Published in final edited form as:

Lab Chip. 2016 January 5; 16(2): 353–359. doi:10.1039/c5lc01004g.

Optofluidic FRET Lasers Using Aqueous Quantum Dots as Donors

Qiushu Chen[†], Alper Kiraz^{†,‡}, and Xudong Fan[†]

Qiushu Chen: akiraz@ku.edu.tr; Xudong Fan: xsfan@umich.edu

[†]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA

[‡]Department of Physics, Koç University, Rumelifeneri Yolu, Sariyer, 34450 Istanbul, Turkey

Abstract

An optofluidic FRET (fluorescence resonance energy transfer) laser is formed by putting FRET pairs inside a microcavity acting as gain medium. This integration of optofluidic laser and FRET mechanism provides novel research frontiers, including sensitive biochemical analysis and novel photonic devices, such as on-chip coherent light sources and bio-tunable lasers. Here we investigated an optofluidic FRET laser using quantum dots (QDs) as FRET donors. We achieved lasing from Cy5 as the acceptor in the QD-Cy5 pair when excited at 450 nm where Cy5 has negligible absorption by itself. The threshold was approximately 14 $\mu\text{J}/\text{mm}^2$. The demonstrated capability of QDs as the donor in a FRET laser greatly improves the versatility of optofluidic laser operation due to the broad and large absorption cross section of QDs in the blue and UV spectral region. The excitation efficiency of the acceptor molecules through FRET channel was also analyzed, showing that the energy transfer rate and the non-radiative Auger recombination rate of QDs plays a significant role in FRET laser performance.

Keywords

Optofluidic laser; FRET; Quantum dot; FRET laser

Introduction

The optofluidic laser has recently emerged as an enabling tool that integrates optical microresonators with microfluidic channels and different gain media in a liquid form.^{1,2} The optofluidic lasers has great potential in sensitive biochemical detection due to its capability to amplify small changes in analyte emission intensities caused by underlying concentration changes or conformational changes in biomolecules.^{3–6} It is also promising for novel on-chip photonic devices, such as on-chip coherent light sources and bio-tunable lasers.^{1,2,7–9} An optofluidic laser is usually excited by tuning an external pump laser (such as optical parametric oscillator – OPO) directly into the gain medium absorption band. Alternatively, it can also be excited indirectly using fluorescence resonance energy transfer (FRET), where an acceptor laser can be achieved by the excitation funneled from the donor molecules through the FRET channel.^{6–9} FRET is a widely used technology in biological research to sensitively analyze biomolecules, as the Förster distance of a FRET pair is around 5–10 nm, close to the characteristic dimension of a biomolecule. Incorporating FRET with the

optofluidic laser will lead to a number of new research frontiers. On one hand, the optofluidic FRET laser that measures the FRET process inside a laser cavity using laser outputs provides a means to analyze biomolecules with orders of magnitude higher sensitivities than traditional fluorescence based FRET detection, as recently shown experimentally and theoretically.^{6,10,11} On the other hand, FRET significantly enhances the versatility and flexibility of an optofluidic laser by providing another pump mechanism that can extend the optofluidic laser spectral coverage and increase the pumping efficiency.¹² In addition, it enables precise control and modulation of the optofluidic laser via biomolecular interactions.⁸

In the past few years, a wide variety of biochemically related fluorophores have been explored as the optofluidic laser gain medium, including organic dyes,^{3,11} biomaterials (fluorescent proteins,^{10,13,14} vitamins,¹⁵ luciferins¹⁶), and products of enzyme-substrate reaction⁵, many of which can also work as the donor or the acceptor in an optofluidic FRET laser^{10,11,16}. Semiconductor colloidal quantum dots (QDs) are another type of fluorophore that possess unique photo-physical properties such as color tunability by size, high brightness, and good photostability that are of great importance for the development of the optofluidic laser technology.^{17–20} In particular, due to high light absorption capability in the blue and UV region, QDs can collect the excitation light more efficiently, resulting in a significantly lower lasing threshold.²⁰ More recently, optofluidic lasers using biocompatible QDs in an aqueous environment have been demonstrated, paving the way to actual biochemical applications with optofluidic QD lasers.^{18,20} In addition to directly acting as the gain medium, QDs have the potential to work as the donor in a FRET laser. In fact, in the context of traditional fluorescence based detection, QDs have been extensively studied as the donor^{21–26} or acceptor²⁷ in a FRET pair for various biosensing applications. QDs are especially well suited as donors due to their broad absorption bands, tunable emission bands for controllable spectral overlap with acceptors, and large Stokes shifts for suppression of direct excitation of acceptors.^{28,29} However, despite extensive studies of QDs in fluorescence based FRET, up to date the performance of QDs in an optofluidic FRET laser has rarely been explored.³⁰

