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FRET lasing from self-assembled DNA tetrahedral nanostructures suspended in optofluidic droplet resonators

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Abstract. We demonstrate Förster resonance energy transfer (FRET) lasing from self-assembled tetrahedral DNA complexes labeled with Cy3 and Cy5 dyes and suspended as a gain medium in aqueous microdroplet cavities deposited on a superhydrophobic surface. Threshold fluence and differential efficiency are characterized for DNA complexes containing 1Cy3-3Cy5 and 3Cy3-1Cy5. We demonstrate that at a constant Cy5 concentration, average threshold fluence is reduced 3 to 8 times and average differential efficiency is enhanced 6 to 30 times for 3Cy3-1Cy5 as compared to 1Cy3-3Cy5. Using 3Cy3-1Cy5 nanostructures, FRET lasing is observed at very low concentrations down to $\sim 1\,\mu$M. This work shows that optofluidic microlasers based on droplet resonators can be combined with DNA nanotechnology to explore applications in bio/chemical sensing and novel photonic devices.

1 Introduction

Miniature optofluidic systems that employ fluids to serve simultaneously for creating integrated, dynamically reconfigurable optical components and for transporting suspended molecules and/or cells are attracting an ever-increasing attention for analytical and sensing applications [1,2]. Among the various optofluidic platforms that have been utilized for biological and biomedical detection to date, optofluidic biolasers, which use the target bio-analyte as the laser gain medium, play a special role. The unique position of biolasers in sensing applications derives from the high sensitivity of the nonlinear optical processes associated with lasing to minute

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perturbations of the gain medium and/or the laser cavity [3]. In combination with advanced biotechnologies based on self-recognition and self-assembly of biological molecules (proteins, polynucleotides, or DNA), the sensing potential of lasers with biological gain media has been demonstrated in highly specific discrimination between DNA molecules with a single base-pair mismatch [4] or analysis of DNA melting [5].

Various experimental strategies of creating optical resonators for integrated optofluidic lasers have been explored. Typically, the resonator is formed from a solid-state material; this includes miniaturized versions of conventional Fabry-Perot cavities [6], distributed-feedback grating resonators [7], and optofluidic ring resonators (OFRRs) based on thin-wall capillaries that can support whispering gallery modes (WGMs) with extremely high quality factors (Q-factors) of $\sim 10^7$ and small modal volumes localized in the proximity of the resonator surface [4,5,8]. While all these approaches provide functional optical cavities, they require microfabrication and/or alignment steps which bring additional experimental challenges to the process of creating a working optofluidic sensor.

In the simplest possible approach, liquid alone – without any additional external solid structures – can be used to build the resonator. This is enabled by the tendency of liquids to minimize their interface area which transforms small liquid parcels into perfectly spherical droplets. Such liquid droplets with a refractive index higher than that of the surrounding medium (either air or a liquid immiscible with the droplet liquid) can then serve as alternative self-assembled ring resonators hosting high-Q WGMs [9–11]. In contrast to the OFRR where WGMs residing inside the capillary wall interact with the analyte only through evanescent mode coupling, in a liquid droplet resonator, analytes are placed directly into the cavity. Thus, the analyte molecules that are located near droplet surface can interact with the peak WGM fields, achieving very strong light-matter coupling. Moreover, aqueous microdroplets provide natural environmental conditions for biological molecules and live cells. These features make microdroplets ideally suited for sensing of biological species [12]. However, droplet-based cavities have only found a limited use in practical sensing application to date, mostly because of the difficulties with controlling and stabilizing the droplets.

In this Letter, we report miniature optofluidic biolasers formed by microdroplets generated from an aqueous solution of fluorescently-labeled DNA complexes and deposited on a superhydrophobic surface which stabilizes the droplet position and shape [11]. Active medium of the presented lasers consists of self-assembled tetrahedral complexes of complementary DNA strands labeled with Cy3 and Cy5 fluorescent dyes which form a donor and acceptor pair for the Förster resonance energy transfer (FRET). Attaching the FRET dye pair to the DNA scaffold with a precisely defined geometry preserves the distance of the donor and acceptor molecules and, thus, fixes the efficiency of FRET, making it independent of the concentration of DNA complex in the droplet liquid [13]. This characteristic feature of FRET microlasers based on DNA scaffolds differs from the behavior of bulk FRET microlasers that display concentration-dependent FRET efficiency [14]. We show that the lasing threshold and efficiency of the DNA-FRET biolasers can be efficiently tuned by adjusting the ratio of the donor-to-acceptor dye molecules on an individual tetrahedral DNA complex.

