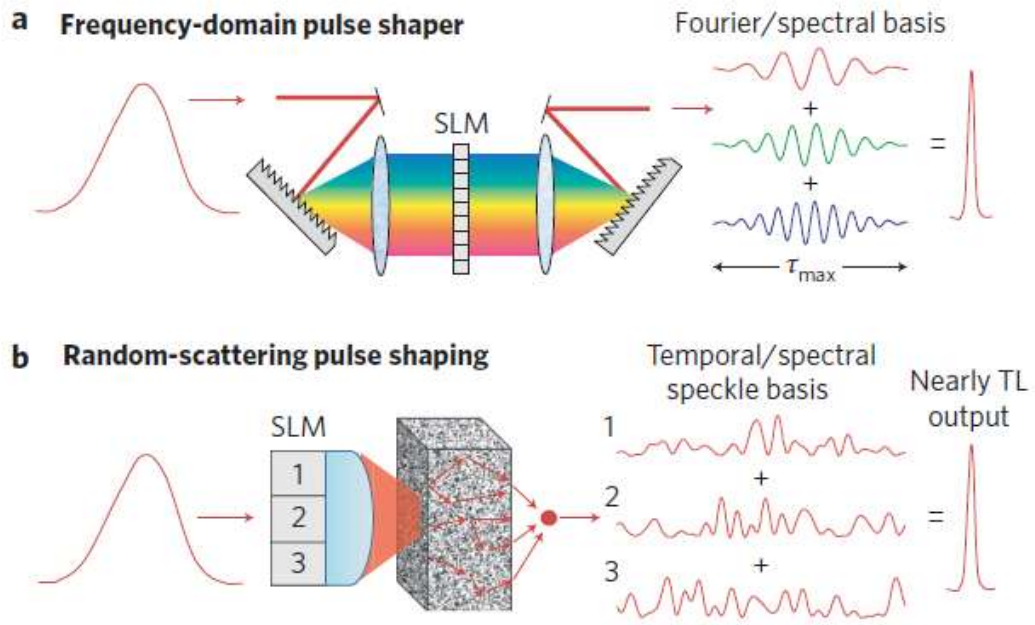



**Figure 3 | Mechanism for temporal compression using only spatial degrees of freedom and random scattering. a,b**, Much like a Fourier (frequency domain) pulse shaper[32] (**a**), where the pixels of the SLM are coupled to the spectral degrees of freedom by scattering from a grating; coherent scattering in a random medium (**b**) couples each SLM pixel to a different linear combination of the spectral (also temporal) degrees of freedom, forming a new random spectral basis that is phase-controlled by the SLM. In both cases, the maximum controllable delay $\tau_{max}$ dictates the shaping spectral resolution $\Delta f = 1/\tau_{max}$, and is determined by the optical path lengths differences in the medium/shaper.

