# cellpose Release 0.7.2

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cellpose is an anatomical segmentation algorithm written in Python 3 by Carsen Stringer and Marius Pachitariu. For support, please open an issue.

We make pip installable releases of cellpose, here is the pypi. You can install it as pip install cellpose[gui].

You can try it out without installing at cellpose.org. Also check out these resources:

Cellpose 2.0

- paper on biorxiv
- twitter paper
- human-in-the-loop training protocol video

#### Cellpose 1.0

- paper on biorxiv (see figure 1 below) and in nature methods
- twitter thread
- Marius's talk

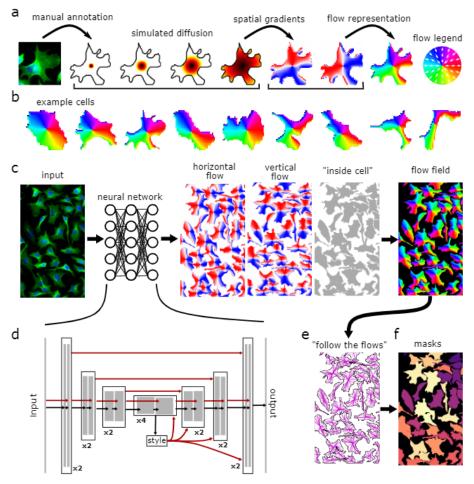


Figure 1: Model architecture. a, Procedure for transforming manually annotated masks into a vector flow representation that can be predicted by a neural network. A simulated diffusion process started at the center of the mask is used to derive spatial gradients that point towards the center of the cell, potentially indirectly around corners. The X and Y gradients are combined into a single normalized direction from 0° to 360°. b, Example spatial flows for cells from the training dataset. cd, A neural network is trained to predict the horizontal and vertical flows, as well as whether a pixel belongs to any cell. The three predicted maps are combined into a flow field. d shows the details of the neural network which contains a standard backbone neural network that downsamples and then upsamples the feature maps, contains skip connections between layers of the same size, and global skip connections from the image styles, computed at the lowest resolution, to all the successive computations. e, At test time, the predicted flow fields are used to construct a dynamical system with fixed points whose basins of attraction represent the predicted masks. Informally, every pixel "follows the flows" along the predicted flow fields towards their eventual fixed point. f, All the pixels that converge to the same fixed point are assigned to the same mask.

ONE

### INSTALLATION

For basic install instructions, look up the main github readme.

## 1.1 Built-in model directory

By default, the pretrained cellpose models are downloaded to \$HOME/.cellpose/models/. This path on linux would look like /home/USERNAME/.cellpose/, and on Windows, C:/Users/USERNAME/.cellpose/models/. These models are downloaded the first time you try to use them, either on the command line, in the GUI or in a notebook.

If you'd like to download the models to a different directory, and are using the command line or the GUI, before you run python -m cellpose ..., you will need to always set the environment variable CELLPOSE\_LOCAL\_MODELS\_PATH (thanks Chris Roat for implementing this!).

To set the environment variable in the command line/Anaconda prompt on windows run the following command modified for your path: set CELLPOSE\_LOCAL\_MODELS\_PATH=C:/PATH\_FOR\_MODELS/. To set the environment variable in the command line on linux, run export CELLPOSE\_LOCAL\_MODELS\_PATH=/PATH\_FOR\_MODELS/.

To set this environment variable when running cellpose in a jupyter notebook, run this code at the beginning of your notebook before you import cellpose:

```
import os
os.environ["CELLPOSE_LOCAL_MODELS_PATH"] = "/PATH_FOR_MODELS/"
```

## 1.2 Common issues

If you receive the error: No module named PyQt5.sip, then try uninstalling and reinstalling pyqt5

```
pip uninstall pyqt5 pyqt5-tools
pip install pyqt5 pyqt5-tools pyqt5.sip
```

If you have errors related to OpenMP and libiomp5, then try

:: conda install nomkl

If you receive an error associated with **matplotlib**, try upgrading it:

pip install matplotlib --upgrade

If you receive the error: ImportError: \_arpack DLL load failed, then try uninstalling and reinstalling scipy

pip uninstall scipy
pip install scipy

If you are having issues with the graphical interface, make sure you have python 3.7 and not python 3.8 installed.

