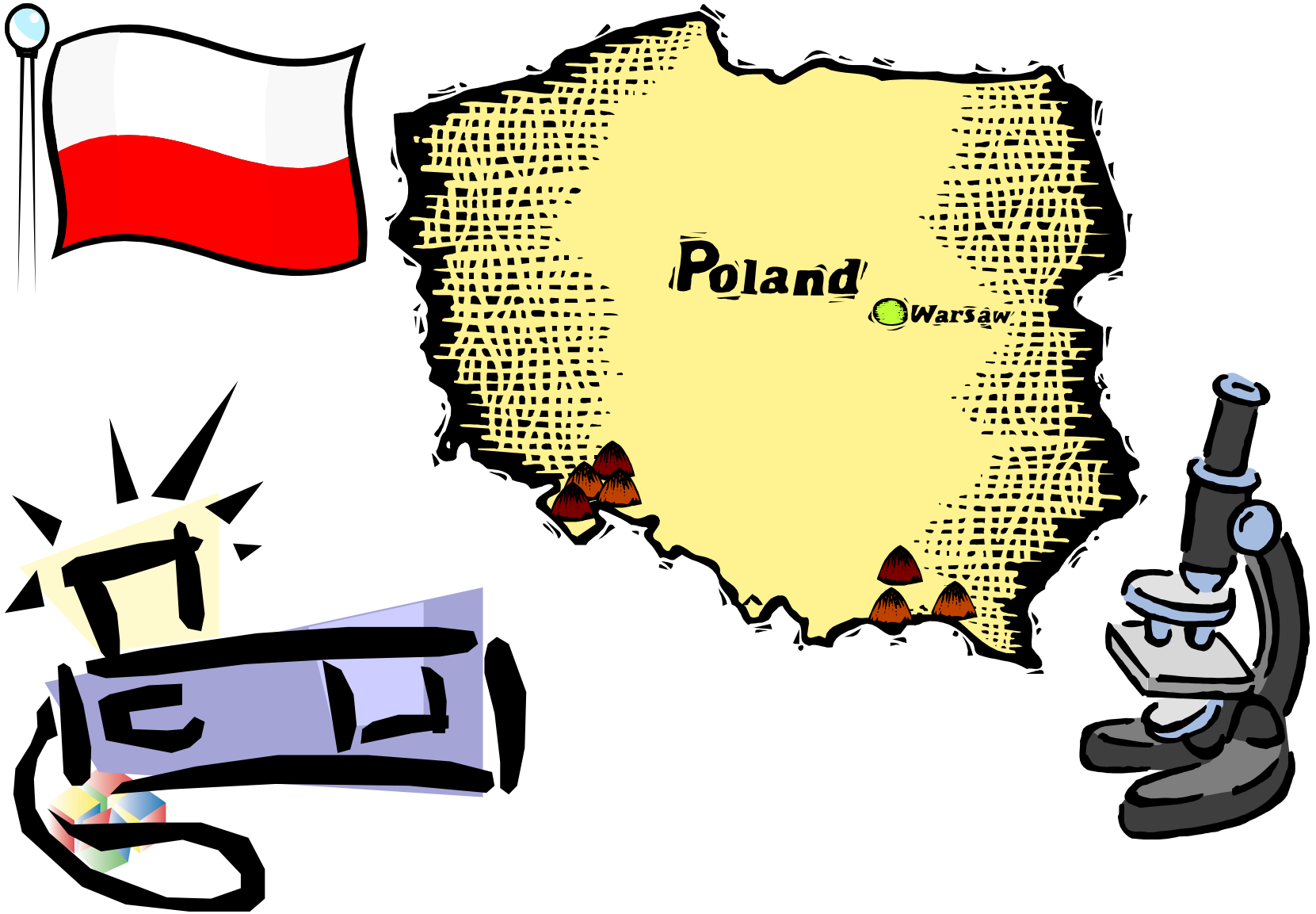
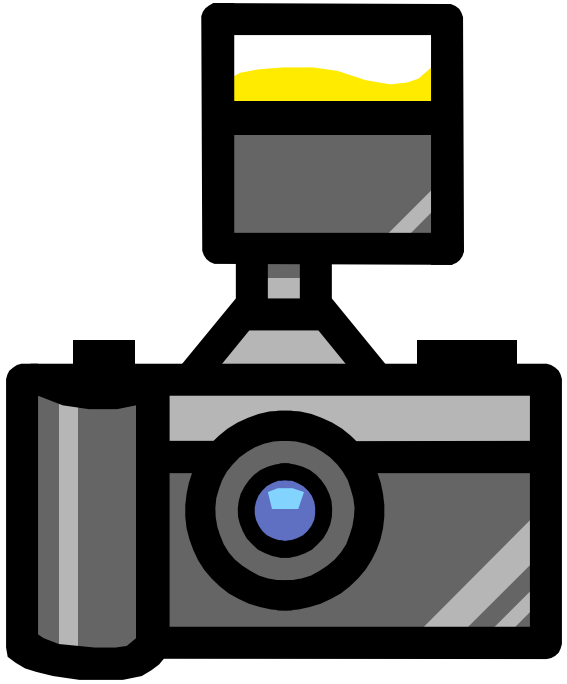
The Digital Image



Overview

- Introduction to Digital Imaging
  Images Sources / Terminology / Image Formats
- Basic Image Processing Techniques
  LUT's / Convolution / Morphology / Deconvolution
- Image Analysis Overview
  Detection / Measurement
- Widefield Fluorescence Imaging
  Software and cameras



Sources of Digital Images – 1

- Consumer Cameras / Web-cameras
  - Huge variety of models and range of resolutions
  - Increasingly popular in general day to day use
  - Devices becoming cheaper and cheaper
  - No optimisation for optical microscopy
- Video Cameras
  - Universal interface from microscope – the C-Mount
  - Medium resolution (750 x 580)
  - Needs a frame-grabber in PC
  - 1-CCD and 3-CCD options
  - Different video standards – US (NTSC) and RoW (PAL)
  - Different size chips in cameras

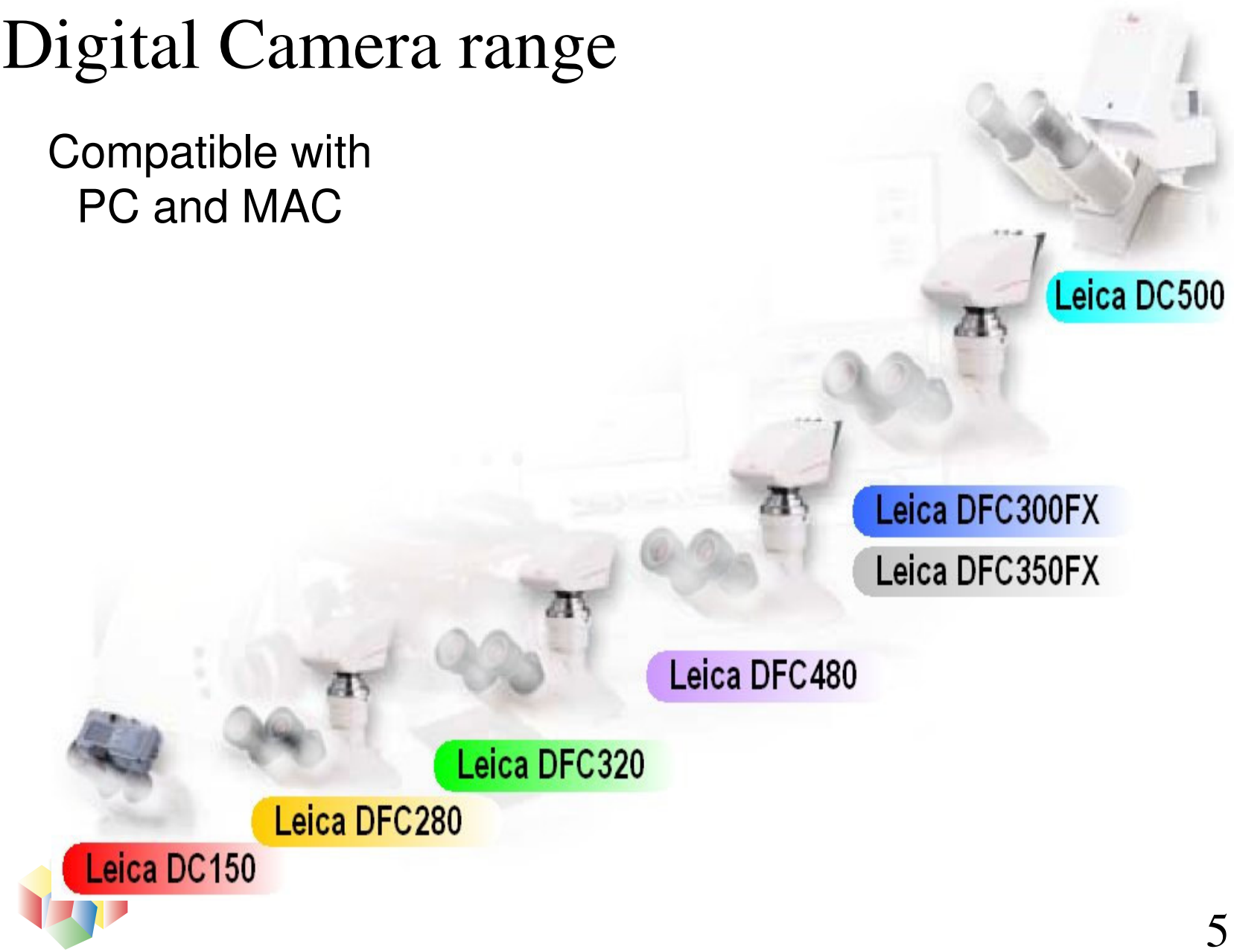
Sources of Digital Images - 2

- Custom Designed Microscope Cameras
  - Often use Firewire interface (to either Mac or PC)
  - Different models with range of resolutions
  - Rapidly replacing Polaroid / 35 mm camera attachments
  - Generally fitted with C-Mounts
  - Specific features for microscopy – White balance, accurate colour rendition, shading correction, long exposure times, pixel shifting
  - Leica offers the DFC range

- Other sources of digital images
  - Flat Bed Scanners
  - Radar / Sonar
  - NMR Scanners / Ultrasound
  - And also the Confocal Scanner (much more later !)

Digital Camera range

Compatible with
PC and MAC



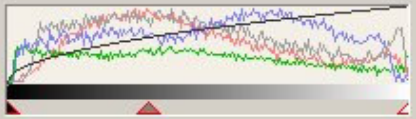
DFC 320 R2, 019930904

Acquire

Exposure time: 46.2 ms

Gain: 1.0 x

Color Saturation: 1.50 x



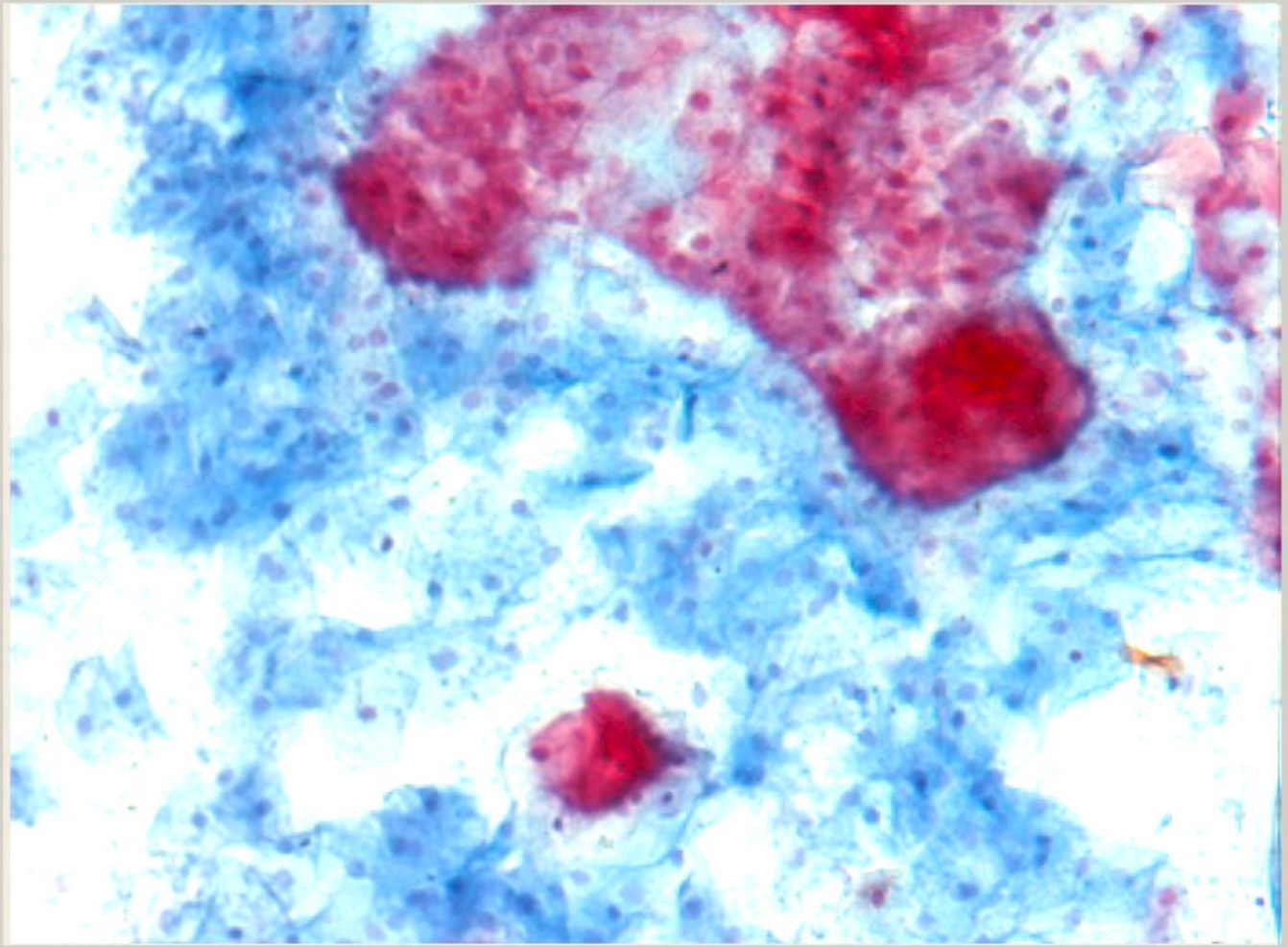
Active Camera	DFC 320 R2, 0199
Configuration	(Last used)
Captured Image	
2088 x 1550	Full Frame HQ
White Balance	(Temporary)
Shading	(None)
Color depth	8 Bit/Channel
Image Type	Color
Scaling	1.00
Sharpen	Off
Crop to ROI	
Live Image	
696 x 514	3x3 Color Binning
Mode	Standard

