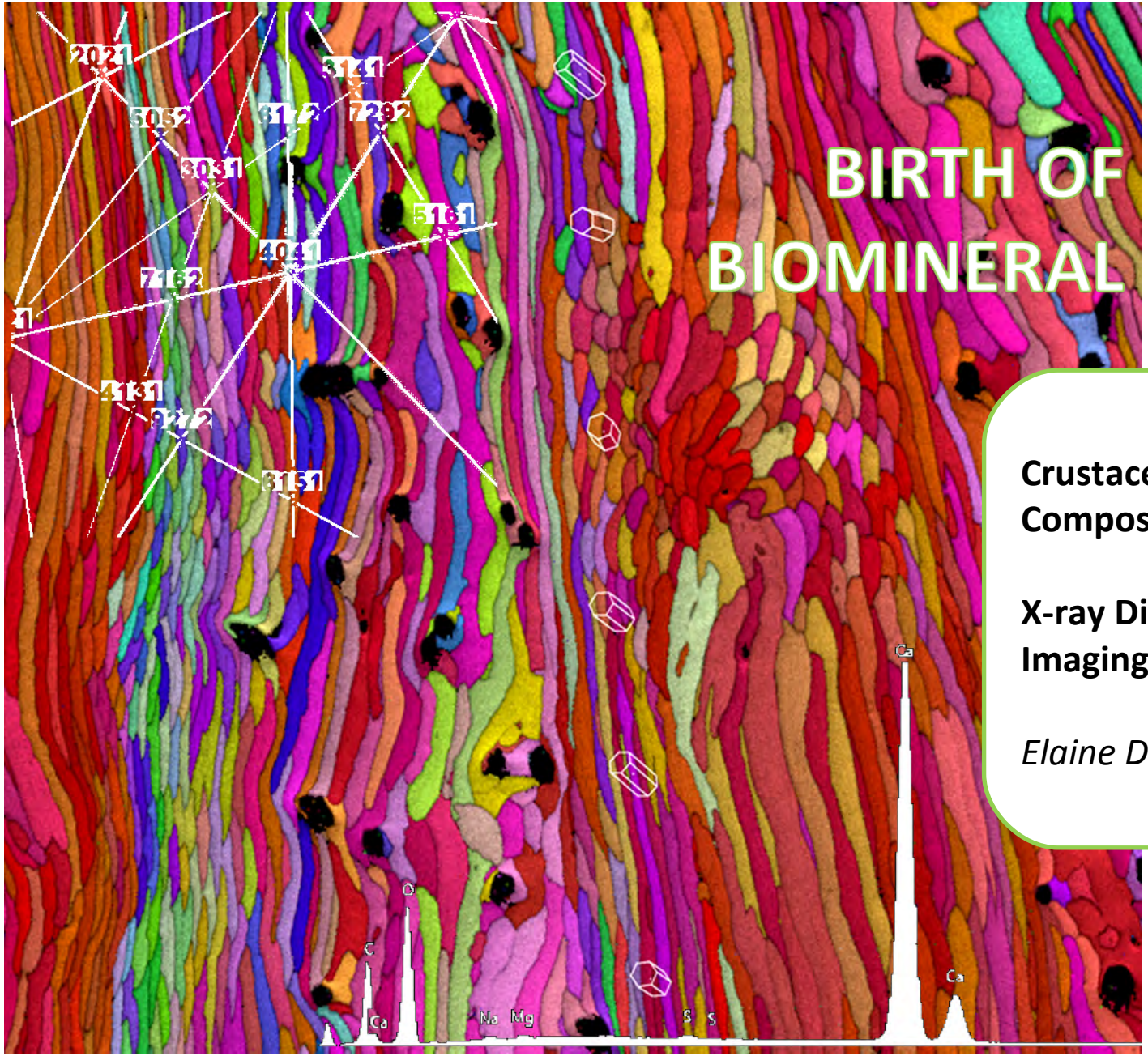

Crustaceans as Composite Materials

X-ray Diffraction Imaging with a Twist

Elaine DiMasi, BNL

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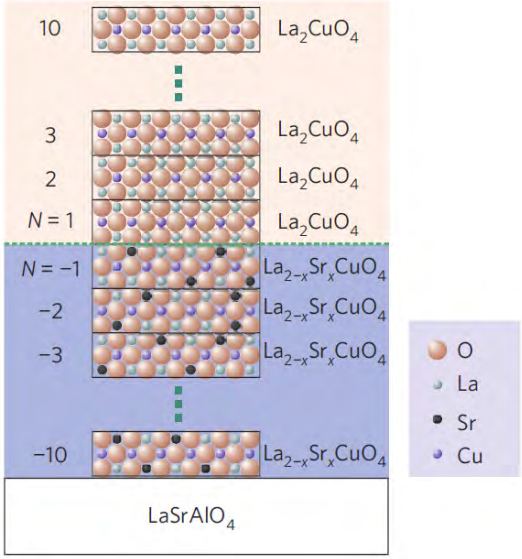
cross-cutting science, cross-cutting techniques



Interface superconductivity in $La_{1.55}Sr_{0.45}CuO_4 – La_2CuO_4$ bilayers

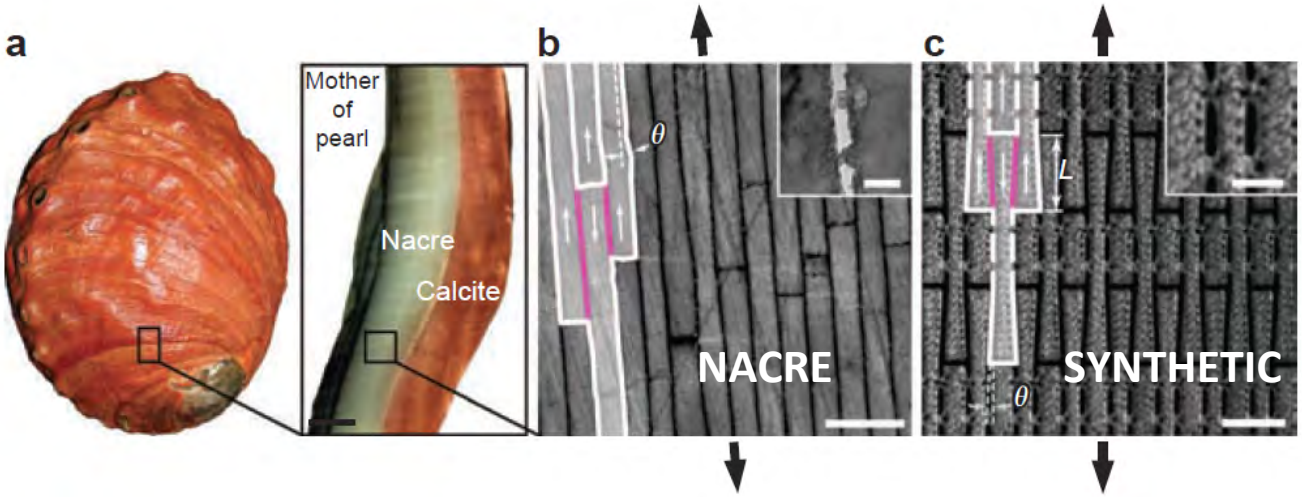
(Wu et al, Nature Materials 2013)

Next step requires combination of ALL-MBE with ARPES spectroscopy and STM imaging (**CFN**).

Interface hardening in Abalone Nacre

(Espinosa et al, Nature Communications 2011)

AFM fracture experiments combined with SEM and digital image correlation. Features on submicron scales.



