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Comparison of visualisation tools for single-cell RNAseq data

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Abstract

In the last decade, single cell RNAseq (scRNAseq) datasets have grown from a single cell to millions of cells. Due to its high dimensionality, the scRNAseq data contains a lot of valuable information, however, it is not always feasible to visualise and share it in a scientific report or an article publication format. Recently, a lot of interactive analysis and visualisation tools have been developed to address this issue and facilitate knowledge transfer in the scientific community. In this study, we review and compare several of the currently available analysis and visualisation tools and benchmark those that allow to visualize the scRNAseq data on the web and share it with others. To address the problem of format compatibility for most visualisation tools, we have also developed a user-friendly R package, *sceasy*, which allows users to convert their own scRNAseq datasets into a specific data format for visualisation.

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Introduction

In just a decade, the number of cells profiled in each scRNAseq experiment has increased from approximately 1,000 cells to millions of cells (Svensson et al. 2017), thanks to the advent of sequencing protocols, from well-based (Deng et al. 2014; Picelli et al. 2014; Ramsköld et al. 2012) to droplet-based (Macosko et al. 2015; Klein et al. 2015), and the ever-decreasing cost of sequencing. In parallel, many computational methods have been developed to analyse and quantify scRNAseq data (Kiselev et al. 2017; Butler et al. 2018; Kiselev et al. 2018; Lee et al. 2019; La Manno et al. 2018; Stuart et al. 2019). A typical scRNAseq analysis pipeline starts from the raw reads, which are processed to create an expression matrix, containing the expression values of every gene in every cell. Further downstream analysis is then performed where cells are clustered and the cluster-specific marker genes are identified to annotate cells with corresponding cell types. The results are then visualized using non-linear embedding methods, such as tSNE (van der Maaten and Hinton 2008) or UMAP (McInnes et al. 2018) usually in a two-dimensional space where each cell gets a pair of X-Y coordinates defining its position on the visualisation plot. Finally, the visualisations are used to asses the obtained cell types by highlighting the cell metadata (information about cells in a given experiment, e.g. batch, donor etc.) or the expression of specific genes across the cell types. This assessment can only be performed in an interactive manner. However, when the results are shared as a report or published in a paper format (a static 2D image), it is only possible to see a snapshot of the analysis corresponding to a single gene and a single set of cell metadata. Therefore, recently the ability to analyse, visualise the data in an interactive way has attracted a lot of attention, and advances in web technologies have led to the development of multiple tools for sharing the analysis results via a web interface.

In this paper we attempt to give an overview of a number of currently available tools to help researchers choose a tool for their visualisations. We compared 13 popular interactive analysis and visualisation tools for scRNAseq data by means of their features, performance and usability. Firstly, we looked at their general characteristics and properties. Secondly, we selected those tools that provide web interface functionality and benchmarked them against each other by means of their performance on datasets of different sizes (from 5,000 to 2 million cells). We also evaluated user experience (UX) features of the tools with a web interface. Finally, since all of the tools have different input requirements, we developed an R package, *sceasy*, for flexible conversion of one data format to another.

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Results

	ASAP	Bbrowser	cellxgene	Granatum	iSEE	Loom viewer	Loupe Cell Browser	SCope	scSVA	scVI	Single Cell Explorer	SPRING	UCSC Cell Browser
Web Interface			✓		✓	✓		✓	✓		✓	✓	✓
Interactivity	✓	✓	✓		✓		✓	✓	✓		✓	✓	✓
Docker	✓		✓		✓			✓	✓		✓		
Cloud Support				✓				✓	✓			✓	
Loom						✓		✓	✓	✓	✓		
h5ad		✓	✓						✓	✓			✓
SCE					✓								
Seurat		✓									✓		✓
csv/txt	✓	✓		✓					✓	✓	✓	✓	✓
Platform	Java/ R	Desktop	Python	R	R	Python	Desktop	Python	R	Python	Python	Python	Python
Last updated*	>3 m	<1 m	<1 m	>6 m	<1 m	>3 m	>3 m	<1 m	>6 m	<1 m	>3 m	>6 m	<1 m
Number of contributors	1	?	19	3	7	5	?	5	2	24	1	3	6
Number of active developers (last 2 m)	0	?	6	0	3	0	?	2	0	4	0	0	2

