# Supporting Information

# Fundamental studies on droplet throughput and the analysis of single cells using a downward-pointing ICP-time-of-flight mass spectrometer

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*Downward ICP*

A downward ICP-MS was reported recently.[1] A detailed rendering of the technical features of the ICP is shown in **Fig. S1**.

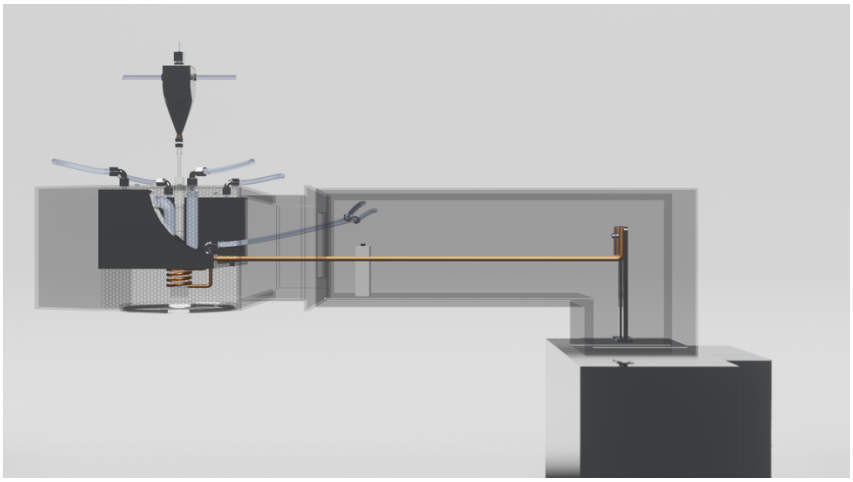

**Fig. S1:** Reprinted with permission from *Anal. Chem.* **2021**, 93, 1001−1008; DOI: 10.1021/acs.analchem.0c03831. Copyright 2021 American Chemical Society. The RF generator of a commercial ELAN 6100 DRC$^{plus}$ ICP-MS (PerkinElmer/SCIEX, Toronto, Canada) was modified so that the plasma torch pointed vertically-downward. In addition, the torch box was equipped with an in-house developed cooling system comprised of several cooling plates. In this study, the quadrupole mass spectrometer was replaced by a prototype time-of-flight mass analyzer which was equipped with the ELAN-type interface which allowed a direct coupling of the MS unit to the ICP. (see also **Fig. 1**) More details of the prototype time-of-flight instrument can be found in Borovinskaya *et al.*[2]

*Cell staining and preparation.*

A single cell suspension sampled from mouse lung tissue was enriched for CD45+ positive cells.[3, 4] Antibody labelled magnetic particles (MACS beads & column, Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany) bind to CD45+ cells that can be further concentrated by magnetic separation. Further preparation steps were adapted from the Maxpar Cell Surface Staining protocol[5] and from Wagner *et al*.[6] The cell number concentration of the cell suspension was determined using a hemocytometer followed by resuspension in cell staining medium. (CSM, PBS with 0.3% saponin, 0,5% bovine serum albumin (Sigma Aldrich)). Per aliquot, approximately 1 Mio. cells were transferred into a 1.5 mL single-use polypropylene tube and incubated with FcR Blocking Reagent for 15 min. at RT. Then, the antibody mixture containing CD45 (final conc. 3 µg $mL^{-1}$), CD4 (final conc. 5 µg $mL^{-1}$) and CD11b (final conc. 4 µg $mL^{-1}$) was added obtaining a total volume of 100 µL and the cells were incubated for 60 min. at 4°C followed by three washing steps with CSM. For cell fixation, the cells were resuspended in 1 mL neutral buffered 4% formalin (approx.1% formaldehyde and PBS) and incubated for 20 min. at RT. After fixation and for staining with Cell-ID Intercalator-Ir, the cells were resuspended in 1 mL Maxpar Fix and Perm Buffer containing 125 nM Intercalator-Ir and stained overnight at 4°C. The cells were resuspended in 1 mL neutral buffered 4% formalin (approx. 1% formaldehyde and PBS) and could be stored for several days at 4°C until analysis. Prior to analysis, the cells were washed twice with ultrapure $H_2O$ and filtered through a 20 µm mesh filter-cap (FACS). The cells were then resuspended in 3 mL ultrapure $H_2O$ containing 10% EQTM Four Element Calibration Beads (Fluidigm Inc. Toronto, Canada) and 30 µg/L Cs droplet tracer using 4 mL vials (narrow-mouth bottle, PP, ThermoScientific, USA). The cell suspension was then sampled into the glass capillary of the Autodrop Pipette.

