



# Regularized phase retrieval algorithms for X-ray phase tomography of 3D bone cell culture analysis

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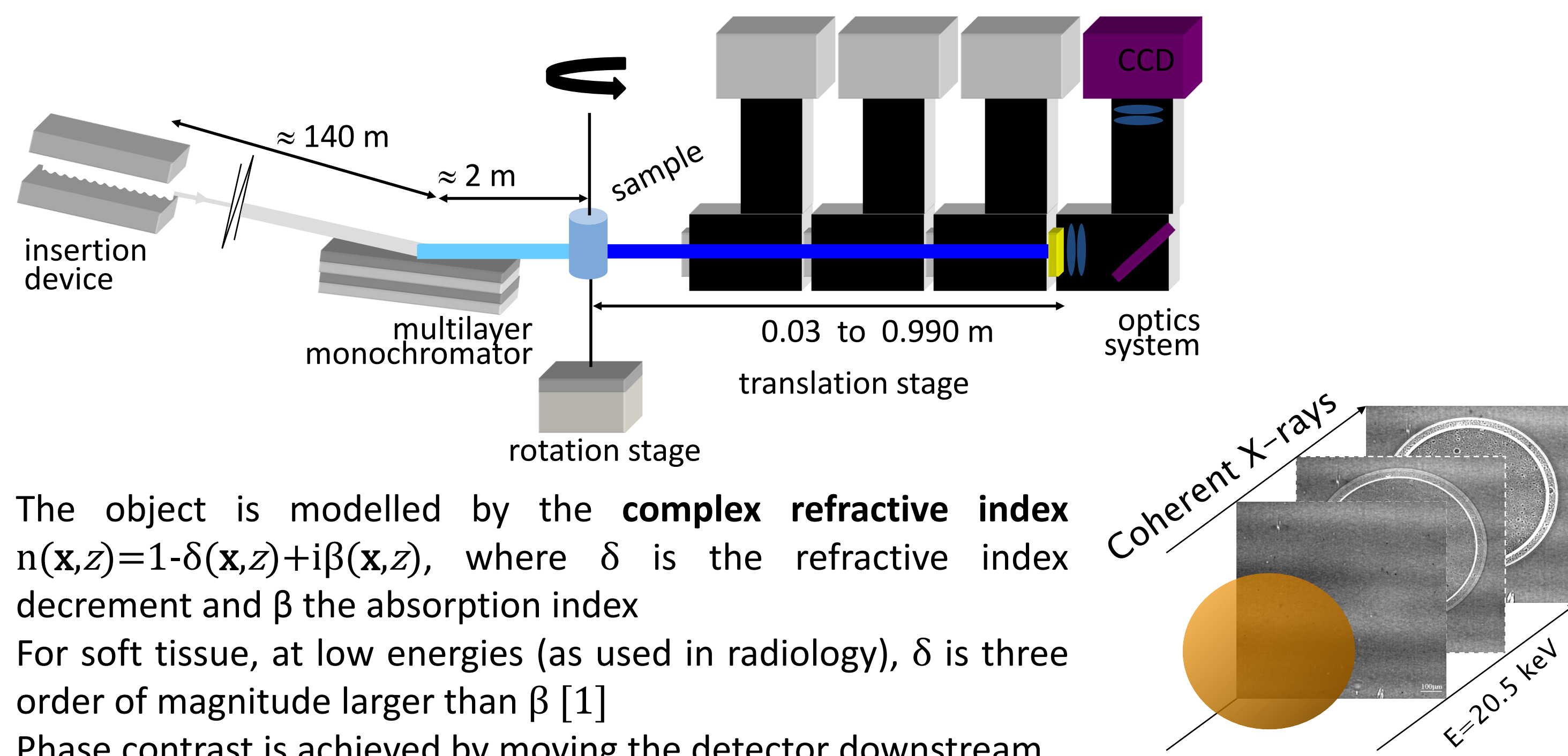
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## In-line phase contrast tomography

