"PEAK-TRACKING CHIP" (PTC) FOR BULK REFRACTIVE INDEX SENSING AND BIOARRAY SENSING

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ABSTRACT

Resonant techniques are of wide interest to detect variation of effective refractive index at a chip surface. Both Surface Plasmon Resonance (SPR) and dielectric resonant waveguide (RWGs) can be exploited. Through their design, RWGs allow more flexibility (size of the biomolecule to detect, detection angle…). Using specially designed RWG "Peak-tracking chip", we propose to use spatial information from a simple monochromatic picture as a new label-free bioarray technique. We discuss robustness, sensitivity, multiplex detection, fluidic integration of the technique and illustrate it through bulk refractive index sensing as well as specific recognition of DNA fragment from gyrase A.

KEYWORDS

Bioarray imaging, label-free, refractive index sensing, resonance, grating

INTRODUCTION

Imaging techniques are intrinsically multiplex and have a large range of application in the field of biodetection. Imaging of biomolecules may involve the use of label (fluorophores, particles, quantum dot…). Through resonant properties, it is possible to detect the change of refractive index induced by biomolecules by measuring the intensity change, and therefore perform label-free detection. This has significant advantage as the use of labels induces extra time and consumables, and labels may also infer with biological reactions. Bioarray imaging using resonant properties has been widely developed using surface plasmon resonance imaging (SPRi) [1]. An alternative to plasmonic resonance is guided wave in dielectric structures. However, despite significant advantages (tunability considering the size of the molecule to detect, optical configuration), resonant waveguide grating imaging (RWGs)) has seen less development. While grating fabrication is no longer a limiting factor, this is explained by background issues in highly monochromatic imaging. Direct imaging on RWGs has therefore been demonstrated only in dry phase on reflective substrate [2]. Indeed, background contributions in real-time imaging with fluidic integration renders difficult to extract tiny variations of reflectivity in the order of $\Delta R \sim 10^{-4}$.

These difficulties may be encompassed by the use of intensity sequences. Till now, this has been implemented through spectral or angular sequences, involving costly instrumentation. Angular sequences are obtained by highly precise scanning [3]. Spectrum may be measured either by through monochromatic images sequences by incident wavelength scanning using tunable light source [4] or by using spectro-imager [5]. An alternative way to obtain robust measurement using direct 2D imaging is to exploit spatial information of a specially designed "Peak-tracking chip" (PTC).

"PEAK-TRACKING CHIP" (PTC) PRINCIPLE

Our technique is based on chip with adjacent micropads, of slowly evolving resonant position. For multiplex purpose, micropads are grouped in "tracks", and chip with several "tracks" can be imaged using simple camera. In Figure 1, we give (A) a scheme of the chip and its imaging set-up, with 6 tracks on the surface, as well as (B) detailed track scheme being a sequence of micropads of period Λ_i and filling factor $f_i=d_i/\Lambda_i$, with d_i the width of the groove as illustrated in (C).

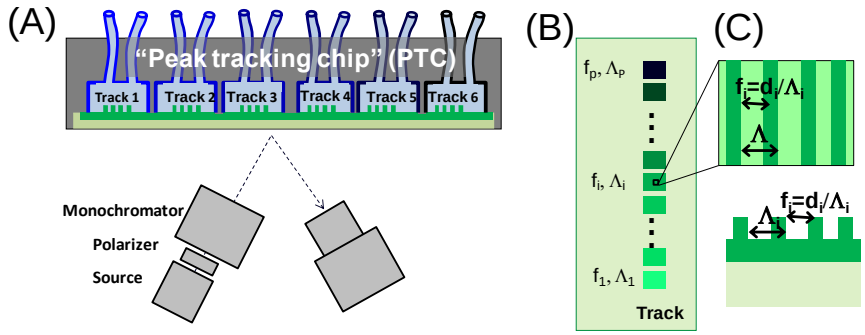

Figure 1: (A) Scheme of the chip and imaging set-up
(B) Top-view of a "track" composed of micropad units with neighboring resonant conditions.
(C) Micropads top view and side view with period Λ_i and filling factor f_i

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With the possibility of using a 2D M×N geometry to use the full capability of bidimensionnal imaging few tens of tracks can be imaged in parallel (taking into account track area ~1mm×200µm and distance between them ~2mm). Units of neighboring resonant condition can be obtained varying resonant waveguide grating geometry. We will discuss detection with chip of varying period and varying filling factor. Bulk experiments will be demonstrated with water/glycerol medium of refractive index varying between 1.333 and 1.474. Biological application will be presented through 100bp~30 kDa DNA strand detection in the framework of mutation in gyrase A observed in resistance to the antibiotic ciprofloxacin in evolution studies [6].

