Immunosensing of cancer markers through surface-enhanced photoluminescence on nanostructured silver substrates

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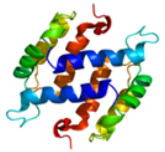
Ovarian cancer

Ovarian carcinoma → one of the most common gynaecologic malignancies worldwide with a high mortality rate

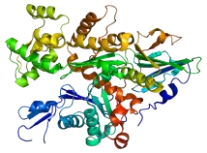
Early diagnosis and monitoring are **essential** for the effective treatment and survival of patients

Diagnosis → Development of analytical tools for cancer diagnosis and prognosis
Detection of ovarian cancer biomarkers



Carbohydrate antigen 125 (CA125)

- One of the earliest identified OC biomarkers
- The gold standard for diagnosis and monitoring of OC
- Normal concentrations ≤ 35 U/mL



Human epididymis protein 4 (HE4)

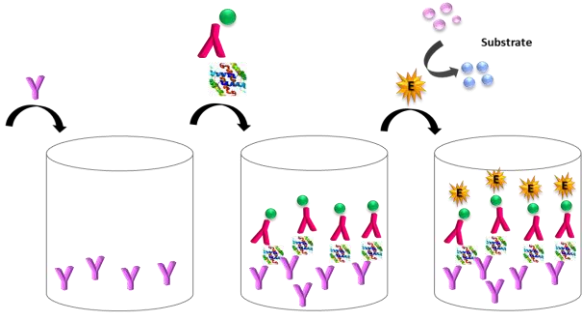
- Novel OC biomarker
- More specific clinical marker for the diagnosis of OC compared to Ca125
- Normal concentrations ≤ 90 pmol/L (2.25 ng/mL)

Biomarkers detection

Need of **specific** and **sensitive** methods for the reliable detection of Ca125 and HE4.

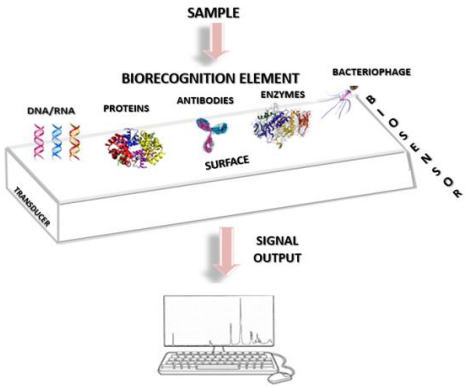
Immunochemical methods

Antigen – antibody interactions (ELISA)



