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SUNBIM evolution: new tools for a reliable (GI)SAXS/(GI)WAXS data reduction

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Abstract: SUNBIM (Supramolecular and sUbmolecular Nano- and Biomaterials X-ray IMaging) is a scientific package for X-ray imaging of nano- and biomaterials using SAXS, WAXS, GISAXS and GIWAXS techniques. With SUNBIM it is possible to perform a number of functions for data analysis such as: centering, q-scale calibration, two-dimensional to one-dimensional folding of small- and wide-angle X-ray scattering (SAXS/WAXS) data, also in grazing-incidence (GISAXS/GIWAXS); indexing of two-dimensional GISAXS frames and extraction of one-dimensional GISAXS profiles along specific cuts; quantitative scanning microscopy in absorption and SAXS/WAXS (SWAXS) contrast. New tools have been developed to enrich SUNBIM suite. The main novelty is the possibility to perform a deeper data reduction including dark current subtraction, background evaluation and subtraction and absorption normalization of the SAXS/WAXS map. The previous release of the software has already been used successfully to analyse several nano-structured samples. We are confident that the new features will allow a more correct and extensive analysis of the (GI)SAXS/(GI)WAXS data.

Keywords: computer programs; tools for crystal and crystallographic issues; SAXS/WAXS; imaging; microscopy

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SUNBIM @ XMILAB

SUNBIM is entirely developed at the Institute of Crystallography of Bari and more specifically at XMI-L@b. Those are all the people involved directly and indirectly with SUMBIM. Most of the codes are written by Dritan Siliqi and me, but the whole group cures the theoretical and the experimental aspects as they are the first testers of the program.

https://www.facebook.com/xmilab

http://www.ic.cnr.it/ic4/en/x-ray-microimaging-laboratory-xmi-lb-ic-bari/

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SUNBIM: a package for X-ray imaging of nano- and biomaterials using SAXS, WAXS, GISAXS and GIWAXS techniques

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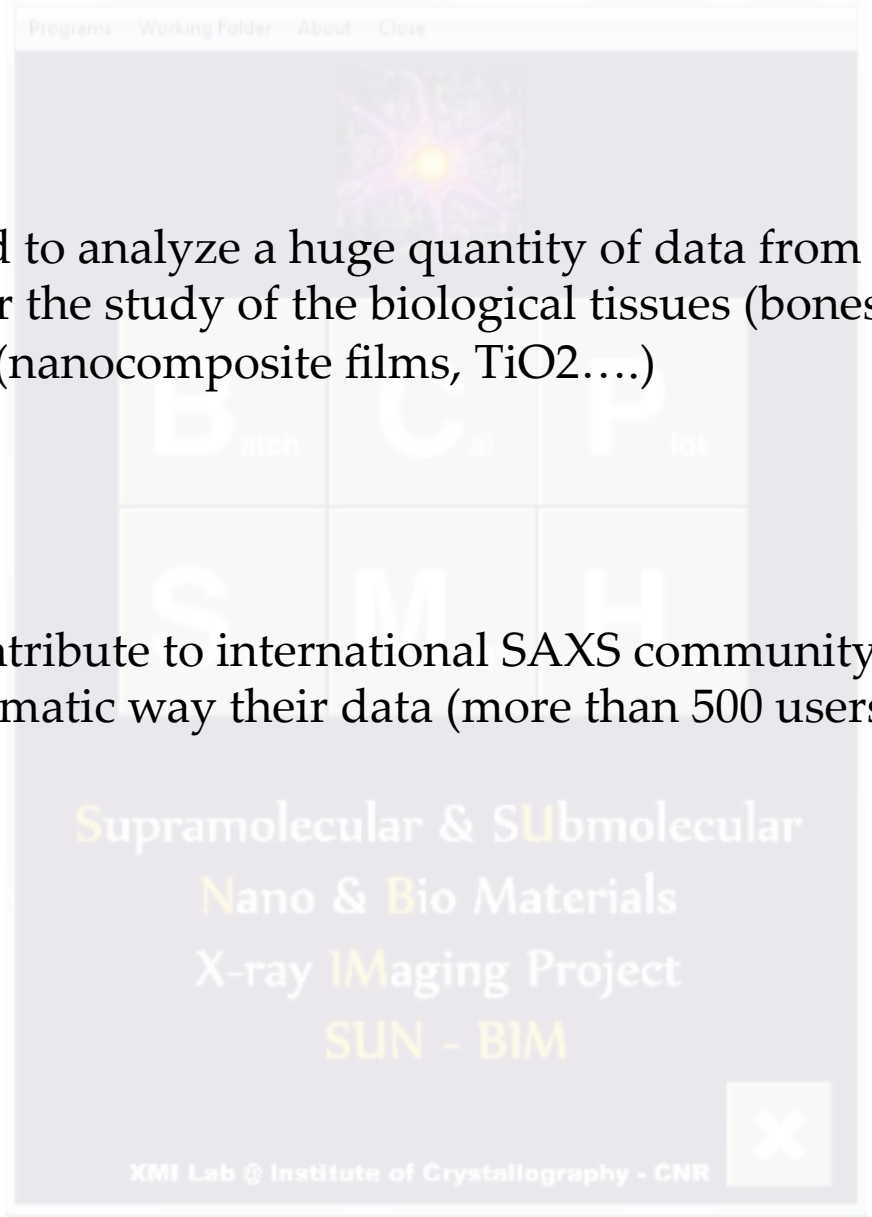
Why SUNBIM?

- To perform and to analyze a huge quantity of data from SWAXS experiments for the study of the biological tissues (bones, cornea, …) and nanomaterials (nanocomposite films, TiO2….)

- To give our contribute to international SAXS community an useful tool to process in automatic way their data (more than 500 users up to now)



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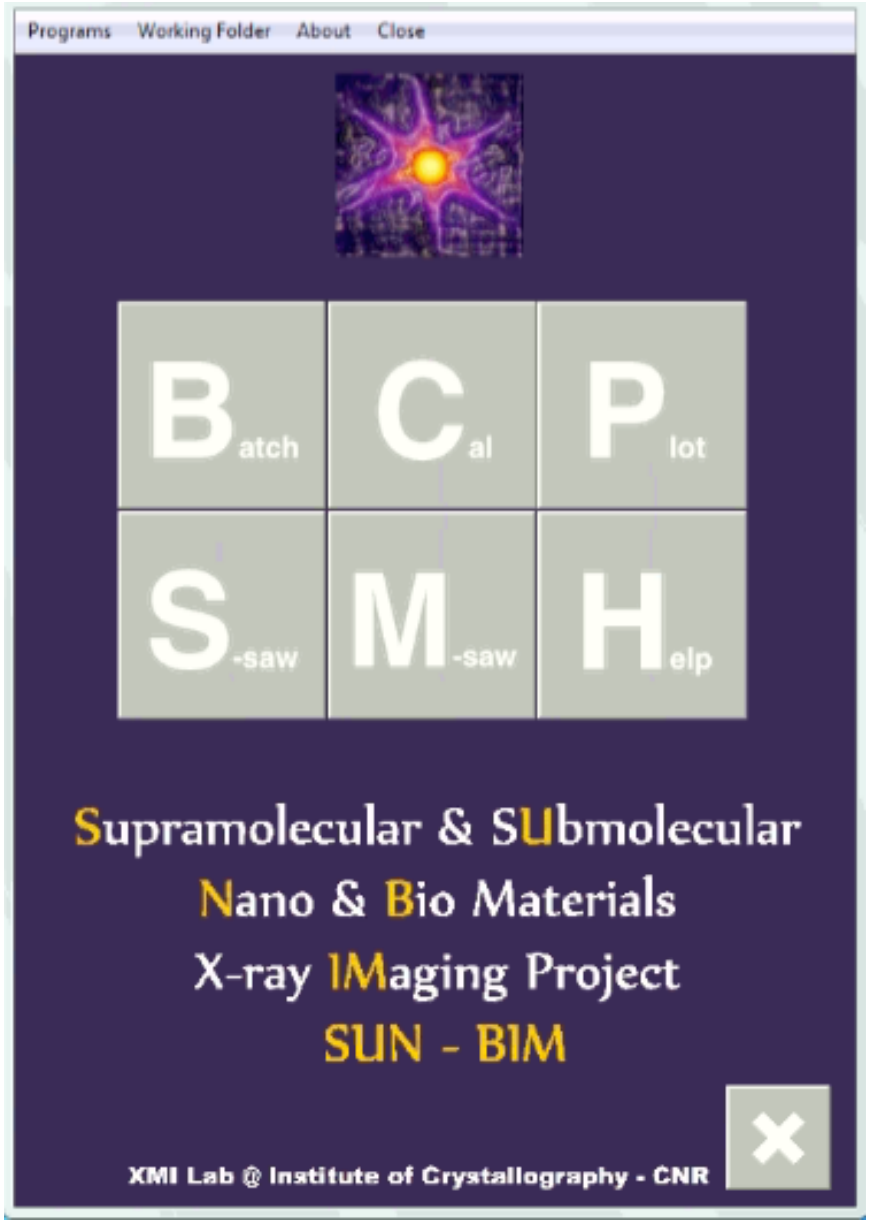
Why SUNBIM?