Table 1. Overview of the visualisation tools and their capabilities. *Latest GitHub commit (checked on 20/12/19). **Web Interface** corresponds to the ability of hosting and sharing a webpage with a data visualisation. **Interactivity** corresponds to the ability of exploring the data in an interactive way as opposed to static images. **Docker** indicates whether a docker image with the tool is provided by the developers. **Cloud Support** indicates whether the authors provided instructions on how to deploy their web interface on a public cloud. **Loom**, **h5ad**, **SCE** (SingleCellExperiment), **Seurat**, **csv/txt** are different input formats.

We reviewed and compared 13 popular scRNAseq analysis and visualisation tools: ASAP (Gardeux et al. 2017), BioTuring Single Cell Browser (Bbrowser) (BioTuring n.d.), cellxgene (Chan Zuckerberg Initiative n.d.), Granatum (Zhu et al. 2017), iSEE (Rue-Albrecht et al. 2018), loom-viewer (Karolinska Institutet n.d.), Loupe Cell Browser (10X Genomics n.d.), SCope (Davie et al. 2018), scSVA (Tabaka et al. 2019), scVI (Lopez et al. 2018), Single Cell Explorer (Feng et al. 2019), SPRING (Weinreb et al. 2018) and UCSC Cell Browser (UCSC n.d.). Table 1 compares these tools in terms of cloud support, containerisation, supported input formats, and developer activity.

The tools vary in the ability to use different input file formats (green colour in Table 1). The csv/txt format is the most accepted one and can be used by eight tools. More specialized formats such as h5ad and loom are accepted by five tools each. SingleCellExperiment (SCE) and Seurat are accepted by one and three tools, respectively. To make it possible for the users to visualize their

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datasets in different ways we have developed the *sceasy* R package for file format conversion, which is available on Github at https://github.com/cellgeni/sceasy.

In terms of the web features (blue colour in Table 1), some of the tools are provided with the cloud-specific deployment instructions (Granatum, SCope, scSVA and SPRING) whereas others (ASAP, cellxgene, iSEE, SCope, scSVA, Single Cell Explorer) only have Docker images which facilitate the deployment, but imply more work on the user side. Note that not every web-friendly tool has the ability to host and share a webpage with a data visualisation (**Web Interface** row in Table 1). For example, ASAP and Granatum provide a full analysis pipeline which include some visualisations, however, they do not allow for sharing them with others.

We selected the tools which can be used for hosting and sharing a webpage with a data visualisation and benchmarked them against each other (except SPRING, see Methods and Fig. 1 for details). We specifically measured how the memory (RAM) usage and the start-up time of the web application depend on the size of the input dataset.

