

Plan

Basics

Image?
Camera
Histograms
Bitdepth

Colour

RGB
multi channel
LUT
Lightness
LUT

Acquisition

what goes in
guidelines
Signal to noise
Resolution

Fundamentals of Microscope Image Analysis

David Miguel Susano Pinto

Micron Advanced Microscopy Course, 2019

Microscope Image Analysis in 3 parts

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

- 1 What is in a microscope image
 - What is in a image?
 - Image display
 - Image acquisition
- 2 Careful with your data
 - File formats
 - OMERO
 - Figure preparation
- 3 Images as N dimensional numeric arrays
 - N dimensional images
 - Spatial filters
 - Morphology
 - Connected components
 - Tools

What is an image?

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

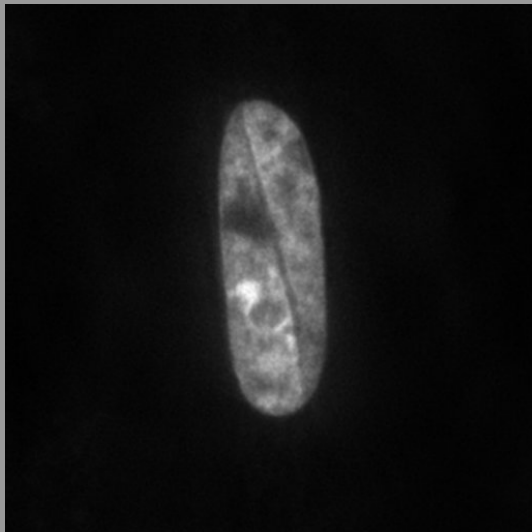
Acquisition

what goes in

guidelines

Signal to noise

Resolution



What is an image?

Pixel data



Metadata

- emission wavelength 535nm
 - excitation wavelength 500nm
 - exposure time 2s
 - objective Olympus 100X/1.40
 - pixel size 64.4×64.4 nm
-
- deconvolved
 - 8bit conversion after contrast adjustment
 - full range displayed
-
- horse fibroblast cell line
 - transient transfection of H2B-GFP
 - treated with sugar lumps

File \Rightarrow my-lovely-horse-H2B-GFP_01_R3D_D3D.dv

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

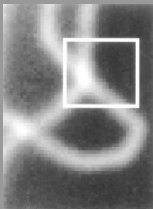
Acquisition

what goes in

guidelines

Signal to noise

Resolution



63	84	119	172	219	225	182	135	79	51	36	24	23	19	15	-1	14	14	8	0	-4	7	18
78	84	104	170	223	224	196	118	84	49	36	28	16	11	25	4	15	13	8	-4	9	11	7
61	80	115	153	209	204	170	113	73	46	41	29	9	17	11	11	0	12	-2	2	2	3	23
72	98	121	160	190	207	178	116	68	50	29	22	17	19	7	27	15	9	-3	-4	14	5	8
64	90	132	167	210	214	180	115	71	37	36	31	13	15	9	8	15	6	0	5	-14	4	12
75	93	124	169	216	229	196	107	71	56	19	18	22	24	7	5	15	11	8	-1	12	6	7
97	87	128	193	210	225	193	111	85	47	27	27	21	12	5	2	-1	4	1	-3	7	2	-10
103	108	134	180	201	233	185	115	55	38	26	25	15	20	18	6	2	2	1	4	-3	-13	0
142	132	161	216	238	223	160	90	59	45	17	10	9	13	10	11	4	-9	5	2	7	0	5
172	162	175	231	239	238	155	88	48	28	24	17	15	13	0	14	0	11	-3	4	9	0	-10
226	219	230	260	265	236	161	92	43	31	31	11	5	11	7	13	19	9	18	-11	-9	-2	8
234	247	256	302	311	253	174	97	48	27	12	15	7	7	0	16	8	5	3	-4	0	-6	4
260	263	297	346	349	303	196	126	65	27	30	24	3	6	7	1	12	3	9	0	-2	-13	2
244	293	340	388	399	321	223	130	74	29	24	30	17	4	3	11	0	8	7	-3	-2	-2	-2
209	273	359	423	436	365	264	141	80	57	32	45	13	3	18	8	-7	0	-6	4	-1	-2	-3
176	253	342	430	443	394	291	161	86	59	37	23	18	5	0	7	8	11	1	-3	13	-5	-2
152	218	311	425	470	420	326	208	111	66	52	29	28	9	4	7	8	4	-7	11	-18	-13	-2
129	199	294	413	469	441	384	257	148	111	69	34	20	20	6	3	15	4	-2	-6	-3	-10	9
140	206	294	385	439	442	365	310	223	157	114	76	45	28	9	21	5	15	-4	-13	0	-5	-1
173	233	309	354	392	375	333	303	261	214	135	92	51	47	18	12	13	12	20	-9	4	1	15
221	278	300	321	306	293	286	279	250	231	184	142	108	67	41	18	13	5	8	-8	0	7	5
267	302	291	244	228	211	201	215	241	227	205	184	136	110	68	51	26	11	8	3	0	8	-3
284	279	257	202	133	129	137	151	183	213	209	188	187	155	109	69	49	26	25	8	8	18	-4
275	248	191	143	95	85	87	98	122	166	184	192	206	194	176	135	98	50	44	19	21	0	1

