



National Center for CryoEM Access and Training

Workshop on graphene grids for cryoEM

AUGUST 24, 2021

NOTES FOR GRAPHENE GRID PREPARATION

1. Protect graphene with MMA.

Graphene on both side of cooper foil (Cu substrate). Keep the side for future supporting face-up. Spin-coat the upside graphene with MMA (2500 rpm for 1 min).

2. Remove backside graphene.

Flip the MMA coated graphene on copper foil with backside graphene up. Argon/Oxygen glow-discharge/plasma cleaner for 30s – 60s (depends on local setting) to remove backside graphene.

3. Etch off Cu substrate

Cut "MMA/graphene/Cu stack" in to small pieces/squares (slightly larger than a grid. Recommend one piece at a time). Always keep the MMA side up and float the small pieces on 0.5-1M APS. Wait 20-30 min to etch off the Cu substrate.

4. Clean MMA/graphene bilayer film.

Use glass-slide to transfer the MMA/graphene film (keep MMA side up) to DI water, wait for 10-min to clean. Repeat this process twice.

5. MMA-coated graphene grid.

Scoop out the MMA/graphene film using Quantifoil Au R1.2/1.3 300 mesh grids with carbon side and air dry with MMA side up.

6. 1st annealing of graphene grid.

Bake the grid on a hot plate at 130°C for 20 mins and cool it down.

7. Remove MMA protect layer.

Soak the grid into acetone vertically for 30 mins to dissolve MMA and transfer it to another fresh acetone to further clean. *Do not let acetone dry during the transfer.

8. Remove acetone.

Transfer the grid to Isopropanol (IPA) to remove the acetone residue for 20 mins. Take out the grid one by one and air dry with graphene side up.

9. 2nd annealing of graphene grid. Same as step 6.

10. Surface treatment/functionalization of graphene by UV/ozone.

Protocol Reference:

<https://doi.org/10.1073/pnas.1919114117>

Reagents used in this study:

Single Layer Graphene on Copper foil: 2"x2"
<https://graphene-supermarket.com/Single-Layer-Graphene-on-Copper-foil-2inch.html>

MMA(8.5)MAA EL 6

<https://www.microresist.de/en/produkt/pmma-copolymer-series/>

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