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Measuring the density of DNA films using ultraviolet-visible interferometry

Małgorzata A. Śmiałek*
Atomic Physics Division, Department of Atomic Physics and Luminescence, Faculty of Applied Physics and Mathematics, Gdańsk University of Technology, 80-233 Gdańsk, Poland

Nykola C. Jones and Søren Vrønning Hoffmann
ISA, Department of Physics and Astronomy, Aarhus University, DK-8000 Aarhus C, Denmark

Nigel J. Mason
Department of Physical Sciences, The Open University, Milton Keynes, MK7 6AA, United Kingdom
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In order to determine a proper value for the density of dry DNA films we have used a method based upon the measurement of interference effects in transmission spectra of thin DNA layers. Our results show that the methodology is effective and the density of DNA in this state, 1.407 g/cm^3, is much lower than the commonly used 1.7 g/cm^3. Obtaining accurate values for the DNA film density will allow the optical constants for DNA to be recalculated, which were previously obtained assuming a higher DNA density. Furthermore, since our recent investigations have shown a strong dependence of the sample composition on DNA film formation and thus on its density, such a method will be important in characterizing particle interactions with DNA film and their dose dependence.

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During the past decade research on the mechanisms of radiation-induced DNA damage has expanded dramatically, with an increasing recognition that such damage must be understood at the nanoscale based upon the underlying fundamental atomic and molecular interactions [1]. Such studies have required the preparation of dry DNA films as targets. In order to characterize interactions between DNA molecules in a film and incident particles it is therefore necessary to measure both the optical and dielectric properties of DNA [2] and, in particular, determine the film density. Measurements of the density of dry DNA films have previously been performed using a variety of methods, with the result strongly related to the method used (see Table I) [2–5]. The most commonly used value of the density of calf thymus (CT) DNA and other genomes (including human) as well as plasmid DNA is reported to be approximately 1.7 g/cm^3 [6–11]. This value was measured using the buoyant density technique, which is commonly used to determine the unknown density of a component against the known density of a solvent. This technique resolves a sample into components of differing buoyant densities upon centrifugation. The term "buoyant density" refers to the fluid density, in which the sample particles manifest no tendency either to float or to sediment. The drawback of this technique is that it strongly depends on the solvent [12] and the temperature at which the procedure is carried out [13]. Moreover, the density of DNA established using this method refers to molecules in an aqueous environment, which is obviously not the case for the films prepared either under vacuum or air dried. The accuracy of the measurement of DNA density is important when other parameters such as refractive index, extinction coefficient, oscillator strength [2,14], energy loss function [15–18], electron mean free path [19], effectiveness of DNA strand breakage [20], electron [21] and proton [17] stopping power, or various cross sections for DNA-electron and photon interactions [21–24] are to be determined.

To date there are no consistent measurements of the density of dried DNA films, but the density is expected to be between that in solution and the density of powders of nucleosides or nucleotides (e.g., adenosine 0.998 g/cm^3). The values obtained from gravitometric measurements [3,4] seem to be slightly too high and were also measured in the presence of a solvent. The value closest to the one presented in this Rapid Communication was also obtained by interferometric measurement, but no errors of measurement were quoted for this value, therefore the accuracy is unknown [2].

In order to determine a new value for the density of dry DNA films we have used a method based on the measurement of interference fringes of transmission spectra from thin DNA layers. A weakly absorbing uniform thin film of thickness d is formed on a transparent substrate with a thickness several orders of magnitude larger than the film (Fig. 1). The different refractive indices of the elements in this system—air, film, and substrate—give rise to multiple reflections from the interfaces. Interference effects due to the film, as the reflected waves constructively and destructively interfere, give rise to fringes in the resulting transmission spectrum (Fig. 1). The refractive index of air that surrounds the investigated system is $n_0 = 1$. The incident beam of transmission $T_1 = 1$ passes through the film supported on the substrate and the signal measured is T.

In a model originally developed by Manaficier [25] and later improved upon by Swanepoel [26], the thickness d of such a film, perpendicular to the incident beam and on the same axis as the detector, can be determined using

$$d = \frac{\Delta i}{2} \frac{\lambda_1 \lambda_2}{\lambda_1 n_2 - \lambda_2 n_1}, \quad (1)$$

*Also at Department of Physical Sciences, The Open University, Milton Keynes, MK7 6AA, United Kingdom; smialek@mif.pg.gda.pl

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TABLE I. Comparison of values for CT DNA density that can be found in the literature; bdg: buoyant density gradient.

