Molecular Diagnostics at the Point-of-Care by Centrifugal Microfluidics

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11.12.2018

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Hahn-Schickard-Gesellschaft für angewandte Forschung e.V.

Applied research, development + fabrication based on microengineering

- Earnings 2018: ~ 26 M€ (~ 9 M€ from industry)
- Staff members 2018: 210 FTE (225 persons)
- Member of Innovationsallianz Baden-Württemberg innBW

Institut für Mikroaufbautechnik Stuttgart

Institut für Mikro- und Informationstechnik Villingen-Schwenningen

Institut für Mikroanalysesysteme Freiburg
Diagnostics in centralized facilities

- Fully automated instruments
- ~2000 tests/hour
- ~700 different assay reagents available on demand
- Costs per test are extremely low

Still thinking about increasing throughput and reducing costs

Roche cobas® 6000 analyzer series
Diagnostics at the **Point of Care** ...

- Diagnostics at emergency settings
- Diagnostics at doctors offices
- Diagnostics at the point of need
- Microfluidics enables miniaturization, integration, parallelization, automation and fast time to result

**Challenges**

- Easy to use & robust
- Sample to answer systems
- Same precision required as in centralized labs
- Complex biochemical assays
- **Highly cost sensitive**

**Opportunities:** mass markets

**Our vision:**

Development of real MicroTAS with sample-in answer-out capability for fast diagnostics of complex diseases

Movie "Outbreak" (1995)
Prominent microfluidic platforms

- Lateral Flow Tests (LAT)
- Linear Actuated Devices
- Laminar Flow Platform
- Microfluidic Large Scale Integration
- Segmented Flow Microfluidics
- Massively Parallel Assay Platforms
- **Centrifugal Microfluidics**
- Electrokinetic Platform
- Electrowetting Platform
- Surface Acoustic Wave Platform
- ...

S. Haeberle, R. Zengerle; Lab. Chip., 2007, 7, 1094 ff
Why centrifugal microfluidics?

- No pumps & no tubings needed (easy interfaces)
- No issue with gas bubbles
- Easy to use @ Point of Care
- Enables implementation of complex assays
The history of centrifugal microfluidics

Web of Science; 19.06.2018
Search for: centrifug* AND (microfluid* OR analyzer* OR analyser)

For more info refer to …
O. Strohmeier et al; Centrifugal microfluidic platforms: advanced unit operations and applications, Chemical Society Reviews, 44, 2015, 6187-6229
A selection of centrifugal microfluidic POC devices

- Abaxis
- Samsung Labgeo
- Roche Cobas 101
- UNIST – Dr. Cho
- Hahn-Schickard
- SpinDiag
- ...

Hahn-Schickard / SpinDiag
LabDisk platform

Application specific disc

- Specific panels for sepsis, respiratory diseases, antibiotic resistant bacteria, etc.

LabDisk player (1st generation)

- Process control: frequency, angular acceleration rate, temperature, magnetic field, read-out, etc.
LabDisk platform

Application specific disc

- Specific panels for sepsis, respiratory diseases, antibiotic resistant bacteria, etc.

LabDisk player (2nd generation)

- Fully automated disk handing
- Fast thermocycling
- Fast read-out, etc.

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Testing for infectious diseases by **genotyping**

Is there a pathogen among billions or even trillions of other cells?

What is its exact identity?

Is genetic material of #1, #2, … #20 present (yes/no)?

→ **Genotyping**
Microfluidic platform that offers ...

- solutions for all unit-operations which ...
- need to be easily combinable ...
- within a low cost fabrication technology ...
- ideally in a monolithic fashion!

In our view centrifugal microfluidics is the most attractive platform solving all those issues in Point-of-Care Scenarios

Simplicity is a key!

- O. Strohmeier et al; Centrifugal microfluidic platforms …, Chemical Society Reviews, 44, 2015, 6187-6229
- D. Mark et al; “Microfluidic lab-on-a-chip platforms …”; Chemical Society Reviews, 39, 2010, pp. 1153-1182
## Liquid reagent storage and release from stick packs

<table>
<thead>
<tr>
<th>Lancing</th>
<th>Sample take up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample transfer</td>
<td>Sample injection</td>
</tr>
<tr>
<td><strong>Reagent supply</strong></td>
<td></td>
</tr>
<tr>
<td>Valving</td>
<td>Liquid transport</td>
</tr>
<tr>
<td>Timing</td>
<td>Switching</td>
</tr>
<tr>
<td>Aliquoting</td>
<td>Metering</td>
</tr>
<tr>
<td>Bead handling</td>
<td>Mixing</td>
</tr>
<tr>
<td>Incubation</td>
<td>Rehydration</td>
</tr>
<tr>
<td>Readout</td>
<td>Thermocycling</td>
</tr>
</tbody>
</table>

