

Fig. S1. Non-uniform distribution of PAR proteins at the cell cortex in a one-cell *C. elegans* zygote. Representative time-lapse images of a one-cell stage *C. elegans* zygote co-expressing GFP::PAR-2 and mCherry::PAR-6. mCherry::PAR-6 was initially localized throughout the cortex. Upon symmetry breaking, it became segregated to the anterior cortex during polarization. GFP::PAR-2 translocated from the cytoplasm onto the posterior cortex. mCherry::PAR-6 and GFP::PAR-2 at the cortex showed a mutually-exclusive pattern throughout the first cell division. Zygotes are oriented with the posterior to the right in this and all subsequent figures. Scale bar, 5 μ m.

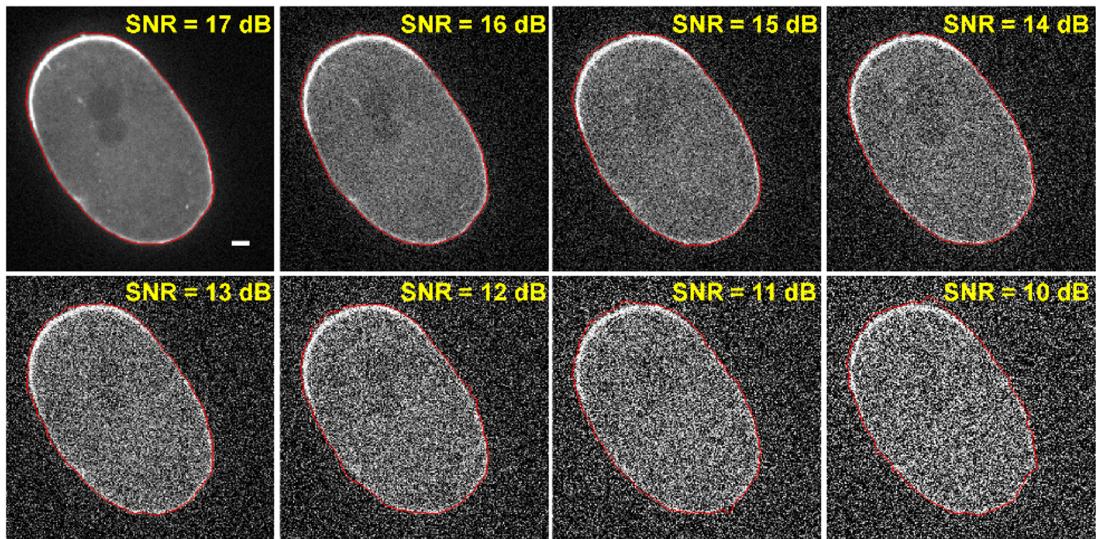


Fig. S2. Sensitivity analysis based on a single image frame. Representative images of a one-cell stage *C. elegans* zygote (merged channels of GFP::PAR-2 and mCherry::PAR-6) with introduced noise of SNR from 10 dB to 17 dB. Scale bar, 5 μ m.

