Oberflächensensorik mit hoher räumlicher Auflösung Mittels Whispering Gallery Modes

Netzwerktreffen Tribologie, Bad Sachsa

11.03.2022

L. Dähne, M. Olszyna, G. Dähne,
M. Himmelhaus,
C. Guernth-Marschner, M. Kirschbaum,

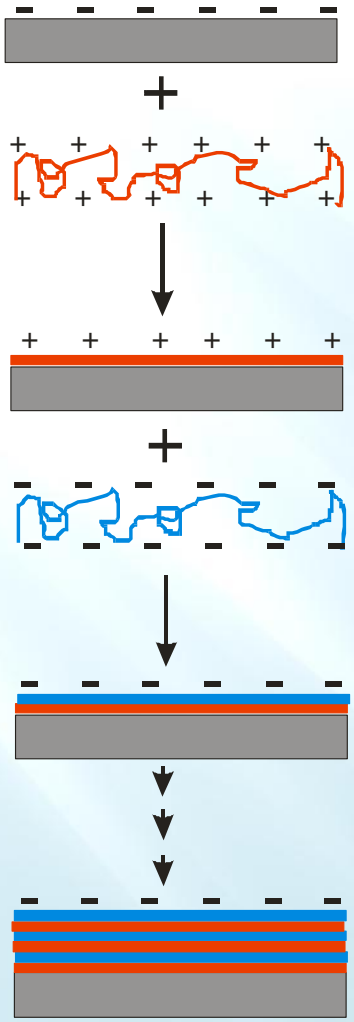
Surflay Nanotec GmbH
NanoBioAnalytics
Fraunhofer Institute for Cell therapy and immunology (ICI)

The Companies

Surflay (**Surf**ace **Lay**ers) founded 2008 in Berlin

- Privately owned Research company, no investors
- 2022: 9 scientists, 1 technician
- Interdisciplinary team: Polymer-, Synthetic-, and Physical Chemists, Pharmacist, Biotechnologists, Engineer
- Surface (bio-) functionalization by Layer-by-Layer LbL-technology
- Monodisperse Nano- and microparticles; Microsensors for pH, T, O_2
- Microsensors for solvent traces in water
- Fluorescence labeled polymers and biomolecules

Controlled complex formation:Layer by Layer (LbL-technology)



Charged Substrate (planar, colloidal, porous, surface structured)

Polycation in excess, aqueous solution 1g/l,
Control of pH, ion strength

Self-limited adsorption, charge reversal
(ζ-potential + 60 mV), removal excess polyelectrolyte

Polyanion in excess

Thickness per double layer
3 nm for PAH/PSS

Layer by Layer (LbL)
coated substrate

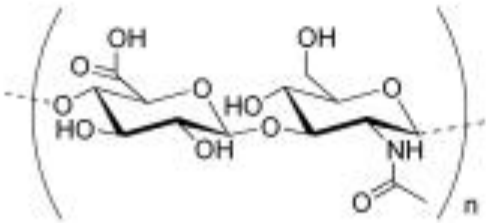
G. Decher 1991, reviewed in
Science 277 (1997) 1232

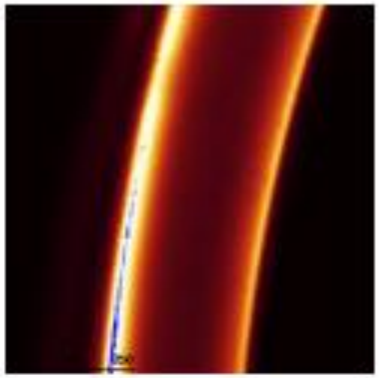
Outermost layer highly charged and hydrated

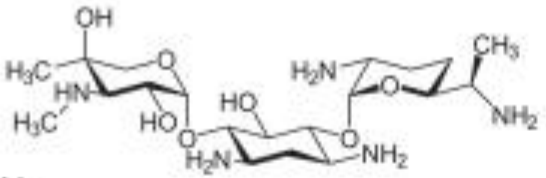
Layer-by-layer coating of the HyperCL

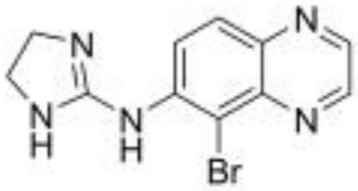
SURFLAY

- A labelled coating polymer was applied
  - Uniformly distributed 12 nm layer
  - The polymer penetrates slightly the CL
  - charged drugs for embedding







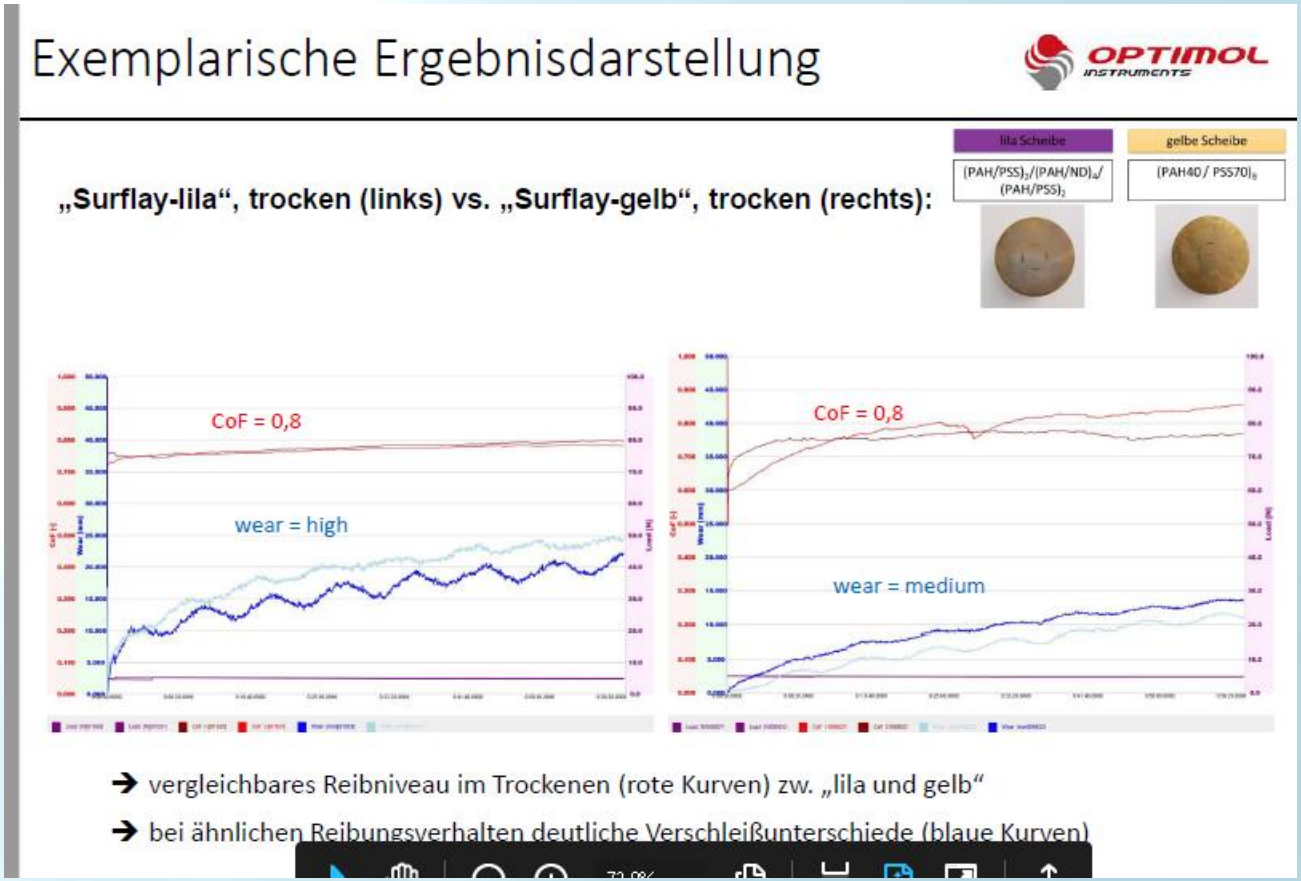


Fraunhofer IAP

Selected LbL Applications: Hair coloration (P&G)

- Covalent linkage of different dyes with polycations
- 4 layers on hairs (two polycations) after washing

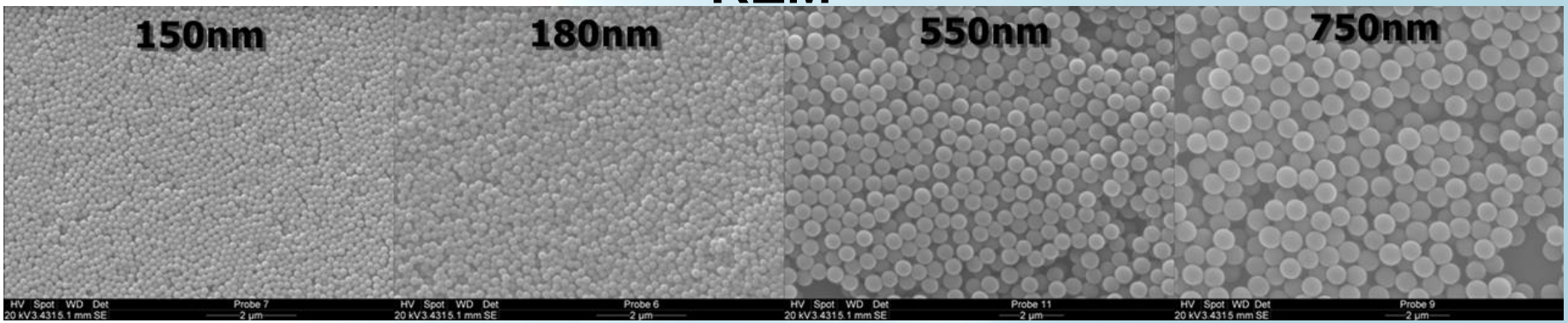
Selected LbL Applications: Friction (Optimol)



