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Multipurpose Silicon-chip Flow Cell for Liquid Transmission Electron Microscopy and Related Analysis

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We describe the development and applications of a flow cell chip for liquid transmission electron microscopy (TEM) for imaging nanoparticles, biological samples, and dynamic phenomena such as the freezing and melting of liquids, and Raman scattering measurements. The liquid flow cell (LFC) consists of two silicon chips with silicon nitride windows that sandwich two flexible microtubes. Notably, our LFC can be removed from the TEM holder for analysis with other instruments, whereas conventional LFC cannot be removed from the TEM holder for post-experiment analysis. Furthermore, currently available commercial solution holders only allow a portion of the flowing solution to enter the cell, but our LFC has observation windows in the flow channel that make it easier to control the experimental conditions. Importantly, we also describe the results of using our LFC chip for imaging the freezing and melting of water that was slowly cooled by a Peltier element to initiate freezing or heated to induce melting. This research shows that our LFC is a powerful component for imaging the temperature dependence of materials at the nanoscale for a wide range of applications including combining TEM with light scattering analysis of materials such as Raman spectroscopy.

1. Introduction

Recent advances in silicon microfabrication technology have led to innovative approaches to direct imaging of specimens immersed in liquids by the so-called liquid cell transmission electron microscopy (LC-TEM) with applications that include synthesis of nanoparticles, observation of living cell phenomena, corrosion of metals, and nanoscale mapping of the

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elemental composition of nanostructures.[1–4] In typical silicon liquid cells, the samples are sandwiched between two silicon microchips with silicon nitride membrane windows for transmitting electron beams through the liquid for both closed and liquid flow cells (LFCs).[5,6]

However, despite recent developments in silicon liquid cells for LC-TEM, conventional technology still has shortcomings including the following: (1) TEM holders require extensive and cost-prohibitive modifications to integrate sample cells into TEM systems; (2) liquid cells are limited to applications inside TEM systems and cannot be easily removed and loaded into other analytical instruments for comprehensive research on materials; and (3) a simple and inexpensive method of imaging temperature-dependent phenomena with LC-TEM systems remains unavailable.

Here, we report on the development of silicon chip LFCs that can be integrated into conventional TEM holders with only slight modifications and also have the functionality of integration with other analytical systems without exposing liquid samples to air. We also describe the integration of the LFC into a dedicated variable-temperature TEM holder with specifications of operation between −50 to 100 °C, and in our experiments, we used the range from −10 °C to room temperature according to the thermocouple in the shaft of the TEM holder. Our LFC was used for imaging a wide range of samples including bacteriophage T7, freezing and heating of water, and laser Raman scattering of alcohol solutions.

2. Materials and Methods

LFCs were fabricated using readily available photolithographic processing methods used for fabricating MEMs devices. For variable-temperature measurements, an LFC was mounted into a modified TEM holder of Kitano Seiki Co., Ltd., Japan, which enabled temperature control using a Peltier element.[7] The holder was connected to a temperature controller to vary the temperature of the LFC in real time during imaging. The temperature can be varied from −20 to +100 ºC. The TEM holder contained a copper strip that thermally connected the LFC to the Peltier module located at the opposite end of the holder. TEM imaging was carried out in a JEOL JEM-2100 system operated at 200 kV. All the images described in this paper are raw and unprocessed.

2.1 Liquid cell fabrication

Figure 1 shows the process used for fabricating the LFCs using 300-μm-thick (100) Si wafers covered on both sides with silicon nitride with an initial thickness of approximately 200 nm.

Fabrication of silicon nitride electron beam windows

The fabrication process is illustrated in Fig. 1: (a) A silicon wafer was covered with a 200-nm-thick silicon nitride by chemical vapor deposition. (b) Photolithography was conducted to pattern and define window regions through an AZ 5214E photoresist. (c) Reactive ion etching using SF_6 was carried out to remove the silicon nitride layer through the windows in the photoresist. (d) Finally, KOH was used to etch silicon until the silicon nitride on the opposite side of the Si wafer was exposed, leaving a <111> plane facet in the silicon.

