









REVIEW

A Hitchhiker's guide through the bio-image analysis software universe

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Modern research in the life sciences is unthinkable without computational methods for extracting, quantifying and visualising information derived from microscopy imaging data of biological samples. In the past decade, we observed a dramatic increase in available software packages for these purposes. As it is increasingly difficult to keep track of the number of available image analysis platforms, tool collections, components and emerging technologies, we provide a conservative overview of software that we use in daily routine and give insights into emerging new tools. We give guidance on which aspects to consider when choosing the platform that best suits the user's needs, including aspects such as image data type, skills of the team, infrastructure and community at the institute and availability of time and budget.

Keywords: bio-image analysis; open-source; software

Scientific bio-image analysis software plays a key role in modern life sciences [1]. New insights are virtually impossible without computational methods for image acquisition, processing, segmentation, feature extraction and visualisation. In the past decade, biologists have increasingly applied statistical data analysis of imaging data and machine learning for image processing and particularly for image segmentation, as these allow overcoming the limitations of purely descriptive

methods. We also perceive that tools and methods are converging: if a single software platform provides image processing, feature extraction, statistical analysis and visualisation, it is superior and preferred to software that is only good at one of those tasks, at least from a user's perspective. Possible applications are highly diverse and spread across multiple sub-disciplines such as developmental biology, cancer research, immunology, cell and molecular biology,

Abbreviations

ANT, advanced normalisation tools; cryoEM, cryo-electron microscopy; CSV, comma-separated values; GUI, graphical user interface; ITK, Insight Toolkit; JSON, JavaScript object notation; NEUBIAS, Network or European Bio-Image Analysts; OpenCV, open computer vision; RELION, Regularised Likelihood Optimisation; XML, extensible markup language; YAML, yet another markup language.

biophysics, agronomy, bioengineering and biomaterials. Often software solutions are created to address a particular analysis challenge in one of those sub-disciplines. As it becomes increasingly hard to keep an overview of existing software, corresponding key applications and targeted scientific questions, we provide a detailed overview of current state-of-the-art software, upcoming next-generation tools and give hints as to which aspects to consider when deciding among the many available software solutions for current bio-image analysis questions.

An early career scientist searching for the right software for their image analysis might have the hardest decision to make. Even if most of them know search engines specialised for bio-image analysis software such as <https://biio.eu> or <https://bio.tools> [2,3], for beginners in the field it is hard to make any decisions as they often do not know the right search terms yet. Hence, the glossary provided below may be a good starting point to get an overview of available software and related use-cases. Furthermore, we recommend attending institutional image-analysis courses, for example, for PhD students in their first year. In addition, getting in touch with senior scientists in their own group, with collaborators and local light or electron microscopy facilities is a good opportunity to find out which software is used in similar projects on campus.

Glossary

Inspired by Adams and Lloyd [4] we give an overview of the bio-image analysis software universe by means of a glossary of software routinely used by bio-image analysts. To further classify those software-related terms, we refer to previously defined groups of software [5]: Firstly, image/data analysis algorithms provided in a sustainably reusable fashion are referred to as ‘components’. Secondly, software libraries and standalone applications that combine multiple components are ‘collections’. Thirdly, software that combines multiple components, potentially from multiple collections to solve a given variety of image analysis questions in a standardised form are referred to as ‘workflow templates’. If the software is specific for solving particular scientific questions using given components in one specific assembly, these are called ‘workflows’. We extend this classification with ‘frameworks’ of scientific software which are collections upon which many other software solutions are built. We add ‘programming languages’ that allow assembling components into workflows. We furthermore categorise the presented software in additional categories such as open-source, free of charge and major application categories such as

acquisition, registration, segmentation and statistical analysis in Table S1. The table also contains properties of the listed software such as preferred dimensionality of input image data and typical imaging modality. The software in the following were selected to reflect long-term available, reliable, sustainably maintained and supported software solutions. We analysts often have to have a conservative perspective on existing software as we need to rely on established, reliable and maintained software to build workflows for our collaborators and trust the given software to be still available in 5–10 years allowing reproducible image data analysis. To this end, the number of citations served as a criterion to select software but we also considered software packages that have been available for about 5–10 years with continuous maintenance and reliable support by a vivid community. A less formal criterion that we applied for selecting software was considering the knowledge of which tools would have allowed a starting bio-image analyst to follow a conversation at one of the Network of European Bio-Image Analysts (NEUBIAS) meetings. The given description of the glossary items highlights the main application of the software and its relationships with other glossary items. While the glossary focuses on general software and terms used in the field, this should not hide the large number of software or plugins developed for a specific task or context. In the domain of plant science, the quantitative plant initiative [6] proposes a curated list of software solutions that may also be useful to know. Similarly, in the microscope hardware and control oriented context, a list of useful software was published [7]. Also for lightsheet microscopy, there is a specific list of software for acquisition and analysis available [8]. There are a large number of software tools and applications that have been specifically developed for the cryo-electron microscopy (cryoEM); a comprehensive list of software for the cryoEM community can be found from wikibooks [9].

3D IMAGEJ SUITE [10] is a collection of IMAGEJ plugins for filtering, segmentation and analysis of geometry, shape and spatial organisation of objects in 3D images.

3D SLICER [11] is an image processing software based on the ITK library focused on medical imaging with 3D surface extraction, rendering and analysis capabilities. It is increasingly used for visualising and analysing 3D structures such as cells, tissues and organs in microscopy data.

ANTS [12], or Advanced Normalisation Tools, is a collection of methods for image registration, segmentation, and analysis, mostly developed in the context of neuro-imaging and the comparison of cohorts. ANTS

depends on ITK, an image processing library to which ANTs developers contribute.

ARIVIS VISION 4D (Arivis AG, Rostock, Germany) is an image analysis software for processing multi-channel 2D, 3D and 4D data, focused but not limited to microscopy data. It is scalable, supports processing big image data, and has intuitive image stitching and alignment tools.

AMIRA-AVIZO (Thermo Fisher Scientific Inc., Waltham, MA, USA) is a 2D–5D image processing, visualisation and analysis software. It can be customised using PYTHON and MATLAB and offers additions for incorporating artificial intelligence.

BIGDATAVIEWER [13] is an n -dimensional image viewer component for slicing volumes in arbitrary directions. The FIJI plugin can handle terabyte-sized image data composed of multiple channels and time points.

BIGSTITCHER [14] is an automated and interactive image registration/fusion FIJI plugin capable of handling terabyte sized image data. It is based on the BIGDATAVIEWER.

BIOFORMATS [15] is an image file format interoperability library which serves multiple image analysis software applications such as FIJI, OMERO and QUPATH, and programming environments such as MATLAB, JAVA and PYTHON to load image data from many formats and vendors.

BLENDER [16] is a 3D surface rendering, modelling and visualisation software with PYTHON scripting, simulation and video editing capabilities. The home of BLENDER is in design and arts, and it is increasingly used for microscopy image data visualisation.

BONEJ [17] is a collection of image processing operations and IMAGEJ plugins for skeletal/bone image analysis. It is used often in the soil, food and materials science communities. Some of the tools were updated to work with IMAGEJ2.

C/C++ are programming languages traditionally used in computing. Most operating systems are programmed in C and C++. Furthermore, many PYTHON and also some JAVA libraries contain components and collections of processing routines written in these languages because C and C++ offer higher performance.

CATMAID [18] is a web application to navigate, share and collaboratively annotate massive volume image data sets.