### Stick Packs

- T. van Oordt et al., *Miniature stick-packaging* ..., Lab Chip, 2013, 13, 2888
Liquid reagent storage and release from stick packs

Stick Packs

- T. van Oordt et al., Miniature stick-packaging ..., Lab Chip, 2013, 13, 2888
Liquid transport by centrifugal force

**Basic principle**

\[ f_\omega \sim \omega^2 \]

**Design parameter**
- Channel width
- Channel length
- Radial position
- ...

<table>
<thead>
<tr>
<th>Lancing</th>
<th>Sample take up</th>
<th>Centrifugal force</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample transfer</td>
<td>Sample injection</td>
<td>Capillary force</td>
</tr>
<tr>
<td>Reagent supply</td>
<td>Liquid transport</td>
<td>Pneumatic force</td>
</tr>
<tr>
<td>Valving</td>
<td>Switching</td>
<td></td>
</tr>
<tr>
<td>Timing</td>
<td>Metering</td>
<td></td>
</tr>
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<td>Rehydration</td>
<td></td>
</tr>
<tr>
<td>Incubation</td>
<td>Thermocycling</td>
<td></td>
</tr>
</tbody>
</table>
Pneumatic pumping

- Lancing
- Sample transfer
- Reagent supply
- Valving
- Timing
- Aliquoting
- Bead handling
- Incubation
- Readout

- Sample take up
- Sample injection
- Liquid transport
- Switching
- Metering
- Mixing
- Rehydration
- Thermocycling
- Centrifugal force
- Capillary force
- Pneumatic force

- S. Zehnle et al., *Centrifugo-dynamic inward pumping* ..., 2012, Lab Chip, 12, 5142

- low resistance
- high resistance

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Pneumatic pumping

<table>
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</tbody>
</table>

Transfer efficiency
91% (in 2012) ~ 100% (now)

---

Employing pneumatics is promising because …

- … pneumatic forces are typically a magnitude larger than capillary forces
- no additional fabrication steps required

- S. Zehnle et al., *Centrifugo-dynamic inward pumping …*, 2012, Lab Chip, 12, 5142
Nucleic acid amplification by PCR

- Lancing
- Sample transfer
- Reagent supply
- Valving
- Timing
- Aliquoting
- Bead handling
- Incubation
- Readout
- Sample take up
- Sample injection
- Liquid transport
- Switching
- Metering
- Mixing
- Rehydration
- Thermocycling

Temperature (°C)

Cycle number

Target DNA

\[ N = (1+E) \text{ cycle number} \]

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Fluorescent readout

- Geometric multiplexing → different PCR reactions simultaneously in each cavity
- Lower limit of detection for nucleic acid testing by combining PCR with fluorescent readout ~ 1 molecule

PCR chambers with primers/probes pre-loaded to specifically amplify DNA fragments of interest
Pathogen detection by genotyping

LabDisk Fabrication
- Thermoforming / injection moulding
- Functionalization
- Sealing

380 µm

RT-PCR
Lyophilisates
Primers
Probes
Stick-packs
Magnetic beads
Hahn-Schickard pilot line

- Forming, functionalization, sealing, packaging
- Cleanroom free of bacterial contamination
- DIN ISO 9001:2015 and DIN ISO 13485
## LabDisk application examples & technology readiness levels

### Sample-to-result pathogen testing

<table>
<thead>
<tr>
<th>Apps</th>
<th>Panel</th>
<th>Targets</th>
<th>Technology readiness level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal sepsis</td>
<td>13 pathogens</td>
<td></td>
<td></td>
<td>Lab Chip, 2015, 15: pp 3749</td>
</tr>
<tr>
<td>Food pathogens</td>
<td>6 pathogens</td>
<td></td>
<td></td>
<td>Anal. Methods, 2014, 6: pp 2038</td>
</tr>
<tr>
<td>Respiratory</td>
<td>18 + 4</td>
<td></td>
<td></td>
<td>Lab Chip, 2016, 16: pp 199</td>
</tr>
<tr>
<td>Malaria &amp; Dengue</td>
<td>10 pathogens</td>
<td></td>
<td></td>
<td><a href="http://www.DiscoGnosis.eu">www.DiscoGnosis.eu</a></td>
</tr>
</tbody>
</table>

