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Original article

Towards portable, real-time, integrated fluorescence microarray diagnostics tools

De futurs outils de diagnostics : les biopuces à fluorescence aptes à la détection en temps réel, intégrées et portables

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Abstract

We present recent results on biochips slides with high fluorescence efficiency and their associated readout systems. To obtain the full performance made possible by such slides, the chemical surface functionalization has to be improved, as formerly unobservable defects of the functionalization are now made observable through the improved efficiency. The systems are based on the integration of the hybridization and readout functions into a single machine instead the usual two separate systems, quite cumbersome and expensive. Ultimate performance is reached with systems using standard imaging circuits, CCD or CMOS, as the biochip substrate. In this case, one can obtain a remarkable miniaturization of the full optical system and the integrated hybridization chamber/readout head can be reduced to the size of a webcam.

Résumé

Nous présentons des résultats récents sur les biopuces à support à haut rendement de fluorescence et les systèmes de detection associés. Pour bien obtenir toute la performance rendue possible par ces supports, la chimie de surface fonctionnalisant la biopuce doit être améliorée, l'augmentation de rendement rendant visible les défauts habituellement cachés de cette fonctionnalisation. Les systèmes sont basés sur l'intégration des fonctions d'hybridation et de lecture, habituellement effectuées par des machines séparées, chères et encombrantes. La performance ultime est atteinte avec des systèmes utilisant un imageur standard, de type CCD ou CMOS, comme support de la biopuce. On atteint là une miniaturisation remarquable, puisque l'ensemble chambre d'hybridation–tête de lecture a une taille de l'ordre d'une webcam.

Keywords: Fluorescence; Microarrays; Biochips; Diagnostics; Genomics; Proteomics

Mots clés : Fluorescence ; Microarrays ; Biopuces ; Diagnostic ; Génomique ; Protéomique

1. Introduction

The need to measure molecular interactions and obtain genomic information is a major driver of molecular recognition systems such as biochips [1]. Applications are numerous: basic research in genomics and proteomics, clinical studies, diagnostics systems. Biochips are desirable tools for diagnostics and prognosis of numerous forms of cancers or of developing infectious species such as SARS, nosocomial pathogens (such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) or *Clostridium difficile*), avian influenza, MDR tuberculosis. . .There are, however, a number of issues to be solved for mass applications in the field, at the practitioner's office or in the hospital, notably nonsupervised operation, low-cost, compact-

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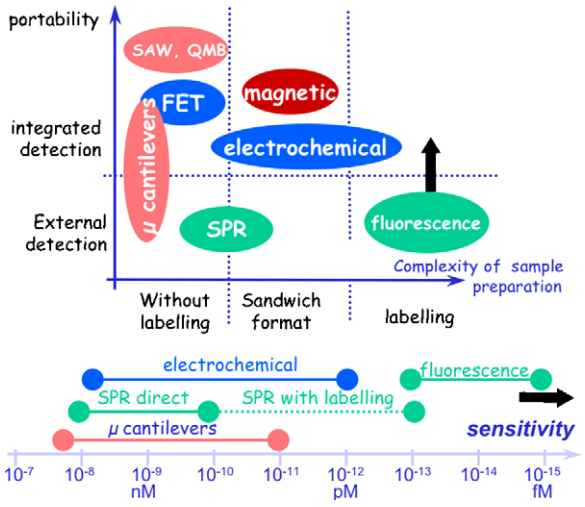

Fig. 1. Schematical comparison of the most common biosensing techniques used in biochips along various parameters: the top diagram compares integration/portability, complexity of sample preparation (labeling or not); the bottom chart and scale indicates the various sensitivities (after CEA-LETI. P. Puget (2003), unpublished; P. Puget in Nanosciences: Nanobiotechnologies et Nanobiologie, Belin, 2007.). The black arrows indicate the improvements to the fluorescence technique discussed in the text.

ness, low reagent consumption, fast measurement, sensitivity, reliability... We describe how our advanced optical architectures make high performance portable systems compatible with these demands, provided that chemistry reliability is well assessed. In the next section, we compare fluorescent biosensing to other techniques. In Section 3, we, then, give some key elements for chemistry and its reliability in this context. In the subsequent sections, we first address amplifying slides and innovative chip reading systems, enabling in particular real-time hybridization, while we describe in the last section miniature integrated biochips.

