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Correlating single-molecule localization microscopy and cryo-electron tomography

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References

- 1 Dubochet, Jacques. "Early cryo-electron microscopy." Nobel acceptance lecture. December 8, 2017. <https://www.nobelprize.org/uploads/2018/01/dubochet-lecture.pdf>
- 2 Abadi, M. *et al.* Large-Scale Machine Learning on Heterogeneous Distributed Systems. *arXiv* (2015).
- 3 Abbe, E. Beiträge zur Theorie des Mikroskops und der mikroskopischen Wahrnehmung. *Archiv für Mikroskopische Anatomie* **9**, 413–468 (1873).
- 4 Abendstein, L. *et al.* Complement is activated by elevated IgG3 hexameric platforms and deposits C4b onto distinct antibody domains. *Nat Commun* **14**, 4027 (2023).
- 5 Abràmoff, M. D., Magalhães, P. J. & Ram, S. J. Image processing with ImageJ. *Biophotonics international* **11**, 36–42 (2004).
- 6 Abramson, J. *et al.* Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **630**, 493–500 (2024).
- 7 Ader, N. R. *et al.* Molecular and topological reorganizations in mitochondrial architecture interplay during Bax-mediated steps of apoptosis. *eLife* **8**, e40712 (2019).
- 8 Afonine, P. V. *et al.* Real-space refinement in PHENIX for cryo-EM and crystallography. *Acta Crystallogr D Struct Biol* **74**, 531–544 (2018).
- 9 Aihara, Y. *et al.* Green fluorescence from cnidarian hosts attracts symbiotic algae. *Proceedings of the National Academy of Sciences* **116**, 2118–2123 (2019).
- 10 Al-Amoudi, A. *et al.* Cryo-electron microscopy of vitreous sections. *The EMBO journal* **23**, 3583–3588 (2004).
- 11 Anderson, K., Nilsson, T. & Fernandez-Rodriguez, J. in *Correlative Imaging* 23–35 (2019).
- 12 Ando, T. *et al.* The 2018 correlative microscopy techniques roadmap. *Journal of physics D: Applied physics* **51**, 443001 (2018).
- 13 Andresen, M. *et al.* Photoswitchable fluorescent proteins enable monochromatic multilabel imaging and dual color fluorescence nanoscopy. *Nature Biotechnology* **26**, 1035–1040 (2008).
- 14 Arnold, J. *et al.* Site-specific cryo-focused ion beam sample preparation guided by 3D correlative microscopy. *Biophysical journal* **110**, 860–869 (2016).
- 15 Arnold, S. A. *et al.* Blotting-free and lossless cryo-electron microscopy grid preparation from nanoliter-sized protein samples and single-cell extracts. *Journal of Structural Biology* **197**, 220–226 (2017).
- 16 Assaiya, A., Burada, A. P., Dhingra, S. & Kumar, J. An overview of the recent advances in cryo-electron microscopy for life sciences. *Emerging Topics in Life Sciences* **5**, 151–168 (2021).
- 17 Au - Vyas, N. *et al.* Cryo-Structured Illumination Microscopic Data Collection from Cryogenically Preserved Cells. *JoVE*, e62274 (2021).
- 18 Bai, X.-C., McMullan, G. & Scheres, S. H. How cryo-EM is revolutionizing structural biology. *Trends in biochemical sciences* **40**, 49–57 (2015).
- 19 Bajar, B. T. *et al.* Improving brightness and photostability of green and red fluorescent proteins for live cell imaging and FRET reporting. *Sci Rep* **6**, 20889 (2016).
- 20 Baldwin, P. R. *et al.* Big data in cryoEM: automated collection, processing and accessibility of EM data. *Curr Opin Microbiol* **43**, 1–8 (2018).
- 21 Bankhead, P. *et al.* QuPath: Open source software for digital pathology image analysis. *Sci Rep* **7**, 16878 (2017).

- 22 Baumlér, W., Regensburger, J., Knak, A., Felgentrager, A. & Maisch, T. UVA and endogenous photosensitizers--the detection of singlet oxygen by its luminescence. *Photochem Photobiol Sci* **11**, 107-117 (2012).
- 23 Beck, M. & Baumeister, W. Cryo-electron tomography: can it reveal the molecular sociology of cells in atomic detail? *Trends in cell biology* **26**, 825-837 (2016).
- 24 Belevich, I., Joensuu, M., Kumar, D., Vihinen, H. & Jokitalo, E. Microscopy image browser: a platform for segmentation and analysis of multidimensional datasets. *PLoS biology* **14**, e1002340 (2016).
- 25 Bepler, T. *et al.* Positive-unlabeled convolutional neural networks for particle picking in cryo-electron micrographs. *Nat Methods* **16**, 1153-1160 (2019).
- 26 Berger, C. *et al.* Plasma FIB milling for the determination of structures in situ. *Nature Communications* **14**, 629 (2023).
- 27 Berger, C. *et al.* Cryo-electron tomography on focused ion beam lamellae transforms structural cell biology. *Nature Methods* **20**, 499-511 (2023).
- 28 Berman, H. M. *et al.* The Protein Data Bank. *Nucleic Acids Research* **28**, 235-242 (2000).
- 29 Betzig, E. Proposed method for molecular optical imaging. *Opt. Lett.* **20**, 237-239 (1995).
- 30 Betzig, E. *et al.* Imaging Intracellular Fluorescent Proteins at Nanometer Resolution. *Science* **313**, 1642-1645 (2006).
- 31 Bieber, A. *et al.* Precise 3D-correlative FIB-milling of biological samples using METEOR, an integrated cryo-CLEM imaging system. *Microscopy and Microanalysis* **27**, 3230-3232 (2021).
- 32 Bindels, D. S. *et al.* mScarlet: a bright monomeric red fluorescent protein for cellular imaging. *Nat Methods* **14**, 53-56 (2017).
- 33 Birtasu, A. N. *et al.* The molecular architecture of the kidney slit diaphragm. *bioRxiv*, 2023.2010.2027.564405 (2023).
- 34 Boltje, D. B. *et al.* A cryogenic, coincident fluorescence, electron, and ion beam microscope. *eLife* **11**, e82891 (2022).
- 35 Boltje, D. B. *et al.* Thickness and quality controlled fabrication of fluorescence-targeted frozen-hydrated lamellae. *bioRxiv*, 2024.2007.2004.602102 (2024).
- 36 Bourgeois, D. & Adam, V. Reversible photoswitching in fluorescent proteins: A mechanistic view. *IUBMB Life* **64**, 482-491 (2012).
- 37 Brown, H. & Hanssen, E. Accurately measuring ice thickness quickly and quantitatively on a screening TEM. *Microscopy and Microanalysis* **27**, 1158-1160 (2021).
- 38 Buchholz, T.-O., Jordan, M., Pigino, G. & Jug, F. in *2019 IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019)*. 502-506 (IEEE).
- 39 Buckley, G. *et al.* Automated cryo-lamella preparation for high-throughput in-situ structural biology. *Journal of structural biology* **210**, 107488 (2020).
- 40 Burnley, T., Palmer, C. M. & Winn, M. Recent developments in the CCP-EM software suite. *Acta Crystallographica Section D* **73**, 469-477 (2017).
- 41 Calcrafft, T. & Rosenthal, P. B. Cryogenic electron microscopy approaches that combine images and tilt series. *Microscopy* **71**, i15-i22 (2022).
- 42 Carter, S. D. *et al.* Distinguishing signal from autofluorescence in cryogenic correlated light and electron microscopy of mammalian cells. *J Struct Biol* **201**, 15-25 (2018).

