

An SEM Analysis Of Amphipleura pellucida with New Findings

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Introduction

For many years, as long ago as 1872[1], the Amphipleura pellucida (Ap) diatom striae and punctae have been an intense object for measuring the quality of resolution of the light microscope (LM). More recent publications have also referenced Ap and the difficulty of resolving its punctae[2]. Thus, from 1872 to 1947, much attention was directed towards Ap but without paying much attention to its detailed valve structure. This was probably due to lack of resolution of LM to reveal the details for the Ap punctae (pores). Work by Stoermer and Pankratz did TEM analysis of Ap in 1964. While their results and mine do not agree, this could be attributed to specimen preparation and the source of the diatoms. However, there is an heretofore unresolved issue about the physical differences of Ap from different geographic locations. My current work should resolve this issue. This started as a challenge about whether Ap could be selectively oriented inside up or inside down.

Earlier SEM analysis of Ap was done by Rene with a Cambridge SEM in the 1980 timeframe[3],[4]. My currently reported work on Ap specimens from the UK and from Mallorca, images and records the resulting values of pitch (horizontal and vertical dimensions from pore centers) and pore dimensions. In addition to obtaining good measurements, the dimensions from the UK and Mallorca diatoms were fundamentally the same. Thus, there does not seem to be a significant difference in Ap characteristics from different locations. Consequently, the TEM and current SEM differences in results remain an unresolved issue.

My new work also shows that there is a definite perpendicular orientation between pores on the concave (inside) and convex (outside) sides of the diatom and why Ap is a good resolution standard for SEM. It also shows why it is not a significant or useful standard for LM. Furthermore, it shows that the dimensions of the outside and inside pores are significantly different. The measured dimensions of the outside pores strongly suggest that only deep UV LM will be able to resolve their details. UV is required to resolve the striae into individual dots or pores. For more detailed observation, such as of the pores, SEM is necessary[8].

In the context of this paper, the linear axis is taken to be that of the longest dimension of the diatom.

Discussion

Three sets of diatoms were obtained from Klaus Kemp[6]. The first set shown in Figure 1 are all of the concave face of the UK diatom. The second set shown in Figure 2 are all of the convex face.

The specimens were prepared by Klaus Kemp without mounting medium. The diatoms were placed on the top of a cover slip which was then attached to a 12mm SEM pin stub using a sticky tab and sputter coated with Pd. These were then imaged in a Zeiss Supra 55VP using the in-lens detector (10KV, 30μ, low current, 4mm WD).

The ability to resolve the pores or a pore of Ap even with high NA objectives and an aplanatic condenser is frustrated by the extremely small pitch between pores. This is shown in Figure 3.

