論 文 の 内 容 の 要 旨

論文題目　Multifunctional MEMS Devices for
in situ TEM Liquid-Phase Experimentation
（液相ＴＥＭによるその場実験用多機能ＭＥＭＳ素子）

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Transmission Electron Microscopy (TEM) is a powerful imaging technique that is often limited by the particular environment required for samples to be investigated. Conventionally, TEM cannot be applied to liquid-phase samples or suspensions due to the stringent requirements of the microscope vacuum chamber. The application of so-called “liquid cell” devices for TEM enables the direct imaging of fluid samples by encapsulating them within a specialized holder for use in the TEM vacuum chamber, but their adoption is relatively limited due in part to their high cost and limited ability to interact with the sample during observation. In this work, a fully-sealed liquid cell device is developed that is appropriate for use with non-specialized electrical-contact TEM holders and is capable of performing *in situ* manipulation of the loaded sample during observation both electrically, thermally, and physically in the form of fluid flow initiation. The development of this and related devices and their comparability to existing liquid cell techniques is described along with various examples of their application for biochemical studies.

The use of Transmission Electron Microscopy (TEM) in the sciences is widespread and represents an essential imaging technique enabling the investigation of samples with both high resolutions and increased throughput as compared to alternative methods. The use of an imaging electron beam mitigates the diffraction-based resolution limits of optical microscopy while also allowing for real-time imaging by avoiding the lengthy sampling durations required for direct-probing techniques such as Atomic Force Microscopy (AFM). Furthermore, precise configuration of the beam, magnetic lenses, and detectors can enable not only sub-nanometer imaging resolutions, but also the derivation of crystallographic and spectroscopic information about the sample. The use of such analytical techniques, combined with real-time, high-resolution imaging capabilities makes TEM a powerful and versatile tool. However, the use of TEM requires that the sample be compatible with the high-vacuum maintained in the microscope chamber that is necessary for a coherent imaging electron beam. Additionally, the sample itself must be thin enough to allow the imaging electrons to transmit though to the detector below while interacting sufficiently with the sample to generate a contrast. These strict limitations on the nature of the sample prevent most liquid-phase systems from be investigated using conventional TEM.

While TEM is very useful for the study of dry solids, its utility for biological and chemical sciences is often inhibited due to the high-vacuum requirements of the technique. When a given observational target that exists naturally within a liquid medium is to be viewed in TEM, e.g. the organelles of a cell, it must first be prepared to both survive in the vacuum and generate a useful contrast. This can involve desiccating the sample and often also requires microtoming of the specimen to produce a sufficiently thin target in addition to staining of the sample to increase the visibility of desired features. This processing can allow the observation of the static structure of the sample, but necessarily removes any dynamic information about its activity in its natural state. The observation of such samples directly within a liquid medium would be ideal.

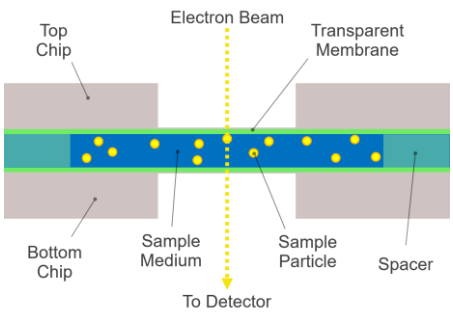

Figure 1: Schematic cross-section of TEM liquid cell

In order to view samples in liquids using TEM, a device known as a “liquid cell” can be used to encapsulate the sample and prevent evaporation in the vacuum chamber. Liquid cells are designed to sandwich the liquid medium between thin membranes that allow the transmission of the electron beam through the medium and enable interaction with the observation target within while isolating it physically from the surrounding vacuum. A cross-sectional diagram of such a device is shown in Figure 1. Because of the strict sealing requirements for such a device to protect the sample and vacuum chamber, these systems often involve custom TEM sample holders that provide mechanical sealing pressure to two encapsulating chips that contain the liquid. This

hermetic sealing also limits the user's ability to interact with the sample during observation. A chemist or biologist hoping to observe a certain reaction involving the mixing of two samples, for example, would require a highly specialized and expensive TEM holder containing a flow conduit that passes through the vacuum chamber and into the sealed cell and out again, all while maintaining a perfect hermetic seal within the chamber. If the experiment also requires applying an electrical signal to the sample while controlling its temperature, ever more complex and expensive holders become necessary, along with auxiliary control equipment outside the TEM. Such significant investments are difficult to justify for researchers who do not already heavily utilize TEM, and the extremely specialized nature of the products and their use cause them to often be overlooked by electron microscopy centers in favor of more conventional systems without liquid-phase capabilities. As such it is necessary to develop a TEM liquid cell device that is both capable of complex electrical, thermal, and flow-based sample manipulation while also being compatible with more conventional and readily-available TEM holders. Doing so will help to make liquid-phase TEM observation more viable as a quantitative analytical technique for researchers who previously found it prohibitively complex or inaccessible.

