The current economics and throughput of single cell proteomics by liquid chromatography mass spectrometry

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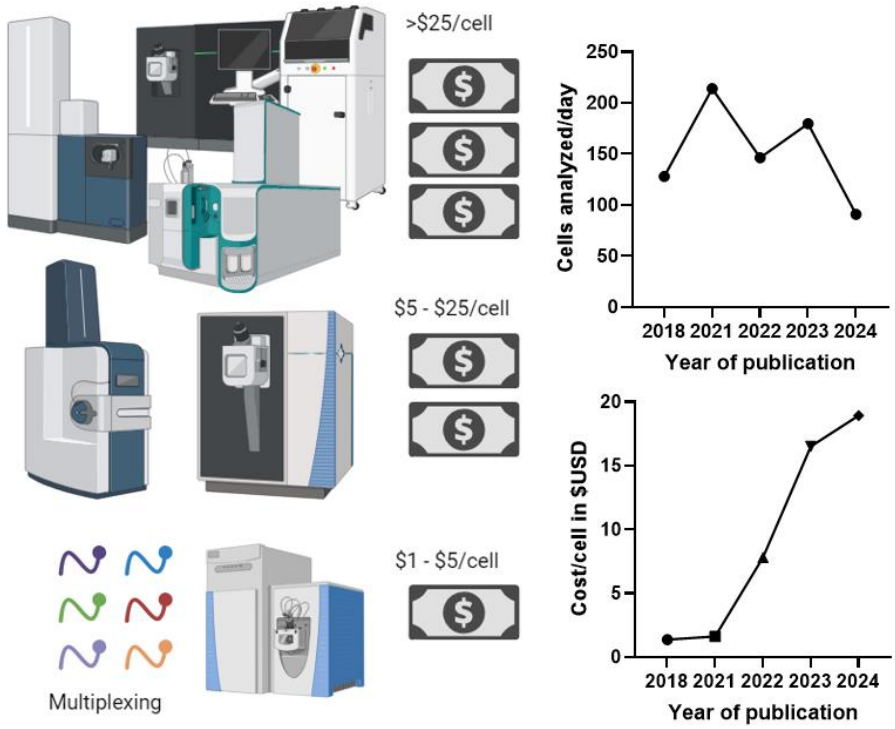
Abstract

Single cell proteomics by mass spectrometry (SCP) is an emerging field of study that has captured the interest and imagination of biologists in a wide array of disciplines. In the pursuit of this new field a dizzying array of technologies and techniques have demonstrated the ability to quantify hundreds to a few thousand proteins in single mammalian cells of typical size. One striking characteristic of these methods is the wide range of relative costs associated with the analysis of each single cell. We have attempted to estimate the cost per cell across 17 different studies based on quotes we have obtained for hardware, reagents and instrument support plans in relation to number of cells that can be analyzed per day. Before including labor or facilities, we find that the cost to analyze a single mammalian cell of typical size can range from less than <$2 per cell to over $50 per cell. The increase in cost appears directly related to the decrease in relative throughput as measured in theoretical maximum number of cells analyzed per day. Perhaps the most surprising observation is that the average number of single cells analyzed across these studies appears to decrease each year. This is striking when compared to the emergence of single cell RNA sequencing where throughput increased, and cost/cell decreased exponentially over the first 7 years of the field's emergence. While we have made many assumptions to obtain these estimates, we hope that these will be informative for scientists interested in obtaining SCP data and for mass spectrometrists who are considering entering this new field. We have provided a spreadsheet with a simple calculator in the supplemental data to allow others to adjust our calculations based on other variables and new methods which will inevitably be described in the future.

Abstract Graphic



Introduction

Single cell proteomics by mass spectrometry (SCP) is an exciting new field of research which promises to unlock new insights in all corners of biology.[1–5] While other single cell technologies, such as single cell sequencing (scSeq) are largely mature with data available on billions of cells, these data have well characterized limits.[6,7] The most important one to most people is that transcript counts and protein concentrations do not have any meaningful quantitative relationship.[8–10] Furthermore, in much of biology the abundance of a protein has little biological meaning as many proteins don't do anything interesting until they are activated by post translational modifications (PTMs) such as phosphorylation or proteolytic cleavage events.[11–14] SCP has the promise to bridge this gap by providing quantitative information on both proteins and protein PTMs in each cell across a studied population.[3,15] In addition, methods for SCP have demonstrated that other molecules of interest can also be quantified in each single cell, which may give rise to true single cell multi-omics in the near future.[16,17] One reflection of this increase in relative interest in SCP is by searching for publications on PubMed for "single cell proteomics", as shown in **Figure 1A**.

