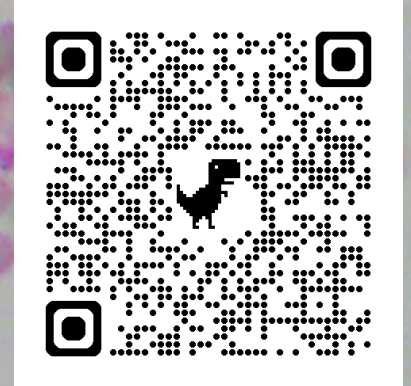


# Honey Sources via Microscopy

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Sean Stephenson and Jenny Morgan



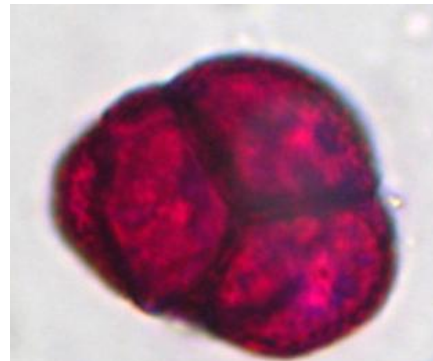
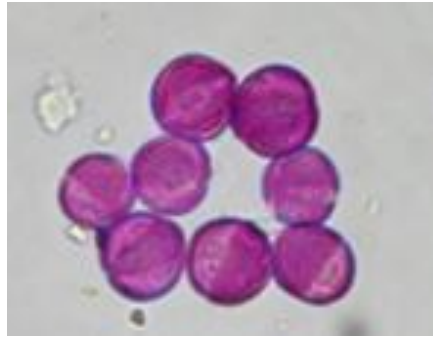
sean@resms.co.uk

# Plan for the day

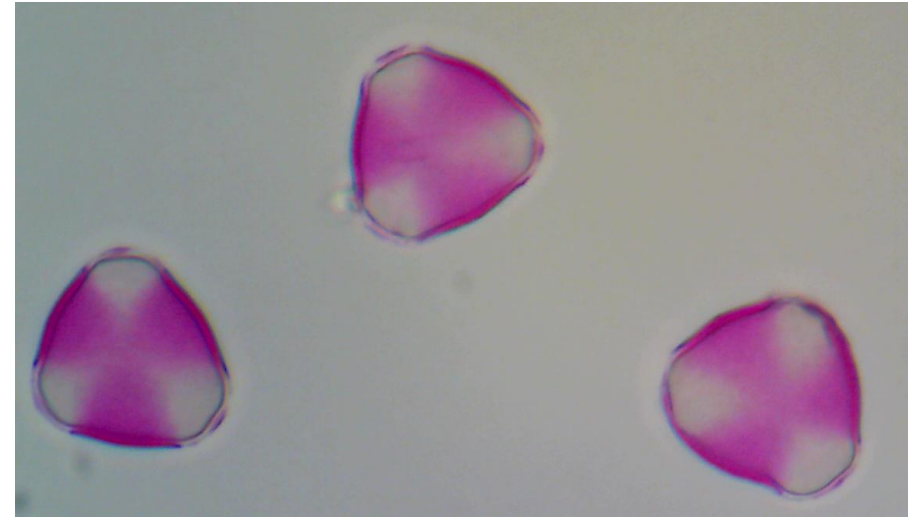
Set-up and understand the compound microscope