If you are on Yosemite Mac OS or earlier, PyQt doesn't work and you won't be able to use the graphical interface for cellpose. More recent versions of Mac OS are fine. The software has been heavily tested on Windows 10 and Ubuntu 18.04, and less well tested on Mac OS. Please post an issue if you have installation problems.

## **1.3 Dependencies**

cellpose relies on the following excellent packages (which are automatically installed with conda/pip if missing):

- pytorch
- pyqtgraph
- PyQt5
- numpy (>=1.16.0)
- numba
- scipy
- scikit-image
- natsort
- matplotlib

#### TWO

### GUI

### 2.1 Starting the GUI

The quickest way to start is to open the GUI from a command line terminal. You might need to open an anaconda prompt if you did not add anaconda to the path:

python -m cellpose

The first time cellpose runs it downloads the latest available trained model weights from the website.

You can **drag and drop** images (.tif, .png, .jpg, .gif) into the GUI and run Cellpose, and/or manually segment them. When the GUI is processing, you will see the progress bar fill up and during this time you cannot click on anything in the GUI. For more information about what the GUI is doing you can look at the terminal/prompt you opened the GUI with. For example data, See [website](http://www.cellpose.org). For best accuracy and runtime performance, resize images so cells are less than 100 pixels across.

For multi-channel, multi-Z tiff's, the expected format is Z x channels x Ly x Lx.

**Note:** The output file with the masks is in the same folder as the loaded image with \_seg.npy appended. The GUI automatically saves after you draw an ROI but NOT after running a model for segmentation and NOT after 3D mask drawing (too slow). Save in the file menu or with Ctrl+S.

**Note:** Since the output file is in the same folder as the loaded image with \_seg.npy appended, make sure you have WRITE access in the folder, otherwise the file will not save.

## 2.2 Using the GUI

The GUI serves two main functions:

- 1. Running the segmentation algorithm.
- 2. Manually labelling data.
- 3. (NEW) Fine-tuning a pretrained cellpose model on your own data.

Main GUI mouse controls (works in all views):

- Pan = left-click + drag
- Zoom = scroll wheel (or +/= and buttons)

- Full view = double left-click
- Select mask = left-click on mask
- Delete mask = Ctrl (or Command on Mac) + left-click
- Merge masks = Alt + left-click (will merge last two)
- Start draw mask = right-click
- End draw mask = right-click, or return to circle at beginning

Overlaps in masks are NOT allowed. If you draw a mask on top of another mask, it is cropped so that it doesn't overlap with the old mask. Masks in 2D should be single strokes (if *single\_stroke* is checked).

If you want to draw masks in 3D, then you can turn *single\_stroke* option off and draw a stroke on each plane with the cell and then press ENTER (cellpose 1.0 only currently). 3D labelling will fill in unlabelled z-planes so that you do not have to as densely label.

## 2.3 Segmentation options

SIZE: you can manually enter the approximate diameter for your cells, or press "calibrate" to let the model estimate it. The size is represented by a disk at the bottom of the view window (can turn this disk off by unchecking "scale disk on").

use GPU: if you have installed the cuda version of mxnet, then you can activate this, but it won't give huge speedups when running single images in the GUI.

MODEL: there is a cytoplasm model and a nuclei model, choose what you want to segment

CHAN TO SEG: this is the channel in which the cytoplasm or nuclei exist

CHAN2 (OPT): if cytoplasm model is chosen, then choose the nuclear channel for this option

### 2.4 Training your own cellpose model

Check out this video to learn the process.

