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UV-Vis Spectrophotometric Analysis of DNA Retrieval for DNA Storage Applications

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Abstract: Informational Deoxyribonucleic Acid (iDNA) has gained the attention of many researchers and pioneer companies for the development of novel storage systems for the long-term and high-density storing of information. This research focuses on the physical storage of iDNA strands to address some of the current challenges by evaluating the accuracy of the process of iDNA retrieval from the surface after the dehydration process. For this aim, a UV-Vis spectrophotometric technique was used to measure the concentration of the DNA samples. Although spectroscopy has been widely employed for the evaluation of DNA concentration and contamination in a solution, it has not been used to investigate dry-state DNA, which is one of the preferred storage formats for the long-term retention of information. These results demonstrate that the UV-Vis spectrophotometric technique can be used to accurately measure dry-state DNA before the retrieval and its residues after the DNA retrieval process. This paper further examines the storage/retrieval process by investigating the relationship between the storage time and the amount of retrieved DNA or the DNA residue left on various surfaces. Based on the experimental results demonstrated and discussed in this paper, UV-Vis spectrophotometry can be used for monitoring dry-state DNA with a high accuracy larger than 98%. Moreover, these results reveal that the hydrophilicity and hydrophobicity of the surface do not significantly affect DNA retrieval over a one-month time period.

Keywords: DNA storage; UV-Vis spectrophotometer; DNA retrieval

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1. Introduction

With the rapid growth of the amount of digital data, a huge global demand has emerged for data storage that has motivated cutting-edge research to achieve new technologies for extremely high-capacity memories. The Internet Data Corporation (IDC) has predicted that worldwide data storage will grow up to 175 zettabytes by 2025 [1]. Incumbent storage technologies such as magnetic tape, hard disk drives, and solid-state drives will not be able to meet this need at the costs that we are currently accustomed to. Moreover, the data stored in these media are sometimes unrecoverable in the long term due to obsolescence and corruption [2]. Additionally, the current data storage devices have short life spans lower than 7 years [3]. Therefore, there is an urgent need to find alternative storage media. Novel strategies, particularly DNA data storage technologies, have recently been proposed for encoding and storing data in new physical media at the molecular or atomic level [4]. DNA molecules have shown the potential of storing the encoded data in both wet and dry forms and their strands represent promising media that could preserve data due to their longevity and immense storage density [5–8].

An astonishingly small amount of DNA can be used to store a significant amount of data (215 petabytes in a gram [9]) for thousands of years. Major technology companies such as Microsoft, Apple, Google, Amazon, and Facebook have paid much attention to DNA memory as a promising technology due to the superiority of DNA for storing digital data and are now making their initial investments into the commercialization of this cutting-edge technology. For example, Microsoft is developing a proto-commercial DNA-based storage device with the intended size of a commercial Xerox copier for pilot utilization in one of its data centers [10].

The workflow of the DNA storage system, as depicted in Figure 1, includes encoding the binary digital file to the sequence of four DNA bases of Adenine (A), Guanine (G), Cytosine (C), and Thymine (T), synthesizing the related information DNA (iDNA), and organizing and storing the products using a reliable physical storage system with the capability of random access to the desired sequences [7]. In order to read the information, the stored DNA oligonucleotides are sequenced using a sequencer and this is followed by an eventual decoding to obtain the initial binary codes. The synthesis [11] and sequencing processes act in a similar way to writing and reading tasks in conventional digital data memories.

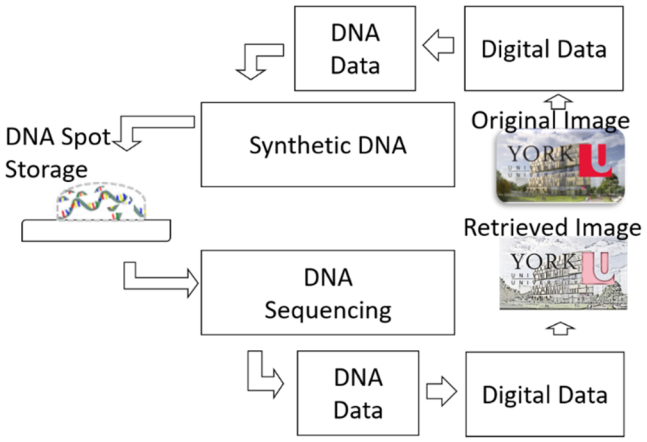


Figure 1. Illustration of the DNA storage process.

Many challenges have limited the applicability of DNA storage systems [12]. These challenges include costly synthesis and sequencing steps, a long turnaround time, a high rate of errors, and the absence of efficient storage systems for the long-term preservation and storage of DNA [13]. Among these challenges, this paper considers the physical storage of a small amount of DNA material. The improper physical storage of the DNA may result in DNA loss as well as an increased risk of contamination in the device which can consequently lead to a loss of information [10].

One of the notable solutions to address this challenge was reported in the research by Newman et al. [14] where a dehydration/hydration process was employed for DNA immobilization/mobilization. An illustration of this idea is presented in Figure 2a–f. A micropipette may be used to drop iDNA on an active digital microfluidics (DMF) surface which allows the droplet to move toward a target position using the dielectrophoresis method [15] (Figure 2a,b). The dehydration of DNA samples results in the deposition of iDNA on the DMF system (Figure 2c). Another droplet of water can be added (Figure 2d) to hydrate and retrieve the DNA sample (Figure 2e). The DNA residual (Figure 2f) can be considered as contamination that can potentially damage the iDNA. This paper further investigates this challenge using UV-Vis spectrophotometry to study DNA retrieval. A UV-Vis spectrophotometer is routinely used for measuring the concentration of DNA samples in wet mode. However, the advantages of this technique for the assessment of dry-state DNA have not been reported yet, and this paper, for the first time, demonstrates, discusses, and compares the analysis of DNA in the dry and wet modes for DNA storage applications.

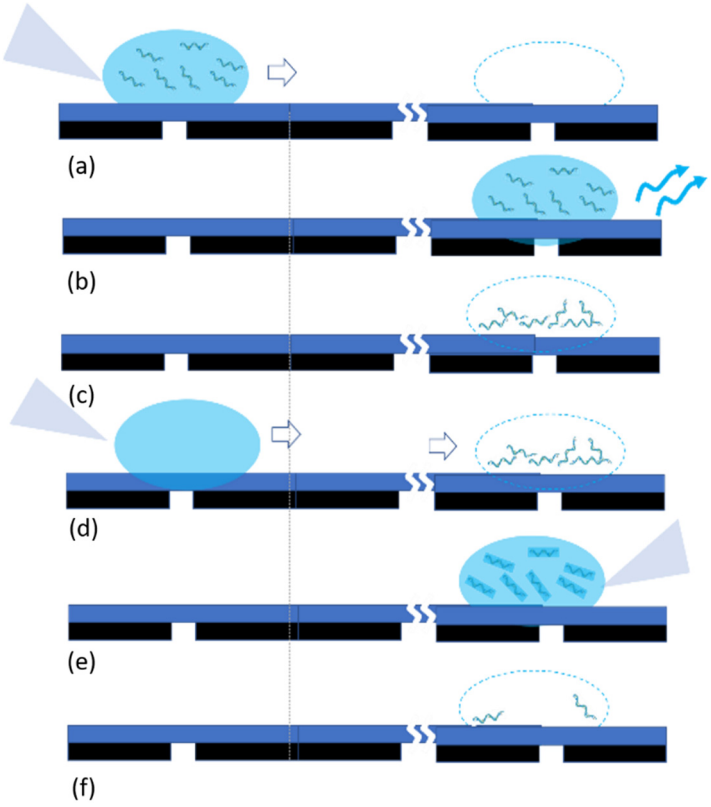


Figure 2. The principle of DMF-based DNA storage includes: (**a**) Adding a DNA droplet on DMF; (**b**) Moving to the target position; (**c**) Dehydration of the DNA droplet; (**d**) Putting a water droplet on DMF; (**e**) Taking the retrieved DNA; The residue on the DMF is the (**f**) non-retrieved DNA.