- [ ] Recording optimized
- [ ] Under/over exposure
- [ ] Zoom focus
- [ ] Find Focus
- [ ] Spot Exposure
- [ ] Check Color
- [ ] Apply shading correction

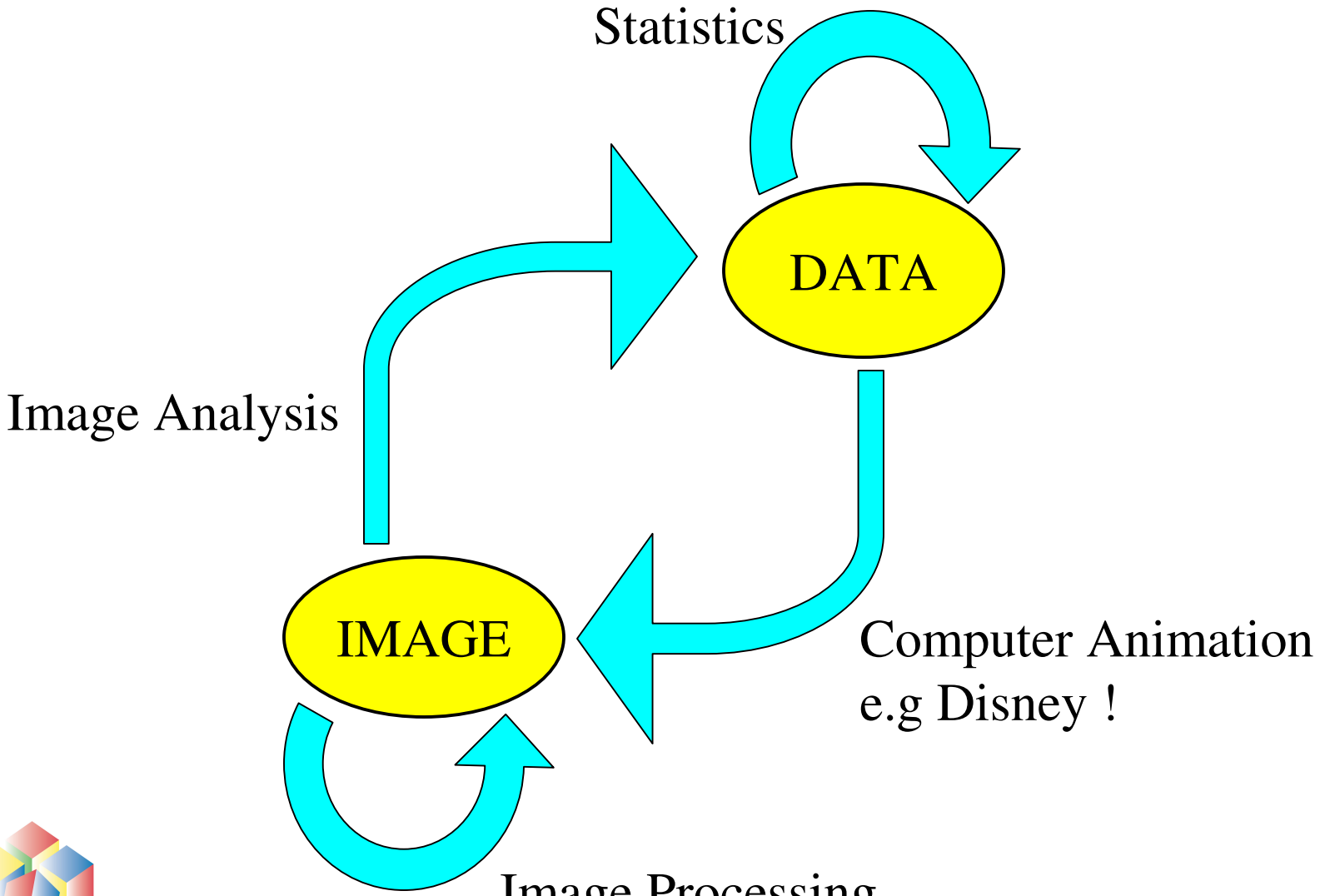
Extra

- [x] Always live
- [ ] Flip Vertical
- [ ] Flip Horizontal
- [ ] Color circle always visible
- [ ] Close after acquire

About...



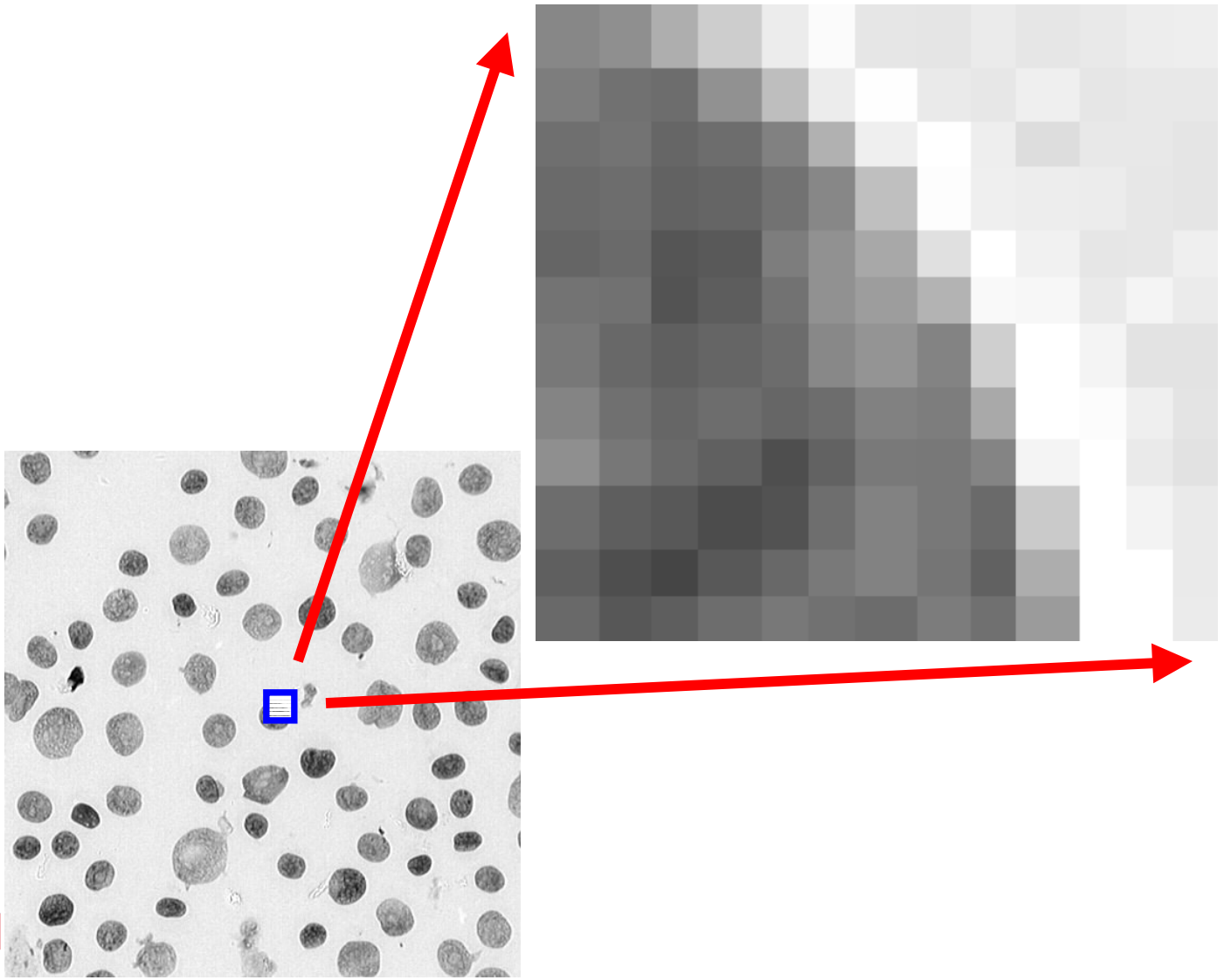
Some terminology



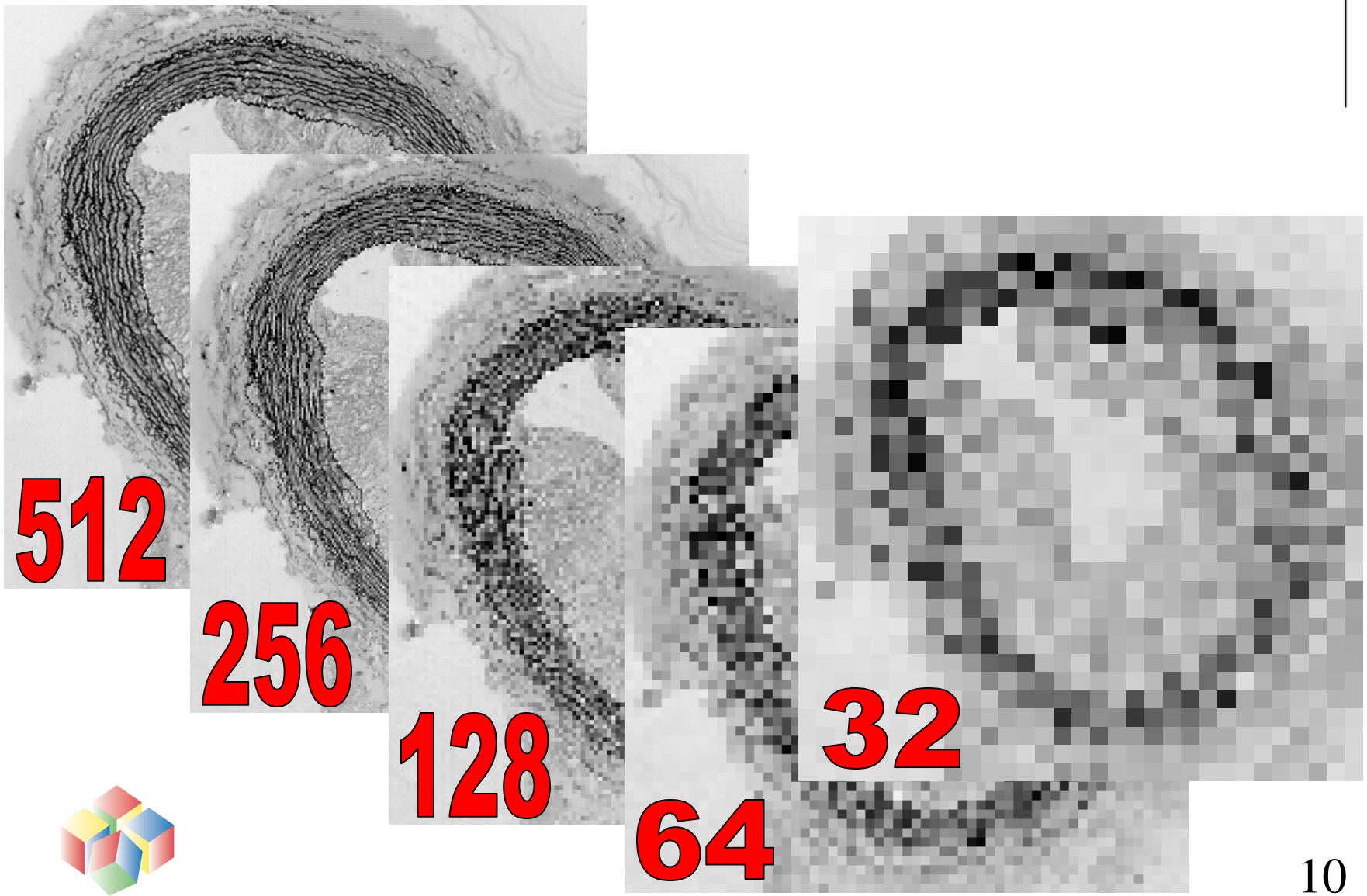
What is a pixel ?

