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Application of two-photon polymerization technique for resonator-based biosensors fabrication

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Abstract

An approach to produce resonator-based biosensors able to perform real-time biochemical component detection based on spectral changes of whispering gallery modes by using two-photon polymerization is discussed. The main emphasis in this paper has been made on the manufacturing of active resonators doped with CdSe/ZnS core-shell quantum dots (QDs). Efficiency of direct introduction of active units and preprocessed QDs embedding techniques are compared. Experimental data on detection of bovine serum albumin protein solution with active resonators is represented.

Keywords: Two-photon polymerization, biosensor, resonator, processing, quantum dot;

1. Introduction

Detection and express analysis of biochemical agents is a rapidly growing area, whose development is primarily caused by the active use of various types of sensors based on optical detection techniques. Among so-called label-free detection technique the detectors based on optical resonance of whispering gallery modes (WGM) are in specific interest due to their superior sensing capabilities [1-4]. The common approach to fabricate WGM resonators is to apply UV or electron beam lithography [5]. They can be doped with a fluorescent material (be “activated”) in order to avoid sophisticated technique of evanescent light coupling and thus to simplify application of sensors and to enhance detection limit for biosensing [5].

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First attempts to produce WGM resonators by applying two-photon polymerization (2PP) technique were performed in [6, 7, 8]. Mixing of a photoresin with a laser dye enabled production of active resonators, but with decreased lasing efficiency due to photobleaching [8]. Photobleaching resisting emitters like quantum dots (QDs) can be incorporated as additional layers inbetween [9] or above the host material [10] layers during the production cycle requiring additional post-processing of the WGM sensors or additional steps in production.

In this paper we propose an approach to fabricate QDs-doped WGM-based biosensors in one step applying two-photon-polymerization technique.

2. Two-photon polymerization

A femtosecond near-IR laser source with a high peak intensity can be used for two-photon absorption (2PA). This process can be only induced in the area restricted in three space directions close to the focal point. Common liquid photoresin materials have high absorption in the UV range but they are transparent in the visible and near-IR spectral range. Thus, 2PA allows to produce 3D objects of arbitrary forms by changing focal point position along all space directions. Process is called direct laser writing or two-photon photopolymerization.

Low feature sizes below the diffraction limit [11] is possible for 2PP due to the fact that photochemical process responsible for the voxel (smallest unit of the polymerized area) formation has a threshold response to the light excitation. It means that the focal point size does not limit the voxel size and it is determined by light intensity distribution near the focus of the objective and light energy already absorbed by the photoresin.

To sum up, 2PP has several advantages for WGM resonators fabrication:

- Possibility to construct structures of arbitrary form.
- Near-IR laser light is insignificantly absorbed and scattered by photoresin and does not influence the polymerization process. High peak power of the femtosecond pulses allows initialisation of the 2PP, but does not results in blistering of the polymer due to low average power.
- No mask is needed. Sample preparation time is reduced.
- Lateral spatial resolution up to 120 nm and transversal resolution around 300 nm are achievable.

3. Resonator fabrication

3.1. Sample preprocessing

Liquid negative-tone hybrid photoresin has been used in this work. For negative photoresins, light absorption causes cross-linking of the polymer chains allowing the unexposed resin to be washed out. Appropriate intensity and exposure time need to be determined for the polymer molecules binding and successful polymerization. The common way is to determine these parameters experimentally.

We have chosen CdSe/ZnS hydrophilic QDs with central wavelength λ = 600±5 nm as a gain material for WGM resonators due to their high resistance to photobleaching. Two possibilities of active units embedding during production cycle are explored in this paper: mechanical localization of the QDs in the polymer material and chemical coupling to the polymer chain. In order to ensure chemical bound of the active units with the growing polymer chain, QDs are preprocessed with polyethylene glycol (PEG) methyl ether thiol (729108) according to the following scheme:

- Purification of the QDs from the excess of organics via precipitation of the QDs in the chloroform-methanol solution.
- Mixing for one hour of the QDs solution with PEG methyl ether thiol in proportion 3 mg to 1 ml in order to perform the ligand exchange.
- Removing of the unbounded PEG via precipitation in acetonitrile.
- Mixing of the photoresin with the PEG-processed QDs.

