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Measurement of changes in impedance of DNA nanowires due to radiation induced structural damage*

A novel approach for a DNA-based radiosensitive device

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Abstract. The ability of DNA to conduct electric current has been the topic of numerous investigations over the past few decades. Those investigations indicate that this ability is dependent on the molecular structure of the DNA. Radiation-induced damages, which lead to an alteration of the molecular structure, should therefore change the electrical impedance of a DNA molecule. In this paper, the damage due to ionising radiation is shown to have a direct effect on the electrical transport properties of DNA. Impedance measurements of DNA samples were carried out by an AC impedance spectrometer before, during and after irradiation. The samples comprised of DNA segments, which were immobilized between gold electrodes with a gap of 12 μm. The impedance of all DNA samples exhibited rising capacitive behaviour with increasing absorbed dose.

1 Introduction

As DNA is the hereditary material in almost all organisms, any damage to it can play a key role in the development of various diseases [1]. DNA damages can occur due to endogenous as well as external effects. Ionizing radiation is the main external source for DNA damages. Since different kinds of radiation interact differently with DNA, their potential hazardousness can vary considerably. For this reason, the concept of the relative biological effectiveness (RBE) was introduced as a weighting factor to account for the damage potential of different types of radiation [2]. The premise for the use of the RBE is that the type and energy of the radiation has to be known. In practice, however, the spectral distribution and composition of radiation fields are not always known. This problem may be avoided if the DNA itself is used as the detector material.

Numerous research has shown that the electrical properties of DNA strongly depend on its molecular structure [3–6]. Since the direct measurement of structural changes are difficult, the electrical resistance was used as an indicator for the degree of radiation damage to DNA. The samples comprised DNA molecules that were attached between gold electrodes on a silicon chip. These samples were characterized by AC impedance spectroscopy before, during and after the radiation exposure. Since the applied voltage did not exceed 100 mV_{pp}, the measurement was not expected to cause any structural changes in the DNA [7]. DNA, however, is known to degrade over time, even in the absence of radiation. Other factors known to influence the electric properties of DNA are the temperature and humidity of the environment [8,9]. In order to separate the aging and environmental effects from radiation effects, a non-irradiated sample was measured simultaneously under the same laboratory conditions. The measurements were conducted with two different radiation sources: an Americium-241 Isotope (^{241}Am), which emitted 5.5 MeV alpha particles, and a microbeam that provided 8 MeV alpha particles.

2 Instrumentation

The setup for the impedance spectroscopy consisted of a signal generator (Agilent 33220A) and a lock-in amplifier (Ametek Signal Recovery 7230). The circuits are shown in Figure 1. The signal generator acted as the voltage source and the lock-in amplifier as the ammeter. The applied

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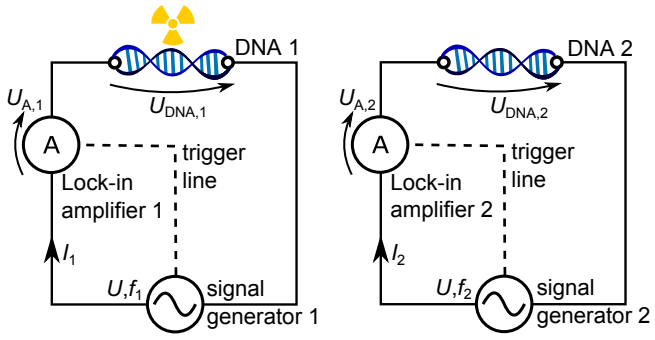

Fig. 1. Circuits for the AC impedance spectroscopy. The signal generator provided a synchronizing trigger signal to the corresponding lock-in amplifier, which was used as an external reference signal. The measurements were performed with DNA nanowires immobilized between gold nanoelectrodes on a Silicon chip. The two circuits operated at different frequencies, to prevent coupling effects.

