Spatially Resolved Transcriptomics Mining in 3D and Virtual Reality Environments with VR-Omics

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Abstract

Spatially resolved transcriptomics (SRT) technologies produce complex, multi-dimensional data sets of gene expression information that can be obtained at subcellular spatial resolution. While several computational tools are available to process and analyse SRT data, no platforms facilitate the visualisation and interaction with SRT data in an immersive manner. Here we present VR-
Omics, a computational platform that supports the analysis, visualisation, exploration, and interpretation SRT data compatible with any SRT technology. VR-Omics is the first tool capable of analysing and visualising data generated by multiple SRT platforms in both 2D desktop and virtual reality environments. It incorporates an in-built workflow to automatically pre-process and spatially mine the data within a user-friendly graphical user interface. Benchmarking VR-Omics against other comparable software demonstrates its seamless end-to-end analysis of SRT data, hence making SRT data processing and mining universally accessible. VR-Omics is an open-source software freely available at: https://ramialison-lab.github.io/pages/vromics.html
Introduction

Spatially resolved transcriptomics (SRT) is an emerging technology allowing the capture of spatial gene expression at unprecedented resolution\(^1\). Several technologies are currently commercially available with different cellular resolution and transcriptome coverage. These include sequencing-based SRT technologies (e.g., Visium\(^2\) from 10X Genomics, Tomo-seq\(^3\), GeoMx from Nanostring, STOmics\(^4\) from BGI) usually allowing full-transcriptome coverage and imaging-based technologies (e.g., MERFISH\(^5\), Xenium\(^6\) from 10X Genomics, CosMx from Nanostring\(^7\)) typically limited to a gene panel but providing sub-cellular resolution. Concomitantly, several novel algorithms have been developed to process and mine SRT data\(^8\)–\(^10\).

Most current SRT pipelines or programs typically require computational knowledge skills to process the data delivered by the service providers (e.g., Scanpy\(^11\), Squidpy\(^12\), SpatialDE\(^13\)). Data visualisation and mining is usually supported by a platform specific for each technology. These are often proprietary (Visium Loupe Browser\(^14\), Xenium Explorer\(^15\), MERSCOPE Web Vizualiser\(^16\)) and sometimes subscription-based (AtoMX\(^17\)). Databases warehousing SRT experiments also offer useful portals for the mining and exploration of existing SRT data (SpatialDB\(^18\), STOmicDB\(^19\), SODB\(^20\)). However, no platform currently exists as a one-stop shop to process, analyse and visualise SRT data directly from the manufacturer to a visualisation interface, that is compatible with all SRT technologies. We and others have previously shown that high-complexity of -omics datasets greatly benefit from analysis using an immersive environment\(^21\),\(^22\). The lack of such software restricts the user base for SRT technologies due to the heavy computational processing and the inability to perform cross-platform comparisons, especially when different SRT technologies are used to tackle the same biological problem.

Here we present VR-Omics, a free open-source software application to process, analyse and visualise SRT data, all through a graphical user interface (GUI). Data generated by the SRT service providers can be directly loaded into VR-Omics for processing. SRT data analysis is then performed locally, and the results are visualised in an interactive user-friendly interface. VR-Omics currently supports both sequencing and imaging-based SRT technologies such as Visium\(^2\) and Xenium\(^23\) from 10X Genomics, MERFISH\(^24\) from Vizgen, STOmics\(^25\) from BGI and open-source technologies such as Tomo-seq\(^26\). A unique feature of VR-Omics is that it also supports custom SRT data. VR-Omics architecture is based on Unity that incorporates a purpose-built Python environment\(^21\),\(^22\). VR-Omics is the first software to enable the analysis and visualisation
of any SRT data in virtual reality (VR). This feature also facilitates the analysis of 3D SRT datasets. Indeed, while most platforms are able to visualise individual sections from a single SRT experiment, VR-Omics enables the visualisation and analysis in 3D through multiple sections (e.g., serial sections of the same tissue). Benchmarking VR-Omics against most common SRT data analysis and visualisation tools using publicly available 2D and 3D datasets from mouse, human and zebrafish demonstrate that VR-Omics provides tailored data analysis and visualisation options, unavailable through current SRT tools, to deepen biological understanding of processed tissues. These unique properties (user-friendly GUI, full automated workflow, VR, cross SRT platform compatibility and modularity) mean that VR-Omics is a robust and sustainable solution for the end-to-end analysis of SRT data for all users.

Results

VR-Omics consists of two integrated components (Fig. 1): the Automated Workflow (AW) that processes and mines the raw SRT data (SRT Input Data) (Fig. 1a, b) and the Visualiser that takes the processed data as input and provides an interactive 3D interface to visualise and further mine the data for 3D display in a desktop or virtual reality (VR) environment (Fig. 1c,d).

The Automated Workflow enables to automatically process raw SRT data

Users can process and analyse their raw data end-to-end within the VR-Omics GUI (Supplementary Video 1). This is enabled in the backend by the AW which was designed to pre-process and spatially analyse SRT raw data generated from different commercialised and open-source SRT platforms and custom-data (Fig. 1a).

Across these platforms, different terms are used for the smallest individual capture area of each technique, e.g., spots, locus/loci, bins, or locations. For consistency we will use the term locations from here on, always referring to the respective SRT methods smallest individual capture area.

Visium, MERFISH and Xenium SRT data are analysed by the AW using the Scanpy\textsuperscript{16} and Squipy\textsuperscript{12} Python libraries (Supplementary methods) (Fig. 1b). For example, for Visium data, the AW takes as input the feature/barcodes matrix and aligned tissue/barcode images generated
from the Space Ranger pipeline using the Space Ranger count function. Following this, users can perform a variety of operations to tailor data analysis (Fig. 1b, 2).

