EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

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.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal 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:** Nanoparticles dynamics in Drosophila synsytial embryos	**Experiment number**: LS-2305
Beamline: ID16A-NI	**Date of experiment**: from: 27-09-2014 to:03-10-2014	**Date of report**: 03/10/2016
Shifts: 18	**Local contact(s)**: CLOETENS Peter/ JOITA PACUREANU Alexandra	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Paolo Ghigna (Università di Pavia, Italy)*

Patrizia Sommi (Università di Pavia, Italy)*

Umberto Anselmi Tamburini (Università di Pavia, Italy)*

Ilenia Tredici (Università di Pavia, Italy)*

Ceccone Giacomo (Laboratory Joint Research Centre – ISPRA)

Vittorio Ricci (Università di Pavia, Italy)

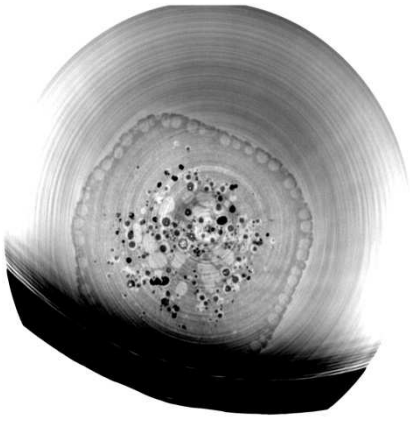
Report:

Background Nanoparticles (NP) have received large attention for their interaction with biological systems and the resulting implications in nanomedicine (in diagnostics or as nanocarriers for drug delivery) and in nanotoxicology. Despite the wide available literature, several aspects of this interaction are still unclear (Cha et al., 2007). The difficulty in defining what makes NP more likely to interact with cells is related to the poor knowledge of the mechanisms of NP internalization on one side and of the effects that NP might have on cell physiology and biochemistry on the other. Toxicological studies have investigated the main aspect of NP impact on cellular processes (Lewinski et al., 2008), but so far no attention has been devoted to their effects on the mitotic process and genomic instability, which eventually could lead to cancer. There are evidences that TiO2 NPs, widely present in commonly used commercial products, can interact *in vitro* with tubulin and microtubules (MTs) by acting on the MT polymerization rate (Gheshlanghi et al., 2008). Specifically, TiO2 NPs have been shown to affect the rate and extent of MT assembly and change their conformation. From this derives the importance of a detailed study that could clarify whether NP, once internalized, either free in the cytoplasm or compartmentalized in lysosomes and/or other intracellular structures, could interfere with the mitotic spindles and the related highly dynamic microtubules (MTs) (Downing, 2000). In our research, we hypothesize that once NP enter the cell, they can interfere with the spindle MTs possibly affecting the highly choreographed sequence of motility events that characterize spindle assembly, thus preventing the completion of cell division and eventually favor defects in chromosomes segregation.

Experimental description To address the interference of NPs with the mitotic process, we performed X-ray synchrotron microtomography on the *Drosophila* syncytial blastoderm-stage embryo, a biological model which is a well-documented system for mitosis research (Brust-Mascher et al., 2011). *Drosophila* embryos (length 500μm, width 200μm) were injected with different types of NPs (gold, SiO2,) with a size of 15-100 nm. Each NP was suspended in a proper solvent and directly injected into the embryo in order to reach a cytoplasmic concentration ranging between 50 and 200μg/ml. To allow NP diffusion and possibly interaction with the spindles. the embryo were then fixed 20 to 60 minutes after the injection. The fixed embryos were then placed in quartz capillary for observation.

Results The outcome of the experiment was only partially positive. It was indeed possible to obtain low resolution 3D reconstruction of the internal structure of the embryo (Figure 1), but, due to some technical limitations, it was not possible to take full advantage of the beamline resolution. Two aspects were critical: 1) the radiation damage was too severe when the maximum resolution was attempted, resulting in sample deformation during the acquisition; 2) the presence of reconstruction rings that reduced that possibility to evidence the finer details of the images including the single NPs (Figure 2). Although we have been able to identify the presence of NP aggregates in the embryo, we could not prove their relation with the internal structures and mitotic spindles. It was not possible to clarify whether all the NPs were present only in agglomerated form, as a result of their interaction with the cytoplasm, or whether a significant fraction of non-agglomerated, individual nanoparticles where also present in proximity of the mitotic spindles.

However, a preliminary test performed on the same Drosophila samples after the recent introduction of significant beamline improvements evidenced the possibility to overcome both limitations. The addition of a cryostat, minimizing the radiation damage, and the introduction of improved strategies for the image acquisition allow, in fact, to remove almost completely the presence of the reconstruction rings and to improve drastically the quality and the resolution of the scan reconstruction obtained at the maximum resolution.



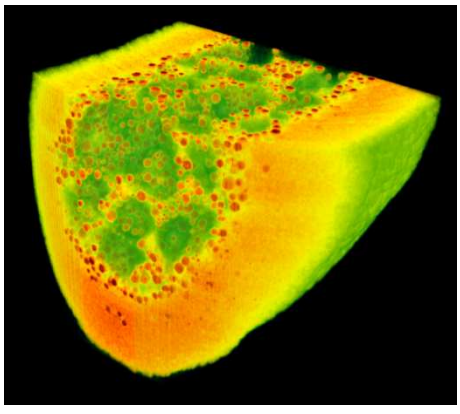


Figure 1 Examples of low resolution images. On the left, example of single plane reconstruction; on the left, embryo 3D reconstraction The dark/red vesices are embryo's yolk and not nanoparticle aggregates.

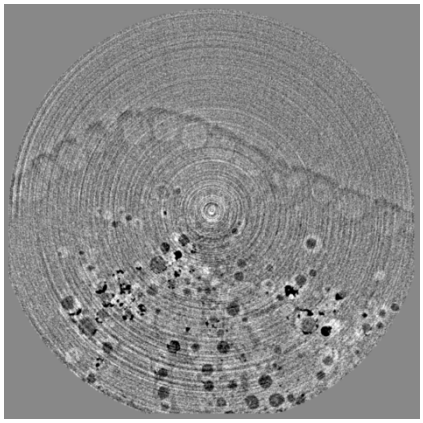


Figure 2 Example of an high resolution image. Note, the dark vesices are embryo's yolk and not nanoparticle aggregates.

Cha K.E. and Heejoon M. (2007) *J. Microbio. Biotechnol.* 17:573–1578.
Lewinski N., Colvin V., and Drezek R. (2008) *Small* 4:26-49.
Gheshlaghi Z.N., Riazi G.H., et al. (2008) *Acta Biochim. Biophys. Sin.* 40:777-782.
Downing K.H. (2000) *Annu. Rev. Cell Dev. Biol.* 16:89–111.

Brust-Mascher I., Scholey J.M. (2011) *Biochem. Soc. Trans.* 39:1149-1153