Imaging techniques and troubleshooting

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Topics:
• Intro to stacking
• Specimen preparation
• Choosing a background
• Microscope objectives
• Imaging larvae in ethanol
What is image stacking?
Specimen cleaning

- Don’t nit-pick over tiny dust
- Minimize touching with hands
- Limit cleaning on type material
Cleaning supplies

• Stage for specimen
• Fine-tip forceps (removing pin labels, hairs, etc.)
• Paintbrush for gentle cleaning
• “Rocket” duster to blow off particles
• Diluted ethanol to remove dust and clean off grease
Imaging setup and light diffusers

- Stage with neutral background (18% grey cardstock)
- Frosted acrylic sheets
- Modified paper lantern
- Your imagination
Lighting

• Flash bulbs are essential for crisp, high-resolution images

• Many different flash setups are available for a range of budgets

• White-balance each time your lighting changes using a neutral spot in your image

• White backgrounds can wash out colors
Background comparisons
Background comparisons

Grey

White

Blue

Black
Minimizing specimen movement is critical.

- 5x, 10x, and 20x objectives can capture tiniest details.
General guidelines

• Cleaning isn’t essential but makes for nice photos
• A stable base is critical
• Shutter speed minimum 1/100s – can be faster with flash bulb setup
• Use neutral grey background whenever possible – no colors!
  • Some exceptions for black and white backgrounds
Imaging alcohol specimens

- Petri-dishes or glass beakers to fit your specimen
- 30%-70% ethanol
- Glycerin
- Specimen pins
- Forceps
- Patience
Beaker or petri dish large enough for specimen, but not wasting glycerin or ethanol

Glycerin – personal lubricant is thicker than pure glycerin and soluble in ethanol
Specimen positioning

- Settle specimen on top of glycerin
- Anterior/posterior views may require more of specimen to be submerged
- Gently pour ethanol to cover specimen
- Pins or forceps can be used to gently remove bubbles