Diffraction Analyses of Mineralized Tissue

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Outline

- Bone and tooth – hierarchy of structures
- Internal strain measurements – approach in mineralized tissues - carbonated hydroxyapatite (cAp) - based.
- Example - Internal strains vs position across the dentino-enamel junction (DEJ) & applied load.
- Example - Elastic modulus vs anatomical position.
- Diffraction tomography – approach.
- Example - trabecular bone sample.
- Examples – Al/SiC composite, mineralized byssus.
- Future

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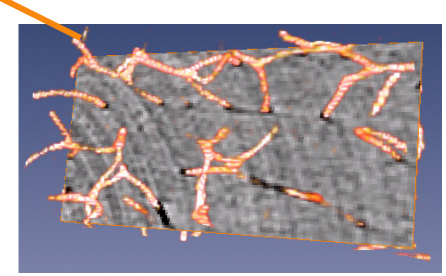
Why care about bone (tooth)?

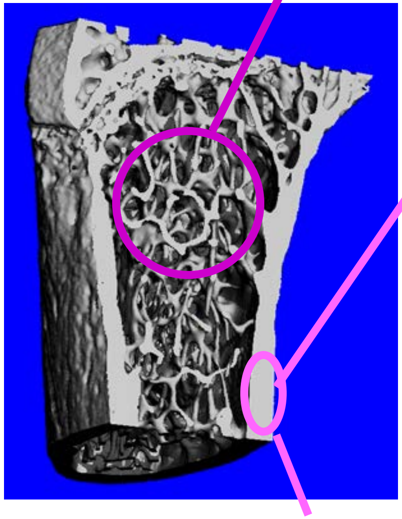
- Osteoporosis major morbidity, mortality issue for aging populations.
- Critical sites (trabecular bone primarily):
  - Femoral neck, head
  - Vertebrae (collapse)
- Clinical assessment: Bone mineral density (BMD) predicts only a fraction of fractures.
- Add bone microarchitecture: Improved prediction.
- Stochastic, environmental effects: NO.
- Bone “quality” invoked. Largely undefined.
- Tooth: How does dentino-enamel junction (DEJ) work (~3.5x difference in moduli)?

Hierarchy of structures in bone

3D canal network

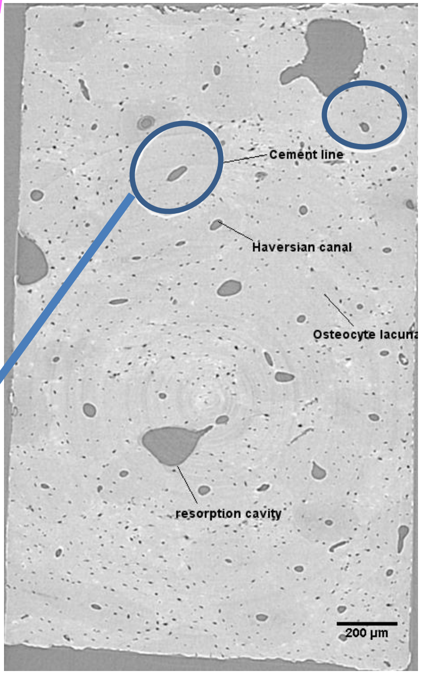


spongy

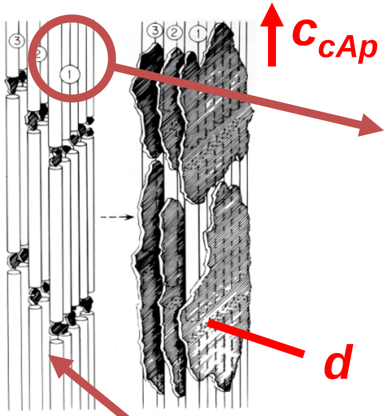


compact

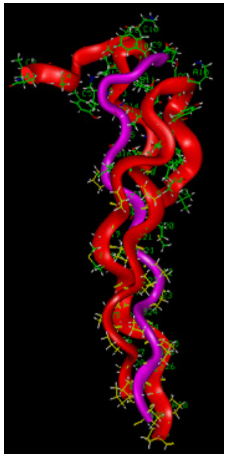
remineralized osteons



nucleation (left) & growth (right)



collagen triple helix



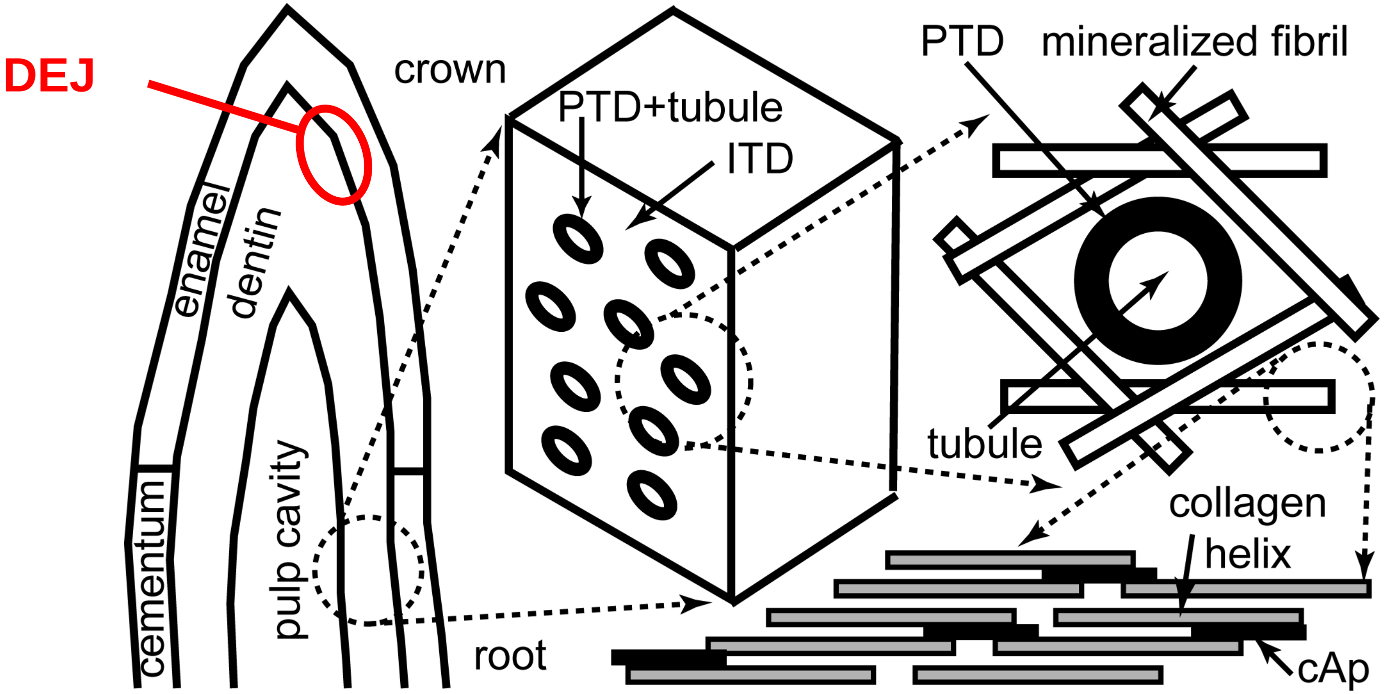
collagen fibrils (tubes)

D period

\+ apatite (cAp) xtals (plates)

