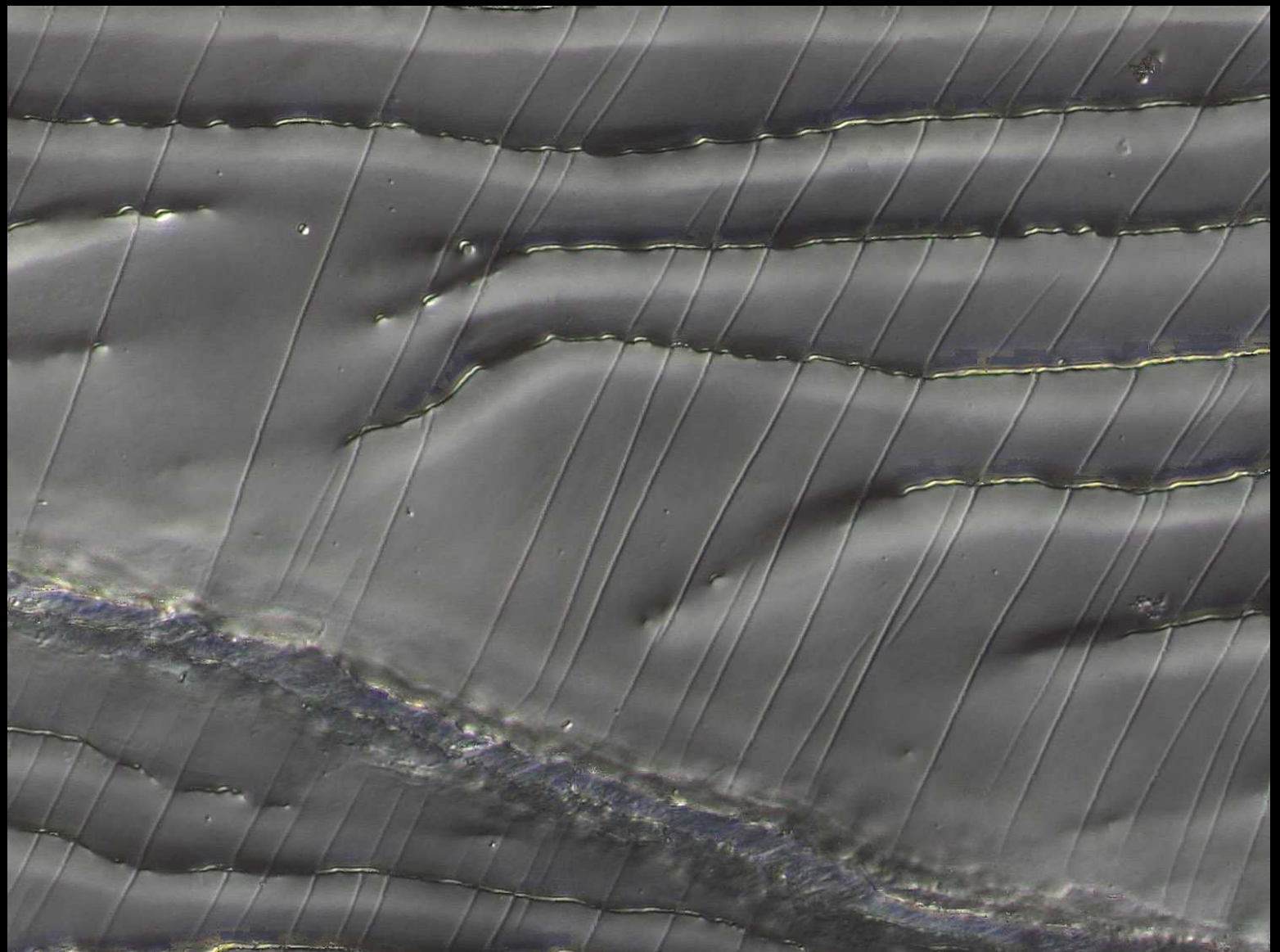
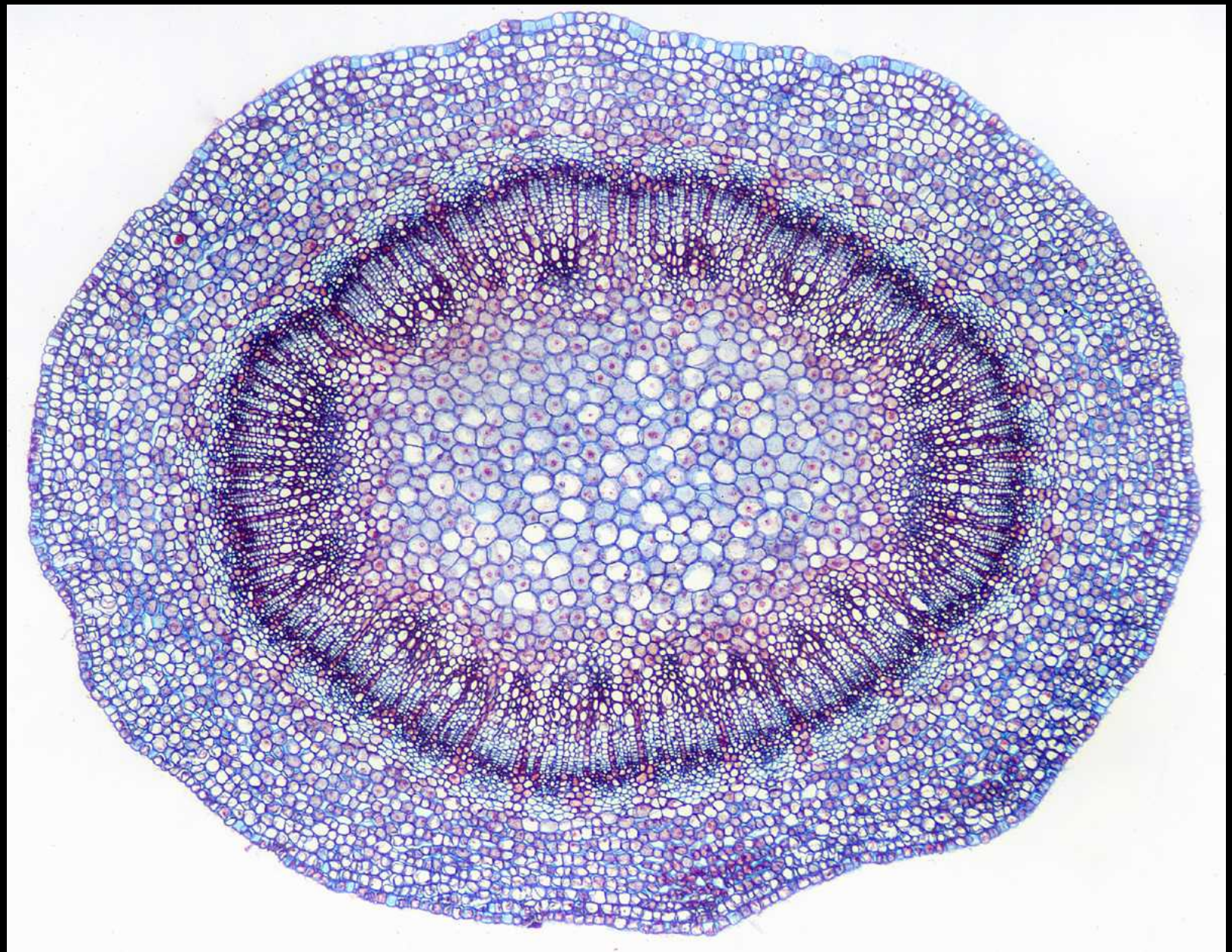
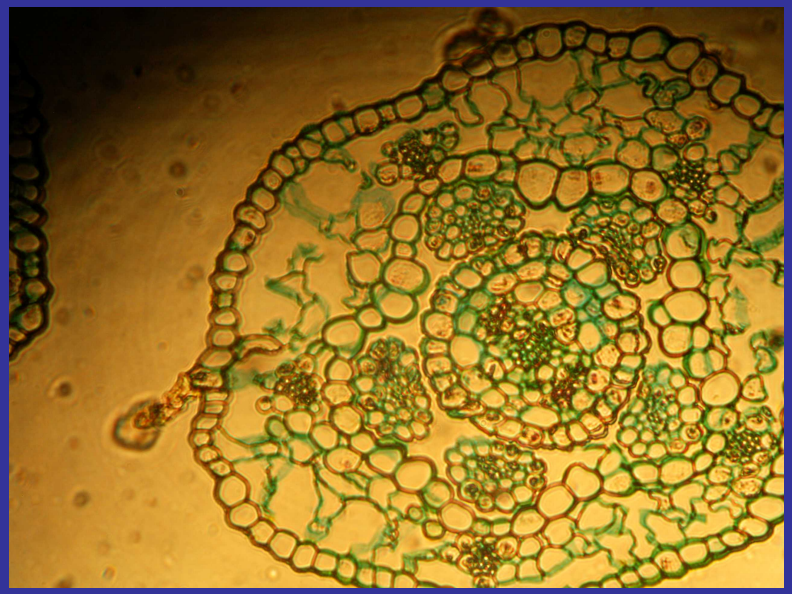
Leeds Microscopical Society.

