PROGRAMUL DE COOPERARE ELVEȚIANO-ROMÂN
SWISS-ROMANIAN COOPERATION PROGRAMME

TUMOURANALYZER

INTERNATIONAL CENTRE OF BIODYNAMICS (ICB)

LABORATORY OF ANALYTICAL AND PHYSICAL ELECTROCHEMISTRY (LEPA)
ECOLE POLYTECHNIQUE FEDERALE DE LAUSANNE (EPFL)

Project number IZERZO_142236/1

PROIECT CO-FINANȚAT PRINTR-UN GRANT DIN PARTEA ELVEȚIEI PRIN INTERMEDIUL CONTRIBUȚIEI ELVEȚIENE PENTRU UNIUNEA EUROPEANĂ EXTINSĂ

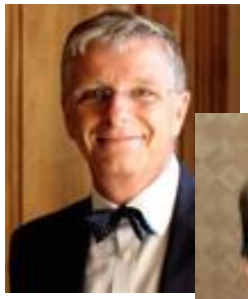
Laboratory of Physical and Analytical Electrochemistry (LEPA)

LEPA
Laboratoire d'Electrochimie Physique & Analytique

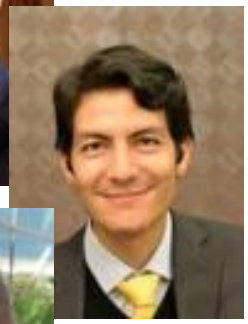
ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

VALAIS WALLIS

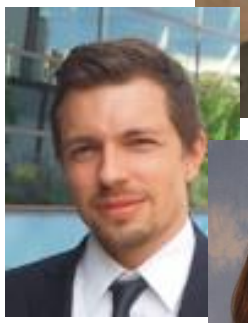
Prof. Hubert Girault



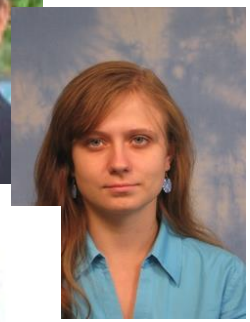
Dr. Fernando Cortés Salazar



Dr. Andreas Lesch



Ms. Alexandra Bondarenko



Ms. Tzu-En Lin

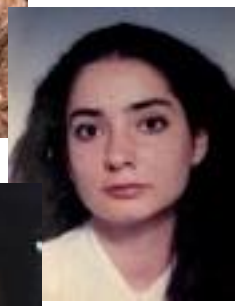
Dr. Horst Pick

International Centre of Biodynamics (ICB)

Prof. Eugen Gheorghiu

Dr. Sorin David

Dr. Szilveszter Gaspar

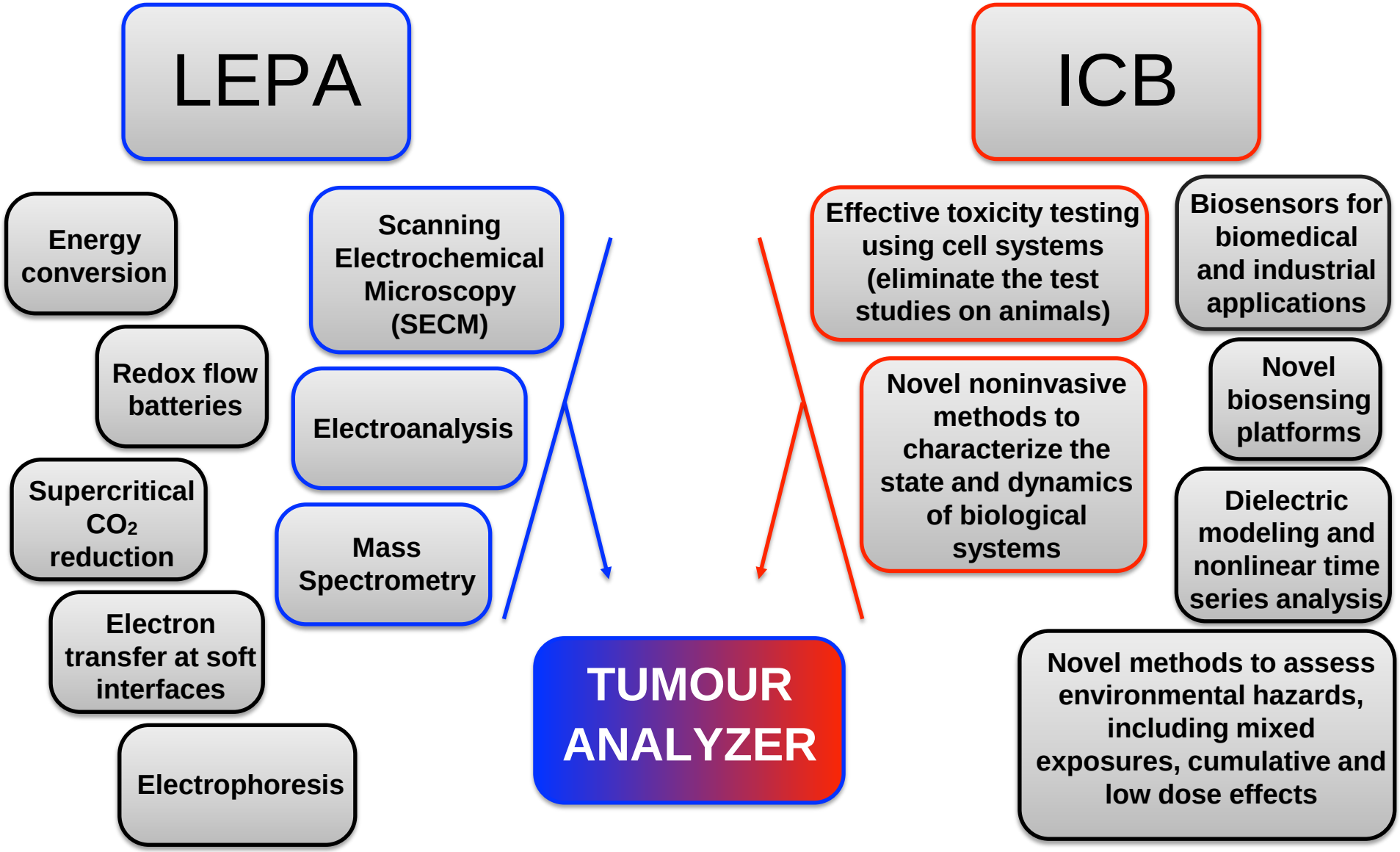


Dr. Mihaela Gheorghui

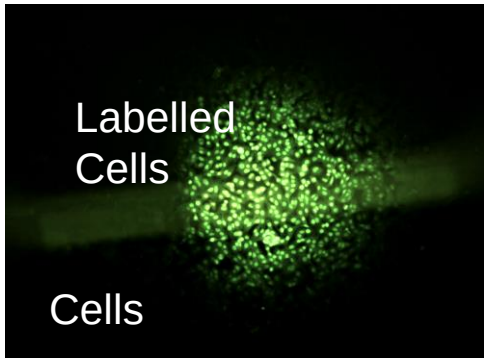
Dr. Cristina Polonschii

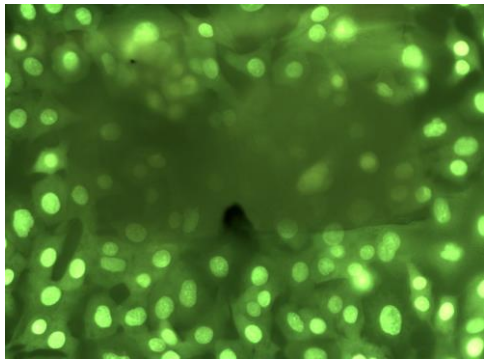
Ms. Luciana Stanica

Who are we?

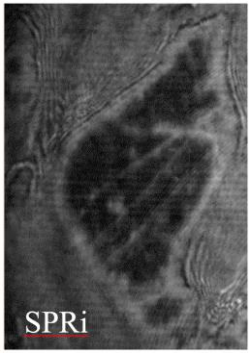

LEPA
ICB
Energy conversion
Redox flow batteries
Supercritical CO_2 reduction
Electron transfer at soft interfaces
Electrophoresis
Scanning Electrochemical Microscopy (SECM)
Electroanalysis
Mass Spectrometry
Effective toxicity testing using cell systems (eliminate the test studies on animals)
Novel noninvasive methods to characterize the state and dynamics of biological systems
Biosensors for biomedical and industrial applications
Novel biosensing platforms
Dielectric modeling and nonlinear time series analysis
Novel methods to assess environmental hazards, including mixed exposures, cumulative and low dose effects
TUMOUR ANALYZER

