Joseph G. Kunkel, Melissa Rosa, Brian Tarbox, Sabine Hild, Michael J. Jercinovic, Ali N. Bahadur
(2018)

Supplementary Material for **Recognizing Incipient Epizootic Shell Disease Lesions in the Carapace of the American Lobster, *Homarus americanus* H. Milne Edwards 1837.**

Bulletin of Marine Science.

Supplementary Table S1. Raman spectral regions Wavenumber(s) of interest (i – vi) covering PO_4^{3-}, CO_3^{2-}, and chitin and carbohydrate polymers. Critical types mentioned in the text and highlighted in figs 2D and S4D are given lower case Roman numbers.

Critical Type	Bond vibration	Wavenumber(s)	Comments	refs
i	ν1 PO_4^{3-}	957, 961, 962, 966	apatite, hydroxyapatite	[1,2,3]
ii	ν2 PO_4^{3-}	432, 445	[1,3]	[1,3]
iii	ν4 PO_4^{3-}	579, 590, 609		[1,3]
iv	ν1 symmetric CO_3^{2-} stretching, ν1 PO_4^{3-}	1073, 1085	calcite, carbonate apatite	[1,2]
v	ν2 CO_3^{2-} translational lattice vibration	281	calcite (not aragonite)	[2]
	Various amide III (β-sheet, α-helix), Glycine, >CH2, proline-sidechain	1200-1300, 1337	Various amide III	[1,2]
	Protein: CH deformation	1318	canal lumen	[2]
	Various amide II	1544, 1554		[2]
	Various amide I	1634-1690		[1,2,3]
vi	CH stretch (sym), C-H str (Fermi-Resonance) of >CH2 CH stretch (asy), amine	2883, 2935, 2965	chitin, carbohydrate polymers, protein sidechains	[2]
	ν (=C–H) stretch of lipids	3011	lipid	[2]
	O–H and N–H stretching vibrations	3232	-OH and amine stretch	[2]
	O–H stretching vibrations	3350 – 3550	-OH stretch	[2]

