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### **IT-4-P-3088 STEM in SEM imaging of gold nanoparticles in tissular ecotoxicity experiments**

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Because of their prospective widespread use, gold nanoparticles (AuNPs) will certainly account for a considerable and persistent nanomaterial input to environmental systems. Therefore ecotoxicological risks in non target organisms associated with AuNPs are showing increasing consideration. Location of AuNPs has been previously studied in our laboratory analyzing slices of gills and digestive gland tissues of the bivalve *Ruditapes philippinarum* after "in vivo" exposure experiments. Analysis was carried out by TEM of ultrathin tissue's slices (80 nm) operating at 80 kV [1].

In this communication we present the results of investigating the use of an "in vitro" methodology associated to the optimization of the STEM-in-SEM technique for the use of a scanning electron microscope (SEM-FEG) in transmission mode and operated at 20-30 kV.

The advantages of STEM-in-SEM over TEM are discussed [2, 3]. The localization of high Z nanoparticles in low Z tissue matrices is presented here by using the STEM-in-SEM coupled to EDX analysis as a powerful technique. In addition we have optimized the measurements with the goal of working with thicker slices. The work with thick samples also avoid the NPs displacement during cutting and increase the possibility of finding NPs when working with low NPs doses (environmental relevant concentrations).

For the optimization of measurements conditions, the resolution in our SEM-FEG has been estimated using Fast Fourier Transform (FFT) algorithms on specific images of our tissue slices. We have used the SMART macro running inside the "SCION Image" program under windows [4, 5]. Working at magnifications over 100 kx, for slices thicknesses of 200-300 nm and operating voltages of 20-30 kV, leads to resolutions below 10 nm (an adequate value for analyzing AuNPs of 23 nm average diameter).

Figure 1 shows a representative image of AuNPs accumulated into the gill tissues after "in vitro" exposures. From the obtained images it was possible to localize AuNPs (see also Figure 2) associated with vesicles (it can be a large phagosome or also exocytosis). Nanoparticles were also found in residual bodies (exocytosis).

In summary this communications presents new results for "in vitro" fast testing and STEM-in-SEM imaging of engineered AuNPs in a tissular ecotoxicity model.

#### References

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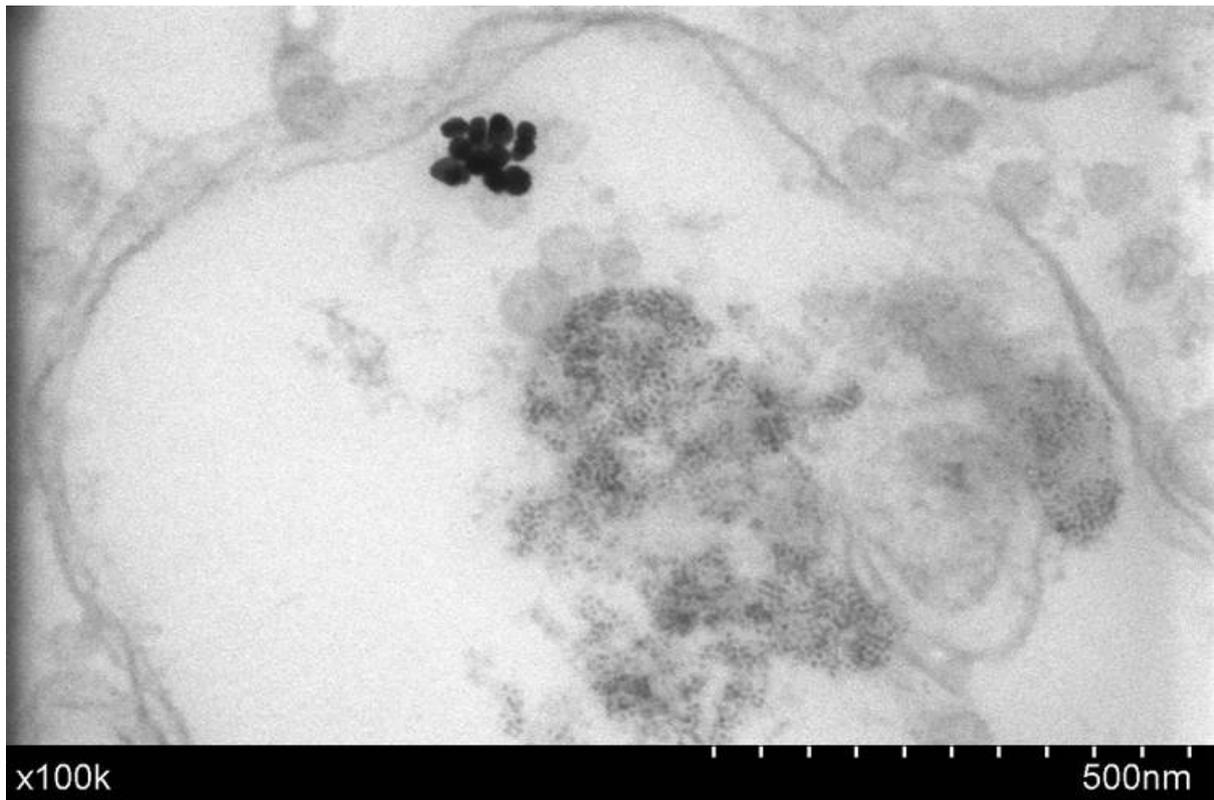


