High-sensitivity screening of cancer biomarkers in cell lysates using one dimensional photonic crystal based biosensors

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OUTLINE

- What is and Why (1D)Photonic Crystals
- The Biosensor platform
- The surface chemistry
- Bioassay Optimization and Results

Photonic Crystals - Block Surface Waves and Band Structure

Photonic Band Structure

$$K_B(k_z,\omega) = \cos^{-1}\left[\frac{1}{2}\mathrm{Tr}\left(T_{N,N-2}\right)\right]$$

Propagating waves (permitted bands)

$|1/2\ \mathrm{Tr}\ (T_{N,N-2})| < 1$

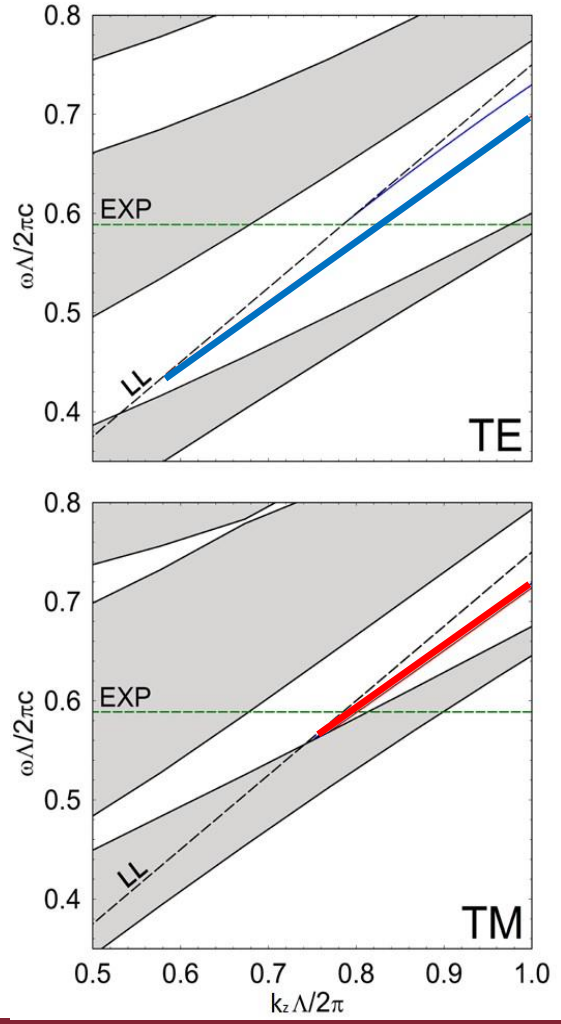
Real K_B

Evanescent waves (forbidden bands)

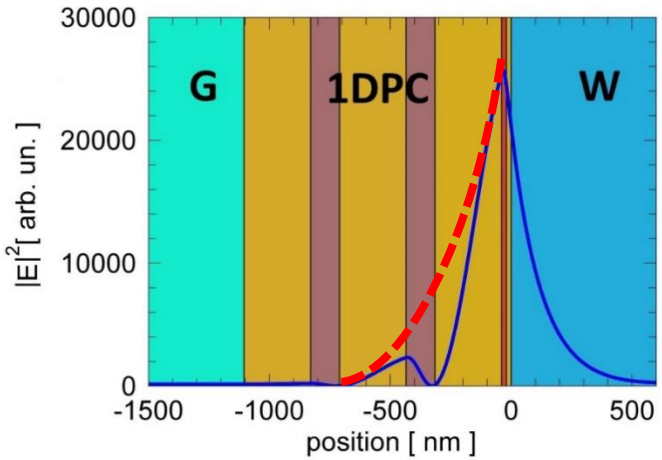
$|1/2\ \mathrm{Tr}\ (T_{N,N-2})| > 1$

Imaginary K_B

$K_B = (m\pi/L) + jK_i$



Truncated 1DPC

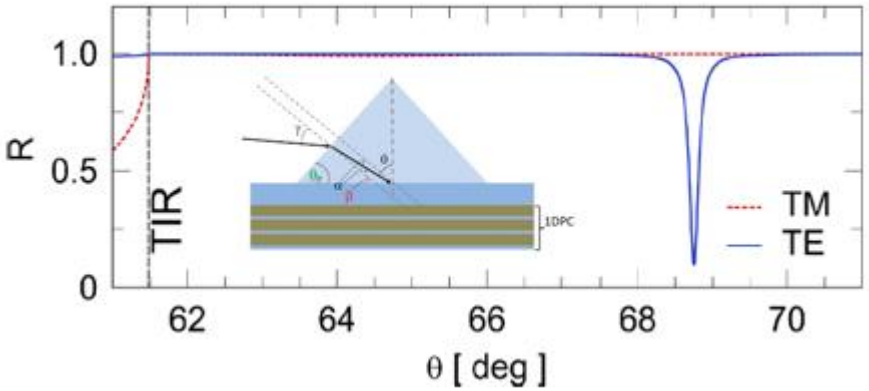


$$E(x) = \begin{cases} \alpha e^{q_e x}, & x \le 0, \\ E_{K_B}(x) e^{jK_B x}, & x \ge 0. \end{cases}$$

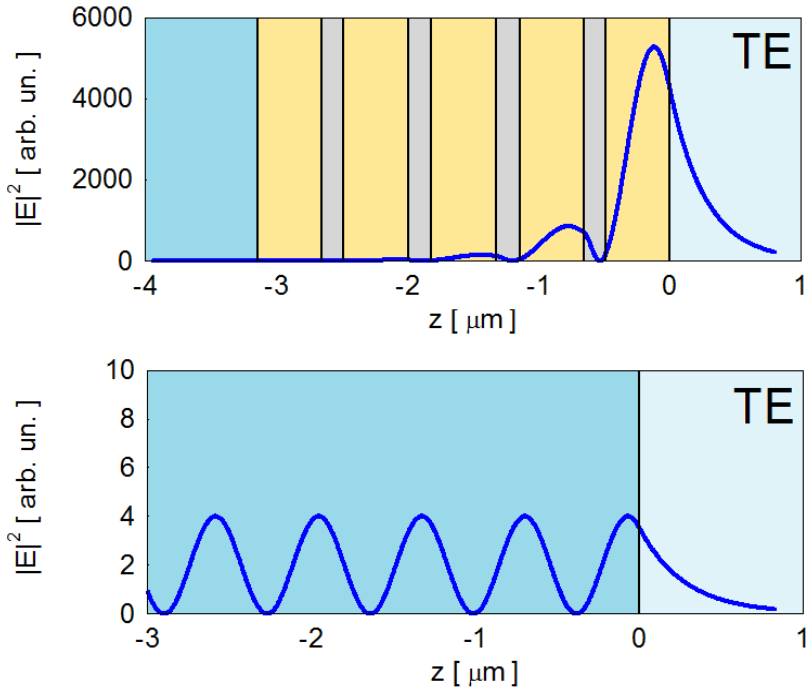
$$q_e = \left\{k_z^{\,2} - \left[\frac{\omega}{c} n_e\right]^2\right\}^{1/2}$$