- Digital images are made up from many thousands of separate points called pixels or pels
- Each usually assigned a value between 0 [black] and 255 [white]. This spread is 2^8 or 256 grey levels
- Sometimes for fluorescence cameras extend range of grey levels to 2^{14} or 16384 grey levels
- Pixels are normally square, but not always
- European video cameras gives 760 x 568 pixels
- Often images are sized in powers of two, e.g. :- 2^7 [128^2], 2^8 [256^2], 2^9 [512^2], 2^{10} [1024^2]
- This is known as an image's spatial resolution

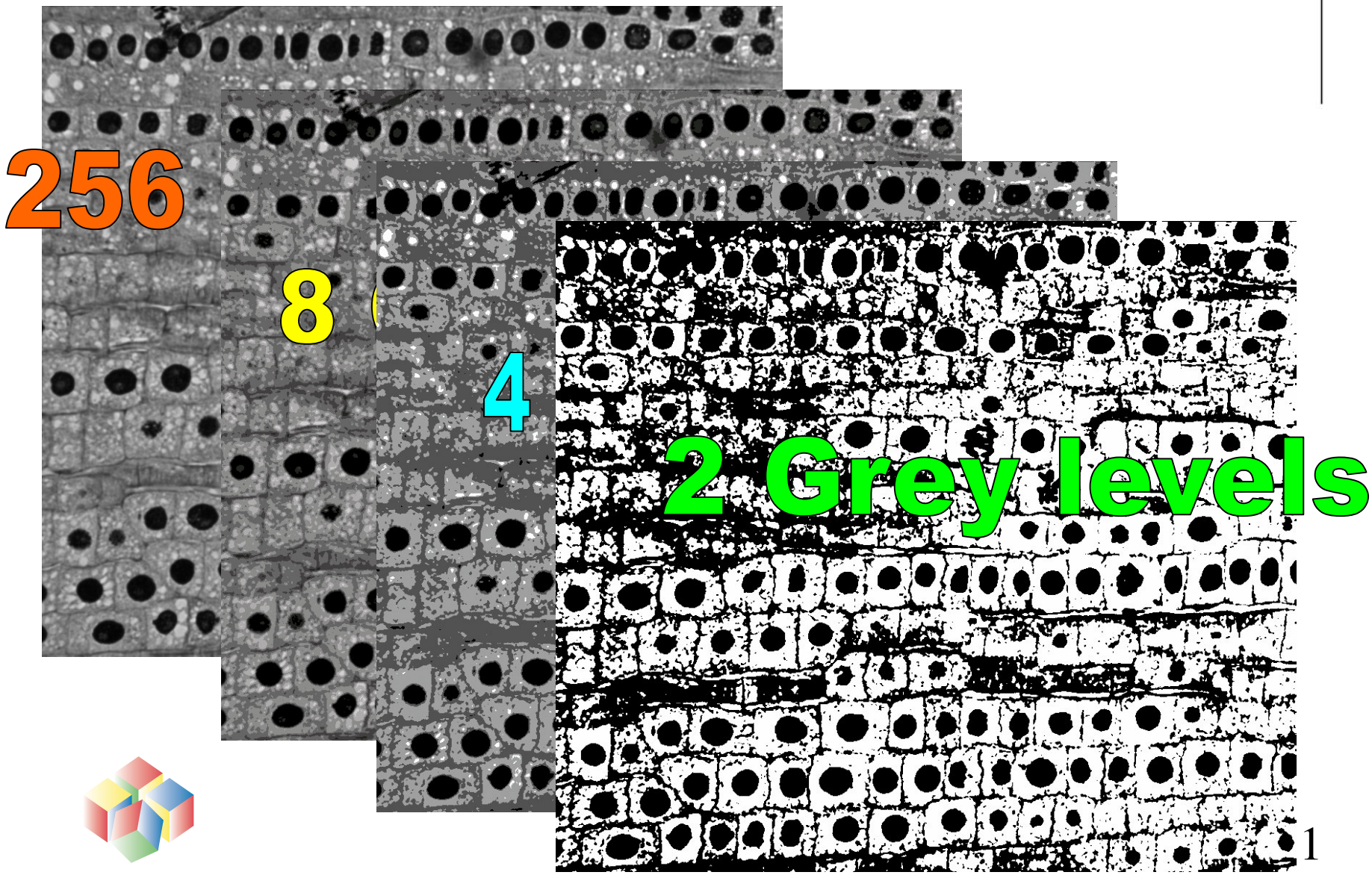
What is a pixel – 2 ?



Spatial resolution examples



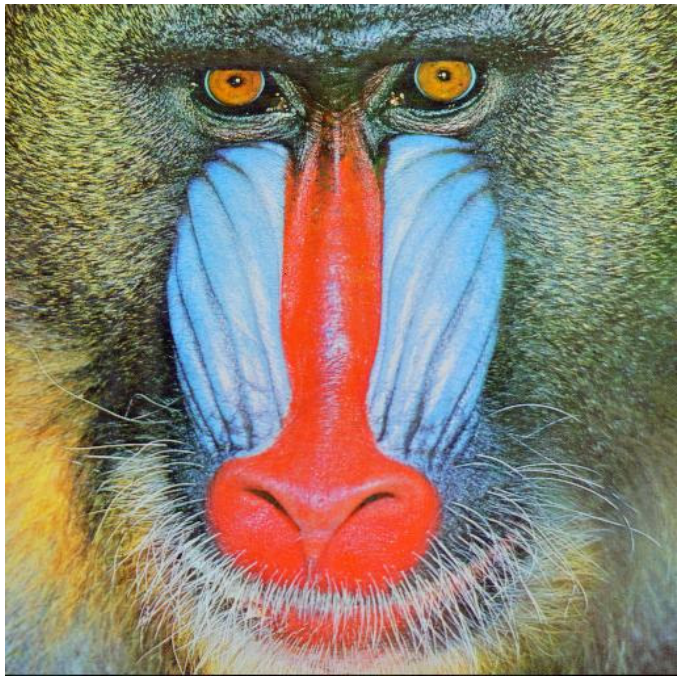
Grey resolution examples

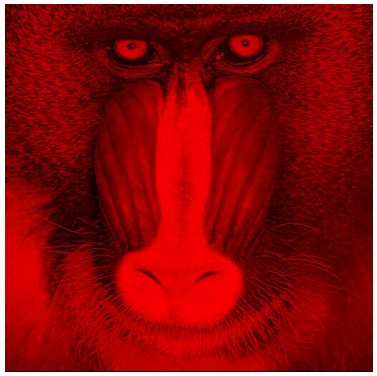


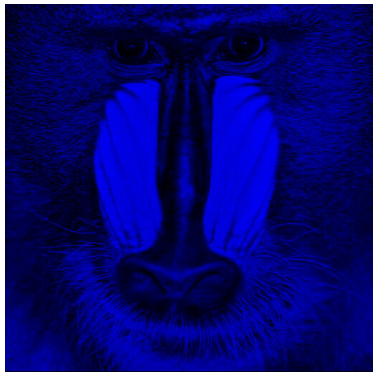
Colour Images

- Usually red green and blue images are superimposed to give a colour image
- Three 256 grey level images give 256^3 or 16,777,216 colour combinations !
- Colour can also be defined in terms of Hue, Saturation and Intensity [HSI], which is often useful for image analysis applications
- Pseudo colours or LUT's are often used to add color to a mono image, e.g. in an SEM or when using a mono camera for capturing fluorescence images