Fig. 1: SEM-FEG image (transmission mode) of a 200 nm slice of gills tissue

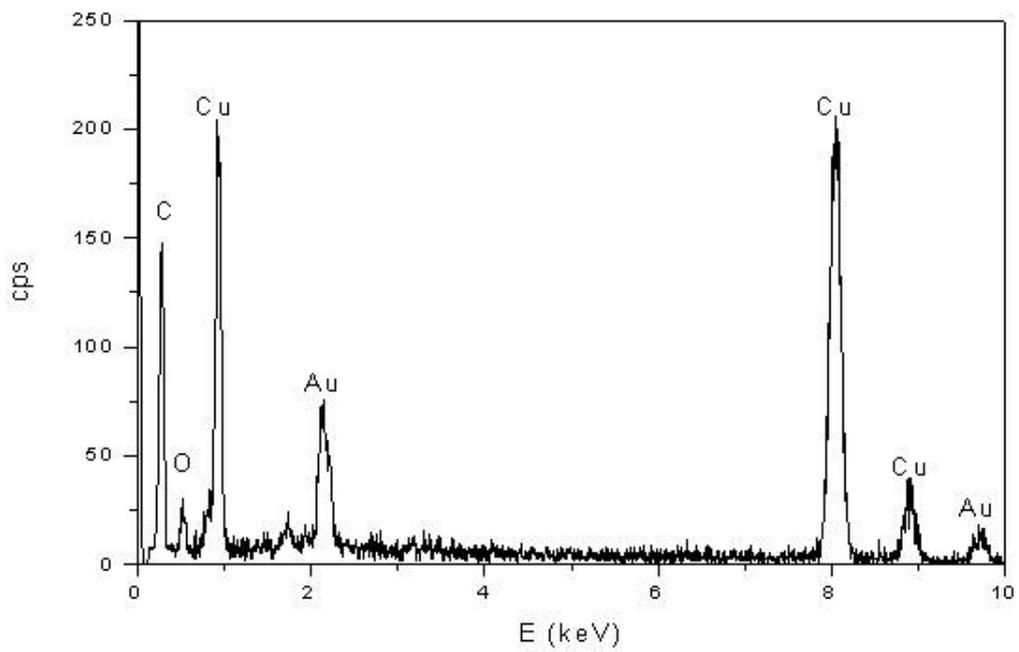


Fig. 2: EDX spectrum from the area containing the AuNPs