Biosensors

Analytical devices that convert a biological response into an electrical signal

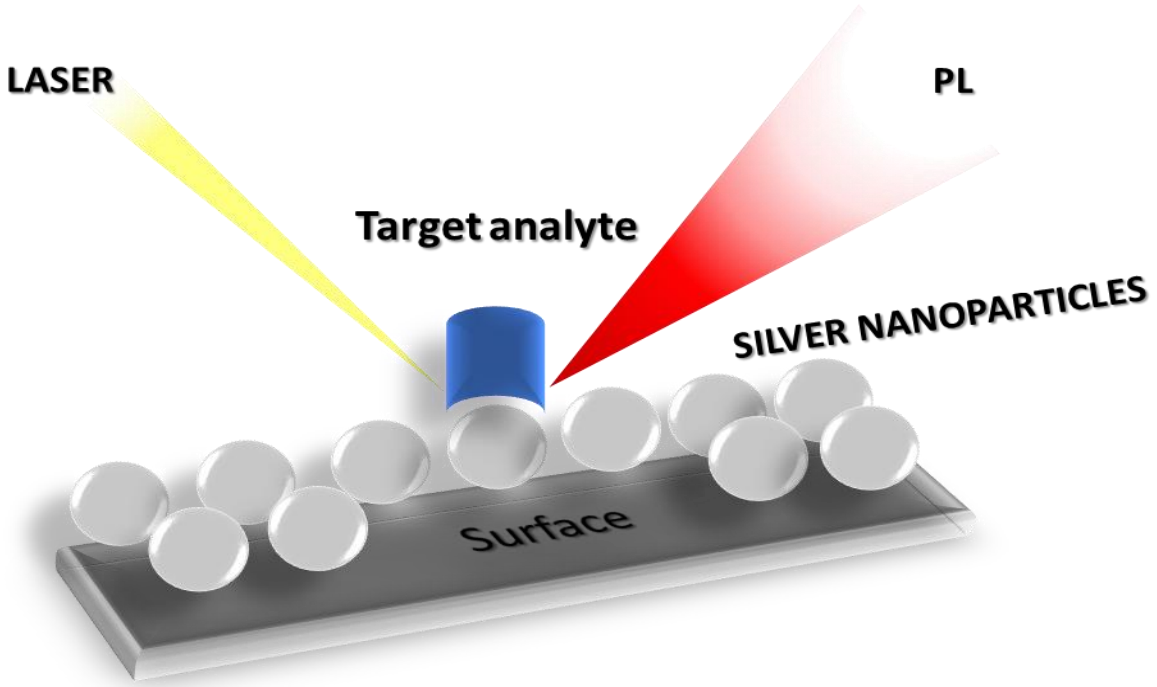


Immunosensors

- The antibody-antigen binding provides the ***specificity of detection.***
- The use of noble metals such as Ag or Au on surfaces ***enhance*** the signal through the electromagnetic field obtained from molecules that are immobilized on the surface increasing their ***detection sensitivity***.

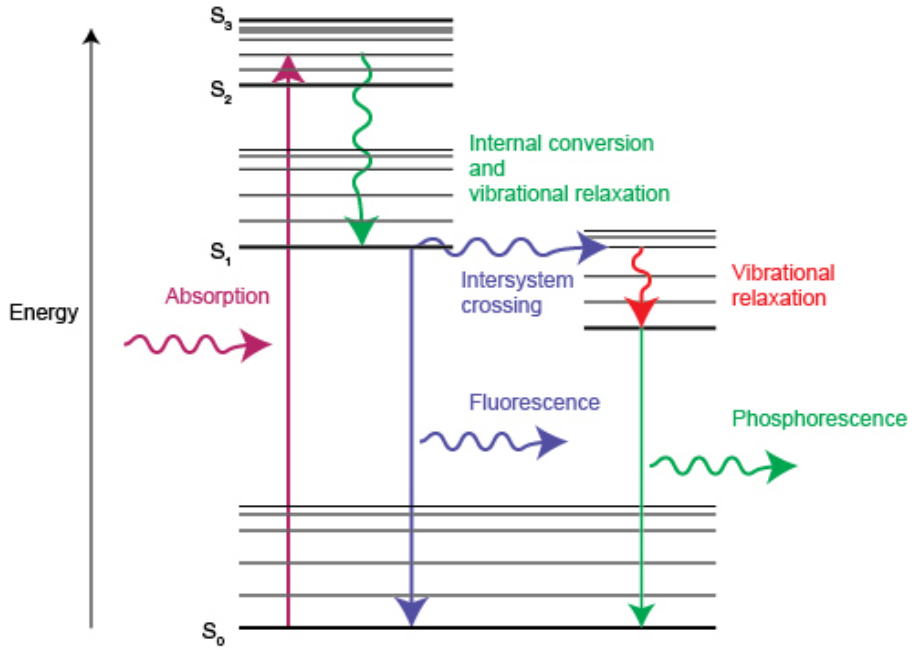
Aim

Evaluation of nanostructured silver surfaces as substrates for the immunochemical detection of CA125 and HE4 through photoluminescence

