Detection of breast cancer biomarkers by means of Bloch Surface Wave-based biosensors

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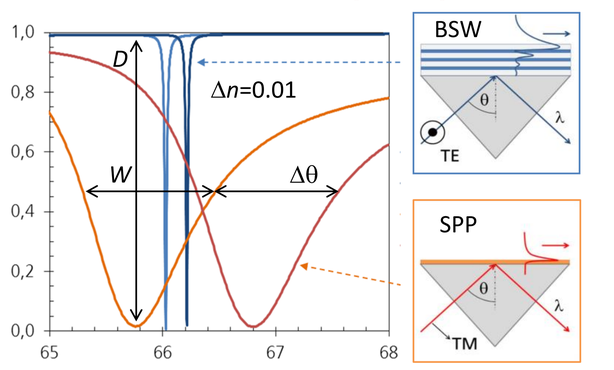
Introduction

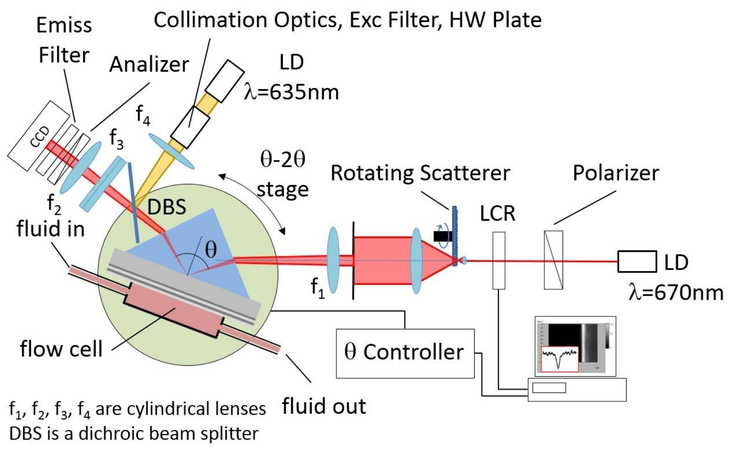
Optical label-free biosensing approaches based on the excitation of Bloch Surface Waves (BSW) at the surface of one dimensional photonic crystals are a practical route to enhanced resolution and constitute an attractive alternative to surface plasmon resonance (SPR). Compared to SPR, the main advantages of BSW lie in the favourable properties of the 1DPC such as the small absorption of the dielectric materials and the tunability of the 1DPC to operate at any wavelength range. Besides, the use of BSW in fluorescence-based bio-sensing does not suffer from quenching of the fluorophores emission at the 1DPC surface. In this work, a BSW biochip and a combined label-free (**LF**) and fluorescence (**FLUO**) read-out system are implemented and tested in a pre-clinical enviroment.

Bloch Surface Waves (BSW) on 1DPC



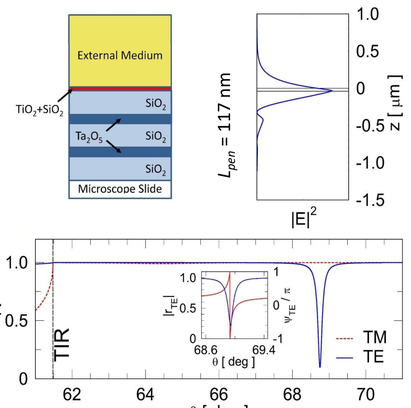
$$S_V = \frac{\Delta\theta}{\Delta n} \qquad FoM = \frac{S_V \cdot D}{W} \qquad LoD = \frac{\sigma}{S_V} \propto \frac{1}{FoM}$$

Proof of concept - Laboratory test bench



f_1, f_2, f_3, f_4 are cylindrical lenses
DBS is a dichroic beam splitter

Optimized 1DPC (VIS range)



