

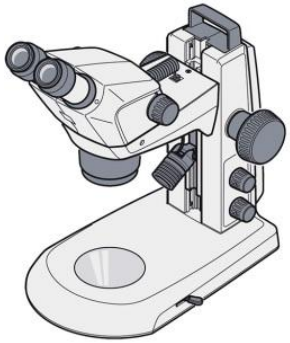
Manchester Microscopical & Natural History Society

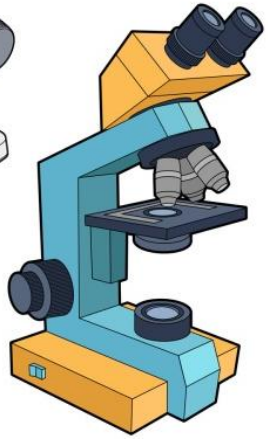
Established 1880

www.manchestermicroscopical.org.uk

More on Focus Stacking ….

For Micro & Macro

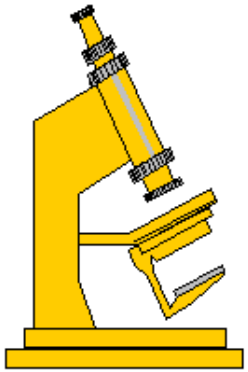








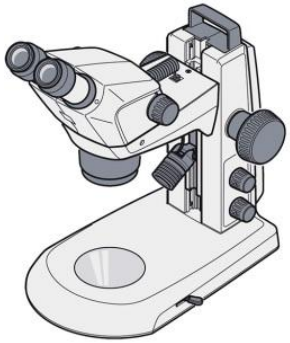
***Mike Mahon**, October 24th, 2026*

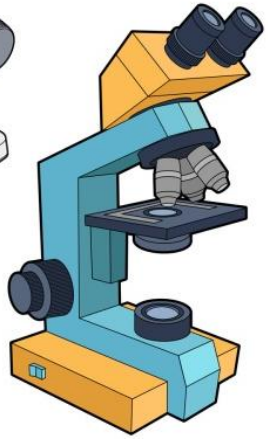


Manchester Microscopical & Natural History Society

Previously !

Micro … Macro … or both …?





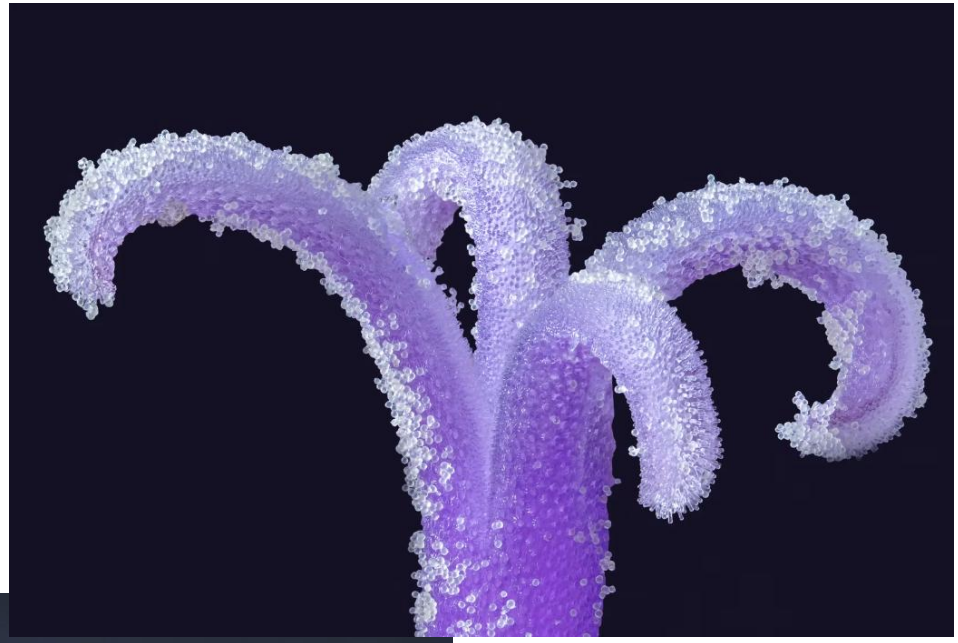




***Mike Mahon**, March 14th, 2024*

Last Month ….
Art of Ultramacro by Tony North

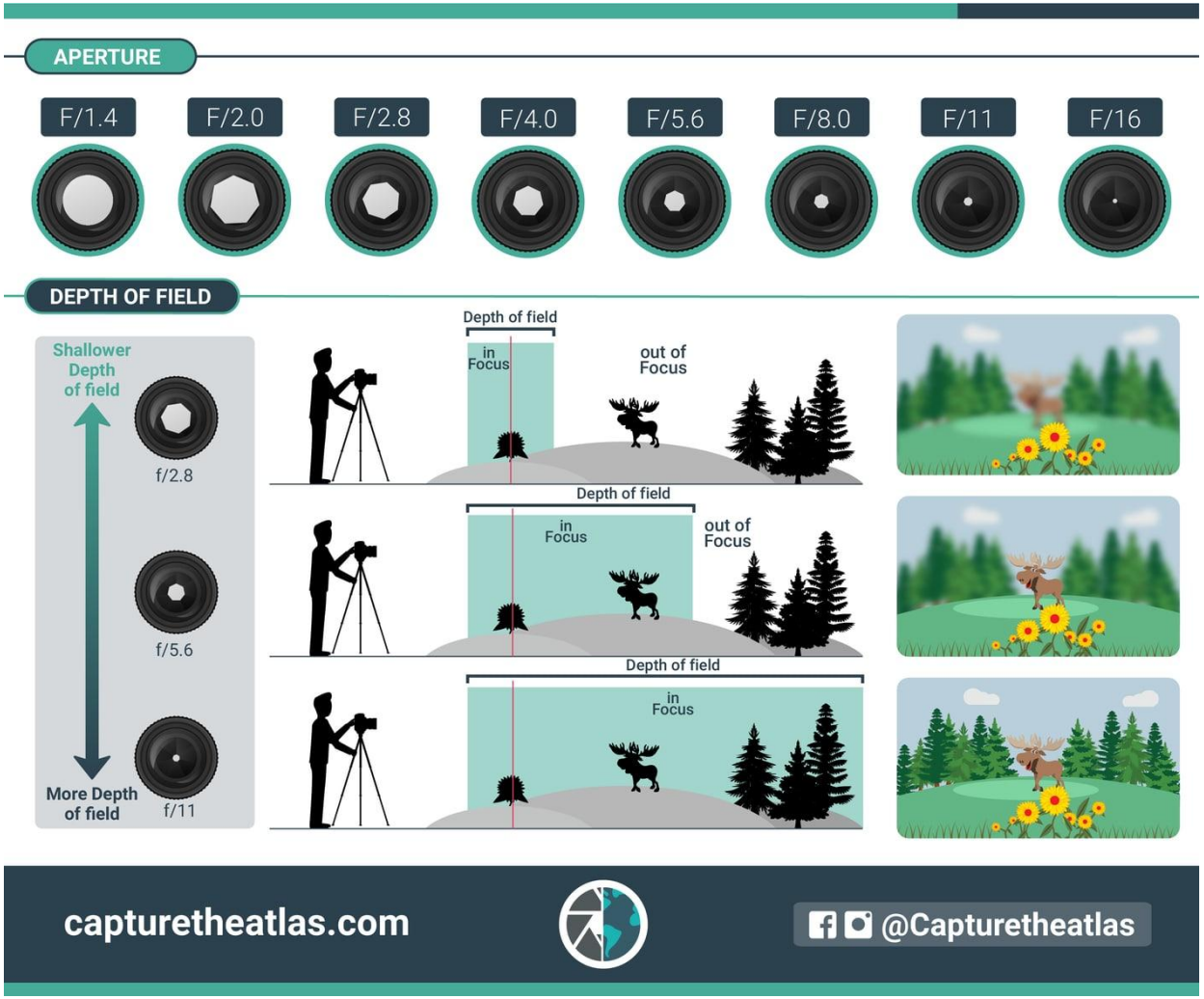






[Macro photography courses and talks | Manchester | Art of Macro]

