Label-free NanoBio Chemical Imaging of Cells and Tissues for New Bio-medical Applications

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Funding: MOST, MOCIE, KRISS,

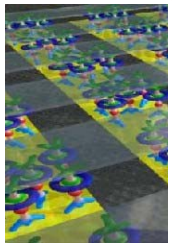
Outline: Our strategy of nano-bio fusion
Present status of nanobio imaging methodology at KRISS
A case report on Atherosclerosis with cardiovascular lipid, cell adhesion, and collagen ECM imaging
Visions in the near future

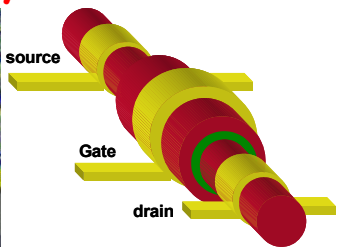
How to utilize NT to solve Biomedical Issues through noble methodologies

1948
First Transistor

Future
15 years
Non-classical CMOS

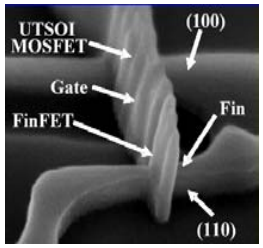
Beyond CMOS





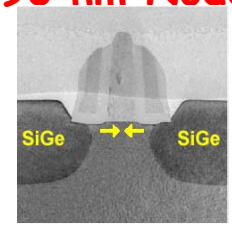
Molecular Switches ?
Nanowire Transistor ?

Tomorrow

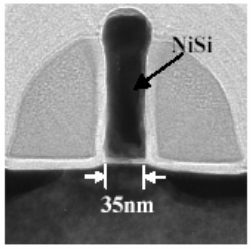


CMOS
pMOS
FINFET

Today
90 nm Node

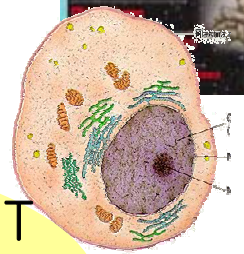


Strain
Enhanced Mobility



New Materials

Nano-Bio Fusion



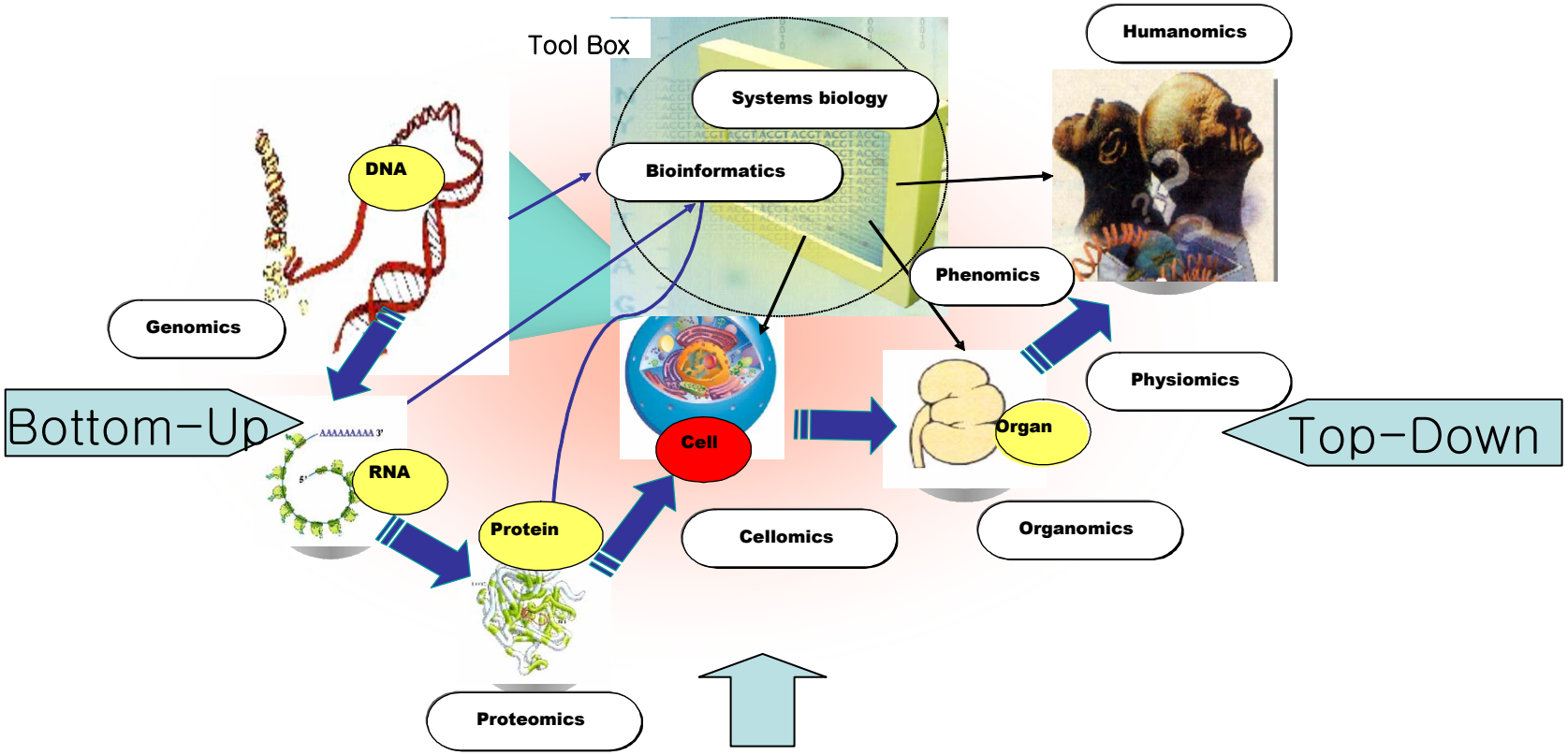
Solving Bio Issues with NT
High throughput
Noble analysis & manipulation

STM/AFM, TEM/SEM, XRD,
PES/AES, SIMS, RBS/MEIS,
Raman, ALD, QD, FIB, ...

Analysis Demands from Bio-Medical R&D

: in-vivo/in-vitro, biochemical imaging, dynamics
sensitivity & selectivity, general methodology

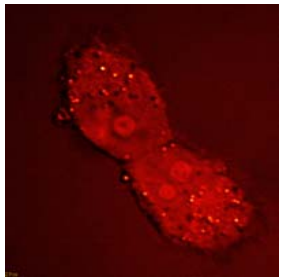
Label-free single cells/tissue biochemical imaging for medical & pharmaceutical applications



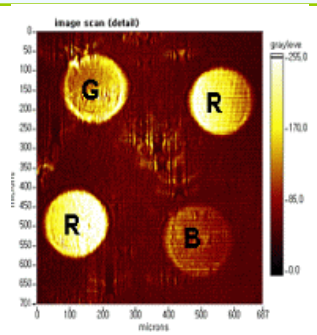
Large Gap between Molecular Biology and Medical Applications

Label-free Single Cells/Tissue Chemical Imaging R&D at KRISS

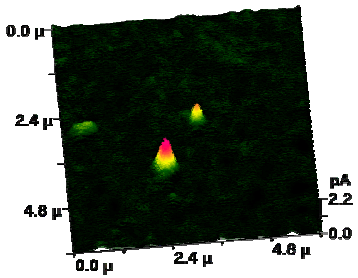
Non-linear Optics:
CARS microscopy
- 3D dynamic biochemical imaging

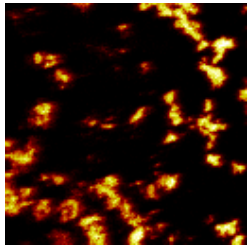


Polarized Microscopy:
SPR imaging
- Cell membrane interface



Single Cells
Tissue
Biochemical
Imaging



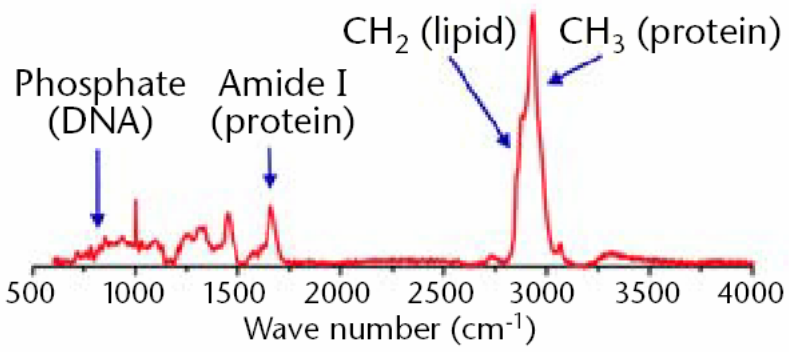


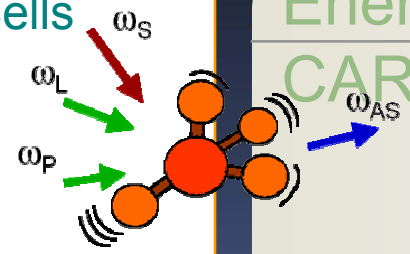
Electrochemical AFM:
Scanning ion conductance microscope (SICM)
- Ion channel monitoring

Bio-molecular mass imaging
SIMS/MALDI imaging
- ex-situ, molecular information

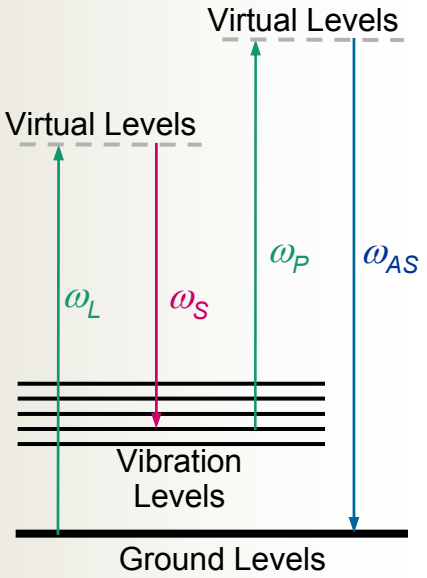
CARS (Coherent Anti-Stokes Raman Scattering

