From RNA Extraction to Microarray Based Readout:

Biofunctionalization of a Microfluidic Cartridge for Fully Integrated microRNA Analysis

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SCIENION AG
SCIENION AG: Excellence in low volume liquid handling

KEY FACTS

• Founded in Berlin in 2001
• Spin-off from the Max Planck Institute for Molecular Genetics
• Life science company focused on ultra-low volume liquid handling systems and solutions
• Four locations: Berlin, Dortmund, Monmouth Junction, NJ (USA; since December 2011), Lyon (France, since May 2016)
• 56 people
• ISO 9001 certified
SCIENION AG: Excellence in low volume liquid handling

TECHNOLOGY

• Core competence: Handling and dispensing pico- and nanoliters
  ➢ Biofunctionalization of microfluidic chips, biosensor surfaces, membranes, microplates
  ➢ Array/Spot based analytics
• Lead product sciFLEXARRAYER: system for automated ultra-low volume liquid handling of biological samples in diagnostics, genomics and proteomics
• Non-contact dispensing technologies: sciDROP PICO, sciDROP NANO
• Consumables leveraging instrument performance
• Integrated portfolio comprising array printer, reader, consumables and services
Small droplets are all around us – typically microliters
Small droplets with big impact in (Bio)Science

Fertilization

Bead handling

Drug delivery

Biosensors

Microarrays

PCR / Library preparation

Sample Prep

Single cell handling

POCT
Spots on Slides, Wafers, Biosensors, ...

**Microarrays**
- arrays of spots in a predefined area

**Biosensors**
- arrays of spots exactly at a predefined position

**Microplates**

**Wafer**

**Slides**

**Microfluidic Cartridges**

CCD Images of the loaded electrodes of a CMOS sensor
Application: Microfluidic Cartridge for miRNA Analysis

Polymer-based microarrays → Clinical Background: Breast cancer diagnostics → Assay: RNA amplification → System integration

Clinical Background:
Breast cancer diagnostics

Assay:
RNA amplification

System integration

Polymer-based microarrays

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System integration

miRNA + universal NASBA

mRNA + universal NASBA
Biofunctionalization of polymer materials

sciPOLY3D immobilization technology

- Enables covalent immobilization of biomolecules on non-activated surfaces – such as microfluidic chips
- No wet chemistry processes
- Water-soluble and simply added to the printing media
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Polymer-based microarrays

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System integration
Background: Personalized healthcare

Tailor-made therapy
- Efficacy
- Side effects
- Cost-efficiency

More detailed diagnostic
- Genetic predisposition
- Therapeutic response

Roche PHC Brochure, 2011.
Background: Tumor diagnostics

Example: breast cancer

- Heterogeneous disease
- Therapy depends on subtyping
  - Phenotypical features
  - Immunohistochemistry
- Estimated ~80% of patients are treated suboptimal

Tumor tissue IHC of HER2 protein (200x magnification)

Cytostatic drugs
- Surgery
- Hormonotherapy
- Radiation
- Immunotherapy

Background: Tumor diagnostics

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More detailed diagnostics based on nucleic acids

Background:
Tumor diagnostics

Nucleic acids biomarker

- SNP (mutation)
- mRNA (gene expression)
- microRNA (regulation of gene expression)

- Risk of disease
- Prediction
- Prognosis

Project Aim & Strategy

Aim
Parallel measurement of multiple biomarker classes in a single test

Strategy

Combination of

- sensitivity and specificity of *in-vitro* nucleic acid amplification

with

- multiplex capacity of microarrays
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RNA Amplification Assay

**NASBA: Nucleic Acid Sequence-Based Amplification**

- Transcription-based
- 3 enzymes
  (AMV Reverse Transcriptase, RNase H, T7 RNA Polymerase)

**Alternative for PCR**

- Isothermal (41 °C)
- Direct amplification of RNA
- Tolerates gDNA
- Produces single stranded RNA

RNA Amplification Assay

Reverse Transcription
universal NASBA (RT-uNASBA)

- Introducing universal sequences
- Integration of miRNA in RT-uNASBA

- RNA
- cDNA
- RT-Primer 1 with universal sequence 1 and T7 promoter
- RT-Primer 2 with universal sequence 2
- stem-loop RT-Primer with universal sequence 2
- NASBA-Primer universal 1 with T7 promoter
- NASBA-Primer universal 2

universal NASBA
multiplex RT
Parallel amplification and detection of mRNA and miRNA

- Up to 14-plex mRNA / miRNA tested
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Polymer-based microarrays

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System integration
Integration in fully automated µTAS

- Isothermal assay procedure
- Functionalization of unmodified plastics
- Combination with on-chip RNA extraction

Lab Chip, 2010, 10, 610-616
Integration in fully automated µTAS
Integration in fully automated µTAS

All-in-one system

Actuation
- Sample transport
- Blister control
- Turning valves

Temperature control

Detection
- 2-channel fluorescence
Summary

Microarray fabrication with sciPOLY3D

Biofunctionalization of unmodified polymer substrates

Isothermal assay for multiplex RNA analyses

Parallel amplification of multiple miRNA and mRNA sequences

Integration in fully automated μTAS
Thank you very much for your attention

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Tools and Service for Microarray Based Application

**Products**
- sciFLEXARRAYER
- sciREADER
- sciPLEXPLATE
- sciCHIP
- sciBUFFER

**Key Technologies**
- Contact Free Spotting
- Surface Functionalization
- Microarray Applications

**Services**
- Product / Process Development & Manufacturing