d-spacing

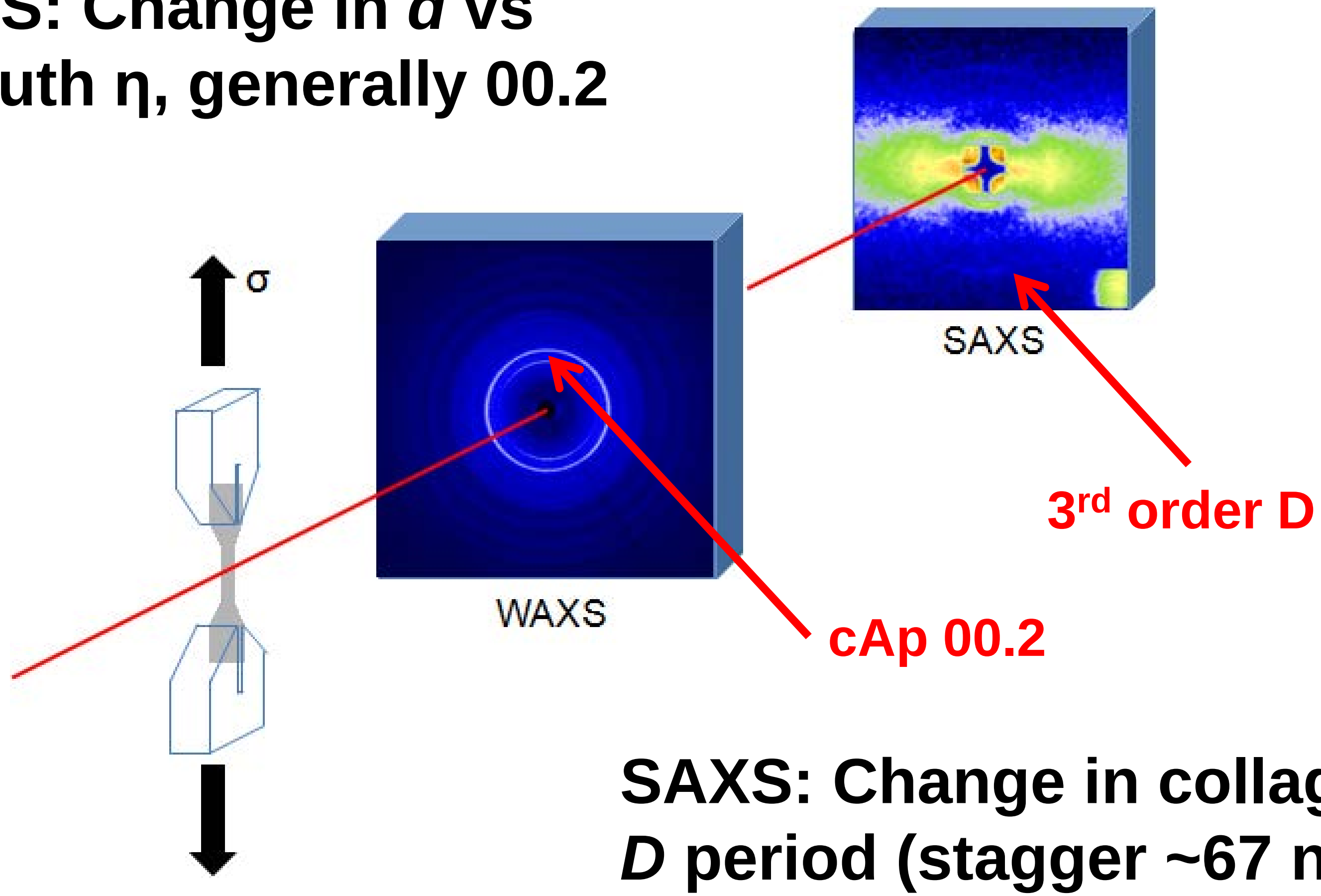
Tooth structure



- Enamel – cAp ceramic. Dentin – collagen/cAp composite.
- Intertubular dentin (ITD) and peritubular dentin (PTD).
- PTD nominally hypercalcified relative to ITD.
- Tubules 1-2 µm diameter, 5-10 µm spacing.
- Fibril orientations → cAp 00.2 preferred orientation.

High energy x-ray scattering: WAXS + SAXS

WAXS: Change in *d* vs azimuth η, generally 00.2

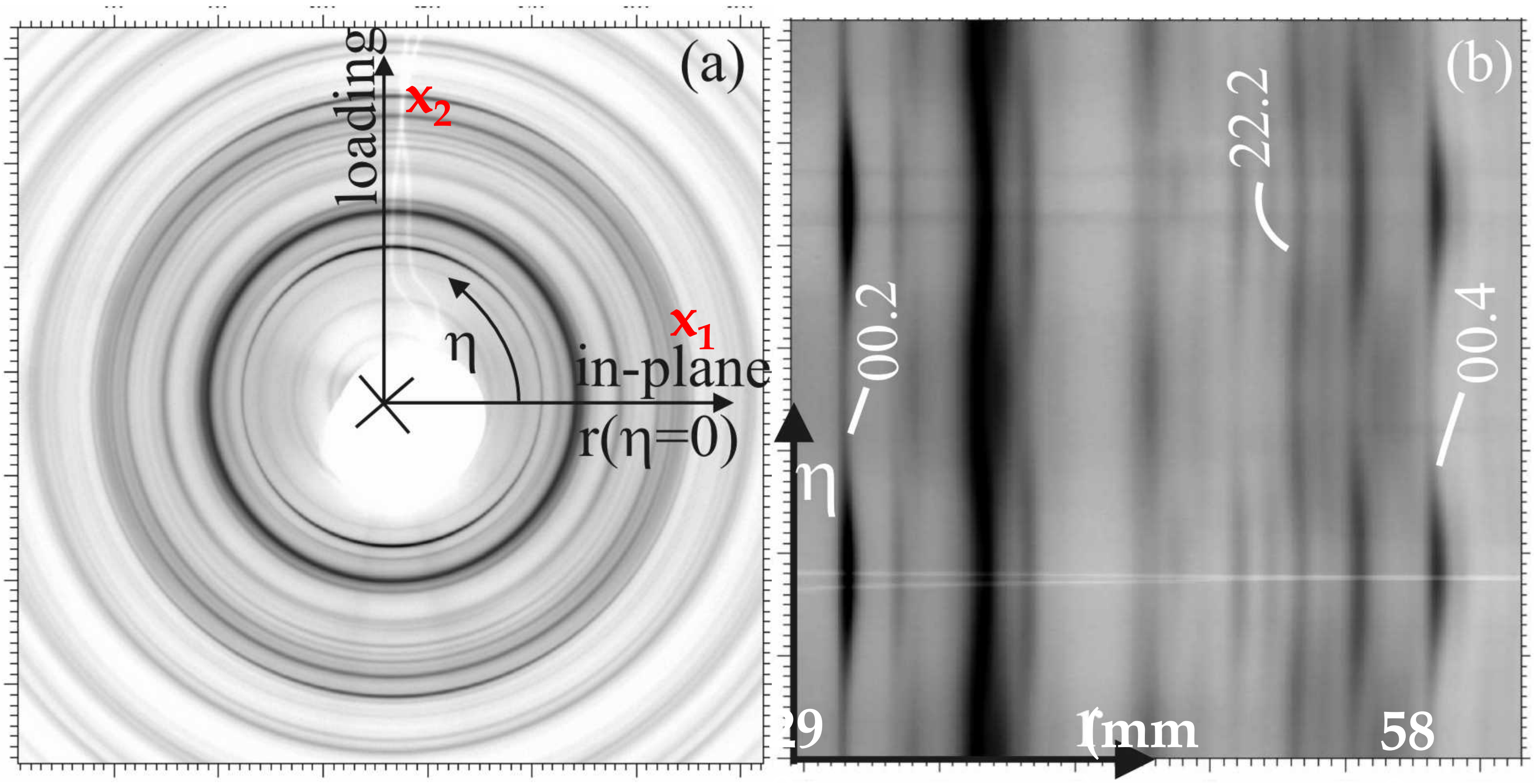


3rd order D

cAp 00.2

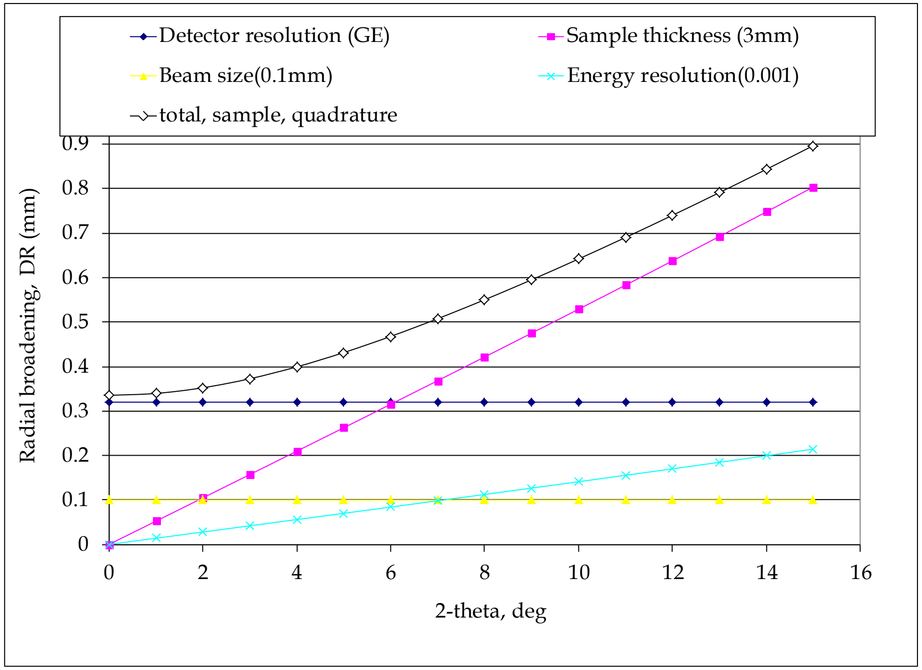
SAXS: Change in collagen *D* period (stagger ~67 nm)

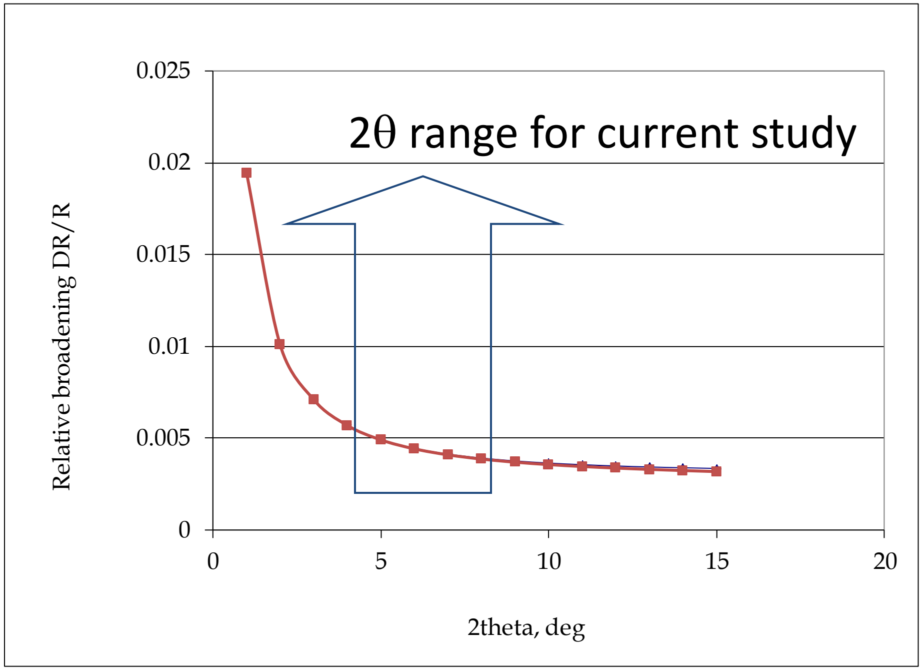
2D detector collection and transformation



