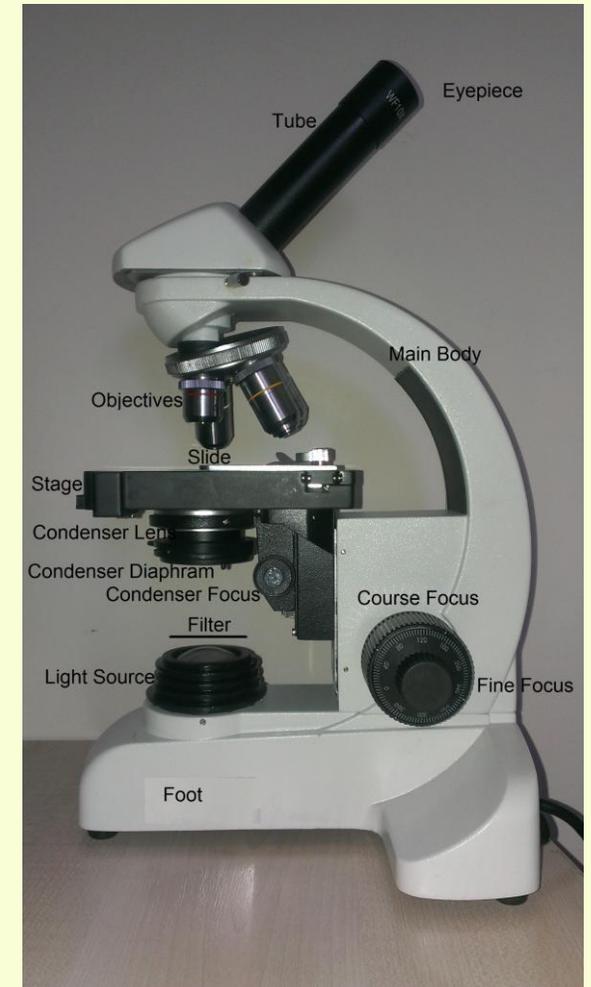


Nosema Testing and Preparing Pollen Slides

Sean Stephenson

Compound Microscope

- **Stage**
 - Hold the object to be examined and to move the object under the lens. Usually called a mechanical stage.
- **Condenser**
 - Illumination of object, produce a cone of light (focused on the top of the slide) to exploit the objective
- **Diaphragm**
 - Controls the amount of light passing through to the object, reduced light improves the contrast
- **Eyepiece**
 - Balance of the magnification e.g. x10 in eyepiece x x10 in objective = x100 magnification
- **Objective**
 - Lens with a short focal length, large aperture, produces the real image
- **Course and Fine Focus**
 - Course focus controls large approximate movement of objective
 - Fine focus, focuses in on the object
 - Focus stop prevents the objective touching the slide



Setting up the microscope

- **Set Focus Stop**

- Write initials on centre of slide and place on the stage
Select the highest magnification objective
Move the course until the objective nearly touching the coverslip, view from the side of microscope
Set the Focus Stop

- **Focus on slide**

- Start with lowest power objective
With brightness about 80%, condenser in uppermost position and diaphragm fully open
Place a slide with an ink mark on the top surface of the slide on the stage (no coverslip)
Focus on the edge of the image

- **Focus the condenser**

- Move the filter holder to one side
Place needle or fine object on the light source
Adjust the focus of the condenser until the image of the needle comes into focus
Do not touch the focus of the objective
Should end up with both in focus
Repeat with each objective up to the magnification you intend to use

- **Set up the diaphragm**

- Remove the slide from the stage
Remove the eyepiece
Open the condenser diaphragm
From a distance look down the eye tube
Close the diaphragm slowly until the edges of the diaphragm can be seen, blocking out about 10% of the light

