Living-cell imaging using a photonic crystal nanolaser array

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Abstract: We proposed and demonstrated a label-free imaging method for living cells using a GaInAsP H0-type photonic crystal nanolaser array. We integrated 441 nanolasers in an arrayed configuration and achieved photopumped lasing with a 100% yield. Then, we attached HeLa cells on it, measured the wavelengths of all nanolasers and used them as pixel information. We acquired cell images, which partially corresponds to optical micrographs and probably reflects the attachment condition of the cells. We improved the refractive index resolution from $\sim 10^{-2}$ to $2 \times 10^{-3}$ by incorporating a nanoslot into each nanolaser and compensating the nonuniformity of each index sensitivity.

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OCIS codes: (230.5298) Photonic crystals; (140.3948) Microcavity devices; (280.4788) Optical sensing and sensors; (170.3880) Medical and biological imaging.

References and links


1. Introduction

Living cells are usually observed with fluorescent labels, but the complicated procedure of preparing samples and the invasiveness of labels are undesirable. Therefore, label-free methods have been studied including those using Raman spectroscopy [1] and optical resonance [2–8]; unfortunately, Raman signals are very weak and the optical resonances of the surface plasmon and diffraction grating have low sensitivity or low resolution. In a previous study, we developed an air-bridge semiconductor photonic crystal nanolaser operated by photopumping [9]. Its fabrication process is straightforward, and its operation using free-space optics is quite simple. Since the cavity of this nanolaser is exposed to air, a laser wavelength shift $\Delta \lambda$ occurs due to the refractive index change $\Delta n$ of the environment. We applied this nanolaser to sensing liquids and biomolecules. We obtained a high sensitivity $A \equiv \Delta \lambda/\Delta n$ of 400 nm/RIU, with an index resolution on the order of $10^{-5}$ RIU for liquids in a single device, and ultralow detection limit concentrations for the adsorption of standard proteins [10,11]. Furthermore, since the effective modal area of a single device is less than 1 $\mu$m$^2$ at $\lambda \sim 1.55$ $\mu$m, a large number of devices can be integrated on a chip as a high-density array [12].

In this study, we propose to use the nanolaser array as an image sensor for living cells and experimentally demonstrate its preliminary operation. Here, nanolasers are aligned into a two-dimensional square lattice with a pitch large enough to prevent mutual mode coupling, and cells are cultured on this array, as shown in Fig. 1. Each nanolaser exhibits a $\Delta \lambda$ value according to the $\Delta n$ value induced by the cell attachment. Real-time tracking of the $\Delta \lambda$ values of all devices are mapped into continuous images, which display some behaviors of the cell. In the following, we first describe such $\Delta \lambda$ imaging, showing its correspondence to optical micrographs, temporal evolution, and reaction against infecting a reagent. Then, we discuss the conversion of a $\Delta \lambda$ image into $\Delta n$ image. Although the sensitivity and resolution of a single device are as high as those mentioned above, the instability of each laser wavelength and the nonuniformity in the array add noise and distortion to the $\Delta n$ image, degrading the...
resolution and hampering its ability to detect the delicate behaviors of the cells. We reduce these problems by structural optimization and calibration of the sensitivity and obtained images whose effective index resolution was $\sim 2 \times 10^{-3}$.

