genewave
FAST MOLECULAR DIAGNOSTICS
FOR TAILORED ANTIBIOTHERAPIES

Presentation d'une PME

Genewave: multiplex, point-of-care diagnostics

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Kickoff meeting NanoSaclay
28 novembre 2011

Genewave geneSpress platform

- Molecular diagnostics based on nucleic acid testing
- Lab-on-chip disposable cartridges
  - On-chip purification, amplificatior hybridization, detection
- High multiplex capability (20-50+)
- < 3-hour turnaround time

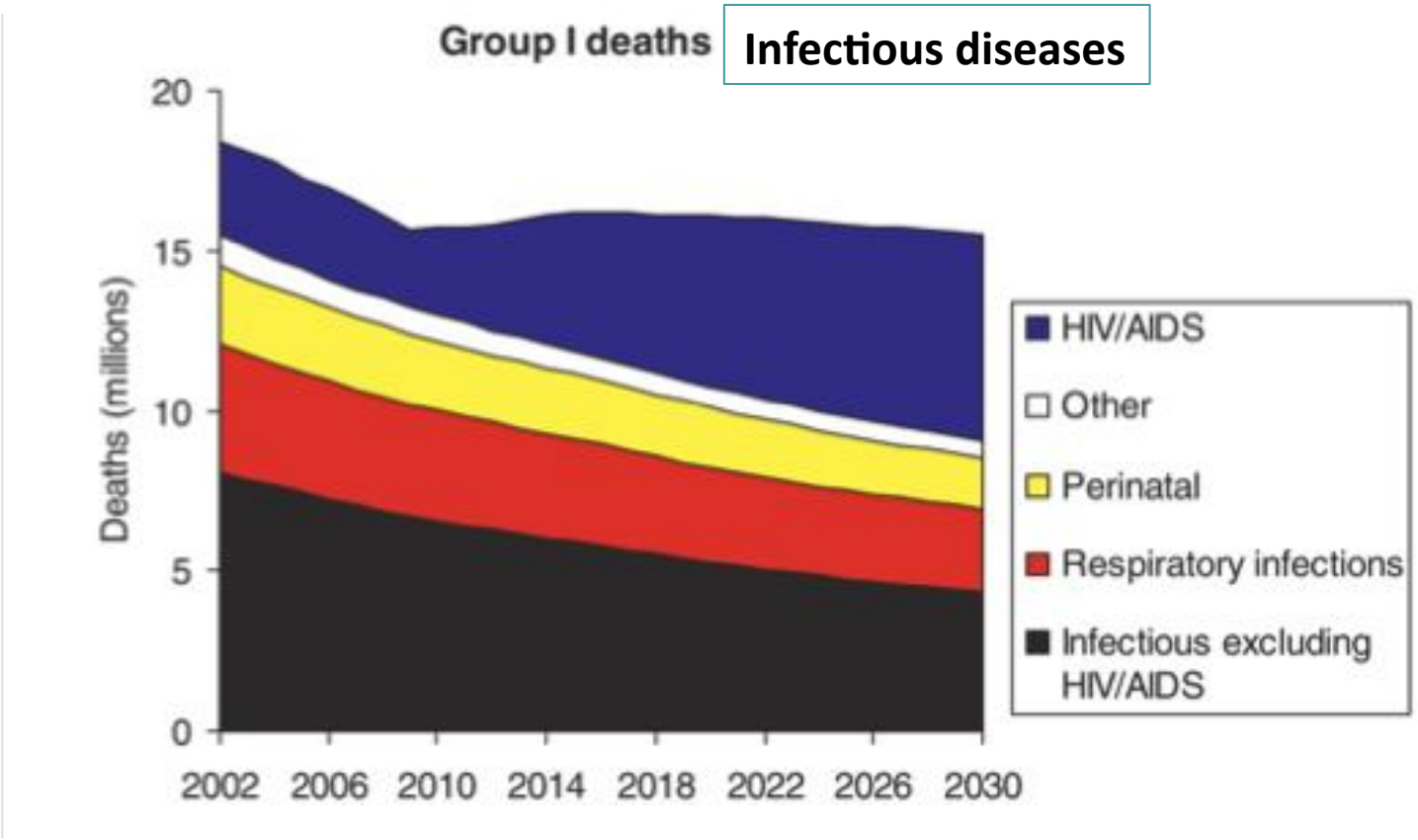
Importance of infectious desease diagnostics

Projections of Global Mortality of Disease from 2002 to 2030

PLoS Medicine | www.plosmedicine.org

Colin D. Mathers*, Dejan Loncar

Evidence and Information for Policy Cluster, World Health Organization, Geneva, Switzerland



Long term decrease due to better diagnostics, better and wider treatments

Maladies infectieuses : des besoins non satisfaits

- Un diagnostic précis nécessite de cribler un large panel de pathogènes potentiels; les symptomes à plusieurs types d'infections, et il faut identifier le bon traitement

(i.e. donner l'antibiotique specifique au lieu de donner a l'aveugle un cocktail d' antibiotiques ce qui contribue à developper de nouvelles souches resistantes.

- Les technologies actuelles fournissent :
  - Un crible large avec des tests longs:
    - *e.g.,* ~15-plex pour les virus respiratoires
  - Des tests rapides à couverture réduite:
    - *e.g.,* ~2-plex pour MRSA/SA

- Besoin: des tests diagnostics rapides, très sensibles, couvrant un large panel (> 6 marqueurs)

Tests multiplexes rapides

L'identification des gènes des bactéries ou virus indique la souche infectieuse, c.a.d. la maladie, et permet d'identifier la meilleure médication

Donne aussi des indications sur la résistance possible de la souche et permet ainsi de prescrire l'antibiotique spécifique pour la souche.

- Tests respiratoires (nécessitent 10-40+-plex)
  - Virus respiratoires
  - Pneumonies associées à la ventilation mécanique (PAVM)
  - Pneumonies communautaires
- Résistance aux antibiotiques(30-40)
- Septicémie (40+)

Genewave

- Société privée focalisée sur le développement et la commercialisation de solutions technologiques innovantes pour le diagnostic moléculaire basé sur la reconnaissance de séquences d'acides nucléiques caractéristiques des infections
- Fondateurs:

  Claude Weisbuch
  - Directeur de Recherches, Ecole Polytechnique
  - Professeur, University of California, Santa Barbara

  Henri Benisty
  - Professeur, Institut d'Optique IOGS
- 22 employés
- Située à Paris et a Evry

Genewave at a glance (business)

Business Model

Development of an Automated, Fast Multiplex Molecular Diagnostics system: GeneSpress™ and associated kits that will be:

- Sold to Hospital Laboratories
- 100% In-House Manufactured (Cost Control)
- Promoted by Direct Sales and Distributors

Structure

Two business sites:

- Paris: R&D, Admin, R&D&M (750 m^2)
- Evry: Manufacturing (350 m^2)

- Workforce: 22 people, mostly engineers and PhDs in various disciplines (Molecular Biology, Physics, Chemistry, Engineering)
- Several prestigious partners: Pasteur Institute, Paris Hospitals, Ecole Polytechnique, CNRS, CEA, Génopôle, Curie Institute, University of Bonn, GE-Whatman, DKFZ, University of Lausanne, University of Geneva, …

Milestones and pipeline

- Q2 2011: GeneSpress® Proof of Concept.
- Q3 2012: GeneVAP™ Market Authorization
- Q1 2014: GeneScreen™ Market Authorization

Panorama Genewave

- Ingénieurs stricto sensu : 7
  - généralistes, chimie, optique, mécanique, électronique
- Universitaires assimilés ingé (bio): 2 (PhDs+postdocs)
- Techs bio : 4
- PhD : 9 (physique, chimie, bio, informatique)
- Expérience internationale : 8 (6 PhD + 2 ingé)
- Boite à dominante R&D (phase start up) → beaucoup de PhD aux postes à responsabilité (4 managers sur 7, 3 chefs de projet sur 4)

Entreprise encore plus pluridisciplinaire que d'habitude (tout projet industriel l'est) par le sujet mélangeant ingéniérie, physique, chimie, biologie, médical, …

Un défi majeur: définir un langage commun pour travailler et progresser ensemble.

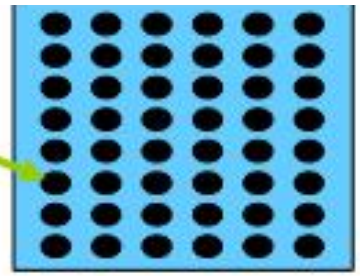
Principe des biopuces

Analyse rapide et à haut débit de biomolécules (parallèlisme)

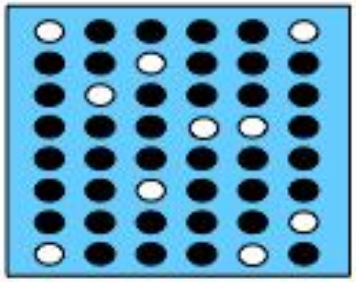
Plusieurs domaines d'applications:

- Génétique
- Pharmaceutique
- alerte bioterrorisme

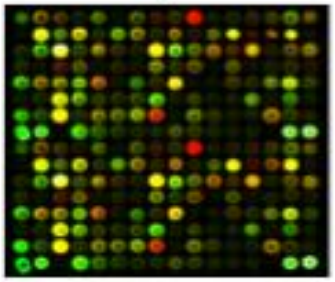
Ensemble de sondes connues liées à un substrat
(ADN, peptides, anticorps)



Interaction avec les espèces biologiques cibles inconnues dans un échantillon



Visualisation, et identification de milliers de cibles



Puces à ADN

Sondes
Séquence complementaire de celle de la cible visée

cibles

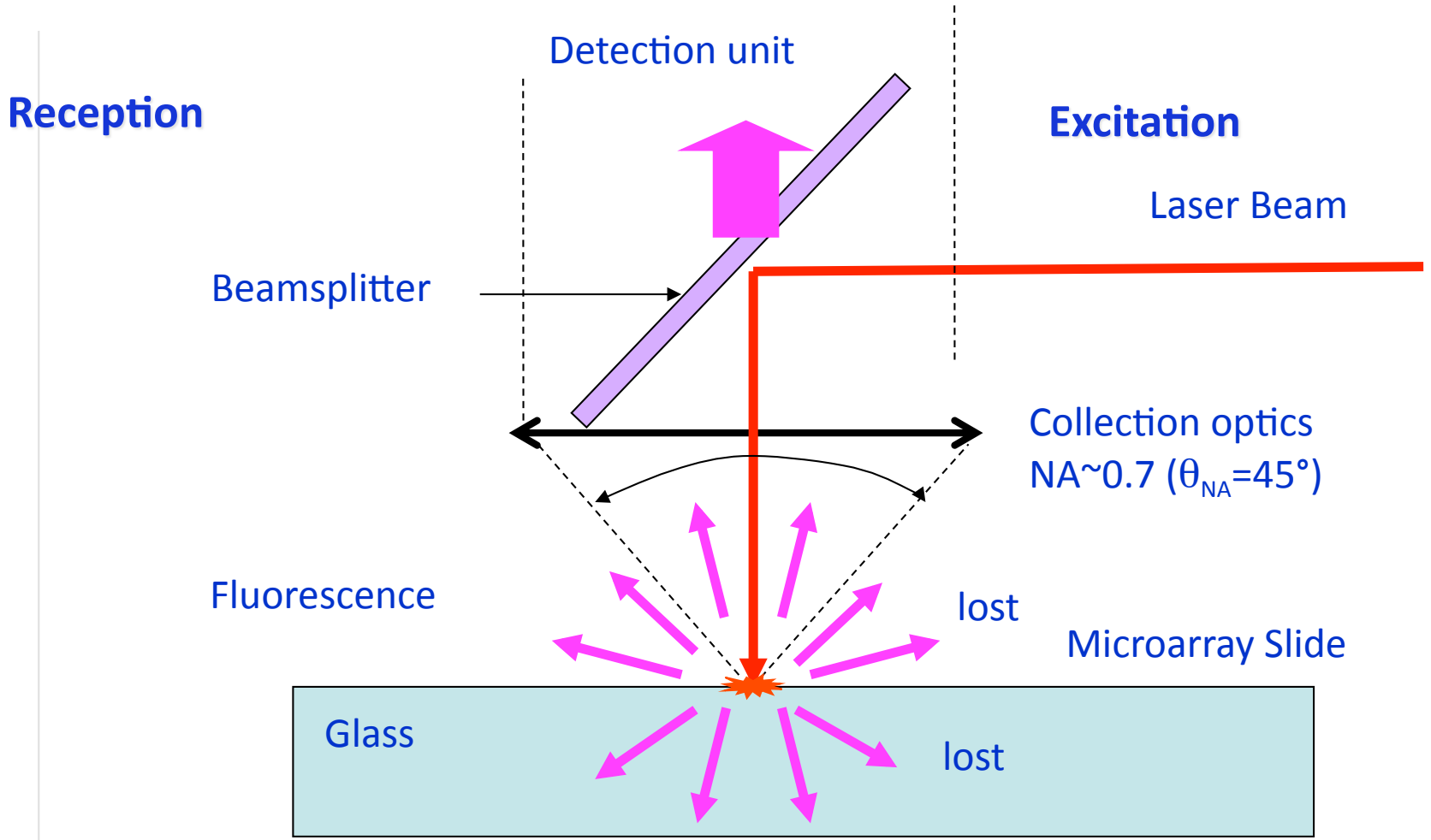
Couche de fonctionnalisation

substrat

immobilisation

From Larbi Touahir

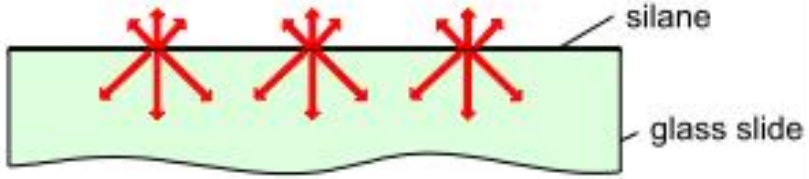
Origine de l'idée sous-jacente de Genewave: What happens in a standard confocal scanner? (i.e. Genepix)