1. Mandair and Morris, 2015.
2. Movasaghi et al. 2007.
3. Kozielski et al. 2011.

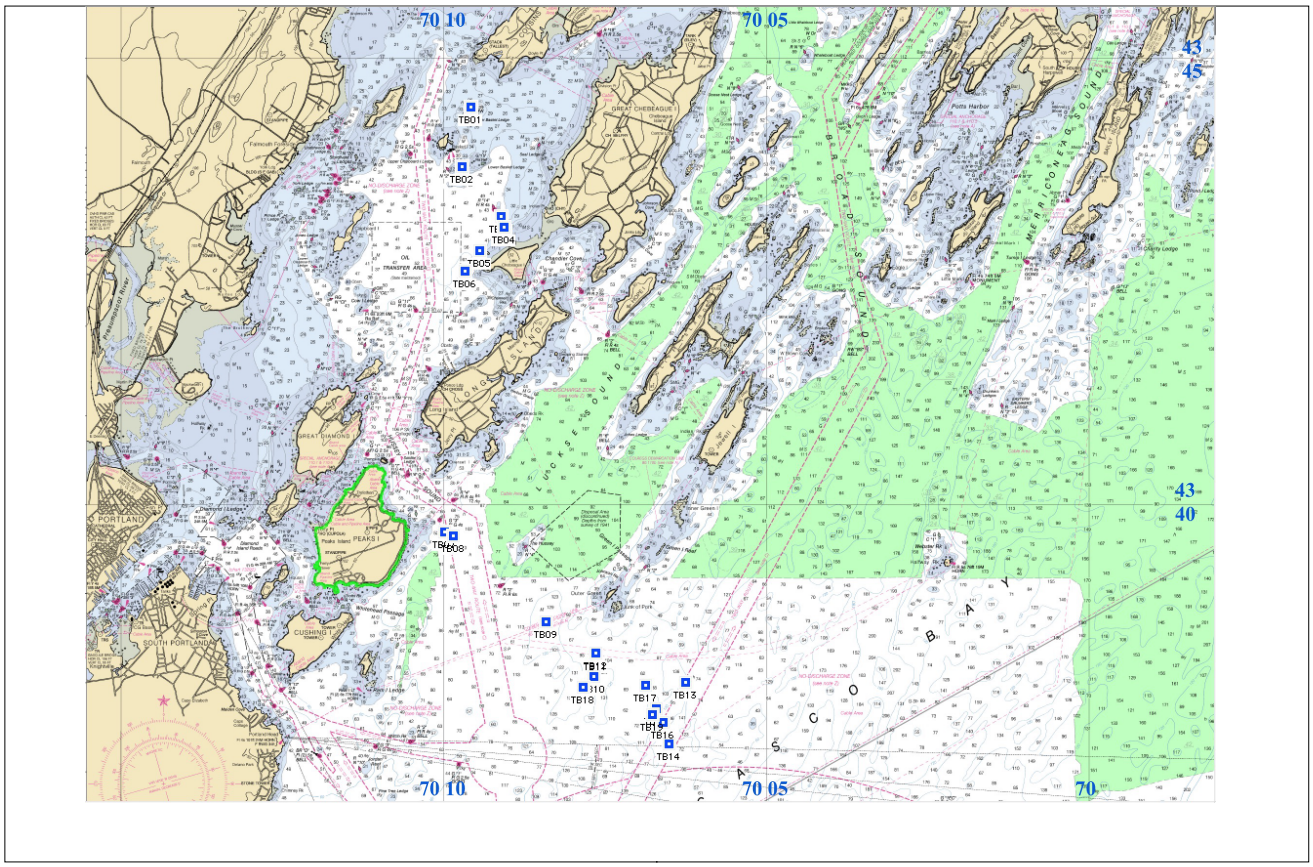

Figure S1. Lobster sampling sites in Casco Bay, Maine. Trap locations. Traps BT01-06 are referred to as Inner Casco Bay. Traps BT09-19 are referred to as Outer Casco Bay.