In this work, we developed and studied an optofluidic QD FRET laser using commercial water-soluble QDs (donor) and dye molecules (acceptor) as a model system. We showed that QDs can assist acceptor dye lasing with an excitation wavelength far from the dye absorption band thanks to FRET. We also investigated the efficiency of our QD FRET laser in comparison with the directly excited dye laser and revealed that the limiting factors of the FRET laser performance are the FRET energy transfer rate and the non-radiative Auger recombination. This study has multiple implications. First, to the best of our knowledge, it is the first demonstration of a FRET laser using aqueous QDs as the donor. The integration of optofluidic laser technology with QD FRET that is conceptually demonstrated here has great potential in building ultra-sensitive, versatile, and robust platforms for biochemical sensing applications. QDs, with their unique photo-physical properties, are expected to improve the performance of optofluidic FRET lasers and bridge the gap between laboratory technology and real applications. Second, the role of QDs in a FRET lasers is of general scientific interest. The optofluidic QD FRET laser studies can also be instrumental in understanding the photo-physical properties of QDs as the laser gain medium and as the donor, and in

revealing their similarity to and dissimilarity from organic dyes in FRET processes, which will be important for us to take advantage of QDs and overcome their limitations.

Experimental

Water-soluble QDot 655 (Invitrogen) and Cy5 were chosen to be the FRET donor and acceptor in this work. QDot 655 has an organic PEG coating that has active amino groups on the surface. Its emission peak is centered at 655 nm, which has significant overlap with the Cy5 absorption band. Cy5 was purchased from Sigma-Aldrich, which has NHS ester that can react with amines for labeling (Cy5 NHS ester). In addition, Cy5 labeled on a DNA (Cy5-DNA) was purchased from Integrated DNA Technologies, which served as a negative control in our studies. The reason to use Cy5-DNA, instead of pure Cy5, is that Cy5-DNA can dissolve in PBS buffer much more efficiently than pure Cy5.

The covalent immobilization method was used to link Cy5 NHS ester to the QD surface, which, along with the sample characterization, is described as follows. After changing the buffer of QDot 655 from original borate buffer to PBS with 100K Amicon Ultra-0.5 centrifugal filter, 100 μ L 6.25 μ M QDot 655 was mixed with 150 μ g Cy5 NHS ester and incubated for 1 hr. Then the mixture was filtered with a resin column that separates free Cy5 NHS ester from QD-Cy5 conjugation. Multiple tests were performed to characterize the resulting sample. According to the absorption test with NanoDrop 2000c UV-Vis spectrophotometer (Fig. 1(A)), the concentration of QD and Cy5 was calculated to be 3.3 μ M and 29 μ M, respectively, corresponding to a labeling ratio of 9 (*i.e.*, 9 Cy5 molecules covalently linked to one QD on average). The existence of FRET in the QD-Cy5 conjugation was verified by photoluminescence excitation (PLE) conducted with FluoroMax-4 spectrofluorometer, in which emission intensity at 720 nm from Cy5 was monitored while scanning the excitation wavelength from 400 nm to 690 nm. A similar pattern was observed in the PLE spectrum compared to the absorption spectrum, as shown in Fig. 1(B), indicating that photons absorbed by QDs can be transferred to Cy5 emission through FRET. Emission spectrum in Fig. 1(C) further shows a peak centered around 665 nm, red-shifted from original QD peak at 655 nm, which is due to the combination of contribution from QDs and Cy5 when excited at 430 nm where Cy5 has negligible absorption. Further clues for FRET can be derived from lifetime measurements. The measured lifetime of the original QDot 655 sample is 34.6 ns (Fig. S1 and Table S1). In the conjugate sample, QDot 655 lifetime is significantly reduced to 4.4 ns, indicating the existence of the fast FRET energy transfer decay channel. A FRET efficiency of 87% can be calculated from the reduced lifetime of QDs in the conjugate sample (see details in the Supplementary Information)