### Antibiotic resistance screening (25)

<table>
<thead>
<tr>
<th>Apps</th>
<th>Panel</th>
<th>Targets</th>
<th>Technology readiness level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary tract</td>
<td>5 pathogens</td>
<td></td>
<td></td>
<td>Unpublished</td>
</tr>
</tbody>
</table>

### Genotype

<table>
<thead>
<tr>
<th>Apps</th>
<th>Panel</th>
<th>Targets</th>
<th>Technology readiness level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>12 resistances</td>
<td></td>
<td></td>
<td>Lab Chip, 2010, 10: pp 2519,</td>
</tr>
<tr>
<td>KRAS</td>
<td>7 point mutations</td>
<td></td>
<td></td>
<td>Microchim Acta, 2014, 181: pp 1681</td>
</tr>
<tr>
<td>Animal ident</td>
<td>14 species</td>
<td></td>
<td></td>
<td>PLoS ONE, 2015, 10(7): e0131845</td>
</tr>
<tr>
<td>Others</td>
<td>xxx</td>
<td></td>
<td></td>
<td>Coming soon</td>
</tr>
</tbody>
</table>

## Technology readiness level (definition of the European Commission)

- 1 principle
- 2 concept
- 3 proof of concept
- 4 lab validation
- 5 field validation
- 6 demonstration
- 7 system prototype
- 8 system qualified
- 9 manufacturing
Automatic generation of **dilution series** for qPCR - standards, controls, references

- Fully automated decadal dilution series up to **1:100,000** with **triplicates** in each dilution stage
- Additional chambers enable water and healthy donor negative controls, as well as quantification of patient and reference gene samples
- Filtered venting outlets prevent contamination

P. Juelg et al; submitted, under review
Automatic generation of dilution series for qPCR - standards, controls, references

- Fully automated decadal dilution series up to 1:100,000 with triplicates in each dilution stage
- Manual operation time
  - LabDisk ~ 1 min
  - Manually ~ 15 min

P. Juelg et al; submitted, under review
Monitoring minimal residual disease (MRD) for Leukemia patients (ALL)

One single disk, integrating …

- … three simultaneously processed dilution series (1:100,000 with triplicates),
- … three quantification structures (Target 1 – 4 + Reference)
- … three negative controls of water and the markers
How can we get faster?
... and more precise at smaller chip size?
Motivation for merging droplet microfluidics with centrifugal microfluidics

Classical assays

- Absolute quantification needs calibration
Digital assays

Classical assays
- Absolute quantification needs calibration

Digital assays
- Split sample into more compartments than molecules of interest in your sample
- Fast (isothermal) and highly non-linear amplification schemes applicable
- Absolute quantification possible by counting positive droplets
Aliquoting

- Lancing
- Sample transfer
- Reagent supply
- Valving
- Timing
- Aliquoting
- Bead handling
- Incubation
- Readout

Metering & Valving  
Centrifugal step emulsification  
Centrifugo-pneumatic multi-liquid aliquoting

- Droplet diameters
  ~ 100 μm, ~ 30 μm (new!)

- Size depending on nozzle geometry and interfacial tensions
- Droplet size independent of flow rate
- Homogenous droplets (CV 2–4%)
- Works with limited amount of stationary oil phase
- ~ 2.800 droplets/s per nozzle

F. Schuler et al., „Centrifugal step emulsification …“; Lab Chip, 2015, 15, 2759
Laboratory verification of emulsification chip with different nucleic acid amplification systems

**dPCR** (Polymerase chain reaction)
- 5 cp. µl⁻¹
- 500 cp. µl⁻¹
- cystic fibrosis mut. p.Phe508del
- prenatal testing

**ddLAMP** (Loop mediated isothermal amp.)
- NTC
- 15 cp. µl⁻¹
- 475 cp. µl⁻¹
- E. coli gene

**ddRPA** (Recombinase polymerase amp.)
- NTC
- 700 cp. µl⁻¹
- Listeria monocytogenes gene

**Lab Chip, 2016, 16: pp 208**
**Anal. Methods-uk, 2016, 8: pp 2750**
**Lab Chip, 2015, 15: pp 2759**
FROM NASAL SWAB TO DIGITAL ANSWER

Antibiotic resistance screening on a single cell level
Centrifugal microfluidic cartridge

Sample inlet:
Wet nasal Swab-Sample

Liquid reagent pre storage:
- RPA assay buffer
- Oil for droplet generation

→ Stick-Packs are opened during centrifugation

Dry reagent pre storage:
RPA oligonucleotides; polymerases; lysis enzyme

Droplet generation unit
Centrifugal microfluidic cartridge

**Sample inlet:**
Wet nasal Swab-Sample

**Liquid reagent pre storage:**
- RPA assay buffer
- Oil for droplet generation
  → Stick-Packs are opened during centrifugation