- 43 Castano-Díez, D., Kudryashev, M., Arheit, M. & Stahlberg, H. Dynamo: a flexible, user-friendly development tool for subtomogram averaging of cryo-EM data in high-performance computing environments. *J Struct Biol* **178**, 139–151 (2012).
- 44 Castaño-Díez, D., Kudryashev, M. & Stahlberg, H. Dynamo Catalogue: Geometrical tools and data management for particle picking in subtomogram averaging of cryo-electron tomograms. *Journal of Structural Biology* **197**, 135–144 (2017).
- 45 Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W. & Prasher, D. C. Green fluorescent protein as a marker for gene expression. *Science* **263**, 802–805 (1994).
- 46 Chandran Suja, V., Sentmanat, J., Hofmann, G., Scales, C. & Fuller, G. G. Hyperspectral imaging for dynamic thin film interferometry. *Scientific reports* **10**, 11378 (2020).
- 47 Chang, Y.-W. *et al.* Correlated cryogenic photoactivated localization microscopy and cryo-electron tomography. *Nature Methods* **11**, 737–739 (2014).
- 48 Chen, F., Tillberg, P. W. & Boyden, E. S. Expansion microscopy. *Science* **347**, 543–548 (2015).
- 49 Chen, X., Zaro, J. L. & Shen, W. C. Fusion protein linkers: property, design and functionality. *Adv Drug Deliv Rev* **65**, 1357–1369 (2013).
- 50 Cheng, Y. Single-particle cryo-EM—How did it get here and where will it go. *Science* **361**, 876–880 (2018).
- 51 Cho, H.-J. *et al.* Measurement of ice thickness on vitreous ice embedded cryo-EM grids: investigation of optimizing condition for visualizing macromolecules. *Journal of Analytical Science and Technology* **4**, 1–5 (2013).
- 52 Chua, E. Y. *et al.* Vitrocam: A simple low cost Vitrobot camera for assessing grid quality. *bioRxiv*, 2022.2006.2016.496351 (2022).
- 53 Clarke, D. N. *et al.* Fluorescent proteins generate a genetic color polymorphism and counteract oxidative stress in intertidal sea anemones. *Proceedings of the National Academy of Sciences* **121** (2024).
- 54 Cleeve, P. *et al.* OpenFIBSEM: A universal API for FIBSEM control. *Journal of Structural Biology* **215**, 107967 (2023).
- 55 Collinson, L. & Verkade, P. Probing the future of correlative microscopy. *Journal of Chemical Biology* **8**, 127–128 (2015).
- 56 Colville, M. J., Park, S., Zipfel, W. R. & Paszek, M. J. High-speed device synchronization in optical microscopy with an open-source hardware control platform. *Scientific Reports* **9**, 12188 (2019).
- 57 Consortium, T. w. EMDB—the Electron Microscopy Data Bank. *Nucleic Acids Research* **52**, D456–D465 (2023).
- 58 Creekmore, B. C., Kixmoeller, K., Black, B. E., Lee, E. B. & Chang, Y.-W. Ultrastructure of human brain tissue vitrified from autopsy revealed by cryo-ET with cryo-plasma FIB milling. *Nature Communications* **15**, 2660 (2024).
- 59 Croll, T. I. ISOLDE: a physically realistic environment for model building into low-resolution electron-density maps. *Acta Crystallogr D Struct Biol* **74**, 519–530 (2018).
- 60 Cruz-León, S. *et al.* High-confidence 3D template matching for cryo-electron tomography. *Nature Communications* **15**, 3992 (2024).
- 61 Dahlberg, P. D. & Moerner, W. E. Cryogenic Super-Resolution Fluorescence and Electron Microscopy Correlated at the Nanoscale. *Annu Rev Phys Chem* **72**, 253–278 (2021).

- 62 Dahlberg, P. D., Perez, D., Hecksel, C. W., Chiu, W. & Moerner, W. E. Metallic support films reduce optical heating in cryogenic correlative light and electron tomography. *Journal of Structural Biology* **214**, 107901 (2022).
- 63 Dahlberg, P. D. *et al.* Identification of PAMKate as a Red Photoactivatable Fluorescent Protein for Cryogenic Super-Resolution Imaging. *Journal of the American Chemical Society* **140**, 12310–12313 (2018).
- 64 Dahlberg, P. D. *et al.* Cryogenic single-molecule fluorescence annotations for electron tomography reveal in situ organization of key proteins in *Caulobacter*. *Proceedings of the National Academy of Sciences* **117**, 13937–13944 (2020).
- 65 Dandey, V. P. *et al.* Spotiton: New features and applications. *Journal of Structural Biology* **202**, 161–169 (2018).
- 66 Danev, R., Yanagisawa, H. & Kikkawa, M. Cryo-EM performance testing of hardware and data acquisition strategies. *Microscopy* **70**, 487–497 (2021).
- 67 Danial, J. S. H. *et al.* Constructing a cost-efficient, high-throughput and high-quality single-molecule localization microscope for super-resolution imaging. *Nature Protocols* **17**, 2570–2619 (2022).
- 68 Danita, C., Chiu, W. & Galaz-Montoya, J. G. Efficient manual annotation of cryogenic electron tomograms using IMOD. *STAR Protocols* **3**, 101658 (2022).
- 69 Darrow, M. C. *et al.* Enabling a Paradigm Shift in CryoEM Sample Preparation with chameleon. *Microscopy and Microanalysis* **27**, 524–525 (2021).
- 70 de Boer, P., Hoogenboom, J. P. & Giepmans, B. N. G. Correlated light and electron microscopy: ultrastructure lights up! *Nature Methods* **12**, 503–513 (2015).
- 71 Depelteau, J. S. *et al.* UVC inactivation of pathogenic samples suitable for cryo-EM analysis. *Communications Biology* **5**, 29 (2022).
- 72 DeRosier, D. J. Where in the cell is my protein? *Quarterly Reviews of Biophysics* **54**, e9 (2021).
- 73 Dertinger, T., Colyer, R., Iyer, G., Weiss, S. & Enderlein, J. Fast, background-free, 3D super-resolution optical fluctuation imaging (SOFI). *Proc Natl Acad Sci U S A* **106**, 22287–22292 (2009).
- 74 TensorFlow (2022).
- 75 Dickson, R. M., Cubitt, A. B., Tsien, R. Y. & Moerner, W. E. On/off blinking and switching behaviour of single molecules of green fluorescent protein. *Nature* **388**, 355–358 (1997).
- 76 Doyle, J. Beyond the spherical cow. *Nature* **411**, 151–152 (2001).
- 77 Dubochet, J., Lepault, J., Freeman, R., Berriman, J. & Homo, J. C. Electron microscopy of frozen water and aqueous solutions. *Journal of Microscopy* **128**, 219–237 (1982).
- 78 Dubochet, J. & McDowell, A. Vitrification of pure water for electron microscopy. *Journal of Microscopy* **124**, 3–4 (1981).
- 79 Dung, N. H. T. *et al.* Serialized On-grid Lift-In Sectioning for Tomography (SOLIST). *bioRxiv*, 2023.2005.2011.540146 (2023).
- 80 Duong, V. Q. *Characterisation of photo-switching in fluorescent molecules at low temperatures*, Staats- und Universitätsbibliothek Hamburg Carl von Ossietzky, (2023).
- 81 Edelstein, A., Amodaj, N., Hoover, K., Vale, R. & Stuurman, N. Computer control of microscopes using µManager. *Curr Protoc Mol Biol* **Chapter 14**, Unit14.20 (2010).
- 82 Edelstein, A. D. *et al.* Advanced methods of microscope control using µManager software. *J Biol Methods* **1** (2014).

