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# Contrast enhancement of UV absorption and improved biochip imaging

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**Abstract:** Biochip using UV absorption for selective DNA or proteins imaging may take advantage of sensitivity enhancement thanks to either multilayer structures or grating structures. We discuss the interest of coupled angular and spectral illumination.

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## 1. Introduction

UV imaging of biochips exploits DNA and proteins absorption respectively at 260 nm and 280 nm [1]. The imaging set-up includes a UV source, illuminating a biochip coated with biological spots of diameter 100  $\mu\text{m}$  whose reflectivity depends on surface absorption. The reflected signal is measured with a spectrally selective AlGaIn detector developed for biosensing application [2], ( $\lambda_{\text{max}} = 278 \text{ nm}$ ,  $\Delta\lambda = 20 \text{ nm}$ ), selecting the bandwidth of protein absorption around 280 nm. In conjunction with LED sources or large spectrum sources, it avoids the use of lasers often awkward for imaging. Fig. 1(b) gives the responsivity spectrum of our detector and of a UV LED ( $\lambda_{\text{max}} = 280 \text{ nm}$ ,  $\Delta\lambda = 12 \text{ nm}$ ) [3], superposed to the absorption spectrum of a methionyl-tRNA synthetase protein (MetRS).

Considering a thin absorbing layer of absorbing coefficient  $\alpha$  and of thickness  $\ell$  on a reflective multilayer chip of reflectivity  $R_{\text{chip}}$ , the absorption induced contrast  $C$  is defined by equation (1).

$$C = -\frac{\Delta R}{R_{\text{chip}}} = -\frac{R_{\text{chip}} - R_{\text{protein}}}{R_{\text{chip}}} > 0 \quad (1)$$

Using perturbation analysis, the contrast  $C$  in this configuration is  $C = \alpha \ell (1 + \sqrt{R_{\text{chip}}})^2 / \sqrt{R_{\text{chip}}}$ , where  $\alpha \ell$  is the absorption in transmission. For a reflectivity  $R_{\text{chip}} = 0.72$  (quarter-wave silica layer on aluminum), the contrast enhancement  $F = C / \alpha \ell$  is 4.0 (ref [1] and more particularly footnote in ref. [20]), which is already sufficient to detect a monolayer of proteins of absorption of order  $10^{-3}$ . Fig. 1(c) gives an image of a multilayer biochip coated with the MetRS protein after specific recognition with an antibody (immunoglobulin G). To attain real-time detection of molecular binding events, the sensitivity has to be further increased. This is achieved by using structured biochips.

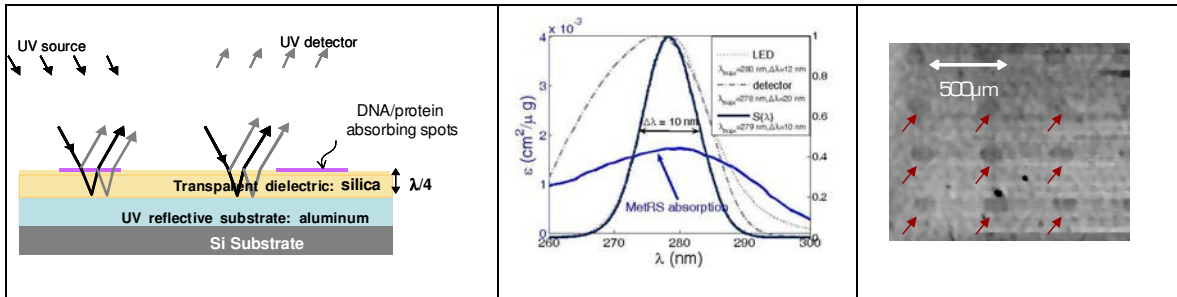


Fig. 1. (a) Experimental set-up; (b) AlGaIn responsivity spectrum and LED emission spectrum superposed to the absorption spectrum of MetRS protein; (c) MetRS coated biochip imaged after specific recognition with IgG antibody. Biological spots are indicated by arrows.

## 2. Contrast enhancement using structured biochips

Structured surfaces enables a largely enhanced light coupling (manifested classically by reflection anomalies, Fano shapes...) with the absorbing layer at the chip surface, translating into an enhancement of the absorption contrast. The highest sensitivity occurs at resonance condition, given by Eq. 2 for grating structures of period  $\Lambda$ , where  $N$  is the effective index of the mode,  $\theta_r$  the incidence coupling angle, and  $\lambda_r$  the resonance wavelength.

