DIAGNOSTIC IMAGING

Lasing cancer biomarkers

Laser light emitted by fluorescently stained human tissue inside a laser cavity can be used to diagnose cancer.

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Fluorescent biomarkers, which are typically imaged by confocal microscopy or super-resolution imaging, are extensively used in biomedical research and in particular in diagnostics. Recently, a radical new approach for the detection of fluorescence has been devised[1]: a biological laser can be formed by introducing cells producing green fluorescent protein[2] or stained with a fluorescent marker[3] inside a laser cavity or in close contact with the cavity, or by inserting a laser cavity into cells and tissues[4]. The light is amplified inside the cavity via optical feedback, generating high-intensity, highly directional emission with sharp spectral lines. The amplification enables the detection of subtle changes in fluorescence intensity at substantially lower background noise. Reporting in *Nature Biomedical Engineering*, Xudong Fan and colleagues now show that by inserting a fluorescently stained histological section into a laser cavity and then analysing the laser output, healthy and cancerous tissues can be discriminated[5]. They name this imaging device a laser-emission-based microscope (LEM).

Fan and colleagues sandwiched a fluorescently stained tissue between two mirrors, spaced 15 μm apart. The mirrors are transparent to the excitation light but highly reflective in the emission-wavelength range of the staining fluorophore (Fig. 1). Akin to fluorescence microscopy, the fluorophore is excited by a wavelength that matches its maximum absorption. To achieve laser emission (which only occurs above the so-called lasing threshold), the excitation source has to be a pulsed laser with sufficient peak power so that enough fluorescent molecules are elevated to their excited states, and so that there is more amplification by stimulated emission than there are losses in the laser cavity. Also, the laser cavity only supports light with certain frequencies, giving rise to the emission of sharp spectral lines. In addition, the lasing threshold is a highly advantageous feature of laser emission. Below the threshold, the emission of fluorescent light is low, owing to the highly reflective mirrors. However, when the threshold is exceeded as a result of light amplification, the emission becomes strong and highly directional. Therefore, lasing occurs only at spots in the tissue where the concentration of fluorophores is high enough. Hence, the LEM provides a higher signal-to-background ratio than normal fluorescence microscopes (Fig. 2).

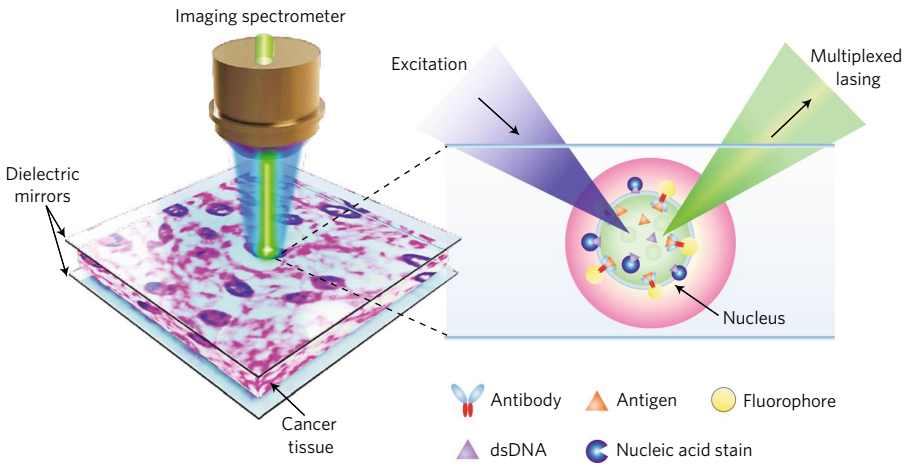

Fig. 1 | Working principle of the laser-emission-based microscope. A fluorescently stained tissue section is sandwiched between two dielectric mirrors. The sample is illuminated by a pulsed laser through a microscope objective, and the laser light generated is collected by the same objective and sent to a camera or spectrometer. Arrows depict the direction of light emission and lasing. dsDNA, double-stranded DNA. Figure adapted from ref. [5], Macmillan Publishers Ltd.

Fan and co-authors used the LEM to detect nucleic acids and nucleic proteins in healthy and cancerous tissues from several patients with lung and colon cancer. The authors stained the tissues with YO-PRO-1 iodide (YOPRO), which becomes fluorescent on binding to nucleic acids. The authors found that, when observing only fluorescence, whole cells were uniformly bright because the YOPRO dye stains both the nucleus and, to a lesser extent, the cytoplasm. However, when observing the cells within the lasing regime, bright spots with a minimum diameter of 700 nm, which is also the typical spatial resolution of this technique, and with one or more narrow (<0.16 nm) spectral peaks within a spectral width of 10 nm were localized in cell nuclei only, because the concentration of stain in the cytoplasm was not high enough to support lasing and therefore did not contribute to background fluorescence. By moving the sample via a motorized stage, the laser excitation beam scanned across a larger area of the sample to compose a 'lasing image'. Importantly, the lasing spots appeared only in cancer cells. The authors show that cancerous cells have a relatively lower laser threshold than healthy cells, and attributed this to the high nucleic-acid concentration in cancer cells, which results from their fast proliferation. When the excitation laser was operated above the lasing threshold of cancerous cells yet below the lasing threshold for healthy cells, only cancerous parts of the sample emitted laser light, thus enabling cancer-cell detection with high sensitivity. However, additional studies are required to better understand why these bright lasing spots arise preferentially in cancerous cells, and what