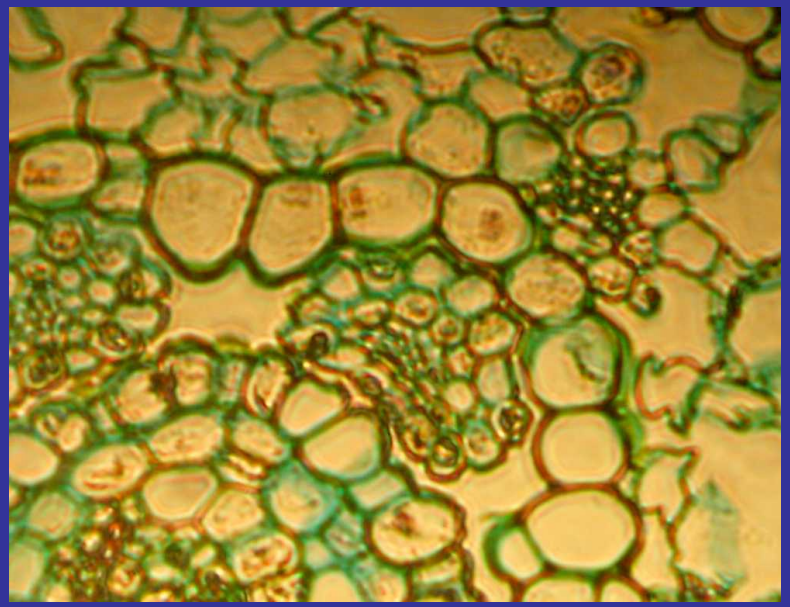
Photomicrography. Part 2.

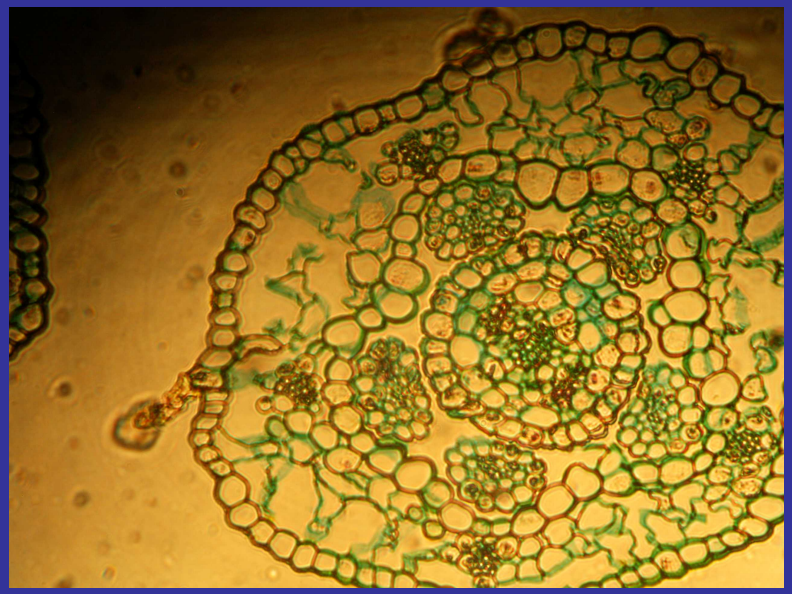


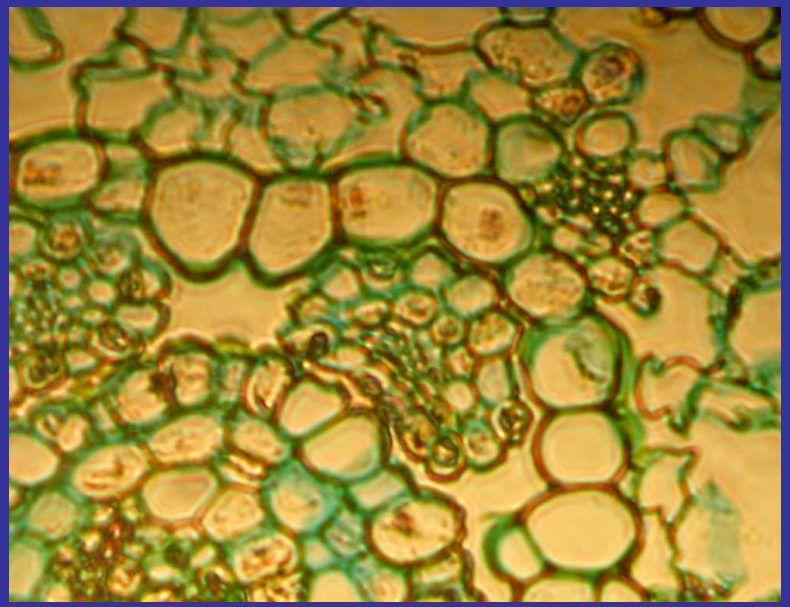
How to make a good permanent image.