**Dry reagent pre storage:**
RPA oligonucleotides; polymerases; lysis enzyme

**Droplet generation unit**
Swab input into the cartridge

- Swab input
- Sample release
- Sample metering
- Reagent release
- Reagent metering
- Pneumatic pumping
- Monolayer assembly
- Droplet generation
- Valving
- Mixing
- Reagent transfer
- Dry reagent rehydration
- Lysis
- Incubation
- Readout

Time [min]

Rotation

T [°C]

f [Hz]
Sample release from swab and Stick-Pack opening

- Swab input
- Sample release
- Sample metering
- Reagent release
- Reagent metering
- Pneumatic pumping
- Monolayer assembly
- Droplet generation
- Valving
- Mixing
- Reagent transfer
- Dry reagent rehydration
- Lysis
- Incubation
- Readout

Time [min]

\[ f \text{ [Hz]} \]

\[ T \text{ [°C]} \]

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Metering of the released bacterial suspension

- Swab input
- Sample release
- Sample metering
- Reagent release
- Reagent metering
- Pneumatic pumping
- Monolayer assembly
- Droplet generation
- Valving
- Mixing
- Reagent transfer
- Dry reagent rehydration
- Lysis
- Incubation
- Readout

Graph:
- Frequency (f [Hz]) vs. Time (min)
- Temperature (T [°C])

Diagram:
- Mixing chamber
- Buffer
- Oil
Metering of the RPA assay buffer

- Swab input
- Sample release
- Sample metering
- Reagent release
- Reagent metering
- Pneumatic pumping
- Monolayer assembly
- Droplet generation
- Valving
- Mixing
- Reagent transfer
- Dry reagent rehydration
- Lysis
- Incubation
- Readout

- 25 µl RPA assay buffer
- Pneumatics

Graph:
- Time [min]
- Frequency [Hz]
- Temperature [°C]

- 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
- 0 20 40 60 80
- -20 0 20 40 60 80
Transfer of fluorinated oil to the droplet generation unit

- Swab input
- Sample release
- Sample metering
- Reagent release
- Pneumatic pumping
- Reagent metering
- Monolayer assembly
- Droplet generation
- Valving
- Mixing
- Reagent transfer
- Dry reagent rehydration
- Lysis
- Incubation
- Readout

Time [min]

- 50 µl Oil

50 µl Oil

Droplet generation unit

0 1 2 3 4 5 6 7 8
0 20 40 60
-20 20 40 60

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Pneumatic pumping of the RPA assay buffer and dry reagent rehydration

25 µl RPA assay buffer

Reaction reagents

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Time [min]

f [Hz]

T [°C]
Mixing of the bacterial suspension with the assay components
Valving of the mixed reaction mix to the droplet generation unit

- Swab input
- Sample release
- Sample metering
- Reagent release
- Reagent metering
- Pneumatic pumping
- Monolayer assembly
- Droplet generation
- Valving
- Mixing
- Reagent transfer
- Dry reagent rehydration
- Lysis
- Incubation
- Readout

Chart showing frequency (f [Hz]) and temperature (T [°C]) over time (Time [min]).

Droplet generation unit
Mixing chamber
Droplet generation by centrifugal step emulsification

- Swab input
- Sample release
- Sample metering
- Reagent release
- Reagent metering
- Pneumatic pumping
- Monolayer assembly
- Droplet generation
- Valving
- Mixing
- Reagent transfer
- Dry reagent rehydration
- Lysis
- Incubation
- Readout

- Lysis
- Swab input

- Time [min]: 0, 20, 40, 60
- Frequency [Hz]: 0, 20, 40, 60, 80
- Temperature [°C]: 0, 20, 40, 60, 80

- 20000 droplets
- 500 droplets/s
Droplet monolayer assembly

- Swab input
- Sample release
- Sample metering
- Reagent release
- Reagent metering
- Pneumatic pumping
- Monolayer assembly
- Droplet generation
- Valving
- Mixing
- Reagent transfer
- Dry reagent rehydration
- Lysis
- Incubation
- Readout
- Sample metering
- Reagent release
- Reagent metering
- Pneumatic pumping
- Monolayer assembly
- Droplet generation
- Valving
- Mixing
- Reagent transfer
- Dry reagent rehydration
- Lysis
- Incubation
- Readout

Graph:
- f [Hz]
- T [°C]
- Time [min]
- 0 1 2 3 4 5 30
- Ø 170 µm
- CV < 5%

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Enzymatic lysis and RPA inside the droplets