Structural and Functional Hierarchies: Strategy and Science for NSLS-II

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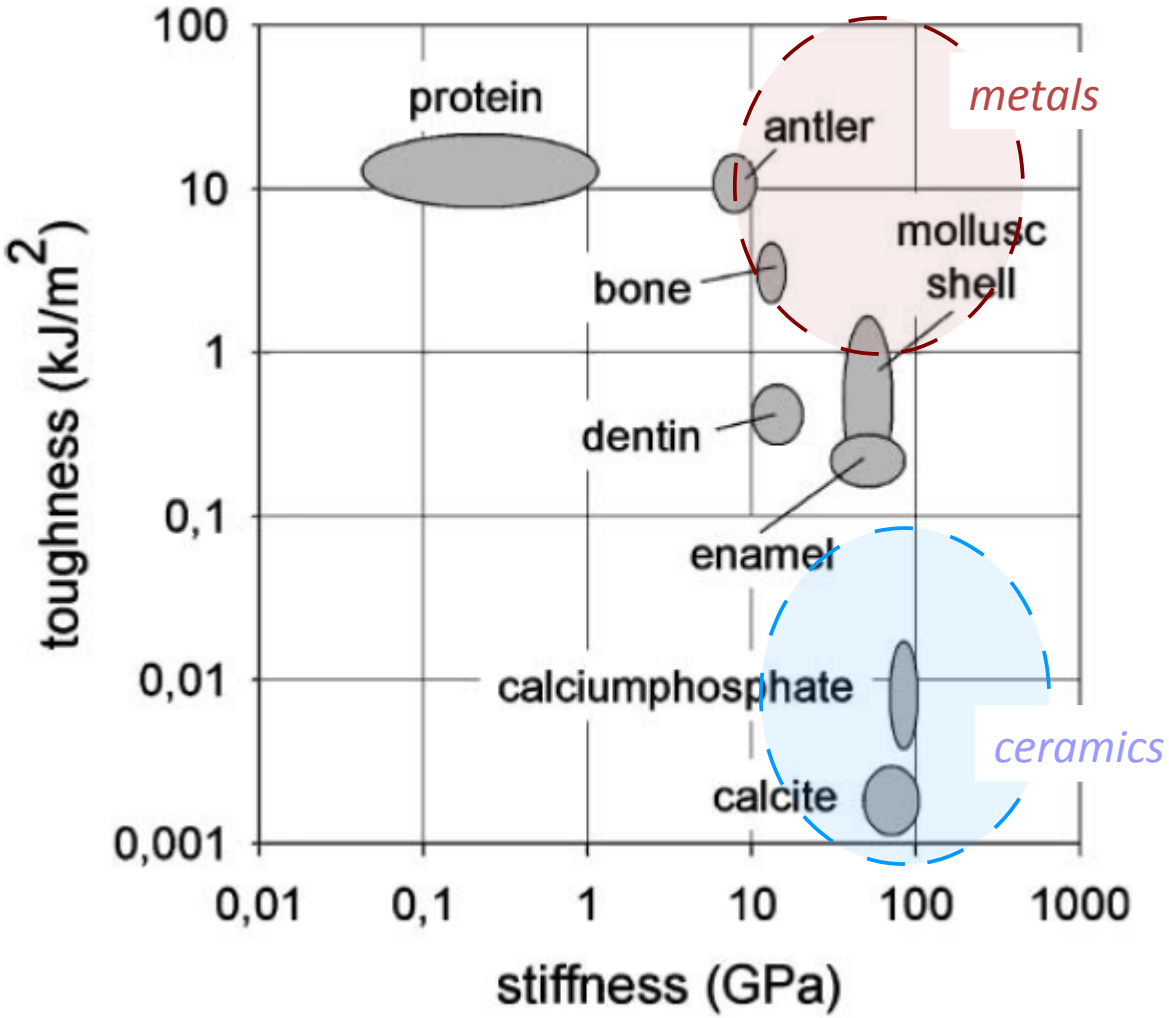
biological hierarchical structural materials



Biominerals:
Tough and Stiff
(high Young's Modulus)
(Fratzl et al 2004)

Components:
Mineral; organic; porosity.

"Porosity" is particularly interesting here because the pore space may have a biological function AND a physical function.

How is biomineral "born"?

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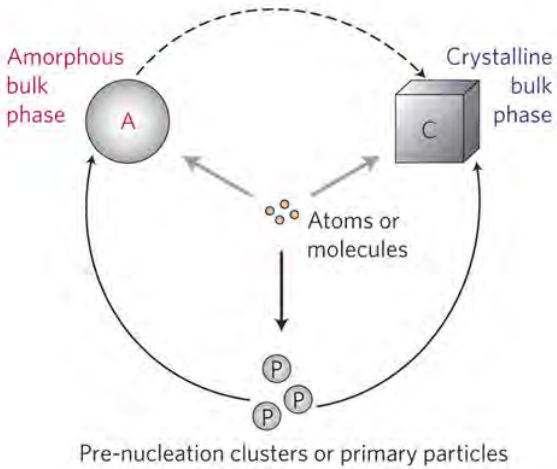
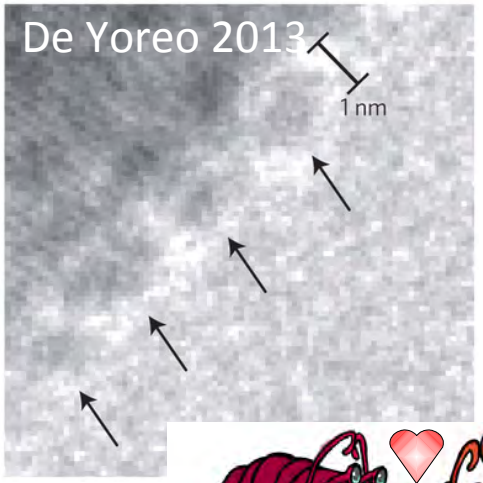
birth of biomineral: scale of approach





Chemically and Physically.
Mineral nucleation in biological environment:

- Concentrations of ions, reactants
- Additives (enzymes, poisons)
- Confinement, templates, gradients..

Development of the hierarchy.
Chemical and physical arguments at each step:

- When do superstructures "grow onto" supporting structures?
- When do substructures "assemble into" superstructures?

via biological Evolution. Some of the most interesting large research programs now look for convergent evolution across taxa.
It is a sign of excellent design.

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birth of biomineral: tissues in crustaceans

Scattering and spectroscopy

Crustacean Cuticle:

- Lobster chitin and calcite
- Mantis shrimp dactyl club

Structural correlations from microbeam x-ray diffraction analysis lead to hypotheses about *tissue growth*.

Chemically and Physically.
Mineral nucleation in biological environment:

- Concentrations of ions, reactants
- Additives (enzymes, poisons)
- Confinement, templates, gradients..

Development of the hierarchy.
Chemical and physical arguments have to be applied to each step and hypotheses tested:

- When do superstructures "grow onto" supporting structures?
- When do substructures "assemble into" superstructures?

via biological Evolution. Some of the most interesting large research programs now look for convergent evolution across taxa.
It is a sign of excellent design.

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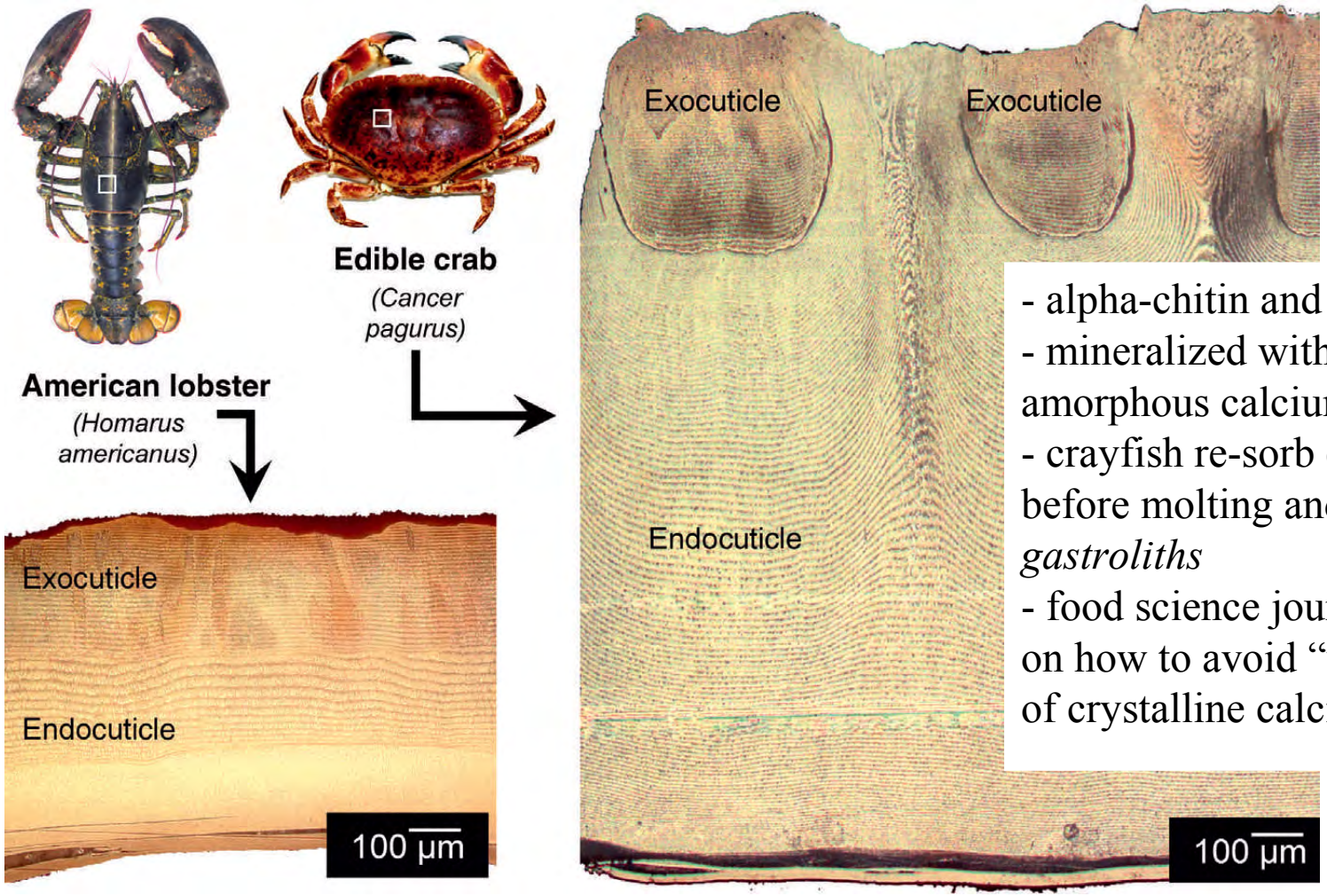
crustacean cuticle basics



- alpha-chitin and proteins
- mineralized with soluble amorphous calcium carbonate
- crayfish re-sorb calcium before molting and store in *gastroliths*
- food science journals publish on how to avoid "white flakes" of crystalline calcite in shells

Mineralization is controlled by the organism. How?

