EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal: *https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do*

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report")*,
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** **Exploring the colloidal structure of emulsion-based paints using nano-holotomography**	**Experiment number**: HG-222
Beamline: ID16B	**Date of experiment**: from: 08 September 2023 to: 11 September 2023	**Date of report**: 24 Nov. 2023
Shifts: 9	**Local contact(s)**: Julie Villanova	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Côme THILLAYE DU BOULLAY[1*], Floriane GERONY[1,2*], Emeline POUYET[1*], Laurence de VIGUERIE[1*]

[1] Laboratoire d'Archéologie Moléculaire et Structurale (LAMS) – UMR 8220 Sorbonne-Université - CNRS

[2] Laboratoire de PHysique des Electrolytes et Nanosystèmes InterfaciauX PHENIX – UMR 8234 Sorbonne-Université – CNRS

Report:

Background summary: Emulsions prepared with egg yolk and linseed oil were likely used as paint binders during the 15th century. This painting technique recently gained the interest of the scientific community, but little is known about the structure at the microscopic scale of such emulsion-based paints. This experiment aims at using X-Ray nano-holotomography to investigate this structure on dry mock-up paint samples with various binder compositions and pigments. Preliminary tests conducted on ID16B at 17 keV on such samples demonstrated the ability of holotomography to discriminate the continuous and dispersed phases in a dried emulsion, despite their small difference in density.

Experimental methodology: Mock-up paint samples were prepared in advance by dispersing a pigment (chosen among azurite $Cu_3(CO_3)_2(OH)_2$, lead-tin yellow Pb_2SnO_4 or hollow silica spheres) in a binder (egg yolk, raw linseed oil, linseed oil heated with lead oxide PbO, oil-in-water o/w or water-in-oil w/o emulsions prepared with egg yolk and either raw linseed oil or linseed oil heated with PbO). Reference binders, without dispersed pigment, were also prepared. These samples were mounted on Kapton loops, 100 or 200µm in diameter, and left to dry for 7 weeks. An additional historical paint microfragment from Colantonio's *St Jerome in his study* (ca. 1445) was mounted on a Kapton loop. The beam energy was 29.6 keV. 2505 projections were acquired over 360°, with an exposure time between 60 and 240 ms, depending on the sample. First a single distance CT was acquired with a field of view of 526 x 526 x 526 µm^3 (pixel size 257 nm) and reconstructed using PyNX. A smaller ROI, 100 µm one each side, was then identified, and a four-distance holotomography, with a pixel size of 50nm, was then acquired on this ROI. A fast reconstruction was performed on site using PyNX to check data quality. Reconstructed slices were visualized using ImageJ. A more advanced reconstruction using an inhouse procedure was later performed, to get the final reconstructed volume. Repeated acquisitions were also conducted on two samples and showed no beam damage.

