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

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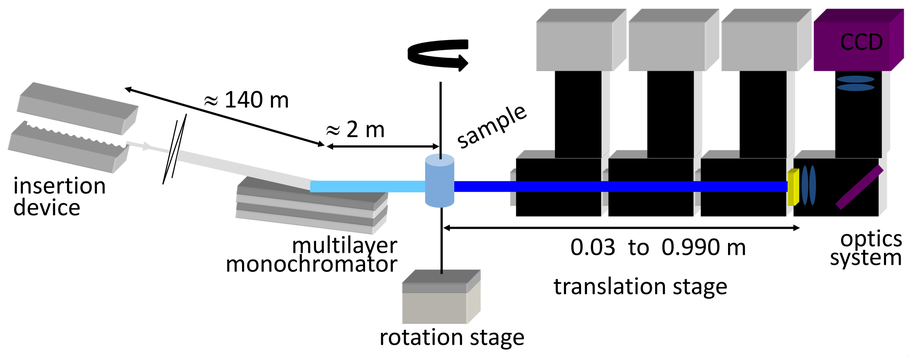
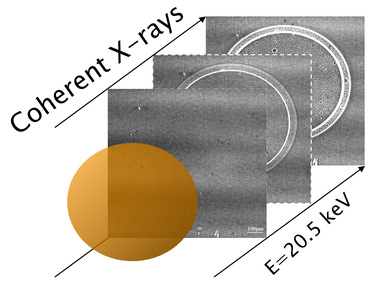
In-line phase contrast tomography

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 δ is the refractive index decrement and β the absorption index
- For soft tissue, at low energies (as used in radiology), δ is three order of magnitude larger than β [1]
- Phase contrast is achieved by moving the detector downstream
- Attenuation B and phase shift φ 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 $\boldsymbol{f}=(f_x, f_y)$ 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 θ, 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 θ 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 $\{I_{\theta,D}(\mathbf{x}), D=D_1...D_n\}$
    - ➔ 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 θ
    - ➔ Usually solved by filtered back-projection (**FBP**).

Phase retrieval methods

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

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

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

Homogeneous objects

- Can be introduced in projections
- Paganin[3]: proportionality between phase and attenuation **(method P)**

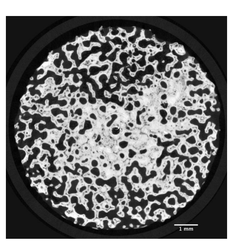
$$\varphi(\mathbf{x})=-\frac{\delta}{2\beta}\ln\left(\mathcal{F}^{-1}\left\{\frac{\mathcal{F}\{I_D(\mathbf{x})\}}{1+\frac{\lambda D\delta}{4\pi\beta}\|\boldsymbol{f}\|^2}\right\}\right)$$

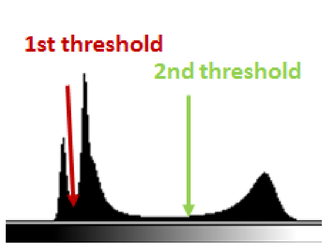
- Langer[4]: *prior* introduced in the regularization **(method A)**

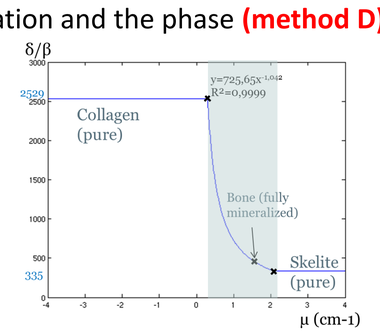
$$\varphi_{\theta,0}(\mathbf{x})=f(\mathbf{x})\frac{\delta}{2\beta}\ln[I_{\theta,D}(\mathbf{x})]$$