- 83 Eichler, M., Lavi, R., Shainberg, A. & Lubart, R. Flavins are source of visible-light-induced free radical formation in cells. *Lasers in Surgery and Medicine* **37**, 314–319 (2005).
- 84 Eisenstein, F., Fukuda, Y. & Danev, R. Smart parallel automated cryo-electron tomography. *Nature Methods*, 1–4 (2024).
- 85 Eisenstein, F. *et al.* Parallel cryo electron tomography on in situ lamellae. *Nature Methods* **20**, 131–138 (2023).
- 86 Ermantraut, E., Wohlfart, K. & Tichelaar, W. Perforated support foils with pre-defined hole size, shape and arrangement. *Ultramicroscopy* **74**, 75–81 (1998).
- 87 Faoro, R. *et al.* Aberration-corrected cryoimmersion light microscopy. *Proceedings of the National Academy of Sciences* **115**, 1204–1209 (2018).
- 88 Faro, A. R. *et al.* Low-temperature switching by photoinduced protonation in photochromic fluorescent proteins. *Photochemical & Photobiological Sciences* **9**, 254–262 (2010).
- 89 Floris, D. & Kühlbrandt, W. Molecular landscape of etioplast inner membranes in higher plants. *Nature Plants* **7**, 514–523 (2021).
- 90 Förster, F. & Briegel, A. *Cryo-electron Tomography: Structural Biology in Situ*. (Springer, 2024).
- 91 Fox, F. <<https://www.mikro-foto.de>>
- 92 Frangakis, A. S. *et al.* Identification of macromolecular complexes in cryoelectron tomograms of phantom cells. *Proceedings of the National Academy of Sciences* **99**, 14153–14158 (2002).
- 93 Fung, H. K. *et al.* Genetically encoded multimeric tags for intracellular protein localisation in cryo-EM. *bioRxiv*, 2022.2012.2010.519870 (2022).
- 94 Furubayashi, T. *et al.* Three-Dimensional Localization of an Individual Fluorescent Molecule with Angstrom Precision. *Journal of the American Chemical Society* **139**, 8990–8994 (2017).
- 95 Galaz-Montoya, J. G., Flanagan, J., Schmid, M. F. & Ludtke, S. J. Single particle tomography in EMAN2. *J Struct Biol* **190**, 279–290 (2015).
- 96 Gemmer, M. *et al.* Visualization of translation and protein biogenesis at the ER membrane. *Nature* **614**, 160–167 (2023).
- 97 Gilles, M. A. & Singer, A. A Bayesian framework for cryo-EM heterogeneity analysis using regularized covariance estimation. *bioRxiv* (2023).
- 98 Goddard, T. D. *et al.* UCSF ChimeraX: Meeting modern challenges in visualization and analysis. *Protein Sci* **27**, 14–25 (2018).
- 99 Godman, G. C., Morgan, C., Breitenfeld, P. M. & Rose, H. M. A CORRELATIVE STUDY BY ELECTRON AND LIGHT MICROSCOPY OF THE DEVELOPMENT OF TYPE 5 ADENOVIRUS : II. LIGHT MICROSCOPY. *Journal of Experimental Medicine* **112**, 383–402 (1960).
- 100 Gorelick, S. *et al.* PIE-scope, integrated cryo-correlative light and FIB/SEM microscopy. *eLife* **8**, e45919 (2019).
- 101 Greenfield, D. *et al.* Self-Organization of the Escherichia coli Chemotaxis Network Imaged with Super-Resolution Light Microscopy. *PLOS Biology* **7**, e1000137 (2009).
- 102 Grotjohann, T. *et al.* rsEGFP2 enables fast RESOLFT nanoscopy of living cells. *eLife* **1**, e00248 (2012).
- 103 Grünewald, K., Medalia, O., Gross, A., Steven, A. C. & Baumeister, W. Prospects of electron cryotomography to visualize macromolecular complexes inside cellular compartments: implications of crowding. *Biophysical chemistry* **100**, 577–591 (2002).

- 104 Guaita, M., Watters, S. C. & Loerch, S. Recent advances and current trends in cryo-electron microscopy. *Current Opinion in Structural Biology* **77**, 102484 (2022).
- 105 Guérin, C. J., Liv, N. & Klumperman, J. in *Correlative Imaging* 1–21 (2019).
- 106 Gustafsson, M. G. L. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. *Journal of Microscopy* **198**, 82–87 (2000).
- 107 Gustafsson, N. *et al.* Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations. *Nature Communications* **7**, 12471 (2016).
- 108 Gwosch, K. C. *et al.* MINFLUX nanoscopy delivers 3D multicolor nanometer resolution in cells. *Nature Methods* **17**, 217–224 (2020).
- 109 Hagen, W. J. Light ‘Em up: Efficient Screening of Gold Foil Grids in Cryo-EM. *Frontiers in Molecular Biosciences* **9**, 912363 (2022).
- 110 Hagen, W. J. H., Wan, W. & Briggs, J. A. G. Implementation of a cryo-electron tomography tilt-scheme optimized for high resolution subtomogram averaging. *J Struct Biol* **197**, 191–198 (2017).
- 111 Haguenau, F. *et al.* Key Events in the History of Electron Microscopy. *Microscopy and Microanalysis* **9**, 96–138 (2003).
- 112 Hampton, C. M. *et al.* Correlated fluorescence microscopy and cryo-electron tomography of virus-infected or transfected mammalian cells. *Nat Protoc* **12**, 150–167 (2017).
- 113 Harris, C. R. *et al.* Array programming with NumPy. *Nature* **585**, 357–362 (2020).
- 114 Harris, J. R. Transmission electron microscopy in molecular structural biology: A historical survey. *Archives of Biochemistry and Biophysics* **581**, 3–18 (2015).
- 115 Hass, G. & Salzberg, C. D. Optical properties of silicon monoxide in the wavelength region from 0.24 to 14.0 microns. *JOSA* **44**, 181–187 (1954).
- 116 Hattne, J. *et al.* Analysis of Global and Site-Specific Radiation Damage in Cryo-EM. *Structure* **26**, 759–766.e754 (2018).
- 117 Heebner, J. E. *et al.* Deep learning-based segmentation of cryo-electron tomograms. *JoVE (Journal of Visualized Experiments)*, e64435 (2022).
- 118 Heiligenstein, X., Paul-Gilloteaux, P., Raposo, G. & Salamero, J. in *Methods in cell biology* Vol. 140 335–352 (Elsevier, 2017).
- 119 Hell, S. W. & Wichmann, J. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. *Opt. Lett.* **19**, 780–782 (1994).
- 120 Hellström, K., Vihinen, H., Kallio, K., Jokitalo, E. & Ahola, T. Correlative light and electron microscopy enables viral replication studies at the ultrastructural level. *Methods* **90**, 49–56 (2015).
- 121 Henderson, R. & Alsarib, M. History of Cryo-EM. *Scientific Video Protocols* **1**, 1 (2020).
- 122 Hirano, M. *et al.* A highly photostable and bright green fluorescent protein. *Nature Biotechnology* **40**, 1132–1142 (2022).
- 123 Hodgson, L., Nam, D., Mantell, J., Achim, A. & Verkade, P. in *Methods in cell biology* Vol. 124 1–21 (Elsevier, 2014).
- 124 Hoffman, D. P. *et al.* Correlative three-dimensional super-resolution and block-face electron microscopy of whole vitreously frozen cells. *Science* **367**, eaaz5357 (2020).
- 125 Hohle, M. M. *et al.* Ice thickness monitoring for cryo-EM grids by interferometry imaging. *Scientific Reports* **12**, 15330 (2022).

