<u>Report MD-901:</u> Studies to localize raw and purified carbon nanotubes in mesothelial cells by X-Ray fluorescence and to monitor the chemical changes produced by exposure.

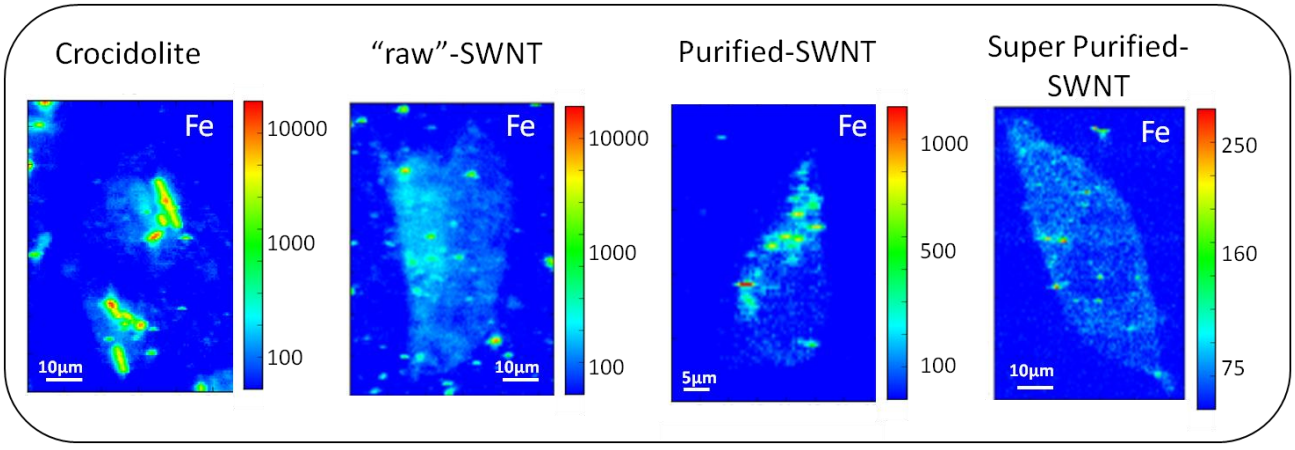
The main objective of the present study is to investigate by synchrotron μXRF carbon nanotubes (CNTs) interaction and internalization in mesothelial cells (MeT5A, ATCC cell line) and possibly reveal altered metabolism of endogenous elements. A particular attention was be given to iron metabolism change, that was expected for analogy to what seen with CNTs and asbestos fibres in other cells [1].

As-prepared, "raw" SWNT usually contain significant amounts of ultrafine metal particles derived from production process. Iron, nickel and cobalt are the most common catalyst particles primarily used during CNTs manufacture. A number of studies have attempted to investigate the role of metals in CNT induced toxicity and early studies demonstrated that the metal content, particularly iron, of CNT can contribute to biological effects probably via the induction of an oxidative stress [2].

The present beamtime activity allowed to reveal the localization of nanomaterials (following iron signal) and evidenced morphological changes of treated cells (following sulphur and phosphorus signals). XRF analyses demonstrated that the "raw" SWNTs alter more the endogenous iron distribution and concentration than partially purified and highly purified SWNTs, as shown in iron bidimensional map reported in the figure below.

Differently the attempt to resolve nickel (Ni) presence in mesothelial cells was not greatly successful, because we noted that the sensibility of ID21 is limited for this element.

However the beamtime was successful and the present study demonstrated the potential of advanced synchrotron-based X-ray technique for studying *in vitro* the cellular response of mesothelium (lining of lung tissue) to the nanomaterial exposure. Moreover, the experiment we confirmed that partially purified SWNTs and highly purified SWNTs have a lower presence of iron traces respect to "raw"-SWNTs highlighting a greatly reduced healthy risk.



References

[1] Donaldson K. et al. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Part Fibre Toxicol 2010, 7:5.

[2] Helinor J. Johnston, Gary R. Hutchison, Frans M. Christensen, Sheona Peters, Steve Hankin, Karin Aschberger, Vicki Stone: A critical review of the biological mechanisms underlying the in vivo and in vitro toxicity of carbon nanotubes: The contribution of physico-chemical characteristics. Nanotoxicology, June 2010; 4(2): 207–246.