

Time-Resolved Fluorescence Spectroscopy of Dopamine in the Single Cells

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Abstract

Dopamine hydrochloric acid salt in aqueous solution was excited at 266 nm Al₂O₃:Ti laser and the sufficient fluorescence emission peaking at 330 nm was detected with a streak camera. The fluorescence decay curve was fitted by 1-exponential functions, with the lifetime of approximately 0.80 ns. The influence of deep-UV laser excitation on cells is also discussed for the direct observation of dopamine in the living cells. In addition, it is needed to detect the dopamine fluorescence in the living cell sensitively, and separately from emission of other fluorescent species. When instrumental arrangement and time-resolved spectral analysis can make it possible to solve such problems, direct visualization of the secretion process of individual cells will be achieved by the laser-induced native fluorescence imaging microscopy, without using any additional fluorescent probes. This quantitative imaging technique will provide a useful noninvasive approach for the study of dynamic cellular changes and the understanding of the molecular mechanisms of information transporting processes.

Keywords: Dopamine, Fluorescence, Lifetime, Time-space resolved laser microscopy

1. INTRODUCTION

In these recent years, it has been reported that dopamine (3-hydroxytyramine) plays an important role in transporting a signal/information in the nerve cells of the brain.^{1,2} Dopamine, which is dissolved in aqueous solution as an ion, is transported between the nerve cells. To further elucidate the mechanism, many real-time methods for monitoring this process have been tried to apply: for example, Raman spectroscopy,³ infrared spectroscopy⁴ and so on. There has been no technique to monitor the behavior of the dopamine molecule itself in real time. It is important to develop methods that can follow real time transportation or secretory processes with both high temporal and high spatial resolution.

For the observation of the cells, the fluorescence probing technique is popular. Some fluorescent dyes have been used successfully as a probe in microscopic cell imaging, although applications are limited to a few intracellular species such as calcium and other ions.⁵ At the same time, the native fluorescence of some proteins and neurotransmitters excited by a ultraviolet (UV) laser light has been shown to be a powerful probe for single cell analysis with capillary electrophoresis.^{4,6} There are several advantages of direct native fluorescence detection. No chemical derivatization with fluorescent dyes is needed so that no contamination or additional background will be introduced. Uncertainties about the efficiencies of the derivatization reaction are eliminated to ensure fast and quantitative response without incomplete equilibrium. The biological intensity of the cells will not be unnecessarily disturbed by having additional reagents or from exposure to artificial environments.

On the other hand, in the field of electronic or photonic materials, many kinds of spectroscopic technique are now employed. We have been developed the spectroscopic technique which possesses temporal and special resolution by combining pico-second pulsed laser system with the optical microscope.^{7,8} It serves as a powerful method to excite the dynamic behavior of exciton in nano or microstructures such as quantum dots. This spatial resolution 1-5 μm is suitable for observing a single cell. We have been trying to apply this spectroscopy for the direct observation of dynamics in cell.

In the present study, the fluorescent measurements for the dopamine were performed to apply to study the dynamics in a single cells using a time-space-resolved laser spectroscopy.

2. EXPERIMENTAL PART

2.1. Materials.

Dopamine hydrochloric acid salt (Tokyo Kasei Co.) was used without further purification. Figure 1 shows a chemical structure of dopamine.

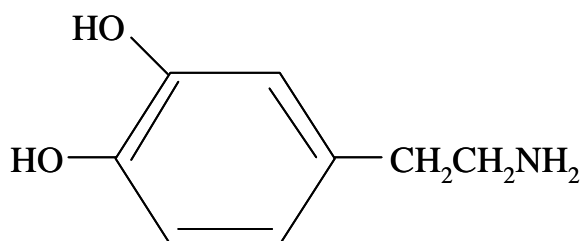


Figure 1. Chemical structure of Dopamine

2.2. Fluorescence Measurements.

The experimental setup of the time-space resolved spectroscopy is schematically shown in Figure 2.^{7,8} This system consists of three components: the ultrafast pulsed laser, the optical microscope, and detection apparatus. For excitation light, the frequency-doubled or tripled beam of mode-locked $\text{Al}_2\text{O}_3:\text{Ti}$ laser pumped by a cw Ar^+ laser was used. Pulse width, wavelength, and repetition rate were 1.5 ps, 266 nm, and 80.00000 MHz, respectively. This pulsed laser beam was expanded by the beam expander and focused to 5 mm diameter by the objective lens on the samples. The emission images were observed by the microscope and detected by the color CCD camera with 2000000 pixels. At the same time, time

