3D-Image analysis platform monitoring relocation of pluripotency genes during reprogramming

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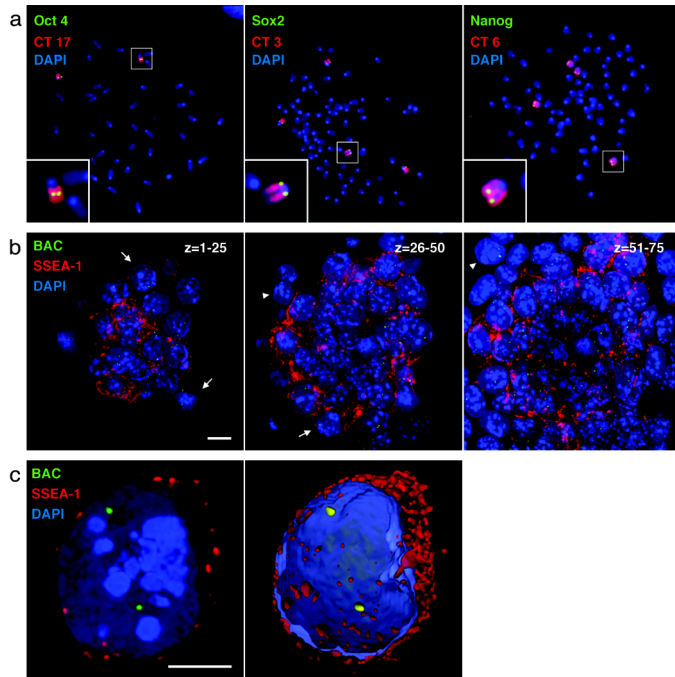[2] Max Delbrück Center for Molecular Medicine, Berlin, Germany

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Figure S1. Probe specificity control and iPS selection strategy. (**a**) Hybridization of BACs containing *Oct4* (left), *Sox2* (middle) or *Nanog* (right) together with the respective chromosome paints on metaphase spreads of MEF cells stably transfected with lentiviral vectors containing *Oct4*, *Klf-4*, *Sox2*, and *c-Myc* show distinct signals only at the expected genomic sites. (**b**) Three subsequent blocks of z-projections (optical serial sections 1-25, 26-50, 51-75) of a typical iPS cell colony. Arrows exemplify SSEA-1 positive cells that were chosen for further analysis. Arrowheads show SSEA-1 negative cells that were excluded from analysis. Scale bar indicates 10 µm. (**c**) Example of a post-processed cell, which has been cut out of its neighboring cells (left). Right image shows a 3D reconstruction of the same cell. Scale bars 5 µm.
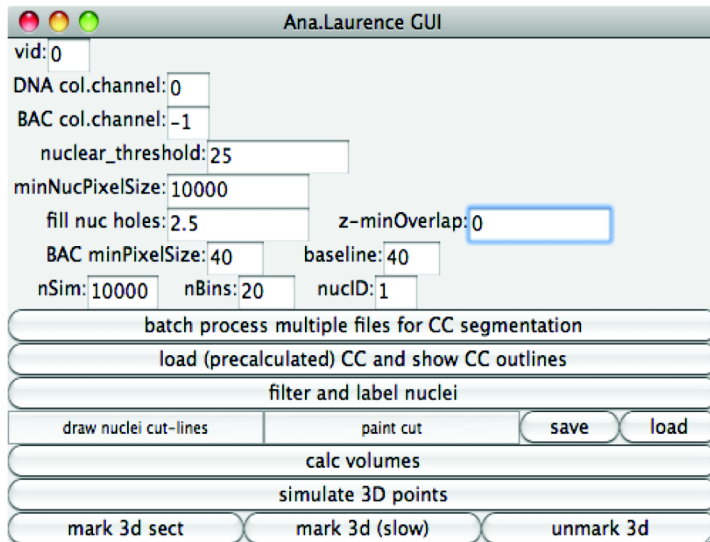
Figure S2. User interface of randomizer software. The user interface window of the randomizer software provides many options to adjust parameters to meet the needs of individual experiments (detailed information in methods and step-by-step tutorial **Movie 1**).

