Portable Antigen Detector Using Blue Laser Diodes and Quantum Dots

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Portable Antigen Detector Using Blue Laser Diodes and Quantum Dots

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We developed a portable antigen detecting system based on laser illuminated fluorescence microscopy. The introduction of small and reliable semiconductor laser and charge coupled device (CCD) camera enable to make a compact antigen detecting system. The 532 nm green laser used as light sources can excite the 655 and 705 nm quantum dots attached to antibody connecting to target antigen. The light emitted by the excited quantum dots forms an image in CCD camera. The fluorescence image successfully delineates the antigen distribution in the specimen. We demonstrate the same performance using a 405 nm laser diode which can excite wider range of emission wavelength of quantum dots. [DOI: 10.1143/JJAP.46.1763]

KEYWORDS: laser diode, quantum dot, antigen, confocal microscopy

1. Introduction

Fluorescence labeling is one of the most widely used techniques in biology and medical science. For instance, DNA sequencing, DNA chip, and protein chip are the main application field of the fluorescence tagging. Fluorescent material attached to antibody is generally used to trace the antigen such as a protein or a particular chemical. Therefore, tracing antigen through detecting fluorescence basically depends on optics which is also well known to have a rich toolbox for the precise measurements. Especially, the recent progress in optical device can improve the existing fluorescence equipments in terms of performance, cost and size.

Along these lines, we find a motive to modify a fluorescent microscope and a fluorescent confocal microscope to meet the present demand in the application field. A fluorescent microscope is a specialized microscope for observing the magnified image after staining the sample with fluorescent materials.[1)] A fluorescent confocal microscope is also a kind of fluorescence microscope except that it enhances resolution greatly and provides three dimensional imaging capability by introducing pinhole, laser and precision stage with other very expensive mechanisms.[2,3)] Although these two devices come up with impressive functions, they share a common weakness. They are too bulky to carry with. In case of fluorescent microscope, it employs combination of incandescent lamp and highly sensitive charge coupled device (CCD) camera. Since lamp cannot focus light into a small area, the detection system should be more sensitive to make up for the light loss. High sensitivity of detector usually requires bulky cooling equipment and high electrical power consumption as well as high cost of system. As to a confocal microscope, it uses sophisticated components in every aspects starting from light source to detector system. Therefore, it is much more expensive and bulkier than a fluorescent microscope. For both microscopes, the lack of portability prevents them from testing samples in the outdoor field in spite of their outstanding functionality. However, detecting on the spot is getting inevitable in emergency such as a biochemical terror attack or an epidemic. In addition to improving portability, if we calibrate the image intensity properly, a portable fluorescent microscope can work for measuring the quantity of target material as well as imaging the distribution.

In this paper, we present the results about a small packaged antigen detector based on semiconductor laser to obtain fluorescent image and find the antigen distribution in the tissue. The laser diode (LD) replaces bulky gas/solid-state lasers which are main source of increasing the volume and power consumption of the existing equipment. Low-power consumption of a laser diode makes the antigen detector battery-operated which is a crucial advantage for portable devices. At the same time, quantum dot (QD) is chosen as a fluorescent material to compensate the lowered sensitivity of detection part and enable single laser source for multi-color labeling. By constructing a real system, we demonstrate the feasibility of portable antigen quantity analyzer which makes possible on-spot diagnosis in the future.

2. Components and Functions of an Antigen Detector

A laser, dichroic mirror, objective lens, filters, sample, CCD camera and the other components are arranged as shown in Fig. 1. The 532 nm light emitted from green laser is reflected by a dichroic mirror, which is designed to be used at a 45° angle of incidence and will reflect green light while transmitting red light. The reflected green light is

