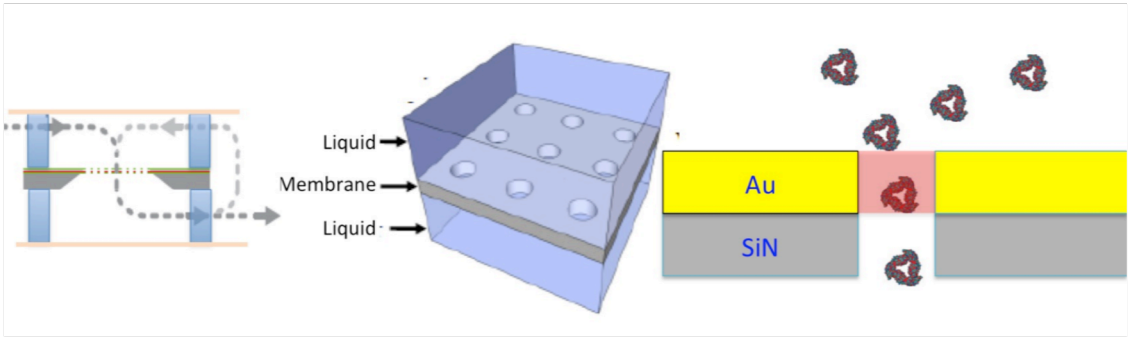
Plasmonic Sensing in Biomimetic Nanopores



Schematic of the plasmonic biosensor integrated into a microfluidic device. Si_3N_4 membrane is integrated into a PDMS/glass sandwich with liquid inlet and outlet (left). Solutions containing low analyte concentrations fill the nanopores (middle). An enhanced optical near-field (red) detects single molecules as they pass through an Au nanopore (right).

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Background and Motivation

Plasmonic biosensors have emerged as a powerful method of label-free and remote sensing. Their sensing mechanism is based on the sensitivity of plasmon resonances on the effective refractive index of local environment. Various types of plasmonic nanostructures have been intensively studied, providing novel detection mechanisms or better sensitivity [1, 2]. The most-commonly used and commercially available method is using functionalized Au thin films and exciting surface plasmon polaritons for detection over large areas. On the other hand, plasmonic nanostructures such as metal nanoparticles or nanoholes/nanopores localize and enhance the light in a nanoscale volume, providing extreme sensitivity in a very small detection volume. Indeed, many groups have demonstrated, for instance, single protein binding detection sensitivity in recent years [3]. In addition to detection of the local changes of effective refractive index due to binding molecules by observing the plasmon resonances, surface plasmons are also used successfully to enhance the signals of fluorescence and Raman by several orders of magnitude [4, 5].

In most of the studies on biosensing with plasmonic nanostructures, the active surface is brought in contact with the solution with analyte. Although this shows the effectiveness of plasmonics for sensing, significant problems remain unresolved for real-life applications. Since the detection range of the plasmonic nanostructure or surface is about 100 nm or less, only the molecules that are bound the surface or enter randomly into the detection volume are detected. This certainly limits the detection sensitivity. Moreover, the response time of such sensors is significantly low. Even in the case of a microfluidic device, where the flow is laminar, binding to the surface is dominated by diffusion, and therefore, the response times are still limited. Therefore, methods that will improve the response time as well as increase the concentration levels on the active area can make plasmonic devices competitive with the established techniques and can extend the application areas of plasmonic biosensors. In addition, if such sensors are integrated into microfluidic devices, i.e. lab-on-a-chip, this will enable plasmonic sensing with multiplexing, increased functionality and response time.

Objectives and Research Plan

We propose developing a plasmonic device that is integrated into a microfluidic device. Au nanoporous arrays are fabricated on Si_3N_4 membranes, which are sandwiched between two glass covers using PDMS as shown in the TOC Figure. By inserting inlets and outlets in PDMS, compartments on both sides of the membrane are filled with water (i.e., buffer) and low concentration analyte solution. Molecules passing through the pores lead to shift of the plasmon resonance. At the saddle point of the plasmon resonance, the molecules will lead to a contrast in the light transmission, which is detected by the CCD coupled to the microscope. This contrast is proportional to the size of the molecules and therefore will be sufficient for single-molecule detection typically for large molecules. Therefore we need functionalization of the surfaces so that molecules are captured in the pore. Alternatively, we will also study plasmon-enhanced fluorescence for single molecule detection. For the fluorescence enhancement the plasmon resonance has to be optimized for the highest enhancement of the near-fields at the excitation and emission wavelengths.

The objectives of the project are as follows:

1. Fabrication of Au nanopores on glass substrates (initially) and on membranes. Si_3N_4 membranes will be patterned with e-beam lithography (initially) and extreme ultraviolet interference lithography at SLS (for large area and high throughput fabrication). On glass substrates evaporation of Au and lift-off lead to Au nanopores. On membranes, etching pores and metal deposition lead to Au nanopores. Integration of the plasmonic structures into a microfluidic device.
2. Numerical simulations of the far-field and far-field properties of Au pore arrays with different diameters and periods. Optical measurements of plasmonic structures using transmission spectroscopy. Optimization of the structural parameters (period of the array, pore diameter, metal thickness) in order to obtain highest near-field enhancement for fluorescence detection. Optimization of the

plasmon resonance in order to obtain highest contrast at the detection wavelength upon entrance or absorption of molecules in the nanopore.

3. Plasmonic biosensing experiments: The microfluidic device with the Au nanopore array will be excited with a HeNe laser and the transmission will be imaged with the CCD where each nanopore is projected to a single or a few pixel of the CCD. To evaluate sensor response time, we will construct a biomimetic nuclear pore complex (NPC) [6] within the nanopore either by tethering (i) simple PEG polymer chains; and/or (ii) NPC proteins (FG nucleoporins) along the pore walls so as to slow analyte diffusion.
4. Fluorescence experiments: Similar to the objective-3, fluorescence of the molecules will be imaged with a fluorescence filter. Again using the biomimetic NPC, we will monitor fluorescently labeled nuclear transport receptor molecules (NTR i.e., as analytes) that will interact with the FG nucleoporins.

Impact

Development of the plasmonic device integrated into a microfluidic device proposed in this project can enable a biosensor with a better sensitivity and response time than the state-of-the-art. Such devices can be used in various applications where detection of extremely low concentrations is needed. These include single molecule detection in nanopores used for molecular sorting [6], DNA sequencing [7], etc.

Infrastructure and Expertise

Nanofabrication of devices will be performed at the cleanroom facility of the Laboratory of Micro- and Nanotechnology at the PSI. Characterization of the devices, determining their plasmonic response, and studying the biosensing performance will be carried out at PSI where we have microscope coupled to a spectrometer with a cooled EM-CCD, which can reach down to single-photon detection with high detection speeds. Electromagnetic simulations will be performed at PSI and UB using the already available codes such as Comsol. Choice and preparation of the biomaterials will be done at UB. The PhD student will be mainly located at PSI and will spend at least one day weekly in UB in order to establish a strong collaboration.

Both groups have strong and complementary experiences and infrastructure for the success of the project. *Y. E.* is an expert in nanooptics, nanofabrication, plasmonic nanostructures, plasmonic biosensing, and fluid dynamics. He has published more than 60 papers and the majority of them are relevant to this project. *R. L.* is an expert in biophysical transport phenomenon through nanopores with an emphasis on biomimetic pores that mimic physiological function (e.g., the nuclear pore complex). He has published over 30 articles in world-renown journals such as *PNAS*, *Science* and *Nature Nanotechnology.*

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