### Setup and image formation



- The object is modelled by the **complex refractive index**  $n(\mathbf{x}, z) = 1 - \delta(\mathbf{x}, z) + i\beta(\mathbf{x}, z)$ , where  $\delta$  is the refractive index decrement and  $\beta$  the absorption index
- For soft tissue, at low energies (as used in radiology),  $\delta$  is three order of magnitude larger than  $\beta$  [1]
- Phase contrast is achieved by moving the detector downstream
- Attenuation  $B$  and phase shift  $\varphi$  induced by the object described as projections perpendicular to the propagation direction  $\vec{z}$  (note that  $\mathbf{x} = (x, y)$  represents the coordinates in the real domain and  $\mathbf{f} = (f_x, f_y)$  the coordinates in the Fourier domain).

$$B(\mathbf{x}) = \frac{2\pi}{\lambda} \int \beta(\mathbf{x}, z) dz \text{ and } \varphi(\mathbf{x}) = \frac{2\pi}{\lambda} \int \delta(\mathbf{x}, z) dz$$

- At each angle  $\theta$ , the interaction between the objet and the X-ray can be described as a transmittance function

$$T_\theta(\mathbf{x}) = \exp[-B_\theta(\mathbf{x}) + i\varphi_\theta(\mathbf{x})]$$

- The free space propagation over a distance  $D$  can be modelled by the Fresnel propagator  $P_D$  (**Fresnel diffraction**)

$$T_{\theta,D}(\mathbf{x}) = (T_\theta * P_D)(\mathbf{x})$$

- The intensity recorded by the detector at a distance  $D$  is

$$I_{\theta,D}(\mathbf{x}) = |T_{\theta,D}(\mathbf{x})|^2$$

- In **holotomography** [2], images are acquired at:
  - **several source-to-sample distances**
  - **under different angles of rotations**
- Phase projections** are obtained by combining these multi-distance projections for each angle  $\theta$  using phase retrieval.

### Image reconstruction

- A two-step process: phase retrieval and tomographic reconstruction
  - Phase retrieval:** calculate the phase shift  $\varphi_\theta(\mathbf{x})$  from the phase contrast images at different propagation distances  $\{I_{\theta,D}(\mathbf{x}), D=D_1 \dots D_n\}$ 
    - Ill-posed inverse problem, which requires regularization
    - Usually solved by least-square method
  - Tomographic reconstruction:** reconstruct the 3D refractive index image from the estimated phase shifts  $\hat{\varphi}_\theta(\mathbf{x})$  at different projection angles  $\theta$ 
    - Usually solved by filtered back-projection (FBP).

### Phase retrieval methods

- Least-square method (LSQ)**, based on the Contrast Transfer Function

$$\tilde{\varphi}(\mathbf{f}) = \frac{\sum_D \sin(\pi \lambda D |f|^2) [\tilde{I}_D(\mathbf{f}) - \delta_{Dirac}(\mathbf{f})]}{[\sum_D 2 \sin^2(\pi \lambda D |f|^2)] + \alpha}$$

- Advanced regularized method ("quasi-iterative methods")**

- $\hat{\varphi}_\theta(\mathbf{x}) = \arg \min_{\psi} \sum_D [|\tilde{I}_{\theta,D}(\mathbf{x}) - I_{\theta,D}(\mathbf{x})|^2 + \alpha |\psi_\theta(\mathbf{x}) - \psi_{\theta,0}(\mathbf{x})|^2]$
- Designing the prior

