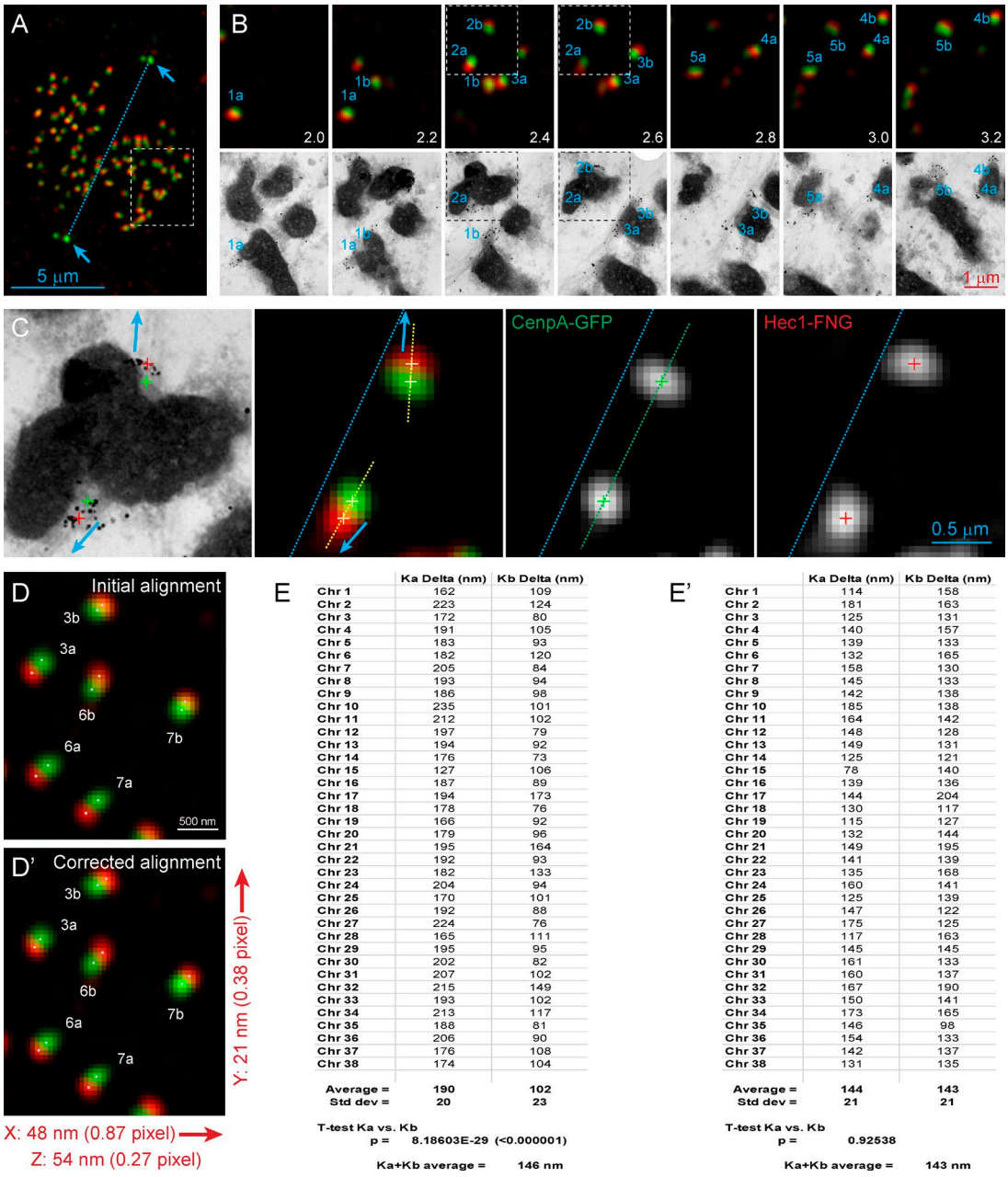
Magidson et al., http://www.jcb.org/cgi/content/full/jcb.201412139/DC1



