Low-cost biosensors with printed interdigitated graphene electrodes

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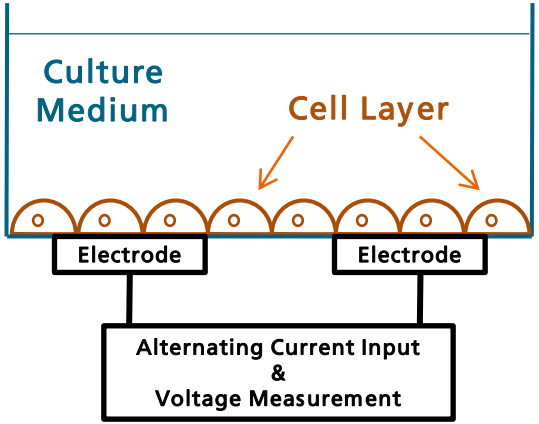
Outline

- Motivation
  - What do we want to measure
  - Drawback of the state of the art
- BIOGRAPHY approach
- Results
  - Printing
  - Biology
- Summary and outlook

What do we want to measure

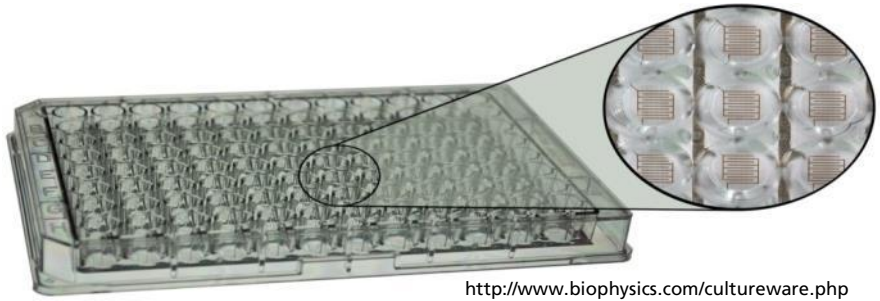
- Sensor for toxicity testing
  - Reaction of living cells to exposure to substances

- Sensor for validation of antiviral substances
  - Cells insulate electrodes
  - Addition of viruses → viruses destroy cells (cytopathic effect ‚CPE')
  - Addition of viruses and antiviral substances → CPE is inhibited



Drawback of commercial systems

- High costs due to
  - Production process for printed circuit boards
  - Biocompatible metals (gold, platinum)

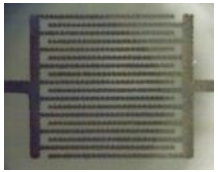


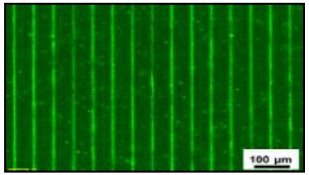
http://www.biophysics.com/cultureware.php

→ Cost effective sensor production method is required

BIOGRAPHY approach

Rotogravure printed
Graphene/protein micro pattern





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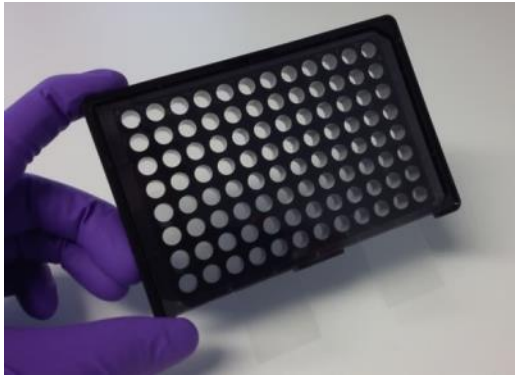
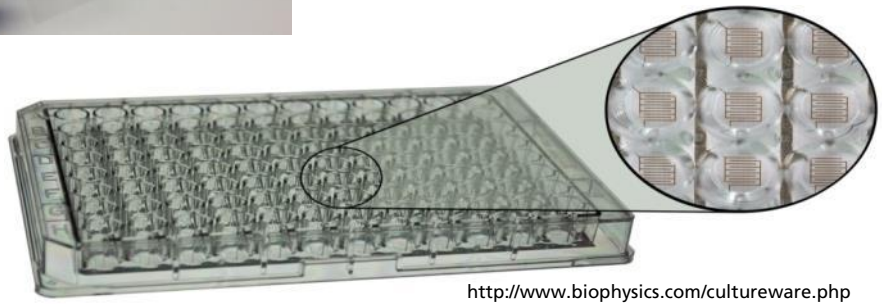