First users can filter the data by: number of counts in locations, percentage of mitochondrial gene expression, and number of cells expressing a certain gene. Plots visualising the data according to these filtering metrics may also be generated (Fig. 2a). Visualisations dependent on these metrics can help elucidate regions of higher counts along the tissue section, bringing to light potential intra-tumour heterogeneity (Fig. 2a). Data normalisation and log transform follow before highly variable genes (HVGs) are labelled in the data.

Subsequently, principal component analysis is performed, neighbours are computed and UMAP coordinates generated prior to performing Leiden clustering (Fig. 2b). This can help visualise, for examples, layers of the human cerebral cortex (Fig. 2b).

Crucial insights for the spatial examination can be obtained by identifying spatially variable genes (SVGs). SVGs can play a role in cell differentiation, tissue organisation and development and progression of disease. The AW allows for identification of SVGs using the package SpatialDE (Fig. 2c).

Finally, users can access all output files stored locally, where each analysis is recorded as a different sample (Figs. 1d, 2a-d). VR-Omics offers the unique option to simultaneously load multiple samples if they represent serial sections of the same tissue, in order to visualise the tissue in 3D (Fig. 2e). For this, VR-Omics provides a straightforward alignment process consisting of selecting multiple Visium sample folders that have been transformed and/or analysed with the AW (Fig. 2e). The selected datasets will be shown to the user in unison, allowing the user to set the correct anatomical order from front to back of the slides. Next, all Haematoxylin and Eosin (H&E) images will be overlapped in a simple user interface that allows the user to rotate the slides individually, correcting misalignments of the samples on the capture areas (Fig. 2eii). Furthermore, the user can set the distances between each slide to align the proportions (Fig. 2eiii). Finally, the user can visualise the merged dataset rendered from the selected slides with the set distances and rotations as a 3D object (Fig. 2eiii). The datasets are ready for further downstream analysis or visualisation in the Visualiser.
The Visualiser enables SRT data mining in 2D, 3D and VR

Datasets processed through the AW can be directly uploaded to the Visualiser. Using the Visualiser, gene expression data from all platforms can be systematically explored and compared across samples in a 2D desktop and VR (Fig. 3,4). Once processed data and associated metadata have been uploaded, the Visualiser offers several features (Fig. 3, Supplementary Fig. 1) to facilitate biological interpretation across the different technologies (Supplementary Table 1). Throughout the development of VR-Omics we focused on developing an easy-to-use GUI for users to navigate through all features and using shortcut key-bindings to further improve the handling. Most of these functions are supported across all SRT input formats whereas some features are unique to a specific SRT method (Supplementary Table 1).

Location information

All datapoints representing the locations are visualised as an interactive data structure. Each location is visualised as a sphere, although, the symbol to represent the locations can be switched to a cube. Users can interact with each single location using either a combination of mouse and keyboard for the desktop solution or controllers in VR. Location information can be accessed, containing the locations barcoded ID, the coordinates, the dataset name, or the current normalised gene expression values. More detailed information can be displayed if the VR-Omics AW was used to analyse a Visium dataset, providing information such as total count per location, mitochondrial count, or the cluster information (Supplementary Fig. 1a). Users can seamlessly zoom into the data up to single location visualisation (Supplementary Fig. 1b).

Gene search

Users can use the search function to retrieve a gene of interest (Fig. 3a). When a gene is selected, its expression is visualised using a heatmap representing its relative expression at each location. Alternatively, the dataset can also be visualised in a binary "gene-is-on" manner, in which locations with non-zero expression of the selected gene are coloured with a uniform intensity. Moreover, users can adjust the size of the locations or filter out locations with low expression levels. This filtering can be applied to the heatmap visualisation (Supplementary Fig. 1c) or to the binary “gene-is-on” option (Supplementary Fig. 1c_ii). All expression levels are normalised across the entire dataset to facilitate comparison between genes and locations. This can help gain understanding of overall expression of important markers, such as EPCAM in breast cancer which is linked to poor prognosis26, to help associate severity of disease in tissue (Fig. 3a).
Data overlay

Data interpretation is facilitated by allowing super-imposition of other metadata generated such as H&E staining when available (Fig. 3b) or Leiden clusters calculated in the AW (Fig. 2b). Users can visualise clusters super-imposed on the tissue image. This allows the user to quickly determine how closely clustering or other signals results in the analysis are in line with the histology displayed by the tissue, such as the clusters found within the mouse brain (Fig. 3b).

When serial slides taken through the same sample are available (Fig. 2e), a 3D-object can be uploaded to map these slides onto the object (Fig. 3c) more accurately portraying the underlying tissue structure. Overlaying the datasets with a 3D model provides additional anatomical orientation. In the case of the developing human heart, an overlay of the 3D model gives the user a more intuitive understanding of which clusters correspond to anatomical structures within the heart, allowing for easier selection of biologically relevant ROIs (Fig. 3c). The user can define the origin of the 3D object and its default rotation. If the 3D model needs additional adjustment, VR-Omics offers a variety of options to help align the 3D model with the dataset within the Visualiser. These include scaling and rotating the model in all three dimensions, as well as moving it in three directions. VR-omics supports most standard 3D objects provided in file formats supported by Unity including .fbx, .dae, .3ds, .dxf and .obj files.

ROI selection

Users can select regions of interest (ROI) or specific spot locations to further explore them in the Visualiser (Fig. 3d). User can export specific information pertaining to the locations that are grouped using the lasso tool. For example, lasso tool applied on the mouse brain section using the MERFISH platform shows selection of a group of genes that belong to a cluster of interest (Fig. 3d). Up to four groups can be created simultaneously, and the selections made with the lasso tool can be exported or re-imported through the context menu. Users can also export the ROI groups, the gene expression values of the current gene selection and the barcoded IDs. This allows users to keep track of their research and continue analysis from previous findings.

