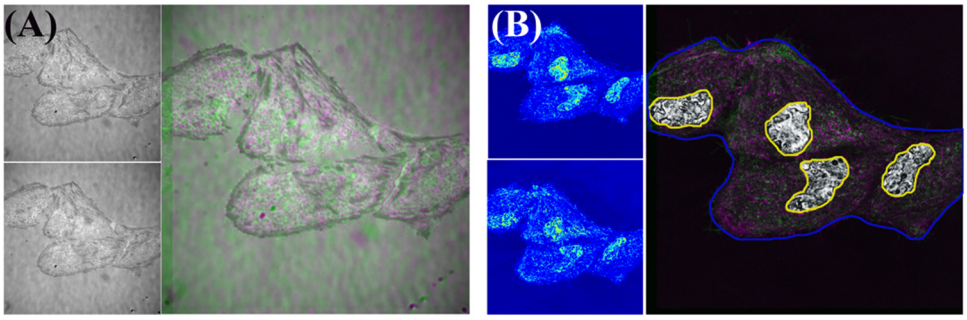
S.I.

Image Alignment and Segmentation Method

For image alignment, we applied translational and rotational transform and used the combination that renders the largest cross-correlation coefficient of the two bright-field images (SI Fig 1 (A)). For nucleus segmentation, we first used the transformation matrix from the image alignment and aligned the PWS Σ map. Then we manually cropped out the nucleus region and cytoplasm region with care on the false color overlaid image (SI Fig 1 (B)) for quantitative analysis.

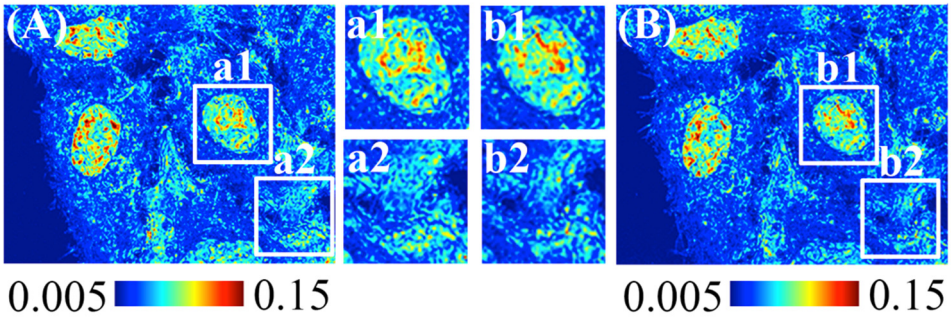


SI Fig 1 Example of image alignment and segmentation. (A) Bright field reflection images of live *HeLa* (upper left), *HeLa* after 20 minutes 4% PFA fixation (lower left). The false color image overlay (right) showed good match after alignment. (B) PWS Σ map of the same cells. After alignment, nucleus region (yellow contour) and cytoplasm region (blue contour) were cropped out manually on the false color overlaid image.

PWS Visualization of Mass-Density Re-distribution Due to Normal Cell Dynamics

Ideal fixation, such as high-pressure freezing, will "freeze" the cell structure and dynamics at a glance. Under ideal circumstances, the mass-density distribution after fixation and right

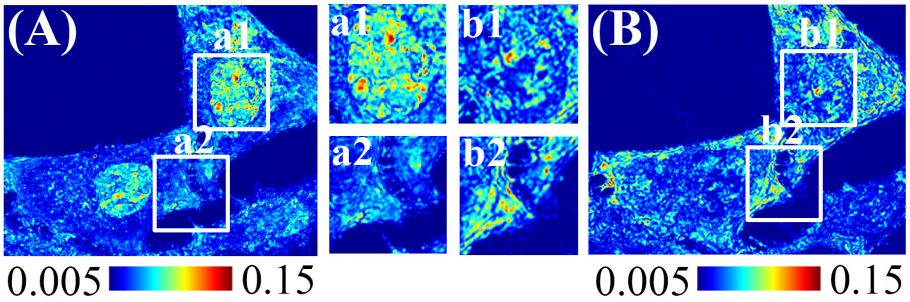
before fixation should be the same. Thus we employed PWS measurements of the same cells under incubator condition 1 minute apart (SI Fig 2) to estimate the goodness of fixation.