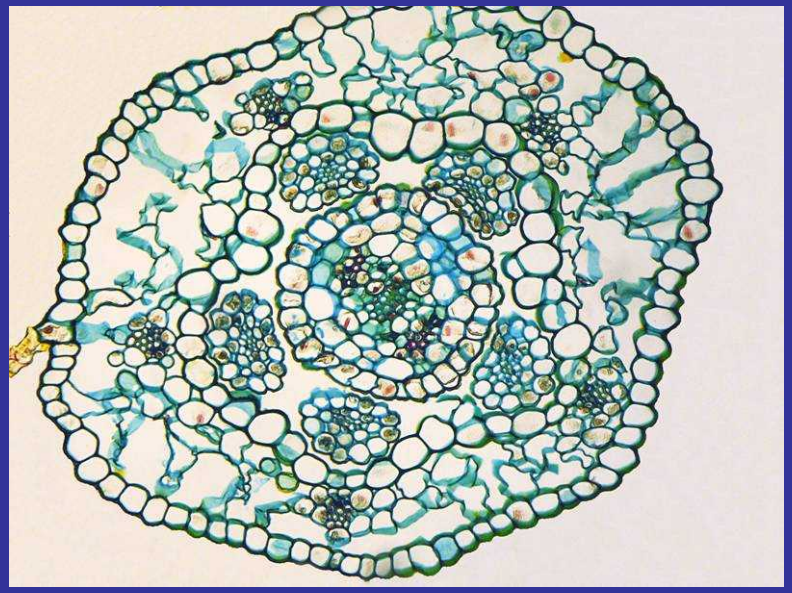


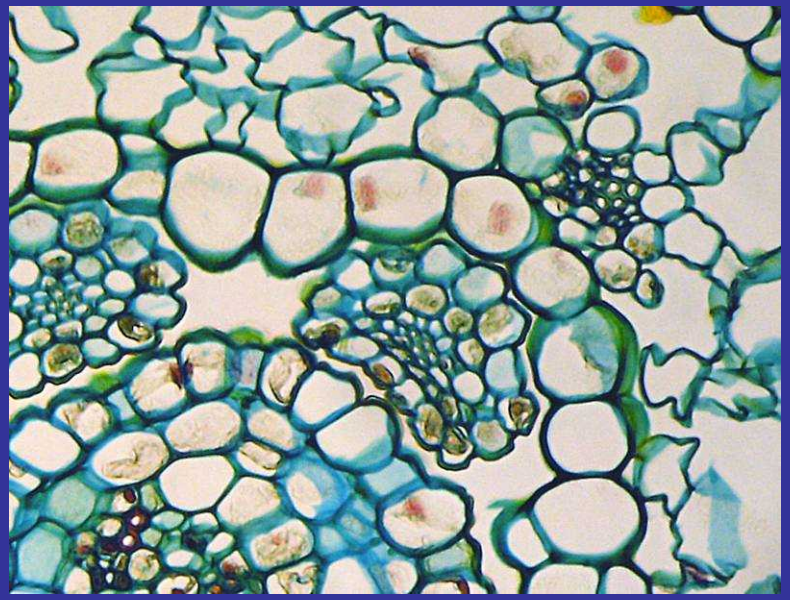












Hints for acquiring an image.

- Basic camera knowledge.

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- Viewing a live image in the camera.

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- Setting the light level.

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- Setting the exposure.
- Firing the shutter.

Hints for acquiring an image.

- Basic camera knowledge.
- Viewing a live image in the camera.
- Setting the light level.
- Focusing the image in the camera.
- Setting the colour balance.
- Setting the exposure.
- Firing the shutter.
- Image acquired!

Basic camera knowledge.

- Shutter speeds and lens aperture settings.

These each vary by halving or doubling adjacent values.

Shutter speeds – the length of time in whole or fractions of a second that the sensor is exposed to the light.

1-2-4-8-15-30-60-125-250-500-1000

Basic camera knowledge.

- Lens aperture settings.

These each vary by letting through a half or twice that of adjacent values.

They are traditionally represented by “f-stops”. The f-stop (correctly: “focal ratio”) is calculated by dividing the lens’ focal length by the diameter of the diaphragm opening, e.g. 100mm focal length lens with 50mm diaphragm = f2.

1.4 – 2 - 2.8 – 4 - 5.6 – 8 – 11 – 16 - 22

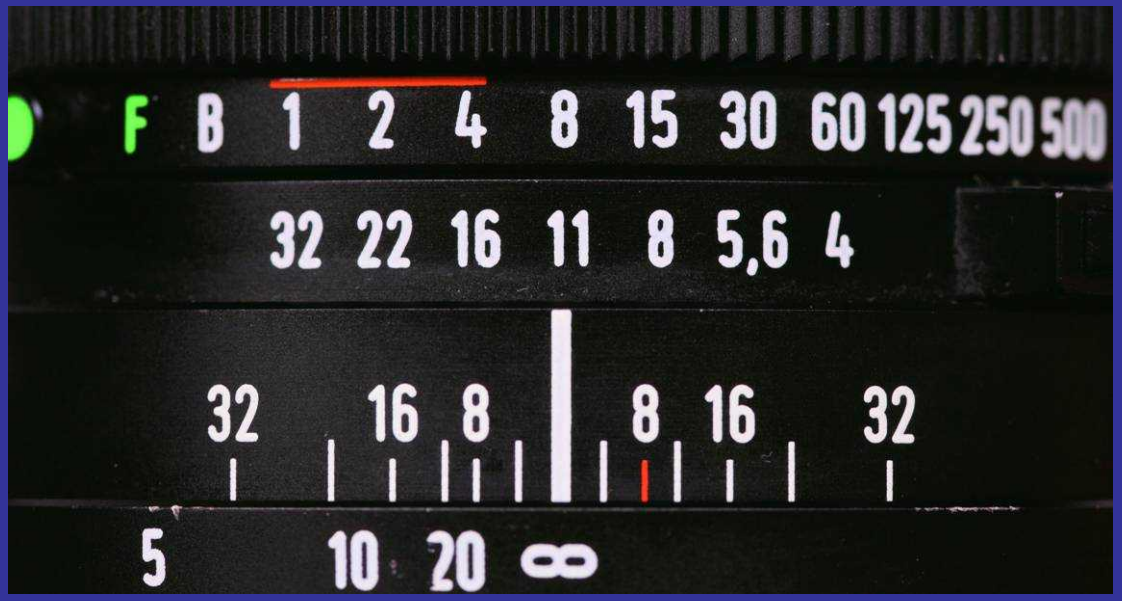
Basic camera knowledge.

Combining shutter speed and f-stop.

You can halve one and double the other. That way, there are several combinations to choose from.

