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Supporting Information

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Distinguishing DNA by Analog-to-Digital-like Conversion by Using Optofluidic Lasers**

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I. THEORETICAL ANALYSIS

A. Conventional fluorescence-based SNP detection using molecular beacon (MB)

The hybridization process of DNA with the MB can be described as:

$$MB_{close} \overset{K_b}{\Leftrightarrow} MB_{open}, \qquad \text{(S1)}$$

$$MB_{open} + DNA \overset{K_d}{\Leftrightarrow} MB_{open} - DNA, \qquad \text{(S2)}$$

where MB_{close} and MB_{open} represent the MB in the close and free open state, respectively. "DNA" represents either perfectly matched (PM) DNA or single-base mismatched (SM) DNA. MB_{open}-DNA is the duplex formed through MB and DNA hybridization. K_b and K_d are the dissociation constant and are given by

$$K_b = [MB_{open}]/[MB_{close}], \qquad \text{(S3)}$$

$$K_d = [MB_{open}]\cdot[DNA]/[MB_{open} - DNA], \qquad \text{(S4)}$$

where [.] denotes the corresponding concentration. K_b and K_d can be calculated from

$$K = \exp(\frac{-\Delta G}{RT}), \qquad \text{(S5)}$$

where ΔG is the free energy change associated with the process described in Eqs. (S1) and (S2). R is the universal gas constant, and T is temperature.

The fluorescence signal from the MB, F, can be written as:

$$F \propto [MB_{open}] + [MB_{open} - DNA] + \delta[MB_{close}] = \frac{[MB_{open}]^2 + (s_0 + K_d)\cdot[MB_{open}]}{[MB_{open}] + K_d} + \delta[MB_{open}]/K_b, \qquad \text{(S6)}$$

where s_0 is the original DNA concentration added to the MB solution. δ is the residual fluorescence from the MB in the close state. $[MB_{open}] = -\frac{P}{2} + \frac{1}{2}\sqrt{P^2 + 4Q}$, where

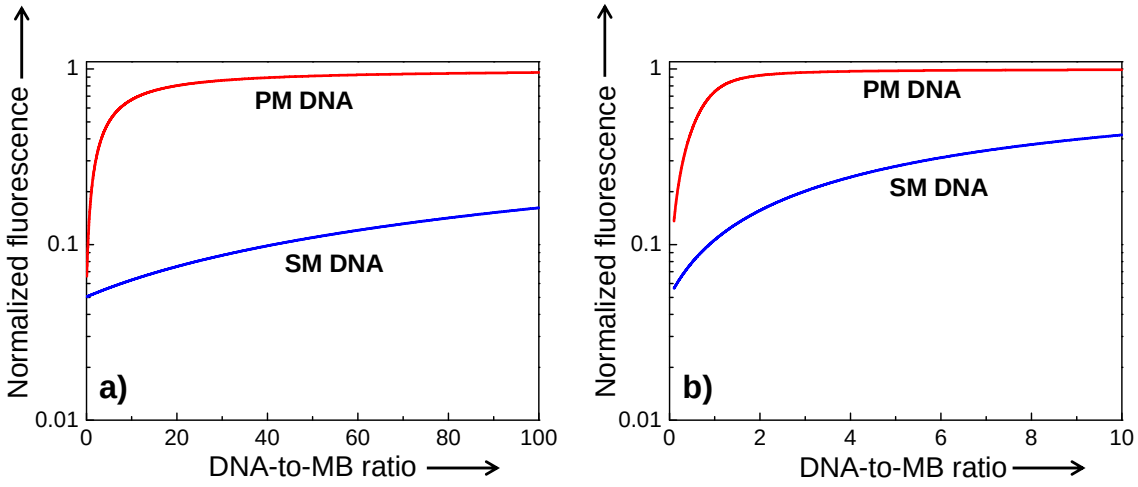

Fig. S1. Normalized fluorescence from the MB for different DNA-to-MB ratios. **a,** n_T=1 μM. **b,** n_T=50 μM. K_b=4x10^{-4} M. K_d=2x10^{-9} M (for PM DNA) and 3x10^{-7} M (for SM DNA). δ=0.05. MB and DNA sequences are listed in Table S1.

