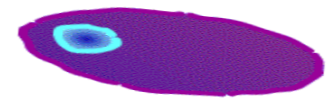
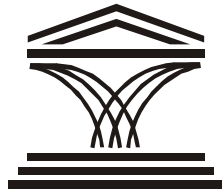


Cellular Biosensors: towards a unitary, integrated (nano-bio) analytical platform

International Centre of Biodynamics



Outline

- Why developing cellular platforms ?
 - Reveal interaction mechanisms & effective biosensing (e.g. detection & cytotoxicity assays)
- Deployed methods
 - **E**lectrochemical (**I**mpedance **S**pectroscopy & **A**mperometry) & **E**lectrophysiological (**T**ransepithelial **I**mpedance and **N**oise **S**ystem) & **A**tomic **F**orce **M**icroscopy & **S**urface **P**lasmon **R**esonance & **F**luorescence **M**icroscopy (**T**otal **I**nternal **R**eflection & **E**pi **F**luorescence)
- Recent Results
 - Cellular systems: Adhered & in Suspension
 - Biomimetic systems (lipid membranes)
- Summary



Why cellular platforms?

The concept of sensing and detection has to be readdressed in view of the huge number of analytes to be assayed to comply with **Registration Evaluation Authorisation Chemicals** requirements for analysis, labeling and cytotoxicological assessment.

- Kinetics - real time Monitoring (molecular) Events at Cellular level
- Live cell multi-parametric studies
- Appraisal of interaction mechanisms; gentle, non-lethal effects
- Ethics problems (alternative to animal tests)

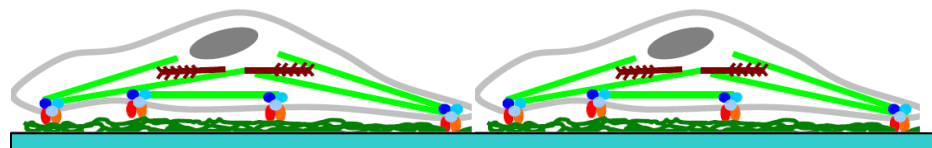
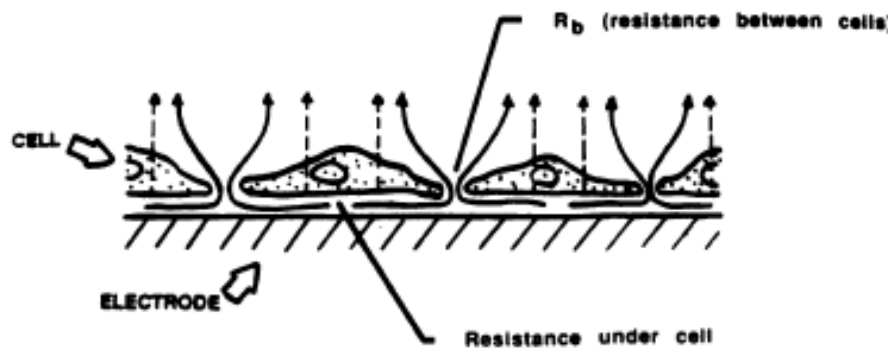
Challenges

- ❑ Stable, selective cell attachment
- ❑ Multi-parametric assays
 - complementarity
 - integrable in the same platform
 - amenable to miniaturization
- ❑ Ability to derive cell dynamics
- ❑ Focus on bio-interface processes
- ❑ Label free...(if possible)

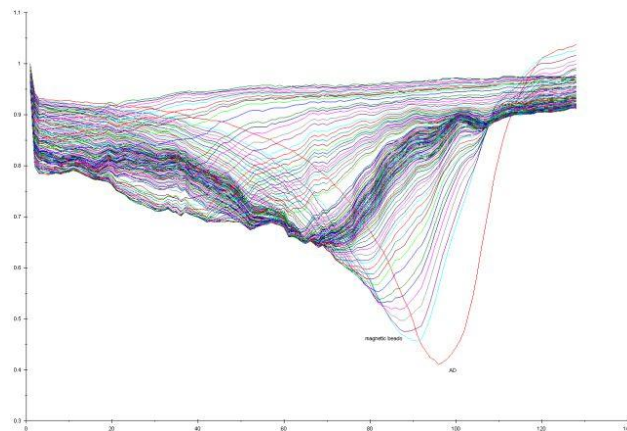


Why combined assays?

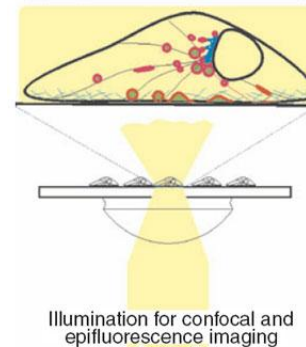
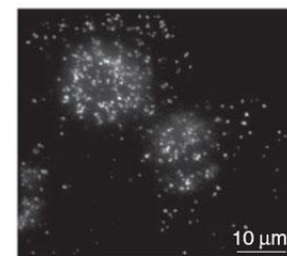
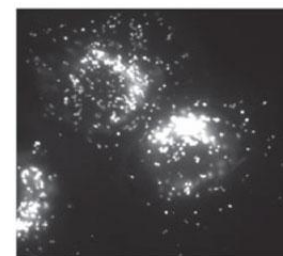
- IS



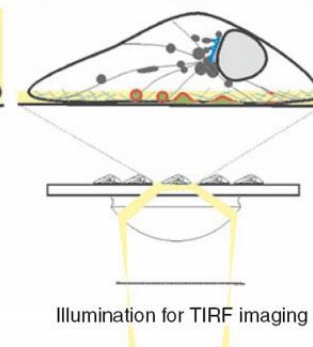
- SPR



- TIRFM

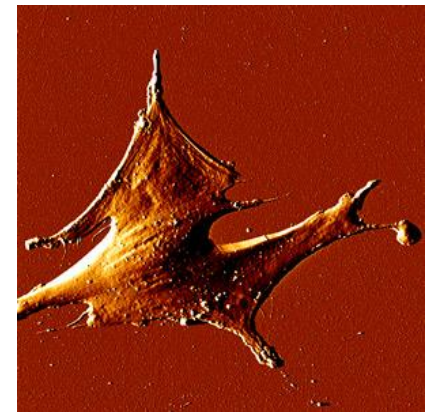
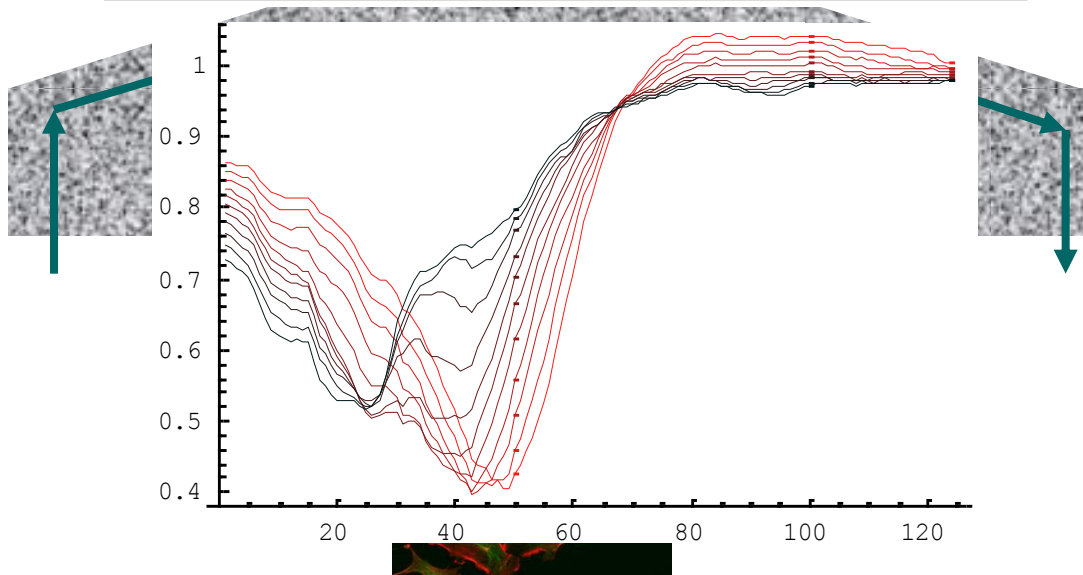
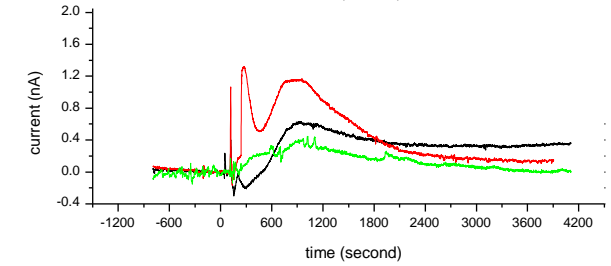
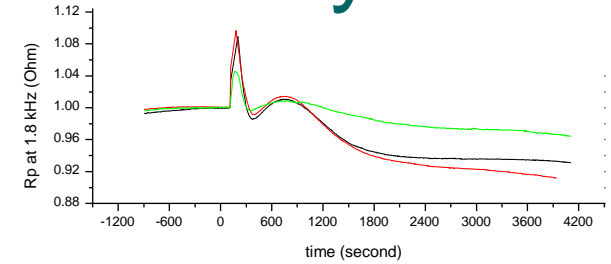
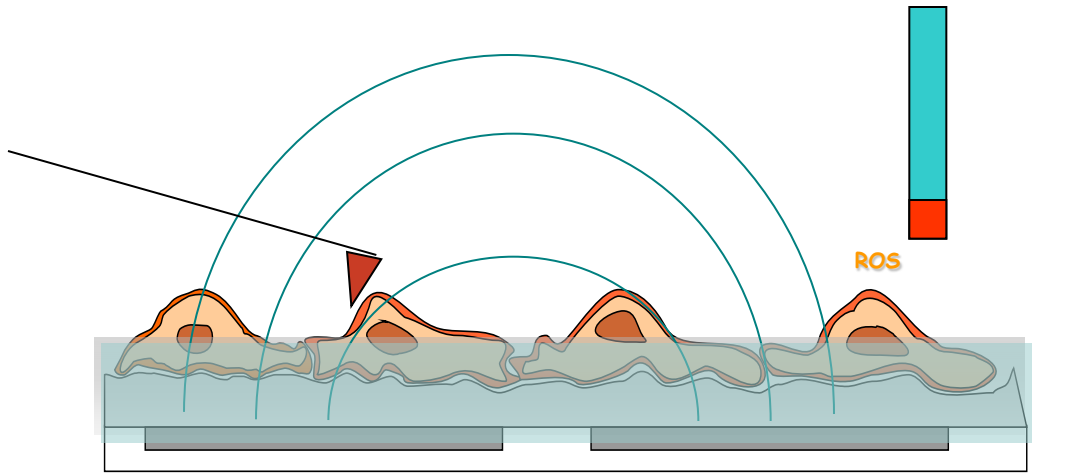


