Microfluidic DNA combing for parallel single-molecule analysis

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Abstract
DNA combing is a widely used method for stretching and immobilising DNA molecules on a surface. Fluorescent labelling of genomic information enables high-resolution optical analysis of DNA at the single-molecule level. Despite its simplicity, the application of DNA combing in diagnostic workflows is still limited, mainly due to difficulties in analysing multiple small-volume DNA samples in parallel. Here, we report a simple and versatile microfluidic DNA combing technology ($\mu$DC), which allows manipulating, stretching and imaging of multiple, microliter scale DNA samples by employing a manifold of parallel microfluidic channels. Using DNA molecules with repetitive units as molecular rulers, we demonstrate that the $\mu$DC technology allows uniform stretching of DNA molecules. The stretching ratio remains consistent along individual molecules as well as between different molecules in the various channels, allowing simultaneous quantitative analysis of different samples loaded into parallel channels. Furthermore, we demonstrate the application of $\mu$DC to characterise UVB-induced DNA damage levels in human embryonic kidney cells and the spatial correlation between DNA damage sites. Our results point out the potential application of $\mu$DC for quantitative and comparative single-molecule studies of genomic features. The extremely simple design of $\mu$DC makes it suitable for integration into other microfluidic platforms to facilitate high-throughput DNA analysis in biological research and medical point-of-care applications.

Keywords: microfluidics, DNA combing, DNA damage, single-molecule imaging, genome mapping

(Some figures may appear in colour only in the online journal)

1. Introduction
Optical mapping is a powerful method for DNA analysis at the single-molecule level, and is widely applied in fast-developing fields such as detection of genes, screening of epigenetic modifications, analysis of genomic structure, mapping of DNA-protein interactions and detection of DNA damage sites [1–5]. A key step in DNA optical mapping is to stretch the DNA molecules from their entropy-favoured coiled form to an extended linear form. Many methods using various technologies have been developed for this purpose, including nano-channels [6–8], fluid force [9, 10] or electrophoretic force in microfluidic devices [11], flow on micro-structured [12–14] or positively-charged surfaces [15–17], DNA combing [18, 19], etc. In all DNA stretching methods, the stretching ratio and uniformity are key factors for high
quality single-molecule analysis, especially for high-throughput or comparative studies. Among these methods, DNA combing is considered one of the most versatile, convenient and efficient methods to stretch and immobilise DNA molecules by retracting the meniscus of aqueous DNA solution on a hydrophobic surface [19, 20]. Moreover, DNA combing under suitable conditions can uniformly overstretch DNA molecules to about 1.6 times of their B-form contour length [21], providing higher optical resolution and stretching uniformity than most other stretching methods. Over the last decades, DNA combing has become a widely used method in DNA optical mapping for analyses of regulation binding sites [4], replication origins [20], physical maps [22], etc. Despite its extensive application, DNA combing is still inadequate for several required applications, such as manipulating different DNA samples in parallel and handling small sample volumes at sub-microliter level for single cell analysis [19, 23], and its integration into automatic sample preparation process for effective automation of high-throughput experiments is still challenging.

Microfluidics, or lab-on-a-chip, has been rapidly developed and widely applied in biological studies, such as high-throughput analysis of DNA at the single-molecule and single-cell level [1, 24]. Typical microfluidic methods for DNA stretching include nano-channel electrophoresis, hydrodynamic or electrophoretic micro-flow [9–11, 25] and flow-based DNA stretching on surface [16, 17, 26–28]. Nano-channel electrophoresis shows good stretching homogeneity of individual DNA molecules, but the fabrication of high-throughput nano-channels is costly and technically demanding [29]. Microfluidic chips using fluid or electrophoretic force to stretch DNA are not suitable for high-resolution imaging, as the molecules fluctuate in solution and the linear conformation of DNA is transient. Another common method of microfluidic DNA stretching is using capillary flow [16], electrophoresis [30] or micro-flow [10] to immobilise DNA molecules on modified glass surfaces carrying net positive charge. However, the stretching ratios of these methods are typically around 0.6–1.2 times of the B-form contour length [26, 30], which are lower and more variable compared to DNA combing on hydrophobic surfaces [19, 21].

