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CellProfiler plugins – An easy image analysis platform integration for containers and Python tools

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Abstract

CellProfiler is a widely used software for creating reproducible, reusable image analysis workflows without needing to code. In addition to the >90 modules that make up the main CellProfiler program, CellProfiler has a plugins system that allows for the creation of new modules which integrate with other Python tools or tools that are packaged in software containers. The CellProfilerplugins repository contains a number of these CellProfiler modules, especially modules that are experimental and/or dependency-heavy. Here, we present an upgraded CellProfiler-plugins repository, an example of accessing containerised tools, improved documentation and added citation/reference tools to facilitate the use and contribution of the community.

KEYWORDS

CellProfiler, image analysis, plugin, Python, software, software container, workflow

1 | INTRODUCTION

Bioimage analysis has become increasingly popular over the last 30 years, with approximately ten times as many citations per year as 20 years ago and three times as many as a decade ago (Figure 1). The number of open-source image analysis tools has both driven and been driven by this increase – since the Scientific Community Image Forum (forum.image.sc)¹ was formed in 2018, the number of participating software tools has grown from 2 to 56. A recent overview of open-source image analysis tools or platforms identified 82 tools, platforms or languages,² and the BioImage Informatics Index (BIII)³ lists 1388 available tools, components and workflows.

While too many tools is certainly preferable to too few, this increase in software tools and methods means users must wade through an increasingly large tool ecosystem to find the best tool for their needs, which can be especially overwhelming for non-expert users. Deep learning-based tools, also booming in popularity, often have particularly complicated installations. A recent analysis of posts at forum.image.sc implies that possibly as many as 1 in 10 threads is started due to installation issues and as many as one in five touch on installation at some point.⁴

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Since image analyses rarely are composed of single steps,⁵ in addition to tools that specialise in performing a single task well, the bioimage analysis space consists of a number of generalist 'no-code' (or 'code-optional') software workflow platforms that collect other tools and/or help create workflows, including Fiji,⁶ Icy,⁷ KNIME⁸ and CellProfiler.^{9,10} Since their role is at least in part to bring groups of tools together, such workflow platforms typically accept outside contributions or 'plugins' created by other groups. It is worth noting that in a recent survey about image analysis in the life and physical sciences, tool users frequently mentioned both 'platforms' and 'plugins' when asked about what tool developers could do to make user experiences easier.¹¹

Of the four tools listed above, Fiji, Icy and KNIME are all implemented in Java; CellProfiler is the sole tool written in Python. Though Java has long been dominant in bioimage analysis due to tools like ImageJ,^{12,13} Python-based tools are becoming increasingly popular, partially though not exclusively due to Python's dominance in deep learning. The recent creation of the PyImageJ¹⁴ library helps integrate Java and Python tools in code-friendly spaces like Jupyter notebooks¹⁵ and napari¹⁶; nevertheless, a Python-based image analysis workflow tool is an important part of the bioimage analysis ecosystem. As such, while CellProfiler has for a number of years allowed users to implement their own custom plugins,^{9,10} we herein report on efforts to make CellProfiler plugins easier to find, install, develop and use.

2 | RESULTS

2.1 | What are CellProfiler plugins?

CellProfiler enables the flexible creation of image analvsis workflows by providing almost 100 modules that each performs an image or object processing function that are arranged and configured by a researcher into a pipeline specific to their analysis task. Plugins are modules that extend CellProfiler's capabilities but are not installed in CellProfiler by default. They allow CellProfiler to be customised and expanded beyond the capabilities of a single development team and provide a platform for other developers to share their work with the community, making CellProfiler more extensible and collaborative. The CellProfiler-plugins repository stores and shares these CellProfiler modules. Version 1.0.0 of the repository contains 15 plugins (Table 1). Decisions about incorporating plugins into the main program are frequently revisited by the CellProfiler development team in consultation with plugin creators. The two primary, nonmutually exclusive reasons a module might be distributed

Practitioner points

- 1. CellProfiler plugins expand the kinds of reproducible analyses that can be performed inside CellProfiler.
- 2. Plugins allow for integration of niche, experimental or cross-language tools into a central image processing workflow.
- 3. Containerisation is particularly useful in allowing access to tools written in different languages and/or with conflicting dependencies.

as a plugin are narrow audience suitability and library dependencies.

