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Dedicated to
Neha Arun, Arun Balanpillai
and
Santhosh P George

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Meera Kanakamma Mohan

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Publications

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Conference Papers

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3. Meera Kanakamma Mohan, Manish Arora, Utkur Mirsaidov, Claus-Dieter Ohl. "Dynamics and stability of nanobubbles inside the Transmission Electron Microscope (TEM)". In Institute of Physics Meeting, Singapore (2014).
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6. Beng Hau Tan*, Hongjie An*, Zahava Barkay, Utkur Mirsaidov, Meera Mohan, Claus-Dieter Ohl*: Insights from the dynamic imaging of nanobubbles. In Institute of Physics Meeting, Singapore (2016).

Declaration of authorship

I, Meera Kanakamma Mohan, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work. I have acknowledged all main sources of help; where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Contributions by others to the thesis

Dr. Zhava Barkay contributed with ESEM images (Fig. 12 and Fig. 13) of my samples taken at Tel Aviv University.

Dr. Michael Bosman contributed with EELS spectrum (Fig. 14) and STEM images (Fig. 18) of my samples. The images recorded together with me at IMRE, A-Star, Singapore.

Dr. Sudhiranjan Tripathy contributed with Raman spectrum (Fig. 21 and 22) of my samples. The spectrum recorded together with me at IMRE, A-Star, Singapore.

Dr. Zhang Qi contributed with Raman spectrum (Fig. 23) of my samples. The spectrum recorded together with me at CBC, NTU, Singapore.

Nordin Bin Abdul Kassim contributed with SEM images (Fig. 11) of my samples. The images recorded together with me at MAE, NTU, Singapore.

Beng Hau Tan contributed to the analysis of data which is documented in Chapter 4.

List of Acronyms

AFM	Atomic Force Microscopy
CVD	Chemical Vapour Deposition
EELS	Electron Energy Loss Spectroscopy
EDX	Energy Dispersive Spectroscopy
ESEM	Environmental Scanning Electron Microscope
FEG	Field Emission Gun
FTIR	Fourier Transform Infra-Red
HOPG	Highly Oriented Pyrolytic Graphite
OTS	Octadecyl Trichloro Silane
STEM	Scanning Transmission Electron Microscope
STXM	Scanning Transmission X-Ray Microscopy
TEM	Transmission Electron Microscope
TIRF	Total Internal Reflection Fluorescence Spectroscopy

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List of Symbols

λ	mean free path of electrons
β	collection semi angle
ρ	density of water
α	relativistic factor
θ	scattering angle
γ	surface tension
η	dynamic viscosity
τ	time scale for coalescence
v	drift velocity
μ	electron mobility

Abstract

The study of nanoscale phenomena using in-situ transmission electron microscopy (TEM) has received increasing attention due to its ability to image at both high spatial and temporal resolution. In this study, nanobubbles forming within graphene liquid cells are characterised in TEM. It was observed that nanobubbles shrink quickly under illumination by an electron beam, but are stable for an hour or more in its absence. By observing the merger of nanobubbles, it was deduced that the apparent viscosity of the confined water in the graphene liquid cell is much larger than bulk water, and this reduces the diffusion constant to an extent that supports the long lifetime of the bubble. Apart from these observations, we also characterize instances of bubbles rupturing, oscillating, merging and non-coalescence events.

Key Words: Nanobubble, Transmission Electron Microscope, nanobubble stability, bubble merger, bubble shrinkage, bubble rupture, bubble oscillations, diffusion, non-coalescence.

Chapter 1

Nanobubbles

1.1 Introduction

The physical properties of materials at the nanoscale are amazingly different from its macroscopic counterparts. In particular fluid dynamics and that of their interfaces shows a rich regime in an emerging research area termed interfacial liquids. Here we are focusing on the properties of vapor and gas filled voids in liquids, i.e. bubbles. These so-called nanobubbles differ from bubbles observed at larger scale. There is a keen interest in their studies as the variations of properties and the smallness makes the nanobubbles useful in other fields of science and technology such as for the treatment of water, in biomedical engineering and general industrial applications. Among the applications of nanobubbles, a remarkable one is in medical therapy where a plasmonic induced *transient* nanobubble may destroy (cancer) cells [1-5]. *Stable* nanobubbles are currently thought as a means to improve floatation in the mining industry [6, 7]. A cousin to the free floating (shelled) nanobubble and the transient cell killing vapour nanobubble are the nanometer sized surface attached bubbles [8, 9] which have received a lot attention due to the unexpected stability and shape.

The presence of gas leads to free shear interfaces at the nanoscale and at the solid-liquid interface may affect large scale flows, drag, and sensing; in particular nanobubble nucleation has been found important for mineral flotation [10], and for the understanding the hydrodynamic slip length [11]. In 20th century, Blake et al., proposed a long range force being orders of magnitude stronger than the van der Waals force [12, 13]. While explaining the long range attractive forces between hydrophobic surfaces [14], Parker et al., suggested the existence of surface attached nanobubbles for the first time in 1994. Attard concluded the possibility of long range attractive forces as a result of air bubble bridging between macroscopic hydrophobic surfaces and the nanobubble research field established [15]. The above research ideas were supported by a de-aeration experiment [16, 17]. It was manifested that the range of attraction decreases in de-aerated water. Later, the presence of these tiny bubbles was confirmed in a number of studies [18-21].

1.2 Significance of nanobubbles

Theoretically, the gaseous content of nanoscale bubbles (bubbles having diameter at the order of 10^{-9}m) will be at a high Laplace pressure, and therefore would lead to their fast dissolution [22]. But experimentally, they are stable for hours. A number of theories explain this stability: In 2003, Attard [23] proposed that an impermeable liquid ‘skin’ at the nanobubble interface hinders the diffusion of gas and this hypothesis was later supported by Ducker [24]. A different explanation was proposed by Seddon et al. [25]: gas escaping from the bubble is recirculating from its apex back to the bubble’s triple contact line thereby stabilizing the bubble against dissolution. Later, the flow necessary to support this dynamic stability was found not to exist by Chan and Ohl [26].

Although there were sufficient experimental evidences for the existence of nanobubbles significant objections remained. Theoretically, according to the Young- Laplace equation, the gas in the nanobubbles will be at a higher pressure than partial pressure of the gas in the surrounding liquid. This will lead to transport of gas through the bubble interface. The partial pressure is proportional to the concentration of that gas and is given by Henry's law. In short, the situation that the gas inside the nanobubble is at high pressure, while the liquid is not highly supersaturated leads to an unstable situation. Therefore, even moderately gas supersaturated liquids cause nanobubbles to shrink. The dynamics of bubble shrinkage can be estimated by solving the diffusion equation for the appropriate geometry and boundary conditions. According to literature [27-29] and for commonly sized nanobubbles in water with experimentally measured gas concentrations, this should happen within milliseconds if not faster. Yet, nanobubbles exist for hours [27].

1.3 Various methods for imaging nanobubbles

Nanobubble research is a very wide field and various laboratories uses different methods to image nanobubbles. Among them, Atomic Force Microscopy (AFM) is the most commonly used method besides Total Internal Reflection Fluorescence (TIRF) spectroscopy, Scanning Transmission Electron Microscopy (STEM) and Transmission Electron Microscopy (TEM).

1.3.1 Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM) is a high resolution Scanning Probe Microscopy (SPM), which helps us to analyze structures effectively on the nanometer scale.

Ishida et al. [20] was the first to observe nanobubbles while imaging hydrophobized silicon wafers immersed in water in tapping mode AFM. Nearly at the same time, Lou et al. [30] reported the nanobubbles production on top of water immersed and atomically flat solid surfaces. Also, they used the AFM in tapping mode and found an unexpected stability of the nanobubbles for hours. Early on, some controversy was established as not all groups could follow Ishida's result. There are reports [31-37] confirming the experiments as well as reports [38-41] where nanobubbles were not seen. In summary, to date AFM is the most widely used method to image nanobubble formation. They have been found on hydrophobic water-solid interface where a typical experiment utilizes Highly Oriented Pyrolytic Graphite (HOPG) with the bubbles being nucleated through a solvent exchange process[30, 42-45], a change in liquid temperature [45, 46], or by the electrolytic production of hydrogen [47].

There are several modes to image the nanobubbles with an AFM like tapping mode, contact mode, frequency modulation mode, force mapping, and peak force quantitative nanoscale mechanical characterization mode. The popularity of the AFM method for nanobubble imaging is due to its advantages to obtain high resolution in all 3-dimensions from which important characteristics such as the contact angle can be extracted. Yet, AFM has its share of disadvantages, in particular its invasiveness as the AFM tip interacts with the sample and may disturb the shape or position of the object under investigation. Also AFM measurements are not specific to liquid or gaseous objects which may lead to a misrepresentation of contamination to nanobubbles [48]. Some non-AFM based methods have confirmed the existence of nanobubbles [49, 27], but lead to a cautious interpretation of AFM-only measurements.

1.3.2 Optical microscopy

Optical microscopy is much quicker, and allows recording the dynamics of nanobubbles on a shorter time scale as compared to AFM. Several modes exist to image the nanobubbles and nanobubble nucleation such as interference enhanced reflection microscopy and Total Internal Reflection Fluorescence (TIRF) microscopy. These optical methods have in common that their lateral resolution is limited to about half the wavelength of light, i.e. about 200nm. In interference enhanced reflection microscopy [49], a silicon wafer is covered with a 300nm thick silicon oxide coating and used as the substrate which is further coated with a monolayer of hydrophobic material to allow creation of the nanobubbles through

the solvent exchange method. Among these, TIRF is most efficient to offer diverse information like nucleation, dynamics and growth of nanobubbles [26].

1.3.3 Scanning Transmission X-Ray Microscopy (STXM)

STXM is a less invasive method than AFM with high spatial resolutions to image nanobubbles having small length scale which are not possible to image with optical microscopy. STXM utilizes a synchrotron to generate soft X-rays which are used for imaging [50].

