Supporting Information

Eltsov *et al.* 10.1073/pnas.0810057105

SI Text

Mask Design. Cutting of vitreous material causes an artificial relief on the surface of the sections (1). Two major components of these relief, knifemarks and crevasses, are clearly distinguishable in the image shown in Fig. S1*A*. In the Fourier representation of the image, the information generated from knifemarks and crevasses was localized into 2 discreet symmetrical sectors. These sectors can be removed by masking without influencing on the global spatial frequencies distribution, as chromosomes are orientated randomly in the images and show no preferential direction.

Fourier masking of the images was performed in ImageJ 1.38a (http://rsb.info.nih.gov/ij/), with a custom mask created in SPIDER. A blank mask of $4{,}096^2$ pixels was generated in SPIDER (http://www.wadsworth.org/spider_doc/spider/docs/spider.html); 4 isosceles triangles were inserted with the PT command, to generate a crude mask aligned to the knifemarks and crevasses. The use of triangular masks allows equal filtering of low and high spatial frequencies in Fourier space. The angle of the mask was determined by direct measurement on Fourier transforms derived from sections of pure dextran with abundant knifemarks and crevasses. A further 4 right angle triangles were added to the mask file to cover the corners of the square. Last, high- and low-pass filters, with cut-offs of 3 and 160 nm, respectively, were added to the wedge shaped masks to remove boost contrast and reduce noise.

Deconvolution and Generation of Averaged 1-DRAPS. The images shown in Fig. S1 were taken at high-defocus levels to generate high-contrast images. However, the effects of the CTF resulted in numerous phase reversals in their Fourier transforms and greater attenuation of higher spatial frequencies. To correct for these effects, we first calculated individual power spectra for the images, in SumPS (2). The program produces a 1-DRAPS for each of the power spectra generated; these were plotted in KaleidaGraph to produce graphs of spatial frequency against log amplitude. Initially, we determined the Gaussian parameters to correct the signal decay and attenuation of the higher spatial frequencies, we excised all of the visible minima and the tail, before fitting a Gaussian curve (2). Once we had determined the Gaussian parameters, we applied them to the raw 1-DRAPS and measured the position of the minima, to enable the calculation of an accurate defocus level in CtfZeros (2). The accurate determination of the defocus level allows the best correction of the CTF. After characterizing the CTF of each image, they underwent a full CTF correction by deconvolution in CTFMIX (2), which resulted individual images corrected for phases and amplitudes. We also performed the CTF corrections with batches of 10 images for larger datasets; in this case, the program calculates an average power spectrum and parameters for the data set of 10 images, after which we calculated the average 1-DRAPS for each larger dataset, typically, 50 images. In our hands, this approach was as successful as averaging the 1-DRAPS of corrected individual images, for datasets with imaging similar conditions, such as magnification and defocus.

1. Dubochet J, *et al.* (2007) How to ''read'' a vitreous section. *Methods Cell Biol* 79:385–406.
2. Conway JF, Steven AC (1999) Methods for reconstructing density maps of ''single'' particles from cryoelectron micrographs to subnanometer resolution. *J Struct Biol* 128:106–118.

