Bio-switchable optofluidic lasers based on DNA Holliday junctions†‡

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Bio-switchable optofluidic lasers based on DNA Holliday junctions were demonstrated. Nearly 100% wavelength switching was achieved through reversible conformational change of the Holliday junction controlled by magnesium ionic strength.

Optofluidic lasers synergize microfluidics and laser technology, and have unique capabilities in handling liquid of picoliter to microliter volumes.1–4 As such, there is currently a great deal of interest in adapting optofluidic lasers for compact, dynamically tunable laser light sources and micro-total-analysis systems. To date, various optofluidic lasers have been demonstrated, including Fabry-Perot lasers,5,6 distributed feedback (DFB) lasers,7,8 and optofluidic ring resonator (OFRR) lasers.9,10 In those lasers, wavelength switching is usually implemented by flowing liquid droplets (or plugs) containing different dyes through the microfluidic channel, with an excitation laser tuned into the absorption band of each dye.5,11 Fluorescence resonance energy transfer (FRET) provides another effective means to achieve wavelength switching, in which the donor is excited and transfers energy to the acceptor for it to lase.9,12,13 As compared to the directly excited laser, the FRET laser is versatile by extending the laser emission range without changing the excitation laser and has a low lasing thresholds due to the significantly reduced self-absorption effect from the dye. The FRET laser can be achieved by co-flowing the donor and the acceptor in free solution through the optofluidic laser cavity.12,13 More recently, a FRET laser controlled by biomolecules (such as DNA scaffolds) has also been demonstrated, thus opening the door to tune/switch the laser wavelength through a plethora of well-studied biological processes.9 In the above DNA-controlled FRET laser, the donor and the acceptor were conjugated with linear DNA sequences with a predetermined ratio and spatial configuration. Through DNA hybridization, the donor and the acceptor are brought to close proximity. However, the FRET process and hence wavelength switching demonstrated so far using either free solution or linear DNA sequences are irreversible. Achieving a wavelength reversible bio-controlled optofluidic laser will not only considerably enhance the laser performance and enable development of novel photonic devices, but also have significant implications in bio/chemical sensing applications, as well as in DNA origami, computation and nanomachines.

The DNA Holliday junction, first discovered in 1964, is a critical intermediate in homologous genetic recombination.14–16 As illustrated in Fig. 1(A), the Holliday junction has four branched double-helical arm structures.14 Due to the strong electrostatic repulsion among the backbone phosphate groups, the arms of the Holliday junction are held apart, and this configuration is highly resistant to further twist. However, as magnesium ions are added to the solution, the junction begins to fold into a stacked X-structure, which is more compact and stable. This conformational change is mediated by the electrostatic interactions between the phosphate groups and the magnesium ions, which pull the arms of the Holliday junction together, leading to a reduction in the overall surface area. As magnesium ions are removed, the junction unfolds back to its original state. This reversible conformational change can be used to control the optical properties of the Holliday junction, such as its refractive index and birefringence, which can in turn affect the lasing characteristics of the optofluidic laser.

![Figure 1](https://example.com/figure1.png)

**Fig. 1** (A) The ion-dependent folding of the DNA Holliday junction. In the presence of Mg$^{2+}$ ions, the junction folds into the stacked X-structure (Conformation II and III). After Mg$^{2+}$ ions are removed by EDTA, the junction unfolds back to Conformation I. (B) Schematic of the bio-switchable laser based on the optofluidic ring resonator (OFRR).
The Holliday junction used in the experiment is detailed in Table S1 of the ESI.† It was formed by four 40-base single-stranded DNA. Each arm of the Holliday junction was conjugated with Cy3 or Cy5 as the donor/acceptor pair. With the increased Mg2+ concentration, the Holliday junction folds gradually, enabling the energy transfer from Cy3 to Cy5. Upon addition of tetrasmium ethylenediamine tetraacetate (Na4 EDTA), Mg2+ ions are removed from the Holliday junctions and the Holliday junction unfolds. Note that, for the folded junction, there exist two possible conformations (see Conformation II and III in Fig. 1(A)). The conformation bias depends on the DNA sequences.17,18 Although the DNA used in our experiments favors Conformation II, the donor and acceptor arrangement in our experiment ensured that efficient energy transfer could occur between Cy3 and Cy5 regardless of the conformation bias. Details of the sample preparation are described in the ESI.†

