The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific
and Cultural Organization

International Atomic
Energy Agency

SMR.1670 - 23

INTRODUCTION TO MICROFLUIDICS

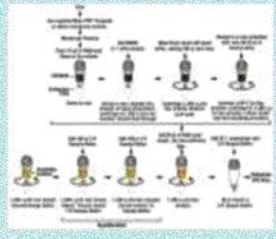
8 - 26 August 2005

Sampling for Bioanalysis

R. Luttge
University of Twente, Enschede, The Netherlands

Topics in this lecture

Biological samples
What's special?



Impact of miniaturization in medical field
The introduction of miniaturized sensors and Lab-on-a-Chip devices in clinical research and routine check-up are…

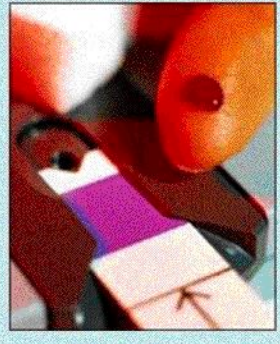
Applications
Critical care, point-of-care and cell based systems are introduced

6. Sampling for bioanalysis

- Introduction
- Strategic developments of sample preparation methods
- Integrated sample treatment
  - Diffusion, extraction and cell filtration
- Bioanalysis systems
  - Bodyfluid sampling and sensor stack
  - Cell containing samples
- Outlook: Future developments
- Summary

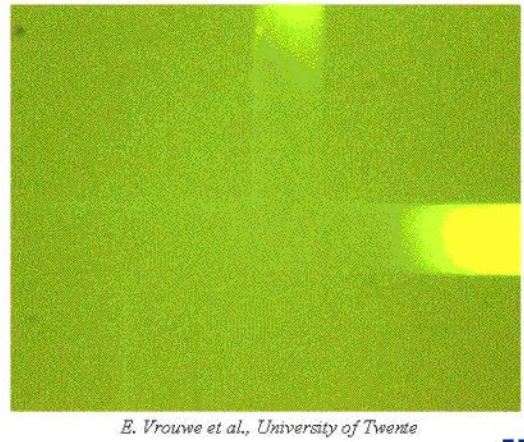
Topics in this section

Biosample plug forming and sample retrieval



Introduction

- Biotechnology
- Pharmacology
- Medical diagnostics

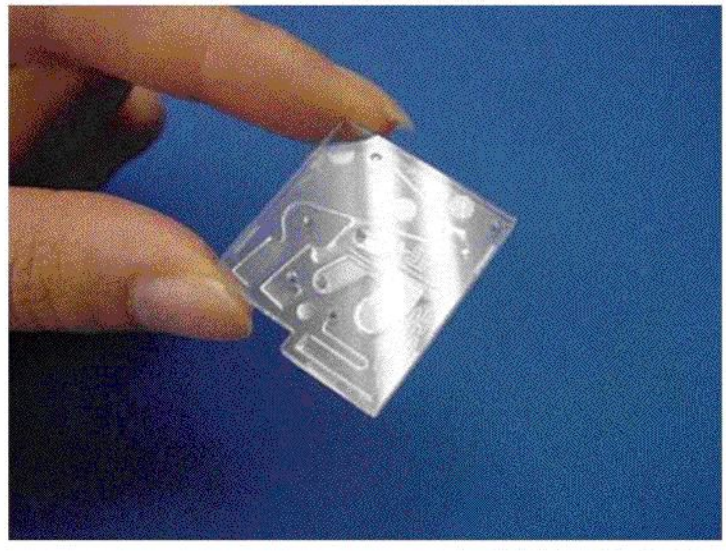


E. Vrouwe et al., University of Twente

Biotechnological sample monitoring

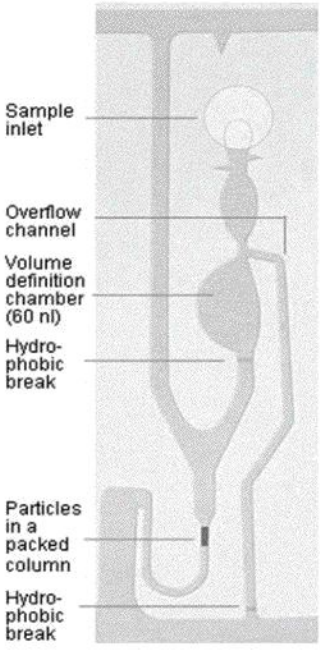
- Detection and quantification of components often using electrical means to reduce operator influence as known from optical microscopy, e.g. in the hospital environment.
- Integration of the information obtained with other process parameters available in real-time and thus utilization for process control loops.

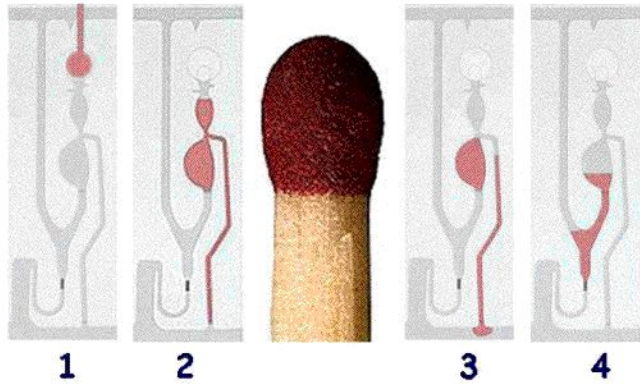
Multiple functions in a single biochip



www.ushio.co.jp

Defined sample volume (plug)



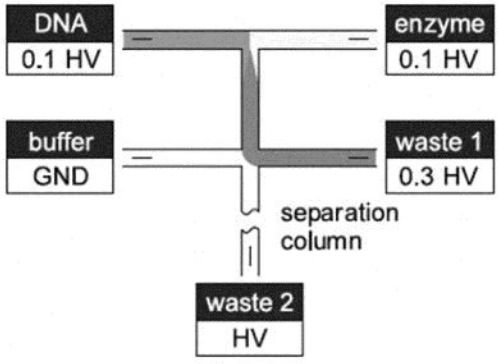


1. Liquid drawn in by capillary action.
2. Overflow channel activated at a low spin speed, removing excess liquid.
3. Volume (60 nl) defined within the chamber.
4. Defined volume moves through packed column by increasing spin speed.

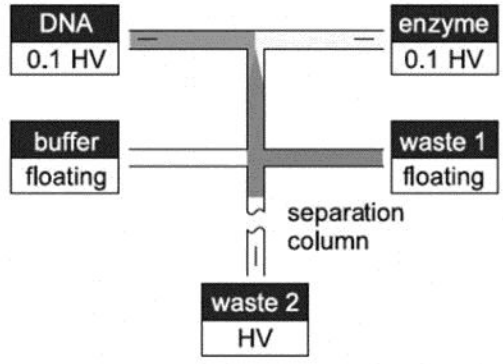
www.gyros.nl

Electrokinetic sample plug definition

Filling of the reaction chamber

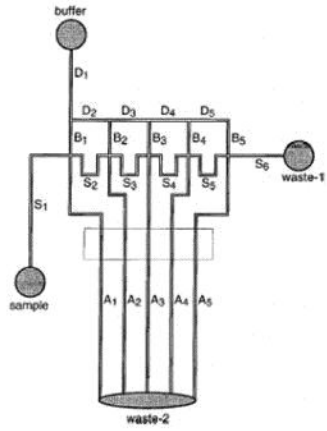


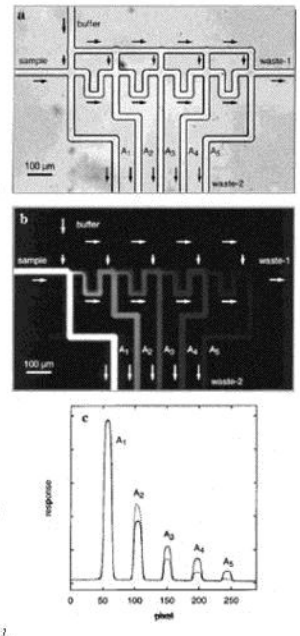
Gated injection for separation



S.C. Jacobson, J.M. Ramsey, Anal. Chem. 68 (1996) 720–723

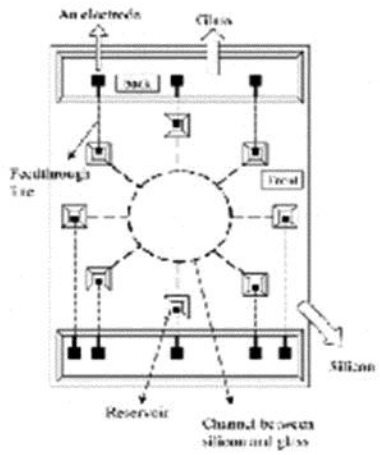
Advanced Sample Manipulation





Microfluidic Devices for Electrokinetically Driven Parallel and Serial Mixing

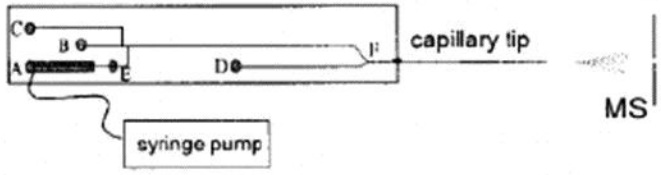
S.C. Jacobson et al., Anal. Chem.1999, 71, 4455-4459

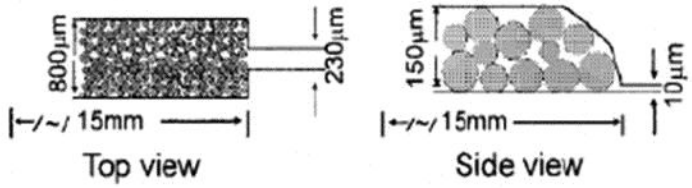


Methodology for device mniaturization and insulation on a silicon substrate

Y. Jeong et al.,
Lab on a Chip, 2001, 1, 143–147

On-chip bead-based digestion-separation-MS





Beds of immobilized trypsin on beads:

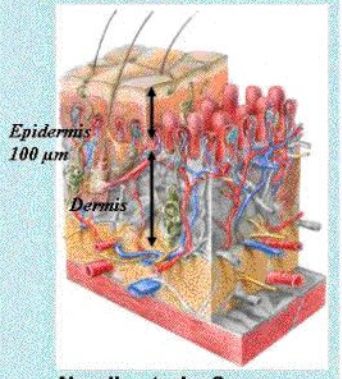
- Increased trypsin:protein ratios → enhanced reaction rates
- Higher trypsin concentrations possible → it doesn't eat itself!