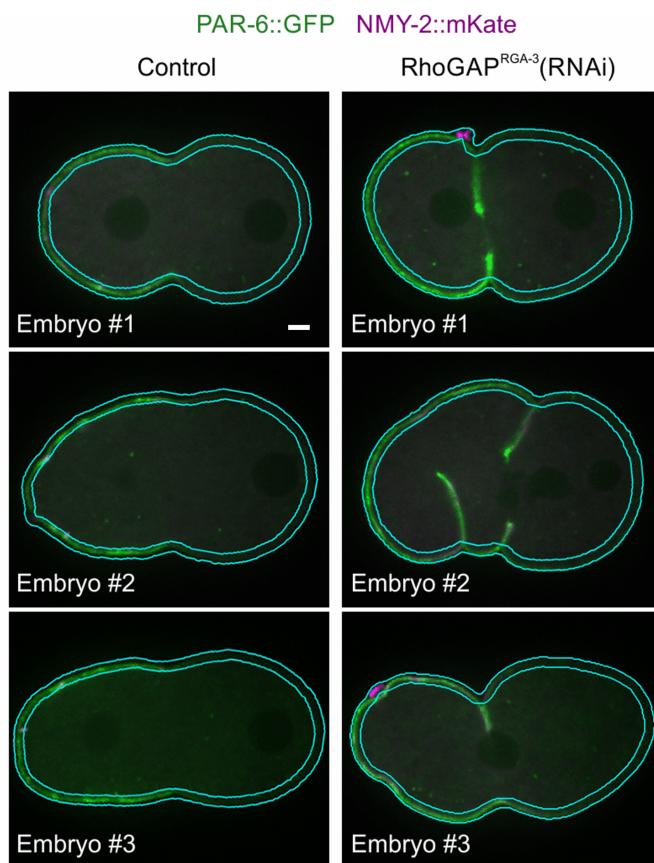


Fig. S3. Analysis of cortical protein dynamics in embryos with hyper-contraction of the cell cortex. Representative images of one-cell stage zygotes expressing PAR-6::GFP and NMY-2::mKate under control and *rga-3*(RNAi) conditions. Cyan lines indicate the inner and outer contours of segmented cell cortex. Scale bar, 5 μ m.

Table S1. Comparison of ImaEdge with open-source tools for cell segmentation in other publications

| Software | ADAPT | KoreTech | QuimP | CellGeo | CellX | CellSegm | CellTracer | CellCognition | ImaEdge |
|---|-----------------------------------|---------------|----------------|---------------------------------|-----------------|-----------------|-----------------|---------------------------------|--------------------------------------|
| Focus | Protrusions during Cell migration | Cell blebbing | Cell migration | Filopodia during cell migration | Clustered cells | Clustered cells | Clustered cells | Morphological dynamics of cells | Cell cortex during Cell polarization |
| Automatic detection for optimal segmentation threshold(s) | No | No | Yes | No | No | Yes | No | No | Yes |
| Energy function for progressive segmentation | No | No | Yes | No | No | No | No | No | No |
| Adaptive Segmentation | No | No | Yes | No | No | Yes | No | Yes | Yes |
| Automatic detection of the cell cortex | Yes | Yes | Yes | Yes | No | Yes | No | No | Yes |
| Systematic test on resistance to image noise | No | No | No | No | No | No | No | Yes | Yes |
| Dependence on a single threshold for image binarization | Yes | Yes | No | Yes | Yes | No | Yes | No | No |
| Information correlation between consecutive frames | Yes | Yes | Yes | No | No | No | Yes | Yes | Yes |
| Analysis of images of multiple cells | Yes | No | No | No | Yes | Yes | Yes | Yes | No |

Table S2. Related publications of open-source tools for cell segmentation

| Software | References |
|---------------|---|
| ADAPT | Barry, D. J., Durkin, C. H., Abella, J. V. and Way, M. (2015). Open source software for quantification of cell migration, protrusions, and fluorescence intensities. <i>J Cell Biol</i> 209, 163-80. |
| KoreTech | Biro, M., Romeo, Y., Kroschwald, S., Bovellan, M., Boden, A., Tcherkezian, J., Roux, P. P., Charras, G. and Paluch, E. K. (2013). Cell cortex composition and homeostasis resolved by integrating proteomics and quantitative imaging. <i>Cytoskeleton (Hoboken)</i> . |
| QuimP | Bosgraaf, L. and Van Haastert, P. J. (2010). Quimp3, an automated pseudopod-tracking algorithm. <i>Cell Adh Migr</i> 4, 46-55. Dormann, D., Libotte, T., Weijer, C. J. and Bretschneider, T. (2002). Simultaneous quantification of cell motility and protein-membrane-association using active contours. <i>Cell Motil Cytoskeleton</i> 52, 221-30. Zatulovskiy, E., Tyson, R., Bretschneider, T. and Kay, R. R. (2014). Bleb-driven chemotaxis of Dictyostelium cells. <i>J Cell Biol</i> 204, 1027-44. |
| CellGeo | Tsygankov, D., Bilancia, C. G., Vitriol, E. A., Hahn, K. M., Peifer, M. and Elston, T. C. (2014). CellGeo: a computational platform for the analysis of shape changes in cells with complex geometries. <i>J Cell Biol</i> 204, 443-60. |
| CellX | Dimopoulos, S., Mayer, C. E., Rudolf, F. and Stelling, J. (2014). Accurate cell segmentation in microscopy images using membrane patterns. <i>Bioinformatics</i> 30, 2644-51. |
| CellSegm | Hodneland, E., Kogel, T., Frei, D. M., Gerdes, H. H. and Lundervold, A. (2013). CellSegm - a MATLAB toolbox for high-throughput 3D cell segmentation. <i>Source Code Biol Med</i> 8, 16. |
| CellTracer | Wang, Q., Niemi, J., Tan, C. M., You, L. and West, M. (2010). Image segmentation and dynamic lineage analysis in single-cell fluorescence microscopy. <i>Cytometry A</i> 77, 101-10. |
| CellCognition | Held, M., Schmitz, M. H., Fischer, B., Walter, T., Neumann, B., Olma, M. H., Peter, M., Ellenberg, J. and Gerlich, D. W. (2010). CellCognition: time-resolved phenotype annotation in high-throughput live cell imaging. <i>Nat Methods</i> 7, 747-54. |

