TURNING VISIONS INTO REALITIES

Microscopy Workshop

Northwick Park
2010

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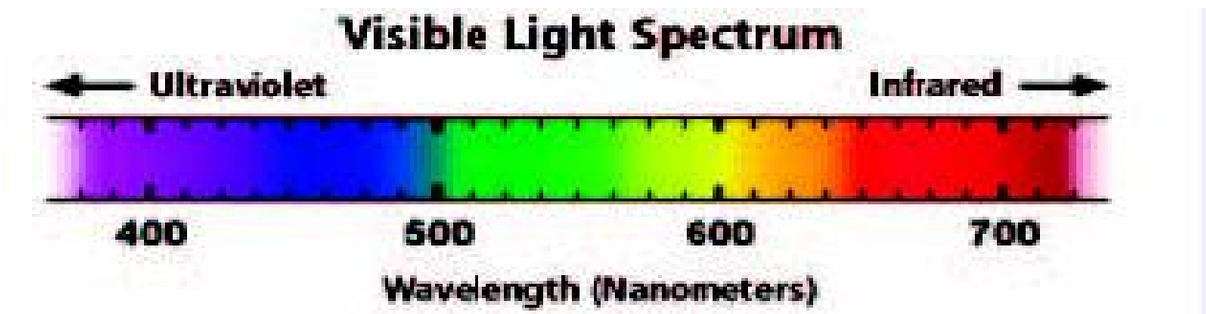
Agenda

- Breakdown Jargon-Explanation of Terms.
- The Microscope- What do you need and why.
- Digital Imaging.

Jargon

- There are only 2 things that the eye can see
- Colour-Represented by Wavelength



Jargon

- Intensity-Represented by Amplitude

- So we can only see our specimen if it has a different **Colour** or **Intensity** than the background

Jargon

- **Resolution-** Resolution can be defined as the least distance between 2 points at which they can still be recognised as 2 separate entities
- For the eye, this is 70 microns, when the object is 250mm away
- For light microscopy, this is 0.24 microns
- **Contrast-**The phenomena that allows you to distinguish relevant information from irrelevant. Either by colour or intensity
- Contrast and Resolution are inversely proportional

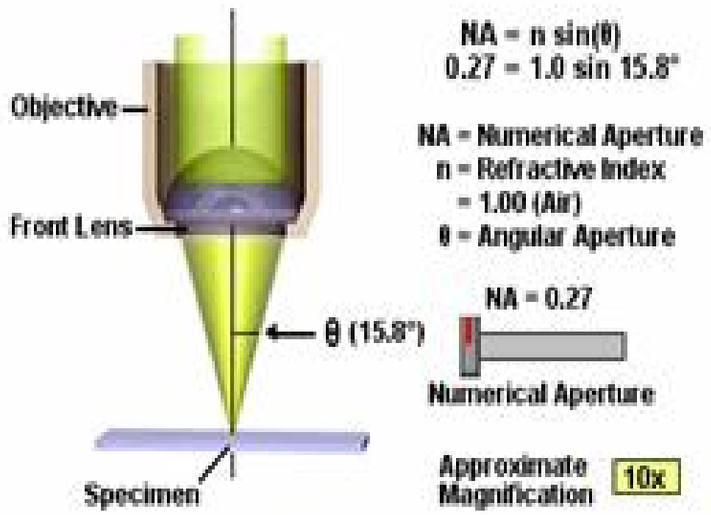
Jargon

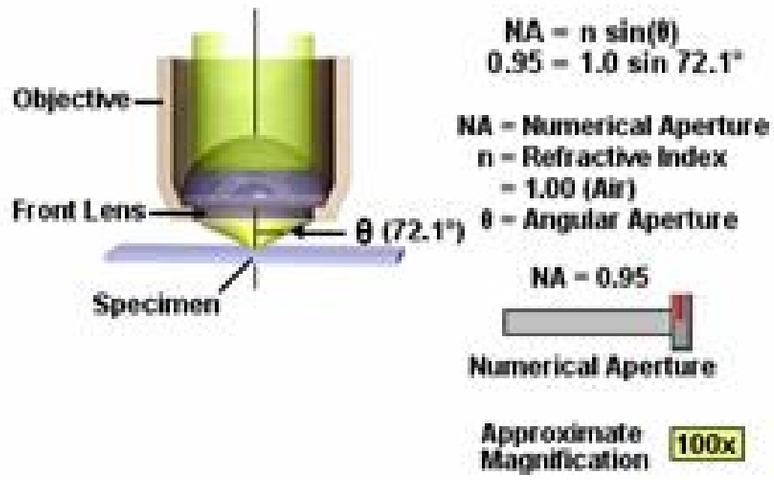
- Working Distance- The distance between the specimen and the front lens of the objective
- Depth of Focus-This is the maximum depth of a specimen for which the whole layer can be held in focus at the same time
- Field of View- This is the area of the specimen in view down the eyepieces. It is dependant on the magnification and the F.N.(field number) of the eyepiece.

