

CONFOCAL MICROSCOPY OF WATER UNDER STATIC PRESSURE

M. D. McCluskey^{1,2}, B. D. Riley¹, A. M. Perenchio¹, and M. Knoblach³

¹*Department of Physics and Astronomy, Washington State University, Pullman WA 99164-2814*

²*Institute for Shock Physics, Washington State University, Pullman WA 99164-2816*

³*School of Biological Sciences, Washington State University, Pullman WA 99164-4236*

Abstract. Developments in confocal microscopy have revolutionized the imaging of samples. Unlike conventional microscopes, which illuminate a wide area, confocal microscopes focus laser light onto a single spot on the sample. The laser spot is scanned, data are collected point by point, and an image is reconstructed from the collected data. The sample can be translated vertically, allowing one to obtain three dimensional (3D) image reconstructions. We have used confocal microscopy to obtain high-quality images of water freezing in a moissanite anvil cell. This technique could prove useful for a variety of equation-of-state investigations.

Keywords: Static high pressure, water:freezing, imaging.

PACS: 07.35.+k, 62.50.+p

INTRODUCTION

Optical imaging techniques are important for determining the equation of state of materials, such as polymers, for which x-ray diffraction is impractical. Results on poly(dimethylsiloxane) have shown the utility of optical microscopy and imaging analysis [1]. In that work, the vertical compression of the sample was assumed to be equal to the in-plane compression, a reasonable assumption for isotropic media. In this paper, we describe a method to obtain a 3D image reconstruction of a sample in a high-pressure cell, using confocal microscopy. This technique could prove useful for determining the equation of state for a wide range of materials.

To test this experimental approach, we investigated water freezing under static pressure. At room temperature, water undergoes a liquid-solid phase transition at ~ 1 GPa. The images obtained by this method provide complementary information to images obtained in shock-induced freezing experiments [2].

EXPERIMENTAL PROCEDURE

A piston-cylinder [3,4] moissanite anvil cell [5] was used with a 400 μm thick copper beryllium gasket. The moissanite anvils had a culet diameter of 1.5 mm and a height of 2.6 mm. A 660 μm hole was drilled through the gasket after pre-indenting to a thickness of ~ 200 μm . High-purity water (HPLC) was loaded into the hole, along with ruby chips for pressure calibration. For the confocal microscope images shown in this paper, the ruby chips were omitted in order to minimize the background fluorescence.

Standard microscopy images were obtained with an Olympus SZ40 microscope and Olympus 3040 digital camera. Confocal microscopy was performed with a Zeiss 510 META confocal laser scanning microscope with a 488 nm excitation wavelength and x5 objective with numerical aperture (NA) of 0.12. Trace amounts of Lucifer Yellow dye were added to the water. Light emitted by the dye passed through a 510-570 nm bandpass filter and was detected by a photomultiplier tube.



Figure 1. Standard microscope image of ice crystals in liquid water, at a pressure of 0.9 GPa and room temperature. A ruby chip is on the right side of the gasket hole.

RESULTS AND DISCUSSION

Standard microscopy and pressure measurements

The pressure was increased steadily, over a period of several hours, by tightening the Allen screws on the cell. At pressures below the phase transition, the liquid water was transparent. At a critical pressure of ~ 1.0 GPa, ice crystals were observed to form. The small ice crystals coalesced into several larger crystals (Fig. 1), over a period of 5-30 min. Coincident with the formation of this ice-liquid mixture, the pressure decreased from ~ 1.0 to 0.9 GPa (Fig. 2). The solid line in Fig. 2 is a least-squares exponential fit,

$$P = A \exp(-t/\tau) + B, \quad (1)$$

with the following values: $A = 0.13 \pm 0.01$ GPa, $B = 0.891 \pm 0.003$ GPa, and $\tau = 10.6 \pm 1.3$ min.

According to Ref. [6], the room-temperature transition from liquid water to ice VI occurs at a pressure of 0.96 GPa. Therefore, at time zero in Fig. 2, the water was in a supercooled state. When the ice crystals nucleated, the pressure relaxed to 0.89 GPa. It is not clear why the pressure appeared to fall to a value slightly below 0.96 GPa. We are currently investigating this discrepancy.

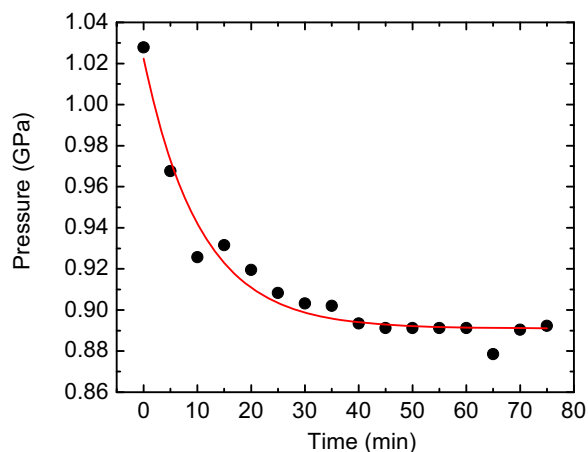


Figure 2. Pressure versus time for water. Time zero is defined as the moment when freezing was first observed.

Confocal microscopy.

The fluorescence confocal microscope system is illustrated schematically in Fig. 3. Collimated, monochromatic light from a laser is reflected off a dichroic mirror and focused by an objective lens onto the sample. The sample contains a dye that fluoresces at a longer wavelength than the laser light. The emitted fluorescence travels through the dichroic mirror and is focused onto a screen with a pinhole. The photons pass through the hole and are detected by a photomultiplier tube (PMT).