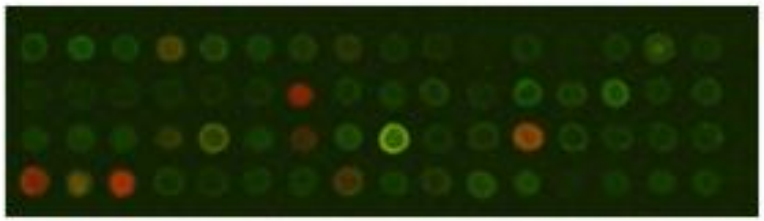
L'essentiel de la lumière est capturée dans le substrat. Même avec une grande ouverture, seule 10-20% de la lumière est récupérée. L'intensité incidente est aussi diminuée (a 64%) juste sur la surface par interférence destructive ave le faisceau excitateur réfléchi

Resultat: AmpliSlide™ Technology

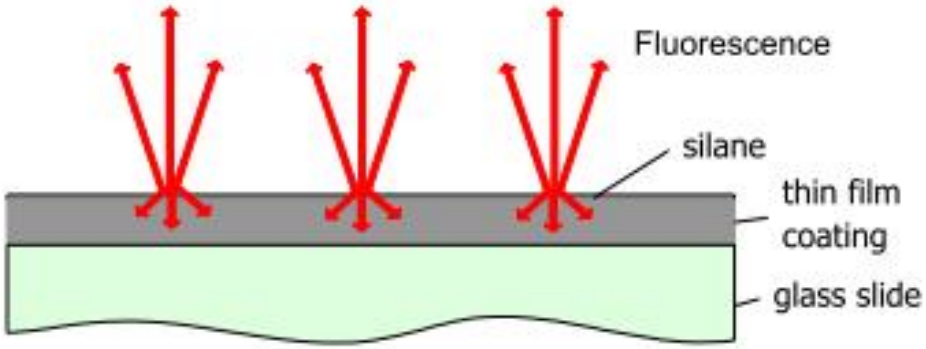
Glass Substrate



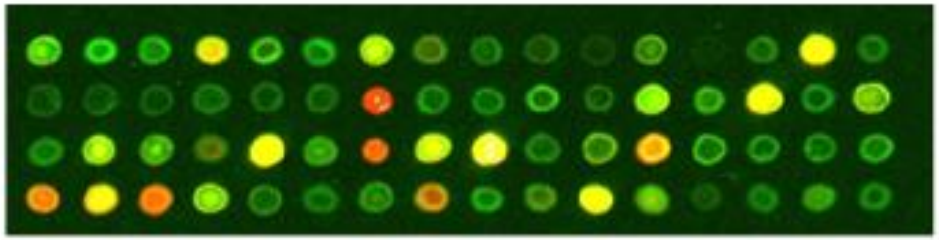
A large part or the fluorescence escapes in the glass substrate



AmpliSlide™



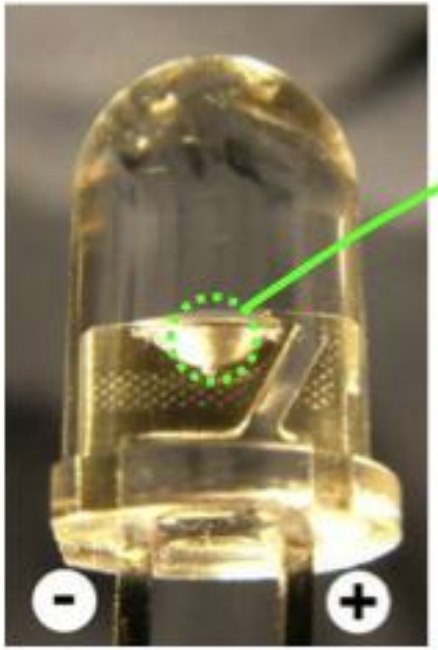
Thanks to the thin film coating, fluorescence is amplified to enhance sensitivity. There is less background fluorescence from the glass substrate.

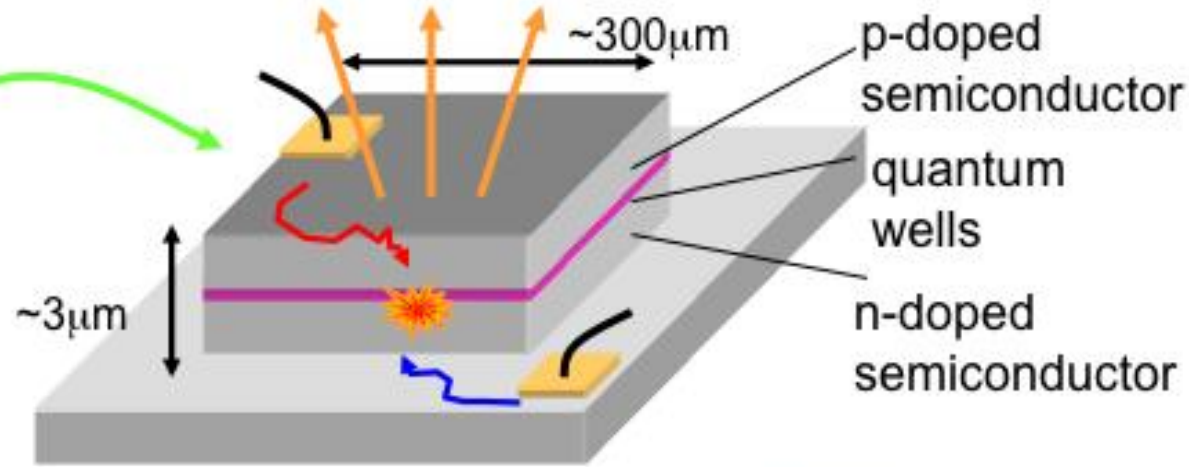


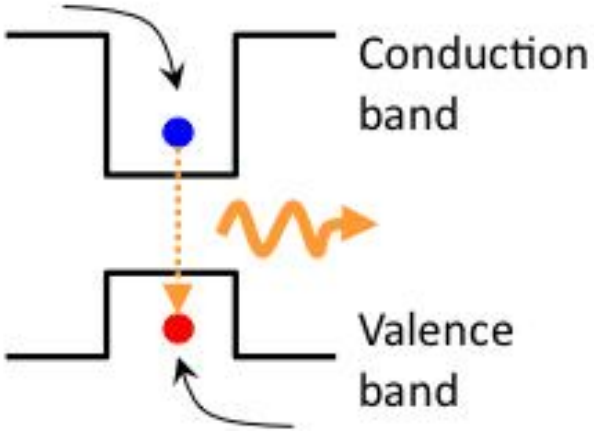
Challenges: - uniformity/reproducibility physical properties
- uniformity/reproducibility chemical properties (competition between Signal and background)

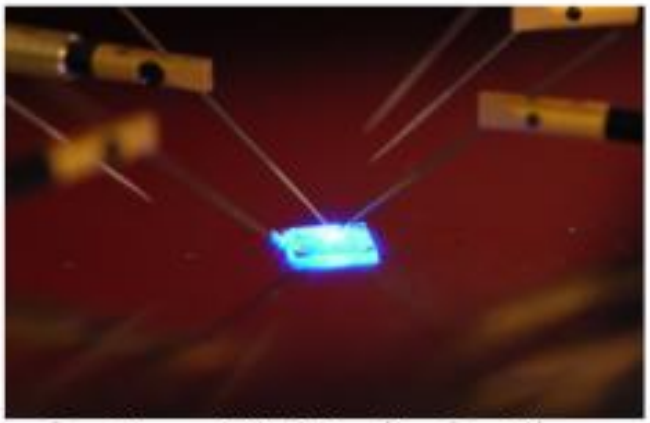
D'ou sont venues nos idées? De nos travaux sur l'extraction de lumière dans les diodes émettrices de lumière

101. Light Emitting Diodes



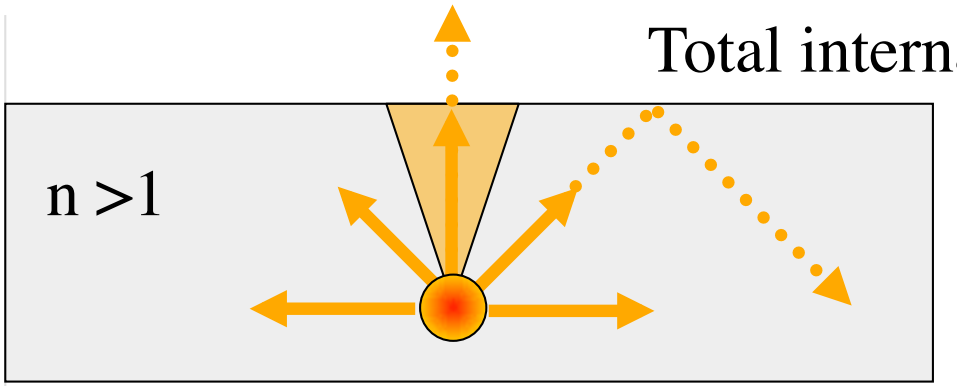






Gallium Nitride (InGaN) LED

Our background: Microcavity and photonic crystal LEDs: The basic idea
Usually light remains in the LED due to total internal reflexion

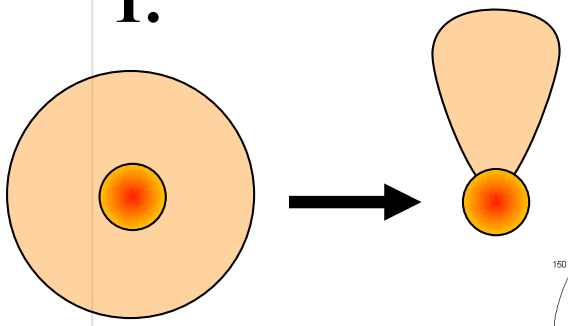


Total internal reflexion

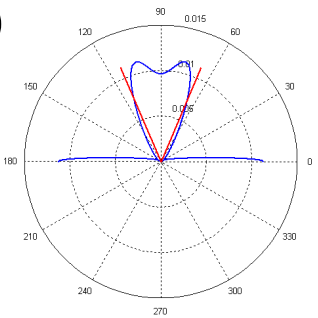
~ 6 % of light extracted
~ 94 % trapped in high-index material

- Geometrical solutions…such as mirrors
- Use the wave nature of light

1.

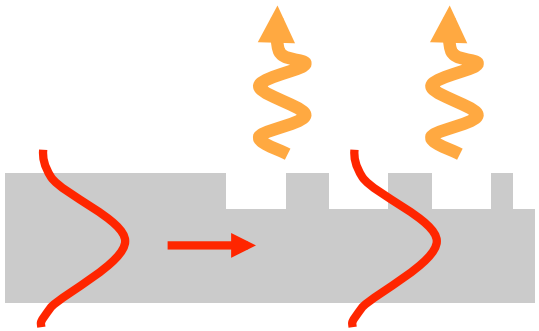


Modification of emission pattern:
MCLEDS



Maximum 40%

2.

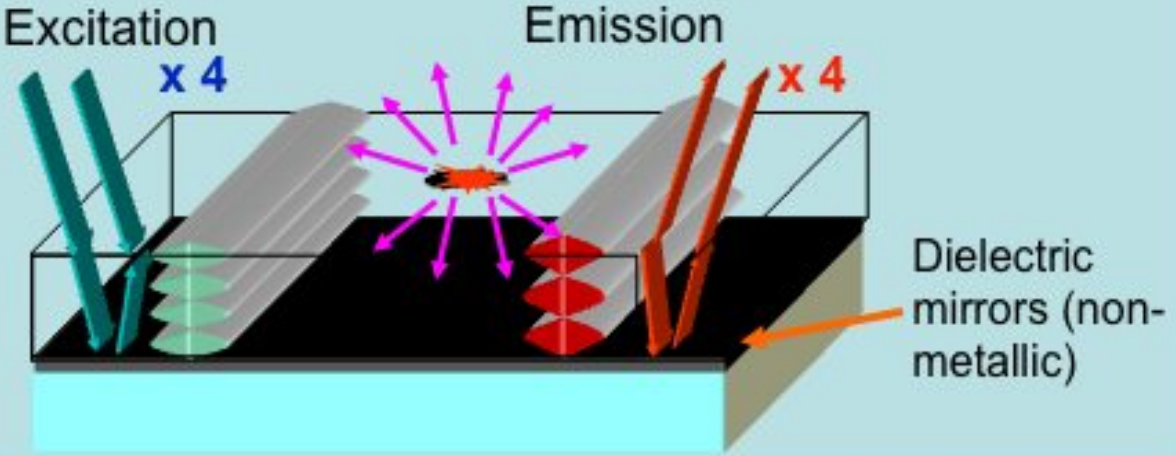


Diffraction of trapped light:
Photonic Crystals

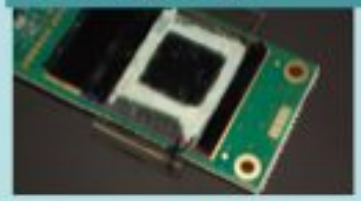
Aim at 80%+ efficiency

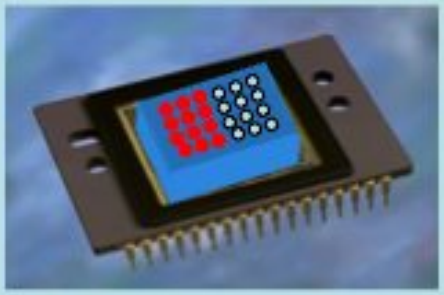
Our solutions: Improving fluorescence efficiency

