

# MorphCol Supplement #10 - Diaphragm Test for AMOR2

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## Introduction:

Visual inspection during imaging with AMOR2 has led to the suspicion, that unfavorable opening of the diaphragm influences the outline and so alters the precision of morphometric measurement. In order to have a better control of the illumination the present test was carried out, where only the diaphragm opening was changed while all other conditions remained constant.

## Experimental set up:

Camera: Sony DXC-390P.

Cmount 1x.

Leica MZ 6 binocular microscope using zoom body (zoom from 0.63x to 4x).

Achromat 1x objective.

Cross-polarized light (polarizing set from Volpi).

Illumination using Volpi 4000-1, light at 4 (fully open).

Imaging system: AMOR2 (PC-based).

Tests were done using specimen 502\_0100CCK0201 from the study of Knappertsbusch (2007) (specimen in field no. 30, keel-position).

AMOR2 was started up, after initialization steps the single mode operation was chosen.

The field 30 with the test specimen appeared then. The specimen was centered and put into focus. A magnification of 1.62x (reading in AMOR2) was set and the specimen was then oriented using autotilt. No autorotate was applied. For final imaging and storage to disk as tiff files (640x480 pixels) a magnification of 2.43x was set for all diaphragm positions.

A small stripe of millimeter-paper was attached on the microscope along the engraved marks of the diaphragm, so that diaphragm readings are more precise.

The mm readings and the marks from Leica follow a linear relationship (for data see Table 1) with the equation:

$$\text{Diaphragm (in mm)} = 6.7857 * \text{Mark} - 0.35714 \quad r^2=0.999$$

Diaphragm Mark (MZ6)	Diaphragm (in mm)
0	0 mm
1	6 mm
2	13 mm
3	20 mm
4	27 mm
5	34 mm
6	40 mm

**Table 1:** Markings of the diaphragm of the Mz6 microscope and corresponding readings in mm.

## Note:

Diaphragm settings in previous tests (for example MorphCol Supplements #'s 8 and 9 were realized at Mark 3.5 ("Blende 3.5"), which corresponds to 24 mm.

## The experiment and post-processing:

During the experiment only the diaphragm was changed with all other parameters (light, x and y tilt, magnification) remaining constant. Images were taken at the following suite of diaphragm openings: 6mm, 10mm, 13mm, 15mm, 20mm, 24mm, 25mm, 27mm, 30mm, and 34mm.

All tiff images were binarized to black- and white raw images using the *automation* macro in *Nih-Image*, thereafter the images were saved as raw files in *Adobe Photoshop*. Before outline extraction the magnification of 2.43x from AMOR2

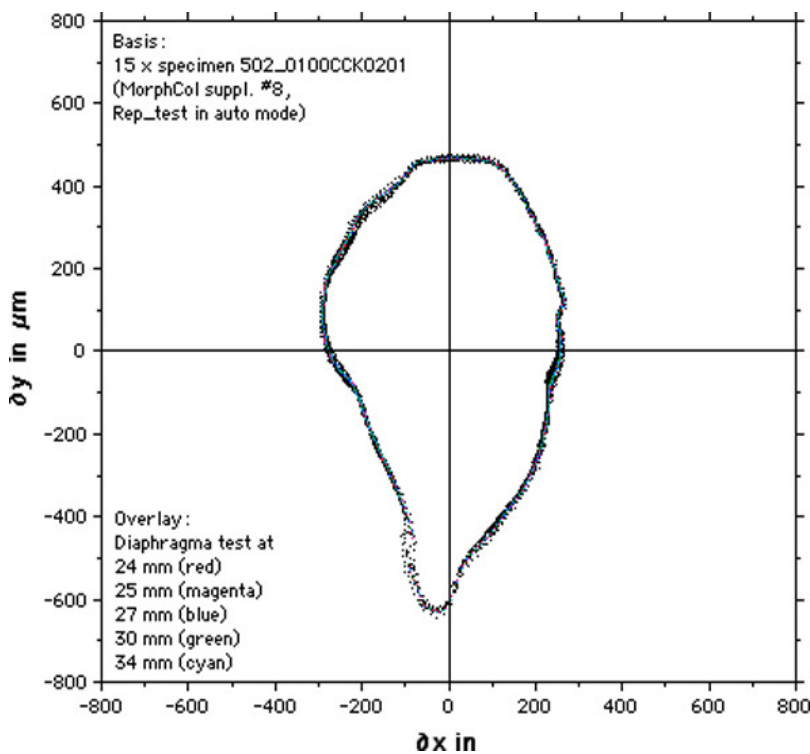
was corrected to 2.36x using the *MagCorr1.out* program. Outline extraction was done using program *Trace\_AMOR1\_batch.out* at magnification of 2.36x. Traced files were then interpolated and keel-view areas were determined using *Sprep53.out*. The image taken at 6mm was excluded from this procedure because the resulting image was entirely black, showing no object.

