Title: **Application of polydimethylsiloxane-based optical system for measuring optical density of microbial culture.**

Running Head: **Measuring optical density by PDMS-based system**

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Abstracts

The performance of recently-developed polydimethylsiloxane (PDMS)-based optical system was tested for measuring optical density of microbial culture. The data showed that PDMS-based spectrometer is superior to "one drop" spectrometers in the accuracy, and has an advantage over conventional spectrometers in measuring dense culture without dilution.

Keywords:
polydimethylsiloxane-based optical system; optical density; growth curves; *Escherichia coli*

Measuring optical density (OD) is routine work for life science in general. Because conventional spectrometers require relatively large volume (~1 mL) for one measurement, researchers often use flasks, instead of daily-used test tubes, to secure enough volume for monitoring growth of culture. Otherwise, they prepare many replicate tubes for one culture and use one tube for one measurement, but growth (e.g. length of lag phase) of each tube sometimes change slightly and make growth curves distorted. Although this problem has been partially solved by microplate readers and/or spectrometers for "one-drop" measurement, the instruments cannot be moved where researchers wish to do measurements (e.g. inside of clean bench), because of their size, weight, and fragility of the optical system. Alternatively, OD can be measured without disturbing the culture using automated OD meters such as OD-monitor (TAITEC) and TVS062CA (Advantec). But introducing such systems is relatively expensive, and limits the shape of the container (e.g. baffled flasks cannot be used because baffles scatter the light).

Recently, a polydimethylsiloxane (PDMS)-based optical technology (1), the

light path of which is filled with a composite structure of a carbon–PDMS compound to suppress intense background radiation, makes a spectrometer to be compact, portable, and inexpensive. This technology has been already commercially available as an instrument mainly designed for protein assay. The required sample volume is small (30 µL at minimum), and solution in a single PCR tube can be directly measured without warmup time. Here I report the accuracy and linearity of OD measured by PDMS-based optical system compared with conventional spectrometer and "one-drop" spectrometers. With this system, I successfully obtained high-quality and reproducible growth curves, and compared the growth of *Escherichia coli* in various disposable tubes by monitoring single culture.

E. coli SCS1 (Agilent Technologies #200231) was grown in LB medium (10 g/L of tryptone, 5 g/L of yeast extract, 10 g/L of NaCl, pH7.0) (2) for overnight, then harvested and resuspended in 1×PBS(-) (140 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4). The density of cells was adjusted as OD_{600} = 10 by using a conventional spectrometer (UV-2450, SHIMADZU; with the length of light path as 1 cm) and then serially diluted to make OD_{600}=0.01, 0.025, 0.5, 0.75, 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, 10. From each suspension, 30, 50, and 100 µL were transferred to single PCR tube (RS-PCR-1F, RIKAKEN) respectively in triplicates, and optical densities were measured by PDMS-based portable spectrometer (PAS-110, USHIO) with the following parameters: LED output, 20%; sensor integration time, 100 ms; color sensor, Red (575-660 nm, maximum sensitivity at 615 nm).

At first, from five times of repetitive measurement of the same tubes, I confirmed that variability of measurement was so small (standard error < 2.2%) that I can use a value from single measurement per one tube in the all range I tested (adjusted OD_{600} from 0.01 to 10). Subsequently, I checked the reproducibility among the three independent samples and effect of sample volume on accuracy (Fig. 1A-1C). Although

R-squared value of the smallest sample volume (30 μL) was already larger than 0.985, the value increased as the sample volume became larger, indicating that larger sample volume makes the results more stable.

I also confirmed that increasing sensor integration time (100 ms, as twice as default setting) increased the quantitative range (Fig. 1A and 1D). With the default setting (50 ms), the instrument could not measure highly dense ($OD_{600} > 5$) and dilute ($OD_{600} < 0.25$) suspensions correctly, which results in lower R-squared value (although the value was 0.9975 in the range of $0.25 \leq OD_{600} \leq 5$, if calculated in $0.1 \leq OD_{600} \leq 10$ the value was 0.9808).

