MorphCol Supplement 3

Michael Knappertsbusch, 8.5.2007

3.2.1.1 Analysis of variation in shape and size due to repeated manual positioning of a single microfossil into the same position

Accurate and consistent manual positioning of the specimens during imaging always introduces variation in the measurements. This variation includes effects from positioning the specimen into the same orientation and tilt angle, and moving it to the same virtual coordinate system on the monitor. A second source for variation in the outlines is the consistent application of LUT operations when the grey-level images are transformed to binary black and white images (the latter is influenced by fluctuations in illumination, background reflections and signal to noise ratio of the original image).
In order to estimate the repeatability (sensu ARNQVIST & MARTENSSON, 1998) a single specimen was imaged 30 times and processed to outline coordinates and derived morphometric parameters under identical conditions, with the same method and the same equipment. For the spiral height (dX) and axial diameter (dY) of the shell standard deviations of 1.2 micrometers and 4.7 micrometers were estimated respectively, which is 0.2% and 0.4% from the mean dX and mean dY. For the estimation of the absolute error range due to manual positioning the 250 rays of each of the 30 interpolated outlines were transformed to polar coordinates (rho,theta) and the variation in ray lenth (rho) due to manual repositioning was analyzed at each angle theta (with incremets of Dtheta =360°/250=1.44°). The average of the absolute differences between maximum and minimum ray-lenth for each ray for the 30 measurements in this experiment is 18.6 micrometers (see results shown further below), which is 1.6% of the length of the shell in keel view (dY=1117 micrometers for this specimen). These experiments demonstrated that variation of final measurements due to orientation handling is negligible with respect to the overall shell-size variation seen from specimens in samples at different geological times.

Method:
The system consisted of a digital (CCD) camera from Kappa, model CF 11/2 mounted on the Leica MZ 6 binocular microscope with a 0.63-4x zoom body, a 1x achromatic objektiv, and a 1x C-mount. Illumination was done *without polarizers*. The computer was a PowerMacintosh 8500, Mac Os 8, imaging software was Nih-Image 1.61 from Wayne Rasband. The magnification was held constant at the 2.5x marking of the magnification knob.

A single specimen was taken from DSDP sample 502-1H-CC, 0-4cm (= sample No. 502_0100CC, see Figure 1). The specimen was 0201 (e.g. the first specimen in field no. 2).

Imaging was done in two sets: A first set of 15 images was taken during 29. August 2002, whereby the specimen was 15 times manually re-oriented into keel view, after each orientation an image was taken (640x480 pixels, grey levels). These images were numbered through from 502_0100CC0201-01 to 502_0100CC0201-15.

