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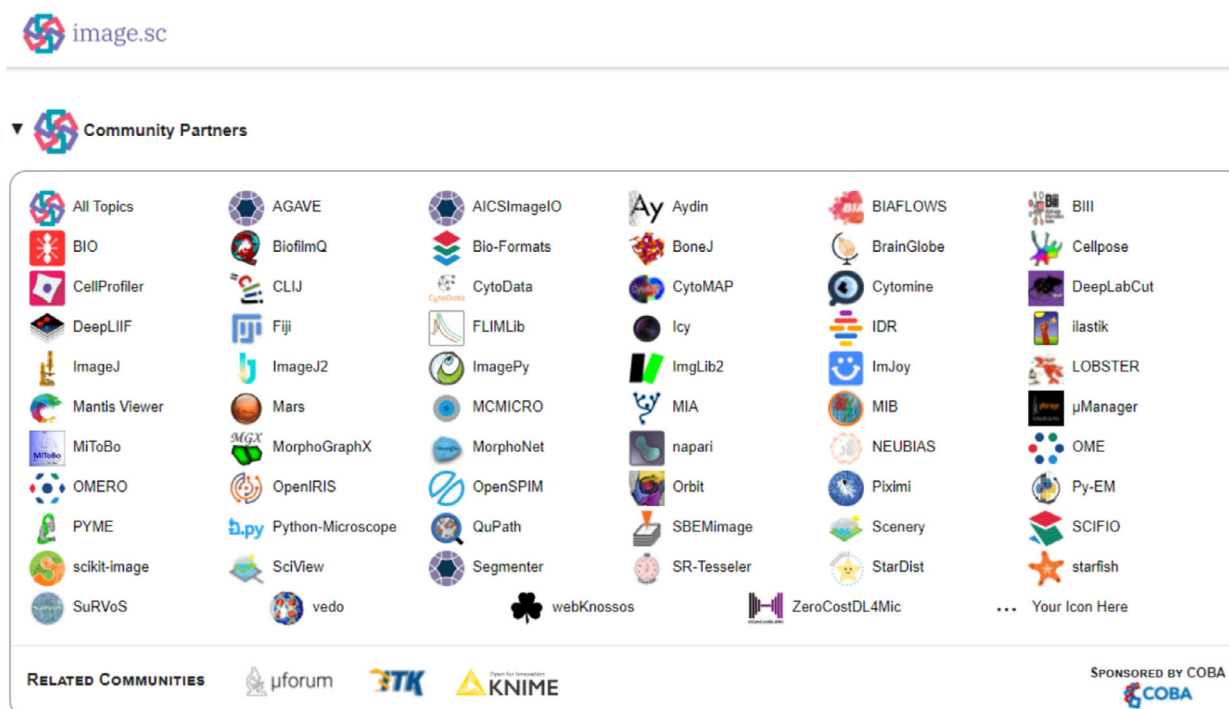
Fig. 1. Screenshot of the image.sc forum in April 2022 showing the logos of the community partners and related communities. Listed open-source projects provide online support for their software on this platform, which might be a key criterion when deciding which software to use.

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 MOR-PHOGRAPHX 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 plug-ins 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.

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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.