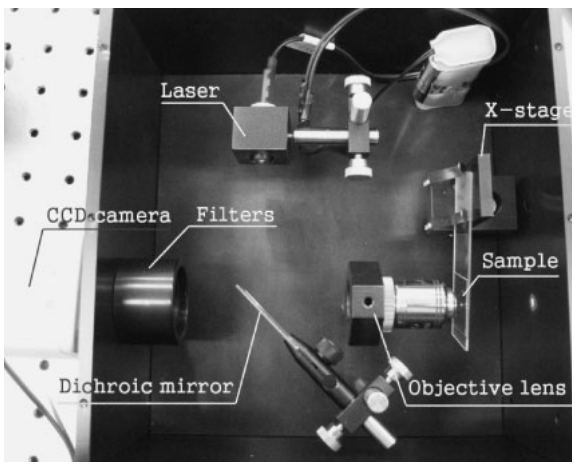

Fig. 1. Photograph of an antigen detector. The dimension of the package is $20 \times 20 \times 10\,\mathrm{cm}^3$.

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focused into the sample by objective lens. The fluorescent material in the sample absorbs the green light and re-emits the red light. The emitted fluorescent light is collected into a photo-detector via optical filter to cut the remaining laser light. Finally, the light at detector is converted to electrical signal. In this way, the amount of biological molecules in the liquid can be measured.

To select an appropriate light source for an antigen detection system, we consider three types of LDs. They are a red LD at 650 nm, a green laser at 550 nm and a blue LD at 405 nm. A red LD is cheap and power-efficient, but the excitable fluorescent materials are restricted to the ones emitting longer wavelength than 650 nm. A blue LD has relatively short wavelength, so that we can choose a wide range of emission wavelength and hence a wide range of QDs.[4–7] However, it is still expensive and in a limited sale. The green laser can be a compromise between the two. In the experiment, a green laser emitting at 532 nm with output power of 5 mW is used. As a matter of fact, the green laser is not a LD, but a diode pumped solid-state laser (DPSSL). Comparing to a green helium-neon laser in a confocal microscope, the green laser is smaller and more economical. As for the dimension of the laser including the housing, it is 40 mm in length and 11 mm in diameter. Besides, the very low power consumption of the green DPSSL has a great advantage as a portable device. The operation voltage of the green laser is 3 V which can be powered by two 1.5 V batteries. After considering all these factors, the green DPSSL is a suitable light source for a portable antigen detector especially in developing stage.

However, there is an obstacle to overcome before applying the green DPSSL to fluorescence equipment. It has two satellite emission peaks other than the main peak at 532 nm. Although the intensities of the satellite peaks are much less than that of the main one, they can be mixed with fluorescence signal and generate a significant level of noise. The satellite peaks located at 805 nm and 1064 nm relate to the fundamental mechanism of green DPSSL. A high power diode laser emitting at 805 nm optically pumps Nd:YAG (yttrium aluminum garnet, $Y_3Al_5O_{12}$) solid state laser. The pumped Nd:YAG laser emits light at 1064 nm.[8] The 1064 nm light enters a KTP (Potassium Titanyl Phosphate, $KTiOPO_4$) crystal and produces light at 532 nm by second harmonic generation (SHG). SHG is the consequence of the nonlinear interaction of the electromagnetic radiation with the crystal lattice.[9] Therefore, we have to filter out the satellite peaks to reduce the noise.

Since the light at 532 nm is used mainly for exciting QDs in the sample, the laser light after illuminating the sample should be removed in the final fluorescent light. A 45° dichroic mirror is placed between the sample and the detector. The dichroic mirror causes strong reflection around 499–555 nm while it transmits light over 569–730 nm. The laser light is focused into the sample by an objective lens to excite QD fluorescent materials in the tissue cells. In tradition, the fluorescent tag is attached to biological molecules to trace the very small amount of the tagged material. When the tagged biological molecules in the sample absorb the short wavelength laser light, the fluorescent material re-emits the light at longer wavelength. Qdot 655 and Qdot 705 are used in the experiment.[10] Fundamentally, Qdot nanocrystals are fluorophores that absorb photons of light and re-emit photons at a different wavelength. However, they exhibit some important differences from traditional fluorophores such as organic fluorescent dyes and naturally fluorescent proteins. Qdot nanocrystals are nanometer-scale atom clusters which contain a few hundred or a few thousand atoms of a semiconductor material such as CdSe.

