The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific
and Cultural Organization

International Atomic
Energy Agency

SMR.1670 - 22

INTRODUCTION TO MICROFLUIDICS

8 - 26 August 2005

Optical Detection

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University of Twente, Enschede, The Netherlands

Topics in this lecture

Sensing principles
From visualizing devices to quantitative optical detection. Its superiority in electrically decoupling has paved its way as a workhorse method in chemical and life-science applications.

Miniaturization
Integrated into microfluidic devices. From classical microscopy to parallel monitoring, e.g. in spinning disc applications, or as an integrated photo-diode many devices and components are established.

Applications
To emphasis on the aspects of integration waveguide technology is primarily presented.

5. Optical detection

- Introduction
- Strategic developments of optical detection methods
- Tackling integration
- Examples of optically integrated microfluidics
- Commercial activities
- Outlook: Future developments
- Summary

Topics in this section

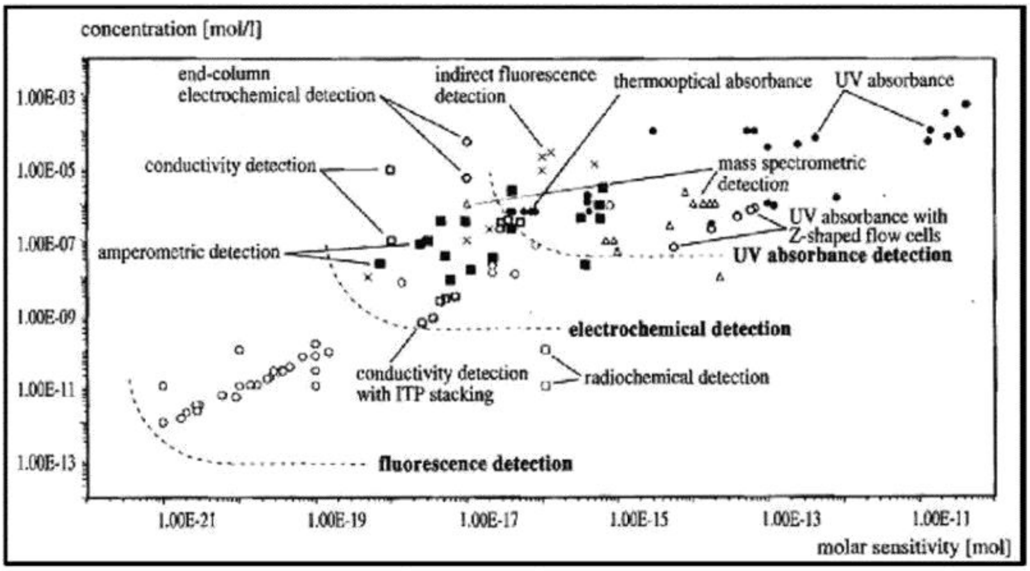
Working principle

Miniaturization efforts
Fibre optics was an important step towards optical integrated systems

Introduction

- Optical detection
- Most popular optical analysis methods in microfluidic chip operation
  - Fluorescence
  - Absorption
- Fibre-integrated systems

Optical detection spectrum

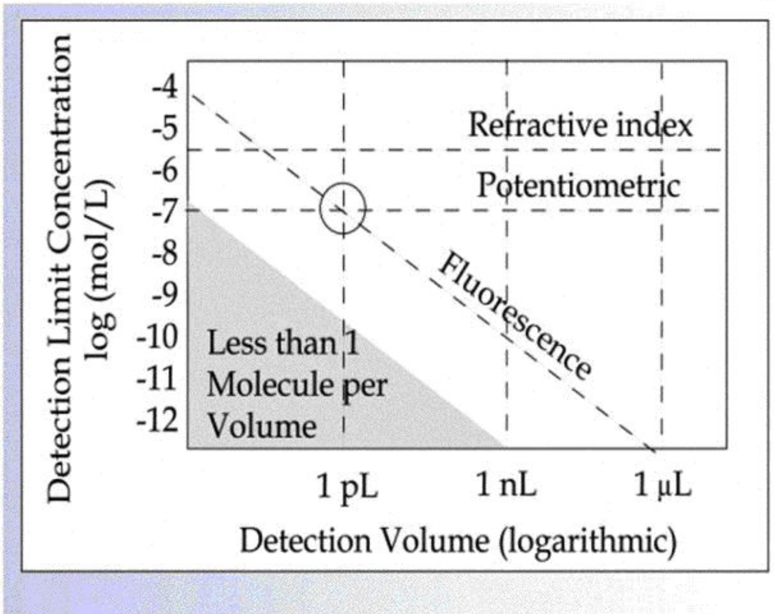


*From: J.P. Landers, ed., **Handbook of capillary electrophoresis**, 2nd ed., 1997, CRC Press, Inc., ISBN 0-8493-2498-X*

Optical methods

- Detection of molecules after chromatographic separation, during chemical reaction, in living cell, etc. ~ fixed wavelength, fixed position.
- Characterization of fluid flow ~ time dependence, correlation.
- Spectroscopy to identify molecules ~ wavelength dependence.

Detection in miniaturized systems

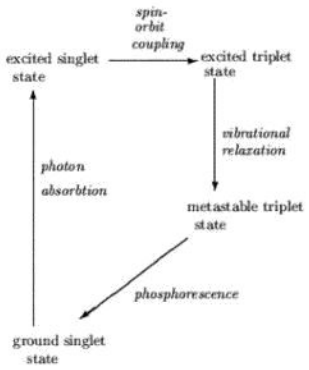


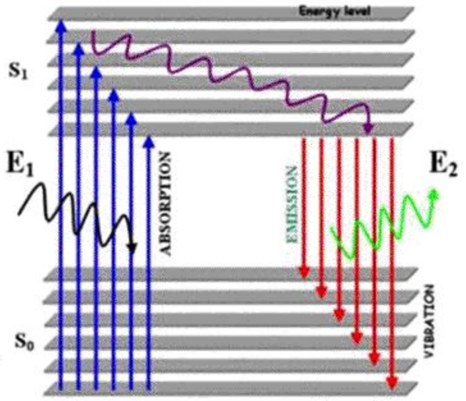
adapted from Manz et al., Adv. Chromatogr. **1993**, *33*, 1

Optical effects suitable for detection

- absorption (transmittance, reflectivity, ...)
- emission (luminescence, fluorescence, phorphorescence, ...)
- scattering
- modulation (interference, ...)
- refraction (refractive index change)
- polarisation
- ......

How does fluorescence detection work?



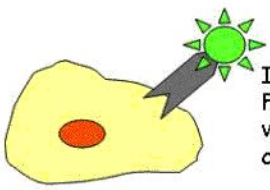


Phosphorescence: transition involving a change in the spin multiplicity of a molecule; because of this change the radiative transition is delayed and the phosphorescent material glows a while after the incident illumination stops.

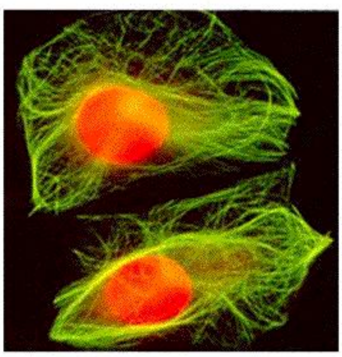
Fluorescence: molecule absorbs high-energy photon, and re-emits as lower-energy photon; energy difference ends up as molecular vibrations (heat)

Fluorescence labeling and analysis

- Fluorescent dyes allow sophisticate analysis of phenotype of living organisms and their metabolic processes, here:
  - Mammalian cells treated with an anti-tubulin antibody (green) to stain the microtubule cytoskeleton.
  - Propidium iodid (red) used to stain the nucleus.



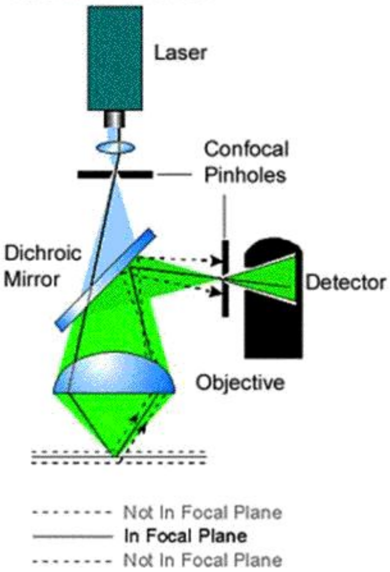
Immunofluorescence: Fluorophore or other tag for visualization is directly conjugated to the antibody!

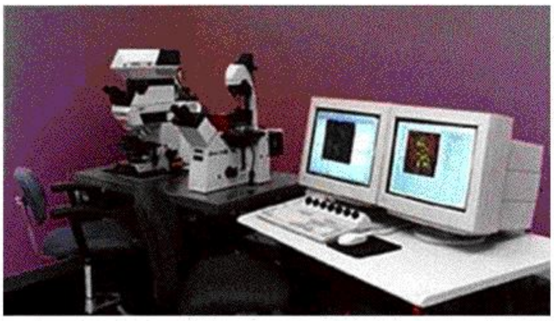


F. Cabral, University of Texas, Houston Medical School

Optical inspection systems

Microscope set-up:

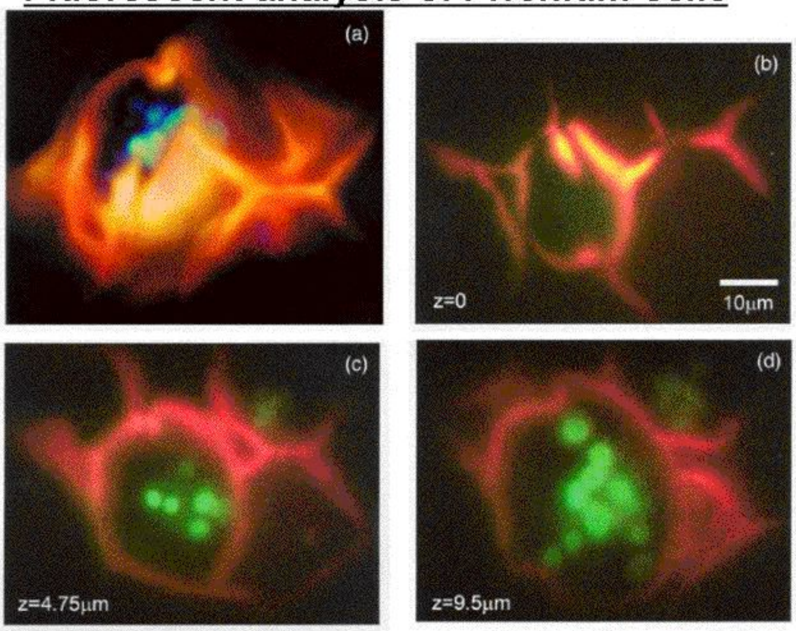




www.leica.com

Multifocal multiphoton microscopy

Fluorescent analysis of *Prionium* cells

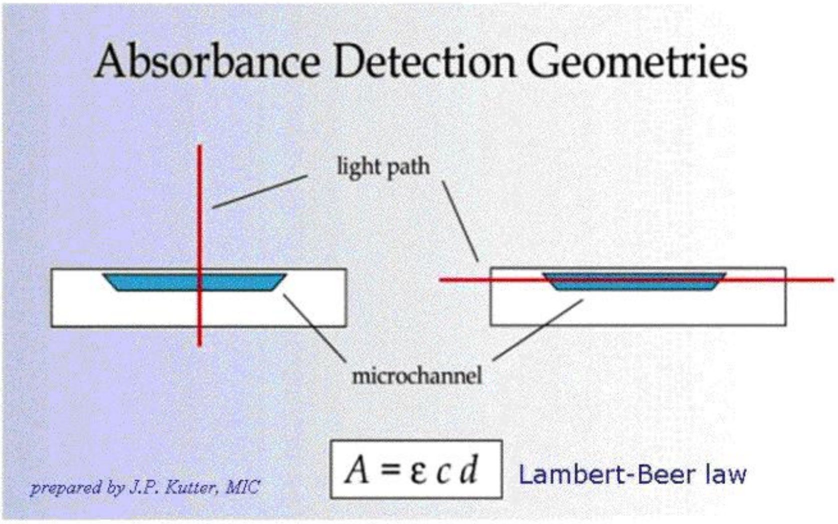


- Fast and efficient tool for 3-D fluorescence imaging.
- Three-dimensional reconstruction (a)
- and three representative XY-slices (b-d).

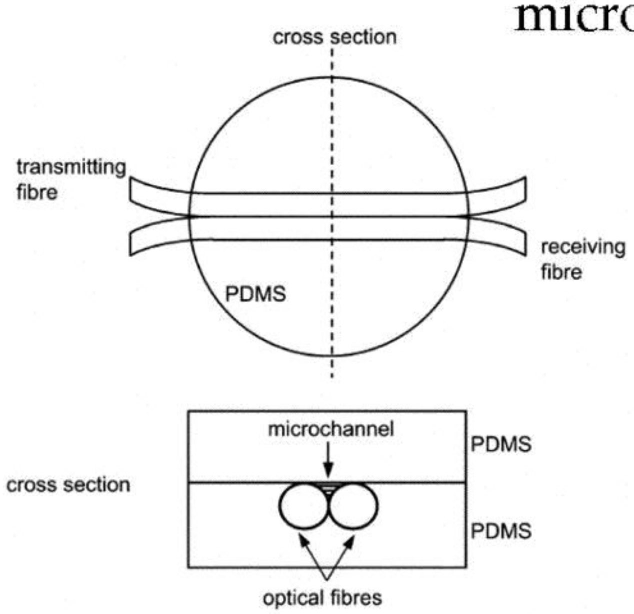
M. Straub and S.W. Hell, Bioimaging **6** *(1998) 177–185*

How does absorbance work?

- Technique that features a history in chemical reaction engineering.



