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Three-Dimensional Positioning and Structure of Chromosomes in a Human Prophase Nucleus

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The work presents a high-resolution three-dimensional spatial structure of a partial human prophase nucleus using a 3D electron imaging method: serial block-face scanning electron microscopy. The structure reveals cylindrical chromatids aligned in a conserved parallel conformation.

Abstract

The human genetic material is packaged into 46 chromosomes. The structure of chromosomes is known at the lowest level, where the DNA chain is wrapped around a core of eight histone proteins to form nucleosomes. Around a million of these nucleosomes, each about 11nm in diameter and 6nm thick, are wrapped up into the complex organelle of the chromosome, whose structure is mostly known at the level of visible light microscopy to form a characteristic cross-shape in metaphase. However, the higher order structure of human chromosomes, between a few tens and hundreds of nanometres, has not been well understood. Here we show a three dimensional (3D) image of a human prophase nucleus obtained by serial block-face scanning electron microscopy (SBFSEM), with 36 out of the complete set of 46 chromosomes captured within it. The acquired image allows us to extract quantitative structural information about the nucleus and of the preserved, intact individual chromosomes within it, including their positioning and full spatial morphology at a resolution of around 50nm in 3D. The chromosome positions were found, at least partially, to follow the pattern of chromosome territories previously observed only in interphase. The 3D conformation shows parallel, planar alignment of the chromatids, whose occupied volumes are almost fully accounted for by the DNA and known chromosomal proteins. We also propose a potential new method of identifying human chromosomes in three dimensions, based on measurements of their 3D morphology.
Introduction

Since the very beginning of optical microscopy, investigations have been made into the different levels of structure found in human chromosomes and nuclei. The interpretation of information about the higher order structure of human chromosomes, at the level of fine structure introduced by the formation of nucleosomes [1-3], still remains controversial in the literature [4-8]. The familiar image of chromosomes, showing an X-shape due to the two arms of the sister chromatids joining at the centromere is, at least partly, caused by the standard preparation method of “dropping” the nuclear extract onto a flat surface. However, if the arms were free to flex, unconstrained by the mounting surface, they would be expected to follow relatively random directions pointing away from the tethering point of the centromeres. When the cell is entering metaphase, the DNA condensation process is believed to occur by self-assembly of more loosely coiled DNA originating in interphase onto a protein scaffold structures [9-11]. Without a scaffold, they might be expected to coil around each other [10], which would cause topological problems in the ability of the chromatids to separate at mitosis. The role of the scaffolding proteins avoids such undesirable coiling.

When chromosome "spreads" are generated, usually by dropping whole prophase or metaphase nuclei onto glass slides, the resulting chromosomes lie down flat after drying in almost all cases with the two chromatids side by side. This suggests, but does not prove, that the mitotic nuclear state of the chromosomes has parallel alignment of the chromatids. In order to resolve the question, we need 3D high-resolution images of the nuclei without flattening them for viewing under a microscope.

To answer these structural biological questions about human chromosomes and nuclei, we have developed new protocols for preserving the 3D structure of whole nuclei (with chromosomes) [12], so they can be imaged by powerful new 3D microscopy methods [13-15]. Here, a human prophase nucleus was imaged in three dimensions by serial block-face
scanning electron microscopy (SBFSEM) which uses a scanning electron microscope (SEM) with a built-in serial-sectioning microtome for 3D registration [13, 16].

The obtained 3D structural images provide unique and vital information because they allow the relative positions of the chromosomes to be determined within the internal space of the nucleus. This raises the question of how the chromosome territories (CT), known to form in interphase, collapse upon condensation of the chromatin and how the chromosomes are positioned in the nucleus before the formation of the spindle structure and the kinetochore. It is not believed that there are any sorting mechanisms active within the nucleus before spindle formation; hence, it would be surprising if the CT positioning information was lost upon the chromatin condensation, which leads to compact chromosomes just before prometaphase during the cell cycle [17-20].