Ref: C. Wang et al. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1377-83.

Slide prepared by S. Verpoorte, RUG, The Netherlands

Topics in this section

Skin penetration mechanism



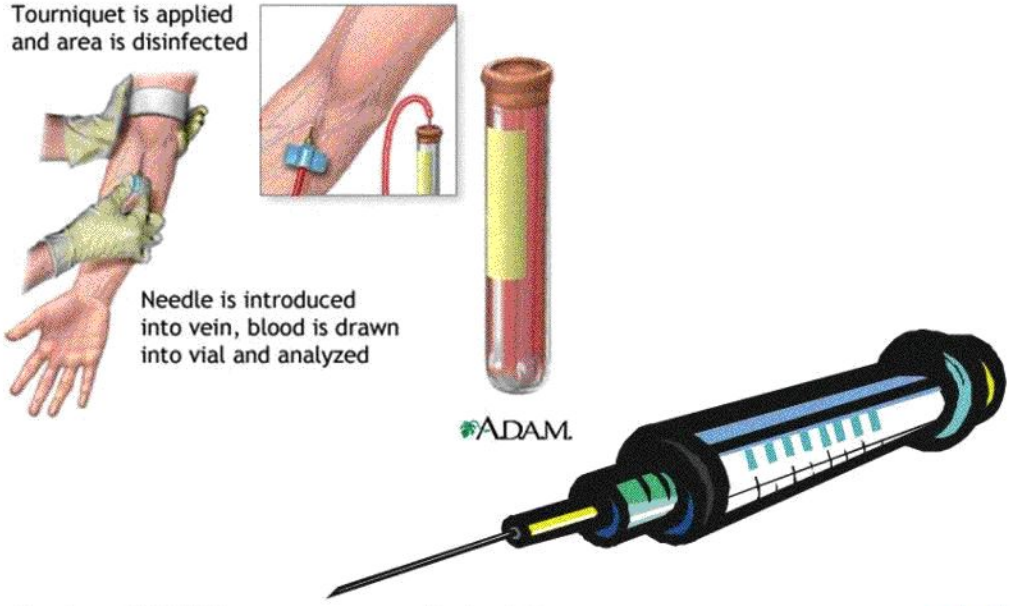
Needles today?

Strategic developments of sample preparation methods

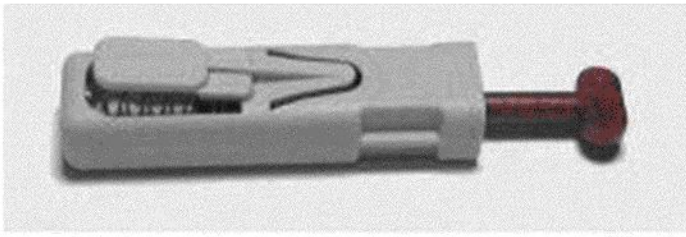
- Retrieving samples through skin
- Blood sample requirements
  - Good efficacy
  - High quality of life
  - Low cost
- Technological solutions

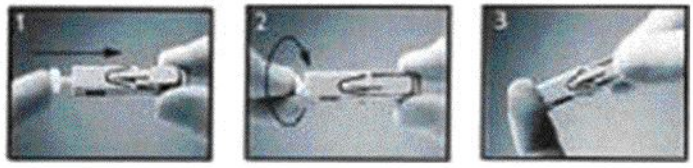
Human skin

Blood Sampling Standards



Various types of "fine" lancets





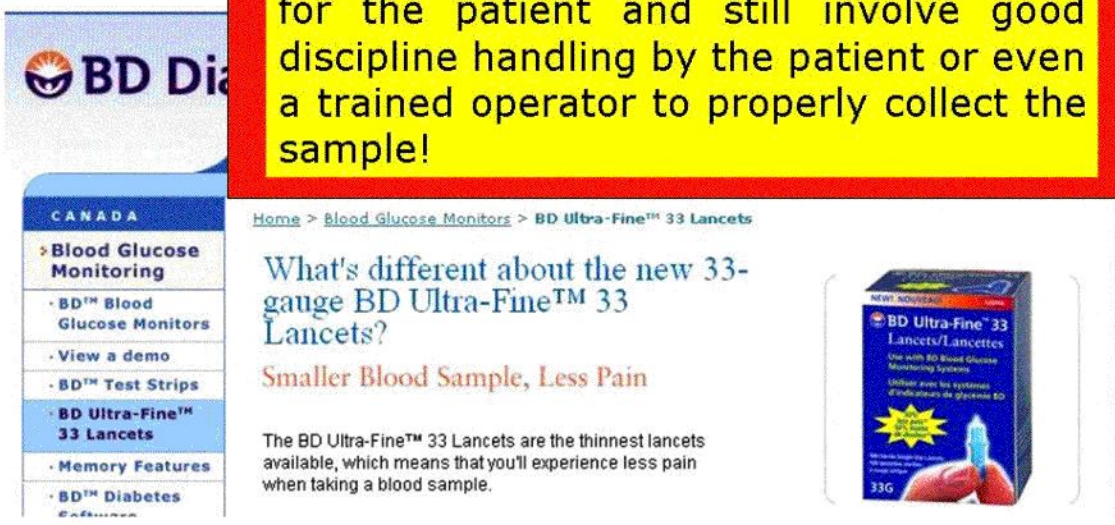
Automatic lancet

Automatic lancet (Bayer)

Vacuum lancet (Bayer)

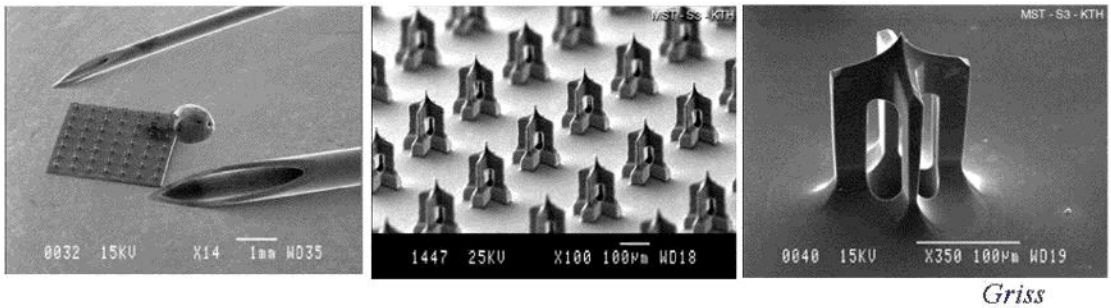
Finer Needles?

Lancets still cause pain / inconvenience for the patient and still involve good discipline handling by the patient or even a trained operator to properly collect the sample!



Micromachined needles

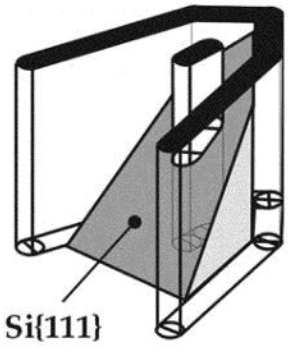
- Claiming painlessness (?)
- Mechanically highly resistant needle
- Low flow resistance (+/-)
- Large exposure area by dense arrays (+/-)
- Inject 100 µl in 2 sec using a 3x3 mm² chip would cause a pressure drop of 2 kPa.



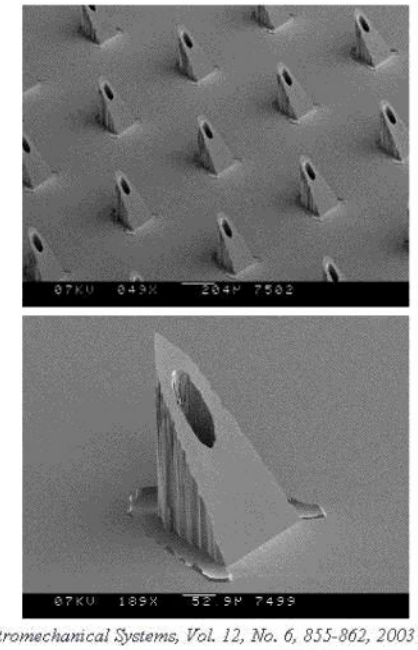
Griss

Silicon Microneedles as Sampler ?

