Correlative Microscopy Techniques for the Analysis of Particles in Safeguards Environmental Samples

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Abstract. This paper presents a novel approach to environmental particle analysis for safeguards by means of a combination of micro-analytical techniques. It includes the tandem utilization of two separate light microscopes, a scanning electron microscope and a femtosecond laser-ablation ICP-MS. These are: a light microscopy automated particle relocation device (Zeiss Z2m); an optical-microscopy-based laser micro-dissection system (IX83 MML+Olympus); a focussed ion beam scanning electron microscope equipped with a time-of-flight mass spectrometer extension (Tescan Lyra3) and a fs LA-ICP-MS (J200 from Applied Spectra Inc. and Thermofisher Scientific iCap Q). The samples examined in this contribution are analysed for their nuclear material signatures, in particular the presence of uranium isotopes.

Keywords: Correlative microscopy, Safeguards, Laser micro-dissection, LA-ICP-MS, FIB-SEM-TOF, Uranium isotopes,

1. Introduction

Environmental samples are processed and analysed in the Environmental Sample Laboratory (ESL), which is part of the Safeguards Analytical Laboratories (SGAS) of the International Atomic Energy Agency, Seibersdorf, Austria. The samples are exclusively handled in cleanroom facilities where all measurements are conducted using micro- and ultramicro-analytical techniques. Advanced instruments such as the LG-SIMS, TIMS, ICP-MS, LA-ICP-MS and SEM are routinely used alongside established radiochemical methods for the bulk and particle analysis of uranium-containing swipe material. [1] [2]

Correlative microscopy has a growing number of applications in the biological [3] [4] [5] [6] [7] and materials [8] [9] [10] sciences. The basic idea is to define a site of interest and then relocate the same area in another instrument for further sample characterization. This paper describes recent efforts in the relocation of 1-5 µm particles from a single sample between two optical microscopes, a scanning electron microscope and a LA-ICP-MS instrument. In addition to correlative microscopy, this contribution goes beyond and demonstrates a method whereby sites of interest were physically isolated and transferred unto a separate substrate for further microprobe analyses steps. In the field of Safeguards, no single microscopy or mass spectrometry technique can provide a complete assessment of an environmental swipe sample (ESS) which is why a particle tracing scheme is needed for facilitating combined analysis from several techniques.

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An analytical procedure was developed for the systematic processing of ESS that includes (Figure 1.):

1. Deposition of sample particles from a swipe onto a catcher;
2. Identification of Particles-Of-Interest (POIs) on the catcher;
3. Isolation and transfer of POIs onto a harvester;
4. Microprobe analysis of POIs;

![Figure 1: Analytical procedure steps: 1. Collection of particles from swipe; 2.a) Dispersion and laser microdissection of POIs; 2.b) isolation of POIs 3. transfer of POIs onto a harvester; 4. Microprobe analysis of POIs (FIB-SEM-TOF U-238 m/q signal is depicted.)](image1)

2. Sample Preparation for Microprobe Analysis

2.1. Deposition of particles onto a catcher (dispersion)

MMI membrane slides with stainless steel rims were used as the catcher substrate. These were marked with three fiducial points to be used for later relocation of POIs [3]. The markings are shown in Figure 2. and covered an area of 10mm x 17 mm. The slide was coated with clear varnish (Daler-Rowney Ltd, Bracknell, Berkshire, USA) by means of a spin coater. After this, the sample was placed inside a shock-wave disperser for deposition of particles from the swipe onto the varnish-coated membrane slide. The test material was a homogenized and sieved DU contaminated soil. The loading of material on the shock wave disperser was ~ 40 μg. Details of the dispersion method are discussed elsewhere [4].

![Figure 2: Laser markings for relocation. The fiducial points are: on the triangle, the end of the line bisecting the upper angle; square - centre of cross; circle - centre of cross.](image2)

2.2. POI identification.

After dispersion, the sample was allowed to settle for 24 hours before coating with Au. Six particles containing DU were identified by a TESCAN Lyra3 SEM. Figure 3. displays an EDX spectra from a single DU-particle (X Max-50, Oxford Instruments). The coordinates of the three reference points and six POIs was recorded for relocation in other instruments.

![Figure 3: EDX spectra of a single DU particle](image3)

2.3. Relocation of POIs

An overview image of the fiducial points and POIs was made using a Zeiss Z2m light microscope along with its accompanying software (see Figure 4.) About 80 individual images taken at 5x magnification (1.268 μm/pixel resolution) were used to build the fused sample collector image. The objective used was an EC Epiplan-Neofluar 5x/0.13 HD M27 with a working distance of 15.1 mm. The images were stitched by the native AxioVision SE64 software using stage coordinates and an image overlap of 10%. No filtering was conducted to enhance
image features and the images were only corrected for background illumination during the acquisition (flat-field correction). The relocation accuracy was ~ 20 µm.

2.3.1. Laser micro-dissection of POIs.
The laser microdissection device (LMD) used was an MMI IX83 with Olympus scope. A 3-point-based algorithm [11] for the transfer of fiducial points and POIs was used to generate a POI relocation file with MMI stage coordinates. The POIs were found in the LMD device with an accuracy of ~ 50 µm.

