

Lasing from *Escherichia coli* bacteria genetically programmed to express green fluorescent protein

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We report on lasing action from colonies of *Escherichia coli* bacteria that are genetically programmed to synthesize the green fluorescent protein (GFP). When embedded in a Fabry–Perot type cavity and excited by ns-pulses of blue light (465 nm), the bacteria generate green laser emission (~520 nm). Broad illumination of pump light yields simultaneous lasing over a large area in bacterial colonies. © 2011 Optical Society of America

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Lasers play an important role in many parts of modern life, with applications ranging from materials processing over optical communication to medicine. In order to tailor the properties of lasers to the specific needs of these applications, a wide range of different device configurations and materials has been tested. Today, materials in all of the three aggregate forms—gaseous, liquid, and solid—are widely used as optical gain materials. Until recently, however, little work has been done on using biological materials as the active parts of lasers, although biocompatible and bioderived lasers might enable previously unthinkable approaches for biosensing, light-based diagnosis and therapies as lasers based on biologically grown components can be more naturally integrated into biological systems. Several reports have shown that biological tissue can support random lasing when infiltrated with concentrated laser dye solutions [1–4]. Recently, DNA strands were used as nanoscale scaffolds to adjust the separation between two different fluorescent dye molecules in bioinspired microfluidic lasers based on Förster resonance energy transfer (FRET) [5]. However, all of these structures relied on synthetic optical gain materials.

We have recently reported the generation of laser light from a fluorescent protein and have shown that single mammalian cells that are transiently transfected to express the green fluorescent protein (GFP) can form the active component of a microlaser [6]. GFP is a highly fluorescent macromolecule that is synthesized by numerous genetically engineered organisms [7]. In this Letter, we show lasing from colonies of GFP-expressing *Escherichia coli* (*E. coli*) bacteria.

E. coli are rod-shaped bacteria that are commonly found in the intestine of humans and animals; most strains are nonharmful. *E. coli* also plays an important role in modern biotechnology and synthetic biology [8]. The bacteria can be genetically manipulated to produce valuable substances, such as human insulin [9] and bio-fuels [10], so that these compounds can be harvested in large quantities at low cost for use in medicine and energy. In general, genetic engineering of bacteria tends to be more robust and more efficient than for mammalian cells. In contrast to our previous work on mammalian

cells, the genetic transformation of the bacteria used here with a plasmid encoding for GFP expression was stable. This means that the capability to synthesize fluorescent protein is maintained during cell replication. Stable transformation allows for long-term use of biological lasers, for instance, by fully harnessing their self-healing nature, the ability to replenish the optical gain-material in response to photo-bleaching of GFP.

We followed standard transformation techniques to genetically program *E. coli* of the BL21 strain to produce GFP [11]. In short, the bacteria were planted on agar plates and grown overnight at 37 °C. The next day, individual colonies were selected and suspended in 200 μ l of a CaCl₂ solution that contained 50 ng of plasmid DNA encoding for GFP and for resistance against the antibiotic ampicillin (pFluoroGreen, Edvotek). The bacteria were subsequently exposed to a heat/cold shock protocol to transfer the plasmid across the cell wall barrier (10 min incubation on ice, 90 s incubation at 42 °C, 5 min incubation on ice). Transformed cells were selected by plating them onto agar plates that were supplemented with ampicillin and isopropyl-beta-D-thiogalactopyranoside (IPTG). The latter is required to turn on the *lac* promoter that controls GFP expression. Plates were incubated overnight at 37 °C. After 12–15 hours of incubation, the plates typically contained 10–100 colonies of *E. coli* expressing GFP, as identified by bright green fluorescence under UV-A light.

To estimate the average amount of GFP per bacteria cell, we measured the brightness of individual cells with a fluorescence microscope (40 \times objective) equipped with a CCD camera (Olympus IX51, SPOT RT3). The measurement was calibrated against the brightness of a 1 mM recombinant GFP solution held between two microscope slides with a defined separation. We found an average GFP concentration of around 600 μ M, with a ± 200 μ M variation between bacteria cells. This corresponds to $\sim 3 \times 10^5$ GFP molecules within each bacteria cell (cell volume is approximately one femtoliter).

A single colony of GFP-expressing bacteria was picked from a plate and transferred with an inoculating loop onto the surface of a dielectric mirror with high-reflectivity (>99.5%) in the 530 nm range of the spectrum

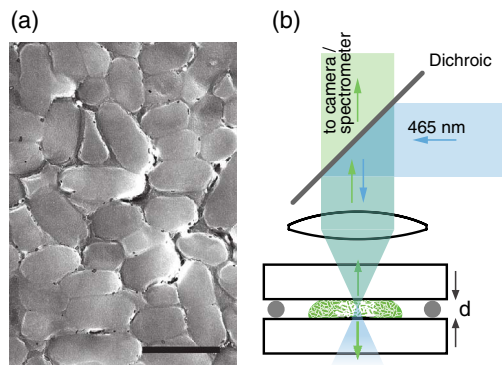


Fig. 1. (Color online) (a) Scanning electron microscope (SEM) image of a colony of GFP-expressing *E. coli* that forms the active gain medium of the bacteria laser. Scale bar $2\ \mu\text{m}$. (b) Schematic of the bacteria laser and the measurement setup. 465 nm pump light is reflected and focused into a microcavity filled with GFP-expressing bacteria (cavity length, $d = 18\ \mu\text{m}$). Laser emission from the structure is collected through the same lens, transmitted through the dichroic mirror, and analyzed by a camera or spectrometer.