- Electrically conductive
- Biocompatible

- Improves cell adhesion



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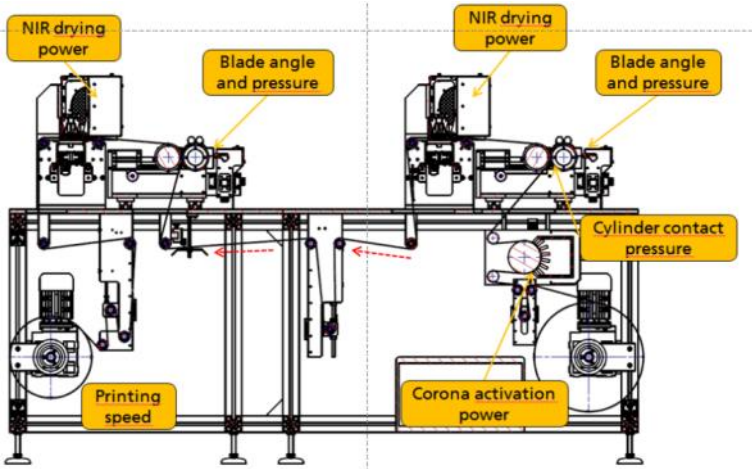
Roll-to-roll gravure printing machine R²R-300

Roll-to-roll gravure printing machine R²R-300

- Two printing units: each with an integrated NIR drying unit
- Power of corona unit: Up to 600 W
- Printing speed: Up to 40 m/min
- Web width: 300 mm
- Width of printable area: 250 mm
- Doctor blade pressure and angle are independently adjustable



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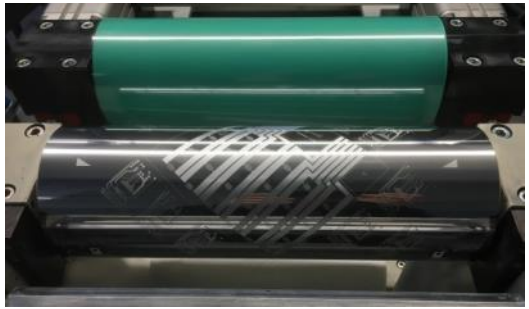
Gravure printing cylinders

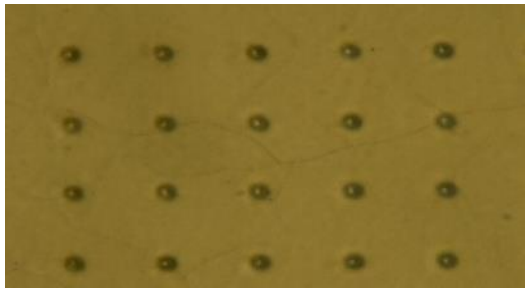
Cylinder for graphene printing

- Masking and chemical etching
- → 50 µm deep cells, 80 l/cm; high ink transfer (low electrical resistance)



Cylinder for protein printing

- Direct engraving in copper with ultrashort pulse laser
- → microstructures < 10 µm
- Alternative: 20 µm deep cells; 120 l/cm



Directly structured dots with diameter 8 µm

Graphene ink

- Basis: screen printing graphene ink (HDPlas IGSC02002)
  - Surface-functionalised Graphene Nanoplatelet (GNP) material (using Haydale's patented HDPlas® plasma-based technology)
  - Viscosity: ~ 5.5 Pa*s
  - Thickness before / after drying: ~ 13 µm / 7 µm
  - Sheet resistivity: ~ 12 Ω/sq. (normalised to 25 µm)

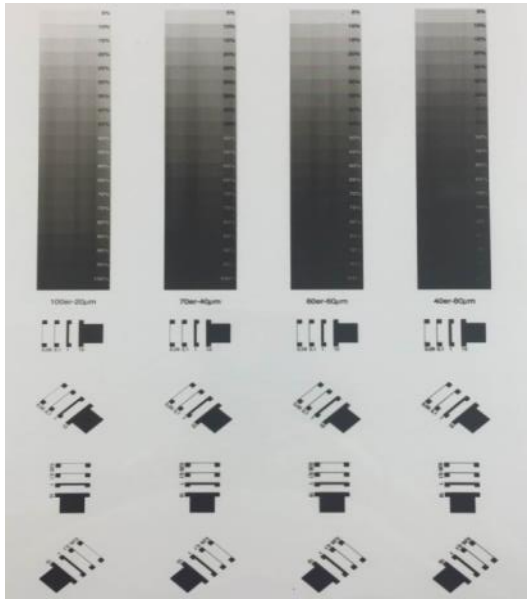
- Modifications
  - Solvent content
  - Resin content
  - Milling procedure