In recent years, researchers have been exploring the potential of implementing DNA combing in microfluidic platforms. Cécilia A P Petit studied the patterning and orienting of stretched DNA by molecular combing in micro-channels [28]. Hirotoshi Yasaki demonstrated DNA stretching on zigzag-shaped microgrooves by microfluidic transfer of a liquid interface [31]. Despite these pioneering efforts, the performance of these methods, such as DNA stretching ratio and uniformity, has not been quantitatively assessed, and to the best of our knowledge, these technologies have yet not been used in biological or medical applications. Therefore, it is of particular interest to develop and optimise a simple and robust microfluidic DNA combing (µDC) system, which is suitable for parallel manipulation of microliter-scale sample volume, and then to apply it for high-resolution DNA analysis, such as optical mapping of DNA damage induced by UV irradiation.

Here, we report a simple, cost-effective and robust DNA stretching technology, namely µDC, which enables uniform stretching of different DNA samples in parallel. The method is suitable for assessing picogram amounts of DNA using only microliter volumes. This technology comprises a custom-designed microfluidic device placed on top of a hydrophobic surface to generate multiple parallel channels for DNA combing. In order to rigorously assess the DNA stretching performance of µDC, we developed a DNA molecular ruler with periodic fluorescent markers to allow quantification of the stretching ratio and stretching uniformity along individual molecules, between different DNA molecules, and between DNA molecules in parallel microfluidic channels. Our results show that µDC generates high stretching ratios, allowing better optical resolution for DNA analysis, while maintaining excellent stretching uniformity not only within individual molecules but also between different DNA molecules in different channels. Additionally, µDC shows high robustness against perturbation of experimental parameters such as the speed of sample droplets. Therefore, µDC has potential for large-scale DNA optical mapping studies, such as high throughput single cell analysis, genomic research of mixed populations, and detection of epigenetic markers and DNA damage in multiple DNA samples [32–34].

To demonstrate its application in biological or medical research, we used µDC to study UV-induced damage on human DNA. Quantitative methods are lacking in this field, which is not only important for understanding the molecular mechanisms governing DNA damage and repair, but also for developing new therapeutics for lethal diseases such as skin cancers [35–37]. Beyond quantifying the levels of DNA damage, it is of great interest to study the location of damage lesions and its correlation with other genomic features [38, 39]. Several methods for UV-induced DNA damage analysis have been reported, including enzymatic approaches [40–43], immunological methods [44, 45], liquid chromatography associated with mass spectrometry [46] and high-throughput sequencing based method [47]. However, the spatial correlation of lesion sites at a distance longer than 1000 base pairs, which matches the scale of chromatin, has barely been reported. This is mainly because most methods for analysis of UV damage require PCR amplification [41, 42, 48], thus precluding analysis of large DNA sequences. In this context, DNA optical mapping offers unique advantages for studying long distance correlation of DNA damage sites.

In our proof of concept experiment, we irradiated human embryonic kidney (HEK) cells by five different doses of UVB light [32], and studied the amount and spatial correlation of damage sites caused by UVB irradiation. The samples were simultaneously stretched in a µDC chip, and imaged using single-molecule fluorescence microscopy. Our method well quantified the relationship between DNA damage site density and UV dose inflicted on the cells. Furthermore, we analysed the spatial correlation of these sites along the irradiated DNA by measuring the distances between neighbouring sites, showing no spatial correlation at the optical resolution (∼600 bp). The µDC-based UV damage detection allows high throughput parallel analysis of DNA samples with different irradiation doses of UV light at the single-molecule
2. Results and discussion

2.1. Fabrication and manipulation of μDC

A detailed description of the design and fabrication of the μDC manifold device is provided in the experimental section. Briefly, the μDC chip consists of two essential parts, a polydimethylsiloxane (PDMS) block with parallel microfluidic channels and a modified glass surface (figure 1(a)). The PDMS block was fabricated using soft lithography to consist a manifold of parallel micro-channels containing multiple inputs and a single output to allow parallel combing experiments with multiple DNA samples (figure 1(b)). After comparing the performance of μDC with different structure parameters, we chose 400 μm as the optimal value for both the width and the height of the microfluidic channels. The cross section of every channel was simply designed as a square for fabrication convenience, and the main areas of the channels were parallel to each other to facilitate automatic fluorescence imaging after DNA stretching. The glass coverslips were carefully cleaned and coated with hydrophobic polymers. The PDMS block and the modified glass coverslip were pressed together to form the μDC device.

