Scanning Electron Microscopy Project Portfolio
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12/9/15

Submitted for
MCR 785 Scanning Electron Microscopy
Fall 2015
N.C. Brown Center for Ultrastructure Studies
Part 1

A portfolio of micrographs demonstrating the following techniques:

• Critical Point Drying or TMS drying
• Depth of Field
• Backscatter
• Low voltage image of uncoated sample
• High Magnification (>50,000) - double coated
• Stereo Pair
• Cryofracture
Fig 1. Microscope Parameters: SEI
- Spot Size: 10 chevrons
- Aperture: 1
- Working Distance: 9mm

Critical point drying:
Mushroom gill
Fig 2a. Microscope Parameters: SEI
- Spot Size: 13 chevrons
- Aperture: 1
- Working Distance: 10mm

Depth of Field:
Mushroom gills
Fig 2b. Microscope Parameters: SEI

- Spot Size: 13 chevrons
- Aperture: 1
- Working Distance: 17mm
- Tilt: +2.8°

Depth of Field:
Mushroom gills
Fig 3a. Microscope Parameters: SEI

- Spot Size: 16 chevrons
- Aperture: 2
- Working Distance: 12mm
- Tilt: -5.8°

Backscattered Electron Imaging:
Tungsten/Silicon
Fig 3b. Microscope Parameters: BEI

- Spot Size: 16 chevrons
- Aperture: 2
- Working Distance: 12mm
- Tilt: -5.8°

Backscattered Electron Imaging:
Tungsten (light)/Silicon (dark)
Low voltage of uncoated sample:

Molybdenum

Fig 4. Microscope Parameters: SEI

- Spot Size: 16 chevrons
- Aperture: 1
- Working Distance: 15mm
High Magnification:
Basidiospores and sterigmata

Fig 5a. Microscope Parameters: SEI
- Spot Size: 12 chevrons
- Aperture: 1
- Working Distance: 8mm
- Tilt: ±29.4°
High Magnification:
Basidiospores and sterigmata

Fig 5a. Microscope Parameters: SEI
- Spot Size: 12 chevrons
- Aperture: 1
- Working Distance: 8mm
- Tilt: +29.4°
Fig 6. Microscope Parameters: SEI, 5kV

- Spot Size: 11 chevrons
- Aperture: 1
- Working Distance: 14mm
- Tilt: +8°

Stereo Pair:
Powdery mildew hyphal network
Cryofracture:
Cross-section of an Oak Leaf

Fig 7a. Microscope Parameters: SEI
- Spot Size: 12 chevrons
- Aperture: 1
- Working Distance: 9mm
Fig 7b. Microscope Parameters: SEI

- Spot Size: 12 chevrons
- Aperture: 1
- Working Distance: 9mm

Cryofracture:
Cross-section of an Oak Leaf
Part 2
Biological Sample

• Micrographs were taken of three different types of biological tissue; fungal, insect, and plant

• Preparation:
  – Fungal and insect tissue was fixed using 2.5% Glutaraldehyde in PBS for 1 hr. Samples then placed in 1% OsO₄ as a post-fixative, 1 hr. Dehydration with EtOH in a series from 30-100% post OsO₄. Critical point drying was accomplished from 100% EtOH. Samples then placed in carbon paint on an Al stub and immediately sputtercoated with a Au/Pd target for 60 seconds at 20 mA.
  – Plant tissue was frozen in liquid nitrogen (aprox. 20 sec) and then freeze fractured and placed in carbon paint on an Al stub and immediately sputtercoated with a Au/Pd target for 30 seconds at 20 mA, rotated 180°, and then coated again under the same parameters for a total of 60 seconds at 20 mA.

• Microscope parameters were set with a TEM grid as control and are placed under each figure in the following series.
Fig 1. Microscope Parameters:

- Spot Size: 10 chevrons
- Aperture: 1
- Working Distance: 9mm

**Biological Sample: Mushroom gill**
Biological Sample: Mushroom gill

Fig 2. Microscope Parameters:
- Spot Size: 10 chevrons
- Aperture: 1
- Working Distance: 9mm

Basidium 5.0kV x4500 2μm
Biological Sample: Grasshopper head

Fig 3. Microscope Parameters:
- Spot Size: 11 chevrons
- Aperture: 1
- Working Distance: 13mm
Biological Sample:
Longitudinal cryofracture of an oak leaf stem

Fig 4. Microscope Parameters:
- Spot Size: 12 chevrons
- Aperture: 1
- Working Distance: 10mm
Fig 5. Microscope Parameters: SEI

- Spot Size: 12 chevrons
- Aperture: 1
- Working Distance: 10mm
- Tilt: +5.8°

Biological Sample: Mushroom gill
Part 2
Non-Biological Sample

• A series of micrographs were taken of a pair Gillette® razorblades comparing fresh vs. used.
• Parameters were set with a TEM grid as the control, represented by the first micrograph in the series.
• Microscope Parameters for all NBS micrographs:
  – Samples placed in carbon paint on Al stub, uncoated
  – Accelerating Voltage: 10kV
  – Spot Size: 12 chevrons
  – Aperture: 1
  – Working distance: 10mm
Nonbiological Sample
Fig 2.

Nonbiological Sample
Nonbiological Sample

Fig 3.
Nonbiological Sample

Fig 4.

Fresh Razor  10.0kV  x650  20μm
Nonbiological Sample

Fig 5.
Fig 6.

Nonbiological Sample
Nonbiological Sample

Fig 7.

Used Razor 10.0kV x1900 5μm
Nonbiological Sample