Development of a cryo-FIB technique for the 3D structural characterization of liquid samples

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Abstract. The observation of three-dimensional distributions in some liquids or colloids is important, for example in cosmetics, functional paints and a catalyst. Furthermore, there is increasing demand to microscopically investigate the structures inside a liquid colloid, such as the interface of a dispersoid and dispersant. In order to meet these requirements, we have developed a cryo-transfer holder which is fully compatible between FIB and TEM/STEM. The cross section of frozen emulsions and a thin film sample were made by FIB-SEM (NB5000). As a result, the dispersion state of a dispersoid was observed by sequential FIB and SEM. Furthermore a 100nm thick sample was made by FIB and observed by 200 kV STEM (HD-2700). The dispersoid (TiO_2 and Fe_2O_3) morphology and distribution were shown at nanometer level.

1. Introduction

The focused ion beam (FIB) method is a well-established and useful technique for the preparation of cross sectional samples for SEM or TEM thin foils from solid materials such as semiconductor, ceramics, metals, and so on. But in recent years the FIB application field has been extended into macromolecules and biological materials. Due to the usually low thermal conductivity of such materials, the heat generated by ion beam causes a rise in sample temperature which eventually might prove fatal for the sample [1]-[2]. Thus, the demand for cryogenic sample cooling during FIB processing and subsequent SEM or TEM observation has increased [3] - [5].

In order to respond to those demands, we have developed a cryo-transfer holder (Fig.1) for Hitachi FIB and FIB-SEM which enables processing of temperature sensitive samples without heat damage. With this holder cryogenic sample applications can be directly executed in all Hitachi FIB and FIB-SEM systems equipped with the TEM compatible goniometer stage and on Hitachi TEM/STEM instruments, without any further modification required to the instrument. In this paper we report an application example for three-dimensional structure observation of a liquid sample by FIB-tomography using this cryo-transfer holder, and subsequent high-resolution observation in STEM

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2. Materials and methods

2.1. Thermometry of sample temperature using thermocouple holder

Two types of cryo-transfer holder were developed (Fig.1). One of them is a cryo-transfer holder for "bulk" specimens, and it is fully compatible with FIB and SEM. The other is for thin film specimens and is fully compatible holder with FIB, SEM and STEM or TEM systems.

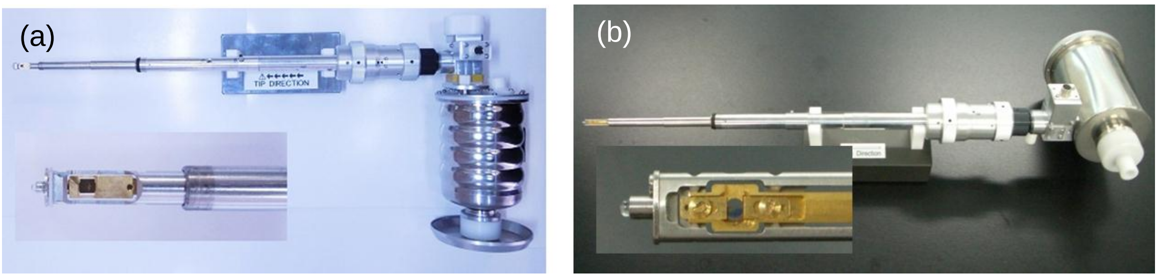

Figure 1. General view of the cryo-transfer holders. (a) For bulk sample. (b) For thin film sample.

To evaluate the cooling effectiveness of the cryo-transfer holder, we measured the temperature change of the sample surface under ion beam irradiation. The basis of the experimental instrumentation is the FB2200 FIB, together with a thermocouple holder (Hitachi, Japan) [6]. In the sample thermometry, the thermocouple holder can put into the micro-sampling stage of FB2200 and the thermocouple can be manipulated to directly contact the sample surface. We used copper as a sample for thermometry. Figure 2 shows the result of the thermometry. In the case of cryo-transfer holder for bulk specimen, the sample temperature fell to a minimum of -138.8 ℃ without ion beam irradiation. When applying a 40 kV ion beam with 64 nA beam current, the temperature change was small. In the case of the cryo-transfer holder for film specimens, the sample temperature fell to a minimum temperature -149.8 ℃ without ion beam irradiation. When applying a 40 kV ion beam with 64 nA beam current, the temperature increased to -147.8 ℃.