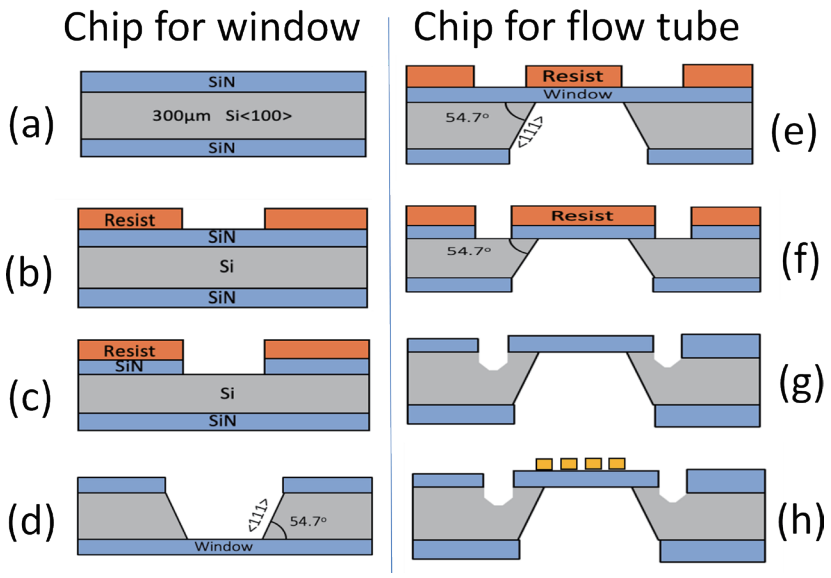


Fig. 1. (Color online) (a)–(h) Steps of the semiconductor photolithography-based dry and wet etching used for fabricating silicon LFCs. The <111> refers to the orientation of the silicon etched by the KOH.

Fabrication of flow tube side of the chip

(e) An AZ 5214E photoresist was used to define the areas for inserting flow tubes. (f) This was followed by SF_6 reactive ion etching of the silicon nitride layer through the windows of the photoresist. (g) The silicon layer was etched with KOH. (h) An array of 5 μm^2 gold pads (thickness of 50 nm) was deposited to aid focusing during measurements.[9]

Finally, the two silicon chips [Figs. 1(d) and 1(h)] were glued together sandwiching two 360-μm-diameter PEEK microtubes, as shown in Fig. 2. A typical LFC consisted of two PEEK microtubes, a microfluidic channel, and a 50 μm^2 SiN window. Other LFC designs consisted of four SiN windows but without etched microfluid channels (Fig. 3).

3. Examples of Measurements of Liquid Flow Transmission Electron Microscopy

3.1 Nanoparticles and biological samples

Figure 4(a) shows the flow of 250 nm silicon oxide particles over gold pads on the lower SiN membrane. We also tested the possibility of imaging mixtures of biological and inorganic samples. Figure 4(b) shows images of fetal bovine serum that adhered to a gold pad after 5 s.

Figure 5 shows another example of biological imaging showing the movement of approximately 60-nm-diameter bacteriophage T7 (T7 phage) in pure water. Initially, the T7 phages overlapped, but within 5–10 s, the flow of the water moving through the LFC moved them until their shapes became delineated and visible as individual circular forms characteristic of the T7 phage. The images are approximately at one second intervals and captured from a real-time video of the measurements.