#### Homogeneous objects

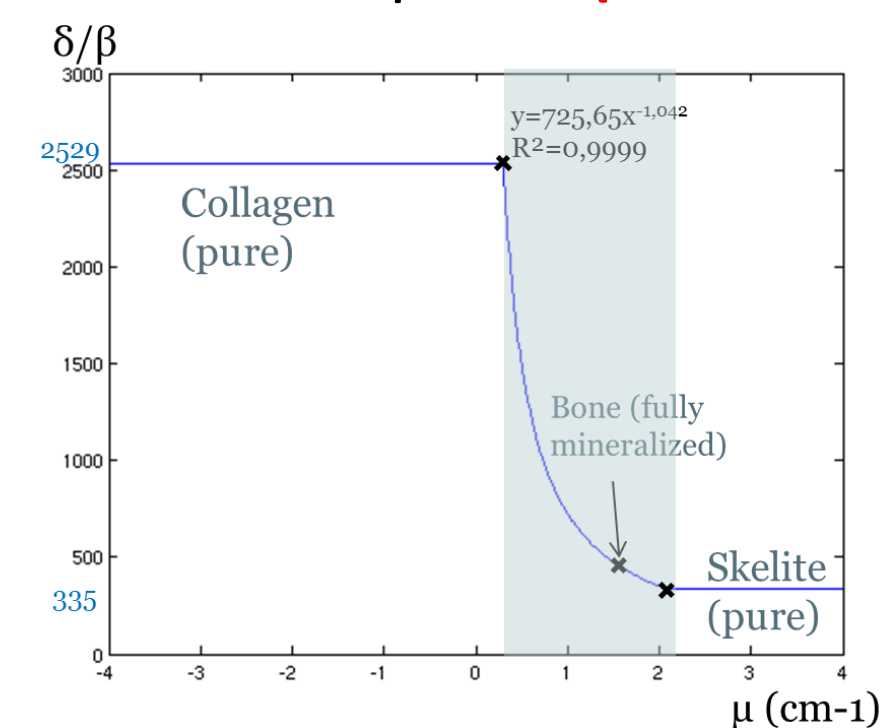
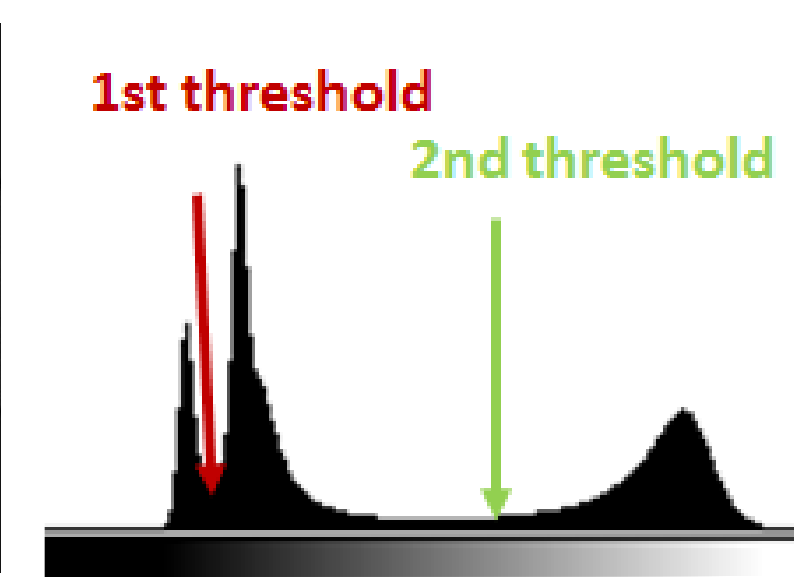
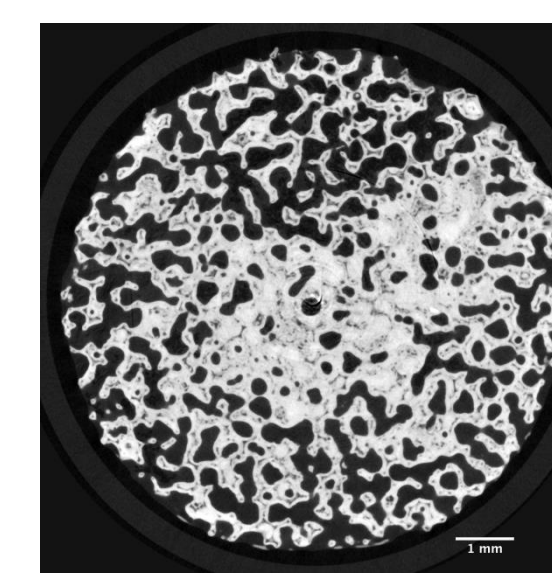
- Can be introduced in projections
- Paganin[3]:** proportionality between phase and attenuation (**method P**)
- Langer[4]:** *prior* introduced in the regularization (**method A**)

$$\varphi(\mathbf{x}) = -\frac{\delta}{2\beta} \ln(\mathcal{F}^{-1} \left\{ \frac{\mathcal{F}\{I_D(\mathbf{x})\}}{1 + \frac{\lambda D \delta}{4\pi\beta} \|f\|^2} \right\})$$

$$\varphi_{\theta,0}(\mathbf{x}) = f(\mathbf{x}) \frac{\delta}{2\beta} \ln[I_{\theta,D}(\mathbf{x})]$$