CCP-EM [19] the Collaborative Computational Project for electron cryo-microscopy is a community guiding the users of CRYO-EM software tools as well as developers of software packages and file formats.

CCPI [20] the Collaborative Computational Project in Tomographic Imaging provides a collection of

software tools for tomographic imaging and reconstruction.

CELLPOSE [21] is a deep-learning based segmentation algorithm for biological structures such as cell and cell nuclei in microscopy images. It is accessible as a PYTHON library and standalone application. CellPose plugins exist for CELLPROFILER, QUPATH and FIJI.

CELLPROFILER [22] is an image analysis software application with graphical user interface (GUI) for user-friendly configuration of standardised image analysis workflows focusing on high-throughput microscopy imaging data of cells with capabilities for extracting tabular image feature data in high-performance-computing environments.

CELLPROFILER ANALYST [23] is a data exploration software for further visualisation and analysis of tabular data produced with CELLPROFILER. It offers advanced plotting, dimensionality reduction and machine learning based object classification for dealing with big data as it is common in pharmaceutical research.

DECONVOLUTIONLAB2 [24] is a collection of image deconvolution algorithms accessible as standalone command-line interface and as user-friendly IMAGEJ plugin.

DRAGONFLY (ORS, Montréal, QC, Canada) is a powerful standalone software featuring an extensive set of tools for image processing, segmentation and 3D visualisation.

DRISHTI [25,26] is a visualisation tool for 3D pixel data, which has been extended with segmentation and measurement tools.

ELASTIX [27] is a standalone command-line tool for registration of 2D and 3D image data based on the ITK library. A PYTHON compatible interface, SimpleElastix, is available as well.

EMAN2 [28] is a software application focusing on cryoEM, covering techniques such as single particle analysis, cryo-electron tomography or sub-tomogram averaging.

FAIRSIM [29] is an IMAGEJ plugin for reconstructing structured illumination microscopy super-resolution images from raw data.

FIJI [30] is an image analysis software based on IMAGEJ and a collection of IMAGEJ- and IMAGEJ2 compatible plugins focusing on general-purpose image analysis in the life sciences. It is scriptable using multiple programming languages compatible with the Java ecosystem, extensible and capable of handling big image data through integration of components such as IMGLIB2 and BIGDATAVIEWER.

GROOVY [31] is a scripting language that can be used for automating image analysis routines in QUPATH and FIJI.

GWYDDION [32] is a modular program for scanning probe microscopy data visualisation and analysis, primarily focused on the analysis of altitude maps such as obtained by atomic force microscopes.

HUYGENS (Scientific Volume Imaging B.V., Hilversum, the Netherlands) is an image processing software dedicated to deconvolution of 3D stacks from fluorescence microscopy, potentially multi-channel and time-lapse data.

ICY [33] is an image analysis software focusing on general purpose image analyses in the life sciences compatible with **IMAGEJ**. **ICY** is scriptable using Java-Script and a visual programming approach using so called protocols.

ILASTIK [34] is an image analysis software offering easy-to-use machine learning capabilities for image segmentation, object classification, object tracking and statistical analysis of microscopy image data. **Ilastik** classifiers can be used from **FIJI** and **CELLPROFILER**. Furthermore, it supports execution on high-performance-computing clusters.

IMAGEJ [35] is an image analysis software and framework for image analysis algorithms integrated in **FIJI**, **ICY**, **MICROMANAGER**, **QUPATH** and others. We conservatively estimate tens or hundreds of thousands of plugins and scripts have been developed in its 20+ year history making it one of the most important platforms for image analysis in the life sciences.

IMAGE J2 [36] is a modern rewrite of the **IMAGEJ** codebase with focus in scientific image processing and analysis of big image data. It serves as an extensible platform underlying **FIJI** and other software platforms in the life sciences.

IMAGEJ MACRO [37] is a limited programming language specific to the **IMAGEJ** platform useful for automating image processing routines.

IMAGE.SC [38] is an online discussion forum based on the Discourse platform [39] that serves as a questions and answers forum for many open source projects from the image science field. It plays a key role in knowledge exchange and community support for many open source bio-image analysis software projects. See Fig. 1 for a list of community partners.

IMARIS (Oxford Instruments, Oxon, UK) is an image processing and visualisation software supporting 3D volume rendering and quantitative analysis. Through extra modules it is interoperable with **FIJI**, **PYTHON** and **MATLAB**.

IMGLIB2 [40] is an image processing framework and collection of algorithms. It is the basis for software such as **BIGDATAVIEWER**, **BIGSTITCHER**, **IMAGEJ2**, **FIJI**, **KNIME** and others to handle terabyte-sized big image data.

IMOD [41] is an image processing, modelling and visualisation software collection for electron microscopy. Aside from command line tools for image processing, it offers a GUI for reconstruction, registration and segmentation of data.

ITK [42] is an image registration and segmentation algorithm collection and library with a long history in medical imaging. It is the underlying framework for tools such as **3D SLICER**, **ELASTIX**, **ANTs**, and **ITK SNAP**.

ITK SNAP [43] is a software application specifically for segmentation and surface rendering of 3D medical imaging datasets based on **ITK**.

JAVA is the programming language **ICY**, **IMAGEJ**, **FIJI**, **QUPATH** and compatible plugins are written in. It is also interoperable with **IMARIS** and **MATLAB**.

JAVASCRIPT is a scripting language used for automation of image analysis routines in **ICY**, **IMAGEJ** and **FIJI**. It is also the most popular web programming language world wide [44].

JUPYTER NOTEBOOKS [45] is an interactive, cloud compatible programming environment suitable for image data analysis, statistics and scientific plotting. It is a key component for reproducible data science in the scientific **PYTHON** ecosystem and is extensively used for documentation and training.

JYTHON is a Java-compatible scripting language based on the syntax of **PYTHON 2**. It can be used for automation of image analysis routines in **FIJI** but is technically not compatible with **NUMPY**, **SCIPY**, **SCIKIT-IMAGE** and other **PYTHON**-based libraries. It is compatible with Java-based components.

KNIME [46] is a visual and interactive programming environment focusing on data science with image analysis and machine learning capabilities. Its image processing capabilities are based on **IMAGEJ**, **IMAGEJ2**, **SCIJAVA** and **IMGLIB2**.

KNOSSOS [47] is an image visualisation and annotation software for large connectomics (electron microscopy) data extensible using **PYTHON** modules.

LEICA APPLICATION SUITE X (Leica Microsystems GmbH, Wetzlar, Germany) is a software for microscope control, image acquisition, visualisation and analysis. It offers modules for computational clearing and deconvolution (lightning), Fluorescence lifetime, FRET, and FCS analysis, CARS calculations, 2D and 3D measurements.

MATLAB (Mathworks, Natick, MA, USA) is a software environment for numeric computing that provides a multi-paradigm programming language and a number of dedicated applications and toolboxes, for example, for image processing, computer vision, statistics and machine learning. It can be extended using Java and Python libraries.

