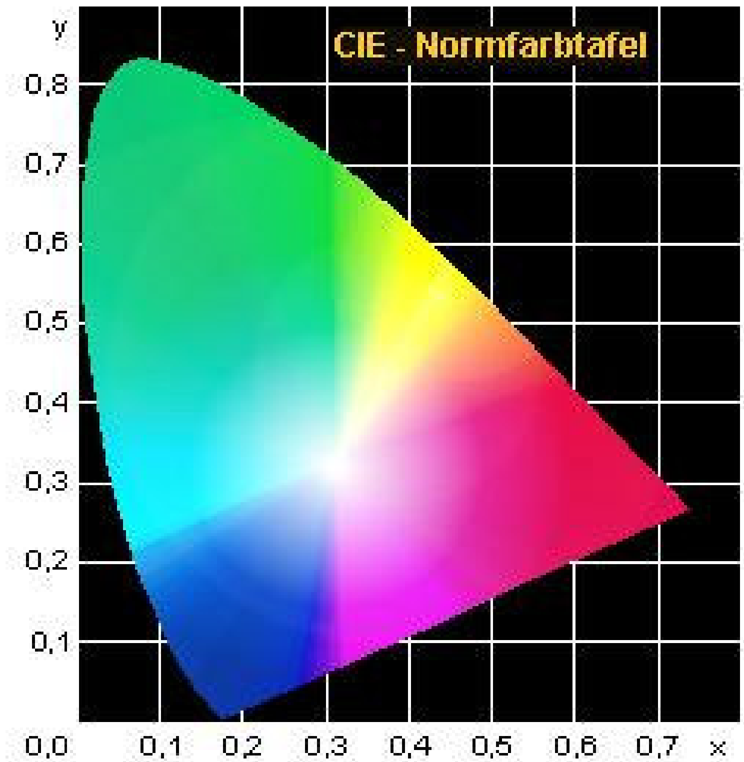
Perfecting Colour Images

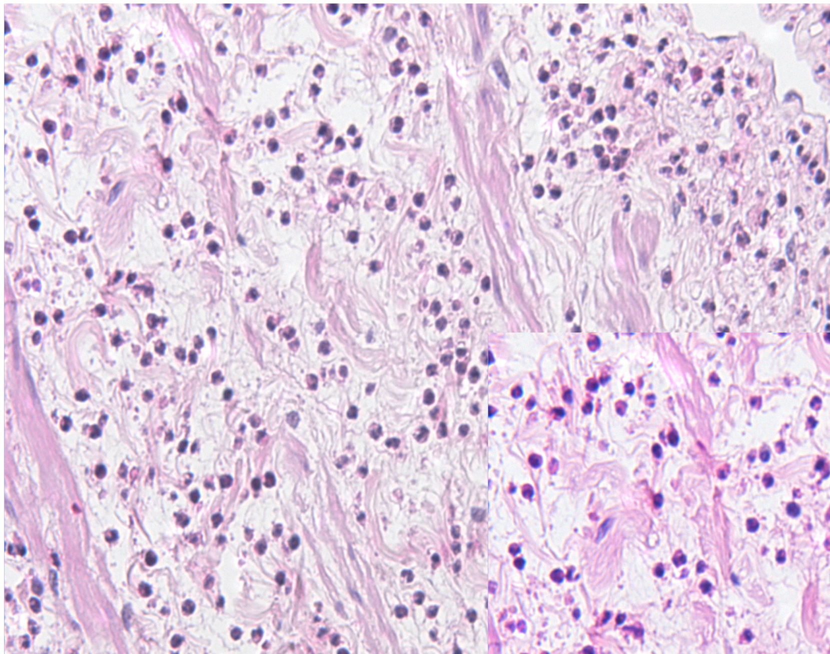
- Colour Issues
- Color Enhancement
- Step-by-step guide



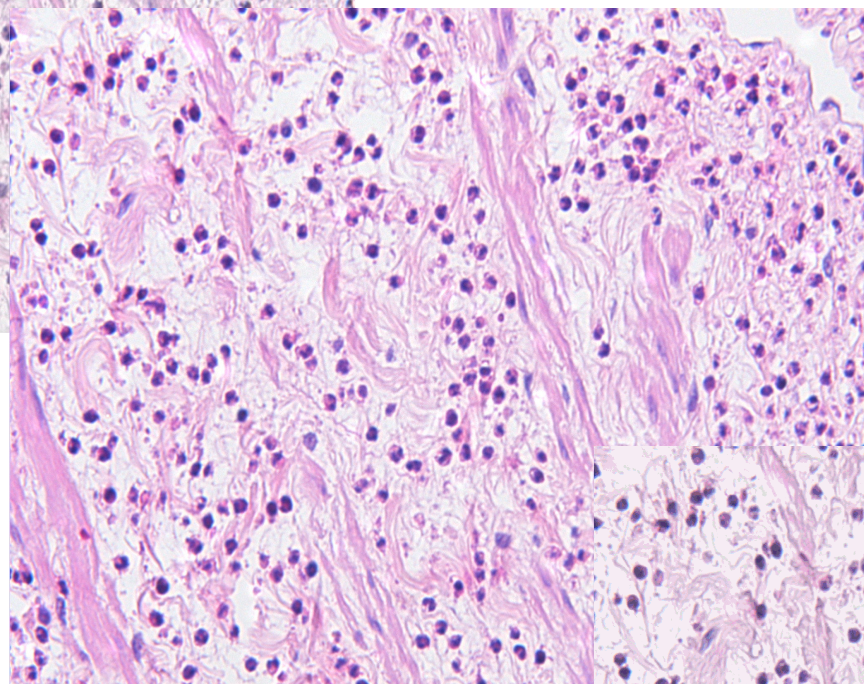
Colour Issues

- Contrast method
  - We are using transmitted brightfield as this is widely used. The same principles apply to other methods as well.
- Microscope illumination
  - Colour temperature varies with voltage
  - White balance is used to correct
- Eye – monitor – camera sensor
  - All components have different response
  - Can these be matched?
- Colour model
  - Understanding the way colour is represented can help us but we don’t have time for the maths here