2.1.1 | Modules not suitable for a majority of CellProfiler users

The existing CellProfiler application contains more than 90 individual modules, and user feedback has consistently shown that the program's complexity is a hurdle to adoption. By consciously including only new modules with a high threshold for reuse, we slow the growth of CellProfiler's complexity; thus, modules such as CallBarcodes, which decodes one-hot-exponentially multiplexed barcodes such as in in situ sequencing¹⁷ and seqFISH,¹⁸ are typically designated as plugins because, in our experience, only a small fraction of CellProfiler users are performing this type of experiment. This class also includes modules we feel have a high potential for accidental misuse: histogram equalisation and normalisation (performed in HistogramEqualization and HistogramMatching modules) are both image manipulations that are undeniably useful in certain contexts but can introduce hard-to-trace effects if used before image quantification. Thus, these modules are only available to users who seek them out.

2.1.2 | Modules that require external dependencies

The existing CellProfiler application contains a number of popular scientific Python libraries like NumPy,¹⁹ SciPy,²⁰ scikit-image²¹ and scikit-learn.²² It does not, however, contain dataframe handling tools such as pandas²³ nor libraries typically used for deep learning such as TensorFlow²⁴ or PyTorch²⁵ due to added complexity for users and to keep the CellProfiler application at a reasonable file size. If the libraries needed to support a particular



FIGURE 1 PubMed search results for 'bioimaging' (A) and 'bioimage analysis' (B) from 1992–2022, plotted as absolute citation counts (blue) and citation counts normalised to all PubMed citations (orange). Increase in both terms is substantial over this time window.

module are not currently contained in CellProfiler, the module must be used as a plugin, either by using specially installed versions of CellProfiler or by using software containers (see below). If the module seems to be suitable for the majority of CellProfiler users, the dependencies may be added in future versions of CellProfiler, subject to considerations such as library size, compatibility with existing libraries and licensing constraints.²⁶

2.2 | Using CellProfiler plugins

Plugins can be used by simply downloading the entire plugins repository (or any individual plugin) from the CellProfiler-plugins GitHub repository at https://github. com/CellProfiler/CellProfiler-plugins. In the CellProfiler 'preferences' menu, the user sets the CellProfiler-plugins directory to the folder containing the plugin(s) they wish to use. When CellProfiler is next opened, it will attempt to load all plugins found in that folder; note that it will not recursively search subfolders. Successfully loaded plugins will be available in the 'Add Modules' menu alongside CellProfiler native modules. Plugins relying only on CellProfiler-provided libraries (see Table 1) can be loaded in CellProfiler whether the CellProfiler application was downloaded from the CellProfiler.org website (sometimes referred to as installing 'built') or whether CellProfiler was installed in Python on the user's computer (sometimes referred to as 'from source').

When the built CellProfiler application does not contain all of a plugin's dependencies, additional installation of these dependencies or an alternative approach that circumvents local installation requirements must be used in order for the plugins to work; three methods are described in the paragraphs following. Some of the most impactful plugins add new dependencies: bringing new functionality to CellProfiler and bridging gaps in the core library (e.g. modules with Deep Learning functionality such as RunCellpose or RunStarDist), even crossing boundaries to other languages and tools (e.g. RunImageJScript). As seen in Figure 2, Cellpose²⁷ is a highly requested plugin for Cell-Profiler, due to its easy-to-tune segmentation networks.

2.2.1 | Installation via Python

CellProfiler can be installed in a system's local Python installation by cloning from GitHub or by directly installing from the Python Package Index (PyPI) in Python versions 3.8 or 3.9. Users may find dependency management easiest if they use Python virtual environment tools such as pyenv,³¹ or Anaconda³² and related tools such as mamba.³³ Users can then add additional libraries (as laid out by the plugin author) via pip or conda per their package manager's instructions, though problems may arise when plugins have conflicting dependencies (e.g. Plugin 1 requires a library to be at version 1.5, but Plugin 2 only works with that library at version 2.0). Solving such dependency conflicts typically requires manual review of the dependencies; help is available in the plugins documentation as well as on the image.sc forum, and we provide a setup file in the CellProfiler-plugins repository to help ease pip installation of CellProfiler and each plugin's dependencies. This is the method that we have historically recommended, though it can be admittedly challenging for non-developer users.

TABLE 1 CellProfiler Plugins in plugins repository version 1.0.0.