$K_B = m\pi/\Lambda \pm jK_i$ ϵ C → **BSW live in the forbidden bands**

Kretschmann-Raether coupling configuration



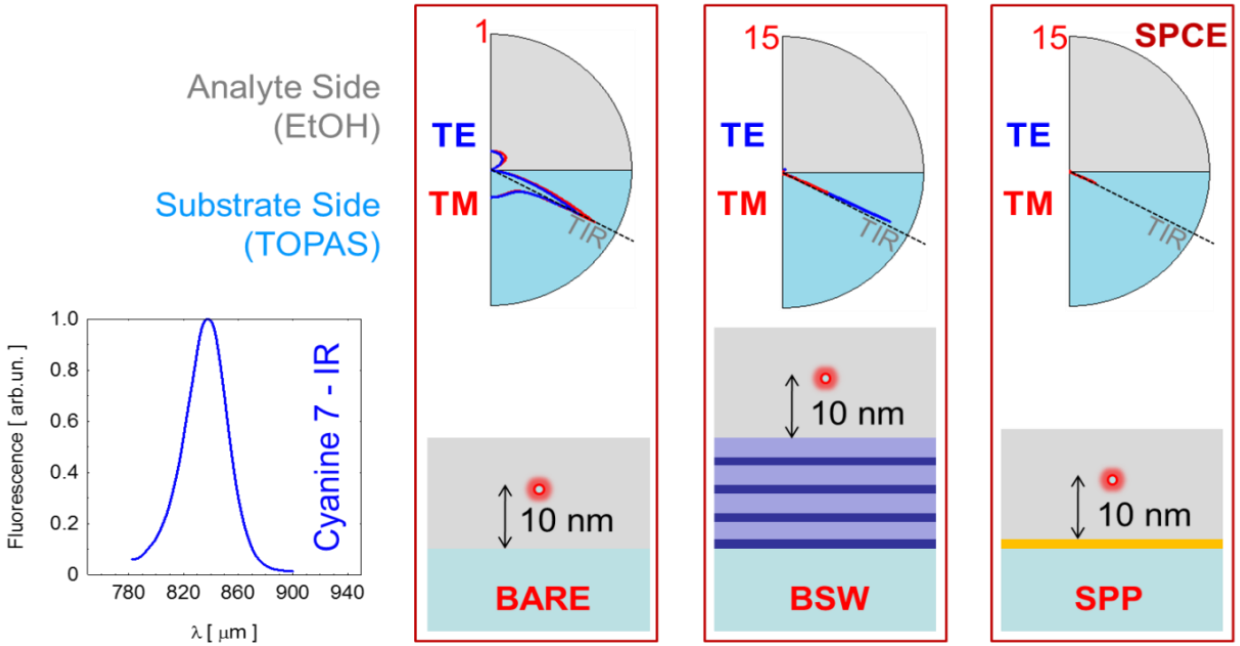
Photonic Crystals – Field Enhancement and Fluorescence Channeling



Field Enhancement :

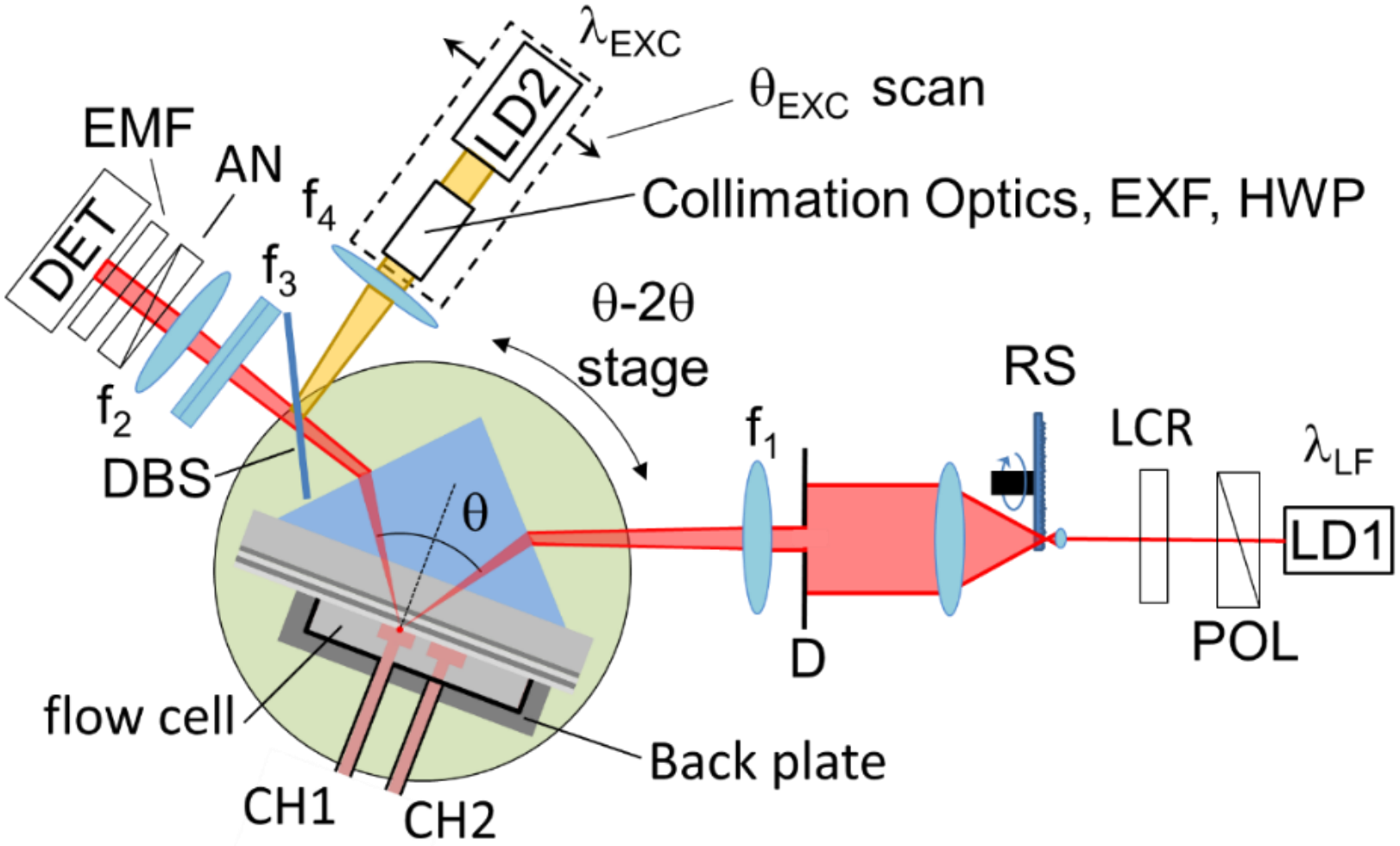
- ~10^2 compared to SPP
- ~10^3 compared to bare surface