#### Heterogeneous objects

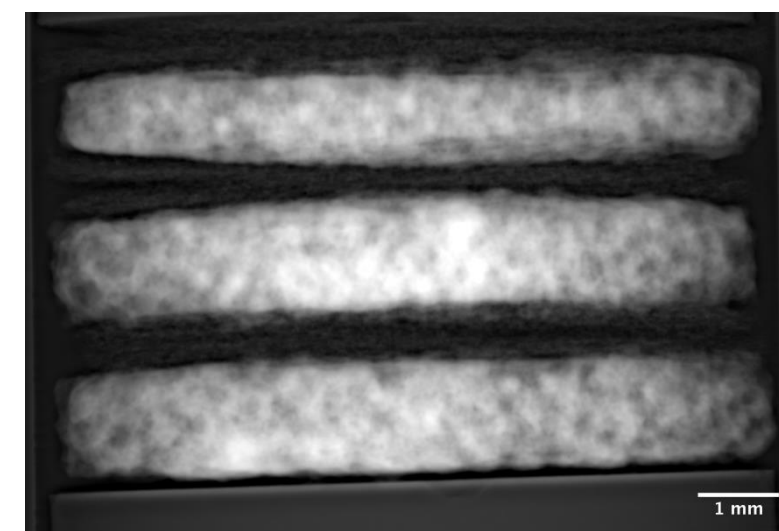
- Must be introduced in object domain
- Langer[5]:** Thresholding of the attenuation scan → **Bi-material object (methods B & C)**
- Langer[6]:** **Functional relationship** between the attenuation and the phase (**method D**)



