Lab on Chip: Micro- e nanotecnologie in microbiologia e virologia

Tecnologie, Materiali e Microsistemi

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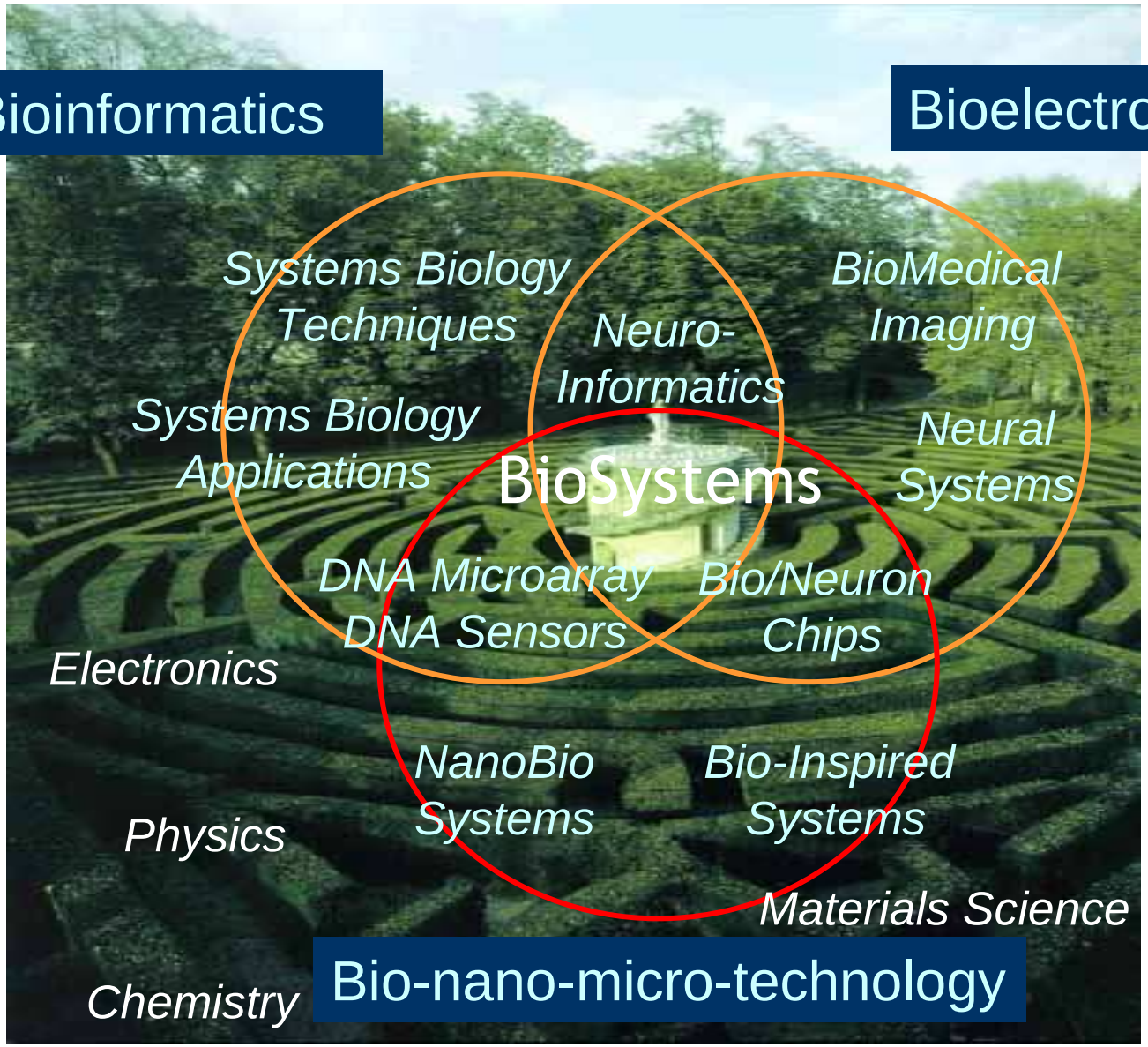

Bioinformatics
Bioelectronics
Systems Biology Techniques
Neuro-Informatics
BioMedical Imaging
Systems Biology Applications
BioSystems
Neural Systems
DNA Microarray
DNA Sensors
Bio/Neuron Chips
Electronics
NanoBio Systems
Bio-Inspired Systems
Physics
Materials Science
Chemistry
Bio-nano-micro-technology

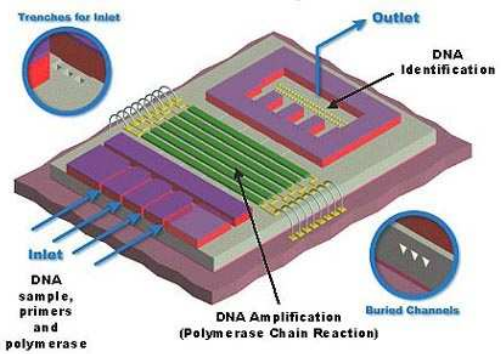
Outline

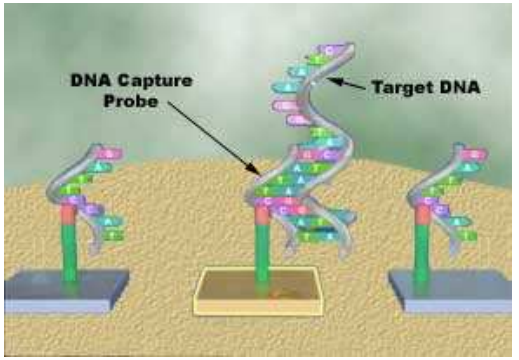
Lab-on-a-chip

1. Biosensors

DNA sensors

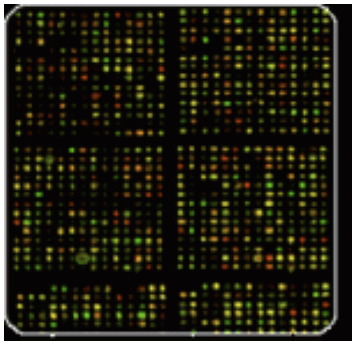
Protein Sensors





2. Microarray

High throughput analysis

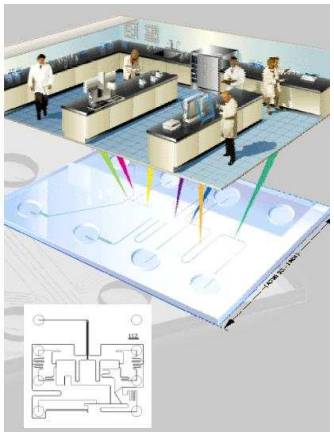


3. Microfluidics

Sample handling

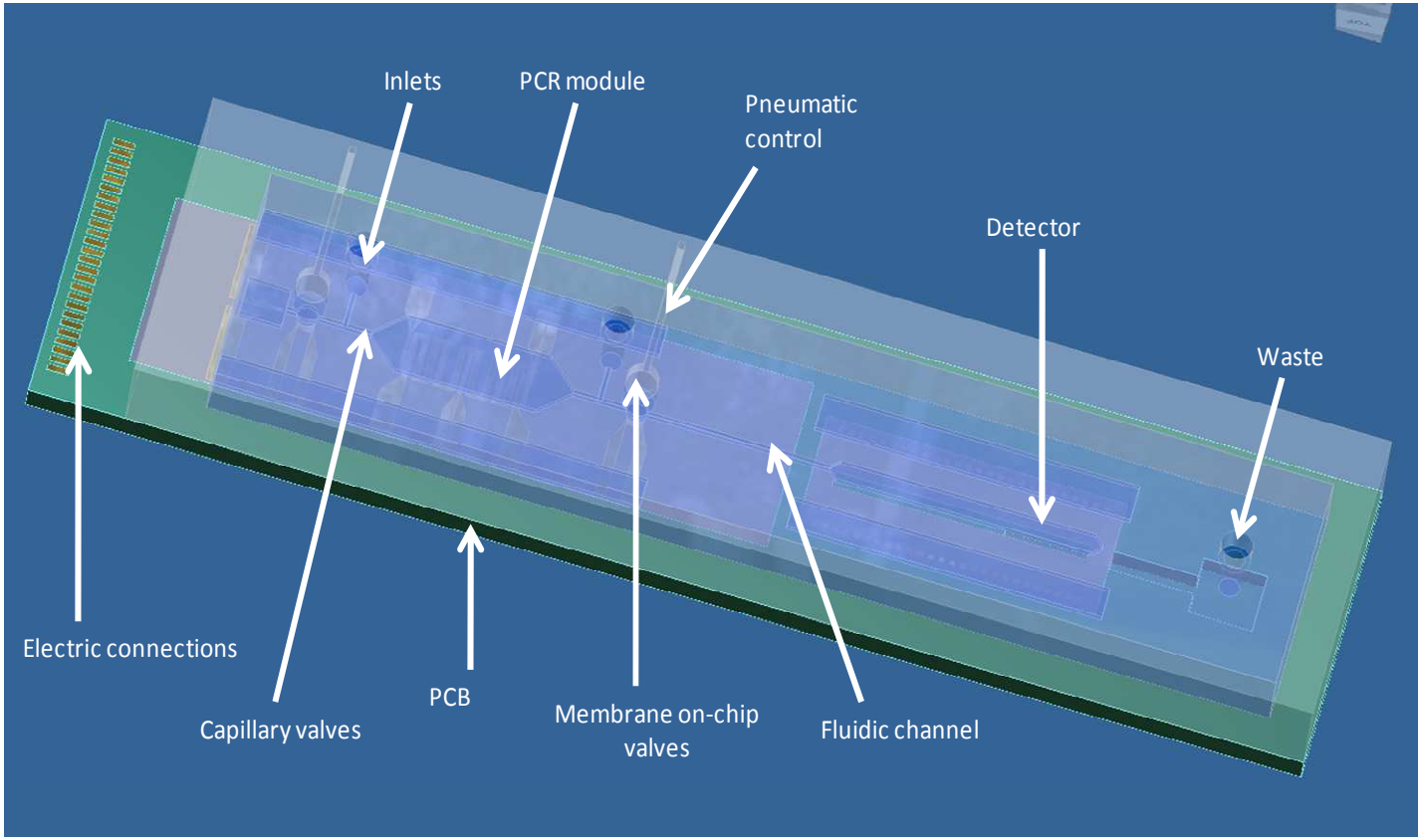
Lab-on-a-chip

- Features
  - Miniaturisation
  - Automation
  - Integration
- Benefits
  - Data Quality/Reproducibility
  - Reagent savings

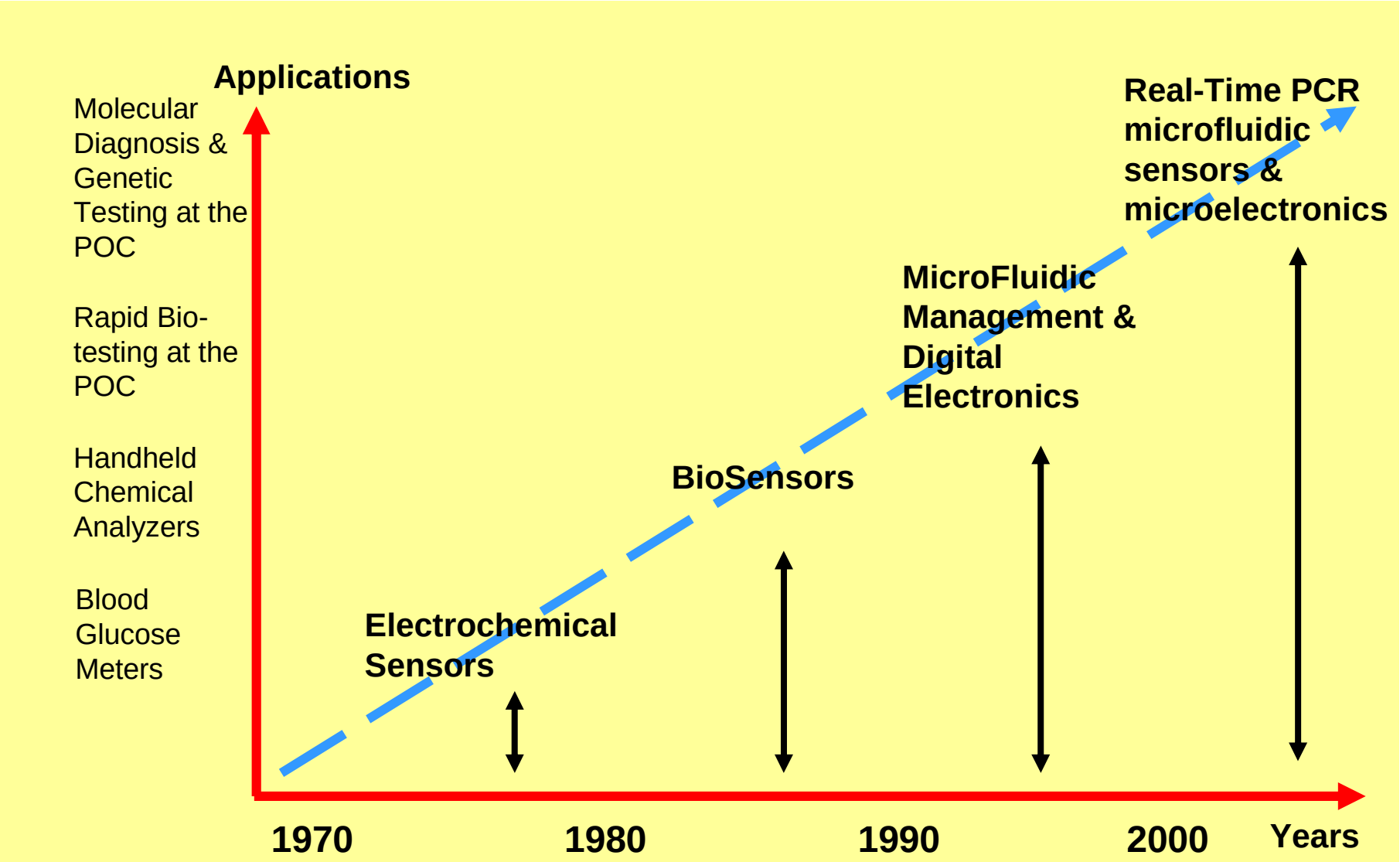


Point of Care (POC) analysis systems

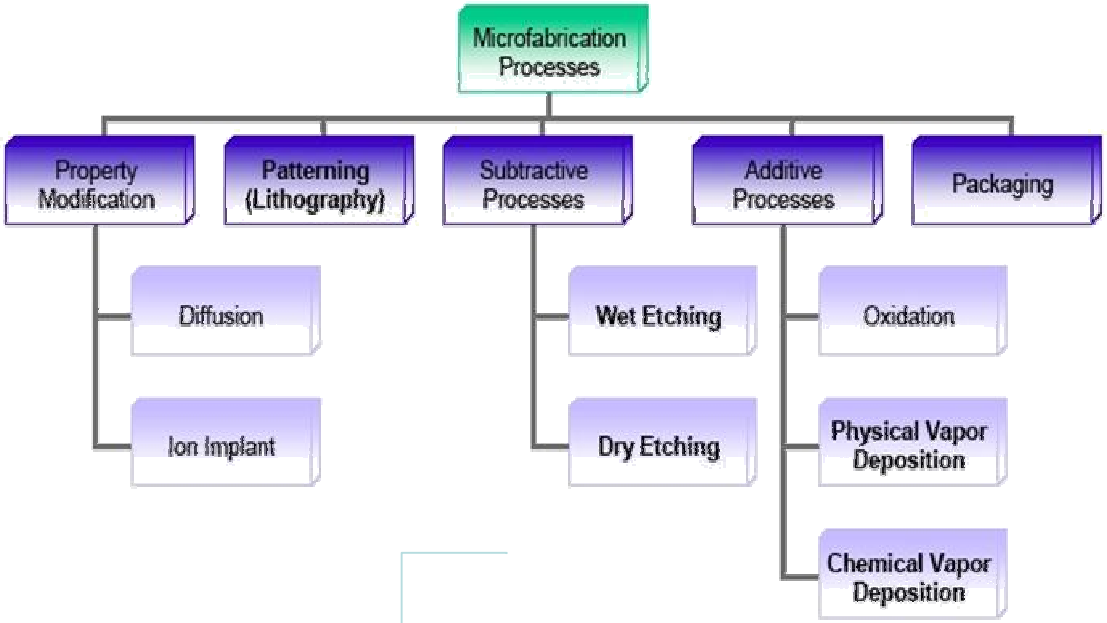
- The Lab on Chip technologies are capable of performing a wide range of proteomic and genomic tests by using a sample of blood or other body fluid such as saliva. These tests aim to facilitate healthcare at preferred environments and point of care disease diagnosis at primary healthcare level.

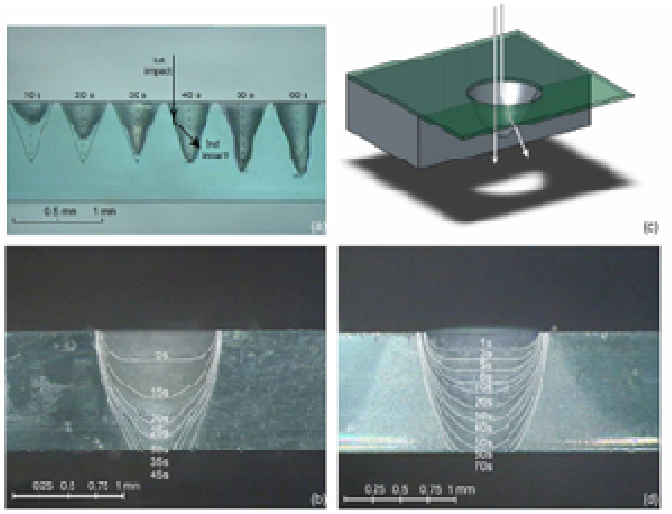


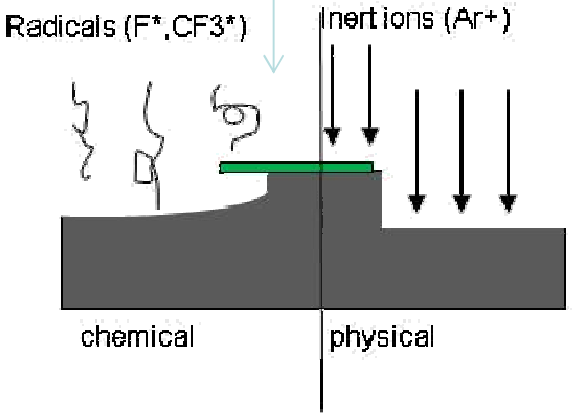
Evolution of point of care systems

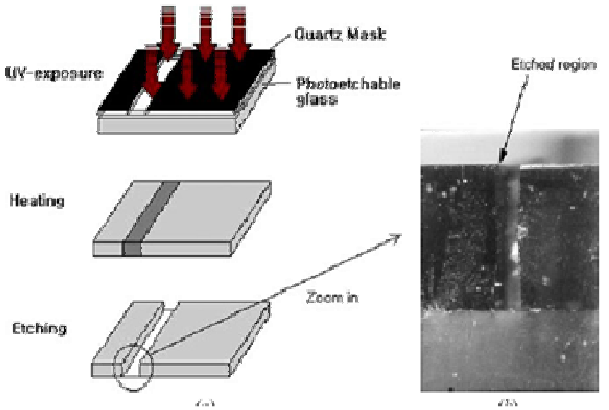


Fabrication techniques: silicon and glass









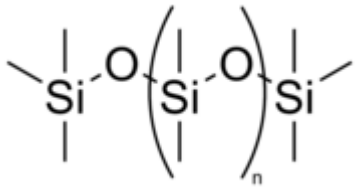
1) *Yamahata C. et al., Microelectronic Engineering , n.78-79, pp.132-137 (2005).*

2) *Park S. and Kim M., J.Micromech. Microeng. n.10, pp. 410–414 (2000)*

Fabrication techniques: polymers

Thermosetting plastics (thermosets) are polymer materials that irreversibly cure form. The cure may be done through heat (generally above 200 degrees Celsius), through a chemical reaction (two-part epoxy, for example), or irradiation such as electron beam processing.