E

	Ka Delta (nm)	Kb Delta (nm)
Chr 1	162	109
Chr 2	223	124
Chr 3	172	80
Chr 4	191	105
Chr 5	183	93
Chr 6	182	120
Chr 7	205	84
Chr 8	193	94
Chr 9	186	98
Chr 10	235	101
Chr 11	212	102
Chr 12	197	79
Chr 13	194	92
Chr 14	176	73
Chr 15	127	106
Chr 16	187	89
Chr 17	194	173
Chr 18	178	76
Chr 19	166	92
Chr 20	179	96
Chr 21	195	164
Chr 22	192	93
Chr 23	182	133
Chr 24	204	94
Chr 25	170	101
Chr 26	192	88
Chr 27	224	76
Chr 28	165	111
Chr 29	195	95
Chr 30	202	82
Chr 31	207	102
Chr 32	215	149
Chr 33	193	102
Chr 34	213	117
Chr 35	188	81
Chr 36	206	90
Chr 37	176	108
Chr 38	174	104
Average =	190	102
Std dev =	20	23

T-test Ka vs. Kb
p = 8.18603E-29 (<0.000001)

Ka+Kb average = 146 nm

E'

	Ka Delta (nm)	Kb Delta (nm)
Chr 1	114	158
Chr 2	181	163
Chr 3	125	131
Chr 4	140	157
Chr 5	139	133
Chr 6	132	165
Chr 7	158	130
Chr 8	145	133
Chr 9	142	138
Chr 10	185	138
Chr 11	164	142
Chr 12	148	128
Chr 13	149	131
Chr 14	125	121
Chr 15	78	140
Chr 16	139	136
Chr 17	144	204
Chr 18	130	117
Chr 19	115	127
Chr 20	132	144
Chr 21	149	195
Chr 22	141	139
Chr 23	135	168
Chr 24	160	141
Chr 25	125	139
Chr 26	147	122
Chr 27	175	125
Chr 28	117	163
Chr 29	145	145
Chr 30	161	133
Chr 31	160	137
Chr 32	167	190
Chr 33	150	141
Chr 34	173	165
Chr 35	146	98
Chr 36	154	133
Chr 37	142	137
Chr 38	131	135
Average =	144	143
Std dev =	21	21

T-test Ka vs. Kb
p = 0.92538

Ka+Kb average = 143 nm

Figure S1. **Correlative LM/EM approach to characterization of kinetochore organization.** (A) Maximum-intensity projection of an RPE1 cell prepared for correlative microscopy. Inner and outer layers of kinetochores are labeled via CenpA-GFP expression (green) and immunostaining for Hec1 (Fluoro-NanoGold, red). Spindle poles (centrioles) are labeled via expression of Centrin1-GFP (blue arrows). The blue line connecting mother centrioles indicates the spindle axis. (B) Individual focal planes from an LM Z series (top) and corresponding EM sections depicting five centromeres from the area boxed in A. White numbers correspond to the distance (in µm) from the coverslip to each section or focal plane. Gold particles (black dots) in the immuno-EM images delineate Hec1. (C) Higher-magnification view of areas boxed in sections 2.4 and 2.6 (minimum-intensity projection for EM sections and maximum-intensity projection for fluorescence). Crosses mark centroids of Hec1 and CenpA fluorescence, and blue arrows indicate orientation of the attached K fibers. Green and yellow dashed lines mark orientations of the centromere axis and intrakinetochore axes, respectively. Blue dashed line is the spindle axis. The angle between the green and blue lines is cTilt; the angles between the yellow and green lines are kTilts of individual kinetochores. (D and D′) A high-magnification view of three pairs of sister kinetochores before (D) and after (D′) correction for residual chromatic aberration by shifting the red channel to make the global centers of mass for each channel coincidental. The exact values and directions of shifts are indicated. Notice that visually the raw and corrected images appear quite similar. (E and E′) Delta values for individual kinetochores oriented toward the opposite spindle poles from the cell shown in D and D′. The populations of left (Ka) versus right (Kb) kinetochores are statistically different when Delta is measured in raw images (E), but they become indistinguishable after chromatic correction (E′). Notice the prominent changes in Delta values for individual kinetochores before and after correction.