It is also vital to identify the chromosomes themselves. We do not yet have multicolor fluorescence in-situ hybridization (M-FISH) type probes [21] available for electron microscopy, so we have to rely instead on the chromosome size and shape information to identify the chromosomes. This is analogous to flow-cytometry karyotyping, where chromosomes are identified by the strength of the fluorescence signal from a dye that is quantitatively bound to the DNA. For complete identification, two dyes that bind differently to AT- and GC-rich sequences are needed [22, 23]. However, from our 3D imaging results, the chromosomes can be ranked according to their volumes/sizes and can be grouped by this information fairly reliably. The centromere positions provide secondary identification information, which is also used in our classification.
Fig. 1 Two slices from the acquired SBFSEM stack of 300 backscattered electron (BSE) micrographs of the nucleus with zoomed-in insets of two selected chromosomes. (A) Slice No. 36 out of 300. (B) Slice No. 127 out of 300. The insets in panels (A) and (B) are a broken chromosome and an identified intact chromosome A1 (assigned chromosome 1, see explanation in the text). The pixel size of the BSE micrographs is 11nm × 11nm, and the sectioning thickness is 20 nm.

Results

SBFSEM Micrographs of a Human Prophase Nucleus

Fig. 1 shows two slices from the series of 2D backscattered electron (BSE) micrographs acquired by the SBFSEM measurements. It clearly presents the distribution of condensed individual chromosomes in the nucleus [24-26] with a nearly circular envelope. Two of the chromosomes are highlighted in the zoomed-in insets of Fig. 1. A distinct porous network structure has been revealed in the imaged chromosomes, and the cross-sections of the chromatid pairs of some of the chromosomes are visible in the original BSE micrographs. These features are easily distinguishable in the 3D maps without any further processing due to the high contrast in the BSE micrographs. As the DNA chain wraps around the proteins in
the chromatin, we believe that the black regions in the imaged chromosomes are protein-DNA complex “dyed” with Pt stain since, SBFSEM does not have sufficient resolution to distinguish the DNA from the chromosomal proteins, although the Pt stain is nominally DNA-specific [27].

Fig. 2 3D characterization and analysis of the imaged chromosomes within the human prophase nucleus. (A) 3D rendering of all the observed chromosomes in the nucleus with its envelope in transparent blue; the intact chromosomes are in yellow or green, and the broken ones are in red. (B) 3D rendering of the intact chromosomes only in the nucleus. (C) Measured chromatid volumes versus the number of base pairs of the accordingly assigned
chromosomes from the human genome sequence, with a linear fit. (D) Correlation between the measured chromatid volumes and the radii of the assigned chromosomes from the centre of the nucleus. (E) Correlation between the gene density of measured chromosomes and their radii from the centre of nucleus. The chromosomes have been labelled by their assigned chromosome numbers in panel (E).

3D Spatial Structure of the Human Prophase Nucleus (with Chromosomes)

Fig. 2A-B and Supplementary Videos S1 and S2 demonstrate individual chromosomes and their 3D spatial distribution in the human prophase nucleus with its envelope revealed by SBFSEM. In total, 36 out of the 46 chromosomes were captured; 19 of them were intact and the other 17 were broken. The intact chromosomes are in yellow or green, and the two green ones are the chromosomes that have close contact with other chromosomes. The broken chromosomes in red are those chromosomes that are not fully visualized either because they were already partially cut away during the sample preparation (before the measurement) or because they were not completely sectioned by the SBFSEM system. From Fig. 2A-B and the videos, we can see that the distribution of the chromosomes in the nucleus is not random, but grouped towards the two sides of the nucleus. Table 1 presents the volume statistical results of all the intact chromosomes identified within the nucleus, and the lengths and diameters of some of them are listed in Table 2. The volumes of the broken chromosomes are listed in Supplementary Table S1 as a reference. Compared with the known distribution of DNA sizes from the human genome [28], it allows the classification of the measured intact chromosomes into groups of similar sizes following the international convention [29] based on their volumes/sizes (in three dimensions): group A for chromosomes 1-3, group BC for chromosomes 4-7 and X, and groups C and D for chromosomes 8-12 and 13-22 & Y, respectively. As expected, the distribution of volumes/sizes of the chromosomes indicates
that the sampling [30] of the 36 chromosomes out of the full set of the 46 is approximately random.

Fig. 3 Rendering of the identified intact individual chromosomes. (A) An SBFSEM slice of the chromosome A1, assigned as chromosome 1. This is a different slice from the inset in Fig. 1b. (B) 3D rendering of the chromatid separated chromosome A1 showing cavities in solid light green, green and blue. (C) 3D rendering of the cavity network in chromosome A1 viewed through a transparent chromosome surface. Bottom row: 3D rendering of the surface views of the chromatid separated chromosomes A2 (D), A4 (E) and BC1 (F), which are assigned as chromosomes 2, 3 and 6. The main scale bars in panels b-f are 500nm in each direction. The insets at the bottom-right corners of panels (D), (E) and (F) are the SBFSEM slices of the corresponding chromosomes A2, A4 and BC1, the scale bars in these are 1 micron.