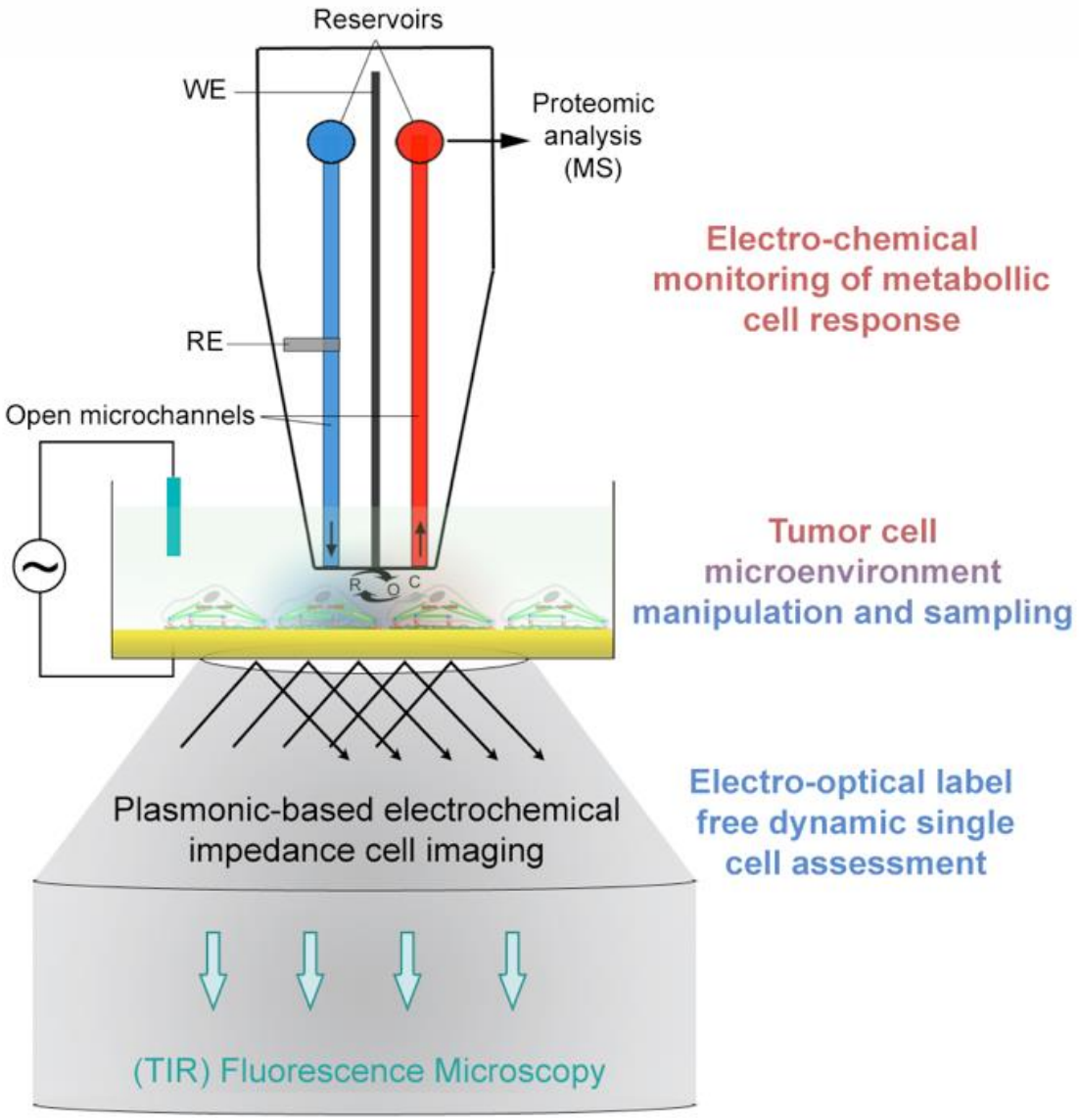
Tumouranalyzer





SPRi



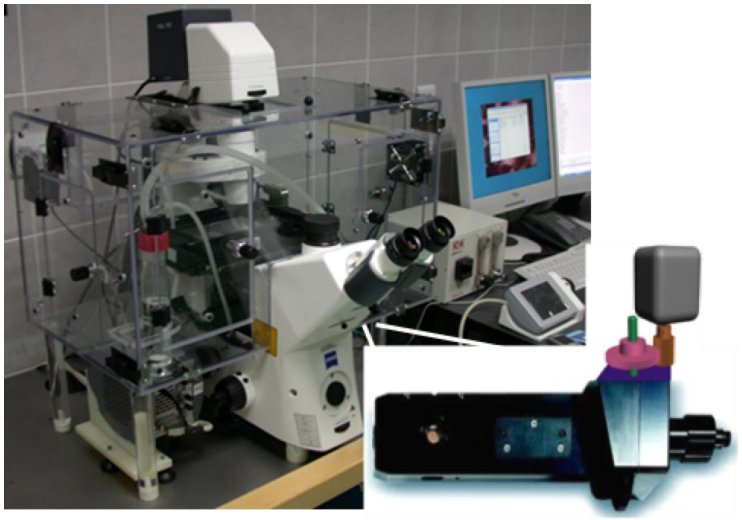


Collaboration aspects

A **Kick-off meeting in Switzerland (Lausanne)**
February 14th 2013
Visitors: Prof. Eugen Gheorghiu, Dr. Mihaela Gheorghiu, Dr. Szilveszter Gaspar

B **Joint experiments in Romania (Bucharest)**
June 22nd 2013 to July 7th 2013
Visitors: Ms. Alexandra Bondarenko, Dr. Fernando Cortés Salazar

B **Joint experiments in Romania (Bucharest)**
March 31st 2014 to April 10th 2014
Visitors: Ms. Alexandra Bondarenko, Dr. Fernando Cortés Salazar



A **Final Tumor Analyzer workshop in Switzerland (Sion)**
November 26th to 27th 2015
Visitors: Prof. Eugen Gheorghiu, Dr. Mihaela Gheorghiu, Dr. Szilveszter Gaspar

Research output: Publications

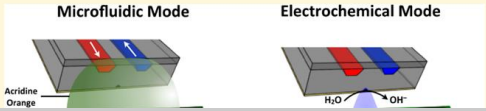
analytical chemistry
Article
pubs.acs.org/ac

Electrochemical Push–Pull Probe: From Scanning Electrochemical Microscopy to Multimodal Altering of Cell Microenvironment

Alexandra Bondarenko,[†] Fernando Cortés-Salazar,[†] Mihaela Gheorghiu,[‡] Szilveszter Gáspár,[‡] Dmitry Momotenko,[†] Luciana Stanica,[‡,§] Andreas Lesch,[†] Eugen Gheorghiu,[‡,§] and Hubert H. Girault*[,†]

[†]Laboratoire d'Electrochimie Physique et Analytique, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland
[‡]International Centre of Biodynamics, 1B Intrarea Portocalelor Street, 060101 Bucharest, Romania
[§]Faculty of Biology, University of Bucharest, 91–95 Splaiul Independentei, 050095 Bucharest, Romania

Supporting Information



GDCh Communications Angewandte International Edition Chemie

Melanoma Diagnosis

International Edition: DOI: 10.1002/anie.201509397
German Edition: DOI: 10.1002/ange.201509397

Monitoring Tyrosinase Expression in Non-metastatic and Metastatic Melanoma Tissues by Scanning Electrochemical Microscopy

*Tzu-En Lin, Alexandra Bondarenko, Andreas Lesch, Horst Pick, Fernando Cortés-Salazar, and Hubert H. Girault**

Abstract: *Although tremendous progress has been made in the diagnosis of melanoma, the identification of different stages of malignancy in a reliable way remains challenging. Current strategies rely on optical monitoring of the concentration and spatial distribution of specific biomarkers. State-of-the-art optical methods can be affected by background-color interference and autofluorescence. We overcame these shortcomings by employing scanning electrochemical microscopy (SECM) to map the prognostic indicator tyrosinase (TyR) in non-metastatic and metastatic melanoma tissues by using soft-stylus microelectrodes. Electrochemical readout of the TyR distribution was enabled by adapting an immunochemical method. SECM can overcome the limitations of optical methods and opens unprecedented possibilities for improved diagnosis and understanding of the spatial distribution of TyR in different melanoma stages.*

melanin can resemble the color of the chromogen 3,3′-diaminobenzidine (DAB), which is commonly employed in IHC (Figure S1 in the Supporting Information).[3] Alternatively, fluorescent tagging could be impeded by cellular autofluorescence or photobleaching.[4]

Electrochemical methods may represent a promising alternative since they rely exclusively on the electrochemical detection of redox-active species related to the presence of biomarkers. Scanning electrochemical microscopy (SECM) is a surface reactivity mapping tool with high spatial resolution and sensitivity that has been used widely for studying living cell cultures[5] but rarely for tissues. For instance, enzymatic activity and oxygen production/consumption in plant tissues and microtissues have been monitored.[6] SECM has also been applied to study molecular transport through skin samples.[7] The lateral dimensions of tissue samples can approach square

analytical chemistry
Article
pubs.acs.org/ac

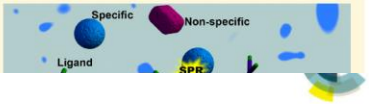
Complementarity of EIS and SPR to Reveal Specific and Nonspecific Binding When Interrogating a Model Bioaffinity Sensor; Perspective Offered by Plasmonic Based EIS

Cristina Polonschii,[†] Sorin David,[†] Szilveszter Gáspár,[†] Mihaela Gheorghiu,[†] Mihnea Rosu-Hamzescu,[†,‡] and Eugen Gheorghiu*[,†,‡]

[†]International Centre of Biodynamics, 1B Intrarea Portocalelor, 060101 Bucharest, Romania
[‡]University of Bucharest, 4-12 Regina Elisabeta Blvd., 030018 Bucharest, Romania

Supporting Information

ABSTRACT: The present work compares the responses of a model bioaffinity sensor based on a dielectric functionalization layer, in terms of specific and nonspecific binding, when interrogated simultaneously by Surface Plasmon Resonance (SPR), non-Faradaic



Analyst

ARTICLE

Aluminum Foil as Single-Use Substrate for MALDI-MS Fingerprinting of Different Melanoma Cell Lines

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

A. Bondarenko,[a] Y. Zhu,[a] L. Qiao,[a] F. Cortés Salazar,[a] H. Pick[b] and H. H. Girault[a]

Herein, we present the intact cell matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) for the fingerprinting of human melanoma cancer cell lines grown on aluminium foil. To perform the MALDI-MS assay, melanoma cells were cultured on a flat and thin foil, which was directly transferred to the target plate of MALDI-MS for analysis. The influence of a wide range of cell fixation protocols (i.e. formalin-based and alcohol-based methods) and MALDI matrices on the obtained characteristic spectra was investigated. For the optimization of the MALDI-MS protocol, the MS fingerprints of the melanoma WM-239 cell line with and without an overexpressed enhanced green fluorescent protein were employed. The fingerprints obtained from WM-239 cells grown on aluminum foil were compared with intact cell MALDI of cell pellet and presented higher sensitivity in high m/z range. The optimized protocol was subsequently applied to characterise melanoma cell lines derived from different cancer stages and allowed the identification of unique MS signals that can be used for the differentiation between the studied cell lines (i.e. molecular weight equal to 10.0 kDa and 26.1 kDa).