Ability of "Molding the Flow of Light" *

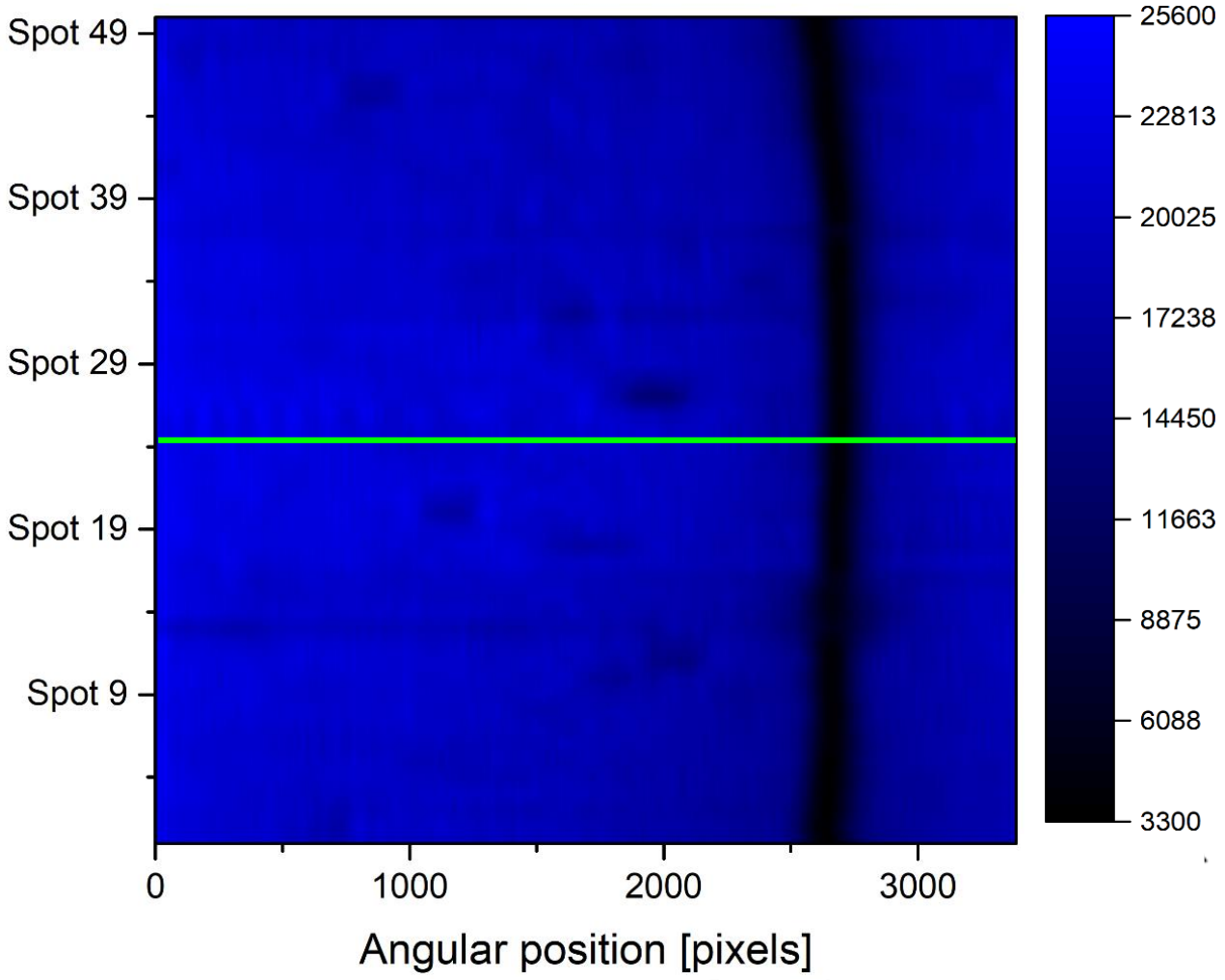


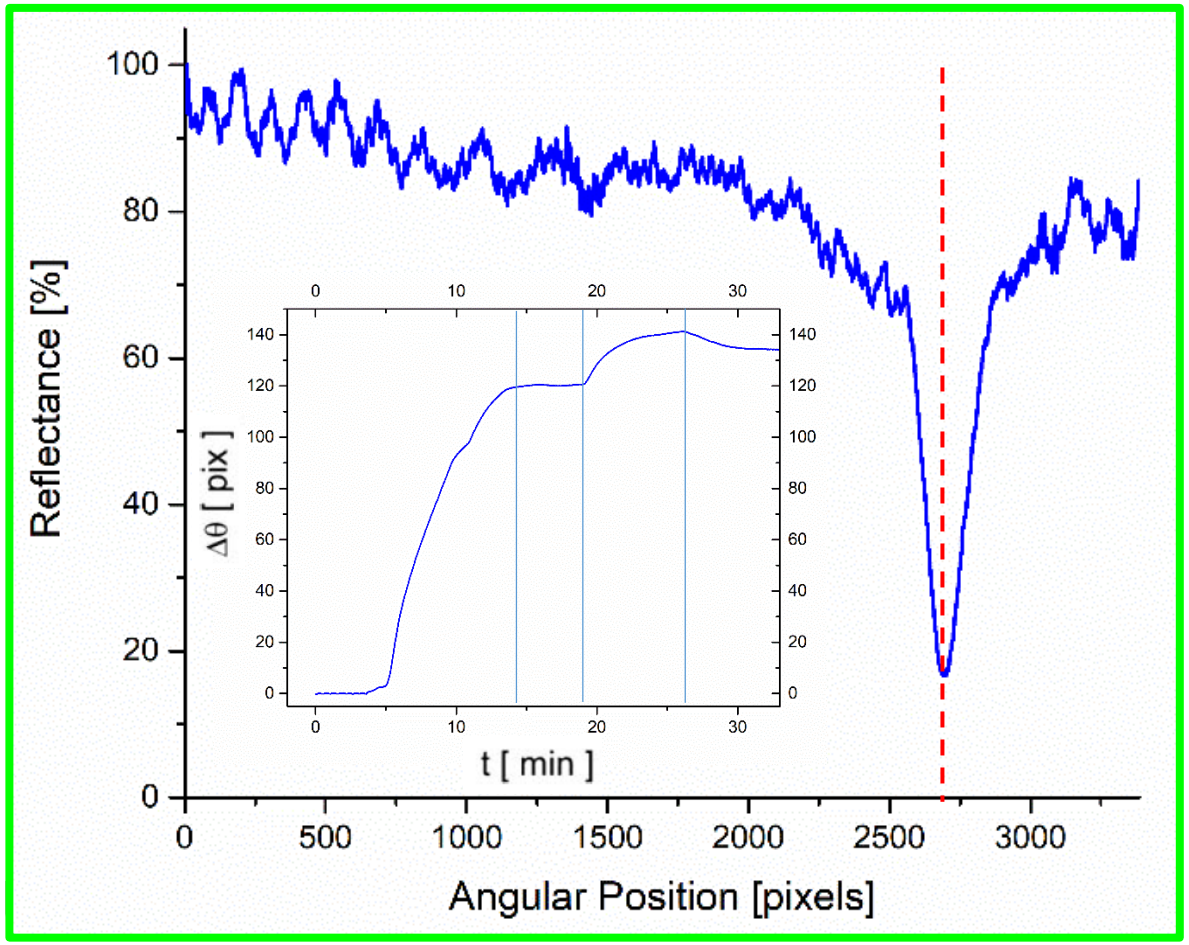
*Joannopulos, J. D.; Photonic Crystals: Molding the flow of light, Second Edi.; Princeton University Press; Princeton, New Jersey, USA, **(2008)**.