UV-Vis spectrophotometers have been reported to estimate the concentration of DNA in liquid form [16] with the help of the Beer-Lambert law calculation [17]. Researchers have reported the application of UV-Vis spectrophotometry to biological species in dry form and there are also very few reports about impure DNA in dry form. For example, N. Rani et al. [18] detected a methanolic extract in dried meizotropis pellita and N. Jovanovic et al. [19] detected myoglobin in a dried supercritical fluid with the help of a UV-Vis spectrophotometer. In another effort, H. Weigmann et al. [20] reported an almost linear relationship between the mass of dry corneocytes on a stripping tape and the obtained absorbance peak height at 430 nm of the UV-Vis spectroscopy tests. Similarly, a linear variation in the 260 nm absorbance peak has also been seen with the increase in the number of poly-L-lysine mixed DNA layers [21]. Another UV-Vis spectrophotometry of DNA mixed in a phosphate buffer solution has been reported in [22]. However, the spectrophotometry of dry DNA in pure form is of interest in many applications. Herein, we apply UV spectroscopy to investigate the absorption spectrum and to develop a relationship between the expected 260 nm peak value and the amount of pure DNA in dry form.

In the remainder of this paper, the materials and methods are described in Section 2. The experimental results are demonstrated and discussed in Section 3 followed by discussions in Section 4. Our conclusions are drawn in the final section.

2. Materials and Methods

2.1. Sample Preparation and Analysis

2.1.1. DNA Sample Preparation

The single-stranded DNA (ssDNA) used in this work was purchased from the Sigma-Aldrich company (cat# W4502) which had a concentration of 10.0 ± 1.0 mg/mL in ultrapure (DNase-free, RNase-free, deionized, distilled) water and was stored at -20 °C. The ssDNA

solution with a concentration of 10 mg/mL was diluted with ultrapure water and achieved a primary concentration of 387.63 ng/μL. The microcentrifuge tubes including the diluted sample were placed in the refrigerator at −80 °C for 2 days before the experiments. Afterward, the desired dilution achieved concentrations of 193.7, 193, 129, 106.23, 97, 77.5, 48, 24, 12, 6, and 3 ng/μL were achieved by adding ultrapure water to the primary ssDNA dilution. After dehydration, 7.5 μL of nuclease-free water which is equal to the volume of the deposited DNA sample, was dropped on the DNA spot. Ideally, it was expected to achieve the same DNA sample concentration. To evaluate the accuracy of the process, the retrieved samples were taken to the NanoDrop analyzer and UV-Vis spectrophotometer for further concentration analysis in the wet and dry mode, respectively.

2.1.2. NanoDrop Analysis

A standard UV-Vis spectrophotometer called NanoDrop was used for measuring the concentration and purity of the sample. This optical spectrometer can accurately read the optical signals with a wavelength (λ) between 190 nm and 840 nm. Therefore, it is suitable for the assessment of ssDNA samples used in this work with a wavelength of about λ = 260 nm. In this work, the ultrapure water was initially used to measure the baseline of the concentration, and then the concentration of the DNA samples was measured before the drying procedure using a Thermo Scientific NanoDrop 2000/2000c Spectrophotometer. Then, after the retrieval of the dry DNA spots, the recovered DNA samples from various substrates were evaluated for concentration measurement.

2.2. UV-Vis Spectrophotometer Setup

A UV-Vis spectrophotometer consists of a light source, a sensor, and a sample holder of quartz material. The source emits a monochromated light beam that passes through the sample's substrate, and the sensor or detector detects the transmitted light from the sample. While passing through the sample beam's light photons of specific wavelengths and weakened light beam, a piece of software plots the absorbance spectrum across a range of defined wavelengths. Therefore, the absorbed light of the 260 nm wavelength absorption spectrum of DNA in the 220 to 320 nm wavelength exhibits a peak at 260 nm wavelength. The height of the absorbance value of this peak at 260 nm, with respect to the absorbance value at 320 nm, can be attributed to the DNA amount.

In order to obtain an excellent absorbance response of the DNA solution, the use of a container or cuvette with transparent walls of material such as quartz, which has excellent transmittance, is inevitable. Therefore, a quartz cuvette and a quartz substrate were used to investigate the DNA concentration in the wet mode. However, a flat surface of quartz material was used to investigate the amount of DNA in the dry mode.

Each UV spectrophotometric test was performed between starting and ending wavelength values of 220 nm and 320 nm, respectively, separated by a large number of 0.6 nm wavelength steps. Each step value was averaged and recorded 1000 absorbance values in an integration time interval of 10 ms. Moreover, the absorbance peak height at 260 nm followed the subtraction of the peak absorbance value from the absorbance value at the 320 nm wavelength of the spectrum.

Every spectrophotometric test was followed by a thorough cleaning of the flat quartz substrate with acetone before it was dried. In order to obtain the light absorption of the dry DNA material, a 2 μL droplet of DNA solution of a known specific concentration was deposited and dried on this clean quartz substrate. The drying process followed 8 min of heating under a temperature of 30 °C. Afterward, the quartz substrate with the dried DNA spot was placed in the spectrophotometer at the proper position and tested.

Every dried DNA sample was retrieved, and the remnant DNA was again UV-tested after retrieval in the dry form. For a successful retrieval process by mixing DNA with water, a 2 μL deionized water drop was pipetted four times at the trace of the dry-state DNA on a quartz substrate before the final retrieval. The remnant on the substrate was dried again for

1 to 2 min at a temperature of 30 °C before being tested. The second retrieval also followed the same procedure as the first.

A total of 2 µL droplets of four different DNA concentrations of 387.5 ng/µL, 193.7 ng/µL, 129 ng/µL, and 77.5 ng/µL were investigated after being completely dried, and five droplets of each concentration were dried and UV tested.

Similarly, different concentrations of DNA solutions were also investigated. For this purpose, a quartz cuvette of 2 mm thickness was filled with the DNA solution and UV-tested. As a result, DNA concentrations of 387 ng/µL, 193 ng/µL, 97 ng/µL, 48 ng/µL, 24 ng/µL, 12 ng/µL, 6 ng/µL, and 3 ng/µL were investigated in liquid form.

3. Results

3.1. Wet-DNA Retrieval Assessment

Each experiment was dedicated to a specific surface (e.g., Teflon, glass, etc.) and consisted of sample preparation, DNA deposition using a micropipette, dehydration, water deposition, and hydration as well as sample retrieval and measurement. The measurements were performed using NanoDrop spectrophotometry.

