APPLICATION NOTE

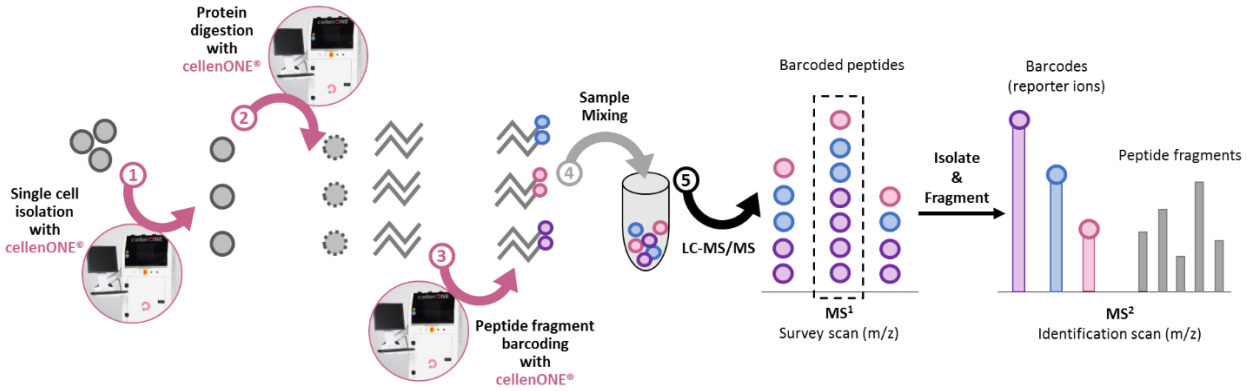
cellenONE®: all-in-one solution for single cell proteomics using LC-MS/MS sample preparation

David Nikolaus Hartlmayr[1], Claudia Ctortecka[1], Karl Mechtler[1,2], Holger Eickhoff[3], Guilhem Tourniaire[4], Anjali Seth[4]

1. IMP-Research Institute of Molecular Pathology, Campus-Vienna-Biocenter 1, 1030 Vienna, Austria
2. IMBA -Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Dr. Bohr Gasse 3, 1030 Vienna, Austria
3. SCIENION, Volmerstraße 7, 12489 Berlin, Germany, 4. Cellenion SASU, 60 Avenue Rockefeller, 69008 Lyon, France

Abstract

The analysis of single cell proteomes using LC-MS/MS is highly relevant to complement single cell RNA-seq analysis for confirmation of actual protein expression. In this study, we aim to reproduce SCoPE2 using cellenONE®, a novel instrument combining single cell isolation and nanolitre dispensing, to perform every step of the sample preparation using a single device.



Materials and Methods

HeLa cells were cultured using standard method (DMEM supplemented with 10% FBS, 1% PenStrep, 1% L-Glutamine) in 10 cm dishes. Prior to single cell isolation, cells were detached using trypsin, dissociated, washed, and resuspended in PBS (200 cells/µL).

SCoPE2 method (Specht et al., 2019) combines single cells (128N/C, 129 N/C 130N/C, 131) and a dominant "carrier" sample (100x - 126) with a reference sample (5x – 127N) barcoded with tandem-mass-tags (TMT) for decreased sample losses, improved precursor selection and enhanced peptide identification.

cellenONE® F1.4 an automated single cell isolation and nanolitre dispensing device, was used for isolating cells into 384 microwell plates (Cellenion). The cellenONE was set to dispense one single HeLa cell per well using the following isolation parameters: cell diameter 10-25 µm, circularity max 1,08 and elongation max 1,8.

Sample preparation. The sample preparation was adapted from the mPOP method (Budnick et al., 2018). In brief: single cells and the reference sample were dispensed into water-prefilled wells, subjected to a freeze-heat cycle (-80°C to 90°C). Digested in 100mM TEAB with 10ng/µL trypsin for 4 hours at 37C. Labelled with ten-plex TMT in acetonitrile for 1 hour at RT followed by quenching with 0.5% hydroxylamine for 30 minutes at RT according to manufacturer's instructions. The carrier channel was prepared in bulk, labelled with ten-plex TMT 126 and added to the prepared single cells within step 5 of the SCoPE2 workflow.

MS analyses and data processing. Pooled SCoPE2 samples were separated by reversed phase chromatography (75 µm x 250 mm PepMap C18 column, Thermo Fisher Scientific), developing a linear gradient from 2% to 80% acetonitrile in 0.1% formic acid within 60 minutes (RLSC nano, Thermo Fisher Scientific) and analysed by MS/MS, using electrospray ionization tandem mass spectrometry (Thermo Scientific™ Q Exactive™ HF-X hybrid quadrupole-Orbitrap™ Mass Spectrometer). The instrument was operated using the published SCoPE2 method for direct comparisons, the following parameters were adapted: MS1 resolution 120k, NCE 35, MS2 resolution 60k.