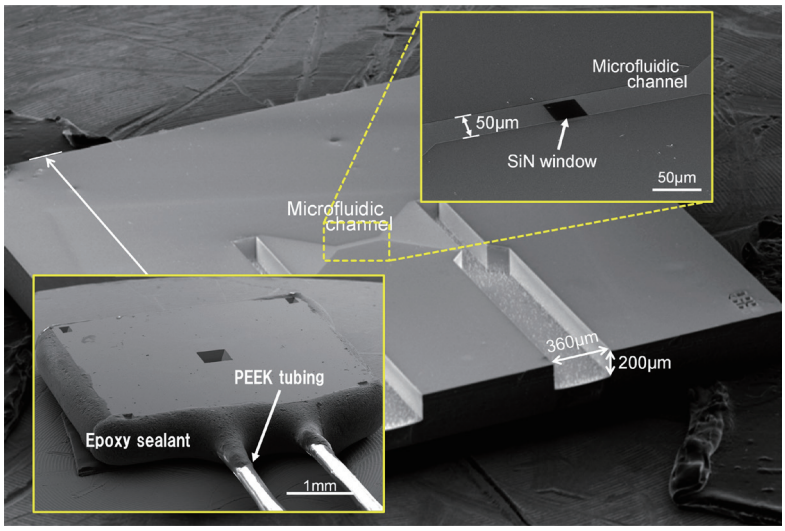


Fig. 2. (Color online) SEM image of the main parts of a typical LFC showing a flow channel and SiN window.

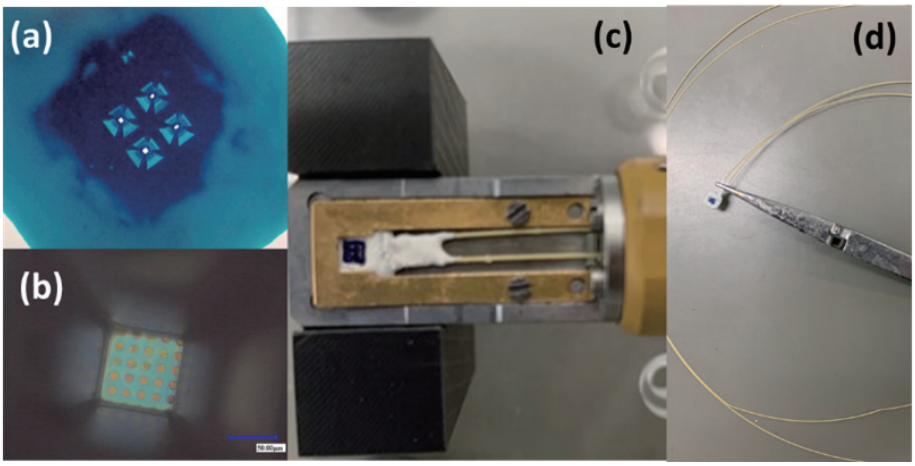
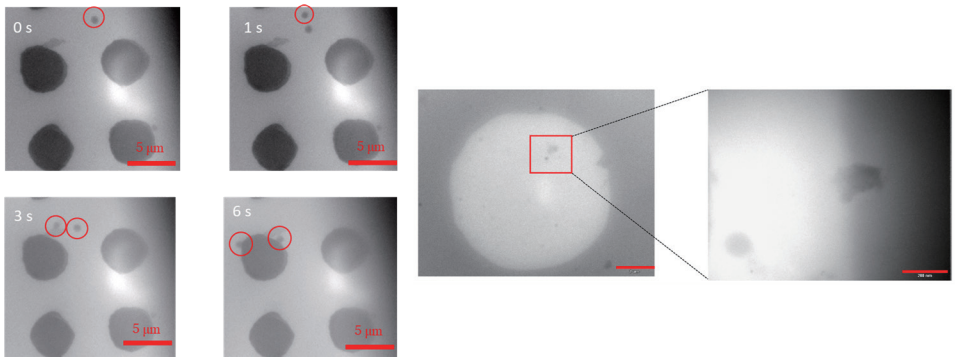


Fig. 3. (Color online) Photographs of another design of the LFC with (a) light shining through four SiN windows each 50 μm in diameter; (b) 5-μm-diameter Au pads on the surface on the lower SiN membrane used to aid focusing during TEM measurements; (c) an LFC chip mounted into a JEM-2100 TEM holder. The white area of the photogragh is epoxy. (d) Typical LFC after completion of the fabrication process. The PEEK microtubes carry liquids through the action of atmospheric pressure from the outside of TEM systems into the LFC that is under vacuum conditions in the TEM system.

3.2 Variable-temperature imaging of freezing and melting of water

A recent paper described the *in situ* TEM imaging of ice crystal formation on the surfaces of TiO_2 nanoparticles in a liquid closed graphene cell.[8] Although the resolution of our LFCs is not as high as that of graphene cells, our flow chips are easy to produce, robust during sample preparation, and enable the injection of different liquids into the same chip, features that offer greater potential for obtaining wide ranging information about pure and mixed liquids during single measurements.