Exposure time ~ 1-5 sc

Resolution limits for 2D detector setup



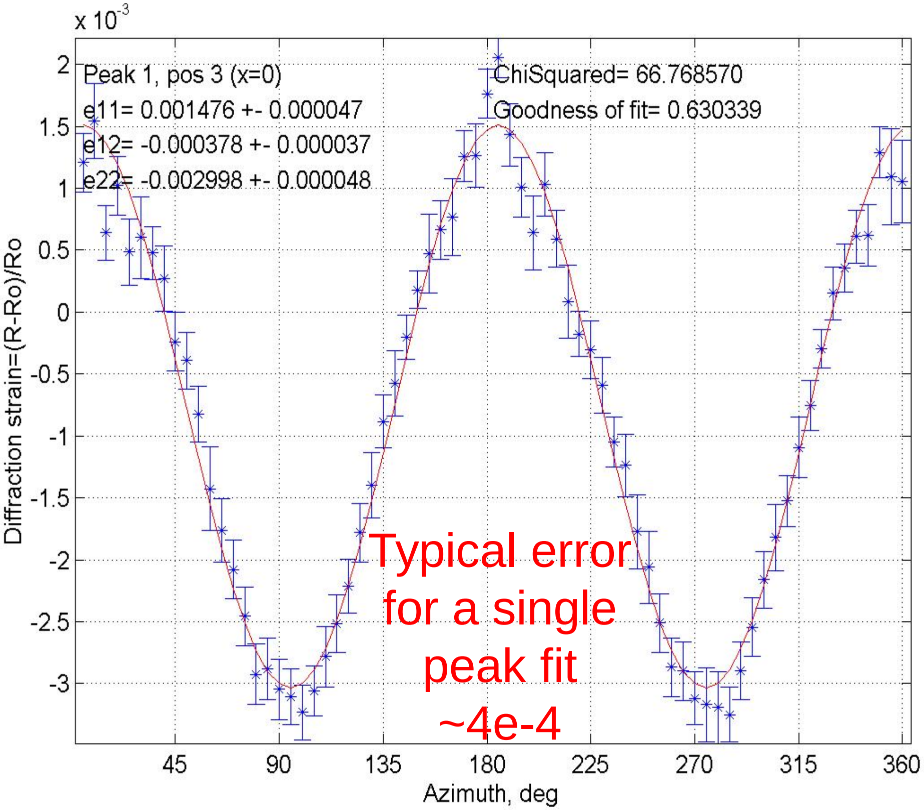

2θ range for current study

- 4 instrumental broadening contributions shown
- Typical values for these experiments provided (1m s-d distance)
- Quadrature broadening matches standard well (ceria or LaB6)

- ΔR/R~Δd/d gives instrumental ‘strain’ resolution
- Sample broadening will degrade this best-case resolution
- Peak fit accuracy is ~10x better than this resolution
- Typically Δd/d~10-4 for 2θ>5 deg

Peak fitting versus azimuth

R(002) vs azimuth, single load.
Normalized Data (blue) and fit (red) shown.



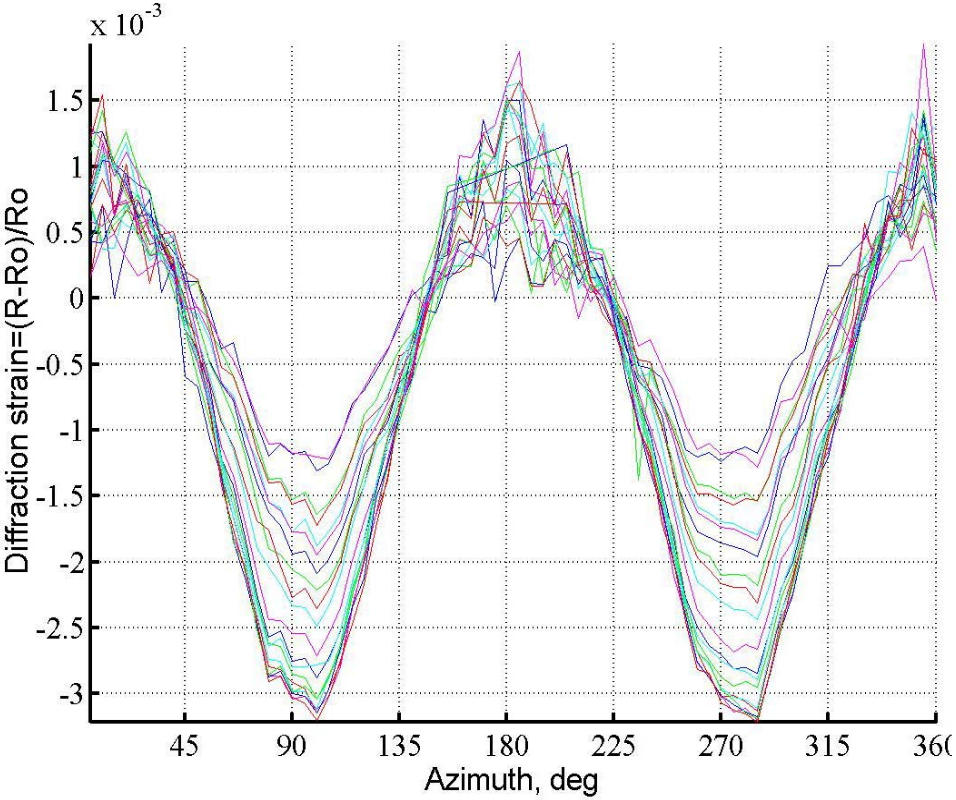
Typical error for a single peak fit ~4e-4

- **Data from 4 quadrants: azimuth η ~ ψ**
- **(small angles).**

- **Red line fits data, assumes linear *d* vs. $\sin^2\psi$ – good assumption here.**

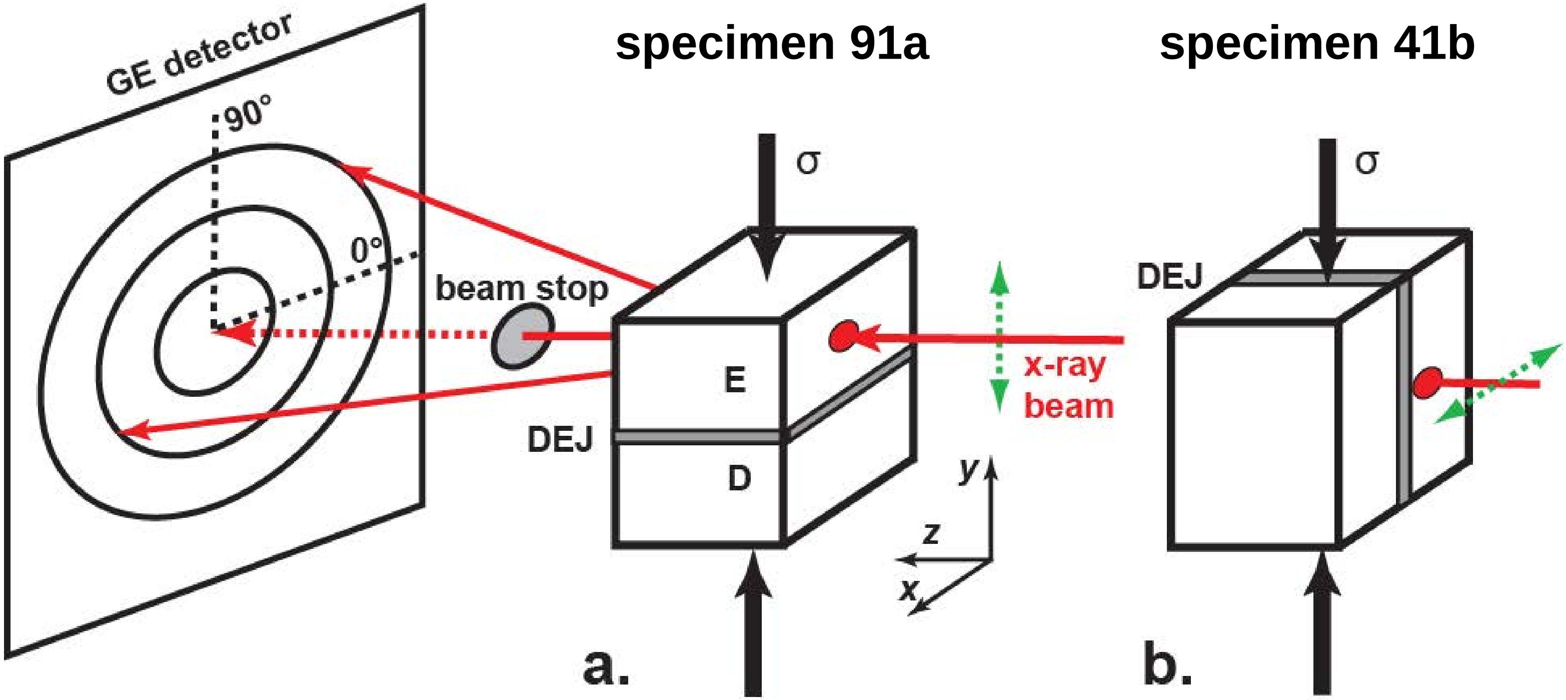
- **Use of many data pts (η) gives good precision,**
- **here ~5e-5**

Normalized R(002) vs azimuth, multiple loads (data only).