Fibre optic coupler as a detector for microfluidic applications

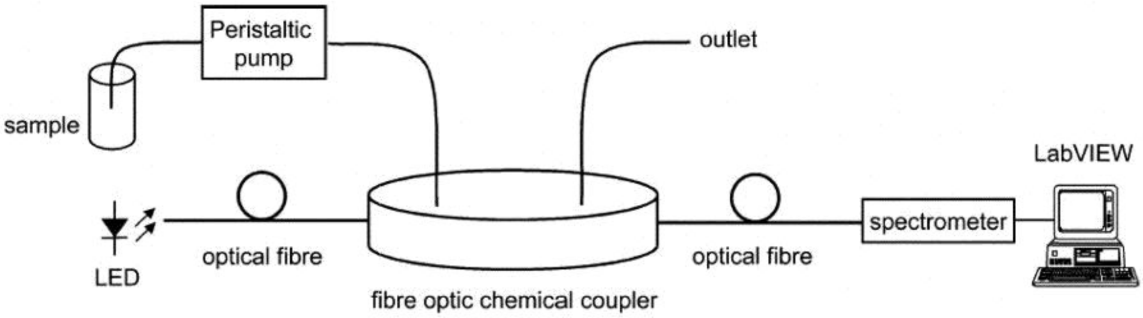


PMMA fibre, diameter: 1 mm

- The main working principle is the change of the refractive index of the medium, which is placed between the fibres. Here, the work is based on the use of a laser beam directed onto the working channel and detecting the interference pattern.
- The fibre optic coupler was fabricated in a block of PDMS to make it compatible with microfluidic structures.

D. Stadnik and Artur Dybko Analyst, 2003, 128, 523–526

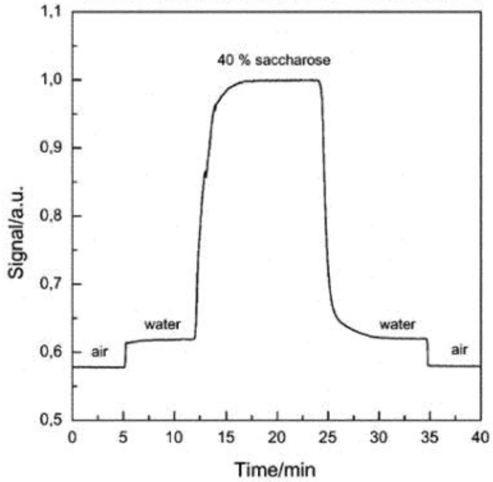
Measurement set-up

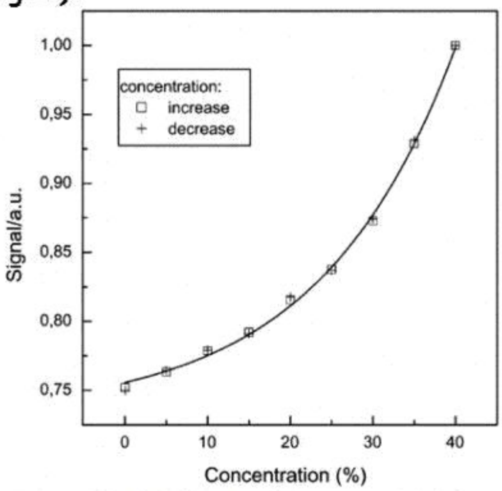


D. Stadnik and Artur Dybko Analyst, 2003, 128, 523–526

Calibration curves

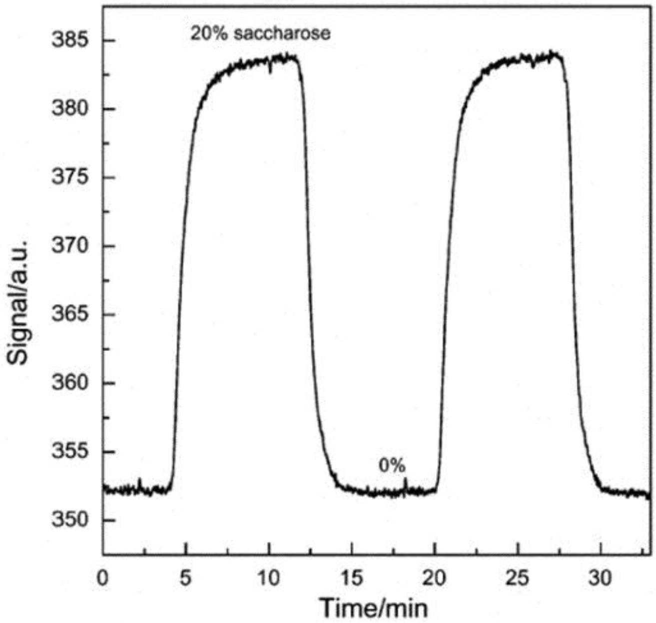
- Signal *versus* time characteristic for different media pumped through the coupler (left).
- Calibration curve of the fibre optic coupler for different concentrations of saccharose (right).





D. Stadnik and Artur Dybko Analyst, 2003, 128, 523–526

Reproducibility test



- Water and 20% saccharose solution were alternatively pumped into the structure. Ten cycles were done and no observable change in the signal values was noticed.

D. Stadnik and Artur Dybko Analyst, 2003, 128, 523–526

Topics in this section

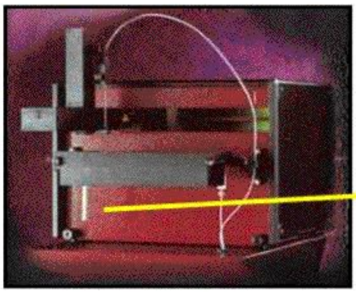
Semi-integrated system and components there of
Research approaches and commercial activities.

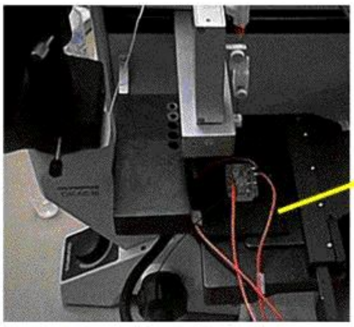
Source: Infineon

Strategic developments

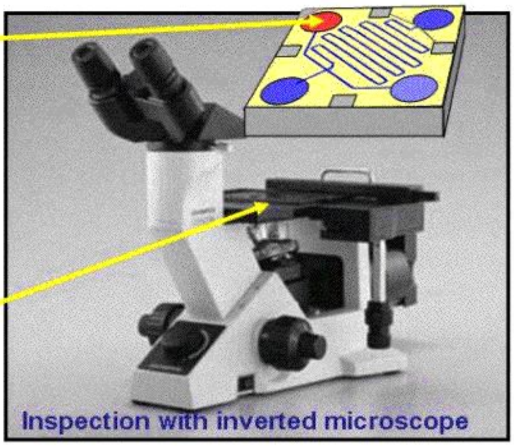
- Methodology of system integration
  - Classical microscopic detection of Lab-on-a-chip events
  - Optical interface to microfluidic channel
    - Discrete optical components (hybrid)
    - Semi-integrated (waveguides, diodes)

Laboratory driven systems





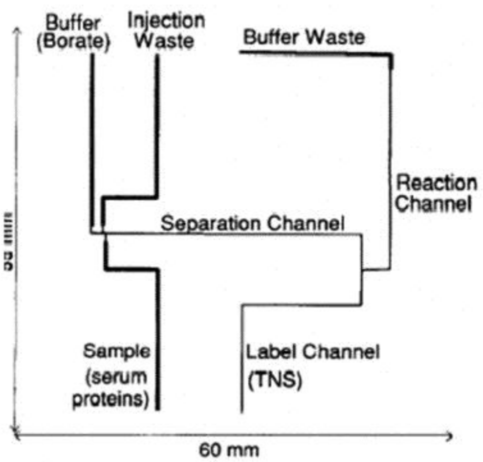
- Experiment utilizing classical microscopy set-up.

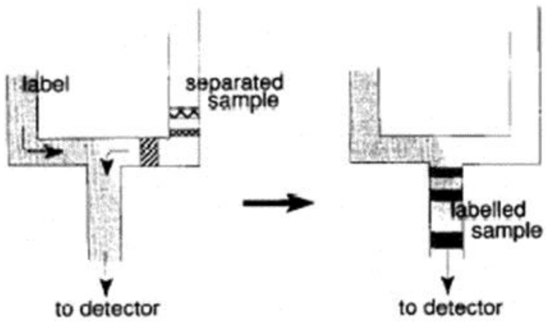


Source: R. Schasfoort, University of Twente

Example: clinical research

- Capillary electrophoresis -on-chip with post column derivatization reaction for identification by confocal microscopy.

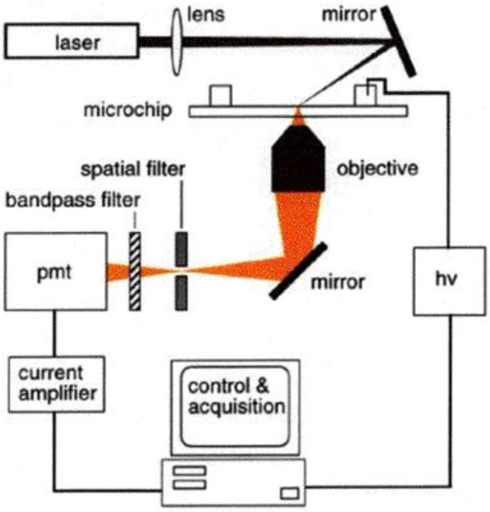




C.L. Colver et al. / J. Chromatogr. A 781 (1997) 271-276

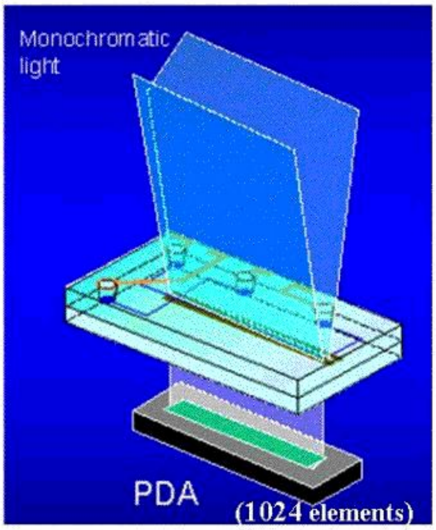
Semi-integrated platforms

Chip set-up with single-point detection using fluorescence



Source: S. C. Jacobson, ORNL Oak Ridge, USA

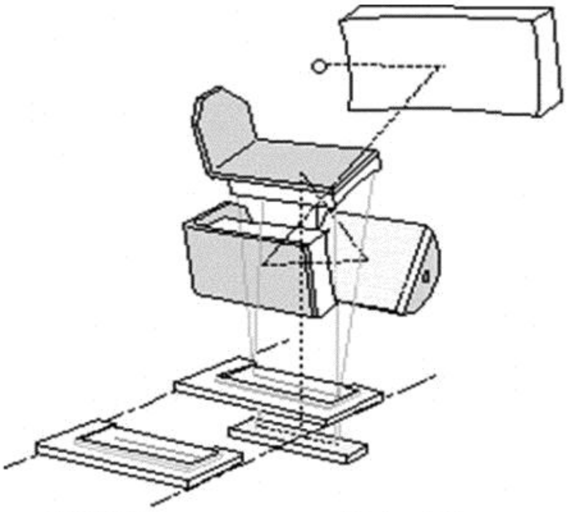
Linear UV-imaging

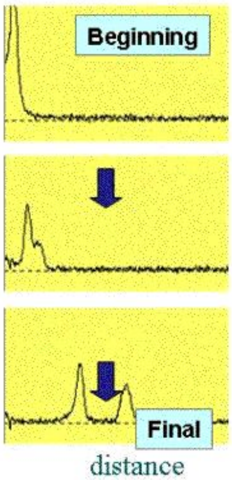


UV absorption Shimadzu MCE-2010

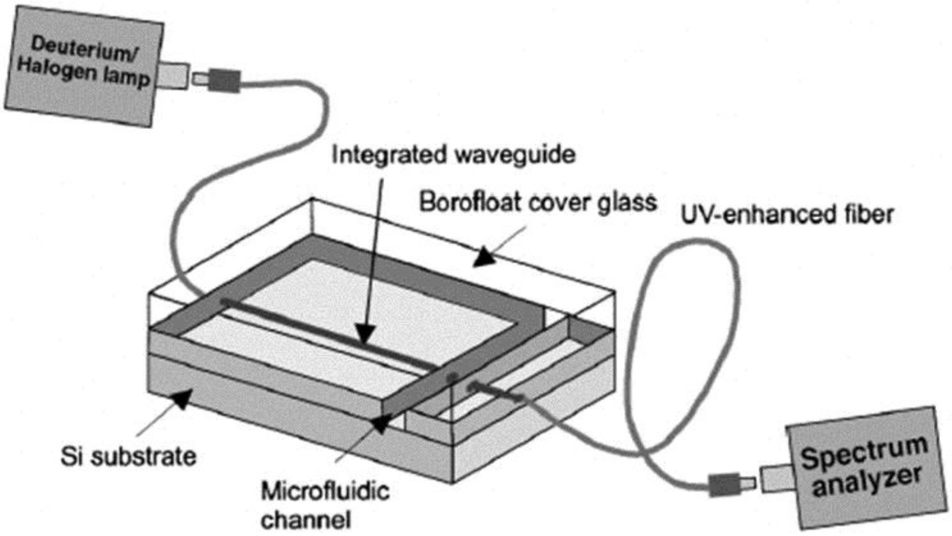
Shimadzu linear imaging UV detection

- Improving signal-to-noise ratio by signal averaging using 1024-element PDA
- Real-time imaging throughout the separation channel



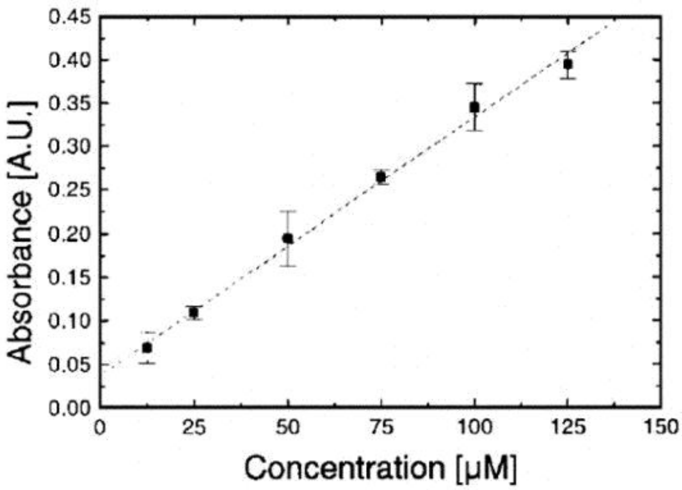


Planar integrated UV-waveguides



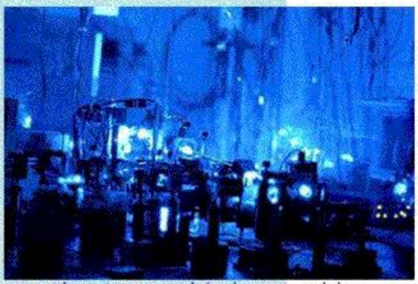
Source: K.B. Mogensen et al. Opt. Lett. 26, 2001, 716

UV-waveguides measurements

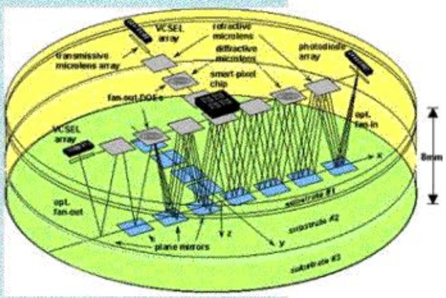


- Absorbance vs. concentration of propranolol averaged from 212 to 215nm with a scan time of 400 ms.
- Lowest detected concentration was 13 mM (signal/noise ratio, 2).