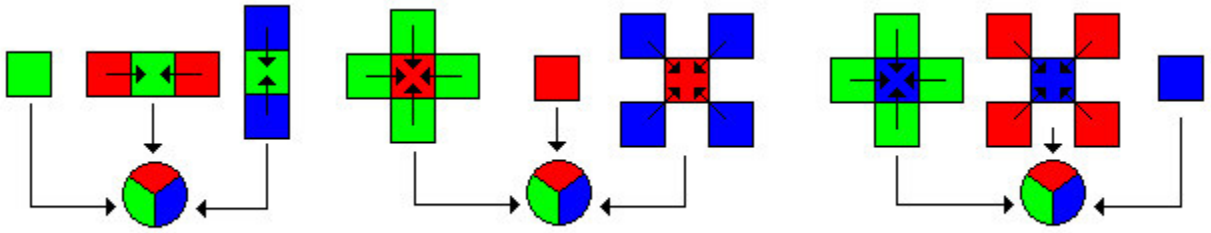
Colour - 1

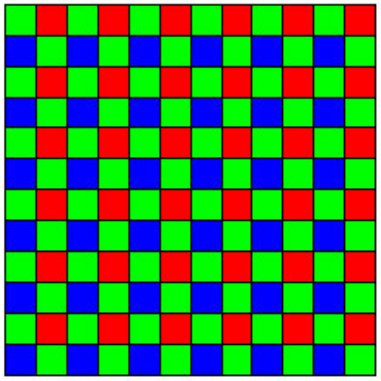






RGB Interpolation



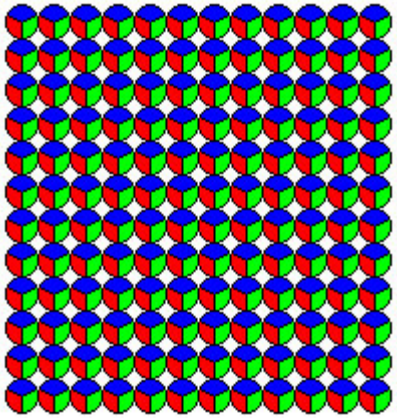


Bayer filter applied on CCD chip

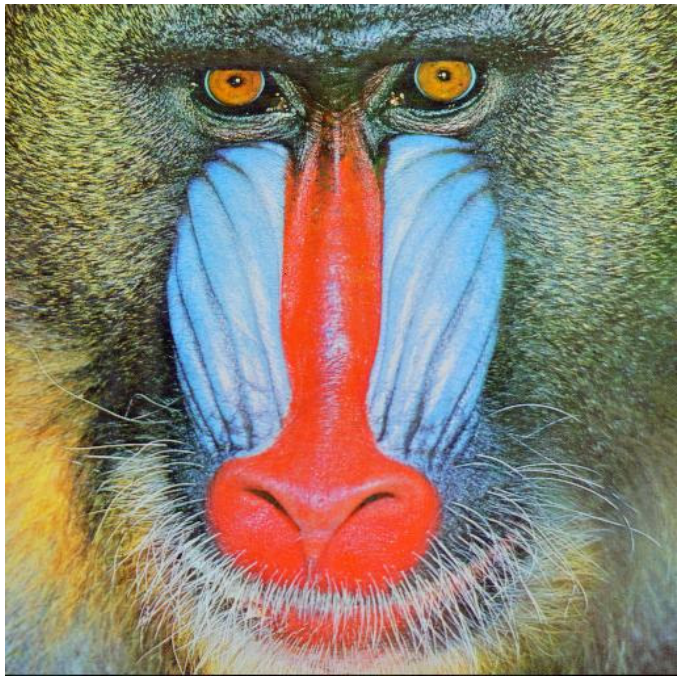
50% are green

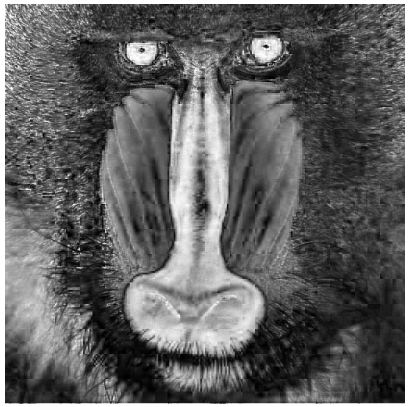
25% are red

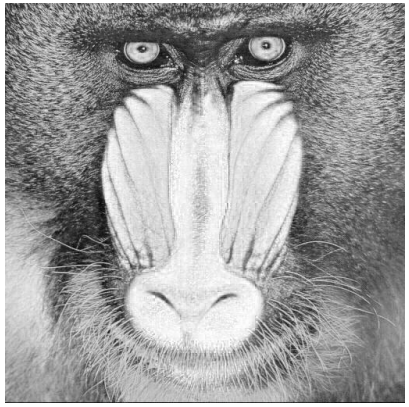
25% are blue



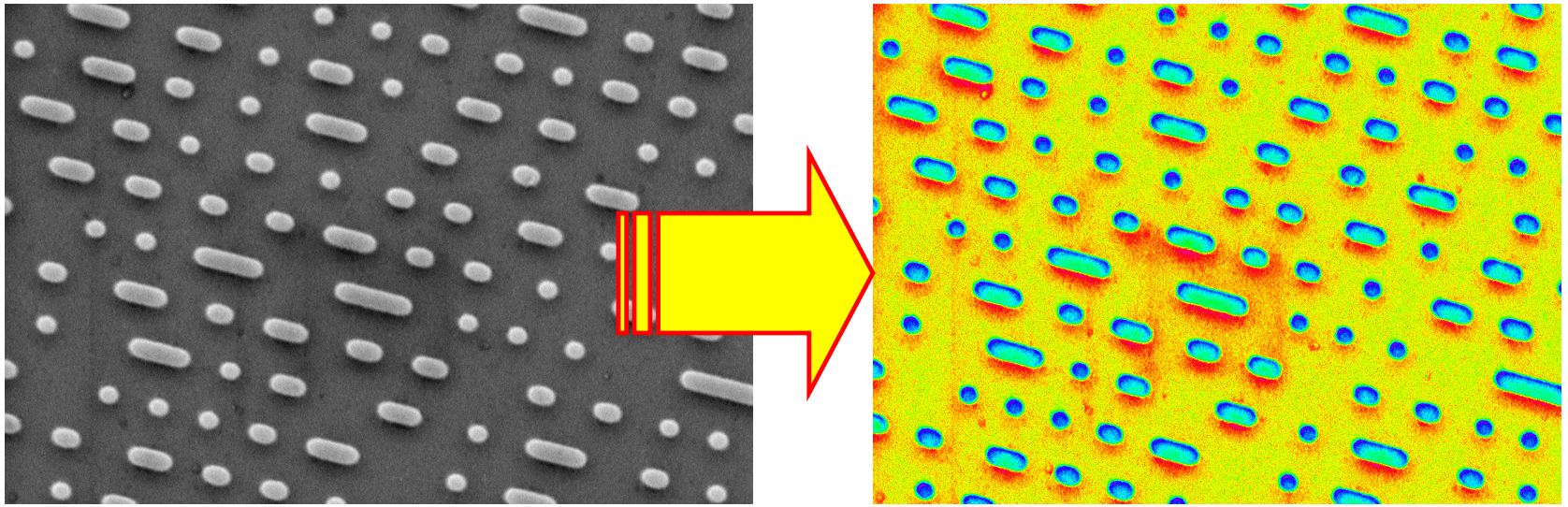
Colour - HSI







Examples of pseudo colour



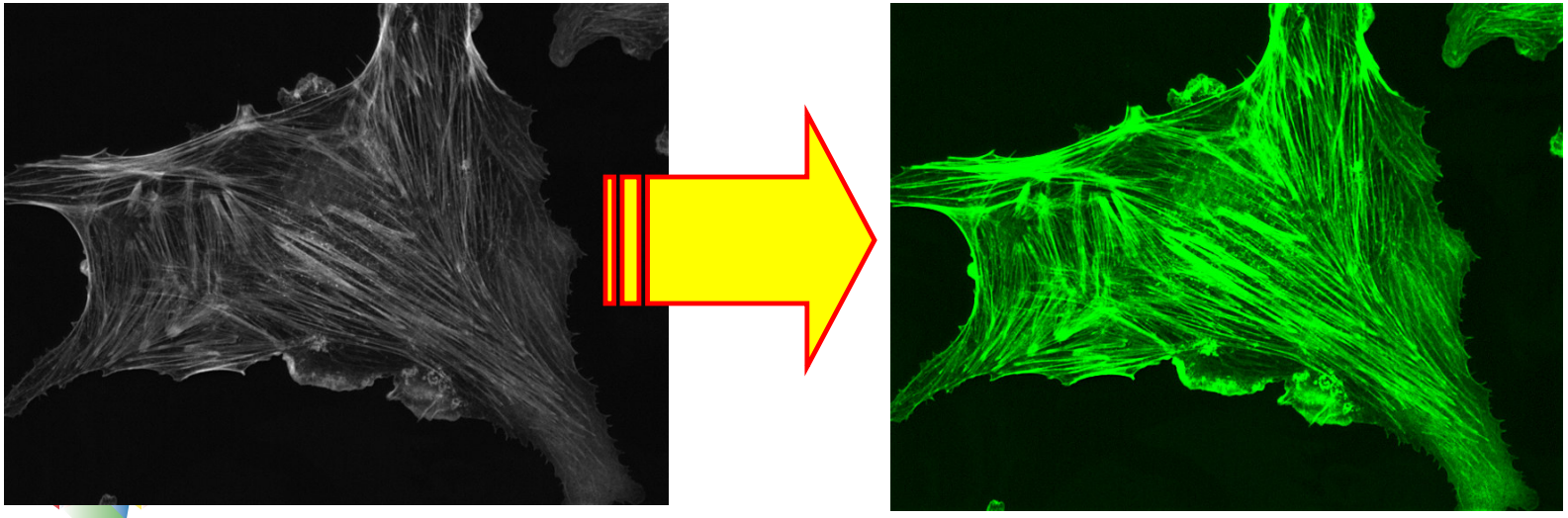


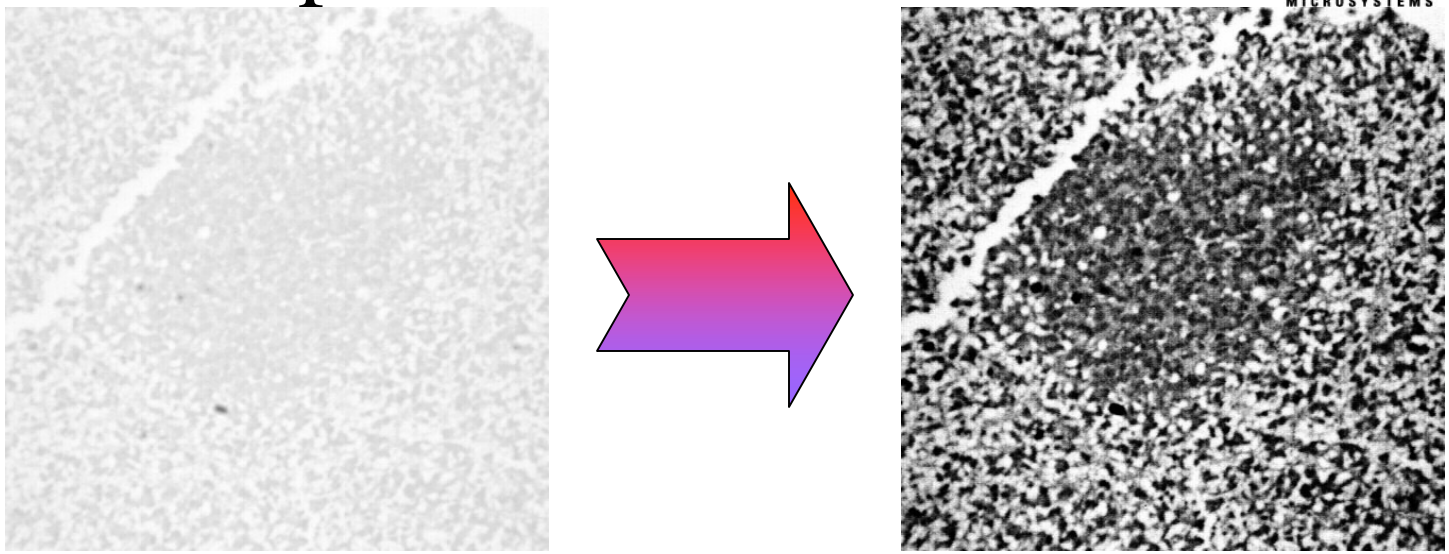
Image Formats

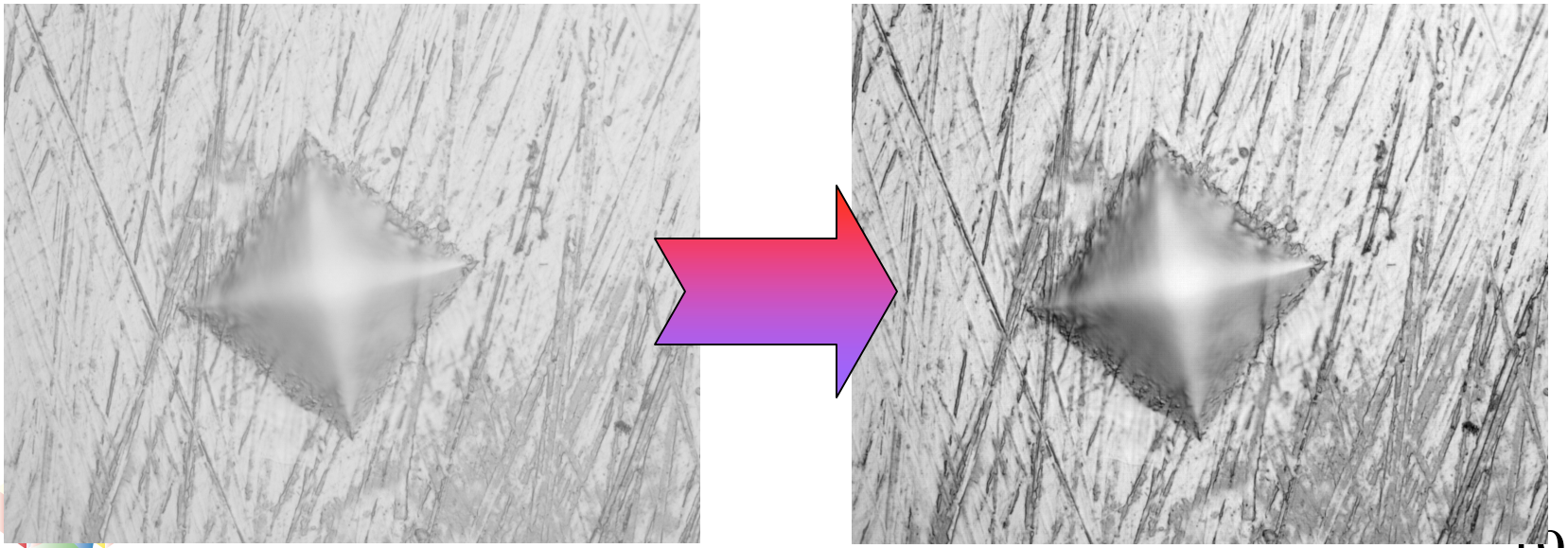
- TIF – Universal 24 bits/ pixel. No compression
- PCX – Dos format 24 bits / pixel. No compression
- BMP – Microsoft image format. No compression
- PICT – Format used by Macs. 24 bit / pixel. No compression
- JPG – Popular format. Good compression to 5%
- IPTC / DICOM – Image data held in header. Used in medical field
- J2K – New improved jpeg compression format
- . . . . and many many others ! ! !

Look-up-table transformations

- The contrast is amended by reviewing the entire grey histogram
- Each grey value from 0 to 255 is mapped to a new output value
- LUTs are often used to ensure image has maximum contrast and is neither under nor over exposed
- Each pixel is handled individually, nearby or neighbouring pixels are not considered
- Common examples include :-
  - Contrast stretch / Auto contrast
  - Invert
  - Histogram equalisation
  - Gamma correction

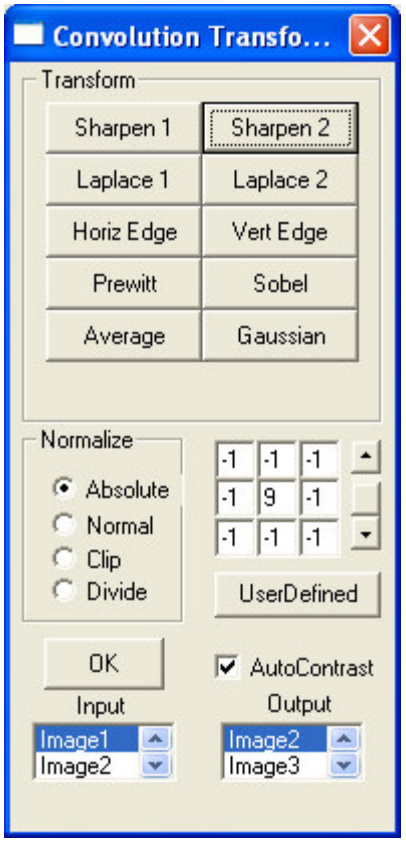
LUT examples



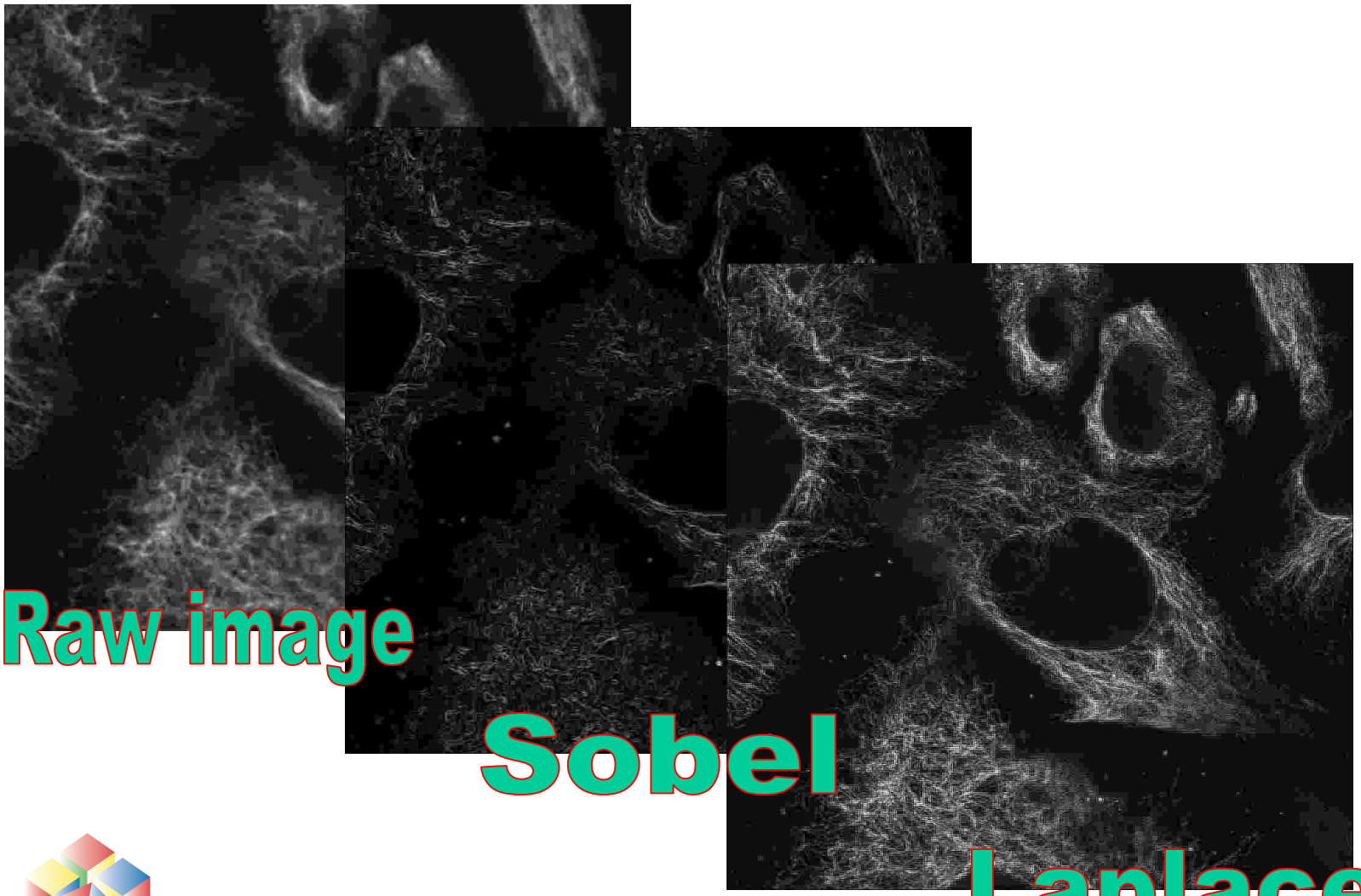


Convolution

- Kernels passed over the image point by point
- Usually 3x3, 5x5 or 7x7
- Example shown here →
- Different kernels used for edge detection, sharpening, smoothing
- Common terms are sobel, laplace, gaussian, prewitt, unsharp masking
- All pixels used in calculation of output image