SU-8



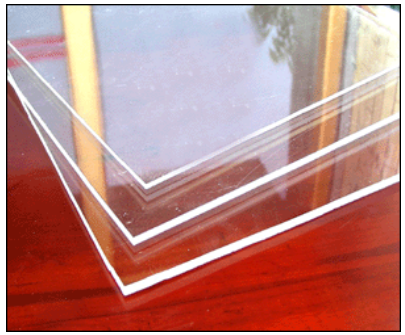
PDMS

A **thermoplastic** is a polymer that turns to a liquid when heated and freezes to a very glassy state when cooled sufficiently

COC

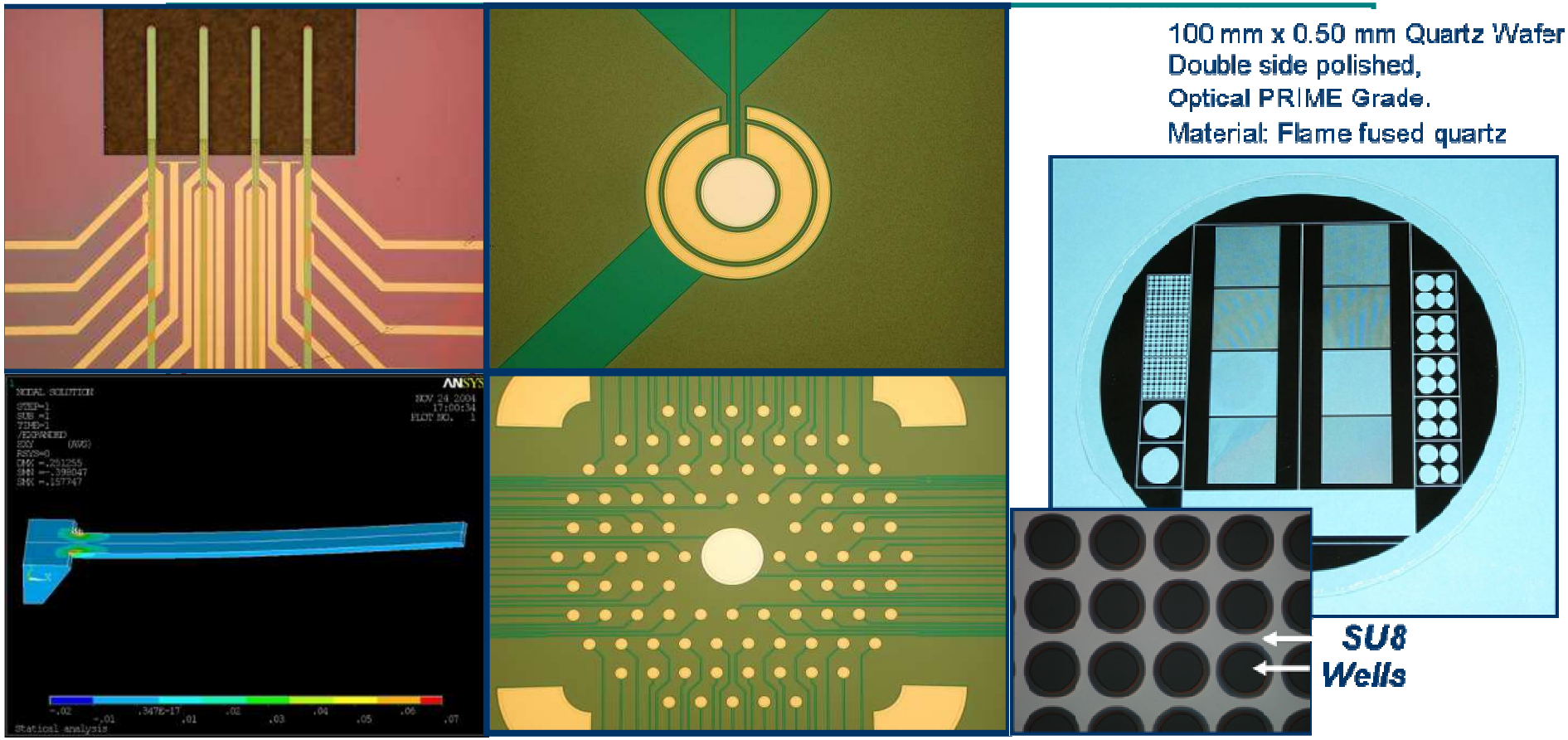
Polycarbonate

PMMA



Biosensors

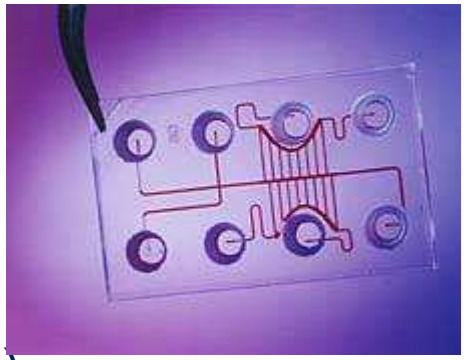
Biosensors are '*analytical devices that combine a biologically sensitive element with a physical or chemical transducer to selectively and quantitatively detect the presence of specific compounds in a given external environment*'.



Adapted from R. Bashir. Advanced Drug Delivery Reviews 56 (2004) 1565–1586

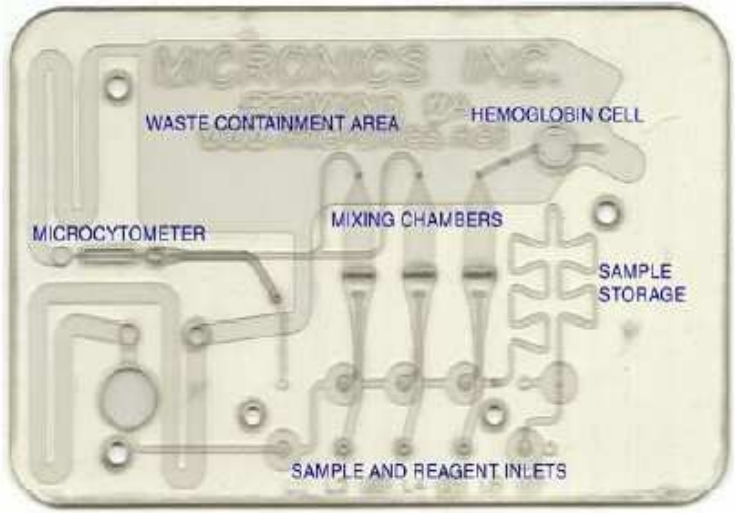
Microfluidics

• **Microfluidics:** behavior, precise control and manipulation of microliter and nanoliter volumes of fluids:
• displacement of a fluid mass
• mixing
• separation
• analysis
• chemical reaction and physical transformation
(phase transition, thermal cycles, electromagnetic interaction etc.)



• **Multidisciplinary field**: physics, chemistry, engineering and biotechnology, with practical applications to the design of systems in which such small volumes of fluids will be used.

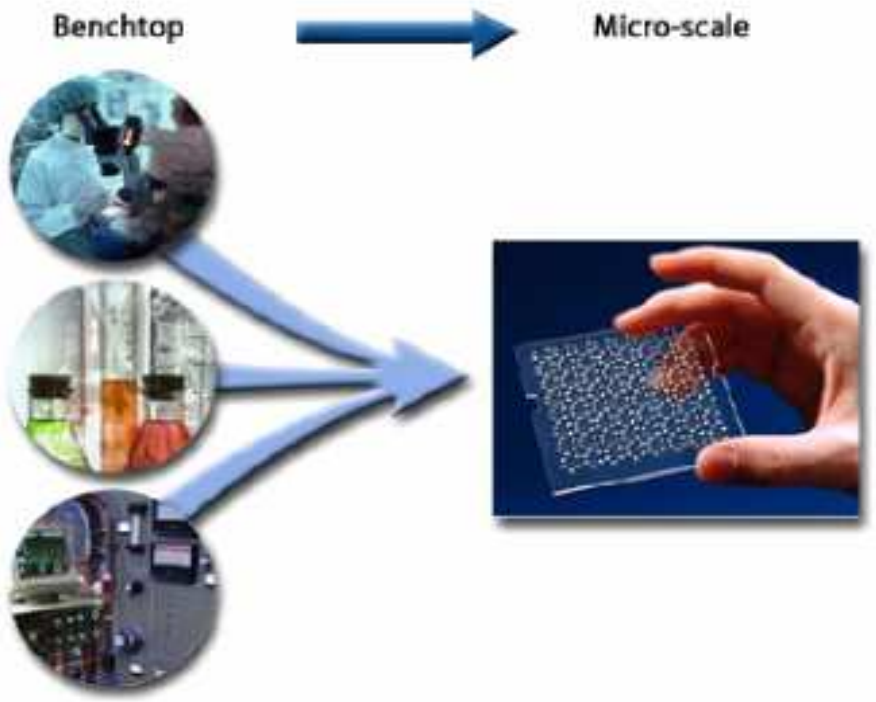
• Microfluidics has emerged only in the 1990s and is used in the development of DNA chips, micro-propulsion, micro-thermal technologies, and lab-on-a-chip technology.



Microfluidic disposable cartridge for blood analysis - Micronics Inc.

Why Being Small ?

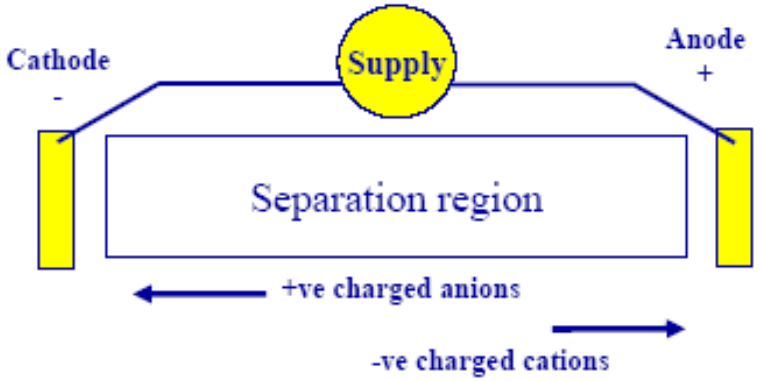
- Savings in time & cost
  - *Less materials and samples*
  - *Short processing time*
- Disposable
- Parallel processing
- Integration/Automation
- Portability - *in-situ* operation (POC)
- Scale economy

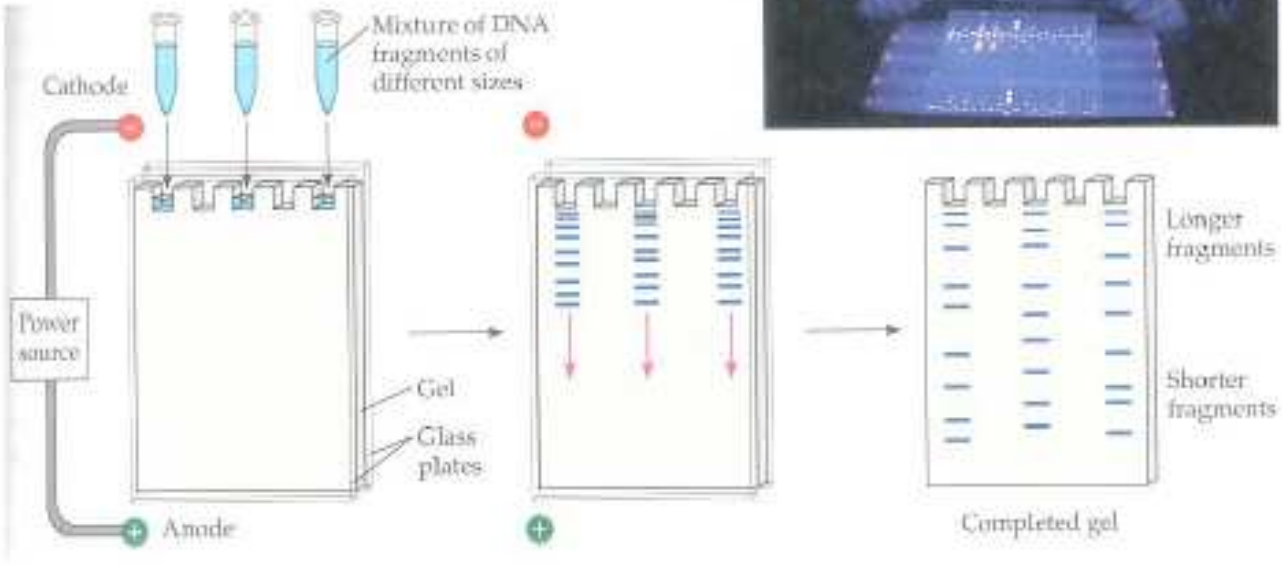


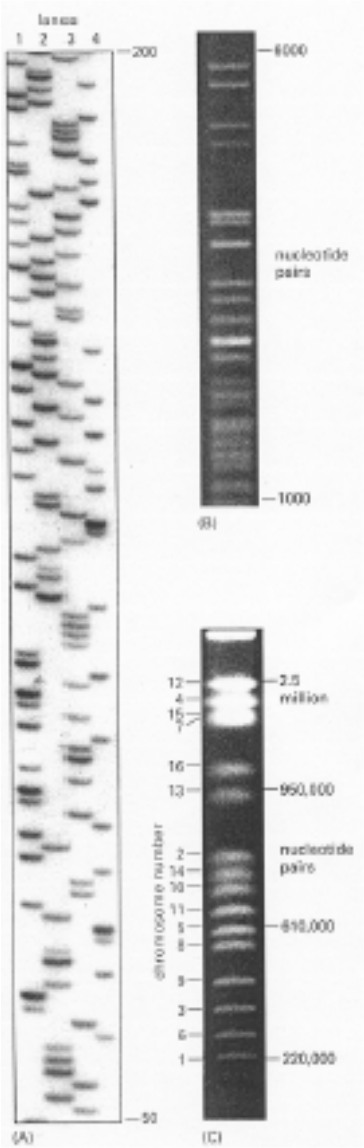
Gain from the unique microscopic features

- Laminar Flow
- High surface to volume ratio
- Small thermal mass
- Strong fields such as electric fields

Microfluidics: electrophoresis







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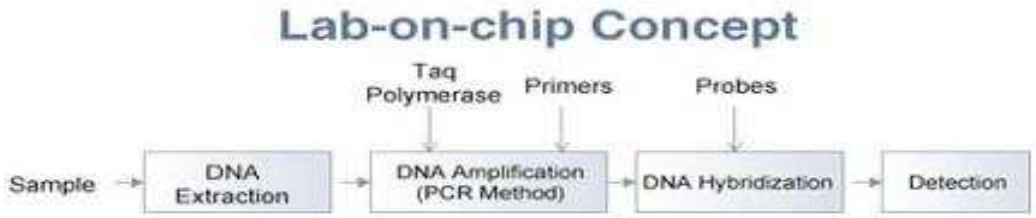
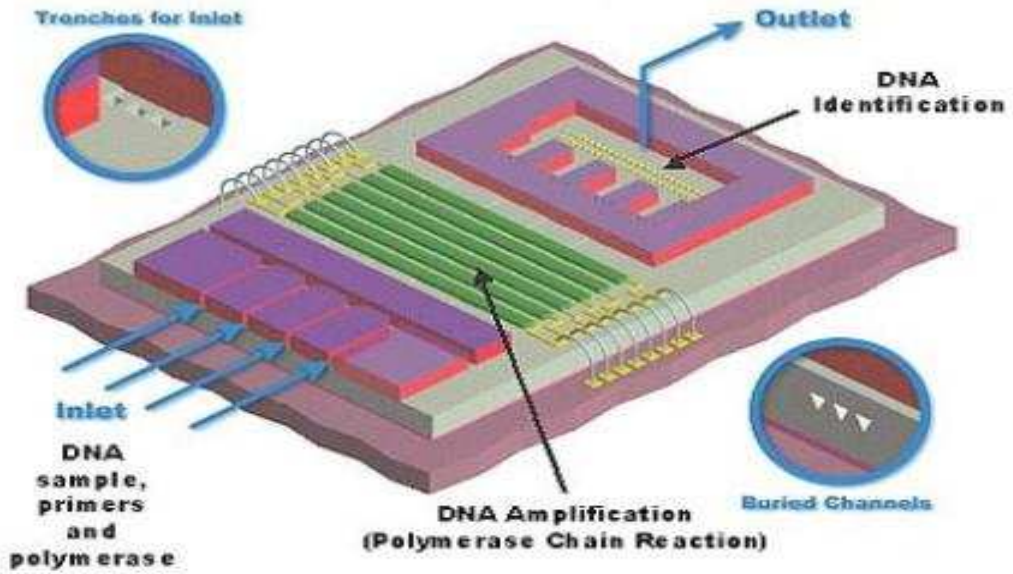
Lab on Chip

Lab-on-a-chip and PCR





- **Continuously flowing sample**
  - well-defined temperature controlled zones
- **Pattern of chip**
  - determines the relative time sample is exposed to each temperature zone
- **Multiple channel**

Lab on Chip can be defined as '*microelectronic-inspired* devices that are used for delivery, processing, analysis, or detection of biological molecules and species' [Bashir, 2004]. These devices are used to detect cells, microorganisms, viruses, proteins, DNA and related nucleic acids, and small molecules of biochemical importance and interest.

Lab on Chip: applications

μTAS: micro Total Analysis System

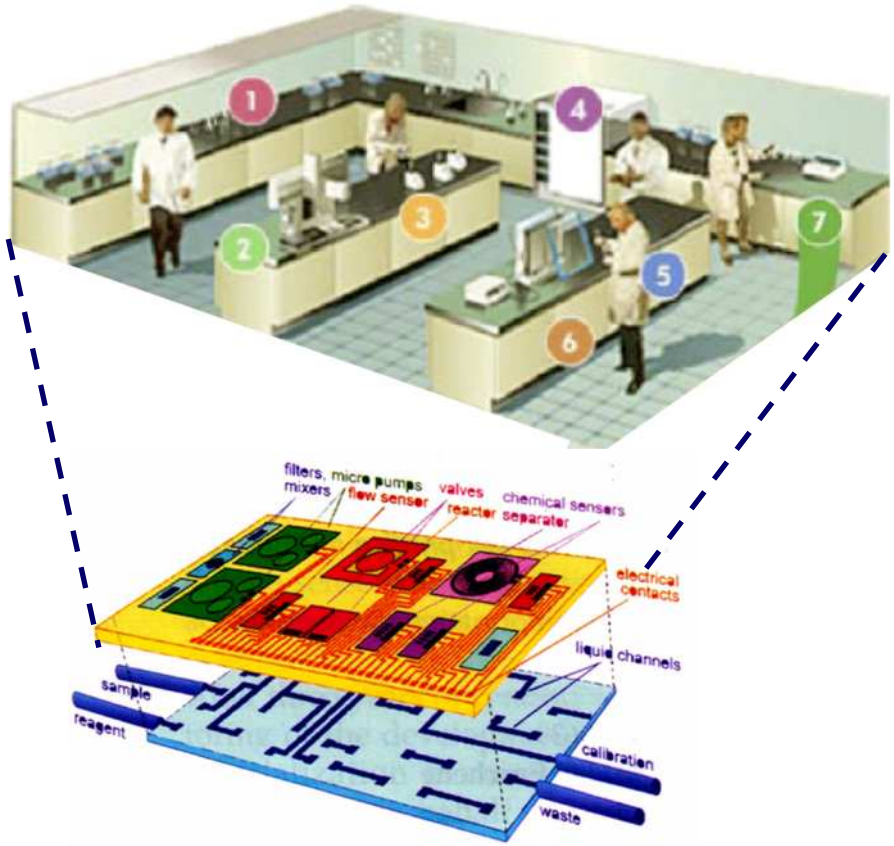
Integrated, miniaturized chemical analysis systems, that include sample preparation, separation and detection system on a small, single chip.