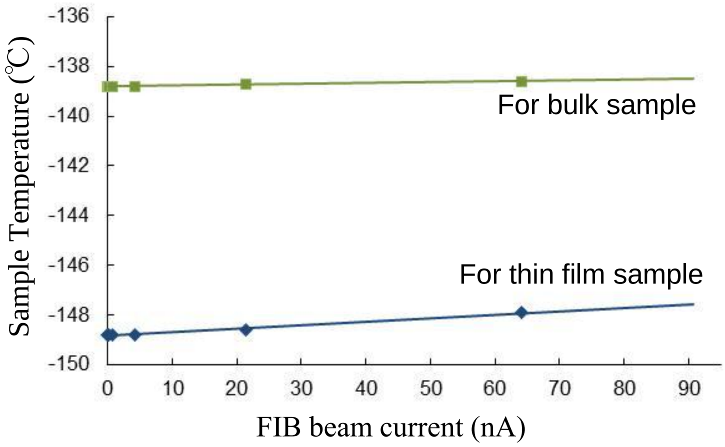

Figure 2. Thermometry of two types of cryo-transfer holder.
Sample: Cu
Holder Temperature:
Thin film: -170℃
Bulk: -149℃
FIB Vacc. :40 kV
FIB irradiation area: 37 μm×25 μm

2.2 Application of the cryo-transfer holder to a liquid sample

As a sample for our experiment we used a commercially available emulsion. This emulsion included mainly water, cyclomethicone, ethanol, glycerin and some metal oxides. Figure 3 shows the process of inserting the sample into the FIB-SEM for each cryo-transfer holder. Two experiments were performed. Firstly, cross sectional SEM observation of a "bulk" specimen and secondly, high resolution STEM observation of a thin film. In the first experiment, the sample was applied to a hydrophilized Cu stub / grid. After the sample was frozen by rapid freezing (ethane plunge), it was mounted on the pre-cooled cryo-transfer holder. The cryo-transfer holder was inserted into the

NB5000 FIB-SEM under constant cooling to -140 °C. During holder transportation the sample area was protected from air contact and frost formation by a retractable shielding capsule. We then applied the focused ion beam to produce a cross-section of the emulsion by using this cryo-transfer holder.
In the second experiment, we milled part of the sample in the Cu grid thinly and transferred it to HD-2700 200 kV STEM (Hitachi, Japan) for high resolution observation.

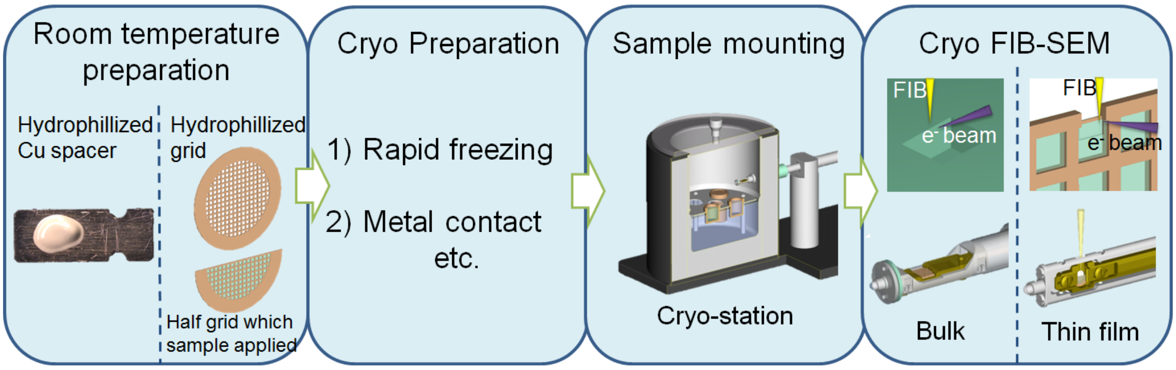

Figure 3. The procedure of the cryo-FIB thinning and STEM observation.

3. Results

Figure 4. shows a cross-sectional SEM image of the frozen emulsion. We can distinguish the dispersant (yellow arrow) from dispersoid (white arrow) in this image. We also observe that the surfaces of the dispersoid particles are coated with fine particles. Next, we obtained a series of 60 consecutive SEM images by using the automatic FIB milling and SEM observation cycle function of the NB5000. Figure 5 is a reconstructed 3D image of emulsion based on the serial section SEM images.