Illumination for confocal and epifluorescence imaging



Illumination for TIRF imaging

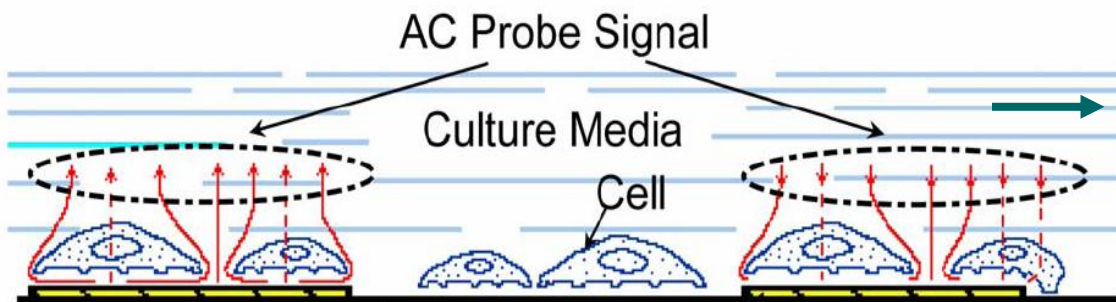
Combined electro-optical assays



Electrochemical Impedance Spectroscopy (EIS)



Assessment of layers of interconnected cells



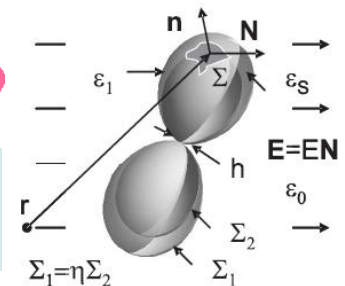
- Cell-cell communication
 - cell state
 - cell response
- Cell number / morphology
 - cell growth
- Cell spreading and attachment
 - cell state
 - cell response to various cues



Dielectric behavior of interconnected cells has been approached using a Microscopic model



T. Sandu, D. Vrinceanu and E. Gheorghiu
(2010) Phys Rev E81, 021913 1-11



Non-invasive appraisal of cells in suspension

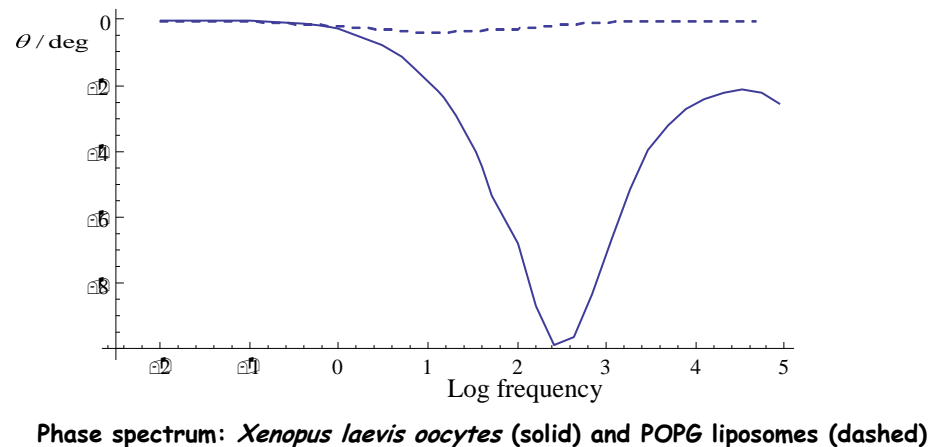
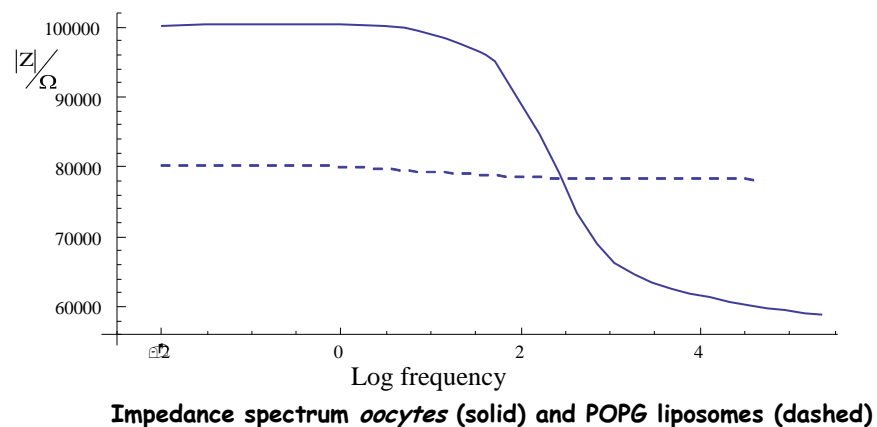
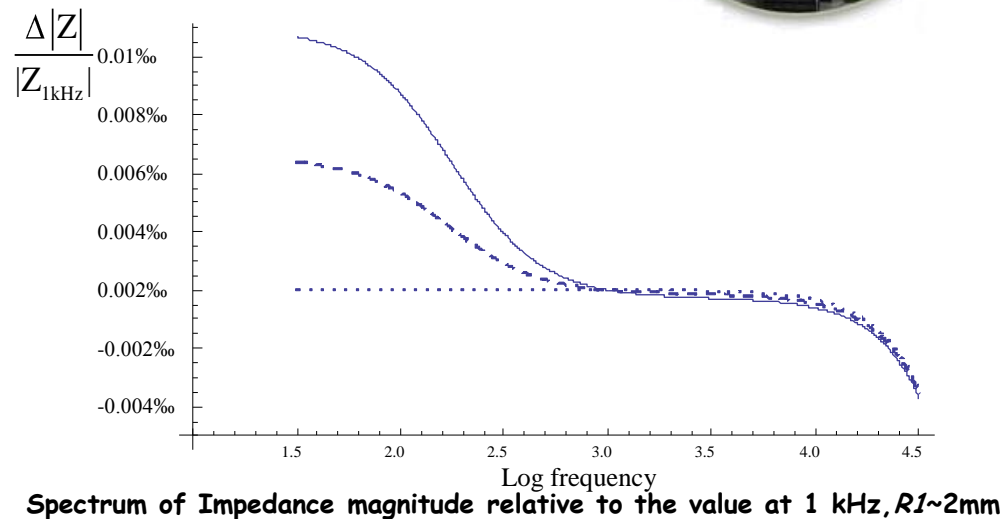
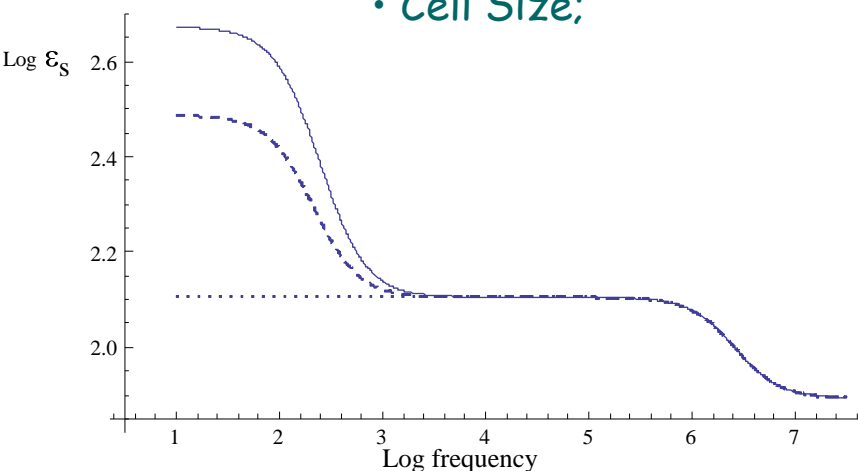
EIS



