Sensorsysteme für die Charakterisierung und das Monitoring von biologischen Zellen und Geweben in der medizinischen Biotechnologie und klinischen Diagnostik

10th LEIBNIZ CONFERENCE OF ADVANCED SCIENCE
- SENSORSYSTEME 2010 -
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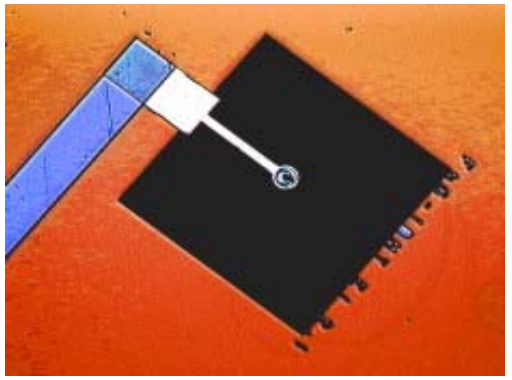

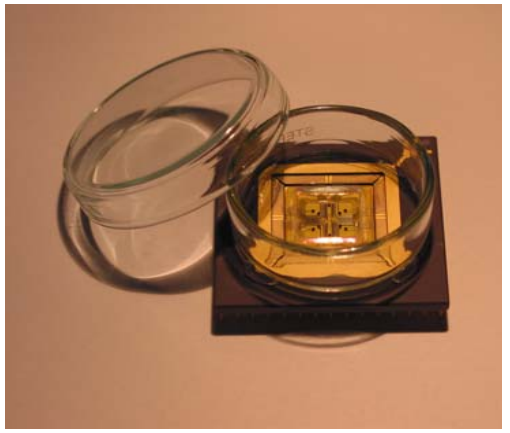

Fraunhofer IBMT – Locations in Germany

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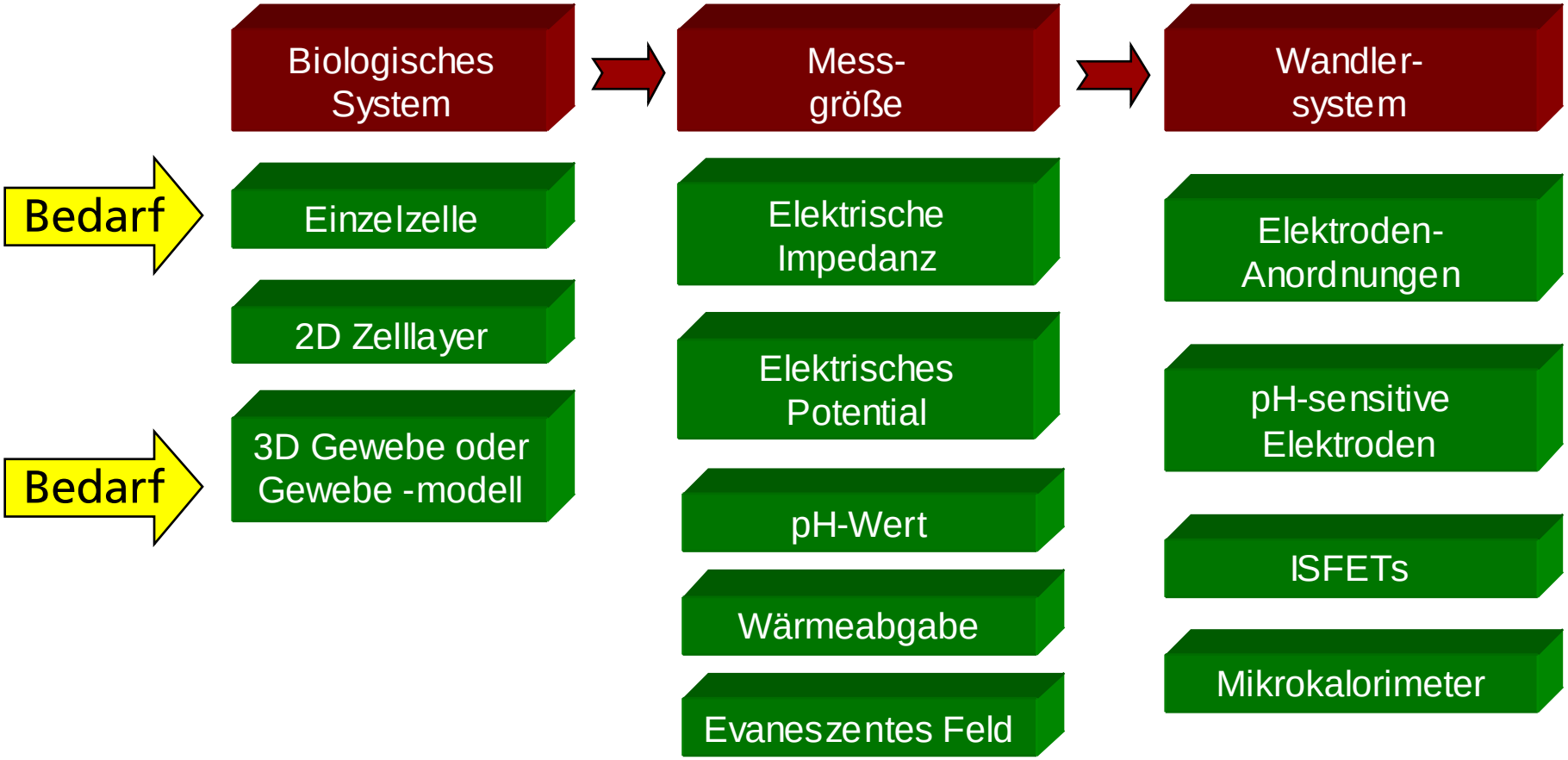
Overview

- Introduction
- Label free measurement methods for cell-based sensors and test systems
- Impedance spectroscopy for cell- and tissue-based biosensors
    - 2D,
    - 3D,
    - single cell
- Examples for applications of cell-based biosensors and test systems
    - Regenerative medicine / cell therapy
    - Drug discovery / development
    - Gene therapy
    - Engineered nanoparticles

Non destructive characterization of cells

- Cell characterization is needed for
  - cell-based test systems (drug development, toxicology)
  - cellular and tissue engineering (quality control)
  - Safety (monitoring of environment, work place environment)
- **Problem:** Currently used techniques characterize the cells indirectly *(label techniques)*
  - Influencing of cell physiology (no long-term tests)
  - High effort for characterization of 3D *in vitro* models and tissues
  - Characterization of single cells is difficult
  - Sensor application not possible with invasive labels
- Micro and nanotechnologies offer new possibilities for label free test techniques & cell-based biosensors

Cell-based sensors and label free measurement methods



Cellparameter and electrical properties

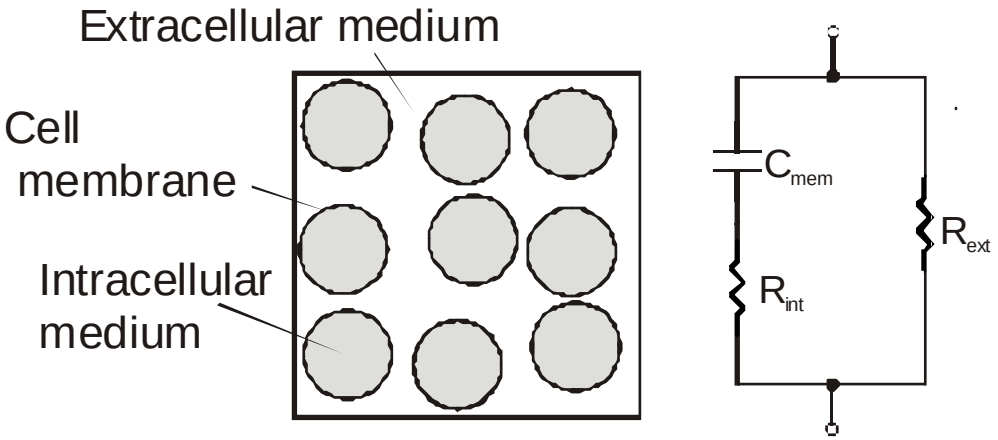
Mechanisms responsible for electrical properties of biological matter

dispersion/ frequency range	responsible for dispersion
α: α_1,α_m,α_t up to 1 kHz	counterions, glycocalyx, membrane ion channel, tubular system
β: β,β_1,β_2 1 kHz to 100 MHz	membrane, organelles, proteins, (amino aces)
γ: γ, Δ 10 MHz to 100 GHz	water, bound water, polar subgroups

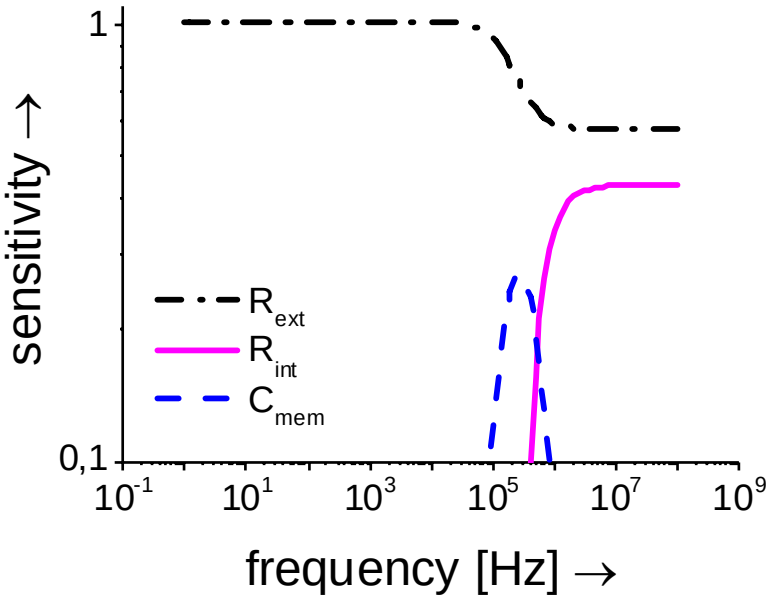
- **Correlation between biological effects and electrical properties**
- **Physiological properties can be determined by**:
  - Recording of electrical impedance over a frequency range (impedance spectroscopy)

Sensitivity analysis for a small tissue sample

Simple tissue model (for β-Dispersion)