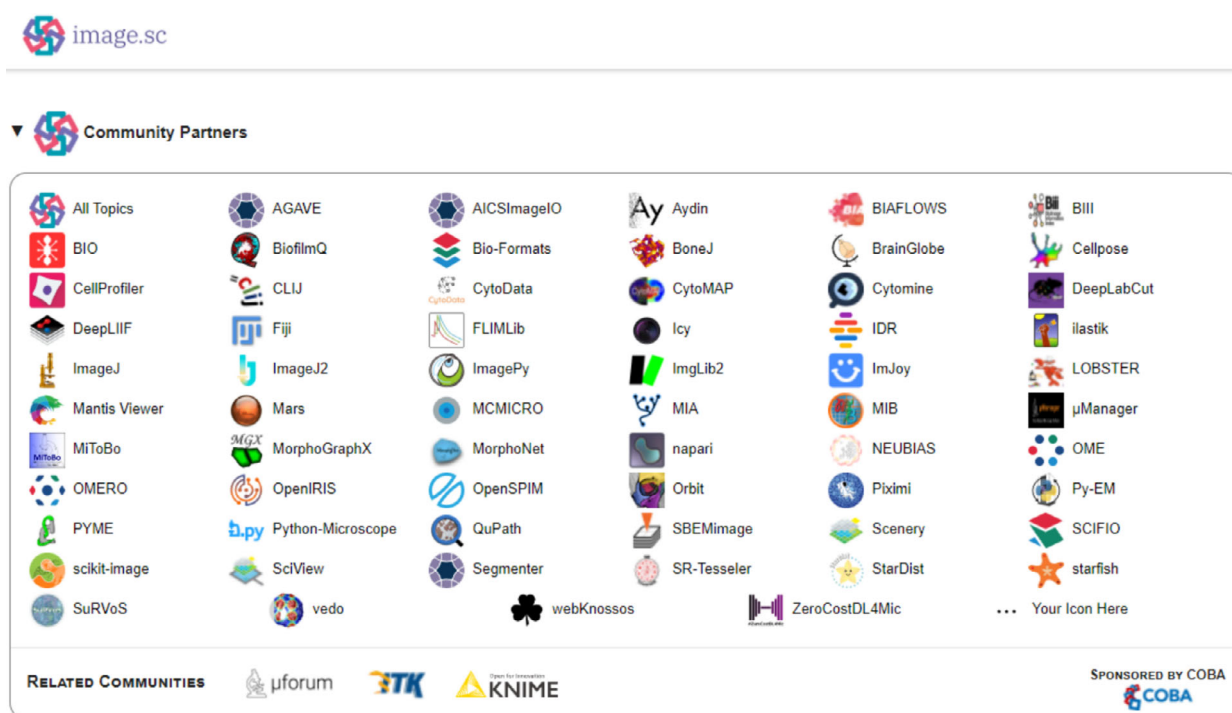


Fig. 1. Screenshot of the image.sc forum in April 2022 showing the logos of the community partners and related communities. Listed open-source projects provide online support for their software on this platform, which might be a key criterion when deciding which software to use.

MATPLOTLIB [48] is a scientific plotting and image visualisation collection commonly used for image data science by the **PYTHON** community.

MICROMANAGER [49] is a microscope control software with built-in image processing capabilities based on **IMAGEJ**. It can be scripted using the BeanShell language and recently using **PYTHON** [50].

MICROSCOPY IMAGE BROWSER [51] is a **MATLAB**-based software for advanced image processing, segmentation, quantification, and visualisation of multi-dimensional light and electron microscopy datasets. It works with **BIOFORMATS**, allows batch processing operations and can be directly linked to **Fiji**.

MORPHOGRAPHX [52] is a software for visualisation and analysis of 4D datasets. It focuses on the analysis of organ growth from 4D live-imaging confocal data of plants. Various algorithms implemented in **MORPHOGRAPHX** extract surfaces from 3D data and post-process the intensities along those surfaces, which can be seen as an efficient 2.5 dimensional approximation of 3D quantification.

MORPHOLIBJ [53] is a collection of methods and plugins for **IMAGEJ** implementing mathematical morphology operations such as dilation, opening, watershed and

reconstruction as well as methods for quantitative analysis of label images.

NANOJ [54] is a toolbox of **IMAGEJ** plugins for super-resolution microscopy processing and analysis tasks, including drift correction and channel registration. It also incorporates the widely used **SRRF** method for live-cell super-resolution image reconstruction [55].

NEURONJ [56] is an **IMAGEJ** plugin for neurite tracing and analysis.

NIS-ELEMENTS (Nikon, Tokyo, Japan) is a software for microscope control, computer-assisted image acquisition and analysis. It integrates artificial intelligence solutions for de-blurring, segmentation and image restoration. Image analysis components can be combined to a workflow within a visual programming environment.

NUMPY [57] is a **PYTHON** library and a collection of efficient array processing algorithms. It is among the most used **PYTHON** libraries in the world [58] and the basis for many image processing components and collections in the **PYTHON** ecosystem.

PARAVIEW [59] is a software for vector and surface data analysis and visualisation based on the **ITK** library.

PYTHON is a programming language, potentially the most popular language in science and surely among the top used programming languages in general [44]. It is commonly used to assemble various image processing, data analysis and visualisation libraries in scientific workflow.

ORIENTATIONJ [60] is an **IMAGEJ** plugin to characterise the orientation and isotropy properties of regions of interest in images.

OMERO [61] is a research data management solution for microscopy image data. It was initially developed to facilitate analysis of large amounts of high-throughput imaging data. **OMERO** can be used as a remote-server storing image data that is highly interoperable with other software such as **CELLPROFILER**, **FIJI** and **QUPATH**.

OPENCV [62] (open computer vision) is a collection of image analysis components that includes several hundred computer vision algorithms. **OPENCV** focuses on 2D+time imaging data acquired with video cameras and has also many applications in microscopy.

QUPATH [63] is an image analysis software for quantitative pathology. It allows visualisation and analysis of large 2D slide scanner imaging data of histological slices. Its user-friendly GUI offers tools for manual annotation, machine-learning based tissue classification and deep-learning based cell segmentation. It is extensible using **JAVA**-based plugins and scriptable using the **GROOVY** programming language. It is interoperable with **OMERO** and **BIOFORMATS**.

R [64] is a programming language for statistical computing and plotting. It is commonly used for the downstream statistical analysis of the output of image analysis packages. **R**-packages also exist for image processing [65].

RELION [66], or **REgularised Likelihood Optimisation**, is a software package for cryo-EM structure determination processing data from single particle or tomography experiments.

RSTUDIO [64] is a standalone application allowing interactive programming using the **R** language. Users can view existing variables, manipulate tables and plots.

SCIJAVA [67] is a collection of image analysis data structures and algorithms such as **IMGLIB2** and serves as the basis for **IMAGEJ2**.

SCIKIT-IMAGE [68] is a general purpose collection of scientific image analysis algorithms based on **NUMPY** and **SCIPY**. Image analysis workflows using **scikit-image** can be written in **PYTHON** and it is commonly used with **JUPYTER NOTEBOOKS**.

SCIKIT-LEARN [69] is a collection of **PYTHON**-based algorithms for machine learning commonly used in the

context of image for pixel, object and image classification.

SCILS (Bruker, Billerica, MA, USA) is a software for analysis of mass-spectrometry imaging (MSI) data, including machine learning algorithms and tools for visualising ion images and mass spectra.

SCIPY [68,70] is a collection of algorithms for scientific data processing, simulation, optimisation and analysis. It serves as the basis for other software such as **SCIKIT-IMAGE**.

SERIALEM [71] is an acquisition software for a variety of transmission electron microscopes. It provides different means of automation through navigation, a built-in scripting language and **PYTHON** integration. Typical applications are electron tomography, large areas for 3-D volume imaging from serial sections or single-particle cryoEM.