Color Improvement

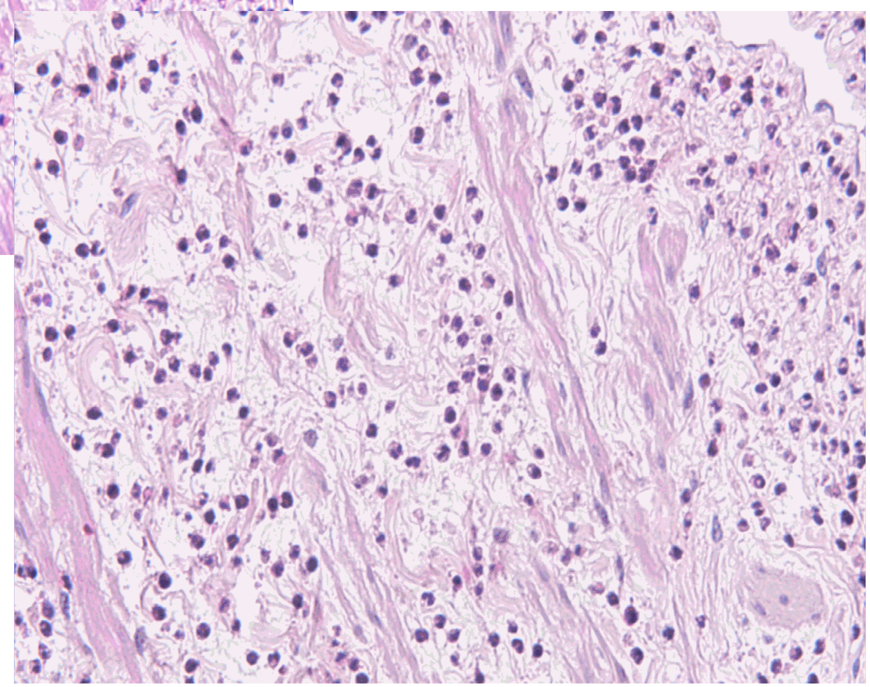


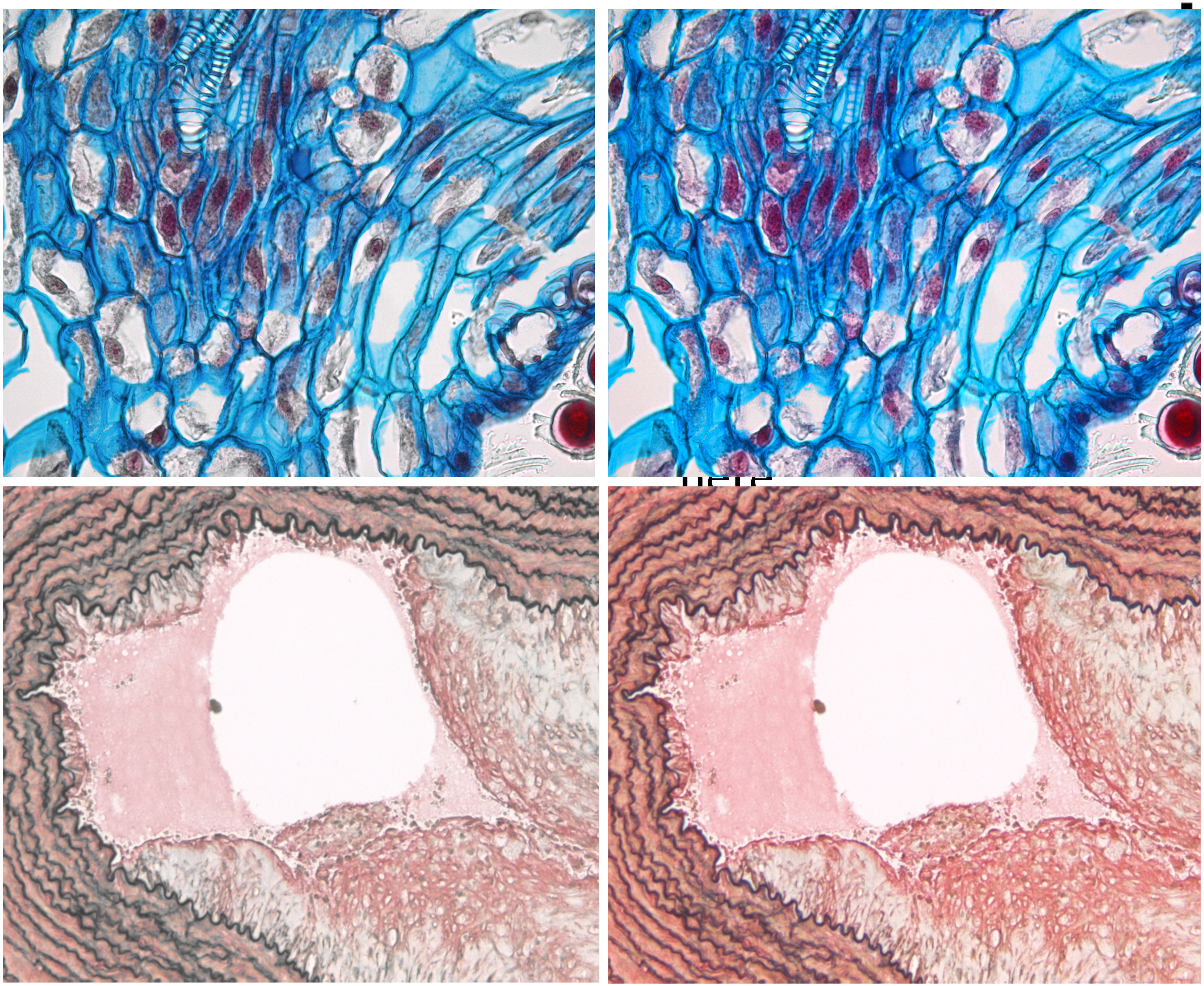
Before



After

Before and after alternate



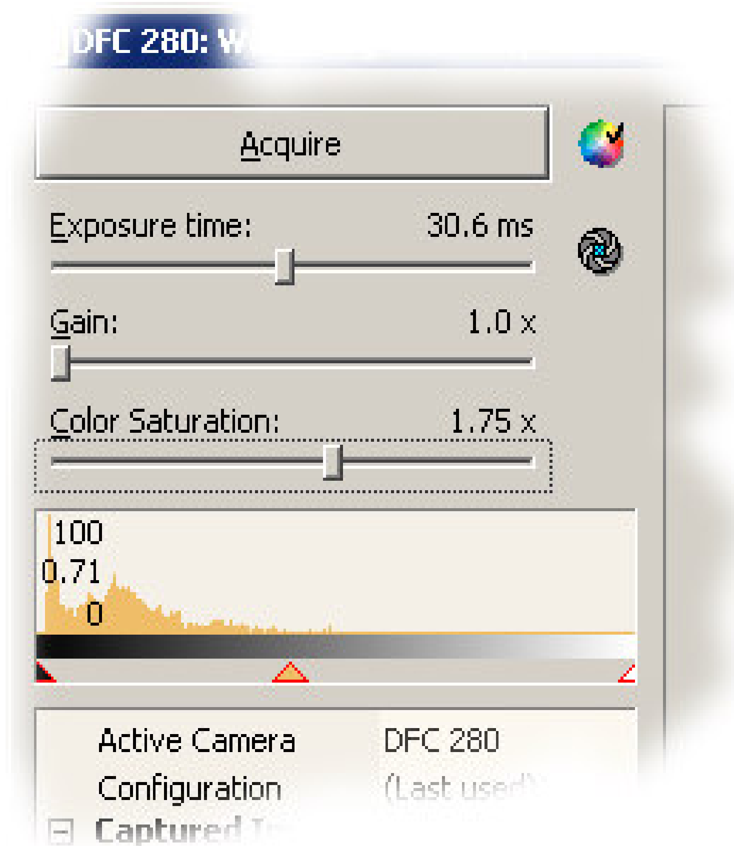


Step 1 - Microscope

- Check for dust on sensor and gently remove any you find. Do NOT wipe the sensor. Even small particles will ruin an image.
- There are rarely problems with the optics – but please check you have an appropriate ‘C’-mount to reduce vignetting. Go for a 2/3” minimum or 1” if possible.
- Make sure that the apertures are focused and have the correct opening.
- Use a DLF – daylight filter. This lessens the demand on white balance
- Set the lamp voltage for comfortable viewing in the eye-piece. It should be >8 volts. If less try to add an ND filter to allow the volts to be increased.