SETUP AND OPERATION – OPTICAL SCHEME

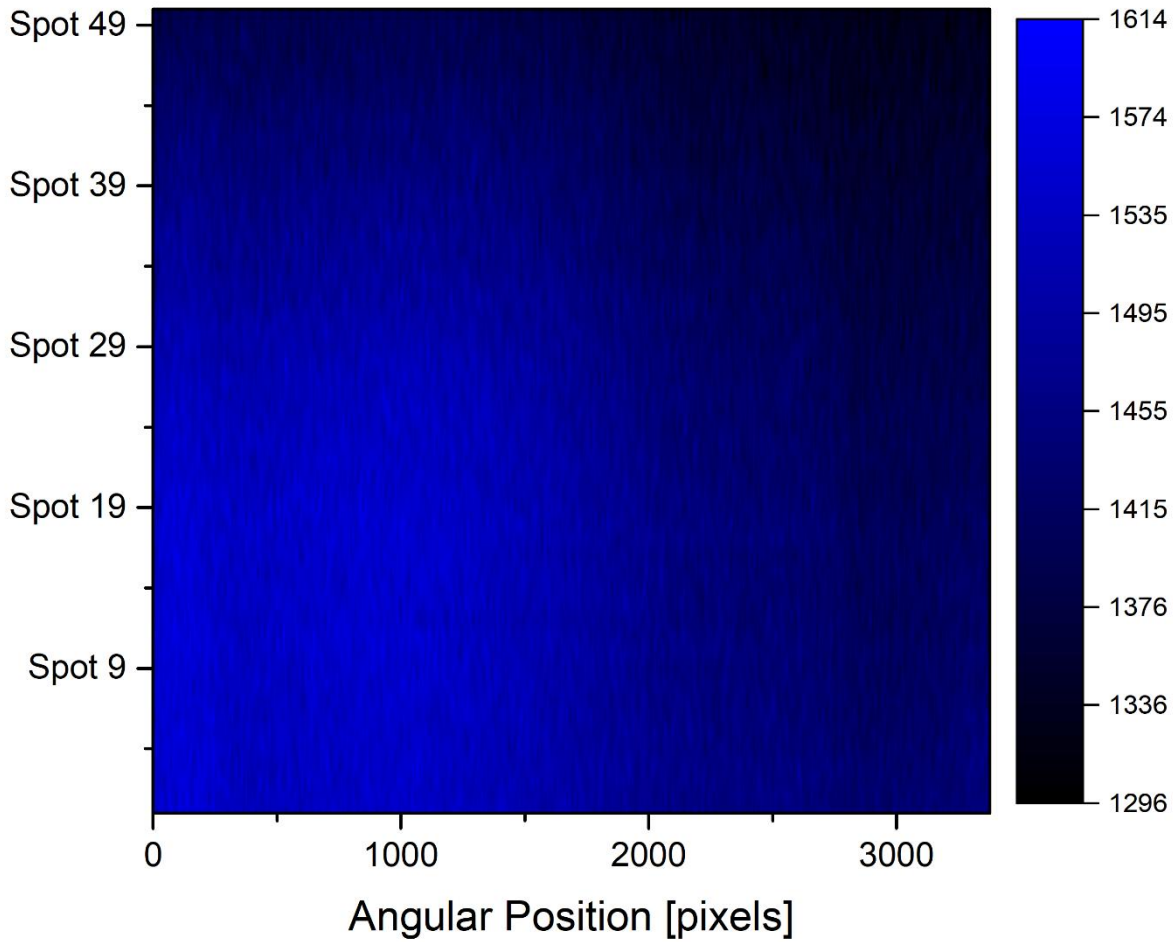


SETUP AND OPERATION – LABEL FREE

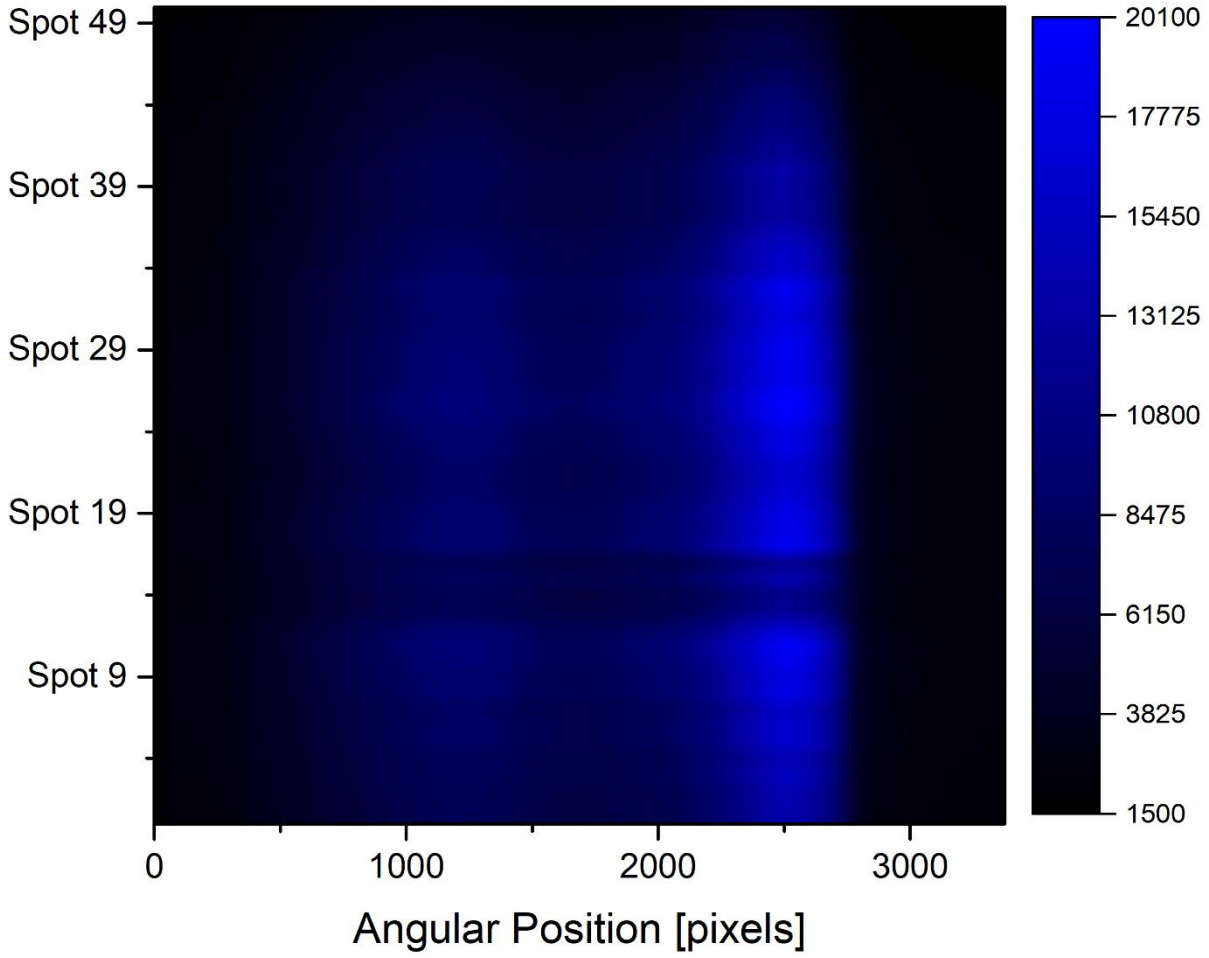




SETUP AND OPERATION - FLUORESCENCE

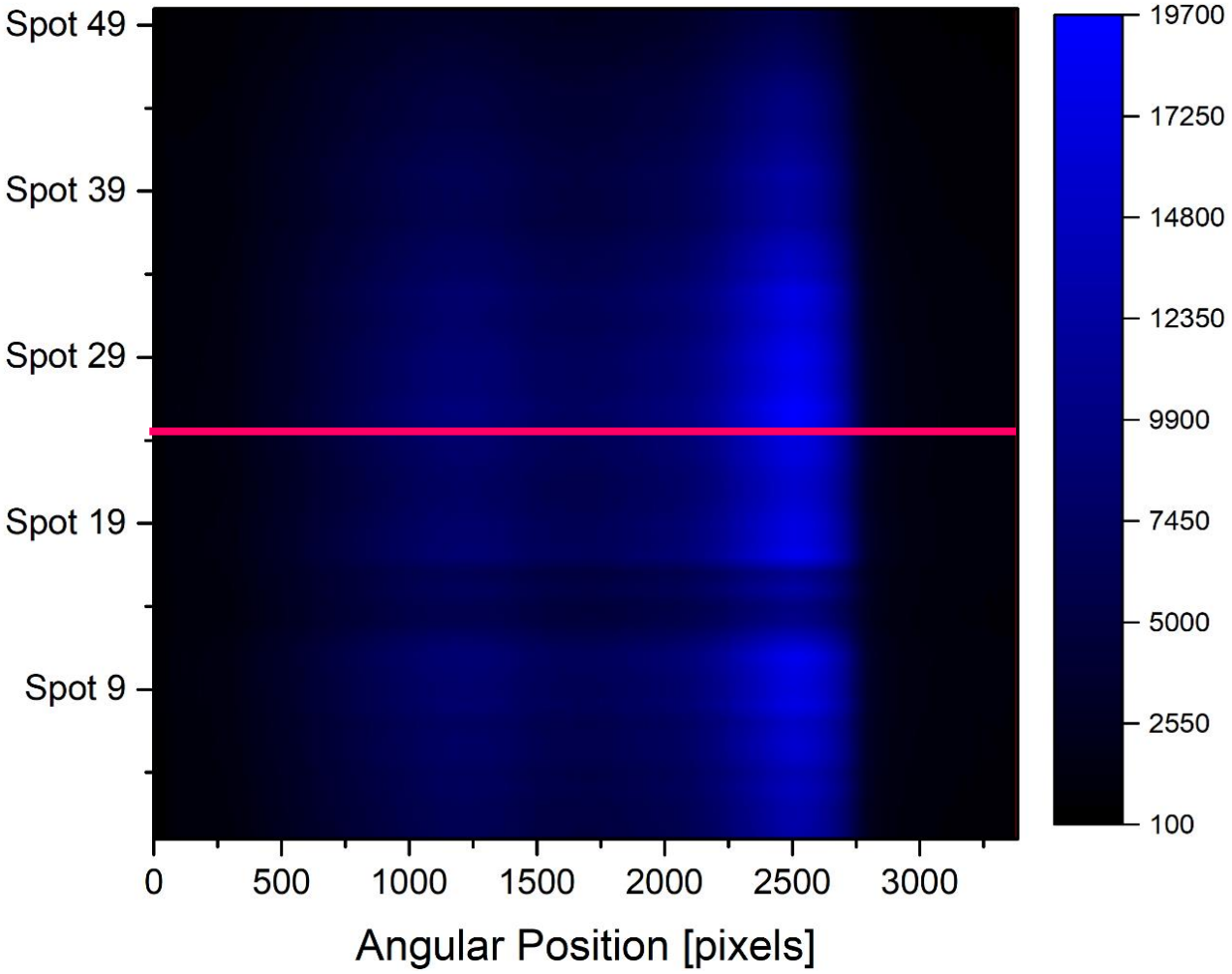


Background

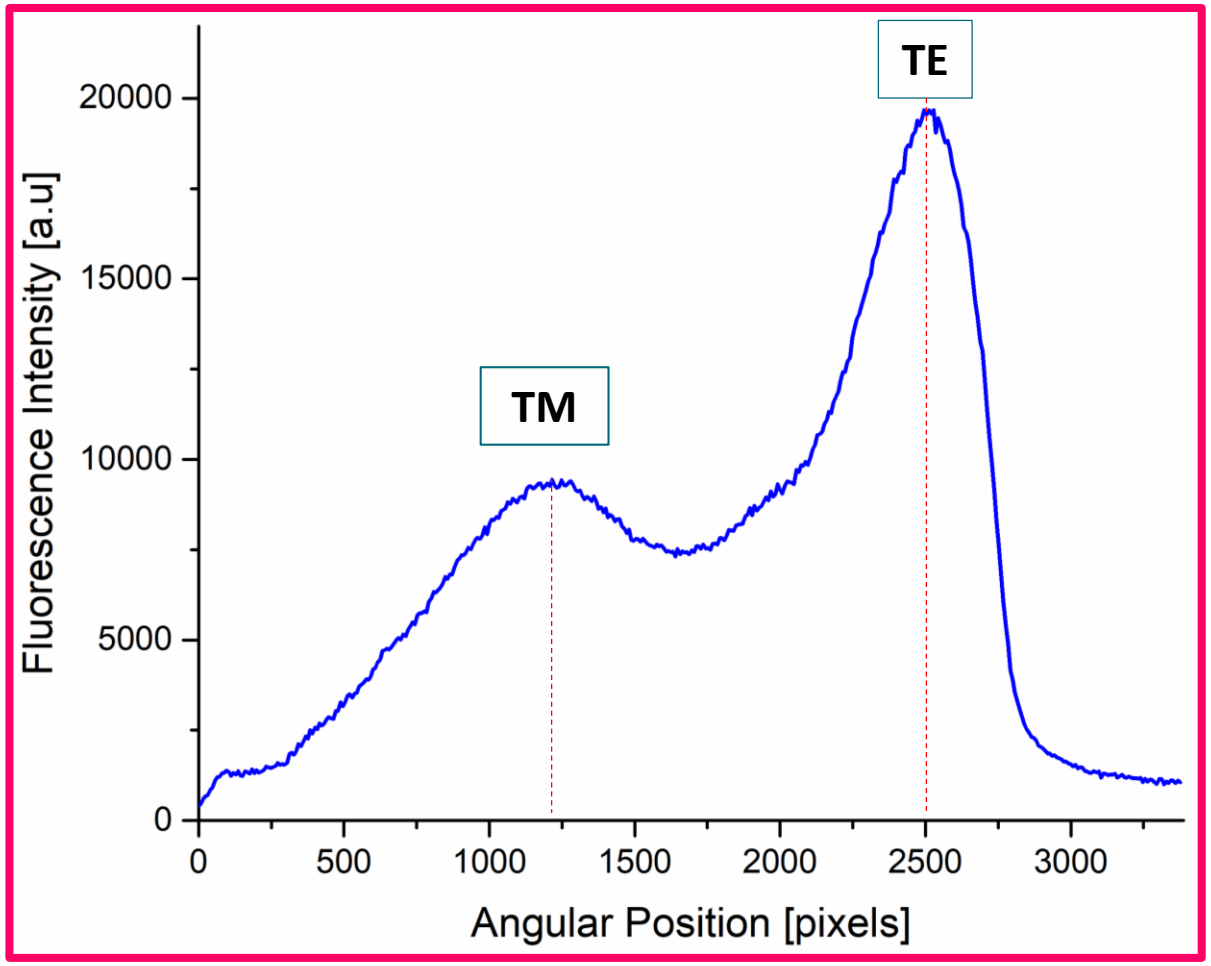


Final fluorescence signal

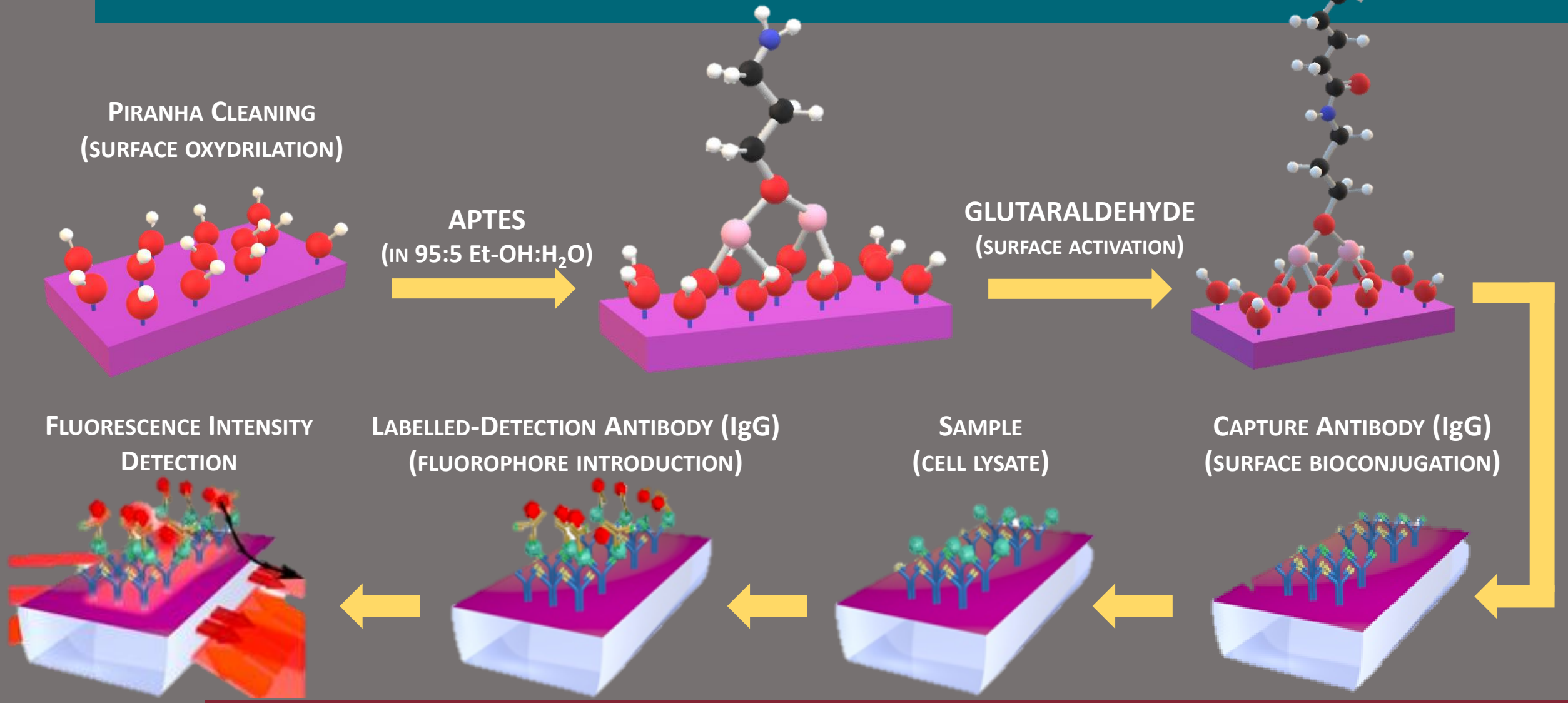
SETUP AND OPERATION - FLUORESCENCE