For 3D datasets of multiple layers, users can select 3D ROIs by grouping locations throughout the depth of the dataset (Fig. 3d). The user can rotate individual slides around their centre or move them along the x or y axis for more precise control over the spatial alignment of the slides. When working with a merged dataset from multiple Visium slides, the user can select the desired slide to operate on.
**Side-by-side comparisons**

VR-Omics makes it possible to visualise two different gene expression profiles next to each other (Fig. 3e) as well as vector-based differences of genes, showing where the gene expression profiles contain differences or similarities. The user can choose to apply the next gene selection to either the original or the duplicate slide. This allows for the comparison of two different gene expression profiles in close proximity. For example, this feature facilitates the comparison of different markers linked to poor prognosis or tumour aggressiveness, such as *EPCAM* and *ELF3*\(^{26,28}\), to better understand if they are affecting the same regions or cells within a tumour (Fig. 3e). Users can highlight ROIs in the original slide, with the selection being automatically transferred to the duplicated slide. This allows for precise ROI delineation while ensuring that the exact same area is analysed in both slides. If the side-by-side feature is deactivated while two different genes are currently being visualised, the user has the option to merge the expressions of both genes (Fig. 3f). This results in the removal of the duplicate slide and a normalised, vector-based difference calculation of the two gene expressions being performed at each location. Regions of high expression indicate a significant difference in the gene expression of the two gene profiles, while regions of low expression indicate a small difference (Fig. 3f).

**Virtual reality**

The Visualiser supports data analysis in a fully immersive VR environment (Fig. 4a-d). Connecting a VR device such as a head-mounted display (HMD) allows easy switching between the desktop 2D visualiser and VR during runtime. By running the Visualiser’s features in VR (Fig. 3, Supplementary Fig. 1, Supplementary Video 1), data interpretation is facilitated by the ability to visualise and explore it in its natural 3D context (Fig. 4a), to understand patterns of gene expression (Fig. 4b), clusters across the tissue (Fig. 4c) and tissue organisation (Fig. 4d). In addition, VR also reduces artefacts that occur while visualising 3D data on a 2D desktop display\(^29\). This occurs as a 3D object needs to be morphed and flattened to be visualised in a 2D space, which can distort the data and create the visual artefacts\(^29\). VR-Omics is compatible with the majority of current head-mounted display devices that supports the Unity software (e.g., Meta Quest 2, HTC Vive and Valve Index).
Benchmarking

In order to assess VR-Omics’ performance, we compared its features against those offered by existing SRT visualisation tools, most of which are compatible with data generated from a single SRT platform. Tools assessed included: Loupe Browser, Xenium Explorer, MERSCOPE Web Vizualizer, Stereopy and SODB (through its visualiser SOView) (Supplementary Table 2).

Data analysis in 3D

While all platforms provide similar features for location analysis, the unique feature of VR-Omics is the ability to comprehensively analyse and visualise 3D-SRT data (Supplementary Table 2). This includes loading of multiple sections along with tools facilitating 3D operations such as alignment. Operations developed for single sections are also applicable across multiple sections (for example ROI selection across multiple slides) (Fig. 3d). While tools for working with z-stacked SRT datasets in 3D such as STich3D are available, it is more limited in its functionality than VR-Omics. It has currently only demonstrated compatibility with sequencing-based SRT platforms and does not offer a GUI. Users will require knowledge of Python to run the bioinformatics analysis of datasets prior to visualisation within STich3D, a task handled in its entirety by the AW in VR-Omics. VR-Omics offers additional levels of analysis such as identification of SVGs within the AW while STitch3D requires the user to output the results and load them into a separate package for analysis.

To assess the 3D data analysis capability, we further compared our VR-Omics pipeline with STich3D, VR-Cardiomics, Loupe Browser, Xenium Explorer, MERSCOPE Web Vizualizer, Stereopy and SODB using real biological data with multiple slices from serial sections on the human embryonic heart at 6 dpc. One typical biologist-driven workflow is to identify genes that are differentially expressed between regions, for instance between the right atrium and the right ventricle. This workflow involves multiple-sliced SRT data and includes these steps: (1) input SRT data; (2) cluster the spots/locations; (3) assemble the slices; (4) manually select two regions of interest (ROIs); (5) generate a region-specific list of genes that can be used to calculate the top differentially expressed genes (Supplementary Table 3). To make the comparison fair for proprietary-data-only applications (Xenium Explorer, Loupe Browser, MERSCOPE Web Vizualizer), we used a standard “location by count” matrix as input, allowing all software to accept the input and progress to the next step. An application is given a score of 1
if it completes the step fully, 0 if not, and 0.5 if only completes partially (e.g., if it can only select 2D region in a 3D object) (Fig 5a, Supplementary Table 4). As this is a 3D-data scenario, only VR-Omics, VR-Cardiomics and STich3D can handle the data in its native 3D format (Fig. 5aiii), while the other applications only uptake individual slices. However, STich3D can align the slices (step 3) but not further steps (4 and 5), which were subsequently completed in the R programming environment. Our previous work, VR-Cardiomics can execute steps 3-5 (Fig. 6aiii), but users cannot easily provide their own input data without modifying the source code (steps 1-2). In brief, only VR-Omics successfully reveals significant biological pathways (via Metascape32) connecting the top differentially expressed genes between the right atrium and the right ventricle (Fig. 5aiv), upon completing all 5 steps of this biological case study (Fig. 5ai,5av)32.