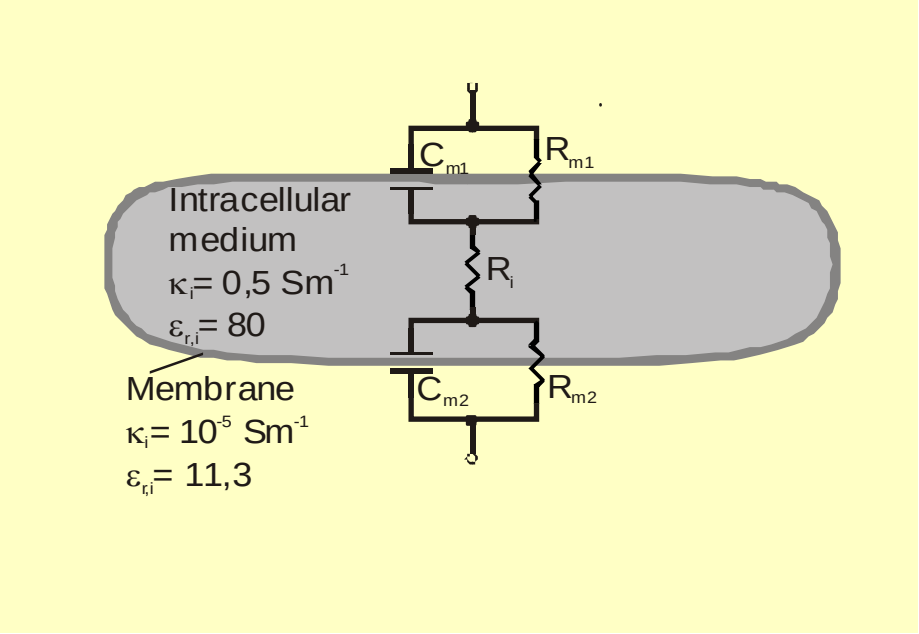


Simulated sensitivities of impedance to R_{ext}, R_{int} und C_{mem}

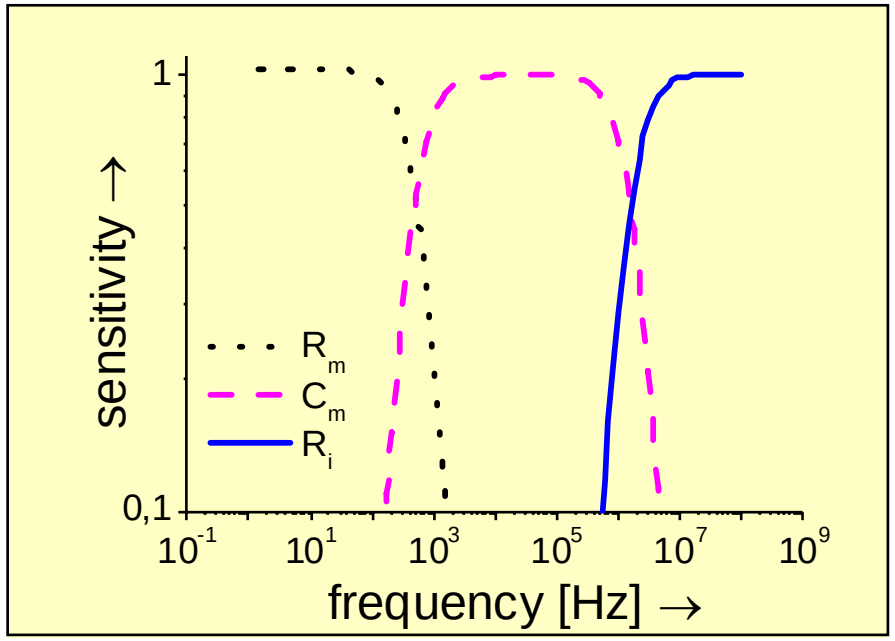


Sensitivity analysis for a single cell

Simple model of a cell (for β-dispersion)



Simulated sensitivities of impedance to R_m, R_i und C_m



Characterization of cell layer

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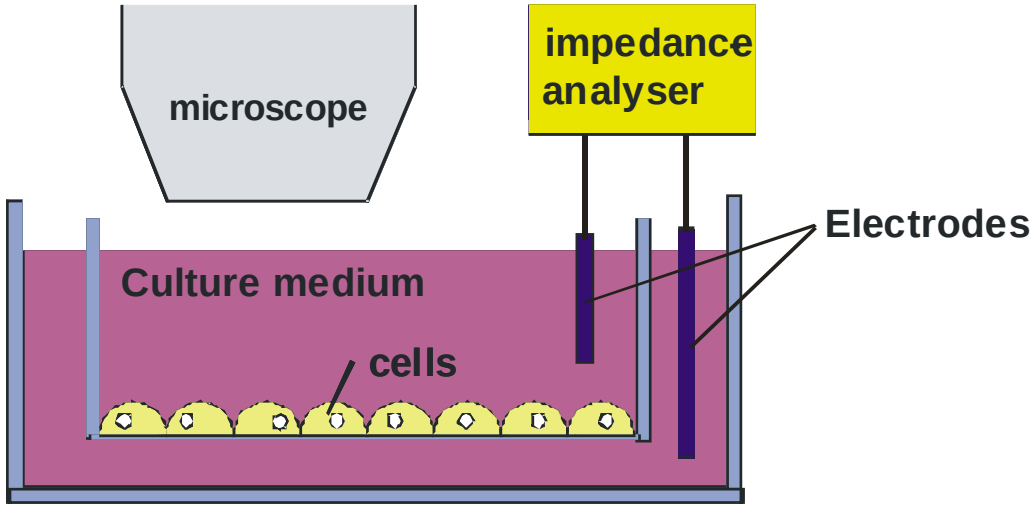
Characterization of 2D cell layer

Trans Epithelial Electric Resistance (TEER) Measurements

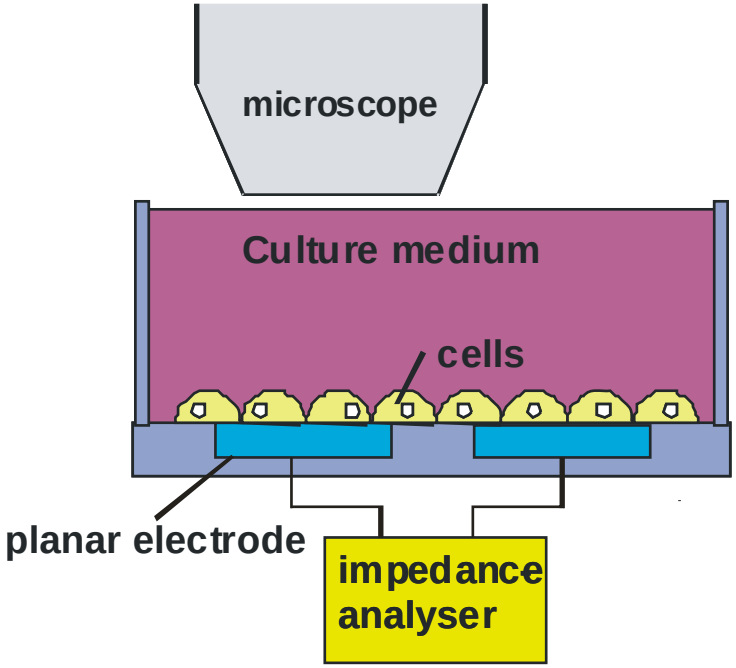
Measurement principle



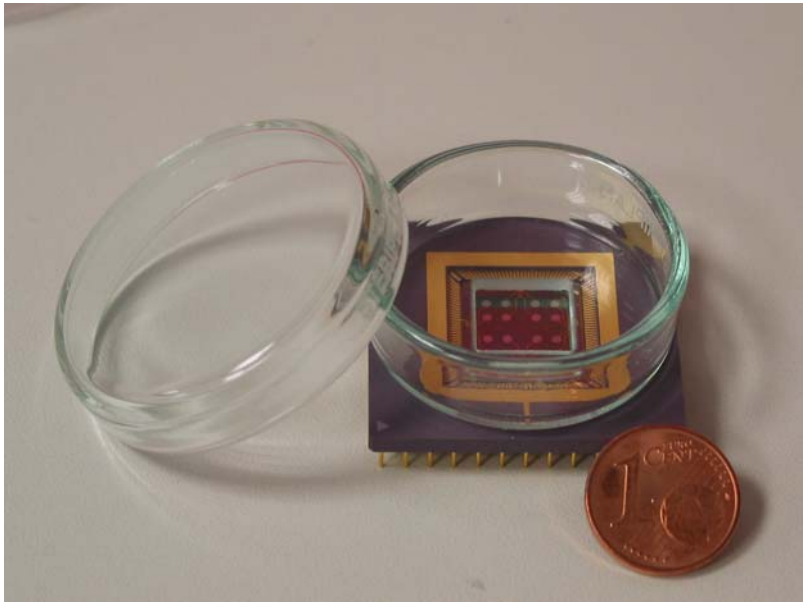
Quelle: http://www.pharmaceutical-int.com/