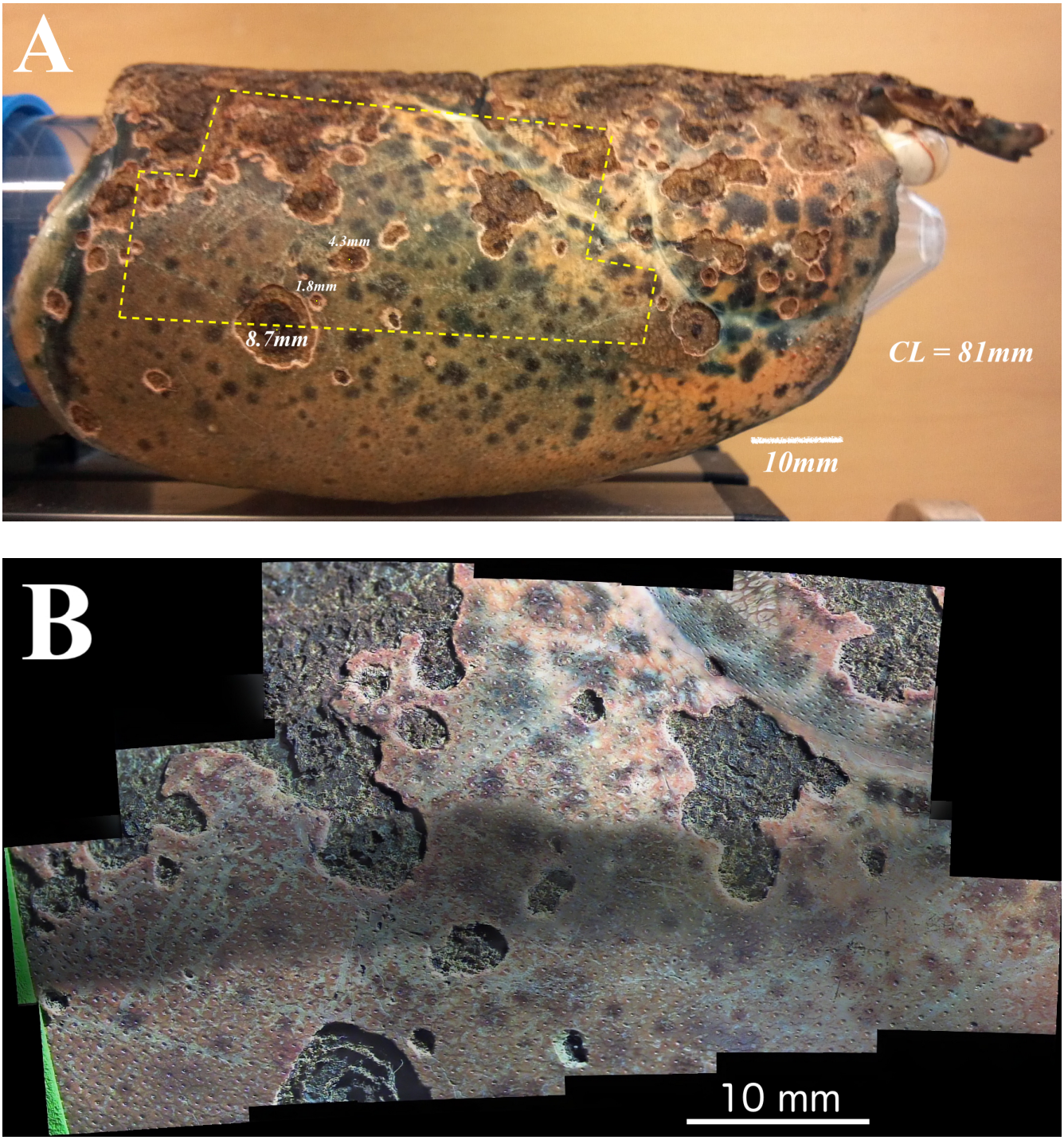

Figure S2. Exemplar ESD shell diseased lobster S6. **A.** Carapace (right half) molted from an advanced ESD infected American lobster. ImageJ calibration from CL = 81 mm allowed the sizes of lesions and the calibration bar to be provided. A yellow dash outlines the area that was the source of the 22 light microscope (LM) images used to create panel B. **B.** Montage of 22 hi-res LM images grabbed from center region of S6-A lobster combined via PhotoSticher with organules and large and small lesions. It is difficult to differentiate the smallest ESD lesions from large high-complexity organules at this level or resolution. The raw PhotoSticher output file is available to judge the visibility of small lesions at URL: http://www.bio.umass.edu/biology/kunkel/LabWiki/images/c/c8/Montage_stereograph100.jpg … to allow evaluation of effectiveness of viewing at this resolution.