K..B. Mogensen et al./ Opt. Lett. 26, No. 10, 2001, 716



www.photonics.cusat.edu/ celos_research.htm



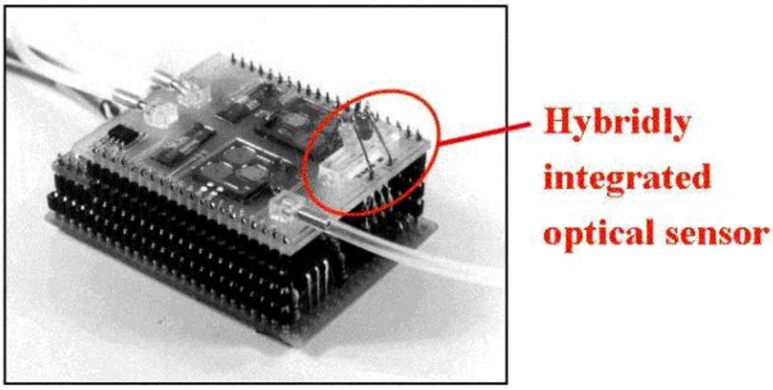
http://microoptics.hanyang.ac.kr/home/purpose.html

Tackling integration

- Hybrid integrated microsystems
  - Packaged systems
    - Modular print board
- Planar integrated components
  - Waveguides, diodes, lenses

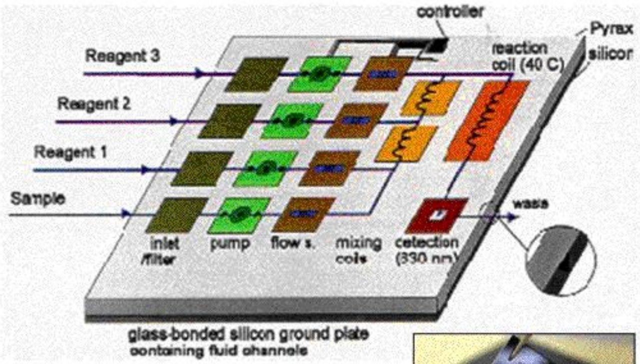
Hybridly integrated optical detection

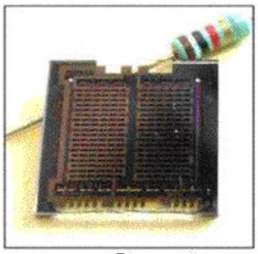
- *A great many case studies exist applying optical detection to integrated systems.*
- *Telecommunication puts down the knowledge base.*



T. Lammerink University of Twente, Modular micro chemical analysis system - 1994

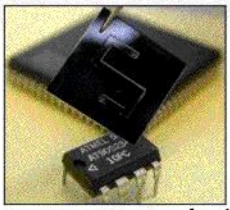
From μ-Total Analysis System to Lab-on-a-Chip

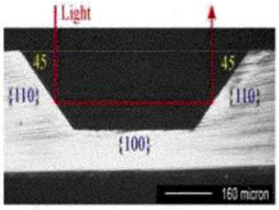




Reactor

Pump

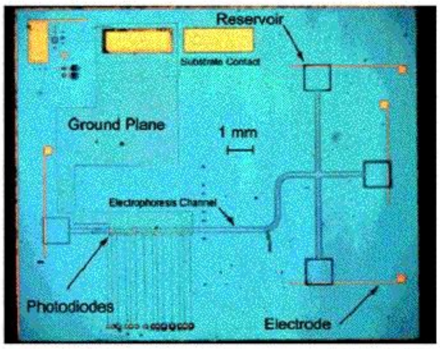




Optical absorbance cell

Source: Ph.D. thesis T. Veenstra (2001), University of Twente

Monolithic CE device with fluorescence detector and off-chip excitation



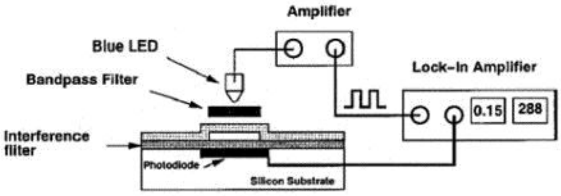
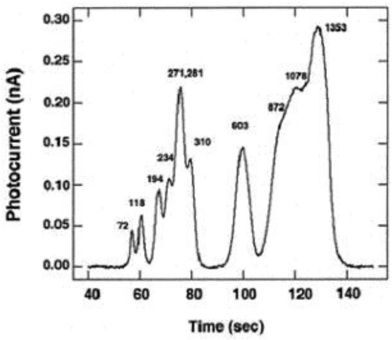
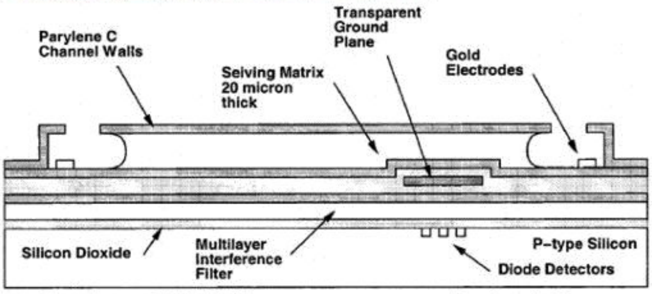


Figure 4. On-chip fluorescence detection system.





J.R. Webster et al. / Anal. Chem. 73, 2001, 1622

Integrated lens arrays

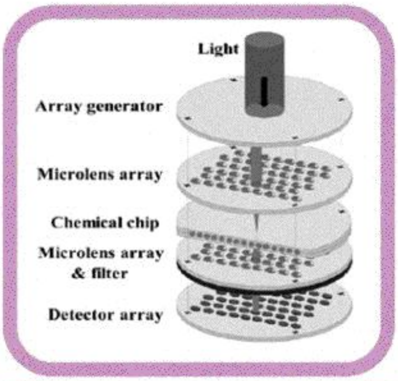
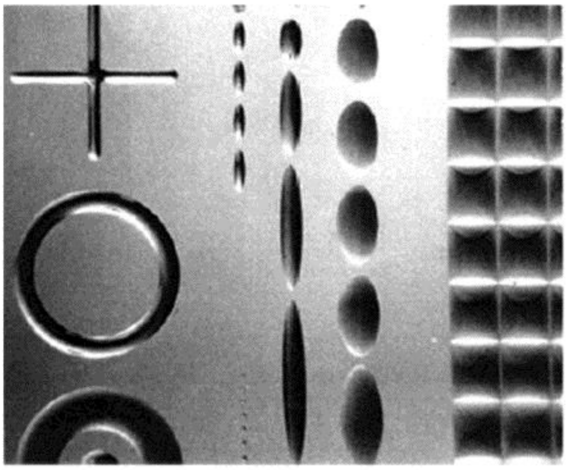


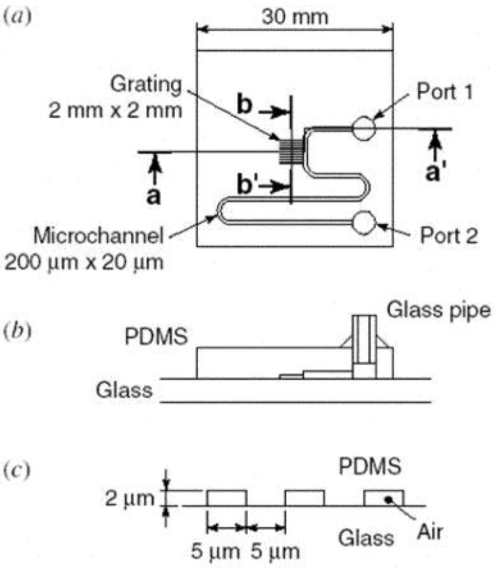
Fig. 2 Concept for the construction of microsystems combining microfluidic and micro-optical functions. In this case, it was of interest to address multiple channels with stacked arrays of optical elements. (Courtesy of J.-C. Roulet and R. Völkel, Institute of Micotechnology, University of Neuchâtel, Switzerland).

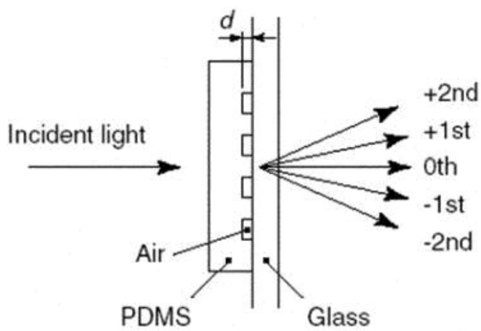


E. Verpoorte, Lab Chip 3, 2003, 42N

5.3. Tackling integration

Integrated optical grating





Deformable diffraction grating to measure pressure in microfluidic chip.

K. Hosokawa e.a., J. Micromech. Microeng. 12, 2002, 1

Dealing with short path length

- Besides improving optical singnal-to-noise ratio by guiding the light over a longer channel distance (which also means that the sample volume is larger) an alternative method is multireflection.
- Increases the path length from 10-30 μm to 50-272 μm by multireflections in a fixed sample volume.

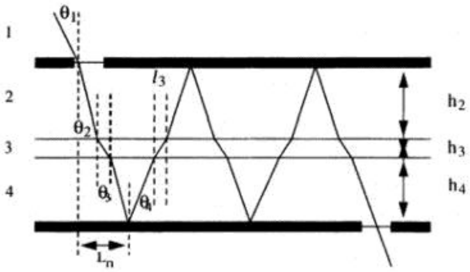
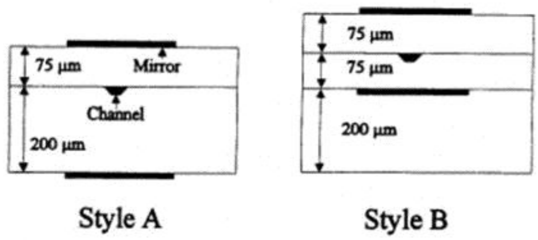
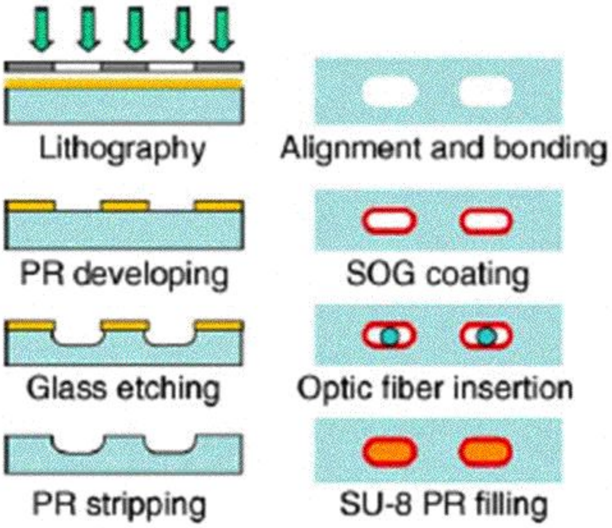


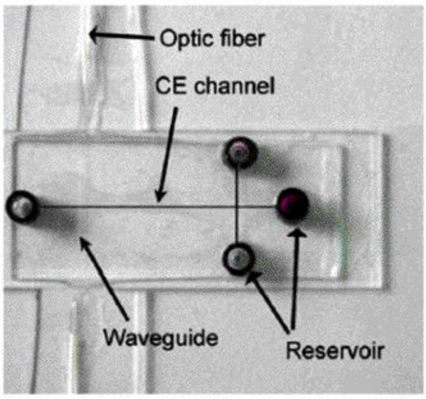
Figure 1. Diagram showing relevant dimensions and angles for determining optical path length in a planar multireflection absorbance cell. The narrowest region inside the cell is the fluid flow path.