1.3.4 In-situ Electron Microscopy

Compared to all other methods, in-situ electron microscopy provides the highest spatial resolution while offering sufficient temporal resolution to resolve dynamics of nanobubbles. Transmission electron microscopy with and without scanning (TEM and STEM) allow to image very small amounts of liquids. On the downside, the method is generally invasive as electrons can induce chemical reactions which may lead to residual gases. Transmission electron microscopy has been introduced by Regan et al. [51] in 2011 for imaging pre-existing nanobubbles in water within a silicon nitride liquid cell. A set of studies utilizing STEM and TEM to create nanobubbles with high and low voltage electrons has been reported since then [52-54].

1.4 Aims and outlook of the work

The aim of this work is to study nanobubbles and their dynamics in confined liquid using transmission electron microscopy. The combination of nanoconfinement and TEM imaging environment can cause drastic alterations of the basic properties of the liquid. The rapid shrinkage of nanobubbles in the *presence* of the beam and contrary to this, much slower shrinkage in the *absence* of the beam is observed. Having the ability to resolve the small scales, we can then relate the dynamic observations to the fundamental properties of confined liquids and compare that to the bulk value. The viscosity enhancement of confined liquid is studied widely with AFM [55-57] but this thesis stresses the confinement of the liquid, imaging environment, electric field effects due to the electron beam and the resultant changes in liquid properties. More specifically, this thesis reports on the in-situ study of various nanobubble dynamics in approximately 20-50 nm thick layer of

water with a spatial resolution of $\sim 0.4\text{nm}$ and a minimum temporal resolution of 0.05 s . This thesis answers some open questions about the confined liquid properties in the nanoscale, diffusion problems and bubble dynamics at the nanoscale in the presence of a beam of high-energy electrons.

1.5 Organization of the thesis

Chapter 2 gives a detailed description about the instrumentation, the sample preparation and how the imaging is conducted. Chapter 3 describes about various observations. In this chapter, possible beam effects and beam-sample interactions are also discussed in detail. Chapter 4 contains the analysis of the observations and results along with the physical explanations of dynamics. Chapter 5 concludes the thesis with the future plans.

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Chapter 2

Liquid Cells and Transmission Electron Microscopy

2.1 Transmission Electron Microscope (TEM)

As the name indicates, a transmission electron microscope uses transmitted electrons to create the image of ultrathin specimens. It is almost similar to an inverted optical microscope, with the main difference that TEM uses high energy electrons for imaging while an optical microscope uses visible light. Due to its shorter wavelength, a much higher diffraction limited magnification can be achieved. For my experiments, I employed an Environmental Transmission electron microscope (ETEM) from FEI TECNAI model T12. Unlike normal TEM, Environmental TEM allows wet, uncoated samples inside the chamber. All the imaging was done by 120 kV except for a few cases mentioned in Chapter 3. A very detailed description of the TEM can be found in the Appendix.

Fig. 1. FEI Tecnai T12 transmission electron microscope (Cryoelectron Microscopy Facility at the Centre for Bio Imaging Sciences, National University of Singapore). The middle screen controls the operation of the microscope and the

screen at the right, controls the camera and it displays the recorded images of the specimens.

2.2 The electron beam interaction with sample

When an electron beam interacts with a sample, two main effects are observed namely elastic and inelastic interactions. During elastic interactions electrons do not transfer energy to the sample or in other words, the incident energy of the electron is same as the emitted energy of the electron. More accurately, the Coulomb interaction of the electron with the positive potential of the electron cloud causes the electron beam to deflect from its path. This gives rise to negligible energy loss for the beam. These elastically scattered electrons in the beam are direct electrons which contribute to the transmitted image formation. Contrary to this, during inelastic interaction, the incident electron transfers some energy to the sample and as a result, there is energy loss of the electrons which is accompanied by the emission of x-rays, phonons (by heating), bremsstrahlung or continuum radiation, cathodoluminescence (visible light fluorescence), secondary electrons (Auger electrons or ejection of outer shell electrons) and plasmons. These signals can be measured during electron microscopy and provides information about the composition of the specimen, its topography, the local electrical potential, local magnetic field and crystalline structure.

2.3 Liquid cells

Specially fabricated imaging cells are required to image liquids in TEM. This is because the low pressure (high vacuum) environment required for TEM would cause rapid evaporation of the liquid. Traditionally, TEMs do not permit loading of liquid samples into the chamber [1], e.g. to image biological samples they need to be frozen first. But the main limitation of frozen state is the lacking of data about in-situ dynamics. A major advance in imaging liquids in TEM was introduced by Williamson et al. [2], who demonstrated that liquids can be imaged in TEM by trapping the liquid within a confining cell thus isolating it from the vacuum environment. The cells used for imaging the liquid have been termed as ‘liquid cells’. Since this pioneering work, liquid cells have developed into a sophisticated platform for imaging liquids for a wide range of fundamental and applied research, including nanocrystalline growth [3, 4], cellular function at the molecular level [5], nanorod growth [6], colloid aggregation and growth dynamics [7], nanoparticle dynamics [8, 9, 10], nanoparticle electrodeposition [11], and e-beam manipulation of gold nanoparticles [12]. The most common liquid cells are micro-fabricated and

sealed silicon cells with electron transparent silicon nitride membrane windows. These windows have to be sufficiently thin to generate enough contrast to visualize the entrapped liquid. Another method is to trap microliters of liquid in between two flat sheets of graphene. These graphene sheets are supported by a TEM sample grid. The advantage of graphene liquid cells over silicon cells is their higher electron transparency [13] and therefore a greatly increased contrast. Details on these particular liquid cells will be provided in the coming sections.

2.4 Various types of liquid cells

2.4.1 Silicon nitride liquid cells

Silicon nitride liquid has been utilized for the study of nanocrystal growth [3-9, 13], nanoparticle dynamics [8-10, 16] bubble dynamics [14-18] etc. These cells can be adopted in a lot of ways to the necessity of the experiment.

The silicon nitride liquid cell consists of two silicon chips (top chip and bottom chip) each with thickness 100 μ m, with a silicon nitride membrane deposited on top (20nm). Then an indium spacer (100nm) is patterned on top of the bottom chip in order to provide space for the water. Then the top and bottom chips are etched with KOH solution. After etching, both chips are aligned and sealed with resins. Then, experimental liquid is filled within the cell with the syringe and the cell is shut with copper ring as in Fig 2. Now the liquid inside the cell is completely isolated from the surrounding and is ready for imaging.

Fig. 2. Sketch and SEM image of silicon nitride liquid cell (A) Planar section view of bottom chip (2.6mm x 2.6mm) (B) Planar section view of top chip (2.6mm x 2.6mm). Inner square dimensions are 0.6mm x 1.2mm and the membrane at the middle is (1 μ m x 50 μ m) (C) Planar section view of copper sealing (D) Alignment of the cell (E) SEM view of liquid cell after sealing the top and bottom pieces (F) SEM view of liquid cell after filling it with liquid and sealed with the copper ring along with the magnified view of the membrane (1 μ m x 50 μ m) (G) Cross-sectional view of the silicon nitride liquid cell

The fluid dynamics inside the cell and the interaction of the electron beam with the liquid is intriguing. Plenty of nanoscale interface phenomena have been observed with the aid of these tiny cells. As an example, Mirsaidov et al. [19], explained a surprising stick and slip movement of water nanodroplets caused by the e-beam irradiation demonstrates, a variety of forces working on water within an Environmental Transmission Electron Microscope (ETEM) and at the nanoscale. The dynamics of the nanoscale water as a result of beam sample interaction reveals the surface energy change in the droplet by the beam is the reason for droplet deformation. The nanoscale dynamics studies of water can help to understand the phase conversions and also the energy dissipations. Another example is that a

microfluidic system that can maintain a flow of liquid inside the cell by Ring and Jonge [20]. Here, the liquid flow cell allows liquid exchange that can be utilized for diverse applications in biology and fundamental studies in nanofluidics.

2.4.2 Graphene liquid cells

In 2012, Alivisatos et al. [4] introduced another type of liquid cell, with increased contrast and high resolution, while sustaining the liquid conditions by sealing the liquid solution within two graphene sheets. This graphene liquid cell was used to study the growth kinetics of colloidal nanocrystals. The same group successfully studied the three dimensional dynamics of double stranded DNA with the same platform. For the 3D studies, they utilized reconstruction of the 2D projected TEM images [21].

We reported the observation of nanobubbles inside the graphene liquid cells for the first time along with its stability for hours in the absence of the beam [22]. Shin et al. [18] reported about merging bubbles inside the graphene liquid cells and calculated the contact angle of the bubble from the side view of the folded graphene liquid cell.

Mirsaidov et al. [23] proposed another type of liquid entrapped cells from scrolling of graphene. It appears that the etchant contributes to some regions of graphene rolling up into what they called graphene nanoscrolls. The nanoscrolls form as a consequence of the scrolling of planar graphene layers induced by water due to the capillarity of water and elasticity of graphene. These nanoscrolls can be used to wrap Fe_3O_4 nanoparticles using liquid nitrogen by cold quenching. The hybrid structure created in this procedure can be used in lithium-ion batteries and other energy storage applications [24]. As it is a new topic and the sample preparation is challenging, there is not much research reported in this field yet.

In my current work, I use graphene liquid cells to study the nanobubble dynamics as a result of beam-sample interaction.

2.5 Sample preparation

The crucial part in the experiment is sample preparation. The quality of graphene is the central element that decides the success of the experiment. Usually, chemical vapour deposited (CVD) graphene is used for sample preparation.

2.5.1 TEM sample grid (Gold grid with quantifoil deposited on top)

The traditional TEM sample holder can hold a TEM grid with a diameter of 3mm and thickness of $25 \pm 5 \mu\text{m}$. We are using gold grids having wide holes of area ($90 \mu\text{m} \times 90 \mu\text{m}$) (Fig.7b). These large holes cannot give sufficient support for the sgraphene sheets. Thus for a better support a holey carbon foil (quantifoil) with square holes of area $7 \mu\text{m} \times 7 \mu\text{m}$ is deposited on to the base grid (Electron Microscopy Sciences, QUANTIFOIL; 200#; 7X7 μm GOLD), see Fig.3.

