VTT TECHNICAL RESEARCH CENTRE OF FINLAND

Microfluidistics for drug development and diagnostics

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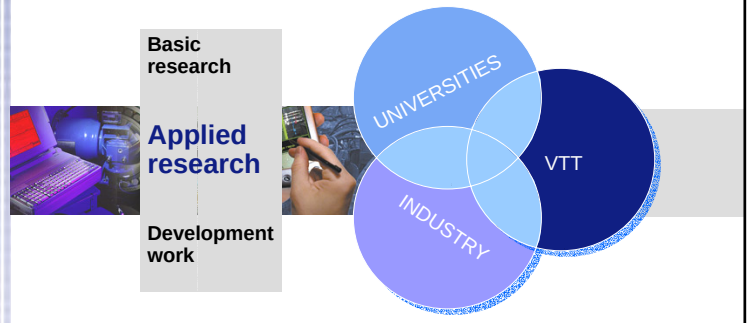
Medical Biotechnology

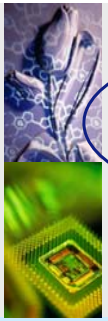
Teknologiasta liiketoimintaa

VTT's status as performer of R&D work



VTT in brief 2008

Customer sectors
- Biotechnology, pharmaceutical and food industries
- Electronics
- Energy
- ICT
- Real estate and construction
- Machines and vehicles
- Services and logistics
- Forest industry
- Process industry and environment



Focus areas of research:
- Applied Materials
- Bio- and Chemical Processes
- Information and Communication Technologies
- Microtechnologies and Electronics
- Industrial Systems Management
- Energy
- Technology in the Community
- Business Research

Personnel: 2 740 (31.12.2007)
Turnover: 241 M€ (budget for 2008)

VTT's operations
Research and Development ■ Strategic Research ■ Business Solutions ■ Ventures ■ Expert Services ■ Corporate Services

Some of the issues to be considered

- **what added value microtechnologies can provide?**
  - value for the customer
  - must be at least as good as the present methods
  - clear added value needed to generate market share
- **how are the various technologies integrated to a system level?**
  - customer interface plays a central role
  - technology must be robust enough for production, use and service
  - availability of components can be an issue
- **can microtechnologies provide additional information?**
  - does a lower limit of detection provide additional information
  - or does micro scale allow massive parallel measurements and provide additional information

Driving forces towards BioMEMS

- **Medical applications**
  - Integration of diagnostic devices with therapeutic devices
  - Drug delivery, implantable devices (passive / responsive)
  - Tissue engineering (hybrid artificial organs or parts of them)
  - High throughput, high content cell based screening
  - Genome wide biology (-omics) calls for new technologies

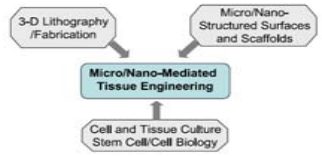
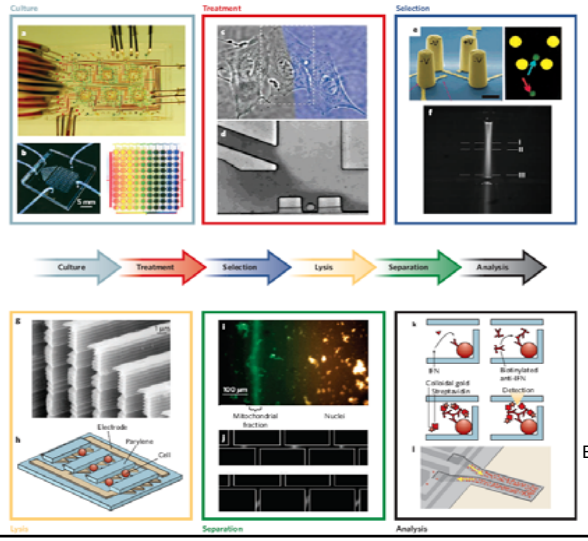


Fig. 17. Opportunities in micro/nano-mediated tissue engineering.