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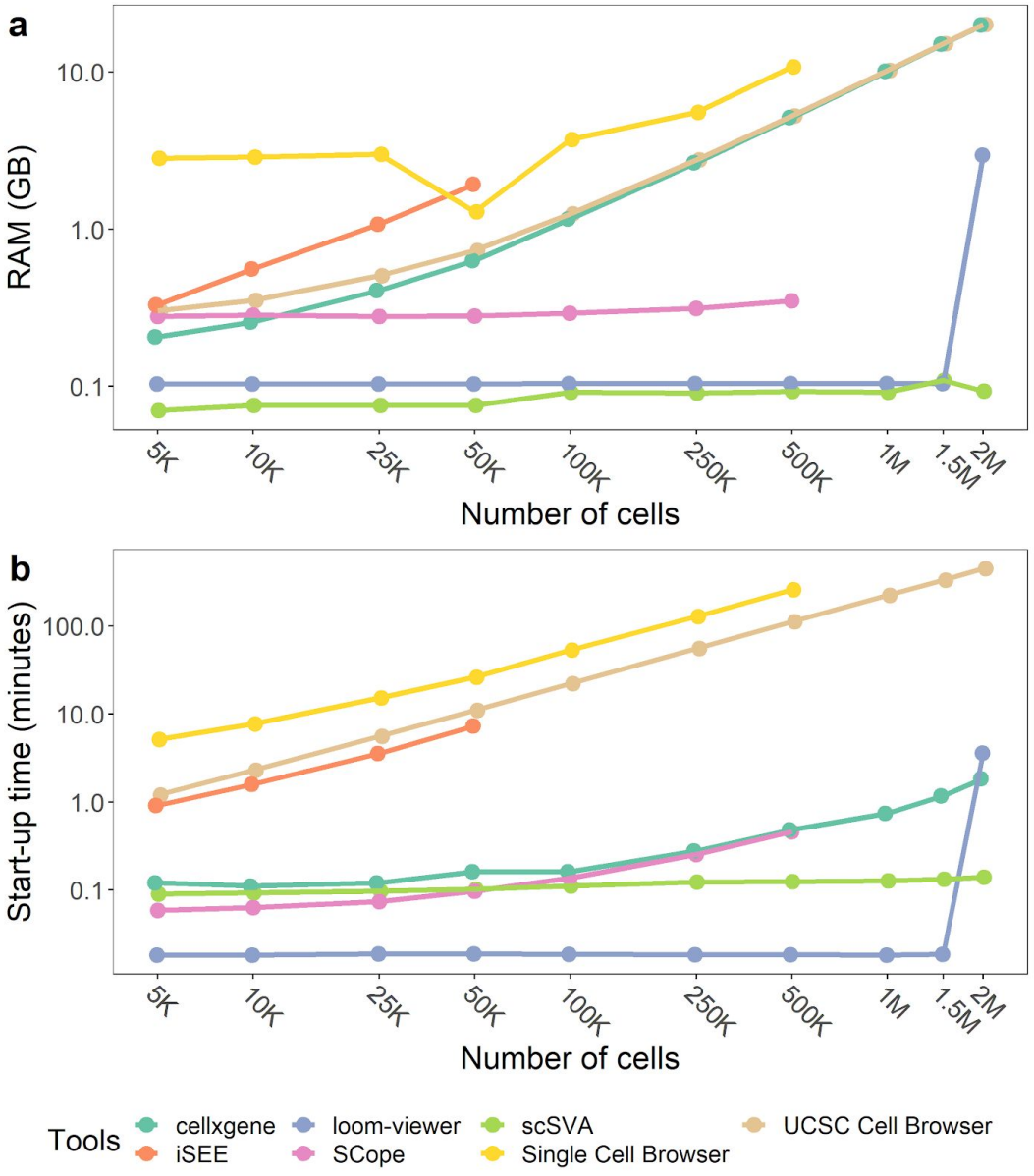

Figure 1. Maximum RAM usage (a) and start-up times (b) of the visualisation tools. The points on the plots represent median times across 5 independent runs. Start-up times include data importing process and visualisation time of each tool.

Fig. 1 summarizes the benchmarking results. The tools can be split into two distinct groups according to the way they handle data loading, and this is reflected in the benchmark results.

The first group (loom-viewer, scSVA and SCope) represents the tools for which the performance is almost independent of the size of the input dataset. This is due to on-demand loading, i.e. the necessary data is only loaded to RAM when it is needed by the application. In this case, at the start the tools only use X-Y tSNE or UMAP coordinates of the cells without loading other input data into memory. Interestingly, there is a sudden drop of loom-viewer efficiency at 2M cells. This effect was consistent across all 5 runs and we could not explain it. Similarly, there is a consistent drop in

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RAM usage by the Single Cell Browser at 50K cells, which we also could not explain. In addition, we were not able to run SCope and Single Cell Browser for datasets larger than 500K cells.

