MorphCol supplement#17 - Depth of focus estimates for the Achromat 1x objective of the Leica MZ 6 binocular

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Introduction

Knowledge of the depth of focus of the Leica MZ6 binocular is needed for various reasons, such as the application of extended focus imaging (for example with AutoMontage software (Knappertsbusch, 2002; Knappertsbusch et al., 2006) or with CombineZ software), or for automated imaging of microfossils with AMOR (Knappertsbusch et al., 2009, Knappertsbusch, 2009).

The depth of focus is the vertical difference within which a structure appears in focus and is a function of the magnification and/or the numerical aperture of the lense. In the following experiment, the depth of focus was estimated for the 1x achromat objective for the Leica MZ 6 binocular.

Experimental set up

For imaging a digital color video-camera from Kappa (model CF 11/2) was used. The camera was mounted on the Leica MZ6, that is equipped with an AX stand and using a 1x Cmount connection peace. The imaging system was a Macintosh 8500, the imaging software was Nih-Image from Wayne Rasband.

An equal-sided standard glass prism at angles of 60° was used, with a thin straight engraved scratch line on its surface was applied to obtain a defined object, that regularly extends over the full range of the depth of focus (see Figure 1). The scratch-line was created on the surface of the prism using a thin diamond-knive.

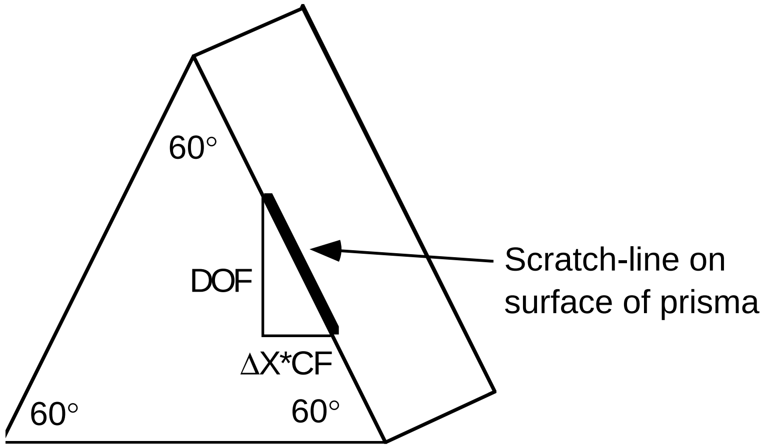

Figure 1. Equalateral triangular glass prisma with a straight scratch-line on the surface to estimate the depth of focus of a microscope lense.

The imaging settings in the Nih-Image program were: NTSC (640x480 pixels), S-Video, gray-scale.
Processing: Average frames 16x, multiplication of images by 1.25, the application of a sobel filter.

Measurement of sharpness and DOF estimates

The scratch-line was oriented horizontally on the monitor and placed in the middle of the frame to the line at 240 pixels. The sequence of image processing operations at each magnification was as follows:

Raw image x 1.25
Sobel [5x5]
Select a 60 x 640 pixel stripe to enclose the scratch line
New window 60 x 640
Paste
Variance image on new window (4 x 4 mask)
Read x-coordinates at 50% of variance height to determine the portion in focus
Past variance image into (Raw image x 1.25)

The portion of the scratch line, that appeared in focus was then measured in pixels at varying magnifications, and the pixels were converted to micrometers. Using the trigonometric formula

$$DOF = DX*CF * \tan 60°$$

it was possible to calculate the depth of focus. DX is the horizontal distance of the scratch, that appears in focus. CF is a factor, that is a function of the magnification (MAG), and that converts pixels into micrometers (see calibration of the Trace program for the Kappa camera used on a Macintosh).

$$CF=1/Xprec \text{ in } \mu m \text{ per pixel, with}$$
$$Xprec = 0.0020061 + 0.11829 * MAG$$

The measurement of the sharp portion of the scratch-line was somewhat arbitrary and was done in three ways: 1.) Estimation of sharpness at 50% of the grey-level variance (in a variance image low variance indicates defocussed conditions, high variance indicates good focus conditions); 2.) Interpretation of the sharp portion of the scratch line by considering the 50% grey-level variance and the original image; and 3.) Interpretation of the image only. The results for the three methods are given in the three tables 1 through 3 below.

