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Development of Laser Excitation Thermal Lens Spectroscopy by Deep Ultraviolet Excitation (LETLS-213)

Aira KAMITO*, Miki ISODA, Akira HARATA
Department of Molecular and Material Sciences, IGSES, Kyushu University, Kasuga city, Fukuoka, Japan
Email: kamito.aira.753@s.kyushu-u.ac.jp

Abstract: *A laser excitation thermal lens spectroscope is developed by using a deep ultraviolet laser for highly sensitive detection and microanalysis of amino acids. The analytical method of all kind molecules in solution without chemical modification is strongly required for advance of biochemistry. Laser induced fluorescence method is major as a highly sensitive analytical method of molecular in solution, but the method is limited to detect target by fluorescence.*

Keywords: Deep ultraviolet, 213 nm, Micro-fluid device, Thermal lens spectroscopy, Amino acid

1. INTRODUCTION

Laser induced fluorescence (LIF) is the highly sensitive analytical method for fluorescent molecules in solution [1]. Species of high fluorescence quantum yield suit LIF method, but low or non-fluorescent species need to derivatize a fluorescenct functional group and there is a possibility of changing physical properties by chemical modification. However, most biochemical species are non-fluorescent and absorb light only in ultraviolet (UV) region (400 nm >λ). Especially, amino acid that is the source of protein absorb light only in deep UV region (240 nm >λ).

This study is focused on highly sensitive detection and microanalysis without chemical modification. Laser excitation thermal lens spectroscopy [2-4] is developed using by deep UV laser (λ_1= 213 nm) (LETLS-213) to detect amino acids. For microanalysis, a sample cell is replaced by a micro-fluid device that can pour these types of solutions from three inlets at the same time to control the width of flow pass, and the thermal lens (TL) signal is detected. Experimental conditions are optimized by evaluating signal to background (S/B) ratio.

2. EXPERIMENTAL METHOD

2.1 Materials and micro-fluid device

The target amino acid used is L-tryptophan (optical absorption wavelength= 220 nm), the solvent is 80/20 (v/v%) acetonitrile aqueous solution. Two kind of solutions forming three liquid layer flow into the micro-fluid device (flow pass width= 300 µm, depth= 100 µm, quartz) from each syringe (5 mL) as shown in Fig. 1. Middle layer is sample solution (1.0×10^{-3} M L-tryptophan solution of the solvent), and both side of middle layer are sheath solution (solvent).

For checking laminar flow in the micro-fluid device, rhodamine 110 chloride and rhodamine B is used as fluorescent reagents.

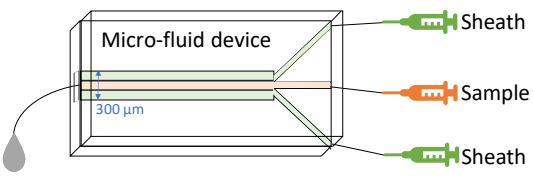

Fig. 1. The flow of solution in the micro-fluid device.

2.2 Experimental setup

Experimental setup of LETLS-213 is schematically shown in Fig. 2. The excitation beam that are emitted by a high repetition rate solid-state diode pumped Q-switched laser (Nd:YVO_4 laser, λ_1= 213 nm, IMPRESS 213, Xiton Photonics) pass a chopper for intensity modulation (chopping frequency f= 100 Hz). The excitation beam combines with the probe beam (He-Ne laser, λ_2= 633 nm) by a dichroic mirror. Two beams pass a pinhole (φ= 0.6 mm) and a condenser lens (focal length f_{213}= 83.05 mm, f_{633}= 97.56 mm), then two beams irradiate the flowing sample in the micro-fluid device. The target amino acid in middle layer absorbs the excitation beam and excite to a high energy state. The refractive index of target sample around excitation beam irradiation changes by TL effect [5]. Only the probe beam pass through a band pass filter (transmission wavelength λ_T= 632±20 nm), is detected with a photodiode (PD), and gives TL signal.

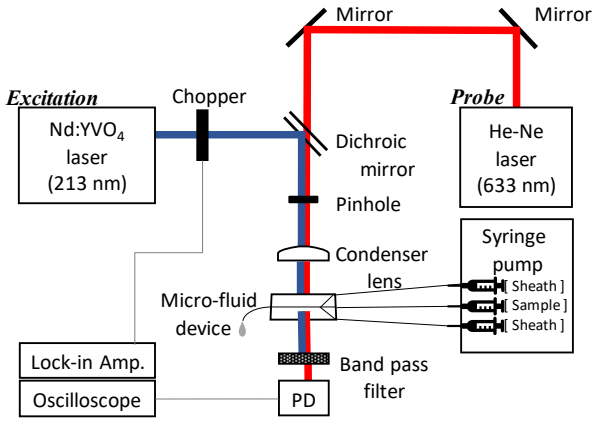

Fig. 2. Experimental setup of LETLS-213.

2.3 Experimental conditions

2.3.a The dependence of middle layer width

The sample flow of middle layer and the sheath flow of both outer layers are changed to make the total flow rate of three layer in the micro-fluid device 0.3 mL/min. The micro-fluid device is fixed 100 mm behind the condenser lens, the photodiode is fixed 100 mm behind the micro-fluid device. In the same flow condition, laminar flow is checked by a fluorescent microscope.