The computational costs of the second group of tools (iSEE, Single Cell Browser, UCSC Cell Browser and cellxgene) grow exponentially with the size of the dataset. This is due to loading of the full data in memory. Single Cell Browser exhibits the highest RAM usage and longest start-up time. iSEE has the steepest RAM usage growth with the number of cells and failed to start for datasets larger than 50K cells. Among these four tools cellxgene has the shortest start-up times.

	cellxgene	iSEE	Loom-viewer	scSVA	SCope	Single Cell Explorer	UCSC Cell Browser
Data loading	5	5	4	2	3	1	1
Ease of cell selection	5	1	1	3	5	5	3
Zoom in/out	2	2	0	2	2	0	2
Multiple embeddings	2	2	2	0	2	0	2
Highlight gene expression	2	2	0	2	2	2	2
Highlight metadata	2	2	2	2	0	2	2
Extra analysis	2	0	0	2	0	2	0
TOTAL	20	14	9	13	14	12	12

Table 2. UX scores of the visualisation tools. The range in the first two rows is from 1 to 5, where 1 is the lowest and 5 is the highest score, representing a scale of fitness. The other rows show absence or presence of a capability, scored as zero and two respectively. This weighting choice does not affect the general trend in the total scores.

In addition to the performance we also scored the benchmarked tools by their user experience as shown in Table 2. Data Loading was scored based on how easy and fast the data could be loaded. The tools that require a single file and could be run from a command line (cellxgene and iSEE) were given the maximum score of 5. The other tools require loading of data via a graphical user interface (GUI) with multiple mouse clicks and involve some waiting time, and therefore scored lower.

For statistical analysis and comparison of groups of cells users need to select cell populations of interest. The tools were scored based on the type of selection they provide. The highest score 5 was given to the tools with lasso selection (cellxgene, SCope and Single Cell Explorer), which allow the user to select the cells by drawing a free shape curve around the cells of interest. Score 3 was given to the tools with rectangular selection (scSVA and UCSC Cell Browser), where the user is limited to drawing a rectangle around the cells of interest. Finally, score 1 was given to iSEE and loom-viewer because they do not provide any method of selection.

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The ability to zoom in and out can be crucial to visually analyse and validate the data. Most of the tools have zooming functionality (and therefore were scored as 2) except loom-viewer and Single Cell Explorer (scored as 0). Similarly, the ability to switch between multiple embeddings (e.g. between tSNE and UMAP) can be very useful and help with the analysis. Again most of the tools scored 2, except scSVA and Single Cell Explorer, which do not support this functionality.

One of the most important features every single-cell visualisation tool must have is the capability to highlight specific information. The user may want to highlight either gene expression levels (continuous scale) or cell metadata (usually on a discrete scale). Not surprisingly, this functionality is available in almost every tool with the exception of loom-viewer (for gene expression) and SCope (for cell metadata).

A useful feature of a visualisation tool is the option of performing extra analysis on user-selected cells, such as e.g. cell-type annotation, differential expression analysis or marker gene identification. Only three of the benchmarked tools (cellxgene, scSVA and Single Cell Explorer) have this functionality.

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Methods

Datasets

For benchmarking we utilised a mouse embryo development scRNAseq dataset (Cao et al. 2019) which contains 2.07 million cells. Ten datasets of different sizes (with 5,000, 10,000, 25,000, 50,000, 100,000, 250,000, 500,000, 1,000,000, 1,500,000 and 2,000,000 cells) used for performance benchmarking were created by randomly subsampling cells of the original dataset.

Profiling

Benchmarking tests were done on a virtual Ubuntu OS 16.04 with 23GB of RAM and 2GHz Intel Xeon Processor with 16 cores.

iSEE and scSVA are both R packages and therefore were tested by using *profvis*, a package for profiling R scripts (Chang et al. 2019). The highest value of the *"memalloc"* slot with the label of *"shiny::runApp"* was considered as RAM usage, and the last value in the *"time"* slot was considered as start-up time.