Cross-SRT platform comparisons and performance

Only VR-Omics and SODBView20 offer unique comparative tools such as side-to-side comparison of two SRT slides (Supplementary Table 2). Both platforms are the only ones to support multiple SRT technologies, which gives the opportunity to directly compare datasets obtained from different technologies in the same framework (Fig. 5b). VR-Omics has the advantage of performing these comparisons locally the user’s own datasets. With the opportunity for analysis and visualisation of custom datasets generated across different platforms (Supplementary Table 2), users have the unique opportunity to explore and validate their experimental results given data from similar SRT platforms (Fig. 5b). This allows for investigation and better understanding of the level of resolution needed for a user to identify important cell clusters or signature within their tissue, thus better informing future experiments. VR-Omics is currently the only software that allows this direct comparison between user and public data, without being limited by the platform used for data generation; while ensuring the analysis performed across datasets is consistent.

Currently, VR-Omics is the only platform that support custom-datasets (Supplementary Table 2, Supplementary Fig. 2b). This expands on the capabilities of SODB as the user is not limited to what is provided by a matched database20.

Generation of SRT data will become more commonplace, leading more groups to sequence multiple sections in tandem for 3D data, and the datasets to grow as resolution improves and
costs decrease. Therefore, it is critical to have sufficient computational resources to support a fluid visualisation experience. To account for this, as a standalone application VR-Omics is currently the only software capable of leveraging the full power of GPU when available to the user (Supplementary Table 2). Other visualisation tools available through a web portal are hindered in their access to the additional power provided by the GPU through the limited capabilities of the web browser. This is dependent on VR-Omics being installed locally, to enable its ability to run across platforms for analysis and visualisation, and so is currently unavailable in a web portal format, requiring a small initial set up cost from the user.

In summary, the combination of AW, Visualiser features, 2D, 3D and VR interactions place VR-Omics at the forefront of user-friendly SRT data processing tools.

Methods

VR-Omics is a desktop application that can be downloaded at https://ramialison-lab.github.io/pages/vromics.html, and runs on a Windows 7 (SP1+) and Windows 10, 64-bit versions. Required graphics are API: DX10, DX11, and DX12-capable GPUs. Complete documentation on installation is available on the same GitHub page.

VR-Omics architecture

VR-Omics was developed using the cross-platform game development engine Unity, by Unity Technologies (version 2019.4.26f1). The Unity version was later updated to version 2021.3.11f1 (LTS) to fully support the VR toolkit plugin (XR Interaction Toolkit version 2.0.3)33,34. New scripts were written in Visual Studio using C# (Supplementary Methods).

The AW is implemented in Python (version 3.8.5). The spatial analysis using the AW is performed using the Python library Scanpy for Visium, and Squidpy12 for MERFISH and Xenium data (Supplementary Methods). To integrate the Python workflows in Unity, a single executable with all dependencies for each script was generated with PyInstaller v5.8.035. This allows the AW to be started the Unity environment. The AW comes with dependencies preinstalled and requires no additional installation or configuration of Python libraries. The AW is a standalone Python environment embedded into VR-Omics. Both applications share a common directory which is used to share data and outputs between the AW and Visualiser.
VR-Omics can optionally use GPU instancing to improve the performance while visualising the locations as a mesh. This way, we can visualise high resolution data such as the imaging-based datasets from MERFISH or Xenium. For development, an Alienware m15 R4 laptop with an Intel® Core™ i7-10870H CPU, 32.0 GB RAM, and a NVIDIA GeForce RTX 3070 graphics card were used. This setup is capable of visualising datasets with more than 350,000 locations in VR, while using the Visualiser features to interact with the data. With a device with no GPU, VR-Omics will automatically switch to CPU instancing, allowing the application to be used as desktop visualiser for non VR-compatible devices and datasets of lower resolution.

Platform-specific data processing
Sample data included with VR-Omics comprises of data generated from mouse, human and zebrafish tissues, across a variety of organs and includes both healthy and tumour tissues sections (Supplementary Fig. 2c). A wide-range of test datasets are provided to run the AW component from raw (Visium) or pre-processed files (Xenium, STOomics, Tomo-seq and MERFISH) (Fig. 1a,b) (Supplementary Fig. 2c).

Visium
Visium data is released in a standardised raw format after performing top end analysis of the spatial gene expression data using the software Space Ranger\textsuperscript{15}, which generates a feature/barcode matrix and aligned images used as the raw input for VR-Omics\textsuperscript{15}. In addition to loading datasets from the user’s local machine, publicly available and processed datasets can be accessed through the 10X Genomics website (https://www.10xgenomics.com/resources/datasets). The AW can be used to easily download those datasets directly within the application using the Scanpy\textsuperscript{16} toolkit (Fig. 1b). The datasets are often stored in a compressed h5 file format. Using the AW, the user can select from all publicly available datasets for download via VR-Omics. Using the Python environment, VR-Omics obtains a list of all available datasets from the 10X Genomics website accessible within Scanpy when the Python executable was generated. Downloading datasets via VR-Omics also ensures the right Space Ranger output files are downloaded to continue the spatial analysis using VR-Omics AW.

Visium datasets are commonly stored in a compressed HDF/h5 file format, which is not supported by Unity. Therefore, we implemented a HDF reader in Unity programmed in C# using
the Assembly netstandard and UTF-8 buffer encoding to allow reading variable length data. This allows VR-Omics to read SRT datasets commonly stored in an HDF/h5 file format.

The gene count per spot values are stored in a sparse matrix within the HDF file. Only expressed values are stored in an array like format with two pointers where one stores the original position of the expression value and one the end of each spot row. To read a sparse matrix, we implemented a sparse matrix reader in C#.

