Microstructure Physics and Metal Forming
Prof. Dr. D. Raabe

Influence of Sample Preparation and Anisotropy on Lobster Claw studied by LOM, SEM and TEM

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INTRODUCTION

The exoskeleton (cuticle) of all arthropods is composed of chitin organized in fibrils embedded in a matrix of various proteins. In addition, the crustacean exoskeleton contains significant amounts of inorganic salts, mainly calcium carbonate [1].

The architecture of cuticle is helicoidal and most probably this building plan is responsible for its extraordinary mechanical, thermal and physiological properties. In this helicoidal structure, chitin is in the form of crystalline filaments and proteins play the role of the matrix. Although chitin is a simple polysaccharide, the second constituent of cuticle, proteins, present a great variety [2].

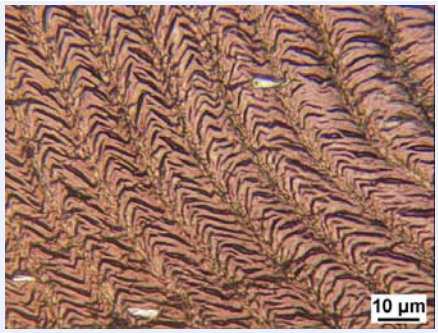
The cuticle consists of three layers: epicuticle with functional properties, exocuticle thicker than the epicuticle, hard and durable and endocuticle which has similar structure than the exocuticle. Both exo and endocuticle play a mechanical role [3,4].

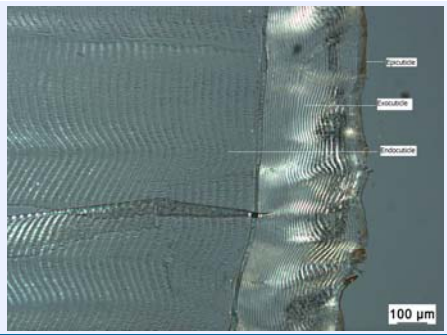
SAMPLE PREPARATION

1. POLISHED SAMPLES. Embedded in cold mounting resin and polished with SiO_2. Coated with a thin layer of gold except the 3D imaged sample which was Au-20Pd coated..
2. TRANSMISSION LIGHT SAMPLES. Sections of 5 µm using a rotary microtome Leica RM 2165.
3. TRANSMISSION ELECTRON MICROSCOPY SAMPLES. Fixed with glutaraldehyde, decalcified with EDTA and stained and fixed with OsO_4. Final staining with uranyl acetate and lead citrate for contrast. Afterwards, samples were ultramicrotomed to 60 nm.
4. FRACTURED SAMPLES. Loads applied to the material until breaking.

MICROSCOPES

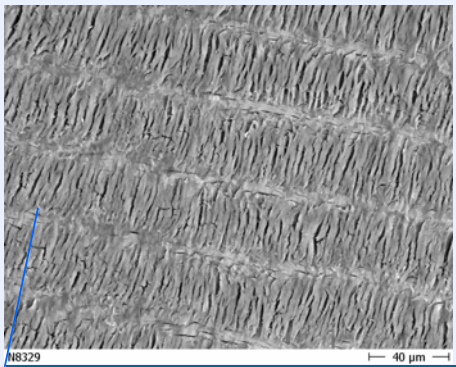
- LIGHT OPTICAL MICROSCOPY. Leica DM 4000B with normal and transmission light.
- SCANNING ELECTRON MICROSCOPY. CamScan 4 and Hitachi s-4700 Field Emission SEM fitted with an Autrata Yttrium Aluminium Garnet (YAG) backscattered electron (BSE) detector.
- TRANSMISSION ELECTRON MICROSCOPY. Hitachi H600 fitted with a Gatan camera.

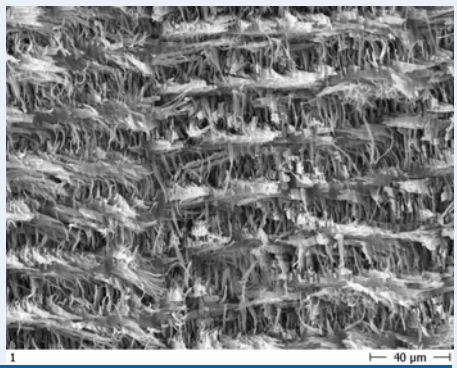




Light optical micrographs of a cross-sectional cut, polished material (left) and a 5 µm thickness cut (right)