- 126 Holm, T. *et al.* A Blueprint for Cost-Efficient Localization Microscopy. *ChemPhysChem* **15**, 651–654 (2014).
- 127 Huang, B., Wang, W., Bates, M. & Zhuang, X. Three-Dimensional Super-Resolution Imaging by Stochastic Optical Reconstruction Microscopy. *Science* **319**, 810–813 (2008).
- 128 Huff, J. The Airyscan detector from ZEISS: confocal imaging with improved signal-to-noise ratio and super-resolution. *Nature Methods* **12**, i–ii (2015).
- 129 Hulleman, C. N., Li, W., Gregor, I., Rieger, B. & Enderlein, J. Photon yield enhancement of red fluorophores at cryogenic temperatures. *ChemPhysChem* **19**, 1774–1780 (2018).
- 130 Icha, J., Weber, M., Waters, J. C. & Norden, C. Phototoxicity in live fluorescence microscopy, and how to avoid it. *Bioessays* **39** (2017).
- 131 Isola, P., Zhu, J.-Y., Zhou, T. & Efros, A. A. Image-to-Image Translation with Conditional Adversarial Networks. *2017 IEEE Conference on Computer Vision and Pattern Recognition (CVPR)*, 5967–5976 (2017).
- 132 Iudin, A. *et al.* EMPIAR: the electron microscopy public image archive. *Nucleic Acids Research* **51**, D1503–D1511 (2023).
- 133 Jacquemet, G., Carisey, A. F., Hamidi, H., Henriques, R. & Leterrier, C. The cell biologist's guide to super-resolution microscopy. *Journal of Cell Science* **133** (2020).
- 134 Jeon, M. *et al.* CryoBench: Diverse and challenging datasets for the heterogeneity problem in cryo-EM. *arXiv preprint arXiv:2408.05526* (2024).
- 135 Jumper, J. *et al.* Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 (2021).
- 136 Jun, S. *et al.* Direct visualization of HIV-1 with correlative live-cell microscopy and cryo-electron tomography. *Structure* **19**, 1573–1581 (2011).
- 137 Jun, S. *et al.* Advances in Cryo-Correlative Light and Electron Microscopy: Applications for Studying Molecular and Cellular Events. *The Protein Journal* **38**, 609–615 (2019).
- 138 Kaufmann, R., Hagen, C. & Grünewald, K. Fluorescence cryo-microscopy: current challenges and prospects. *Current Opinion in Chemical Biology* **20**, 86–91 (2014).
- 139 Kaufmann, R. *et al.* Super-resolution microscopy using standard fluorescent proteins in intact cells under cryo-conditions. *Nano Lett* **14**, 4171–4175 (2014).
- 140 Khatler, H., Myasnikov, A. G., Natchiar, S. K. & Klaholz, B. P. Structure of the human 80S ribosome. *Nature* **520**, 640–645 (2015).
- 141 Kikuchi, K., Adair, L. D., Lin, J., New, E. J. & Kaur, A. Photochemical mechanisms of fluorophores employed in single-molecule localization microscopy. *Angewandte Chemie* **135**, e202204745 (2023).
- 142 Kimanius, D., Dong, L., Sharov, G., Nakane, T. & Scheres, S. H. W. New tools for automated cryo-EM single-particle analysis in RELION-4.0. *Biochem J* **478**, 4169–4185 (2021).
- 143 Kitagawa, K. Thin-film thickness profile measurement by three-wavelength interference color analysis. *Appl. Opt.* **52**, 1998–2007 (2013).
- 144 Klein, S. *et al.* Post-correlation on-lamella cryo-CLEM reveals the membrane architecture of lamellar bodies. *Communications Biology* **4**, 137 (2021).
- 145 Klumpe, S. *et al.* A modular platform for automated cryo-FIB workflows. *eLife* **10** (2021).
- 146 Knoll, M. & Ruska, E. Das Elektronenmikroskop. *Zeitschrift für Physik* **78**, 318–339 (1932).
- 147 Kofman, V., He, J., ten Kate, I. L. & Linnartz, H. The refractive index of amorphous and crystalline water ice in the UV-vis. *The Astrophysical Journal* **875**, 131 (2019).

- 148 Koning, R. I. & Koster, A. J. Cryo-electron tomography in biology and medicine. *Annals of Anatomy-Anatomischer Anzeiger* **191**, 427-445 (2009).
- 149 Koning, R. I., Koster, A. J. & Sharp, T. H. Advances in cryo-electron tomography for biology and medicine. *Annals of Anatomy - Anatomischer Anzeiger* **217**, 82-96 (2018).
- 150 Koning, R. I. *et al.* Automated vitrification of cryo-EM samples with controllable sample thickness using suction and real-time optical inspection. *Nature Communications* **13**, 2985 (2022).
- 151 Kremer, J. R., Mastronarde, D. N. & McIntosh, J. R. Computer visualization of three-dimensional image data using IMOD. *Journal of structural biology* **116**, 71-76 (1996).
- 152 Kremers, G.-J., Gilbert, S. G., Cranfill, P. J., Davidson, M. W. & Piston, D. W. Fluorescent proteins at a glance. *Journal of Cell Science* **124**, 157-160 (2011).
- 153 Kuba, J. *et al.* Advanced cryo-tomography workflow developments – correlative microscopy, milling automation and cryo-lift-out. *Journal of Microscopy* **281**, 112-124 (2020).
- 154 Kühlbrandt, W. The Resolution Revolution. *Science* **343**, 1443-1444 (2014).
- 155 Kukulski, W. *et al.* Correlated fluorescence and 3D electron microscopy with high sensitivity and spatial precision. *Journal of Cell Biology* **192**, 111-119 (2011).
- 156 LaFrance, B. J. *et al.* Structural transitions in the GTP cap visualized by cryo-electron microscopy of catalytically inactive microtubules. *Proceedings of the National Academy of Sciences* **119**, e2114994119 (2022).
- 157 Lam, V. & Villa, E. Practical Approaches for Cryo-FIB Milling and Applications for Cellular Cryo-Electron Tomography. *Methods Mol Biol* **2215**, 49-82 (2021).
- 158 Lambert, T. J. FPbase: a community-editable fluorescent protein database. *Nature Methods* **16**, 277-278 (2019).
- 159 Last, M. G., Voortman, L. M. & Sharp, T. H. Building a super-resolution fluorescence cryomicroscope. *Methods in cell biology* **187**, 205-222 (2024).
- 160 Last, M. G., Voortman, L. M. & Sharp, T. H. Imaging intracellular components in situ using super-resolution cryo-correlative light and electron microscopy. *Methods in cell biology* **187**, 223-248 (2024).
- 161 Last, M. G. F., Abendstein, L., Voortman, L. M. & Sharp, T. H. Ais: streamlining segmentation of cryo-electron tomography datasets. *eLife* (2024).
- 162 Last, M. G. F., Noteborn, W. E. M., Voortman, L. M. & Sharp, T. H. Super-resolution fluorescence imaging of cryosamples does not limit achievable resolution in cryoEM. *Journal of Structural Biology* **215**, 108040 (2023).
- 163 Last, M. G. F., Tuijtel, M. W., Voortman, L. M. & Sharp, T. H. Selecting optimal support grids for super-resolution cryogenic correlated light and electron microscopy. *Scientific Reports* **13** (2023).
- 164 Last, M. G. F., Voortman, L. M. & Sharp, T. H. Measuring cryo-TEM sample thickness using reflected light microscopy and machine learning. *Journal of Structural Biology* **215**, 107965 (2023).
- 165 Last, M. G. F., Voortman, L. M. & Sharp, T. H. scNodes: a correlation and processing toolkit for super-resolution fluorescence and electron microscopy. *Nat Methods* **20**, 1445-1446 (2023).
- 166 Lavoie-Cardinal, F. *et al.* Two-color RESOLFT nanoscopy with green and red fluorescent photochromic proteins. *Chemphyschem* **15**, 655-663 (2014).