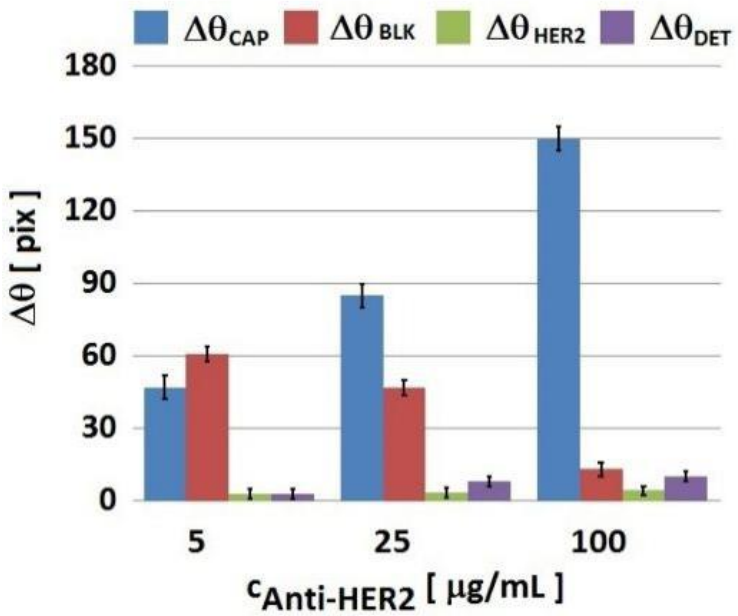
Background subtracted fluorescence map

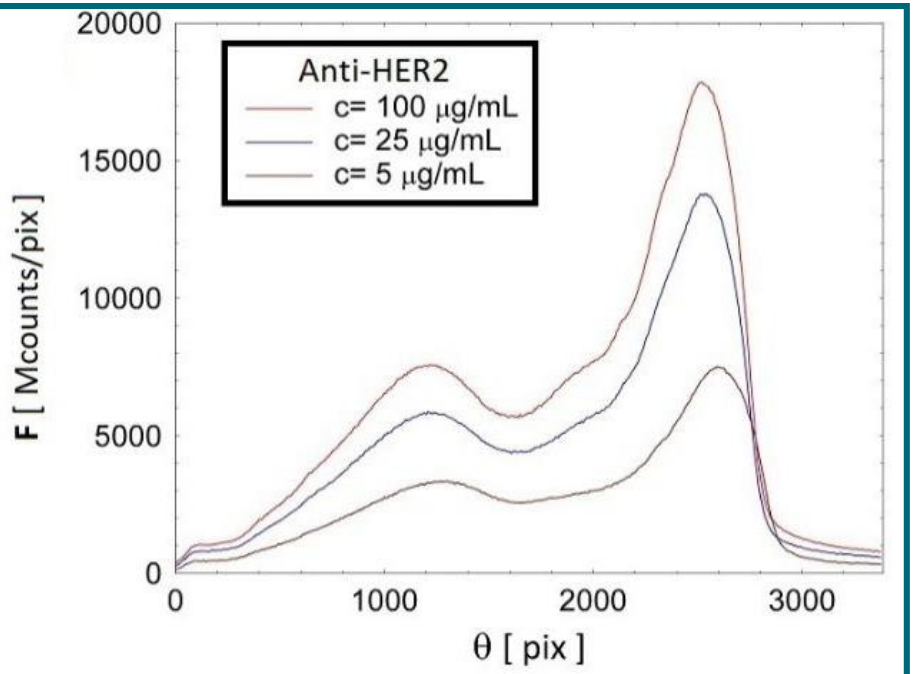


Surface Chemistry and the Sandwich Assay



BIOASSAY OPTIMIZATION – CAPTURE ANTIBODY

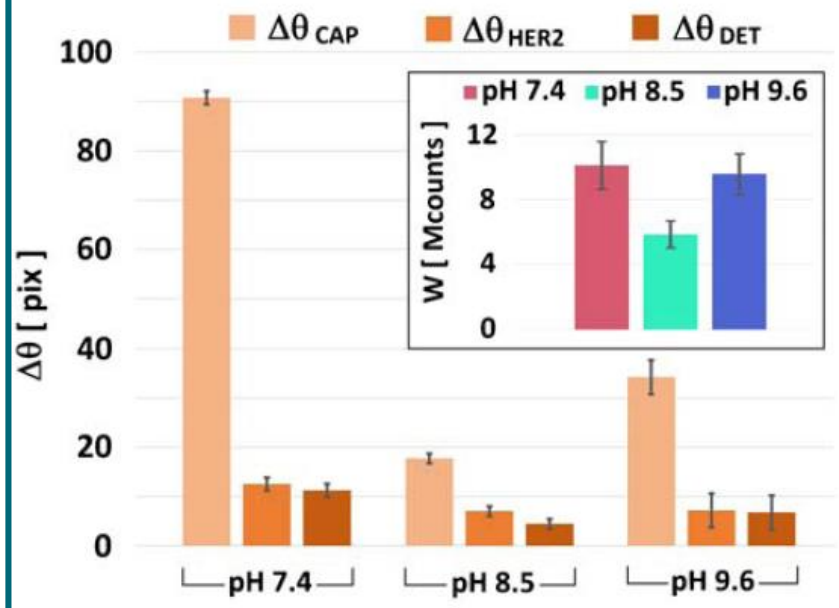




CAPTURE ANTIBODY CONCENTRATION

100 μg/mL Capture Antibody:

- Maximized assay efficacy in steps 3 and 4
- Reduced amount of free binding sites onto chemically activated surface

