

Real-time detection and quantification of small molecules drugs by T-LSPR



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Introduction

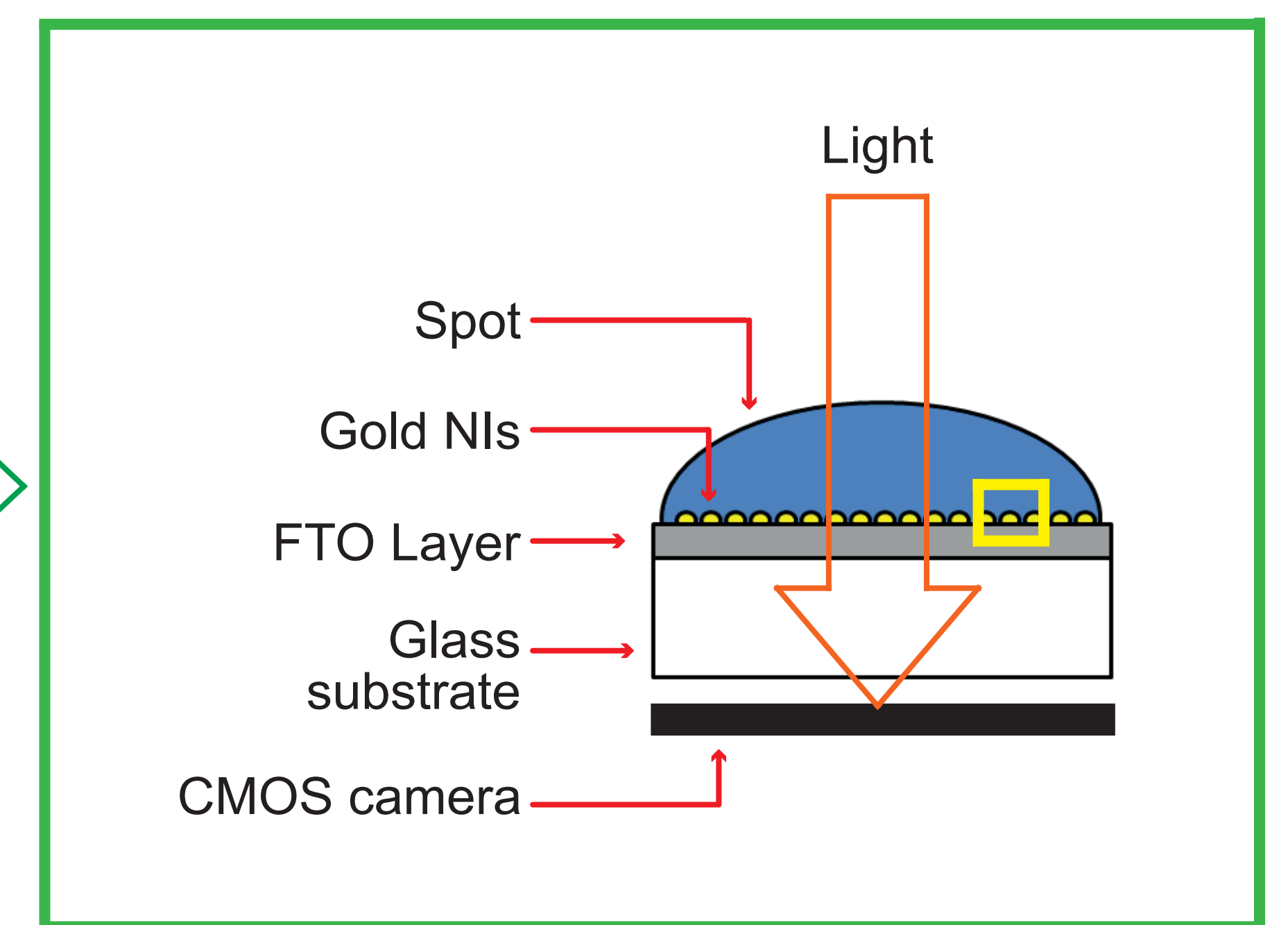
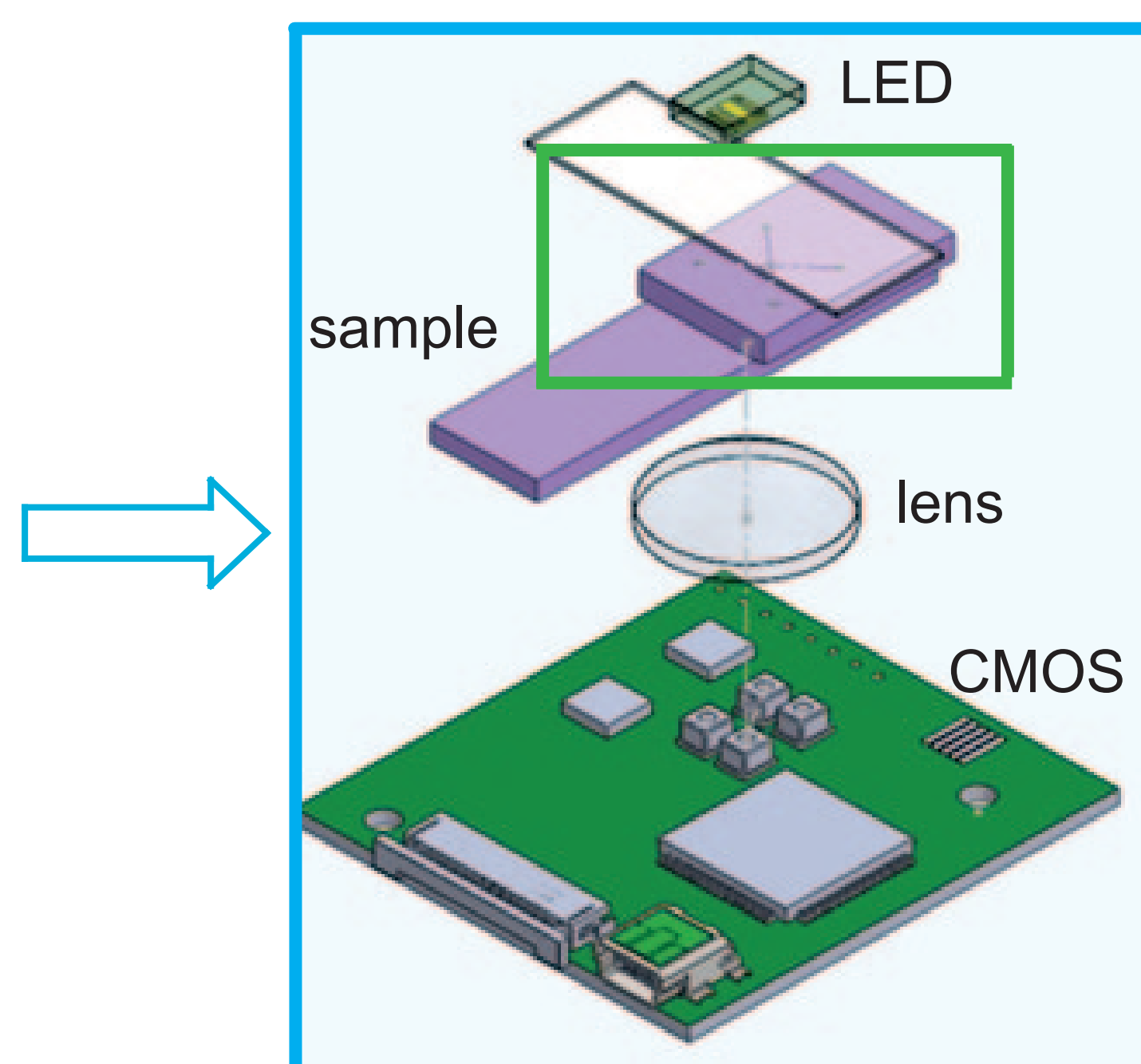
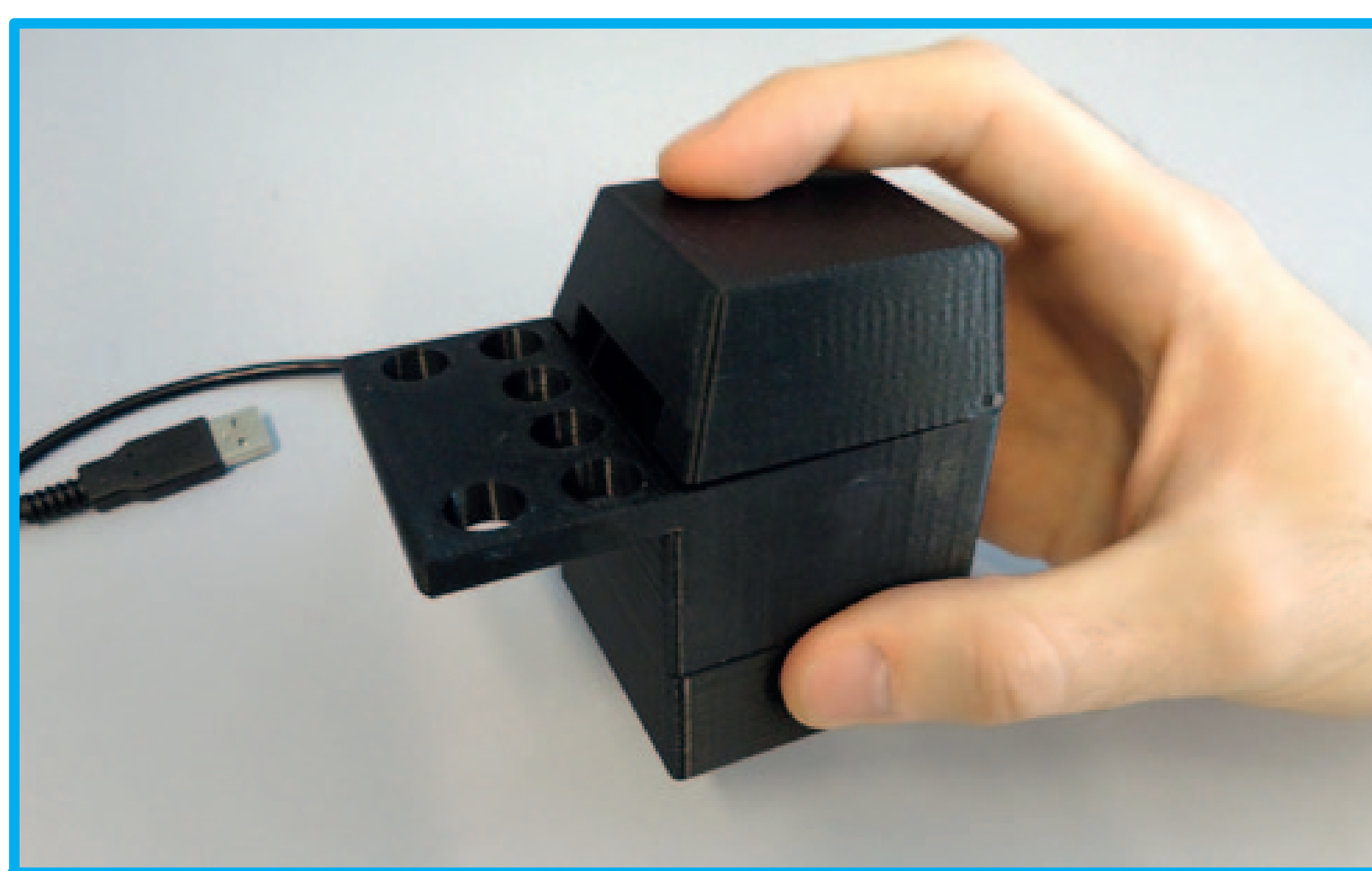
Point-of-Care (POC) systems are rapidly expanding thanks to their simplicity to use and portability that allows an in site diagnosis, thus improving the quality of the treatment. The necessity of integrating multiple functions on the same device is an important requirement, common characteristics of POCs are high throughput, low cost, sensitivity and material compatibility. Our group is working on the development of miniaturized components for a POC system based on Localized Surface Plasmon Resonance in a Transmission configuration (**T-LSPR**) for drug monitoring and dose adjustment tailored to the patient. We focused on the study of Tobramycin (MW: 467 Da), an antibiotic used to treat lung infections. The ability to detect **small molecule drugs** has always been a challenge, due to the low molecular weight and the small change in the refractive index they cause, leading often to competitive assays, sandwich assays or other combinations. Furthermore the discrimination between non specific absorption and the difficulty of discriminate those particles in more complex media, such as serum, make the small molecules a new topic of interest in biosensing systems. A variety of selected **DNA aptamers** to specifically recognize Tobramycin have been tested in **real-time** in a **100% Serum** matrix.

