Single Cell-Asymmetrical Flow Field-Flow Fractionation/ICP-Time of Flight-Mass Spectrometry (sc-AF4/ICP-ToF-MS): An efficient alternative for the cleaning and multielemental analysis of individual cells

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Supplementary material

4.2. Method Development (additional info)

During the rinsing step (14 min), the detector flow is not expected to correspond to the given 1 mL/min. That is because of the opening of the purge valve, which offers a path of less backpressure for the liquid phase. During that phase, the channel pressure is noticeably lower, and the background of the ToF is also observably lowering. The overall duration of the procedure is 20 minutes.

4.3. Data Processing (extended)

The result of the developed measurement procedure for this work, monitoring P for cell detection, is depicted in Figure 3a (see manuscript), where the Laser Light Scattering (LLS) data (orange) match the corresponding ToF raw data (blue). The LLS data are briefly found out of the detector's range (~7.5-9.5 min), due to the recording program's limitations. Two different approaches were tested for the processing of the resulting single-cell data: First, they were processed utilizing the particle processing module that is embedded in the operating software of the 2R ICP-ToF-MS instrument by TOFWERK. This processing is explained thoroughly by Meili-Borovinskaya *et al*[1]. In order to obtain more control over the handling of the data, and the ability to access every individual step of the data processing and being able to monitor and altering it as well, a python-based script was developed. This was deemed necessary by the special nature of the data, due to the separation of the steps and the concentration of majority of the sample within a subsection of the dataset. The normalized cell histograms produced by the 2R software and the newly developed python script, with their corresponding gaussian fits, are depicted in Figure 3b and Figure 3c respectively (see manuscript).

The 2R software detected 393 cell related phosphorus events, with a mean value±standard deviation of 1.10±0.21 or 12.46±1.60 cts. The in-house developed script detected 403 cell related phosphorus events, with a mean value of 1.10±0.20 or 12.44±1.58 cts. In terms of comparability, the two approaches are performing similarly, which means that the development of the script was successful. That fact was also validated for the rest of the presented results, as they were evaluated with both approaches. Ergo, only the results from the newly developed approach will be presented.

The basic schematic algorithm of the python script is depicted in Figure 4 (see manuscript). As mentioned before, each individual step can be altered to the user's discretion. First, the exported raw data are imported in the notebook on which the script is running, and the desired analytes are chosen. Then, the threshold (Th) for the event's determination is chosen – for this work, Th = mean + 2.72 + 3.29*SD (mean = average of the dataset, and SD = standard deviation)[2]. The next step is defining the repetitive function that will facilitate the processing of the data, as mentioned by Pace *et al*[3]: the given

dataset is copied (to be preserved), its mean and SD are calculated, and the threshold is determined. Every datapoint that exceeds that threshold is then removed from the given dataset, and the process is repeated with the new dataset, setting the threshold anew. When no more datapoints can be excluded, the final threshold value is set, and the given dataset is split in two datasets: one contains the cell datapoints, and one that contains the background datapoints. Following the defining of the function, it is applied to the original dataset as follows: the whole dataset is segmented into smaller pieces – in this work, 1,000 datapoints long, that can be individually accessed – and the function is applied to each of them individually. In this manner, any effects due to changing in the background (drifting, increase or decrease in intensity, etc) can be minimized. This approach is derived from the work of Meili-Borovinskaya *et al*[1]. Finally, the separated cell and non-cell datasets from each segment are combined into one complete cell and one complete non-cell datasets and can be treated individually. In this manner, information about the cells (number, intensities etc) and the background (intensity, drifting etc) can be obtained unobstructed.

The multielemental analytical capabilities of the ToF mass analyzer allow for the simultaneous determination of more than one element, within the same pulse. From the perspective of single cell analysis, that can be a huge advantage, as the metal content of cell populations can be directly determined and correlated with each individual cell's size. Therefore, it is imperative for newly developed approaches to be able to distinguish which pulses contain the elements of interest, *i.e.*, cell related signals overlapped by metal related signals. Towards that goal, a second coding part was also developed, that is able to identify such overlapping signals, and therefore enable the safer study of crucial aspects of environmental research, eliminating the possibility of falsely identified signals.