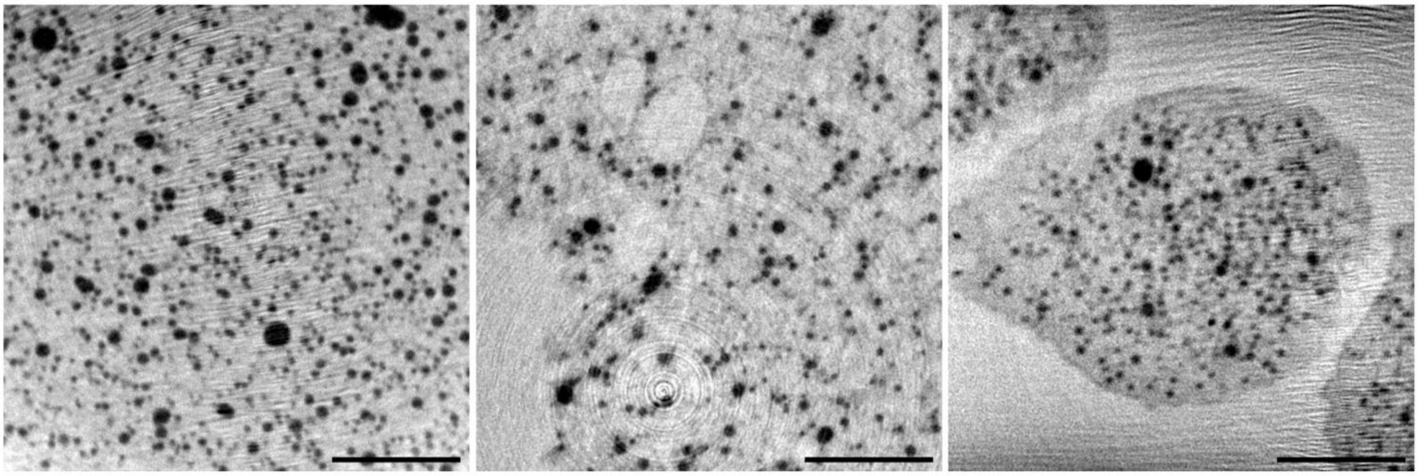

Fig. 1: reconstructed holotomography slices of dry binders. Left : egg yolk. Middle : o/w emulsion. Right: w/o emulsion. Pixel size : 50nm. Scale bar is 10 µm.

Results :

Binders : egg yolk and linseed oil can clearly be distinguished in CT images. In particular, egg yolk displays two distinct phases, dense spherical structures with diameters in the 0.3-2 µm range, identified as the egg yolk granules, *i.e.* protein-rich aggregates, dispersed in a less dense continuous phase, the egg yolk plasma (*cf.* Fig.1, left). These charateristic features allow further differentiation between egg and oil in dried emulsions, and show that the structure of the binder (o/w or w/o), previously studied on fresh emulsions using confocal microscopy, is kept during drying (*cf.* Fig. 1, middle and right). However, despite the difference between egg yolk and oil, the contrast in these images remains lower than during preliminary tests conducted at 17 keV.

Binders + pigments : for all the mock-up paints, pigments can easily be discriminated from the binder thanks to their higher density. However, the contrast in the organic phase is unsufficient to distinguish granules, plasma and linseed oil: after segmentation of the pigments, a single population can be seen in the pixel intensity histogram of the binder (*cf.* Fig. 2). Image analysis, in particular investigation of the binder structure in the presence of pigment particles, is therefore limited at this point. Further developments are underway to try to enhance the contrast within the organic phase.

Historical sample : The various pictorial layers of Colantonio's paint fragment, previously identified using Scanning Electron Microscopy (SEM), can be identified in CT images (*cf.* Fig 3) thanks to the pigment nature (pigment density and morphology). Again, information on the binder are limited at this point.

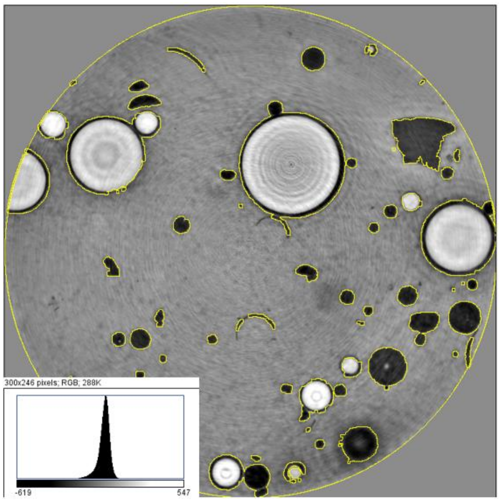

Fig. 2: mock-up paint with hollow glass-spheres dispersed in an o/w emulsion. Image is segmented between particles and binder based on pixel intensity values. Inset: pixel values distribution in the binder.

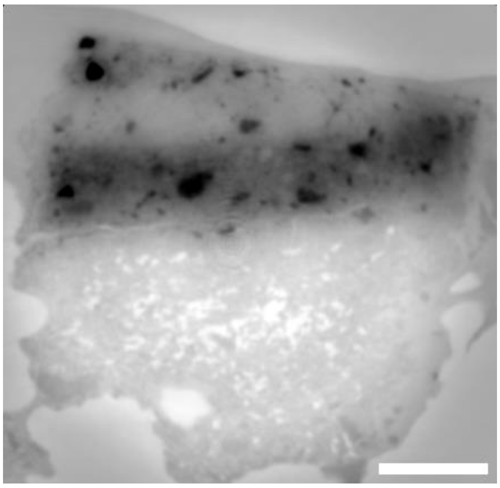

Fig. 3: historical paint fragment. Several pictorial layers are visible, on top of the preparation layer. Pixel size : 257 nm. Scale bar is 50 µm.

Conclusions:

The whole set of samples (29 mock-up binders and paints + 1 historical fragment) have been analyzed using X-Ray nano-holotomography. Egg yolk distinctive structure can be used to investigate the microscopic organization of dried emulsions. Data acquired on samples containing pigments could not be fully exploited yet due to poor contrast in the binder. More results on this particular point may come in the following months thanks to ongoing work in the data reconstruction.