The tissue samples are prepared by the following procedure. 7- to 10-week-old female Balb/c mice were maintained in the lab animal facility and used for experiments. For a general immunology study (CD11c antigen is popularly used in the immunology field), 7- to 12-week old mice are usually used for the tissue samples of lymphoid organs. Mice outside this range are too young or old. Female mice are generally used since they are easier to care for than male mice.

For bleeding before making tissue section, mice were sacrificed by exsanguination of jugular vein under diethyl ether anesthesia. Spleens were dissected and embedded in OCT compound (Tissue-tek, Sakura Fine Technical, Tokyo, Japan) and transferred into liquid nitrogen. They were immediately plunged into isopentane solution chilled with liquid nitrogen. The frozen tissues were sectioned at 5–10 μm thickness using a cryostat (Leica, Nussloch, Germany) and kept at −20 °C until used. The sections were dried at room temperature (RT) for more than 20 min and fixed in 4% paraformaldehyde at RT for 20 min. 5% normal goat serum (Pierce Biotech, Rockford, IL, U.S.A.) in phosphate buffered saline (PBS) was used for blocking tissues at RT for 30 min. Tissues were incubated with a biotinylated anti-mouse CD11c monoclonal antibody (clone HL3, BD Biosciences, San Jose, CA, U.S.A.) at a concentration of 1 μg/ml. Antibody was diluted in PBS containing 2% normal goat serum. Subsequently, they were incubated with Qdot streptavidin conjugate 655 or 705 nm (Invitrogen, Carlsbad, CA, U.S.A.) at a dilution of 1 : 100. Finally, tissues on slides were mounted with a fluorescent mounting medium (Dako, Glostrup, Denmark). In preliminary experiments, we optimized the conditions for immunohistochemistry, such as the concentration level of the first antibody, Qdot streptavidin conjugate, tissue thickness and wavelength of Qdot for a detector.

The emitted fluorescent light is focused into a photo-detector via optical filter and turns into an electrical signal. Fluorescent light is not the only detected signal. The laser light reflected by a slide glass on which sample lies also goes into a detector. Although the dichroic mirror diminishes the laser light significantly, it does not remove the laser light completely. Moreover, the remaining laser light is not weaker than the fluorescent light. In order to cut off the laser light, several filters are placed in front of the detector. At first, a red additive dichroic filter is used to transmit the light with wavelength longer than 600 nm. A large amount of laser light is cut down, but the laser light still survives in the signal. Another red additive filter reduces the noise further. However, the third red additive filter does not help to lower the noise level. As mentioned before, a green DPSSL has a very small amount of light at 805 nm and at 1064 nm. In order to eliminate these infrared lights, infra-red (IR) cutoff filters are used. The IR filters pass only shorter wavelength

than 700 nm. Two IR cutoff filters completely remove the noise due to the satellite peaks.

As a fluorescence detector, we select CCD camera even though photo-multiplier tube (PMT) has much higher sensitivity. It is mainly because PMT occupies relatively large space and the exposure to the ambient light easily damages the system. Since QDs enhance the fluorescence more than 10 times compared to the conventional fluorescent probes, CCD has no problem with obtaining the image. In addition, CCD has an advantage of getting the two dimensional image over PMT.

The used CCD camera has pixel values of 410,000 and offers black-and-white image. The size of the CCD chip is $4.4 \times 3.3\,\mathrm{mm}^2$ and the minimum detection level is 32 fW/cell. The CCD camera is connected to PC using a media interface. And an application program can show live images from a camera and capture them as well. Visualizing fluorescence distribution is a powerful function of the detecting system. The resolution is limited by the objective lens and is usually high enough to delineate the distribution of antigen tagged with fluorescent materials. Furthermore, analyzing the captured images enables to determine the antigen quantity.

