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Gold nanoparticle-mediated fluorescence enhancement by two-photon polymerized 3D microstructures

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ABSTRACT

Fluorescence enhancement achieved by functionalized microstructures made by two-photon polymerization (TPP) is reported for the first time. Microstructures of various shapes made of SU-8 photoresist were prepared and coated with gold nanoparticles (NP) of 80 nm. Localized fluorescence enhancement was demonstrated by microstructures equipped with tips of sub-micron dimensions. The enhancement was realized by positioning the NP-coated structures over fluorescent protein layers. Two fluorophores with their absorption in the red and in the green region of the VIS spectrum were used. Laser scanning confocal microscopy was used to quantify the enhancement. The enhancement factor was as high as 6 in areas of several square-micrometers and more than 3 in the case of local enhancement, comparable with literature values for similar nanoparticles. The structured pattern of the observed fluorescence intensity indicates a classic enhancement mechanism realized by standing waves over reflecting surfaces. With further development mobile microtools made by TPP and functionalized by metal NPs can be actuated by optical tweezers and position to any fluorescent micro-object, such as single cells to realize localized, targeted fluorescence enhancement.

1. Introduction

Numerous modern medical, biotechnological and biological research applications rely on fluorescence measurements. The performance of the approach is limited by the sensitivity and spatial resolution of the applied technique. Improvement of these properties promises new possibilities in identification and characterization of specific biomolecules [1]. Metal-enhanced fluorescence (MEF), achieved by metal nanoparticles (NPs) in proximity to a fluorophore, has been applied recently in cell imaging observing otherwise weakly detectable signals [2–4]. The size, shape and arrangement variability of the NPs gave rise to a wide variety of spectral characteristics [5–8] resulting in signal enhancement in various parts of the visible spectrum. Enhancement has been realized in a broad size range as well: there are examples of single NPs to enhance the signal of single fluorophores [9] of microscopic area NP clusters [10] and of macroscopic area NP layers for enhanced cell imaging [11].

Movable enhancers in the range of 100 μm have been created before and shown to induce fluorescence enhancement of nearly a factor of 10 in cell imaging as determined with confocal microscopy by Radha et al. [10]; micromirrors were also generated of gold NPs by Kim and Osterloh with possible enhancement application [12]. However, so far no fluorescence enhancers have been introduced that is in the micrometer size range and mobile enough to be positioned at any desired position in a controlled manner.

Two-photon polymerization (TPP) is an efficient tool to produce complex, micrometer-sized 3D objects of arbitrary shape with nanometer features [13–15]. Such structures can serve as platforms for optical tweezers actuated microtools since their surface can easily be coated with metal nanoparticles [16]. Our laboratory has already polymerized structures of the negative-tone SU-8 photoresist that were coated with gold NPs with amino-silane linker molecules [17]. In these experiments we could control the surface NP density on the SU-8 layer over a wide range. Such polymerized structures can easily be actuated by optical tweezers as we demonstrated with mobile polymerized optical waveguides where localized fluorescence excitation was realized [18].

Abbreviations: APTES, (3-Aminopropyl)triethoxysilane; bBSA, Biotin-conjugated bovine serum albumin; BSA, Bovine serum albumin; CAN, Ceric-ammonium nitrate; fSTA, Fluorescent streptavidin conjugate; LSCM, laser scanning confocal microscope; MEF, Metal enhanced fluorescence; NP, Nanoparticle; PEG-diamine, Poly(ethylene glycol)bis(amine); SEM, Scanning electron microscope; TPP, Two photon polymerization; VIS, Visible.

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Here we show that micrometer-sized structures, called enhancers throughout this paper, shaped by TPP and covered with gold NPs, can perform localized fluorescence enhancement even on an area less than 1 μm^2. These tools are the forerunners of a new class of micrometer-sized mobile enhancers that can be actuated by optical tweezers for targeted fluorescence enhancement. Such a microdevice could be used in cell studies where measuring fluorescence at precisely localized positions at a reduced level of excitation is needed. We designed the shape of these objects with two goals in mind: (i) to perform enhancement over a sub-micrometer area and (ii) to demonstrate the distance–dependence of the enhancement. We performed the fluorescence enhancement on single layer of fluorophores by positioning the gold-coated enhancers over them and carried out the excitation and detection from below, from the direction of the supporting glass. We produced enhancer structures with four distinct shapes including two that have tips to obtain localized enhancement and to study distance dependence of the fluorescence. In order to explore the origin of the enhancement, we created enhancers with a reflective surface that is tilted relative to the substrate to allow precise control of the distance between the NP and fluorophore layers in the 0–5 μm range.

