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Critical evaluation of fast and high resolved elemental distribution in single cells using LA-ICP-SFMS.

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Abstract

The analytical potential of a nanosecond laser ablation inductively coupled plasma mass spectrometer (ns-LA-ICP-SFMS) system, equipped with an ultra-fast wash-out ablation chamber, is critically investigated for fast and high spatially resolved (~ μm) qualitative elemental distribution within single cells. Initially, a low surface roughness (< 10 nm) thin In-SnO_2 layer (total coating thickness ~ 200 nm) deposited on glass is employed to investigate the size, morphology and overlapping of laser-induced craters obtained at different laser repetition rates, making use of Atomic Force Microscopy (AFM). Conical craters with about 2 μm of surface diameter and depths about 100 nm were measured after a single laser shot. Furthermore, the influence of the sampling distance (e.g. distance between sample surface and inner sniffer of the ablation chamber) on the LA-ICP-MS ion signal wash-out time is evaluated. A significant decrease of the transient $^{120}Sn^+$ ion signal is noticed after slight variations (± 200 μm) around the optimum sampling position. Ultra-fast wash-outs (< 10 ms) are achieved reducing the aerosol mixing from consecutive laser shots even when operating the laser at high repetition rates (25-100 Hz). Fast and high spatially resolved images of elemental distribution within mouse embryonic fibroblast cells (NIH/3T3 fibroblast cells) and human cervical carcinoma cells (HeLa cells), incubated with gold nanoparticles (Au NPs) and Cd-based quantum dots (QDs), respectively, are determined at the optimized operating conditions. Elemental distribution of Au and Cd in single cells is achieved using high scanning speed (50 μm/s), and high repetition rate (100 Hz). Results obtained for the distribution of fluorescent Cd-based QDs within the HeLa cells are in good agreement with those obtained by confocal microscopy. Size, morphology and overlapping of laser-induced craters in the fixed cells are also investigated using AFM, observing conical craters with about 2.5 μm of surface diameter and depths about 800 nm after a single laser shot.

Keywords: LA-ICP-SFMS, ultra-fast wash-out, crater size and morphology, single cell, Au NPs, Cd-based quantum dots, fast imaging, elemental distribution, atomic force microscopy.

Introduction

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Advance analytical techniques have been developed for fast microanalysis in biological applications, including fluorescence imaging, electrochemistry, microscopy and mass spectrometry [1-3]. Additionally, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has become a mature technique for qualitative and quantitative elemental imaging with high lateral resolution [4-10].

Typically, a pulsed (e.g. ns or fs) laser is fired at a repetition rate of about 10 Hz while the stage with the mounted sample is moved underneath the laser beam (e.g. laser raster mode). Laser spot sizes are usually equal to or bigger than 4 μm (nominal value of the crater spot size) and laser scan speeds equal to or lower than 15 μm/s [11-14]. The image is then constructed by ablating parallel lines on the sample surface. The laser-induced aerosol is evacuated from an air-tight ablation chamber to the ICP-MS with a continuous flow of inert gas (typically He). Wash-out times for conventional ablation chamber are in the order of 0.5–30 s. However, recently developed low-dispersion laser-ablation chambers, including the TwoVol2 with a dual concentric injector (DCI) (Electro Scientific Industries), the tube chamber from ETH-Zürich, or the HelEx II with an aerosol rapid introduction system (ARIS) (Teledyne CETAC Technologies), have significantly reduced the duration of single LA signals to less than 30 ms, improving the signal to background ratios [15-17]. In all these ablation chambers, laser-induced aerosol needs to be extracted in an efficient way into the laminar flow of the connecting tube to the ICP plasma torch. In this context, the distance between the sample surface and the extraction tube or inner volume is critical, and should be carefully optimized.