Characterization of 2D cell layer II

Measurement principle

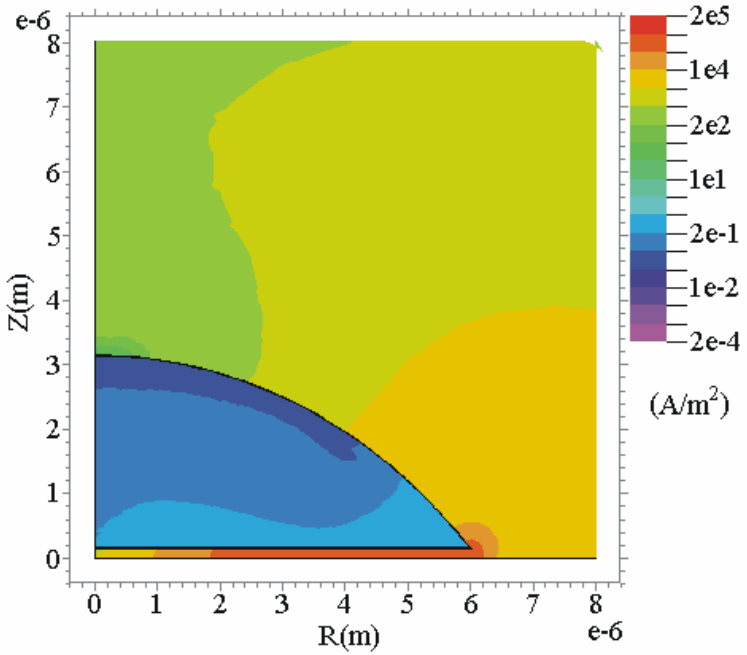


Chip unit

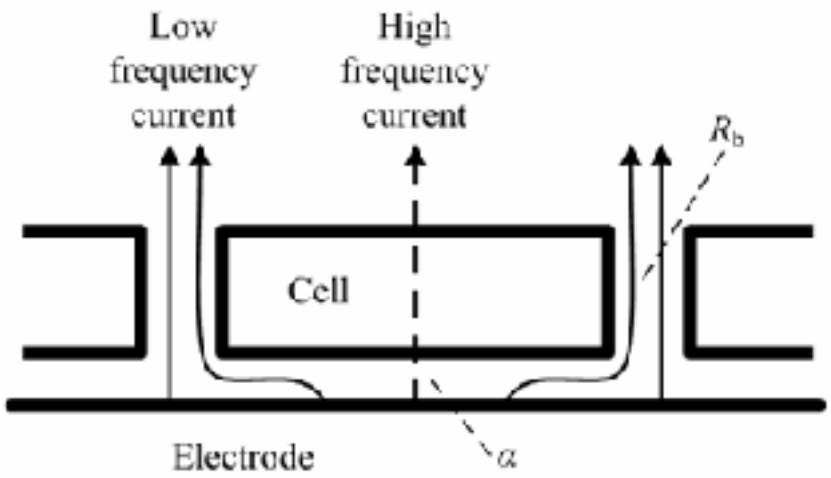


Electrical field distribution and current paths for an adherent cell layer

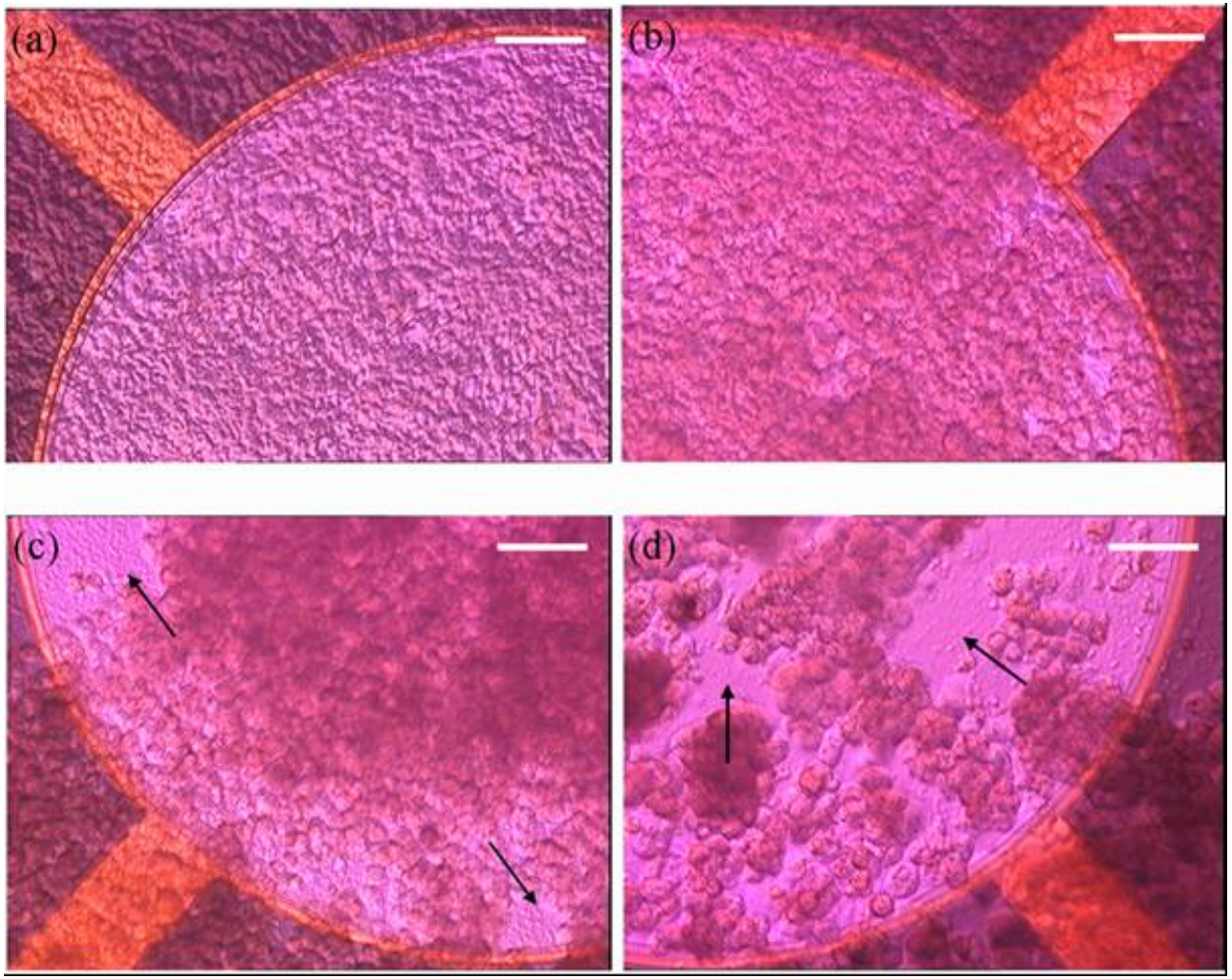
Field distribution



Current paths

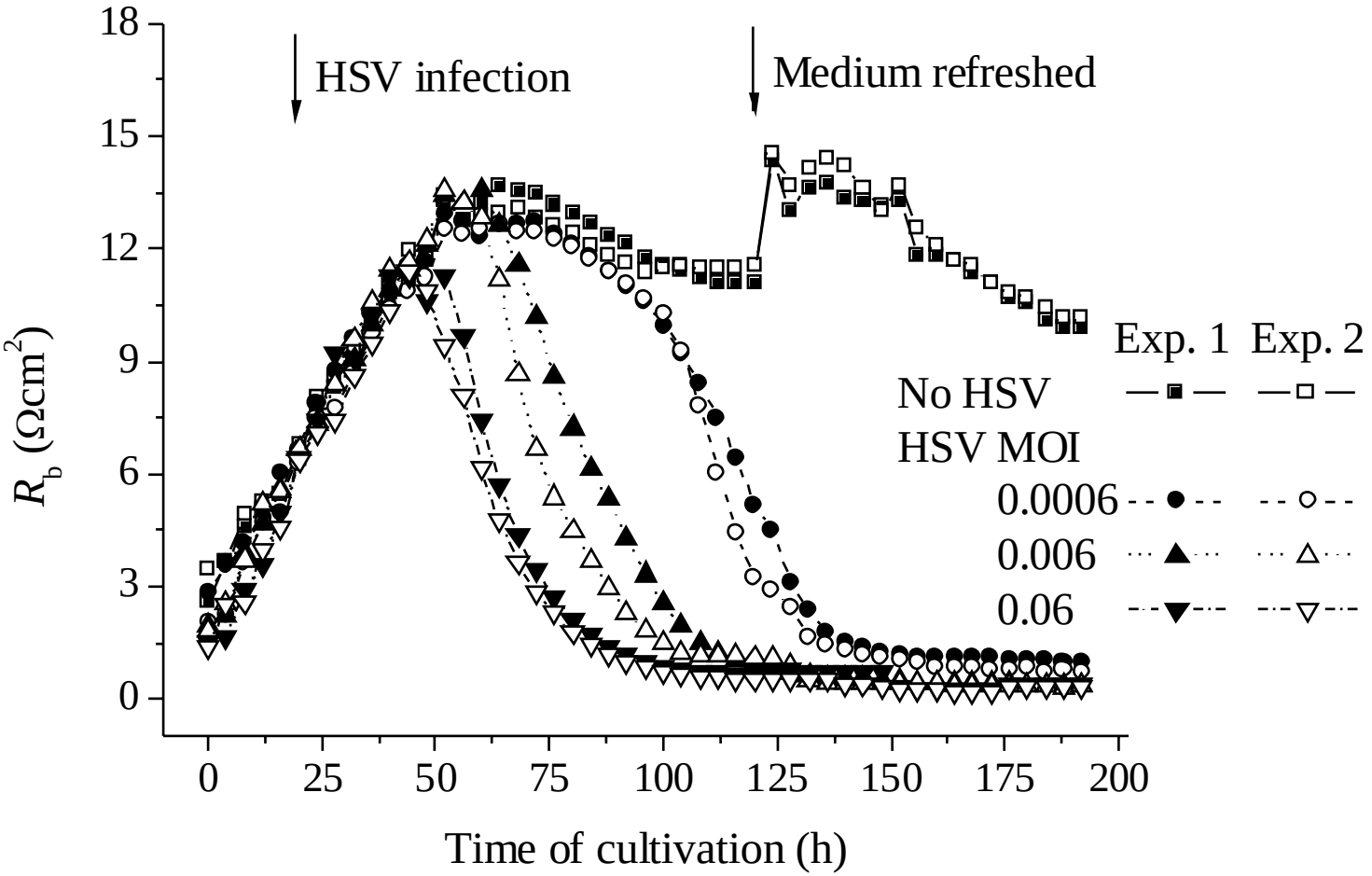


Vero cells infected with HSV virus after 102 h

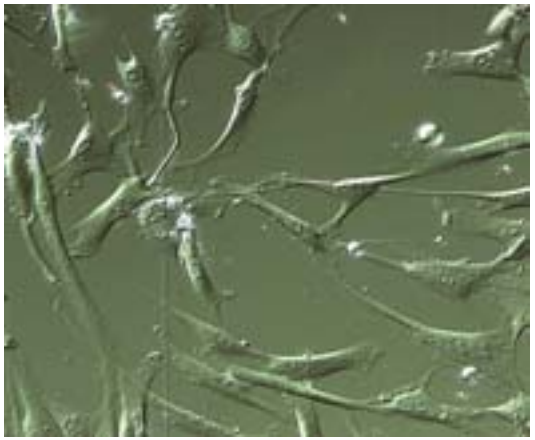


MOI
(a) Control
(b) 0.0006
(c) 0.006
(d) 0.06

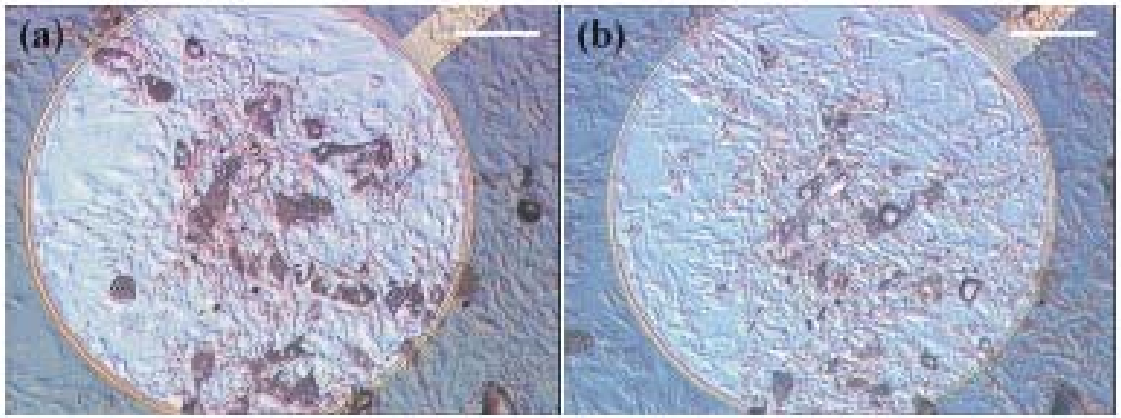
Results of impedance measurement on infected cells

