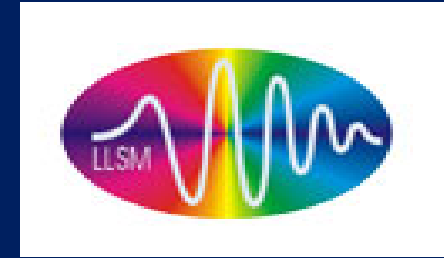


# OBRAZOWANIE RAMANA

Obrazowanie medyczne, st. 1., sem. 6, specjalność CHEMIA  
BIOMEDYCZNA



MIĘDZYRESORTOWY INSTYTUT TECHNIKI RADIACYJNEJ  
WYDZIAŁ CHEMICZNY POLITECHNIKI ŁÓDZKIEJ



## CEL

Obecnie stosowane metody obrazowania są bardzo często ograniczone ze względu na niedostateczną czułość, swoistość a także rozdzielczość przestrzenną oraz spektralną.

Właściwości fal elektromagnetycznych zależą od długości fali. Promieniowaniem elektromagnetycznym o różnej długości fali są fale radiowe, mikrofałe, podczerwień, światło widzialne, ultrafiolet, promieniowanie rentgenowskie i promieniowanie gamma. Każdy zakres spektralny promieniowania jest związany z określonym rodzajem spektroskopii.

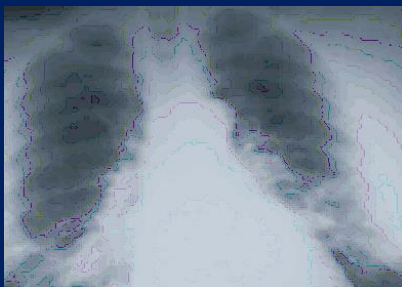
MRI



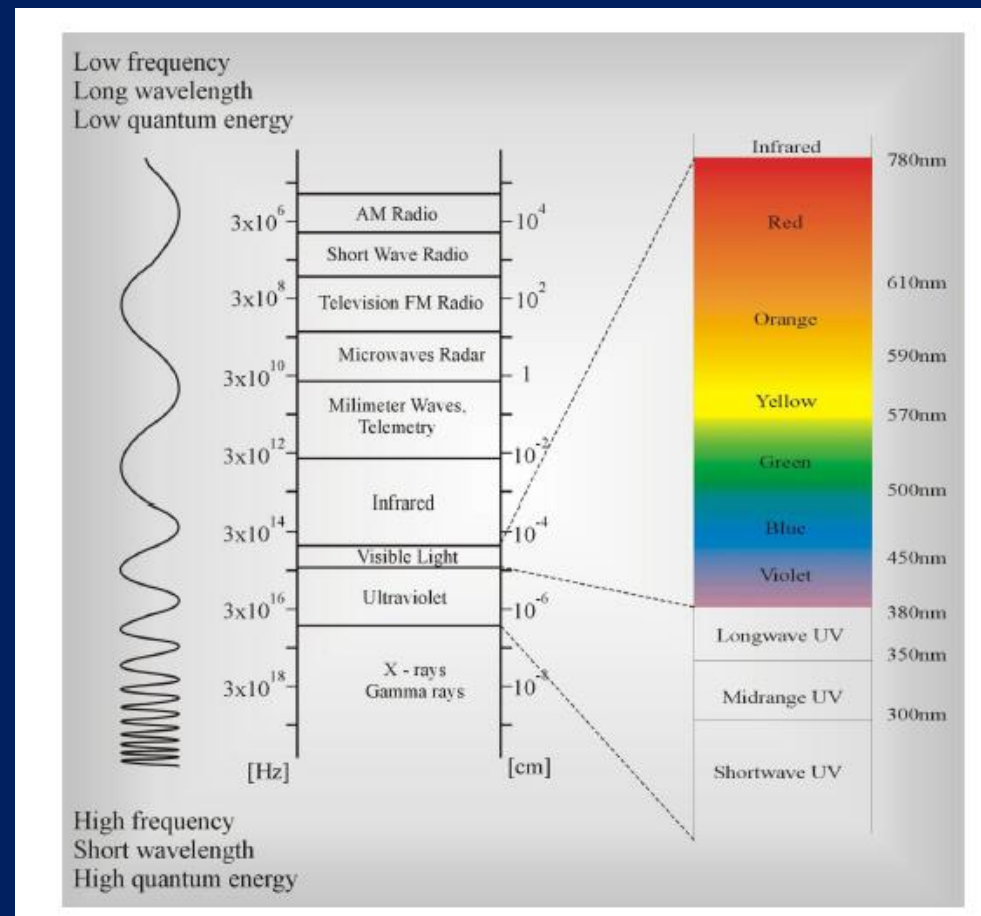
TECHNOLOGIA 5G



Raman



Roentgen ,  
Tomografia



Frequency SPECTRUM

0 Hertz	Steady direct current
50-60 Hertz (5000Km)	AC power
-----USA-----	
16-16.000 kHz	Audio frequencies
10-30 kHz 30-10Km	v.l.f -very low frequency
30-30000 KHz	Radio Frequencies
30-300 kHz 10-1Km	l.f. low frequency
30-35 kHz	Marine com & navigation, aero nav.
300-3000 kHz 1000-100m	m.f medium frequencies
535-1635 kHz	AM broadcast bands
1800-2000 kHz	160 meter band
-----Football_Field-----	
3-30 MHz 100-10m	h.f. - high frequencies
3.5-4 MHz	80 m e1erband
7-7.3 MHz	40 band
14-14.35 MHz	20 meter band
21-21.45 MHz	15meterbend
26.85-27.54 MHz	Industrial, Scientific, medical
28-29.7 MHz	10 meterband
26.86-27.455 MHz	Citizens Band Class D
-----Human-----	
30-300 MHz 10-1m	very high frequencies
44-49 Mhz	Analog cordless phone
30-50 MHz	Police,fire,highway,railroad
50-54 MHz	6 meter band
54-72 MHz	TV channels 2 to 4
72-76 MHz	Government, Aero,Marker 75MHz
76-88 MHz	TV channels 5 and 6
88-108 MHz	FM broadcast band
108-118 MHz	Aeronautical navigation
118-136 MHz	Civil Communication Band
148-174 MHz	Government
144-148 MHz	2 meter band
174-216 MHz	TV channels 7 to 13
216-470 MHz	Amateur, government. CB Bend
	non-gov ,fixed or mobile ,aero navigate
220-225 MHz	Amateur band. 1-1/4 meter
225-400 MHz	Military
420-450 MHz	Amateur band, 0.7 meter
462.5-465 MHz	Citizens Band
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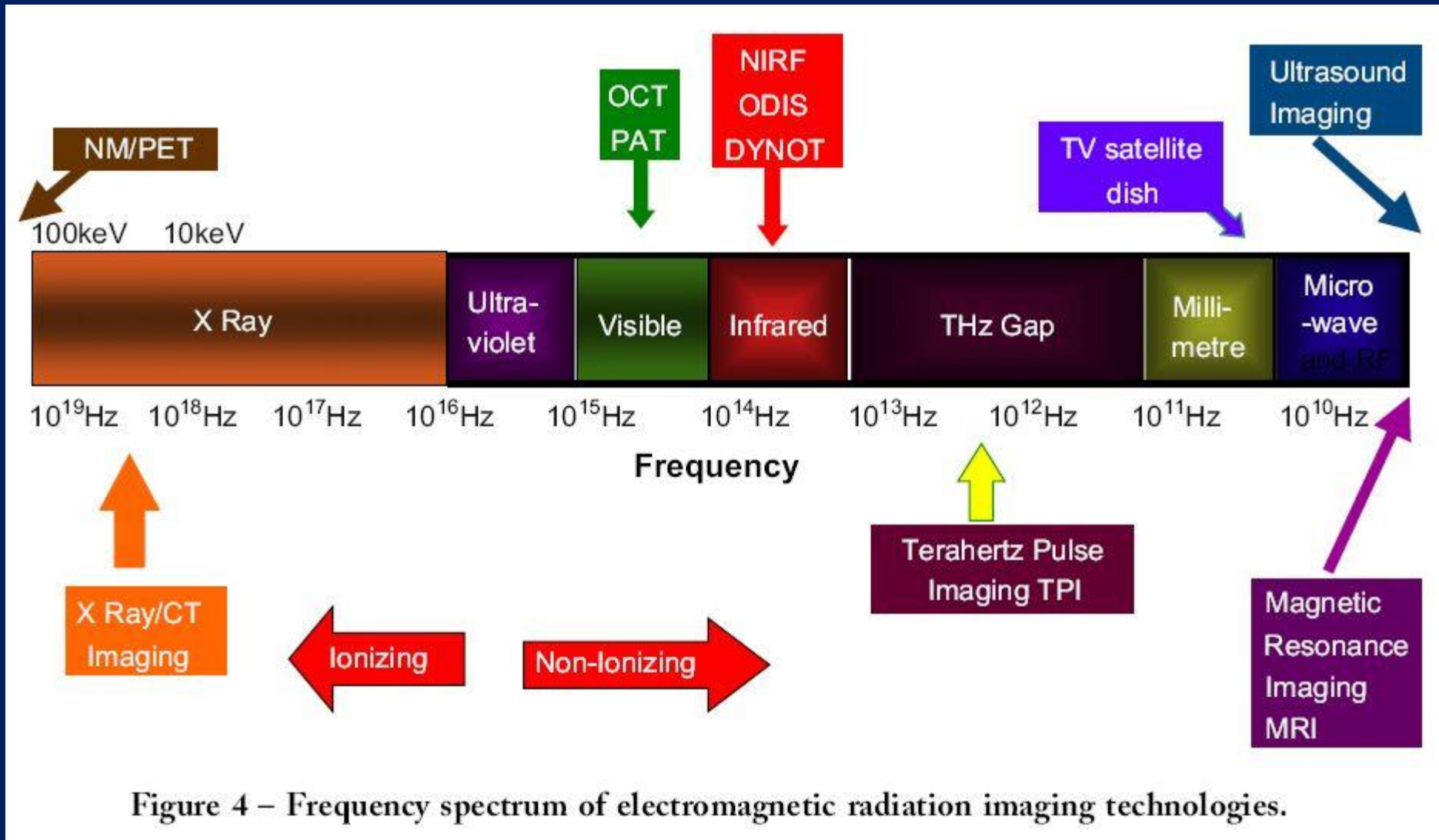


Figure 4 – Frequency spectrum of electromagnetic radiation imaging technologies.

**Obrazowanie** nie jest metodą leczenia samą w sobie, ale może ono pomóc w wyborze najkorzystniejszego sposobu leczenia. Niektóre metody obrazowania mogą być przydatne również w diagnozowaniu chorób nowotworowych, mogą one pomagać w określeniu jak zaawansowana jest choroba, jak działają dostarczane do organizmu leki i jak efektywna jest zastosowana procedura leczenia.

## Zastosowanie obrazowania

### ➤ **Badania przesiewowe nowotworów**

Obrazowanie może być wykorzystywane w celu sprawdzenia czy w organizmie pacjenta występują podejrzanym struktury mogące mieć charakter nowotworowy.

**Mammografie** są przykładem bardzo dobrze znanej metody obrazowania używanej do badania raka piersi. Badania przesiewowe są szczególnie polecane osobom z grup podwyższonego ryzyka (ze względu na wiek, historię zachorowań w rodzinie czy też styl życia) w celu zdiagnozowania różnych typów nowotworów.

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## ➤ Diagnostyka

Obrazowanie może być wykorzystywane do określenia gdzie zlokalizowany jest nowotwór i jaki jest stopień rozwoju choroby. W tym sensie obrazowanie może pomóc w określeniu stopnia zaawansowania choroby i stwierdzeniu czy nowotwór jest umiejscowiony w środku, na zewnątrz czy też w pobliżu ważnych organów wewnętrznych lub naczyń krwionośnych. Jeśli konieczna jest biopsja (pobranie małego fragmentu z guza do celów diagnostycznych) obrazowanie jest przydatne w precyzyjnej lokalizacji nowotworu i pobraniu wycinka do analizy.

## ➤ Dobór metody leczenia

Obrazowanie może być także przydatne w kontekście sterowania leczeniem by było one jak najmniej inwazyjne i skupione na tkance nowotworowej. Np. ultrasonografia, MRI czy CT mogą być używane do bardzo dokładnego zlokalizowania guza dzięki czemu leczenie może obejmować prawie wyłącznie tkanki guza jak najmniej uszkodzając tkanki otaczające.

### ➤ **Nadzorowanie efektywności podjętego leczenia**

Obrazowanie może także pozwolić na śledzenie postępów stosowanej terapii. Dzięki obrazowaniu można śledzić czy guz ulega zmniejszeniu czy też rozrasta się. Zastosowanie znajdują tutaj, wykonywane regularnie badania technikami takimi jak: prześwietlenia promieniami X, MRI czy CT. PET i inne techniki medycyny nuklearnej są wykorzystywane do badań mających stwierdzić czy guz atakuje najważniejsze życiowe narządy. MNR jest zaś stosowany do badań mających na celu śledzenie zmian chemicznych zachodzących w nowotworach.

### ➤ **Monitorowanie nawrotów choroby nowotworowej**

Obrazowanie może być używane także do diagnozowania nawrotów choroby nowotworowej i śledzenia jej rozprzestrzeniania się w organizmie.



# KONTRAST OBRAZOWANIA

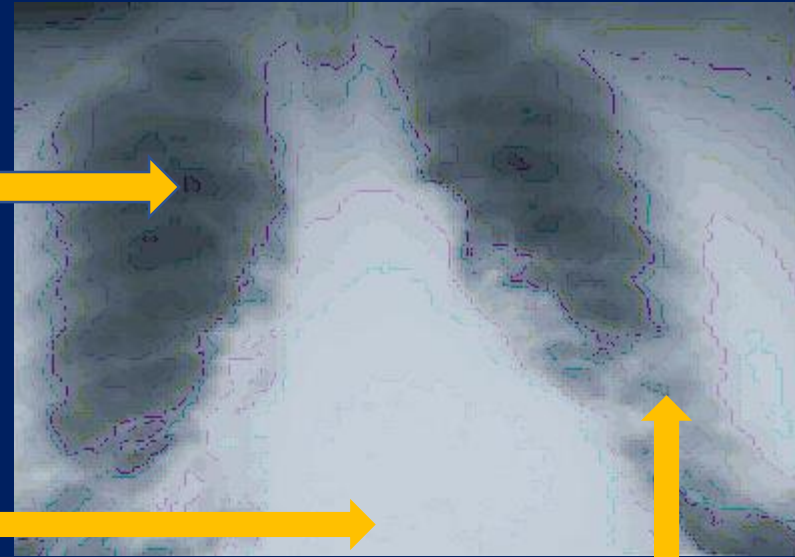


# KONTRAST OBRAZOWANIA

- **Egzogenne mechanizmy:**
- **Absorpcja, odbicie, transmisja**
  - **Hiperpolaryzacja spinu**
  - **Relaksacja magnetyczna**
  - **Podatność magnetyczna**
- **Transfer namagnesowania**
  - **Transfer nasycenia**
    - **Izotopy**
    - **Fluorescencja**
    - **Bioluminescencja**
    - **komórkowe pH**
- **Niedotlenienie (Hypoxia)**

## Obrazowanie promieniowaniem X (X-ray imaging)

Prześwietlenie promieniowaniem X jest najprawdopodobniej jedną z najpopularniejszych metod obrazowania

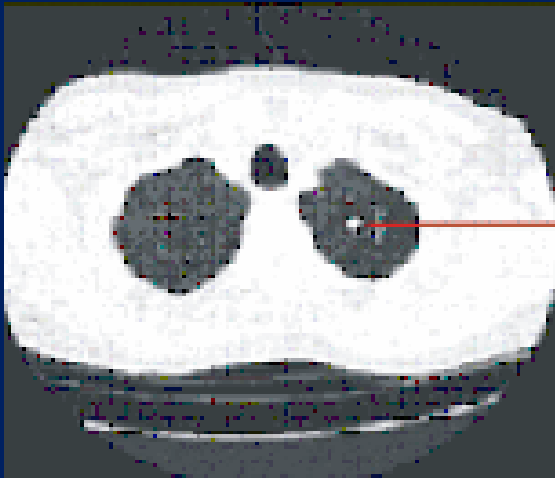


Obrazy powstają podczas prześwietlenia na skutek odmiennej absorpcji promieniowania przez różne typy tkanek. Wapń zawarty w kościach absorbuje promieniowanie najsilniej i dlatego kości widoczne na zdjęciu są białe. Tłuszcz oraz inne tkanki absorbują słabiej i dlatego mają kolor szary. Najslabiej promieniowanie absorbują płuca z tego powodu odpowiada im kolor czarny.

Najpopularniejszym zastosowaniem prześwietlenia rentgenowskiego jest badanie złamanych kości. Metoda ta ma jednak zastosowanie również w badaniu nowotworów. Analiza zdjęć pozwala bowiem na stwierdzenie czy guz występuje, a jeśli tak to czy rozrasta się w płucach lub innych obszarach klatki piersiowej.

Mammografia wykorzystuje promieniowanie X do wyszukiwania guzów i obszarów podejrzanych o charakter nowotworowy w piersiach.

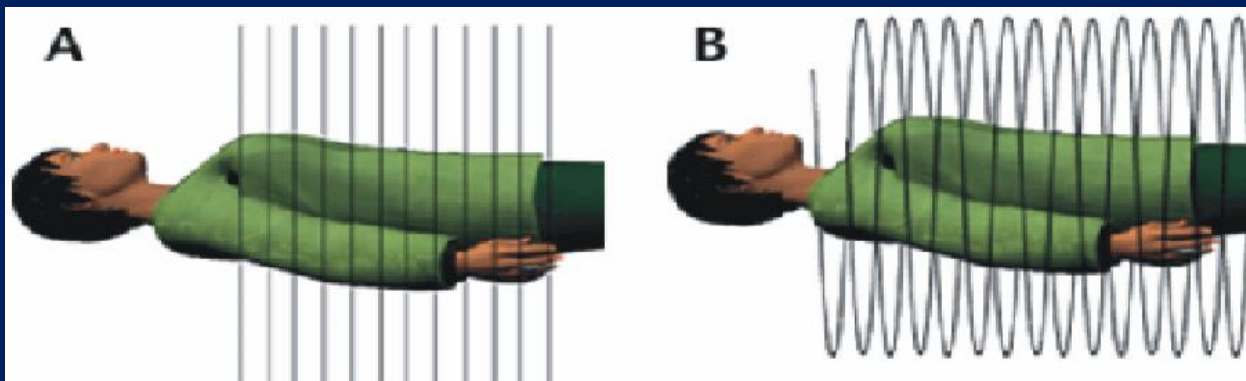
## Tomografia komputerowa CT (computed tomography)



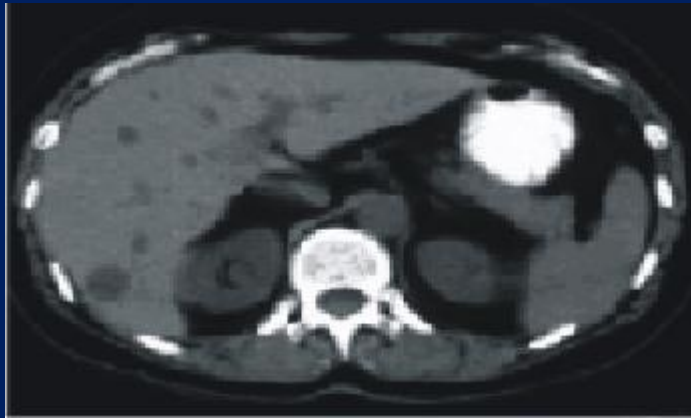
A. P. Reeves, Cornell University

Tomografia komputerowa (CT lub CAT) wykorzystuje komputerowo kontrolowane promieniowanie X do kreowania obrazów ciała. Zastosowanie promieniowania X zbliża CT do prześwietlenia rentgenowskiego jednakże zdjęcie rentgenowskie i skany CT pozwalają pozyskać całkiem odmienne informacje. Chociaż doświadczony radiolog potrafi również ze zdjęcia rentgenowskiego wywnioskować o trójwymiarowej lokacji guza prześwietlenia rentgenowskie w przeciwieństwie do CT są z natury dwuwymiarowe.

Prześwietlenia rentgenowskie ramienia lub klatki piersiowej nie są w stanie dostarczyć informacji jak głęboko penetrujemy ciało w poszukiwaniu zmian. Skany CT są trójwymiarowe. Dzięki zarejestrowaniu i zanalizowaniu trójwymiarowych „plastrów” ciała pacjenta lekarz nie tylko może stwierdzić obecność guza, ale także jak głęboko jest on umiejscowiony. Skany CT są trójwymiarowe, ponieważ obrazowanie następuje nie na płaskiej kliszy a w pamięci komputera.



A- konwencjonalna CT  
B- spiralna CT



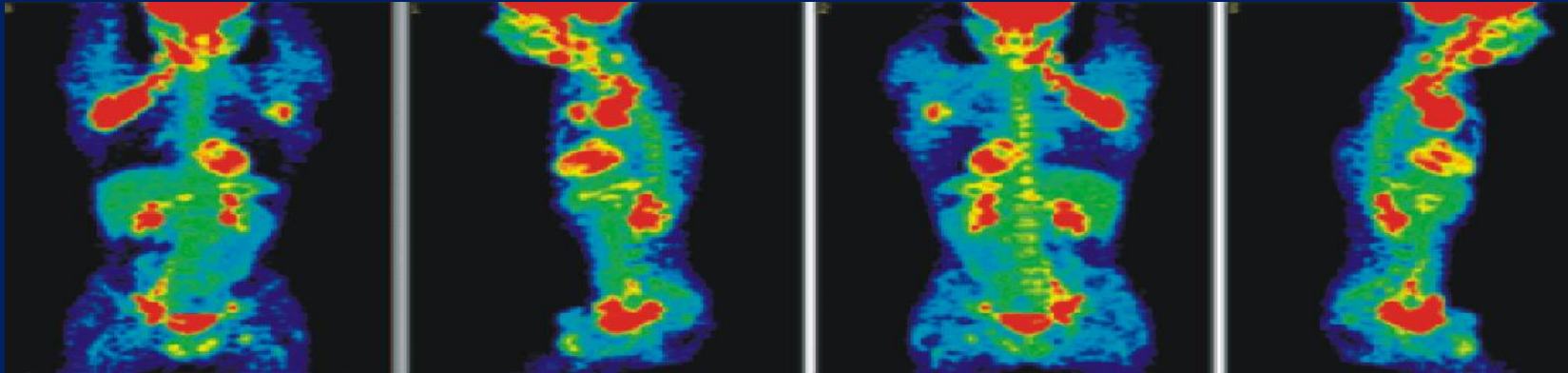
Konwencjonalny skan CT bez kontrastu ukazujący guz wątroby.



Konwencjonalny skan CT z kontrastem tego samego pacjenta ukazujący guz wątroby.

Autor:Dr. Peter Choyke,  
Department of Radiology, Clinical Center, National Institutes of Health

# OBRAZOWANIE JĄDROWE (PET i SPECT)



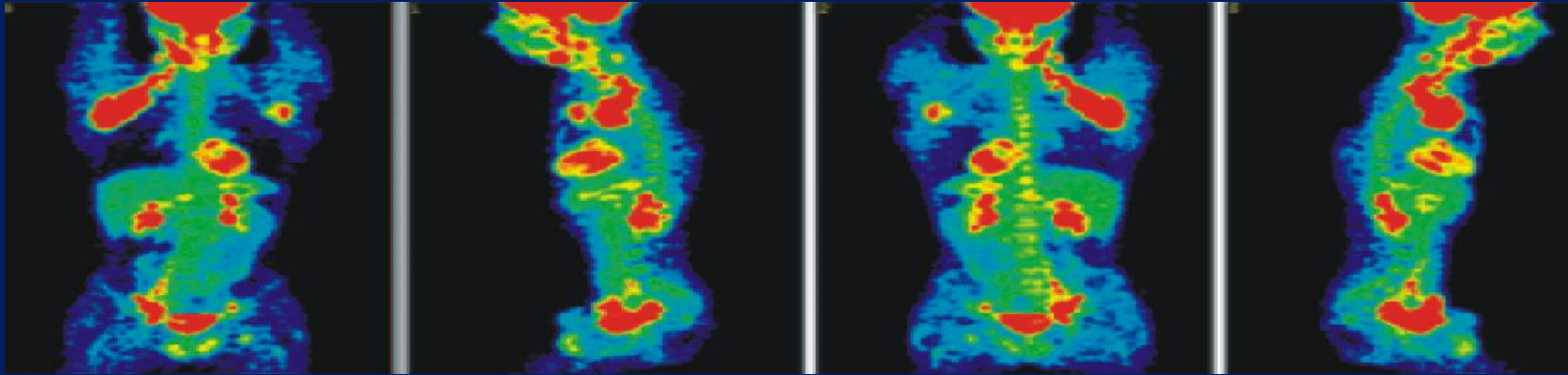
## **Obrazowanie jądrowe (PET and SPECT)**

Obrazowanie jądrowe wykorzystuje małe dawki znaczników radioaktywnych, które zostają wprowadzone do ciała pacjenta. Odpowiednio skonstruowana aparatura śledzi następnie gdzie i kiedy następuje koncentracja znacznika. Najpopularniejsze metody z tej grupy to **PET i SPECT**.

## **Pozytonowa tomografia emisyjna (PET)**

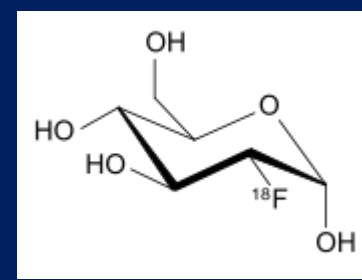
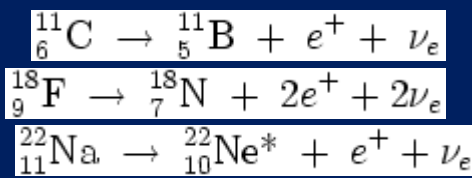
rejestruje komputerowe obrazy zmian metabolizmu cukrów, jakie mają miejsce w tkankach. Standardowo pacjent otrzymuje zastrzyk substancji będącej połączeniem cukru i cukru znaczonego izotopowo. Cukier znaczone izotopowo pozwala na zlokalizowanie guza ponieważ komórki guza pochłaniają cząsteczki cukru efektywniej niż inne tkanki organizmu. Po otrzymaniu cukru znaczonego izotopowo pacjent leży przez około 60 min podczas gdy znaczonego cukru krąży w jego ciele. Jeśli w organizmie występuje guz cukier powinien efektywnie się w nim zakumulować.

## Pozytonowa tomografia emisyjna (PET)



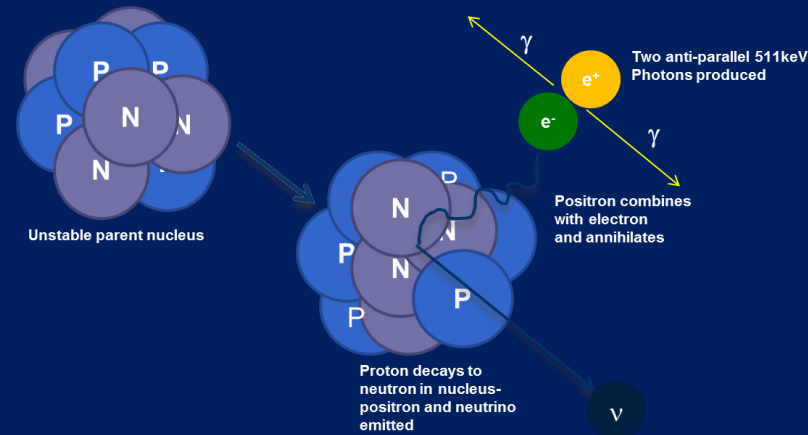
Następnie pacjent umieszczony na stole stopniowo wprowadzany jest do skanera PET 6-7 razy w ciągu 45-60min. Skaner PET używany jest do określenia rozmieszczenia cukru w guzie i innych tkankach ciała. Dzięki analizie skanów CT i PET można osiągnąć lepszą zdolność opisu guza. Obraz obrobiony komputerowo analizowany jest przez radiologa.

PET może wykryć guz nawet gdy inne techniki wskazują na normalny charakter tkanki. PET pozwala także na diagnostykę wznowień nowotworowych i określenie efektywności leczenia-gdy guz zmniejsza się jego komórki metabolizują coraz mniej cukru.



**Fludeoksyglukoza (18F)**

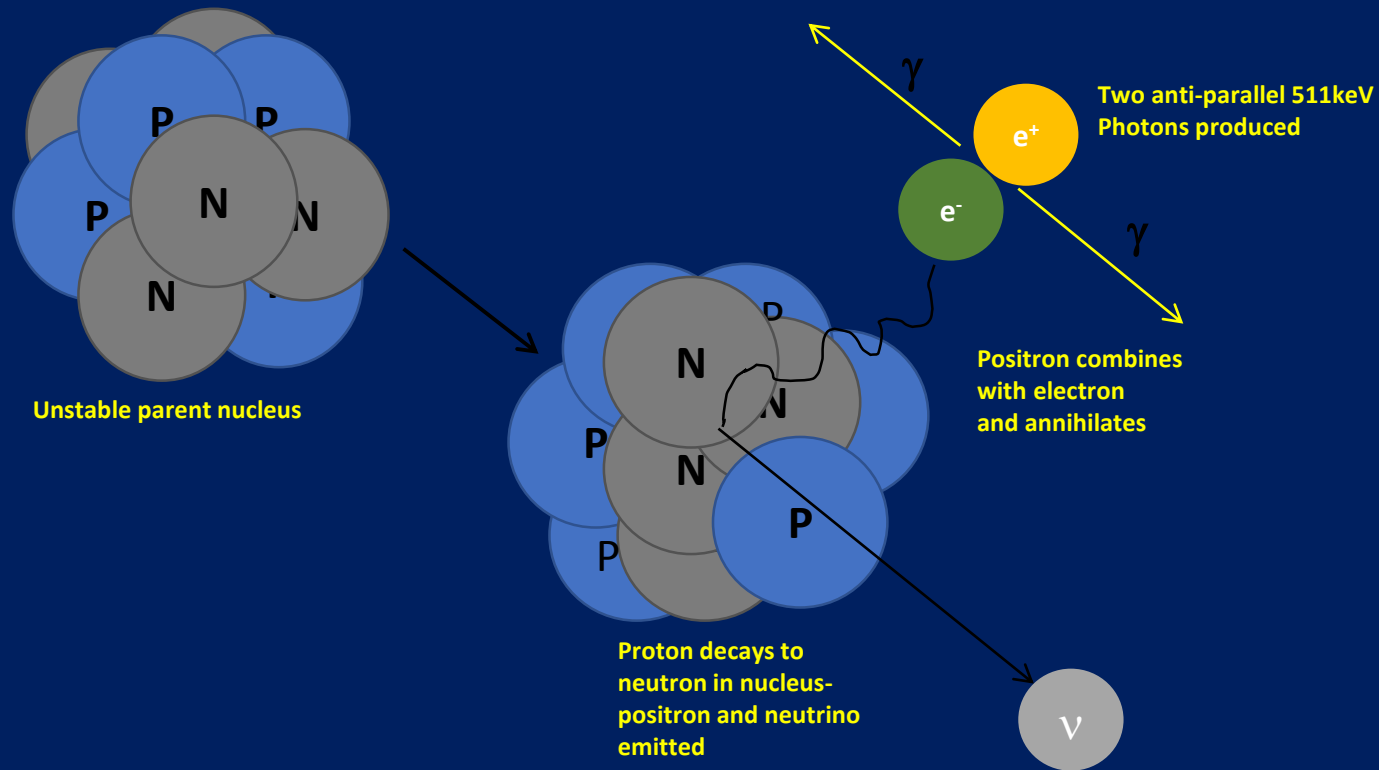
**Rozpad beta plus (przemiana  $\beta^{+}$ )** - reakcja jądrowa, w której emitowana jest cząstka  $\beta^{+}$  (zwana pozytonem lub antyelektronem) oraz neutrino elektronowe. Przykłady izotopów, które ulegają rozpadowi beta plus:  ${}^{11}\text{C}$ ,  ${}^{13}\text{N}$ ,  ${}^{15}\text{O}$ ,  ${}^{18}\text{F}$  i  ${}^{22}\text{Na}$ .



Powstające w rozpadzie promieniotwórczym pozytony, po przebyciu drogi kilku milimetrów, zderzają się z elektronami zawartymi w tkankach ciała, ulegając anihilacji. W wyniku anihilacji pary elektron–pozyton powstają dwa kwanty promieniowania elektromagnetycznego (fotony) o energii 511 keV każdy, poruszające się w przeciwnych kierunkach (pod kątem  $180^{\circ}$ ). Fotony te rejestrowane są jednocześnie przez dwa z wielu detektorów ustawionych pod różnymi kątami w stosunku do ciała pacjenta (najczęściej w postaci pierścienia), w wyniku czego można określić dokładne miejsce powstania pozytonów. Informacje te rejestrowane w postaci cyfrowej na dysku komputera, pozwalają na konstrukcję obrazów będących przekrojami ciała pacjenta, analogicznych do obrazów uzyskiwanych w tomografii NMR.



. The detector records pair of gamma rays emitted by a positron-emitting radioactive substance. To recall, proton-rich isotopes like are may decay via positron emission, in which a proton in the nucleus decays to a neutron, a positron (positive electron) and a neutrino. Understanding this phenomenon of detection requires recalling that there is antimatter in the universe, and when antimatter (positron) meets matter (electron), then both kinds of matter are annihilated, and pure energy (gamma radiation) is formed. PET radioactive substance emits positrons which annihilate with electrons up to a few millimeters away, causing two gamma photons to be emitted in opposite directions

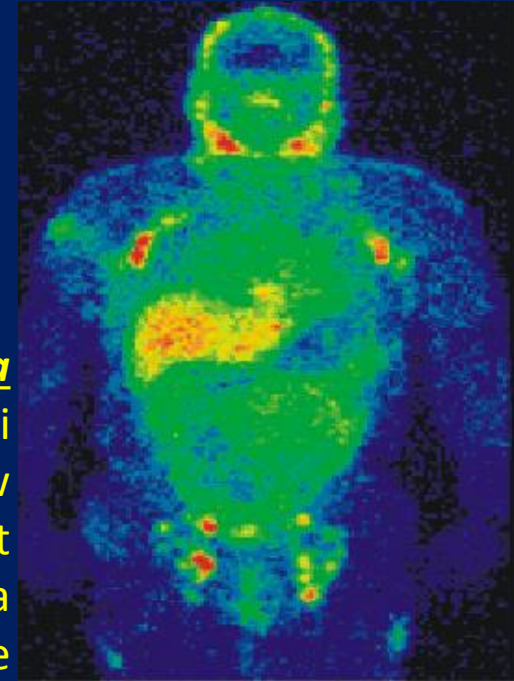


# SPECT

*single photon emission computed tomography*

Podobna do techniki PET komputerowa tomografia emisyjna pojedynczego fotonu SPECT wykorzystuje radioaktywne znaczniki i skaner do rejestrowania dwu- lub trójwymiarowych obrazów ciała . Mała porcja radioaktywnego znacznika podawana jest dożylnie, a następnie wykonywane jest skanowanie ciała pacjenta podczas gdy znacznik jest wchłaniany przez tkanki. **SPECT** może dostarczyć informacji o przepływie krwi w tkankach i metabolizmie zachodzącym w ciele pacjenta.

W procedurze tej antyciała mogą być połączone z substancją radioaktywną. Gdy w ciele pacjenta występuje guz antyciała mogą łatwo przyłączyć się do komórek nowotworowych. Skany SPECT pozwalają więc na lokalizację guza.



## Ultradźwięki

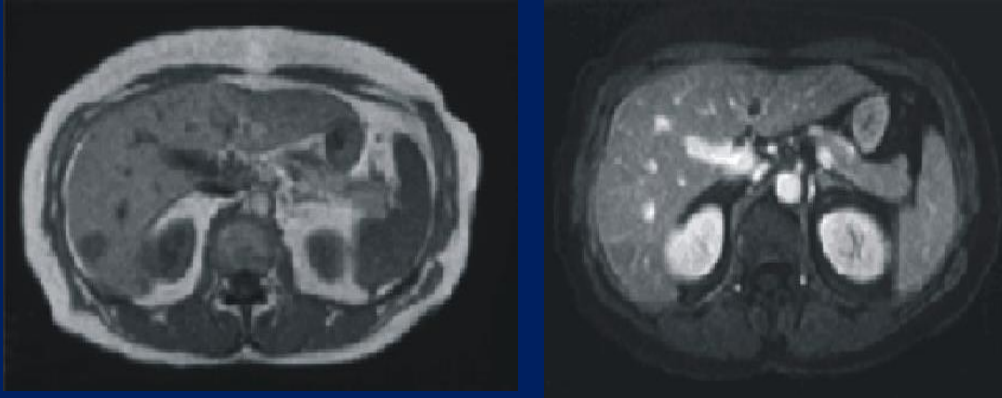
Ultrasonografia wykorzystuje fale dźwiękowe o częstościach powyżej wykrywalnych przez ucho ludzkie. Przetwornik wytwarza fale, które przenikają do ciała badanego pacjenta i ulegają odbiciu od organów oraz tkanek pozwalając na wytworzenie obrazu organów wewnętrznych. Ultradźwięki mogą być wykorzystywane do lokalizacji nowotworów i są pomocne podczas wykonywania biopsji



Ultrasound image of the liver; dark areas by arrows show possible tumors. Image courtesy of Dr. Thomas Shawker, National Cancer Institute.

# Magnetic Resonance Imaging (MRI)

## Obrazowanie jądrowym rezonansem magnetycznym



MRI scan without contrast showing possible tumor in the liver.  
MRI scan of the same patient using contrast.  
Images courtesy of Dr. Peter Choyke,  
Clinical Center, NIH.

Obrazowanie rezonansem magnetycznym wykorzystuje fale radiowe do badania pacjentów umieszczonych w silnym polu magnetycznym, a uzyskany obraz powstaje w oparciu o fale radiowe wyemitowane przez badane tkanki.

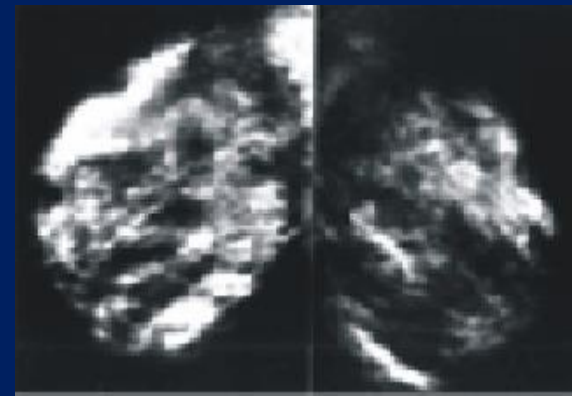
Różne tkanki włączając także guzy emitują mniej lub bardziej intensywne sygnały w zależności od ich chemicznego otoczenia, a obraz ciała pacjenta może zostać zapisany w postaci danych komputerowych. Obrazowanie rezonansem magnetycznym podobnie jak CT dostarcza obrazów dwu- i trójwymiarowych czasem jest jednak o wiele czulszą metodą szczególnie w rozróżnianiu tkanek miękkich.

