EUROPEAN SYNCHROTRON RADIATION FACILITY
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Experiment Report Form

	Experiment title: Submicron XRF Imaging of the Cisplatin Distribution in Peritoneal Carcinomatosis Tumors Treated with Intraperitoneal Chemotherapy	**Experiment number**: LS 2444
Beamline:	**Date of experiment**: from: 29/10/2015 to: 02/11/2015	**Date of report**:
Shifts:	**Local contact(s)**: Julie Villanova	*Received at ESRF:*

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Report:

Ovarian cancer, being one of the most common types of cancer in women worldwide, only has an overal cure rate of 30 %.[1,2] Intraperitoneal (IP) chemotherapy with cisplatin has been proven clinically to be an effective treatment for this type of cancer.[3] However, up-to-now little research has been performed on the optimization of IP protocols. Furthermore, it has been found the cancer cells develop resistance towards the drug relatively quickly, urging for more in-depth research on the tumor-drug interactions.[4]

The nano-XRF measurements at ID16B were part of our attempts to unravel the resistance mechanism of cancer cells towards cisplatin, while a second goal was to assess the influence of temperature on the penetration depth of the cisplatin drug. At Ghent University Hospital, nude-foxn1nu female mice were injected with ovarian cancer cells in a matrigel matrix. These mice were treated with cisplatin IP (at two temperatures: 37°C and 41°C) two weeks after injection. The tumors were resected immediately after the IP treatment, fixed in paraformaldehyde and imbedded in parafin. Slices of 2 µm thickness 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 such as the cell membrane, the nucleus or the extracellular matrix.

Two types of XRF experiments have been performed on the tumor sections.
Linescans covering the entire width of the samples were measured in order to obtain the penetration depth of the cisplatin drug in the tumor tissue (stepsize 100 nm). It could be shown thet IPC treatment at elevated temperatures leads to a higher penetration depth. The high sensitivity of the ID16B instrument was crucial in detecting the small Pt concentrations, which would have been undetectable using a laboratory setup. In view of our goal to optimize the IP protocol, the enhancing effect of elevated temperatures on the penetration depth of cisplatin drugs is of high relevance..

Secondly, high resolution maps (50 nm) of the tumor tissue were made. The XRF maps are used to study the elemental distribution of Pt and trace metals in the tumor tissue. In order to effectively kill the cancer cells, the drug must interact with its DNA in the cell nucleus. Hence, the submicron distribution of Pt revealed by the XRF images will be of huge importance when investigating its activity. The correlations between Pt and others metals are also of interest, because of possible synergetic effects. Figure-1 represents three XRF maps of P, Zn and Pt respectively. The P signal clearly reveals the outlines of the tumor cells, due to it being present in the pospholipid membranes of the cell structure. Zn is present in multiple cell structures and is notably concentrated in the nucleus. Comparing the P and Zn map with Pt, it is clear the regions with high Pt concentration are lying outside the tumor cells. Further investigations are needed in order to clarify the reason why Pt resides preferentially in the extracellular matrix (ECM) of the tumor tissue, hypothesises being a too short reaction time, high affinity the specific structures of the ECM or the resistance mechanism of the tumor cells preventing it from entering the cells. Information on the chemical structure of the Pt will be crucial when assessing this question. Furthermore, it was discovered Pt shows a strong correlation with Br and to a lesser extent with S. The Pt-S correlation might be due to interactions of the cisplatin drug with certain proteins present in the ECM. Br is a known trace element in human tissue, while its comparable chemical structure to Cl (both being halogens) could explain its affinity towards cisplatin, since Pt is complexed with Cl in this molecule. Once again, further research is needed to clarify the interactions Pt with these two elements.

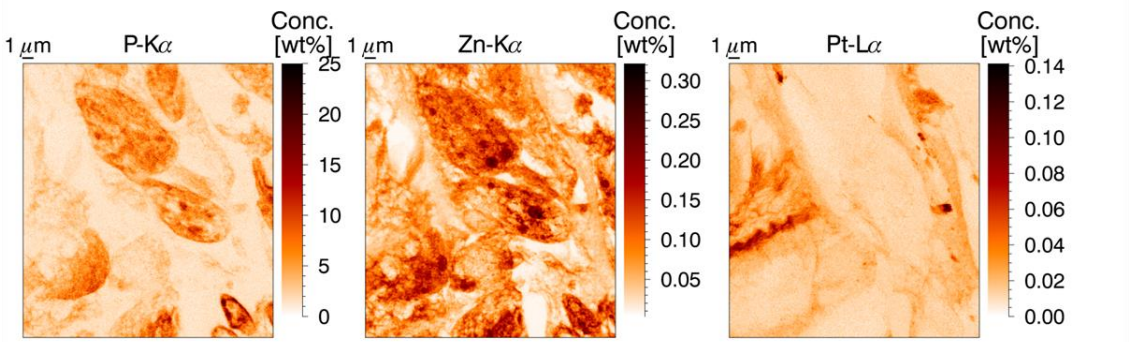

Figure-1 XRF maps of P, Zn and Pt (50 nm step size, 0.1 s dwell time) concentration were determined using a Monte Carlo simulation aided quantification procedure

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.