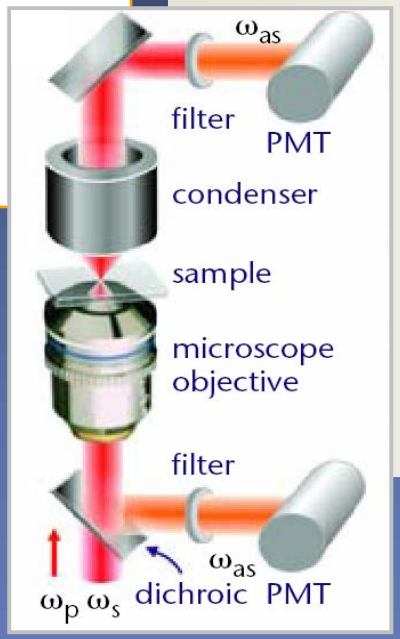
Raman Fingerprints of Biological Cells





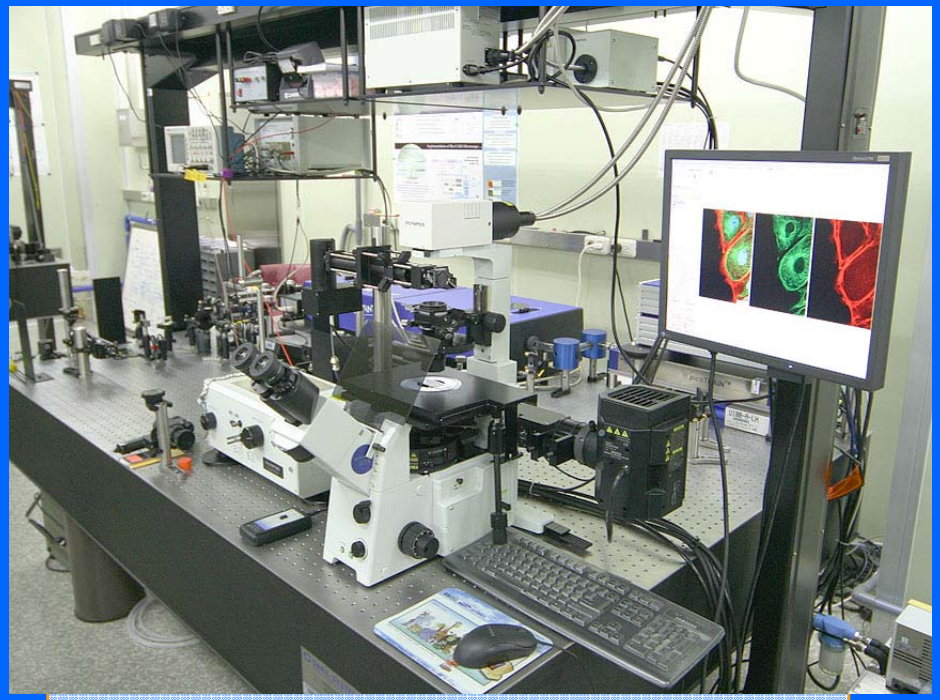
Energy diagram of CARS





- **Label-free biochemical imaging**
  - **no biological disturbance**
- **high sensitivity (x> 10^4 Raman)**
- **high spatial resolution (300 nm)**
- **3D dynamic imaging**
  - **in-vivo/in-vitro environment**

CARS Microscope at KRISS



1064 nm Modelocked ps laser

750 – 960 nm NIR synchronously pumped ps OPO

Laser beam/pulse diagnostics and overlap control

Dichroic beam coupling and signal decoupling

Non-descan CARS signal detection Optics

Relay optics and optimal microscope objective

Galvano-mirror laser scan inverted optical microscope

CARS Excitation Source

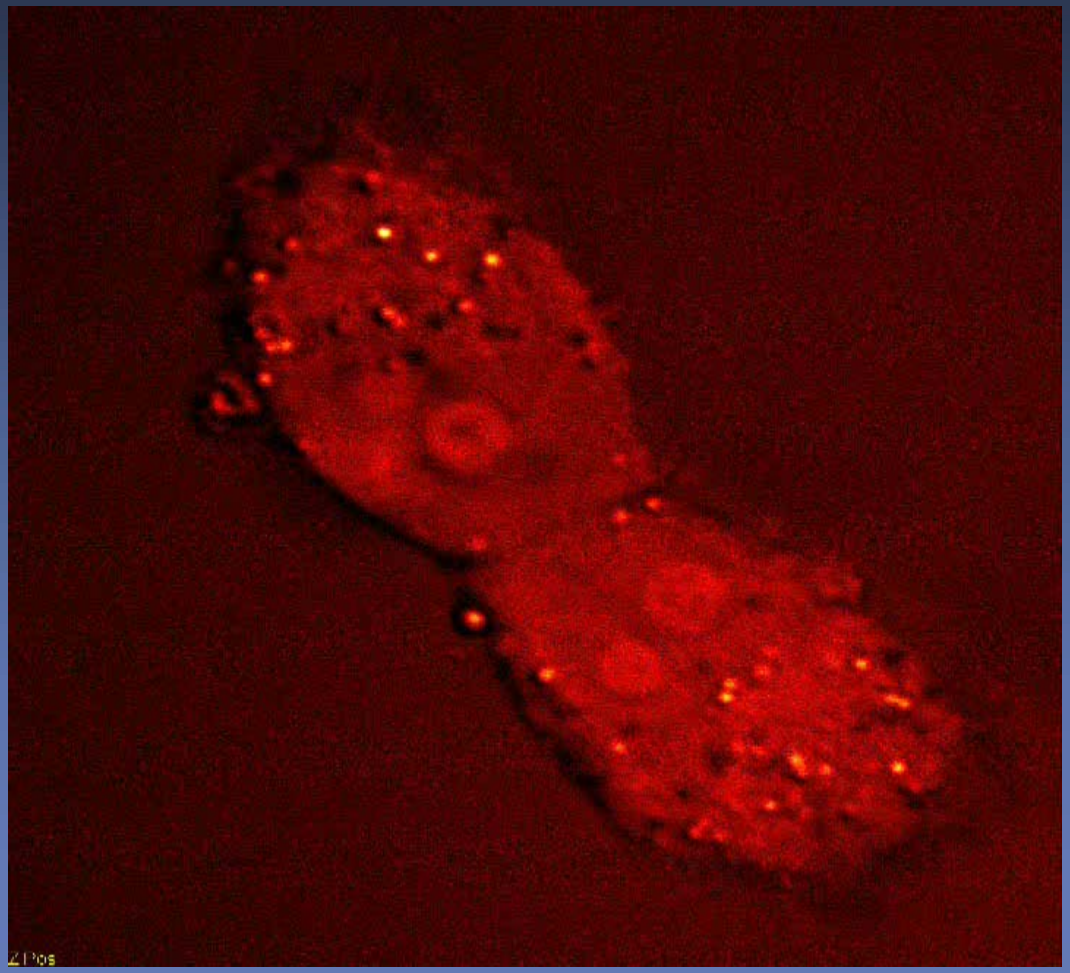
Stokes Laser	*1.5 W @ 1064 nm fixed*
Pump/Probe Laser	*2 W @ 725 – 960 nm*
Rep. Rate	*76 MHz*
Pulse Width	*7 ps*
Bandwidth	*0.38 nm / 6 – 7 cm^{-1}*
Raman shift coverage	*1500 – 3500 cm^{-1}*
Sample Irradiation	*~ 100 mW in total*

Image Acquisition

Imaging Area	*250 x 250 μm^2*
Pixels	*1024 x 1024*
Frame Rate	*10 image/s*
Z- section Range	*500 μm*
Z- section Step	*0.1 μm*
Spatial Resolution	*Lateral ~ 300 nm*
	Axial ~ 900 nm

+ Multiplex Raman capability : 200 cm^{-1}~ 1500 cm^{-1}

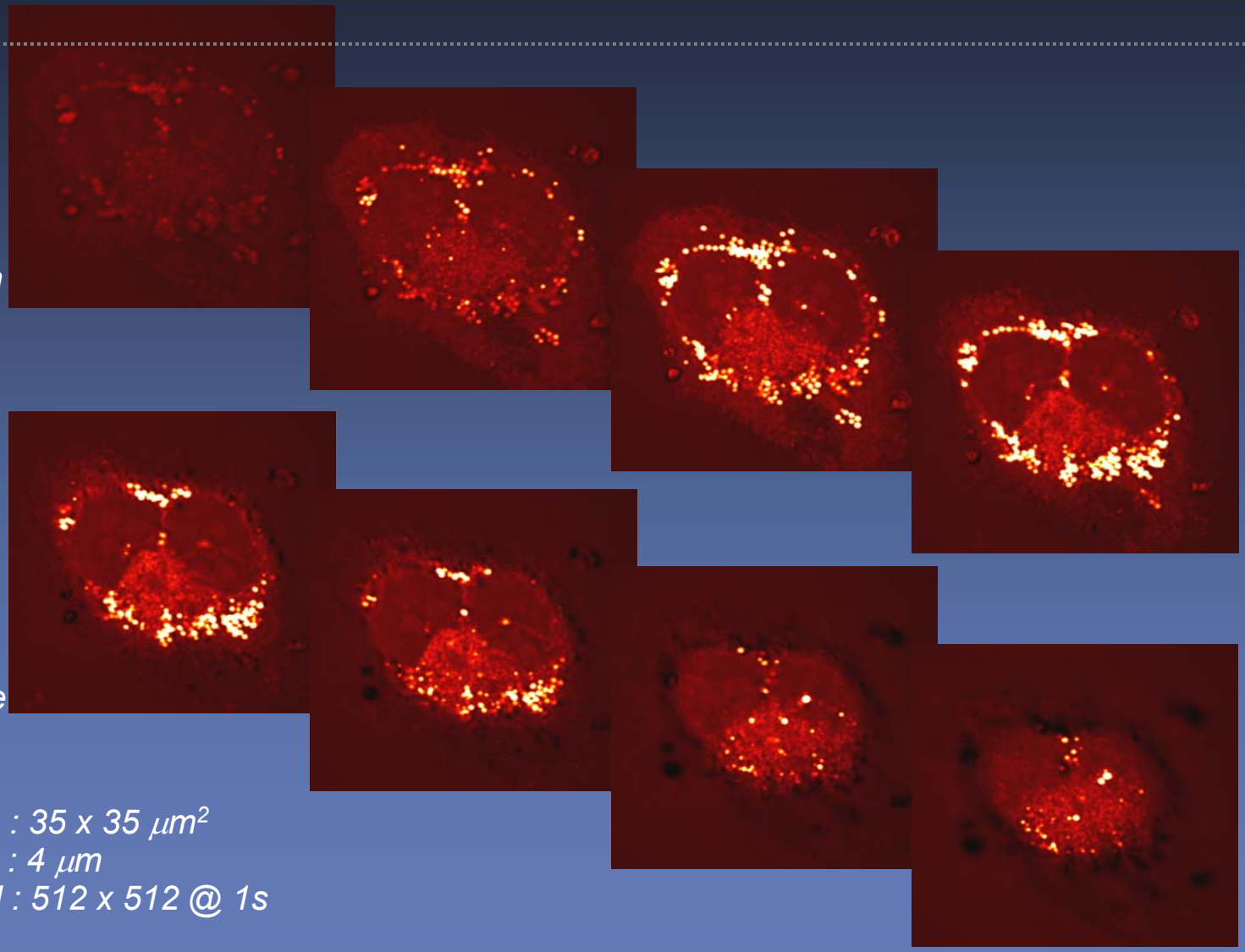
Real Time CARS images of an alive Hela Cell