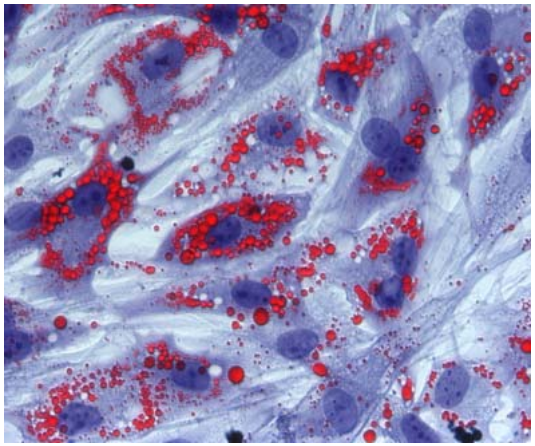


Adipogenic differentiation of hMSCs

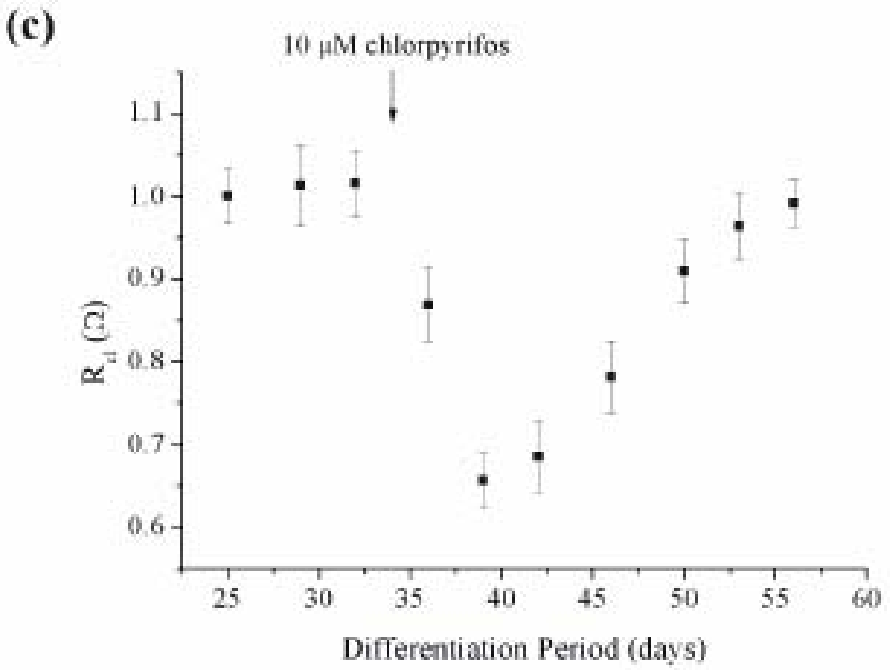


MSC



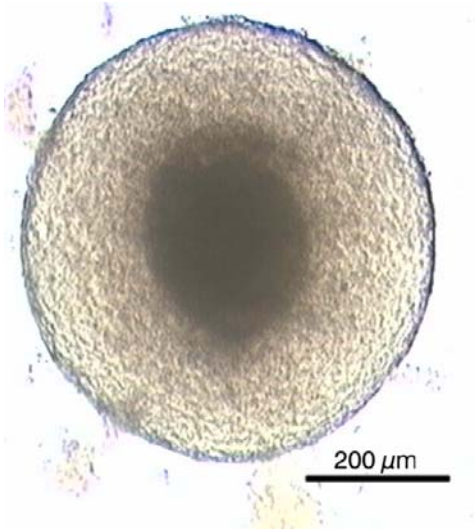


Preadiopocytes (oil red)

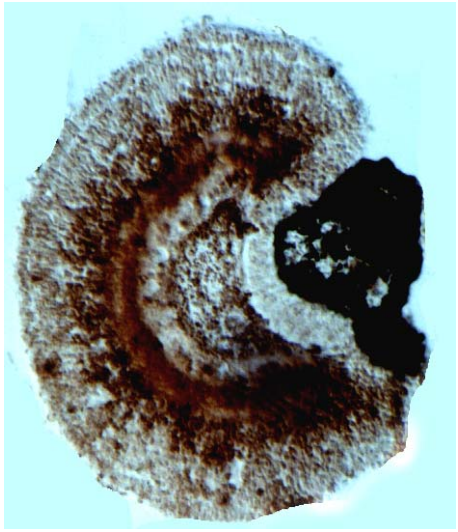


Characterization of 3D cell aggregates

3D in vitro-tissue models



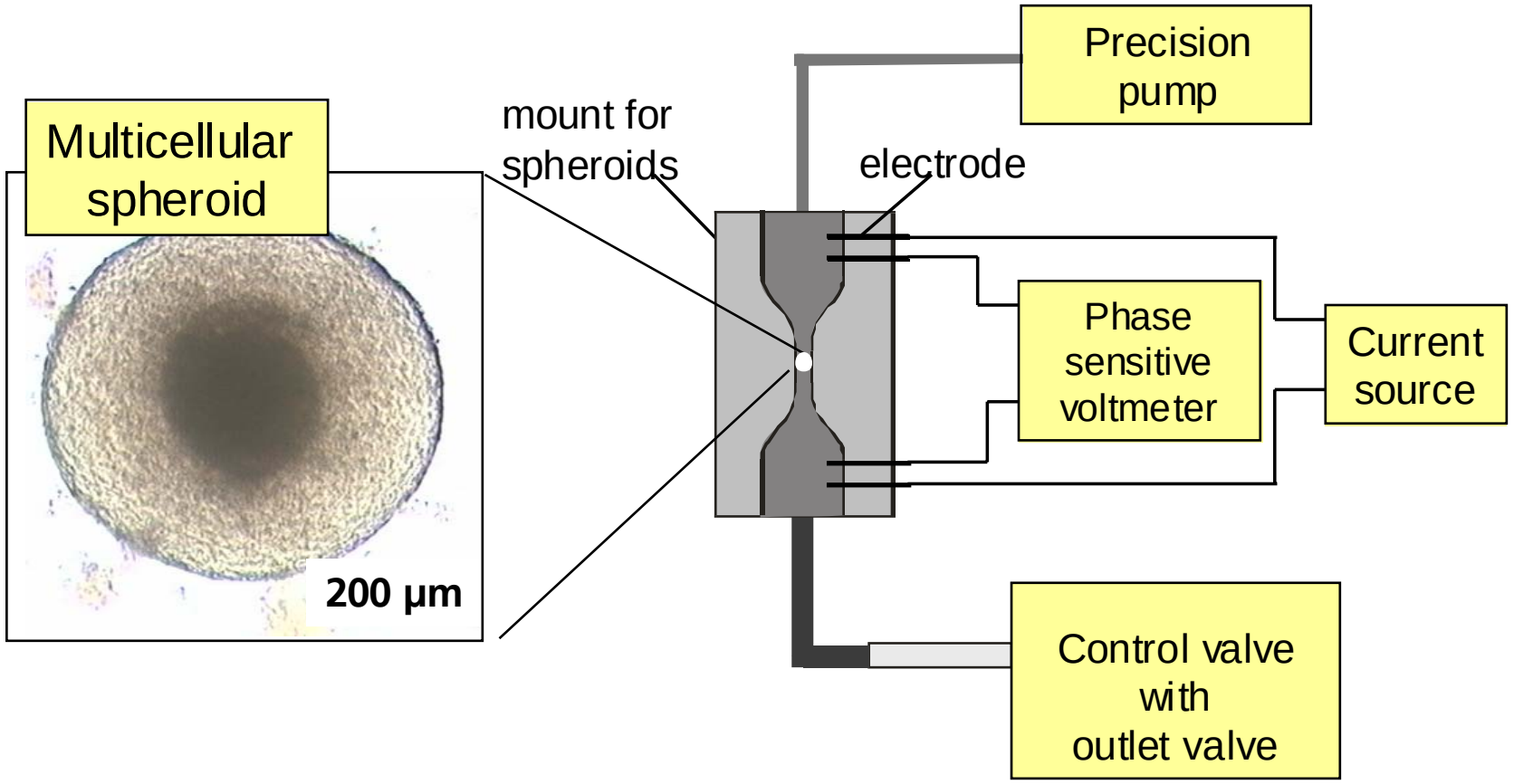
T47D-tumor spheroid



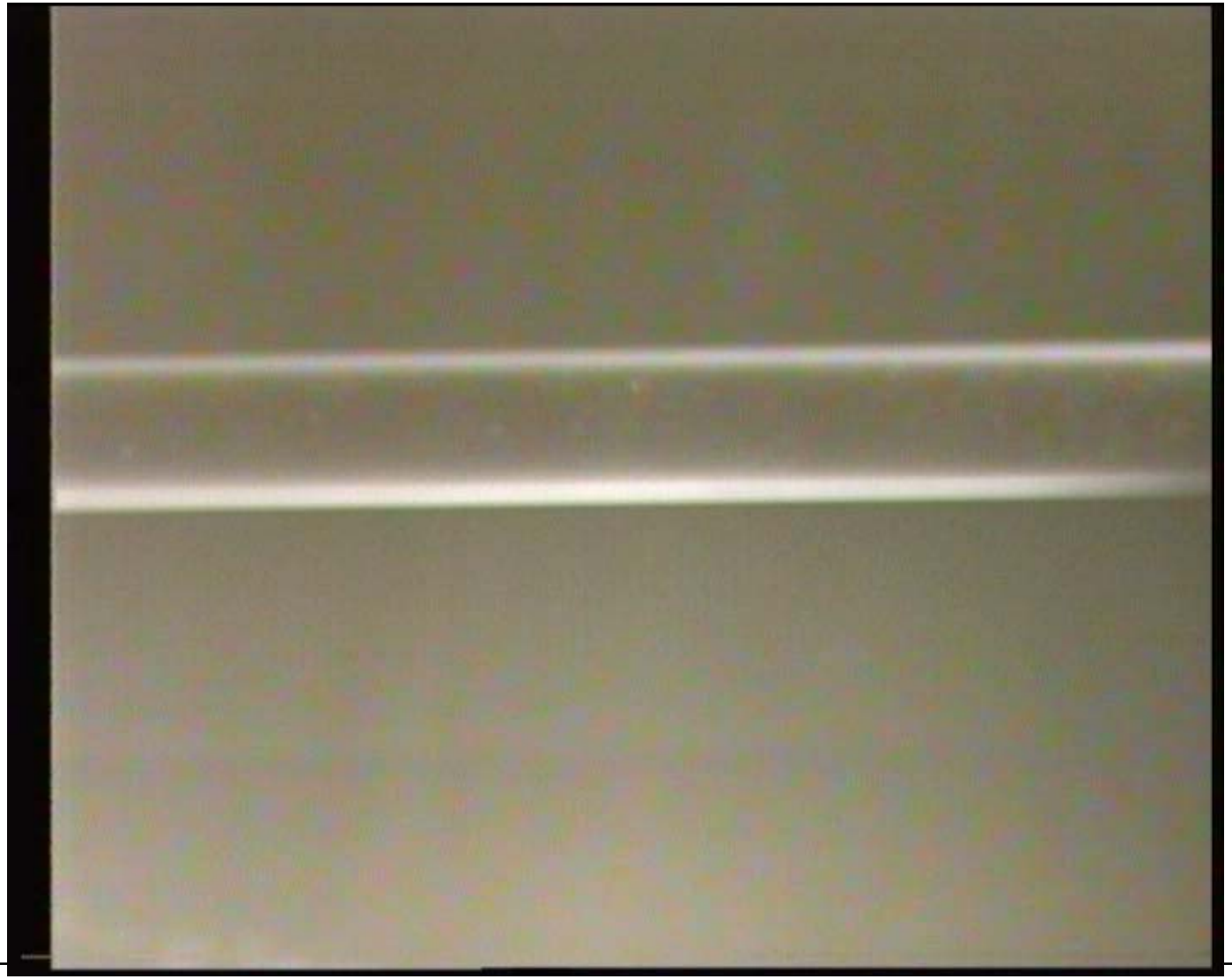
in vitro-retina (Stratospheroid)