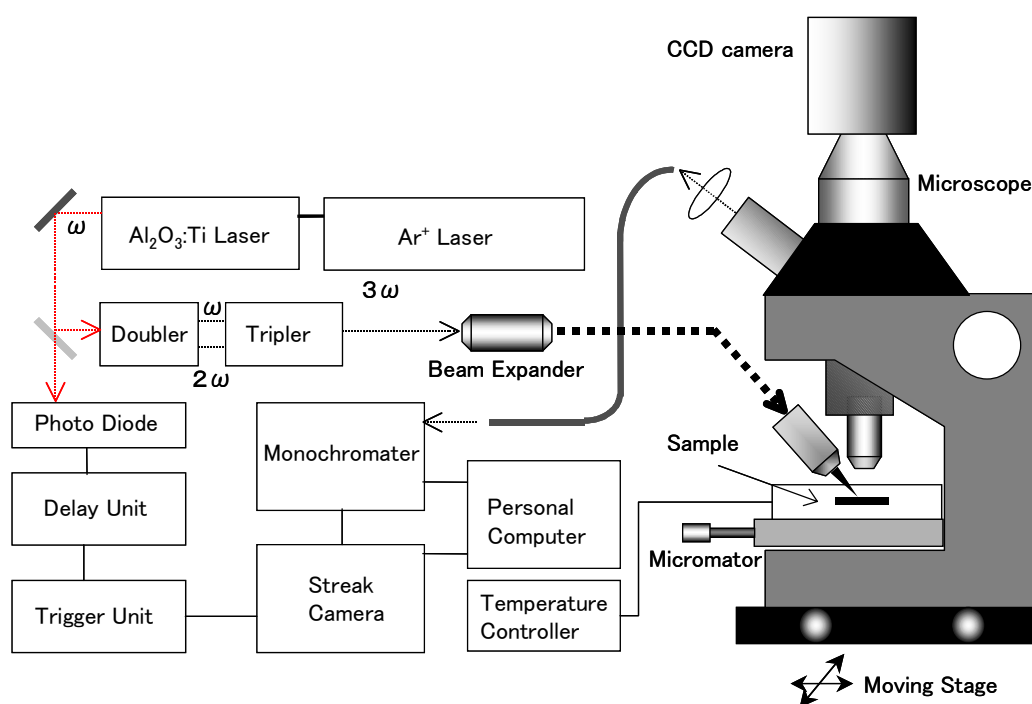


Figure 2. Schematic illustration of the experimental setup for time-space resolved spectroscopy.

resolved fluorescence spectra were measured by the synchroscan streak camera in conjunction with monochromator (25 cm, 150 lines/mm grating). The measurements were performed at the room temperature, 25 °C. Figure 3 shows an example of measurement: a microscopic fluorescence image of a nerve cell of the mouse with Hoechst dye and spatial-resolved fluorescence spectrum for the same sample.

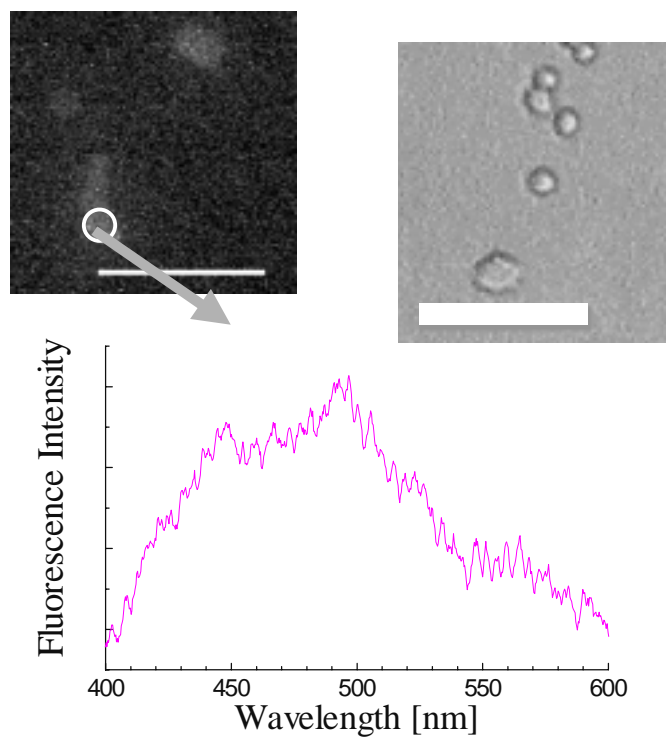


Figure 3. (left) The microscopic fluorescence image; (center) spatial-resolved fluorescence spectrum, for a nerve cell of the mouse with Hoechst dye.; (right) optical microscope image for a nerve cell of the mouse *in vitro*. The scale bar is 100 μm .