To perform parallel multi-sample DNA combing, we injected 2 μl droplets of various DNA samples into the inlets of parallel microfluidic channels to generate uniformly sized droplets. A peristaltic pump was connected to the outlet to provide negative pressure as the driving force to manipulate the droplets. Compared with pushing droplets with positive pressure, dragging with negative pressure can prevent separation between the PDMS block and the modified glass surface, and therefore decrease the chance of sample contamination and experiment failure. While the droplets advanced through the micro-channels, the DNA molecules were attached to the hydrophobic glass surface and stretched by the receding contact line of the liquid–air interface (figure 1(c)). By virtue of the small sample volume and parallel manipulation, a typical μDC stretching experiment can be carried out in less than one minute. After completion of the μDC stretching process, images of the immobilised DNA molecules were acquired using fluorescence microscopy.

Figure 1(d) shows a typical fluorescence image of stretched DNA (linearised 48.5 Kbp genomic DNA of λ bacteriophage) stained with YOYO-1 on a Zeonex modified hydrophobic glass coverslip. The length of the stretched λ DNA molecules using μDC was 28.4 ± 1.8 μm, in accordance with the results of macro-scale DNA combing on a surface (figure S1 available online at stacks.iop.org/NANO/30/045101/mmedia) [19, 21]. These results show that μDC offers easy operation for parallel DNA combing with high efficiency and performance.

2.2. Stretching uniformity analysis

In order to extract valid genomic information from DNA combing experiments, it is necessary to obtain a precise measurement of the molecular distances between genomic markers along DNA molecules. Therefore, stretching uniformity is a key property of any DNA extension method. There are three different kinds of stretching uniformity at three different levels: intra-molecular, inter-molecular and inter-channel level. The intra-molecular uniformity along a single molecule is crucial in order to achieve an accurate estimation of the distances between specific sites on the DNA. The inter-molecular stretching uniformity between different molecules in the same channel is important for quantitative analysis and reliable statistics of the DNA sample. The inter-channel uniformity, or the homogeneity of average stretching ratios of DNA between different microfluidic channels, is crucial for comparing between samples or experiments. Up to now, most studies did not address the uniformity issues, especially the intra-molecular uniformity, largely due to the lack of methods to precisely measure the local stretching ratio along individual DNA molecules. In this study, we developed a molecular ruler (see below for details) of DNA with repetitive fluorescently-labelled markers to precisely measure the DNA stretching uniformity in the three contexts.
To quantify the different aspects of stretching uniformity, we defined the apparent stretching ratio (ASR), namely the ratio between the measured length of the stretched molecule and the contour length of native B-form DNA, as follows:

$$\text{ASR} = \frac{l_{\text{physical}}}{l_{\text{contour}}}.$$  \hfill (1)

where the $l_{\text{physical}}$ is the physical length of the stretched segment as measured in the image and $l_{\text{contour}}$ is the theoretical contour length of this segment (0.34 nm bp$^{-1}$ for B-form DNA) [19].