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| RunImageJScriptRunImageJScript allows you to run any supported ImageJ script directly within CellProfiler using PyImageJ.14 It is significantly more performant than RunImageJMacro and is also less likely to leave behind temporary files.RunOmniposeRunOmnipose allows you to run Omnipose29 within CellProfiler. Omnipose is a general image segmentation tool that builds on Cellpose.RunStardistRunStarDist allows you to run StarDist30 within CellProfiler. StarDist is a machine-learning algorithm for object detection with star-convex shapes making it best suited for nuclei or round-ish cells. You can use pre-trained StarDist models or your custom model with this plugin. | RunDeepProfiler | RunDeepProfiler allows you to create DeepProfiler ²⁸ features within CellProfiler. DeepProfiler is a comprehensive suite of tools designed to leverage deep learning techniques for the analysis of imaging data in high-throughput biological experiments. | |
| RunOmniposeRunOmnipose allows you to run Omnipose29 within CellProfiler. Omnipose is a general image segmentation tool that builds on Cellpose.RunStardistRunStarDist allows you to run StarDist30 within CellProfiler. StarDist is a machine-learning algorithm for object detection with star-convex shapes making it best suited for nuclei or round-ish cells. You can use pre-trained StarDist models or your custom model with this plugin. | RunImageJScript | RunImageJScript allows you to run any supported ImageJ script <i>directly</i> within CellProfiler using PyImageJ. ¹⁴ It is significantly more performant than RunImageJMacro and is also less likely to leave behind temporary files. | |
| RunStardist RunStarDist allows you to run StarDist ³⁰ within CellProfiler. StarDist is a machine-learning algorithm for object detection with star-convex shapes making it best suited for nuclei or round-ish cells. You can use pre-trained StarDist models or your custom model with this plugin. | RunOmnipose | RunOmnipose allows you to run Omnipose ²⁹ within CellProfiler. Omnipose is a general image segmentation tool that builds on Cellpose. | |
| | RunStardist | RunStarDist allows you to run StarDist ³⁰ within CellProfiler. StarDist is a machine-learning algorithm for object detection with star-convex shapes making it best suited for nuclei or round-ish cells. You can use pre-trained StarDist models or your custom model with this plugin. | |

2.2.2 | Installation of Python dependencies into a built application

A developer who wishes to add support for a particular plugin to CellProfiler could add their dependencies directly to the built CellProfiler application and then distribute the modified application, simplifying plugin use for non-developer users. Further explanation is given in the plugins documentation at https://plugins.cellprofiler.org. Since the extra dependencies will be operating system dependent, and since the source of such a modified version should be extremely well trusted before a user attempts to use it, this approach does not scale widely, but could be useful in the context of an individual laboratory or core facility.

2.2.3 | Access of external dependencies via containers

Since recent analyses suggest users who spend more of their time on 'imaging' than 'image analysis' self-report



FIGURE 2 Wordcloud of terms used in ~200 forum.image.sc posts tagged with 'cellprofiler' from February to May of 2023. 'plugin' (top right), 'install' (bottom center), 'StarDist' (top left) and 'cellpose' (center left) are all visible.

lower computational comfort than those who spend more time on image analysis,¹¹ and installation issues can be a particular pain point in working with bioimage analysis software,⁴ solutions that require Python installation may exclude a sizable fraction of the users of bioimage analysis tools, especially beginning users. Software containers such as Docker³⁴ and Singularity³⁵ are increasingly used to distribute open-source software tools; the Biocontainers repository³⁶ contains more than 1000 software tools packaged into containers. Containers simplify dependency management (since packages are installed once and only once during creation of the container) and especially version conflicts between packages (since each tool in a workflow can live in a separate container or application). A built copy of CellProfiler that can access containers can therefore, in theory, access any other software that has been containerised. With this in mind, we have recently converted the RunCellpose module to optionally use a Cellpose Docker container so that Python and Cellpose installation are no longer required, which makes our RunCellpose plugin accessible to even the most computationally novice users as the only additional installation it requires is the one-click download of Docker. We are currently working to bring the optional use of Docker to other plugins which are compatible with CellProfiler 4 (the current version), and to modules packaged with CellProfiler 5.