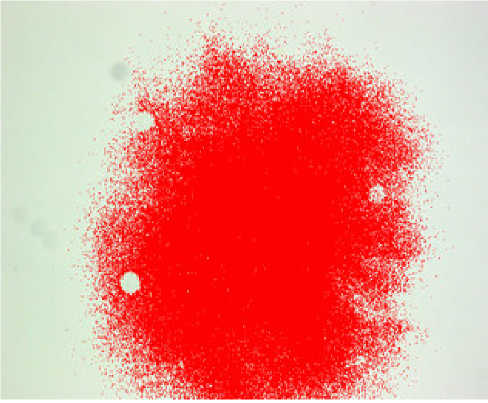
Step 3 – Colour Adjustment

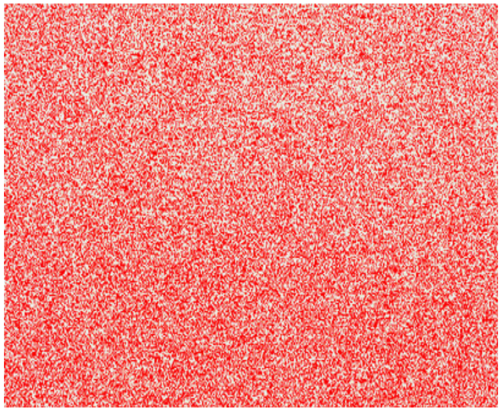
- Next you need to set the white balance. Find a part of the image that is empty of specimen detail. Use the mouse to drag a box in this region and select ‘White balance’ from the pop-up menu.
- The empty space should go white. If you get a message saying that the white balance failed, decrease the exposure by 2 steps and try again.
- The saturation control allows more vibrant colours to be displayed and is particularly useful on tissue sections, H&E. The change is subtle but significant.
- Adjust Saturation in range 15 to 2 and gamma in range 0.45 to 0.7 until the image is pleasing.