### 2.3. Stretching uniformity along a single molecule

We used a bacterial artificial chromosome (BAC) containing a repetitive array from human chromosome 4 as a molecular ruler. This DNA molecular ruler contains 23 fluorescently labelled repetitive units with constant molecular length (3400 base pairs), allowing precise measurement of the stretching ratios of different segments along a single DNA molecule. To visualise single DNA molecules, we stained the DNA with the fluorescent dye YOYO-1, which can increase the contour length of the DNA when intercalated between the base pairs [49–51]. To minimise the potential stretching inhomogeneity along DNA molecules caused by uneven distribution of the fluorescent molecules, we used a high concentration of YOYO-1 (a YOYO-1:DNA bp ratio of 1:1) to oversaturate their intercalation sites [49]. A typical ruler, stretched by μDC on a Zeonex modified hydrophobic glass coverslip, is shown in figures 2(a)–(c). The distance between two neighbouring peaks in the repetitive fluorescence intensity profile was measured as $l_{\text{physical}}$ of a single repetitive unit in a stretched DNA ruler, allowing to calculate the ASR for each repetitive unit (ASR$_{\text{rep}}$) according to equation (1) (figure 2(d)). The stretching variation along a single molecule ($\Delta$SR$_{\text{rep}}$) is defined as the standard deviation of all ASR$_{\text{rep}}$ of a specific molecule divided by the mean ASR$_{\text{rep}}$ of that molecule. For instance, ASR$_{\text{rep}}$ for the ruler molecule shown in figure 2(b) was 1.782 ± 0.135 (mean ± standard deviation) and $\Delta$SR$_{\text{rep}}$, the stretching variation, was 7.6%. Next, we repeated the process for 14 other stretched rulers in the same channel to get a statistical single-molecule stretching variation. The corresponding average stretching variation of all 15 rulers was 5.1%. This variation could arise from various effects such as non-uniformity of the hydrophobic surface and speed perturbation of the DNA droplets.

### 2.4. Stretching uniformity in a single channel

In order to assess the stretching uniformity between different DNA molecules in a single sample, we measured the ASR of the entire repetitive region for each DNA molecule (ASR$_{\text{DNA}}$) in a given micro-channel and calculated the corresponding ASR variation ($\Delta$SR$_{\text{DNA}}$) between them. The ASR$_{\text{DNA}}$ data from seven parallel channels are shown in figure 2(e). For instance, the ASR$_{\text{DNA}}$ in channel 1 was 1.728 ± 0.123, with a stretching variation of 7.1%. The average stretching variation across all seven channels was 5.9%, similar to previous reports for DNA combing [19, 21].

### 2.5. Stretching uniformity between channels

Finally, we measured the stretching ratio variation between different channels. The results (figure 2(e)) show that the standard error of ASR$_{\text{DNA}}$ between the seven channels was only 0.034, indicating good stretching uniformity between channels. Furthermore, we used chi-square nonparametric hypothesis test for multiple samples to examine whether the ASR$_{\text{DNA}}$ distributions in the different channels (figure 2(e)) correspond to the same general distribution. The result of this test shows that all the different distributions correspond to a same distribution with a significance level $\alpha = 0.05$ (figure 2(e) and supplementary information).

To summarise, by using DNA ruler as the measurement, we show that the μDC technology allows DNA stretching with high ASR ratio (about 1.7), similar to the best performance of previously reported DNA combing techniques and much better than flow-based surface stretching methods [26, 30]. Moreover, our results show that μDC allows parallel DNA combing experiments with high stretching uniformity at the intramolecular, intermolecular and inter-channel level.

### 2.6. Robustness of μDC against change of droplet speed

In general, perturbation of experimental parameters may have a strong impact on the performance and validity of DNA combing. This is especially true for pressure driven experiments where variation in flow speed may occur between different experiments, and in our case also between different channels. In order to test the robustness of μDC to such inevitable experimental perturbations, we measured the relation between the ASR$_{\text{DNA}}$ and the average droplet speed. Overall, as shown in figure 3, the average ASR$_{\text{DNA}}$ tended to increase with increasing average droplet speed. However, with the droplet speed increasing over 10-fold, from 1 to 11.5 mm s$^{-1}$, the ASR$_{\text{DNA}}$ only increased by 6.3%, from 1.59 to 1.69. Therefore, we conclude that the stretching ratio of μDC is highly robust against the perturbation of droplet speed within the above-mentioned speed range, indicating high reliability and flexibility.