(a) (b)

Fig. 4. (Color online) (a) Flow of 250 nm SiO_2 particles over gold pads. (b) Test using biological samples. The images show fetal bovine serum that adhered to the Au pads after flowing for 5 s. The scale bar for the left image is 2 μm and that for the image on the right is 200 nm.

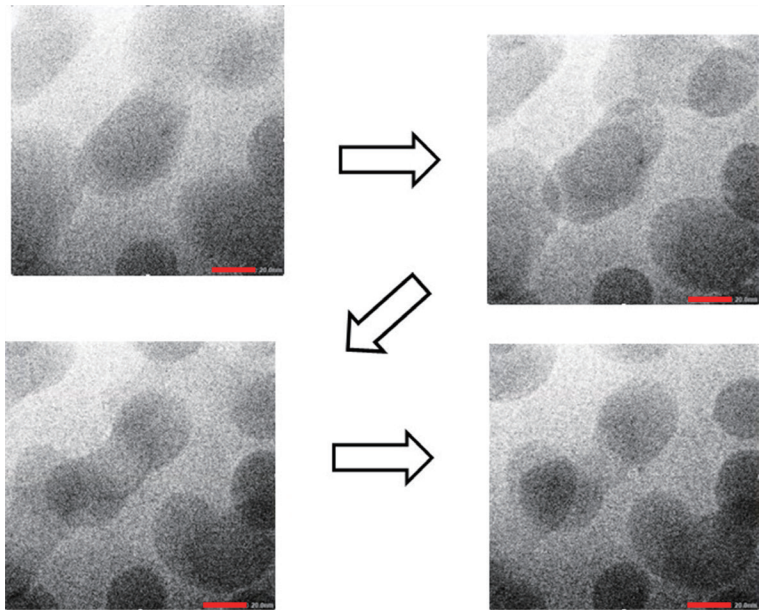


Fig. 5. (Color online) Series of images taken at 120 kV showing the movement of ca. 60-nm-diameter T7 phage. After 5–10s, the overlapping T7 phages move under the action of the flow of the water and evolve into individual circular forms characteristic of the T7 phage. The scale bars in the images are 20 nm.

The following series of experiments demonstrate the range of imaging possible by combining our LFCs and a Peltier-device-based temperature-variable TEM holder for the direct observation of freezing and melting water. The measurements were carried out in a JEM-2100 system at an acceleration voltage of 200 kV in the range from −10 °C to room temperature (20 °C). We injected deionized water containing polystyrene particles with nominal diameters of 3 and 1 μm through our flow cell. The particles acted as dynamic markers as a visual aid to observe the

onset of freezing and melting of water when they stopped and started moving, respectively. Figures 6(a)–6(e) are a sequence of images taken during water cooling from room temperature to −10 °C [Fig. 6(e)]. We observed that the microparticles slowly stopped moving as the water froze. Figures 7(a)–7(c) are images of water being heated from −10 °C to room temperature. The white circles indicate ice-like structures that slowly became smaller and eventually melted as the temperature returned to room temperature.

Importantly, video images of real-time observations showed the square particles indicated by yellow triangles flashing on and off during measurements, strongly suggesting that the particles are crystalline (Fig. 8). The variable temperature observations were made using an objective aperture of 60 μm diameter, and only electrons scattered below about 23 mrad were captured for