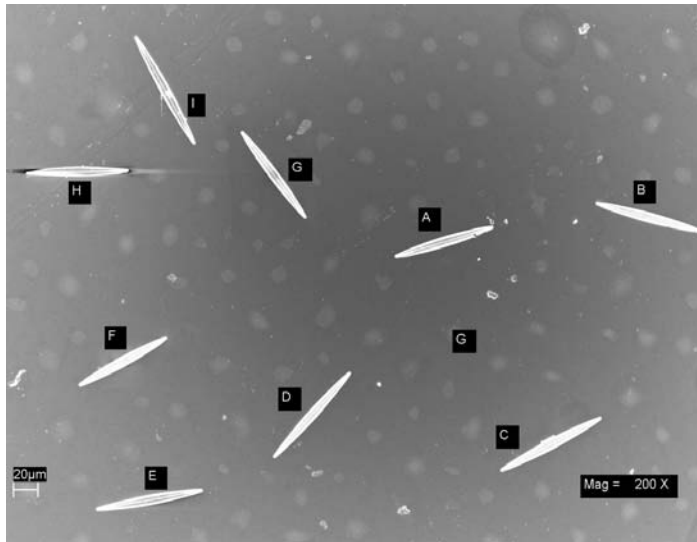


Figure 1: Field view of concave face of UK diatoms

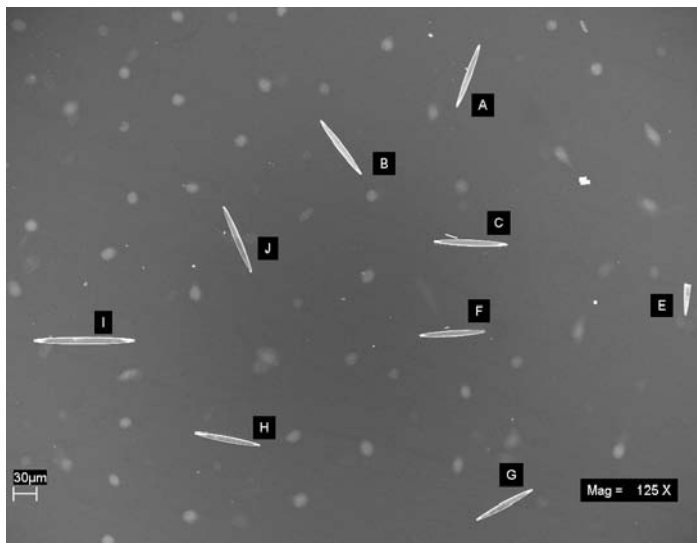


Figure 2: Field view of convex face of UK diatoms

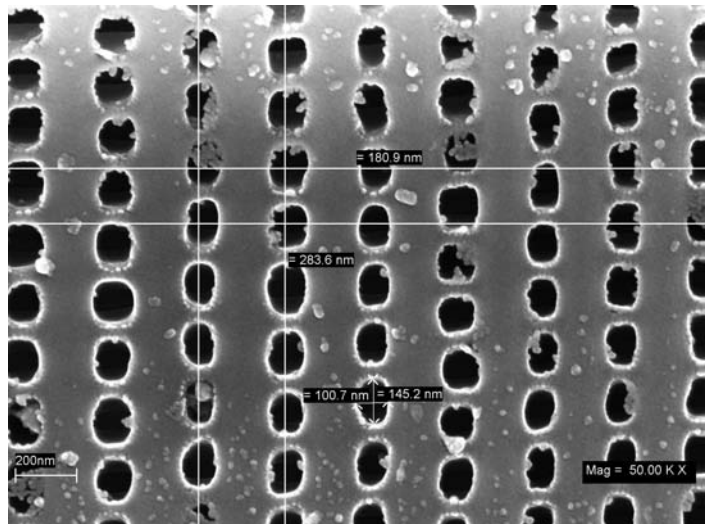


Figure 3: Horizontal and vertical pitch (Diatom F) showing the inside view of the valve

From this figure, one can see that the basic pitch is 280nm by 180nm. Consequently, only UV LM would be able to resolve striae into pores. Choosing the 180nm pitch, to resolve this according to