Aliphatic C-H @Δ = 2837 cm^{-1}

Dynamic Imaging of Vesicles

Depth-Resolved Images of an unstained HeLa Cell

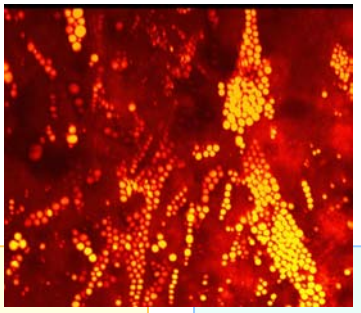


Area : 35 x 35 μm^2
Step : 4 μm
Pixel : 512 x 512 @ 1s

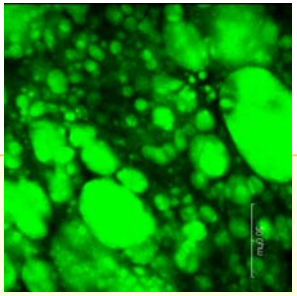
Tissues

Single Cells

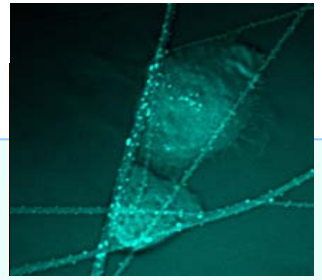
Atherosclerosis



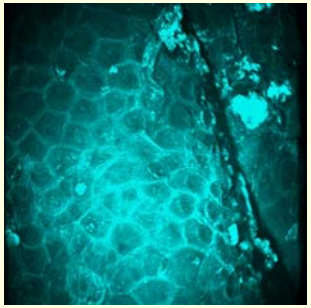
Fat Liver Tissue



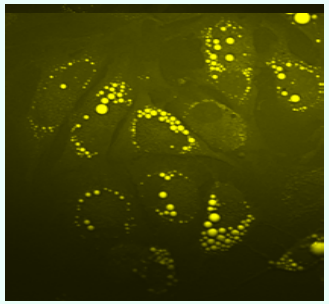
Focal Adhesion
& Migration



Skin
Stratum Corneum

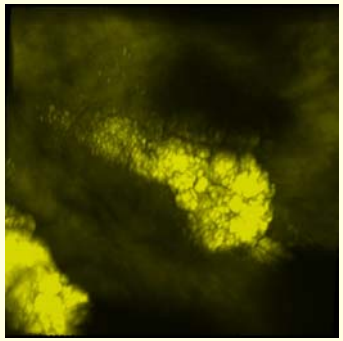


Stem Cell
Differentiation

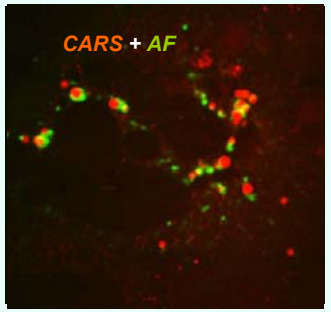


μ-CARS
Potential

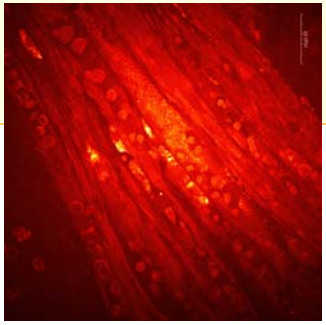
Sebaceous Gland



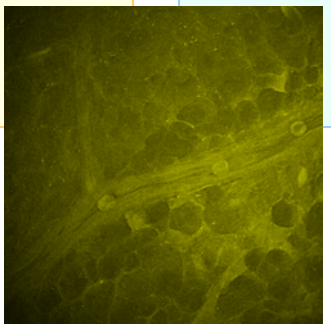
HCV-LD Collocalization



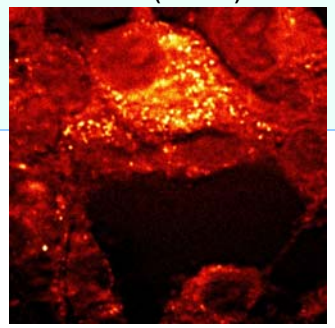
Hyaloid Vessel



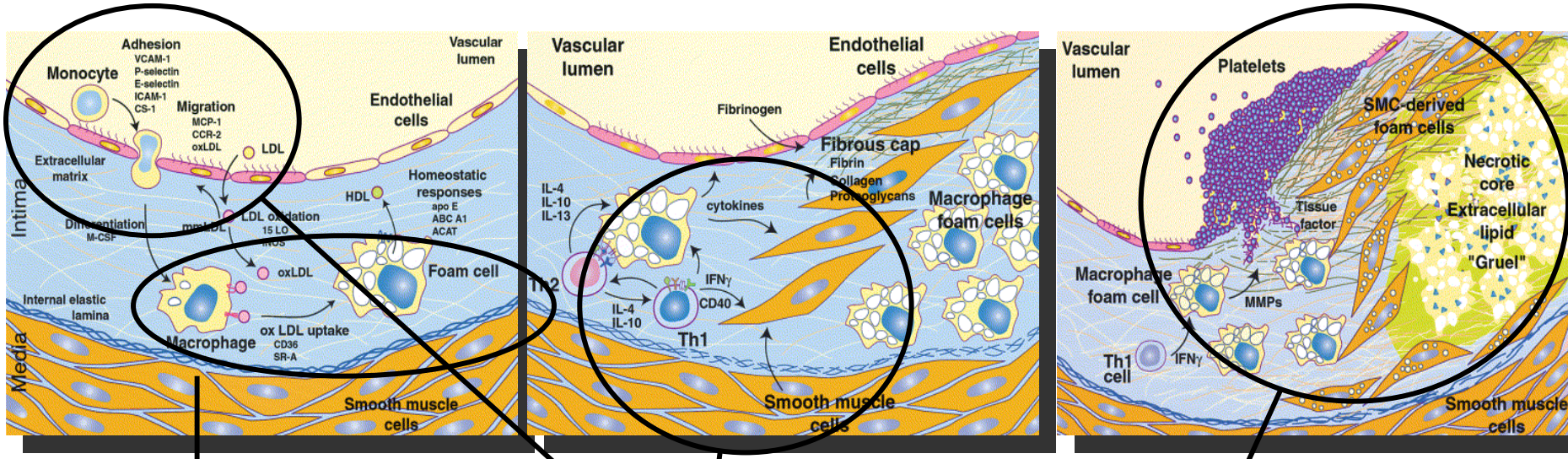
Retinal Tissue



Live Cell (NIH3T3)



From Cellular basic studies to Medical interests in Atherosclerosis



lipid uptake by macrophages & its differentiation to foam cells (CARS)

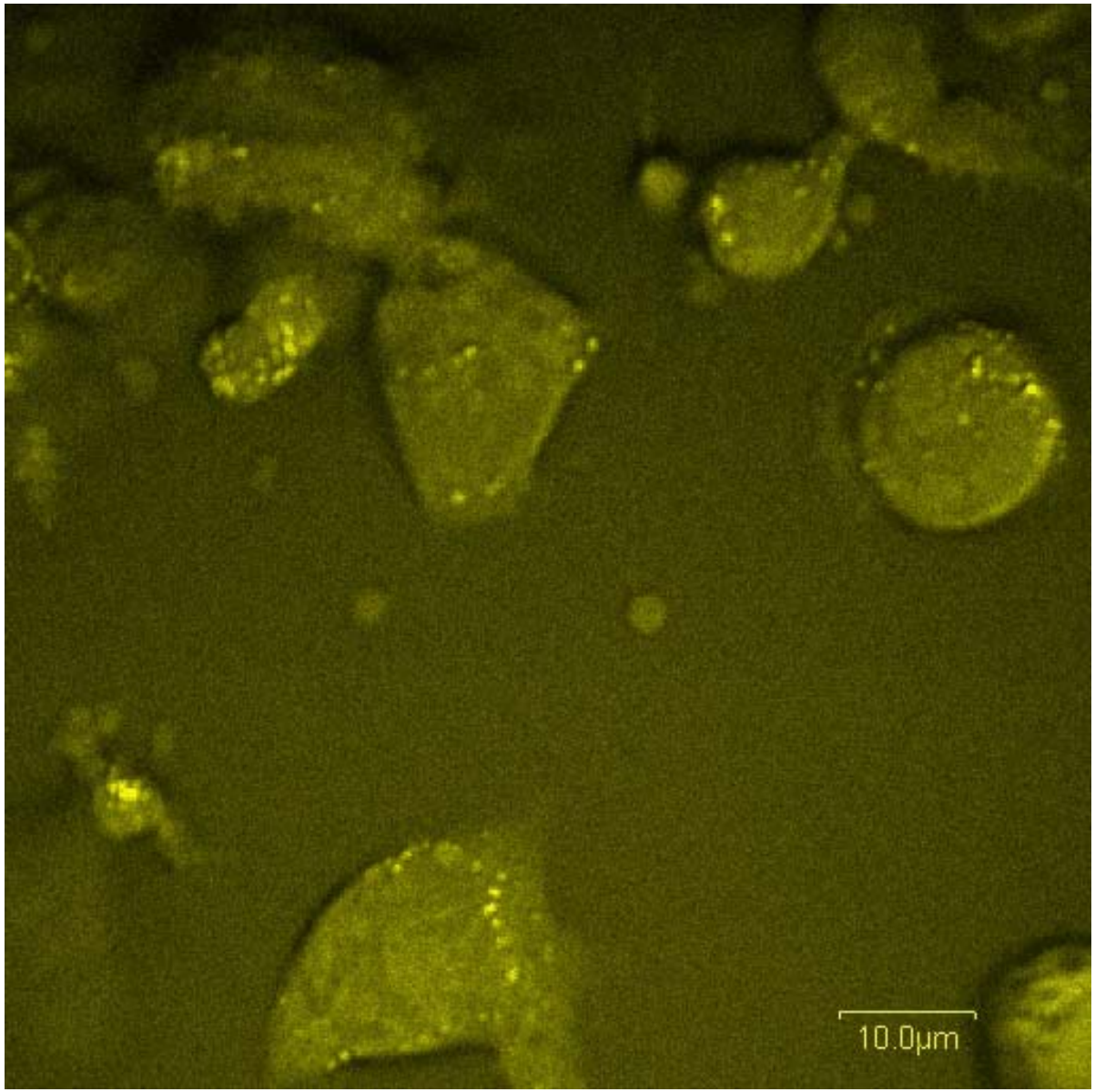
cell–cell, cell–ECM adhesion & migration (SPR, SIMS, SICM)

imaging plaques and its stabilization (CARS & SIMS)

↕

US, CT, MRI, PET

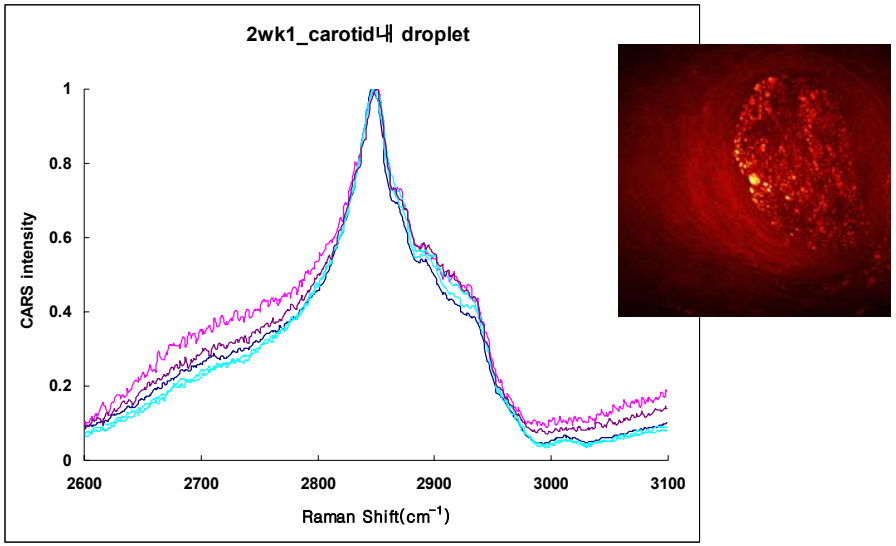
CARS images for lipid vesicle uptake processes in the differentiation of human monocytes (THP-1) to macrophages