Jargon

- **Numerical Aperture-** This indicates the resolving ability of an objective. Larger N.A.= Greater resolution and also brighter fluorescence signal. However larger N.A.= less depth of field and shorter working distance. NA= nSinA, where n= refractive index of medium and A is the angle at which light enters the objective.

Numerical Aperture





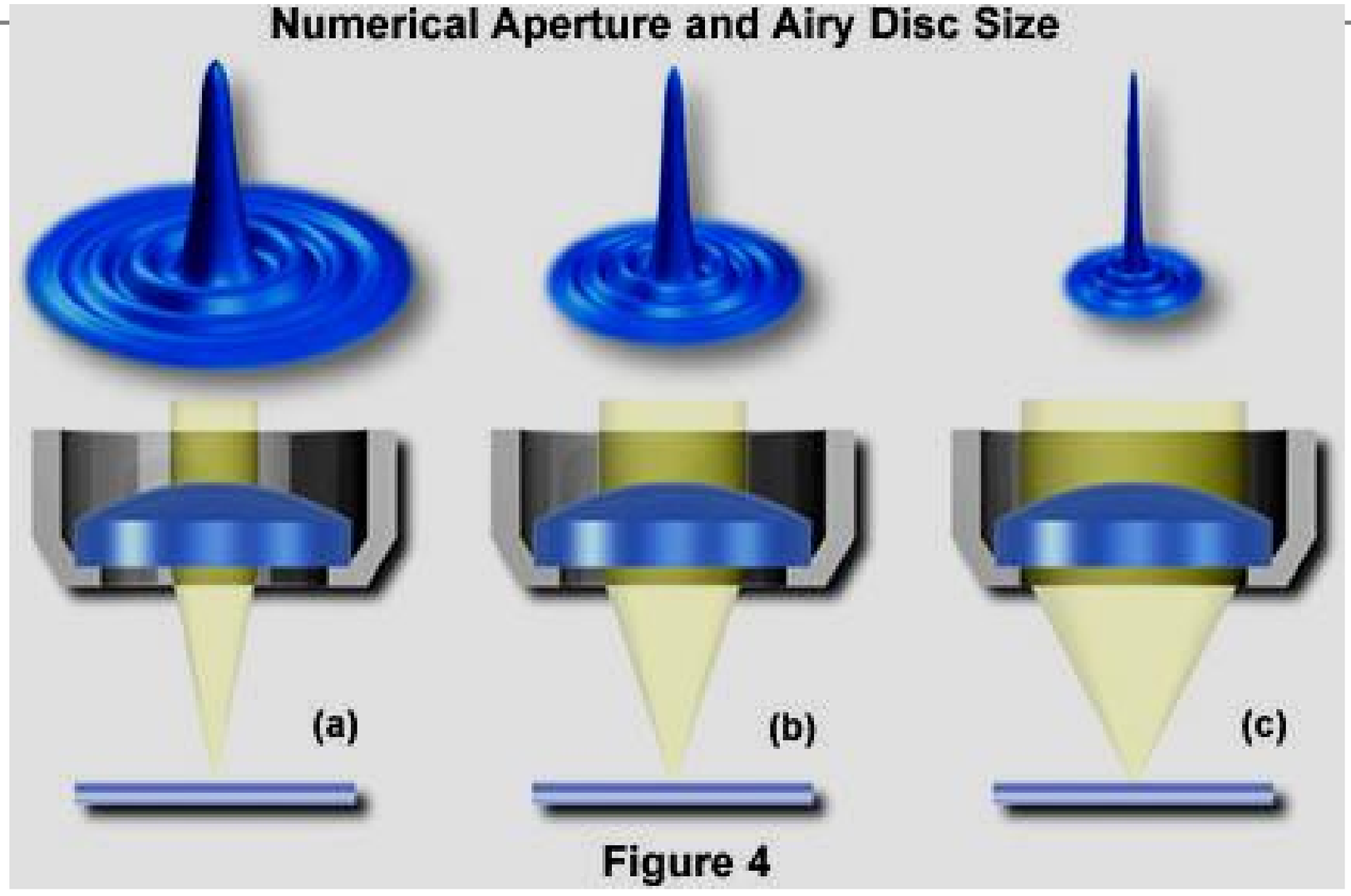
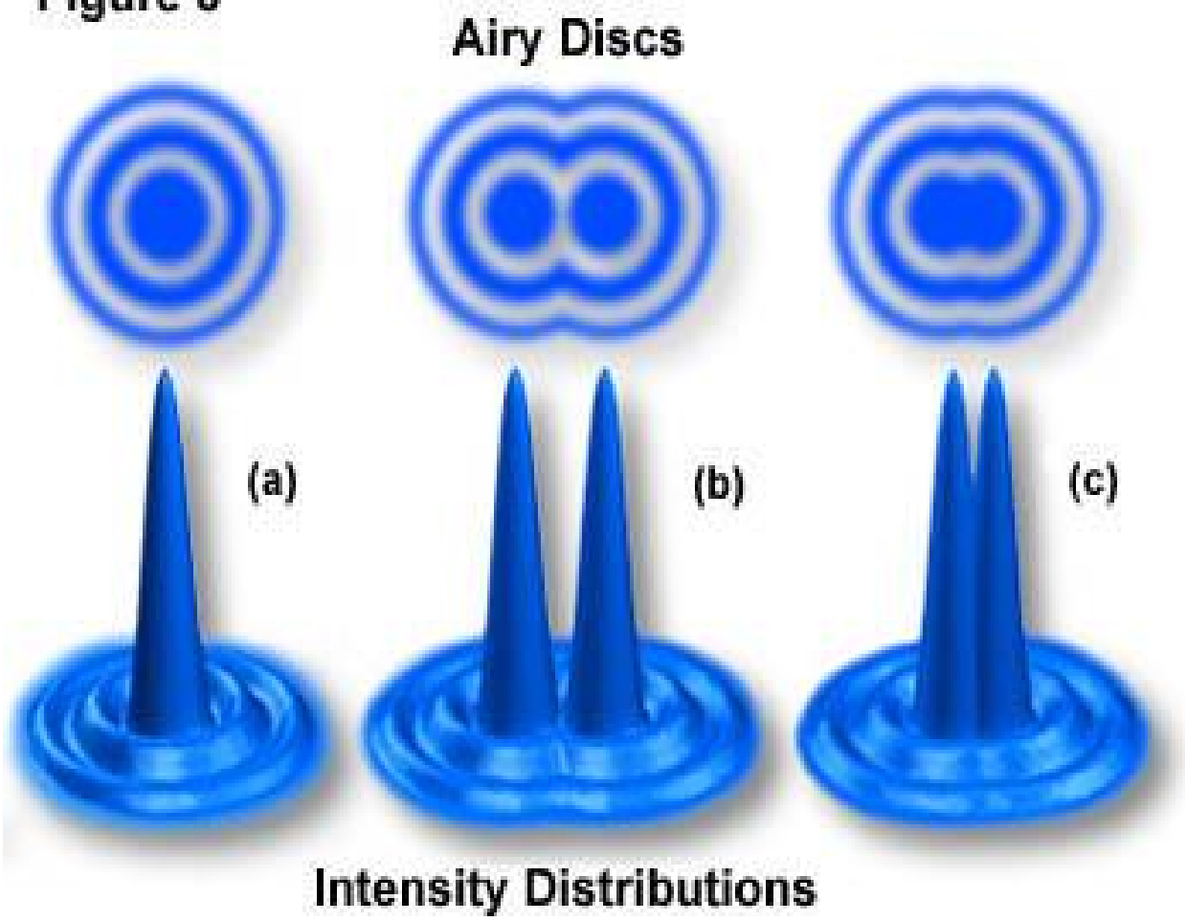