For optofluidic laser studies, we used the capillary-based optofluidic ring resonator (OFRR) as the platform due to its high quality factor optical resonances, simple fabrication, and intrinsic microfluidic characteristic for easy sample delivery.^{31,32} The OFRR had an outer diameter of approximately 100 μ m and a wall thickness of a few micrometers. The circular cross section of the OFRR supports the whispering gallery mode (WGM) that circulates along its circumference. Due to the thin wall (a few micrometers), the WGM has a sufficient evanescent field in the core and provides optical feedback for the gain medium flowing

inside the capillary to lase.^{7,32} The preparation of the OFRR was described elsewhere.^{31–33} A typical confocal setup was used to excite the sample and collect emission light (Fig. 2). A pulsed OPO laser (pulse width: 5 ns, repetition rate: 20 Hz) was used as the excitation source. Pump intensity was adjusted by a continuously variable neutral density filter. The laser beam was focused on the OFRR using a 20 mm lens. The emission light was collected through the same lens and sent to the spectrometer (Horiba iHR550) for analysis.

Results and discussion

As a control experiment, we first ran a 30 μM Cy5-DNA sample in PBS buffer in the system and studied its lasing performance, which had almost the same Cy5 concentration as in the QD-Cy5 conjugate sample. When excited at 450 nm, no lasing could be observed within the system excitation power capability (up to 550 $\mu\text{J}/\text{mm}^2$). As exemplified in the red curve in Fig. 3(A), only featureless emission spectrum is observed. In contrast, when the QD-Cy5 conjugate sample was flowed through the same capillary immediately after the experiments with the Cy5-DNA, Cy5 lasing peaks emerged around 730 nm (Fig. 3(A), black curves) with excitation at the same wavelength (450 nm). The measured linewidth of the lasing peaks was 0.32 nm, much sharper than fluorescence emission. This linewidth is limited by the resolution of our spectrometer (0.05 nm with 600 g/mm grating) and by the fact that each peak may contain multiple lasing modes that have slightly different lasing wavelengths. The red shift of the lasing peak relative to the fluorescence emission peak of Cy5 is typical for dye lasers,³² caused by the self-absorption of Cy5 and additional absorption of QDs. Fig. 3(B) plots the Cy5 laser output as a function of pump intensity. A threshold pump intensity of 14 $\mu\text{J}/\text{mm}^2$ is derived by fitting the lasing output above threshold. Beyond the threshold, the Cy5 lasing emission increases linearly with the increased pump intensity, but levels off when the pump intensity is above 100 $\mu\text{J}/\text{mm}^2$, which indicates saturation in excitation. From these measurements, it can be concluded that through conjugation with QDs, which has strong absorption around 450 nm, Cy5 lasing emission can be achieved via FRET.

We further performed a photostability experiment of FRET lasing with the QD-Cy5 sample. Fixing pump intensity at 176 $\mu\text{J}/\text{mm}^2$, we recorded the lasing spectrum and derived lasing and fluorescence intensities from different spectral regions over 49 consecutive excitation pulses (Fig. 4(A) and (B)). After 49 excitation pulses, fluorescence intensity experienced a decrease of less than 20% while a 50% decrease was recorded for lasing emission. This result is consistent with the non-linear nature of laser mechanism.³⁴ The relatively rapid photo-bleaching of Cy5 laser emission is due to the high excitation intensity used in the experiment. In contrast, when a lower excitation intensity (36.7 $\mu\text{J}/\text{mm}^2$) was applied, significantly improved photostability was observed (Fig. 4(C) and (D)). In practice, the laser does not need to be operated in high excitation intensity for biosensing. In fact, single pulse excitation at an intensity slightly above the threshold is sufficient.