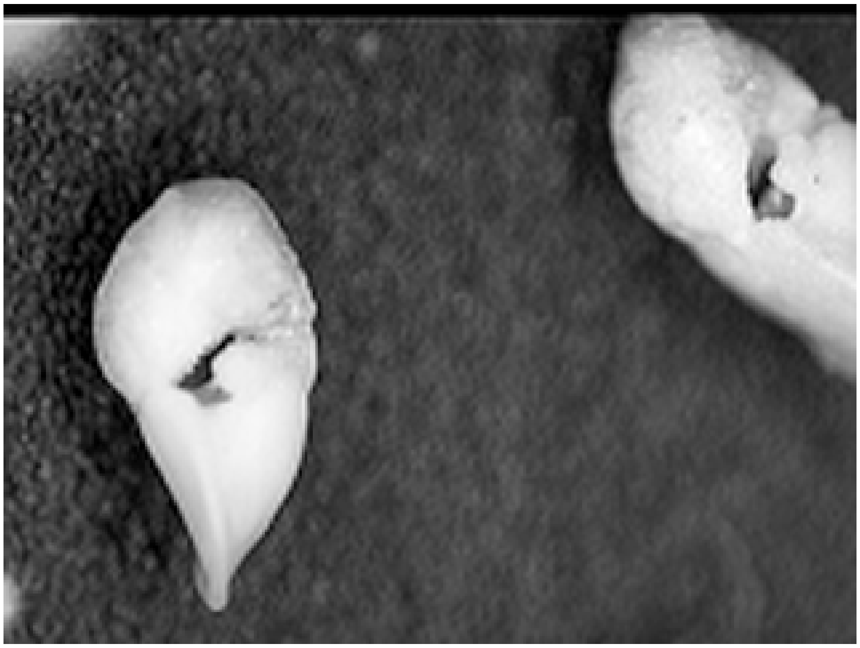

Figure 1. Specimen 502_0100CCK0201

A second test was done on 21 november 2002 and using the same specimen after replacement of the previous monitor by a new monitor from Trinitron (all other conditions were held identical). The new set of images were numbered through from 502_0100CC0201-16 bis 502_0100CC0201-30, so that a total of 30 images were available to test the repeatability (n.b. the new monitor had no recognizable influence on the measurements of the outlines).

All images were then converted to black and white images, the outline coordinates determined using Trace33_batch.out, and the outlines transformed into outlines with 250 points each and centered into the origin of coordinate system using program Sprep53.out as desribed in the previous chapters of MorphCol. The extracted outlines are deposited in folder "Traced", the transformed files are in folder "Sprep". All original Tiff and Raw images and data are available from CD-Rom, *Morphometry, CD #16*.

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Results:

a.) Analysis of the cartesian form of the outline
Figure 2 shows an overlay of the 30 outlines of the same microfossil after repeated