Nanostructures and POC for label-free detection

We present a POC able to perform small molecules drug detection in 100% Serum at relevant clinical concentrations, without any alteration of the proteins and thanks to a selected DNA aptamer developed in our lab.

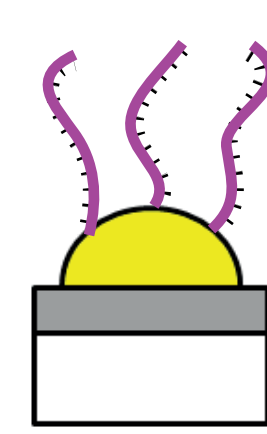
The system is based on a white LED light source and a CMOS camera as a detector, that enables to perform real-time binding experiments.

The sensing area is made of gold Nanolands formed on Fluorine-doped Tin Oxide (FTO) coated glass slides. FTO coated slides are characterized by excellent adhesion properties while showing high optical transmittance and conductivity.

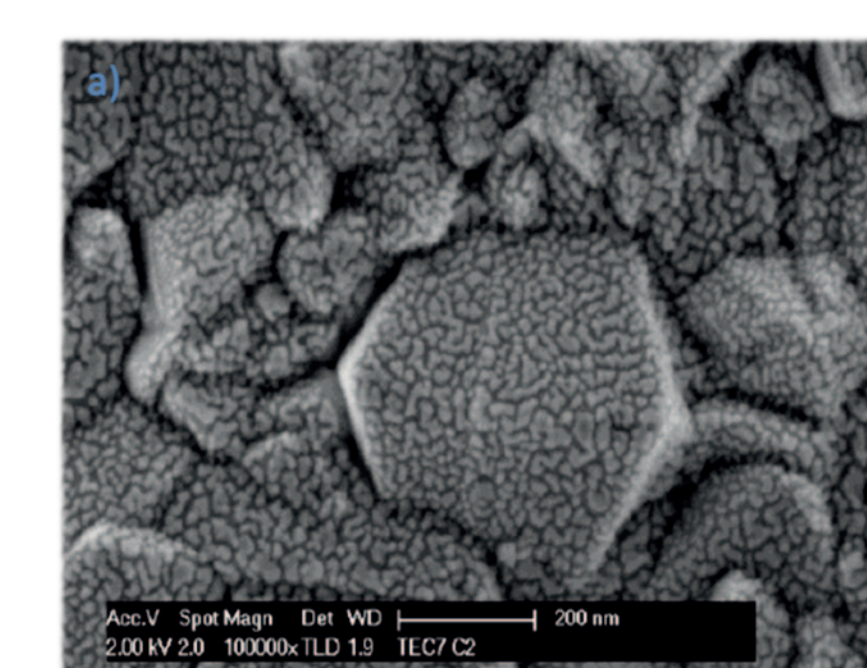
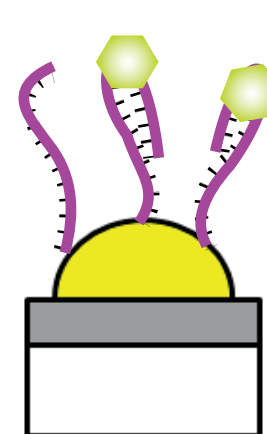


Real-time association and dissociation of Tobramycin (MW: 467 Da) with DNA aptamers (blue line) immobilized on the surface at one end. During the association step, the binding of Tobramycin is masked by the bulk effect of the Serum. The amount of Tobramycin bound to the DNA aptamer can be quantified in the dissociation step. The green line represents the control measurement performed with a layer of mercaptohexanol on the nanoislands, which allows to cancel out the signal of the bulk effect and to verify the occurrence of non-specific binding.

