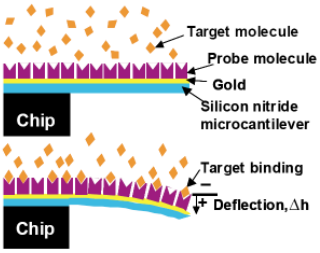
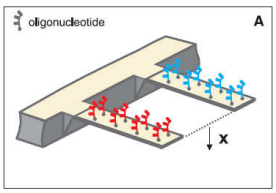
Welcome to Lecture 14

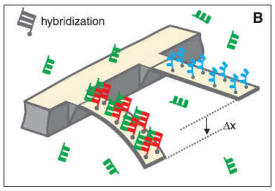
Last time: AFM and microcantilevers



Questions: where does the stress come from?

- Bioreceptors are always immobilized on one side of the cantilever
- In some assays one cantilever is referenced against another





Plan for today:

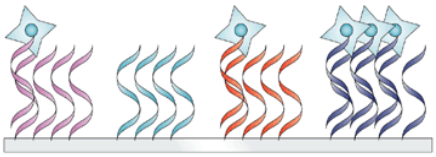
- More on chips
- Talk about some cool stuff and
- Wrap it all up

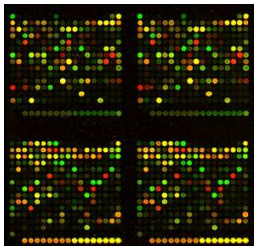
There will be presentations by students

Biochips

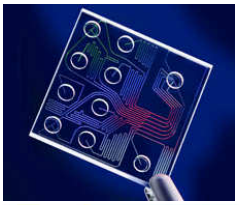
At the last two lectures we explored the biochips technologies

Microarrays e.g. hybridization





Lab-on-the-chip or Micro Total Analysis Systems (μTAS)



Both are often called biochips

What is the difference?

Microarrays are massively parallel assembly of "detection spots" which are often (but not necessarily) biosensors

Labs-on-the-chip are exactly what the name implies – miniaturized devices to carry out laborious/time consuming lab operations

Why miniaturize?

Cost efficiency for the end user

- Sample
- Reagents **Time = $$$**
- Space

i.e. doing massively parallel analysis becomes practical and affordable

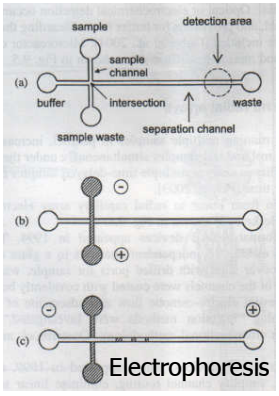
Also, going to a very small scale offers some new technical opportunities

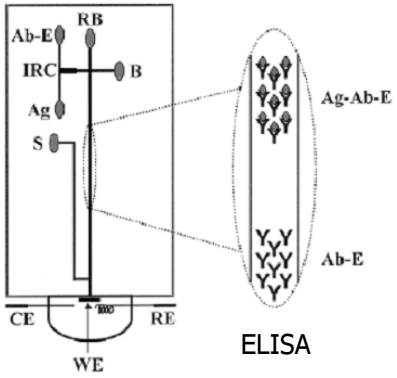
and for manufacturers

e.g. the more chips can be cut from a wafer, the better ☺

How good are these devices

Last time we have seen examples of what they can do





Microfluidic devices – can manipulate minute amounts of fluids

Today we'll take more in depth look at microfluidic biochips

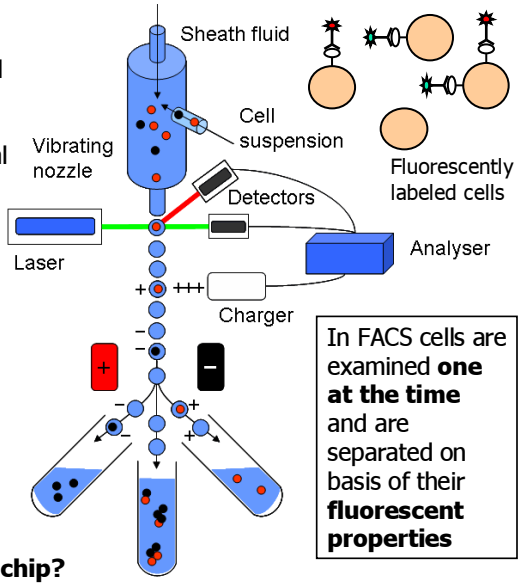
What else can we do?