SINGLE NEURITE TRACER [72] is a **FIJI** plugin for processing three-dimensional, multi-channel, timelapse data to trace neurites including analysis and plotting.

SMAP [73] is a **MATLAB**-based framework for 2D and 3D single-molecule localisation microscopy analysis encompassing tasks such as molecule localisation, image rendering and quantitative analysis.

SR-TESSLER [74] is a standalone software for quantitative analysis of localisation-based super-resolution microscopy data.

STACKREG [75] is an **IMAGEJ** plugin for 2D + time image registration. It is also commonly used for other types of image registration, for example, for alignment of slices in 3D image stacks.

STARDIST [76,77] is a deep-learning based **PYTHON** library for segmenting star-shaped objects such as cell nuclei which is also available as plugins for **CELLPROFILER**, **FIJI** and **QUPATH**.

THUNDERSTORM [78] is an **IMAGEJ** plugin for automated processing, analysis and visualisation of data acquired by single-molecule localisation microscopy. **THUNDERSTORM** is at the moment of writing not actively maintained and may in the future be replaced by other solutions.

TOMOPY [79] is an open-source **PYTHON** package for processing tomography data and image reconstruction. It is mainly used for X-Ray tomography.

TOMVIZ [80] is a software package tailored for processing, visualisation, and analysis of 3D tomographic data acquired with transmission electron microscopy. It is compatible with **PYTHON** scripting to accommodate custom algorithms.

TRACKMATE [81] is a **FIJI** plugin for object tracking in 2D+t and 3D+t image data. It comes with advanced plotting, track visualisation and cell lineage tree visualisation tools. It is extensible using **Java**-based plugins

and scriptable using advanced scripting languages in FIJI such as Groovy, JavaScript and Jython.

TRAINABLE WEKA SEGMENTATION [82] is a user-friendly IMAGEJ plugin for pixel classification using various machine learning techniques based on the Waikato Environment for Knowledge Analysis [83].

TRAKEM2 [82,84] is a FIJI plugin for registration, stitching and management of large-scale electron microscopy data which offers tools for segmentation and reconstruction of objects such as neurons in 3D.

ZEN (Zeiss AG, Oberkochen, Germany) is a software and collection of components for microscope control, image acquisition, visualisation and analysis. Its integration of image analysis and microscope control allows feedback microscopy applications.

Emerging software

Apart from our conservative view on the field, we also perceive recent software developments which presumably will become part of the above glossary within the next 5–10 years. Most prominently, deep learning approaches are flooding our field with promising image processing components especially for image restoration [85–87] and cell/nuclei segmentation [21,76,77,88], classification and tracking [89] within complex scenes. Readers interested in an extended list of new applications and ready-to-use software [2,3] are referred to [90] and the bioimage model zoo online repository [91]. Those deep-learning-based components rely on technical frameworks such as TensorFlow [92] and PyTorch [93] which are not directly accessible to end-users. Multiple user-friendly GUIs were recently developed offering modern deep-learning tools to a wide target audience [94–97]. User-friendly deep-learning-based image processing is also already integrated within some of the applications listed in the glossary, namely ILASTIK, MICROSCOPY IMAGE BROWSER, almost all of the listed commercial software packages. New commercial solutions, such as AIVIA (Leica Microsystems GmbH) and APEER (Zeiss AG), focusing on machine learning for microscopy image analysis are arising as well.

In the same context, the NAPARI project [98] is bridging the PYTHON community towards the life scientists community by offering automatically generated, user-friendly GUIs to the most recent deep-learning and data science components and strives to become a major framework of the bio-image analysis community. From a PYTHON community perspective, napari is already a game changer as it brings widely usable *n*-dimensional viewing to the otherwise scripting centred PYTHON community [99]. From a wider perspective, more image visualisation tools have been published

recently and show high potential to become major players within the next decade since, compared to current default solutions, they provide opportunities to processing big image data and applying deep learning to microscopy image data [100–102].

Processing big image data, in the form of large 3D image stacks, long 3D+time data or large collections of 2D or 3D image data sets, is also a hot topic where new tools developed using remote-data, remote-computing and network-based approaches are emerging [103–109] and also semi-commercial solutions are appearing such as APEER (Zeiss AG). Graphics-Processing-Unit (GPU)-accelerated classical image processing [110–113] will play a major role for overcoming current limitations concerning processing times for large image data. From our perspective, such big-data capable solutions will also facilitate analysing spatial relationships in biological specimen and tissues using modern data-science approaches in the context of spatial-omics and transcriptomics [114–119].

For single molecule localisation microscopy analysis, improved methods for molecule detection and localisation are in active development [120]. In the field of super-resolution microscopy more broadly, there is also a focus on developing user-friendly methods for ensuring the fidelity of reconstructed images [121–123].

Last but not least, new file formats and solutions for research image data management [124–126] are under development and we expect those to have a huge impact on how analysts handle image data within the next decade.

Aspects to consider when choosing bio-image analysis software for your research

The choice of the right bio-image analysis software is closely related to the purpose of a given research project and more broadly to the field a research group is working in. We suggest becoming confident in a single software that broadly fits the planned research needs instead of switching the used software from project to project just because a single feature may be more accessible or more accurate in another software. Getting to know software and maintaining expertise comes at a high cost when numerous potentially incompatible platforms are used.

Interoperability between software is another key feature to consider. For example, we discourage using software that comes with proprietary custom file formats and suggest using broadly available file formats instead. Most prominently, there are some software packages with custom formats for project files. We

recommend making sure these project files can be accessed with common text editors and contain human-readable text that is based on standard formats such as XML, JSON, YAML or CSV. It also appears beneficial if software has capabilities for workflow automation or, in the case of plugins, can be automated using the platform they can be integrated with. For example, software with a great built-in segmentation algorithm can become a major bottleneck if the algorithm cannot be integrated with other software, for example, for pre-processing, post-processing, feature-extraction and statistical analysis.

Striving for reproducible bio-image analysis workflows with minimal manual interaction steps is key for analysing large amounts of image data leading to insights cemented by appropriate measurements exhibiting statistical power. If the software supports forming and properly documenting such automated workflows, reproducibility and interpretability of results can be ensured [127].

Other technical aspects such as big data capabilities play a key role, especially when new microscopy techniques potentially producing more and more data are published every year. Many software packages claim the ability to work with big data, but often refer to visualisation only or refer to big data as many images with a size of megabytes to gigabytes each. On the other hand, software packages capable of processing big volumetric image data in the range of terabytes and petabytes to produce quantitative analysis results are still rare and often limited in other aspects, for example, image data dimensionality or imaging modality. We see more and more web-based solutions being published diminishing the need to buy expensive computational hardware, to train and execute neural network architectures. When using web-based solutions in the cloud, institutional, national and international laws have to be respected. Additional technical burdens hinder the wide adoption of cloud computing at the moment. For example, uploading multiple terabytes of imaging data from a European institute to an American computing server is not just challenging from a legal but also from the file transfer bandwidth perspective. We assume these burdens will fall in the next decade and the technology will become available to more and more researchers as the benefits of using it outweigh the risks. Hence, choosing a software that is interoperable with cloud-computing and cloud-storage technologies appears a future-proof approach.