- 1. Drag and drop an image from a folder of images with a similar style (like similar cell types).
- 2. Run the built-in models on one of the images using the "model zoo" and find the one that works best for your data. Make sure that if you have a nuclear channel you have selected it for CHAN2.
- 3. Fix the labelling by drawing new ROIs (right-click) and deleting incorrect ones (CTRL+click). The GUI autosaves any manual changes (but does not autosave after running the model, for that click CTRL+S). The segmentation is saved in a \_seg.npy file.
- 4. Go to the "Models" menu in the File bar at the top and click "Train new model..." or use shortcut CTRL+T.
- 5. Choose the pretrained model to start the training from (the model you used in #2), and type in the model name that you want to use. The other parameters should work well in general for most data types. Then click OK.
- 6. The model will train (much faster if you have a GPU) and then auto-run on the next image in the folder. Next you can repeat #3-#5 as many times as is necessary.
- 7. The trained model is available to use in the future in the GUI in the "custom model" section and is saved in your image folder.

## 2.5 Contributing training data

We are very excited about receiving community contributions to the training data and re-training the cytoplasm model to make it better. Please follow these guidelines:

- 1. Run cellpose on your data to see how well it does. Try varying the diameter, which can change results a little.
- 2. If there are relatively few mistakes, it won't help much to contribute labelled data.
- 3. If there are consistent mistakes, your data is likely very different from anything in the training set, and you should expect major improvements from contributing even just a few manually segmented images.
- 4. For images that you contribute, the cells should be at least 10 pixels in diameter, and there should be **at least** several dozens of cells per image, ideally ~100. If your images are too small, consider combining multiple images into a single big one and then manually segmenting that. If they are too big, consider splitting them into smaller crops.
- 5. For the manual segmentation, please try to outline the boundaries of the cell, so that everything (membrane, cytoplasm, nucleus) is inside the boundaries. Do not just outline the cytoplasm and exclude the membrane, because that would be inconsistent with our own labelling and we wouldn't be able to use that.
- 6. Do not use the results of the algorithm in any way to do contributed manual segmentations. This can reinforce a vicious circle of mistakes, and compromise the dataset for further algorithm development.

If you are having problems with the nucleus model, please open an issue before contributing data. Nucleus images are generally much less diverse, and we think the current training dataset already covers a very large set of modalities. Additionally, you can run a non-nuclear model on nuclear data such as cyto.

| Keyboard shortcuts | Description   |
|--------------------|---|
| CTRL+H             | help  |
| =/+ // -           | zoom in // zoom out   |
| CTRL+Z             | undo previously drawn mask/stroke   |
| CTRL+0             | clear all masks   |
| CTRL+L             | load image (can alternatively drag and drop image)                            |
| CTRL+S             | SAVE MASKS IN IMAGE to _seg.npy file  |
| CTRL+T             | start model training using _seg.npy files                                     |
| CTRL+P             | load _seg.npy file (note: it will load automatically with image if it exists) |
| CTRL+M             | load masks file (must be same size as image with 0 for NO mask, and 1,2,3 for |
|                    | masks)  |
| A/D or LEFT/RIGHT  | cycle through images in current directory                                     |
| W/S or UP/DOWN     | change color (RGB/gray/red/green/blue)  |
| R / G / B          | press to toggle RGB and Red or Green or Blue                                  |
| PAGE-UP / PAGE-    | change to flows and cell prob views (if segmentation computed)                |
| DOWN               |   |
| X                  | turn masks ON or OFF  |
| Ζ                  | toggle outlines ON or OFF   |
| ,/.                | increase / decrease brush size for drawing                                    |

## 2.6 Keyboard shortcuts

THREE

### INPUTS

You can use tiffs or PNGs or JPEGs. We use the image loader from scikit-image. Single plane images can read into data as nY x nX x channels or channels x nY x nX. Then the channels settings will take care of reshaping the input appropriately for the network. Note the model also rescales the input for each channel so that 0 = 1st percentile of image values and 1 = 99th percentile.