- Collaboration of
  - Transducer, Science and Technology Group, BIOS (MESA+), and Nanopass Technologies Ltd., Israel



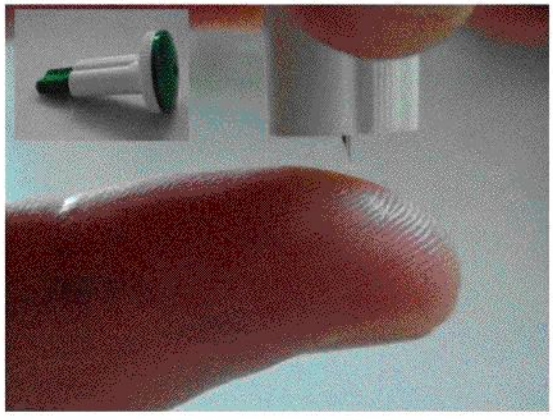
3-D masking for KOH etching

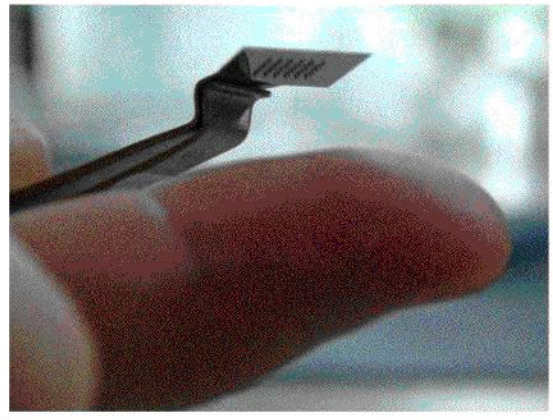


Gardeniers et al, Journal of Microelectromechanical Systems, Vol. 12, No. 6, 855-862, 2003

Standard lancet versus silicon array

- Advantages/disadvantages

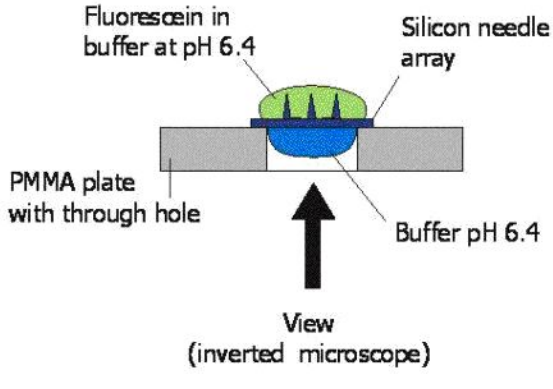


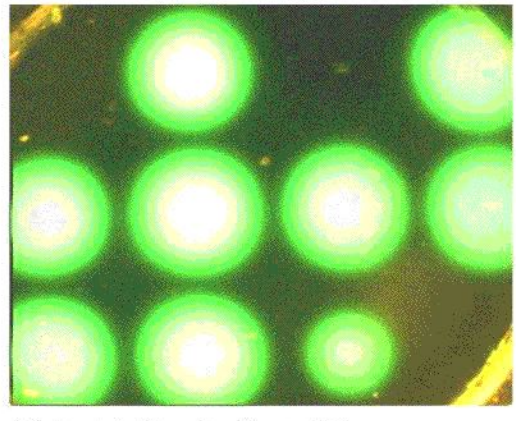


R. Luttge et al., University of Twente, 2002

Transport of "Sample"

- Fluorescent dye testing

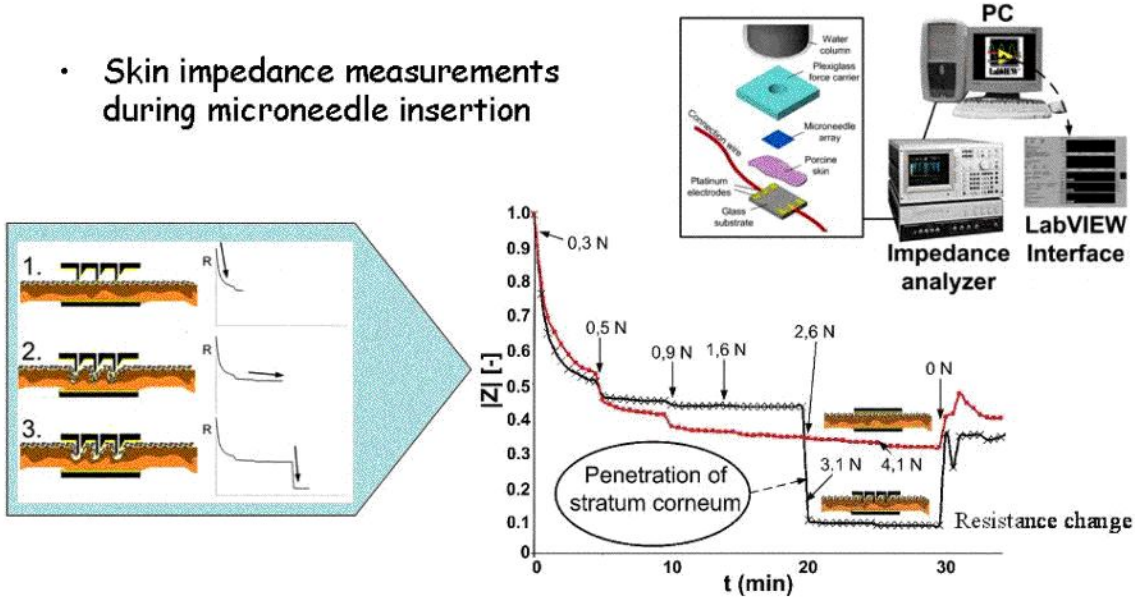




R. Luttge et al., University of Twente, 2003

Electrical penetration sensing

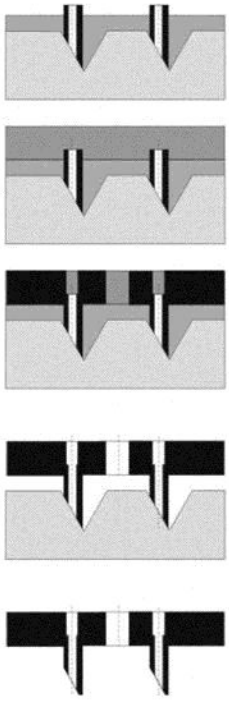
- Skin impedance measurements during microneedle insertion



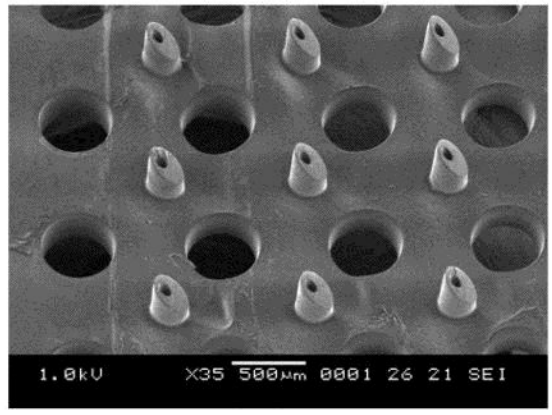
P. Loeters et al, μTAS2004

6.2. Strategic developments of sample preparation methods

SU-8 microneedles

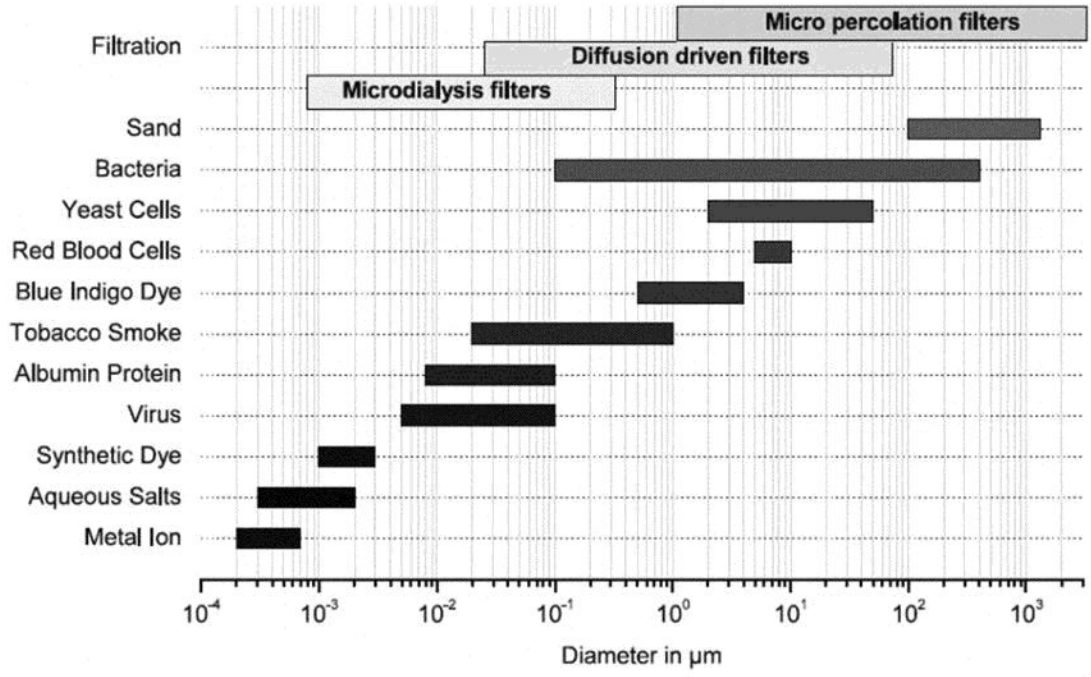


- Lithography on pre-patterned substrates

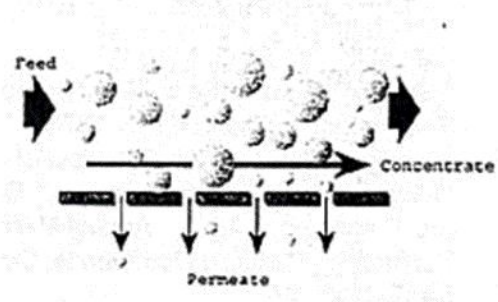


D. Alpeter et al. , University of Twente, 2003
Nanopass Ltd., patent pending

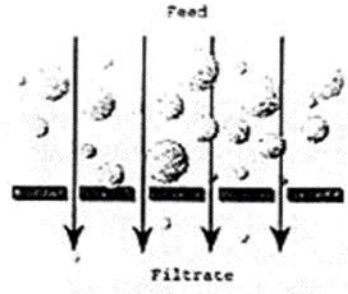
The "Filtration Spectrum"