SI Fig 2 PWS Σ map of the same live *HeLa* cells 1 minute apart. The insets showed higher magnification of the mass-density mismatch of nucleus and cytoplasm region. For both regions, mass-density underwent re-distribution due to normal cell dynamics. (A) At time zero, (B) at 1 minute after.

PWS Measurement of *HeLa* Cells After Resin Infiltration

We compared the PWS measurements between live cells and resin-infiltrated cells. The cells had been processed followed the TEM resin embedding protocol. The range of PWS Σ map is smaller after resin infiltration than that right after serial ethanol dehydration. This is because the density of Spurr's resin (1.1g/cm^3) is similar to the density of protein (~1.2 g/cm^3). As a result, the absolute value in mass-density mismatch is suppressed as well.



SI Fig 3 PWS Σ map of the same *HeLa* cells before and after resin infiltration. (A) Live-cell and (B) infiltrated by resin following the TEM resin-embedding protocol. The insets are higher magnification images of nucleus and cytoplasm region. Dramatic change was shown in the nucleus region.

Tables of Statistical Parameters Calculated from PWS Σ Map

Multiple *HeLa* cells were measured by PWS: 61 for PFA, 114 for ethanol, 69 for TEM fixation, 75 for live cell dynamics control, and 22 for TEM resin infiltration. Statistical parameters were calculated and tabulated for time lapse live cells (SI Table 1), PFA fixation (SI Table 2), ethanol fixation (SI Table 3), TEM resin-embedding fixation without resin embedding (SI Table 4), and TEM resin-embedding fixation with resin infiltration (SI Table 5).

SI Table 1 Statistics of Cells Captured 1 Minutes Apart

(75 Cells)

Nucleus	Average		Cytoplasm	Average	
	Time 0	1 Min Later		Time 0	1 Min Later
Mean	0.055±0.005	0.054±0.004	**Mean**	0.031±0.003	0.031±0.003

CV	0.41±0.02	0.41±0.02	**CV**	0.48±0.02	0.48±0.02
Kurtosis	3.25±0.29	3.30±0.30	**Kurtosis**	5.11±1.07	5.20±0.84
Skewness	0.55±0.13	0.56±0.15	**Skewness**	1.17±0.21	1.21±0.19
Entropy	14.96±0.38	14.96±0.38	**Entropy**	17.37±0.25	17.37±0.25
CCC	1	0.49±0.07	**CCC**	1	0.53±0.07

For live cells measured 1min apart (SI Table1), all parameters were stable regardless of the rearrangement of mass-density distribution caused by the vibrant cell dynamics. In other words, the bulk properties of PWS **Σ** map are only structural dependent.

SI Table 2 Statistics of Cells Before and After PFA Fixation

(61 Cells)

Nucleus	**Average**		**Cytoplasm**	**Average**	
	Live	PFA		Live	PFA
Mean	0.055±0.006	0.048±0.004	**Mean**	0.031±0.005	0.035±0.005
CV	0.407±0.027	0.414±0.019	**CV**	0.50±0.03	0.47±0.03
Kurtosis	3.32±0.23	3.48±0.46	**Kurtosis**	5.03±1.11	5.35±1.43
Skewness	0.58±0.12	0.80±0.13	**Skewness**	1.07±0.24	1.16±0.25
Entropy	12.15±0.02	11.95±0.18	**Entropy**	11.80±0.24	11.87±0.26
CCC	1	0.23±0.06	**CCC**	1	0.35±0.07

In 4% PFA Fixation, for nucleus region, the more than 10% drop in mean value and almost 38% increase in skew suggested the dominance of smaller mass clusters after fixation and a

relatively more homogeneous overall distribution. The last point was also validated by the slightly decreased value in entropy. However, the kurtosis and CV of the nucleus region were consistent, with less then 2% decrease respectively, which indicated the PFA fixation didn't change the symmetry of the mass-density distribution.

The effects of PFA on the cytoplasm are not exactly the same as on the nucleus. For example, the mean value and CV in the cytoplasm both increased after PFA fixation. This showed clearly that the mass-density had more fluctuations, probably due to the cross-linking process. The kurtosis and the skewness increased while the entropy remained almost constant.