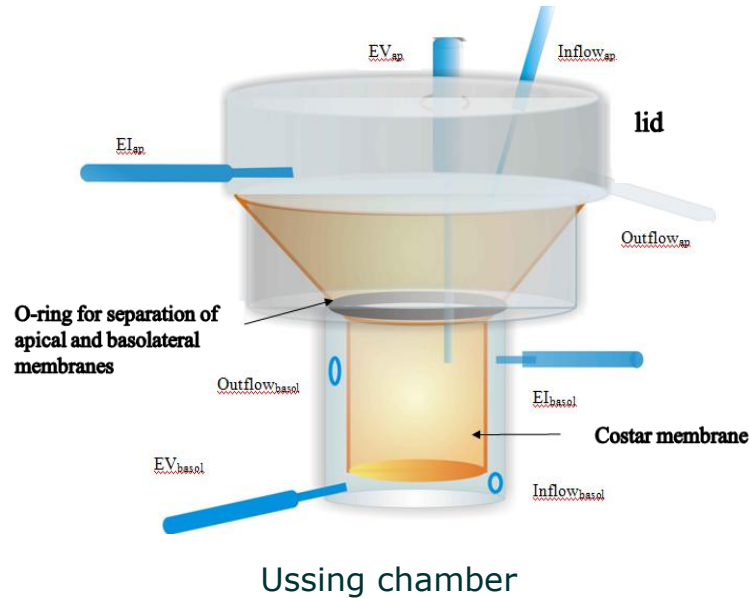
Xenopus laevis Oocytes and liposomes

Reveal the dependence of Impedance/Dielectric spectra on:

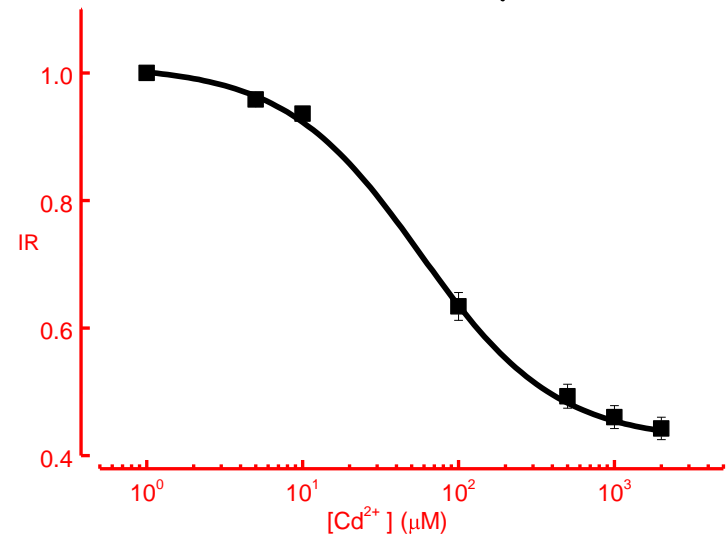
- Membrane potential & Membrane conductivity;
- Diffusion effects in extracellular medium;
- Cell Size;



Transepithelial Impedance and Noise System



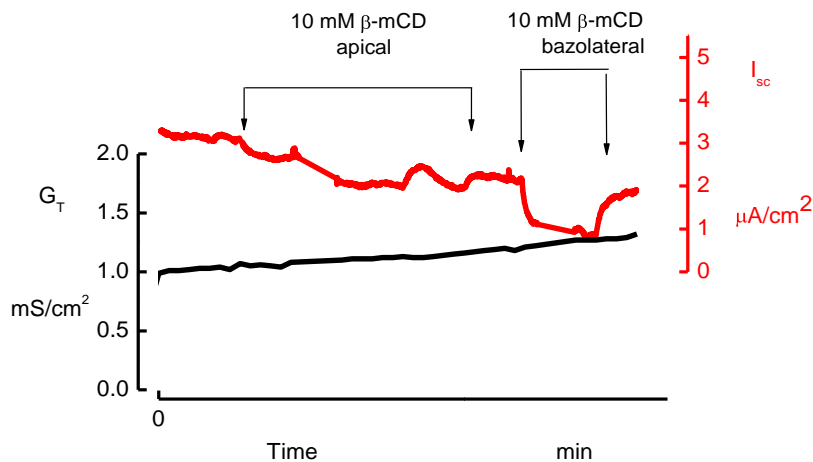
• Assessment of heavy metals



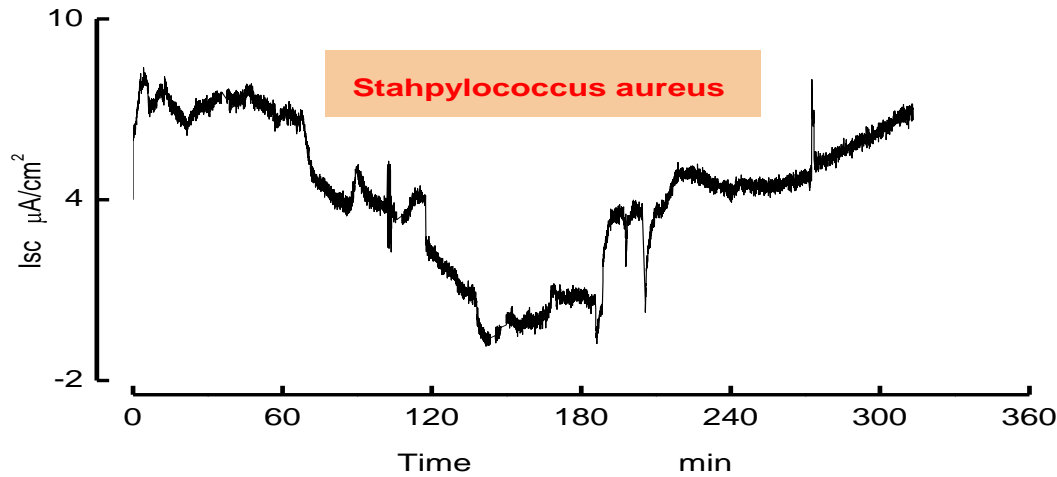
Dose-response curve of Cd²⁺ on relative currents from renal epithelial cells, A6

• Changes in cholesterol composition

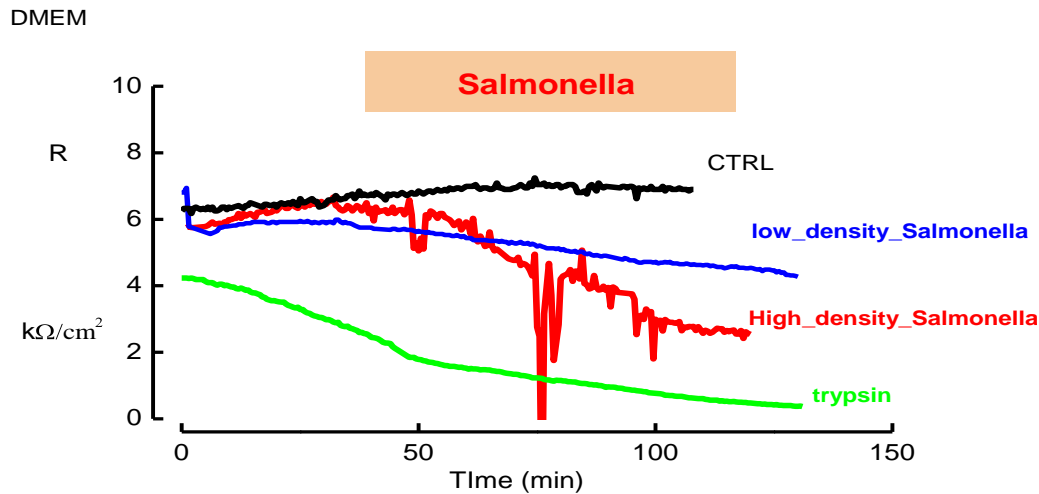
• Analysis of flavonoids



Transepithelial Impedance and Noise System



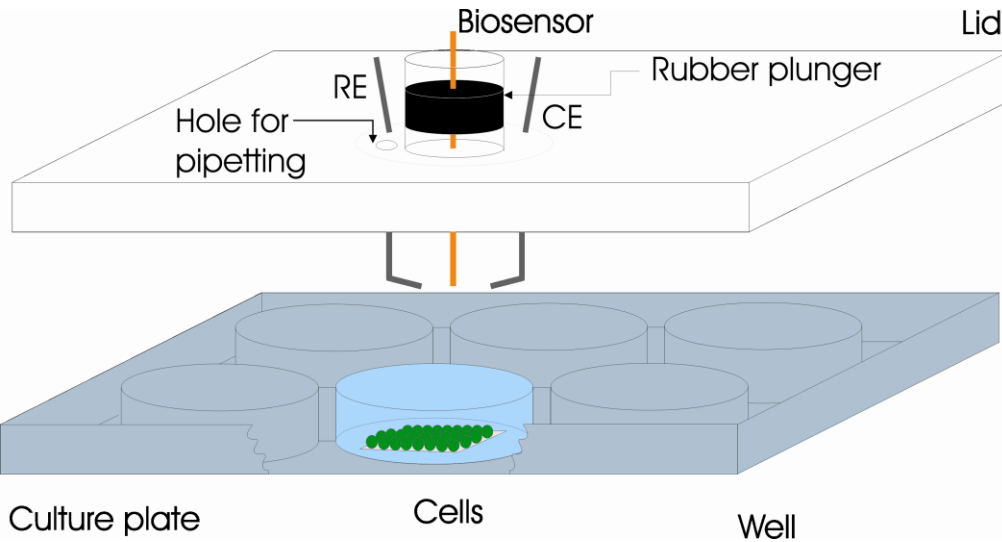
- Assessment of pathogens
- Renal A6 cells