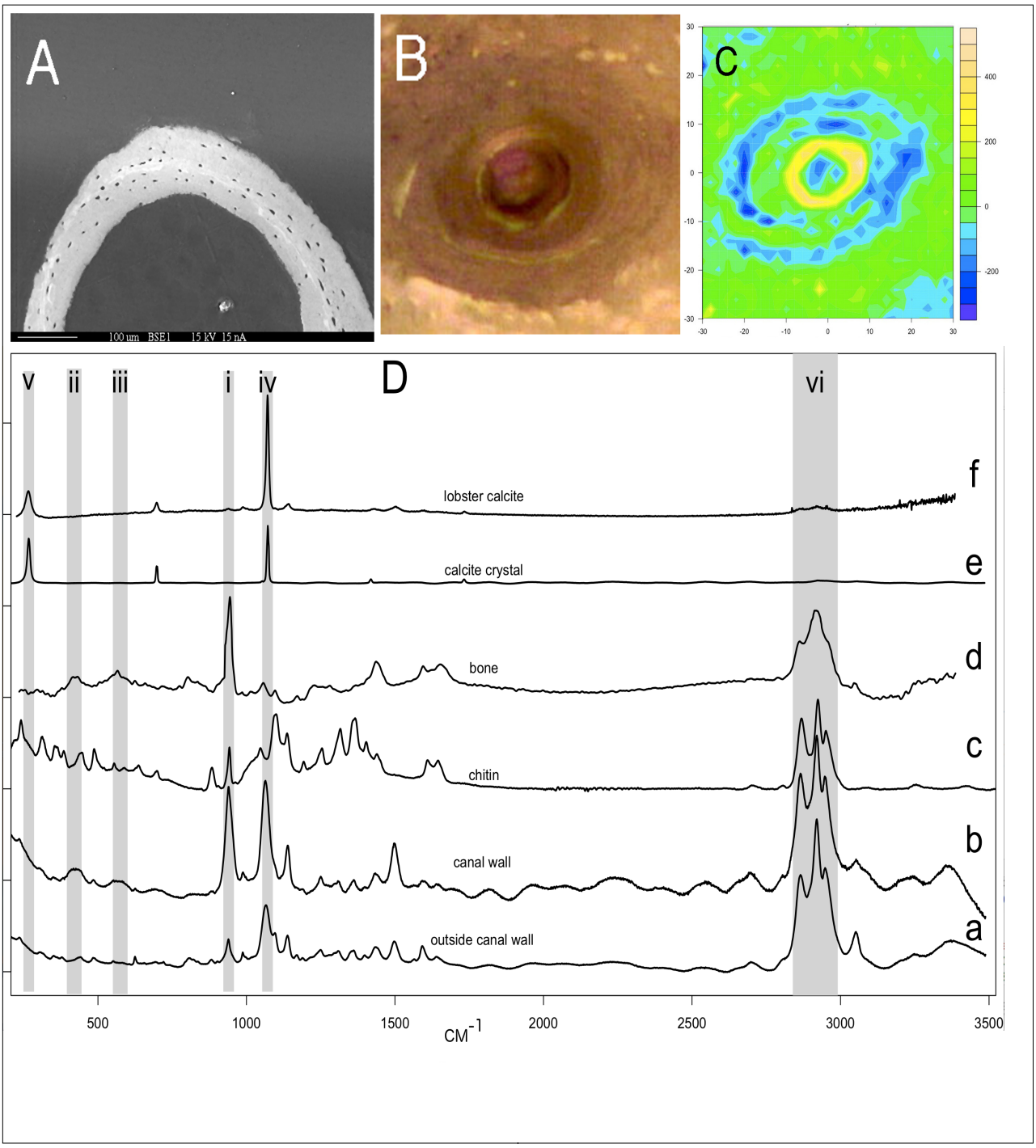

Figure S3. Raman spectra of selected areas of lobster cuticle in tangental polished section including the 2800-3400 μm^{-1} polymer region. A. Neonatal mouse cancelous bone. B. LM image of dermal canal. C. svd V7 contrasting canal wall to other surrounding structure. D. Raman Spectra a-f as intensity vs cm^{-1}: a. Outside canal wall. b. Canal wall. c. alpha-chitin crystal. d. neonatal mouse bone. e. calcite crystal. f. Lobster calcite layer (Thermo Fisher DX). Grey highlighted zones: *i.* v1 PO_4, *ii.* v2 PO_4, *iii.* v3 PO_4 , *iv.* v1 CO_3, *v.* v2 CO_3; *vi.* v3 CO_3.