Much of science today is driven by commercial interests and liquid chromatography mass spectrometry (LCMS) is no exception.[18] Instrument vendors facing this interesting new field are optimizing and developing new technologies to make entering this field faster and easier than ever before. Some of these include extremely low loss sample preparation methods,[16,19–22] lower flow chromatography[23,24] and more sensitive mass detectors.[25,26] However, when reviewing the proteomes of single cells in public repositories at the date of this writing, it is clear that the methods which have resulted in the largest numbers of analyzed cells are not from these new technologies. While this may change in the future, the vast majority of SCP data on ProteomeXchange partner repositories[27] has been produced by previous generation instruments employing multiplexing (MSX) reagents. For example, the gold standard for SCP today utilized an improved version of the SCoPE-MS technique[28] developed by pioneers Budnik *et al.,* titled SCOPE2 and characterized mouse macrophage differentiation in nearly 1,500 single cells.[29] These groundbreaking studies utilized an LTQ Orbitrap "Elite" and Q Exactive "Classic" system, which were first launched in 2010 and 2012, respectively.[30,31] Similar methods by Vegvari *et al.,*[32] Schoof *et al.,*[33] and in the Orsburn lab[34,35] utilized instruments a generation or two behind the state-of-the art to analyze several thousand cells in aggregate.

Perhaps prior to, but largely coinciding with the publication of the SCoPE-MS methods, pioneering work at Pacific Northwest National Labs by Ryan Kelly, Ying Zhou and colleagues began describing label free methods for SCP by pushing the best hardware of the day to the absolute limits.[36–38] These teams developed custom robotics solutions for processing single cells in the smallest volumes possible and coupled those with labor intensive direct column loading and low flow chromatography. By leveraging methods with the lowest possible sample loss between cell isolation and ionization over 1,000 proteins could be quantified per cell, but only on a few cells per calendar day.[37]

It is tempting to compare the emergence of SCP with that of single cell RNA sequencing approximately a decade prior. As best reviewed by Valentine Svennson *et al*., in the clearly titled "Exponential scaling of single-cell RNA-Seq in the past decade" the number of single cells analyzed in each published study increased markedly over time with multiple studies of more than 10,000 cells appearing within 7 years of the first study of a single mammalian cell in 2009.[7]

In stark contrast to the emergence of scSeq, SCP appears to have generally moved away from sample multiplexing and embraced the path forged at PNNL. High accuracy sample robotics, increasingly lower

flow chromatography and label free proteomics have been the star of more recent publications. [19,20,22,39,40] While faster chromatography[41] and clever instrument[25,40,42–44] and data processing methods[45–49] continue to emerge and evolve the field, at the date of this writing, no label free study has yet to achieve a higher relative throughput than the original SCoPE-MS study published in 2018.[28]

As summarized in **Figure 1B**, while we've had to estimate cell numbers in published SCP studies, we can say with certainty no exponential increase in cell numbers has yet to be described for SCP. In fact, no increase in study size at all appears to have occurred since the SCOPE2 paper was published.[29] This curious discrepancy between the emergence of these two seemingly similar fields suggested that further analysis was warranted. To better understand how cost and throughput interplay with SCP study size, we have summarized 17 studies from different labs where single cells of typical size and protein concentration were isolated and analyzed by LCMS techniques in the context of relative throughput and estimated cost considerations.