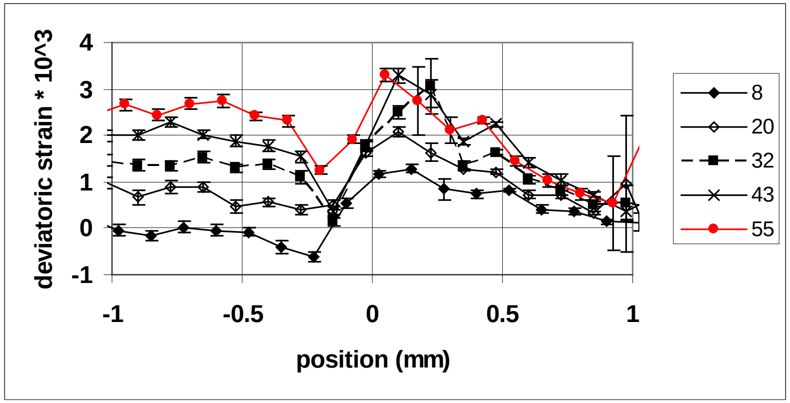
- **Overlaying different stress levels on same specimen allows cross-over to be established.**
- **Provides zero-point ‘strain’ reference to separate e11, e22**
- **Reduces systematic errors – good accuracy.**

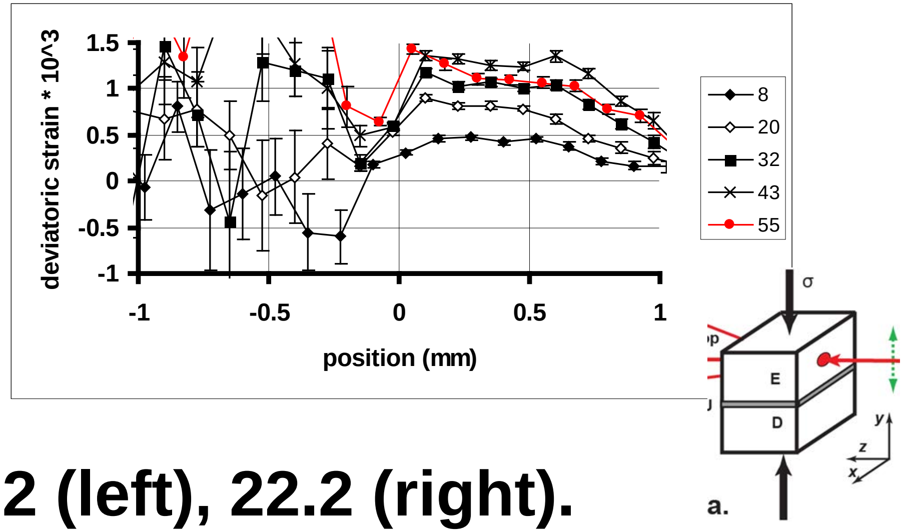
Strain gradients vs. applied stress across bovine dentinoenamel junction (DEJ)



J Biomech **43** (2010) 2294

Deviatoric strain vs position vs σ_{appl}

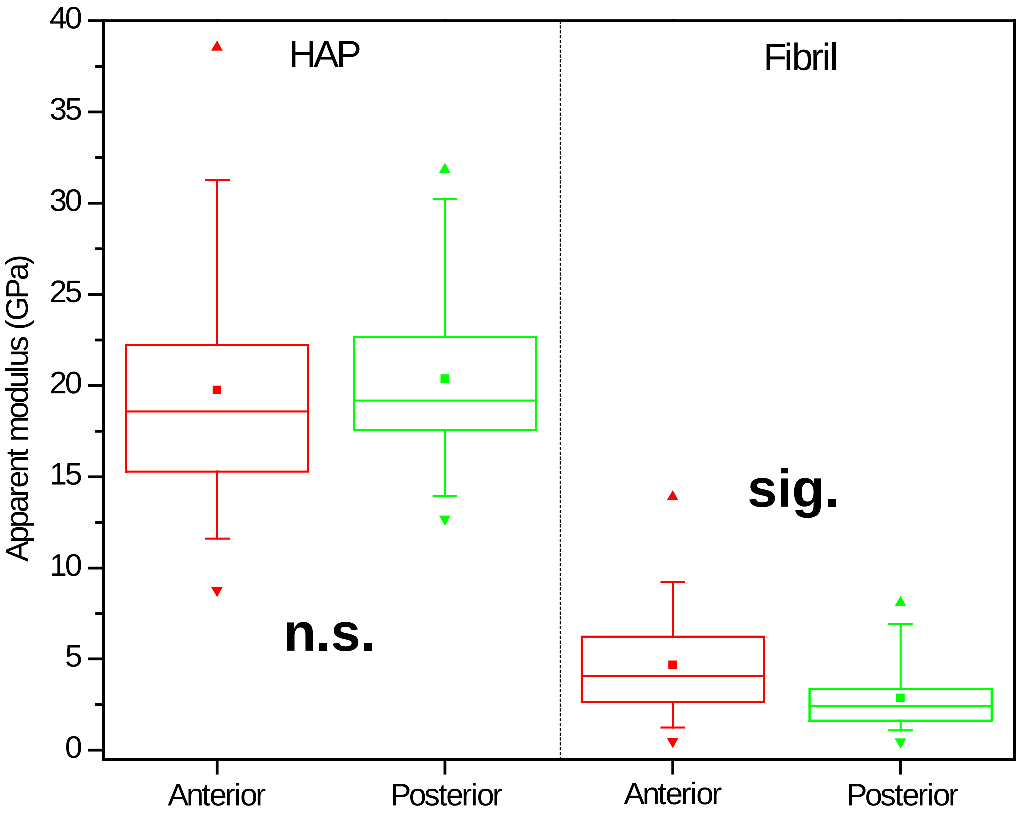




- Specimen 91a, cAp 00.2 (left), 22.2 (right).
- Positions -1 to 0 mm, dentin; 0 to 1 mm, enamel.
- 00.2: enamel, strong gradients rise with σ_{appl}; dentin, uniform increasing strain.
- 22.2: enamel, uniform rising strain near DEJ for $\sigma_{appl} \leq 43$ MPa, then drops (cracking?); dentin ?
- 00.2 dentin: E_{dentin} ~ 24 GPa.
- 22.2 enamel: E_{enamel} ~ 82 GPa.

Variation of cAp, fibril moduli with anatomical position in bovine femur

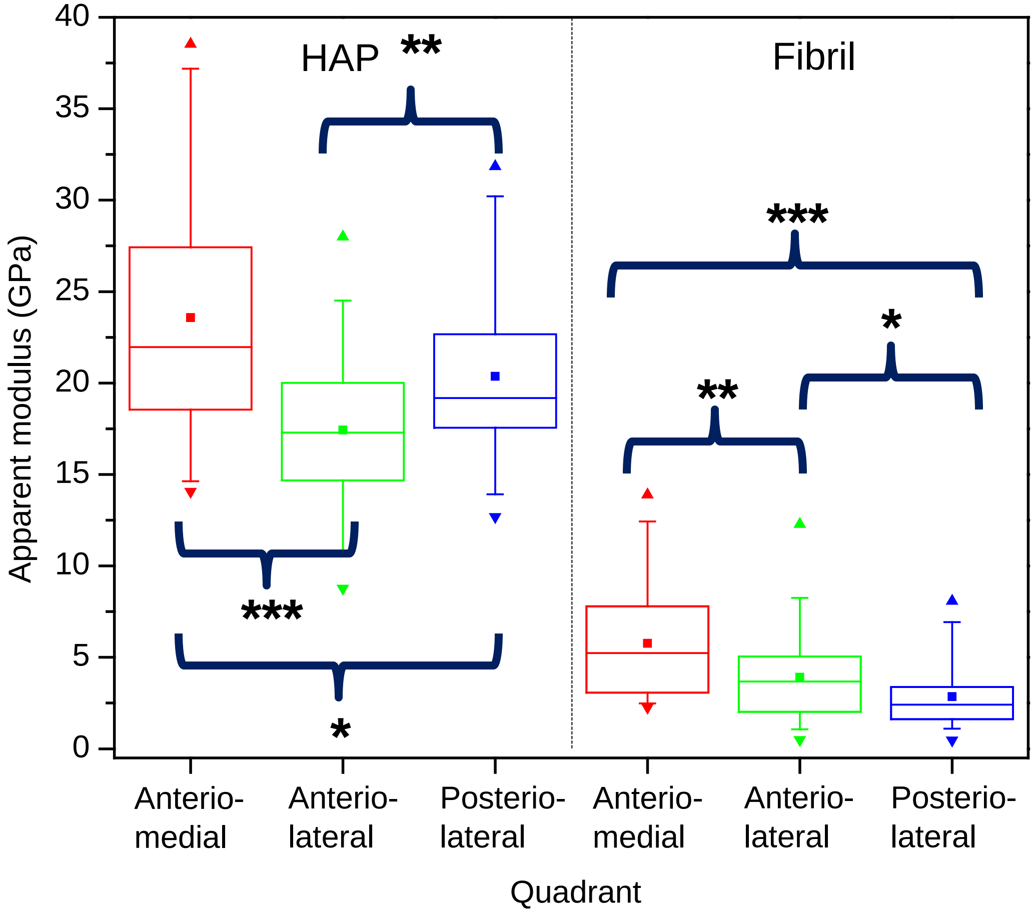
- HAP = cAp.
- Singhal et al. Adv Eng Mater 15 (2013) 238
- Apparent moduli: $E_{apparent} = \sigma_{applied} / \varepsilon_{x\text{-}ray}$ for cAp (WAXS) and fibril (SAXS).
- Measure multiple specimens from different quadrants of the same bone.
- Similar study on bovine dentin: Deymier-Black et al. J Mech Behav Biomed Mater 5 (2012) 71.



N = 80 45 68 33

Apparent moduli for anterior and posterior samples. Box = 75th, 50th and 25th percentiles, whiskers 95th and 5th percentiles, (■) mean, (▲▼) max and min values.

	p
***	**< 0.001**
**	**< 0.01**
*	**< 0.05**



N =	30	50	45	28	40	33

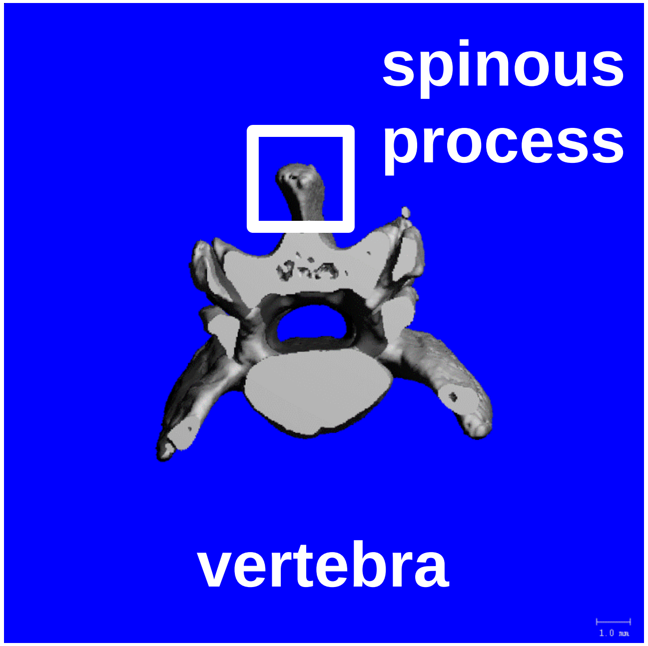
Quadrant-wise comparison of HAP and fibrillar apparent moduli.