If the automated analysis tool (AW) was used to perform spatially variable gene analysis on the Visium data, in the Visualiser the resulting button to select a specific gene will be coloured in cyan to indicate that the gene was identified as spatially variable. Additionally, a table containing a list of all SVGs together with their respective p-values can be displayed (Supplementary Fig. 1d).

**Xenium**

The AW automatically processes Xenium datasets from 10X Genomics Xenium platform into a format readable by VR-Omics. The workflow was adapted to the first publicly available dataset of the Xenium platform that can be downloaded from the 10X Genomics website (https://www.10xgenomics.com/products/xenium-in-situ/preview-dataset-human-breast). The Xenium download folder contains the list with the panel of available genes within the dataset, the location meta data and a matrix (.mtx) file with the gene count information. VR-Omics uses the AW to perform the spatial analysis including filtering the data set according to the users input parameters, performing clustering using the Squidpy\(^{12}\) package and finally performs spatial analysis by calculating the Moran’s I Results.

The .mtx data format is not directly supported by Unity, therefore, VR-Omics uses the AW to translate the matrix file into a gene-count by locations .csv file. The Xenium output folder contains the cell feature matrix (.h5) file and a cells (.csv) file. These files serve as input for the VR-Omics AW and for spatial analysis. Moreover, the AW optimises the visualisation of the data by transposing the cell by gene counts file (.csv). Finally, the user can visualise the processed dataset from the Load Xenium context menu. This includes features to display the information unique to each location when selected in the visualiser, such as its coordinates and overall expression value (Supplementary Fig. 1a).
Publicly available data from Vizgen (vizgen.com/data-release-program/) was used to adapt the AW to support the MERFISH platform. To process MERFISH data, the AW requires three input files: cell by genes (.csv), cell metadata (.csv), and detected transcripts (.csv). Like the Xenium output, the MERFISH cell by gene count (.csv) file is transposed to enhance performance. Similarly, if the spatial analysis was performed on the Xenium or MERFISH data, the results of the Moran’s I statistics will be displayed once a gene is selected (Supplementary Fig. 1dii).

The VR-Omics AW for STOmics data is designed using publicly available datasets from the STOmics database (db.cngb.org/stomics). Sample data included with VR-Omics comprises of data generated from mouse, human and zebrafish tissues, across a variety of organs and includes both healthy and tumour tissues sections (Supplementary Fig. 2c).

The STOmics gene count data is stored in a compressed sparse matrix which can be read using the VR-Omics HDF and Sparse reader classes (Supplementary Methods, HDF and Sparse Matrix Reader). The data is commonly stored in a .h5 file which each location represented by a row and each gene expression value represented by a new column. Commonly in the .h5 files, the data is stored using one row for each location and each column representing a gene expression value. However, reading the gene expression value for one gene requires reading every location row to find the individual gene expression value. Hence, to improve performance, we use the VR-Omics AW to transpose the .h5 matrix. This way each row represents one gene allowing us to read a single row to find the gene expression values for each location.

Tomo-seq is an open-source available procedure and can be used to obtain spatial gene expression information of a sample using a grid-based dissection method. The resulting sequencing data is stored in three .csv files, each referring to the specific direction in which the sample was sectioned. For our provided example Tomo-seq data in VR-Omics, we precomputed the 3D spatial gene expression data from Junker et al. (Methods). VR-Omics takes the three .csv files of the different cutting directions, as well as a folder containing 3D reconstructed gene files as .txt files (Supplementary Fig. 2a). For the visualisation of Tomo-seq data, VR-Omics generates a grid based on the number of slices in each direction of the Tomo-seq data (anterior to
posterior, ventral to dorsal, and left to right). This creates a 3D cube-like grid of intersections. By overlaying this grid with a 3D bitmask of the sample, the 3D structure can be generated. Gene expression can be visualised using 3D reconstructed gene expression profiles, as described in the Tomo-seq protocol\(^3\). In addition to the visualisation of gene expression profiles, VR-Omics provides graphs showing expression values for each selected gene per slice (Supplementary Fig. 2a). This additional information of the gene expression for each of the sections aids in the interpretation of the visual gene expression patterns of the 3D model.

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**Custom data**

Other custom pre-processed data can be loaded and stored locally. Like longer-standing sequencing-based methods such as bulk RNA-seq, custom-data may be provided as a feature-by-location count matrix. A second matrix of coordinates is attached to the location dimension of the count matrix to represent spatial information. Imaging-based approaches typically consist of microscope images and signal quantification for specified probes. Custom data in that space can be transformed into gene-by-location count matrix and coordinates similar to sequencing-based technologies (Supplementary Fig. 2b).

**Immersive environment**

The VR-Omics Visualiser enables easy switching between a 3D visualised and HMD immersive environment and *vice versa*. If VR-Omics detects a valid HMD device switching between 3D and immersive modes is enabled by a single button press. Upon start of the application, VR-Omics will be launched as a 3D desktop application.

In Unity, a canvas serves as a container for UI elements, such as text, buttons, or panels. The canvas also determines how this interface is displayed in the final application. Typically, canvases are displayed in Screen Space Overlay, rendering them on top of the scene independent...
of the user’s field of view, which is represented as a camera in Unity. However, for VR use, the
canvas must be placed in 3D space instead to avoid obstructing the user’s view. Therefore, VR-
Oomics calculates the relative dimensions of the canvas to the user’s point of view and places the
canvas accordingly in 3D space.

To enable VR interaction, the fixed camera is replaced with an interactive user template.
Therefore, we instantiate a first-person prefab in the scene representing the user’s position and
point of view within the virtual environment. Additionally, a new behaviour is added to the
canvas detecting the interaction of the VR controller instead of the mouse input. Ray casting is
used to detect collisions between a ray generated from the VR controller in a linear path, and the
collisions between the ray and the SRT data or the canvas can be understood as user input.