greyscale

denoise

blurr

unsharpen mask

threshold

FI

µm
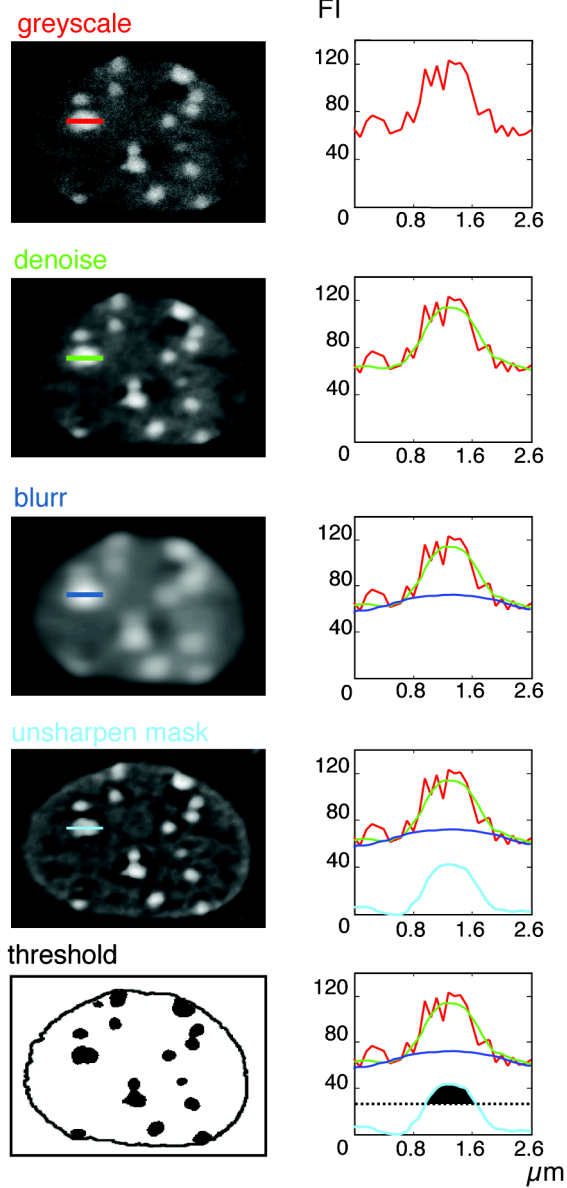
Figure S3. 3D image segmentation procedure. Starting with a grayscale image stack of DAPI stained mouse nuclei (left column) image processing routine included denoise and blurring as a prerequisite for an unsharpen mask calculation. Basic global thresholding results in a 3D binary picture of chromocenter structures (detailed information in methods). The effect of each filtering step is highlighted exemplarily