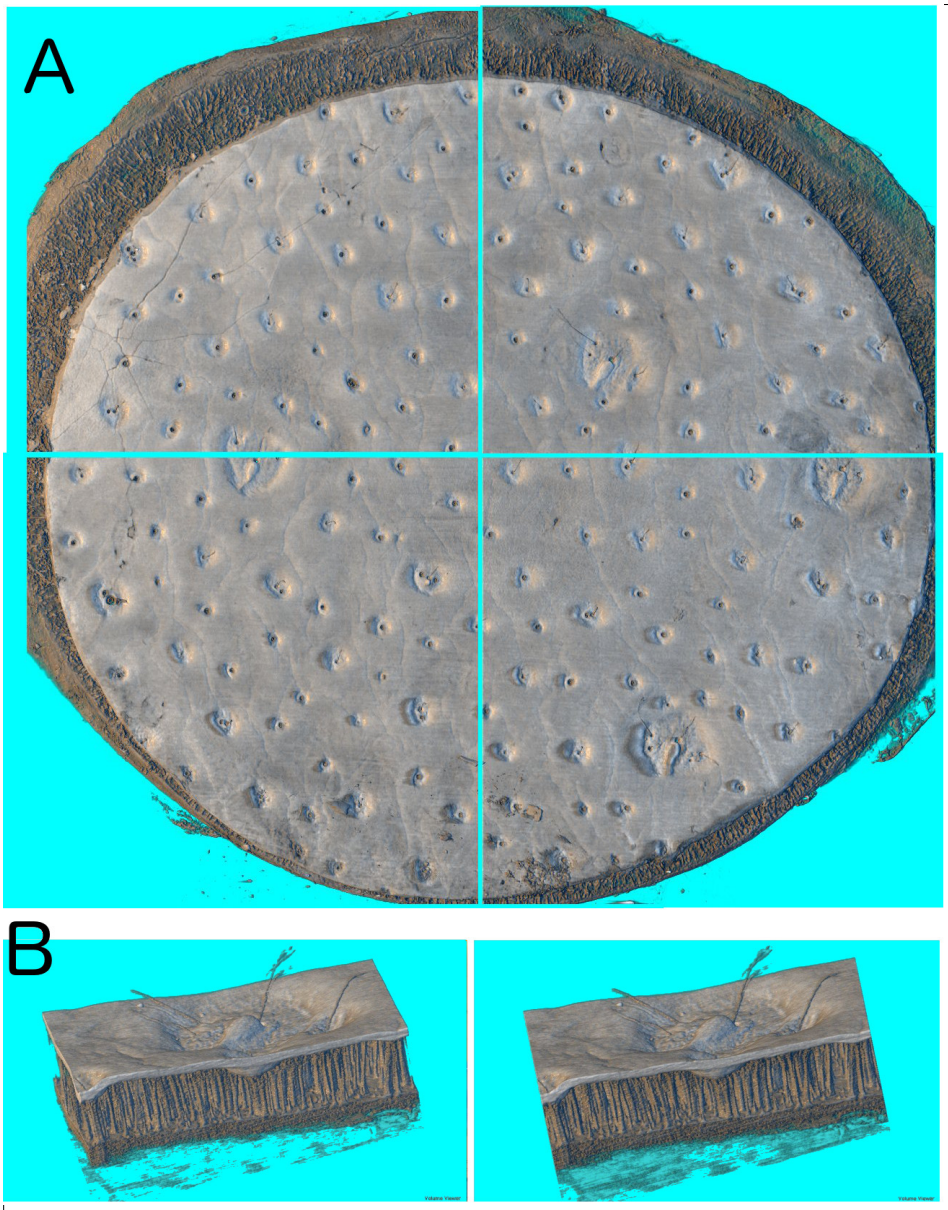

Figure S4. One-week post molt carapace cuticle medallion. **A.** Four sectors of data were analyzed separately using identical protocols in ImageJ VolumeViewer because the full 2.5 µm voxel resolution 6 mm diameter medallion 16 bit data set was too large to be analyzed together. The resultant images were saved and combined with GIMP software. **B.** A ROI of the same medallion scanned at 1 µm voxel resolution showing the organule of Fig. 3 and Fig. 4B as a stereo pair which allows one to see more clearly the organule canal freeze-fixed-secretion streaming in the space above the organule depression onto the epicuticle surface. The stereo pair can be viewed effectively by uploading into a phone browser and viewing with a VR viewer or by cross-eye viewing. Various size choices are offered ... URL:
http://www.bio.umass.edu/biology/kunkel/LabWiki/images/thumb/a/a0/Ha7_1um_b_stereo.jpg

A video of an organule with a bristle is instructive:
http://www.bio.umass.edu/biology/kunkel/LabWiki/images/3/38/Ha7_2.5_crop2A_ThMacrCx0.6.avi

Another video illustrates applying the ImageJ Bone/thickness plugin to Fig. 4C medallion sections:
http://www.bio.umass.edu/biology/kunkel/pub/lobster/3D/AVI/Ha5_2.5_aX300center.avi

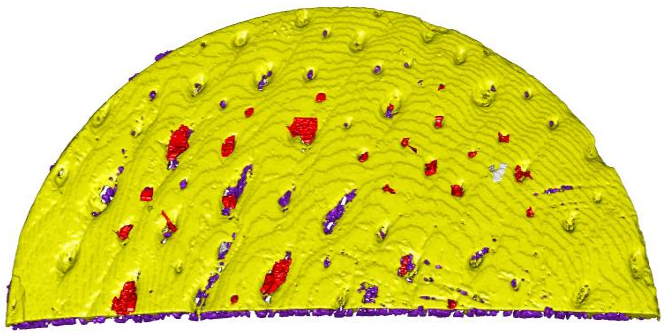