Fig. 3. (a) Schematic diagram of TEM gold grid with holey carbon mesh

(b) SEM image of the gold grid with its magnified image to show the dimensions of hole meshes. Gold squares having dimensions ($90 \mu\text{m} \times 90 \mu\text{m}$) and Quantifoil is ($7 \mu\text{m} \times 7 \mu\text{m}$)

2.6 Methodology

The single atomic layer CVD graphene deposited on one side of the copper foil ($18 \mu\text{m}$) is used for the preparation of the sample (Cat# Monolayer Graphene on Cu

(60 mm x 40 mm), Graphenea). The method of preparations is indicated in Fig. 4. The graphene sheet attached with the grid after etching can also be seen in Fig. 5.

Fig. 4. Method of preparation of graphene liquid cells

Fig. 5. Graphene sheet attached to the gold grid during sample preparation (rectangular dark area under the gold grid shows graphene sheet)

2.6.1 Procedures

- I. The graphene on Copper foil is placed on top of a 4% aqueous Ammonium persulfate ($(\text{NH}_4)_2\text{S}_2\text{O}_8$) solution with graphene facing air.
- II. The gold grid with Quantifoil (Electron Microscopy Sciences, quantifoil; 200#; $7 \times 7 \mu\text{m}$ Gold) was then gently placed on top of the graphene-copper foil.

- III. After the complete etching of the copper film with ammonium persulfate, the graphene adheres to the TEM grid. Next, the “TEM grid-graphene complex” was rinsed in a water beaker for overnight.
- IV. On an individual basis, a second “graphene-Cu” foil was etched with a 4% ammonium persulfate solution. During this process a drop (~1 μ l) of deionized water was laid gently on top of the floating “graphene-Cu” foil. Next, the “TEM grid-graphene structure” prepared earlier is placed on top of the drop. The water trapping completes within this phase.
- V. After three hours of etching, the copper foil on the bottom was removed and the resulting “TEM grid-graphene-graphene complex” was transferred to a beaker with water for overnight to remove ammonium persulfate completely. The grid was dried to remove the water that is not encapsulated inside the sheets before loading the grid onto the TEM holder.

The whole etching process with $(\text{NH}_4)_2\text{S}_2\text{O}_8$ solution and the clean-up process with DI water is done inside covered beakers to prevent contaminations through air flow.

2.7 Imaging nanobubbles with TEM

Uchida et al. [25] are likely the first to report on TEM imaging of micro and nanobubbles. They used a freeze-fracture method to replicate the surface of oxygen bubbles in waste water and interestingly, they found adsorbed impurities on the bubble surface. However, we emphasise that the observation of these bubbles is not dynamic, and therefore the dynamical aspects of nanobubbles cannot be understood with the method from Uchida et al. We focus on in-situ observations of nanobubbles inside the liquid cells and their dynamics.

2.7.1 Observations of nanobubbles inside silicon nitride liquid cells

The nanobubble dynamics in silicon nitride liquid cells probed by the electron beam were first proposed by White et al. [13]. They reported about the bubble nucleation inside the cell due to electrical pulses passing through a platinum wire fabricated on top of the silicon nitride membrane. In the absence of an applied potential in the platinum wire, the bubbles were unstable and rapidly collapsed by the electron beam. These bubbles were about 50-100 nm and dissolved into the

water within 5-10 seconds. In 2013, Grogan et.al [15] offered a model for radiolysis inside the TEM which is based on a reaction-diffusion model. This radiolytic decomposition of liquid from the electron beam causes the bubble nucleation, growth, and migration. The production of hydrogen gas as a by-product of radiolysis forms the bubbles. The formation of hydrogen nanobubbles created by the radiolysis of KLH (keyhole limpet hemocyanin) protein, was proposed by Fu-Rong Chen group [16]. They suggested that the gas inside the nanobubbles is a Knudsen gas and they reported about the observations of anti-Ostwald ripening and bubble growth. Liu and Dillon [17] reported about the electrolytic production of hydrogen near gold electrodes. Their observations clarified that the bubble formation happens near the electrodes and not always on the electrode. The electron-liquid interaction and further decomposition of the liquid is a complex process that needs more analysis and clarification.

2.7.2 Observations of nanobubbles inside graphene liquid cells

Graphene is unique in its transmissivity over a broad energy range [26-29], excellent mechanical strength [30, 31], and impermeability to gases and liquids [32-34]. Thus graphene is a good candidate for the TEM liquid cell to acquire high resolution for in-situ observations. However, graphene liquid cells are not free from drawbacks. The intractable properties of graphene like scroll formation [23], creation of folds [34, 35] and multiple layer formations during sample preparation creates complications. In addition to this, the 2-D images and videos recorded from TEM is another main limitation of this work. The 3-D picture of graphene liquid cells and 3-D information about bubbles is lacking. Shin et al. [20] claimed that the bubbles inside graphene liquid cells are cap shaped surface nanobubbles having the contact angle of approximately 70 degrees. It is rather unlikely to obtain hemispherical bubbles with the above mentioned contact angle. A possible hypothesis will be elucidated in Chapter III. Over all, the current work is promising and relevant to understand the bubble dynamics and liquid properties inside a confined system, the role of viscosity enhancement of water in nanoscale along with the bubble diffusion problems.

2.8 Various instruments used for detailed analysis

2.8.1 Scanning Electron Microscope (SEM)

In order to study the topography of the liquid cell, the samples were imaged with SEM (Hitachi 3500N located on Micro Machines Lab, Mechanical and Aerospace

Engineering, Nanyang Technological University and JEOL JSM-6700 F, School of Physical and Mathematical Sciences, NTU). Unlike TEM, SEM scans the samples and produces the images with a focused beam. The electron beam interacts with the top of the sample and creates SEM images with the secondary electrons and back scattered electrons. The 2-D scan of the focused probe across the specimen surface row by row and line by line creates grid element pixels which are known as “raster” and each image pixel has a specific gray scale value. The magnification controls the raster size.

2.8.2 Environmental Scanning Electron Microscope (ESEM)

In order to analyze the sample topography and bubble morphology, the samples were imaged with ESEM (Quanta 200 FEG, Wolfson Applied Materials Research Centre, Tel Aviv University, Israel). Like Environmental Transmission Electron Microscope (ETEM), ESEM also provides wet, uncoated samples inside the chamber. The ESEM can also work in transmission mode and is able to record the correlated images of bubble dynamics with its transmission mode and topography of the liquid cells during the bubble dynamics with a good contrast. The ESEM analysis of the liquid cell played an important role in the analysis.

2.8.3 Scanning Transmission Electron Microscope (STEM)

The low voltage analysis of bubbles were done with STEM (FEI Titan) and Electron Energy Loss Spectroscopy studies are undertaken with the STEM to check the presence of gaseous species. STEM is modified form of TEM with some additional coils and detectors for scanning and mapping. The STEM uses focused beam like SEM and it can give atomic resolution images.

2.9 Spectroscopy Analyses used for the experiment

2.9.1 Electron Energy Loss Spectroscopy (EELS)

As the name indicates, EELS is the detection of the loss of energy (inelastically scattered electrons) during its interaction with various elements presents in the sample. The electron spectrometer detects how much energy loosed by the electron beam when it transfers kinetic energy to the electron clouds of atoms during interaction. The spectrum depicts the loss of energy due to the presence of various elements and this energy loss is the energy utilized to remove the inner shell electrons of the element. For example, if the electron loses 532eV during its interaction with the sample, which is the energy needed to remove an electron from

inner shell orbit of an oxygen atom. This will be record in the spectrum as the 532 eV peak.

2.9.2 Electron Dispersive Spectroscopy (EDS)

Electron Dispersive Spectroscopy or Electron Dispersive X-Ray spectroscopy (EDX or EDS or XEDS) collects the information about the presence of a particular element and its proportions. An EDX spectrometer detects the characteristic x-rays emitted from an entire scan area of the sample as a result of beam sample interaction. When an electron beam having a certain frequency interacts with the sample, it initiates the removal of an electron from the inner shell of an atom and creates a vacancy or hole. This hole is filled with an upper shell electron and x-rays are emitted with the same energy as the difference in the electron energy.

The main disadvantage of EELS and EDX is as they use the interaction with the K-shell electrons, both of them are not sensitive to lighter elements such as hydrogen and helium.

2.9.3 Raman spectroscopy and mapping

Raman spectroscopy analysis is the best way to analyze the crystal structure of graphene because it is sensitive, nondestructive, and a fast method. When a laser light of certain frequency in the visible or UV range interacts with the vibrational modes or phonons of an atom in a lattice, its frequency is shifted either up or down. This “Raman shift” gives the information about the crystal structure [36-40]. In this study, the radiation damage (knock-on damage) of graphene from the high energy electron beam has been tested with Raman spectroscopy and mapping of the sheet.

A typical Raman spectrum mainly consists of the D band, the G band and the 2D band. The D band lies around 1345 cm^{-1} . The D-bands are called defect band or disorder band because according to the double-resonance model, the D-band will be active in the spectrum if there is a disorder in the crystal structure. The D-band is caused by the breathing modes of sp^2 atoms. The A_{1g} symmetry phonons near the K - zone boundary gives rise to a D - band. The G peak lies at 1560cm^{-1} range and the phonons corresponds to G-peak are the E_{2g} phonons at the Brillouin zone [39, 40]. According to Tunistra and Koenig [36], the in-plane crystalline size is

inversely proportional to the ratio of the intensities of the D band and the G band. The second order D-band or the overtone of D-band is called 2D band or G' band or D*band and it will lay around 2700cm^{-1} [39]. The 2D peak originates when the momentum conservation is satisfied by two phonons having opposite wave vectors and these peaks will be present in all the graphene spectrums, unrelated to the defect [40]. It can give information about strains present in the crystal structure.