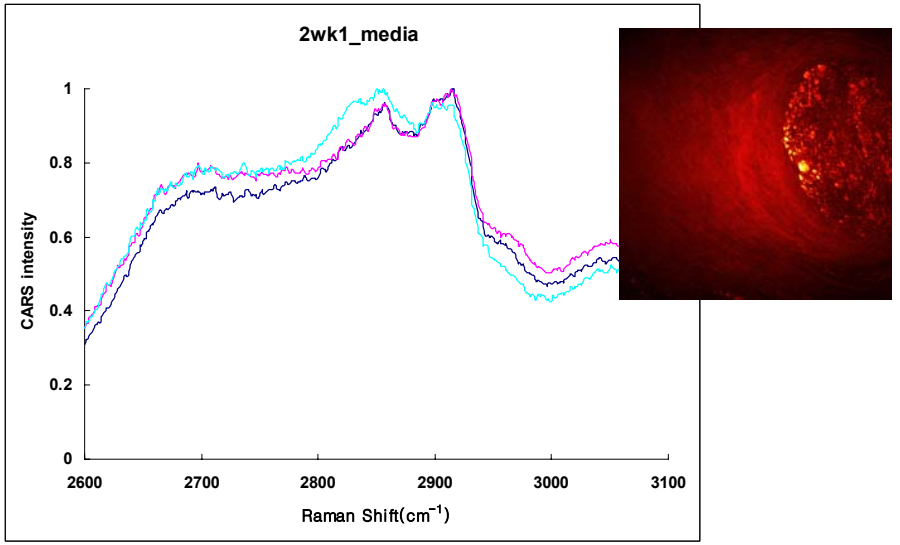


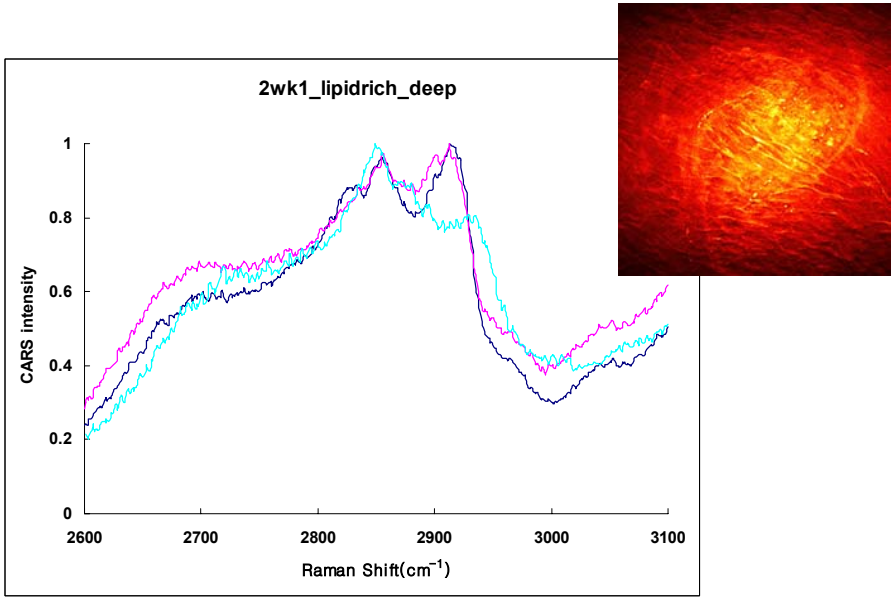
PMA in 10% serum media

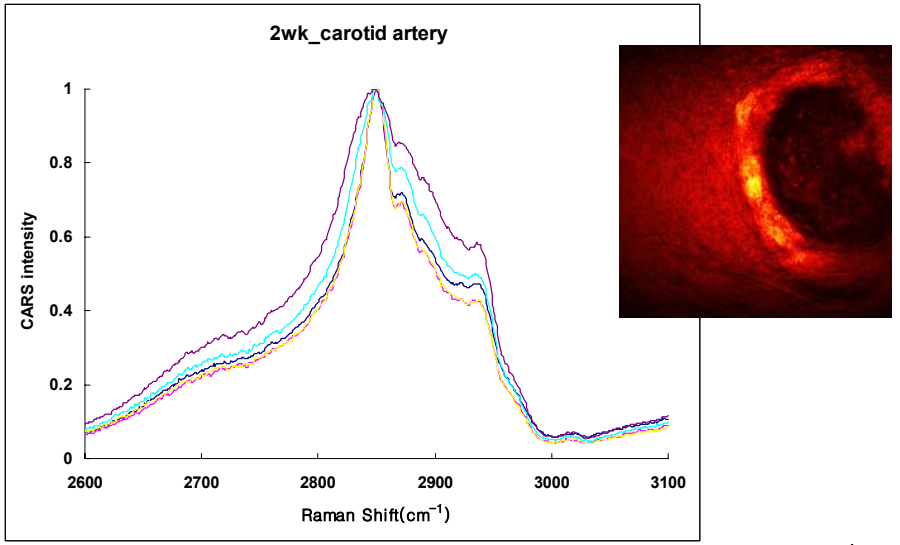
duration:
2 hours

CARS spectra for biochemical characterization of lipids from a mouse atheroma tissue



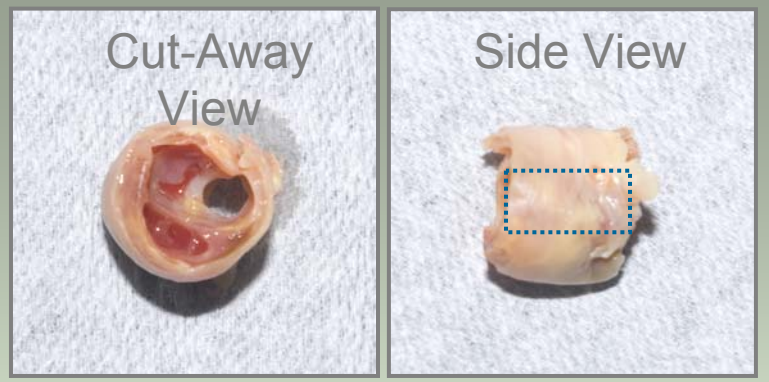






- Collaboration with Samsung Medical Center

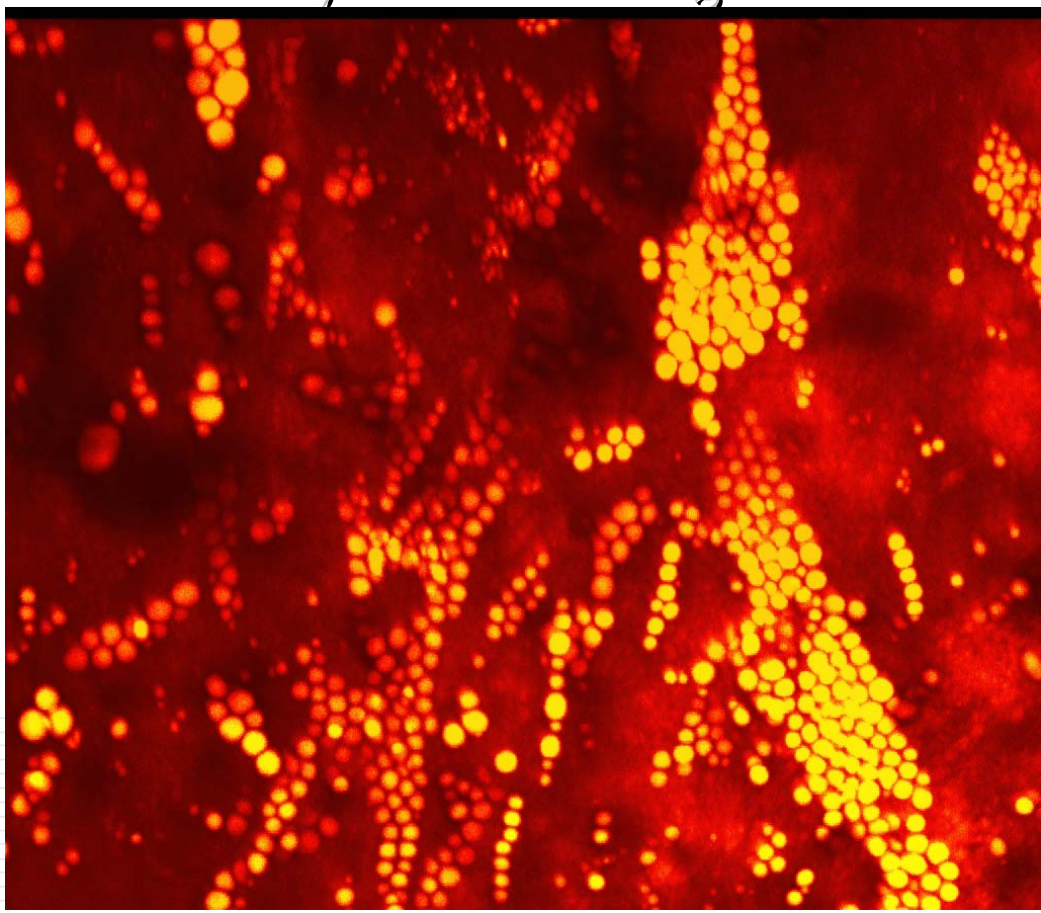
ex vivo Atherosclerosis Cardiovascular CARS Imaging



Cardiovascular Imaging

- in vivo US/SPECT/PET/NIR :
  - *Agents required*
  - *Low resolution*
- ex vivo Biopsy of atheroma tissue :
  - *Cryosection*
  - *Foam cell staining with oil red-O dye*