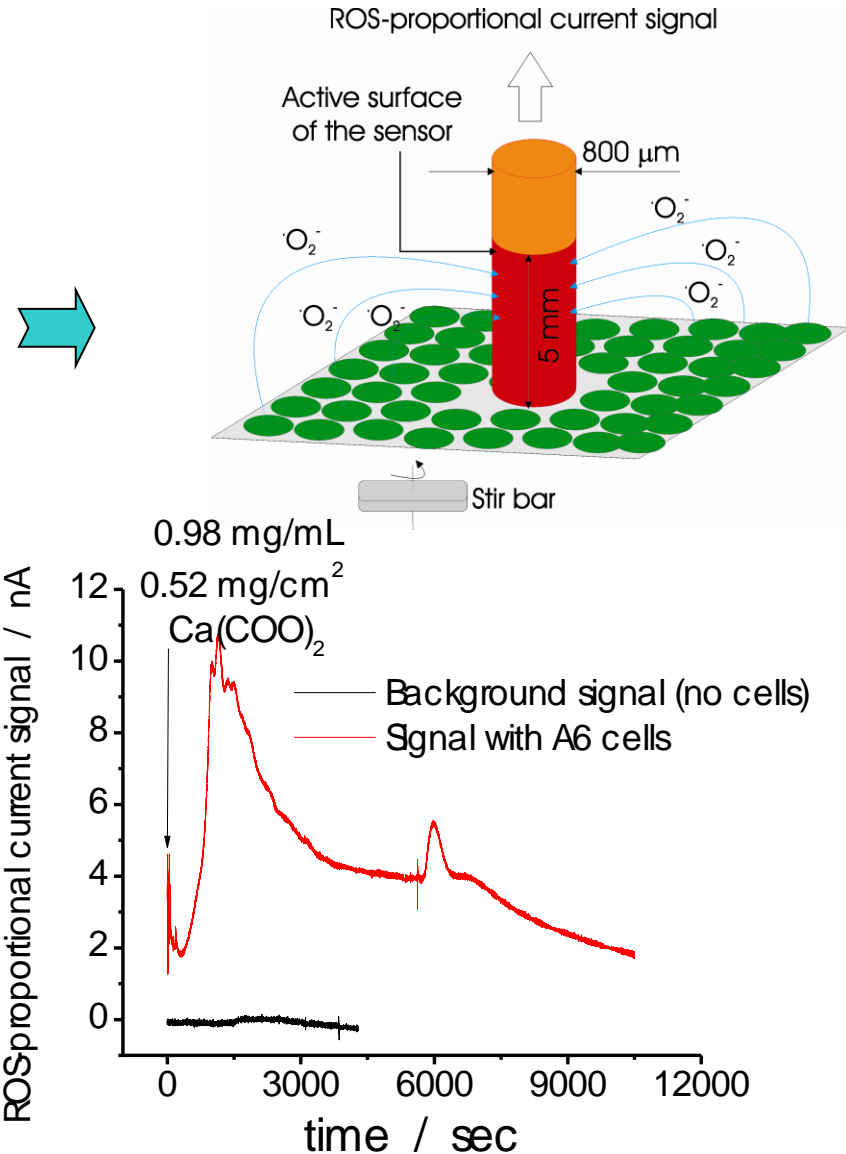
- Intestinal CaCo-2 cells

Amperometry

Assessment of ROS secretion by renal cells challenged with calcium oxalate

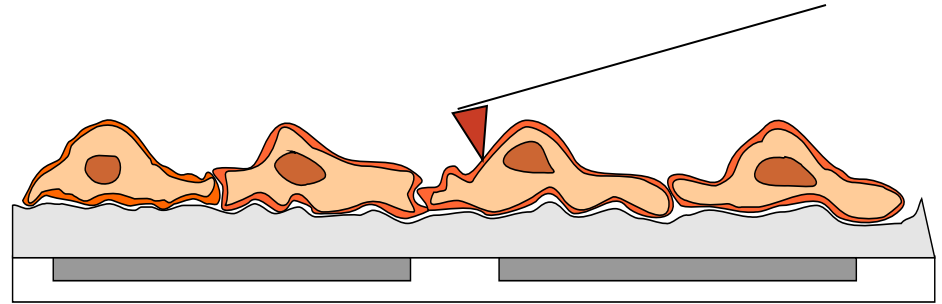
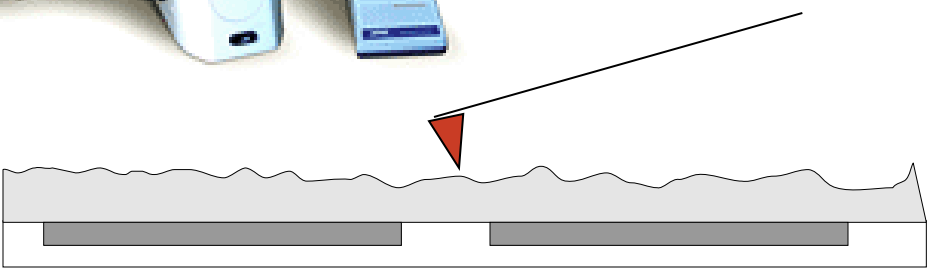


Gáspár *et al.*, (2010) *Biosens. Bioelectronics*



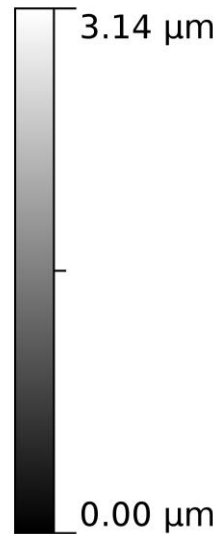
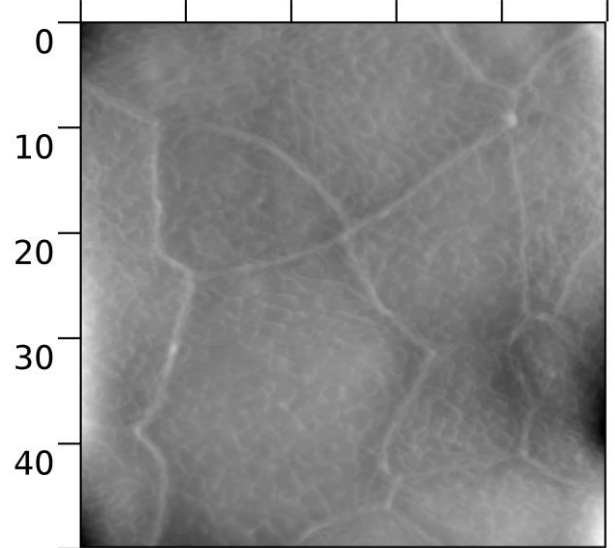
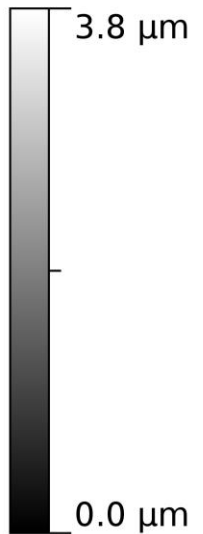
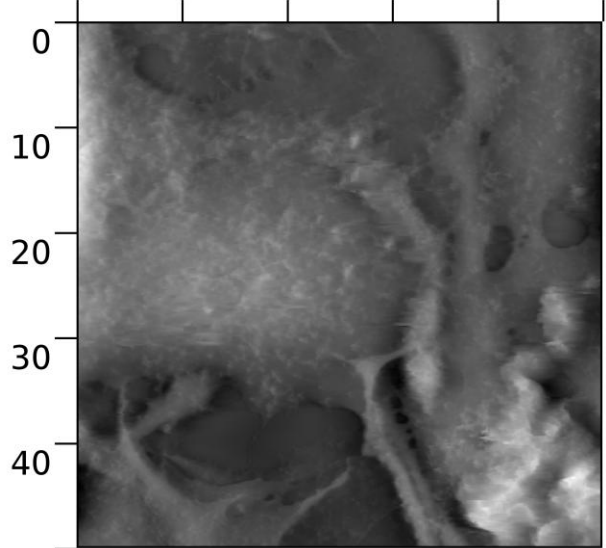