In addition to modifying the user interface and camera, the SRT data must be adjusted to fit the
VR environment. When used as a desktop application, the SRT data is displayed within the user’s
field of view based on monitor dimensions. In VR mode, the dimensions must be translated
relative to the user’s position in 3D space and the canvas as a reference for the total data size. To
achieve this, we save the SRT data's relative positions of each location as an array of 3D vectors,
which are adjusted to the new relative distances in 3D space upon launching the VR
environment.

Data export

The user can create screenshots and recordings within the Visualiser (Supplementary Fig. 1e).
These features are useful for collaboration and for creating material for publications and
presentations. VR-Oomics also provides several customisation options such as changing the
background colour, changing the symbol representing the locations as well as their size.

Differential gene expression analyses

Differential gene expression analysis was performed in R v.4.2.2\textsuperscript{36} using the \textit{voom/limma}
packages\textsuperscript{37} between right atrium and right ventricle spots in the human embryonic heart at 6 dpc
dataset\textsuperscript{31}. The sets of differentially expressed genes (fold-change $> 2$, FDR-adjusted p-value $<$
0.05) were processed for pathway enrichment using Metascape\textsuperscript{32} with default parameters. 3D
visualisation figures were generated using the \textit{rgl} R package\textsuperscript{38}. 

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\textsuperscript{26} Data export

\textsuperscript{36} Differential gene expression analyses
Discussion

VR-Omics is an application suited for different SRT data technologies, enabling users unfamiliar with computational workflows to analyse, visualise and explore their data in a user-friendly 3D desktop interface or virtual reality. Based on the exponential growth of SRT based publications in the recent years, it is expected that the number of datasets and algorithms for SRT data analysis will follow a similar pattern. The supplied Python environment enables VR-Omics to incorporate any publicly available Python modules dedicated for the processing of SRT data. The modular nature of the VR-Omics architecture facilitates the rapid incorporation of new SRT platforms such as Nanostring (GeoMX and CosMX), BayesSpace or STAGATE for clustering and PASTE for improved alignment of 3D slices.

VR-Omics’ AW is a powerful processing and analytical pipeline which allows users to perform data processing and analysis in an intuitive GUI that is customable without requiring extensive computational knowledge. VR-Omics supports standard SRT files generated by the respective technologies or custom SRT data as raw input. We envision expanding the AW to include pre-processing pipelines for data from SRT platforms beyond Visium, Xenium and MERFISH (e.g., Stereopy package for STOmics and a cell segmentation pipeline for image based technologies). While VR-Omics supports the analysis and visualisation of datasets generated by both sequencing and imaging-based methods, some features such as cell segmentation and visualisation of cell boundaries for imaging-based methods are currently unavailable. While packages developed specifically for visualisation of imaging-based methods offer these features (Supplementary Table 2), VR-Omics will incorporate these features in future releases.

VR-Omics’ Visualiser is a user-friendly interface that allows users to navigate through the datasets (Fig. 3). VR-Omics provides several unique overlay options for various data types (e.g., H&E staining, cluster visualisation, 3D model). In time, more features will be added to the Visualiser such as full gene correlation pre-processing analysis, to enable real-time differential gene expression exploration.

The space of SRT data analysis urgently requires 3D applications to align, visualise and process multi-sliced data. To the best of our knowledge, only STich3D and our previous work VR-Cardiomics have this 3D capability. However, both lack certain features such as no loading of user’s own data for VR-Cardiomics and generation of region-specific gene lists required in a
biological case study. Thus, we have developed VR-Omics to bridge this gap, allowing users to interact with their 3D data in an intuitive manner.

Finally, the use of VR improves natural understanding of 3D data, reduces artifacts when 3D data is displayed on a 2D desktop, and improves data interaction with 3D data by enabling higher accuracy and better understanding. We plan to expand support to the HoloLens family of HMD from Microsoft thus providing augmented reality features as well.

VR-Omics is designed from the ground up to be SRT platform agnostic and aims to enable users to perform cross-platform multi-omics spatial analyses. As the user base for these technologies continue to expand, so will the need for appropriate tools for mining and visualisation, to ensure the data most accurately captures the underlying biology to drive novel insight. The VR-Omics platform is open-source, modular and continually maintained, making it well-placed to achieve these functions for the spatial biology community.

**Figures**

**Figure 1**

**Figure 1: VR-Omics architecture.** a, VR-Omics will accept as input sequencing and imaging-based SRT data of different technologies. b, The automated workflow (AW) processes, filters and performing spatial analysis and producing output files. c, Data can be explored using VR-Omics as a 3D Visualiser in a virtual environment or desktop application as shown here with human embryonic heart at 6 dpc. d, Output files, screenshots and video recordings are generated from the Visualiser.

**Figure 2**

**Figure 2: Automated Workflow.** a, Data processing and filtering. Accepted SRT data types for analysis, processing and filtering using the AW include Visium, MERFISH and Xenium. Analysis steps available to all SRT data types and menu with examples of available visualisations after data analysis. On the right, an example of heatmap of read counts generated for invasive ductal carcinoma breast tissue data generated with Visium from 10X Genomics. b, Leiden clustering analysis and visualisation of clustering output using Visium data of the human cerebral cortex. c, Identification of SVGs is performed using SpatialDE for Visium data and...
by calculating Moran’s I statistic for MERFISH and Xenium. d, Examples of output files generated by AW workflow with example data from coronal mouse kidney section generated by Visium\textsuperscript{47}. e, The user interface allows for alignment of multiple, serial Visium slides (e\textsubscript{i}) uploaded in tandem (e\textsubscript{ii}) so they can be combined into a 3D object (e\textsubscript{iii}), demonstrated here with data from the human embryonic heart at 6 dpc\textsuperscript{31}. The tissue images are used for reference.