Types of filtration



CROSSFLOW

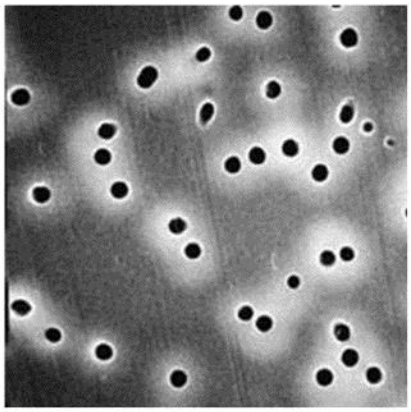


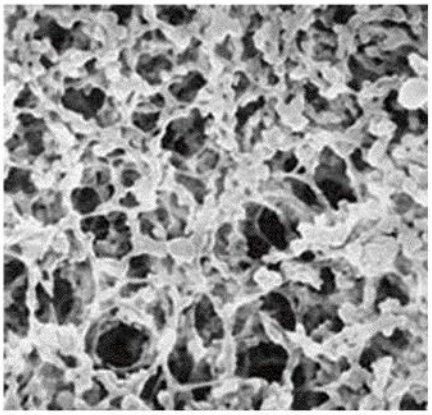
NORMAL FLOW

http://www.gewater.com

Filter types

- Screen membrane filters (capillary pore), left
- Depth membrane filters (tortuous filter), right

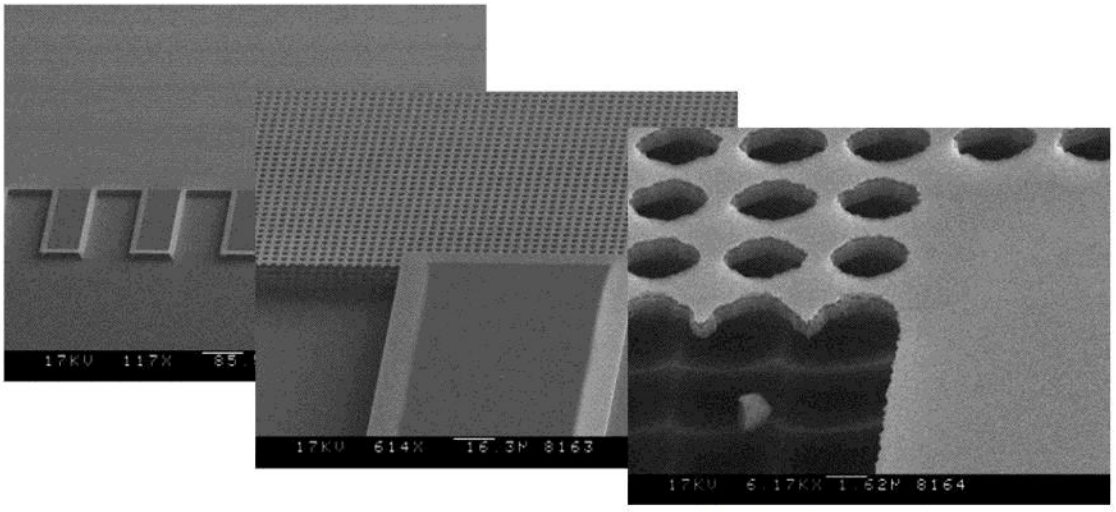




http://www.gewater.com

Micromachined filter membranes

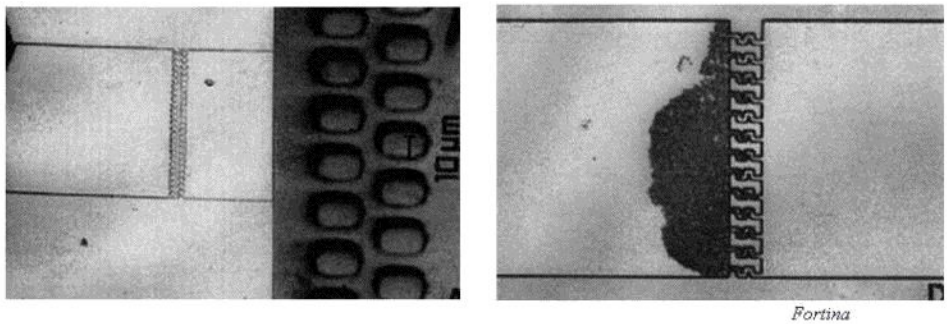
- White blood cells are stopped
- Red blood cells squeeze through



van den Berg, University of Twente

Channel integrated filters

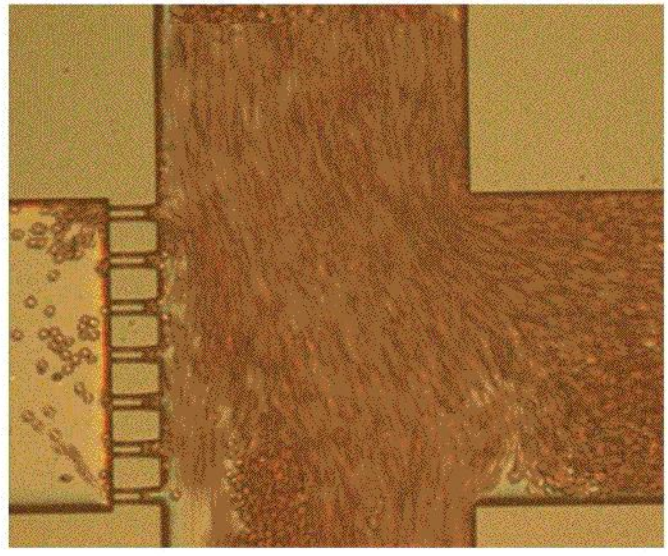
- Silicon micropost-type filter
- Wire-type filter
- White blood cells are isolated from whole blood. Genomic targets can then be directly amplified.



Fortina

Studying cells on microchip

- Characterize cellular flow properties
- Control cell flow
- Capture single cells
- Manipulate cells by e.g. electrical fields



Valero et al., Nanoscan STW-project

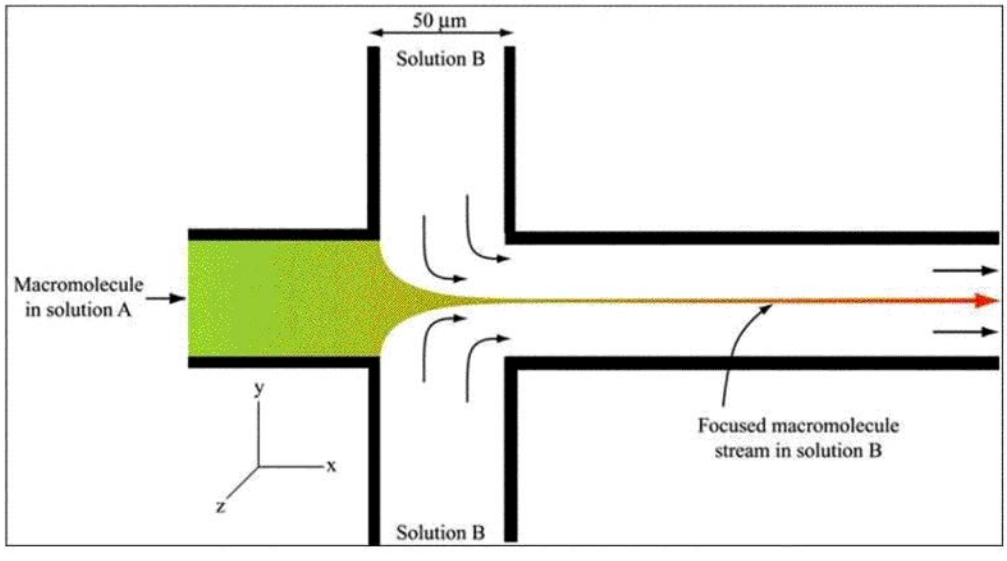
Topics in this section

Sample preparation/ clean up

Integrated sample treatment

- Reducing the complexity of a sample
  - Diffusion based systems
  - Solid phase extraction

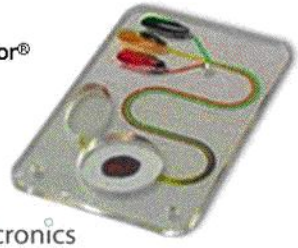
Sheath flow- mixing by diffusion

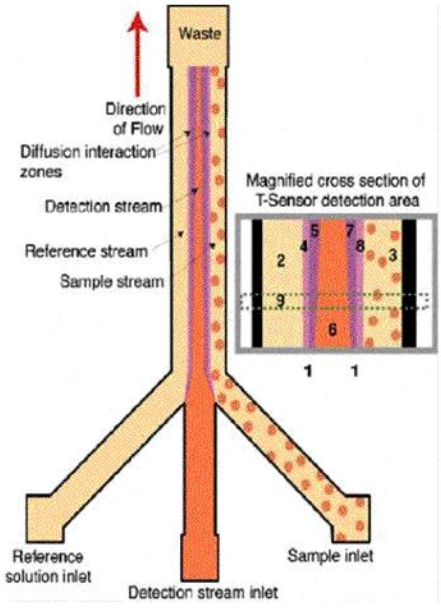


Diffusion-based reaction

- Reference and sample solution are broad into contact with a detection solution downstream.
- Reactions occur in the interdiffusion (mixed) zones.