A. Bondarenko, T.-E. Lin, A. Lesch, F. Cortés Salazar, P. Stupar, H. Pick and H. H. Girault.
Scanning Electrochemical Microscopy of Alive, Fixed and Permeabilized Adherent Melanoma Cells. In preparation

L. Stanica, M. Gheorghiu, S. Gaspar, C. Polonschii, M. Stan, A. Dinischiotu, E. Gheorghiu
Electro-optical platform for quantitative assessment of specific carbonic anhydrase inhibitors effect on live hypoxic cells. In preparation

Research output: Conferences

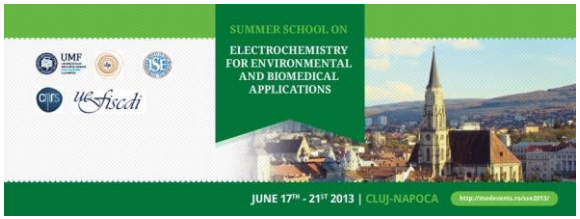
NanoBioTech 2015 (Montreux, Switzerland)

8th International Workshop on Scanning Electrochemical Microscopy 2015 (Xiamen, China)

ElecNano 2014: Electrochemistry in Nanoscience – 6 (Paris, France)

ISE 2015: 66th Annual International Society of Electrochemistry Meeting (Taipei, Taiwan)

ISE 2013: 64th Annual International Society of Electrochemistry Meeting (Santiago de Queretaro, Mexico)



SMOBE 2015: Summer meeting on bioelectrochemistry (Antwerp, Belgium)

Summer School on Electrochemistry for Environmental and Biomedical Applications, 2013 (Cluj-Napoca, Romania)

Research output: PhD degree

Dr. Alexandra Bondarenko

PhD thesis entitled:

"Electrochemical Sensing and Imaging of Biological Samples"

under the supervision of

Prof. Hubert H. Girault
Dr. Fernando Cortés Salazar

date of the defence:

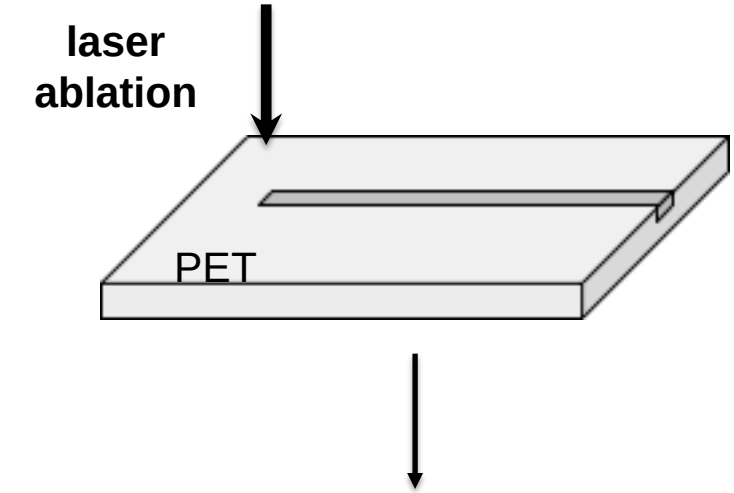
17 of December 2015

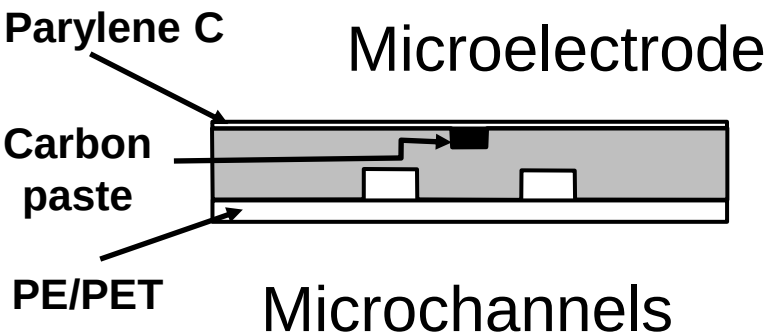
Joint experiments during the Swiss-Romanian cooperation program Bucharest

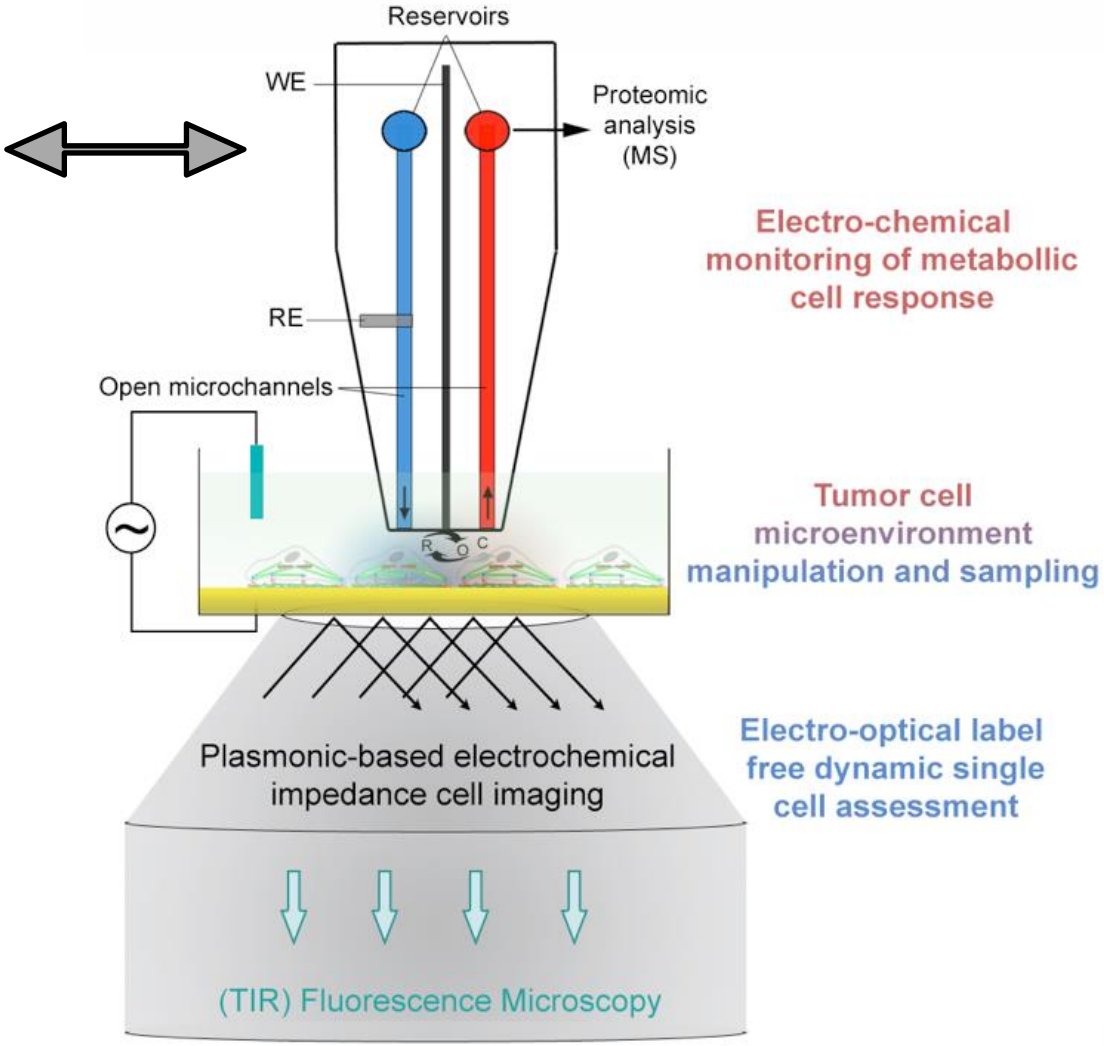
* Electrochemical push-pull probe
* TUMOURANALYZER setup
* *In vitro* cells experiments

Tumouranalyzer

Electrochemical Push-Pull Probe



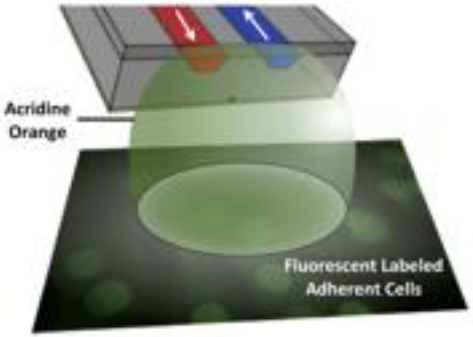


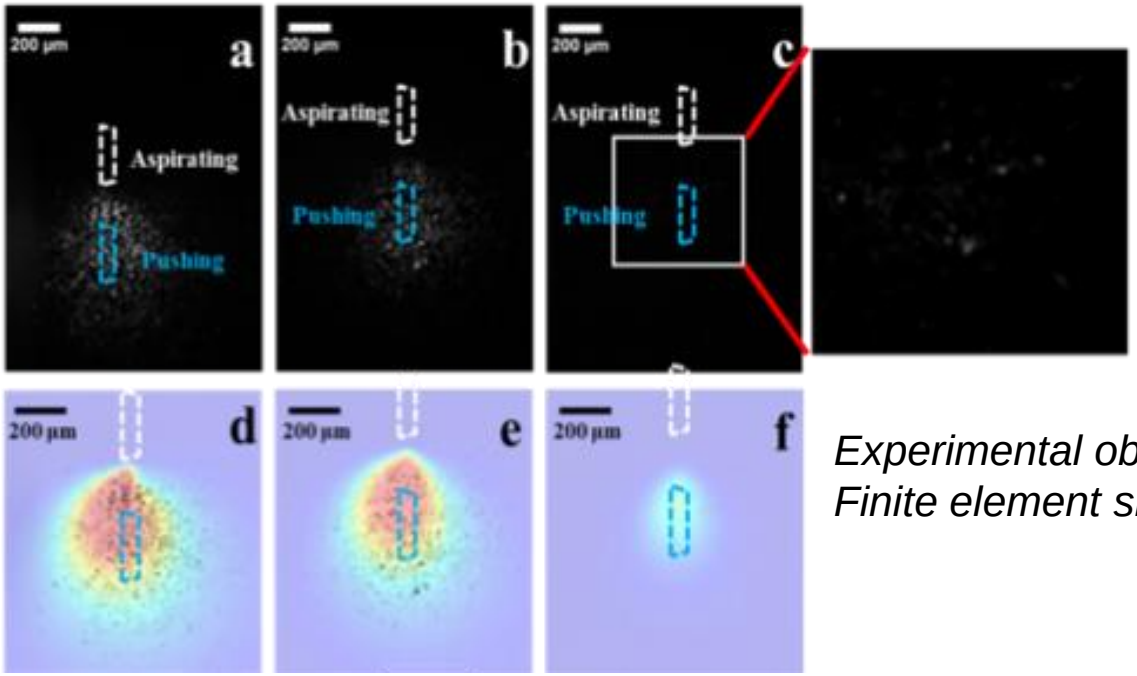


Multimodal altering of cell microenvironment

Microfluidic mode

* **delivery of acridine orange for local labelling of adherent cells**



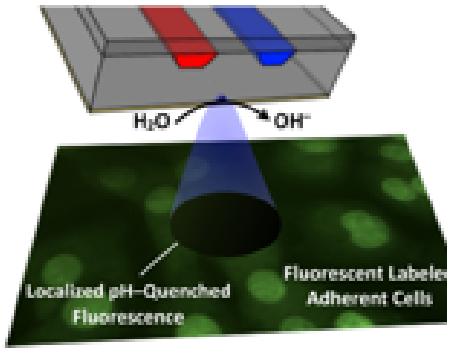


The working distance d was:
50 µm (a) and (d);
100 µm (b) and (e);
250 µm (c) and (f).