voltage U was split between the impedance of the DNA sample and the internal resistance of the ammeter, such that

$$U = U_{\mathrm{DNA}} + U_{\mathrm{A}}. \tag{1}$$

All samples in this work had an electrical impedance in the order of several MΩ. The internal impedance of the lock-in amplifier was lower than 2.5 kΩ, according to its data sheet [10]. This value was many orders of magnitude lower than the DNA impedance. It was therefore assumed that the full voltage U was applied to the DNA. The absolute value of the impedance was calculated using Ohm's law

$$|Z| = \frac{U_{\mathrm{rms}}}{I_{\mathrm{rms}}}. \tag{2}$$

U_{rms} and I_{rms} represent the root mean square values of the sinusoidal voltage U and the current signal I, respectively. The samples were not exposed to high voltages in order to prevent the DNA from being influenced by the applied electric field [7]. Hence, the measurement of very low currents $I_{\mathrm{rms}} < 100$ nA was necessary. The lock-in method allowed the measurement of such low currents and their separation from the background noise. This enabled an accurate measurement of the amplitude and phase of the electrical signal. Since two samples were measured simultaneously, the frequencies of the applied voltages differed by a few percent to prevent coupling effects between the two circuits. The amplifiers were capable of measuring the impedances at frequencies up to 120 kHz. However, the frequency dependent impedance of the measuring system can lead to high uncertainties at high frequencies. In combination with the capacitance of the DNA samples, this parasitic impedance can cause different frequency dependences at higher frequencies. Therefore, values over 5 kHz were not shown in the following spectra.

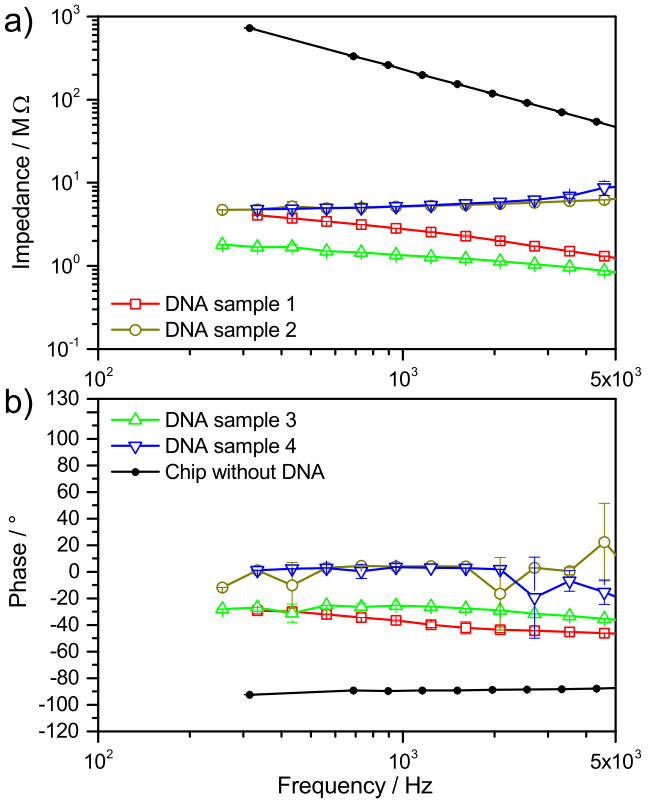

Fig. 2. Impedance (a) and phase (b) as a function of the frequency with and without the presence of DNA. The solid line shows the response of the unloaded chip, which behaved like a capacitor with $C = 0.7$ pF. The chips with DNA between the electrodes, on the other hand, exhibited a more ohmic-like behaviour.

3 DNA samples

The DNA samples were produced by the San Diego State University [11,12]. A sample comprised a silicon chip with a SiO_2 surface layer for better insulation. The gold electrodes were manufactured on the chip, by photolithography and ion-beam sputter deposition. The gap between the electrodes was 12 μm. The electrodes were covered with thiol, on which two different oligonucleotides were attached [13,14]. The DNA was then immobilized between the electrodes making use of its self-assembly properties [12].

The measured frequency dependencies of a few DNA samples are shown in Figures 2a and 2b. These results are in good agreement with those measured by Kassegne et al. [11]. In the case of the DNA immobilisation technique employed by Kassegne et al., the arrangement and the number of immobilised DNA strands can vary. This causes differences in the sample resistances and reactances. The frequency response of an unloaded chip, i.e. a chip containing electrodes but no DNA, is also shown in Figure 2. This unloaded chip acts like a capacitor with a capacitance C of 0.7 pF. The reactance of a capacitor decreases proportionally to the frequency, while the phase stays constant at –90°.