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)**



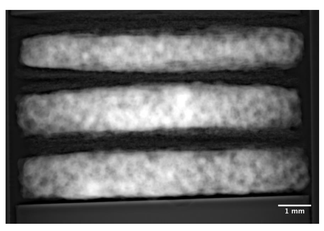




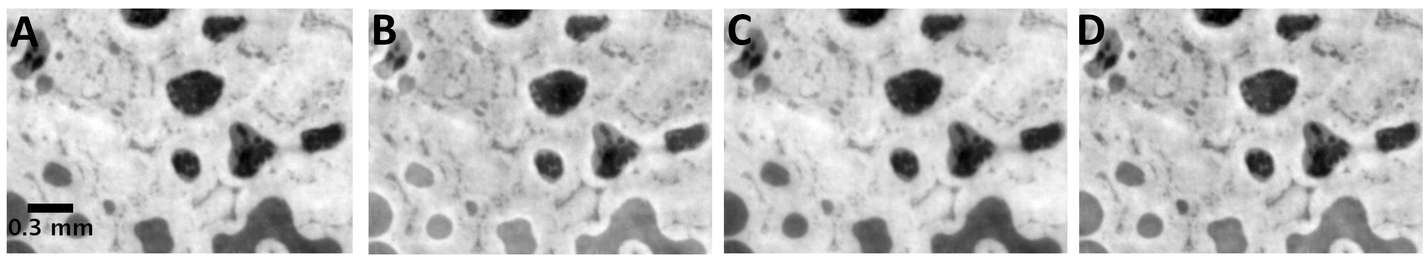
Application to 3D cell cultures

Material and methods

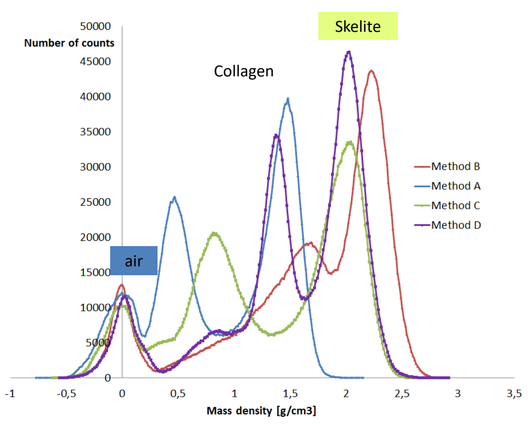
- Biomaterial (Skelite®) used for 3D cell culture and artificial bone scaffolds in surgery (coll. R Cancedda, University of Genova) [7]
- Composed of Si-TCP(67%) and β-TCP/HA (33%)
- 9 scaffolds imaged: discs of 9 mm diameter, seeded with bone cells
- **Experimental conditions:** 2000 projections, 30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



Results



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

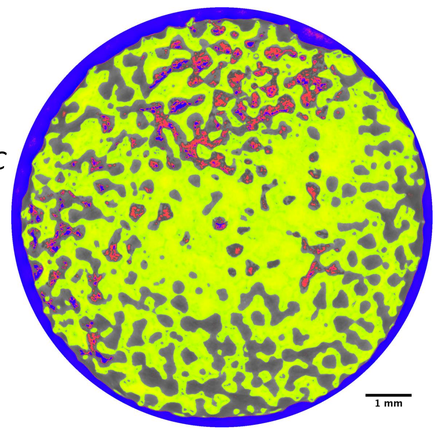


Histograms of volumetric mass densities of the reconstructed slice with the 4 methods

Theoretical vol. mass densities:

- ρ(air)=1.3 x 10^{-3} g/cm³
- ρ(Collagen)=1 g/cm³
- ρ(Skelite®)=1.8 g/cm³

Thresholded slice reconstructed with method C

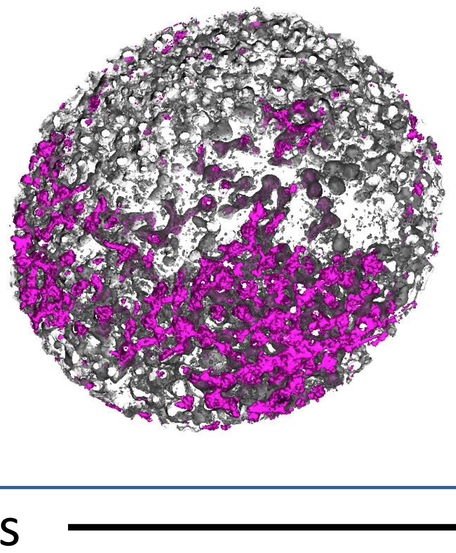


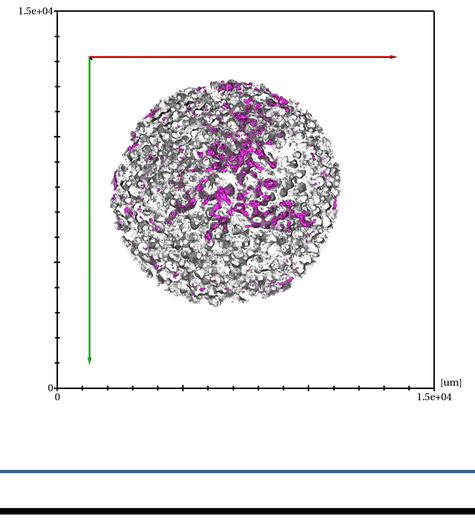
- Only 3 modes are visible using methods A and B, while we can distinguish 4 modes with methods C and D.
- Method A presents fringes around soft tissue, and phase contrast artefacts are visible using methods B and D.
- Method C seems more quantitative, and image quality is improved with regards to the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

	Fraction (in %)			Local thickness (in µm)		
Sample	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.

[1] A. Snigirev, I. Snigireva, V. Kohn, S. Kuznetsov, & I. Schelokov, *Review of Scientific Instruments*, *66*(12), 1995.
[2] P. Cloetens, W. Ludwig, J. Baruchel, D. Van Dyck, J. Van Landuyt, J. P. Guigay, and M. Schlenker, *Appl. Phys. Lett.*, p. 2912, 1999.
[3] D. Paganin, S.C. Mayo, T.E. Gureyev, P.R. Miller, S.W. Wilkins, Journal of Microscopy, pp.33-40, 2002.
[4] M. Langer, P. Cloetens, and F. Peyrin, *IEEE Trans. Image Process.*, pp. 2428–36, 2010.
[5] M. Langer, P. Cloetens, A. Pacureanu, and F. Peyrin, *Opt. Lett.*, pp. 2151–3, 2012.
[6] M. Langer, P. Cloetens, B. Hesse, H. Suhonen, A. Pacureanu, K. Raum, and F. Peyrin, *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.*, 2014.
[7] M. Langer, Y. Liu, F. Tortelli, P. Cloetens, R. Cancedda and F. Peyrin, *Journal of Microscopy*, *238*(3), 2010.

Acknowledgements

This work was supported by the LABEX PRIMES (ANR-11-LABX-0063) of Université de Lyon, within the program "Investissements d'Avenir" (ANR-11-IDEX-0007) operated by the French National Research Agency (ANR).