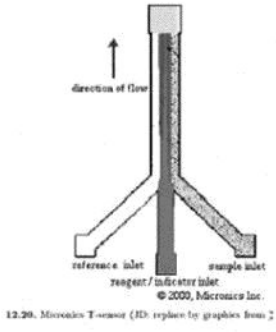
T-sensor®





Source: P. Yager, Univ. of Washington, USA

Determination of human serum albumin



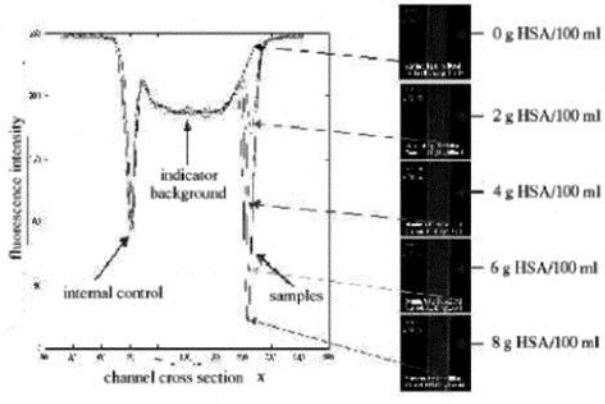
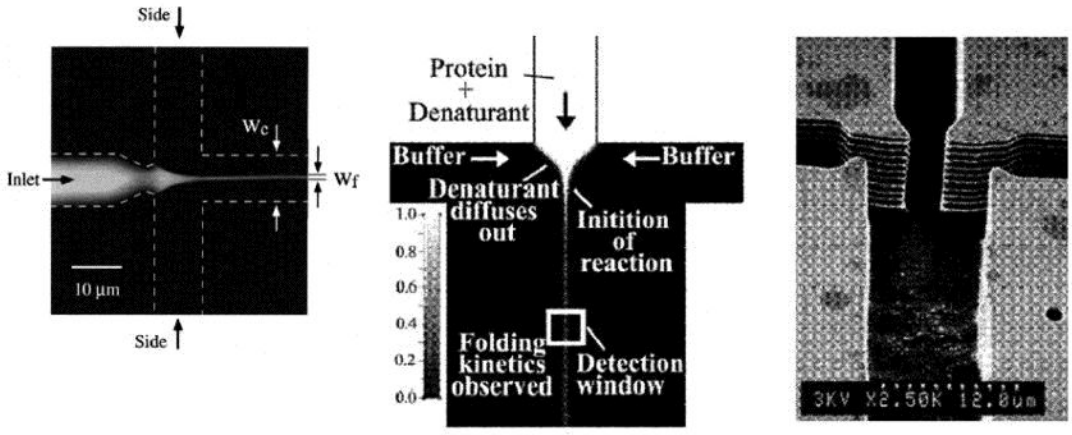


Fig. 12.21. Example T-Sensor assay. Fluorescence micrographs of a detection channel section containing a control, indicator, and sample streams during a determination of human serum albumin, and a graph displaying the corresponding light intensity profiles across the width of the channel

Microsecond mixing/de-mixing by hydrodynamic focusing

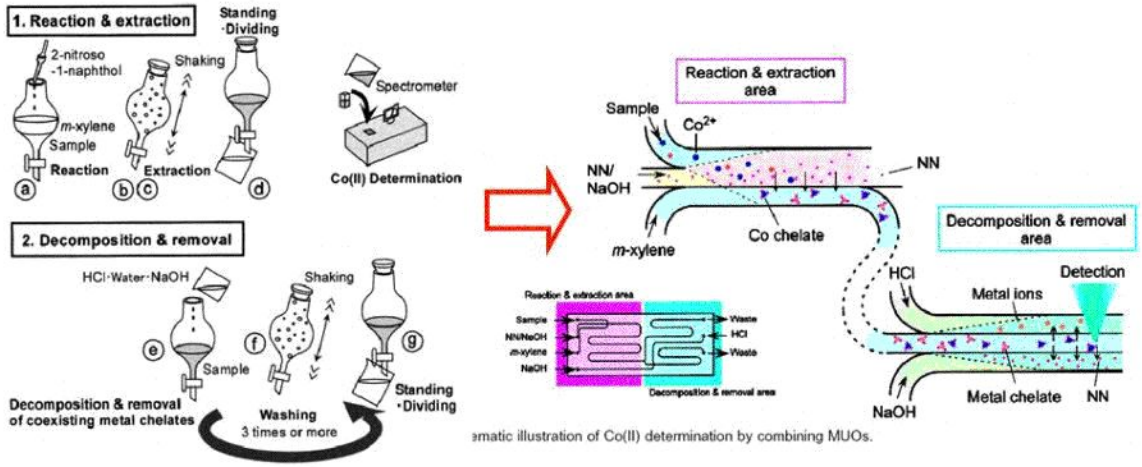


J.B. Knight, et al. / Phys. Rev. Lett. 80, 1998, 3863-3866
D.E. Hertzog, et al. / Proc. Micro Total Analysis Systems Conf. 2003, Squaw Valley, Oct. 5-9, 2003, pp. 891-894

6.3. Integrated sample treatment

Continuous-flow chemical processing

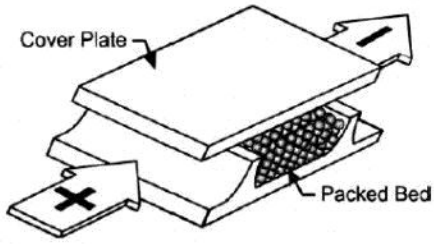
- Combining microunit operations and multiphase flow network
- Co(II) conc. detection limit: 18 nM, absolute amount detectable 0.13 zmol.



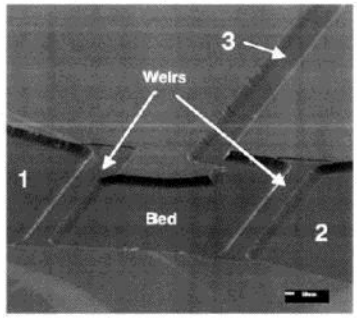
ematic illustration of Co(II) determination by combining MUOs.

M. Tokeshi et al., Anal. Chem. 74, 2002, 1565

Solid-phase packed bed extraction



a)

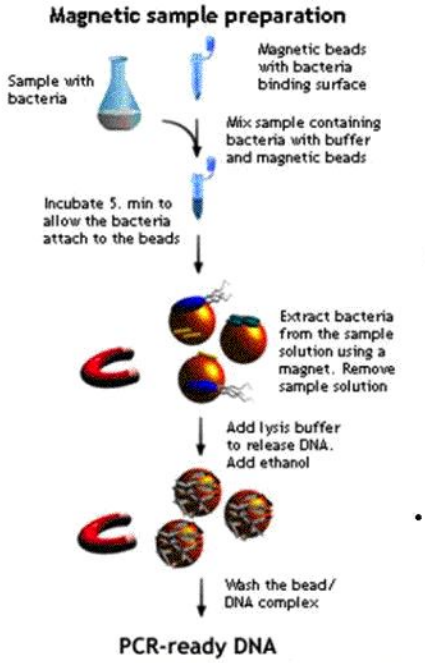


b)

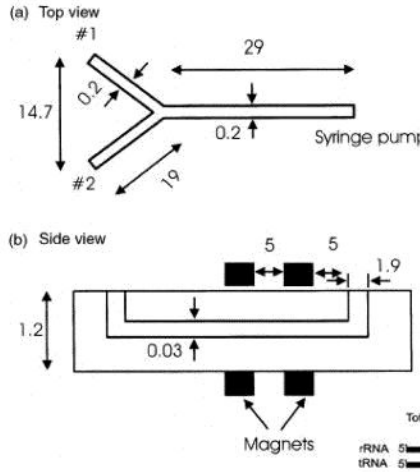
- Analyte-bead interaction may be:
  - Physical
  - Chemical (surface modification)

R.D. Oleschuk, A.B. Jemere, L.L. Schultz-Lockyear, F. Fajuyigbe, D.J. Harrison, Proceedings of the Micro Total Analysis Systems Symposium 2000, Kluwer Academic, Dordrecht, 2000, pp. 11–14.

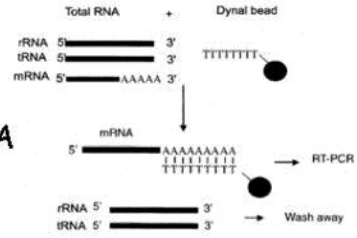
Magnetic bead extraction



http://www.genpoint.com/Media/sampleprep.gif



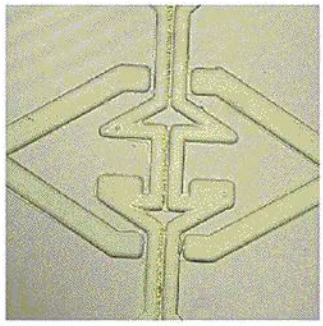
- mRNA isolation for the purpose of cDNA library construction



G. Jiang, D.J. Harrison, Analyst 125 (2000) 2176–2179

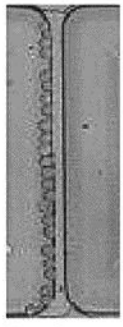
Docking system for cell analysis

(a)



- Control of cell transport and docking using a 'Parallel dam' structure (5 μm)
- Generation of concentration gradient by controlled fluid distribution and diffusive mixing

(b)



- Made of quartz with PDMS seal
- Successfully measured the ATP-dependent calcium uptake in HL-60 cells.