## Mammografia cyfrowa

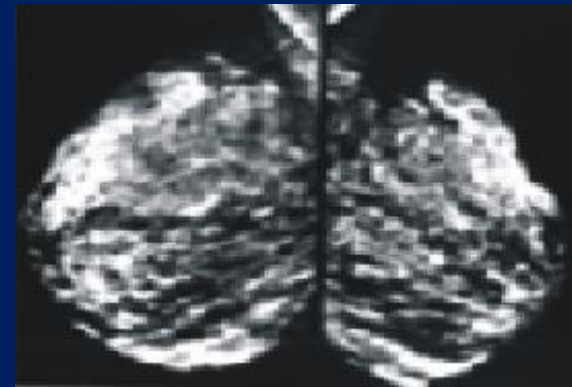
Mammografia konwencjonalna wykorzystuje promieniowanie X w celu identyfikacji zmian nowotworowych lub obszarów podejrzanych o charakter nowotworowy w piersi.

Mammografia cyfrowa również wykorzystuje promieniowanie X, ale dane gromadzone są w pamięci komputera zamiast na kliszy fotograficznej. Pozwala to na komputerowe opracowywanie obrazów w tym ich wzmacnianie czy też powiększanie.

A



B



A- konwencjonalna  
mammografia piersi.

B- mammografia  
cyfrowa  
tej samej pacjentki.

Autor Dr. Laurie Fajardo,  
Johns Hopkins  
Medical Institutions

## Table of Links

1

<http://dev1.cancer.gov/imaginginformation/CancerImagingClinicalTrials>

2

<http://www.radiologyinfo.org/index.cfm?bhcp=1>

3 <http://www.medicalimaging.org>

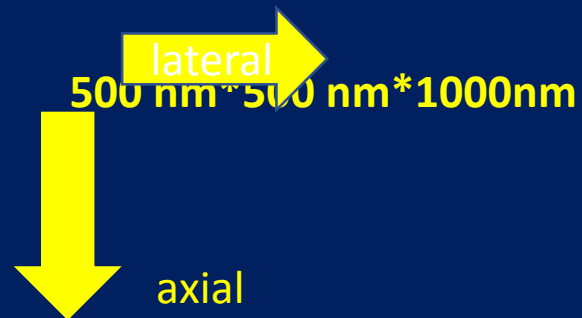
4 <http://www.cc.nih.gov/drd/colonoscopy.html>

5

<http://digestive.niddk.nih.gov/ddiseases/pubs/colonoscopy/index.htm>

# LASEROWA KONFOKALNA MIKROSKOPIA FLUORESCENCYJNA

- Laserowa konfokalna mikroskopia fluorescencyjna stała się standardem dla obrazowania optycznego oferując możliwość konstrukcji trójwymiarowych obrazów o rozdzielczości określonej przez kryterium Rayleigha, które określa lateralną rozdzielczość jako równą długości fali  $0.61\lambda$  światła próbującego, zaś rozdzielczość aksjalną jako  $2\lambda$
- Typowa próbkowana objętość w zakresie widzialnym wynosi więc około  $500\text{ nm} * 500\text{ nm} * 1000\text{ nm}$



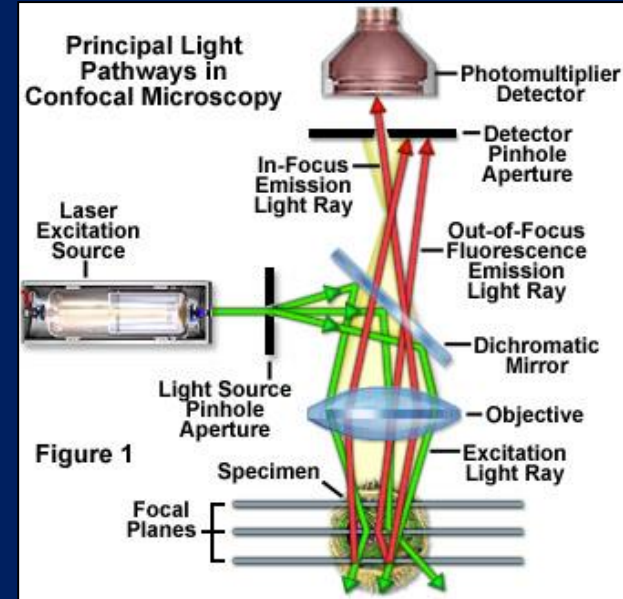
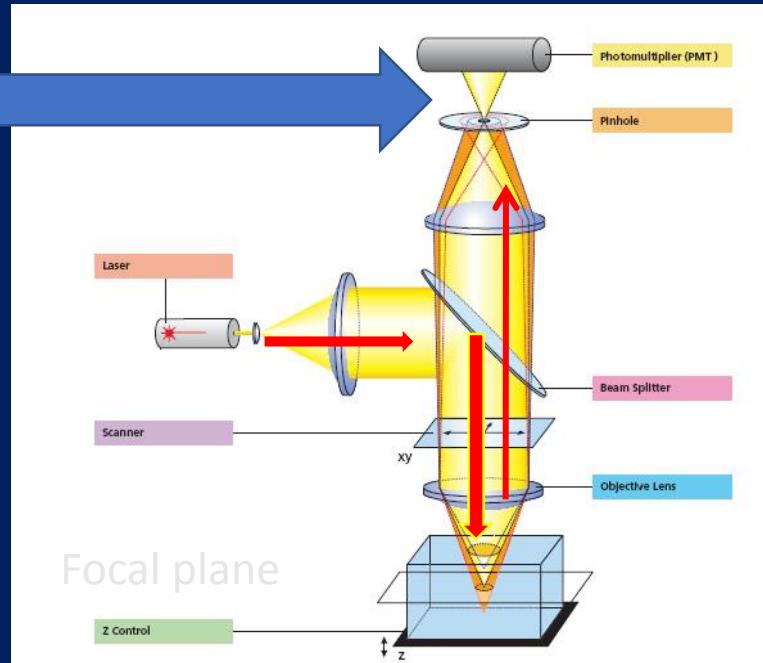
# MIKROSKOPIA KONFOKALNA

$$\delta_{lat} = 0.61 \lambda / NA$$

lateral

$$\delta_{ax} = 2 \lambda n / (NA)^2$$

axial



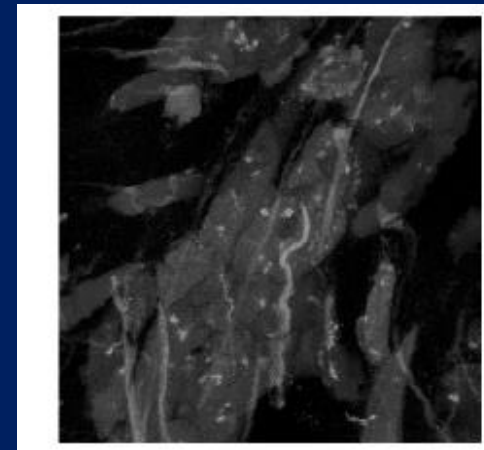
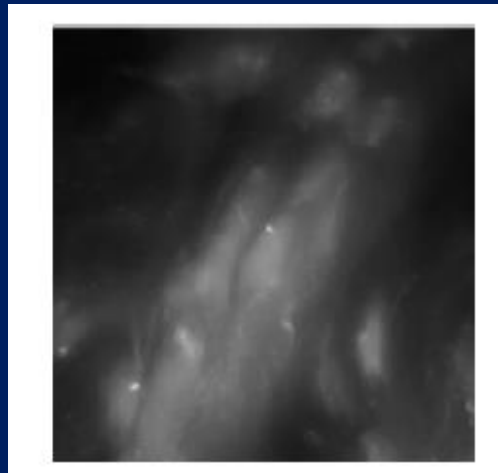
3D imaging and deep profiling due to confocal configuration



The lateral and axial spatial resolution,  $\delta_{lat}$  and  $\delta_{ax}$ , are determined by the diffraction limit, as discussed before:  
 $\delta_{lat} = 0.61 \lambda / NA$                        $\delta_{ax} = 2 \lambda n / (NA)^2$   
 where  $\lambda$  is the wavelength of exciting light,  $n$  the refractive index, and  $NA$  the numeric aperture of the objective.



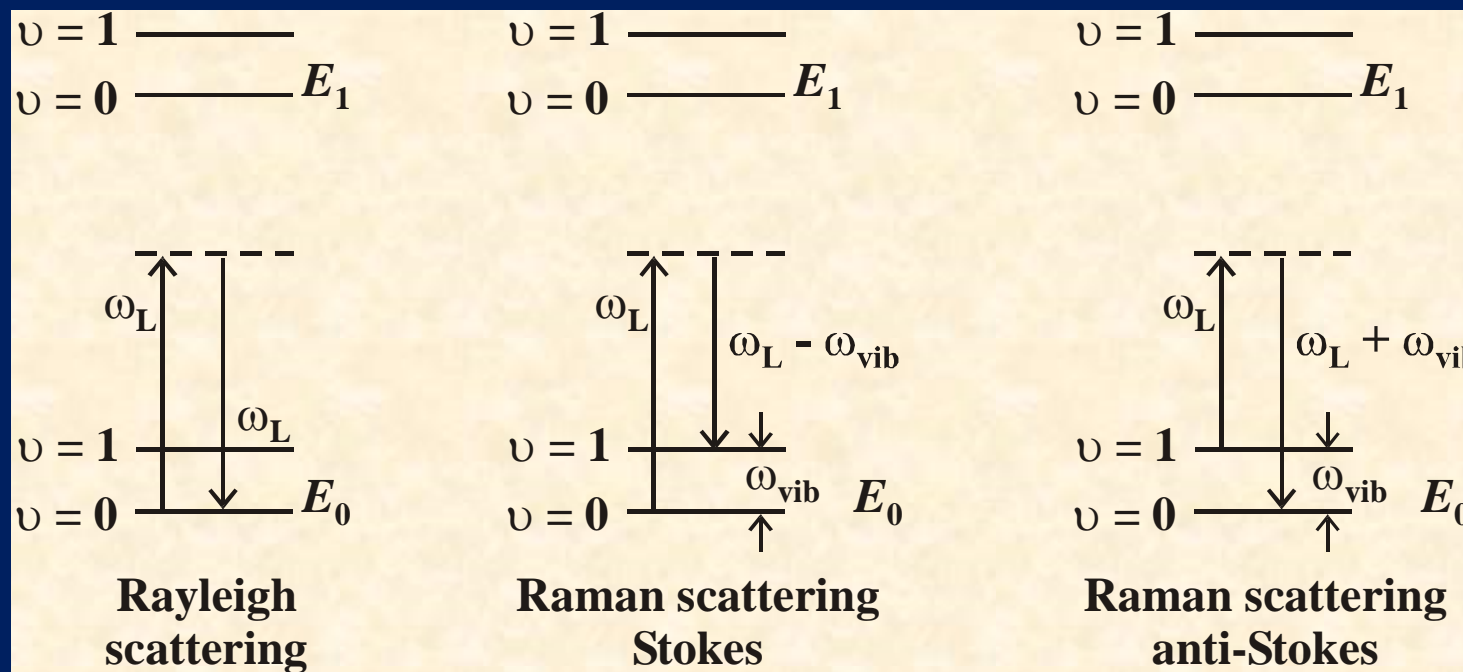
*Fig. 3 Non-confocal (top) and confocal (bottom) image of a double-labeled cell aggregate (demonstration object). In the non-confocal image, specimen planes outside the focal plane degrade the information of interest from the focal plane, and differently stained specimen details appear in mixed color. In the confocal image (bottom), specimen details blurred in non-confocal imaging become distinctly visible, and the image throughout is greatly improved in contrast.*



[http://www.hopkinsmedicine.org/micfac/refman/Zeiss/LSM510Meta/Principles\\_of\\_Confocal\\_Microscopy.pdf](http://www.hopkinsmedicine.org/micfac/refman/Zeiss/LSM510Meta/Principles_of_Confocal_Microscopy.pdf)

# RAMAN IMAGING

## OBRAZOWANIE RAMANA





# No technology has proven effective for detecting invasive cancer

- Surgical removal of tumors is the best treatment, but current methods do not address the invasion of cancer cells into healthy tissue. And until now, no technology has proven effective for detecting invasive cancer.
  - Existing clinical technologies—including state-of-the-art neurosurgical microscopy, fluorescence-guided surgery and magnetic resonance imaging (MRI, used either pre- or intra-operatively)—cannot detect the full extent of cancer invasion.
- 
- *BioOptics World, 09/13/2017*
  - By Barbara Gefvert

# Visualize cancer in real time as doctors operate

- For these reasons, a great need exists for practical tools that fit into the current surgical practice, and that enable surgeons to visualize cancer in real time as they operate.



There is an urgent need to improve the conventional methods of cancer diagnostics. For example, it has in fact been found that 70–90% of mammographically detected lesions are found to be benign upon needle biopsy.

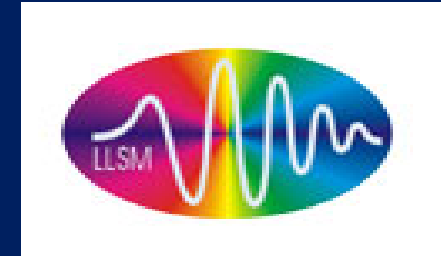
Current imaging methods are often limited by inadequate sensitivity, specificity, spatial and spectral resolutions.

MRI- limited spatial resolution

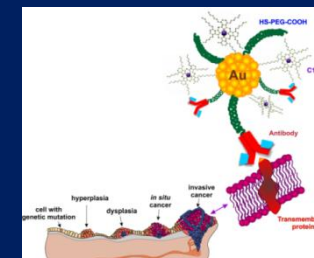
FLUORESCENCE- limited spectral resolution

# Obrazowanie Ramana/AFM/SNOM/TERS

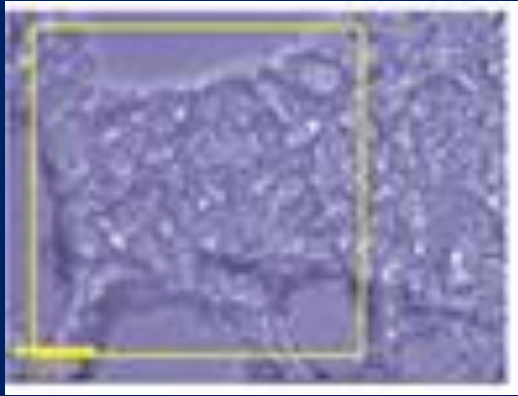
CEL



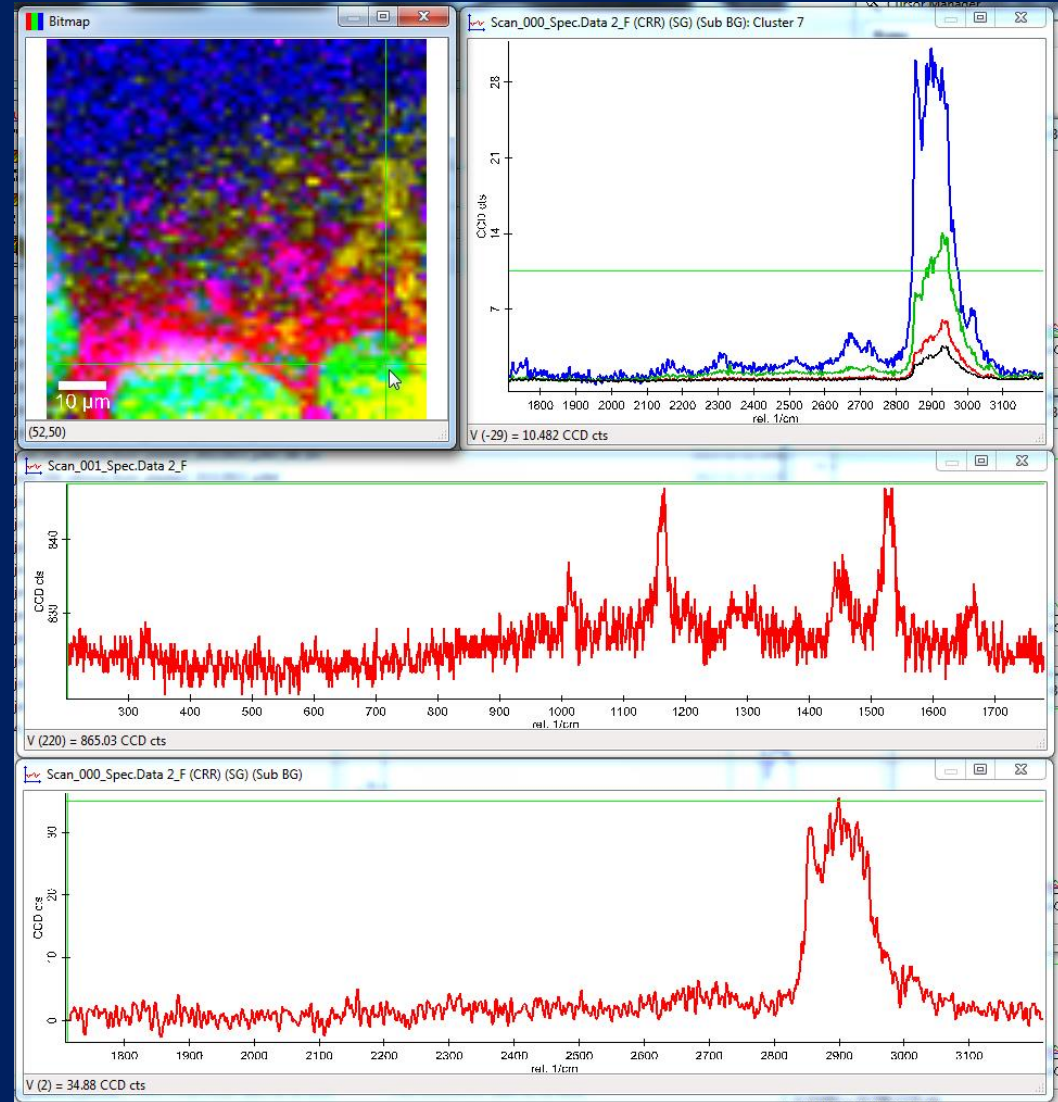
- **Obrazowanie Ramana w połączeniu z ultraszybką spektroskopią femtosekundową może dać nadzieję w rozpoznawaniu nowotworów. Zastosowanie takiego połączenia oferuje niedoścignione możliwości czasowo-przestrzenne, czułość oraz możliwość multipleksacji.**



# OBRAZOWANIE RAMANA

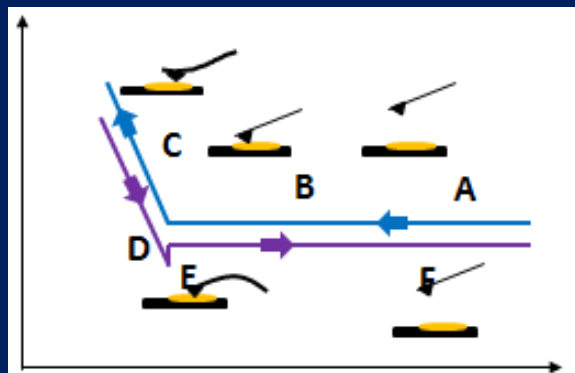


Spektroskopia i obrazowanie Ramana mogą identyfikować składniki tkanki, takie jak komórki nabłonkowe i macierz zewnątrzkomórkowa, która tworzy środowisko, w którym proliferują komórki nowotworowe. Dlatego obrazowanie Ramana jest doskonałą metodą badania składu biochemicznego, w tym zmian, które prowadzą do transformacji nowotworowej i umożliwia wykrywanie nieprawidłowości genetycznych w komórkach nowotworowych bardziej precyzyjnie i kompleksowo niż metody klasyczne.

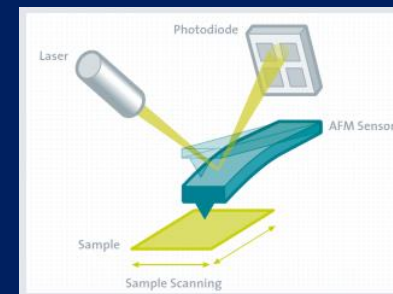




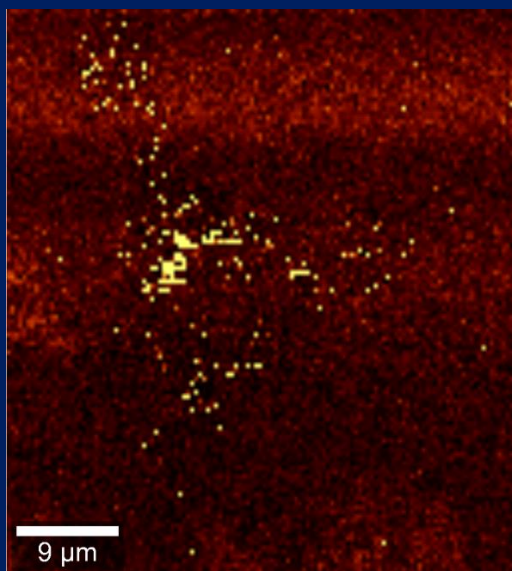
MIĘDZYRESORTOWY INSTYTUT TECHNIKI RADIACYJNEJ  
WYDZIAŁ CHEMICZNY POLITECHNIKI ŁÓDZKIEJ



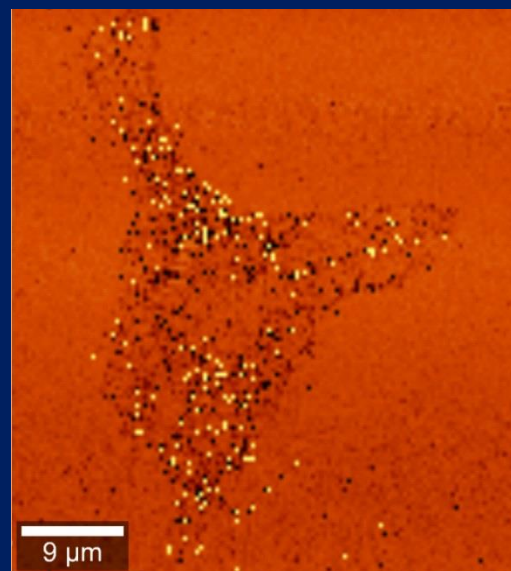
OM



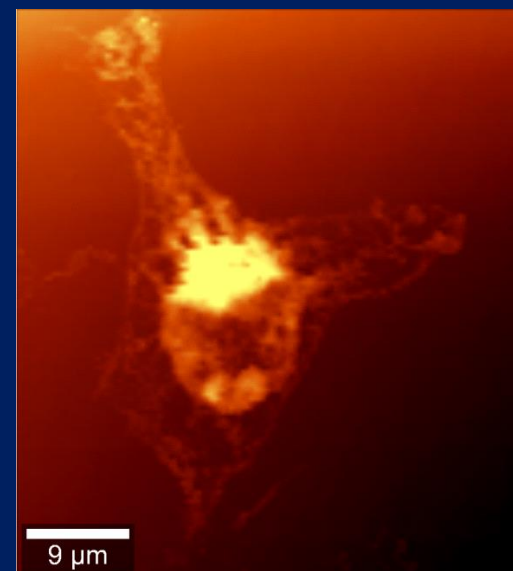
mitr.p.lodz.pl/raman



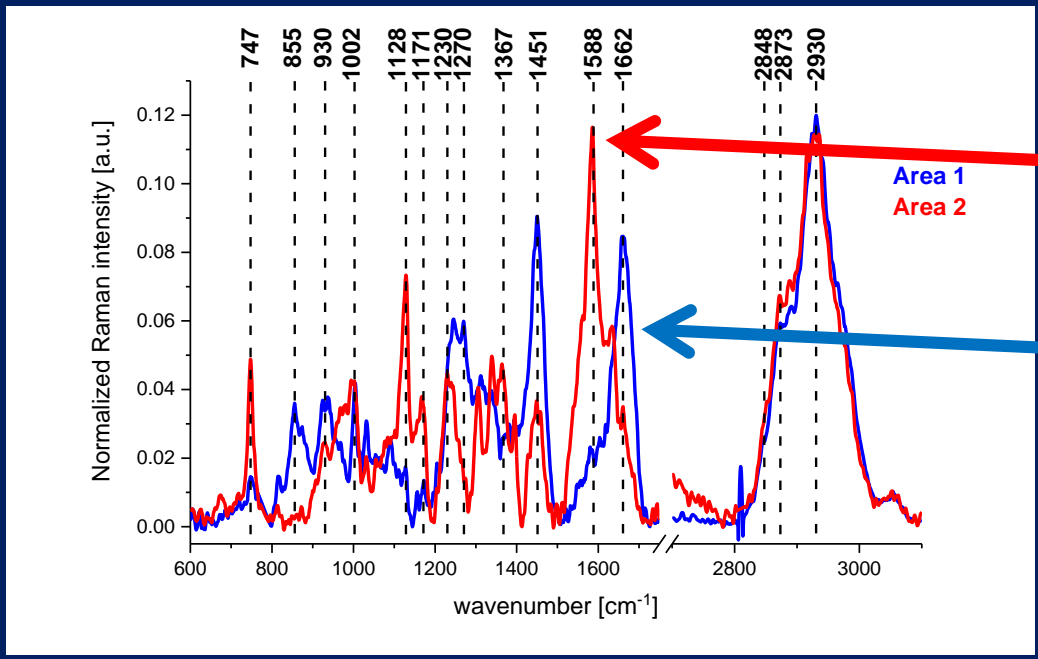
adhezja



szttywność



topografia



guz

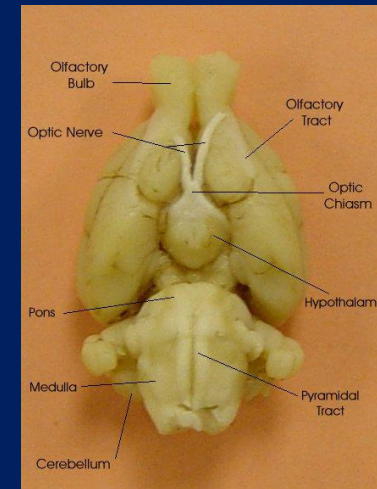
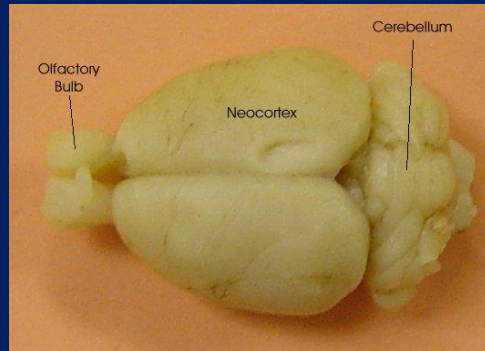
Zdrowa/  
ew.przerosnieta  
komorkami  
nowotworowym  
i

**Glioblastoma multiforme**  
Odrost po pierwszym usunieciu

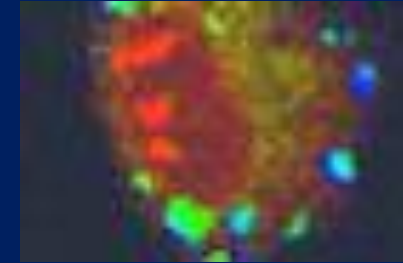
**Raman spectroscopy has proven effective for detecting invasive cancer (our results LLSM)**



# IN VIVO RAMAN OPTICAL BIOPSY on RAT BRAIN IN LABORATORY OF LASER MOLECULAR SPECTROSCOPY



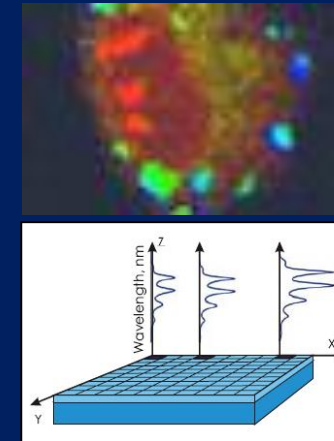
# Raman imaging



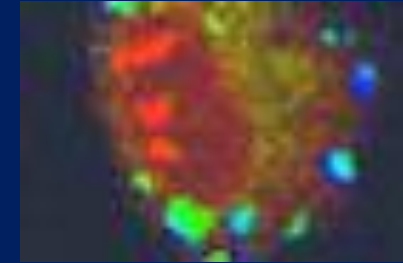
- Raman spectrometer
- Confocal Raman microscope
- Collection of spatially resolved spectroscopic data by raster-scanning the focused laser beam over the sample, or by moving the sample through the laser focus in a raster pattern via a high resolution microscope stage
- The resulting spectral data can be converted into spectral images
- Improvement of spectral images with different statistical methods, Principal Component analysis (PCA), Vertex Component Analysis (VCA), Hierarchical Cluster Analysis (HCA)

# Raman imaging

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Typically, the sample in Raman imaging is illuminated with ca. 10 mW of the laser power focused into a spot of ca. 200 nm radius that gives a power density of  $10^7$  W/cm<sup>2</sup> which is about a limit of damage for the biological tissue.

# KONFOKALNA MIKROSKOPIA RAMANA

## 1.5 Confocal Raman Microscopy

Confocal microscopy requires a point source (usually a laser), which is focused onto the sample. The reflected light (Raman, fluorescence) is collected with the same objective and focused through a pinhole at the front of the detector (Fig. 3). This ensures that only light from the image focal plane can reach the detector, which greatly increases image contrast and with the proper selection of pinhole size, slightly increases resolution (max. gain in resolution: factor  $\sqrt{2}$ ).

For Raman microscopy, the enhancement of image contrast and depth resolution is very important. An enhancement of the lateral resolution in confocal microscopy requires extremely small pinhole diameters and will therefore decrease the detection efficiency to a level usually unacceptable in most experiments (Fig. 4).

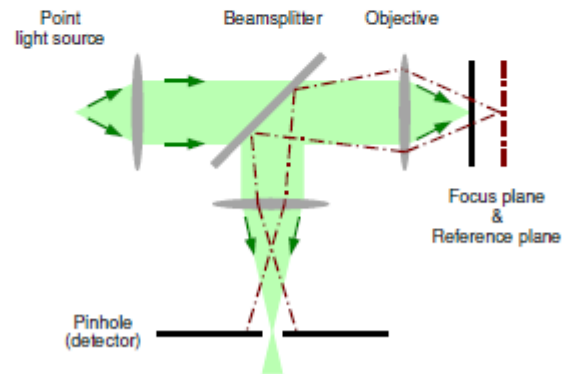


Fig. 3: Principal setup of a confocal microscope

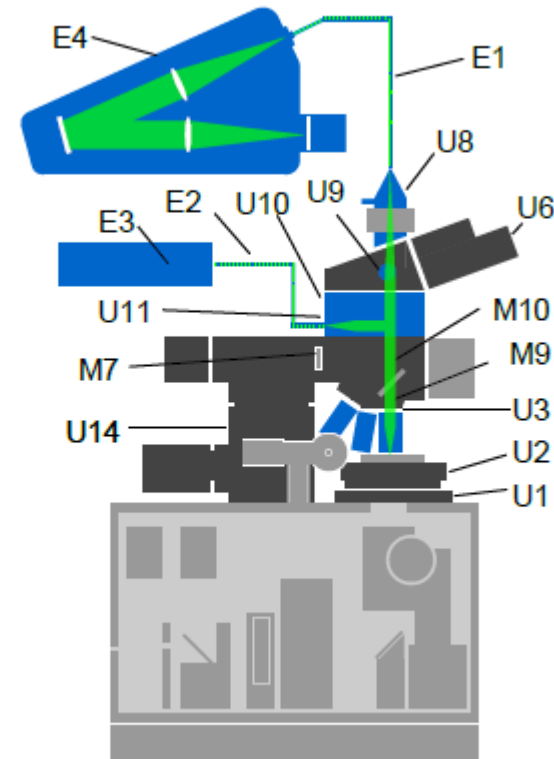


Fig. 8: Schematic illustration of the beam path for confocal Raman microscopy.

# SNOM

## MIKROSKOPIA BLISKIEGO POLA PRZEŁAMUJE LIMIT DYFRAKCYJNY!

### 3.1 SNOM AC in transmission configuration

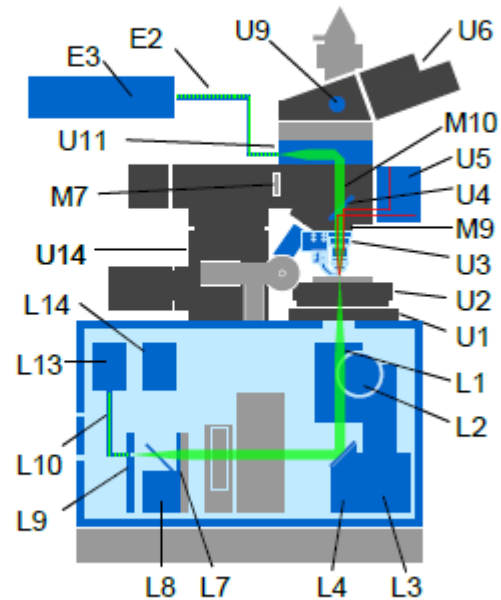
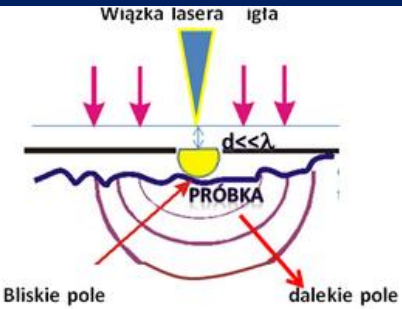
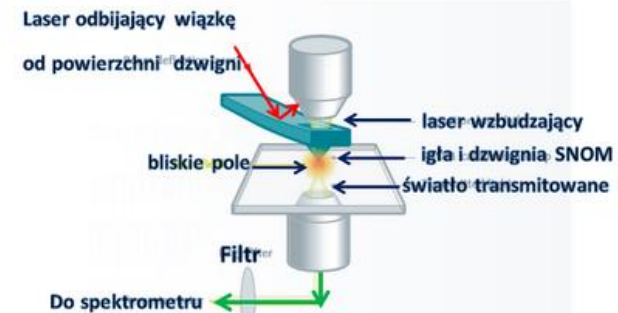


Fig. 5: Schematic illustration of the beam path for Scanning Near-field Optical Microscopy in AC transmission mode.



a)



b)

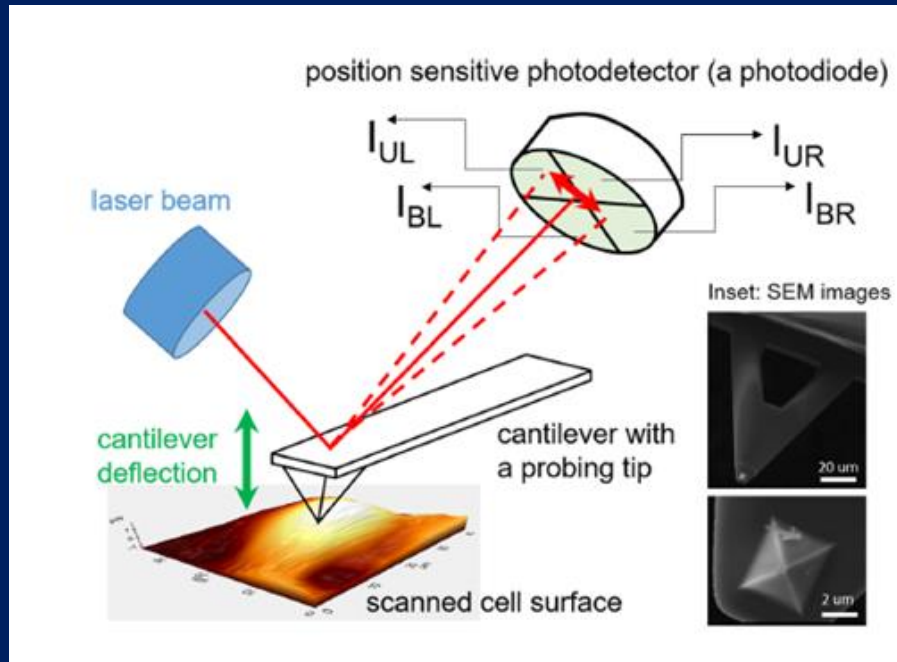
Rys. 3 Ilustracja idei pomiarów SNOM (a) i zasada działania na podstawie instrukcji Witec near-field-Raman imaging, Ulm, Germany (b).

# AFM (MIKROSKOPIA SIŁ ATOMOWYCH)

AFM nie jest oparty na rozdzielności przestrzennej ograniczonej dyfrakcją. Rozdzielczość przestrzenna

zależy od rozmiaru ostrza i jest znacznie poniżej granicy dyfrakcji.

AFM to typ mikroskopii z sondą skanującą (SPM) o bardzo wysokiej rozdzielczości, z rozdzielczością rzędu ułamków nanometra, ponad 1000 razy lepszą niż granica dyfrakcji optycznej.



Wykrywanie poziomego światła laserowego w oparciu o matrycę fotodiodową

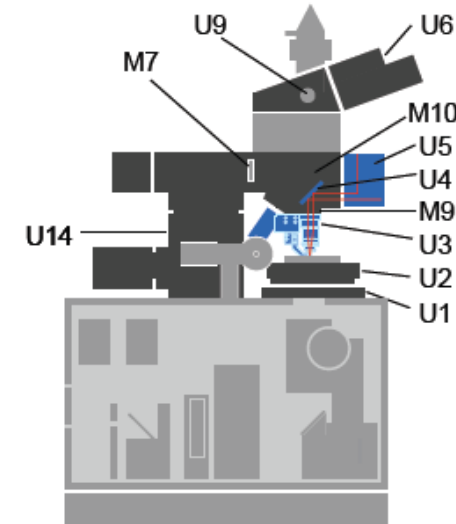


Fig. 5: Schematic illustration of the beam path for AFM AC Mode.