Experimental observation (spots)
Finite element simulation (colours)

Anal. Chem., 2015, 87, 4479–4486

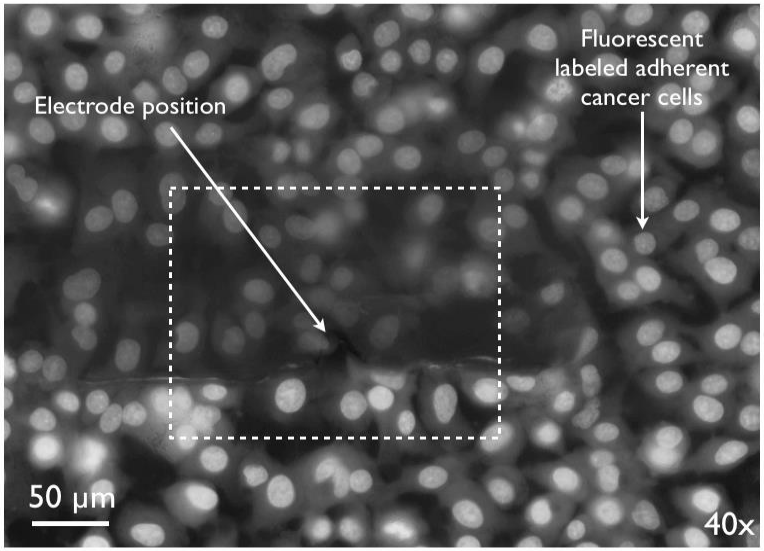
Multimodal altering of cell microenvironment



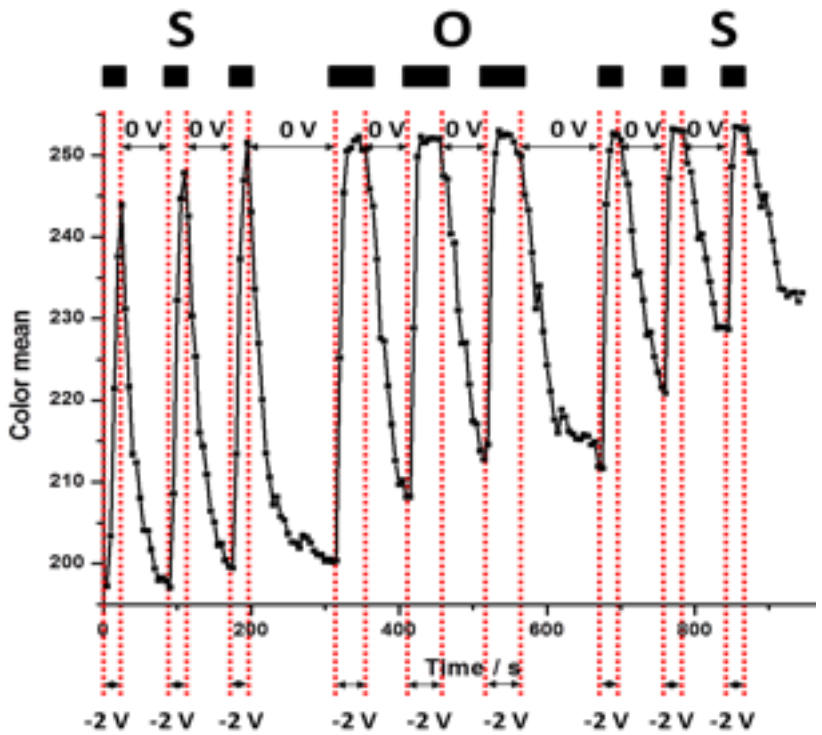
Electrochemical mode

* potential-modulated pH variation for quenching acridine orange fluorescence

Cell-Generated *S-O-S* Morse Signal



Fluorescent microscope video (speed 40x) of adherent cancer cells perturbed by using the MPPD. 90° probe tilt, E_{tip} = –2 V, d = 10 µm.



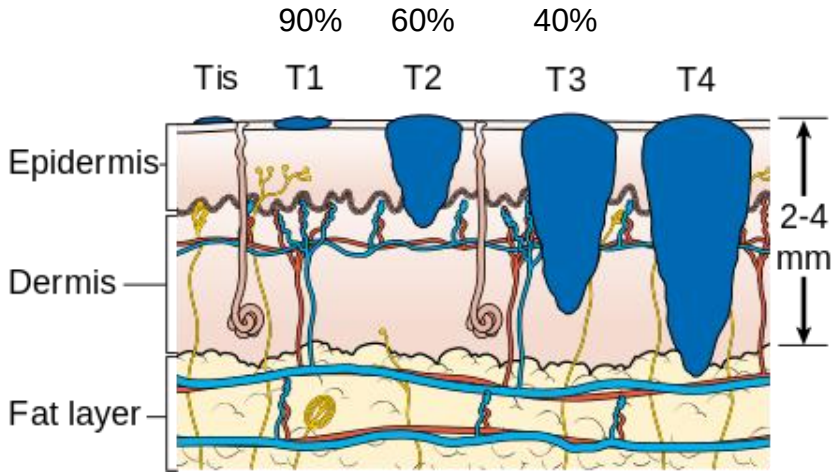
Anal. Chem., 2015, 87, 4479–4486

Thanks to the Swiss-Romanian cooperation program, LEPA moved towards a new direction, *i.e. in vitro* characterisation of cells and tissues

* SECM of tissues
* SECM of cells
* MS of cells

Stage 1 : Radial growth phase
Stage 2 : Vertical growth phase
Stage 3 : Metastatic

