HITACHI SCIENTIFIC INSTRUMENT
TECHNICAL DATA
SEM

SHEET NO. 96

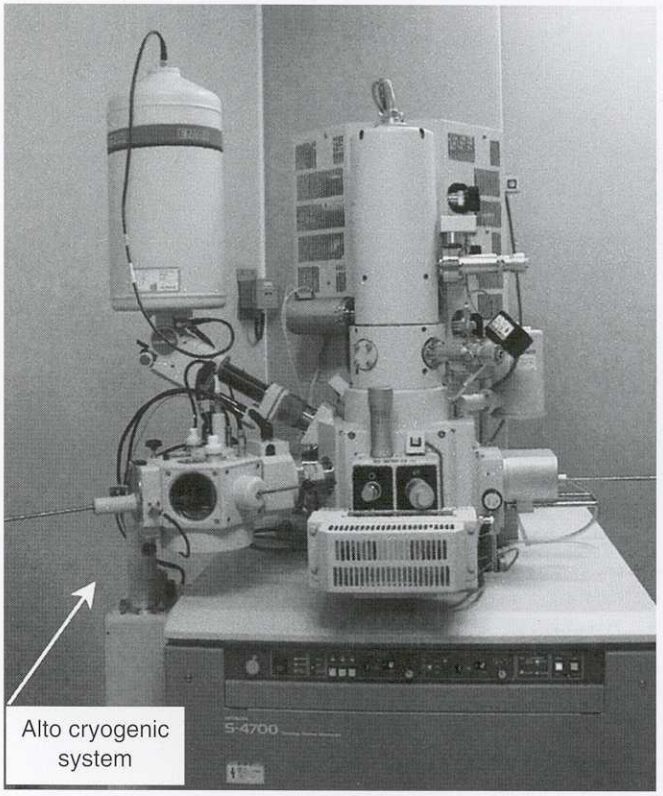
SUBJECT: HIGH RESOLUTION CRYOGENIC MICROSCOPY USING THE S-4700 FE-SEM

INSTRUMENT: THE S-4700 COLD FIELD EMISSION SEM

CONTENTS

1. INTRODUCTION

Cryogenic scanning electron microscopy allows observation of water-containing samples in a frozen condition that permits sample morphology to be maintained in high vacuum conditions and close to their natural state. This technique has been widely used in food, biology and pharmaceutical fields for a long time. Recently it has been applied for high resolution cryogenic microscopy of protein particles. We have tested the capabilities of the S-4700 coupled with an Oxford cryogenic system using yeast, colloid and other samples. The S-4700 has a snorkel objective lens and achieves a high resolution of 2.1 nm at a low operating voltage of 1 kV. The Oxford cryogenic system allows operation of the S-4700 at short working distances that are preferred for high resolution work. We report here on the features of this combined system and some initial applications.



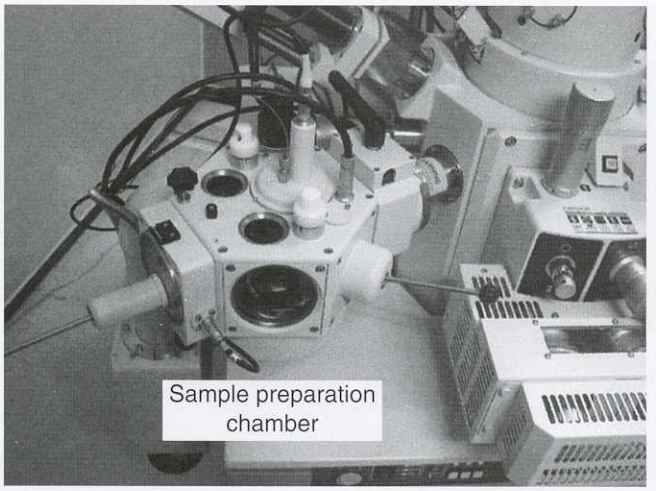


Fig. 1 A general view of the S-4700 FE-SEM(Type 1) with Alto 2500 cryogenic system

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2. A SYSTEM CONFIGURATION AND FEATURES OF THE CRYOGENIC SYSTEM

Fig. 2 shows a system configuration of the S-4700 FE-SEM with Oxford's Alto 2500 cryogenic system.