- 167 Lawson, C. L. *et al.* EMDatabank unified data resource for 3DEM. *Nucleic Acids Research* **44**, D396–D403 (2015).
- 168 Le Gros, M. A., McDermott, G., Uchida, M., Knoechel, C. G. & Larabell, C. A. High-aperture cryogenic light microscopy. *Journal of Microscopy* **235**, 1–8 (2009).
- 169 Lelek, M. *et al.* Single-molecule localization microscopy. *Nature Reviews Methods Primers* **1**, 39 (2021).
- 170 Li, H. & Vaughan, J. C. Switchable fluorophores for single-molecule localization microscopy. *Chemical reviews* **118**, 9412–9454 (2018).
- 171 Li, S. *et al.* HOPE-SIM, a cryo-structured illumination fluorescence microscopy system for accurately targeted cryo-electron tomography. *Communications Biology* **6**, 474 (2023).
- 172 Li, S. *et al.* ELI trifocal microscope: a precise system to prepare target cryo-lamellae for in situ cryo-ET study. *Nature Methods* **20**, 276–283 (2023).
- 173 Li, W. *et al.* Integrated multimodality microscope for accurate and efficient target-guided cryo-lamellae preparation. *Nature Methods* **20**, 268–275 (2023).
- 174 Li, W., Stein, S. C., Gregor, I. & Enderlein, J. Ultra-stable and versatile widefield cryo-fluorescence microscope for single-molecule localization with sub-nanometer accuracy. *Opt. Express* **23**, 3770–3783 (2015).
- 175 Liu, B. *et al.* Three-dimensional super-resolution protein localization correlated with vitrified cellular context. *Scientific Reports* **5**, 13017 (2015).
- 176 Liu, P. Y. *et al.* Cell refractive index for cell biology and disease diagnosis: past, present and future. *Lab on a Chip* **16**, 634–644 (2016).
- 177 Liu, W. *et al.* Breaking the Axial Diffraction Limit: A Guide to Axial Super-Resolution Fluorescence Microscopy. *Laser & Photonics Reviews* **12**, 1700333 (2018).
- 178 Liu, Y.-T. *et al.* Isotropic reconstruction for electron tomography with deep learning. *Nature Communications* **13**, 6482 (2022).
- 179 Liv, N., Fermie, J., ten Brink, C. B. M., de Heus, C. & Klumperman, J. in *Methods in Cell Biology* Vol. 177 (eds Kedar Narayan, Lucy Collinson, & Paul Verkade) 301–326 (Academic Press, 2023).
- 180 Loginov, S. V., Boltje, D. B., Hensgens, M. N. F., Hoogenboom, J. P. & van der Wee, E. B. Depth-dependent scaling of axial distances in light microscopy. *Optica* **11**, 553–568 (2024).
- 181 Luengo, I. *et al.* SuRVoS: Super-Region Volume Segmentation workbench. *J Struct Biol* **198**, 43–53 (2017).
- 182 Mantel, I., Sadiq, B. A. & Blander, J. M. Spotlight on TAP and its vital role in antigen presentation and cross-presentation. *Molecular immunology* **142**, 105–119 (2022).
- 183 Mantovanelli, A. *Development of Fluorescent Markers for Super-Resolution Microscopy at Cryogenic Temperature*, Université Grenoble Alpes [2020–....], (2023).
- 184 Mantovanelli, A. M. *et al.* Photophysical studies at cryogenic temperature reveal a novel photoswitching mechanism of rsEGFP2. *Journal of the American Chemical Society* **145**, 14636–14646 (2023).
- 185 Marko, M., Hsieh, C., Moberlychan, W., Mannella, C. & Frank, J. Focused ion beam milling of vitreous water: prospects for an alternative to cryo-ultramicrotomy of frozen-hydrated biological samples. *Journal of microscopy* **222**, 42–47 (2006).
- 186 Martens, K. J. A. *et al.* Visualisation of dCas9 target search in vivo using an open-microscopy framework. *Nature Communications* **10**, 3552 (2019).

- 187 Martynowycz, M. W., Clabbers, M. T., Unge, J., Hattne, J. & Gonen, T. Benchmarking the
ideal sample thickness in cryo-EM. *Proceedings of the National Academy of Sciences*
118, e2108884118 (2021).
- 188 Masich, S., Östberg, T., Norlén, L., Shupliakov, O. & Daneholt, B. A procedure to deposit
fiducial markers on vitreous cryo-sections for cellular tomography. *Journal of*
Structural Biology **156**, 461–468 (2006).
- 189 Masters, B. R. The development of fluorescence microscopy. *Encyclopedia of life*
sciences, 1–9 (2010).
- 190 Mastronarde, D. N. & Held, S. R. Automated tilt series alignment and tomographic
reconstruction in IMOD. *J Struct Biol* **197**, 102–113 (2017).
- 191 Masullo, L. A. *et al.* An alternative to MINFLUX that enables nanometer resolution in a
confocal microscope. *Light: Science & Applications* **11**, 199 (2022).
- 192 Mathews, C. K. The cell-bag of enzymes or network of channels? *Journal of*
bacteriology **175**, 6377–6381 (1993).
- 193 Matthies, D., Bartesaghi, A., Merk, A., Banerjee, S. & Subramaniam, S. Residue Specific
Radiation Damage of Protein Structures using High-Resolution Cryo-Electron
Microscopy. *Biophysical Journal* **108**, 190a (2015).
- 194 Matz, M. V. *et al.* Fluorescent proteins from nonbioluminescent Anthozoa species.
Nature Biotechnology **17**, 969–973 (1999).
- 195 Maurer, V. J., Siggel, M. & Kosinski, J. PyTME (Python Template Matching Engine): A fast,
flexible, and multi-purpose template matching library for cryogenic electron
microscopy data. *SoftwareX* **25**, 101636 (2024).
- 196 Merzlyak, E. M. *et al.* Bright monomeric red fluorescent protein with an extended
fluorescence lifetime. *Nat Methods* **4**, 555–557 (2007).
- 197 Metzger, M. *et al.* Resolution enhancement for low-temperature scanning microscopy
by cryo-immersion. *Opt. Express* **24**, 13023–13032 (2016).
- 198 Mills, D. J. Setting up and operating a cryo-EM laboratory. *Quarterly Reviews of*
Biophysics **54**, e2 (2021).
- 199 Milo, R. & Phillips, R. *Cell biology by the numbers*. (Garland Science, 2015).
- 200 Mironov, A. A. & Beznoussenko, G. V. in *Methods in Enzymology* Vol. 504 (ed P. Michael
conn) 201–219 (Academic Press, 2012).
- 201 Moerner, W. E. Microscopy beyond the diffraction limit using actively controlled single
molecules. *J Microsc* **246**, 213–220 (2012).
- 202 Mohammadian, S. *et al.* High accuracy, fiducial marker-based image registration of
correlative microscopy images. *Scientific Reports* **9**, 3211 (2019).
- 203 Moser, F. *et al.* Cryo-SOFI enabling low-dose super-resolution correlative light and
electron cryo-microscopy. *Proc Natl Acad Sci U S A* **116**, 4804–4809 (2019).
- 204 Nahmani, M., Lanahan, C., DeRosier, D. & Turrigiano, G. G. High-numerical-aperture
cryogenic light microscopy for increased precision of superresolution
reconstructions. *Proceedings of the National Academy of Sciences* **114**, 3832–3836
(2017).
- 205 Nakane, T. *et al.* Single-particle cryo-EM at atomic resolution. *Nature* **587**, 152–156
(2020).
- 206 Naydenova, K. *et al.* On the reduction in the effects of radiation damage to two-
dimensional crystals of organic and biological molecules at liquid-helium
temperature. *Ultramicroscopy* **237**, 113512 (2022).