Fluorescence-activated cell sorting (FACS) is a specialized flow cytometry technique used for characterization and separation (sorting) of individual cell types from mixtures

It is a complex instrument that requires trained personnel for operation and maintenance

~$0.5mln

Becton Dickinson FACS

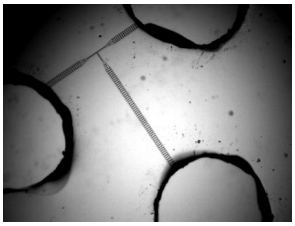


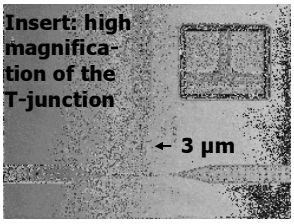
In FACS cells are examined **one at the time** and are separated on basis of their **fluorescent properties**

Can we do FACS on a micro-chip?

Cell sorting on a chip: µFACS

Optical micrograph of the µFACS device:
fabricated from a mold using soft lithography

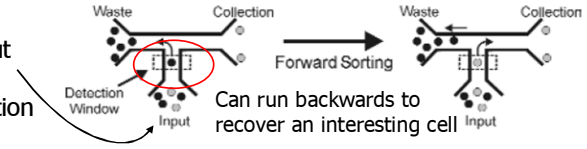




The device has channels that are 100 µm wide at the wells, narrowing to 3 µm at the sorting junction

The channel depth is 4 µm, and the wells are 2 mm in diameter

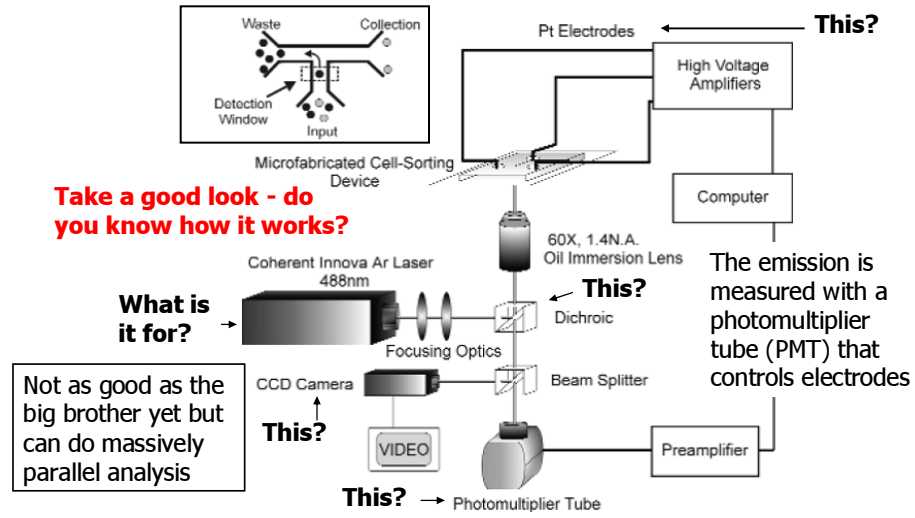
A buffer solution is introduced at the input channel and fills the device by capillary action



Can run backwards to recover an interesting cell

Schematic diagram

The chip is mounted on an optical microscope, and fluorescence is excited near the T-shaped junction with a focused laser beam



The emission is measured with a photomultiplier tube (PMT) that controls electrodes

Not as good as the big brother yet but can do massively parallel analysis

µFACS performance

Single pass sorting on µFACS

Table 1. Results of sorting red from blue fluorescent beads (forward mode and reverse mode) and of sorting GFP-expressing HB101 *E. coli* from wild-type HB101 *E. coli* (forward mode)[a]

	Input well		Collection well		Waste well	
Bead color	Blue	Red	Blue	Red	Blue	Red
Forward-mode bead sorting	0.925	0.074	0.160	0.840	0.998	0.002
Reverse-mode bead sorting	0.988	0.012	0.043	0.957	0.999	0.001

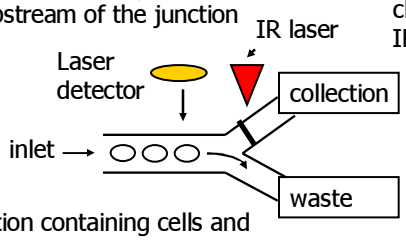
Does not look as good as the big brother perhaps, but it was the first device constructed back in 1999

- The sorting can be repeated many times over by using the recovered cells as a starting material
- In principle, µFACS sorters can do massively parallel analysis with very little extra cost

Fu et al (1999) Nature Biotech 17, 1109

Another way to skin the cat

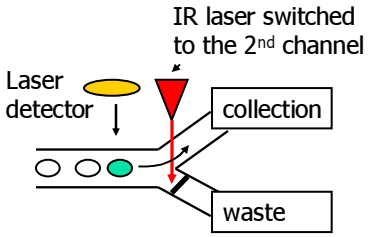
The window for detecting the fluorescence signal is located upstream of the junction