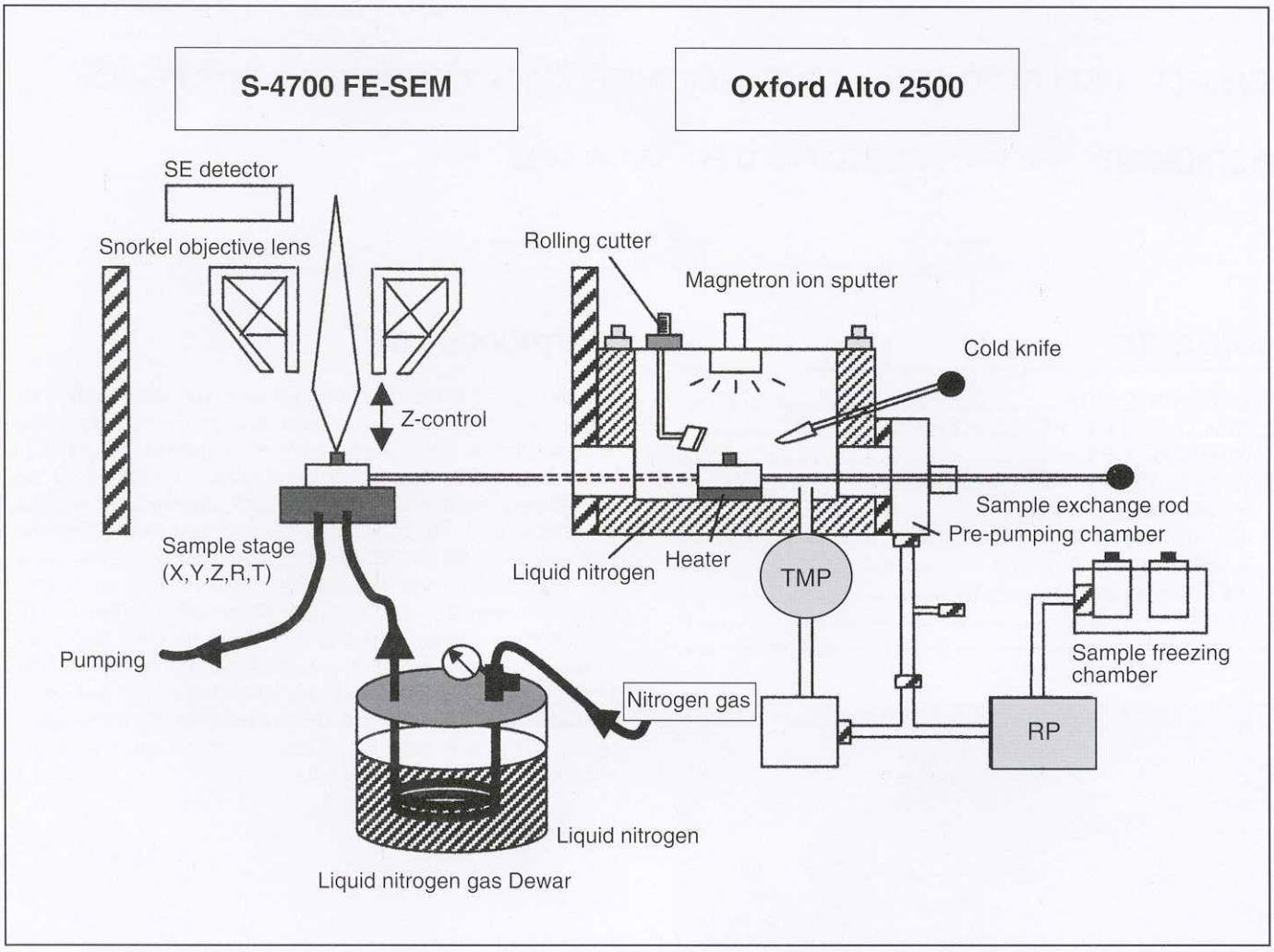


Fig. 2 A system configuration

Features

(1) Operation at short working distances allows high resolution cryogenic microscopy at low operating voltages and good S/N ratio.

(2) A new secondary electron detection of the S-4700 is effective for minimizing sample charging artifact for cryogenic work. It is also effective for BSE work at low operating voltages.

(3) The sample stage is cooled using nitrogen gas which has been cooled down to liquid nitrogen temperature. This design is advantageous for excellent stage stability free from bubbling of liquid nitrogen. It allows stable operation for long hours.

(4) In the sample freezing chamber, samples are frozen using slush of nitrogen which allows a rapid freezing time and minimizes sample damage by crystal growth. Frozen samples can be transferred to the sample processing chamber all under vacuum conditions so that samples are protected from ice forming.

(5) Temperature control is available for both the sample processing chamber and the sample stage. This design allows sample preparation such as fracturing, etching, coating (using a magnetron sputter coater) simply and repeatedly.

3. MICROSCOPY

- Rapid freezing (Sample freezing chamber)
  - ↓ Vacuum transfer
- Fracturing using a cold knife in the sample processing chamber (Sample temperature: About –170°C)
  - ↓
- Sublimation of ice (Sample temperature: –105°C ~ –90°C)
  - ↓ Non-conductive sample ← Pt coated in the sample processing chamber (Sample temperature: –115°C)
- Microscopy (Sample temperature: –120°C maintained)

For samples that have a high water content, an ethanol substitution technique was employed prior to rapid freezing for preventing icing.

4. APPLICATIONS

4.1 Microscopy of agar gel

Fig. 3 shows micrographs of agar gel. Water concentration of this sample is high or about 99%. An ethanol substitution was employed for preventing icing problems. Both of these micrographs show fine net structures of fibers (10 nm or smaller) clearly.