**WITec**  
focus innovations

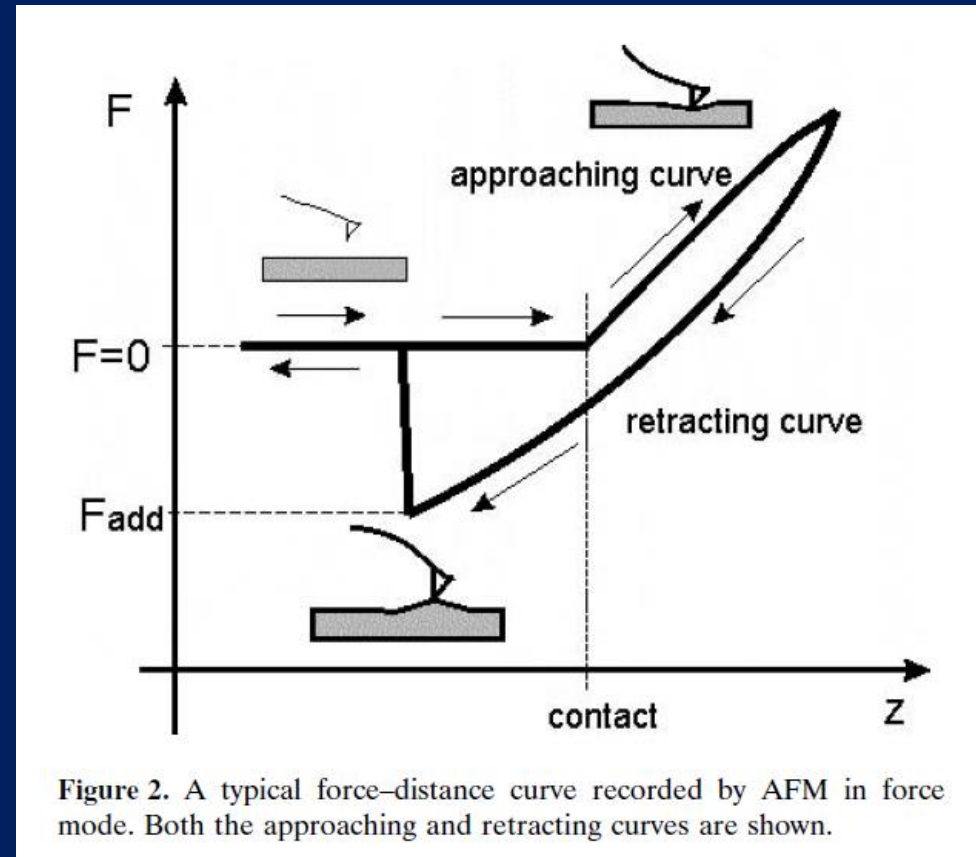
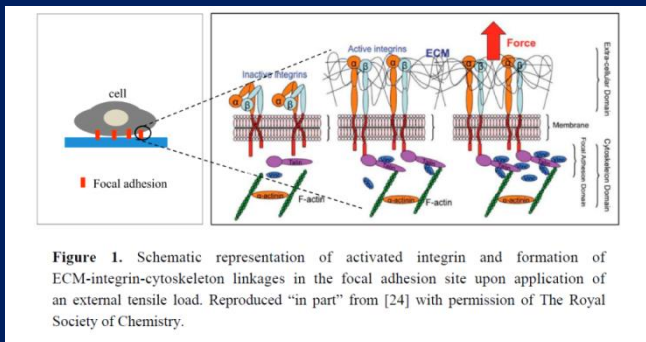
## AFM IN AC MODE

- U1 XY positioner
- U2 Scan stage
- U3 Objective turret with objectives including the inertial drive and the SNOM tip
- U4 Dichroic mirror
- U5 Beam deflection unit
- U6 Binocular tube with ocular camera
- U9 Pushrod
- U14 Microscope Z stage with stepper motor

# ADHEZJA

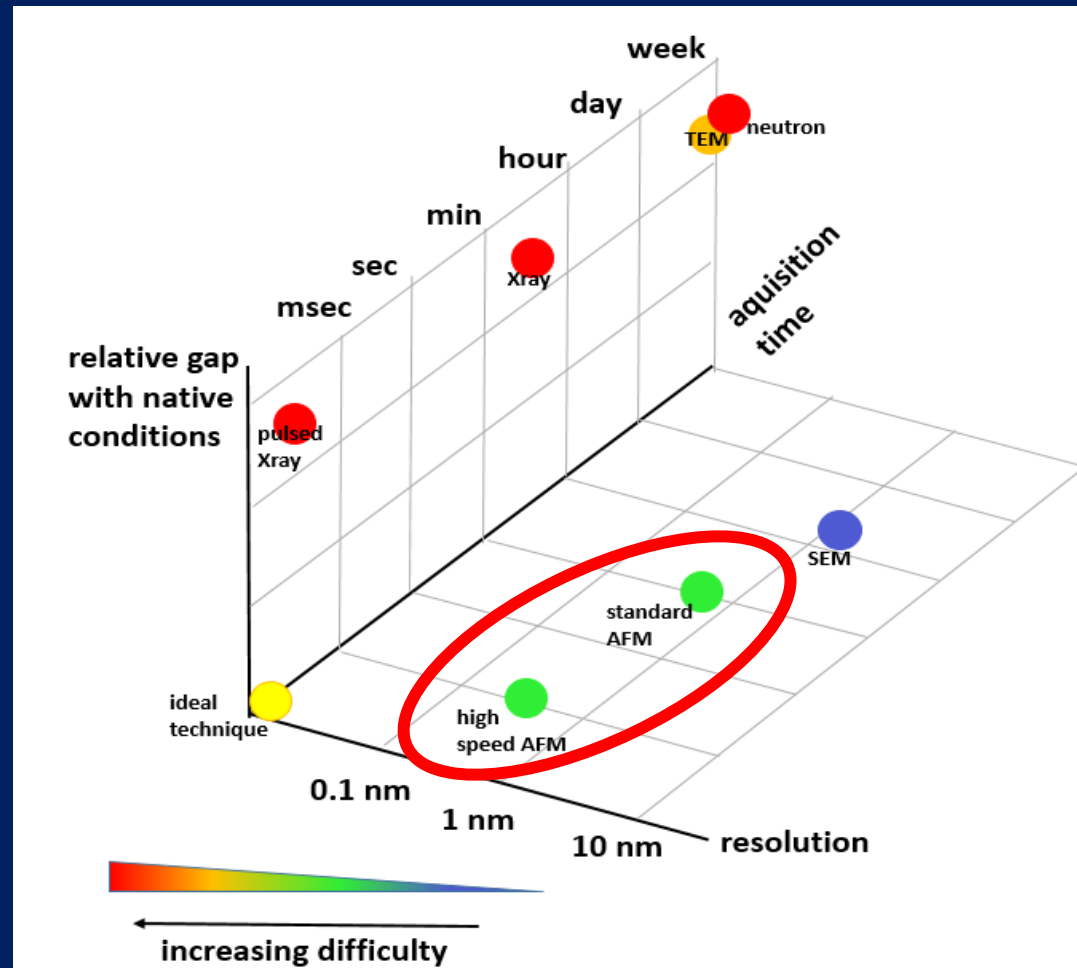
- Kolejną interesującą cechą krzywej retracting jest niezerowa siła wymagana do oderwania końcówki sondy od powierzchni. Jest to tak zwana siła adhezji. Pojawia się z powodu słabych sił (takich jak siły van der Waalsa) działających między końcówką sondy a powierzchnią próbki podczas kontaktu

*Int. J. Mol. Sci.* 2015, 16

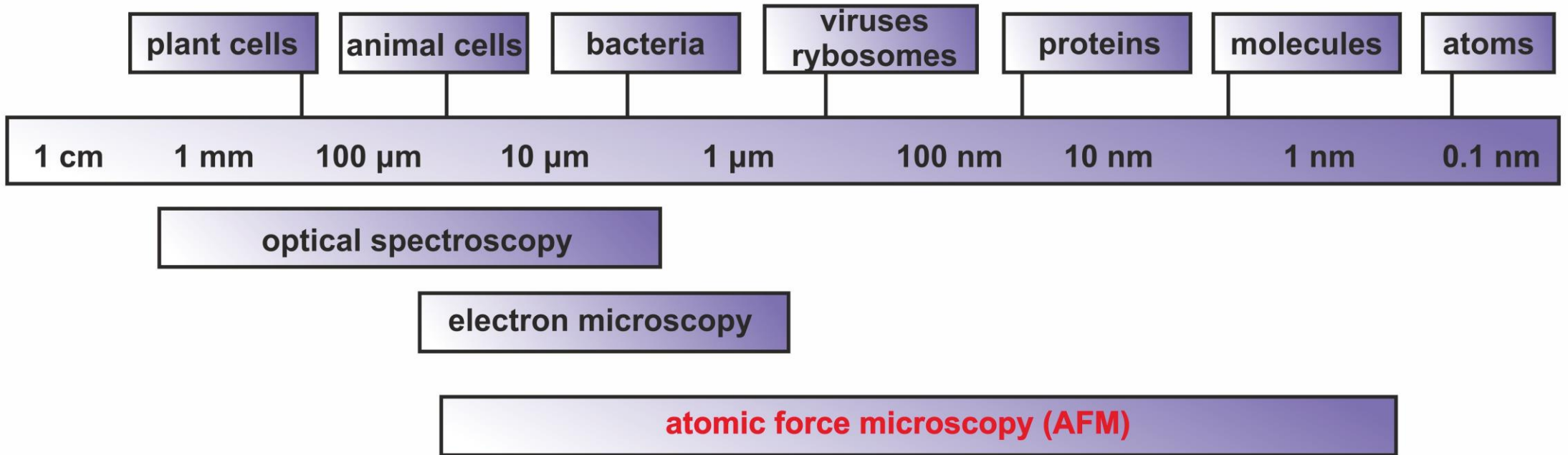




# ROZDZIELCZOŚĆ PRZESTRZENNA RÓŻNYCH METODY OBRAZOWANIA

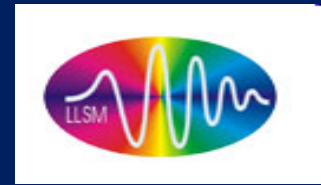


Zatem pomyslane obserwacje dynamiki nanostrukturalnej w żywych neuronach mogą w najbliższej przyszłości otworzyć możliwość wizualizacji morfologii plastyczności synaps w rozdzielczości nanometrycznej w czasie rzeczywistym.



AFM jest przydatna do uzyskiwania informacji topograficznej 3D próbek o rozdzielczości poprzecznej (w płaszczyźnie x / y) do 0,3 nm i rozdzielczości pionowej (w osi z) do 0,1 nm [26]. Próbkami te obejmują skupiska atomów i cząsteczek [27], pojedyncze makrocząsteczki [28] i cząsteczki biologiczne (komórki, DNA, białka) [29, 30].

# NANOONCOLOGICAL PLATFORM in our laboratory



- **CONFOCAL IMAGING RAMAN MICROSCOPY (High spatial resolution )**
- **SNOM MICROSCOPY (far below the diffraction limit, SNOM)**
- **IR IMAGING MICROSCOPY**
- **ATOMIC FORCE MICROSCOPY IMAGING (topography, stiffness and adhesion imaging)**
- **FEMTOSECOND PUMP-PROBE SPECTROSCOPY (High temporal resolution)**
- **The real-time *in vivo* neurosurgical Raman system**

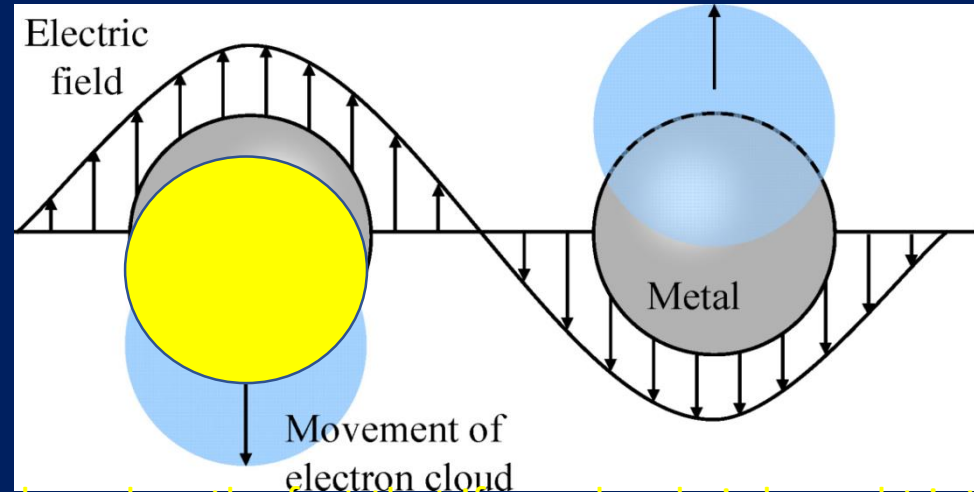
# SERS methods

- Despite the high specificity (vibrational fingerprint) , traditional Raman spectroscopy was considered limited because of the very poor efficiency of the inelastic scattering processes and thus the relatively weak signal.
- Surface enhanced Raman spectroscopy or SERS helps to solve this problem allowing detection of chemical species at parts per million (ppm) levels and the improvement of SERS by resonance conditions

Schematic of plasmon oscillation for small metallic spheres Journal of Innovative Optical Health Sciences Vol. 1, No. 2 (2008) 267–284

**a diameter much smaller than the wavelength of the excitation light.**

**plasmon**



The SERS technique is based on the fact that if a molecule is brought into close proximity with a metal (Au, Ag) nanostructure or nanoparticle that results in significant increase in the intensity of the Raman spectra.

The enhancement mechanism for SERS comes from intense localized fields arising from surface plasmon resonance in metallic (e.g. Au, Ag, Cu) nanostructures with sizes of the order of tens of nanometers, **a diameter much smaller than the wavelength of the excitation light.**

The amplification of Raman scattering in SERS effect is generally generated by two mechanisms: (i) electromagnetic-field enhancement through the localization of optical fields in metallic nanostructures, and (ii) chemical or electronic enhancement due to the increase of Raman cross-section when the molecule or lattice is in contact with metal nanostructures.

By immobilising a coloured molecule (Raman reporter) onto a suitably roughened metal surface of the nanoparticle, extremely strong SERRS signals can be obtained with an overall enhancement factor of up to  $10^{14}$  enabling monitoring the genetic and immunological responses in biological systems.

# SERS combined with nanoparticles

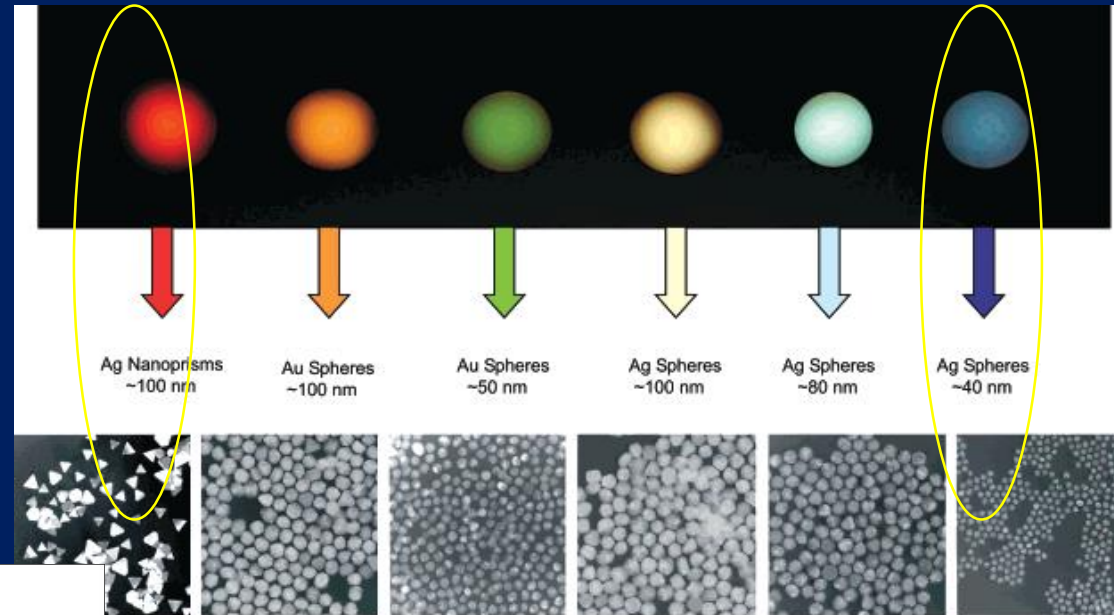
What we presented so far it is all that physics and molecular spectroscopy can do on its own – go to the edge of the non-diffraction limited imaging.

As we said the enhancement mechanism for SERS comes from intense localized fields arising from surface plasmon resonance in metallic (e.g. Au, Ag, Cu) nanostructures with sizes of the order of tens of nanometers, a diameter much smaller than the wavelength of the excitation light.

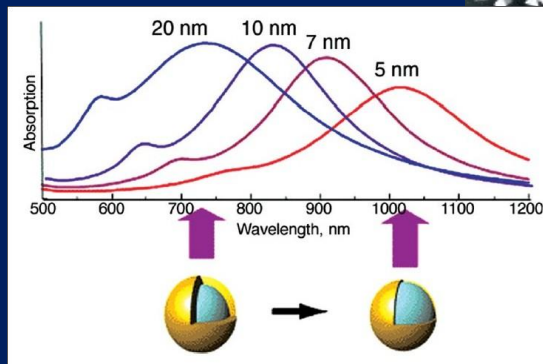
The plasmon resonance can be obtained at the roughened metal surfaces, vapor deposited films, electrodes and nanoparticles. Silver and gold nanoparticles are used in SERS because they have plasmon frequencies in the visible. When we attach a coloured molecule to the nanoparticle we obtain further enhancement – in resonance Raman conditions SERRS.

Next step is to enhance the SERS signal with help of nanotechnology.

Sizes, shapes, and compositions of metal nanoparticles can be systematically varied to produce materials with distinct light-scattering properties

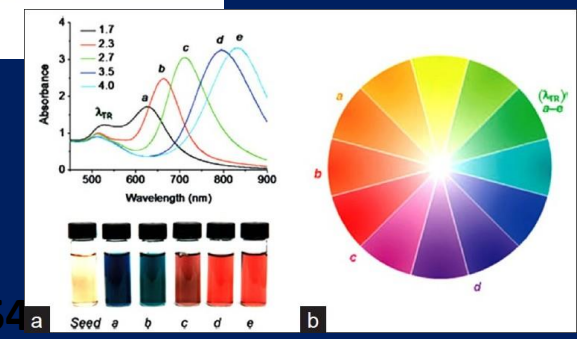


Gold nanoshell plasmon resonances for a 120-nm core with indicated shell thickness



Journal of Pharmacy & BioAllied Sciences

200nm (same for all the images)



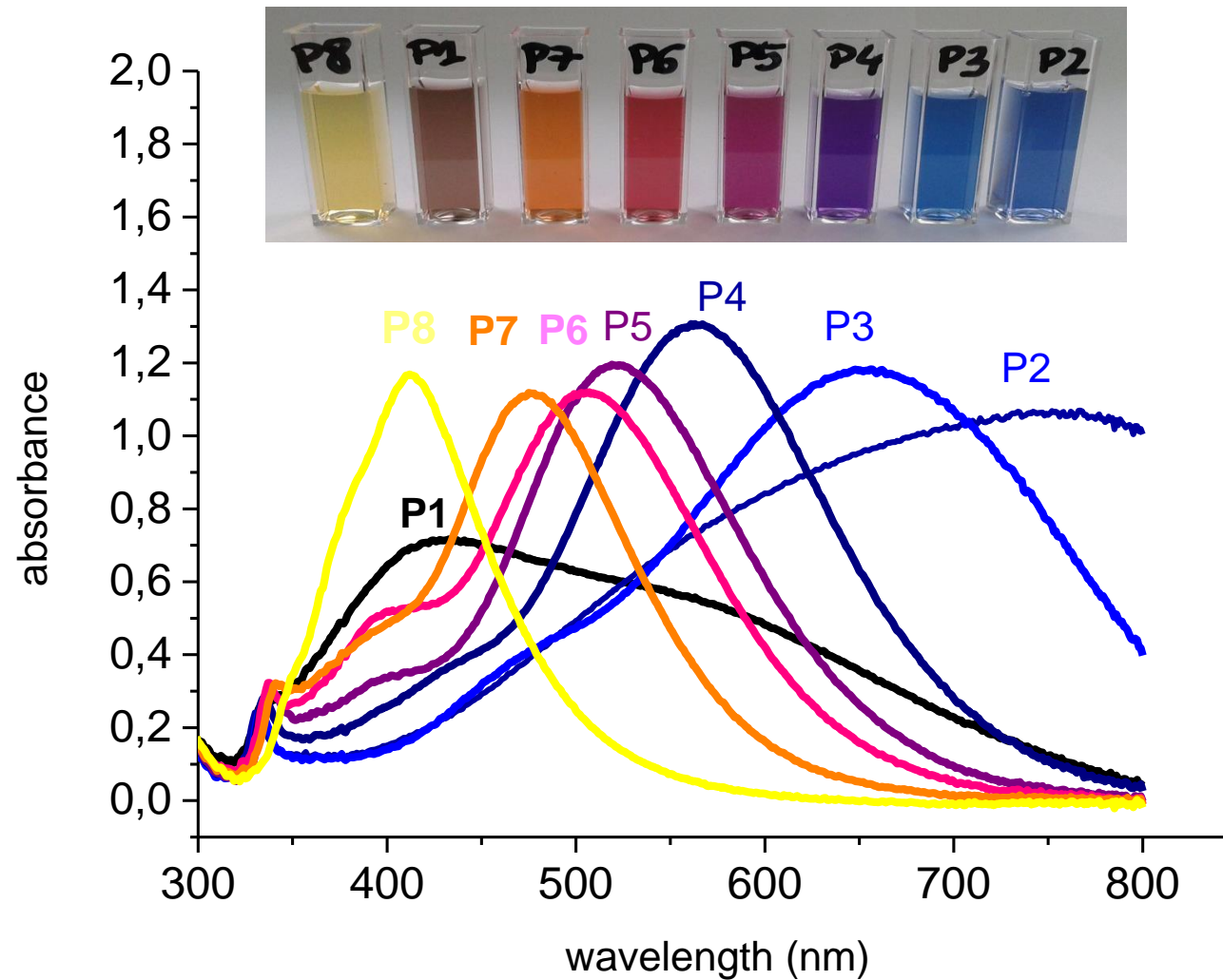
Chemical Reviews, 2005, Vol. 105, No. 4 154

Gold nanorods

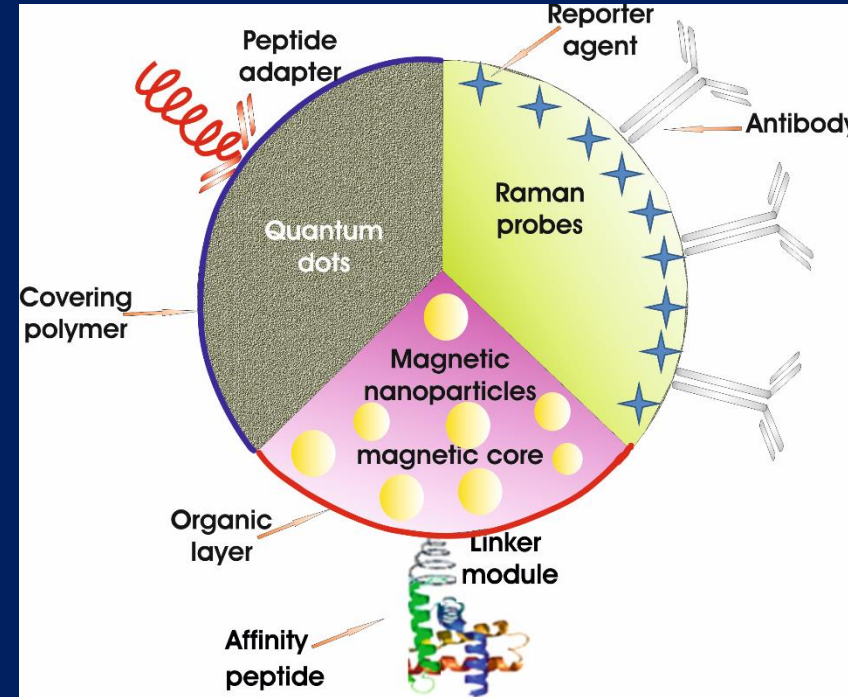
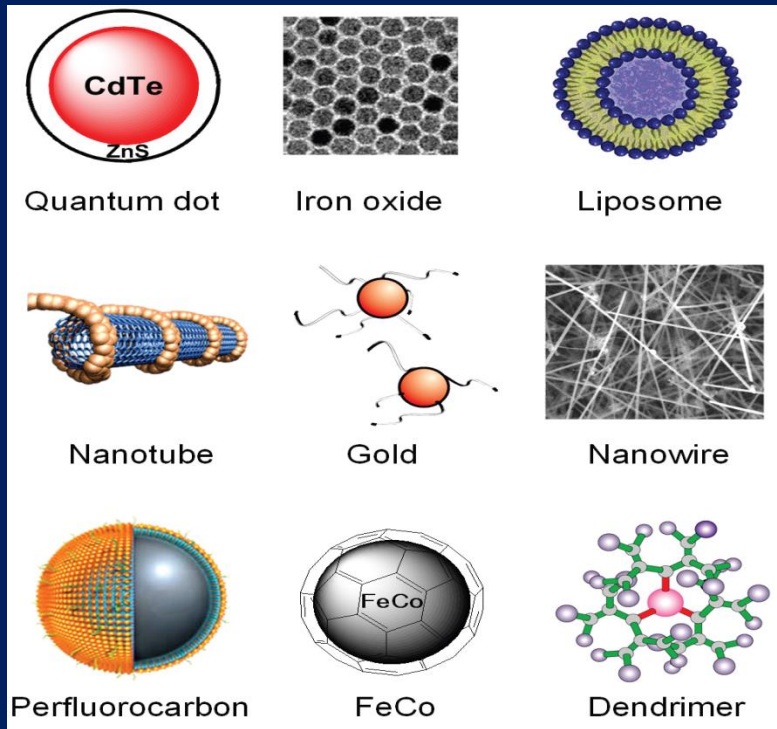
Localized surface plasmons (LSP) resonance can be tuned on a spectral range from the visible up to the far IR in the case of gold nanostructures



# Nanoparticles produced in our lab



Many nanoparticles have been investigated for biomedical applications targeting cancer.



- **inorganic quantum dot (QD) nanocrystals**
- (Bruchez et al., 1998; Chan and Nie, 1998;Michalet et al., 2005)
- **Gold and silver nanoparticles**
- (Sokolov et al., 2003; Reinhard et al., 2005)
- **core–shell-type nanoparticles**

gold nanoshells (Lal et al., 2008; Wang et al., 2007; Loo et al., 2005), nanocrescents (Lu et al., 2005) and hollow gold nanospheres (HGNs) (Schwartzberg et al., 2006a,b; Sun et al., 2003)

*Lancet Oncol 2006; 7: 657–67*

SERS reporters and biosensors detecting the genetic,  
immunological or cell based markers associated with  
cancer  
sensitivity  
specificity  
~~multiplexing~~

There are a few recognition schemes that may involve DNA hybridization, aptamer recognition, supramolecular host-guest complexation, or antibody-antigen pairing

# RECOGNITION SCHEMES

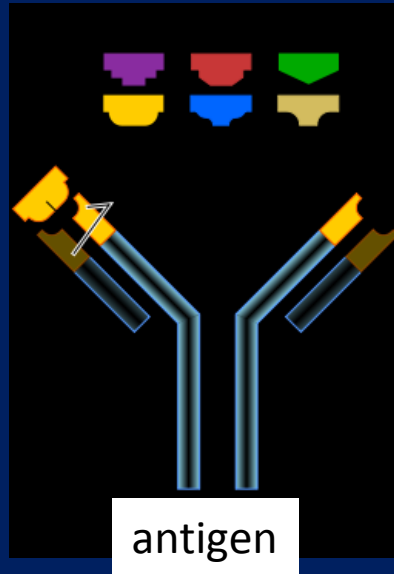
- **DNA hybridization**
- **aptamer recognition**
- **supramolecular host-guest complexation**
- **antibody-antigen pairing**

How to reach selective interaction?

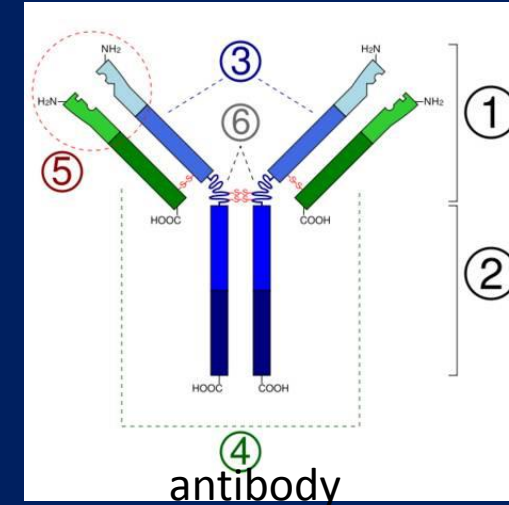
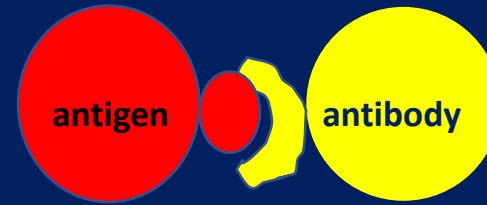
binding specificity

Answer: antibody-antigen interactions

Each antibody binds to a specific antigen;  
interaction is similar to a lock and key.



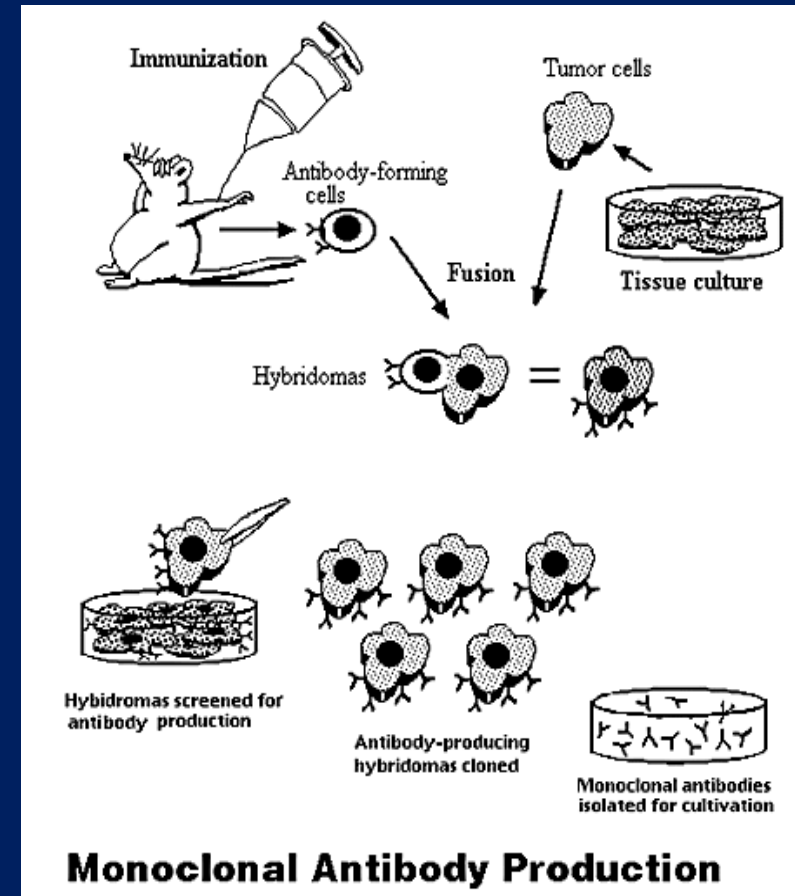
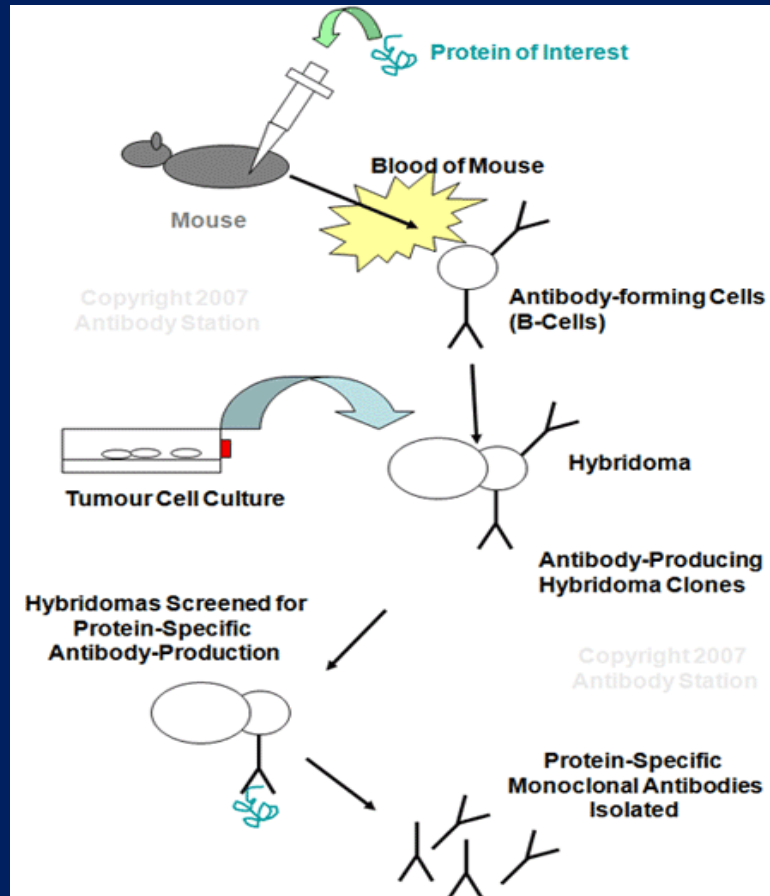
An antigen is a protein molecule that  
triggers antibody generation



Fortunately, nature provides a solution.  
The antibody has an unique ability to bind  
with high specificity to the antigen. Each  
antibody binds to a specific antigen; an  
interaction is similar to a lock and key.

An antibody is a protein molecule (immunoglobulin) that has one or more combining sites called paratopes. An antigen is a general term for a molecule that may trigger **antibody generation**, with potentially many different features surface. Antigenic determinants are those surfaces features of the antigen that are complementary to an antibodies combining site.

# Monoclonal Antibody Production

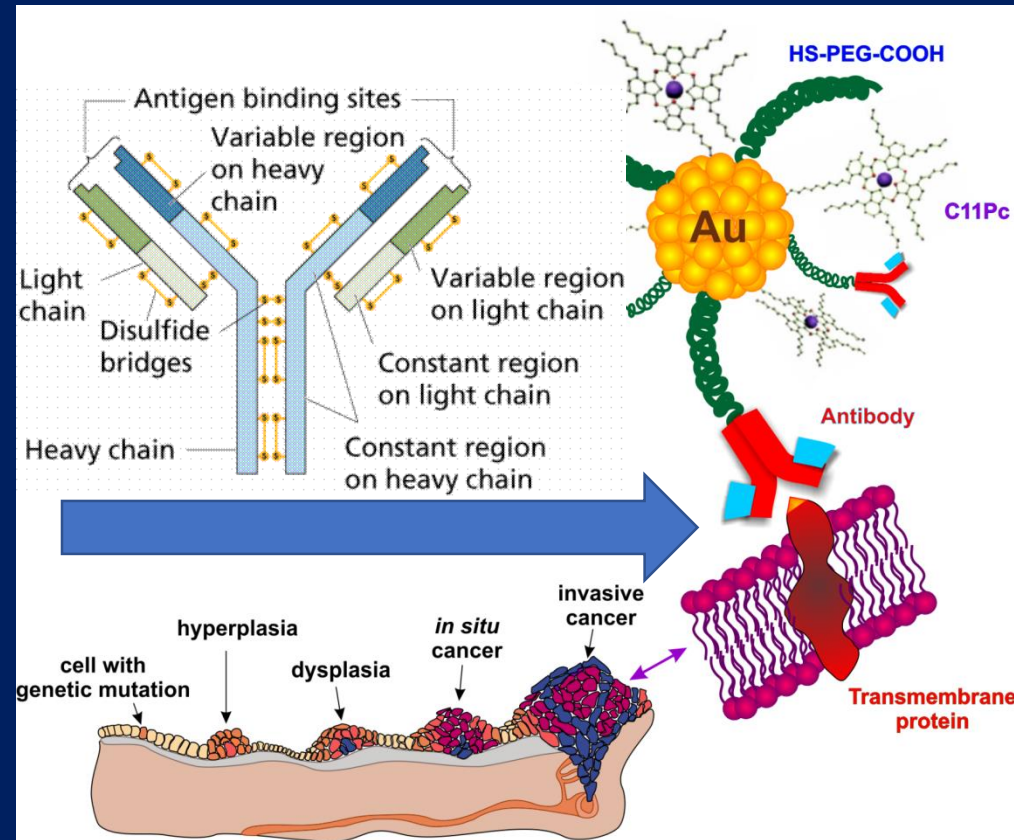


Molecular biology knows a range of methods to produce any kind of antibodies

# Biocojugates

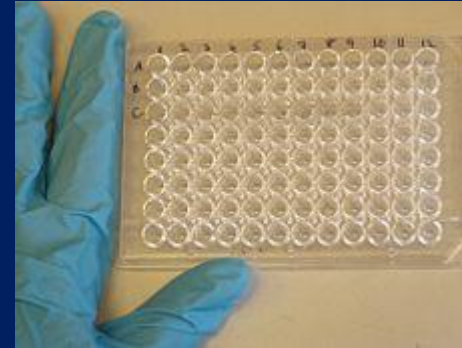
There are a number of formats used to provide Raman signal. Currently, a promising way to catch cancer lesions early is to use Raman reporters coupled with nanoparticles and antibodies that recognise and bind to cancer cells.

When conjugated with biomolecular targeting ligands such as monoclonal antibodies, peptides or small molecules, these nanoparticles can be used to target malignant tumors with high specificity and affinity.





# ELISA



A 96-well microtiter plate being used for ELISA

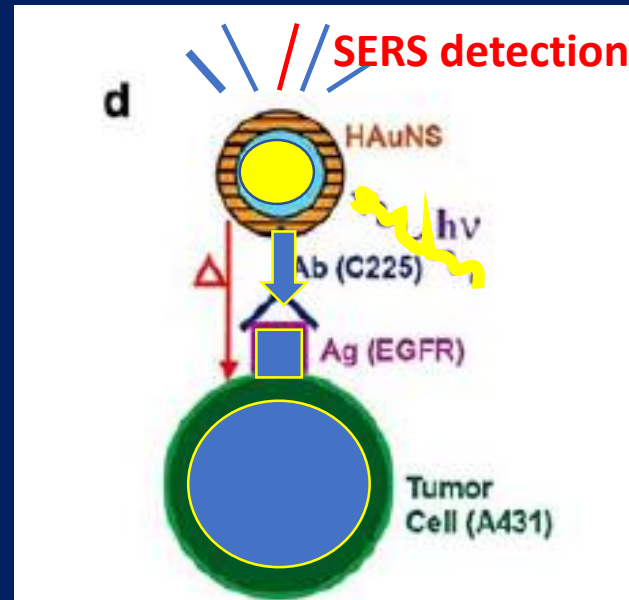
**Enzyme-linked immunosorbent assay (ELISA)**, also known as an **enzyme immunoassay (EIA)**, is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.

Traditional ELISA typically involves chromogenic reporters and substrates that produce some kind of observable color change to indicate the presence of antigen or analyte. Newer ELISA-like techniques utilize fluorogenic, electrochemiluminescent, and real time PCR reporters to create quantifiable signals. These new reporters can have various advantages including higher sensitivities and multiplexing. In technical terms, newer assays of this type are not strictly ELISAs, as they are not "enzyme-linked" but are instead linked to some non-enzymatic reporter. However, given that the general principles in these assays are largely similar, they are often grouped in the same category as ELISAs.