If you want to run multiple images in a directory, use the command line or a jupyter notebook to run cellpose.

### 3.1 3D segmentation

Tiffs with multiple planes and multiple channels are supported in the GUI (can drag-and-drop tiffs) and supported when running in a notebook. Multiplane images should be of shape nplanes x channels x nY x nX or as nplanes x nY x nX. You can test this by running in python

```
import tifffile
data = tifffile.imread('img.tif')
print(data.shape)
```

If drag-and-drop of the tiff into the GUI does not work correctly, then it's likely that the shape of the tiff is incorrect. If drag-and-drop works (you can see a tiff with multiple planes), then the GUI will automatically run 3D segmentation and display it in the GUI. Watch the command line for progress. It is recommended to use a GPU to speed up processing.

When running cellpose in a notebook, set do\_3D=True to enable 3D processing. You can give a list of 3D inputs, or a single 3D/4D stack. When running on the command line, add the flag --do\_3D (it will run all tiffs in the folder as 3D tiffs if possible).

If the 3D segmentation is not working well and there is inhomogeneity in Z, try stitching masks in Z instead of running do\_3D=True. See details for this option here: stitch\_threshold.

If drag-and-drop doesn't work because of the shape of your tiff, you need to transpose the tiff and resave to use the GUI, or use the napari plugin for cellpose, or run CLI/notebook and specify the channel\_axis and/or z\_axis parameters:

channel\_axis and z\_axis can be used to specify the axis (0-based) of the image which corresponds to the image channels and to the z axis. For example an image with 2 channels of shape (1024,1024,2,105,1) can be specified with channel\_axis=2 and z\_axis=3. If channel\_axis=None cellpose will try to automatically determine the channel axis by choosing the dimension with the minimal size after squeezing. If z\_axis=None cellpose will automatically select the first non-channel axis of the image to be the Z axis. These parameters can be specified using the command line with --channel\_axis or --z\_axis or as inputs to model.eval for the Cellpose or CellposeModel model.

FOUR

### SETTINGS

The important settings are described on this page. See the Cellpose class for all run options.

Here is an example of calling the Cellpose class and running a list of images for reference:

You can make lists of channels/diameter for each image, or set the same channels/diameter for all images as shown in the example above.

### 4.1 Channels

There are two channels inputs. The first channel is the channel you want to segment. The second channel is an optional channel that is helpful in models trained with images with a nucleus channel. See more details in the models page.

- 1. 0=grayscale, 1=red, 2=green, 3=blue
- 2. 0=None (will set to zero), 1=red, 2=green, 3=blue

Set channels to a list with each of these elements, e.g. channels = [0,0] if you want to segment cells in grayscale or for single channel images, or channels = [2,3] if you green cells with blue nuclei.

On the command line the above would be -- chan 0 -- chan 2 -

## 4.2 Diameter

The cellpose models have been trained on images which were rescaled to all have the same diameter (30 pixels in the case of the *cyto* model and 17 pixels in the case of the *nuclei* model). Therefore, cellpose needs a user-defined cell diameter (in pixels) as input, or to estimate the object size of an image-by-image basis.

The automated estimation of the diameter is a two-step process using the *style* vector from the network, a 64-dimensional summary of the input image. We trained a linear regression model to predict the size of objects from these style vectors on the training data. On a new image the procedure is as follows.

- 1. Run the image through the cellpose network and obtain the style vector. Predict the size using the linear regression model from the style vector.
- 2. Resize the image based on the predicted size and run cellpose again, and produce ROIs. Take the final estimated size as the median diameter of the predicted ROIs.

For automated estimation set diameter = None. However, if this estimate is incorrect please set the diameter by hand.

Changing the diameter will change the results that the algorithm outputs. When the diameter is set smaller than the true size then cellpose may over-split cells. Similarly, if the diameter is set too big then cellpose may over-merge cells.