For all the other tools (except cellxgene) the characteristics were measured by running Linux command `/usr/bin/time -v`, and using "*Maximum resident set size (kbytes)"* output for RAM usage and using the sum of *"User time (seconds)"* and *"System time (seconds)"* outputs for start-up times. For cellxgene the *gnomon* command was used with elapsed-total option to measure the startup times. For SCope the start-up time was profiled only from the server side (the timed process was `scope-server`). The start-up times included both the internal data import time (only for UCSC Cell Browser and Single Cell Explorer) and visualisation time. Both UCSC Cell Browser and Single Cell Explorer spent a considerable amount of time on the data import as they need to convert the data into other structures (json files and MongoDB database, respectively) before being able to visualize it. For UCSC Cell Browser, scanpy's (Wolf et al. 2018) `scanpy.external.exporting.cellbrowser` was used to perform the conversion. For Single Cell Explorer, `ProcessPipline.insertToDB` function from the `scpipline.py` library provided by the authors was used.

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Discussion

The size and volume of the scRNAseq data has been exponentially increasing in the last decade and this has opened up new avenues of scientific discovery and understanding. Consequently, there is a great need of being able to quickly and easily explore these data. There is a need among scientists to communicate their data to collaborators and colleagues for quick and easy exploration. The burden of computational resources and bioinformatics skills required to do this should ideally be removed from the recipients of the data.

Single-cell interactive analysis and visualisation tools have been widely adopted by the research community. They make data import, public data access and analysis much easier for the users and accelerate the science. Furthermore, tools now exist (those with **Web Interface** functionality in Table 1) that allow the user to host and share their scRNAseq data visualisation with others on the web, due to recent advances in web technologies. These make it possible to share analysis results with others in a user-friendly manner, allowing for much faster scientific development. We believe that high complexity and dimensionality of scRNAseq data can only be revealed via comprehensive, interactive, and user-friendly tools that can provide shareable visualisations via the web. This is supported by the recent developments of scRNAseq visualisation portals at large scientific institutes:

- Single-Cell Expression Atlas (Papatheodorou et al. 2020) at the European Bioinformatics Institute - https://www.ebi.ac.uk/gxa/sc/home
- Single-Cell Portal at the Broad Institute - https://singlecell.broadinstitute.org/single_cell
- Cell Browser at the University of California Santa Cruz - https://cells.ucsc.edu/

To understand the current landscape of interactive analysis and visualisation tools we compared (Table 1) several of the most popular based on their general qualities. Our results show that each tool has particular advantages and disadvantages and as such a simple ranking cannot be achieved. We looked specifically at the tools suitable for sharing an interactive visualisation of results via a web interface (the **Web Interface** row in Table 1). Again, in this case there is not one tool that stands out as best in all categories. From our personal experience and partly supported by benchmarking, we currently recommend using cellxgene for publishing and sharing your data.

Cellxgene performs well in terms of both memory and startup times (Fig. 1) and leads by the UX scores (Table 2). It also has a thriving community, is the most supported (Table 1) with 24 contributors and has the highest developer activity in the last 2 months. We have developed a detailed tutorial (https://cellgeni.readthedocs.io/en/latest/visualisations.html) for using cellxgene and how to convert data into the required input format.

Single-cell sequencing technologies are still in rapid development and we expect the dataset sizes (number of cells per dataset) to further grow in the next few years. Tools that use on-demand

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loading with linear or sublinear memory usage relative to cell count are best positioned to cope with this growth. Other tools will have to adapt and optimise in order to stay competitive.

Those entering the visualisation competition now will need to not only think about efficiency and scalability of visualisation but also about additional features that can enrich the data visualisation and provide more scientific insights. An example is the user-friendly integration of a dataset under consideration with public scRNAseq data. This is already happening in commercial products, e.g. Bbrowser provides, for a selected group of cells, a suggestion of cell type based on publicly available data. It also provides the ability to search for specific cells from public data similar to the selected ones. It is worth noting that there are a lot of command line tools with exactly the same or similar functionality. However, putting this functionality into an interactive user-friendly interface allows more researchers to use it and facilitates scientific progress.

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