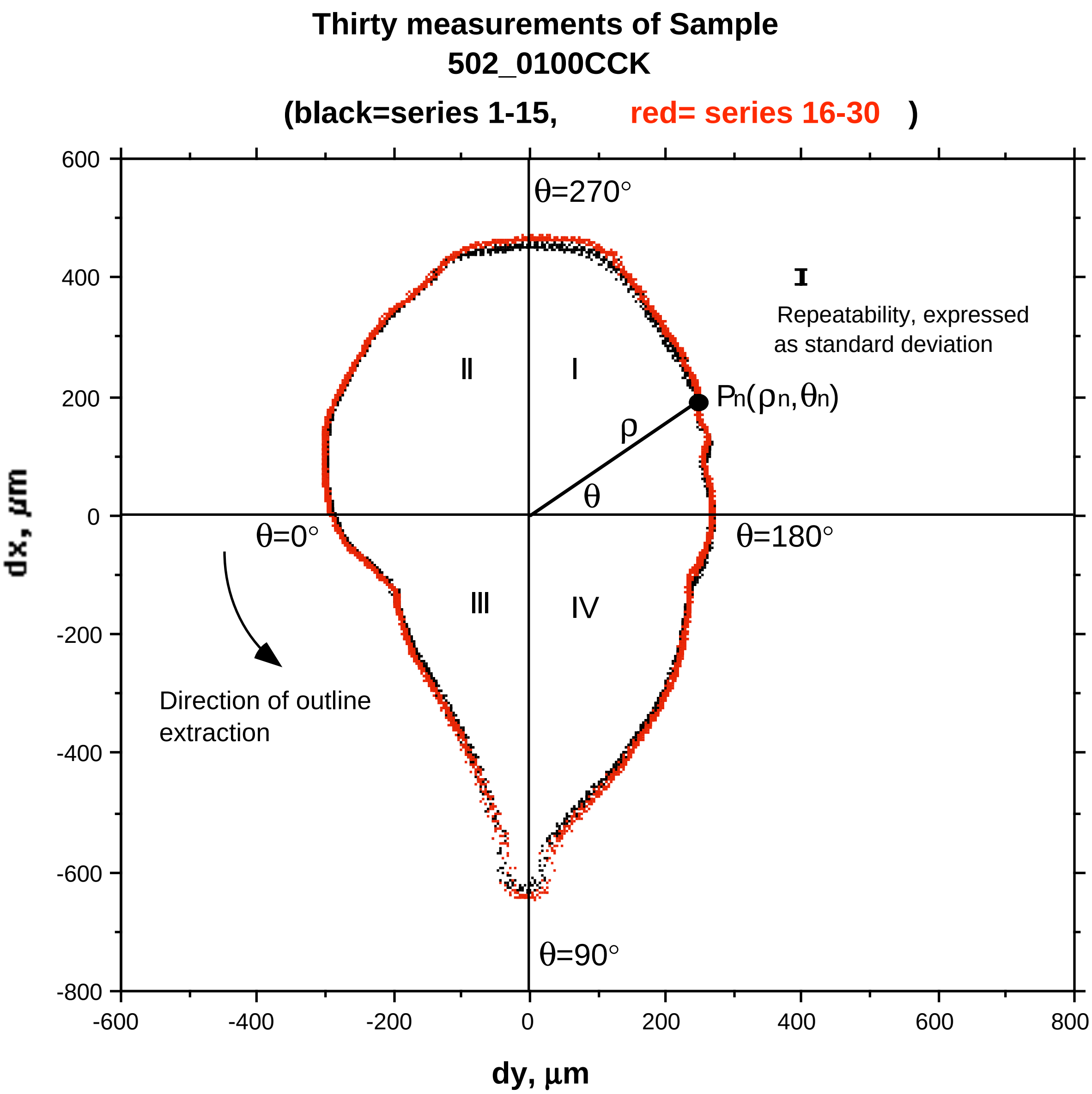

Figure 2

manual repositioning, imaging, processing to binary images, outline extraction and transformation [250 points, centered to origin of coordinate system] of the shell in keel view. Outline extraction was performed in counter-clockwise direction and started at theta ≥ 270° (3rd quadrant, just below of the x-axis. The data show the interpolated outlines at 250 equiangular steps, e.g. the angular increment is 360°/250=1.44°. The figure illustrates, that variations are highest in the upper and lower keel region.
Table 1 shows the variation of the resulting 30 outline curves as represented by the means of dX, dY, R, Ar, Phi1 and Phi2, and the respective 95% confidence intervals about

the means. The average vertical length of the shell in keel view (dY) for the 30 outlines, for example, is 1097µm. These data show, that the deviation from individual positioning and processing the images to outlines is minimal with respect to the size of the specimen.

Table 1

dX in µm	dY in µm	R=dX/dY	Ar in mm2	Phi_1°	Phi_2°
568.0 ± 1.2	1097.4 ± 4.7	0.518 ± 0.002	0.397 ± 0.002	70.1 ± 0.4	44.9 ± 0.2

b.) Analysis of the polar form of the outline

In order to better investigate the variation of shape due to different positioning the polar form of the interpolated (250 points) outlines was determined, e.g.

$$X_n = Rho_n * \cos Theta_n$$
$$Y_n = Rho_n * \sin Theta_n$$
$$Rho_n = SQRT(X_n**2 + Y_n**2)$$
$$Theta_n = arcta_n(Y_n/X_n)$$

This conversion into (Rho_n,Phi_n) coordinates was performed with program XY_to_PhiD1.out (for the listing see the appendix). For every single outline the polar coordinates were then imported into Excel and then the pairs sorted by Theta. In their polar notation each outline is represented by a "sine-like" curve as shown in Figure 3 for all 30 outlines. This figure shows the deviation of the microfossil shape from a circle (because a circle in that notation is represented by a horizontal line with the Rho-intercept being the radius of the circle).

This polar notation is advantageous because it allows to separately analyze the variation in ray length (Rho) and the associated angular argument (Theta) for every value of Theta, which is performed below.