Applications

1. Bioscience and Bioengineering
(genetics, proteomics, bioinformatics, cell biology)

2. Medical science
(diagnostics, drug delivery/discovery)

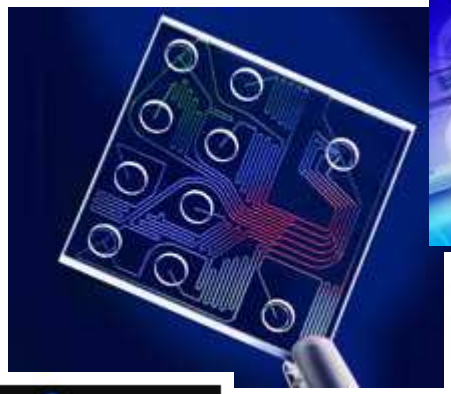
3. Homeland security
(chemical warfare agent detection)



Lab on Chip: applications

DNA Analysis and Genomics

- Microfabricated electrophoresis chips for high –speed genotyping
- Microfluidic chip for genomics
- DNA hybridization assays in microfluidic chips



Protein Analysis and Proteomics

- Protein separation in microchips
- Microfluidic systems for immunoassays

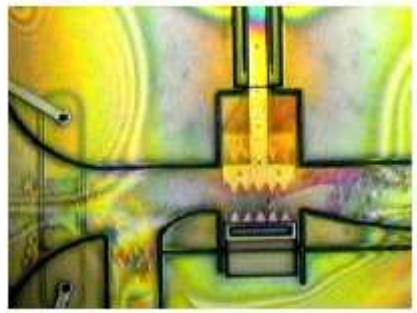
Biofluids components analysis

- Determination of electrolytes, hemoglobin, bloodgases, pH, pO_2

(b)

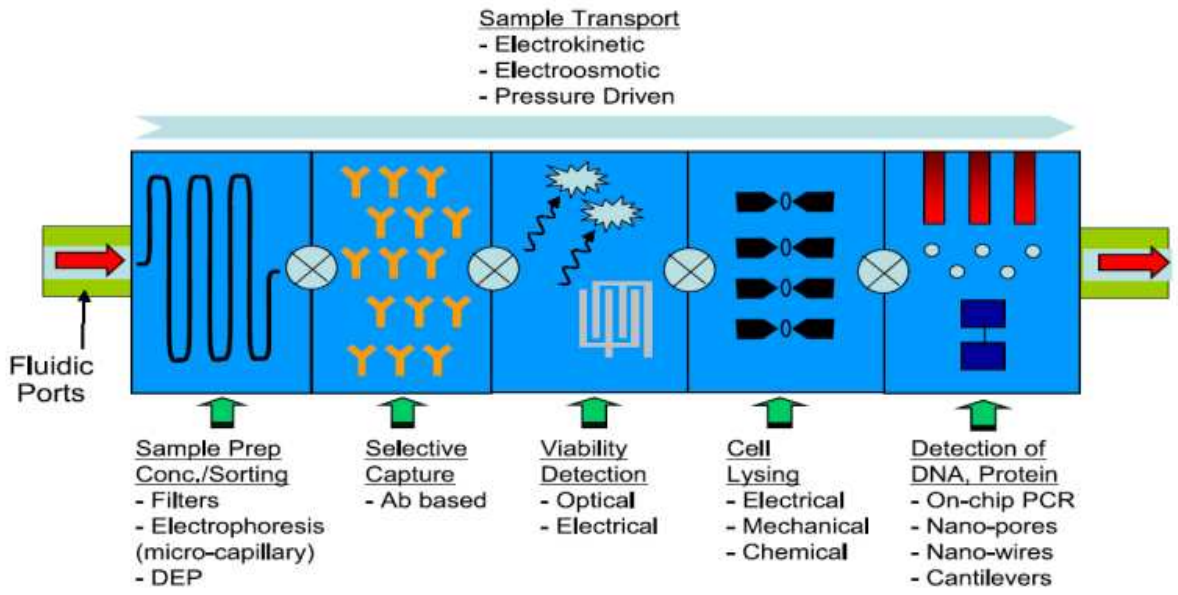
Microcytometry

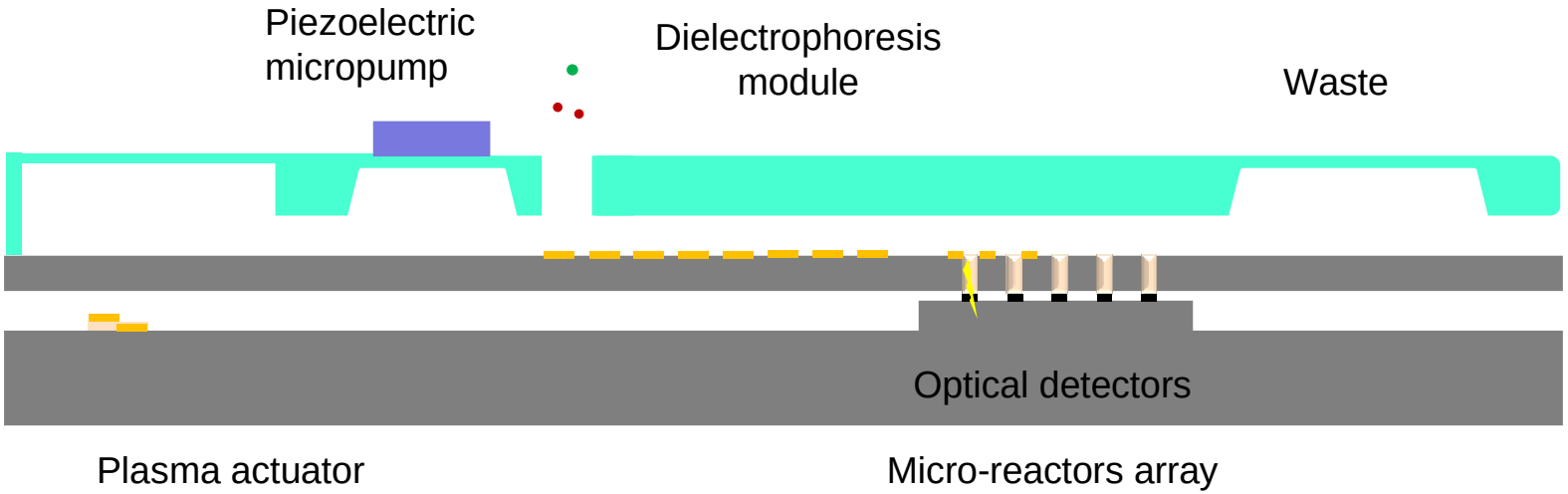
- Flow cytometer for rapid analysis of multiple characteristics of single cells



(b)

The LOC Concept



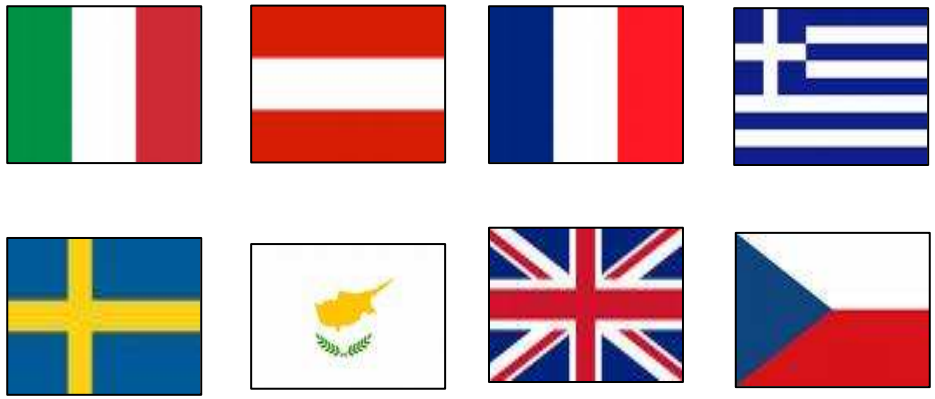


Pocemon

Point-Of-Care MONitoring And Diagnostics For Autoimmune Diseases

SEVENTH FRAMEWORK PROGRAMME

eHealth

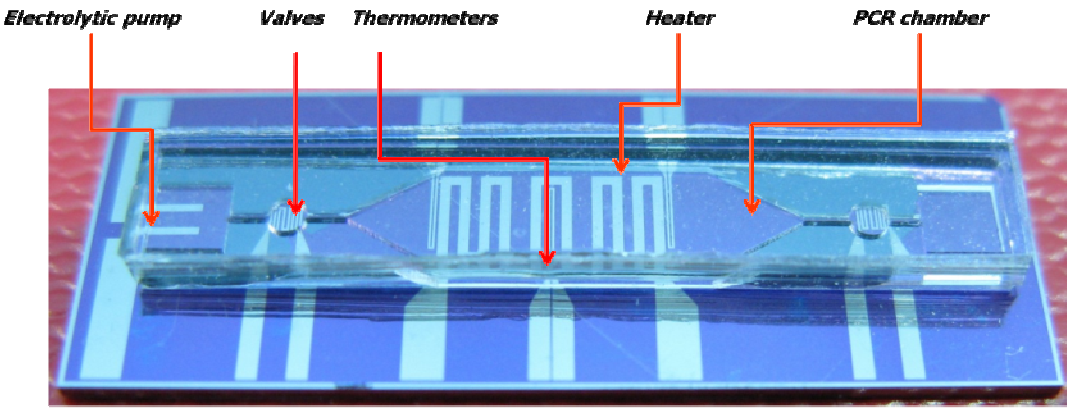


Lab on Chip for DNA amplification module

- Disposable miniaturized Polymerase Chain Reaction (PCR) modules have been integrated in an innovative Lab on a Chip (LOC) platform to detect genetic profiling.

- The amplification system consists of a micro-chamber reactor with a hybrid silicon-polymer structure.

- The temperature control system has been implemented by means of Platinum microheaters and thermometers integrated on a silicon substrate and the reaction chamber has been completely made of polydimethylsiloxane (PDMS) since it is biocompatible, transparent and easily moldable.



Main advantages of the chamber type:

- ✓ No need for complex fluidic
- ✓ Reduced sample absorption on the channel walls
- ✓ Easier fabrication
- ✓ Possibility of parallelization (multi chamber)
- ✓ Increased flexibility over the cycles number

- The final micro reactor will be coupled with a label-free Single Nucleotide Polymorphisms (SNP) microcantilever array.
- The coupling of the proposed PCR module with a cantilever-based detector would provide a really portable and automated multifunctional system able to fulfill a wide number of critical clinical practices.

Polymerase Chain Reaction or PCR

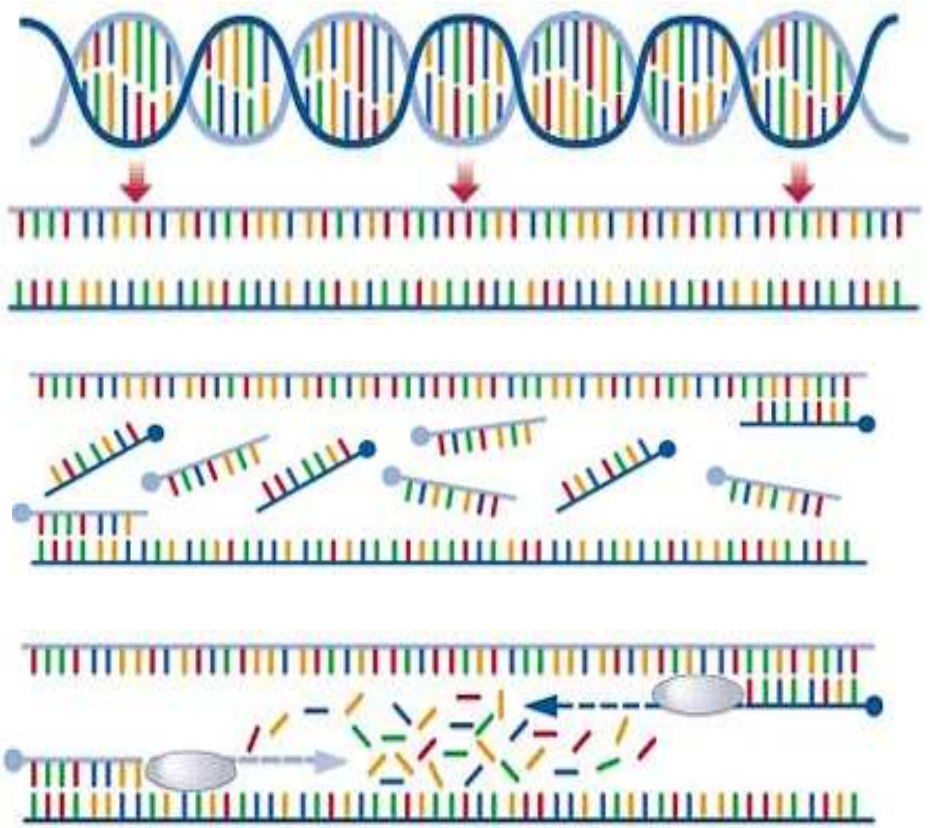
A process which "Amplifies" or "Copies" a piece of DNA repeatedly until there is an amount which is great enough to observe visually..

PCR is an exponential processes ($y=e^x$)
step 1

Denaturation (optimal temperature is 94℃): By heating the DNA, the double strand melts and open to single stranded DNA.
step 2

Annealing (optimal temperature is 60℃) The single-stranded primers bind to their complementary single-stranded bases on the denaturated DNA.
step 3

Extension 72℃ is the ideal temperature for the Taq polymerase to attach and start copying the template. The result is two new helixes in place of the first.

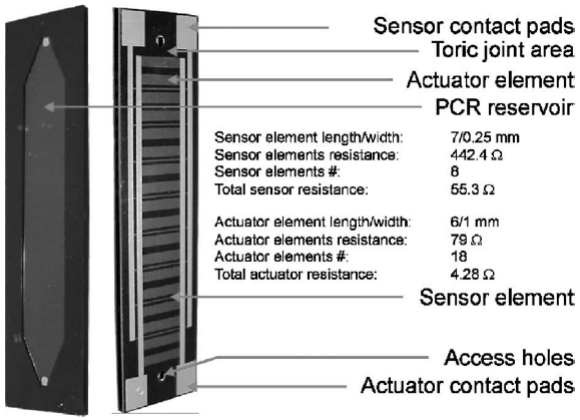


http://www.youtube.com/watch?v=_YgXcJ4n-kQ&feature=related

State of the Art

Biochemical analysis and optimization of inhibition and adsorption phenomena in glass–silicon PCR-chips

Erill et al. – Sensors and Actuators B- 96 - 2003



- **Chamber type PCR realized on silicon and glass. On the chamber bottom, an array of polysilicon heater is used for temperature control**
- **PCR protocol:**

  2min @ 95ºC
  40-45 cycles of (1s @ 95.5ºC,10s @ 61ºC, 19s @ 72ºC)
  1min @ 72ºC
  Test with agarose gel

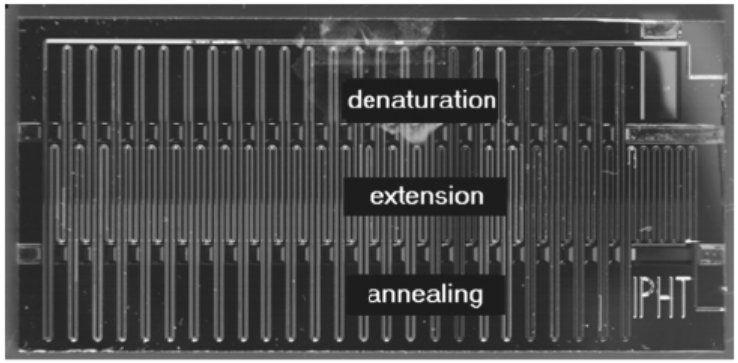
The chip was used to study the inhibition and absorption phenomena of different materials.

Miniaturized flow-through PCR with different template types in a silicon chip thermocycler

Schneegass et al. – Lab on chip – 1 - 2001

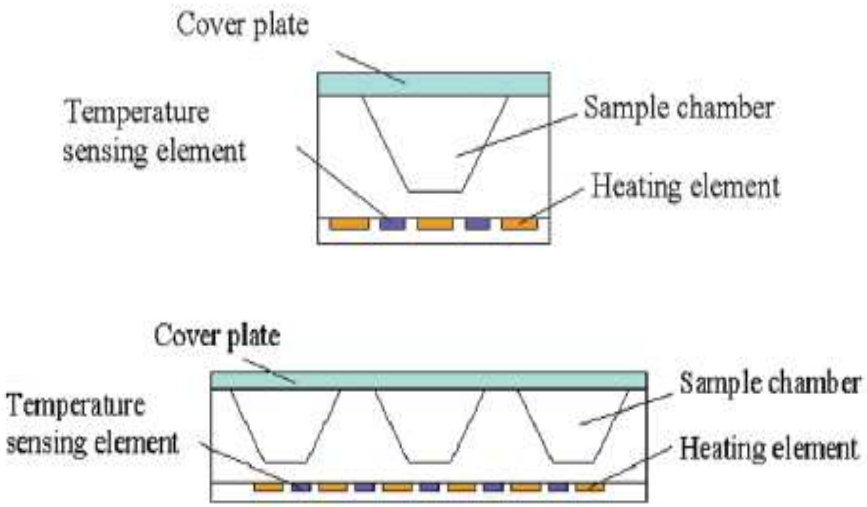
- **Continuous flow type PCR made of silicon and glass, consisting of a reaction channel etched on glass and a cover chip made on silicon with platinum heaters and sensors.**
- **PCR protocol:**

  Syringe injection: flow rate 1µL/min
  Sample volume: 30µL
  1 cycle @ 94ºC
  25 cycles of (94ºC, 58ºC, 72ºC)
  1 cycle 72ºC
  Time: 35 min
  Test with agarose gel



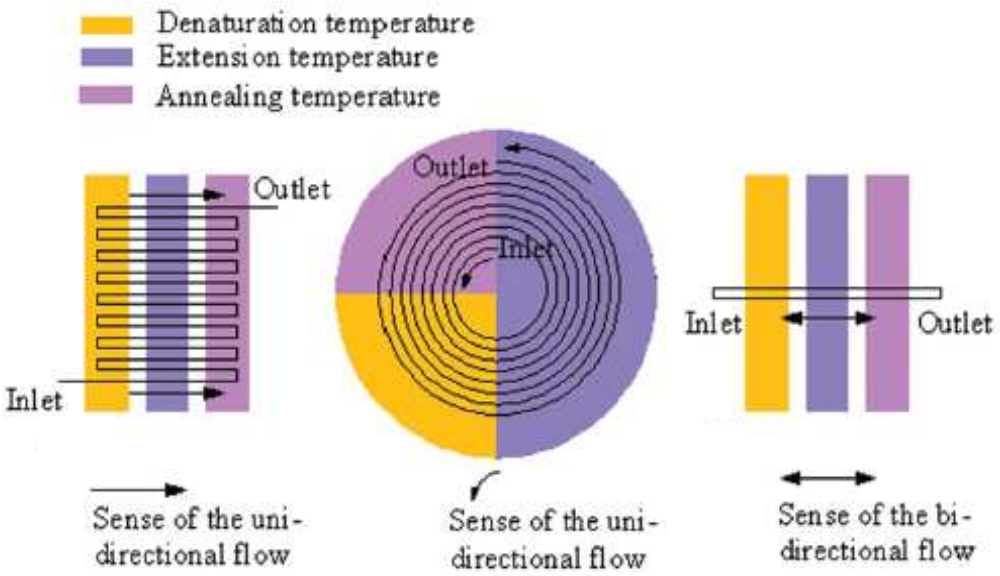
Architecture for PCR chips

Stationary chamber-based PCR



- ✓Static approach
- ✓Slow thermal cycles
- ✓Flexible number of cycles
- ✓Arrays configuration
- ✓Requires microfluidics for LOC integration