SI Table 3 Statistics of *HeLa* sells before and after 95% ethanol fixation

(114 Cells)

Nucleus	**Average**		**Cytoplasm**	**Average**	
	Live	ETOH		Live	ETOH
Mean	0.043±0.001	0.200±0.039	**Mean**	0.026±0.003	0.144±0.028
CV	0.44±0.02	0.39±0.04	**CV**	0.47±0.02	0.44±0.04
Kurtosis	3.15±0.23	3.15±0.31	**Kurtosis**	8.17±1.62	4.21±0.58
Skewness	0.59±0.09	0.59±0.10	**Skewness**	1.59±0.16	0.84±0.15
Entropy	12.14±0.10	13.82±0.20	**Entropy**	11.43±0.21	13.82±0.21
CCC	1	0.13±0.03	**CCC**	1	0.04±0.07

For 95% ETOH fixation (SI Table 3), the most dramatic change was in mean values with magnitudes of increasing for both nucleus and cytoplasm region. This can be explained by the shrinkage in volume and resulting condensed density caused by EtOH. However, the kurtosis and

skewness showed opposite trend for nucleus and cytoplasm: the skewness and kurtosis increased for the nucleus but dropped significantly for the cytoplasm. For the nucleus, this indicated that the mass-density distribution started from a normal distribution (kurtosis =3) and ended with a less normal distribution, with more small mass clusters than big ones. For the cytoplasm, this decreased skewness and kurtosis suggested the dominance of a small density mismatch. This might be the result of deposition of small particles during ethanol fixation.

SI Table 4 Statistics of Cells at Different Stages of Preparation for TEM Resin Section (69 Cells)

Nucleus	Live	GA and FA	OsO_4	Serial ETOH
Mean	0.048±0.005	0.046±0.003	0.078±0.008	0.062±0.007
CV	0.41±0.03	0.40±0.03	0.43±0.02	0.45±0.02
Kurtosis	3.15±0.34	3.92±0.63	3.89±1.25	4.81±1.32
Skewness	0.49±0.23	0.85±0.15	0.85±0.19	1.11±0.24
Entropy	11.96±0.27	11.87±0.27	12.50±0.41	12.28±0.36
Absolute CCC	1	0.28±0.09	0.19±0.07	0.07±0.06
Relative CCC	1	0.28±0.09	0.36±0.10	0.25±0.08
Cytoplasm	**Live**	**GA and FA**	**OsO_4**	**Serial ETOH**
Mean	0.030±0.006	0.043±0.008	0.083±0.018	0.078±0.014
CV	0.47±0.02	0.51±0.02	0.56±0.04	0.57±0.03
Kurtosis	5.46±1.98	6.59±1.84	6.71±2.27	7.05±3.55
Skewness	1.14±0.35	1.39±0.34	1.43±0.37	1.41±0.39

Entropy	11.66±0.31	12.19±0.31	13.16±0.37	13.15±0.28
Absolute CCC	1	0.35±0.07	0.35±0.07	0.31±0.06
Relative CCC	1	0.35±0.07	0.49±0.07	0.55±0.06

For TEM resin-embedding protocols, the fixatives were added in a specific sequence. First of all, the GA and FA fixation changed the nucleus regional mean value and CV by only less than 5% difference compared with live cells. With 22% increase in kurtosis and 73% increase in nucleus skewness, we can deduce the distribution of mass-density has experienced dramatic change. For Cytoplasm, major changes occurred after GA and FA fixation and remained relatively stable in the following steps. The mean value increased significantly (43%) after GA and FA fixation. The increased mass-density in cytoplasm as a result of volume loss during fixation can serve as a possible explanation. The obvious increase in kurtosis and skewness indicate a more peaked and asymmetric structure with small mass dominance even before OsO_4 fixation and ethanol dehydration.

Secondly, the OsO_4 fixation enhanced the overall contrast in PWS with almost doubled mean value (70% increase), but the overall distribution didn't change much, as the CV, kurtosis and skewness were relatively stable. It seems that the GA and FA efficiently cross-linked the protein and nucleic acids, and OsO_4 stained that entire cell evenly.