Many image analysis tasks have a substantial number of solutions that have been developed, and it can often be unclear which solution is most appropriate for a user's specific problem. Several image analysis

fields have established benchmarking challenges, whereby software is applied to exemplar datasets and performance is automatically and independently assessed. Such challenges exist for cell tracking [128,129], cell segmentation [130], electron microscopy image segmentation [131] and single molecule localisation [120]. These provide users with a quantitative comparison of the state-of-the-art, along with test datasets and guidance for quality reporting.

Community aspects should also be taken into account when choosing the right image analysis software. A key role in bio-image analysis for microscopy is played by the Image Science community <https://image.sc>, an online forum where developers and users of most software listed in the glossary are actively supporting each other by providing support and feedback (Fig. 1). Before using a software mid-/long-term, users may want to explore this forum and other online platforms to figure out how actively supported the software is by a broader community. Furthermore, some software communities hold regular virtual community meetings, where users and plugin-developers can get in touch with core-developers to exchange ideas, use-cases and receive support. The weekly community meeting of the napari community and the open office hours of the CellProfiler community shall be highlighted here as well-appreciated examples. For staying up-to-date with new developments in bio-image analysis software, following new media channels such as the NEUBIAS Academy YouTube channel [132] and the @Talk_BioImg Twitter bot which retweets posts containing the #BioImageAnalysis hashtag should be considered as well for an audience with general interest in the field, for example, postdocs and core-facility staff working on applied bio-image analysis and image data science. In addition, the Global Bioimaging infrastructure is organising image analysis courses and providing a training resource for core facility staff and image analysis community.

From a group leader's and an institutional decision maker's perspective, guiding scientists towards using a common software platform makes sense. The more local collaborators work with the same software, and maybe just use different plugins, the more they can support each other and exchange knowledge. If it is apparent that a majority of the group or institute members are using the same software, an institute can strengthen this community by inviting the developers of that software annually for courses and seminars. Building this bridge between users and developers is of mutual benefit: While the users receive support and training from the experts, the experts can establish collaborations with power users leading to scientific

publications. These applied-science publications are key to grant applications and sustainable maintenance of research software.

Conclusions and perspectives

Comparing the bio-image analysis software universe one decade ago to its current state clearly shows that it is expanding. Multiple huge ecosystems grew from a small number of general-purpose software developed more than a decade ago. Recently, many of such platforms are building bridges among them, which is appreciated from our bio-image analysis workflow designers' perspective. Hence, users are well advised to use interoperable, established, sustainably maintained software packages for their research. It is also important to stay alert on recent developments, especially in the deep learning context, even though one should stay a brave scientist and question those new methods. Such critical thinking is necessary to solidify knowledge about new methods before eventually adopting them as community standards. Last but not least we want to emphasise that the decision on which software to use for specific research projects should be made in groups. Getting in touch with local, regional and global experts and discussing advantages and disadvantages is the right path in a growing universe of bio-image analysis software.

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Data accessibility

Supplementary Table 1 contains all data referred to this document.

References

- 1 Levet F, Carpenter AE, Eliceiri KW, Kreshuk A, Bankhead P, Haase R. Developing open-source software for bioimage analysis: opportunities and challenges. *FI000Res*. 2021;**10**:302.
- 2 BioImage Informatics Index. 2022 [cited 2022 Jul 20]. Available from: <https://biii.eu>
- 3 Elixir Community. bio.tools. 2022 [cited 2022 Jul 20]. Available from: <https://bio.tools/>
- 4 Adams D, Lloyd J. The meaning of Liff. London: Pan; 1983.
- 5 Bioimage data analysis workflows. Berlin: Springer Nature; 2019.
- 6 Lobet G, Draye X, Périlleux C. An online database for plant image analysis software tools. *Plant Methods*. 2013;**9**:38.
- 7 Hohlbein J, Diederich B, Marsikova B, Reynaud EG, Holden S, Jahr W, et al. Open microscopy in the life sciences: Quo Vadis? *arXiv*. 2021. <https://doi.org/10.48550/arXiv.2110.13951>
- 8 Gibbs HC, Mota SM, Hart NA, Min SW, Vernino AO, Pritchard AL, et al. Navigating the light-sheet image analysis software landscape: concepts for driving cohesion from data acquisition to analysis. *Front Cell Dev Biol*. 2021;**9**:739079.
- 9 Software tools for molecular microscopy. 2006 [cited 2022 Jul 20]. Available from: https://en.wikibooks.org/wiki/Software_Tools_For_Molecular_Microscopy
- 10 Ollion J, Cochenne J, Loll F, Escudé C, Boudier T. TANGO: a generic tool for high-throughput 3D image analysis for studying nuclear organization. *Bioinformatics*. 2013;**29**:1840–1.
- 11 Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin JC, Pujol S, et al. 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magn Reson Imaging*. 2012;**30**:1323–41.
- 12 Avants BB, Tustison NJ, Song G, Cook PA, Klein A, Gee JC. A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage*. 2011;**54**:2033–44.