To compare performance of PDMS-based spectrometer with existing spectrometers, the suspensions were also measured by conventional spectrophotometer (UV-2450) and two types of "one-drop" spectrophotometer (NanoDrop 1000, Thermo Scientific; BioDrop μLite, BERTHOLD THCHNOLOGIES) (Fig. 1E and 1F). As results, the measurable range ($0.1 \leq OD_{600} \leq 10$) was almost the same between PDMS-based and "one drop" spectrometer (Fig. 1E), but R-squared value of PDMS-based was higher than those of "one drop" spectrometers. For conventional spectrometer (Fig. 1F), while R-squared value calculated in the range of $0.01 \leq OD_{600} \leq 1$ was the highest and it was the only spectrometer among I tested which could measure highly dilute suspensions ($OD_{600} < 0.1$) correctly, the linearity greatly decreased for dense suspension ($OD_{600} > 1$). Combining the data described above, it was shown that PDMS-based spectrometer is superior to "one drop" spectrometers in the accuracy, and has an advantage over conventional spectrometer in measuring dense suspension without dilution.

Next, I monitored the growth of *E. coli* by PDMS-based spectrometer for practical trial. As shown in Fig. 2, I could obtain high-quality growth curves. From the measurement of six replicate culture in the same condition (Fig. 2A), the resultant

97 growth curves overlapped each other. From monitoring cultures in different container
98 (i.e. different aeration conditions) (Fig. 2B), I could detect reproducible difference of
99 growth. While the culture in baffled flask showed logarithmic growth during 0-4 hours
100 after inoculation and entered stationary phase quickly, the growth rates of cultures in the
101 three types of tubes (50 mL conical, 15 mL conical, and glass test tubes) were smaller
102 than that in baffled flask, and gradually decreased during 4-18 hours after inoculation.
103 Among the three tubes, the growth rate also differed each other; the culture in 50 mL
104 conical tube was the fastest in reaching full growth and that in test tube was the latest.
105 To strengthen reliability of the method and the reproducibility of the results, I
106 also monitored the growth of *E. coli* in M9 minimal medium (2), which limit growth
107 rates slower than those on LB medium (Fig. 3). As results, reproducible difference of
108 growth in different aeration conditions could be detected also in defined minimal
109 medium. The growth rate of cultures in baffled flask was the fastest, which is consistent
110 with the growth in LB medium. On the other hand, the growth rates of cultures in the
111 three types of tubes (50 mL conical, 15 mL conical, and glass test tubes) did not show
112 clear difference each other, which might due to the widen interval of sampling (from
113 once per 1 hour to once per 2-5 hours). Because the cultures in all the four container
114 show almost the same growth curves until their mid exponential phase both in LB and
115 M9 medium, it was suggested that the aeration condition initiate to limit the growth
116 after the growth reach their late exponential phase in the culturing condition used in this
117 study.
118 It was notable that by using PDMS-based spectrometer which can be put close
119 to sampling and does not required sample dilution, one person could measure 26
120 cultures every one hours (the all data in Fig. 2 were obtained in the same day with other
121 tubes not shown in this paper). Moreover, the properties of PDMS-based spectrometer
122 that culture in the closed PCR tube can be directly measured will not only reduce

contamination risk of biohazardous bacteria but also enable to recover sample after measurements. By using this portable instrument, it will be also possible to measure OD of environmental water just after sampled at site.

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Author contribution

Yurika Takahashi conceived, designed, and performed the experiments, and analyzed data, and wrote the paper.

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Disclosure statement

No potential conflict of interest was reported by the author.