In general, the equations for the acceptor laser are given as follows:⁶

$$\frac{dn_d}{dt} I_p \sigma_{d,a} (N_d - n_d) - \frac{n_d}{\tau_d} - k_F n_d \quad (1)$$

$$\frac{dn_a}{dt} = k_F n_d - \frac{n_a}{\tau_a} \quad (2)$$

$$\frac{dq_a}{dt} = \frac{c}{m} \sigma_{a,e}(\lambda_L) n_a - \frac{c}{m} \sigma_{a,a}(\lambda_L) (N_a - n_a) - \frac{q_a}{\tau_{cavity}} \quad (3)$$

where n_d and n_a are the donor and acceptor concentration in the excited state, respectively. N_d and N_a are the total concentration for the donor and acceptor, respectively. q_a is the photon number density in the cavity emitted by the acceptor. τ_d , τ_a , and τ_{cavity} are the lifetime for the donor, the acceptor, and the photons in the cavity mode. τ_{cavity} is related to

the empty cavity Q-factor, Q_0 , by $\tau_{cavity} = \frac{Q_0 \lambda}{2\pi c}$. c is the light speed in vacuum. k_F is the energy transfer rate from the donor to the acceptor. $\sigma_{d,a}$, $\sigma_{a,e}$, and $\sigma_{a,a}$ are the donor absorption cross section, the acceptor emission cross section, and the acceptor absorption cross section, respectively. I_p is the pump intensity. m is the refractive index of the cavity mode. λ_L is the lasing wavelength. Eqs. (1) and (2) describe the donor and acceptor concentrations in the excited state, respectively, whereas Eq. (3) describes the number density of photons inside the cavity. Under the steady-state condition, we have

$$n_d = \frac{I_p \sigma_{d,a}}{I_p \sigma_{d,a} + 1/\tau_d + k_F} N_d \quad (4)$$

$$n_a = \tau_a k_F n_d \quad (5)$$

$$\sigma_{a,e}(\lambda_L) n_a - \sigma_{a,a}(\lambda_L) (N_a - n_a) - \frac{2\pi m}{Q_0 \lambda_L} = 0 \quad (6)$$

Eq. (6) leads to the threshold condition for the acceptor concentration, $n_{a,th}$, which is

$$\gamma_{th} = \frac{n_{a,th}}{N_a} \approx \frac{\sigma_{a,a}(\lambda_L) N_a + 2\pi m / (Q_0 \lambda_L)}{N_a \sigma_{a,e}(\lambda_L)}. \quad (7)$$

For the case where QDs are used as the donor, Eq. (1) may need to be modified to reflect multi-exciton excitation and extremely fast relaxation processes of multi-excitons,^{35,36} which are on the order of tens to hundreds of pico-second, shorter than the energy transfer time ($1/k_F$) of a few nano-second. To further understand the energy transfer between the QD donor and the dye acceptor, we chose QDot 655 and Alexa Fluor 680 (AF680) as another donor/acceptor pair. Due to smaller spectral overlap between QDot 655 and AF680 (see Fig. S2 in the Supplementary Information), energy transfer efficiency is lower than that for QDot 655 and Cy5 (see Fig. S3 in the Supplementary Information). Therefore, only distinct fluorescent peaks of the donor and the acceptor were observed using exactly the same experimental conditions as in the QD-Cy5 case, as plotted in the inset of Fig. 5, which allows us to study the energy transfer between the QD donor and dye acceptor without interference from the acceptor laser emission.

Fig. 5 plots the fluorescence from both QDot 655 and AF680 measured simultaneously with various pump intensities. Initially, the fluorescence from both QDot 655 and AF680 increases concomitantly and is linearly proportional to the pump intensity, indicating that more QDs are excited through direct excitation and more AF680 are excited through FRET. Between 10 and 100 $\mu\text{J}/\text{mm}^2$, the QD emission starts to show certain degree of saturation, *i.e.*, its intensity still grows with the increased pump intensity, but with a smaller slope. Accordingly, the AF680 emission has a similar pattern of saturation. When the pump intensity is above 100 $\mu\text{J}/\text{mm}^2$, the QD emission completely levels off, suggesting that no more photons can be emitted by the QDs, despite the increased pump intensity. Meanwhile, the AF680 increases with a very slow slope. Detailed analysis in Fig. S4 in the Supplementary Information shows that this slight increase in AF680 emission results from the small direct excitation of AF680 at 450 nm pump whereas the emission of AF680 through the energy transfer from the QDs should completely saturate, as suggested by the QD emission. Indeed, after the correction for the emission from the direct excitation, the AF680 emission exhibits the corresponding saturation behavior that follows that in the QD emission (see Fig. S4).

