MICROFABRICATED GRAPHENE LIQUID CELLS FOR LIQUID PHASE ELECTRON MICROSCOPY OF MICROTUBULES & PROTEINS

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Liquid cell electron microscopy

Cryo-Electron Microscopy has produced high resolution 2D and 3D reconstructions of proteins and entire cells, yet this technique always provides a static snapshot of the system. Liquid phase EM extends this into the temporal dimension, where material is imaged in their native state in liquid water and has the potential to reveal nanoscale dynamics for biological systems. To achieve this, the liquid sample has to be separated from the high TEM vacuum by thin membranes. 2D materials such as graphene are the thinnest material that can still be vacuum-tight and are therefore the gold standard. Graphene liquid cells have been demonstrated to reduce beam damage, but existing applications all suffer from either (1) a very small imaging window, massively hindering throughput, or (2) a lack of control of pocket formation, making it impossible to get reproducible conditions or quickly find a location of interest.

Wafer-scale microfabricated GLC

We are working on 4-inch wafer-scale production of graphene liquid cells (GLC) in the Kavli Nanolab cleanroom, with designs that have a large imaging area, and a well defined geometry for liquid pocket formation. We grow graphene in a transfer-free way through patterned thin film. Currently we are testing Molybdenum[2] for suitability as catalyst for our design, with promising results. Wafer-scale production can make liquid cell EM experiments more routine by increasing availability and decreasing cost. The precise control offered by cleanroom equipment allows designs to be tuned for the specific system at hand (e.g. larger or smaller liquid pockets, thicker or thinner liquid layer).

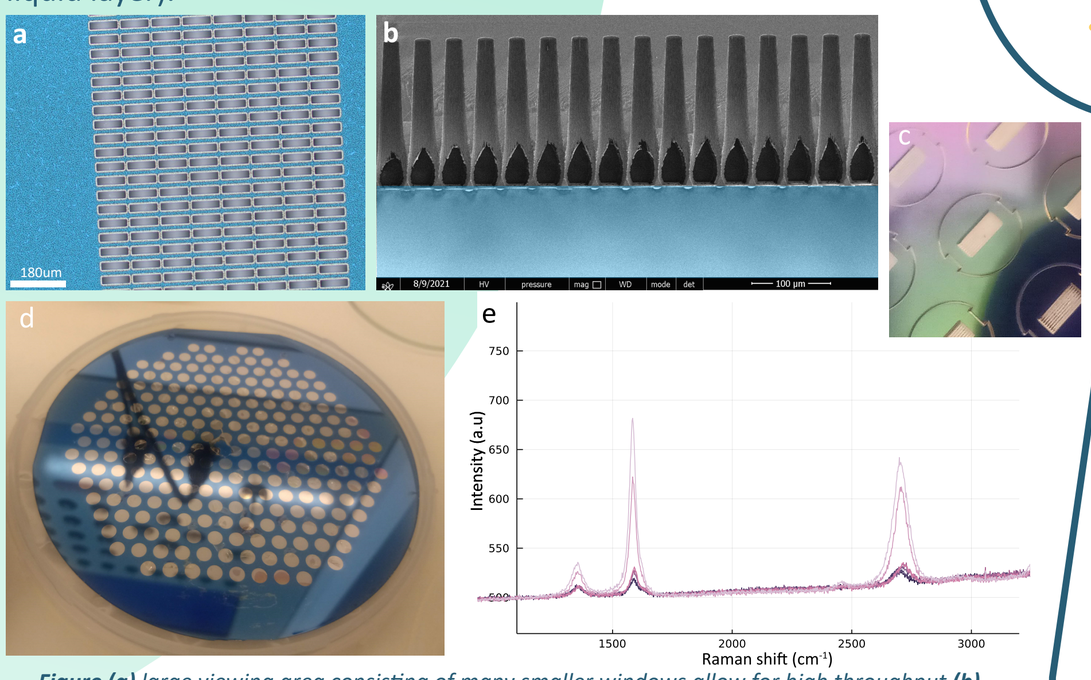

Figure (a) *large viewing area consisting of many smaller windows allow for high throughput* ***(b)*** *cross-section of (a)DRIE etched openings (for viewing area) in silicon* ***(c)*** *chip outline* ***(d)*** *patterened molybdenum thin film on wafer* ***(e)*** *raman signal after CVD on molydenum patches.*

Transfer-free GLC channels

We also explore simpler ways to make transfer-free GLCs. To this end, we evaporate a thin layer of gold directly on the catalyst (copper) after CVD to serve as a spacer and then selectively etch away the copper in APS to be left with suspended graphene structures without any transfer. Gold has proven to be a challenging material to evaporate due to its high mobility, and we are exploring alternative strategies to ensure higher yield.