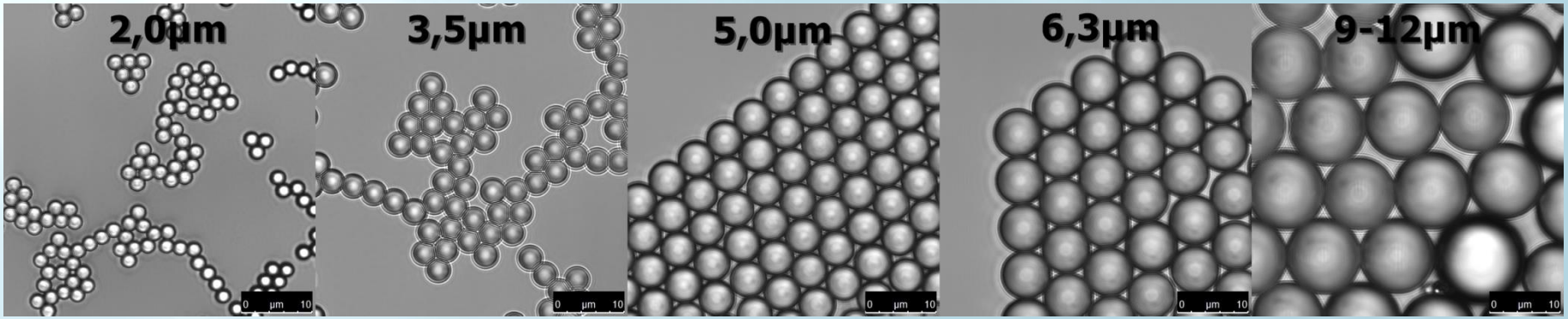
- different layer combinations:
- due to high osmotic pressure less friction in water

Particle preparation (Polystyren, PMMA, MF)

REM



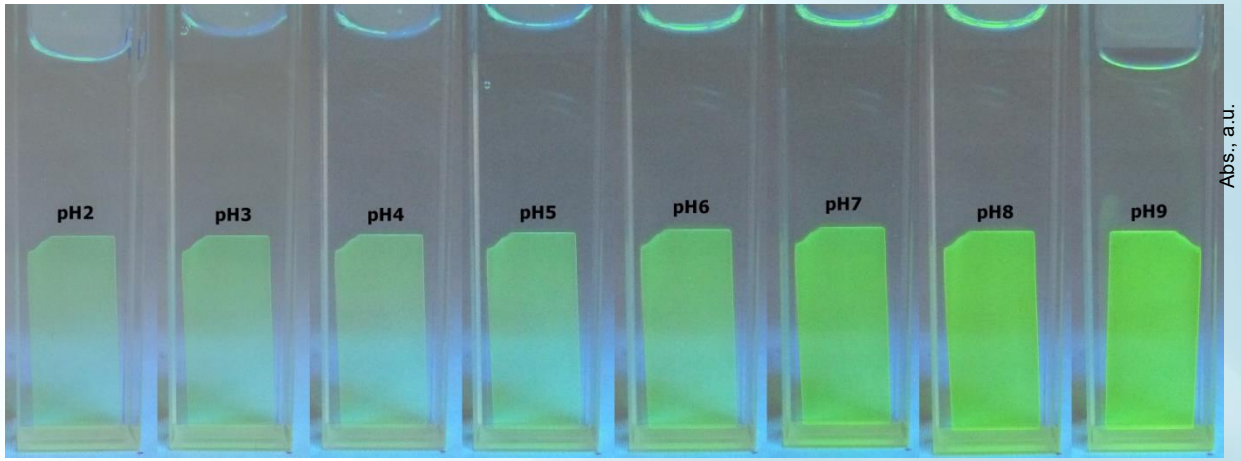
CLSM



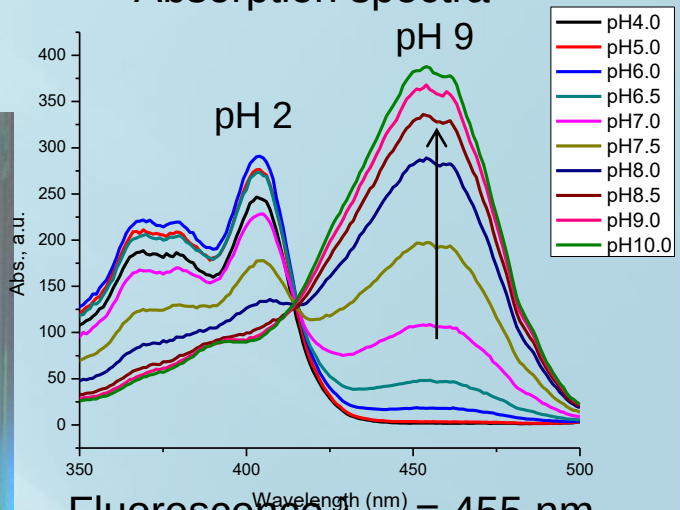
→ PS-Partikelgrößen (monodispers) : 100nm – 100 µm

LbL-immobilized pH and oxygen sensors (Glas of Prof. Enke, Leipzig)

→ increasing pH value

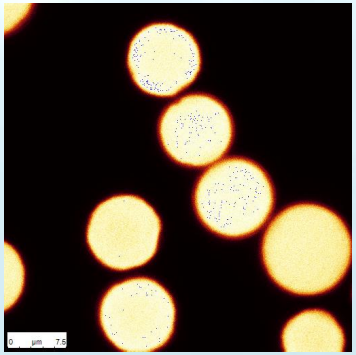


Absorption spectra



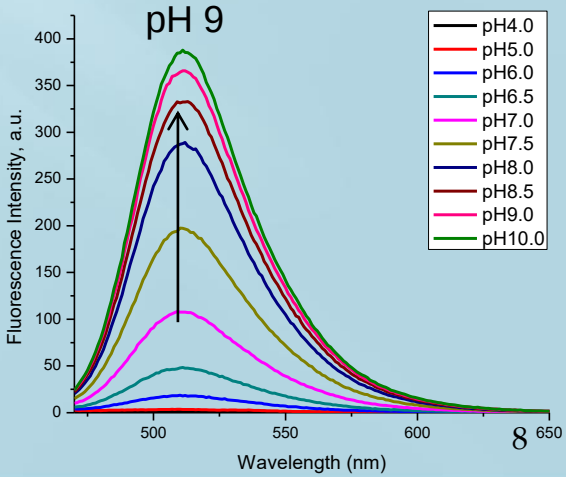
Fluorescence λ_{exc} = 455 nm

Fluorescence of porous Glas-Slights (20x8x0.5 mm³ coated with PEI-HPTS/PSS from pH2 til pH9



PEI-HPTS coated porous SiO_2-Particles 10 µm

Fluorescence: internal Reference necessary: Adding Cy5 dye pH independent



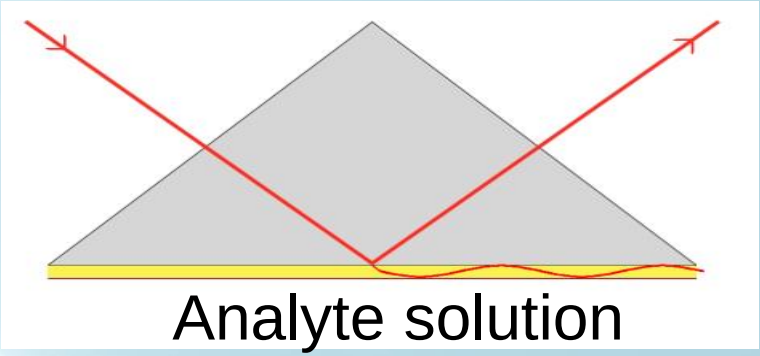
Label free analysis of specific Interactions on surfaces

- Surface Plasmon Resonance (SPR)
• Biacore, Chips 60 - 400 €

- Quartz Crystal Microbalance
• Chipsensor 25-100 €

-**Ellipsometry**
• large planar surface

- **Reflection Interferometry RIFs**
• smaller planar tips

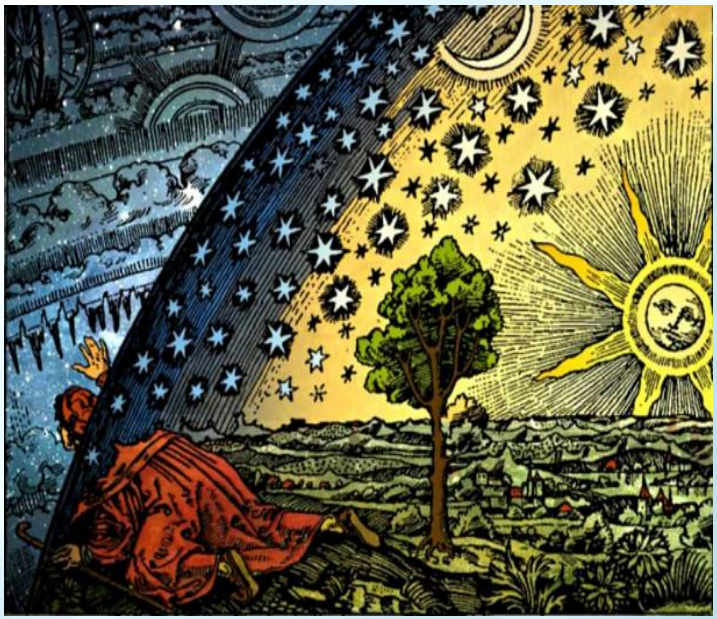


Planar wave on gold coated
Surface area, area 16 mm^2

Miniaturization of sensor??