A solution containing cells and thermoreversible gelation polymer (TGP) is introduced into the Y-shaped microchannel via inlet

In the absence of fluorescence signal, the collection channel is plugged and flow directed to the waste channel

The polymer gelation (sol gel) is induced locally one of the micro-channel (collection) by site-directed IR laser irradiation

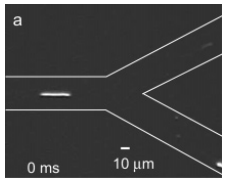


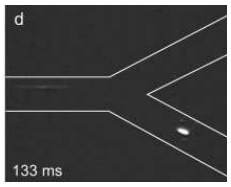
Upon detection of a fluorescence signal, the entrance to the waste channel is plugged by switching the position of the laser; this directs the flow to the collection channel

Shirasaki et al (2006) Anal Chem 78, 695

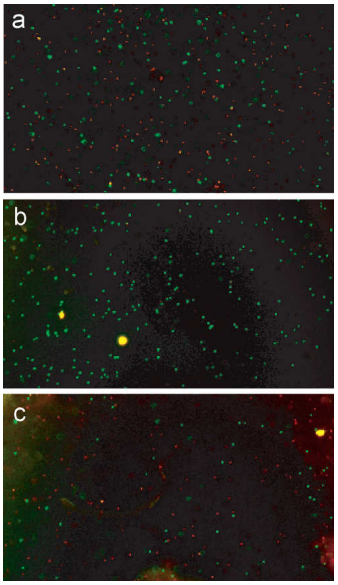
On-chip TGP cell sorter

A fluorescently labeled cell redirected to collection channel ↓





Cells expressing GFP (green) and DsRed (orange) before and after cell sorting →



Inlet reservoir ←

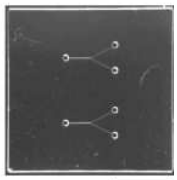
Collection reservoir ←

Waste reservoir ←

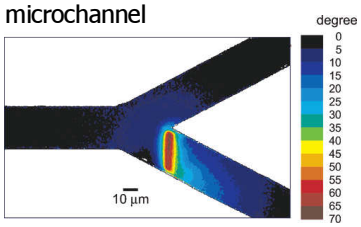
Cool things work on a microscale

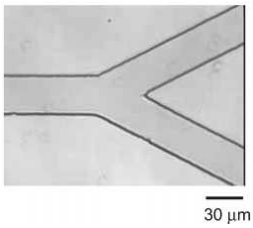
The gel functions as a microvalve to switch the flow between the microchannel

Thermoreversible gelation of the sol gel polymer occurs with a response time of of 3 ms



Distribution of temp elevation in a microchannel

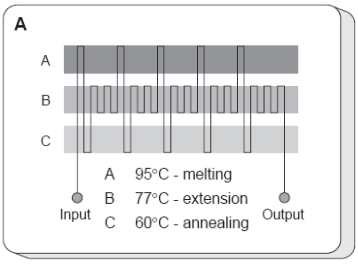




This is only possible because the device is so small

OK, let's see what else one can do on a microscale

A PCR chip



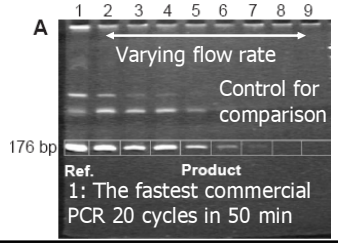
- Continuous flow at high speed
- Very small sample can be run

A 20-cycle PCR amplification of a 176–base pair fragment resulting in total reaction times of 90 seconds to 18.7 mines with heating and cooling times are each less than 100 ms

Kopp, et al (1998) Science 280, 1046

The individual steps are simply performed by heating or cooling the sample to characteristic temp:

- A: 95°C for dsDNA melting
- B: 50° to 65°C for primer annealing (binding of the specific primers to their target sites)
- C: 72° to 77°C for primer extension at the optimum enzyme temperature



Taking blood apart

Fractionation of whole blood components and isolation of blood plasma with no dilution

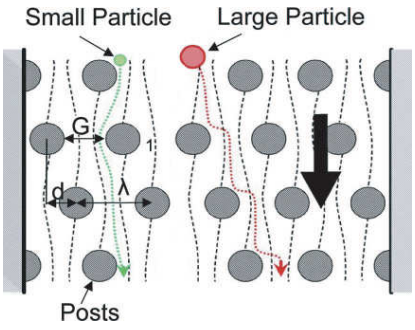
- Human blood is a highly complex fluid containing objects of many different sizes and shapes (cells, proteins, etc)
- Magnetic cell sorting (MACS) and flow cytometry (FACS) require labeling

Basic principle: particles below a critical hydrodynamic diameter (Dc) move downward with flow direction, but bigger ones move at an angle