- Swab input
- Sample release
- Sample metering
- Reagent release
- Reagent metering
- Pneumatic pumping
- Monolayer assembly
- Droplet generation
- Valving
- Mixing
- Reagent transfer
- Dry reagent rehydration
- Lysis
- Incubation
- Readout

Graph:
- Time [min]: 0, 1, 2, 3, 4, 5, 30
- Temperature [°C]: -20, 0, 20, 40, 60, 80

- Swab input to Sample release
- Sample release to Sample metering
- Sample metering to Reagent release
- Reagent release to Reagent metering
- Reagent metering to Pneumatic pumping
- Monolayer assembly to Droplet generation
- Droplet generation to Valving
- Valving to Mixing
- Mixing to Reagent transfer
- Reagent transfer to Dry reagent rehydration
- Lysis
- Incubation
- Readout
Fluorescence readout

Total processing time from swab input to readout: 30 min
Verification of complete protocol from sample to digital answer

1) Bacterial recovery after fluidic processing
   - Extraction of bacterial suspension from disk after fluidic processing, plating and colony counting
   → Recovery rate ~ 70 %
Verification of complete protocol from sample to digital answer

1) Bacterial recovery after fluidic processing
   - Extraction of bacterial suspension from disk after fluidic processing, plating and colony counting
     → Recovery rate ~ 70 %

2) Amplification efficiency
   - RPA with synthetic DNA template (Bi-plex - species and resistance gen)
     → Amplification efficiency ~ 56 %
Confirmation of complete protocol from sample to digital answer

1) Bacterial recovery after fluidic processing
   - Extraction of bacterial suspension from disk after fluidic processing, plating and colony counting
     → Recovery rate ~ 70%

2) Amplification efficiency
   - RPA with synthetic DNA template (Bi-plex - species and resistance gen)
     → Amplification efficiency ~ 56%

3) Combined amplification and lysis efficiency
   - RPA with bacteria and lysis inside the droplets
     → Combined assay efficiency ~ 30%

→ Currently under development: Lysis- and amplification efficiency
Various form factors of centrifugal cartridges (1/2)

LabDisk

LabSlice

LabCard

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Summary

Centrifugal microfluidics
- No pumps & no tubings
- No issues with gas bubbles
- Easy to use
- Negligible dead volume
- Digital assays via droplet generation

From the MicroTAS perspective …
- Sample-to-answer analysis
- Cross contamination free liquid handling
- Validated with pathogens of clinical relevance
- Digital assays for fast and accurate results
- Scalable & monolithic fabrication
- Platform approach reduces R&D effort
Acknowledgements

Centrifugal microfluidics team

- Oliver Barth, Daniel Baumann, Lisa Becherer, **Dr. Nadine Borst**, Stefan Burger, **Dr. Gregor Czilwik**, Dr. Katharina Dormanns, Dr. Lisa Drechsel, Dr. Susanna Früh, Jacob Hess, Sebastian Hin, Dr. Ana Homann, Dr. Tobias Hutzenlaub, Dr. Michael Jehle, Benita Johannsen, **Peter Jülg**, **Dr. Mark Keller**, Elena Kipf, Jan-Niklas Klatt, Dominique Kosse, Harald Kühnle, Dr. Michael Lehnert, Dr. Jia Li, **Dr. Daniel Mark**, Martin Meyer, Dr. Konstantinos Mitsakakis, Guido Müller, **Dr. Nils Paust**, **Christelle Robelin**, Dr. Markus Rombach, Benjamin Rutschinski, **Martin Schulz**, Ingmar Schwarz, Dr. Frank Schwemmer, Dr. Mara Specht, **Dr. Felix von Stetten**, Dr. Rouven Streller, **Dr. Oliver Strohmeier**, Philipp Tepper, **Steffen Zehnle**, Dr. Roland Zengerle, Yunpeng Zhao, …

Microfluidics and BioPrinting team

- Björn Gerdes, Ludwig Gutzweiler, Dr. Csaba Jeney, Sabrina Kartmann, Fritz Koch, Dr. Peter Kolay, Julian Riba, Dr. Lutz Riegger, Cheng-Han Tsai, Dr. Stefan Zimmermann

Alumni

- Dr. André Groß, **Dr. Friedrich Schuler**, Dr. Jonas Schöendube, **Dr. Fabian Stumpf**, Dr. Laurent Tanguy, Dr. Thomas van Oordt, Dr. Sandeep Kumar Vashist, Dr. Simon Wadle, Mirjam Weil, Dr. Azmi Yusof
Tank you!

Appendix