Microfabricated Sample Carrier for Cryogenic Electron Microscopy

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Cryo-electron microscopy (CryoEM) has been proven as invaluable tool for small biomolecule structure determination ranging from proteins to viruses and DNA. Nevertheless. since the introduction of TEM and cryoEM the sample carrier (grid) has remained largely unchanged. Meanwhile microfabrication has made leaps due to the semiconductor industry. In this abstract we present a silicon-based sample carrier made with microfabrication techniques.

Current sample carriers consist of grid made by soft metals typically copper or gold and covered by a holey carbon film or lately higher end carriers are covered by a gold film[1] . Despite their desirable high thermal conductivity, soft metals carriers are very easy to bend, and require gentle handling . Clipping the grid (i.e. to create AutoGrids) is required to be compatible with autoloaders. In addition, due to their low robustness, their surface is typically wavy (not flat), making sample preparation far from ideal, especially for applications such as cryo-tomography. The carbon film transfer typically happens on an individual carrier level, resulting in surface inconsistencies between carriers even from the same fabrication batch. Furthermore, any process in the individual carrier level lengthens the fabrication process and hence the lead times.

Our proposed carriers use nanotechnology materials and, via the introduction of microfabrication techniques, we aim to improve all the aforementioned limitations of the current carriers. Our microfabricated devices consist of a silicon frame, holey silicon nitride membrane and a monolayer Graphene layer (Figure 1). Silicon offers a rigid and robust frame, making the handling and sample preparation much more forgiving. Our carriers can be clipped in the standard AutoGrid following the same process and tools as the current grids, but our microfabrication process enables the possibility to produce carriers with the geometry of an assembled AutoGrid, which would help to skip the tedious process of clipping. By meticulously controlling the deposition of silicon nitride on the silicon substrate, we produce a highly reproducible, atomically flat surface. In addition, microfabrication allows precise control material properties such as internal stress that can improve robustness. A large amount of literature reports the advantages of graphene for cryoEM [2-5], ranging from eliminating one air water interface to enabling affinity grids and controlling ice thickness via graphene hydrophilicity treatment. In order to guarantee the scalability of our production, we have developed the

processes to introduce graphene on the wafer level, ensuring consistent quality and surface properties for carriers from the same batch.

Initial characterization of the carriers has been performed, with more extensive experimental results expected in the coming months. The first results are very promising. The Graphene coverage, which depends on the hole size, showed a coverage of 66% for 6µm holes, 85% for 3.5µm holes and 96% for 2µm holes. We have confirmed the compatibility of our chips with different vitrification methods (i.e. plunge freezing and jetting). The acquired ice quality results, obtained with an unoptimized protocol using a Vitrobot, indicate amorphous ice of a uniform thickness of approximately 40nm (Figure 2). Several methods for the hydrophilization of graphene have been investigated depending on the users equipment, ranging from O2 & H2 plasma and glow discharge, to annealing and chemical modification of graphene.

We believe that silicon-based sample carriers are the logical next step to enhance and advance the capabilities of cryoEM. Leveraging microfabrication technologies provides us with unprecedented possibilities to customize the layout/geometry of the chip, allowing us to meet the experiment´s specific requirements precisely.

Graphic:

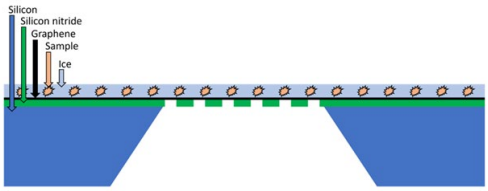

Fig. 1. Schematic of the Sample Carrier. The image shows the resulting architecture of our Silicon-based devices. As observed, a monolayer Graphene lays over a holey Silicon Nitride film.

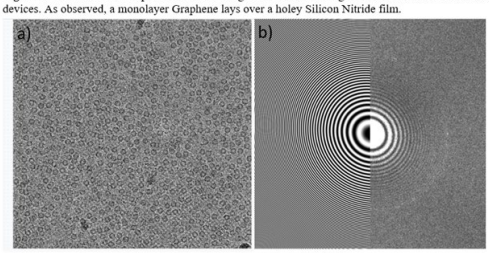

Fig. 2. Ice quality results. a) a monolayer of apoferritin with good distribution can be seen. B) The FFT indicates good quality of amorphous ice

Keywords:
CryoEM, graphene, Silicon based carriersReference:

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