The main advantage of the fast wash-out chambers is the reduced aerosol dispersion, which avoids intermixing of the ablated material generated from consecutive laser shots even when operating the laser at high repetition rates (> 25 Hz). Moreover, high ion signal to background ratios are achieved as the aerosol from individual laser shots reaches the ICP-MS in a narrow time slot. Combining high laser repetition rate, high scanning speed and small laser spots, results in fast and high spatially resolved analysis with adequate sensitivity for the determination of major and minor elements in solid samples, including biological or geological samples.

The main drawback of the fast wash-out chambers in LA-ICP-MS for multielemental analysis is the requirement of a fast detector (e.g. high acquisition rate mass spectrometer). ICP-Time-of-flight MS might be the most adequate candidate as it is able to provide complete mass spectra at acquisition rates ≥ 30 kHz [18]. Nevertheless, sequential mass spectrometers, such as ICP-Sector Field (SF)MS, might be optimized for fast and high spatially resolved single elemental analysis, which could be of great interest in different application fields, including analysis of Nanoparticles (NPs) or Quantum Dots (QDs) in single cells.

In this work, the analytical potential of a representative ultra-fast wash-out ablation chamber is critically evaluated. A low surface roughness thin In-SnO_2 layer deposited on glass, is employed to optimize the distance between sample surface and inner sniffer, and to evaluate its influence on the peak ion signal and FWHM value. Additionally, size, morphology and overlapping of laser-induced craters measured by Atomic Force Microscopy (AFM) are reported. This high speed and high spatial resolution LA-ICP-SFMS method is investigated for the determination of elemental distributions within single cells, which is crucial to study intracellular and intercellular processes as well as their function in a cell system (e.g. tissue or organ) [19-21]. Distribution and visualization of the cellular uptake of gold nanoparticles (Au NPs) and Cd-based quantum dots (QDs) in two established cell lines, such as mouse embryonic fibroblast cells (NIH/3T3 fibroblast cells) and human cervical carcinoma cells (HeLa cells), are here investigated.

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Experimental

LA-ICP-SFMS

A NWRimage laser ablation system from Elemental Scientific Inc. (ESI) has been used in these experiments. This instrument consists of a diode pumped Nd:YAG laser at a wavelength of 266 nm (4th harmonic). It provides repetition rates from 1 Hz up to 100 Hz, fluence at the sample surface up to 20 J/cm^2, and nominal spot sizes from 1 µm to 60 µm of diameter. An ultra-fast wash-out ablation chamber (Bloodhound) combined with a Dual Concentric Injector (DCI) provides washout times shorter than 30 ms. Helium gas (99.999 % minimum purity) from Linde (Germany) was introduced in the chamber through two different inlet lines placed at the bottom part. The total carrier gas was held constant at 1.5 L/min. The laser-induced aerosol was transported from the sniffer (inner micro sampling chamber) to the ICP-SFMS through a flexible silicon tube (< 1 m length and 1.7 mm i.d). Figure 1 shows a picture of the sniffer (inner micro sampling chamber in Bloodhound design) and a schematic draw of the ablation chamber and tube connection to ICP-MS.

The detection was carried out by an Element XR ICP-SFMS (Thermo Fisher Scientific, Bremen, Germany). Operation conditions in the ICP-MS (e.g. ion optics, gas flows) were daily optimized. The ion optic was adjusted to obtain the maximum sensitivity monitoring $^{137}Ba^+$ and $^{232}Th^+$ signals produced by the ablation of the standard reference material SRM NIST 612 in raster mode at 50 Hz, 60 µm spot size, 10 µm/s and maximum fluence (20 J/cm^2). Additionally, the makeup gas flow was also adjusted to keep the ratios $^{238}U^+/^{232}Th^+$ and $^{248}OTh^+/^{232}Th^+$ below 120 % and 0.5 %, respectively. High purity Ar (99.999 % minimum purity) from Linde (Germany) was employed as plasma and make up gas. Ar was coaxially mixed with the He carrier gas inside the DCI before entering the ICP. The calibration of the digital and analogous detectors was carried out daily to warrant a linear response in the detection.