Continuous-flowPCR

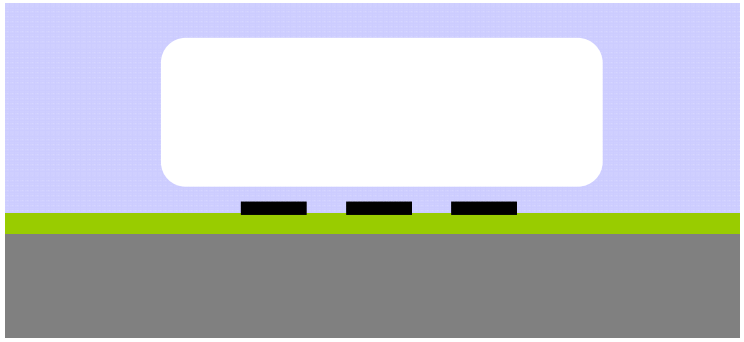


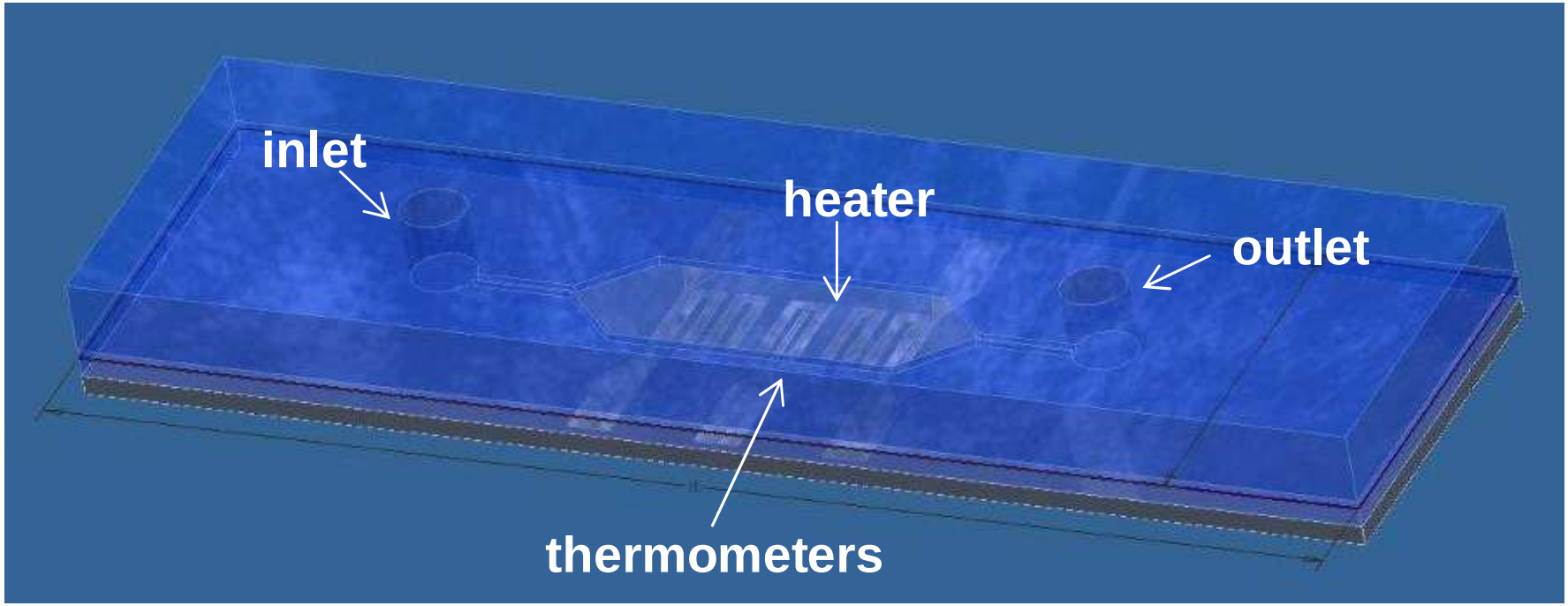
- ✓Dynamic approach
- ✓Fast thermal cycles
- ✓Fixed number of cycles
- ✓Temperature gradients
- ✓Progressive sample dispersion

The PCR microdevice

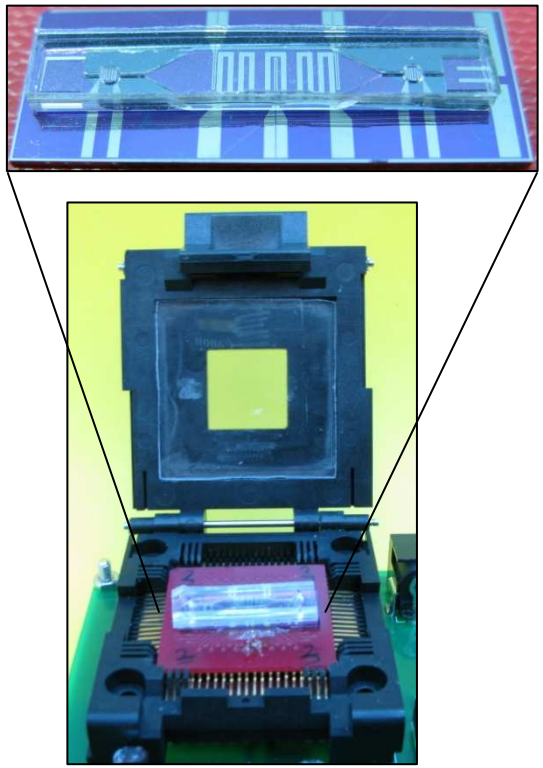
- Stationary chamber-based PCR
- **Hybrid structure:**

✓***Silicon*** for the heating system
✓***PDMS*** for its biocompatibility, transparency, inexpensive fabrication techniques



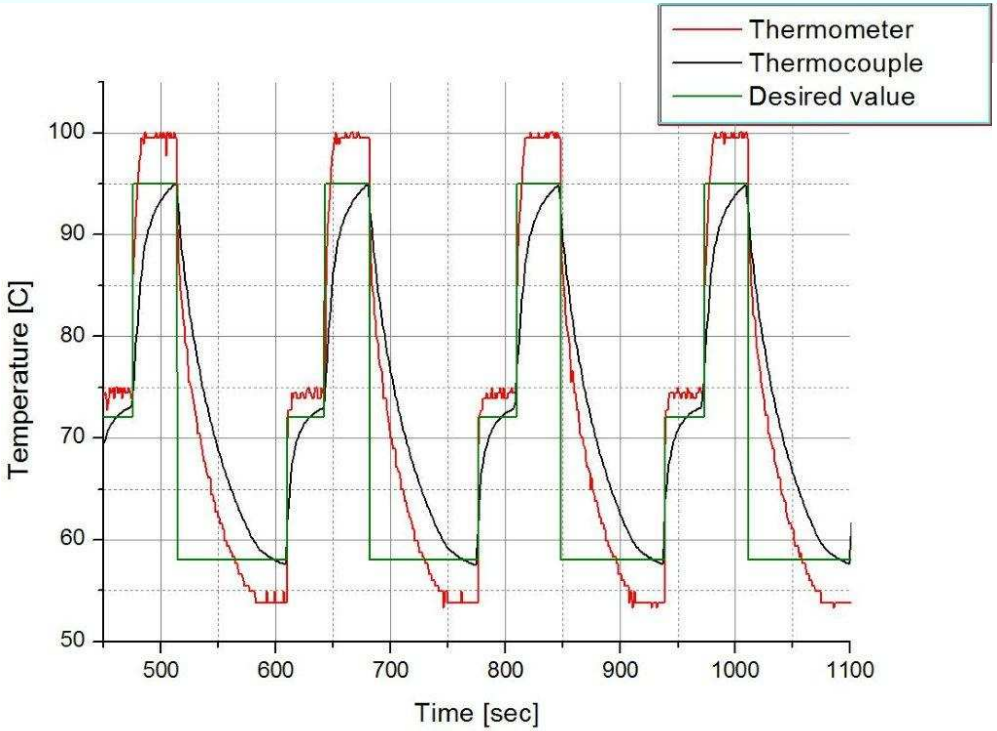


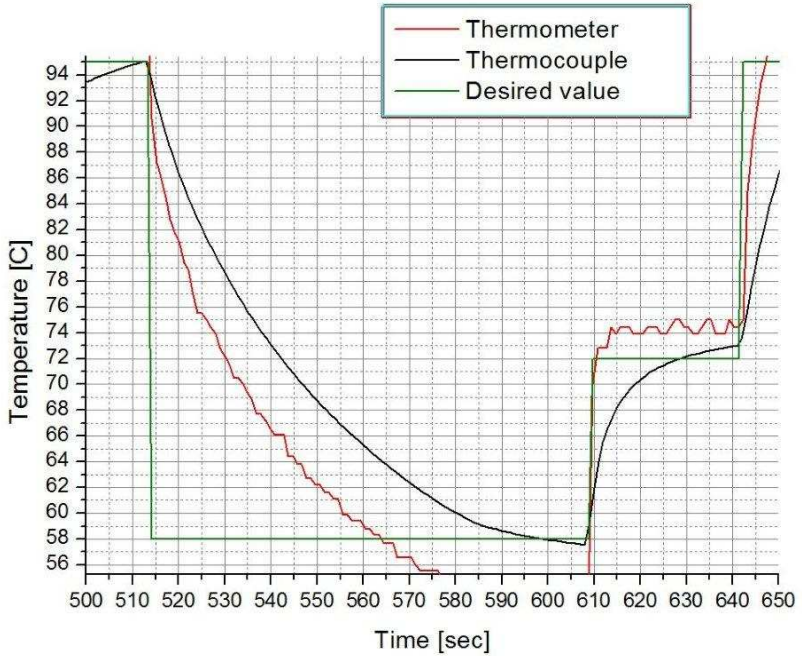
The PCR microdevice



Mechanical clamp:
- ✓good sealing of inlet and outlet to prevent evaporation
- ✓contact between Silicon chip and PDMS microchamber
- ✓disposable microchamber

The PCR Thermal curves

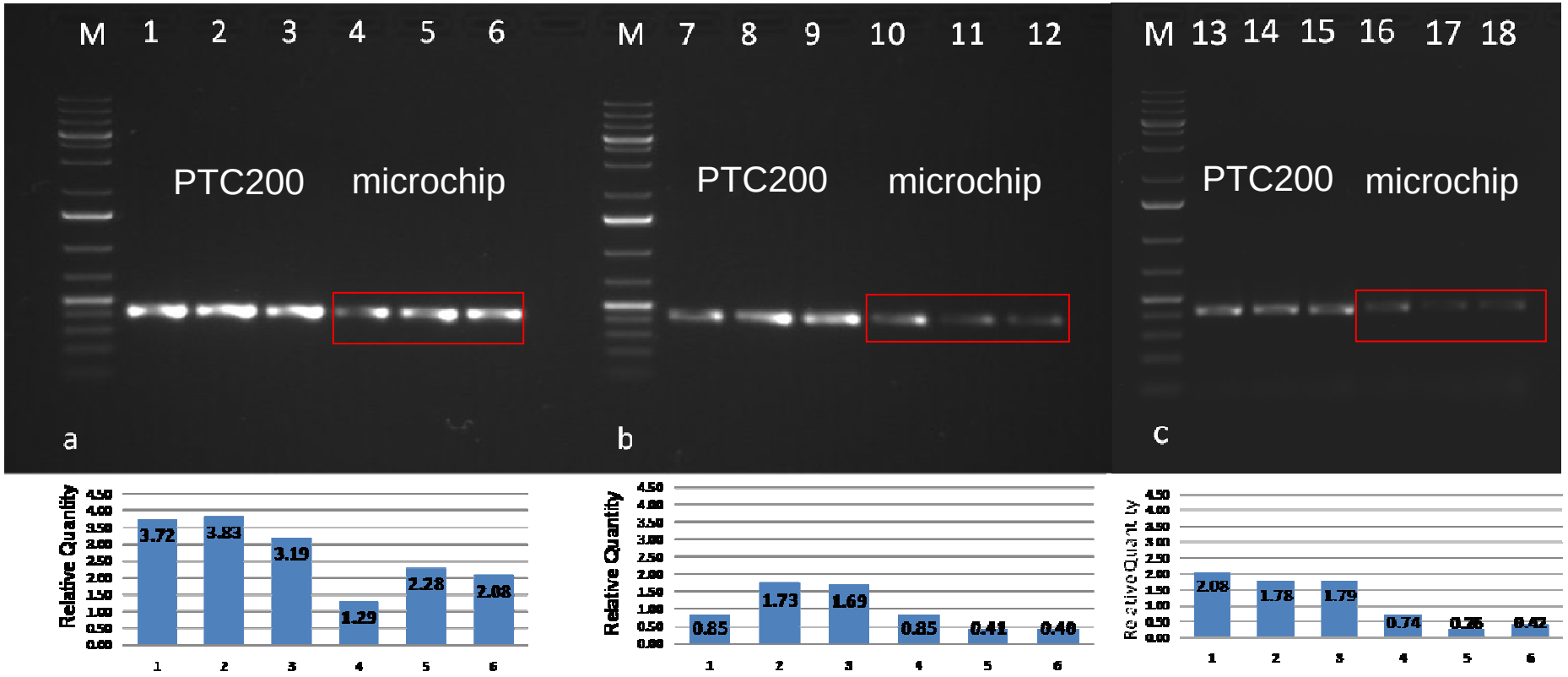




Thermal steps	PTC200-PCR		Microchamber-PCR	
	Temperature (℃)	Time (sec)	Temperature (℃)	Time (sec)
Initial denaturation	95	180	95	180
Denaturation	95	10	95±0.5	10
Annealing	58	10	58 ±0.5	16
Extension	72	10	72 ±0.5	9
Final Extension	72	180	72 ±0.5	300

Validation tests*

Comparison of PCR efficiency and reproducibility with different gDNA S. cervisiae strains concentrations



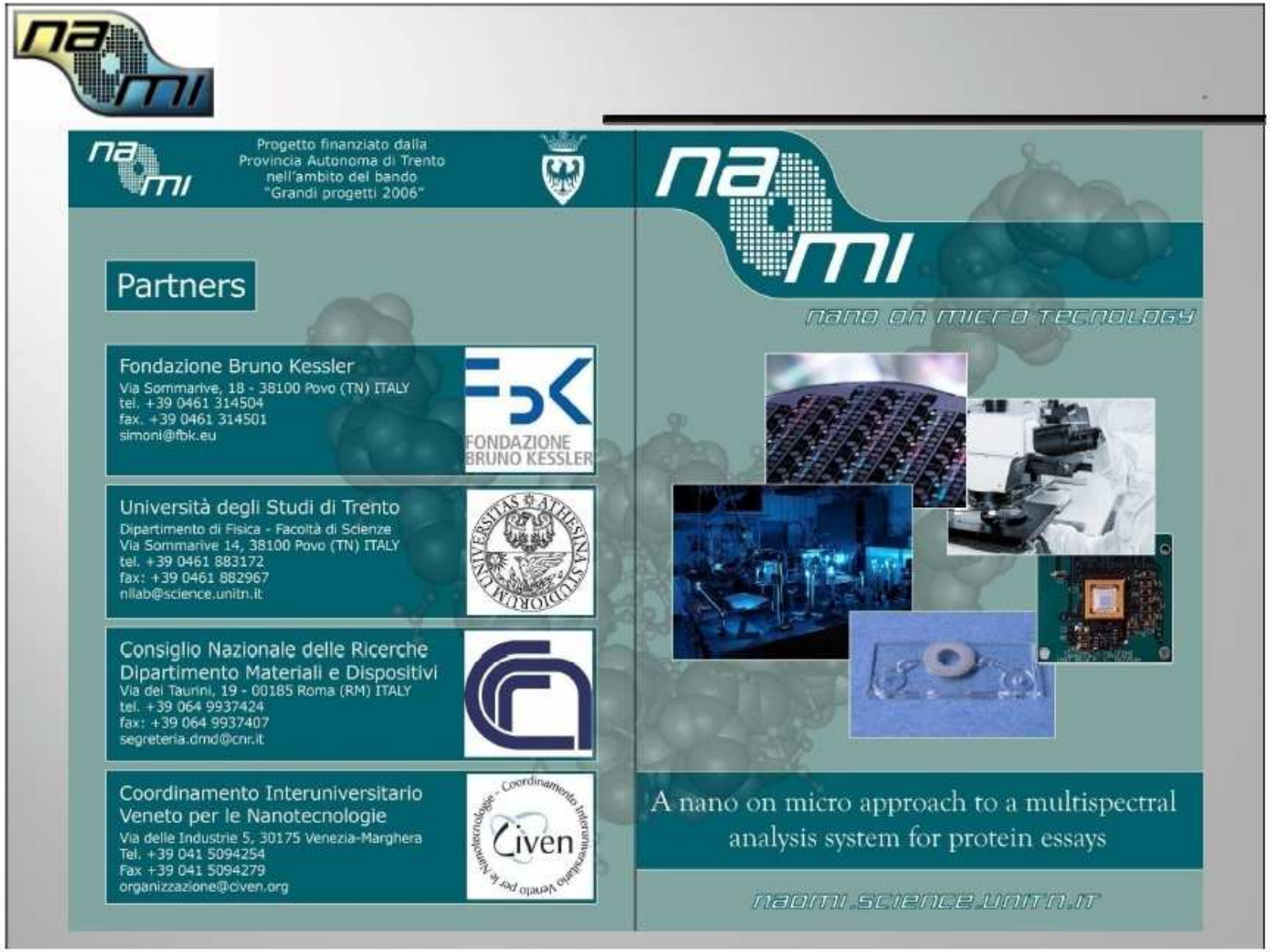
gDNA dilutions 1:10 1:100 1:1000

microchip-PCR was successful even with the most diluted template sample

DNA amplification of 10-fold serial dilution of yeast purified genomic DNA (a. 177ng; b. 17.7; c. 1.77ng

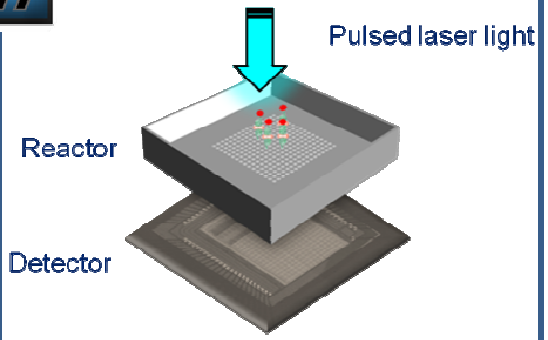
(*) by Cristina Ress (FBK-CMM, Trento), Annalisa Ballarini (CIBIO, Univ. Trento)

