Submission ID #: 62225
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18978673

Title: Studying the Effects of Temperature on the Nucleation and Growth of Nanoparticles by Liquid-Cell Transmission Electron Microscopy

Authors and Affiliations: Abdelali Khelfa, Jaysen Nelayah, Guillaume Wang, Christian Ricolleau, Damien Alloyeau

Laboratoire Matériaux et Phénomènes Quantiques, Université de Paris, CNRS

Corresponding Author:
Damien Alloyeau
damien.alloyeau@univ-paris-diderot.fr

Co-authors:
abdelali.khelfa@gmail.com
Jaysen.nelayah@univ-paris-diderot.fr
guillaume.wang@univ-paris-diderot.fr
Christian.Ricolleau@u-paris.fr

© 2021, Journal of Visualized Experiments

Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured video files to your project page as soon as possible.

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **27**

© 2021, Journal of Visualized Experiments

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Damien Alloyeau**: Temperature control is a recent development that provides an additional degree of freedom in studying nanochemistry by liquid cell transmission electron microscopy, notably the formation of gold nanoparticles in solution **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Abdelali Khelfa**: This methodology allows imaging of the dynamics of individual nanostructures in liquid with great control over the composition and temperature of the environment under realistic synthesis conditions **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: last 2 ones

OPTIONAL:

1.3. **Damien Alloyeau**: Interestingly, this method can be used to study the effects of temperature on the structural evolution of soft, hard, or biological nano-objects in liquid environments by mimicking their formation and application medium **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: one before last

OPTIONAL:

1.4. **Abdelali Khelfa**: The key success factors for liquid TEM experiments are a clean sample preparation and a consideration for the electron beam effects on the nanoparticle dynamics **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

© 2021, Journal of Visualized Experiments

Protocol

2. Liquid Cell (E-Chip) Preparation

2.1. For liquid cell preparation, first fill one glass Petri Dish with acetone and another with methanol in a fume hood **[1]**.

2.1.1. WIDE: Talent filling dish with acetone and/or methanol, with dishes and acetone and methanol containers visible in frame NOTE: take 2

2.2. Place one small and one large E-chip into the acetone for 2 minutes **[1]** before moving both chips into the methanol for 2 minutes **[2]**.

2.2.1. Talent placing chips into acetone, with acetone container visible in frame
2.2.2. Talent placing chips into methanol, with methanol container visible in frame

2.3. After the methanol wash, use an air pistol and tweezers to dry the cells **[1]** and use a binocular magnifier or an optical microscope to verify the integrity of the silicon nitride window **[2]**.

2.3.1. Chip being dried
2.3.2. LAB MEDIA: Figure 2 *Video Editor: please emphasize Figure 2A chip image*

2.4. If the chips are intact, plasma clean the E-chips with a mixture of argon and oxygen gas for 2 minutes **[1-TXT]** and load the gasket O-rings into the liquid cell holder **[2]**.

2.4.1. Talent starting plasma clean **TEXT: See text for plasma cleaning setup details**
2.4.2. Talent loading O-rings into holder NOTE: take 2

2.5. Place the small E-chip into the liquid cell holder **[1]** and drop approximately 2 microliters of the liquid sample of interest onto the chip **[2-TXT]**.

2.5.1. Talent placing chip into holder *Videographer: Important step* NOTE: take 3
2.5.2. Sample being added to chip *Videographer: Important step* **TEXT: e.g., 1 mM $HAuCl_4$ in H_2O** NOTE: take 2

2.6. Using a sharply cut piece of filter paper, remove any excess liquid from the chip until the liquid droplet forms a flat dome **[1]** and place the big E-chip onto the small E-chip front side facing down **[2]**.

2.6.1. Liquid being wicked *Videographer: Important/difficult step* NOTE: take4,

© 2021, Journal of Visualized Experiments

including 2.5.2

2.6.2. Chip being placed *Videographer: Important/difficult step*

2.7. Slide the lid back onto the liquid cell holder **[1]** and gradually tighten each screw **[2]**.

2.7.1. Talent sliding lid *Videographer: Important/difficult step* NOTE: take 2
2.7.2. Talent tightening screw *Videographer: Important/difficult step*

2.8. Use filter paper to remove any excess liquid from the chips, rotating the liquid cell holder around its axis to make sure that all of the liquid is captured **[1]**.

2.8.1. Liquid being removed *Videographer: Important step*

2.9. Test the vacuum sealing of the liquid cell in a pumping station **[1]**. If the vacuum level of the pump reaches 5 x 10^{-2} Pascals, verify the integrity of the silicon nitride window one last time **[2]** and load the liquid cell holder into the microscope **[3]**.

2.9.1. Talent testing seal NOTE: MED and CU
2.9.2. Talent checking integrity
2.9.3. Talent loading cell into microscope

3. Flow Mode Setup

3.1. To set up the flow mode, load one syringe with the solution of interest **[1]** and connect two external PEEK (peek) tubes to the syringe **[2]**.

3.1.1. WIDE: Talent loading syringe, with solution container visible in frame
3.1.2. Talent connecting tube(s) to syringe

3.2. Place the syringe onto the syringe pump **[1]** and insert the external PEEK tubes into the entries of the liquid cell holder **[2]**. Insert one additional external PEEK tube for the output of the liquid cell holder **[3]**.