$P = K_d + \frac{s_0 - n_T}{1+1/K_b}$ and $Q = \frac{K_d n_T}{1+1/K_b}$. n_T is the total MB (or dye) concentration. Fig. S1 illustrates the fluorescence (normalized to the highest signal when all MBs are open) for different PM and SM DNA concentrations.

B. MB-OFRR laser for SNP detection

B.1. Lasing condition

For the dye attached to a MB, it can be in one of the following states:

1. MB open, dye in the excited state
2. MB open, dye in the ground state
3. MB closed, dye in the excited state
4. MB closed, dye in the ground state

For the dye molecule in State #3, due to static quenching or rapid non-radiative recombination, we can assume that it does not participate in the lasing process. The MB in the open states (#1 and #2) can be in the free open state (*i.e.*, MB_{open}) or in the hybridized form (*i.e.*, MB_{open}-DNA). For the lasing analysis, they both contribute to the lasing action.

When placed in the OFRR, the MB (or more precisely, the dye labeled on the MB) becomes the gain medium of the OFRR laser and has evanescent-wave coupling with the OFRR resonant modes (see Fig. 1b). The corresponding population inversion condition for such a four-energy-level laser system can be written as:[1-3]

$$\eta n_1 \sigma_{dye,e}(\lambda_e) \geq \eta(n_T - n_1)\sigma_{dye,a}(\lambda_e) + \eta n_T \sigma_{quencher,a}(\lambda_e) + \frac{2\pi m}{\lambda_e Q_0}, \qquad \text{(S7)}$$

where n_T is the total concentration of the dye (*i.e.*, summation of State #1-#4). n_1 is the concentration of the dye in State #1. $\sigma_{dye,e}$ ($\sigma_{dye,a}$ and $\sigma_{quencher,a}$) is the dye emission cross section (dye absorption cross section and quencher absorption cross section) at the lasing wavelength (λ_e). η is the fraction of the light in the evanescent field. Q_0 is the OFRR empty-cavity Q-factor. m is the effective refractive index of the circulating optical mode. Here we assume that each MB has only one dye and one quencher attached. At the threshold, Eq. (S7) becomes:

$$\gamma \approx \frac{\sigma_{dye,a}(\lambda_e) + \sigma_{quencher,a}(\lambda_e)}{\sigma_{dye,e}(\lambda_e)} \cdot [1 + \frac{Q_{abs}}{\eta Q_0}], \qquad \text{(S8)}$$

where $\gamma = n_1/n_T$ and

$$Q_{abs} = \frac{2\pi m}{\lambda_e n_T [\sigma_{dye,a}(\lambda_e) + \sigma_{quencher,a}(\lambda_e)]} \qquad \text{(S9)}$$

is the Q-factor related to the dye and quencher absorption.

Note that γ depends on the dye concentration as well as the emission/absorption cross sections of the dye and the quencher. For our OFRR system (n_T=50 μM, m=1.40), $Q_{abs} >> \eta Q_0$. Therefore, Eq. (S8) can be simplified as:

$$\gamma \approx \frac{1}{\sigma_{dye,e}(\lambda_e)} \cdot [\frac{2\pi m}{\lambda_e n_T} \frac{1}{\eta Q_0}]. \qquad \text{(S10)}$$

With $\eta Q_0=10^5$ and $\sigma_{dye,e}=4x10^{-16}$ cm^2 that are typically obtained with the OFRR and the dye[3-5], γ of approximately 10% is obtained, which is similar to that obtained with other dye laser systems (~1-10%).[1, 3] In the subsequent simulation, we used γ=10%.