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

Photons \Rightarrow Charge \Rightarrow Digital number

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

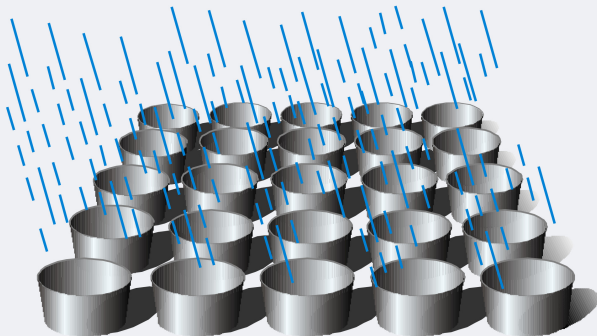
Acquisition

what goes in

guidelines

Signal to noise

Resolution



Array of Discrete Photodetectors

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

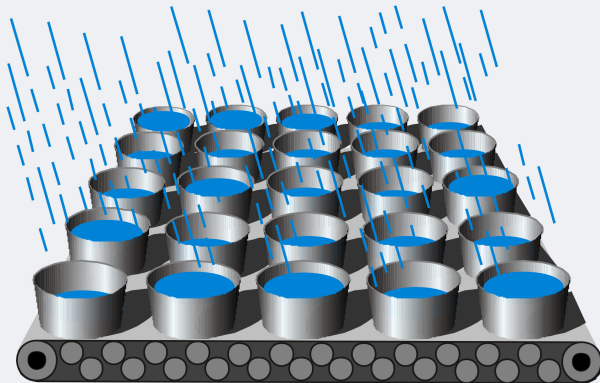
Acquisition

what goes in

guidelines

Signal to noise

Resolution



CCD Operation
Integration of Photo-Induced Charge

Histogram of pixel values

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

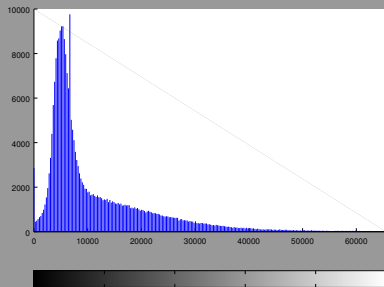
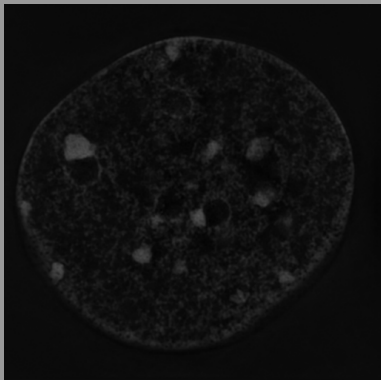
Acquisition

what goes in

guidelines

Signal to noise

Resolution



Adjust contrast/levels, see effect on display and pixel values.

Bit depth / dynamic range

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution



Bit depth / dynamic range

Plan



Basics



Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

Bit depth / dynamic range

Plan



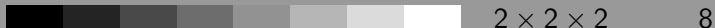
Basics

Image?



Camera

Histograms



Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

Bit depth / dynamic range

Plan



Basics



Image?