Problem = Depth of Field





- DOF is proportional to f-value & distance/magnification
- small dof v large dof but ….. diffraction
- use mid f + stacking

Problem is Depth of Field (DOF)



Single



Close-up

Stacked



Single



Macro

Stacked

Micro



Single



Stacked



Limited depth of field



Stacked – 5 shots

Copyright © by Enrico Savazzi, 2009-2010 (www.savazzi.net). DoF equation originally published by Lefkowitz (1979, p. 258), equation used in this sheet is an equivale

Table 1 (below): DoF and effective aperture (E) based on nominal aperture and magnification.

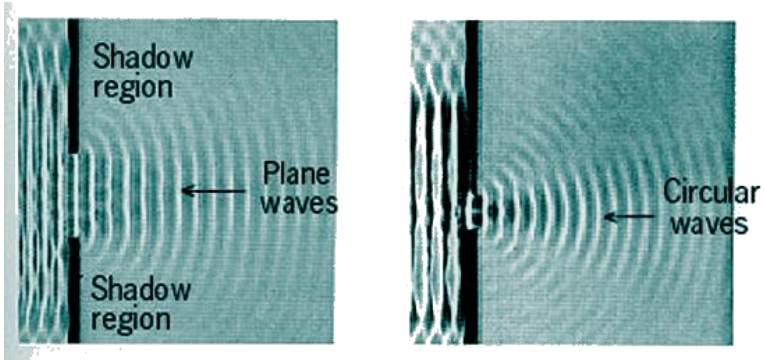
Green: optimal settings, resolution is limited by CoC (*C* ≥ *Airy*). The best compromise between DoF and diffraction is the cell at the bottom of each green column.

Yellow: use if higher-than-optimal DoF is required of if the image will be printed at a small absolute size. Resolution is slightly limited by diffraction (*C* < *Airy* < 2*C*).

Red: avoid, resolution is visibly reduced by diffraction (2*C* < *Airy*).

Nom. aperture→	1		1.2		1.4		2		2.8		4		5.6		8		11		16		22		32	
Magnification↓	*DoF*	*E*	*DoF*	*E*	*DoF*	*E*	*DoF*	*E*	*DoF*	*E*	*DoF*	*E*	*DoF*	*E*	*DoF*	*E*	*DoF*	*E*	*DoF*	*E*	*DoF*	*E*	*DoF*	*E*
0.1	8.3	1.1	9.9	1.3	12	1.5	17	2.2	23	3.1	33	4.4	46	6.2	66	8.8	91	12	130	18	180	24	260	35
0.2	2.3	1.2	2.7	1.4	3.2	1.7	4.5	2.4	6.3	3.4	9	4.8	13	6.7	18	9.6	25	13	36	19	50	26	72	38
0.3	1.1	1.3	1.3	1.6	1.5	1.8	2.2	2.6	3	3.6	4.3	5.2	6.1	7.3	8.7	10	12	14	17	21	24	29	35	42
0.4	0.66	1.4	0.79	1.7	0.92	2	1.3	2.8	1.8	3.9	2.6	5.6	3.7	7.8	5.3	11	7.2	15	11	22	14	31	21	45
0.5	0.45	1.5	0.54	1.8	0.63	2.1	0.9	3	1.3	4.2	1.8	6	2.5	8.4	3.6	12	5	17	7.2	24	9.9	33	14	48
0.75	0.23	1.8	0.28	2.1	0.33	2.5	0.47	3.5	0.65	4.9	0.93	7	1.3	9.8	1.9	14	2.6	19	3.7	28	5.1	39	7.5	56
1	0.15	2	0.18	2.4	0.21	2.8	0.3	4	0.42	5.6	0.6	8	0.84	11	1.2	16	1.7	22	2.4	32	3.3	44	4.8	64
1.5	0.083	2.5	0.1	3	0.12	3.5	0.17	5	0.23	7	0.33	10	0.47	14	0.67	20	0.92	28	1.3	40	1.8	55	2.7	80
2	0.056	3	0.068	3.6	0.079	4.2	0.11	6	0.16	8.4	0.23	12	0.32	17	0.45	24	0.62	33	0.9	48	1.2	66	1.8	96
2.5	0.042	3.5	0.05	4.2	0.059	4.9	0.084	7	0.12	9.8	0.17	14	0.24	20	0.34	28	0.46	39	0.67	56	0.92	77	1.3	110
3	0.033	4	0.04	4.8	0.047	5.6	0.067	8	0.093	11	0.13	16	0.19	22	0.27	32	0.37	44	0.53	64	0.73	88	1.1	130
4	0.023	5	0.028	6	0.033	7	0.047	10	0.066	14	0.094	20	0.13	28	0.19	40	0.26	55	0.38	80	0.52	110	0.75	160
5	0.018	6	0.022	7.2	0.025	8.4	0.036	12	0.05	17	0.072	24	0.1	34	0.14	48	0.2	66	0.29	96	0.4	130	0.58	190
7.5	0.011	8.5	0.014	10	0.016	12	0.023	17	0.032	24	0.045	34	0.063	48	0.091	68	0.12	94	0.18	140	0.25	190	0.36	270
10	0.0083	11	0.0099	13	0.012	15	0.017	22	0.023	31	0.033	44	0.046	62	0.066	88	0.091	120	0.13	180	0.18	240	0.26	350
15	0.0053	16	0.0064	19	0.0075	22	0.011	32	0.015	45	0.021	64	0.03	90	0.043	130	0.059	180	0.085	260	0.12	350	0.17	510
20	0.0039	21	0.0047	25	0.0055	29	0.0079	42	0.011	59	0.016	84	0.022	120	0.032	170	0.043	230	0.063	340	0.087	460	0.13	670
30	0.0026	31	0.0031	37	0.0036	43	0.0052	62	0.0072	87	0.01	120	0.014	170	0.021	250	0.028	340	0.041	500	0.057	680	0.083	990
40	0.0019	41	0.0023	49	0.0027	57	0.0038	82	0.0054	110	0.0077	160	0.011	230	0.015	330	0.021	450	0.031	660	0.042	900	0.062	1300

Diffraction ….