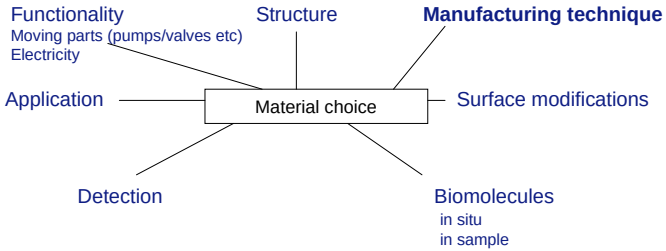
Bashir (2004)



El-Ali et al 2006

Material choices

- Factors effecting the choice of material



Ion channel R&D

Industrial demand

Increasing share of development within the drug discovery industry targeting ion channels [1]. (hypertension, diabetes, anaesthetics, mental disorders…)

Research demand

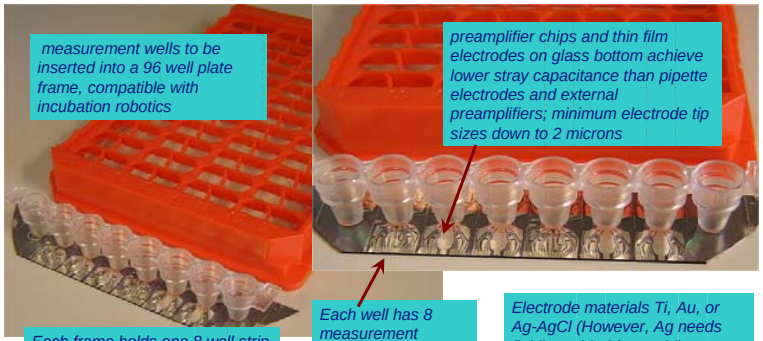
- *Most commercial parallel patch clamp systems intended for rapid screening, so they are less useful for:*
  - *Inter-cell signalling or long term studies, e.g. LTP, (long term potentiation) in epilepsy, short term memory research, etc.*
  - *Neural stem cell differentiation that requires stimulation and measurements for verification.*
  - *Currently lots of research in chemical signalling, but much less in verification of the proposed electrical models of even the simplest neural cell nets.*

1. C. Woods et al., Drug Discovery Today, vol. 9, No. 10, May 2004

R&D for ion channel measurements

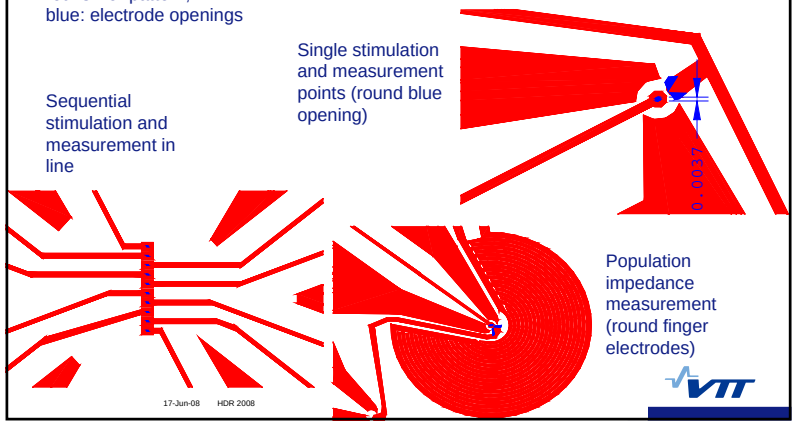
- *design for measurement of functional confluent or near confluent populations, (i.e. not for rapid ion channel screening)*
- *facilitates studies of new phenomena beyond present parallel patch clamp systems´ capability:*
  - *long term effects of target substances on ion channels*
  - *inter-cell signalling, e.g. synaptic plasticity, signal transfer over synapses, and other analyses within functional populations*
  - *simultaneous stimulation and measurement*
  - *population impedance variations when subjected to target molecules*

CellElectro System

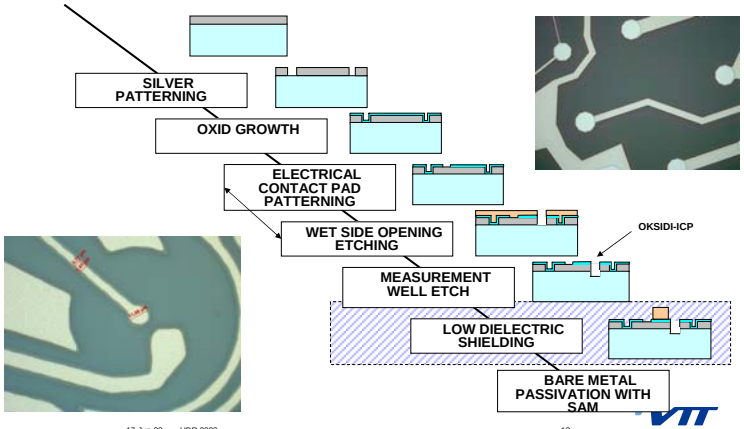


Detailed functional patterns (photomask CAD)