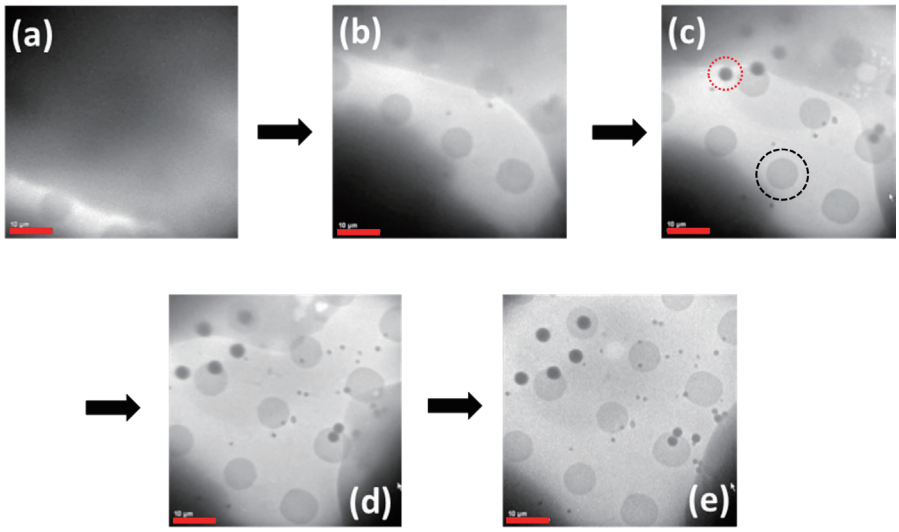


Fig. 6. (Color online) (a)–(e) Liquid flow cell images of water freezing from room temperature (a) to −10 °C (e). The 1-μm-polystyrene particles slowly stopped moving and became stationary in (e). The scale bar is 10 μm. The red broken circle is a 3 μm particle and the black circle shows a gold pad.

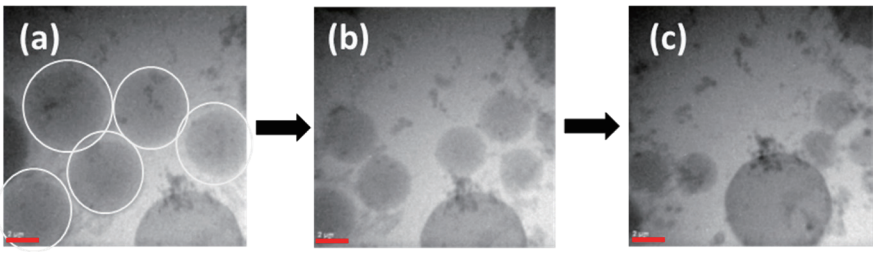


Fig. 7. (Color online) (a)–(c) Liquid flow cell images of frozen water being heated. The white circles show the formation of ice structures as the water melted. The structures slowly decreased in size and completely disappeared above approximately 10 °C, as measured with a thermocouple. The scale bars are 2 μm.

imaging. That is, when a crystal faces a certain direction and satisfies the Bragg condition, it appears to shine brightly. In addition, there were many particles that flickered whilst moving. These are all microcrystalline particles of ice. During the experiments, we were not able to obtain clear electron beam diffraction patterns due to the rather large thickness of water and SiN membrane in the observation channel. This is a topic for future research.

3.3 Other potential applications: Raman scattering of solutions of ethanol and water

We also studied the possibility of expanding the potential applications of the LFC to material analysis without removing samples from the cells. As a trial experiment, we conducted Raman scattering measurements to monitor the dilution of ethanol with deionized water (DIW) using the LFCs. Figure 9 shows an LFC with four 50 × 50 μm^2 silicon nitride transmission windows installed inside a laser Raman system (JASCO NRS-3100) with a syringe (inset) attached for injecting liquids. The measurements were carried out with a 532.1 nm laser beam that was focused into one of the nitride windows.