# Antibody-antigen interaction protein-protein interaction

C225- human-mouse  
chimerized  
monoclonal antibody  
directed against the  
epidermal growth  
factor receptor (EGFR)

EGFR-epidermal  
growth factor  
receptor



Society]. **d** Schematic illustration of the concept of SERS detection and photothermal therapy using HGNS based on antibody (C225)-antigen (EGFR) interaction that targets A431 carcinoma cancer cells (Schwartzberg and Zhang 2008) [Adapted with permission from the American Chemical Society]

HAuNs -  
hollow gold nanoparticles

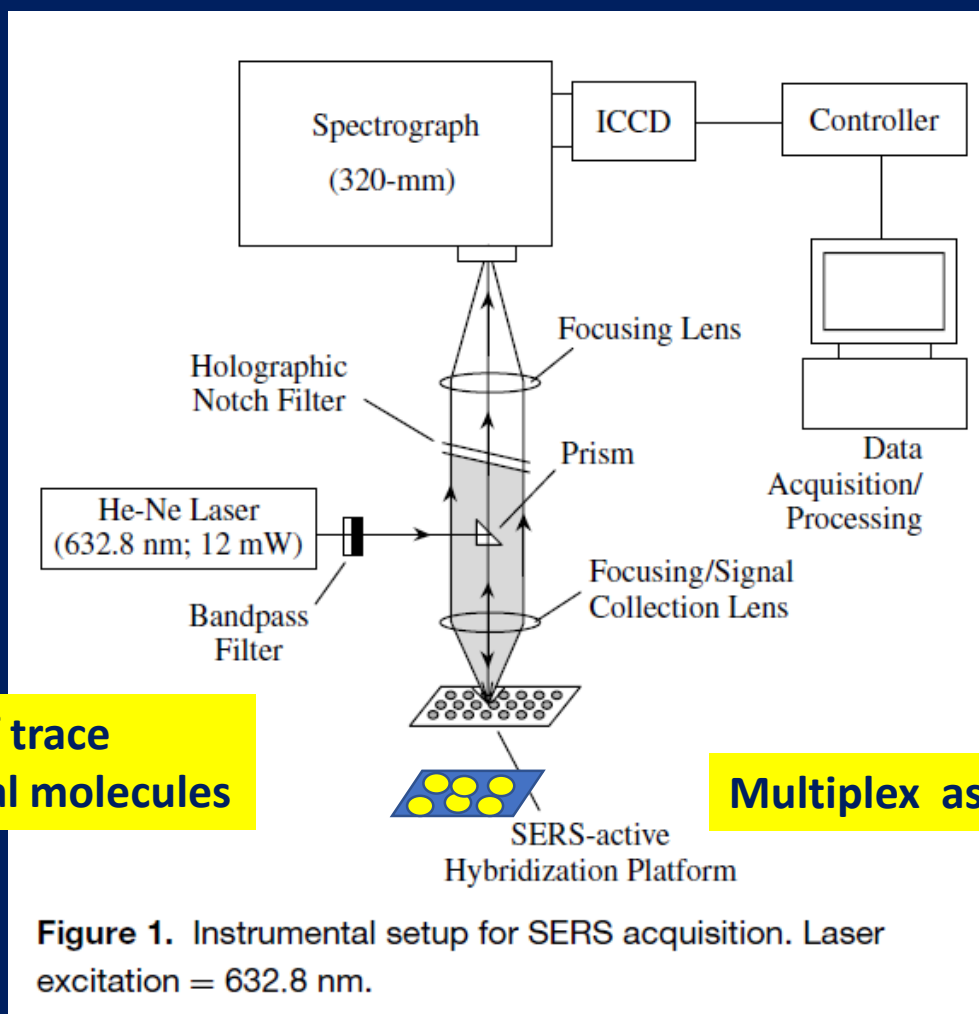
Antibody- C225

Antigen- EGFR-HER2

Mutations affecting EGFR  
expression or activity could  
result in cancer

In a few papers HGNS have been used as sensitive imaging agents for detection in cancer cells. As an optical imaging target, MCF7 cancer cells (MCF7) expressing human epidermal growth factor (HER2) markers on their surface membrane were used. HER2 is a clinically significant molecular marker of breast cancer (Ueda et al., 2004).

# Technologies for multiplexed SERS detection



a rapid detection of trace amount of biological molecules

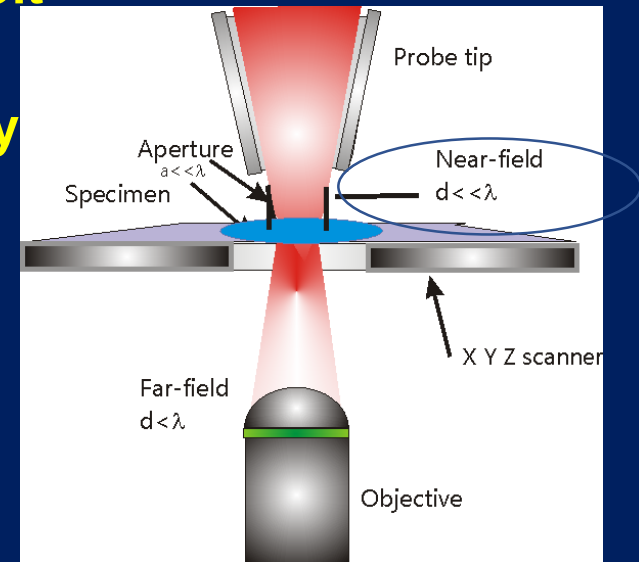
Multiplex assay (microarray )

**Figure 1.** Instrumental setup for SERS acquisition. Laser excitation = 632.8 nm.

A **multiplex assay** is a type of laboratory procedure that simultaneously measures multiple analytes (dozens or more) in a single assay . It is distinguished from procedures that measure one or a few analytes at a time.

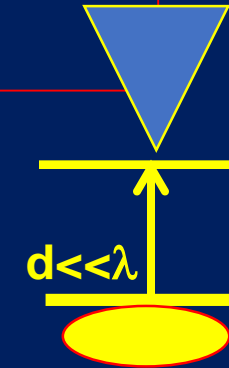
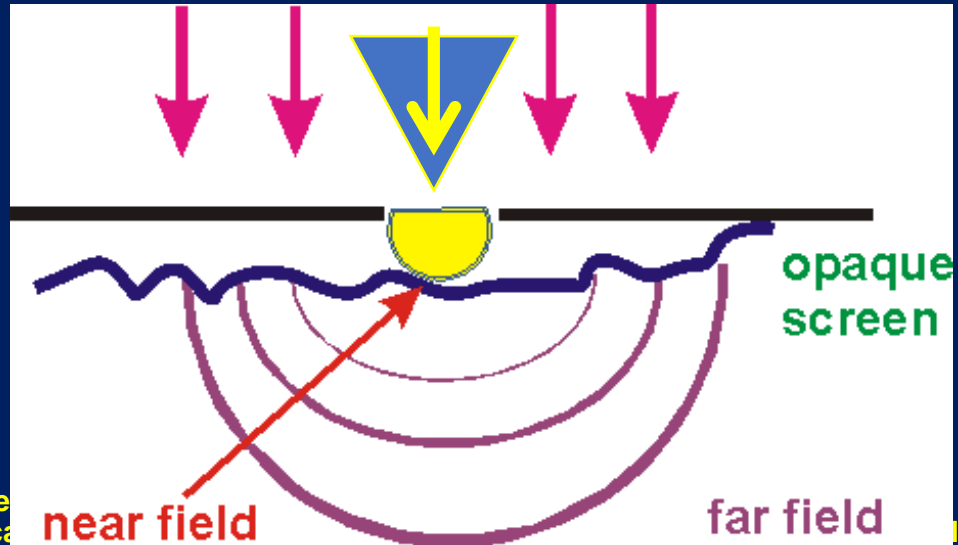
# SERS combined with scanning near-field microscopy (SNOM)

- There are many types of hybrid systems that exploit the SERS effect:
- surface-enhanced resonance Raman spectroscopy (SERRS)
- near IR FT-SERS microspectroscopy
- Surface-enhanced Raman scattering–Scanning near-field optical microscopy (SERS–SNOM)
- tip-enhanced Raman spectroscopy (TERS)



- In the last two methods near-field scanning optical microscopy (SNOM) is employed. Near-field imaging occurs when a sub-micron optical probe is positioned a very short distance from the sample and light is transmitted through a small aperture at the tip of this probe.

# SERS combined with scanning near-field microscopy (SNOM)



spatial resolution  $< 100\text{nm}$ .

In the last two me  
sub-micron optical  
transmitted through a small aperture at the tip of this probe.

near field

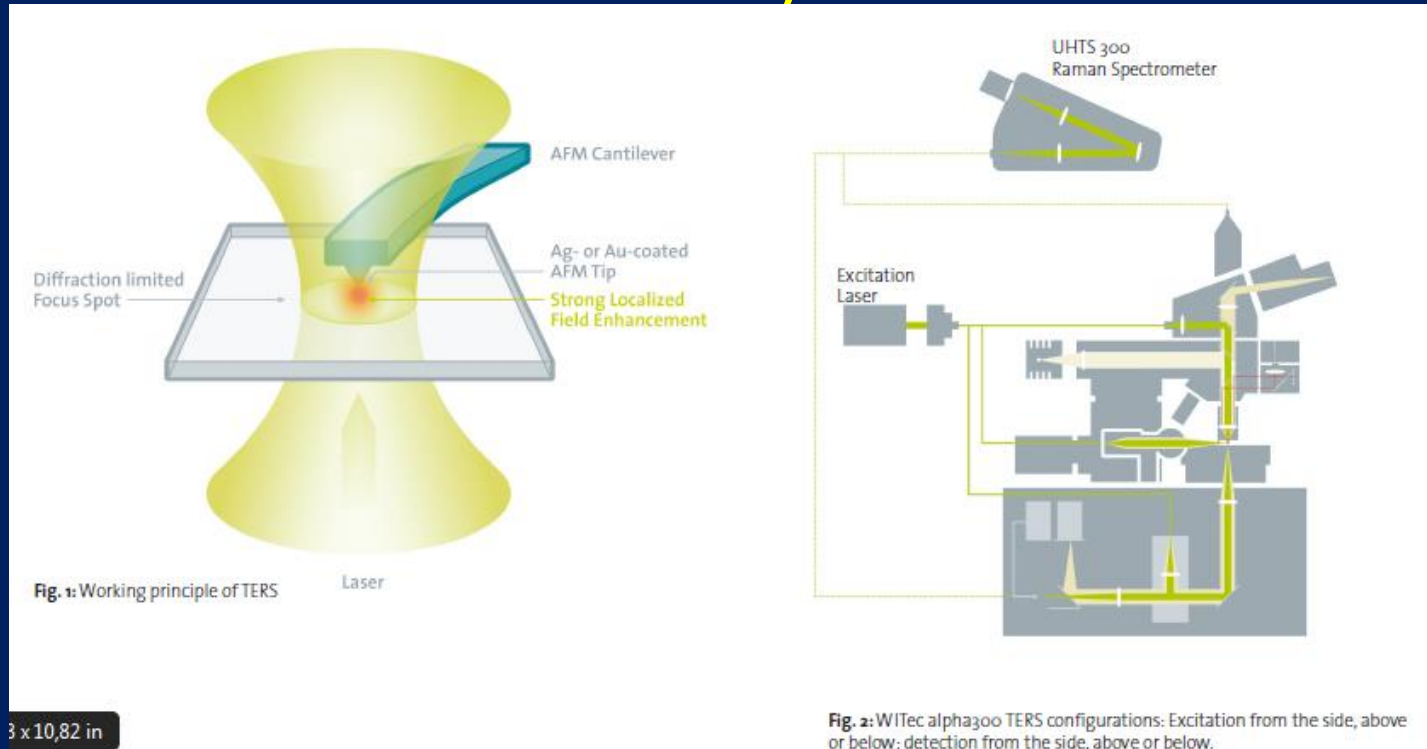
far field

Near-field imaging occurs when a  
light is

The near-field is defined as the region above a surface with dimensions less than a single wavelength of the light incident on the surface. Within the near-field region light is not diffraction limited and nanometer spatial resolution is possible. This phenomenon enables non-diffraction limited imaging of a sample that is simply not possible with conventional optical imaging techniques.

The next step to material analysis on a smaller scale has been the combination of Raman spectroscopic analysis with near field optics and an Atomic force microscope (AFM). Such systems allow tip enhanced Raman scattering to be explored, making true NanoRaman achievable, with spatial resolution  $< 100\text{nm}$ .

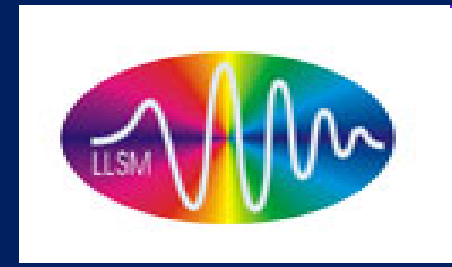
# Tip-enhanced Raman spectroscopy (TERS) (cantilever based SNOM)



3 x 10,82 in

As an alternative approach to achieve lateral resolution far below the diffraction limit Tip-enhanced Raman Spectroscopy can be applied. In a typical TERS experiment a Au- or Ag-coated AFM tip is used as a nanostructure to produce Raman signal enhancement on a sample surface once the excitation laser is focused on the apex of the tip with the tip brought into close proximity with the surface. The tip radius, which defines the lateral resolution of an AFM measurement, is typically in the range of 10-20 nm. In the TERS experiment the lateral resolution depends on the size of the hot-spot therefore one can expect resolution in the range of 20-50 nm for Raman spectroscopy and imaging measurements. The TERS tip-apex must be illuminated with the excitation laser from either above, below, or the side.

# What?



**Human normal and cancerous breast tissue, brain, neck and head , intestine tissues**

**Cell human Lines: breast normal (MCF-10A, ) and cancerous epithelial : (MCF-7, MDA-MB-231) and brain glial cells: NHA Astrocytes CC2565 ), astrocytoma (CCF-STTG1 (ATCC CLR1718) and glioblastoma (U87MG) (ATCC<sup>®</sup> HTB-14)**

**Drugs ( temodal,erlotinib) and photosensitizers in cancer therapy**

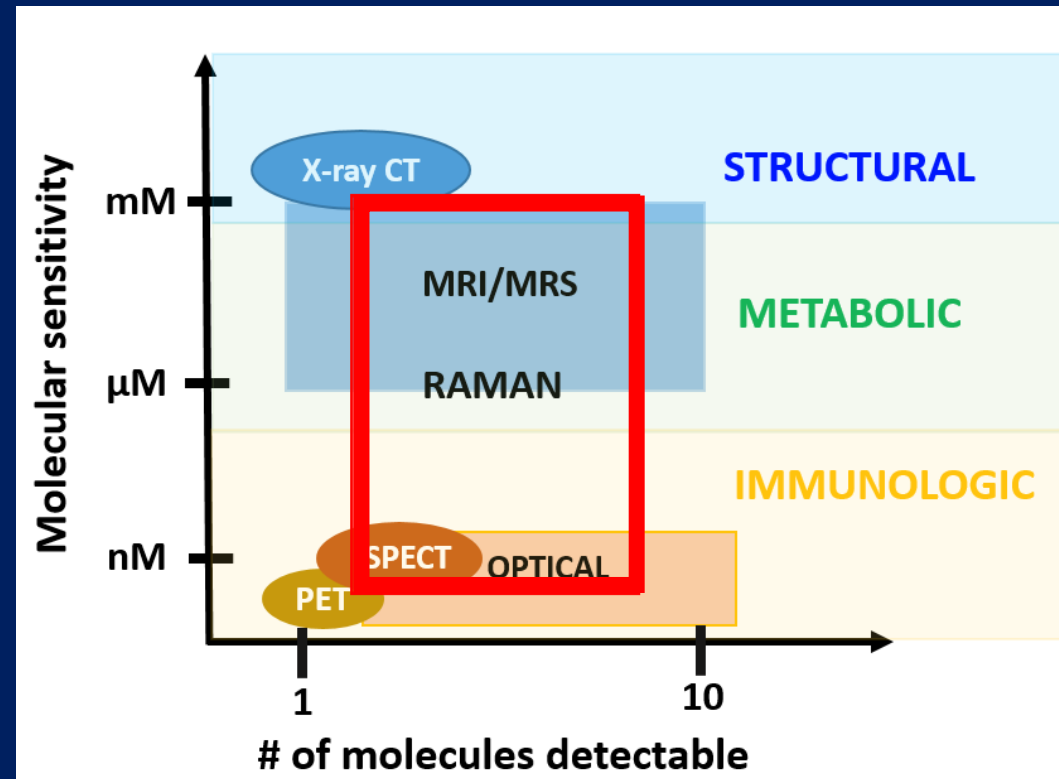
# HOW DOES RAMAN SPECTROSCOPY AND IMAGING BENEFIT CANCER RESEARCH?

- RAMAN BIOMARKERS OF CANCER
- RAMAN OPTICAL BIOPSY
- the emergence of Raman-driven proteomics, lipidomics, glycomics, and epigenetics research
- EPIGENETIC MODIFICATIONS
- POLARITY OF CELLS
- GLYCOME PROFILE



# molecular sensitivities of various imaging modalities

Scientists are also working to develop molecule-activated and quantitative probes that produce a signal only when they bind to the intended receptor. These probes are prime targets for optical imaging, including Raman scattering because optical switch mechanisms can be inherently electronic or vibrational.

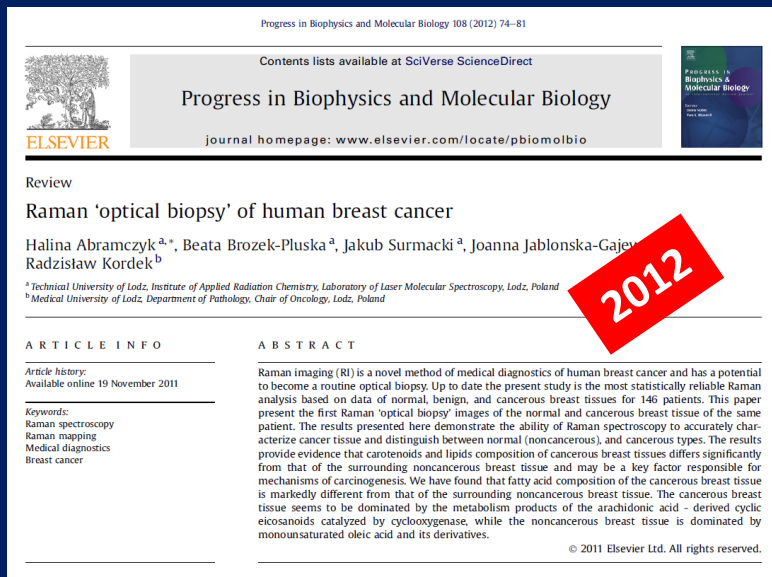


Pogue et al., Adapted from Am. J. Roentgen 195, 321 (2010)

Molecular imaging requires sensitivity and specificity. Imaging sensitivity to low molecular concentrations determines its use. Optical imaging has a nanomolar-level of sensitivity, parallel to nuclear methods such as PET and SPECT, and has the capability to detect over a dozen different molecular species.

# HOW DOES RAMAN SPECTROSCOPY AND IMAGING BENEFIT CANCER RESEARCH?

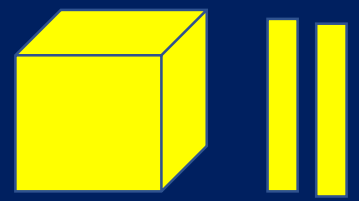
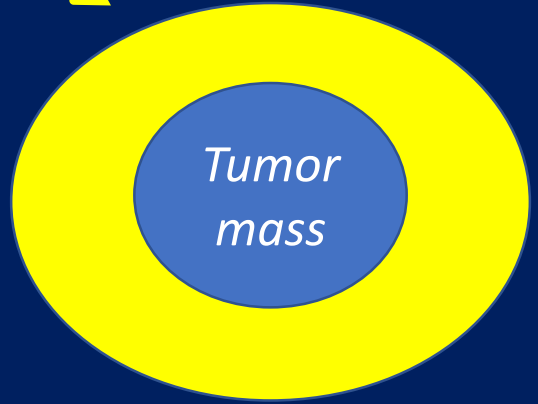
## • RAMAN OPTICAL BIOPSY



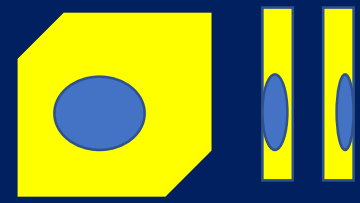
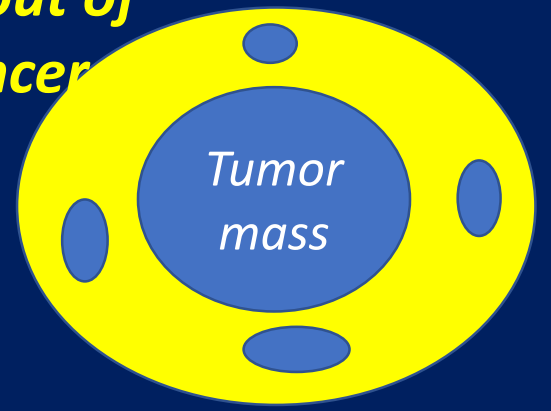
H. Abramczyk, B. Brozek-Pluska, J. Surmacki, J. Jablonska-Gajewicz, R. Kordek, *PBMB* 108 (2012) 74-81

The completeness of the surgical resection is a key factor in the progress of patients with cancers. The safety margin can be positive which means that not all cancer cells have been removed in the surgery. Patients with a positive margin often require more surgery to make sure that all the cancer is removed. The advantage of the 'Raman biopsy' is that it provides direct biochemical information (vibrational fingerprint) in real time, it is not prone to subjective interpretations, and it monitors biological tissue without any external agents, in contrast to histopathological assessment.

**Negative safety margin**  
**No cancer cells are found by histology**

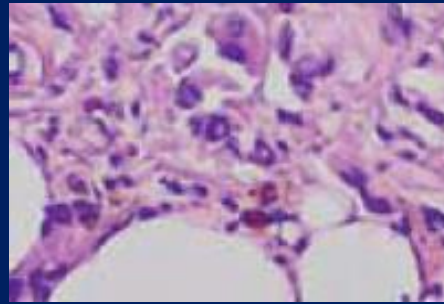


**Positive safety margin**  
**cancer cells extend out of the tumor mass, cancer cells are found by histology**

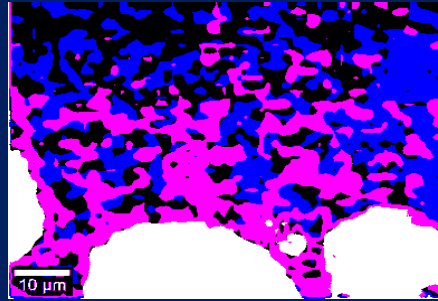


adjacent sections of 5  $\mu$ m

# VIRTUAL RAMAN HISTOPATHOLOGY IMAGE



STANDARD H&E HISTOPATHOLOGY

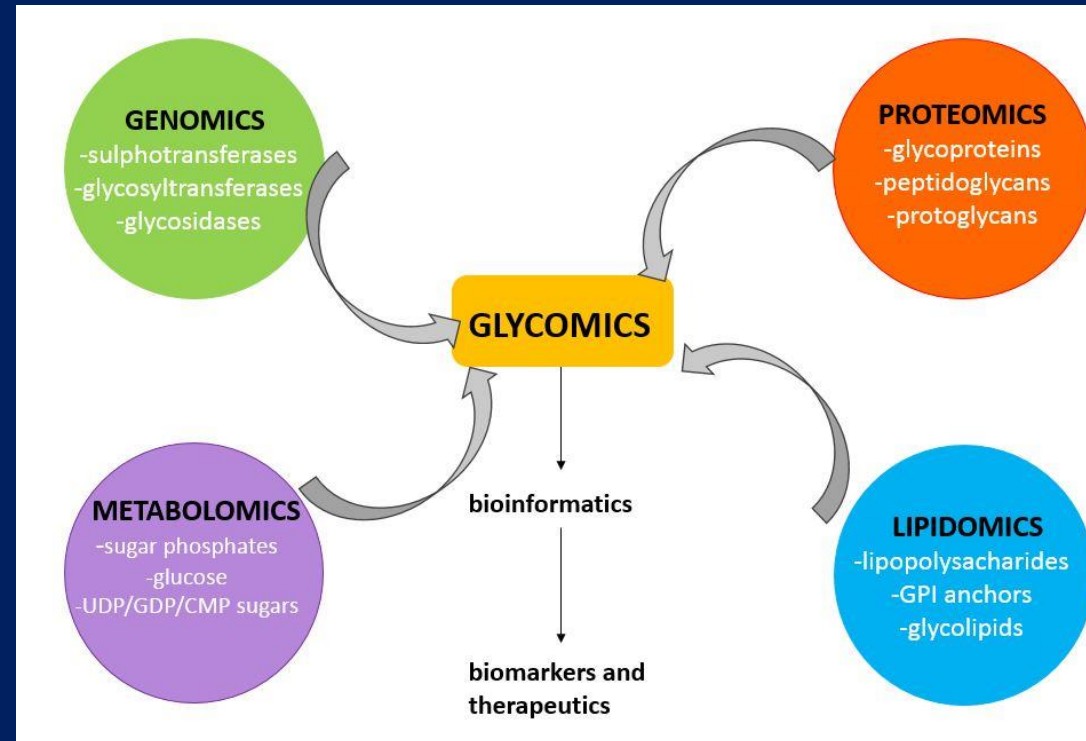


RAMAN HISTOPATHOLOGY IMAGE

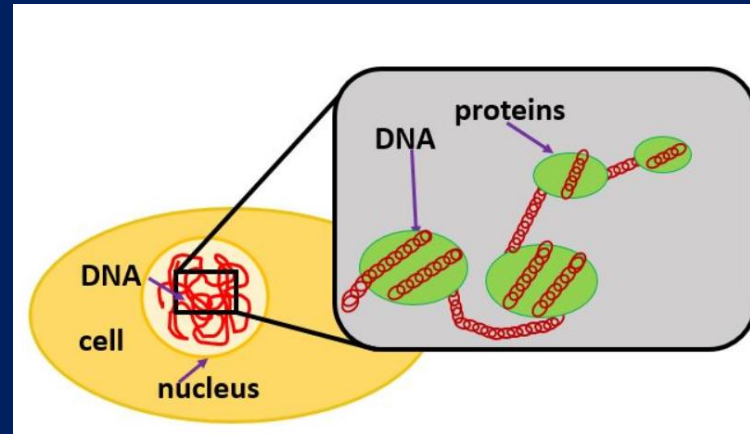
Abramczyk H et al.,  
patent application

- **Fast histopathological analysis for clinical practice**
- **Label-free histopathological analysis (without any staining procedures)**
- **Real time diagnostics to access the safety margin during operation by Raman-guided surgery**
- **High spatial resolution (small cancer changes can be easily identified)**
- **Objective diagnosis (without human interpretation, Raman spectra)**
- **Discrimination of grades with high specificity and sensitivity (c.a. 90%)**
- **Monitoring of tumor tissue heterogeneity**

# FUTURE DIRECTIONS OF CANCER RESEARCH

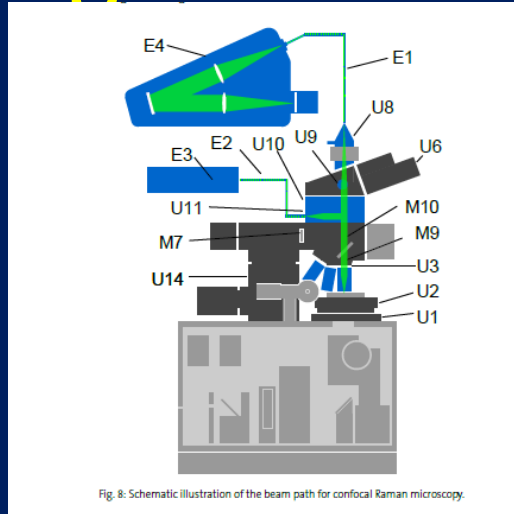


# Conventional molecular biology

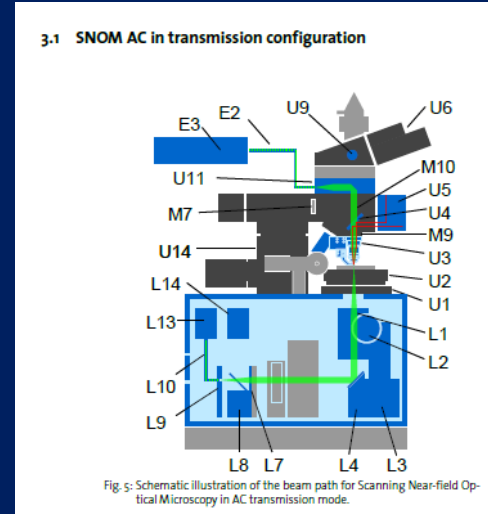


- **Isolation of DNA from Cells and Tissues**
- DNA can be extracted from many types of cells. The first step is to lyse or break open the cell. This can be done by grinding a piece of tissue in a blender. After the cells have broken open, a salt solution such as NaCl and a detergent solution containing the compound SDS (sodiumdodecyl sulfate) is added.
- **Isolation of Mitochondria from Cells and Tissues**
- Mitochondrial isolation protocols involve two processes – cell disruption to break open the cells and release the cellular structures, and differential centrifugation to recover fractions that are enriched for mitochondria.

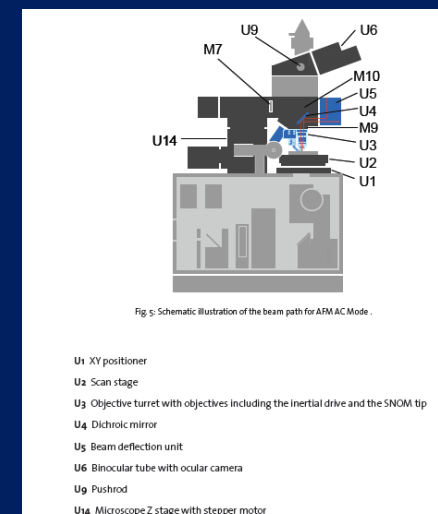
# Methods of molecular and laser microspectroscopy imaging



Raman imaging



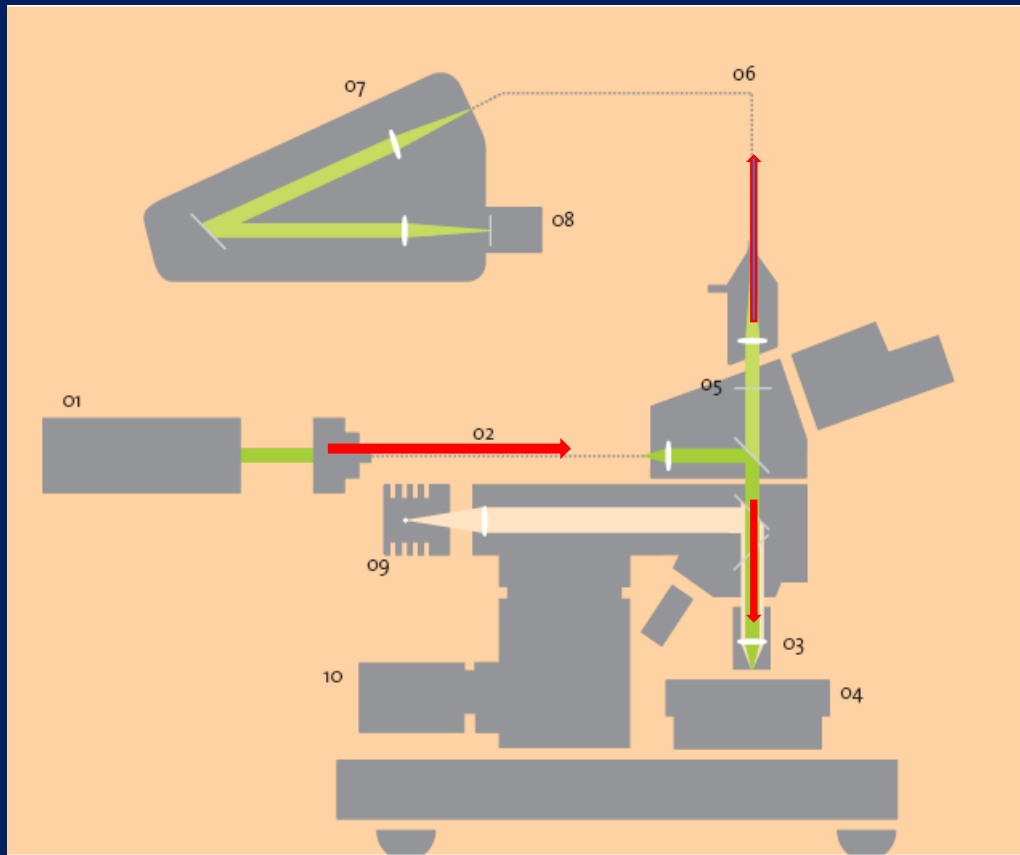
SNOM imaging



AFM imaging

We do not need to disrupt cells to break open the cells and release the cellular structures

<http://www.witec.de/en/download/Raman/alpha300Rflyer.pdf>



**alpha300 R Beam Path**

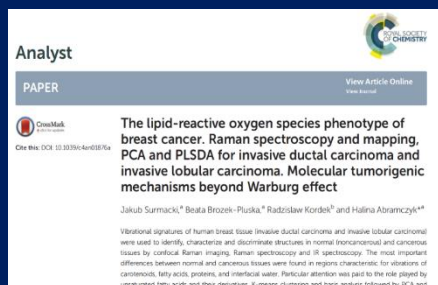
- 01. laser
- 02. single mode fiber
- 03. objective
- 04. scan table
- 05. filter

- 06. multi mode fiber
- 07. lense based spectroscopy system UHTS 300
- 08. CCD detector
- 09. white light illumination
- 10. Z-stage for focusing



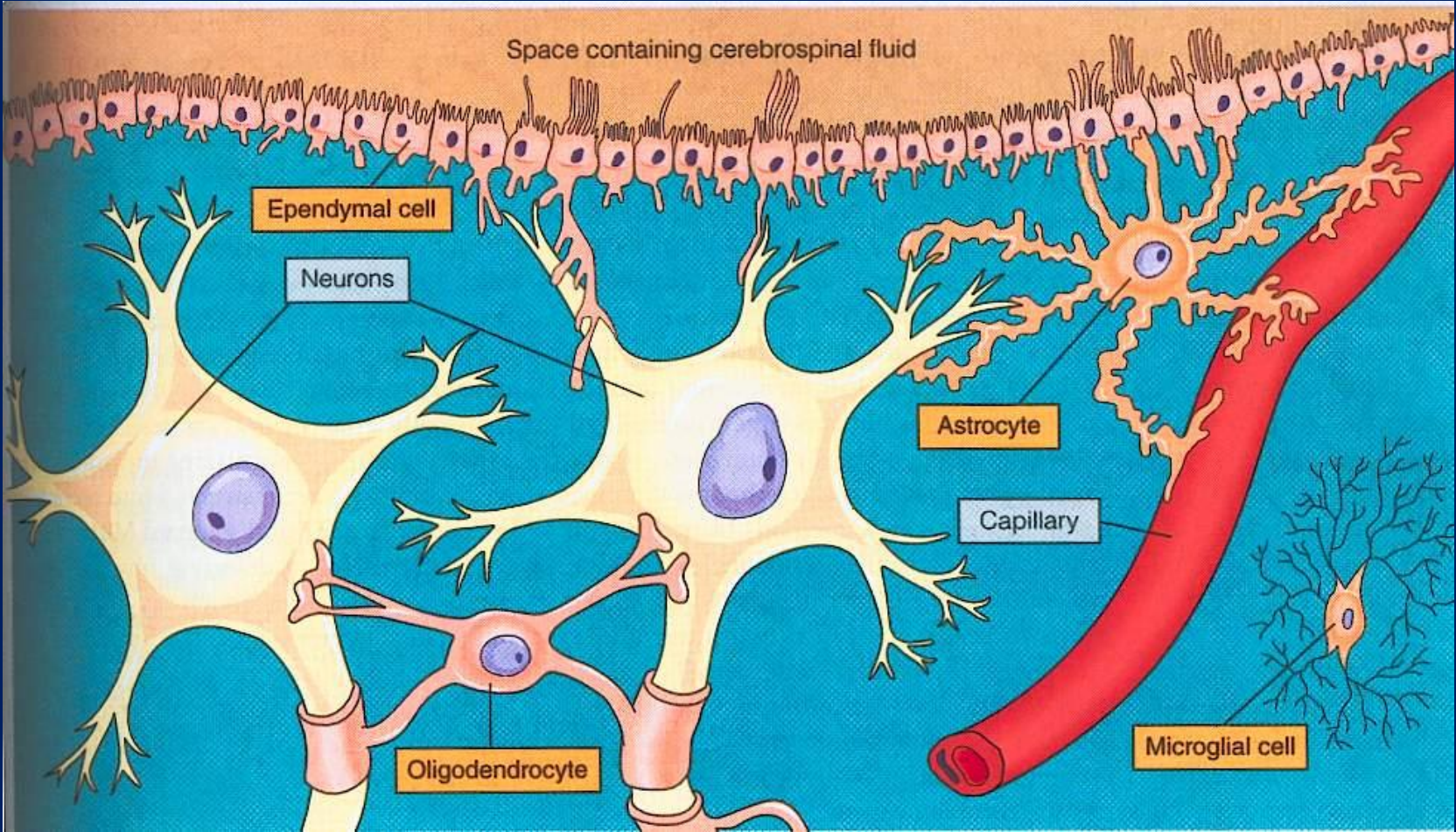
# HOW DOES RAMAN SPECTROSCOPY AND IMAGING BENEFIT CANCER RESEARCH?