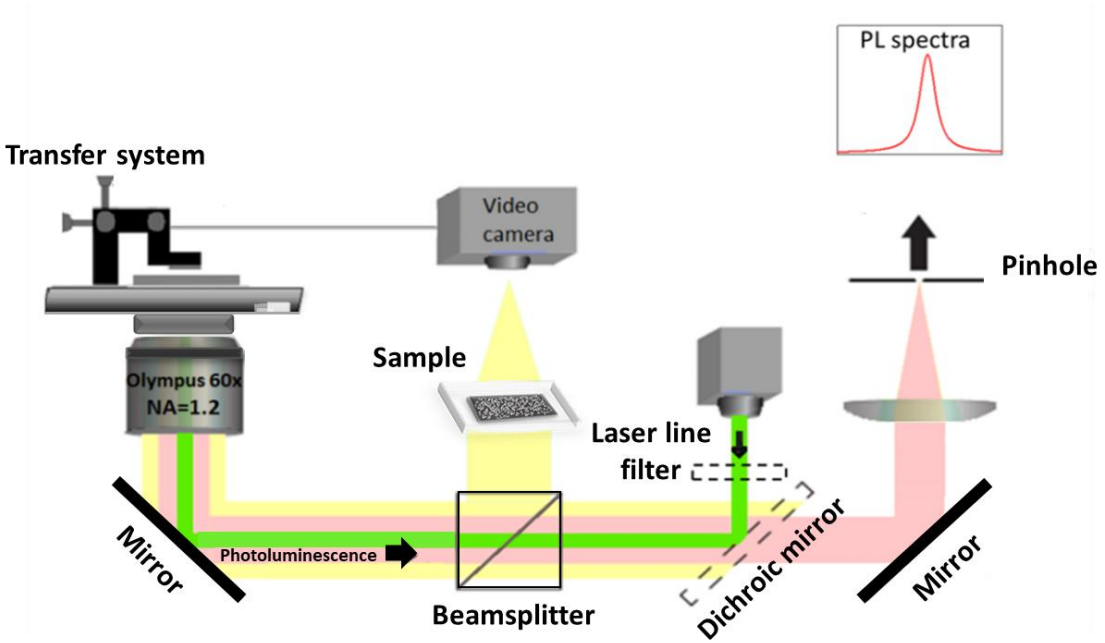


Photoluminescence spectroscopy

Photoluminescence (PL), including **fluorescence** and **phosphorescence**, is a form of light emission spectroscopy in which light is emitted when the electrons within a material return to their ground state after they have moved to excited states via adsorption of light of appropriate wavelength.