NAOMI Protein Chip



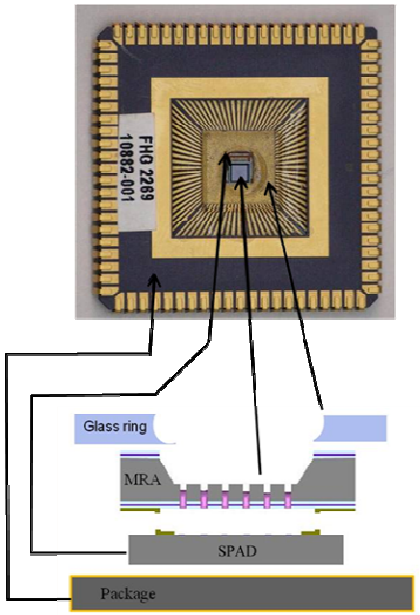
Micro-reactor array for fluorescence-based bioaffinity assays

Principle

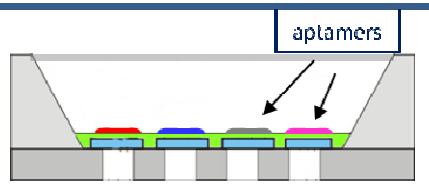


SPAD detector
CMOS Single-Photon Avalanche Diode
64-pixel linear array

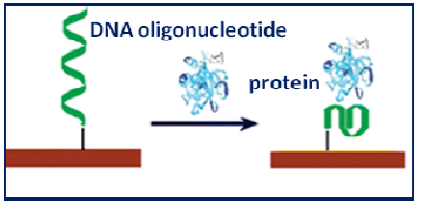
Microsystem



Biological Model

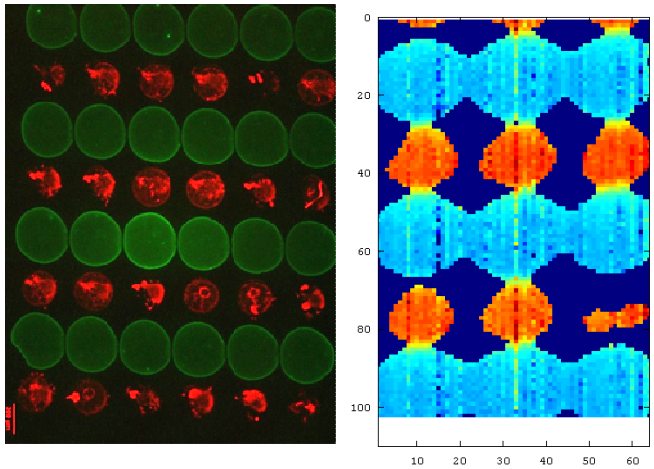


Aptamer-based sensor



Thrombin (cardiovascular diseases, inflammation and tissue repair at the vessel wall)
Vascular Endothelial Growth Factor (tumors, cronical ischemia, retinopathy)

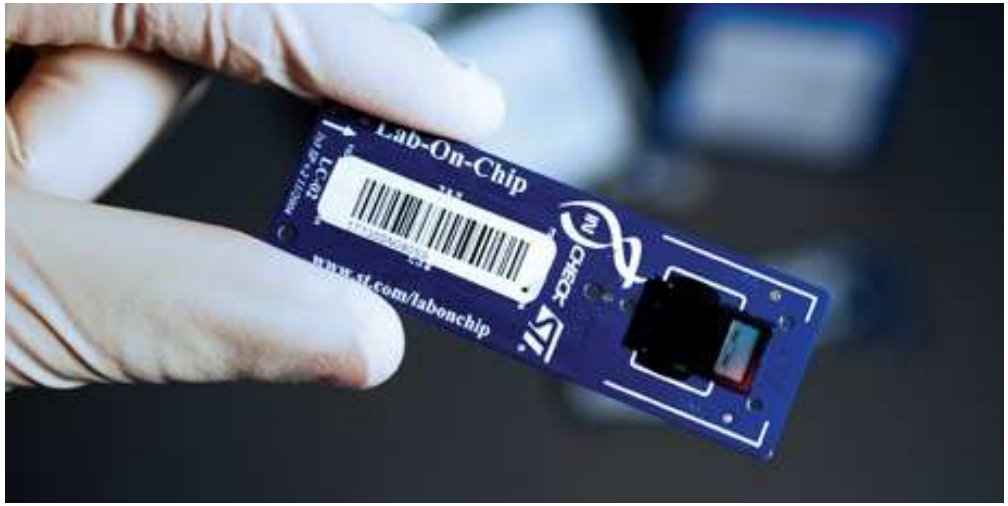
Confocal microscope image



Lifetime image

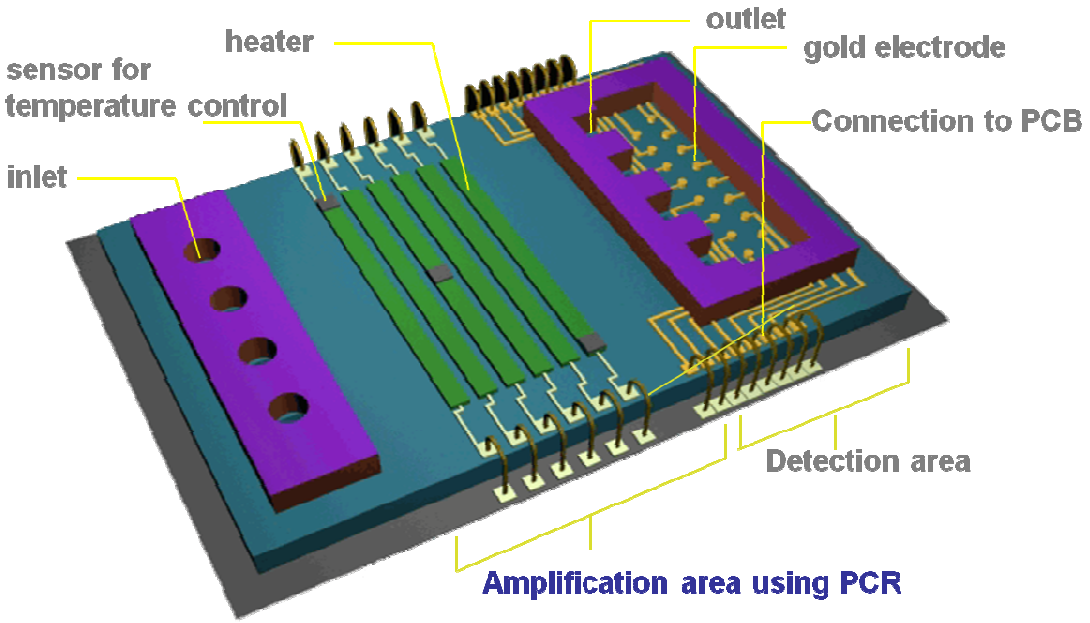
Array of Avidin-AlexaFluor488 (15mM) and Quantum Dots

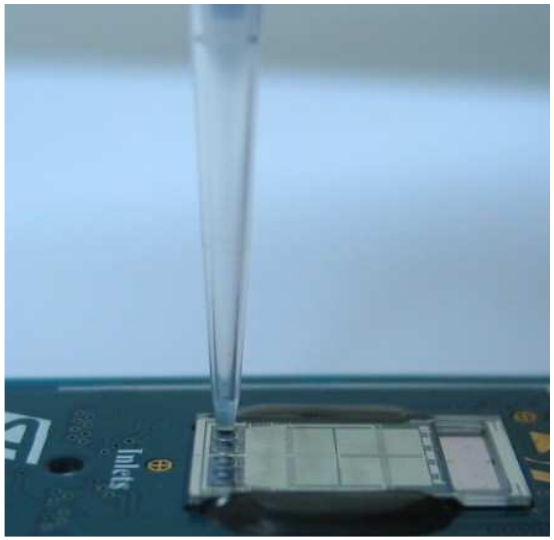
VereFlu Lab-on-chip

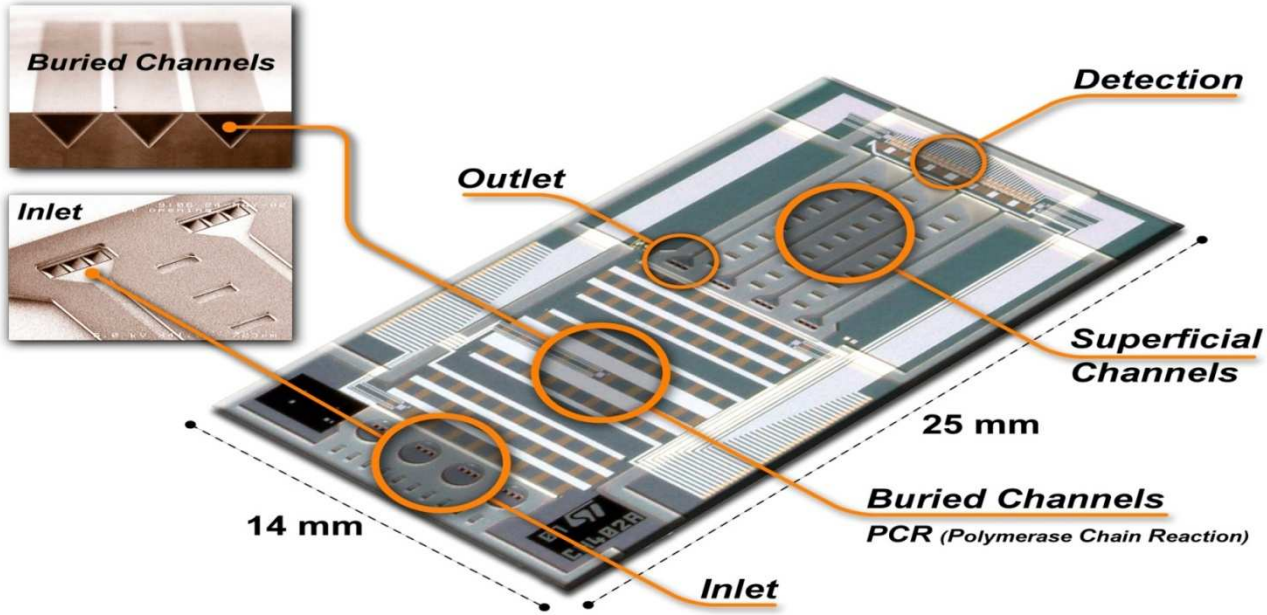


Veredus Laboratories Pte Ltd, Singapore, and STMicroelectronics, Geneva, have joined to offer VereFlu™, a portable application for rapid detection of all major influenza types at the point of need. It integrates two key molecular biological applications in a lab-on-chip the size of a fingernail, and identifies and differentiates human strains of influenza A and B viruses, including the Avian Flu strain H5N1, in a single test. The molecular diagnostic test detects infection with high accuracy and sensitivity within 2 hours, providing genetic information about the infection. Its automation allows users outside the traditional lab environment to perform tests at the point of need. A miniature lab on a chip, ST's In-Check platform allows users to process and analyze minute patient samples—human blood, serum, or respiratory swabs—on one disposable, thumbnail-sized chip. **www.vereduslabs.com**

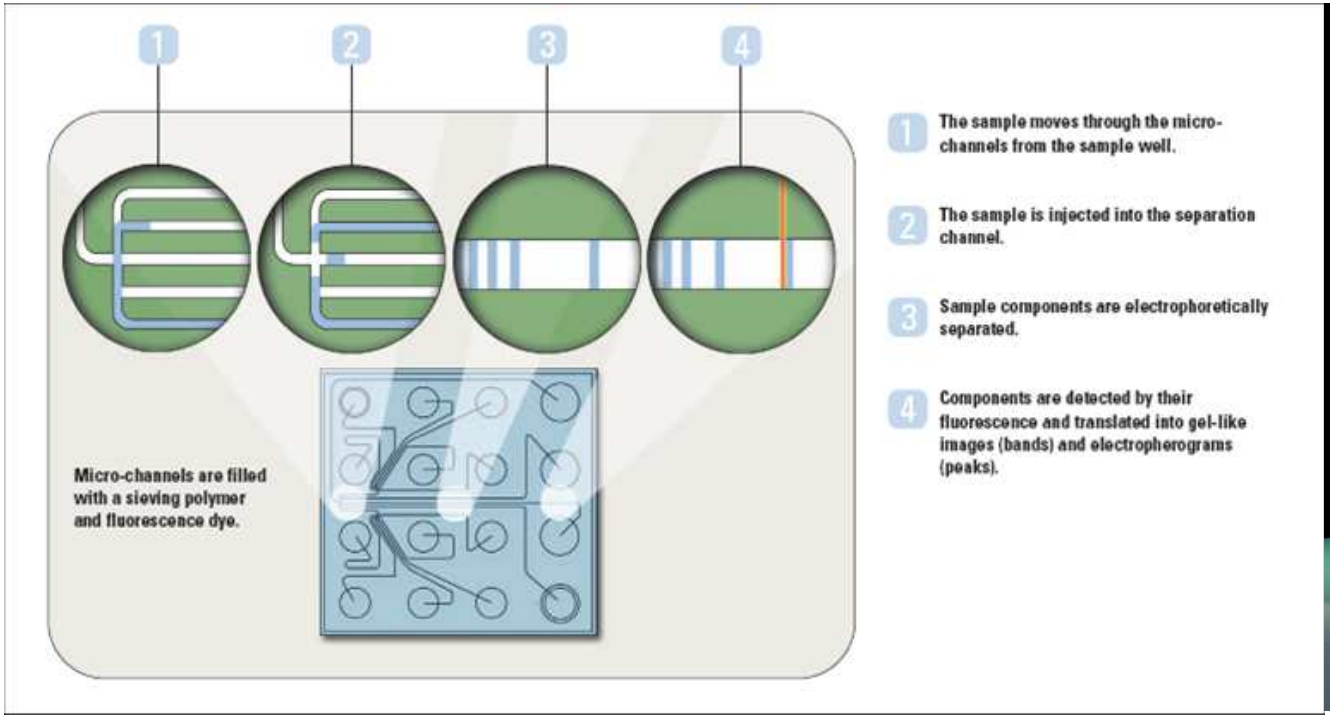
VereFlu Lab-on-chip







Agilent 2100 Bioanalyzer



Quantitative real-time polymerase chain reaction (qPCR) and microarray analysis have become essential for elucidating variations in gene expression. While guidelines that define the minimum information required for interpretation of microarray data have been available since 2001, similar specifications for qPCR experiments have been developed only recently. In early 2009, a consortium of leading scientists who use qPCR, established specifications for the minimum information that you must report for a qPCR experiment that you wish to publish. These are the MIQE guidelines (for minimum information for publication of quantitative real-time PCR experiments).

Conclusions and future perspectives

Genomic Test

Environment Friendly – Easy – Safe – Painless – Less wastes - Fast

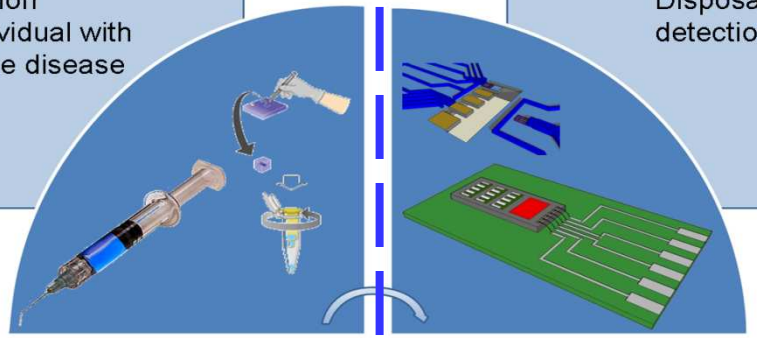
Users

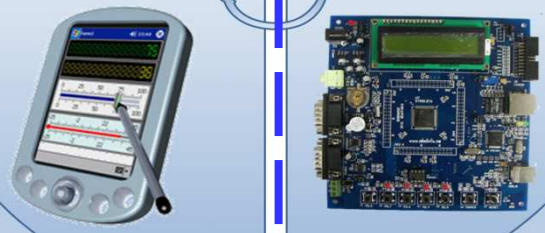
Individual with or without the disease

Primary Care GP

DNA extraction from an Individual with or without the disease

Disposable LOC detection module





PDA – management and handling of the genomic diagnostic tests

µController - control unit of the LOC device Non-disposable module

LOC Device

Microfluidics & µ-technology

Automated

Portable

"Smart"

Light-weight

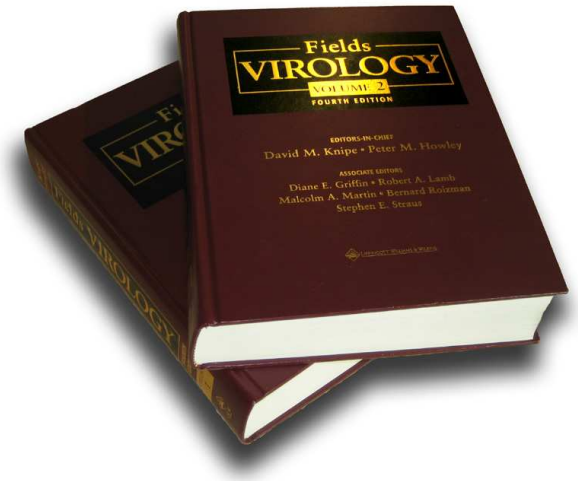
Low power supply

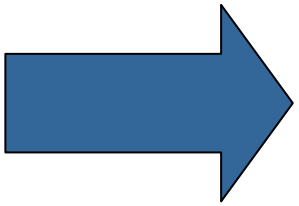
Artificial Intelligent Algorithms

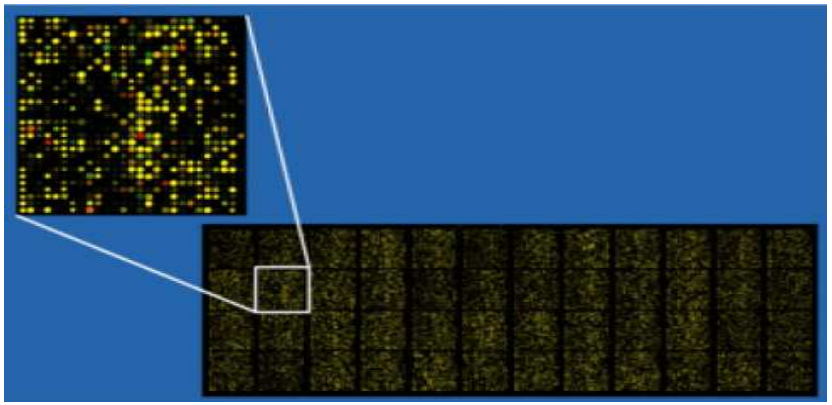
Decision Support – Early Prognosis – Diagnosis

LAB-ON-A-CHIP: MICRO E NANOTECNOLOGIE IN MICROBIOLOGIA E VIROLOGIA

APPLICAZIONI IN MICROBIOLOGIA CLINICA



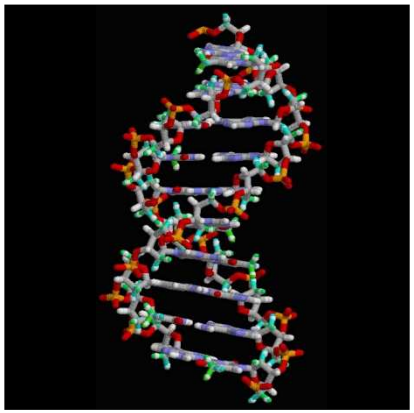




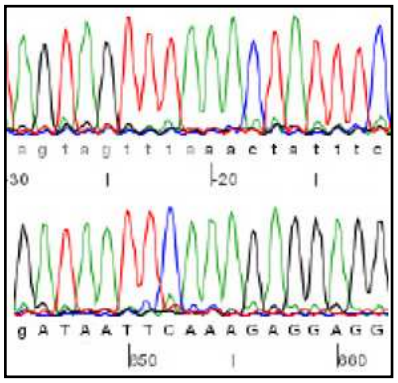
Paolo Lanzafame

- I microarrays sono caratterizzati da una potenziale capacità di rilevazione ed identificazione di migliaia di geni microbici
- Sebbene inizialmente applicati alla ricerca, ed in particolare a studi di espressione genica, il loro utilizzo nei laboratori di microbiologia clinica sta diventando una realtà destinata, nell'immediato futuro, a modificare la diagnostica microbiologica
- La capacità di rilevare un gran numero di patogeni e/o monitorare la variabilità delle popolazioni microbiche e l'approccio metagenomico potrebbero modificare la nostra capacità di comprensione delle malattie infettive

