

Multiplex protein imaging in tumour biology

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Tool style	Open-source Tools	Analysis Process										Reference	Summary
		Image Correction Normalization	Stitching Registration	Cell/Nuclei Segmentation	Clustering Classification	Cell-cell Interaction	Deconvolution Annotation	Visualization	Neighborhood Analysis	Trajectory	3D Reconstruction		
Basic	Fiji	✓	✓	✓				✓			✓	doi:10.1038/nmeth.2019	An open-source software imageJ focused on biological-image analysis. Fiji uses modern software engineering practices to combine powerful software libraries with a broad range of scripting languages to enable rapid prototyping of image-processing algorithms.
	Napari						✓	✓				doi:10.5281/ZENODO.3555620	A multi-dimensional image viewer for Python
	QuPath			✓	✓	✓	✓	✓				doi: 10.1038/s41598-017-17204-5	Open source software for digital pathology and whole slide image analysis, which incorporates extensive annotation and visualization tools
GUI	CellProfiler 4	✓		✓				✓			✓	doi.org/10.1186/s12859-021-04344-9	An open-source image analysis program which allows users without specific training to automate their image analysis by using modular processing pipelines, that simultaneously measures the size, shape, intensity and texture of a variety of cell types in a high throughput manner
	BaSIC	✓										DOI: 10.1038/ncomms14836	An image correction method that achieves high-accuracy with significantly fewer input images, works for diverse imaging conditions and is robust against artefacts
	Ilastik			✓	✓							doi.org/10.1038/s41592-019-0582-9	An easy-to-use interactive tool that brings machine-learning-based (bio)image analysis to end users; contains pre-defined workflows for image segmentation, object classification, counting and tracking.
	Cytokit		✓	✓			✓	✓			✓	doi.org/10.1186/s12859-019-3055-3	A collection of open source tools for quantifying and analyzing properties of individual cells in large fluorescent microscopy datasets. It provides GPU-accelerated implementations of cycle registration and image deconvolution as well as an interactive user interface for characterizing the phenotypic features of cells alongside their spatial distribution.
	Orbit			✓	✓		✓	✓				doi.org/10.1371/journal.pcbi.1007313	An open-source whole slide image analysis tool that consists of a generic tile-processing engine which allows the execution of various image analysis algorithms provided by either Orbit itself or from other open-source platforms using a tile-based map-reduce execution framework.
	HistoCAT				✓	✓		✓	✓			doi.org/10.1038/nmeth.4391	An open-source, computational histology topography cytometry analysis toolbox (histocat) to enable interactive, quantitative, and comprehensive exploration of individual cell phenotypes, cell–cell interactions, microenvironments, and morphological structures within intact tissues
Script	CytoMAP				✓	✓	✓	✓	✓	✓	✓	doi.org/10.1016/j.celrep.2020.107523	A user-friendly, comprehensive, and broadly applicable spatial analysis toolbox for analysis of 2D or 3D quantitative imaging datasets, which excels at utilizing high-dimensional imaging data to reveal complex tissue features based on cellular phenotypic heterogeneity and spatial patterning.
	DeepCell			✓								doi.org/10.1038/s41587-021-01094-0	A deep-learning-enabled segmentation algorithm that generalizes to the full diversity of tissue types and imaging platforms in TissueNet, and achieves human-level performance.
	UnMICST			✓								doi.org/10.1038/s42003-022-04076-3	Optimized universal models maximizing the accuracy of image segmentation by machine learning algorithms in multiplexed tissue images containing common imaging artefacts.
	ASHLAR		✓									doi.org/10.1101/2021.04.20.440625	An open source Python tool for coordinated stitching and registration of individual multiplexed images to generate accurate whole-slide mosaics.
	Facetto				✓	✓		✓	✓			doi.org/10.1101/722918	A scalable visual analytics application that is used to discover single-cell phenotypes in high-dimensional multi-channel microscopy images
	Minerva							✓				doi.org/10.1101/2020.03.27.001834	A single-page web application that uses client-side JavaScript and is easily deployable on standard commercial clouds, which supports interactive views of derived single-cell features within the image space
	Steinbock			✓								doi.org/10.1101/2021.11.12.468357	A user- friendly, customizable, and interoperable workflow for processing and analyzing data generated by highly multiplexed imaging technologies, that supports image pre-processing, segmentation, feature extraction, and standardized data export.
