1440
doi:10.1017/S1431927622005864
Microsc. Microanal. 28 (Suppl 1), 2022

Advanced In-situ Liquid Phase Transmission Electron Microscopy: A Powerful Tool for Pharmaceutical Studies and Life Science Applications

Hongyu Sun[1], Cliff Mathisen[2], Eva Bladt[1], and H. Hugo Pérez-Garza[1]*

[1.] DENSsolutions B.V., Delft, Zuid Holland, The Netherlands
[2.] Nanoscience Instruments, Alexandria VA, USA
* Corresponding author: hugo.perez@denssolutions.com

We introduce our technology for in-situ liquid phase studies inside the Transmission Electron Microscope. The system relies on a Micro Electro-Mechanical System (MEMS)-based device as a smart sample carrier, which contains an integrated set of biasing electrodes (i.e. to perform liquid biasing, or bio-electrochemical studies) or an integrated microheater (i.e. to perform liquid heating experiments). These capabilities enable in-situ pharmaceutical and life science studies.

Conventionally, the TEM studies are limited to work under static conditions. Similarly, liquid phase studies have been experiencing considerable limitations. When working within these environments, the uncontrolled liquid layers affect the imaging resolution and hinder analytical techniques such as electron diffraction, EDS or EELS. Furthermore, controlling the microfluidic environment around the sample (i.e. pressure, flow rate) has proven to be extremely challenging, and the interaction of the electron beam with liquid results in the formation of several chemical radicals that are known to attack your biological sample.

In order to provide meaningful results and address these historical challenges, our MEMS device controls the flow direction and ensures the liquid containing the sample (e.g. proteins, vesicles, viruses, etc) will always pass through the region of interest (i.e. where the electron transparent window is) preventing the liquid-air interface, making it a powerful tool for single particle analysis. Similarly, the device contains chemical scavenging capabilities to mitigate the effect of the chemical radicals. Furthermore, the developed system offers the opportunity to define the mass transport and control the biokinetics of the reaction, as well as the unique opportunity to control your biochemistry on-the-fly.

The system allows to control the liquid thickness well below the beam broadening threshold, enabling resolutions that can go even down to 2.15 Å [1], as it has been reported before for a 100nm liquid thickness. Such control of the liquid thickness enables elemental mapping, allowing users to distinguish the spatial distribution of different elements in liquid. We believe that our developments will play a fundamental role in addressing many of the research questions within life science. Furthermore, it will provide unique insights into the biochemistry that governs certain reactions, as well as the unique possibility to visualize biological processes in real time with angstrom resolutions, without the need of vitrifying the biological specimen [2, 3].

The system provides users with the capability to visualize exciting dynamics (i.e. biological processes) in the biosample´s own liquid native environment as a function of different stimuli. This opens up several possibilities such as in-vivo studies of protein-protein interactions and single particle analysis, pharmaceutical studies [4, 5] and fundamental studies in biophysics and biochemistry [6, 7]. We believe that this technique will become a very powerful complementary technique to cryo electron microscopy.

Figure 1. Plug-and-play system for Liquid Phase TEM. In order to enable meaningful experiments, the Nano-Cell (a MEMS-based sample carrier) is inserted into the liquid holder. Once inside the TEM, the user can accurately control pressure, flow rate, liquid thickness using the Liquid Supply System.

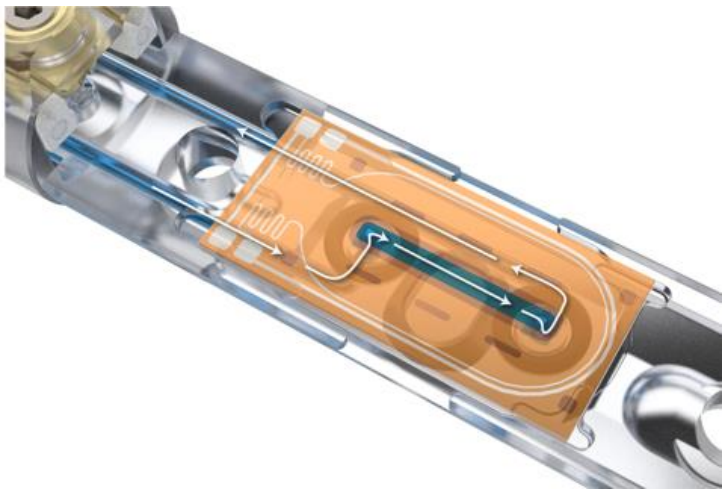

Figure 2. Well-defined microfluidic channel. When flowing the liquid, it will enter the Nano-Cell via an inlet and the liquid (depicted in blue) will flow through the well-defined microfluidic channel, existing via the outlet. Particularly for life science and pharmaceutical applications, this capability enables high throughput and avoids the liquid-air interface.

https://doi.org/10.1017/S1431927622005864 Published online by Cambridge University Press

References:

[1] A F Beker et al, "*In situ* electrochemistry inside a TEM with controlled mass transport" Nanoscale, 12, 22192-22201 (2020)
[2] G Battaglia et al, "4D imaging of soft matter in liquid water" (2021),
DOI: 10.1101/2021.01.21.427613
[3] L Ruiz-Pérez et al, "Imaging protein conformational space in liquid water" (2021), DOI: https://doi.org/10.21203/rs.3.rs-701802/v1
[4] J Cookman, V Hamilton, L S Price, S R Hall and U Bangert, "Visualising early-stage liquid phase organic crystal growth via liquid cell electron microscopy" Nanoscale, 7 (2020).
[5] J Cookman , V Hamilton, S R Hall et al. "Non-classical crystallisation pathway directly observed for a pharmaceutical crystal via liquid phase electron microscopy" Scientific Reports, 10, 19156 (2020). https://doi.org/10.1038/s41598-020-75937-2
[6] A Ianiro, H Wu, M M J van Rijt, et al. "Liquid–liquid phase separation during amphiphilic self-assembly", Nature Chemistry, 11, 320–328 (2019), https://doi.org/10.1038/s41557-019-0210-4
[7] A Rizvi, J T Mulvey, J P Patterson, "Observation of Liquid–Liquid-Phase Separation and Vesicle Spreading during Supported Bilayer Formation via Liquid-Phase Transmission Electron Microscopy", Nano Letters, 21, 24, 10325–10332 (2021).