Camera

Histograms

Bitdepth



Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

Bit depth / dynamic range

Plan		2	2
Basics		2×2	4
Image?		$2 \times 2 \times 2$	8
Camera		2^4	16
Histograms		2^5	32
Bitdepth			
Colour			
RGB			
multi channel			
LUT			
Lightness			
LUT			

Acquisition

- what goes in
- guidelines
- Signal to noise
- Resolution

Bit depth / dynamic range

Plan		2	2
Basics			
Image?		2×2	4
Camera			
Histograms		$2 \times 2 \times 2$	8
Bitdepth		2^4	16
Colour			
RGB		2^5	32
multi channel			
LUT		2^6	64
Lightness			
LUT		2^7	128
Acquisition		2^8	256
what goes in			
guidelines			
Signal to noise			
Resolution			

Bit depth / dynamic range

Plan		2	2
Basics			
Image?		2×2	4
Camera			
Histograms		$2 \times 2 \times 2$	8
Bitdepth		2^4	16
Colour			
RGB		2^5	32
multi channel		2^6	64
LUT		2^7	128
Lightness		2^8	256
LUT		2^9	512
Acquisition			
what goes in		2^{10}	1024
guidelines		2^{11}	2048
Signal to noise			
Resolution		2^{15}	32768
		2^{16}	65536

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

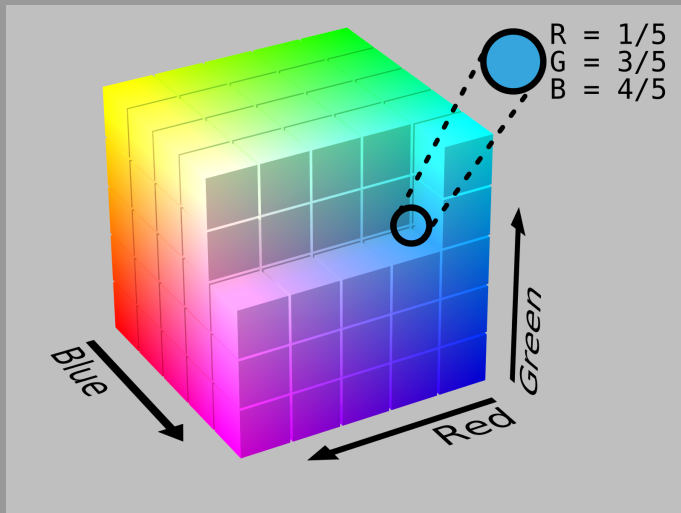
Acquisition

what goes in

guidelines

Signal to noise

Resolution



Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

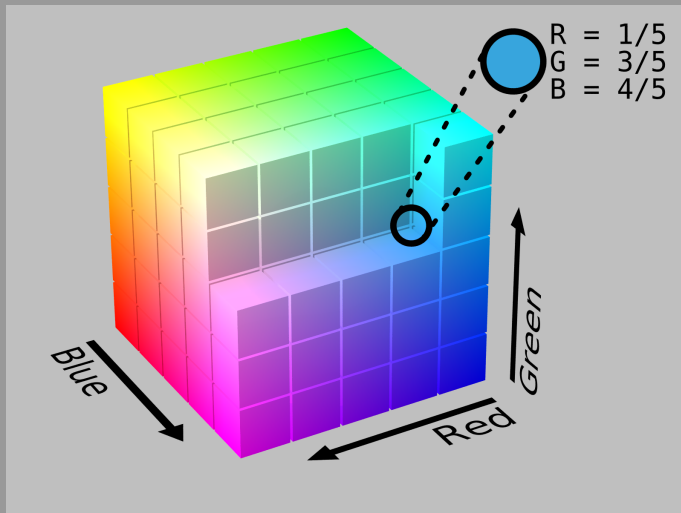
Acquisition

what goes in

guidelines

Signal to noise

Resolution



If you ever get an RGB image, you did something wrong.

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

Multichannel image is a series of grayscale images.

Lookup tables or colormaps

Plan

Basics

- Image?
- Camera
- Histograms
- Bitdepth

Colour

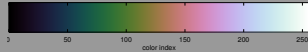
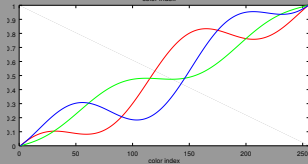
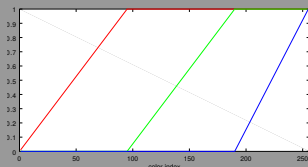
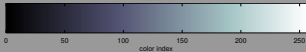
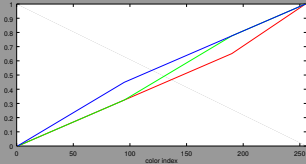
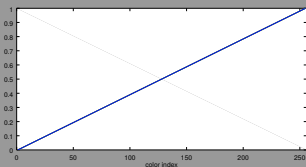
- RGB
- multi channel