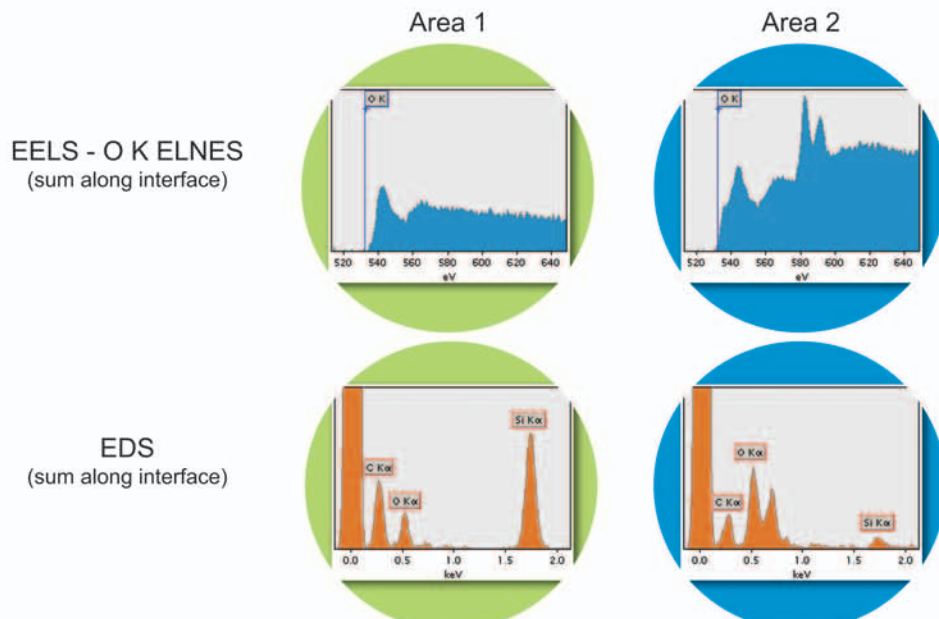
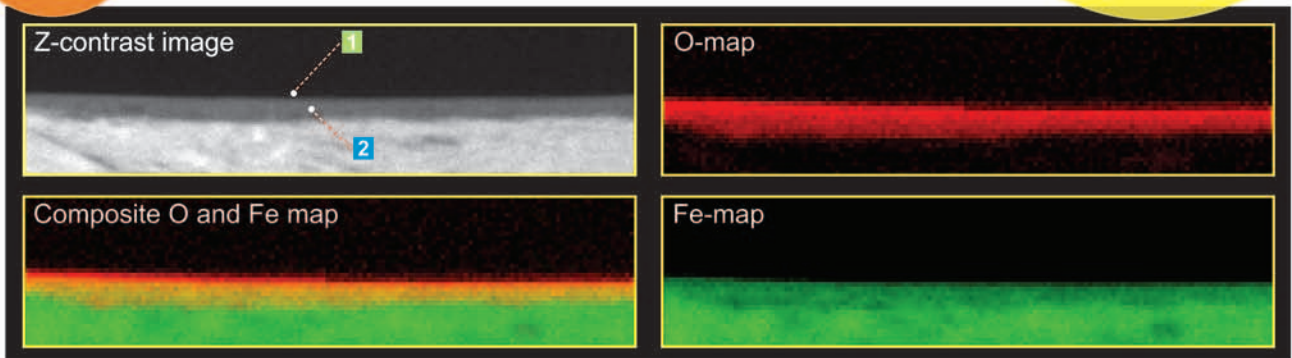
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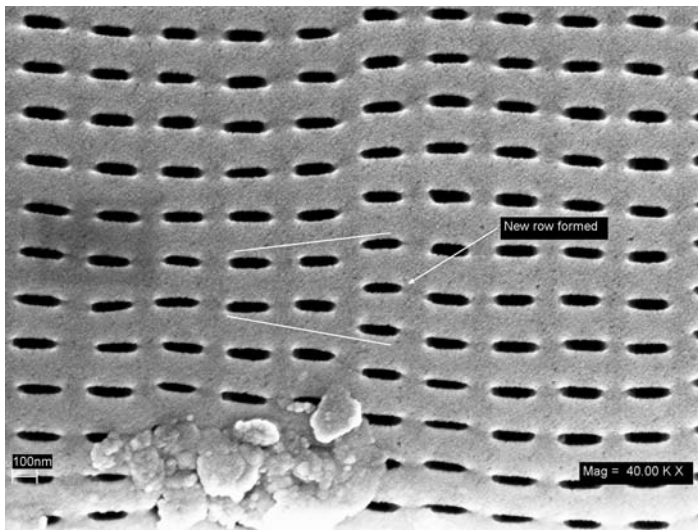


Figure 4: Offset in the rows of pores on the convex side of the diatom's valve

the Raleigh Criteria with NA=1.4 would require a Lambda of about 400nm. This resolves the pores as points but does not resolve their detail. Consequently, "resolution" takes on a whole new meaning with these very small feature size specimens. This directly extends to resolving minute details of modern day microcircuit devices. It further engages the evolving area of nanotechnology[9].

If one images the opposite (convex) side of the diatom, the dimensions of the pores are dramatically different as seen in Figures 5 and 6.

Figure 4 shows the growth of a new set of pores that are offset by a linear set of pores that diverge. Our materials science colleagues will recognize the extra row of pores as a biological analog to an end-on view of a crystallographic edge type dislocation.

The other interesting observation is that the pores from inside to outside are not parallel to one another but rather perpendicular.

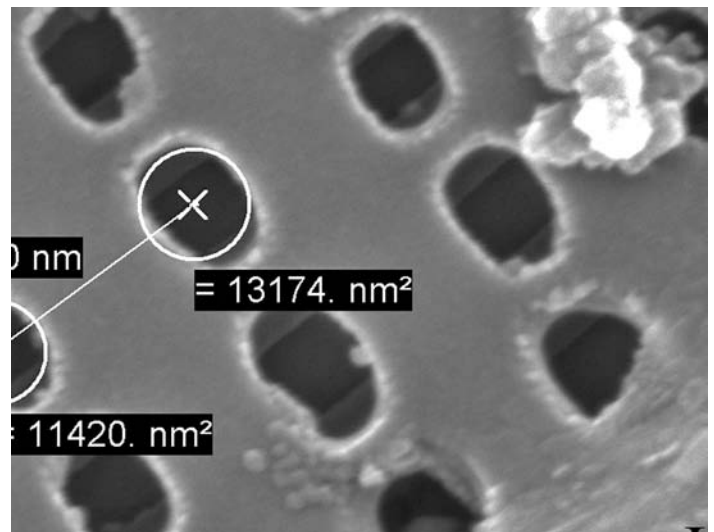


Figure 7: View from inside of valve to outside of valve. The hint of this can be seen in Figure 7.