Step 4 – Shading Correction

- Next is to check if the image appears brighter in the centre of the image compared to the edge
- Switch on the under/over exposure display. Is you image like to top one here? You may need to adjust the exposure to see this.
- If you do have shading, find a perfectly clear region of the specimen but which otherwise has the same cover glass and mounting medium. Slightly defocus the image and set the shading. The under/over display should now be random red spots.
- Switch off the under/over display. Move back to the specimen and refocus.
- Make final adjustments to gamma and saturation

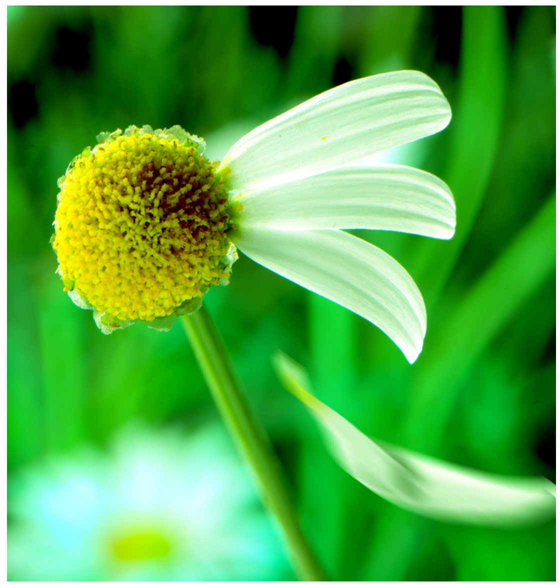




And finally

- Even with taking all this care you may find that when you look at you images on a different monitor or on a printer, the results are not what you expect.
- This is because monitors and printers have massive variations in colour response.
- Check that you know the ‘colour profile’ of the monitor you took the images, and select this same profile on the monitor you are going to use.
- Setting up monitors and printers is a story for another day.

Microscanning Principle Revealed



Microscanning revealed

Microscanning Principle Revealed

4-Shot mode 1300x1030 4x col

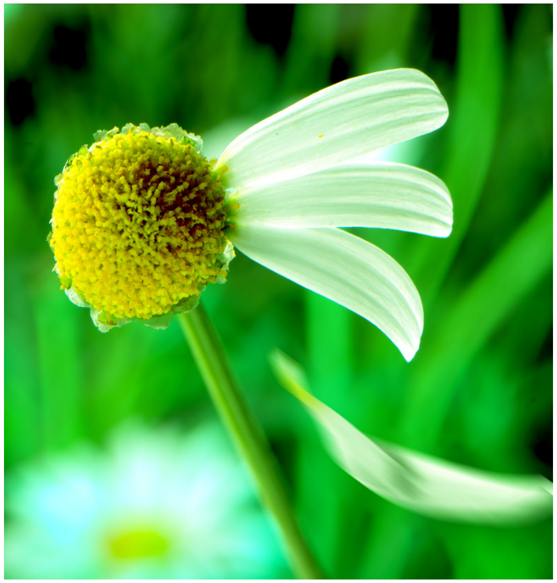
The 4-Shot mode of the LEICA DC 500 increases color resolution, because color is measured for each single pixel.

The image looks more detailed and correct, because no interpolation errors are introduced in image information.

However, spatial resolution is not increased in this mode.