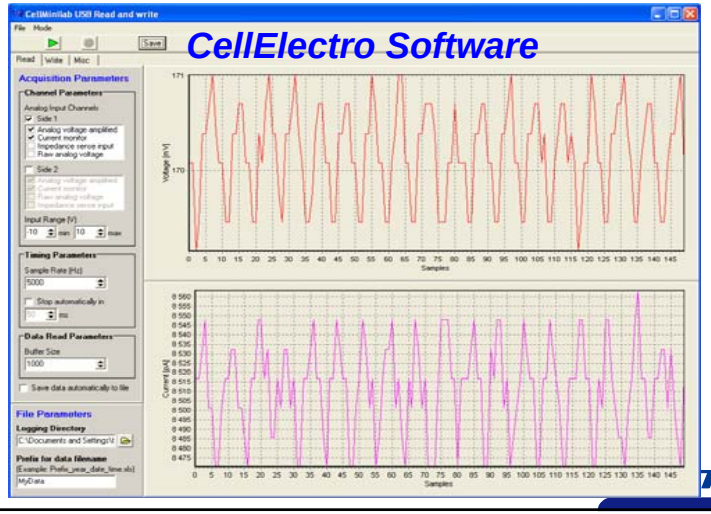
dimensions in mm,
red: silver pattern,
blue: electrode openings



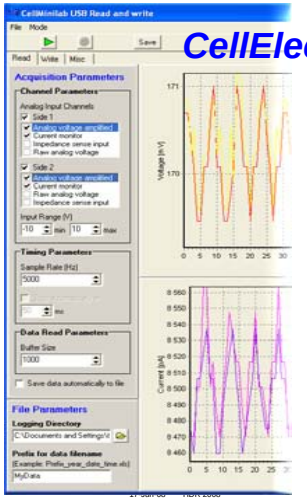
Chip process in wet etch



CellElectro Software



CellElectro Software

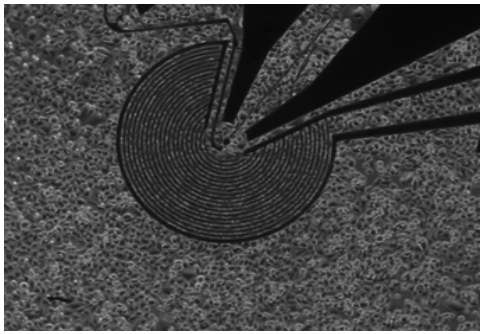


- voltage and current measurements displayed simultaneously; selectable from 16 channels on side1 and 16 on side2
- Three different measurement modes: patch clamp, impedance measurement and diagnostics

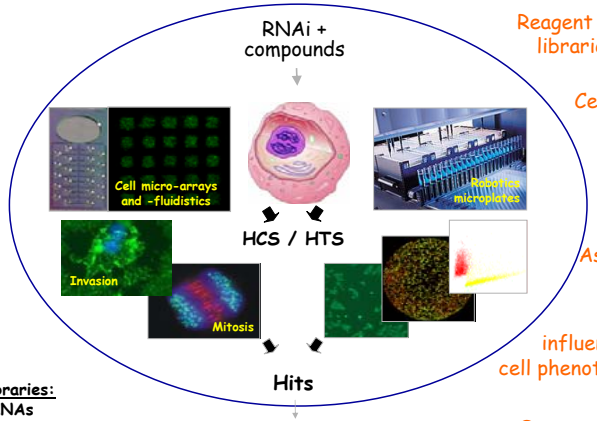
Measurement results

- ongoing work, so far successful growth on fused silica - Ag surfaces

Cytomyx Ltd. hERG-CHO K1 Recombinant cell line grown on the chip (octanethiol SAM passivation on Ag surfaces)