Effect of radiation dose

- WAXS, SAXS patterns recorded in ~ 1s at 80 keV. Dose ~ 0.3-0.4 kGy.
- Currey et al. [1] sterilization protocol
  - ^{60}Co γ (1.17 + 1.33 MeV): Doses 17, 30, 95 kGy
  - Even 17 kGy, 50% reduction in work to fracture
- Kinney et al. [2] in vivo synchrotron microCT (rat)
  - 25 keV, 0.9 Gy/data set, bone response not affected
- Barth et al. [3] dose – response synch. X-rays
  - 20 keV, ~ 70 kGy dose suppresses plasticity

- [1] J Orthop Res 15 (1997) 111; [2] JBMR 10 (1995) 264. [3] Bone (in press)

Diffraction tomography of porcine spinous process



Cut piece of process

1-ID, APS: E=70 keV

beam 0.1mm (H) x ~0.05mm (V)

80 x @ 0.1mm/step, 6º rotation steps over 180º

5s/pt, ~10s between points

Hydra configuration: 4 GE panels + ion chamber (trans.)

GE1&3 – vertical & GE 2&4 – horizontal component

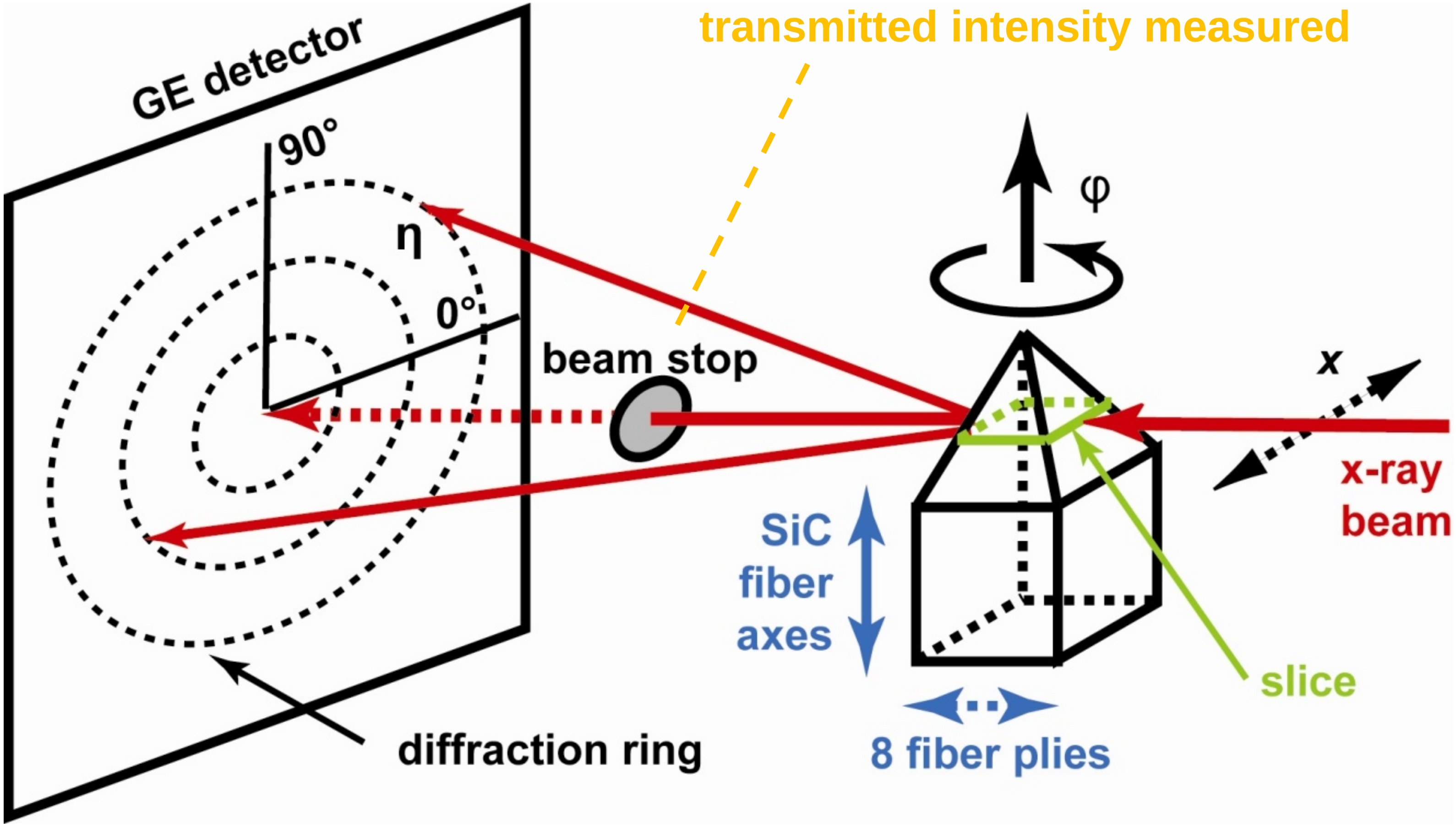
Reconstruct 3 cAp peaks for panel 3, 4: 22.2, 31.0, 00.4

Compare to lab microCT

High energy x-ray diffraction tomography

Use integrated diffracted intensity

Reconstruct with filtered back projection



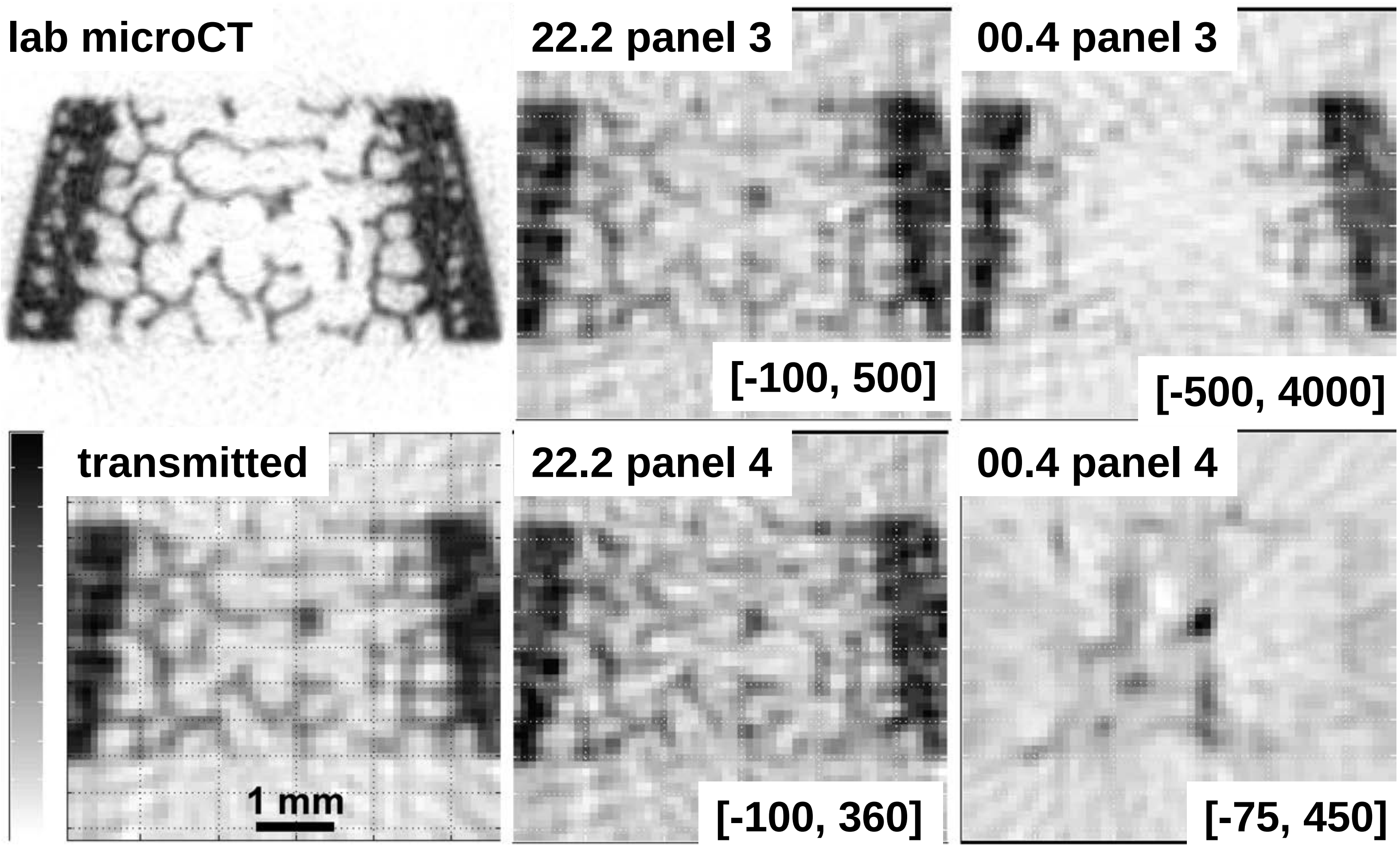
X-ray scattering tomography literature

- 1985. CT phantom. Harding et al. Phys Med Biol **30,** 183-186.
- 1998. Lamb chop (muscle, fat, bone). Kleuker et al. Phys Med Biol **43,** 2911-2923.
- 2001. Synthetic hydroxyapatite bone phantom. Barroso et al. Nucl Instrum Meth A **471,** 75-79.
- 2008. Bone. Stock et al. J Struct Biol **161,** 144-150.
- 2008. Carbon. Bleuet et al. Nature Mater **7**, 468-472.
- 2010. Cement. Artioli et al. Anal Bioanal Chem **309,** 2131-6.