3. Results

At first, we have to check whether the light detected by the CCD camera is emitted by fluorescent materials or by the green laser. We inspected the location where fluorescent materials are absent so as to see if filters can completely eliminate the laser light. Figure 2(a) shows that the green laser light reflected from the slide glass exceeds the fluorescent light in the absence of filters. If a red additive dichroic filter is added, a 22 μm diameter spot is seen as in Fig. 2(b). If two filters are inserted, the spot becomes 18 μm in diameter as shown in Fig. 2(c). Additional filters do not reduce the neither size nor the intensity of the spot.

When an IR cutoff filter is added to the two red additive filters, the spot becomes even smaller as in Fig. 2(d). Another IR cutoff filter offers total darkness as in Fig. 2(e). As a result, two red additive dichroic filters and two IR cutoff filters thoroughly remove the noise due to the laser. Therefore, we are convinced that the image taken by the CCD camera with those filters is attributed to fluorescent emission.

The CCD images display the density of fluorescent materials in the specimen. Although the amount of fluorescent light at detector is normally very small, CCD camera offers relatively clear images. We prepared the two samples with two different concentrations of QDs. The first sample is treated with five time stronger QD solution than the second one. The former QD fluid is prepared by diluting the original QD fluid 100 times. The second QD solution is made by diluting the first solution again by 5 times. The CCD images with two different QD concentrations are shown in Figs. 3 and 4, respectively. The photos show the detailed feature of positive cells in tissue. It implies that QDs have higher density at cells. It agrees with the fact that the target protein, CD11c was detected by using biotin-labeled anti-CD11c monoclonal antibody. Generally, CD11c molecules are expressed in dendritic cells, the most potent antigen-presenting cells as critical immune cells and mainly located on the surface of cells. The white region in the photos seems to be caused by the detector saturation due to high intensity of fluorescence.

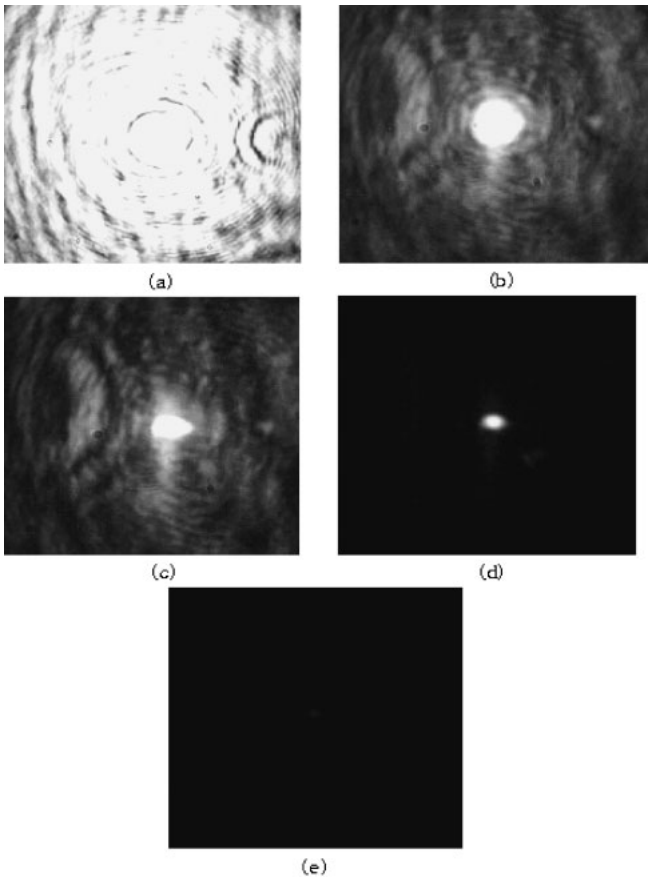

Fig. 2. CCD images of the bare substrate with filters.