3.1.1. The Effect of Time on the Retrieved DNA from Teflon

Figure 3a shows the measurement results of 36 samples of 1 µL of rederived DNA about 30 min after dehydration. In other words, for each spot, the experiments were repeated 36 times and compared, as shown in this figure. Similarly, Figure 2b–d show the measurements when the storage time (ST) was 72, 96, and 192 h using a Teflon substrate. It is noteworthy that the storage time is defined as the time duration between the dehydration step and the DNA retrieval step. As seen in these results in Figure 3, the concentration of the retrieved DNA varied from 80 to 110 ng/µL. There were several sources of errors (see Section 4.1.1) that were unavoidable and affected the measurement results. This is why a large variation can be observed in these results. Despite this, a statistical analysis of these results and their comparison with the results achieved using other types of surfaces provide us with useful and conclusive information as discussed in this section.

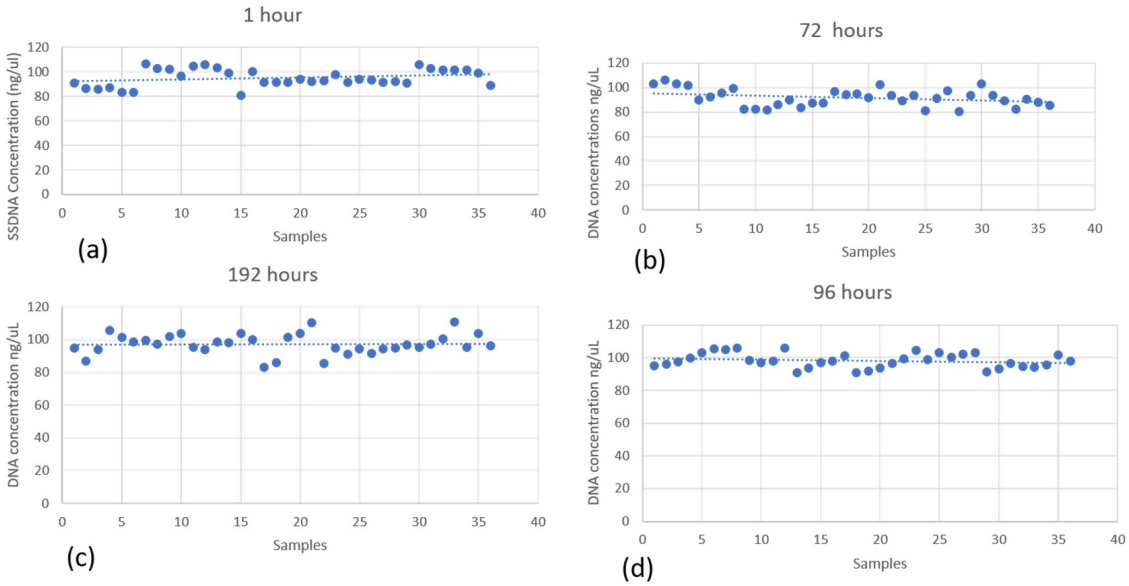


Figure 3. DNA retrieval from the surface of Teflon after (**a**) 1 h, (**b**) 72 h, (**c**) 96 h, and (**d**) 193 h.

3.1.2. Retrieved DNA from Various Surfaces after Different Times

The retrieved DNA from the surfaces of glass, silicon, polystyrene, and PDMS were measured and the results of 36 samples in each one of four different storage times associated with four different substrates (~576 measured data) are shown in Figure 4a–d. The

distribution of measurements in glass and silicon coated with native silicon oxide layer was higher than the ones in PDMS and polystyrene. However, the storage time over eight days did not significantly affect the DNA retrieval from any of the surfaces. These results will be discussed in Section 4.

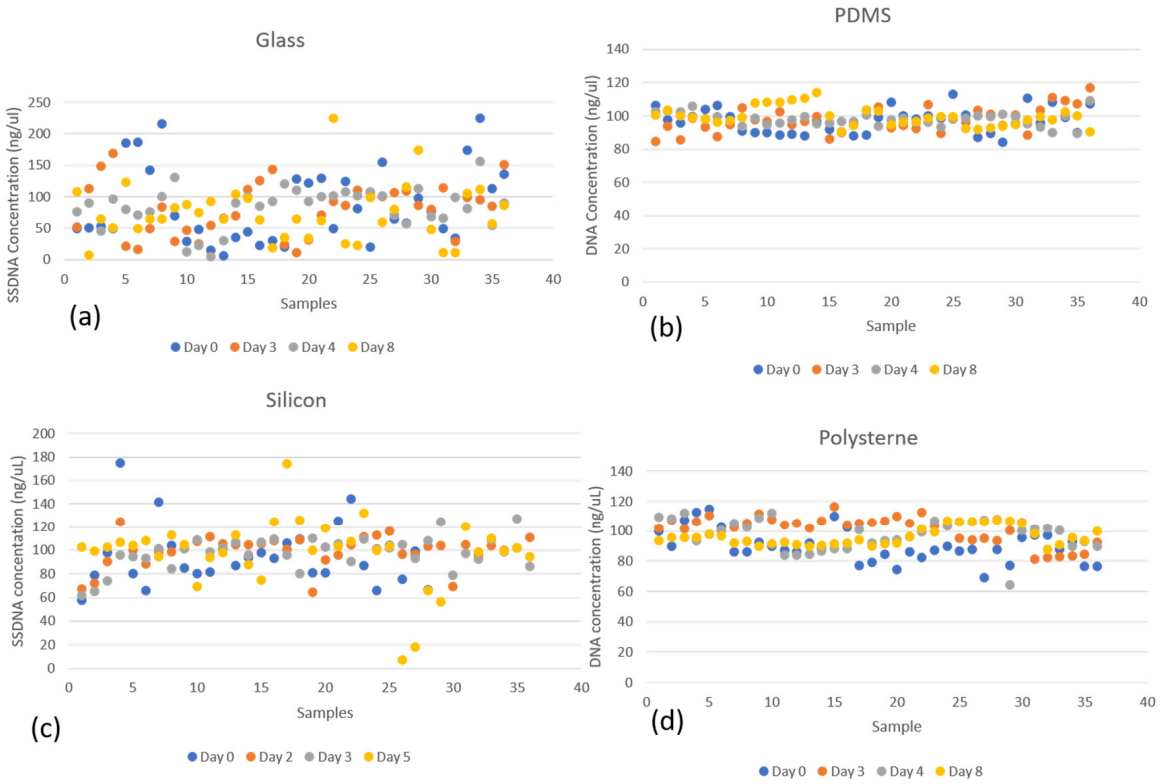


Figure 4. The effect of hydrophilicity and hydrophobicity of surfaces at different times: (**a**) Glass; (**b**) PDMS; (**c**) Silicon; (**d**) Polystyrene.

The mean values of the measured results in Figure 4a–d can be seen in Figure 5. In this figure, the standard errors associated with measurement results can also be seen. Based on these results, the DNA retrieval rate from all surfaces over eight days of storage did not show significant change. Moreover, one can argue that the type of surface did not significantly affect the variation of DNA retrieval rate from short to long storage time. However, the dispersion of the measured data on hydrophilic surfaces such as glass (see Figures 3 and 4a) was significantly higher than the dispersion on the Teflon surface, as seen in Figures 4a and 5.