Atomic Force Spectroscopy



0 μm 10 20 30 40

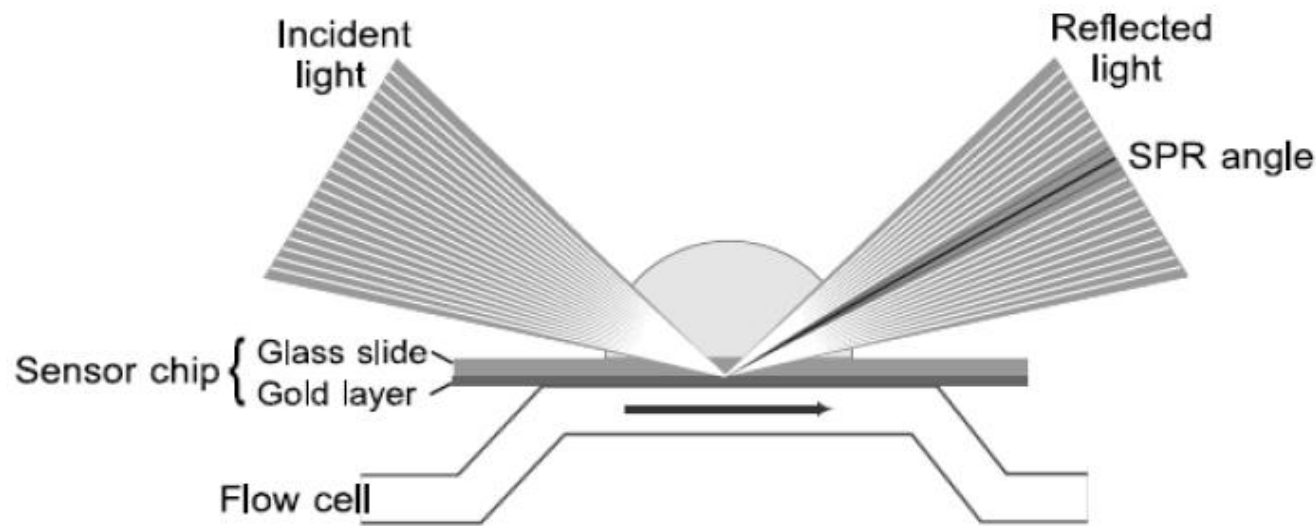
0 μm 10 20 30 40



After 1 day

After 18 days

Surface Plasmon Resonance



Assessment of:

1. Cell-Substrate interaction/adherence
2. Multi-phase interaction process between pore forming compounds (antimicrobial peptides) and lipid membranes

Gheorghiu et al, Biosensors and Bioelectronics (2009)

Olaru et al, Phys Chem B (2009)

3. Evaluation of chip quality

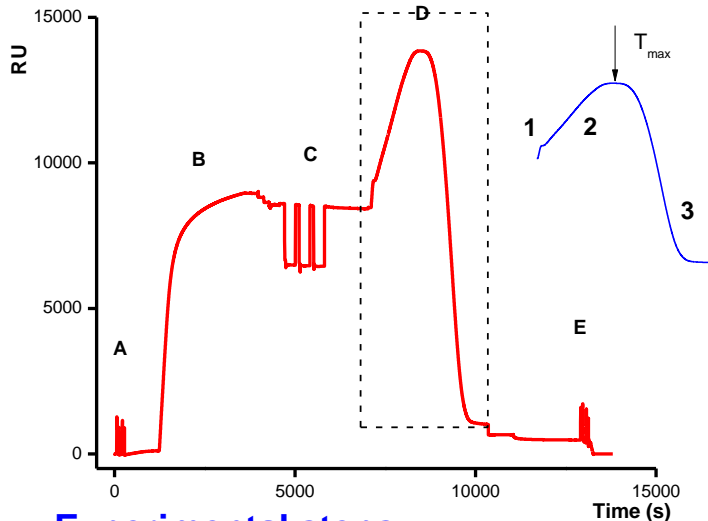
Assessment of the multi-phase interaction process between Melittin and a lipid membrane



Melittin (26 AA)



Biacore 3000



• Experimental steps

- A - cleaning the sensor surface
- B - lipid membrane formation (POPC)
- C - removal of loosely bound structures
- D - melittin interaction with the lipid membrane
- E - regeneration of sensor surface

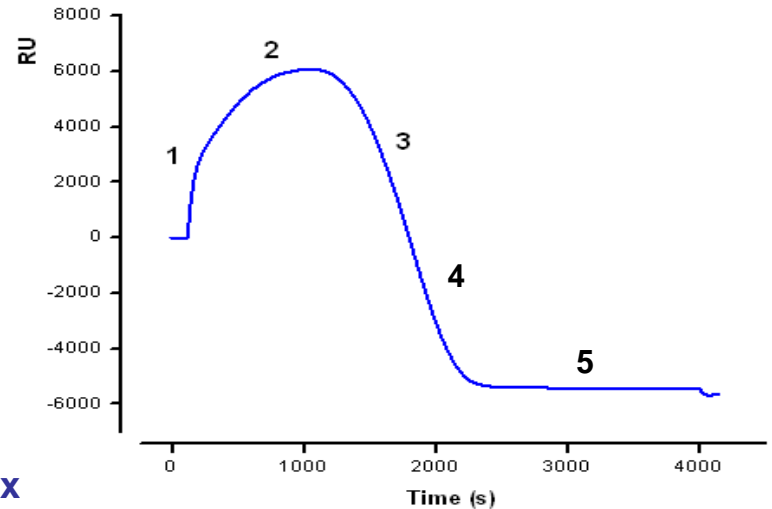
Kinetic model

+

Transfer matrix approach



Quantitative (kinetic) parameters



1. M-L Association
2. M Insertion into lipid membrane
3. Pore formation
4. Lipid layer destruction
5. M- chip association

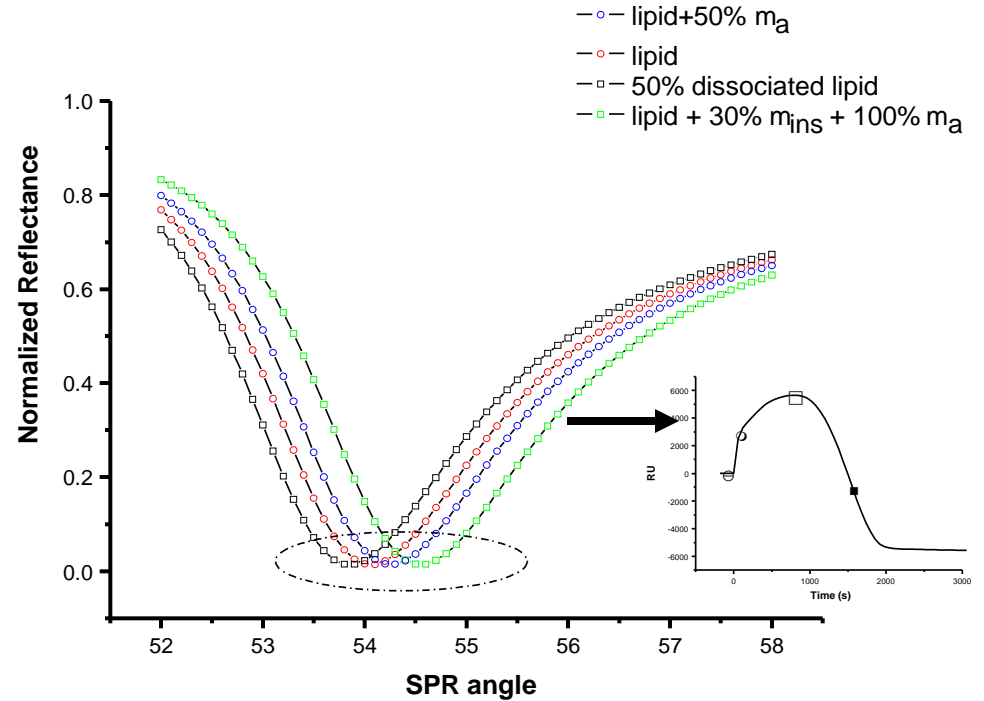
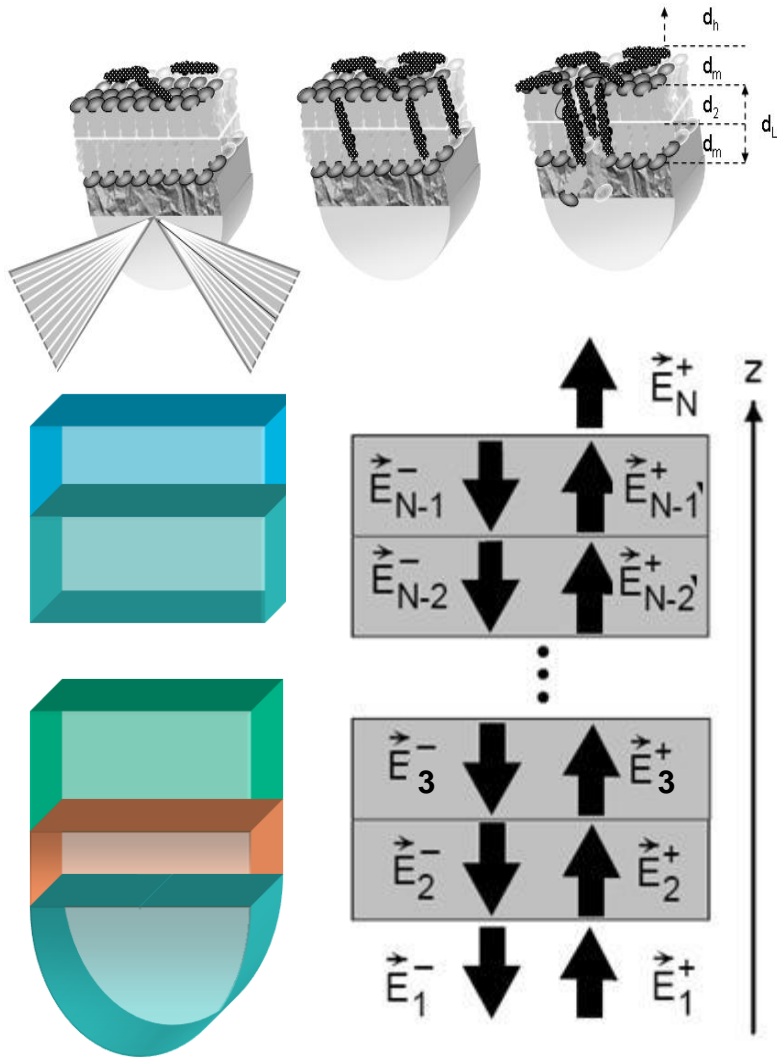
• Association constants