- 207 Nevers, Y., Glover, N. M., Dessimoz, C. & Lecompte, O. Protein length distribution is
remarkably uniform across the tree of life. *Genome Biology* **24**, 135 (2023).
- 208 Ng, C. T. & Gan, L. Investigating eukaryotic cells with cryo-ET. *Mol Biol Cell* **31**, 87-100
(2020).
- 209 Nicholson, D. J. Is the cell really a machine? *Journal of theoretical biology* **477**, 108-126
(2019).
- 210 Nicovich, P. R., Walsh, J., Böcking, T. & Gaus, K. NicoLase—An open-source diode laser
combiner, fiber launch, and sequencing controller for fluorescence microscopy. *PLOS*
ONE **12**, e0173879 (2017).
- 211 Nifosì, R., Storti, B. & Bizzarri, R. Reversibly switchable fluorescent proteins:“the fair
switch project”. *La Rivista del Nuovo Cimento*, 1-88 (2024).
- 212 Ovesný, M., Křížek, P., Borkovec, J., Švindrych, Z. & Hagen, G. M. ThunderSTORM: a
comprehensive ImageJ plug-in for PALM and STORM data analysis and super-
resolution imaging. *Bioinformatics* **30**, 2389-2390 (2014).
- 213 Palade, G. E. The fine structure of mitochondria. *Anat Rec* **114**, 427-451 (1952).
- 214 Palade, G. E. A SMALL PARTICULATE COMPONENT OF THE CYTOPLASM. *The Journal of*
Biophysical and Biochemical Cytology **1**, 59-68 (1955).
- 215 Palmer, C. V., Modi, C. K. & Mydlarz, L. D. Coral Fluorescent Proteins as Antioxidants.
PLOS ONE **4**, e7298 (2009).
- 216 Parsons, D. F. & Johnson, H. M. Possibility of a Phase Contrast Electron Microscope.
Appl. Opt. **11**, 2840-2843 (1972).
- 217 Paul-Gilloteaux, P. et al. eC-CLEM: flexible multidimensional registration software for
correlative microscopies. *Nature methods* **14**, 102-103 (2017).
- 218 Peddie, C. J. et al. Correlative super-resolution fluorescence and electron microscopy
using conventional fluorescent proteins in vacuo. *Journal of Structural Biology* **199**,
120-131 (2017).
- 219 Peplow, M. Cryo-Electron Microscopy Reaches Resolution Milestone. *ACS Central*
Science **6**, 1274-1277 (2020).
- 220 Perez, D. et al. Identification and demonstration of roGFP2 as an environmental sensor
for cryogenic correlative light and electron microscopy. *Journal of structural biology*
214, 107881 (2022).
- 221 Petrov, P. N. & Moerner, W. E. Addressing systematic errors in axial distance
measurements in single-emitter localization microscopy. *Opt. Express* **28**, 18616-
18632 (2020).
- 222 Pettersen, E. F. et al. UCSF ChimeraX: Structure visualization for researchers, educators,
and developers. *Protein Sci* **30**, 70-82 (2021).
- 223 Phillips, M. A. et al. CryoSIM: super-resolution 3D structured illumination cryogenic
fluorescence microscopy for correlated ultrastructural imaging. *Optica* **7**, 802-812
(2020).
- 224 Pletneva, N. V. et al. Crystal Structure of Phototoxic Orange Fluorescent Proteins with
a Tryptophan-Based Chromophore. *PLOS ONE* **10**, e0145740 (2015).
- 225 Prakash, K., Diederich, B., Heintzmann, R. & Schermelleh, L. Super-resolution
microscopy: a brief history and new avenues. *Philosophical Transactions of the Royal*
Society A: Mathematical, Physical and Engineering Sciences **380**, 20210110 (2022).
- 226 Prasai, B. et al. The nanoscale molecular morphology of docked exocytic dense-core
vesicles in neuroendocrine cells. *Nat Commun* **12**, 3970 (2021).

- 227 Pražák, V., Grünewald, K. & Kaufmann, R. in *Methods in Cell Biology* Vol. 162 (eds Thomas Müller-Reichert & Paul Verkade) 253–271 (Academic Press, 2021).
- 228 Przybylski, A., Thiel, B., Keller-Findeisen, J., Stock, B. & Bates, M. Gpufit: An open-source toolkit for GPU-accelerated curve fitting. *Scientific Reports* **7**, 15722 (2017).
- 229 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nature methods* **14**, 290–296 (2017).
- 230 Purnell, C. *et al.* Rapid synthesis of cryo-ET data for training deep learning models. *bioRxiv* (2023).
- 231 Rai, V. & Srivastava, A. Correlation between optical and morphological properties of nanostructured gold thin film. *JSM Nanotechnol Nanomed* **4**, 1039 (2016).
- 232 Rane, L. *et al.* Light-Induced Forward and Reverse Intersystem Crossing in Green Fluorescent Proteins at Cryogenic Temperatures. *The Journal of Physical Chemistry B* **127**, 5046–5054 (2023).
- 233 Ratz, M., Testa, I., Hell, S. W. & Jakobs, S. CRISPR/Cas9-mediated endogenous protein tagging for RESOLFT super-resolution microscopy of living human cells. *Scientific Reports* **5**, 9592 (2015).
- 234 Reinhardt, S. C. M. *et al.* Ångström-resolution fluorescence microscopy. *Nature* **617**, 711–716 (2023).
- 235 Remington, S. J. Green fluorescent protein: A perspective. *Protein Science* **20**, 1509–1519 (2011).
- 236 Rigort, A. *et al.* Micromachining tools and correlative approaches for cellular cryo-electron tomography. *Journal of Structural Biology* **172**, 169–179 (2010).
- 237 Rigort, A. & Plitzko, J. M. Cryo-focused-ion-beam applications in structural biology. *Archives of biochemistry and biophysics* **581**, 122–130 (2015).
- 238 Rohou, A. & Grigorieff, N. CTFFIND4: Fast and accurate defocus estimation from electron micrographs. *J Struct Biol* **192**, 216–221 (2015).
- 239 Ronneberger, O., Fisher, P. & Brox, T. U-Net: Convolutional Networks for Biomedical Image Segmentation. *arXiv* (2015).
- 240 Russo, C. J. & Passmore, L. A. Ultrastable gold substrates for electron cryomicroscopy. *Science* **346**, 1377–1380 (2014).
- 241 Rust, M. J., Bates, M. & Zhuang, X. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nature Methods* **3**, 793–796 (2006).
- 242 Salih, A., Larkum, A., Cox, G., Kühl, M. & Hoegh-Guldberg, O. Fluorescent pigments in corals are photoprotective. *Nature* **408**, 850–853 (2000).
- 243 Sartor, A. M., Dahlberg, P. D., Perez, D. & Moerner, W. Characterization of mApple as a red fluorescent protein for cryogenic single-molecule imaging with turn-off and turn-on active control mechanisms. *The Journal of Physical Chemistry B* **127**, 2690–2700 (2023).
- 244 Sartor, A. M., Dahlberg, P. D., Perez, D. & Moerner, W. E. Characterization of mApple as a Red Fluorescent Protein for Cryogenic Single-Molecule Imaging with Turn-Off and Turn-On Active Control Mechanisms. *The Journal of Physical Chemistry B* **127**, 2690–2700 (2023).
- 245 Sartori, A. *et al.* Correlative microscopy: Bridging the gap between fluorescence light microscopy and cryo-electron tomography. *Journal of Structural Biology* **160**, 135–145 (2007).