3.1.3. Pipetting Processes

The sample preparation and measurement methods are very time-sensitive so that any delay in retrieving DNA may result in the evaporation of water and can thus lead to a change in DNA concentration. Figure 6 compares the measurement results of DNA retrieval from the surface of Teflon using two different methods. In the first method, nine droplets of water were introduced to nine DNA spots, and then, one by one, the DNA retrieval and measurement were performed. In the second method, the droplet of water was added to each spot after the DNA measurement of the previous spot was finished. Indeed, the first method allows more evaporation of droplets and consequently, the measured concentration becomes higher. As seen in Figure 6, the results in the second method were very consistent with fewer errors.

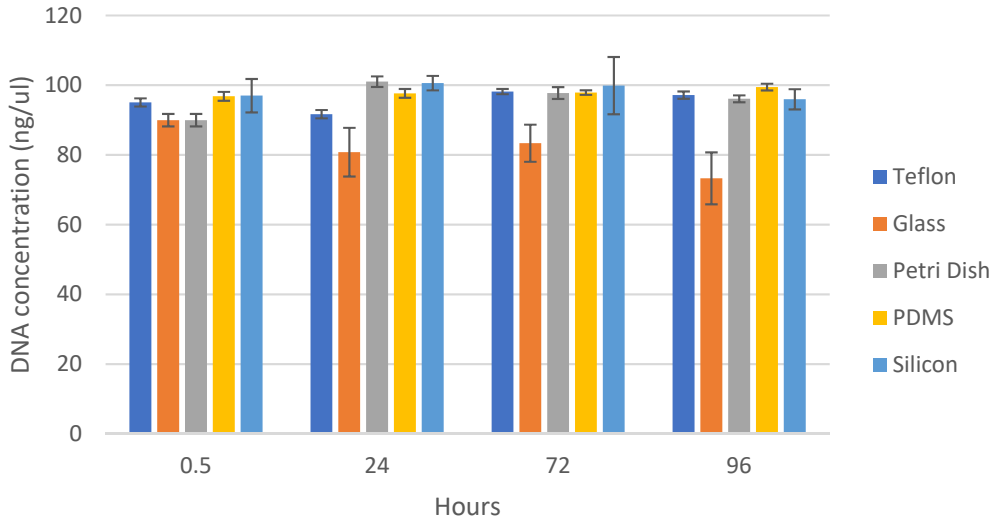


Figure 5. The mean values of the retrieved DNA from various surfaces at different times.

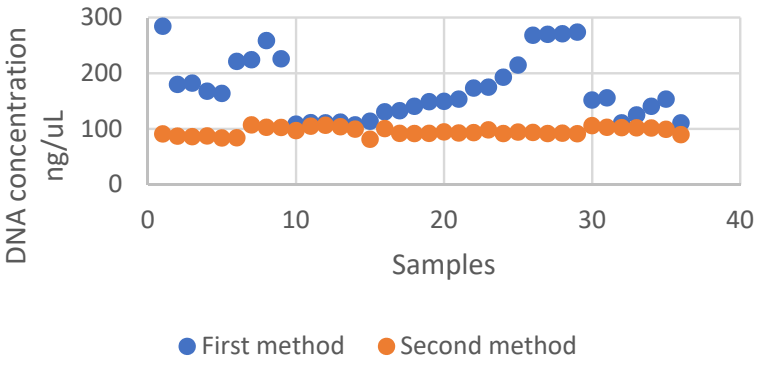


Figure 6. Comparison of the measurement results using the first and second methods of introducing water droplets.

3.2. Dry DNA UV Spectrophotometry

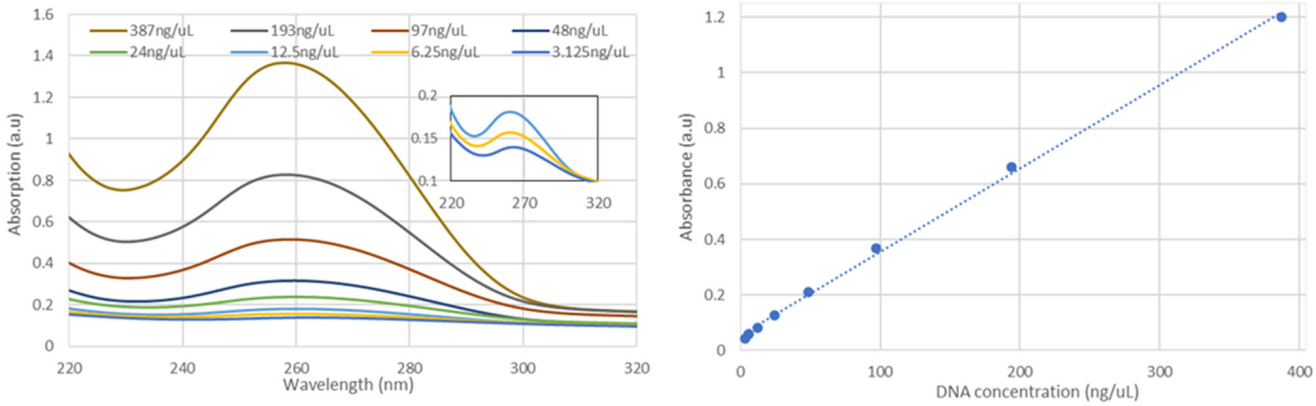
3.2.1. Liquid DNA in a Cuvette

Figure 7a shows the UV spectrophotometric results of different concentrations in a quartz cuvette in liquid mode. All spectra exhibited broad peaks at 260 nm, which is characteristic of light absorption in the DNA material present in the solution. Thus, a decrease in the DNA concentration led to a drop in this absorbance peak. Figure 7b shows the 260 nm absorbance peaks against their concentrations following a linear behavior which has also been seen before [23].

3.2.2. Dry DNA

A 2 µL droplet of liquid DNA placed on the quartz substrate under a room environment was found in a completely dry mode after 12 min and formed a spot. Figure 8a shows the absorption spectra of the spotted DNA obtained after time intervals of 3 min during the drying process. The absorption spectrum of the completely dried DNA spot after 12 min, compared with the other spectra in Figure 8a, was similar to DNA in water (Figure 8a), which exhibits a characteristic peak at 260 nm. In another experiment, the absorption spectrum taken even after a wait time of 11 days showed this characteristic peak. The irregular 260 nm peak of the absorption wet-mode DNA spectra in Figure 8a before being dried can be attributed to the physical nature of evaporation and the unstable physical characteristics of the droplet and the DNA strand during the 13 min time. When evaporation is taking place, the droplet size, the position of the unbonded-DNA strands in the solution, and subsequently their absorption characteristics change until the droplet

is completely evaporated. The DNA strands attach to the surface and remain in a steady state from physical and biochemical perspectives. Hence, the variation in the absorption wet- and dry-mode DNA spectra could be attributed to the nature of the drying process, not the DNA-surface bonding. In the dry mode, these DNA molecules come to rest, form a spot of around 2 mm diameter, and exhibit a characteristic UV spectrum of DNA. This spectrophotometric response is the outcome of DNA molecules contained in 775 ng amount of mass because the concentration of deposited 2 µL liquid DNA droplet was 387.5 ng/µL. This spectrophotometric response of the dry-state DNA highlights the significance of the method. To better understand this, three more dry-state DNA amounts of 387.5 ng, 258 ng, and 155 ng as well as their five repeats were UV tested. The corresponding absorption spectra are shown in Figure 8b.