MAG	Sharp distance (horizontal pixels)	DX, pixels	CF in µm per pixel	DOF in µm @ 50% of Variance
0.63x	264 to 368	104	13.0669	2354
0.8x	291 to 343	52	10.3479	932
1.0x	221 to 284	63	8.3128	907
1.25x	252 to 308	56	6.6725	647
1.6x	259 to 302	43	5.2282	389
2.0x	210 to 273	63	4.1914	457

2.5x	284 to 332	48	3.3587	279
3.2x	248 to 284	36	2.6279	164
4.0x	215 to 254	39	2.1045	142

Table 1. DOF estimated from 50% grey-level variance.

MAG	Sharp distance (horizontal pixels)	DX, pixels	CF in µm per pixel	DOF in µm @ 50% of Variance
0.63x	264 to 368	130	13.0669	2942
0.8x	291 to 343	74	10.3479	1326
1.0x	221 to 284	65	8.3128	936
1.25x	252 to 308	64	6.6725	740
1.6x	259 to 302	43	5.2282	389
2.0x	210 to 273	40	4.1914	290
2.5x	284 to 332	38	3.3587	221
3.2x	248 to 284	39	2.6279	178
4.0x	215 to 254	27	2.1045	98

Table 2. DOF estimated from 50% grey-level variance and by watching the image.

MAG	Sharp distance (horizontal pixels)	DX, pixels	CF in µm per pixel	DOF in µm @ 50% of Variance
0.63x	264 to 368	114	13.0669	2580
0.8x	291 to 343	93	10.3479	1667
1.0x	221 to 284	66	8.3128	950
1.25x	252 to 308	59	6.6725	682
1.6x	259 to 302	53	5.2282	480
2.0x	210 to 273	49	4.1914	356
2.5x	284 to 332	47	3.3587	273
3.2x	248 to 284	37	2.6279	168
4.0x	215 to 254	35	2.1045	128

Table 3. DOF estimated by eye only.

Results

Table 4 gives the mean DOF as a function of MAG combining Tables 1, 2 and 3 and Figure 2 illustrates the DOF estimates from Tables 1 through 4.

MAG	Average DOF, µm
0.63x	2625
0.8x	1308
1.0x	931
1.25x	690
1.6x	419
2.0x	368
2.5x	258
3.2x	170
4.0x	123

Table 4. Mean DOF (in µm) as a function of MAG.