H. Salimi-Moosavi et al., Electrophoresis 21, 2000, 1291

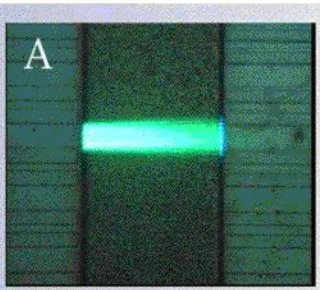
Buried fibre-optical waveguides

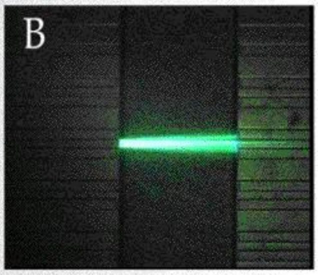


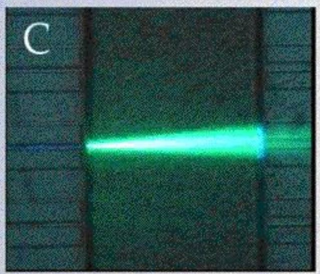


C.H. Lin et al., Sens. Act. A 107, 2003, 125

Coupling integrated waveguides to microfluidic channel cross section







Waveguide widths:

A. 100 µm

B. 24 µm

C. 6 µm

Source: P. Friis, MIC , slide prepared by J.P Kutter, MIC

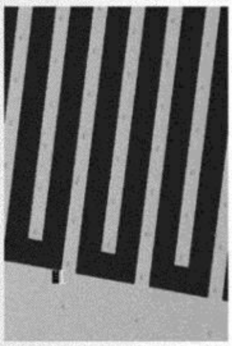
Active integrated optical components

prepared by J.P Kutter, MIC

Back-side photodiodes

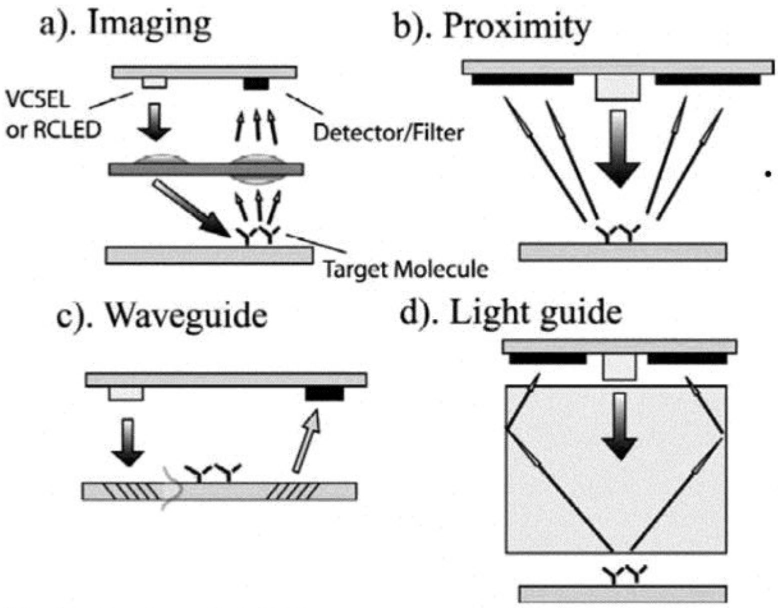
schematic of a waveguide, a coupling structure, and a back-side photodiode

microphotograph of a diode with inter-digitated fingers of alternating polarity



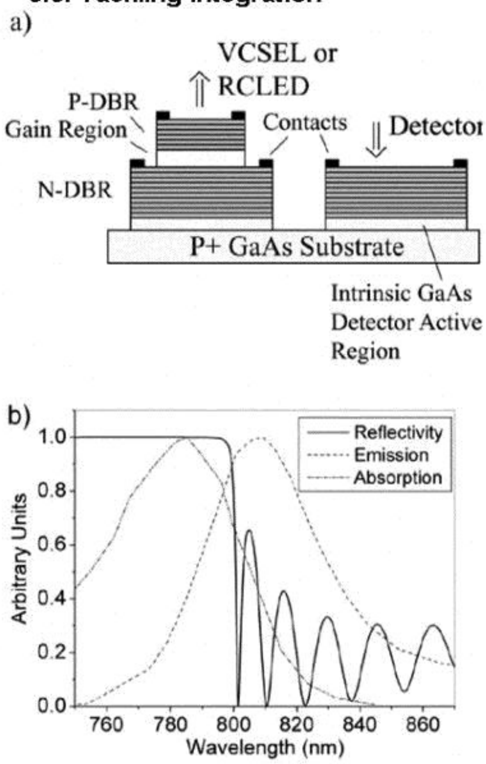
Source: A. M. Jørgensen, MIC, slide

Integrated VECSEL excitation and imaging

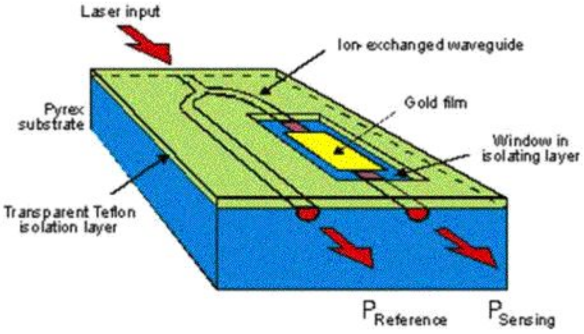


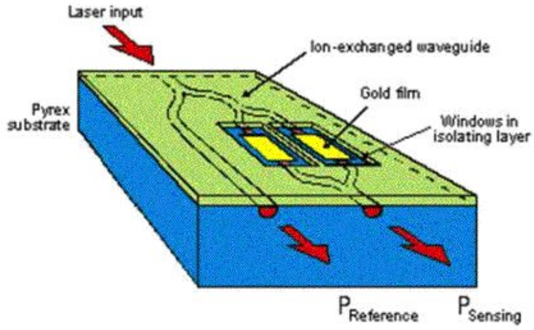
- Optical system design: Filter through the many possible design alternatives to find the most optimal and practical solutions.
- Sensor architectures that are possible with vertical oriented optical devices, such as VCSELs, PIN photodiodes and emission filters. The imaging architecture (Fig. 1a) utilizes micro-optics, refractive or diffractive, for focusing the laser beam and collecting the fluorescence.

Optical performance

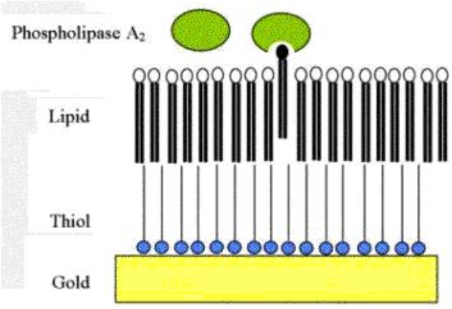


Refractive index changes bio-layer probing



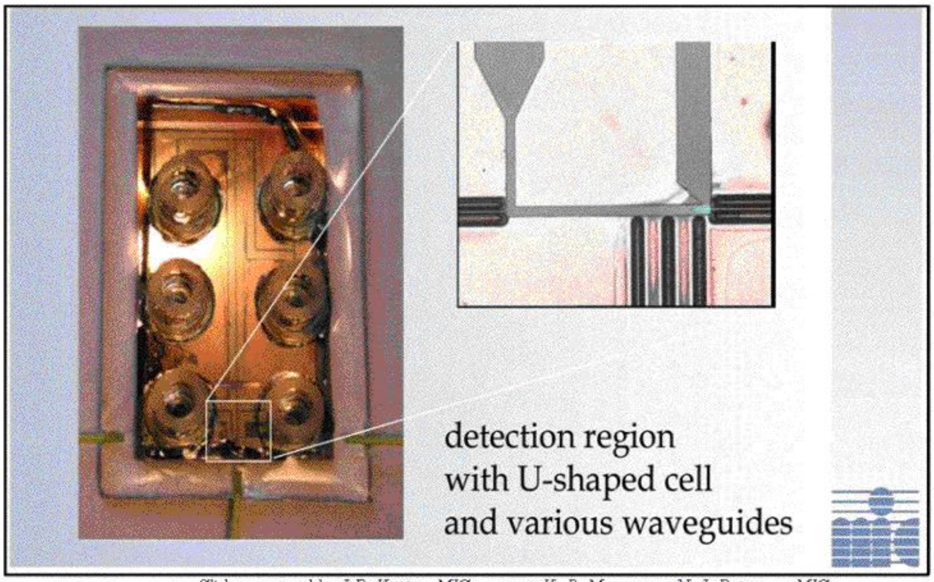


- Mach Zehnder integraded detection



www.orc.soton.ac.uk/ ioms/sensors1.php

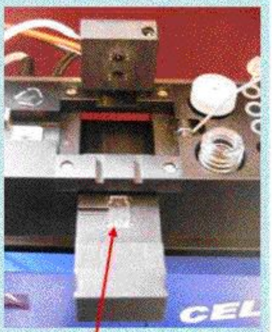
Next generation electrophoresis chip with integrated waveguides



Slide prepared by J.P. Kutter, MIC, source: K. B. Mogensen, N. J. Petersen, MIC

Topics in this section

Integrated optical microsystems
Packaging needs to reach microelectronics standard, however, development efforts in research approaches are often financially out of reach. Therefore many hybrid device platforms exist.



Chip (1 cm x 2 cm)

Examples of optically integrated microfluidics

- Case study.
- Examples of optical techniques other than fluorescent and absorption in brief.

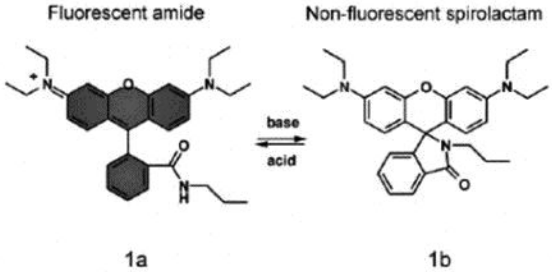
5.4. Examples of optically integrated microfluidics

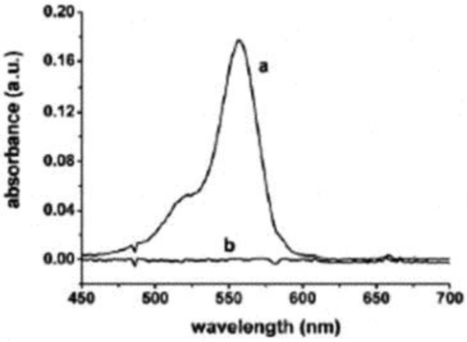
Case study I:

New microfluidic-driven experimental studies exploiting optical phenomena for the detection of physical parameter

Molecular optical dye probing

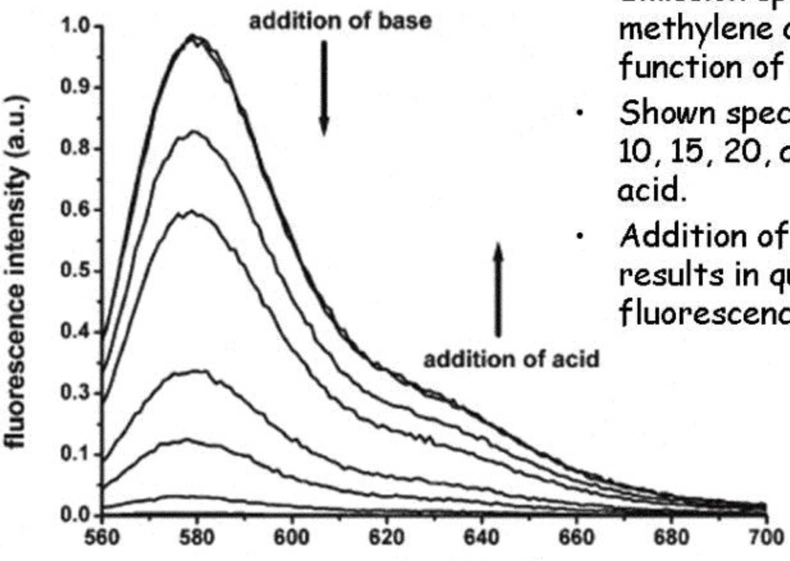
- Application demonstrates a monolayer-functionalized microfluidics device for optical sensing of acidity.
- System is sensitive enough to detect the addition of 1% of acetic acid and reaches maximum fluorescence intensity after addition of ca. 20% of acetic acid.





P. Mela et al., Lab Chip, 2005, 5, 163–170

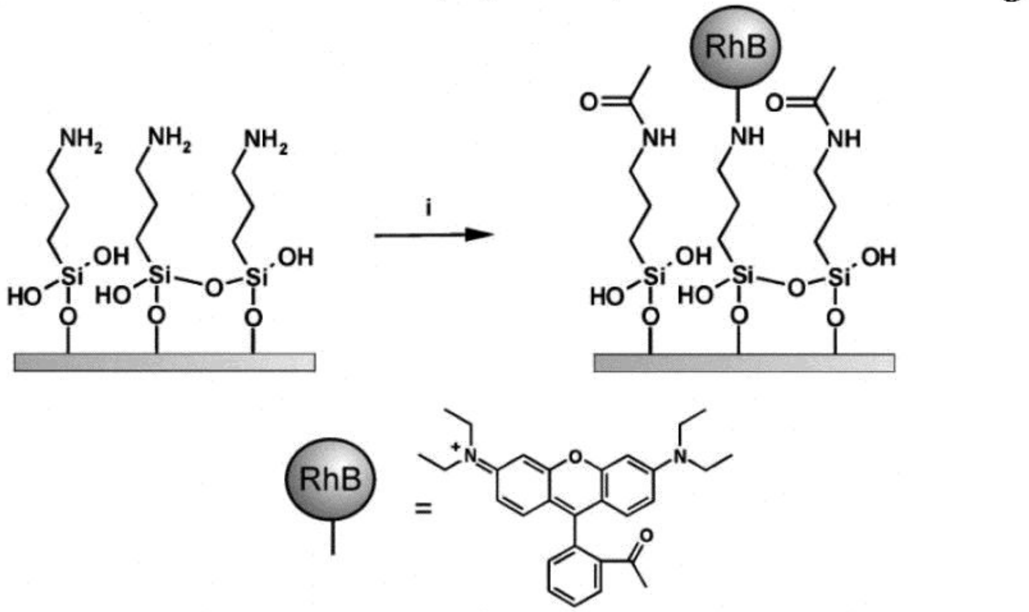
Intensity shift



- Emission spectra of 1 in methylene chloride as a function of added acetic acid.
- Shown spectra contain 1, 3, 5, 10, 15, 20, and 25% of acetic acid.
- Addition of triethylamine results in quenching of the fluorescence (data not shown).