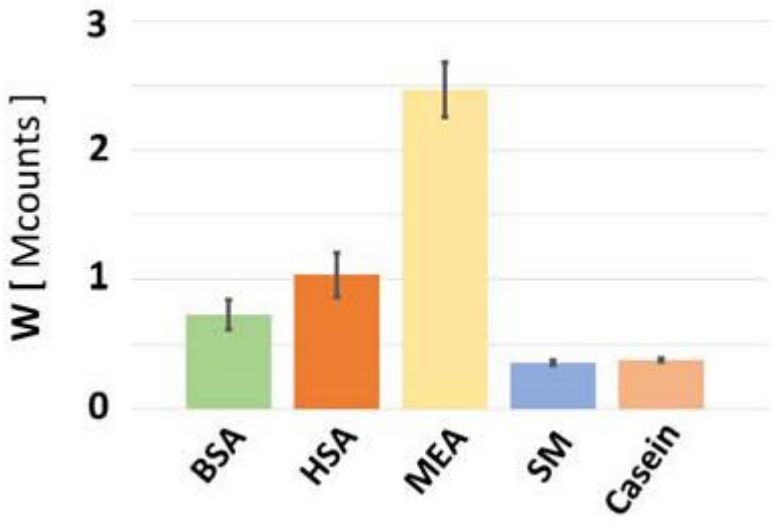


CAPTURE ANTIBODY IMMOBILIZATION BUFFER pH

Immobilization buffer pH ≈ pI – 1 maximizes immobilization efficiency

Sinibaldi, A., Pileri, T. *et al.* Bioassay engineering: a combined label-free and fluorescence approach to optimize HER2 detection in complex biological media. *Anal Bioanal Chem* **412,** 3509–3517 (2020).

