BIOPHOTONICS

Living lasers

Green fluorescent protein — an important biological imaging tool for many years — now forms the foundation of the first cellular laser.

Steve Meech

In the July issue of *Nature Photonics*, Malte C. Gather and Seok Hyun Yun report the first observation of laser action in a living system[1]. Their gain medium consisted of a single mammalian cell, measuring 15 μm in diameter, that had been transfected with a mutant form of green fluorescent protein (GFP). A suspension containing the cells was placed in a resonator cavity consisting of two highly reflective distributed Bragg reflectors (Fig. 1).

The researchers found that optically pumping the suspension with nanosecond blue (465 nm) pulses from an optical parametric oscillator caused a cell within the cavity to lase on several longitudinal modes in the wavelength range of 516–520 nm. They estimate the threshold for laser operation to be around 1 nJ.

The spectrum and mode structure of the beam emitted by this living laser are well-matched to theoretical mode simulations (Fig. 2). Interestingly, the transverse laser mode patterns were found not to resemble Hermite–Gaussian or Laguerre modes, which are common in lasers, but rather the Ince–Gaussian mode. The cell remained alive even after several minutes of laser action or exposure to higher pump energies of 50 nJ per pulse.

It is currently unclear what applications lie in store for cellular lasers, although the authors suggest possible applications for the three-dimensional imaging of cells. Whatever the eventual applications, the advent of GFP in photonics certainly marks an exciting new avenue of research for this extraordinarily versatile protein.

GFP was discovered in 1962 by the Japanese scientist Osamu Shimomura, who isolated and characterized it during his investigations into the bioluminescence from the jellyfish *Aequeorea victoria* (*A. victoria*)[2]. During his research he discovered a protein that exhibited strong green fluorescence when irradiated with ultraviolet light. Such an inherently fluorescent protein is unique; whereas most coloured proteins bind their chromophores by van der Waals forces, the chromophore of GFP is formed in a chemical reaction between three adjacent amino acid residues and is covalently bound to the protein backbone.

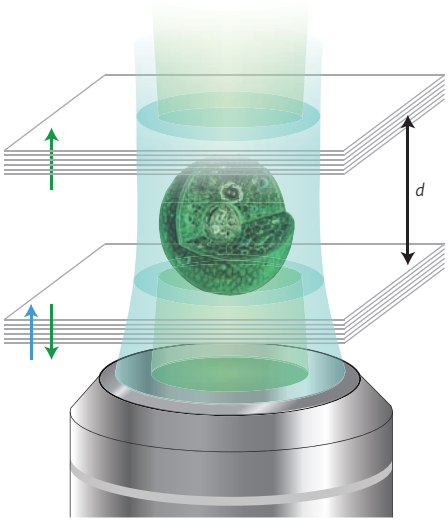

Figure 1 | Schematic of the cellular laser, showing a cell transfected with GFP between two reflectors that form an optical resonator.

Chalfie and co-workers were the first to realize the potential of GFP for imaging live cells[3]. The fluorescent dyes employed at the time had limited biological specificity and were often toxic. In contrast, the gene that encodes for GFP can be spliced into the DNA of an organism in the region that encodes for a particular protein. The target protein is then expressed by the organism as a fusion protein to which GFP is attached, thus allowing the location of the target protein to be followed by fluorescence microscopy. The importance of this development in the field of cell biology cannot be overemphasized. For the first time, the formation, function, transport and eventual demise of a specific protein could be recorded in space and time within a living cell.

The images obtained by Chalfie and co-workers initiated a revolution in the application of GFP, sparking the need for better, brighter proteins than that produced by *A. victoria*. In particular, Tsien and co-workers demonstrated the ability to tune the optical properties of GFP through mutagenesis[4]. Gather and Yun[1] employ an enhanced GFP (EGFP) mutant as their laser gain medium; EGFP is a much stronger absorber of visible light than GFP derived from *A. victoria*, thus making the laser easier to pump.

A rainbow of fluorescent proteins soon became available following the research of Tsien and others. This enabled many proteins to be imaged simultaneously by using filters and multiple photodetectors. Many important biological functions require the self-assembly of protein complexes. Such protein–protein interactions can be detected by labelling each of a pair of target proteins with different fluorescent proteins (blue and yellow, for example) that are capable of undergoing Förster energy transfer. Optical excitation of the blue transition results in yellow fluorescence only when the two proteins are within the Förster radius, which is typically a few nanometres. This allows the location and lifetime of protein complexes to be selectively imaged from the yellow emission. The great importance of these developments was recently recognized by the 2008 Nobel Prize for Chemistry awarded to Shimomura, Chalfie and Tsien, for which the recipients thoughtfully gave due credit to *A. victoria*.

