1

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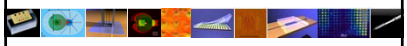
The Microfluidic probe: a non-contact scanning microfluidic technology

Emmanuel Delamarche, Robert Lovchik
Govind Kaigala, Julien Cors
http://www.zurich.ibm.com/st/bioscience/



2

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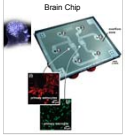
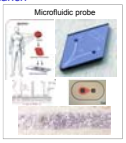
Experimental Biosciences @ IBM Research – Zurich

Chips for Life

Brain Chip



Microfluidic probe



3

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What is special about the microfluidic probe?

"classical" closed microfluidic system

"A nanoliter-scale nucleic acid processor with parallel architecture" Stephen R Quake et al., Nature Biotechnology 22, 435 - 439 (2004)

***The Microfluidic Probe (MFP)**: a non-contact, scanning microfluidic system*

"Multipurpose microfluidic probe" D. Juncker, H. Schmid, E. Delamarche, Nature Materials, 2005, 4, 622-628.

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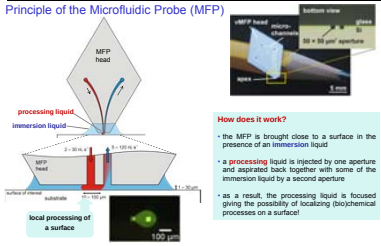
Principle of the Microfluidic Probe (MFP)



How does it work?

- the MFP is brought close to a surface in the presence of an **immersion** liquid
- a **processing** liquid is injected by one aperture and aspirated back together with some of the immersion liquid by a second aperture
- as a result, the processing liquid is focused giving the possibility of localizing (bio)chemical processes on a surface!

G. Kaigala, R. Lovchik, U. Drechsler, E. Delamarche, "A **vertical** microfluidic probe," *Langmuir*, 2, 2011, 5686–5693.
D. Juncker, H. Schmid, E. Delamarche, "Multifunctional **microfluidic probe**," *Nature Materials*, 4, 2005, 622–628.

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Video showing the confinement as a function of Qa/Qi

confinement volume << 1 nL !!

video available at:
http://www.zurich.ibm.com/st/video/nmat1435-s1.mov

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MFP – Research Landscape

David Juncker
McGill, Canada (3)

Owe Orwar and Aldo Jesorka
Chalmers, Sweden (2)

Tomokazu Matsue
Tohoku University, Japan (1)

1. H. Shiku *et al.*, "Microfluidic dual capillary probe to collect messenger RNA from adherent cells and spheroids," *Analytical Biochemistry*, 2009, 385, 138 -142.
2. A. Arida *et al.*, "A Microfluidic Pipette for Single-Cell Pharmacology," *Analytical Chemistry*, 2010, 82, 4529-4536.
3. M. A. Qasaimeh, T. Gervais, and D. Juncker, "Microfluidic quadrupole and floating concentration gradient," *Nature Commun.*, 2, 2011, 464.

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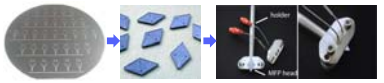
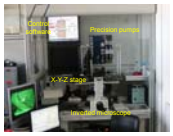
Our specific MFP implementation



Key points

- compatible to standard silicon manufacturing processes
- 100s of MFP heads / wafer
- many designs co-fabricated
- reasonably easy to set-up and use system
- requires standard inverted microscope



G. Kaigala, R. Lovchik, U. Drechsler, E. Delamarche, "A vertical microfluidic probe," *Langmuir*, 2, 2011, 5686–5693.

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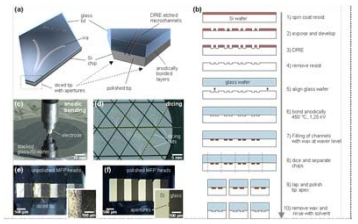
Microfabrication of vertical MFP heads



G. Kaigala, R. Lovchik, U. Drechsler, E. Delamarche, "The vertical microfluidic probe," *Langmuir*, 2011, 2, 5686–5693.

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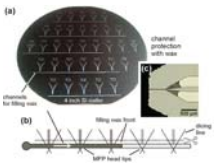
Protecting channels during fabrication – key challenge!



- For improved yield in fabrication wax is filled in the channels to minimize clogging of with debris generated during fabrication and packaging (dicing, polishing).
- Wax could be left in the channel post-processing for packaging and shipping of the MFP heads.

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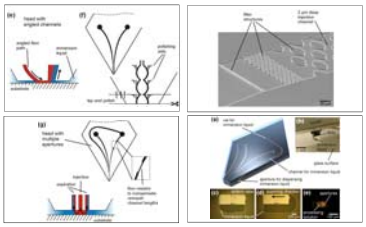
MFP head - Advanced designs



G. Kaigala, R. Lovchik, U. Drechsler, E. Delamarche, "The vertical microfluidic probe," *Langmuir*, 2011, *2*, 5686–5693.

