# MiniReview-High order mitotic chromosome structure investigation using 3D microscopy and future direction.

Yusuf M[1,3], Kaneyoshi K[2], Fukui K[2*] and Robinson IK[1,5]

1) London Centre for Nanotechnology, University College London, London WC1H 0AH, UK

2) Laboratory of Dynamic Cell Biology, Department of Biotechnology, Graduate School of Engineering, Osaka University, Suita, Osaka, Japan

3) Centre for Regenerative Medicine and Stem Cell Research, Aga Khan University, Karachi, Pakistan

4) Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan

5) Brookhaven National Lab, Upton NY 11973, USA

**Abstract**

The enigma of how a metaphase chromosome is constructed remains unclear with the higher order structure still under investigation. It is clear that this is a 3D structural problem requiring the development of new technologies necessary to answer this long standing mystery. This mini-review highlights new and recent, advanced 3D imaging methods that have been used to image mitotic human chromosomes. These 3D methods have advantages and limitations that are both discussed, Furthermore chromosome sample preparation and future imaging directions are also highlighted.

## Chromosome structure

The DNA in chromosomes holds the genomic information of all eukaryotes. Throughout the cell cycle progression, chromosomes condense into the higher order compact structures until they reach mitosis. The biological implications of this chromosome higher-order structure is for packaging the long DNA molecules into chromatin fibers within chromosomes facilitating upon cell division the separation of the chromatids for transportation of DNA fibers evenly to two new daughter cells without damage (1). The higher order structure of mitotic chromosomes has been under investigation for more than these three centuries with the full structural details still remaining unknown. Our current understanding is that the 2 nm thick DNA with 146 base pairs (bp) wraps twice around an octamer of histone proteins forming a series of nucleosomes; this protein-DNA complex is called chromatin (2). The X-ray crystal

structure of the nucleosome has been resolved down to Angstrom resolutions (3, 4) with the largest assembly to date being the tetranucleosome at 2.9 Å (5, 6). Recently a cryoelectron microscopy (EM) study using 12 x 187 bp and 12 x 177 bp nucleosomes with reconstituted fibers in presence of histone H1 has also shown repeating tetra-nucleosomal structural units (7). These nucleosomes form a “bead on a string” like structure having 11 nm diameter (8). The binding of the linker histone (H1 or H5) organises the nucleosome arrays into a more condensed 30 nm chromatin fiber (9). Linker histone (e.g., H1 or H5) bound to a single nucleosome is known as a chromatosome and have shown to be involved in the 30 nm structure that is compacted further into a mitotic chromosome. Even though several models have been proposed for the 30nm fiber such as the one-start helix/solenoid model (10) and zigzag mode (11, 12), many studies even question the existence of this structure (13-15). Proteins play an important role in the compaction of mitotic chromosome with over 158 proteins identified on chromosomes including the histones (16). The backbone structure of chromosomes after depletion of histone proteins is known as the chromosome scaffold (17). This consists of non-histone proteins, so-called scaffold proteins (18, 19). To fully understand the inner structure of the chromosome, both the chromatin fiber and the chromosome scaffold need to be understood (20). Different scientific approaches that have contributed hugely towards understanding how chromosomes are folded, compacted and organised, have been employed. Chromosome conformation capture techniques such as Hi C have been recently used to understand the contacts between chromatin fibres (21, 22). Great efforts have also been made towards imaging chromosomes using different microscopy methods (20, 23, 24). It is clear that 3D techniques are needed to study the intact mitotic chromosome. In this mini-review, we discuss the 3D microscopy approaches used for investigating the higher order chromosome structure. The limitations and future directions are also discussed.