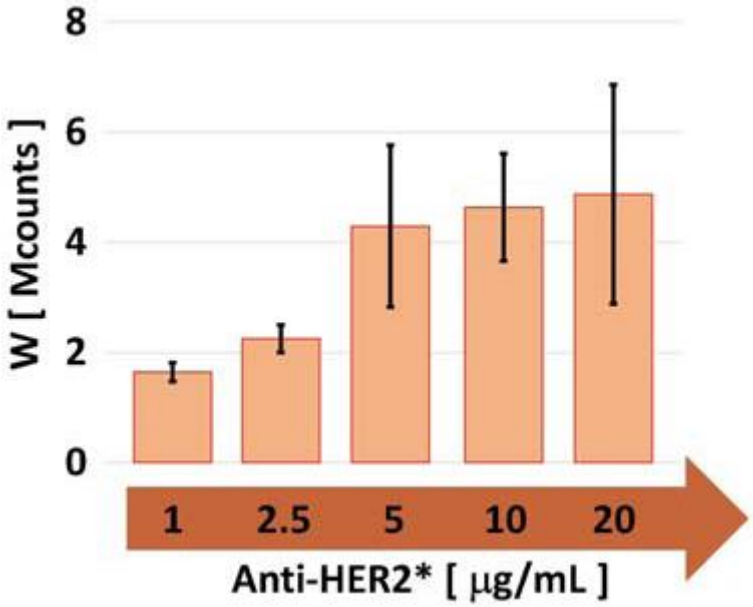
Bioassay Optimization – Passivation Solution and Detection Antibody



Passivation Solution Composition

Bovine Serum Albumin (BSA) is selected among different passivating strategies because:

- Minimizes non specific fluorescence signal
- Do not contaminate the assay with biotin* (contained in Skim Milk and Casein)



Detection Antibody Concentration

Reduced N/S in a range of 1÷2.5 μg/mL

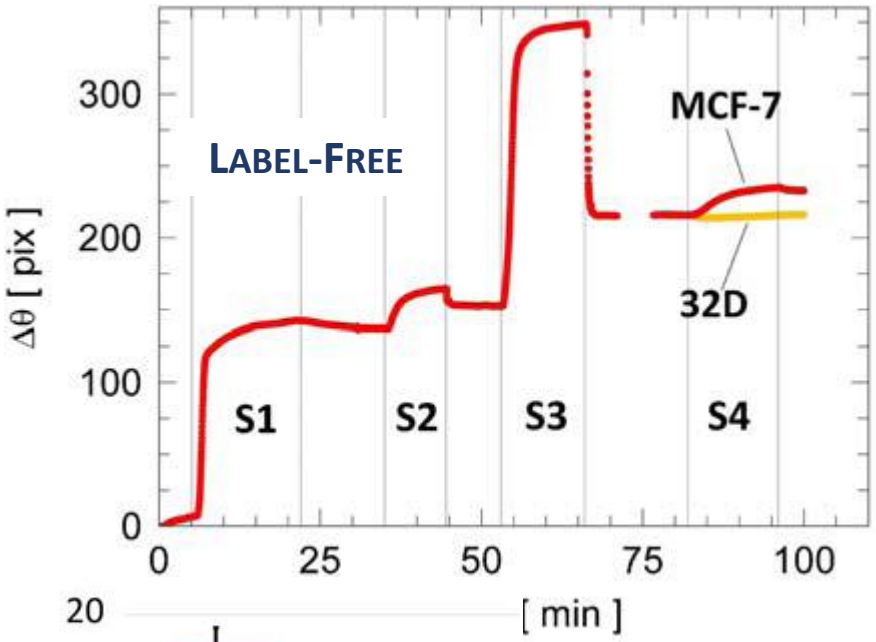
Confirmed by FACS results

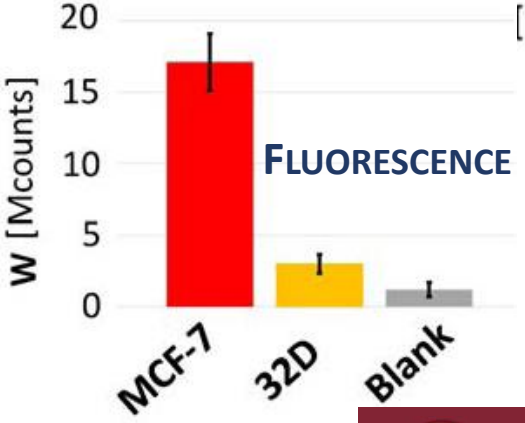
*Antibody-fluorophore complexes are usually conjugated via biotin-streptavidin extraordinary affinity

Sinibaldi, A., Pileri, T. *et al.* Bioassay engineering: a combined label-free and fluorescence approach to optimize HER2 detection in complex biological media. *Anal Bioanal Chem* **412,** 3509–3517 (2020).

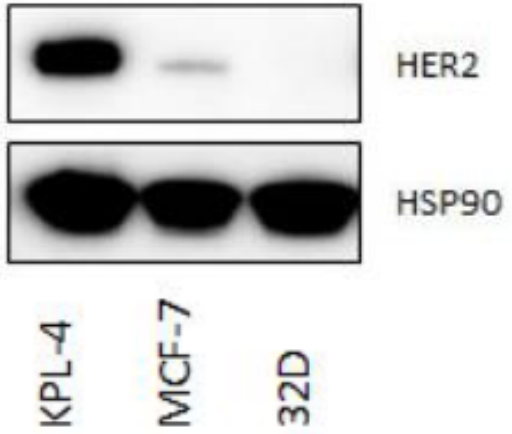
RESULTS

OUR SETUP

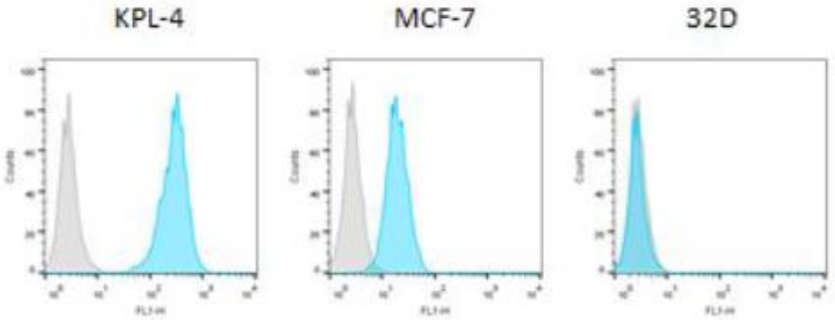




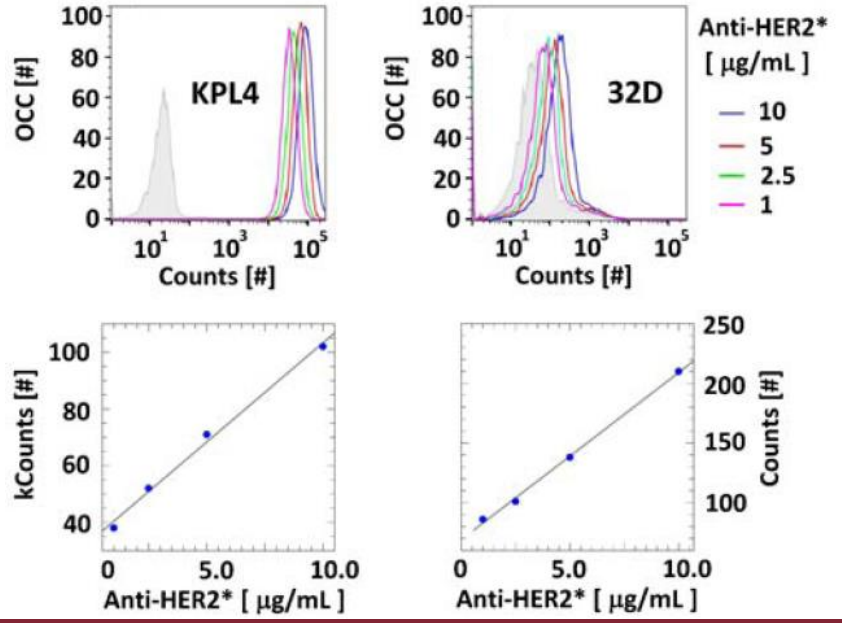
WESTERN BLOT



CYTOFLUORIMETRY



Fluorescence-Activated Cell Sorting (FACS)



