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Meeting-report

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Cryogenic electron microscopy (cryo-EM) provides powerful insights into the study of biological systems by revealing molecular structures in their close-to-native environments. Single particle analysis (SPA) has enabled structure determination of purified macromolecular complexes up to atomic resolution [1-2]. Cryogenic electron tomography (cryo-ET) combined with subtomogram averaging (STA) [3-4] has been demonstrated to offer distinct perspectives of the arrangement and function of structural proteins within intact cells. Compared to SPA, cryo-ET with STA, despite its powerful potential to offer much richer information, is still an emerging technique that has only achieved high resolution for a handful of biomolecular structures thus far. At the Chan Zuckerberg Institute for Advanced Biological Imaging (CZ Imaging Institute), our objective is to contribute to the ongoing refinement of this technique through joint efforts. This includes collaborative hardware development such as the laser phase plate [5] and direct electron detectors, and algorithm development, including but not limited to machine-learning-based denoising and automated annotation, and the establishment of a data portal to catalyze the development of machine-learning methods [6].

Currently, one another challenge that cryo-ET faces is reduced resolution when imaging thick cellular specimens, where increased inelastic scattered signals result in increased chromatic blurring. Energy-filtered TEM (EFTEM) removes this blur caused by inelastic scattering but reduces signal potential. In response, we are exploring the potential advantages of an emerging technique enabled by 4D scanning transmission electron microscopy (4D-STEM): tilt-corrected bright-field (tcBF-)STEM [7-8] (also known as parallax [9]). In tcBF, each pixel within the bright-field (BF) disk functions as a virtual coherent detector with a small collection angle. The resulting virtual BF images, depending on the aberration function, exhibit image shifts, and these shifts are measured and corrected on a pixel-by-pixel basis. In principle, tcBF has an advantage over EFTEM for thick samples. It allows a coherent image to be retained and uses almost all the forward-scattered incident beam - i.e., a similar dose efficiency to TEM. The post-specimen lenses for TEM are the image-formation lenses, so chromatic aberrations there degrade the image resolution. But for tcBF the post-specimen lenses simply transfer an image of the diffraction pattern, so chromatic aberrations result in a small loss of angular resolution instead - i.e., a reduction in coherence. In our preliminary observations, we noticed improved contrast in large organelles and intact bacterial cells when imaged with tcBF-STEM as compared to EFTEM. Additionally, on a thin specimen of purified proteins, we demonstrate sub-nanometer resolution SPA maps with tcBF-STEM at a nominal resolution of 7 Å. This suggests the potential feasibility of employing this technique for biomolecular structure determination using cryo-ET and STA. However, more efforts are needed in both algorithm development and experimental design to match the state-of-the-art performance of EFTEM on thin samples at high resolution. In summary, our observations on both thick and thin samples indicate that combining 4D-STEM phase imaging techniques with cryo-ET for studying biological systems may provide additional information by enabling imaging on thicker and denser samples. [10]

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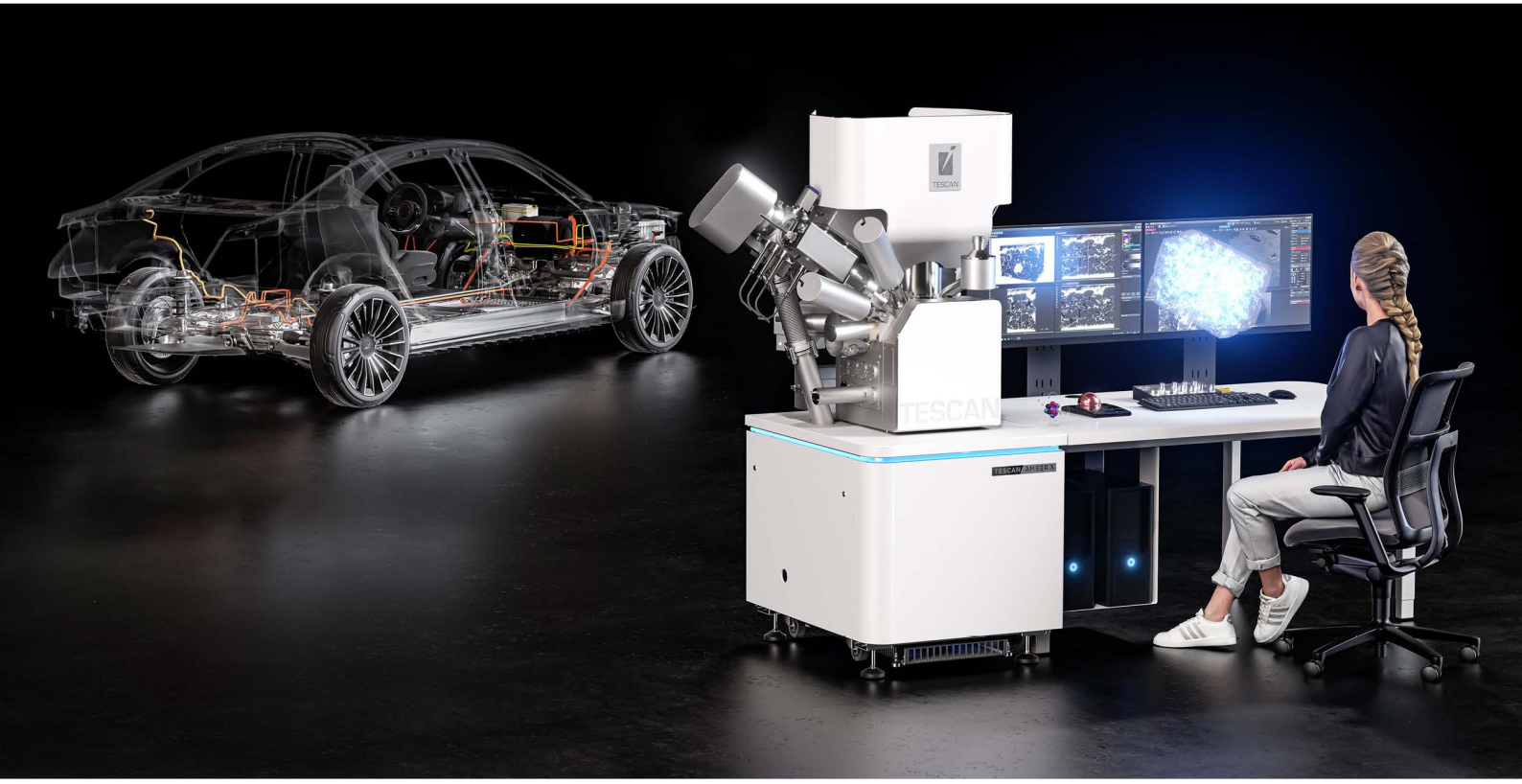

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