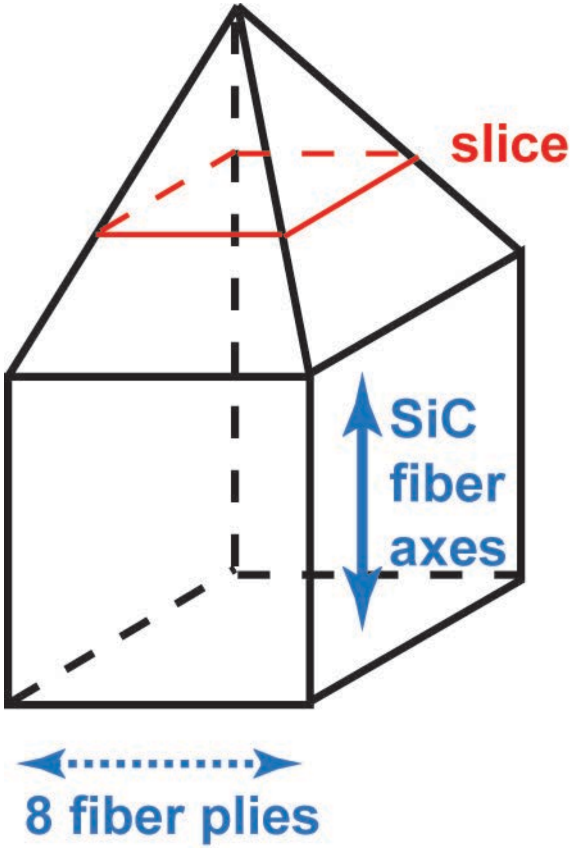
…

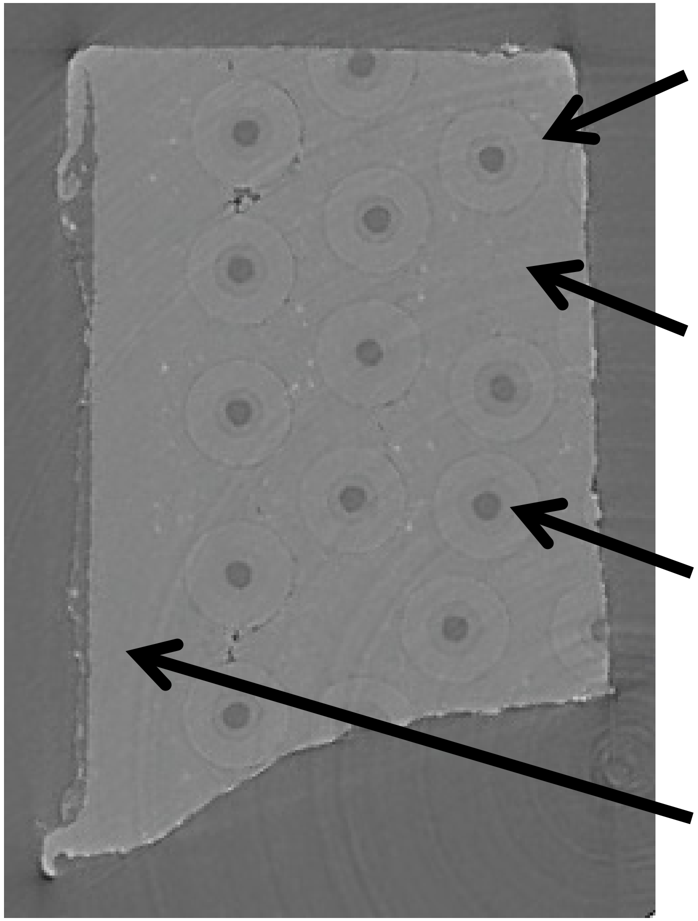
- 2012. Al/SiC. Stock, Almer, J Appl Cryst **47,** 1077-83.
- 2012. Review. Alvarez-Murga et al. J Appl Cryst **47,** 1109-24.

dark = greater signal, linear gray scale [min, max]



Al - SiC uniaxially aligned monofilaments





SiC sheath
~140 µm dia.

AA 6061
matrix

C core
~30 µm dia.

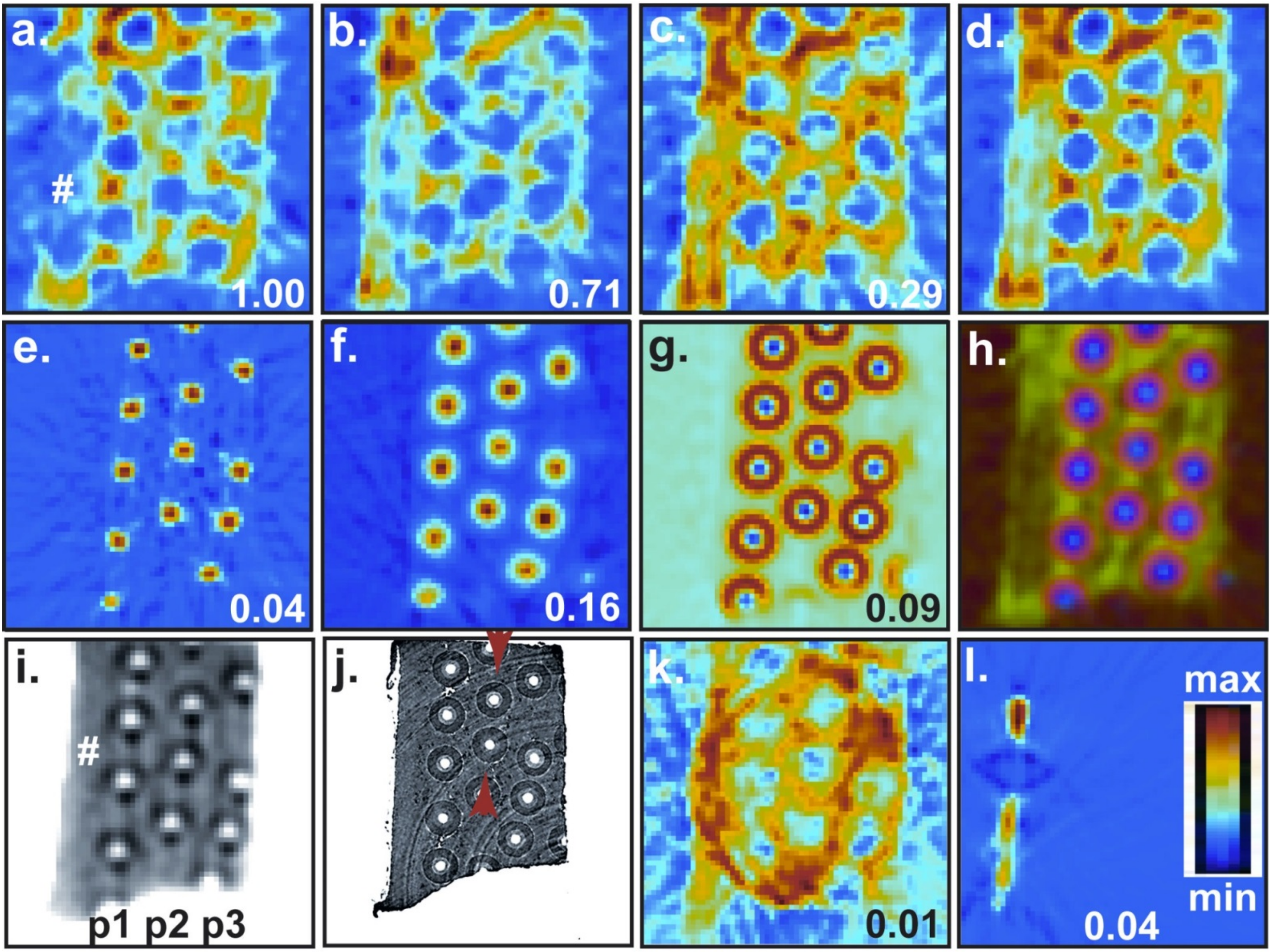
AA 1100
cover sheet

Stock, Almer, J Appl Cryst **47** (2012) 1077-1083.

62 x 62 voxels (15 µm in-plane).