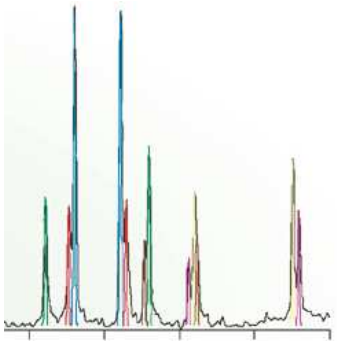
Metagenomics for microbial detection / discovery



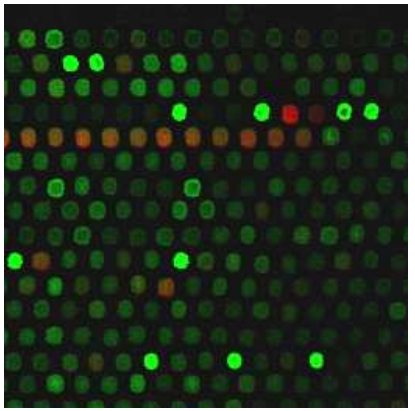
Genomics



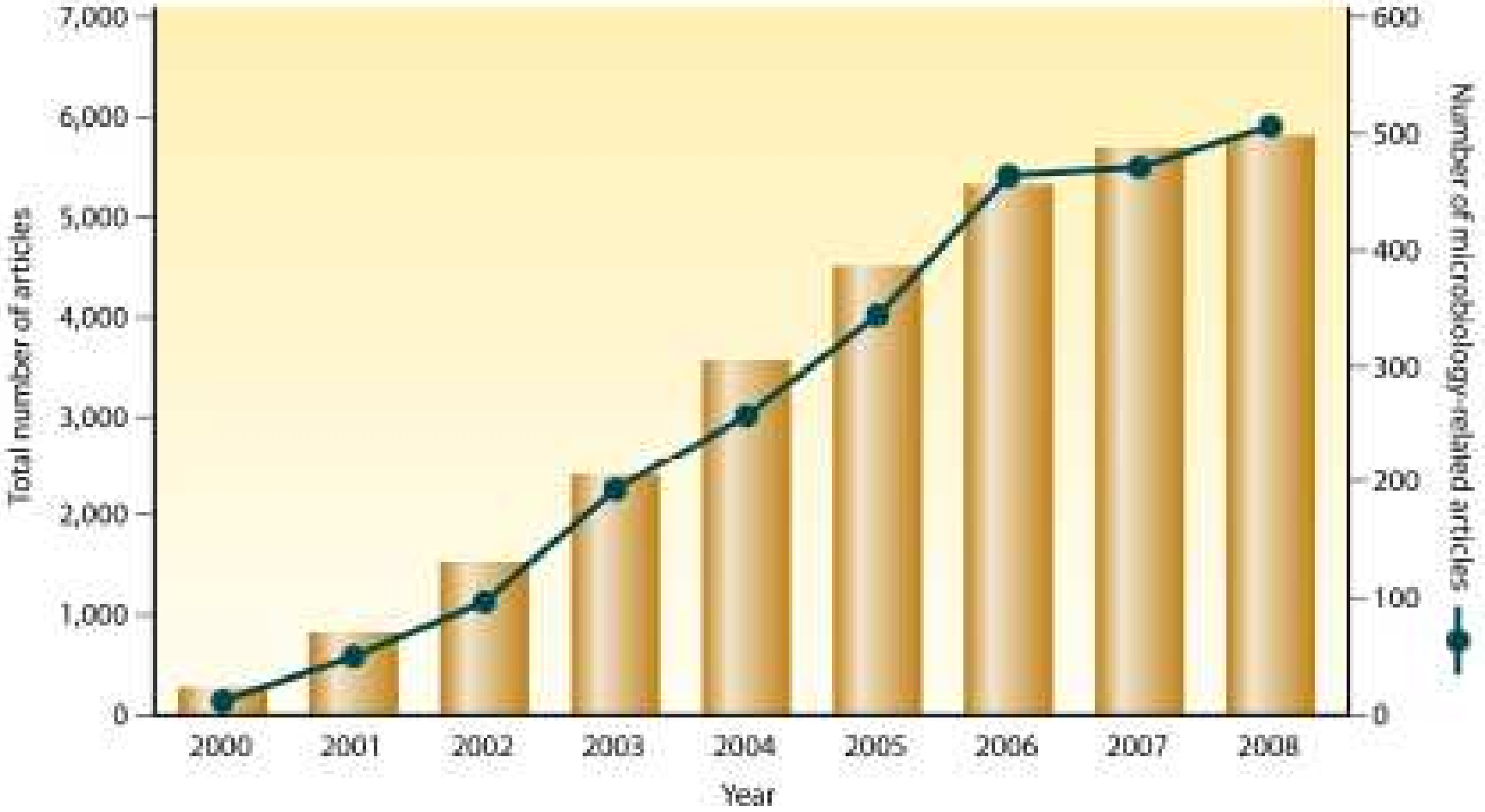
Deep Sequencing



Massively Parallel PCR and mass spectrometry (PLEX-ID)



Microarrays



Microarray publications. The number of primary manuscripts published using microarray technology (bars) and the number of microarray publications that have infectious disease and/or microbiology applications (line) are depicted.

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0893-8512/09/$08.00+0 doi:10.1128/CMR.00019-09

Vol. 22, No. 4

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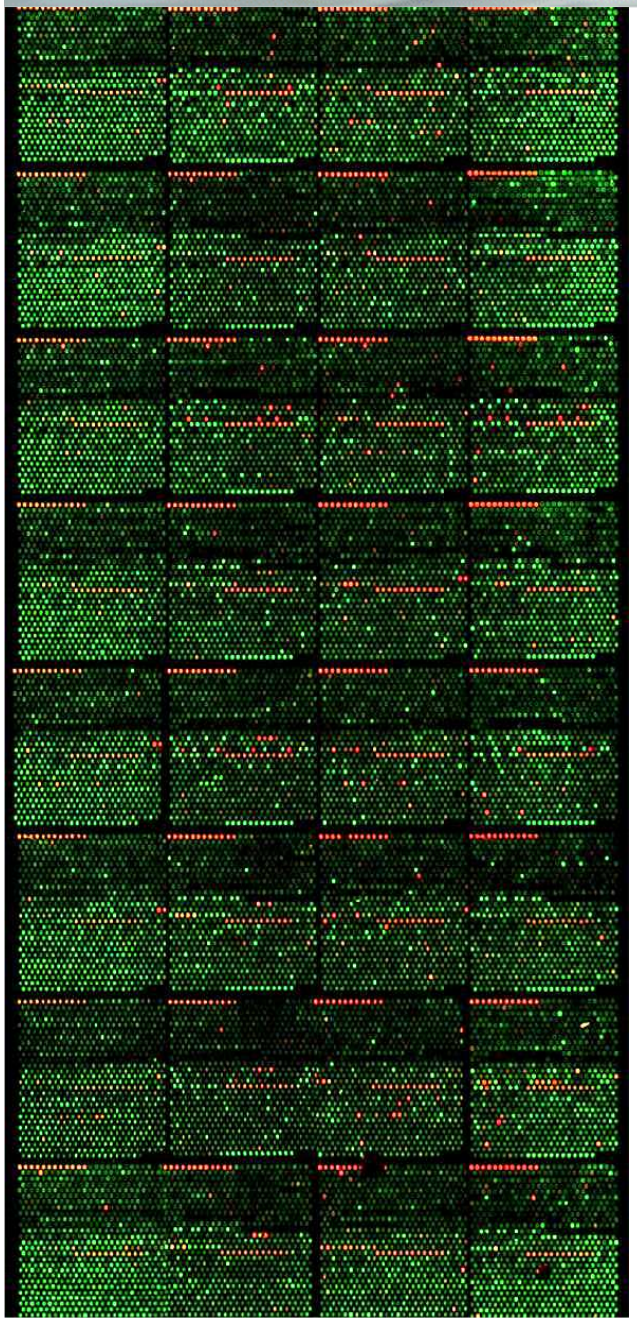
Department of Pathology and Laboratory Medicine, School of Medicine, University of North Carolina, Chapel Hill, North Carolina,[1] and Departments of Medicine and Pathology, Vanderbilt University School of Medicine, Nashville, Tennessee[2]



Evoluzione di Virochip™

v1.0
- respiratory virus detection only (~3,000 probes; Aug 2002)

v2.0
- most conserved viral sequences (~10,000 probes; Sep 2003) – SARS coronavirus

v3.0
- expansion for broad coverage of viral taxonomy (~20,000 probes; Jun 2004) – XMRV retrovirus, HTCV cardiovirus

v4.0
- Clinical diagnostic focus – human / animal viruses (~15,000 probes; Jun 2006)

v5.0
- shift to commercial platform (Agilent); targeted microarrays with enhanced subtyping capability; expanded coverage (~36,000 probes; Dec 2008)

Applicazioni in Microbiologia Clinica

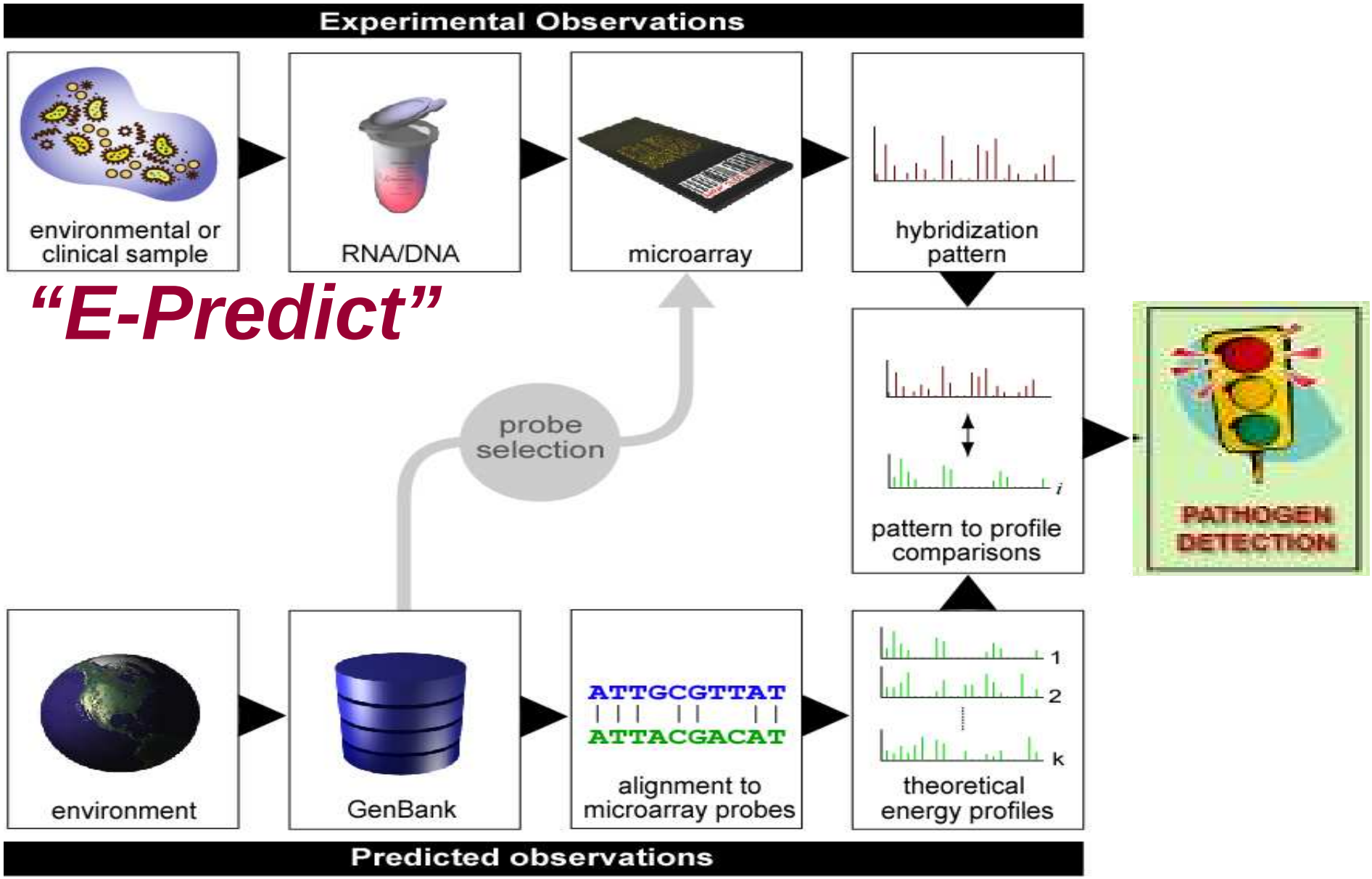
1. Rilevamento ed identificazione microrganismi
2. Rilevamento geni di resistenza ai farmaci antimicrobici
3. Microbial typing
4. Studio dei profili di espressione genica dei microrganismi
5. Studio dei profili di espressione genica dell'ospite in corso di infezione
6. Determinazione dei polimorfismi genomici dell'ospite

Applicazioni in Microbiologia Clinica

1. Rilevamento ed identificazione microrganismi

Probabilmente l'area di maggiore sviluppo e di potenziale utilizzo.

- **Batteri e funghi da emocolture positive (Anthony 2000 e Marlowe 2003)**
- **Batteri patogeni intestinali, 40 microrganismi rilevati, (Wang 2004, You 2008)**
- **B. anthracis (2004)**
- **HPV (rilevazione e tipizzazione)**
- **Rilevazione ed identificazione di infezioni fungine in pazienti neutropenici (Spiess 2007)**
- **HIV 1 + HBV + HCV in donatori (khodakow 2008)**
- **Mycobacterium tuberculosis (rilevazione, tipizzazione e resistenze)**
- **Infezioni da virus respiratori**
- **MRSA (StaphPlex, MVPlex)**
- **Meningoencefaliti batteriche e virali (Boving 2009)**



(Urisman A, et al. *Genome Biology* 2005)