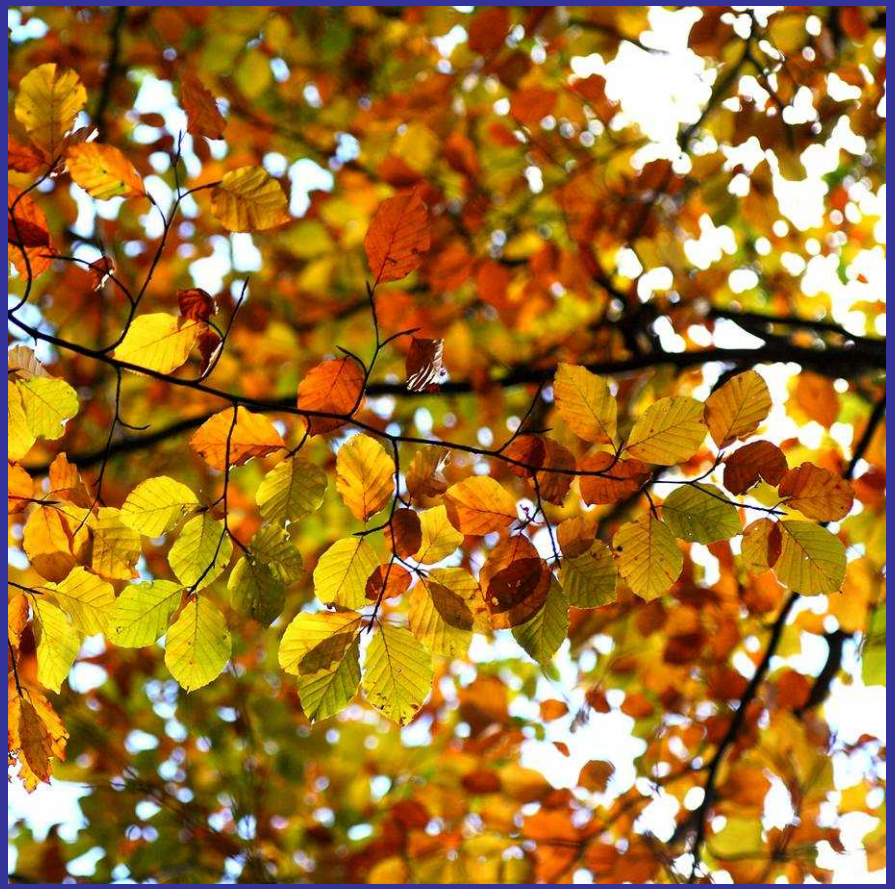
Briefly – apertures control depth of field whilst shutter speeds control freezing of motion either in scene or in camera.

A “stop” is the halving or doubling of light to the sensor via either aperture or shutter speed.



Basic camera knowledge.

Combining shutter speed and f-stop.



f4 1/500 sec

f11 1/60 sec

Basic camera knowledge.

ISO speeds.

A doubling of the ISO factor increases the camera's sensitivity twice.

Keeping shutter and aperture the same, this would allow twice as much light on the image.

Only increase the ISO if you can't increase lens aperture further but a short exposure is still required.

Basic camera knowledge.

ISO speeds.

Increasing ISO causes the signal from each pixel of the sensor to be more amplified (increased “gain”).

This produces unwanted “noise” in the image – a kind of granularity, ultimately blurring the image.

Standard ISO: 50 – 100 – 200 – 400 – 800 - 1600

Basic camera knowledge.

- Camera lenses.

Most compact digital cameras have a zoom lens.

A zoom lens has variable focal length whilst holding focus.

Basic camera knowledge.

- Camera lenses.

  Compact digital camera lens with focal length range of 5.8 to 17.4 mm, a “zoom ratio” of 3:1.

Unless you know the size of the sensor and can calculate the angle of view, these are relatively meaningless.

Basic camera knowledge.

- Camera lenses.

Recent convention to express focal lengths in 35mm camera terms:

15-35mm = wideangle 40-60mm = normal perspective 85-1000mm = telephoto.

Basic camera knowledge.

- Camera lenses.

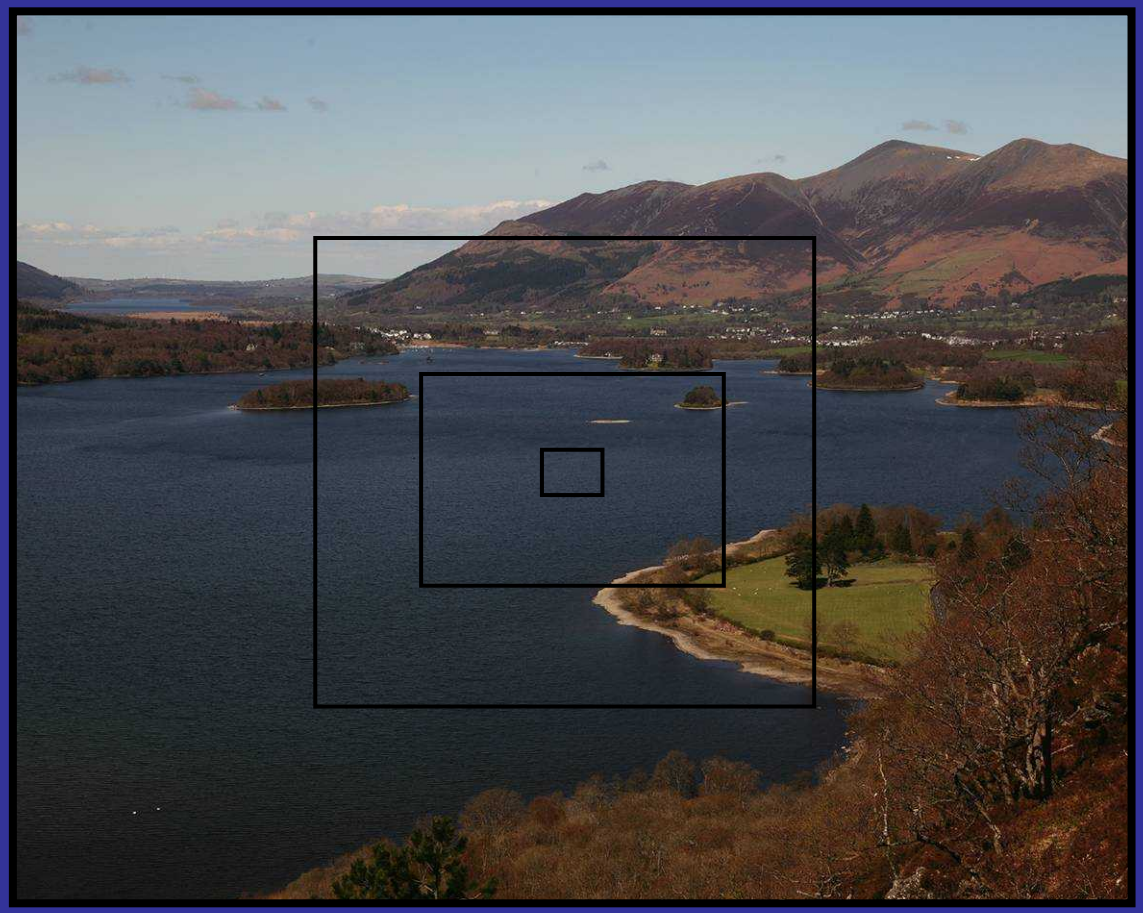
Views with a 50mm lens on:

5”x4” film

6cm x 6 cm

35mm

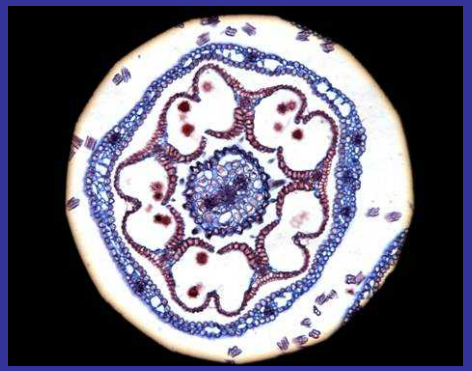
Compact digital



Setting the zoom lens when used on a microscope.