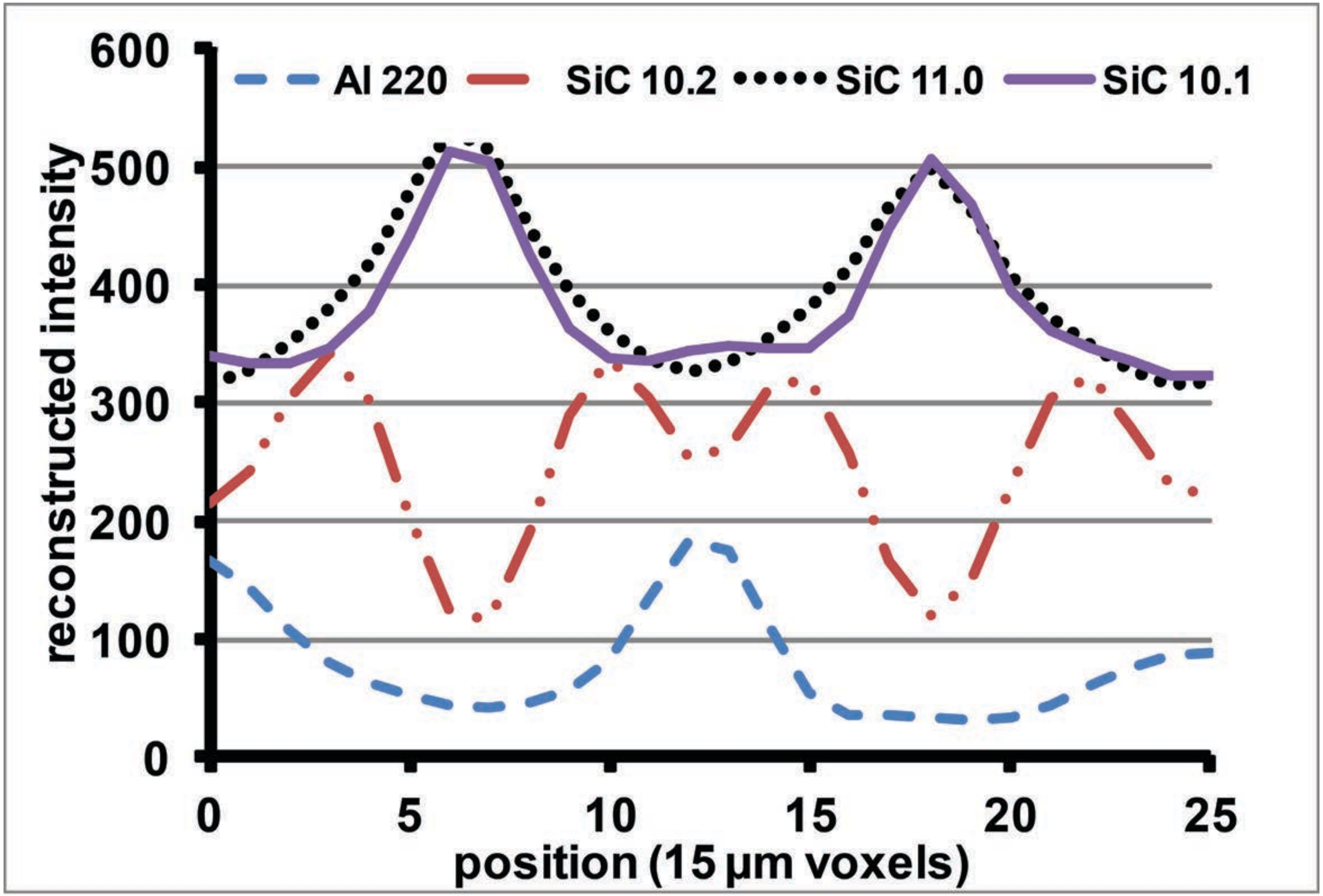
Linear color bar shown in (l).

Relative “intensities” given in lower right corner of each panel.



(a) Reconstruction with Al 111; (b) Al 200; (c) Al 220; (d) Sum of the (a-c) Al slices. (e) Reconstruction with SiC 10.1; (f) SiC 11.0; (g) SiC 10.2; (h) Sum of Al slices (green) and SiC 11.0 slice (blue) and SiC 10.2 slice (red). (i) Transmitted intensity slice. (j) 2-BM, APS, matching slice (1.45 µm voxels). In (i-j), black highest, white lowest absorption. (k) Reconstruction with *d* = 2.3 Å and (l) *d* = 4.15 Å (impurities?).

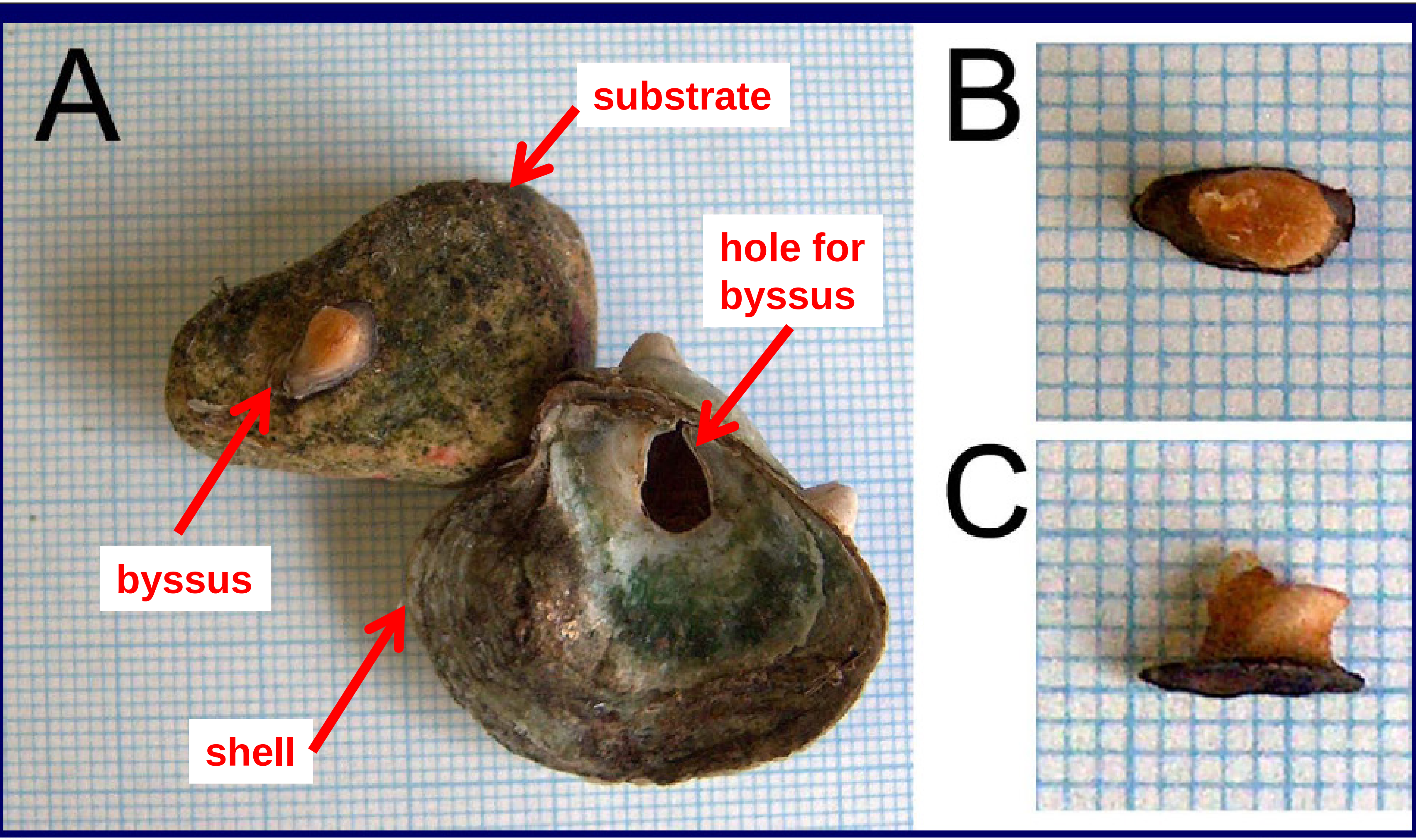
Profiles across two fibers. SiC 10.2 shows outer fiber texture. SiC 11.0 and 10.1 show inner fiber texture. C cores obscured by long direction of beam.



Diffraction tomography of mineralized byssus (attachment system for *Anomia*)

- H. Leemreize, H. Birkedal, Aarhus Univ.; J. D. Almer, APS; SRS
- Underwater attachment: challenging materials issue.
- Many bivalves use protein byssi.
- *Anomia* uses mineralized byssus; combination of calcite and aragonite, two forms of calcium carbonate.
- Mg content of calcite varies spatially.
- Control of polytypes in *Anomia* of may provides information on biomineralization process.
- Use diffraction tomography.