Very old Idea

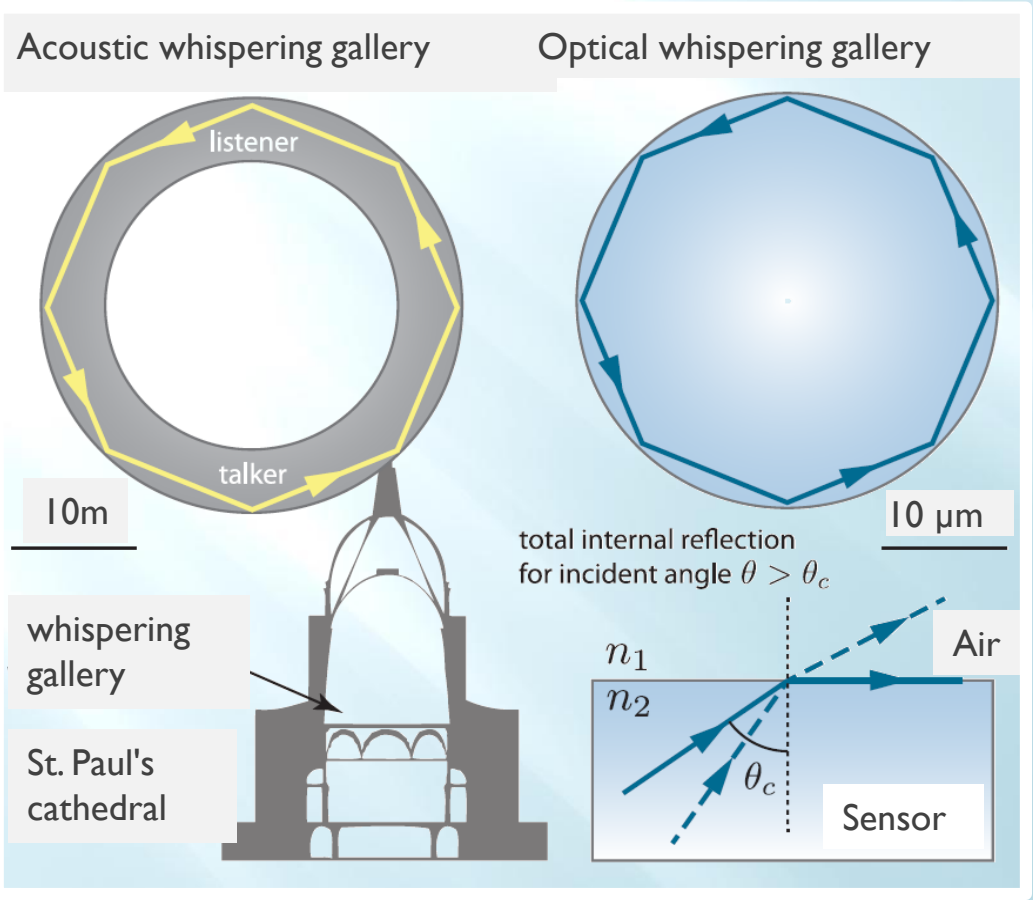
Ancient times:
FLAT is good



Kopernikus:
ROUND is better

- **Transformation of planar in circular waves**
  → Whispering Gallery Modes WGM

Principle Whispering Gallery Modes (WGM)



WGM: Reflexion and Interference from waves in circular cavities of high RI

Acoustic waves: Middle age: Secret listening of important talks

Saint Pauls Cathedral London;

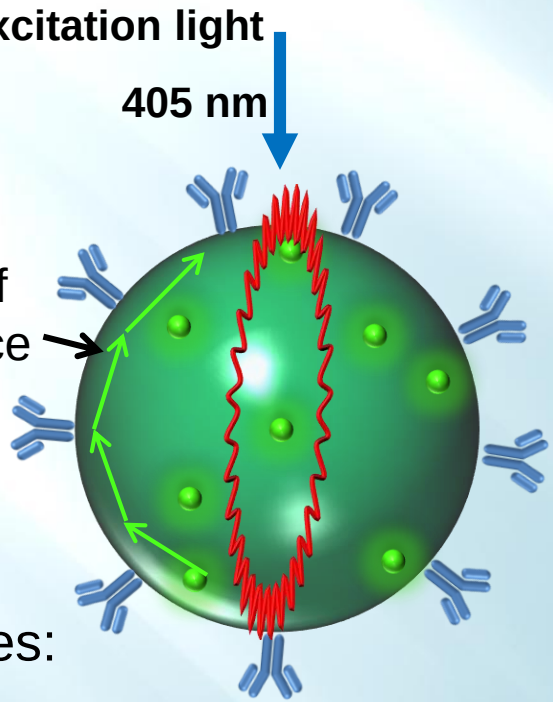
Optical waves: smaller circular space

Light wave totally reflected inner surface,

→ only resonant waves circulates

How to immobilize light waves into media of higher refractive index:

a) Tunneling, b) Low Q WGM in fluorescent particles



Surface area of 10 µm Particle

0.0006 mm^2 (SPR 16 mm^2)

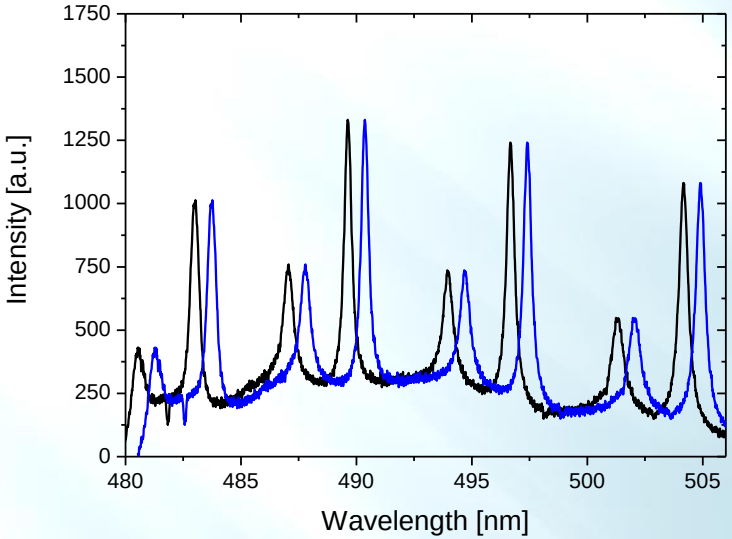
Advantages:

→Tiny sensors → measurements in small cavities

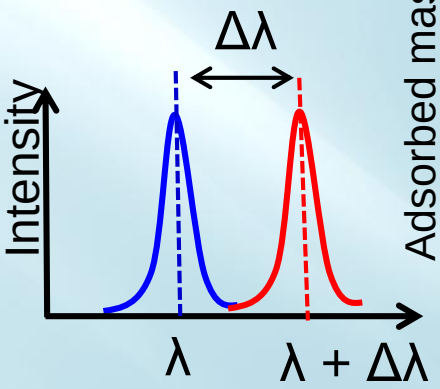
→ No connection to measuring unit necessary → simple use of microfluidic

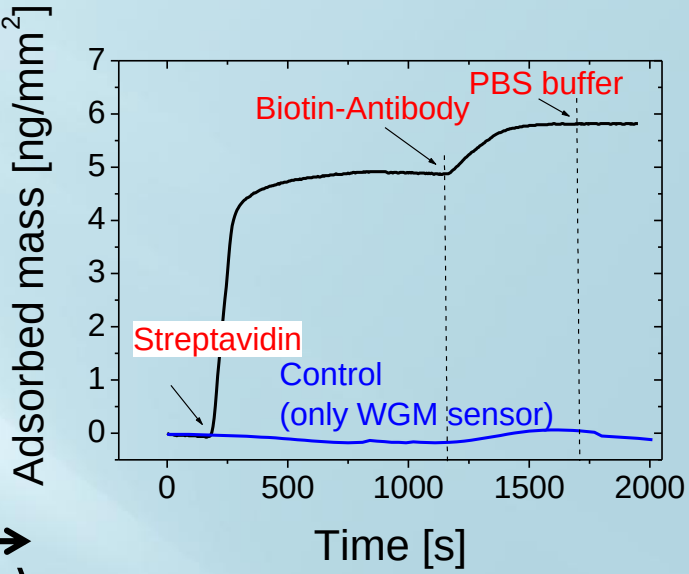
→ Very small amount of analyte molecules needed

Measured signal



Emission spectrum of 1 particle
Before and after adsorption



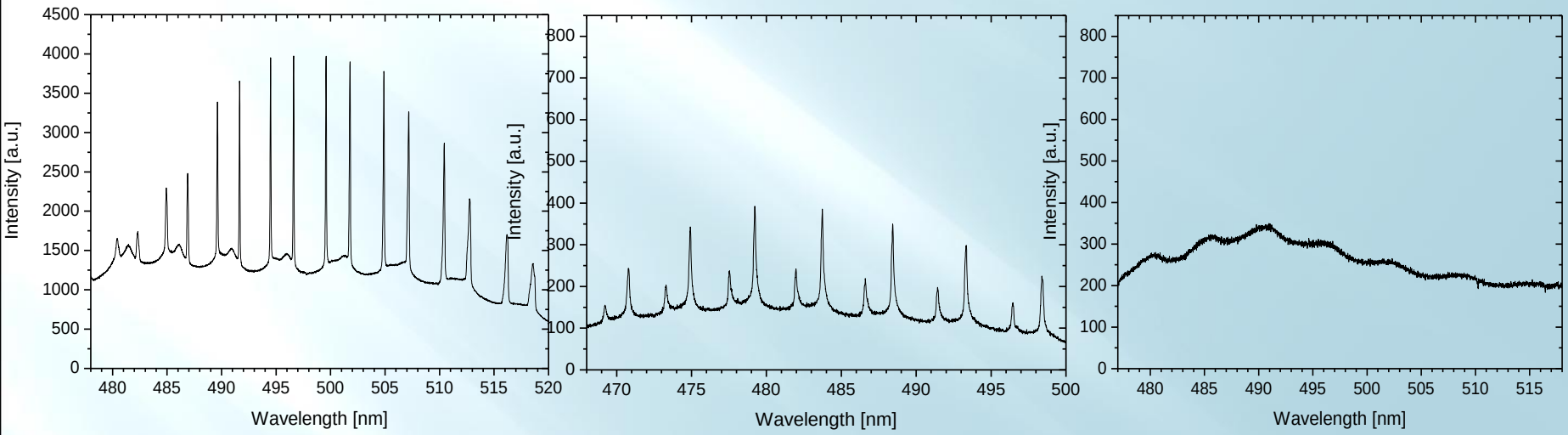


Signal shift transformed
in adsorbed mass
→ Typical adsorption kinetics

- FWHM WGM peaks 150 pm for 10 µm particle
- Shift for monomolecular (antibody) coverage around 400 - 800 pm
- Instrumentation for measuring (WhisperSense) resolution 6-10 pm

Further Miniaturization??