2.3.b Dependence of distance between the condenser lens and the micro-fluid device

The sample flow and the sheath flow per a layer are 0.1 mL/min. The condenser lens and the photodiode are fixed on this experiment. The micro-fluid device is moved between the condenser lens and the photodiode.

3. RESULTS and DISCUSSION

3.1.a The dependence of middle layer width

In each experiment from (a) to (e), the flow velocity in the micro-fluid device is constant by keeping the total flow rate as a constant in table 1. As the middle layer width is wide, the S/B ratio increase as in Fig. 3. The result shows that the wider flow width, the more amino acid is excited. Laminar flow is also confirmed for all conditions of flow rates as shown in Fig. 4.

Table 1. Flow rate of each experiment.

	Flow rate (mL/min)		
	Sample	Sheath	Total
a	0.250	0.025	0.300
b	0.200	0.050	0.300
c	0.150	0.075	0.300
d	0.100	0.100	0.300
e	0.050	0.125	0.300

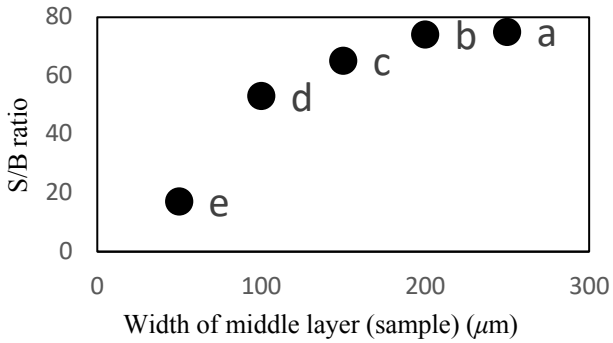

Fig. 3. Dependence of S/B ratio on the middle layer width in the micro- fluid devise.

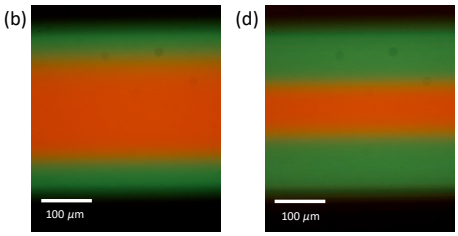

Fig. 4. Laminar flow in the micro fluid device under the condition of (b) and (d).

3.1.b Dependence of the TL signal intensity on the position of the micro-fluid device

The strongest TL signal is obtained at the distance of 104 mm as in Fig. 5. The position of the strongest TL signal is behind the focal points of excitation beam and probe beam as in Fig. 6. According to theory, strong TL signal is obtained near the focal point of excitation beam in front of the focus of probe beam. However, this optical system causes beam loss because the probe beam is too large, and the irradiation area is too narrow. Therefore, the best position of the micro-fluid device for this system is behind of two focal points without beam loss at the distance of 104 mm.

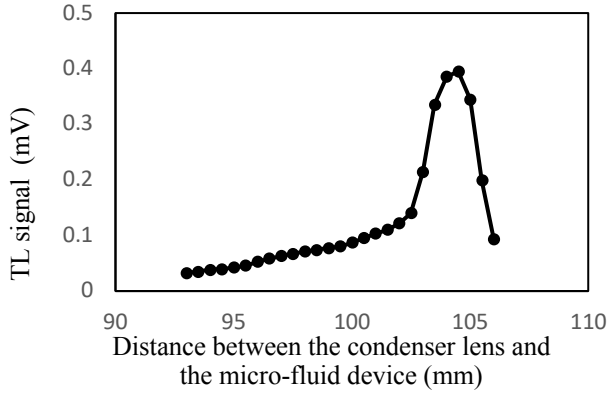

Fig. 5. The dependence of TL signal intensity on the position of the micro-fluid device.

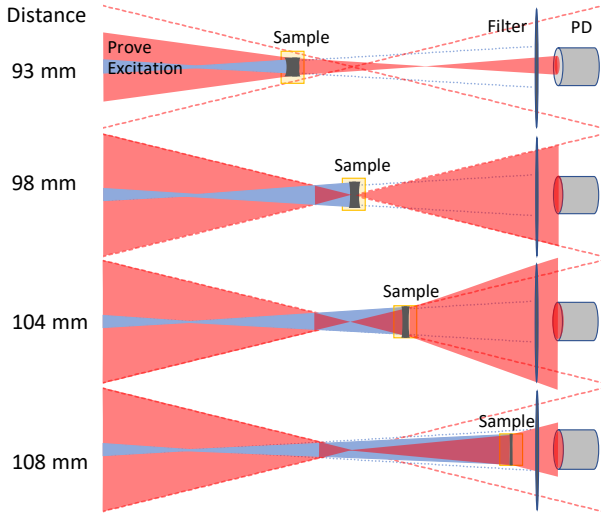

Fig. 6. Schematic illustration of prove beam divergence change by TL effect for four different positions of the sample. The exciting beam and the probe beam pass through sample.

4. SUMMARY

The setup of LETLS-213 is optimized for highly sensitive detection and microanalysis without chemical modification. The micro-fluid device can control the width of the sample flow remaining laminar flow. The wider is the width of middle layer, the stronger the TL signal. The micro-fluid device has an optical position of 104 nm for this system.

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