Human Prophase Chromosome Structure in Three-Dimensions

We can see that the chromosomes imaged in our experiment are tightly packed without a great portion of “empty” space (Fig. 1 and 3), since, on average, only about 6% of the
observed chromosome volume appears as cavities, in the form of less-dense regions in the imaged chromosomes, and the remaining 94% (Table 1) is the “dyed” chromosomal protein-DNA complex. Also, the larger chromosomes, in groups A/B, appear to have slightly denser compaction (fewer cavities) than the smaller ones, in groups C/D, as the first 7 chromosomes in Table 1 have on average 95.6% of their volume occupied by chromosomal protein-DNA complex, and the later 12 chromosomes have about 93.3% of this value. As stated above, we believe that the black regions in the BSE micrographs of the chromosomes are chromosomal protein-DNA complex. In chromosome A1 (Fig. 3A-C), a typical example, there are a few cavities (in blue) connected to the external space surrounding the chromosome, and the majority of cavities (in light green and green) are totally sealed inside the chromosome. The latter cavities need to be counted as part of the internal structure of the chromosomes, and we think they correspond to either unstained proteins or cytoplasm-filled empty space within the chromosomes. The cavities are observed to distribute evenly along the two sister chromatids of the chromosome (Fig. 3C) and are positioned near the central axis of the chromatids. Those cavities, in green in 3D images or in white in the middle of Fig. 3A, which are at the interface region of the two sister chromatids (transition colour region in the middle of Fig. 3B-C) are generally larger than the cavities fully sealed inside the single chromatids. This is not hard to understand since the paired chromatids are structured for their ultimate separation into two single chromatids in metaphase, and the separation appears to be already starting to take place to generate more space in between.

In most cases, as shown in Fig. 3 and 4A-C, the chromosomes were observed to have two parallel chromatids with roughly the same (curved) cylindrical shape. In some cases, particularly for smaller chromosomes (Supplementary Fig. S1), the interface between the two chromatids is indistinct, which makes it hard to determine and to separate the chromatid pairs. However, in the majority of the examples, it was possible to segment the images into pairs of
chromatids reliably (Fig. 3 and 4). To verify the similarity in conformation between the two sister chromatids, a superposition calculation was carried out in 3D to overlay the pairs through volume registration using Avizo software (Fig. 4D-F). As seen, the chromatid pairs in each chromosome match with each other in both shape and size with an average volume difference of about 5%. Despite the fact that the segmentation of the sister chromatids is a semi-automatic work with some subjective processing, the resulting volume differences between the sister chromatids are narrowly distributed from 0.31% to 9.13% (Table 2). Examples are provided in Table 2 with the lengths, cross-sectional areas and diameters of the chromatids measured using image tools provided by Avizo. The diameters of the chromatid cross-sections are notably narrowly distributed with most of the variation in chromosome sizes/numbers accounted for by their lengths. On average, the diameter of the chromatids is about 765nm. This suggests that a highly orchestrated folding of the sequence takes place which is uniformly conserved across all the chromosomes. We can also see from Table 2 that whenever the two sister chromatids do not have identical sizes, the longer ones always have smaller cross-sections. This indicates that the two sister chromatids with identical amounts of DNA appear to conserve volume, but probably do not preserve their morphologies exactly during the chromatin condensation.
Fig. 4 Characterization and analysis of chromatid pairs of selected chromosomes. Left, middle and right columns show chromosomes A3, BC3 and D2 respectively, which are the assigned as chromosomes 3, 7 or X and 16 accordingly to the scheme of Table 1. (A), (B) and (C) 3D rendering of the chromatid-separated chromosomes. (D), (E) and (F) 3D superposition of the chromatid pairs through volume registration using Avizo. The scales are 500nm in each direction.