Yang (2002) Analytical Chemistry, 74, 3991-4001.

Suggested reading

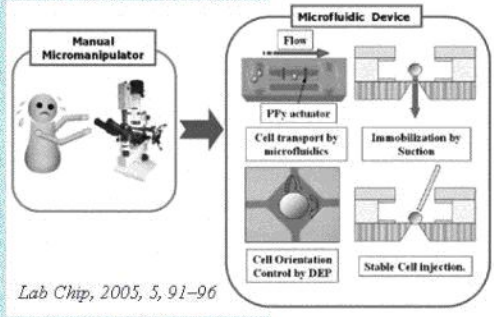
- James P. Brody and Paul Yager, A Diffusion-based extraction in a microfabricated device, Sensors and Actuators **A** 58, 1997, 13-18.
- Jan Lichtenberg, Nico F. de Rooij, Elisabeth Verpoorte, REVIEW, Sample pretreatment on microfabricated devices Talanta 56, 2002, 233-266.

Topics in this section

Integrated microfluidic bioanalysis

Infineon

Cellular analysis



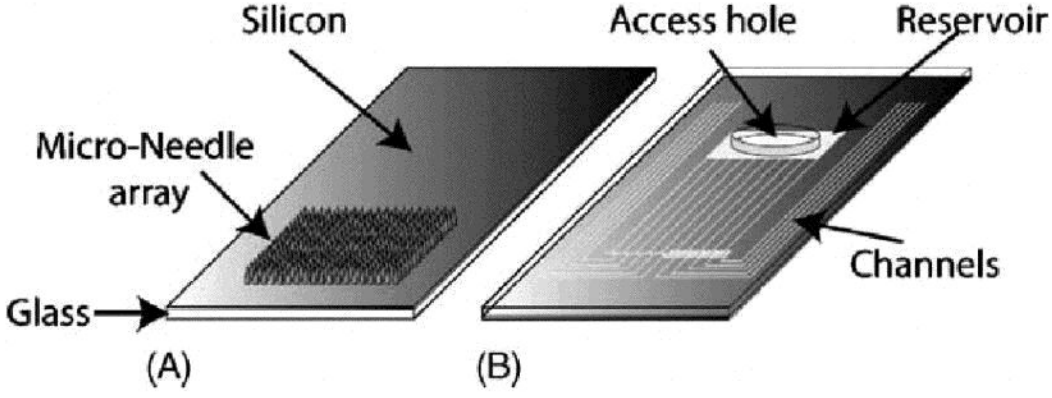
Lab Chip, 2005, 5, 91–96

Bioanalysis systems

- Bodyfluid sampling

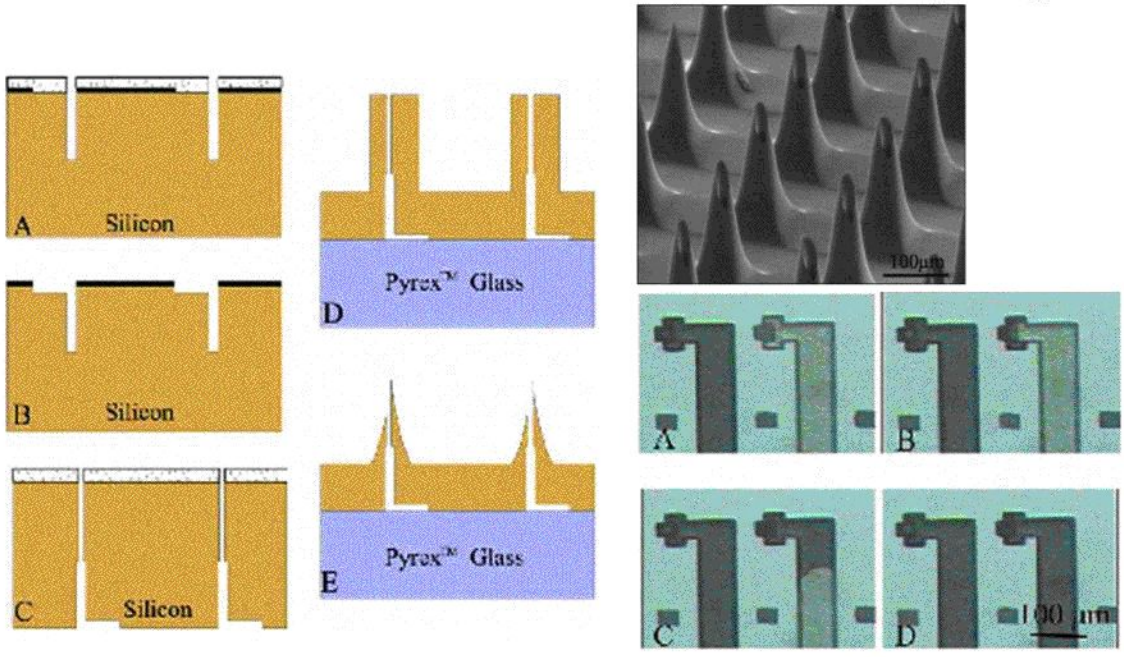
6.4. Bioanalysis systems

Microneedle integrated glucose sensor



Mukerjee et al., S&A A (2004)

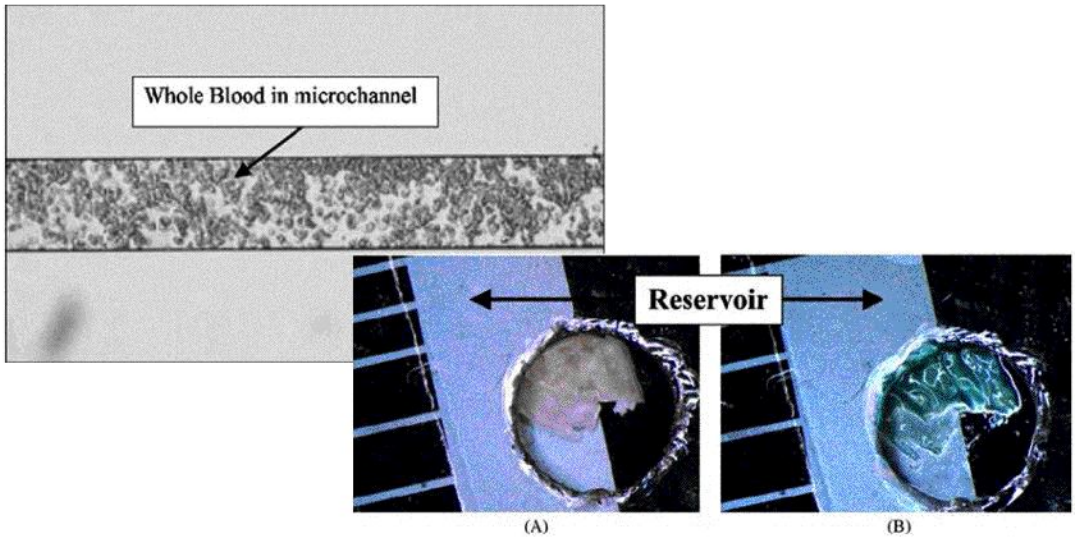
μ-needle biofluid sampling



Mukerjee et al., S&A A (2004)

6.4. Bioanalysis systems

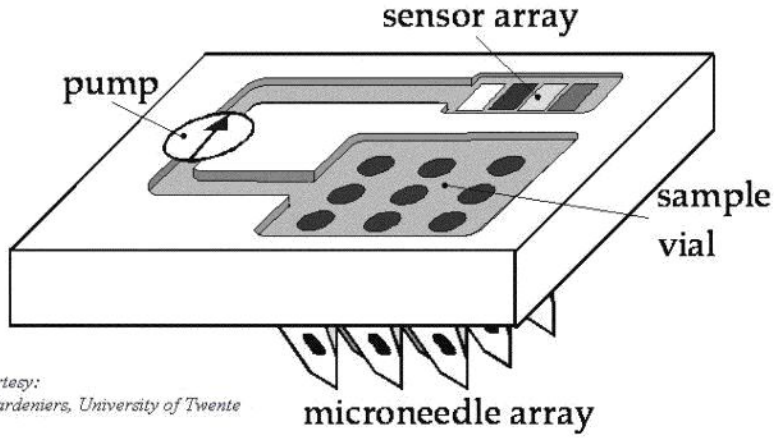
Blood and interstitial fluid sampled by microneedle array



Mukerjee et al., S&A A (2004)

Integrated sampling – selective sensing

- Basic layout of a carry-on system to monitor the health status of a person, by using information based on the measurement of constituents in the body fluid of the person.