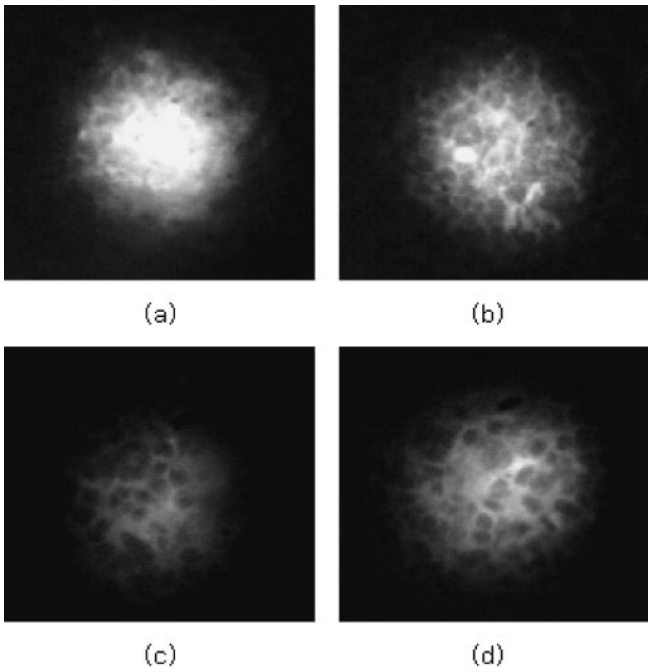

Fig. 3. The specimen labeled with 655 nm QDs of high concentration.

When the laser beam is focused into a spot by objective lens, the spot diameter is about 5 μm as in Fig. 2(d).

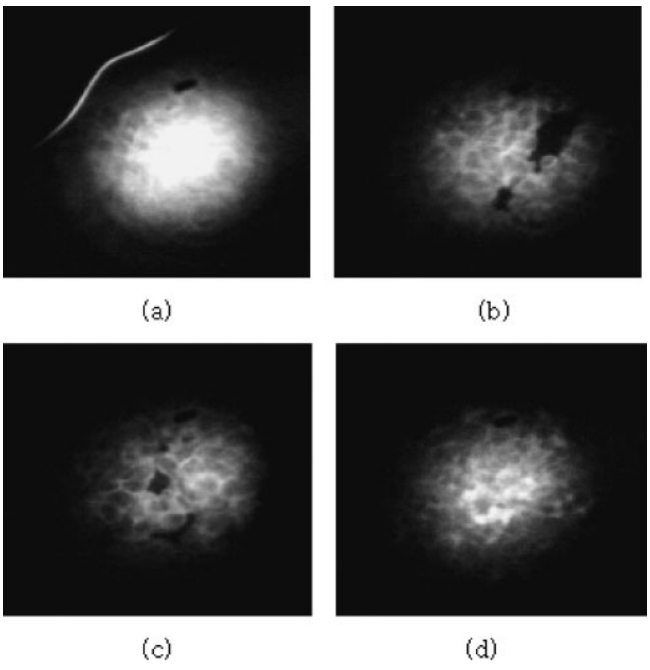

Fig. 4. The specimen labeled with 655 nm QDs of low concentration.