A harvester was made out of MMI microdissection chambers (diameter 35 mm) with a silicone bottom. The transparent silicone was removed and trimmed to fit the slit on the membrane slide and carefully fixed onto a standard microscopy glass slide (76 mm x 26 mm). The harvesting was done by placing the catcher on top of the harvester so that the silicone was under the membrane slide and the deposited particles were on top, see Figure 5.) This prevented unwanted particle transfer to the harvester, see Figure 6. for laser micro-dissection illustration). A 50 µm circle was cut around each particle (see Figure 8. for an image of the catcher after LMD cuts and detachment of the harvester). The cut parameters were: cut velocity 27 µm/s, laser focus 2667 µm and 80% laser energy. During the laser-cutting, the membrane with the POIs would stick onto the melting silicone. New markings were made around the POIs for facilitating relocation in other instruments (see Figure 7. for an SEM image of a relocated DU particle and Figure 8. for an overview of the catcher post cutting). Additional material including videos of the dissection can be found online.

Figure 4: Overview of relocation area. Laser markings are shown: circle (bottom left, diameter 117 µm), square (right, 190 µm side length) and triangle (top left, base length

Figure 5: (top) harvester with silicone; (bottom) MMI membrane slide was placed on top of the harvester.

Figure 6: (top) side-view of catcher and harvester sandwich; (bottom) laser-microdissection of POI.

Figure 7: A relocated POI with membrane material. The image was taken in a Lyra3 SEM prior to FIB-SEM-TOF analysis.

3. Results of Microprobe Analysis

Prior to transfer into an FIB-SEM-TOF, the harvester and catcher were detached. All particles were transferred successfully onto the harvester. The TOF measurement was conducted in a Lyra3 FIB-SEM-TOF instrument – see Figure 1 Part 4 for a laterally resolved signal of a single DU particle (the U-238 mass spectra signal is shown). The uranium isotopic composition of a 1 µm particle obtained from the FIB-SEM-TOF was evaluated at 0.0046 (U-235/U-238).
The harvester with the remaining five POIs was transferred into a J200 LA/LIBS system (Applied Spectra Inc.). These particles were ablated with the following parameters: 3 shots @ 30 µm spot size, 40 Hz repetition rate, 100% output energy, 0.7 l/min Ar flow and 0.7 l/min He flow. The J200 LA system was connected to a quadrupole mass spectrometer - iCap Q (Thermofisher Scientific) to obtain simultaneous mass spectrometry measurements of the five POIs. It was found that the mean isotopic ratio of uranium for the five particles was 0.0020 U-235/U-238 (naturally occurring U is found in the isotope ratios: 0.0072 U-235/U-238) [12]. See Figure 9 for an overview image of the harvester post laser-ablation.

4. Conclusion

The proposed analytical procedure for the isolation and transfer of six 1-5 µm particles was found to be successful. A mixture of soil particles with depleted uranium were deposited onto a thin membrane slide and six particles containing DU were identified by means of their EDX spectra in an SEM. Their positions were recorded with reference to three fiducial marks for later relocation in a LMD device. Once transferred, they were cut out and collected onto a silicone-based harvester. All particles were harvested successfully. One was analysed by means of a FIB-SEM-TOF and five by LA-ICP-MS. All micro-probed particle measurements found the U isotopic signature of the particles to be of depleted uranium (i.e. less than 0.0072 U-235/U-238).

5. References

[1] D. Donohue, *Analytical Chemistry*, vol. 74, no. 1, pp. 28A-35A, 2002.
[2] G. Voigt and M. Scheland, IAEA Internal SGAS Report, Vienna, Austria, 2013.
[3] A. Sartori, R. Gatz, F. Beck, et al, *Journal of Structural Biology*, vol. 160, no. 2, pp. 135-145, 2007.
[4] J. Plitzko and A. L. A. Rigort, *Current Opinion in Biotechnology*, vol. 20, no. 1, pp. 83-89, 2009.
[5] P. Kempen, M. Kircher, A. de la Zerda, et al, *Micron*, vol. 68, pp. 70-76, 2015.
[6] C. Fonta and B. Humbel, *Archives of Biochemistry and Biophysics*, 2015.
[7] D. Kong and J. Loncarek, *Methods in Cell Biology*, p. online, 2015.
[8] Carl Zeiss, *Tools & Techniques*, vol. 12, no. 10, p. 47, 2009.
[9] A. Fejfar, M. Hylv, A. Vetuschka, P. Pikna, et al, *Solar Energy Materials and Solar Cells*, vol. 135, no. EMRS 2014 Spring Meeting – Advanced materials and characterization techniques for solar cells II, pp. 106-112, 2015.
[10] M. Herbig, P. Choi and D. Raabe, *Ultramicroscopy*, vol. 153, pp. 32-39, 2015.
[11] U. Admon, et al., *Hot Particles Released from Different Nuclear Sources*, Yalta, Crimea, 2007.
[12] S. Richer, A. Alonso, et al, *International Journal of Mass Spectrometry*, vol. 193, no. 1, pp. 9-14, 1999.
[13] U. Admon, E. Boblil, E. Chinea-Cano and N. Dzigal, *IAEA Safeguards Symposium*, Vienna, 2014.

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