## •RAMAN BIOMARKERS OF CANCER

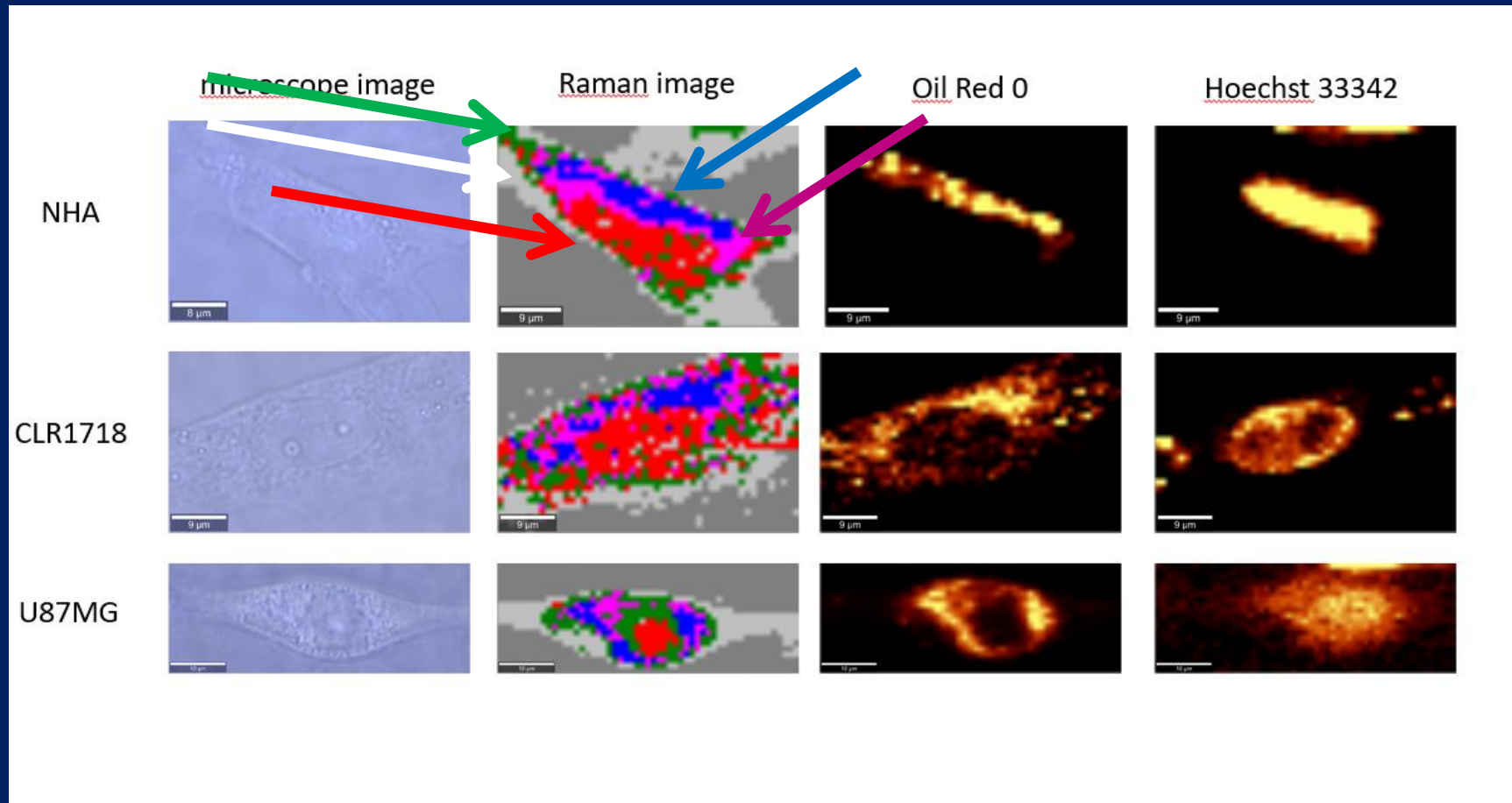


Surmacki J, Brozek-Pluska B, Kordek R, Abramczyk H,  
The lipid-reactive oxygen species phenotype of breast cancer. Raman spectroscopy and mapping, PCA and PLSDA for invasive ductal carcinoma and invasive lobular carcinoma. Molecular tumorigenic mechanisms beyond Warburg effect, Analyst, 2015, 140, 2121 – 2133, (IF=4.2)

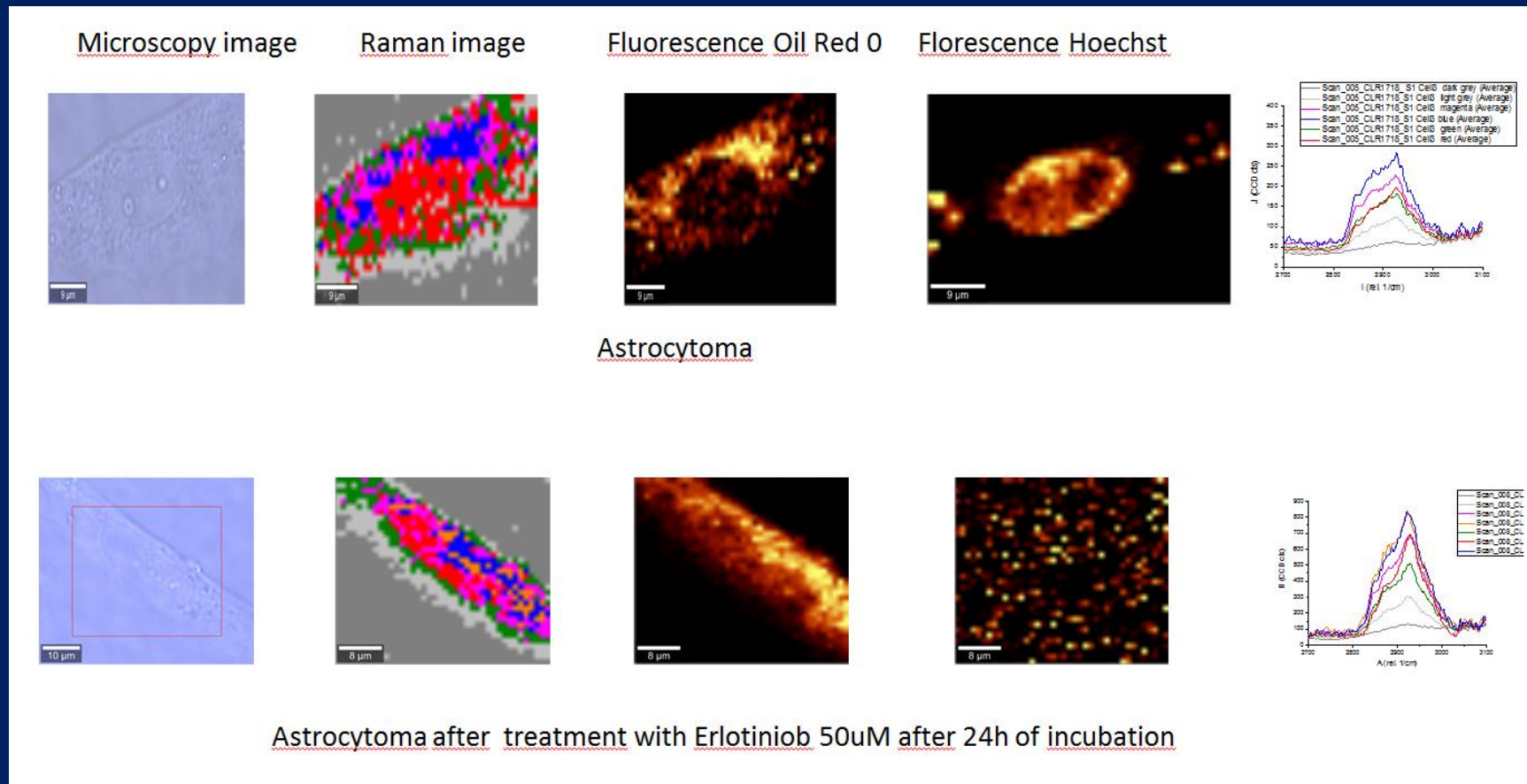
# BRAIN



In Raman imaging we do not need to disrupt cells to break open the cells and release the cellular structures to learn about their biochemical composition

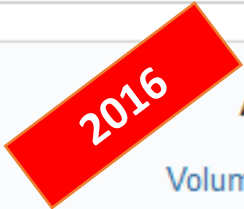



and to learn about their biochemical composition after drug treatment






# New look inside human breast ducts with Raman imaging





Analytica Chimica Acta

Volume 909, 25 February 2016, Pages 91–100



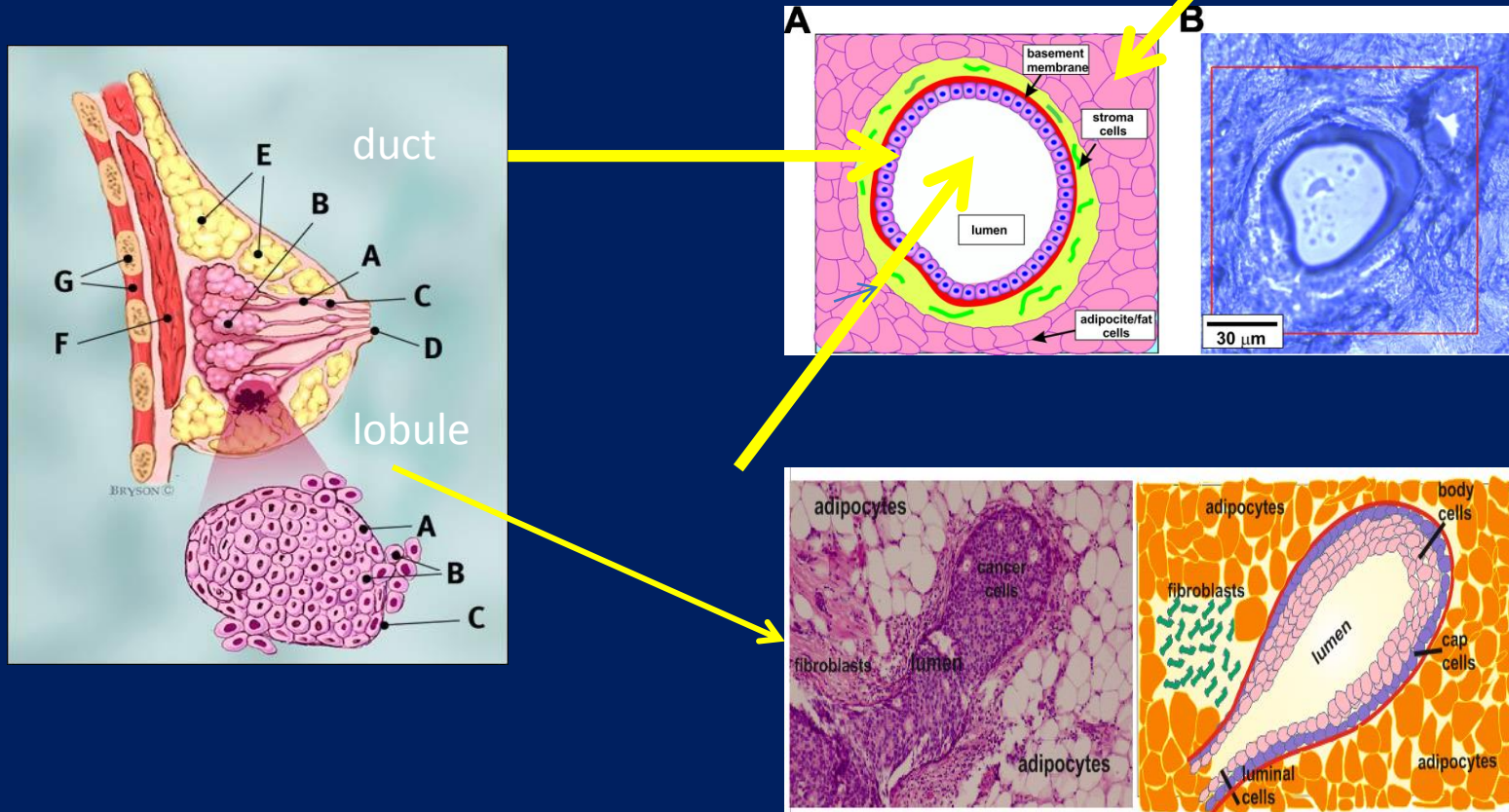
New look inside human breast ducts with Raman imaging.  
Raman candidates as diagnostic markers for breast cancer prognosis: Mammaglobin, palmitic acid and sphingomyelin

Halina Abramczyk  ·  , Beata Brozek-Pluska

[+ Show more](#)

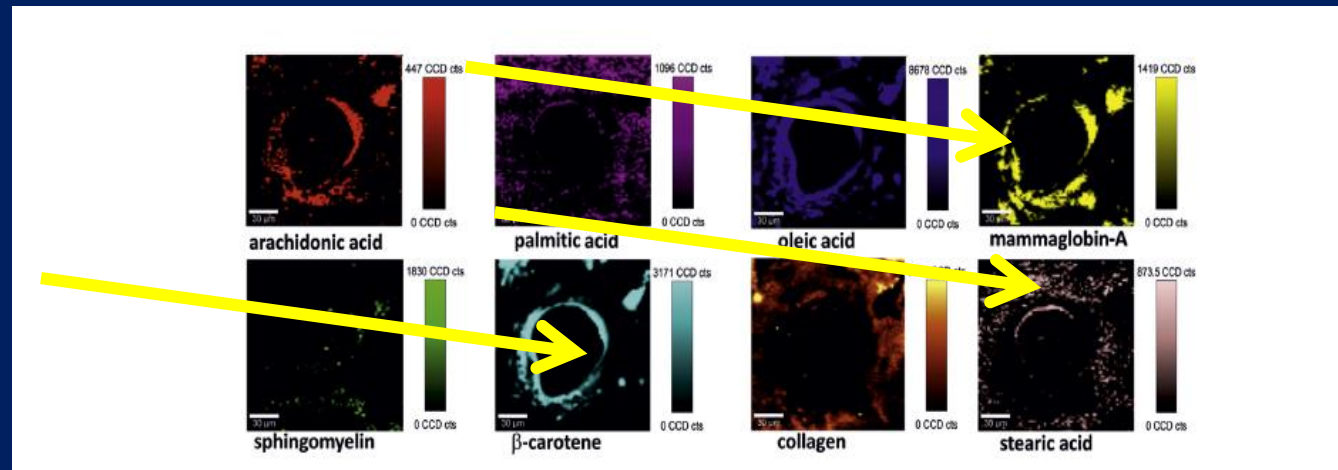
We will look inside human breast ducts answering fundamental questions about location and distribution of various biochemical components inside the lumen, epithelial cells of the duct and the stroma around the duct during cancer development.

# Breast morphology and biochemistry by Raman imaging

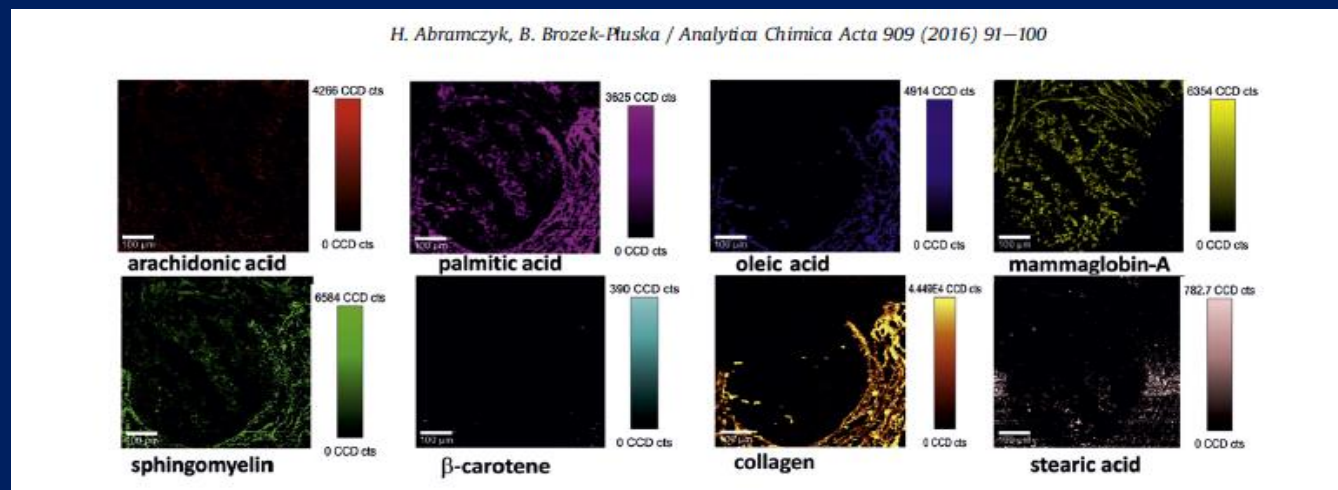


We will look inside human breast ducts answering fundamental questions about location and distribution of various biochemical components inside the lumen, epithelial cells of the duct and the stroma around the duct during cancer development.

# Comparison of Raman images: cross section through the normal and cancerous breast duct



normal duct



cancerous duct



Carotenoids, oleic acid: candidates as diagnostic markers for normal breast prognosis.

Mammaglobin, palmitic acid, and sphingomyelin: candidates as diagnostic markers for breast cancer prognosis.

# HOW DOES RAMAN SPECTROSCOPY AND IMAGING BENEFIT CANCER RESEARCH?


## •POLARITY OF CELLS

The cause-and-effect relationship between metabolic/epigenetic changes and polarity disruption of tissue structure is not clear





Journal of Molecular Liquids

Available online 1 June 2017  
In Press, Accepted Manuscript






Apical-basal polarity of epithelial cells imaged by Raman microscopy and Raman imaging: Capabilities and challenges for cancer research

H. Abramczyk  , B. Brozek-Pluska

H. Abramczyk, B. Brozek-Pluska, Apical-basal polarity of epithelial cells imaged by Raman microscopy and Raman imaging: Capabilities and challenges for cancer research, J Mol Liq, 2017, S0167-7322(17)30910-8





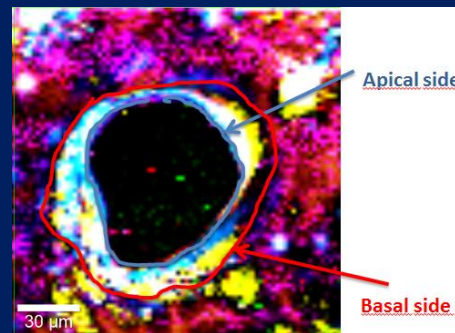
# POLARITY OF EPITHELIAL CELLS

    
Journal of Molecular Liquids  
Available online 1 June 2017  
In Press, Accepted Manuscript

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Apical-basal polarity of epithelial cells imaged by Raman microscopy and Raman imaging: Capabilities and challenges for cancer research

H. Abramczyk  , B. Brozek-Pluska



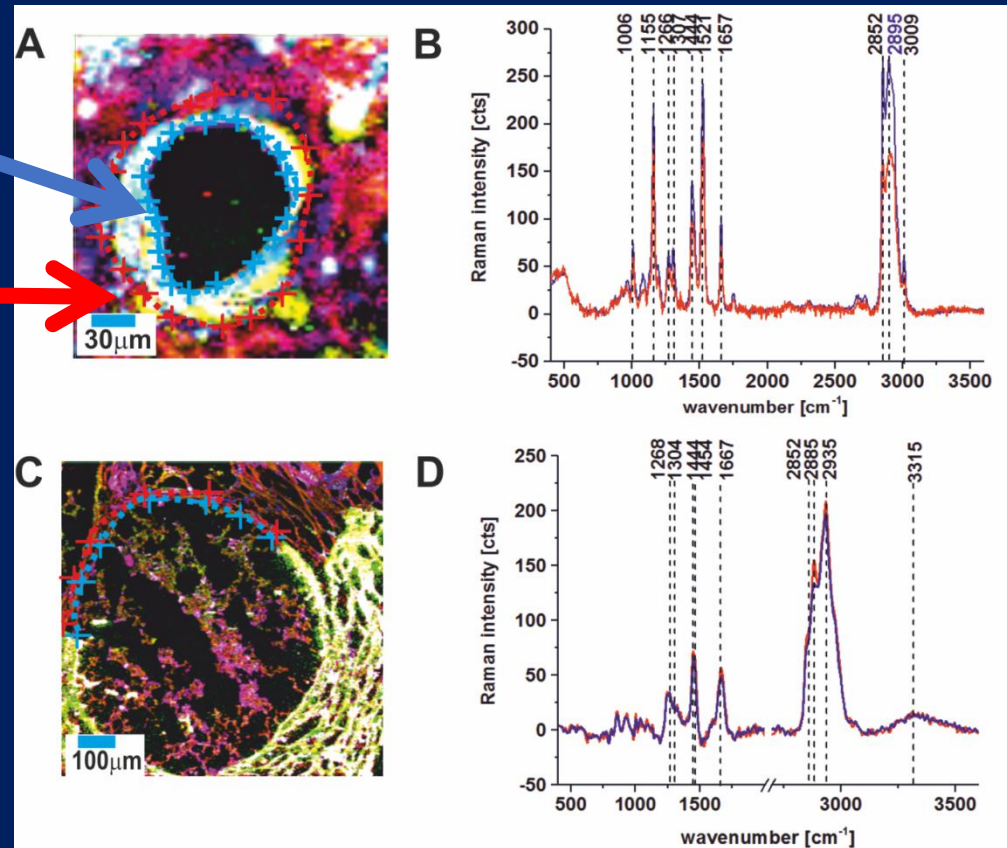
The common feature of cells is their polarity which refers to cellular asymmetry that reflects the asymmetric organization of the cell including the cell surface, intracellular organelles and the cytoskeleton. Cell polarity is a major regulatory mechanism for controlling spatially restricted signaling, reorganization of the cytoskeleton, and polarized membrane trafficking.

It is worth emphasizing that the Physics definition of polarization is different from the Cell Biology term. In Cell Biology polarity refers to cellular asymmetry that reflects the asymmetric organization of the cell, in physics- the asymmetry in the charge distribution.

# APICAL-BASAL POLARITY OF EPITHELIAL CELLS BY RAMAN IMAGING

APICAL

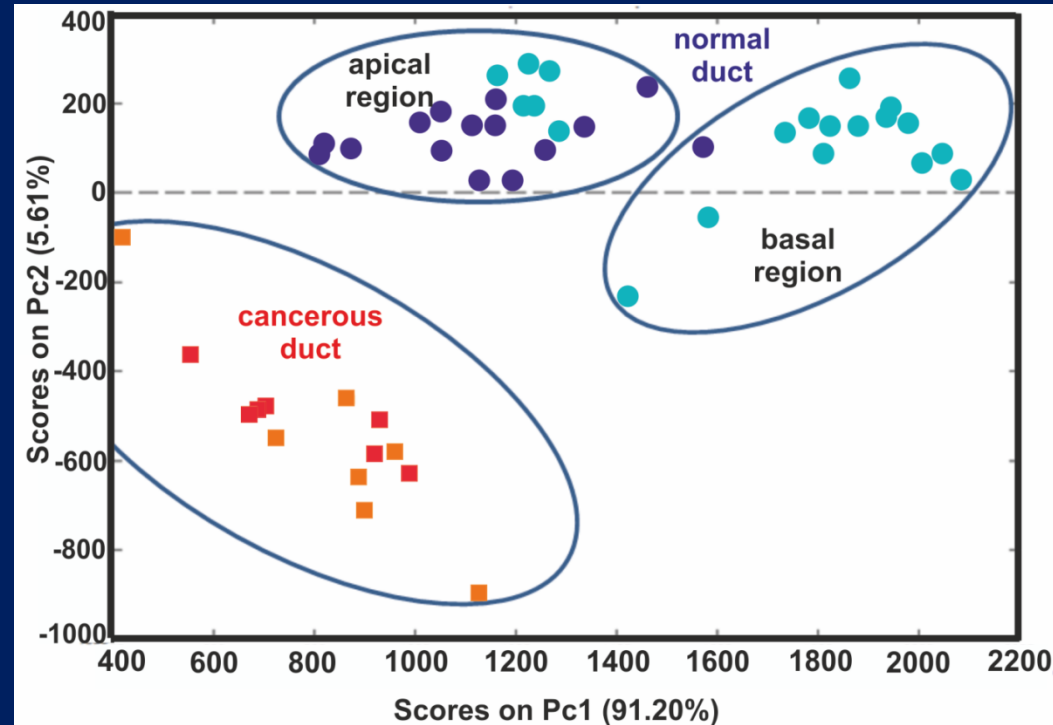
BASAL



Apical - basal polarity of epithelial cells is critical in the correct functioning of an organism and constitutes an important parameter to assess epithelial integrity and homeostasis. Loss of cell polarity results in epithelial tissue disorganization and is a hallmark of cancer development.

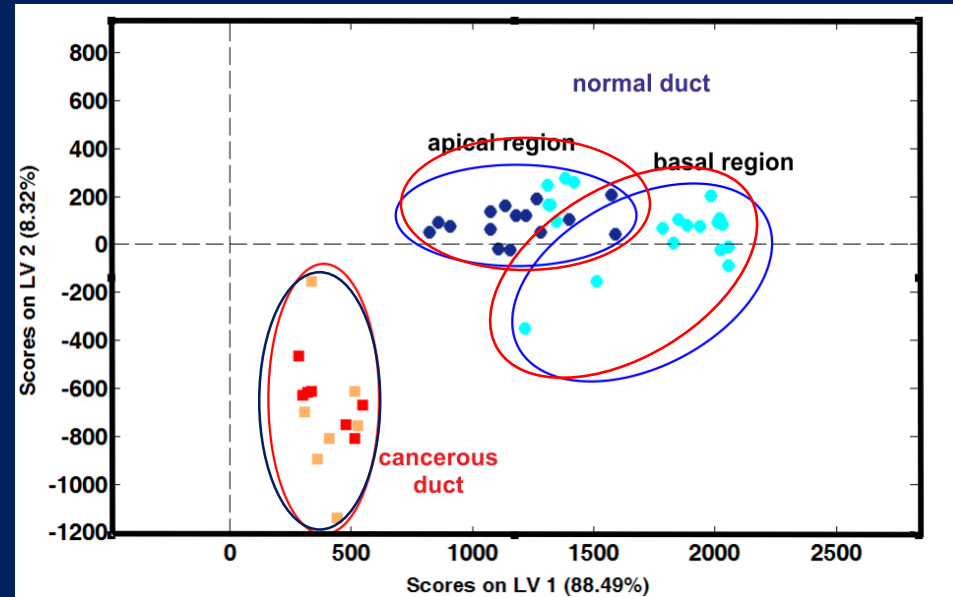
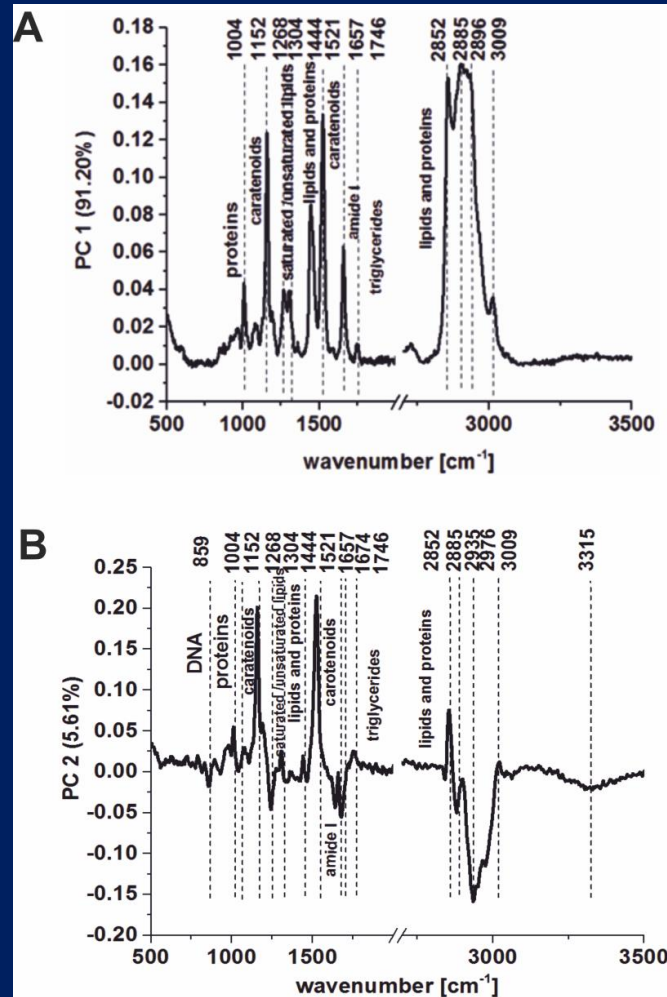
The common feature of cancer development is the increased loss of cell polarity that results in epithelial tissue disorganization. Despite intense interest in determining the origin of tumor tissue disorganization, the function of cell polarity remains unclear. In the study we traced the epithelial cell asymmetry and concentration gradient of main constituents of the cells along the apical-basal axis and monitored ordering at the apical membranes compared to basal membranes in normal and cancerous human breast duct.

# APICAL-BASAL POLARITY OF EPITHELIAL CELLS BY RAMAN IMAGING



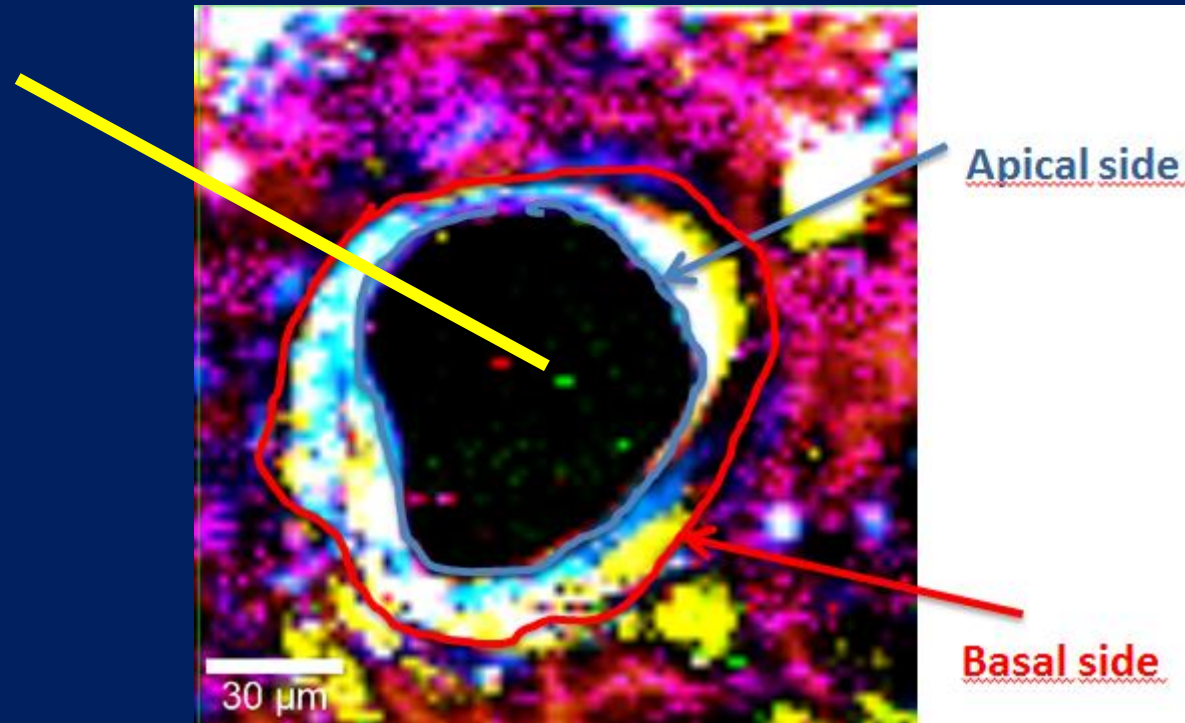
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# APICAL-BASAL POLARITY OF EPITHELIAL CELLS BY RAMAN IMAGING



The results of the PCA reveal separation of the ApM and BaM in normal duct (upper side of the PCA plot). The differences are clearly visible by grouping the Raman spectra of the normal duct in two separate clusters (navy blue and blue points) corresponding to the ApM and BaM samples, where the spectra with similar or identical Raman vibrational properties for the samples from the ApM (navy blue points) are grouped in the upper left area while the samples from the BaM (blue points) are grouped in the upper right area of the plot. The PCA separation indicates that statistically the normal duct is polarized with asymmetric distribution of lipids, proteins, carotenoids, triglycerides on the ApM and BaM sides. An inverse situation occurred in cancerous duct where there is no separation between ApM and BaM, both samples from the ApM (red points) and BaM (orange points) are grouped in the lower area of the PCA plot. It indicates that there are much more similarities than differences between ApM and BaM, which reflects lack of differentiation in the cancerous duct that lost apical polarity upon cancer development.

# APICAL-BASAL POLARITY OF EPITHELIAL CELLS BY RAMAN IMAGING

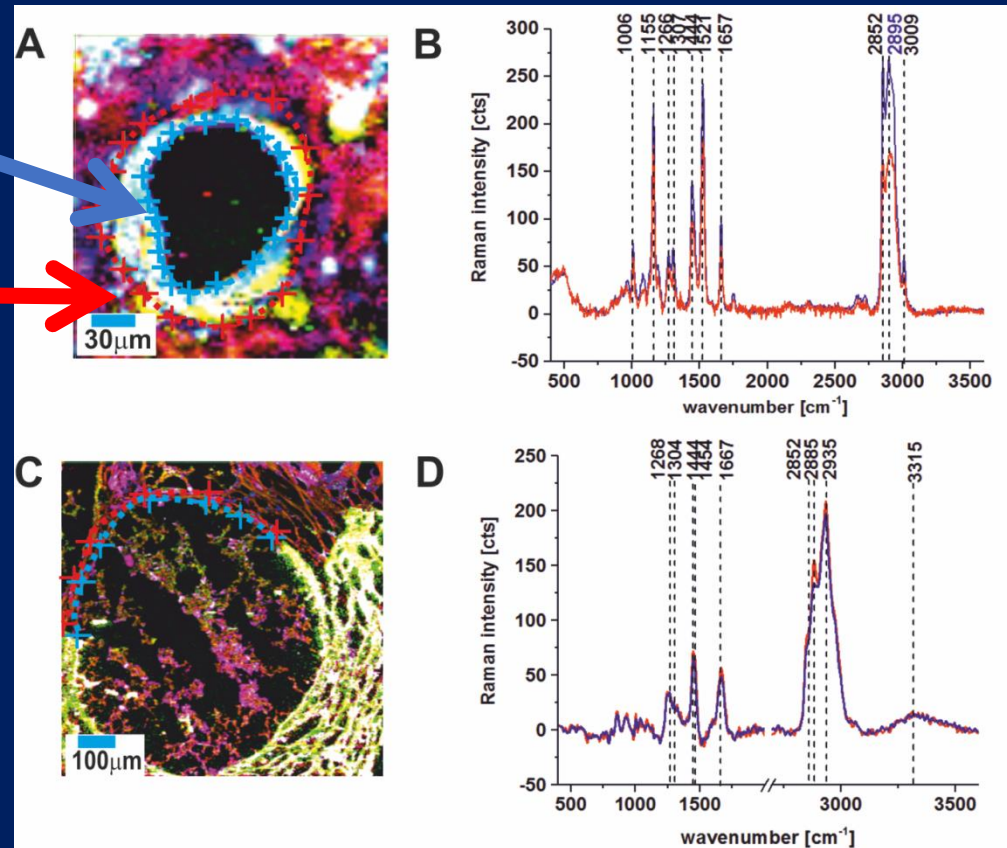


Here, we show the concentration gradient of main constituents of the cells along the apical-basal axis.

# APICAL-BASAL POLARITY OF EPITHELIAL CELLS BY RAMAN IMAGING

APICAL

BASAL



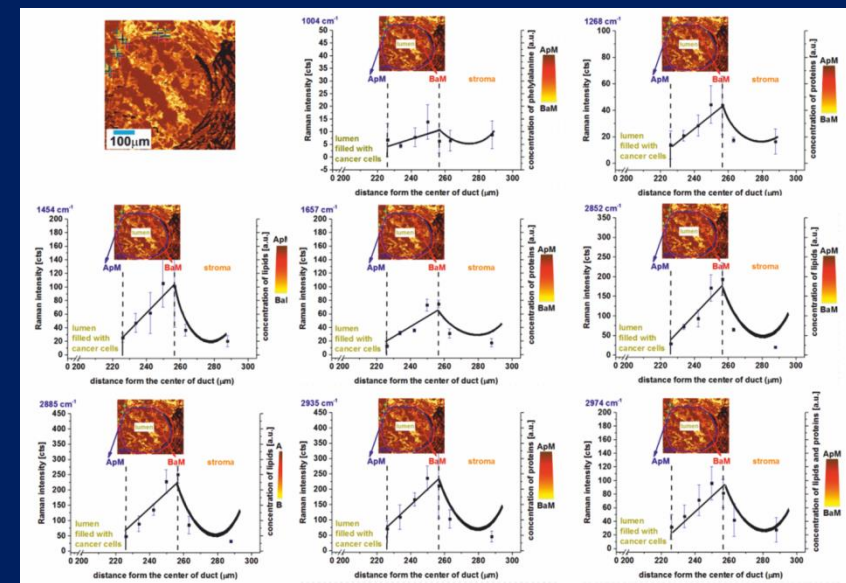
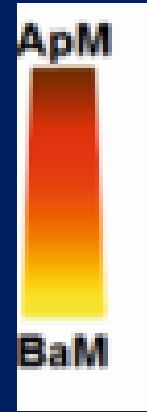
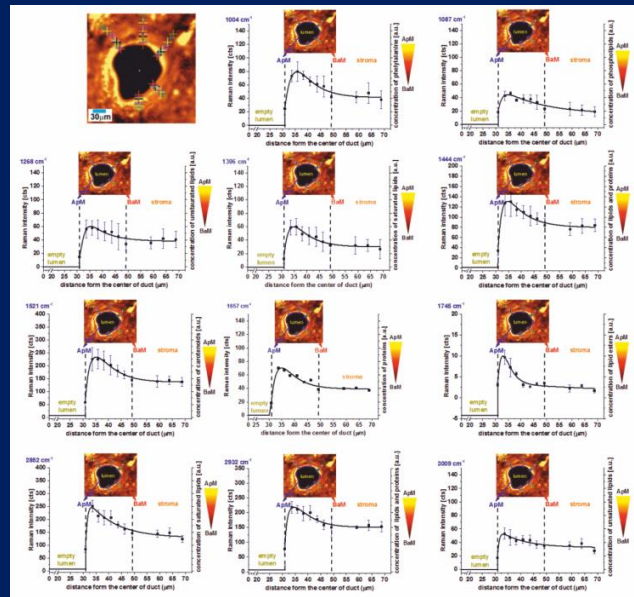
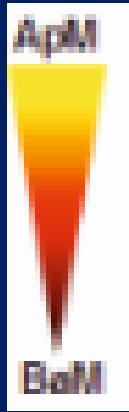
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Abramczyk H. J. Mol. Liquids, 2017

The common feature of cancer development is the increased loss of cell polarity that results in epithelial tissue disorganization. Despite intense interest in determining the origin of tumor tissue disorganization, the function of cell polarity remains unclear. In the proposal we will monitor the epithelial cell asymmetry and concentration gradient of main constituents of the cells along the apical-basal axis and monitor ordering at the apical membranes compared to basal membranes in normal and cancerous human cells.

# APICAL-BASAL POLARITY OF EPITHELIAL CELLS BY RAMAN IMAGING

## NORMAL CELLS

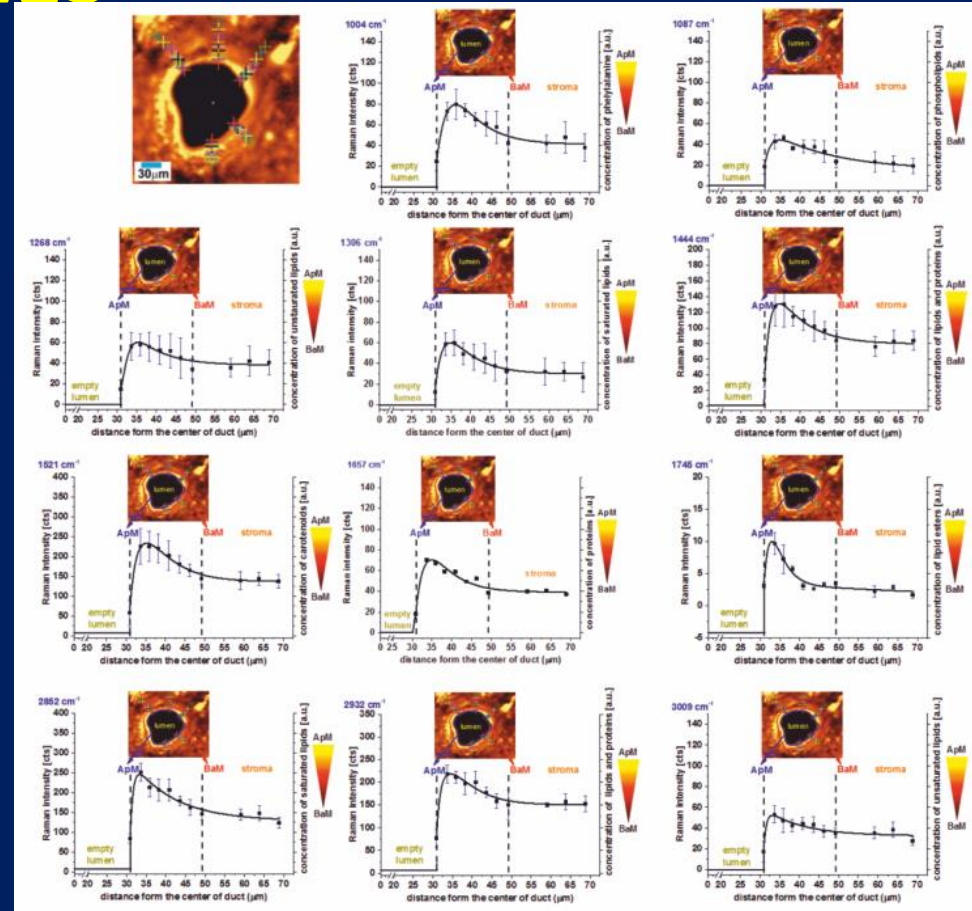
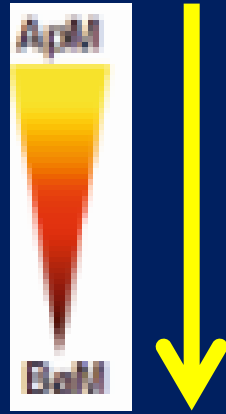


## CANCEROUS CELLS

Here, we show the concentration gradient of main constituents of the cells along the apical-basal axis. The radial distribution functions of concentration of major components of the normal and cancerous epithelial cells are presented. The concentration of these chemical components decreases along the apical-basal axis. The apical region of the normal duct is rich in unsaturated triglycerides derived from oleic acid (glyceryl trioleate), carotenoids and proteins.

Our results reveal that the normal duct is polarized with asymmetric distribution of lipids, proteins, carotenoids, triglycerides and that an inverse situation occurred in duct that lost apical polarity upon cancer development.