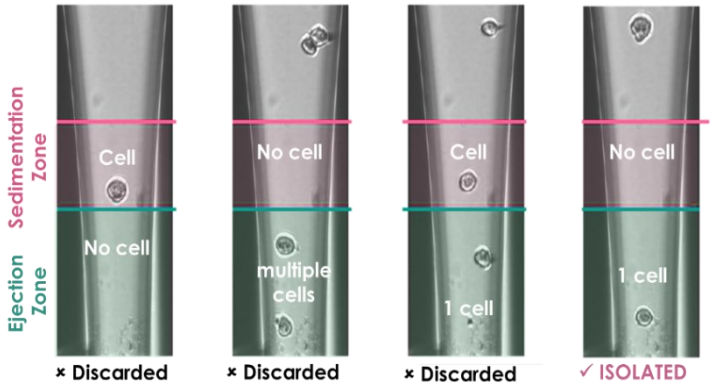


Figure 1. cellenONE® single cell isolation based on optical detection. Fluid containing cells circulating through the capillary tip is segmented into two zones: ejection zone (green, i.e. what will be in the next generated droplet) and sedimentation zone (pink, i.e. a safety zone considering possible sedimentation in the capillary). If sedimentation zone is empty and ejection zone contains a single cell the next droplet will be isolated insuring the accuracy on single cell isolation.

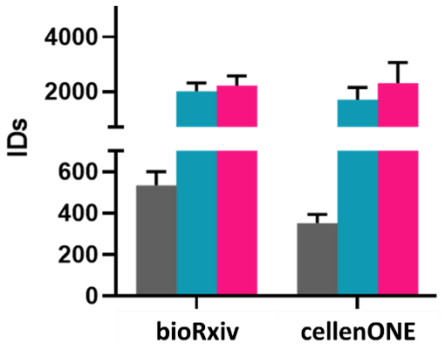
All MS/MS scans were processed and analysed using Proteome Discoverer 2.3 (Thermo Fisher Scientific) searched with MSAmanda v2.0.0.14114 (Dorfer et al., 2014) against the homo sapiens database, using the following parameters: max. missed cleavages 2, with iodoacetamide derivative on cysteine and peptide N-terminal ten-plex TMT (fixed mod.); oxidation on methionine, ten-plex TMT on lysine (variable mod.). Peptide mass tolerance: ±5 ppm; fragment mass tolerance: ±15 ppm. Filtered to 1% FDR on protein and peptide level using percolator and reporter ions were quantified using IMP Hyperplex (in-house developed software).

Results and Discussion

SCoPE2 experiments performed with the cellenONE result in similar protein, peptide and spectra identifications compared to the published datasets across three replicates (Fig. 2). Single cell reporter ion intensities of between the published and the datasets prepared using the cellenONE are highly akin (Fig. 3 A & A'). This, suggests comparable sample losses throughout the sample preparation workflow of the two methods.

Additionally, correlating single cell channel reporter ion intensities confirming reproducible single cell preparation within one analytical run (Fig. 3 B).

Future sample preparation advances using the cellenONE will allow to reduce sample volumes and therefore drastically increase enzyme to substrate ratios and isobaric label to peptide ratio, reducing sample losses and the costs per cell analysed.



SCoPE2-MS	BioRxiv	cellenONE
Protein Groups	460	325
	566	327
	578	401
Peptide Groups	1664	1646
	2157	1293
	2224	2182
PSMs	1826	2578
	2393	1457
	2461	2896

Figure 2. Protein, peptide and spectra identifications of cellenONE and published SCoPE2 datasets. Experiments are performed in technical triplicates.