Paths of particles smaller and larger than the critical threshold are depicted with green and red dotted lines, respectively Small particles stay within a flow stream and large particles are displaced at each obstacle

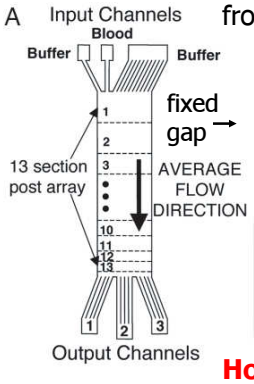
The bump array:

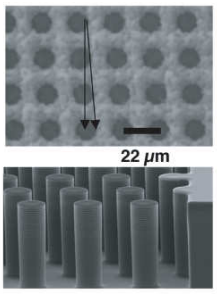
The fluid flows through an array of microposts, in which each row of posts is slightly offset laterally with respect to the previous row above it



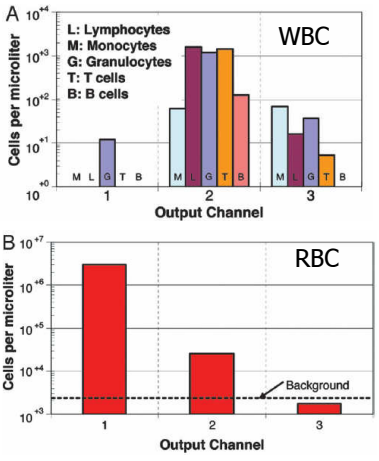
Separation of blood components

Device designed to separate WBCs from RBCs and platelets





How are they made?



The 13 regions differ in positioning of the posts

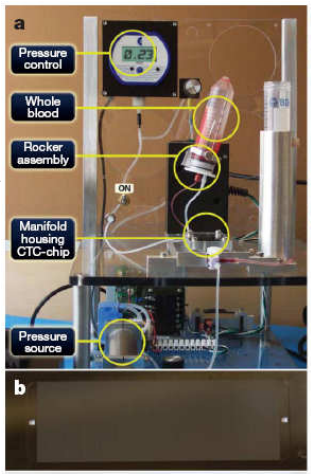
Flowing blood flows through three successive arrays (different geometries) to remove cells of decreasing size – enables to obtain plasma from a very **small sample and practically with no dilution**

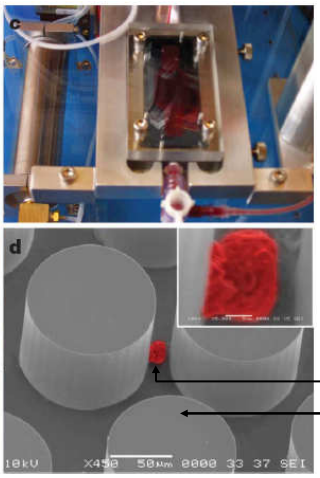
Davis et al, PNAS 2006

Cell recovery from blood

CTC-chip for the isolation of rare circulating tumor cells

The overall work-station setup →





Whole blood flowing through the chip ←

Scanning electron microscope image of a captured NCI-H1650 lung cancer

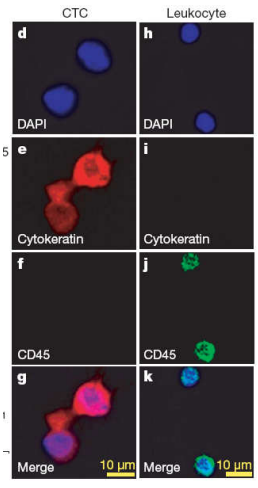
You know what this is and how it's made ☺

*anti-epithelial-cell adhesion-molecule

EpCAM*-mAbs are immobilized on the posts to fish out epithelial cells from blood and CD45 Abs for blood cells

Detection of cancer

Isolation of rare circulating tumour cells in cancer patients by microchip technology



CTCs were identified in 115 of 116 (99%) patient samples; the single negative was a specimen with a small volume (0.9 ml)

Cohort	Total no. of samples	Range of CTCs per mL				Samples with >5 CTC per ml (%)
		5–20	20–50	50–100	>100	
		No. of samples (%)				
Healthy subjects	20	0	0 (0)	0 (0)	0 (0)	0
Lung cancer	55	11 (20)	11 (20)	11 (20)	22 (40)	100
Prostate cancer	19	3 (16)	8 (42)	1 (5)	7 (37)	100
Localized prostate cancer	7	0 (0)	3 (43)	0 (0)	4 (57)	100
Pancreatic cancer	15	1 (7)	3 (20)	2 (13)	9 (60)	100
Breast cancer	10	2 (20)	0 (0)	4 (40)	4 (40)	100
Colon cancer	10	0 (0)	2 (20)	4 (40)	3 (30)	90