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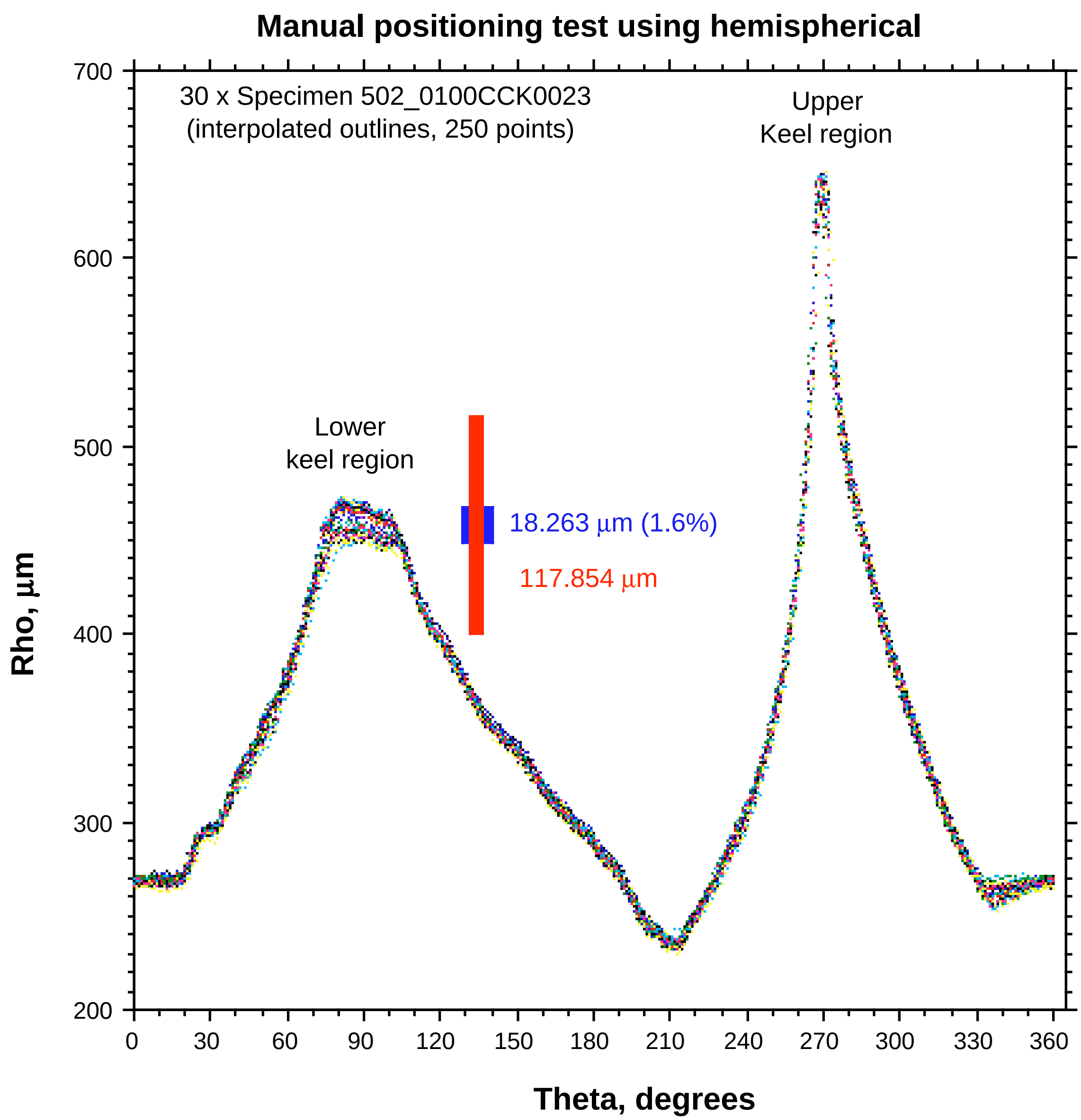

Figure 3
The 30 outlines represented by polar coordinates Rho,Theta

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c.) Mean variation and error in ray length (Rho)

Figure 4 shows the average values for Rho from the 30 experiments for every individual ray position (n=1 through 250).