SENSITIVITY

S_V =31.8 deg/RIU

FIGURE OF MERIT

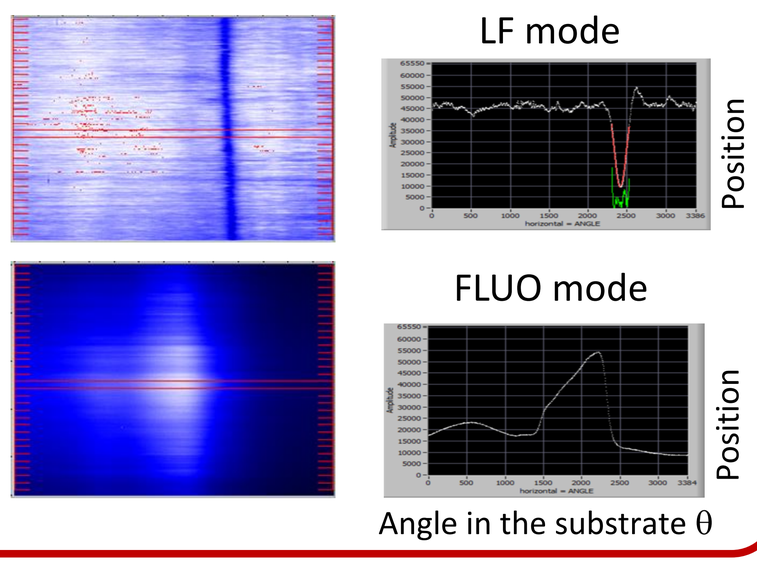
FoM_{BSW} = 290 RIU^{-1}

REFRACTIVE LOD

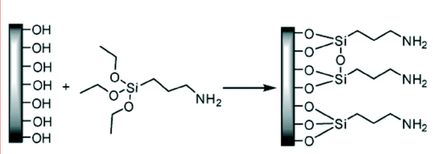
$LoD = 6.5\times10^{-7}$ $RIU/Hz^{1/2}$

$n_{SiO2} = 1.474 + j\ 5\times10^{-6}$ $d_{SiO2} = 275$ nm ($d_{SiO2}^{top} = 20$ nm)
$n_{Ta2O5} = 2.160 + j\ 5\times10^{-5}$ $d_{Ta2O5} = 120$ nm
$n_{TiO2} = 2.28 + j\ 1.8\times10^{-3}$ $d_{TiO2} = 20$ nm

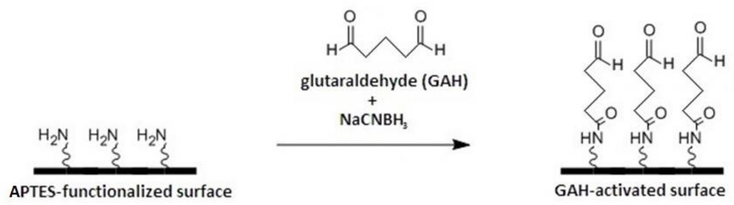
Combined label-free (LF) and fluorescence (FLUO) operation modes



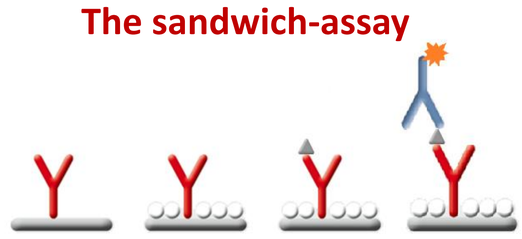
Optimized surface chemistry and biofunctionalization



The 1DPC top layer is cleaned and oxydrilized with piranha solution, silanized with APTES and activated (glutaralaldehyde) to perform amidic bond with primary amines.

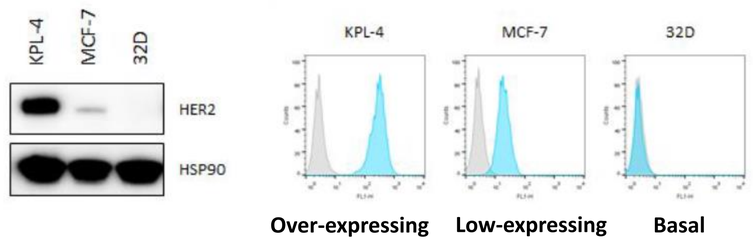


The sandwich-assay



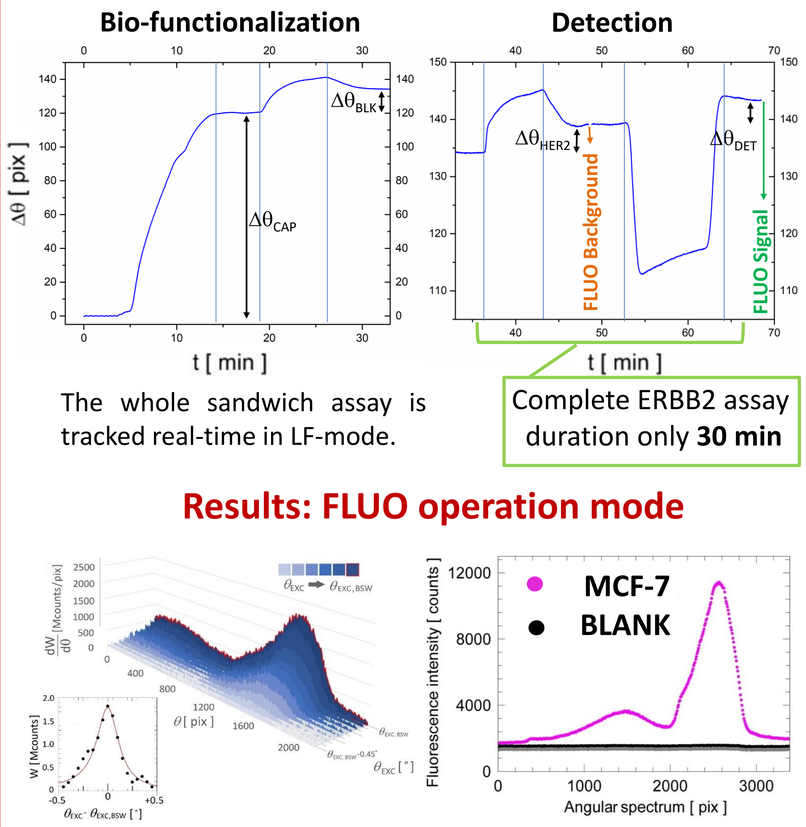
After the bio-functionalization with anti-HER2 mAb (capture), surface is passivated and exposed to lysates. If present, the biomarker is recognized by the primary antibody and furtherly conjugated to a second specific labeled antibody (detection).