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Chapter 3

Nanobubbles- Nucleation, Shape and Effects of the Beam

3.1 Imaging of nanobubbles with TEM

The graphene liquid cell is loaded inside the TEM sample chamber and is imaged with the FEI Tecnai T12. This TEM is located at the Cryoelectron Microscopy facility, National University Singapore. The FEI Tecnai T12 is an environmental TEM which allows wet, uncoated samples inside the chamber. It can operate at different voltages from 20 kV to 120 kV. The experimental observations and results reported in this work are imaged, mostly at 120 kV for better resolution. At lower magnification, the bubble dynamics are hard to interpret, while higher magnification enables good contrasted visualization of nanobubble dynamics. An example of the kind of picture quality possible is depicted in Fig. 6.

Fig. 6. TEM images of the graphene liquid cell for increasing magnifications from left to right. At the highest magnifications nanobubbles become visible. (a) Scale bar is 5 μ m (b) Scale bar is 5 μ m (c) Scale bar is 0.1 μ m (d) Scale bar is 50nm.

Fig. 7. SEM images for increasing magnifications.

(a) At very low magnifications (scale bar 50μm), gold grids and holey carbon meshes are visible.

(b) Holey carbon meshes (7μm x 7μm) and graphene cells attached to the carbon mesh can be seen (scale bar 50μm)

(c) At the highest magnification graphene sheets on carbon mesh become visible (scale bar 3μm)

Fig. 8 ESEM images of graphene liquid cell with bubbles in TEM mode and SEM mode.

(a) TEM mode image of graphene liquid cell

(b) The correlated view of the topography of the graphene liquid cell.

In order to understand the topography, the graphene liquid cells were imaged with a variety of electron microscopes. The scanning electron microscope (Hitachi 3500N located on Micro Machines Lab, Mechanical and Aerospace Engineering, Nanyang Technological University) provides pictures as shown in Fig.7. Due to limitations in magnification, SEM failed to provide sufficient details about the topography of the liquid cell. The ESEM (Quanta 200 FEG at Wolfson Applied Materials Research Centre, Tel Aviv University, Israel) imaging was done by our collaborator Dr Zahava Barkay. These experiments allowed to gather a lot of

valuable information including simultaneous pictures of the liquid cell in transmission and reflection mode as shown in Fig. 8. In particular the motion of the graphene layers as a result of bubble coalescence and dissolution has been obtained. Analysis of the results will be elucidated in the coming sections and chapters.

3.2 Bubble nucleation

There are two likely mechanisms for the nucleation of bubbles. One is radiolysis due to the beam-sample interaction or in other words, radiolytic decomposition of the liquid by the beam. The second mechanism for bubble nucleation are chemical reactions with contamination. These may have been deposited on the graphene surface during the sample preparation.

3.2.1 Radiolysis of water

Radiolysis is the dissociation of a molecule by radiation. The model for the radiolysis of water from an electron beam was first proposed by Weiss in 1944 [1]. This model has been confirmed later by experiments and computer models [2, 3]. The interaction of the electron beam with water having energy greater than the bond energy of a valence electron (~ 10 eV) may generate aqueous electrons and radical species. The primary reactions creates excited water molecules, hydrogen, and hydroxide radicals along with other molecular species [4].

According to the quantity of energy transferred to the electron, the water molecule may undergo

- Ionization (threshold in water ~ 13 eV), [4]
- Excitation (threshold in water ~ 7.4 eV), [4]
- Thermal transfer.

Radical ions and free ‘sub excitation electrons’ are created by ionization of water with the electrons of energy as low as 7.4 eV.

Within 10^{-16} s the energy transfer can generate an excited state of the water molecule, see equation. (3.2).

Due to the dipolar interactions, the electrons captured by water becomes solvated referred as aqueous electrons or solvated electrons (equation 3.3).

This reaction can form radicals also in the solution.

The water radical ion can dissociate to hydroxyl radical and hydrogen ion:

Through bond breakage, the excited water molecule dissipates ~5 eV energy.

So the initial species are H_2O^* , $\text{H}_2\text{O}^{+\cdot}$ and e^- , and the chemically reactive species are located in the vicinity of the original species that caused their creation after $\sim 10^{-12}$ s. The new species created are radicals $\text{H}\cdot$, $\text{HO}\cdot$ and aqueous electrons. Such species are highly reactive. So there will be successive reactions as follows.

And the chemical development will be over by 10^{-6} s. From the above reactions equations (3.7a-g), it is clear that the final product of the reaction is gaseous hydrogen. The experiments and modelling by Grogan et al. [5] demonstrated that the growth of bubbles most likely due to this reason. Contradictory to the silicon nitride liquid cell nanobubbles, the graphene liquid cell nanobubbles shows shrinkage rather than bubble growth. Most likely, the radiolytic reactions inside the graphene liquid cells may be different due to the presence of etchants.

3.2.2 Contaminations

In general, it is nearly impossible to prepare a clean graphene surface by wet etching. The surface is most likely contaminated by the etchants which are needed for sample preparations. As an effect, complex radiolytic reactions form inside the cell. As long as there are no other methods to study the radiolysis inside the liquid cell, only a likely but not definite pathway of the radiolytic reactions can be proposed.

3.2.2.1 Ammonium persulfate ((NH₄)₂SO₄) etchant and sulfate contaminations

As explained in Chapter II, the graphene sheets used for the experiment was deposited by the Chemical Vapour Deposition (CVD) method onto a copper foil. To free the graphene sheet the copper foil is removed by wet etching with Ammonium persulfate ((NH₄)₂SO₄). Ammonium persulfate is a strong oxidizing agent and the dianion can produce radicals by dissociation [6]. So the dissociation of Ammonium persulfate can produce sulfate radicals in the solution.

The Energy Dispersive Spectroscopy (EDS) results in Fig. 9 shows the presence of sulfur and oxygen in the graphene liquid cells at the areas having high topography or folding. Most probably, the sulfur deposits may be produced during the wet etching. Hence the possibility of bubble formation as a result of the reactions between sulfur and/or other sulfur products like sulfate ions, sulfuric acid with the entrapped water has to be taken into account. A likely reaction of ammonium persulphate with water [7] is:

Fig. 9. EDS Spectrum of graphene liquid cells, which shows the presence of sulfur and oxygen. The spectrum also shows the presence of copper, which may be the undissolved copper from the copper substrate where the graphene was deposited.

Electron Energy Loss Spectroscopy (EELS) in Fig.10 shows the presence of oxygen inside the cells along with other gases which supports the possibility of above said reaction which can produce oxygen. But EDX and EELS are not sensitive to lighter elements like hydrogen because both EDX and EELS interacts with the K-shell electrons. Thus final proof that reaction products from the beam with Sulfur leads to oxygen bubbles cannot be given.

Fig. 10. Electron Energy Loss Spectroscopy of water (blue) and bubble (red) shows oxygen K-edge at 532eV. It supports the presence of oxygen inside the cell.

3.2.2.2 Ammonium contaminations

The cells can also contain ammonium cations like NH_4^+ . One possible reactions that can happen inside the cells may be

From the literature [8] the initial radiolytic products of ammonium salts are hydrogen, nitrogen and hydrazine. But the prolonged irradiation can exhaust the hydrazine by back reaction and an equilibrium can establish between N_2 , H_2 and NH_3 .

3.3 Ferric chloride etchant

To interpret the role of contaminations by depositions of the etchants on the graphene surfaces a ferric chloride etchant was used. For all samples (N=4) prepared in the same way as described in Section 2.6, we found *no* bubble formation observed in any of the samples prepared with Iron (III) chloride (Fig. 15). According to literature [9], during the etching process, the Cl^- ions adsorbed on the surface of graphene and these ions may react with the hydrogen ions to form HCl and its radiolysis may not produce gaseous species in contrast to Ammonium persulfate. The absence of bubbles inside the samples that are etched with ferric chloride supports the possibility of bubble formation and subsequent shrinkage by Ammonium persulfate reactions.

Fig. 11. Graphene liquid cells etched with two different etchants shows bubbles and no bubbles in the same magnification (1pixel = 0.64nm) and imaging conditions (dose rate = $2e/A^{0.2}/s$).

- (a) Graphene liquid cells prepared by the Ammonium per Sulfate etchant depicts bubbles within the pockets of water formed by the graphene sheets.
- (b) Graphene liquid cells prepared by the Iron (III) chloride etchant shows no bubbles in similar sized water pockets.

In the above mentioned radiolytic reactions, graphene is considered inert. If we consider that graphene is also taking part in the radiolysis, the possible reactions that can happen inside the graphene liquid cell is the reaction between graphene and atomic and/or molecular oxygen which is the by-product of the radiolysis. Thus, there is a chance of oxidation of graphene. Strong acids, like sulfuric acid which may form by the radiolysis of etchant can also oxidize graphene [10]. In order to bind oxygen with graphene, structural defects and grain boundaries are necessary. The local tensile strain on the defected areas attracts oxygen and new bonds are created [11, 12]. Wang et al. [13] report that for graphene layers, there is a lower reaction barrier in the defected areas as compared to a pristine graphene layer. Their kinetic Monte Carlo simulations confirm that oxygen dynamics on graphene is controlled by the temperature and abundance of oxygen inside the system. In effect, normal graphene is more reactive than pristine graphene. The graphene used in the present experiments is a pristine graphene. Also, Raman spectroscopy and mapping analysis do not reveal considerable defects in the sample. Another favourable condition for GO (Graphene Oxide) formation is the

rise of temperature of the liquid cell. This can happen only, if the sample heating or graphene heating as a result of beam-sample interaction. But the possibility of sample heating is negligible in the case of liquid cells (Refer Section 3.7.1). So the in conclusion, production of graphene oxide is unlikely.

Some other possible reactions involving graphene is the hydrogenation of graphene. When hydrogen reacts with graphene the formation of hydrocarbon called graphane [14-16] may happen. The possibility of chemisorption or physisorption [17-19] of hydrogen on graphene may also occur. Sofo et al. [20] reports that the direct exposure of hydrogen molecule may not create graphane due to the higher binding energy needed for H_2 .

As long as there is no sample heating or higher degrees of damages in the crystal structure, it can be assumed that the graphene is inert in the radiolytic reactions happens in graphene liquid cells and the bubble nucleation is most probably due to the radiolysis of water and etchant.