## 4.3 Resample

The cellpose network is run on your rescaled image – where the rescaling factor is determined by the diameter you input (or determined automatically as above). For instance, if you have an image with 60 pixel diameter cells, the rescaling factor is 30./60. = 0.5. After determining the flows (dX, dY, cellprob), the model runs the dynamics. The dynamics can be run at the rescaled size (resample=False), or the dynamics can be run on the resampled, interpolated flows at the true image size (resample=True). resample=True will create smoother ROIs when the cells are large but will be slower in case; resample=False will find more ROIs when the cells are small but will be slower in this case. By default in versions >=1.0 resample=True.

The nuclear model in cellpose is trained on two-channel images, where the first channel is the channel to segment, and the second channel is always set to an array of zeros. Therefore set the first channel as 0=grayscale, 1=red, 2=green, 3=blue; and set the second channel to zero, e.g. channels = [0,0] if you want to segment nuclei in grayscale or for single channel images, or channels = [3,0] if you want to segment blue nuclei.

If the nuclear model isn't working well, try the cytoplasmic model.

### 4.4 Flow threshold (aka model fit threshold in GUI)

Note there is nothing keeping the neural network from predicting horizontal and vertical flows that do not correspond to any real shapes at all. In practice, most predicted flows are consistent with real shapes, because the network was only trained on image flows that are consistent with real shapes, but sometimes when the network is uncertain it may output inconsistent flows. To check that the recovered shapes after the flow dynamics step are consistent with real ROIs, we recompute the flow gradients for these putative predicted ROIs, and compute the mean squared error between them and the flows predicted by the network.

The flow\_threshold parameter is the maximum allowed error of the flows for each mask. The default is flow\_threshold=0.4. Increase this threshold if cellpose is not returning as many ROIs as you'd expect. Similarly, decrease this threshold if cellpose is returning too many ill-shaped ROIs.

### 4.5 Mask threshold

The network predicts 3 outputs: flows in X, flows in Y, and cell "probability". The predictions the network makes of the probability are the inputs to a sigmoid centered at zero  $(1 / (1 + e^{-x}))$ , so they vary from around -6 to +6. The pixels greater than the cellprob\_threshold are used to run dynamics and determine ROIs. The default is cellprob\_threshold=0.0. Decrease this threshold if cellpose is not returning as many ROIs as you'd expect. Similarly, increase this threshold if cellpose is returning too ROIs particularly from dim areas.

## 4.6 3D settings

Volumetric stacks do not always have the same sampling in XY as they do in Z. Therefore you can set an anisotropy parameter to allow for differences in sampling, e.g. set to 2.0 if Z is sampled half as dense as X or Y.

There may be additional differences in YZ and XZ slices that make them unable to be used for 3D segmentation. I'd recommend viewing the volume in those dimensions if the segmentation is failing. In those instances, you may want to turn off 3D segmentation (do\_3D=False) and run instead with stitch\_threshold>0. Cellpose will create ROIs in 2D on each XY slice and then stitch them across slices if the IoU between the mask on the current slice and the next slice is greater than or equal to the stitch\_threshold.

3D segmentation ignores the flow\_threshold because we did not find that it helped to filter out false positives in our test 3D cell volume. Instead, we found that setting min\_size is a good way to remove false positives.

## OUTPUTS

Internally, the network predicts 3 (or 4) outputs: (flows in Z), flows in Y, flows in X, and cellprob. The predictions the network makes of cellprob are the inputs to a sigmoid centered at zero  $(1 / (1 + e^{-x}))$ , so they vary from around -6 to +6.