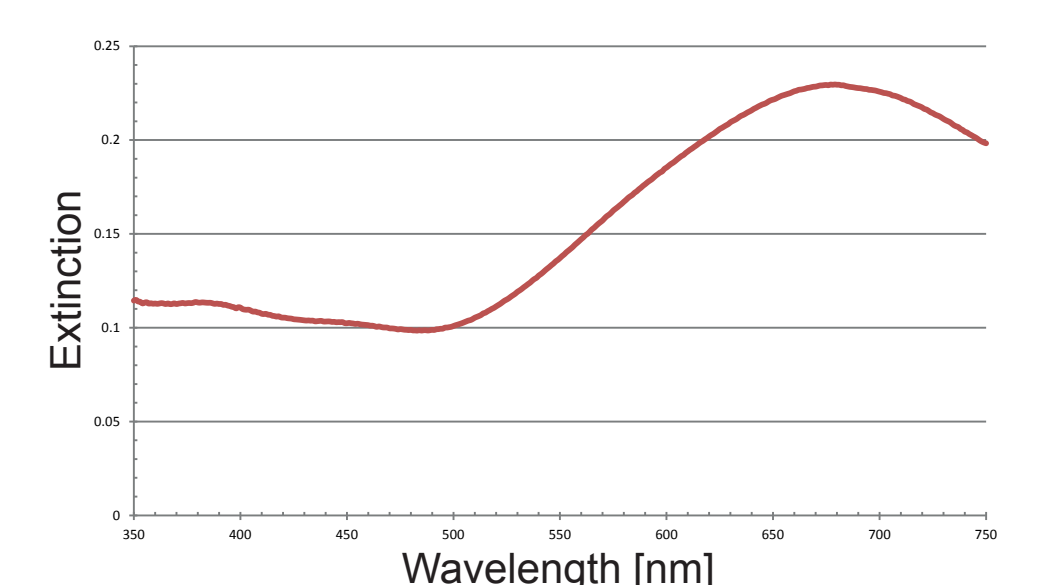
Gold Nanoislands have been functionalized with specifically selected DNA aptamers in order to perform the real-time recognition of Tobramycin.



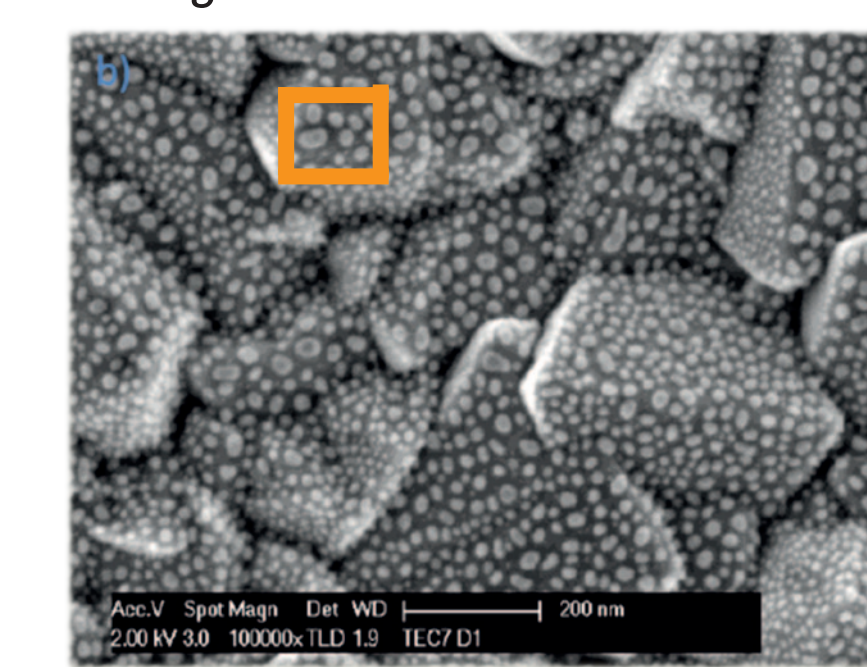
By monitoring the RGB pixels of the CMOS images we can extract quantitative information about the binding process occurring at the surface.