P. Mela et al., Lab Chip, 2005, 5, 163–170

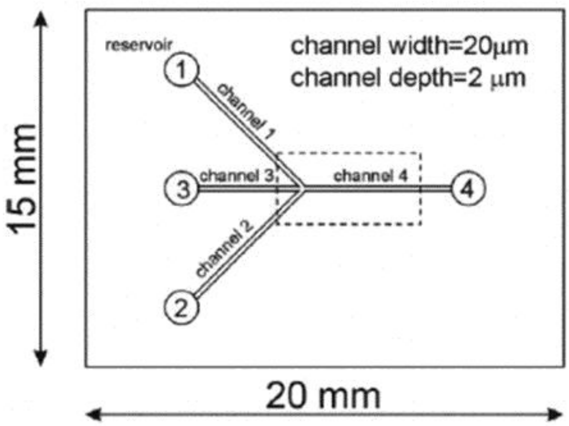
Surface chemistry (SAM) and RhB binding

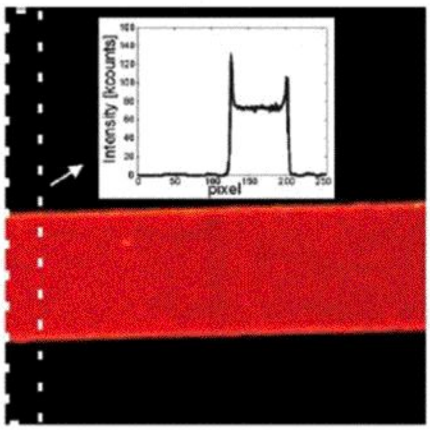


P. Mela et al., Lab Chip, 2005, 5, 163–170

Chip layout and microscopy

- Typical confocal microscopy image (70 x 70 μm^2) and mean fluorescence intensity (inset) measured over 10 scan lines.

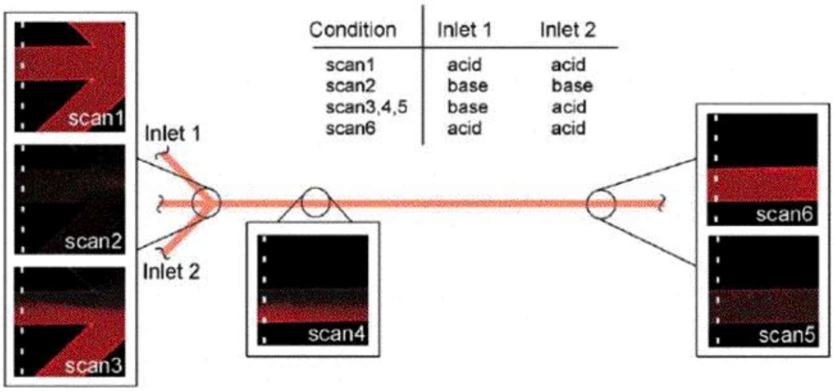


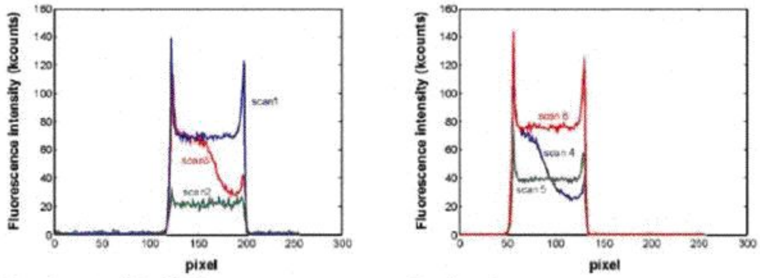


P. Mela et al., Lab Chip, 2005, 5, 163–170

RhB-functionalized microfluidics

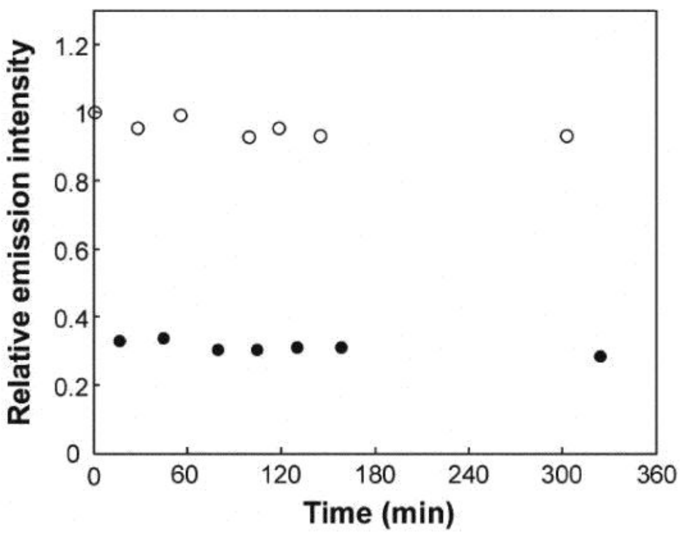
Condition	Inlet 1	Inlet 2
scan1	acid	acid
scan2	base	base
scan3,4,5	base	acid
scan6	acid	acid





P. Mela et al.,
Lab Chip, 2005, 5, 163–170

Repeated on–off switching of RhB

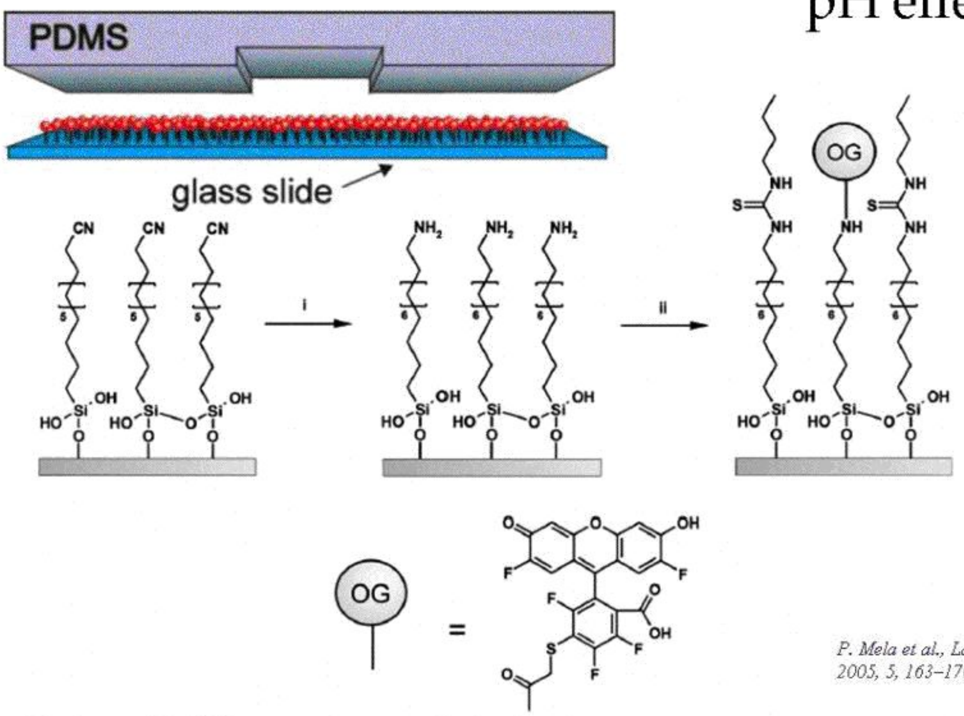


- Relative mean fluorescence intensity after alternating exposure to acid (open dots) and to base (solid dots).
- In between acid-base cycles the channels were rinsed with methylene chloride.

P. Mela et al., Lab Chip, 2005, 5, 163–170

5.4. Examples of optically integrated microfluidics

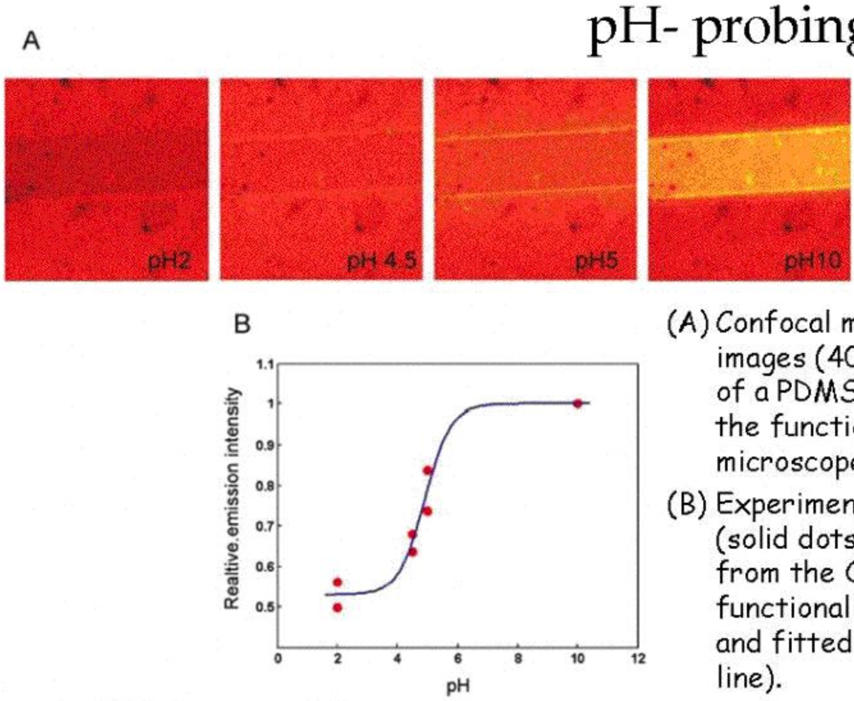
pH effect



P. Mela et al., Lab Chip, 2005, 5, 163–170

5.4. Examples of optically integrated microfluidics

pH- probing results



(A) Confocal microscopy images (40 x 40 μm^2) of a PDMS channel on the functionalized microscope glass slide.

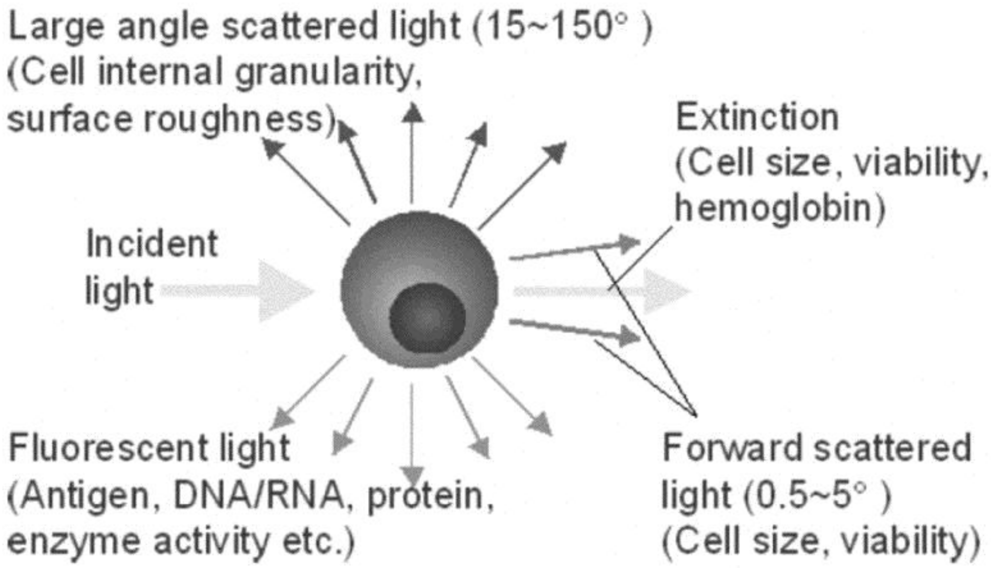
(B) Experimental data (solid dots) obtained from the OG514 functionalized layers and fitted curve (solid line).

P. Mela et al., Lab Chip, 2005, 5, 163–170

Case study II

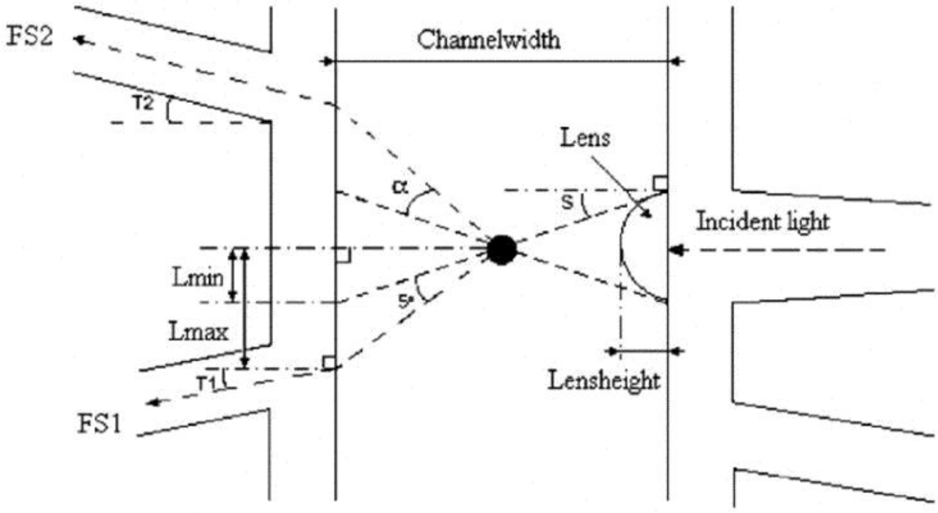
Applying optical phenomena to microfluidic chip-based single cell analysis