Following heavy metal staining, the serial ethanol dehydration reduced the mean value by washing away loosely bonded or unbounded OsO_4. The CV was quite stable for all treatment within the nucleus, but the distribution was largely difference from live cells. Kurtosis and skewness were increased by 52% and by 126%, respectively. This indicates that, as cells are

dehydrated, small chunks of material were re-deposited to the cells and resulting in a very asymmetric distribution peaked at small values.

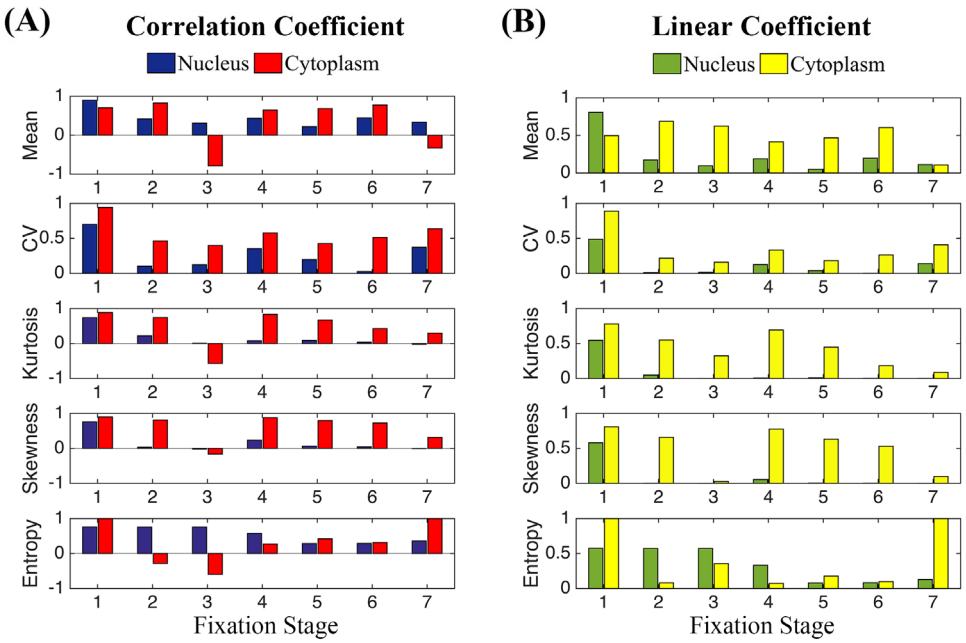
SI Table 5 Statistics of Live Cells and Cells Infiltrated with Resin after Fixation (22 Cells)

Nucleus	Average		Cytoplasm	Average	
	Live	Infiltrated		Live	Infiltrated
Mean	0.053±0.006	0.033±0.004	**Mean**	0.033±0.003	0.044±0.002
CV	0.38±0.05	0.51±0.03	**CV**	0.47±0.03	0.57±0.04
Kurtosis	3.16±0.35	4.58±0.61	**Kurtosis**	4.62±1.30	5.14±1.21
Skewness	0.45±0.28	2.25±0.13	**Skewness**	0.95±0.25	1.16±0.19
Entropy	13.84±0.99	13.84±0.99	**Entropy**	16.44±0.85	16.44±0.85
CCC	1	0.12±0.04	**CCC**	1	0.28±0.07

Lastly, resin infiltration homogenized the mass-density distribution inside nucleus and reduced the correlation between treated cells and live cells even more.

Bulk Property Correlation

We calculated the correlation and linear coefficient of bulk properties in different fixation stage for nucleus and cytoplasm, respectively (SI Fig 4).



SI Fig 4 Correlation of the bulk properties between live cells and cells at different stage of treatments. For both (A) correlation coefficient, and (B) linear coefficient, steps 1 to 7 stand for 1. Live cell sequence 1 min apart, 2. 4% PFA, 3. 95% EtOH, 4. 2.5% GA & 2% FA, 5. 1% OsO_4, 6. Serial EtOH, 7. Resin embedding.