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Why SUNBIM?

SUNBIM is a computer suite of 5 integrated main programs:

① Calibration package

- centering patterns
- q-scale calibration

② S-SAWANA

- from 2D to 1D folding on data
- indexing of 2D GISAXS frames
- extraction of 1D GISAXS profiles along cuts
- **geometric aberration correction for WAXS folded data (NEW!)**

③ M-SAWANA

- **data reduction (NEW!)**
- manage with collections of data
- scanning microscopy in absorption and SWAXS contrast

④ Batch Script

- prepare batch script files (ASCII files) to run a sequential acquisition of two-dimensional frames (in scanning mode)

⑤ One-D Data Analysis Manager (Plot)

- quick plot of 1D profiles
- 1D background evaluation and subtraction
- denoising and deconvolution of the primary beam angular divergence on SAXS/GISAXS profiles

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FOCUS

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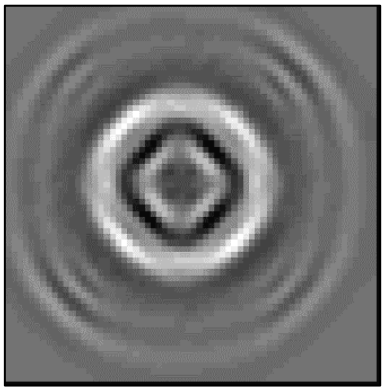
SUNBIM Programs

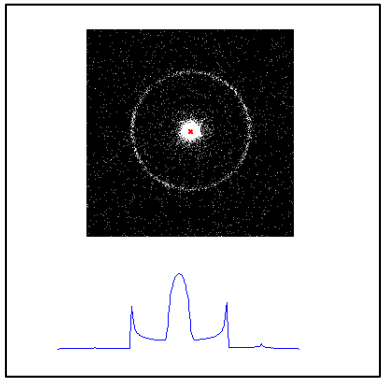
① Calibration: Main Features

Users can calibrate their data using a number of function able to convert diffraction pattern from pixels space into q-space:

- Beam centering calculation
  - manually (click one point directly on the center)
  - selecting three points along a specific diffraction ring
  - automatically (by using two different approaches)



Based on a set of fluid dynamics equations (Landau & Lifshitz, 1987), known as **Shallow Water Equations (SWE)**



Based on the **Integral Radon Transform** (Radon, 1986; Barbano et al., 2009),

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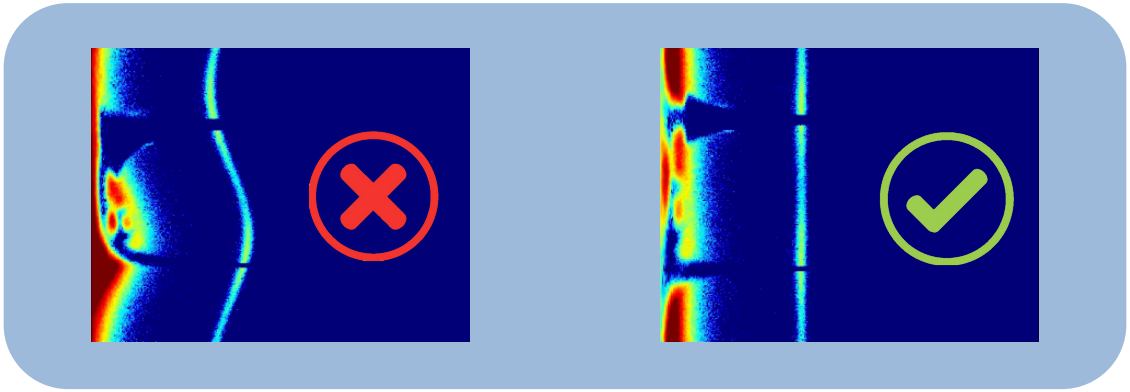
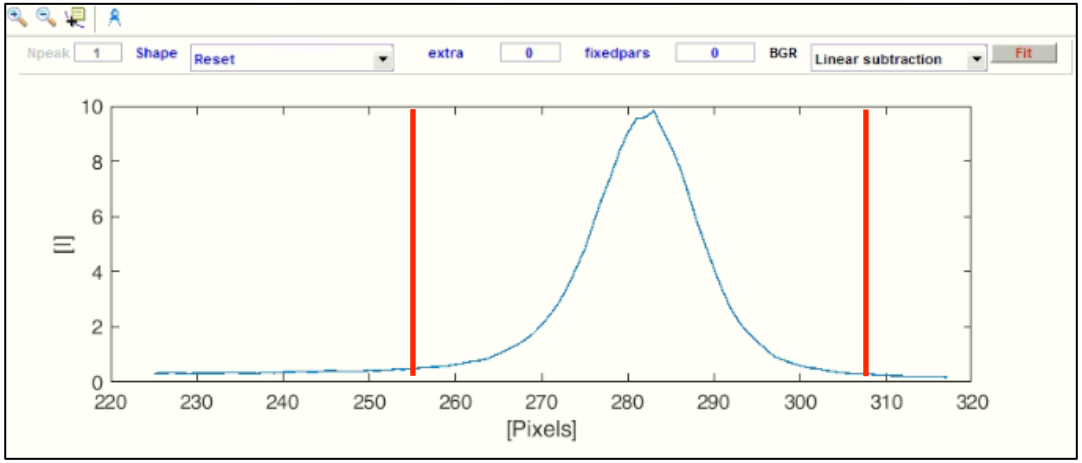
SUNBIM Programs

① Calibration

- Representation in polar coordinates of the diffraction pattern to check the correctness of the center