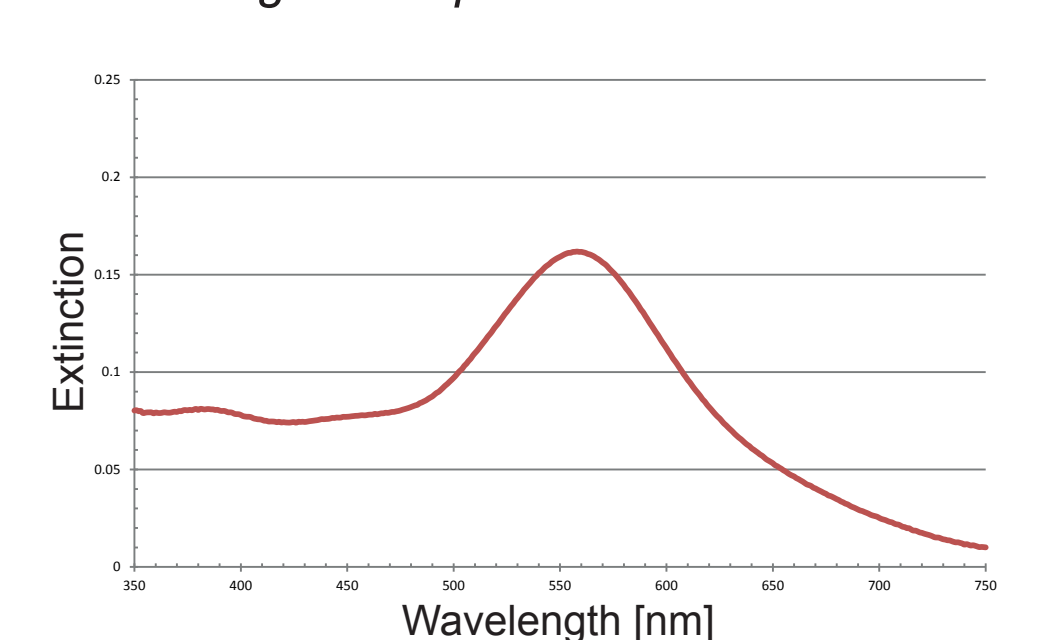
5 nm gold layer evaporated on a FTO glass substrate.



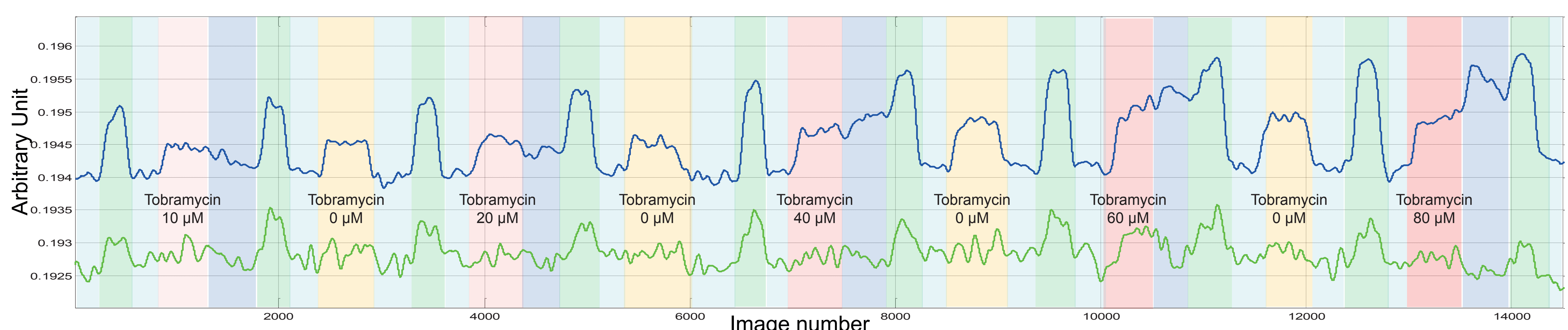
Extinction spectrum of a sample after gold evaporation.



5 nm gold layer evaporated on a FTO glass substrate after mild thermal annealing at 200°C.



Extinction spectrum of a sample after evaporation and subsequent annealing.



■ Association step: injection of Tobramycin in 100% FBS Serum.
 ■ Dissociation step: injection of Buffer.
 ■ Baseline: DNA on surface, injection of buffer.
 ■ Control step: injection of Serum 100% without Tobramycin.
 ■ Washing step: injection of NaCl 1M.