## 5.1 \_seg.npy output

\*\_seg.npy files have the following fields:

- *filename* : filename of image
- *img* : image with chosen channels (nchan x Ly x Lx) (if not multiplane)
- masks : each pixel in the image is assigned to an ROI (0 = NO ROI; 1, 2, ... = ROI labels)
- colors : colors for ROIs
- *outlines* : outlines of ROIs (0 = NO outline; 1,2,... = outline labels)
- *chan\_choose* : channels that you chose in GUI (0=gray/none, 1=red, 2=green, 3=blue)
- *ismanual* : element k = whether or not mask k was manually drawn or computed by the cellpose algorithm
- *flows* [flows[0] is XY flow in RGB, flows[1] is the cell probability in range 0-255 instead of 0.0 to 1.0, flows[2] is Z flow in range 0-255 (if it exists, otherwise zeros),] flows[3] is [dY, dX, cellprob] (or [dZ, dY, dX, cellprob] for 3D), flows[4] is pixel destinations (for internal use)
- est\_diam : estimated diameter (if run on command line)
- *zdraw* : for each mask, which planes were manually labelled (planes in between manually drawn have interpolated ROIs)

Here is an example of loading in a  $\texttt{*\_seg.npy}$  file and plotting masks and outlines

(continues on next page)

(continued from previous page)

```
for o in outlines:
    plt.plot(o[:,0], o[:,1], color='r')
```

If you run in a notebook and want to save to a \*\_seg.npy file, run

```
from cellpose import io
io.masks_flows_to_seg(images, masks, flows, diams, file_name, channels)
```

where each of these inputs is a list (as the output of model.eval is)

## 5.2 PNG output

You can save masks to PNG in the GUI.

To save masks (and other plots in PNG) using the command line, add the flag --save\_png.

Or use the function below if running in a notebook

```
from cellpose import io
io.save_to_png(images, masks, flows, image_names)
```

## 5.3 ROI manager compatible output for ImageJ

You can save the outlines of ROIs in a text file that's compatible with ImageJ ROI Manager in the GUI File menu.

To save using the command line, add the flag --save\_outlines.

Or use the function below if running in a notebook

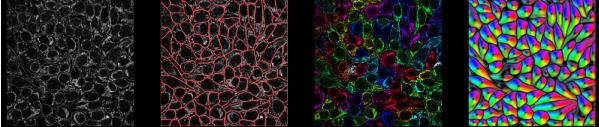
```
from cellpose import io, utils
# image_name is file name of image
# masks is numpy array of masks for image
base = os.path.splitext(image_name)[0]
outlines = utils.outlines_list(masks)
io.outlines_to_text(base, outlines)
```

To load this \_cp\_outlines.txt file into ImageJ, use the python script provided in cellpose: imagej\_roi\_converter.py. Run this as a macro after opening your image file. It will ask you to input the path to the \_cp\_outlines.txt file. Input that and the ROIs will appear in the ROI manager.

## 5.4 Plotting functions

In plot.py there are functions, like show\_segmentation:

```
from cellpose import plot
nimg = len(imgs)
for idx in range(nimg):
    maski = masks[idx]
    flowi = flows[idx][0]
    fig = plt.figure(figsize=(12,5))
    plot.show_segmentation(fig, imgs[idx], maski, flowi, channels=channels[idx])
    plt.tight_layout()
    plt.show()
```



SIX

### MODELS

from cellpose import models

Cellpose 2.0 now has a model zoo and options for user model training. Each model will be downloaded automatically to your models.MODELS\_DIR (see Installation instructions for more details on MODELS\_DIR). See paper for more details on the model zoo. You can also directly download a model by going to the URL, e.g.:

https://www.cellpose.org/models/MODEL\_NAME

### 6.1 Model Zoo

All built-in models were trained with the ROIs resized to a diameter of 30.0 (diam\_mean = 30), except the '*nuclei*' model which was trained with a diameter of 17.0 (diam\_mean = 17). The models will internally take care of rescaling the images given a user-provided diameter (or with the diameter from auto-diameter estimation in full models).

### 6.2 Full built-in models

These models have a size model and 4 different training versions, each trained starting from 4 different random initial parameter sets. This means you can run with diameter=0 or --diameter 0 and the model can estimate the ROI size. Also you can set net\_avg=True or --net\_avg to average the results of the 4 models.