3D Reconstruction of *en face* CARS Images

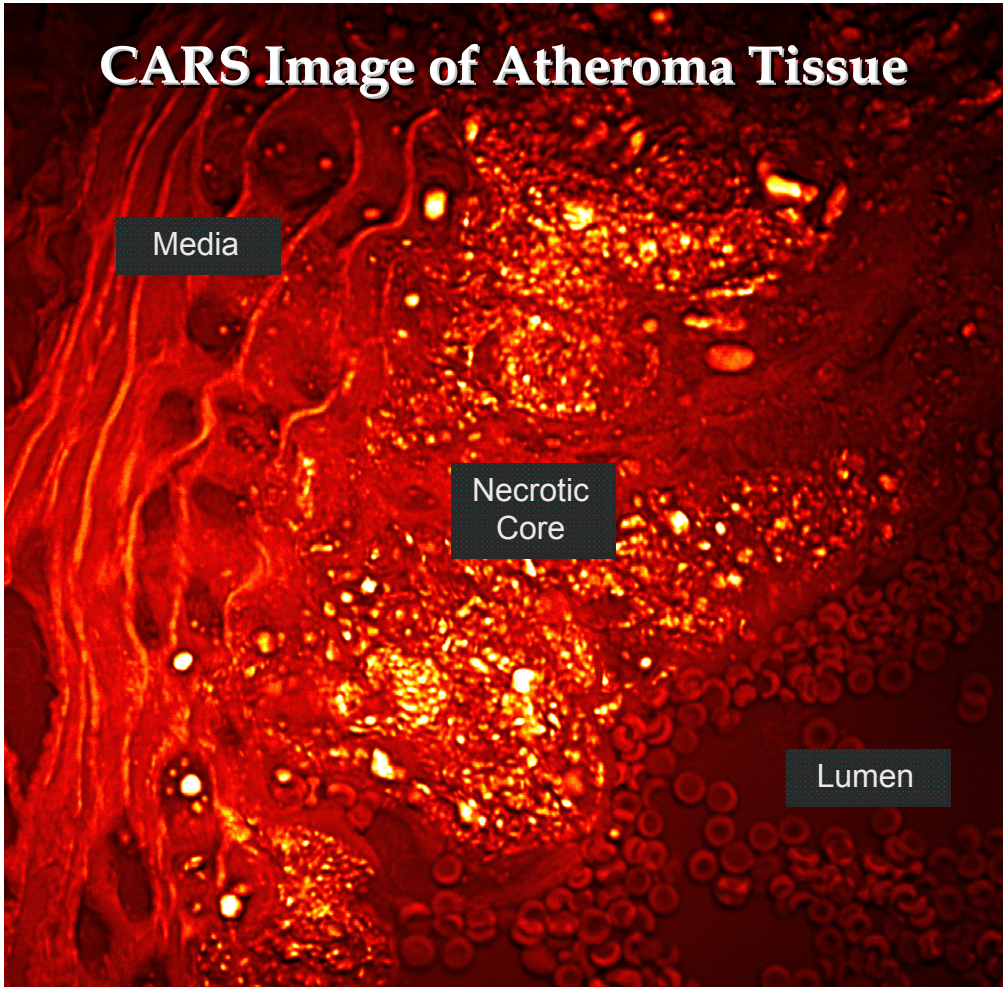


- Collaboration with Samsung Medical Center

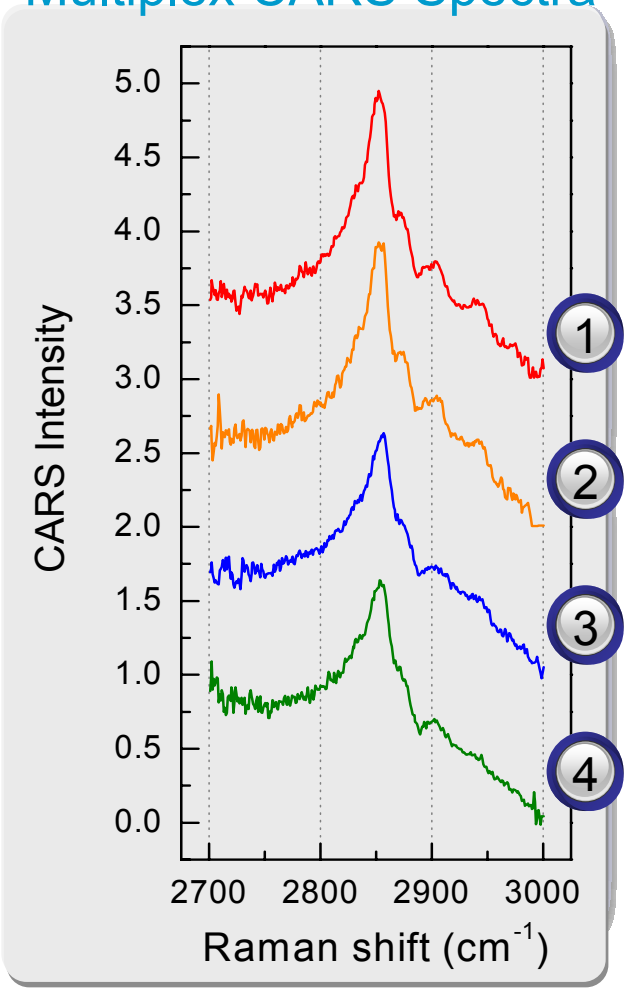
Foam cell differentiation/ Atherosclerosis Diagnosis

Atherosclerosis tissue analysis with multiplex CARS

degree of oxidation/saturation of lipids for plaque stabilization analysis ?

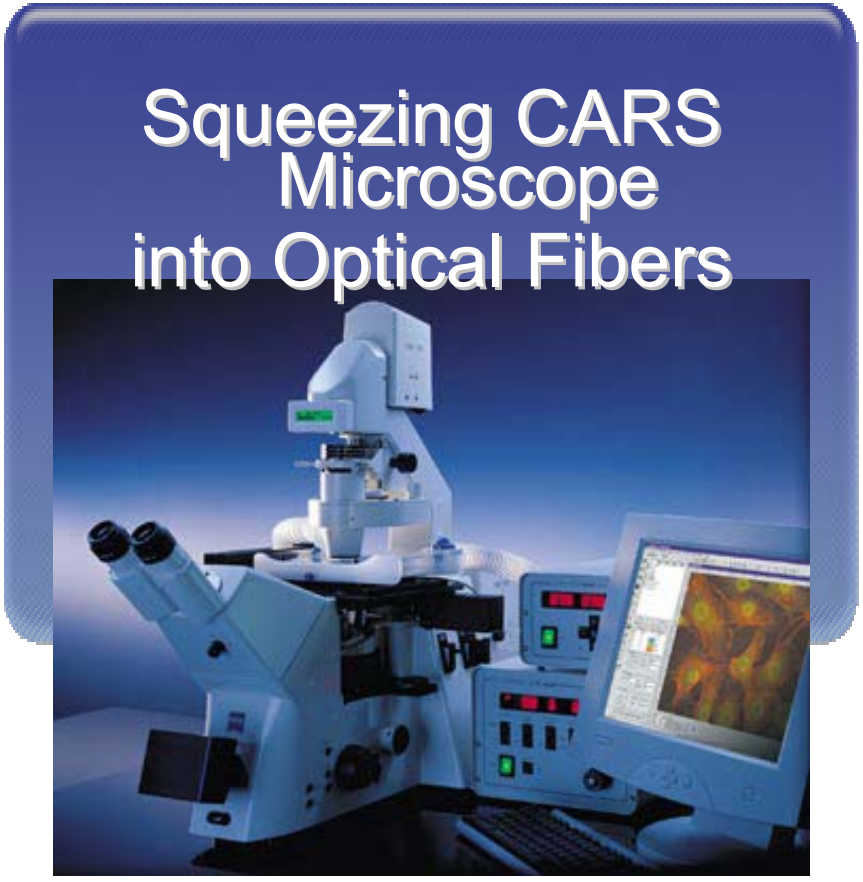


Multiplex CARS Spectra



Vision of CARS Laser Microscopy

in-vivo Medical and/or Animal model Imaging Endoscopy



Biomedicla Imaging &
Diagnostics

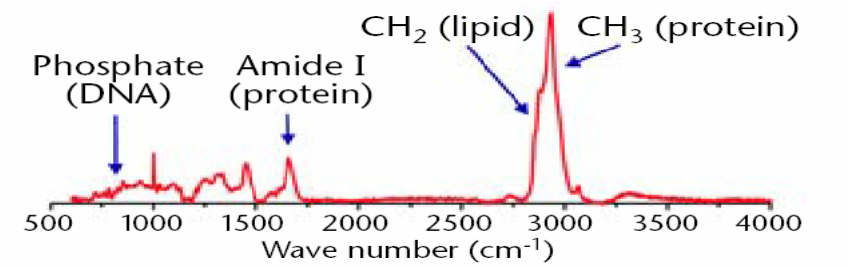
Animal Model Imaging
for Pre-clinicall Screening

Complementary Use of CARS and SIMS/MALDI imaging

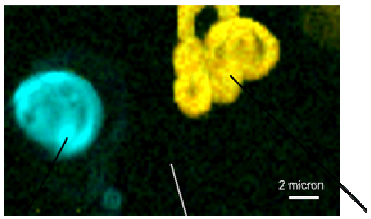
CARS
: overview of biochemical imaging
: in-vitro/in-vivo dynamics
: poor sensitivity and selectivity

Mass Spectrometry (laser/ion beam)
: molecular specificity
: high sensitivity (?)
: high contents biochemical information
: ex-situ, no dynamics

Multiplex CARS

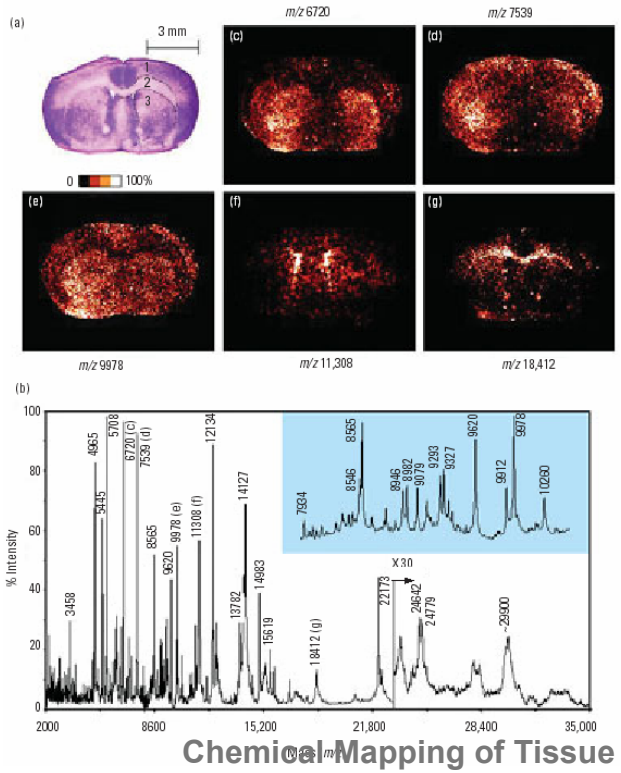


Lipid structure change



C-C skeletal mode @ (~1100 cm^{-1})

Mueller *et al.* JPC B (2002).