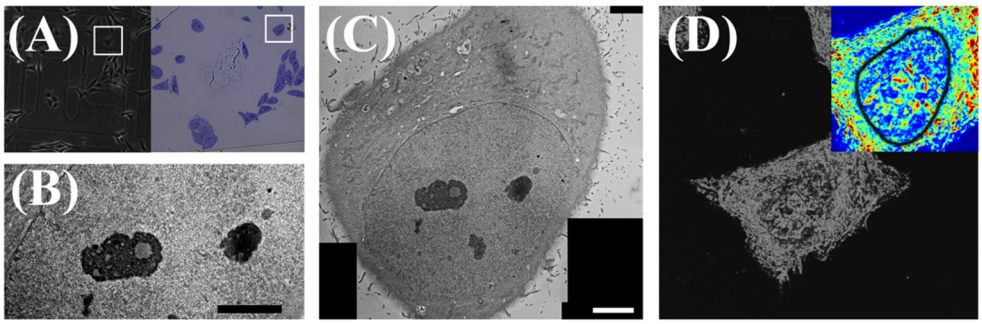
SI Table 6 Correlations of Bulk Statistics

Nucleus	Correlation Coefficient					Linear Coefficient				
	Mean	CV	Kurtosis	Skewness	Entropy	Mean	CV	Kurtosis	Skewness	Entropy
Live (1min Apart)	0.90	0.80	0.74	0.76	0.76	0.81	0.49	0.55	0.58	0.58
PFA	0.42	0.10	0.22	0.033	0.76	0.17	0.011	0.049	0.0011	0.57
ETOH	0.31	0.12	0.01	-0.017	0.76	0.095	0.015	0.00012	0.00028	0.57
TEM (GA&FA)	0.43	0.35	0.075	0.23	0.58	0.19	0.13	0.0056	0.055	0.33
TEM (OsO_4)	0.22	0.20	0.087	0.064	0.28	0.049	0.030	0.0076	0.0040	0.080
TEM (ETOH)	0.44	0.027	0.040	0.043	0.29	0.20	0.001	0.0016	0.0019	0.082

TEM (Resin)	0.33	0.37	-0.026	-0.0035	0.36	0.11	0.14	0.00065	0.000012	0.13
Cytoplasm	**Correlation Coefficient**					**Linear Coefficient**				
	Mean	CV	Kurtosis	Skewness	Entropy	Mean	CV	Kurtosis	Skewness	Entropy
Live (1min Apart)	0.71	0.94	0.88	0.90	1	0.45	0.89	0.78	0.81	1
PFA	0.83	0.46	0.74	0.81	-0.28	0.67	0.22	0.55	0.66	0.08
ETOH	0.79	0.34	-0.57	-0.16	-0.60	0.62	0.16	0.32	0.027	0.36
TEM (GA&FA)	0.65	0.58	0.83	0.88	0.27	0.42	0.33	0.69	0.78	0.073
TEM (OsO_4)	0.68	0.43	0.67	0.80	0.42	0.47	0.18	0.45	0.63	0.18
TEM (ETOH)	0.78	0.51	0.43	0.73	0.31	0.61	0.26	0.18	0.53	0.098
TEM (Resin)	0.33	0.64	0.30	0.31	1	0.11	0.41	0.087	0.099	1

TEM Images of the Same Cells Measured by PWS in Resin Sections

We compared the low magnification phase-contrast images of the live cells in the Petri dish (SI Fig 5 (A)) and cell resin sections stained with toluidine blue (SI Fig 5 (B)) to locate the same cells measured by PWS. Then we took high-resolution TEM images of the same cell on another section on a carbon/Formvar coated TEM grid (SI Fig 5 (C)). Finally, we stitched multiple images taken at high magnification to form the whole cells (SI Fig 5(D)) at 7nm resolution. We were able to recognize nucleoli, mitochondria and vesicles. For finer structures, it was hard to distinguish the two layers of membrane in the nuclear envelope, which was probably smeared by the sample preparation protocol.



SI Fig 5 Images of the same cells in thin section as in Fig 1 (E) to (H). (A) Left: phase contrast image of live *HeLa* cells in a Petri dish. Right: bright field optical micrograph of the resin section containing the same cells stained. The white box enclosed the same cell as in (B) to (D). (B) Part of nucleus and nucleoli at 3000x magnification. (C) Whole cell after stitching, scale bar is 5 μm in both TEM images. (D) Gray scale PWS map of the same cell after serial ethanol dehydration before resin infiltration. The inset shows an enlarged area with a black contour enclosing roughly the region where TEM images (B) and (C) were taken.