MCF7: low-expressing HER2 lysate (unhealthy)

32D: basal-expressing HER2 lysate (healthy)

Blank: total negative sample (used as reference)

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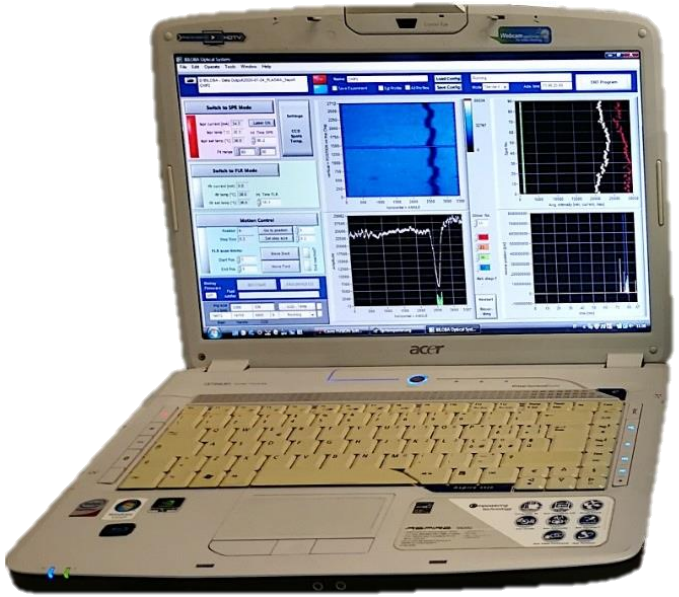
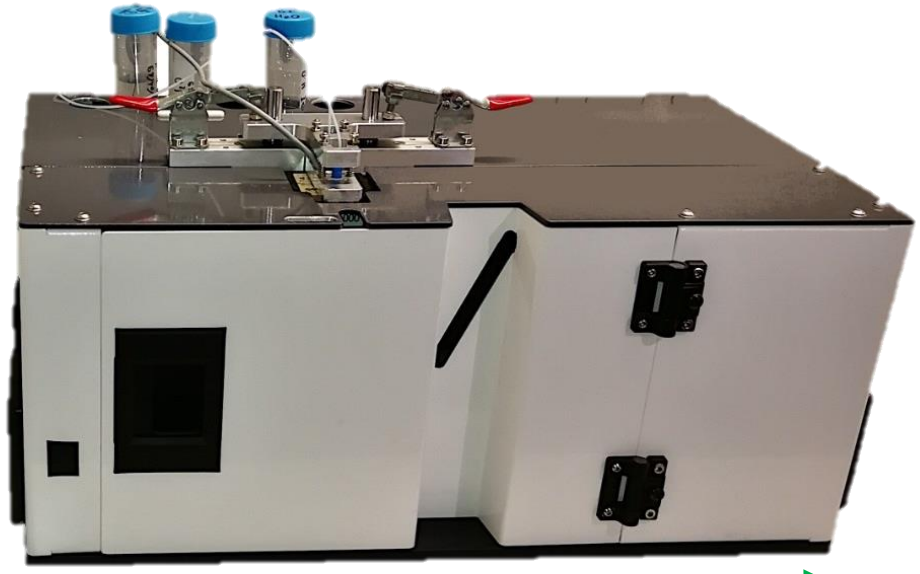
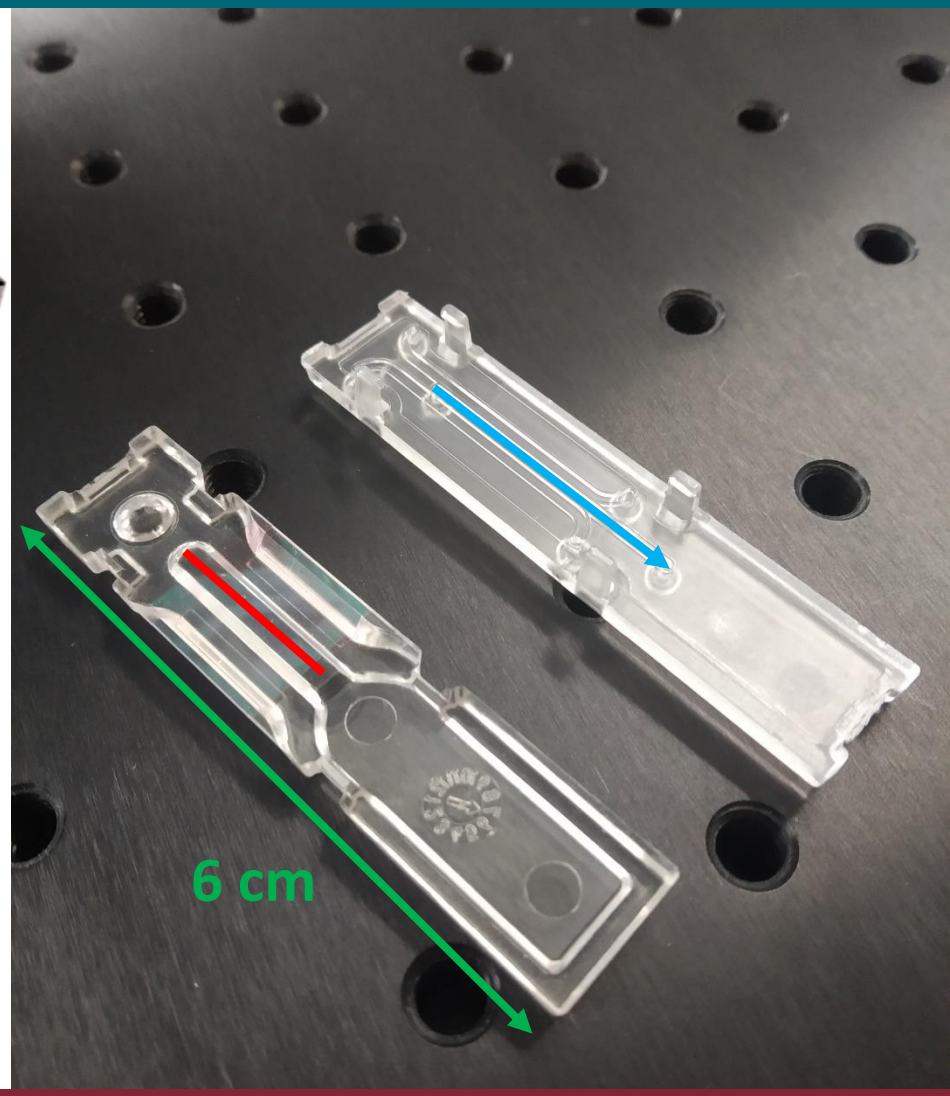
Our Biochip and Readout System





45 cm



- Reduced dimensions
- Nearly fully automatized system
- Fast
- High level of accuracy
  (compared to other existing fast solutions)

→

POC
Point Of Care

Conclusions

Reasons to work with BSW:

- Combined **Label-free** and **fluorescence** operation modes
- Work with **both linear polarizations**: TE and TM
- Enhanced **field intensity** (fluorescence gain) and fluorescence **oriented extraction** without significant losses
- **Tailored** working range tuning layer thickness and composition

From our Setup:

- **Surface localized sandwich assay** is a successful strategy to detect soluble HER2
  - Is likely attractive for a wide range of **proteomic biomarkers**
- **Angular resolution** used for label-free detection yields to **spectral resolution** in fluorescence detection
- **Duration** time for one assay **reduced**: ≈ 40 min instead of few hours required by other techniques (e.g. ELISA ≈ 4.5 h)
- Capability of **discrimination** between **low and basal-expressing** HER2 cell lysates (complex biological media)