- 246 Schaffer, M. *et al.* A cryo-FIB lift-out technique enables molecular-resolution cryo-ET
within native *Caenorhabditis elegans* tissue. *Nature methods* **16**, 757–762 (2019).
- 247 Scher, N. & Avinoam, O. in *Methods in Cell Biology* Vol. 162 (eds Thomas Müller-
Reichert & Paul Verkade) 1–11 (Academic Press, 2021).
- 248 Scher, N., Rechav, K., Paul-Gilloteaux, P. & Avinoam, O. In situ fiducial markers for 3D
correlative cryo-fluorescence and FIB-SEM imaging. *Iscience* **24** (2021).
- 249 Schermelleh, L. *et al.* Super-resolution microscopy demystified. *Nature Cell Biology* **21**,
72–84 (2019).
- 250 Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nature*
Methods **9**, 676–682 (2012).
- 251 Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image
analysis. *Nature Methods* **9**, 671–675 (2012).
- 252 Schnitzbauer, J., Strauss, M. T., Schlichthaerle, T., Schueder, F. & Jungmann, R. Super-
resolution microscopy with DNA-PAINT. *Nature Protocols* **12**, 1198–1228 (2017).
- 253 Schorb, M. *et al.* New hardware and workflows for semi-automated correlative cryo-
fluorescence and cryo-electron microscopy/tomography. *Journal of Structural*
Biology **197**, 83–93 (2017).
- 254 Schröder, D., Deschamps, J., Dasgupta, A., Matti, U. & Ries, J. Cost-efficient open source
laser engine for microscopy. *Biomed. Opt. Express* **11**, 609–623 (2020).
- 255 Schur, F. K. Toward high-resolution in situ structural biology with cryo-electron
tomography and subtomogram averaging. *Current opinion in structural biology* **58**,
1–9 (2019).
- 256 Schwartz, C. L., Sarbash, V. I., Ataullakhanov, F. I., McIntosh, J. R. & Nicastro, D. Cryo-
fluorescence microscopy facilitates correlations between light and cryo-electron
microscopy and reduces the rate of photobleaching. *J Microsc* **227**, 98–109 (2007).
- 257 Seifert, R. *et al.* DeepCLEM: automated registration for correlative light and electron
microscopy using deep learning. *F1000Res* **9**, 1275 (2020).
- 258 Sexton, D. L., Burgold, S., Schertel, A. & Tocheva, E. I. Super-resolution confocal cryo-
CLEM with cryo-FIB milling for in situ imaging of *Deinococcus radiodurans*. *Current*
Research in Structural Biology **4**, 1–9 (2022).
- 259 Shaner, N. C. *et al.* Improved monomeric red, orange and yellow fluorescent proteins
derived from *Discosoma* sp. red fluorescent protein. *Nature biotechnology* **22**, 1567–
1572 (2004).
- 260 Shimomura, O. The discovery of aequorin and green fluorescent protein. *Journal of*
microscopy **217**, 3–15 (2005).
- 261 Shimomura, O., Johnson, F. H. & Saiga, Y. Extraction, purification and properties of
aequorin, a bioluminescent protein from the luminous hydromedusan, *Aequorea*.
Journal of cellular and comparative physiology **59**, 223–239 (1962).
- 262 Shimomura, O., Shimomura, S. & Brinegar, J. H. *Luminous Pursuit: Jellyfish, GFP, and*
the Unforeseen Path to the Nobel Prize. (2017).
- 263 Siegfried, W., Bo, J., Alois, R. & Vahid, S. Cryogenic localization of single molecules with
angstrom precision. *Proc.SPIE* **8815**, 88150D (2013).
- 264 Simonyan, K. & Zisserman, A. Very Deep Convolutional Networks for Large-scale
Image Regognition. *arXiv* (2015).

- 265 Sjollem, K. A., Schnell, U., Kuipers, J., Kalicharan, R. & Giepmans, B. N. G. in *Methods in Cell Biology* Vol. III (eds Thomas Müller-Reichert & Paul Verkade) 157-173 (Academic Press, 2012).
- 266 Skoupý, R. *et al.* Robust Local Thickness Estimation of Sub-Micrometer Specimen by 4D-STEM. *Small Methods* **7**, 2300258 (2023).
- 267 Stiel, A. C. *et al.* Generation of monomeric reversibly switchable red fluorescent proteins for far-field fluorescence nanoscopy. *Biophys J* **95**, 2989-2997 (2008).
- 268 Subach, F. V. *et al.* Photoactivatable mCherry for high-resolution two-color fluorescence microscopy. *Nat Methods* **6**, 153-159 (2009).
- 269 Subach, F. V., Patterson, G. H., Renz, M., Lippincott-Schwartz, J. & Verkhusha, V. V. Bright monomeric photoactivatable red fluorescent protein for two-color super-resolution sptPALM of live cells. *J Am Chem Soc* **132**, 6481-6491 (2010).
- 270 Subach, F. V. *et al.* Red fluorescent protein with reversibly photoswitchable absorbance for photochromic FRET. *Chemistry & biology* **17**, 745-755 (2010).
- 271 Subach, O. M. *et al.* A photoswitchable orange-to-far-red fluorescent protein, PSmOrange. *Nature methods* **8**, 771-777 (2011).
- 272 Subach, O. M. *et al.* LSSmScarlet, dCyRFP2s, dCyOFP2s and CRISPRed2s, Genetically Encoded Red Fluorescent Proteins with a Large Stokes Shift. *International Journal of Molecular Sciences* **22**, 12887 (2021).
- 273 Susano Pinto, D. M. *et al.* Python-Microscope – a new open-source Python library for the control of microscopes. *Journal of Cell Science* **134**, jcs258955 (2021).
- 274 Swulius, M. T. *et al.* Structure of the fission yeast actomyosin ring during constriction. *Proceedings of the National Academy of Sciences* **115**, E1455-E1464 (2018).
- 275 Szegedy, C., Ioffe, S., Vanhoucke, V. & Alemi, A. Inception-v4, Inception-ResNet and the Impact of Residual Connections on Learning. *arXiv* (2016).
- 276 Szegedy, C. *et al.* Going deeper with convolutions. *arXiv* (2014).
- 277 Tacke, S. *et al.* A streamlined workflow for automated cryo focused ion beam milling. *Journal of Structural Biology* **213**, 107743 (2021).
- 278 Tang, G. *et al.* EMAN2: An extensible image processing suite for electron microscopy. *Journal of Structural Biology* **157**, 38-46 (2007).
- 279 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nature Methods* **16**, 1146-1152 (2019).
- 280 Thevenaz, P., Ruttimann, U. E. & Unser, M. A pyramid approach to subpixel registration based on intensity. *IEEE Transactions on Image Processing* **7**, 27-41 (1998).
- 281 Thomas, C. I. *et al.* Cryo-Confocal Imaging for CLEM Mapping in Brain Tissues. *Microscopy Today* **29**, 34-39 (2021).
- 282 Tian, B., Xu, X., Xue, Y., Ji, W. & Xu, T. Cryogenic superresolution correlative light and electron microscopy on the frontier of subcellular imaging. *Biophysical Reviews* **13**, 1163-1171 (2021).
- 283 Tillu, V. *et al.* Precision in situ cryo-correlative light and electron microscopy of optogenetically-positioned organelles. *Journal of Cell Science*, jcs. 262163 (2024).
- 284 Trewin, A. J. *et al.* Light-induced oxidant production by fluorescent proteins. *Free Radic Biol Med* **128**, 157-164 (2018).
- 285 Tuijtel, M. W., Koster, A. J., Faas, F. G. A. & Sharp, T. H. Correlated Cryo Super-Resolution Light and Cryo-Electron Microscopy on Mammalian Cells Expressing the Fluorescent Protein rsEGFP2. *Small Methods* **3**, 1900425 (2019).