We achieved the bio-switchable laser using the OFRR based on a thin-walled fused silica capillary (57.5 μm in outer diameter and 2 μm in wall thickness), whose cross section forms the ring resonator that supports the whispering gallery modes (WGMs).9,10 The WGM (Q ~ 10ˇ7)9,10 circulates along the ring resonator circumference and interacts with the gain medium flowing through the capillary to provide the optical feedback for lasing. The experimental setup is illustrated in Fig. 1(B). The Holliday junction solution was flowed through the capillary at a flow rate of 1 μL min−1 and was pumped by an optical parametric oscillator at 518 nm (5 ns pulse width and 20 Hz repetition rate), a wavelength within the Cy3 absorption band but far away from that of Cy5. The emitted light was collected by a multimode fiber and was subsequently transmitted to a spectrometer (spectral resolution: 0.4 nm). Details of the experimental procedures can be found in the ESI.†

In the absence of Mg2+, the arms of the Holliday junction were fully extended into a planar square conformation as a result of strong electrostatic repulsion. The distance between Cy3 and Cy5 was approximately 9.6 nm. Based on the energy transfer efficiency given by R0=4(R0=3 + r), where R0 and r are the Förster distance and donor/acceptor distance, we estimate that the energy transfer efficiency between Cy3 and Cy5 was only 5.6% (R0 = 6 nm for Cy3 and Cy5). Fig. 2 shows the lasing spectra for both Cy3 and Cy5 at a DNA concentration of 100 μM (the lasing threshold curves are shown in Fig. S1). A strong lasing peak emerges at approximately 560 nm, corresponding to the laser emission from Cy3. In contrast, no lasing peak was observed in the emission band of Cy5 due to the insufficient energy transfer from Cy3 to Cy5.

Fig. 3 shows the evolution of lasing emission spectra for Cy3 and Cy5 when Mg2+ was varied. With the addition of Mg2+, the electrostatic repulsion between the arms was suppressed and thus the distance between Cy3 and Cy5 decreased. The increased energy transfer between Cy3 and Cy5 led to emergence of Cy5 lasing emission at approximately 680 nm (Fig. 3(A)). Meanwhile, due to the loss of excitation, the lasing intensity of Cy3 declined rapidly. When the concentration of Mg2+ reached 7.02 mM (Fig. 3(B)), the maximal lasing of the Holliday junction was achieved, in which conformation the two arms formed a 60° angle.19–21 The corresponding energy transfer efficiency was approximately 32%. Accordingly, the Cy5 lasing emission reached the highest, whereas the Cy3 lasing emission was completely quenched due to the large energy transfer efficiency.

To reverse the lasing wavelength, EDTA was added (Fig. 3(C)–(F)). Mg2+ ions react strongly with EDTA to form chelates, thus reducing free Mg2+ ions in the Holliday junction solution. In the presence of 1.74 mM of EDTA, the free Mg2+ concentration dropped to 5.22 mM. A significant reduction in Cy5 lasing intensity is observed due to the increased distance between Cy3 and Cy5, although the lasing emission from Cy3 is still completely quenched. With the further increase in the EDTA concentration, the lasing emission of Cy3 re-emerges whereas Cy5 lasing emission diminishes. When all the Mg2+ ions were removed, strong Cy3 lasing emission was restored with concomitant disappearance of Cy5 lasing, indicating that the Holliday junction fully returned
back to the original conformation. Note that in the above wavelength switching cycle only very small volumes of magnesium and EDTA solutions were added (about 7% in total volume; see the ESI for details!), which had little effect on the DNA (and hence Cy3/Cy5) concentration. In addition, the Holliday junctions can easily be separated from magnesium/EDTA solution by the ethanol precipitation method; thus the DNA samples can be collected and re-used in the bio-switchable laser.

In Fig. 4, we analyze the wavelength switching ratio, $I' = (I_{Cy3} - I_{Cy5})/(I_{Cy3} + I_{Cy5})$, where $I_{Cy3}$ and $I_{Cy5}$ denote the emission intensity of Cy3 and Cy5, respectively. A ratio of 1 or −1 refers to a complete wavelength switching from Cy3 to Cy5 or vice versa. According to Fig. 4, complete wavelength switching can be accomplished with a change of only 5 mM of Mg$^{2+}$. Fig. 4 also shows that the laser signal is over one order of magnitude more sensitive to the DNA conformational change than the conventional fluorescence method to study small conformational changes in DNA/protein structures triggered by environmental changes.22 Finally, as all the materials used are bio-compatible, the bio-switchable optofluidic lasers may be realized in a cell, which is one step closer to intracellular sensing, cytometry and imaging.26

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References


![Fig. 4](image-url) $I'$ for both laser and fluorescence signal at various concentrations of free Mg$^{2+}$ ions based on the spectra in Fig. 3 and Fig. S2. Solid lines are the linear fit for the linear part of response.