3.4 Shape of the entrapped bubble

The experimental evidence to demonstrate the shape of the bubble is still lacking due to the limitations of the TEM imaging. From our ESEM observations (Fig.3), we found that the liquid cells are freestanding and flexible. So most likely, the smaller bubbles may be unpinning and in spherical shape and larger ones may be ellipsoidal. Mirsaidov et al. [21] reported that the bubbles in graphene nanoscrolls are unpinning which supports the above said hypothesis. In a recent publication, Shin et al. [22] claimed that the bubbles inside the graphene liquid cells prepared with ammonium persulfate etchant is pinned hemispherically on the graphene surface having a contact angle of 60-90 degree. They evaluated the contact angle by folding the liquid cell. As already discussed, the flexible and freestanding [23, 24] graphene liquid cells wouldn't be able to provide any plausible estimate about the contact angle of the bubbles inside it because of the possible deformations that can occur during the process of folding. Further, the quality of the images taken from folded liquid cell does not unambiguously demonstrate the contact angle of a hemispherical shape. From the literature [25, 26] it is clear that the hemispherical bubbles are pinned strongly on the substrate and their shrinkage is in such a way that the base radius keeps constant and height decreases in time [27, 28]. If the nanobubbles in the graphene liquid cells are surface attached, the contrast of the TEM image at the gas region has to be changed with respect to the liquid region and the radius must be keep constant (refer Fig. 13). Also, if they are weakly

pinned, the shrinkage curve of the nanobubble inside the graphene liquid cell may show a step-wise shrinkage due to unpinning and re-pinning and not the smooth curve [25] (details of the shrinkage curve can be seen in Chapter 4). The sketch of a likely shape of the bubble is as shown in Fig. 12(a). The ESEM images (Fig. 12(b)) of topology and transmitted view of the liquid cell shows the bulging and collapse of the graphene liquid cells during as a result of the bubble's volume dynamics. An expanded bubble bulges the sheets while a shrinking bubble buckles the sheet.

Fig. 12 (a) Schematic cross-section of the graphene liquid cell.

Fig. 12(b) Correlated images taken from ESEM (Voltage 20KV) which shows the topographical change of liquid cells during bubble shrinkage (on left) and their

correlated TEM view(on right). The liquid cell is bulged where bubbles agglomerate and it buckles when the bubbles are dissolved.

3.5 Shrinkage of bubble during imaging- An example

The most common bubble dynamics ensued inside the graphene liquid cell during imaging is the shrinkage of bubbles present right from the first picture taken with the camera. Thus already 0.1s after the beam is switched on these bubbles are present. Fig. 13 shows the shrinkage of a nanobubble having an initial cross-sectional radius of ~50nm. When the beam is switched on, plenty of bubbles of different sizes can be seen and these bubbles start to shrink during imaging. The detailed mechanism of bubble shrinkage will be discussed and modelled in Chapter 4.

Fig. 13. An example of most common bubble dynamics inside the liquid cell. The shrinkage of a bubble with an initial radius of 50nm.

3.6 Thickness of liquid inside the graphene liquid cell

The thickness measurements are difficult in the graphene liquid cell due to the inconsistency of water thickness around the bubble. But an estimate of the thickness of water is possible utilizing the mass-thickness contrast quantified from the Log-Ratio method proposed by Malis et al. [29]:

$$\frac{I_{out}}{I_0} \approx \exp\left(-\frac{t}{\lambda}\right) \quad (3.11)$$

$$\text{so,} \quad t = -\lambda \ln\left(\frac{I_w}{I_b}\right) \quad (3.12)$$

$$\text{where} \quad \lambda = \frac{106F \frac{E_0}{E_m}}{\ln\left(2\beta \frac{E_0}{E_m}\right)} \quad (3.13)$$

and
$$F = \frac{\left(1 + \frac{E_0}{1022}\right)}{\left(1 + \frac{E_0}{511}\right)^2}; \quad (3.14)$$

Also,
$$E_m = 7.6 Z^{0.36} \quad (3.15)$$

Here I_{out} and I_0 are the intensities of the output and incident beam and λ is the mean free path of electrons in water and $\beta=11$ mrad is the collection semiangle. The incident energy of the electron is E_0 , Z is the atomic number of the material and E_m is the average energy loss per elastic collision. The calculated values for $E_m=13.3$ eV and the relativistic factor is $F=0.733$ and $E_0=120$ keV.

The mean free path of electron in water calculated as 132.7 nm for the existing experimental conditions. If, I_w/I_b ranges from ~0.7 to 0.8, resulting to a water film thickness $t \sim 20$ -50 nm.

3.7 Possible Effects caused by the beam-sample interaction

The interaction of the electron beam may lead to the heating of the sample, increase in pressure, and damage of the graphene structure. In this section we evaluate these effects and discuss their importance for the later analysis of the bubble dynamics.

3.7.1 Sample heating

There may be a possibility of sample heating by the beam which is caused by the energy loss of electrons due to inelastic scattering [34]. The energy loss within the sample can be estimated by the Bethe function as given below [30].

$$\frac{dE}{dx} = 2\pi Z N_0 q^4 \rho \frac{Z}{A} \frac{1}{E} \ln\left(\frac{\alpha E}{I}\right) \quad (3.16)$$

where E is the energy of the electron having charge q . And the atomic number of the material is Z and its atomic weight is A and ρ is the density of the material. Here, N is Avogadro's number. The relativistic factor is α , which is calculated as 1.16.

$$I = 9.76Z + \frac{58.8}{Z^{-1.19}} \quad (3.17)$$

where I is the mean excitation energy for energy loss in the material.

The energy loss (dE/dx) of 120 kV in water is 0.2 eV/nm and for graphene is 1.22 eV/nm. Let us assume that this energy loss may be transferred into heat. Two dimensional thermal conduction is the most likely way for heat dissipation resulted

in sample heating [31-34]. At steady state, the heat conduction equation in axisymmetry is,

$$-\kappa_g \left(\frac{d^2 T}{dr^2} + \frac{1}{r} \frac{dT}{dr} \right) = J, \quad (3.18)$$

where κ_g is the thermal conductivity of graphene and T is the temperature distribution as a function of r , where J is the heat flux density. The flow of heat is from the beam irradiated area having radius R_b to the sink at the edge of the graphene liquid cell water pocket and R_m is the carbon mesh length, which is approximately 90 μ m assuming that the sink temperature is ambient temperature which is $T = T_0$. It is also considered that $J=0$ outside the irradiated area ($R_b \leq r \leq R_m$). The boundary conditions are $T(R_m) = T_0$ and $\frac{dT(R_b)}{dr} = \frac{Q}{2\pi\kappa_g t_g}$ where R_b is the radius of the irradiated area and Q is the input heat power and t_g is the thickness of graphene. The solution of equation (3.18) gives

$$T = T_0 + \frac{Q}{2\pi\kappa_g t_g} \ln\left(\frac{R_m}{r}\right) \quad (3.19)$$

Applying boundary conditions between the irradiated and non-irradiated area we get the expression for the temperature at the irradiated area as,

$$T_b = T(R_b) = T_0 + \frac{Q}{2\pi\kappa_g t_g} \ln\left(\frac{R_m}{R_b}\right) \quad (3.20)$$

Within the irradiated area heat flux density $J = \frac{Q}{\pi t_g R_b^2} = n \left(\frac{dE}{dx} \right)$ where n is the number of electrons hitting the sample per unit area per unit volume or simply it is dose rate. So the solution for equation (3.18) gives the temperature at the irradiated area which is,

$$T = T_b + \frac{1}{4\kappa_g t_g} J_g (R_b^2 - r^2) \quad (3.21)$$

At the centre of the irradiated area, $r=0$ we are substituting the value of T_b from Eq. 3.20, we will get the graphene temperature as,

$$T_g = T_0 + \frac{Q}{2\pi\kappa_g t_g} \ln\left(\frac{R_m}{R_b}\right) + \frac{1}{4\kappa_g t_g} J_g R_b^2 \quad (3.22)$$

The heating of graphene by electron beam at different dose rate is shown in the plot given below.

Fig. 14. Plot of the temperature dependence of the graphene as a function of the electron dose rate.

Now, let us imagine that the heat flows from graphene to the liquid layer through the interface layer which is in contact with the graphene surface having thickness t_0 . Then, the heat transfer equation will be,

$$-\kappa_g \left(\frac{d^2 T}{dz^2} \right) = 0 \quad (3.23)$$

The solution of Eq. (3.23) will be the temperature of the liquid layer, which is,

$$T_w = \frac{Q}{\kappa_w A} z + T_g \quad (3.24)$$

In the assumption that the water layer transfers heat across the contact layer and the heat spreads over the thickness of water t_w and the whole water pocket which can be assumed as a micrometer sized droplet having area πR_w^2 . The heat power $Q \approx J_w t_w \pi R_w^2$. Thus the final expression for water temperature due to the heating by the beam is,

$$T_w = T_0 + \frac{J_g R_b^2}{2\kappa_g} \ln \left(\frac{R_w}{R_b} \right) + \frac{1}{4\kappa_g} J_g R_b^2 - \frac{1}{\kappa_w} J_w t_w t_0 \quad (3.25)$$

The thermal conductivity of suspended graphene is 1500-2500 W/mK [34-37] and of water is 0.6 W/mK. Using Eq. (3.22) we plot the temperature increase of

graphene as a function of the electron dose rate in Fig. 14. The graph shows that only minute heating is expected. This finding is in agreement with previous reports [31-34], i.e. where samples having a thermal conductivity of more than $0.1 \text{ Wm}^{-1}\text{K}^{-1}$ causes a rise of temperature of less than 9 K. From the calculations, the temperature difference between water layer and graphene ($T_w - T_g$) is negligibly small due its prodigious difference in thermal conductivity. Hence the possibility of sample heating from the electron energy loss can be ruled out.

