Toward a modern interpretation of the American lobster shell using X-ray tomography

From: Joseph G. Kunkel, Melissa Rosa and Ali Bahadur

The American lobster shell has been studied as a material structure by many investigators using various techniques. Older studies were typically 2-dimensional using light, electrons, electron generated X-rays and Raman and FTIR spectroscopy to analyze the density or chemistry of thin sections or polished surfaces of cuticle specimens. These 2-D views have provided a basis of numerous theories of the lobster shell structure which have been reviewed and modeled (Nikolov et al. 2010; Kunkel, Nagel & Jercinovic 2012; Kunkel & Jercinovic, 2013; Kunkel, 2013). The prior analytic techniques are informative about structure and chemistry but, being inherently two dimensional, are time and labor intensive, taking many days of sample preparation while not providing a 3-dimensional view of structure. Those types of data collection from individual specimens take long valuable operator and machine time. This delay in sample preparation and data gathering often inhibits the ability to perform a time series within an animal, or results in small and limited sample sizes. These results are due to the practicality of operator-time, effort and machine-time, thus limiting a comprehensive understanding of shell development. None of these previous analytical techniques have allowed us to study some of our most pressing problems about the role of the mineral and polymer structure during the changing stages and different phases of the molting cycle. Now, a new instrument is available that fits many of the needs for rapid analysis of the complex structure of the lobster shell. As it has done in human medicine, X-ray tomography allows structures to be viewed and interpreted in 3-dimensions. This approach is now available on a micro scale and can be applied at the dimensions of interest in lobster cuticle structure. The technique of micro-CT scanning produces massive amounts of data, which can be both a curse and a blessing. For instance a 6 mm cube sample is the largest one that we can practically scan and achieve 0.5 um resolution densities of the component cubes, called voxels. But a 6 mm cube produces 1.7×10^{12} voxels (the 3-D analog of pixels) of density, a formidable amount of data to analyze. However, this amount of data is a blessing in that it can be selectively analyzed *post facto* of gathering, similar to how an MRI allows medical analysts to see all sorts of unsuspected issues in the human spine or surrounding tissue. A production grade machine, the Bruker **Skyscan 1272**, could analyze 2 dozen cuticle samples overnight at 0.5 μm resolution. Samples need no special embedding or fixation. We excise 6 mm cuticle medallions with a coring drill and the hole is repaired with a plastic sheet epoxied to the rim, Fig. 1, allowing further timed samples following development.

Figure 1. Producing medallions of lobster cuticle from a single or multiple lobsters. **A.** Lobster carapace with drill cored medallion removed and fixed with a clear patch applied to the remaining hole (picture after 4 months). **B.** Five 6 mm medallions after a freeze fix in acetone can represent replicates or sequential samples from one animal in time or space.

Our cored medallion of the carapace was plunged into -50º C acetone to freeze fix and dehydrate the medallion. It is ready in a day to be placed into the **Skyscan 1272** in its carousel auto-sampler that provides unattended X-ray tomography measurement of all samples overnight. As with MRIs, unfixed, live samples are possible! If 24 medallions were scanned 6 mm diameter by 1 mm thick it would produce 3.4 x 10^{11} voxels of data. The challenge is to develop approaches to study that amount of data efficiently. Bruker has developed software for handling such data in general and we have further developed R-scripts for further analyzing the data to make it intelligible to lobster scientists. Fig. 2 illustrates one small 1000 x 1000 x 1000 = block of 10^9 voxels developed using NIH ImageJ and our R-scripts that interpret a span a cuticle thickness, outer epicuticle to the inner epidermal surface, including 2 primary organules, with their canals running from the surface down to the gland cell (not shown) in the epidermis. The entire 4700x 2024x 3100 pixel original data set can be viewed as a movie of slices in any of the 3 dimensions, or rotated and viewed at any angle using free software from Bruker.

Our sample analytic-interpretation of a small volume of the data in Fig. 2 illustrates discovery of several new structural features missing from prior models of the lobster cuticle (Kunkel, Nagel & Jercinovic 2012; Kunkel & Jercinovic, 2013; Kunkel, 2013). Other analyzed volumes of this same intermolt cuticle medallion of Fig. 2, not shown here, illuminate additional new features of secondary and tertiary organule architecture. The newly revealed structures will need to be coordinated with information from other physical techniques which address the chemical nature of the structures. So, as in human health and mouse research, micro-CT potentially provides a wealth of new information about lobsters. The genesis and development of these newly described structures need to be correlated with our knowledge of the molting process, such as the diagnostic regional softening of the cuticle in the approach to molting (Waddy et al. 1995), and, as well, demand changes in the *ab initio* models of the lobster-shell composite structure (Nikolov et al. 2010).