2. Materials and methods

2.1. Chemicals

Glass coverslips (24 × 40 mm) were purchased from Menzel Glaser (Germany); methanol, ethanol and isopropanol from Molar Chemical KFT (Budapest, Hungary). The SU-8 2007 photoresist and SU-8 developer (mr-Dev 600) were purchased from Micro Resist Technol. (Germany). Ceric-ammonium nitrate (CAN) (cat. No.: 22249), poly(ethylene glycol)bis(amine) (PEG-diamine) (2000 M_W, cat. No. 14501), (3-Aminopropyl) triethoxysilane (APTES) (cat. No.: 440140), bovine serum albumin (BSA) (cat. No.: A7906), biotin-conjugated bovine serum albumin (bBSA) (cat. No.: A8549) and gold (III) chloride hydrate ($HAuCl_4$) were purchased from Sigma–Aldrich. Tri-sodium citrate 2-hydrate and nitric acid were obtained from Reanal Ltd (Budapest, Hungary). Fluorescent streptavidin conjugate (fSTA) (cat No. 84547, DyLight 650, λ_{exc} = 652 nm, λ_{em} = 670 nm) and EZ-Link Sulfo-NHS-Biotin (cat No. 21217) was purchased from Thermo Fisher Scientific Inc. (USA). Fluorescent streptavidin conjugate (cat. No.: SA1010, Cy3, λ_{exc} = 554 nm, λ_{em} = 570 nm) was purchased from Life Technologies (Carlsbad, CA, USA).

2.2. Two-photon polymerization

The two-photon polymerization of the fluorescence enhancers was carried out as earlier [17–19]. Briefly the laser beam (C-Fiber A 780, Menlo Systems GmbH, Germany, $\Delta\tau$ = 100 fs, λ = 785 nm, 100 MHz repetition rate) was focused into an ~18 μm thick layer of soft-baked SU-8 2007 photoresist by a 100× oil immersion objective (NA = 1.25). During illumination the sample was moved relative to the focus by a 3D piezo translation system. Then it was baked again for 10 min at 95 °C to complete polymerization, developed in SU-8 developer and finally rinsed with ethanol. We designed two types of flat enhancer structures, as shown in Fig. 1(a): one with parallel surfaces (E1), one with rounded sides (E2) and two types with two (E3) or four tips (E4). Fig. 1(a) illustrates how the enhancer structures were polymerized onto their substrate and their largest area side as well as the tips are accessible for the gold NP suspension. The largest dimensions of each structure measured 15 μm and 20 μm while their thickness varied between 3 μm and 5 μm. The type E2 and E3 blocks were designed to visualize the distance dependence of the enhancement measured between the fluorophore and the NP layers. The projection of type E2 consists of two circular arc-shaped sides with a radius of curvature of 50 μm. The structures equipped with tips of 3–8 μm length enable the localization of the fluorescence enhancement into a region less than 1 μm^2. The end profiles of the tips

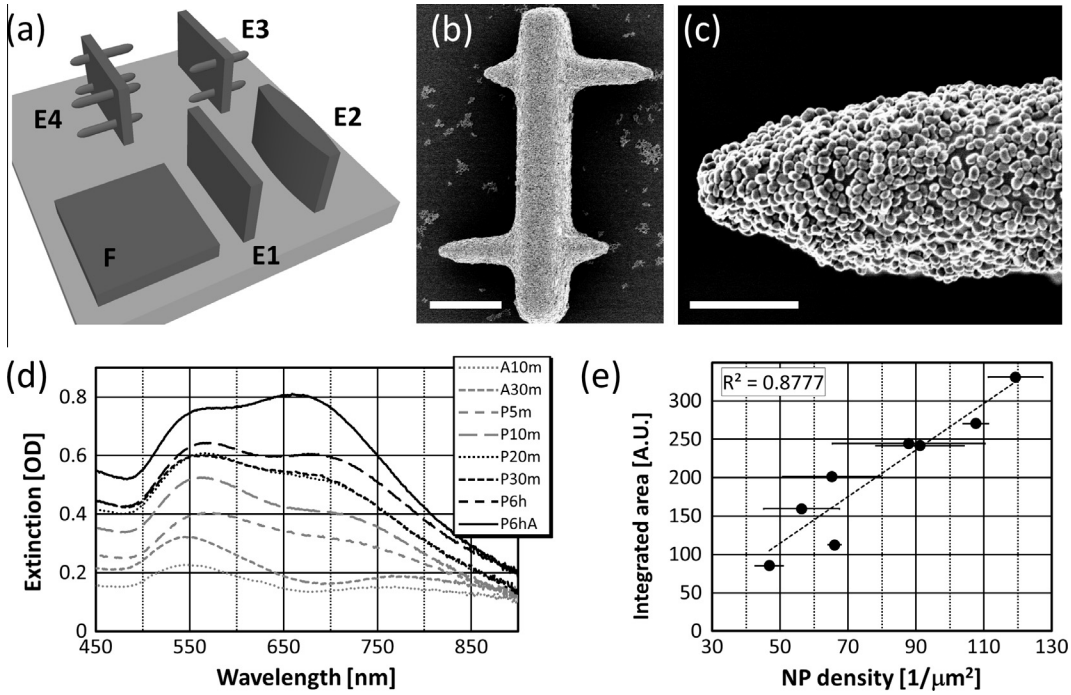

Fig. 1. Gold NP-coated TPP enhancers. (a) Schematic drawing of the four types of TPP enhancer structure (E1–E4) and the one used for microspectroscopy (F). SEM image of a gold NP-coated microstructures with tips (type E4) (b). (c) SEM image of a gold NP-covered tip. Scale bars: 5 μm (b) and 1 μm (c). (d) VIS extinction spectra of 80 nm gold NP layers on type F SU-8 microblocks prepared with various incubation times and linker molecules (for sample names see Table 1).