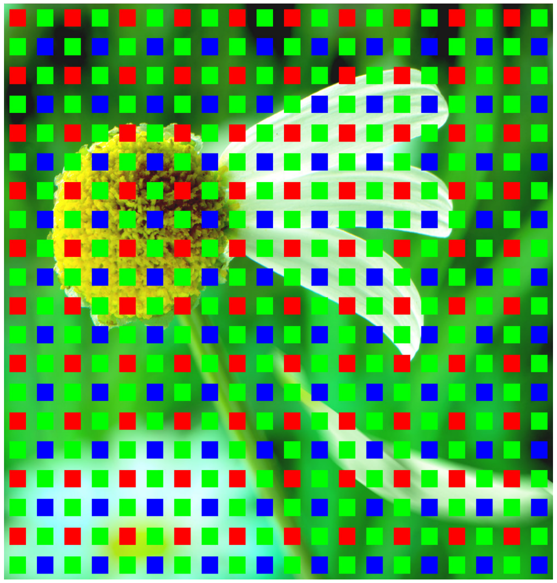
Microscanning Principle Revealed

4-Shot mode 1300x1030 4x col



Microscanning Principle Revealed

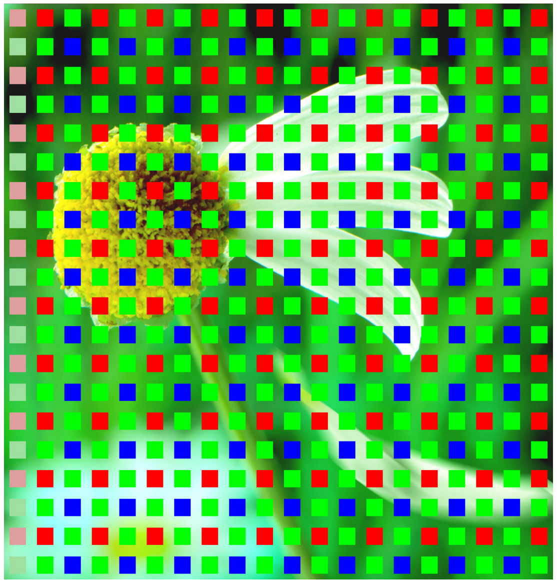
4-Shot mode 1300x1030 4x col



1. snap

Microscanning Principle Revealed

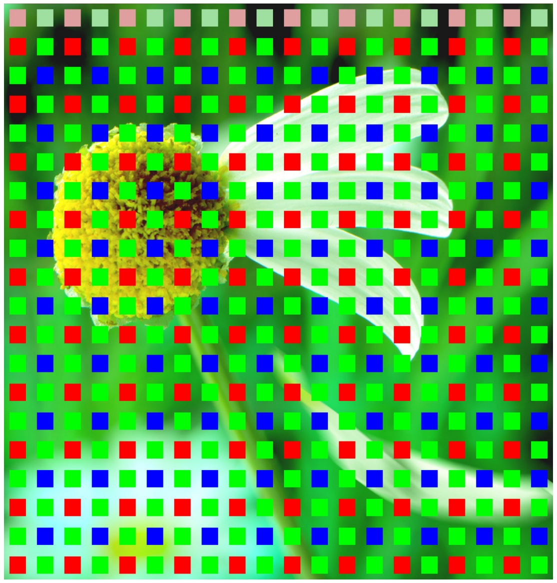
4-Shot mode 1300x1030 4x col



2. snap

Microscanning Principle Revealed

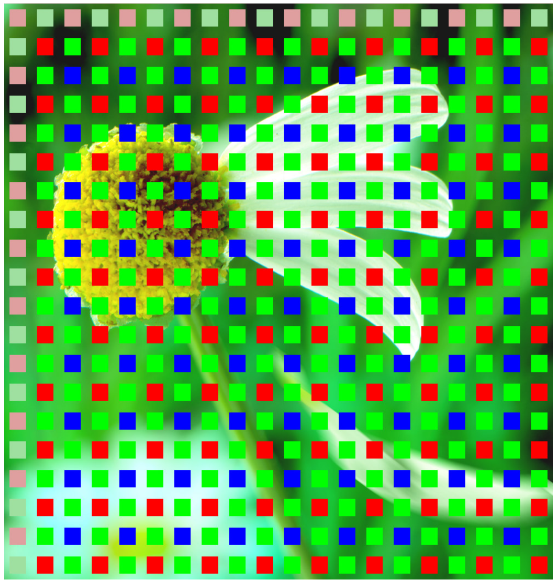
4-Shot mode 1300x1030 4x col



3. snap

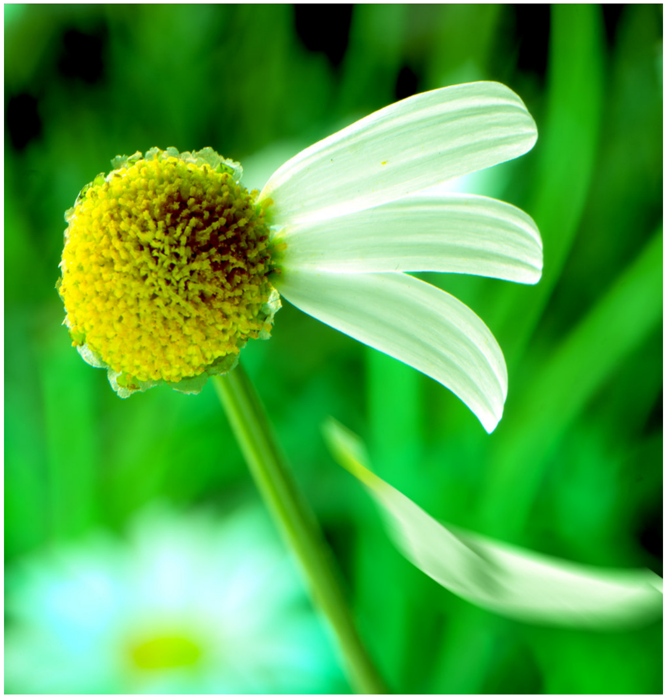
Microscanning Principle Revealed

4-Shot mode 1300x1030 4x col

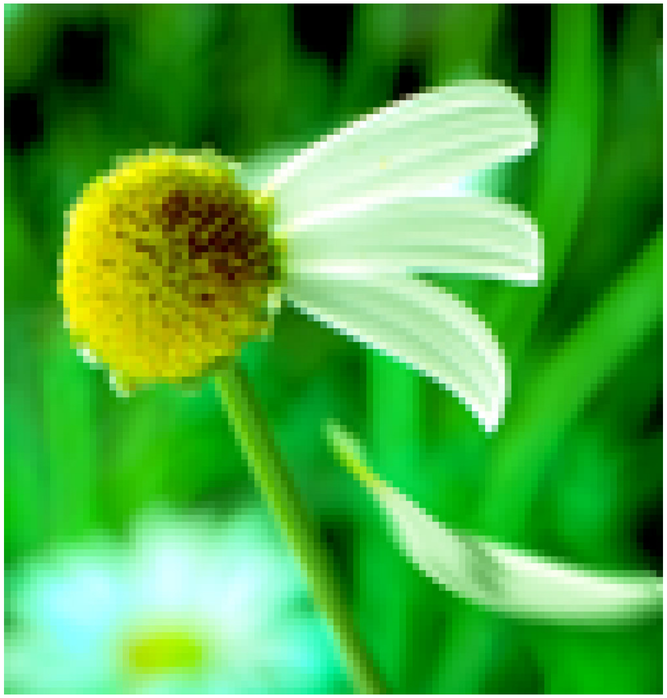


4. snap

Microscanning Principle Revealed



original



result

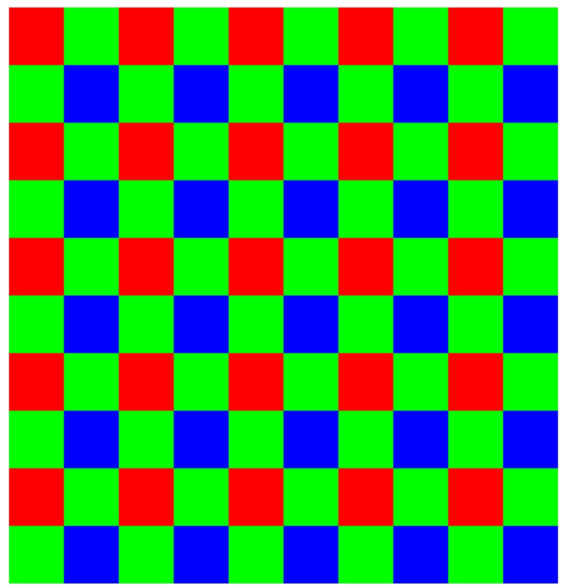
Microscanning Principle Revealed

Interline CCD basics

This short introduction to the architecture of interline transfer CCDs will motivate the benefits of Microscanning technique.