!.amplifying slides Interferences

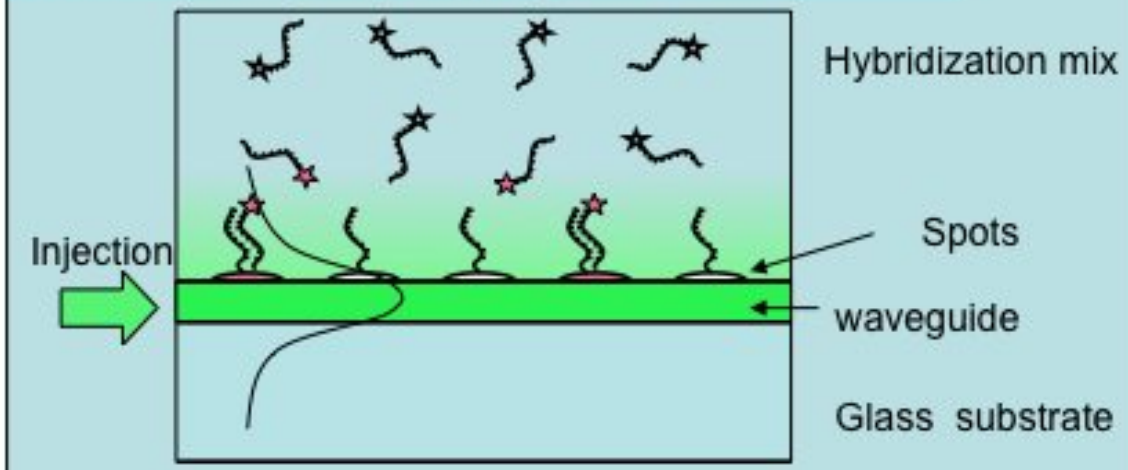


2 Direct imaging





3.evanescent wave excitation and direct imaging

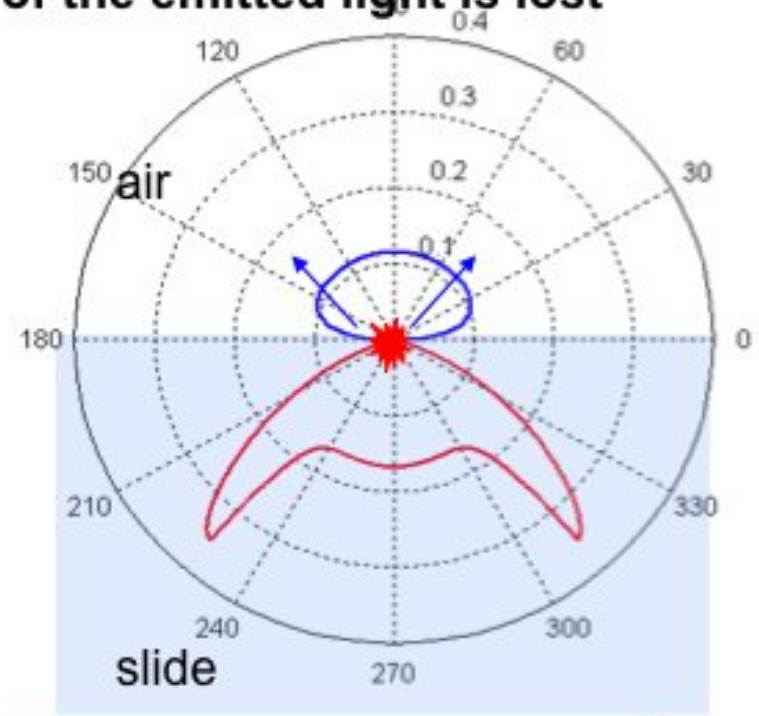


Comparing emission on Glass or AmpliSlide™ substrate

- Glass slide:

  Fluorescence escapes to the glass substrate and does not enter into the collection optics:

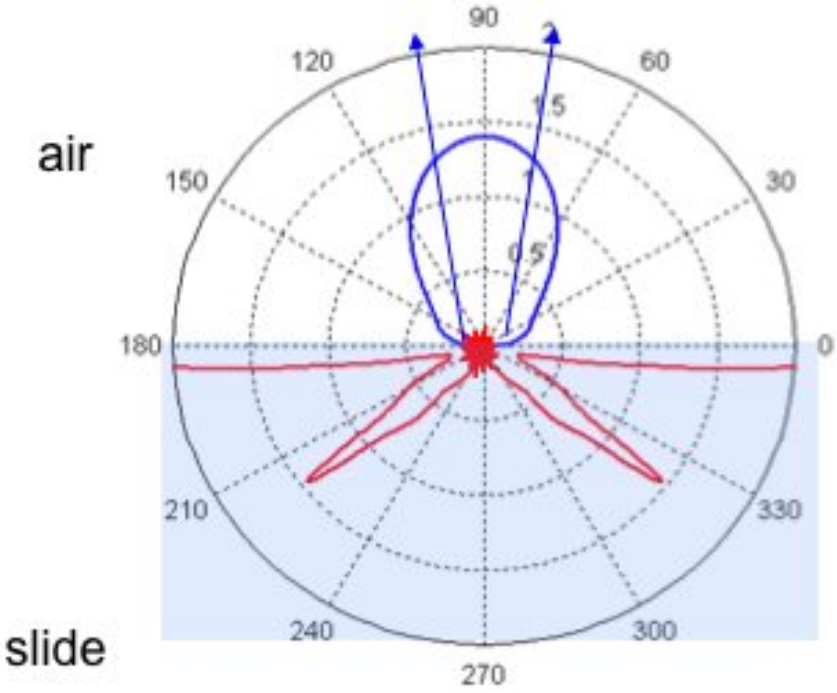
  Most of the emitted light is lost



Low collection efficiency

- AmpliSlide™:

  Fluorescence is avantageously **redirected and enhanced** upward to the collection optics



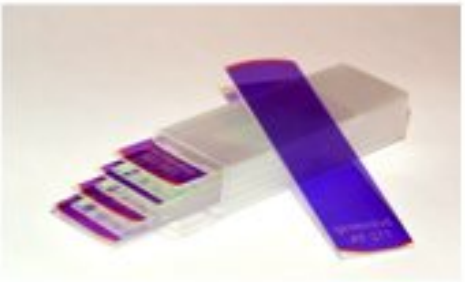
High collection efficiency

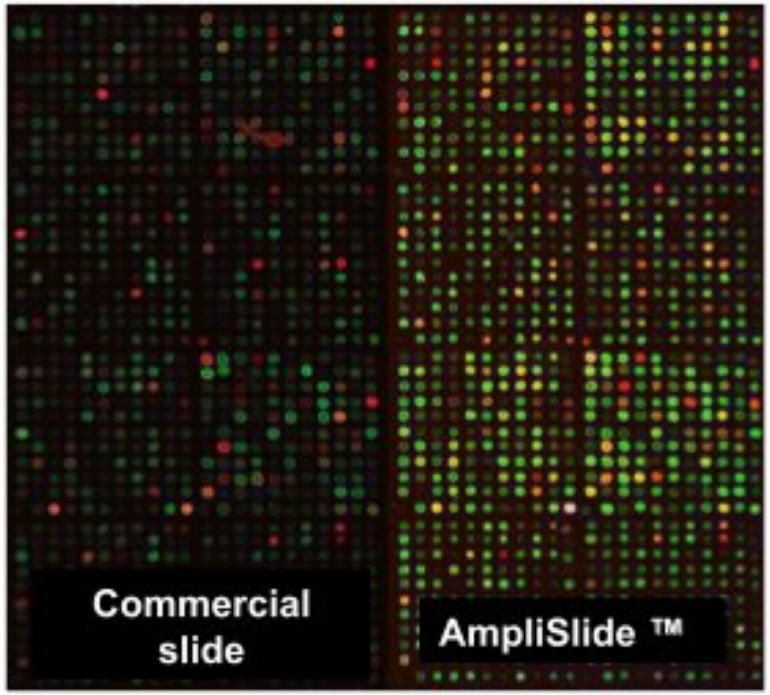
H. Choumane et al., Appl. Phys. Lett. 87, 03110 2005

AmpliSlide

Fluorescence amplifying slides

- Up to **30 fold signal fluorescence amplification**
- **Fully compatible** with commercial equipment (spotters, hybridizers and scanners) and microarray surface chemistries





Benefits:

More signal and/or **more sensitivity** to:

- Lower detection threshold
- See low expressed genes
- Explore new research fields
- Use less biological material
- Reduce the Costs
- Enhance quality and reliability
- Reduce Scanning duration

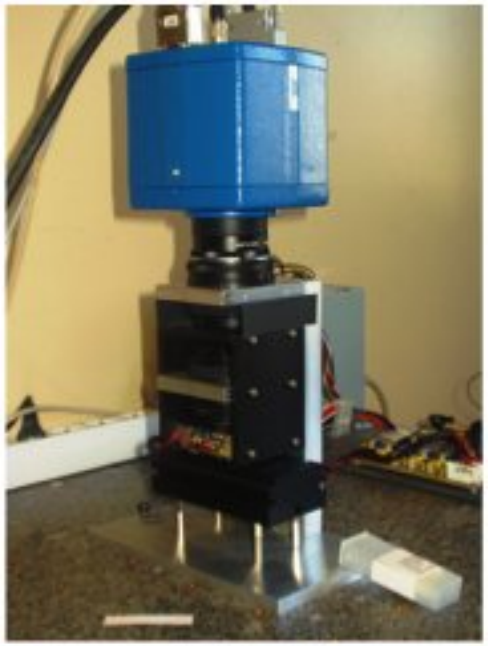
CNRS Plateform Gif, Nicolas Maunoury AmpliSlide amino, cDNA array

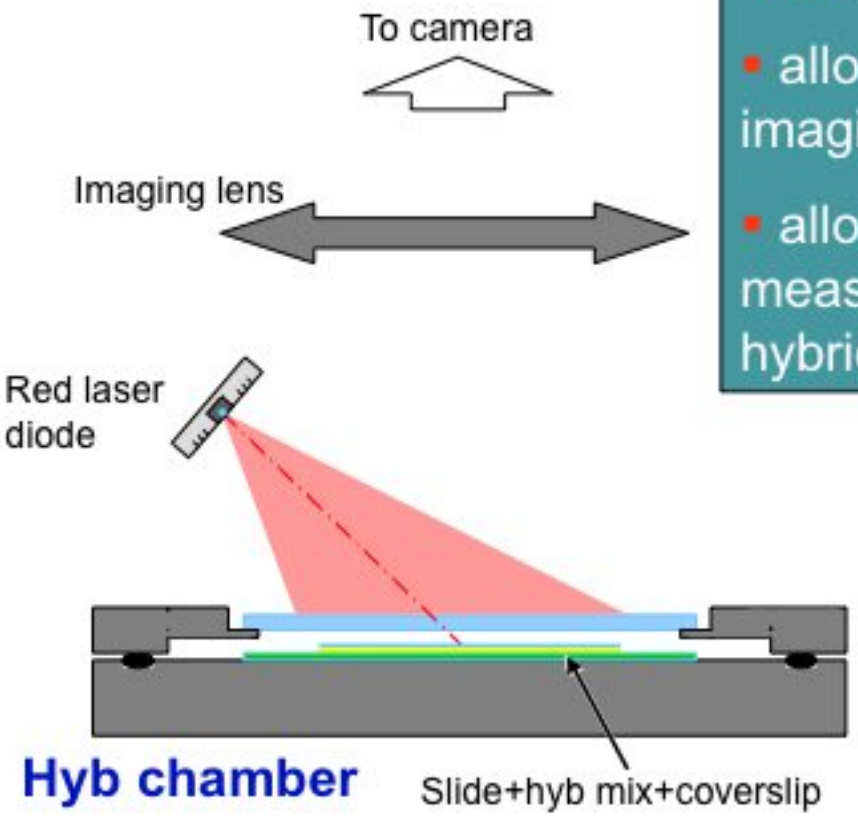
Hyblive™

Integrated hybridization chamber & real-time reader

Amplifying slides

- Allow good collection at small aperture
- can image large field
- allows real time imaging of many spots
- allows real time measurement of hybridization





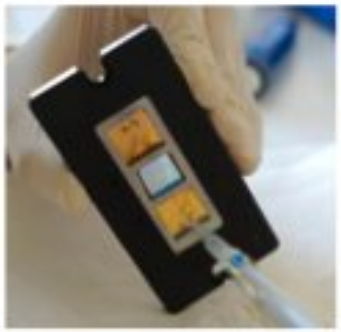
Benefits:

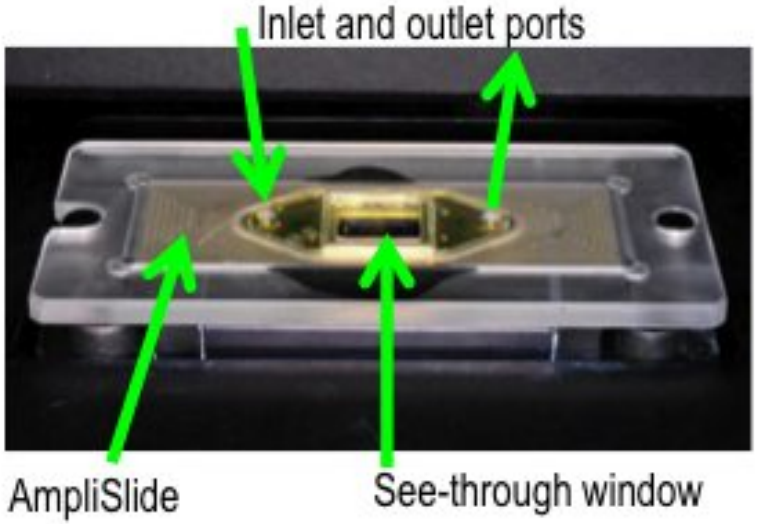
- Develop a better understanding of DNA binding to spot
- Access to hybridization kinetics
- High - quality data and error estimates

instrumentation scientifique: la station d'hybridation en temps réel : HybLive™

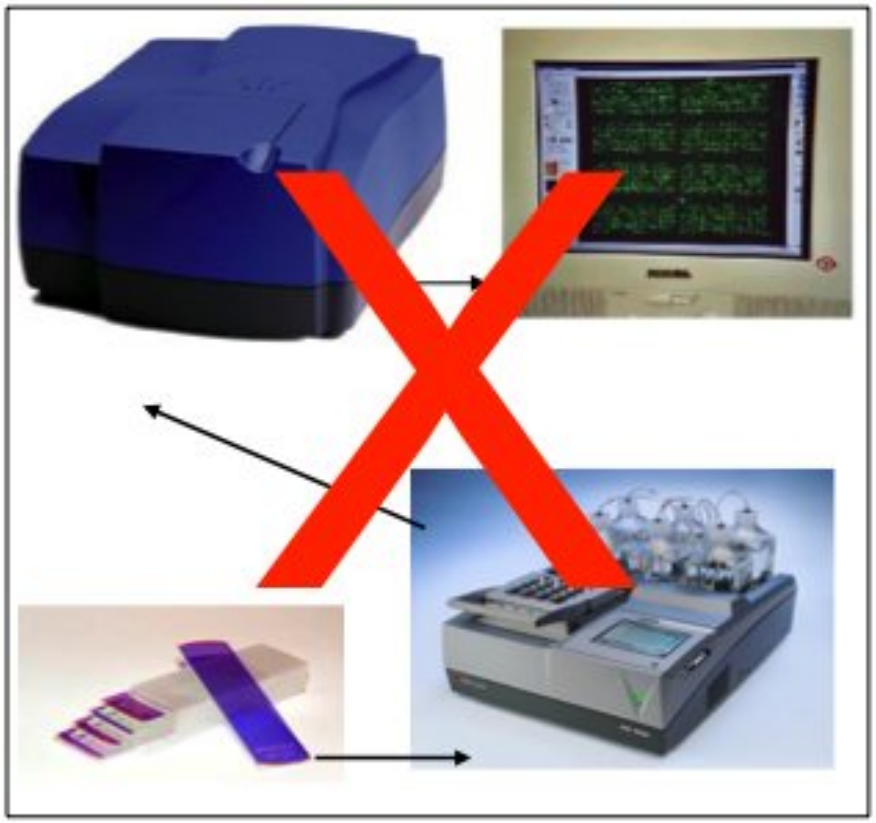
- Station d'hybridation, de lavage et lecteur de fluorescence intégré
- Système intégré de mixage
- Contrôle de température (20-70°C)
- 3 bouteilles de lavage + 1 bouteille d'eau
- Résolution de 10 µm
- Jusqu'à 3 images par minute
- Logiciels de contrôle et d'analyse