It was also striking that the three-dimensional shapes of the chromosomes were far from “flat”; a selection of examples is shown in Fig. 3 and 4 with the chromatid pairs colour coded. The common shape for the larger chromosomes was not X-shaped, but S-shaped (chromosomes A2 and A3) or C-shaped (chromosomes A1 and A4), while the “classical” X-shapes were seen in the smaller chromosomes (chromosomes BC3 and D2). This is probably because to form a S- or C-shape, the chromosomes need to be long enough to bend a couple of times; however, to form a X-shape just needs one knot point between two chromatids (see Fig. 4C). In all cases, the two sister chromatids follow a very similar shape, as if they were bonded together all along their length (Fig. 3 and 4). It is apparent that the overall structure is more flexible transverse to the pairing direction than along it, with a flat plane of separation.
between the chromatids. It is also significant that no examples of twisted chromatid pairs were found either.

**Discussion**

Looking further into all these findings, the volume information from Table 1 and the morphology details from the Figures not only allow us to designate the chromosome groups as shown above, but also to roughly identify all the intact individual chromosomes and to obtain the volume per base pair of the imaged chromosomes accordingly. After a global comparison of the volumes of all the 36 measured chromosomes, both the intact and broken ones, we can assume that the biggest measured intact chromosome, A1, is very likely to be human chromosome 1 or 2. After checking the centromere positions, we assign the imaged chromosome A1, to be chromosome 1. We then assigned all the other imaged chromosomes to the chromosome numbers with closest agreement of relative volume to relative genome size. The full assignment (in Table 1 and Fig. 2) was finished after a combined consideration of the centromere positions and shapes of the chromosomes. The known distribution of sizes among the human chromosomes 1-23 from both flow cytometry [3] and the human genome sequence [28] is well represented by the 19 measured examples in Fig. 2C. The slope of the linear relationship of Fig. 2C, 6.69±0.087 (with r²=0.997) provides the volume per base pair from our experiment as 6.69nm³. To check for ambiguity in the assignment, if we choose the biggest chromosome A1 to be chromosome 2 instead, and follow the same assignment process, the fitting gives a slope of 7.16±0.15, and r²=0.991 (Supplementary Table 2 and Fig. 2). This demonstrates that the first assignment is a better solution.

We can also calculate the expected volume per base pair. The base pairs in the DNA double helix structure can be considered as a cylinder which is 2nm in diameter with a spacing of 0.34nm, thus occupying a volume of 1.07nm³ per base pair [31, 32]. The nucleosome, which contains about 146 base pairs [3, 33], can be considered also to have a cylindrical shape with
a diameter of 11nm and thickness of 6nm [3]; hence, this histone-DNA complex has an aggregate volume of $3.91\text{nm}^3$ per base pair. It is about four times the size of the DNA itself because of the solvent-filled spaces and the eight histone proteins making up its core particle [1]. It has been determined that histones make up about 60% of the total protein mass of chromosomes in metaphase [34], so we can estimate that if the histone protein complement takes up $(3.91-1.07)\text{nm}^3=2.84\text{nm}^3$, then the remaining non-histone chromosomal proteins would occupy $(1-0.60)/0.60\times2.84\text{nm}^3=1.89\text{nm}^3$, so the chromosomal protein-DNA complex total volume is $(3.91+1.89)\text{nm}^3=5.80\text{nm}^3$ per base pair. According to this, the estimated volumes of a single chromatid of a human chromosome can be calculated, and the volumes of assigned ones are listed in Table 1. Our experimentally measured volume per base pair, $6.69\text{nm}^3$, is 15% higher than the calculated value of $5.80\text{nm}^3$. It suggests that our interpretation of the experimental results is reliable with respect to the chromosome size and shape measurement and confirms that the volumes of the 3D images we have obtained are largely accounted for by the complement of DNA and known proteins, with very little “empty” space. We can speculate that the measured volume is larger than the calculated one because the Pt stain (in the chromosomes) and additional cavities, which are too small to be resolved by SBFSEM. It is also known that the genome sequence length is less than the full DNA length.

The observed 3D shapes and tight parallel structure of the chromatid pairs without twisting (Fig. 3 and 4) can be understood by assuming inter-chromatid interactions effectively bonding each other along the entire length of the chromatids. If there were explicit contacts between the chromatids, these would impede any lateral flexing of the structure, but still allow it to flex in the non-paired direction. There are considerable implications for the nature of the scaffold structure if it is not allowed to twist; it may be that intertwining of the chromatids is prevented by anisotropies of the scaffold structure, or that the inter-chromatid
contacts are built in early in the condensation and serve to guide the construction of the scaffold. With further consideration of the porous network structure of chromosome A1 (Fig. 3A-C), we hypothesise that the appearance of cavities distributed along the central axis of the chromatids might be due to unstained scaffolding protein structures.