… to overcome depth of field limitations

Photo stacking / Z stacking / Focus stacking / Focus Bracketing / Compositing / EDoF

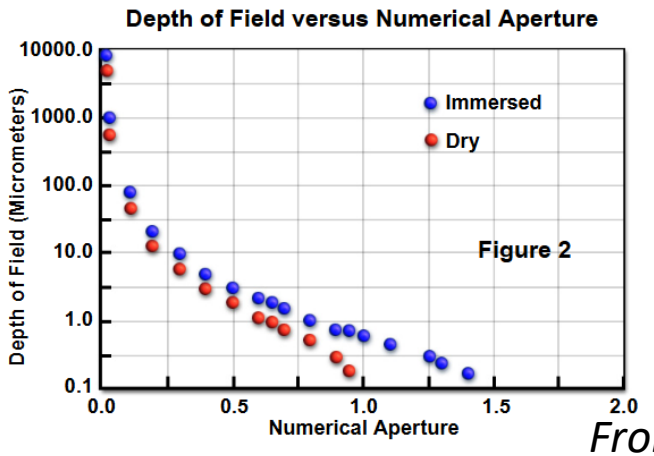
- Photography
  - Landscapes 3
  - Interiors 6
- Macro 5-20
- Ultramacro/Stereomicro 20-100
- Micro 10-3

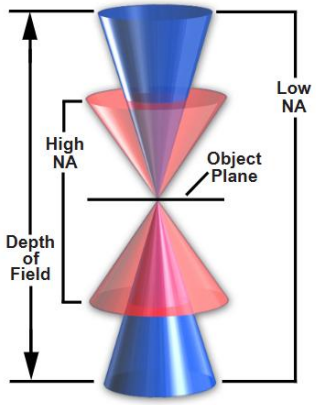
  WD 0.15 cm x100 oil obj dof = 0.1 µm, ? Section thickness

Astronomy … to overcome exposure, noise, time, … Sequator software

MM working guide …

Subject		Mag	f	NA	ef	DOF	N (images)
5 cm	Closeup	x 0.5	8		12	4 mm	12
1 cm	Macro	x 1	8		16 *	1.2 mm	10
		x 2	8		24 *	0.5 mm	20
		x 2.5	8		28 *	0.3 mm	30
10 µm	Micro	x 4		0.1		50 µm	1
		x 10		0.25		5 µm	2
		x 40		0.65		0.5 µm	20
		x 100		1.3		0.1 µm	100





From Nikon Microscopy U

* Or use f4

X1 ef8 0.6mm N=20

X2 ef12 0.2mm N= 50

X2.5 ef14 0.17mm N=60

Hardware

+ digital magnifier

- Camera with Tele Lens + close up filter
  Macro Lens + focus rail or Focus Bracketing
- Camera with adapters and microscope objective + rail
- Microscope with camera & adapters
- Microscope with digital sensor