WGM Spectra of different bead diameter



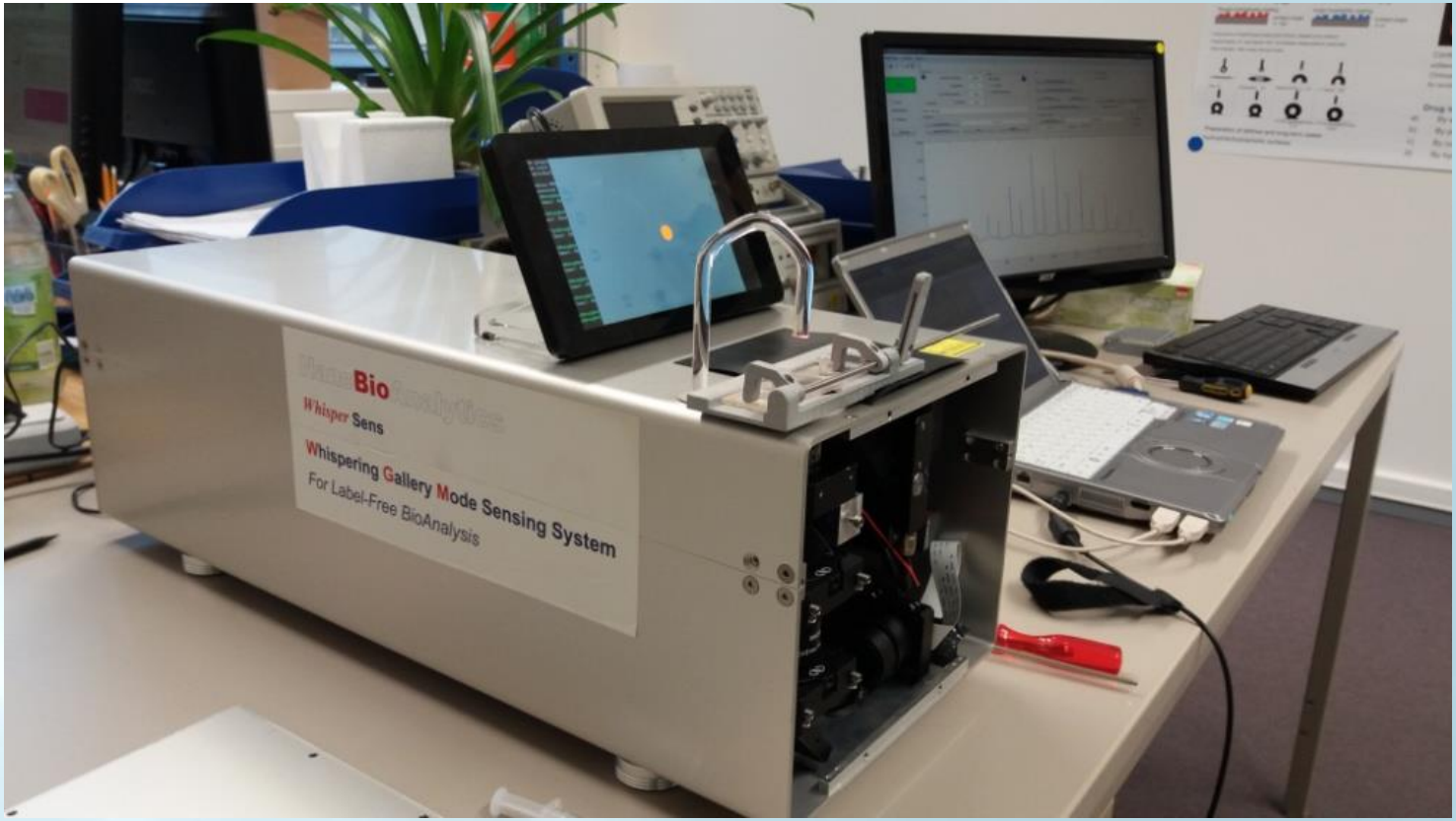
Particle diameter

9.5 µm 7.2 µm 5.8 µm

Decreasing Q-Value →

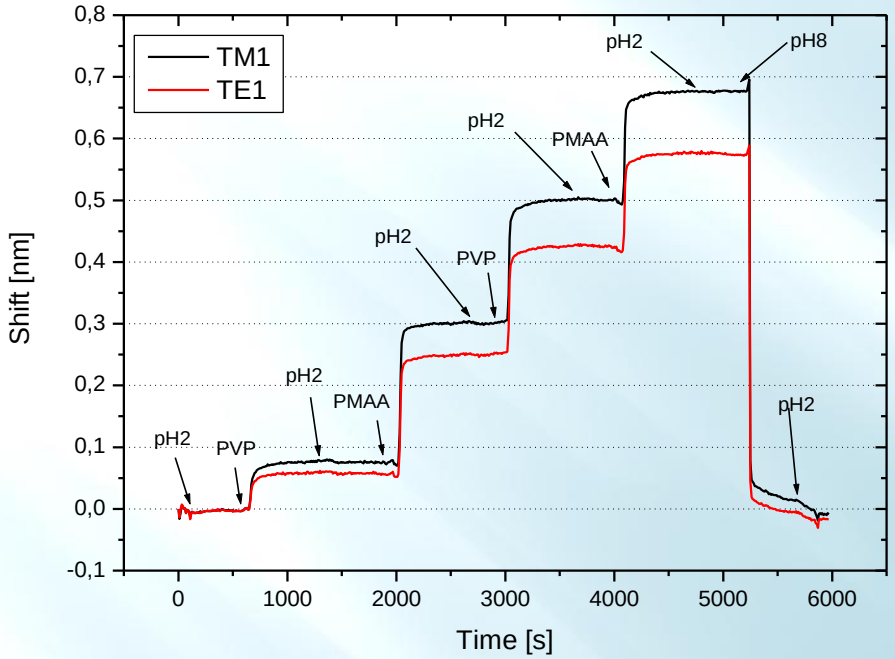
Circ. 10 000 < 3 000 < 500

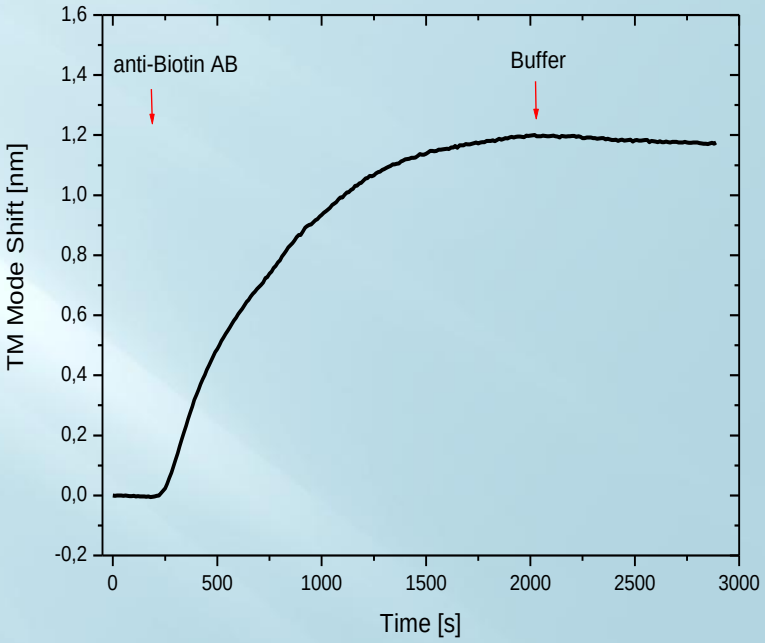
Instrumentation “WhisperSense” (Surflay, NanoBioAnalytics)



Box (25 cm x 30 cm x 58 cm) integrates excitation laser, spectrometer, camera, computer and automatic xyz stage; Wavelength resolution 6-10 pm

Application Examples:





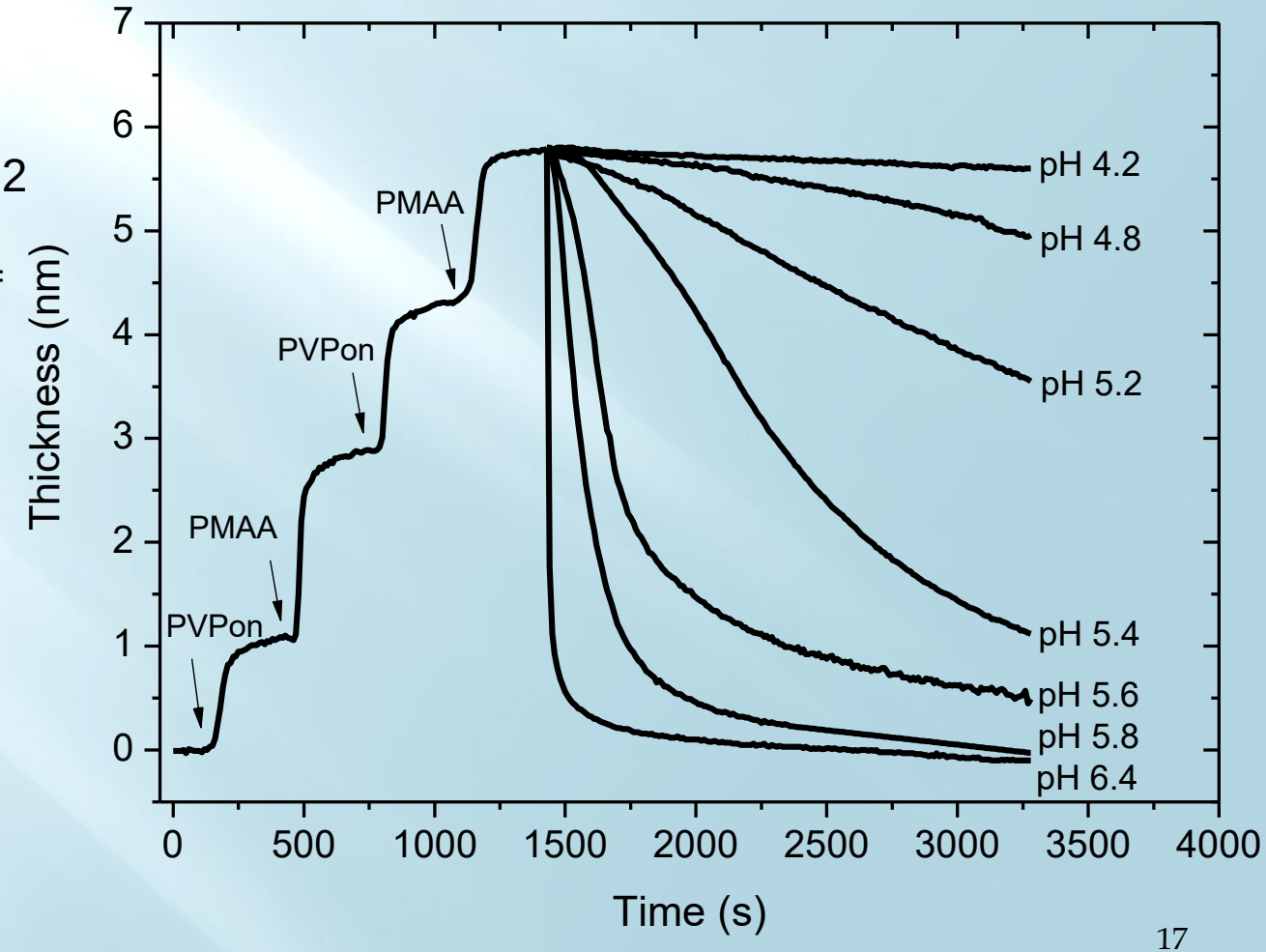
Stepwise Layer-by-Layer Coating of approx.1 nm thick polymer layers + final complete removal of layers

Coupling of antibody anti-Biotin (25 µg/ml) on biotinylated surface

- Typical adsorption kinetics as for SPR or QCM

Application: Release of polymers/drugs from surfaces

- Hydrogen bonded Films: PVP/PMAA pH =2
- pH > 4 deprotonation of PMAA (pKa) = 6.2
- Decreasing H-Bonds
- Increasing negative charge → repulsion.



Difficulty: Immobilisation of sensor particles

→ Even for apparently monodisperse particles: Each particle has individual WGM pattern

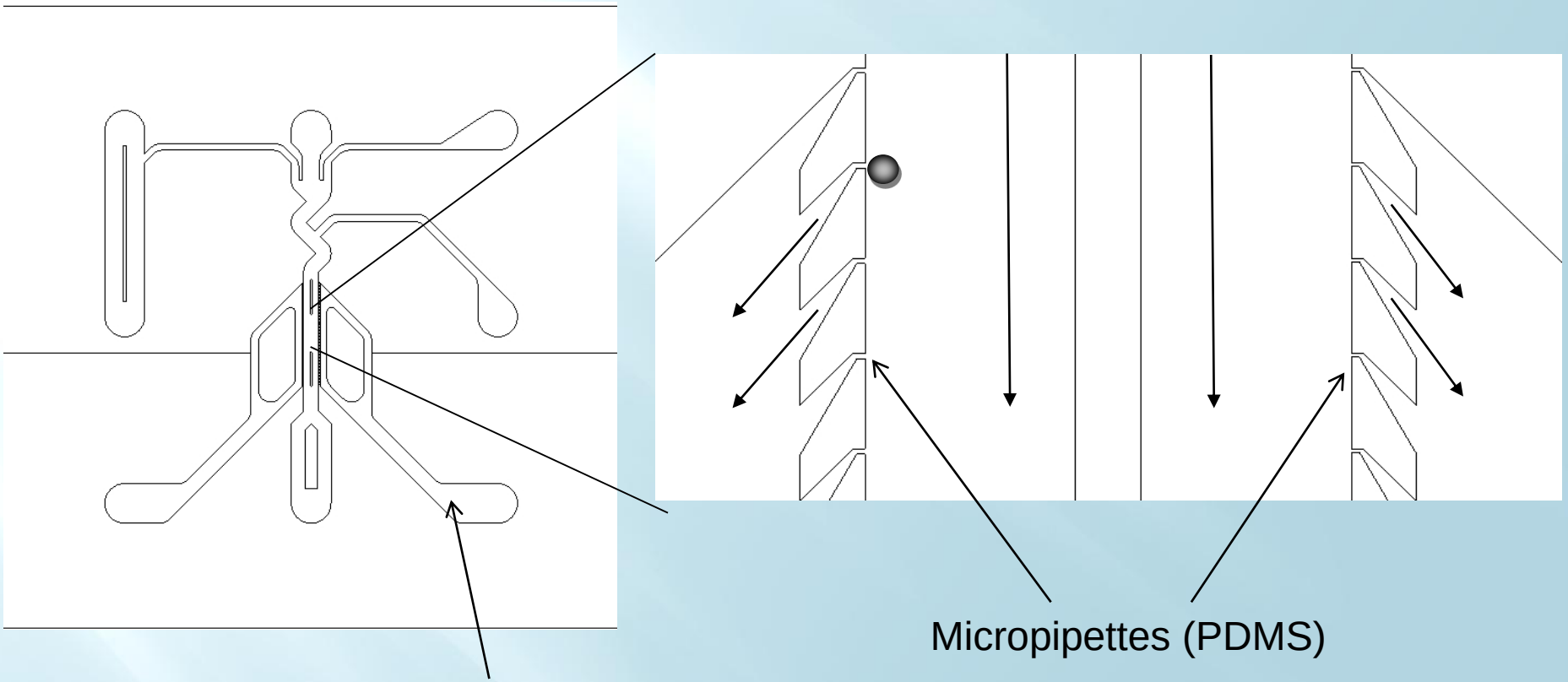
→ measurement on **one and same** sensor particle necessary

→ Requires immobilisation of 10 µm particles in analyte stream

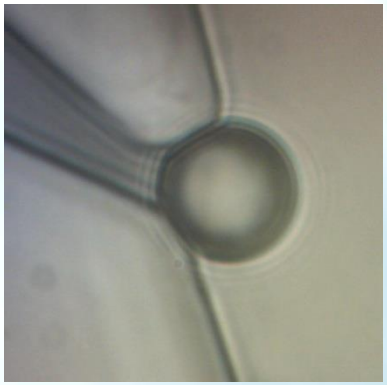
Possible (microfluidic) approaches:

1. Micropipettes
2. Pillar structures
3. Laser tweezer
4. Dielectrophoretic immobilization
5. Well arrays
6. Water droplets in oil phase
7. Between cells in cell culture