(**a**) (**b**)

Figure 7. (**a**) UV absorption of different concentrations of DNA in liquid form; (**b**) Peak absorbance values obtained for these concentrations.

All spectra showed the same DNA characteristic curves with a broad absorbance peak at 260 nm and a decrease in peak height value with the decrease in the amount of dry-state DNA. There was a slight change in the peak heights of the five absorption curves obtained for the same amounts of dry-state DNA, which is attributable to the experimental conditions. However, the average of these five peak absorbance values depicted a linear change with the change in the amount of dry-state DNA and therefore a straight-line plot was obtained (Figure 8c). This direct correlation between the amount of dry-state DNA and the peak absorbance values was similar to the liquid DNA peak absorbance response to different concentrations (Figure 7b). Similar to the liquid mode, the amount of unknown dry-state DNA can be estimated with the help of this straight-line plot. Figure 8d–h demonstrate the different iterations of 775ng dry DNA after the first and second retrievals.

3.2.3. DNA Retrieval

Figure 9a–d shows the spectrophotometry results of the five repeats obtained after the first and second retrievals of four DNA amounts of 775 ng, 387.5 ng, 258 ng, and 155 ng dried at the surface of the quartz substrate, respectively. All the curves of 775 ng and 387 ng of DNA exhibited very broad peaks at 260 nm, whereas the curves for 258 ng and 155 ng were less broad. More profound peaks can be seen in the second retrieval curve for 155 ng.

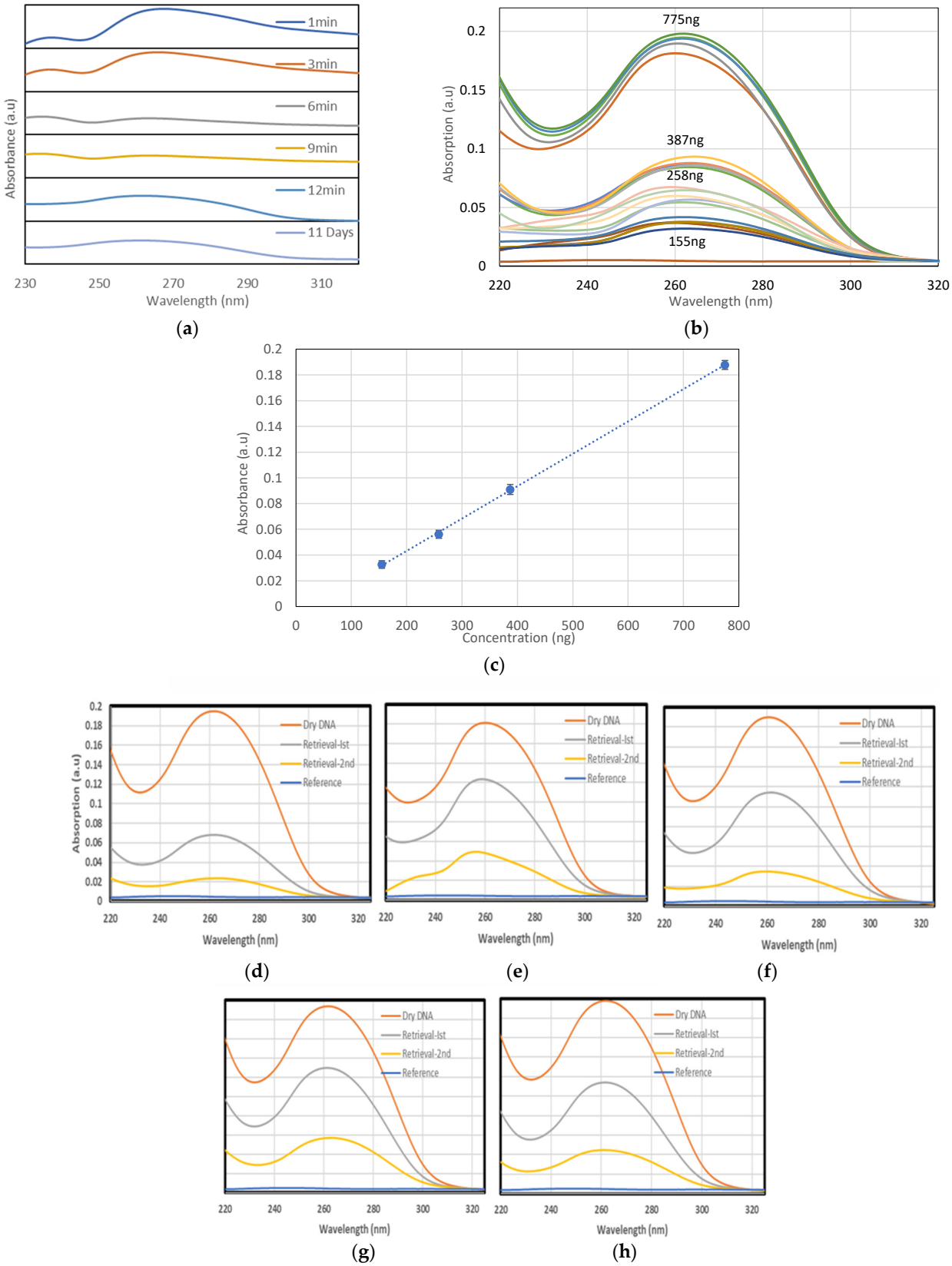


Figure 8. (**a**) Absorption spectra at different timings of the 2 µL droplet during the drying process at room temperature; (**b**) UV absorption of different amounts of dry-state DNA; (**c**) Peak absorbance values obtained for these concentrations; (**d**–**h**) Absorption spectra after drying, first and second retrieval of 775 ng DNA (**a**) first iteration, (**b**) second iteration, (**c**) third iteration (**d**) fourth iteration, and (**e**) fifth iteration.