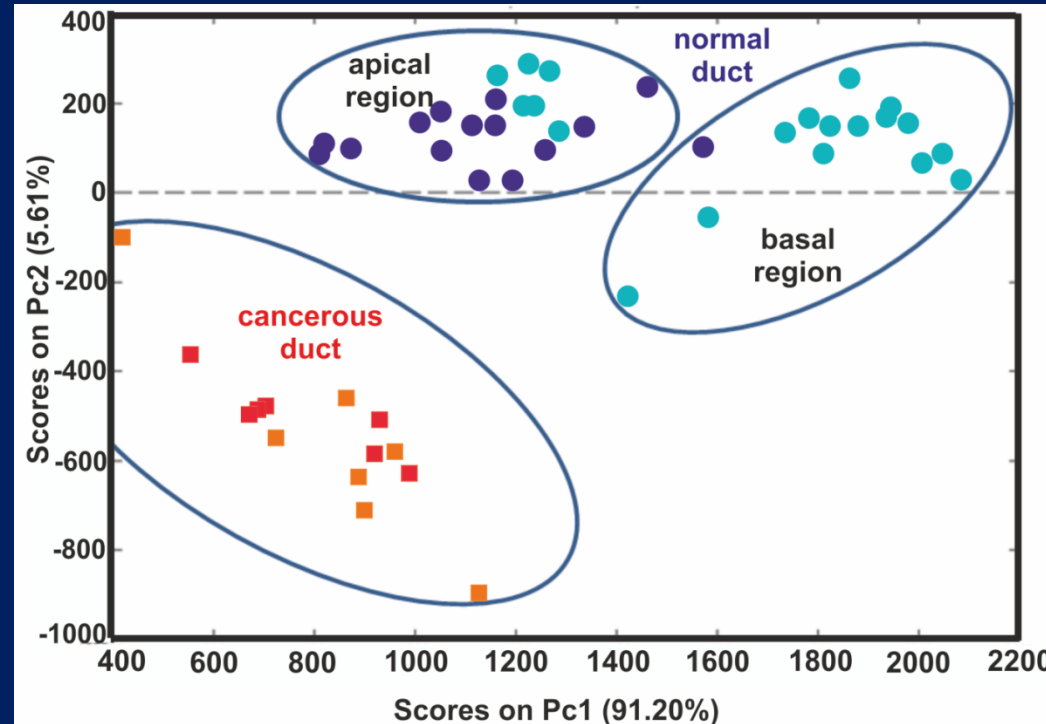
# APICAL-BASAL POLARITY OF EPITHELIAL NORMAL CELLS



The concentration of these chemical components decreases along the apical-basal axis for the normal epithelial cells. The apical region of the normal duct is rich in monounsaturated triglycerides derived from oleic acid (glyceryl trioleate), carotenoids and proteins.



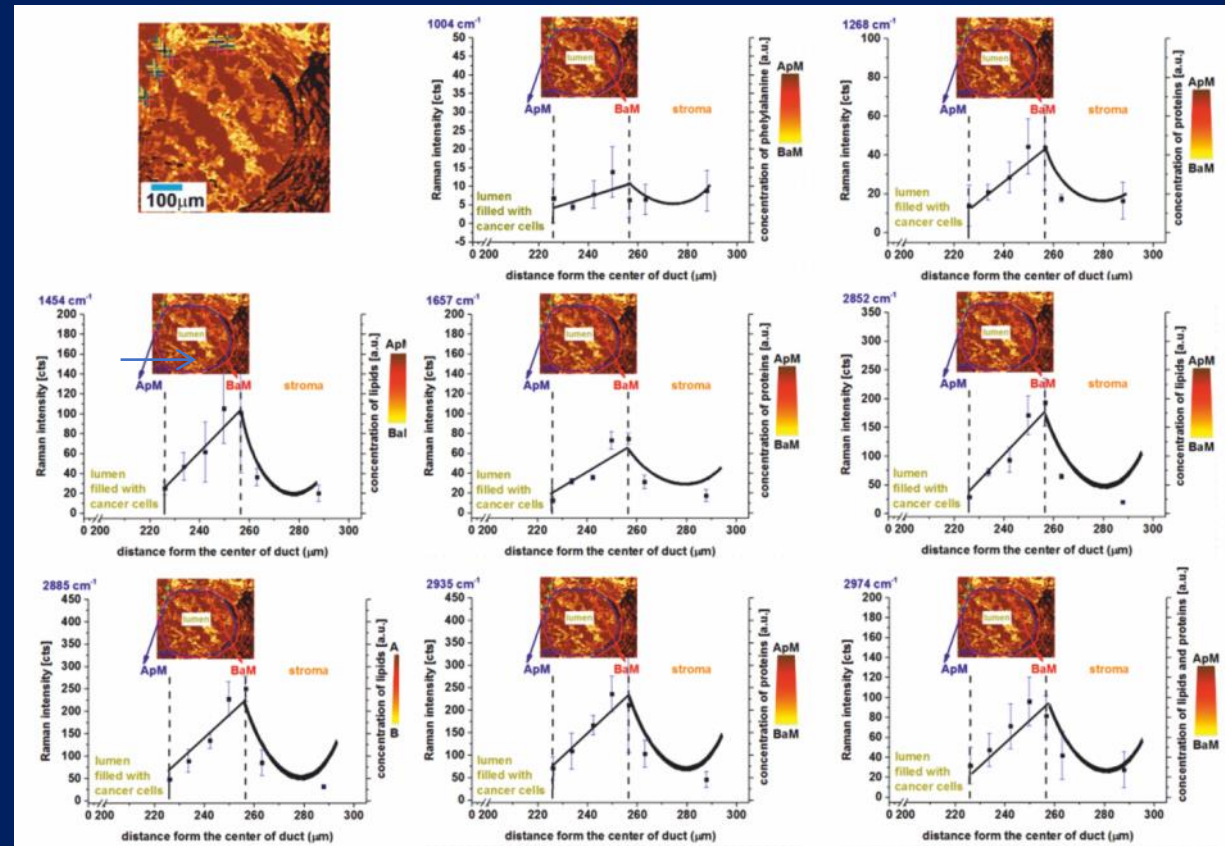
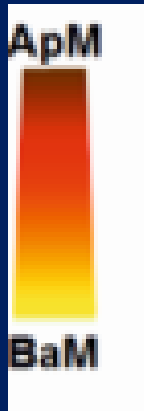
# APICAL-BASAL POLARITY OF EPITHELIAL CELLS BY RAMAN IMAGING



Abramczyk H. J. Mol. Liquids, 2017

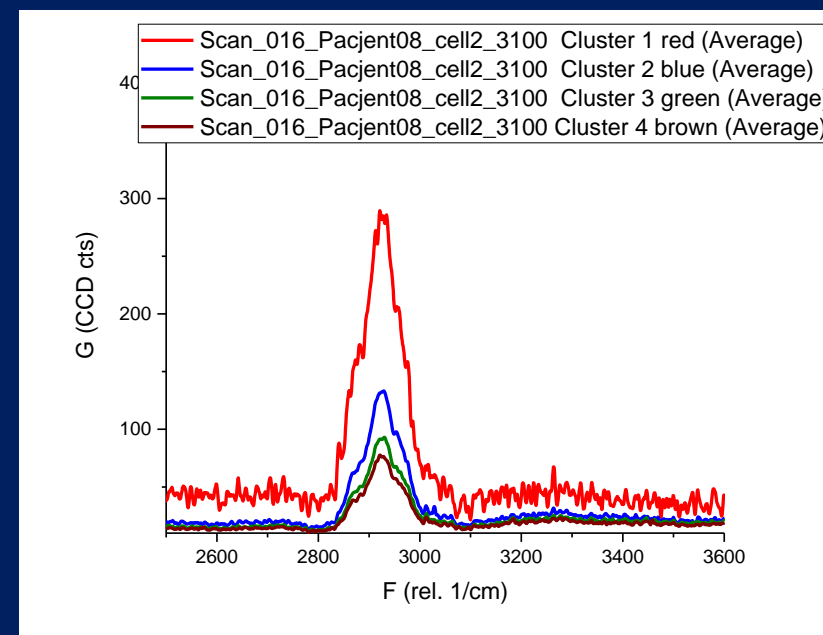
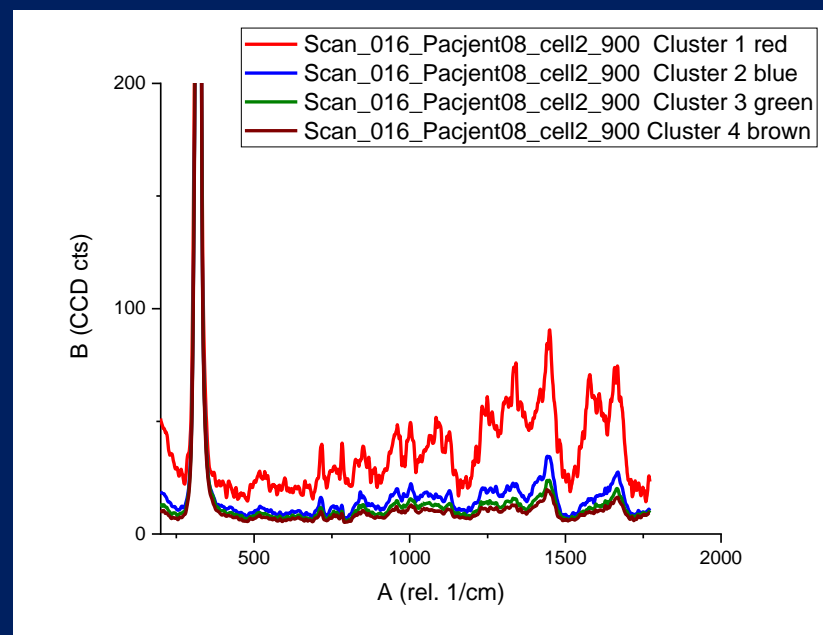
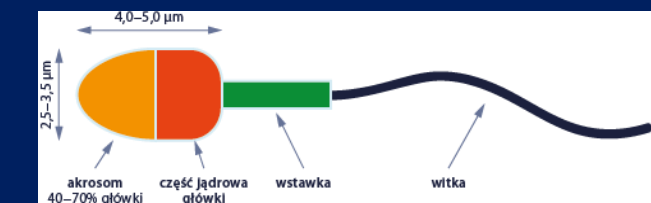
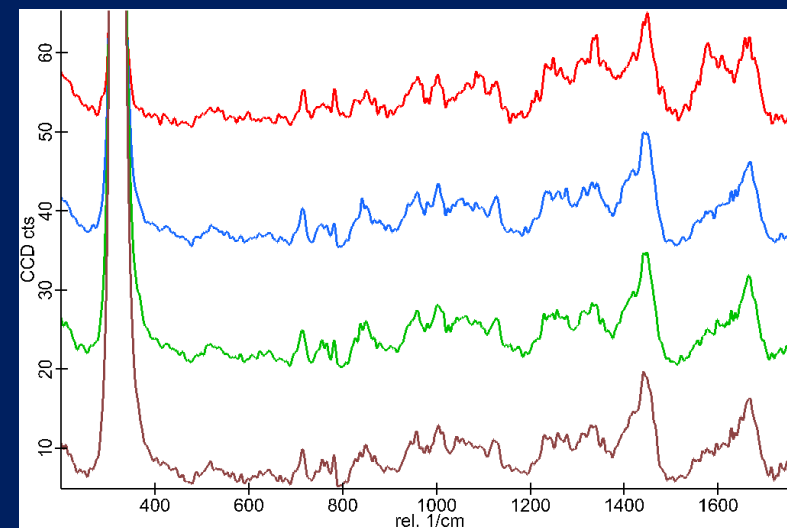
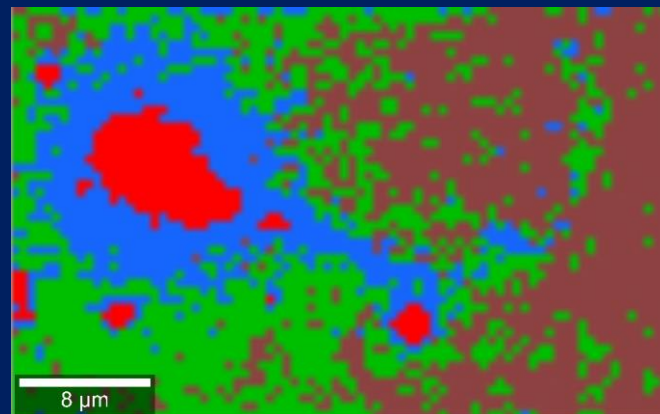
The common feature of cancer development is the increased loss of cell polarity that results in epithelial tissue disorganization. Despite intense interest in determining the origin of tumor tissue disorganization, the function of cell polarity remains unclear. We traced the epithelial cell asymmetry and concentration gradient of main constituents of the cells along the apical-basal axis and monitored ordering at the apical membranes compared to basal membranes in normal and cancerous human breast duct.

# APICAL-BASAL POLARITY OF EPITHELIAL CANCER CELLS



An inverse situation occurred in duct that lost apical polarity upon cancer development. The concentration of these chemical components increases along the apical-basal axis for the cancer epithelial cells.

# spermatozoid fertility



# ELUSIVE OUTCOMES IN THE WAR ON CANCER

In a recent study, TCGA reported that over 30,000 mutations were identified in breast cancer tissue (Cancer Genome Atlas Network, 2012). The evidence seems to suggest that science based only on cancer genomics, cannot be continued.

# TRENDS IN CANCER RESEARCH

- Presently, a growing number of reports have initiated a discussion about the benefits of metabolic regulation in cancers. The Warburg effect, a great discovery approximately 90 years ago, addresses the “universality” of cancer characteristics. For instance, most cancer cells prefer anerobic glycolysis instead of mitochondrial respiration.

Biomol Ther (Seoul) 2015; 23(2): 99-109 <https://doi.org/10.4062/biomolther.2015.013>

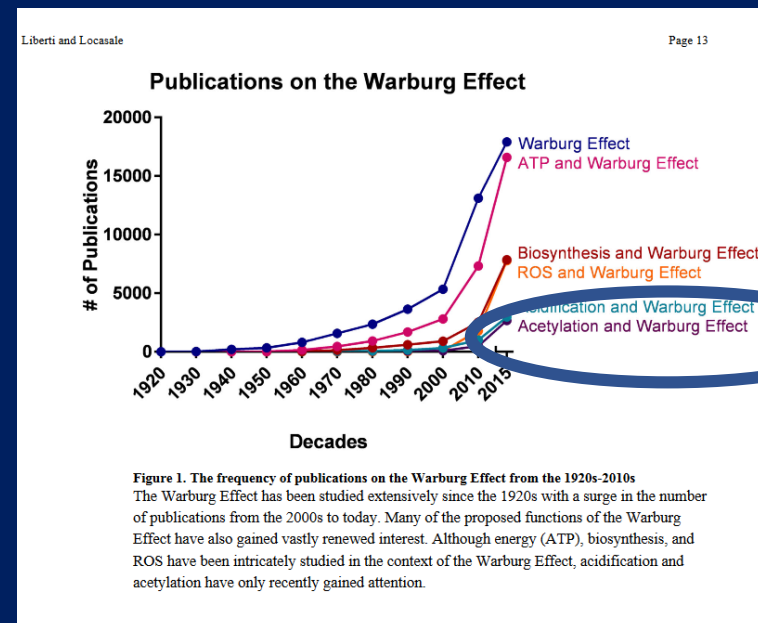
Cancer Metabolism: Strategic Diversion from Targeting Cancer Drivers to Targeting Cancer Suppliers

# Current ideas in cancer metabolism

- The Raman –driven oncological platform will help to analyze proposed biological explanations for the Warburg effect and enhanced fatty acid synthesis de novo, emphasize their rationale, and discuss their controversies.

Although energy (ATP), biosynthesis, and ROS have been studied in the context of the Warburg Effect, epigenetic modifications (acetylation) have only recently gained attention.

*Trends Biochem Sci.* 2016 March ; 41(3): 211–218



# Rapid ATP synthesis

Proposal: Increases access to a limited energy source

Questions:

- Why are ATP demands not limiting for proliferation?
- Why are there other mechanisms for rapid ATP synthesis?

# Biosynthesis

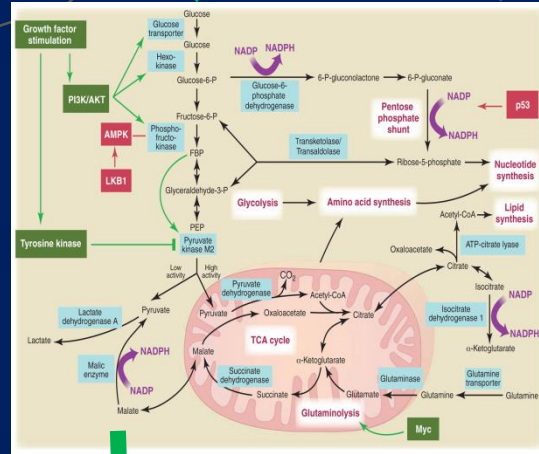
Proposal: Promotes flux into biosynthetic pathways

Questions:

- Why is most glucose not retained?
- Why does optimal biosynthesis not require aerobic glycolysis?

# Function of the Warburg Effect and Fatty Acids de novo synthesis?

## Glucose



# Tumor microenvironment

Proposal: Enhances disruption of tissue architecture

Questions:

- Why do unicellular organisms and cultured cells use aerobic glycolysis?
- Why do oncogenes induce the Warburg Effect cell-intrinsically?

LIPIDS Fatty acids de novo

# Cell

# Signaling

Proposal: Allows for signal transduction through ROS and/or chromatin modulation

Questions:

- Why is specificity unclear?
- Why would metabolite levels be influenced by flux?

# CANCER PHENOTYPE

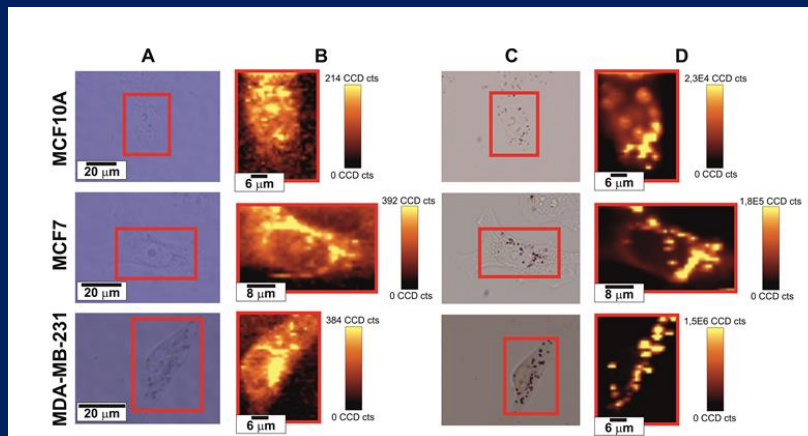
## Lipid metabolic reprogramming in cancer cells

Warburg proposed that dysfunctional mitochondria is the root of aerobic glycolysis. Warburg further hypothesized that this event is the primary cause of cancer. A higher rate, but lower yield, of ATP production may gain a selective advantage when competing for shared and limited energy resources

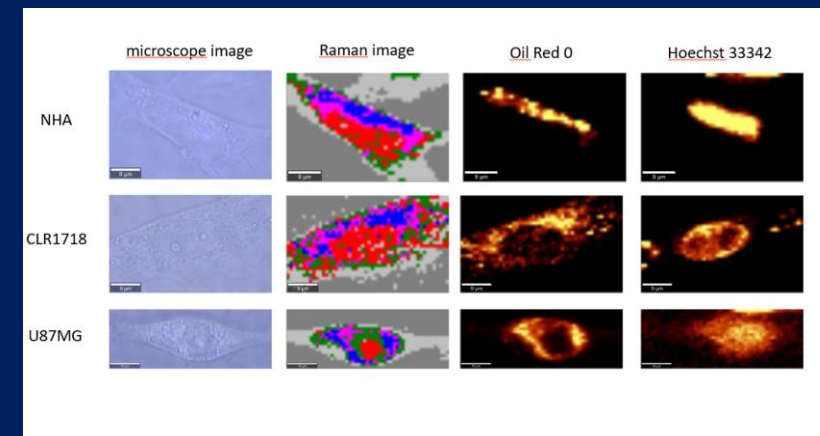
**We want to verify the hypothesis that metabolic adaptation in tumours extends beyond the Warburg effect and that commonly disregarded in the past alterations in lipid metabolic reprogramming encountered in tumors are very important in cancer development.**



To address these important questions on lipid phenotypic alterations we will study compositional mapping of cellular compartments, such as lipid droplets (LDs), mitochondria, nucleus in cell cultures



Abramczyk et al. Analyst , 2015

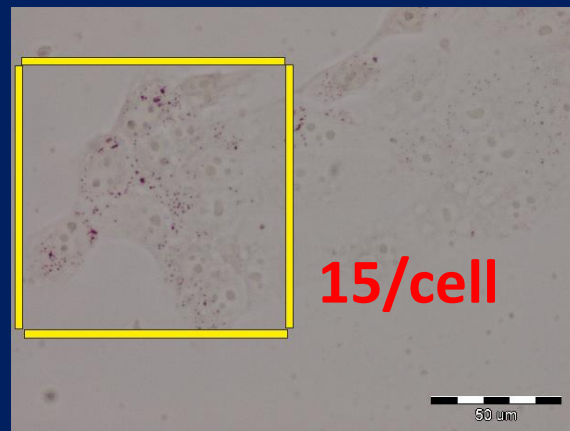


Abramczyk et al. LLSM, 2018

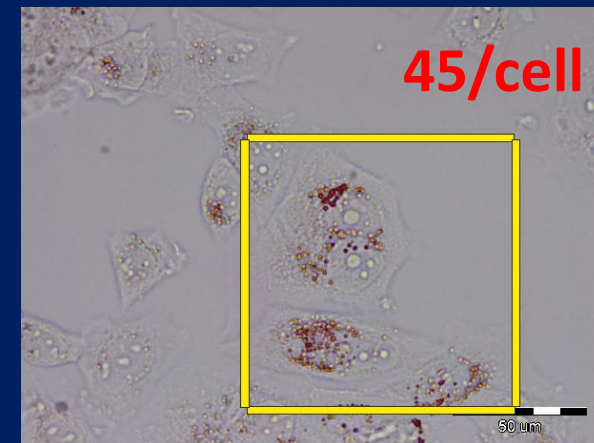
- Recent advances in lipid analytical and imaging technologies, including Raman scattering microscopy, have greatly progressed such lipidomic analysis. Raman-based imaging offers lipid compositional mapping of cellular compartments, such as LDs. These complementary approaches provide crucial information on tumor lipid phenotype, in particular abundance, FA composition and spatial distribution of lipid classes within tumors

# LIPID DROPLETS IN NORMAL MCF10A EPITHELIAL CELLS OF BREAST vs MILDLY MALIGNANT MCF7 AND AGGRESSIVELY MALIGNANT MDA-MB-231

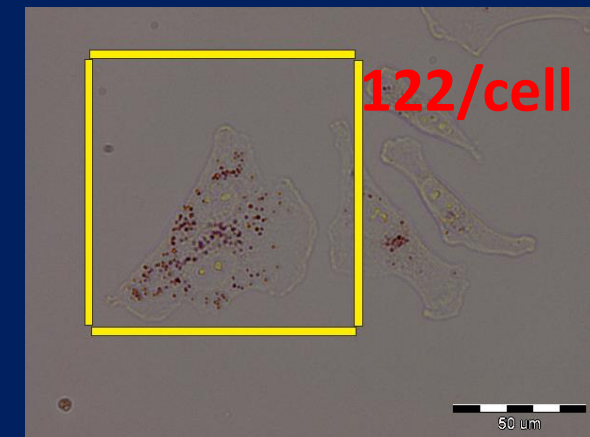
MCF10A - normal cells



MCF7 - mildly malignant

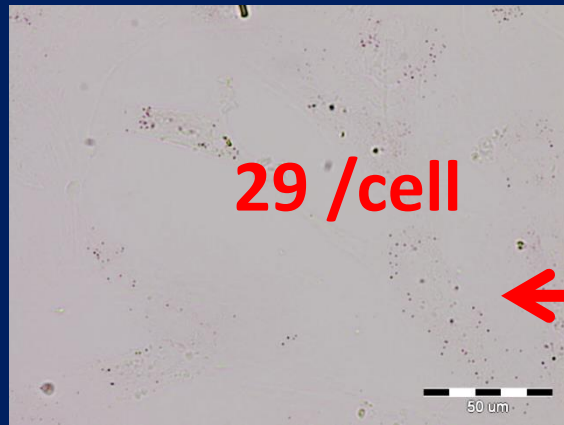


MDA-MB-231 - Aggressively malignant

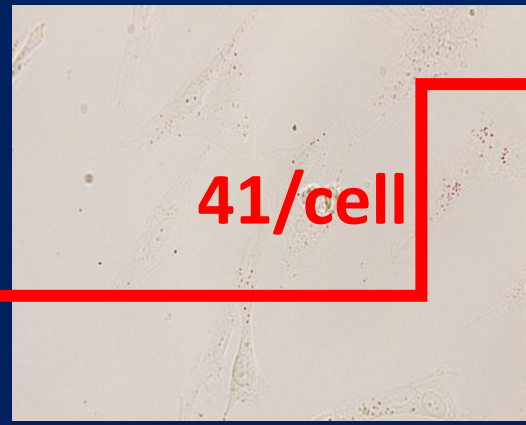


The goal of our study will be to assess the impact of cancer aggressiveness on the amount of cytosolic lipid droplets and their chemical composition in non-malignant and malignant human epithelial cell lines.

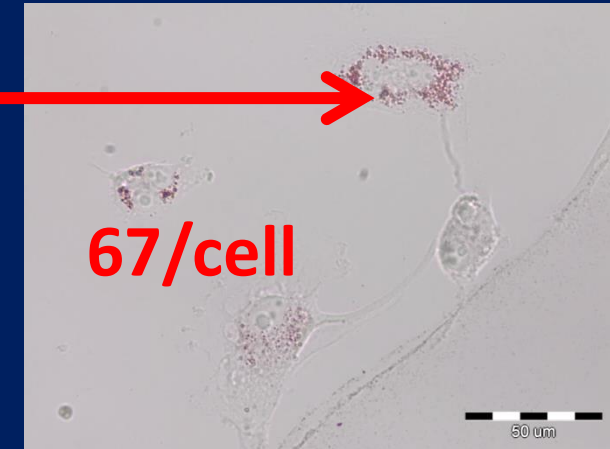
# LIPID DROPLETS in Astrocytes vs Glioblastoma U89



**Astrocytes –normal cells**



**Astrocytoma –malignant cells**



**Glioblastoma U89- highly malignant cells**

- Cancer cells contain increased numbers of lipid droplets compared with normal cells.
- Increased amount of lipid droplets correlates with increased aggressiveness of cancer.
- The increased amount of cytoplasmic lipid droplets in the human cancer cells may be closely related to increased rate of lipid synthesis in cancerous tissues.

# HOW DOES RAMAN SPECTROSCOPY AND IMAGING BENEFIT CANCER RESEARCH?

- **EPIGENETIC MODIFICATIONS**

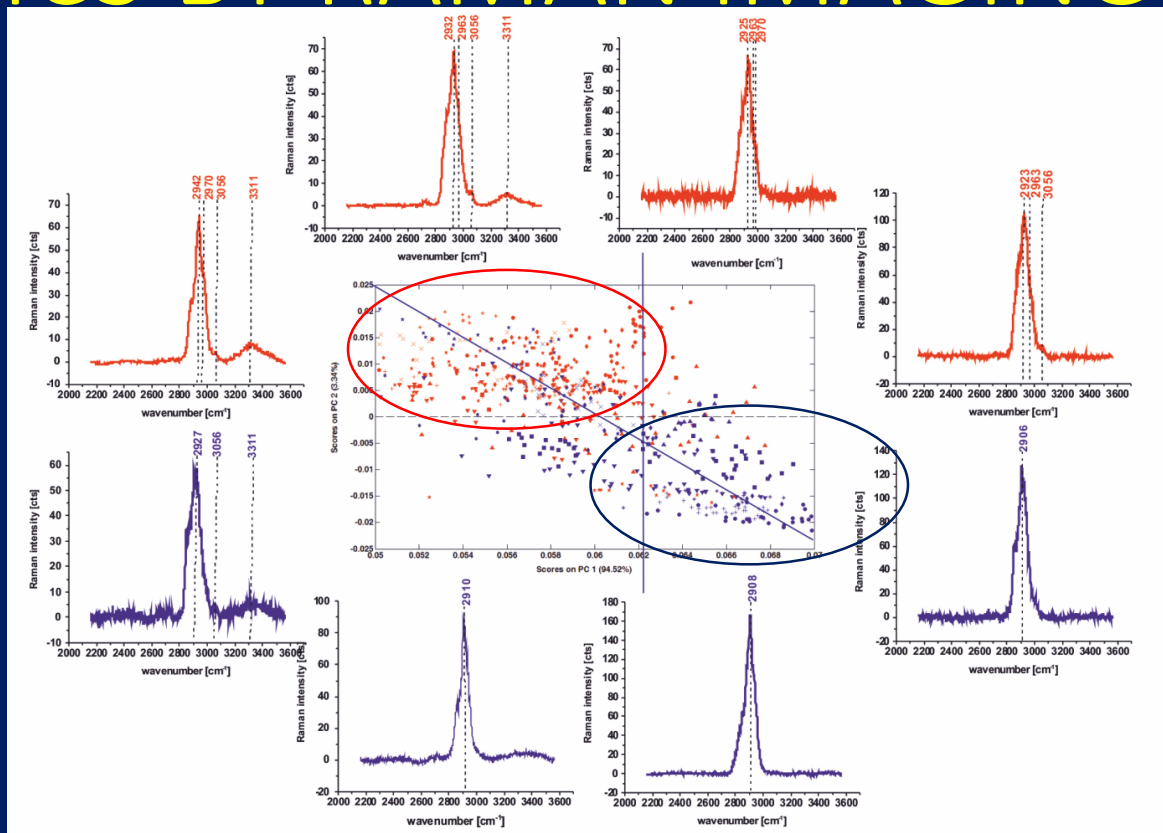
Reprogrammed metabolic pathways that are essential for cancer cell survival and growth affect epigenetics.

# EPIGENETICS BY RAMAN IMAGING

- **We will develop a novel Raman based alternative for currently existing epigenetics research approaches. The proposed Raman approach can 'upgrade' cancer epigenetic tests and answer many questions by monitoring the biochemistry of cancer cells.**

# EPIGENETICS BY RAMAN IMAGING

In order to visualize chemical similarities and differences in level of acetylation and methylation in cancerous and normal tissues we have evaluated the predictive validity and potential of Raman spectroscopy by using multivariate statistical methods for data interpretation.



The results of the PCA reveal separation of the cancer and normal tissues. The similarities and differences are clearly visible by grouping the Raman spectra in two separate clusters, where the spectra with similar or identical vibrational properties for the samples from the tumor mass (red points) are grouped in the upper area while the samples from the safety margin (blue points) are grouped in the lower area of the plot.

PCA score plot (model: area normalized to 1) for the Raman spectra from the region (2000 cm<sup>-1</sup> -3700 cm<sup>-1</sup>) of the human breast tissue samples from the tumor mass (red points) and the safety margin (blue points), integration time 0.5 s, laser power 10 mW; The characteristic spectra corresponding to the various coordinates of the PC1-PC2 score plot.

[B. Brozek-Pluska, M. Kopec, H. Abramczyk, Development of a new diagnostic Raman method for monitoring epigenetic modifications in the cancer cells of human breast tissue, Anal. Methods, 2016, 8, 8542-8553](#)

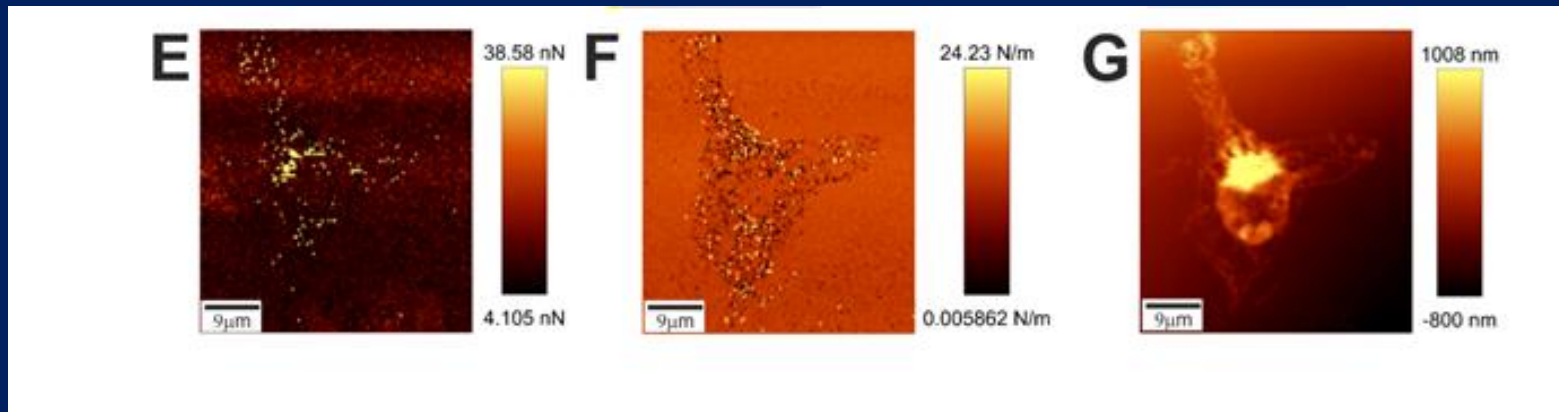
No technology has proven effective for detecting polarity changes in cancer

- **The biochemistry of cancer due to metabolic or epigenetic alterations is related to second important feature of cells – polarity of cells**
- There is also a significant need to develop ways to define and quantify polarity changes in cancer. The conventional method of monitoring the localization of membrane proteins such as E-cadherin, ZO-1 and mucins to monitor apical-basal polarity is misleading and inconclusive [Annu Rev Cell Dev Biol. 2012 ; 28: 599–625].

# Nanomechanical signatures of cancer

The ability of epithelial cells due to polarity to interact with each other and with the surrounding environment to maintain tissue organization that is lost in cancer is related to next important feature of cells – stiffness and adhesion.

U-87 MG cell (glioblastoma - brain)



adhesion image (E), stiffness image (F) and topography image (G) of air-dried cell



# AFM stiffness and adhesion of cancer cells and tissue by DPFM

To calculate the stiffness of the sample the information on the slope of the rising part of the DPFM curve is needed. Fig. II. presents the force-distance curve recorded by DPFM mode, the fitting range (in light blue) and the fitting curve (in dark blue).

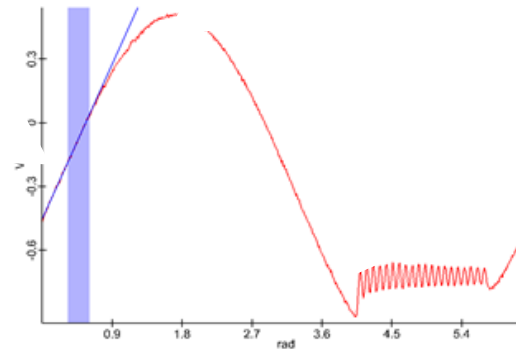


Fig. II. The force-distance curve recorded by DPFM, the selected range of interest with the fitting curve.

We will measure cancer cells and tissues by DPFM to learn how rigid, how sticky, how adherent cancer cells are.

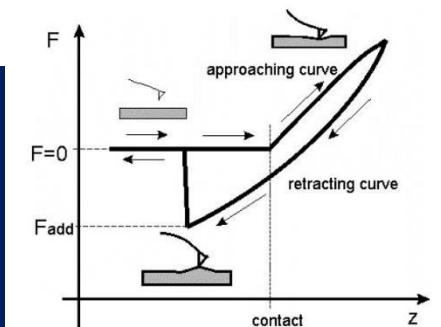
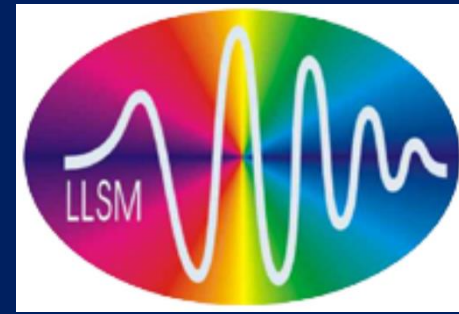


Figure 2. A typical force-distance curve recorded by AFM in force mode. Both the approaching and retracting curves are shown.



# Novel strategies for brain tumor research

2017

[www.impactjournals.com/oncotarget/](http://www.impactjournals.com/oncotarget/)

Oncotarget, Advance Publications 2017

## Novel strategies of Raman imaging for brain tumor research

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<sup>2</sup>Polish Mother's Memorial Hospital Research Institute, Department of Neurosurgery and Neurotraumatology, 3-338 Lodz, Poland

**Correspondence to:** Abramczyk Halina, **email:** [abramczy@mitr.p.lodz.pl](mailto:abramczy@mitr.p.lodz.pl)

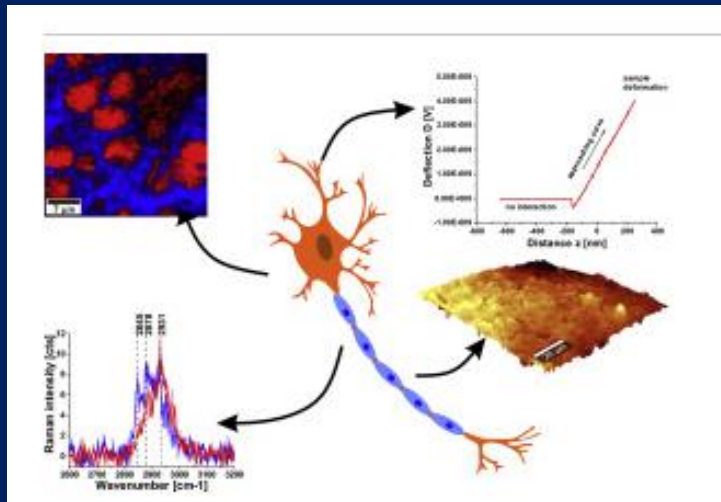
**Keywords:** Raman spectroscopy, Raman imaging, brain tumor, CNS, iodine number

**Received:** January 21, 2017

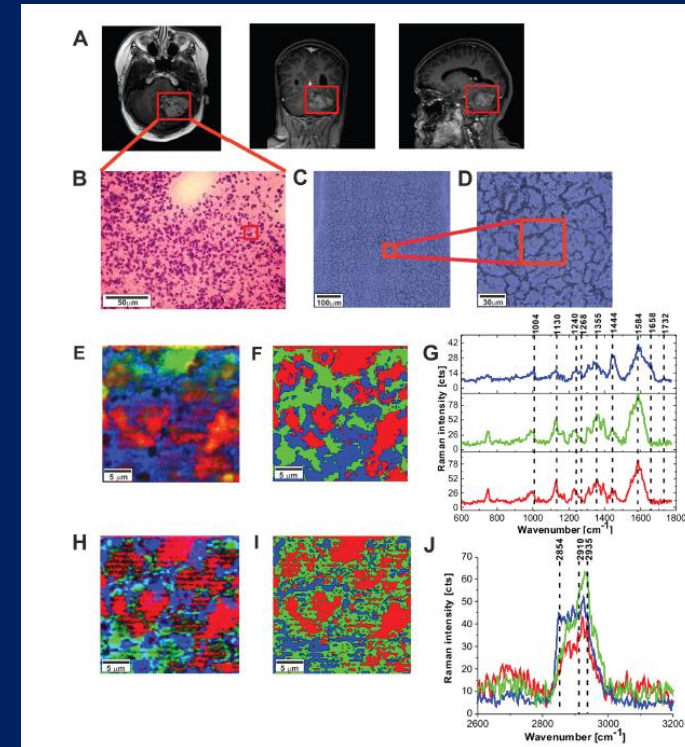
**Accepted:** April 29, 2017

**Published:** July 28, 2017

# The biochemical and nanomechanical signatures of brain cancer



A. [Imiela, B. Polis, L. Polis, H. Abramczyk, Novel strategies of Raman imaging for brain tumor research, Oncotarget, 2017, 8:85290-85310](#)



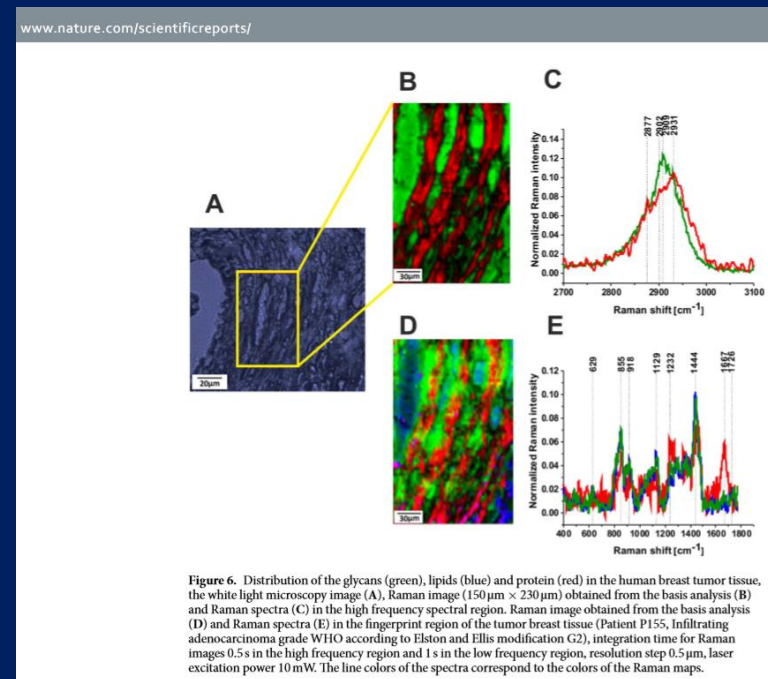
**Figure 5:** The MRI image (A) H&E-stained histological image (B) stitching microscopy image (520 μm x 520 μm) (C) microscopy image (155 μm x 155 μm) (D) Raman images (50 μm x 50 μm) obtained by basis analysis (E) and cluster analysis (F) and the characteristic vibrational Raman spectra in the fingerprint frequency region (G) Raman images (50 μm x 50 μm) obtained by basis analysis (H) and cluster analysis (I) and the characteristic vibrational Raman spectra in the high frequency region (J) of the tumor CNS (medulloblastoma, grade WHO IV, infratentorial. Left cerebellar hemisphere) (P9). The line colors of the spectra correspond to the colors of the Raman maps. Raman integration time for images 0.5 s, resolution step: 1 μm, laser excitation power: 10m W.