3.2.1. Talent placing syringe onto pump
3.2.2. Talent inserting tube(s) NOTE: take1, MED, take2, CU
3.2.3. Talent inserting additional tube

3.3. Then inject the solution into each inlet at a flow rate of 5 microliters/minute **[1]**.

3.3.1. Solution being injected

4. Liquid Environment Heating

© 2021, Journal of Visualized Experiments

4.1. To heat the liquid environment, open the **Heating** software and power up the power supply **[1]**.

4.1.1. WIDE: Talent opening software, with monitor visible in frame NOTE: CU at end

4.2. Click the **Device Check** button and open the **Experiment** tab. Click **Manual** to activate the manual heating mode and select the targeted temperature to change the temperature rate as appropriate to the experiment. Then click **Apply** to heat the E-chips to the targeted temperature **[1]**.

4.2.1. SCREEN: vid4.2.1.

5. High-Angle Annular Dark-Field-Scanning Transmission Electron Microscopy (HAADF-STEM) Imaging and STEM Single Nanoparticles Nanodiffraction

5.1. To image the radiolysis-driven formation of gold nanoparticles with a good signal to noise ratio **[1]** in STEM HAADF *(stem H-A-A-D-F)* mode, identify a pristine area of the sample near a corner of the observation window in which the liquid thickness is at a minimum **[2]**.

5.1.1. WIDE: Talent at computer, locating area
5.1.2. SCREEN: vid5.1.1. *Video Editor: only enough action as necessary for VO*

5.2. Note the imaging conditions, including the spot size, the condenser aperture size, and the magnification to allow subsequent calculation of the electron and cumulative electron doses irradiating the analyzed area **[1]**.

5.2.1. Conditions being noted in experience notebook

5.3. Then acquire videos of the nanoparticle growth at different temperatures using the same imaging conditions **[1]**.

5.3.1. LAB MEDIA: 5.3_image_diffraction.png

5.4. For single nanoparticle nanodiffraction, acquire a STEM HAADF image of several nano-objects **[1]** and use STEMx software to acquire the diffraction pattern of individual nanoparticles within the image **[2]**.

5.4.1. SCREEN: vid5.4.1_selection_diff.avi
5.4.2. LAB MEDIA: Figures 6B and 6C

Results

6. Results: Representative Temperature Effects on Nanoparticle Nucleation and Growth as Observed by Liquid-Cell Transmission Electron Microscopy (TEM)

6.1. As observed in these two STEM HAADF image series **[1]**, the growth of a very dense assembly of small nanoparticles can be observed at low temperatures **[2]**, while at high temperatures, a few large and well facetted nanostructures are obtained **[3]**.

6.1.1. LAB MEDIA: Figure 5
6.1.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize top row of images*
6.1.3. LAB MEDIA: Figure 5 *Video Editor: please emphasize bottom row of images*

6.2. As the contrast of STEM HAADF images is proportional to the gold nanoparticle thickness, two populations of objects formed during these growth experiments can be observed **[1]** - highly contrasted 3D nanoparticles **[2]** and large 2D nanostructures with a triangular or hexagonal shape and a lower contrast **[3]**.

6.2.1. LAB MEDIA: Figure 5
6.2.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize red arrows/nanoparticles indicated by red arrows in top 80.01 s image*
6.2.3. LAB MEDIA: Figure 5 *Video Editor: please emphasize red arrows/nanoparticles indicated by red arrows in bottom 80.01 s image*

6.3. Automated video processing as demonstrated in this method allows measurement of the nucleation and growth rates of nanoparticles **[1]**.

6.3.1. LAB MEDIA: Figure 8

6.4. At low temperatures, more than 800 nanoparticles are formed within in a few tens of seconds of observation **[1]**, while only 30 nanoparticles are formed in the same amount of time at a high temperature **[2]**.

6.4.1. LAB MEDIA: Figure 8 *Video Editor: please emphasize red and blue data lines*
6.4.2. LAB MEDIA: Figure 8 *Video Editor: please emphasize yellow data line*

6.5. Conversely, the mean surface area of the nanoparticles **[1]** increases 40 times faster at 85 degrees Celsius than at 25 **[2]**.

6.5.1. LAB MEDIA: Figure 9 *Video Editor: please emphasize 85 °C data line*
6.5.2. LAB MEDIA: Figure 9 *Video Editor: please emphasize 25 °C data line*

© 2021, Journal of Visualized Experiments

6.6. Here the diffraction pattern of two gold nanoparticles that have been selected directly from a typical STEM image can be observed **[1]**. The face-centered cubic structure of gold oriented along the 001 **[2]** and 112 zone axes can be identified **[3]**.

6.6.1. LAB MEDIA: Figure 6A *Video Editor: please emphasize red arrows*
6.6.2. LAB MEDIA: Figure 6 *Video Editor: please emphasize Figure 6B*
6.6.3. LAB MEDIA: Figure 6 *Video Editor: please emphasize Figure 6C*

© 2021, Journal of Visualized Experiments

Conclusion

7. Conclusion Interview Statements

7.1. **Damien Alloyeau**: Studying the effects of temperature on the nucleation and growth of nanoparticles requires a comparison of videos acquired with the same electron dose rate, as radiolysis has an impact on nanoparticle formation **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (5.1., 5.2.)

7.2. **Abdelali Khelfa**: Ex situ SEM or TEM characterizations can be performed after unsealing the liquid cell to further analyze nano-object structures **[1]**.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

7.3. **Damien Alloyeau**: Temperature controlled LCTEM provides an opportunity to investigate the effects of temperature on the many other chemical reactions that occur at the interface between solids and liquids, opening many avenues in material, life, and earth sciences **[1]**.

7.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

© 2021, Journal of Visualized Experiments