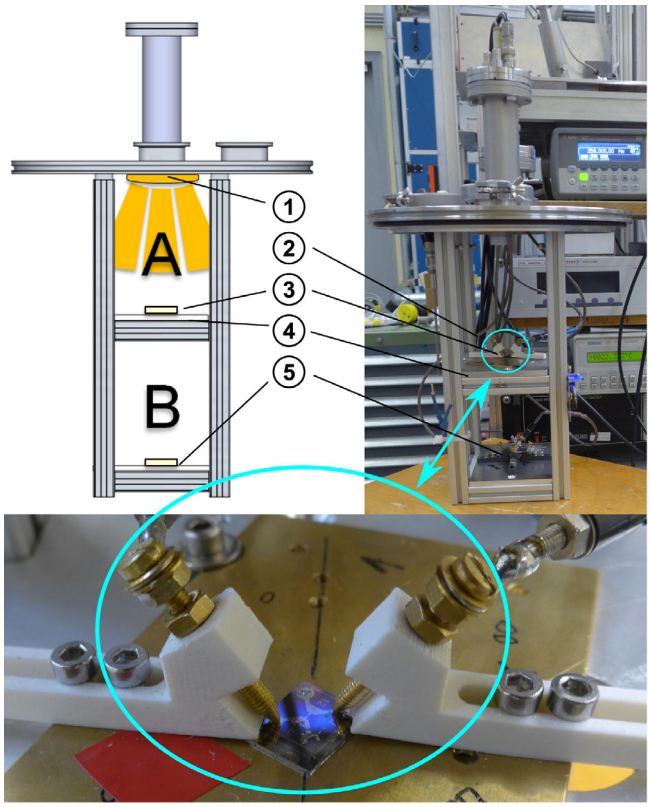

Fig. 3. Sketch and photograph of the setup for the ^{241}Am experiment. 1. ^{241}Am source with adjustable height. 2. Sample holder with electrical contacts. 3. Irradiated sample. 4. Aluminium plate. 5. Reference sample.

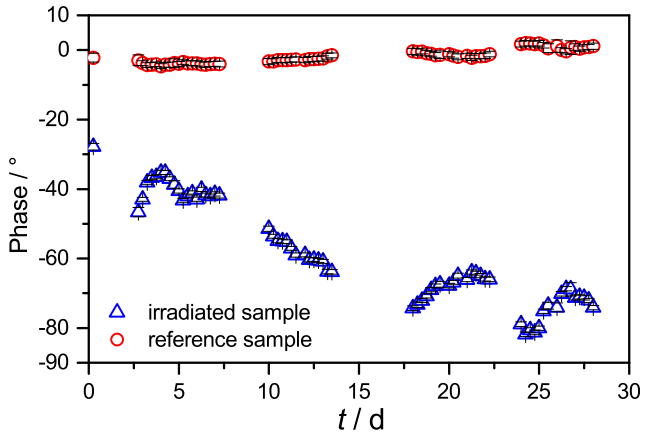

Fig. 4. Phase response during irradiation with the ^{241}Am source. The plotted values represent the average value of the phase response for frequencies ranging from 250 Hz to 350 Hz. Gaps in the timeline were caused by instrument failure due to technical difficulties.

4 Irradiation with ^{241}Am source

4.1 Setup

As illustrated in Figure 3, the experimental setup comprised two DNA samples, which were measured simultaneously. The first sample was irradiated by an ^{241}Am source, whilst the second sample was shielded against the source by an aluminium plate. Apart from that, the instrumental setups were identical. The DNA samples were mounted in an aluminium rack together with the ^{241}Am source. The distance between the DNA sample and the alpha source was 2.1 cm. This was the minimum distance limited by the size of the sample holders. The energy of the alpha particles impinging on the DNA was 1.5 MeV. The reduction in energy resulted from an energy loss in the air gap. This energy loss was calculated using SRIM simulation [15]. The rack was designed in such a way that measurements within a vacuum chamber are possible. However, in the present experiment, the measurements were carried out under ambient conditions.

The ^{241}Am source had an activity A of 0.5 MBq. The radiation was emitted isotropically. Assuming a point source, the average rate of incoming particles r is given by

$$r = A \cdot \frac{F_{\mathrm{DNA}}}{F_{\mathrm{sphere}}}. \quad (3)$$

F_{DNA} is the area of the DNA sample. F_{sphere} is the surface area of a sphere with a radius equal to the distance between the source and the sample. The DNA sample was assumed to have a length and width of 12 μm and 10 nm, respectively. Therefore, r was approximately 1.08×10^{-7} s^{-1}, which equates to roughly one particle hitting the DNA every 24 h in average. The samples were irradiated for a period of 28 days. The measurements were conducted in air at room temperature.