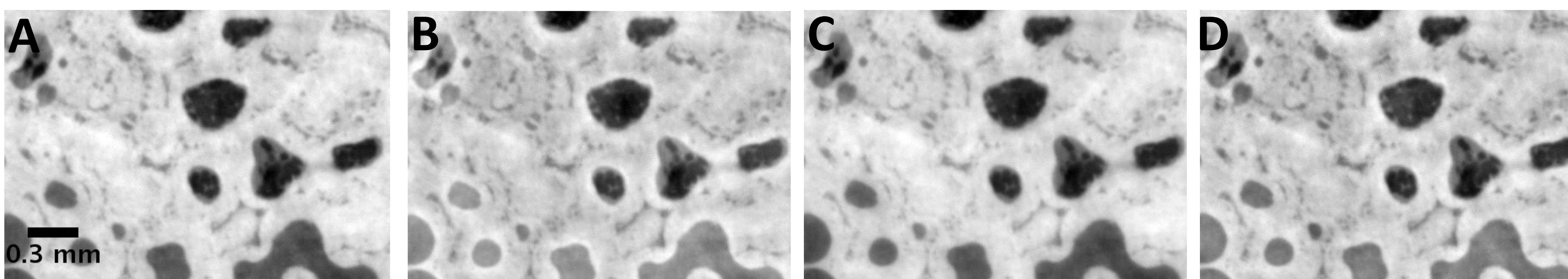
## Application to 3D cell cultures

### Material and methods

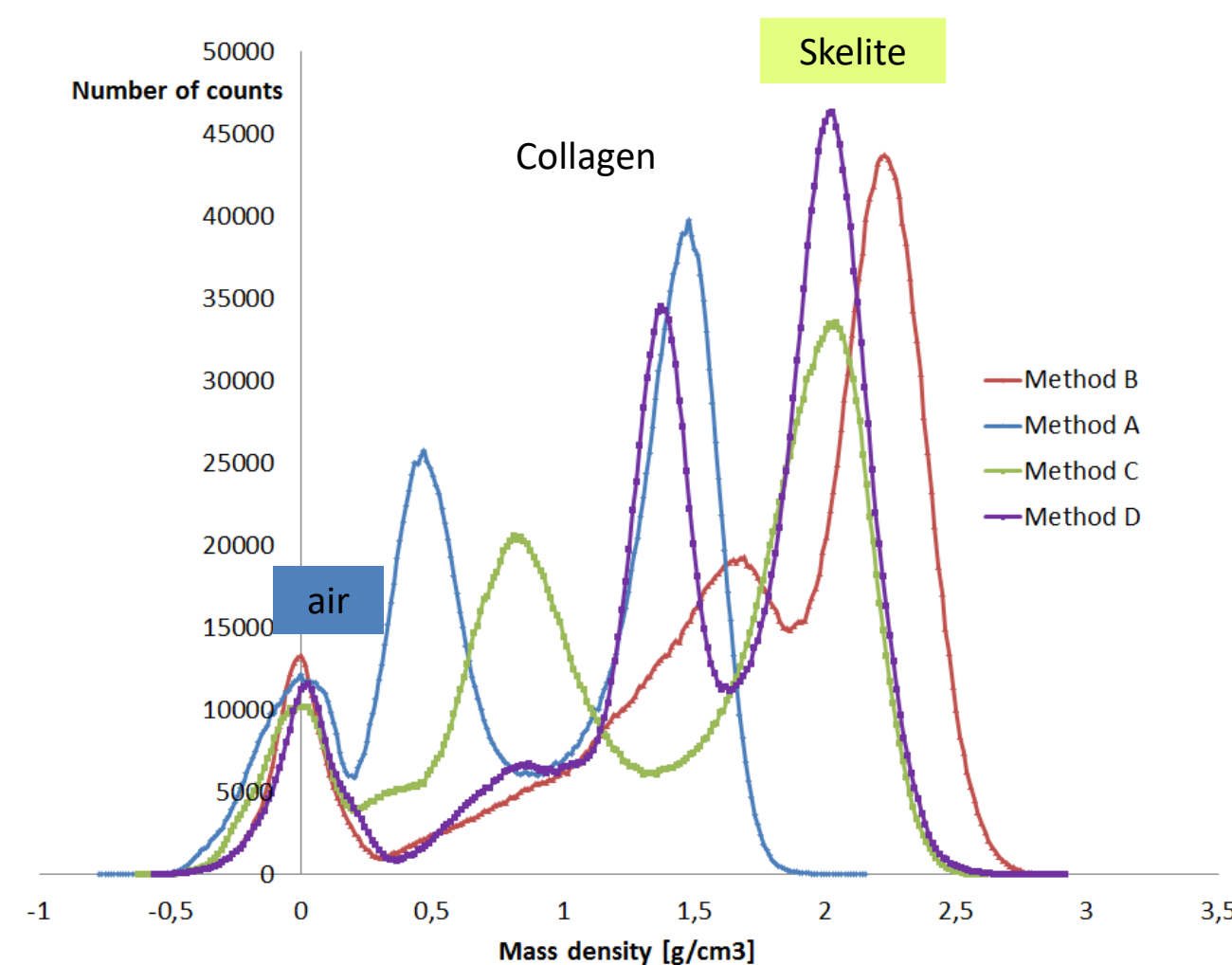
- Biomaterial (Skelite®) used for 3D cell culture and artificial bone scaffolds in surgery (coll. R Cancedda, University of Genova) [7]
- Composed of Si-TCP(67%) and  $\beta$ -TCP/HA (33%)
- 9 scaffolds imaged: discs of 9 mm diameter, seeded with bone cells
- Experimental conditions:** 2000 projections, 30 keV, 4 sample-to-detector distances, pixel size=5  $\mu$ m, Field of view = 10.2 mm
- Reconstruction methods** : see above



### Results



Crop of a reconstructed slice, using homogeneous approach [A], heterogeneous approach with 1<sup>st</sup> threshold [B] or 2<sup>nd</sup> threshold [C], and heterogeneous approach with a functional relationship [D].