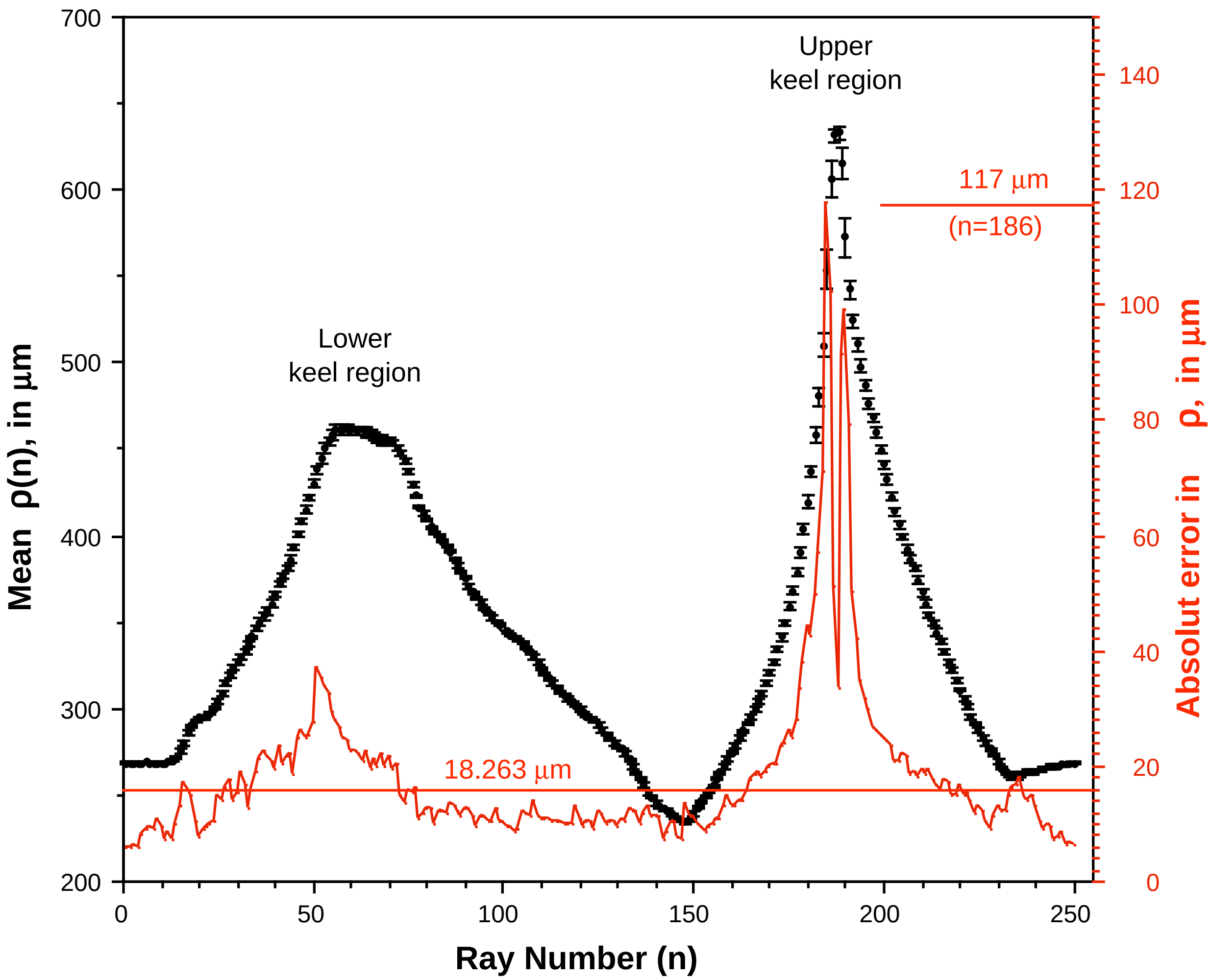

Figure 4
Variation of the mean ray length from the 30 outlines at every ray number (black curve). The black vertical bars indicate ±95% confidence intervals about the mean for every ray. The red curve indicates the absolute error in rho at every position of n. The absolute error of rho was calculated as follows:

$$[\mathrm{Max}(\rho_{1,n}, \rho_{30,n}) - \mathrm{Min}(\rho_{1,n}, \rho_{30,n})]_{n=1,...,250}$$

The red line indicates the mean error over the 30 outlines as a function of the position of the 250 rays (n or theta).

The 95% confidence interval about the overall mean for all rays is ±1.6503 µm, which is 0.5% of the mean ray length (=345.523 µm). The mean standard deviation over all rays is 4.6116 µm. The red horizontal line in Figure 3 indicates the average error (18.263 µm) over all rays from n=1 to 250. The maximum absolute error is 117 µm and occurs at the ray with n=186, which is close the theta=270° (e.g. in the lower keel region). These considerations show, that the manual position is in general quite precise, and that most variation in arriving at the correct keel position occurs within the keel regions of the shells.

d.) Mean variation in the angular argument theta

Theoretically, values of theta for the corresponding rays throughout the 30 outlines should show no variation at all. However, small deviations were observed from the ideal value. The ideal value of theta for every ray with the number n is calculated by $0° + (n-1)*1.44°$, with n ranging from 1 through 250. The reason for this variation is not fully clear, but it could result from the process of digitization of the outline from the binary black/white image with program Trace or the subsequent interpolation into equiangular points with program Sprep52. Figure 5 shows the value of Theta for each of the 30 outlines at every ray n.