Since the 3D structure of the partial nucleus was acquired (Fig. 1, 2A-B), the positions of all the imaged chromosomes inside the nucleus are retrievable from the 3D image. This presents us with the opportunity to follow the destiny of the chromosome territories (CTs) [35] established during interphase, earlier in the cell cycle. The correlation between the volumes of chromatids and the radii of the (assigned) chromosomes from the centre of nucleus is presented in Fig. 2D. The radius here equals the distance between the centre of mass of each chromosome and the centre of mass of the measured partial nuclear envelope. The observed positive correlation demonstrates that the smaller chromosomes are generally closer to the centre of the nucleus and that the larger chromosomes are nearer to the nuclear periphery, except for the biggest chromosomes. However, the preferred position of the chromosomes seems to have a stronger relation to their gene density (Fig. 2E). In agreement with a previous report [36], the assigned gene-rich chromosome 19 (chromosome D5) is found to be located closest to the nuclear centre, while the assigned gene-poor chromosome 18 (chromosome D4) is one of the chromosomes staying furthest from the nuclear centre (Fig. 2E). Since these non-random trends in the locations of chromosomes in the measured prophase nucleus agree with the CT information in interphase revealed by previous research [35-37], this provides evidence to suggest that the CT arrangement in the nucleus is at least partially preserved from interphase up to the prophase of the cell cycle. This also suggests that the chromatin condenses locally within its interphase CT before the chromosomes start to move to the equatorial plate of the cell. In a future study, the condensation process of the chromosomes
could be investigated in more detail by comparing the 3D structure of chromosomes between the earlier and later stages of prophase.

In summary, our analysis of the 3D spatial structure of a human prophase nucleus containing condensed chromosomes has revealed that the chromosomes have a porous network structure and that, in 3D space, they can be S- and C- shaped and not only X-shaped. The larger chromosomes were more likely found to show S-shape or C-shape, while the smaller chromosomes tend to be X-shaped. Experimentally, the measured volume per base pair is found to be \(6.69\pm0.087\text{nm}^3\), which is accounted for by the calculated volumes of DNA and known chromosomal proteins, with a 15% excess. The sister chromatids have curved cylindrical shapes with a well-conserved diameter of around 765nm. The chromatids are about 2-3\(\mu\)m long and remain in contact with each other all along their length without twisting, possibly because of interaction with or support from a scaffold “backbone”. The size analysis of the sister chromatid pairs indicates that while they are not exactly identical in morphology during the chromatin condensation process, the two sister chromatids have similar volumes. The measured chromosomes can be roughly identified, even without the full set of 46 chromosomes, by the analysis of their volume/size distributions in combination with their centromere positions in space. This method could be used in future for identifying human chromosomes through their 3D imaging approaches, avoiding the need for M-FISH type tools. The characteristic non-random positioning of the chromosome territories in prophase resembles the pattern found in interphase [35-37]: the smaller chromosomes are found closer to the centre of the nucleus while the larger ones are nearer the periphery. The radial position of the chromosomes within the nucleus also seems to correlate with their gene density, with the gene-rich chromosomes near the nuclear centre and gene-poor ones near the periphery. This suggests that the chromatin condenses locally, and the chromosome territories are at least partially maintained from interphase into prophase of the cell cycle.
**Materials and Methods**

