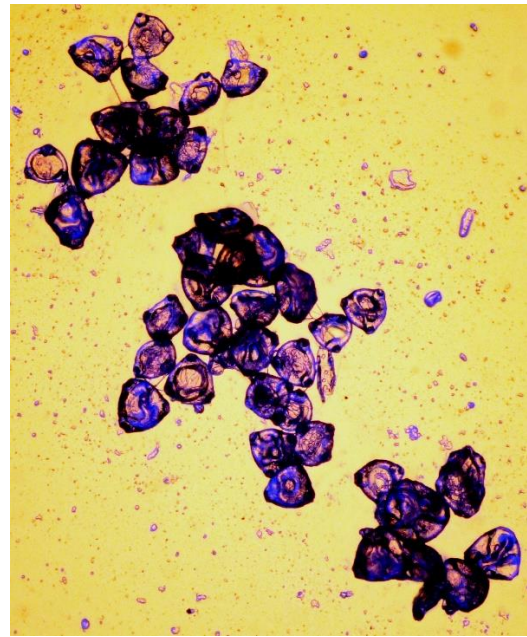
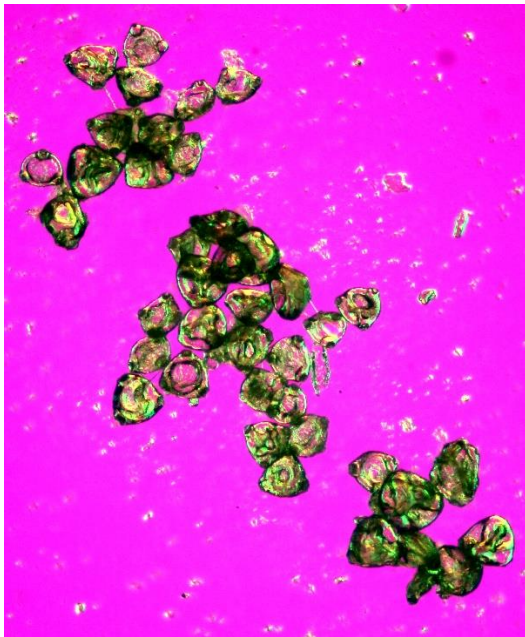


Rheinberg Illumination.

A method of 'staining' and enhancing microscopic specimens with coloured light.

I was introduced to this technique at a meeting of the Leeds Microscopical Society about two years ago. We stamped out discs of different sizes from coloured plastic film. These were introduced into the illumination entering the condenser underneath the stage of the microscope which supports the specimen being examined. The effect was startling as the specimen was illuminated in one colour eg. purple, while the background was a contrasting colour eg. yellow. This made the structural features stand out. In the case of pollen (mounted in water) the exine, pores and furrows were clear enough to help with identification without resorting to staining with fuchsin and glycerine jelly.



Fuchsia pollen (unstained) Rheinberg illumination using different combinations of coloured filters.

(Photos John Chandler)

Not only do small specimens such as pollen show up well but even comparatively large objects such as Varroa are enhanced by this technique.



A combination of blue light (background) and red and yellow light (specimen) helps to pick out features on Varroa. Photo John Chandler

Julius Rheinberg presented his “Multiple Colour Illumination” to the Quekett Club in 1896, but because of the difficulty in making coloured glass discs it is a method of illumination that was largely ignored until the advent of plastic film.

Rather than cutting out discs from plastic film with a metal stamp, it is now possible to print them onto an overhead projector (OHP) film using an inkjet or laser home printer and free software.

A full description of this method can be read on the Quekett Microscopy web site.

<http://www.quekett.org/resources/making-rheinberg-illumination-discs>

I will summarise the procedure below, but if you are going to try this you would be well advised to study the excellent article by Carel Sartory (President of the Quekett Microscopical Club).

1. You need to know how big to make your filters and you need to know where to place them.

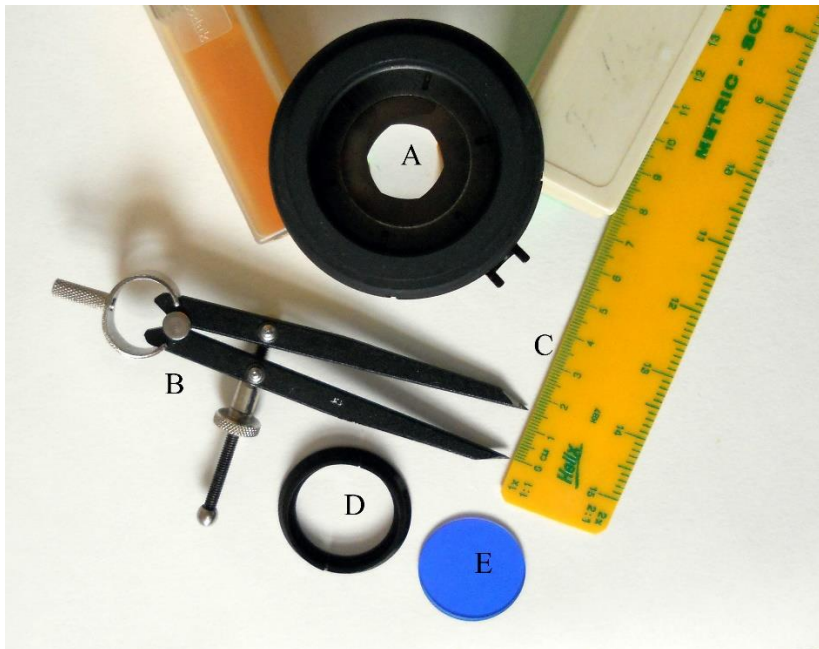
The filters must go under the iris of the microscope condenser. Most condensers have a swing-out filter holder. The discs that you make must fit into the holder so this is the first measurement that you will require.