- Lames de format standard
- Fenêtre de 1 cm² (2000 spots)
- Chambre d'hybridation de 70 µl (mm^3)

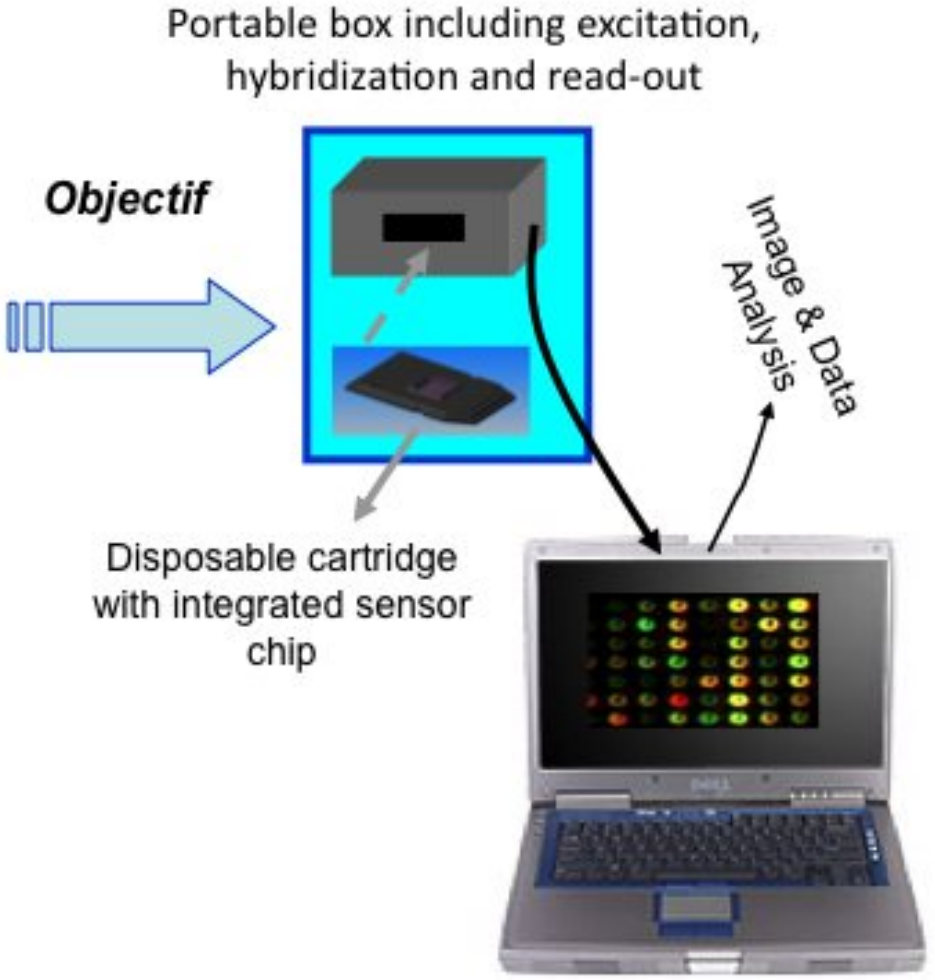




Solution sans optique
la vision du biocapteur silicium intégré



Applications
- Field Analysis
- Pathogens Identification
- Analysis Automation
- Diagnosis kit development
- Lab-On-a-Chip

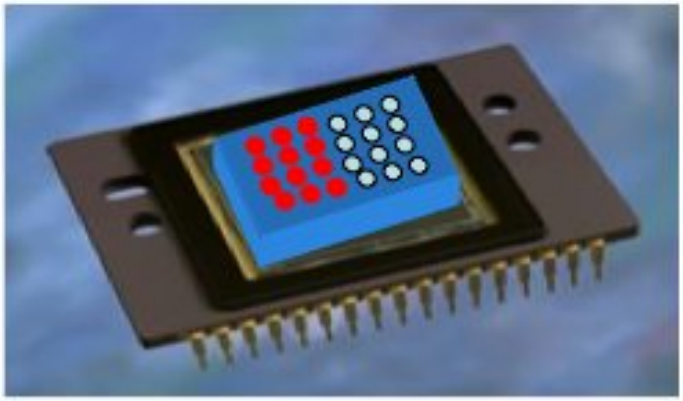


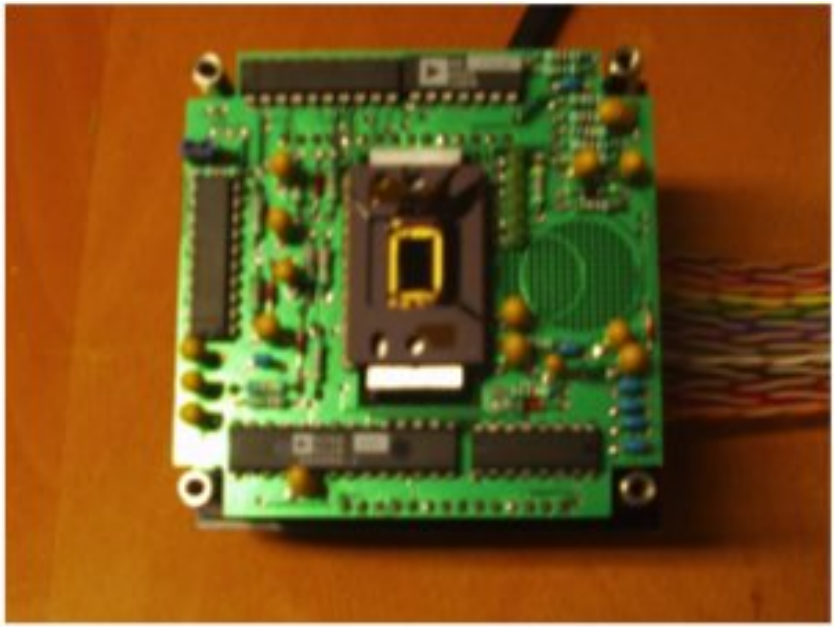
Biocapteur silicium intégré: imagerie par contact

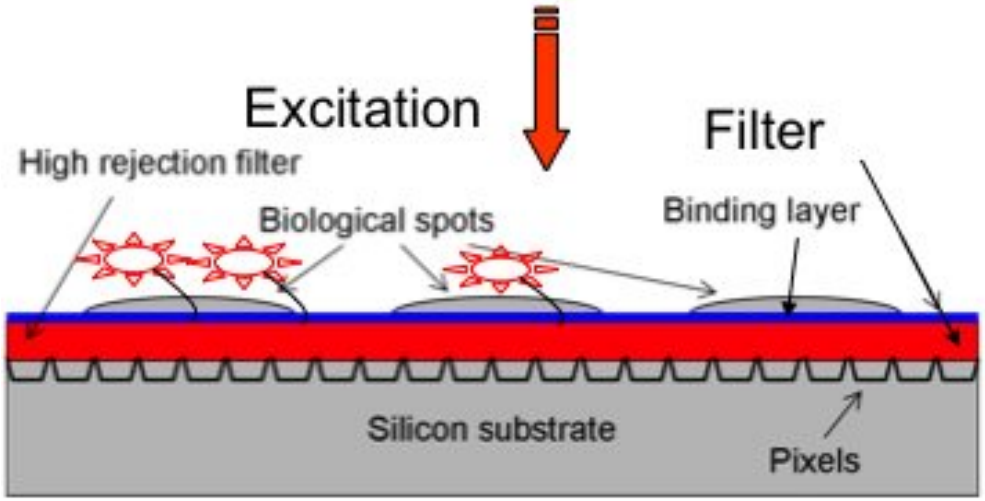
Integration on a CCD Imager

Miniaturization (about 20x)

Sensitivity enhancement (about 30x)





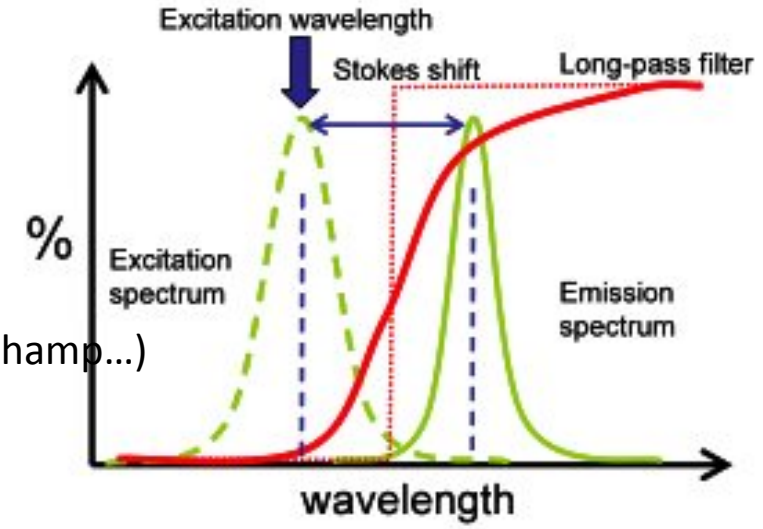


Demand on high rejection filter:

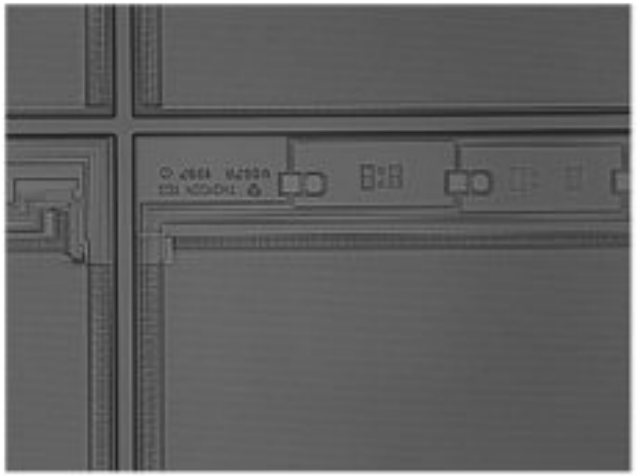
- High rejection
  - Low excitation transmission
  - High fluorescence transmission
- Omnidirectional (not interference)
- Low intrinsic fluorescence
- Low fabrication temperature

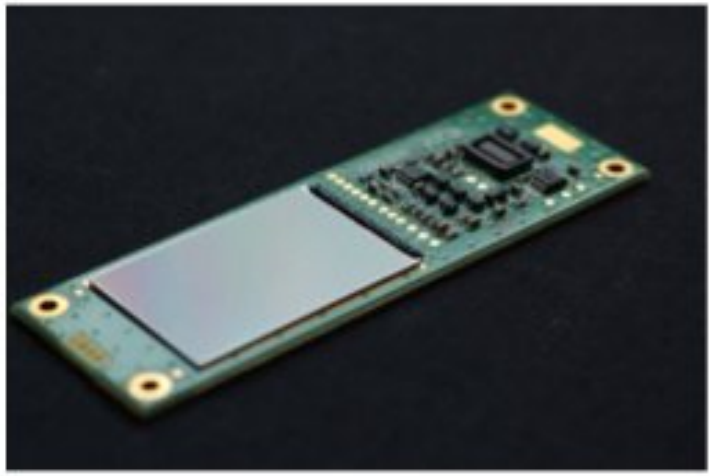
Filtre absorbant

- Spec sur la réjection excitation / émission
  - R > 10^7
- Imageur grand champ
  - Filtre interférentiel
    - R > 10^5 (faisceau parallèle… angle de champ…)
  - Géométrie champ sombre
    - R ~ 10^3
- Filtre absorbant
  - R > 10^{7+} (plus mesurable au spectrophotomètre)
- Impose des contraintes sur le choix du fluorophore