Nosema examination

Remove the abdomens of 30 bees

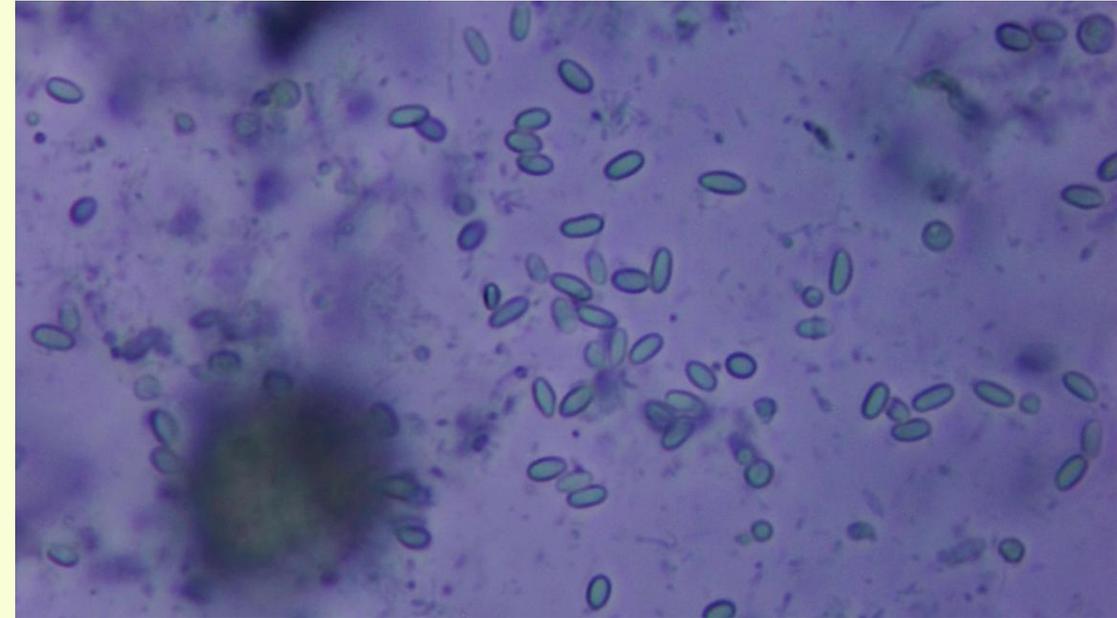
Place in plastic bag

Add 15ml water and crush

With glass rod dab 3 times on slide

Add coverslip

Examine under microscope@ x400



Preparing a pollen slide – from stored pollen

- Place anther with minimum of debris in watch glass, add 100% isopropanol and mix by swirling
- Allow most of isopropanol to evaporate and remove the excess debris
- With a glass rod dab liquid from the watch glass in the centre of a clean slide and place on a warming plate to dry and allow to cool
- Place a small square of glycerine with fuchsin on pollen, with clean coverslip above
- Place on warm plate and allow stain to flow to the edge of the coverslip
- Allow to cool, if good specimen label with date made, scientific name, common name and approximate size
- After 24 hours seal coverslip with clear nail varnish

Preparing a pollen slide – from anther

- Collect flower in bud, put in water in light warm room, when the anthers are dehiscent, cut away petals, stigma etc.
- Prepare a clean slide by putting a drop of 50/50 water/isopropanol in the middle of the slide
- Dab the anther on the liquid, allow to dry (use hot plate)
- Run 100% isopropanol over slide to degrease pollen and allow to dry and cool
- Place a small square of glycerine with fuchsin on pollen, with clean coverslip above
- Place on warm plate and allow stain to flow to the edge of the coverslip
- Allow to cool, if good specimen label with date made, scientific name, common name and approximate size
- After 24 hours seal coverslip with clear nail varnish

Preparing a pollen slide – from pollen load

- Break up one load in a watch glass with isopropanol to make a soup
- With a glass rod dab liquid from the watch glass in the centre of a clean slide and place on a warming plate to dry
- Place a small square of glycerine with fuchsin on pollen, with clean coverslip above
- Place on warm plate and allow stain to flow to the edge of the coverslip
- Allow to cool, if good specimen label with date made, scientific name, common name and approximate size
- After 24 hours seal coverslip with clear nail varnish

Work practice

- Avoid contamination
 - Glycerine
 - Tools
 - Microscopes
- Do not rush
- Practice makes perfect
 - Make several slides at the same time
- Hand back equipment as it was found

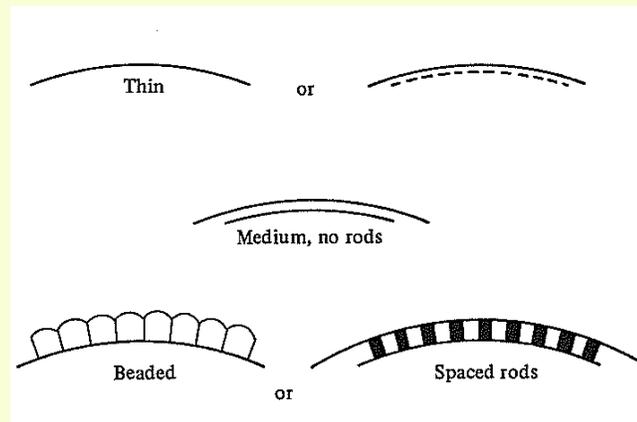
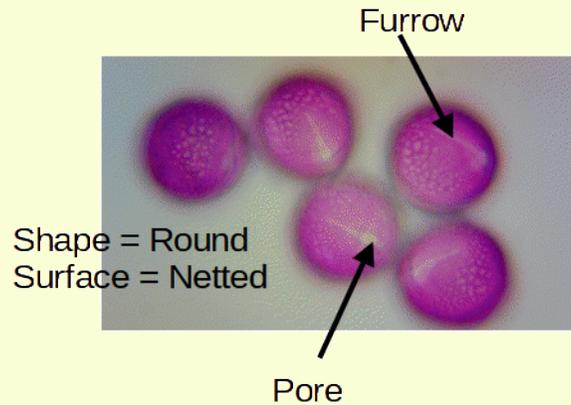
Worksheet