- Tool for Sample-to-Detector distance calculation



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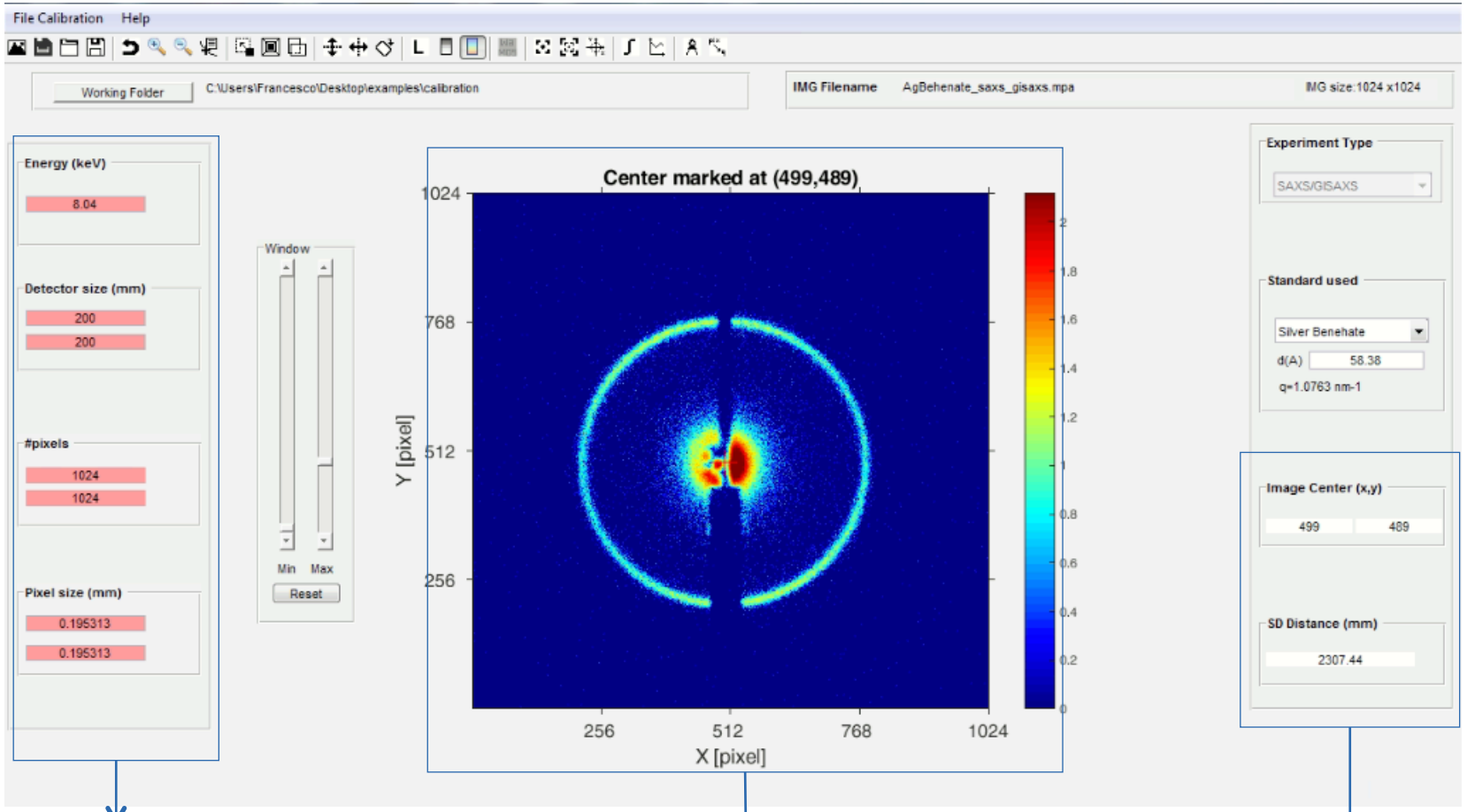
SUNBIM Programs

① Calibration Window



Experimental Settings

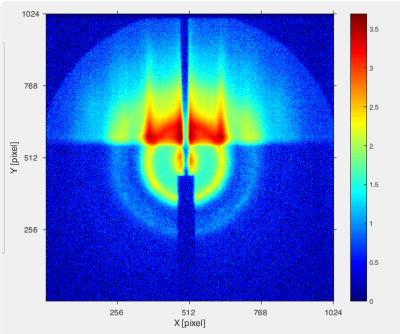
Diffraction Pattern of the standard sample

Calibration Results

SUNBIM Programs

② S-SAWANA (Single scan SWAXS and Data Analysis): Main Features

- Load and visualize a single two-dimensional frame



Relevant tools

- change the colour map or colour scale
- shift, rotate and transpose the pattern
- import calibration data
- extract one or more cross sections and plot them (vertical, horizontal, radial)

and more…..

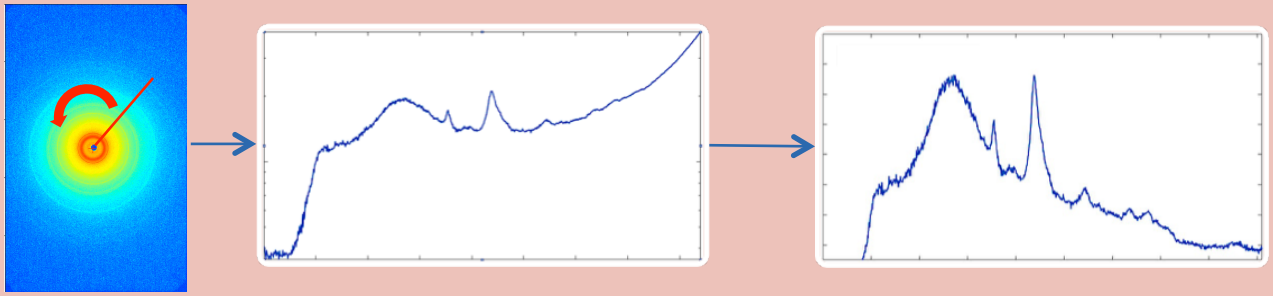
- Fold the two-dimensional data (radial integration)

Relevant tools

- Radial Integration (full circle or angular sectors)
- Automatic Background Subtraction for WAXS data

NEW FEATURE

Geometric aberration for small sample-to-detector distance could affect WAXS data.
For this reason, we developed an automatic evaluation (directly from the 2D pattern) and subtraction (if necessary) of the background from the 1D profile of the radial integration to enhance peak visibility at large scattering angles.



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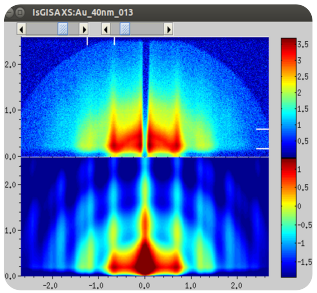
SUNBIM Programs

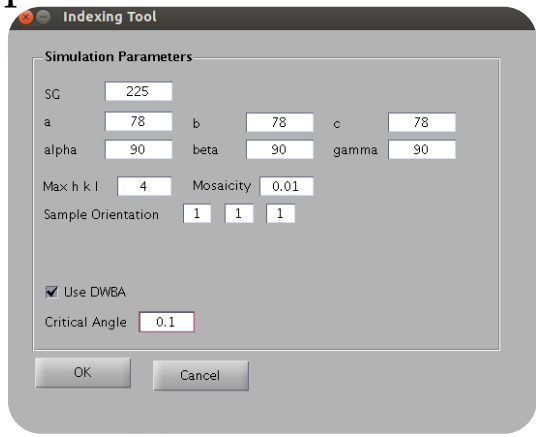
② S-SAWANA (Single scan SWAXS and Data Analysis): Main Features

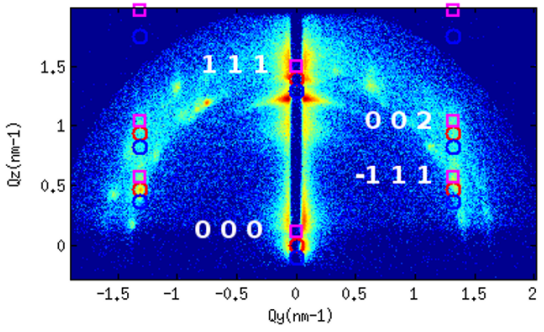
- Export data for simulation into:
  - IsGISAXS program format[1]
  - ASCII text file (free format)

The simulated (IsGISAXS) image can be uploaded and compared with the experimental one[2]



- Indexing GISAXS/GIWAXS data: simulate the pattern produced by a collection of nanoparticles assembled on top of a substrate according to the space-group symmetry, the unit-cell size, the orientation and the disorder in the sample[3]





1 Lazzari, R. (2002). J. Appl. Cryst. 35, 406–421
2 Corricelli M et al. J. Phys. Chem. C, 2014, 118, 7579-7590.
3 Tate M. P. et al. (2006). J. Phys. Chem. B, 110, 9882–9892.