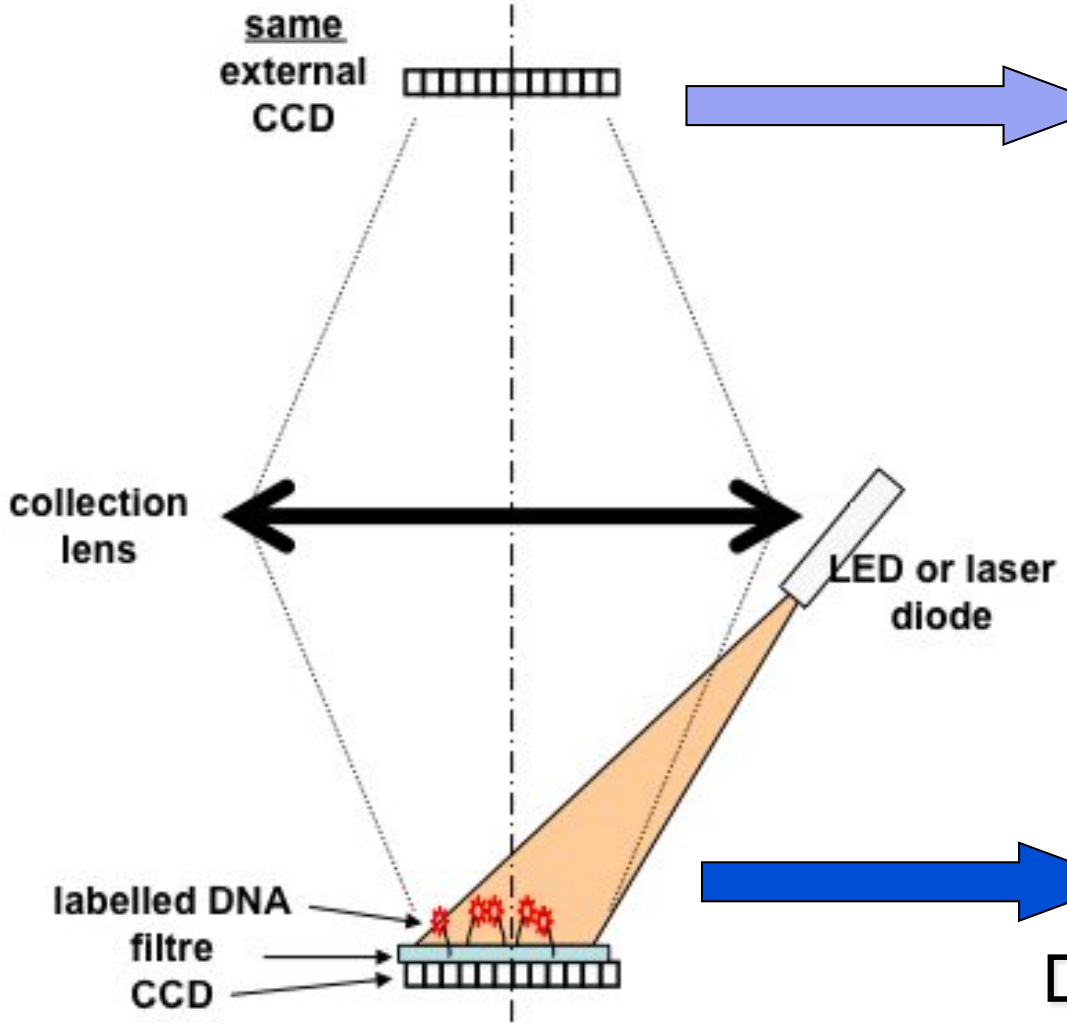


1cm

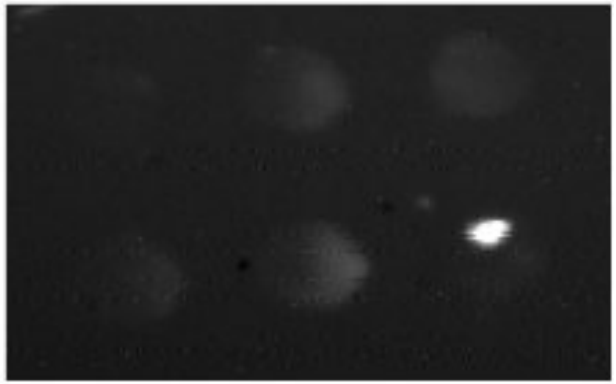


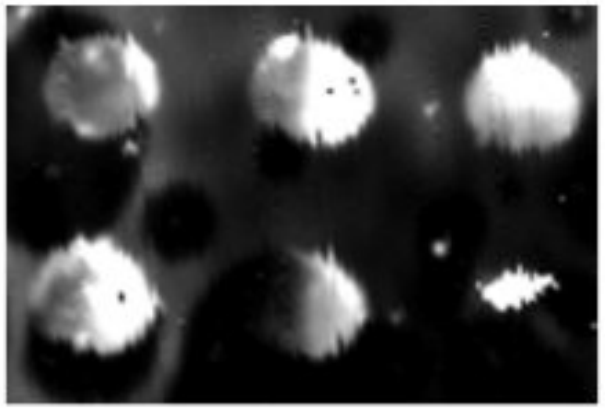


Integrated Silicon Biosensor performance: observation des memes spots par imagerie lointaine ou par contact



far-field Image



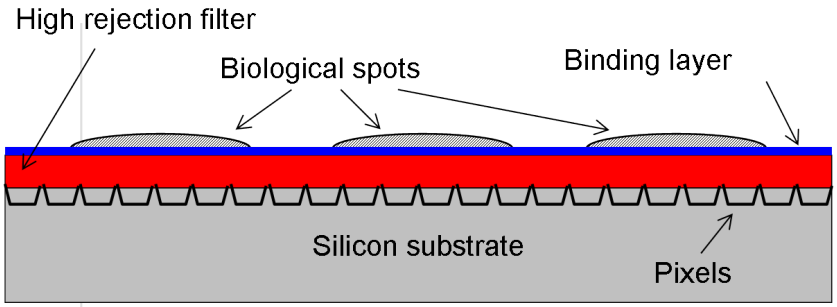


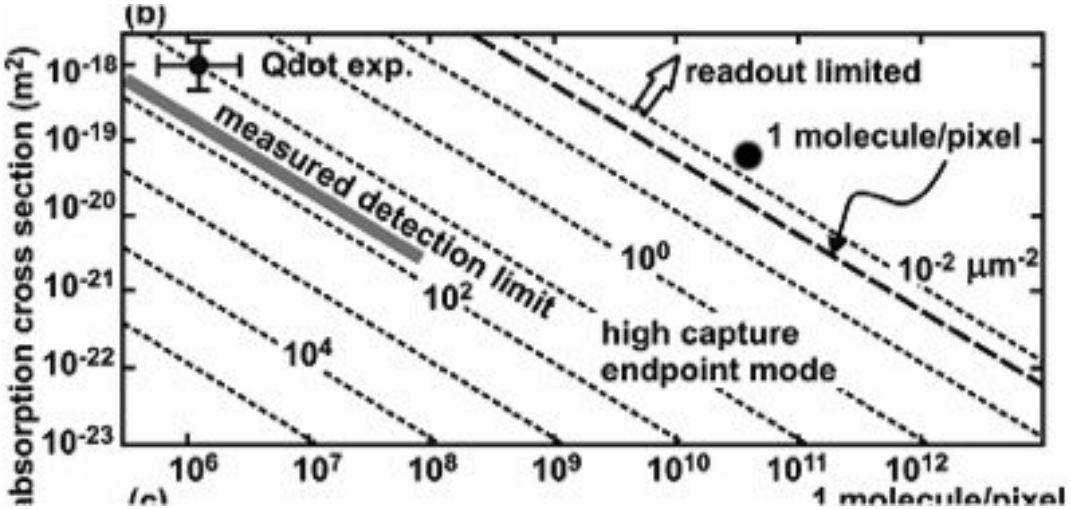
Direct Image : signal X 30

Polyimide layer doped with a chromium-based dye
t ≈ 6 μm, dye concentration ≈ 50% polymer volume solution
T ≈ 10^{-6} at λ_{exc} and a 60% transmission at λ_{emi}

Smaller is better!

Sensitivity limit: impact of the high collection efficiency





I= 5 mW/cm^2

Excitation photon flux ~10^{16} ph cm^{-2}s^{-1}
Pixel surface 10x10µm = 10^{-6}cm^{-2}
Background = rejection * ph 10^{10} s^{-1}/pixel
(for **rejection =10^{-6}, bkg=10^4 ph s^{-1}/pixel**

Effective section (Qdots) ~10^{-14} cm^2
Quantum efficiency (Qdots) ~10%
Emission per fluorophore ~ 10 ph s^{-1}
For 10 fluorophore/ µm^2, per pixel
Emission per 10X100 (area) ~ **10^4 ph s^{-1}/pixel**

For 10 fluorophore/ µm^2 sensitivity rejection must be 10^6

Intrinsic detector noise does not set the limit

Multiple single molecule detection level can be reached in low-cost imaging system

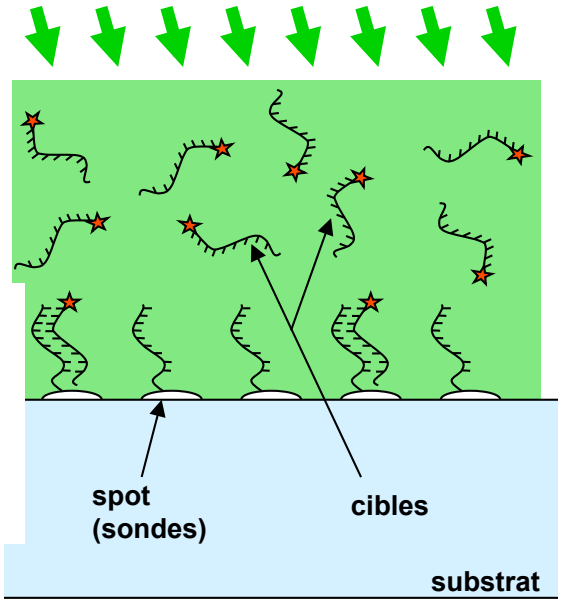
*L. Martinelli et al., Appl. Phys. Lett .**91**, 083901 2007*

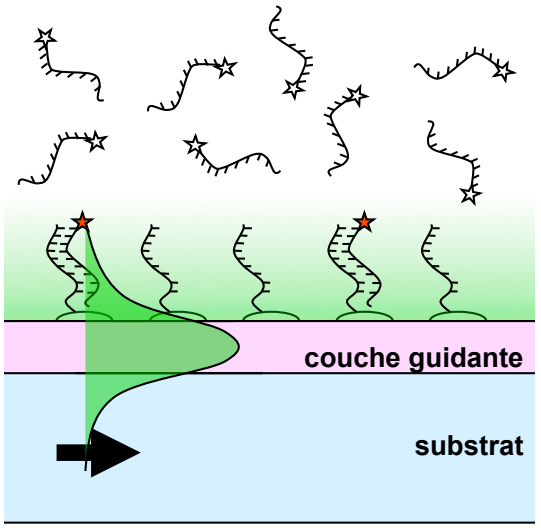
Excitation par ondes evanescentes: SmartSlides: Principe

Excitation externe

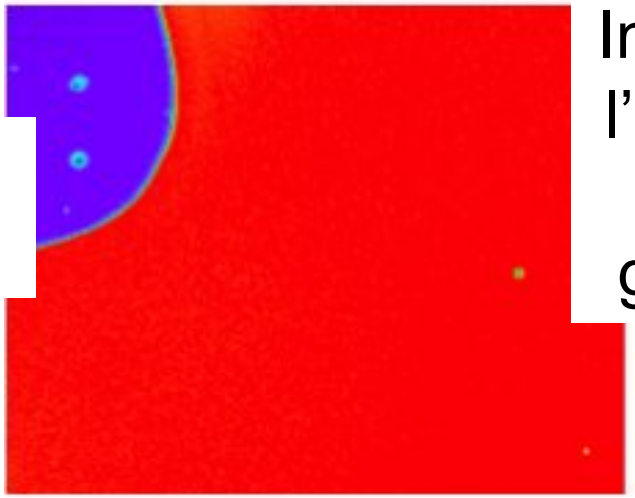
Excitation par ondes évanescentes

Vue sur la tranche

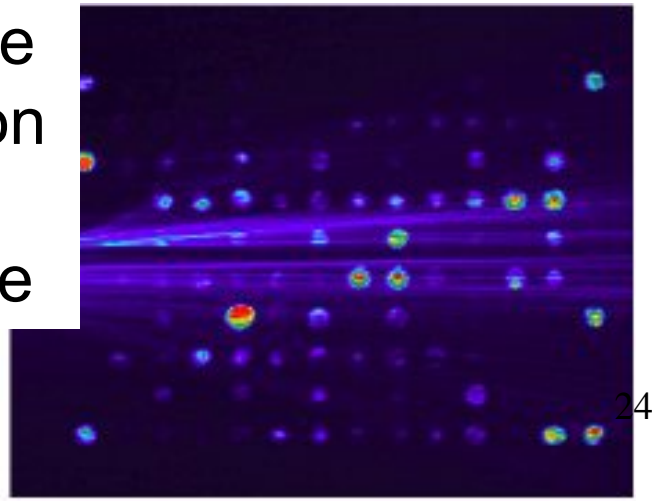




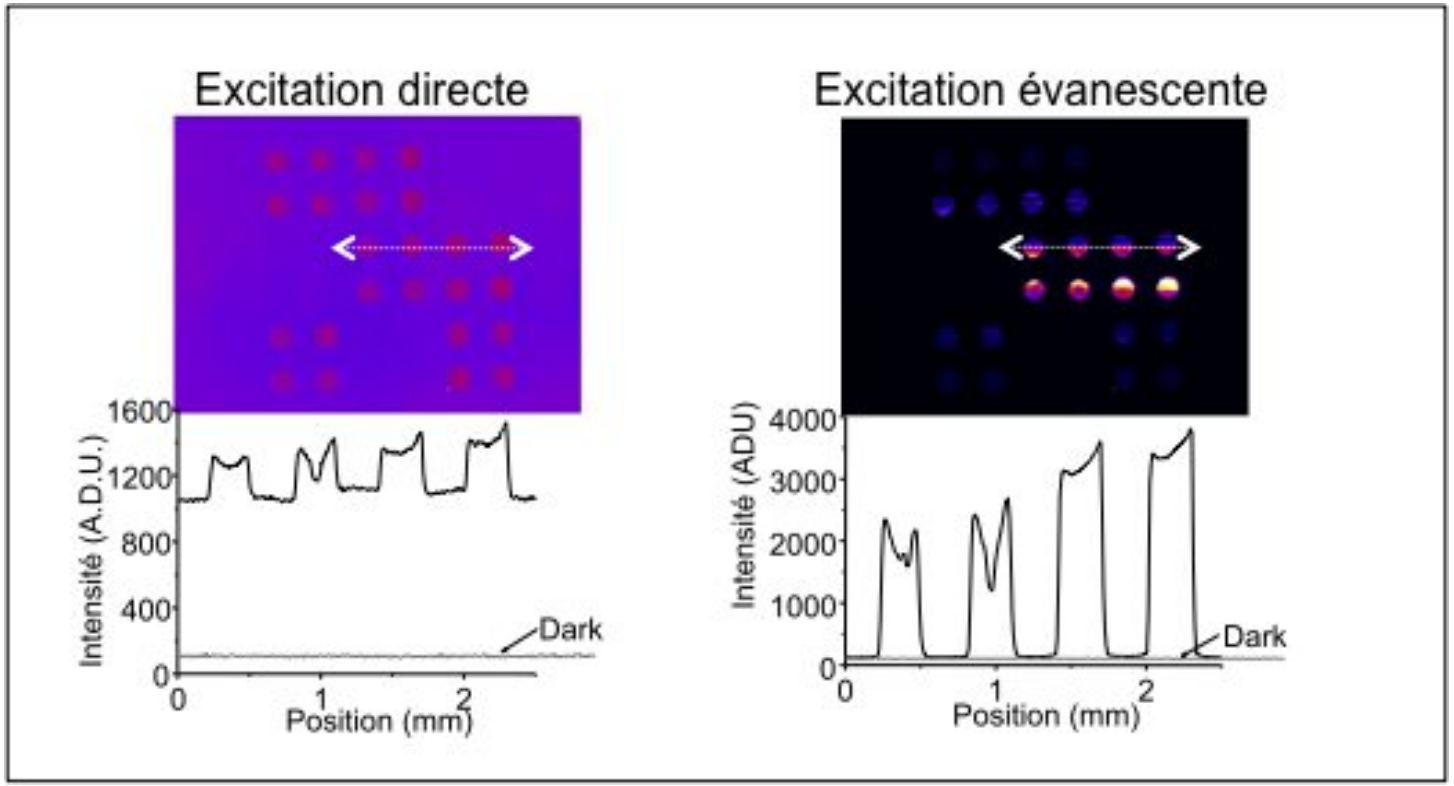
Vue de dessus



Imagerie de l'hybridation d'ADN génomique



SmartSlides: Résultats



- Intérêts :
  - protocoles simplifiés :
    - avant hybridation, pas de purification
    - après hybridation, pas de lavage
  - accès aux constantes d'association
  - facteur d'amplification du S/B (jusqu'à 10 000)