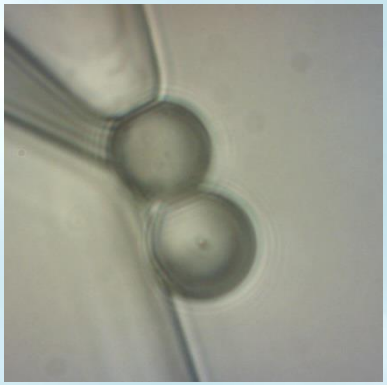
1. Micropipettes



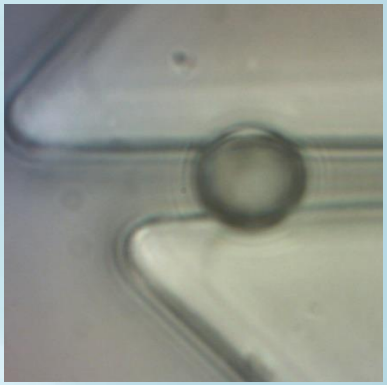
Micropipettes



I



II

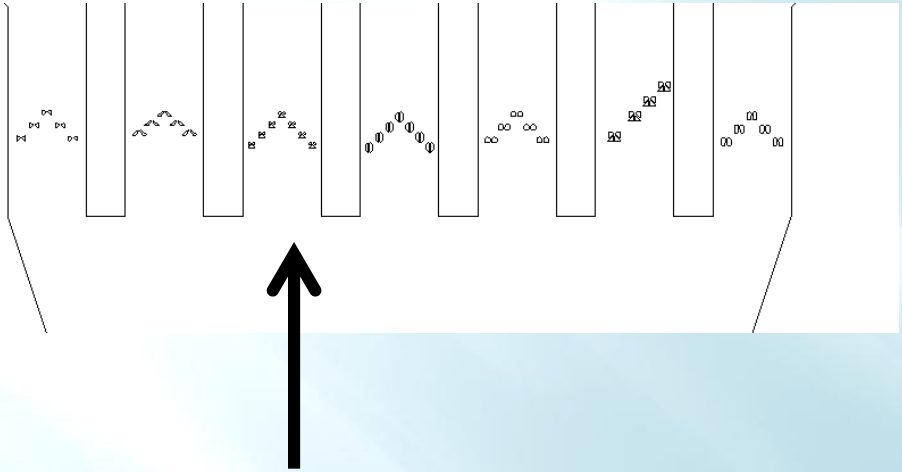


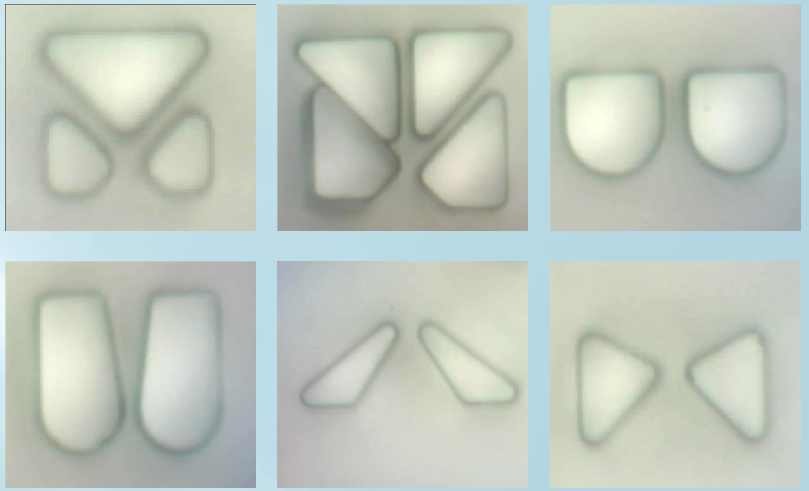
III

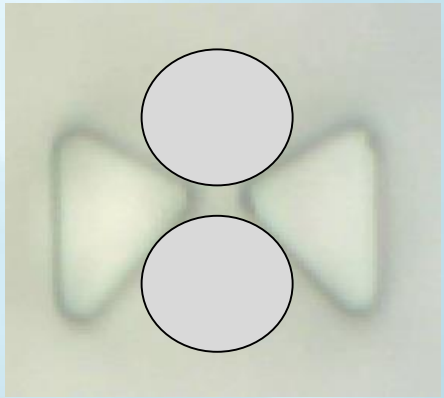
PDMS too soft, Contact phase to particle too large

→ Disturbing WGM signal

2. Pillar blocking structures

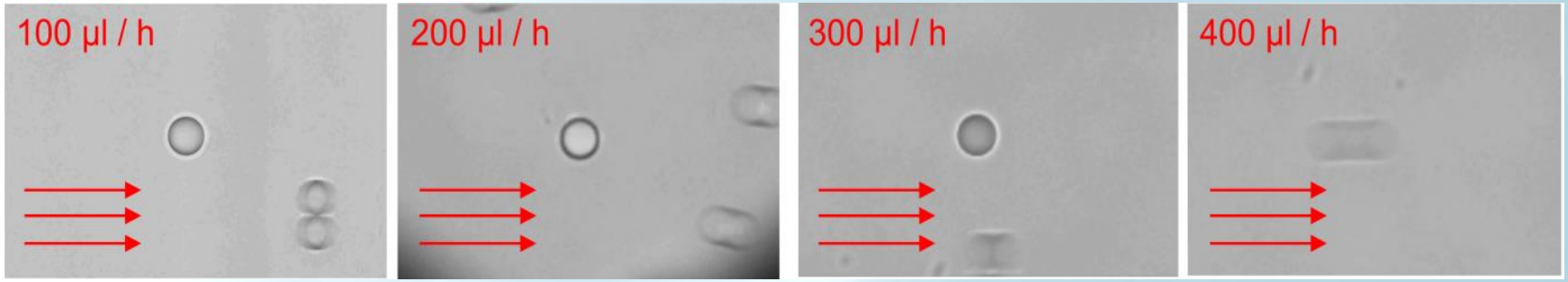




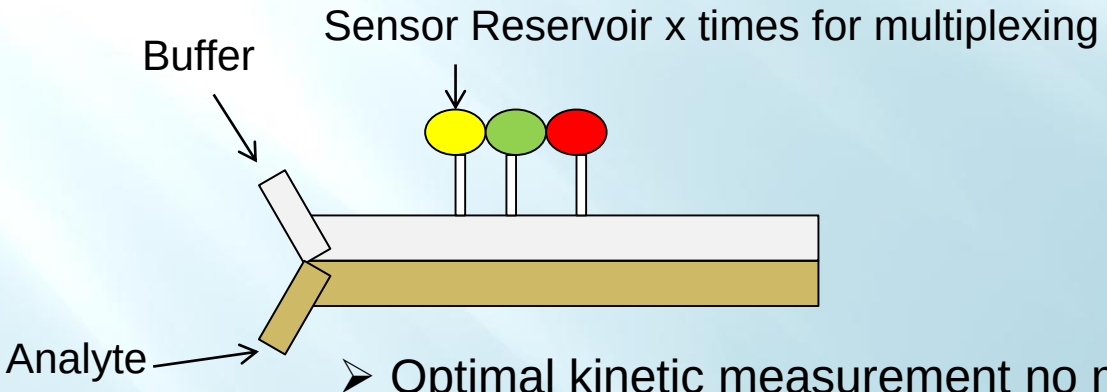


- Pillars of PDMS too weak
- Stream from backside
- Removal of used particles not possible by backflushing

3. Laser Tweezers

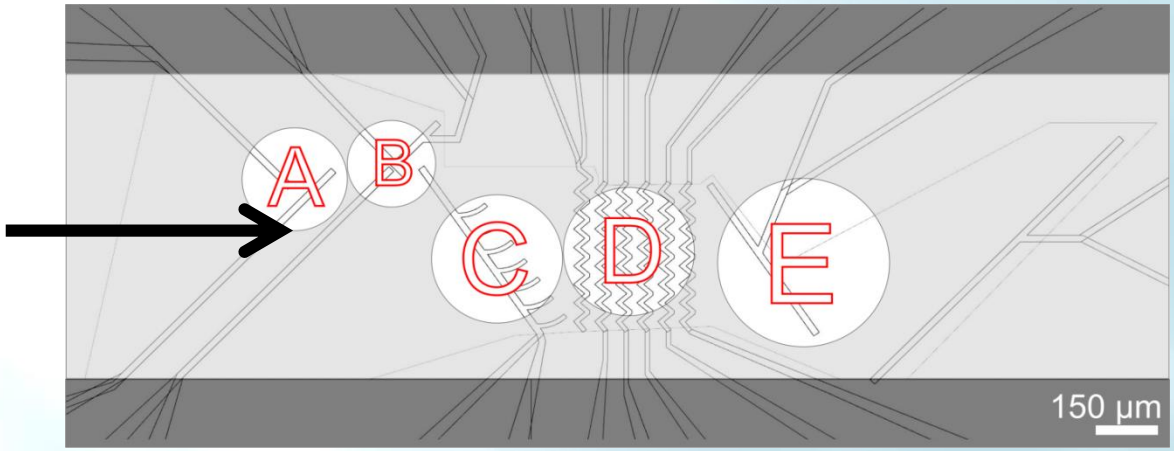


Continuous long term measurement

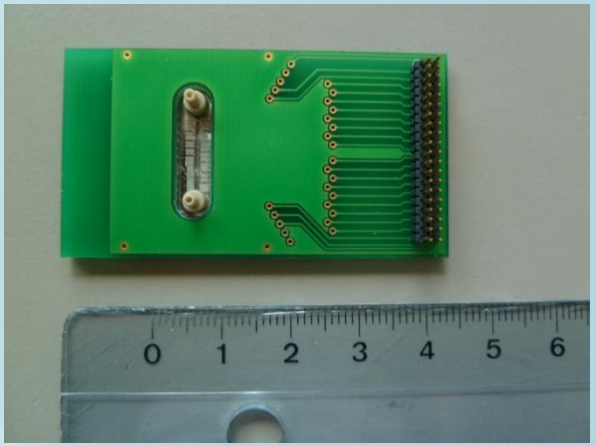


- Optimal kinetic measurement no mixing effects in microfluidic
- WGM Signal not influenced by Laser tweezer
- Promising technology
- Implementation in WGM instrument exceeds our present capacity