Then measure the size of the central stop or colour patch. This can be tricky. Set up your microscope with your X10 objective and use a prepared slide to focus on the specimen. Remove an eyepiece and look down the microscope at the rear of the objective lens while at the same time adjusting the iris. When closed you will see a very small circle of light. Slowly open the iris until it just disappears and the objective is fully lit.

Carefully remove the condenser without touching the iris lever and measure the diameter of the iris. You can do this with a pair of school dividers and a ruler.

Repeat this procedure for different objectives. (NB this technique is not suitable for high power objectives above X40.)

For a standard microscope, you may find that the outer diameter of the filter will be, say, 32mm and the central coloured stop will be about 15mm for X5 objective, 18mm for X10 and as much as 23mm for X40 objectives.



Measurements needed: (A) Diameter of Iris in condenser for a specific objective and (D) the diameter of the lens holder. A typical standard blue filter (E) is shown – the diameter can be measured easily. I use a pair of dividers (B) and a ruler (C) to measure accurately. (Photo John Chandler)

2. You need to download a free postage label making program.

I have used the programme that is recommended by Carel Sartory which is a free to download programme by Avery Dennison for designing postage labels. It is called **DesignPro 5.5 Lite Edition** and it makes the production of a range of coloured filters on OHP transparent sheets fairly straightforward.

Use the template for a sheet of 21 labels 63.5 X 38.1 mm in size for each filter and patch. Load your label template and use the design label screen with 1 mm horizontal and vertical scales and the grid of blue dots set 1 mm apart.

Ignore the 'Master' tab. The filter and patch size are designed on the 'Label 1' tab.

Draw a circle the same diameter as the filter using the drawing ellipse tool and the rulers and blue dot grid. Use the 'centre vertically and horizontally' commands to centre your label. Repeat the process for the patch, but set a wider line thickness of about 1mm.

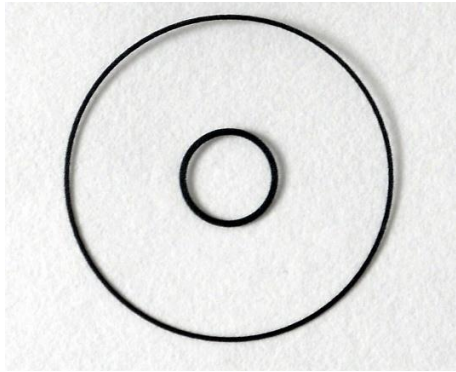
You now have a template label with two correctly sized circles. Using the 'copy tab' command make as many copies of the label as you require - up to 20 for one transparency A4 sheet.

With the dimension and line thickness set, you are able to change the colour of the central patch and the peripheral area for each label.

Remember that label 1 is your template so start with label 2 and set the periphery colour by right clicking on the outer circle and select 'fill colour' to choose an appropriate colour. Repeat the process for the inner patch circle and choose a contrasting colour.

Move to label 3 and ignore the periphery but fill the inner patch with the same colour as label 2. You now have the first Rheinberg filter ready to cut out and assemble.

Repeat the process for labels 4 and 5 etc. but use different colour combinations until your sheet is full. NB. To make darkground stops choose black as the patch colour and a clear uncoloured periphery.