Intensive acetone washing and baking process at 200 °C for 5 min for the glass coverslip were performed previously to placing of the drop of the photoresin. Sample was kept at room temperature for approx. 24 hours to evaporate the solvent. Comparison of the QDs embedding efficiency for chemical coupling and mechanical localization is shown in the Figure 1. Results show, that the fluorescence intensity for the chemically coupled QDs is approx. one order of magnitude higher than for the localized in the structure active units thus a possibility to reach lasing conditions is higher. In addition, chemical coupling of the QDs increases homogeneity of the particle distribution in the structure due to reduction of the probability for the aggregation formation. Therefore, fabrication of the active WGM resonators with chemically coupled QDs via direct laser writing technology is preferred.

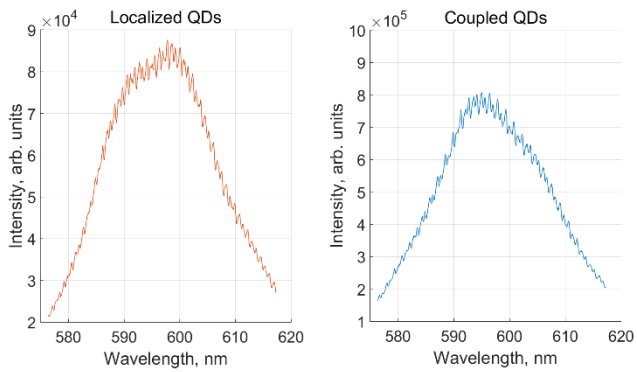

Fig. 1. Fluorescence of the 2PP manufactured structures (woodpile [11], rod thickness and distance 0.1 µm, total size 10 µm)

3.2. Experimental setup

Experimental setup used for two-photon polymerization is represented in the Figure 2. A mode-locked Ti:Sa laser system (Tsunami, Spectra Physics) with a repetition rate of 82 MHz, pulse width of 90 fs, and wavelength of 780 nm is used to generate ultrashort laser pulses (1). This laser system is pumped with Nd:YAG pulsed laser (0) operating in the doubled-frequency regime. Power adjustment is ensured by two components: λ/2 retardation plate (2), which is fixed in the rotation mount and polarizing beam splitter (3). Beam splitter selects one of the orthogonal components of the linearly polarized light. An acousto-optical modulator (AOM) (4) is used as a shutter. Experimental setup contains also several mirrors (5) for light redirection towards the galvoscanner (Scanlab, Hurryscan II) (7) with two piezo-controlled mirrors providing scanning ability in the XY plane. An additional lens tube (6) has been introduced in order to enlarge the laser beam profile matching the objective rear aperture for efficient light focusing. Oil-immersed 100× objective (8) with numerical aperture of 1.4 is connected from the bottom side to the scanner. The objective focuses

the light onto the sample (drop of the photoresin) which is placed under the glass coverslip. Sample movement in XYZ directions is provided by three orthogonal motorized linear stages (Aerotech Inc., USA) (9) that uses air bearings. CCD camera (10) has been introduced into the system in order to enable real-time 2PP production monitoring.

Samples were manufactured with the following laser light properties: 15mW average power of laser system, 1 m/s travel speed of the galvoscanner. After photoresin illumination, samples were wet-chemically processed using OrmoDev (MicroResist Gmbh.) developer for 30 min.