3.7.2 Radiation pressure

Next we consider radiation pressure which is the pressure exerted by the electron on the sample. This pressure may act in the same way as a static pressure and accelerate the dissolution of the bubble. The radiation pressure exerted by the electron beam on the sample and is given by [34],

$$P = \frac{F}{A} = \frac{1}{A} \frac{N_{scatt} \Delta P}{\Delta t} = \frac{N_{scatt}}{A \Delta t} \sqrt{2m_e E (1 - \cos \theta)}$$

$$= \frac{N_{tot}}{A \Delta t} \left(1 - \exp\left(-\frac{t}{\lambda}\right)\right) \sqrt{2m_e E (1 - \cos \theta)} \quad (3.26)$$

where θ is the scattering angle and m_e is mass of the electron which possess an energy of 120KeV. The dose rate is $\frac{N_{tot}}{A \Delta t}$. If we assume complete back scattering that can impose maximum radiation pressure (which is an ideal case for suspended graphene), the estimated value is only 0.27Pa. This value is negligible compared to the Laplace pressure of a spherical bubble, e.g. for a radius 50nm it is several orders of magnitude larger:

$$P = \frac{2\gamma}{R} = 2.8 \text{ MPa} \quad (3.27)$$

Thus we can disregard the potential effects of radiation pressure in the case of graphene liquid cell nanobubbles.

3.7.3 Raman spectroscopy analysis of graphene radiation damage

According to literature [38-42] the crystal structure of carbon allotropes may be damaged if it is exposed to a high voltage electron beam above 80kV [43]. Above this voltage, the incoming high energy electrons are sufficient to break the bonds between the atoms and the atom can be “knocked” out of the lattice as a result of

recoil energy transfer between the atom and the electron beam [44]. As we are using high operating voltage of 120kV, which is twice as the knock-on damage threshold voltage of graphene, we would like to confirm the radiation damage of the graphene liquid cells from the beam. The knock-on damage can create defects in the crystal structure, which can also cause the shrinkage of the bubbles due to the leakage of gaseous species from this atomic defects. As it can be avoided by lower voltage imaging several of our samples were imaged at lower electron energies to confirm for similar dynamics. During our low voltage imaging with the Scanning Transmission Electron Microscope (STEM) and the TEM at 40kV and 60kV, shrinkage of bubbles were observed as shown in Fig.15 and Fig.16. It was observed that the low voltage imaging with STEM, fastened the bubble dynamics.

(a)

(b)

Fig. 15(a). Low voltage imaging at 40 kV shows the shrinkage of bubbles

(b) Low voltage imaging at 60 kV shows the shrinkage of bubbles.

All scale bars have a length of 100nm.

Fig. 16. Low voltage imaging with STEM at 60 kV shows the shrinkage and dissolution of bubbles.

Raman spectroscopy allows us to understand if the structure has indeed been damaged during imaging. We analyzed three sets of samples with Micro-Raman spectroscopy with a JYT-64000 having a spatial resolution 1mm, a lateral resolution 2mm and spectral resolution 0.2cm^{-1} located in IMRE, A-Star, Singapore. The first sets of samples (termed “untreated”) were not exposed to the electron beam or in other words, they were only transferred to the grid from the copper foil and have not been placed into the TEM. Another set was imaged only one time with the beam (“one time treated”) and the last set of samples were many times (more than 3 times) imaged (“many times treated”). The graphene on copper was also imaged to check the quality of graphene (Fig.17). All samples were imaged with Visible –Raman having a wavelength 488nm. As we can see from Fig. 17, the peaks which are activated by the defects called D-peak are present in the spectrum of graphene on the copper foil and D-peaks are present after the transfer to the grid. No clear trend as a function of imaging is seen from this visible Raman spectrum.

Fig. 17. The D and G peak in graphene spectrum that shows the defect starts from the transfer stage

In order to get a better confirmation, the samples analyzed again with UV-Raman at a wavelength of 325nm to check the significance of the D-band after multiple imaging and interestingly, the D-band became prominent after multiple imaging and it shows the sample damages more after repeated imaging as we can see from the spectrums given in Fig. 18.

Fig. 18. UV-Raman spectrum (325nm) of many times treated graphene liquid cell at three different spots in the same sample which shows the prominent D-band after multiple imaging and also it shows that the intensity of the defect is different in different regions. The defects are mostly localized.

The ratio of the intensities of G-peak and D-peak increases from the untreated sample to the many times treated sample. This shows the change in crystal structure [45]. The peak broadening is also seen in the sample and it is the indication of strain as a result of deformation of the crystal. It was also confirmed from the analysis that the graphene liquid cell has multiple layers at some points. The sharp G-peaks indicate the presence of multiple layers of graphene [46]. The peak broadening shows the deformation in the crystal structure due to strain [47].

As the defects are localized, we imaged some areas with Raman Mapping (LabRAM HR Raman instrument located in CBC-NTU) (Fig.19). From the Raman mapping, the ratio of intensities of G-peak and D-peak increases due to imaging confirming above results.

Fig. 19. Raman mapping observations of three different samples on its surface having a beam area of 80 μ m X 80 μ m. The ratio of peak intensities quantifies the average defects in the crystal structure and it shows that the graphene is not much damaged by the electron beam.

The peak intensity ratios of defect D- peak and G-peak *didn't* show sufficiently high values [42, 48] that can be considered as a serious damage in the crystal structure. So from the analysis with Raman mapping and Raman imaging, we confirmed that the defects likely do not play a crucial role for the bubble shrinkage.

3.8 Conclusions

This chapter summarizes that the shrinkage of the bubble is not caused by heating, radiation pressure or damage of the graphene. We are therefore left with a fluid mechanical mechanism which is detailed in Chapter 4.

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Chapter 4

Shrinkage, Coalescence and Stability of Nanobubbles- A pathway to the properties of nanoconfined water

4.1 Observations

4.1.1 Behavior of bubbles inside the graphene liquid cell- An overview

When the sample is loaded and the electron beam is switched on, plenty of bubbles and dynamics can be seen as in Fig. 20. As mentioned in Chapter 3, the bubbles may be created by the radiolysis reactions of the electrons with the etchant ions. Once observed, the dissolution of a bubble can happen in an average time of 50-60 seconds or less than that, depending on the dose rate. Also the exact time can depend on many factors such as diffusive shielding from nearby bubbles (Fig. 20(a) and (b)). This chapter discusses about some aspects of the bubbles in detail, such as the merger and shrinkage dynamics and their explanations in terms of diffusion constant.

(a)

(b)

Fig. 20. Nanobubble dynamics in the graphene liquid cells

- (a) It is observed that the bubbles appear once the beam is switched on and all the bubbles dissolved and disappeared in the surrounding liquid after 50s of imaging. (Scale bar 0.1 μ m)
- (b) The bubbles coalesced and the daughter bubble started to shrink during imaging. The is scale bar is 50nm.

4.1.2 Shrinkage of nanobubbles

Shrinkage of surface attached nanobubbles have been widely studied with AFM [1-3]. Unlike AFM, the possibility of surface pinned nanobubbles may not be valid in the case of TEM nanobubbles. In most of the samples imaged with TEM,

shrinkage is widely observed. The radius of the bubble decreases with time and at last the bubble dissolves into the surrounding liquid (Fig. 21).

(a)

Fig. 21. A bubble having radius $\sim 20\text{nm}$ took 6.5s for complete shrinkage. The scale bar is 50nm.

4.1.3 Stability of the bubble in the absence of the beam

According to Young-Laplace equation, internal pressure of a bubble is inversely proportional to its radius. So a spherical bubble of radius 10 nm should dissolve into the solution within nanoseconds [4]. Contrary to the theoretical explanation, nanobubbles nucleated inside the liquid cells are stable for hours in the absence of the beam. In order to check the stability, the nanobubble was imaged for 25s and observed that the bubble get shrunk by the beam. Then, the sample was left inside the TEM while the beam was switched off for one hour. After one hour, switched on the beam and found that the bubble didn't dissolve in the liquid, but very slight shrinkage was observed (Fig. 22). When starting to image it again, the bubble resumed its former behaviour and dissolved into the liquid within 20s. This behaviour of the bubble confirms that the beam interaction drives the shrinkage.

Fig. 22. The stability graph of a bubble. The bubble imaged for 25s and left inside the TEM for 1hour without the beam and imaged again for 20s.

4.1.4 Coalescence of nanobubbles

Another widely observed class of bubble dynamics within the liquid cells are coalescence of nanobubbles. In general, bubble coalescence means, when two bubbles come closer, they merge together to form a single bubble (Fig 23(a) and (b)). It is remarkable that the high resolution of graphene liquid cell images permits to measure the merger bridge thickness even at very low values (Fig 23 (b)).

(a)

(b)

12

Fig. 23. The coalescence of Nanobubbles

- (a) The coalescence of nanobubbles at a time scale of 0.1s. The scale bar is 5nm
- (b) The coalescence of nanobubble at a time scale of 0.1s. The bridge formation between the bubbles and the widening of the bridge is clearly visible.

4.2 Analysis of shrinkage and stability in terms of diffusion constant of the bubble

4.2.1 Effective viscosity of confined water

Three distinct phenomena are observed in liquid cells:

1. Slow coalescence of nanobubbles.
2. The shrinkage of bubble in presence of the beam
3. Mostly stable (slowly shrinking) bubbles in the absence of the beam.

From the above observations (Fig. 23), the merging of the bubbles seems to be rather slow, as if the gas bubbles are merging with each other within a highly viscous medium. Some previous studies already reported that the viscosity inside the TEM liquid cells is higher than its bulk counterparts [5, 6]. As the dynamics of the coalescence of the air bubbles are well understood, the effective viscosity of the confined liquid inside the liquid cell can be estimated using the merging of nanobubbles. Let us assume that the merger between the bubbles in the liquid cell confined system is dominated by a capillary-viscous balance. The merging process is initiated by the formation of a high-curvature nanometer sized neck and the radius of the neck r is smaller than the radius of the merging bubble R_0 . The Laplace pressure of the curved gas-liquid interface is given by,

$$p \sim \frac{2\gamma R_0}{r^2} \quad (4.1)$$

where γ is the surface tension.