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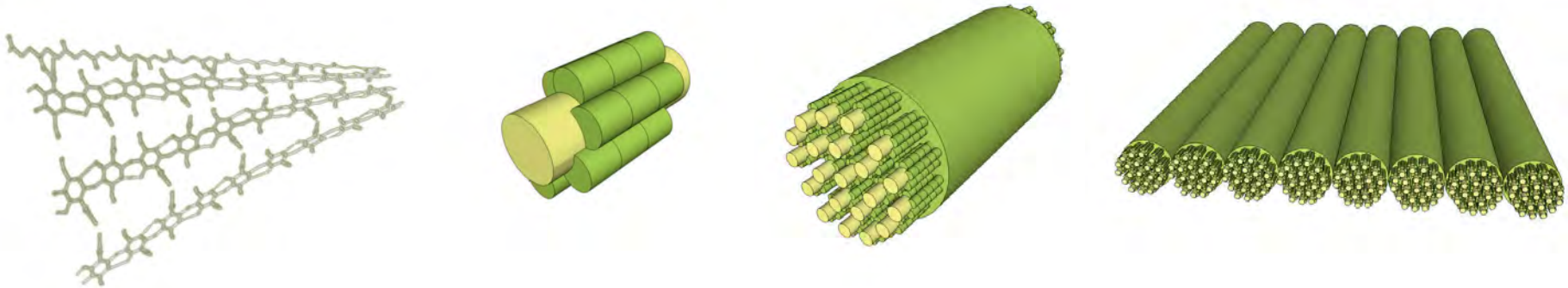
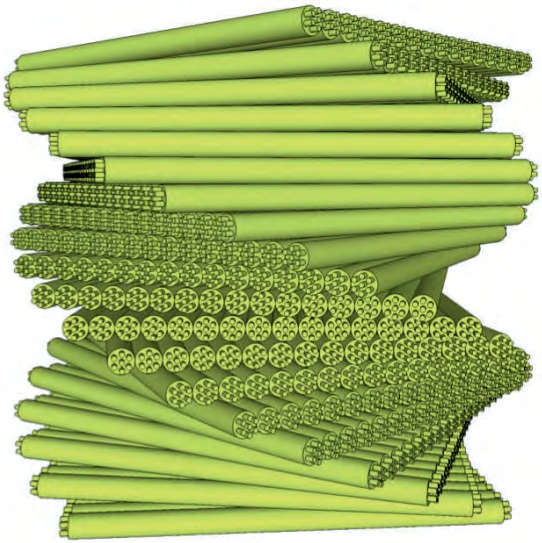
the chitin template in crustacean cuticle

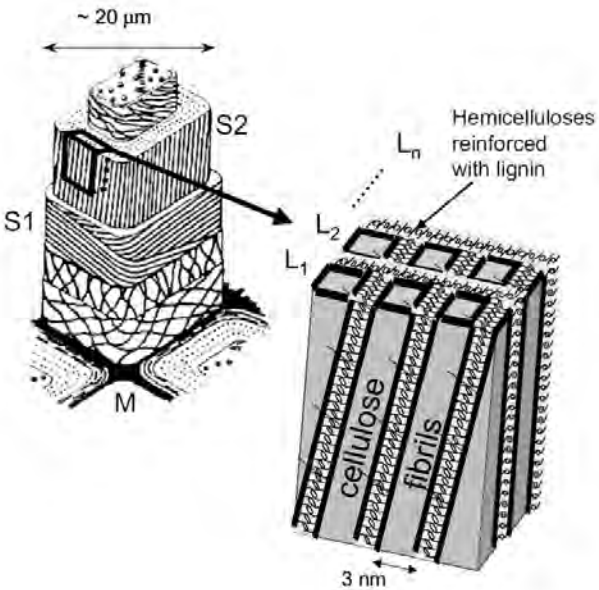


alpha-chitin is crystalline; coated with protein in **nanofibrils**; bundled in **fibers**; **layered**; assembled into **"twisted plywood"** structure. Tissues perforated with **pores**.(micron scale).



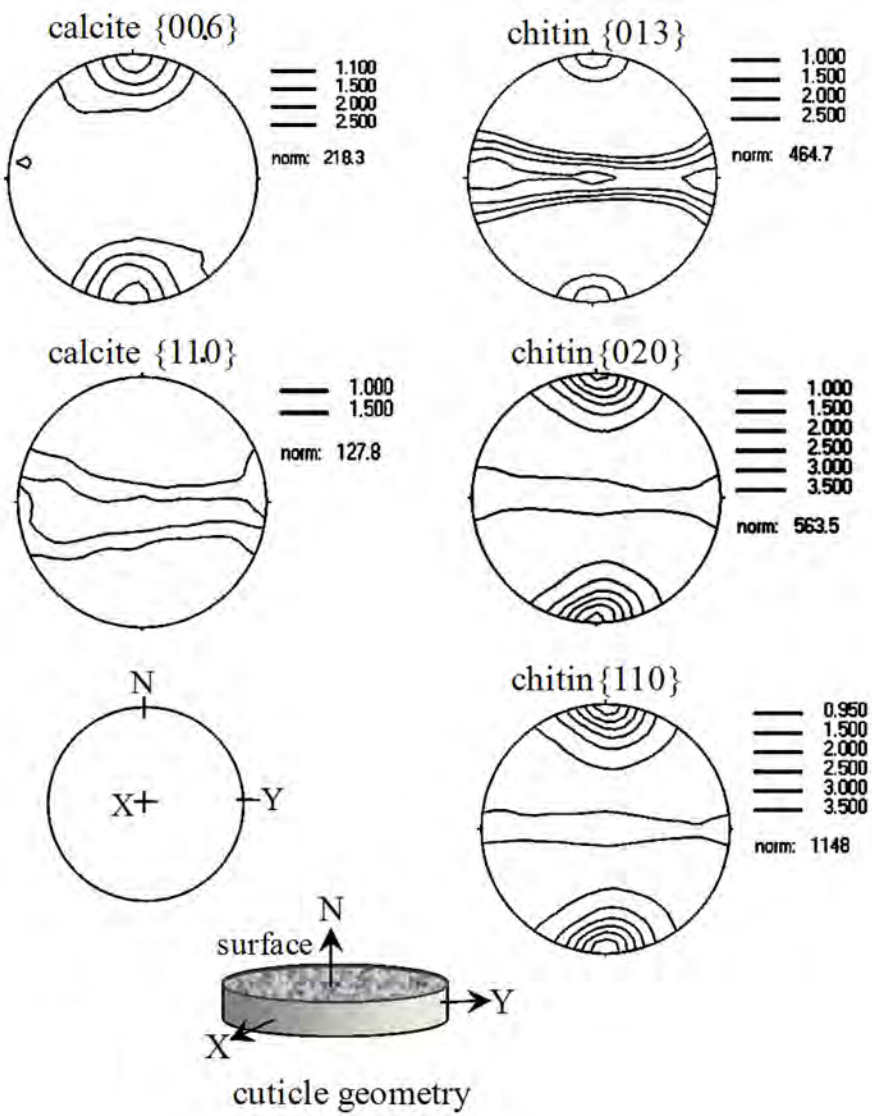
Superstructure resists shear.
Why and how does it mineralize?
and...

... it shares features with cellulose fiber organization in wood?



[images from a Max Planck Research publication, P. Fratzl]

calcification in American lobster cuticle



Mineral-Organic Co-Organization.
Is calcite formation in the (mostly ACC) cuticle systematic or accidental? [Al-Sawalmih et al, Adv. Func. Mater. **18** (2008) 3307]

- XRD pole figures measured
- One orientation for calcite
- Two for chitin: in-plane (layers) and out-of-plane (along the pores)

Could the calcite be associated with just one of the chitin structures?

How is it possible to know? The nm-scale layers are much smaller than the X-ray beam.