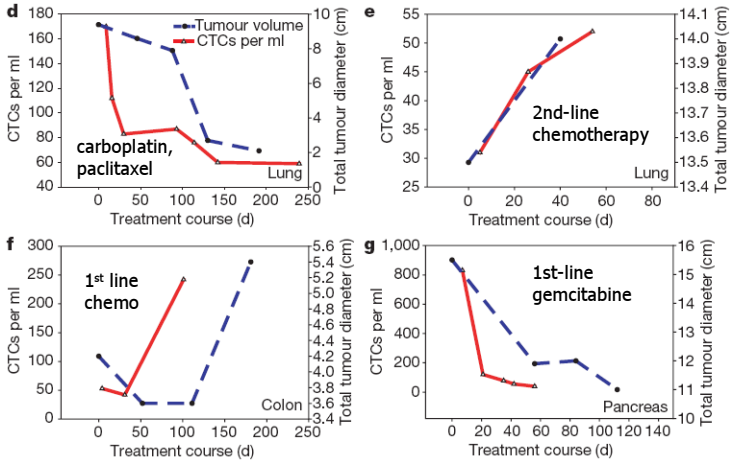
Note the range of cancers detected

← Anti-cytokeratin Abs for epithelial cells and CD45 Abs for blood cells are used for staining

Nagrath et al Nature, December 2007

Disease management

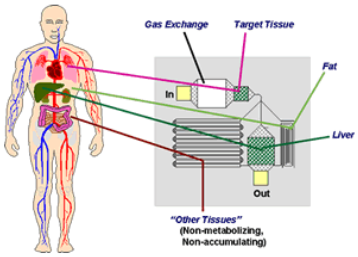
Correlation between CTC quantity (red), and tumor size (blue) measured during the course of treatment



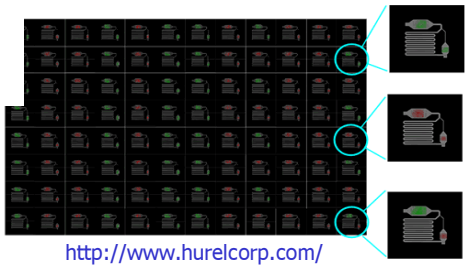
CTC is a leading indicator – should work great for detection of metastases

Anything for pharma?

Human organs on a chip ☺



HμREL® chip is a microfluidic circuit originally developed at Cornell Unv, NY to determine interactions among multiple tissues and pharmacologic compounds

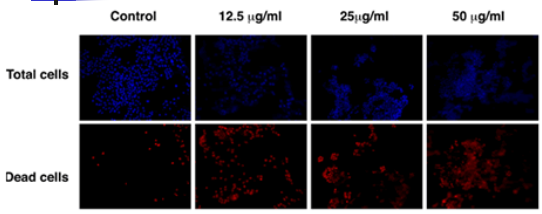


http://www.hurelcorp.com/

The chip comprises separate, but microfluidically connected "organ" or "tissue" compartments, each comprising living cells that are representative of this organ or tissue function

Function: to provide safety and efficacy test for (pro)drugs under development

Validation studies: tegafur



Tegafur is anti-cancer pro-drug

It is inactive by itself and requires metabolic activation by cytP450 (liver) to generate the pharmacologicaly active metabolite 5'-fluorouracil, 5-FU

- Both Tegafur and 5-FU were tested with hepatocytes as "liver" and colonocytes as "colon" compartments
- The drug was effective against colon cancer in the chip, but not in a traditional assay because Tegafur is converted to 5-FU in liver
- Cytotoxicity of both Tegafur and 5-FU against other tissues can be assessed in the same experiment

Applications: screening and assay for hepato- and multi-organ toxicity, efficacy/toxicity of compounds and their metabolites, absorption and bioavailability, metabolic inactivation, etc

Forbes, 2005

Meet the stripped down rat

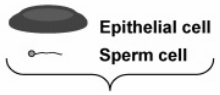
Are wafers of silicon that support cells cultured from vital organs the future of drug testing and toxicology? Roxanne Khamsi talks to the pioneers creating model animals — and humans — on a chip.

Nature, 2005

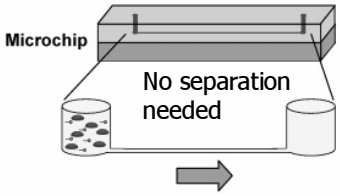
Forensic: sexual assault evidence

Forensic DNA analysis in sexual assault cases requires separation of DNA from epithelial (victim) and sperm (perpetrator) cells

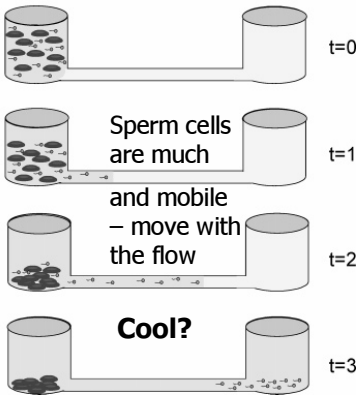
Conventionally, differential extraction is used: laborious and time-consuming process



Better on a chip?