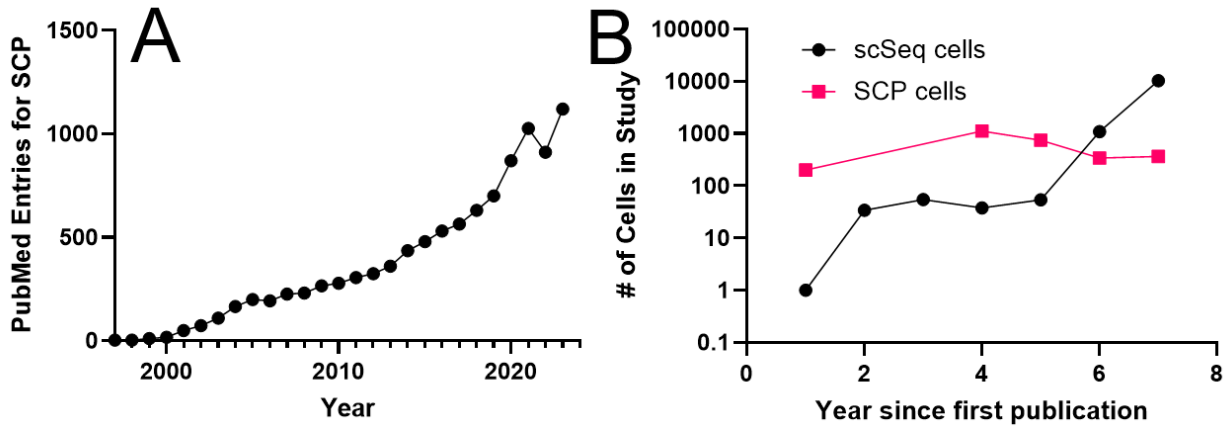


Figure 1. Chronological observations for single cell -omics. A. The number of publications each year in Pubmed acquired with the search term "single cell proteomics". **B.** A summary of the estimated number of cells analyzed in representative studies each year following the first description of global single cell analysis. scSeq data was plotted from a previous review[7] and estimates for SCP studies are from the 17 studies of focus of this current review (Table 1).

Methods

Estimations of relative costs

As previously noted by others, a number of factors complicate the calculation of the costs – and sometimes hidden costs – of shotgun proteomics.[50] Many LCMS labs are subsidized through university or government resources such as the NIH S10 program[51], NSF large equipment grants and through collaborative programs such as IDEA and EPIC-XS.[52] However if we assume that the total costs for instruments, service plans and reagents are ultimately covered – and by whom doesn't matter – we can start to make some general assumptions.

Assumption 1: An LCMS system has a total usable lifespan of approximately 7 years

At many US universities, capital equipment depreciation is amortized assuming an instrument life cycle of 7 years.[18] While there are many LCMS systems out there in the world operating at beyond 7 years, those

of us that have them are not operating without challenges. Instrument vendors will often downgrade instrument service licenses to "best attempt" to maintain instrument operations and downgrade to "not eligible for support" a few years after. Again, the calculations here are based on round number assumptions.

Assumption 2: Service costs equal are approximately 10% of service price annually

Capital equipment sales are typically competitive businesses and promotions abound, particularly at the end of fiscal quarters or financial years. A common tactic for vendors may be to include multiple years of discounted service. For the assumptions in this review, we will assume that each instrument list price will contain one year of warranty service support. The following 6 years of instrument operation costs will be assumed to be 10% of the instrument list or purchase price.[50] For studies where the current cost of the instrument, such as the Orbitrap Elite or Q Exactive Classic is quite low due to the instrument no longer being manufactured, we have inserted a minimum service cost value. The lowest current service estimate for a manufactured instrument was $40,800/year and this was used as a proxy for the total package service costs. A summary of our total calculations is provided in **Supplemental Data X** under the "Cost Calculations" sheet.

Assumption 3: Reagents and consumables are used at a uniform rate by all methods

The wide array of single cell LCMS analysis methods require an equally wide array of reagents employed. While we attempt to take these into account we will assume that reagents are utilized at 100% efficiency. For example, we have recently calculated that a single TMTPro16 reagent kit can be used to label 60,000 single human cells,[53] which is considerably more than the total number of human cells analyzed by LCMS in aggregate at the date of this writing. In addition, as all single cell studies will require the use of digestion reagents and chromatography columns, we have chosen to assume these costs will be uniform for the sake of this analysis.