**Chromosome sample preparation procedures**

Chromosomes are generally prepared from live cells after growing in cell culture (25). At metaphase they are at the most compact state making them easier for isolation and analysis. Cells are treated with a mitotic inhibitor e.g. colcemid or nocodozole (26) that disrupts the spindle fibers. After treatment with a hypotonic solution such as ?M KCl that swells the cellular volume, the sample is then fixed using (most commonly) methanol-acetic acid (MAA) that preserves the chromosomes (27). Such preparations,

prepared on glass slides, are used routinely in clinical cytogenetic laboratories for disease investigation (25, 27). Fixation using MAA causes partiel denaturation and precipitation of nucleic acid and proteins (28) therefore other types of chromosome preparations have been developed (29, 30). The in-solution preparation method known as polyamine chromosomes is similar to the MAA method for preparation. After the KCl hypotonic treatment, the cells are burst using centrifugation. The chromosomes are transferred into a polyamine solution, containing spermine and spermidine. These chromosomes are more compact and only stable in the solution (31). They are widely used for a number of applications including flow cytometry (32) and microfluidic applications (33).

**Imaging of chromosomes using 3D methods**

Chromosome size, number and morphology have been well-characterised using standard 2D optical microscopy (27). Banding methods allows investigation of structural alterations and numerical abnormalities (34). G-banding that distinguishes the states of chromatin being hetrochromatin and euchromatin can be identified showing as dark and light bands on the chromosome respectively (27). The use of fluorescent dyes further allowed genes to be localised using *in situ* hybridisation (35). Determination of the higher order mitotic chromosome structure using the optical microscope is not possible as the resolution is limited to the diffraction limit of light (~200 nm), making it difficult to resolve the smaller structures (36).

Ultra-high resolution microscopy has been used to investigate mitotic chromosomes at high resolution. Even though the TEM has Angstrom resolution, it is not a suitable microscopy for studying intact mitotic chromosomes because chromosomes are too thick for the electrons to penetrate through biological samples over one micron thick (37). Whole human chromosomes have been imaged and reconstructed in 3D by TEM tomography after the sample was chemically prepared (38, 39). 41 projection images were obtained after tilting the sample from -60 to + 60 degrees to reconstruct. This led onto the reconstruction of a 3D chromosome in which internal structure showed 26 nm to 58 nm features and clearly showed distribution of 30 nm fibre that are consistent with the published looping model (38).

Scanning Electron Microscopy (SEM) images the sample surface by scanning it with

a focused electron beam and collection of backscattered electrons. The major limitation has been due to sample preparation as MAA chromosomes show an artificial surface "skin" layer over the chromosomes following air-drying (40). A "drop-cryo" method was developed for barley chromosomes that allowed routine investigation of the surface structure information. On the arms of the chromosomes, chromomeres of around 200-300 nm were observed that had a knot-like structure representing highly condensed regions while the centromeres showed parallel fibers (41, 42). Removal of nucleoplasm from the surface of MAA human chromosomes has been investigated after treating the chromosomes using a commercial enzyme product, cytoclear [40].

Barley chromosome structure has been investigated after Focussed Ion Beam SEM (FIB-SEM) imaging (43). After performing the drop-cryo method the samples were critical-point dried. Pt-blue staining was performed and the phosphorylated histone H3 (serine 10) was labelled with immunogold. Slices were taken using a $Ga^+$ beam from the sample surface after it was tilted 54 degrees. The dissected chromosome showed many cavities within the cross-section. A full 3D reconstructed chromosome was achieved from a series of 198 cross-sections at resolutions between 1.5-3 nm but blurred by a labelling diameter between 15-30 nm (see Figure 1); (43). This method has a great advantage to obtain images with high resolution brought from direct observation of exposed inner structure, however, sample damage caused by accelerated $Ga^+$ beam cannot be ignored.

Serial Block Face SEM (SBF-SEM) allows 3D information and is similar to FIB-SEM but uses a diamond knife instead of focused gallium ions to cut the sample (44). The sample is first embedded into a resin and dissected by ultra-microtome within the microscopy chamber, allowing serial inner structure images by SEM. Human chromosomes have been investigated using this methods that allowed full 3D images of polyamine preparations (see Figure 2); (45). SBF-SEM has been used on a human prophase nucleus where 36 out of 46 were analysed. Chromosomes showed porous network structure after MAA treatment and platinum blue staining. With a resolution of around 50 nm in 3D, the internal structure could not be determined however chromosomes showed parallel arrangement of chromatids. Sister chromatids showed curved cylindrical shapes with a well-conserved diameter of around 765 nm that were 2 to 3 μm long (46). A correlative approach known as 3D Combined light microscopy and serial block-face scanning electron microscopy (CLEM) was used to study the mitotic chromosomes. After analysis of wild-type and Ki-67- depleted chromosomes

it was shown that the periphery was 30%–47% of the entire chromosome volume with more than 33% of the protein mass of isolated mitotic chromosomes determined by quantitative proteomics. This study concluded that the chromatin made a small percentage of the total mass of metaphase chromosomes (47). SBF-SEM and FIB-SEM both involve serial cutting of the sample that destroys the original sample. Both the Diamond knife and Ga ion beam can leave an amorphous layer after sectioning and also leave damage.