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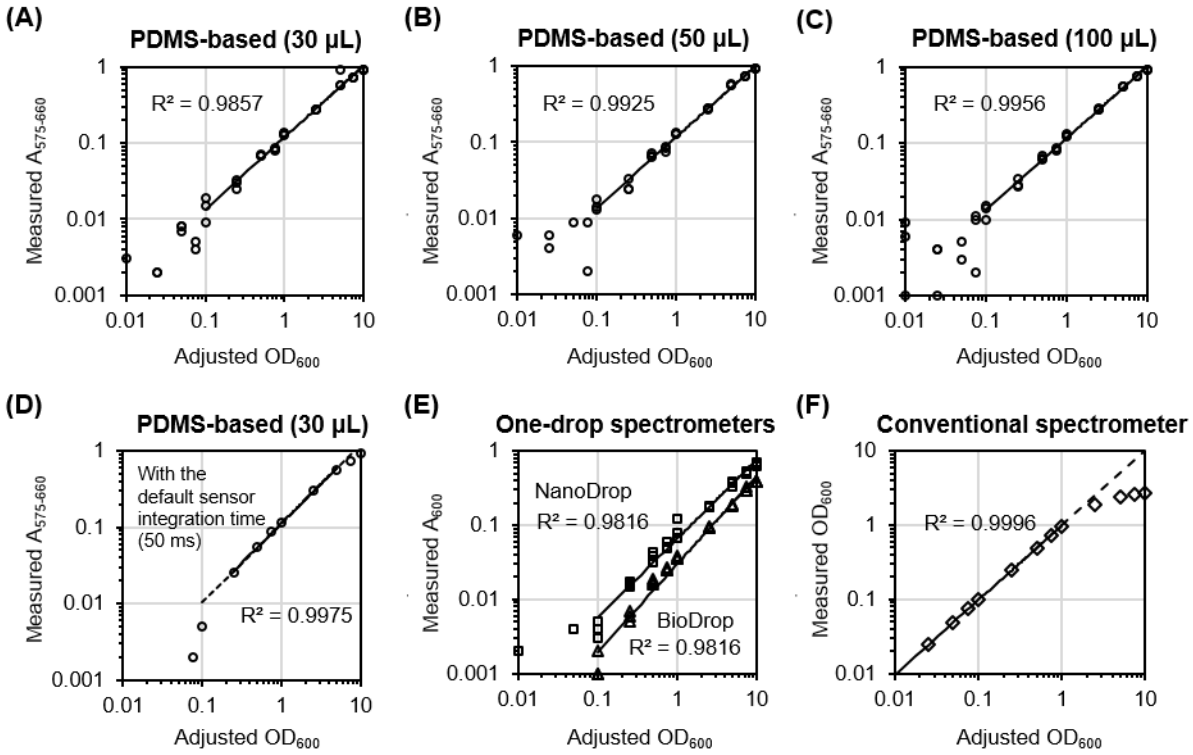

Fig. 1. Accuracy and linearity of optical density measured by PDMSbased optical system compared with "one-drop" spectrometers and conventional spectrometer. Serial dilutions of *E. coli* suspension were measured by PDMS-based optical system (PAS-110, USHIO) with increased sensor integration time (100 ms) using 30 µL (A), 50 µL (B), and 100 µL (C), respectively. The suspensions were also measured by PDMS-based optical system with default sensor integration time (D), two types of "one-drop" spectrometers (NanoDrop 1000, Thermo Scientific, "Cell Culture Mode"; BioDrop µLite, BERTHOLD THCHNOLOGIES) (E), and conventional spectrometer (UV-2450, SHIMADZU) (F).