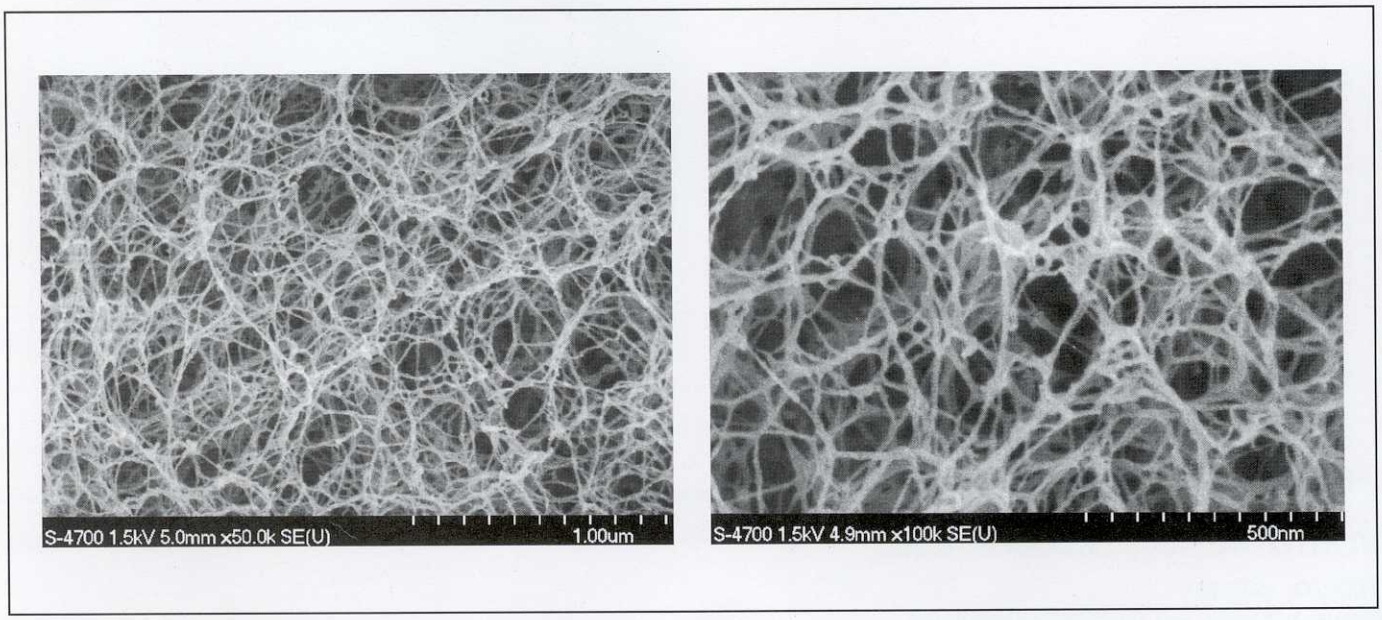


Fig. 3 Microscopy of agar gel

4.2 Microscopy of processed cheese

The sample was quickly frozen using slush of nitrogen in the sample freezing chamber. It was then freeze-fractured at –170°C in the sample preparation chamber, sublimated at –95°C, Pt-coated at –115°C. Fig. 4 shows the recorded micrographs. Fat balls (→) and protein particles of about 10 nm around it are clearly resolved.

4.3 Microscopy of colloid

Beam sensitive colloid samples were observed as shown in Fig. 5. A fluid of colloid was quickly frozen, freeze-fractured and observed at –120°C. Both low molecular weight(a) and high molecular weight(b) show bonding conditions (→) of colloid particles like fibers.