- 286 Tuijtel, M. W., Koster, A. J., Jakobs, S., Faas, F. G. A. & Sharp, T. H. Correlative cryo super-
resolution light and electron microscopy on mammalian cells using fluorescent
287 proteins. *Scientific Reports* **9**, 1369 (2019).
- 288 Turk, M. & Baumeister, W. The promise and the challenges of cryo-electron
tomography. *FEBS Letters* **594**, 3243–3261 (2020).
- 289 Uriarte, L. M. *et al.* Structural information about the trans-to-cis isomerization
mechanism of the photoswitchable fluorescent protein rsEGFP2 revealed by
multiscale infrared transient absorption. *The Journal of Physical Chemistry Letters* **13**,
1194–1202 (2022).
- 290 van den Dries, K., Fransen, J. & Cambi, A. Fluorescence CLEM in biology: historic
developments and current super-resolution applications. *Febs Letters* **596**, 2486–
2496 (2022).
- 291 van den Hoek, H. *et al.* In situ architecture of the ciliary base reveals the stepwise
assembly of intraflagellar transport trains. *Science* **377**, 543–548 (2022).
- 292 van der Walt, S. *et al.* scikit-image: image processing in Python. *PeerJ* **2**, e453 (2014).
- 293 van Driel, L. F., Valentijn, J. A., Valentijn, K. M., Koning, R. I. & Koster, A. J. Tools for
correlative cryo-fluorescence microscopy and cryo-electron tomography applied to
whole mitochondria in human endothelial cells. *European Journal of Cell Biology* **88**,
669–684 (2009).
- 294 van Leeuwenhoek, A. Part of a letter from Mr. Antoni van Leeuwenhoek, concerning
the worms in Sheeps livers, gnats, and animalcula in the excrements of Frogs.
Philosophical Transactions of the Royal Society (1700).
- 295 Virtanen, P. *et al.* SciPy 1.0: fundamental algorithms for scientific computing in Python.
Nat Methods **17**, 261–272 (2020).
- 296 von Borries, B., Ruska, E. & Ruska, H. Bakterien und Virus in Übermikroskopischer
Aufnahme. *Klinische Wochenschrift* **17**, 921–925 (1938).
- 297 Vulović, M., Voortman, L. M., van Vliet, L. J. & Rieger, B. When to use the projection
assumption and the weak-phase object approximation in phase contrast cryo-EM.
Ultramicroscopy **136**, 61–66 (2014).
- 298 Wagner, J., Schaffer, M. & Fernández-Busnadiego, R. Cryo-electron tomography—the
cell biology that came in from the cold. *FEBS letters* **591**, 2520–2533 (2017).
- 299 Wan, W. & Briggs, J. A. Cryo-electron tomography and subtomogram averaging.
Methods in enzymology **579**, 329–367 (2016).
- 300 Wan, W., Khavnekar, S. & Wagner, J. STOPGAP: an open-source package for template
matching, subtomogram alignment and classification. *Acta Crystallographica
Section D: Structural Biology* **80** (2024).
- 301 Wang, L. *et al.* Solid immersion microscopy images cells under cryogenic conditions
with 12 nm resolution. *Communications Biology* **2**, 74 (2019).
- 302 Wang, S. *et al.* CryoFIB milling large tissue samples for cryo-electron tomography.
Scientific Reports **13**, 5879 (2023).
- 303 Watson, M. L. The nuclear envelope; its structure and relation to cytoplasmic
membranes. *J Biophys Biochem Cytol* **1**, 257–270 (1955).
- 304 Weber, M. *et al.* MINSTED nanoscopy enters the Ångström localization range. *Nature
Biotechnology* **41**, 569–576 (2023).
- Weis, F., Hagen, W. J. H., Schorb, M. & Mattei, S. Strategies for Optimization of Cryogenic
Electron Tomography Data Acquisition. *JoVE*, e62383 (2021).

305 Weisenburger, S. *et al.* Cryogenic optical localization provides 3D protein structure
data with Angstrom resolution. *Nature Methods* **14**, 141-144 (2017).

306 Weissenberger, G., Henderikx, R. J. M. & Peters, P. J. Understanding the invisible hands
of sample preparation for cryo-EM. *Nature Methods* **18**, 463-471 (2021).

307 Wiedenmann, J. *et al.* A far-red fluorescent protein with fast maturation and reduced
oligomerization tendency from *Entacmaea quadricolor* (Anthozoa, Actinaria). *Proc
Natl Acad Sci U S A* **99**, 11646-11651 (2002).

308 Wieferig, J.-P., Mills, D. J. & Kühlbrandt, W. Devitrification reduces beam-induced
movement in cryo-EM. *IUCrJ* **8**, 186-194 (2021).

309 Williams, D. B. & Carter, C. B. in *Transmission Electron Microscopy: A Textbook for
Materials Science* 371-388 (Springer US, 2009).

310 Wolff, G., Hagen, C., Grünewald, K. & Kaufmann, R. Towards correlative super-
resolution fluorescence and electron cryo-microscopy. *Biology of the Cell* **108**, 245-
258 (2016).

311 Wolff, G. *et al.* A molecular pore spans the double membrane of the coronavirus
replication organelle. *Science* **369**, 1395-1398 (2020).

312 Wu, G. H. *et al.* CryoET reveals organelle phenotypes in huntington disease patient
iPSC-derived and mouse primary neurons. *Nat Commun* **14**, 692 (2023).

313 Xu, X. *et al.* Ultra-stable super-resolution fluorescence cryo-microscopy for
correlative light and electron cryo-microscopy. *Science China Life Sciences* **61**, 1312-
1319 (2018).

314 Yang, F., Moss, L. G. & Phillips, G. N. The molecular structure of green fluorescent protein.
Nature Biotechnology **14**, 1246-1251 (1996).

315 Yang, J. *et al.* Precise 3D Localization by Integrated Fluorescence Microscopy (iFLM)
for Cryo-FIB-milling and In-situ Cryo-ET. *Microscopy and Microanalysis* **29**, 1055-1057
(2023).

316 Yang, J. E., Larson, M. R., Sibert, B. S., Shrum, S. & Wright, E. R. CorRelator: Interactive
software for real-time high precision cryo-correlative light and electron microscopy.
Journal of Structural Biology **213**, 107709 (2021).

317 Yip, K. M., Fischer, N., Paknia, E., Chari, A. & Stark, H. Atomic-resolution protein structure
determination by cryo-EM. *Nature* **587**, 157-161 (2020).

318 Yuste, R. Fluorescence microscopy today. *Nature Methods* **2**, 902-904 (2005).

319 Zanetti-Domingues, L. C. *et al.* in *Methods in Cell Biology* Vol. 187 (eds Thomas Müller-
Reichert & Paul Verkade) 249-292 (Academic Press, 2024).

320 Zeng, X., Yang, X., Wang, Z. & Xu, M. in *State of the Art in Neural Networks and their
Applications* 63-72 (Elsevier, 2021).

321 Zhang, T. *et al.* Rapid and robust two-dimensional phase unwrapping via deep
learning. *Opt. Express* **27**, 23173-23185 (2019).

322 Zheng, S. *et al.* AreTomo: An integrated software package for automated marker-free,
motion-corrected cryo-electron tomographic alignment and reconstruction. *Journal
of Structural Biology: X* **6**, 100068 (2022).

323 Zheng, S. Q. *et al.* MotionCor2: anisotropic correction of beam-induced motion for
improved cryo-electron microscopy. *Nat Methods* **14**, 331-332 (2017).

324 Zhong, E. D., Bepler, T., Berger, B. & Davis, J. H. CryoDRGN: reconstruction of
heterogeneous cryo-EM structures using neural networks. *Nature methods* **18**, 176-
185 (2021).

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Appendices

Supplementary Information

The supplementary information to Chapters 2 – 8 can be found accompanying the original online publications, which are accessible via the following links:

Chapter 2 – *Building a super-resolution fluorescence cryo-microscope:*

doi.org/10.1101/2023.11.21.567712

Chapter 3 – *Imaging intracellular components in situ using super-resolution cryo-correlative light and electron microscopy*

doi.org/10.1101/2023.11.19.567713

Chapter 4 – *scNodes: a correlation and processing toolkit for super-resolution fluorescence and electron microscopy*

doi.org/10.1038/s41592-023-01991-z

Chapter 5 – *Ais: streamlining segmentation of cryo-electron tomography datasets*

doi.org/10.7554/eLife.98552.2

Chapter 6 – *Selecting optimal support grids for superresolution cryogenic correlated light and electron microscopy*

doi.org/10.1038/s41598-023-35590-x

Chapter 7 – *Super-resolution fluorescence imaging of cryosamples does not limit achievable resolution in cryoEM*

doi.org/10.1016/j.jsb.2023.108040

Chapter 8 – *Measuring cryo-TEM sample thickness using reflected light microscopy and machine learning*

doi.org/10.1016/j.jsb.2023.107965