As the confined water is much more viscous than the bulk water, let us assume that the merger of the bubbles in the liquid cell are dominated by capillary-viscous balance.

So the viscous stress at the interface is,

$$p_s \sim \eta \frac{\partial u}{\partial r} \sim \frac{\eta}{r} \frac{dr}{dt} \quad (4.2)$$

where η is the viscosity

Balancing the two equations leads to,

$$r \frac{dr}{dt} = \frac{2\gamma R_0}{\eta}. \quad (4.3)$$

And the solution of this differential equation gives

$$\frac{r^2}{R_0} = \frac{2\gamma}{\eta} t, \quad (4.4)$$

$$\frac{r}{R_0} = \sqrt{\frac{2\gamma R_0}{\eta}} t^{\frac{1}{2}} = k \sqrt{\frac{t}{\tau_m}}, \quad (4.5)$$

Where, R_0 is the radius of the merging bubble, k is a constant of order 1. Where $\tau_m \equiv \frac{\eta R_0}{\gamma}$ is the time scale for merging. The merging of millimeter sized gas bubbles [7], in liquids having varying viscosities agrees with equation (4.4) in the viscosity limits (the viscous relation $\frac{r}{R_0} \sim \sqrt{\frac{t}{\tau_m}}$ holds very well in the limit of very viscous liquids ($\eta > 0.1$ Pa s). So the viscosity of the liquid in a TEM environment can be estimated with the viscous time scale of merger bubbles. For a merger timescale of 1-2 frames, we have $\tau \sim 0.1$ s. For a bubble with size ~ 10 nm, having surface tension $\gamma = 72 \times 10^{-3} \frac{N}{m}$, so the effective viscosity will be,

$$\eta_T \sim \frac{R_0 \tau}{\gamma} \sim 7 \times 10^5 \text{ Pa s} \quad (4.6)$$

The estimated viscosity is almost 8 orders of magnitude larger than the bulk value of the viscosity of water. According to recent literatures [3] about the TEM study of gold nanoparticles in 30 nm thick layers of water reported the enhancement of viscosity indirectly by measuring the mean-squared displacement of gold nanoparticles under the electron beam. In that study the viscosity was inferred to be about 2×10^6 Pa s, which is very close to our estimate of 7×10^5 Pa s. Compared to the method of Lu et al [5], the estimate in our study is a direct measure of viscosity from the liquid properties.

4.2.2 Measurement of diffusion constant

From the merger time scale, the effective viscosity inside the liquid cells are estimated. Now, let us consider the contrast behavior of the bubble in presence and absence of the beam. The stability of the bubble in the absence of the beam may be due to the viscosity enhancement. Let us consider the beam induced shrinkage of the bubble.

Consider a Brownian particle having radius R' propagating within a liquid.

According to Stokes-Einstein relation,

$$D = \frac{k_B T}{6\pi\eta R'}, \quad (4.7)$$

Where η is the dynamic viscosity of the liquid and R' is the radius of the particle.

If we assume that the Stoke-Einstein relation is valid for confined water too, the diffusion constant of confined water will be reduced by the enhanced viscosity. The diffusion constant of water will be,

$$D_c = \frac{\eta_b D_b}{\eta_c} \quad (4.8)$$

Where the dynamic viscosity of bulk water is η_b and D_b is the diffusion constant of bulk water and η_c is the dynamic viscosity of confined water.

After substituting the values, the diffusion constant of confined water was obtained as $10^{-18} \text{ m}^2 \text{ s}^{-1}$ which is nine orders smaller than the bulk diffusion constant of bulk water which is $D_b = 2 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$.

4.2.3 Stability of the bubble in the absence of the beam

The stability of the bubble in the absence of the beam is also explained with the diffusion constant. Using the equation 4.8, it can be calculated that the diffusion constant D of the bubble during switch off time as $\sim D_{off} = 10^{-18} \text{ m}^2 \text{ s}^{-1}$ and the shrinkage rate of a bubble having radius of 20nm (R_0) will be estimated as $0.85 R_0$ after 30 minutes of switch-off time and $0.7 R_0$ for an hour of switch-off time. This estimate also agrees with the experiment (Fig. 24)

Fig. 24. Stability data of nine bubbles which are imaged and their switch off time Δt is plotted against the ratio of foot print areas A_0 (before switch-off) and A (after switch-off). There is not so remarkable change in area after switch off time Δt .

4.2.4 Diffusion constant variation with and without the beam

Consider two bubbles having initial radius R_0 of 20nm and 15nm. The diffusion constant for the two bubbles are estimated as $D_{on} = 1.5 \times 10^{-16} m^2 s^{-1}$ and $D_{on} = 1.75 \times 10^{-16} m^2 s^{-1}$. There is also good agreement with theory and experiments (Fig 25). From the figure it is observed that the smaller bubble (red) shows good agreement than the bigger one may be due to the thickness estimate is not much accurate due to the inconsistency of water layer and also due to the flexibility and movement of the graphene membrane during imaging causes some effects in the gas- liquid interface .

Fig. 25. Radius-time curve for two nanobubbles having initial sizes 20nm and 15nm. The dot lines represents the experimental data and the solid line represents the fitting for $D_{on} = 1.5 \times 10^{-16} m^2 s^{-1}$ (red) and $D_{on} = 1.75 \times 10^{-16} m^2 s^{-1}$ (blue).

The diffusion constant during the imaging D_{on} is almost two orders of magnitude higher than D_{off} (beam is OFF). Most likely, this change may arise due to the

variation of Brownian mobility of the particle by the irradiation. During the switch-OFF stage, the viscosity is dominated the mobility of the Brownian particles and in the presence of the beam (during imaging) the electric field becomes dominates over the viscosity. The fast dissolution and coalescence of the bubble by high dose rate also supports this hypothesis.

The drift velocity of the gas molecules which are charged is given by,

$$v_d = \mu E = \frac{\mu V}{d} \quad (4.9)$$

If we assume that the electric field length scale d of 10nm and an electric field of $10^{-7}V^{-1}m^{-1}$ the mobility will be,

$$\mu = \frac{D_1}{k_B T} \sim 10^4 m^2 V^{-1} s^{-1} \quad (4.10)$$

Thus the effective mobility will be of the order of $10^{-3}m^2V^{-1}s^{-1}$ which is several magnitudes higher than the bulk water.

4.3 Discussion

The properties of water confined to the nanoscale is drastically different from that in the bulk. Plenty of literature available about the variations in basic properties of nanoconfined liquids. Especially, the viscosity of nanoconfined water is elevated some orders of magnitude than bulk water is widely reported. [8-12]. There are stronger intermolecular forces and local electric field inside all polar liquids unlike non-polar liquids. So the ordering of water molecules occurs at the interface due its change in intermolecular bonding [13-16] unless the water does not contain any salts (pH neutral) and the solid surface which is in contact with the liquid has to be smooth [17]. Sedner et. al. [18], reports that only hydrophilic confinement causes viscosity enhancement, which is not present in the case of hydrophobic surfaces. As far as the presence of etchants in the liquid cells are confirmed and the non-uniformity of the free standing graphene surface [19], the possibility of viscosity enhancement of water trapped between the graphene layers are valid. The hydrophobicity of graphene is still a question and the multiple layer formation [20] and the presence of electric field can alter the hydrophobicity of graphene [21]. So in effect, a lot of factors which supports the viscosity changes and unusual behavior of nanobubbles inside the liquid cells.

The theoretical calculations are in good agreement with the experimental results which supports the argument that the viscosity is enhanced in nanoconfined liquid

in a TEM environment which stabilises the bubble in the absence of the beam. During imaging, the Brownian motion induced by the electric field comes to play and causes the shrinkage of the bubble. The origin of high viscosity arises due to the special conditions of TEM imaging. The graphene liquid cells are prepared in atmospheric pressure and are imaged in high vacuum conditions, which may create strong pressure and hence forces in the liquid cells which may enhance the viscosity of the liquid. Zangi and Mark [22] reported that under a strong directed force, monolayer water become packed into ice. The liquid cell inside the TEM may also be experience analogous force.

4.4 Future works

In addition to shrinkage and coalescence, some other interesting bubble dynamics observed inside the liquid cells during the whole study. They are liquid thin film retraction (bubble rupture), bubble oscillation and non-coalescence of nanobubbles. Most of this dynamics were not so frequent during imaging. So it is too hard to explain due to the limited availability of the results, even though, a future study about the observations can unfold the mechanism or it can support the viscosity enhancement which is already explained by the slow merger.

4.4.1 Liquid thin film retraction (Rupture of bubble)

The study of thin film rupture is relevant in Colloidal and Biophysics. During the imaging of TEM nanobubbles, the thin films rupture processes were observed and the film retraction was appeared as slow as compared to the bulk water (Fig.26). The most probable reason may be the enhanced viscosity of confined water, as explained in the former chapter. More results needed for a better outlook.

Fig. 26. The liquid thin film retraction inside the liquid cell. The whole process took 0.56s for a complete retraction. The scale bar is 20nm.

4.4.2 Bubble Oscillation

Bubble oscillations were observed in two ways. In some cases, bubbles oscillates at the initial stages of imaging and then they started to shrink. But in another case, they continuously oscillated for a short period time of approximately 60s (Fig. 27). The oscillation of the bubbles and the reason for its shrinkage needs a further study as it can see often in some of the samples.

Fig. 27. The oscillation of the bubbles at different time periods. The scale bar is 50nm.

4.4.3 Non-Coalescence of Nanobubbles

Evenif, the bubble coalescence is widely observed in almost every sample, non-coalescence is also present (Fig. 28). Why some bubbles are coalescing and some not, in the same conditions and the proximities is still in question. More analysis is needed for a better outlook. Some former studies [23]reported non coalescence of bubbles in presence of electric field. Ristenpart et.al. detailed a critical electric field strength above which the bubbles do not coalesce. But in graphene liquid cells, under the same electric field strength, some bubbles get coalesced and some does not. This result is really confusing and more detailed study is needed to make some possible hythpothesis.

(a)

(b)

Fig. 28. Non-coalescence of the bubbles.