Looking carefully into this side's pores, one can see the opposite structure which is smaller and perpendicular to the inside punctae. As discovered, the pores do not have parallel walls but rather are 90 degrees rotated and concave from inside to outside. This is graphically illustrated in Figure 10. From this figure, the factor of "rotation" actually means that the long sides of the rectangular punctae on the concave face are perpendicular to the longitudinal axis while the pores on the convex (outer) face are parallel to it.

Therefore, the outside pores are about 30nm x 100nm rectangular, while the inside pores are about 100 x 145 nm. These pores overlie each other and are separated by 290 nm along the long axis of the diatom and by 230 nm in the direction perpendicular to it.

For the outside pores measuring about 30nm x 100nm rectangular, Raleigh Criteria would require Lambda of about 60nm. Consequently, one might be able to image and possibly resolve the inside of the diatom if they knew that this was the face that they were viewing since these pores are larger. However, if one were viewing the outside of the diatom, it would be a huge challenge (impossible?) for LM--even at deep UV. The only other known method is flattening of the diatom when placed on the cover slip[7].

Using Raleigh's Criterion for a NA of 1.4 one needs a wavelength of 527 nm to resolve the rows of pores spreading perpendicularly from the longitudinal axis into striae and 500 nm to separate the striae into individual pores. The term striae is used because it is difficult to separate the pores in a direction perpendicular to the axis and the pores appear as fine lines rather than as series of dots in most observations. Separating the striae into dots is more difficult because it requires a shorter wavelength and significant enhancement of contrast.

The above measurements were shown on one diatom. The dimension tables are for an average of at least five individual diatom pores. It is significant that measurements on several diatoms provide dimensions which differ and provide additional explanation why it is more difficult to separate striae into dots, than to separate the striae from one another. For this, one needs to look at the variability of pitches measured on different individuals of Ap. Table 1 provides such an overview. It gives the distance between striae and the distance between pores measured along the striae for different diatoms depicted in Fig. 1.