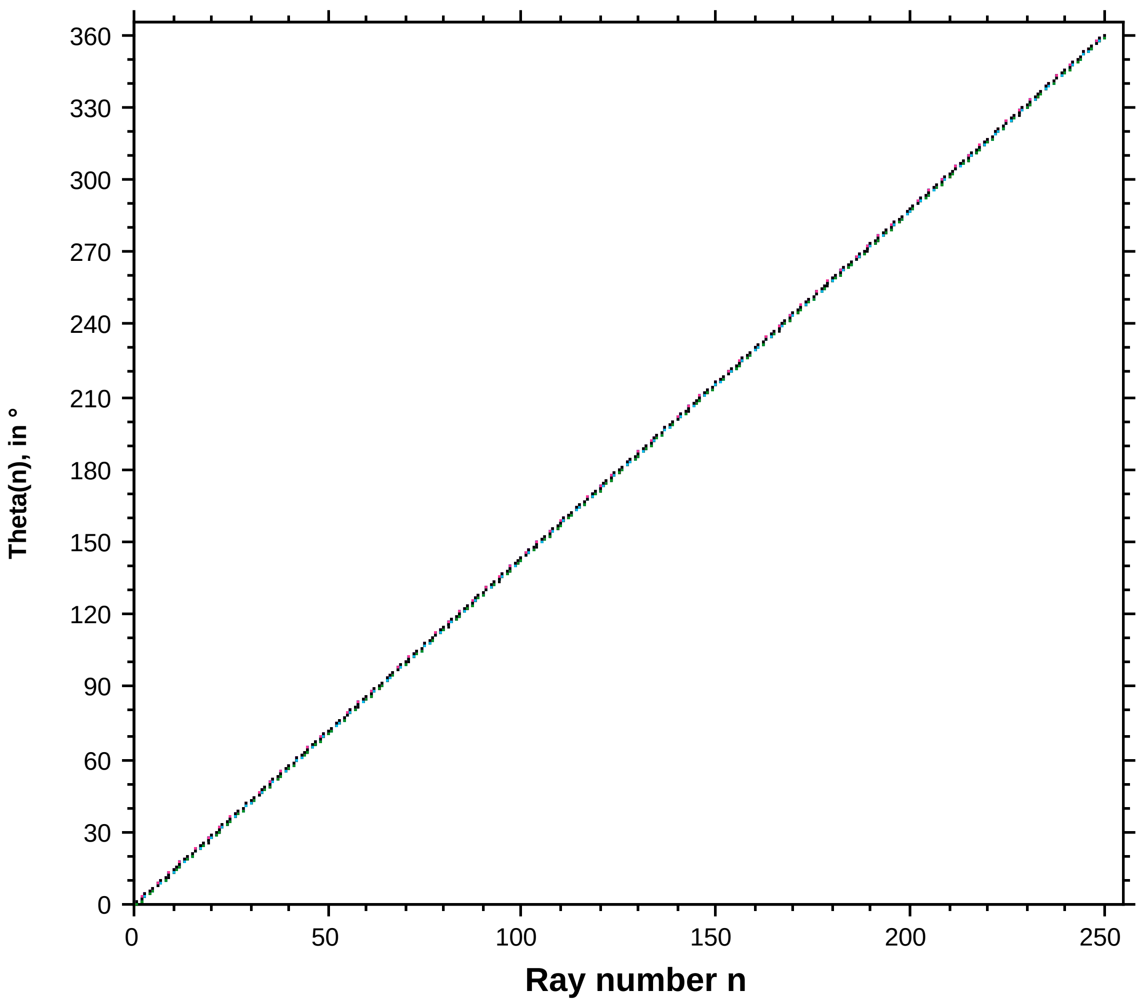

Figure 5. Theta for each of the 30 outlines at every ray n.

The overall 95% confidence interval about the mean for $Theta_1$ through $Theta_{250}$ from the 30 outlines is ±0.144°. The observed mean angular increment from one ray to the next is 1.44° as should be by theory. These observations show, that the variation of $Theta_n$ between outlines is really negligible as a source for error in shape variation of the outlines due to manual positioning of the shells.

e.) Summary

In conclusion, the analysis of the polar notation of the 30 test outlines provides an error box of 18.263µm in mean Rho and 0°±0.144° in the deviation of Theta around each averaged point of the digitized and interpolated outline. In comparison to the dimension of the shell (mean dY=1097µm), these variations are small (e.g. ≤ 1.66%) and are considered negligible.
The degree of variation depends on the position of the ray around the outline. The absolute variation is biggest in the upper keel region (average Rho_{186}=117.854µm) and lower keel region. This variation occurs to the trial of the operator to "wobble" the shell into the position with the highest areal extent of the shell.

References

ARNQVIST G. & MARTENSSON T. (1998). - Measurement error in geometric morphometrics: Empirical strategies to assess and reduce its its impact on measurement of shape. - *Acta Zoologica Academiae Scientiarum Hungaricae*, Budapest, v. **44**, N° 1-2, p. 73-96.