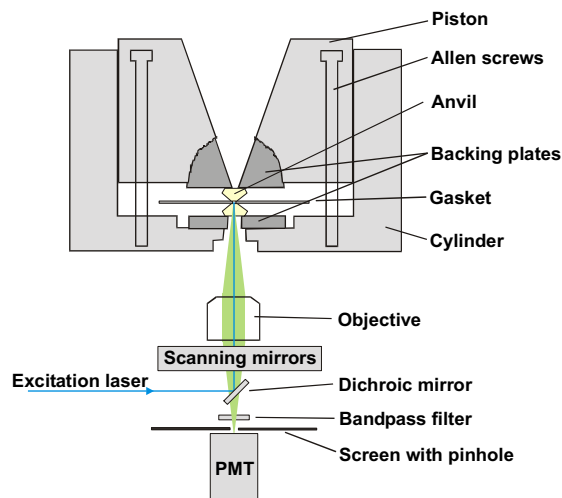


Figure 3. Schematic diagram of the high-pressure confocal microscope system.

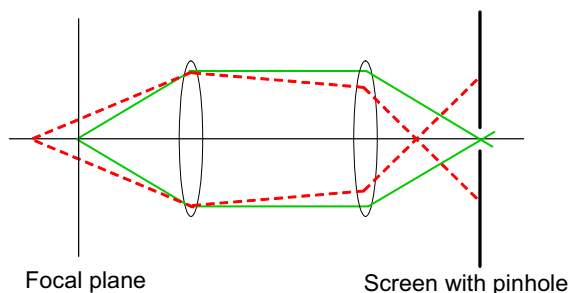


Figure 4. Illustration of optical sectioning. Light emitted from the focal plane (solid line) travels through the pinhole and is detected. Most of the light emitted out of the focal plane (dashed lines) is rejected.

The focusing of the laser causes most of the fluorescence to be emitted in a small, diffraction-limited spot. However, fluorescence will also occur from within the cones of light above and below the spot. To suppress the background “haze” that arises from out-of-focus light, the fluorescence is focused onto a screen with a pinhole (Fig. 4). Photons emitted from the focal point are focused onto the screen and pass through the pinhole. Photons emitted away from the focal point are blocked by the screen. The image created by this process is a planar slice through the sample, called an *optical section*. By obtaining a series of optical sections, a 3D image can be reconstructed. (See Ref. [7] for an introduction to confocal microscopy.)

Figure 5 shows a top-view image of the cell at a pressure of 0.9 GPa. The pinhole width was opened such that the width of the optical section exceeded the sample thickness. The fringes arise from interference of the excitation laser caused by the nonplanar moissanite surfaces and thickness variations in the ice crystals.

To obtain a 3D reconstruction, the pinhole width was reduced such that the light passing through the optical section came from an optical section 46 μm thick. A total of 47 optical sections were taken at increments of 5 μm , for a total thickness of 230 μm . A cross sectional image of the gasket was generated using ImageJ with the Volume Viewer plug-in [8] (Fig. 6). The image in Fig. 6 shows that one of the ice crystals, indicated by the arrow, is near the top of the cell.

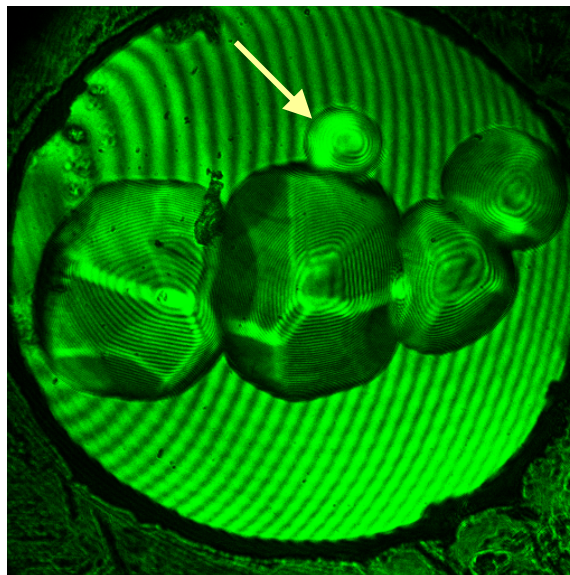


Figure 5. Confocal microscope image of ice crystals in water under pressure. The diameter of the gasket hole is $\sim 660 \mu\text{m}$. The fringes arise from interference of the excitation laser caused by the nonplanar moissanite surfaces and thickness variations in the ice crystals.

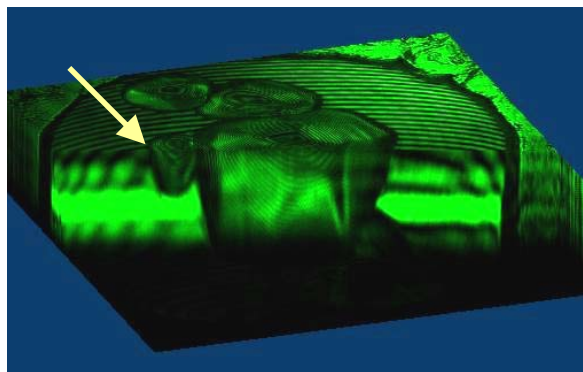


Figure 6. 3D reconstruction of ice crystals in water under pressure. The thickness of the cross-sectional image is 230 μm . The arrow indicates a small ice crystal near the top of the gasket hole.

CONCLUSIONS

High-quality images of ice crystals in water under pressure were obtained with confocal microscopy and a moissanite anvil cell. This technique is promising for equation-of-state investigations of materials under pressure. In the future, the depth resolution will be improved by increasing the NA of the objective.

ACKNOWLEDGEMENTS

Support was provided by the National Science Foundation under Grant DMR-0704163 and the Institute for Shock Physics under DOE Grant DE-FG03-97SF21388 (MDM).

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