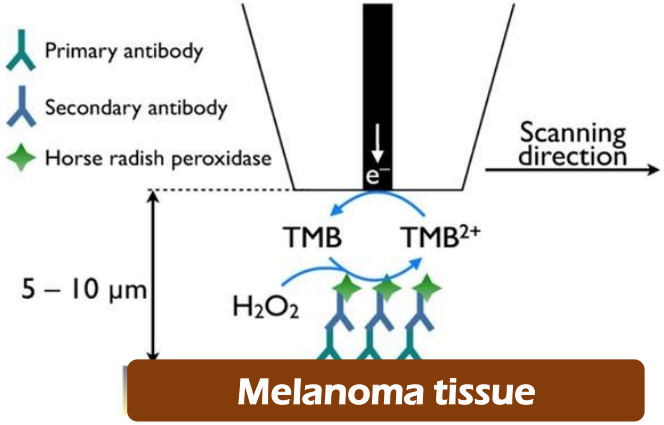
Melanoma



Wikipedia

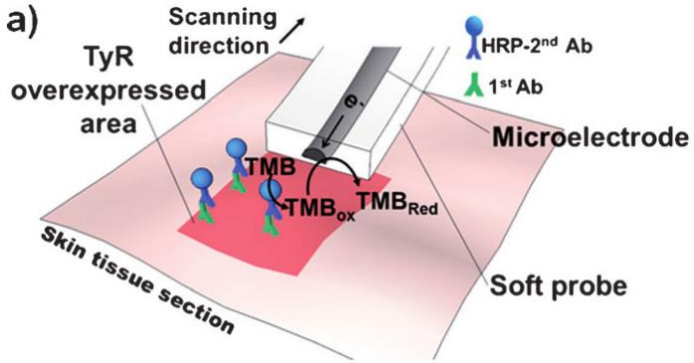
As seen on TV…

SECM of melanoma

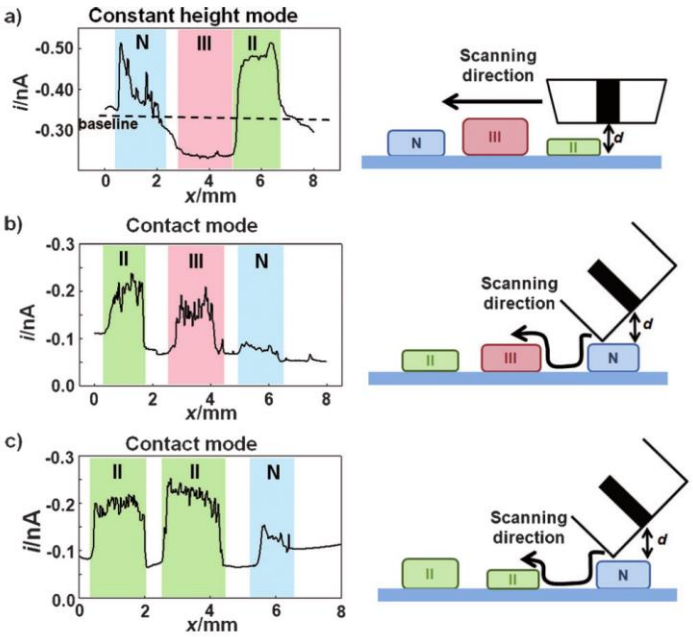


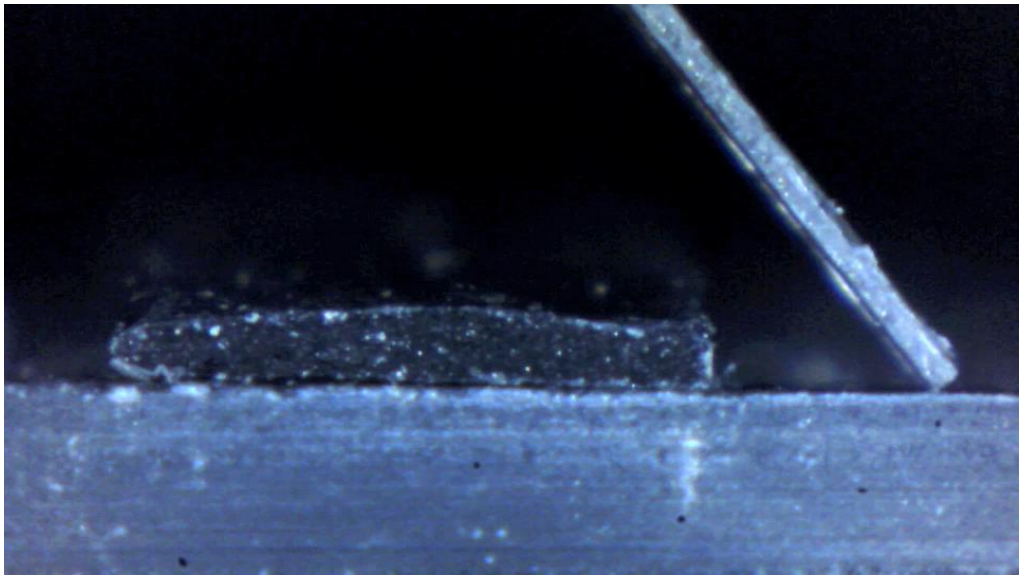
The dream: Image a melanoma directly on the skin…
and even develop electrodynamic therapy

SECM for melanoma diagnostic



Schematic representation of the immunoassay-based detection strategy to map the tyrosinase (TyR) distribution in tissue sections by using a soft SECM probe and TMB as the redox-active species.

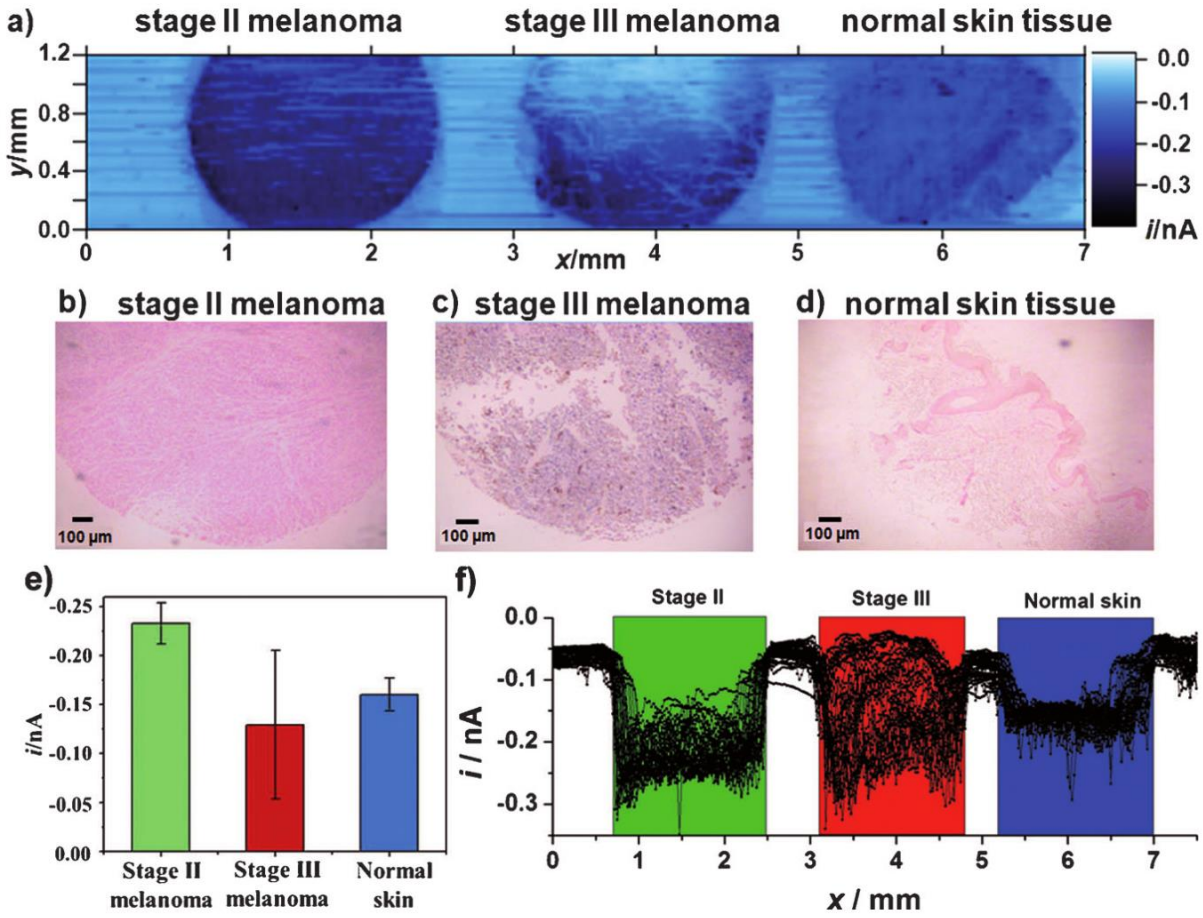




Soft probes allow contact mode brushing of samples without damaging delicate substrates.

Angew. Chim. Int. Ed. 2016, 55, 3813–3816

SECM for melanoma diagnostic



Contact-mode SECM image of stage II and stage III melanoma and normal skin tissues after immunostaining of TyR

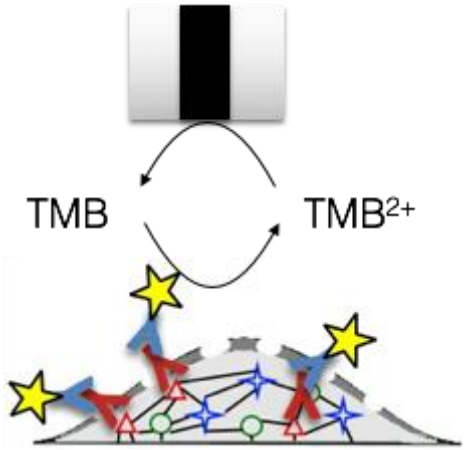
Optical images of tissues obtained by IHC

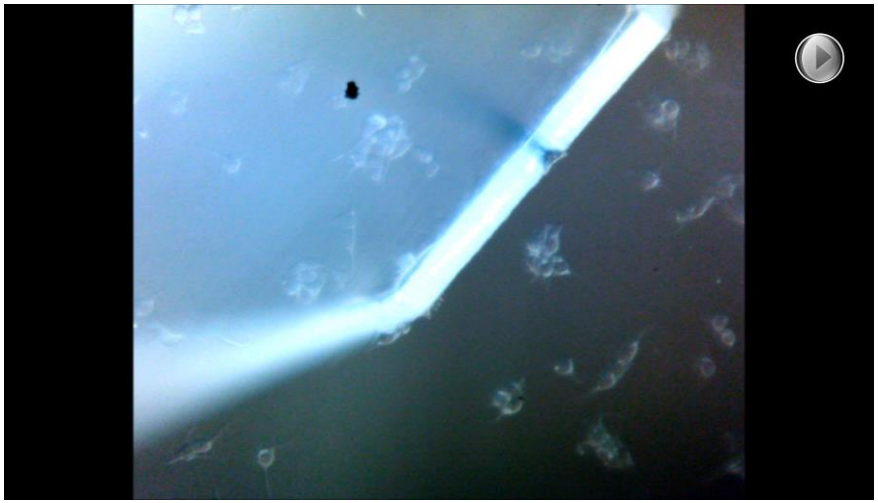
2D plot of all line scans of the SECM

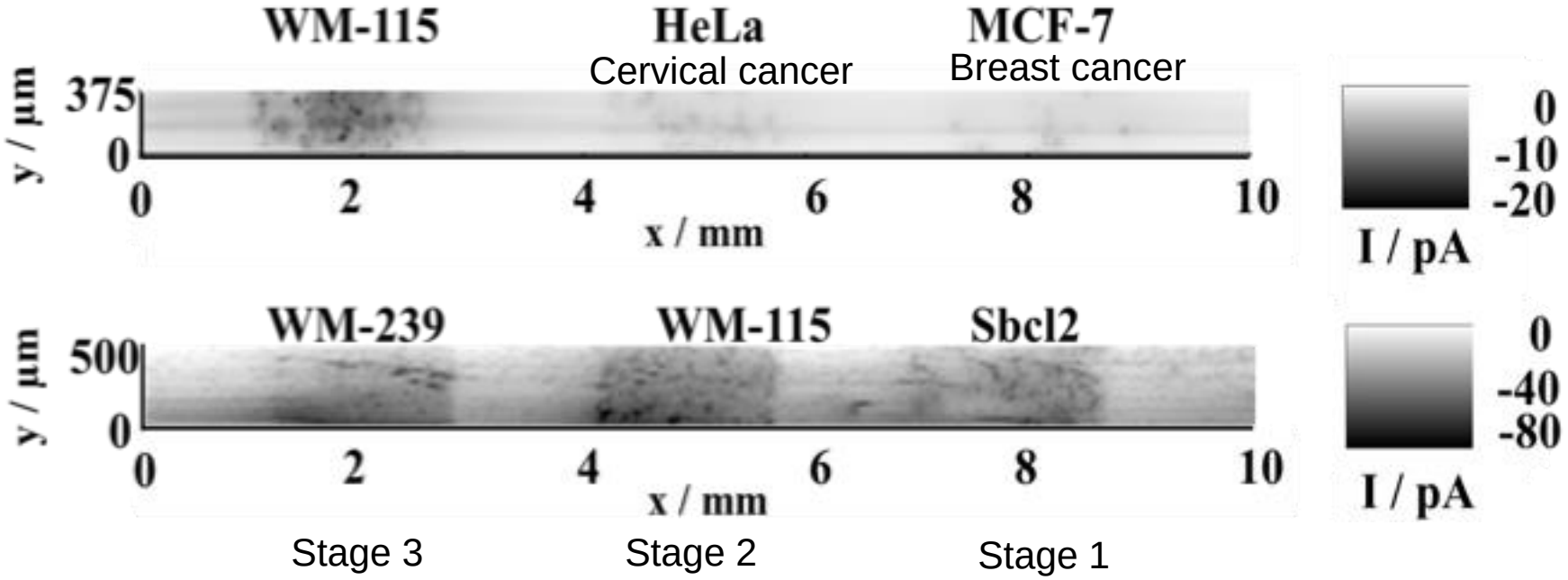
Average currents of normal skin and stage II and stage III melanomas. Nine current values were extracted and averaged from each tissue section

SECM can overcome the limitations of optical methods and opens new possibilities for improved diagnosis and understanding of the spatial distribution of TyR in different melanoma stages.