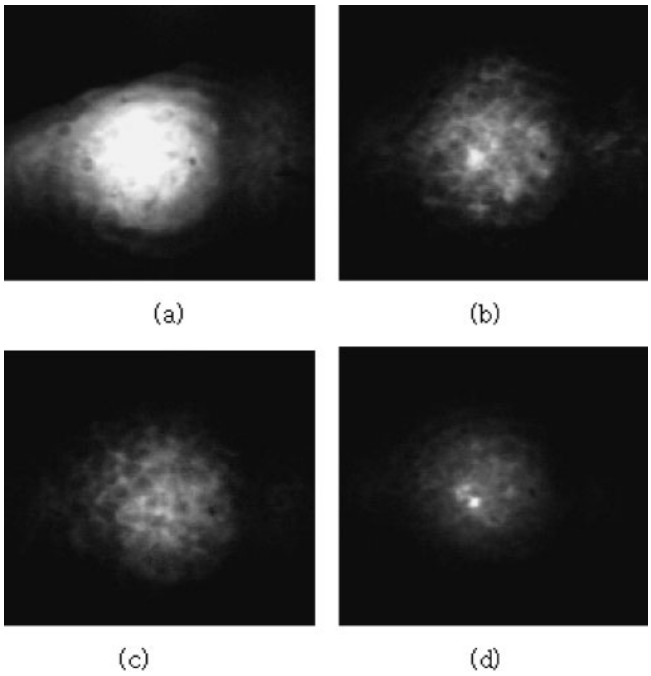

Fig. 5. The specimen labeled with 705 nm QDs of high concentration.

However, the bright region in Fig. 3(a) is about 57 μm in diameter. The fluorescent image is 11 times larger than the size of laser spot. The laser light reflects and scatters back and forth between the slide glass and the cover glass. The spread laser light excites the nearby fluorescent materials. The edge of the image is darker than the center not because of lower density of fluorescent materials, but because of weaker laser illumination at boundary. It can be confirmed by moving the sample with the focal spot fixed. Therefore, the laser illumination has a certain profile where intensity gradually decreases with radius. We also compare the degree of brightness between the images using image processing software. The maximum intensity in Fig. 3(a) is twice that in Fig. 3(d). In this way, we can digitize the brightness at each point of the image and analyze the relative density of QDs.

Figure 4 shows the sample treated with 5 times more dilute QD. However, there is no meaningful difference between Figs. 3 and 4 except the difference in cell structure. The average brightness is about the same regardless of the concentration of QDs. From this result, we can infer that the number of QDs even in low concentration solution is greater than the number of antigen to which QD can stick. In other words, the density of QDs in the specimen is not limited by the concentration of QDs in solution but by density of antigen.

We also check if the detection system can work with the QDs emitting at other wavelength. When the sample is treated with 705 nm QD, the fluorescent images in Fig. 5 are obtained. There is no significant difference in brightness between 655 and 705 nm. 600 nm QDs are also detected by CCD when they are placed on the substrate without specimen. It means that the antigen detector can work at least with 600–705 nm QD. Comparing performances between standard fluorescence microscopy and our detection system can be done by estimating illumination power and the produced fluorescence. The use of a laser diode and quantum dots increases the amount of fluorescence 4,000 times as compared to the standard fluorescence microscopy where a 75-W xenon arc-discharge lamp and a conventional fluorophore are employed. Improvements mainly come from the small focal spot of the laser and the high absorption cross-section of quantum dot, however, since standard fluorescence microscopy adopts the CCD camera whose noise level is usually 10,000 times lower than that used in our system, the overall performance is about the same.

We test a 405 nm LD as a light source for the antigen detector. With the change of light source, the dichroic mirror should have a different reflectance range from that used for the green laser. The dichroic mirror used in the detector has high reflection band over 350–500 nm. Another modification is made to the filter set which contains only red additive filters without IR cutoff filters since a blue LD emits the light only near the band gap. The fluorescent images obtained from a blue LD are displayed in Fig. 6. The four pictures correspond to different operation currents of blue LD. Even though the image maintains good resolution, the brightness drops lower than that of the green laser. The loss of intensity is mainly ascribed to the low output power of blue LD. The used blue LD generates only 0.7 mW at maximum while the green laser goes beyond 5 mW. The weak signal is detected when the operation current is about 38 mA with output power of 0.27 mW. As the optical output power of the blue LD increases, the intensity of the image increases. Better image quality is expected in the future since the performance of the blue LD improves a lot recently due to the commercialization of Blu-ray. The strength of the antigen detector using blue LD comes from the fact that it can excites the wider range of QDs as long as the emission wavelength is longer than the band gap of the laser.