Appendix

```
      PROGRAM XY_to_PhiD
C
C     Version 1, by Michael Knappertsbusch, 4.5.2007
C
C     INPUT:
C     Given is a series of input files, each containing an outline represented by cartes
C     X,Y coordinates.
C     Also given is a list containing the names of these input files.
C
C     The program converts the coordinates of each outline into polar coordinates phi,rh
C     and writes each polar form into a separate file ending with _C (for converted).
C
C
      DOUBLE PRECISION PI,X,Y
      DOUBLE PRECISION RHO,PHIR,PHID      ! PHIR in radians, PHID in degrees
      CHARACTER*20 LIST       ! Name of list with input filenames
      CHARACTER*19 INPUT            ! Names of input files
      CHARACTER*21 OUTPUT           ! Names of output files
      CHARACTER*1 TAB
C
      PARAMETER(PI=3.14159265)
      TAB=CHAR(9)             ! Tabulator, only valid on Macintosh computers.
C
C
      WRITE(9,*) '*************************************'
      WRITE(9,*) '*                                   *'
      WRITE(9,*) '*        Program XY_to_PhiD         *'
      WRITE(9,*) '*                                   *'
      WRITE(9,*) '*    Conversion of cartesian to     *'
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```
      WRITE(9,*) '*         polar coordinates       *'
      WRITE(9,*) '*                                 *'
      WRITE(9,*) '* Version 1, by M. Knappertsbusch *'
      WRITE(9,*) '*           4.5.2007              *'
      WRITE(9,*) '*                                 *'
      WRITE(9,*) '***********************************'
C     Input file handling:
C
      WRITE(9,*) '. . .Enter name of list with input file names'
      WRITE(9,*) '           (max. 20 chars)'
      READ(9,1) LIST
 1    FORMAT(A20)

      OPEN(13,FILE=LIST,STATUS='OLD')
 3    READ(13,5,END=99) INPUT
 5    FORMAT(A19)
C
C     Determine corresponding name of output file from input file name and open output f
C
      OUTPUT=INPUT//'_C'
      OPEN(14,FILE=OUTPUT,STATUS='NEW')
C
C
C     Now, opening INPUT file and reading data from it:
C
      OPEN(16,FILE=INPUT,STATUS='OLD')
 10   READ(16,*,END=88) X,Y
C
      CALL POLAR(X,Y,RHO,PHIR)
C
C     Convert PHIR from radians into degrees:
C
      PHID=PHIR*180.0/PI
C
C     Write polar coordinates PHID,RHO to screen and to output file:
C
C     WRITE(9,70) PHID,TAB,RHO
      WRITE(14,70) PHID,TAB,RHO
 70   FORMAT(F5.1,A1,F8.3)
      GOTO 10
C
 88   REWIND(16)
      CLOSE(16)
      GOTO 3
C
 99   WRITE(9,*) 'All files converted'
      PAUSE 100
 100  CONTINUE
c
      STOP
      END
```

```
C***************************************************************************
      SUBROUTINE POLAR(US,VS,RS,THETAS)
C***************************************************************************
C
C     Subroutine taken from program SPrep52.f
C
C     Converts cartesian coordinates US,VS into polar coordinates
C     RS and THETA. Origin of local coordinate systems is at 0,0.
C     The calling unit gives US and VS as input. RS and THETAS are
C     returned to the calling unit. THETAS in Radians.
C
      DOUBLE PRECISION US,VS,RS,THETAS
      DOUBLE PRECISION ARG,PI
C
      PARAMETER(PI=3.14159265)
C
      RS=SQRT(US**2+VS**2)
C
C     First special cases:
C
      IF (US.GT.0.AND.VS.EQ.0) THEN
        THETAS=0
      ELSE IF (US.EQ.0.AND.VS.GT.0) THEN
        THETAS=PI/2
      ELSE IF (US.LT.0.AND.VS.EQ.0) THEN
        THETAS=PI
      ELSE IF (US.EQ.0.AND.VS.LT.0) THEN
        THETAS=3*PI/2
      END IF
C
C     For all other cases:
C
      IF (US.GT.0.AND.VS.GT.0) THEN
        ARG=ABS(VS/US)
        THETAS=ATAN(ARG)
      ELSE IF (US.LT.0.AND.VS.GT.0) THEN
        ARG=ABS(US/VS)
        THETAS=ATAN(ARG)+PI/2
      ELSE IF (US.LT.0.AND.VS.LT.0) THEN
        ARG=ABS(VS/US)
        THETAS=ATAN(ARG)+PI
      ELSE IF (US.GT.0.AND.VS.LT.0) THEN
        ARG=ABS(US/VS)
        THETAS=ATAN(ARG)+3*PI/2
      END IF
C
      RETURN
      END
```