# A Simple Approach to Biological Single-Cell Lasers Via Intracellular Dyes

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Cell lasers have potential applications in biology and medicine, including in biomolecular sensing and cytometry.[1,2] So far, cell lasers have been realized either by using an extracellular semiconductor gain medium or by intracellular fluorescent proteins.[3–5] Fluorescent proteins were found to be an attractive option for providing optical gain in cells as they offer biocompatibility, high quantum yield, and good photostability. However, the transfection required to trigger expression of fluorescent proteins in cells is a time-consuming process, typically taking more than 24 h. Such long preparation times are inconvenient for applications not requiring genetically encoded gain.

In the wider context of visualizing cells and studying intracellular processes, synthetic fluorescent molecular probes that can penetrate the membrane of live cells are widely used.[6,7] Compared to fluorescent proteins, a much greater variety of such small organic dyes are available, providing a wide range of different biochemical and spectral properties.[8] In general, these probes are detected via their fluorescence, i.e., spontaneous emission.[9] However, here, we show that integrating these probes into an optical resonator turns them into an optical gain medium supporting efficient stimulated emission with distinct spectral characteristics. Advantageously, this approach provides a fast and simple method to obtain lasing from normal cells within less than 1 h by using a biocompatible cell-tracker dye that becomes highly fluorescent upon entering the cytoplasm, thus forming a localized gain volume. We demonstrate lasing with this approach from both spherical cells in suspension and from elongated, adherent cells grown and stained directly on one of the reflectors forming the cavity. Fluorescent dyes offer a convenient method for transforming normal cells into biolasers for a variety of applications in cell-culture and lab-on-a-chip settings.

In the present study, we have exploited 5-chloromethylfluorescein diacetate (CMFDA, CellTracker Green CMFDA, Invitrogen) to investigate the feasibility of using fluorescent probes for intracellular gain as a convenient alternative to fluorescent proteins. The cell tracker dye CMFDA is widely used to stain cells for tracking and monitoring.[10] Besides bright fluorescence and good photostability, it offers two further advantageous features in the context of providing optical gain in cells. First, CMFDA molecules are originally nonabsorbing and nonfluorescent, but transform into a highly fluorescent form once they enter the cytosol of a cell. This property is due to cleavage of the acetate groups from the molecular core by intracellular esterases, which produces a highly bright, fluorescein derivative, 5-chloromethylfluorescein (CMF) (**Figure 1**a). Second, a subsequent reaction of the chloromethyl group of CMF with the thiol groups of intracellular proteins yields a form that can no longer penetrate the cell membrane, thus confining the fluorescent form of the dye to the inside of the cell. We confirmed that CMF is well retained within the cytosol for over 72 h. By contrast, conventional dyes, such as fluorescein, leak out of cells within 1 h (Figure 1b). In terms of its spectral properties, the intracellular form of the dye is similar to the green fluorescent protein that was previously used for cell lasers, its excitation and emission spectra peak at 493 and 522 nm, respectively (Figure 1c).

The dramatic shift in cell membrane permeability of CMDFA helps achieve high intracellular concentration (Figure S1, Supporting Information), which can provide high gain and, therefore, facilitate lasing. We incubated HeLa cells with $100 \times 10^{-6}$ M of CMFDA dye in serum-free medium containing 1% dimethyl sulfoxide (DMSO) for 30 min under standard culture conditions. This concentration is four times higher than the

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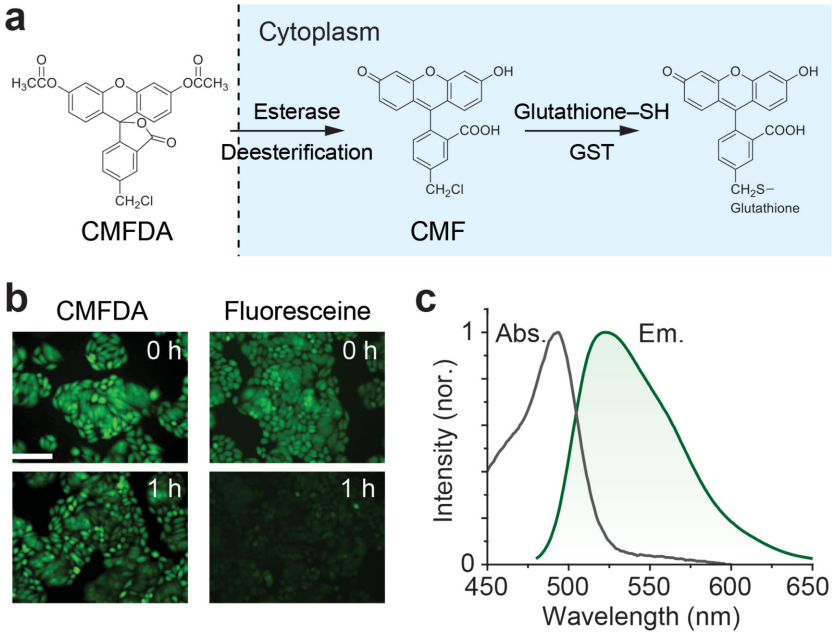