2. Fabrication and measurement

Figure 2 shows the fabrication process for the nanolaser array. We prepared a commercially available GaInAsP/InP single-quantum-well wafer with a photoluminescence peak around 1.50–1.51 $\mu$m and a total GaInAsP thickness of 180 nm. Then, triangular lattice hole patterns of the photonic crystal were formed by e-beam lithography and hydrogen iodide (HI) inductively coupled plasma etching [9]. The hole diameter was 250 nm and the lattice constant was 500 nm. The laser cavity consisted of a submicron space, which was formed by shifting two or four adjacent air holes (lattice-shift-type H0 nanocavity). In addition, the diameters of some holes are slightly modified to orient the laser emission toward the vertical direction [11,13]. The fabrication process of the nanolaser array is similar to that reported in Ref [12]; the GaInAsP layer with the photonic crystal pattern was bonded onto a glass substrate with a polydimethylsiloxane (PDMS) resin. Even assuming PDMS ($n = 1.4$) and water ($n = 1.321$) around the device, the cavity $Q$ was calculated by finite-difference time-domain (FDTD) method to be 7000, which is sufficient for lasing. In such a simple process, however, the resin passed through the holes and covered the device surface after removing the InP substrate, which hampers the array's ability to sense the attachment of the cells. Therefore, the holes were buried by silica deposited from the hydrolysis of tetraethyl orthosilicate, and then the processed surface was bonded onto the glass substrate. The InP substrate and silica were removed successively by selective wet etchings using HCl solution and buffered HF, respectively. Finally, the wafer surface was oxidized by heating at 180°C for 1 h to protect the semiconductor surface from chemicals in the culture fluid used in the cell-sensing experiments shown later. In principle, there are no limitations on the size of the nanolaser array using this process.

Fig. 2. Fabrication process. (a) Epitaxial wafer. (b) E-beam lithography. (c) HI inductively coupled plasma etching. (d) Silica deposition into holes. (e) Bonding using PDMS resin. (f) Wet etchings.

In the measurement, the device chip was fixed by glass plates on its sides or set in a PDMS microchannel so that it can be immersed in liquids and photopumped from above by 980 nm pulsed laser light (with 1 $\mu$s length and 10 kHz repetition) through a 50 x objective lens. Laser emission from the nanolaser was detected by the same lens, observed by an InGaAs infrared camera, and measured by an optical spectrum analyzer (OSA), Advantest Q8383 (spectral resolution is 0.2 nm), or a spectrometer, Roper SP2760-3 (spectral resolution is 0.139 nm) with an InGaAs array detector, Roper OMA-V1024. Figure 3 shows the lasing characteristics of a single nanolaser. The intensity of the single mode peak is more than 30 dB (maximally 40 dB) higher than the background spontaneous emission level, indicating the
laser emission. The spectral width was typically 0.6 nm even above the threshold, which is known to be caused by the thermal chirping under the pulsed pumping condition [9].

Fig. 3. Pulsed lasing characteristics of single nanolaser formed on PDMS and immersed in water. Right lowest panel shows the spontaneous emission spectrum at unpatterned slab on PDMS, which was obtained by pumping harder with a larger pump spot. It indicates that the spontaneous emission peak was around 1.50 μm and that the noisy background in the second lowest panel arose from the spontaneous emission.

Fig. 4. Fabricated nanolaser array. (a) Optical micrograph of nanolaser array, SEM image of one nanolaser, and pumping scheme. (b) Near-field pattern of laser emission from the 441-nanolaser array.

In the first experiment of the nanolaser array, we fabricated a 21 × 21 = 441 array with ~5 μm pitch and a 5 × 5 = 25 array with ~2.5 μm pitch. Figure 4(a) shows the optical micrograph of the nanolaser array and the scanning electron micrograph (SEM) image of a single nanolaser. The silica embedded in the holes suppressed the penetration of PDMS, and a uniform surface was obtained after bonding. Figure 4(b) shows the near-field pattern of laser emission from 441 nanolasers in water. The lasing of all devices was confirmed; although emission intensities were not uniform, they were sufficient to measure the laser spectrum in the OSA. In the measurement of lasing wavelength, each nanolaser was pumped and the measurement was repeated by scanning the pump position using the computer-controlled stage, as illustrated in Fig. 4(a). It took 12 min to measure all λ values. Here, one might be concerned by mode coupling between the nanolaser that is mainly being pumped and its surrounding nanolasers; an additional concern may be unwanted detection of the emission...
from surrounding nanolasers, which may hamper the system’s ability to identify the wavelength of the mainly pumped nanolaser. To avoid these problems, a much larger pitch between nanolasers may be necessary, but this limits the integration density and consequently the spatial resolution of the Δλ image. Therefore, we considered $3 \times 3 = 9$ neighboring nanolasers as a unit and designed their $\lambda$ values to be slightly different from each other by adjusting the diameter of the shifted holes in the H0 cavity. Figure 5(a) shows the dependence of $\lambda$ on this diameter. Neglecting the mode hopping at $2r < 160$ nm, $\lambda$ changed monotonically from 1.59 to 1.51 μm with increasing 2r. Thus, we set different values of the diameter of each of the nine nanolasers in Fig. 4(a). We expected the wavelengths to converge into nine values, but each wavelength fluctuated owing to disorder in the fabrication, and they were consequently distributed almost continuously in the range 1.57–1.59 μm, as shown in Fig. 5(b). If this fluctuation is suppressed by some process optimization, we may pump nine nanolasers simultaneously using a larger high-power pump spot and measure their $\lambda$ values in a single spectral detection, thus reducing the total measurement time to ~1.4 min. If only nine nanolasers were used for constructing the images, the time will be less than 1 s.

