Palmtop spectrophotometer for DNA and protein measurement in micro-nanoliter assays

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Abstract. Spectrophotometer, an important tool in life science, medicine, and analytical fields, usually uses an optical path of 10 mm or more for absorbance measurement of UV light. This corresponds to a sample consumption of \( \geq 50 \mu L \) in volume and a narrow measuring range of 0.5-50 ng/µL for nucleic acid samples and 0.05-2 mg/mL for protein samples. Higher concentrations must be diluted for measurement. In this paper, we developed an advanced palmtop spectrophotometer for the measurement of both DNA and protein concentrations in micro-nanoliter assays. We constructed a fiber transmission and a fiber reflection absorbance detection scheme illuminated by either UV-LED or deuterium lamp. The sensitivity of 0.5 ng/µL and a wide measuring range of 0.5-2000 ng/µL in concentrations were obtained for DNA, and the sensitivity of 0.05 mg/mL and a wide measuring range of 0.05-100 mg/mL were also obtained for protein. However, sample consumption is only 1 µL in volume for fiber transmission detection scheme and 500 nL for fiber reflection detection scheme. The linear correlation coefficient of measured concentrations to theoretical concentrations is greater than 0.99. With the profit of this work, a miniaturized spectrophotometer with better sensitivity and wider measuring range can be produced for analytical applications.

1. Introduction

Spectrometry is an instrumental analysis method that measures the intensity and wavelength of light emission, absorption, or scattering. Ultraviolet-Visible (UV-Vis) spectrophotometry, based on molecular absorption spectrum caused by electronic transition, is with a wide wavelength range from 200nm to 800nm [1]. It is commonly used in quantitative analysis of organic compounds [2-4]. In ordinary molecular biology experiments, determination of nucleic acid or protein concentration is an essential step. UV-Vis spectrophotometry is the most common, faster and more cost-effective way than other analytical methods [5-6]. Taking microarray as an example, DNA extraction is essential to probe preparation and hybridizing samples preparation. High purity DNA template is the premise of the subsequent steps, which is usually measured by UV-Vis spectrophotometry [7].

Though traditional UV-Vis spectrophotometer [8], using 10mm×10mm quartz cuvette, has high sensitivity, it has the following disadvantages. 1) Large sample consumption, specifically tens of micro-liter or even more volume is required, which loses the suitability for precious biological
samples’ detection. 2) The linear range is so narrow that high concentration samples have to be diluted and the operation becomes complicated. 3) After each measurement, washing of used cuvette is also time and labor intensive. 4) Traditional instrument, with complicated structures inside, occupies a large area of the already crowded laboratory. All of these problems make the traditional spectrophotometer unsuitable for the high-throughput detection of precious biological samples.

Facing these problems, several micro-UV-Vis spectrophotometers were developed for micro-liter biological samples. Typical commercially available products are Nanodrop ND-1000 [9], GE NanoVue [10]. They take advantage of liquid surface tension to contain samples between two surfaces instead of the traditional cuvette, which not only decreases the sample consumption, but also broadens the measurement range and simplifies the operations. However, some problems still remain unsolved, such as low precision and reproducibility, complicated instrument structure, and high cost of instrument.

Fiber-optic biosensors have attracted great attention because of their potential for sensitive measurements in biological and chemical applications. In our previous work, we have developed several opt-biosensors for detection of interactions between biological moleculars [11-13]. These opt-biosensors are capable of label-free detection of micro-nano-liter biological samples reaction; however, they cannot determine the net concentration of initial samples. The integration of three steps, namely sample preparation, chemical reaction, and results detection on a chip is the ultimate goal to develop a micro-Total-Analysis-System (μTAS) or Lab-on-a-Chip (LOC) for point-of-care diagnostics [14]. As a result, the research of fiber-optic biosensor for measuring initial sample concentration quickly, accurately, and label-freely becomes crucial.

In this paper, we report an advanced palm-top spectrophotometer for UV spectrophotometric detection of micro-nanoliter DNA and protein samples. Taking advantage of surface tension and fiber reflection method, the linear range, detection limit, and reproducibility of the system were proved to be suitable for normal analytical applications. The sample consumption is much less (500 nL minimum) compared with current commercial instruments (2 μL minimum), which is essential for precious biological samples detection. The novel system illuminated by ultraviolet light emitting diode (UV-LED) facilitates simple fabrication process and lower cost. Moreover, without the restriction of UV transparent material, the system is more suitable for integration on μTAS or LOC.