EXPERIMENT

- Set-up and chip structure

The set-up is composed of a white light high power LED illuminating our "Peak-tracking chip", and a simple commercial camera to image the chip (Figure 1). The incident light is TM polarized by using linear glass polarizer, and filtered angularly and spectrally with a monochromator and adapted optical set-up to illuminate the chip under the wavelength λ=547 nm with spectral resolution $\Delta\lambda$=0.2 nm and angle θ=17 ° with angular resolution $\Delta\theta$=0.008 °. The chip has been optimized for imaging in green under an incidence angle close from normal. The central period of the chip is Λ=450 nm and filling factor f=0.5, on a guiding layer SiN of index n~2 and thickness t=0.28Λ etched on h=0.15Λ. The chip is mounted on a fluidic holder, itself placed on an (3 rotations-3 axis) optical holder for fine adjustment of the chip position.

- Bulk experiment

Media of different composition in water-glycerol 100:0, 80:20, 60:40, 40:60, 20:80, 0:100 are introduced in the different chambers. Their refractive index varies between n =1.333 and n =1.474 by step Δn=0.028.

We test refractive index sensing both with period variation PTC (micropads of period from Λ_1=440 nm to Λ_P=460 nm at constant filling factor f=0.5 by step of 2 nm), and with filling factor variation PTC (from f_1=0.3 to f_p=0.7 at constant period Λ=450 nm with step Δf=0.0089). The steps are limited by our e-beam fabrication system. In Figure 2(A) we give the picture of the track with period variation used for sensing the medium n=1.388. The corresponding profile is given in Figure 2(B) with bold line. Profiles of other media with n=1.333 to 1.474 are also given. We then test the filling factor variation PTC. The track picture for the sensed index n=1.388 is given in Figure 2(C) and the corresponding profile in Figure 2(D). From these profiles, we also see that the chosen range for parameters of the PTC (either with period or filling factor variation) allows coverage of the whole range Δn=[1.333-1.474]. Refractive index span for various liquid media is therefore not a limitation of the technique. Filling factor variation PTC allows better sampling of the profiles. For biological sensing, the shift resulting from a ~nm thick layer of induces, changes to detect are in fraction of micropad unit. For accurate and sensitive sensing, biological detection is realized with the filling factor variation PTC design.

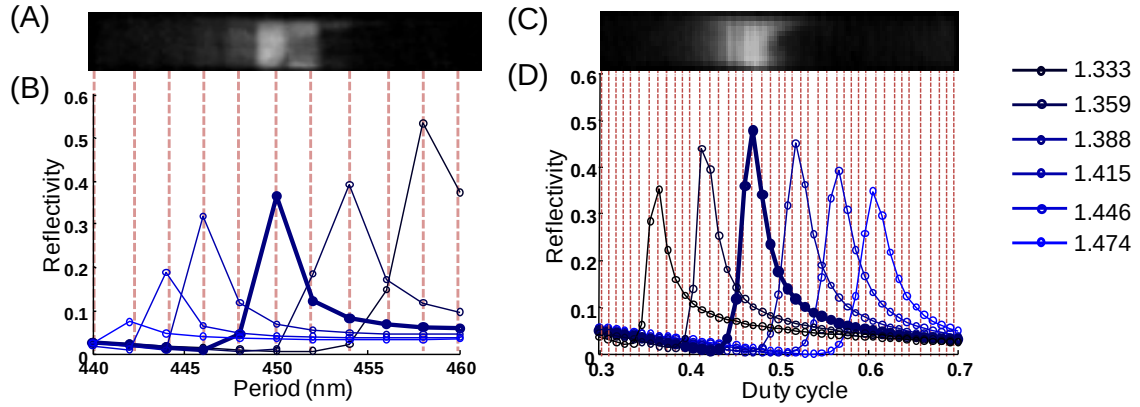

Figure 2. (A) Experimental picture for chip with bulk medium of index 1.388 (B) Profiles for period variation with index between 1.33 and 1.474 (C) Experimental picture for chip with bulk medium of index 1.388 (D) Profiles for period variation with index between 1.33 and 1.474

- Single nucleotide polymorphism (SNP)

The phenomenon of resistance to antibiotic is a growing problem. Studies in a microfluidic device (silicon chip) designed to mimic the spatial heterogeneity of human body demonstrated that emergence of resistance might occur within 10 hours [6]. These studies were realized on *E. coli* model, using ciprofloxacin antibiotic. Whole genome sequencing of resistant colonies after 24 hours experiments demonstrated 4 reproducible SNPs in genes that might be associated with resistance (e.g. gyrA, rbsA, marR). Although dynamics and genotypic characterization already answered some key phenomenon of evolution processes, there is still a lack of information in the time dependence of mutations as well as in the spread of resistance to the population. Evolution of resistance to antimicrobial drugs has concentration, spatial, temporal, bacterial mobility components dependence, which need more detailed characterization. Whole genome sequencing is not appropriate technology for statistical population analysis. Such analysis may benefit of targeting known mutations on a biochip.