- Set up the microscope
- Nosema examination
 - Positive sample
 - Unknown sample
- Make pollen slides
 - Stored pollen
 - Anther
 - Pollen load
- Describe slides in terms of Sawyer

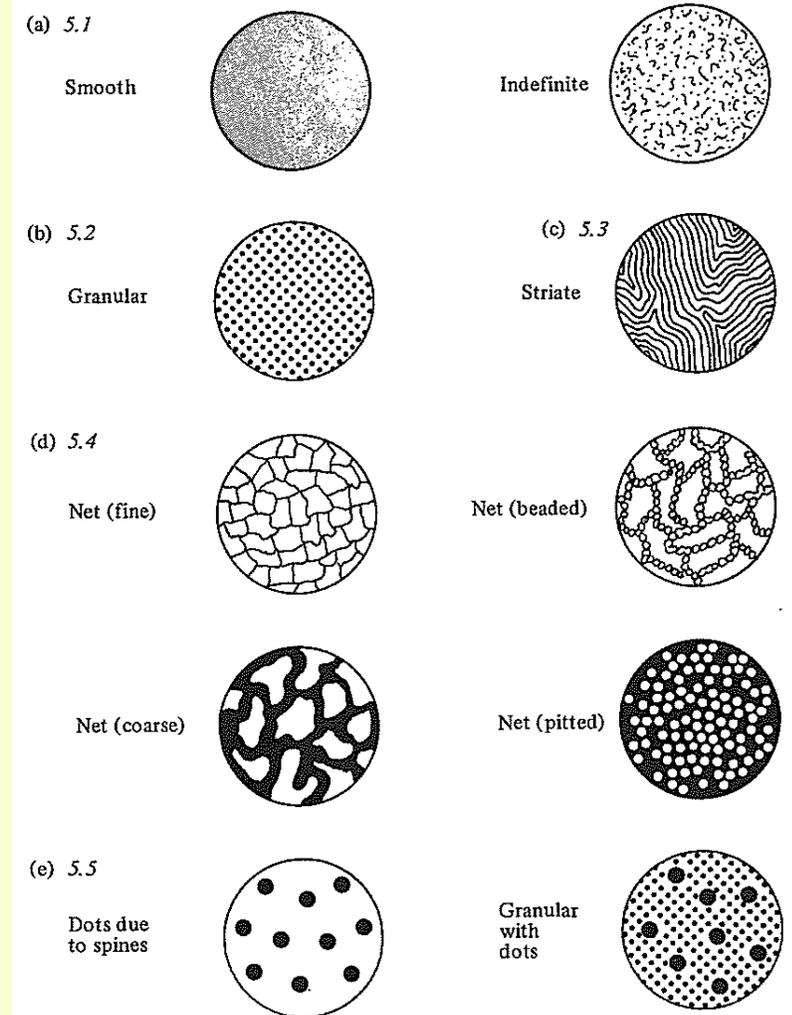
Pollen Key

Size	<ol style="list-style-type: none"> 1. Very Small <20µm 2. Small 20-30µm 3. Medium 30-50µm 4. Large 50-100µm 5. Very Large >100µm
Shape	<ol style="list-style-type: none"> 1. Round or irregularly Round 2. Oval, flattened 3. Oval, elongated 4. Long 5. Triangular 6. Semi-circular or boat shaped 7. Multi-sided or irregular
Aperture Numbers	<ol style="list-style-type: none"> 1. 0 or indefinite 2. 1-2 3. 3 4. 4-6 5. 7-12 6. >12
Aperture Type	<ol style="list-style-type: none"> 1. Pores only 2. Furrows only 3. Furrows with pores 4. United or irregular furrows may occur
Surface	<ol style="list-style-type: none"> 1. Smooth or indefinite 2. Granular 3. Striate 4. Net or pitted 5. Isolated dots to spines or other projections
Exine, Section	<ol style="list-style-type: none"> 1. Thin 2. Medium, no rods 3. Medium with spaced rods or beaded 4. Medium or thick with coarse external rods 5. Layer of close, thin rods 6. Long, thin spines 7. Large, broad based spines 8. Small or very small spines or warts 9. Other projections

Extracts from: Rex Sawyer Pollen Identification for Beekeepers



Exine



Surface

Only 3 books needed to get started with Microscopy