4.2 Results

Figure 4 shows the temporal development of the phase response of the samples with and without irradiation. The reference sample did not exhibit a change in behaviour. The phase of the irradiated sample, however, dropped to –90°, which suggests that the irradiated DNA was destroyed. Hence, only the frequency response corresponding to that of the unloaded chip was measured. This is supported by Figures 5a and 5b, which show the impedance and phase response of the irradiated sample (Rad) and the reference sample (Ref) before and after irradiation, respectively. Post-irradiation, the reference sample exhibited a higher impedance, which was probably caused by aging of the DNA strands. This aging leads to an increase of the ohmic resistance. In the case of a high ohmic resistance, capacitances play a dominant role already at low frequencies. Such behaviour is responsible for the drop in phase and impedance that can be seen above 1 kHz. The irradiated sample, on the other hand, showed a capacitive behaviour similar to that of an unloaded chip. This indicates that the reference sample as well as the irradiated sample had suffered degradation over time. The irradiated sample, however, degraded at a much faster rate, barely conducting current after the irradiation. This change in electrical properties is demonstrated in Figure 6. Diagrams (a) and (b) show the equivalent circuits of the measurement

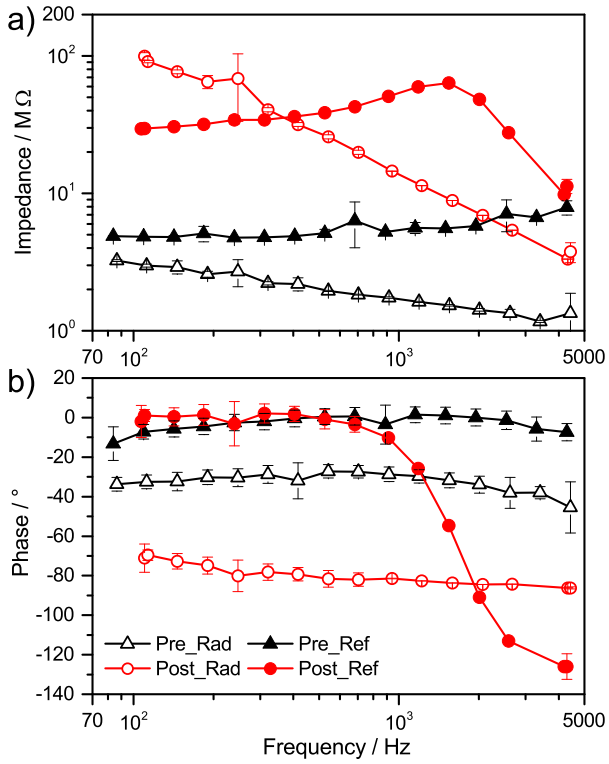

Fig. 5. Frequency dependence of the impedance (a) and phase (b) of the irradiated (Rad) and reference (Ref) sample before and after irradiation with the ^{241}Am source. The duration of the irradiation was 672 h.