Microscanning Principle Revealed

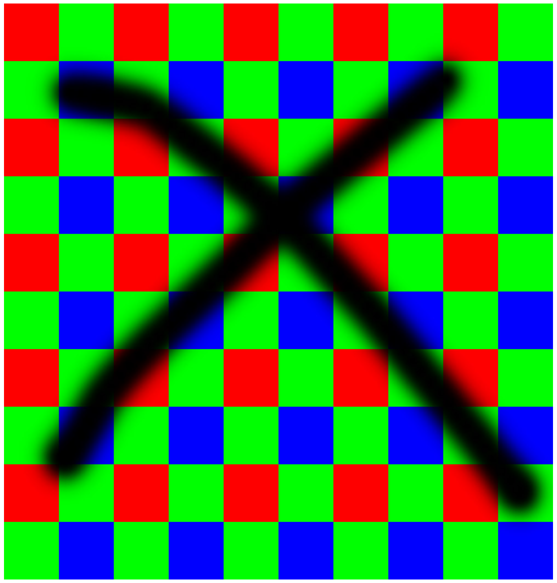
Interline CCD basics



A RGB checkerboard pattern is often referred to as the on-chip color filter set, dubbed „Bayer mosaic mask“.

Microscanning Principle Revealed

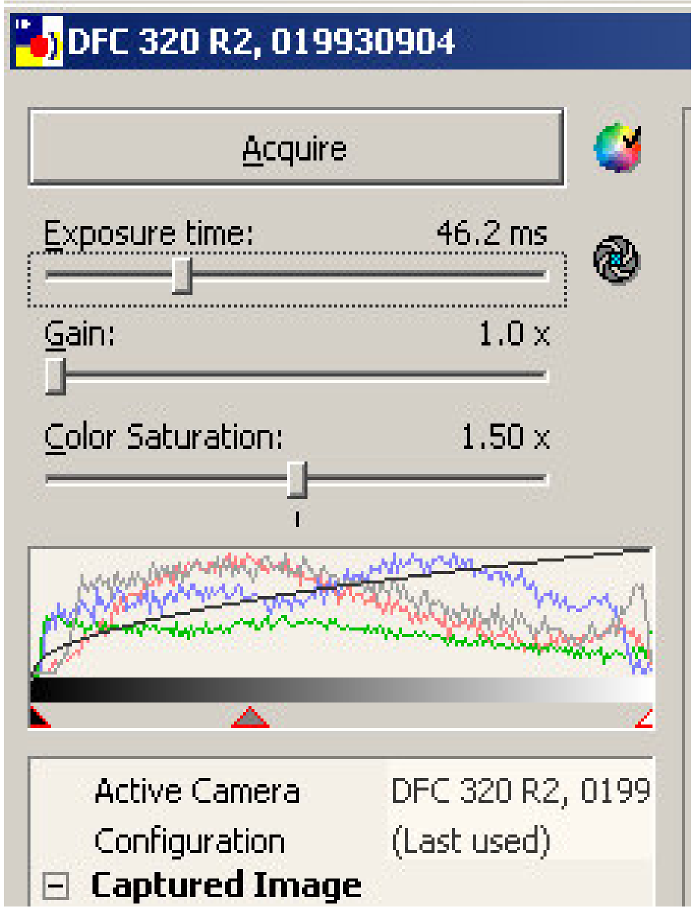
Interline CCD basics



Unfortunately it‘s not as easy as that.

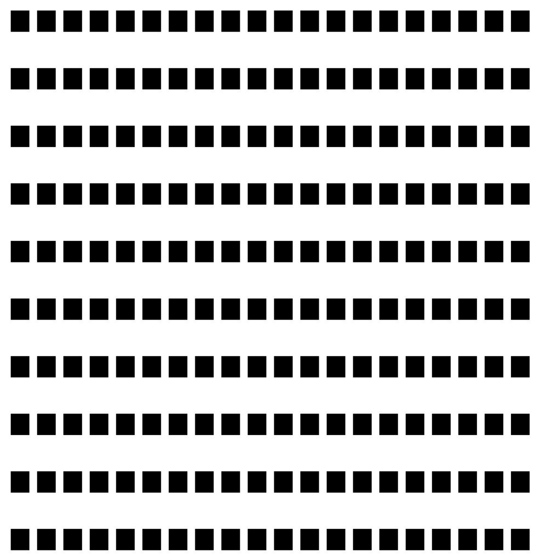
Step 2 – Camera Set-up

- Using DFC Twain focus on the specimen. Reset black and white levels to 0 and 100%. Gamma to 0.6 and not on ‘Auto’. Gain always = 1.
- Select your live image resolution – x2 binning will give a fast refresh rate for 320 and 480.
- Switch on Auto-exposure and set brightness to 90%. Switch off auto-exposure.
- Check that the background regions of the image are not over-exposed as this will hide the fine detail. Adjust the exposure +/- 1 or 2 steps until you achieve this. Your exposure should be <50 msec. If not check the microscope is set-up correctly



Microscanning Principle Revealed

Interline CCD basics



Actual pixels are far less dense packed, as illustrated above.

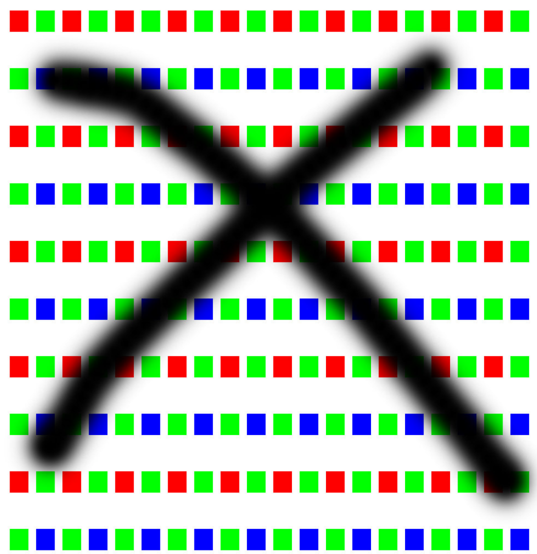
Microscanning Principle Revealed

Interline CCD basics

Then lets now place a color filter in front of each pixel.