B.2. Lasing threshold and output power

Using the rate equations for a four-energy-level system, n_1 can be estimated by[4]:

$$\frac{n_1}{n_{open}} = \frac{I}{I+1}, \qquad \text{(S11)}$$

where

$$n_{open} = [MB_{open}] + [MB_{open} - DNA] \qquad \text{(S12)}$$

is the concentration of the dye molecules on the open MB (*i.e.*, the summation of State #1 and #2). Here we assume that the dye attached to the closed MB does not contribute to the population of the emissive excited states (*i.e.*, n_1) due to the strong quenching effect. I is dimensionless and is linearly proportional to the pump intensity.[4] Rewriting Eq. (S11), we arrive at an important lasing threshold condition:

$$I_{th} = \frac{\gamma}{\Gamma - \gamma}, \qquad \text{(S13)}$$

where

$$\Gamma = \frac{n_{open}}{n_T} \qquad \text{(S14)}$$

is the fraction of the MBs that are in the open state. Fig. S2 shows the normalized lasing threshold for PM and SM DNA based on Eqs. (S12-S14).

During the laser operation, the lasing emission is a few orders of magnitude stronger than the spontaneous emission.[4] The laser output power is linearly proportional to the pump intensity, I_{pump}, above the lasing threshold:[4]

$$I_{output} \propto (\frac{I_{pump}}{I_{th}} - 1). \qquad \text{(S15)}$$

Considering Eqs. (S13) and (S15), we arrive at

$$I_{output} \propto I_{pump}(\frac{\Gamma}{\gamma} - 1) - 1. \qquad \text{(S16)}$$

Eq. (S16) enables quantitative measurement of PM DNA concentration. At the fixed pump intensity, the laser output is proportional to Γ and hence the DNA concentration.

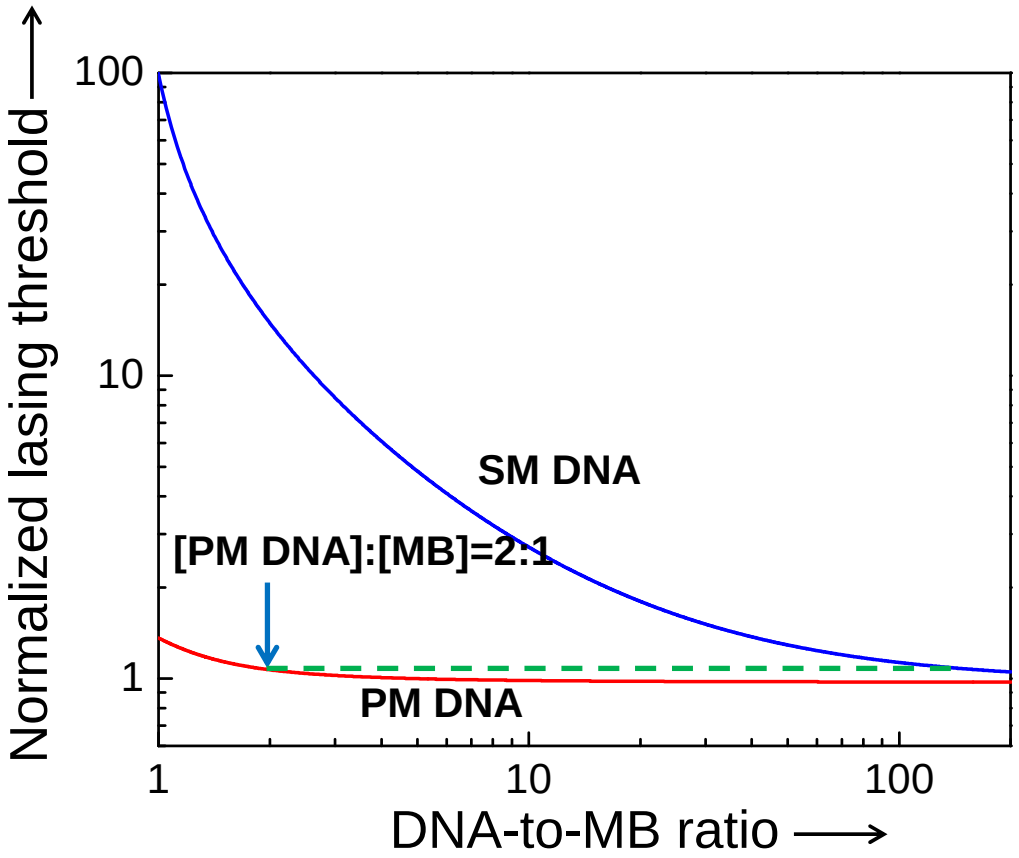

Fig. S2. Lasing threshold for various concentrations of PM DNA and SM DNA based on Eqs. (S12-14). The lasing threshold is normalized to the threshold for [PM DNA]:[MB]=5:1 (for the purpose of comparison with the experimental results). The dashed horizontal line illustrates that the lasing threshold can be achieved with [PM DNA]:[MB]=2:1, but not until when [SM DNA]:[MB] is exceeds 100:1. γ=10%. Other related parameters are the same as in Fig. S1b.