High throughput screening with living cells



Reagent libraries
Cell types
Plat-forms
Assays
Hits influencing cell phenotypes
Outcomes

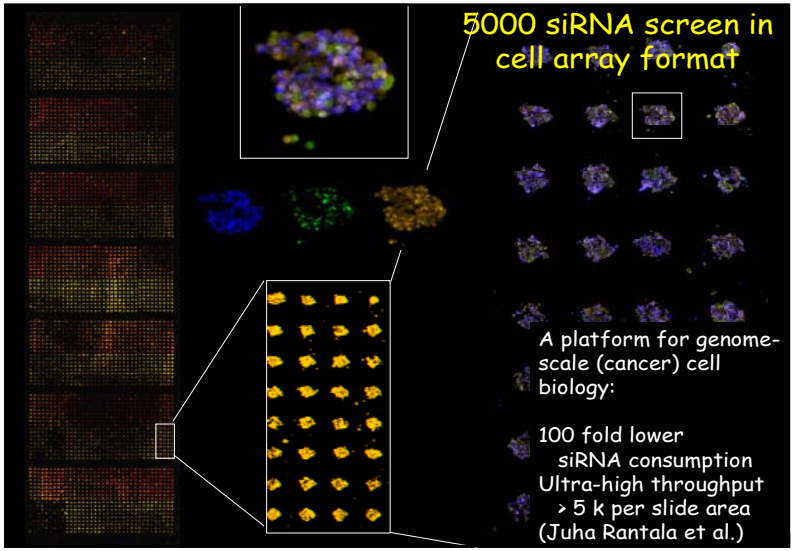
Reagent libraries:
44,000 siRNAs
Matching cDNAs
miRNAs
70,000 compounds
In-house libraries

Targets
Lead molecules
Pharmacogenomics

New technologies for high-throughput RNAi screening

1-2 M€ infrastructure
Liters of reagents (RNAi, medium, assay)
10-50 k€ per screen
Up to 1€/well expenses
2-3 screens per week

Minimal infrastructure
100-fold decrease of reag.
0.5-5 k€ per screen
<5 c per spot
20-30 screens per week

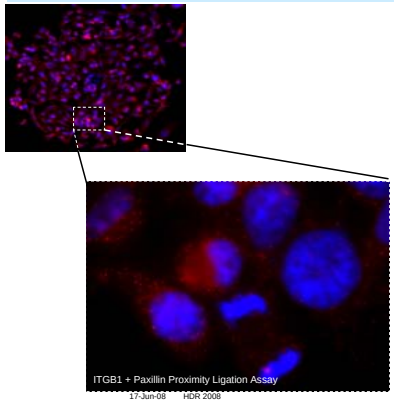


A platform for genome-scale (cancer) cell biology:

100 fold lower siRNA consumption
Ultra-high throughput
> 5 k per slide area
(Juha Rantala et al.)

Current assay development status

56 cell lines tested and used with cell arrays.
25 antibody based assays optimized for screening.



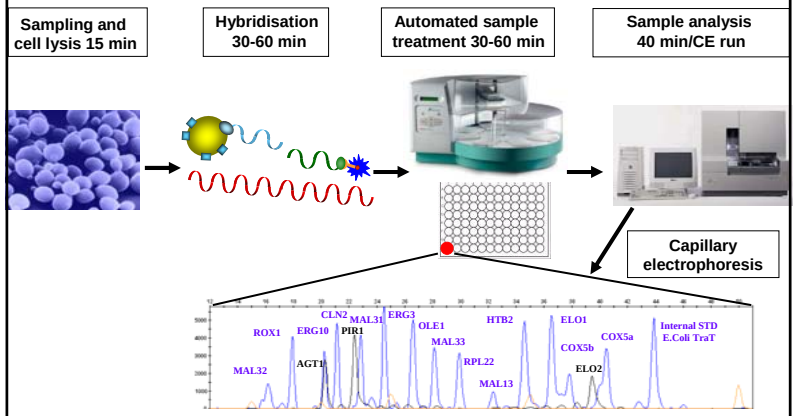
Antibody	Array use
actin	+++
Ki-67	+++
H2Ax	+++
PCNA	+++
Casp-3	+++
MDR-1	+++
Vinculin	+++
cPARP	+++
HUTS21/ITGB1	+++
1936/ITGA2	+++
12G10/ITGB1	+++
9EG7/ITGB1	+++
DDX21	+++
HEY1	+++
AR	+++
Paxillin	+++
PSA	+++
NCOA3	+++
H3K9me2	+++
H3K18ac	+++
H3K4me2	+++
H4K5ac	+++
H4K16ac	+++
Histone H3	+++
pH3	+++
KPNB1/Importin B1	+++