# Obrazowaniem w ośrodkach silnie rozpraszających

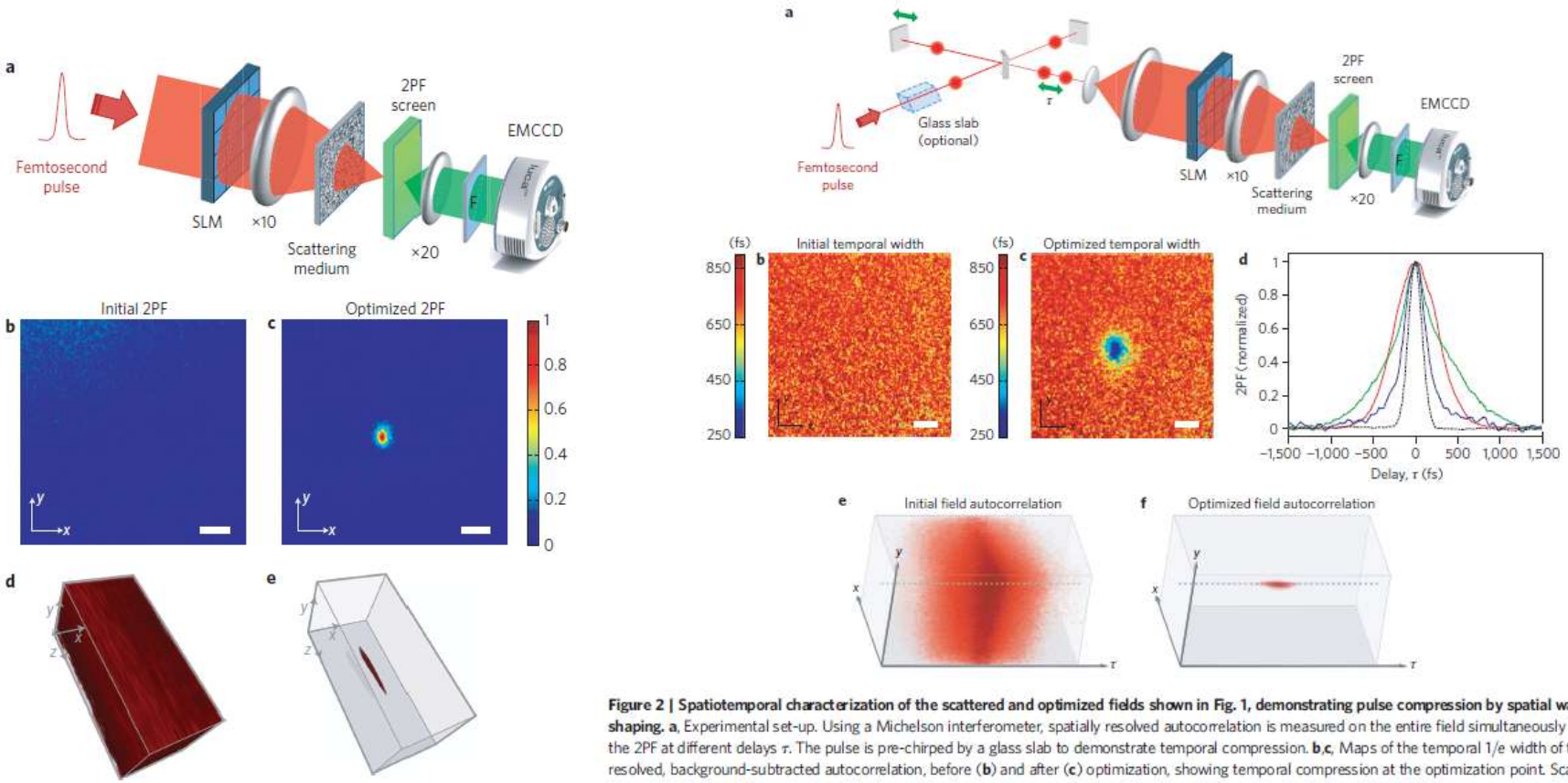
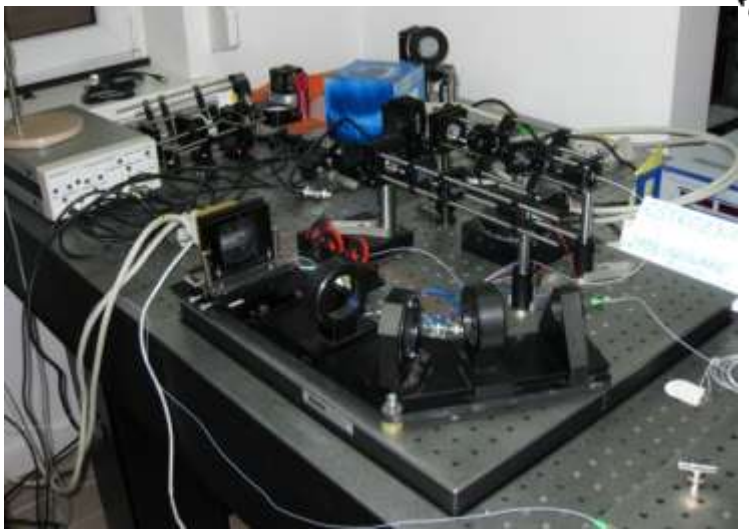



**Figure 2 | Spatiotemporal characterization of the scattered and optimized fields shown in Fig. 1, demonstrating pulse compression by spatial wavefront shaping.** **a**, Experimental set-up. Using a Michelson interferometer, spatially resolved autocorrelation is measured on the entire field simultaneously by imaging the 2PF at different delays τ. The pulse is pre-chirped by a glass slab to demonstrate temporal compression. **b**,**c**, Maps of the temporal 1/e width of the spatially resolved, background-subtracted autocorrelation, before (**b**) and after (**c**) optimization, showing temporal compression at the optimization point. Scale bars, 25 μm. **d**, Measured fringe-averaged, background-subtracted autocorrelation of the chirped input pulse (red; 1/e width, 715 fs), the non-optimized scattered pulse (green; 1/e width, 875 fs), the optimized pulse (blue; 1/e width, 370 fs), and the transform-limited pulse (dashed-black; 1/e width, 230 fs). **e**,**f**, Spatiotemporal renderings of the measured autocorrelations in the non-optimized (**e**) and optimized (**f**) cases (Supplementary Video 1). Dashed green and blue lines in **e** and **f** are the locations of autocorrelations plotted in corresponding colours in **d**. Rendered spatiotemporal volume, 200 μm × 200 μm × 2,200 fs.

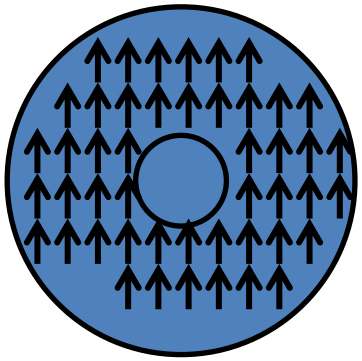
# Kształtowanie przestrzennego rozkładu fazy

**Założenie:**
Uzyskanie efektu analogicznego do mikroskopii STED bez wykorzystania fluorescencji – w procesie rozpraszania światła



Zalety:
Mikroskopia superrozdzielcza z bardzo czułą i szybką detekcją, pomiary 3-D przyżyciowe

Wady:
Brak selektywności molekularnej



**Przekrój zmodulowanej wiązki**

European Young Investigator Award (EURYI)

# Dziękuję za Uwagę