	Cellpose			✓							✓	doi.org/10.1038/s41592-020-01018-x	A generalist, deep learning-based segmentation method, which can precisely segment cells from a wide range of image types and does not require model retraining or parameter adjustments.
	HEMnet	✓	✓					✓				doi.org/10.1038/s41698-022-00252-0	A new automated approach that utilizes immunohistochemistry as an initial molecular label for cancer cells on a H&E image and trains a cancer classifier on the overlapping clinical histopathological images.
	CODA		✓	✓			✓				✓	doi.org/10.1038/s41592-022-01650-9	A method to reconstruct exceptionally large (up to multicentimeter cubed) tissues at subcellular resolution using serially sectioned hematoxylin and eosin-stained tissue sections.
	nucleAIzer	✓		✓								doi.org/10.1016/j.cels.2020.04.003	A deep-learning approach aiming toward a truly general method for localizing 2D cell nuclei across a diverse range of assays and light microscopy modalities, which uses an image-to-image translation using a pixel-wise mapping from one image to the other that ensures the generated synthetic output image resembles the original as closely as possible.
	NaroNet				✓	✓	✓	✓	✓			doi.org/10.1016/j.media.2022.102384	A deep learning framework that models the multi-scale tumor microenvironment from multiplex-stained cancer tissue images and provides patient-level interpretable predictions using a seamless end-to-end learning pipeline.
	Cytomapper							✓				doi: 10.1093/bioinformatics/bta1061	A computational tool written in R, that enables visualization of pixel- and cell-level information obtained by multiplexed imaging
	CIRCLE			✓								doi.org/10.1101/2022.02.27.482183	A cell identification pipeline that combines classical and modern machine learning-based computer vision algorithms to address the shortcomings of current cell segmentation tools for 2D images.
	Squidpy	✓		✓	✓			✓	✓		✓	doi.org/10.1038/s41592-021-01358-2	A Python framework that brings together tools from omics and image analysis to enable scalable description of spatial molecular data, such as transcriptome or multivariate proteins. It provides efficient infrastructure and numerous analysis methods that allow to efficiently store, manipulate and interactively visualize spatial omics data.
	CellSighter				✓			✓				doi.org/10.1101/2022.11.07.515441	An ensemble of convolutional neural networks (CNNs) to perform supervised classification of cells
	CELESTA				✓		✓	✓				doi.org/10.1038/s41592-022-01498-z	An unsupervised machine learning cell type identification method, which does not involve manual gating or clustering and instead leverages the marker expressions and spatial information of cells with minimal user dependence.
	MCMICRO	✓	✓	✓	✓	✓	✓	✓	✓			doi.org/10.1038/s41592-021-01308-y	A scalable, modular and open-source image processing pipeline that performing the sequential steps needed to transform whole-slide images into single-cell data. The pipeline is based on widely accepted software standards and interoperates with any programming language through the use of software containers.
	Giotto				✓	✓		✓	✓			doi.org/10.1186/s13059-021-02286-2	A comprehensive and open-source toolbox for spatial data analysis and visualization. The analysis module provides end-to-end analysis by implementing a wide range of algorithms for characterizing tissue composition, spatial expression patterns, and cellular interactions.
	TissueSchematics								✓			doi.org/10.1016/j.cels.2021.09.012	A conceptual and algorithmic framework for quantifying architectural units in a tissue from multiplex imaging data.
	STELLAR						✓					doi.org/10.1038/s41592-022-01651-8	A geometric deep learning method for cell-type discovery and identification in spatially resolved single-cell datasets.
	Pixie						✓					doi.org/10.1038/s41467-023-40068-5	Quantitative annotation of pixel-level features that captures phenotypes independent of traditional cell segmentation masks
	Spatial-LDA								✓			doi: 10.1089/cmb.2019.0340	A method to identify characteristic cell types overrepresented in neighborhoods that serve as proxies for microenvironment
	LisaClust				✓							doi.org/10.1002/cyto.a.24729	A method for identifying consistent spatial organization of multiple cell types in an unsupervised way, implemented in R.