Heart muscle spheroid

Monitoring system for small tissue samples

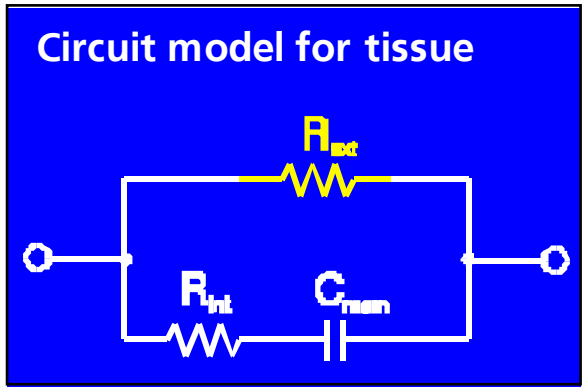


Positioning and removal of a multicellular spheroid

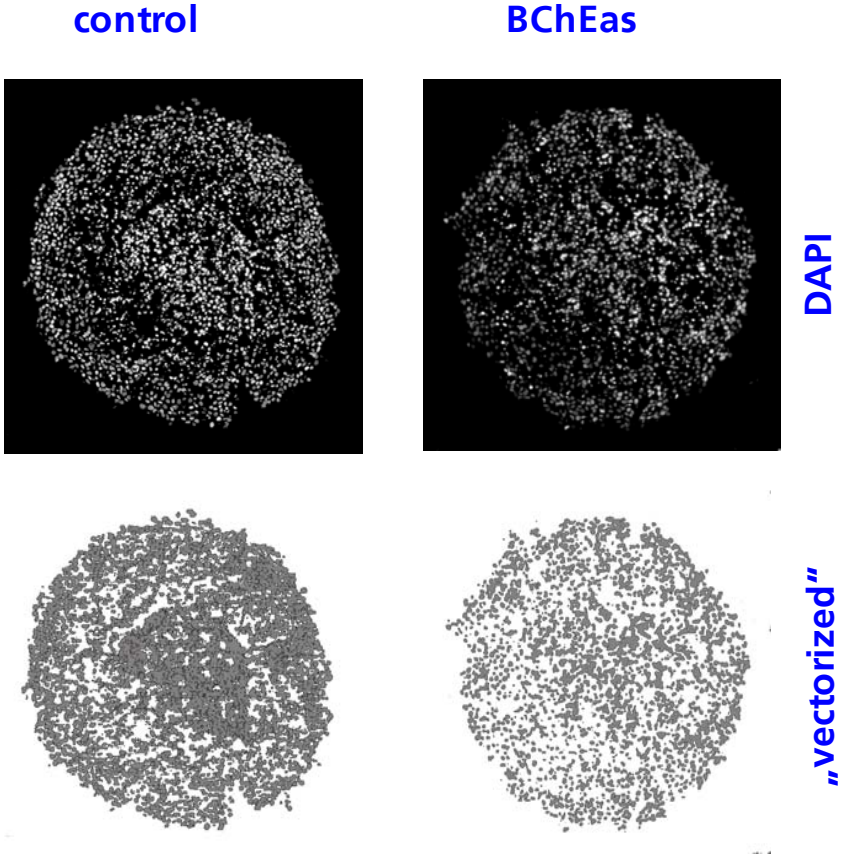


Example: Anti Cancer Therapy

Aim of cancer therapeutics	*Effect on R_{ext}*
stop of proliferation	↓ decrease
induction of apoptosis	↓ decrease

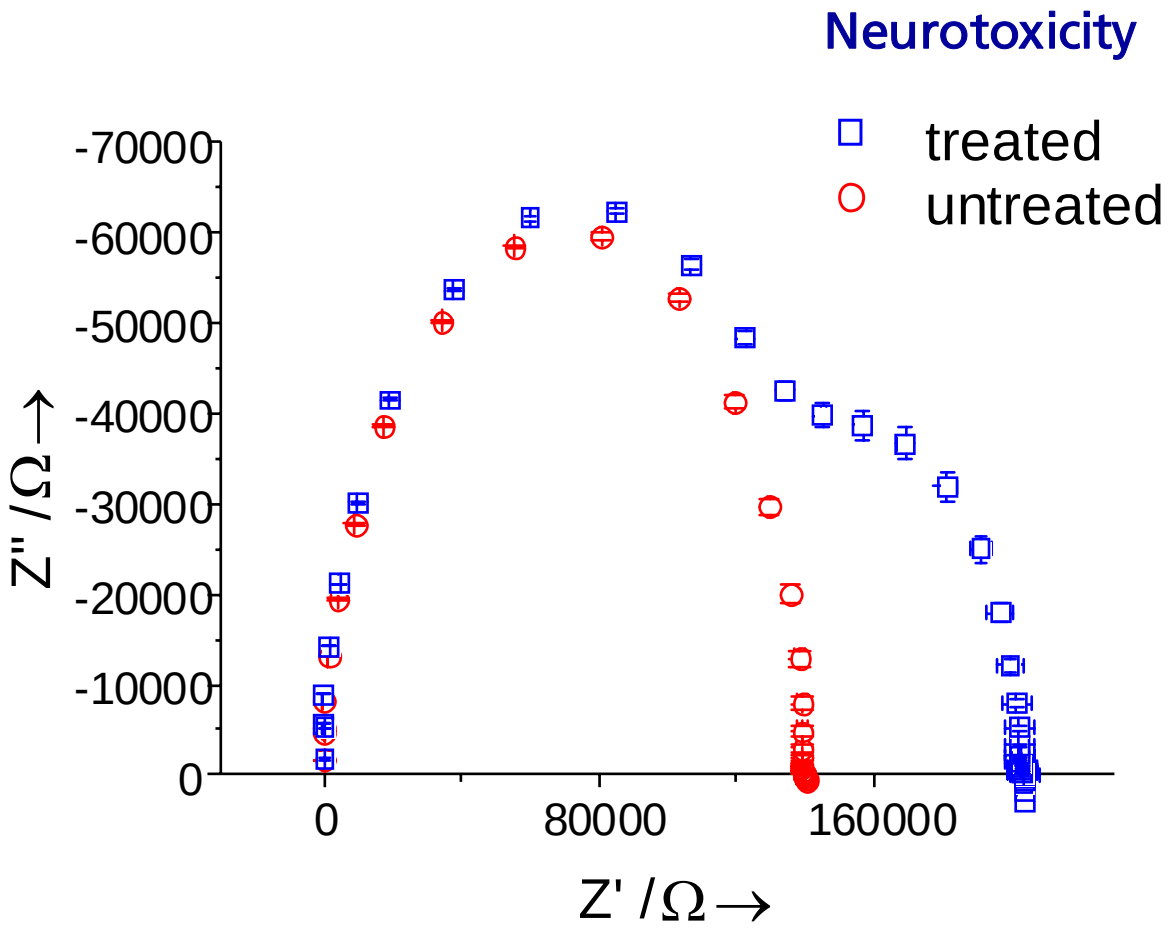


Cryosections of spheroids



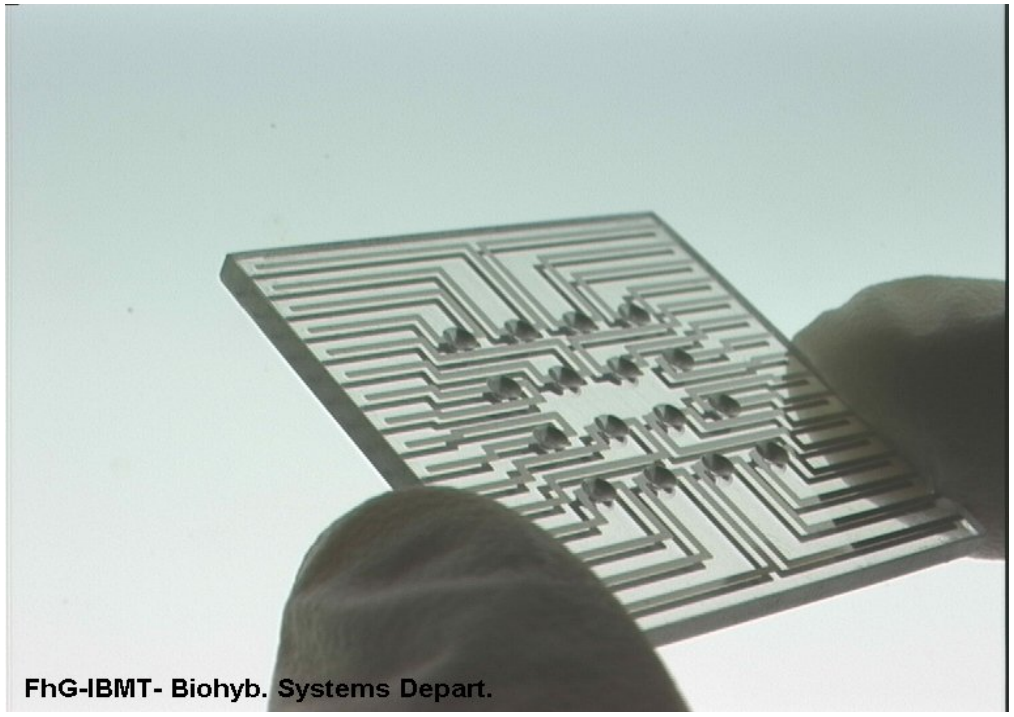
Thielecke *et. al.*:.
Biosens. Bioelectron. 16, 261-269 (2001).