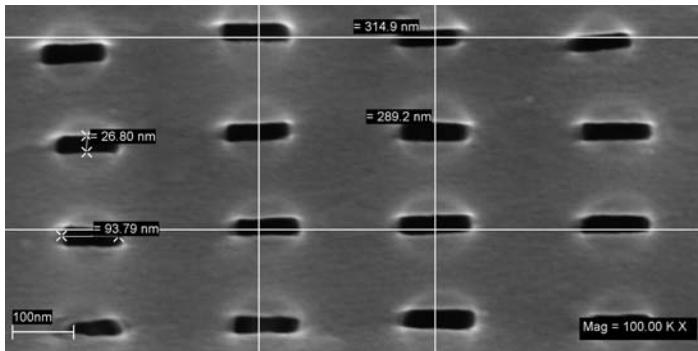


Figure 5: Outside of diatom A from Figure 2 with dimensions

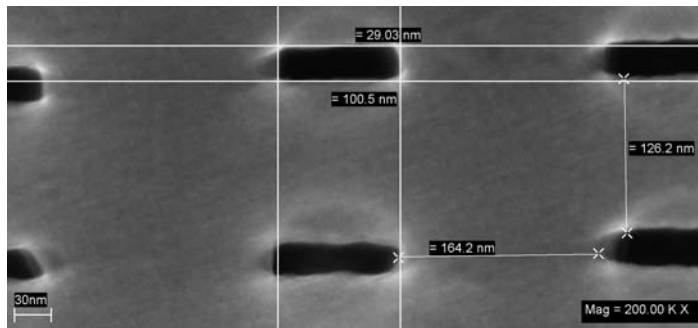
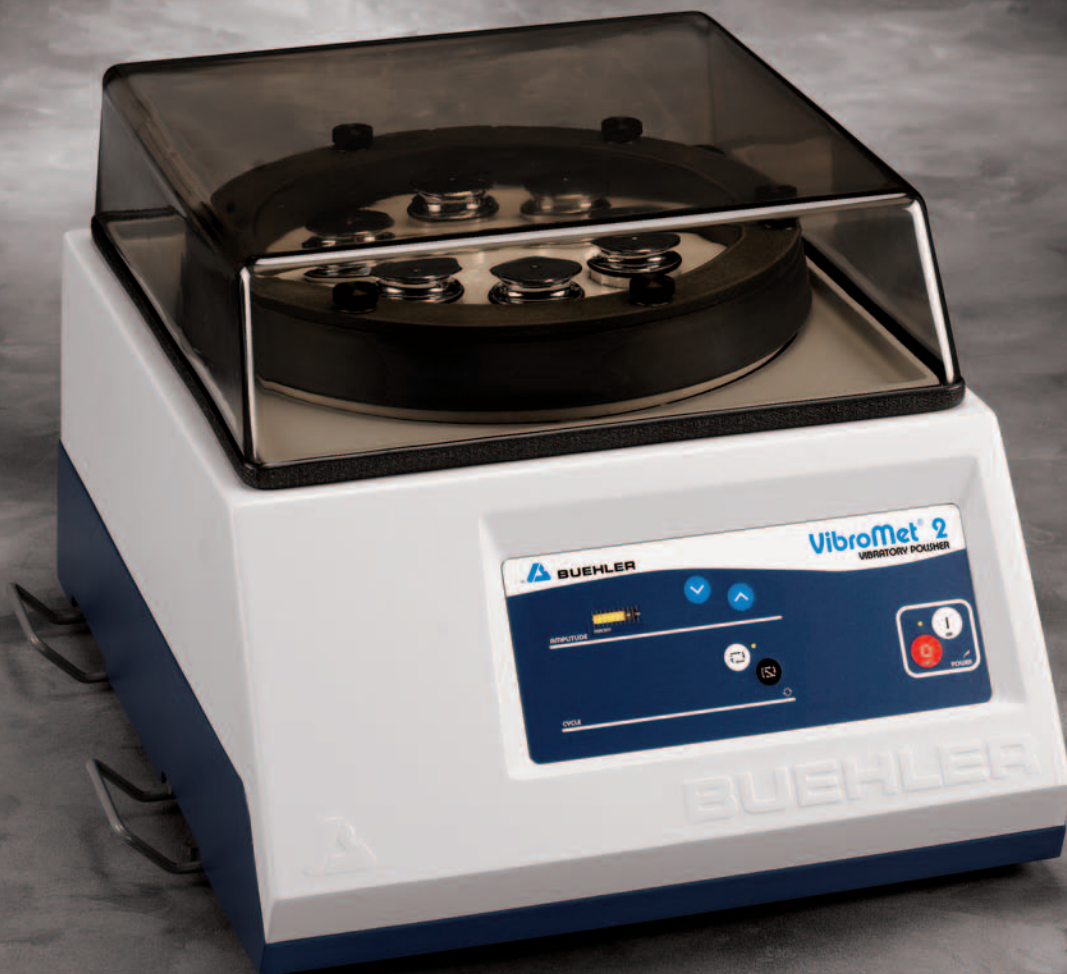


Figure 6: Outside of diatom A from Figure 2, with different portion being dimensioned



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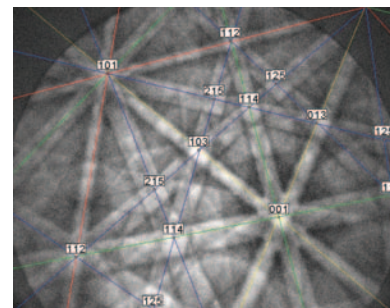
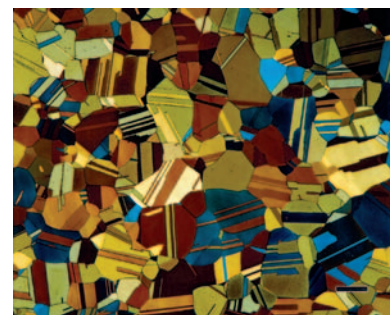
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Table 1: Overview of the variability of UK Diatom parameters of Figure 1 (concave faces)

Diatom ID	Distance between striae (nm)	Distance between pores (nm)	Pore dimensions (nm)
A	271	200	135x107
D	263	184	146x111
E	280	173	plugged
F	285	185	136x112
I	270	178	137x102

Table 2 lists the same type of measurements for the convex face.