Mechanism of photoluminescence light emission

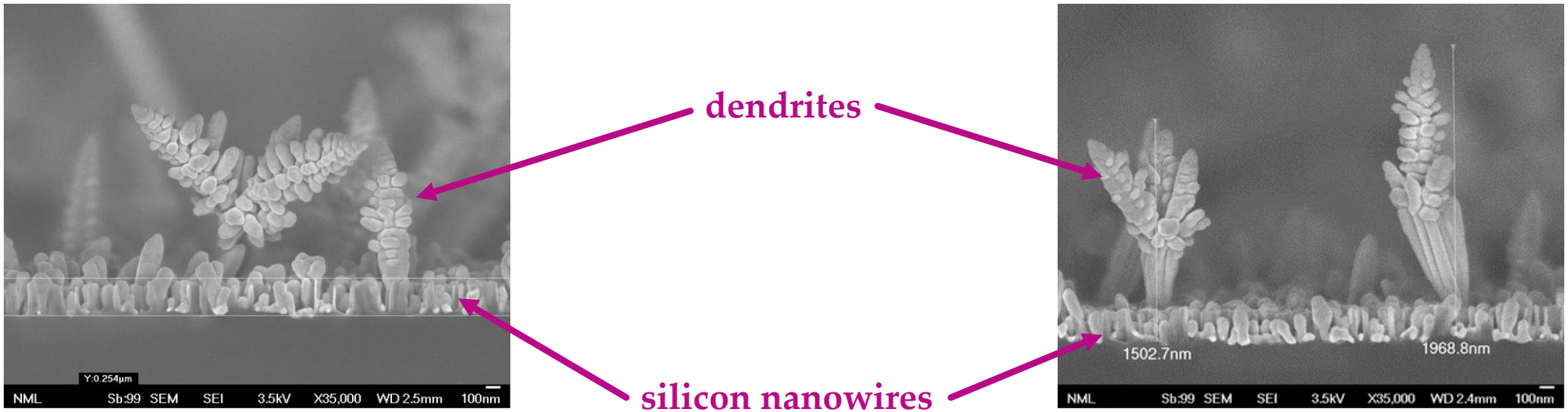


Main components of experimental set-up for photoluminescence detection

PL substrates

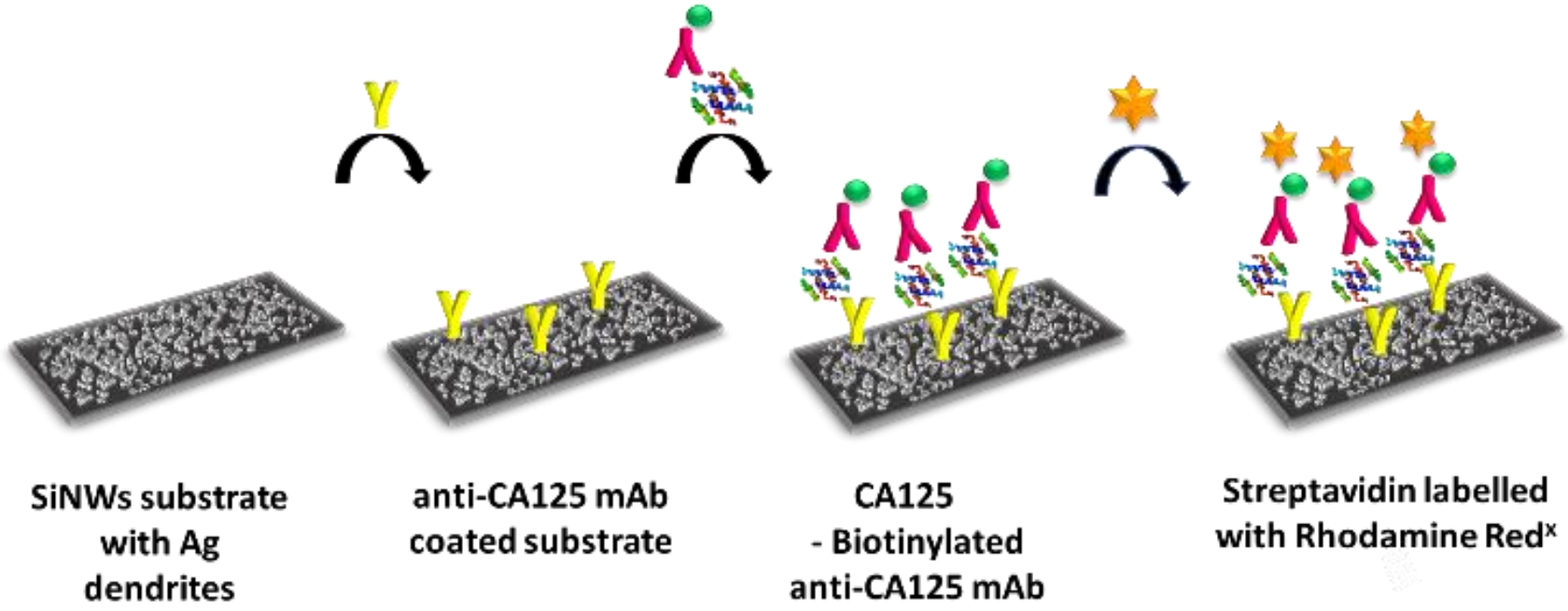
- The PL substrates were fabricated by a single metal-assisted chemical etching (MACE) method to form silicon nanowires (SiNWs) decorated with silver dendrites on the top.
- The Si wafer was immersed into an aqueous solution of **$AgNO_3$/HF** for 3.5 min to create **SiNWs** (mean length 200-300 nm) decorated with **Ag dendrites** (mean height 1500-2000 nm).