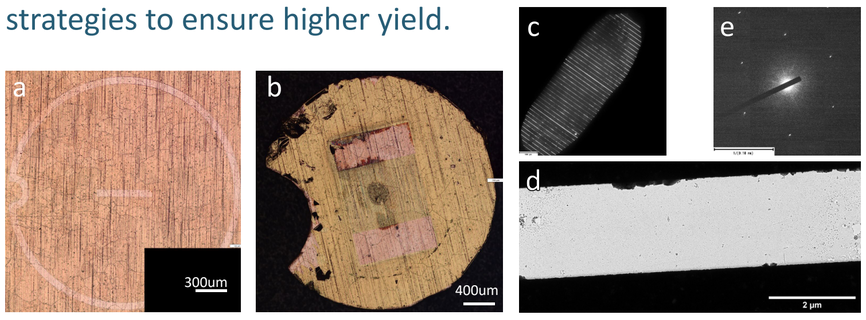

Figure (a) *'backside' of chip showing photoresist etchmask (dark).* ***(b)*** *front side of chip, showing patterned gold* ***(c)*** *TEM image of gold channels* ***(d)*** *suspended transfer-free graphene* ***(e)*** *diffraction pattern of around 100nm section of suspended graphene.*

Microtubules in liquid phase EM

Microtubules are hollow protein filaments with an outer diameter of around 24nm, and have a diverse set of essential physical roles in eukaryotic cells. Fundamental questions remain about their characteristic growth and spontaneous rapid disassembly behaviour known as *dynamic instability.* We aim to capture these nanoscale dynamics with liquid cell EM. In work done in collaboration with **VitroTEM BV**, we have been able to look at stable microtubules in liquid. We find that microtubules are (partially) flattened by the attractive forces of two graphene sheets, but can retain their tubular shape when the graphene sheets are separated by a bigger templating object.