Anomia simplex byssus and shells

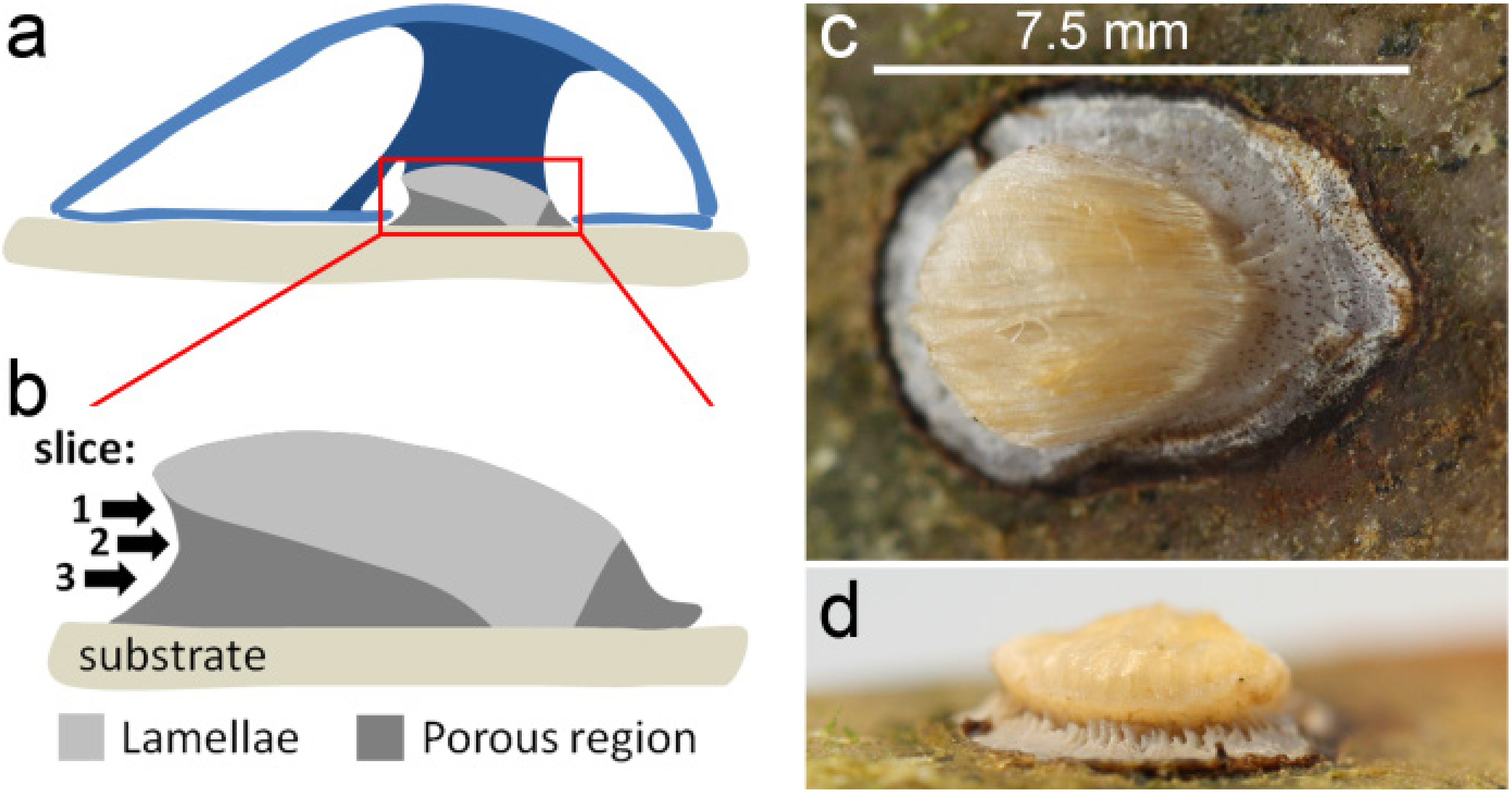


Eltzholtz & Birkedal *J. Adhesion*, **2009**, *85*, 590

Mineralized byssus of *Anomia sp.*

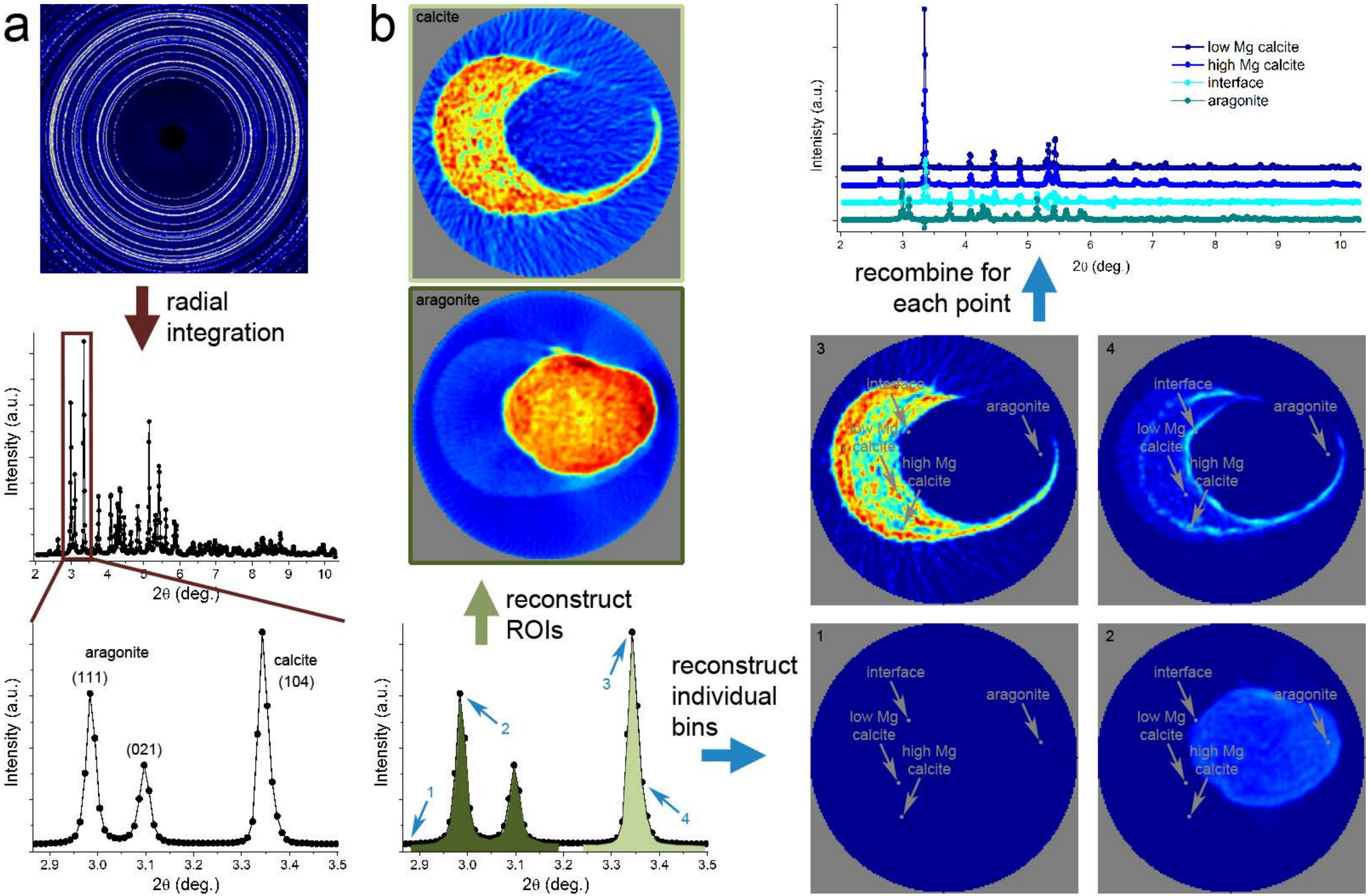
in (a) muscle dark blue, shell light blue

in (c,d) lamellae yellow, porous region gray



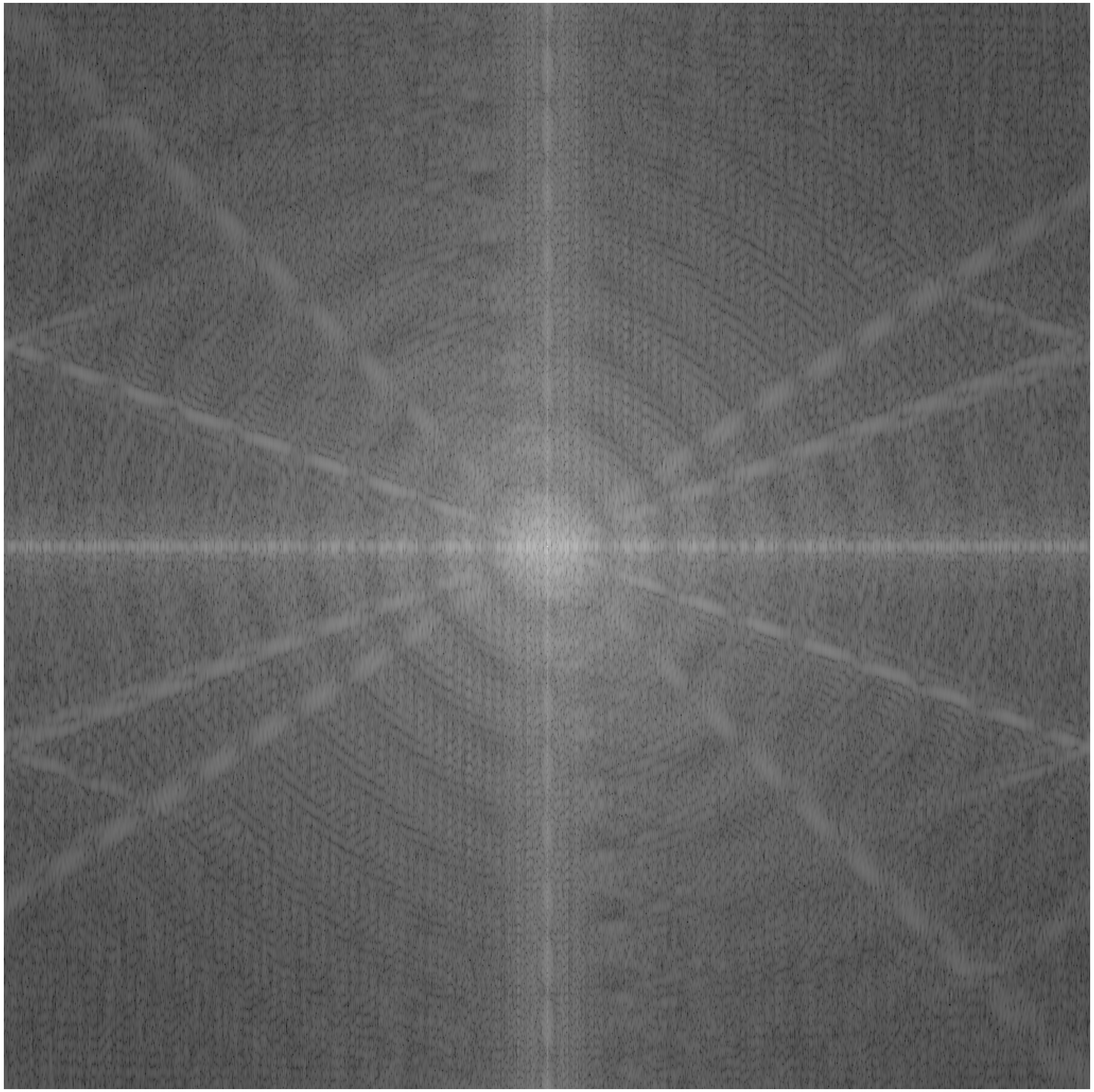
Reconstruction of diffraction pattern pt. by pt.

In calcite, Mg ↑, *d* ↓ and 2θ ↑.



Discussion, Future

- Not a strictly valid reconstruction approach. Different grains diffract at different angles.
- Seems to work OK if there is not too much texture and if adequate numbers of grains are present.
- Local Mg content can be determined.
- Just collected byssi data with 20 µm, 10 µm voxels. Reconstruction underway.
- Can we extract strain and texture vs position? (Ex. of diffraction tomography of trabecular bone).
- Would algebraic reconstruction technique be better?



Thank you.