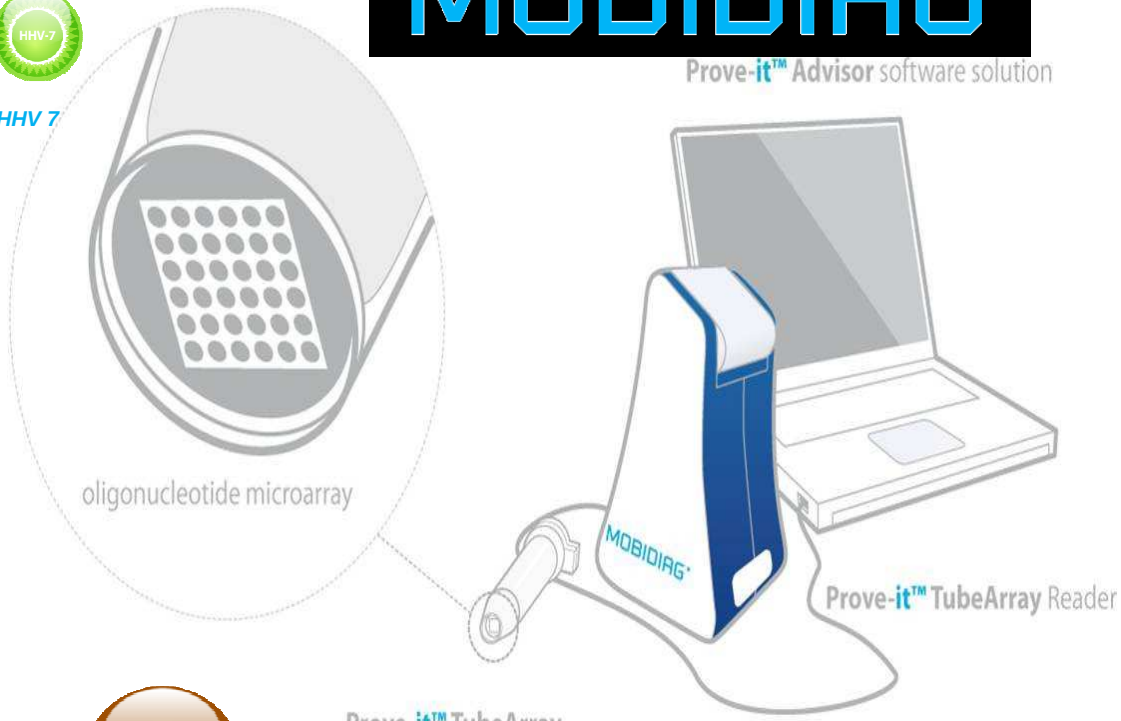
Prove-it® Herpes

Seven identified viral targets

HSV 1 *HSV 2* *VZV* *EBV* *CMV* *HHV 6* *HHV 7*

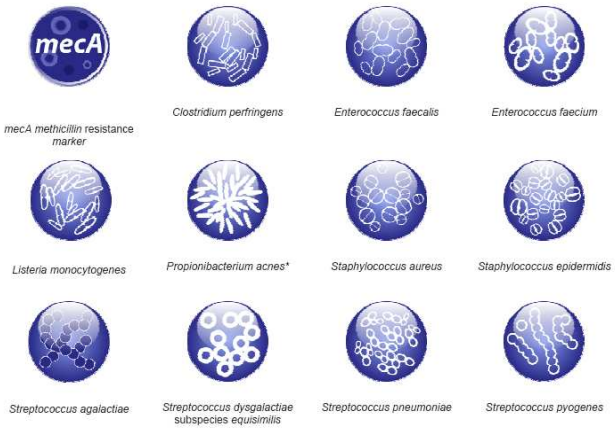
MOBIDIAG®

Prove-it™ Advisor software solution



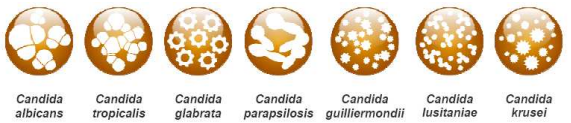
Prove-it® Sepsis

Gram +

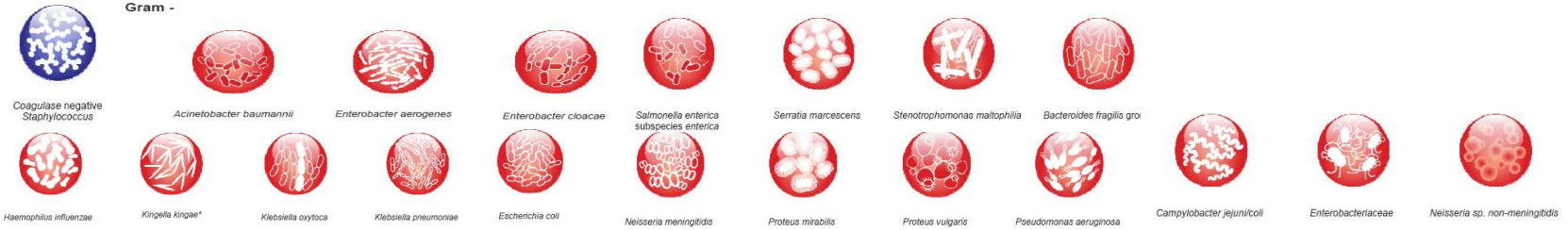


Prove-it® Fungi

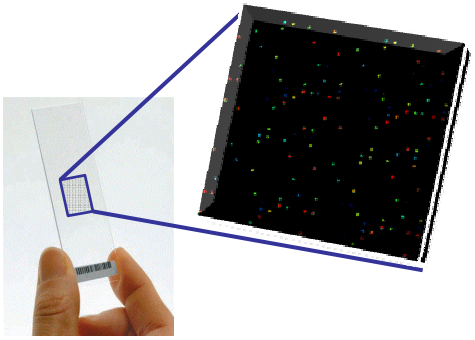
Prove-it™ TubeArray



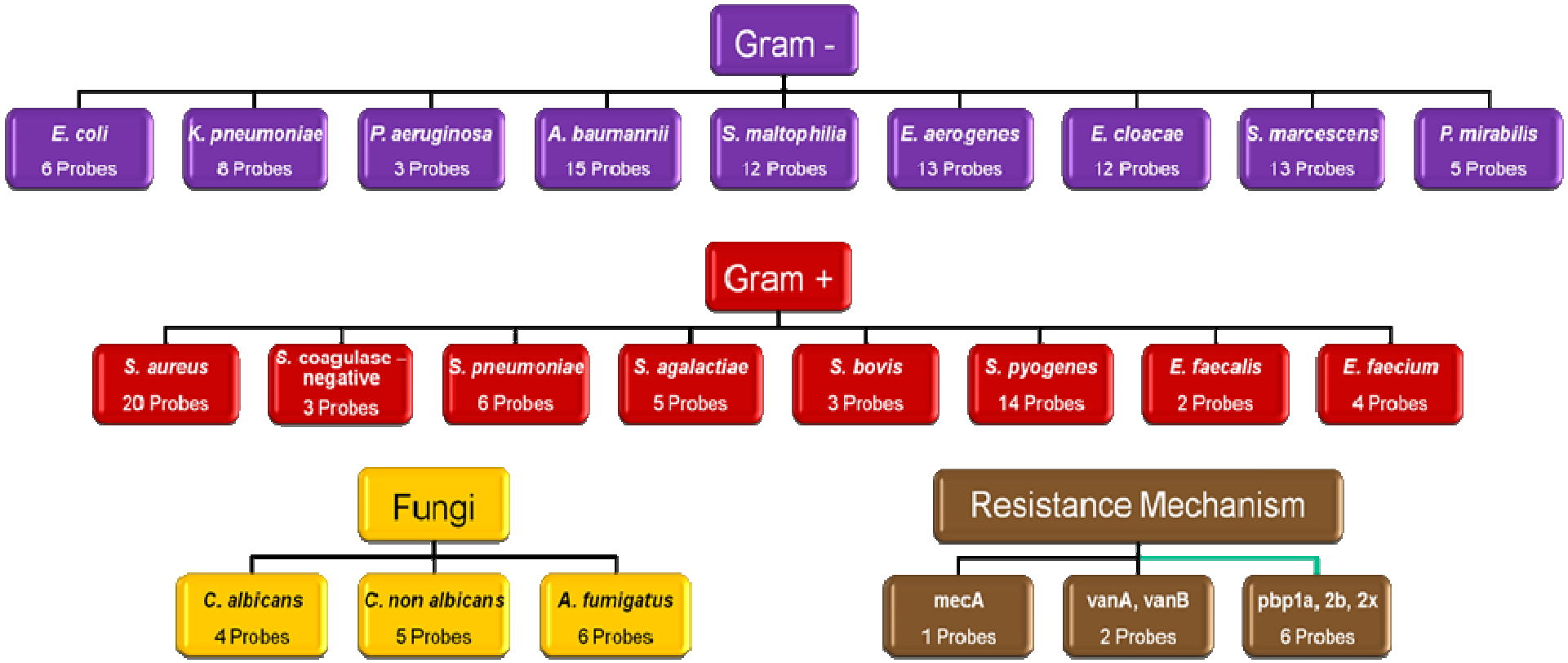
Gram -



Molecular Stamping



Sepsi-1H DNA microarray



Sepsi-1H DNA microarray

High Efficiency

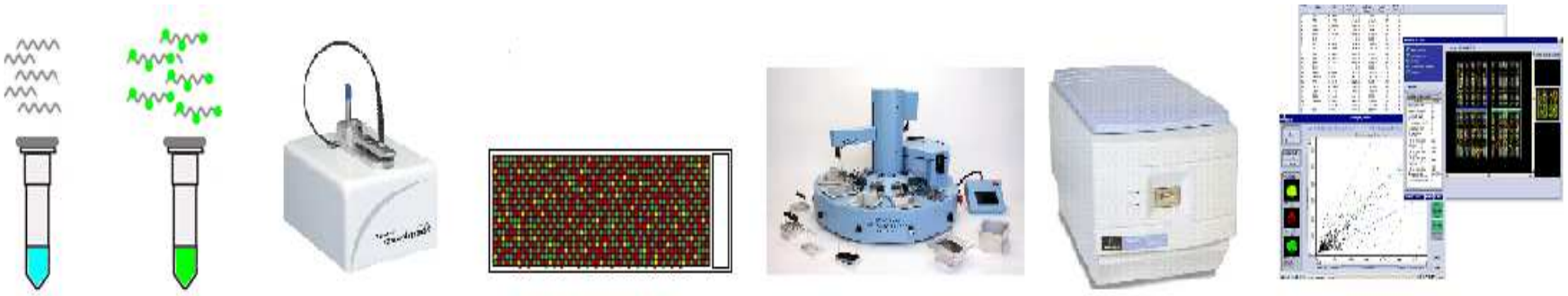
- DNA microarray for multiple patients analysis (up to 4 per run)
- NO bacterial culture required
- NO PCR amplification required
- 2µg total genomic DNA extracted from patient serum
- Detects up to 0.1% pathogen DNA

MAIN STEPS

1.gDNA fragmentation and labeling
2.Sample QC
3.Hybridization
4.Post hybridization processing
5.Scanning
6.Data acquisition

SEPSI PROTOCOL

From DNA to data. This analysis protocol comprises sample preparation, labeling, hybridization, post hybridization processing, scanning and data acquisition. First of all needs to perform gDNA fragmentation and labeling, afterwards we check reaction yield and fluorophore incorporation efficiency. Hybridization is carried out with automated hyb station or hyb chambers, depending on experimental design. After hybridization, slides are scanned with confocal laser scanner. Obtained image are analyzed and data acquisition performed using suitable software .



Fragmentation **Labeling** **Sample QC** **Hybridization** **Post hyb. processing** **Scanning** **Data acquisition**

AutoGenomics

INFINITI™

L'utilizzo di BioFilmChip Microarray, rende possibile la ricerca contemporanea di 24 virus respiratori, inclusi i sottotipi.

- **Influenza** **A e B**
- **Parainfluenza Umana (HPIV)** **1, 2, 3, 4**
- **Rinovirus** **A e B**
- **Enterovirus** **A, B, C, D**
- **Coronavirus** **HKU1, OC43, NL63, 229E**
- **Metapneumovirus Umani (HMPV)** **A e B**
- **Virus Respiratori Sinciziali Umani (HRSV)** **A e B**
- **Adenovirus** **A, B, C, E**

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TABLE 2. Comparison of commercially available, microarray-based kits for detection and identification of respiratory viruses[a]

Product	Company	Viruses and/or genotypes detected	Amplification platform(s)	Microarray platform	Characteristic(s)	Reference(s)
Infiniti RVP	AutoGenomics, Inc. (Carlsbad, CA)	Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, PIV-4, RSV-A, RSV-B, hMPV-A, hMPV-B, RhV-A, RhV-B, EnV, CoV, and Adv	Multiplex PCR and RT-PCR	Infiniti analyzer (solid chip)	The detection step by the Infiniti analyzer is completely automatic	160
MultiCode-PLx RVP	EraGen Biosciences (Madison, WI)	Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, PIV-4, RSV, hMPV, RhV, AdV, and CoV	Multiplex PCR and RT-PCR	Luminex (liquid chip)	Universal beads used in detection employ EraCode sequences	109, 143
ResPlex II assay	Qiagen (Valencia, CA)	Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, PIV-4, RSV-A, RSV-B, hMPV, RhV, EnV, and severe acute respiratory CoV	Multiplex RT-PCR (Tem-PCR)	Luminex (liquid chip)	A unique Tem-PCR allows large numbers of targets included in one reaction without significant loss of sensitivity	18, 112
NGEN respiratory virus ASR	Nanogen (San Diego, CA)	Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, and RSV	Multiplex RT-PCR	NanoChip (solid chip)	Probe labeling, target capture, and detection are accomplished using electronic microarray technology	112, 185
xTAG RVP	Luminex Molecular Diagnostics (Toronto, Ontario, Canada)	Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, PIV-4, RSV-A, RSV-B, hMPV, AdV, EnV, CoV, and RhV	Multiplex PCR and RT-PCR	Luminex (liquid chip)	TSPE is used in combination with universal detection beads	126, 132, 148

[a] Abbreviations: Tem, target-enriched multiplex; Flu, influenza virus; PIV, parainfluenza virus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; RhV, rhinoviruses; EnV, enteroviruses; CoV, coronavirus; RT, reverse transcription.

Applicazioni in Microbiologia Clinica

2. Rilevamento geni di resistenza ai farmaci antimicrobici

- M. tuberculosis: resistenza a rifampicina, isoniazide, streptomicina, etambutolo (QIAplex: 24 mutazioni geniche), kanamicina e pirazinamide
- M. tuberculosis: resistenza a fluorchinoloni (Antonova 2008)
- HIV 1
- H. pylori: resistenza a metronidazolo (Albert 2005)
- TEM beta-lattamasi (Grimm 2004)
- E.coli resistenza ai fluorchinoloni (Yu 2004)
- Resistenze nei G+ (90 geni di resistenza: Perreten 2005)
- Vibrio spp. (Vora 2005)
- Staphylococcus spp. (Zhu 2007)

Congresso NEWMICRO
Biotecnologie e diagnostica molecolare nella diagnosi delle malattie infettive
Poster Presented At The Clinical Virology Symposium May 2006

T-PM57: Identification of *Mycobacterium tuberculosis* and genotyping of *rpo*B and *kat*G genes for drug resistance using the automatic microarray INFINITI™ Analyzer

W. Wu[1], P. Kim[1], V. Mahant[1], N. Dattagupta[1], G. Washabaugh[2], C. R. Peter[2]
[1]AutoGenomics Incorporated, Carlsbad, CA; [2]San Diego County Public Health Laboratory, San Diego, CA.

The INFINITI analyzer is a self-contained molecular diagnostics platform which automates all steps required to detect PCR amplified products, including fluidics, hybridization, thermal cycling and optical detection. The INFINITI analyzer can detect single nucleotide mutations in *rpo*B gene and *kat*G gene of *M. tuberculosis*. The MTBDR Rif/INH RUO™ assay provides MTB identification plus drug resistant genotyping with the INFINITI analyzer. Amplified PCR products are detected following a single multiplexed PCR amplification. Amplicon is detected using detection probes which contain primers that are extended with polymerase which incorporates fluorescent labels into the complimentary strand. Following primer extension, hybridization to the microarray is achieved via capture oligonucleotides previously spotted onto the BioFilmChip™ microarray.
758 Pulmonary TB patients were evaluated with cultures, drug susceptibility tests, and questionnaires. Drug resistance was found in 41% of the BC *Mycobacterium tuberculosis* complex isolates and 20% of the SDC isolates.
The MTBDR Rif/INH assay on the INFINITI analyzer using MTB crude cell lysates, successfully demonstrated MTB identification with simultaneous detection of point-mutations in *rpo*B and *kat*G genes associated with drug resistance. On a single microarray chip, we reported rifampin sensitive genotypes from five codons (511, 516, 526, 531, 533) of *rpo*B gene, and rifampin resistant genotypes (L511P, D516V, H526Y, L531L, L533P); the isoniazid sensitive genotype from *kat*G gene codon 315, and isoniazid resistant genotype S315T. When the MTBDR assay was applied to fresh decontaminated sputum samples, all smear positive sputum samples received satisfactory results. The ability to rapidly provide MTB drug resistance information directly from sputum samples without the need for culture, will assist physicians in providing appropriate treatment options to MTB patients.

MTB ID 100%

RP - R 88,9%

INI - R 90%

Applicazioni in Microbiologia Clinica

3. Microbial typing

La capacità dei microarray di rilevare simultaneamente una grande varietà di genomi consente l'uso di questa tecnologia per la tipizzazione microbica e l'investigazione epidemiologica, eventualmente in associazione con altre biotecnologie.

Questa capacità è stata utilizzata anche in sostituzione di tecniche tradizionali:

- **DNA serotyping patogeni enterici (Li 2006)**
- **Fast DNA serotyping *E.coli* (Ag O e H) (Barlann 2005)**
- **Genotipizzazioni virali (HPV, Norovirus, Astrovirus, Rotavirus, etc.)**

AutoGenomics

INFINITI™

1. **Separare HPV16 e HPV18, i due tipi di HPV con maggior rischio oncogenico, dagli altri tipi di HPV oncogenici, potrebbe aiutare ad identificare, fra le donne HPV positive, quelle più probabilmente destinate a progredire verso ≥ CIN3, potrebbe quindi essere più importante dell'ASC o anche della citologia LSIL per predire un futuro CIN3 e carcinoma**
2. **La strategia di screening può aiutare a ridurre il numero delle donne che vengono inviate alla colposcopia a causa di un test HPV positivo.**
3. **I tipi HPV 16, 18, 59 e 33 sono quelli associati a più alto rischio per adenocarcinoma.**
4. **Circa 8% delle pazienti HPV positive hanno infezioni multiple, infezioni multiple HPV aumentano il rischio di carcinoma cervicale a cellule squamose.**
5. **Il rischio di cancro alla cervice nelle portatrici di HPV tipo 16 più un altro tipo di HPV ad alto rischio è 617.4 (odds ratio), rispetto al rischio 4.3 delle portatrici di HPV tipo 6.**
6. **Il rischio più elevato per adenocarcinoma cervicale è legato ai tipi 18,16, 59 e 33.**

AutoGenomics

INFINITI™

1) Ricerca contemporanea di HPV umani per 4 diversi pazienti su un unico BioFilmChip (HPV Quad)

Alto Rischio	16,18, 31, 33, 45, 35/68, 39/56, 58/52, 59/51
Basso rischio	6/11

2) Ricerca contemporanea di 26 tipi di HPV (HPV genotipo)

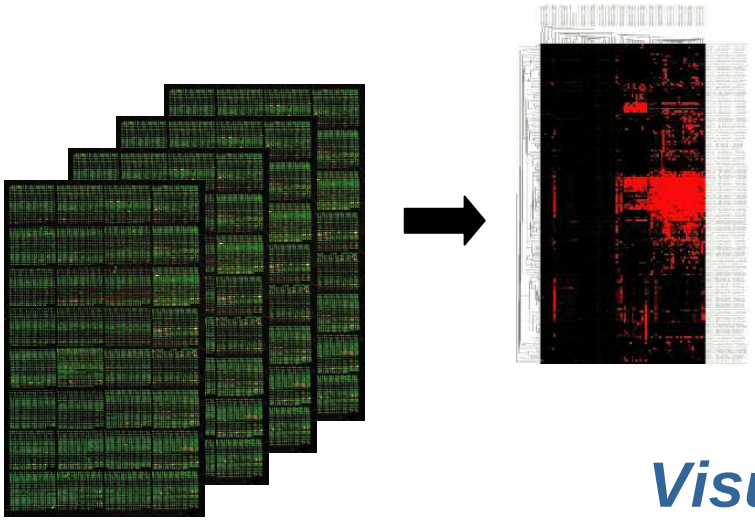
Alto Rischio	16,18,31,33,34,35,39,45,53,58,59,66,68
Rischio Alto/Intermedio	26,51,52,56,67,69,73,82
Basso Rischio	6,11,30,70,85

Monkey Outbreak

- Outbreak of fulminant pneumonia and hepatitis in an indoor pen of 55 titi monkeys (*Callicebus cupreus*)
- New World monkeys from South America, commonly used in social behavior studies
- High mortality rate (83%)
- Conventional diagnostic testing for pathogens negative

Image of California National Primate Research Center

Identification of Viral Signatures

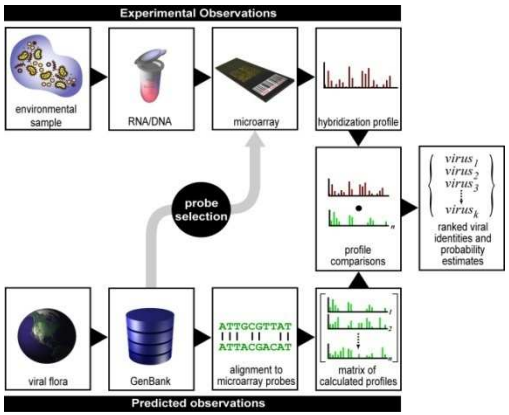


Hierarchical cluster analysis

viral signature detection and diversity

Visual inspection / Z-score analysis

“quick-and-dirty” analysis



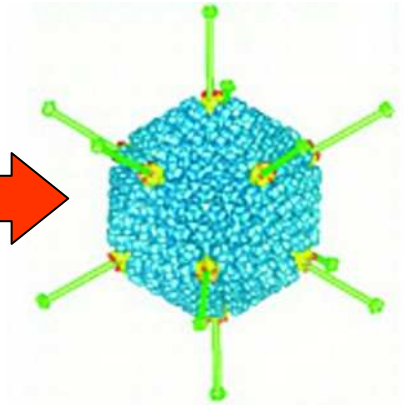
E-predict

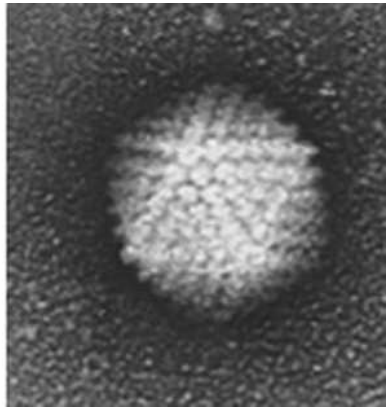
viral signature similarity to known viruses

Adenovirus

- **Linear, dsDNA genome, large, ~33 kB**
- **Known to cause a variety of clinical syndromes, including gastroenteritis, respiratory disease, conjunctivitis,**
- **51 serotypes, split into 6 species hAd A-F**
- **Simian and human adenoviruses very similar by sequence but are thought to be species-specific**

Deep Sequencing (Metagenomic approach)





Novel monkey Adenovirus

Chiu and Stewart, 2003

Applicazioni in Microbiologia Clinica

4. Studio dei profili di espressione genica dei microrganismi
5. Studio dei profili di espressione genica dell'ospite in corso di infezione
6. Determinazione dei polimorfismi genomici dell'ospite

- Finora riservati quasi esclusivamente all'uso nella ricerca
  - M. tuberculosis, Salmonella, Shigella (4)
  - Sepsi (5)
  - SNPs (B19v, HCV) (6)

CLINICAL MICROBIOLOGY REVIEWS, Oct. 2009, p. 611–633 Vol. 22, No. 4
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Basic Concepts of Microarrays and Potential Applications in Clinical Microbiology

Melissa B. Miller[1]* and Yi-Wei Tang[2]
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TABLE 3. Selected sepsis studies using microarray-based host gene expression