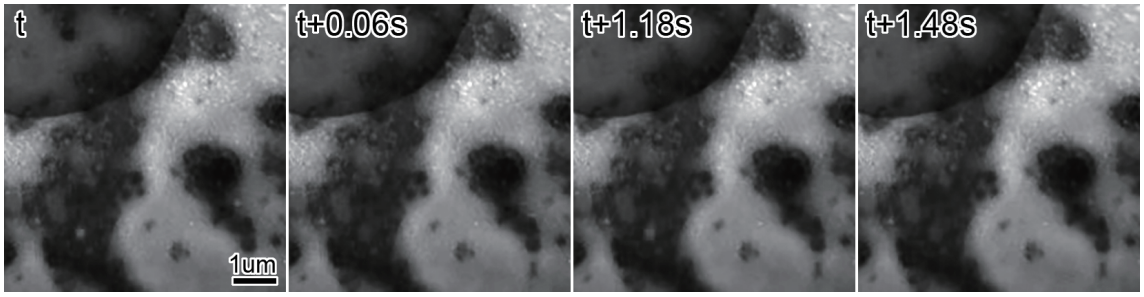


Fig. 8. (Color online) Time lapse images of water crystals at −10 °C. The shape of the particles indicated by yellow triangles is almost square. Our video measurements (Ref. 11) showed them to flash on and off during observation, suggesting that the particles are crystalline. The observations were made using an objective aperture of 60 μm diameter, and only electrons scattered below about 23 mrad were used. That is, when a crystal faces a certain direction and satisfies the Bragg condition, it appears to shine brightly.

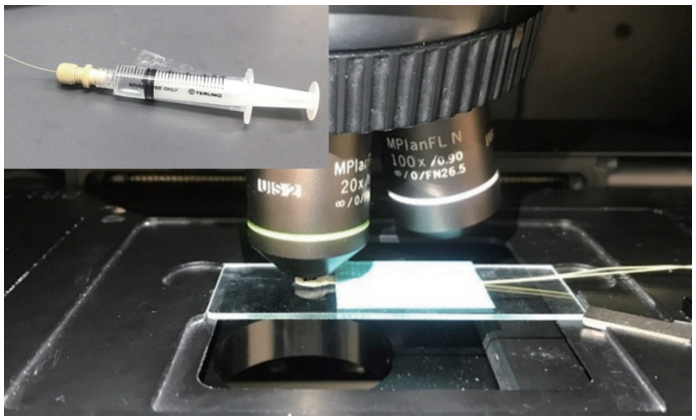


Fig. 9. (Color online) An LFC with four nitride windows was positioned below the lens in a Raman spectroscopy system. Liquids were injected into the LFC via microtubes connected to a syringe (inset).

Figures 10(a)–10(f) show the evolution of Raman scattering spectra starting with a solution of 100% ethanol to the final solution of DIW only prepared by diluting the initial solution in 20% steps by injecting DIW. The Raman spectra of a mixture of ethanol and water are well known.[10] The main peaks in the ethanol spectrum are in the range of 2800–3000 cm^{-1} attributable to the stretching vibration between C–H bonds, the 880–1450 cm^{-1} range attributable to the bending vibration between C–H bonds, and around 1000–1300 cm^{-1} is attributable to the vibration between C–O bonds. For water, the main peaks are between 3000 and 3600 cm^{-1} attributable to O–H stretching vibrations.