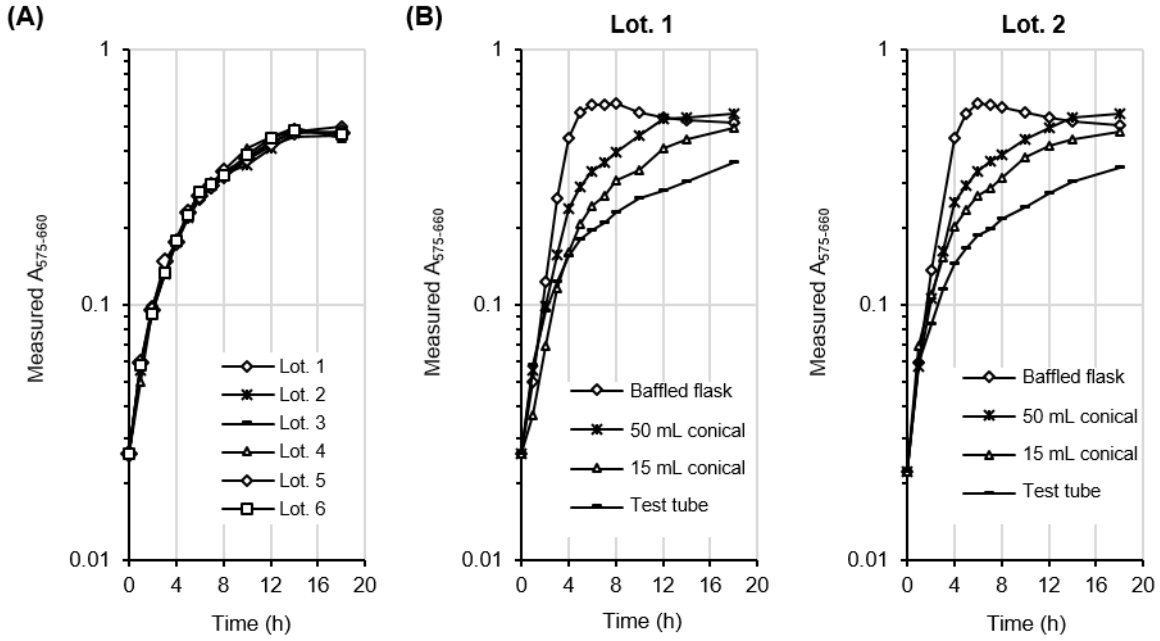

Fig. 2. Growth curves of *Escherichia coli* SCS1 grown in LB medium measured by PDMS-based spectrometer with the following parameters: LED output, 20%; sensor integration time, 100 ms; color sensor, Red (575-660 nm, maximum sensitivity at 615 nm). The pre-culture was inoculated to fresh LB medium to obtain initial $A_{575-660}$ at 0.02, and 100 µL (0-8 hours after inoculation) or 50 µL (10-18 hours after inoculation) of culture was sampled for one measurement. All cultures were incubated at 37°C, 200 strokes/min, except for baffled flask (120 rpm). (A) Consistency of measurement of six replicates in the same condition (2.5 mL in two-position cap tubes, φ16×100 mm, SARSTEDT, code: 55.459.725S). (B) Reproducible difference of growth in different containers. The culture volume in each container was 30 mL (baffled flask with capacity 200 mL capped with aluminum foil), 2.5 mL (15 mL conical tube), 10 mL (50 mL conical tube), and 5 mL (glass test tube, φ16×160 mm).

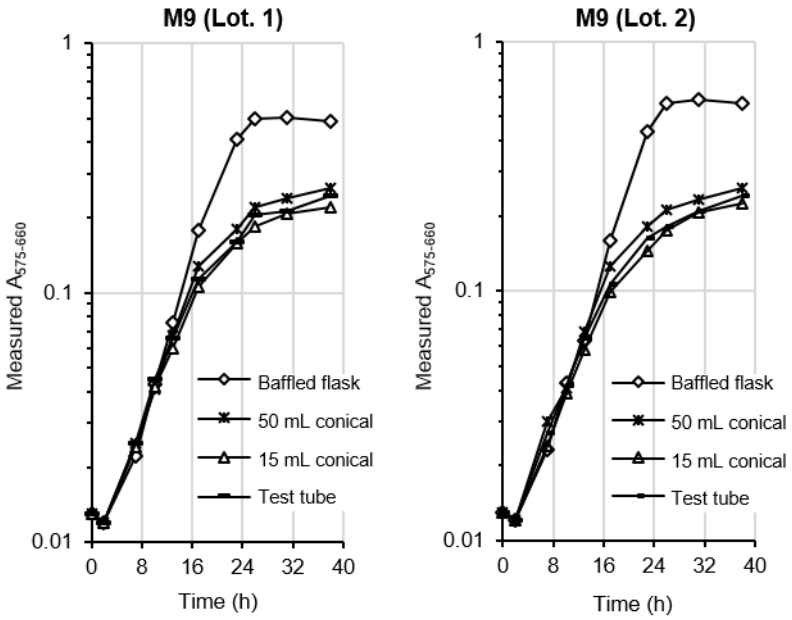

Fig. 3. Growth curves of *Escherichia coli* SCS1 grown on M9 minimal medium measured by PDMS-based spectrometer with the following parameters: LED output, 20%; sensor integration time, 100 ms; color sensor, Red (575-660 nm, maximum sensitivity at 615 nm). The cells were pre-cultured in LB medium, and once washed by glucose -free M9 medium and then resuspended in M9 medium to obtain initial $A_{575-660}$ at 0.02. 50 μL of culture was sampled for one measurement. All cultures were incubated at 37°C, 200 strokes/min, except for baffled flask (120 rpm). The culture volume in each container was the same with that in Fig. 2(B).