A mixture of sperm and epithelial cells is added to the inlet reservoir of the chip



The epithelial cells are shown settling to the bottom of the inlet reservoir; flow is induced to mobilize the sperm cells, while the epithelial cells remain in the inlet reservoir

Horsman et al (2005) Anal Chem 77, 742-749

Not the whole story though…

DNA from sperm must be recovered and amplified for analysis

and there are chips for doing this too

Ultrasensitive PCR and Real-Time Detection from Human Genomic Samples Using a Bidirectional Flow Microreactor

Lin Chen, Jonathan West, Pierre-Alain Auroux, Andreas Manz, and Philip J. R. Day

Anal. Chem., **2007**, 79 (23), 9185-9190 • DOI: 10.1021/ac701668k

A fully integrated microfluidic genetic analysis system with sample-in–answer-out capability

Christopher J. Easley*, James M. Karlinsey*, Joan M. Bienvenue*, Lindsay A. Legendre*, Michael G. Roper*, Sanford H. Feldman†, Molly A. Hughes‡, Erik L. Hewlett‡, Tod J. Merkel§, Jerome P. Ferrance*, and James P. Landers*¶||

Integrated microfluidic device capable of accepting whole blood as a crude biological sample with the endpoint generation of a genetic profile

Upon loading the sample, the glass microfluidic genetic analysis system device carries out on-chip DNA purification and PCR-based amplification, followed by separation and detection

www.pnas.orgcgidoi10.1073pnas.0604663103

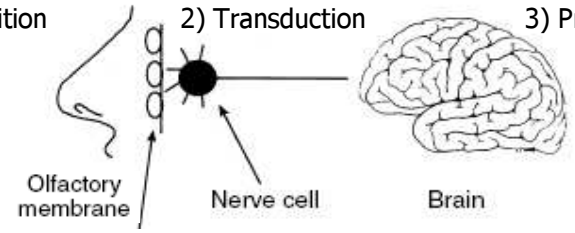
And finally - the human nose

1) Recognition

2) Transduction

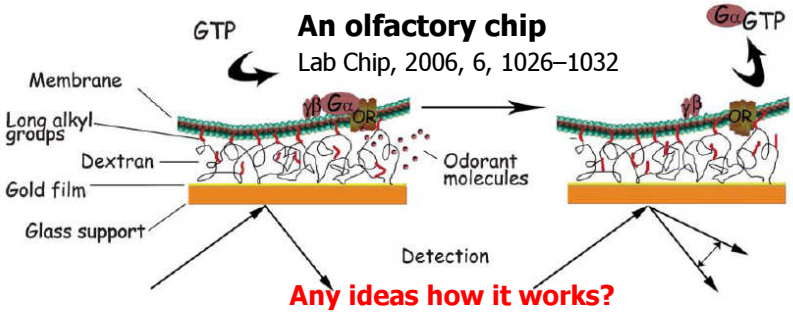
3) Processing

Remember where we started? ☺



An olfactory chip

Lab Chip, 2006, 6, 1026–1032



Any ideas how it works?

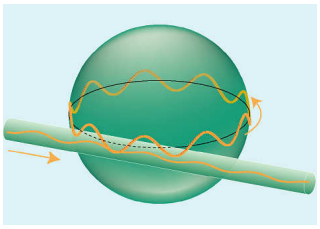
See Donna's presentations

Olfactory perception and e-nose

What's up

- More miniaturization

WGM sensor: Can it be miniaturized?



You bet!

In fact, the folks are working on it

- Walking biosensors?

Robosapien™

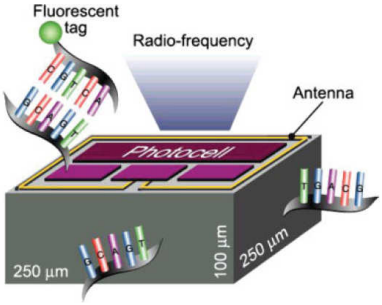
A sophisticated fusion of technology and personality. Loaded with attitude and intelligence, Robosapien is the first robot based on the science of applied biomorphic robotics. With a full range of dynamic motion, interactive sensors and personality

http://www.wowwee.com/

And RFIDs of course

The Miniature RFID Electronic Chips

- Upon activation by light, each MTP* provides a unique RF identification (ID) signal that matches a chip to the specific biological material attached to it
- The MTP is powered by a photocell and has an antenna that transmits the signal



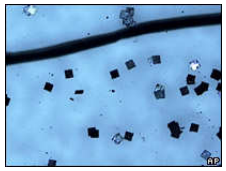
A single-stranded DNA covalently linked to the MTP surface (probe)

When it binds to complementary DNA in a sample (target) which has been fluorescently tagged – the chip transmit a unique ID

*Microtransponders i.e. miniature RFID

Remember how big the smallest tags are?