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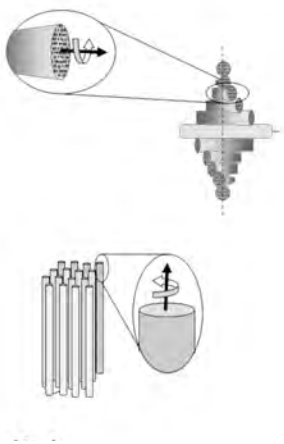
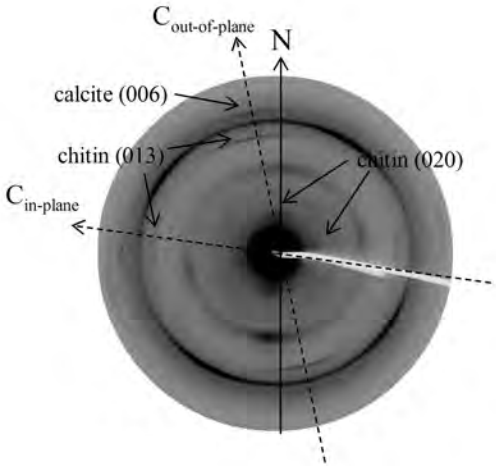
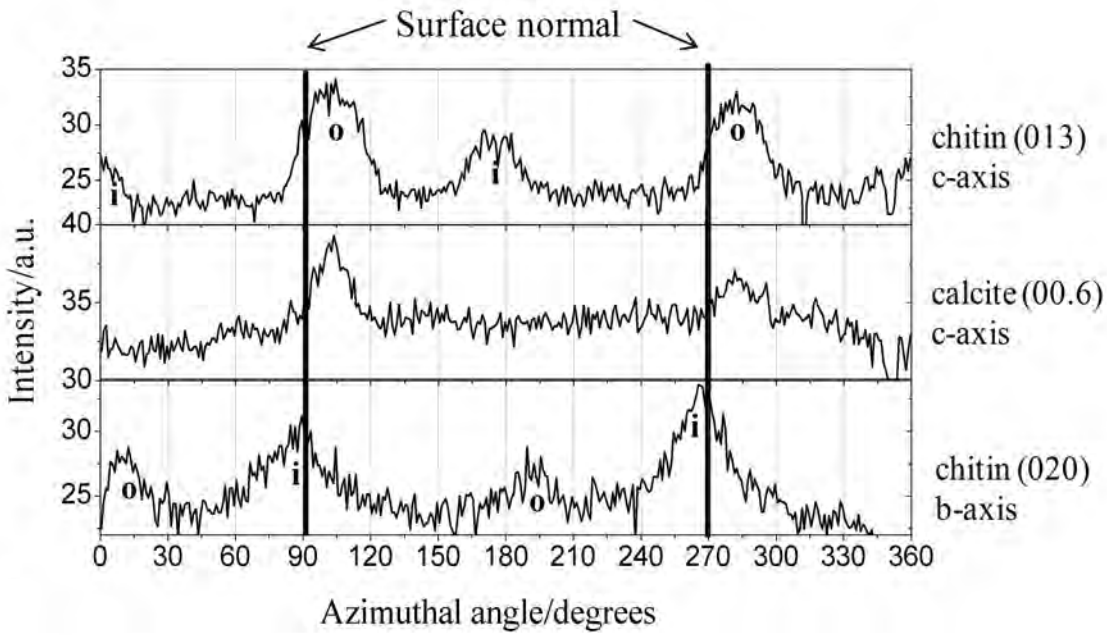
calcification in American lobster cuticle

In-plane fibers (upper scheme) and **out-of-plane fibers** (lower scheme) contribute to the pattern.

Rotation of the fibers in-plane has a "fiber symmetry" of its own; **pseudo fiber pattern** superimposed.







Azimuthal dependence of scattered intensity now clearly distinguishes between the in-plane and out-of-plane chitin.

Calcite crystals are aligned with out-of-plane chitin association with **pores**.

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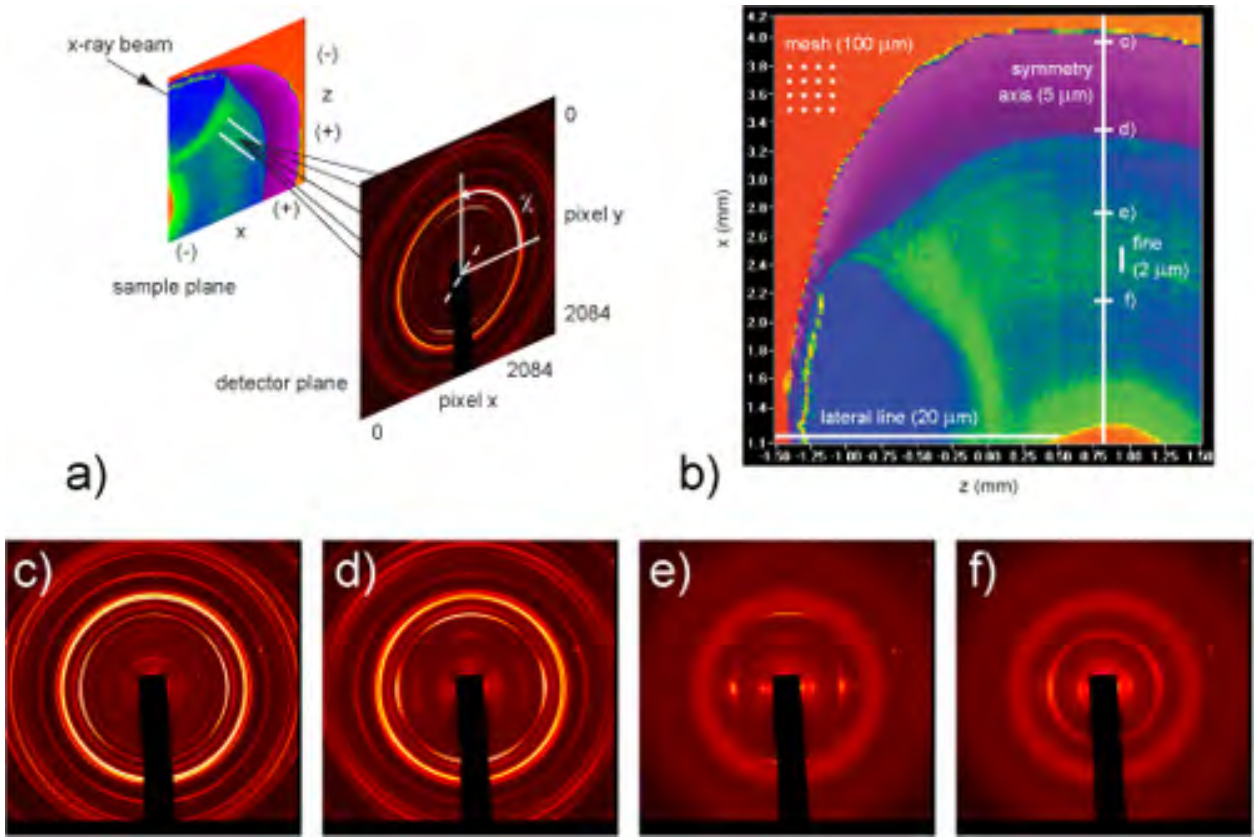
calcification in the Stomatopod (mantis shrimp)

"X-ray Scattering with a Twist" part 2.

- Mineralization of the dactyl club
- X13B micro-diffraction
- Necessity of multiple techniques
- The power of imaging

[Weaver et al Science 336 (2012) 1275]



X-ray absorption image = powerful tool for the µXRD experiment.

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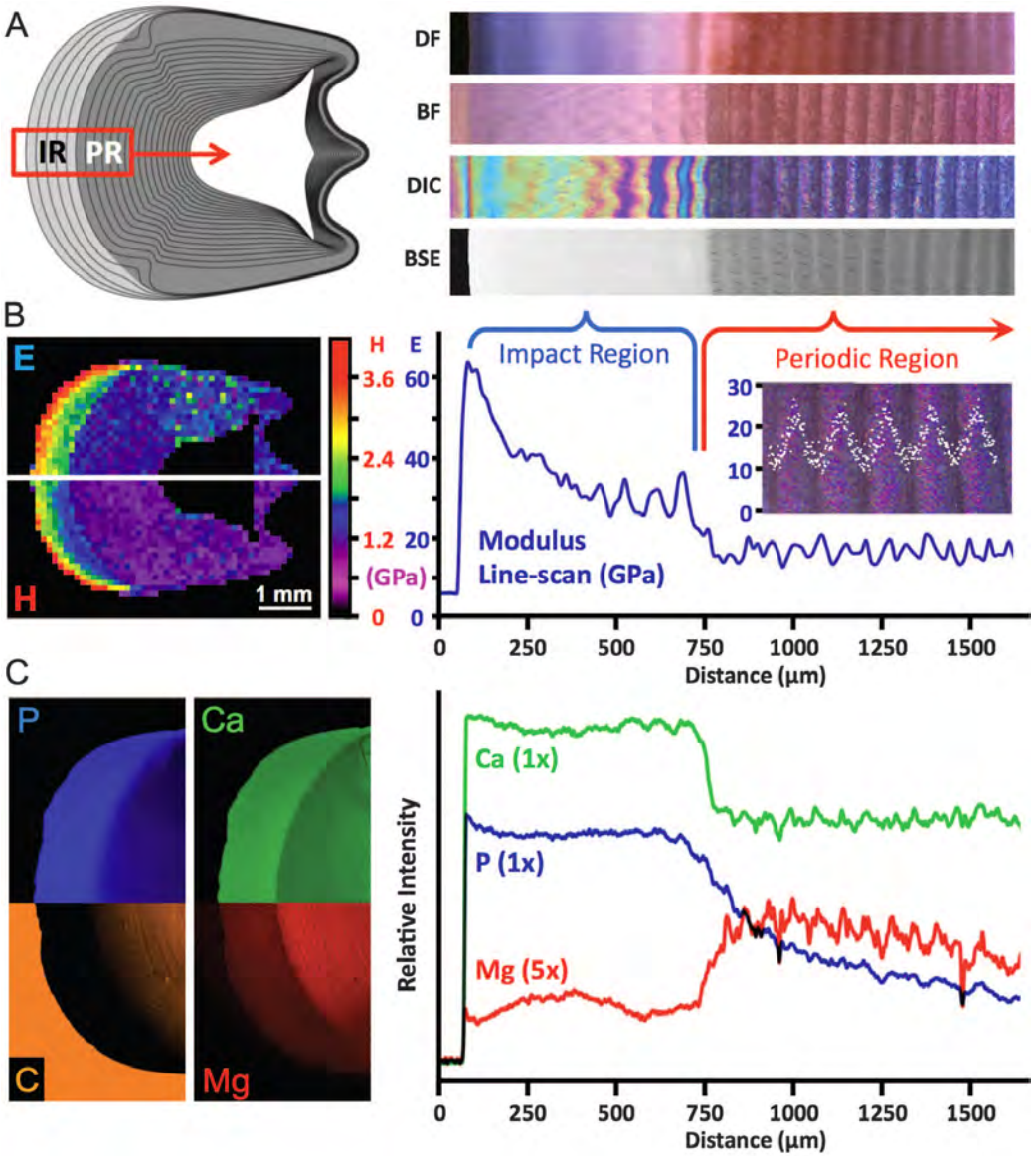
Hierarchical tissues via multiple techniques



Impact Region of the smashing club is x-ray dense, electron-dense, Ca- and P- rich, and has maximum hardness and stiffness.