2.3 | Creating new CellProfiler plugins

For developers wishing to create new CellProfiler plugins, a number of resources are available. Module templates are available in the main CellProfiler repository at https://github.com/CellProfiler/CellProfiler/tree/master/ cellprofiler/modules/plugins; the plugin templates walk developers through the various steps required to create several classes of CellProfiler modules (such as 'image processing' and 'measurement'). Additional documentation about plugins and the plugin repository submission process is available at https://plugins.cellprofiler.org. Finally, a video in the NEUBIAS academy YouTube series³⁷ walks developers through the process of designing and creating a new plugin, including incorporating module settings, design decisions, and how to incorporate the actual run code into the module, which is typically the simplest part of writing a new plugin.

While the above approaches are suitable for addition of everything from simple library calls to large external tools to CellProfiler, developers of the latter may reasonably be concerned that by distributing their tool inside a CellProfiler plugin, the end user may not fully appreciate that it is an independent piece of software and forget to credit and/or cite it. To alleviate these concerns, CellProfiler has added a 'doi' property to modules, and added Digital Object Identifier (DOI) citations to all existing plugins relying on

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external libraries. These DOIs feed into a citation generator tool in CellProfiler that allows users to easily export a list of citations required by their pipeline. This functionality is currently available in CellProfiler's source code and will be widely available in the upcoming CellProfiler 5 release.

3 | DISCUSSION

In the 17 years since the original CellProfiler paper³⁸ was published, CellProfiler has been cited or mentioned in more than 15,000 academic papers. After a code-free pipeline creation process that the user can tune to their own data, it is designed to multiprocess small-to-medium datasets locally on the user's computer or, with a few clicks, it can export files allowing a locally tuned pipeline to be used on clusters³⁸ or the cloud.⁹ These abilities have earned CellProfiler a reputation as a friendly and easy-to-use platform.³⁹ In contrast to CellProfiler's early days, however, we now live in a scientific world where new bioimage analysis libraries and tools become available weekly or even daily. We argue that having central workflow platforms that make it easy for developers to share their work and users to easily try it facilitates adoption and knowledge sharing, accelerating the field as a whole.

CellProfiler is certainly not the only such workflow platform - Fiji notably contains more than 10,000 externally contributed plugins, and other tools exist in this space, some of which require minimal or no code (Icy, KNIME) and some of which require code for many or most applications (Jupyter, napari). Within this ecosystem, CellProfiler specialises in shareable, code-free, linear image analysis workflows; users should choose the platform that best fits their needs. Efforts to make multiple deep learning models available, such as DeepImageJ⁴⁰ and the Bioimage Model Zoo,⁴¹ while not workflow tools, also help users find image analysis solutions, and to try a number of different deep learning tools side by side. However, CellProfiler's module templates and ability to use containerised tools help ease the lift for developers in creating easily disseminated plugins, and contribution to a centralised repository helps both developers and users with discovery of new tools. These changes, along with improved ease of citation, we believe make CellProfiler's plugin system a valuable part of the image analysis workflow ecosystem.

4 | CONCLUSION

Bioimage analysis is now a firmly established part of the bioimaging universe; qualitative conclusions drawn from human observations are slowly but steadily being replaced by quantitative measurements of particular

image or object properties. This replacement process can be accelerated by giving software users friendly, interactive workflow tools that give them beginning-to-end solutions for their image analysis tasks. Plugins play an important role in this process as they allow users to perform specific but necessary steps to customise and extend their analyses. Plugin creation allows for rapid and straightforward integration of new tools, components and approaches, while allowing users continuity using workflow tools they are already familiar with. We hope the improvement of the CellProfiler-plugins repository encourages developers to contribute plugins, and encourages users to integrate these plugins into their CellProfiler pipelines. These approaches will lead to faster workflow creation and more reproducible workflows for all those using these tools to solve their image analysis problems.

5 | MATERIALS AND METHODS

Current modules in the CellProfiler-plugins repository are written for Python⁴² 3.8 and designed to be used with Cell-Profiler 4^{10} – the repository also contains unmaintained plugins designed for CellProfiler 2^{43} and CellProfiler 3.⁹ Dependencies for each plugin are indicated in the repository's setup.py file.

Figures in this paper were generated in Jupyter notebook¹⁵ 6.4.12 running Python 3.8.16 using the matplotlib⁴⁴ 3.7.1, pandas²³ 1.5.2, seaborn⁴⁵ 0.12.2 and wordcloud⁴⁶ 1.9.2 libraries.

AUTHOR CONTRIBUTIONS

Co-authors BDR, CTC, DRS, EW, FG-F and RAS all contributed equally to the work presented in this manuscript, and each has the right to list themselves first in author order on their CVs.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no competing interests associated with the manuscript.

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