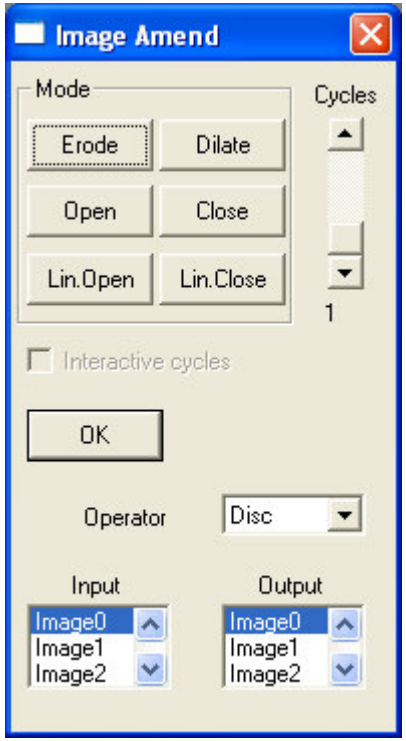


Convolution examples

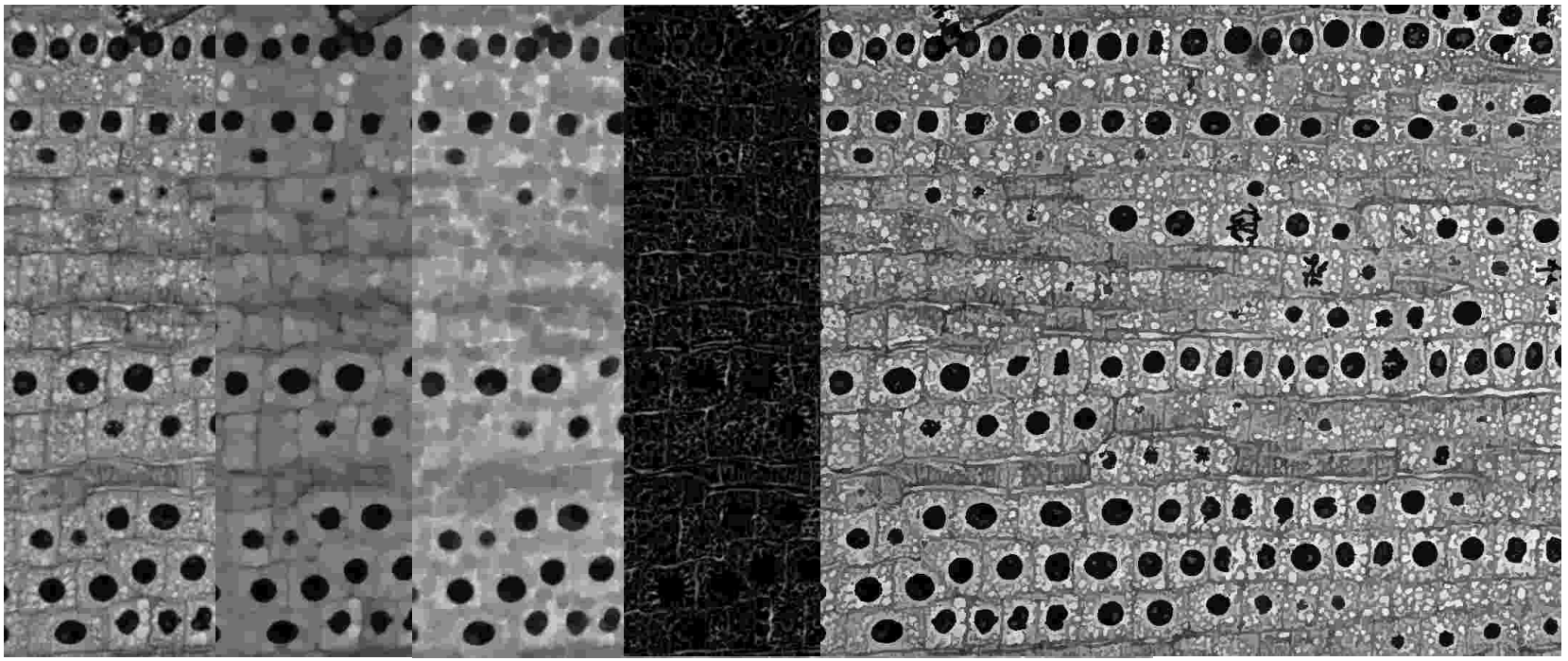


Morphology

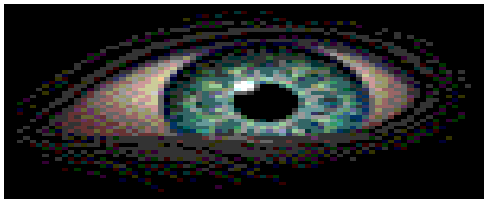
- Concept broadly similar to convolution
- Mask or operator passed over the image, pixel by pixel
- Difference is brightest or darkest pixel are selected while others are discarded
- Basic operations are erosion, dilation, open and close
- Many effects can be created from these basic operators, e.g. top hat, sharpen, gradient, delineation
- Careful choice of operator can be used to extract features of interest



Morphology examples



Human visual system



- Extraordinarily powerful at finding interesting detail
- Limited accuracy for precise measurements
- Suffers from monotony and is error prone
- Needs rest and has suspect objectivity

Image Analysis benefits

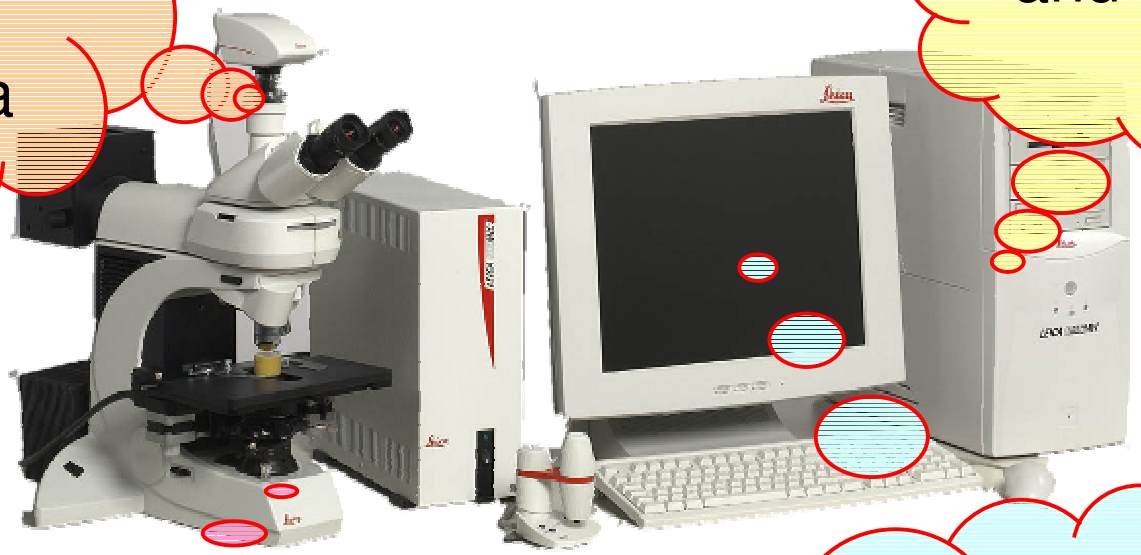
- Is reliable and tireless
- Improves throughput
- Makes exact measurements
- Doesn't get bored
- But is not intelligent

IA system components

Leica MICROSYSTEMS

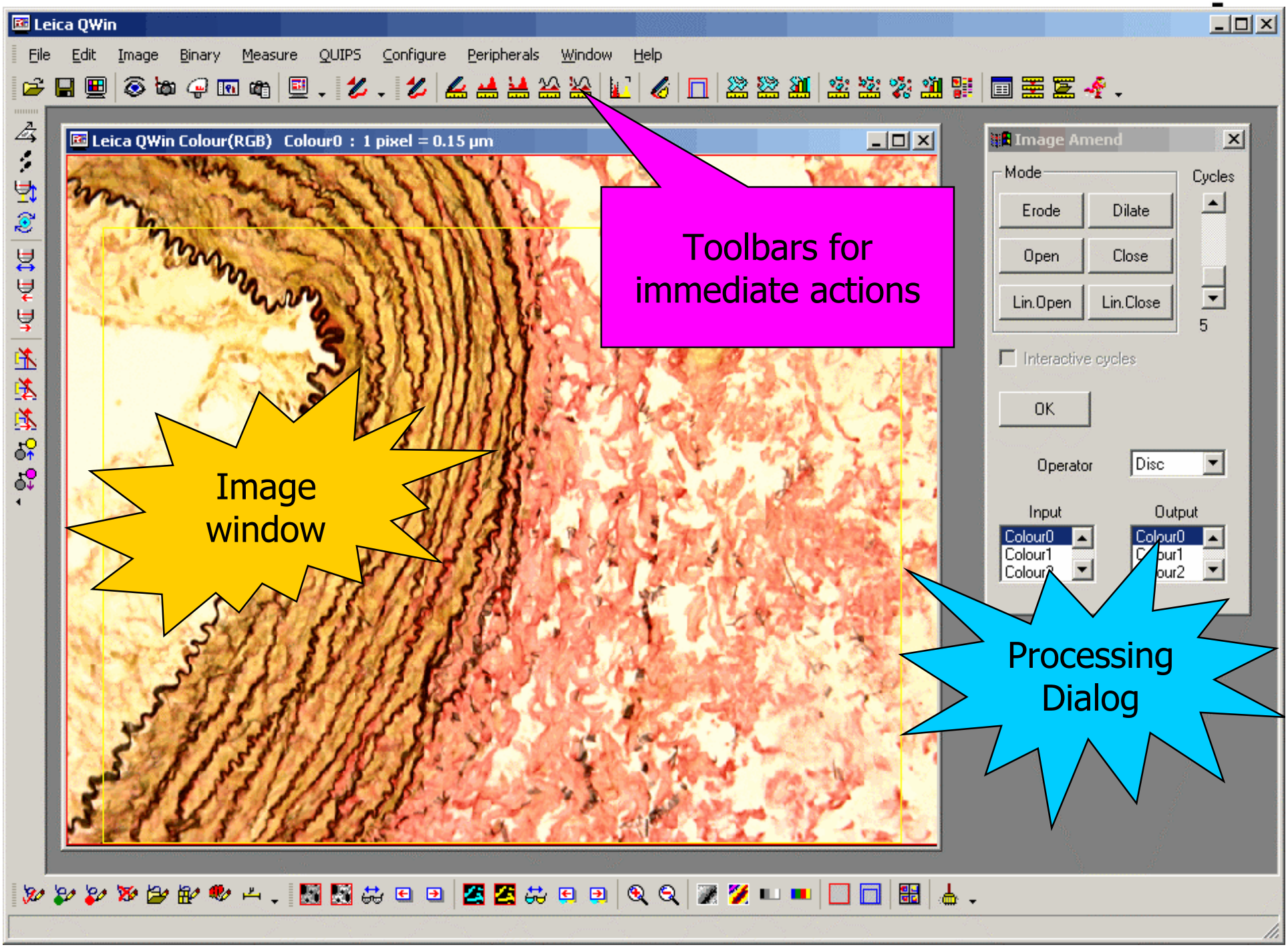
Video or Digital Camera

Computer and display



Microscope

Image Analysis Software


Leica QWin
File Edit Image Binary Measure QUIPS Configure Peripherals Window Help
Leica QWin Colour(RGB) Colour0 : 1 pixel = 0.15 µm
Toolbars for immediate actions
Image window
Image Amend
Mode
Erode
Dilate
Open
Close
Lin.Open
Lin.Close
Cycles
5
Interactive cycles
OK
Operator
Disc
Input
Output
Colour0
Colour1
Processing Dialog

Choose the imaging technique that gives the best contrast and detail . .