Assumption 4: Instruments could achieve 100% usage in single cell proteomics over instrument lifespan

It is no secret that precision analytical instruments require cleaning, tuning, maintenance and frequent testing to ensure they are operating optimally with quality control or quality assurance experiments. Add in the fact the typical preventative maintenance on an LCMS system occurs between 1 and 2 times per year, considerable hours per year are dedicated to not running samples. In addition, for some workflows where the number of cells that can be analyzed by LCMS per day is larger than the total number of cells that can be prepared, there may be many unused hours of instrument time. However, for these estimates we have assumed that the goal of every lab will be for every instrument to be acquiring single cell data at every hour of every calendar day.

Assumption 5: Instruments are being purchased for the rate at which we can purchase them

There is likely a large range of actual purchase prices for LCMS instruments that will have dramatic effects on these calculations. We have obtained recent quotes for every instrument in every study that has been subjected to a cost analysis here and we will assume that the purchase price for any researcher will be the same as it would be for us.

Results and Discussions

Study reference	Type	Cells/day	Instrument	Cell isolation	$USD/cell

Budnik et al., 2018[28]	MSX	128	LTQ Orbitrap "Elite"	FACs	$1.18
Specht et al., 2021[29]	MSX	308	Q Exactive "Classic"	FACs	$0.57
Schoof et al., 2021[33]	MSX	120	Exploris 480 FAIMS	FACs	$2.26
Orsburn et al., 2022[15]	MSX	224	TIMSTOF Flex	FACs	$2.52
Vegvari et al., 2022[32]	MSX	175	Orbitrap Fusion 2	FACs	$1.71
Brunner et al., 2022[26]	LFQ	40	TIMSTOF SCP EvoSep	FACs	$16.14
Ctortecka & Hartlmayer et al., 2023[19]	MSX	308	Exploris 480 FAIMS	CellenOne	$4.62
Petrosius et al., 2023[55]	LFQ	72	Orbitrap Fusion 3 FAIMS	FACs	$6.47
Orsburn, 2023[56]	MSX	700	TIMSTOF SCP	FACs	$2.81
Straubhaar et al., 2023[57]	MSX	256	Q Exactive HF-X	FACs	$0.82
Sanchez-Avila et al., 2023[24]	LFQ	24	Exploris 480	Tecan UNO	$13.20
Guise et al., 2023[58]	LFQ	9	Exploris 480	LCM	$55.45
Orsburn, 2023[c17]	LFQ	32	TIMSTOF SCP	FACs	$17.67
Matzinger et al., 2023[39]	LFQ	36	Exploris 480 FAIMS	CellenOne 384 well	$13.86
Ctortecka & Clark et al., 2024[20]	LFQ	80	TIMSTOF SCP/Ultra	CellenOne 96 well EvoSep	$15.81
Montes et al., 2024[59]	MSX	153	Q Exactive Plus	FACs	$1.13
Ye et al., 2024[25]	LFQ	40	Orbitrap Astral EvoSep One	CellenOne/Uno 96 well	$32.45

Table 1. A summary of 17 recent single cell studies with a final estimate from this analysis in cost/cell if the method described was ran 24/7 for the instrument lifecycle. Abbreviations: Multiplexed, MSX; Label free quantification, LFQ; Fluorescence activated cell sorting, FACS; Laser capture microdissection, LCM

The earliest multiplexed single cell proteomics strategies are the most cost effective

While this will come as no surprise to anyone familiar with shotgun proteomics or genomics sequencing, multiplexing is a clear winner for both throughput and relative cost. The SCOPE2 study not only stands out as the first large scale SCP study, but this pioneering work remains the lowest cost SCP study performed to date.[29,60] It is worth noting that in these studies the authors estimated their total costs per cell with numbers approximately double those we have calculated here. In their analyses the authors included reagent costs and the cost of instrument time at their respective core facilities as an hourly rate.[28] In addition, new multiplexing reagents have been developed which allow a larger number of cells to be analyzed simultaneously.[61,62] For the sake of this analysis we have assumed that the maximum currently available reagent (18-plex) could be employed in all studies.