Periodic region is less dense; softer; higher Mg content; ~75µm period in chemical and physical features.

Nanoindentation done on a point by point basis; all other techniques are well known imaging modes now.

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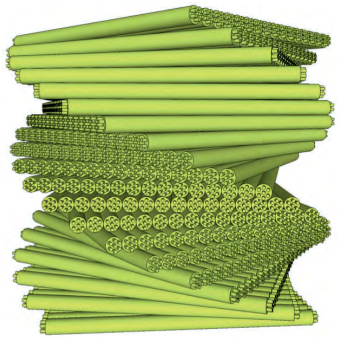
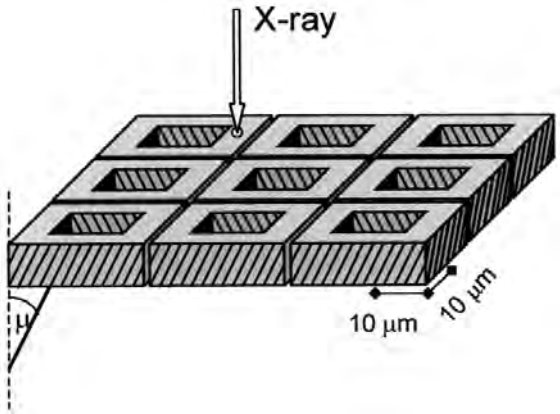
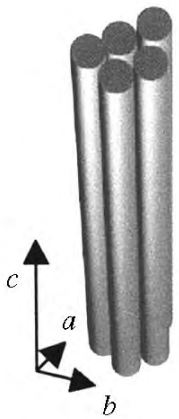
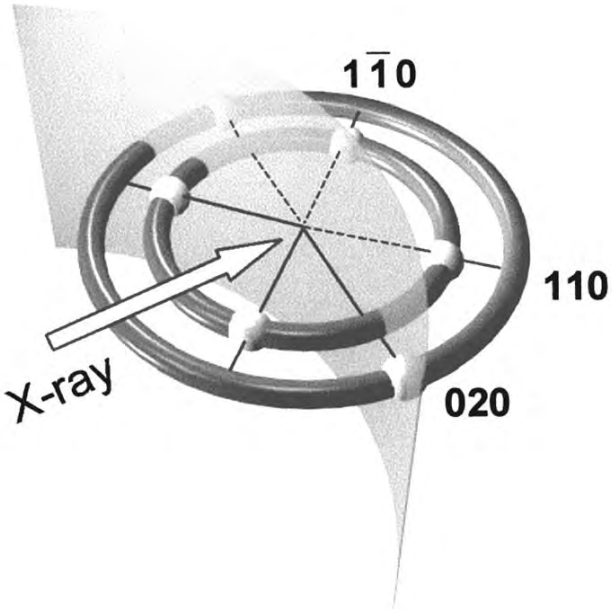
X-ray microbeam imaging of fiber orientation



Lobster cuticle layers (left) were too small to image with microbeam... but **cellulose fibers** in wood (right) were not *[Lichtenegger 1999]*.







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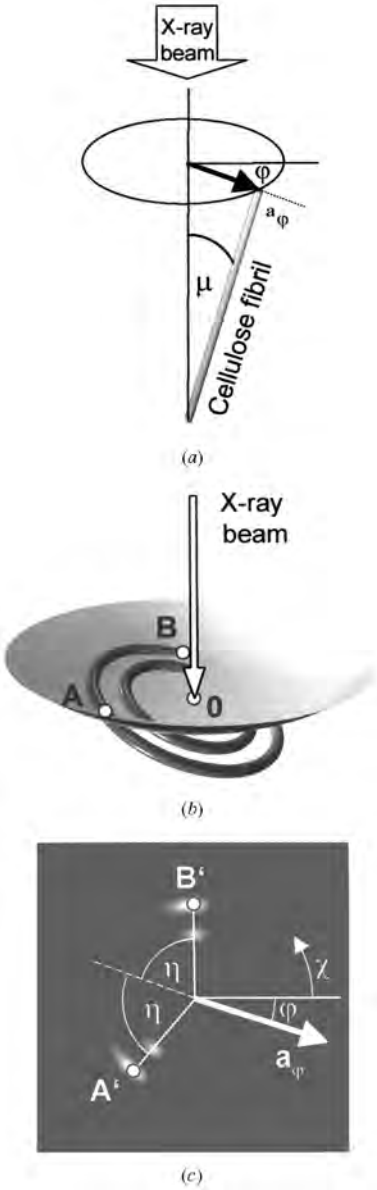
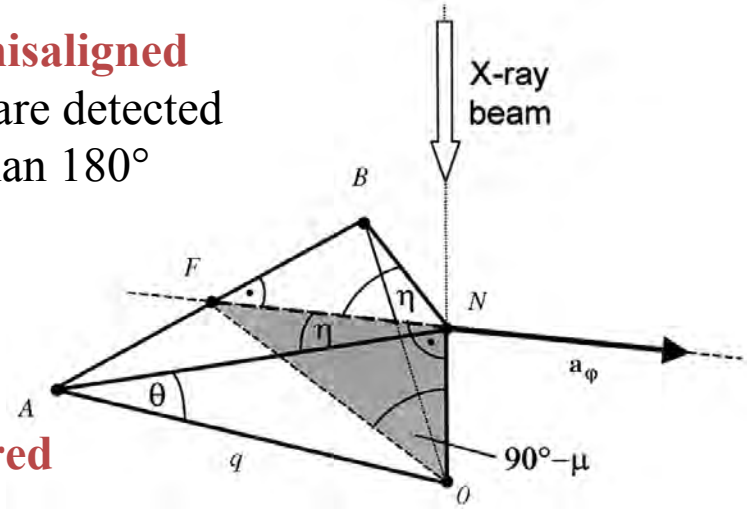
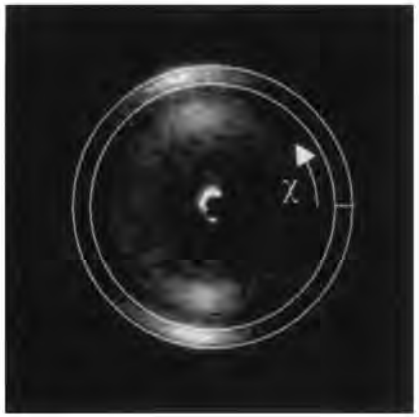
X-ray microbeam imaging of fiber orientation



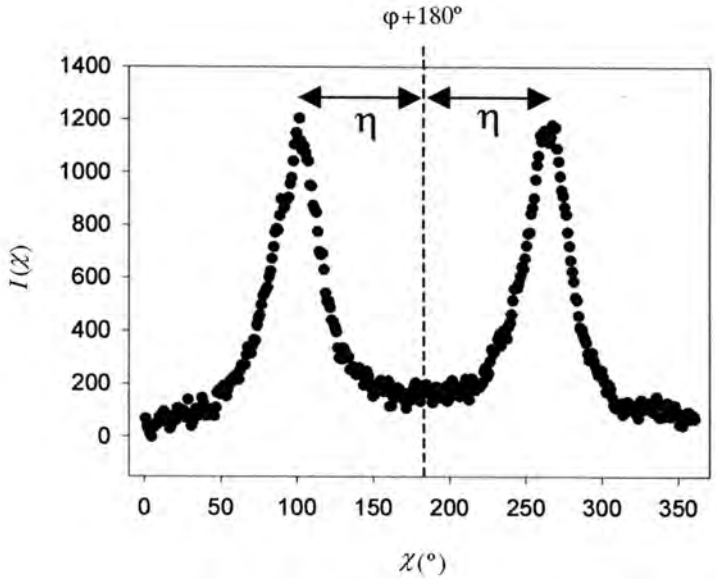
With fiber axes **slightly misaligned** to the X-ray beam, peaks are detected at azimuthal angles less than 180° apart.