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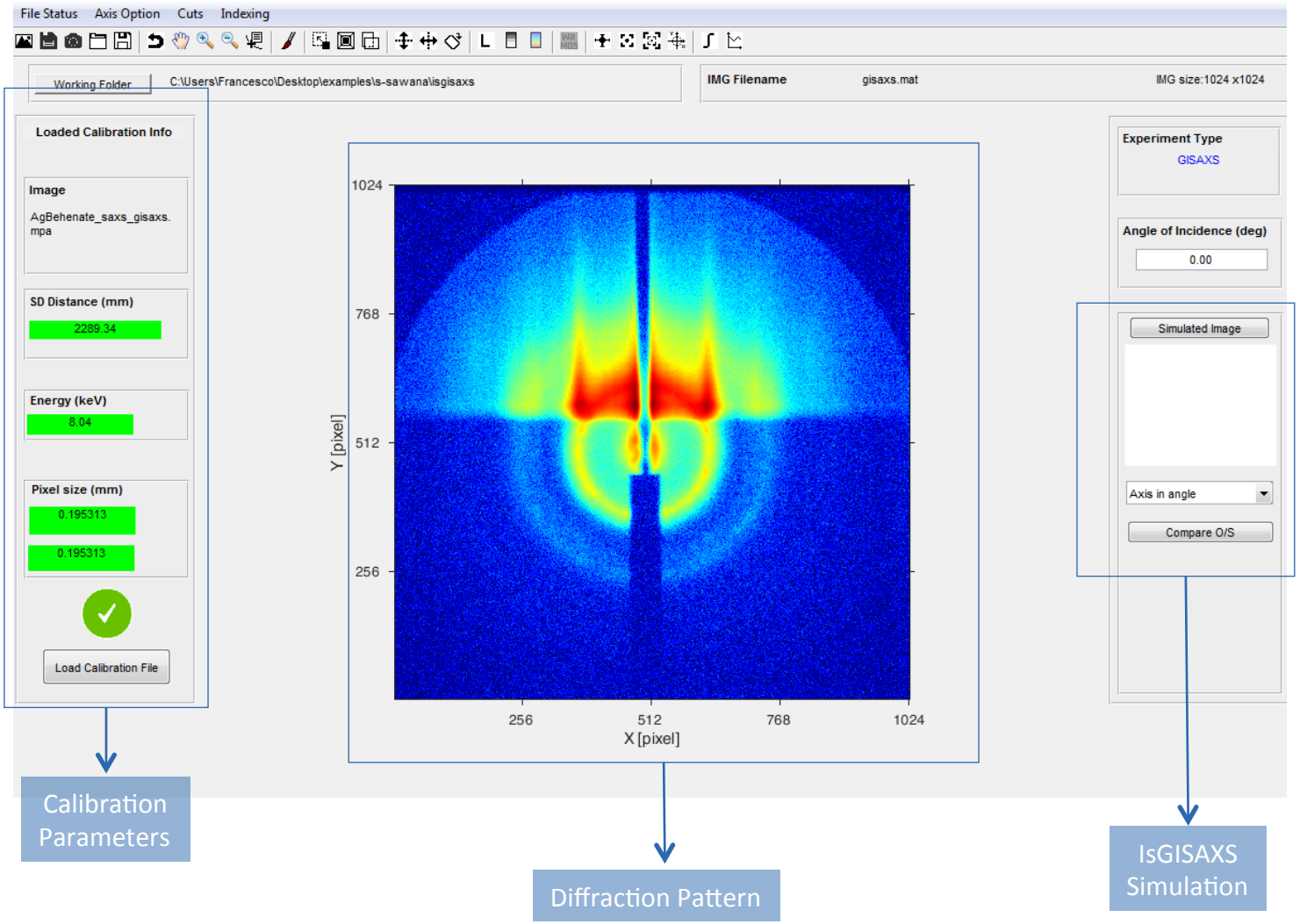
SUNBIM Programs

② S-SAWANA Window



SUNBIM Programs

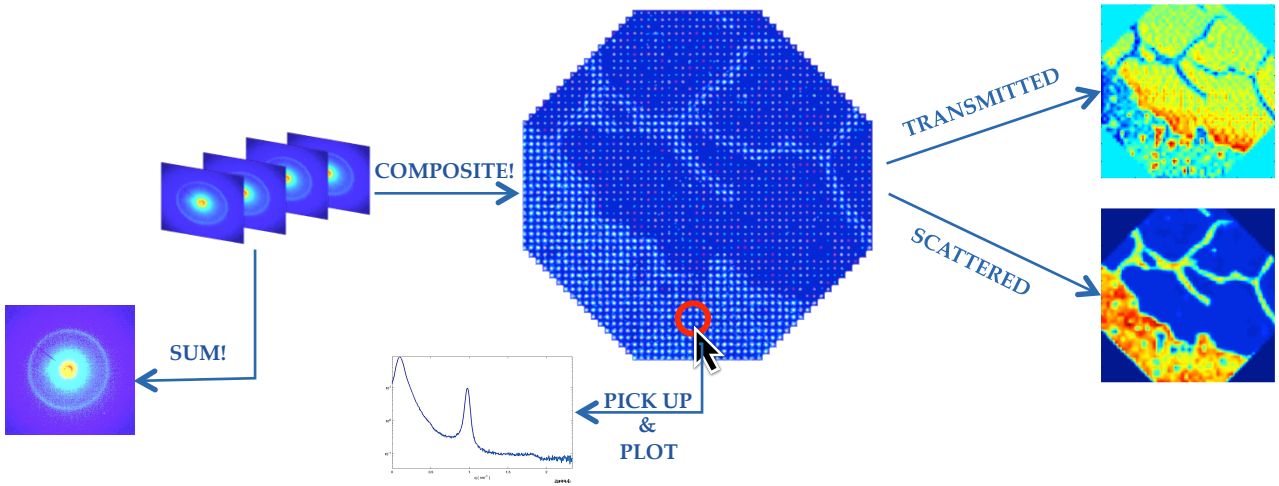
③ M-SAWANA (Multi scan SWAXS and Data Analysis): Main Features

This tool is useful when scanning SWAXS has been performed. Multi scan SWAXS gives point by point a 2D signal that can be arranged in colour maps by SUNBIM.