II. SAMPLE PREPARATION

DNA samples and their preparation. MB, PM single stranded DNA, and SM single stranded DNA were purchased from Integrated DNA Technology (see Table 1 and 2 for details). For comparison purposes, all the DNA sequences followed those used in the previous studies in Ref. [6, 7] and were checked using mfold software to minimize possible secondary structure formation.[6, 8] The MB was first dissolved in hybridization buffer (PerfectHybTM Plus, Sigma-Aldrich) to a concentration of 200 μM and then diluted to the desired concentration with TRIS acetate-EDTA buffer (pH=8.3) (Sigma-Aldrich). PM DNA or SM DNA was dissolved in TRIS acetate-EDTA buffer (pH=8.3). For the detection in serum, the samples were dissolved in serum/TRIS acetate-EDTA buffer (v:v=1:1). DNA hybridization was carried out at room temperature (~27 ^{o}C) for 20 minutes. Finally, the hybridized sample was placed in a cuvette for regular fluorescence based detection or was flowed through the OFRR with a syringe pump for intra-cavity based detection.

Table S1. MB and DNA sequences used in the experiments

Sample name	Sequence
Molecular beacon (MB)	5'-/**6-FAM**/ CGCTC-TTTTTTTTTTTTTTT-GAGCG /**DABCYL**/-3'
PM DNA	5'-AAAAAAAAAAAAAAG-3'
SM DNA	5'-AAAAAAA**C**AAAAAAG-3'

Table S2. MB and DNA sequences used in the experiments

Sample name	Sequence
Molecular beacon	5'-/**6-FAM**/ CCTAGCC-CCTATGTATGC TCTTTGTTGT-GGCTAGG /**DABCYL**/-3'
PM DNA	5'-TAAC-ACAACAAAGAGCATACATAGG-GTTT-3'
SM-M DNA	5'-TAAC-ACAACAAAGA**A**CATACATAGG-GTTT-3'
SM-E DNA	5'-TAAC-ACAACAAAGAGCATACAT**G**GG-GTTT-3'

III. EXPERIMENTAL SETUP AND RESULTS

A. Experimental setup and results for conventional fluorescence based detection

Hybridization of the MB with PM DNA or SM DNA (see Table 1 for sample details) took place in a micro-quartz cuvette by mixing 35 µL MB with 35 µL DNA sample to the desired final concentrations at room temperature (~27 ^{o}C). A laser at 480 nm was used for MB excitation. The fluorescence spectra were recorded by a USB 4000 Miniature Fiber Optic spectrometer (Ocean Optics, FL) for post-analysis. The MB fluorescence from PM DNA and SM DNA is presented in Fig. S3.