• Dissociation constants

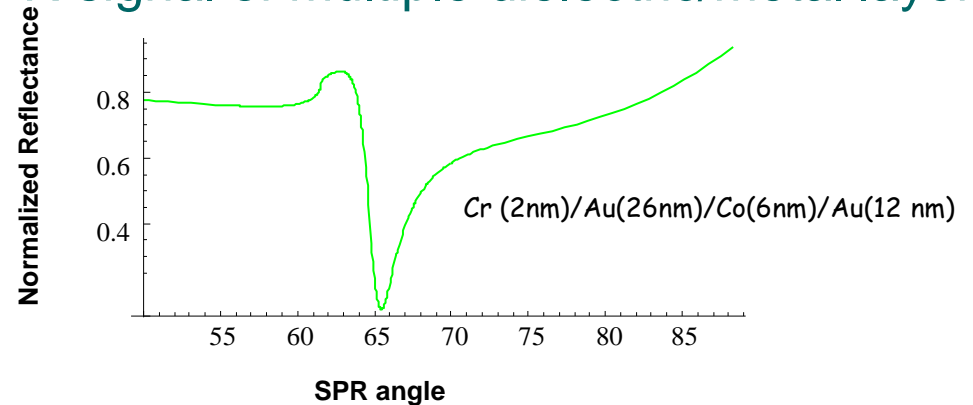
• Threshold values

• Actual P:L ratios

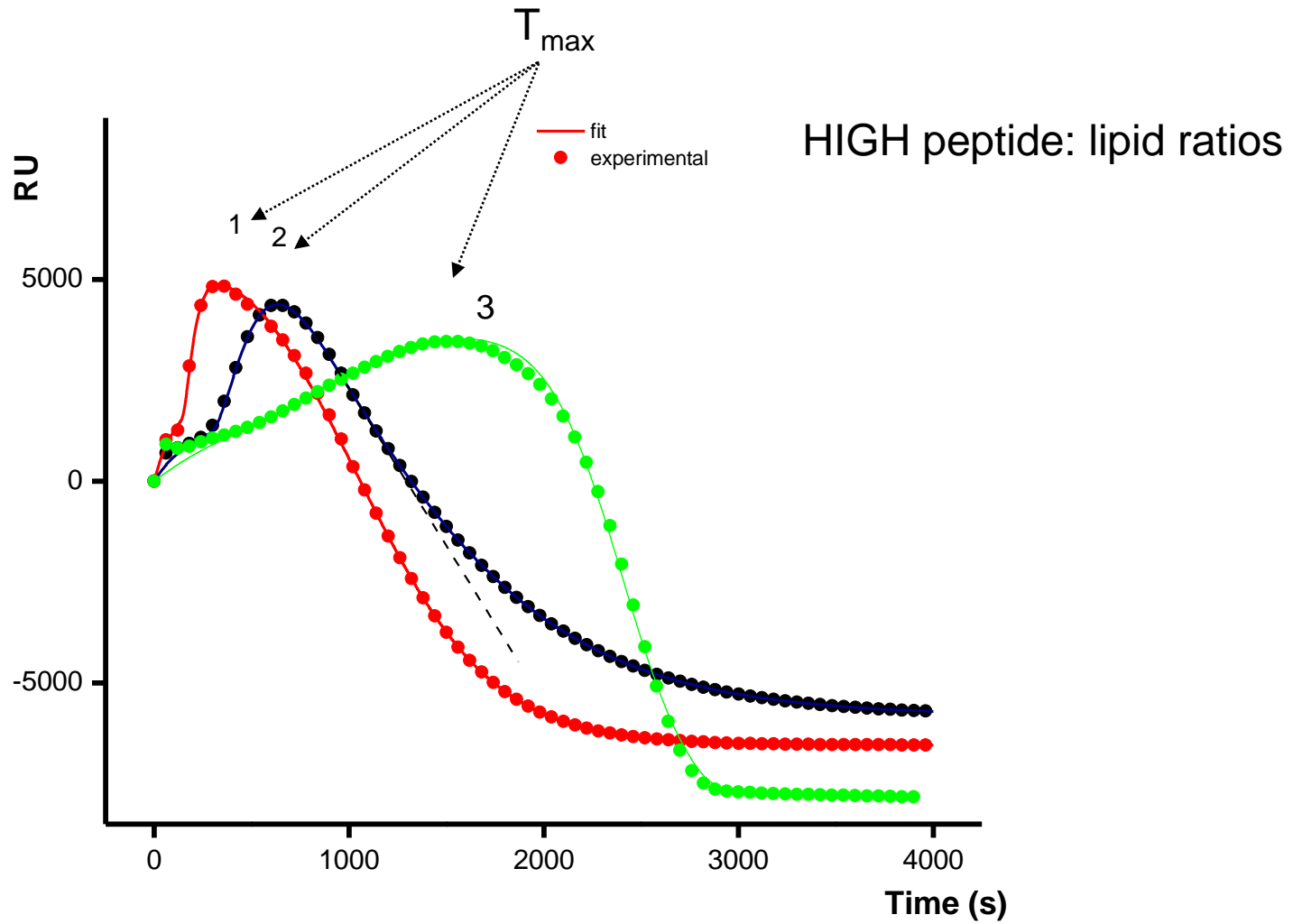
Deriving the reflectance of a multilayer system by Transfer Matrix Approach



SPR signal of multiple dielectric/metal layers

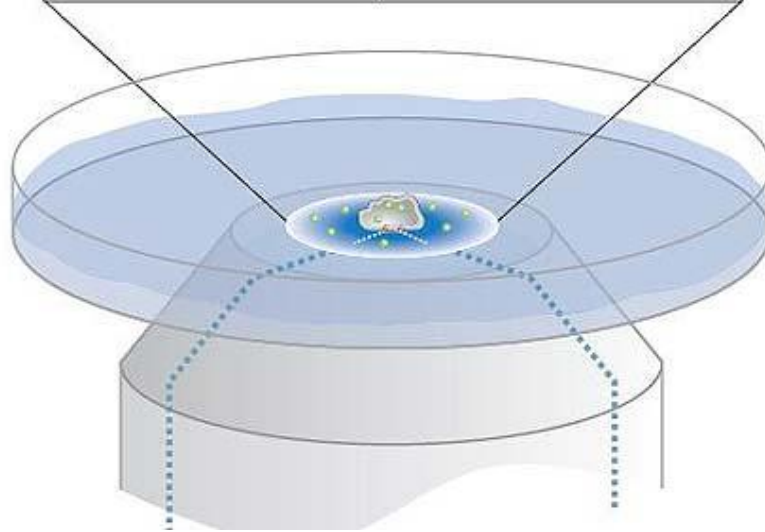
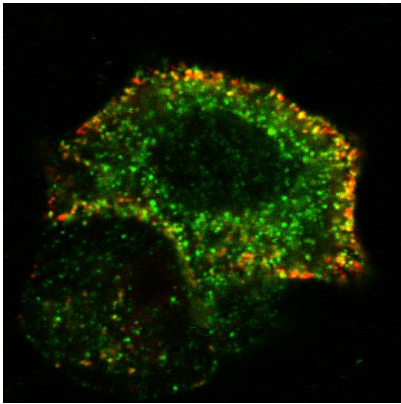
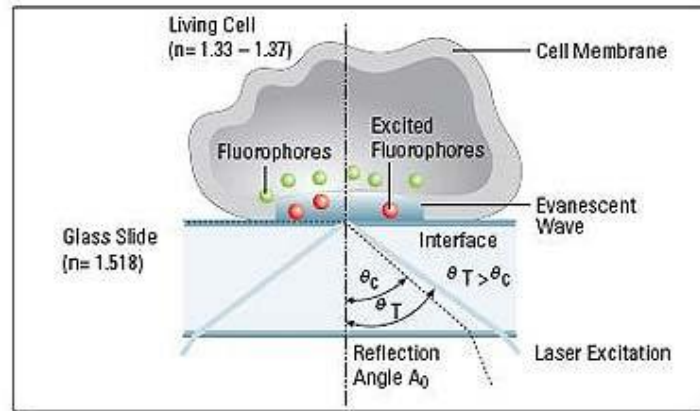