2 Materials and methods

Self-assembled DNA complexes containing Cy3 (donor) and Cy5 (acceptor) dye molecules were prepared as described previously [13]. Two different samples containing either three Cy3 molecules and one Cy5 molecule (3Cy3-1Cy5) or one Cy3 molecule and three Cy5 molecules (1Cy3-3Cy5) per tetrahedron were studied in the experiments. Aqueous microdroplets containing 35% w/w glycerol/water with 1X TAE

buffer plus 12.5 mM $MgCl_2$ and the fluorescent DNA complexes were deposited on cover glasses with superhydrophobic surface layer. These superhydrophobic surfaces were produced by spin-coating hydrophobic silica nanoparticles (Aeroxide LE1; Degussa AG) dispersed in ethanol on cleaned microscope cover glasses [11]. Thus prepared superhydrophobic surfaces are transparent to visible light and provide a high contact angle ($>150°$) for aqueous microdroplets [15]. To deposit micron-sized droplets on the superhydrophobic surface, aerosols of the droplet liquid were generated using a compact ultrasonic nebulizer (JIH50, Beuer) and sprayed over the surface. This procedure provided surface-supported microdroplets with diameters ranging from 1 μm to 100 μm. All the data reported in this Letter were recorded from droplets with a narrow size distribution (droplet diameter between 14–17 μm) in order to minimize the variation in the observed FRET lasing properties due to the droplet size. Upon droplet deposition, slow evaporation was observed as the droplets were exposed to the ambient atmosphere with approximately 50% relative humidity. All the experiments reported in this Letter were performed during approximately the first 10 minutes after the droplet generation. During this time, reduction in droplet size was measured to be less than 4%, coresponding to the maximal increase of the dye concentrations by ∼12% with respect to the initial values. We note that all dye concentrations reported in this Letter refer to those in the initial solutions used for droplet generation.

Experimental setup employed in the reported DNA-FRET lasing studies was built around an inverted optical microscope [14]. A 532 nm green beam generated by a home-built, passively Q-switched, frequency-doubled Nd-YVO_4 laser (20 ns pulse width and 33 kHz repetition rate) was used for optical pumping of the surface-supported microdroplets. Overall pump power was controlled by a polarizing beam splitter combined with a half-wave plate. The pump beam was directed towards the sample by reflection from a dichroic mirror and, subsequently, the beam was focused by a water-immersion objective with a high numerical aperture (Nikon 60×, NA=1.2) at the rim of a selected microdroplet. In order to reduce the sensitivity of the observed spectra to the precise position of the pump beam focus with respect to the microdroplet rim, the pump beam was focused relatively loosely to a focal spot of $\sim 1.6\,\mu$m diameter. Fluorescence emission from the optically pumped microdroplet was collected by the same microscope objective, transmitted through the dichroic mirror, and dispersed by a monochromator (focal length 500 mm; Acton Research) on the chip of a cooled CCD detector (Pixis 100; Princeton Instruments). A shutter was added to the pump laser beam path to prevent fast photobleaching of the dye molecules in the microdroplets. This shutter was only opened during the spectrum acquisition time and provided trigger signal for the CCD detector to synchronize the data recording. Microdroplets were imaged with an independent CCD camera placed at the other exit port of the microscope.

3 Results and discussion

Figure 1 illustrates typical FRET lasing spectra recorded from a 15.6 μm diameter droplet containing 8.3 μM 1Cy3-3Cy5 (part a) and a 16.4 μm diameter droplet containing 25 μM 3Cy3-1Cy5 (part b). For both microdroplets, the total Cy5 concentration was equal to 25 μM and the spectra were acquired with a constant pump fluence of 58 mJ/cm^2 which was well above the lasing threshold in both cases. The corresponding integral intensity of the lasing emission as a function of the pump beam fluence is shown in Fig. 1c. Here, the integral lasing intensity was obtained by integrating the recorded spectra over the spectral interval from 720 to 760 nm. For the two studied droplets containing 1Cy3-3Cy5 and 3Cy3-1Cy5, respectively,