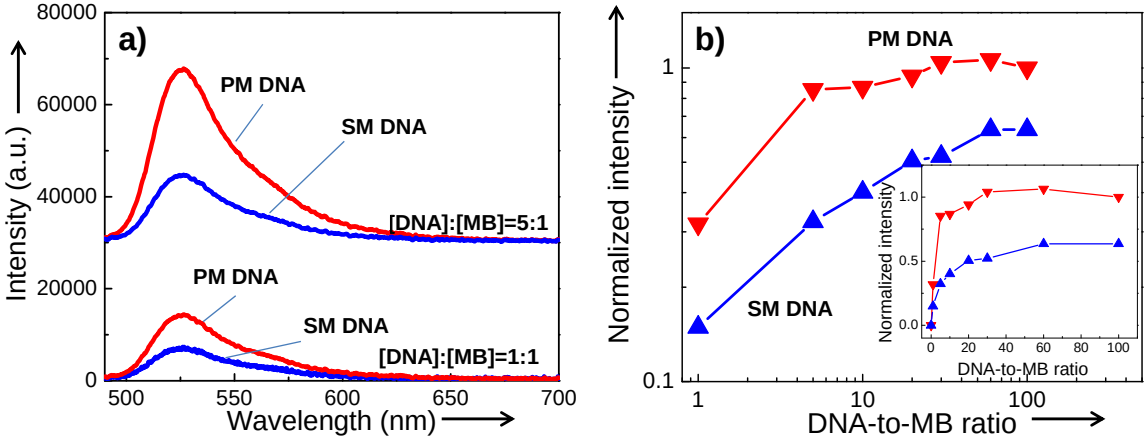

Fig. S3. MB fluorescence from PM DNA and SM DNA. **a,** Fluorescence spectra for two different [DNA]:[MB] ratios. Curves are vertically shifted for clarity. **b,** MB fluorescence signal vs. DNA-to-MB ratio. The signal is normalized to that for [PM DNA]:[MB]=100:1. Inset, the corresponding curves in the linear-linear scale. In all experiments, [MB]=1 µM. MB background is subtracted from all spectra. MB and DNA sequences are listed in Table S1.

B. Experimental setup and results for the OFRR laser based detection

B.1. OFRR fabrication

The details of OFRR fabrication have been reported previously.[9] Briefly, a fused silica capillary preform (Polymicro Technologies TSP700850) was first etched with diluted hydrofluoric acid (HF) overnight and then rapidly stretched under CO_2 laser irradiation. The resulting OFRR capillary was approximately 75 µm in outer diameter and approximately 3 µm in wall thickness. The Q-factor exceeded 10^7 .

B.2. Experimental setup for the MB-OFRR laser based detection

The overall experimental setup is illustrated in Fig. S4. The OFRR laser was optically pumped with a pulsed optical parametric oscillator laser (OPO) (Continuum Surelite, approximately 5 ns pulse width, 20 Hz repetition rate). The OPO wavelength was fixed at 490.7 nm. The pump power was adjusted by a continuously variable neutral density filter, and then loosely focused through a cylindrical lens to excite a 2 mm portion of the OFRR capillary.

Lasing emission from the OFRR (about 100 μm long within the 2 mm illuminated OFRR capillary) was focused through another lens in the direction perpendicular to the incident OPO laser beam, and collected in free space through a multimode fiber connected to a spectrometer (iHR550, Horiba Jobin Yvon, spectral resolution=0.12 nm). The sample solution containing MB and DNA was delivered into the OFRR capillary with a syringe pump applying a constant pressure at the capillary outlet at a flow rate of 5 μL/min. The MB concentration was fixed at 50 μM. All the experiments were carried out isothermally at room temperature (~27 ^{o}C).

Note that the sample does not have to be flowed through the OFRR. The same detection scheme can be carried out as long as the sample fills only small part of the capillary of a detection volume on the order of 1 nL (70 μm x 70 μm x 100 μm).[10] Since the lasing emission can be achieved with the excitation of even a single OPO pulse, the photo-bleaching of the dye molecule is not a concern.

