Supporting information (SI)

A simple metal staining procedure for identification and visualization of single cells by LA-ICP-MS

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Abstract

High lateral resolution of metal detection in single cells by use of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) demands powerful staining methods. In this work different staining procedures for the single cell analysis with LA-ICP-MS were optimized. An iridium intercalator was utilized to stain the cell nuclei whereas the whole cell was stained by the use of maleimido-mono-amide-DOTA (mDOTA) complexing lanthanide(III) ions. The content of the artificially introduced metals per cell was quantified using a matrix matched calibration approach based on cellulose membranes onto which standards were spotted by a microarray spotter. Absolute metal stain amounts in the range of 2.34 to 9.81 femtomole per cell were determined.
The metal staining procedures allow direct identification and visualization of single cells and their cell compartments by element microscopy without the use of bright field images of the sample.

3. Results and discussion:

3.4 Combination of Ir-intercalator and mDOTA(Tm) for cell visualization by element microscopy

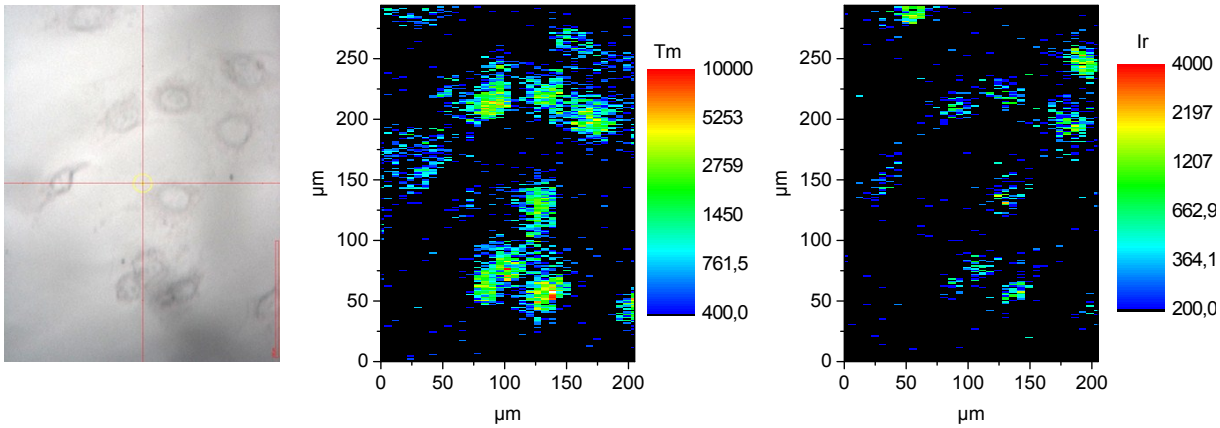

Figure S-1: Left: bright field image of single 3T3 cells. Right: corresponding 2D-itensity profiles for the two isotopes ^{169}Tm and ^{193}Ir detected with LA-ICP-MS. Pixel size: 6 x 0.4 µm.

3.5 Quantification experiment

For calibration, a solution with various concentrations of all metals of interest was spotted onto a nitrocellulose membrane using the validated microarray system and measured by LA-ICP-MS. Each calibration point was spotted three times with a solid pin (3 * 0.61 nL) and a total volume of 1.83 nL of each calibration solution was transferred to the membrane. For calibration, the amount of element/spot was calculated and plotted against the area/spot.

Table S-1: Calibration data from the NC-membrane measured via LA-ICP-MS.

	standard 1	standard 2	standard 3	standard 4	standard 5
calibration ^{159}Tb					
area/spot [cps]	20150	39087	153223	330869	1642092
amount metal/spot [pg]	0,09	0,19	0,93	1,86	9,30
calibration ^{191}Ir					
area/spot [cps]	4444	11110	53682	118123	607218
amount metal/spot [pg]	0,03	0,07	0,35	0,69	3,47
calibration ^{193}Ir					
area/spot [cps]	7323	15453	91457	194085	998878
amount metal/spot [pg]	0,06	0,12	0,58	1,17	5,83