Prepare Pollen slides

- from pollen
- from pollen loads
- from honey



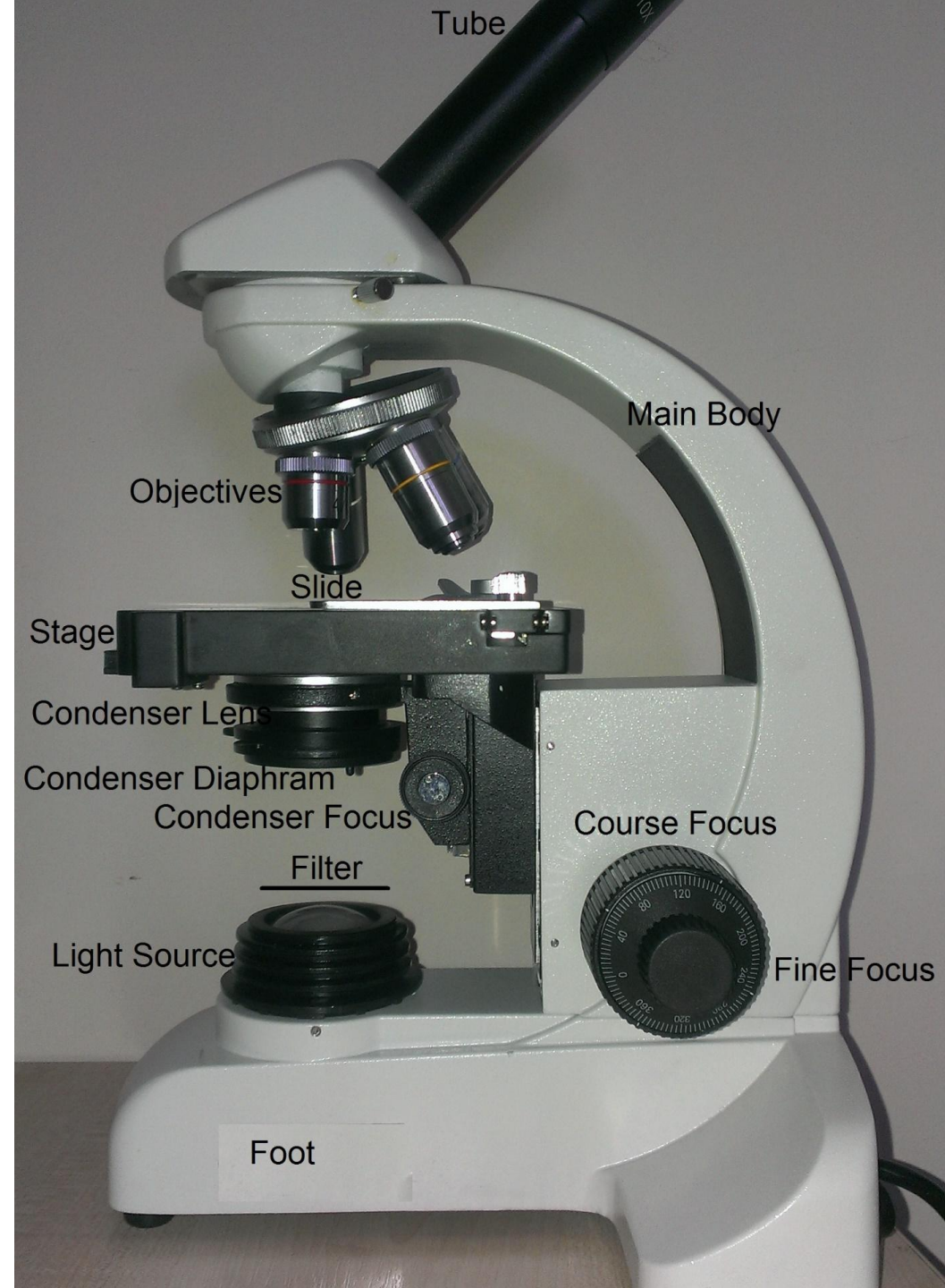
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# Compound Microscope

- **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

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# Setting up the microscope

- **Set Focus Stop**

Place a slide with cover slip 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**

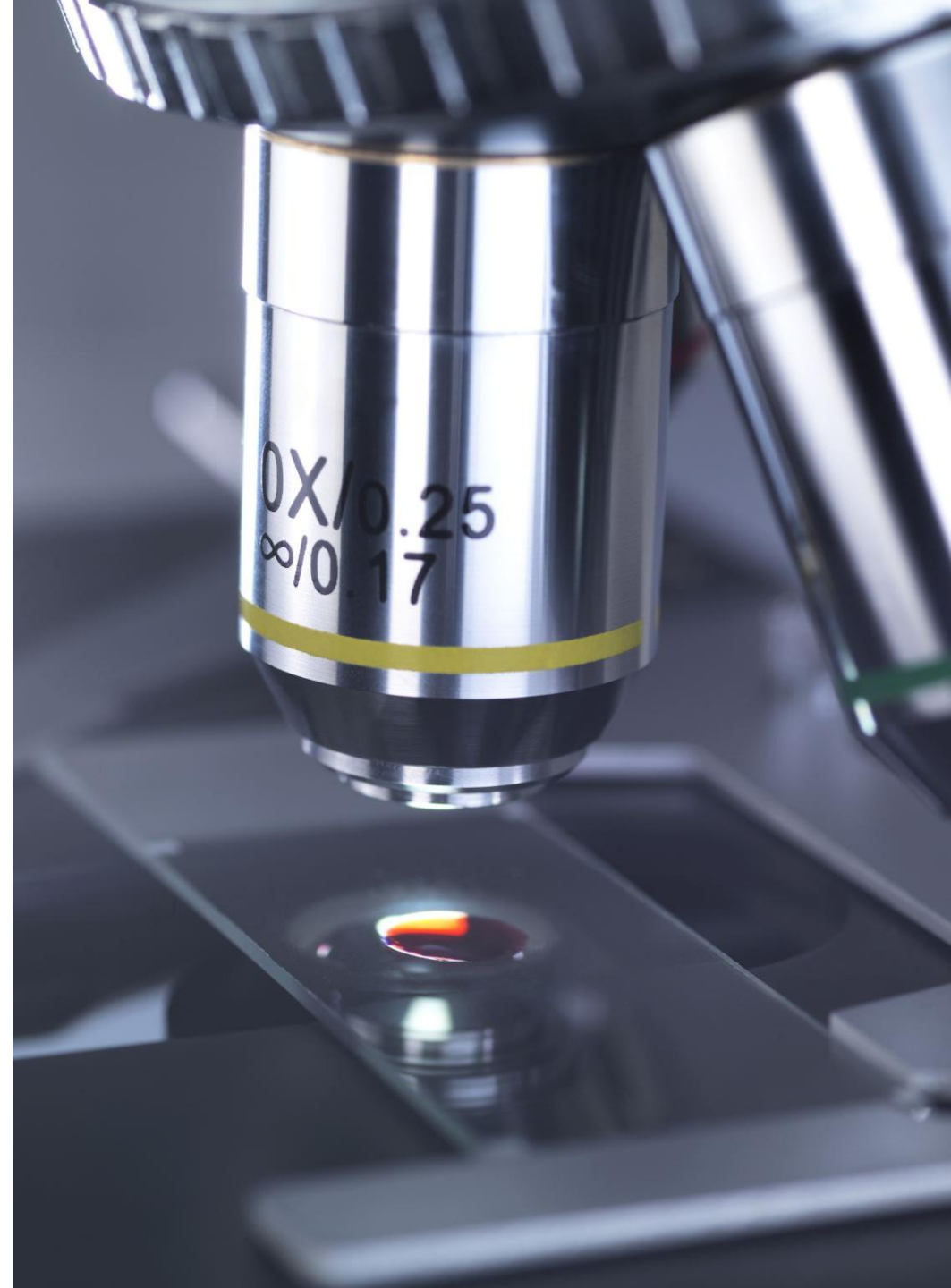
Start with lowest power objective  
With brightness about 80%, condenser in uppermost position and diaphragm fully open  
Place a slide with an ink mark on the top surface of the slide on the stage (no coverslip)  
Focus on the edge of the image