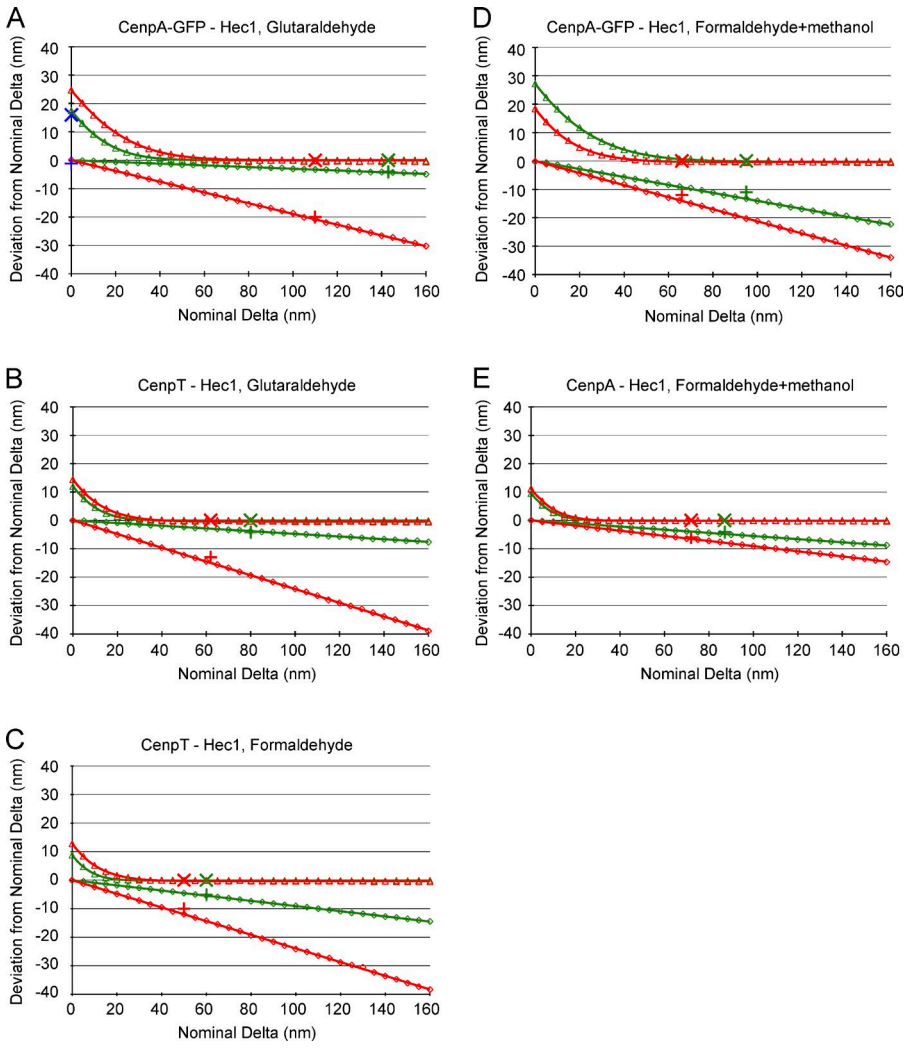


Figure S2. **Accuracy of Delta values calculated by methods I and II.** (A–E) Simulation curves of the predicted deviation from the nominal Delta as a function of nominal Delta for method I (diamonds) and method II (triangles). Simulations are based on observed values of kTilt and observed variance of Delta and kTilt for untreated (green) and taxol-treated (red) metaphase RPE1 cells under the conditions indicated for each plot. Mean values of Delta experimentally observed in control (green) and taxol-treated (red) cells are indicated by + (method I) and × (method II). Experimental Delta values at zero nominal Delta in (blue + and × in A) were measured for Hec1 stained both green and red. Notice that the experimental values are not statistically different from the model predictions.