Chemical Mapping of Tissue

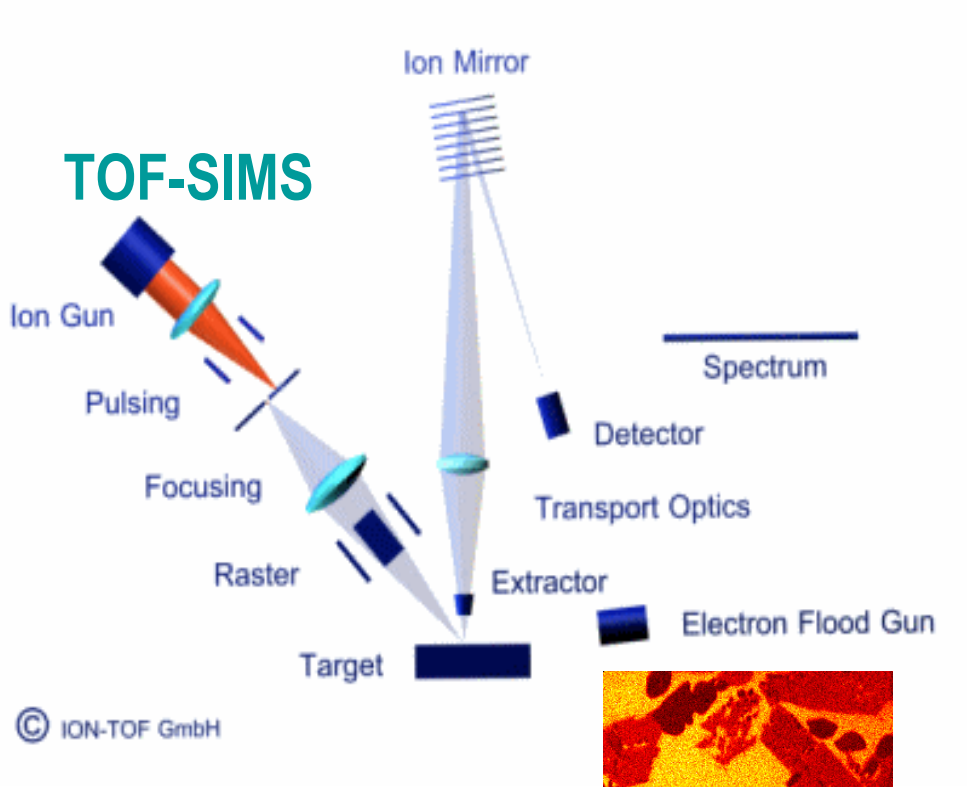
Anal. Chem. [Feature Article] (2004)

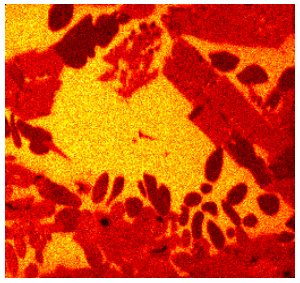
Secondary Ion Mass Spectrometry (SIMS)

: unique for semiconductor dopant analysis

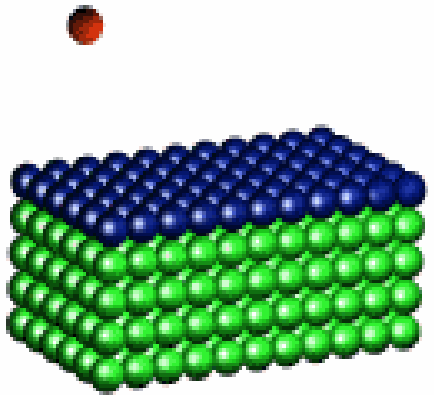
➡ Can SIMS be useful for biochemical imaging of tissues ?

Can it beat traditional staining optical microscopy & bio-SEM/TEM ?



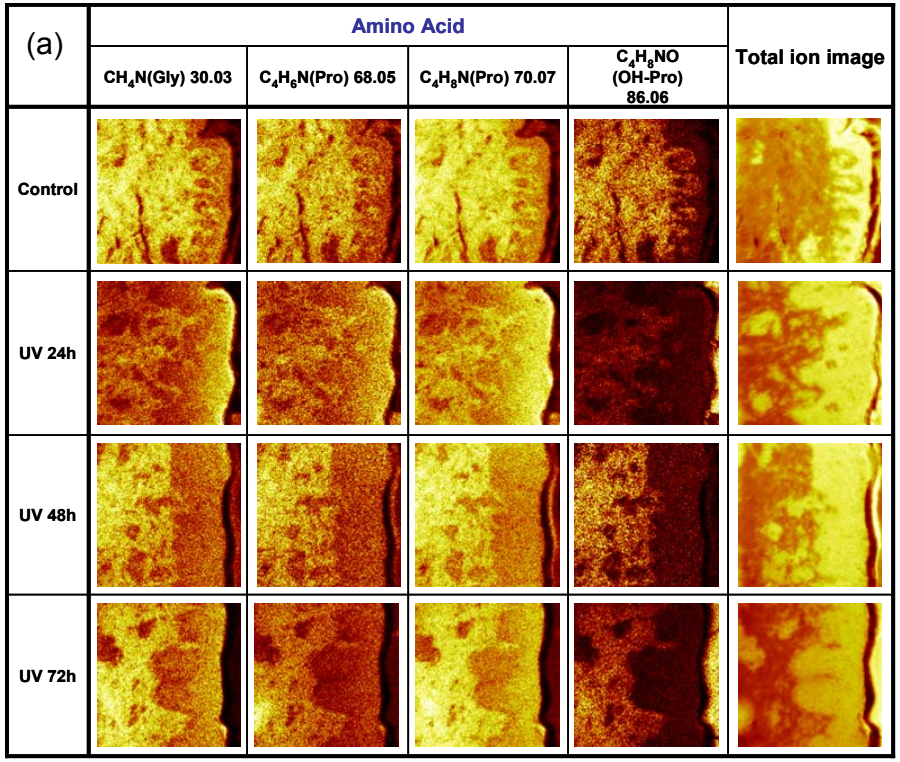


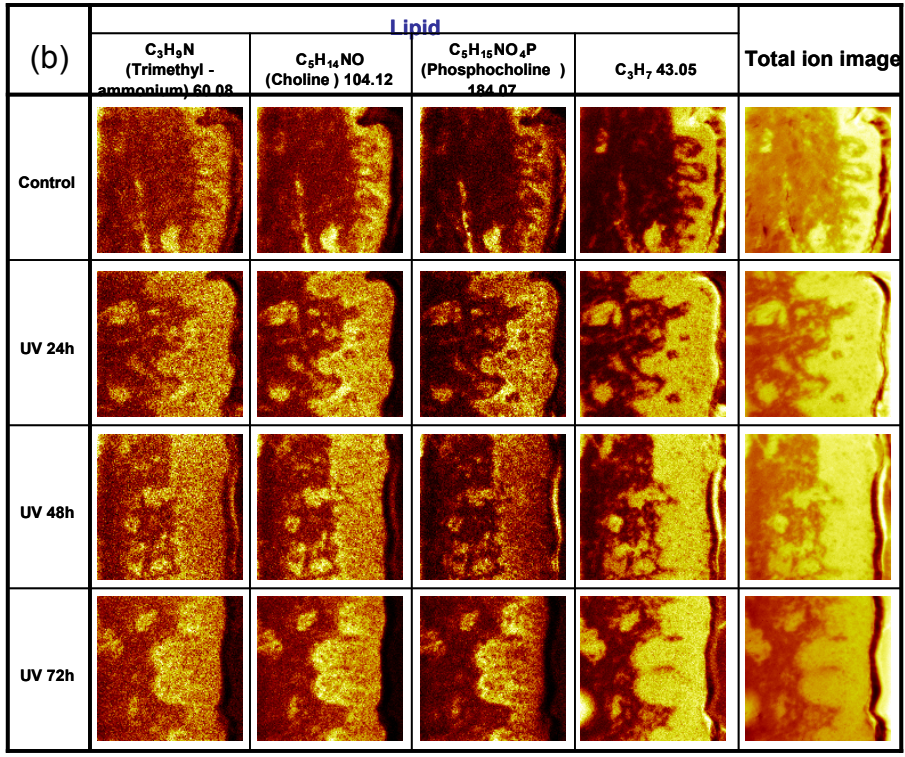
image



SIMS studies on Photoaging Effects of Skin by UV irradiation

25 keV Bi_3^+ imaging after C_{60}^{++} cleaning:





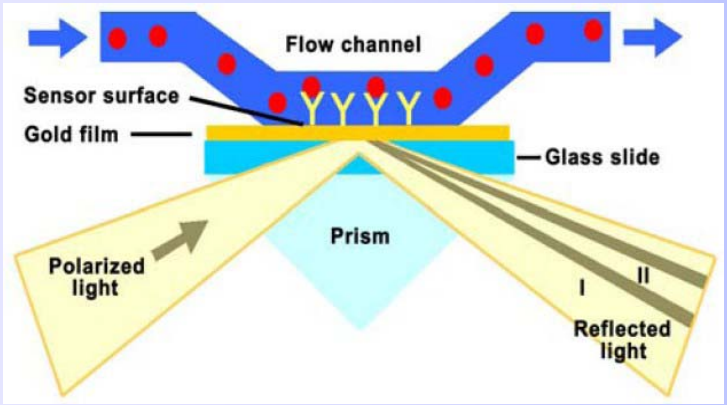
(collaborations with SNU Medical School, Dermatology, J.H. Chung)

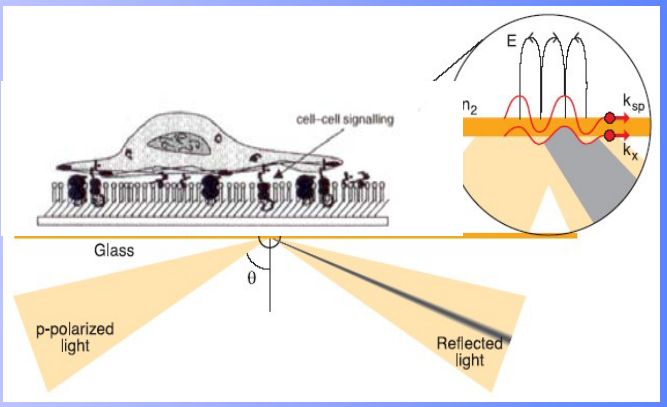
Is he happy ? **Maybe, No for proteins, Yes for lipids. Good for CV imaging**

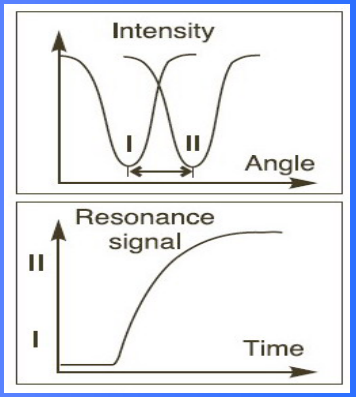
Is he excited ? **No. Why ??? >> insufficient molecular ions**