The misalignment is calculated from the data. **Full 360° azimuth required for WAXS detector!**



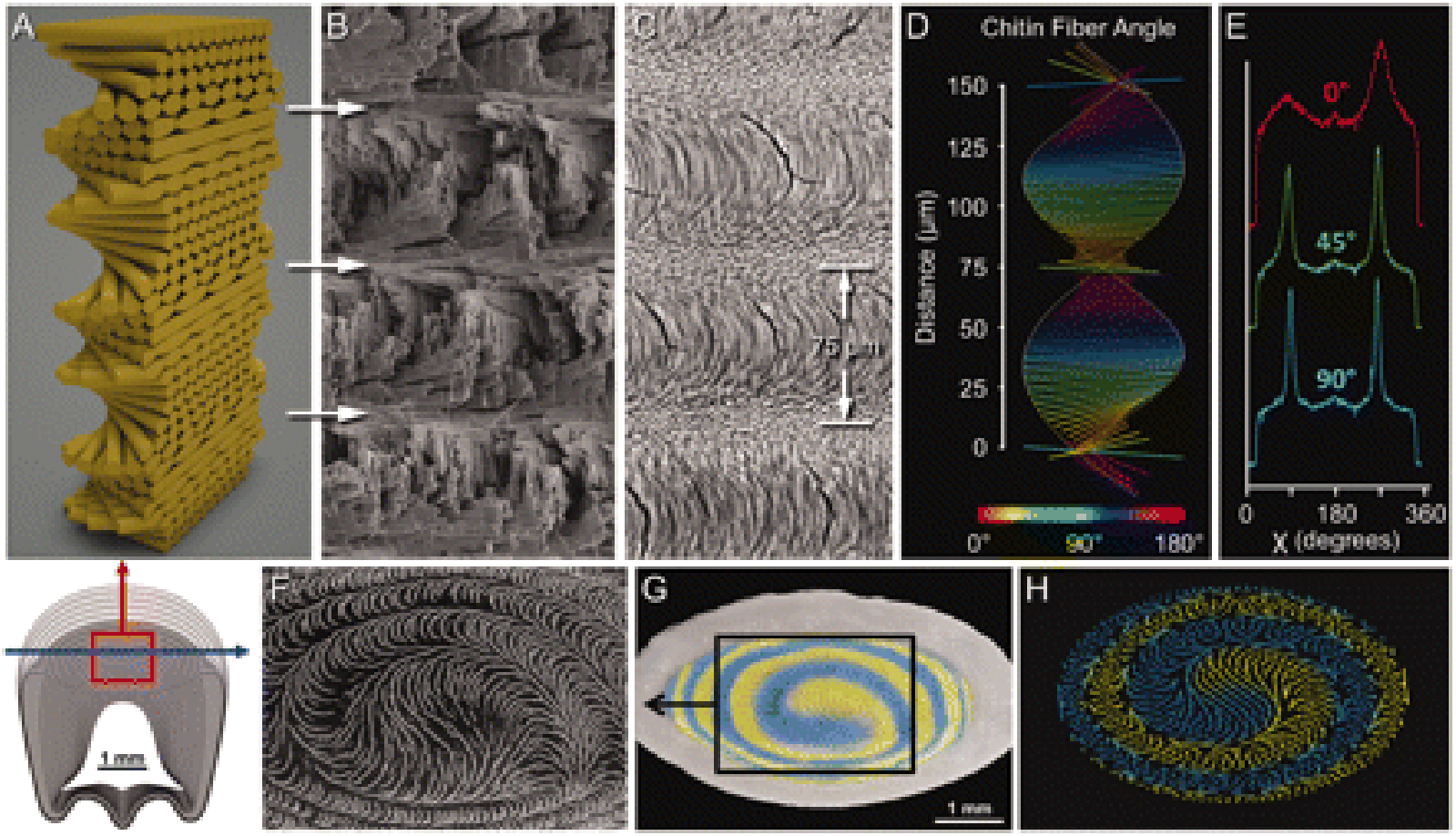


(a)



(b)

twisted plywood structure in Stomatopod dactyl club



Curved shape at macroscale + helical microstructure = maze for cracks

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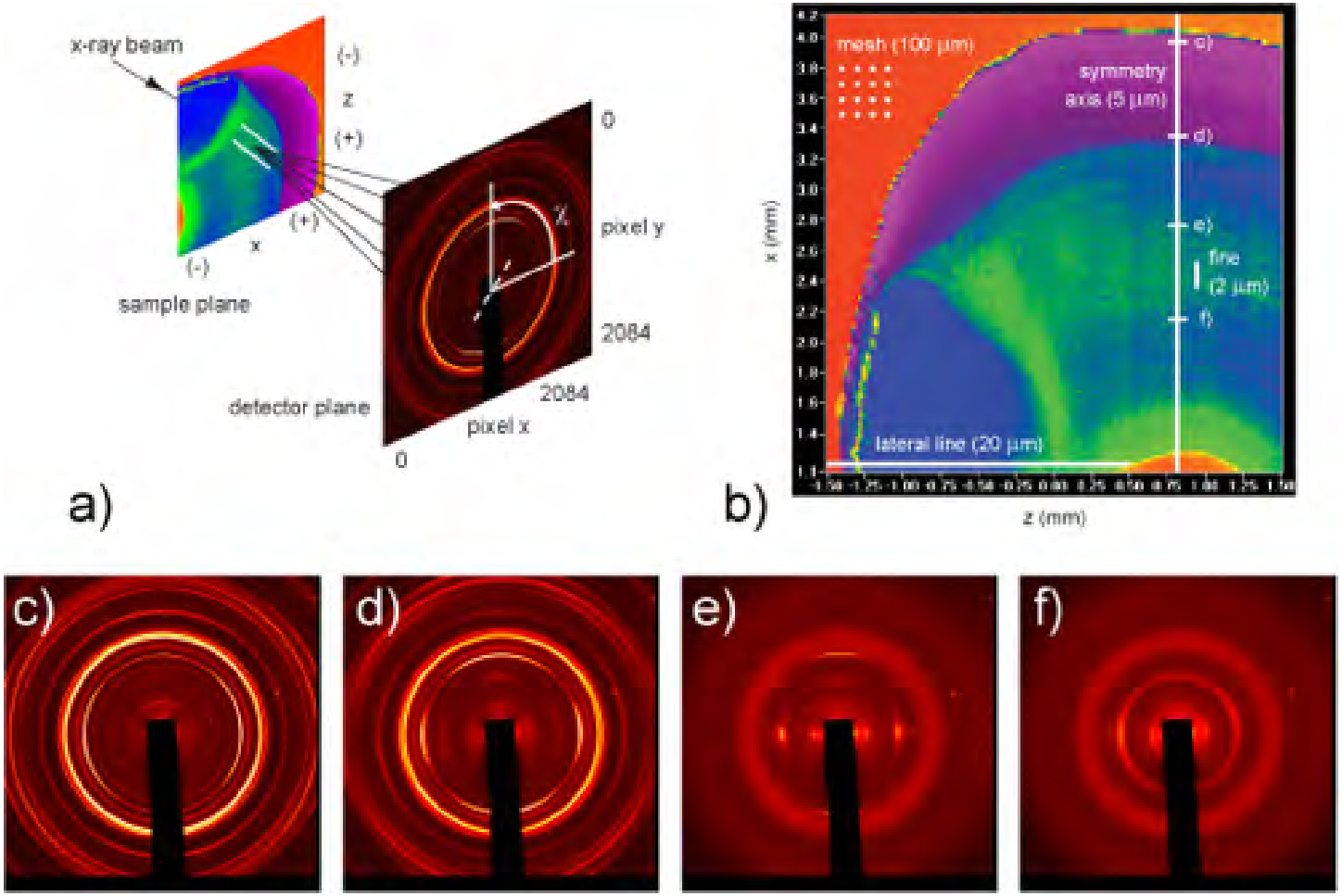
amorphous mineral in the periodic region?



X13B, 5 µm spot: What does a diffraction map look like?

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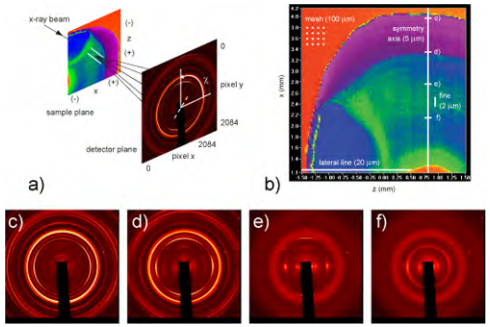
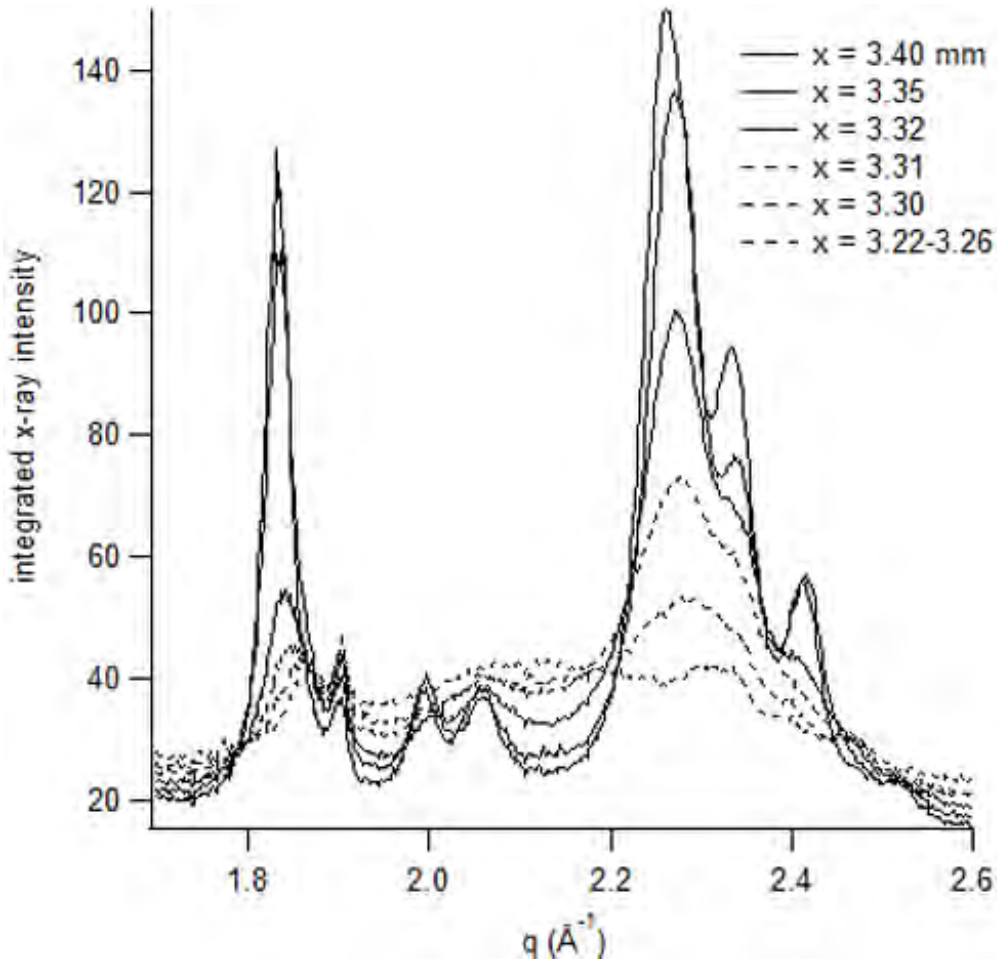
amorphous mineral in the periodic region?