**Figure 1.** a) The molecular structures of CMFDA dye and the chemical reaction taking place upon internalization of CMFDA by a cell. b) Confocal microscopy images of HeLa stained with CMFDA and fluorescein. The intracellular concentration of CMFDA is invariant over 1 h after the application of the dye, whereas a considerable amount of fluorescein escapes the cell under identical incubation conditions. Scale bar, 100 μm. c) Optical absorption and emission profiles of CMFDA.

manufacturer's recommendation of $25 \times 10^{-6}$ M for cell tracking experiments.[10] The latter is, however, a rather conservative recommendation, set to avoid with great certainty any effect on cell function.[11] We have performed viability essays at $100 \times 10^{-6}$ M CMFDA concentration (using ethidium homodimer-1) and found that more than 98% of stained cells remain viable in culture for at least 24 h after incubation with CMFDA in the DMSO solution (1%). By measuring the intensity of the cell fluorescence after incubation with CMFDA, the intracellular concentration was estimated to be $1 \times 10^{-3}$ M, i.e., tenfold higher than the initial extracellular concentration, demonstrating that over time the dye accumulates within cells as expected due to the formation of the non-membrane-permeable form within cells.

For laser experiments, dye-stained HeLa cells were detached from their substrate by trypsinization and placed between a pair of dichroic mirrors separated by about 20 μm (**Figure 2**a). The mirrors had a reflectivity of >99.5% in the spectral range of 500–560 nm. Pump pulses (10 ns, 475 nm, 10 Hz) provided by an optical parametric oscillator (OPO) were focused onto an individual cell through one of the mirrors. The spot size on the cell surface was approximately 4 μm, thus illuminating a substantial fraction of the cell volume. Imaging the cell fluorescence upon excitation with low energy pulses shows that the dye is distributed rather homogeneously throughout the cell. However, the emission pattern changed drastically at higher pump pulse energy (Figure 2b). In addition, the