Fig. 5. Spectral characteristics of the nanolaser array. (a) Wavelength controlled by changing diameters of the shifted holes in a test sample in water. (b) Laser spectra of the 441 nanolasers in water, where the intensities are normalized.

Fig. 6. Observation of resist pattern on a nanolaser array. (a) Optical micrograph of resist pattern and organic substance. (b) Δλ image.

In this study, the Δλ image was constructed offline after removing the attached medium, measuring the reference $\lambda$, and mapping Δλ at each position. As a test experiment, we partially covered the device with a Zeon resist, ZEP520A, and an organic substance (which remains after culturing the cells) and obtained the Δλ image, as shown in Fig. 6. The resist and organic substance in the optical micrograph were clearly reconstructed in the Δλ image.

3. Cell imaging

In the cell imaging experiments, fabricated device chips were first washed by ethanol and phosphate-buffered saline, and then successively placed into a vessel filled with a culture fluid known as Dulbecco’s modified Eagle medium (DMEM), GIBCO, which included HeLa cells (human cervical carcinoma line) and is supplemented with 10% fetal bovine serum, GIBCO, and 1% penicillin streptomycin, GIBCO. The vessel with the device chips were then placed in an incubator overnight at 37°C with 5% CO₂ to culture cells on the devices. After

#240970

Received 15 May 2015; revised 16 Jun 2015; accepted 16 Jun 2015; published 19 Jun 2015
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29 Jun 2015 | Vol. 23, No. 13 | DOI:10.1364/OE.23.017056 | OPTICS EXPRESS 17060
incubation, the device chips were put out together with the fluid from the incubator and set in the abovementioned setup, controlled at 37°C. Then, each nanolaser was operated and its \( \lambda \) values were measured. Since CO2 was not fed into the setup in this experiment, the activity of the cells was maintained only within several hours. For a longer observation, we employed another culture fluid known as Leibovitz’s L-15 medium, Gibco, which suppresses the deficiency in CO2. Since the optical absorption of pump light at 980 nm and emission at \( \sim 1.57 \) \( \mu m \) by the cells was almost negligible, the damage to the cells was considered minimal. Finally, we removed the cells by injecting trypsin, which hydrolyzes the protein of the cell membrane, and measured the reference \( \lambda \).