➢ Composite

Relevant tools

- composite of the as-collected two-dimensional SAXS frames into a single image
- transmitted and/or scattered single mesh image of intensities in the whole q range
- average pattern calculated over the whole set of the data or over a particular ROI to increase SNR



Interactive Composite

- pick up and plot one or more specific one-dimensional profile (and the corresponding 2D image) corresponding to a scan
- find a scan position into the mesh
- free-hand draw area (ROI) and calculating comparatives parameters (vertex coordinates, area size, Δq and integrated intensity)

SUNBIM Programs

③ M-SAWANA (Multi scan SWAXS and Data Analysis): Main Features

➢ Analysis Section (1/2)

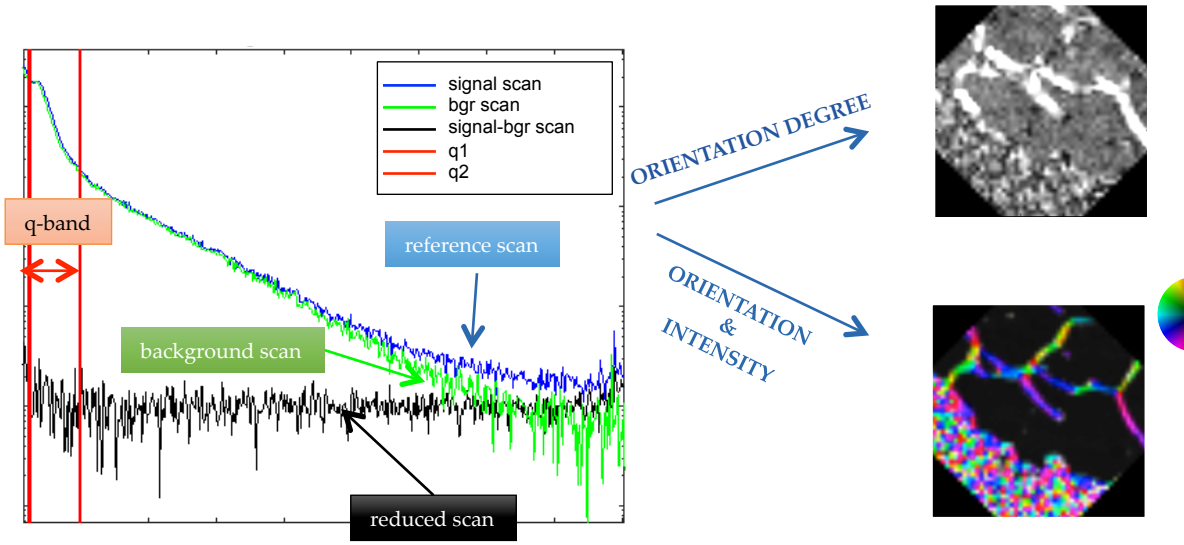
Multi-modal imaging approach: transform a mesh of two-dimensional SAXS frames into microscopy images*

Relevant tools

- each 2D frame of the mesh is folded into a one-dimensional profile
- from a specific 1D profile, properly selected from the composite map, the user has to choose one or more diffraction peaks (a q range)

Orientation & Intensity

- Intensity distribution along the azimuth of the selected rings is analysed for each 2D frame and transformed into microscopy images.
- For each monitored peak, the subtracted background can be evaluated by polynomial interpolation or using an experimental background scan



*Bunk et al. New J. Phys. 11, 123016, 2009

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SUNBIM Programs

③ M-SAWANA (Multi scan SWAXS and Data Analysis): Main Features

➢ Analysis Section (2/2)

CCA (Canonical Correlation Analysis) map

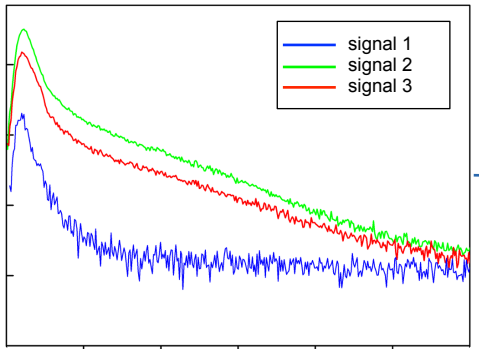
Extract from 1D mesh profiles a few data which can characterize the investigated sample.

CCA is a statistical technique (Hotelling,1936) to assess the relationship between two sets of variables.

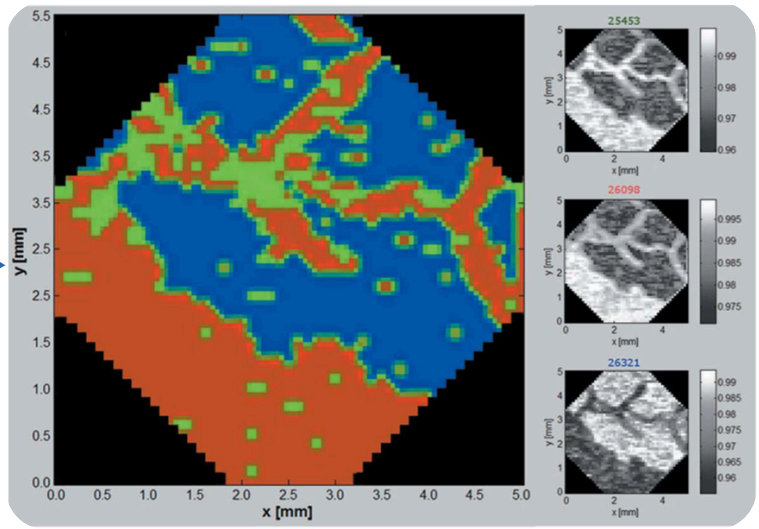
CCA quantifies the relationship between two random variables, x and y, by means of the so-called correlation coefficient.

Exploit the spatial information characterizing the composite SAXS/WAXS data set:

- the variable x is a multivariate vector with components representing the set of acquired diffraction profiles of the composite map
- the variable y also consists of a multivariate vector having few components; these are (usually three) models extracted from the composite map by means of different methods (i.e. adaptive binning), which represent the less correlated SWAXS profiles among the entire set of data