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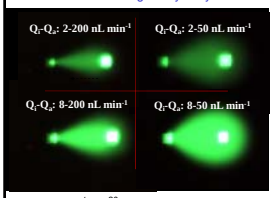
Parameters determining the hydrodynamic flow confinement



variable parameters:
- Q_a and Q_s (nL/min)
- gap between the MFP and substrate (µm)
- writing speed (mm/s)

apertures: 20 µm
distance between the apertures: 120 µm
gap: 2 µm

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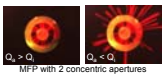
Parameters determining the hydrodynamic flow confinement

- fixed parameters
  - size and shape of the apertures
  - number of apertures
  - relative position of the apertures



MFP with 2 concentric apertures

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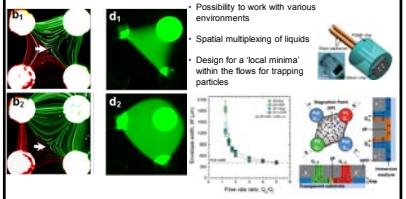
Microfluidic quadrupole and floating concentration gradient



- Possibility to work with various environments
- Spatial multiplexing of liquids
- Design for a 'local minima' within the flows for trapping particles

M. A. Qasaimeh and D. Juncker, 1844-1846, *Proceedings microTAS*, 2010
M. A. Qasaimeh, T. Gervais, D. Juncker, Nat. Commun. 2011, *2*, 464.

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Writing protein patterns using a MFP

- Proteins in the processing liquid deposit on the scanned surface
- no drying artifact due to the presence of the immersion liquid (biological buffer)
- moving quickly the MFP makes the immersion liquid inserting below the processing liquid → **non-writing mode!**

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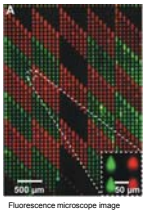
Patterning proteins on a surface using a MFP



Fluorescence microscope image

- 2 types of antibodies were subsequently patterned on an activated glass slide
- array has 1384 spots spaced 80 µm apart
- ~130 pL of antibody solution and 0.3 s writing time per spot
- array needed 300 nL of antibody solution and 15 min writing time

- depositing, removing, delivering and creating protein gradients on surfaces in a way that preserves the protein function
- functionalize surfaces to study advanced cell-surface interactions

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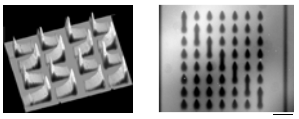
Patterning and removing Proteins onto/from a Surface



- surface-density gradients of proteins on a glass surface
- gradient was formed by varying the writing speed of the MFP

- fluorescently labeled proteins removed from a surface
- the processing liquid contained a surfactant, a high pH and high ionic strength
- proteins adsorbed on a glass slide are removed by the processing liquid

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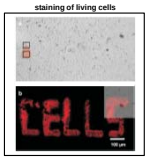
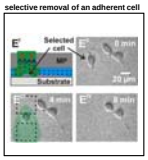
Staining cells & picking cells

staining of living cells



selective removal of an adherent cell



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Microscale tissue staining

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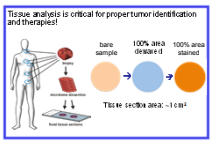
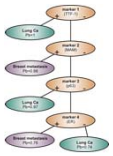
Tissue analysis: Needs and challenges

Tissue analysis is critical for proper tumor identification and therapies!



Strong desire for more and better "quality" information
- multiplexing
- adaptive staining conditions
- use/need for lesser amount of tissue
- supply data for complex decision algorithms
- use/need for lesser amount of reagents (antibodies)



Need for more sample!

H. Ahmadzadeh, L. V. Thompson and E. A Arriaga, *Anal.Bioanal.Chem.*,384, 2006,169.

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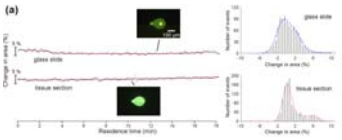
Flow stability and staining contrast

- flow confinement is stable for tens of minutes with area variations < 5% of its mean
- tissue section is not delaminated from glass slide by shear stress from the flow



Human thyroid (normal) tissue section, 4 µm thick; processed with anti-thyroglobulin (anti-TGB)

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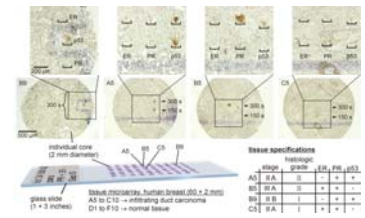
Tissue Microarrays

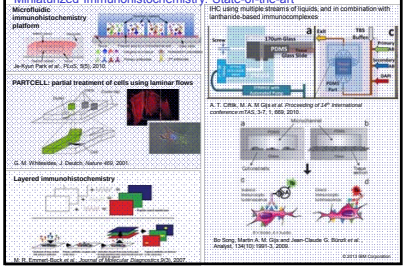
- breast cancer tissue cores (30 individuals)



R. D. Lovchik, G. V. Kaigala, M. Georgiadis, E. Delamarche,"Micro-immunohistochemistry with the microfluidic probe" *Lab on a Chip*, 12, 2012, 1040–1043.