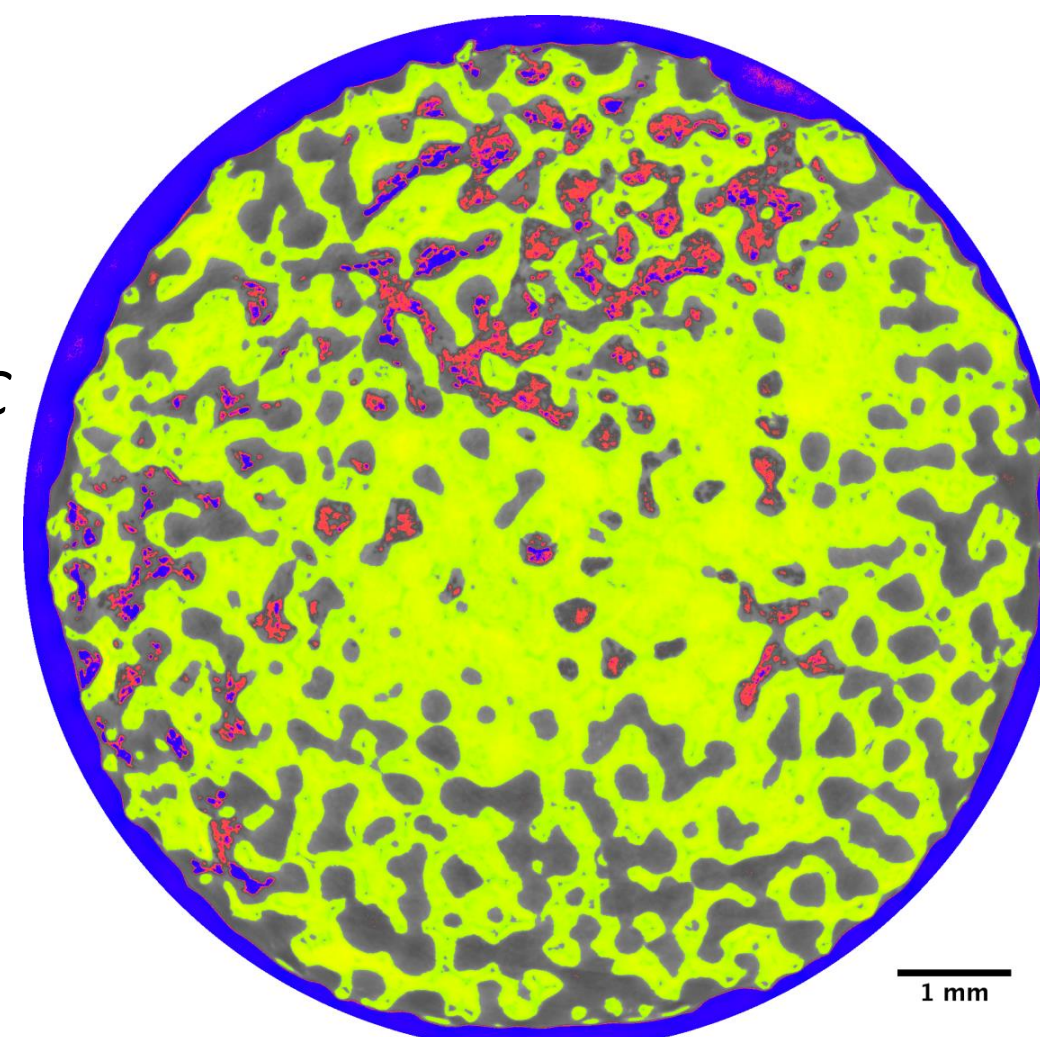


Histograms of volumetric mass densities of the reconstructed slice with the 4 methods

Theoretical vol. mass densities:

- $\rho(\text{air}) = 1.3 \times 10^{-3} \text{ g/cm}^3$
- $\rho(\text{Collagen}) = 1 \text{ g/cm}^3$
- $\rho(\text{Skelite}^\circ) = 1.8 \text{ g/cm}^3$