$$n_a \sin(\theta) = N(\theta_r) - m_g \frac{\lambda_r}{\Lambda} \quad (2)$$

Here, the resonance condition  $(\lambda_r, \theta_r)$  follows a quasi linear trend in the region of interest. We propose that selecting  $(\lambda_r, \theta_r)$  couples all along this line provides an optimal sensitivity as it realises the best possible

combination of the resonance anomaly with an optimal use of the source spectrum. To evaluate the measured signal  $R \equiv R(\alpha)$  (Eq. 3), we integrate over the resonance by convolving the signal with the instrumental sensitivity  $S(\lambda)$  (including both spectral sensitivity of the detector and emission spectrum of a deep-UV LED, Fig.1), and the angular resolution with a Gaussian  $G(\theta)$ .

$$R = \int_{\lambda} \int_{\theta(\lambda_r)} R_0(\theta_r, \lambda_r) G(\theta) S(\lambda) d\theta d\lambda \quad (3)$$

Experimentally, the resonance region can be extensively excited by taking inspiration from existing configurations based on diffracting optical elements (prisms, gratings) [4,5]. Such optical configurations will be discussed in the presentation.

We now evaluate quantitatively the advantage of this “resonance-tracking” integration for our contrast enhancing chip, composed of a silicon mirror, covered with transparent dielectrics: silica ( $n \sim 1.5$ ) and silicon nitride ( $n \sim 2$ ), designed to be highly sensitive to an absorbing layer at 280 nm. In Fig.2, we show the zero<sup>th</sup> order reflectivity  $R_0$  (specular reflection), and the induced contrast in this diffraction order. The reflectivity is calculated using a scattering matrix formalism [6], and includes dispersion laws of the materials as determined from ellipsometry measurements. To model the sole absorption effect, the absorbing layer index is  $1+0.02i$ . The width of the resonance depends on the corrugation thickness. For an angular resolution of  $0.5^\circ$ , we determine that a corrugation of 20 nm and a period of 278 nm are reasonable parameters to obtain a contrast enhancement of 50, becoming 40 when integrating across the angular resolution. Nonetheless, the integration on  $\lambda$  leads to a photon flux 10.6 times higher than the one in 1 nm spectral bandwidth, whereas the contrast is kept at the same value as for this narrow-bandwidth resonant configuration. Conversely, setting  $42^\circ$  illumination for all wavelengths (collimated “white” beam), the contrast enhancement does not exceed 12. Hence, our proposed illumination combination is very interesting to retain a high flux with the associated low noise or quick detection, while not compromising sizably the resonant character of the system, nor the excellent imaging conditions provided by LEDs.

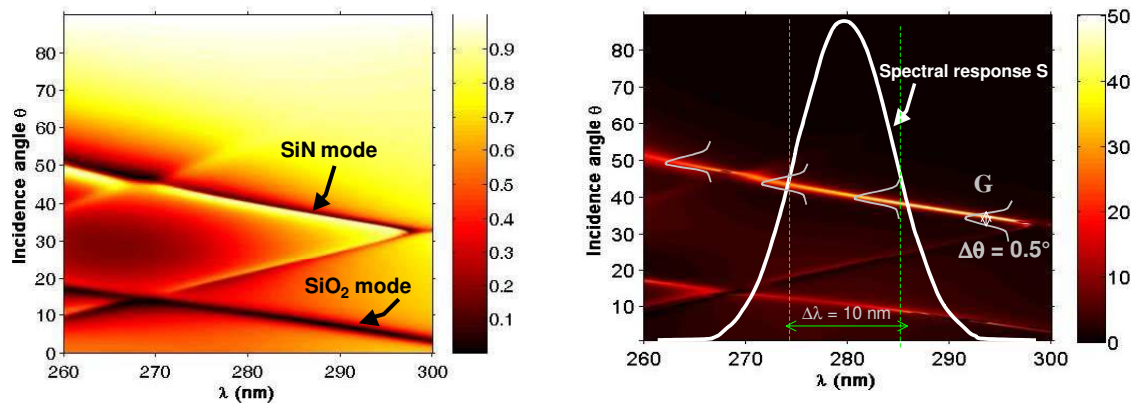


Fig. 2. (a) Reflected signal in the zero<sup>th</sup> order. Modes lying in silica and silicon nitride are indicated by arrows (b) Contrast enhancement  $G=C/\alpha \ell$ . The mode lying in SiN is more sensitive to the absorbing layer, since this layer is located on its surface.

### 3. Conclusion

In this paper, we address structures that are highly-sensitive to UV absorption of biological spots (multi-layers or resonant structures). Specific optical set-up for imaging structured biochips at resonant sensitivity condition ( $\theta_r, \lambda_r$ ) but avoiding the cost and speckle problems associated with lasers will be presented. For instance, configurations including LEDs or broad sources, associated with spectrally selective AlGaIn detectors will be shown to combine the advantage of the large resonant sensitivity with that of a high photon flux, and thus a low noise. Experimental results and numerical evaluations of the sensitivity enhancements will be given.

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