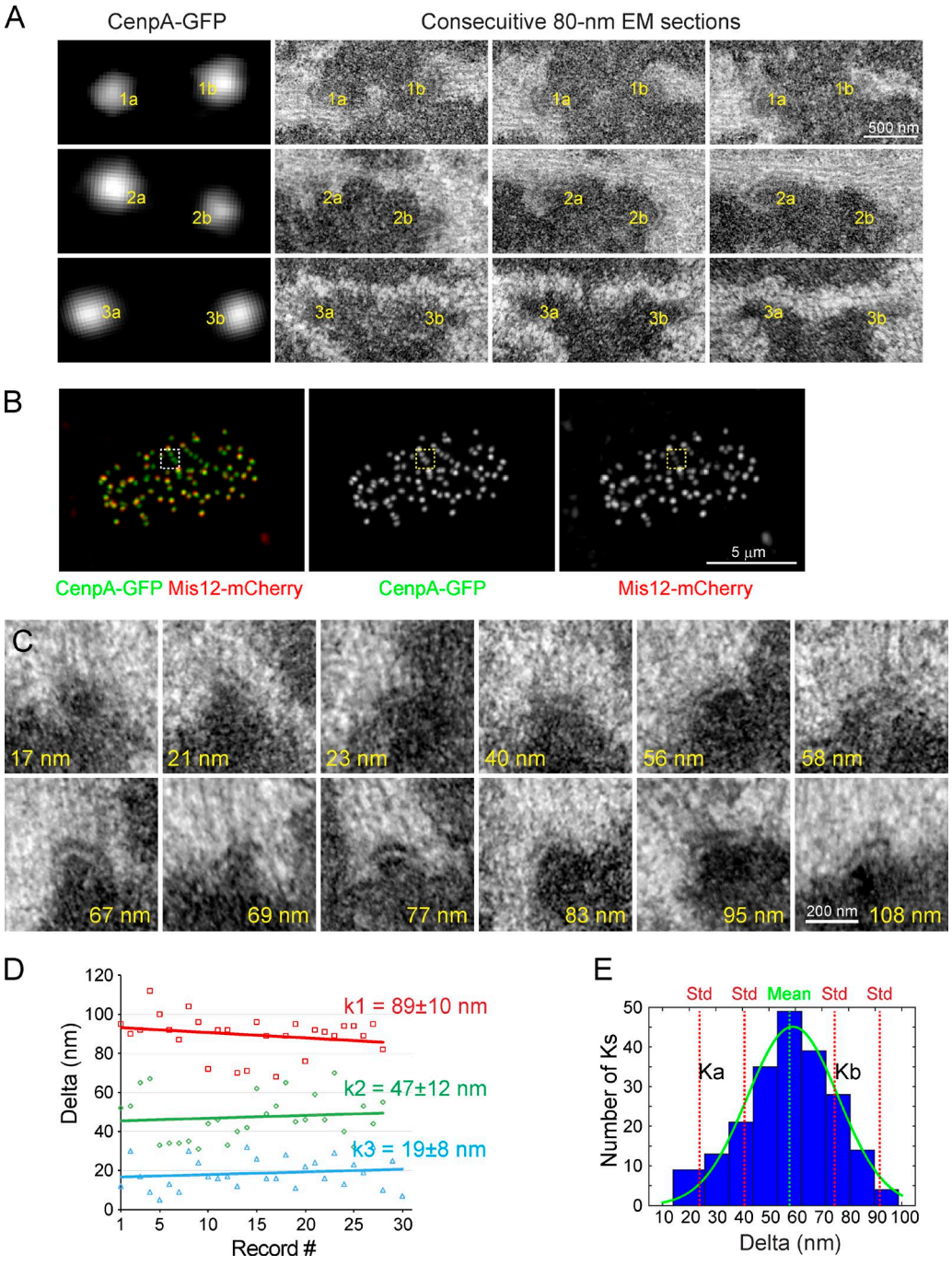


Figure S3. **Morphologic variability of kinetochores and accuracy of individual Delta measurements.** (A) Correlative LM/EM images of three pairs of coplanar sister kinetochores from an RPE1 cell expressing CenpA-GFP. CenpA-GFP fluorescence of the sister kinetochores after fixation (left). The remaining panels are EM images of the regions corresponding to the fluorescent objects. Notice the morphologic variability among the kinetochores. The plates of sister kinetochores are often bent (kinetochores 1a and 1b and 2a and 2b) and sometimes are not recognizable as plates (kinetochores 3a and 3b). (B) Maximum-intensity projection of an untreated metaphase RPE1 cell tagged with CenpA-GFP and Mis12-mCherry. Boxed area indicates the sister kinetochores illustrated in Fig. 3 (A and B). (C) EM appearance of kinetochores with various values of $Delta_{CenpA\text{-}GFP\text{–}Mis12\text{-}mCherry}$. Values of delta are marked on each image. (D) Repetitive measurements of Delta for three individual kinetochores in a glutaraldehyde-fixed cell. Linear regression (solid lines) demonstrates that the mean values of Delta for each kinetochore remains stable in series of repetitive measurements. However, there is a significant variability among individual values. Individual measurements can yield overlapping Delta values for kinetochores with intermediate (green circles) and high (red squares) or for intermediate and low (blue triangles) mean Delta. Because the kinetochores are chemically fixed, variability of measurements is solely caused by the noise in recording of relatively weak fluorescence. Values of mean ± SD are shown for each kinetochore. (E) Distribution of Delta values for 212 kinetochores (106 pairs in three metaphase cells). Green line indicates the mean Delta value (58 nm), and the red lines are positions of 1 (±17 nm) and 2 (±34 nm) SDs on either side of the mean. The sister kinetochores illustrated in Fig. 3 are approximately two SDs below (Ka) and one SD above (Kb) the mean. Such a separation between Delta values determined in a single measurement is sufficient to identify these kinetochores as members of low and high Delta classes. Std, standard.