Cytometry: using scattered signals



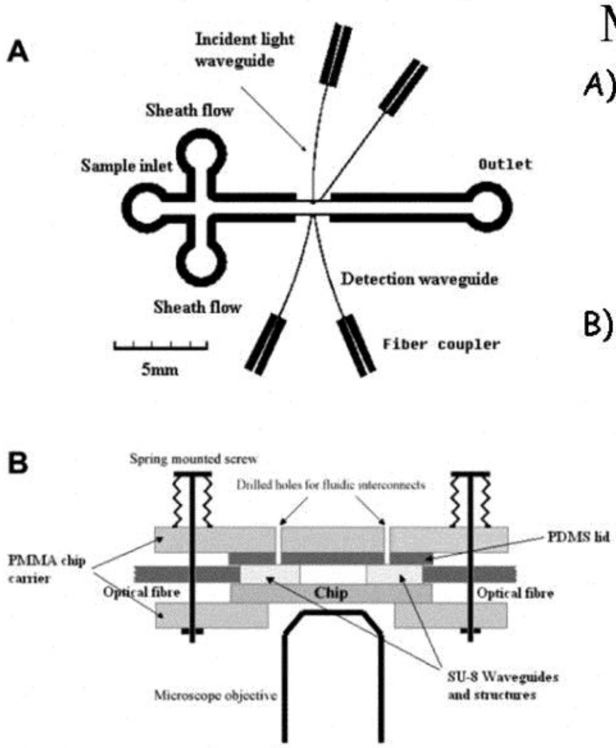
Z. Wang et al., Lab Chip, 2004, 4, 372–377

Microchip cytometer layout



Z. Wang et al., Lab Chip, 2004, 4, 372–377

Microchip cytometry

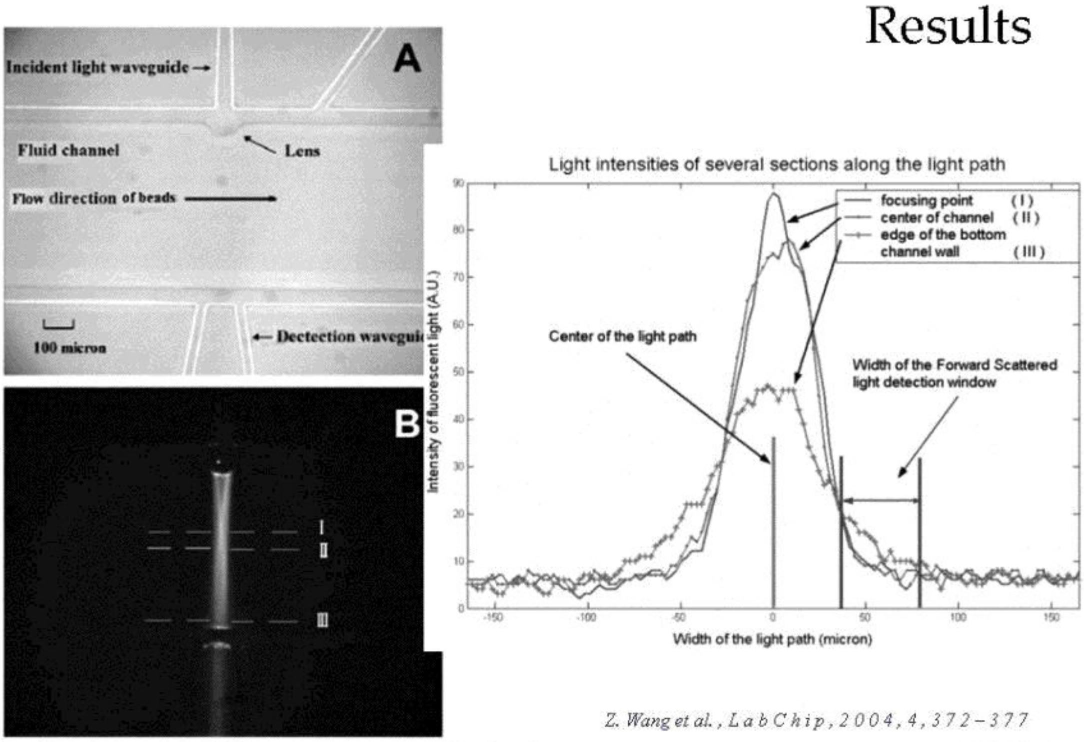


A) Design of the microchip. All the optical elements, the microfluidic system, and the fibre-to-waveguide couplers were defined in one layer of polymer.

B) Schematic of the chip's packaging. The chip is placed in a holder and a PDMS lid (n = 1.4) was utilized to seal the flow channels and to serve as top cladding layer. The thickness of the SU-8 layer was adjusted to readily accommodate 70 μm outer diameter optical fibres in the fibre couplers.

Z. Wang et al., Lab Chip, 2004, 4, 372–377

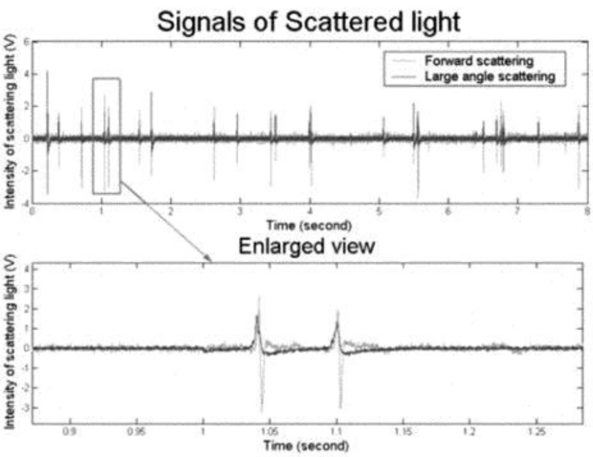
Results



Z. Wang et al., Lab Chip, 2004, 4, 372–377

Time-dependent intensity plots

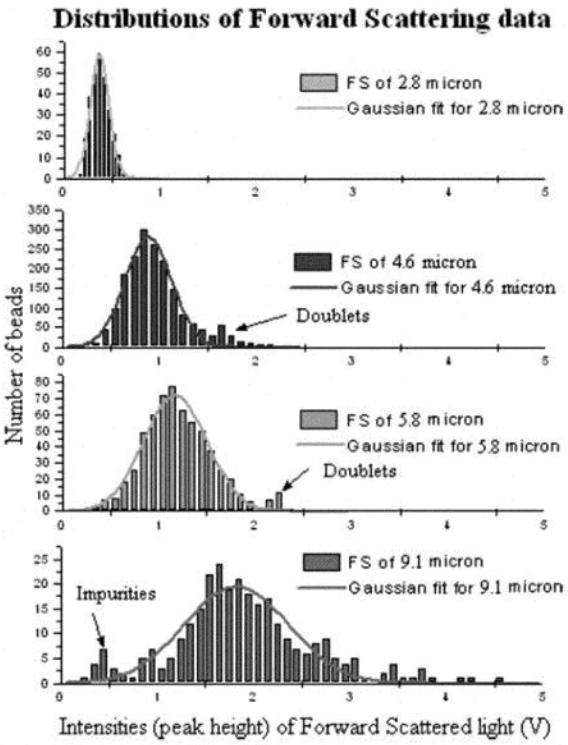
- Typical scattered light signals. When a bead passes through the light beam a positive forward scattered light (FS) peak is detected by the FS waveguide first, followed by a negative extinction (EX) peak as a result of the bead blocking part of the incident light.



- Simultaneously, large angle scattered light (LS (90°)) was detected vertically through the microscope objective.

Z. Wang et al., Lab Chip, 2004, 4, 372–377

Scattering data

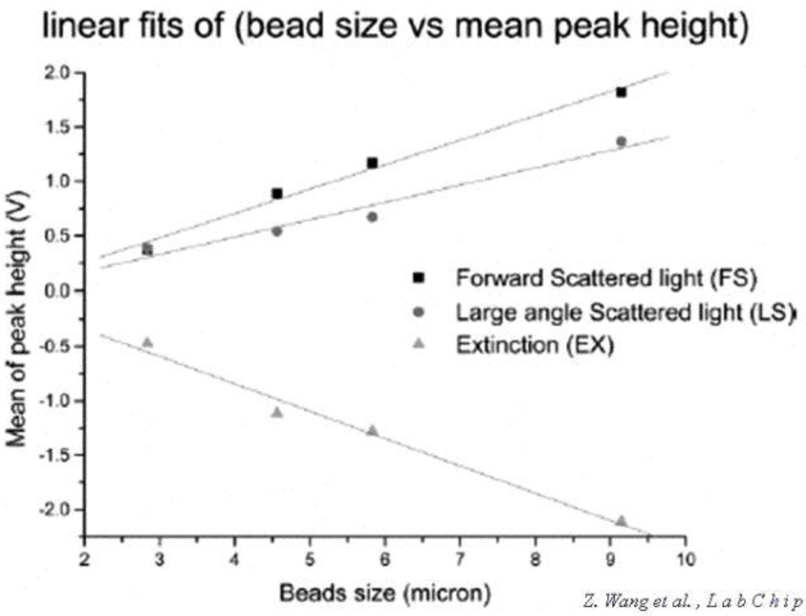


- Distribution of forward scattered light intensities (peak height) for four different bead sizes.
- In the histogram for 9 μm beads an extra peak is seen in the low intensity area. This is due to an impurity of smaller particles in the beads sample.

Z. Wang et al., Lab Chip, 2004, 4, 372−377

MESA+

Fitting the data



Z. Wang et al., Lab Chip, 2004, 4, 372−377

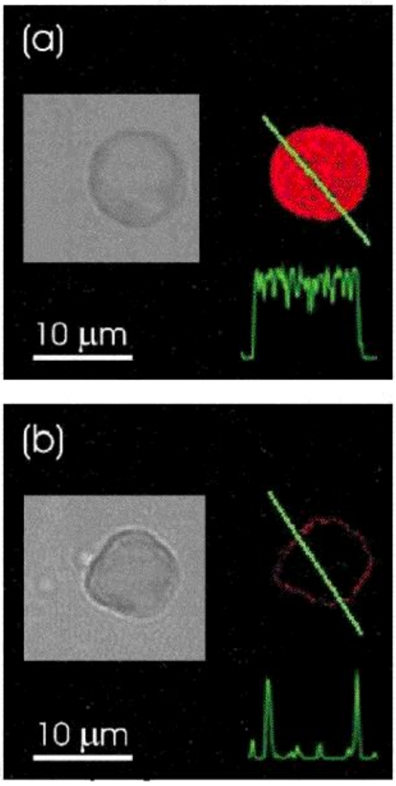
MESA+

Special optical techniques in brief:

Introducing optical integrated systems and phenomena to monitor molecular interactions and microfluidics operation

5.4. Examples of optically integrated microfluidics

Remote release of encapsulated beads

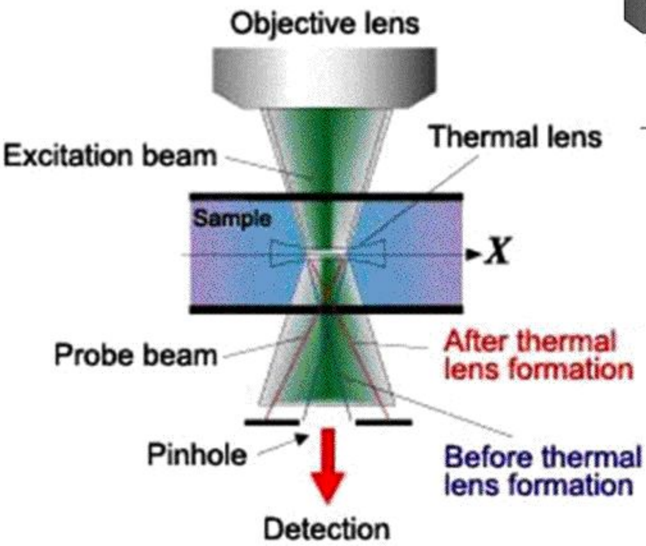


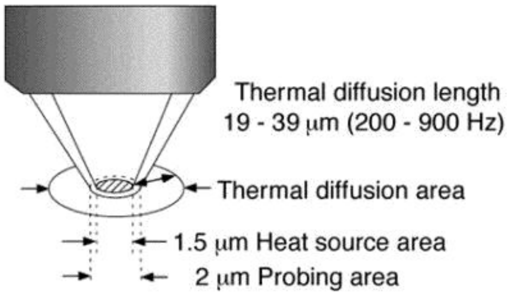
- Confocal fluorescent analysis during optically-induced particle release from a encapsulated fluorescently labelled polymers from nanoengineered polyelectrolyte multilayer capsules.
- Incident intensity of laser diode operating at 830 nm was set at 50 mW

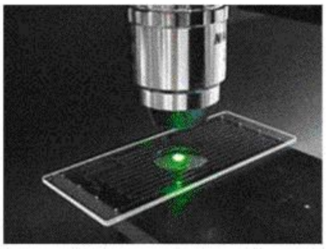
A.G. Skirtach et al., Nano Lett., Vol. 5, No. 7, ***2005, 1371-1377***

W Regina Luttge

Thermal lens detection



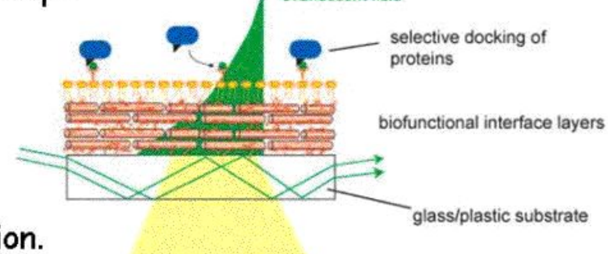




K. Uchiyama et al., Jpn. J. Appl. Phys. 39, 2000, 5316

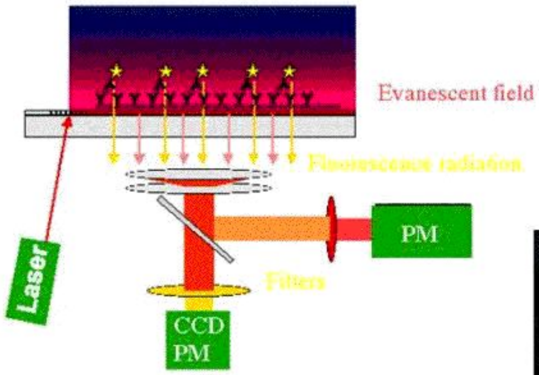
Evanescent field detection

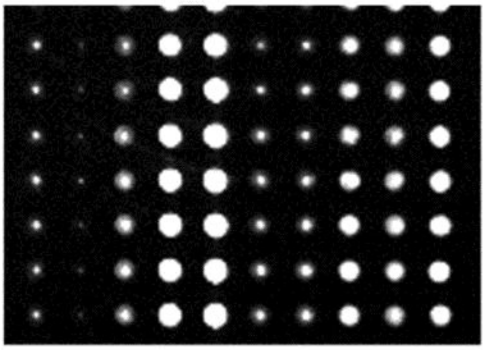
- Many affinity based biochip applications depending on the properties of the biofunctional interface layer.
- Ultrahigh sensitivity for signal detection on a microdot:
  - Minimal sample volumes required.
- Capability to perform real-time monitoring of binding events:
  - Kinetic studies can be performed .
- No background interference from the bulk medium:
  - No necessity of washing steps to remove non-bound tracer molecules,
  - Measurements in light scattering media, e.g. serum, blood, etc., can be performed. No or reduced sample preparation.