### 2.7. Quantification of UVB induced DNA damage using μDC

As a proof of concept for its application, we employed μDC for quantitative study of the extent of DNA damage induced by UVB irradiation and the spatial correlation of damage sites at the single-molecule level. In living cells, the self-repairing system, three-dimensional structure of genomic DNA and interaction between genomic DNA and proteins may influence both the level of UVB-induced DNA damage and the spatial correlation of damage sites. To mimic the realistic situation that UVB exposure on human beings, we investigate the UVB-induced DNA damage in living cells. Total genomic DNA was extracted from human HEK cells [32] immediately after UVB radiation at: 0, 70, 140, 700 and 1400 J m$^{-2}$. The group of cells with 0 J m$^{-2}$ UVB exposure is a control group to rule out DNA damage caused by other factors. UVB damage sites on purified genomic DNA were labelled with ATTO647-dUTP fluorescent nucleotides as previously
reported [32], and DNA backbone was stained with YOYO-1. Different samples were loaded on the μDC device in parallel for fluorescence imaging (figure 4). The degree of UVB damage was quantified by counting the number of damage sites on each DNA molecule and dividing this amount by the total length of sampled DNA (supporting information). Our results show an increase in DNA damage site density upon UVB irradiation in a dose-dependent manner until saturation is reached at 700 J m$^{-2}$ (figure 5(a)), in agreement with
probability of the distance between neighbouring damage sites follows a decreasing exponential distribution, indicating no spatial correlation between damage sites. To validate that this exponential distance distribution corresponds to a pure stochastic damage process, we performed in silico simulations, in which we randomly set damage sites onto a virtual DNA molecule and measured the corresponding distribution of the distance between neighbouring sites (figure 5(b) and supporting information). The excellent match between the experimental data and the simulation reinforced the conclusion that there is no significant spatial correlation between damage site locations. Notably, given the optical resolution of the system, our conclusion is restricted to distances above 348 nm (or 600 bp). Further study with super resolution imaging is required to decipher whether there is spatial correlation between DNA damage sites below this scale.

3. Experimental section

3.1. Microfluidic device design and fabrication

We prepared hydrophobic glass coverslips by spin-coating a hydrophobic polymer solution on clean glass coverslips. Glass coverslips (22 × 22 mm, Marienfeld, Germany) were soaked in a mixture of nitric acid 70% w/w and hydrochloric acid 37% w/w at a ratio of 2:1 overnight in a fume hood to corrode any fluorescent contaminants. The cleaned glass coverslips were carefully washed from both sides with DI water and then with ethanol. The glass coverslips were dried by N2 gas. Zeonex (480R, Zeon, Japan) was dissolved in chlorobenzene overnight to prepare a 1.5% w/v solution. Poly(methyl methacrylate) (PMMA) was dissolved in chloroform to prepare a 1.5% w/v solution. A 60 μl droplet of Zeonex or PMMA solution was applied to the centre of a dry glass coverslip by glass transfer pipette and spin coated at 2500 rpm for 90 s (WS-650HZB-223NPP, Laurell, USA). Another hydrophobic surface used in this work was a commercially available vinyl silane modified glass coverslip (CombiCoverslips, Genomic Vision, France). Microfluidic channels were prepared by solidifying PDMS in an aluminium mould machine according to the design in figure 1(b). PDMS oligomers (184 silicone elastomer base, Sylgard, USA) and cross-linking agent (184 silicone elastomer curing agent, Sylgard, USA) were mixed at a 10:1 mass ratio to prepare PDMS pre-polymer solution. This solution was first centrifuged to remove air bubbles, then poured into the aluminium mould and cured at 80 °C for 4 h in an oven (Thermo Scientific, Hera Therm). The solidified PDMS was pulled out from the aluminium mould and inlet/outlet holes were punched through using a flat-top syringe needle.

To assemble the microfluidic device, the PDMS block with microfluidic channels was placed on top of a Zeonex, PMMA or vinyl silane modified glass coverslip and pressed slightly for tight sealing of the device (figure 1(a)). A peristaltic pump (Lead Fluid, China) was connected to the outlet hole of the device and used to drive droplets of DNA solution into the system.

Figure 5. Quantification of UVB damage in genomic DNA extracted from cells treated with various UVB doses. (a) Density of UVB damage sites as a function of UVB irradiation dose. The ratio of the total number of damage spots to the total length of sampled DNA in kbps versus the amount of radiation is shown. (b) Experimental distributions of distances between neighbouring damage sites (full marks) and simulated random distribution (solid lines). Different colours correspond to different irradiation intensities, as indicated.