Complementary use of SIMS & MALDI imaging of tissues with matrix controls

Surface Plasmon Resonance for cell adhesion & migration imaging



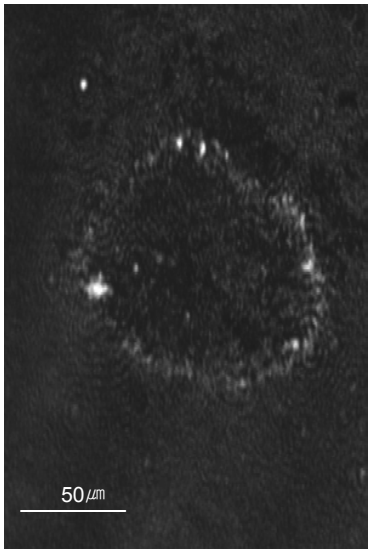




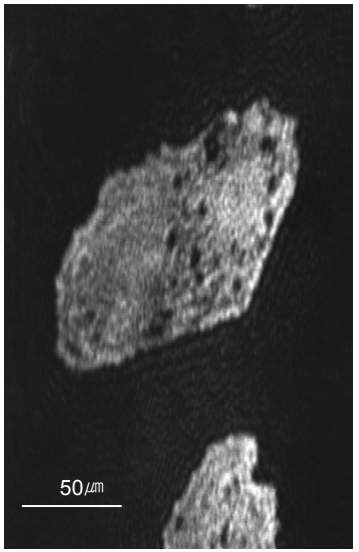
SPR applications

quantitative analysis of biomolecules on surface

- biomolecule adsorption dynamics
- antibody-antigen, DNA-DNA interactions

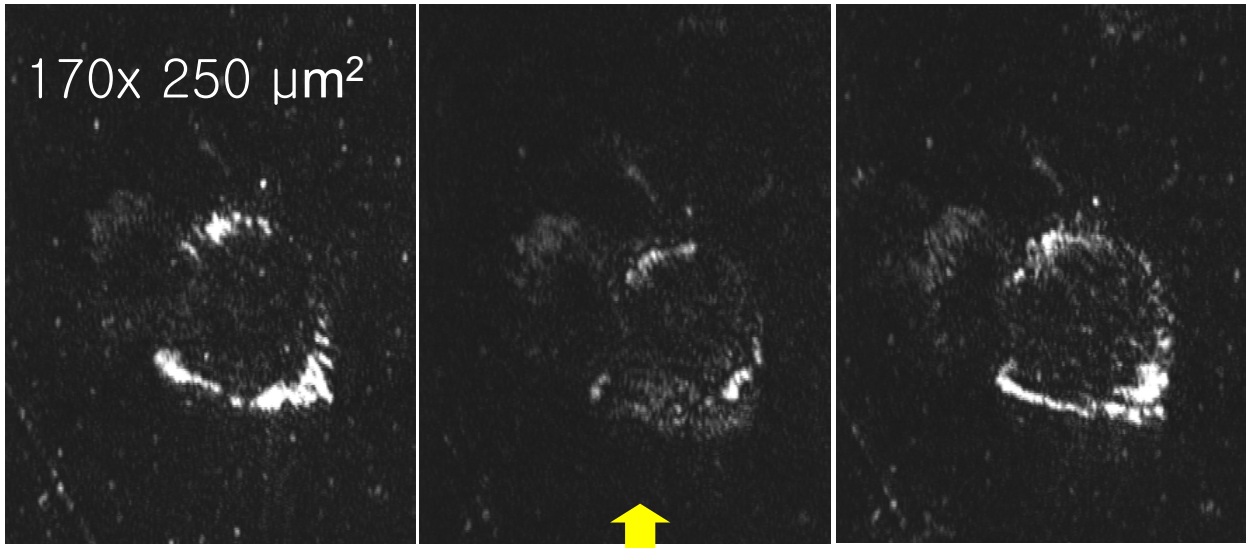


A10 SMC on collagen



HUVEC on fibronectin

The Effect of Flow Rate to A10 SMC Adhesion on Collagen



flow rate: 1 cm/s

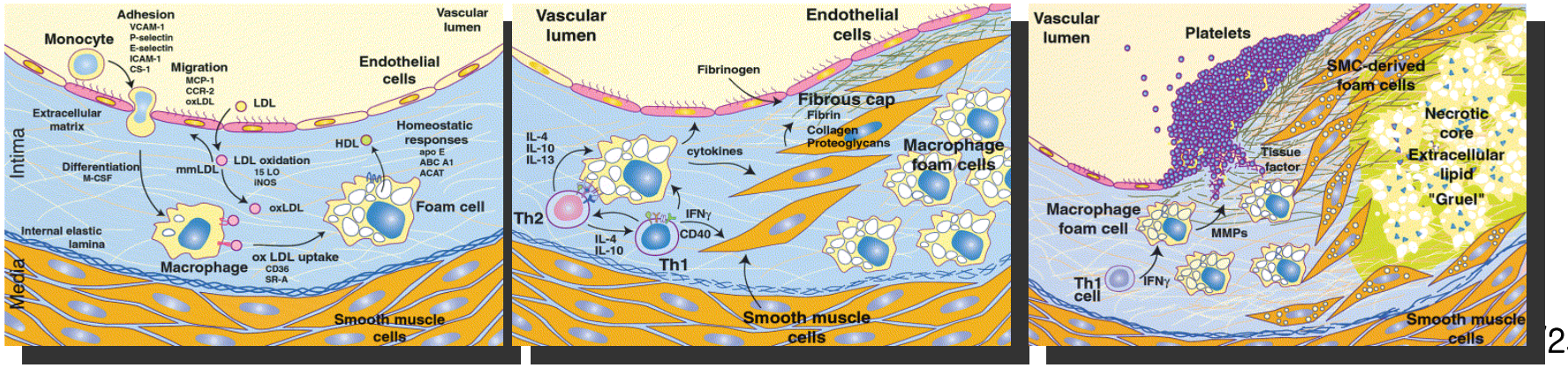
1 hour

flow rate: 27 cm/s

5 hours

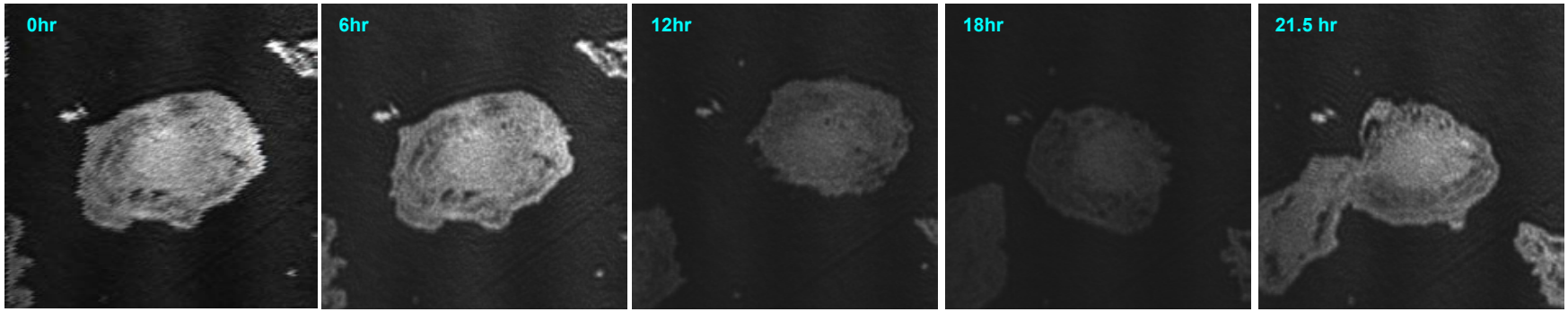
flow rate: 1 cm/s

6 hours

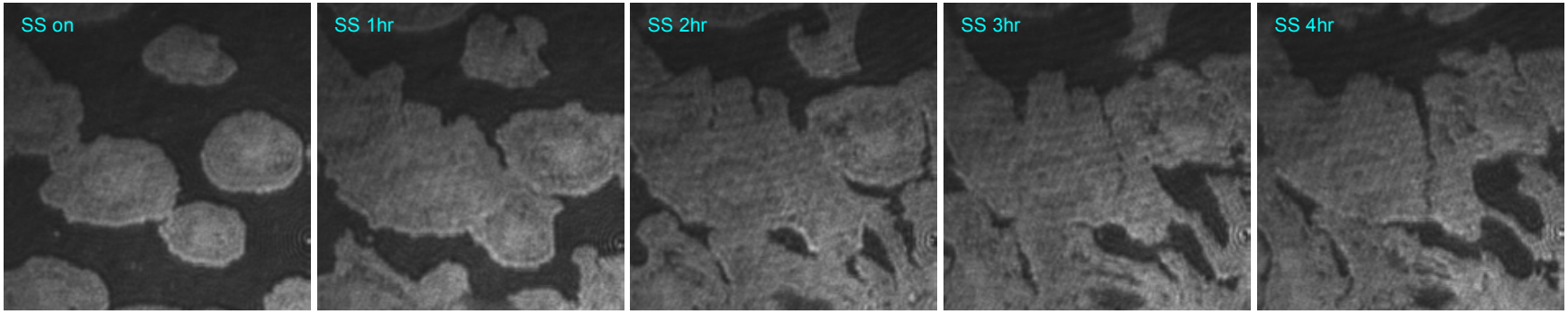


SPR dynamic imaging of HUVEC adhesion on fibronectin & the Shear Stress Effect

no shear stress

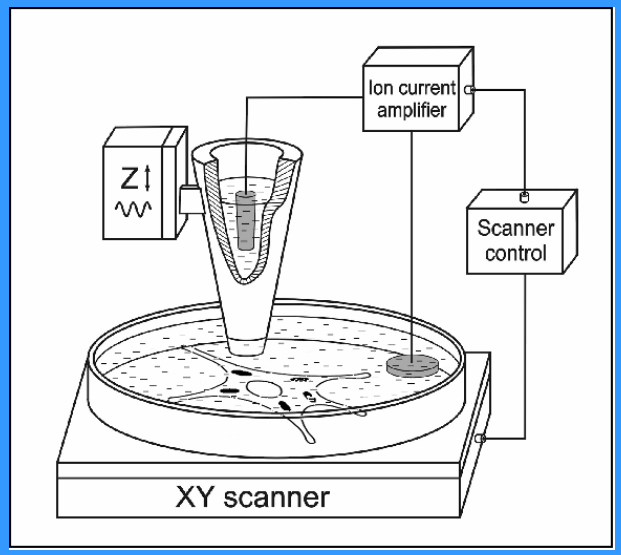


1.2 Pa shear stress

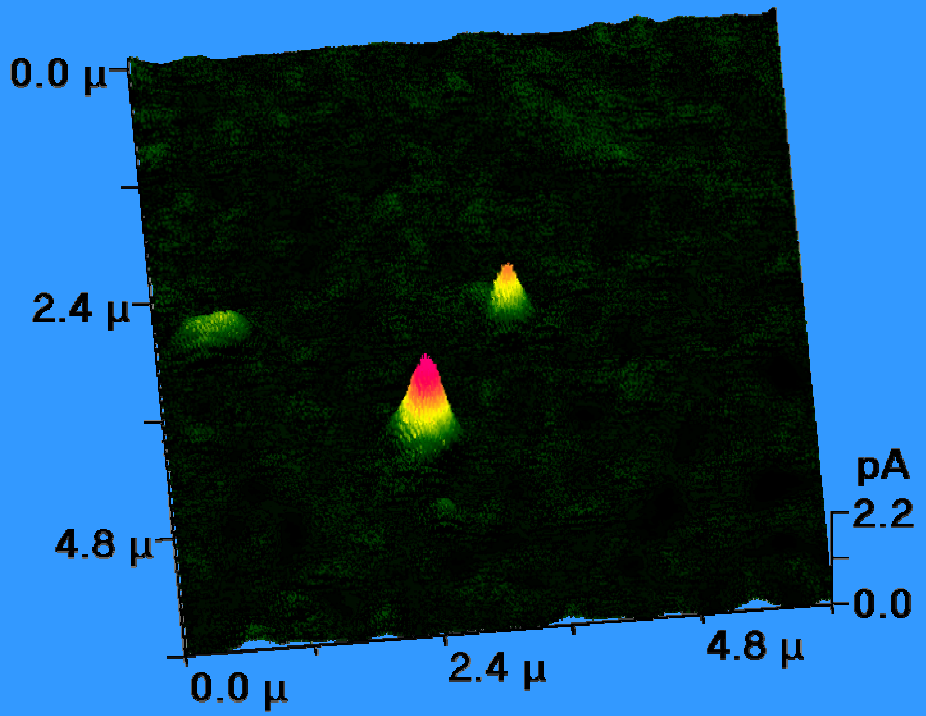


dynamics movies

Scanning Ion Conductance Microscope (SICM)