**Figure 3**

**Figure 3: Features of the Visualiser in VR-Omics.** a, Gene search. a\textsubscript{i}, Gene search bar options for visualisation of selected gene expression patterns across a tissue. a\textsubscript{ii}, A scale of gene expression can be visualised with a colour map indicating the relative expression levels of the selected gene at each location. The colour map uses a blue to red gradient, with lower expression levels corresponding to colours on the blue end of the spectrum and higher expression levels corresponding to colours on the red side. The image represents the *EPCAM* gene expression in a breast tumour section (Xenium)\textsuperscript{6}. a\textsubscript{ii}, Binary expression of the gene can be visualised, displayed here for *EPCAM* using a Xenium breast tumour section\textsuperscript{6}. b, Example of orientation of H&E image overlay, displayed with coronal mouse Visium brain slice\textsuperscript{46}. The Visualiser allows the user to adjust the opacity of the H&E image, displayed with coronal mouse Visium brain slice\textsuperscript{46}. c, 3D model overlay. c\textsubscript{i}, Required inputs for 3D model overlay. 3D-model can be overlayed over single or multiple SRT slides in the Visualiser then aligned and orientated appropriately. c\textsubscript{ii}, Example of 3D model overlay displayed here with data from the developing human heart\textsuperscript{27}. c\textsubscript{iii}, Accepted file formats for 3D model. c\textsubscript{iv}, Alignment movements available for 3D model in the Visualiser. d, Selection of ROIs throughout slices and output. d\textsubscript{i}, User-defined selection of ROIs within 2D slide with option to select same ROI across serial 3D sections or to select up to 4 ROIs within an object. The information of selected ROIs can be exported to csv files then re-imported for subsequent analysis. d\textsubscript{ii}, Visualisation of clusters in MERFISH adult mouse brain\textsuperscript{47} and zoomed individual clusters. When serial slices or 3D objects are available, up to 4 separate ROIs can be selected across serial slices for export allowing subsequent, in-depth analysis. e, Side-by-side comparison. e\textsubscript{i}, Side-by-side feature allows comparison of two different gene profiles within identical copies of the same section for comparison of marker genes. e\textsubscript{ii}, Side-by-side comparison displayed using a Xenium breast tumour section\textsuperscript{6}. Expression of *EPCAM* is displayed in the left panel and expression of *ELF3* in the right panel. f, After side-by-side comparison has been selected, deselecting this option will ask the user to merge the slides. This creates a
comparison of gene expression within the tissue section. Areas with more similar expression between the two genes will be displayed in cool colours, with areas of difference in warm colours. Example data displayed here is Visium human lymph node\textsuperscript{27}.

**Figure 4**

**Figure 4: Data analysis in virtual reality.** a, Visium breast cancer tumour section\textsuperscript{44} visualised in VR coloured by regions of high read counts\textsuperscript{47} b, MERFISH adult mouse data\textsuperscript{47} displayed in VR, coloured by normalised gene expression with areas of low expression coloured in blue and regions of higher expression in red. Additional information for selected spots will be visualised on the panel on the right-hand side. c, Xenium breast tumour section\textsuperscript{6} visualised in VR and coloured by Leiden clustering results. d, Example of 3D object overlay of serial sections in VR for data of human embryonic heart at 6 dpc\textsuperscript{31}.

**Figure 5**

**Figure 5: Cross-platform comparisons.** a, Benchmarking of 3D-datasets. ai, Data from human embryonic heart at 6 dpc\textsuperscript{31} visualised in VR in VR-Omics with spots corresponding to the right ventricle highlighted in red and spots corresponding to the right atrium in blue. aii, Data from human embryonic heart at 6 dpc\textsuperscript{31} aligned and in STich3D. Selection of specific ROIs to highlight the right atrium in black and right ventricle in red performed by a custom R script outside of the STich3D framework. aiii, Example of mouse heart displayed in VR-Cardiomics\textsuperscript{21}, right atrium displayed in blue and right ventricle displayed in red. aiv, Metascape\textsuperscript{32} results using the differential expressed genes (fold-change>2, FDR-adjusted p-value < 0.05) between the right ventricle and atrium. av, Radar plot displaying performance of benchmarked tools on performing the following steps: (1) input SRT data; (2) cluster the spots/locations; (3) assemble the slices; (4) manually select two regions of interest (ROIs); (5) generate gene lists to calculate the top differentially expressed genes. b, Comparison of a coronal mouse brain sections generated separately by the bi, Visium\textsuperscript{46} and bi, MERFISH\textsuperscript{47} platforms.
**Supplementary information**

**Supplementary Figure 1.**

**Supplementary Figure 1: Location analysis features. a,** By selecting a specific spot additional location-specific information can be displayed and exported as a text file. **b,** Within the Visualiser, seamless zoom functionality allows the user to more closely inspect particular areas of the tissue, such as in the MERFISH adult mouse data. **c,** After searching and selecting for a gene, a minimum expression threshold can be set to only display spots with a normalised expression above set threshold. Displayed here for Xenium breast tumour section. **ci,** scale of expression with cooler colours denoting lower expression and warmer colours denoting higher expression. Displayed here with gene STC1. **cii,** Alternatively once the threshold has been set, expression of a gene such as HARCR2 displayed here, can be displayed in a binary manner as being ‘on’ or ‘off’ above the threshold. **d,** SVG display. **di,** Statistically significant SVGs will appear in the search blue highlighted in blue and a table containing additional information of all identified SVGs can be accessed in the Visualiser, displayed for the coronal mouse Visium brain slice. **dii,** Example of additional information related to SVGs identified by Moran’s I statistic for Xenium and MERFISH data displayed for Xenium breast tumour section. **e,** Selection of screenshot function within the Visualiser for export of .png files, displayed here with 3D data from human embryonic heart at 6 dpc.