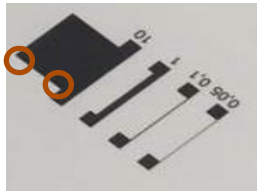
Protein ink

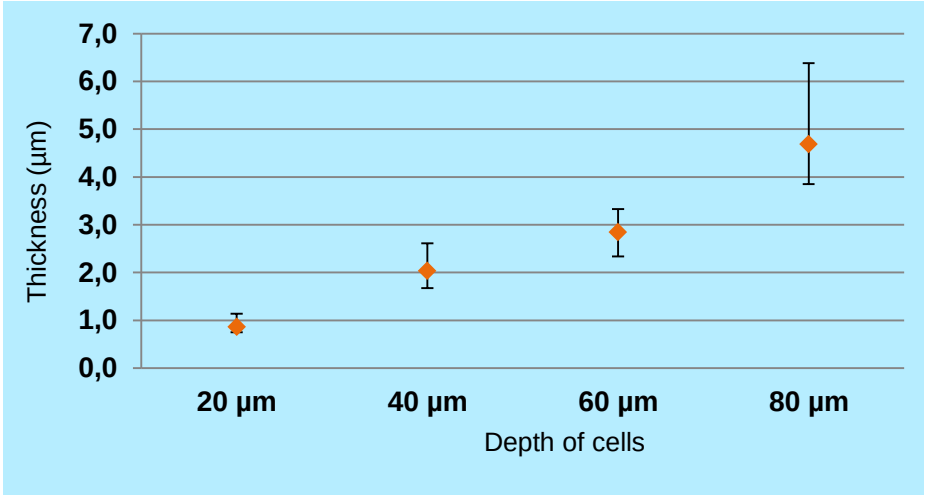
- Composition
  - Matrigel, dissolved in DMEM/F12
  - Ammonium acetate buffer
  - Carboxymethylcellulose sodium salt, dissolved in DI water
  - X

Roll-to-roll gravure printing with graphene ink

- Printed graphene patterns with test cylinder







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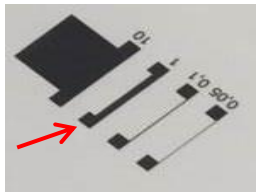
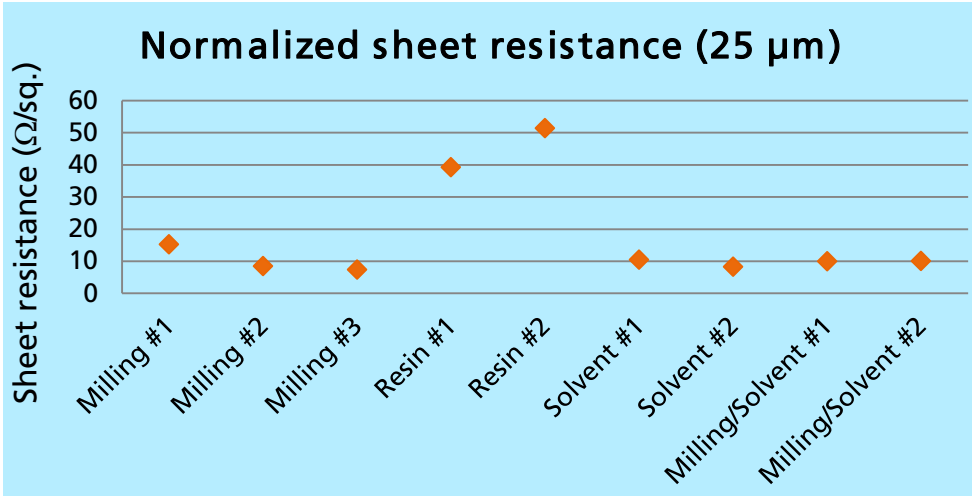
Electrical characterization

- Resistivity measurements
- Line width: ~1.05 mm, length: 10 mm
- Angles: 45° and 135°, cell depth: 60 µm
- Graphene thickness: ~ 4 µm

$R_{sq.}$ = 10 – 20 Ω/sq.