3. RESULTS AND DISCUSSION

3.1. Fluorescence Properties of Dopamine.

Although several catecholamines are known to show a weak fluorescence, there have been few reports to the fluorescence of dopamine itself. In this measurement, we could obtain a clear, sufficient fluorescence spectrum peaking at 330 nm when excited at 266nm light. Figure 4 shows a fluorescence decay curve for a dopamine hydrochloric acid salt in aqueous solution observed at 318 nm. The curve was successfully fitted by a 1-exponential function with the lifetime of 0.813 ns. Figure 5 shows time-resolved fluorescence spectra by a streak camera and the lifetime data as a function of observing wavelength. The spectra and lifetime data indicate that the dopamine fluorescence decays with keeping the shape of its spectrum in the wavelength range lower than 360 nm. Thus, the species of the lifetime 0.81 ± 0.01 ns is the one from excited single dopamine ion which is isolated in a dilute solution. On the other hand, in the wavelength range higher than 360 nm, the lifetime was a little larger: 0.84 ns. The excitation spectrum coincided with the absorption spectrum of dopamine, even if it was monitored at the higher wavelengths than 360 nm. This means that this long-lived species in 360-380 nm is originated from dopamine molecules. It is assigned to the emission from exciplex formed with trace amounts of unknown acceptors.

It is needed to detect the dopamine fluorescence in the living cell sensitively, and separately from emission of other fluorescent species. By using these data for the basic properties of dopamine, selectively detection of a trace amount of dopamine flowing in the living cell can be achieved without using additional fluorescent probes.

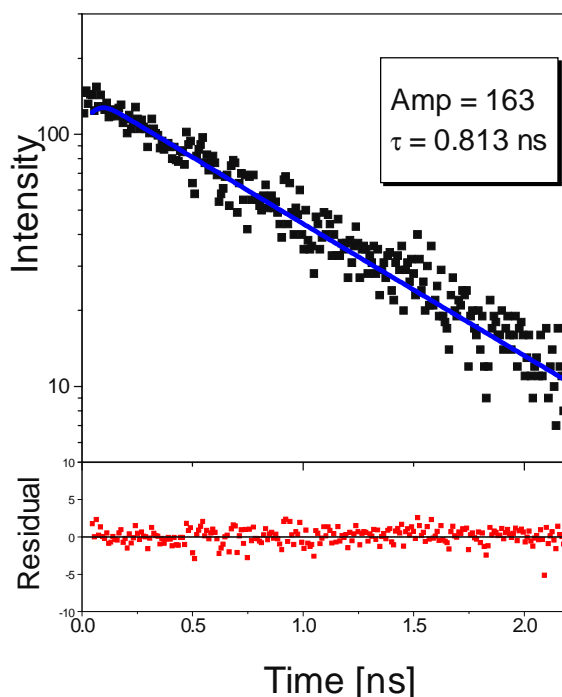


Figure 4. Fluorescence decay curve of the dopamine chloride acid salt (in water) at 318 nm. The solid line shows a fitting curve.