In order to address the limitations in current TEM liquid cell technology, new devices compatible with conventional TEM holders that also feature the experimental complexity required by biological and chemical researchers must be designed. Specifically, the device must be fully contained without any complex sealing or flow mechanism requiring a specialized holder. As a result of this, the device must be able to function using only electrical interconnects to the outside that are provided by more generic and accessible TEM holders. For its experimental utility, the device must also be capable of allowing the user to manipulate the contained sample in real time. In particular, the device must allow for electrical biasing, temperature control, and the manipulation of sample concentration in the form of flow control.

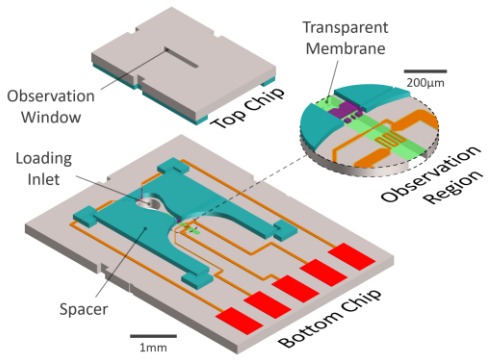

Figure 2: Layout of two-chip liquid cell device

In this work, the design and development of a liquid cell device aimed at achieving the above-mentioned capabilities is described. The device, referred to as the "two-chip" device, is based on a conventional static liquid cell structure that involves two micromachined chips which, when bonded together, encapsulate the sample liquid for observation in the TEM. The device features thin silicon nitride membranes which prevent leakage but enable the transmission of the electron beam. Furthermore, the chip can be patterned with electrodes and other surface-features that allow for the active manipulation of the sample. A schematic of the device is shown in Figure 2. In addition to the two-chip device, another

structure developed in collaboration with Dr. Edin Sarajlic of the University of Twente is also introduced. Known as the "nanochannel device", this structure achieves liquid encapsulation not through sandwiched chips, but rather using a continuous suspended silicon nitride channel. The use of a suspended observation channel is advantageous in that it enables the use of electron-based spectroscopy techniques such as Electron Energy Loss Spectroscopy (EELS) to investigate sample composition. In this work, the general structure, fabrication process, and use of these devices is described in detail.

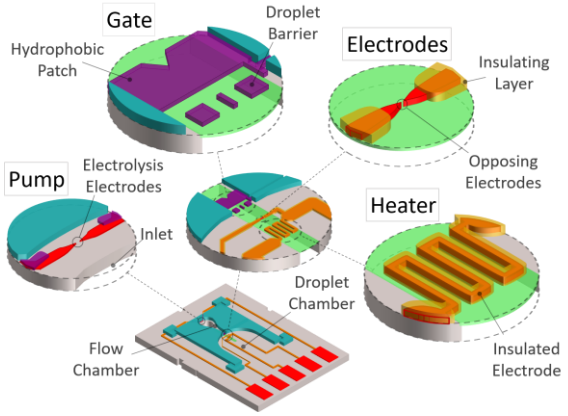

Figure 3: Two-chip device active MEMS components

In addition to the basic liquid cell framework, the development of active features to augment the device to be capable of electrical biasing, temperature control, and directed flow is also described. Each of these functionalities for active experimentation was first developed and demonstrated on individual liquid cell devices and subsequently integrated onto a single, unified device. The features enabling these functionalities on a single device are illustrated in Figure 3. Electrical biasing and heating capabilities were achieved using basic electrodes patterned onto the surface of the liquid cell chip. While biasing of the sample solution simply requires the application of the desired voltage across the electrodes, heating involves Joule heating with a feedback loop in which the changing resistance of the heating electrode is used to monitor and control its temperature and thereby the temperature of the observed sample. Flow initiation is achieved through the use of a hydrophobic "burst-valve" coupled with upstream pressurization through electrolysis of the sample medium itself. The achievement of this flow initiation in a fully-encapsulated liquid cell represents the first of its kind in the field. The fabrication, characterization, and utilization of liquid cell devices integrating these three experimental features is detailed in the main text.

In addition to the development of active MEMS features on the liquid cell device, observation and experimentation was performed with various scientific samples including zeolite structures and metallic nanoparticles functionalized with calixarene and DNA in order to demonstrate the viability of the device as an investigative platform. By achieving experimental capabilities similar to commercial liquid cell setups without the need for a dedicated TEM holder, the devices developed here represent a step towards making liquid-phase TEM experimentation more accessible and applicable to a wider range of researchers who can benefit from the many advantages of the technique.