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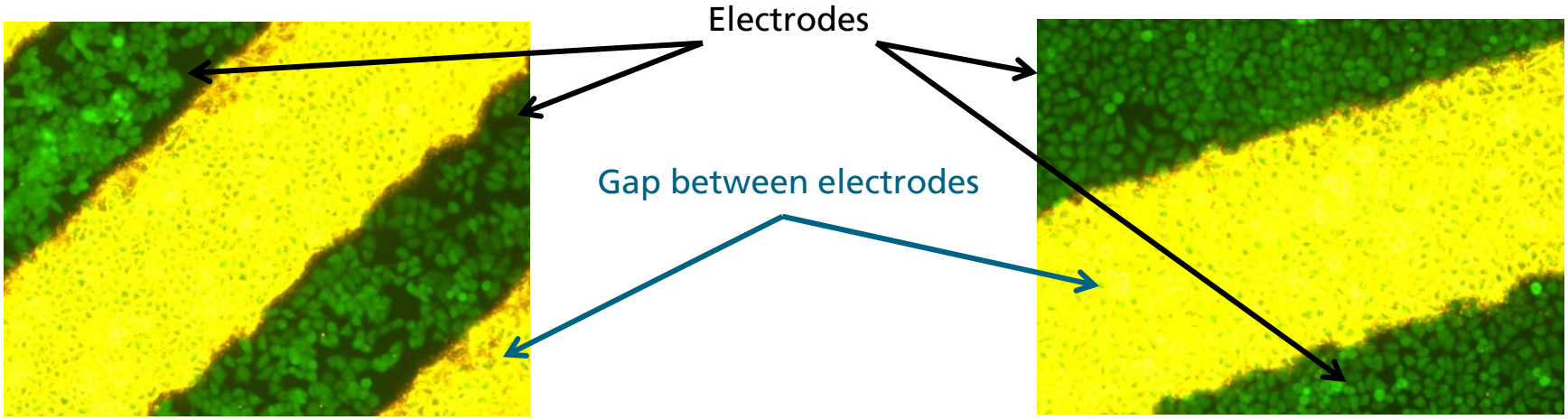
Cell adhesion and proliferation on graphene electrodes

- FDA (fluorescein diacetate) stained TZM-bl cells on graphene after 48 h incubation



Cytotoxicity testing of graphene ink (ISO 10993)

- Cytotoxicity of PET foil as well as of graphene ink printed PET foil was analysed in more than ten different cell lines
- Cells are from
  - different species (human, mouse, rat)
  - different organs and tissues (liver, kidney, heart, nerve system, skin and blood)

- No evidence that PET foil or graphene ink has any cytotoxic potential

Final printing parameters (graphene ink)

- PET foil: thickness 50 µm, surface energy < 40 mN/m
- Surface energy of graphene ink ~ 45 mN/m
- Corona activation (600 W) → surface energy ~ 60 mN/m

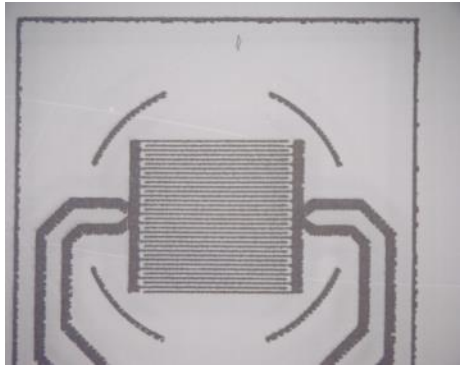
- Speed: 40 m/min
- Doctor blade contact pressure: 4.0 bar
- Cylinder contact pressure: 3.5 bar
- NIR drying power: 90% (of 3.5 kW)

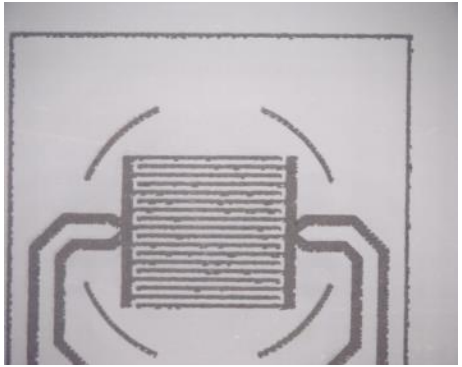
Final printing parameters (protein ink)