Microscanning Principle Revealed

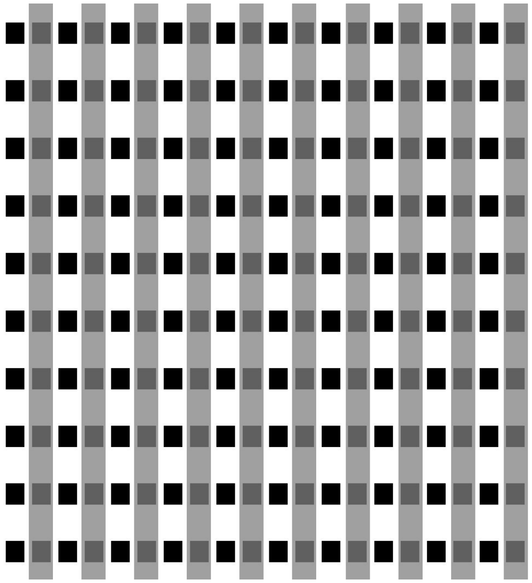
Interline CCD basics



Doesn‘t work, unless we use an external shutter.

Microscanning Principle Revealed

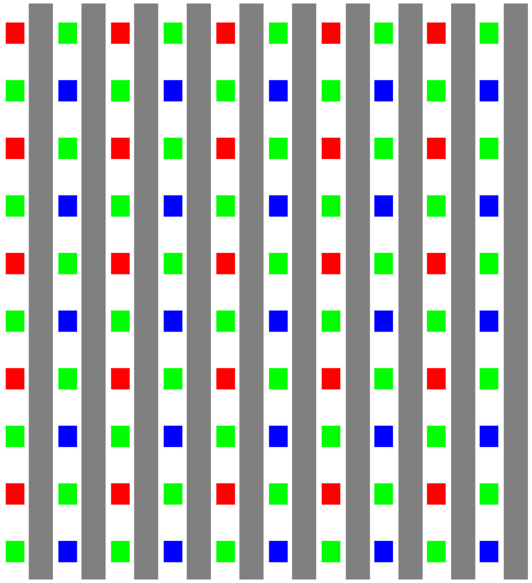
Interline CCD basics



Interline transfer sensors have an integrated electronic shutter Called a „dark register“. Pixel charge is transfered to this after exposure waiting to be read from the sensor.

Microscanning Principle Revealed

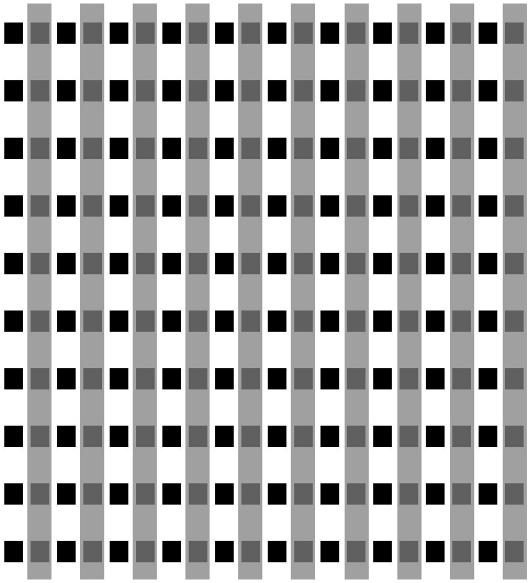
Interline CCD basics



We now could place a small color filter in front of each pixel.
But this would leave a large part of the sensor area inactive.

Microscanning Principle Revealed

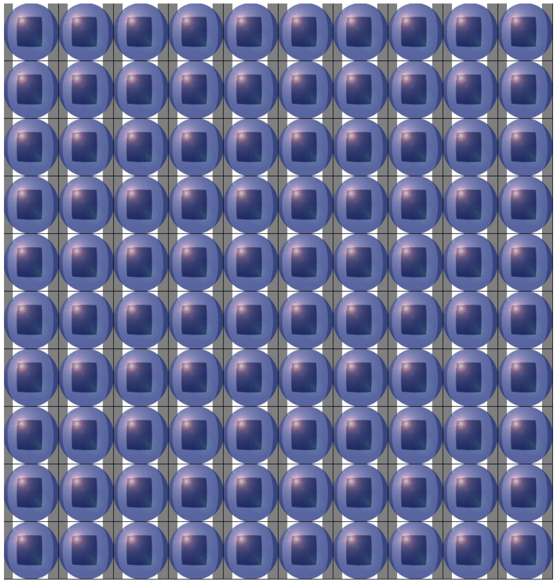
Interline CCD basics



Let‘s go back to the tiny „naked“ pixels...

Microscanning Principle Revealed

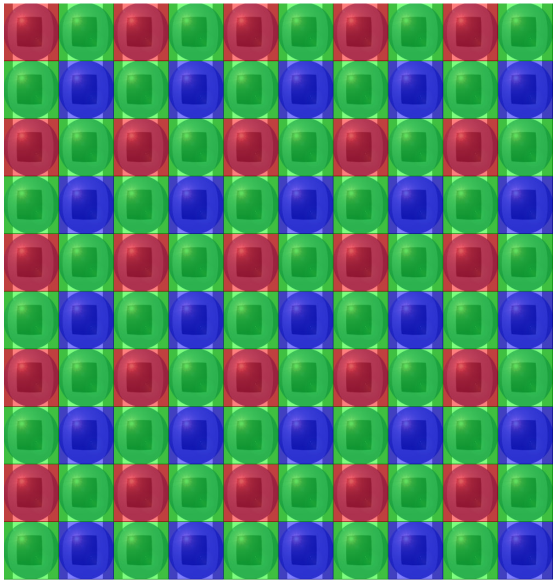
Interline CCD basics



...and put a tiny lens („microlens“) in front of each.
This will focus the light otherwise lost in the drains between the pixels to the actual pixels.