- (a) The bigger bubble dissolved and the other still persists in the almost same size as the beginning. The scale bar is 50nm. A slight displacement of the undissolved bubble can be seen.
- (b) The Bigger bubble dissolved and smaller one exists almost in the same size. The Scale bar is 50nm

4.5 Conclusion

The stability of nanobubbles in the absence of the beam and its shrinkage in the presence of the beam were the contradictory results observed throughout the experiments along with the observations of slow coalescence. The enhanced viscosity of the liquid which is estimated from the coalescence time scale, stabilizes the bubble in the absence of the beam. The variation of diffusion constant during switch ON and switch OFF, affirms the changes in the dominating factor due to the presence of the beam which is the electric field over viscosity.

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Chapter 5

Conclusions and Outlook

5.1 Conclusions

In this work liquid cell Transmission Electron Microscope (TEM) imaging has been used to study nanobubbles. The method used in this thesis is a high-resolution, in-situ method. Using graphene liquid cells, various bubble dynamics were imaged with the TEM and discussed. Chapter 1 introduced nanobubbles, their significance and methods in TEM. Chapter 2 elucidates the necessities of liquid cells for TEM imaging, types of liquid cells and their preparation. It also introduces various methods used in the experimental chapters such as Scanning Electron Microscope (SEM), ESEM (Environmental Scanning Electron Microscope), EDS (Energy Dispersive X-Ray Spectroscopy) and EELS (Electron Energy Loss Spectroscopy).

In Chapter 3, the observations of nanobubbles are reported; we suggest a possible reaction pathway for radiolysis of the etchant to produce gas in the nanobubbles. The presence of various gaseous species was studied with EDS and EELS spectroscopy. The topology of graphene studied with ESEM and the plausible morphology of the bubble also explained. Beam-sample interaction and other associated effects like charging, radiation pressure, sample heating, and radiation damage of graphene were discussed and it is concluded that none of these play a significant role for the dynamics of nanobubbles. Bubble oscillations and the slow retraction of liquid film were documented.

In Chapter 4, we clarified that the bubbles are likely nucleated by the radiolysis of water and etchant by the beam and these bubbles persist over a timescale of ~ 10 s, though the exact time depends on the dose rate and the diffusive shielding from the neighbouring bubbles. The bubbles were stable at least for an hour in the absence of the beam. The physical explanation of bubble dynamics with the diffusion constant and enhanced viscosity is done and concluded in Chapter 4. From the timescale of merging between bubbles, it was found that confined water within the graphene liquid cell has an apparent viscosity that is about 8 orders larger than the bulk value. Apart from slowing down the dynamics of merger, the large viscosity also stabilises the bubble against diffusion. We find that the diffusion constant switches to a value about two orders of magnitude larger in the presence of an electron beam. A likely explanation has been put forward that this variation occurs due to a change in the diffusion constants. The electric field created by the beam

affects the Brownian mobility of the particle and resulted in the increase of diffusion constant two orders of magnitude higher than that of the absence of the beam.

5.2 Outlook

Imaging graphene liquid cells with TEM provides excellent spatial and temporal resolution to study the dynamics of liquids, gases and solids at the nanoscale. As in-situ imaging of confined water is still very new, there are many nanoscale biological and physical phenomena which can be explored using this technique. The confined liquids imaged with TEM can also be used to study the reaction chemistry inside the TEM as a result of beam-sample interaction. I like to propose some studies for the future.

1. Design a radiolytic model for detailed analysis of the decomposition of liquid samples during imaging, as the radiolysis plays a significant role in the study.
2. A detailed study about various novel nanobubble dynamics such as thin film retraction and bubble oscillations within the liquid and its physical explanations.
3. As the radiolysis plays a vital role in bubble dynamics, it will be interesting to image various liquids having variable pH values and study their radiolytic reactions.
4. The colloidal samples like gold can also be used for imaging to study about the colloidal assembly inside the graphene liquid cells.
5. The mechanical strength and properties of boron nitride is close to graphene. So graphene can be replaced by boron nitride to prepare the nanoscrolls and trapped, confined liquid dynamics can be studied with such a system too to check the variations in morphology and fluid dynamics.

Appendices

The Environmental Transmission Electron Microscope (ETEM) is a complex device. The various parts of the ETEM can be divided from top to bottom as given below.

2.1.1 The electron gun

At the top the electron gun is the source of electrons for the sample illumination. It is one of the most significant components of the TEM. Nowadays, two types of electron guns are available and chosen depending on the imaging requirements: thermionic guns and Field-Emission Guns (FEG). Thermionic sources are based on lanthanum hexaboride (LaB_6) crystals or tungsten filaments, while in FEGs, filaments are tungsten needles. Thermionic sources are electron producers by heating and FEGs produce electrons due to the large potential difference between the cathode/electron gun and an anode. The FEI Tecnai T12 is equipped with a LaB_6 filament, see Fig. I(a). This electron gun is able to emit electrons with a minimum voltage of 20keV and a maximum of 120 keV. The advantage of this filament is its high melting point (2210°C) so it is stable at high temperature. Because of its low work function of 3eV of the material it delivers a high electron emissivity.

Fig. I. (a) Electron gun along with Wehnelt and anode.

(b) Electron source of filament

The thermionic gun/ triode/self-biasing gun consists of three parts as shown in Fig. I. The main parts are the cathode or filament, Wehnelt and anode. The cathode or

filament is a LaB_6 crystal which is bonded to a rhenium metal wire that is resistively heated to produce thermionic emission. Wehnelt is a biased grid, which is more negative than the gun, so that the difference in potential helps the beam to cross over at the anode hole. This crossover acts as the electron source for the optics within the microscope. The filament current controls the beam current.

2.1.2 The lens system

In 1926, Hans Busch successfully focussed electrons using electromagnetic lens and that was incorporated by Ernst Ruska in his first TEM. These electromagnetic lenses consist of a magnetic circuit and an electric circuit, as shown in Fig. II. The electric circuit consists of a coil through which electric current flows and the magnetic circuit consist of a piece of specifically shaped magnetic alloy. The flow of current through the coil generates a magnetic field. Due to the interruption in this circuit by the gap creates a lens field that is used to focus the electron beam. The shape of the pole pieces is the determining factor of efficiency of the lenses. The magnetic field can be changed by the current through the coil. A separate water circulation system provides the necessary cooling for the lens coil. Although the constructions of lenses for electrons are very much different from optical lenses both can be described through very similar equations [1].

Fig. II. The structure of an electromagnetic lens

The lens arrangement in a TEM is indicated in Fig. III. The Condenser Lens (CL) is the first lens that is utilized to focus the beam to the specimen in by collecting and focusing the beam. This lens controls the spot size on the specimen. Typically, TEM systems incorporate double condenser lenses (C1 and C2) for better contrast and high coherence. Once the beam has passed the condenser lens, the electron path is controlled by the condenser aperture (CA). Its purpose is to protect the sample from excessive irradiation (in case of sensitive samples). The CA also filters electrons that are scattered into high angles, thus reducing spherical and chromatic aberrations in the later imaging step.

Fig. III. The optical components inside the TEM.

The image magnification system consists of 5 lenses; in the order of the electron path they are the objective lens, diffraction lens, intermediate lens, and projector lenses 1 and 2. The objective lens magnifies and focuses the image. This lens is close to the sample and creates the initial image. The image quality is determined by the focussing and the alignment of the objective lens, as the objective lens controls the resolving power of the TEM. At lower magnifications, the objective lens is not used, and the diffraction lens is used instead. As with the condenser lens, the objective lens is also combined with an aperture to avoid imaging aberrations.

2.1.3 The sample stage

A typical TEM sample holder is shown in Fig. IV. A TEM sample must be about 3 mm in diameter and should be less than 80 μm in thickness to fit into the holder. As the TEM operates in ultra-high vacuum, the sample holder includes an airlock. The sample holder insertion and removal requires special attention, in order to keep the pressure level inside the column. The specimen chamber is connected to a cold trap. The cold trap is a metal piece cooled with liquid nitrogen. During the insertion of the holder, the gaseous products and water vapour in the sample holder condense on the cold surface and their partial pressures is reduced to prevent contamination in the vacuum system from the sample.

(a)

(b)

(c)

Fig. IV. (a) The image of a TEM sample holder

(b) The structure of a sample holder

(c) The sample clamp and needle

2.1.4 The vacuum system

The TEM operates at very low pressure in order to avoid the unwanted scattering of electrons from residual gas to maximize their mean free path. Generally, the operating pressure within the column is around 10^{-5} Pa. To evacuate air, the TEM incorporates a comprehensive vacuum system with a number of pumps, vessels, gauges and valves. Vacuum system inside the TEM can be divided as two categories, according to the grade of vacuum and the variety of pumps used to achieve a desirable vacuum level for the system. The specimen and gun areas have to be clean and must be free from all kinds of contamination. Air from the upper half of the TEM column is evacuated by an ion-getter pump to provide high vacuum conditions for the gun and specimen stage. The lower half of the TEM is equipped with an oil-diffusion pump and a rotary pump, which provide considerably lesser vacuum than the ion-getter pump. The rotary pump provides preliminary evacuation to 0.1 Pa and serves as a backup for other pumps. The oil diffusion pump provides stronger evacuation, by decreasing the pressure to 10^{-5} Pa.

2.1.5 The fluorescent screen

The image formation in TEM is the result of the beam-sample interaction. The transmitted electrons form images on the fluorescent screen. Dark regions indicate

stronger absorption of electrons, while bright regions indicate lesser absorption of electrons.

2.1.6 The camera system

The camera (Gatan CCD camera) is fixed below the fluorescent screen and is read out by a computer. By lifting the screen, an image forms can be recorded and analyzed with the Gatan digital micrograph software. The videos are saved using screen recording with the Virtual Dub [2] software.

References for Appendices

1. Reimer, L. (2013). *Transmission Electron Microscopy: Physics of Image Formation and Microanalysis* (Vol. 36). Springer.
2. Lee, A. (2001). Virtual Dub home page. *URL: www.virtualdub.org/index*.