Our first efforts concentrate on detecting gyrase A mutations. For this purpose, we choose 3 probes, one corresponding

to the wild type gyrase A DNA fragment, one to the mutated version of DNA fragment, and one negative control. Their sequences are the following: PA: 5'-T_{10}CGCCAGATACCGTGCTAG-3', PAmut: 5'-T_{10}CGCCAGATACTGTGCTAG-3' and PE: 5'-T_{10}TACCGAGCTGTTCCTTGTG-3'. PE corresponds to negative control, specific to unrelated gene from *E. coli* K12 genome. We first immobilize the probes with a hydroxyl based chemistry and amino silane. After appropriate rinsing, the chip and holder are then passivated to avoid unspecific binding. Finally, a 100 base pair DNA target corresponding to the wild type sequence is introduced in the solution. Its sequence TA is 3'-GCGGTCTATGACACGATCGTCCGCATGGCGCAGCCATTCTCGCTGCGTTATATGCTGGT AGACGGTCAGGGTAACTTCGGTTCTATCGACGGCGACTCTG-5'. The calculated melting temperature in 5X SSC buffer solution, is T_m=68.7 ℃ for the exact match case and T_m= 58.6 ℃ for the mismatch, so a difference of 10.1 ℃. Therefore, to be able to detect the targeted SNP, we improved the hybridization buffer by adding 25% formamide and increased the hybridization chamber temperature to 42 ℃. To reduce the background with our transparent chip and have good temperature control, we choose a black anodized aluminum chamber [7]. The chamber is warmed using 6 resistances on the back of the chamber, and the temperature thermo-controlled for proper stabilization. Figure 3(A) gives the scheme of the hybridization experiment. From theoretical modeling, further confirmed on the large index range span; a shift of 0.1 micropad corresponds to a refractive index change of 7×10^{-4} or 0.7 ng/mm^2 which is coherent with our expectation.

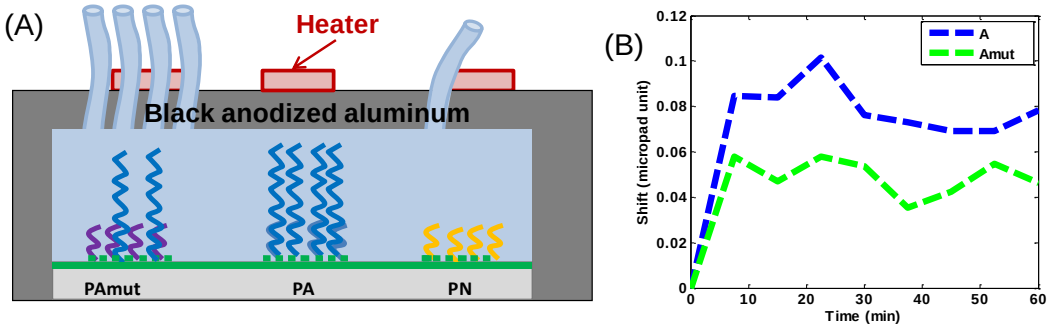

Figure 3: (A) Scheme of the chip which is used as front cover of the fluidic structure with 3 probes: PAmut (mismatch case, single nucleotide polymorphism with the target), PA (mismatch, specific to the target), and PN (negative control) (B) Measured peak shift for the track with probes with matched sequence and mismatch sequence corrected from background contribution

In conclusion, we demonstrated a new label-free imaging method based on RWGs imaged with simple and cheap set-up, and robustness obtained through the "Peak-tracking chip" design. Multiplex aspect and applications to large refractive index span as well as sensitive detection were demonstrated. We demonstrated also the fluidic integration for imaging with dark background and simple implementation of temperature control for real-time SNP detection.

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REFERENCES

[1] "Surface plasmon resonance sensors: review" J. Homola, S. Yee Sinclair, G. Gauglitz, Sens. Act. B 54, 3-15 (1999)

[2] "2D label-free imaging of resonant grating biochips in ultraviolet" K. Bougot-Robin, J-L. Reverchon, M. Fromant, L. Mugherli, P. Plateau, and H. Benisty, Opt. Exp. 18, 11472 (2010)

[3] "Label-free prehybridization DNA microarray imaging using photonic crystals for quantitative spot quality analysis", S. George I.D. Block, S.I. Jones, P.C. Mathias, V. Chaudhery, P., Vuttipittayamongkol, H.Y. Wu, L.O. Vodkin, and B.T. Cunningham, Anal Chem. 2010, 82(20):8551-7.

[4] "Resonant waveguide grating imager for live cell sensing", A.M. Ferrie, Q. Wu, and Y. Fang, Appl. Phys. Lett. 97 (2010)

[5] "A new method for Label-free imaging of Biomolecular interactions" P.Y. Li, B. Lin, J. Gerstenmaier, and B.T. Cunningham, Sens. Act. B, 99, (2004)

[6] "Acceleration of Emergence of Bacterial Antibiotic Resistance in a Microenvironment" Q. Zhang, G. Lambert, D. Liao, H. Kim, K. Robin, C.K. Tung, N. Pourmand, and R.H. Austin, Science 333 (6050), 1764 (2011)

[7] R. Kodzius, K. Xiao, J. Wu, X. Yi, X. Gong, I. G. Foulds, W. Wen, "Inhibitory effect of common microfluidic materials on PCR outcome", Sens. Act. B: Chemical, 161, 349 (2012)

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