In the context of UV induced DNA damage, it is interesting to examine whether the damage event is influenced by different cellular contexts such as the 3D packaging of DNA in the cell nucleus, which would lead to non-stochastic spatial distribution of UV damage sites along the DNA. Along with the comparative measurement of DNA damage density reported above, we were able to quantify the spatial distribution of damage sites on individual DNA molecules and investigate whether there is spatial correlation between UVB damage sites (figure 5(b)). The results show that in all experiments using different doses of UVB irradiation, the previously reported macroscopic measurements of UV induced DNA damage [52].

Glass coverslips (22 × 22 mm, Marienfeld, Germany) were soaked in a mixture of nitric acid 70% w/w and hydrochloric acid 37% w/w at a ratio of 2:1 overnight in a fume hood to corrode any fluorescent contaminants. The cleaned glass coverslips were carefully washed from both sides with DI water and then with ethanol. The glass coverslips were dried by N2 gas. Zeonex (480R, Zeon, Japan) was dissolved in chlorobenzene overnight to prepare a 1.5% w/v solution. Poly(methyl methacrylate) (PMMA) was dissolved in chloroform to prepare a 1.5% w/v solution. A 60 μl droplet of Zeonex or PMMA solution was applied to the centre of a dry glass coverslip by glass transfer pipette and spin coated at 2500 rpm for 90 s (WS-650HZB-223NPP, Laurell, USA). Another hydrophobic surface used in this work was a commercially available vinyl silane modified glass coverslip (CombiCoverslips, Genomic Vision, France). Microfluidic channels were prepared by solidifying PDMS in an aluminium mould machine according to the design in figure 1(b). PDMS oligomers (184 silicone elastomer base, Sylgard, USA) and cross-linking agent (184 silicone elastomer curing agent, Sylgard, USA) were mixed at a 10:1 mass ratio to prepare PDMS pre-polymer solution. This solution was first centrifuged to remove air bubbles, then poured into the aluminium mould and inlet/outlet holes were punched through using a flat-top syringe needle.

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3.2. Surface treatment for μDC

The surface of a DNA combing platform significantly effects its performance [19, 53]. In order to optimize the μDC device, we tested four different substrates for DNA combing in our experimental system. All DNA combing experiments were carried out following the same protocol, and stretching uniformity was assessed. The measured ASRs for DNA ruler molecules on these substrates were 1.63 ± 0.15, 1.61 ± 0.13, 1.54 ± 0.12 and 1.28 ± 0.22, corresponding to Zeonex, PMMA, vinyl silane and amino silane modified cover-slips, respectively. The three hydrophobic glass cover slips showed higher and more uniform stretching ratios relative to the hydrophilic amino silane modified surface. Although all hydrophobic surfaces performed well in terms of stretching, Zeonex modified glass coverslips showed the best performance in terms of optical properties, with the least fluorescent background.

3.3. Preparing the DNA ruler

For preparation of the DNA ruler, we used the CHI6-29A23 BAC containing 23 repetitive units cloned from the D4Z4 macro satellite array in human chromosome 4. E. coli cells containing the BAC were cultured overnight in LB containing 12.5 μg ml⁻¹ chloramphenicol (Sigma-Aldrich, Rehovot, Israel) at 30°C. BAC DNA was purified from the cells using the NucleoBondXtra BAC kit (MACHEREY-NAGEL Inc. Düren, Germany).