Table S3. Parameters in ImaEgde

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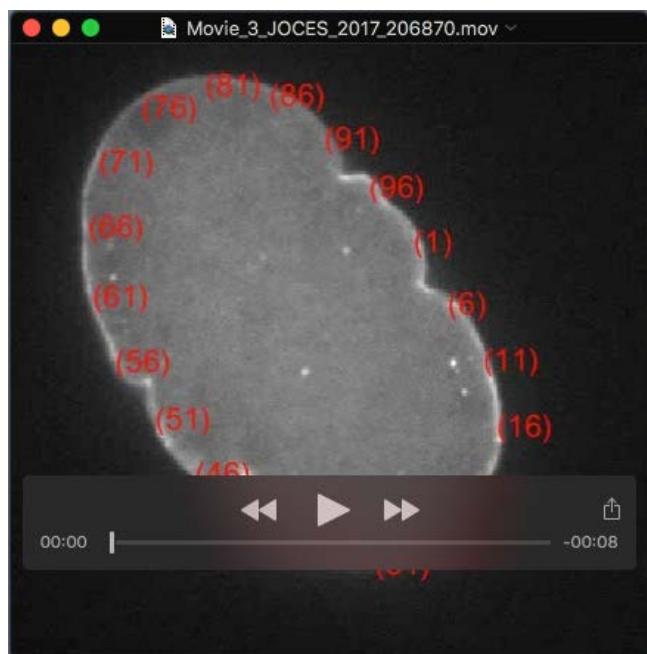
Supplementary Movies



Movie 1. GFP::PAR-2, mCherry::PAR-6, and merged images in a one-cell stage *C. elegans* zygote. Related to Fig. 2.



Movie 2. Sampling windows along the cell cortex for extraction of intensities of GFP::PAR-2 and mCherry::PAR-6 in a one-cell stage *C. elegans* zygote. Related to Fig. 4.



Movie 3. Linkage of cortical sampling windows over time in a movie of a one-cell stage *C. elegans* zygote (merged channels of GFP::PAR-2 and mCherry::PAR-6). Related to Fig. 4.



Movie 4. One-cell stage *C. elegans* zygotes expressing mCherry::PAR-6 and low levels of GFP::PAR-2 transgene. Related to Fig. 7.



Movie 5. One-cell stage *C. elegans* zygotes expressing mCherry::PAR-6 and higher levels of GFP::PAR-2 transgene. Related to Fig. 7.



Movie 6. One-cell stage *C. elegans* zygotes expressing mCherry::PAR-6 and highest levels of GFP::PAR-2 transgene. Related to Fig. 7.



Movie 7. A one-cell stage *C. elegans* zygote expressing NMY-2::mKate and GFP::PAR-2. Related to Fig. 7.



Movie 8. A two-cell stage *C. elegans* wild-type embryo expressing mCherry::PAR-6 and GFP::PAR-2. Related to Fig. 8.



Movie 9 A two-cell stage *C. elegans* zyg-1(RNAi) embryo expressing mCherry::PAR-6 and GFP::PAR-2. Related to Fig. 8.