![Fig. 7. Observation of HeLa cells attached on a nanolaser array. (a) Snapshots for samples A–D. Left and right panels for each sample show the optical micrographs and \( \Delta \lambda \) images, respectively. Dashed lines depict the cell boundaries. (b) Time evolution.](image)

Figure 7(a) shows optical micrographs of the attached cells and the corresponding \( \Delta \lambda \) images for four different samples A–D. In many repeated experiments, flatly attached cells were observed, suggesting strong attachment and low cytotoxicity of the nanolaser material (GaInAsP). Some similarities between the micrographs and \( \Delta \lambda \) images were observed. For example, sample A exhibited two cells attached separately. The \( \Delta \lambda \) image showed a similar but slightly nonuniform distribution within the cells; the nonuniformity was greater than 1 nm in the \( \Delta \lambda \) image. Such nonuniformity was more evident in other samples. Sample B exhibited a complicated image including cell-to-cell adhesion. The round cells give smaller redshifts in the \( \Delta \lambda \) image. Samples C and D exhibited more nonuniform patterns. Since the evanescent field of the nanolasers can only detect the behaviors within the penetration depth of the laser mode from the device surface into the fluid and cells \( \sim 120 \) nm, the \( \Delta \lambda \) image might reflect the attachment condition determined by the footing of the cells.

To support this consideration, we calculated \( \Delta \lambda \) for different attachment conditions using the FDTD method, as shown in Fig. 8. Here, the distance \( d \) between the nanolaser and cell \( (n = 1.355) \) is changed and culture fluid \( (n = 1.337) \) is assumed in-between them. \( \Delta \lambda \) is set at zero in the absence of the cell. As \( d \) is decreased, \( \Delta \lambda \) is increased particularly at \( d < 100 \) nm, which is within the penetration depth of the laser mode. \( \Delta \lambda \) is calculated to be \( 1.5 \) nm at \( d = 0 \).

Received 15 May 2015; revised 16 Jun 2015; accepted 16 Jun 2015; published 19 Jun 2015
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where the cell is uniformly attached on the top surface of the nanolaser. It is further increased when the cell partially penetrates into holes of the photonic crystal slab. These calculations well explain the experimental $\Delta \lambda$ in the cell area in Fig. 7 ranging from 0.5–2.0 nm.

![Diagram](image)

**Fig. 8.** FDTD calculation of wavelength shift for the distance between nanolaser and cell. The distance is set at zero when the cell is attached on the top surface of the nanolaser. The negative distance means that the cell partially penetrates into holes of the nanolaser.

Figure 7(b) shows the time evolution of the $\Delta \lambda$ image. Here, the DMEM was exchanged repeatedly during the measurement to compensate for the evaporation of volatile elements. The time evolution in the $\Delta \lambda$ image of each nanolaser was tracked automatically using a developed computer algorithm that checks if the time derivative of $\lambda$ is reasonable when the wavelengths of two nanolasers become close. When the culture fluid was exchanged, all $\lambda$ exhibited a constant shift owing to slight changes in the concentration and temperature of the fluid. This shift was neglected automatically in the algorithm. As observed, the redshift pattern gradually changed during the 150 min measurement. Three cells A, B, and C exhibited different changes, i.e., small changes, spreading of the pattern, and increase in the redshift, respectively. Referring to the discussion for Fig. 7(a), it is reasonable to believe that the larger change was mainly due to a local change in the attachment condition of the cells.

Figure 9 shows the time evolution of each $\Delta \lambda$ image during the 8000 s measurement. Here, the $\sim\!2.5$ μm pitch $5 \times 5$ array was used to shorten each measurement to 15 s. At 1400 s, 0.80 μM of the reagent actinomycin D (which inhibits the nucleus RNA polymerase [14]), was injected to investigate the reaction in the cells. Before the reagent was injected, $\Delta \lambda$ was less than 0.5 nm. This shows that thermal shifts, evaporation of the fluid, and change of the cells were almost negligible over such a short duration. When actinomycin D was injected, $\lambda$ did not show immediate change because the solution had an index close to that of the culture fluid. Then, $\lambda$ redshifted until $\sim\!3500$ s and became stable, followed by a second redshift from 5000 s. The slope of the first shift was not the same between the nanolasers, whereas that of the second one was almost the same. This suggests that the first shift was mainly due to the reaction in the cell and the second one due to the evaporation of volatile elements.
4. Refractive index image