Figure 4

Figure 3



Jargon

- Chromatic Aberration- When white light passes through a lens, the colours can split and focus at different points causing colour fringing. Optics have corrections to overcome this problem.
- Apochromatic objectives are fully corrected and Achromatic objectives are corrected for red/blue.
- Plan objectives are designed, assuming a flat specimen, to provide a focused image across the whole field of view

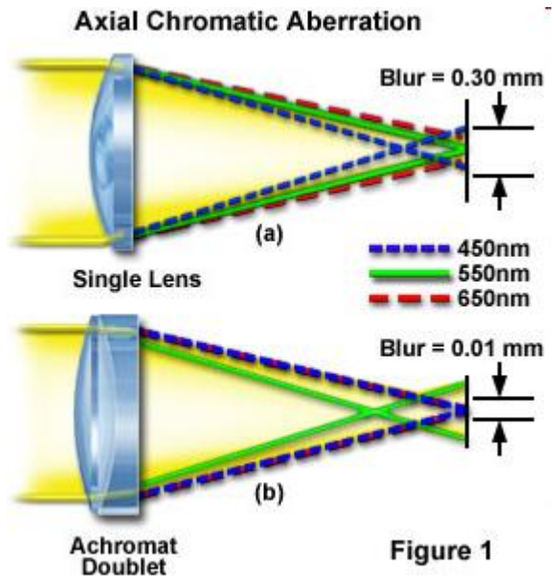

Axial Chromatic Aberration
Blur = 0.30 mm
(a)
450nm
550nm
650nm
Single Lens
Blur = 0.01 mm
(b)
Achromat
Doublet