The Element XR was operated in a fast scanning mode with a short time resolution to measure the transient LA signals using the original detector without modification of the existing electronics. The data acquisition was performed according to Shigeta et al. [22]

Testing samples, chemicals, cell culture and treatment

Low roughness surface samples consisting of Indium Tin Oxide (ITO) layers (~ 200 nm), deposited on a glass substrate using a magnetron sputtering-up physical vapour deposition (PVD) system (ATC Orion 8HV - AJA International, USA), were employed to optimize the LA-ICP-MS operating conditions.

Gold nanoparticles, Au NPs (20 nm citrate capped gold nanoparticles) were obtained from Nanovex Biotechnologies S.L. (Principado de Asturias, Spain). Water-soluble CdSe/ZnS quantum dots (QDs) (Qdot® 605 ITK™ carboxyl quantum dots) consist of CdSe core with a ZnS shell and surface coated with a carboxylic layer (nanoparticle diameter of 15-20 nm), and were purchased from Invitrogen-Molecular Probes (Thermo Fisher Scientific; Eugene, OR, USA).

Human cervical carcinoma cells (HeLa cells; Cat Number # *CCL-2*™) and mouse embryonic fibroblast cells (NIH/3T3 fibroblast cells; Cat Number # *93061524*) were obtained from 'American Type Culture Collection' (ATCC; Manassas, VA, USA) and from 'European Collection of Cell Cultures' (ECACC; Salisbury, UK), respectively. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 2 mM *L*-glutamine, 10 mM HEPES and an antibiotic-antimycotic cocktail containing 100 U/mL penicillin, 10 µg/mL streptomycin and 0.25 µg/mL amphotericin B. All cell culture reagents were purchased from Gibco®-Invitrogen (Thermo Fisher Scientific; Waltham, MA,

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USA) unless otherwise indicated. Both cell lines were grown at 37 ºC in a humidified 5% CO_2 environment.

For LA-ICP-SFMS experiments, cells were seeded at a density of 40000 cells/mL and grown on sterile Nunc™ Thermanox™ coverslips, from Thermo Fisher Scientific, in a 6-well plate. Then, NIH/3T3 fibroblast cells were incubated with 1.5 mL of Au NPs suspension at a concentration of 10^9 particles/mL and HeLa cells were incubated with 1.5 mL of QDs aqueous suspension at a concentration of 15 nM of QDs, both in standard cell culture medium. After an exposure time of 24 h, cells were washed with phosphate buffered saline (PBS) and immediately fixed with 4 % formaldehyde in PBS. After fixation, cells were dehydrated in a graded series of ethanol (70 %, 80 %, 96 % and 100 %; at least 1 min residence time in every solution) and, finally, dried for LA-ICP-SFMS analysis.

AFM

Atomic force microscopy (AFM) measurements were performed in contact mode with a Cypher microscope (Asylum Research, Santa Barbara, CA). The AFM was equipped with a NSC12 silicon cantilever (MikroMasch, Tallinn, Estonia) with elastic constant (as given by the manufacturer) $k_c = 0.3$ N/m. The very low elastic constant of the cantilever allowed for performing measurements in contact mode without damaging the sample.

Confocal microscopy

For confocal microscopy study, HeLa cells were seeded at a density of 40000 cells/mL and grown on sterile Nunc™ Thermanox™ coverslips in a 6-well plate. Then, HeLa cells were incubated with 1.5 mL of the water-soluble QDs at a concentration of 15 nM of QDs in DMEM medium. After an exposure time of 24 h, cells were washed thoroughly with PBS and immediately fixed with 4% formaldehyde in PBS. Cells were then rinsed with PBS and counterstained with 1 μg/mL of 4',6-diamidino-2-phenylindole, DAPI (Sigma-Aldrich; St. Louis, MO, USA), for 5 min at room temperature. The coverslips were finally mounted using both Fluoromount-G (SouthernBiotech; Birmingham, AL, USA). Finally, Leica TCS-SP8X confocal microscope (Leica; Wetzlar, Germany) was used for confocal microscopy study and *ImageJ* program (Bethesda, MD, USA) was used for image processing. The confocal fluorescence microscopy images of the cells were taken under a 405 nm diode laser excitation.