Leica MICROSYSTEMS

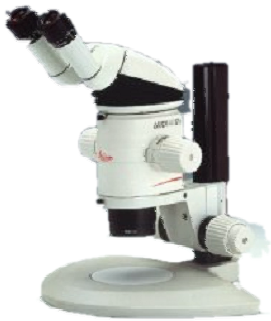
Fluorescence

Brightfield

Polarised

Dark-field

Incident



DIC Phase contrast

Transmitted

Measure- interactively – simply draw on the image

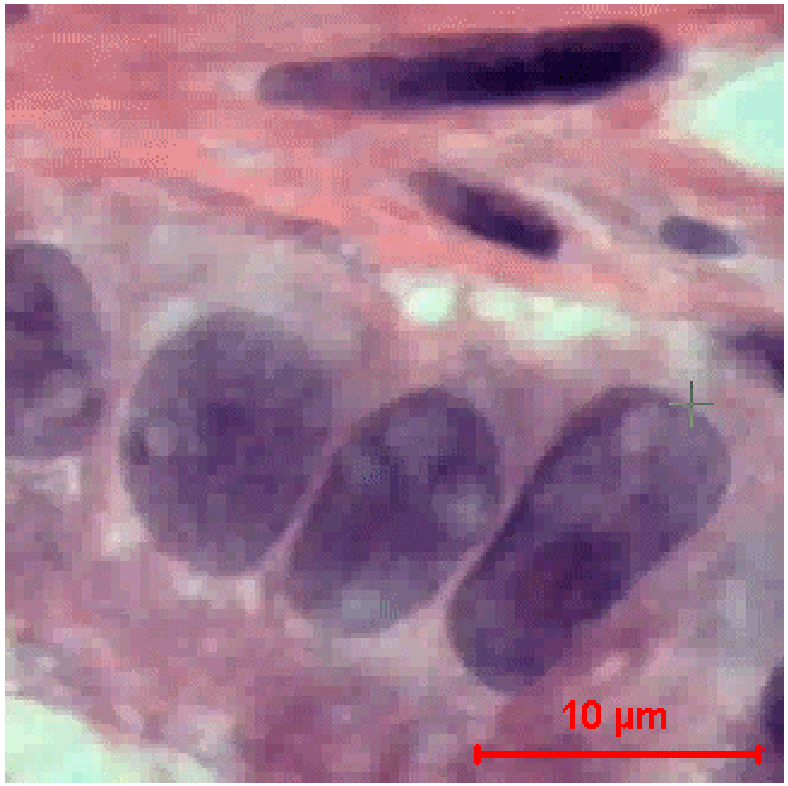
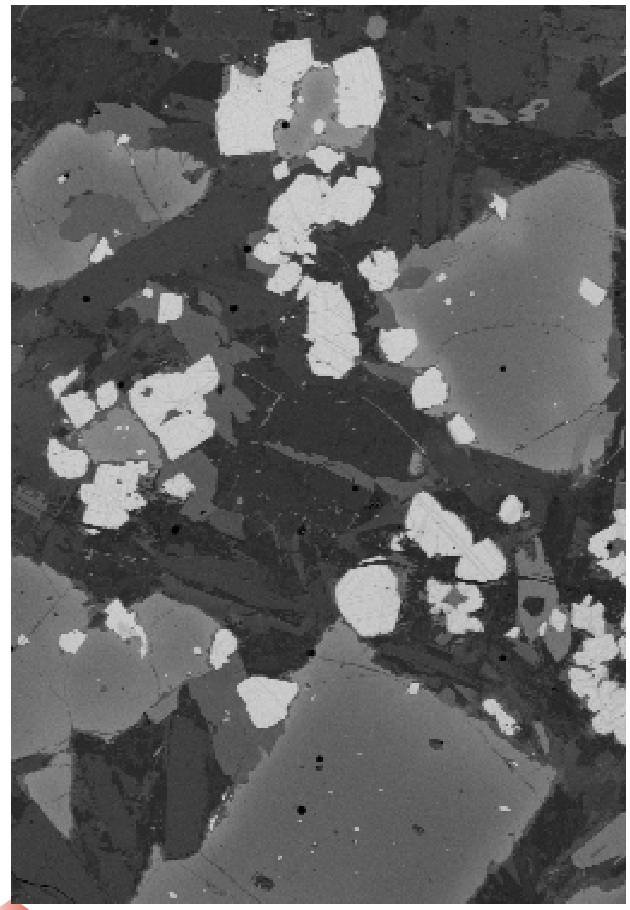
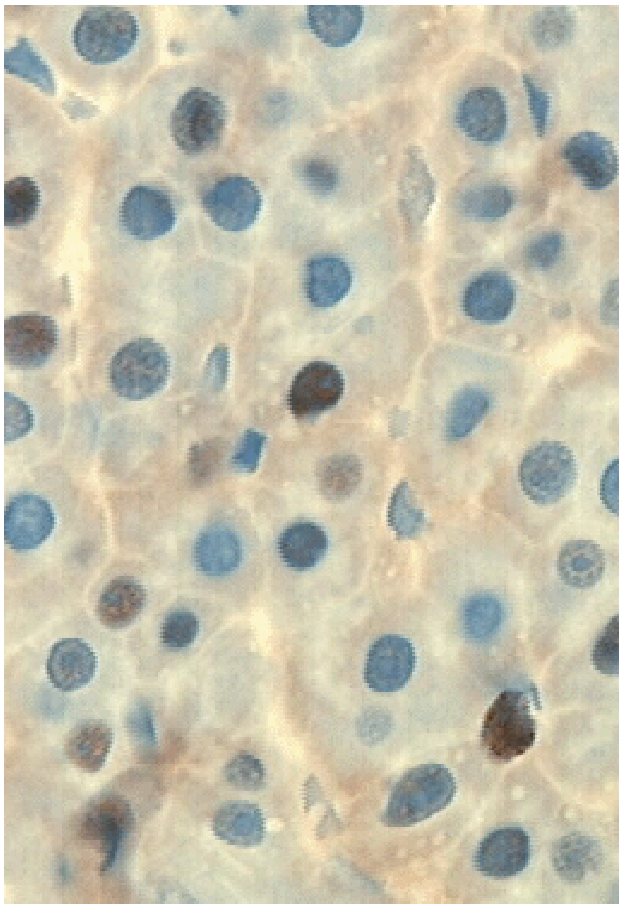


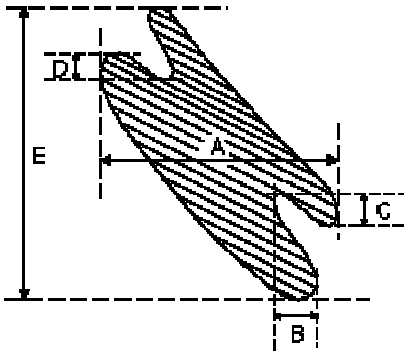
Image detail is identified by colour or intensity



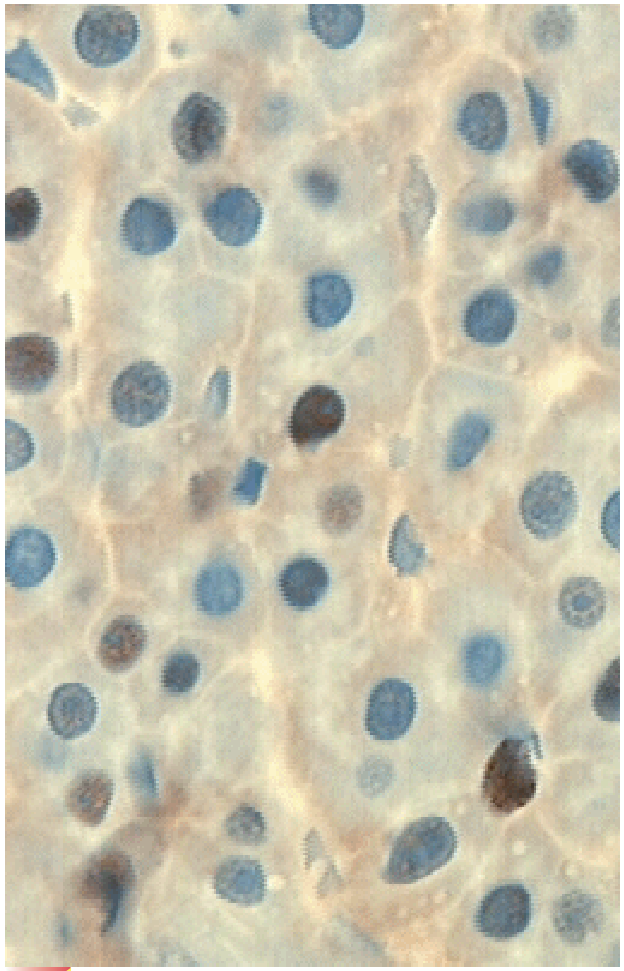


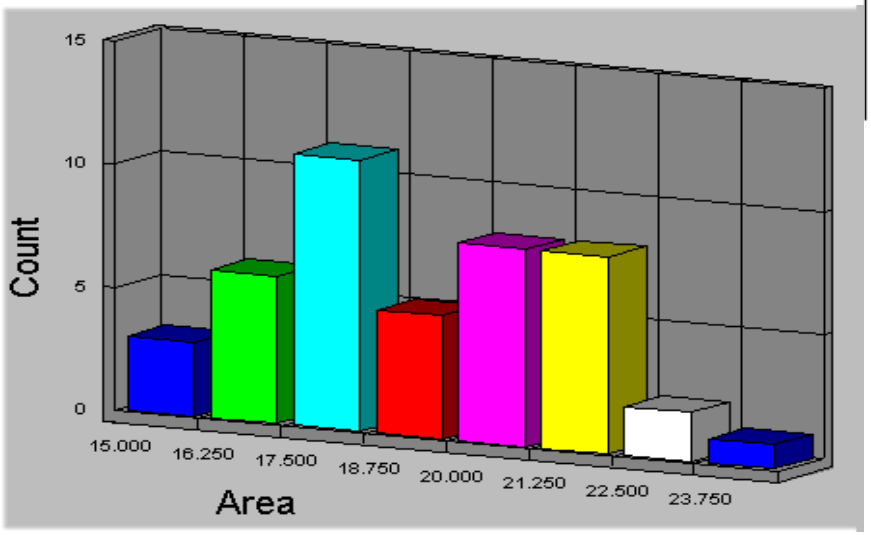
Many types of measurements

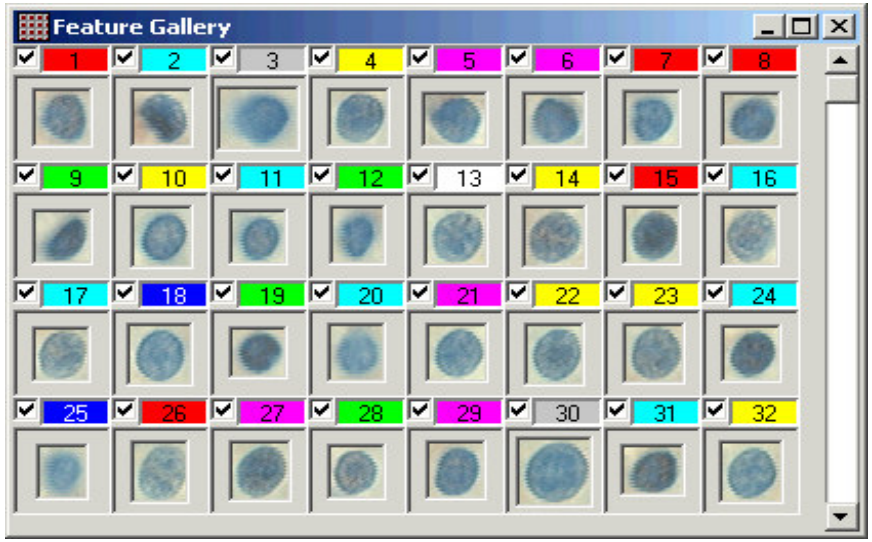
- Count
  - objects in field of view
- Size
  - area, length, volume, layer thickness
- Brightness
  - profile, reflectance, density, colour
- Shape
  - roundness, aspect ratio