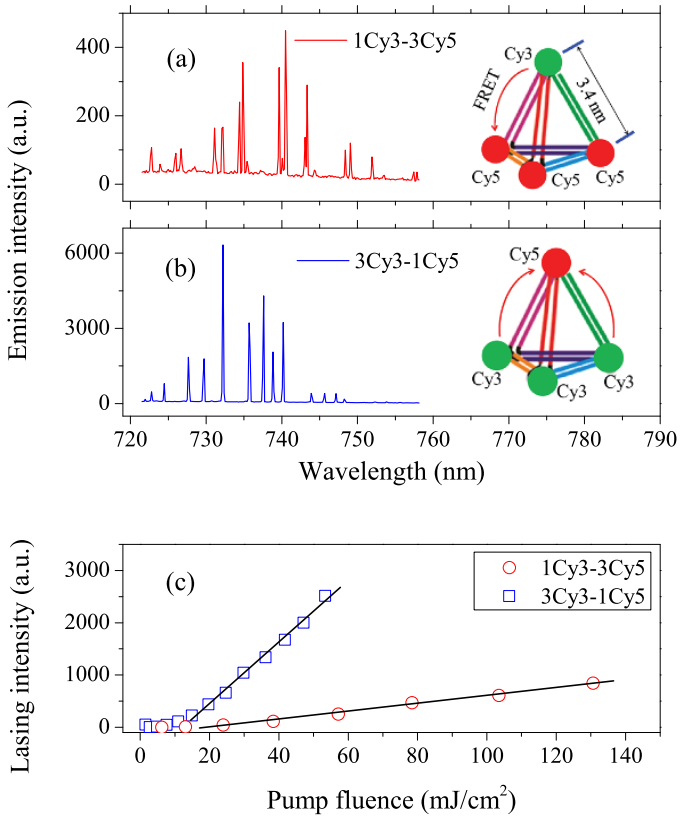

Fig. 1. (a) Lasing spectrum of a 15.6 μm diameter droplet doped with 1Cy3-3Cy5 and (b) lasing spectrum of a 16.4 μm diameter droplet doped with 3Cy3-1Cy5 DNA complexes with identical Cy5 concentration of 25 μM at a pump fluence of 58 mJ/cm^2. (c) Integral intensity of the lasing peaks as a function of the pump fluence for the droplet spectra shown in (a) (circles) and (b) (squares). Line fits to both experimental data sets give the lasing threshold fluences of 20 mJ/cm^2 and 11 mJ/cm^2, and differential efficiencies of 310 cm^2/mJ and 3200 cm^2/mJ, respectively.

lasing threshold fluences of 20 mJ/cm^2 and 11 mJ/cm^2, and differential efficiencies (defined as the slope of the linear fit to the integral lasing intensity) of 310 cm^2/mJ and 3200 cm^2/mJ, were measured. The ratio of threshold fluences and differential efficiencies observed for lasing from 1Cy3-3Cy5 and 3Cy3-1Cy5 are 1.8/1 and 1/10.3, respectively. These values are comparable with the theoretical analysis of the DNA FRET lasing and previous experiments carried out using the OFRR. For the studied system of Cy3 and Cy5 fluorescent dyes with the Förster radius of 6 nm and separation distance of 3.4 nm defined by the DNA scaffold, the threshold fluence of 3Cy3-1Cy5 complex is expected to be 8.8 times lower than the threshold fluence of 1Cy3-3Cy5 complex with the same total concentration of Cy5 [13]. In the OFRR experiments, the ratios of lasing threshold fluences and differential efficiencies for 1Cy3-3Cy5 and 3Cy3-1Cy5 with the same total concentration of Cy5 were measured to be 3.8/1 and 1/28, respectively [13]. Quantitative discrepancy between our results and the previously reported data obtained with the OFRR can be attributed to several factors including different geometry of the used active cavity (direct modal field coupling in the surface-supported microdroplets vs. evanescent mode coupling in the OFRR) and different total concentration of Cy5 dye in the liquid gain medium. In