Chip used	Subject(s)	Main findings and conclusions
Atlas array, Clontech Laboratories (Mountain View, CA)	Mouse	Microarray technology provides a powerful new tool for rapidly analyzing tissue-specific changes in gene expression induced by sepsis
Hu95aVer2 GeneChip, Affymetrix (Santa Clara, CA)	Adult patients	The host inflammatory responses to gram-negative and gram-positive stimuli share some common response elements but also exhibit distinct patterns of cytokine appearance and leukocyte gene expression
Image consortium libraries, Livermore National Laboratory (Livermore, CA)[a]	Mouse	Both gram-positive sepsis and gram-negative sepsis share a final common pathway involved in the pathogenesis of sepsis, but certain genes are differentially expressed under distinct regulation
Arraytor human 500-1 cDNA, SIRS-Laboratory (Jena, Germany)	Adult patients	Microarrays can identify typical gene expression profiles for blood samples from patients with severe sepsis
Hu 133A and 133B GeneChip, Affymetrix	Healthy adult blood leukocytes receiving bacterial endotoxin stimulus	Human blood leukocyte response to acute systemic inflammation includes the transient dysregulation of leukocyte bioenergetics and modulation of translational machinery; these findings provide insight into the regulation of global leukocyte activities as they relate to innate immune system tolerance and increased susceptibility to infection in humans
MGU74Av2 GeneChip, Affymetrix	Mouse	A(2A)R blockade may be useful for treatment of infection and sepsis
HG-U133A GeneChip, Affymetrix	Adult patients	Blood transcriptional profiling is a valuable approach not only for patient stratification but also to identity new genes possibly involved in sepsis pathophysiology
Mouse 430 2.0 GeneChip, Affymetrix	Mouse	T-cell receptor signaling and mitogen-activated protein kinase signaling were significantly altered by sepsis
U74Av2 GeneChip, Affymetrix	Mouse	Sepsis induces common inflammatory response gene changes in mouse leukocyte gene expression that can be used to diagnose sepsis
Adelaide Microarray, Compugen, San Jose, CA)	Adult patients	The signature genes reflect suppression of neutrophils' immune and inflammatory function by sepsis; gene expression profiling therefore provides a novel approach to advance our understanding of the host response to sepsis
430A GeneChip, Affymetrix	Mouse	Sepsis induces alterations in balance of pro- and antiapoptotic transcriptional networks, and bcl-2 overexpression improves survival in sepsis
U133 Plus 2.0 GeneChip, Affymetrix	Pediatric patients	Genome-level alterations of zinc homeostasis may be prevalent in clinical pediatric septic shock
U133 Plus 2.0 GeneChip, Affymetrix	Adult patients	Sepsis has a unique gene expression profile that is different from that for uninfected inflammation and becomes apparent prior to expression of the clinical sepsis phenotype
U133 Plus 2.0 GeneChip, Affymetrix	Adult patients	Toll-like receptors and downstream signaling genes are differentially expressed in critically ill patients developing sepsis compared with those with sterile inflammation; these expression differences occur before phenotype-based diagnosis of clinical sepsis
U133 Plus 2.0 GeneChip, Affymetrix	Adult patients	There was evidence of sepsis-related immunosuppression and reduced inflammatory response in mononuclear cells on a transcriptome level; these characteristic transcriptional changes can be used to aid the diagnosis of sepsis

Retrovirology

BioMed Central

Research

Open Access

Microarray study reveals that HIV-1 induces rapid type-I interferon-dependent p53 mRNA up-regulation in human primary CD4+ T cells

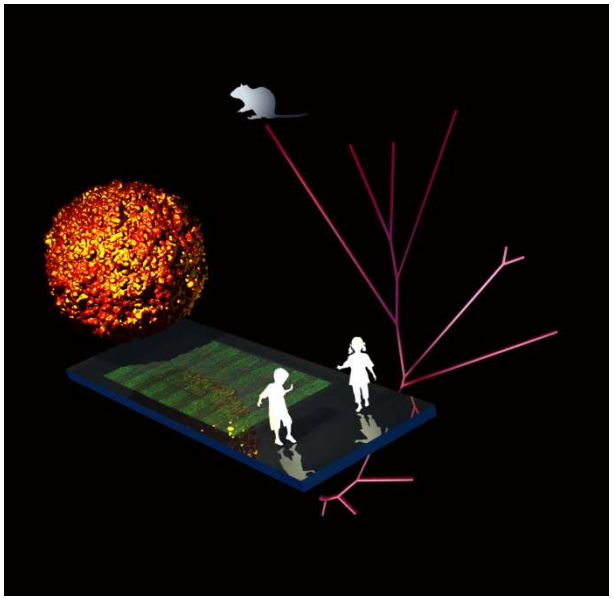
Michaël Imbeault, Michel Ouellet and Michel J Tremblay*

In conclusion, we confirm that microarrays represent a useful tool for elucidating the molecular details of the complex interaction between HIV-1, its target cells and uninfected/bystander cells. We demonstrate that even small scale gene expression profiling can lead to a better comprehension of host-defence strategies, which is essential for the design of a new generation of therapeutic agents.

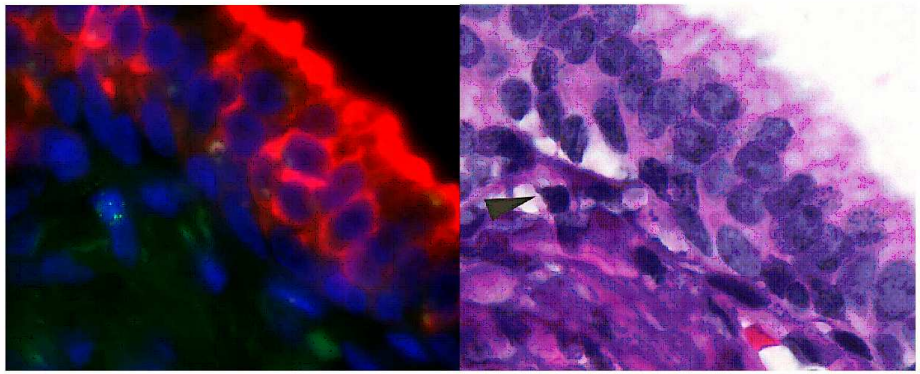
Microarray (Virochip) for Pathogen Discovery

ABV, the likely etiologic agent of PDD (Kistler, et al., 2008, *Virol J*)

HTCV, a novel human cardiovirus in children with respiratory / gastrointestinal infection (Chiu, et al, 2008, *PNAS*)



XMRV, a gammaretrovirus associated a type of hereditary prostate cancer (Urisman, et al, 2006, *PloS Pathogens*)



A Metagenomics Investigation of the 2009 Influenza A H1N1 Pandemic (n=17)

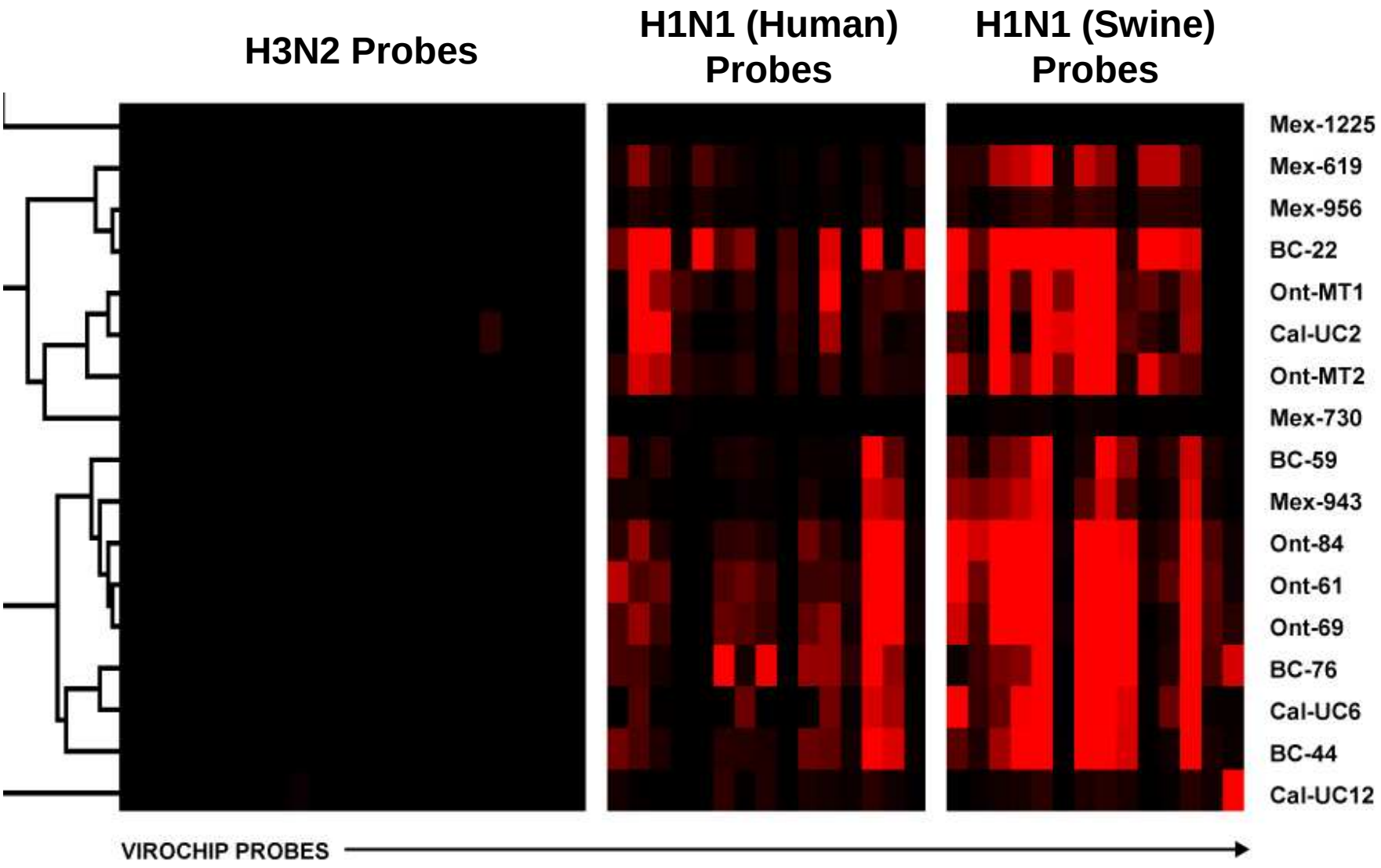
University of Toronto / Ontario Agency for Health Protection and Promotion
Toronto, Canada
5 outpatients
post-DNased

British Columbia CDC
Vancouver, Canada
4 outpatients
post-DNased

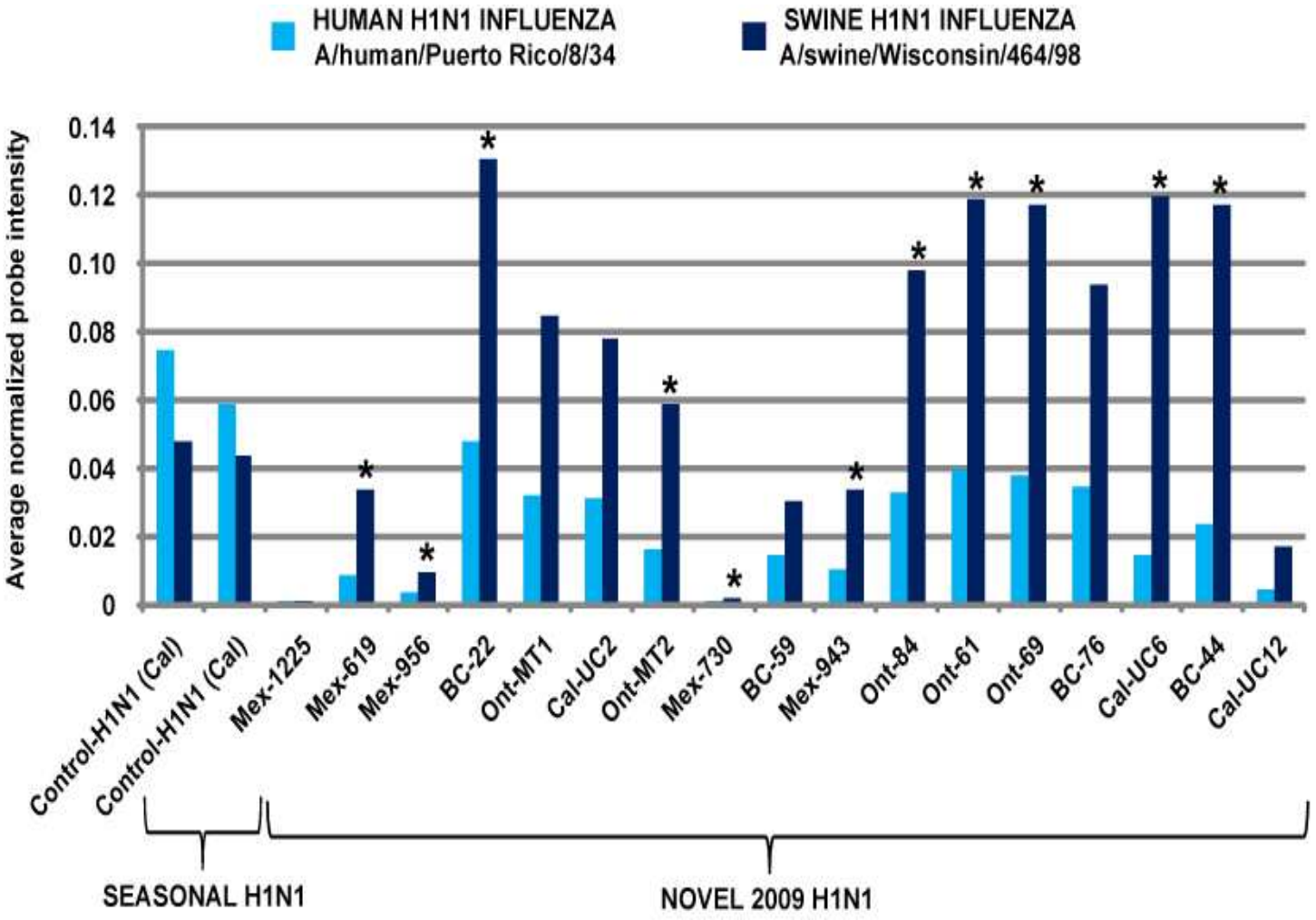
University of California, San Francisco
3 hospitalized patients
pre- / post-DNased

Veracruz State Ministry of Health
Veracruz, Mexico
5 outpatients / hospitalized patients
pre-DNased

Virochip → A/swine/Wisconsin/464/98(H1N1)

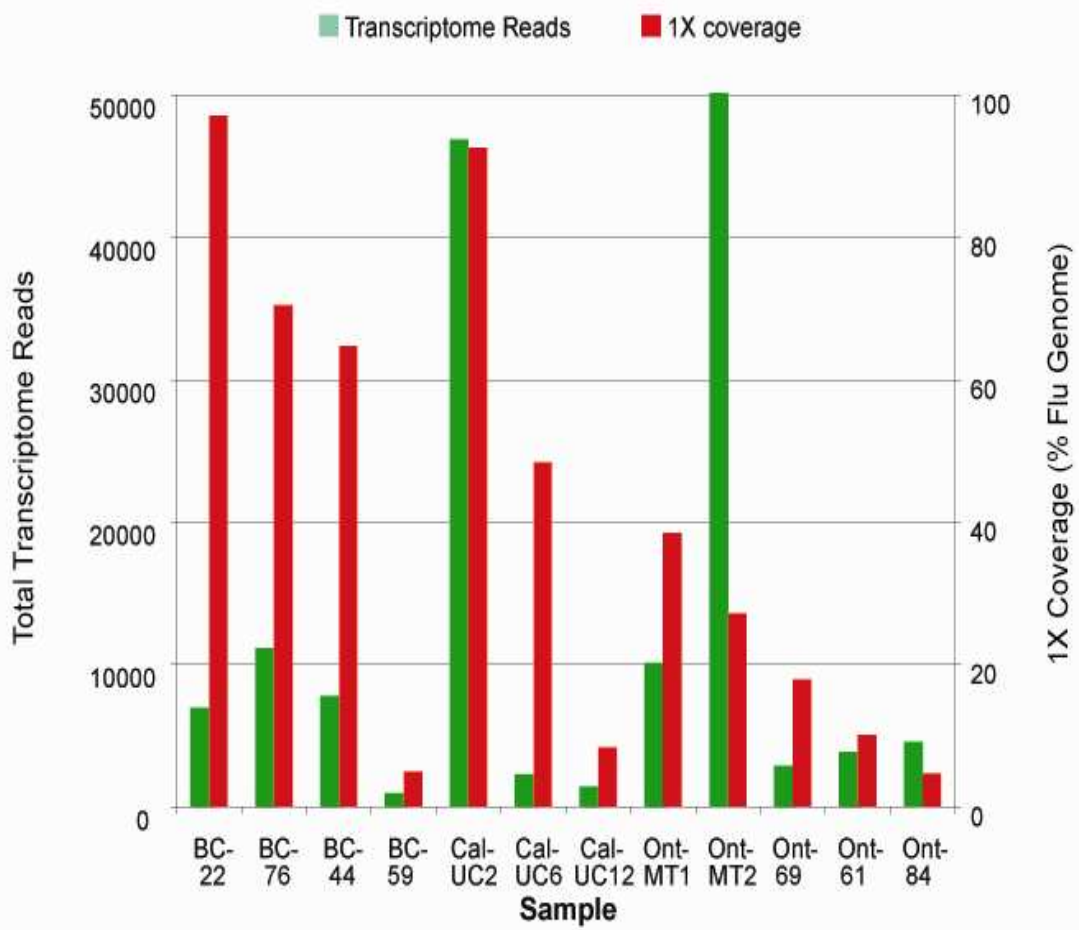


2009 H1N1 is More Similar to Swine than Human Influenza H1N1 by Virochip



Host Gene Expression in Response to H1N1 Infection

Sample	PANTHER Category
BC-22	Interferon-mediated immunity (n=14)
	Immunity and defense (n=45)
	Cell structure and motility (n=37)
	Intracellular protein traffic (n=28)
BC-76	Interferon-mediated immunity (n=10)
	Inhibition of apoptosis (n=9)
	Immunity and defense (n=29)
	Nucleic acid metabolism (n=53)
BC-44	Protein metabolism (n=43)
	Immunity and defense (n=21)
Cal-UC2	Interferon-mediated immunity (n=11)
	Immunity and defense (n=42)
	Cell structure and motility (n=36)
	Protein metabolism and modification (
	Apoptosis (n=18)
Ont-MT1	Endocytosis (n=5)
Ont-MT2	Cell structure and motility (n=23)
	Intracellular protein traffic (n=20)



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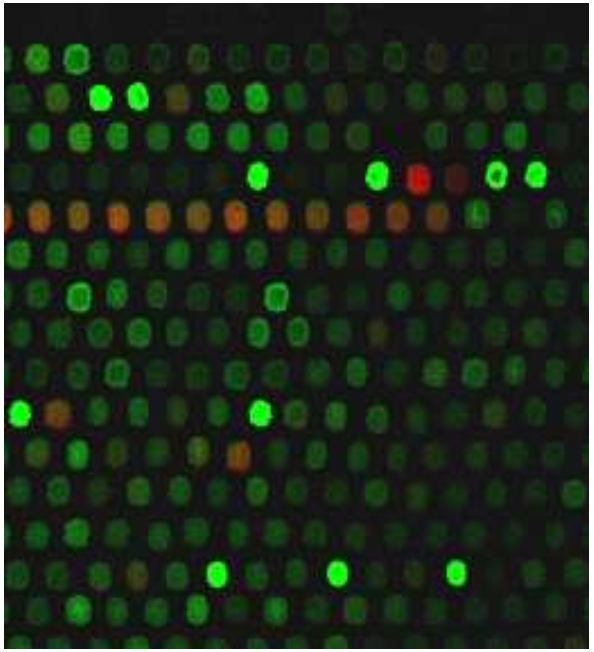

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