Free space whispering gallery mode microlasers as highly sensitive biosensors

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Abstract. High-precision biosensors for single or few molecules detection play a central role in numerous key fields, such as environmental monitoring and healthcare for early-stage disease diagnosis. In the last decade, laser biosensors have been investigated as proofs of concept, and several technologies have been proposed. Here we propose a demonstration of polymeric whispering gallery microlasers as biosensors for detecting proteins at low concentrations. Free space microlasers have the great advantage of working without any need for waveguiding for input excitation or output signal detection. The photonic microsensors can be easily patterned on microscope slides and operate in air and solution. We could detect down to 400 pg of protein without specific binding, and few tens of pg/mL with specific binding.

1 Introduction

Light trapping by total internal reflection inside a hollow micron-sized dielectric cavity gives rise to optical whispering gallery modes (WGM) at the cavity edge [1]. WGM microresonators are highly sensitive to infinitesimal variations in the surrounding refractive index as well as in the cavity geometry, with measurable shifts in the resonance wavelength [2,3]. This makes such cavities particularly suitable as label-free chemical and biological sensors.

Including a gain medium (e.g. a fluorescent dye) in the dielectric cavity results in an active optical cavity that supports stimulated emission modulated by WGM resonances. Using free space WGM microlaser sensors rather than passive resonators offers important improvements such as a more compact and versatile optical setup [4].

WGM microlasers have also inspired promising applications as biosensors within living cells [5,6], for the detection of single virus particles [7], and in advancement of *in vivo* sensing [8]. Our group developed a laser-based biosensor made of single or arrays of dyed polystyrene microspheres of 30 μm diameter patterned on glass substrates [9]. In this study, we investigated their response to variable concentrations of different proteins in terms of emission line shifts. Our microresonators were able to operate both in air and in solution. Furthermore, a protocol to functionalize our spheres for specific binding with interferon gamma (IFN-γ) has been optimised. In presence of proteins, evident emission line shifts in the microlasers spectra were observed. We obtained a limit of detection of 0.38 ng for lysozyme protein in air. This limit is the lowest for bare spheres with non-specific protein bindings. The microlaser biosensor was also tested on τ protein in solution, which is one of the major hallmarks of Alzheimer's disease, obtaining a sensitivity of 148 nm/RIU (refractive index unit). Regarding IFN-γ, our immunosorbent photonic probe could detect the protein down to concentrations of few tens of pg/mL.

2 Results

The optical setup is shown in Fig. 1(a). The microspheres were excited by a 10 Hz, 532 nm pulsed laser beam, whose input energy was modulated by neutral density filters. To excite the whole microsphere, the pump light was focused on the sample to a spot of ~50 μm diameter. The emission from the single microlaser was magnified and residual pump light was filtered out with a notch filter. The filtered light was then half split on a digital camera for imaging, as shown in Fig. 1(b), and on an optical fiber connected to a spectrometer for spectral analysis. Spectra from multiple beads in the same conditions, and from different points of the same bead were recorded.

Great attention was dedicated to sample deposition, as microresonators stability during the whole experiment is of major importance. The slides were functionalized using a 3% ethanol solution of (3-aminopropyl) triethoxysilane,

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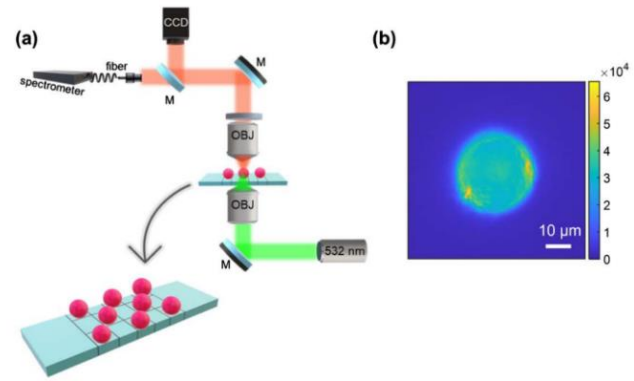

Fig. 1. Optical setup (a) and fluorescence image of a single WGM microlaser (b).

which allowed a good adhesion of polystyrene spheres to the substrate.