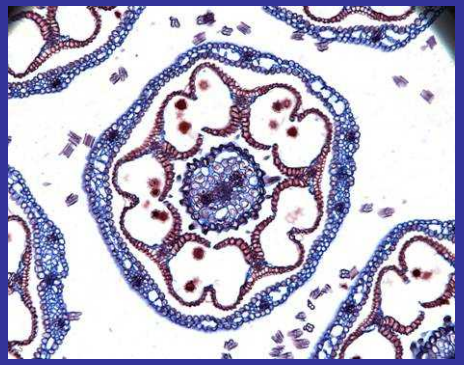
With a compact digital camera, positioning and setting of the zoom lens are crucial.

Camera not close enough to eyepiece.



Zoom setting needs to be more telephoto.

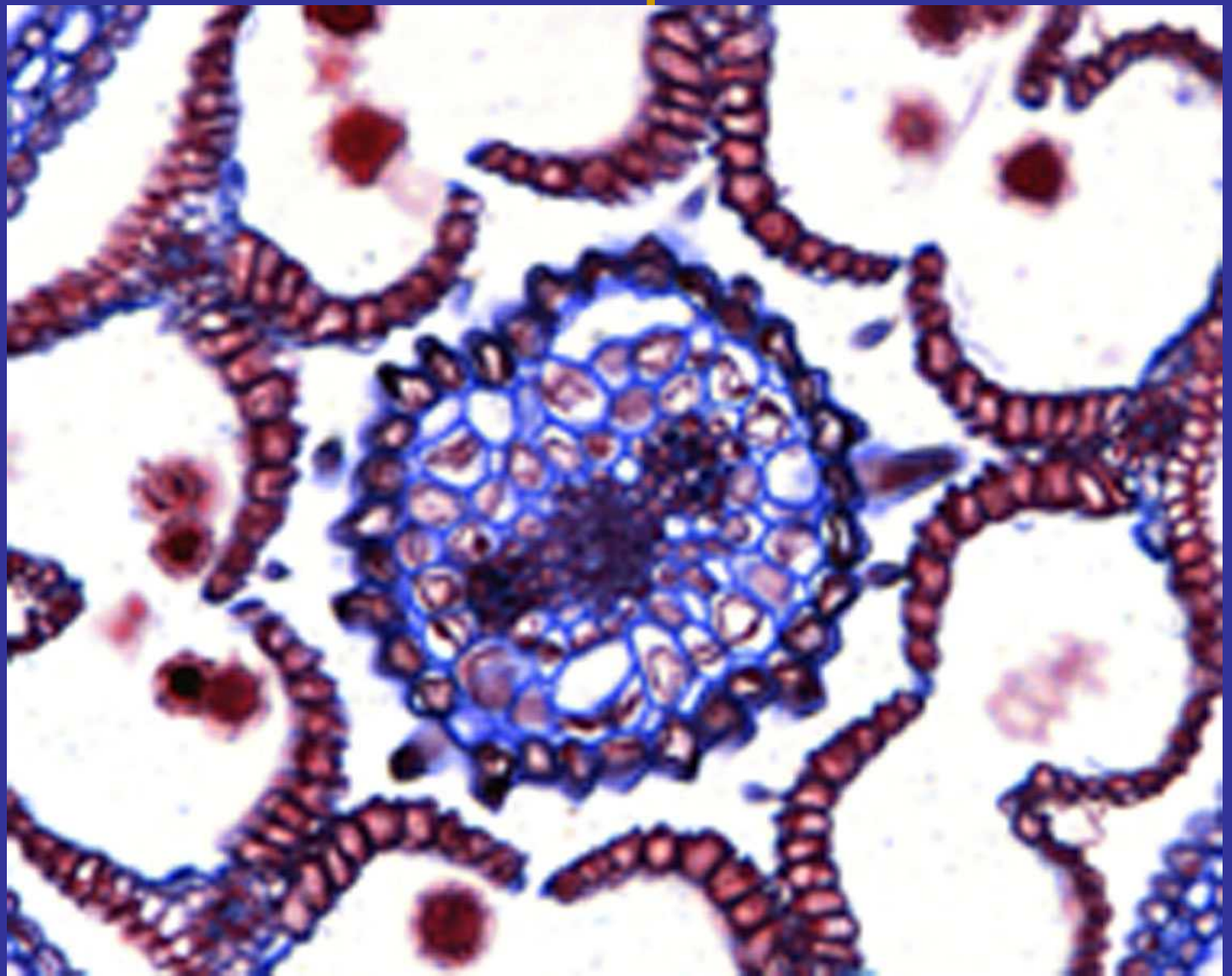
Camera not perpendicular.



Zoom at telephoto end of lens. Maybe camera needs to be even closer or this may be all that is possible.

Setting the zoom lens when used on a microscope.

Here the camera is zoomed in too far to telephoto. Only a small part of the visual image is recorded and the image is short of detail.



The problem of aligning compact camera.

Recording the image – live demo.

1. Check microscope is set up correctly.
2. Set basic illumination level.
3. Make basic adjustments to camera.
4. Focus the image in the camera.
5. Set the colour balance.
6. Set the exposure.
7. Trip the shutter.
8. Check image and repeat until happy.

Recording the image – live demo.

Set basic illumination level

Ideally, the level of illumination seen through eyepieces should be just slightly brighter than ambient room light.

Beware: if illumination level too low and exposure too long noise is produced. If ISO increased to shorten the exposure the noise will be worse.

Note for users of “trinocular” tubes: those tubes that have a shared light split allow light to enter the eyepieces and be passed up into the camera – you get images of lights and windows superimposed on the microscopical image! To prevent this add a cap to eyepieces.

Recording the image – live demo.

Focus the image in the camera.

This is never easy, especially with low power microscope objectives!

1. Compact digital cameras.

Options: a) focus on screen (can't use the optical viewfinder). May be awkward to see in some positions.

Screen resolution low. Screen magnifier helpful.

b) Plug analogue output (usually yellow phono plug) into TV.

c) Do any have digital outputs of live image?

Recording the image – live demo.

Focus the image in the camera.

1. Compact digital cameras.

Live image on television screen.

Acquired image.

Recording the image – live demo.

Focus the image in the camera.

1. Compact digital cameras.

Autofocus is occasionally useable!

On thin specimens where you don't have to choose a focus level within the specimen, e.g., blood film, cell culture, polished metal.

Check that the microscope focus lies within the camera's autofocus range (the camera can't focus the microscope!).

Especially useful at low powers.

Recording the image – live demo.

Focus the image in the camera.

2. Single lens reflex cameras.

Focussing via ground glass screen in viewfinder is easy with low power objectives but becomes very granular and unclear at high powers.