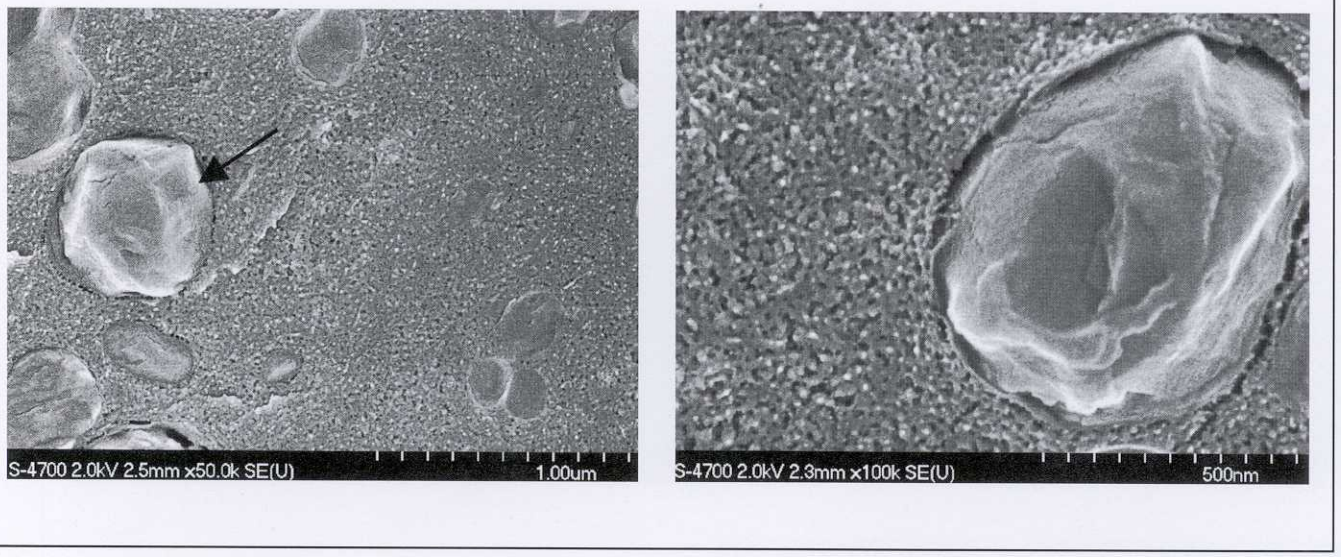
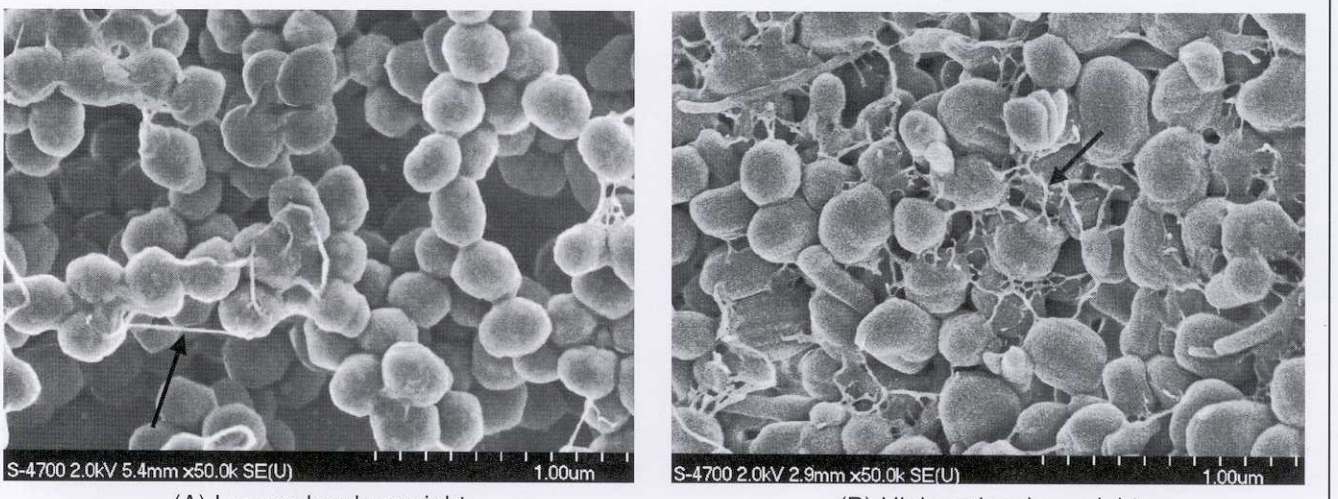


Fig. 4 Microscopy of processed cheese



(A) Low molecular weight

(B) High molecular weight

Fig. 5 Microscopy of colloid

4.4 Microscopy of bread yeast

Commercially available bread yeast was fermented in luke-warm water of about 37°C and about 10 times the yeast in volume. It was then quickly frozen for microscopy as shown in Fig. 6. Invagination of cells and hexagonal array of intramembrane protein particles of 10 nm (→) are clearly observed.