**Chromosomes and Nucleus Embedding**

The chromosomes and nuclei were obtained by bursting grown cells from a registered B-lymphocyte Yoruba cell line (passage 4, male) cultured at 37°C in a 5% CO₂ atmosphere. The cells were thymidine synchronised for 16 hours using 2mM thymidine followed by a 0.2 μg/mL Colcemid (Gibco Life Technologies, UK) treatment for 6 hours and a hypotonic treatment at 37°C using 0.075M KCl for 5min. Then the sample was fixed in three changes of 3:1 methanol:acetic acid, and then chemically fixed again by 2.5% (vol/vol) glutaraldehyde in 0.1M cacodyl ate buffer (pH 7.2) for 1 hour. After fixation, the sample was stained with 5mM platinum blue (self-synthesised in the laboratory) for 30 minutes at room temperature, and was then washed twice with milli-Q water and was dehydrated by ethanol-water solutions (30%, 50%, 75%, 100%) for 15 minutes each. The sample was centrifuged after every washing or dehydration step to remove the treatment solution. After all the above procedures, we expect to obtain isolated nuclei and to remove the general contamination from other organelles as much as possible, which would minimise the consumption of the platinum blue stain and maximise our chance to gain homogeneously fully-stained nuclear specimens containing chromosomes inside. The chromosomes and nuclei were finally embedded in a small volume of a four-component epoxy resin and left to cure overnight at 60°C, subsequently, more fresh resin of the same recipe was added to fill the container to form a second part, and left at 60°C again for about another 20 hours to be fully cured [12]. The epoxy resin made by following the standard recipe of hard resin based on Agar-100 epoxy from Agar Scientific, Elektron Technology UK Ltd.

**SBFSEM Measurement**

The cured sample was trimmed to a pyramid-shaped block with a top face of about 500μm × 500μm using a conventional ultramicrotome (Leica Ultracut UCT) after mechanical polishing.
Finally, the sample was serially sectioned and imaged by the SBFSEM system in an FEI Quanta 250 field emission gun environmental SEM (FEGESEM) at 5kV under a chamber pressure of 60Pa of water vapour. The pixel size of generated BSE micrographs was set to 11nm × 11nm to cover the whole nucleus. The nominal sectioning thickness is 20nm per slice with a diamond knife (horizontal) cutting speed of 0.3mm/s; totally, 300 slices were collected for the measured sample.

Supplementary Materials

Supplementary material accompanies this article is at http://advances.sciencemag.org

Supplementary Video S1 Three-dimensional (3D) rendering of the measured prophase nucleus.

Supplementary Video S2 Three-dimensional (3D) rendering of the measured prophase nucleus from another orientation.

Supplementary Table S1 Volume statistics of all the broken chromosomes in the nucleus.

Supplementary Fig. S1 SBFSEM slices of the measured (smaller) chromosomes D5 (A) and D6 (B).

Supplementary Table S2 Reassignment of all the intact chromosomes as a comparison with the assignment in the main text.

Supplementary Fig. S2 Second linear fitting of the chromatid volumes against their base pair numbers.
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**Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and the Supplementary Materials. Additional data related to this paper may be requested from the authors.
### Table 1. Volume statistics of all the intact chromosomes in the nucleus

| Chromosome (Number) | Chromatid Volume $^*$ /V1 ($\mu$m³) | Cavity Volume $^+$ /V2 ($\mu$m³) | Ratio of $^\dagger$ $V_1/(V_1+V_2)$ (%) | Assigned Chromosome (Number) | Calculated Chromatid Volume $^\ddagger$ ($\mu$m³) |
|---------------------|-----------------------------------|----------------------------------|----------------------------------------|-----------------------------|---------------------------------------------|
| A1                  | 1.655                             | 0.087                            | 95.0                                   | 1                           | 1.446                                       |
| A2                  | 1.650                             | 0.048                            | 97.2                                   | 2                           | 1.411                                       |
| A3                  | 1.328                             | 0.047                            | 96.6                                   | 3                           | 1.148                                       |
| A4                  | 1.263                             | 0.037                            | 97.1                                   | 3                           | 1.148                                       |
| BC1                 | 1.124                             | 0.083                            | 93.1                                   | 5                           | 1.049                                       |
| BC2                 | 1.092                             | 0.076                            | 93.5                                   | 6                           | 0.992                                       |
| BC3                 | 1.056                             | 0.036                            | 96.7                                   | 7 (or X)                    | 0.923                                       |
| C1                  | 0.993                             | 0.102                            | 90.7                                   | 8                           | 0.849                                       |
| C2                  | 0.972                             | 0.053                            | 94.8                                   | 9                           | 0.819                                       |
| C3                  | 0.963                             | 0.074                            | 92.9                                   | 10                          | 0.786                                       |
| C4                  | 0.955                             | 0.054                            | 94.7                                   | 11                          | 0.783                                       |
| C5                  | 0.895                             | 0.049                            | 94.8                                   | 12                          | 0.777                                       |
| D1                  | 0.776                             | 0.054                            | 93.5                                   | 13                          | 0.668                                       |
| D2                  | 0.625                             | 0.066                            | 90.4                                   | 16                          | 0.524                                       |
| D3                  | 0.603                             | 0.037                            | 94.2                                   | 17                          | 0.471                                       |
| D4                  | 0.572                             | 0.045                            | 92.7                                   | 18                          | 0.453                                       |
| D5                  | 0.509                             | 0.029                            | 94.6                                   | 19                          | 0.343                                       |
| D6                  | 0.435                             | 0.035                            | 92.6                                   | 22                          | 0.298                                       |
| D7                  | 0.213                             | 0.014                            | 93.8                                   | 21                          | 0.279                                       |