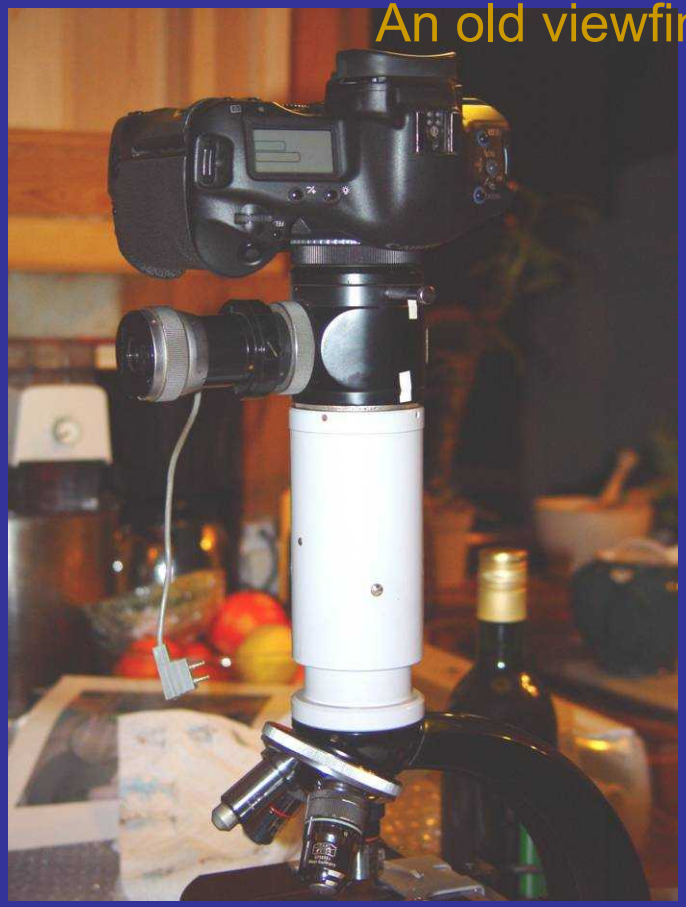
For comfort, an angled viewfinder may help – some have a 2x magnification switch.

Recording the image – live demo.

Focus the image in the camera.

2. Single lens reflex cameras.

An old viewfinder has excellent performance.



Recording the image – live demo.

Focus the image in the camera.

2. Single lens reflex cameras.

"Live View" via computer.

Much better ergonomically.

Shutter operation from computer keyboard (best keep microscope and computer on separate tables).

Live image good but poor detail compared to acquired image, so critical focusing can be a guess when contrast not high.

With Canon, the mirror stays up. With Nikon (?all) the mirror returns for conventional exposure – vibration!

Recording the image – live demo.

Focus the image in the camera.

In all cameras:

A magnification changer (Zeiss Optovar etc) is an excellent way to boost the magnification for critical focusing.

Return to lowest setting (without touching the focus) to acquire the image.

Recording the image – live demo.

Set the colour balance.

Not easy – even with best equipment many people get it wrong.

First step: get the lamp as near daylight colour as possible. Add a blue correction filter and ND filters if necessary.

There is a misunderstanding that you can acquire the image in whatever the colour and correct later. If the specimen is short on blue wavelengths the image will never be as crisp as when acquired in white light.

Recording the image – live demo.

Set the colour balance.

Colour temperature.

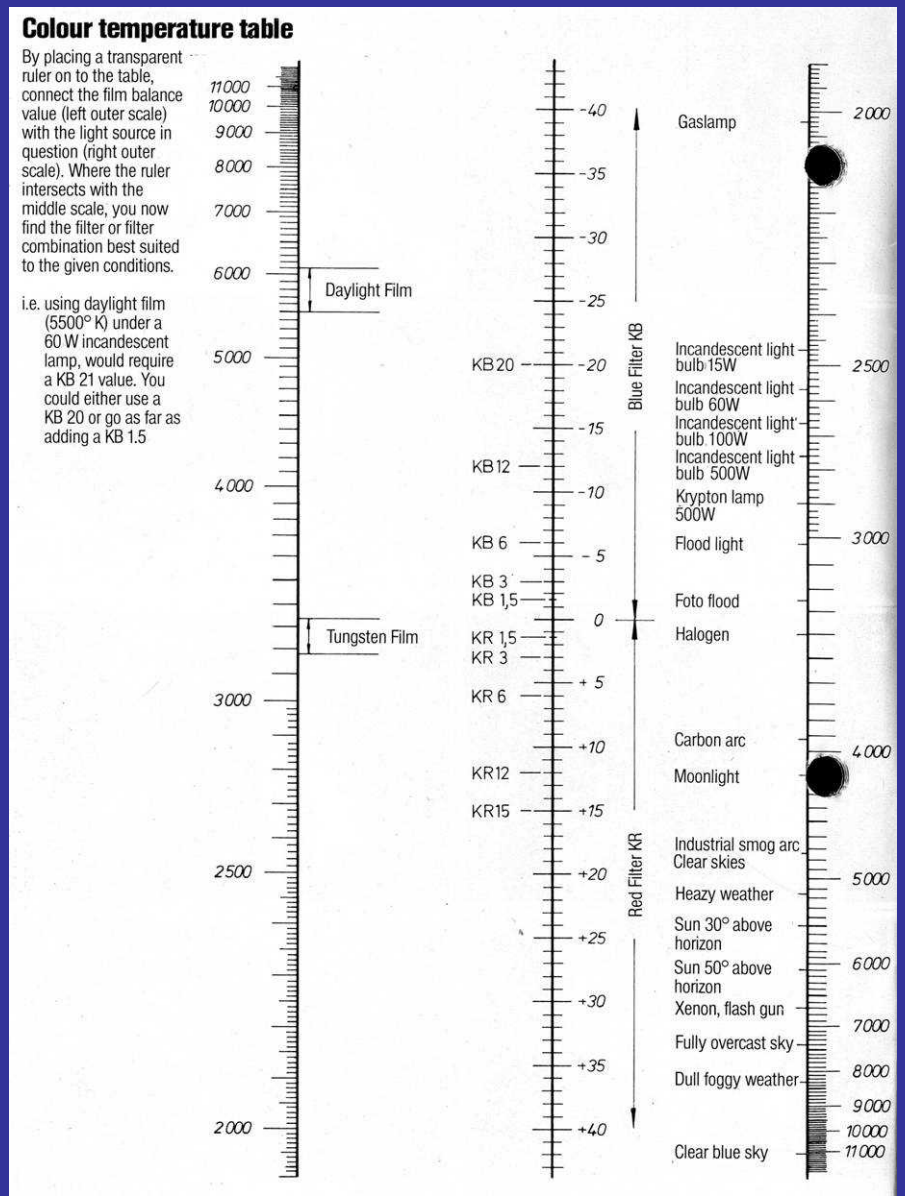
These are the temperatures in degrees Kelvin (or Absolute) of a heated metal at a certain spectrum. A metal heats up to red, through yellow to white and then blue.