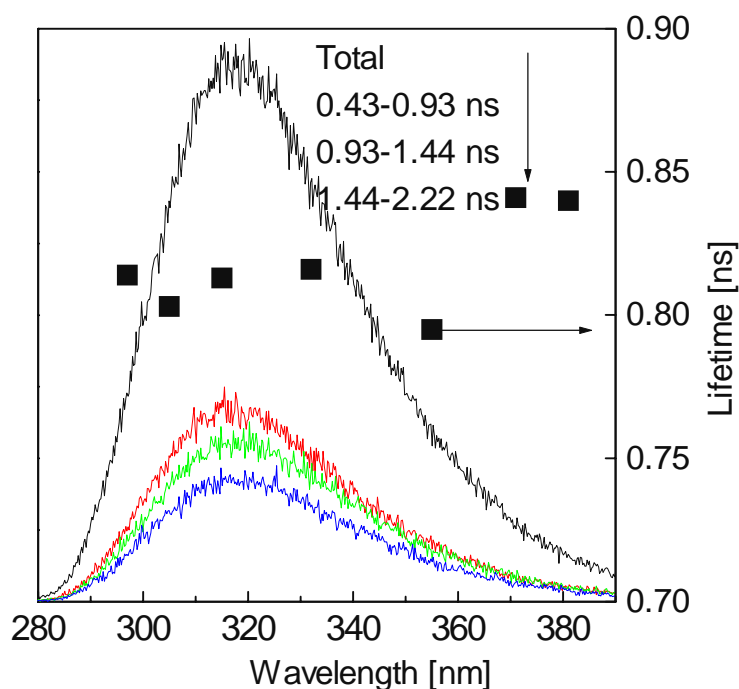


Figure 5. Time-resolved fluorescence spectra by a streak camera and the lifetime data as a function of observing wavelength.

3.2. Influence of UV Laser Light on a Living Cell.

Many biomolecules including amines and proteins will fluoresce naturally when excited with 260-275 nm laser line.^{10,11} It is known that the wavelengths of native fluorescence of these molecules are at about 310 – 350 nm.

Figure 6 shows the influences of laser light on a living cell observed by the optical microscope. Figure 6a shows irradiation dependence on the cells. The circle indicates the laser spot. By the cell in the laser spot gradually lost its apparent softness in the water and its shape decayed. The structure of the cell was disordered immediately by light with the strength of 0.57 mJ/cm^2 , which is about 10 or more times larger than the laser light strength of measurements for normal semiconductor quantum dots or inorganic semiconductor samples. The stronger the laser intensity, the shorter the disordering time of the cell. As shown in Figure 6b, a single cell was kept within 1-2 minutes by the laser light of 0.057 mJ/cm^2 . The cell bore the laser light of 0.024 mJ/cm^2 for 5-10 minutes. The results of the laser lights at other wavelengths are almost the same as this result. Therefore, it was concluded that the fluorescence measurement can be carried out within a few minutes, thus this condition setting for the laser excitation is possible to measure the time-dependent fluorescence spectra without damage.