LUT

- Lightness
- LUT

Acquisition

- what goes in
- guidelines
- Signal to noise
- Resolution



Lookup tables or colormaps

Plan

Basics

- Image?
- Camera
- Histograms
- Bitdepth

Colour

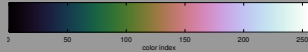
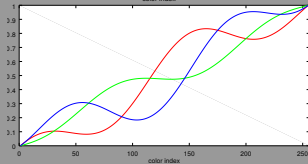
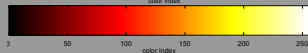
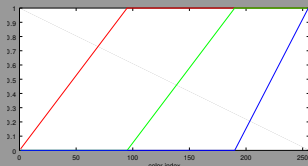
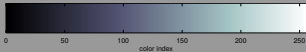
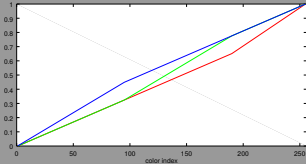
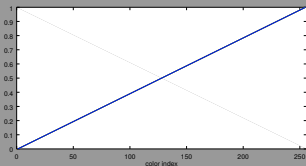
- RGB
- multi channel

LUT

- Lightness
- LUT

Acquisition

- what goes in
- guidelines
- Signal to noise
- Resolution



See ImageJ "Display LUTs"

Lightness perception

Plan

Basics

- Image?
- Camera
- Histograms
- Bitdepth

Colour

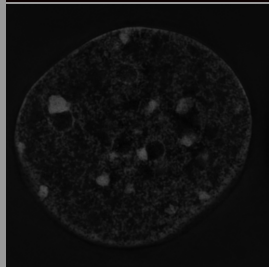
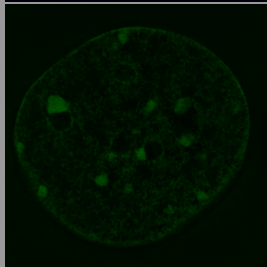
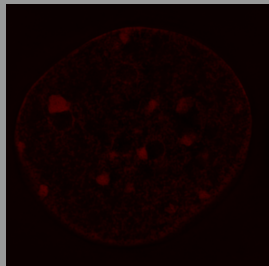
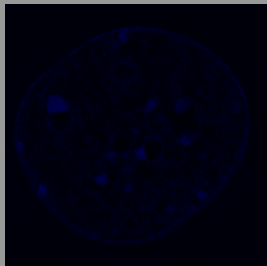
- RGB
- multi channel
- LUT

Lightness

- LUT

Acquisition

- what goes in
- guidelines
- Signal to noise
- Resolution



Lightness perception

Plan

Basics

- Image?
- Camera
- Histograms
- Bitdepth

Colour

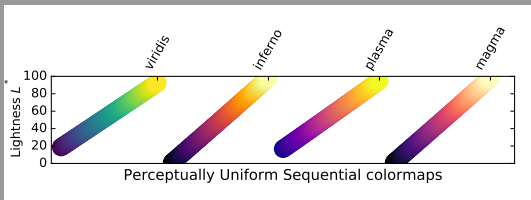
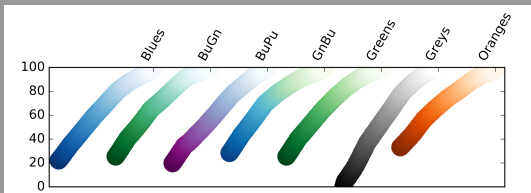
- RGB
- multi channel
- LUT

Lightness

- LUT

Acquisition

- what goes in
- guidelines
- Signal to noise
- Resolution



Qualitative or Categorical

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

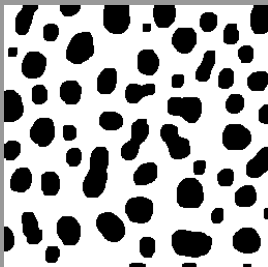
Acquisition

what goes in

guidelines

Signal to noise

Resolution



Qualitative or Categorical

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

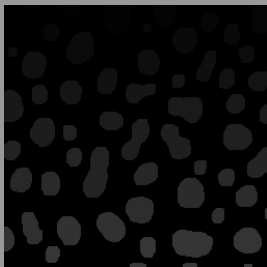
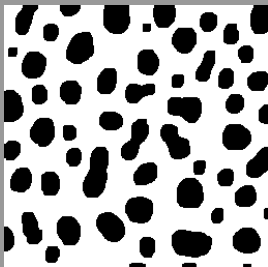
Acquisition

what goes in

guidelines

Signal to noise

Resolution



Qualitative or Categorical

Plan

Basics

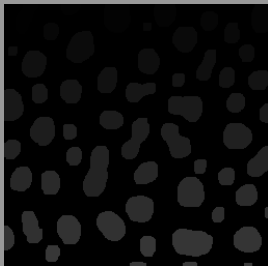
- Image?
- Camera
- Histograms
- Bitdepth