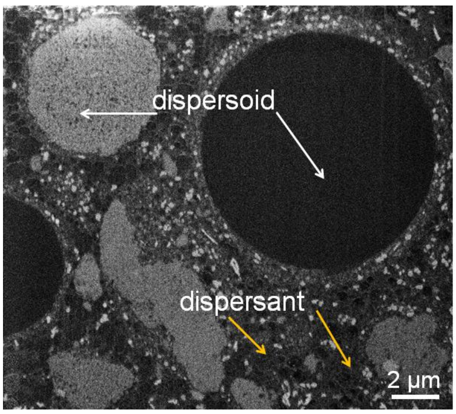

Figure 4. Cross-section image of emulsion. SEM Acc. Volt: 2.5 kV, Signal: Back scattered electron, Holder temp.: -140℃

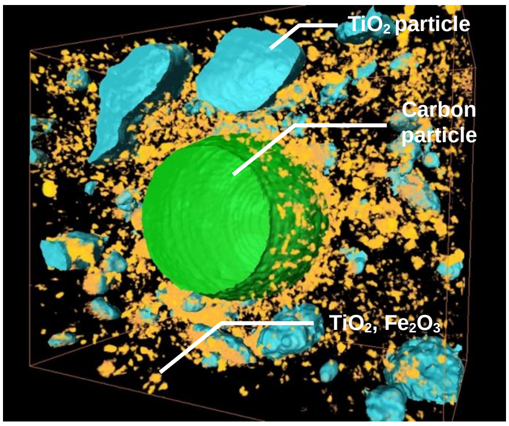

Figure 5. 3D reconstruction image.

The reconstruction volume is approximately 20 μm in width, 17 μm in height and 12 μm in depth. We were able to clearly visualize the three-dimensional distribution of the pigments in the emulsion.

Figure 6 shows the 200 kV STEM image of the thin film specimen (Thickness: approximately 100 nm) of the frozen emulsion. In this result it is possible to clearly see each dispersoid, such as the needle crystals and the granular crystals. The results indicate the low temperature and high stability performance of this cryo-transfer holder.

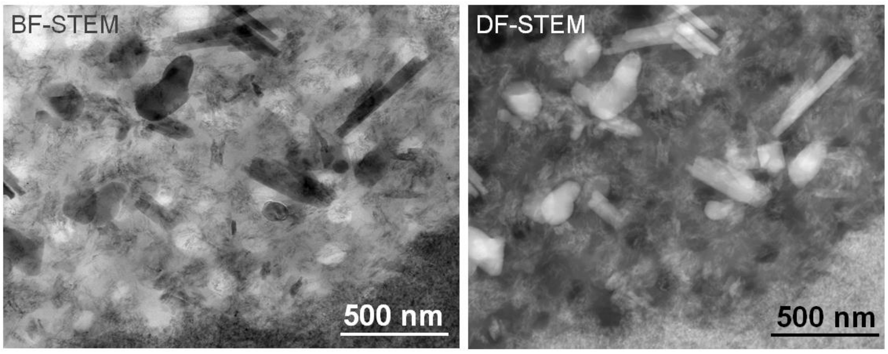

Figure 6. BF and DF-STEM images of emulsion.

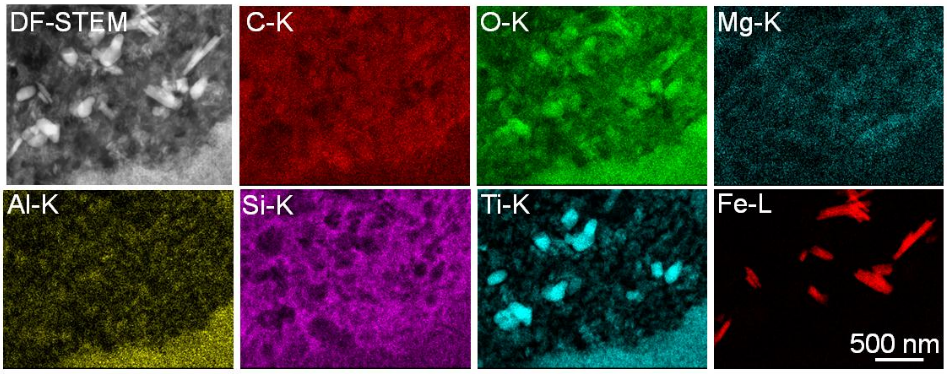

Figure 7. DF-STEM image and EDX maps of emulsion.

Figure 7 shows the EDX maps of emulsion, the needle crystals and granular crystals each represent of Fe and Ti. Very fine crystals also are composed of Ti and most of dispersion medium is made up of Si and carbon materials.

4. Summary

We have developed two types of cryo-transfer holder for FIB-SEM and validated their functionality by applying it to the FIB processing and SEM observation of a rapid frozen emulsion. We succeeded in visualizing the 3-dimentional distribution of the dispersoid using automated serial FIB milling and SEM observation, showing the effectiveness of the sample cooling. In addition, we carried out thinning by FIB and we transferred the thin section to 200 kV STEM and performed STEM observation. The dispersoid morphology and distribution were clearly observed and we were able to undertake detailed studies of the structures and composition. Also, the demand for microscopic examination of interfaces in emulsions is expected to increase in many fields including biomaterials, and a capability and workflow to do that has been demonstrated.

5. References

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