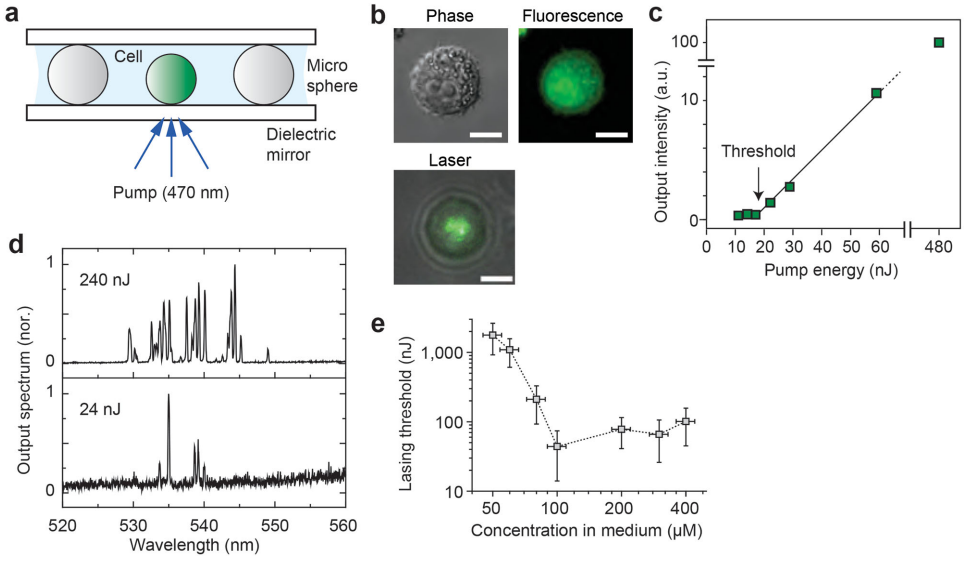


**Figure 2.** a) A schematic of the laser configuration with a floating HeLa cell inside a microcavity formed by two dielectric mirrors separated by size-calibrated microspheres. b) Phase contrast image (left) and fluorescence microscope image (middle) of a single HeLa cell in culture outside the resonator, and merged bright-field and laser-emission images of a cell inside the resonator (bottom). Scale bar, 10 μm. c) The measured laser threshold pump energy for different dye concentrations. Error bars represent measurement uncertainty. d) Laser output energy of a HeLa cell ($100 \times 10^{-6}$ M CMFDA) as a function of pump energy. Line is a linear fit to data above 12 nJ. e) Output spectra of the cell laser at pump energy levels of 24 nJ and 240 nJ, respectively. Vertical error bars represent detector noise and pulse-to-pulse variation. Horizontal error bars represent the variation of the dye concentration in the medium.

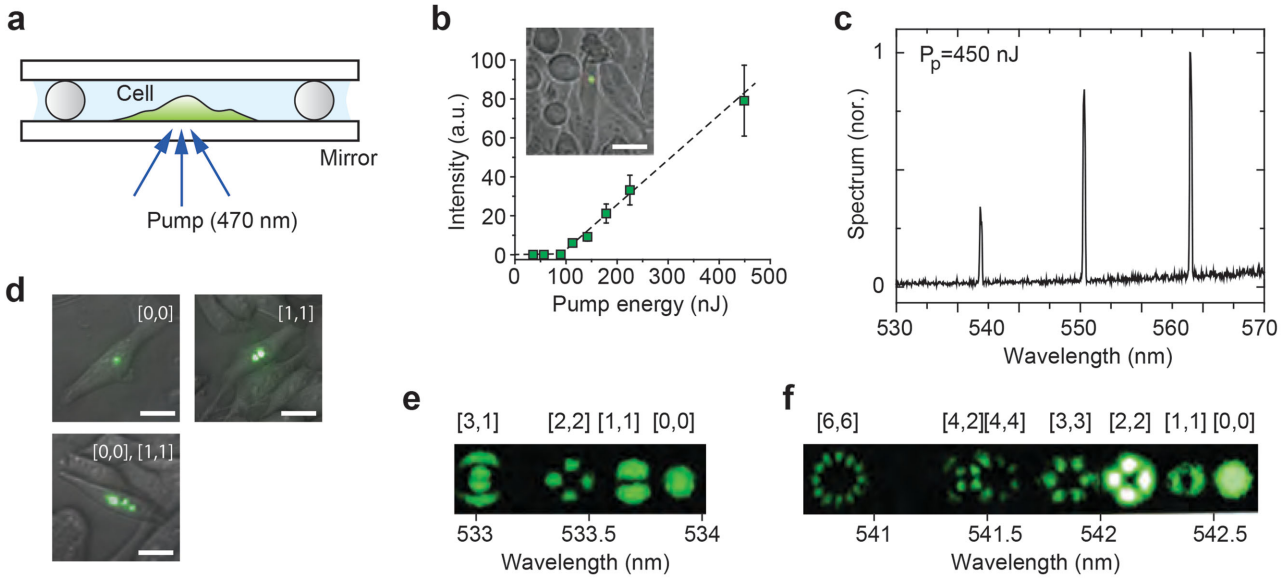


**Figure 3.** a) A schematic of the experimental setup for an attached single HeLa cell. b) Laser output energy as a function of pump energy. Line is a linear fit to data above 89 nJ. Error bars represent detector noise and pulse-to-pulse variation. Inset: merged bright-field and laser-emission images of HeLa cells grown in the laser resonator. c) A typical output spectrum of the cell laser at pump energy of 450 nJ, showing three longitudinal modes separated by 9 nm. d) Representative merged bright-field and laser emission images for different cells. The numbers denote the Ince–Gaussian mode indices [p,m]. Scale bars, 5 μm. e,f) Hyperspectral laser mode image from two different floating cells each supporting multiples of transverse laser modes simultaneously.

emission intensity showed a well-defined threshold behavior with a stark increase in emission for pump pulse energies above 12 nJ (Figure 2c). When excited above this threshold, the emission spectra of the cell within the resonator featured distinct peaks (Figure 2d), corresponding to transverse and longitudinal modes.[4,5] Each peak has a spectral linewidth below the resolution limit of our instrument (0.24 nm). The number of peaks was found to increase with pump energy, supposedly due to net optical gain conditions being reached for an increasing number of cavity modes (Figure 2d).