**Results:**

In images taken at a diaphragma opening at 10mm through 20mm the shell was incomplete or showed strong erosion of the outline. From 24mm onwards, the outline of the shell remained quite constant leading to stable measurements of the enclosed area (see Table 2 and Figures 1 and 2). In Figure 1 the outlines obtained at diaphragma positions 24 mm through 34 mm are superimposed on 15 outlines obtained during the repeatability test described in MorphCol supplement # 8 (imaging with AMOR2, automeasurement mode). This superposition illustrates, that variation of diaphragma openings between 24 mm through 34 mm has no significant influence on the shape of the outline.

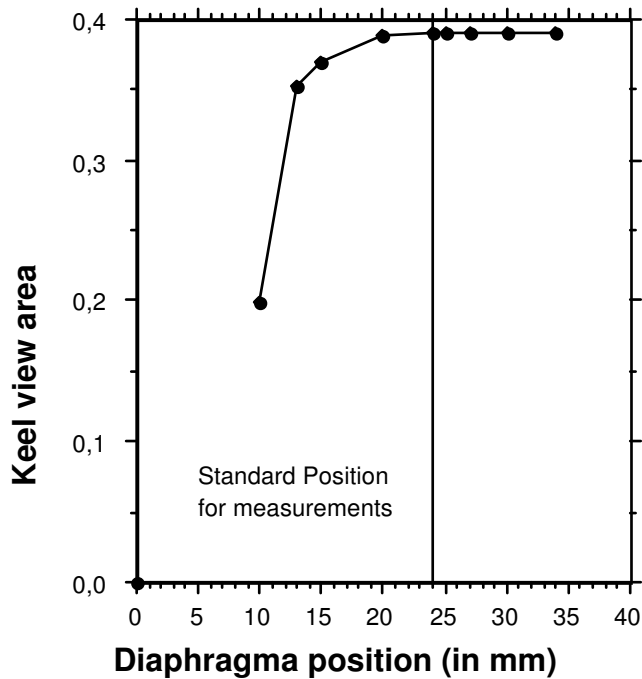
Diaphragma (mm)	# of points	$\delta x$ ( $\mu m$ )	$\delta y$ ( $\mu m$ )	Area (mm <sup>2</sup> )
0	0	0	0	0
10	910	391.3	817.8	0.1996
13	835	521.7	1043.7	0.3522
15	858	547.8	1081.4	0.3702
20	871	557.6	1094.8	0.389
24	872	557.6	1097.5	0.3903
25	873	557.6	1097.5	0.3901
27	874	557.6	1097.5	0.3898
30	874	557.6	1094.8	0.3896
34	875	557.6	1100.2	0.3902

**Table 2:** Extract of the measurements file after *Sprep53.out*



**Figure 1:** Superposition of 15 outlines obtained from the repeatability test with AMOR2 (in auto-mode) and the outlines obtained during the present diaphragma test for diaphragma positions ranging from 24mm through 34 mm. There is no measurable deviation recognizable between the two test series.

Figure 2 (keel view area as a function of diaphragma opening) shows, that a diaphragma opening of  $\geq 24$  mm leads to stable measurements. If the diaphragma is equal or larger than 24mm, the mean area is  $0.39\text{mm}^2$  (min= $0.3896\text{mm}^2$ , max= $0.3903\text{mm}^2$ , range= $\pm 0.00035\text{mm}^2$ ), which means a error of less than 0.09% of the mean area. No disturbing effect was observed when the diaphragma was opened to full maximum.



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**Figure 2:** Keel view area as a function of the diaphragma position in AMOR2.

**Conclusions:**

Testing the influence of the diaphragma opening of the Leica MZ6 for imaging using the AMO2 system shows, that a diaphragma position at  $\geq 24\text{mm}$  (= "Blende 3.5 or larger) provides negligible influence on the keel view area measurements. (less than 0.09% of the mean keel view area). No disturbing effect was observed when the diaphragma was opened to full maximum.

**Literature:**

Knappertsbusch, M. (2007). Morphological variability of *Globorotalia menardii* (planktonic foraminifera) in two DSDP cores from the Caribbean Sea and the Eastern Equatorial Pacific. Carnets de Géologie, Article 2007/04 (CG2007\_A04).

Knappertsbusch, M. (2008). MorphCol Supplement #8 – Repeatability Test with AMOR2. Technical report, NMB, 7.2.-10.3.2008, 19 pages.