Future Prospects of 3D Human Chromosome Imaging by Serial Block Face Scanning Electron Microscopy

Mohammed Yusuf1,2* Bo Chen1,2 and Ian Robinson1,2
1London Centre for Nanotechnology, University College London, London, London, UK
2Research Complex at Harwell, Rutherford Appleton Laboratory, Oxon, UK

Keywords: Chromosome; Serial block-face scanning electron microscopy; Three-dimensional imaging

Commentary

The higher order structure of human chromosomes remains to be elucidated with the 30 nm mystery still remaining. For internal structure determination, transmission electron microscopy (TEM) cannot be used as the chromosomes are too thick (approx. 1.4 microns) and scanning electron microscopy (SEM) is a surface imaging technique. For this purpose, the three-dimensional (3D) serial block face scanning electron microscopy (SBFSEM) was used on imaging mitotic human chromosome for the first time [1].

SBFSEM has been used down to 20 nm sections showing clear signs of internal structure [1]. Even though porous features or cavities were seen on the chromosome arms, the resolution was limited to 11 × 11 × 20 nm. Charging effects and radiation damage were believed to give the current resolution. Despite the 11 nm nominal lateral resolution, no 30 nm structures were seen. New images with 8.3 × 8.3 nm pixel size show consistent staining in each slice (Figure 1a) and in slices further down in the series (Figure 1b) of the same chromosome (150 nm apart), also having porous information. Complementary methods such as focused ion beam scanning electron microscopy (FIBSEM) have provided 3D information on plant chromosomes (not human) due to sample preparation difficulties [6]. 3D X-ray coherent diffraction imaging (CDI) provided 120 nm resolution with little internal fine structure on a human chromosome [7]. Therefore there is no doubt that the SBFSEM method will be further used in the future for exploring higher order structure of the human genome, hopefully providing higher resolution as the techniques are improving.

Figure 1: SBFSEM of a single human mitotic chromosome prepared from a lymphoblastoid cell line (GM18507). 2D sections are stained with platinum blue. (a) shows a section after SBFSEM from a whole chromosome b) is a section further down the series being 150 nm apart from (a). Pixel size is 8.3 × 8.3 nm.

for example employing new generation back scattered electron (BSE) detectors. Various sample preparation procedures need to be developed that would include decondensing the chromatin by removing divalent cations, elimination of hypertonic buffer and imaging chromosomes by directly slicing cells. Currently no cryogenic stage for the instrument is available therefore the samples have to be imaged at room temperature after embedding in resin, however freeze substitution [8] of chromosomes after high pressure freezing would be a positive way forward. This would reduce the damage rate by preventing the diffusion of free radicals. Cryo-FIB has been used for cell imaging [9] and is yet to be experimented on human chromosomes in a single cell.

Acknowledgment

This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC), grant BB/H022597/1. Also we would like to thank Professor George Thompson and Mr Teruo Hashimoto for preparing the resin sample and use of the SBFSEM microscope. This was done at the Corrosion & Protection Centre, School of Materials, The University of Manchester, UK. Therefore the Engineering and Physical Sciences Research Council (EPSRC) is also acknowledged for support of the LATEST2 Programme Grant and the associated imaging facilities.

References


*Corresponding author: Mohammed Yusuf, London Centre for Nanotechnology, University College London, London, Research Complex at Harwell, Rutherford Appleton Laboratory, Oxon, UK. Tel: +44 20 7679 2000; E-mail: yusuf.mohammed@ucl.ac.uk

Received March 14, 2016; Accepted April 05, 2016; Published April 07, 2016


Copyright: © 2016 Yusuf M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.