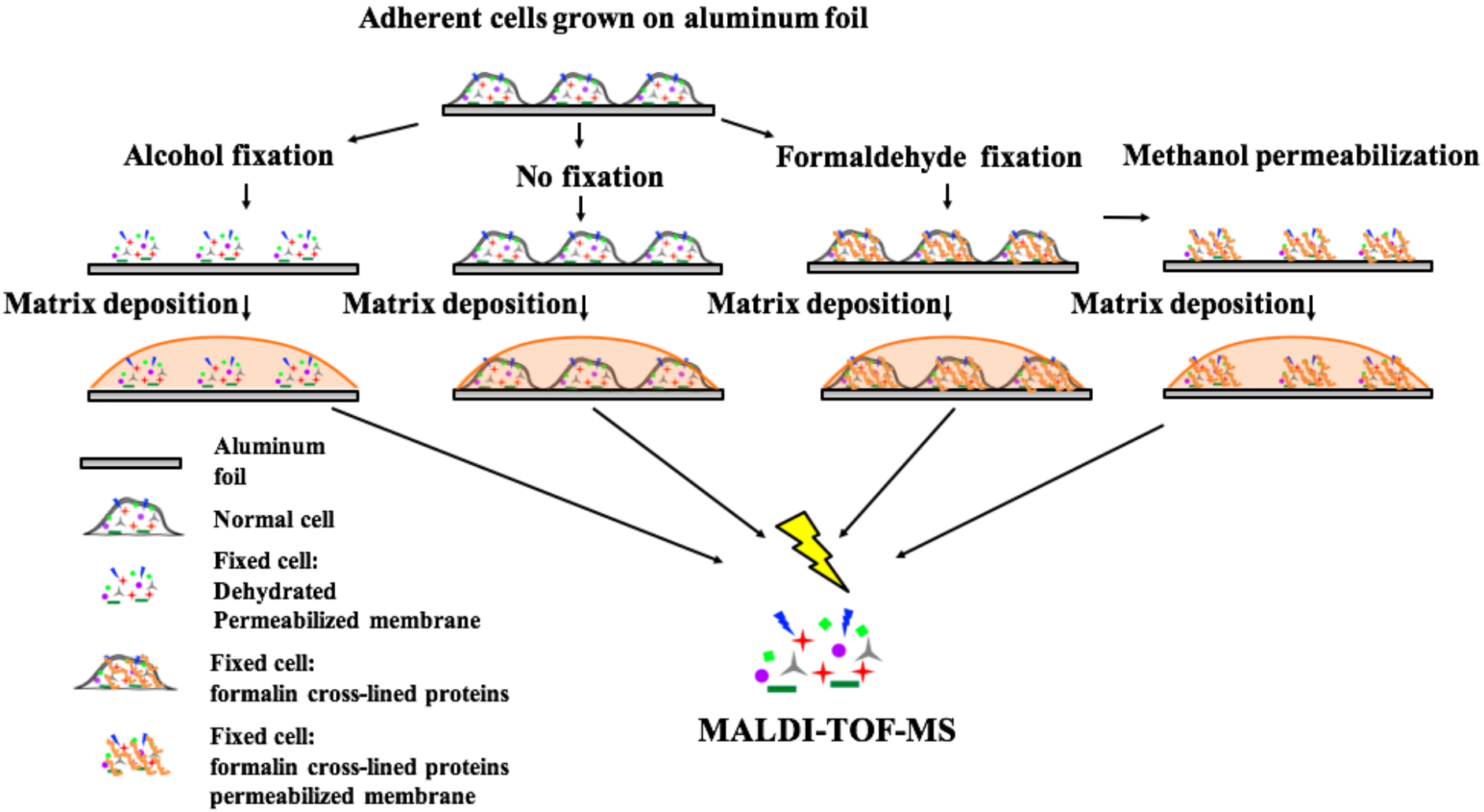
SECM for cancer diagnostic







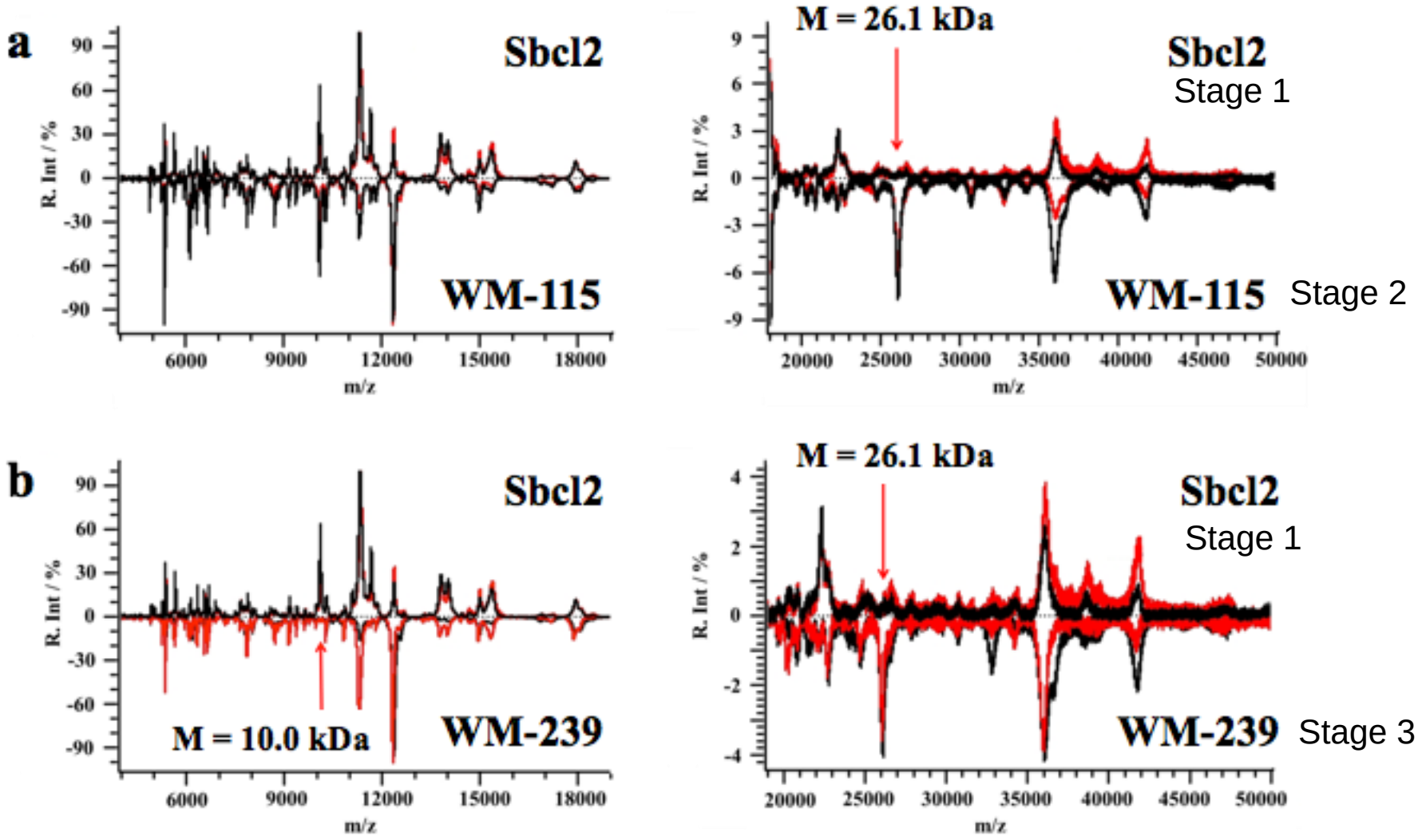
MALDI-MS of melanoma cells



Methanol – acetone fixation protocol; sinapic acid (SA) matrix

Analyst, 2016, DOI: 10.1039/C6AN00126B

MALDI-MS of melanoma cells



m/z = 10000
Distinguish WM-239

m/z = 26100
Distinguish Sbcl2

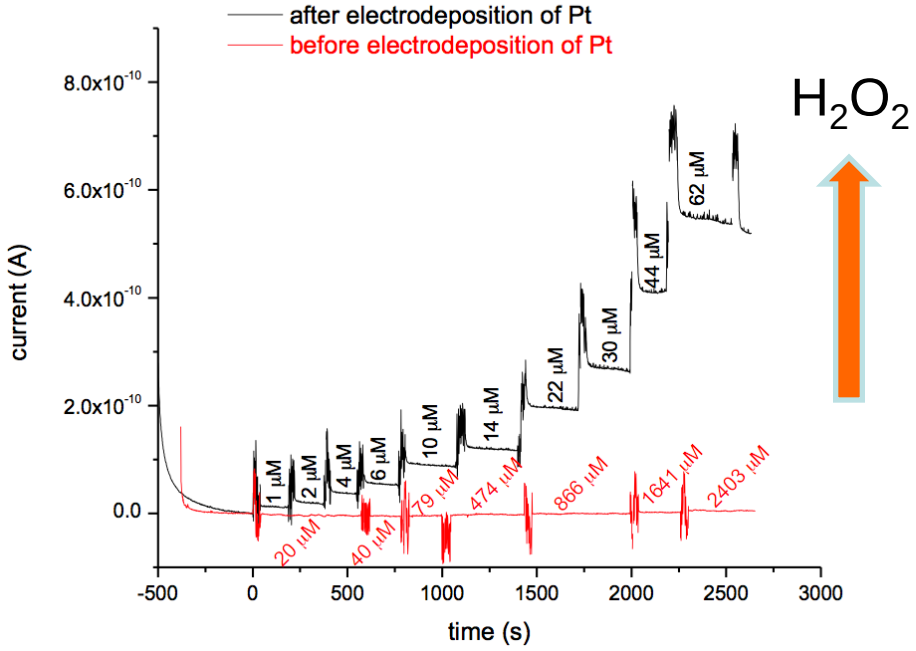
Analyst, 2016, DOI: 10.1039/C6AN00126B

Thanks to the Swiss-Romanian cooperation program, ICB moved towards characterisation of new CA IX inhibitors