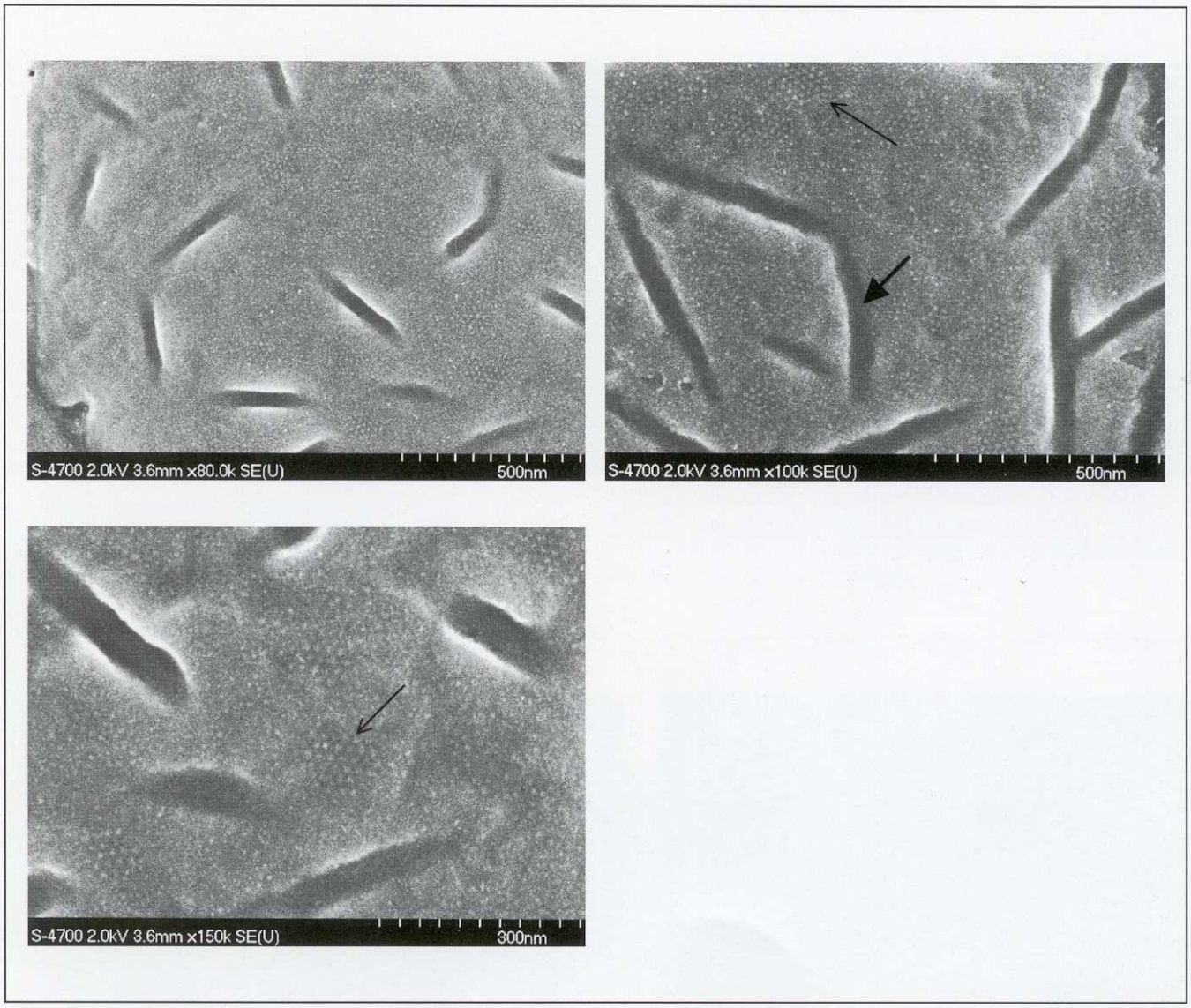


Fig. 6 Microscopy of bread yeast

5. CLOSING

We have reported on some unique capabilities of the S-4700 FE-SEM coupled with Alto 2500 cryogenic system. This combined system has allowed high magnification work at x100,000 or higher at low operating voltages. We wish to examine some more samples such as liquid, biological, and beam sensitive materials which require high resolution images. We hope to report on some more interesting findings in the future.

References

1. A.Robins; Proc. 56th Japan Electron Microscopy, P347 (2000)
2. T. Suzuki et al; Hitachi Technical Data SEM Sheet No.74 (1995)
3. M.Nakagawa et al; Hitachi Technical Data SEM Sheet No.100 (2000)

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