So far, we have shown only the $\Delta \lambda$ image, although the image based on the index change $\Delta n \equiv \Delta \lambda / A$ is more valuable for evaluating cellular behavior. As mentioned, the most attractive feature of the nanolaser sensor was its high index resolution on the order of $10^{-5}$ RIU [9], which should be effective for detecting, for example, the emission of secretory proteins from cells. However, when the fluctuation in $\Delta \lambda$ in each nanolaser and the nonuniformity in the sensitivity $A$ between nanolasers are larger than this value, the effective resolution is degraded, minute signals disappear in the noise, and the $\Delta n$ image is distorted. To suppress the noise and guarantee the correspondence between the $\Delta n$ image and the actual $\Delta n$ profile, the fluctuation must be suppressed and nonuniformity must be calibrated.

First, we suppressed the fluctuation in the laser spectrum mainly caused by the thermo-optic (TO) chirping. In a previous study, we reported that the TO chirping broadens the laser spectrum and fluctuates the peak wavelength, particularly under the pulsed pumping condition [9] that was employed in this study to avoid severe heating. We also showed that incorporating a nanoslot (NS) into a nanolaser in water localizes the laser mode and balances the positive TO effect in the semiconductor and the negative one in water, which was confirmed from the spectral narrowing and more stable peak wavelength. We expect similar cancellation even in the device bonded on the PDMS, because the PDMS on the back side of the device has a negative TO coefficient of $-0.5$ RIU/K, similar to that of water [15], and the front side is exposed to water similarly to the previous case. Therefore, for constructing the $\Delta n$ image, we employed NS nanolasers, as shown in Fig. 10(a). We fabricated a $12 \times 12 = 144$ array, setting coarse pitches of $\sim 10 \mu m$ for a wider modal distribution in the presence of the NS. The width of the NS was designed to be $\sim 50 \text{ nm}$, considering high yields of fabrication and laser operation. Figure 10(b) shows the near-field pattern of the laser emission from all nanolasers, indicating a $100\%$ yield and intensities more uniform than that in Fig. 4(b). Figure 11(a) shows all the lasing spectra with and without an NS. Evidently, the NS narrowed the spectrum. Although the averaged full-width at half-maximum was wider than the resolution limit of the OSA used in both cases, that with the NS was much closer to the resolution limit. Moreover, the uniformity of the spectral shape was markedly improved by the NS, resulting in the stability of the peak wavelength.
Fig. 10. Fabricated NS nanolaser. (a) SEM image of a single nanolaser. (b) Near-field pattern of the 144-nanolaser array.

Fig. 11. Sensing characteristics of the nanolaser array with and without an NS. (a) Lasing spectra. (b) Sensitivity. (c) Index absolute accuracy of the index shift after calibrating the sensitivity. Its dispersion determines the index resolution. Green, purple, yellow, and blue plots represent liquid indices of 1.33, 1.35, 1.37, and 1.39, respectively.