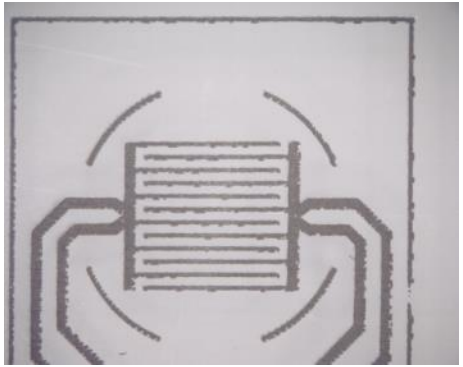
- Speed: 40 m/min
- Doctor blade contact pressure: 3.0 bar
- Cylinder contact pressure: 2.5 bar
- NIR drying power: 60% (of 3 kW)

Printed structures

120 l/cm
30 µm





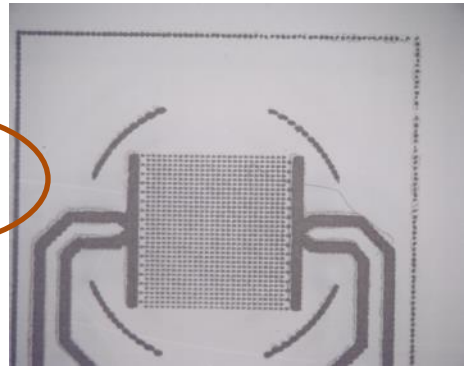


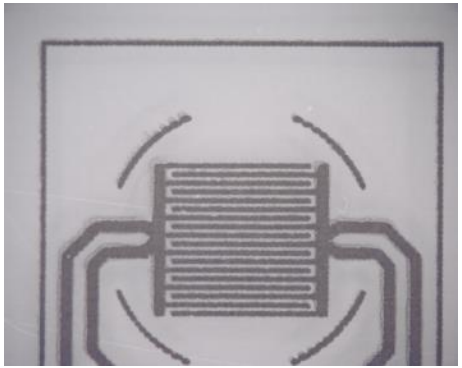
A (52/52)

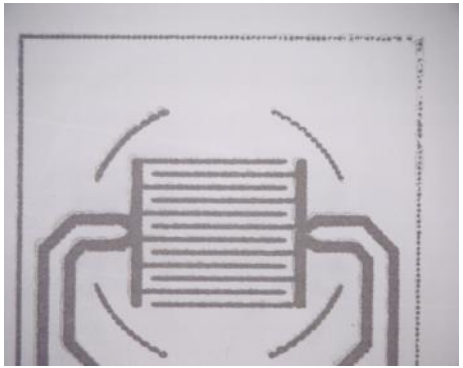
B (100/100)

B1 (100/200)