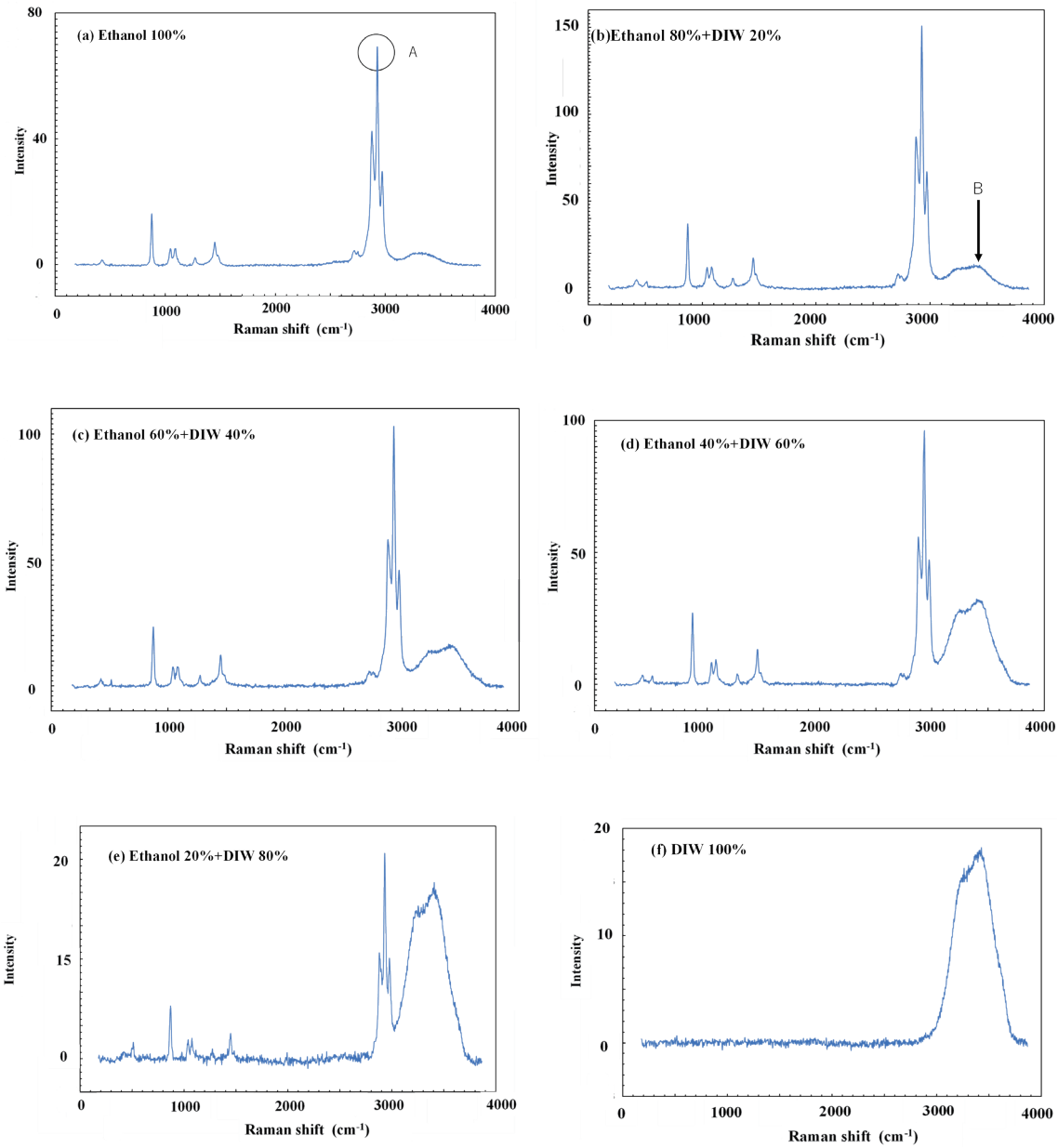


Fig. 10. (Color online) Raman scattering of a solution of ethanol as it is diluted with DIW where (a) is 100% ethanol and (f) is 100% DIW. Peak 'A' is associated with ethanol and peak 'B' with water. The relative intensities of 'A' and 'B' are directly related to the composition of the ethanol/water mixture.

The spectra in Fig. 10 clearly show that the intensity of peak 'B' (associated with water) increased relative to peak 'A' (associated with ethanol) as the percentage of DIW in the solution increased. These results could be used as calibration data for determining the composition of ethanol/water solutions. Moreover, other mixtures of liquids could also be examined with the LFC for not only TEM imaging but also elemental analysis by methods such as energy-dispersive X-ray analysis (EDX) and energy-loss spectroscopy (EELS). This capability to move samples between different analytical instruments without exposing them to air offers a powerful approach to comprehensive materials analysis.

4. Conclusions

We demonstrated the potential of our LFC for TEM with a wide range of materials including the imaging of nanoparticles, biological samples, and the freezing and heating (melting) of water at the nanoscale. LFC-TEM offers new possibilities for studying the dynamics of nanoparticles in solution and observing particle growth, aggregation, and dissolution. Compared with current commercial liquid TEM systems, the SiN chips are inexpensive because they can be mass produced using a standard silicon fabrication process, and we were able to produce approximately 100 functioning chips from a single 4 inch silicon wafer. Furthermore, the ability to observe the freezing and melting of water at the nanoscale has important implications for a variety of scientific fields, including materials science, biology, and environmental science. By using LFC-TEM, researchers can directly observe the mechanisms of ice formation and melting, as well as the behavior of solutes and interfaces during these processes.

Overall, our LFC-TEM is a valuable tool for investigating a wide range of phenomena in liquid environments, including complementary methods such as Raman without exposing specimens to air.

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