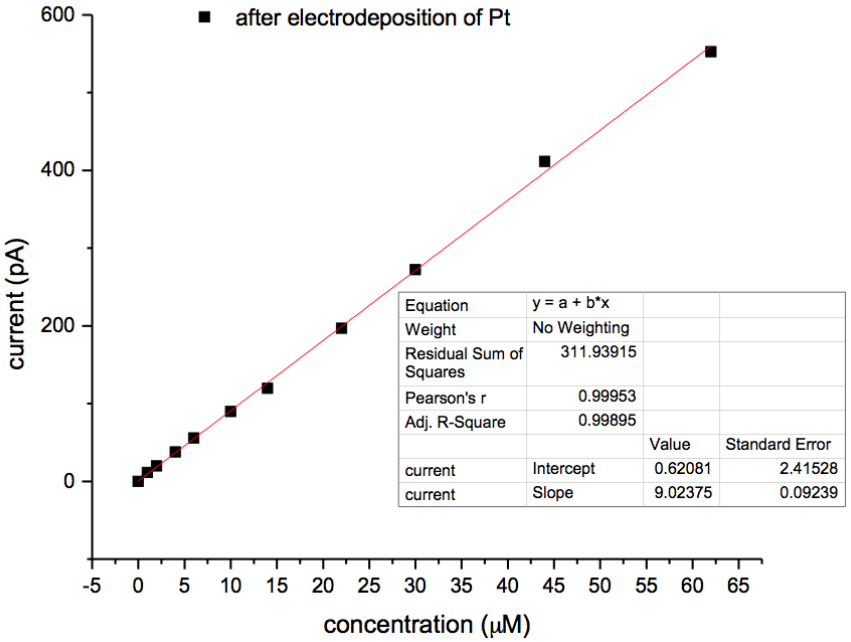
* Electrochemical push-pull probe for H_2O_2 detection for oxidative stress meaurements
* HT29 colon cancer cells in hypoxic conditions
* Testing new Carbonic Anhydrase (CA-IX) inhibitors

Electrochemical push-pull probe for H_2O_2 detection

Turning the microelectrode of the push-pull probe into a hydrogen peroxide sensor by its modification with Pt

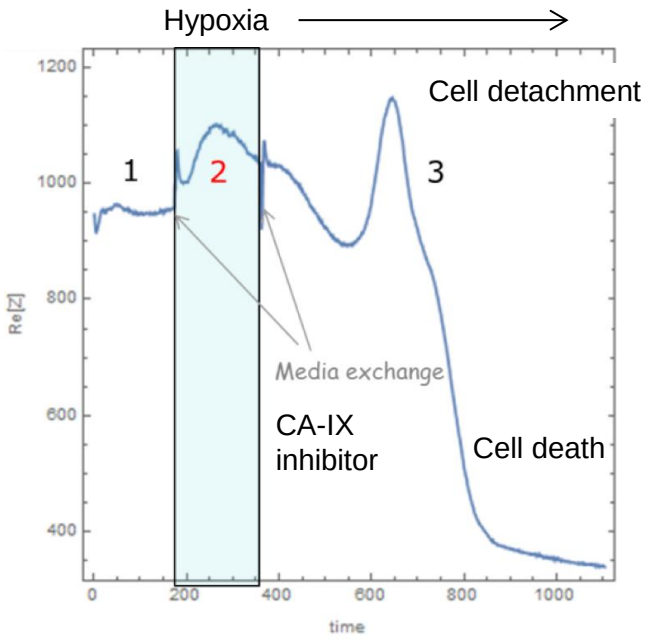


H_2O_2



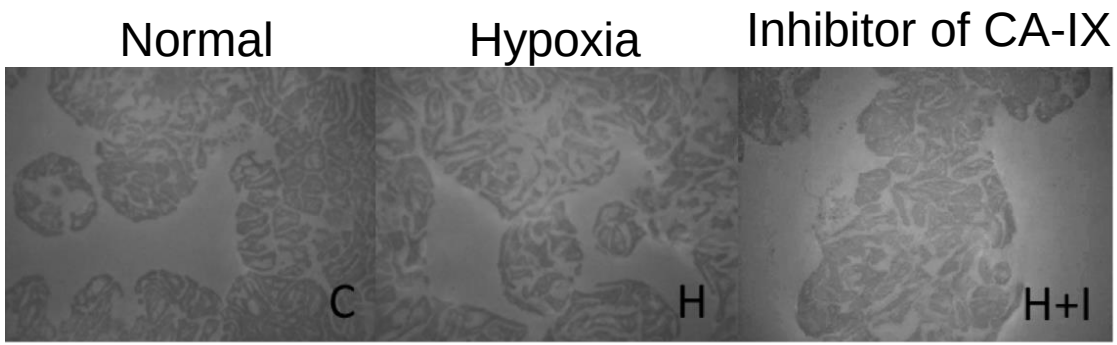
Current signals and **calibration curve** recorded with the microelectrode of the push-pull probe polarized to +750 mV when increasing the hydrogen peroxide concentration step-by-step

HT29 colon cancer in hypoxic conditions

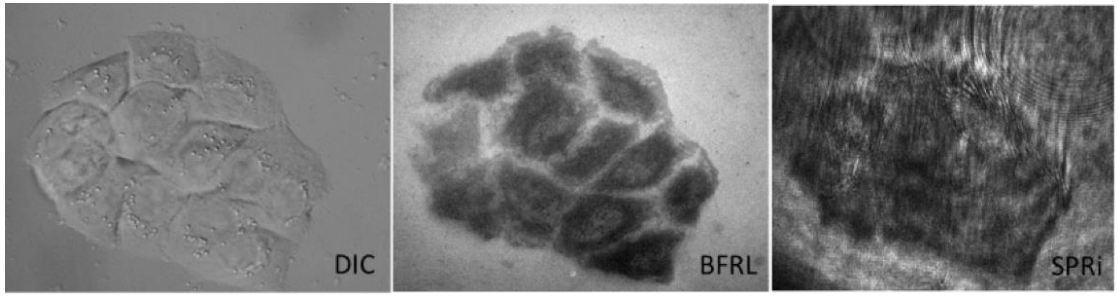


Evolution of the real part of impedance at 1 kHz for confluent cells under:

- normal conditions (1)
- hypoxia (2)
- exposure to CA-IX inhibitors (3)



Cell-surface contacts for control (C), hypoxic (H) and subjected to CAIX inhibitor (H+I) with enlarged and loosely attached cells under hypoxic conditions as revealed by fluorescence microscopy.

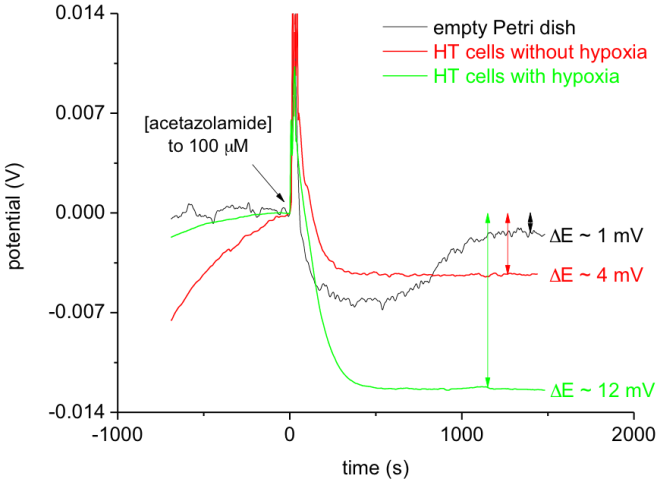


Various imaging formats (Differential interference contrast – DIC, Bright field reflected light – BFRL, Surface Plasmon Resonance imaging - SPRi), enabled within the system implemented in TUMORANALYZER project.

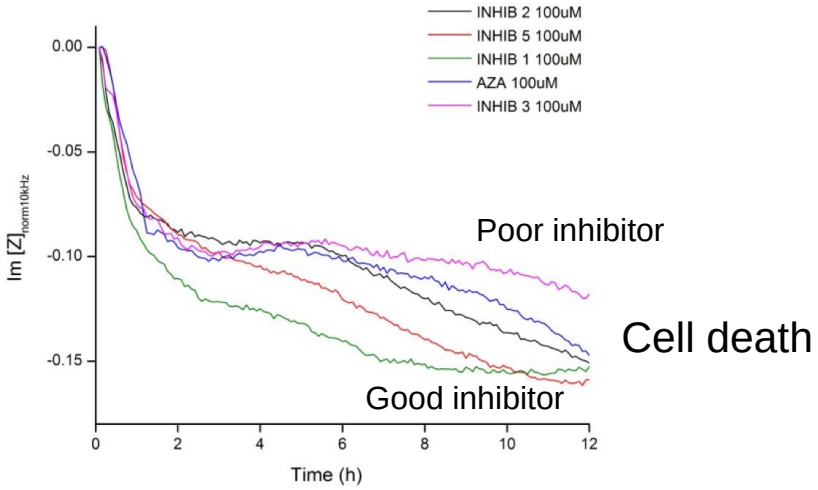
Testing new CA IX inhibitors

CA IX inhibitors:

- AZA - acetazolamide
- INHIB 1 - fluorescein-thioureido-homosulfanilamide
- INHIB 3 4-(2,4,6-trimethylpyridinium-N-methylcarboxamido)-benzenesulphonamide perchlorate
- INHIB 2, INHIB 5 - new compounds with confidential structure provided by Prof. Claudiu Supuran from University of Florence, Italy



pH



Cell death

Open circuit potential of polyaniline-modified microelectrodes before and after exposure of HT cells to a carbonic anhydrase inhibitor (i.e. acetazolamide). The inhibitor concentration in the extracellular space was increased to 100 μM.