Figure 1

Adjustment of Condenser Aperture

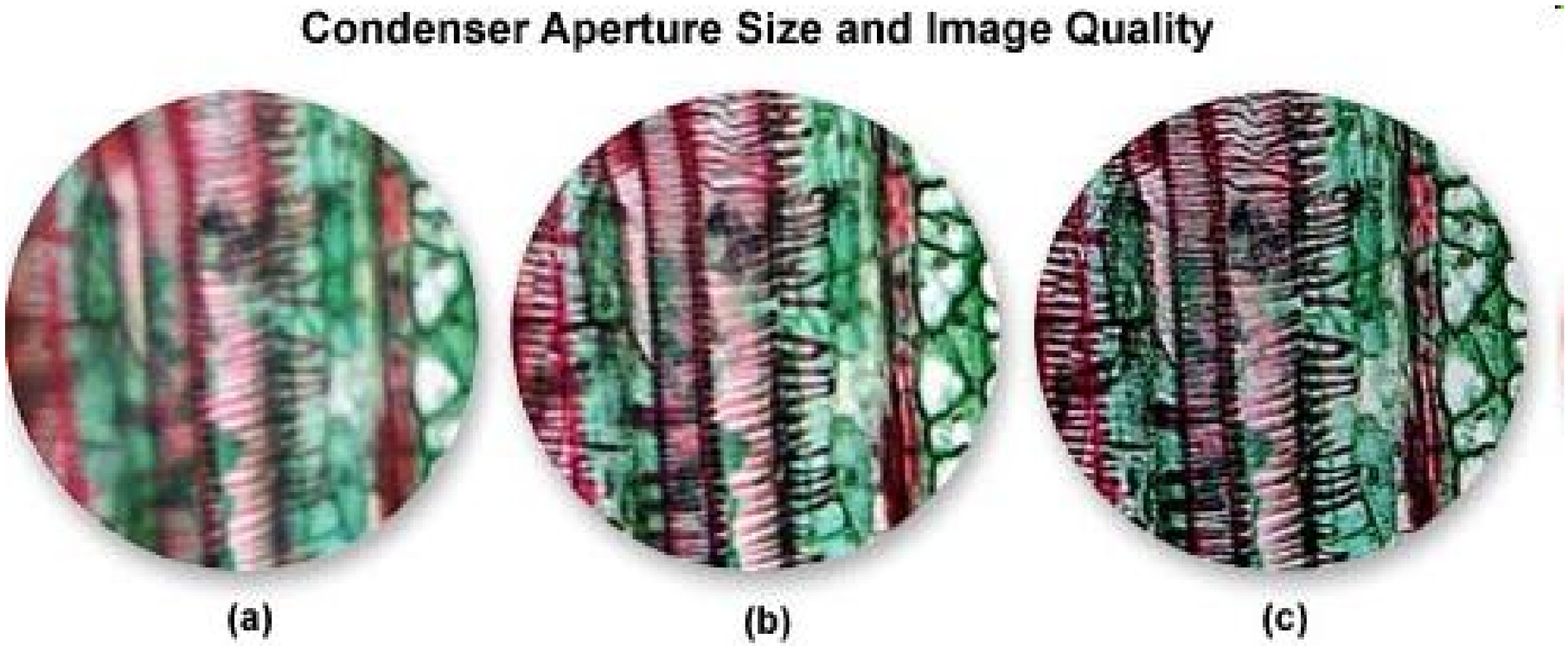


Figure 4

The Microscope

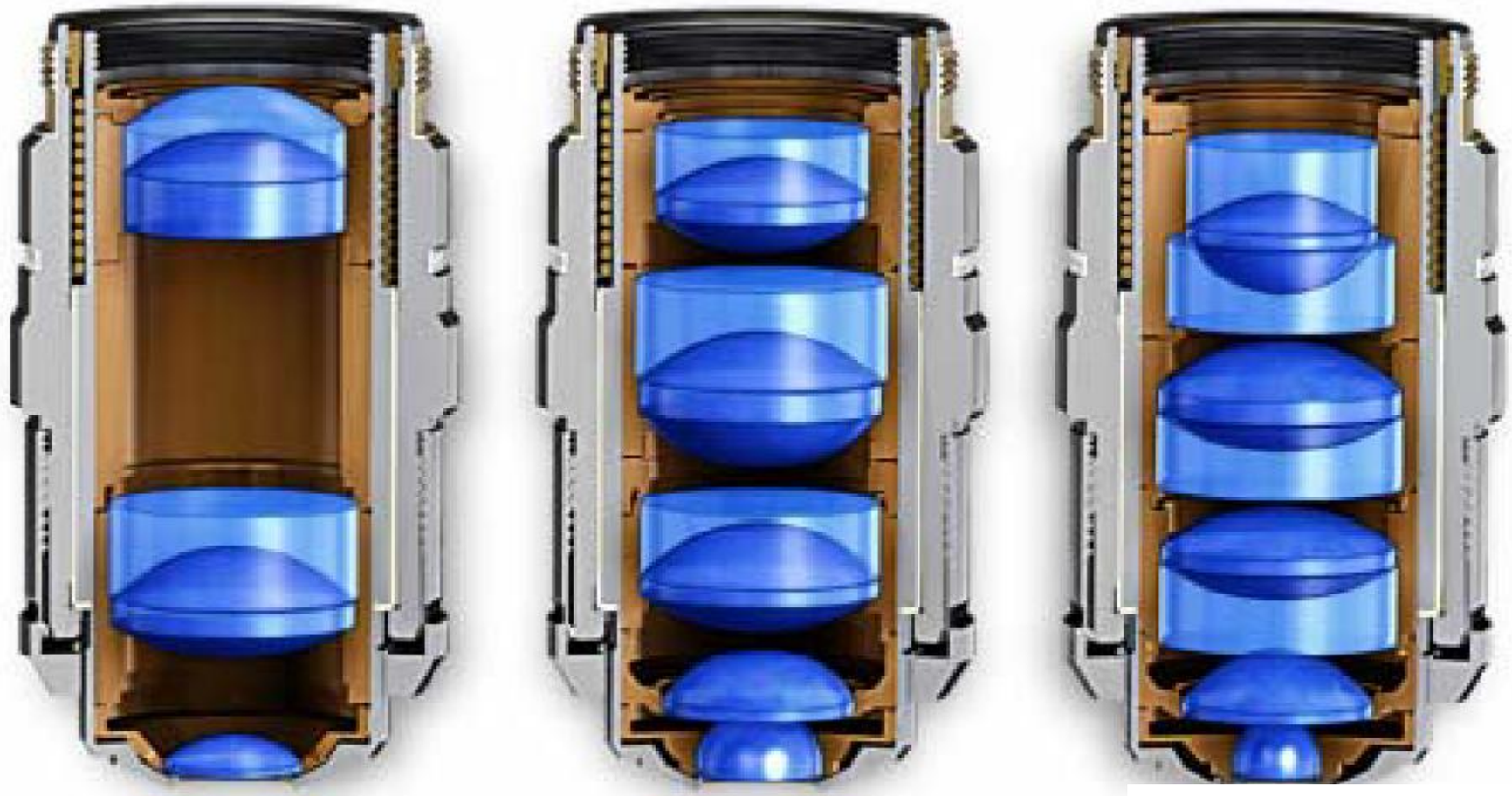
- Location
- Avoid direct lighting - not next to a window.
- Comfortable working area
- Adjustable chair
- Isolate external vibration

Ergonomics

The Microscope

- Requirements- Transmitted Light
- 12v 100w Halogen source- DIC needs a lot of light
- Polariser- needed for DIC, normally part of the condenser
- Universal condenser with slots for DIC prisms
- Focus- ideally sensitive to 1 micron
- Mechanical Stage with drop control or motorised with joystick
- Objectives-ideally highest N.A. at each magnification. Ensure that they are UV compatible