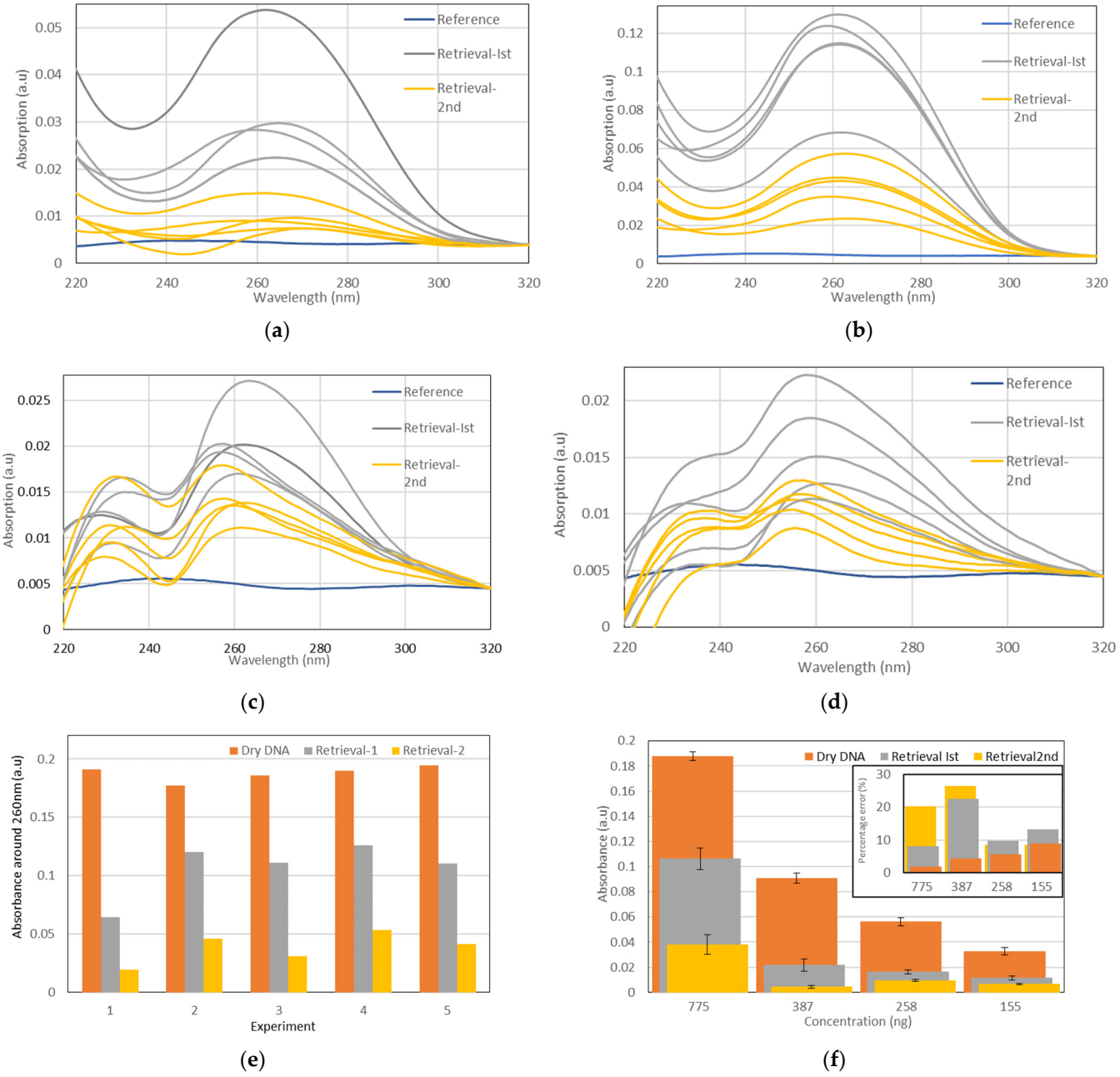


Figure 9. Absorption spectra of dry-state DNA after the first and second retrieval process for (**a**) 775 ng, (**b**) 387 ng, (**c**) 258 ng, and (**d**) 155 ng; (**e**) Obtained peak absorbance values of five 775 ng dry-state DNA; (**f**) Obtained average peak absorbance values of 775 ng, 387 ng, 258 ng, and 155 ng spectra, and their averages after the first and second retrieval processes with (inset) their corresponding percentage errors. Each value is an average of 5 peak absorbances.

Figure 8b shows the absorption spectra of all dry-state DNA samples and their five repeats before the retrieval process and Figure 9a–d show them after the retrieval processes. The peak absorbance values, both before and after the retrieval of the 775 ng samples, are shown in Figure 9e. There was a slight change in the peak values before retrieval compared to those after retrieval. Therefore, the calculated error in the peak values was below 2% in the spectra of five dry-state DNA samples of 775 ng mass obtained before the retrieval. Figure 9f shows a plot of the average peak absorbance values calculated for all dry-state DNA masses before and after the retrievals, and the related errors are shown in the inset of Figure 9f. A 2% error of the peak values of 775 ng spectra increased to 4%, 6%, and 8% for 387.5 ng, 258 ng, and 155 ng, respectively. A steady drop in the average peak absorbance

values is evident in the first and second spectra of 775 ng DNA. This trend can also be seen in Figures 8b and 9a for 775 ng. However, all other average peak values exhibited a large drop in the first-retrieval values compared with the second-retrieval values. Moreover, the inset of Figure 9f shows a very high percentage error of nearly 25% in the first and second retrieval peak absorbances of five 387 ng dry-state DNA. Surprisingly, this was not the case with the peak absorbances of 258 ng and 155 ng dry-state DNA. Percentage errors in the first and second-retrieval peak absorbance values of the 155 ng DNA were close to 10%, which can be seen in the inset of Figure 9f. The corresponding spectra can be seen in Figure 9a–d.

A significant decrease in the absorbances can be seen in the first retrieval of a low amount of DNA. Therefore, compared to 155 ng dry-state DNA, average decreases of 70% and 80% in the peak absorbances after the first and the second retrievals, respectively, are noticeable.

4. Discussions

4.1. DNA Retrieval Assessment Using NanoDrop

In this subsection, before the discussion of the DNA retrieval results shown in Figures 3–6, it is crucial to discuss the sources of errors in Section 4.1.1. A brief discussion of the DNA retrieval is made in Section 4.1.2.

4.1.1. Source of Errors

The measurement results demonstrated in Figures 3–5 suffered from systematic and human errors. The main sources of errors were as follows.

1. Hydrophilicity of surface

A droplet on a hydrophilic surface has a small contact angle. Therefore, during the sample retrieval, the tip of the micropipette should be placed closer to the surface in order to suck the sample (see Figure 10a,b). Consequently, this increases the risk of contacting the surface and causing an error in the volume of the taken solution from the surface.

It is noteworthy that the contact angles associated with the droplets on polystyrene, PDMS, and Teflon were higher than 90°. This high hydrophobicity feature of PDMS and Teflon is because of a bulky non-polar methyl group and $(C_2F_4)_n$, respectively. Additionally, polystyrene only contains carbon-hydrogen (CH) and carbon-carbon (CC) bonds. These covalent bonds do not have an unequal sharing of electrons. Therefore, polystyrene is nonpolar and consequently, it is a hydrophobic material [24]. On the other hand, glass and silicon with a top native silicon oxide layer have hydrophilicity properties because of a polar covalent structure of SiO_2.

2. Non-Homogeneity

As seen in Figure 10c–d, the non-homogeneity of the DNA sample is another source of error. It was assumed that the droplet of water was deposited on the DNA spot. Therefore, the DNA sample was removed from the surface and retrieved into the droplet. Thus, naturally, the DNA sample was not homogenous, as depicted in Figure 10c with two samples, r1 and r2, that have different concentrations.

This challenge was addressed by using a pipetting method to mix the sample and make it more homogenous before the measurement, as discussed in Section 3.1.3 and demonstrated in Figure 10d with two samples, r'1 and r'2, with different concentrations.

The non-homogeneity may be due to the affinity of charged DNA molecules to the surface, as seen in Figure 10e. The sample retrieval from the top of a small droplet above the surface may have included a lower number of DNA fragments due to the settling of DNA on the substrate. Another cause of non-homogeneity is the evaporation of droplets when the sample retrieval becomes lengthy. It is noteworthy that, in the retrieval process, a droplet of 1 µL was added to the surface DNA spots. This allowed a sample of 1 µL to be taken for the NanoDrop. These steps were repeated about four times. Each NanoDrop test

requires 1 min. Therefore, in total, the small sample was exposed to the air for above 4 min that may have resulted in partial evaporation.