Hydroxyapatite XRD patterns in the Impact Region: better crystallized than bone.

Amorphous mineral in Periodic Region, plus alpha-chitin peaks. Want to **map** it.

Amorphous phase still unidentified (ACP+ACC?)

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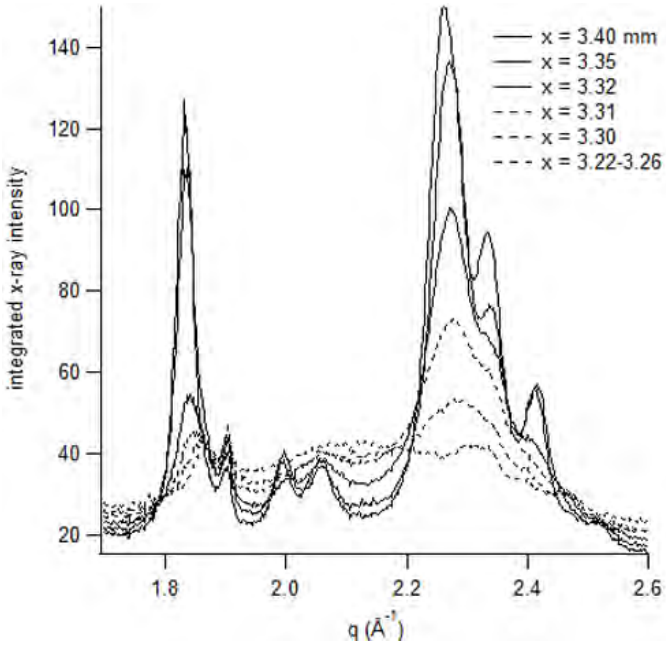
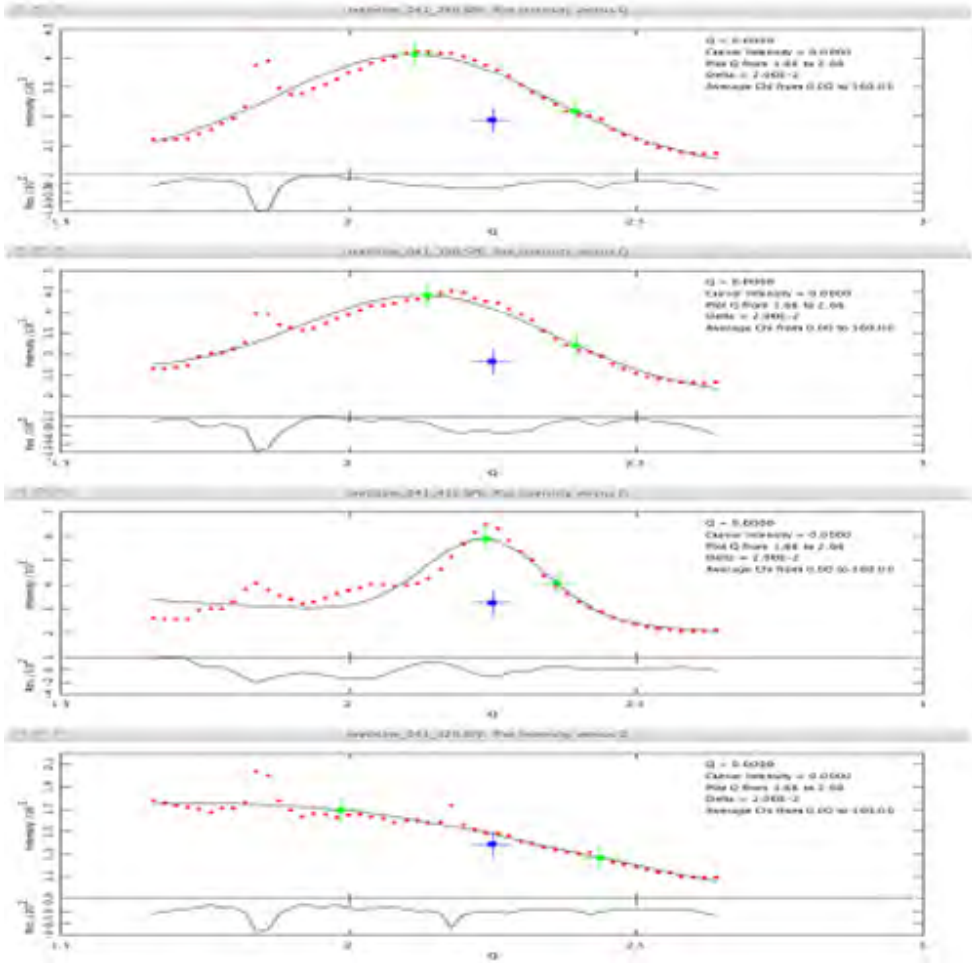
amorphous mineral in the periodic region?





Method: fit the "amorphous peak" ... "badly".

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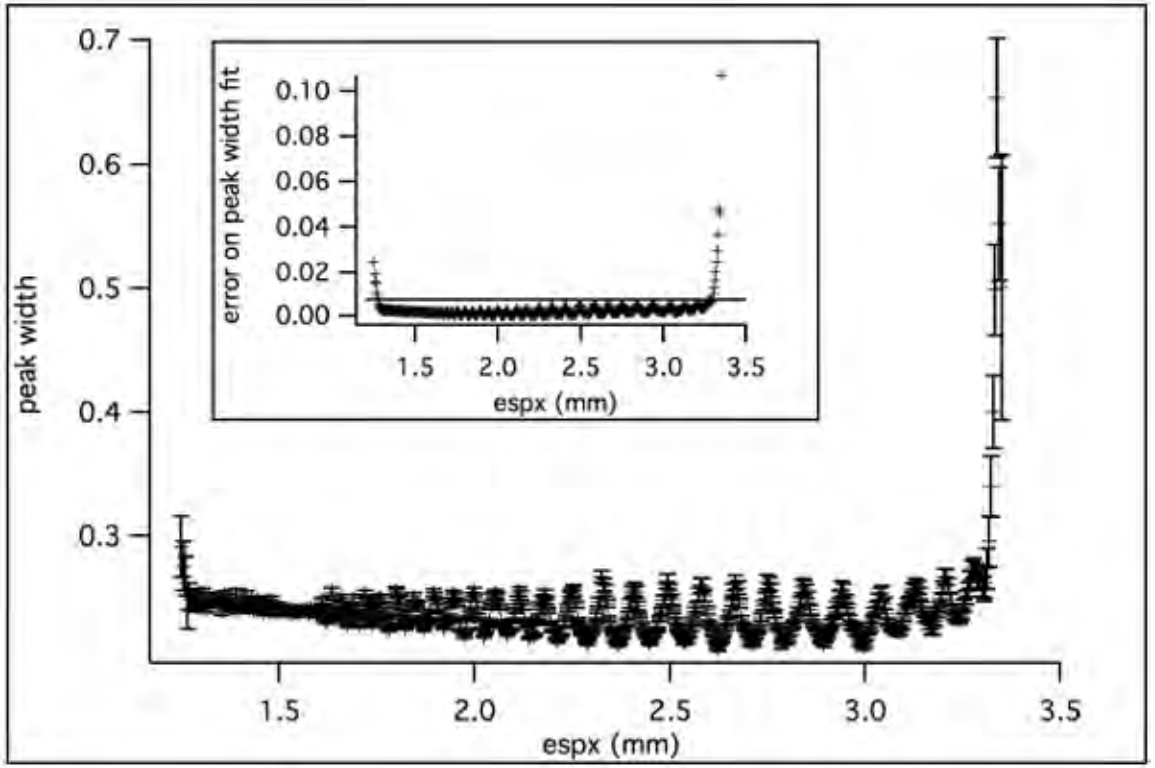
amorphous mineral in the periodic region?

