Supporting Information

Essential movie data

The movie data, in files, will need to be downloaded to the desk-top for ease of access.

First, there is S1, a Quick Time 3-dimentional data stack that is clicked on and displayed in Quick Time, present in all computers, with a bar, that can be displaced, to move up and down the Z axis.

Second, there are the 7 stereo movies, the Rocking Angular Stereo Pair movies (S2-S8) which are played by clicking the file name and looking in stereo as detailed below. These are essential to study the 3-dimentional data that is the basis of the evidence for the interphase chromosome structure.

Supplemental Movie Legend
S1.mov – Master deconvoluted volume data of nucleus for this paper.
S2.mov – Rocking angular stereo pairs of S1 data, coordinates X 356-926, Y 650-1024, Z 180-279
S3.mov – Rocking angular stereo pairs of S1 data, coordinates X 0-356, Y 180-650, Z 66-165,
S4.mov – Rocking angular stereo pairs of S1 data, coordinates X 0-356, Y 180-650, Z 170-169
S5.mov – Rocking angular stereo pairs of S1 data, coordinates X 0-356, Y 180-650, Z 192-291
S6.mov – Rocking angular stereo pairs of S1 data, coordinates X 0-356, Y 180-650, Z 106-205
S7.mov – Rocking angular stereo pairs of S1 data, coordinates X 0-356, Y 650-1024, Z 80-189
S8.mov – Rocking angular stereo pairs of S1 data, coordinates X 0-396, Y 650-1024, Z 173-272

Special Nuclear Structure Visualization Problems and Their Solutions

One of the best ways to study and analyze the nuclear Cryo-electron tomogram chromosome structure is through the use of stereo pairs (1, 2) to take into account the intrinsic 3-dimensional nature of the data. Stereo pairs are best seen as side-by-side images fused after crossing one’s eyes. A tutorial below will outline how to rapidly see stereo by the crossed eyes technique. A second tutorial is provided to further aid in visualizing the 3-dimensional interphase chromosome structures, in the nucleus, once the stereo fused images technique is learned from the first tutorial.

Usually, a small cube of data is computationally cut out of the deconvoluted 3-dimensional image for stereo pair study. The stereo pairs are further modified by rocking the stereo pairs so that the 3-dimensional data can be seen from different angles, typically 180 degrees of tilt. In addition to rocking, the stereo pair data cube is rotated by large different (Euler) angles to further displace the 3-dimensional structures. These stereo pair rocking and large angle views are called rocking angular stereo pairs (defined above) and will be one of the primary ways the chromosome structure will be analyzed.

Further stereo pair modifications may be necessary for structure analysis. Viewing the stereo pair movies can, in many cases, be enhanced by variation of the size (can be modified by movement of the
lower right-side diagonal tab), so that structures can be discerned, differentially, if viewed at a distance which size allows one to do. Motion, rapid rocking of the views, can in addition be used to discern additional structural features (the structure can snap into view with motion). The rocking angular stereo pair movies, because both eyes see features slightly differently, give the impression of enhanced (better) resolution. One usually requires that the same structure is seen at close adjacent (fast) rocking angles, and in many cases that structure can be reconciled/seen at the other large angular views to be believed. The rocking angular stereo pairs turn out to be crucial for the interpretation of the banded large-scale structures. It is worth pointing out that there is a learning curve for the study of the rocking angular stereo pair movies. Movie S2 of the Supporting Information is a guide to seeing the structures in the nucleus rocking angular stereo pair movies. Initially, five to ten minutes of study are usually required to see the banded structures reliably, but subsequently they are rapidly found in abundant places.

Finally, rocking angular stereo pair(s) are studied for each nuclear box in Fig. 1. The detailed large-scale structures banding, and its sub-structure is really only appreciated by these stereo pair movies. In some cases, these nuclear boxes are shifted in Z, giving rise to slightly different rocking angular stereo pair movie(s), for example Table 1 and Table 2 B’–B’’, so that the banded large-scale structures at different Z depths (and their modifications) can be studied.

**Problems for Large Scale Structure Data Interpretation**

Before interpreting the large scale structure and their bands (presented in the Results section) one needs to discuss a number of general interpretation problems. First, EM grey-level data, both for STEM and TEM, represents mass; though, STEM via scattering is rigorous mass (3). However, the grey levels lack specificity, and one cannot say which grey level is which, protein or nucleic acid or their complexes. Instead one uses molecular attributes and organelle locations to define structures, a crude process (4). There are ways to think about this problem, and the solution is in the future (5, 6).

While the chromatin/nucleosome makes up the bulk of the nuclear mass, it is not possible to rigorously specify these structures. Likewise, local densities, slight thickenings, etc. could represent transcription products, transcription factor complexes (usually multi-protein complexes), transcription products, processing/splicing factor complexes, or other structural components. A second problem is that one typically needs to scale the images for intensity, hence mass. The grey levels are in a very large dynamic range that cannot be all displayed. Instead, upper and lower intensity levels are displayed, but in the process some structure/mass is clipped out, thereby missing possible structural features. In several cases, several dynamic ranges are simultaneously studied to remedy some of these problems. Coupled with this problem is the worry that very weak features – such as the 2-nm bare DNA fibers – are not visible because of the scaling; in addition, one worries that structural features are lost because one is at the end of the Cryo-electron tomogram resolution.