In contrast to TEM, X-rays have the ability to penetrate through whole intact chromosomes. Coherent Diffraction Imaging (CDI) X-ray diffraction was achieved on intact mitotic chromosomes in both 2D and 3D (48). The sample preparation involved fixing the chromosomes onto thin silicon membranes without staining the sample. The 2D resolution achieved was 38 nm. 3D was achieved by imaging the sample after tilting to different angles from -70 to +70 degrees at intervals of 2.5 or 5 degrees. For 3D data analysis, coherent diffraction data sets at 38 incident angles were used resulting in 120 nm spatial resolution in 3D. The study showed not only the surface of the chromosome but also high electron density around the centromere and at the q arm. No significant internal structure was observed (See Figure 3 taken from 48). This method has disadvantages because the spatial resolution is often limited by the X-ray radiation damage and/or by statistical precision at high angles (48).

Atomic force Microscopy (AFM) may be another strong tool to visualize 3D chromosome structure. AFM gives us images which are similar to those obtained using SEM by scanning solid sample surface with a sharp probe tip and detecting the interaction force between the tip and the sample to get height information. From this principle, AFM does not require metal coating, and the observation can be performed either in vacuum, air, or liquid condition (49, 50). Human chromosomes isolated by hexylene glycol method were dropped onto a glass slide and observed in hexylene glycol buffer. No fixation was performed. The obtained images showed 400-800 nm thick chromosome whose surface was covered with globular or fibrous structures with about 50 nm in thickness; (51).

3D Structured Illumination Microscopy (SIM), a super-resolution technique, has been performed on mitotic chromosomes (52). SIM allows us to get higher resolution im-

ages by illuminating a sample with a striped pattern of visible light, which is rotated and scanned to give a number of images, obtained in different phase and direction conditions. After processing, the image resolution is said to reach approximately 120 nm (lateral), being twice the optical diffraction limit (53). It has been shown that the axial distributions of scaffold proteins in metaphase chromatids are comprised of two twisted double strands. It was suggested that this allows both chromosomal bending flexibility and rigidity to occur (Figure 4); (54).

**Future direction**

Despite the impressive nature of imaging methods mentioned above, the detailed higher order structure is still under debate. Optical super-resolution microscopy techniques are under development and present an opportunity to study the structure of the chromosomes below the diffraction limit. Such methods should allow the study of live cells that has not been possible with any previous high-resolution method and with development in 3D. Optical super-resolution techniques are already being applied to study chromatin contacts (55) and will prove useful for studying chromosomes. This technique is limited to the number of dyes used and the thickness of the sample. Scanning transmission electron microscopy (STEM) allows visualisation of 3D structures without sectioning the sample and does not require staining. The limitation is in the z-direction thickness. Such promising studies have been done on intact chromosomes showing chromatin fibres with a fibrous structure of 20 to 30 nm (20).

3D studies done to date have tended to require harsh chemical fixation, dehydration and drying steps that may not represent the true nature of the sample under investigation. Cryo-electron microscopy enables us to observe biological samples close to the native state. Chromosome samples are rapidly cooled down to cryogenic temperature that allows samples to be embedded in vitreous ice, which appears to be featureless. In principle, there is no morphology destruction caused by water volume expansion. Development of new technologies that will maintain the chromosome condition as close to its native state would be useful to obtain information of chromosome surface and interior details. Such methods would include 3D Cryo TEM, SEM or X-ray imaging. To date there is no report showing 3D metaphase chromosomes imaged with such techniques but these are under development. 3D Cryo FIB-SEM and Cryo 3D CDI have already been performed on cells (55, 56) and Cryo X-ray Ptychographic Imaging

has been attempted on nuclei (57). Using new and advanced 3D imaging tools, the higher order chromosome structure enigma should be resolved after a century of research.

