EUROPEAN SYNCHROTRON RADIATION FACILITY
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Experiment Report Form

	Experiment title: Assessment of drug-tissue interactions in cisplatin treated ovarian cancer tumors with nano-XRF and XANES	**Experiment number**: LS 2559
Beamline:	**Date of experiment**: from: 9/2/2017 to: 13/2/2017	**Date of report**:
Shifts:	**Local contact(s)**: Remi Tucoulou	*Received at ESRF:*

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Report:

Ovarian cancer is one of the primary cancers in women worldwide. The onlook for patients diagnosed with this disease is not positive, with an overal cure rate of 30%.[1,2] Clinically, it has been proven that intraperitoneal (IP) chemotherapy with cisplatin can be effective against this cancer.[3] Little research has been performed on the biological mechanisms governing the effectniveness of IP protocols. It has been found that cancer cells develop resistance towards the drug quite quickly, a process which is not yet understood.[4]
The nano-XRF and –XANES experiment at the ID16B beamline were performed in the context of our attempts to unravel the resistance mechanism of cancer cells towards cisplatin. Nude-foxn1nu female mice were injected with ovarian cancer cells in a matrigel matrix. These mice were treated with cisplatin IP (at 41°C, the optimal temperature found during a previous experiment at ID16B) two weeks after injection with the cancer cells. After the IP treatment the tumors were immediately resected, fixated in paraformaldehyde and imbedded in parafin. 5 µm thick slices were placed on ultralene® foil and analysed with the ID16B nano-probe. Optical microscopy images were used to correlate the structures found via XRF imaging with cell structures, thus selecting the ROIs for nano-XANES analysis.

The experiments performed on the tumor sections were performed in two steps. First a quich scanning procedure was used to image the elemental composition of the sample in order to find regions with augmented Pt concentrations. Care was taken to minimze exposure time during these scans, in order to avoid beam damage. Figure 1 shows the result of such an overview scan as a red/green/blue (rgb) image. Based on the data of our previous beamtime, Zn was selected as signature element of tumor cells and more specifically their nucleus, where Pt should interact with the DNA in order to kill the malligant cell. However, as before, Pt is found to reside mainly outside the Zn-rich regions (i.e. the cells). The a smaller extent, it was also once more noted S and Pt are most often found in augmented concentrations in the same parts of the scan. These zones were selected for further analysis using XANES.

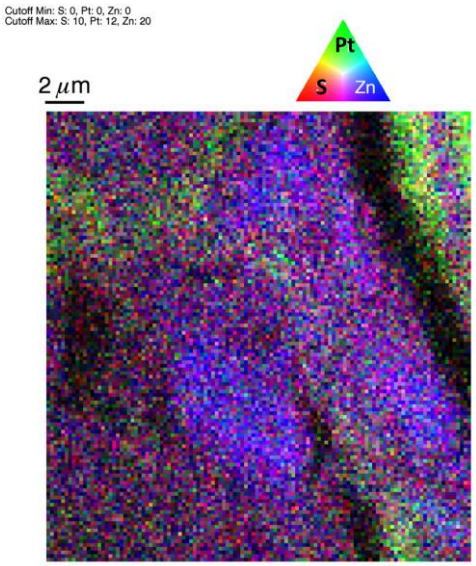

Figure 1 rgb image of S, Pt and Zn. 0.4 ms dwell time, 200 nm step size, 11.71 keV

Once a region of interest was determined, long XANES scans were performed on a single nanoscopic point (± 30s). Instead of illuminating the sample for this long time span in one go, we rather performed a series of sort XANES measurements (~ 500 ms) wich were then summed. This procedure allowed us to monitor possible beam damage, which was noted in some cases after several seconds of measurement by a change in the XANES pattern (i.e. lowering of the white line).
Measurements on several Pt containing reference materials allowed us to draw conclusions on the oxidation state of the detected Pt, however, due to instrumental issues the XANES-spectra were insufficiently detailed for certain identification of the Pt compounds. The KB mirror system of ID16B at the time of this experiment was constructed from a W multilayer. Unfortunately, the tungsten L2 edge (11.544 keV) lies very close to the Pt L3 edge (11.564) we were aiming at, greatly diminishing the sensitivity of our measurements. The future refurbishment of the ID16B beamline with new KB mirrors made from palladium would resolve this issue.

References

(1) Ferlay, J.; Shin, H.-R.; Bray, F.; Forman, D.; Mathers, C.; Parkin, D. M. *International Journal of Cancer* **2010**, *127*, 2893-2917.
(2) Bast, R. C.; Hennessy, B.; Mills, G. B. *Nat Rev Cancer* **2009**, *9*, 415-428.
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The data gathered during this beamtime yielded information for two publications:

Laforce, B.; Carlier, C.; Vekemans, B.; Villanova, J.; Tucoulou, R.; Ceelen, W.; Vincze, L., Assessment of Ovarian Cancer Tumors Treated with Intraperitoneal Cisplatin Therapy by Nanoscopic X-ray Fluorescence Imaging. *Scientific Reports* **2016,** *6*, 29999.
Carlier, C.; Laforce, B.; Van Malderen, S. J. M.; Gremonprez, F.; Tucoulou, R.; Villanova, J.; De Wever, O.; Vincze, L.; Vanhaecke, F.; Ceelen, W., Nanoscopic tumor tissue distribution of platinum after intraperitoneal administration in a xenograft model of ovarian cancer. *Journal of Pharmaceutical and Biomedical Analysis* **2016,** *131*, 256-262.