Cellline	Array use	Tissue
HeLa	+++	-
BT-474	++	Breast
HMEC	---	Breast
MCF-7	+	Breast
MCF-10A	++	Breast
MDA-MB-2	+++	Breast
MDA-MB-2	+++	Breast
MDA-MB-4	+++	Breast
MDA-MB-4	-	Breast
SK-BR-3	+	Breast
T47D	+	Breast
ZR-75-1	+++	Breast
MA11	+++	Breast
PM1	+++	Breast
HCT-116	+++	Colon
SW480	+++	Colon
HT29	+++	Colon
Caco-2	+++	Colon
DLD-1	+++	Colon
LS174T	+++	Colon
HEK293	+++	Kidney
A549	+++	Lung
A549EpoB	+++	Lung
A549EpoB	++	Lung
MDA-MB-4	+++	Melanoma
UACC-257	+++	Melanoma
NIH3T3	++	Mouse fib
1A9	++	Ovarian
1A9/PTX10	++	Ovarian
1A9/PTX22	++	Ovarian
Ascites cel	+	Ovarian
KF28	+++	Ovarian
KF28Tx	+++	Ovarian
KFr13	+++	Ovarian
KFr13Tx	+++	Ovarian
OVCA solid	-	Ovarian
OVCAR-3	+	Ovarian
OVCAR-4	+	Ovarian
OVCAR-5	+	Ovarian
OVCAR-8	+++	Ovarian
OVCAR-8/I	++	Ovarian
22RV1	++	Prostate
DuCaP		Prostate
LnCaP	++	Prostate
PC-3	+++	Prostate
PWR-1E	---	Prostate
RWPE-1	-	Prostate
VCaP	+++	Prostate
WPE-1/NA	-	Prostate
WPE-1/NB	- - -	Prostate
WPM4-1	+++	Prostate
P97E	++	Prostate

Driving forces towards BioMEMS, 2

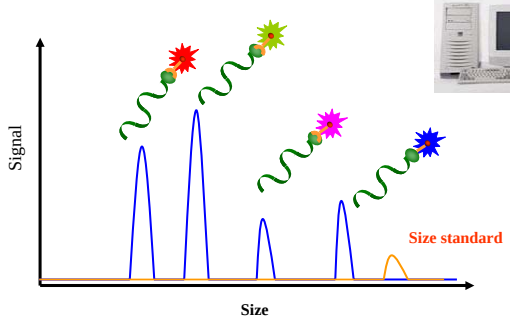
- **Diagnostics**
  - The largest and most researched BioMEMS segment at the moment
  - Demand for high quality medical care:
    - preventive care
    - minimally invasive procedures
    - personalized medicine
    - continuous monitoring
  - Improved performance
  - Low cost

TRAC allows frequent sampling and many replicates



96 samples x 30-plex = 2880 transcription levels in a single assay

Multiplex probe detection



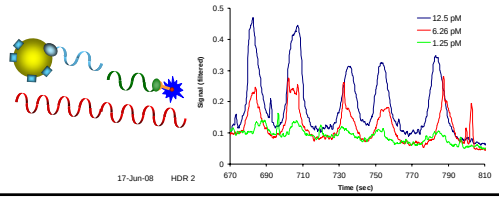
Probes are separated by size and label type

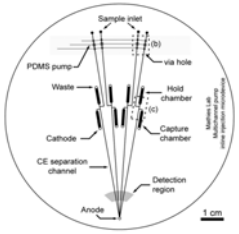
VTT QB3 project - Low Cost Diagnostics

Analysis of gene sets in microfluidistic devices

The 96-lane microfabricated sequencer for high-speed sequencing, developed at Richard Mathies lab. The design incorporates 16-cm folded channels, and fluidically balanced injectors.