Think …

Outdoors or indoors .. ? Lighting, support stands

FOV, Mag, WD ….. Stack depth …

+ Laowa macro x2, x5 x10 +++ + helicoid focus

+ axial / vertical /epi illumination

Objective infinity, no coverslip

Macro



Macro Lens 1:1 1:1.4



Macro: <25mm> or mag x1.4

Flat Objects = very sharp

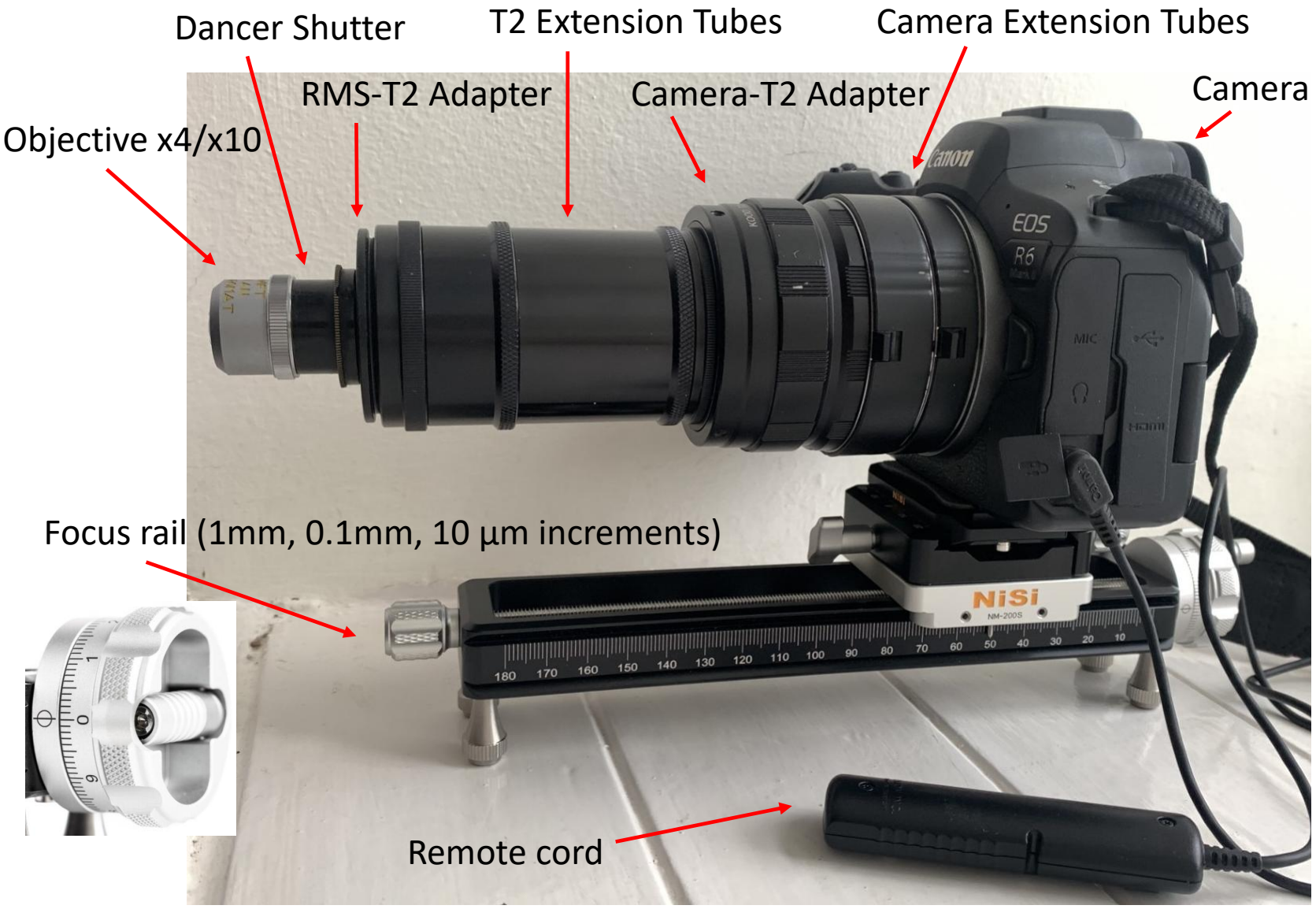
3D objects




SA CONTROL
Canon
100
M-Fn
OFF LOCK ON
AF-ON
20 10 0 10 20
20 10 0 10 20
EOS R
JJC
JJC

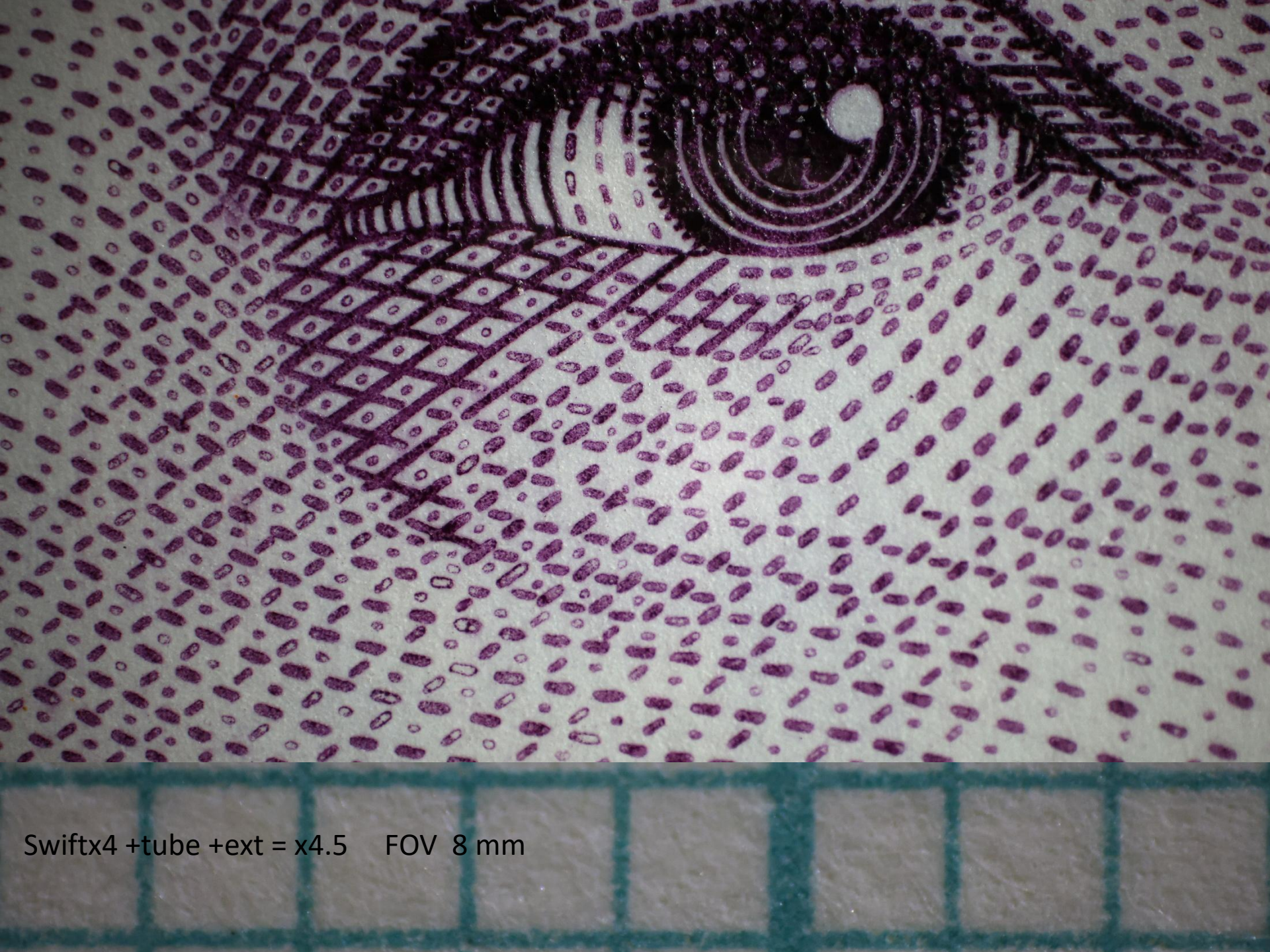
U-Macro

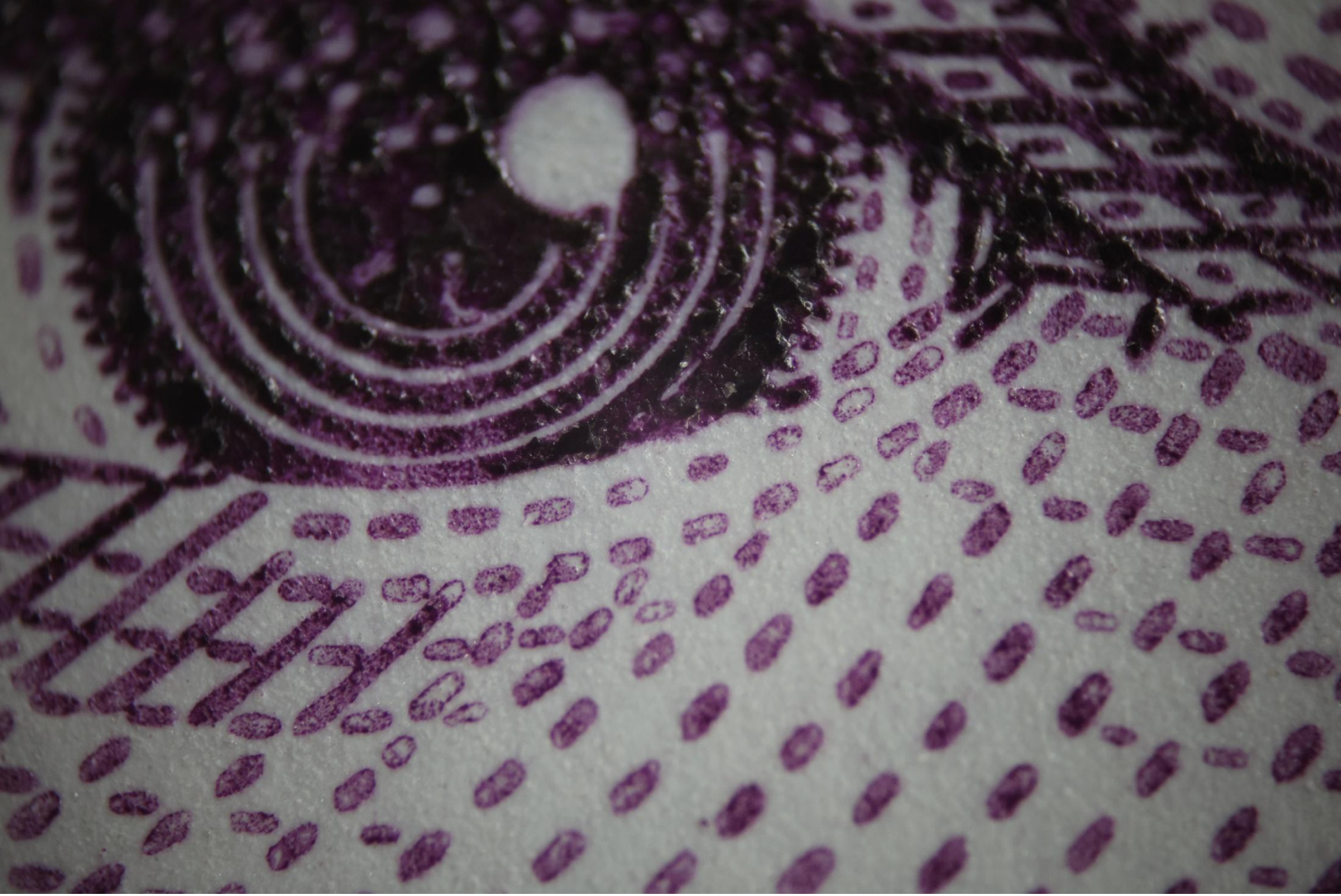
~ 160 mm