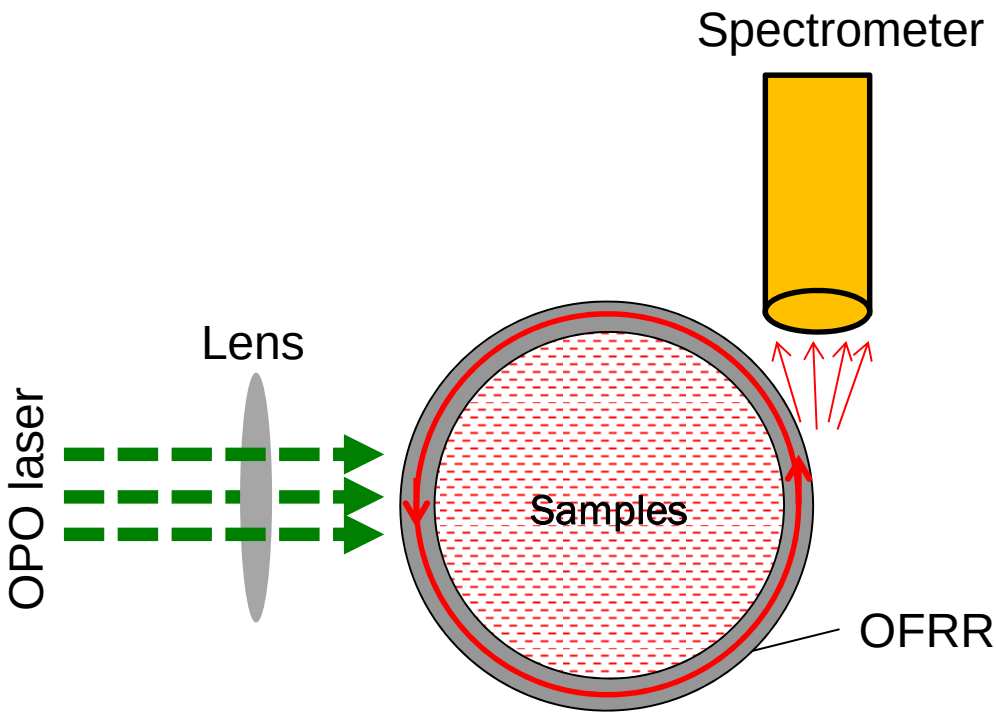

Fig. S4. Illustration of the experimental setup. Pump light from an OPO laser was loosely focused to illuminate a small segment of the OFRR capillary. Sample solution containing MB and DNA was flowed through the capillary by a syringe pump. A multimode fiber was placed near the edge of the capillary cross section to collect the lasing emission in free space. Dimensions are not to scale.

IV. TESTS WITH CLINICALLY RELEVANT SAMPLES

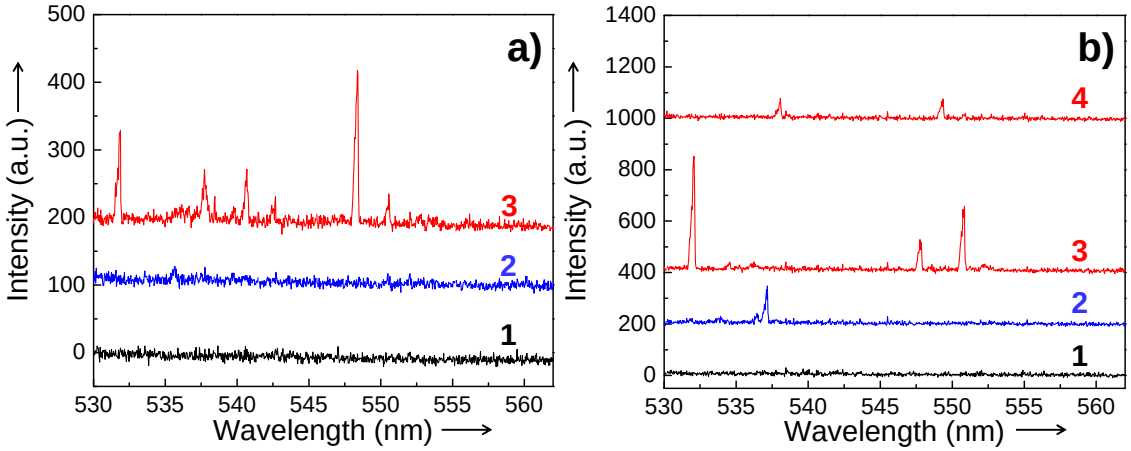

Fig. S5. Comparison of the MB emission spectrum in the presence of PM DNA and of SM DNA. **a,** [DNA]:[MB]=1:1. Pump energy density was 3 μJ/mm^2 for Curves 1-3. **b,** [DNA]:[MB]=5:1. Pump energy density was 1 μJ/mm^2 for Curves 1-3 and 3.2 μJ/mm^2 for Curve 4. In both **a** and **b**, Curve 1: SM-M in buffer. Curve 2: SM-E in buffer. Curve 3: PM DNA in buffer. Curve 4: PM DNA in 50% serum. MB concentration was fixed at 50 μM. Curves are vertically shifted for clarity.