4. Dielectrophoretic immobilisation



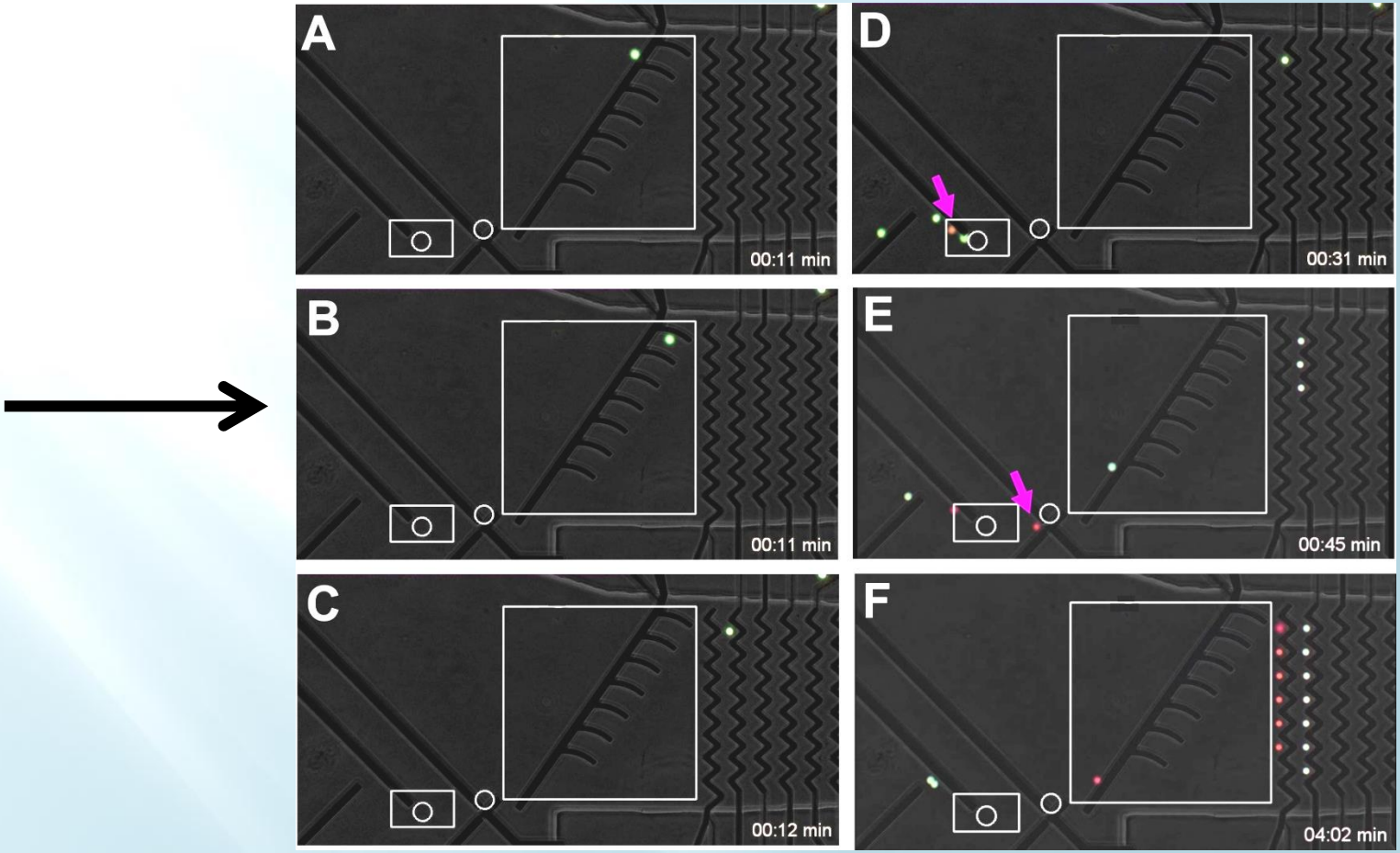
Arrangement of electrodes for particle sorting



Dielectrophorese-Chip

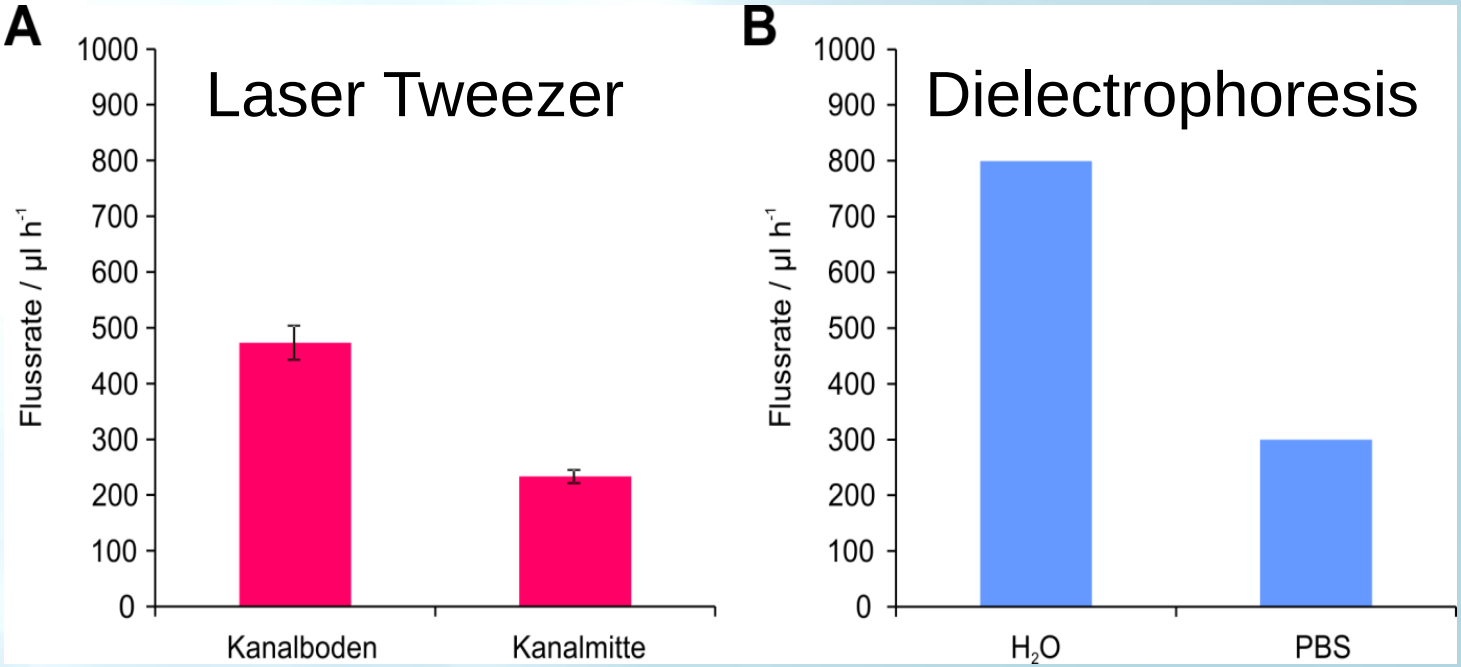
- Dielectrophorese chip for continuous particle handling
- Electrode structures for particle handling
- Separate switch On/Off

Dielectrophoretic immobilisation



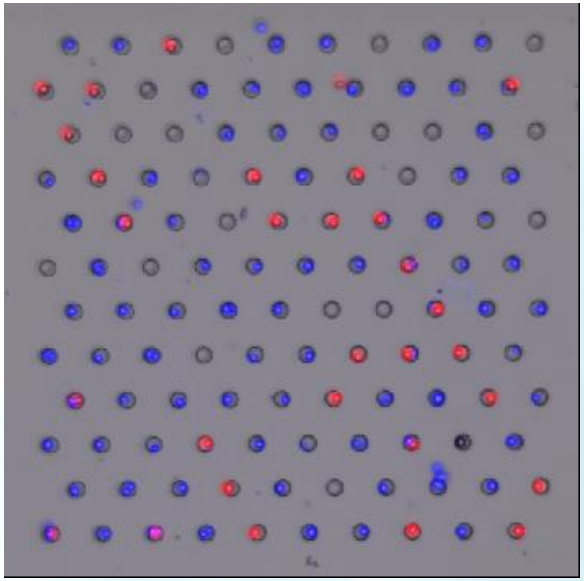
SURFLAY

Possible Flux rates

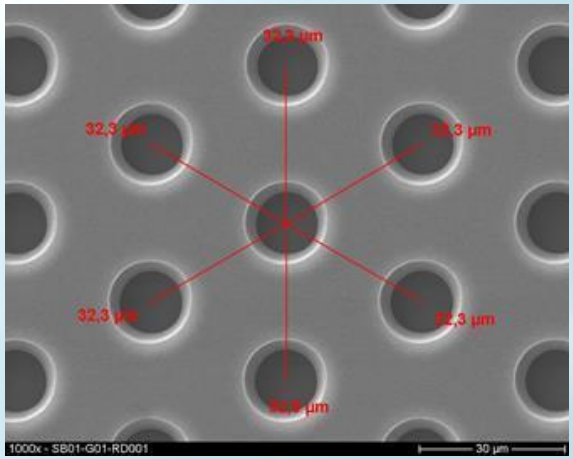


Flux rates limited, approx. 20 times less than Biacore

Multiplexing: Immobilisation of particles in microfluidic well arrays



500 µm x 500 µm



Bottom

Stratec Consumables GmbH

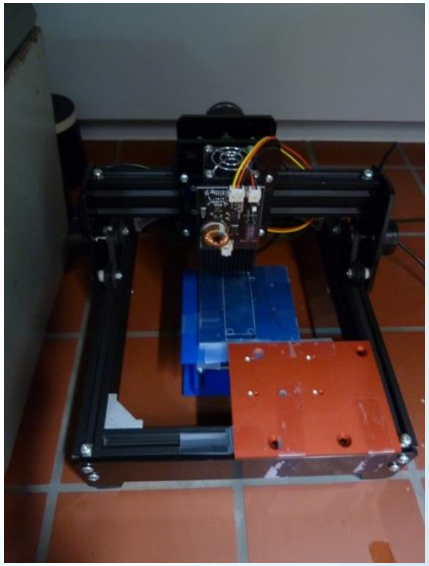
Excellent structures, d = 12 µm

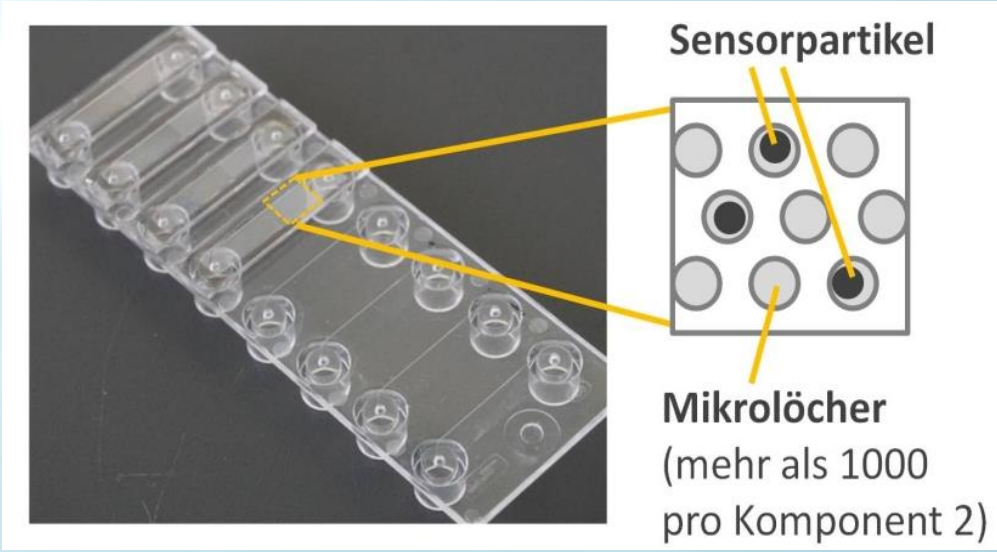
1 000 well array

Microscopic image of immobilised Beads in cylindric wells,

Only 1 particle/well possible

Microfluidic cells





Connection of Stratec Well array with Lids of ChipShop;
Laser welding, 6 channels a 1000 sensors
→ Enables Multiplexing and High statistic safety