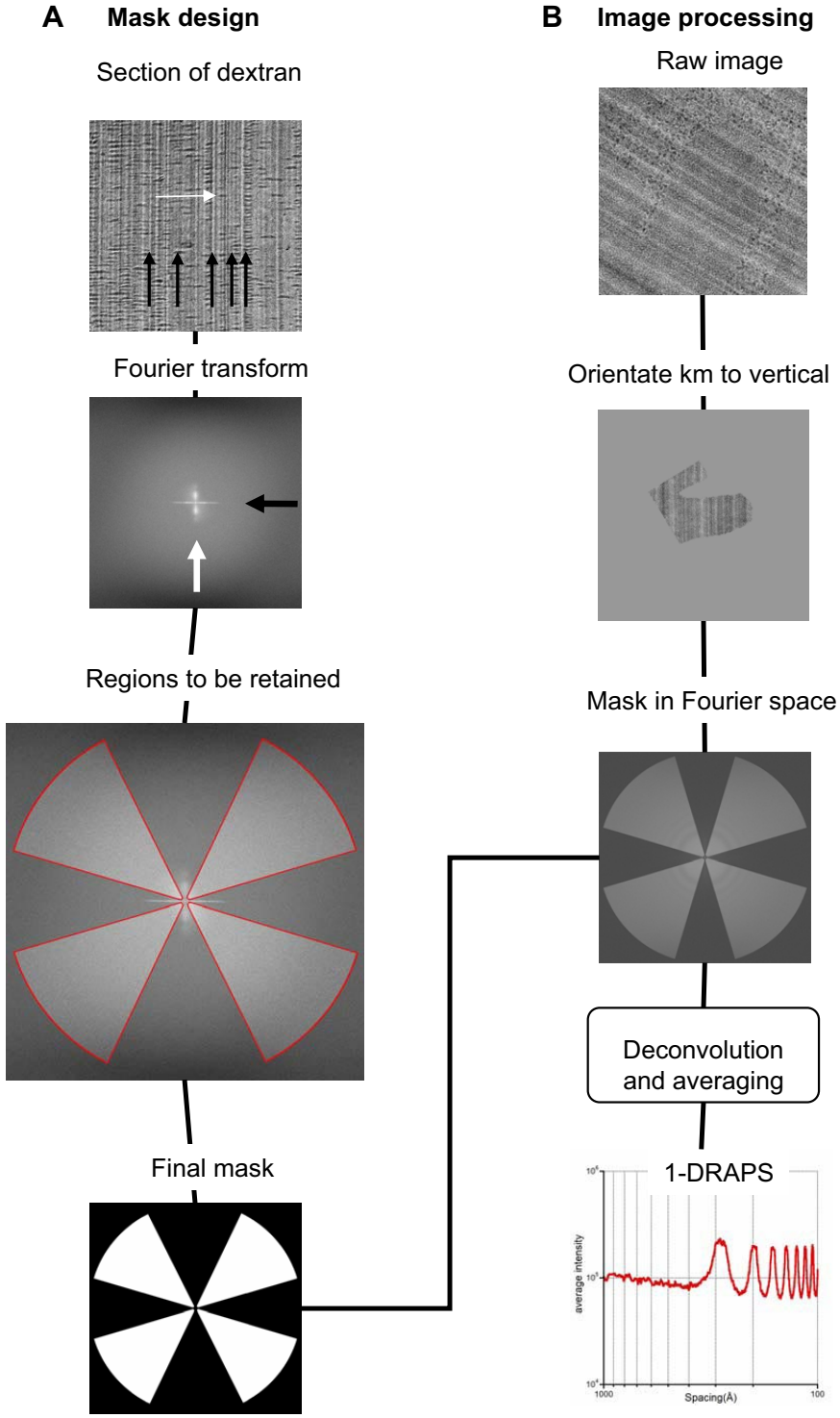

Fig. S1. Flow diagram for generation of averaged 1-DRAPS. (*A*) the design of mask for knifemarks and crevasses. (*Top*) image of pure dextran was padded into box of 4096^2 pixels and orientated so knife marks were horizontal (*Upper Middle*). A Fourier transform of the padded image was created (*Lower Middle*). Knifemarks (white arrow) and crevasses (black arrow) were visible on the Fourier transform. A mask consisting of 4 isosceles triangles, 4 right angle triangles was generated in Spider. Low-pass and high-pass masks with cutoffs of 3 and 160 nm were added to create the final mask (*Lower Middle*). The mask was applied to the padded and orientated image (*Bottom*) before deconvolution of the image and calculation of 1-DRAPS (*Bottom Left*). (*B*) image processing. Images were padded and orientated before calculation of Fourier transform. The knifemark and crevasse mask was applied to the images, which were retransformed to check the fit of the mask. Masked images were deconvoluted and 1-DRAPS were calculated for the images.