## Monitoring glycosylation metabolism in brain and breast cancer by Raman and AFM imaging

- Our recent results opened a new window for exploration of processes occurring in non-cellular space - glycocalyx –a coat on the external surface of their plasma membranes of epithelial cells consisting of several carbohydrates, several carbohydrate moieties of membrane glycolipids and glycoproteins, which serve as backbone molecules for support.

# Visualization techniques of glycocalyx- Raman imaging wins once again

- Because of the functional importance of the epithelial glycocalyx, development of direct visualization techniques is crucial to establish its exact role.
- The glycocalyx can be labeled by administration of specific markers that attach to one or more of its components, making them fluorescent or detectable, but Raman imaging wins once again.

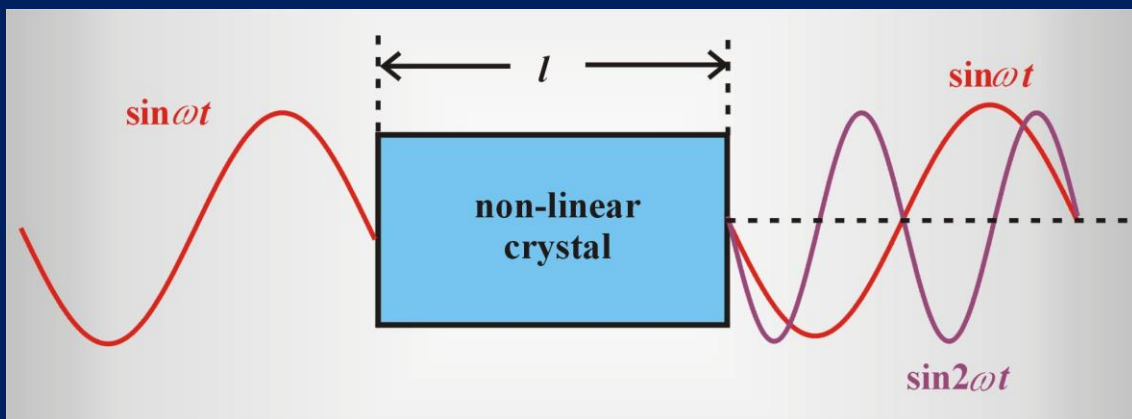


M. Kopeć, A. Imiela, H. Abramczyk, Monitoring glycosylation metabolism in brain and breast cancer by Raman imaging, Scientific Reports, Nature, 2019

# ZJAWISKA NIELINIOWE DRUGIEGO RZĘDU

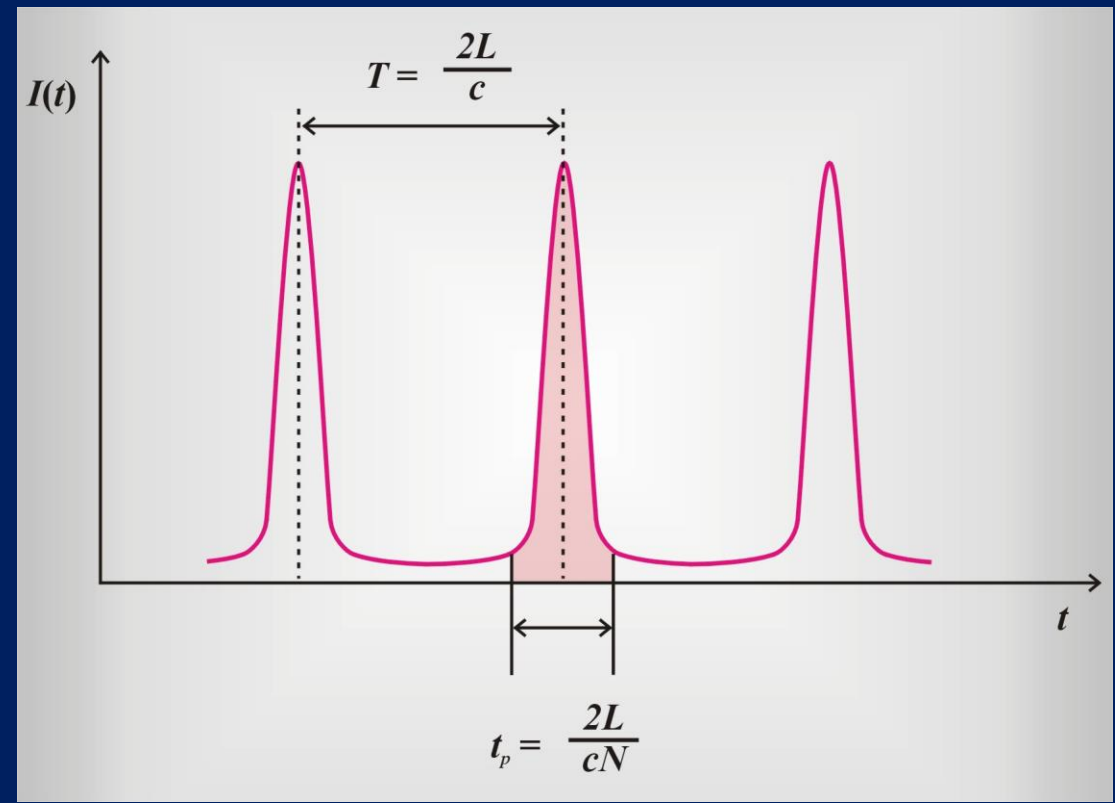
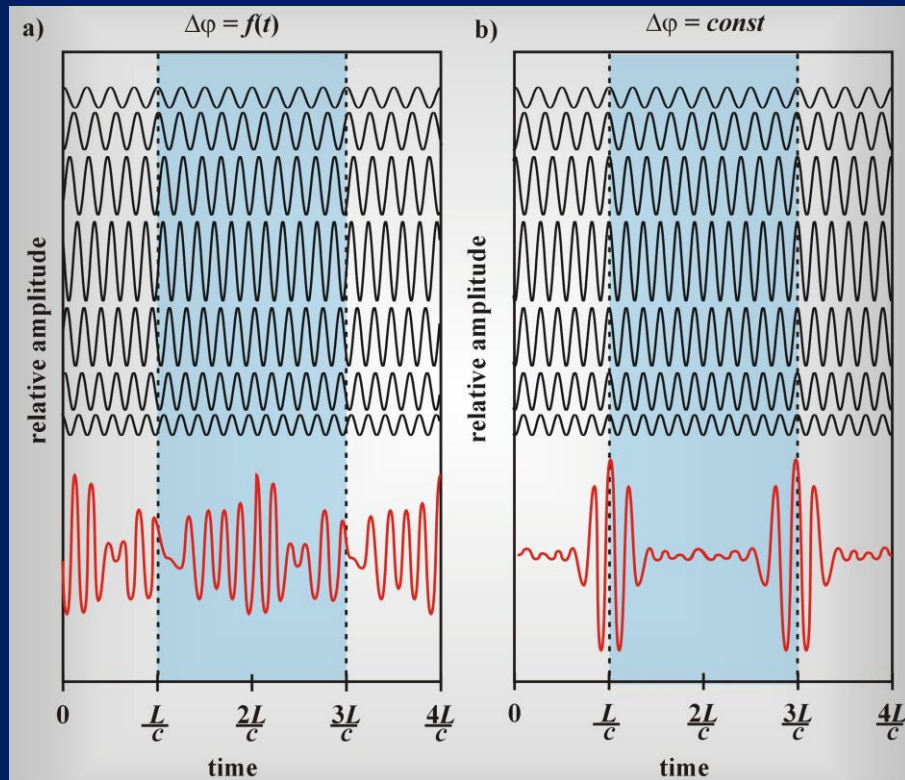
$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$

**GENERACJA DRUGIEJ  
HARMONICZNEJ (SHG)**



# ZALETY OPTYKI NIELINIOWEJ W BADANIACH NOWOTWORÓW

# METODY GENEROWANIA ULTRAKRÓTIKICH IMPULSÓW-SYNCHRONIZACJA MODÓW



Aby zbadać interakcje nieliniowe, potrzebujemy laserów impulsowych o wysokich mocach szczytowych



# ZALETY OPTYKI NIELINIOWEJ W BADANIACH NOWOTWORÓW

OPTYKA NIELINIOWA	OPTYKA LINIOWA
<p>Wywołanie zjawiska i odczyt dla oddziaływań nieliniowych uzyskuje się za pomocą co najmniej dwóch impulsów o dobrze określonych właściwościach: wiązka pompująca i wiązka sondująca. Różnica w stosunku do innych nieliniowych metod obrazowania optycznego, takich jak fluorescencja i niespójna mikroskopia Ramana.</p>	
<p>Odczyt wymuszony tłumi procesy niespójne, takie jak emisja spontaniczna</p>	<p>Emisja spontaniczna</p>
<p>Połączenie wysokiej mocy lasera i czułej detekcji skutkuje wysoką wydajnością interakcji nieliniowej</p>	
<p><b>Umożliwia to szybkie uzyskiwanie obrazów</b></p>	<p><b>Długie czasy akwizycji</b></p>
<p><b>Krótki czas naświetlania przez laser redukuje foto-uszkodzenie próbki spowodowane przez laser o dużej mocy</b></p>	
<p>Cząsteczki znakujące nie są potrzebne do przygotowania próbki, jak ma to miejsce w obrazowaniu fluorescencyjnym</p>	
<p>Jednym z kluczowych celów obecnych badań jest skrócenie czasu potrzebnego do uzyskania obrazu. Jest to często bezpośrednio związane z czułością wykrywania. Dzięki nowoczesnemu mikroskopowi skaningowemu czas akwizycji do 30 fs jest możliwy dla obrazu 512 na 512 pikseli</p>	

## OPTYKA NIELINIOWA

Zastosowanie femtosekundowych impulsów laserowych zamiast szeroko stosowanych impulsów pikosekundowych w kolejnej innowacyjnej funkcji mikroskopu. Dzięki zwiększonej szerokości pasma wzbudzenia krótszych impulsów femtosekundowe impulsy laserowe są w stanie lepiej wzbudzać izolowane rezonanse wibracyjne

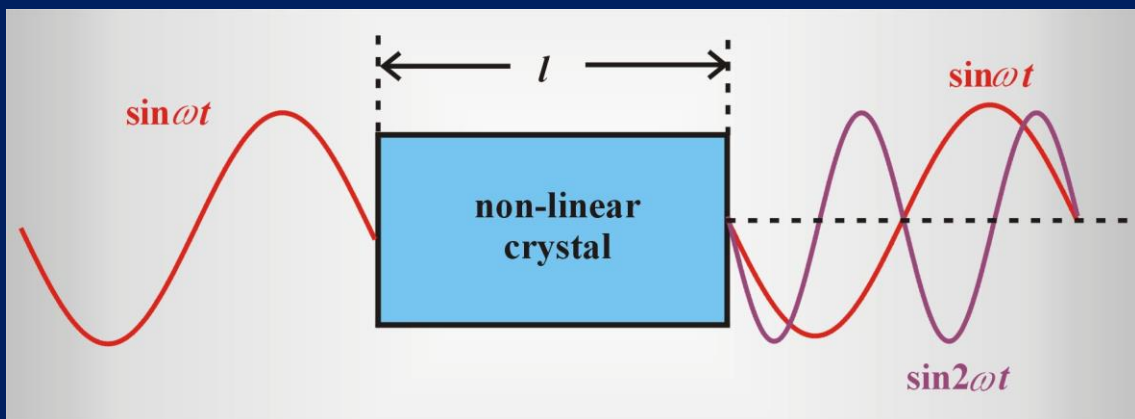
Ważnym zagadnieniem ze względu na wysokie moce wiązek laserowych jest fotouszkodzenie próbki podczas analizy. Musi nastąpić zbalansowanie mocy wiązki wzbudzającej – dla impulsów zakresu NIR do około 40mW. Na takim poziomie niebezpieczeństwo uszkodzenia próbki jest relatywnie niższe niż np. dla mocy rzędu 10mW dla światła z zakresu widzialnego. Obrazowanie może być rejestrowane z wysoką czułością i szybkością. Ekspozycja pojedynczego pixela na wiązkę laserową jest poniżej 4 ms.

## OPTYKA LINIOWA

# ZJAWISKA NIELINIOWE DRUGIEGO RZĘDU

$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$

GENERACJA DRUGIEJ  
HARMONICZNEJ (SHG)



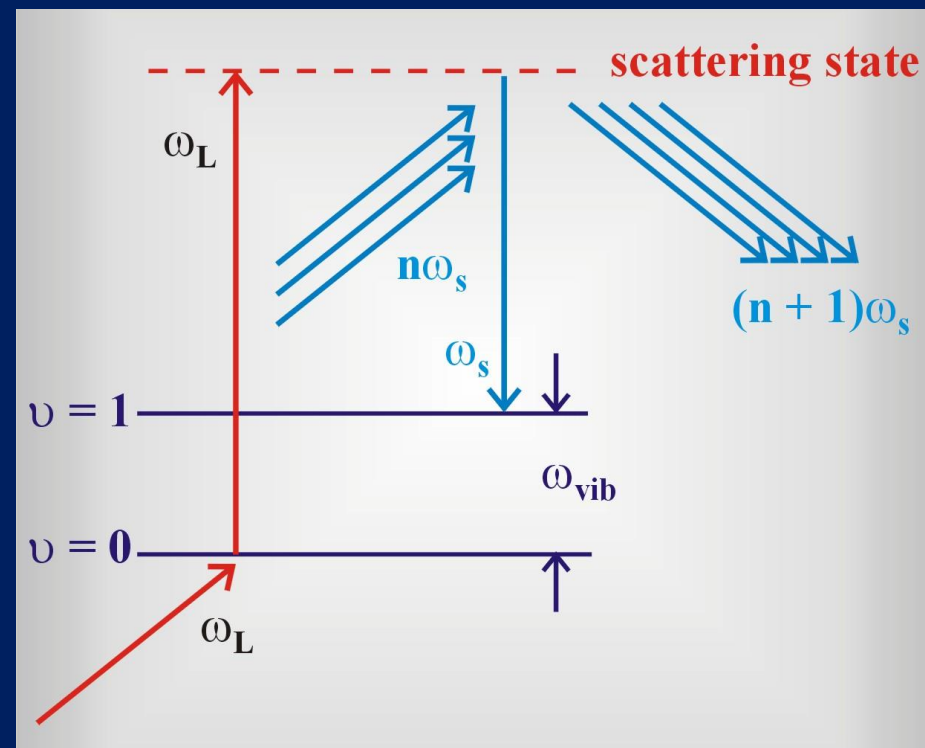
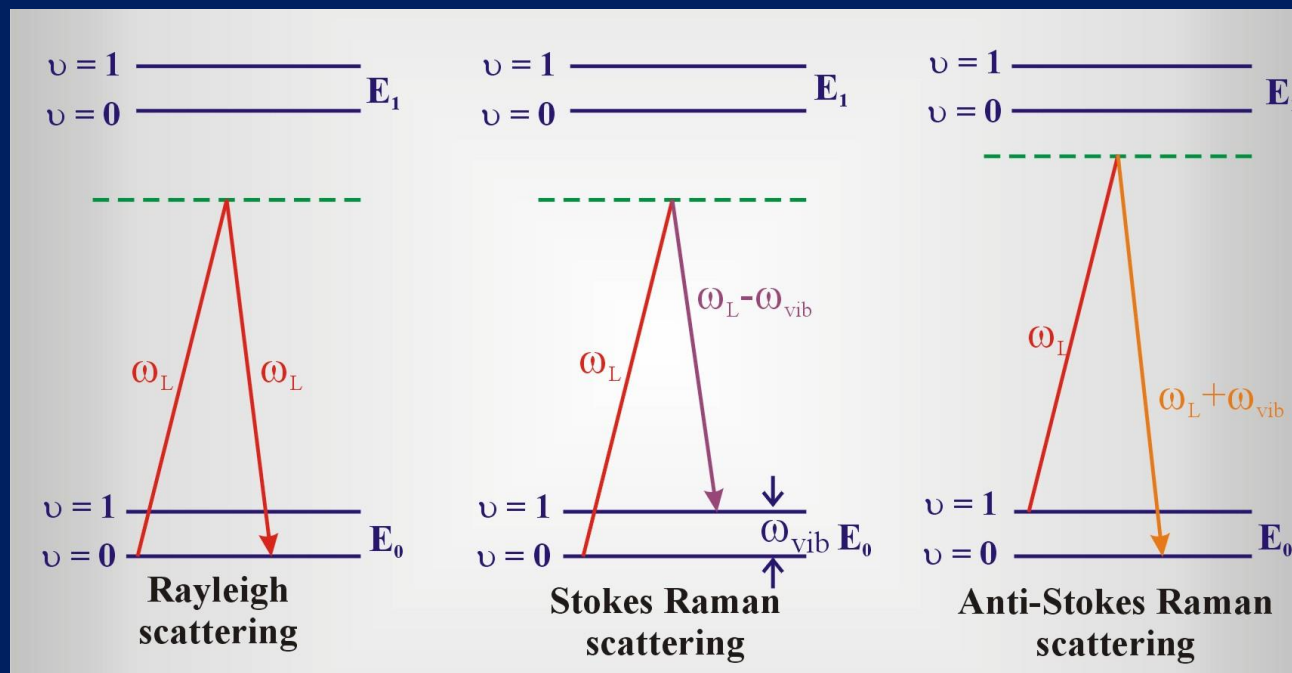
# ZJAWISKA NIELINIOWE TRZECIEGO RZĘDU

$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$

**WYMUSZONE ROZPRASZANIE RAMANA**

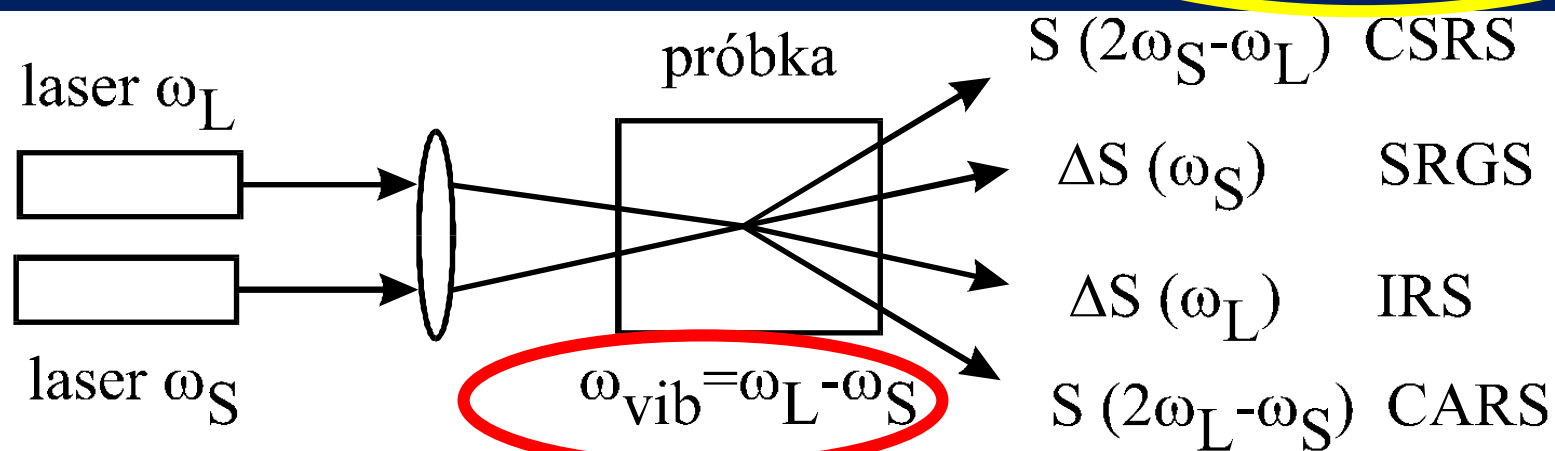
**SPEKTROSKOPIA ABSORPCJI PRZEJŚCIOWEJ  
METODĄ WIĄZKI POMPUJĄCEJ I SONDUJĄCEJ**

# WYMUSZONE ROZPRASZANIE RAMANA



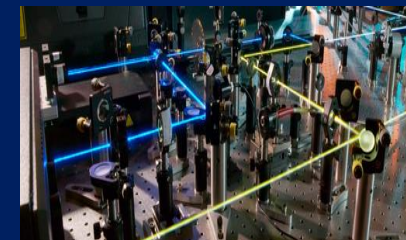
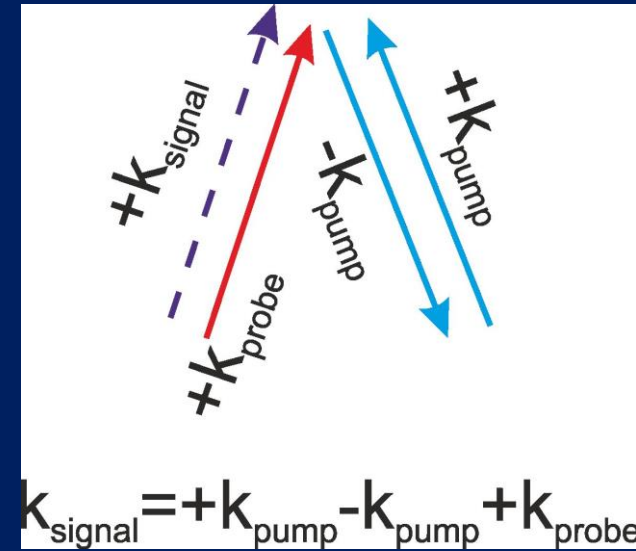
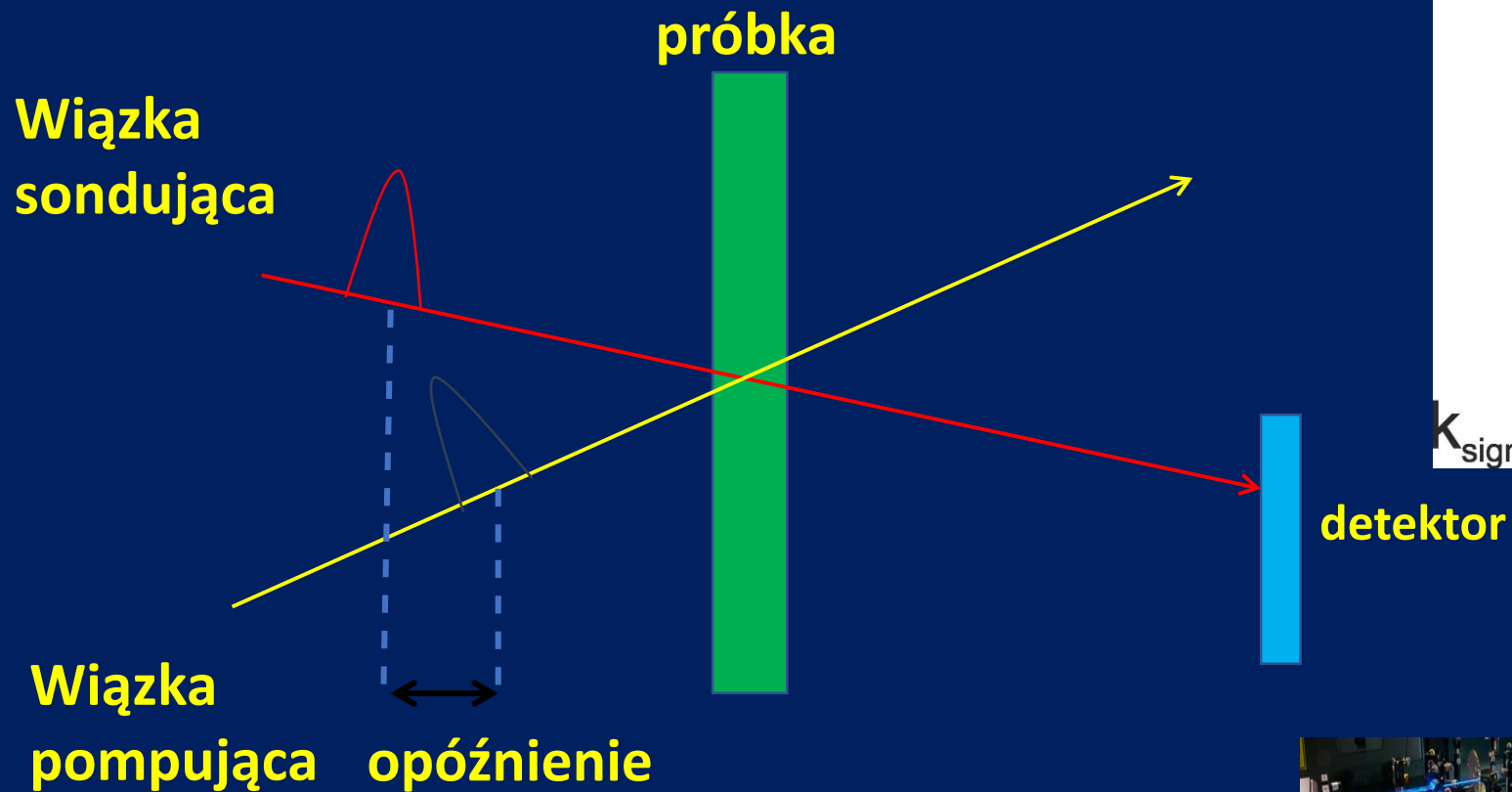
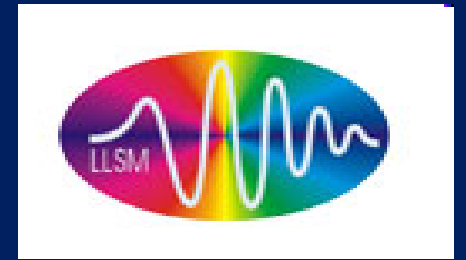
# ZJAWISKA NIELINIOWE TRZECIEGO RZĘDU

$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$

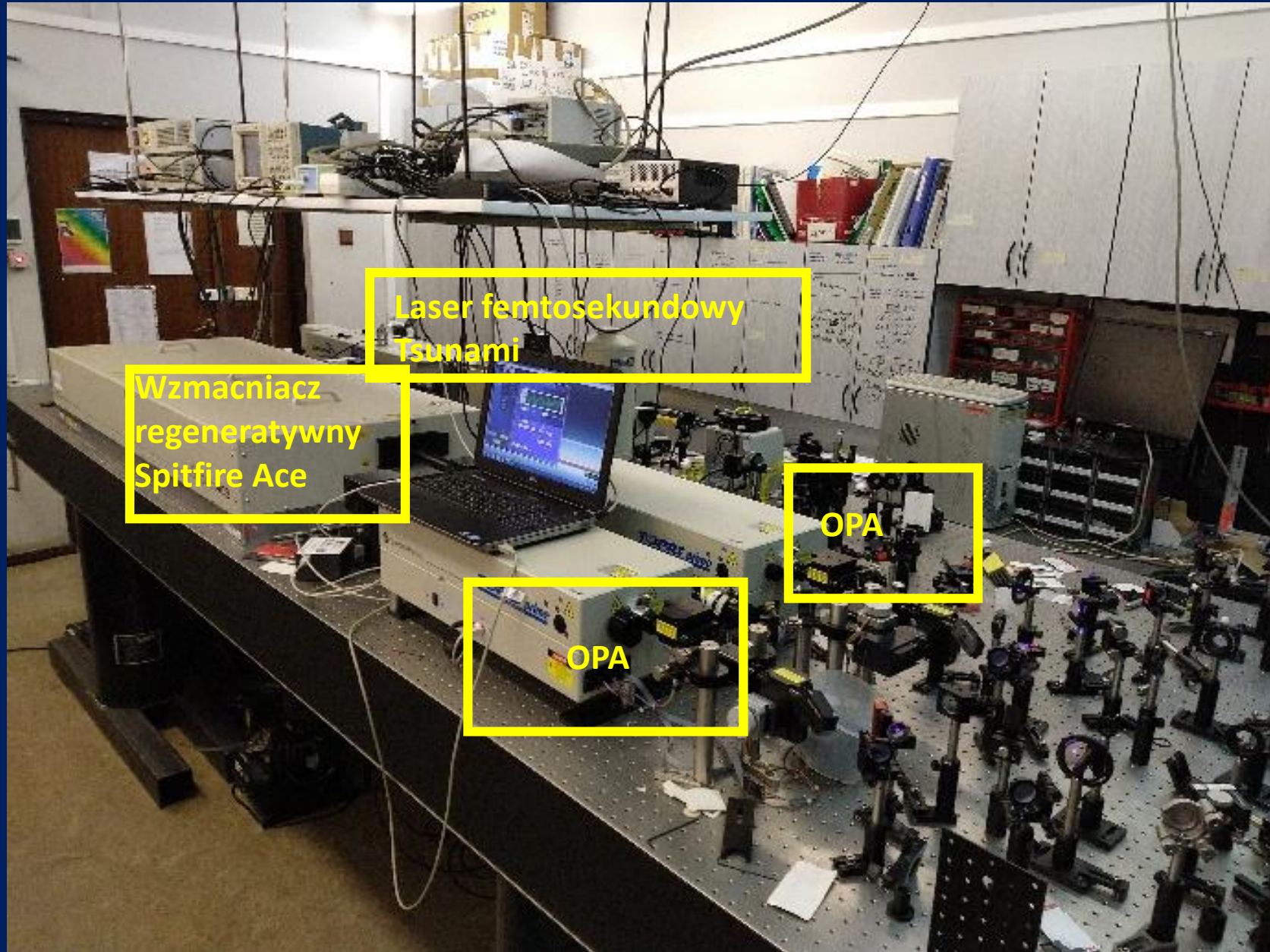


Rys. 6.19. Schematyczny diagram najczęściej stosowanych technik nieliniowego wymuszonego rozpraszania Ramana

# SPEKTROSKOPIA ABSORPCJI PRZEJŚCIOWEJ METODA WIĄZKI POMPUJĄCEJ I SONDUJĄCEJ



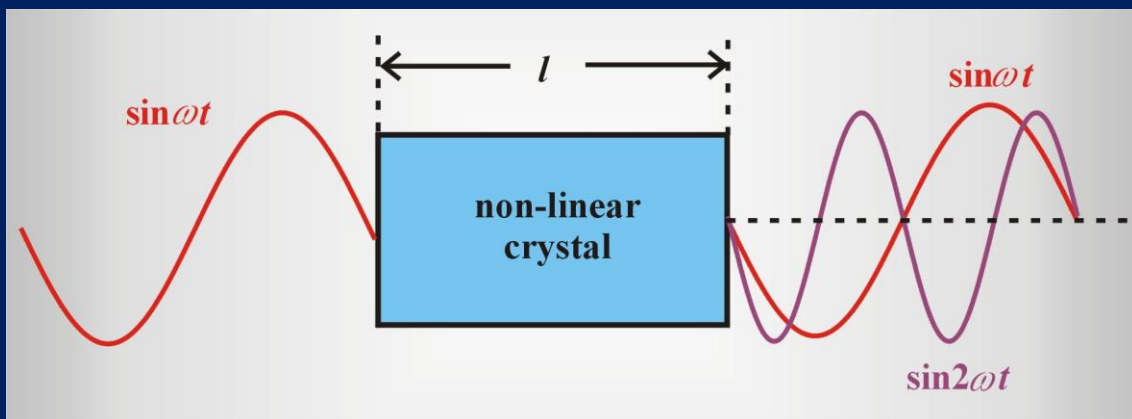




# ZJAWISKA NIELINIOWE DRUGIEGO RZĘDU

$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$

**GENERACJA DRUGIEJ  
HARMONICZNEJ (SHG)**



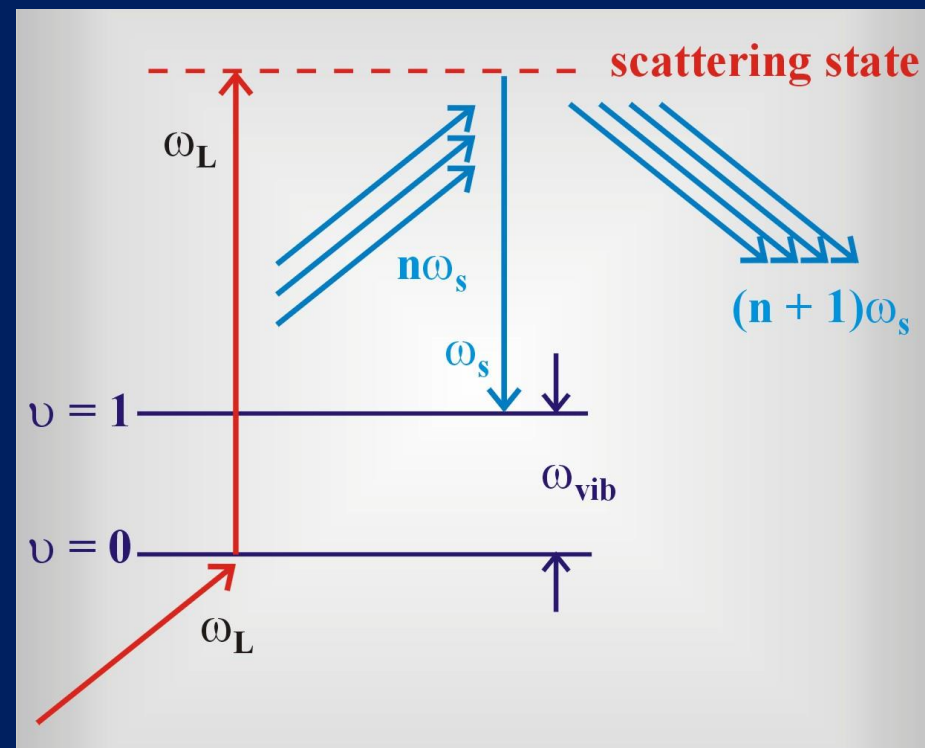
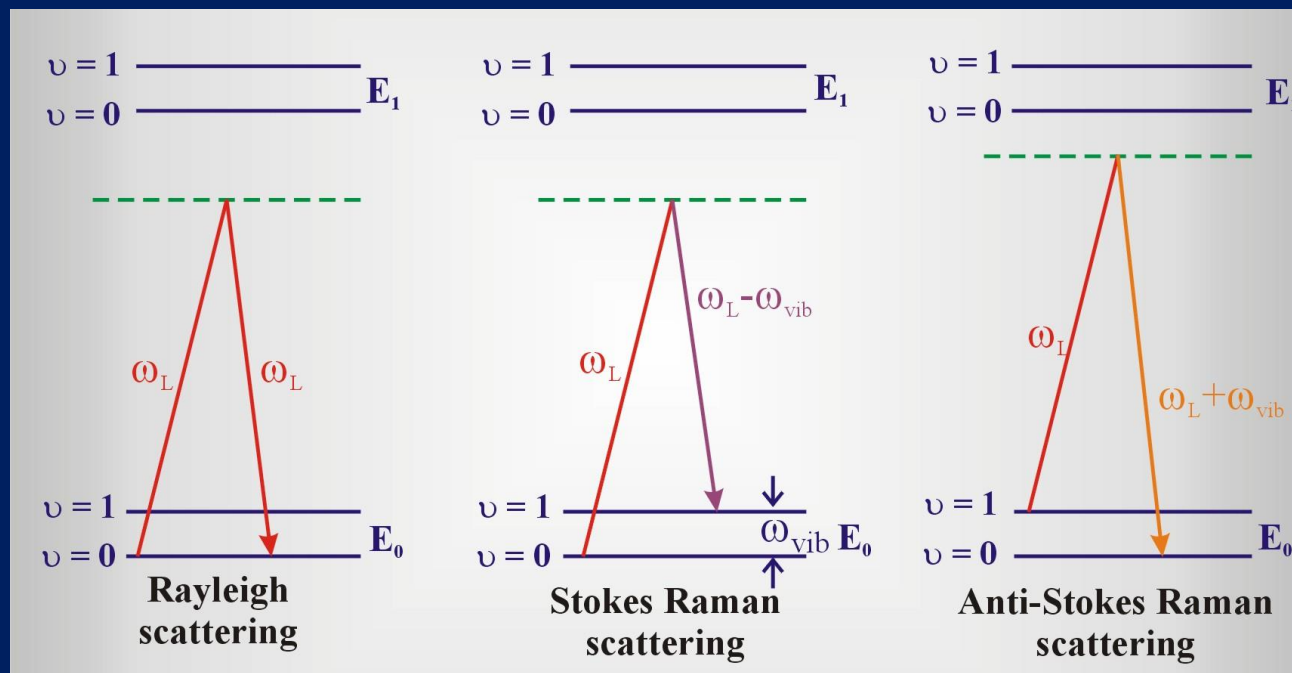
# ZJAWISKA NIELINIOWE TRZECIEGO RZĘDU