Sx4 Mag x3.6 FOV 10 mm


Swiftx4 +tube +ext = x4.5 FOV 8 mm



Sx10 FOV 4mm Mag x9


0 cm 1 2 3 4 5 6 7 8 9 10 11 12



Swiftx4 +tube +ext = x4.5



Swiftx4 +tube +ext = x4.5 Stacked (10)

Micro



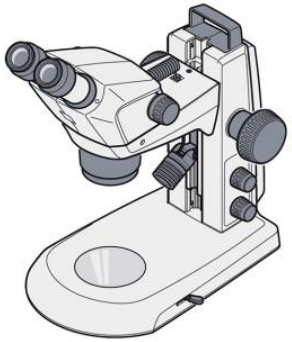


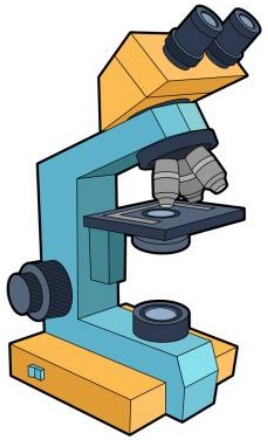

ASAHI PENTAX
MICROSCOPE ADAPTO
LOCK


OLYMPUS


MTV-3
CCD CAMERA ADAPTER







Focus Stacking Software

- In camera ….Olympus, Canon, Fujifilm, (Nikon, Sony)

- Helicon Focus £
- Zerene Stacker £
- Combine ZP 0
- Photoshop £
- Canon DPP 0
- Photopea.com 0
- Stacklab ?