Time evolutions of cell impedance (imaginary part) at 10 kHz for hypoxic cultures exposed to 100 μM of CA inhibitors: acetazolamide and 4 new inhibitors developed by the group of Claudiu Supuran

Our dream

Can we develop a probe to assist surgeons to image tissues at the micron resolution and kill target cells using microfluidics and microelectrodes?

Microfinger probe array for
scanning melanoma tumors

- made by LEPA-EPFL -

lepa.epfl.ch

Acknowledgements

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SWISS-ROMANIAN COOPERATION PROGRAMME

SWISS NATIONAL SCIENCE FOUNDATION

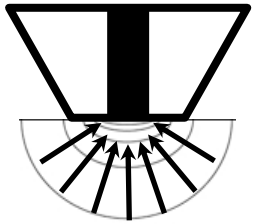
Timothy Ryan, Monica Cruceru

Thank you all for your attention!

Additional slides/ Drafts

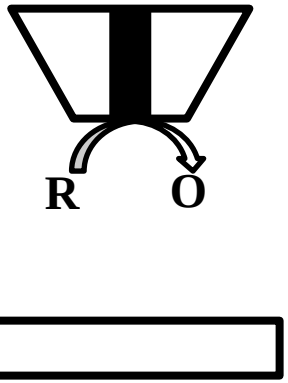
Scanning Electrochemical Microscopy

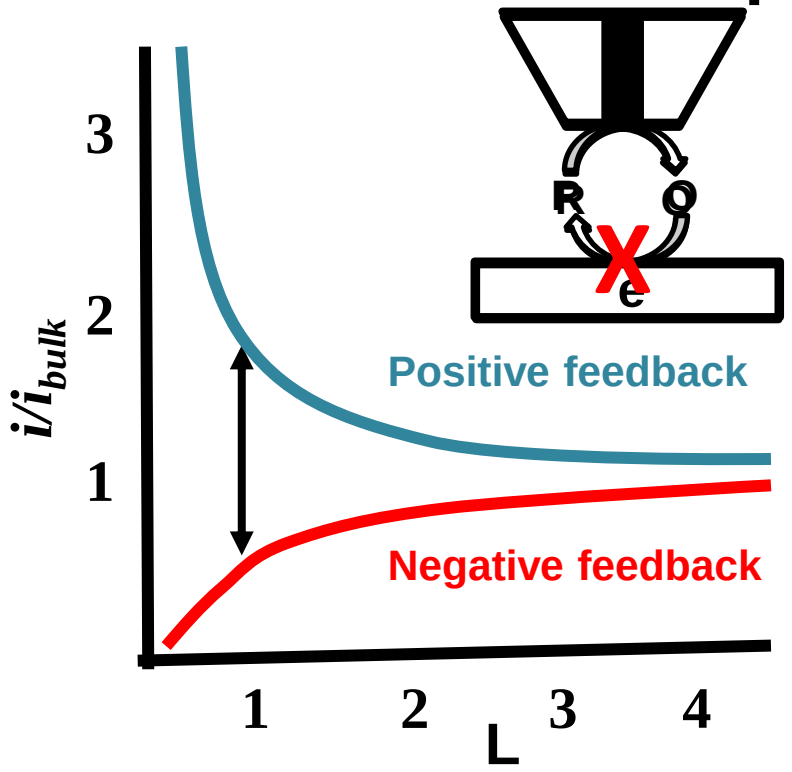
Microelectrode



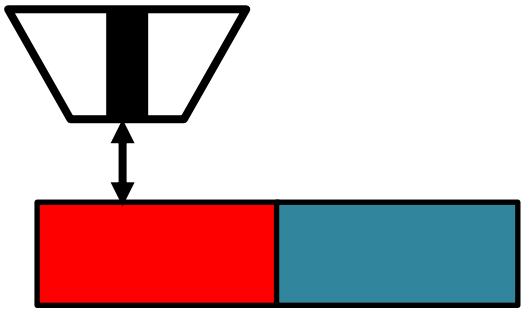
$$i_{bulk} = 4nFDaC$$

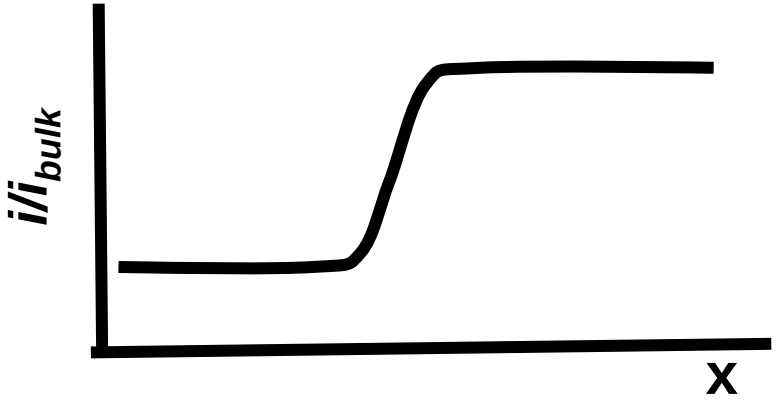
Approach curve





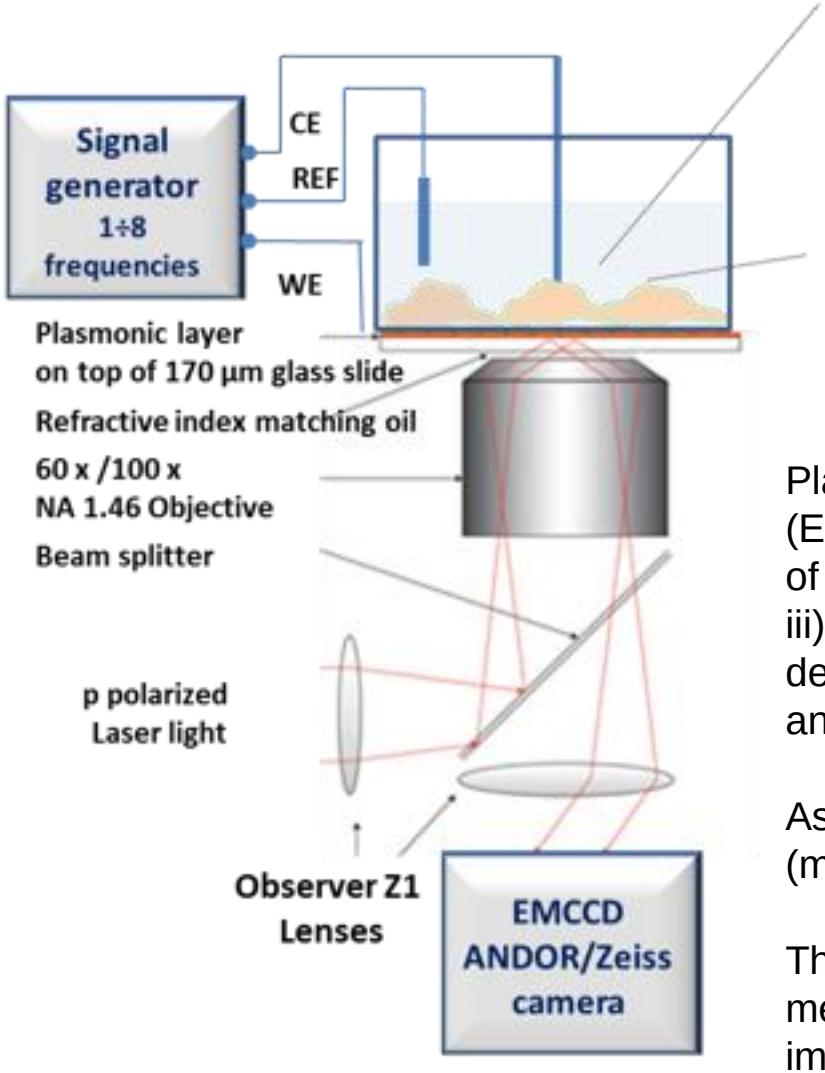
Line scan

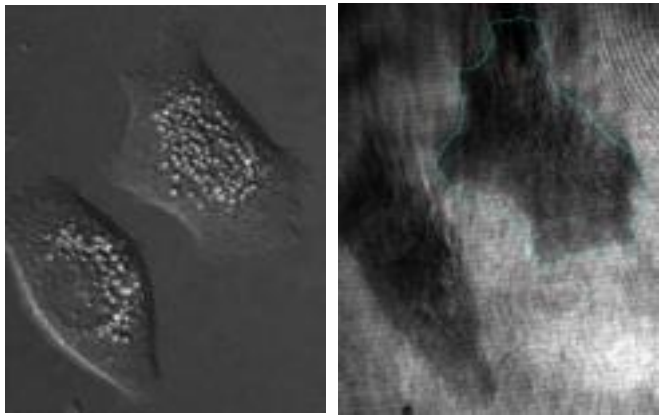




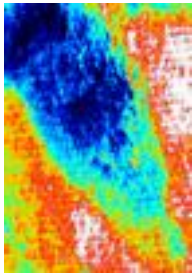
- **Working distance**
- **Surface properties**

Plasmonic-based electrochemical impedance cell imaging





Cell- interface &



Intracellular

Plasmonic-based Electrochemical Impedance Microscopy (EIM) involves: i) selection of an appropriate incidence angle of the ***p*** polarized laser light, ii) application of an AC signal iii) fast acquisition of the related reflectance signal and iv) deriving spatial impedance amplitude and phase maps by analyzing each pixel in a photodetector array.

As such, cell impedance is measured using SPR imaging (microscopy) and not electrically.

This combination between an electrical and an optical method is characterized by excellent spatial resolution of impedance data at cell interface, achieved simultaneous with classical optical information.