Measure cells and show gallery

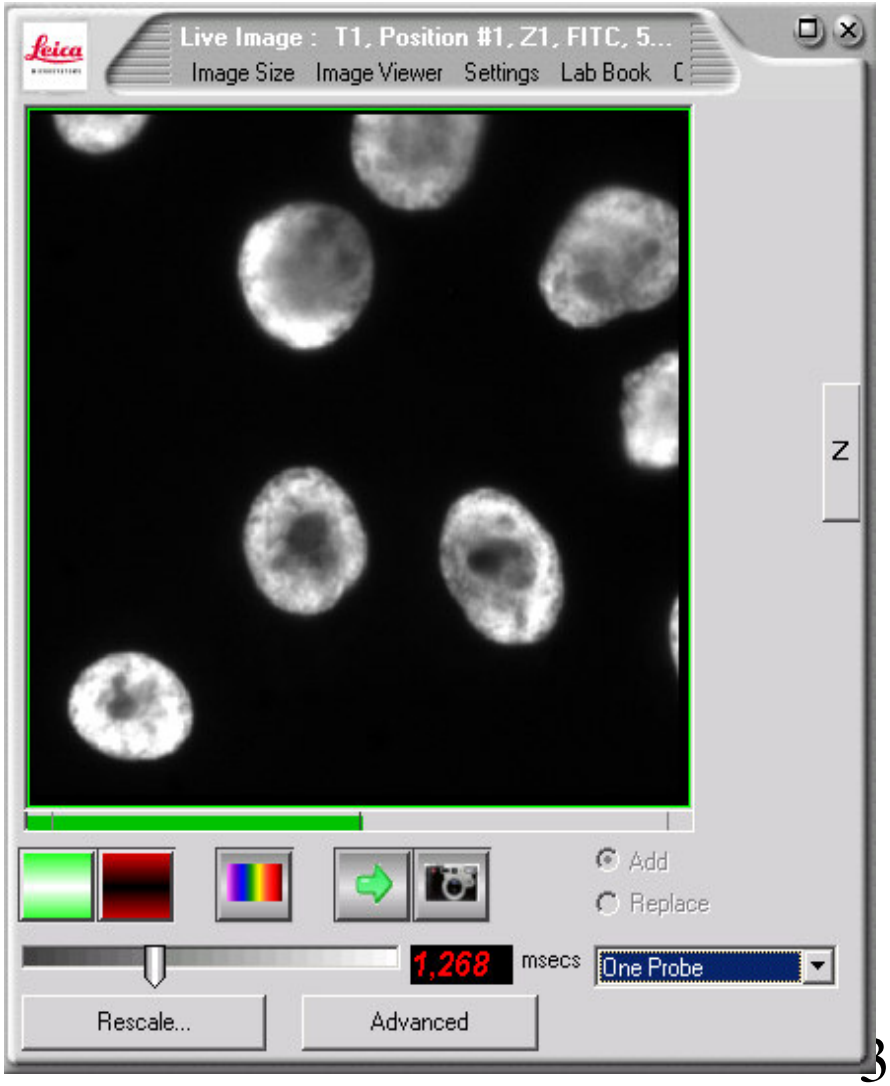




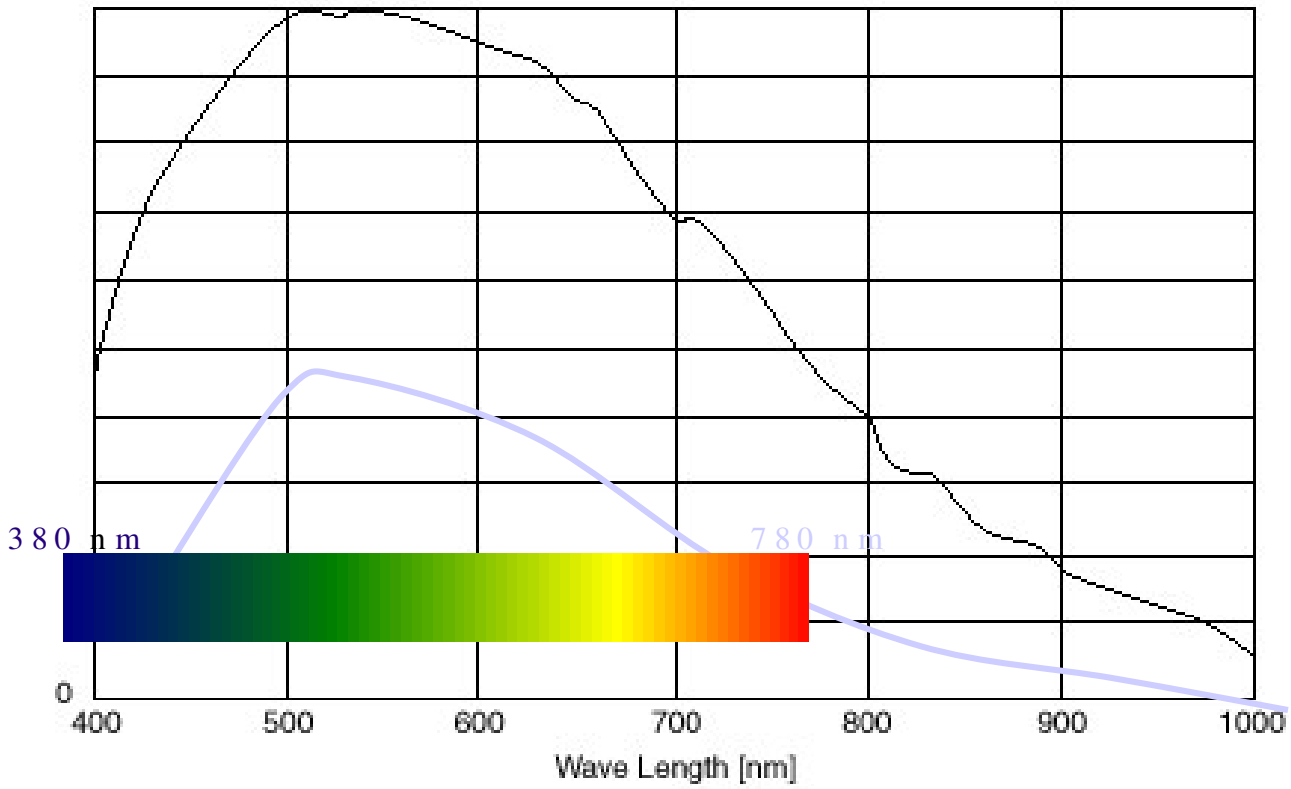


Fluorescence Digital Imaging

- Camera concepts
- Software
- FW4000



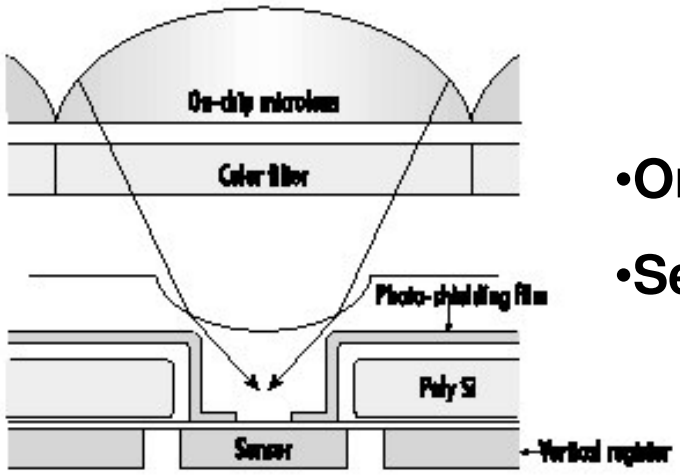
Excellent quantum efficiency



EXview HAD CCD™

Excellent quantum efficiency - 2

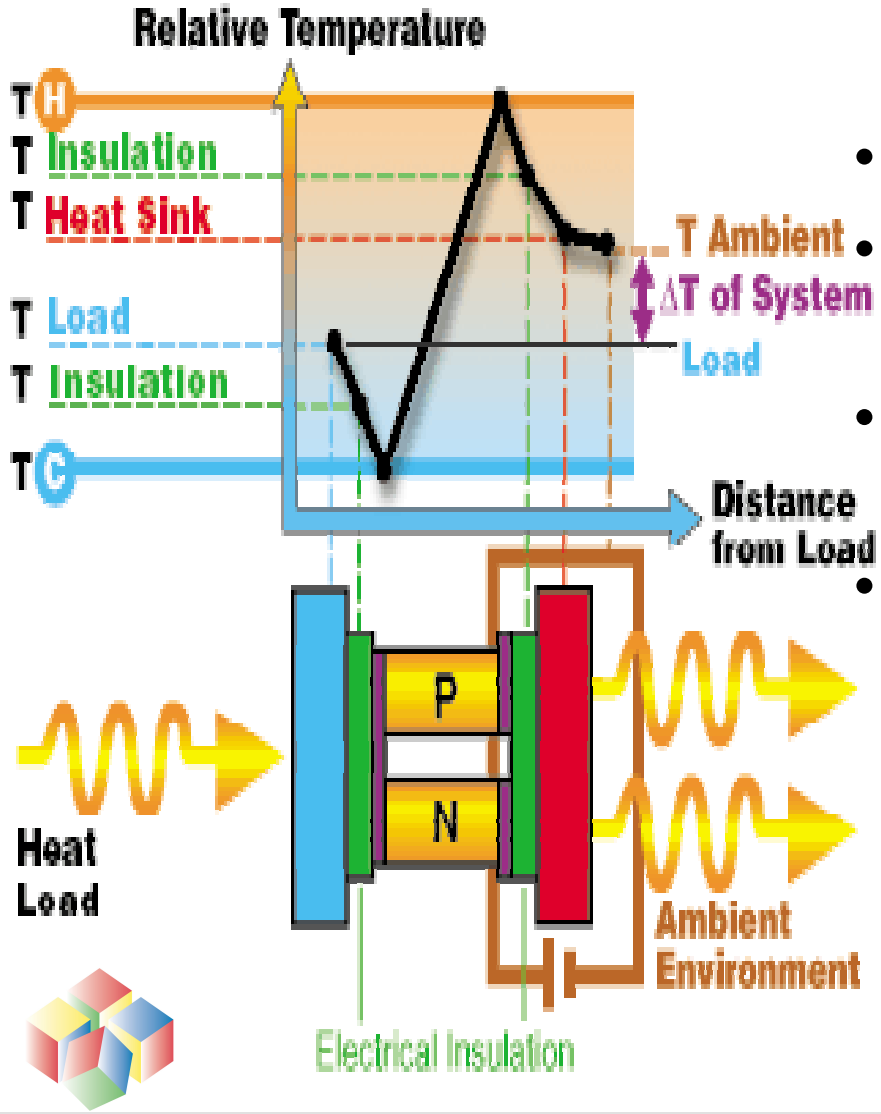
- Genetics work with :-
  - Cy3 570nm
  - Cy5 670nm
  - Cy7 767nm



Super HAD CCD Sensor

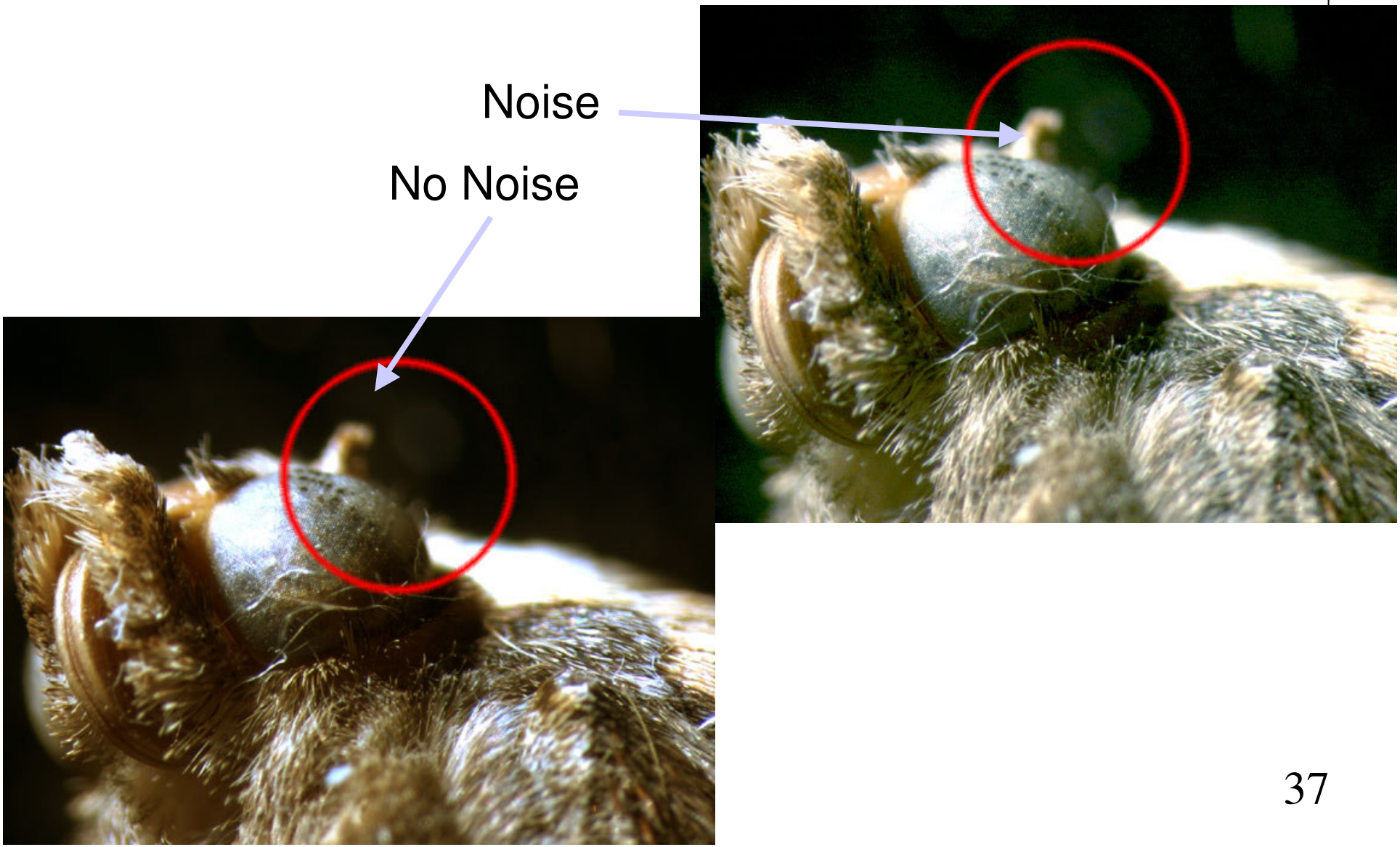
- **On chip microlenses**
- **Second layer of microlenses for sensitivity**

Peltier cooled

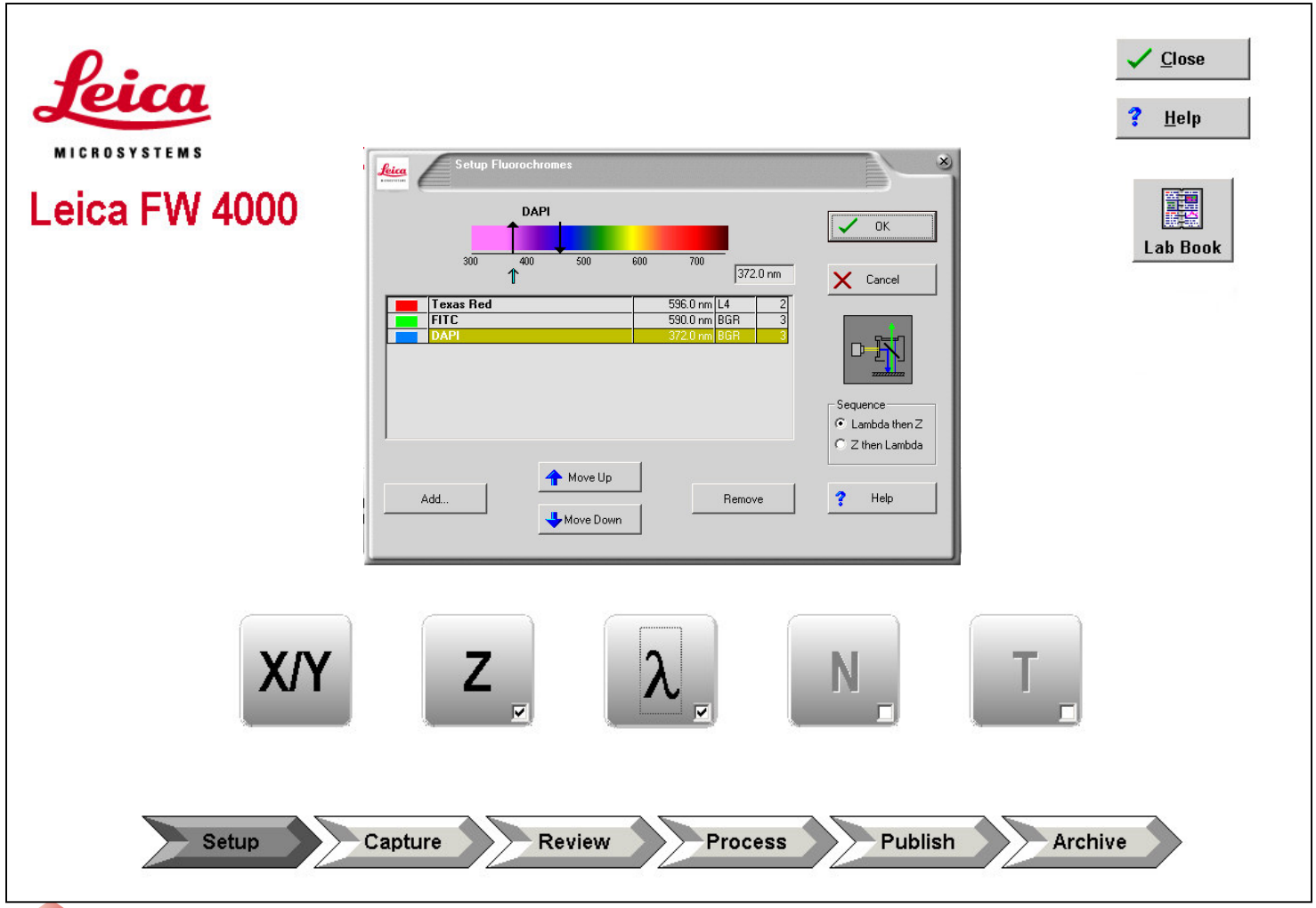


- Reduces dark current
- Allows exposure times of up to 600 seconds
- Dark current for DFC300FX
  - 0.15 e / p / s
- Dark noise depends on cooling temperature