Detection - T_{max}

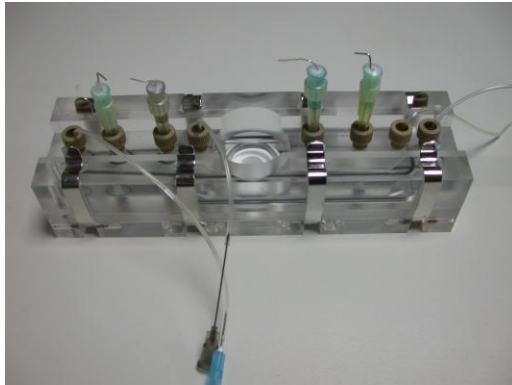


- 1) 3.62 μ M melittin for 6500 RU of lipids
- 2) 2.81 μ M melittin for 5800 RU of lipids
- 3) 2.35 μ M melittin for 8600 RU lipid coverage

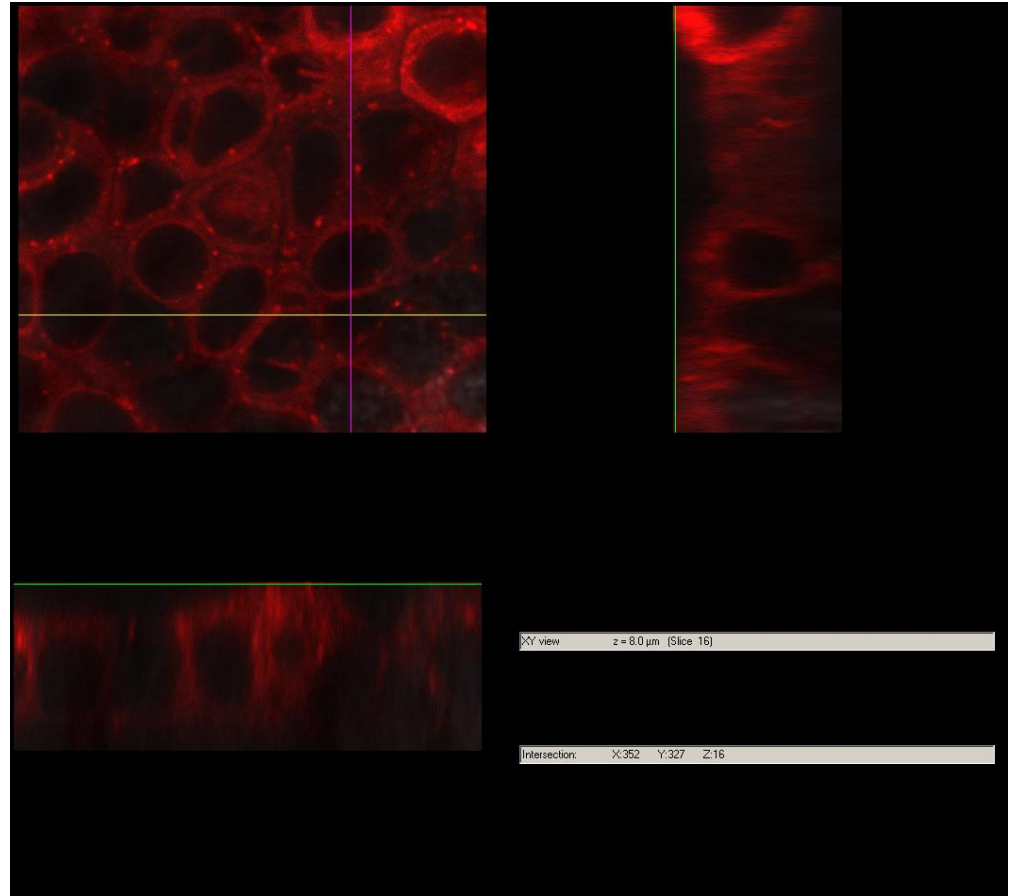
Total Internal Reflection Fluorescence Microscopy



Combined set-up

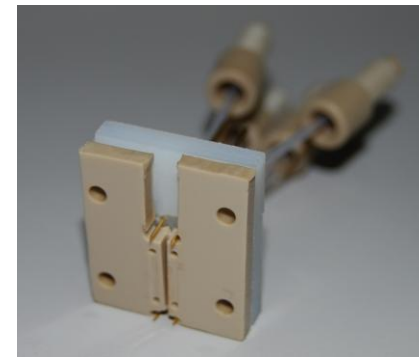
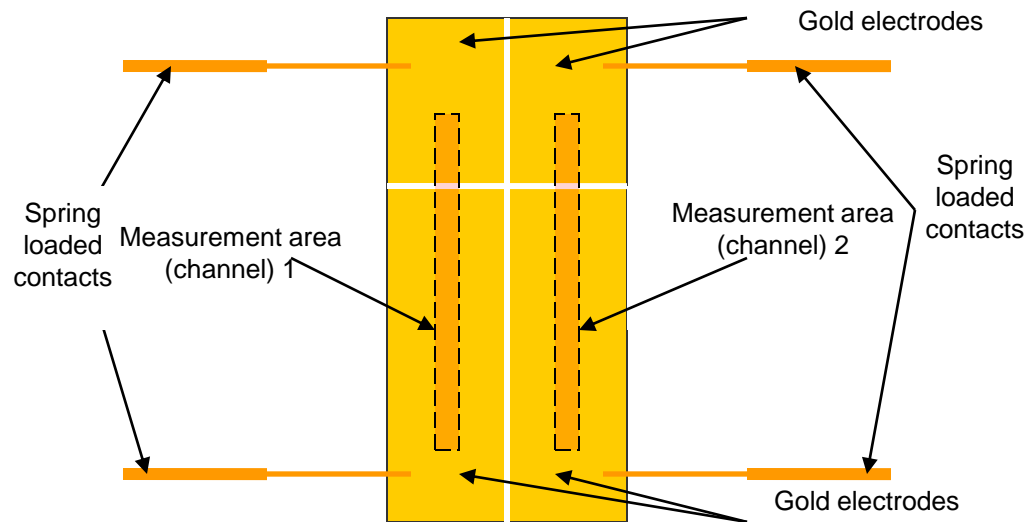


Confocal / impedance
Membranes / cell monolayers



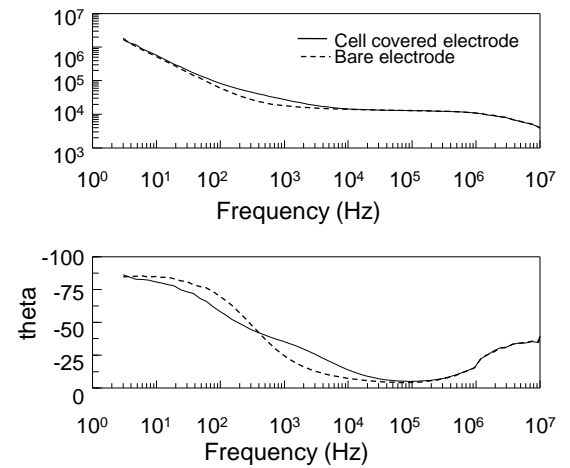
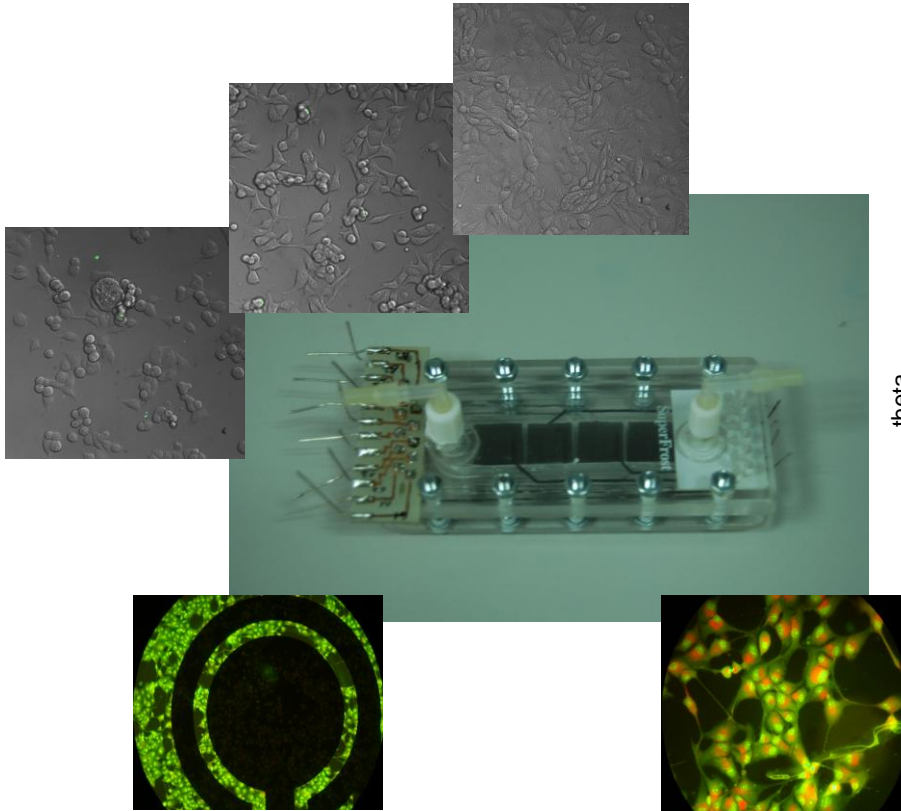
Confocal xy, xz, and yz sections of a three dimensional image stack taken from cells grown on a polycarbonate filter in the sample chamber. For simultaneous, confocal and impedance, assessment of cell monolayer in response to apical and basal changing media.