Courtesy:
H.Gardeniers, University of Twente

Progressive systems towards market

In Vitro Diagnostics

Gold MEDICAL DESIGN EXCELLENCE AWARDS 2000 WINNER

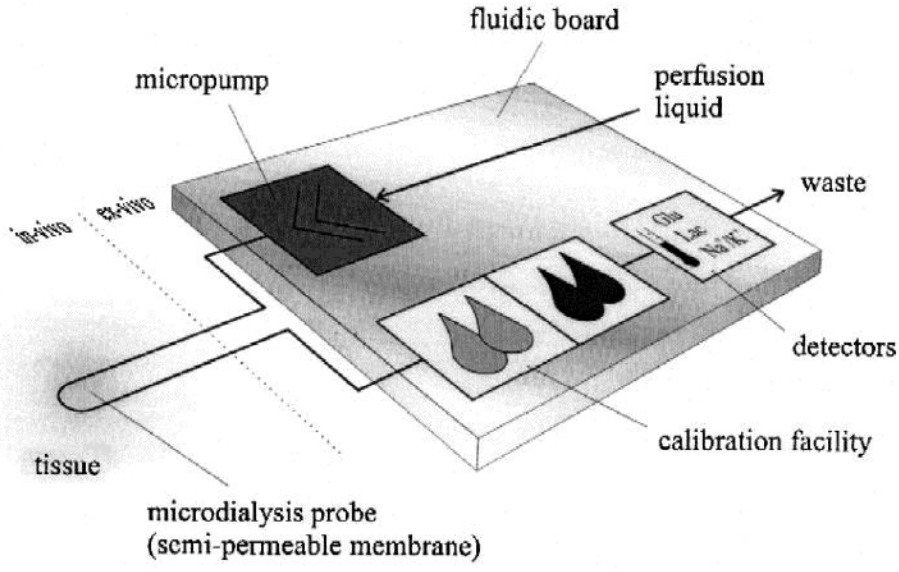
Careside Analyzer blood testing system

Submitted by: Careside, Inc.
Culver City, CA
Designers: John Von Buelow, Barry Sween, David Hoard, (Hauser, Westlake Village, CA); Bill Reid, (UMM Electronics, Indianapolis, IN)
www.careside.com

Outstanding feature: delivers almost instant test results onsite, eliminating the traditional daylong wait for laboratory results.

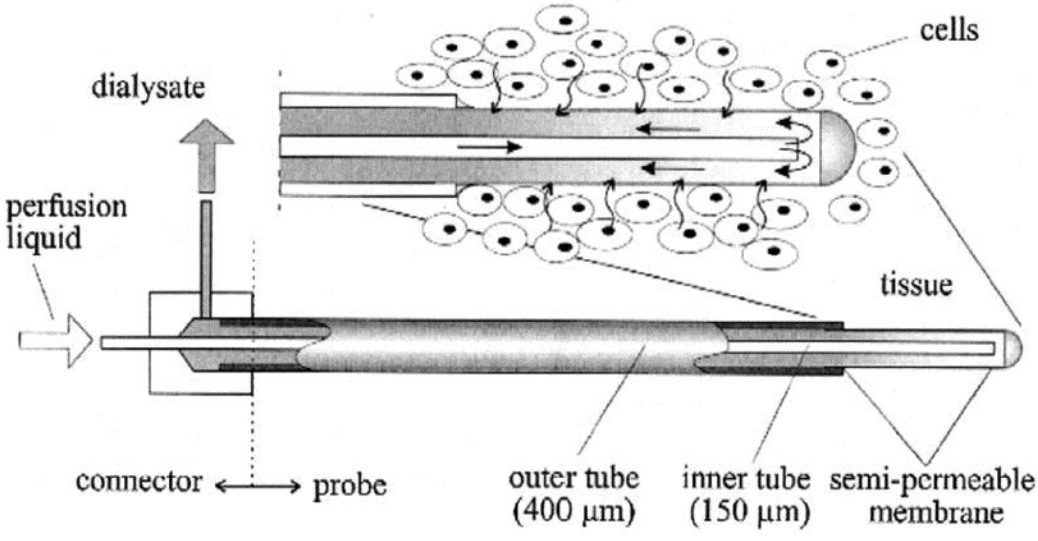
With its capability to provide accurate point-of-care blood test results within 12 to 15 minutes, the Careside Analyzer decentralizes lab operations and eliminates the traditional two to 24-hour turnaround for test information. The system performs chemistry, electrochemistry, coagulation, and immunochemistry tests, and can conduct hematology tests when connected to a separate device. The compact unit has a footprint of less than one square foot and features a graphical user interface designed for

Integrated microdialysis system



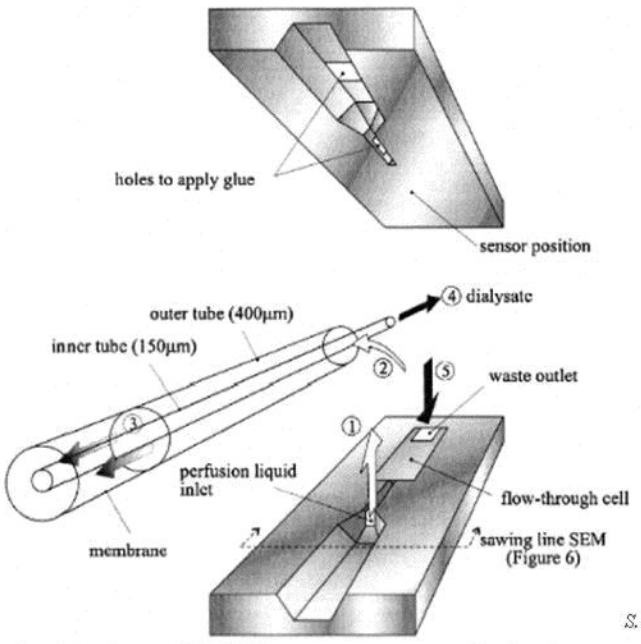
S. Bohm et al., Sens. Actuators B 63 (2000) 201–208

Commercial available double lumen microdialysis probe



S. Bohm et al., Sens. Actuators B 63 (2000) 201–208

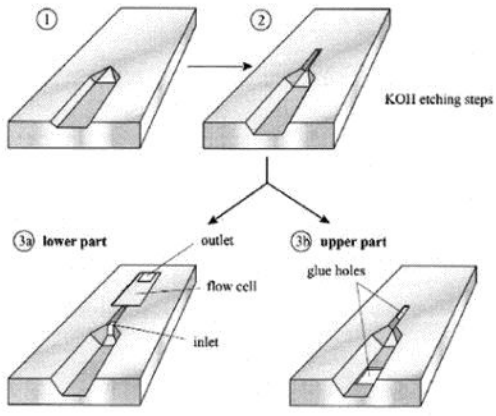
Exploded view of the connector / sensor construction

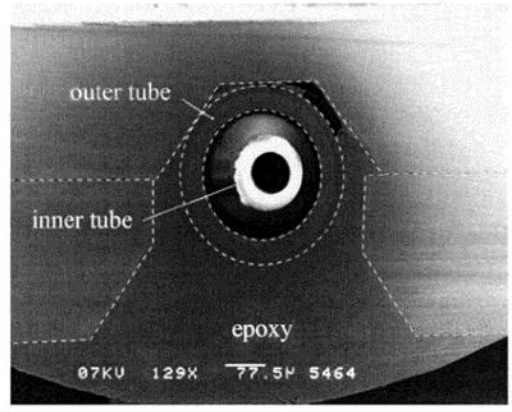


- The fluid flow from inlet to outlet is marked with numbers 1-5:
  - 1-3: perfusion liquid,
  - 4: dialysate entering the flow-through cell,
  - 5: dialysate leaving the system. The 'SEM sawing line' indicates the position and direction where the cross section (figure assembly, next slide) was taken.

S. Bohm et al., Sens. Actuators B 63 (2000) 201–208

General fabrication scheme and cross-section of assembly

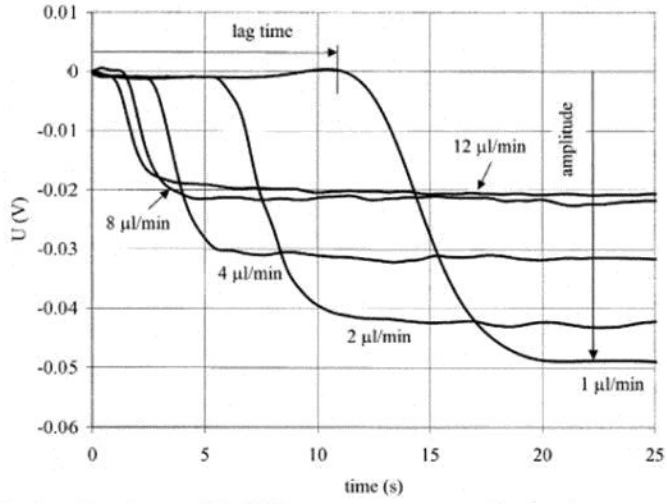




S. Bohm et al., Sens. Actuators B 63 (2000) 201–208

Potentiometric detection at integrated electrodes

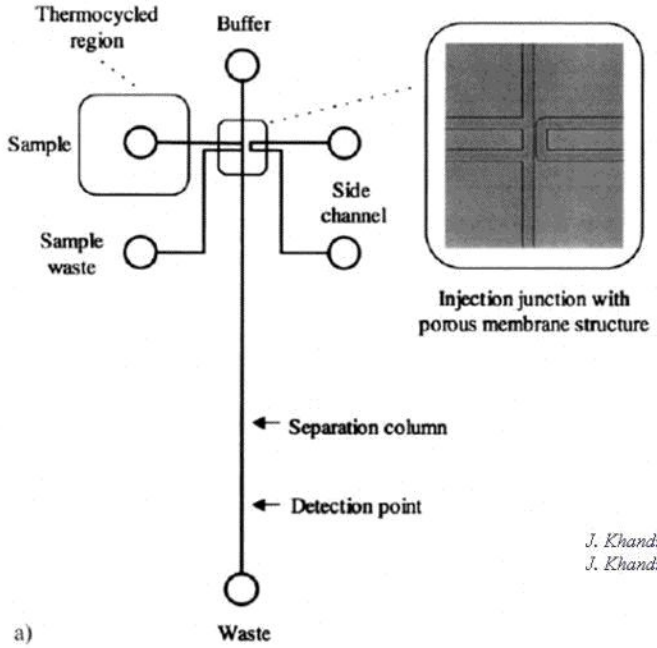
- Measured response to 100 mM KCl for a micromachined probe/sensor membrane length 5 mm.