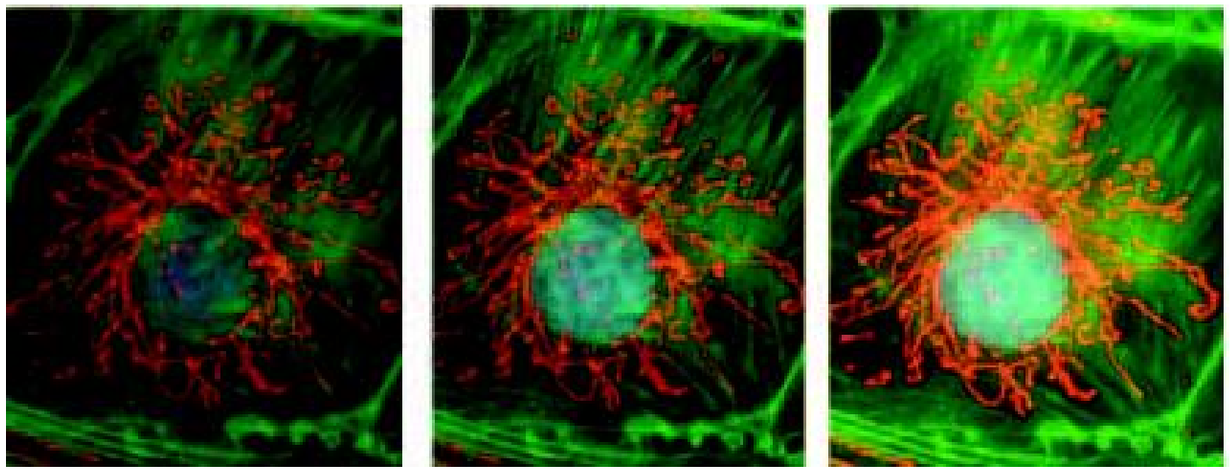
Objectives



Achromat

Plan-Fluorite

Plan-Apochromat

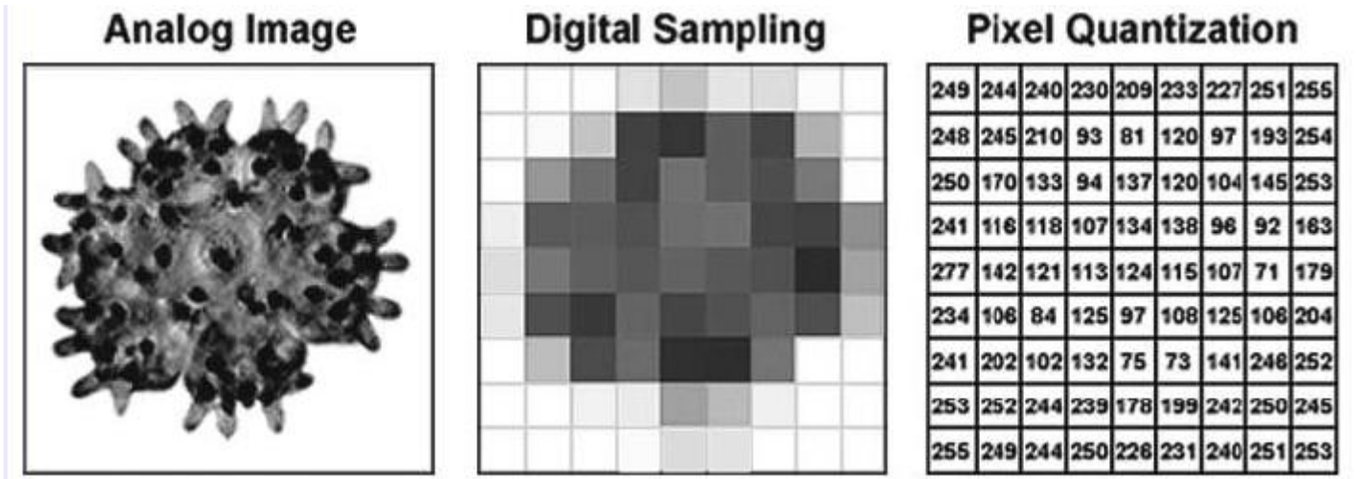


Digital Imaging

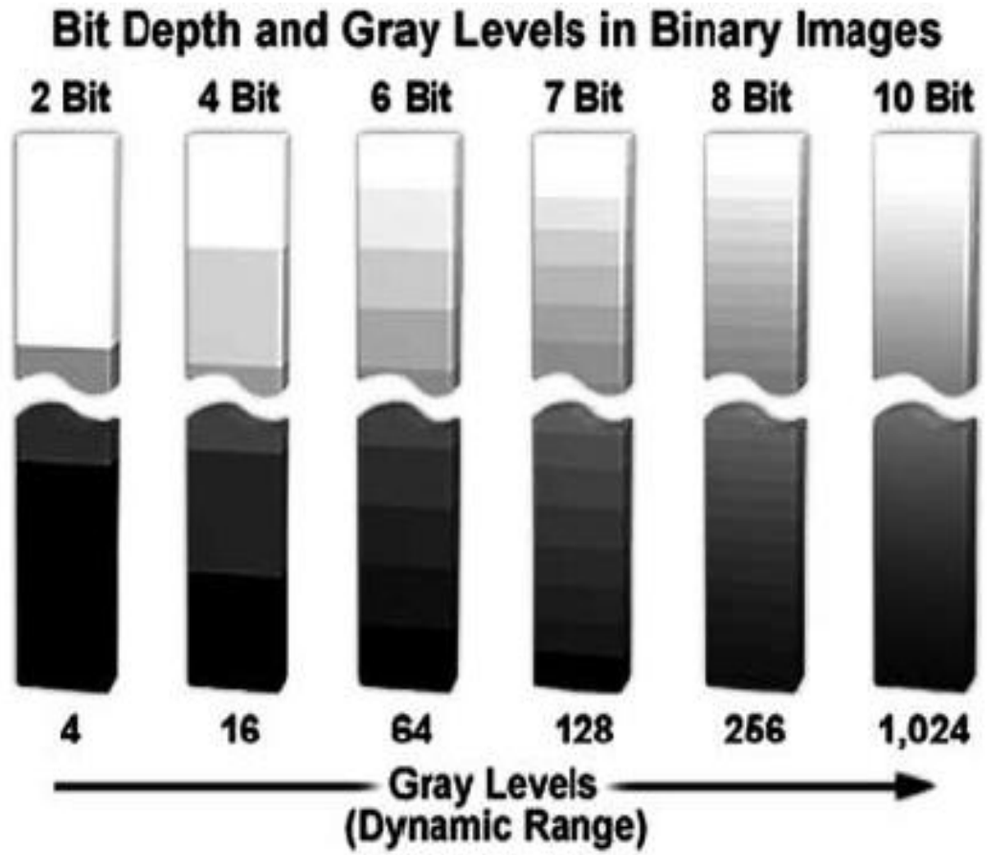
- Older Cameras were Analogue
- They gave a live image on a monitor
- Governed by the PAL system in the UK
- Refresh rate of 25 frames/second
- Limited resolution to 760 x 580
- Limited sensitivity because of frame rate