over a line scan across a chromocenter by overlaying the respective fluorescent intensity (FI) profile with the ones of the previous steps (right column).
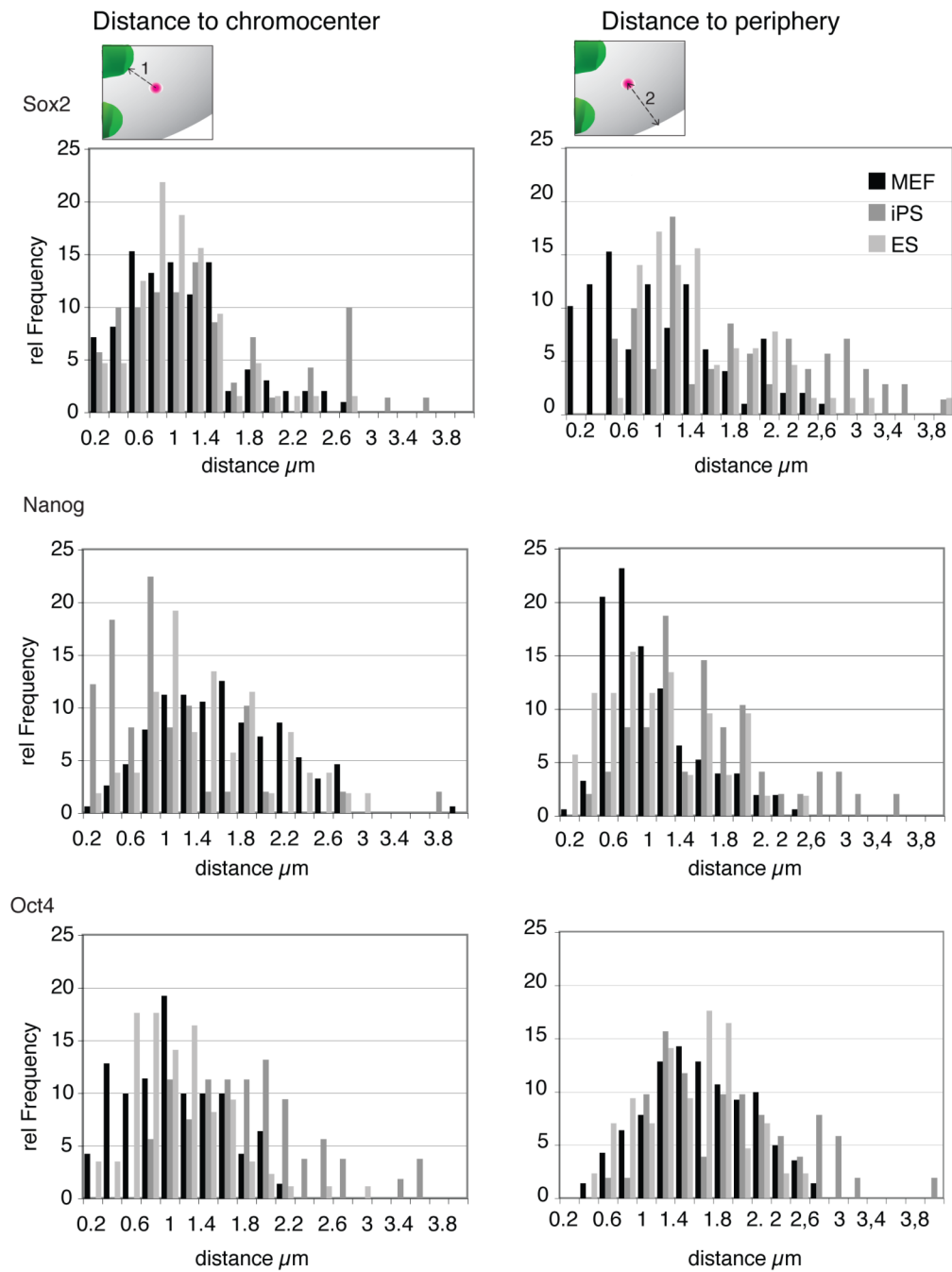
Figure S4. Non-normalized distance distribution for *Sox2, Nanog* and *Oct4* relative to the chromocenters and periphery. The graphs represent non-normalized distances of the genes to the nuclear landmarks in MEF (black), iPS (dark gray) and ES (light gray) cells. (N=52-151)
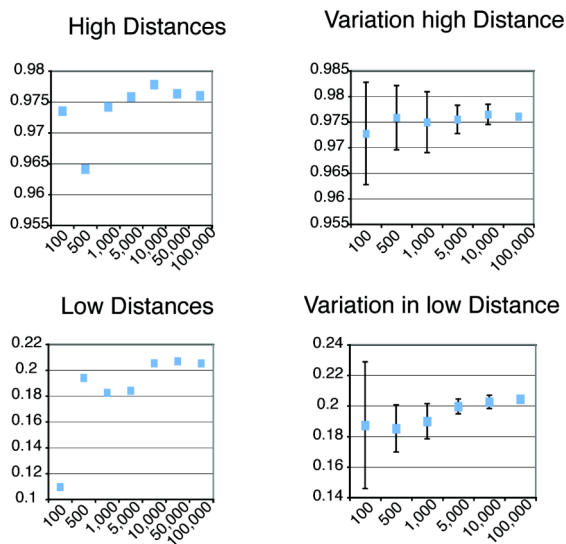
a

