Published in final edited form as:

Nat Struct Mol Biol. 2018 December ; 25(12): 1061–1062. doi:10.1038/s41594-018-0161-2.

Deconstructing transport-distribution reconstruction in the nuclear-pore complex

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Reporting summary

Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

To the Editor —

Nuclear-pore complexes (NPCs) span the nuclear envelope and mediate bidirectional transport between the nucleus and cytoplasm. Macromolecules (>40 kDa) require transport receptors to transit the NPC efficiently, whereas smaller molecules diffuse through the NPC passively¹. The mechanism through which the semipermeable barrier at the center of the NPC regulates selective transport is unknown. Aiming to elucidate this mechanism, Yang and colleagues have investigated spatial cargo distribution within the NPC² by using single-point edge-excitation subdiffraction (SPEED) microscopy^{2–6}.

SPEED microscopy features innovations in optical microscopy as well as data processing. The potential of SPEED to obtain pseudotomographic data prompted us to systematically analyze the technical aspects of the data-processing method. Through a series of simulations, we examined the data requirements (including precision, dataset size, and symmetry constraints) and the limits to which reconstructed transport densities can be interpreted with confidence.

Author contributions

All authors contributed to the study design and data interpretation; L.-C.T., M.H., C.S.S. and Y.-C.C. collected and analyzed data, M.H. led and implemented simulations with support from C.S.S. and Y.-C.C.; Y.-C.C. derived theory with support from L.-C.T. and C.S.S. D.G. initiated the project and provided supervision. All authors wrote, revised, and approved the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41594-018-0161-2>.

The SPEED process uses 2D singlemolecule localizations obtained by high-speed fluorescence microscopy to reconstruct a 3D density distribution. This back-projection transformation assumes that the transported particles are distributed in a cylindrically symmetrical manner, requiring just a single ‘perspective’ to reconstruct the spatial distribution. The symmetry constraint has two important implications: (i) if the underlying transport distribution is not fully cylindrically symmetric, then the reconstructed 3D density does not correspond to the actual 3D density; and (ii) if the 2D localization data used to obtain the 3D density do not reflect the underlying cylindrical symmetry (for example, because of small dataset size or limited localization precision), the accuracy of the reconstructed 3D density will diminish. As such, 3D SPEED does not meet the definition of tomography and is not guaranteed to provide accurate or unique reconstructions (Supplementary Note 1).

We simulated idealized localization datasets with known underlying distributions, performed the SPEED transformation, and compared the reconstructed distributions to the ground-truth input distributions (Fig. 1a–f). The resulting comparisons (Fig. 1g–i) revealed that the quality of reconstruction depends strongly on the size of the dataset and the overall precision of the transport particle localization: average success rates above 75% typically require at least a few hundred localizations and a localization precision below 5 nm for the distributions that we simulated. Registration precision (chromatically and between separate datasets), the precision of detection of NPC rotation, and the degree of symmetry of the transport distribution also critically affected the quality of the reconstruction (Supplementary Notes 2 and 3). Our results indicate that, under the reported experimental conditions (~ 100 localizations and localization precision of $10\text{--}13\text{ nm}$)^{2–7}, the SPEED technique is unable to reliably distinguish among uniform, central, peripheral, and bimodal transport in the central channel of the NPC. Increasing the dataset size and improving localization precision would improve the reliability of SPEED, but to do so would require precision enhancements that are currently out of reach in highly time- resolved live-cell optical microscopy.

In response to our analysis, Yang and colleagues have stressed the importance of bin-size optimization and have presented a different method of distribution characterization. We note that these procedures were not described in their previous publications. Although the choice of bin size is not trivial, especially for small datasets, varying the bin size does not significantly improve overall performance according to our simulations (Supplementary Note 2, Fig. S7). Moreover, by comparing the reconstructed distribution to the exact input distributions, Yang and colleagues incorporate a priori knowledge of the ground-truth distributions used to simulate their dataset. Such information would not be available for experimentally obtained data. Therefore, we believe that our categorization procedure conveys more realistic expectations with regard to the technique’s limitations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was funded by grants 1R01GM123541. D.G. acknowledges support from the National Institutes of Health Common Fund 4D Nucleome Program (grant 1U01EB021238) and NIGMS grant R01GM123541. C.S.S. was supported by a Junior Research Fellowship through Merton College, Oxford, UK. We thank S. Musser for critical feedback on the entire project and M. Hammer for critical reading of theory development. L.-C.T. acknowledges support by the NIH through grant K99 GM126810.

References

1. Wente SR & Rout MP Cold Spring Harb. Perspect. Biol.2, a000562 (2010).
2. Ma J, Goryaynov A & Yang W Nat. Struct. Mol. Biol.23, 239–247 (2016). [PubMed: 26878241]
3. Ma J & Yang W Proc. Natl Acad. Sci. USA 107, 7305–7310 (2010). [PubMed: 20368455]
4. Ma J, Goryaynov A, Sarma A & Yang W Proc. Natl Acad. Sci. USA 109, 7326–7331 (2012). [PubMed: 22529346]
5. Ma J et al. Nat. Commun.4, 2414 (2013). [PubMed: 24008311]
6. Goryaynov A & Yang W PLoS One 9, e88792 (2014). [PubMed: 24558427]
7. Kelich JM et al. Mol. Ther. Methods Clin. Dev.2, 15047 (2015). [PubMed: 26665132]

Fig. 1 | The performance of SPEED depends on the dataset size and overall precision.

SPEED was used to reconstruct radial cargo distribution in the central channel of the NPC by using idealized, simulated data. **a**, Dimensions of the NPC central channel. **b**, Model distributions (dist.) in which the central channel accommodates transport of molecules through its center, periphery, or both. Simul. locs., simulated locations. **c–f**, To quantify the ability of SPEED to distinguish among central, peripheral, bimodal, or uniform distributions, we generated a simulated dataset of transport localizations for each distribution pattern (step 1; shown in color in **c**). Simulated measurement uncertainty was added (step 2), and the localizations were projected to 2D (step 3; gray dots in **c**) to mimic data acquired by microscopy. The projected density profiles were extracted in **d** and **e** (the effect of simulated measurement uncertainty differs between **e** and **d**). The back-projection transformation was subsequently applied (step 4) to the projected density profile in **e** to reconstruct a radial density profile in **f**, which was then fitted to a radial distribution (step 5) and compared with the four model distributions (step 6). The most significant fit was compared to the input distribution of the simulated dataset, thus revealing either successful identification or failure. **g–i**, Bottom charts, success rates (percentage correctly identified, $n = 100$ unique simulations) for each combination of distribution type (central, peripheral, bimodal, and uniform), dataset size (10^2 , 10^3 , and 10^4 localizations) and precision ($\sigma = 2, 4, 6, 10$ nm). Top charts, identities of the incorrectly designated reconstructions (opaque colors) assigned by the SPEED data-processing algorithm for each dataset size at $\sigma = 10$ nm, revealing that reconstructions fail mainly because of a lack of data points in small datasets and insufficient localization precision in large datasets.