Genewave geneSpress platform

Genewave

Il y a 3 ans Genewave

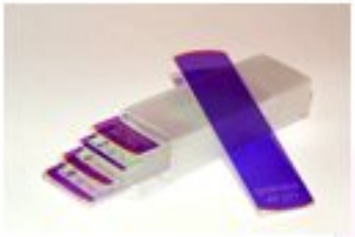
se transforme en une société
de diagnostic

(l'instrumentation en biopuces
ne permet pas à une entreprise
de 20+ personnes de vivre)

Usual tools for multiplex microarray diagnostics
Many machines and manipulations are involved

Microarray fabrication

Microarray subsrates

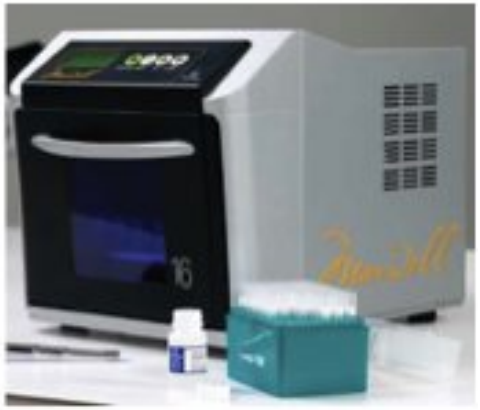


spotter

Spotter needdles

Biochip diagnostics

DNA, RNA extraction



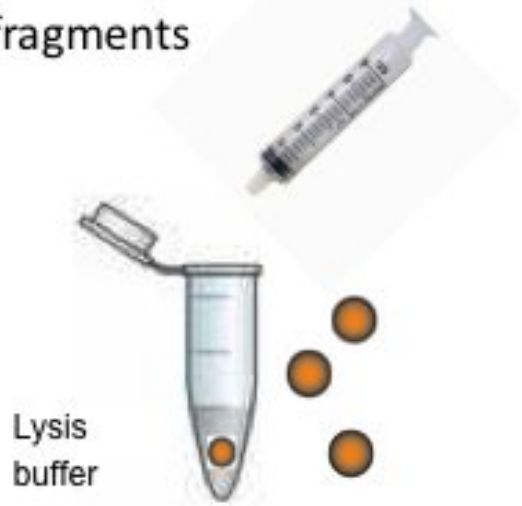
RNA RT, DNA amplification & fluorescent labelling

Scanner

Hybridization chamber

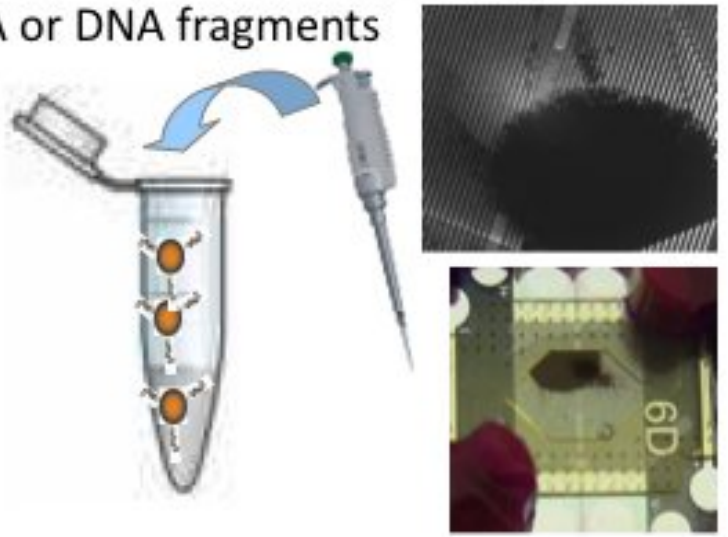
Even the classic viral RNA extraction procedure is complex

1. Lysis step (virus destrcution. Magnetic beads addition to bind RNA or DNA fragments



2. Lysate injection. Purification by washing steps in washing capsule. Magnetic capture

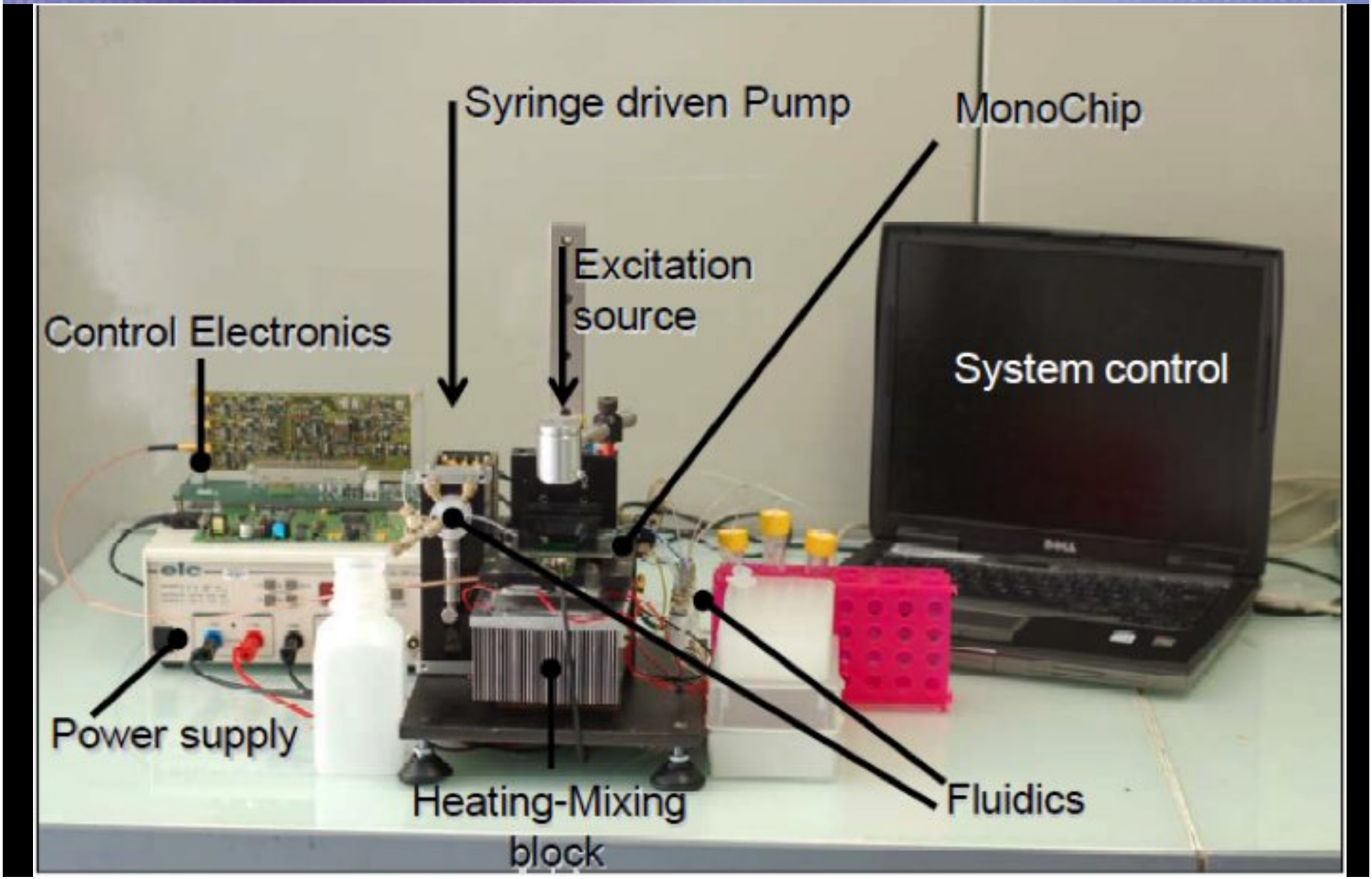
3. Recovery of beads coated by viral RNA or DNA fragments



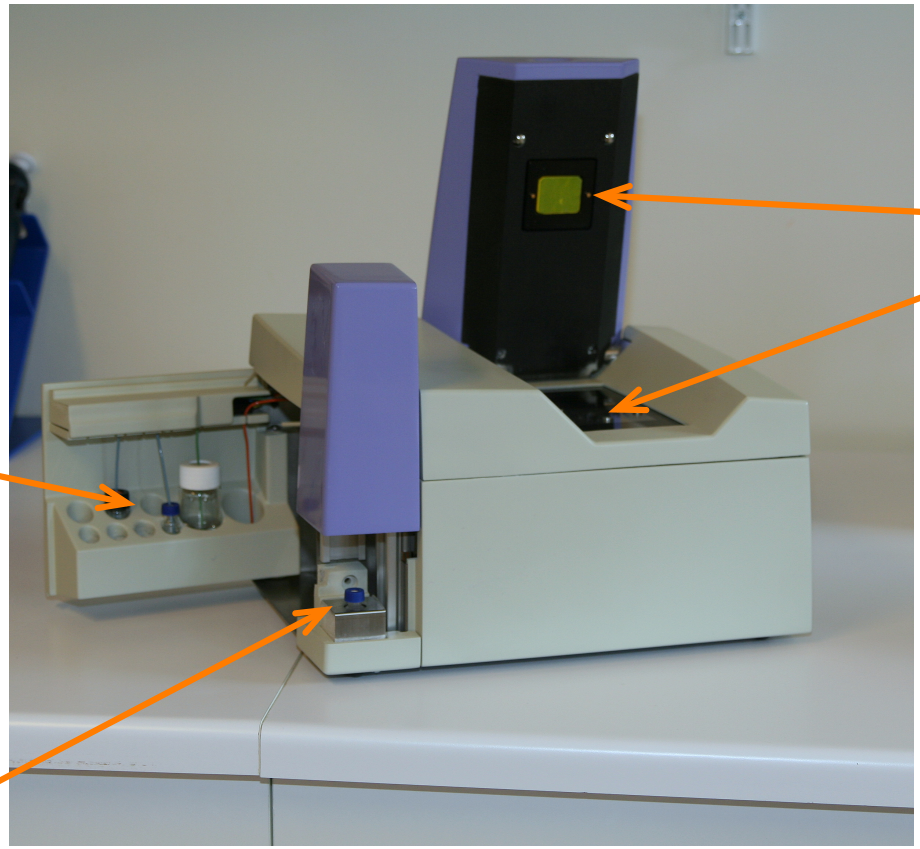
4. RT-qPCR on tube

Gaiker (Zamudio, Spain)
With CIRAD (Montpellier), Ikerlan (Mondragon)
Donostia Hospital

Diagnostics: prototype of integrated system



Prototype of integrated system for optical readout



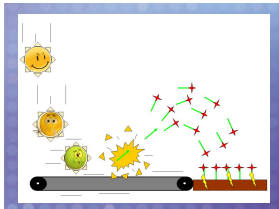
Imaging device

Cartridge

Reagents and waste
(8+1, 2-20mL tubes)

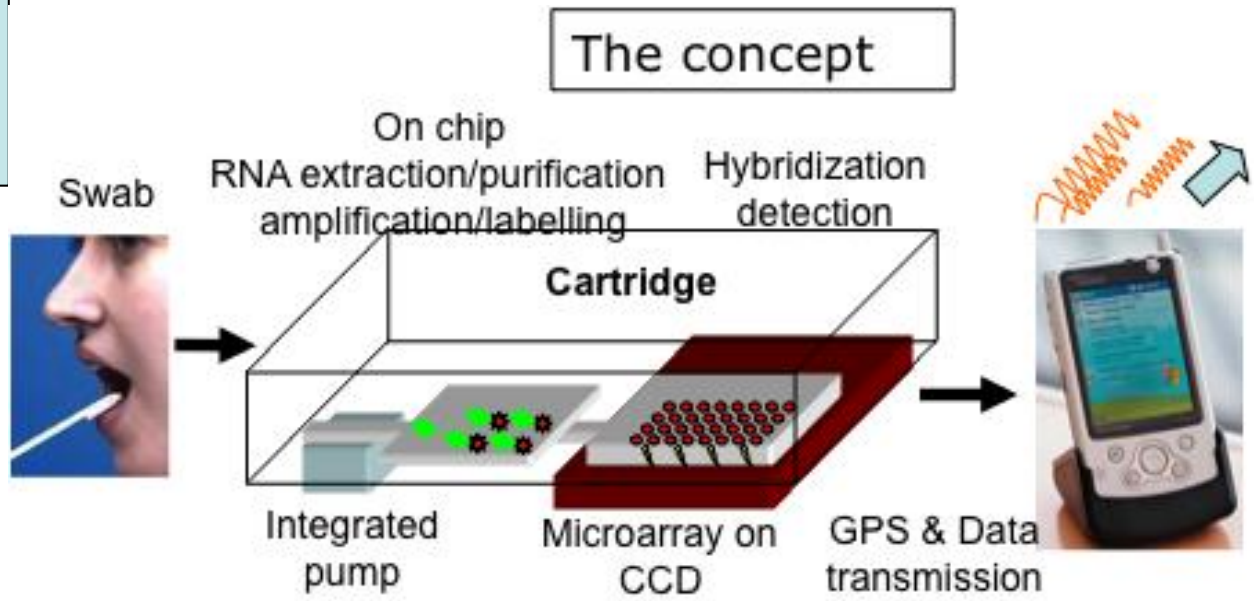
Sample: 300μL-2mL microtube,
automated loading