$^*$Here, the chromatid volume is half of the measured volume of the chromosomes without cavities, i.e. half of the volume of the black region only in the imaged chromosomes with chromatid pairs measured by Avizo;

$^+$Here, the cavity volume is half of the volume of all the cavities in the chromosomes with chromatid pairs;

$^\dagger$Here, the calculated chromatid volumes were obtained by multiplying the sequence length of the assigned human chromosomes (in MBp) from the database by 5.80 nm³. The database is Archive Ensembl Release 68, July 2012. Its permanent link: http://Jul2012.archive.ensembl.org/Homo_sapiens/Location/Chromosome?r=1

The volume per base pair, 5.80 nm³, was obtained by theoretical calculation which is explained in the main text.
Table 2. Statistical analysis of the chromatid pairs of selected intact chromosomes

| Chromosome (Number) | Chromatid Length (μm) | Ave. of Cross-Section Area (μm²) | Ave. Diameter of Cross-sections (μm) | Difference of the Chromatid Pairs in Volume (%) |
|---------------------|-----------------------|----------------------------------|--------------------------------------|-----------------------------------------------|
| SSC1                | 3.241                 | 0.524                            |                                      |                                               |
| A1                  | 2.801                 | 0.530                            | 0.819                                | 1.84                                          |
| Ave.                | 3.021                 | 0.527                            |                                      |                                               |
| SSC1                | 2.869                 | 0.512                            |                                      |                                               |
| A2                  | 3.007                 | 0.492                            | 0.799                                | 6.54                                          |
| Ave.                | 2.938                 | 0.502                            |                                      |                                               |
| SSC1                | 2.828                 | 0.438                            |                                      |                                               |
| A3                  | 2.744                 | 0.470                            | 0.760                                | 3.50                                          |
| Ave.                | 2.786                 | 0.454                            |                                      |                                               |
| SSC1                | 2.766                 | 0.421                            |                                      |                                               |
| A4                  | 2.768                 | 0.443                            | 0.742                                | 0.31                                          |
| Ave.                | 2.767                 | 0.432                            |                                      |                                               |
| SSC1                | 2.236                 | 0.492                            |                                      |                                               |
| BC1                 | 2.531                 | 0.453                            | 0.776                                | 9.00                                          |
| Ave.                | 2.384                 | 0.473                            |                                      |                                               |
| SSC1                | 2.085                 | 0.439                            |                                      |                                               |
| BC3                 | 1.914                 | 0.468                            | 0.760                                | 4.61                                          |
| Ave.                | 2.000                 | 0.454                            |                                      |                                               |
| SSC1                | 1.732                 | 0.375                            |                                      |                                               |
| D2                  | 1.647                 | 0.396                            | 0.701                                | 9.13                                          |
| Ave.                | 1.690                 | 0.386                            |                                      |                                               |

Here, the SSC1 is the abbreviation for single sister chromatid 1; the SSC2 is the abbreviation for single sister chromatid 2; the Ave. is the average value of the above two.

§Here, the chromatid lengths were obtained by measuring the separated two chromatids of the chromosomes, respectively, using Avizo;

‖Here, “Ave. of Cross-Section Area” denotes the average of the areas of a few cross-sections along the central axis of the chromatids, which were carried out using Avizo. “Ave. Diameter of Cross-sections” denotes the diameter calculated from the values of the average of the “Ave. of Cross-Section Area” when we assume the cross-sections are circles;

*Here, the difference was obtained by superposition calculation on the two chromatids by 3D volume registration using Avizo. The difference (in percentage) equals \(2 \times \text{abs}(V_{C1} - V_{C2})/(V_{C1} + V_{C2})\) * 100%, where \(V_{C1}\) is the volume of the single sister chromatid 1; \(V_{C2}\) is the volume of the single sister chromatid 2.