Standard techniques as reference: FACS and Western Blot



Several lysates have been preliminarily characterized by standard techniques like fluorescence-activated cell sorting (FACS) and Western Blot to distinguish them in over, low and basal HER2-expressing.

Results: LF operation mode



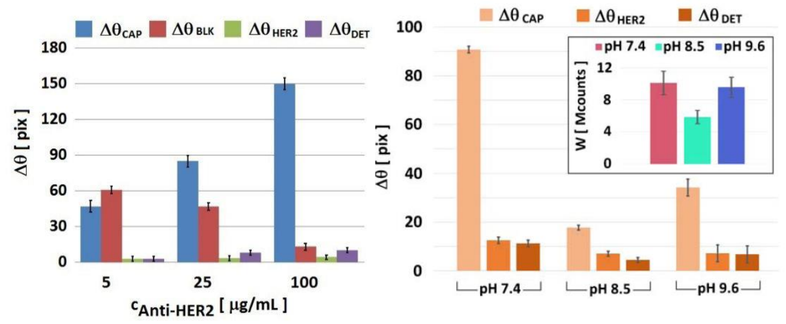
The whole sandwich assay is tracked real-time in LF-mode.

Complete ERBB2 assay duration only **30 min**

Results: FLUO operation mode

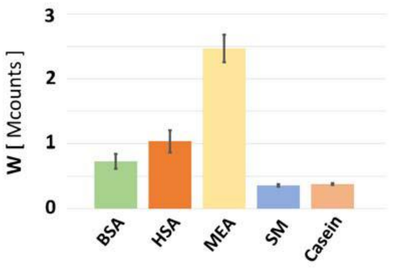
Parameters optimization:

Capture antibody concentration and pH (LF & FLUO)



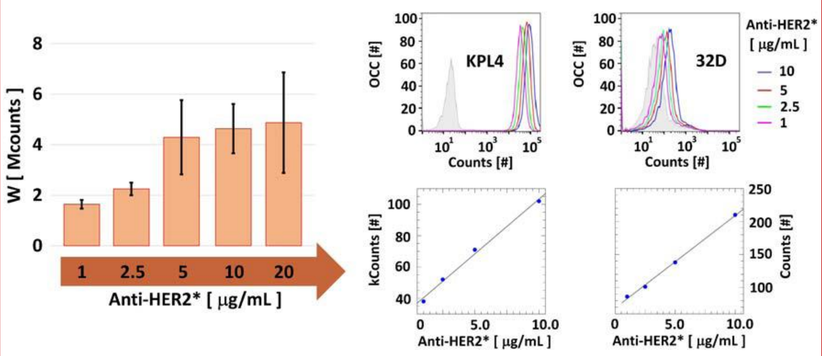
LF residual shifts for the four steps constituting the whole cancer biomarker assay as a function of increasing concentrations of capture Anti-HER2.

Passivation solution (FLUO)



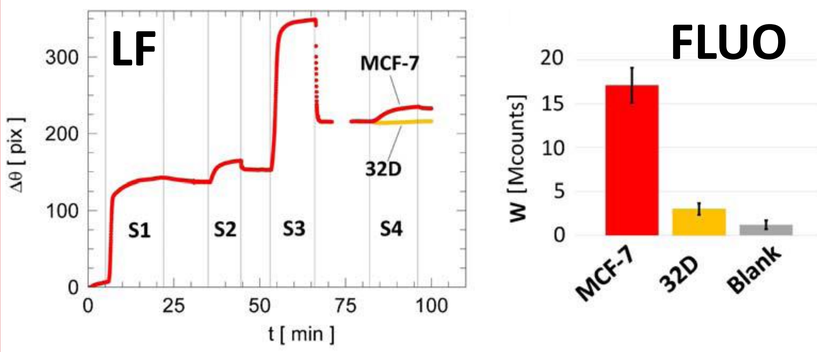
BSA has been selected among several passivation strategies because minimizes non-specific fluorescence signal, without the risk of biotin contamination as for skim milk [SM] and casein).

Detection antibody concentration (FLUO & FACS)



The integrated fluorescence intensity confirms FACS results for detection antibody concentration.

Optimized HER2 detection assay in cell lysates



From the final analysis of the complete (LF) sensorgrams related to a breast cancer assay for HER2 detection and the resulting integrated fluorescence (FLUO) intensities for MCF-7 (red), 32D (yellow), and the blank (grey), we can distinguish low-expressing (32D) lysates from a negative sample (blank).

Conclusions

- BSW approach → Tune resonance properties
  → Enhanced fluorescence
- Angular resolution used for label-free detection yields spectral resolution in fluorescence detection
- Sandwich assay for soluble HER2 detection (likely for a wide range of proteomic biomarkers)
- Duration time for one assay dramatically reduced: only 30 min
- Enabling discrimination between low and basal-expressing HER2 in cell lysates.

References

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