Thresholded slice reconstructed with method C

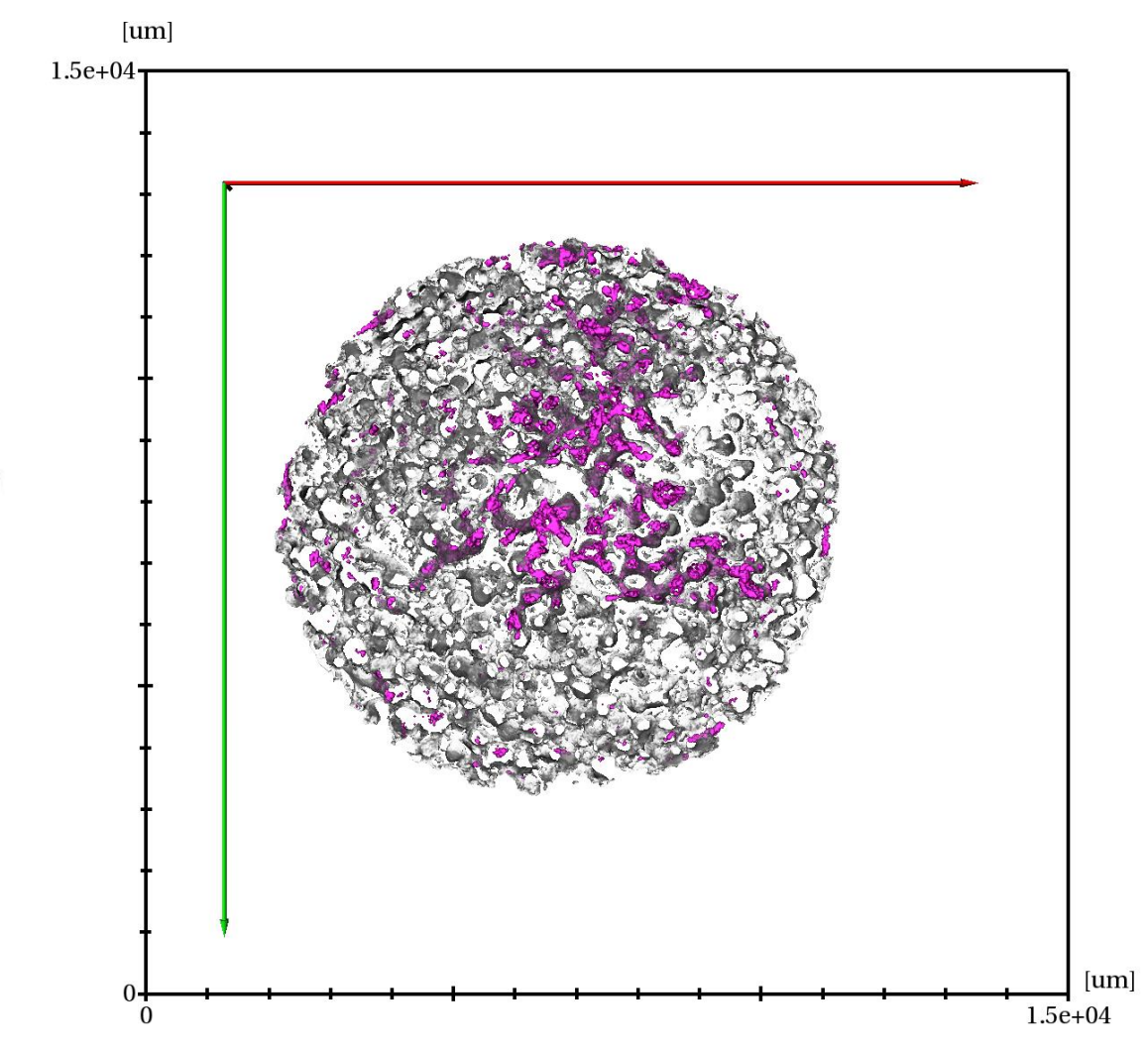
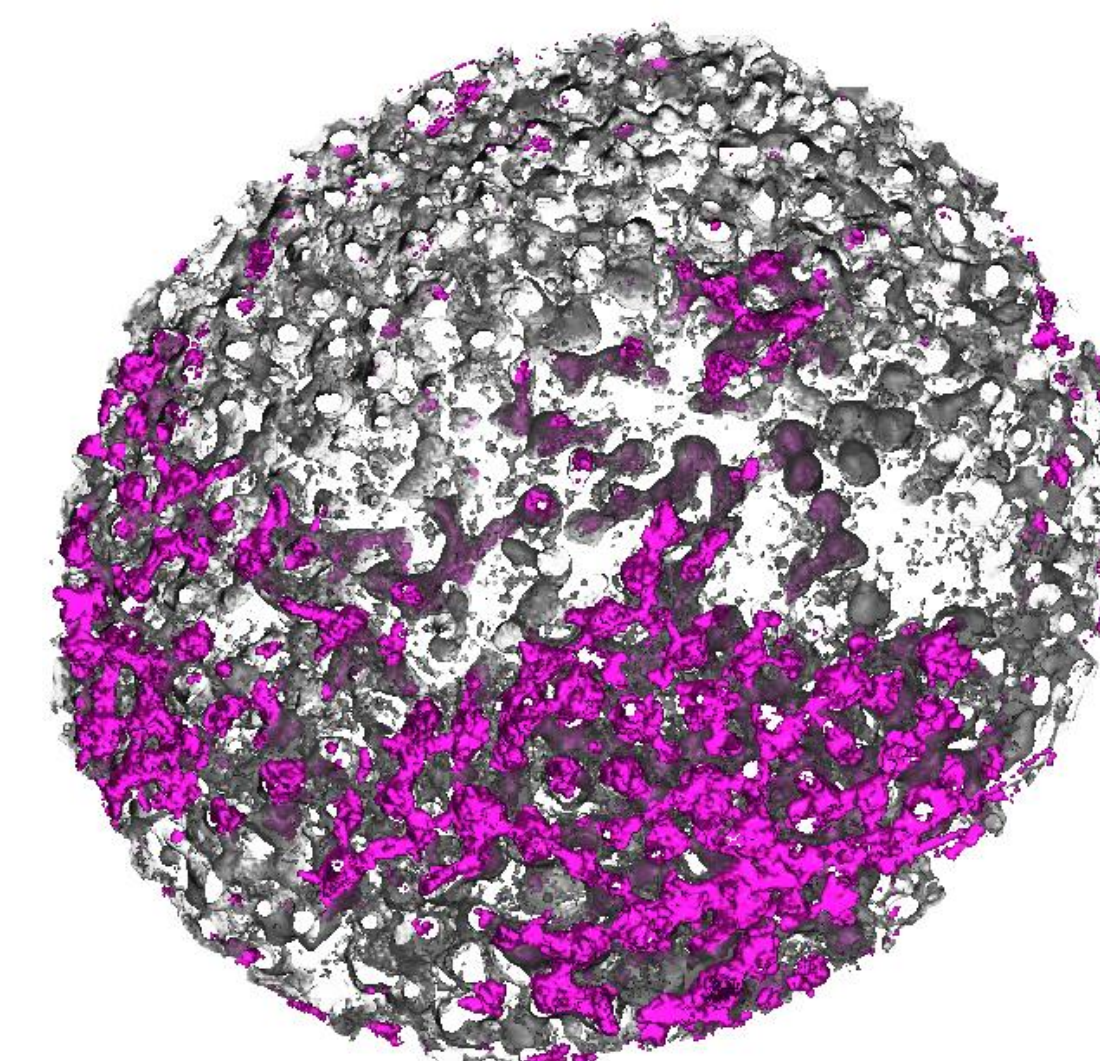