**Tab. S1.** Droplet size, multi-element solution concentration and analyte mass per droplet for Rh, Lu and Ir is provided.

| **Analyte** | **Droplet size [µm]** | **CONCENTRATION [µg $L^{-1}$]** | **Analyte mass per droplet [fg]** |
|---|---|---|---|
| **Rh** | $74.3 \pm 1.5$ | 19.77 | $4.3 \pm 0.3$ |
| **Lu** | $74.3 \pm 1.5$ | 18.82 | $4.04 \pm 0.24$ |
| **Ir** | $74.3 \pm 1.5$ | 18.82 | $4.04 \pm 0.24$ |
| | $54.8 \pm 1.5$ | 18.82 | $1.62 \pm 0.13$ |
| | $80.3 \pm 1.5$ | 18.82 | $5.1 \pm 0.3$ |
| | $92.6 \pm 1.5$ | 18.82 | $7.8 \pm 0.4$ |

**Tab. S2.** Comparison of various physical parameters for Lu and Ir is provided. [7, 8]

| | **Lu** | **Ir** |
|---|---|---|
| **First ionization potential [eV]** | 5.43 | 8.97 |
| **Heat of vaporization [kJ mol$^{-1}$]** | 414 | 564 |
| **Thermal conductivity [W m$^{-1}$ K$^{-1}$]** | 16.4 | 147 |
| **Density [g cm$^{-3}$]** | 9.84 | 22.56 |
| **Boiling point [°C]** | 3402 | 4428 |

$Lu^+/Ir^+$ ratios as a function of the analyte mass for four different droplet sizes are shown in **Fig. S2**.

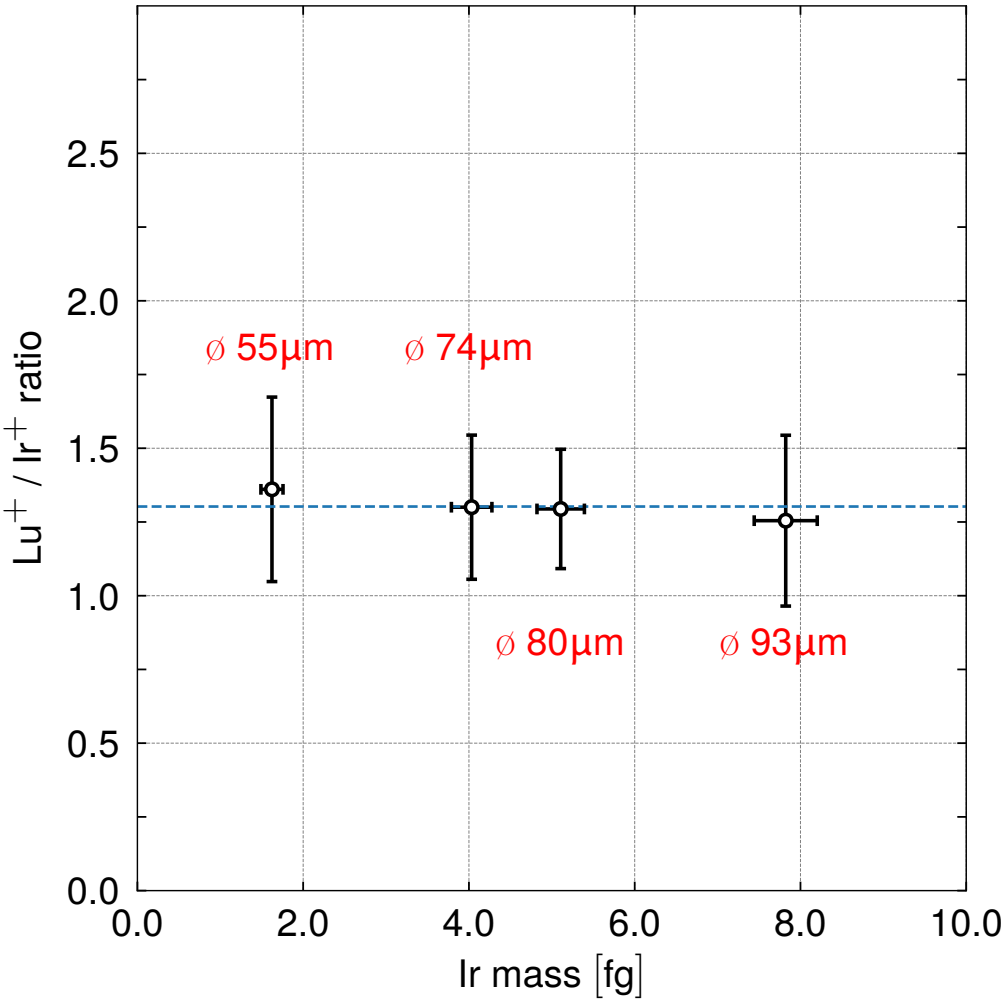


**Fig. S2:** $Lu^+/Ir^+$ ratios and the corresponding standard deviations derived by error propagation are shown as a function of the analyte mass for four different droplet sizes. The mean ratio was calculated to 1.3 and is indicated by the dashed, horizontal line and is in agreement with the mean value obtained in **Fig. 4**. As the droplet size increased from 55 µm to 74 µm, and thus, the volume and the analyte mass accordingly, the obtained ratio decreased from 1.36 to the mean ratio 1.3 (5% deviation). When the droplet size was increased from 80 µm to 93 µm, a ratio of 1.25 (4% deviation). In comparison to the derived standard deviations, the ratios were assumed to be stable.

Literature


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