**Acknowledgements**

This work was supported under grant number BB/H022597/1 by the BBSRC for a "Diamond Professorial Fellowship for imaging chromosomes by coherent X-ray diffraction". Work at Brookhaven National Laboratory was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-SC00112704.

**References**

1) Sumner, A. T. (2003) Chromosomes: Organization and Function, pp. 5–142, Blackwell Publishing Ltd., Oxford

2) Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. Chromosomal DNA and Its Packaging in the Chromatin Fiber. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26834/

3) Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ.Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature 1997; 389 (6648), 251-60

4) Davey CA, Sargent DF, Luger K, Mäder AW, Richmond TJ. Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 Å resolution. J Mol Biol 2002; 319(5), 1097-113.

5) Arents G, Burlingame RW, Wang BC, Love WE, Moudrianakis EN. The nucleosomal core histone octamer at 3.1 A resolution: a tripartite protein assembly and a left-handed. Proc Natl Acad Sci USA 1991; 22:10148–10152.

6) Schalch T, Duda S, Sargent DF, Richmond TJ. X-ray structure of a tetranucleosome and its implications for the chromatin fibre. Nature 2005; 436, 138.

7) Song F, Chen P, Sun D, Wang M, Dong L, Liang D, Xu RM, Zhu P, Li G. Cryo-EM study of the chromatin fiber reveals a double helix twisted by tetranucleosomal units. Science 2014; 344:376–380.

8) Olins AL, Olins DE. Spheroid chromatin units (v bodies) Science. 1974;183:330–332.
9) Robinson PJ, Fairall L, Huynh VA, Rhodes D. EM measurements define the dimensions of the ‘‘30-nm’’ chromatin fiber: evidence for a compact, interdigitated structure. Proc Natl Acad Sci USA 2006; 103:6506-6511.
10) Finch JT, Klug A. Solenoidal model for superstructure in chromatin. Proc Natl Acad Sci U S A 1976; 73:1897–1901
11) Woodcock CL, Frado LL, Rattner JB. The higher-order structure of chromatin: evidence for a helical ribbon arrangement. J Cell Biol 1984; 99:42-52.
12) Dorigo B. Nucleosome arrays reveal the two-start organization of the chromatin fiber. Science 2004. 306:1571–3.
13) Maeshima K, Hihara S, Eltsov M. Chromatin structure: does the 30-nm fibre exist in vivo?Curr Opin Cell Biol 2010; 22: 291–297
14) Nishino Y, Eltsov M, Joti Y, Ito K, Takata H, Takahashi Y, Hihara S, Frangakis AS, Imamoto N, Ishikawa T, Maeshima K. Human mitotic chromosomes consist predominantly of irregularly folded nucleosome fibres without a 30-nm chromatin structure. EMBO J 2012. 31:1644–1653.
15) Fussner E, Ching RW, Bazett-Jones DP. Living without 30 nm chromatin fibers. Trends Biochem Sci 2011; 36:1-6
16) Uchiyama S, Kobayashi S, Takata H, Ishihara T, Hori N, Higashi T, Hayashihara K, Sone T, Higo D, Nirasawa T, Takao T, Matsunaga S, Fukui K. Proteome analysis of human metaphase chromosomes. J Biol Chem 2005; 280(17):16994-7004.

17) Paulson JR, Laemmli UK. The structure of histone-depleted metaphase chromosomes. Cell 1977; 12, 817–828.

18) Earnshaw, WC, Halligan B, Cooke CA, Heck MM, Liu, LF. Topoisomerase II is a structural component of mitotic chromosome scaffolds. J. Cell Biol 1985; 100: 1706–1715.
19) Samejima, K, Samejima, I, Vagnarelli, P, Ogawa, H, Vargiu, G, Kelly, DA, de Lima AF, Kerr A, Green LC, Hudson DF, Ohta S, Cooke CA, Farr CJ, Rappsilber J, Earnshaw WC. Mitotic chromosomes are compacted laterally by KIF4 and condensin and axially by topoisomerase IIα. The Journal of Cell Biology 2012; 199(5):755–770.
20) Fukui K. Contribution of nanotechnology to chromosome science. Chromosome Science 2016; 19:51-56.

21) Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie BR, Mirny LA, Dekker J. Organization of the mitotic chromosome. Science (New York, N.Y.) 2013; 342(6161):948-953.
22) Nagano T, Lubling Y, Stevens TJ, Schoenfelder S, Yaffe E, Dean W, Ernest DL, Tanay A, Fraser P. Single cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature, 2013, 502(7469):59-64.
23 Pollard TD, Earnshaw WC: Cell Biology. Elsevier; 2002.
24) Flors C, Earnshaw WC. Super-resolution fluorescence microscopy as a tool to study the nanoscale organization of chromosomes. Curr Opin Chem Biol 2011; 15(6):838-44.
25) Howe B, Umrigar A, Tsien F. Chromosome Preparation From Cultured Cells. Journal of Visualized Experiments : JoVE 2014; (83), 50203.

26) Moralli D, Yusuf M, Mandegar MA, Khoja S, Monaco ZL, Volpi EV. An Improved Technique for Chromosomal Analysis of Human ES and iPS Cells. Stem Cell Rev 2011; 7(2):471-7.

27) Tobias ES, Connor M, Ferguson-Smith M. Essential Medical Genetics, Includes Desktop Edition Wiley 2011

28) Shihab SNA. Your Easy Way To Chromosomes. AuthorHouse 2012.

29) Sone T, Iwano M, Kobayashi S, Ishihara T, Hori N, Takata H, Ushiki T, Uchiyama S, Fukui K. Changes in chromosomal surface structure by different isolation conditions. Arch. Histol. Cytol 2002; 65:445-455.

30) Lewis CD, Laemmli UK. Higher order metaphase chromosome structure: evidence for metalloprotein interactions. Cell 1982; 29:171-181.

31) Yusuf M, Parmar N, Bhella GK, Robinson IK. A simple filtration technique for obtaining purified human chromosomes in suspension. Biotechniques 2014; 56:257-261.

32) Doležel J, Vrána J. Safár J. Bartoš J, Kubaláková M, Simková H. Chromosomes in the flow to simplify genome analysis. Funct. Integr. Genomics 2012; 12:397-416.

33) Bauer DL, Marie R, Rasmussen KH, Kristensen A, Mir KU. DNA catenation maintains structure of human metaphase chromosomes. Nucleic Acids Res 2012; 40:11428-11434.

34 Kannan TP, Zilfalil B. Cytogenetics: past, present and future. Malays J Med Sci 2009. 16(2): 4–9.

35) Volpi EV and Bridger JM. FISH glossary: an overview of the fluorescence in situ hybridization technique.Biotechniques 2008; 45(4):385-6,

36) Abbe E. Beiträge zur Theorie des Mikroskops und der mikroskopischen Wahrnehmung. Archiv für mikroskopische Anatomie 1873; 9, 413–418.

37) Ris, H. Stereoscopic electron microscopy of chromosomes. Methods Cell Biol. 1981; 22:77-96.

38) Engelhardt P. Electron Tomography of Chromosome Structure In Encyclopedia of Analytical Chemistry. R.A. Meyers (Ed.) John Wiley & Sons Ltd. Chichester 2000; 6:4948-4984.

39) Harauz G, Borland L, Bhar GF, Zeitler E, Hell MV. Three-dimensional reconstruction of a human metaphase chromosome from electron micrographs. Chromosoma 1987; 95:366-374.

40) Shemilt L, Estandarte AKC, Yusuf M, Robinson IK. Scanning electron microscope studies of human metaphase chromosomes. Phil. Trans. R. Soc. A 2014; 372: 20130144

41) Wanner G, Formanek H (2000). A New Chromosome Model. Journal of Structural Biology 2000; 132:147-161

42) Iwano M, Fukui K, Takaichi S, Isogai A. Globular and fibrous structure in barley chromosomes revealed by high-resolution scanning electron microscopy. Chromosome Res 1997; 5:341-349

43) Schroeder-Reiter E, Pérez-Willard F, Zeile U, Wanner G. Focused ion beam (FIB) combined with high resolution scanning electron microscopy: a promising tool for 3D analysis of chromosome architecture. J Struct Biol 2009; 165(2):97-106.

