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 Baumler, 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 Calcraft, 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-Diez, 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. & McDowall, 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 Hendersona, 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 Khatter, 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 Sjollema, K. A., Schnell, U., Kuipers, J., Kalicharan, R. & Giepmans, B. N. G. in *Methods in Cell Biology* Vol. 111 (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 proteins. *Scientific Reports* **9**, 1369 (2019).

287 Turk, M. & Baumeister, W. The promise and the challenges of cryo-electron tomography. *FEBS Letters* **594**, 3243-3261 (2020).

288 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).

289 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).

290 van den Hoek, H. *et al.* In situ architecture of the ciliary base reveals thestepwise assembly of intraflagellar transport trains. *Science* **377**, 543-548 (2022).

291 van der Walt, S. *et al.* scikit-image: image processing in Python. *PeerJ* **2**, e453 (2014).

292 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).

293 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).

294 Virtanen, P. *et al.* SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nat Methods* **17**, 261-272 (2020).

295 von Borries, B., Ruska, E. & Ruska, H. Bakterien und Virus in Übermikroskopischer Aufnahme. *Klinische Wochenschrift* **17**, 921-925 (1938).

296 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).

297 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).

298 Wan, W. & Briggs, J. A. Cryo-electron tomography and subtomogram averaging. *Methods in enzymology* **579**, 329-367 (2016).

299 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).

300 Wang, L. *et al.* Solid immersion microscopy images cells under cryogenic conditions with 12 nm resolution. *Communications Biology* **2**, 74 (2019).

301 Wang, S. *et al.* CryoFIB milling large tissue samples for cryo-electron tomography. *Scientific Reports* **13**, 5879 (2023).

302 Watson, M. L. The nuclear envelope; its structure and relation to cytoplasmic membranes. *J Biophys Biochem Cytol* **1**, 257-270 (1955).

303 Weber, M. *et al.* MINSTED nanoscopy enters the Ångström localization range. *Nature Biotechnology* **41**, 569-576 (2023).

304 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