Table 2: Overview of the variability of UK Diatom parameters of Figure 2 (convex faces)

Diatom ID	Distance between striae (nm)	Distance between pores (nm)	Pore dimensions (nm)
A	286	161	107x30
B	272	175	102x40
F	267	163	111x44
G	269	179	plugged
I	267	182	106x32

One sees that the distance between striae is consistently larger than the distance between the pores along the striae and that the latter did vary from a high of 200nm to a low of 161 nm for the concave side. But the convex face varied much less. The distances between the striae are such that the wavelength required to separate them fall within the range of UV LM. The distance between the pores along the striae however may be large enough for wavelengths in the visible range, but may also be so small as to require wavelengths of only 340 nm. This variability of the distances between the pores might explain why some microscopists are able to resolve the striae into dots or pearls while others have been unable to do so. SEM

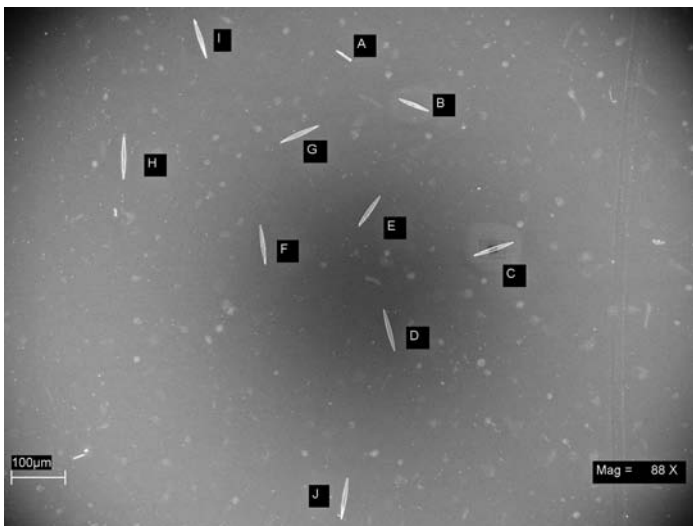


Figure 8: Ap from Mallorca (convex side)

does provide the answer for this apparent discrepancy.

Figure 8 shows the collection of diatoms that originated from Mallorca. The purpose of examining these diatoms was to determine if Ap from one locale were different from Ap from another locale. Table 3 lists the data for the Mallorca diatoms.

Table 3: Mallorca Diatom Dimensions (convex face)

Diatom ID	Distance between striae (nm)	Distance between pores (nm)	Pore dimensions (nm)
B	275	189	165 x 49
C	266	193	plugged
D	278	192	126 x 40
E	279	179	122 x 42
F	285	193	123 x 42
G	278	187	122 x 39
H	277	188	plugged

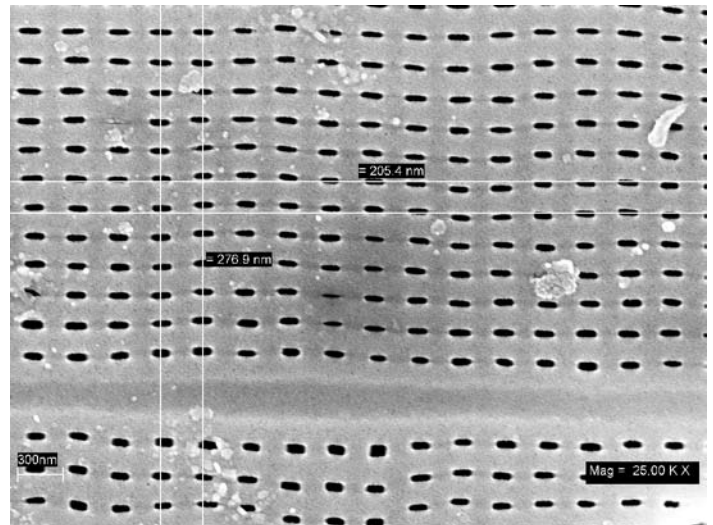


Figure 9: Ap Mallorca diatom F

Based on overall observation of the data, Figure 10 shows the basic structure of a punctae.

The inside and outside of the diatom are remarkably different. Figure 11 shows the outside view while Figure 12 shows the inside. These were taken at 30 degrees tilt, high current.