Next, we calibrated the nonuniformity in $\Delta n$ by evaluating the sensitivity $A$ of each nanolaser in advance. For the evaluation, we measured the $\lambda$ of the all the nanolasers in sugar water with various concentrations whose refractive indices were measured independently by refractometer. $A$ for the liquid index was obtained from $\Delta \lambda/\Delta n$ for all nanolasers, as shown in Fig. 11(b). Without the NS, $A$ is distributed from 50 to 70 nm/RIU, neglecting the most scattered 10% of points. This indicates that a comparably large nonuniformity was included in the $\Delta \lambda$ image. In other words, the nonuniform image of the cells in Fig. 7(a), exhibiting wavelength shifts from 1 to 2 nm, qualitatively showed some actual behaviors of the cells.
although the image is not precise. With the NS, \( A \) was markedly stabilized in the range 95–102 nm/RIU. Some strongly scattered points were due to the mode hops observed with the NS. This might be because of the mode spacing being reduced by incorporating the NS and should be increased by further optimization in future studies. The averaged \( A \) values were 97 nm/RIU and 57 nm/RIU with and without the NS, respectively. The \( A \) values were smaller overall than structure on the PDMS, in which the backside of the photonic crystal slab does not contribute to the sensing, and the laser mode penetration becomes deeper and shallower in the PDMS and liquid than that in the air-bridge device, respectively. The higher average value of \( A \) with the NS was attributed to the stronger light localization inside the NS outside of the semiconductor. Then, \( \Delta n \) was obtained from \( \Delta \lambda /A \) for each nanolaser. The absolute accuracy of \( \Delta n \), defined as the difference between the \( \Delta n \) obtained in the above manner and the value directly measured by the refractometer, is shown in Fig. 11(c). The index resolution was evaluated from the deviation in the absolute accuracy. Without the NS, the resolution was on the order of \( 10^{-2} \) RIU. Such a large dispersion even after the calibration is thought to be due to the spectral broadening and wavelength instability. The NS reduced this scattering range to \( 2 \times 10^{-3} \) RIU, neglecting some points caused by mode hops. The highest index resolution of \( 10^{-5} \) order was evaluated for the individual nanolaser under a cw pumping condition. Compared with this, the TO chirping under the pulsed condition still has room for suppression, which is also a future issue.

Figure 12 shows the \( \Delta n \) image with the NS, where the device chip and HeLa cells were immersed in an L-15 culture fluid and placed in a PDMS microchannel to suppress the evaporation of the fluid. The time evolution of the image partially corresponded to the cellular behaviors in the optical micrograph. When the color scale was changed in Fig. 12(a), we realized that the cell on the left side moved more frequently than that on the right side. Since the lamellipodium on the left side moved out from the observation frame, such frequent movement might reflect the activity of the cell. Figure 12(b) shows the \( \Delta n \) image for the cells that were finally desorbed from the device. The \( \Delta n \) image disappeared gradually from the lower side to upper side of the cell image. This temporal change must be due to the chain reaction of the local adhesion.

5. Summary

We succeeded in observing living cells using a photonic crystal nanolaser array rather than labels. We first fabricated and operated a 441-nanolaser array with a 100% yield and acquired \( \Delta \lambda \) images for the resist coating and cultured HeLa cells, which roughly corresponded to
those observed in optical micrographs. Next, we converted the $\Delta \lambda$ image to a $\Delta n$ image by introducing an NS into each nanolaser. We demonstrated a $12 \times 12 = 144$ array with a 100% yield and improved the index resolution to $\sim 2 \times 10^{-3}$ RIU, which was a five-fold improvement over that without the NS. Cell images thus obtained suggested that they mainly reflected the attachment condition of the cells on the nanolaser, which is usually difficult to observe in the optical micrograph. Further investigation of the correspondence between the image and actual cellular behavior is expected in future studies.

As an image sensor, three constraints must be cleared for progress to continue. The first constraint is the spatial resolution determined by the nanolaser pitch. Without the NS, the laser mode was distributed over a $\sim 2 \mu m$ diameter area and this determines the minimum pitch. This is not sufficiently small for the observation of detailed cellular behaviors, but may be applicable to some macroscopic observations. The second constraint is the readout time of all $\lambda$ values; at present, the readout time of one frame is longer than 10 min for large arrays over 100. An optical system enabling parallel pumping and readout may be a solution for this. Another alternative is to focus on a smaller array with parallel pumping and readout, enabling to capture a rough image of cells at a video frame rate. It will be helpful if the index resolution can be further improved or if the image can detect delicate behaviors such as the emission of secretory proteins. Thus, the third constraint is the index resolution, which is currently limited to $\sim 1 \times 10^{-2}$ RIU without the NS. For a higher index resolution, the NS must be incorporated, although it results in a larger modal area and a lower spatial resolution. However, the resolution is still limited to $\sim 2 \times 10^{-3}$ RIU, for which further optimization of the cavity structure, laser mode, and fabrication process are necessary.

Acknowledgment

This work was partly supported by a Grant-in-Aid of MEXT #24226003.