**Supplementary Figure 2.**

**Supplementary Figure 2: Additional formats. a,** Visualise Tomo-seq data. **ai,** Visualisation of zebrafish embryo and axes for sectioning taken from Junker et al 2014. **aii,** Example of output data files of Tomo-seq data for upload into VR-Omics. **aiii,** Tomo-seq data visualisation using data from Junker et al 2014. **aiiv,** Expression values of a gene from a single direction of sectioning. **b,** Custom data upload. **bi,** Example of format supported for custom data upload and example of processing and visualisation. **bii,** Data from coronal mouse kidney section generated by Visium visualised here. **c,** Available sample datasets that are available within VR-Omics and available for download at the Monash Bridges platform (https://doi.org/10.26180/22207579.v1).
Supplementary Table 1: Overview of all features supported by VR-Omics indicating for which SRT method they are available.

Supplementary Table 2: Comparison of VR-Omics with commonly used visualisation tools for each of the SRT methods supported by VR-Omics.

Supplementary Table 3: List of differentially expressed genes between the right ventricle and right atrium identified in the human embryonic heart at 6 dpc\textsuperscript{31} by VR-Omics.

Supplementary Table 4: Benchmarking use case comparison and scoring between VR-Omics, STich3D, VR-Cardiomics, Loupe Browser, Xenium Desktop Browser, MERSCOPE Web-Vizualizer, Stereopy and SODB.

Supplementary Methods.

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Declarations

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Competing interests

The authors declare the following competing interests: MR, NC, ERP and DAE are recipients of the STOmics Grant Program awarded by BGI. The other authors declare no competing interests.

Ethics approval

N/A

Consent to participate

N/A

Consent for publication

All authors consent on the publication.

Availability of data and materials

For development of VR-Omics publicly available data was used. The Visium data from 10X Genomics is available at the 10X Genomics website: https://www.10xgenomics.com/resources/datasets. The 10X Genomics Xenium dataset is available under: https://www.10xgenomics.com/products/xenium-in-situ/preview-dataset-human-breast. The STOmics database is available at: https://db.cngb.org/stomics. The Vizgen MERFISH data release program can be accessed via: https://vizgen.com/data-release-program/. The Tomo-seq data is available via their publication https://doi.org/10.1016/j.cell.2014.09.038 which also contains the MATLAB code for the 3D data reconstruction. The Visium demo was
adapted from Asp et al. and can be accessed via the related publication\textsuperscript{31} or at
https://data.mendeley.com/datasets/zkzyprdr5z/1.

The demo datasets generated for VR-Omics can be found at:
https://doi.org/10.26180/22207579.v1. The 3D Visium data set of the human developing heart
adapted from Asp et al. can be found within the application and can be accessed from the main
menu following the Visium, Demo context menu.

**Code availability**

The complete standalone version of VR-Omics (containing Python AW and Visualiser) can be
downloaded at https://ramialison-lab.github.io/pages/vromics.html or at
https://doi.org/10.26180/20220312.v1. Alternatively, the code is available at GitHub
(https://github.com/Ramialison-Lab/VR-Omics). To use the GitHub version an installation of
Unity Gaming Engine (version 2021.3.11f1) is required. This version does not include the
Python AW. The Python AW can be accessed at: https://doi.org/10.26180/22207903.v1. More
information of run VR-Omics via Unity can be found in the full documentation accessible at

**Authors’ contributions**

D.B, M.R., F.S., K.K., H.T.N. conceived the study. D.B. implemented the system with
contribution from N.C.. D.G. and D.B. implemented the VR integration and worked on the data
visualisation. S.J.-H. implemented the Python environment for the AW based on the scripts from
N.C. and D.B., N.C. compared the system with available state-of-the-art software, and
contributed to the design of the visualisation features. D.B. created the documentation website.
D.B, S.J.-H., D.G., K.K. and F.S. designed the framework from the computational side. D.B.,
N.C., H.T.N. and M.R. designed the framework from the biological side. M.R., F.S., H.T.N. and
K.K. supervised this study. D.B. and N.C. drafted the manuscript with contributions S.J.-H.,
H.T.N. and M.R. All authors contributed intellectually to the interpretation of the results. All
authors provided critical feedback and helped shape the research, analysis, and manuscript. All
authors read and approved the final manuscript.
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46. Mouse Brain Section (Coronal) - 10x Genomics. https://www.10xgenomics.com/resources/datasets/mouse-brain-section-coronal-1-standard-1-0-0.

48. Mouse Kidney Section (Coronal) - 10x Genomics.
a) Data Filtering
- Visium
- MERFISH
- Xenium

- Filter and processing
- Gene and read count analysis

b) Cluster Analysis
- Leiden cluster analysis using Python

Cluster saved for later analysis

Spatial Variable Genes
- Visium
- MERFISH
- Xenium

Visium: Spatially variable genes analysis (SpatialDE).
Xenium, MERFISH: Moran Results (MR)

View results in the Visualiser
Quick access SVGs in Visualiser
SVGs / MR saved for later analysis

d) AW Output
- Sample Output
- Figures
- Files

- Spatial plots:
- Histogram total counts
- UMAPs

- Compressed h5 output
- Count matrix
- Log files

e) Create 3D Datasets From Multiple Slides

Create and explore 3D datasets using the VR-Omics Visualiser features

Figure 2 - Bienroth et al.
Figure 3 - Bienroth et al.
Figure 5 - Bienroth et al.