The repetitive fluorescence pattern was created by a nick-labelling-repair reaction performed as follows: (a) the nicking enzyme Nb.BsmI, was applied to generate single-strand nicks at its specific recognition site (GAATGC); (b) a DNA polymerase enzyme was used to incorporate fluorescent nucleotides at the nicked sites; and finally, (c) a DNA ligase enzyme was utilised to repair the remaining single-strand breaks. 900 ng of BAC DNA was first reacted with 30 units of the enzyme (New England BioLabs Inc., Ipswich MA, USA) in 30 μl NEBuffer 3.1 for 120 min at 65°C. Next, the DNA was reacted with 15 units of Taq DNA polymerase (New England BioLabs Inc., Ipswich MA, USA) in the presence of the following nucleotides: dGTP, dCTP dATP (Sigma-Aldrich, Rehovot, Israel) and the fluorescent nucleotide dUTP-Atto647 (Jena Bioscience GmbH, Jena, Germany) at a final concentration of 600 nM each. The reaction was carried out in a reaction buffer (ThermoPol buffer, New England BioLabs Inc., Ipswich MA, USA) in a total volume of 45 μl for 60 min at 72°C. Finally, the DNA was reacted with 120 units of Taq DNA ligase (New England BioLabs Inc., Ipswich MA, USA) with 0.5 mM NAD+ (New England BioLabs Inc., Ipswich MA, USA), in a reaction buffer (ThermoPol Buffer, New England BioLabs Inc., Ipswich MA, USA) including 0.5 mM NAD+ (New England BioLabs Inc., Ipswich MA, USA) and 10 μM dNTP mix, in a total reaction volume of 60 μl for 30 min at 45°C.

The whole DNA molecule with fluorescently-labelled repetitive units is referred to here as a DNA ruler and used for the measurement of ASR of DNA molecules. The labelled DNA rulers were stained with YOYO-1 before being stretched and imaged.

3.4. Preparing DNA samples for UVB damage analysis

Samples for DNA damage analysis were prepared as previously reported [32]. Briefly, HEK cells were cultured in petri dishes and exposed to different doses of UVB radiation (70, 140, 700, 1400 J m⁻²) under a UVB lamp (302 nm UV, XX-15M, UVP, USA). Genomic DNA was extracted and purified from the exposed HEK cells immediately after irradiation using ‘GenElute-Mammalian Genomic DNA Mini-prep Kit’ (Sigma-Aldrich, Rehovot, Israel). The UVB damage sites were labelled with fluorescent ATTO 647-dUTPs then stained with the intercalating dye YOYO-1 before imaging in the μDC device.

3.5. DNA stretching and observation

The λ DNA, labelled DNA samples or DNA rulers were stained with YOYO-1 and diluted in MES buffer. The final concentrations were about 0.3 ng μl⁻¹ DNA, 0.5 μM YOYO-1 and 0.01 M MES buffer. 2 μl droplets of DNA solution were inserted into the inlet holes of the μDC device. A peristaltic pump was used to move the droplets into the microfluidic channels by applying negative pressure to the system. The speed of the droplets in the channels was controlled by tuning the flow rates of the pump. DNA molecules were linearly stretched on the surface at the receding line of the moving droplet. After stretching, the device was mounted on an inverted fluorescence microscope (Till photonics GMBH, Germany) and imaged using an EM-CCD camera (Ixon-888, Andor Technology Ltd Belfast, UK).

4. Conclusions

Stretching individual DNA molecules is a crucial step for optical DNA mapping, as it influences the resolution and fidelity of information readout along DNA molecules. Here, we report μDC, a method for parallel stretching of multiple DNA samples with small sample volumes. Using our DNA ruler as a quantification tool, we measured the performance of μDC and demonstrated its application for high throughput comparative analysis of DNA. μDC provides high stretching ratio, good stretching uniformity and is very robust to experimental perturbation. We also used the μDC device to analyse UVB induced DNA damage in HEK cells and showed that the damage density increased with increasing irradiation power until reaching saturation. We measured the distance between adjacent damage sites and found no significant spatial correlation between damage sites, indicating that UV damage is a stochastic process. By applying super-resolution imaging [19] and combining fluorescence methods for genetic and epigenetic labelling [33, 34, 54, 55], μDC could routinely generate rich, high quality genetic and epigenetic maps for research and medical applications. In addition, μDC allows easy integration with existing microfluidic
platforms for sample preparation, such as on-chip single-cell trapping [56], extracting genomic DNA [57, 58], purifying DNA and fluorescence labelling [59]. Such integrated devices can also be tailored for point-of-care biomedical applications and personalised diagnostics.

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Author contributions

LX and YE conceived the research. SW, LX, SZ and YE designed the experiments. SW performed the experiments and data analysis. JJ, AG, NG and HS contributed to sample preparation and data analysis. DT contributed to image analysis software. SW, JJ, DT, LX and YE wrote the paper.

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