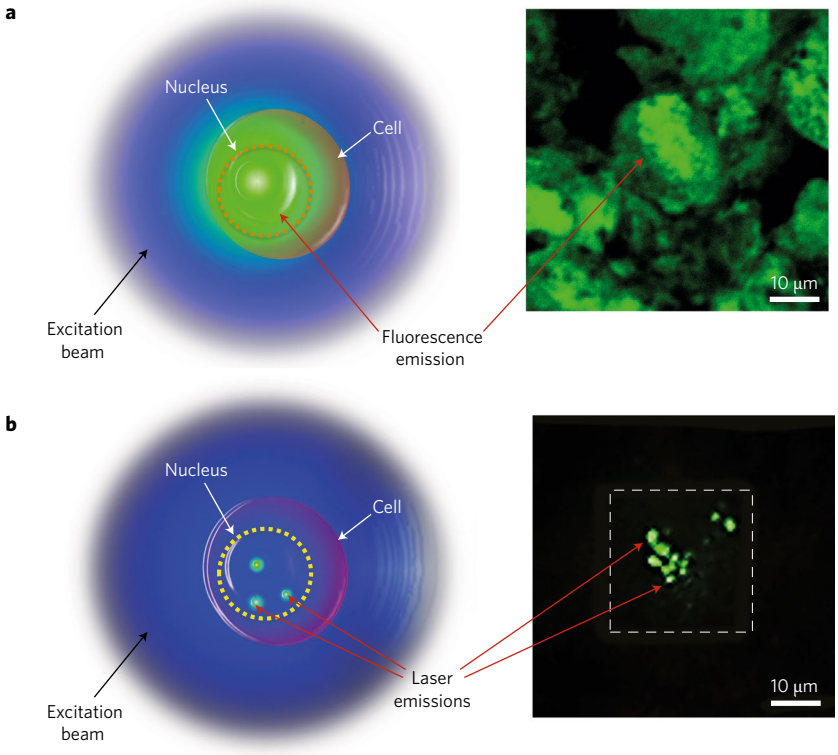

Fig. 2 | Differences between fluorescence emission and laser-light emission. a, In a standard, typically blurry, fluorescence image, fluorescent biomarkers cannot be precisely localized. Schematic (left) and confocal image (right) of lung-cancer cells stained with anti-EGFR-FITC. **b**, In the lasing regime, an image with low background signal shows a few lasing spots, which better represent the location of the biomarkers. Schematic (left) and laser-emission-based microscope image (right) from the same piece of tissue (yet different cells) shown in **a**. The dashed box represents the approximate area illuminated by the laser. Figure adapted from ref. [5], Macmillan Publishers Ltd.

information can be extracted from their number and positions inside cell nuclei.

To increase the specificity of detection, Fan and co-authors used fluorescein isothiocyanate (FITC) dye conjugated to epidermal growth factor receptor antibody (anti-EGFR; EGFR overexpression is associated with a number of cancer types). Although both YOPRO and anti-EGFR-FITC have been simultaneously used to stain tissue, the two fluorophores have closely overlapping emission spectra (with emission peaks separated by only ~10 nm); therefore, they cannot be distinguished via a standard fluorescence microscope. However, when the two dyes are placed inside the laser cavity of the LEM, their lasing wavelengths are clearly separated owing to the narrow spectral emission, and can thus be individually detected.

Standard immunohistochemistry staining procedures can be used with the LEM, which may enable faster adoption for real-life applications in medical diagnosis and in basic research. Still, the implementation of the LEM also presents challenges, as sophisticated sample preparation and special optical equipment are required. For example, the sample needs to be sandwiched between two dichroic mirrors, which are considerably more costly than standard glass slides. Additionally, the mirrors need to be aligned so that they are reasonably parallel to each other, with well-controlled spacing. For the optical set-up, in addition to a microscope and a camera, a pulsed laser is required (a spectrometer is optional). Both pieces of equipment are however widely available. Currently, to obtain the entire tissue image and the emission spectra from a large area, the pump beam needs to be scanned across the sample, which is a slow process. Furthermore, to map the concentrations of fluorescent markers in the sample, the image has to be captured at different intensities of the pump laser. Namely, the LEM can detect subtle changes in fluorophore concentrations, but is only discriminating if the concentration of fluorophores is high enough for lasing to occur at a particular pump intensity. Therefore, for measuring across a wider dynamic range, multiple measurements at different pump intensities are required. In future implementations, the whole tissue sample can be illuminated at the same time, and advanced hyperspectral imaging could be employed to shorten acquisition times.

Although Fan and colleagues' study relied on the presence or absence of laser emission only, there are significant opportunities in the analysis of the intensity, wavelength, spatial distribution and number of lasing peaks. The spectral positions of the peaks are sensitive to the sample's refractive index, which may enable the detection of subtle optical changes in cells and tissue. Moreover, with the LEM, fluorescent probes whose emission-spectrum peak changes when subject to different stimuli can also be used, as these changes can be precisely detected via the intensities of the lasing-emission peaks. For research purposes, the LEM could be extended to employ recombinant fluorescent proteins, or be integrated with Förster resonance energy transfer, fluorescence in situ hybridization, or fluorescence recovery after photobleaching, thereby imparting higher sensitivities to these techniques. In the future, owing to its sensitivity, the LEM could provide a complementary method to standard histopathology to support cancer diagnosis, especially when the difference between abnormal and healthy tissue is small, as is in early-stage cancers. ❐

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