Results and discussion

Evaluation of crater shape and morphology in low surface roughness thin layer.

The spatial resolution achieved by LA-ICP-MS is close related to laser induced crater shape and dimensions. The ideal situation for high spatially resolved analysis of biochemical samples would be the use of consecutive small square laser-induced craters, with flat bottom and no redeposition. Nevertheless, non-uniform laser beam energy distribution, undesired self-focusing of the beam to the bottom of the ablated zone, change of the ablation rate and thermal effects significantly affect crater shape and morphology.

Laser-induced craters, obtained using the NWRimage laser ablation system, were investigated in In-SnO_2 layer deposited on glass using AFM. Laser induced craters were studied in laser raster mode, operating the laser at different laser repetition rates (25, 50 and 100 Hz), while maintaining the laser fluence (~ 0.1 J/cm^2), the scanning speed (50 μm/s) and the laser spot size diameter (2 μm). At 25 Hz, adjacent craters (in contact but without overlapping) should be expected. Figure 2 shows the sequence of craters imaged by AFM that were obtained at the different laser repetition rates. It can be noticed that the craters do not have a perfect cylindrical shape. This is confirmed by the cross-section profiles in the right column of the picture, drawn

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perpendicularly to the line. These profiles show conical shapes. Moreover, redeposition is observed around the crater pit mainly due to thermal effects during ns laser ablation. At increasing laser repetition rate, crater diameter keeps constant while crater depth increases (from about 100 nm to circa 200 nm), as well as the degree of overlapping, which is also visible at 25 Hz. At the right side of the ablation crater, very shallow craters (less than 20 nm) were observed probably due to optical aberrations along the laser optical beam.

Operating conditions in ultra-fast wash-out LA coupled to ICP-SFMS

The ablation process was performed in an ultra-fast wash-out ablation chamber prototype (Bloodhound, ESI) connected to the ICP-SFMS with a Dual Concentric Injector (DCI), resulting in very fast transient ion signals (washouts times < 30 ms). The acquisition conditions of the ICP-SFMS, a relatively slow sequential mass analyzer, were optimized for fast mass spectra acquisition rate (see Table 1). It should be remarked that fast mass spectra acquisition rate in the ICP-SFMS was achieved collecting ion signals (every 2 ms) in low resolution mode within the flat top peak of the corresponding isotope. Fast acquisition conditions might be affected when operating the SF-MS in middle or high resolution.

Thin In-SnO_2 layer was used to optimize the distance between sample surface and the sniffer (inner micro sampling chamber) in terms of ion signal ($^{120}Sn^+$) and wash-out time after each ablation event. This distance can be modified using micrometer screws, which move in z-axis the platform containing the samples. The position of the sniffer is kept constant with respect to the ablation chamber, and the distance between the objective and the surface of the sample is given by the z-value at the focal position in the LA software. Hence, the relative variation of the distance between sniffer and sample surface, which is critical for the laser-induced aerosol extraction efficiency, could be monitored through changes in the z-value at the focal position.

Figure 3 shows the transient $^{120}Sn^+$ ion signals measured by LA-ICP-SFMS by operating the laser at a repetition rate of 25 Hz, scanning speed of 50 μm/s, laser spot size diameter of 2 μm, laser fluence (~ 0.1 J/cm^2), and at different relative position between the sniffer and the surface of the sample. At these operating conditions, it was shown above (see Figure 2 top) that laser induced craters have a depth of about 100 nm but ion signals are observed to get more than $5x10^6$ cps, showing a high signal to background ratio. Simultaneously, ultra-fast wash-outs (< 10 ms) are achieved reducing the aerosol mixing from consecutive laser shots even when operating the laser at high repetition rates. However, it is noticed that slight variations (± 100 μm) around the optimum relative position significantly affect these signal to background ratios (see Figure 3).