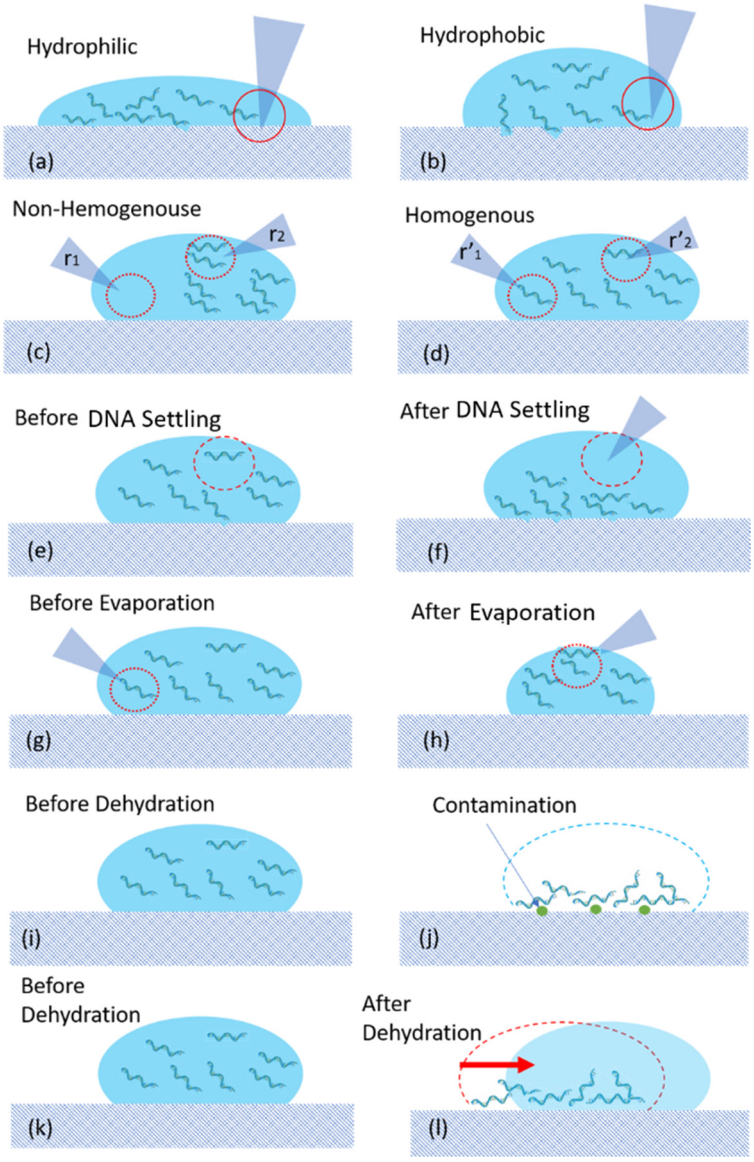


Figure 10. The source of non-idealities in the DNA sample deposition and sample retrieval, (**a**,**b**) Hydrophilicity effect; (**c**,**d**) Non-homogeneity; (**e**,**f**) Deposition of DNA to surface; (**g**,**h**) Evaporation; (**i**,**j**) Contamination; (**k**,**l**) Misalignment.

3. Contamination

The DNA sample might have been contaminated by non-DNA samples such as dust and other chemicals on the surface of substrates. To decrease the risk of contamination, all steps of sample preparation were performed in the biosafety cabinet, and all substrates were placed on the racks covered with lids. The latter prevents the substrate from direct contact with dust or other contaminations. Indeed, the contamination can potentially show an error in the measured concentrations. Despite the above-mentioned techniques, the contamination cannot be canceled or further reduced due to the nature of the experiments. Some of the most likely causes of possible contamination in these experiments are as follows:

- Dehydrated DNA spots are exposed to air containing dust. During the preparation of DNA spots, the experiments were performed under a biosafety cabinet and then

were placed on the racks with a lid. Then, the DNA spot must be carried to another room and the samples were in direct contact with air for the duration time of the experiments (~1 h).

- As described in the last subsections and shown in Figure 10, there is always a risk of direct contact of the tip of the pipette with the substrate, which may increase the risk of contamination.
- The surface of substrates including Teflon tape will be in direct contact with the water and DNA. One may argue the risk of the removal of nanogram material from the surface of the substrate or the possibility of nanogram DNA residual. This can potentially increase the risk of contamination as well.

The contamination measurement results related to the first day of analysis can be seen in Table A1 (see Appendix A). The 260/230 values for "pure" nucleic acid were often higher than the respective 260/280 values. As seen in Table A1, every 260/230 value was higher than the respected 260/230 value. Therefore, the purity of the measurements was acceptable. However, one may argue that the expected 260/230 values were commonly in the range of 2.0–2.2. Even though the measured 260/230 values were almost around this range of 2 to 2.2, any deviation from this range might have been due to contamination as described in this subsection. It is noteworthy that the contamination can be reduced by putting all instruments including the NanoDrop instrument and sample preparation tools in a large biosafety cabinet and performing the experiments in a fully clean environment.

4. Misalignment

After the dehydration of the DNA sample, the position of each DNA spot is not visible and, therefore, the deposition of the droplet of water might not exactly be on the same spot. For this reason, in this work, the location of each spot was marked. Even though this method is not accurate, it can reduce the misalignment between the sample and the DNA spot's location. It is noteworthy that the glare effect of the dehydrated DNA sample on the substrate can slightly help to better place the water droplet on the DNA spot's location.

4.1.2. DNA Retrieval

- DNA storage time

As shown in Figures 3–5, the measurement results reveal that there was not a significant difference between the concentrations of DNA retrieved shortly after dehydration and after a long time (192 h). It is noteworthy that Newman et al. [14] reported a DNA retrieval of above 90% from the surface of Teflon, with a short storage time. As per the results of this work, this high rate can be expected even though the storage time was very long. Additionally, in [14], a PCR process was performed before DNA sequencing. Therefore, the lower concentration level of DNA fragments should not cause a serious issue in recovering the data from the DNA sample. However, in a DNA storage system with a high DNA retrieval rate, the PCR process is not required.

- Dispersion of measurement results

The measurement results show a large spread or dispersion in the measured concentration of the DNA sample taken from hydrophilic surfaces of glass and silicon wafers, likely coated with a thin layer of SiO_2. As shown in Figure 10a,c, the hydrophilicity increases the error. This is most likely because the DNA droplet is spread on the surface. Therefore, the sample retrieval will be difficult and increase the risk of error as seen in Figure 10. Even though the measurement results associated with hydrophobic surfaces showed more consistent results in comparison with the results taken related to the hydrophilic surfaces, a dispersion can also be seen in the results related to hydrophobic PDMS, Teflon, and polystyrene surfaces. This is because other sources of error such as misalignment, contamination, partial evaporation of sample, and non-homogeneity affect the measurement results.

Despite the aforementioned unavoidable errors, the experiments successfully show that the types of materials used in this work, or the DNA storage time did not affect the

DNA retrieval ratio as per the measurement results reported in this paper. The reduction in the retrieval ratio might be due to the spread of the sample on the surface of the substrate or the weak binding between the DNA molecules and the surface. The DNA molecules left on the surface after retrieval or the movement of the droplet in the microfluidic platform (1) can be a source of contamination for the new DNA sample placed on the same location, (2) may cause the loss of data, especially for the DNA samples with a large volume of data. These two hypotheses have not been verified in this work and can be investigated in the future.