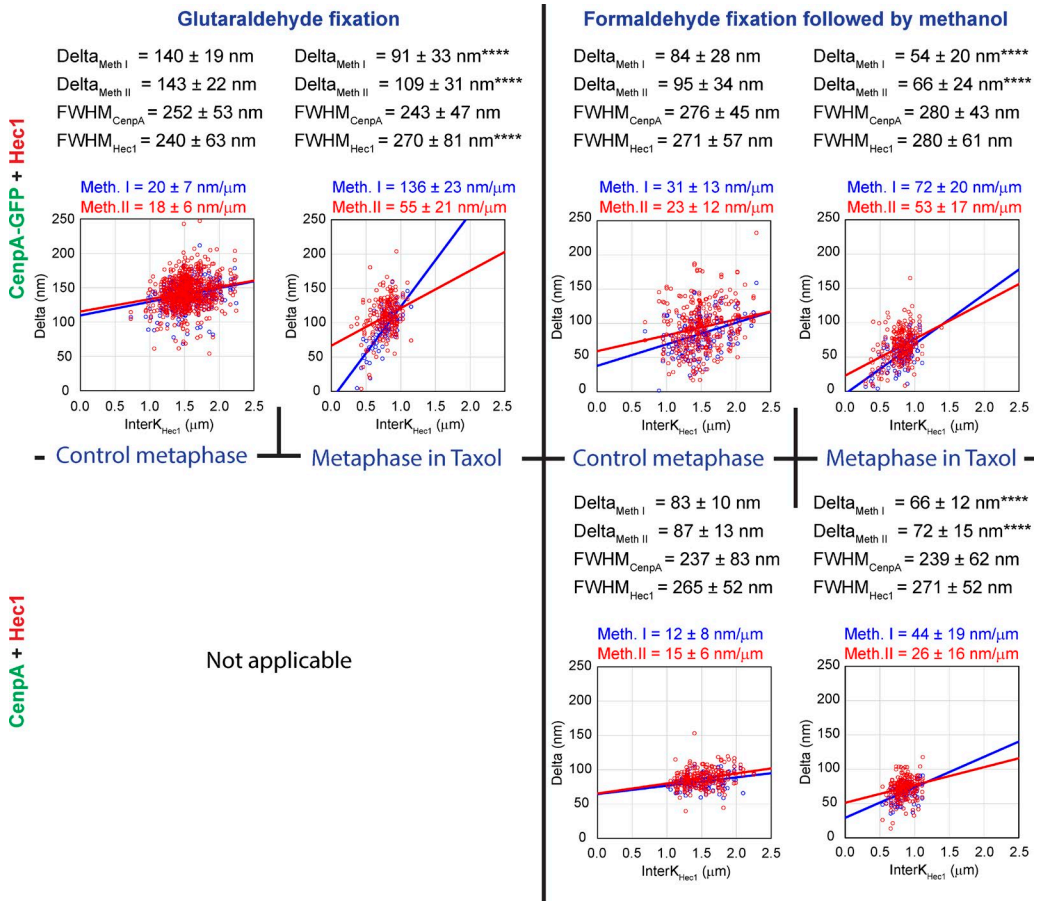


Figure S4. **Effects of various fixations and alternative approaches to chromatic aberration correction on SHREC values for CenpA-GFP, CenpA, and Hec1.** Values of $Delta_{CenpA\text{-}GFP–Hec1}$ and kinetochore compliancy appear to be higher after glutaraldehyde than after formaldehyde/methanol fixation. Because of antigen accessibility problems, endogenous CenpA cannot be detected after glutaraldehyde or formaldehyde-only fixations. Most of the SHREC values determined after formaldehyde/methanol fixation are different from the values obtained for CenpA-GFP–Hec1. Notice that FWHM of Hec1 but not CenpA-GFP is larger in taxol versus control when cells are fixed with glutaraldehyde. FWHM of both CenpA-GFP and Hec1 are larger in formaldehyde/methanol fixed cells, and the values do not change in response to taxol. In contrast, FWHM of endogenous CenpA in both control and taxol-treated cells are smaller. ****, $P < 0.0001$, significant differences between the values in control versus taxol-treated cells. Meth., method.