Reproducibility of the ion signals at low integration time (2 ms) was also investigated. Figure S1 (supplementary information) shows LA-ICP-SFMS transient $^{120}Sn^+$ ion signals obtained a laser repetition rate of 100 Hz (highest value), a scanning speed of 50 μm/s, and a laser spot size diameter of 2 μm. Ion signal related to each laser pulse was estimated (addition of 5 consecutive data points), obtaining an average value after 100 laser shots of 6.7 10^6 counts/peak and a RSD of 20%. Moreover, the moving average (considering 5 neighbor points) was calculated to demonstrate non- increasing or decreasing trend in the ion signals.

Evaluation of crater shape and morphology in fixed cells.

Laser-induced craters, obtained using the NWRimage laser ablation system, were investigated in the NIH/3T3 fibroblast cells incubated with Au NPs. Figure 4 shows an AFM topography image of a fixed NIH/3T3 fibroblast cell, highlighting its shape, morphology and dimensions.

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Laser-induced craters in cells were investigated operating the laser at a repetition rate of 25 Hz, laser fluence of ~ 0.1 J/cm^2, scanning speed of 50 μm/s, and using a laser spot size diameter of 2 μm. Figure 5 shows the sequence of craters obtained on the NIH/3T3 fibroblast cells, measured by AFM. It is noticed that the craters do not show a perfect round shape, their diameter is about 2.5 μm, and the cross-section profiles show conical shapes. Crater depths after a single laser shot were about 800 nm (8 times deeper than in the case of In-SnO_2 thin layers). Redeposition is also observed around the crater pit due to thermal effects using a ns laser at 266 nm.

Elemental distribution of Au NPs and Cd-based QDs in single cells by LA-ICP-SFMS

NIH/3T3 fibroblast cells were incubated with Au NPs and grown as a monolayer on sterile coverslips, fixed and dried before LA-ICP-SFMS analysis at optimized operating conditions. To obtain fast and high spatially resolved images of the metallic nanoparticle distribution in the cells, a laser spot size of 2 μm with a scanning speed of 50 μm/s and a repetition rate of 100 Hz was applied. At these operating conditions the wash-out time (~ 10 ms) is almost matching the laser repetition rate (1 laser pulse every 10 ms). Single NIH/3T3 fibroblast cells were analyzed with this approach to assess the cellular uptake and qualitative elemental distribution of the Au NPs within the cells. Figure 6 displays the LA-ICP-SFMS image of the distribution of Au NPs inside single NIH/3T3 fibroblast cells. The LA-ICP-SFMS image of the $^{197}Au^+$ intensity distribution is superimposed on the bright field image of the fixed NIH/3T3 fibroblast cells in the bottom panel of the figure. The obtained resolution is adequate to distinguish the background, the cell cytosol and, even, the cell nucleus. As can be observed, Au NPs were taken up by cells by endocytosis and were evenly distributed in the cytosol after an incubation time of 24 hours. Moreover, spots of high gold intensity are visible near the cell nucleus. These results suggest that NPs aggregate in the perinuclear region in the course of endosomal maturation and multivesicular fusion, but not inside the cell nucleus. These findings agree with previous results on the distribution of Au NPs in cells [11,12,14]. It is noteworthy that the low signal intensity that was found in the cell nucleus region might be originated from Au NPs located in the cytosol volume above or below the cell nucleus. LA-ICP-SFMS ion signals from $^{197}Au^+$ were significantly higher than background as can be observed in Figure 6 (bottom).

