High-sensitivity screening of soluble ERBB2 in different cell lines using a combined label-free and fluorescence biosensing platform

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ERBB2 (also known as Neu, or HER2) is a tyrosine kinase receptor that acts as master integrator of epidermal growth factor receptor signaling, and regulates a variety of cell proliferation, growth and differentiation pathways. In particular, ERBB2 overexpression occurs in approximately 20–30% of breast cancers and unless treated with ERBB2-targeted therapies this breast cancer subtype is associated with a dismal prognosis, collocating breast cancer as the most common, potentially fatal cancer of women [1].
According to international guidelines (College of American Pathologist, http://www.cap.org), the target therapy based on Trastuzumab is administered when IHC staining either reaches a 3+ intensity, or is at least 2+ and the gene is amplified, as assessed by FISH. This method, although accurate and highly standardized, relies on semi-quantitative, subjective IHC scores, and yields a discontinuous scale. In the present work, we propose a new analytical technique that is able to quantify very low concentrations of ERBB2 cancer biomarkers in biological complex matrices.
In particular, we report on the use of one-dimensional photonic crystal (1DPC) biochips to detect clinically relevant concentrations of ERBB2 expressed in different cell lines. To do that, we have developed an optical platform, combining both label-free and fluorescence detection, which makes use of 1DPC biochips tailored with monoclonal antibodies for highly specific biological recognition. The excitation of a Bloch surface wave (BSW) is obtained by a prism coupling system leading to a dip in the angular reflectance spectrum [2]. Similar to surface plasmon resonance, the measurement of the shift of the position of such a dip, due to refractive index perturbations at the surface, is exploited for biosensing (Label-free mode, LF). Moreover, in the presence of fluorescent labels at the surface, the platform analyses the BSW biochip also in the enhanced fluorescence mode, thus obtaining further information on the cancer biomarker assay and making bio-recognition more robust and sensitive [3]. For fluorescence operation mode (FLUO), a limit of detection below 1 ng/mL, about 10 times lower than label-free approach, has been attained, enabling an ultimate resolution for ERBB2 quantitation that is used to successfully discriminate cell lines over-expressing different amounts of ERBB2. Such a method definitely meets international recommendations (15 ng/mL) for diagnostic ERBB2 assays that in the future may help to more precisely assign therapies counteracting cancer cell proliferation and metastatic spread. Further development of the platform, leading to the fabrication of an integrated point-of-care instrument making use of plastic disposable biochips [4], will be also reported.

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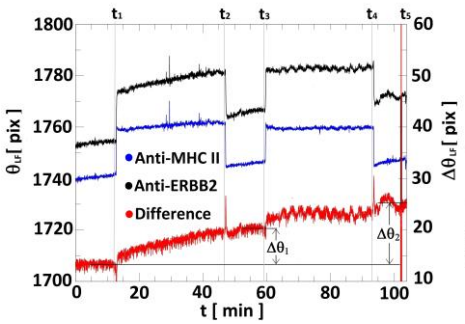

Figure 1. Label-free sensograms recorded during the calibration procedure for the detection of ERBB2 spiked in D-PBS 1X at 20 ng/mL. The curves are for the signal spot (black, left axis), reference spot (blue, left axis) and difference (red, right axis).

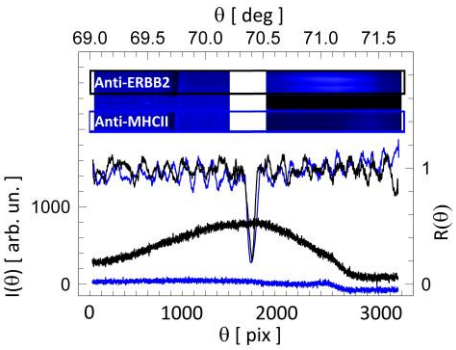

Figure 2. Experimental curves recorded by the CCD camera during the assay with ERBB2 spiked in D-PBS 1X. The curves refer to either LF (top curves) or FLUO (bottom curves) operation for the signal (black) and reference (blue) sensing spots. R(θ) (right axis) shows the BSW resonance in the LF mode. I(θ) shows DyLight 650 fluorescence emission. (Insets) CCD images in the LF (left) and FLUO (right) modes. In both images the top region is the signal spot and the bottom region is the reference spot.