Footprint: A4 size



Integration of diagnostics processes in a european project

Projet Portfastflu FP7
Detection of A & B influenza viruses and identification of H1, H2, H3, N1, N3, H5, H7, H9 subtypes



Coordinator Genewave (Palaiseau, France)
Nottingham Univ. (Nottingham, UK)
Biosensia (Dublin, Ireland)
CIRAD (Montpellier, France)
VIB University of Ghent (Ghent, Belgium)
Ikerlan (Mondragon, Spain)
Gaiker (Zamudio, Spain)
Hospital donostia (San Sebastian, Spain)
Whatman (Maidstone, UK)

The major challenge:
Integrate the various contributions, vastly different in technology and maturity, in a single fully automated system where everything must operate flawless

Evolution des differents prototypes un re-engineering continu

v0

Validation of compact Hybridization and Optical readout prototype with **contact optics**

v1.0

First integration of fluidic functions: Multiplex Amplification Hybridization and detection

v2.0

Further integration of fluidic functionsDNA purification Multiplex amplification Hybridization **HairLoop** Detection

v3.0

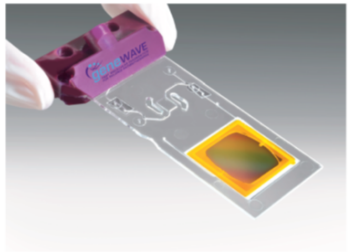
Random access **Evanescent** excitation

Concept geneSpress v3 : 100 steps => 3 steps

Hands-on time : 5-15 min

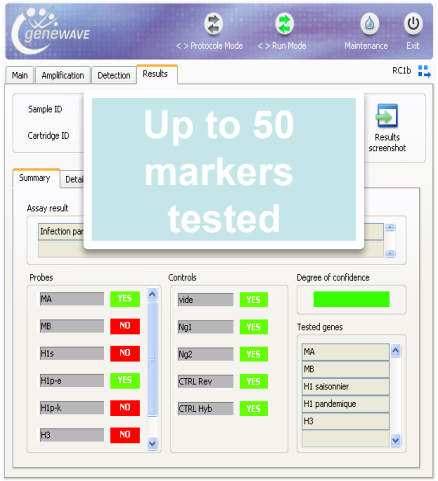
Collecte du specimen

Specimen placé dans un tampon de lyse

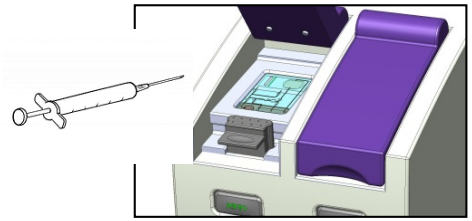


Cartouche laboratoire sur puce et réactifs

De l'échantillon au résultat en moins de 3h



Affichage des résultats

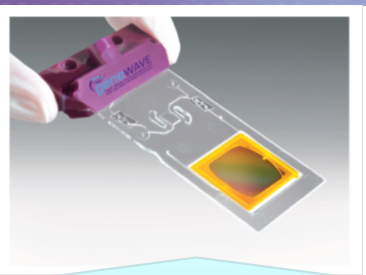


Injection de l'échantillon
Traitement et analyse automatique

Consommables placés dans l'instrument

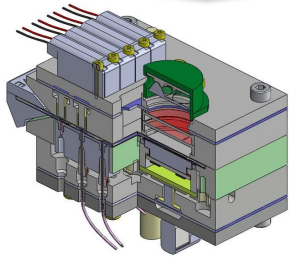
GeneSpress® Platform
Why it works

GeneSpress®
Cartridge



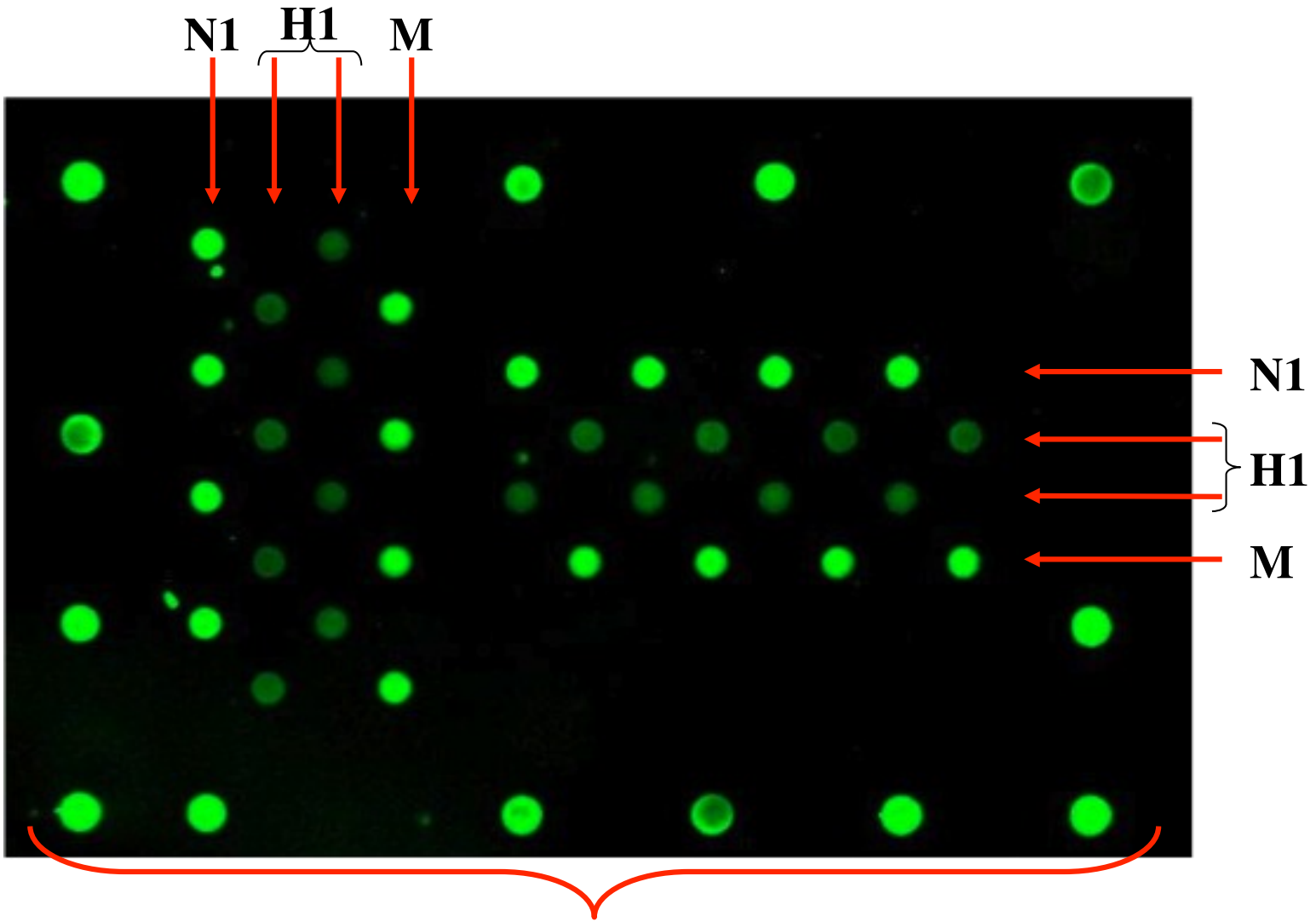
- Simple cartridge design
- On-chip valves
- Direct sample loading
- Robust Assay Design methodology

The core of compact technology:
The GeneSpress®
Docking station



- Valve actuation / Reagents transfer
- Fast and efficient Cycling
- Unique TouchArray fluorescence detection technology
- Expert Analysis Software

Flu microarrays H1N1 detection on Real Time system



Limit of detection: Biosensor DNA analysis

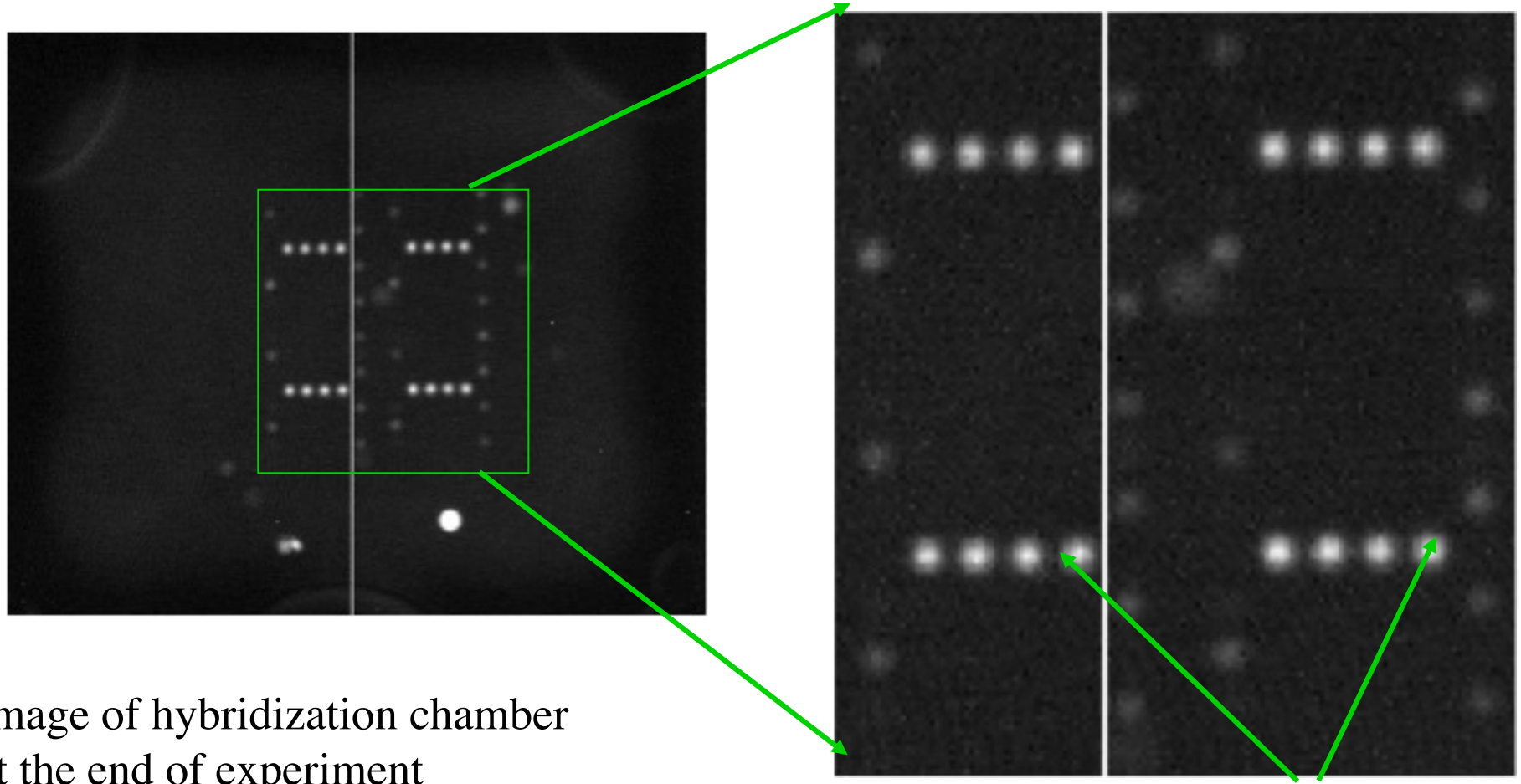


Image of hybridization chamber
at the end of experiment

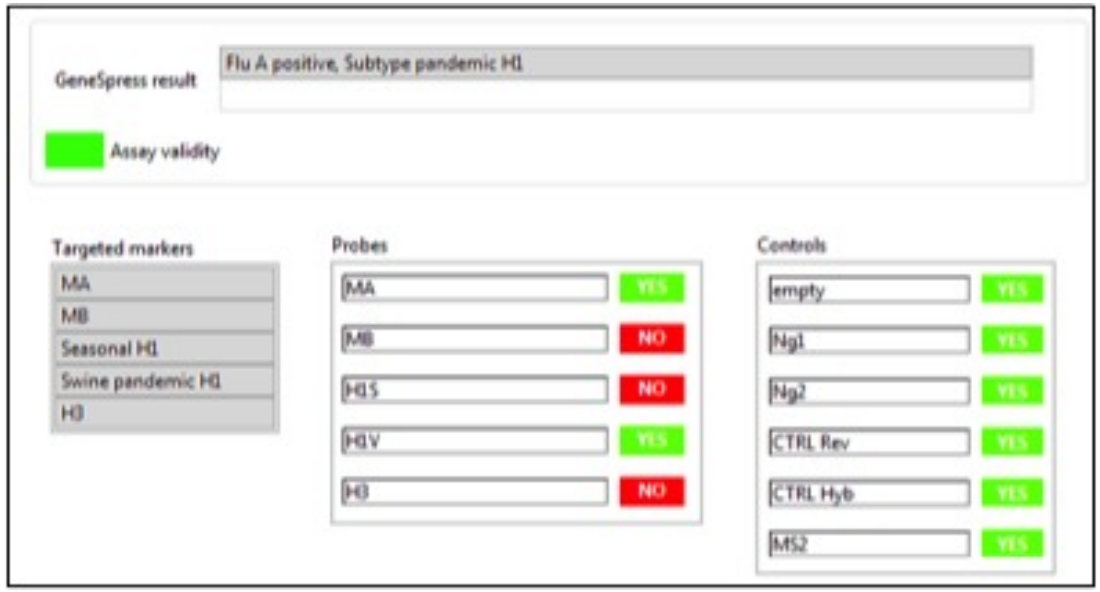
controls

Hybridization of RT-PCR non purified target (biotinylated)
Starting material: 10^{-7} dilution of viral RNA (100 copies)
Detected by integrated biosensor (SNR = 7,5)

GeneSpress® Platform: : Diagnostics Decision call by Expert software (DiagES)