COMPONENTS



CCA MAPS

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SUNBIM Programs

③ M-SAWANA Window

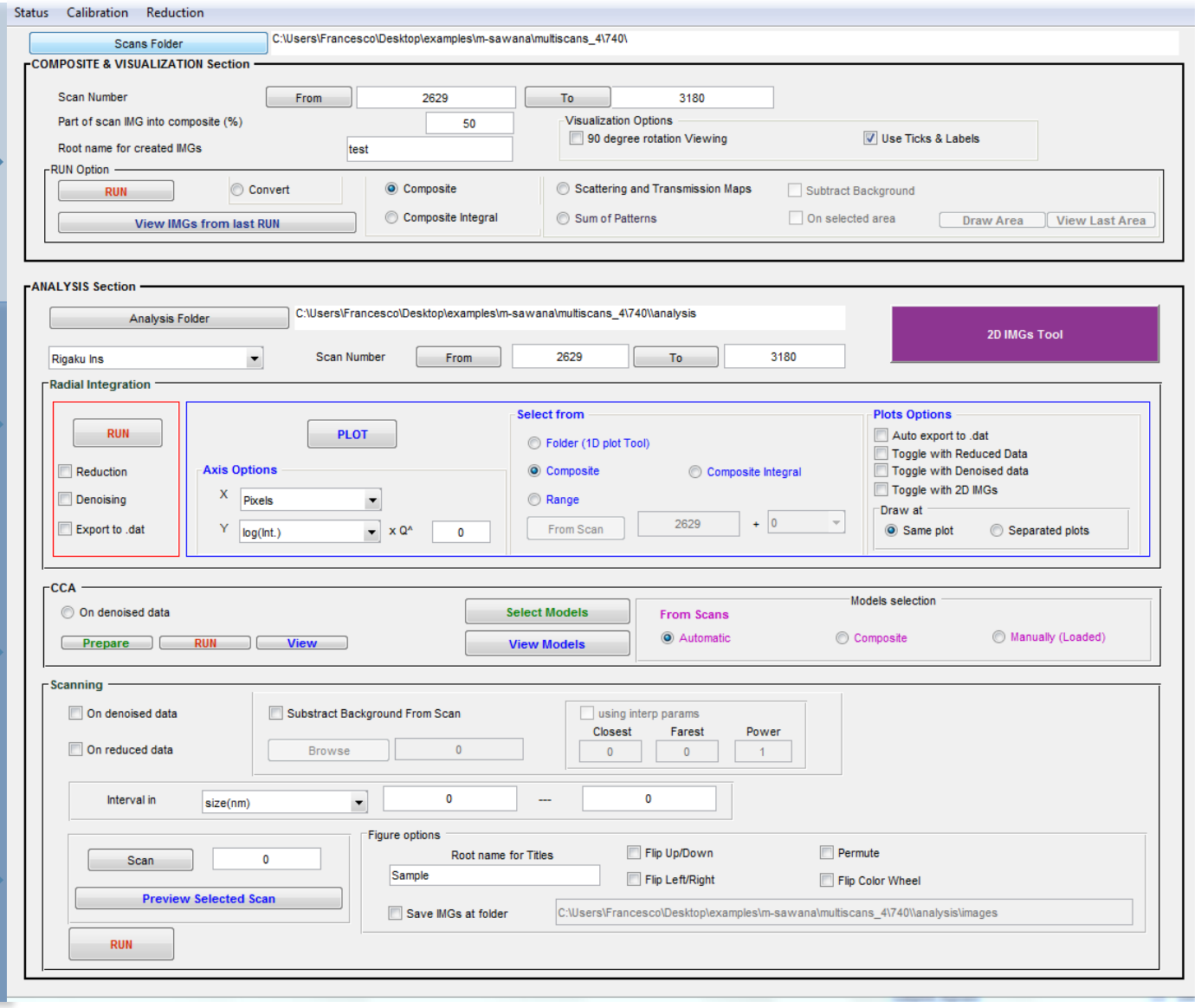
Composite Tool

Analysis Section

Radial Integration & Plot

CCA

Scanning



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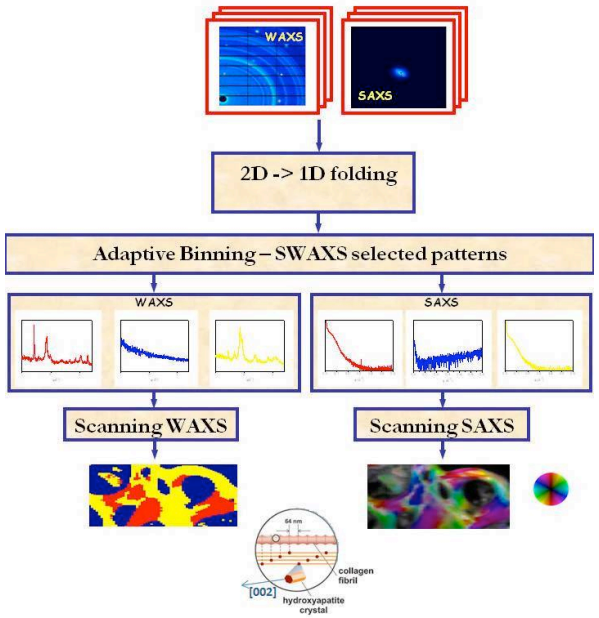
Case Study

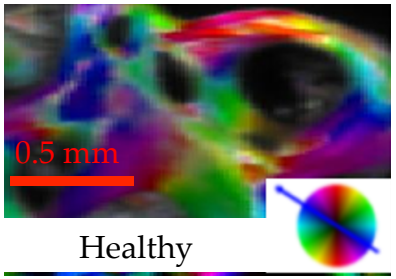
Biological Tissues (1/2)

- Scanning small and wide angle X-ray scattering (scanning SWAXS) experiments were performed on healthy and pathologic human bone sections (Paget's disease and dwarfism).

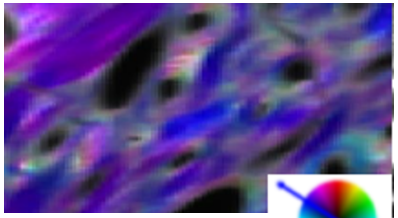
Methods: M-SAWANA Flow Chart



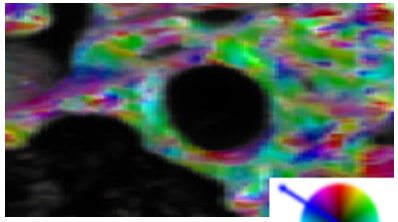
Results: Intensity & Orientation Maps



Healthy



Dwarfism



Paget

RESULT
SWAXS images and analysis allowed extracting information of the mineral nanocrystalline phase embedded (hydroxyapatite), with and without preferred orientation, in the collagen fibrils, mapping local changes at sub-osteon resolution.

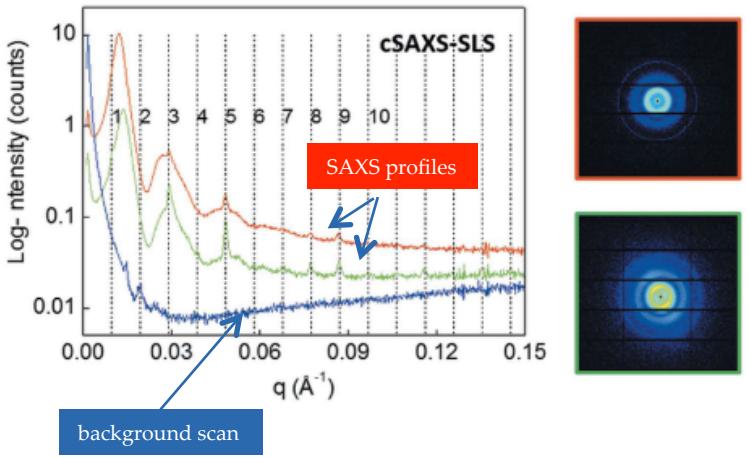
C. Giannini, D. Siliqi, O. Bunk, A. Beraudi, M. Ladisa, D. Altamura, S. Stea & F. Baruffaldi, Scientific Reports 2, 435 (2012)

Case Study

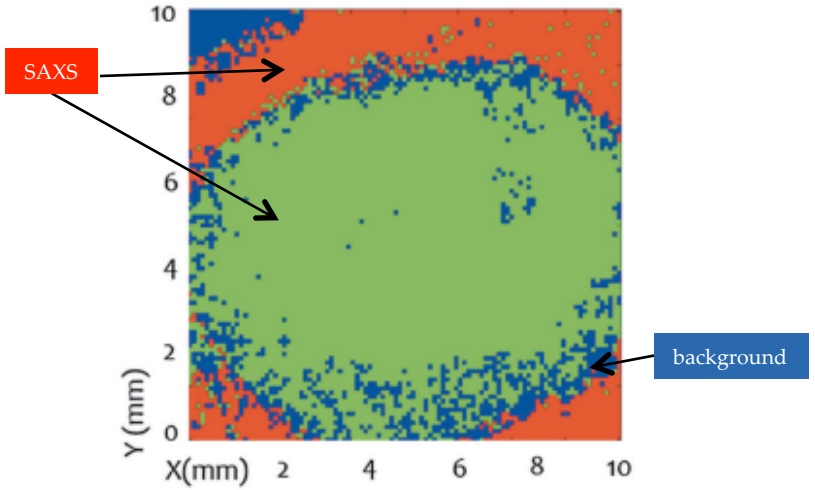
Biological Tissues (2/2)

- Bovine cornea was studied with scanning small-angle X-ray scattering (SAXS)microscopy, by using both synchrotron radiation and a microfocus laboratory source
- A combination of statistical (adaptive binning and canonical correlation analysis) and crystallographic (pair distribution function analysis) approaches allowed inspection of the collagen lateral packing of the supramolecular structure.