- Only 3 modes are visible using methods A and B, while we can distinguish 4 modes with methods C and D.
- Method A presents fringes around soft tissue, and phase contrast artefacts are visible using methods B and D.
- Method C seems more quantitative, and image quality is improved with regards to the other methods.

### Statistical analysis

- 9 samples: 3 samples / type of seeded cells (OBs, pre-OCs, OBs+pre-OCs)
- Quantification of volumes and corresponding thicknesses: Mineralized Volume (MV), Immature bone Volume (IBV), volume of cells (C)
- ANOVA : whether there is a difference between the groups or not
- Tukey's HSD (honest significant difference): which groups are significantly different from each other.
- Statistical analysis showed no influence on seeded cells, except on the Immature Bone Volume.
- /!\ BUT: a very few samples imaged and analyzed.

Sample	Fraction (in %)			Local thickness (in $\mu$ m)		
	MV/TV	IBV/(TV-MV)	C/(TV-MV)	MV.Th	IBV.Th	C.Th
OBs	44,7 +/- 5,0	98,2 +/- 8,9	5,1 +/- 4,3	121,4 +/- 20,3	176,0 +/- 29,0	60,8 +/- 2,1
OBs + pre-OCs	46,4 +/- 1,9	91,7 +/- 14,3	0,45 +/- 0,60	123,66 +/- 20,27	206,23 +/- 43,38	64,71 +/- 26,26
pre-OCs	48,5 +/- 2,7	0 +/- 0	0 +/- 0	150,4 +/- 13,8	0 +/- 0	0 +/- 0



## Conclusions & Perspectives

- Work to be submitted to *Phys. Med. Biol.*
- Heterogeneous object approaches can clearly improve image quality
- Choice of good parameters (threshold, function) is of crucial importance
- Introduce spatial constraints in prior, i.e. reconstruction while getting rid of the glass capillary
- Further quantitative studies using a calibrated phantom for example.

### Acknowledgements

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