The detailed microscopic mechanism of FRET using QDs as the donor can be understood as follows. Upon excitation of multiple excitons, they relax very rapidly down to biexciton and then single exciton through the non-radiative recombination.^{35,36} As those relaxation processes are very fast in comparison with the radiative decay rate, they do not contribute to photon generation. The QD fluorescence emission in Fig. 5 comes mainly from biexciton and single exciton, whose radiative decay rates are comparable to the corresponding non-radiative rates. Both biexciton and single exciton are able to transfer excitation energy to the acceptor dye.²⁶ The energy transfer efficiency for biexciton and single exciton can be written as $\tau_{\text{FRET}}^{-1} / (\tau_{\text{FRET}}^{-1} + \tau_x^{-1})$, where τ_x is the biexciton ($x=2$) and single exciton ($x=1$) lifetime and is the energy transfer time. However, since the biexciton lifetime (<2 ns)²⁶ is much shorter than that of single exciton (~ 34 ns), and the energy transfer time for biexciton and single exciton is similar²⁶, the emission from AF680 comes mainly from the energy transferred from single excitons. The same argument is valid for the QD-Cy5 system, whose energy transfer time is 4.4 ns. Consequently, n_d in Eq. 2 should refer to the concentration of single excitons. At high pump intensity (>100 $\mu\text{J}/\text{mm}^2$), we can assume that the concentration of single excitons is the same as the QD concentration. At relatively low pump intensity (<100 $\mu\text{J}/\text{mm}^2$), the fractional single exciton concentration can be deduced by comparing the AF680 fluorescence at low pump intensity with the saturation fluorescence. Based on the above discussion, for the QD-Cy5 conjugates, the lasing threshold of 14 $\mu\text{J}/\text{mm}^2$ corresponds to n_d of 2 μM ($=3.3$ $\mu\text{M} \times 0.6$, where 0.6 is the ratio of AF680 fluorescence between 14 $\mu\text{J}/\text{mm}^2$ and 100 $\mu\text{J}/\text{mm}^2$). Using $\tau_a = 1$ ns for Cy5 and $k_F = (4.4$ ns) $^{-1}$ in Eq. 5, we arrive at $n_a = 0.45$ μM and $\gamma = n_a/N_a = 1.6\%$. At high pump intensity, the Cy5 laser emission saturates beyond 100 $\mu\text{J}/\text{mm}^2$, consistent with the QD saturation behavior obtained by fluorescence measurement using QD-AF680.

To further examine the γ value, we performed the laser measurement using Cy5-DNA under exactly the same condition as in the QD-Cy5 case, except that the pump wavelength was moved to 500 nm where Cy5 has much higher absorption than at 450 nm. Fig. 6 shows that

the lasing emission can be achieved with a threshold of approximately $13 \mu\text{J}/\text{mm}^2$. For direct excitation, the concentration of Cy5 at the excited state can be calculated by

$$n_a = \frac{I_p \sigma_{a,a}(500\text{nm})}{I_p \sigma_{a,a}(500\text{nm}) + 1/\tau_a} N_a. \quad (8)$$

Using $\sigma_{a,a} = 0.31 \times 10^{-16} \text{ cm}^2$ at 500 nm (see Fig. S1 or Table S1), $\tau_a = 1 \text{ ns}$, and $I_p = 6.5 \times 10^{14}/(\text{cm}^2 \text{ ns})$, we have $\gamma = n_a/N_a = 2.0\%$, which has the same level of Cy5 excitation as in the QD-Cy5 conjugates. Furthermore, in Eq. 7, using $\sigma_{a,e} = 2.5 \times 10^{-16} \text{ cm}^2$, $Q_0 = 2 \times 10^6$, $\lambda_L = 730 \text{ nm}$ and ignoring the first term (*i.e.*, $\sigma_{a,a}N_a$, as it is small compared to the second term related to the cavity loss), we obtain the lasing threshold condition of $n_{a,\text{th}} = 0.41 \mu\text{M}$ and $\gamma_{\text{th}} = 1.4\%$.