- 13 Pietzsch T, Saalfeld S, Preibisch S, Tomancak P. BigDataViewer: visualization and processing for large image data sets. *Nat Methods*. 2015;**12**:481–3.
- 14 Hörl D, Rojas Rusak F, Preusser F, Tillberg P, Randel N, Chhetri RK, et al. BigStitcher: reconstructing high-resolution image datasets of cleared and expanded samples. *Nat Methods*. 2019;**16**:870–4.
- 15 Linkert M, Rueden CT, Allan C, Burel JM, Moore W, Patterson A, et al. Metadata matters: access to image data in the real world. *J Cell Biol*. 2010;**189**:777–82.
- 16 Blender Foundation. blender.org – home of the Blender project – free and open 3D creation software. 2022 [cited 2022 Jul 20]. Available from: <https://www.blender.org/>
- 17 Domander R, Felder AA, Doube M. BoneJ2 – refactoring established research software. *Wellcome Open Res*. 2021;**6**:37.
- 18 Saalfeld S, Cardona A, Hartenstein V, Tomancak P. CATMAID: collaborative annotation toolkit for massive amounts of image data. *Bioinformatics*. 2009;**25**:1984–6.
- 19 Burnley T, Palmer CM, Winn M. Recent developments in the CCP-EM software suite. *Acta Crystallogr D Struct Biol*. 2017;**73**:469–77.
- 20 CCPi tomographic imaging. 2022 [cited 2022 Jul 20]. Available from: <http://www.ccpi.ac.uk/>
- 21 Stringer C, Wang T, Michaelos M, Pachitariu M. Cellpose: a generalist algorithm for cellular segmentation. *Nat Methods*. 2021;**18**:100–6.
- 22 McQuin C, Goodman A, Chernyshev V, Kamensky L, Cimini BA, Karhohs KW, et al. CellProfiler 3.0: next-generation image processing for biology. *PLoS Biol*. 2018;**16**:e2005970.
- 23 Jones TR, Kang IH, Wheeler DB, Lindquist RA, Papallo A, Sabatini DM, et al. CellProfiler Analyst: data exploration and analysis software for complex image-based screens. *BMC Bioinformatics*. 2008;**9**:482.
- 24 Sage D, Donati L, Soulez F, Fortun D, Schmit G, Seitz A, et al. DeconvolutionLab2: an open-source software for deconvolution microscopy. *Methods*. 2017;**115**:28–41.
- 25 Limaye A. Drishti: a volume exploration and presentation tool. In: Stock SR, editor. Developments in X-ray tomography VIII. Bellingham, WA: SPIE; 2012. <https://doi.org/10.1117/12.935640>
- 26 Hu Y, Limaye A, Lu J. Three-dimensional segmentation of computed tomography data using: new tools and developments. *R Soc Open Sci*. 2020;**7**:201033.
- 27 Klein S, Staring M, Murphy K, Viergever MA, Pluim JPW. elastix: a toolbox for intensity-based medical image registration. *IEEE Trans Med Imaging*. 2010;**29**:196–205.
- 28 Tang G, Peng L, Baldwin PR, Mann DS, Jiang W, Rees I, et al. EMAN2: an extensible image processing suite for electron microscopy. *J Struct Biol*. 2007;**157**:38–46.
- 29 Müller M, Mönkemöller V, Hennig S, Hübner W, Huser T. Open-source image reconstruction of super-resolution structured illumination microscopy data in ImageJ. *Nat Commun*. 2016;**7**:10980.
- 30 Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods*. 2012;**9**:676–82.
- 31 The Apache Groovy programming language. 2022 [cited 2022 Jul 20]. Available from: <https://groovy-lang.org/>
- 32 Nečas D, Klapetek P. Gwyddion: an open-source software for SPM data analysis. *Centr Eur J Phys*. 2012;**10**:181–8.
- 33 de Chaumont F, Dallongeville S, Chenouard N, Hervé N, Pop S, Provoost T, et al. Icy: an open bioimage informatics platform for extended reproducible research. *Nat Methods*. 2012;**9**:690–6.
- 34 Berg S, Kutra D, Kroeger T, Straehle CN, Kausler BX, Haubold C, et al. ilastik: interactive machine learning for (bio)image analysis. *Nat Methods*. 2019;**16**:1226–32.
- 35 Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;**9**:671–5.
- 36 Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, et al. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*. 2017;**18**:529.
- 37 Macro language. 2022 [cited 2022 Jul 20]. Available from: <https://imagej.nih.gov/ij/developer/macro/macros.html>
- 38 Rueden CT, Ackerman J, Arena ET, Eglinger J, Cimini BA, Goodman A, et al. Scientific Community Image Forum: a discussion forum for scientific image software. *PLoS Biol*. 2019;**17**:e3000340.
- 39 Discourse – civilized discussion. 2022 [cited 2022 Jul 20]. Available from: <https://www.discourse.org/>
- 40 Pietzsch T, Preibisch S, Tomancak P, Saalfeld S. ImgLib2 – generic image processing in Java. *Bioinformatics*. 2012;**28**:3009–11.
- 41 Kremer JR, Mastronarde DN, McIntosh JR. Computer visualization of three-dimensional image data using IMOD. *J Struct Biol*. 1996;**116**:71–6.
- 42 Yoo TS, Ackerman MJ, Lorensen WE, Schroeder W, Chalana V, Aylward S, et al. Engineering and algorithm design for an image processing Api: a technical report on ITK—the Insight Toolkit. *Stud Health Technol Inform*. 2002;**85**:586–92.
- 43 Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage*. 2006;**31**:1116–28.

- 44 Stack Overflow Developer Survey 2021. Stack Overflow. 2021 [cited 2022 Jul 20]. Available from: https://insights.stackoverflow.com/survey/2021/?utm_source=social-share&utm_medium=social&utm_campaign=dev-survey-2021
- 45 Kluyver T, Ragan-Kelley B, Pérez F, Granger BE, Bussonnier M, Frederic J, et al. Jupyter Notebooks – a publishing format for reproducible computational workflows. In: Loizides F, Schmidt B, editors. Positioning and power in academic publishing: players, agents and agendas. Amsterdam: IOS Press; 2016. p. 87–90.
- 46 Berthold MR, Cebron N, Dill F, Gabriel TR, Kötter T, Meinl T, et al. KNIME: the Konstanz information miner. In: Preisach C, Burkhardt H, Schmidt-Thieme L, De R, editors. Data analysis, machine learning and applications. Berlin, Heidelberg: Springer; 2008. p. 319–26.
- 47 Helmstaedter M, Briggman KL, Denk W. High-accuracy neurite reconstruction for high-throughput neuroanatomy. *Nat Neurosci*. 2011;**14**:1081–8.
- 48 Hunter JD. Matplotlib: a 2D graphics environment. *Comput Sci Eng*. 2007;**9**:90–5.
- 49 Edelstein A, Amodaj N, Hoover K, Vale R, Stuurman N. Computer control of microscopes using µManager. *Curr Protoc Mol Biol*. 2010;**Chapter 14**:Unit14.20.
- 50 Pinkard H, Stuurman N, Ivanov IE, Anthony NM, Ouyang W, Li B, et al. Pycro-Manager: open-source software for customized and reproducible microscope control. *Nat Methods*. 2021;**18**:226–8.
- 51 Belevich I, Joensuu M, Kumar D, Vihinen H, Jokitalo E. Microscopy image browser: a platform for segmentation and analysis of multidimensional datasets. *PLoS Biol*. 2016;**14**:e1002340.
- 52 Barbier de Reuille P, Routier-Kierzkowska AL, Kierzkowski D, Bassel GW, Schüpbach T, Tauriello G, et al. MorphoGraphX: a platform for quantifying morphogenesis in 4D. *Elife*. 2015;**4**:05864.
- 53 Legland D, Arganda-Carreras I, Andrey P. MorphoLibJ: integrated library and plugins for mathematical morphology with ImageJ. *Bioinformatics*. 2016;**32**:3532–4.
- 54 Laine RF, Tosheva KL, Gustafsson N, Gray RDM, Almada P, Albrecht D, et al. NanoJ: a high-performance open-source super-resolution microscopy toolbox. *J Phys D Appl Phys*. 2019;**52**:163001.
- 55 Gustafsson N, Culley S, Ashdown G, Owen DM, Pereira PM, Henriques R. Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations. *Nat Commun*. 2016;**7**:12471.
- 56 Meijering E, Jacob M, Sarria JCF, Steiner P, Hirling H, Unser M. Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images. *Cytometry A*. 2004;**58**:167–76.
- 57 Harris CR, Millman KJ, van der Walt SJ, Gommers R, Virtanen P, Cournapeau D, et al. Array programming with NumPy. *Nature*. 2020;**585**:357–62.
- 58 Python package index download statistics. 2021 [cited 2022 Jul 20]. Available from: <https://pypistats.org/top>
- 59 Ahrens J, Geveci B, Law C. ParaView: an end-user tool for large-data visualization. In: Visualization handbook. Amsterdam: Elsevier; 2005. p. 717–31. <https://doi.org/10.1016/B978-012387582-2/50038-1>
- 60 Püspöki Z, Storath M, Sage D, Unser M. Transforms and operators for directional bioimage analysis: a survey. *Adv Anat Embryol Cell Biol*. 2016;**219**:69–93.
- 61 Allan C, Burel JM, Moore J, Blackburn C, Linkert M, Loynton S, et al. OMERO: flexible, model-driven data management for experimental biology. *Nat Methods*. 2012;**9**:245–53.
- 62 Bradski G. The OpenCV library. *Dr Dobb's J Software Tools*. 2000;**120**:122–5.
- 63 Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD, et al. QuPath: open source software for digital pathology image analysis. *Sci Rep*. 2017;**7**:16878.
- 64 The R Community. The R project for statistical computing. 2022 [cited 2022 Jul 20]. Available from: <https://www.R-project.org/>
- 65 Pau G, Fuchs F, Sklyar O, Boutros M, Huber W. EBImage – an R package for image processing with applications to cellular phenotypes. *Bioinformatics*. 2010;**26**:979–81.
- 66 Scheres SHW. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol*. 2012;**180**:519–30.
- 67 SciJava. 2022 [cited 2022 Jul 20]. Available from: <https://scijava.org/>
- 68 van der Walt S, Schönberger JL, Nunez-Iglesias J, Boulogne F, Warner JD, Yager N, et al. scikit-image: image processing in Python. *PeerJ*. 2014;**2**:e453.
- 69 Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. Scikit-learn: machine learning in Python. *J Mach Learn Res*. 2011;**12**:2825–30.
- 70 Virtanen P, Gommers R, Oliphant TE, Haberland M, Reddy T, Cournapeau D, et al. SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nat Methods*. 2020;**17**:261–72.
- 71 Mastronarde DN. Automated electron microscope tomography using robust prediction of specimen movements. *J Struct Biol*. 2005;**152**:36–51.
- 72 Arshadi C, Günther U, Eddison M, Harrington KIS, Ferreira TA. SNT: a unifying toolbox for quantification of neuronal anatomy. *Nat Methods*. 2021;**18**:374–7.
- 73 Ries J. SMAP: a modular super-resolution microscopy analysis platform for SMLM data. *Nat Methods*. 2020;**17**:870–2.
- 74 Levet F, Hosy E, Kechkar A, Butler C, Beghin A, Choquet D, et al. SR-Tesseler: a method to segment