- Picolay 0 
- Image J 0
- Toupview / Progress Capture ?

+ stitching Photoshop panoramas, Microsoft ICE

Handheld, Tripod, Focus rail (mm,um)

- No movement, >3D, …… video

Cleaning … photoshop, Aiarty

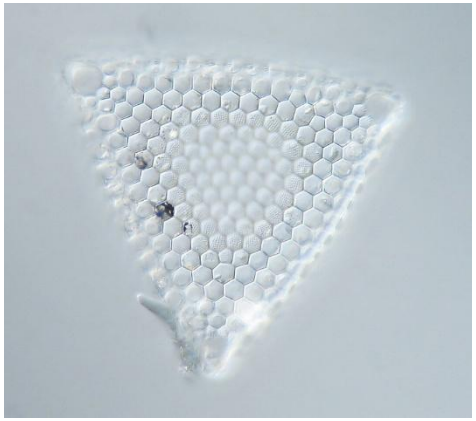
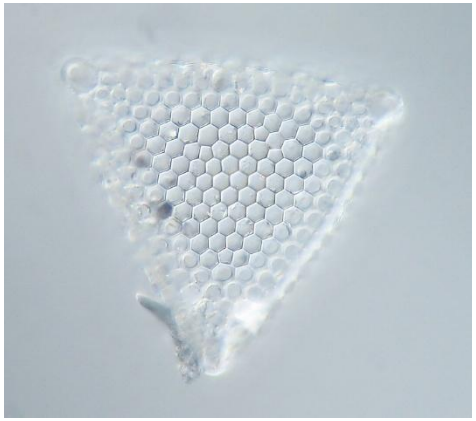
See also for iphone …

Reeflex Pro
Aura HDR Camera

Photoshop

1. File>Scripts>Load files into stack
2. Browse to select required files, #Auto-align images
3. In Layers Window Select all required layers
4. Edit >Autoblend Layers
5. Select Stack images, #Seamless Tones and Colours
6. Crop & Save

Picolay

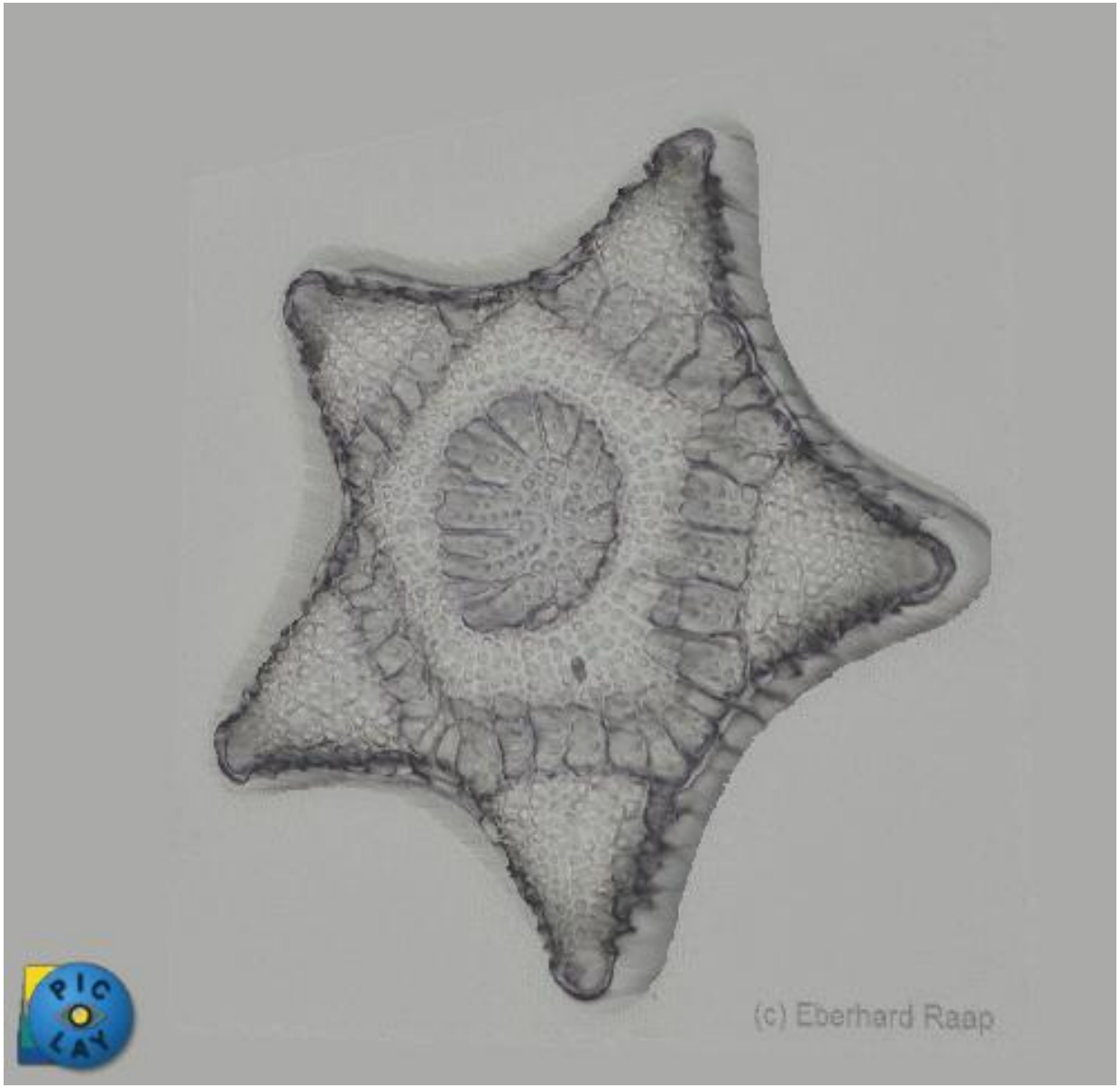
.....

Great for microscopists ..

Includes

Focus stacking
Slideshow set
Animated Gif
Stereo pairs glyph
Interactive Hologram output




(c) Eberhard Raap

My Favourite Stack !!



References

- Artofmacro
- Closeupphotography.com
- Ultramacro.co.uk
- Extreme-macro.co.uk
- Savazzi.net

Examples ....