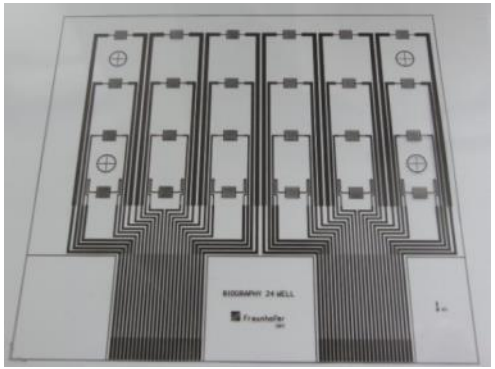
80 l/cm
50 µm

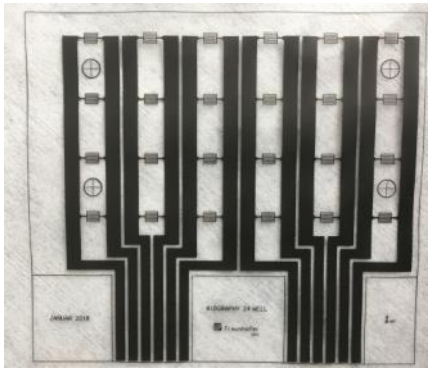


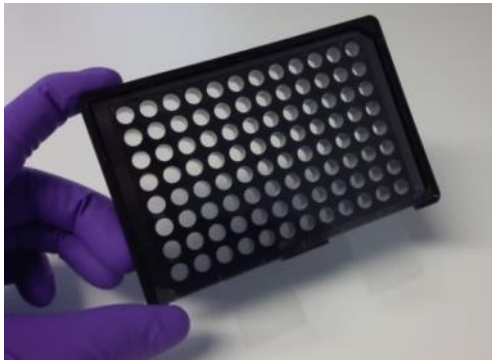


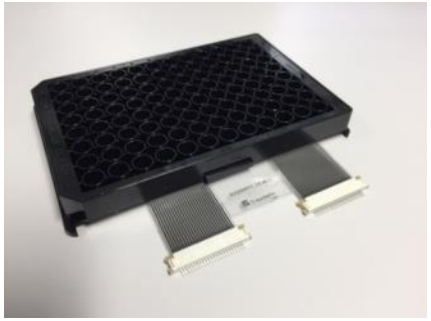


96-well plate set-up with printed circuit adapter









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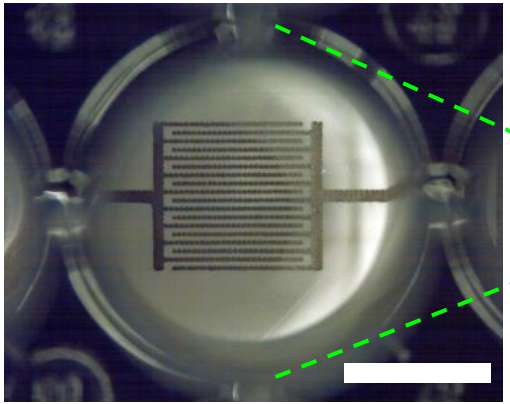
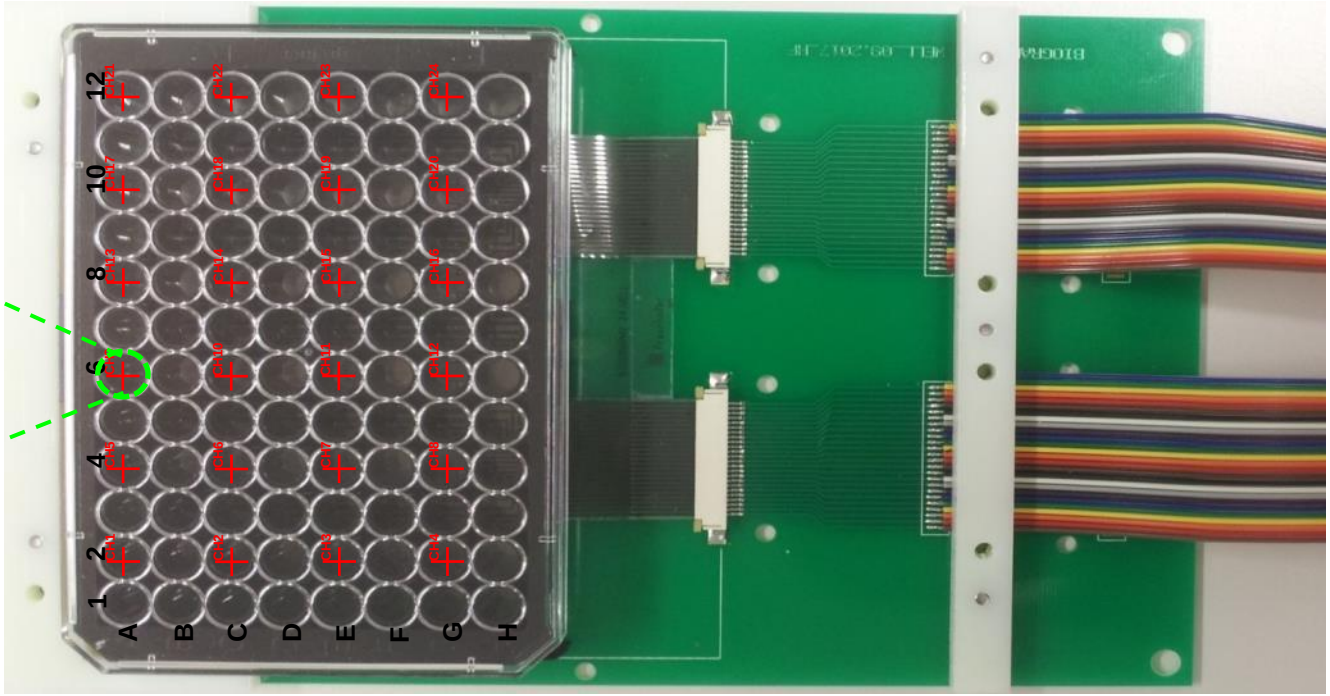