20

Miniaturized Immunohistochemistry: State-of-the-art



Je-Kyun Park et al., PLoS, 5(6), 2010.

G. M. Whitesides, J. Daniel, *Nature* 450, 2007.

M. R. Emmert-Buck et al., *Journal of Molecular Diagnostics* 9(2), 2007

A. T. Cibik, M. A. M Gijs et al. *Proceeding of 14th International conference on* TAS, 3-7, 1, 689, 2010.

Bo Song, Martin A. M. Gijs and Jean-Claude G. Bünzli et al., *Analyst*, 134(10) 1991-3, 2009.

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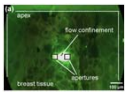
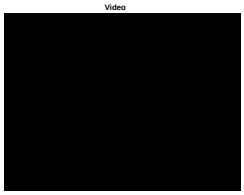
Flow stability on a human cancerous thyroid tissue section



Video



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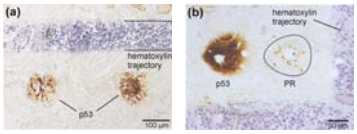
Tumor tissue processing using the vMFP

- breast cancer tissue sections – anti-p53 antibodies (spots), anti-progesterone receptor (PR) antibodies and hematoxylin line



Monoplexed Multiplexed

R. D. Lovchik, G. V. Kaigala, M. Georgiadis, E. Delamarche,"Micro-immunohistochemistry with the microfluidic probe" *Lab on a Chip*, 12, 2012, 1040–1043.

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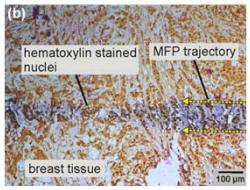
First steps with local staining: Counter staining using a MFP



stain	target
DAPI	nucleus
phalloidin	actin
hematoxylin	nucleus
eosin	cytoplasm

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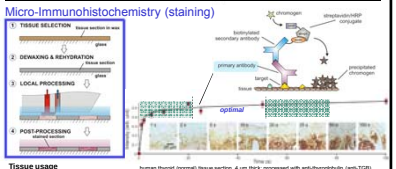
Micro-Immunohistochemistry (staining)



human thyroid (normal) tissue section, 4 µm thick; processed with anti-thyroglobulin (anti-TGB)

Tissue usage

5% area stained
→ each spot 100 ×100 µm²
→ 5% area for optimization
→ 5% of tissue area corresponds to **500 spots**

reduced processing/incubation time probably due to increased convection

R. D. Lovchik, G. V. Kaigala, M. Georgiadis, E. Delamarche,"Micro-immunohistochemistry with the microfluidic probe" *Lab on a Chip*, 12, 2012, 1040–1043.

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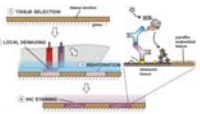
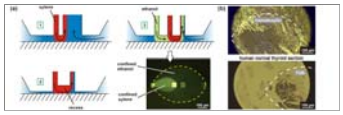
Local dewaxing

- key goal: local staining AND local dewaxing
- a sample is not "spent" after a single analysis
- enabling retrospective analysis taking advantage of the >1.5 billion tissue samples i bio-banks?

Workflow



MFP head designs and local dewaxing



G. Kaigala et al., µTAS 2012 | October 29, 2012

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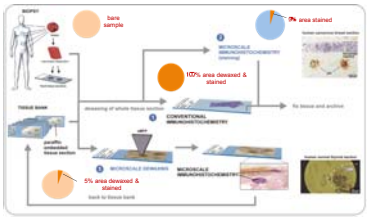
Microscale staining & dewaxing – on-going work



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Thank you!

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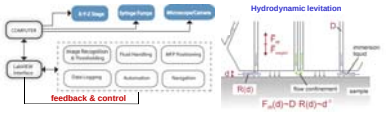
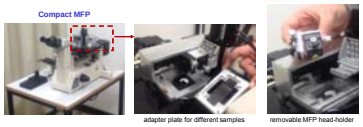
New ideas and concepts for the MFP



Hydrodynamic levitation

Compact MFP



adapter plate for different samples

removable MFP head-holder

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Summary

- The MFP is a non-contact microfluidic technology; it works in the presence of a surrounding liquid (no drying artifacts)
- it can write patterns with ~5 µm accuracy with additive and subtractive surface patterning processes
- it is very versatile
- it represents an exciting opportunity for microscale tissue section processing

Acknowledgements

Marios Georgiadis (now at ETH-Zurich), Martina Hitzbleck
Ute Drechsler, Michel Despont and Walter Riess

IBM ($$$) and "BioProbe" ERC grant (EU) ($)