A third problem is geometry considerations for the large-scale structure. It is a flexible, bending, twisted, dotted (broken up because of sub-structure), and possibly coiled structure. When it is studied by
selecting Z planes or sub-regions, these structures are computationally cut so that different orientations are seen in complex geometries, complicating the analysis. In addition, these structures can be on top of one another in some places in the nucleus. Fourth, there is the problem of where one is in the cell cycle. One does not know if the nucleus is in G1, S, or a mixture, complicating the interpretation of the structures. This issue will be resolved in future Cryo-electron tomogram studies.

**Stereo pair viewing tutorial**

A training stereo pair is shown in Fig. S1. This image is downloaded as a side-by-side horizontal pair about 4–5 inches across separated by 1–2 inches. Next this pair of images are studied/viewed approximately 12–18 inches away (straight above the images), crossing one's eyes until a third picture between the two pictures appears, then gently relaxing one’s eyes, but still slightly crossing them, so that the third image appears in the middle between the two pairs. One concentrates on this third image, and immediately a stereo 3-dimensional, dominant image appears. In the trial Fig. S1, these geometric structures in 3-dimensions are evident, spaced 3-dimensionally. Then one continues to train by seeing stereo rapidly, then experimenting by viewing the stereo images increasing distances away (24+ inches away), and even viewing off axis. Large-scale 3-dimensional structures can be discerned, in some cases, by viewing 6–8 feet away from the stereo pairs. See https://en.wikipedia.org/wiki/Stereoscopy for general stereo viewing.

![Fig. S1. Stereo training geometric models.](https://mathematica.stackexchange.com/a/30015/77273)

A second training image is provided as a firework movie [http://youtu.be/dpyjYWfZESw](http://youtu.be/dpyjYWfZESw) so that time dependent 3-dimensional data are seen in stereo as above.
Stereo viewing tutorial for rocking angular stereo pair movies

Now that it is possible to view stereo in a facile way, we can proceed to the rocking angular stereo pair data movies. Start with rocking angular stereo pair movies and be able to see the different angular views that are rocking by the cursor movement and be able to jump from one view to another in stereo, seamlessly. Be able to reconcile a structure from one rocking and a different angular view. This ability may take a few tens of minutes to accomplish.

Next study features, like heterochromatin, in Fig. 1 and Fig. 1-guide to pick out 3-dimensional chromosome structures. Look for repetitive structures that are perpendicular to the long axis of the large scale structure, that are curving. Next look for sub-structural features like bumps and sub-structure with grey level differences/texture in 3 dimensions. Lastly, study the features in Table 2 using these new visualization techniques. Many additional examples will be discerned.

DC processing; Histogram Trimming Details

The deconvolution post processing and the histogram trimming is now described in detail. First, the deconvoluted 3-dimensional image from (7) had the pathological intensities (~500,000) removed; the intensity values from 8,000 and above were truncated to 8,000 (Fig. S2B.). Second, the low intensity values, less than 1500 and the values greater than 8,000 (the high intensity values come largely from the mitochondria granules), part of the histogram trimming process, were truncated to their set values (1,500 & 8,000), as shown in Fig. S2C. This histogram trimming process is described in detail in Fig. 5 and Fig. S4 in the Supplement of ref. 7. Finally, this deconvoluted image was trimmed in X, Y, and then Z was trimmed above and below the nucleus to center the nucleus as shown in Fig. S2D.

File Data Base Archive

The digital files for the deconvolution and the S1-S8 data movies are achieved in the EMBD data base repository (https://deposit-pdbe.wwpdb.org/deposition), and the numbers are in the small table below:

|   |       |
|---|-------|
| S1 | EMD-14924 |
| S2 | EMD-15057 |
| S3 | EMD-15058 |
| S4 | EMD-15059 |
| S5 | EMD-15060 |
| S6 | EMD-15061 |
| S7 | EMD-15062 |
| S8 | EMD-15063 |
Fig. S2. Different deconvoluted/histogram trimming and post-processing steps.

Fig. S2A shows the deconvoluted image as it came from (7) with the intensity range of ~(-5000) to ~500,000 as shown in the histogram insert. Fig. S2B is A with the intensity values greater than 8,000 truncated to 8,000. Fig. S2C is B with the intensity values lower than 1,500 truncated to 1,500; the intensity range is now 1,500 to 8,000. Fig. S2D has the XYZ borders trimmed (pixels removed) to center, and only included nucleus deconvoluted structures. The intensity values go from 1,500 to 8,000.
Supplement References

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4. E. Villa, M. Schaffer, J. M. Plitzko, W. Baumeister, Opening windows into the cell: focused-ion-beam milling for cryo-electron tomography. Curr. Opin. Struct. Biol. 23, 771–777 (2013).

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