- measured against a standard glass reference electrode using a high impedance amplifier, 10 V input impedance and a digital storage oscilloscope for data acquisition.

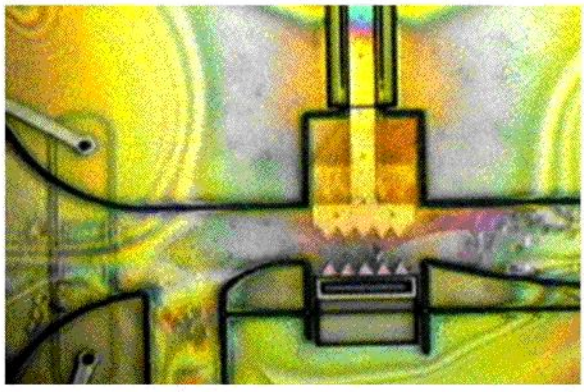
S. Bohm et al., Sens. Actuators B 63 (2000) 201–208

Planar integrated dialysis and sample pre-concentration



J. Khandurina et al., Anal. Chem. 71 (1999) 1815–1819
J. Khandurina et al., Anal. Chem. 72 (2000) 2995–3000.

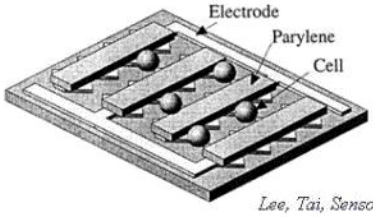
Cell lysis



Mechanical

- 20 μm wide jaw
- 10 cells/second
- Continuous flow
- Teeth with needles
- under development

www.sandia.com



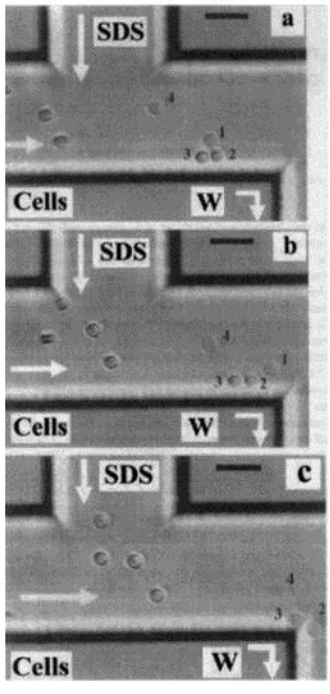
Electrical

- Consists of multi-electrode pairs
- The cells are lysed by a pulsed electric field 1-10 kV/cm

Lee, Tai, Sensors and Actuators, 73, 74, 1999.

6.4. Bioanalysis systems

Chemical Lysing

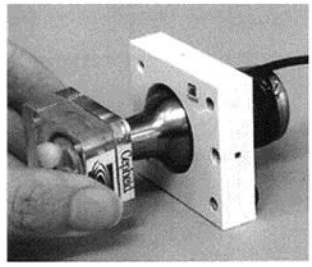


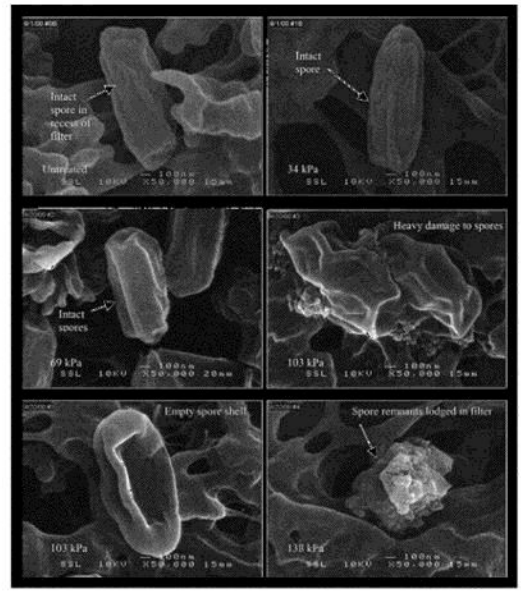
- Electrophoresis/electroosmosis
- Lysing agent:
  sodium dodecyl sulphate (SDS)
- Lysis of erythrocytes

Li, Harrison, Analytical Chem, 69, 1564, 1997.

Microsonicator

- Sonication in conjuction with microfluidics and glass beads
- A fluidic module that capture baterial spores on a filter
- Applying ultrasonic energy through a thin-film flexible interface
- Bacillus spores successfully lysed in less than 30 sec.



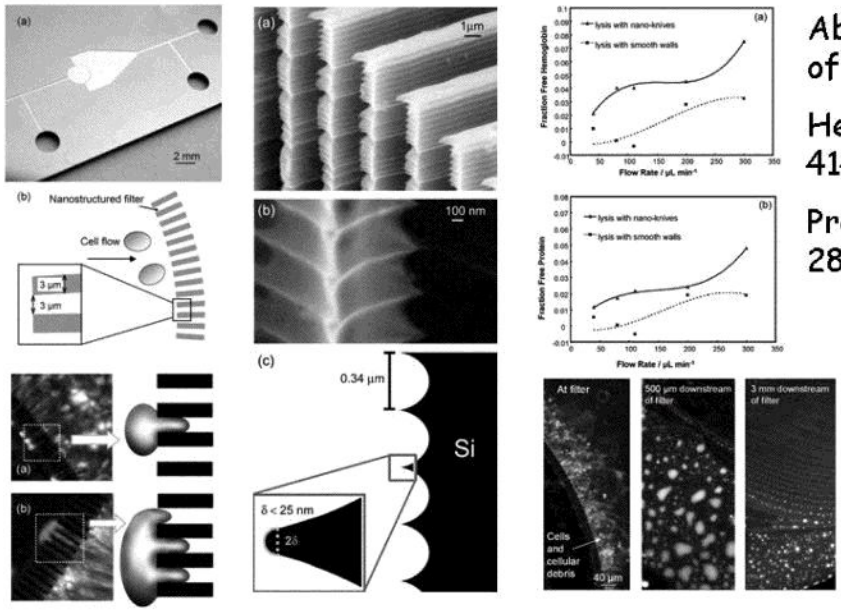


Taylor, M., et al., (2001) Analytical Chemistry, 73, 492-496.

Outlook: Future developments

- Cell based diagnostic systems and screening platforms.
- Miniaturized critical care microfluidic *Lab-on-a-Chip* devices will go to market within the next 10 years.
- From cell population data acquisition systems there will be also systems to retrieve information from singel cells in a highly parallel fashion.

Cell information retrieval



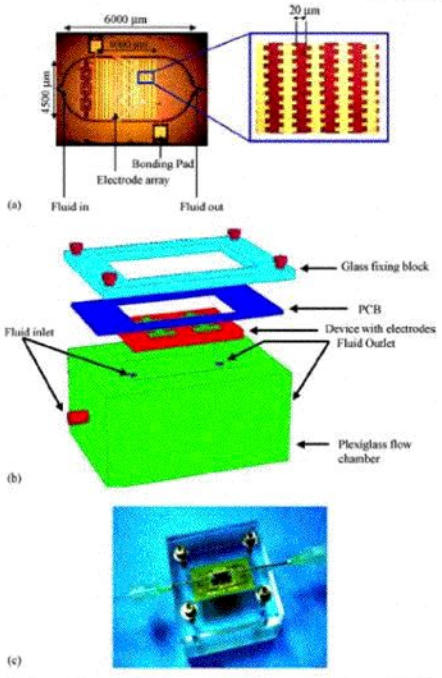
Absorbance of:

Hemoglobin 414 nm

Protein 280 nm

D. D. Carlo et al. / Lab Chip, 2003, 3, 287–291

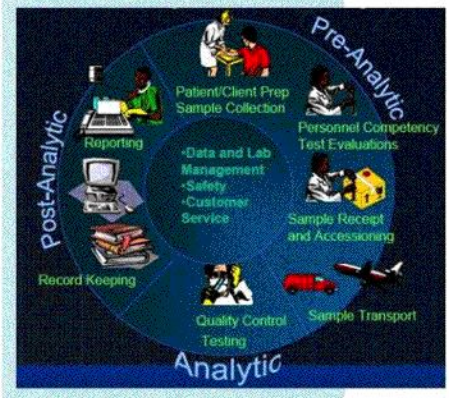
Parallel single cell processors



- High through-put, e.g., simultaneous cell lysis by electroporation
- Higher sensitivity in biomarker diagnostics by integrated single cell lysis and on-line signal amplification (PCR).

Q. Ramadan et al. / Sensors and Actuators B xxx (2005) xxx–xxx

Summary



- The last decade has been marked by a boom in the number and variety of microtechnologies in life science.
- By focusing on predicting patient outcomes, targeted medicine, earlier disease detection and more advanced automation, diagnostics will secure considerable market growth potential.
- "Lab-on-a-Chips" are key technology to feed this market potential.
- The aim is to strive for closed-loop treatment systems (drug delivery and monitoring) which includes sample retrieval, preparation and analysis on one platform.