44) Denk W, Horstmann H. Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLoS Biol 2004; 2:e329.

45) Yusuf M, Chen B, Hashimato T, Estandarte AK, Thompson GE & Robinson IK.

(2014). Staining and embedding of human chromosomes for 3D Serial Block Face Scanning Electron Microscopy. BioTechniques 2014; 1;57(6):302-7.

46 Chen B, Yusuf M, Hashimato T, Estandarte AK, Thompson GE & Robinson IK. Three-Dimensional Positioning and Structure of Chromosomes in a Human Prophase Nucleus. Sci Adv 2017; 21;3(7):e1602231.

47) Booth, DG, Beckett AJ, Molina O, Samejima I, Masumoto H, Kouprina N, Larionov V, Prior IA, Earnshaw WC. 3D-CLEM Reveals that a Major Portion of Mitotic Chromosomes Is Not Chromatin. Molecular Cell 2016; 64(4), 790–802.

48) Nishino Y, Takahashi Y, Imamoto N, Ishikawa T, Maeshima K. Three-dimensional visualization of a human chromosome using coherent X-ray diffraction. Phys. Rev. Lett 2009; 102:018101.

49) Ushiki T, Hitomi J, Ogura S, Umemoto T, and Shigeno M. Atomic force microscopy in histology and cytology. Arch Histol Cytol 1996; 59(5): 421-431.

50) Ushiki T, Shigeno M, and Hoshi O. Techniques for imaging human metaphase chromosomes in liquid conditions by atomic force microscopy. Nanotechnology 2008; 19, 384022.

51) Hoshi O, Shigeno M, and Ushiki T. Atomic force microscopy of native human metaphase chromosomes in a liquid. Arch Histol Cytol 2006; 69(1): 73-78.

52) Carlton PM. Three-dimensional structured illumination microscopy and its application to chromosome structure. Chromosome Research 2008; 16: 351-3

53) Schermelleh L, Carlton PM, Haase S, Shao L, Winoto L, Kner P, Burke B, Cardoso MC, Agard DA, Gustafsson MGL, Leonhardt H, Sedat JW. (2008). Subdiffraction Multicolor Imaging of the Nuclear Periphery with 3D Structured Illumination Microscopy. Science (New York, N.Y.) 2008; 320(5881), 1332–1336.

54) Poonperm R, Takata H, Hamano T, Matsuda A, Uchiyama S, Hiraoka Y, Fukui K. Chromosome Scaffold is a Double-Stranded Assembly of Scaffold Proteins. *Scientific Reports* 2015; *5*, 11916.

55) Wang Y, Ratna P, Shivashankar GV. Superresolution imaging of nanoscale chromosome contacts. Scientific Reports 2017; 7:42422

56) Schertel A, Snaidero N, Han HM, Ruhwedel T, Laue M, Grabenbauer M and Möbius W. Cryo FIB-SEM: volume imaging of cellular ultrastructure in native frozen specimens. J Struct Biol 2013; 184(2):355-60.

57) Yusuf M, Zhang F, Chen B, Bhartiya A, Cunnea K, Wagner U, Cacho-Nerin F, Schwenke J and Robinson IK. Procedures for Cryogenic X-ray Ptychographic Imaging of Biological Samples. IUCrJ 2017; 4(2):147–151.

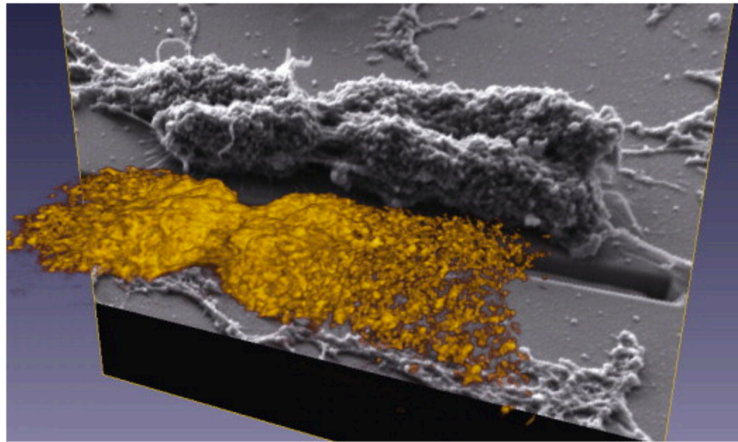

Figure 1. Reconstruction of a Barley chromosome after 3D FIB-SEM. 198 sections were aligned after imaging. The chromosome was labelled with phosphorylated histone H3 (ser 10); (yellow). ( 42)