4.4. Sodium Chloride matrix tackling
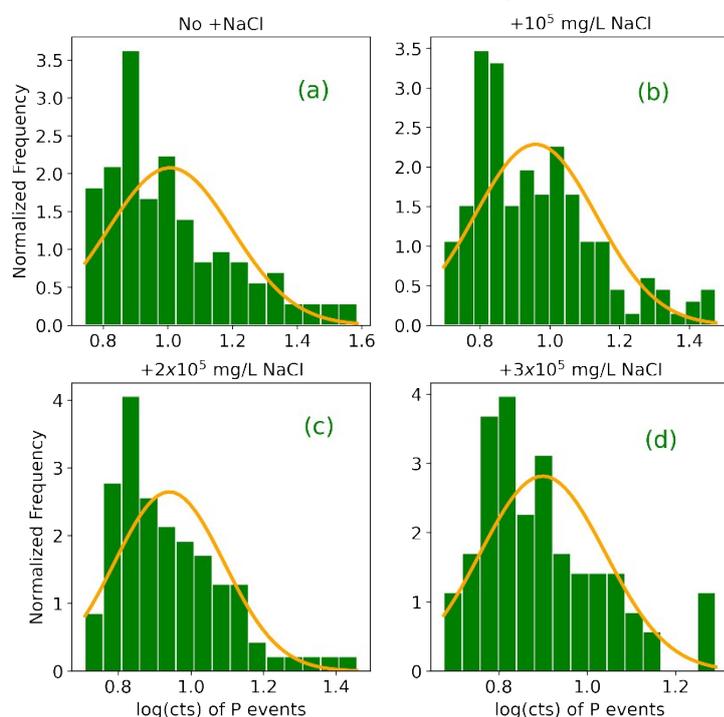
Figure S1. Normalized histograms of P events for a dilution factor of 1,000 for a) no additional b) 10^5 mg/L c) $2x10^5$ mg/L and d) $3x10^5$ mg/L of NaCl.

4.5. Phosphorus matrix tackling
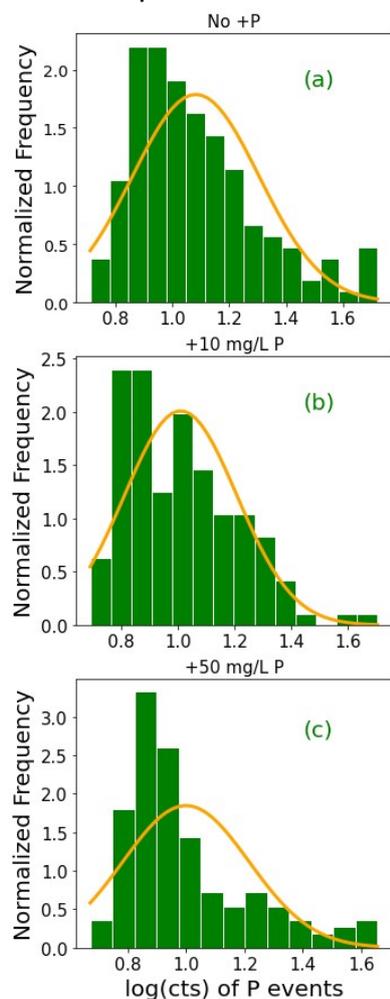
Figure S2. Normalized histograms of P events for a dilution factor of 400 for a) no additional b) 10 mg/L c) 50 mg/L of ionic P.

Literature

1. Meili-Borovinskaya, O. *et al.* Analysis of complex particle mixtures by asymmetrical flow field-flow fractionation coupled to inductively coupled plasma time-of-flight mass spectrometry. *J. Chromatogr. A* **1641**, 461981, doi:https://doi.org/10.1016/j.chroma.2021.461981 (2021).
2. Tanner, M. Shorter signals for improved signal to noise ratio, the influence of Poisson distribution. *J. Anal. At. Spectrom.* **25**, 405-407, doi:10.1039/B921077F (2010).
3. Pace, H. E. *et al.* Determining Transport Efficiency for the Purpose of Counting and Sizing Nanoparticles via Single Particle Inductively Coupled Plasma Mass Spectrometry. *Anal. Chem.* **83**, 9361-9369, doi:10.1021/ac201952t (2011).