Freeze-fracture technique is a method used to visualise membrane surfaces of cell organelles. This method is based on cryo-fixation that stabilizes samples. The sample is rapidly frozen in nitrogen, and cut in the chamber under a vacuum and low temperature. Glycerol is used as a cryoprotectant preserving the fine structure of cells in their native stage. Although, cryoprotectants serve as a substitute for water and protect against ice crystal production, they could also affect the form of fracture through biological membranes. Figure 1 shows structures in a sample frozen in the presence of 25% glycerol. The temperature of the apparatus was not low enough during the process of fracturing and etching the sample. The structure of cells seems to be deformed due to melting glycerol. In contrast, figure 2 shows a replica with fine structure of frozen and proper good form of fracturing. The cells used for this study were human leukemic cells (HL-60). Another artefact is shown in figure 3A, compare with 3B. Each sample has to be fractured with a specific speed of cut. The force used for fracturing the membranes has to be set to optimal conditions, which depend on a hardness of sample and a coherence of drops. Low speed and unstable coherence of drops resulted in sample fragmentation. High speed of cut could cause cross-section of cellular structures, similar to ultrathin sections. Figure 3A shows fragmentation of nuclear membrane. This sample was not fractured, it was fragmented due to unstable coherence of drop. This overview shows how a combination of different conditions including the physical properties of the sample, cryoprotectants used and temperature could affect the form of fractures and hence significantly affect interpretation of morphological structures.

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Fig. 1: Freeze-fracture of HL-60 cells in suspension: structures of fracture in the replica are affected by melting glycerol. Bar 10 um.

Fig. 2: Freeze-fracture of HL-60 cells in suspension: structures of fracture in the replica with fine frozen and optimal fracturing condition. Bar 10 um.

Fig. 3: Nuclear membrane: A) the replica of fragmented structures; B) the replica of optimally fractured structures. Different form of nuclear pores (arrows). In compare with replica of fractured structures, the fragmentation of the sample affected the form of nuclear pores and structure of the membrane. Bar 1 um.