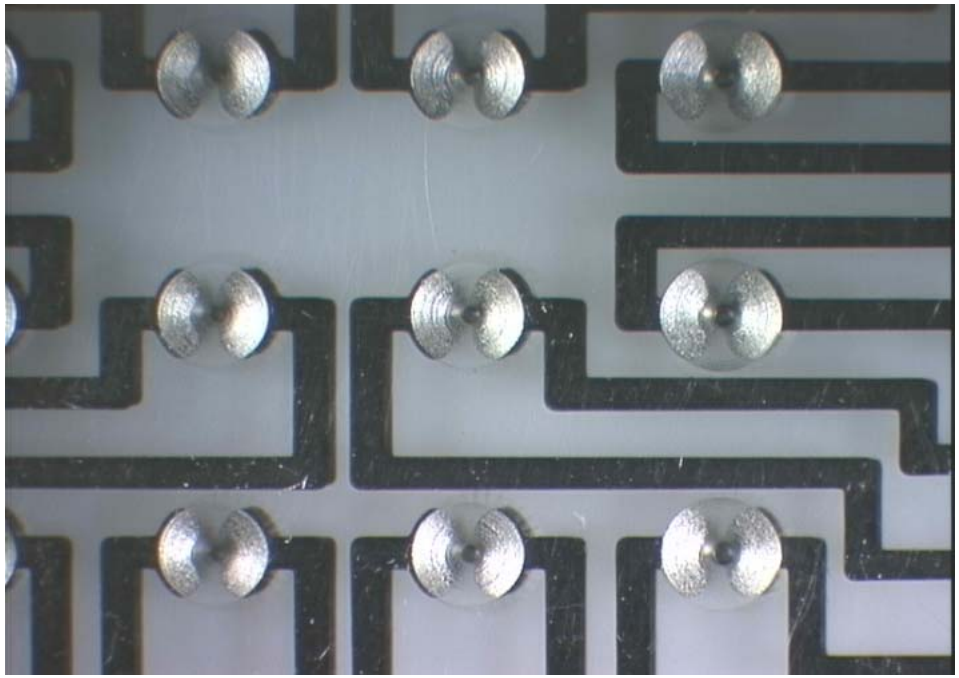
Further example: Homogeneity of cell aggregates



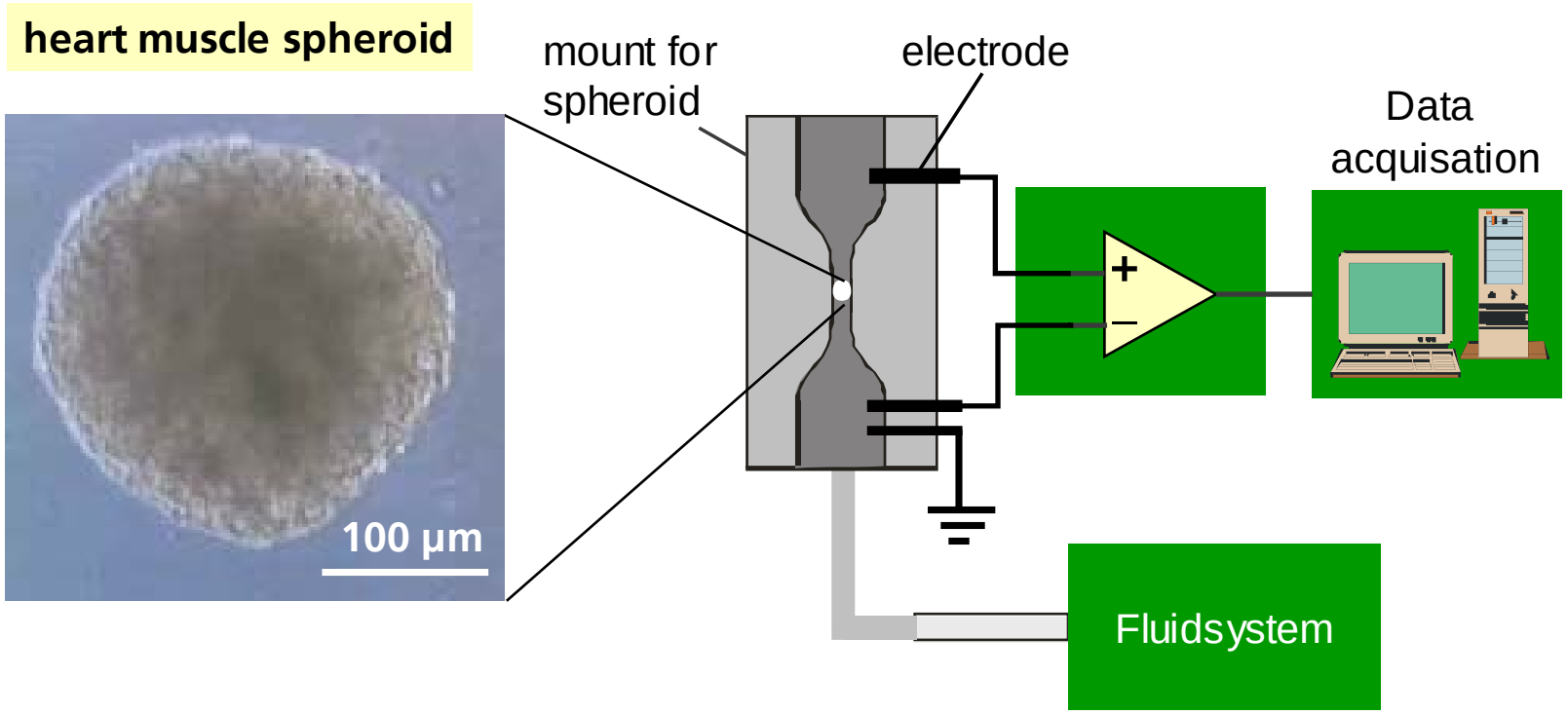
Animation capillary chip

3D capillary

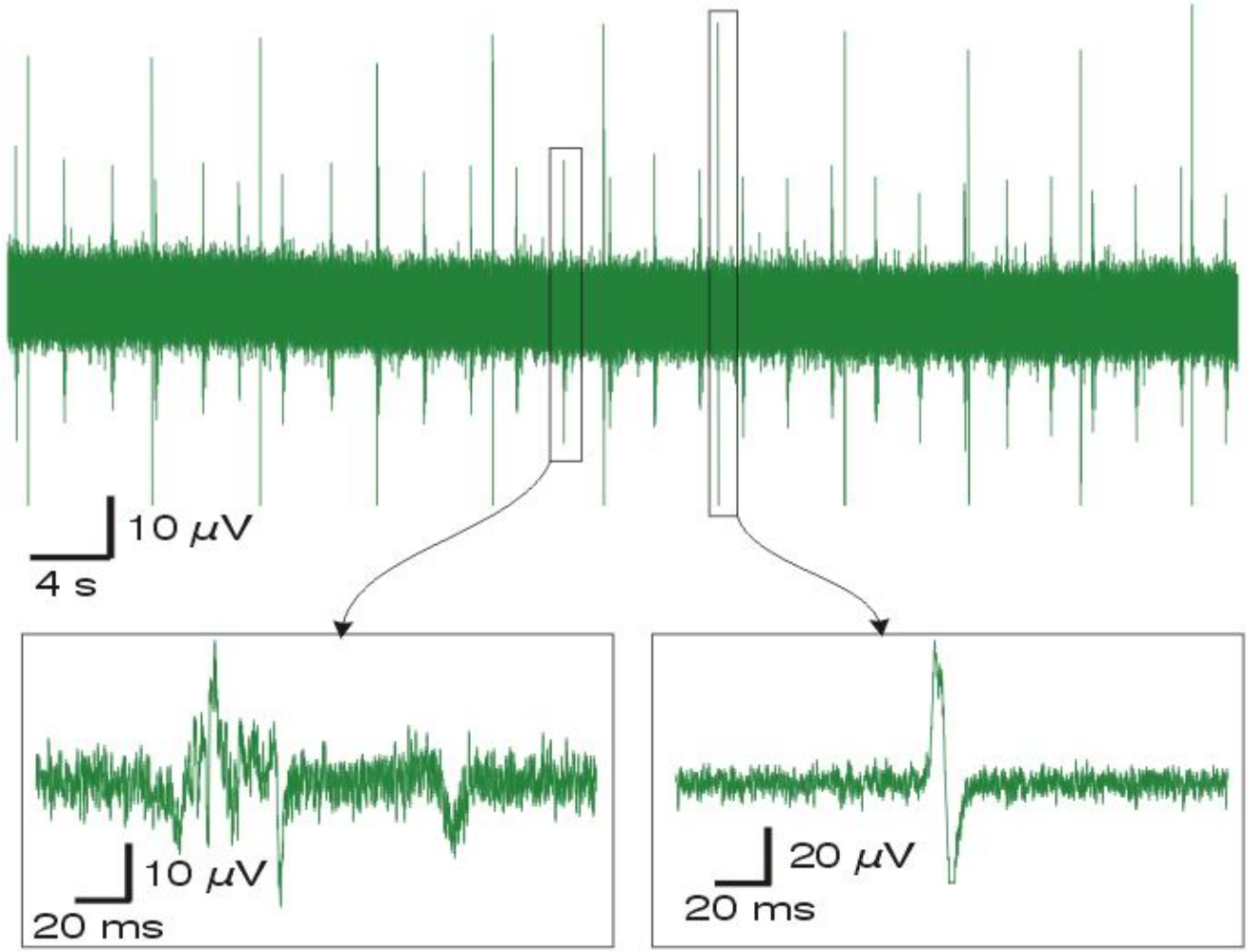




Recording system for small tissue samples



Recording



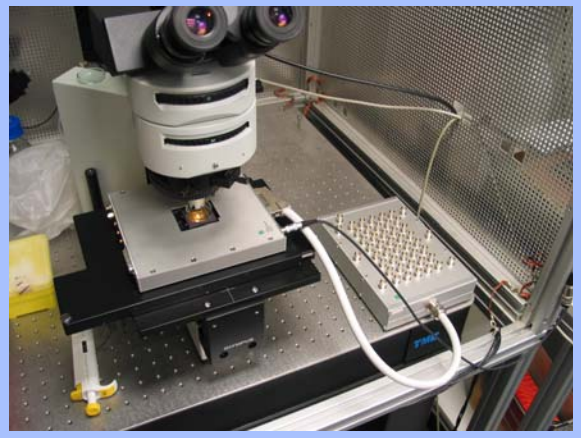
in vitro Test Platform for Cardiovascular Diseases (IBMT)

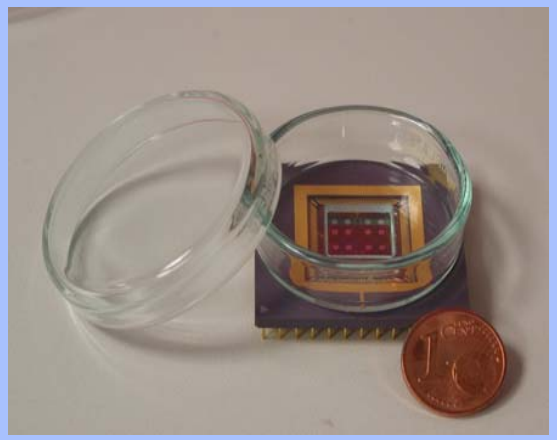
in vitro models

- Cardiomyocytes
- Smooth muscle cells
- Vascular endothel cells
- Stem cell cocultures