Scale bar: 3.5 mm



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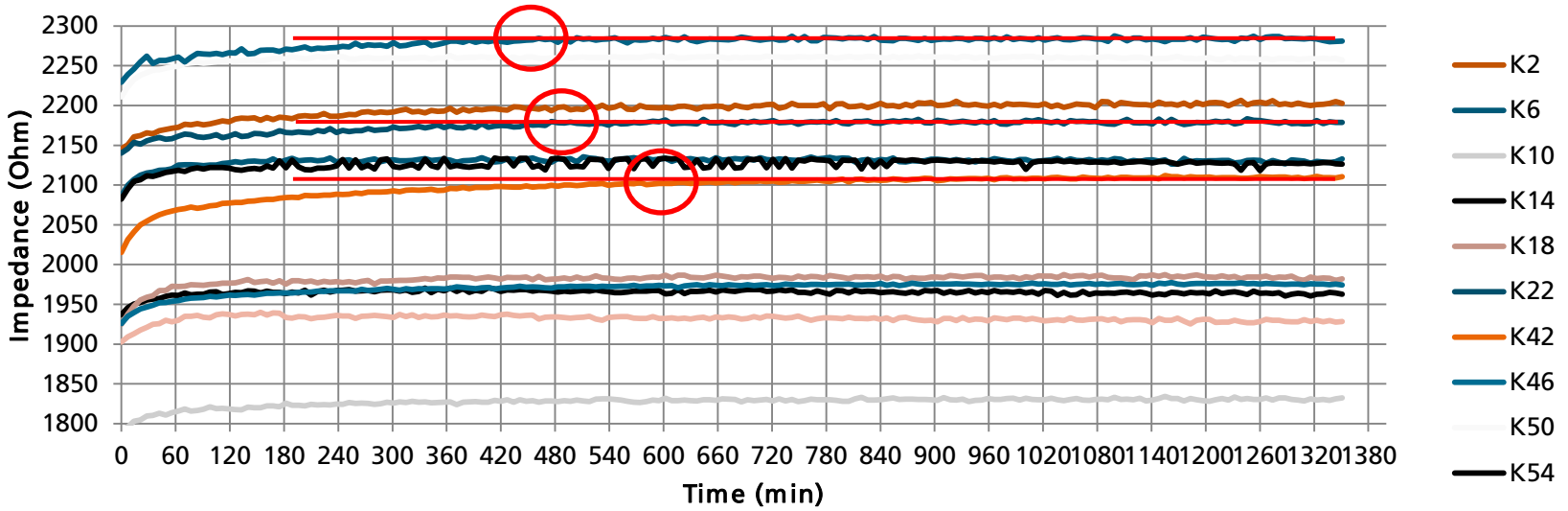
Drift after adding cell culture medium at 37°C



Stable after < 10 hours

Summary

- We have successfully developed
  - Graphene ink for gravure printing
  - Protein ink for gravure printing
  - Two-colour rotogravure printing system
  - Processes for printing graphene electrodes and for printing proteins
- Printed structures are biocompatibel
- Printed proteins improve cell adhesion/proliferation
- Printed sensors are useable with the IMOLA - IVD system of cellasys - R&D
- **Next steps**
  - Validation of biosensors for the two application fields

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Thank you

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(Hall B0 Booth 313)

BIOGRAPHY consortium

M-Era.net project with partners from Germany and UK

Tasks	Name
R2R gravure printing process (graphene and proteins), biocompatibility tests	Fraunhofer IBMT
R2R two-colour printing machine, laser-based method for micro patterning of gravure printing cylinders	SAUERESSIG GmbH + Co. KG
Biocompatible graphene ink for gravure printing	Haydale Ltd.
Application: antiviral drug validation	AiCuris GmbH & Co. KG
Application: repeated dose toxicity	cellasys GmbH R&D