and good transmission for blue-light (Y2 coating, CVI). Figure 1(a) shows a scanning electron microscopy (SEM) image of the bacteria colony and reveals that cells are closely packed. Individual *E. coli* have an elongated cylindrical shape with an average length and diameter of $1.8\ \mu\text{m}$ and $0.8\ \mu\text{m}$, respectively. For the laser experiment, the colony was covered with a drop of glycerol to reduce scattering of light at the cell wall interfaces and then covered with a second mirror to form a Fabry–Perot type laser cavity. Spacer beads (diameter, $d = 18\ \mu\text{m}$) were inserted to protect the bacteria from rupturing due to the weight of the top-mirror and to control the cavity length.

We used the pulsed output of an optical parametric oscillator (OPO) that was tuned to 465 nm to optically pump the GFP-expressing bacteria (Quanta Ray MOPO-700, Spectra-Physics, pulse duration $\sim 5\ \text{ns}$). The energy of the excitation pulses was adjusted with neutral density filters and monitored with an energy meter. The pulses were reflected into the laser cavity described above by a dichroic mirror (500 nm long-pass) and focused with a 35 mm achromatic lens. The pulse energies quoted in the following are the energy of single pulses behind the focusing lens, but before the front mirror of the cavity. The emission from the bacteria culture was collected with the same lens, separated from back-reflected pump light by the dichroic mirror and fed to a camera and a 300 mm spectrograph connected to a cooled CCD camera (Andor). See Fig. 1(b) for a schematic illustration of the setup.

Figure 2 shows the integrated output from the bacteria filled resonator as a function of the energy of the pump pulses. A distinct threshold can be observed at a pump energy of $E_{\text{th}} = 160 \pm 10\ \text{nJ}$. This value is higher than what we reported previously for lasers based on recombinant protein solution and single mammalian cells. We attribute the increased threshold to a combination of two effects: (1) The presence of multiple small *E. coli* cells in the cavity results in higher scattering loss during each round-trip than for a situation where only a single mammalian cell (typical diameter, $15\ \mu\text{m}$) is placed inside

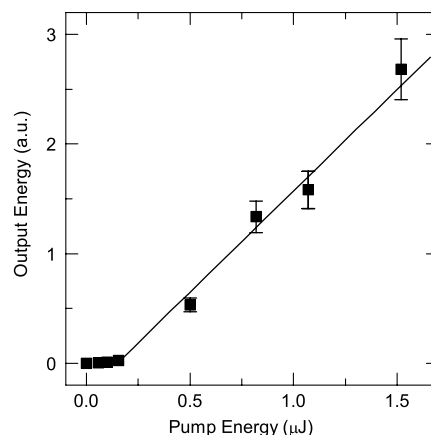


Fig. 2. Input–output characteristics of the bacteria laser. Energy of laser output as a function of the energy per pump pulse. Solid line represents fit to data above lasing threshold ($E_{\text{th}} = 160\ \text{nJ}$).

the resonator. (2) The plane–plane resonator configuration used here is only marginally stable and we do not expect that the closely packed bacteria cells between the mirrors provide an equally efficient lens effect for stabilizing the resonator as a single large mammalian cell [6]. Thus, the gain provided by the GFP-expressing bacteria will have to compensate for substantial diffraction losses.

For pump energies just above the lasing threshold, the output spectrum showed a well-defined mode structure (Fig. 3, top). The wavelength separation between the peaks ($\Delta\lambda_{\text{exp}} \approx 5.1\ \text{nm}$) is consistent with the expected longitudinal mode separation ($\Delta\lambda_{\text{calc}} \approx \lambda^2 / (2dn) = 5.2\ \text{nm}$, where $n \approx 1.475$ is the refractive index of the glycerol filling the cavity). At higher pump energies, however, these lines expanded into an ensemble of closely spaced peaks (Fig. 3, bottom). Such a spectral profile is indicative of simultaneous lasing action in multiple nondegenerate transverse laser modes [12].