It should be noted that although production of the Q Exactive systems ended in 2020, they are still alive and well in the SCP space. Two studies early in 2024 expanded our understanding of the mammalian epithelial mesenchymal transition,[63] and the variability of root cell development in *Arabidopsis thaliana* [59]with this venerable hardware. Mass detectors with higher complexity operations such as the Orbitrap Fusion systems have contributed meaningfully to the multiplexed SCP space as well. Both Schoof *et al*., and Vegvari *et al*., leveraged low cost high throughput fluorescence activated cell sorting technology and multiplexed analysis on Fusion 2 systems.[32,33] In both cases, large numbers of cells were analyzed and revealed new intricacies in cellular systems at relatively low cost per sample. The first studies in our lab

attempted to match these successes by mimicking SCOPE2 with the limitations of time of flight hardware. While fewer cells can be multiplexed in each LCMS experiment, higher relative scan speeds permit shorter chromatography gradients and the same relative proteomic coverage and throughput per cell.[34,35] However, the higher relative purchase costs and associated service fees of the Orbitrap Fusion and the TIMSTOF Flex system used in these works, respectively, leads to a near doubling of the cost per cell analyzed when compared to the Q Exactive systems. However, it is clear that FACs based cell isolation with either manual or semi-automated liquid handlers such as the OpenTrons OT-2 or Mantis are the most cost-effective SCP studies on a per cell basis. One direct comparison of the cost reduction in multiplexing an be seen in two preprints by the corresponding author in 2023. In both bases, the author used the TIMSTOF SCP system. When multiplexing 7 single cells and using a 100 LCMS injection per day method the estimated cost per cell was less than $3. However, when using the same system for a label free study with a maximum throughput of 32 cells/day the cost per cell increased by 6-fold.

Precision robotics such as the CellenOne system dramatically increases the cost per cell

The relative cost per cell even when utilizing multiplexing reagents increased markedly with the introduction of the CellenOne system into the SCP workflow.[19,22] These microscope based cell sorting and picoliter handling robotics systems have many advantages, including both reproducibility and improved sample recovery. The system, however, has only room for two plates on the deck which can allow only a single 96 well or 384 well plate to be loaded with single cells and reagents at a time.[64] The precision of the system does allow sample preparation to occur in a far more condensed space, such as in the innovative nPOP method. [22]In nPOP, over 1,000 single cells can be isolated, lysed, digested, and pooled on a single deck by printing the cells on coated glass slides. Both nPOP and similar methods utilize standard low cost reagents for analysis. Commercial reagents specific for SCP, however, dramatically add to the relative cost for each cell analyzed. The ProteoChip, for example, costs approximately $500 and allows the processing of up to 12 sets of 16 cells. If including no control, blank or carrier channels, 192 cells can be prepared in each ProteoChip and 3 can be loaded on each CellenOne. Using the typical carrier channel and 2 blank lanes, 156 cells can be processed on each chip and 468 cells in a single day on a high resolution LCMS system. On Time of Flight systems, these numbers drop to 84 cells/chip or 252 cells per day as a maximum of 7 cells can be prepared after two blank channels and one carrier. While TOF systems have lower multiplexing capabilities, the increased scan acquisition rate can allow shorter chromatographic gradients and therefore comparable or superior throughput in cells/day than Orbitrap based methods.[15] In our lab, we faced this worst case scenario on our University's CellenOne system with $1,500 in reagents for each 252 single cells prepared, or an additional $5.92/cell on top of the instrument cost. The estimated cost of ownership of a CellenOne system over 7 years is approximately $80,000/year or $219/day. Therefore, integrating the ProteoChip into your workflow for the maximum of 468 single cells prepared per day will add $1,700 per single cell prep/day before reagents and any associated operating costs, when the system is in use 365 days each year.