A common strategy was adopted for all experiments: a reference spectrum was recorded without proteins as blank; after deposition of a droplet of protein solution, further spectra were recorded over time, either in solution or in air, to analyse the temporal evolution of the system. Fig. 2 shows (a) an example of two complete emission spectra of a microlaser in water, used as reference, and τ protein solution, and (b) the time evolution of the central wavelength of the selected peak (highlighted in yellow in Fig. 2(a)), extracted from a gaussian fit of the curve. Conversely, the experiments on lysozyme were performed in air. The protein was deposited in one or more successive droplets from the same water solution (0.3 µg/mL) and was studied after water evaporation. For both these proteins, we waited for their occasional adhesion to beads by diffusion, without any specific chemical bindings. We also carried out immune-functionalized MLs for the detection of purified *in vitro* stimulated human T-lymphocytes. This experiment required specific binding with IFN-γ, thus a human IFN-γ ELISA kit [10] was used to bind antibodies on microresonators and we developed a protocol to obtain a photonic standard curve for the antigen detection which shows higher potential compared to enzyme linked assays.

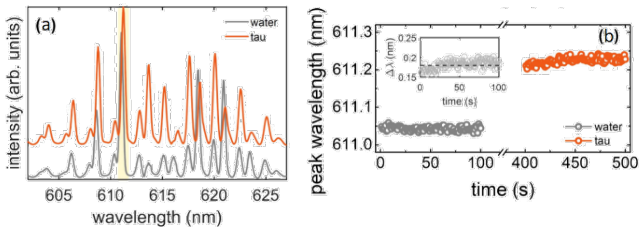

Fig. 2. Complete emission spectra (a) and time evolution of the peak central wavelength (b) of a microlaser in water (grey) and in τ protein suspension (orange).

3 Conclusions

We propose a novel methodology based on the employment of polymeric microlasers as dynamic sophisticated probes for faster and accurate detection of biomolecules secreted by cells, *in vitro*, or directly in biological samples within an all-optical, versatile, and adaptable platform. We demonstrate that our immuno-based biosensor system is highly sensitive in detecting specific biomarkers at low concentration, which is crucial especially at early stages of a disease. We achieved high sensitivity for IFN-γ with concentrations down to 30 pg/mL.

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References

1. K.J. Vahala, Nature **424**, 6950 (2003)
2. L. Cai, J. Pan, Y. Zhao, J. Wang, and S. Xiao, Phys. Status Solidi A **217**, 6 (2020)
3. E. Kim, M.D. Baaske, and F. Vollmer, Lab Chip **17**, 7 (2017)
4. D. D'Ambrosio, X. Zambrana-Puyalto, M. Capezzuto, A. Giorgini, P. Malara, S. Avino, and G. Gagliardi, Phys. Rev. A **104**, 4 (2021)
5. V.D. Ta, S. Caixeiro, F.M. Fernandes, and R. Sapienza, Adv. Opt. Mater. **5**, 8 (2017)
6. M. Schubert, L. Woolfson, I.R.M. Barnard, A.M. Dorward, B. Casement, A. Morton, G.B. Robertson, P.L. Appleton, G.B. Miles, C.S. Tucker, S.J. Pitt, and M.C. Gather, Nat. Photonics **14**, 7 (2020)
7. L. He, Ş.K. Özdemir, J. Zhu, W. Kim, and L. Yang, Nat. Nanotechnol. **6**, 7 (2011)
8. M. Humar, and S.H. Yun, Nat. Photonics **9**, 9 (2015)
9. A. Capocefalo, S. Gentilini, L. Barolo, P. Baiocco, C. Conti, and N. Ghofraniha, Photonics Res. **11**, 5 (2023)
10. R. Hnasko, *Elisa* (2015)