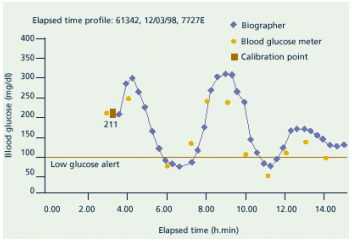
50×50 µm

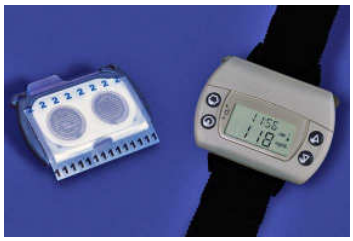


Just a model for now

Non-invasive monitoring

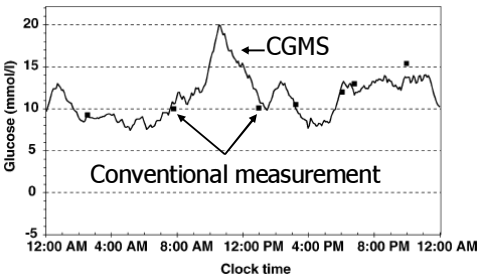
Glucowatch Biographer™





The MiniMed-Medtronic Continuous Glucose Monitoring (CGMS)

A subcutaneously implanted, needle-based amperometric GOX electrode system, coupled to a portable logging device



Implantable sensors – great challenge

Biosensors and Bioelectronics 20 (2005) 1897–1902

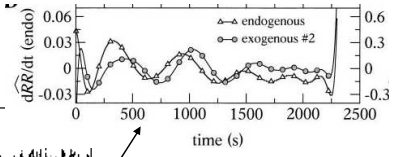
Implants of the future?

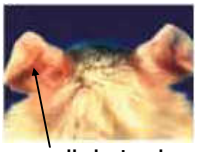
An implantable medical device is required to provide accurate real-time determination of relevant functional physiological needs e.g. a cardiac pacemaker must determine the pacing rate required to supply the body with adequate cardiac output

Can a pacemaker be run by a biosensor?

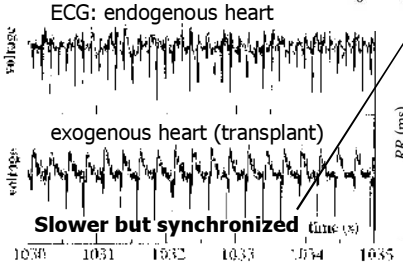
Direct biologically based biosensing of dynamic physiological function

People are working on it

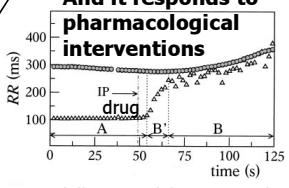




Stem cell-derived cardiac myocytes implanted in the outer part of mouse ear



And it responds to pharmacological interventions

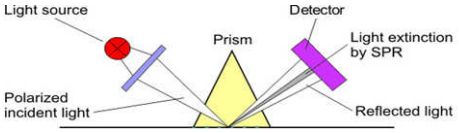


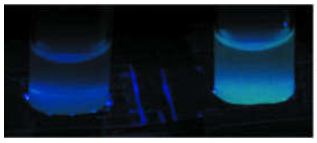
Edelberg et al (2001, 2002)

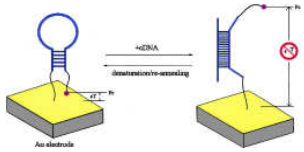
See Eran's presentation

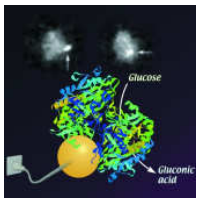
The bionic arm; how it works

It was quite a journey









Useful?

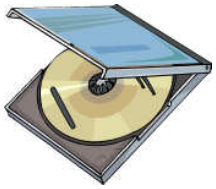
Hope there was something in it for most of you, guys