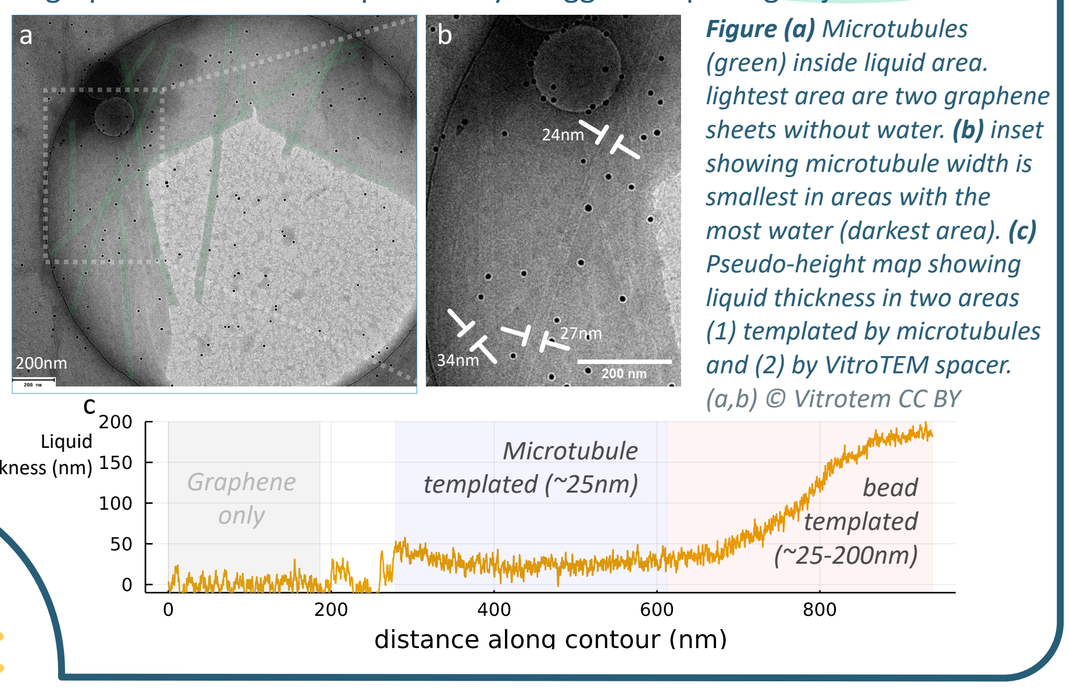

Figure (a) *Microtubules (green) inside liquid area. lightest area are two graphene sheets without water.* ***(b)*** *inset showing microtubule width is smallest in areas with the most water (darkest area).* ***(c)*** *Pseudo-height map showing liquid thickness in two areas (1) templated by microtubules and (2) by VitroTEM spacer.* *(a,b) © Vitrotem CC BY*

Low complexity, high predictability

To compliment the high complexity wafer scale silicon GLC design, we explore designs that do not require expensive facilities, while maintaining large window and high predictability.

By evaporating gold though a reusable 300nm thick Silicon Nitride stencil mask, gold can be patterened on graphene without damage and without the involvement of etchants, which may contaminate the graphene. Subsequent sample application and *Loop-Assisted Transfer[1]* should result in predictable pocket formation.

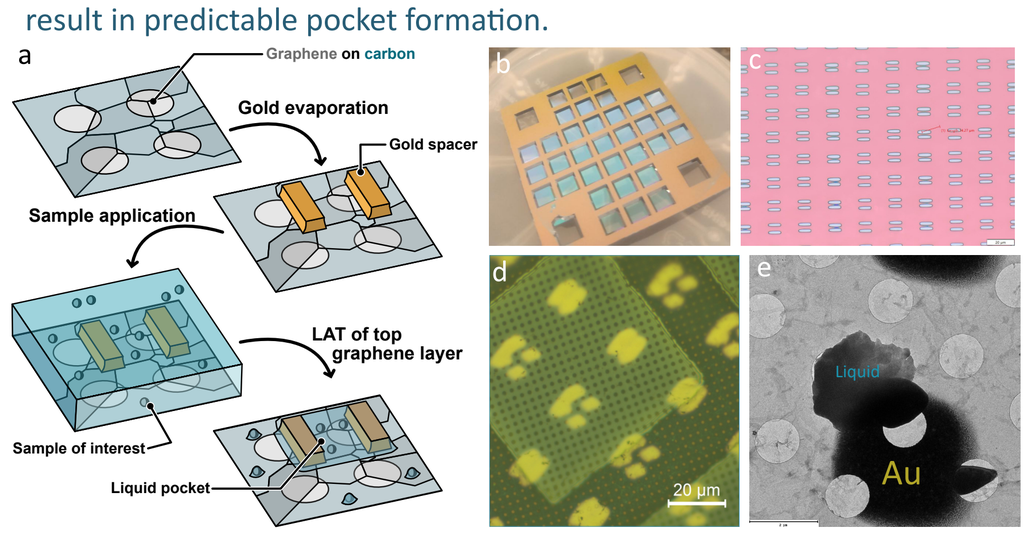

Figure (a) *Fabrication process liquid cells involving a simple evaporation step and LAT.* ***(b)*** *Stencil mask out of LPCVD silicon nitride on a piece of silicon wafer* ***(c)*** *Example of mask pattern* ***(d)*** *resulting structures on graphene-on-quantifoil EM grid* ***(e)*** *early example of liquid pocket formation near gold spacer.*

Outlook

Further work in the wafer-scale and the low complexity designs will create a liquid environment that should be compatible with conditions for microtubule growth, and the observation of their dynamics. Development of image processing tools will be essential to deal with high noise and getting the most out of the complex image data.

References

[1] van Deursen, Pauline MG, et al. "Graphene liquid cells assembled through loop-assisted transfer method and located with correlated light-electron microscopy." Advanced Functional Materials 30.11 (2020): 1904468.

[2] Vollebregt, Sten, et al. "A transfer-free wafer-scale CVD graphene fabrication process for MEMS/NEMS sensors." 2016 IEEE 29th International Conference on Micro Electro Mechanical Systems (MEMS). IEEE, 2016.

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