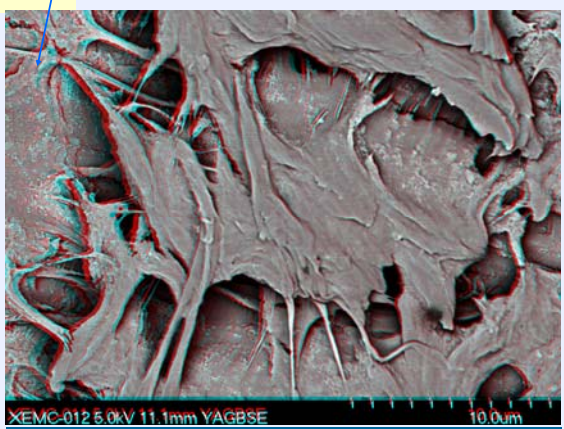
The hierarchical organization of the material is visible already with optical microscopy. In decapods, this level is referred to as *twisted plywood* or *Boulingand pattern* [5]. The horizontal layers consist of bundles of chitin microfibrils and superimposed series of fibril arcs.





Scanning electron micrographs of a cross-sectional cut, polished material (left) and fractured material (right)

The intermediate level below the *Bouligand pattern* is visible in the field of SEM. It reveals a densely organized woven structure consisting on groups of highly oriented crystalline α-chitin fibrils. The polishing has smoothed out the surface, making visible only the arrangment in layers. However, the fractured material clearly reveals bundles of oriented fibers.



Stereoscopic field emission scanning electron micrograph (red: left eye; green: right eye) of a cross-sectional polished sample (*)

The stereoscopic image shows a highly three-dimensional structure where fibers as well as massive amounts of material are visible.The micrograph is a detail of the image n° N8329, after penetrating in the cracks let in the surface by the polishing as it is indicated by the arrow.

(*) Image taken by Mrs. L.C. Baxter. Institute for Biological Sciences, The University of Wales, Aberystwyth, Wales

CONCLUSIONS

• Lobster cuticle presents a pronounced hierarchical structure.

• Depending on the direction of the cut, the structure reveals horizontal layers with a waving pattern (when cross-sectional cuts) or a peacock feather-type pattern (when surface-parallel cuts).

• Already at light optical microscopy level, the three layers of the cuticle, epi, exo and endocuticle can be observed.

• It is necessary to prepare correctly the samples by chemical fixation and decalcination to find out where the mineral can be kept.

LITERATURE REVIEW

[1] M. Nousiainen, K. Rafn, L. Skou, P. Roepstorff, S. O. Andersen. Comp. Biochem. Physiol. Vol. 119, No. 1, pp. 189-199, 1998.

[2] http://bioinformatics.biol.uoa.gr/cuticleDB.

[3] F. J. Vernberg and W. B. Vernberg. The biology of the crustacea. Academic Press, New York, USA. 1983.

[4] J. F. V. Vicent. Structural Biomaterials. Princeton University Press, USA. 1990.

[5] Y. Boulingand. Tissue and Cell. Vol. 18, pp. 621-643, 1986.

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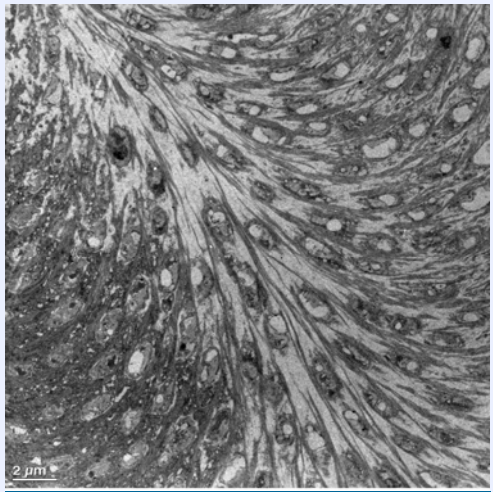
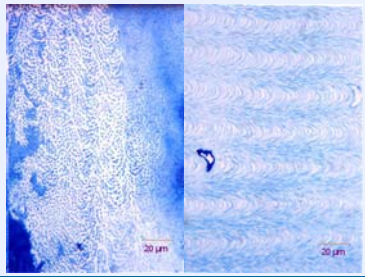
These sections of 1µm thickness show the difference on the arrangement of the chitin-protein matrix. While the cross section shows horizontal layers of chitin-protein bundles, the parallel to the surface cut shows a peacock feather look-a-like structure



Transmission electron micrograph of a surface-parallel cut



Light optical micrographs of a cross-sectional cut (right) and a surface-parallel cut (left)

The TEM image shows in more detail what was observed with optical microscopy. The structure resembles a feather of the peacock tail. Bundles of chitin-protein fibers act as a scaffold that secures calcite clusters.