Source	Method	Density (g/cm^3)
This work	Interferometry	1.41 ± 0.03
Astbury [3]	Gravitometry	1.63
Franklin [4]	Gravitometry	1.625 ± 0.002
Inagaki [2]	Interferometry	1.35
Weidlich [5]	Crystallography	1.64
Votavova [6]	CsCl-netropsin bdg	1.7
Thiery [8]	CsCl bdg	1.7033 ± 0.0002
Macaya [9]	Cs_2SO_4 BAMD bdg	1.7085
Filipski [11]	Cs_2SO_4 bdg	1.697

where Δi is the number of fringes between wavelengths at two maxima or minima of interference (λ_1 and λ_2) and n_1 and n_2 are refractive indices at these wavelengths. This method is valid for measurements carried out in higher wavelength regions, where the film is only weakly absorbing and the substrate is transparent.

The thin film of thickness d has a complex refractive index $\boldsymbol{n} = n + ik$, where n is the real part of the complex index of refraction and k is the refraction coefficient, which can also be expressed in terms of absorption coefficient α. For the substrate-film system an expression for the refractive index can be calculated using values from envelopes describing maximal (T_M) and minimal (T_m) transmission on the interference signal [26] as

$$n = \sqrt{N + \sqrt{N^2 - s^2}}, \tag{2}$$

with

$$N = 2s\frac{T_M - T_m}{T_M T_m} + \frac{s^2 + 1}{2} \tag{3}$$

and s is refractive index of the substrate, which can be found using

$$s = \frac{1}{T_s} + \left(\frac{1}{T_s^2} - 1\right)^{1/2}. \tag{4}$$

T_s is the transmission of the substrate in the absence of the film (Fig. 2, solid line). For the MgF_2 substrates used in this investigation the transmission was measured to be constant over the wavelength range used and the resulting refractive index is $s = 1.375$.

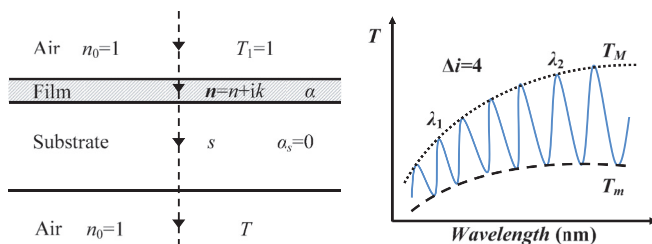

FIG. 1. (Color online) For a light absorbing thin film supported on a thick, finite, transparent substrate (left) the interference effects will give rise to the transmitted signal producing interference fringes, confined by two envelopes T_M and T_m (right). λ_1, λ_2, and Δi according to the text.

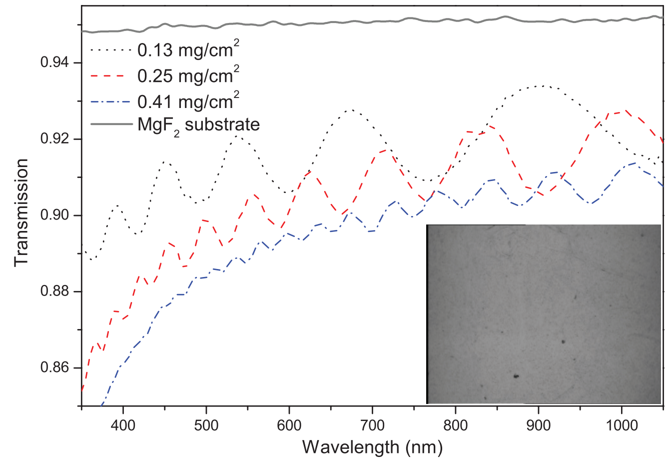

FIG. 2. (Color online) Interference fringes in transmission spectra for calf thymus DNA films of 0.13, 0.25, and 0.41 mg/cm^2, together with transmission measured for the MgF_2 substrate used in the experiments; the inset shows a photograph of the surface of a 0.41 mg/cm^2 film taken on a 40× zoom with an optical microscope.

For this work calf thymus DNA sodium salt was purchased from Sigma-Aldrich, Denmark, and used with no further processing or purification. Magnesium fluoride substrates, 20 mm in diameter and 1 mm thick, were obtained from Crystran, UK.

DNA samples of the desired mass were dissolved in 0.5 ml ultra high purity (UHP) H_2O for 12 h, outgassed, and transferred onto the MgF_2 substrates by pipetting, covering all of their surface. The accuracy of the balance used was 0.1 mg, therefore, in order to avoid large uncertainties at low masses, stock solutions containing five to ten times more DNA than required for one film were prepared and then diluted to give the required mass of DNA. Samples were then placed in a desiccator and the water was slowly pumped away over a 24 h period using a vacuum water pump with a base pressure of 100 mbar. Tests showed that any higher vacuum distorts the film surface and therefore a low-vacuum, slow evaporation preparation protocol was used.