2. Materials and methods

2.1. Materials

Single-strand DNA (ssDNA) standard substances, purchased from National Institute of Metrology (Beijing, China), were Salmon Sperm DNA with concentrations of 2050, 998, 501, 100 and 11 ng/μL, respectively. TE buffer (10mM Tris-HCl, pH 8.0, 1mM EDTA, Capitalbio Corporation, Beijing) was used to dilute the standard substances to a series of 19 concentrations ranging from 2000 to 1 ng/μL.

BSA (Bovine Serum Albumin) was used as protein sample. BSA solid powder (Proliant, Ankeny, IA) of 10 g was dissolved in 1 mL PBS (Phosphate Buffered Saline, Four Ring Sunny Bioscience Co., Beijing) to obtain the highest concentration of 100 mg/mL. Then, a series of 15 samples was diluted to concentrations from 100 to 0.05 mg/mL, and the final concentrations were quantified by Nanodrop ND-1000 (Thermo Fisher Scientific, Wilmington, DE).

2.2. Apparatus

A spectrometer (USB2000+, Ocean Optics, Dunedin, FL) was used for the spectral measurement. Micro-deuterium lamp (DT-Mini-2-GS, Ocean Optics) and two UV-LEDs (central wavelength 265 and 285 nm, bandwidth 10 and 10 nm respectively, Sensor Electronic Technology, Columbia, SC), were used as the light source. Quartz fibers with center diameter 115 μm and 400 μm were also purchased from Ocean Optics. Quartz fiber reflective probes were manufactured by Xingyuan Cooperation (Beijing, China). The probe positions 6 fibers around one central fiber with center distances 220 μm and the fibers’ diameters are all 200 μm.
2.3. **Construction of the Palmtop spectrophotometer**

Figure 1 shows the fiber reflection detection scheme of the palmtop spectrophotometer, which is mainly a mechanical swing-arm structure. Liquid sample is compressed to a thin film and held still by surface tension between upper surface of the reflective probe (4) and the plane mirror (6). The thickness of the liquid film, nearly half of the optical path length, can be adjusted by screw thread micrometer (2) (GCM4002-TM, Daheng Optics and Electronics, Beijing).

UV Light is emitted from micro-deuterium lamp or UV-LED (13), transmits through incoming fiber (11) and liquid sample (5), and after reflected by plane mirror (6) transmits through the sample (5) once more. The reflective light is collected by six outgoing fibers (10) of reflective probe (4), and finally irradiates into spectrometer (12). Computer receives spectrogram by software (Spectrasuite, Ocean Optics), and the concentrations of samples were calculated by readings of the spectrogram. Magnet (7) is attracted with screw on the fixed arm (3) in order to keep the swing arm (1) and the head of screw thread micrometer (2) in close contact.

![Figure 1. Schematic diagram of the miniaturized measurement system](image)

1-swinger arm; 2-screw thread micrometer; 3-fixed arm; 4-reflective probe; 5-liquid sample; 6-plane mirror; 7-magnet; 8-pedestal; 9-shaft; 10-outgoing fiber; 11-incoming fiber; 12-micro-spectrometer; 13-UV-LED; 14-computer

Another approach of the system, the transmission detection scheme, could be easily transformed by changing connectors of optical fibers, so that the transmitting light emitted from 400 μm fiber below
will not be reflected, but received by the upper 115 μm fiber. Figure 2 is a photo of the major part of constructed reflection detection system.

2.4. Absorbance measurement
First, the clearance between the upper surface of the probe and the plane mirror was adjusted to specific distances with a clearance gauge. The deuterium lamp was warmed up. The spectrometer was connected to the computer and sampling parameters were configured. Parameters of spectrophotometer were set up as follows: integration time 0.5 s for deuterium lamp and 30 ms for UV-LED, boxcar 5, average times 3.

After adjustment, the swing arm (1) was swung up, and 0.5-1 μL solvent was added onto the center of the reflective probe (or incoming fiber in transmission scheme). By swinging down the arm to the place as shown in figure 2, a compressed liquid film (5) was formed between the upper surface of the probe (4) and the plane mirror (6), whose thickness, in relation to the measurement path length could be changed by the screw thread micrometer (2).

Background light intensity \( I_b \) was measured, after the shutter of the lamp was closed. Then, the shutter was opened and the reflective light intensity of solvent \( I_0 \) was then measured. After that, the shutter was closed again to protect the user from UV radiation. Both the probe and the mirror (or two fibers’ end in transmission scheme) were cleaned by gauze, and then sample solution was added. Subsequently the reflective light intensity of solution \( I_1 \) was measured by the same steps described above. The path lengths of solution and solvent measurement have to be maintained the same.