From our experimental result and theoretical analysis, we conclude that there are two major strategies to achieve a FRET laser with QD as the donor. The first one is to increase the FRET energy transfer rate k_F (see Eq. (5)). This can be fulfilled by (1) choosing a good FRET pair that the emission band of QD has significant overlap with the acceptor absorption band; (2) carefully tuning the distance between the core of the quantum dots and the acceptor to enable sufficient energy transfer; (3) increasing the labeling ratio. The second strategy is to suppress the non-radiative Auger recombination rate of multi-exciton states of QDs to enable FRET from higher-order exciton states. For this purpose, QDs with better surface chemical designs or type II QDs can be used^{37–40} to further exploit the large absorption cross section of QDs in FRET lasing.

Conclusion

In summary, we have investigated the capability of QDs as the donor for optofluidic FRET laser operation and successfully achieved lasing from acceptor molecules in a QD-Cy5 system when excited at 450 nm where Cy5 has negligible absorption by itself, thus significantly extending the excitation spectral range for the acceptor. The lasing threshold was approximately $14 \mu\text{J}/\text{mm}^2$. The power-dependent fluorescence and laser studies suggest that the excitation of the acceptor comes mainly from the energy transferred from single excitons in QDs. We further reveal the similarities and dissimilarities between organic dyes and QDs as the donor and point out the methods to improve the optofluidic FRET laser performance when using QDs as the donor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Authors acknowledge the support from the National Institutes of Health (1R21EB016783). A.K. acknowledges the support from Fulbright Fellowship and the University of Michigan as a visiting scholar. Authors also thank M.-A. Mycek for QD lifetime measurement, and S. Sivaramakrishnan for the help in sample preparation and characterization.

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Figure 1.

Characterization of the Cy5-conjugated QD sample. (A) 1 mm absorbance of 10× diluted sample. (B) Photoluminescence excitation spectrum of the same sample as in (A). Emission was collected at 720 nm. (C) Normalized emission spectra of pure QD, QD-Cy5 conjugation, and Cy5. Inset: a QD-Cy5 conjugate.

Figure 2.
Optofluidic laser experiment setup.

Figure 3.

(A) Emission spectra of QD-Cy5 and Cy5 when pumped at 450 nm. Spectra are vertically shifted for clarity. (B) Spectrally integrated emission versus pump intensity for QD-Cy5 when pumped at 450 nm. Spectral integration takes place in the range of 730–740 nm. The lasing threshold for QD-Cy5 is approximately $14 \mu\text{J}/\text{mm}^2$. Each data point was collected upon single pulse excitation in order to minimize photo-bleaching. Error bars are obtained with 3 measurements. Concentration of Cy5 in QD-Cy5 in (A) and (B) was $29 \mu\text{M}$.

Figure 4.

QD-Cy5 FRET lasing stability study. (A) Normalized spectrally integrated emission for Cy5 fluorescence (675 – 705 nm) and Cy5 lasing (710 – 740 nm) under $176 \mu\text{J}/\text{mm}^2$ excitation. Each datapoint represents the normalized emission under excitation of a single pulse. (B) The corresponding Cy5 laser emission spectrum for the 1st and 49th excitation pulse. (C) Normalized spectrally integrated emission for Cy5 fluorescence (690 – 700 nm) and Cy5 lasing (710 – 740 nm) under $36.7 \mu\text{J}/\text{mm}^2$ excitation. Each datapoint represents the normalized emission under excitation of 20 pulses. (D) The corresponding Cy5 laser

emission spectrum for the sum of 1st–20th pulses and the sum of 81st–100th excitation pulses.

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Figure 5. Spectrally integrated fluorescence of QD and AF680 from QD-AF680 conjugates for various pump intensities at 450 nm. Each data point is recorded under single pulse excitation. Spectral integration takes place in the range of 650 – 655 nm for QD emission and of 700 – 705 nm for AF680. Inset: emission spectra of QD-AF680 conjugation at different pump intensities. Spectral curves are smoothed with a low pass filter with a cutoff at 0.1 Hz.

Figure 6. Spectrally integrated emission versus pump intensity for Cy5 when pumped at 500 nm. Spectral integration takes place in the range of 715–735 nm for lasing and 680–700 nm for fluorescence (FL). Inset: emission spectrum of Cy 3 pumped at $176 \mu\text{J}/\text{mm}^2$. The lasing threshold for Cy5 is approximately $13 \mu\text{J}/\text{mm}^2$. Concentration of Cy5 was $30 \mu\text{M}$. Experimental conditions were the same as in Fig. 3.