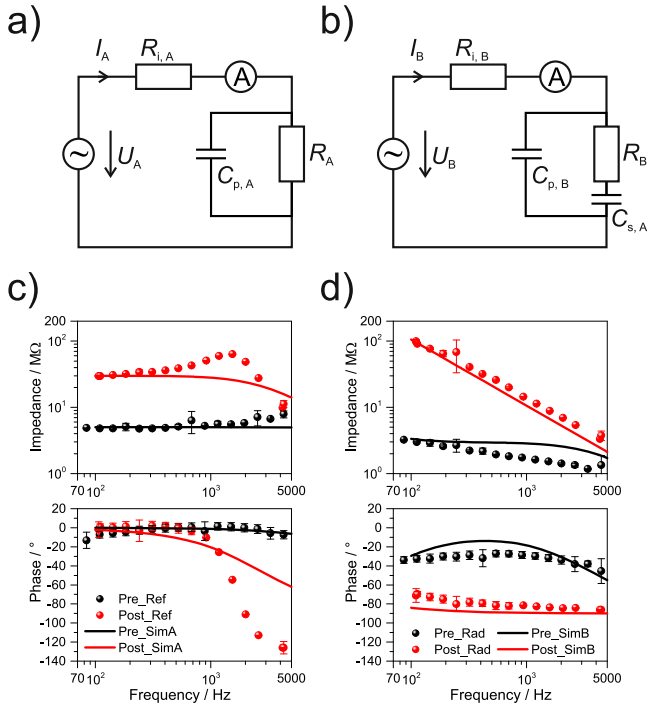

Fig. 6. Simplified equivalent circuits of the measurement setups for the ^{241}Am radiation experiments. Diagrams (a) and (b) represent the setup of the reference sample and irradiated sample, respectively. Here, $R_i = R_{i,A} = R_{i,B} = 100\ \Omega$ represents the input resistances of the lock-in amplifiers measuring the currents I_A and I_B. R, C_p and C_s represent the DNA samples, including the electrode chip. The resistance R_A increased during the measurement time from 5 MΩ to 30 MΩ, while its parallel capacitor $C_{p,A}$ increased from 0.7 pF to 2 pF. Simultaneously, the resistance of the irradiated sample R_B increased from 3 MΩ to 1 GΩ, while $C_{p,B} = 15$ pF and $C_{p,B} = 1$ nF remained the same. Graphs (c) and (d) show the calculated frequency dependencies SimA and SimB, in comparison to the measured data. SimA and SimB were calculated using the SPICE software LTspice.

setups. For better understanding, the circuits have been simplified to qualitatively demonstrate the influence of major components on the output signal. Figures 6c and 6d show the calculated signals SimA and SimB in comparison to the measured data.

5 Microbeam radiation experiments

5.1 Setup

The measurements were conducted with 8 MeV alpha particles produced by a microbeam [16]. AC impedance spectroscopy was once again used to measure the electrical transport properties of DNA samples during irradiation. The experimental setup is shown in Figure 7. The distance between the DNA sample and the vacuum window was 4 cm. After passing through the vacuum window and 4 cm of air, the alpha particles arrived at the sample with energies of 5 MeV. This energy was calculated using SRIM simulation. The samples were mounted on glass slides for better positioning. The reference sample was placed approximately 50 cm away from the irradiated sample so that it was not influenced by the radiation. The beam had a diameter of 6 μm and a rate of 8000 particles per second, such that approximately 120 particles hit the sample per hour. This allowed considerably shorter irradiation times compared to those in the previous experiments with ^{241}Am. The duration of the irradiation for the measurement was 2.5 h.

5.2 Results

The temporal development of the phase response of an irradiated and a reference sample is shown in Figure 8. The response of the irradiated sample showed a steady decline, while the response of the reference sample remained constant. The frequency response of an irradiated and a reference sample before and after irradiation is shown in Figure 9. Unlike the samples in the previous ^{241}Am experiment, the DNA does not appear to be fully destroyed by the radiation. However, a noticeable difference between the response of the irradiated and the reference samples can be seen. During the comparatively short measurement time, the reference sample did not experience a measurable degradation. The irradiated sample, however,

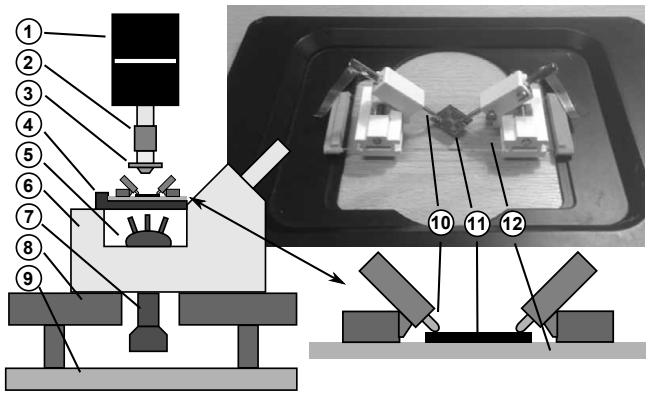

Fig. 7. Sketch of the setup for the microbeam experiment and photograph of the sample holder. 1. Quadrupole doublet. 2. Bellows for vertical-movement. 3. Vacuum window. 4. *xy*-stage. 5. Objective turret and detectors. 6. Inverse microscope. 7. CCD camera. 8. Optical table. 9. Basement floor. 10. Spring contacts. 11. DNA sample. 12. Glass slide.

