3D reconstruction of Golgi stacks in Brefeldin A treated cells. First results using STEM tomography

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The Golgi apparatus plays an important role in packaging and transporting macromolecules to different destinations in the cell [1]. It is a complex intracellular membrane-bound structure that consists of a series of different cisternae and tubules that are aligned in parallel to form stacks. In HeLa cells and Panc-1 cells usually several Golgi stacks are observed within one cell and it is not clear, how these stacks are interconnected. Our goal is to learn more about the complex structure and function of the Golgi.

Brefeldin A (BFA) had been used to investigate disruption and rebuilding of the Golgi apparatus [2 and others]. Human ADP-ribosylation factor (ARF) 1 and 3 are activated by Brefeldin A-inhibited guanine nucleotide-exchange protein and lead to the replacement of ARF-bound GDP with GTP to initiate recruitment of coat proteins for membrane vesicle formation [3]. The disruption of the Golgi apparatus by BFA is a dynamic process, that has been described as a chronological sequence in two dimensions [2]. For this work we have attempted to investigate this process in three dimensions.

For optimal structural preservation and contrast of cells in a physiologically defined state, we used high-pressure freezing and freeze substitution as described [4].

Electron tomography is currently the method of choice for 3D imaging of cellular structures with macromolecular resolution. Imaging dynamic changes in the Golgi with tomography, however, faces a number of challenging problems: Golgi stacks are spread over a large area of the cell. In order to clearly image the membrane bilayer, a pixel size of about 3 nm or less is required. When 1024x1024 pixel images were used, an area of about 3 μm x 3 μm can be recorded (Fig. 1A), which is only a small portion of a cell (Fig. 1B). The estimated thickness of a cultivated cell is about 2 to 5 μm, which is, unfortunately, thicker than can be imaged in a tomogram. It is, therefore, our goal to use sections as thick as possible. Resolution, however, decreases with increasing section thickness, because of the increased amount of inelastic scattering. Inelastic scattering, however, decreases with higher voltage; therefore 1000 kV instruments had been used for Golgi reconstruction [5]. For this study, we used a 300 kV instrument in scanning transmission mode (dark field STEM-mode) since section thickness is thereby not restricted by inelastic scattering (but by beam spreading) and contrast and signal to noise ratio are increased [6] (Fig. 1C). Using this method, we managed to obtain reasonable well-resolved tomograms of sections up to 600 nm thick (Fig. 2). [7]

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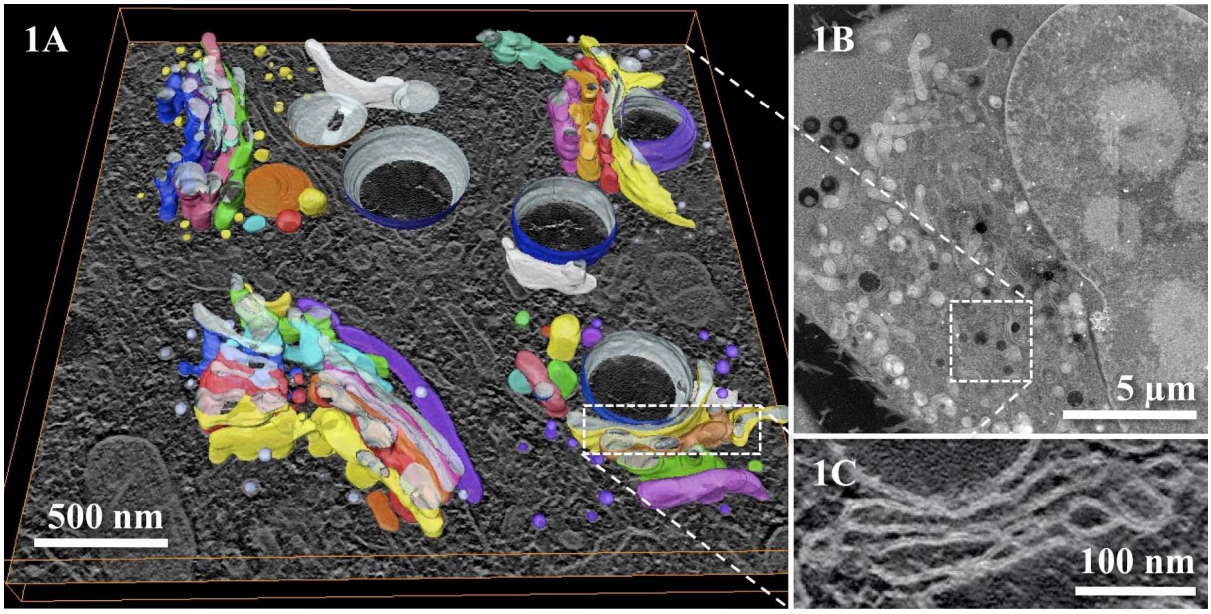

Fig. 1A. STEM-Tomogram of Golgi stacks of a HeLa cell. **Fig. 1B.** Overview of the same cell. **Fig. 1C**. Cutout of the same tomogram demonstrating the good membrane contrast.