Digital Imaging

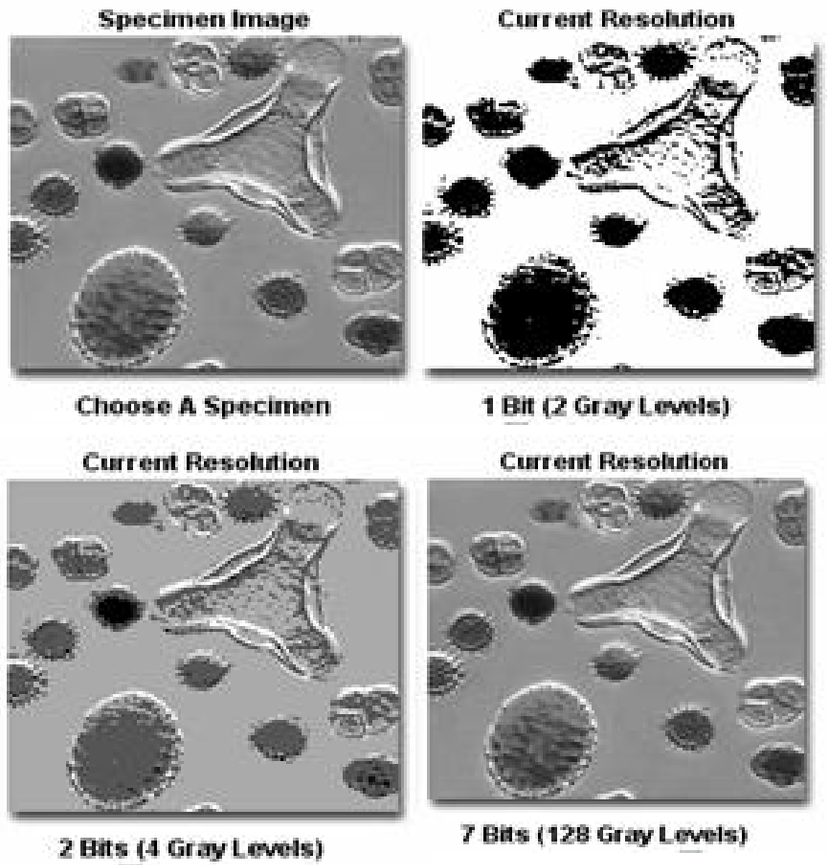
- Most modern cameras are CCD devices
- Image is converted in electrical signal



Digital Imaging

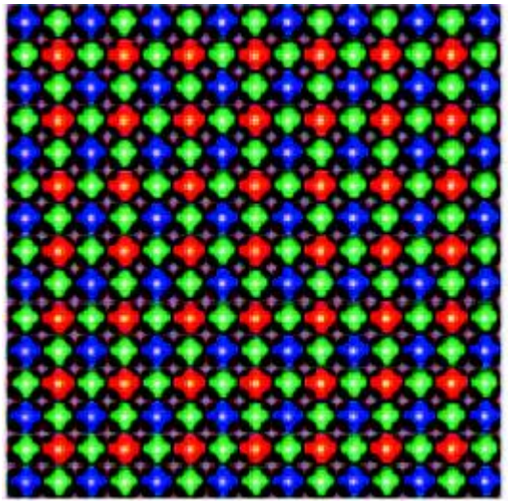


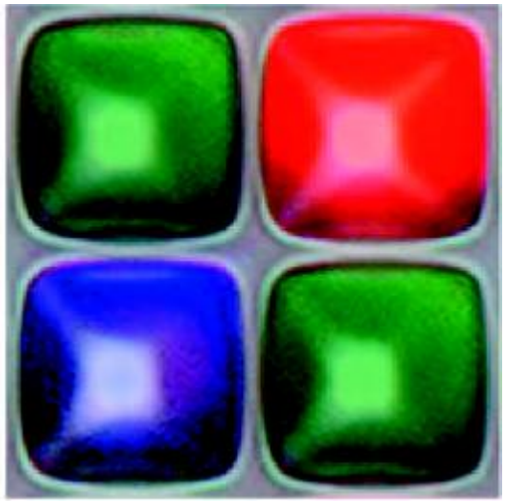
Bit Depth


Specimen Image
Current Resolution
Choose A Specimen
1 Bit (2 Gray Levels)
Current Resolution
Current Resolution
2 Bits (4 Gray Levels)
7 Bits (128 Gray Levels)