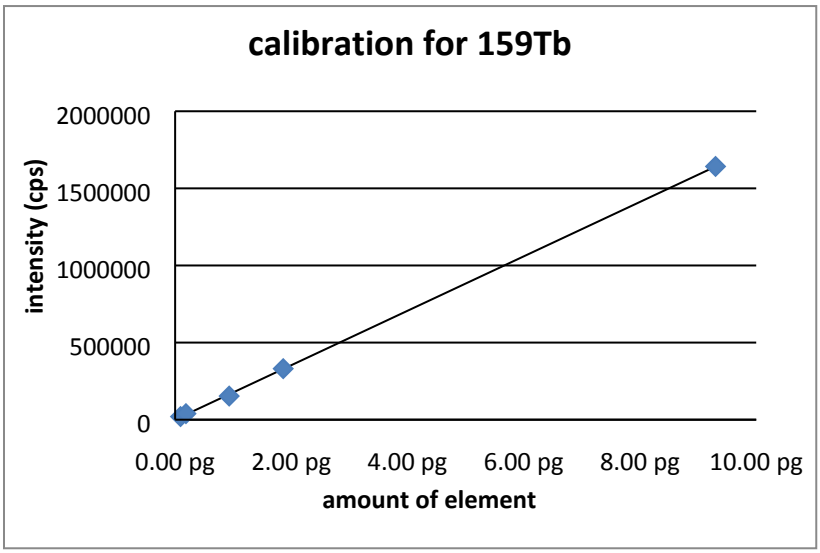

Figure S-2: Calibration curve for ^{159}Tb measured via LA-ICP-MS.

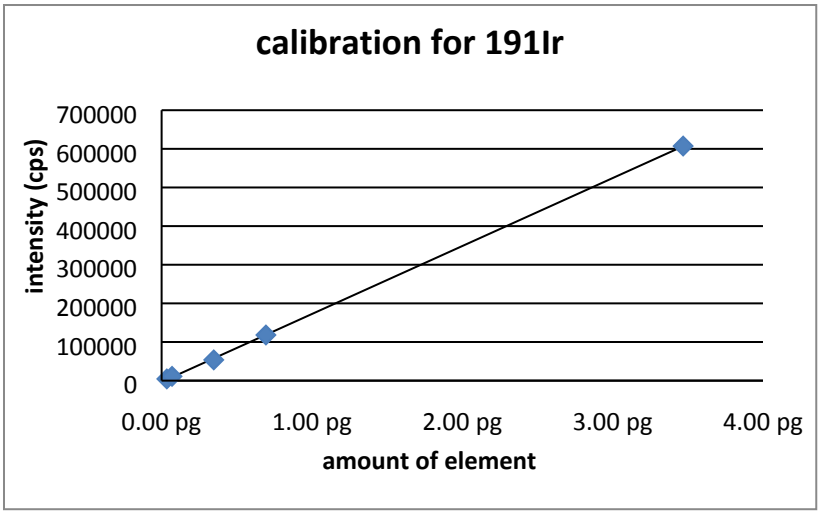

Figure S-3: Calibration curve for ^{191}Ir measured via LA-ICP-MS.

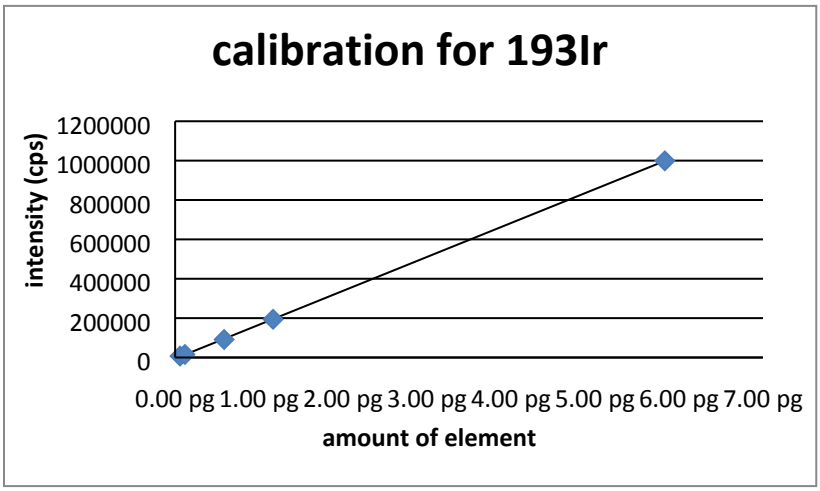

Figure S-4: Calibration curve for ^{193}Ir measured via LA-ICP-MS.

The theoretical instrument detection limit (LOD) for each isotope of interest was calculated using equation (1) and LODs from 28 amol/spot (^{191}Ir), 18 amol/spot (^{193}Ir) to 27 amol/spot (^{159}Tb) were achieved. The R^2 value for all analyzed isotopes is better than 0.9999.

$$\mathrm{LOD} = \frac{3\sigma\ \text{background}}{\text{sensitivity}} \qquad (1)$$

The spotted dilution series on the NC-membrane and the cell samples were analyzed with exactly the same settings. After summation of the peak areas of selected single cells and calibration spots, the Ir and Tb content were determined in the cell samples using the external calibration. An average of ten cells per sample was used for calculation.