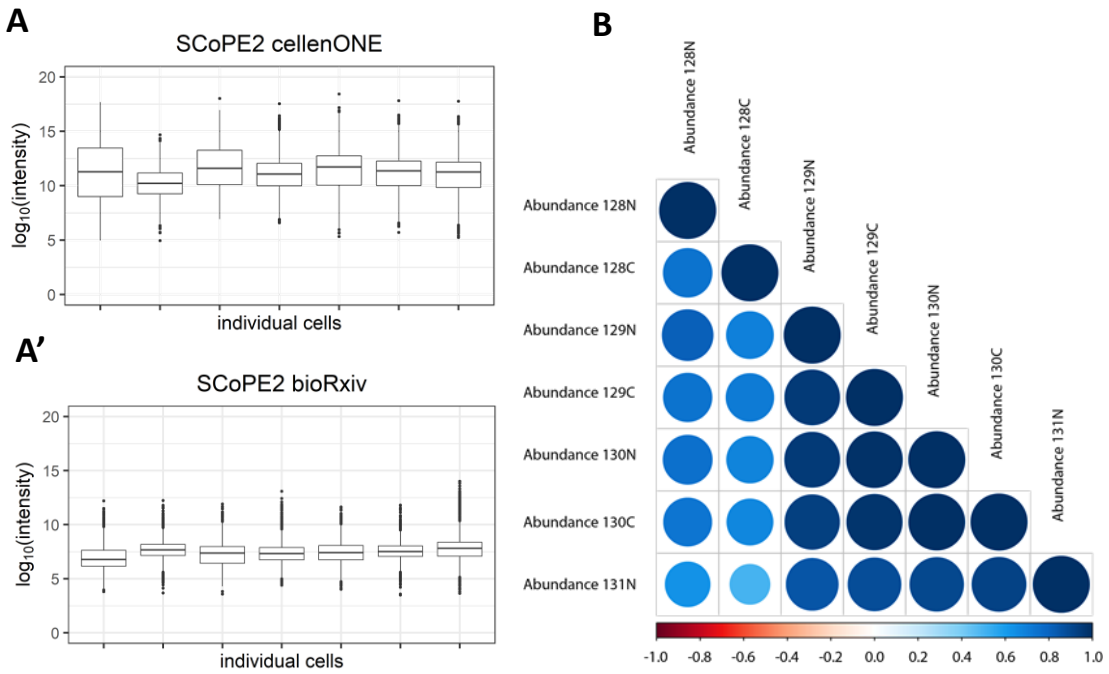


Figure 3. (A & A') Reporter ion intensities of single cell channels over technical triplicates of cellenONE or the published SCoPE2 datasets. (B) Pairwise correlation of single cell channel reporter ion intensities prepared with the cellenONE. One representative file is used.

Additional features (not used in this study)

A dual proteome experiment (yeast/human) was performed to confirm that subsequent dispensing of all TMT labels using one PDC will not introduce a cross contamination bias towards the peptide sample and/or the TMT labels.

In this study, peptide mixtures (yeast and human, PROMEGA/Thermo Fisher) were dispensed in different wells and labelled with different TMTpro labels according to manufacturer's instructions. The analysis shows no overlap from either species or labels (representative PSM shown in Fig. 4), supporting the evidence that cellenONE can be used.

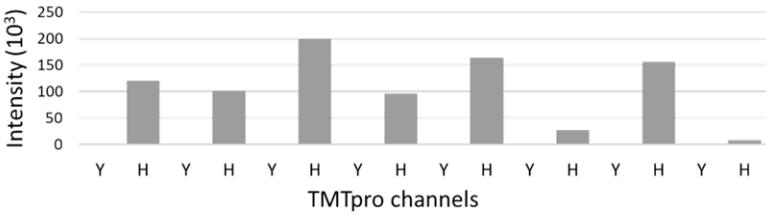


Figure 4. Cross contamination experiment using the TMTpro reagents. Reporter ion intensities of a unique human peptide are shown. Y=yeast. H=human.

Conclusions

In this study, we successfully reproduced the mPOP SCoPE2 workflow using cellenONE® a single instrument for single cell proteomics LC-MS/MS sample preparation. Results are highly comparable to the published SCoPE2 results corroborating the use of cellenONE for an automated single cell isolation and sample preparation usage.

References

1. Specht H., Emmott E., Petelski A., Huffman R., Perlman D., Serra M., Kharchenko P., Koller A., Slavov N. Single-cell proteomic and transcriptomic analysis of macrophage heterogeneity. *bioRxiv*, 2019; 665307, DOI:10.1101/665307
2. Budnik B., Levy E., Harmange G. and Slavov N. SCoPE-MS: mass spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation. *Genome Biology*, 2018; 9:161, DOI:10.1186/s13059-018-1547-5

Contact Us

Cellenion SASU
60 Avenue Rockefeller
69008 Lyon
France
Tel: +33 986 48 70 70
contact@cellenion.com
www.cellenion.com

SCIENION AG
Volmerstr. 7b
D-12489 Berlin
Germany
Tel: +49 (0)30 6392 1700
support@scienion.com
www.scienion.com

SCIENION US, Inc.
2640 W Medtronic Way
Tempe, AZ 85281
United States
Tel: +1 (888) 988-3842
USsalessupport@scienion.com

SCIENION (UK) Ltd
Chichester Enterprise Centre
Terminus Road, Chichester PO19 8TX
United Kingdom
Tel: +44 (0) 1243 88 71 65
support@scienion.com