- VTT has developed technologies for transcriptional profiling based on capture technology linked to capillary electrophoresis and production technology employing roll-to-roll printing on polymer surfaces

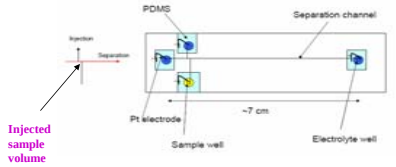


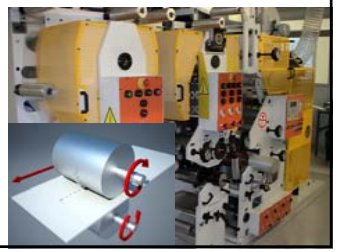


Roll-to-roll printing

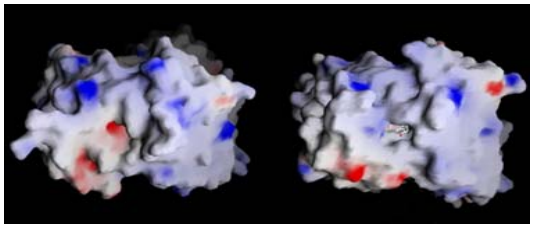
Main aims

- Bringing silicon or glass based microfluidic devices into a low-cost diagnostic platform using roll-to-roll printing
  - Roll-to-roll hot-embossing
  - Flexible thermoplastic materials
  - Integration of functional components (pumps, valves, electrodes, biologicals…) onto the platform by printing





Surface of the antibody binding site

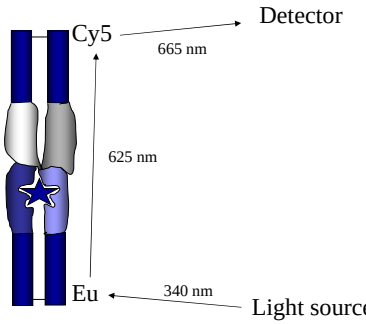


Free antibody Antibody bound to an analyte

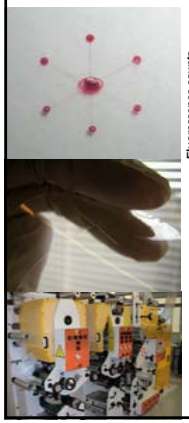
Fluorescence resonance energy transfer (FRET)-based homogeneous non-competitive immunoassay

The distance between fluorophores (10-100 Å) is critical in FRET.

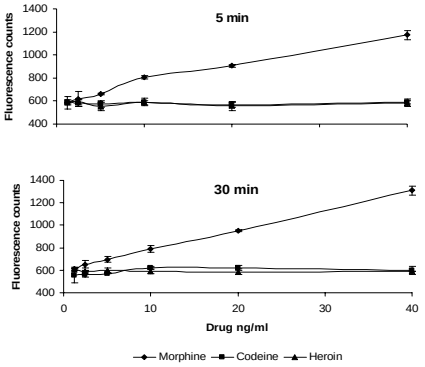
Utilization of recombinant antibody technology.



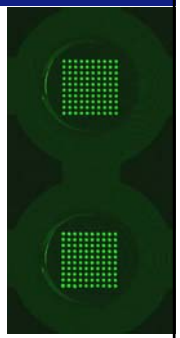
An example of the morphine test based on this technology: the test for morphine is specific, sensitive and fast



50 µl saliva samples, cutoff level 40 ng/ml



Multiplexed Assays



Sample

- Contains one or Several antigens

Label Ab mix

- Multiple Abs
- Each Ab HRP-labeled
- All specificities are mixed together

Array-In-Well

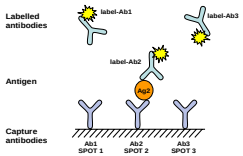
- Multiple spots in well
- One specificity/spot:
  - Myoglobin
  - CRP
  - CK-MB
  - cTnI

Readout:

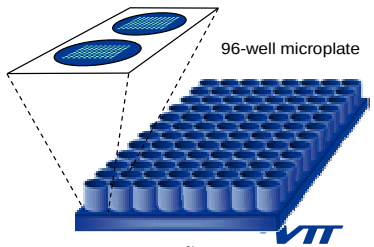
- Ag identified by spot location
- Concentration deduced by signal intensity from standard curve