Throughput in published studies are decreasing over time while the cost per cell is increasing

In stark contrast to the emergence of scSeq technologies, the throughput of single cell proteomics appears to be decreasing over time, rather than increasing. While the reasons for this are likely multi-faceted, it could be indicative of less interest in SCP as an application at this point than scSeq seven years into the development of each field. It may also be that scSeq is so mature at this point that SCP only makes sense to many researchers today as a confirmatory technology and thousands of cells simply aren't considered

necessary. One final possibility we've considered is that SCP may not be developing at the same pace as scSeq due to the relative number of scientists in each field or to the amazing power of the polymerase chain reaction, for which proteomics still has no parallel. What is clear, however is that the decrease in throughput and the utilization of increasingly sensitive and expensive instrumentation is leading to a dramatic overall increase in the costs of each cell analyzed. There are clear outliers to these trends, particularly as new biological studies emerge that are using the SCOPE2 framework with few alterations. For example, the 2024 preprint from Montes *et al.,* closely follows the SCOPE2 method, as FACs sorted plant cells were analyzed on a Q Exactive Plus system following labeling with low cost robotics.[59] This enabled a group's first entry in the world of SCP to be a study of nearly 800 single cells with the lowest estimated cost/cell in any publication reviewed here in the last 6 years. However, the use of high precision instrumentation such as the CellenOne coupled to today's newest instruments for label free analysis compound to increase the relative cost per cell by more than an order of magnitude. There are clearly advantages to label free proteomics. However, to achieve the same proteomic coverage as a carrier enabled multiplexed single cell requires extremely low sample loss and the highest sensitivity chromatography and mass detectors. Multiple groups have demonstrated ways to increase the number of injections per day through use of multi-column systems and shorter gradients enabled by deep learning of artificial intelligence for data analysis. However, it is worth stating again, that no label free SCP study to date has demonstrated a higher throughput in cells analyzed per day than the original SCoPE-MS study.