Conclusions:

Amphipleura pel-lucida is a very difficult diatom to image. This writer does not think that LM users would disagree

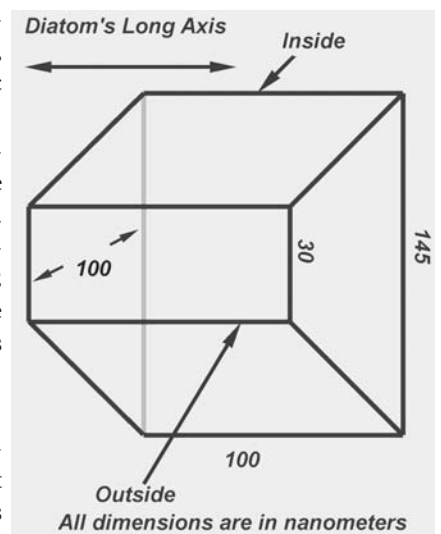


Figure 10: Basic dimensions of diatom punctae

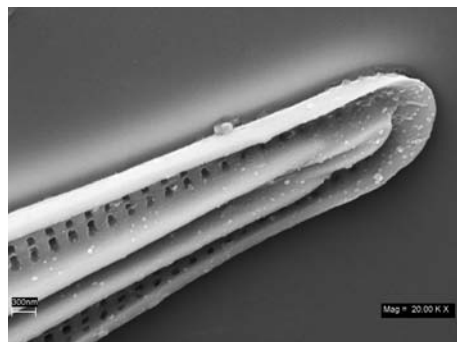
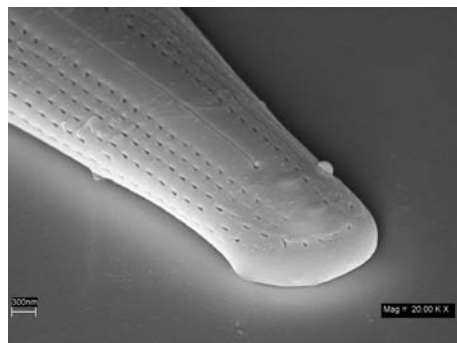


Figure 11 top: Outside view,
Figure 12 bottom: Inside view

with this statement. However, past SEM analysis of Ap has not been all that clear. It is hoped that these new images will provide clarification of the Ap morphology and perhaps close the book on studying Ap. If not, what are the other factors to consider? For environmental, Tungsten filament and LaB6 thermionic SEMs, Ap can be a low cost means of gauging resolution. For high resolution FE-SEM instruments, it can also be a valuable measure of resolution since these specimens, when coated, tend not

to become as contaminated with hydrocarbons that afflicts traditional Au on C standards. Additionally, the diatom specimens are much less costly. The small dimensions of Ap also reveal difficulties in EM alignment and stigmation.

The determination of pore area, in this author's opinion, helps to semi-quantitatively explain the poor contrast of Ap when observed with the LM. If there is agreement that the contrast is related to how much light gets through the portion of the diatom area with pores compared to what comes through the diatom areas without pores, then the contrast for 8% open areas would only be about 4%. Analysis of diatoms with plugged pores using ImageJ concludes that internal pores occupy between 19% to 23% of the diatom area in the absence of plugging while external pores occupy between 6% to 7% of the diatom in the absence of plugging. For the total number of diatoms imaged, plugging can reduce the number of open pores to as little as one third of the surface area or less.

Use of light of short wavelength will only permit the separation into dots, but not a determination of pore shape. To obtain an approximate image of the shape of the pores one must discern the very unequal two dimensions of the outer pores, which measure some 110 nm along the long axis of the diatom but only some 30 nm in the direction perpendicular to the long axis. A wavelength smaller by a factor of 30/279 than 550 nm would have to be used. The resulting wavelength of only 59nm is outside the range of what can be resolved with light microscopy and SEM must be used instead.

Another interesting finding is that the diatoms were very difficult to image using E-T SE. The most effective imaging was accomplished using the in-lens detector. The reason for this is probably due to lack of contrast between the SiO2 diatoms and the glass cover slip. Further work would be to examine Ap according to the criteria established by Hildebrand and Palenik[11] for applications to nano-technology. Additionally, this would include application to self-assembly of nano-structures for military applications as well as other potential uses. ■

Acknowledgements:

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