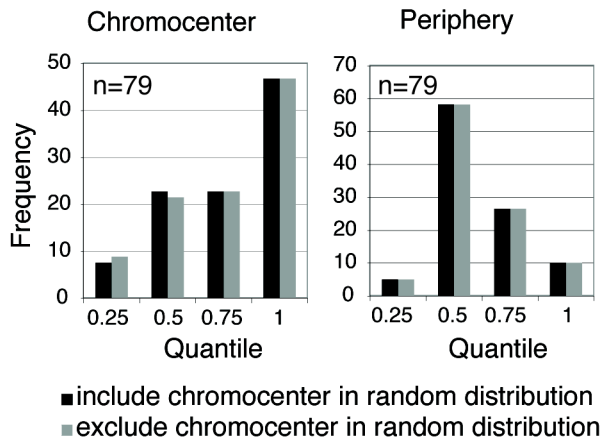Distribution of random points visualized for one section

100 points   500 points   1,000 points

5,000 points   10,000 points   100,000 points
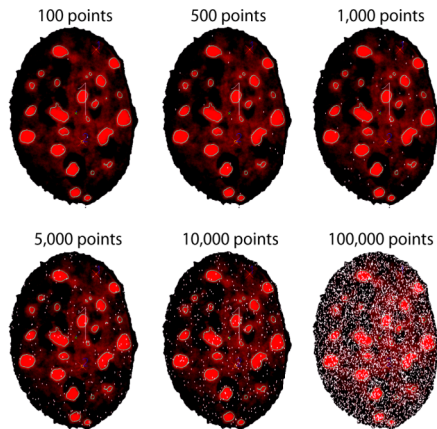
Figure S5. Effect of number of simulated points on quantile variation. (**a**) Visualization of random distribution using increasing numbers of simulated points. For simplicity only one z-section is shown. See **Movie 2** for full z-stack with 10,000 simulated points. (**b**) Calculating the quantile for a small and a large distance measurement with different numbers of simulated points for the random distribution. A plateau is seen at 10,000. Repeating these measurements 30 times and plotting the average quantile with standard deviation shows that the error with 10,000 simulated points is minimal.

b

Variation of quantile result by number of simulated points

High Distances

Variation high Distance

Low Distances

Variation in low Distance

Chromocenter | **Periphery**

■ include chromocenter in random distribution
■ exclude chromocenter in random distribution

Figure S6. Impact of chromocenter inclusion/exclusion on data normalization. Simulations were performed excluding and including random points from the chromocenter volume and normalized data compared. Including/excluding chromocenter volumes for random positioning does not affect normalized data.

— cell type 1
— cell type 2
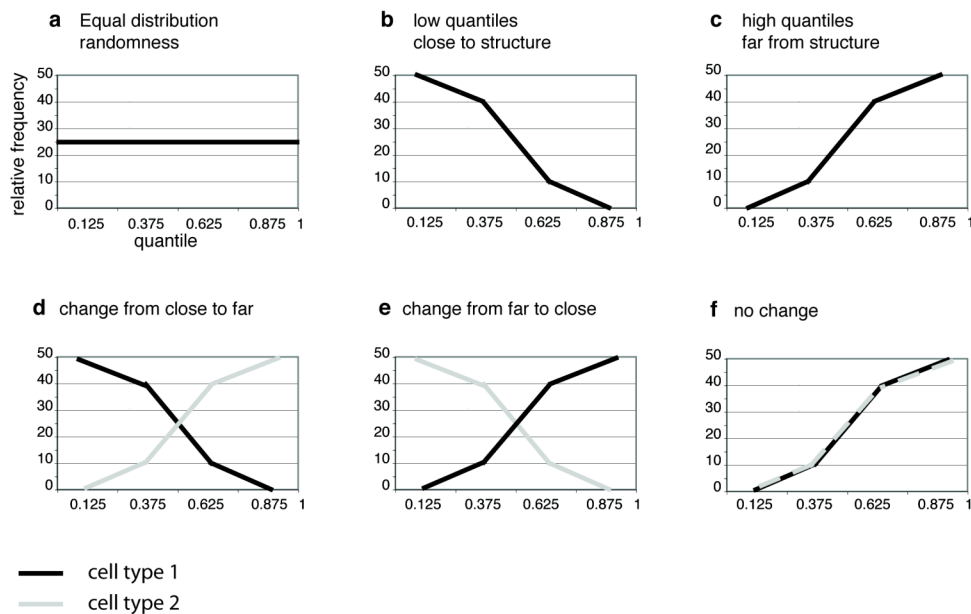
Figure S7. Examples of different quantile distributions. (a) Example of a gene showing no position preference within the nucleus. All quantiles are represented in the same proportion. (**b**) Example of a gene with high preference for proximity to the measured nuclear landmark. The majority of quantiles are in the lower quart. (**c**) Example of a gene with aversion to the measured nuclear landmark. The majority of quantiles are in the upper quart. (**d**) Example of increased distances of a gene in cell type 2 compared to cell type 1. (**e**) Example of decreased distances of a gene in cell type 2 compared to cell type 1. (**f**) Example of no positional change compared to the measured structure. In both situations the gene presents a distal position.

Movie 1. Randomizer software step-by-step tutorial.

Movie 2. Random points simulation in 3D. Visualization of the 10,000 random points simulated for each individual nucleus in 3D. DNA is false colored in red, the nuclear periphery in white and chromocenter borders in light blue. Random points are marked in white. The two yellow circles represent the gene signal whose centroid position is marked by a cross.