Digital Imaging

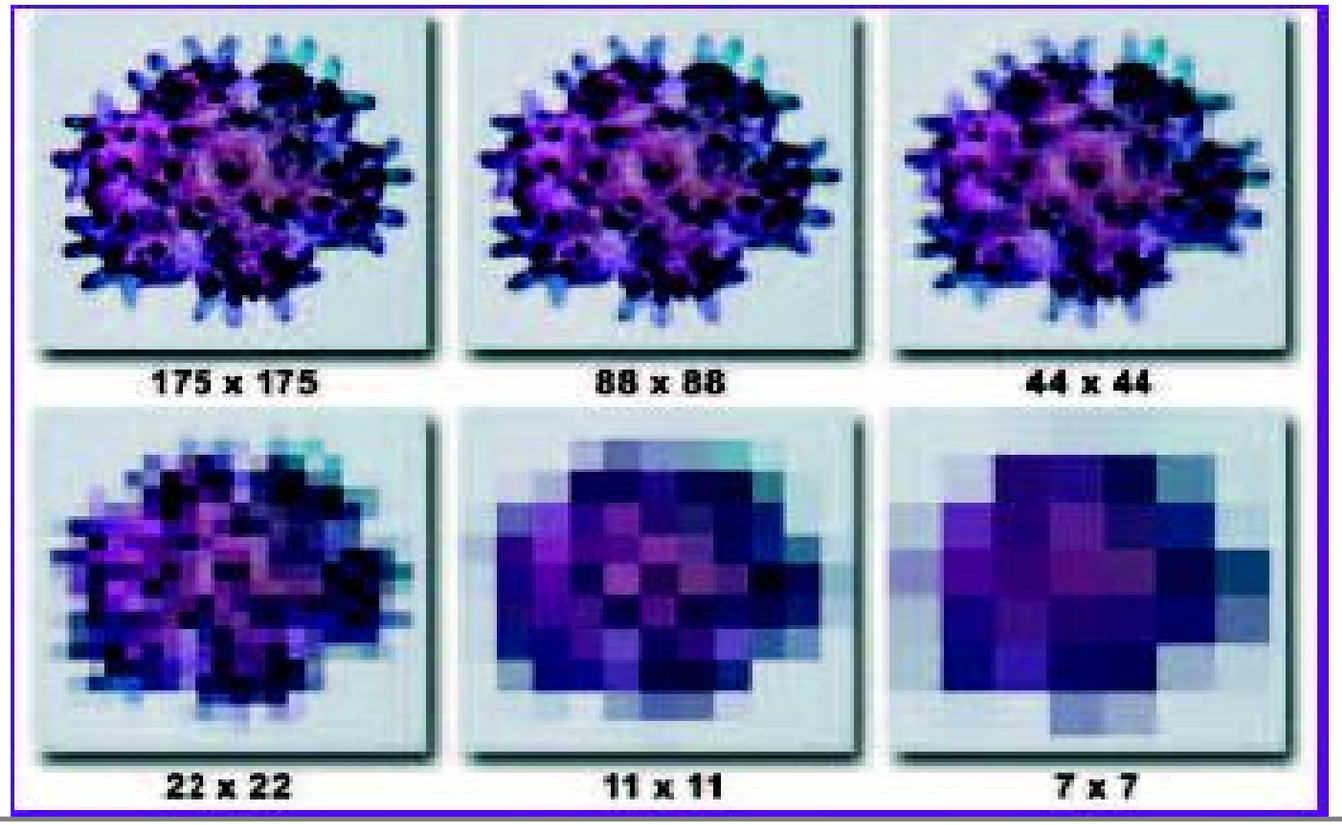
- All CCD`s are Black and White- Colour can be assigned either by software or by use of a filter.





Digital Imaging

- Number of pixels governs image quality



Digital Imaging

- Higher resolution available up to 4000 x 3000
- Up to 16 bit Black and White Cameras
- 65,536 shades of grey
- Variable frame rate to increase sensitivity
- Chip cooling to reduce noise
- Software available for analysis

Digital Imaging

Table 2: the number of pixels a $^1/_2$ inch chip should have to meet the Nyquist criterion (2 pixels per feature) and the optimum resolution (3 pixels per feature).

Objective	Magnification	N.A.	Resolution specimen plan in µm	Resolution image plan in µm	lp/mm	CCD resolution 1/2″ Nyquist limit 2 pixel/lp	CCD resolution 1/2″ Necessaryresolution 3 pixel/lp (Nyquist x 1.5)	
PlanApo	2	0,08	4,19	8,39	119	1526 x 1145	2289 x 1717	2,80
UPlanApo	4	0,16	2,10	8,39	119	1526 x 1145	2289 x 1717	2,80
UPlanApo	10	0,4	0,84	8,39	119	1526 x 1145	2289 x 1717	2,80
UPlanApo	20	0,7	0,48	9,59	104	1335 x 1001	2003 x 1502	3,20
UPlanApo	40	0,9	0,37	14,91	67	858 x 644	1288 x 966	4,97
UPlanApo	100	1,4	0,24	23,96	42	534 x 401	801 x 601	7,99

Microscopy Workshop

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Thank You