- **Focus the condenser**

Open filter  
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

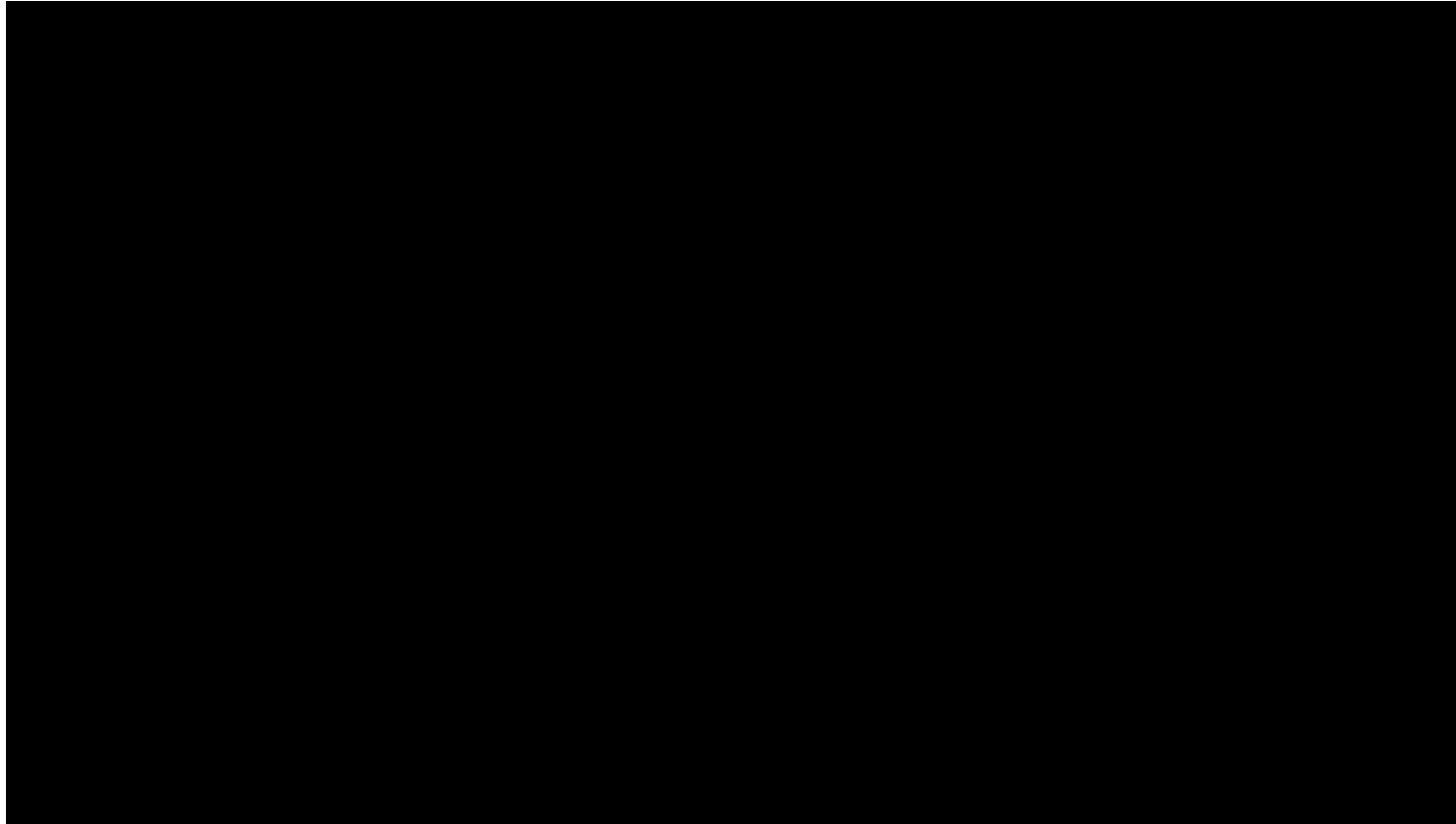


# Work practice

- 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

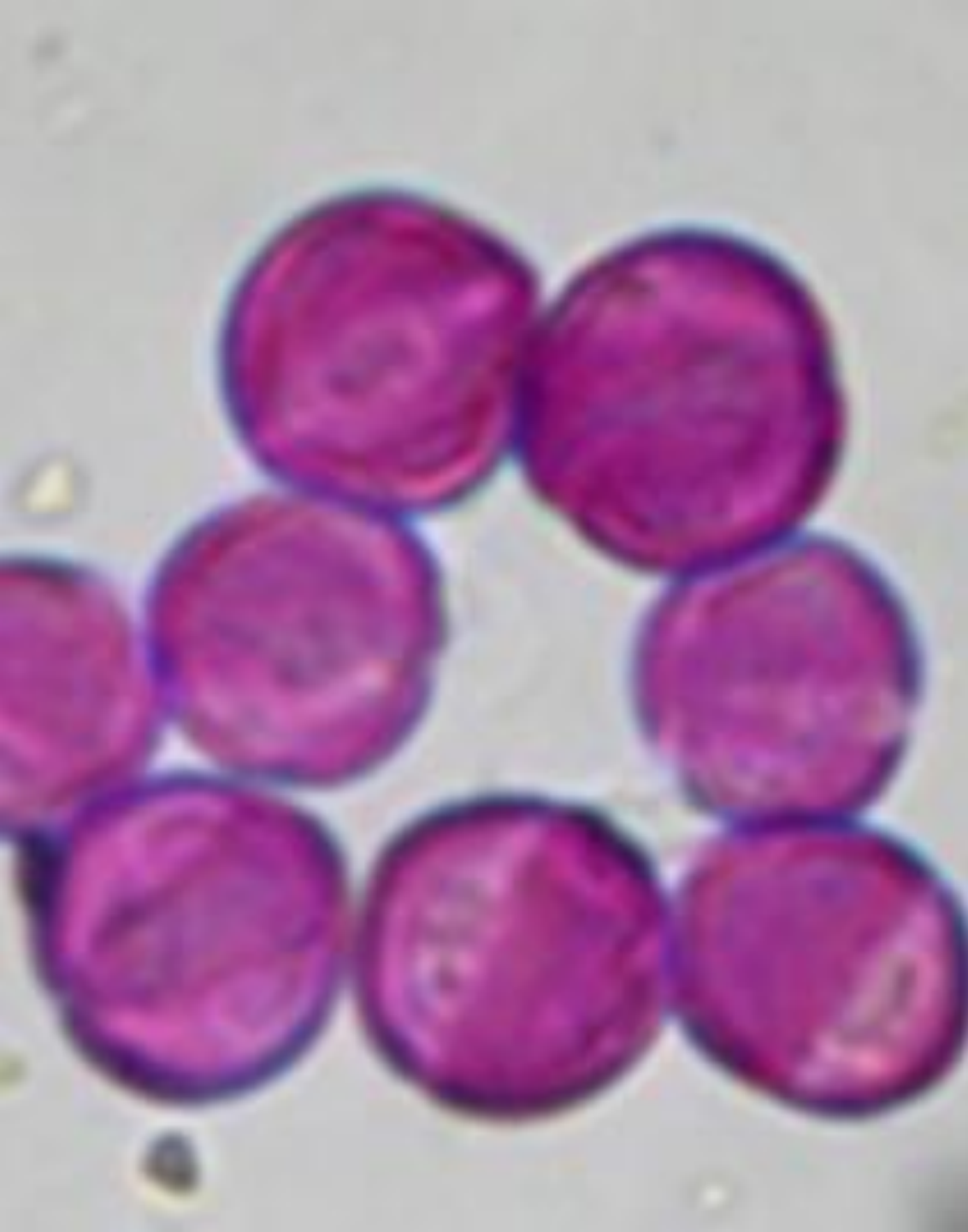


# Preparing a slide



# 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 