Figure 4 shows the spatial output pattern of an *E. coli* cell laser pumped with an extended excitation beam. For this experiment, the 35 mm focusing lens was replaced by a $20\times$ microscope objective and the excitation beam was expanded to a diameter of $\sim 150\ \mu\text{m}$. Figures 4(a) and 4(b) show the output when the structure is pumped close to

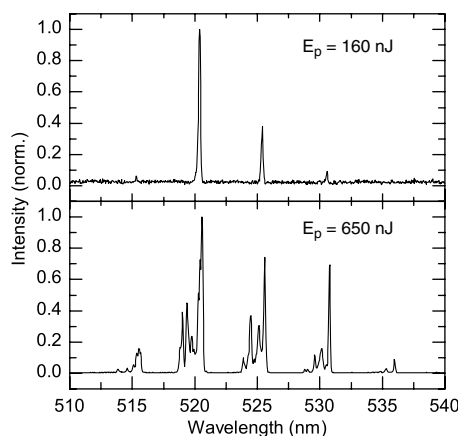


Fig. 3. Output spectrum of bacteria laser. Top, pumped close to the lasing threshold ($E_p = 160\ \text{nJ}$). Bottom, well above threshold ($E_p = 650\ \text{nJ}$).

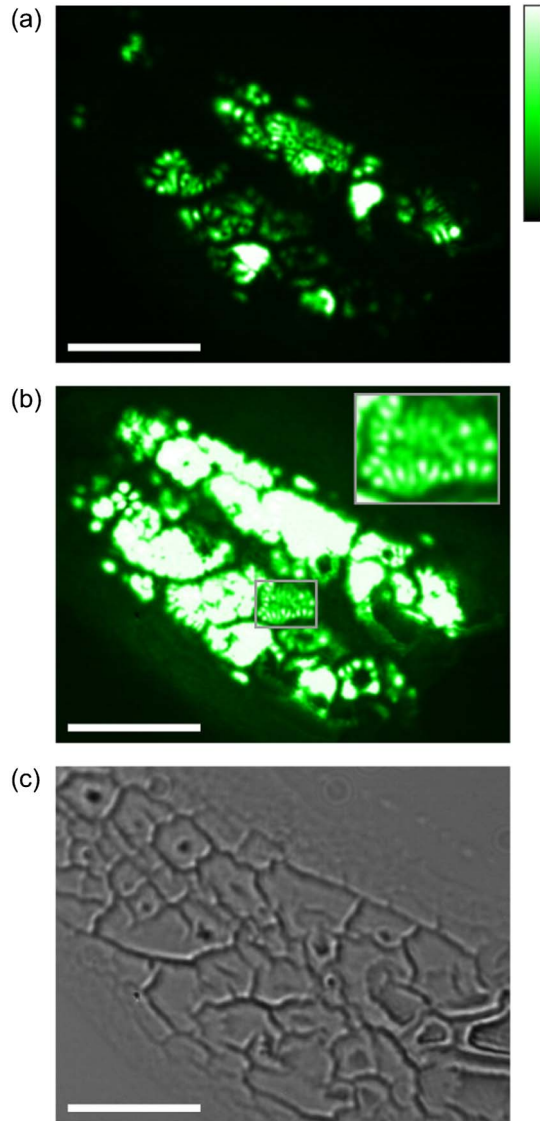


Fig. 4. (Color online) Emission pattern of bacteria laser pumped with an expanded excitation beam covering the entire field of view (pump beam diameter $\sim 150 \mu\text{m}$). (a) Close to the lasing threshold. (b) Pumped $3\times$ above threshold. The inset is a zoom-in of the marked area in the center. (c) Bright field image of same region on sample. Scale bars, $50 \mu\text{m}$.

the lasing threshold ($300 \mu\text{J}/\text{mm}^2$) and $3\times$ above threshold, respectively. Figure 4(c) shows a coregistered bright field image of the bacteria laser. As expected, lasing is observed in regions where bacteria are densely packed and the optical gain provided by GFP molecules is high. The complex and seemingly random output pattern of the laser presumably results from the disordered ensembles of bacteria clusters that cause the laser to operate at higher order transverse laser modes. As the energy of the pump pulses was increased, additional parts of the sample began to lase [compare inset to Fig. 4(b)] while the previously lasing regions substantially gained in

brightness. We conclude that, at high pump energies the observed spatial patterns represent a superposition of several higher-order transverse laser modes. This is consistent with the observation that ensembles of peaks form in the output spectrum of the laser as the pump energy is increased (Fig. 3, bottom). The lobe-size of the modes is in some cases consistent with the size of individual bacteria [inset to Fig. 4(b)]. However, considering the dense and multilayered packing of *E. coli*, we think the lobes were formed by coherent optical interference rather than representing individual bacteria.

In conclusion, we have shown that lasing can be generated from colonies of *E. coli* bacteria genetically transformed to synthesize fluorescent protein. Lasing was evidenced by clear threshold behavior and discrete peaks in the emission spectrum. Demonstration of lasing from bacteria that inherit the capability to synthesize GFP upon cell division is an important step towards large-scale self-sustained biological lasers. We note that the cavity mirrors are critical to the lasing action. In the absence of either of the mirrors, no lasing was observed up to a pump flux of $1 \text{ mJ}/\text{mm}^2$. In the future, it will be interesting to see whether bacteria colonies can be configured to form random or ordered structures providing sufficient optical feedback to produce laser light without external mirrors.

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