In experiments of transmission method, every solution was measured 3 times independently to test the system’s feasibility; while in reflection method, every measurement was repeated 15 times independently in order to get a reliable result on the system’s performance.

2.5. Detection principle and data processing
Lambert-Beer’s law states that absorbance of solution \( A \) is proportional to solute concentration and light path length through the solution\(^{[15]} \), which is written as

\[
A = abc
\]  

where \( c \) is absorption coefficient of the substance determined by the solute, \( b \) is light path length, \( c \) is substance concentration. \( A \) is solution absorbance of specific wavelength (260 nm for DNA and 280 nm for protein), which is defined as

\[
A = \log \frac{I_0 - I_b}{I_1 - I_b}
\]

where \( I_0 \) is the transmitted light intensity of solvent; \( I_1 \) is the transmitted light intensity of solution; \( I_b \) is the background intensity, including stray light and dark noise of detector.

When light intensities \( I_0, I_1, I_b \) are measured, the concentration \( c \) can be calculated using the following derived formula

\[
c = \left( \log \frac{I_0 - I_b}{I_1 - I_b} \right) / (eb)
\]

where absorption coefficient \( e \) is 0.03 μL/(ng·cm) for ssDNA and 0.67 mL/(mg·cm) for BSA.

3. Result and discussion
3.1. Protein measurement using deuterium lamp-fiber transmission method
First, BSA samples were measured using fiber transmission method with a deuterium lamp. The path length was set to 0.5 mm, and the results are shown in figure 3, where the experimental results are plotted against measurements of commercial instruments. As the fitted line approaches a slope of 1, the measurements more closely match the true concentrations.

The reproducibility is satisfied with standard deviation between measurements lower than ±0.02 mg/mL (in range of 0.2 – 10 mg/mL) and ±1.5% (in range of 10 – 80 mg/mL). High linearity is kept in
the range of 1-50 mg/mL, while large negative deviation in high concentration measurement (> 50 mg/mL) was observed. That is because 0.5 mm path length is too large for high concentration detection, in which the intensity of passing light is too low for CCD (charge-coupled devices) to detect.

According to the Lambert-Beer’s law (1) and CCD’s normal detection range (absorbance is between 0.03-3), 0.2 mm path length can be used to measure protein solutions in range of 2.24-223.88 mg/mL. That represents the main range of normal protein concentrations, hence 0.2mm is theoretically the optimized path length.

3.2. Protein measurement using LED-fiber transmission method
In order to fulfill the portability and low cost of a palmtop system, LEDs were tested as light source. As shown in figure 4, standard deviations are lower than ±0.07 mg/mL (in range of 0.2 – 10 mg/mL) and ±0.6% (in range of 10 – 80 mg/mL), which satisfy the basic requirement of spectrophotometry [9, 10]. Compared with former experiment illuminated by deuterium lamp, due to the usage of shorter path length (0.2 mm), the reproducibility was improved in high concentration range. Moreover, the large negative deviation at high concentrations was also smaller using 0.2 mm path length, which agrees with the theoretical prediction of 0.2 mm as the optimal length.

UV-LED was proved to be suitable for absorbance detection in miniaturized system. Because light intensity of UV-LED on 280 nm is much higher than that of deuterium lamp, the integration time of detection was set to 30 ms, less than one tenth of that used for deuterium lamp experiment (500 ms). This not only results in a faster testing time, but also may lead to lower detection limit and higher measurement precision.
3.3. Protein measurement results using LED-fiber reflection method

In contrast with the transmission absorbance detection method discussed above, micro sample was illuminated by one fiber in the center, and the reflective light was received by 6 surrounding fibers. 1 mm and 0.2 mm path lengths were both evaluated.

Because of the large concentration range of samples, results of whole measuring range are shown in figure 5(a). In supplement, the commonly used low concentration range 0.05-4 mg/mL is also shown in figure 5(b). High linearity is kept in the whole range (0.05-100 mg/mL) using 0.2mm and 1mm path lengths with correlation coefficient of 0.9995. Standard deviations are lower than ±0.15 mg/mL (in range of 0.05 – 10 mg/mL) and ±4% (in range of 10 – 100 mg/mL), and the detection limit is 0.05 mg/mL.

Positive deviation at high concentration was observed in figure 5. The reason may lie in the fact that the reflective light received by 6 outgoing fibers travels larger path length than that of calculation. According to formula (3), when smaller path length \( b \) is used in calculation, calculated concentration will reveal a positive deviation.