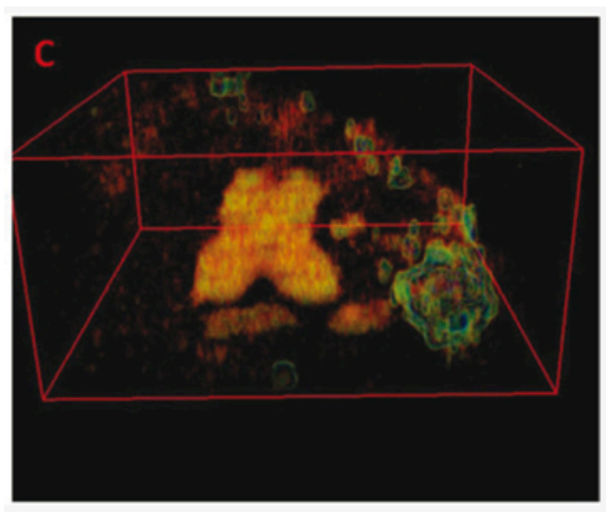


Figure 2. 3D SBFSEM of human mitotic chromosome . X-shaped human mitotic chromosome was reconstructed after 13-nm × 100-nm sections. Box size: 4.253 × 3.741 × 1.6 microns. (44).

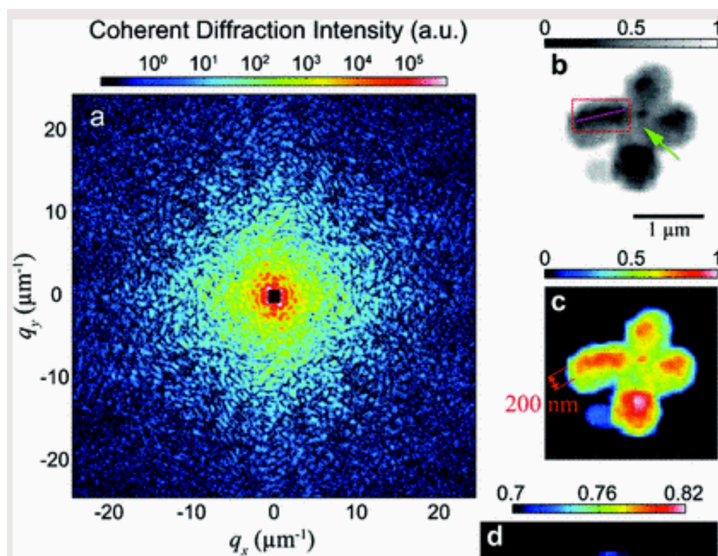


Figure 3. Xray imaging of unstained human chromosome a) Coherent diffraction pattern after Xray CDI experiment b) reconstructed image in gray scale with the centromere on the chromosome highlighted with an arrow (c) in color scale of b and shows a high-intensity region resembling the chromosome axial structure near the center of the chromatids. (47)

**Figure 4 a**–**c**, Maximum intensity projections of z-stack images. Scale bars, 1 µm. **a**, wide-field microscopy applying deconvolution imaging of PA chromosome immunostained for hCAP-E and Topo IIα. **b**, 3D-SIM image of the same PA chromosome as **a**. **c**, 3D-SIM image of HeLa-wt metaphase chromosome immunostained for hCAP-E and Topo IIα. Arrowheads indicate the double strands. Insets show magnified views of the white boxes in **a** and **b** as indicated. Scale bars, 250 nm. Red dotted lines represent double strands of chromosome scaffold. DNA is shown in blue. **d**, RGB line profile of yellow path in **c**.(53)