Figure S5. Video of American lobster 6 mm hemi-medallion viewed in (Kunkel et al. 2018) Fig. 8A,B illustrating a potential subclinical ESD lesion. The calcite layer is yellow, the trabeculae are purple and some canals and organule depression contents were characterized as red. A few structures that did not conform to the criteria of calcite, trabeculae, stalactites or canal contents in the epicuticle and exocuticle region were defined as white and one such large such object is seen at about 2 o'clock on the face of this S5 video, URL:

http://www.bio.umass.edu/biology/kunkel/pub/lobster/3D/XRT/Ha5_2.5/16bit/AVI/interp/Ha5_2.5um_16b_semiMedalionA3.avi

This white object was not fully understood and is a potential subclinical ESD lesion residing close to an organule.

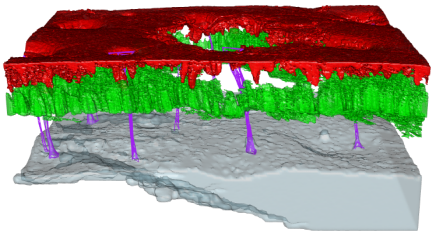

Figure S6. Video of ROI (Kunkel et al. 2018) Figs. 7A, 8C,D and 10A, a 50 µm ESD lesion, based on 1 µm resolution µCT data from American lobster medallion Ha4.

http://www.bio.umass.edu/biology/kunkel/pub/lobster/3D/XRT/Ha4_1um/AVI/interp/Ha4_1um_16b_transp_0-325_237.avi

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2. Movasaghi Z, Rehman S, Rehman IU. (2007). Raman Spectroscopy of Biological Tissues. Applied Spectroscopy Reviews 42(5): 493-541. http://dx.doi.org/10.1080/05704920701551530

3. Kozielski M, Buchwald T, Szybowicz M, Błaszczak Z, Piotrowski A, Ciesielczyk B. (2011). Determination of composition and structure of spongy bone tissue in human head of femur by Raman spectral mapping. Journal of Materials Science: Materials in Medicine 22(7): 1653–1661. https://doi.org/10.1007/s10856-011-4353-0