SEM images of SiNWs decorated with silver dendrites



Immunoassay protocol

The immunoassay for CA125 and HE4 was performed on the PL substrates following a non-competitive immunoassay protocol as shown below for CA125.



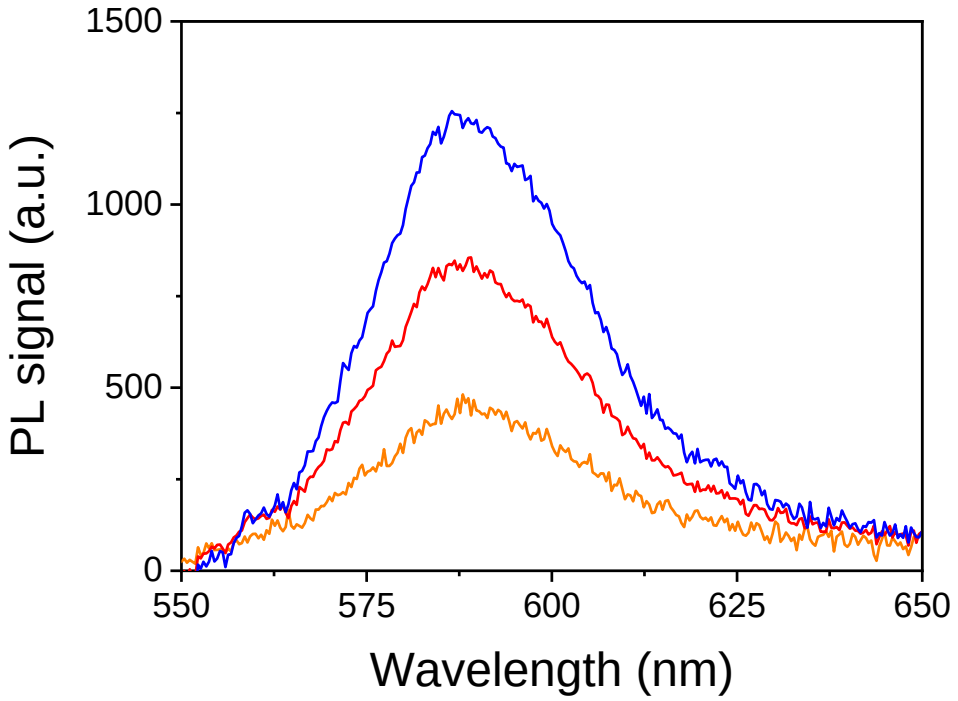
Immunoassay optimization

- capture antibody concentration used for modification of nanostructured silver substrates
- the biotinylated detection antibody concentration
- the assay duration
- the incubation time with the streptavidin-Rhodamine Red-X conjugate