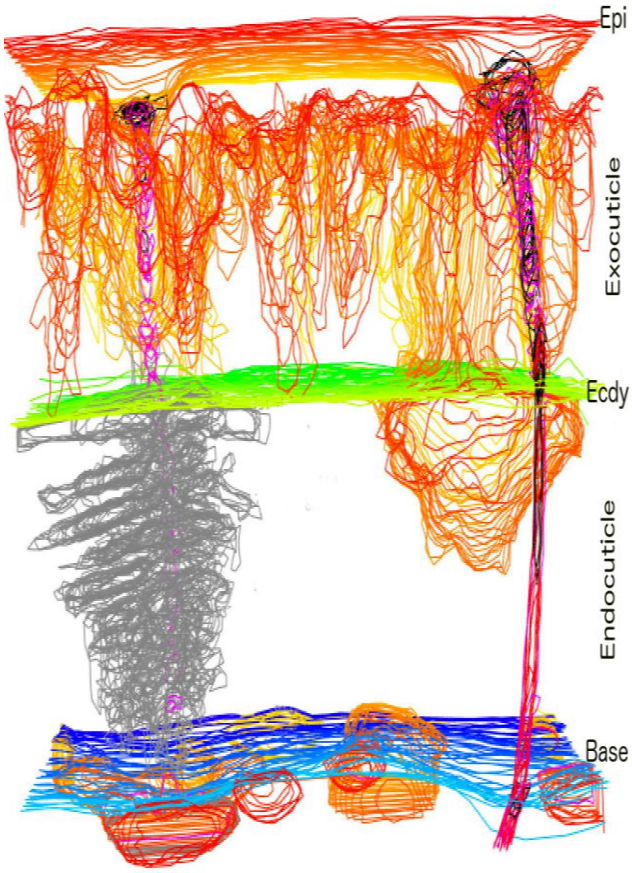

Figure 2. Select contours interpreted from 3D micro-CT voxels of density of Ca CO_3 forming stalactite-like (orange) structures extending down from the surface epicuticle (Epi) into the exocuticle. Black and red outlined canals of two primary organules reach from depressions in the surface to end below the Base layer of the endocuticle (blue). Grey whorls of Ca CO_3 form aligned with the spiral of 'twisted-plywood' chitin lamellae but in groups that suggest they are associated with each epidermal cell. Features with Ca CO_3 like density protrude through the ecdysial line (Ecdy) creating yet another departure from regular structure. Additional formed deposits of dense mineral are accumulated below the Base inner margin of the endocuticle. How do these structures change during the molting cycle? How do they relate to the progress of shell disease? How do they respond to ocean acidification? These questions can only be answered by an easily applied and rapid measurement such as microCT.

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Whale Interactions: Industry adaption to a major social issue

[Abstract of a presentation to the Trans Tasman Rock Lobster Congress 2015]

From: Jason How

The western rock lobster fishery (WRLF) has recently transitioned to a quota-based management system and has consequently seen a progression to year-round fishing as a result of effort-control restriction removal. This has seen fishing now overlapping more with the months when humpback whales (*Megaptera novaeangilae*) migrate along the west coast of Australia. The population of humpbacks which migrate along the West Australian coast from May to November has experienced a large increase in population size. The current population estimates are thought to be over 30 000, although debate exists around the accuracy of this estimate. There is however general agreement that this population is increasing at a rate of about 10% per annum, and hence posing an ever increasing risk of interactions with commercial fishing gear.

The Federal government placed a number of conditions on the Western Rock Lobster Fishery (WRLF) to reduce whale interactions, and this was coupled with pressure from the state regulatory authority (Department of Fisheries Western Australia). Interactions between humpback whales and WRLF gear involve entanglements and other contact with fishing gear. All levels of government acknowledge that WRLF gear interactions with humpback whales do not pose a risk to recovery of the humpback-whale population. Rather they wish to address the social and ethical issues relating to their interactions.

A number of research projects commenced to inform management of options that could be implemented to reduce whale interactions. The primary goal of research was to compile all available information on whale interactions and humpback migration and to test a number of possible gear modifications suggested by industry. The move to year-round fishing had an added financial bonus of between $50-100 million to the WRLF, and therefore options to maintain fishing in the presence of an increasing whale population were examined.

Gear modifications were introduced in June 2014 and were implemented until the end of the whale migration “season” (15 November 2014). Modifications in 2015 were implemented prior to the whale migration (1 May) and conclude on