4.2. Dry DNA Retrieval Assessment

A typical absorption spectrum of DNA exhibits a broad peak at 260 nm wavelength. According to Beer-Lambert, the 260 nm peak absorbance height varies with concentration. Hence, this direct correlation leads to a straight-line plot between absorbance values and known concentrations. This linearity further helps to estimate the unknown concentration of DNA in liquid or wet mode obtained. This linear response is also illustrated in Figure 7b. However, a similar study can also be made about the DNA in dry mode.

All related spectrophotometric results of dry spots containing different amounts of DNA mass (775 ng, 387 ng, 258 ng, or 155 ng each repeated five times) on the quartz surface are depicted in Figures 8 and 9. As with the DNA solution, all the absorption spectra of dry-state DNA exhibited a typical broad peak at 260 nm. No significant change in the spectrum of the dry DNA was seen even after 11 days (Figure 8a bottom). The absorption spectra of all five different dry DNA spots containing the identical DNA amounts of 775 ng showed an error below 2% in their close peak absorbance values at 260 nm, highlighting the significance of these results. Four different dry DNA amounts of 775 ng, 387.5 ng, 258 ng, and 155 ng were UV-tested, repeated five times, and exhibited average peak absorbance values of 0.18, 0.09, 0.056, and 0.032, respectively. This linear peak absorbance response of known dry-state DNA amounts (Figure 8c) was similar to that of liquid-state DNA of known concentrations (Figure 7b). This straight-line plot can be used for the mass estimation of unknown dry DNA. However, accurate results can be obtained by improving experimental conditions such as the drop volume of the pipette and its placement in the path of UV-Vis spectrophotometer beam, drying time, and pipette accuracy.

The DNA retrieval process is a crucial part of DNA storage. The amount of DNA mass removed during a retrieval process is subjected to the DNA molecules' bond with the substrate material at the storage site, affecting the data storage ability. However, in order to analyze the retrieved and leftover DNA masses at the storage site, a suitable analytical method is required. Herein, the developed dry DNA mass estimation by spectrophotometry was successfully further employed to analyze DNA leftover dry mass. A clear change in the absorbance values were observed with the mass change at the storage site after retrieval. Therefore, considering the dry spot of DNA on the quartz substrate as DNA storage site, retrieval was completed with the help of a 2 µL deionized droplet placed at the site and picked with the help of a pipette. Every retrieval process revealed some leftover DNA mass at the site after retrieval, as confirmed by the corresponding typical DNA absorption spectrum recorded after the UV spectrophotometric analysis of the storage site. Moreover, comparing the UV spectrum obtained before and after retrieval, a clear low-value peak was seen at 260 nm wavelength in the after-retrieval spectrum. This peak height was further decreased in the second-retrieval spectrum. However, this peak height variation was not regular because a noticeable peak variation in first- and second-retrieval spectra of the identical amounts of dry-state DNA mass can be seen. Therefore, all five 775 ng dry DNA mass repeats exhibited nearly 10% and 20% errors in the first- and second-retrieval spectra, respectively. These are high errors compared to only a 2% error in the peak height values of the spectra of the five repeats before retrieval. The high percentage error in after-retrieval spectra can be attributed to variable experimental conditions such as the volume of deionized droplets placed at a storage site for retrieval, room, and droplet temperature difference, time duration, and pipetting. Therefore, considering these peak heights and

errors, it can be concluded that average DNA masses of 335 ng and 282 ng were retrieved during the first and second retrievals of the 755-ng dry DNA, respectively. Although the percentage error was high, further retrievals retrieved more mass. Instead of employing quartz substrate for the storage site, using other materials as substrate can also affect the retrieved DNA mass. Similarly, the peak absorbance values of the after-retrieval spectra showed average mass retrievals of 294 ng, 182 ng, and 100 ng during the first retrieval and 73 ng, 30 ng, and 23 ng during the second retrieval for 387 ng, 258 ng, and 155 ng of dry-state DNAs, respectively. So, it can be deduced that large amounts of DNA mass were retrieved during the first retrieval process compared to the second one. The low percentage errors shown in Figure 9f for the 258 ng and 155 ng dry DNA mass amounts may have been because the DNA molecules formed strong bonds with the substrate material.

The mass estimation of dry-state DNA molecules before and after retrieval at the storage site, and other information extracted from this study on completely dry DNA can help to understand the interaction of DNA with the substrate in other related dry-mode DNA storage applications.

5. Conclusions

This paper investigated the advantages of UV-Vis spectrophotometry for DNA storage in dry mode. The experimental results were demonstrated, discussed, and compared with the standard UV-Vis spectrophotometric DNA analysis in wet mode. In this study, the hydrophilicity and hydrophobicity of the surface and the storage time were also considered as the factors affecting DNA retrieval. Based on these results, it can be argued that the hydrophilicity of the substrate increases the measurement error. However, the average of the retrieved DNA remained the same. Furthermore, the results revealed no change in the DNA retrieval rate over the limited time of 8 days. Similar to the wet mode, the dry mode also exhibited typical DNA absorption spectra with a linear relationship between the amount of DNA and the peak absorbance value. Moreover, the retrieval process of high amounts of dry DNA showed no significant change in spectra, but low amounts showed sharp spectra. This paper demonstrated the advantages of UV-Vis spectrophotometry for monitoring DNA storage in the future of automated DNA storage systems.

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Appendix A

In this appendix, the NanoDrop experimental results of 260/230 and 260/280 associated with two different substrates are summarized.

Table A1. The contamination data analysis related to DNA retrieval from various substrates including PETTE, PDMS, Polystyrene, and glass with 35 repetitions.

	Polystyrene		Teflon	
	260/230	**260/280**	**260/230**	**260/280**
1	2.29	1.72	2.12	2.29
2	2.44	1.72	2.16	2.44
3	2.69	1.67	2.18	2.69
4	2.7	1.83	1.96	2.7
5	2.7	1.74	2.18	2.7
6	2.65	1.78	2.2	2.65
7	2.85	1.82	2.21	2.85
8	2.95	1.75	2.26	2.95
9	2.89	1.84	2.27	2.89
10	2.85	1.89	2.17	2.85
11	2.95	1.77	2.09	2.95
12	2.9	1.64	1.95	2.9
13	2.85	1.67	1.05	2.85
14	2.79	1.56	2.21	2.79
15	2.48	1.68	2.27	2.48
16	2.54	1.28	2.01	2.54
17	2.99	1.68	2.29	2.99
18	2.79	1.56	2.01	2.79
19	3.18	1.87	2.25	3.18
20	3.04	1.89	2.23	3.04
21	2.96	1.89	2.01	2.96
23	3.02	1.28	2.28	3.02
24	2.91	1.9	2.23	2.91
25	2.89	1.93	2.44	2.89
26	3	1.26	2.54	3
27	3.12	1.96	2.34	3.12
28	2.89	1.87	2.47	2.89
29	2.94	1.26	2.26	2.94
30	2.92	1.86	2.19	2.92
31	2.92	1.59	2.29	2.92
32	2.91	1.15	2.3	2.91
33	2.9	1.23	2.32	2.9
34	2.89	2.1	2.43	2.89

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