$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$

**WYMUSZONE ROZPRASZANIE RAMANA**

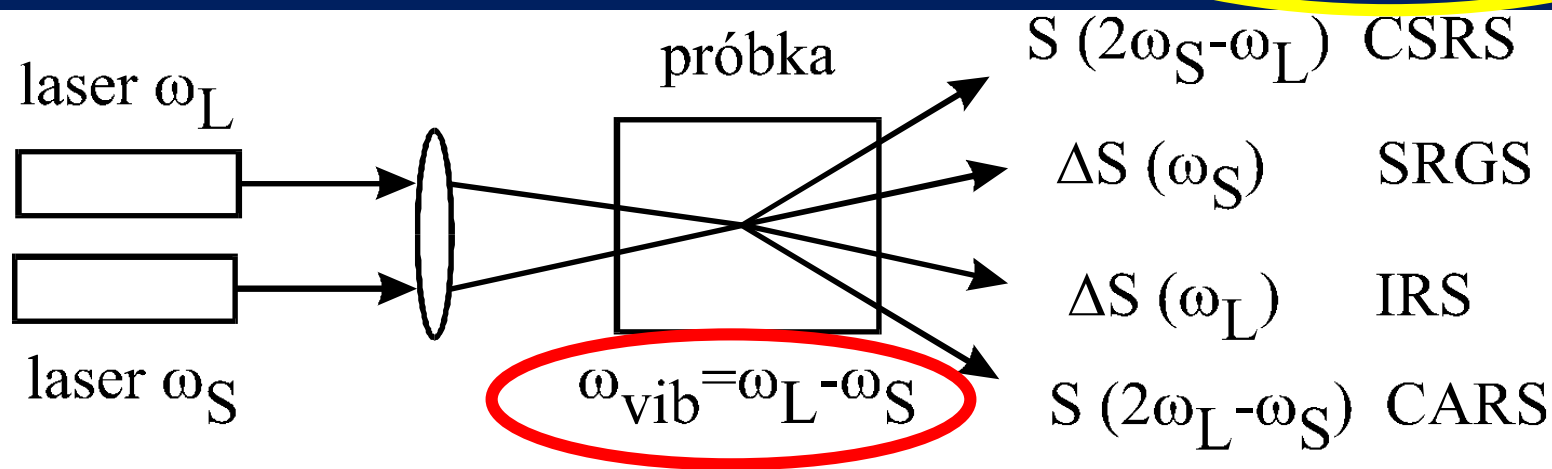
**SPEKTROSKOPIA ABSORPCJI PRZEJŚCIOWEJ  
METODĄ WIĄZKI POMPUJĄCEJ I SONDUJĄCEJ**

# WYMUSZONE ROZPRASZANIE RAMANA



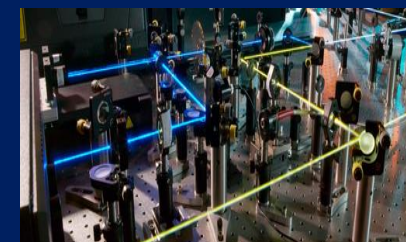
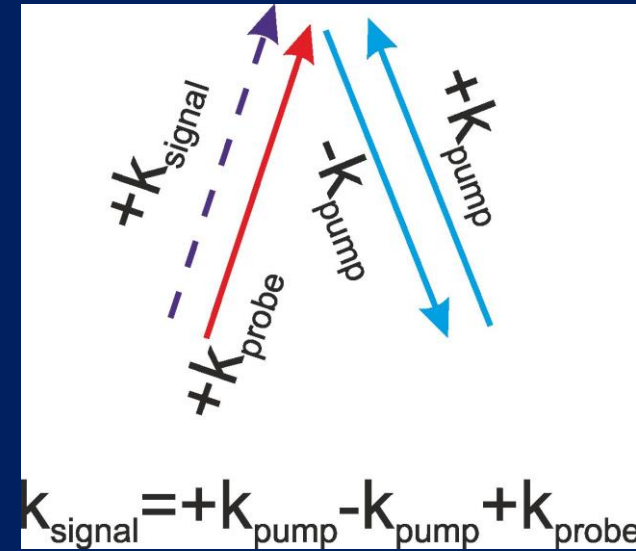
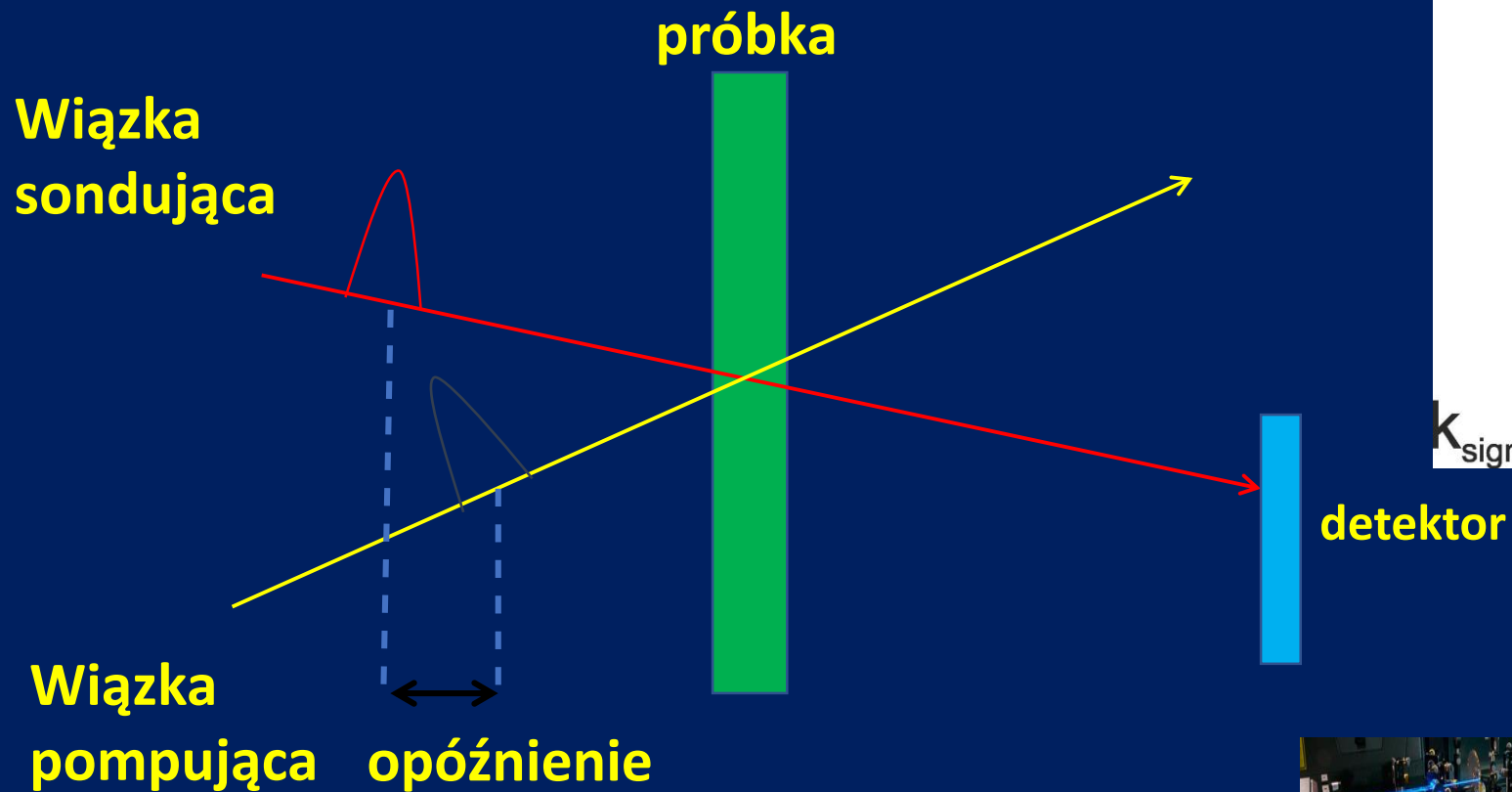
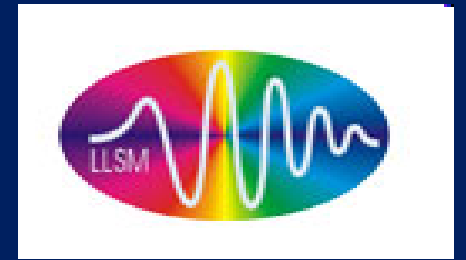
# ZJAWISKA NIELINIOWE TRZECIEGO RZĘDU

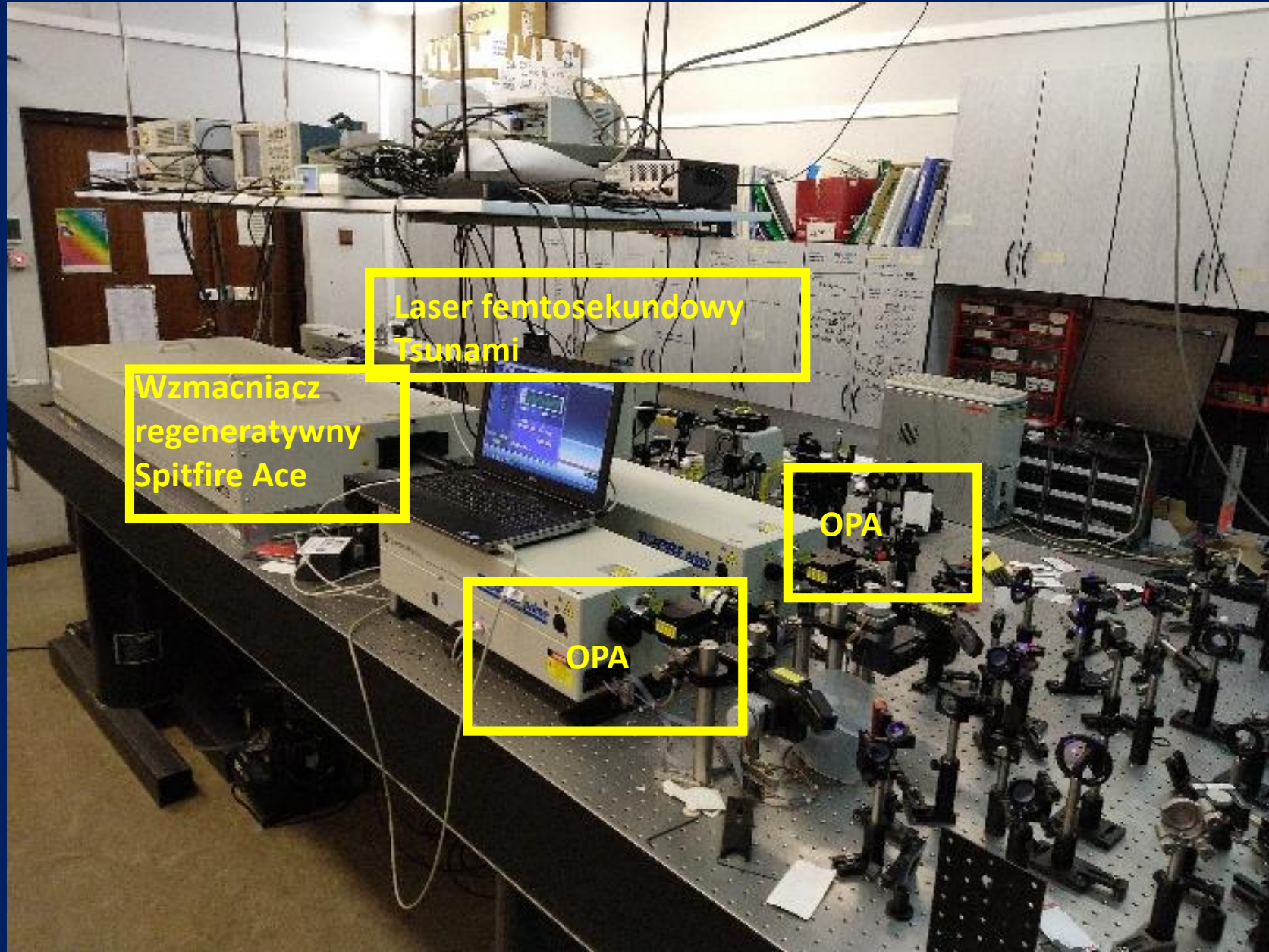
$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$



Rys. 6.19. Schematyczny diagram najczęściej stosowanych technik nieliniowego wymuszonego rozpraszania Ramana

# SPEKTROSKOPIA ABSORPCJI PRZEJŚCIOWEJ METODA WIĄZKI POMPUJĄCEJ I SONDUJĄCEJ





Laser femtosekundowy  
Tsunami

Wzmacniacz  
regeneratywny  
Spitfire Ace

OPA

OPA

# ZJAWISKA NIELINIOWE TRZECIEGO RZĘDU

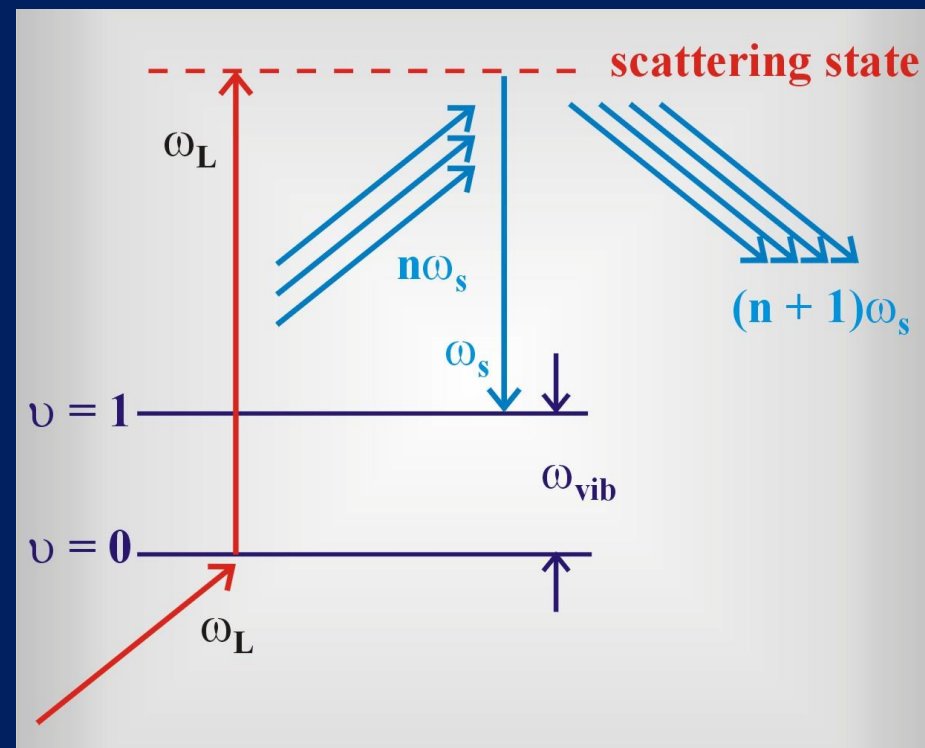
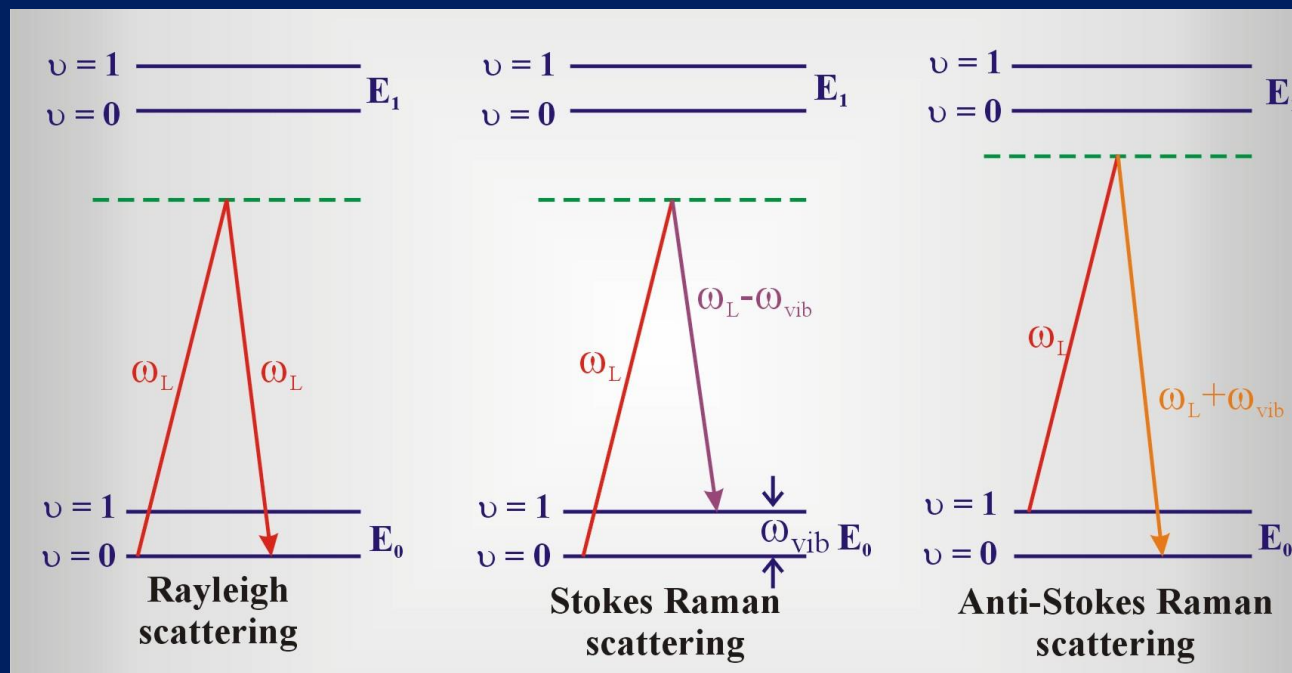
$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$

**WYMUSZONE ROZPRASZANIE RAMANA**

**SPEKTROSKOPIA ABSORPCJI PRZEJŚCIOWEJ  
METODĄ WIĄZKI POMPUJĄCEJ I SONDUJĄCEJ**

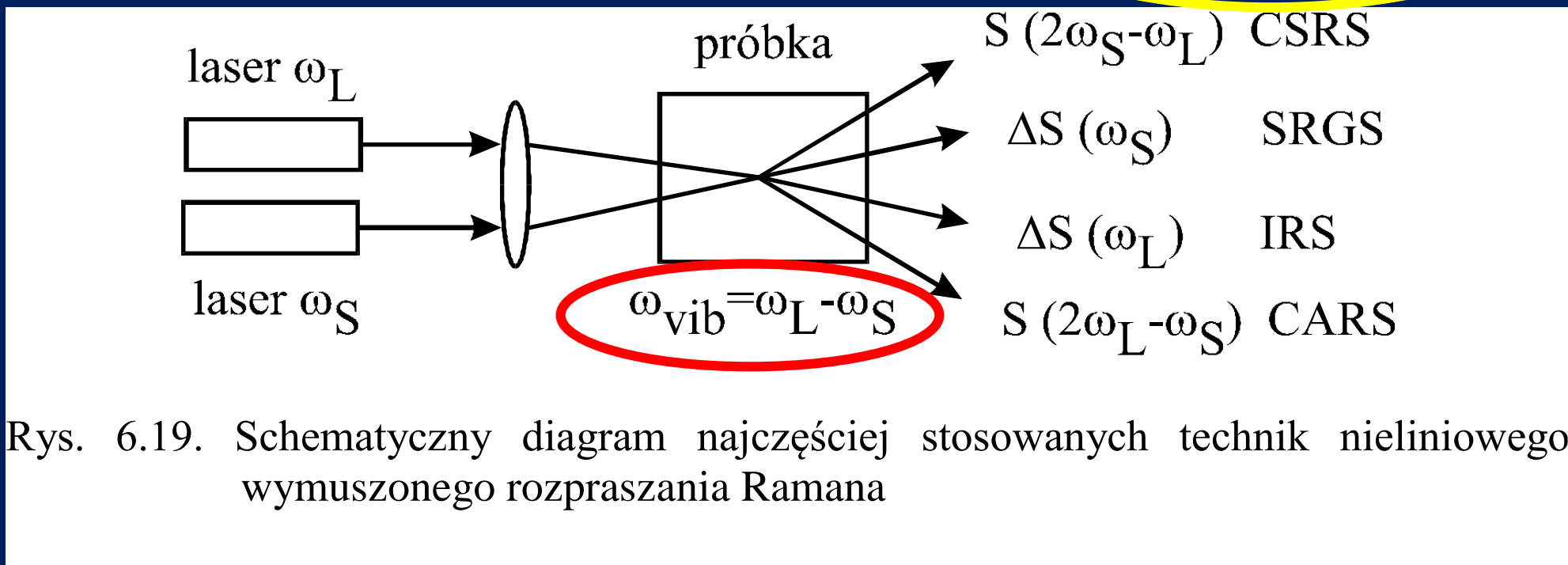


# WYMUSZONE ROZPRASZANIE RAMANA



# ZJAWISKA NIELINIOWE TRZECIEGO RZĘDU

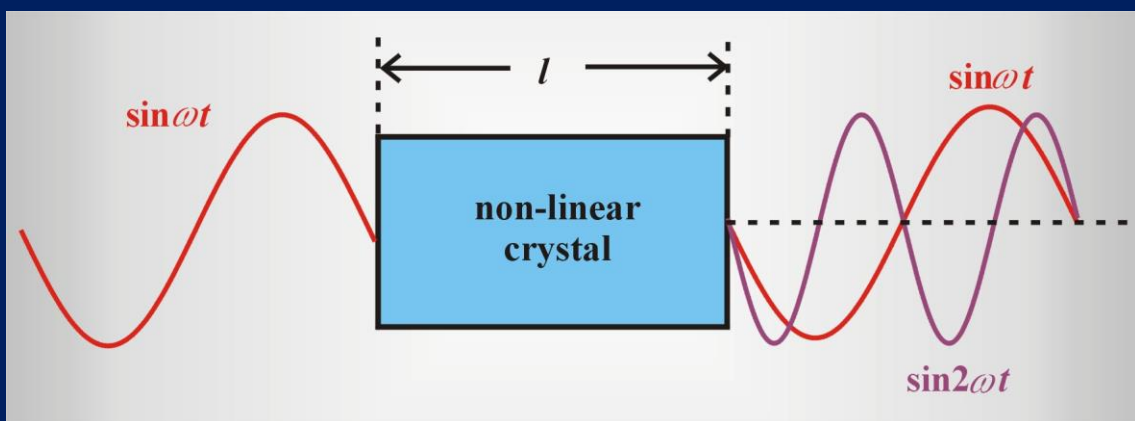
$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$



# ZJAWISKA NIELINIOWE DRUGIEGO RZĘDU

$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$

**GENERACJA DRUGIEJ  
HARMONICZNEJ (SHG)**



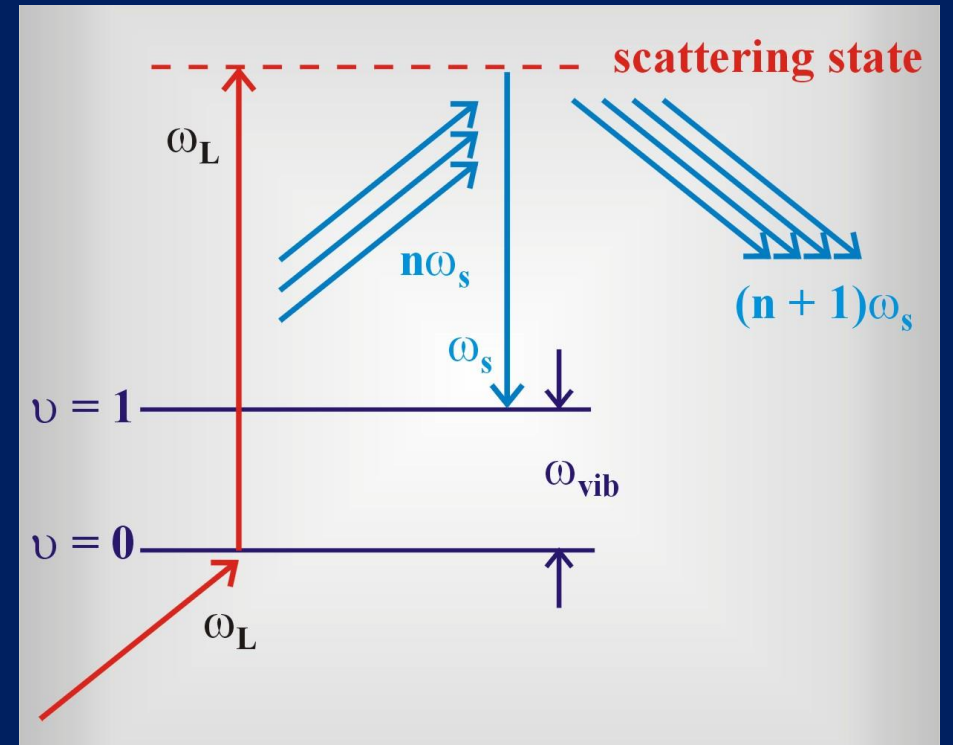
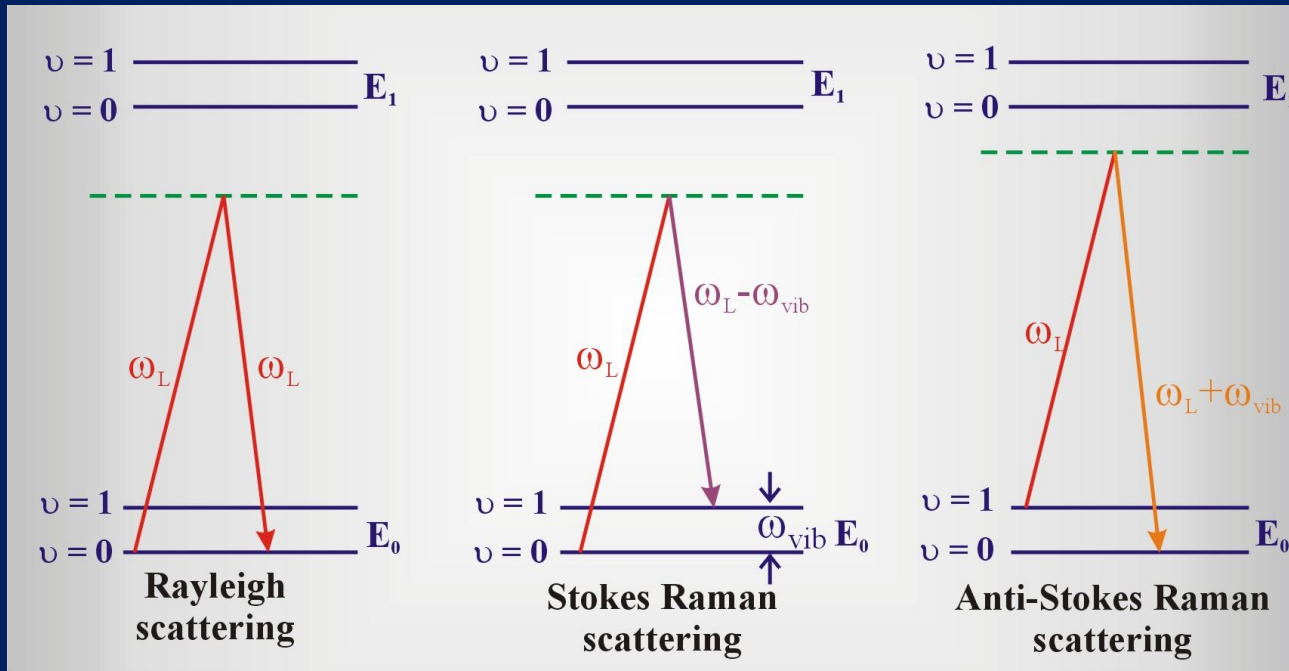
# ZJAWISKA NIELINIOWE TRZECIEGO RZĘDU

$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$

**WYMUSZONE ROZPRASZANIE RAMANA**

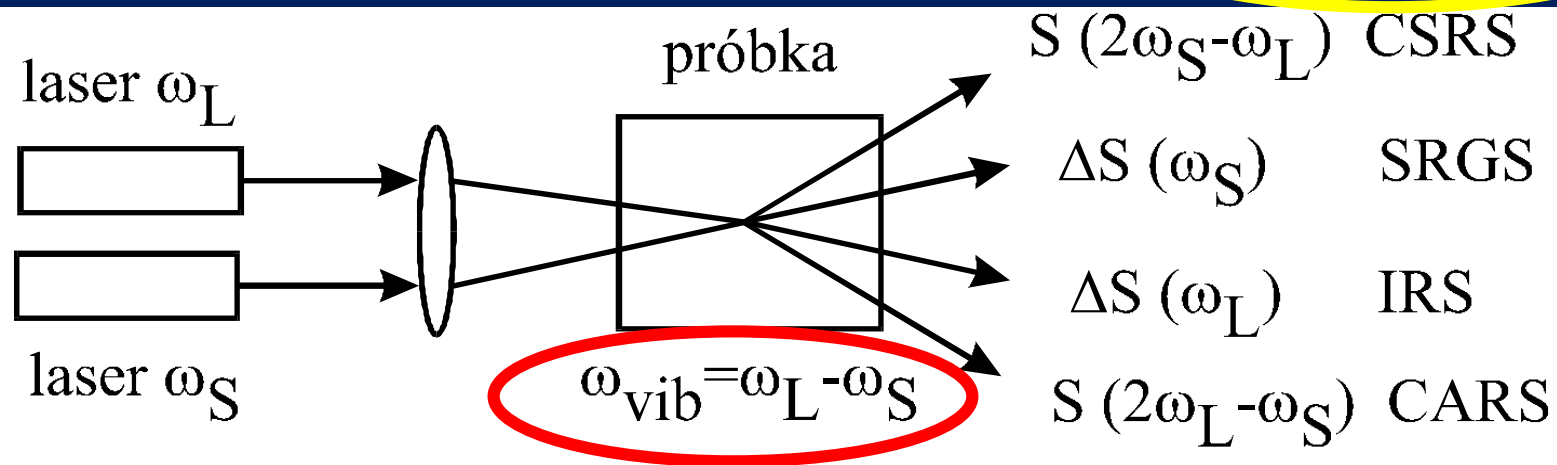
**SPEKTROSKOPIA ABSORPCJI PRZEJŚCIOWEJ  
METODĄ WIĄZKI POMPUJĄCEJ I SONDUJĄCEJ**

# WYMUSZONE ROZPRASZANIE RAMANA



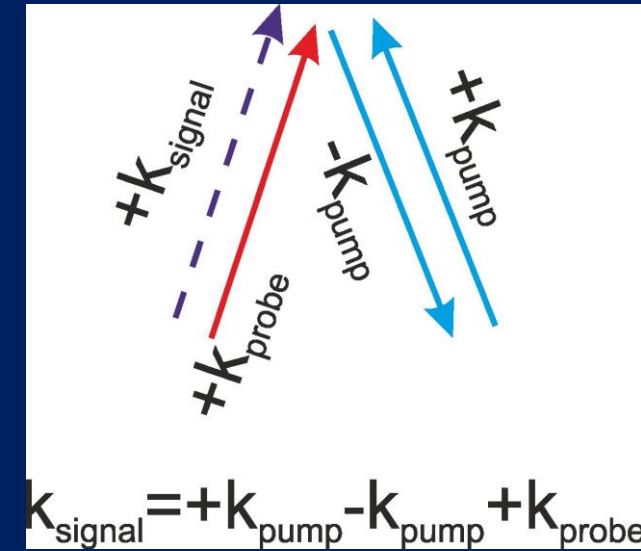
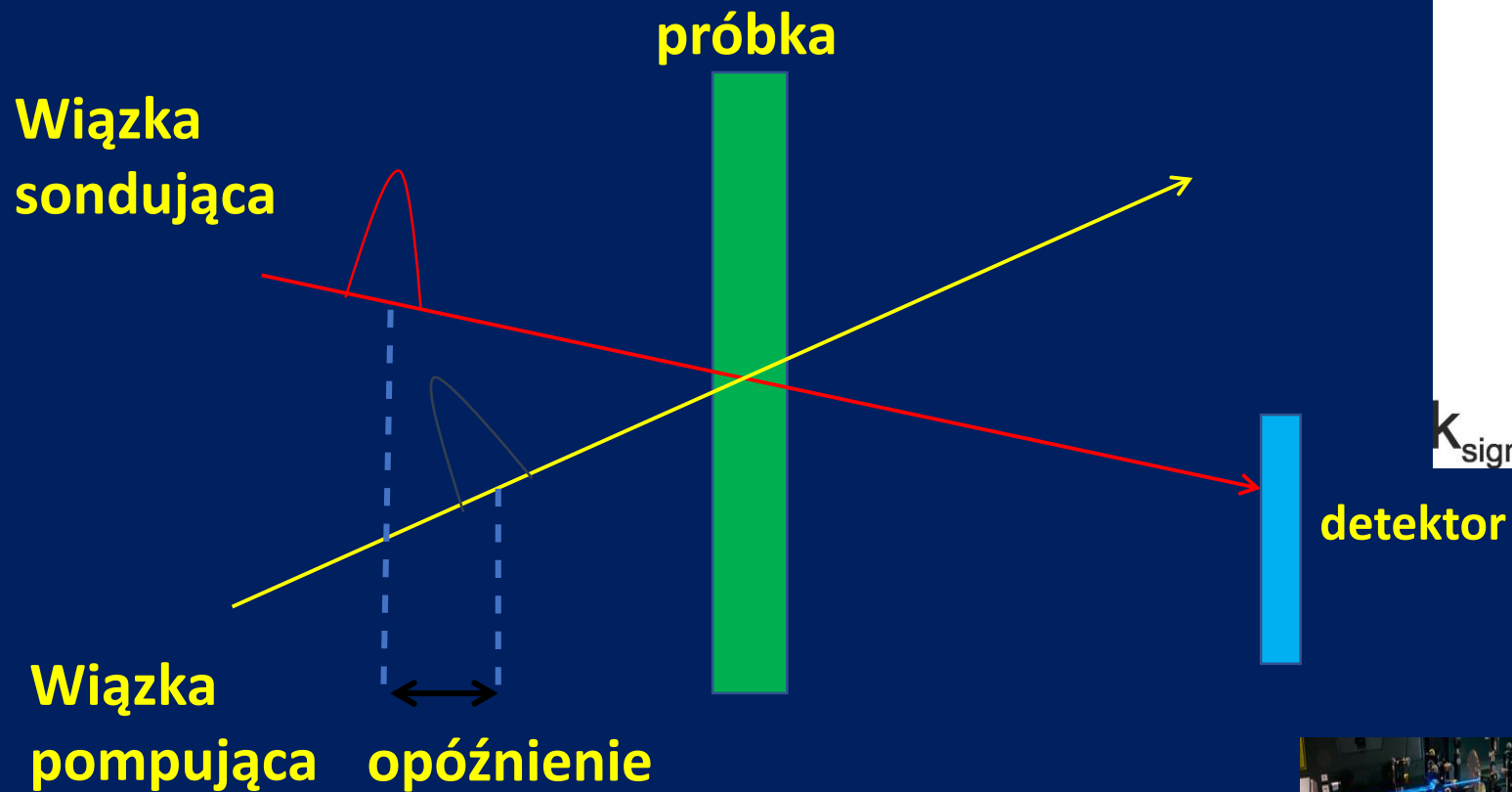
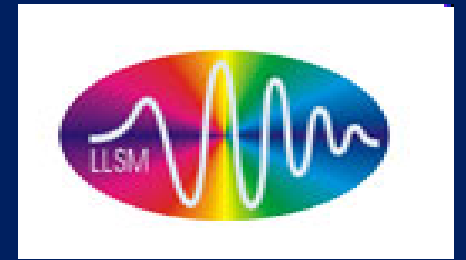
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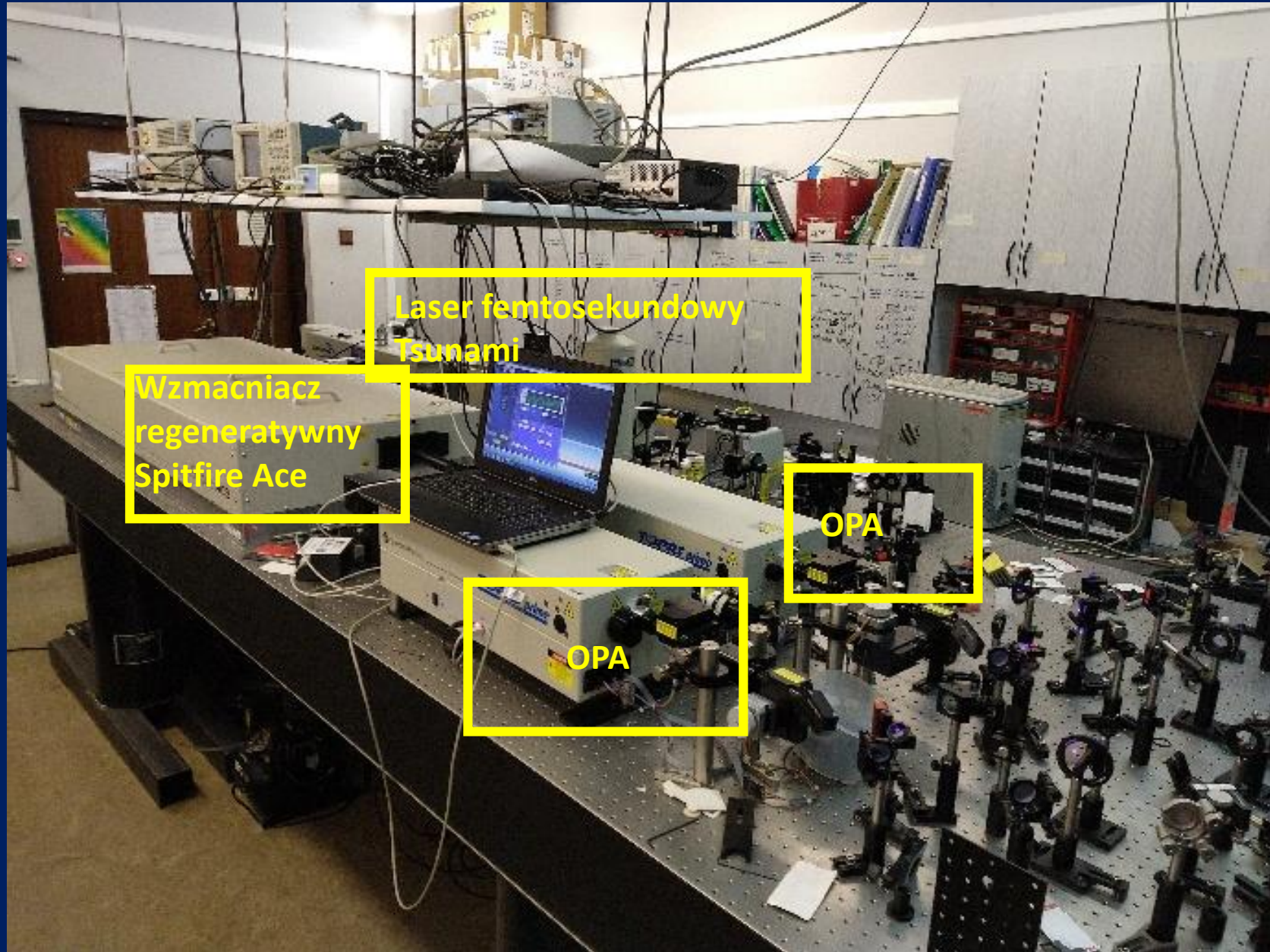
$$P_i = \chi_{ij}^{(1)} E + \chi_{ijk}^{(2)} E_j E_k + \chi_{ijkl}^{(3)} E_j E_k E_l$$



Rys. 6.19. Schematyczny diagram najczęściej stosowanych technik nieliniowego wymuszonego rozpraszania Ramana

# SPEKTROSKOPIA ABSORPCJI PRZEJŚCIOWEJ METODA WIĄZKI POMPUJĄCEJ I SONDUJĄCEJ





Laser femtosekundowy  
Tsunami

Wzmacniacz  
regeneratywny  
Spitfire Ace

OPA

OPA