Sample



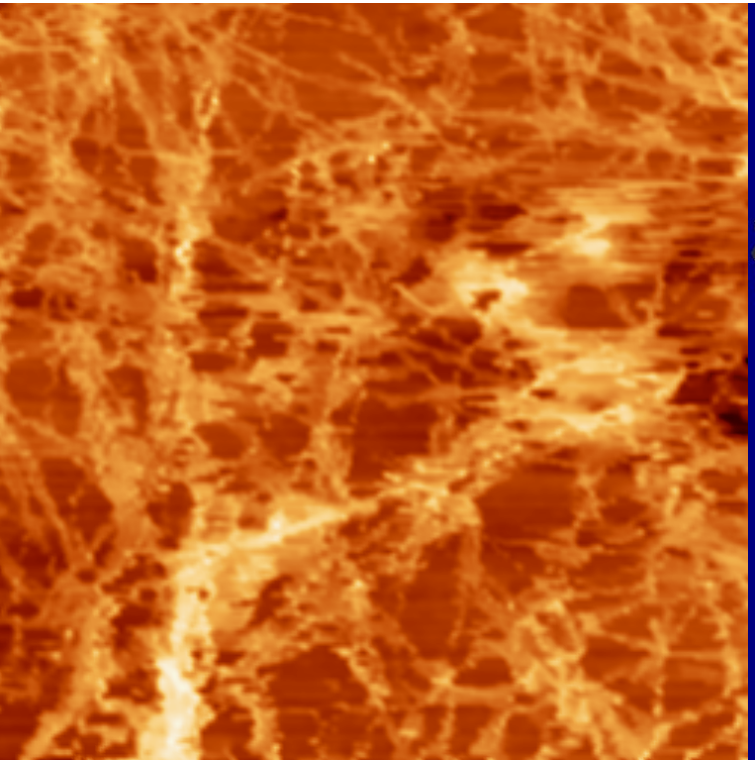
Functional localization of K_{ATP} Channels

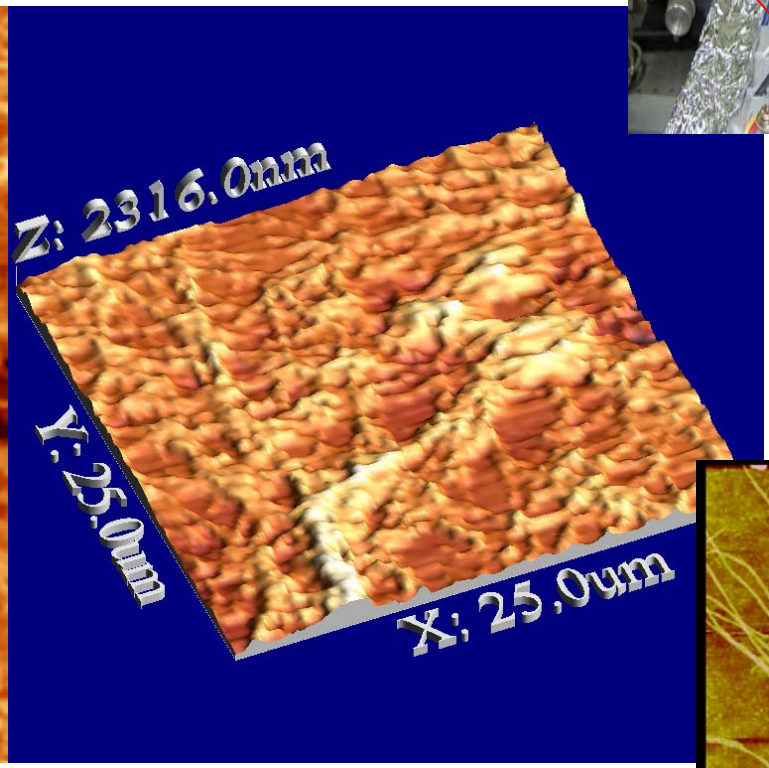
- measurement of cells alive in solution
- cell membrane electrochemical mapping
- ~10 nm resolution, elemental specificity
- single ion channel localization and monitoring

Y. Korchev
Imperial College

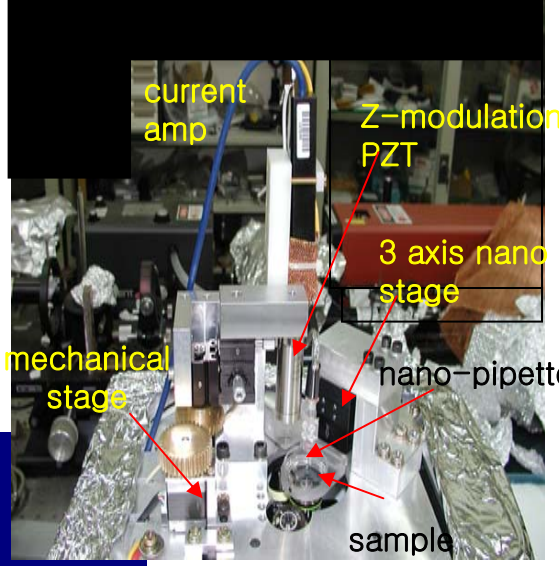
SICM imaging of Collagen ECM morphology in solution

300ug 1 hr incubation



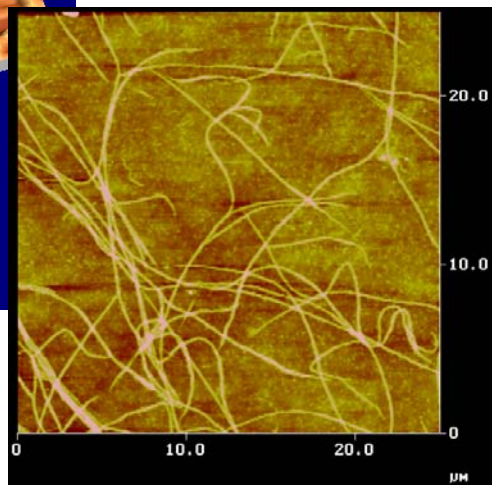


25um x 25 um



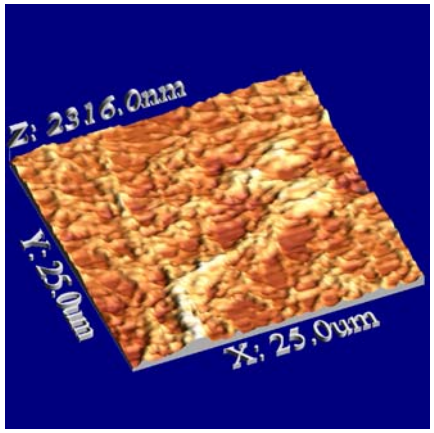
SICM at KRISS

AFM 25um x 25um

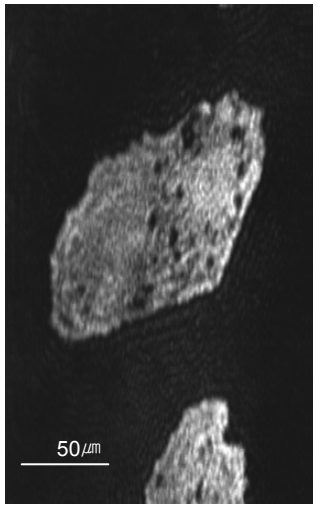


Final Vision:

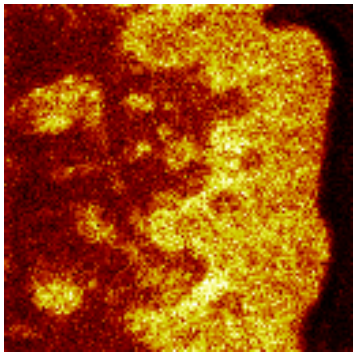
1) Understanding & monitoring atherosclerosis from the subcellular level to the *in-vivo* tissue level
2) by *in-vitro/in-vivo* label free biochemical imaging tools
3) For medical imaging diagnostics and/or animal imaging for pre-clinical screening



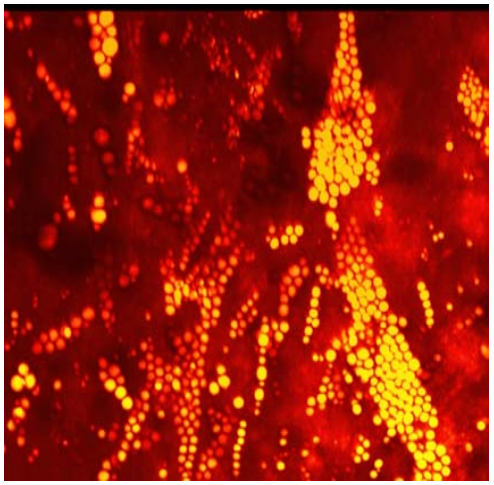
SICM image of collagen fibers



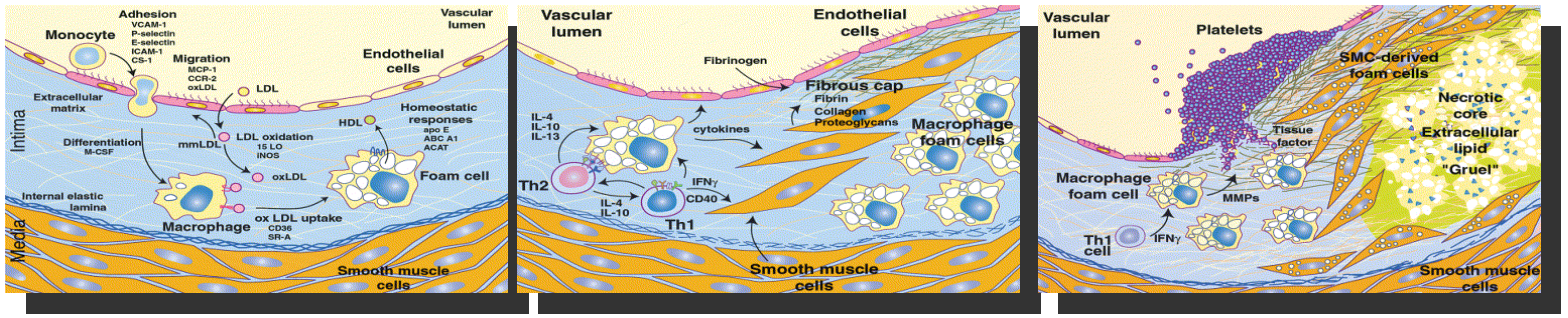
SPR image of **HUVEC on fibronectin**



SIMS lipid choline image of a skin tissue



CARS lipid image of foam cells in a blood vessel tissue



Conclusions

1. **Label-free tools such as CARS, bio-SIMS, SPR, SICM can be used as noble and complementary tools in biochemical imaging of single cells/tissues for cell biology and medical diagnostics.**

2. **If it works nicely for atherosclerosis, it can be extended to study other diseases and to understanding EHS issues of nanomaterials for improvement of the quality of life.**

3. **To tackle these issues, global collaborations are mandatory and beneficial to all of us.**

Why not between Korea and USA !