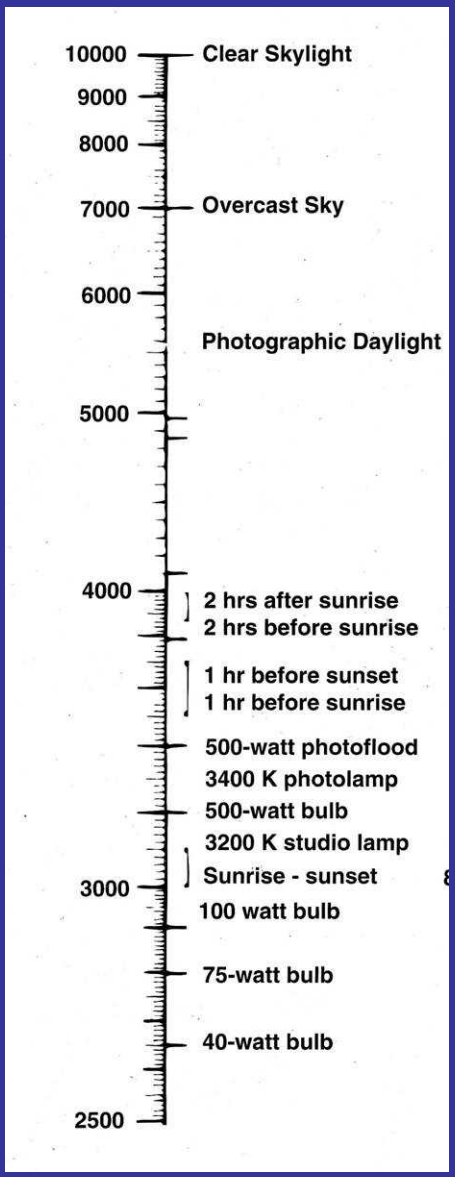
Daylight with sun in clear sky at midday is regarded as 5,500 degrees K.

A 12v 100W halogen bulb run at 12V is 3,200 degrees K.

To convert 3,200 to 5,500 a CB 12 or 85B blue filter is required.

Recording the image – live demo.





Recording the image – live demo.

A lesson from wedding photography.

On a sunny day strong yellow light from the sun plus weak, blue light from the sky make us think the bride's dress is white and the groom's suit is grey.

If these are corrected so that they are so, the shadows (which lack yellow) will be the colour of the sky. On photographs, that looks poor and the bride complains that her dress looks blue. Objects in sunlight, to us, look yellowish – so both suit and dress must look on the photo.

Automatic white balance in cameras would make the dress pure white and the suit pure grey.

Recording the image – live demo.

Automatic white balance (AWB) setting – the problems for microscopists.

1. Areas where strong colours predominate, e.g., a haematoxylin and eosin stained section where little clear background covers the frame. Or where the specimen is not pure grey but entirely covers the frame.
2. Specimens where the stain may have dispersed into the mounting medium, or the mounting medium has discoloured.
3. Polarisation, especially if using a red retardation plate.
4. If colour accuracy vital, camera should be set to 3,200K or 5,500K and the microscope lamp set accordingly.

Recording the image – live demo.

If the acquired image does not look the same colour as what you see through the eyepieces, most likely it is because you are not viewing with daylight white (5,500K), whilst the camera has corrected for this.

Some lens systems may have a yellowish colour and many heat filters have a greenish hue. Our eyes can adapt but the camera can’t.

Electronic sensors are not perfect – most have a tendency to produce too prominent reds.

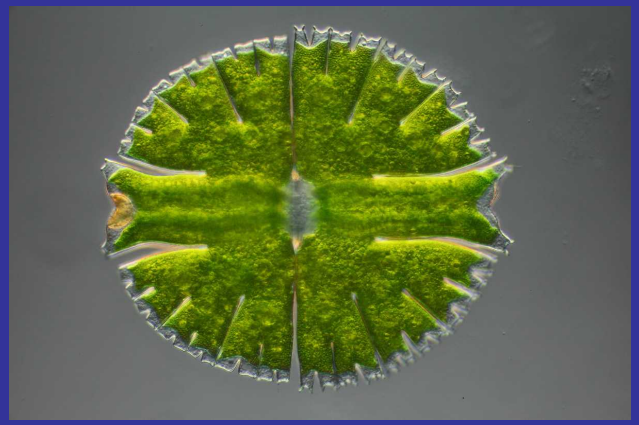
Recording the image – live demo.

Setting the exposure.

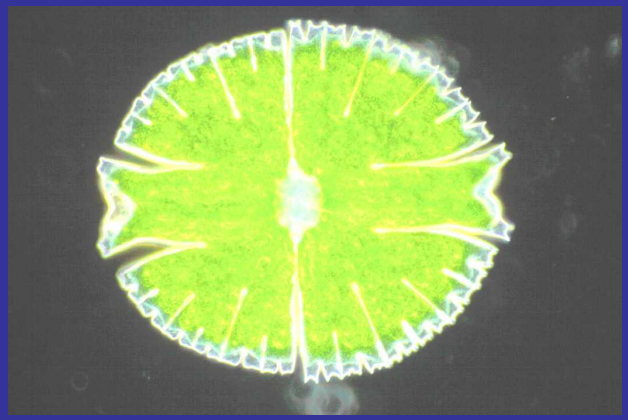
All light meters in all cameras are calibrated against “18% grey” – the lids of Kodak film pots. Generally, it also corresponds to green fields and blue skies or people in mixed clothing

If you leave the camera on auto-exposure, this is what you get!

Brightfield



Phase or DIC



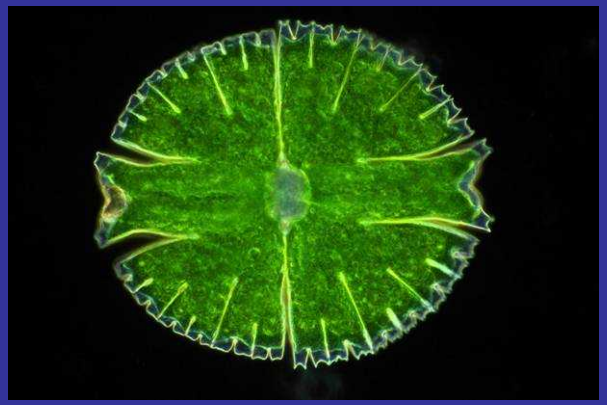
Darkfield

Recording the image – live demo.

Setting the exposure.

Over-expose 2 stops

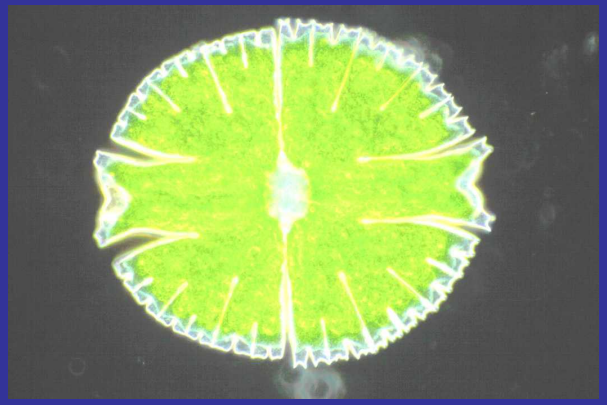
Just a shade darker



Under-expose 2 stops

Brightfield

Phase or DIC



Darkfield

Recording the image – live demo.

Setting the exposure.

How to set the camera.

Manual exposure with aperture fully open.