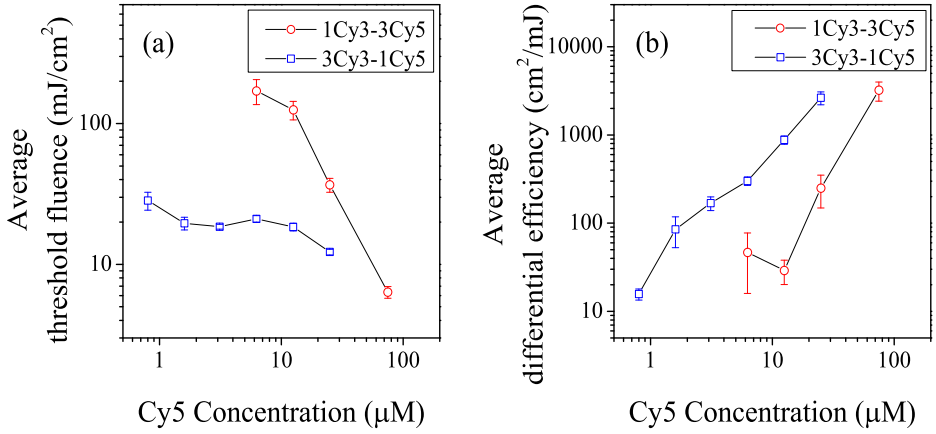

Fig. 2. (a) Average lasing threshold fluence and (b) average lasing differential efficiency for droplets with diameters 14–17 μm containing 1Cy3-3Cy5 (circles) and 3Cy3-1Cy5 (squares) DNA complexes as a function of Cy5 concentration varying between 0.8 μM and 75 μM.

addition, our experiments with 3Cy3-1Cy5 and 1Cy3-3Cy5 complexes were carried out with different droplets and, thus, we could not prevent variations in the droplet size, precise location of the excitation beam focus on the droplet, and Q-factor of the cavity caused by different contact angles of the droplets.

To demonstrate the dependence of FRET lasing characteristics on the total Cy5 concentration in the droplets, we varied the concentrations of 1Cy3-3Cy5 and 3Cy3-1Cy5 from 25 μM down to the sub-μM range and measured the threshold fluence and differential efficiency. In Fig. 2a, mean threshold fluence of lasing from droplets in the size range of 14–17 μm is shown as a function of the total Cy5 concentration for both 1Cy3-3Cy5 and 3Cy3-1Cy5. Here, each data point represents an average calculated from at least 10 measurements carried out with different droplets. Error bars indicate the standard error of the mean (i.e. the standard deviation of the measured values divided by the square root of the number of measurements) for each concentration. Due to the highly efficient FRET pumping mechanism in 3Cy3-1Cy5 complexes, lasing is observed even at very low Cy5 concentrations ($\sim$1 μM). For all Cy5 concentrations, average threshold fluence measured for 1Cy3-3Cy5 is 3 to 8 times larger than that recorded for 3Cy3-1Cy5. Figure 2b shows the lasing differential efficiency of droplets with diameters between 14–17 μm whose lasing threshold fluence was studied in Fig. 2a. Error bars in Fig. 2b also represent the standard error of the mean. In agreement with the previous work by Chen et al. [13], for all Cy5 concentrations, average differential efficiency of 3Cy3-1Cy5 is measured to be larger than that of 1Cy3-3Cy5 by a factor changing between 6 and 30. Hence, we conclude that for the same Cy5 concentration, 3Cy3-1Cy5 displays lower threshold and larger slope efficiency in comparison to 1Cy3-3Cy5. This is due to the high excitation efficiency of a given acceptor molecule when it is surrounded by multiple donor molecules leading to higher overall FRET efficiency. We should note that relatively large variations were observed when the threshold fluences and differential efficiencies were compared for individual studied droplets. These variations stem mainly from the variations in the droplet cavity Q-factor which is influenced by the droplet size and contact angle and from slight differences in the position of the pump beam focus on the droplet.

4 Conclusion

We have demonstrated miniature optofluidic lasers based on surface-supported liquid microdroplets which use self-assembled DNA complexes labeled with fluorescent dyes forming a FRET donor-acceptor pair as the laser gain medium. We have characterized FRET lasing for different concentrations of DNA scaffold structures and shown that the threshold pump fluence and differential efficiency can be tuned over a large range by adjusting the ratio of concentrations of the donor and acceptor dye per single DNA complex and the overall acceptor concentration in the droplet liquid. Since the surface-supported droplets can be generated from minute liquid volumes, our technique allows efficient analysis of samples that are only available in small quantities. Thus, the reported work paves the way for using flexible, programmable, and cell-permeable DNA tetrahedral scaffolds together with surface-supported liquid microdroplets as optical resonant cavities for highly sensitive biological and chemical detection, and for the development of novel photonic devices [16].

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