Hand-Held Photos

Macro – single shot



Macro – in camera Focus Bracketing



Hand- Held



Hand- Held







On tripod



Single

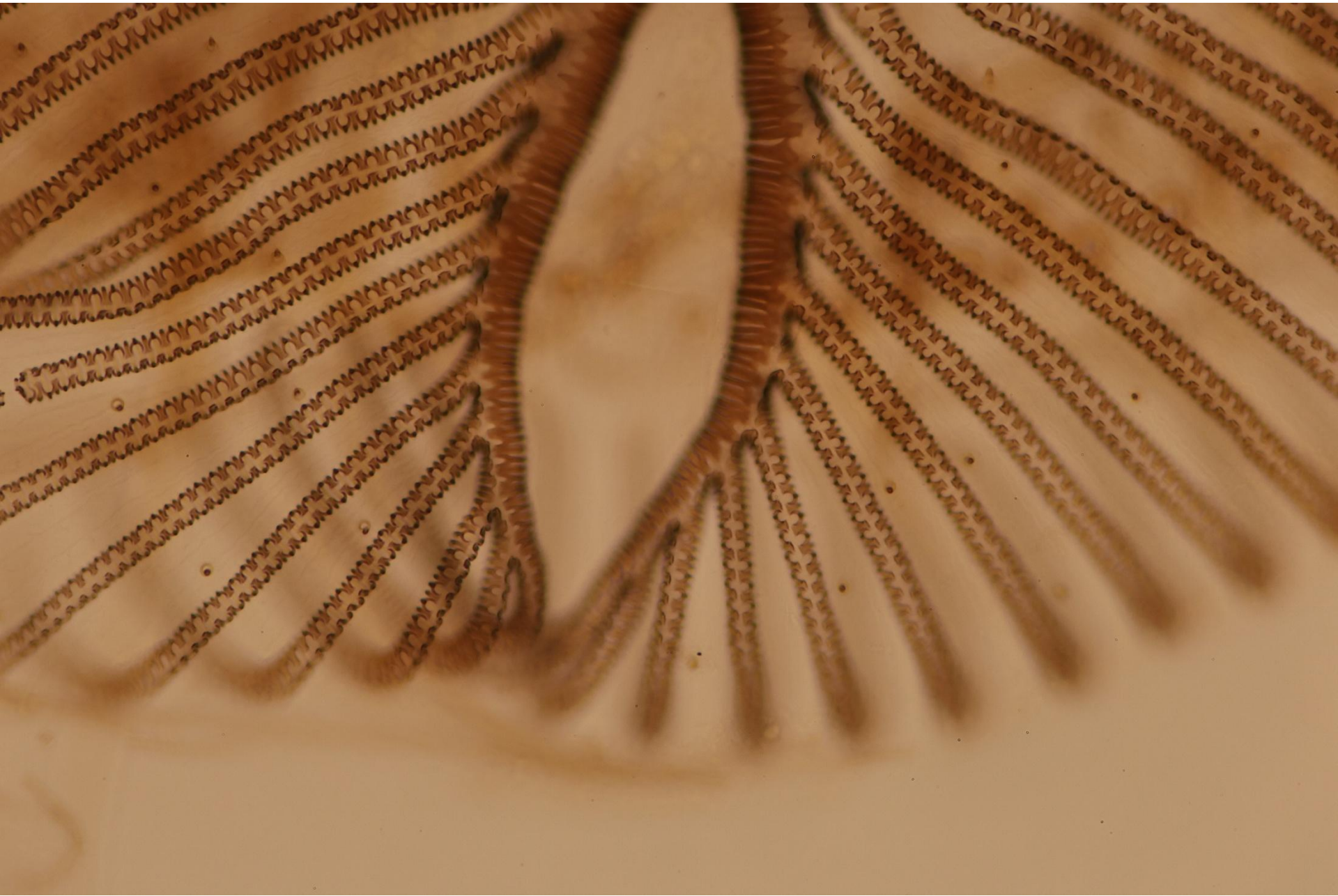


Stack 14

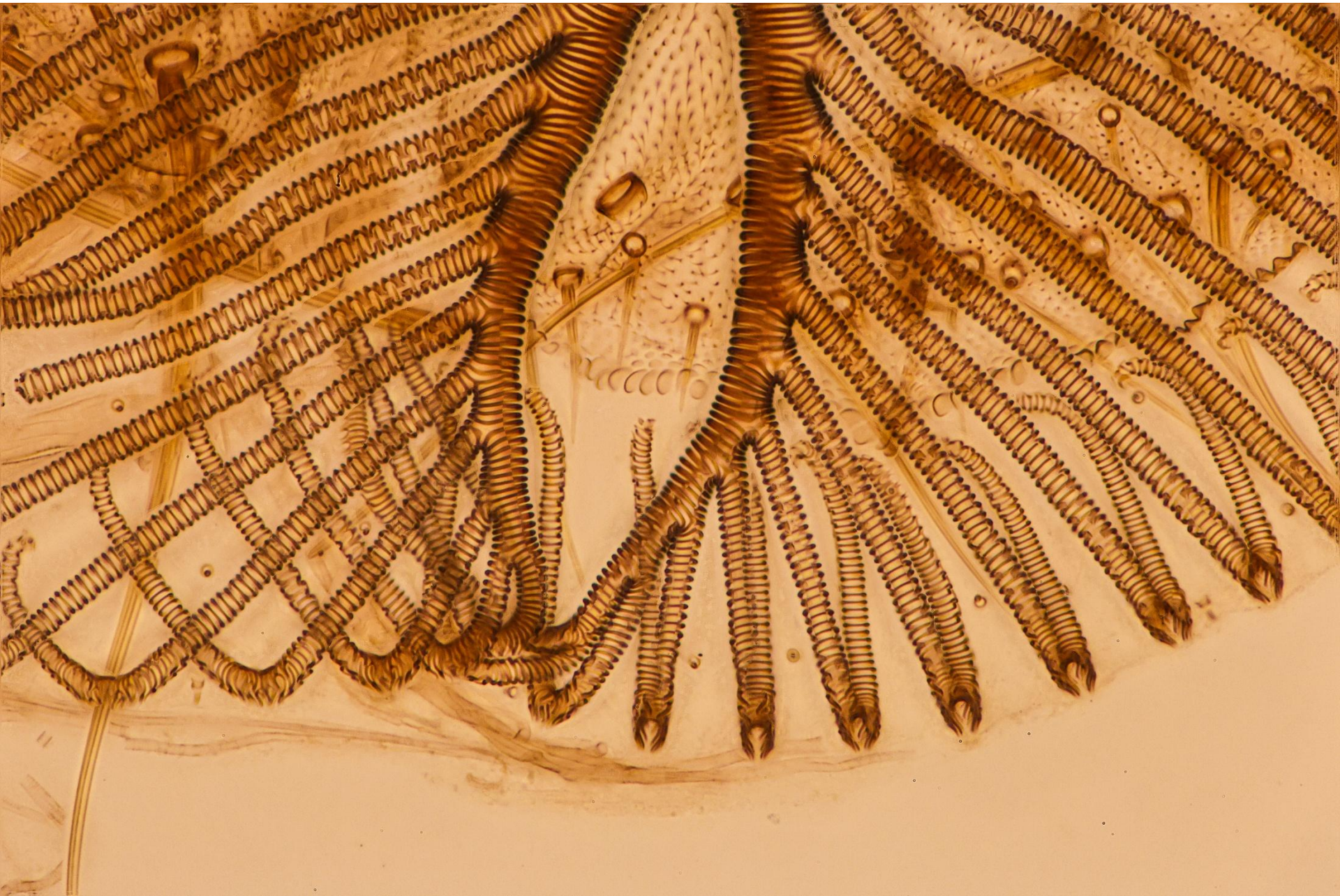


Stack 34

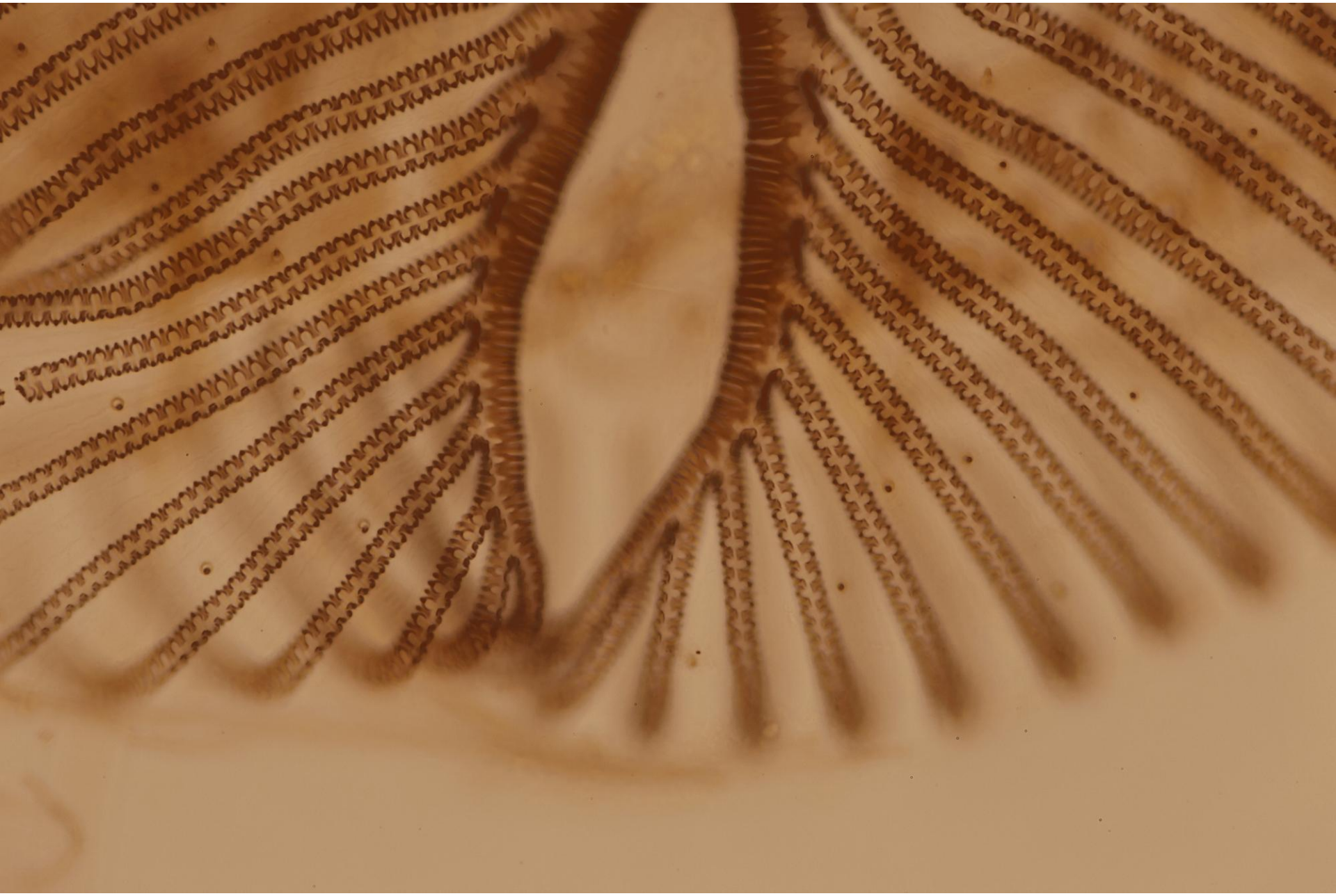
Microscope x2 Single Blowfly proboscis endotrachea