Technology

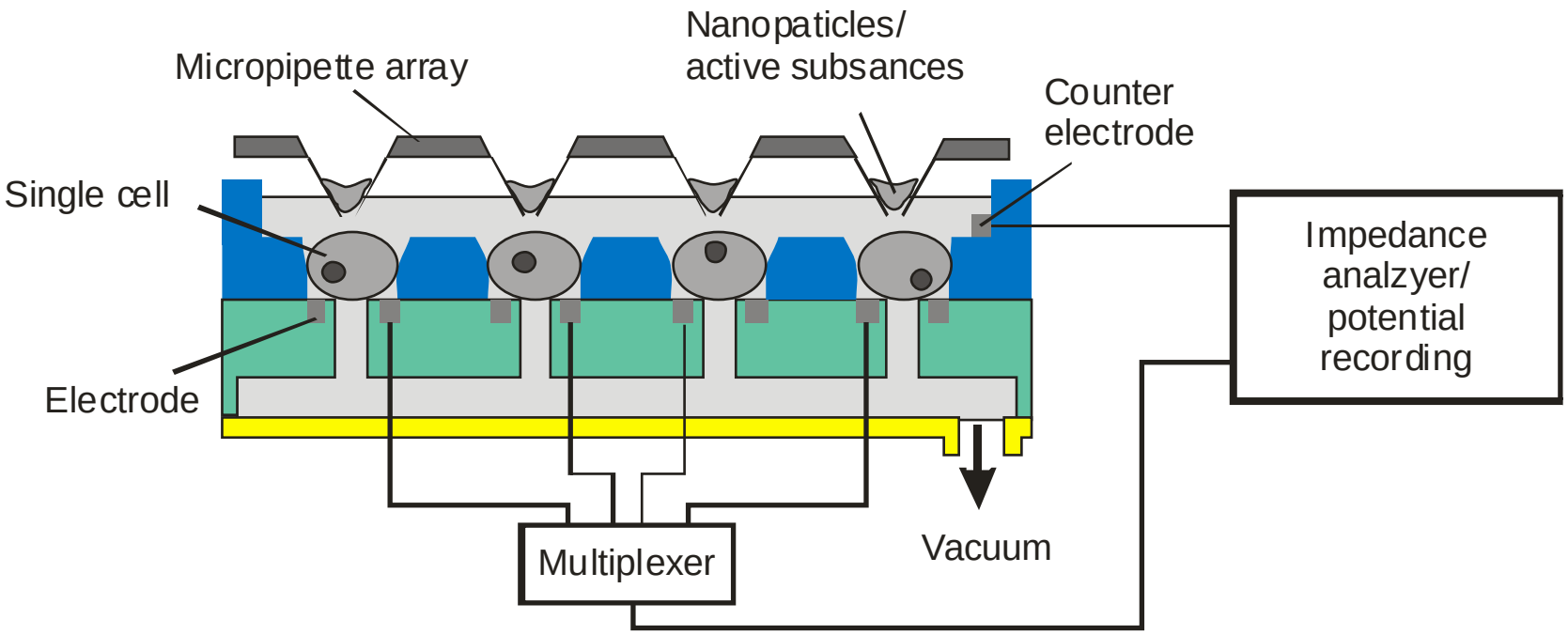
- Potential recording
- Image processing
- Impedance spectroscopy
- Acoustic microscopy