Microscanning Principle Revealed

Interline CCD basics



Now we can coat each of the microlenses with a color filter.

Microscanning Principle Revealed

Interline CCD basics

Result: Sensor sensitivity is increased by the microlens array. Still the resolution depends on the architecture of pixels and microlens array. In most sensors resolution can be Increased.

And that‘s what is done by Microscanning.

Introduction to Deconvolution

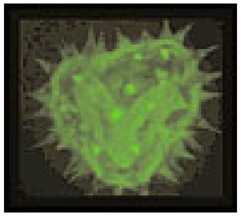
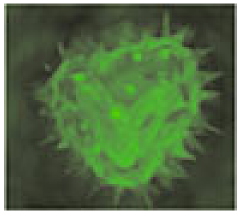
- Also known as "deblurring" or 3D image restoration
- Computational technique for removing out-of-focus haze from stacks of optical sections
- The out-of-focus haze can be mathematically modeled as a "point spread function (PSF)“
- Deconvolution methods can therefore be thought of as methods for inverting the unavoidable and natural blurring effect of the optical system
- The purpose of deconvolution is to remove haze and blur and restore the sharpness and clarity to an image

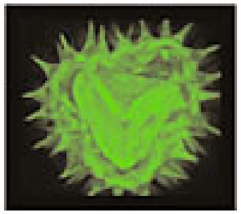
Blind Deconvolution

- Blind Deconvolution is a term that is used to describe methods of deconvolution which do not require the point spread function (PSF) of the system to be explicitly known prior to the deconvolution
- The blind deconvolution used in FW4000 reconstructs an estimate of the PSF concurrently with the deconvolved image. This blind deconvolution adapts to PSF changes within the specimen itself, thus providing superior results when compared to methods that utilize either theoretical or measured PSFs

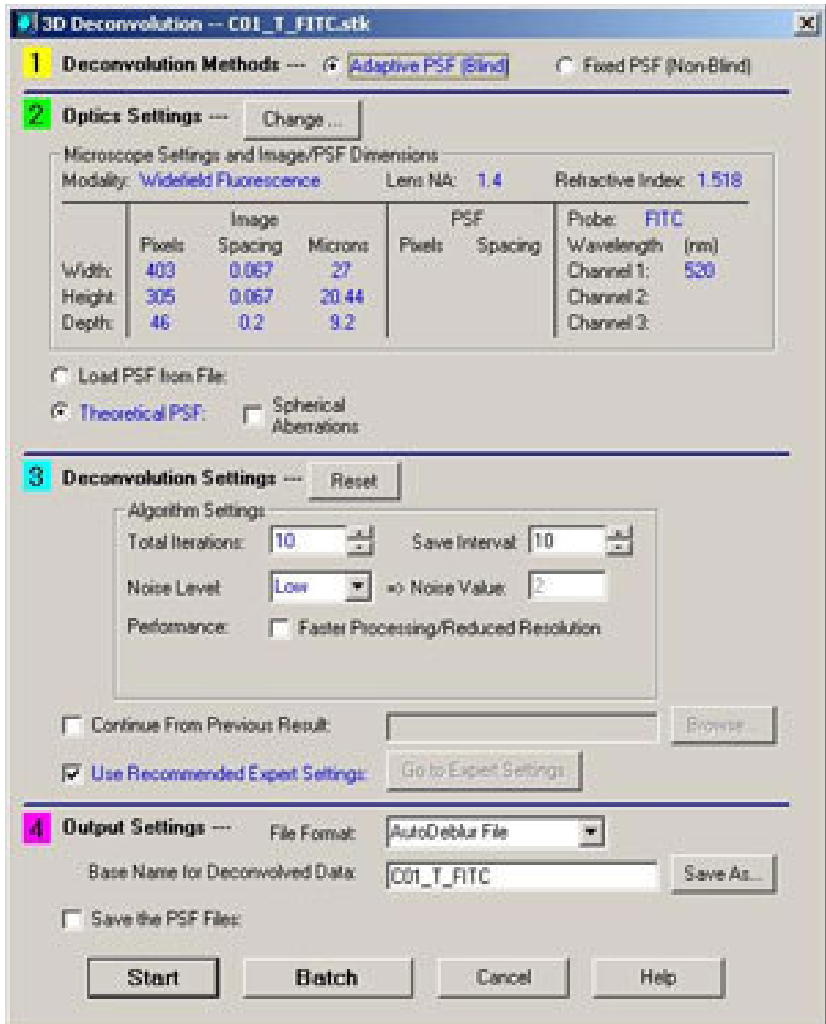
Deconvolution Techniques

- No/Nearest Neighbor algorithms are fast, qualitative and work particularly well on images with strong signal to noise ratios
- Inverse filter created by dividing the captured image by the PSF. Generally better than the no/nearest neighbor algorithms
- Non-Blind deconvolution uses a measured or synthetically acquired PSF which is not modified during the deconvolution. Offers an excellent balance between quality results and processing time
- Adaptive Blind does not require a measured or acquired PSF, but reconstructs both the underlying PSF and best image solution possible from the collected 3D dataset.



Typical deconvolution menu



Conclusions

- Understanding design of camera and matching it to the applications it will be used for is vital
- Extreme care is needed to get consistently good colour rendition
- Pixel shifting is a very useful technique to improve camera resolution
- Once the optimum image is acquired, deconvolution is very useful to remove haze and boost signal to noise ratio

Thanks for you attention

- Any questions ????