- and quantify localization-based super-resolution microscopy data. *Nat Methods*. 2015;**12**:1065–71.
- 75 Thévenaz P, Ruttimann UE, Unser M. A pyramid approach to subpixel registration based on intensity. *IEEE Trans Image Process*. 1998;**7**:27–41.
 - 76 Schmidt U, Weigert M, Broaddus C, Myers G. Cell detection with star-convex polygons. In: Frangi AF, editor. Medical image computing and computer assisted intervention – MICCAI 2018. Cham: Springer International Publishing; 2018. p. 265–73.
 - 77 Weigert M, Schmidt U, Haase R, Sugawara K, Myers G. Star-convex polyhedra for 3D object detection and segmentation in microscopy. In: 2020 IEEE Winter Conference on Applications of Computer Vision (WACV). IEEE; 2020. <https://doi.org/10.1109/wacv45572.2020.9093435>
 - 78 Ovesný M, Krížek P, Borkovec J, Svindrych Z, Hagen GM. ThunderSTORM: a comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution imaging. *Bioinformatics*. 2014;**30**:2389–90.
 - 79 Gürsoy D, De Carlo F, Xiao X, Jacobsen C. TomoPy: a framework for the analysis of synchrotron tomographic data. *J Synchrotron Radiat*. 2014;**21**:1188–93.
 - 80 Levin BDA, Jiang Y, Padgett E, Waldon S, Quammen C, Harris C, et al. Tutorial on the visualization of volumetric data using tomviz. *Micros Today*. 2018;**26**:12–7.
 - 81 Tinevez J-Y, Perry N, Schindelin J, Hoopes GM, Reynolds GD, Laplantine E, et al. TrackMate: an open and extensible platform for single-particle tracking. *Methods*. 2017;**115**:80–90.
 - 82 Arganda-Carreras I, Kaynig V, Rueden C, Eliceiri KW, Schindelin J, Cardona A, et al. Trainable Weka segmentation: a machine learning tool for microscopy pixel classification. *Bioinformatics*. 2017;**33**:2424–6.
 - 83 Witten IH, Frank E, Hall MA, Pal CJ. Data mining: practical machine learning tools and techniques. Burlington, WA: Morgan Kaufmann; 2016.
 - 84 Cardona A, Saalfeld S, Schindelin J, Arganda-Carreras I, Preibisch S, Longair M, et al. TrakEM2 software for neural circuit reconstruction. *PLoS One*. 2012;**7**: e38011.
 - 85 Weigert M, Schmidt U, Boothe T, Müller A, Dibrov A, Jain A, et al. Content-aware image restoration: pushing the limits of fluorescence microscopy. *Nat Methods*. 2018;**15**:1090–7.
 - 86 Krull A, Buchholz T-O, Jug F. Noise2Void – learning denoising from single noisy images. *arXiv*. 2018. <https://doi.org/10.48550/arXiv.1811.10980>
 - 87 Batson J, Royer L. Noise2Self: blind denoising by self-supervision. 2019.
 - 88 Aigouy B, Cortes C, Liu S, Prud'Homme B. EPySeg: a coding-free solution for automated segmentation of epithelia using deep learning. *Development*. 2020;**147**: dev194589.
 - 89 Mathis A, Mamidanna P, Cury KM, Abe T, Murthy VN, Mathis MW, et al. DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat Neurosci*. 2018;**21**:1281–9.
 - 90 Belthangady C, Royer LA. Applications, promises, and pitfalls of deep learning for fluorescence image reconstruction. *Nat Methods*. 2019;**16**:1215–25.
 - 91 BioImageIO. BioImage Model Zoo. 2022 [cited 2022 Jul 20]. Available from: <https://bioimage.io>
 - 92 TensorFlow-Developers. TensorFlow. Zenodo. 2021. <https://doi.org/10.5281/ZENODO.4724125>
 - 93 Paszke A, Gross S, Massa F, Lerer A, Bradbury J, Chanan G, et al. PyTorch: an imperative style, high-performance deep learning library. *arXiv*. 2019; arXiv:1912.01703.
 - 94 Gómez-de-Mariscal E, García-López-de-Haro C, Ouyang W, Donati L, Lundberg E, Unser M, et al. DeepImageJ: a user-friendly environment to run deep learning models in ImageJ. *Nat Methods*. 2021;**18**:1192–5.
 - 95 von Chamier L, Laine RF, Jukkala J, Spahn C, Krentzel D, Nehme E, et al. Democratising deep learning for microscopy with ZeroCostDL4Mic. *Nat Commun*. 2021;**12**:2276.
 - 96 Ouyang W, Mueller F, Hjelmare M, Lundberg E, Zimmer C. ImJoy: an open-source computational platform for the deep learning era. *Nat Methods*. 2019;**16**:1199–200.
 - 97 Belevich I, Jokitalo E. DeepMIB: user-friendly and open-source software for training of deep learning network for biological image segmentation. *PLoS Comput Biol*. 2021;**17**:e1008374.
 - 98 Sofroniew N, Lambert T, Evans K, Nunez-Iglesias J, Bokota G, Peña-Castellanos G, et al. napari/napari: 0.4.12rc2. Zenodo. 2021. <https://doi.org/10.5281/ZENODO.3555620>
 - 99 Perkel JM. Python power-up: new image tool visualizes complex data. *Nature*. 2021;**600**:347–8.
 - 100 Vergara HM, Pape C, Meechan KI, Zinchenko V, Genoud C, Wanner AA, et al. Whole-body integration of gene expression and single-cell morphology. *Cell*. 2021;**184**:4819–4837.e22.
 - 101 Chiaruttini N, Burri O, Haub P, Guet H, Sordet-Dessimoz J, Seitz A. An open-source whole slide image registration workflow at cellular precision using Fiji, QuPath and elastix. *Front Comput Sci*. 2022;**3**. <https://doi.org/10.3389/fcomp.2021.780026>
 - 102 Schmid B, Tripal P, Fraaß T, Kersten C, Ruder B, Grüneboom A, et al. 3Dscript: animating 3D/4D microscopy data using a natural-language-based syntax. *Nat Methods*. 2019;**16**:278–80.
 - 103 Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Čech M, et al. The Galaxy platform for accessible,

- reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Res.* 2018;**46**:W537–44.
- 104 Marée R, Rollus L, Stévens B, Hoyoux R, Louppe G, Vandaele R, et al. Collaborative analysis of multi-gigapixel imaging data using Cytomine. *Bioinformatics.* 2016;**32**:1395–401.
 - 105 Rubens U, Mormont R, Paavola L, Bäcker V, Pavie B, Scholz LA, et al. BIAFLOWS: a collaborative framework to reproducibly deploy and benchmark bioimage analysis workflows. *Patterns (N Y).* 2020;**1**:100040.
 - 106 Tischer C, Ravindran A, Reither S, Chiaruttini N, Pepperkok R, Norlin N. BigDataProcessor2: a free and open-source Fiji plugin for inspection and processing of TB sized image data. *Bioinformatics.* 2021;**37**(18):3079–81. <https://doi.org/10.1093/bioinformatics/btab106>
 - 107 Rubens U, Hoyoux R, Vanosmael L, Ours M, Tasset M, Hamilton C, et al. Cytomine: toward an open and collaborative software platform for digital pathology bridged to molecular investigations. *Proteomics Clin Appl.* 2019;**13**:e1800057.
 - 108 Boergens KM, Berning M, Bocklisch T, Bräunlein D, Drawitsch F, Frohnhofen J, et al. webKnossos: efficient online 3D data annotation for connectomics. *Nat Methods.* 2017;**14**:691–4.
 - 109 Rocklin M. Dask: parallel computation with blocked algorithms and task scheduling. In: Proceedings of the 14th Python in Science Conference. SciPy; 2015. <https://doi.org/10.25080/majora-7b98e3ed-013>
 - 110 Okuta R, Unno Y, Nishino D, Hido S, Loomis C. CuPy: a NumPy-compatible library for NVIDIA GPU calculations. In: Proceedings of workshop on machine learning systems (LearningSys) in the thirty-first annual conference on neural information processing systems (NIPS); 2017 [cited 2022 Jul 20]. Available from: http://learningsys.org/nips17/assets/papers/paper_16.pdf
 - 111 Haase R, Royer LA, Steinbach P, Schmidt D, Dibrov A, Schmidt U, et al. CLIJ: GPU-accelerated image processing for everyone. *Nat. Methods.* 2020;**17**:5–6.
 - 112 Malyshau D, Ninomiya K, Jones B, editors. WebGPU. 2022 [cited 2022 Jul 20]. Available from: <https://www.w3.org/TR/webgpu/>
 - 113 Haase R, Jain A, Rigaud S, Vorkel D, Rajasekhar P, Suckert T, et al. Interactive design of GPU-accelerated image data flow graphs and cross-platform deployment using multi-lingual code generation. *bioRxiv.* 2020. <https://doi.org/10.1101/2020.11.19.386565>
 - 114 Stoltzfus CR, Filipek J, Gern BH, Olin BE, Leal JM, Wu Y, et al. CytoMAP: a spatial analysis toolbox reveals features of myeloid cell organization in lymphoid tissues. *Cell Rep.* 2020;**31**:107523.
 - 115 Palla G, Spitzer H, Klein M, Fischer D, Schaar AC, Kuemmerle LB, et al. Squidpy: a scalable framework for spatial single cell analysis. *bioRxiv.* 2021. <https://doi.org/10.1101/2021.02.19.431994>
 - 116 Axelrod S, Cai M, Carr AJ, Freeman J, Ganguli D, Kiggins JT, et al. starfish: scalable pipelines for image-cryptomics. *J Open Source Softw.* 2021;**6**:2440.
 - 117 Haase R. Image processing filters for grids of cells analogous to filters processing grids of pixels. *Front Comput Sci.* 2021;**3**. <https://doi.org/10.3389/fcomp.2021.774396>
 - 118 Solorzano L, Partel G, Wählby C. TissUMaps: interactive visualization of large-scale spatial gene expression and tissue morphology data. *Bioinformatics.* 2020;**36**:4363–5.
 - 119 Pielawski N, Andersson A, Avenel C, Behanova A, Chelebian E, Klemm A, et al. TissUMaps 3: interactive visualization and quality assessment of large-scale spatial omics data. *bioRxiv.* 2022. <https://doi.org/10.1101/2022.01.28.478131>
 - 120 Sage D, Pham TA, Babcock H, Lukes T, Pengo T, Chao J, et al. Super-resolution fight club: assessment of 2D and 3D single-molecule localization microscopy software. *Nat Methods.* 2019;**16**:387–95.
 - 121 Culley S, Albrecht D, Jacobs C, Pereira PM, Leterrier C, Mercer J, et al. Quantitative mapping and minimization of super-resolution optical imaging artifacts. *Nat Methods.* 2018;**15**:263–6.
 - 122 Ball G, Demmerle J, Kaufmann R, Davis I, Dobbie IM, Schermelleh L. SIMcheck: a toolbox for successful super-resolution structured illumination microscopy. *Sci Rep.* 2015;**5**:15915.
 - 123 Marsh RJ, Costello I, Gorey M-A, Ma D, Huang F, Gautel M, et al. Sub-diffraction error mapping for localisation microscopy images. *Nat Commun.* 2021;**12**:5611.
 - 124 Miles A, Kirkham J, Durant M, Bourbeau J, Onalan T, Hamman J, et al. zarr-developers/zarr-python: v2.4.0. 2020. <https://doi.org/10.5281/zenodo.3773450>
 - 125 Moore J, Allan C, Besson S, Burel J-M, Diel E, Gault D, et al. OME-NGFF: a next-generation file format for expanding bioimaging data-access strategies. *Nat Methods.* 2021;**18**:1496–8.
 - 126 Saalfeld S, Pisarev I, Hanslovsky P, Bogovic J, Champion A, Rueden C, et al. N5: Not HDF5. 2017 [cited 2022 Jul 20]. Available from: <https://github.com/saalfeldlab/n5>
 - 127 Miura K, Nørrelykke SF. Reproducible image handling and analysis. *EMBO J.* 2021;**40**:e105889.
 - 128 Ulman V, Maška M, KEG M, Ronneberger O, Haubold C, Harder N, et al. An objective comparison of cell-tracking algorithms. *Nat Methods.* 2017;**14**:1141–52.

- 129 Cell Tracking Benchmark. 2022 [cited 2022 Jul 20]. Available from: <http://celltrackingchallenge.net/latest-ctb-results/>
- 130 Arganda-Carreras I, Turaga SC, Berger DR, Cireşan D, Giusti A, Gambardella LM, et al. Crowdsourcing the creation of image segmentation algorithms for connectomics. *Front Neuroanat.* 2015;**9**:142.
- 131 Wei D, Lin Z, Franco-Barranco D, Wendt N, Liu X, Yin W, et al. MitoEM Dataset: large-scale 3D mitochondria instance segmentation from EM images. *Med Image Comput Comput Assist Interv.* 2020;**12265**:66–76.

- 132 NEUBIAS. NEUBIAS Academy youtube channel. 2020 [cited 2022 Jul 20]. Available from: <https://www.youtube.com/neubias>

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. List of the software terms from the glossary with categorical information and interactive filtering capabilities.