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

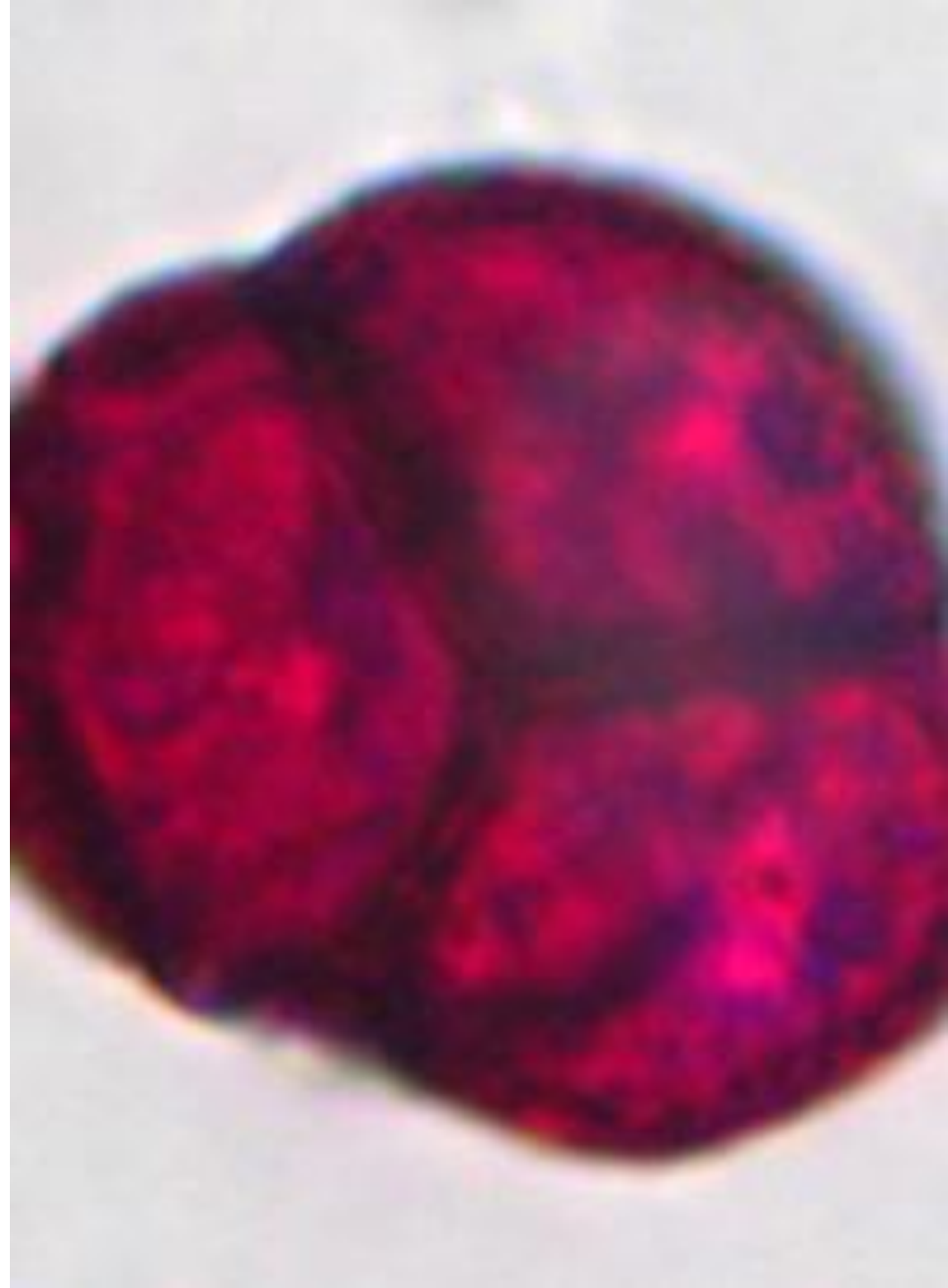
# Preparing a pollen slide – from honey (sedimentation)

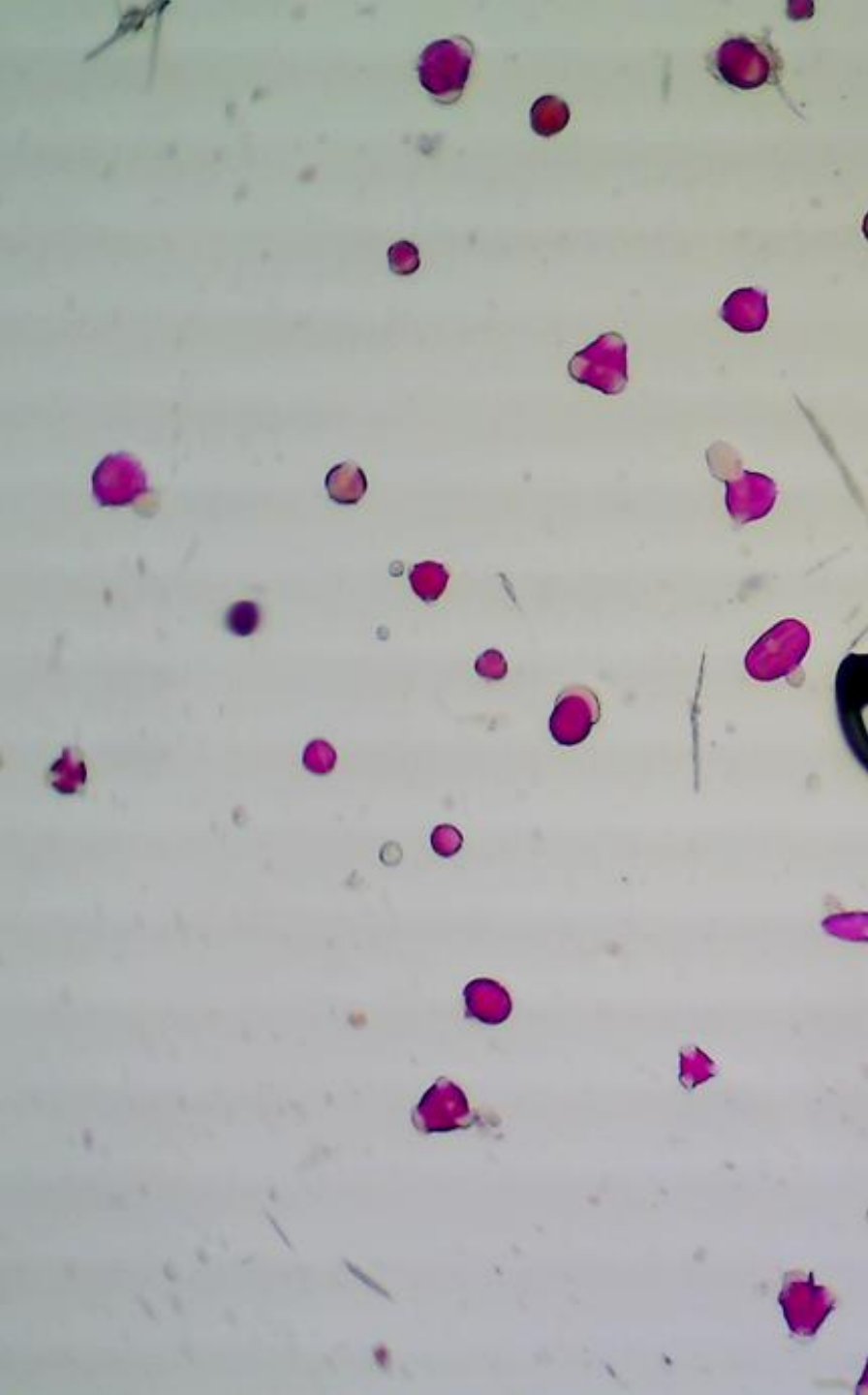
- Place 20g honey in a large test tube and fill with warm water
- Seal and shake
- Place in a warm place for 2 days
- 1st day shake regularly
- 2nd day there should be visible sediment in the bottom of tube
- Syphon off with a pipette as much liquid as possible
- Insert glass rod into sediment and dab liquid onto a slide x 3 before drying on a warming plate
- 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 honey (10µm filter)