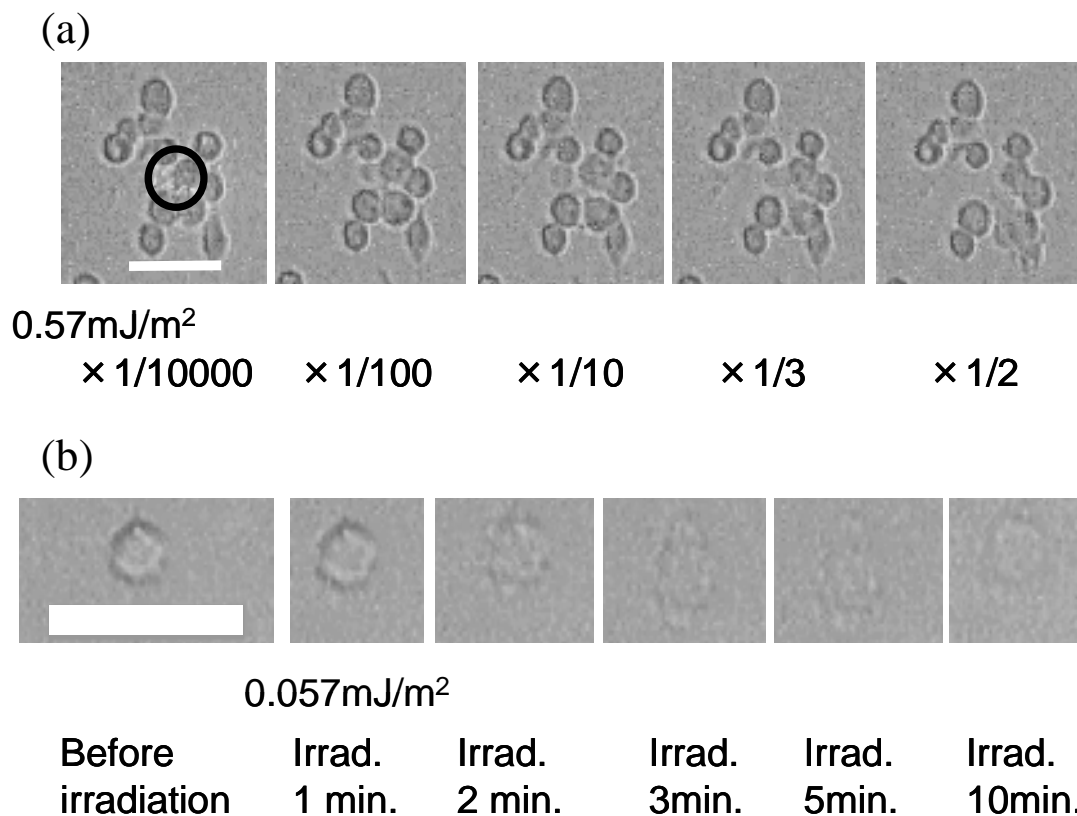


Figure 6. The influence of laser light irradiation on nerve cells of the mouse *in vitro* by the optical microscope; (a) irradiation strength dependence on the cells. A circle denotes the area where laser light was irradiated with spitting. Each image is taken after 1-minute irradiation; (b) irradiation time dependence of the cells. The scale bar: 100 μm .

CONCLUSION

The native fluorescence properties of dopamine were examined aiming the direct visualization of dopamine dynamics in the living cell. The native fluorescence of dopamine in solution was detected with a sufficient intensity by our time-resolved spectroscopy equipment. The influence of deep-UV laser excitation on cells was also discussed and the experimental conditions for observing a living cell without damage were found. The dopamine fluorescence are possible to detected selectively from the cell by examining the direct results of time-resolved spectroscopy and the basic fluorescence properties; contributions from stray light and fluorescence from the background matrix, cell membrane, and cell nuclei are canceled. Thus, in the cell fluorescence image, we can interpret the decrease of fluorescence intensities as being due to the release of dopamine molecules. This direct imaging technique can be developed to apply a quantitative analysis, and can provide a useful noninvasive approach for the study of dynamic cellular changes and the understanding of the molecular mechanisms of secretory or information transportation processes.

At the same time we are investigation the diffusion process of dopamine in solution by using Taylor dispersion technique and transient grating technique. The diffusion properties in solution from these measurements will also serves as a basic data to understanding the dopamine behavior in the living cell.

ACKNOWLEDGEMENTS

This study was partly supported by the Kyoto University-Venture Business Laboratory Project, Research Foundation for Opto-Science and Technology, Konica Imaging Science Foundation and a Grant-in- Aid for Scientific Research from the Japan Society for the Promotion of Science and Ministry of Education, Science and Culture. The authors are grateful to Dr. Tsuyoshi Yaoi (Kyoto Prefectural University of Medicine) for supplying the living cell samples.

REFERENCES

1. H.-T. Chen, S. B. Kandasamy, "Effect of ionizing radiation on in vivo striatal release of dopamine in the rat", *Radiat. Res.*, **146**, 111, 1996.
2. A. Okada H. Nakamura, H. Nakamura, S. Nohara, "Combined effect on whole-body and noise on the dopamine turnover in the rat brain", *J. Acoust. Soc. Am. Suppl. 1*, **83**, S22, 1988.
3. W. K. Kowalchuk, K. L. Davis, M. D. Morris, "Surface-enhanced resonance Raman spectroscopy of iron-dopamine complexes", *Spectrochim. Acta A, Mol. Spectrosc.*, **51A**, 145, 1995.
4. M. Souta, unpublished data.

5. R. Sanders, H. C. Gerritsen, A. Draaijer, P. M. Houpt, Y. K. Levine, "Fluorescence lifetime imaging of free calcium in single cells", *Bioimaging*, **2**, 131, 1994.
6. R. Y. Tsien, *C&EN* 72(29), **34**, 1991.
7. T. Izumi, Y. Narukawa, K. Okamoto, Y. Kawakami, Sg. Fujita, S. Nakamura, "Time-resolved photoluminescence spectroscopy in GaN-based semiconductors with micron spatial resolution", *J. Lumin.*, **87-89**, 1196, 2000.
8. K. Okamoto, H. -C. Ko, Y. Kawakami, Sg. Fujita, "Time-space-resolved photoluminescence from (Zn, Cd)Se-based quantum structures", *J. Crystal Growth*, **214/215**, 639, 2000.
9. J. M. Finnegan, R. M. Wightman, *J. Biol. Chem.*, **270**, 5353, 1995.
10. W. Tong, E. S. Yeung, "Direct visualization of secretion from single bovine adrenal chromaffin cells by laser-induced fluorescence microscopy", *Applied Spectroscopy*, **52**, 407, 1998.
11. F. -H. Schnepel, *Fluorometric Analysis in Biomedical Chemistry*, pp. 69, J. D. Winefordner, Ed. , John Wiley and Sons, New York, 1991.