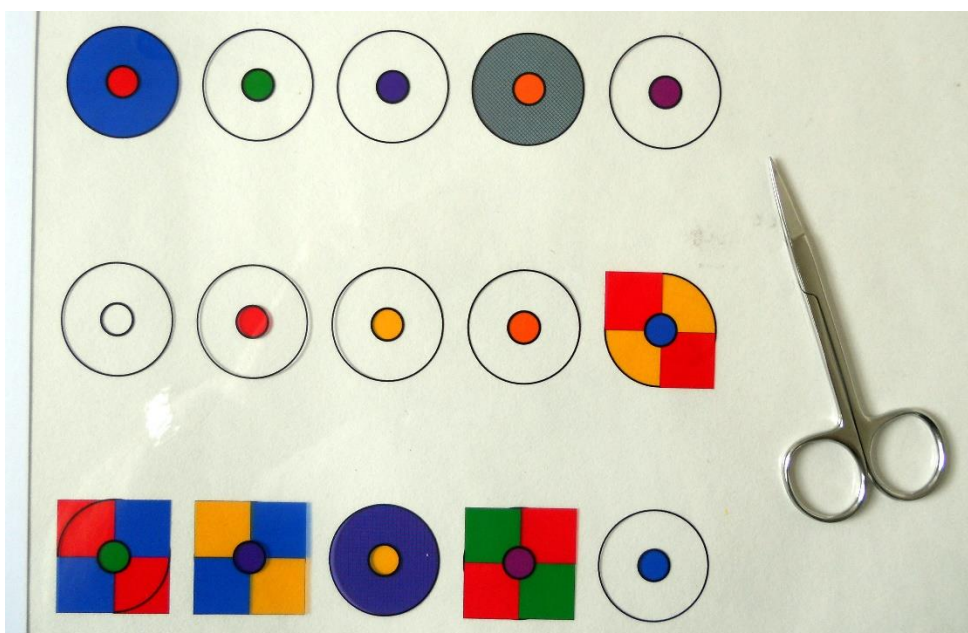
This is the template that is 'label' 1 for a specific objective. The inner ring forms the central patch and the outer periphery ring will need to be a different colour. (Photo John Chandler)

3. **You need to obtain clear printable A4 OHP sheets.**

These must be high quality and not have a mottled surface.

Print your sheets at high quality (as for photos) and take care not to smudge the coloured discs.

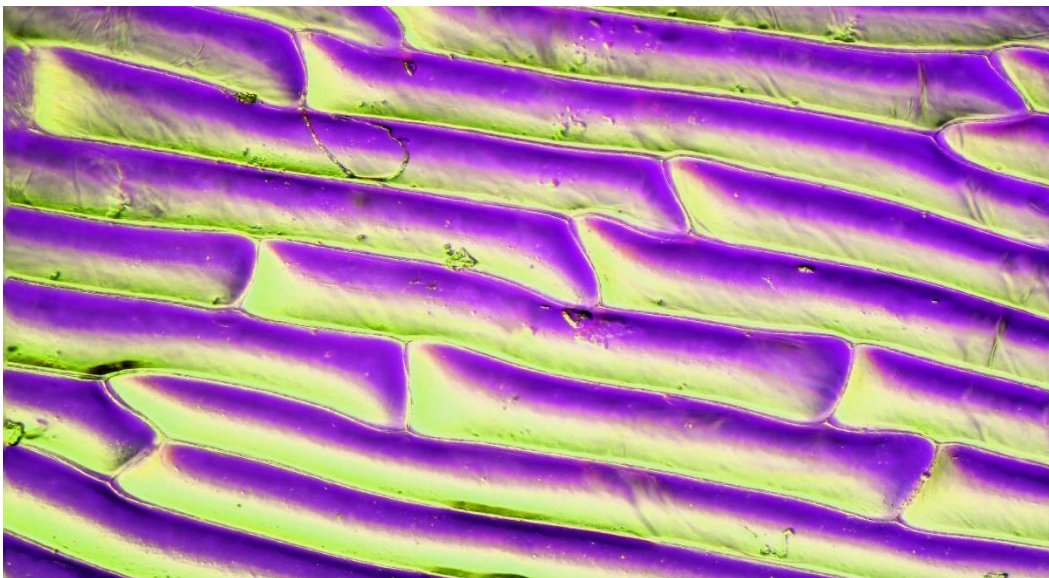
4. Each Rheinberg disc consists of two labels – one with a coloured periphery and central patch and one with a central patch only. **Cut out the pair of labels with sharp scissors and stick them together.** (For inkjet printers breathe on 'inky' side and gently squeeze them together. Take care to 'register' the two labels so they align together accurately.)



A few discs printed onto an OHP sheet ready to be cut out. You can experiment with combinations of colours in the outer discs. The label writing programme allows you to do this. (Photo John Chandler).

5. Use an unstained specimen to try out your Rheinberg filters. Remember that darkground illumination (a simple central black patch) is ideal for hunting for *Nosema* spores. A blue/red and green filter will enhance views of acharine and varroa mites as well as bees' wings.

You can broaden your biological and scientific knowledge by looking at other specimens. Try sugar crystals in honey, thymol crystals and hair from different animals. Using Rheinberg filters I have recently looked at fresh onion skin to observe movement within the cytoplasm and done a comparative study of the hair from Common pipistrelle and Natterer's bats!



Cells of onion skin (Rheinberg illumination). (Photo John Chandler)

I am indebted to Carel Sartory (President of the Quekett Microscopical Club) for the information that I have used in writing this article. I would urge anyone interested in trying this technique to read Carel's article on the subject to be found in the Quekett Club magazine. The web site details can be found above.

John Chandler (Harrogate and Ripon BKA)