2. Comparison of fluorescent biochips with other molecular sensing techniques for biochip readout

Various detection techniques (based on electrochemistry, surface plasmon resonance (SPR), ellipsometry, microcantilevers, magnetic beads, surface acoustic waves (SAW), quartz microbalance (QMB), etc.) have been implemented in biosensors and are being developed according to the various constraints mentioned above (Fig. 1). Among these, fluorescence stands out due to its sensitivity, at least two to three orders of magnitude better than the next best technique. A high detection sensitivity is indeed extremely important in microarray format biochips for analysis of gene expression patterns, patient genotypes, drug metabolism, etc. and especially when RNA or cDNA is involved.

One drawback usually raised about the fluorescence detection scheme, is its lack of integration (separate hybridization and measuring apparatus) and of portability, in particular when considering the new approaches in bioanalytical techniques based on integrated lab-on-chips. Also, the size of reading systems (scanners) can be a deterrent to the search for portable integrated systems

Another often quoted disadvantage of the fluorescence sensing technique is of course the required labeling of targets or hybridized species with fluorescent species, but in spite of the added complexity in sample preparation it also brings some advantage. When dealing with complex, real world samples where one searches for a few targets floating in much greater concentrations of unwanted materials (such as cell materials from the sample), only the labeled species have a strong fluorescence and, therefore, the labeling step adds a significant factor in selectivity and specificity, most important parameters in molecular recognition measurements.

Thus, while nonfluorescence label-free solutions might prove useful in the some of lesser demanding applications, we believe that many others will require performance at the fluorescence limit sensitivity and selectivity. We have developed solutions to the afore mentioned limitations of the fluorescence systems, namely through the integration of the hybridization system with the reading instrument, and in a more revolutionary manner through the monolithic integration of the biochip with its reading system. In both cases, the integration yields a significantly increased sensitivity thanks to better fluorescent light collection strategies. We describe below advances in these aspects of sensitivity, integration, measurement speed and portability.

3. A prerequisite building block: surface chemistry

A typical microarray biochip consists of thousands of spots of known DNA molecular probes immobilized at known locations on a microscope glass slide functionalized, with adequate surface attachment chemistry. Research slides can contain tens of thousands of spots, even a few hundred thousand, while diagnostic slides will rather carry the few hundreds of spots required for the identification of all relevant molecular species for a given diagnostics. Dye-tagged or quantum-dot (QD) tagged DNA targets originating from a sample of interest are specifically hybridized in situ onto those probes, achieving a spatially-resolved molecular recognition.[1] Quite usually, two dyes (or QDs) emitting at different wavelengths are used, for example, green cyanine 3 (Cy3) for the DNA sample of interest and red cyanine 5 (Cy5) for a control target DNA sample serving as a reference. The typical sensitivity of existing scanners for a glass slide is in the range of 10 fluorophores per micrometer square, the typical pixel and spot sizes being 10 and 100 μm, respectively.

The density of fluorophore molecules grafted on DNA strands remains small (1 per oligo–DNA), to limit fluorescence quenching, making the detection of genes with low expression levels, for example, difficult to observe due to the weak signal.

The biological information obtained from a biochip is based on the molecular recognition function performed by the probe molecules bound to the surface. To perform this binding function, one puts a thin layer of chemical binding species, which will

[1] We take as definition of probes and targets the molecules that are attached to the surface and the molecules to be identified, respectively. The converse notation is also sometimes used.