Colour

- RGB
- multi channel
- LUT
- Lightness
- LUT

Acquisition

- what goes in
- guidelines
- Signal to noise
- Resolution



Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

Image \Rightarrow Results

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

Image \Rightarrow Results
crap crap

Speak with us first

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

- ① What you need from experiment
 - resolution
 - on each dimension
 - numbers required for statistics
- ② Check different techniques
 - pros / cons
 - availability
- ③ Check your sample
 - Thickness
 - Labelling
 - Viability

correct image acquisition

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

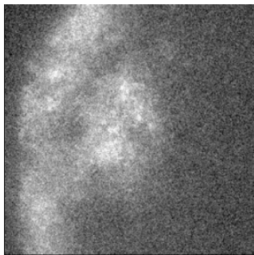
Signal to noise

Resolution

- The system must be correctly set up and aligned. PSF verification.
- The specimen should not cause undue optical aberration.
- Avoid underflow and overflow but fill dynamic range while keeping same settings across all images. Use a HiLo LUT. Beware of automatic intensity caling.
- Check dark signal or background image.
- Be aware of XYZ optical resolution of the system and sample appropriately.
- Take care with signal to noise limitations. Binning, gain, exposure time, fluorophore. Make sure you collect enough light.

increased signal increases S/N = improved contrast

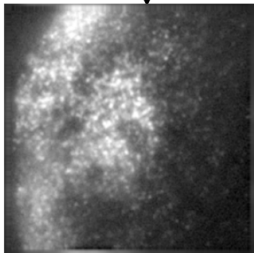
**noisy image
(scaled)**



5x integration time



**increased number
of photons counted**



improved S/N

ImageJ

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

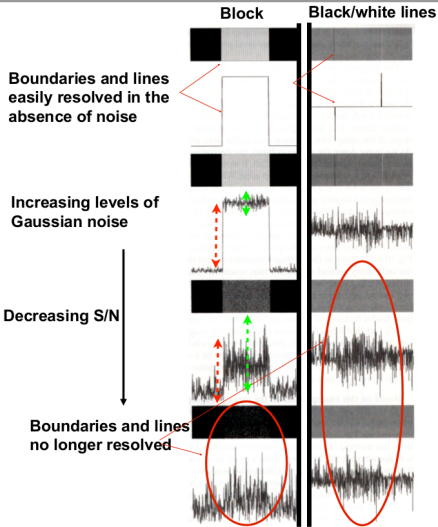
Signal to noise

Resolution

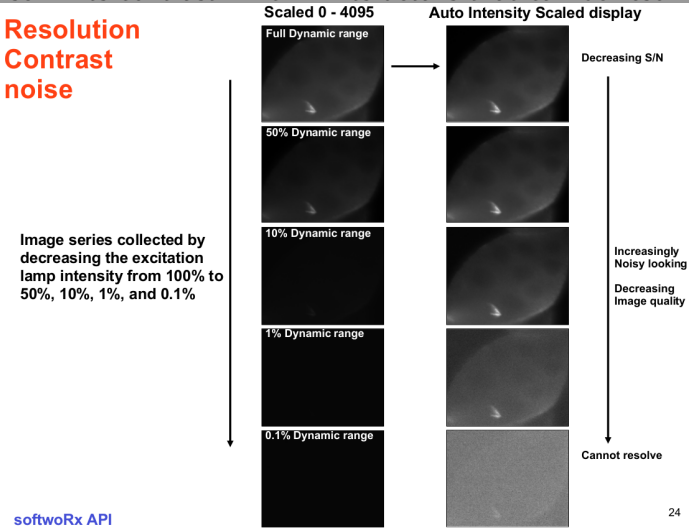
Noise limits contrast which limits details that can be resolved.

Resolution, contrast, noise

The difference between
signal and background
must be at least 3X the
noise to be detectable



Noise limits contrast which limits details that can be resolved.



Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

Resolution is function of NA and wavelength.

Plan

Basics

Image?

Camera

Histograms

Bitdepth

Colour

RGB

multi channel

LUT

Lightness

LUT

Acquisition

what goes in

guidelines

Signal to noise

Resolution

Resolution is meaningless if considered in isolation from noise.