Reference data
(as selected by adaptive binning)



Canonical Correlation Analysis
on the explored area



RESULT
Decrease in the interfibrillar distance and in the shell thickness around the fibrils from the periphery to the center of the cornea; the central area coincides with the region (approx. 10 mm) where the epithelium has been removed.

T. Sibillano, L. De Caro, F. Scattarella, G. Scarcelli, D. Siliqi, D. Altamura,M. Liebi, M. Ladisa, O. Bunk and C. Giannini, J. Appl. Cryst. (2016). 49, 1231-1239

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What's new?

Data Reduction Panel

An accurate data redaction is mandatory on SAXS/WAXS to obtain readable, reliable and quantitative data (even more so if they are acquired by laboratory sources).

STEPS

① The Dark Current (DC) signal (beam off) is subtracted from the SAXS signal to reduce the instrumental noise of the detector

② The resulting signal is normalized to the transmission coefficient T, so as to make it independent of the different degree of absorption of the sample

③ A background signal is subtracted from the normalized signal, taken as an average of the SAXS signal in an area outside the sample.

$$SAXS_{redu} = \frac{SAXS - DC}{T} - BG$$

$$BG = \frac{\sum_{i=1}^{M} (SAXS_i - DC)}{M}$$

DC = Dark-current signal
BG = Background measured outside the sample area
M = number of selected BG patterns

A number of new functions have been implemented in SUNBIM.

A personalized Data Reduction is now possible!

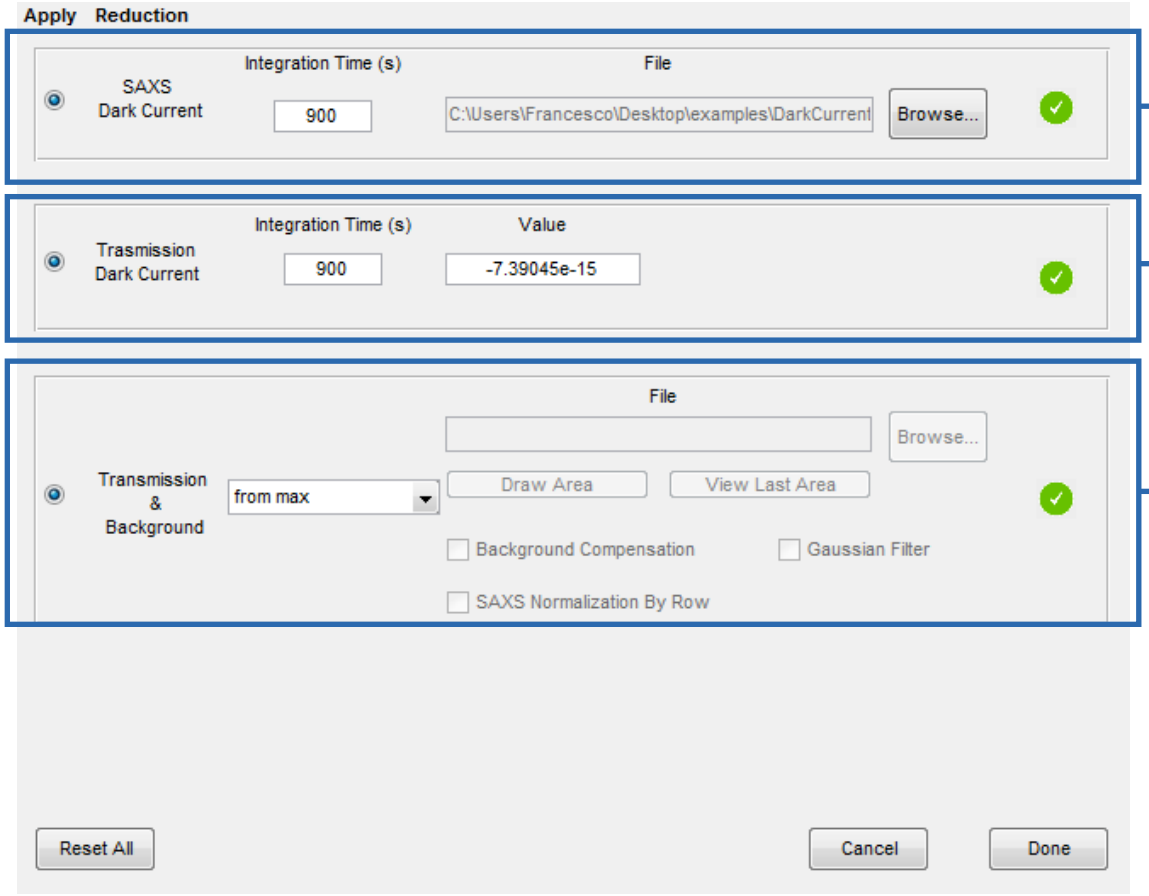
Main Options

- Four options to calculate T map and BG using:
  - max intensity of the map
  - max of each row
  - area (to increase SNR)
  - external file (if measured)
- Post processing functions (to improve maps quality):
  - Background Compensation*
  - Gaussian Filter
  - SAXS signal normalization row by row

*Scattarella et. al, Crystals 7 (7), 186, 2017

What's new?

Data Reduction Panel



SAXS DARK CURRENT SUBTRACTION

TRANSMISSION DARK CURRENT SUBTRACTION

TRANSMISSION COEFFICIENT CALCULATION AND BACKGROUND SUBTRACTION

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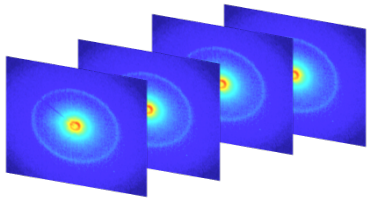
Case Study

Nanomaterials: Whey Protein Concentrate (WPC) Films + TiO_2

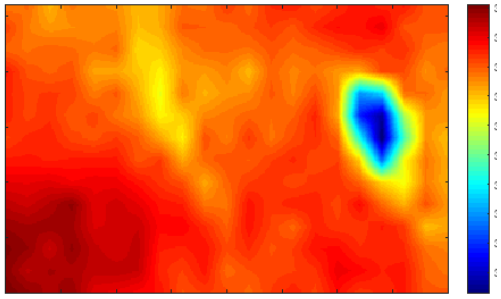
Scanning SAXS at XMI-L@b

1. The sample is moved in steps (~ 200 μm) along the two xy directions and for each step a 2D diffraction pattern is acquired by the SAXS detector.