Once the films had dried, the transmission spectrum of each was measured over the 300–1100 nm wavelength range, using a Thermo Scientific Evolution 300 UV-VIS spectrometer with a specially adapted disk sample holder. Films were made with DNA masses ranging from 0.3 to 2.0 mg per film, and for each amount of DNA three films were prepared and measured. The transmission spectrum of each of these three independently prepared films was measured at three randomly chosen positions of each film, to ensure the uniformity and homogeneity of the surface of the sample. The interference fringes observed in the transmission spectra were then processed in ORIGINPRO 8.5 software. The optical constants were evaluated using the "envelope method."

Figure 2 shows the spectra obtained for films of DNA made using 0.13, 0.25, and 0.41 mg/cm^2. The interference fringes seen in the transmission spectrum indicate that the film thickness is uniform over the film. If the thickness of the film were tapered or not uniform, these fringes would not be observed as all interference effects are destroyed.

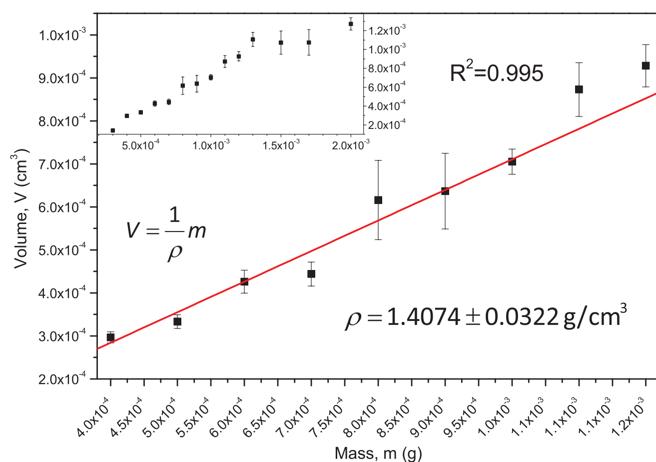

FIG. 3. (Color online) Relationship between the mass of CT DNA deposited over the whole surface of MgF_2 substrates and the calculated volume of the sample, fitted with a weighted least-squares method. The inset shows the data points over the whole range investigated.

The thickness of each of the films was calculated using the model presented above. Using these values the volume of the samples can be easily obtained. The average volume over the nine measurements for each mass of DNA is calculated. The inset in Fig. 3 shows the final results as a function of DNA mass obtained for all of the DNA films. From residual and R-square parameter analysis it was clear that there is a linear relationship between the mass and volume for samples containing between 0.4 and 1.2 mg of DNA spread over the surface of the substrate (Fig. 3), which results in films of thickness between approximately 1 and 3 μm. To obtain a value for the density of the DNA films, ρ, linear fitting was only performed over this range. A value of the density of CT DNA films was obtained using a least-squares method with weighting to yield

$$\rho = 1.41 \pm 0.03\ \text{g/cm}^3. \qquad (5)$$

This value is lower than most of those previously reported in the literature for CT DNA (see Table I for a comparison of values obtained previously) and consistent with the conclusions of our previous studies conducted for DNA films [27,28]. Another result which is consistent with that presented here was obtained by Inagaki and colleagues, who also used an interferometric method. Although the film thickness was not the main target of their studies, the value obtained matches the one shown here within the experimental error. With all other methods the obtained values of density are higher by approximately 20%.

The results presented in this Rapid Communication prove that the methodology is effective and show that the density of dry films of DNA is lower than previously thought. By producing films from a wide range of DNA masses it has been possible to find a range in which film formation is uniform and where the thickness increases linearly with the amount of sample. The saturation effect, seen in Fig. 3, for DNA masses higher than 1.2 mg per sample can be attributed to reaching a critical value for a film, where the large mass of the material starts compressing its structure. Obtaining accurate values for the DNA film density will allow the optical constants for DNA to be recalculated, which were previously obtained assuming a higher DNA concentration. The mass per square centimeter quantities used in this work are similar to those often used in irradiation studies [29], so the value obtained here should be of use in further studies. Furthermore, since our recent investigations have shown a strong dependence of the sample composition on DNA film formation and thus on its density [27,28], such a method will be important in characterizing particle interactions with DNA film and their dose dependence.

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