The potential of the herein developed LA-ICP-SFMS approach was also evaluated in HeLa cells, which are the most commonly used human cell lines in biological and toxicological studies. In this case, HeLa cells were incubated with photoluminescent water-soluble semiconductor nanoparticles (Cd-based QDs). Such type of QDs have been extensively used as improved fluorescent probes for the imaging of biological samples [21]. Figure 7 shows the bright field image of the fixed HeLa cells and the LA-ICP-SFMS image of the $^{114}Cd^+$ intensity distribution (QDs distribution) superimposed on the bright field image of the fixed HeLa cells in the middle panel of the figure. LA-ICP-SFMS ion signals from $^{114}Cd^+$ were significantly higher than background as can be observed in Figure 7 (bottom). QDs were found to be present in the cytosol of the HeLa cells and, as it was already observed for the Au NPs in the NIH/3T3 fibroblast cells, but they did not enter the nucleus.

Validation of the observed behavior for the Cd-based QDs was performed using confocal fluorescence images. Figure 8 shows the nanoparticle distribution obtained by confocal fluorescence microscopy, in which can be observed: (a) the distribution of the fluorescent QDs inside the HeLa cells (red fluorescence), (b) DAPI-stained nuclei (DAPI was used for nuclear visualization), (c) bright field and (d) overlapping images. If both the confocal fluorescence microscopy image (Figures 8c-d) and the LA-ICP-SFMS image (Figure 7) are compared, LA-ICP-SFMS image of the HeLa cells incubated with QDs is not only in good agreement with the nanoparticle distribution observed in the confocal microscopy image, but it also provides a fast and high resolved qualitative elemental distribution image of each single cell just by monitoring

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the Cd present in these nanoparticles. It should be noted that confocal fluorescence microscopy analysis was only carried out for the fluorescent Cd-based QDs, since the nanoparticle distribution of Au NPs can only be done by the herein proposed LA-ICP-SFMS approach as Au NPs are not fluorescent. These examples illustrate that the proposed LA-ICP-SFMS approach allows to perform in a fast way the bioimaging of NPs and QDs in single cells, obtaining high spatially resolved images of the elemental distribution of such NPs or QDs inside the cells.

Conclusions

The analytical potential of a ns-LA-ICP-SFMS system, equipped with an ultra-fast wash-out ablation chamber, was critically investigated for fast (50 μm/s) and high spatially resolved (~ 2 μm) qualitative elemental distribution of NPs and QDs within single cells.

AFM was employed to investigate the size, morphology and overlapping of laser-induced craters obtained at different laser repetition rates. Laser-induced craters showed conical shapes and slight redeposition both in the SnO_2 layer and in the cell cultures. Ablation efficiency at the same operating conditions was different for these samples: (a) penetration rate after single laser shot was about 8 times higher in cell cultures (~ 800 nm) than in the In-SnO_2 layer (~ 100 nm); (b) diameter of the crater in the cell culture after single laser shot was about 2.5 μm, which was slightly higher than the diameter measured in the In-SnO_2 layer (~2 μm).

The influence of the sampling distance (e.g. distance between sample surface and sniffer (inner micro sampling chamber) on the LA-ICP-SFMS ion signal and wash-out times, was evaluated. Slight variations (± 200 μm) significantly affected the measured ion signals, and thereby the wash-out time of the LA chamber. At optimized conditions, $^{120}Sn^{+}$ ion signals measured in the In-SnO_2 layer were in the order of 10^6 cps with a FWHM of less than 10 ms, reducing the aerosol mixing from consecutive laser shots even when operating the laser at high repetition rates (25-100 Hz). Acquisition conditions of the ICP-SFMS were also modified to monitor single ion signals with an integration time of 2 ms.

Fast and high spatially resolved images of elemental distribution within mouse embryonic fibroblast cells (NIH/3T3 fibroblast cells) and human cervical carcinoma cells (HeLa cells), incubated with gold nanoparticles (Au NPs) and Cd-based quantum dots (QDs), respectively, were determined at the optimized operating conditions. Elemental distribution of Au and Cd in single cells was achieved with a resolution of about 2.5 μm (given by the spot size), using high scanning speed (50 μm/s), and high repetition rate (100 Hz). The obtained resolution was adequate to distinguish the background, the cell cytosol and, even, the cell nucleus. Au NPs and Cd-based QDs were taken up by cells by endocytosis and were evenly distributed in the cytosol after incubation time of 24 hours. Moreover, spots of high Au and Cd ion signal were visible near the cell nucleus, suggesting that they aggregate in the perinuclear region in the course of endosomal maturation and multivesicular fusion, but not inside the cell nucleus. Moreover, it was possible to validate the qualitative elemental distribution of QDs in the single cells obtained by LA-ICP-SFMS using confocal fluorescence microscopy images as these QDs are highly fluorescent.