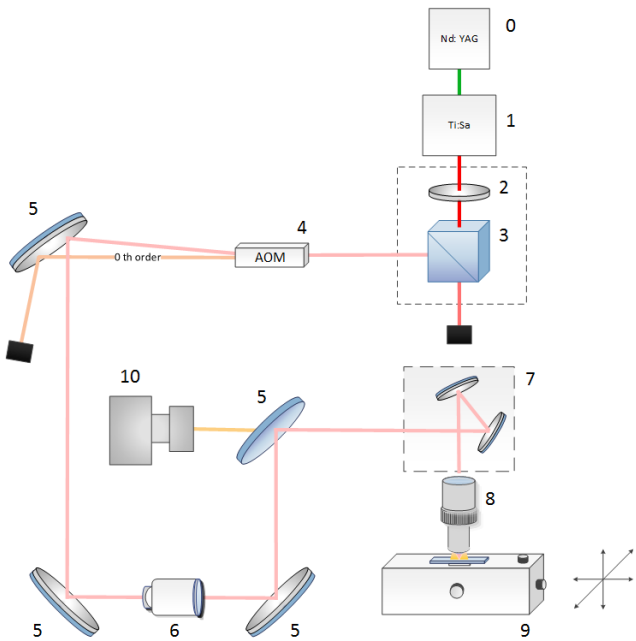

Fig. 2. 2PP experimental setup

4. Biochemical components detection

This paper represents experimental data on detection of 10 mg/ml bovine serum albumin (BSA) protein solution with active WGM resonators (Fig. 3).

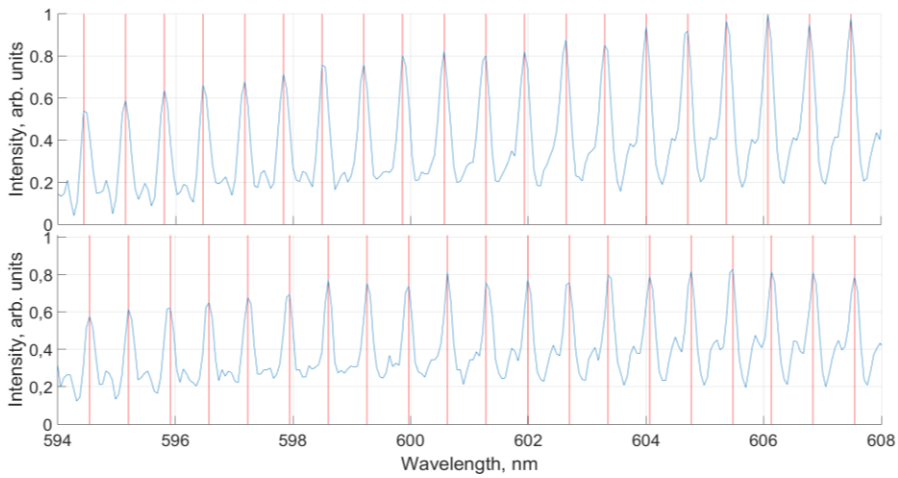

Fig. 3. WGM spectrum for de-ionized water (upper graph) and for BSA solution (bottom graph) environments.

Spectroscope (Renishaw inVia) has been used for detecting the spectrum emitted by the QDs. Sensors on the cover glass are placed into the microfluidic cell according to the scheme discussed in [12], connected to the pump and to the BSA solution container. Resonance fluorescence spectrum of the biosensors has been excited by the continuous wave emission of Nd:YaG laser system with doubled frequency (532 nm) with the following parameters: 5 mW power with one second exposure time and three accumulations were made and averaged in order to define the output spectrum.

Spectra for the initial environment (de-ionized water) as well as for the BSA solution were defined as averaged over the spectra acquired for the 5 minutes. Mean over the all resonant peaks in the observation region WGM spectral response on BSA solution is equal to 67 pm. Spectral shift of the WGM resonance confirms biosensing capabilities of the manufactured active resonators, however due to the spectrometer resolution limitations, the surface density of the albumin protein cannot be exactly determined in the current configuration.

5. Conclusion

A new technique for production of the WGM-based resonators doped with the PEG-preprocessed inorganic fluorescent components (QDs) via two-photon polymerization is discussed. High-resolution below diffraction limit and possibility to construct structures of arbitrary form with controllable parameters may ensure superior reproducibility making this technique attractive for WGM resonator-based biosensors fabrication in general. Proposed approach has a great potential for production of high-throughput and high-density packaged resonator-based biosensing devices.

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