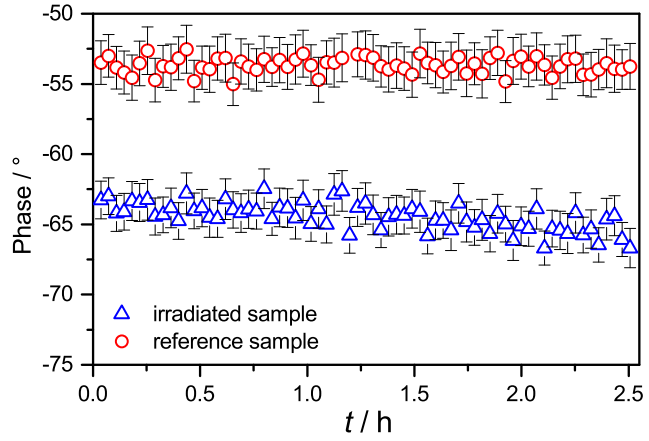

Fig. 8. Phase response as a function of time for the irradiated and reference sample during microbeam irradiation. The plotted data are averaged values of the phase response for frequencies between 250 Hz and 350 Hz.

exhibited a decrease in the phase response which indicates that the DNA was damaged by the radiation.

6 Conclusions and outlook

The DNA samples have been shown to undergo a change in impedance when exposed to radiation. With increasing absorbed dose, the frequency response of the samples became more capacitive. This is due to the increased resistance of the DNA such that the impedance is dominated by the reactance of the electrode chip. These measurements demonstrate the functionality and feasibility of a DNA-based radiosensitive device. Establishing a quantitative correlation between the amount of radiation damage to DNA and its subsequent change in resistance would be the next step in this work. For a such quantitative correlation, a direct measurement of DNA strand breaks (i.e. by means of high-resolution atomic force microscopy) in combination

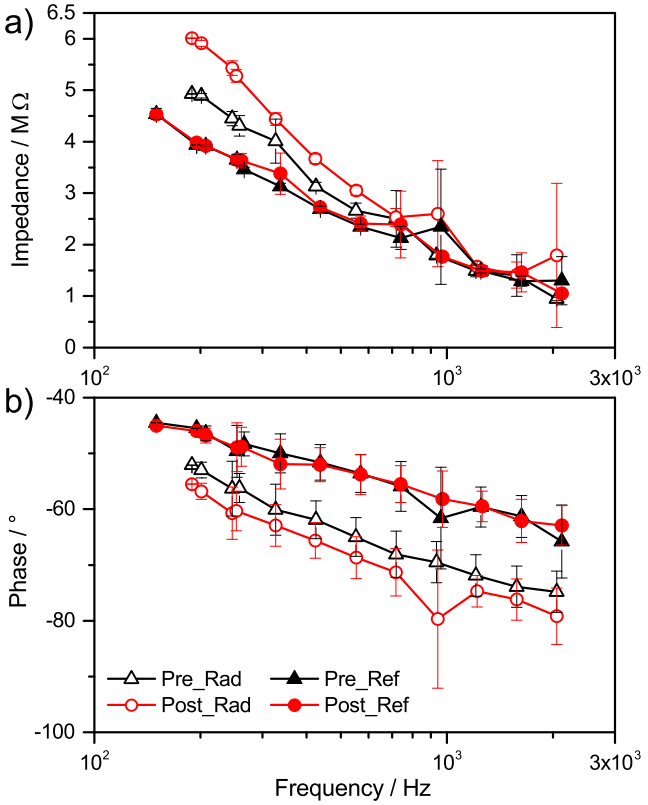

Fig. 9. Frequency dependency of the impedance (a) and phase (b) of the irradiated (Rad) and reference (Ref) sample before and after irradiation with the microbeam. The duration of the irradiation was 2.5 h.

with theoretical modelling is needed. Over the last few decades, different models describing the transport properties of DNA have been developed [17–19]. These models, in combination with molecular dynamics simulation, may be used to establish a correlation between the number of strand breaks and change in electrical resistance. A reliable comparison between theoretical and experimental results would require more reproducible DNA samples to eliminate such variations as seen in Figure 2. This could be achieved by producing samples with AC dielectrophoresis [20], which would not only allow better comparison between different experiments but also between irradiated and reference samples.

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Author contribution statement

Arndt, Baek and Rabus developed the presented idea. Baek and Rabus directed the project. Arndt, Heimbach and Sellner developed the measurement setup and conducted the measurements. Shen and Toppari provided their know-how regarding the sample preparation. Giesen and Langer operated the microbeam facility. Arndt,

Heimbach and Sellner analysed the measurement data with contributions from Giesen, Langner and Nettelbeck. Baek, Shen and Toppari helped interpreting the results. Heimbach took the lead in writing the manuscript with contributions from Arndt, Baek, Giesen, Nettelbeck and Rabus. Baek and Nettelbeck corrected the manuscript. All authors discussed the results and contributed to the final manuscript.

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