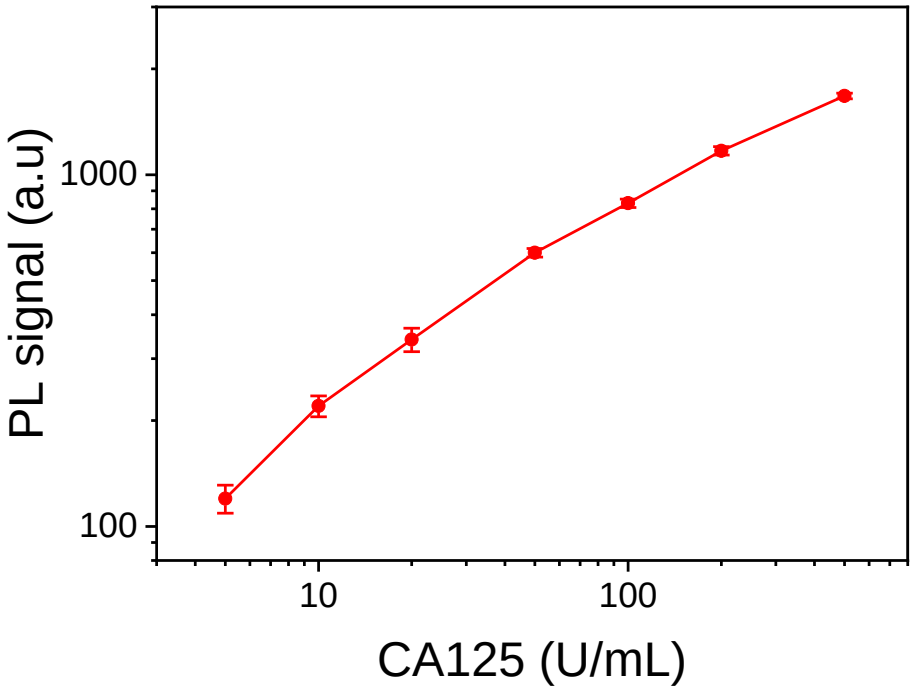
Final optimized immunoassay conditions for each biomarker

Parameters	CA125	HE4
Capture antibody concentration	200 µg/mL	200 µg/mL
Detection antibody concentration	2.5 µg/mL	5.0 µg/mL
Assay duration	60 min	60 min
Streptavidin-Rhodamine incubation	30 min	30 min

Calibration curve for CA125

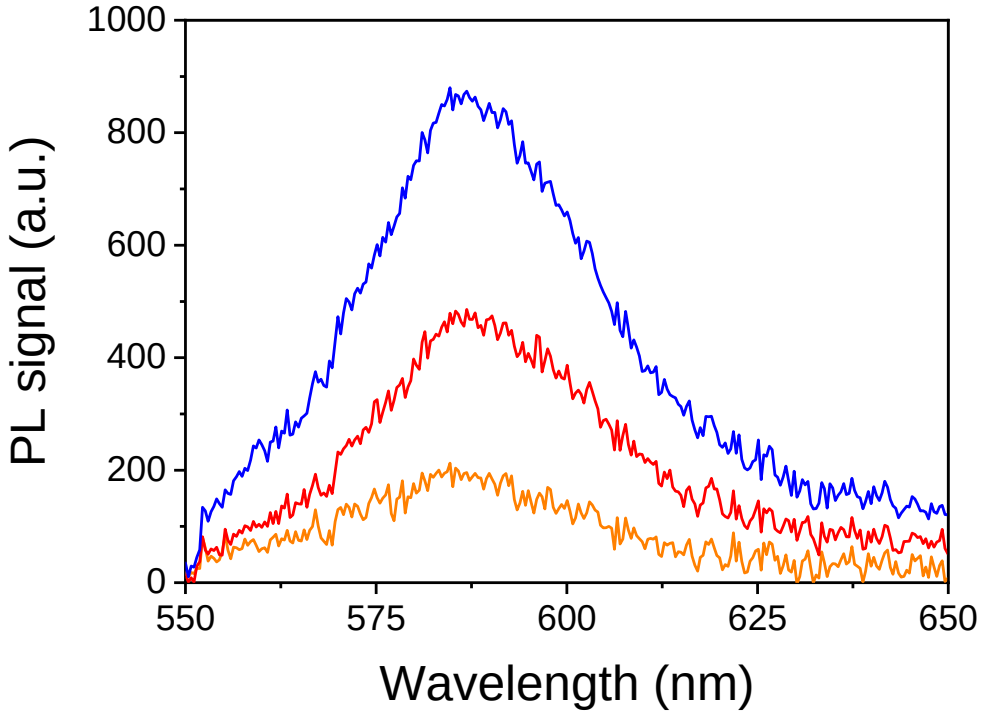


Photoluminescence signal at the spectral range from 550-650 nm received from surfaces with silver dendrites functionalized with an anti-CA125 antibody. Orange line corresponds to zero calibrator (non-specific binding), red line to a calibrator with concentration of 20 U/mL and blue line to a calibrator with concentration of 100 U/ mL.