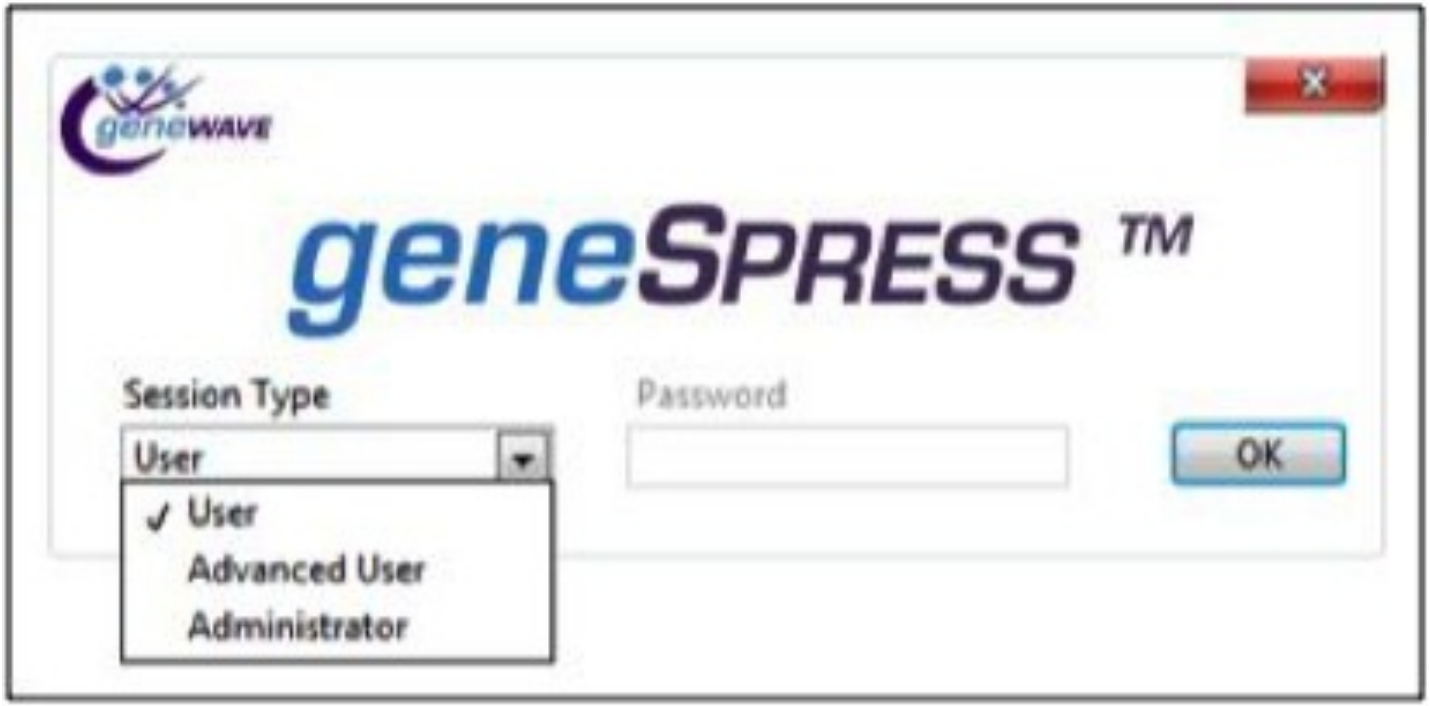
- Statistical determination of the on/off state of each probe, based on replicates analysis

- Result and interpretation of the analysis is given via a truth table



The GeneSpress platform: the control and analysis software

Welcome window and user choice



The GeneSpress platform: the control and analysis software

User's instructions

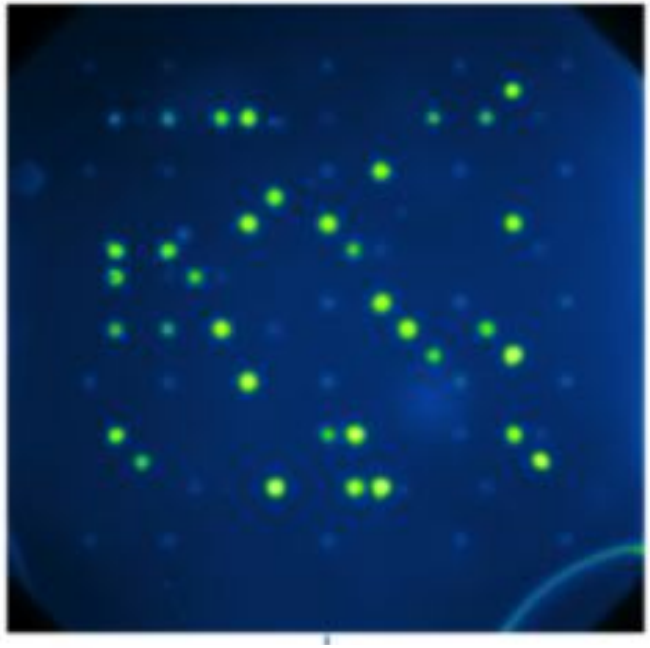
The GeneSpress platform: the control and analysis software

Real time monitoring of process steps

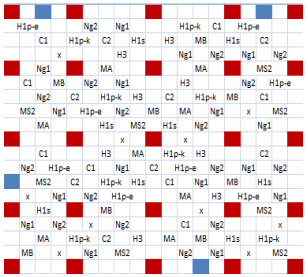
The GeneSpress platform: the control and analysis software

Display of analysis results

Automated Analysis Software



Raw image (false color)



Spotting grid

Autogridding

finding the spots

Segmentation & Quantification

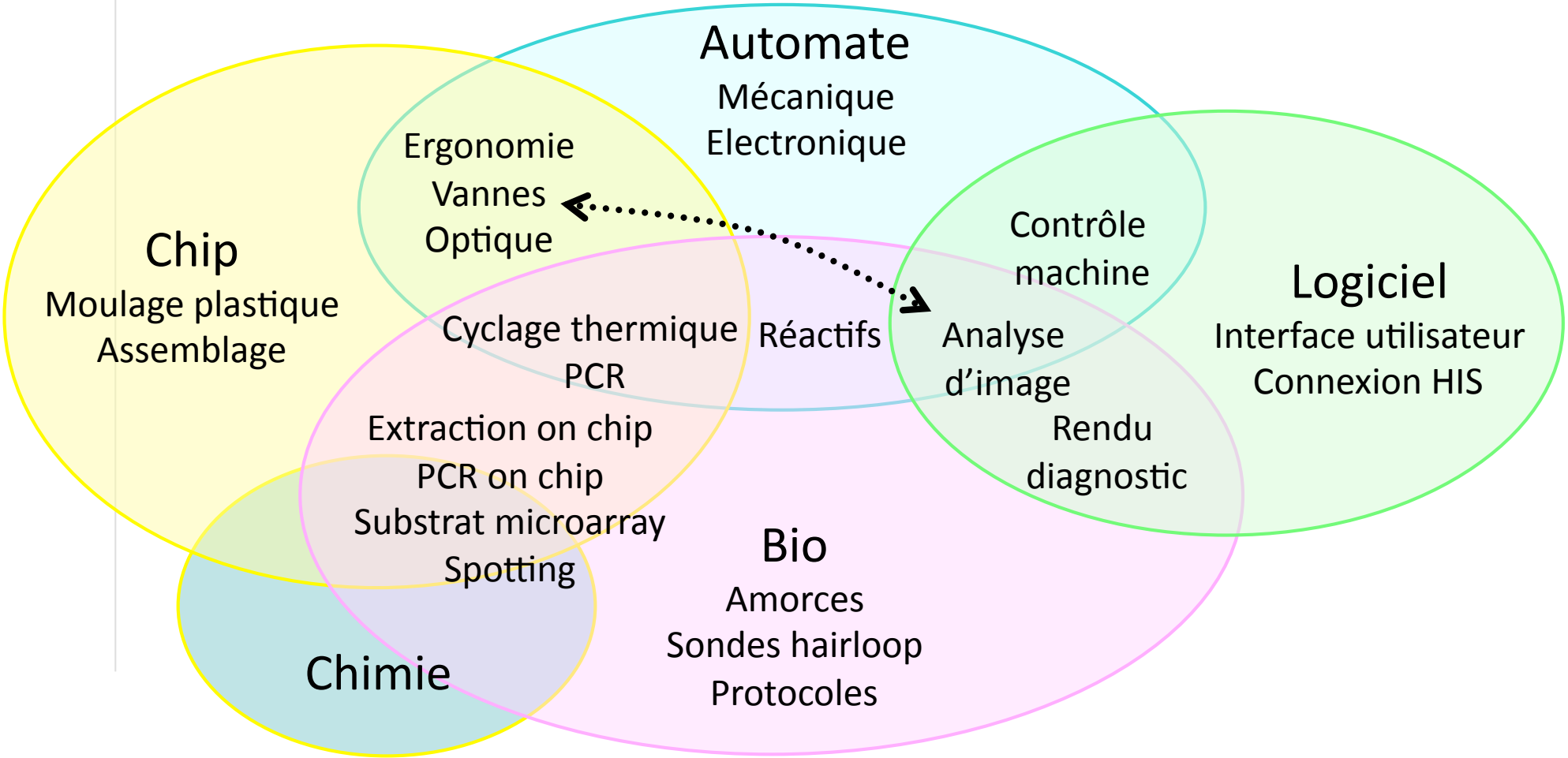
Validity criterion

(counting the correct spots)

Complete result report

Technologies Genewave

La réalisation d'un tel outil n'est possible que par l'intégration de nombreuses technologies, portées par des spécialistes, travaillant dans une organisation ayant une autorité légitime et efficace, la start up.



Messages
Une bonne idée en physique => une start up?

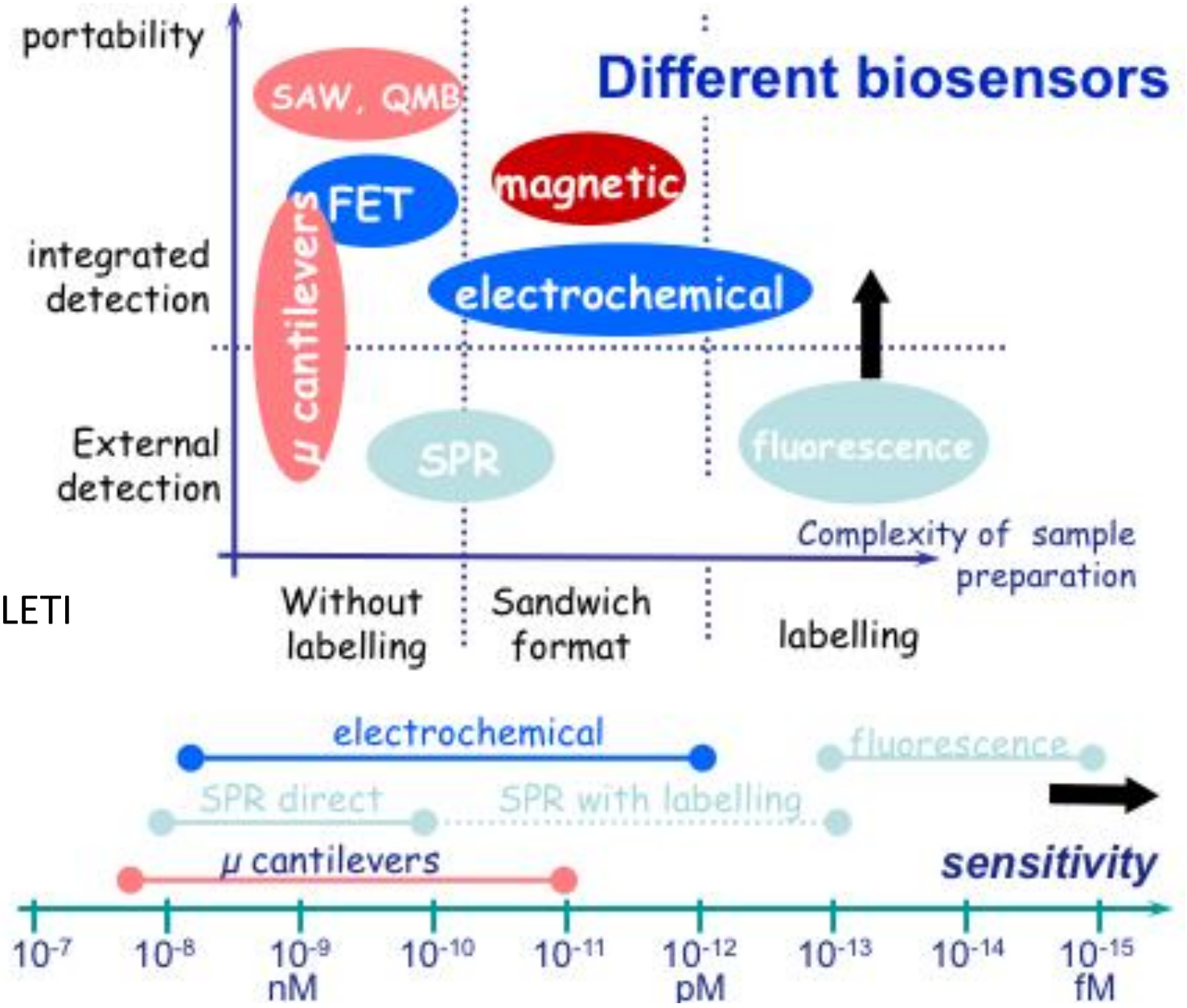
Il faut la d'abord transformer l'idée en produit, $$$$, santé publique : course de haies avec beaucoup de haies, il faut TOUTES LES SAUTER!

Pour diagnostic moléculaire, obstacles et limites physiques variées
(liste non exhaustive):
Fonctionnalisation surface/Rendement d'extraction acides nucléiques/ Purification/inhibiteurs de PCR/Rendement d'hybridation/Rendement de fluorescence/Adsorption non spécifique/Limite de détection/Multiplexage de la PCR- design des amorces/Multiplexage de l'hybridation- sondes pour bonnes sensibilité et spécificité…

Complexité: une seule personne ne connaît pas l'ensemble des points critiques

Conditions nécessaires (et pas suffisantes): réactivité, adaptabilité, travail en équipe avec boucles réaction rapides, compétences techniques, designs multiples continus; capacité à se remettre en cause.

Supplément:
Différents principes de détection d'hybridation



D'apres CEA LETI