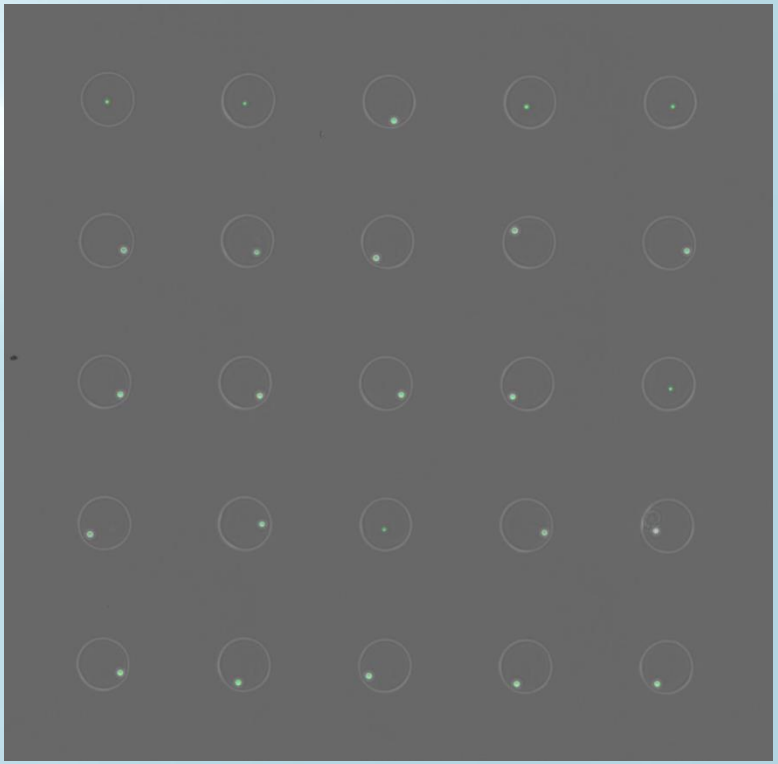
Multiplexing: a) bead position,
b) bead size 6-12 µm
c) fluorescence encoding

Multiplexing by bead position

Requires different beads in defined positions (of STRATEC arrays)

→ New spotting technology of SCIENION enables defined positioning of cells or particles

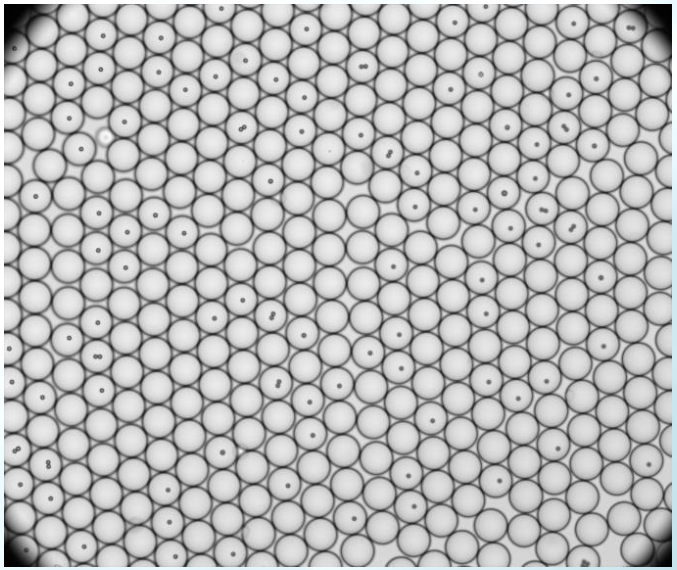
(Image of spotted WGM particles→



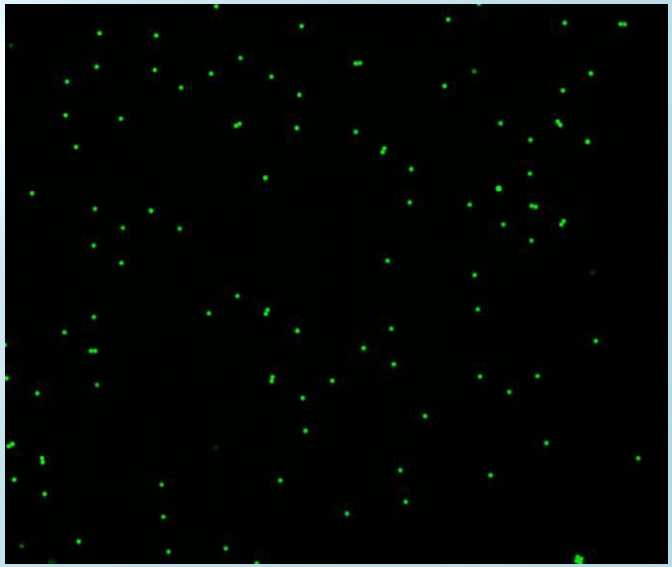
Automatic xy positioning in WGM find particles within the circles)

BMBF Project „ImmunoPlex“ with SCIENION and DIARECT

6. WGM beads in droplets



Transmission



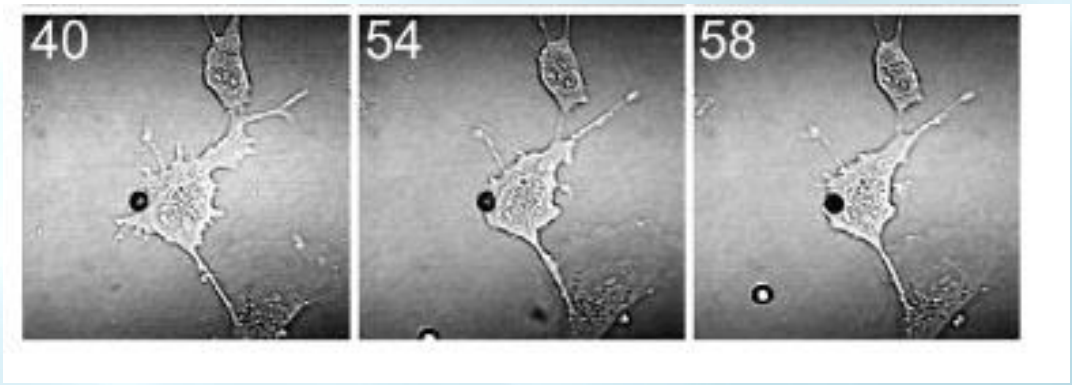
Fluorescence

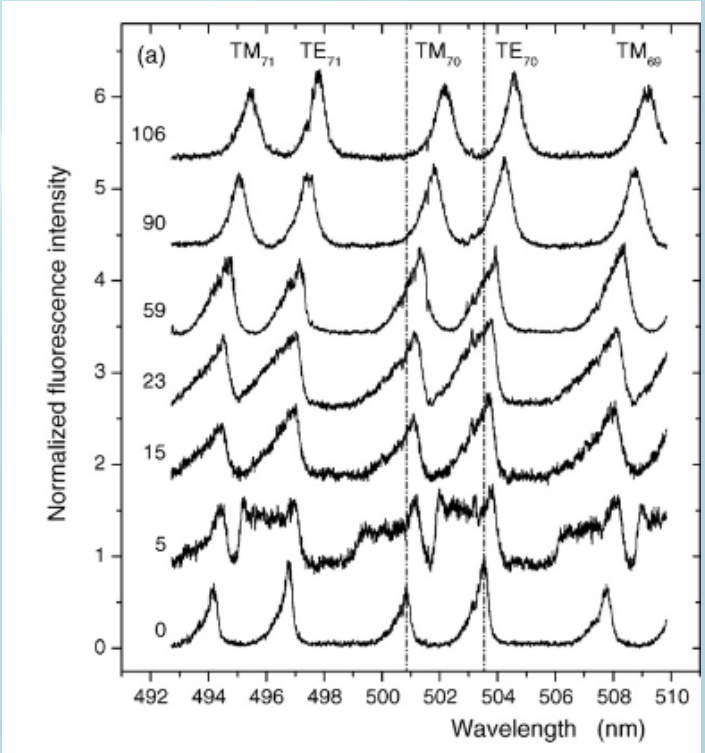
- 200 pL water droplets with partly filled with WGM beads
- Plan: Measurement of released proteins from immobilized single cells

(Partner Oksana Shvydkiv, Hans-Knöll Institut Jena)

7. WGM beads in cell arrays and single cells

- Uptake of 6-7 µm particles from cells possible
- Online measurement of particle uptake in cells (M. Himmelhaus, A. Francois, Biosensors and Bioelectronics, 25 (2009) 418–427)





Summary

- Low Q WGM promising microtechnology for labelfree detection
- Instrumentation developed
- Functional WGM Sensor particle ready
- Biofunctionalization for specific adsorption established
- Microfluidic structures evaluated
- Searching best suited applications

Acknowledgements:

- Dr. M.Himmelhaus (NanoBioAnalytics), Instrumentation
- Dr. I. + A. Prinz STRATEC Consumables GmbH
- Dr. W. Weigel et al SCIENION AG
- BMBF for financing projects „ImmunoPlex“ und „ParFluid“

Thank you for your attention

of Surflay‘s work