![Figure 5. Protein measurement results using LED-fiber reflection method](image)
(a) Whole measuring range: 0.05-100 mg/mL; (b) Low concentration range: 0.05-4 mg/mL

3.4. Nucleic acid measurement results using LED-fiber reflection method

Similar to protein detection, nucleic acid measurement can also be achieved using the same system. In figure 6(a) and 6(b), the system reveals high reproducibility, high sensitivity, and wide detection range in single-strand DNA standard substances tests. The linear range is 0.5 – 2000 ng/µL with correlation coefficient of 0.9992, and standard deviations are lower than ±2.10 ng/µL (in range of 2 – 100 ng/µL) and ±3% (in range of 100 – 2000 ng/µL). In addition, when measuring the sample of 0.5 ng/µL, the concentration measurement is 0.56±0.12 ng/µL, which means the sensitivity reaches 0.5 ng/µL, better than that of commercial instrument.

Positive deviation in high concentration was also observed in DNA measurement. The major reason is the same, namely the path length error in calculation. The concentration error caused by path length error can be derived as follows basically from formula (1).

\[
c_{\text{real}} = \frac{A}{\varepsilon \cdot b} \quad (4)
\]

When there is a path length error during measurement, the concentration can be stated as

\[
c_{\text{meas}} = c_{\text{real}} + \Delta c = \frac{A}{\varepsilon \cdot (b+\Delta b)} \quad (5)
\]

where \( c_{\text{real}} \), \( c_{\text{cal}} \), \( \Delta c \) represent solution’s real concentration, measured concentration and concentration deviation respectively.

Use formula (5) minus (4), then \( \Delta c \) is derived:
Because $\Delta b$ and $b$ are constant during measurement, it is obvious that the positive deviation caused by path length error is proportional to calculated solution concentration.

In experiments, the linear coefficient of concentration deviation to measured concentration of 0.2 mm and 1 mm path length are 0.99 and 0.91 respectively, so the deviation value can be linearly fitted and corrected. The corrected results after using this linear model arithmetic, shown as red line in figure 6(a), are perfectly matched to the theoretical value (maximum $\pm$ 2.8 ng/µL deviation in range of 0.5 – 100 ng/µL, $\pm$ 5% deviation in range of 100 – 2000 ng/µL).

3.5. **Comparison between novel palmtop system and traditional systems**

The novel measurement system is designed to maintain the advantages of micro-spectrophotometer, known as wide test linear range and simple operation. Nevertheless, it offers a different way from traditional systems for spectrophotometric detection. In fiber-reflective system, measuring path length is more than twice as the distance between two solid surfaces, which means that small volume liquid sample is compressed but not stretched to liquid-column so as to reach the path length. Less liquid sample is required for detection (500 nL minimum), which is crucial for precious biological samples detection, and liquid surface tension will not result in non-uniform solute distribution anymore, which facilitates more precise absorbance measurement.

High coaxiality of incoming and outgoing fibers is required in a transmitted measurement system. However, in a reflective system, there is no coaxiality requirement. In addition, calculation by Lambert-Beer’s law is based on the portion of relative intensity of solution and solvent, so that perpendicularity between plane mirror and incoming light is also not so strict. As a result, the structure to maintain liquid thin film is simplified, which leads to looser requirement for manufacturing precision and lower cost of the instrument.

In addition, fiber reflection scheme excludes the restriction of UV transparent substrate material. Commonly used materials for biochip fabrication, i.e. silicon, gold, aluminum, are all not transparent to UV light, but they are able to reflect UV light. Without the requirement of UV transparency, the reflective system can also work with these materials, so that it has the potential of on-chip absorbance measurements integration in Micro Total Analysis System.

4. **Conclusion**

We developed an advanced palmtop spectrophotometer for both nucleic acid and protein measurement in micro-nanoliter assays. A fiber transmission absorbance detection scheme and a fiber reflection scheme illuminated either by UV-LED or by deuterium lamp were both constructed. With the optimized detection path lengths, the sensitivity of 0.5 ng/µL and a wide measuring range of 0.5-2000 ng/µL.
ng/µL were obtained for nucleic acid sample, and the sensitivity of 0.05 mg/mL and a wide measuring range of 0.05-100 mg/mL were also obtained for protein sample. With the profit of this work, a miniaturized spectrophotometer with high reproducibility, sensitivity, and wide measuring range can be produced for analytical applications.

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