4. Conclusions

We demonstrate a small package antigen detection system

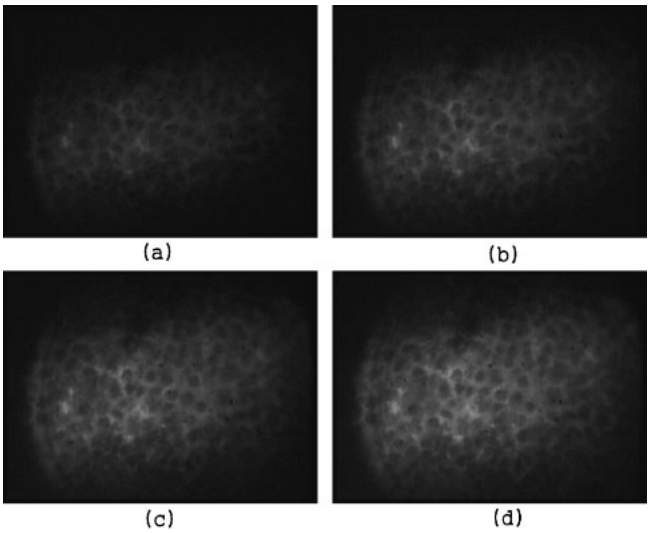

Fig. 6. The specimen is illuminated by a 405 nm LD. 655 nm QDs are used for labeling. The operation currents of LD are (a) 38.3, (b) 41.0, (c) 43.7, and (d) 46.5 mA.

using a green DPSSL and a CCD camera. The dimension of the package is $20 \times 20 \times 10\,\mathrm{cm}^3$ which can be reduced much further by using more compact optics. A small green DPSSL is proved to be a suitable light source for a portable antigen detector since it consumes little power and excites several types of quantum dots. The use of quantum dots compensates the relative loss of sensitivity caused by adopting a CCD instead of a PMT. Although a CCD camera has lower sensitivity than a conventionally used photo-multiplier, it provides two dimensional imaging capabilities which are very difficult to implement with PMT. Image processing of the captured images enables to compare the relative intensity of fluorescence and the corresponding density of antigens in the specimen. The antigen detector provides the clear fluorescence image of the specimen labeled with 655 and 705 nm quantum dots. The detection system also works well with 600 nm quantum dot. In the last step, we replaced the green DPSSL with a 405 nm LD. The system with blue LD exhibits the same performance as a green diode. Since the potential of the blue diode lies on the ability to excite wider range of quantum dots, more technological developments will be expected in the future.

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1) J. G. White, W. B. Amos, and M. Fordham: J. Cell Biol. **105** (1987) 41.
2) N. S. Claxton, T. J. Fellers, and M. W. Davidson: http://www.olympusfluoview.com/theory/LSCMIntro.pdf
3) A. R. Hibbs: *Confocal Microscopy for Biologists* (Kluwer Academic, NewYork, 2004) Chap. 1.
4) S. Nakamura, M. Senoh, S. I. Nagahama, N. Iwasa, T. Yamada, T. Matsushita, and H. Kiyoku: Jpn. J. Appl. Phys. **37** (1998) L309.
5) S. Nakamura, M. Senoh, S. I. Nagahama, N. Iwasa, T. Yamada, and T. Matsushita: Jpn. J. Appl. Phys. **37** (1998) L1020.
6) S. Nakamura, M. Senoh, S. I. Nagahama, N. Iwasa, T. Yamada, T. Matsushita, and H. Kiyoku: Jpn. J. Appl. Phys. **36** (1997) L1568.
7) S. Nakamura, M. Senoh, S. I. Nagahama, N. Iwasa, T. Yamada, T. Matsushita, and H. Kiyoku: Jpn. J. Appl. Phys. **37** (1998) L627.
8) R. Scheps: *Introduction to Laser Diode-Pumped Solid State Lasers* (SPIE Press, Washington, D.C., 2002).
9) W. P. Risk, T. R. Gosnell, and A. V. Nurmikko: *Compact Blue-Green Laser* (Cambridge University Press, Cambridge, 2003).
10) Qdot® nanocrystal technology: http://probes.invitrogen.com/products/qdot/