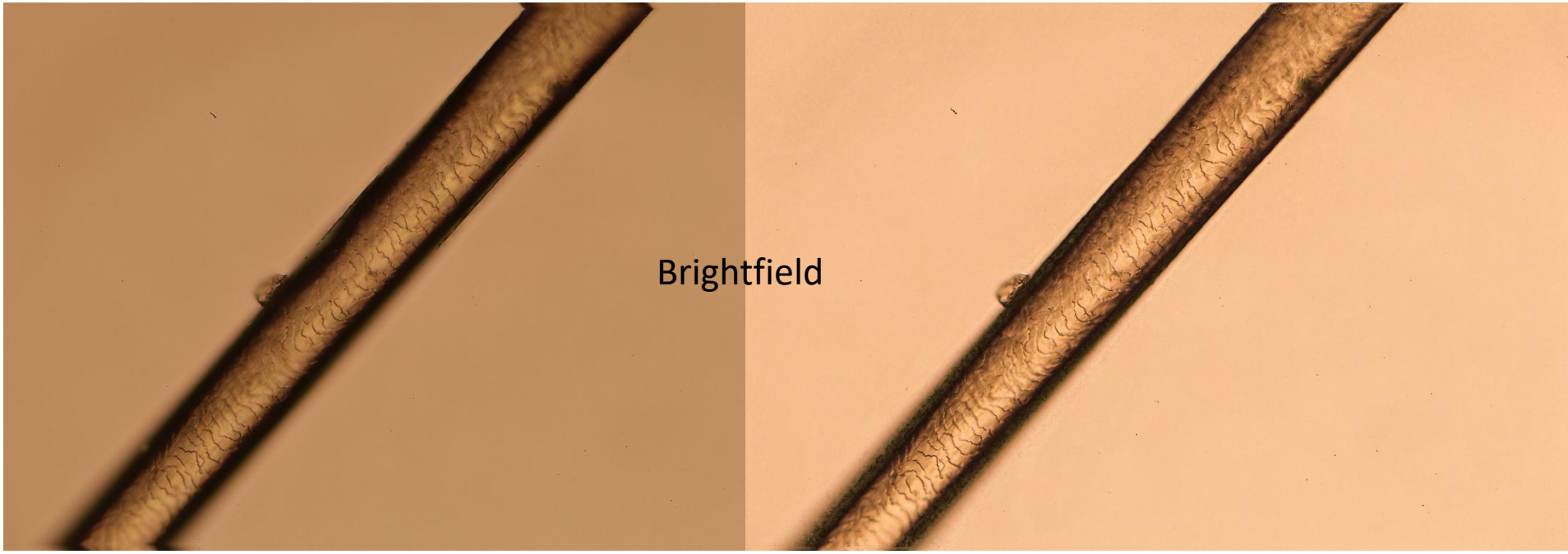
Microscope x2 Stack 4

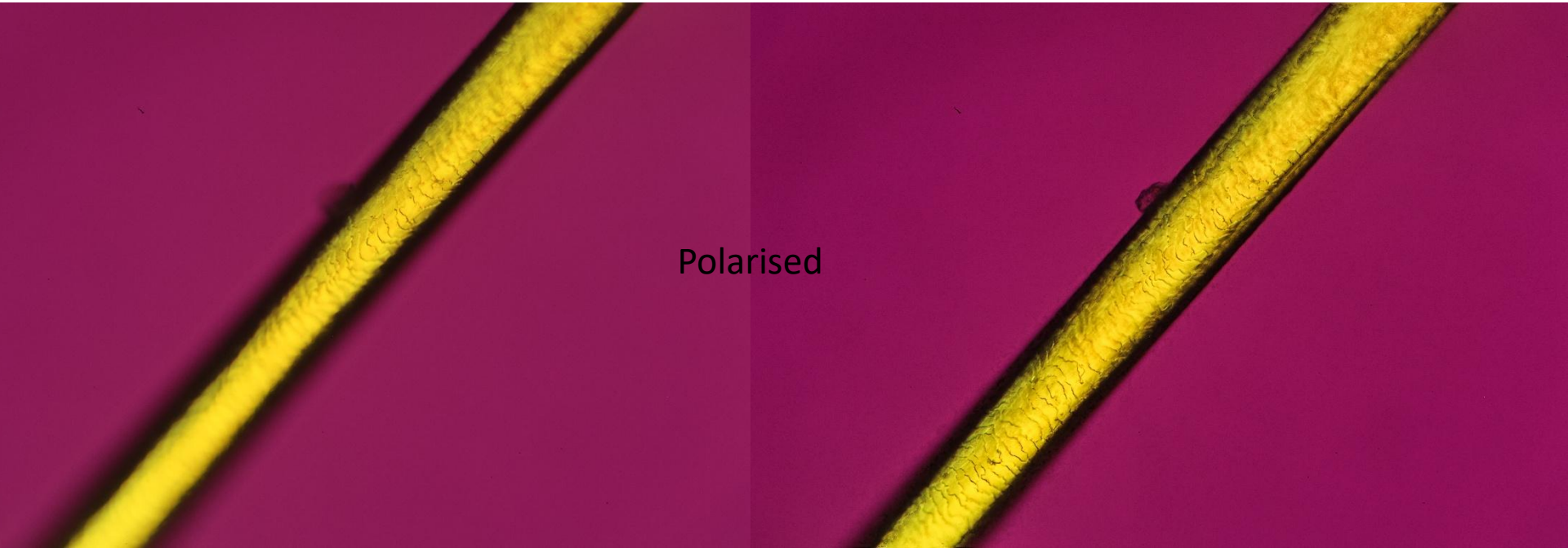


Animated Gif of stack set

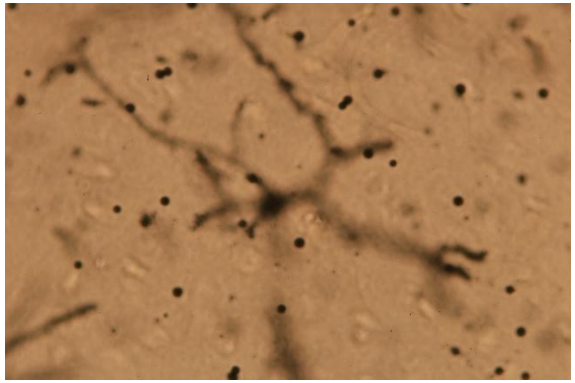
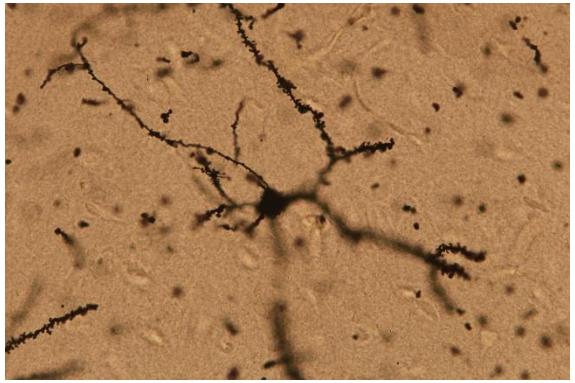
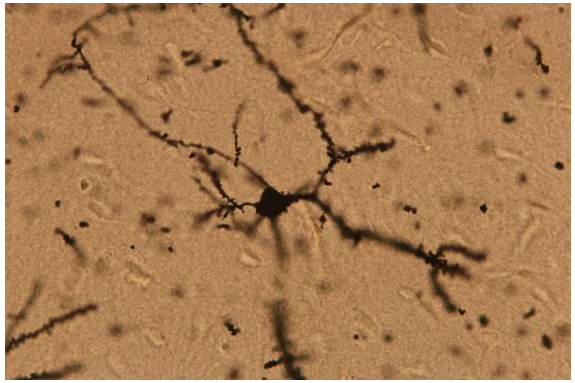
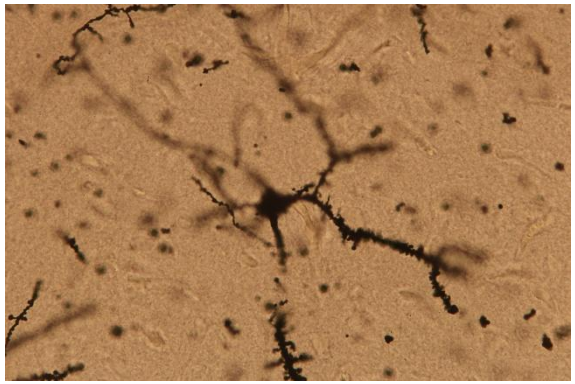
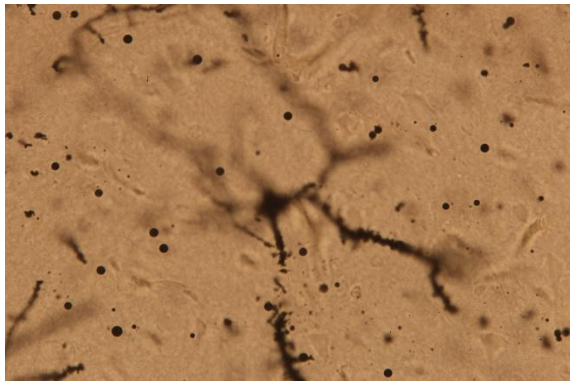


Single Microscope x20 Stacked 5

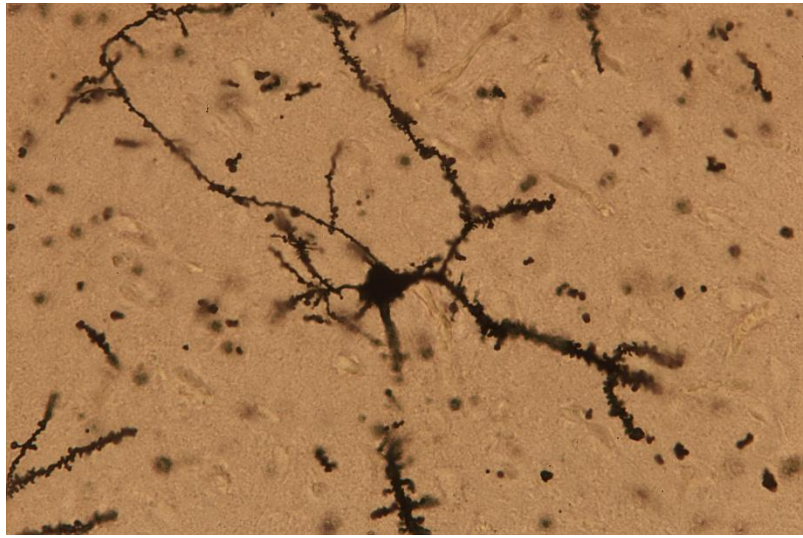




Brain Pyramidal Neurone Microscope x20

Stacked 5-1





Single

Stack 14