- Mix 20g of honey with warm water in large test tube, shake until honey is dissolved
- Pour mixture through filter, rinse with slow running warm water
- Pollen will be visible on the filter, with a glass rod remove and dab onto a clean slide x3 before drying on a warming plate
- 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

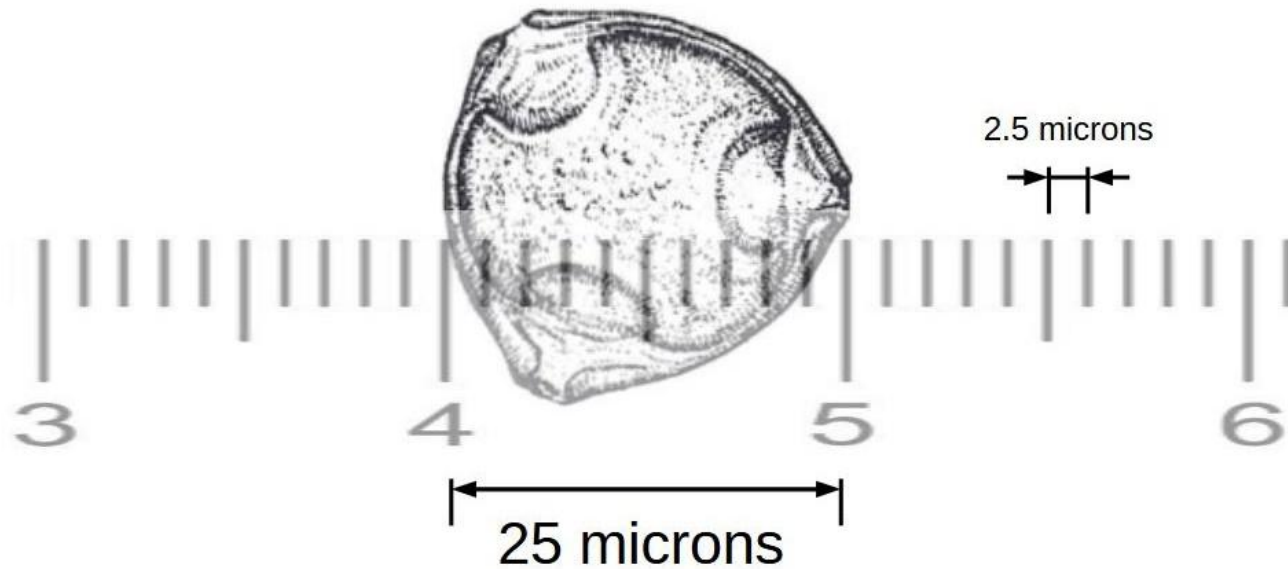




## Preparing a pollen slide – from honey (centifuge)

- Mix 20g of honey with warm water in two large test tube, shake until honey is dissolved
- Place in centrifuge and spin at 2,000 rpm for 10 minutes
- There should be sediment in the base of each tube
- Remove with a pipette as much liquid as possible and merge sediment from both tubes into one
- Top both tubes up with fresh warm water and spin for another 10 minutes
- Pollen will be visible in the base of the tube, remove excess water with a pipette and empty contents on to watch glass
- Dab some sediment onto a clean slide x3 before drying on a warming plate
- 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

# Measuring a pollen @ x400



Note:

X40 major division = 250 microns

x100 major division = 100 microns

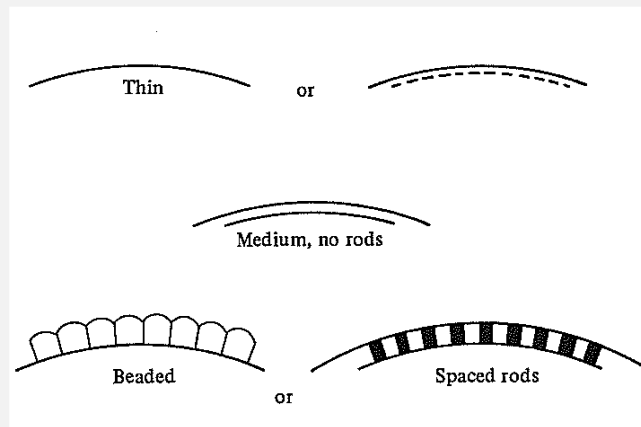
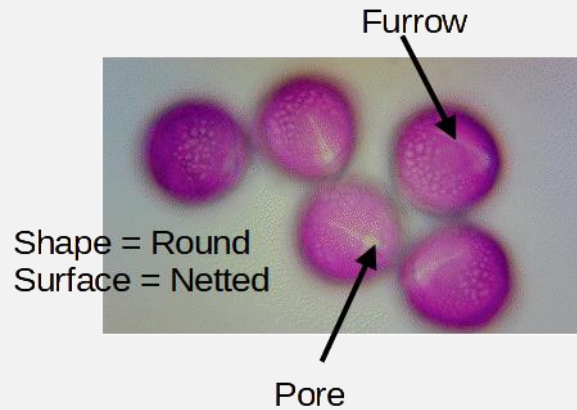
X600 major division = 37.5 microns

X1,000 major division = 10 microns

## Pollen Key

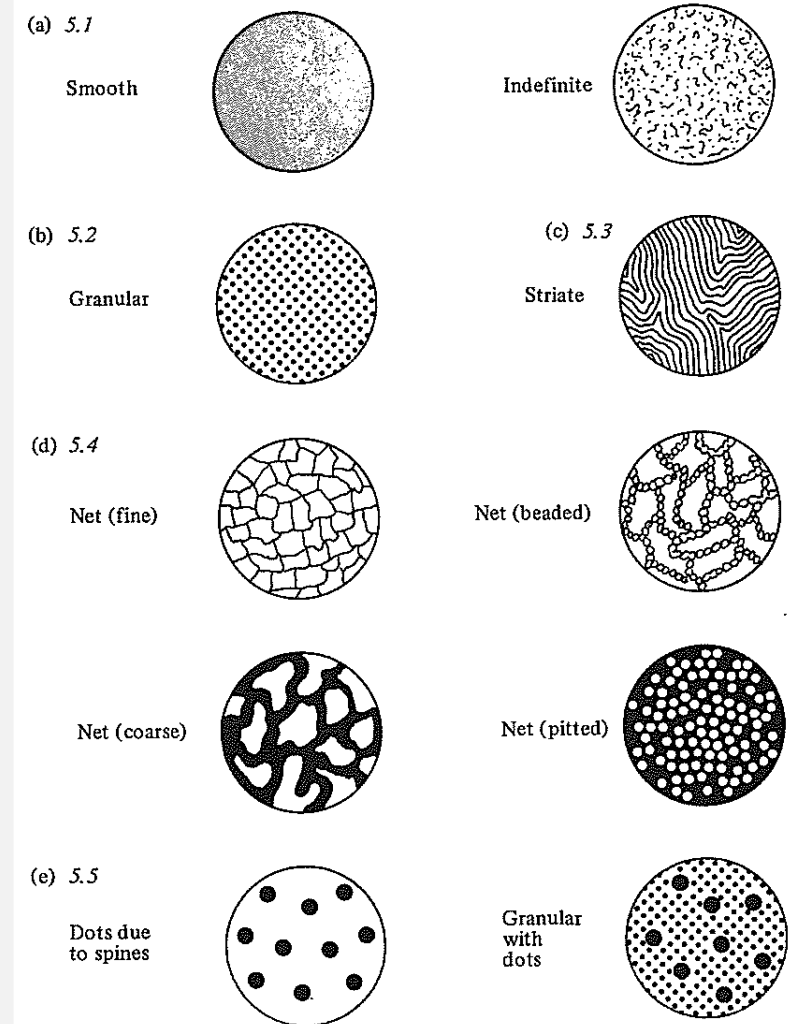
<b>Size</b>	<ol style="list-style-type: none"> <li>1. Very Small &lt;20µm</li> <li>2. Small 20-30µm</li> <li>3. Medium 30-50µm</li> <li>4. Large 50-100µm</li> <li>5. Very Large &gt;100µm</li> </ol>
<b>Shape</b>	<ol style="list-style-type: none"> <li>1. Round or irregularly Round</li> <li>2. Oval, flattened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