Multiplexed immunoassays

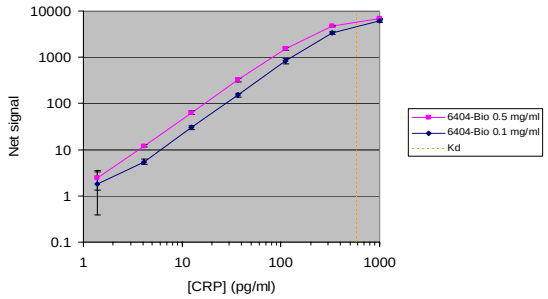
- **Arrays-in-wells**
  - Troponin I, CK-MB, myoglobin and CRP in serum
  - CRP: low sensitivity detection is enough
  - Troponin I: high sensitivity needed



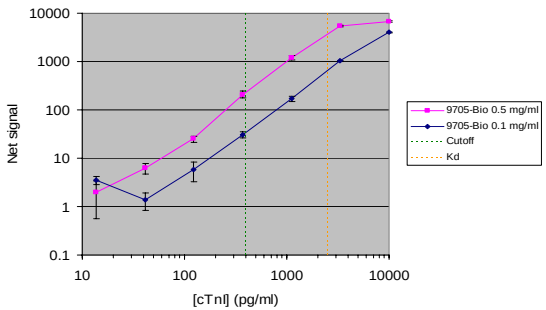
Wellplate Microarray Platform



CRP spots

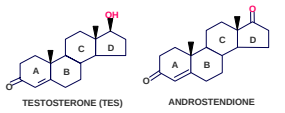


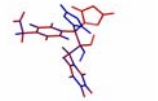
cTnI spots



Antibody chips (AIW and SPE): Challenges

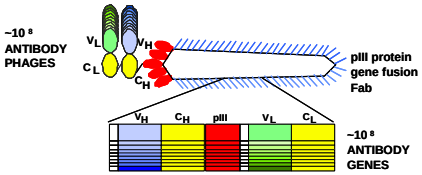
Specific antibodies: affinity, specificity





Stereoisomers of a drug

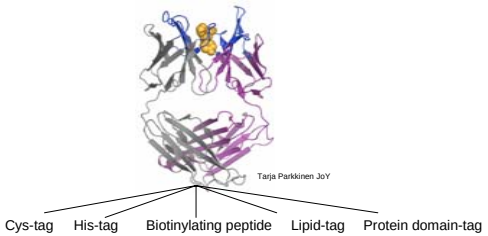
VTT Solution: recombinant antibodies
(antibody gene libraries, engineering, production, purification)



Antibody chips (AIW and SPE): Challenges 2

Antibody immobilisation

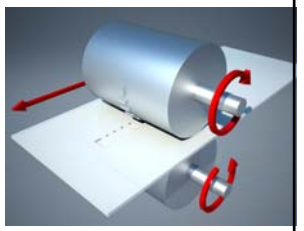
Random (passive absorption, active covalent)
Oriented (for recombinant antibodies via immobilisation tags, fusion proteins

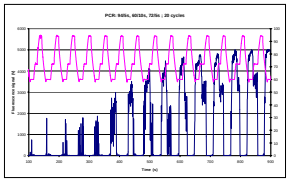


Carrier: direct immobilisation to chips or movable beads

Role of microfluidistics in drug development and diagnostics

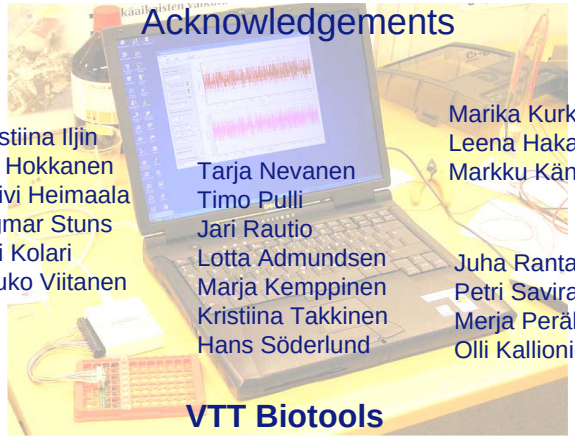
Benefits:
- more information obtained per sample
- small sample consumption
- faster assays
- new concepts possible





Issues:
- technology integration/production
- performance; sensitivity, specificity
- price/result
- clinical validation

Acknowledgements



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