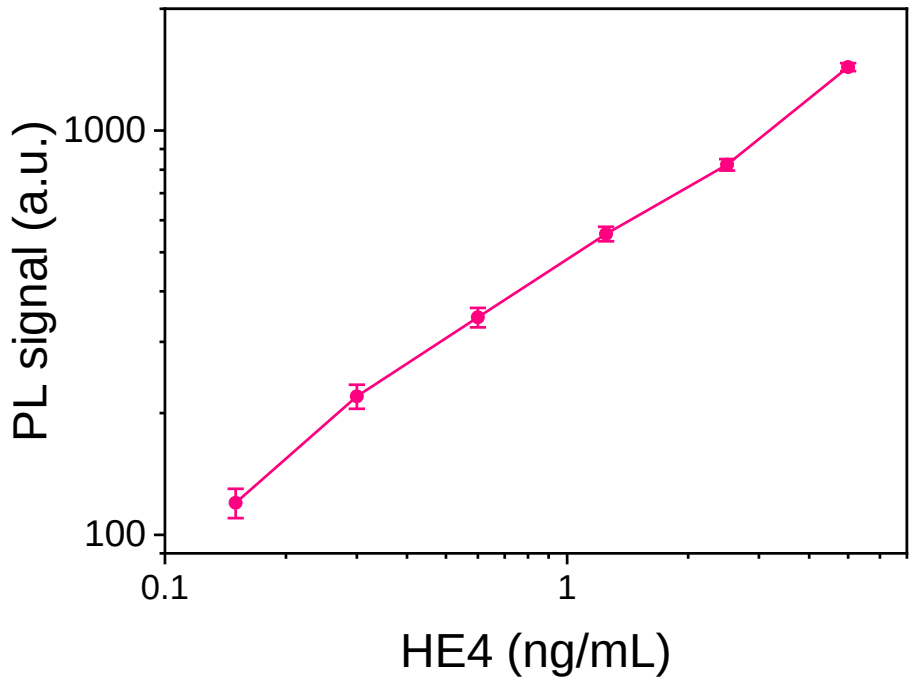


Typical CA125 calibration curve with a **limit of detection 2.5 U/mL**.

Calibration curve for HE4



Photoluminescence signal at the spectral range from 550-650 nm received from surfaces with silver dendrites functionalized with an anti-HE4 antibody.
Orange line corresponds to zero calibrator, blue line to a calibrator with concentration of 0.6 ng/mL and red line to a calibrator with concentration of 1.25 ng/mL.



Typical HE4 calibration curve with a **limit of detection 0.06 ng/mL**.

Conclusions

- Nanostructured silicon surfaces decorated with silver dendrites were prepared and evaluated as PL substrates.
- A sensitive and fast non-competitive immunoassay for the ovarian cancer markers CA125 and HE4 was developed on these substrates for their detection through photoluminescence.
- The detection limit of CA125 was 2.5 U/mL and of HE4 0.06 ng/mL.
- Linear dynamic range extending up to 500 U/mL for CA125 and 5.0 ng/mL for HE4.
- The working ranges of both assays cover the concentrations encountered in both healthy individuals and ovarian cancer patients and thus they can be implemented for the early diagnosis of ovarian cancer.
- The surfaces will be evaluated as substrates for the immunochemical detection of CA125 and HE4 through surface-enhanced Raman spectroscopy employing suitable labels, aiming to develop a portable system for biomarkers detection at the point-of-need.

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Thank you!!!