AiCuris Anti-infective Cures cellasys Fraunhofer IBMT haydale SAUERESSIG

M-era.Net BIOGRAPHY Fraunhofer IBMT

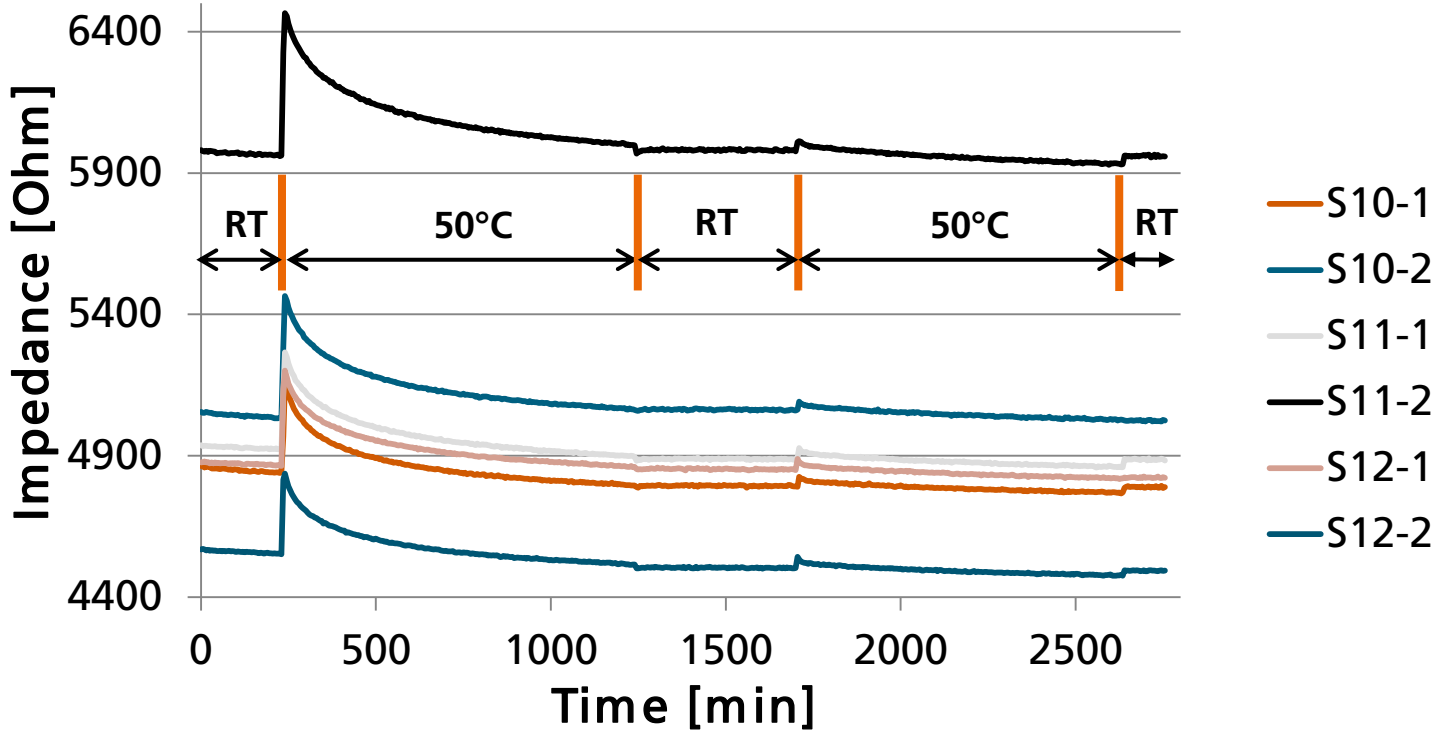
Investigation of impact of the graphene ink on the inhibitory effect of antiviral drugs

- Serial dilutions of two different antivirals were pre-incubated with graphene printed PET foils before a regular antiviral assay was performed

- Antiviral drugs used:
  - BAY 41-4109 (inhibitor for HBV)
  - Efavirenz (inhibitor for HIV)

- There was no evidence that graphene ink has any influence on the antiviral activity of BAY 41-4109 and Evavirenz

Repeated annealing in air at 50 °C



Mechanical wear and impedance

- 5x insertion of sensors into connector to test dependence of impedance values on mechanical wear