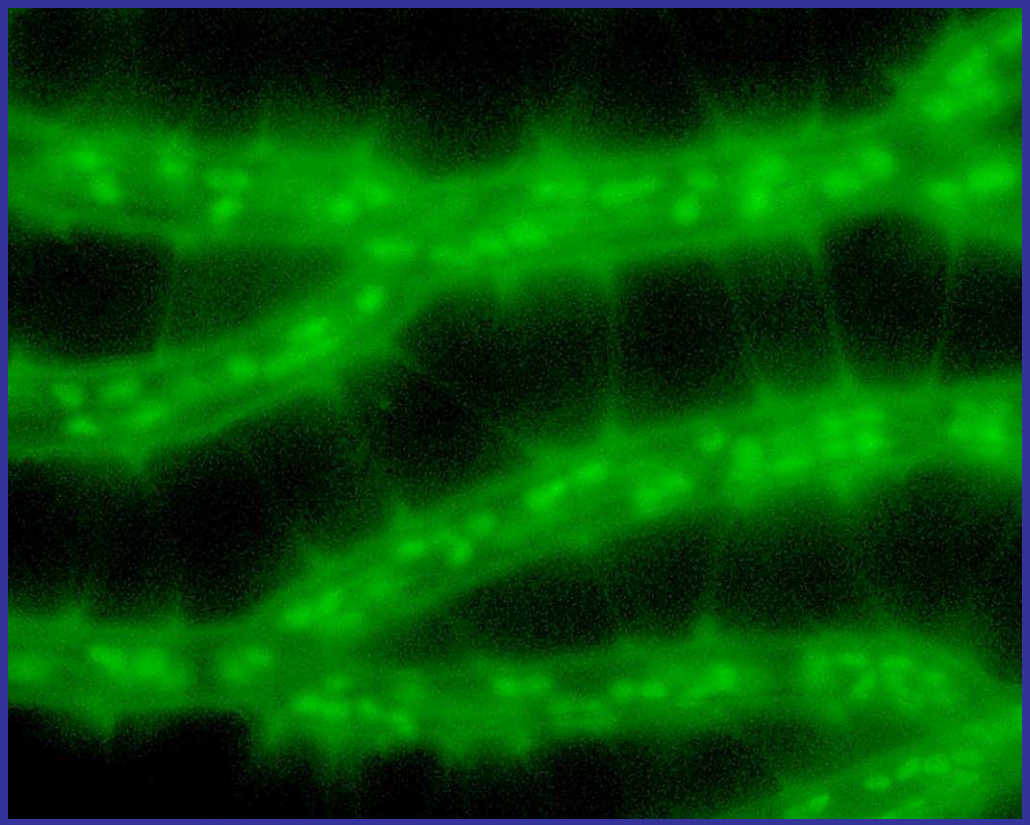
Aperture priority exposure with aperture fully open.

If neither provided, maybe “Sports Mode” would do.

Then use exposure compensation to + or – 1 to 2 stops – may need to check camera’s instructions!

Recording the image – live demo.

Setting the exposure.

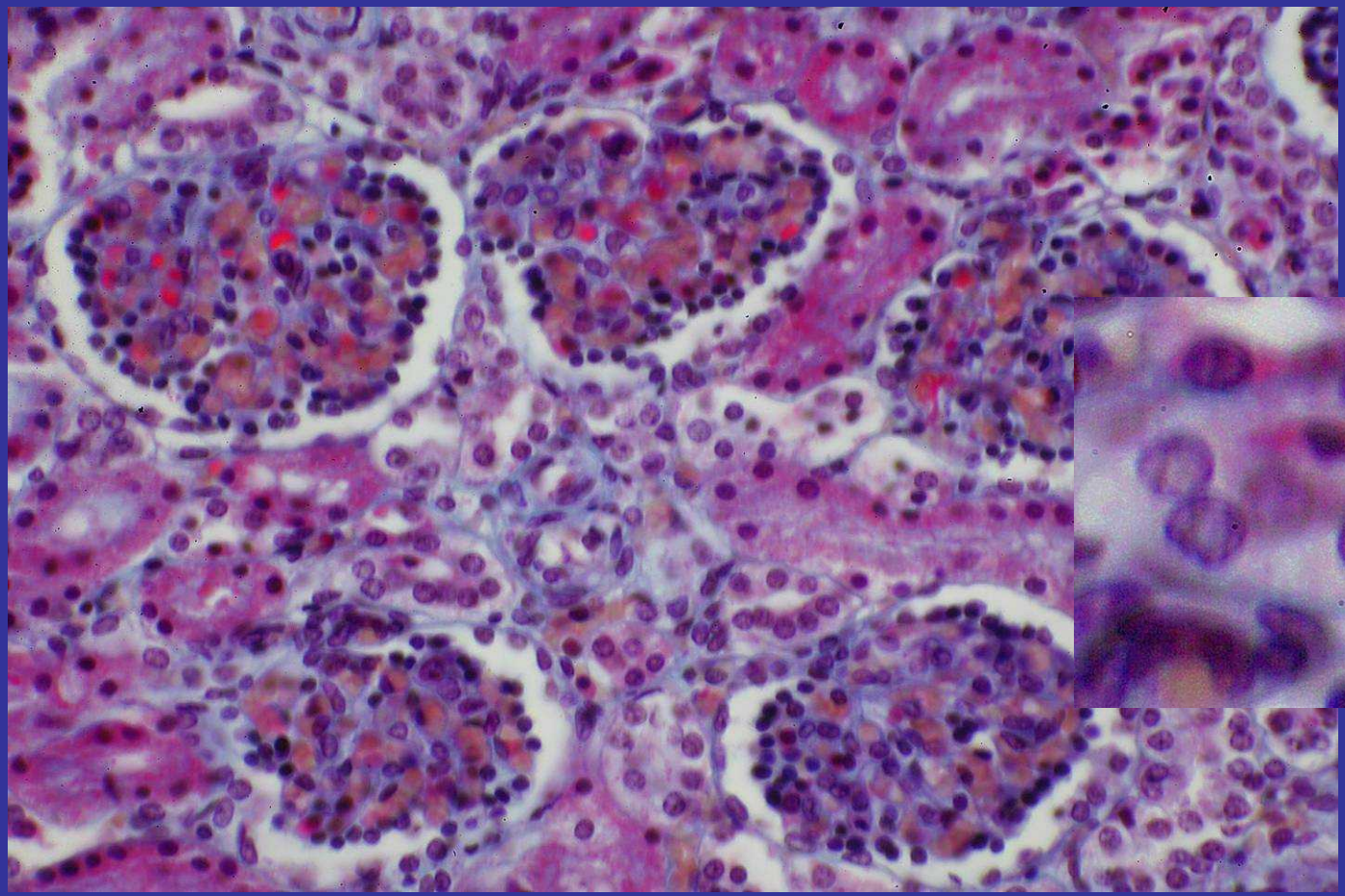


Beware – brightening a dark image at processing will reveal a noisy and dull image. Best to get it right in-camera!

Recording the image – live demo.

Tripping the shutter.

Vibration is your enemy!



SLR + electronic release – mirror vibration.

Recording the image – live demo.

Tripping the shutter.

Vibration is your enemy!

Important to keep your hands off, so use:

Cable release – electronic or mechanical. If no sockets, there are adapters available.

Use self-timer/shutter delay – but not good if you need to choose your moment.

Infra-red trigger.

If triggering from computer keyboard/mouse, preferably use on a different table. Note that this will not stop SLR mirror vibration unless similar to Canon Live View.

Processing the image.

- Factors you will probably need to address:

- File type, file size.
- Brightness, contrast, gamma, “curves”.
- Colour correction, saturation.
- Sharpening, unsharp mask.
- May need to clone out dust-spots etc.
- Output for intended use + keep original.
- “Creative” modifications – some other day!