The wide range of optical properties exhibited by fluorescent proteins prompted detailed investigations into the photophysics of such systems[5]. The chromophores in fluorescent proteins were found to exist in two charge states: a neutral form and a red-shifted deprotonated anionic form. Intriguingly, excitation of the neutral form resulted in emission from the deprotonated form; a clear indication of an excited-state proton transfer (ESPT) reaction. The ESPT reaction in GFP is unique in biology. Time-resolved measurements showed that the proton transfer in GFP occurs in just a few picoseconds[6]. Such an excited-state reaction leads to a four-level system that naturally achieves population inversion because the deprotonated ground-state is only lightly populated. This is a different route to positive gain than that employed by Gather and Yun. Detailed theoretical and spectroscopic studies of the ESPT reaction showed that excitation of the

neutral chromophore initiates a three-step proton relay, which causes the protonation of an amino acid residue several ångström from the original site. Proton translocation is involved in many important biological processes. The initiation of the ESPT reaction in GFP by an ultrafast laser pulse has made it possible to observe and analyse protein proton transfer dynamics[7].

The valuable research that flowed from the discovery of GFP in *A. victoria* also stimulated intense interest in the origin of colouration in other marine organisms. The second revolution in GFP research was initiated by the discovery of fluorescent proteins in corals, which, particularly through the discovery of the coral protein DsRed, greatly extended the palette of available GFP colours. DsRed contains a modified form of the GFP chromophore whose emission spectrum is shifted towards red wavelengths[8]. The serendipitous discovery that some coral fluorescent proteins are photoactive also generated great excitement. The coral protein Kaede was found to undergo a permanent colour change from green to red when irradiated[9]. The origin of the colour change is a photochemical reaction that causes extended π-electron conjugation. The applications of this are clear: irradiating a spatially localized sub-population of proteins allows their dynamics to be observed distinct from the unirradiated population. For example, the complex overlapping structures of neurons can be mapped out by irradiating a single neuron.

Of even greater impact was the discovery of photochromic fluorescent proteins. The coral protein Dronpa can be efficiently and reversibly switched between blue and green absorbing forms, of which only the latter is strongly fluorescent[10]. Consequently, fluorescence due to excitation by green light can be switched on and off through many cycles. Although the switching mechanism is still under investigation, this phenomenon has found an extremely important application in ultraresolution microscopy, where it enables optical imaging at resolutions down to 10 nm — well beyond the Rayleigh diffraction limit[11]. The technique begins with a population of Dronpa fusion proteins in their dark state. A few are switched on and an image of the isolated fluorescent proteins is recorded. The emission from each protein is matched to a Gaussian function, thus allowing the centroid to be located with nanometre precision, after which the population is photochromically turned off. A new random set is then turned on, and a second image is recorded and analysed. The final image is the sum of all the individual 10-nm-resolution sets. Such ultraresolution microscopy has succeeded in revealing entirely new features of cell structure.

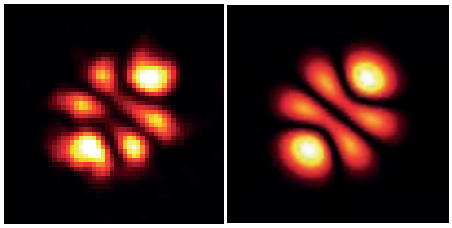

Figure 2 | Cellular laser modes: measured (left) and calculated Ince–Gaussian (right) transverse mode pattern of the single-cell laser.

GFP has revolutionized our understanding of cellular processes and contributed a major improvement to image resolution. The possibilities of photochromic proteins as biological optical data storage media have also been investigated. The work of Gather and Yun suggests that GFP may soon have a significant impact in the field of photonics. It is too early to say what will become of a biological self-generating and self-healing laser medium, but it certainly offers an exciting new range of possibilities for fluorescent proteins. ❐

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PHOTOVOLTAICS

Japan–EU team targets solar record

A four-year collaboration between Japan and Europe aims to increase the efficiency of concentrator solar cells to beyond 45%.

Oliver Graydon

The achievement of power conversion efficiencies of more than 45% would mark not only a world record but also an important milestone in the field of photovoltaics. A recently established collaboration between organizations in Japan and the EU — the first project under the EU–Japan Energy Technology Cooperation Agreement — aims to achieve such efficiencies by improving the technology of concentrator solar cells.

The four-year project, which began on 1 June 2011, is a strategic collaboration between Japan's New Energy and Industrial Technology Development Organization (NEDO) and the European Commission. The initiative is funded with a budget of around ¥650 million from Japan and €5 million from the European Commission, and involves academic and industrial partners from six EU member states working in collaboration with a variety of organizations in Japan.

The Japanese research team is led by Masafumi Yamaguchi from the Toyota Technological Institute and also includes partners from Sharp, Daido Steel, the University of Tokyo and Japan's National Institute of Advanced Industrial Science and Technology (AIST).

The European consortium is led by Antonio Luque from the Technical University of Madrid (Spain), together with the Fraunhofer Institute for Solar Energy (Germany), Imperial College London