Combined set-up



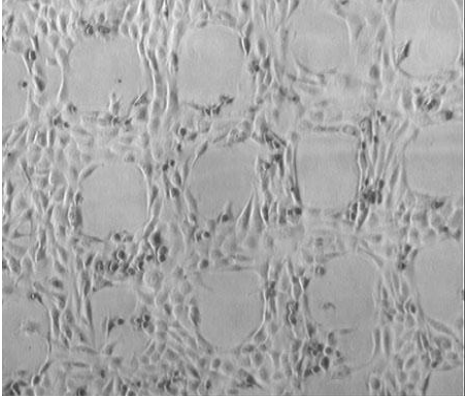
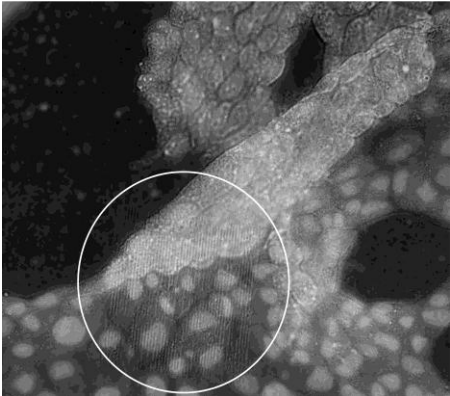
SPR/ impedance
cells, proteins, target analytes
biorecognition platforms with aptamers and antibodies

Combined set-up



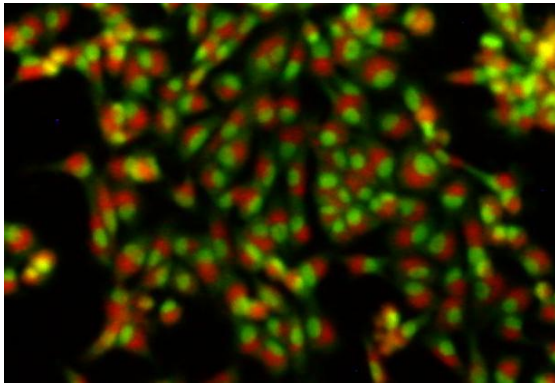
Optical / impedance
cellular dynamics

Controlled cell growth

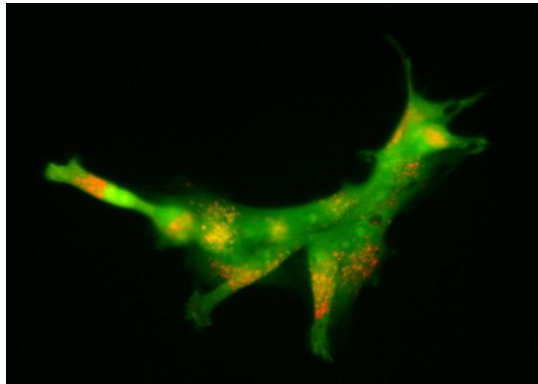


Cell morphology on functionalized substrates

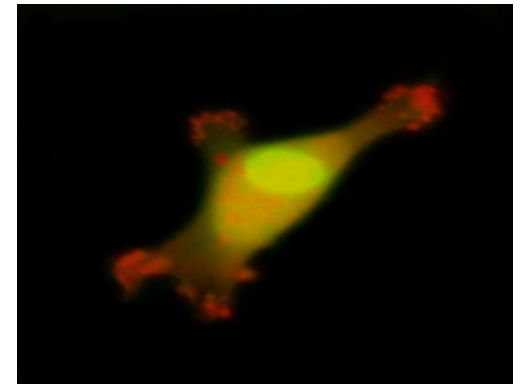
MC3 control



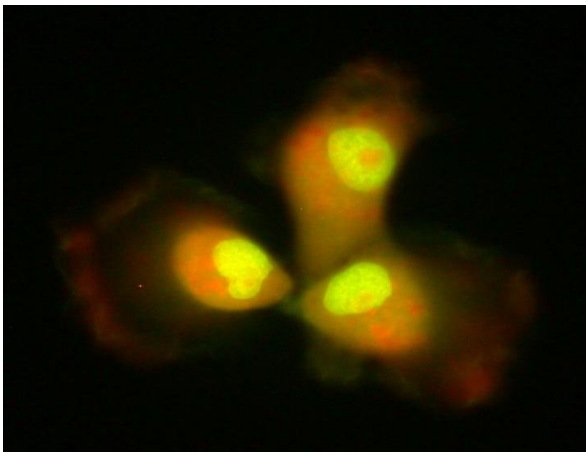
PDMS irradiation O⁺ 30 keV



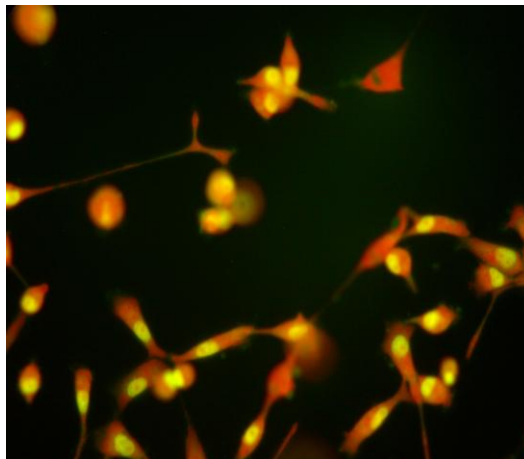
SIOMMA irradiation He²⁺ 50 keV



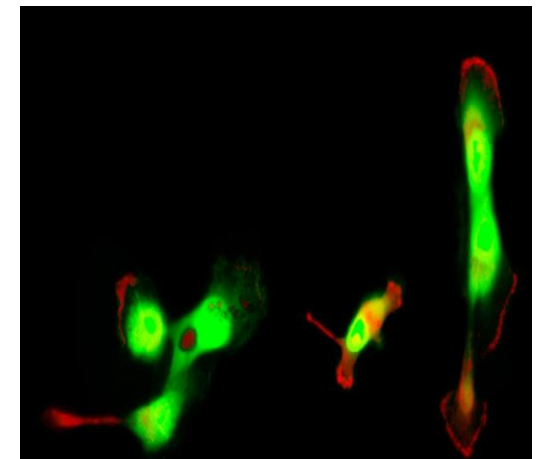
MC3 irradiation O⁺ 30 keV



PDMS irradiation O⁺ 50 keV



SIOMMA irradiation He²⁺ 70 keV



Summary

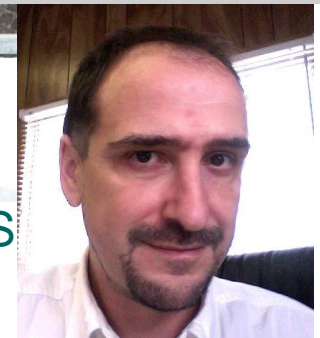
- **Cellular Platforms** investigated by combined Electro-Optical Assays allow for noninvasive access to:
 - Shape, size and electrical parameters of interconnected cells
 - heavy metal, pathogens effect on cell monolayers
 - ROS release following stimulation with $\text{Ca}(\text{COO})_2$
 - Cell-cell and cell-surface interaction
 - bioaffinity detectionproviding efficient tools for nano-cytotoxicity assessment
- A novel procedure to analyze the reflectivity of complex multi-layered structures, including super paramagnetic nanometric-films.
- We assess and quantitatively describe the whole interaction process between a pore forming compound and a lipid membrane and the relationship via transfer matrix to the measured SPR data.
- The response of a biosensor (bioaffinity or cellular platform) to a specific stimulus may not exhibit a monotonous evolution, therefore the entire process should be monitored via multiparametric assays....
biodynamics

Team & Acknowledgments

“Bees” in the hive



Thorsten Wohland
National University of Singapore



Daniel Vranceanu
Texas Southern University

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Support of National Projects:
RONANOMAGMA, PROPETHAD, ELBIOARCH and
DEMENTJUNCTION