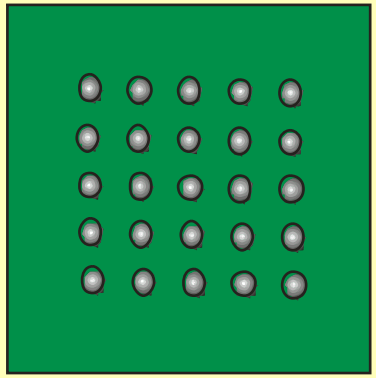
Characterization of single cells

Single Cell Characterization



Hydrodynamic cell positioning

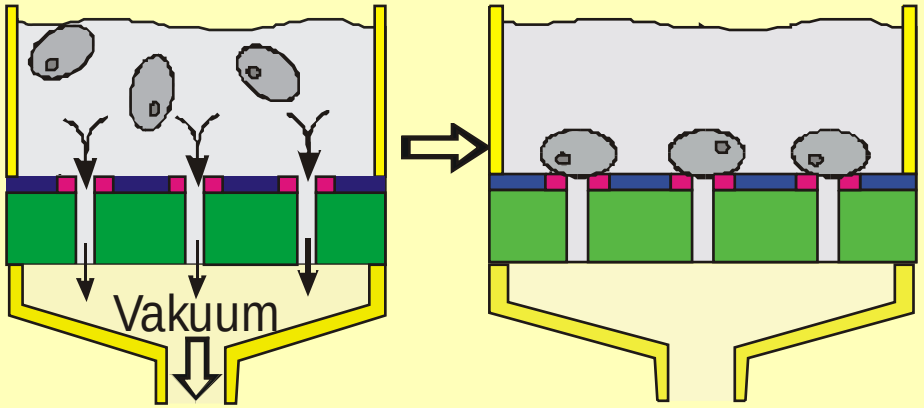
Cells in array structure



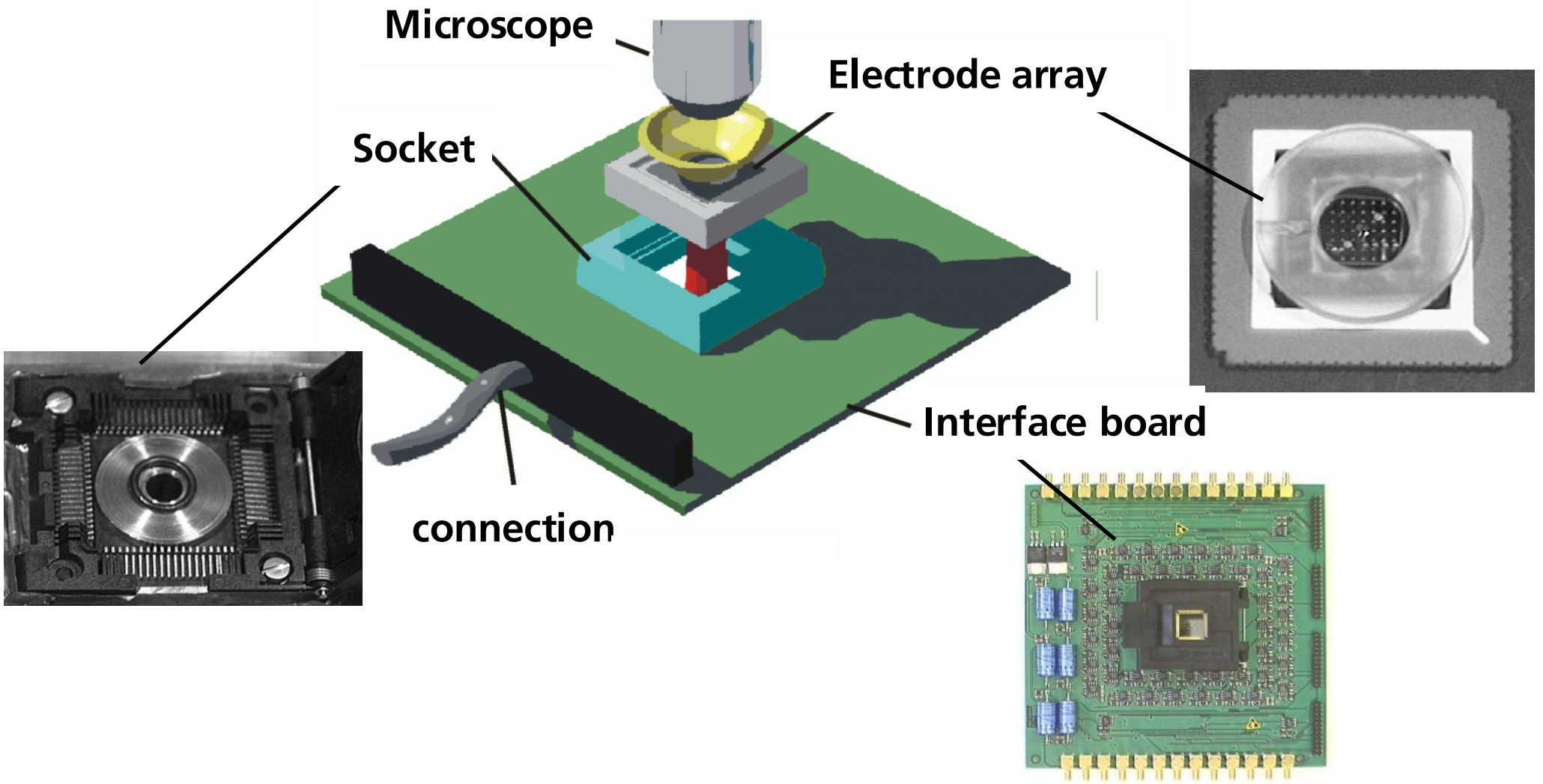
Cells on microelectrodes

Cells in suspension

Cells on electrodes



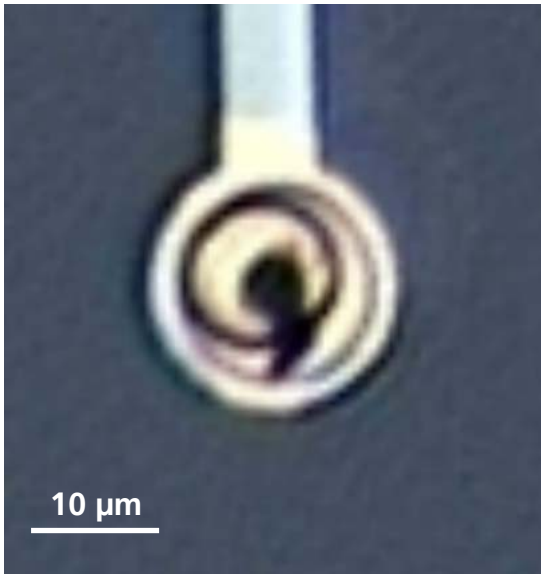
Single Cell Monitoring System

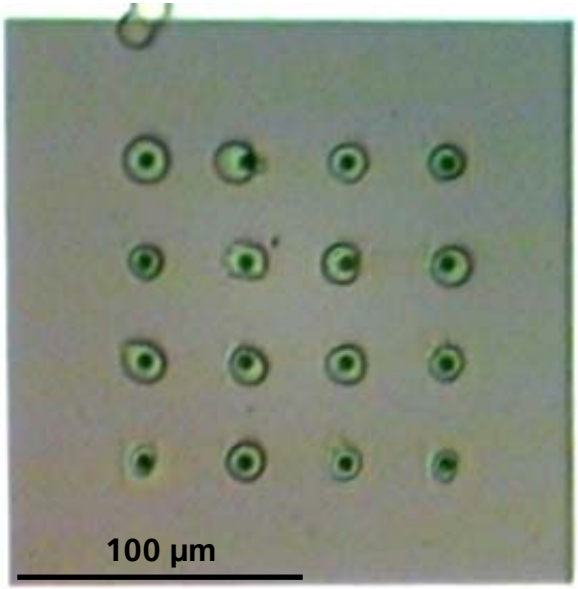


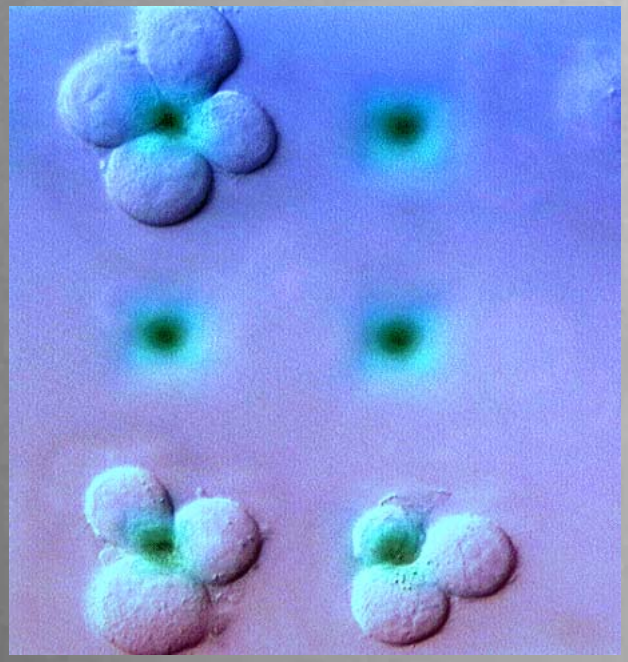
Positioned Cells

Positioned Neuro 2a cells on holes

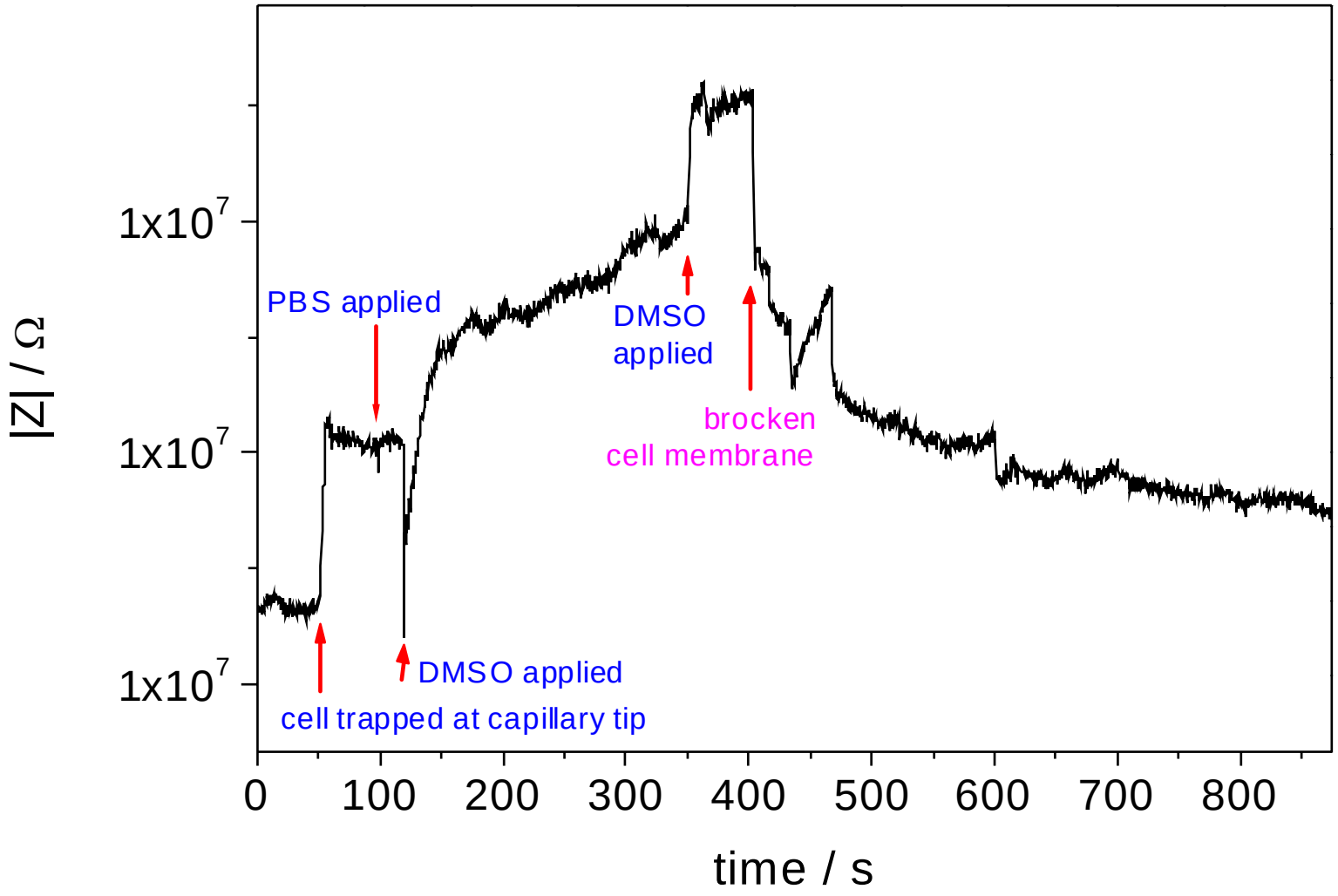
- negative pressure: 60 mbar
- concentration of cells: 5x10^4 cells/ml
- diameter of suction holes: 5 µm



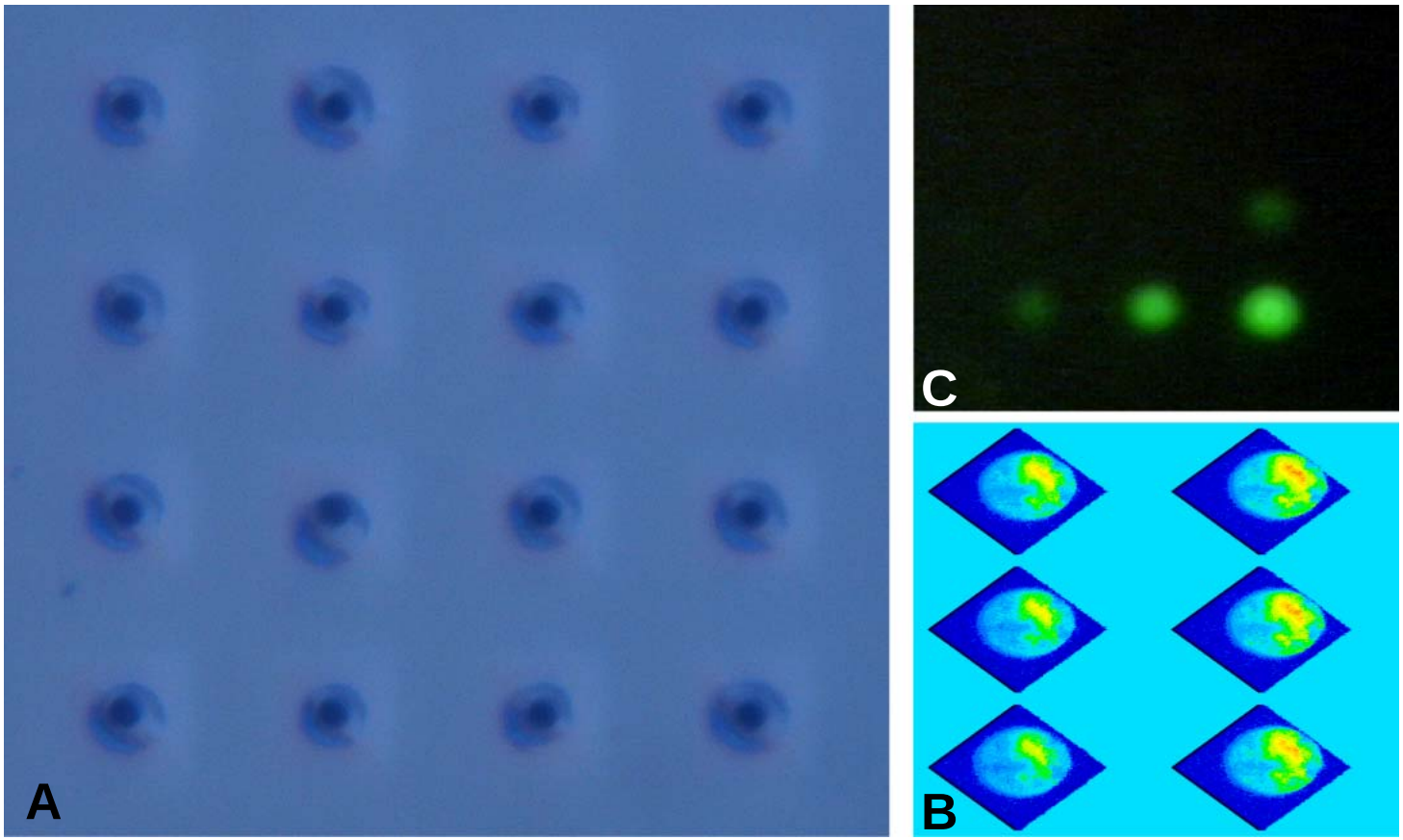




Impedance of a single cell



Cell arrays and optical characterization



A: Cell positioning **B:** Fluorescence of single cells **C:** Fluorescence quantification

BIOspektrum
Das Magazin für Biowissenschaften

Fraunhofer
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EU-projects as examples for different relevant application fields

- **Regenerative Medicine / Cell therapy (OsteoCord)**
  Bone from blood: Optimised isolation, characterisation and osteogenic induction of mesenchymal stem cells from umbilical cord blood.
- **Drug discovery / development (CARDIOWORKBENCH)**
  Drug Design for Cardiovascular Diseases: Integration of *in silico* and *in vitro* Analysis
- **Gene Therapy (PolexGene)**
  Biocompatible non-viral polymeric gene delivery systems for the ex vivo treatment of ocular and cardiovascular diseases with high unmet medical need
- **Engineered nanoparticels (DIPNA)**
  Development of an Integrated Platform for Nanoparticle Analysis to verify their possible toxicity and the eco-toxicity
- **Nanoparticles for Diagnostics (NEMO)**
  Nano based capsule-Endoscopy with Molecular Imaging and Optical biopsy

Conclusions

Impedance spectroscopy as examples for non invasive monitoring

- Cell and tissue parameter are related to electrical properties
- Different tissue parameter are related to different frequency ranges
  → selectivity (sensitivity must be tested for every application!)

Parameter which can be determined in general:

- 2D – coverage, cell/cell contacts, cell adhesion, cell migration
- 3D – cell contents, homogeneity, areas with different cells
- Single cell – membrane integrity, changes of membrane properties

Examples for applications:

- Barrier function
- Non-destructive characterization of cell cultures, long time
- Effects on cell membrane of single cells over time