- Biotechnology
- Biotechnology and Entrepreneurship
- Biomedical Engineering

And you learned a few practical things too ☺

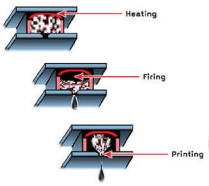
How chips are made

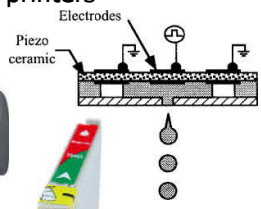
and how CD/CD-ROMs and scanners work



And more ☺

The difference between Cannon and Epson printers



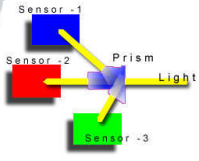


and how HP make money

and how to choose a camera or a camcorder

A big CCD and
quality optics vs
10 megapixels
no-name ***

and above all

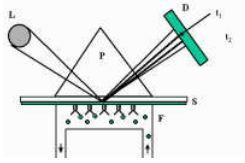


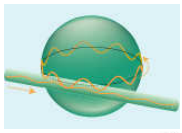
3CCD

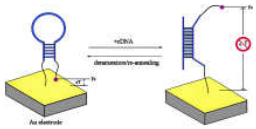
Everyone rises to the level of their incompetence

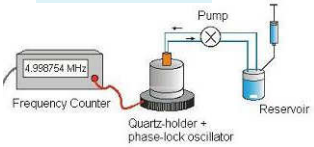
Platforms and applications

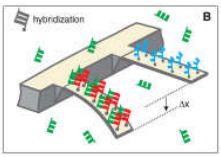
Platforms:





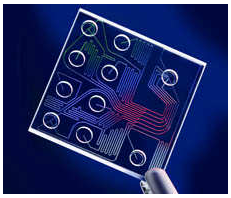




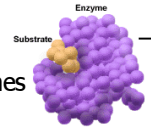


Applications areas:

- Heath care and medical diagnostics
- Defense and forensic
- Food and Agriculture
- Environment
- Research and drug development



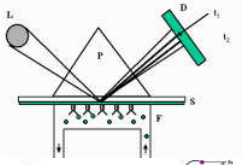
Platforms and bioreceptors



Enzymes

Bioreceptors: ??? Platforms:

Antibodies



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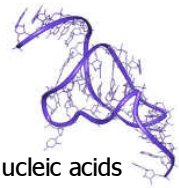
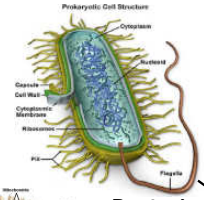
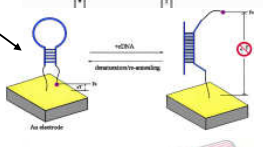


Nucleic acids



Bacteria



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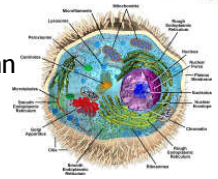
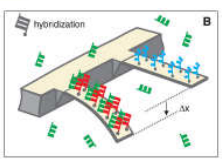


Mammalian cells

???



Designing your sensor

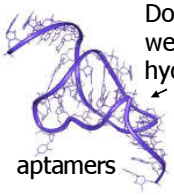
How does one go about doing it?
There must be a need!

A particular analyte?

What kind?

Macromolecule or small molecule?

Hydrophobic or hydrophilic?



Don't work well with hydrophobic

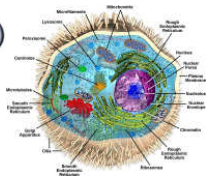
Is there an enzyme?

Amplification, cheap, well established

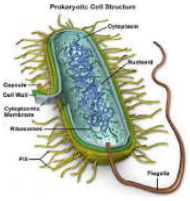
- Sensitivity?
- Cost?
- Customer?
- IP position?

A hazard?

What kind of hazard?



Biodefence or biomedical

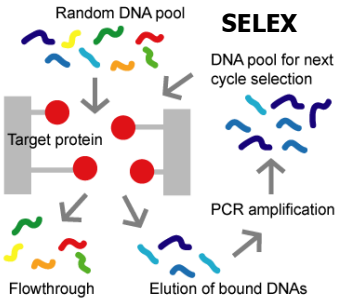


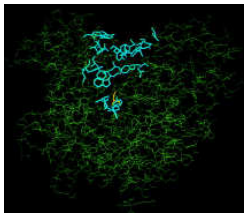
Water supply or environment

And remember you can always improve your bioreceptor too

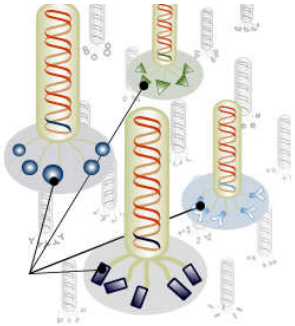
Bioreceptors can be improved

But only by an appropriate method



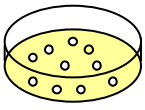


Rational protein engineering

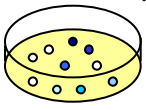


Phage display

Generated library

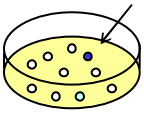


"Normal" assay



Directed evolution of enzymes

Assay at T above the normal T inactivation



OK, let see how you, guys, did in your HW