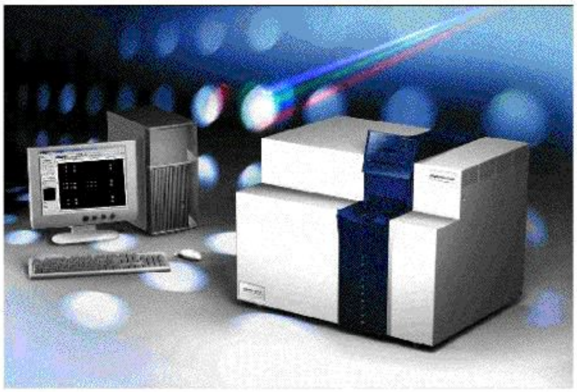


Source: "picture finder Google"

Biochip affinity arrays





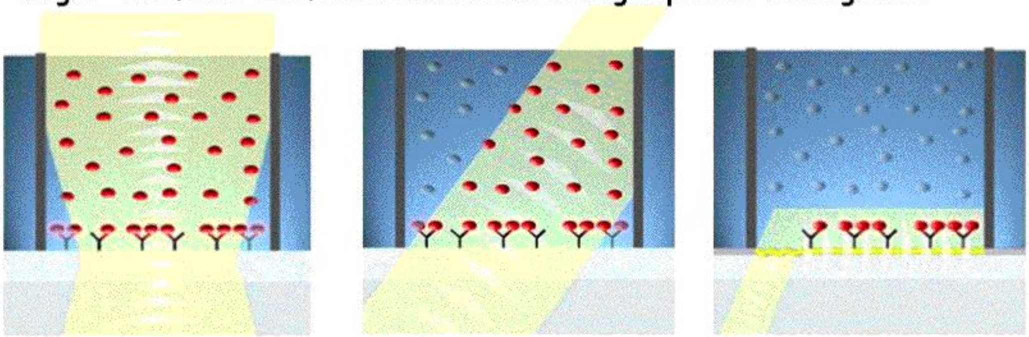


http://www.zeptosens.com/

http://www.textorgroup.ch/

Excitation schemes used for fluorescence excitation

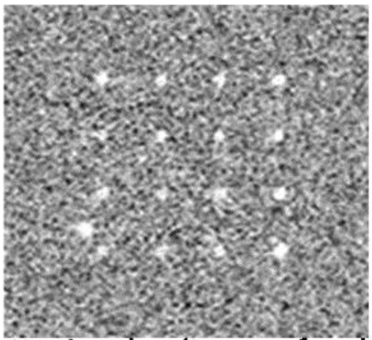
Left: conventional (confocal) excitation.
Center: excitation by an inclined excitation light path.
Right: surface-confined excitation using a planar waveguide.



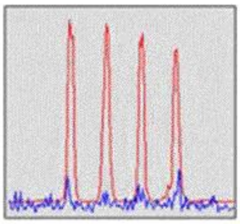
http://www.zeptosens.com/

High sensitivity detection

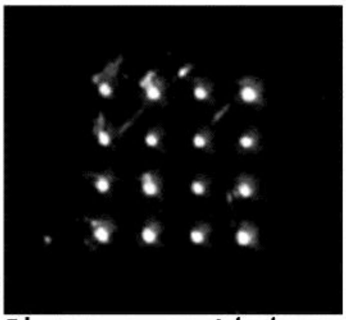
- **Application to microarray sensor chip.**
  - The **outperforming sensitivity** of fluorescence excitation in the evanescent field of a planar waveguide. The example shows the fluorescence signals after hybridization of Cy5-labelled 25-mer oligonucleotides (50pM) with immobilized complementary strands. Superior S/N ratio obtained with the PWG detection system (centre).



Market leading confocal fluorescence scanner



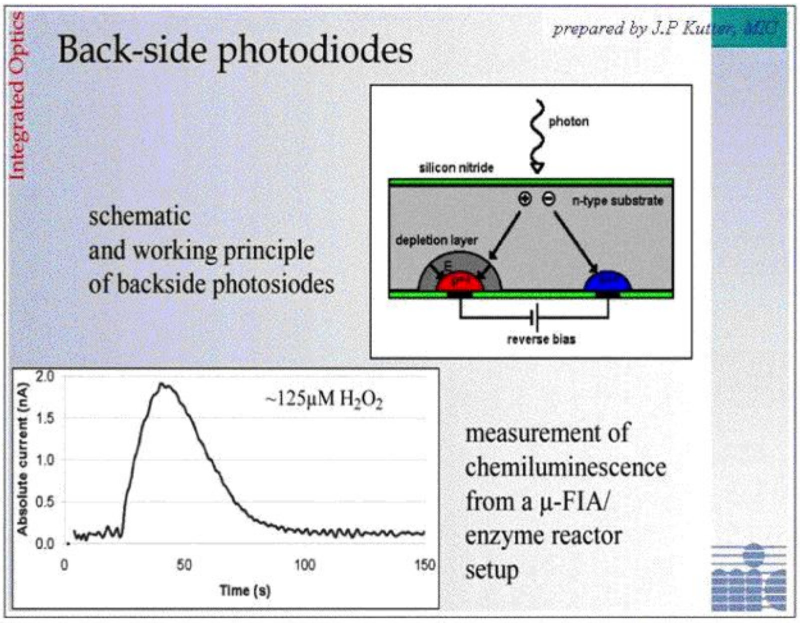
S/N = 2/1
(top: PWG intensity)
S/N = 200/1
(bottom: scanner intensity)



Planar waveguide based detection system

http://www.zeptosens.com/

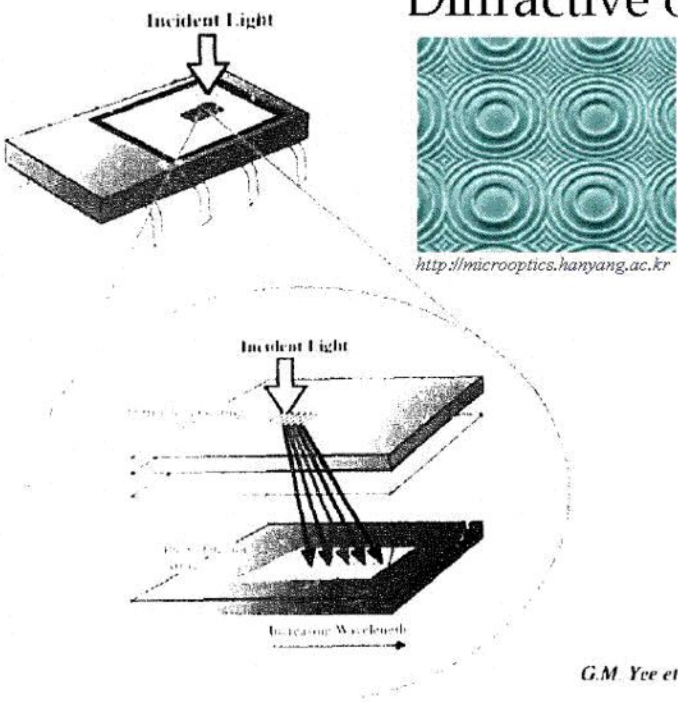
Detection of chemiluminescence



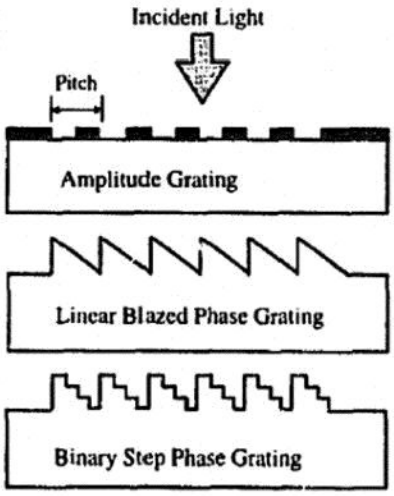
Source: A. M. Jørgensen, MIC, slide

5.4. Examples of optically integrated microfluidics

Diffractive optics integrated spectrometer



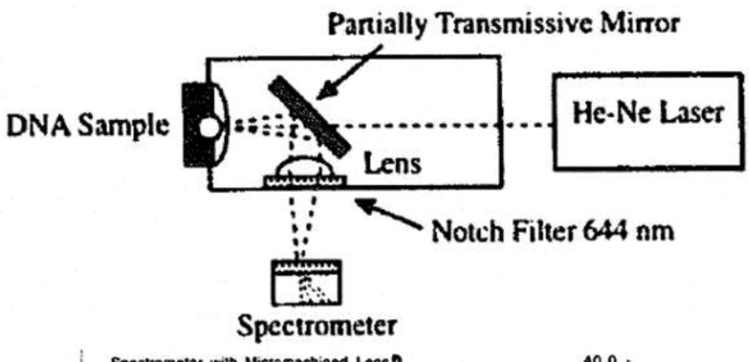
http://microoptics.hanyang.ac.kr

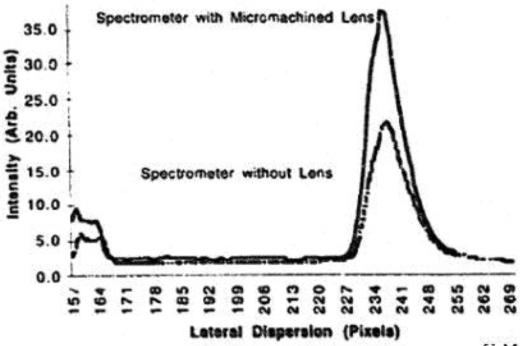


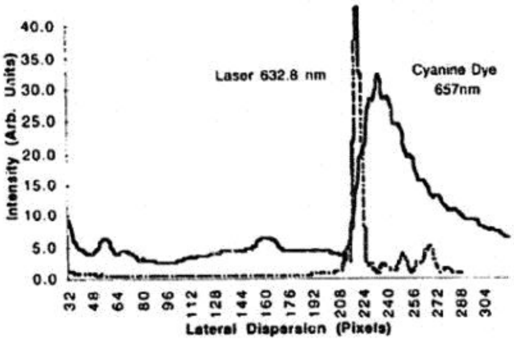
G.M. Yee et al. / Sensors and Actuators A 58 (1997) 61–66

5.4. Examples of optically integrated microfluidics

Performance







G.M. Yee et al. / Sensors and Actuators A 58 (1997) 61–66

Topics in this section

Commercialization

Agilent

Examples of commercial systems using optical detection

- Point-of-Care
  - Colorimetric reagent strips
- Pharmacokinetics and drug screening
- Biomedical research
  - Biomarker, PCR

Reagent strips

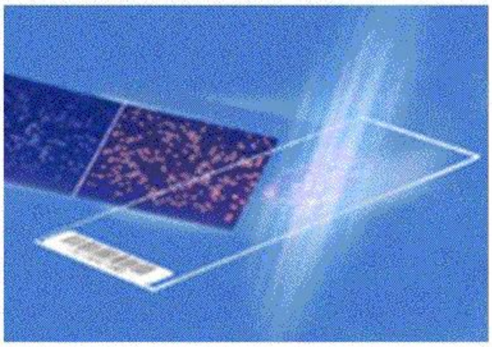
- **MAKROmed Urine strip reader**
  - portable desktop instrument for reading reagent strips for urinalysis. It can be used in a central laboratory or a point-of-care setting and is able to read up to 120 strips per hour. The analyser uses four LEDs (2x 522 nm green, 2x 624 nm orange) as a light source. The reflected light is detected with a photodiode and according to the internal calibration converted to the concentration of the analyte on the test pad.

http://www.makro-med.com/products.htm

Commercial examples of analytical biochemistry chips

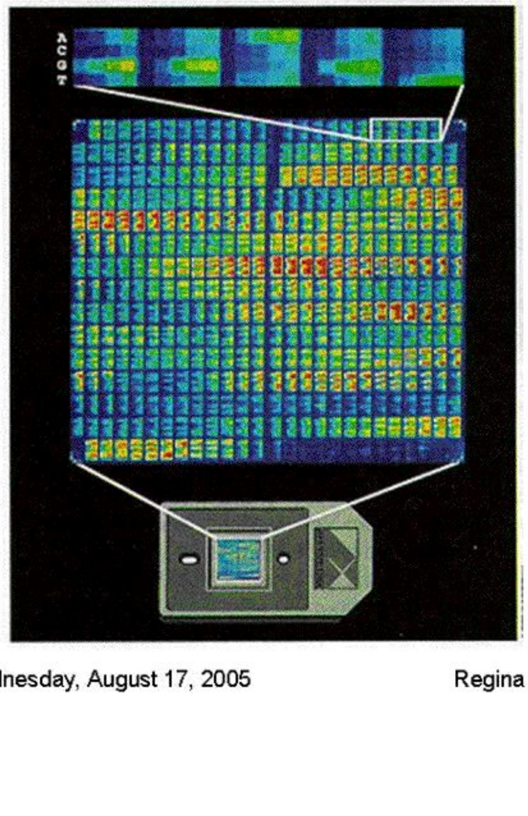
- Exploiting optical detection

Agilent



GeneScan

"Classical" optical-read microarrays

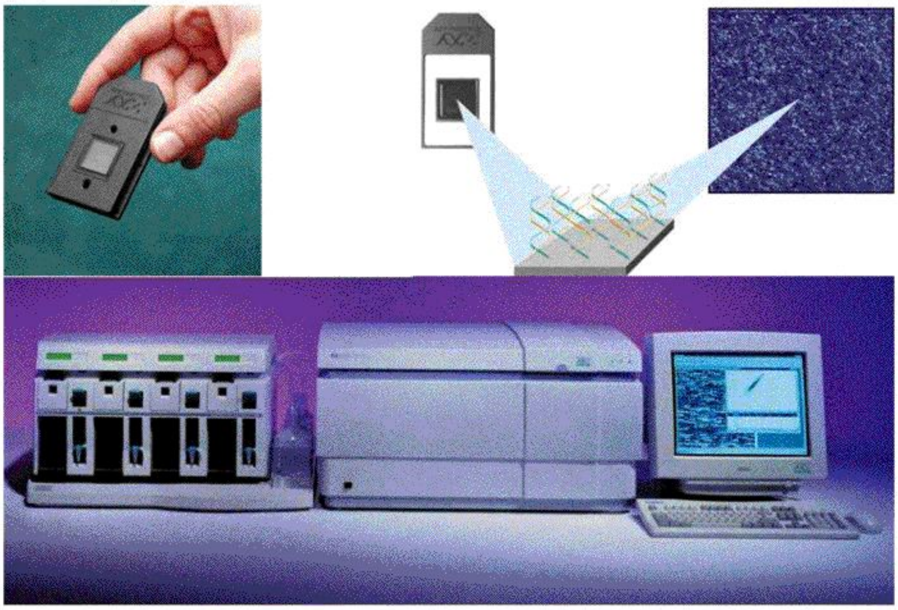


- Optical signal matrix is often specific for a particlular health condition (counts fluorescent intensity per spot).