Tested fits on the high resolution linescan through periodic region.
Decided to ***fix the fit peak position*** and let it struggle.



Inset: plot of fit error. Excellent proxy for amorphous peak...

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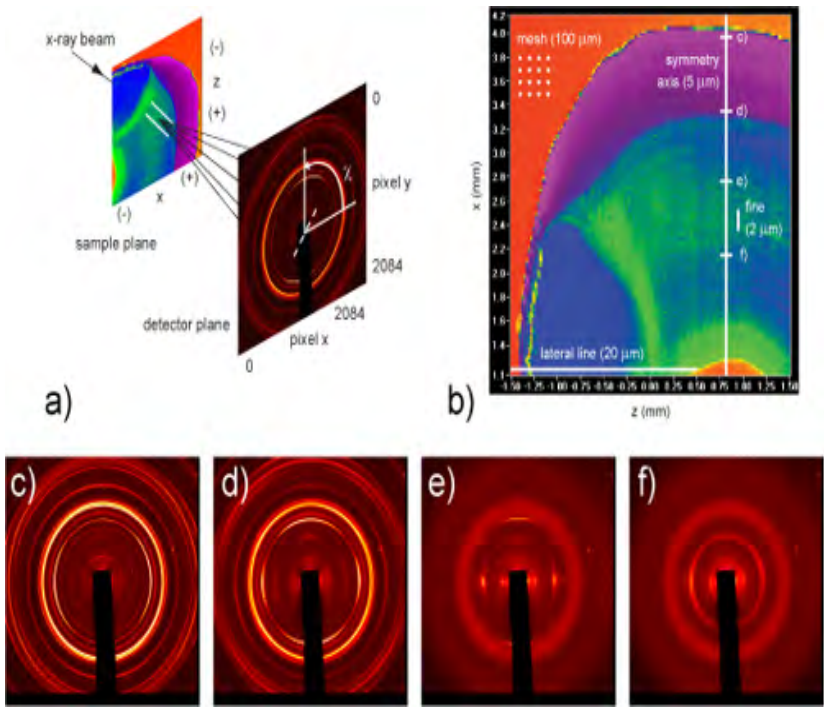
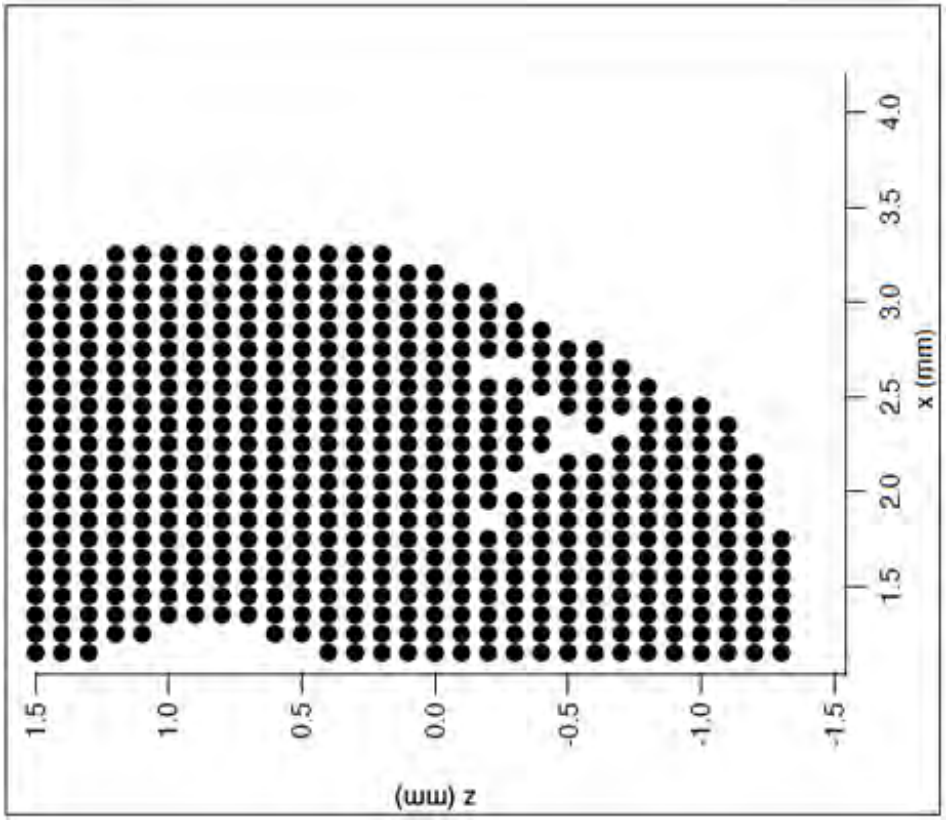
amorphous mineral in the periodic region!





... have mapped the amorphous content for whole area.

Lessons learned: (1) “wrong” fit can answer the right question, and (2) this HAS to be automated at NSLS-II.

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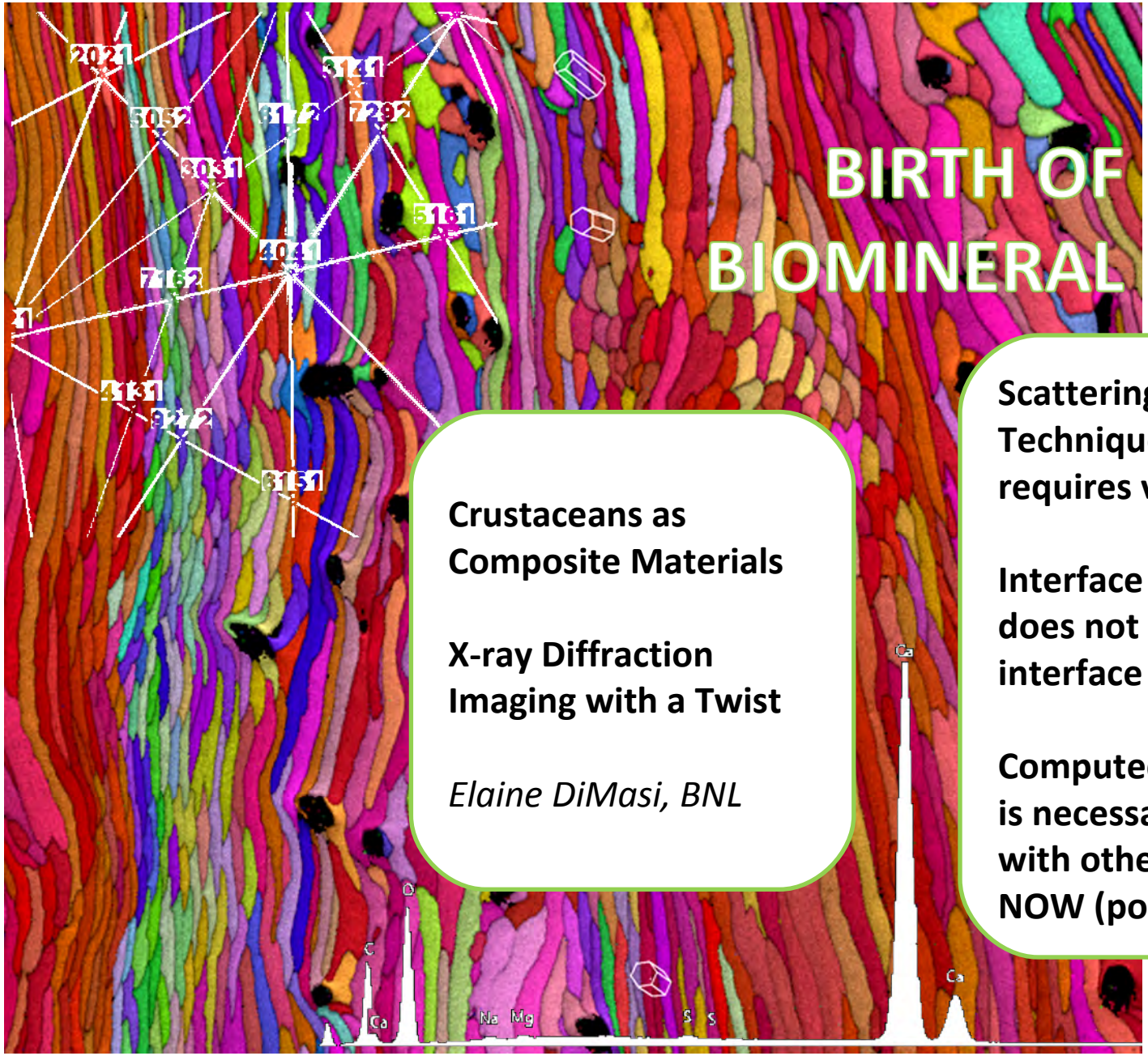

BIRTH OF BIOMINERAL

Crustaceans as Composite Materials

X-ray Diffraction Imaging with a Twist

Elaine DiMasi, BNL

Scattering in the Multi Technique toolkit requires visualization

Interface scattering does not always mean interface geometry

Computed tomography is necessary to catch up with other facilities NOW (pores!)

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