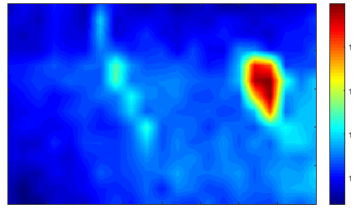
SUNBIM Software

The software associates the collected patterns with the sample positions and reconstructs the spatial distribution of the scattering intensity



2. The instrument also collects data on the absorption of the sample point by point (pin diode).

SUNBIM Software



Intensity Transmitted Map

SUNBIM NEW DATA REDUCTION

It is possible to retrieve a map of the **Transmission Coefficient** **T** of the sample

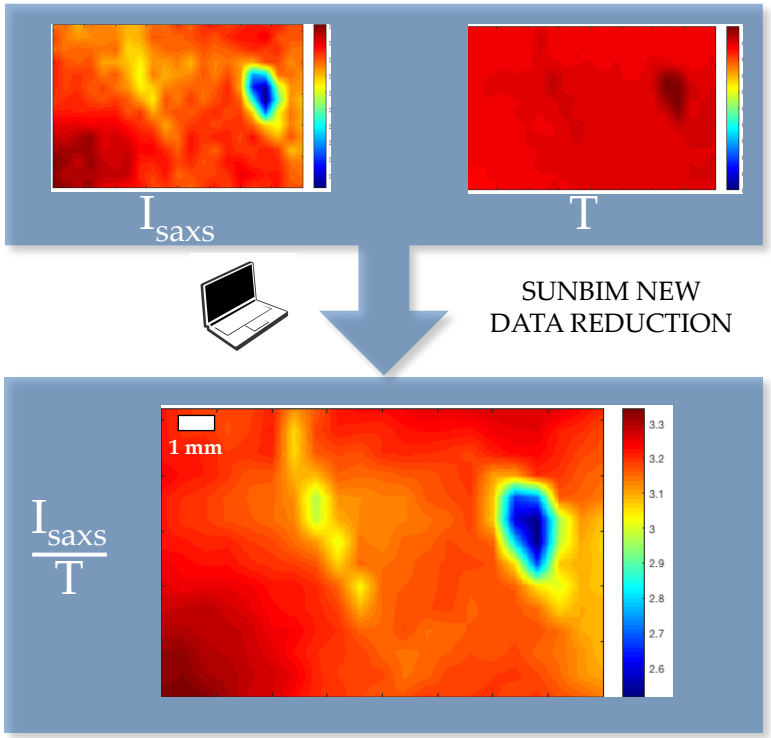
$$T = e^{-\mu t}$$

Lambert-Beer Law

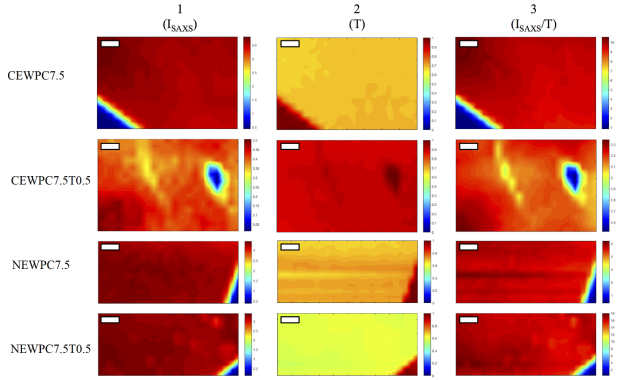
Possible estimation of local sample thickness

Case Study

Nanomaterials: Whey Protein Concentrate (WPC) Films + TiO_2

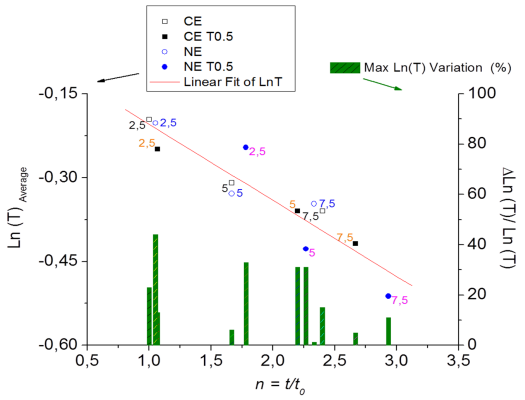


Scattering/Absorption contrast of films CEWPC7.5 with TiO_2 loading. SAXS/T maps lead to an **enhancement** of **contrast** and **resolution** related to thickness/density and structure variations, or a flattening of the inhomogeneities when these are due to bare density/thickness variations*.



- SAXS
- T
- SAXS/T

maps of different films



From Lambert-Beer Law we derived the logarithmic plot of T (average) as a function of the normalized sample thickness $n=t/t_0$ and extrapolated the unknown thickness value for very thin samples

*J. M. Montes-de-Oca-Ávalos, D. Altamura, M. L. Herrera, C. Huck-Iriart, F. Scattarella, D. Siliqi, C. Giannini, R. J. Candal, *Physical and structural properties of whey protein concentrate - corn oil - TiO2 nanocomposite films for edible food-packaging*, Food Packaging and Shelf Life (2020), **in press**

List of Selected papers

- J. M. Montes-de-Oca-Ávalos, D. Altamura, M. L. Herrera, C. Huck-Iriart, F. Scattarella, D. Siliqi, C. Giannini, R. J. Candal, *Physical and structural properties of whey protein concentrate - corn oil - TiO2 nanocomposite films for edible food-packaging*, Food Packaging and Shelf Life **(2020)**, in press
- T. Sibillano, A. Terzi, L. De Caro, M. Ladisa, D. Altamura, A. Moliterni, R. Lassandro, F. Scattarella, D. Siliqi and C. Giannini, Crystals, 10(4), 274, **(2020)**
- Terzi A., Gallo N., Bettini S., Sibillano T., Altamura D., Campa L., Natali M. L., Salvatore L., Madaghiele M., De Caro L., Valli L., Sannino A., Giannini C., Front. Bioeng. Biotechnol., 7, 203 **(2019)**
- A. Terzi, E. Storelli, S. Bettini, T. Sibillano, D. Altamura, L. Salvatore, M. Madaghiele, A. Romano, D. Siliqi, M. Ladisa, L. De Caro, A. Quattrini, L. Valli, A. Sannino, and C. Giannini, Scie. Rep., 8(1), **(2018)**
- J. M. Montes-de-Oca-Ávalos, D. Altamura, R. Jorge Candal, F. Scattarella, D. Siliqi, C. Giannini, M. L. Herrera, Food Research International, 105, 129-139 **(2018)**
- C. Diaferia, N. Balasco , T. Sibillano, M. Ghosh , L. Adler-Abramovich, C. Giannini, L. Vitagliano , G. Morelli, A. Accardo, Chemistry - A European Journal, 24 (26), 6804-6817, **(2018)**
- T. Sibillano, L. De Caro, F. Scattarella, G. Scarcelli, D. Siliqi, D. Altamura, M. Liebi, M. Ladisa, O. Bunk and C. Giannini, J. Appl. Cryst. 49, 1231-1239, **(2016)**
- A. Camposeo, R. D. Pensack, M. Moffa, V. Fasano, D. Altamura, C. Giannini, D. Pisignano and G. D. Scholes, J. Am. Chem. Soc.138 (47), 15497-15505, (**2016)**
- D. Altamura, S.G. Pastore, M.G. Raucci, D. Siliqi, F. De Pascalis, M. Nacucchi, L. Ambrosio, and C. Giannini, ACS App. Mater. Int., 8(13):8728–8736, **(2016)**
- D. Siliqi, L. De Caro, M. Ladisa, F. Scattarella, A. Mazzone, D. Altamura, T. Sibillano and C. Giannini, J. Appl. Cryst. 49, 1107–1114, **(2016)**

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Obtaining & Installation

- Only for Windows (64 bit)
- Download from: http://www.ba.ic.cnr.it/softwareic/sunbim/ (after a valid registration)
- Run the installation file (SUNBIMInstaller.exe) accepting the "license agreement"

SUNBIM is developed on MATLAB and deployed with MATLAB Runtime Compiler R2020a (included in setup file and automatically installed)

- Find the executable run file under "Programs" or as a link on the "Desktop"
- Useful info at:

http://www.ba.ic.cnr.it/softwareic/sunbim/tutorials/ (video tutorials)
http://www.ba.ic.cnr.it/content/sunbim-examples (zip file with few examples)

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