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Tables:

Table 1: Acquisition conditions in the ICP-SFMS.

RF power / W	1350
Guard electrode	Platinum, active
Ar cooling gas flow rate / L min^{-1}	16
Ar auxiliary gas flow rate / L min^{-1}	1.0
Ar sample gas flow rate / L min^{-1}	0.63-0.75
Sample and skimmer cone	Ni
Mass resolution	Low (R = 300)
Scan optimization	Speed
Isotopes monitored	^{120}Sn, ^{197}Au, ^{114}Cd (only one isotope per analysis)
Magnet settling time / s	0.001
Runs x pass	20 x 1
Detection mode	Triple (isotopes measured with SEM)
Sample time / s	0.002
Samples per peak	1000
Segment duration / s	0.4
Mass window / %	20
Search window / %	0
Integration window / %	20
Scan type	E-scan

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Legend of figures:

Figure 1: (Top) Picture of the objective and sniffer (inner micro sampling chamber in Bloodhound design). (Bottom) Schematic draw of the ablation chamber and tube connection to ICP-MS.

Figure 2: Craters obtained in laser raster mode, operating the laser at different laser repetition rates, i.e. 25 Hz (top), 50 Hz (middle) and 100 Hz (bottom). The left column shows AFM topographies, the right column line profiles drawn perpendicularly to the line.

Figure 3: LA-ICP-SFMS transient $^{120}Sn^{+}$ ion signals obtained at different relative position between the inner sniffer and the surface of the sample (z-value at the focal position is changed from 9.33 mm to 9.83 mm). Laser operating conditions: repetition rate of 25 Hz, scanning speed of 50 μm/s, laser spot size diameter of 2 μm.

Figure 4: AFM topography image of a fixed NIH/3T3 fibroblast cell.

Figure 5: AFM topography image of a sequence of crater obtained on NIH/3T3 fibroblast cells.

Figure 6: (Top) Bright field image of the fixed NIH/3T3 fibroblast cells. (Middle) LA-ICP-SFMS image of the smoothed $^{197}Au^{+}$ intensity distribution (distribution of Au NPs) inside single NIH/3T3 fibroblast cells is superimposed. (Bottom) Variation of $^{197}Au^{+}$ ion signal (cps) along line 20th of the LA-ICP-SFMS image of NIH/3T3 cells. Note: Figures 6 (top and middle) were built using Matlab and Image J software. In these images, a 2D median filter using 3-by-3 neighbors was applied to reduce the influence of noise and/or outlier spots.

Figure 7: (Top) Bright field image of the fixed HeLa cells. (Middle) LA-ICP-SFMS image of the smoothed $^{114}Cd^{+}$ intensity distribution (distribution of Cd-based QDs) inside single HeLa cells is superimposed. (Bottom) Variation of $^{114}Cd^{+}$ ion signal (cps) along line 8th of the LA-ICP-SFMS image of HeLa cells. Note: Figures 7 (top and middle) were built using Matlab and Image J software. In these images, a 2D median filter using 3-by-3 neighbors was applied to reduce the influence of noise and/or outlier spots.

Figure 8: Confocal fluorescence images of HeLa cells treated with CdSe/ZnS QDs after an exposure time of 24 h. Images of the nanoparticle distribution of the QDs in the HeLa cells were taken under (a) bright field, fluorescence at an excitation wavelength of 405 nm (b) DAPI-stained nuclei (c) fluorescent CdSe/ZnS QDs, and (d) merge of corresponding bright field and both fluorescence images (scale bar = 10 μm).