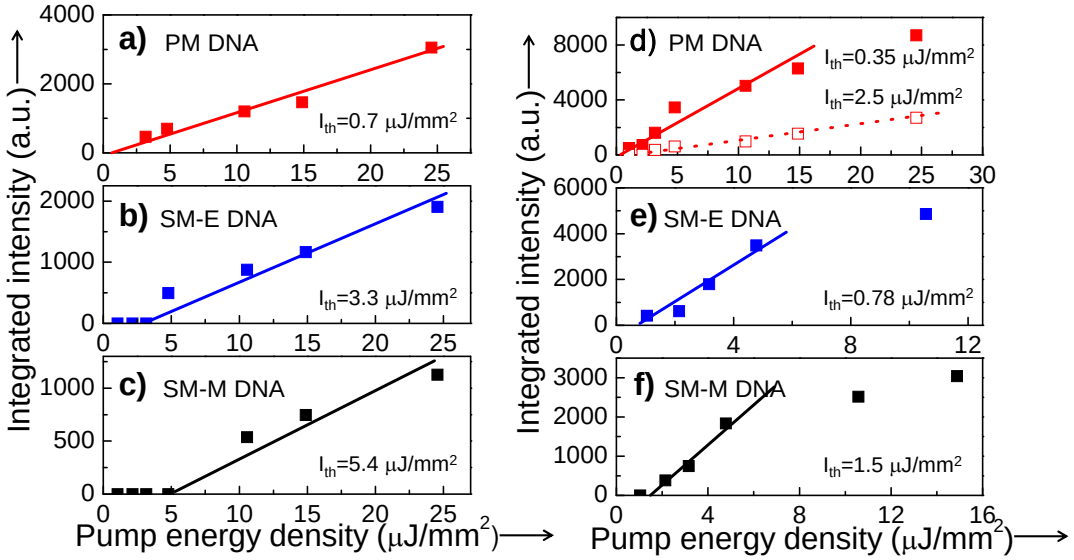

Fig. S6. Spectrally integrated MB emission in Fig. S5 vs. the pump energy density for various concentrations of PM and SM DNA. **a-c,** [DNA]:[MB]=1:1. **d-f,** [DNA]:[MB]=5:1. Spectral integration takes place from 528.2 nm to 556.5 nm. Solid lines are the linear fit for the pump energy density above the lasing threshold. The lasing threshold is labeled near the corresponding curve. Hollow squares in **d** are the integrated intensity obtained when PM DNA and MB ([DNA]:[MB]=5:1) were dissolved in 50% serum. Dotted line is the corresponding linear fit.

References:

[1] H.-J. Moon, Y.-T. Chough, K. An, *Phys. Rev. Lett.* **2000,** *85*, 3161-3164.
[2] Z. Li, D. Psaltis, *Microfluid. Nanofluid.* **2007,** *4*, 145-158.
[3] S. Lacey, I. M. White, Y. Sun, S. I. Shopova, J. M. Cupps, P. Zhang, X. Fan, *Opt. Express* **2007,** *15*, 15523-15530.
[4] A. E. Siegman, *Lasers*. University Science Books: Sausalito, CA, 1986.
[5] Y. Sun, S. I. Shopova, C.-S. Wu, S. Arnold, X. Fan, *Proc. Natl. Acad. Sci. USA* **2010,** *107*, 16039-16042.
[6] B. Dubertret, M. Calame, A. J. Libchaber, *Nature Biotechnol.* **2001,** *19*, 365-370.
[7] A. Hsieh, P. Pan, A. Lee, *Microfluid. Nanofluid.* **2009,** *6*, 391-401.
[8] M. Zuker, *Nucl. Acids Res.* **2003,** *31*, 3406-3415.
[9] S. I. Shopova, H. Zhu, X. Fan, P. Zhang, *Appl. Phys. Lett.* **2007,** *90*, 221101.
[10] W. Lee, Y. Luo, Q. Zhu, X. Fan, *Opt. Express* **2011,** *19*, 19668-19674.