DFC Low-noise example



FW-4000 Application Package

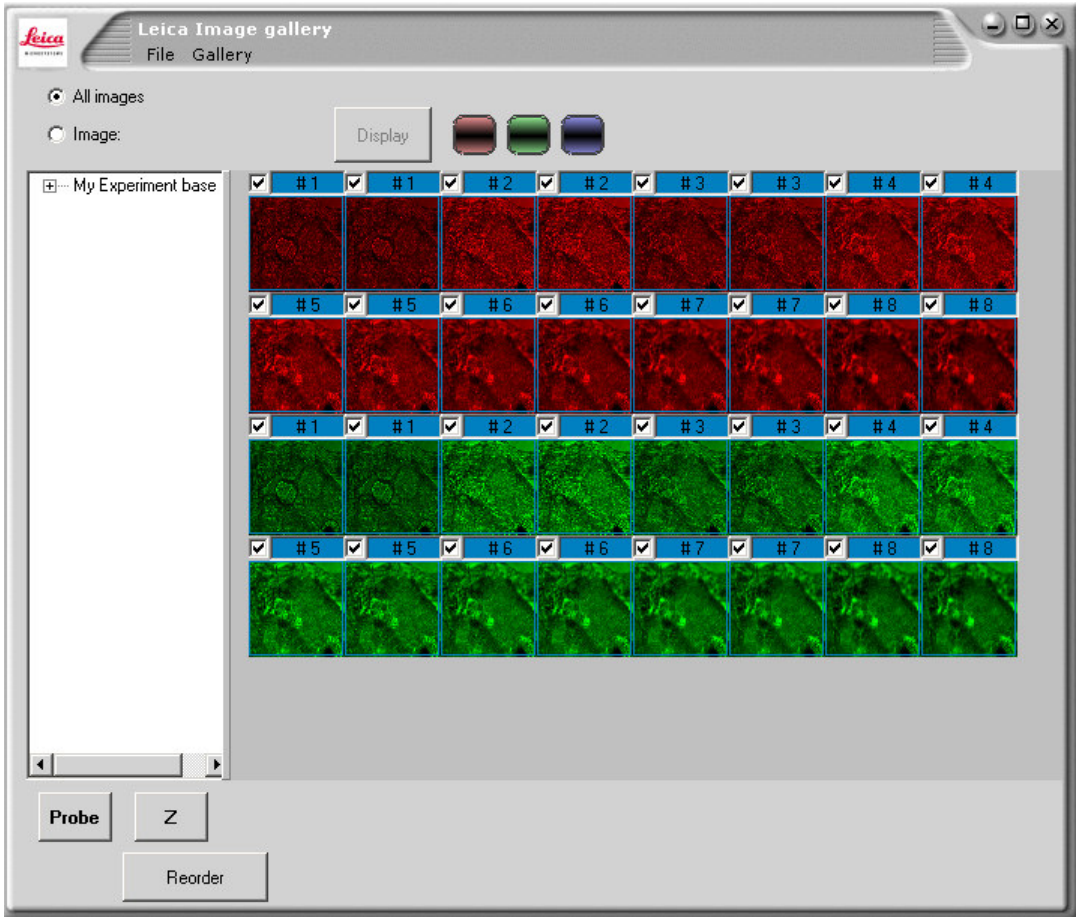


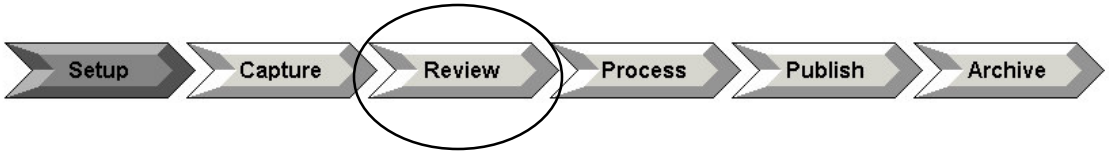
Performing an Experiment

- Overview of the steps

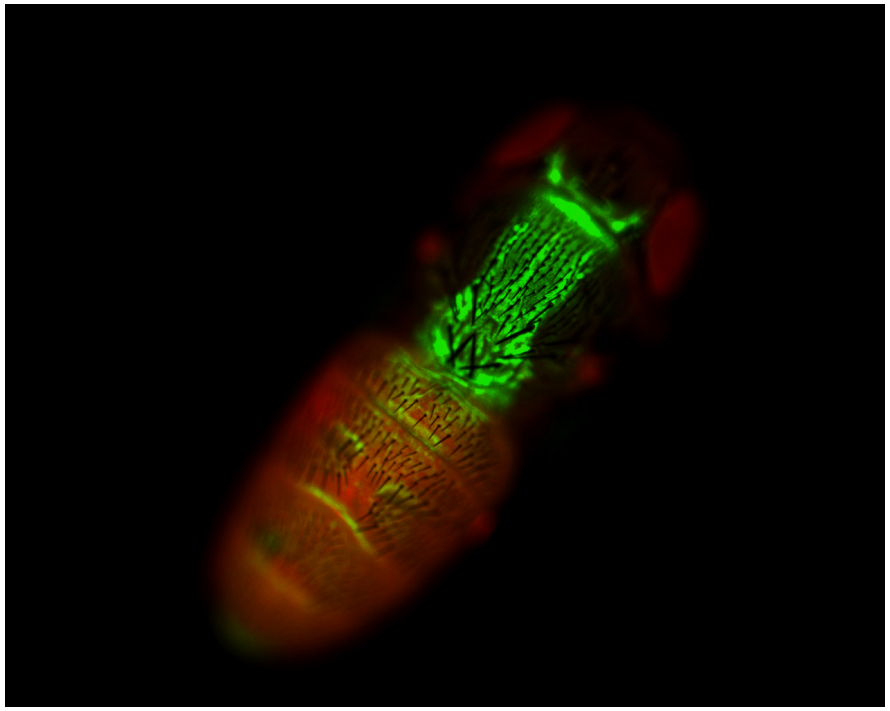
- **Step One –** Set up **your Experiment**
- **Step Two – Automatically** Capture **your Images**
- **Step Three –** Review **your images in the interactive Image Gallery**
- **Step Four –** Process, **Enhance or Measure your Images**
- **Step Five –** Publish **Print, make Web documents or Movies from your Images**
- **Step Six - Archive Transfer your Experiment across a Network or onto Backup Media**
  - Experiment Complete!

Overview of Review





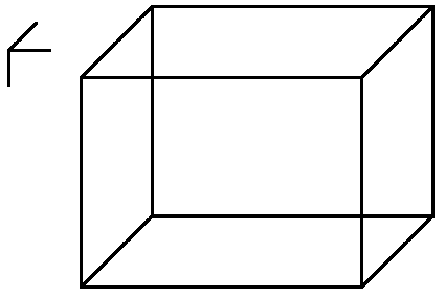
Fluroescence Applications –1



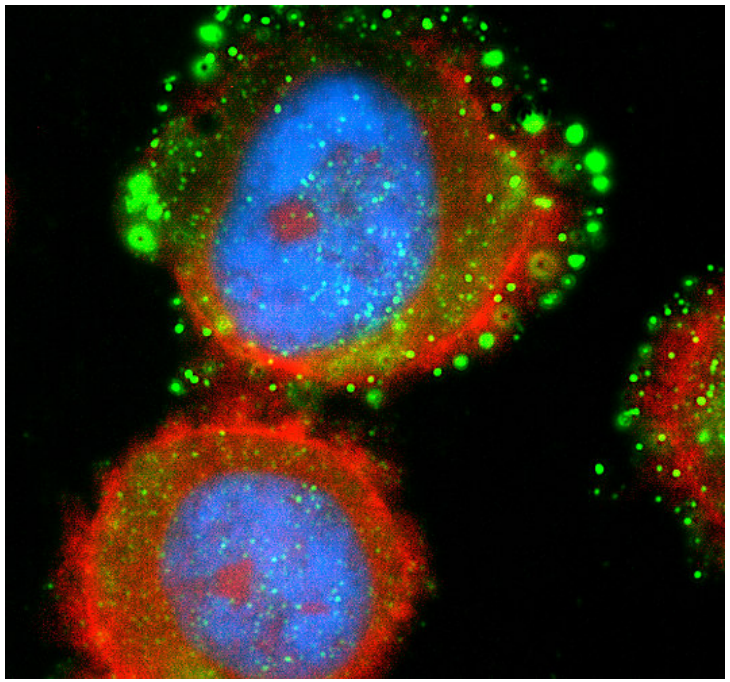
Dual fluorochrome labelling of Drosophila pupa

An example of the use of fluorescence in **DEVELOPMENTAL BIOLOGY** This picture shows expression of a gene called Pannier in green (GFP) and the body auto-fluorescing in red.

This picture has been taken using the Z stack acquisition option.



Fluroescence Applications –2



Triple labelling of human lung cells

An example of the use of fluorescence in Oncology or Cancer research

This picture shows a cellular component called the major vault protein

Using an FITC labelled antibody.
The nucleus of the cell is labelled with DAPI (blue) &
Actin is labelled with TRITC (red)

Deconvolution

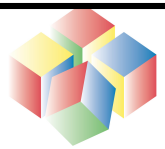
- All microscope objectives have an inherent focal depth and power of resolution
- Stray light from above or below plane of focus will tend to blur the image received at the camera
- Fluorescence beads can be used to measure the Point Spread Function [PSF] of each objective
- Aim of deconvolution is to model the optical axis and to increase contrast and resolution of interesting structures
- Many different methods (e.g. blind, no neighbours, nearest neighbours, inverse filtering, 2-D, 3D, ...)

Deconvolution - 2

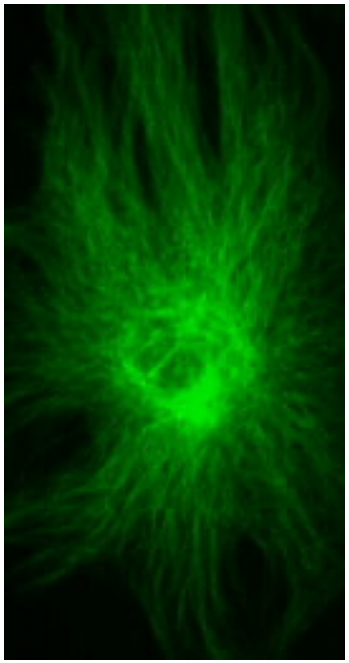
- Since uses a model of the optical axis, advantage is that deconvolution, (when correctly applied), will not introduce false artifacts into the image
- Often use a z-stack of images to get better deconvolution results
- Always image from well above the region of interest to well below
- Boom in last few years since modern algorithms and PC's mean results now take minutes not hours to calculate final results
- Z spacing depends upon Numerical Aperture (NA) of objective

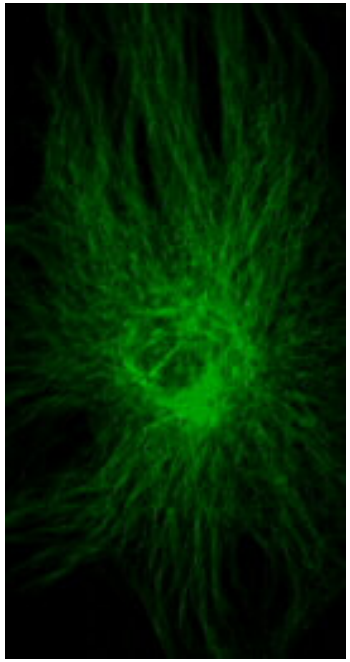
Recommended Z Spacing

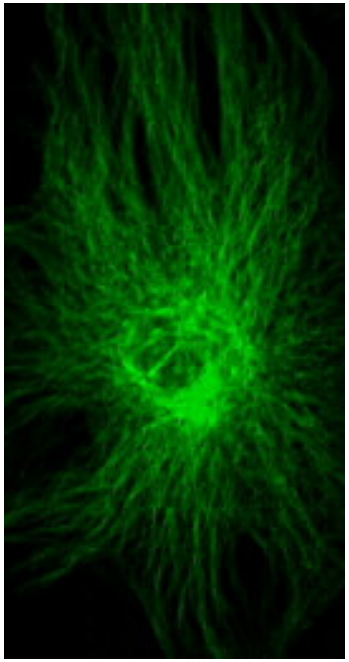
NA	Step (oil η=1.515)	Step Air (η=1)
0.2	18.8	12.3
0.4	4.6	3.0
0.6	2.0	1.2
0.8	1.1	0.6
1.0	0.7	0.25
1.2	0.4	X
1.4	0.25	X



Deconvolution example







Conclusions

- The ‘Digital Image’ is the future of optical microscopy
- Adding a camera and application software to a microscope massively increases its usefulness and flexibility, allowing you to store, archive, process, annotate, print, amend, email and measure images !
- Recent growth of PC performance with high band-pass firewire connectors ensure high quality live images and good processing horsepower are available from latest generation of digital imaging systems
- Microscope imaging is benefiting from consumer digital products, although niche products are available which better meet the requirements of microscope users.

Thank-you for your attention

- Any questions ?