## Extracts from: Rex Sawyer Pollen Identification for Beekeepers



## Exine

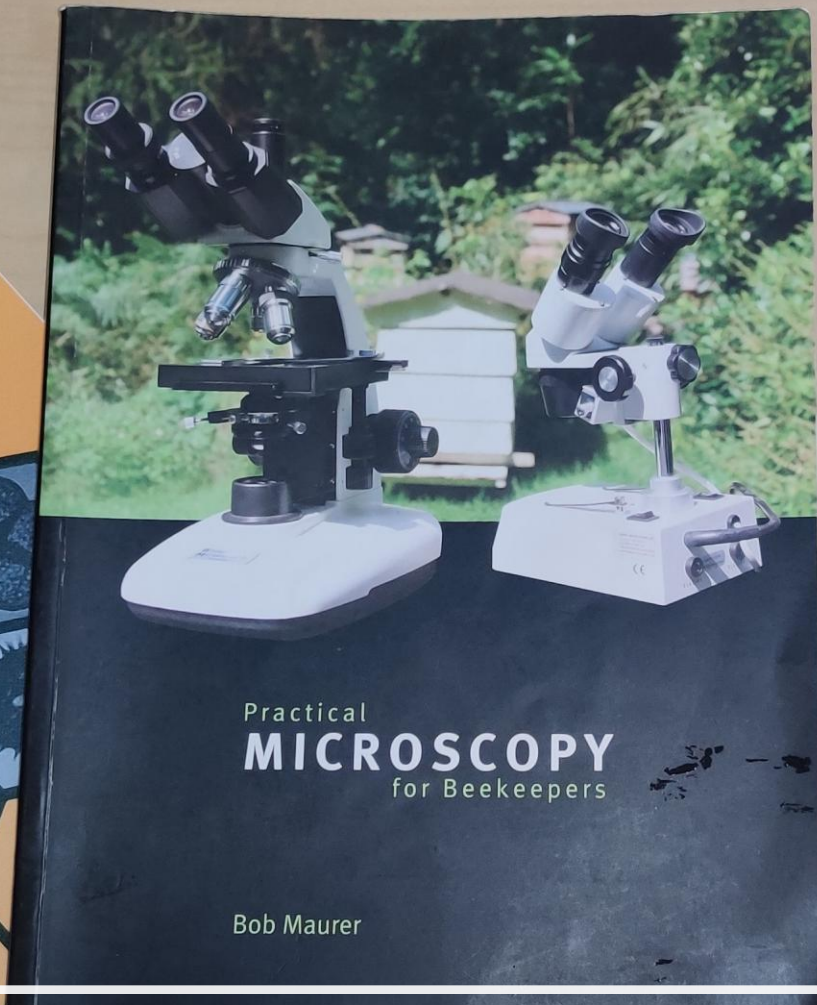
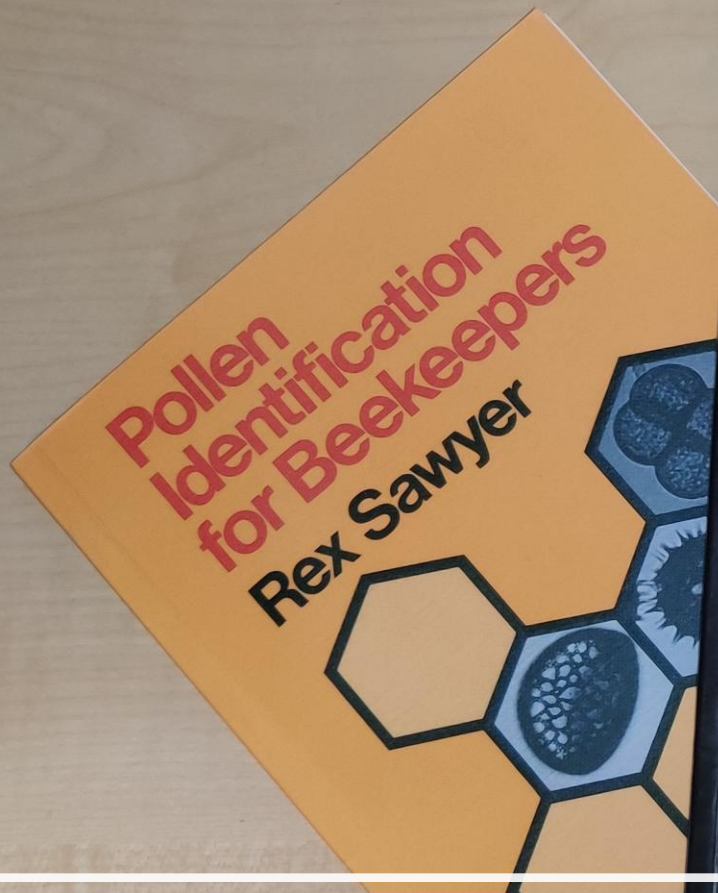
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## Surface

# Rex Sawyer pollen keys for pollens

Willow	<a href="#">Salix caprea</a> 1,1/2,3,2/3,4,3,-,4/5		Rape	<a href="#">Brassica napus</a> 2,1/5,3,2,4,3,-,5	
Heather	<a href="#">Calluna vulgaris</a> 3,7,3,3,1(2),2,1/2,2		Sycamore	<a href="#">Acer pseudoplatanus</a> 3,2/5,3,2,3,2(3),-,1/2	
Hazel	<a href="#">Corylus avellana</a> 2,2/5,3,1,1,2,2/5,5				



**Only 3 books needed to get started with Microscopy**