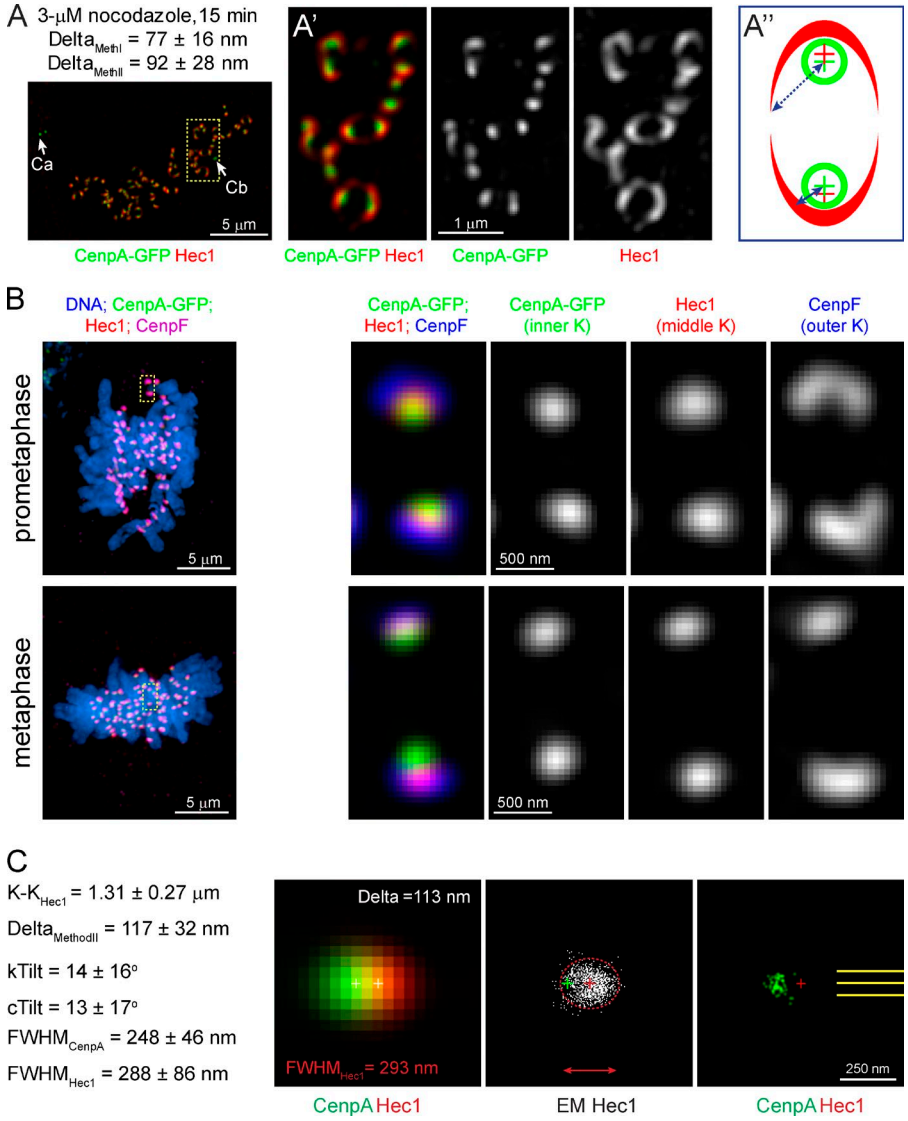


Figure S5. **Radial-segment kinetochore architecture affects Delta values.** (A) Expansion of the outer kinetochore in metaphase cells treated with 3 μM nocodazole. Maximum intensity projection of an RPE1 cell that was treated with nocodazole for 15 min during metaphase. The chromosomes remain in a configuration that resembles the metaphase plate with the centrosomes (Ca and Cb, Centrin-GFP fluorescence) positioned on the opposite sides of the plate. Boxed area in A is shown at higher magnification in A′. Notice that Hec1 forms large crescents so that sister kinetochores largely encircle the centromere. CenpA-GFP remains compact. As illustrated by the cartoon (A″), in this configuration the distance between the green and red centers of mass (crosses) does not correspond to the mean (solid double arrow) or the maximal (dashed double arrow) separation between the green and red components. (B) Radial organization of the outer kinetochore in untreated cells. Typical prometaphase and metaphase (bottom row) RPE1 cells with CenpA-GFP (inner kinetochore) labeled kinetochores. Hec1 (outer kinetochore) and CenpF (outmost peripheral kinetochore component) are stained with antibodies. DNA is visualized with Hoechst 33343. Sister kinetochore pairs from the boxed areas are shown at higher magnification. Crescent-shaped distribution of CenpF indicates a radial organization, especially during prometaphase. (C) The outer kinetochore is expanded during prometaphase. (left) SHREC values calculated for 198 chromosomes from six late prometaphase cells. Notice that $FWHM_{Hec1}$ is significantly larger ($P < 0.0001$), whereas $FWHM_{CenpA\text{-}GFP}$ is similar to that in metaphase cells (Fig. 1 D). (right) Cumulative distribution of immunogold particles in 60 aligned kinetochores (from three cells) also indicates that the Hec1-containing domain is radially expanded during prometaphase (compare with Fig. 2 B). Notice that the values of Delta and $FWHM_{Hec1}$ measured in the averaged images match the values obtained for a larger number of nonaligned kinetochores (left).

Provided online is a ZIP file with custom routines for measuring SHREC parameters and predicting accuracy of delta measurements via alternative approaches.