AFFYMETRIX

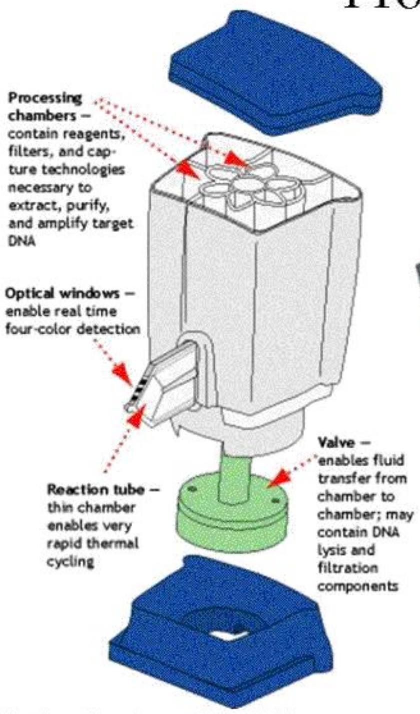
GeneChip®

Products: Affimetrix- GeneChips



www.affymetrix.com

Products: Cepheid "smart cycler"



- DNA Extraction, concentration, and purification from real world samples
  - All in one step

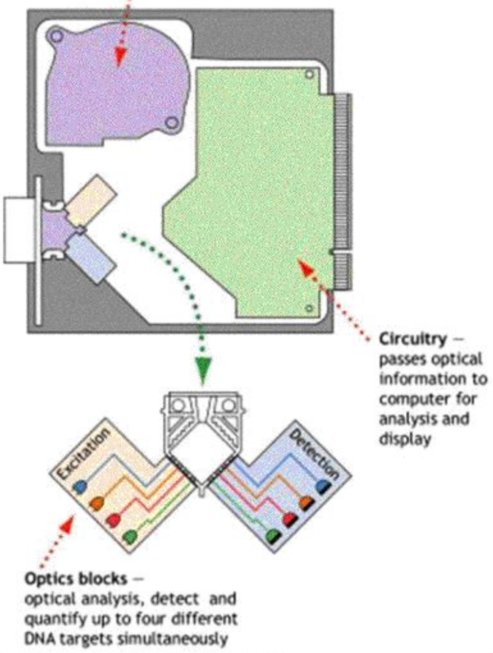
See also sampling session and microarrays

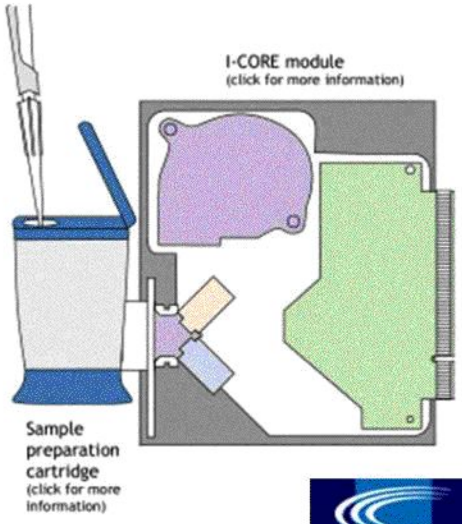
Cepheid

www.cepheid.com

5.5. Examples of commercial systems

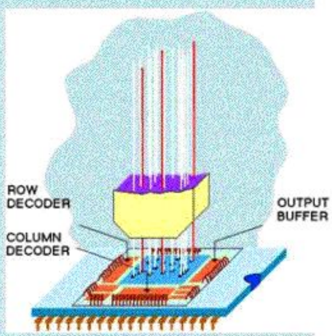
Integrated detection cuvette





www.cepheid.com

Outlook: Future developments

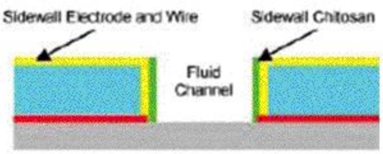


http://www.ph.tn.tudelft.nl/~ed/gifs/optmas.gif

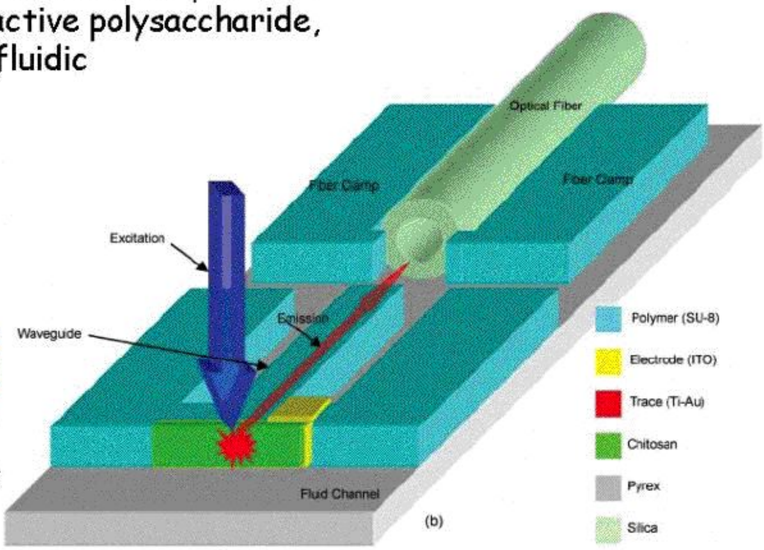
- Bio-MOEMS
  - Unique bioassembly and optical signal collection.
  - Schemes will employ microelectromechanical-assisted optically enhanced techniques in a much more progressive parallel fashion then today.
  - Telecommunication has already paved the way- developments in Lab-on-a-Chip microfluidics will follow.

Unique bioassembly and optical signal collection

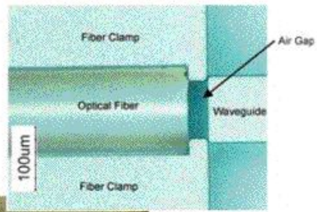
- Scheme employs the electrically-driven deposition of a reactive polysaccharide, chitosan, on microfluidic channel sidewalls.

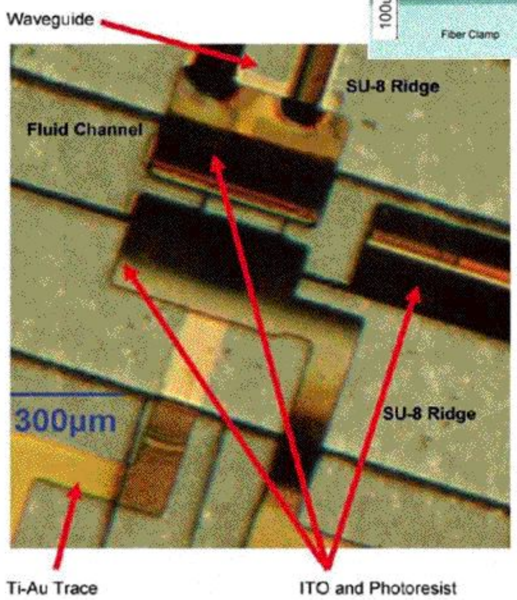


M. A. Powers et al., Lab Chip, 2005, 5, 583–586



Realization

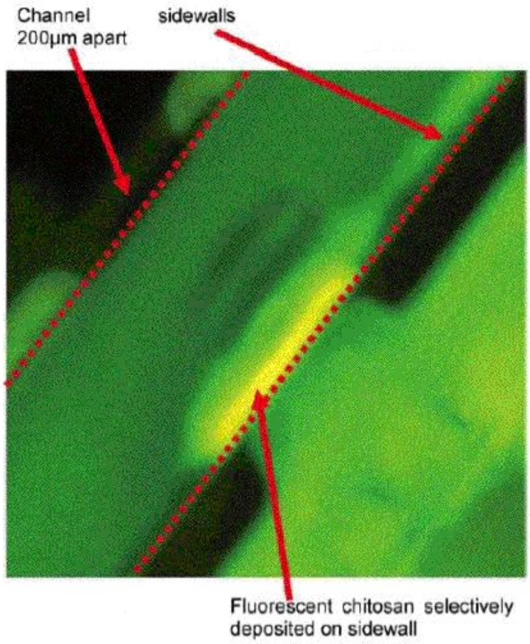




- Electrode configuration in multi-material mix-match assembly resulting in integrated Bio-MOEMS platform.
- Established micro-fabrication techniques meet new operation challenges.

M. A. Powers et al., Lab Chip, 2005, 5, 583–586

Resulting performance

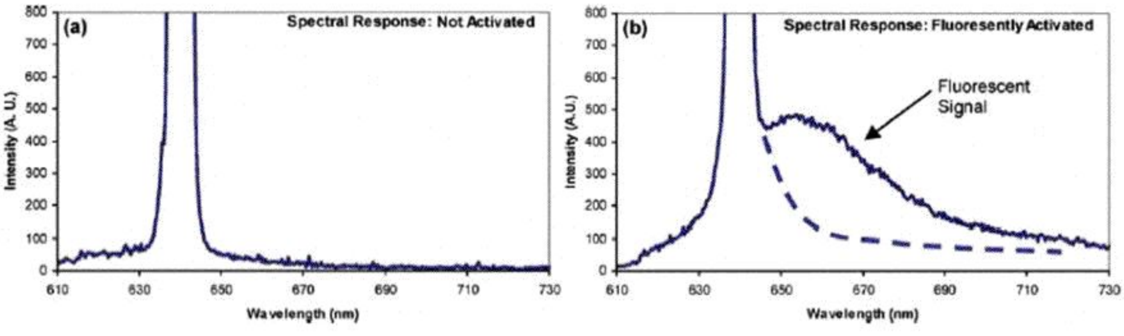


- Advanced in integration techniques on the ever decreasing length scale.
- Will facilitate higher troughput by parallel operation of integrated function on chip.
- Combining physical properties with biological events.

M. A. Powers et al., Lab Chip, 2005, 5, 583–586

Optical signal collection

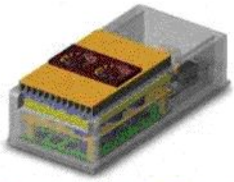
- The chitosan based sensor design and fabrication process is a user-configurable biosensor strategy that is comprised of multifaceted sensing elements arrayed in complex geometries for the simultaneous analysis of multiple analytes, enabling the next generation of micro-biophotonic systems.



M. A. Powers et al., Lab Chip, 2005, 5, 583–586

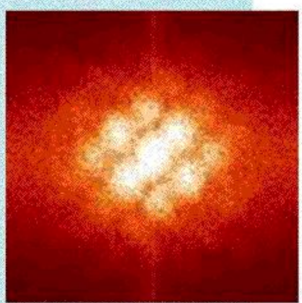
ISGEN: In-Situ Genetics Experiments on Nanosatellites

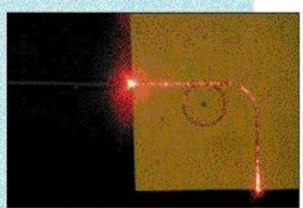
- The miniaturized system telemeters genetic changes in micro-organisms.
- Integrated analytical "cassette", ~ 2" x 4" x 8". It includes:
  - pumps, valves, microchannels, filters, membranes, and wells to maintain the biological viability of various microorganisms.
  - an integrated thermal control system.
  - a suite of sensors.
  - a miniaturized optical detection system.
  - 8 – 24 integrated "microwells."
  - Each 20 – 50 μL microwell contains a population of a model organism, with the option to include replicates and/or genetic variants in the different wells.
  - A permeable membrane covering each well provides gas exchange, and an optical surface on the other face allows (imaging) fluorescence, luminescence, or absorbance-based assay of gene or protein expression, as well as population enumeration via counting or optical density measurement.



http://www.nasa.gov/centers/ames/research/technology-onepagers/isgen.html

Summary





http://www.photonics.com/spectra/tech/XQ/ASP/techid.1602/QX/read.htm

- Many established techniques and methods but no universal approach.
- Upcoming trend to merging technologies.
- Future will benefit from highly parallel processing by adopting telecommunication technologies into microfluidic chip design.