Like all organic fluorophores, the CMFDA dye eventually showed signs of photo-bleaching when excited repeatedly over longer periods of time, while cavity and cells, other components of the biolaser, are stable. At a pump pulse energy of twice the lasing threshold, a noticeable drop of laser output occurred after several hundreds of pump pulses. When pumping 15 times above threshold, the lasing ceased after about 60 pump pulses and we did not observe any physical damage after laser action of cells. While higher stability would obviously be desirable for some applications, this lifetime will be sufficient for those requiring short-term measurements.

We also investigate the dependence of the lasing threshold on the amount of dye present in the cell. In principle, one expects the threshold to decrease with increasing concentration of active dye in the cell. Experimentally, we find that the measured threshold decreases with increasing dye concentration up to a concentration of $100 \times 10^{-6}$ M (as measured in the culture medium during the staining process, Figure 2e). Higher dye concentrations did not lower the threshold further, presumably because the intracellular dye concentration saturates (near $1 \times 10^{-3}$ M). In fact, the active photons that are absorbed and lead to the population inversion and lasing are lower than the input energy, which corresponds to an estimated fraction of approximately 8% of the input energy (see the Supporting Information). Furthermore, we examined the morphology of the cells during laser action as a function of the pump pulse energy and number of pulses, and did not observe any morphological change due to prolonged laser action (3000 pulses, 5 min total duration) at the pumping energy levels of 140 nJ, 660 nJ, and 2.9 μJ, respectively (see the Supporting Information). We started to observe bleb formation, bulging of the plasma membrane, after exposure of 1000 pulses with very high pulse energy above 7.5 μJ, whereas control cells without dyes exhibited no apparent bleb formation. These results indicate that the mechanism of cellular toxicity in cell laser operation is photo-thermal but should be negligible under normal operating conditions.

Next, we investigated the possibility of lasing from adherent cells, i.e., cells attached to one of the mirrors forming the laser cavity. For this experiment, cells were cultured directly on a dielectric laser mirror. We found that administration of CMFDA dye ($100 \times 10^{-6}$ M) dissolved in 10% DMSO solution causes the cells to detach from the mirror surface, but with 2% DMSO solution most of the cells remain attached (Figures S2 and S3, Supporting Information). After incubation for 30 min, a cover mirror was placed on the top of the cells, using spacer beads with a diameter of 10 μm to adjust separation and prevent mechanical damage to the cells (**Figure 3**a). For adherent cells, lasing was typically achieved above a threshold energy level of 90 nJ (Figure 3b). We attribute this increase in threshold compared to the floating cells to the fact that the cells are being spread out on the mirror surface, rather than forming a compact sphere as for the floating cells, which reduces the effective gain length available.

Analysis of the emission spectrum of adherent cells indicates the simultaneous oscillation of several longitudinal modes (Figure 3c) in a single transverse mode. Depending on cell geometry, we clearly observe the oscillation of a single fundamental transverse electromagnetic (TEM) mode [0, 0], the first higher-order mode [1, 1], or combinations of several modes (Figure 3d). To compare the modal characteristics, a hyperspectral imaging configuration enables the analysis of emission from floating cells and it simultaneously shows multiples of higher modes (e.g., [3, 1], [4, 4], etc.) from floating cells (Figure 3e,f).[5] While the floating spherical-shaped cells support both lower- and higher order Ince–Gaussian (IG) eigenmodes due to its spherical symmetry, the elongated-shaped adherent cells function as a spatial filter due to the perturbed symmetry of gain medium and they isolate only lower order laser modes including the fundamental IG [0, 0] and IG [1, 1] modes. In other words, we can generate the same modes with a spherical cell than with an attached cell if we put a rectangular pinhole into the gain medium. Therefore, the adherent cells with nonspherical shapes behave as natural filters that prevent oscillation of higher order transverse modes.

In conclusion, for the first time to our knowledge, we have shown that fluorescent dyes can serve as viable gain medium for cell lasers. Inserting synthetic dyes into normal live cells can be achieved within less than 1 h, which is much quicker than the transient transfection that facilitates sufficiently high levels of fluorescence proteins for lasing. This allowed us to observe both spherical cells in suspension and adherent cells grown and stained directly on one of the reflectors forming the cavity. CMFDA is well suited for biolasers, but in the future many of the fluorescent dyes that have been developed as cellular probes for a variety of purposes may also serve as a gain medium. This will pave the way for lasing from cells in culture, lab-on-a-chip devices, bio-laser microscopy, or flow cytometry type applications.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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