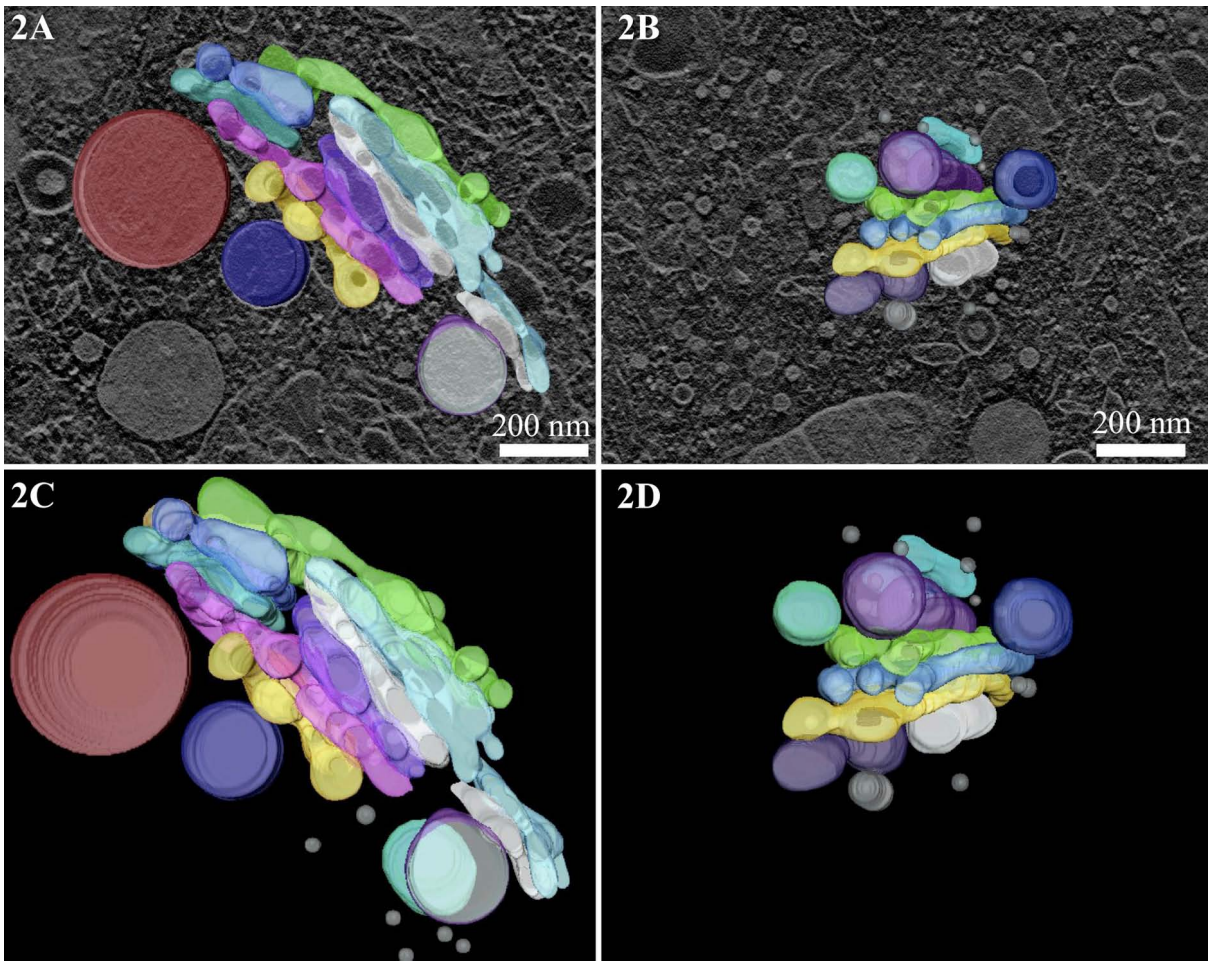

Fig. 2. Tomographic reconstruction of Golgi stacks of Panc 1 cells. **Fig. 2A**. Visualization of the overall morphology of a Golgi stack showing inter-cisternal connections. **Fig. 2B**. A Golgi stack of a Panc 1 cell after treatment with Brefeldin A for 10 minutes. This results in the dispersal of the Golgi stacks. **Figs. 2C and 2D** 3-D-Models of the Golgi stacks visualized with the Amira software.

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