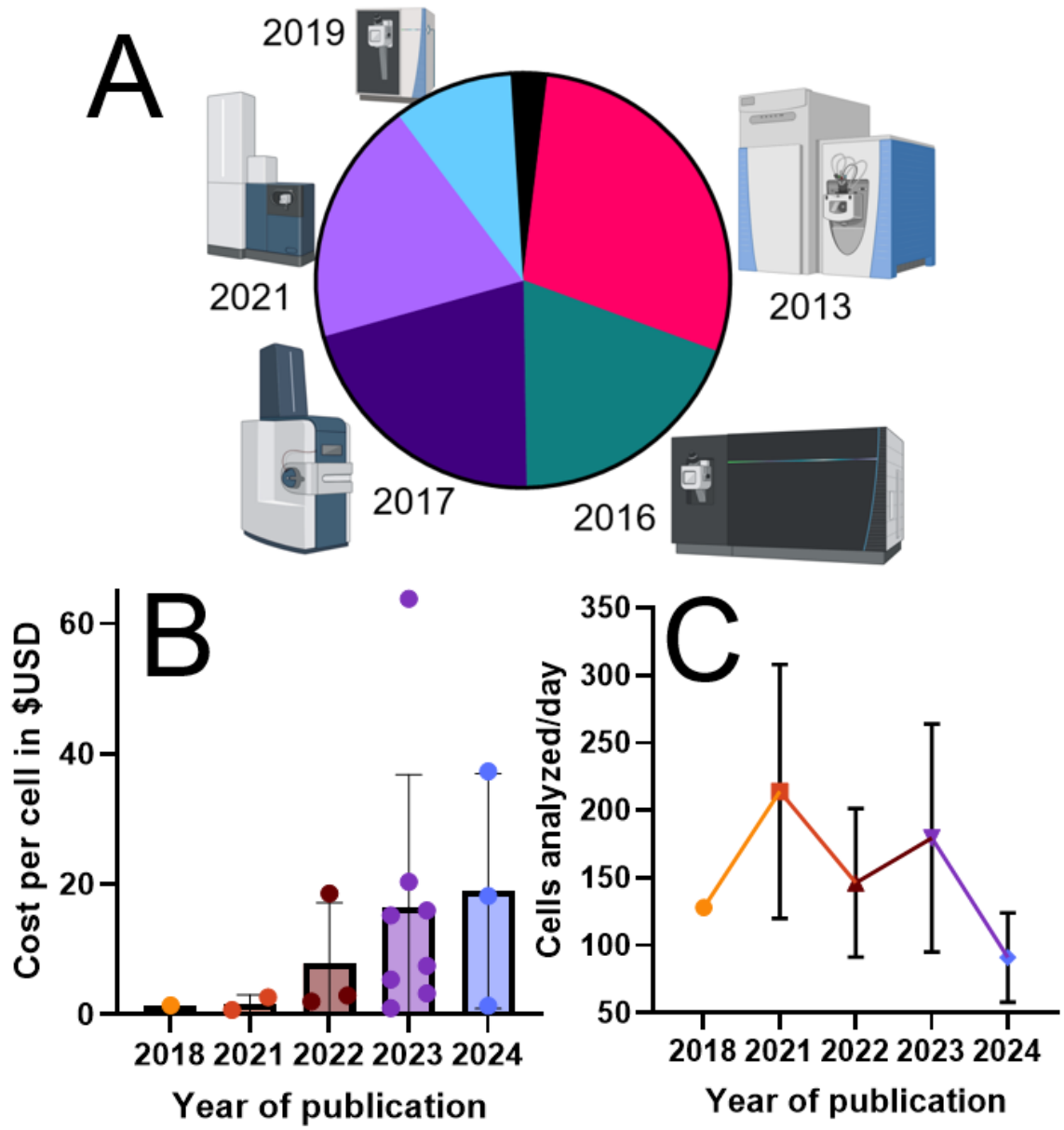


Figure 2. A graphical summary of SCP studies reviewed in this work. A. A pie chart illustrating the number of single human cells currently in the public domain with the instrument used and the original instrument release year. **B.** The mean and standard deviation of the throughput in maximum number of cells analyzed per day from the original SCoPE-MS study through 2024 demonstrating an overall downward trend in throughput. **C.** The mean and standard deviations visualized for the cost per cell as calculated in this review for each single cell analyzed.

Limitations of this current study

Considerable work today is happening today in all single cell analysis fields. As such, it is tough to keep on top of all the new studies that are being preprinted and published across journals. We've sampled studies from the groups we're aware of that are publishing in this field with representative publications of their work. Multiple studies were disregarded for reasons such as the cells analyzed were significantly larger

than the typical human cell[65–67] or because data was not publicly available and could not be obtained from the authors upon request (citations not shown). There is considerable evidence that industry scientists are utilizing SCP but it is impossible to guess whether their motivation is focused on throughput or analysis depth.[25,68] On that final note, it is likely that if we performed an additional analysis on the number of proteins analyzed per cell per day similar to a recent analysis of single molecule detection techniques then the newer and often more expensive methods made provide greater proteomic coverage of each cell.[69,70] However, an attempt to quantify this was quickly abandoned as the wide range of data processing schemes and philosophies such as whether or how to use MS1 based feature matching, rapidly placed the attempt beyond the scope of the study we intended.

Conclusions

We have detailed a strikingly wide range of methods to analyze hundreds or thousands of proteins in single cells. Associated with these methods are a similarly striking range of relative costs. It should be noted that even some of the earliest single cell studies considered relative costs per cell. While we are clearly in the early stages of this new and exciting field, it is still curious to us that no study since SCOPE2 has obtained a lower cost per cell analyzed.[29] In fact, the opposite appears to have occurred with the average cost/cell of the studies included here increasing every year. There are many reasons to use high accuracy robotics for sample preparation, including both increased recovery and improved reproducibility.[19,22,37] It has only been passingly noted, however, that the use of these tools dramatically increases the cost per study.[24] Similarly, there are many reasons for using label free analysis methods. Authors have noted fewer missing values and better quantitative accuracy[21,55,71] as well as the well characterized limits of carrier channel enabled experiments.[68,72] However, if the goal is to compete or fully complement other single cell technologies where the analysis of thousands or tens of thousands of cells are simply required to accurately sample all cell populations present, multiplexing appears to be the only answer.[73] At this point, it may be up to the greater scientific community to determine what shape the field of single cell proteomics will ultimately assume.

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Summary of Supplemental Data

Supplemental Data 1: A summary of all studies reviewed in this work with relevant characteristics

Supplemental Data 2: The step by step calculations for how the cost for analyzing each cell was determined for each study

Supplemental Data 3: Extracted data from Svennson et al., used for scSeq data presented in Figure 1B

Supplemental Data 4: A simple calculator sheet to estimate the cost/cell for other studies.