Application Note:

Arraying of single cells for quantitative high throughput Laser Ablation ICP-TOF-MS

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Abstract

The development of ultra fast and sensitive inductively coupled plasma time-of-flight mass spectrometry (ICP-TOF-MS) systems by TOFWERK enables multiparameter ionomics and metallomics studies at the single cell level. Researchers at BAM successfully developed a novel method combining single cell isolation and arraying by cellenONE® and subsequent isotopic fingerprinting of these single cells by laser ablation ICP-TOF mass spectrometry. They demonstrated how the precise arraying of calibration standards and blanks enabled the quantitative determination of analytes down to ~100 ag.

Materials and methods

Human monocytic leukemia cells (from cell line THP-1) were fixed and dyed with mDOTA-Ho and Ir-DNA-intercalator prior to arraying.

20 µL of cell suspension were aspirated using a PDC70 (coating type 3) with a sciFLEXARRAYER S3 (SCIENION AG) and single cells were arrayed using cellenONE® software (cellenion SASU) as fields of 10 x 10 positions (500 µm center-to-center distances) on glass slides (Superfrost+, Thermo Fisher Inc.). The same device was used to array blanks and matrix adapted standards of mDOTA-Ho and Ir-DNA-intercalator in four different concentrations.

Cells were ablated with 10 laser pulses per printed single spot (d=150µm) with 100 Hz repetition rate and a fluence of 0.36 J cm^{-2} using an Analyte G2 ArF Excimer LA system (193 nm, Teledyne CETAC Technologies) with aerosol rapid introduction system (ARIS) and subsequently measured using an ICP-TOF-MS (icpTOF R, TOFWERK).

Signals from cells, blanks and standards were automatically identified and integrated using the Originlab® peak finder function.

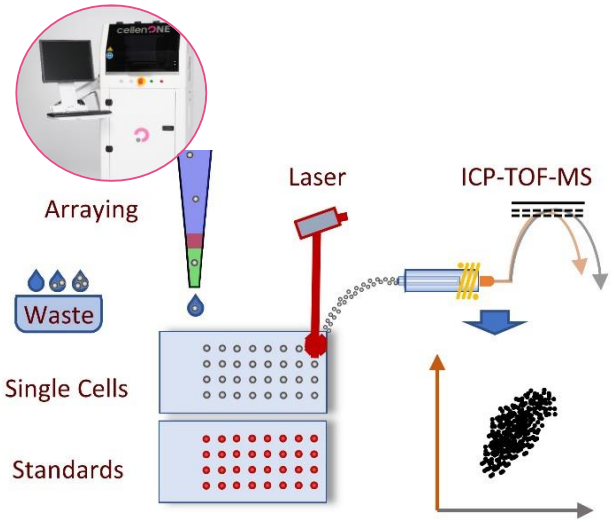

Figure 1. Scheme describing the method for quantitative analysis and isotopic fingerprinting by laser ablation ICP-TOF mass spectrometry with cellenONE®.

Results and Discussions

Using optimized cellenONE parameters, high single cell accuracy (>99%), and high cell recovery (>66%) were achieved. Fully automated LA-ICP-TOF-MS enabled ablation and measurement at a rate of ~1 cell per second. In total, 562 single cells were measured to provide sufficient statistical significance. Moreover, 50 blanks of cell suspension supernatant were measured to enable blank correction.

Matrix adapted standards were successfully used to determine the content of Ir-DNA-intercalator and mDOTA-Ho per single cell. Figure 2 shows the Ir and Ho mass distributions in THP-1 cells. The Ir mass in cells was well above the LOQ (dashed vertical line), while for Ho mass some cells were close to the LOQ of ~100 ag. The histogram of Ho content per cell appears to follow a simple normal distribution, while the Ir-DNA-intercalator approximately correlates with the DNA content distribution. The Ir-DNA-intercalator content was used to track the accumulation of different trace elements (e.g. P and Zn) throughout the cell cycle (not shown here). Moreover, LA-ICP-TOF-MS enabled the detection of naturally occurring isotopes in the whole mass range (Na, Mg, Al, P, S, Ca, Mn, Fe, Cu, Zn, As, Sr, Ba) as fingerprints of individual cells (not shown here).

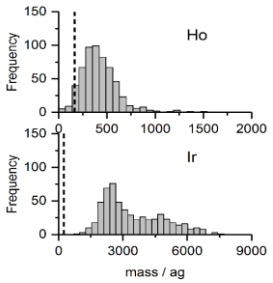

Figure 2. Histograms of Ho and Ir mass per single cell. Dashed lines represent LOQs of 102 ag for Ho and 236 ag for Ir, respectively.

Conclusions

A novel single cell arraying approach was tested as preparation method for high throughput analyses of single cells by LA-ICP-TOF-MS. This automated method was proven to allow quantitative determination of isotopes (both endogenic and exogenic) with detection limits as low as one hundred attogram per single cell which could be used as fingerprints of individual cells.