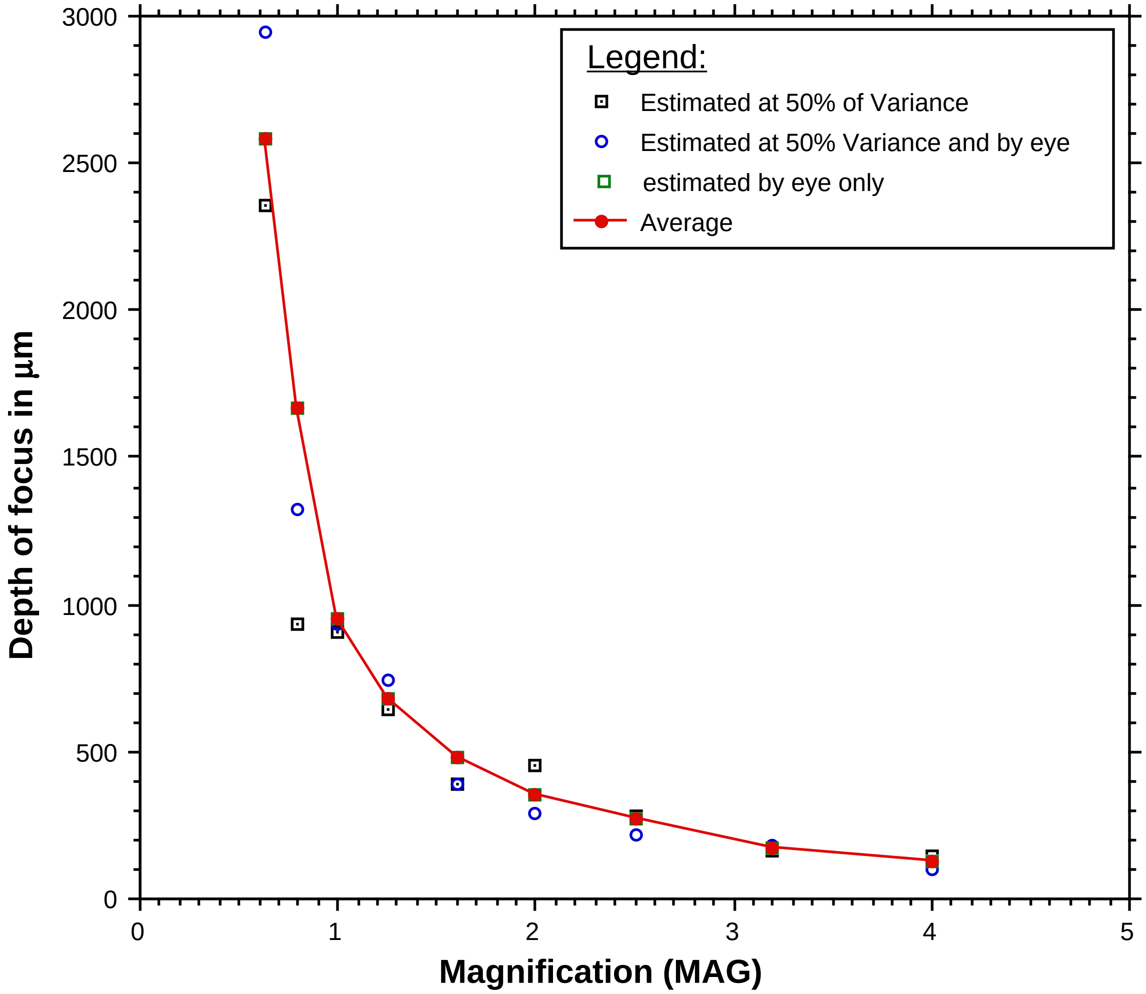

Figure 2. Graph showing the depth of focus (in µm) as a function of the magnification of the Achromat 1x lense of the Leica MZ 6 binocular microscope. The values of MAG correspond to the numerals, that are engraved at the magnification changer at the microscope.

Conclusions

The depth of focus of the Achromat 1x objective for Leica MZ 6 shows a strong non-linear decrease with increasing magnification. At the highest possible magnification (4x) of the microscope the DOF extends over about 123 µm.

References

Knappertsbusch, M. (2002). Stereographic virtual reality representations of microfossils in reflected light microscopy. Palaeontologia Electronica, vol. 5, Issue 3, 11 p., 1.1 MB. http://palaeo-electronica.org/paleo/2002_1/light/issue1_02.htm

Knappertsbusch, M., Brown, K.R. and Rüegg, H.R. (2006). Positioning and enhanced stereographic imaging of microfossils in reflected light. Palaeontologia Electronica, vol. 9, Issue 2, 8A: 10 p., 30.1 MB. http://palaeo-electronica.org/paleo/2006_2/reflect.Index.html

Knappertsbusch, M., Binggeli, D., Herzig, A., Schmutz, L., Stapfer, S., Schneider, C., Eisenecker, J., and Widmer, L. (2009). AMOR - A new system for automated imaging of microfossils for morphometric analyses. Palaeontologia Electronica, vol. 12, Issue 2, 2T: 20 p. http://palaeo-electronica.org/paleo/2009_2/165/index.html

Knappertsbusch, M. (2009). Vertical correction of the auto-focus in AMOR 3.5. MorphCol Supplement #18. Unpublished technical report, 1.10.2009, 10 p.