These models can be loaded and used in the notebook with models.Cellpose(model\_type='cyto') or in the command line with python -m cellpose --pretrained\_model cyto.

These models' names (to download all the models for a class run with --net\_avg): \* 'cyto': cytotorch\_0, cytotorch\_1, cytotorch\_2, cytotorch\_3, size\_cytotorch\_0.npy \* 'nuclei': nucleitorch\_0, nucleitorch\_1, nucleitorch\_2, nucleitorch\_3, size\_nucleitorch\_0.npy \* 'cyto2': cyto2torch\_0, cyto2torch\_1, cyto2torch\_2, cyto2torch\_3, size\_cyto2torch\_0.npy

#### 6.2.1 Cytoplasm model ('cyto')

The cytoplasm model in cellpose is trained on two-channel images, where the first channel is the channel to segment, and the second channel is an optional nuclear channel. Here are the options for each: 1. 0=grayscale, 1=red, 2=green, 3=blue 2. 0=None (will set to zero), 1=red, 2=green, 3=blue

Set channels to a list with each of these elements, e.g. channels = [0, 0] if you want to segment cells in grayscale or for single channel images, or channels = [2,3] if you green cells with blue nuclei.

#### 6.2.2 Nucleus model ('nuclei')

The nuclear model in cellpose is trained on two-channel images, where the first channel is the channel to segment, and the second channel is always set to an array of zeros. Therefore set the first channel as 0=grayscale, 1=red, 2=green, 3=blue; and set the second channel to zero, e.g. channels = [0,0] if you want to segment nuclei in grayscale or for single channel images, or channels = [3,0] if you want to segment blue nuclei.

#### 6.2.3 Cytoplasm 2.0 model ('cyto2')

The cytoplasm 2.0 model in cellpose is trained on two-channel images, where the first channel is the channel to segment, and the second channel is an optional nuclear channel, as the cytoplasm model.

In addition to the training data in our dataset, it was trained with user-submitted images.

### 6.3 Other built-in models

These models do not have a size model and 4 different training versions. If the diameter is set to 0.0, then the model uses the default diam\_mean for the diameter (30.0).

These models can be loaded and used in the notebook with e.g. models. CellposeModel(model\_type='tissuenet') or models.CellposeModel(model\_type='LC2'), or in the command line with python -m cellpose --pretrained\_model tissuenet.

These models' names are the same as their strings in the GUI.

#### 6.3.1 TissueNet models

The 'tissuenet' model was trained on all training images from the tissuenet dataset. These images have a cytoplasm channel and a nuclear channel. The other tissuenet models ('TN1', 'TN2', and 'TN3') were trained on subsets of the tissuenet dataset that had similar characteristics.

#### 6.3.2 LiveCell models

The 'livecell' model was trained on all training images from the livecell dataset. These images only have a cytoplasm channel. The other livecell models ('LC1', 'LC2', 'LC3', and 'LC4') were trained on subsets of the livecell dataset that had similar characteristics.

### 6.4 User-trained models

By default, models are trained with the ROIs resized to a diameter of  $30.0 \text{ (diam_mean} = 30)$  – this is necessary if you want to start from a pretrained cellpose model. If you want to use a different diameter and use pretraining, we recommend performing training yourself on the cellpose dataset with that diameter so the model learns objects at that size. All user-trained models will save the diam\_mean so it will be loaded automatically along with the model weights.

Each model also saves the diam\_labels which is the mean diameter of the ROIs in the training images. This value is auto-loaded into the GUI for use with the model, or will be used if the diameter is 0 (diameter=0 or --diameter 0).

These models can be loaded and used in the notebook with e.g. models.CellposeModel(model\_type='/full/path/to/model') or in the command line with python -m cellpose --pretrained\_model /full/path/to/model.

#### SEVEN

## TRAINING

At the beginning of training, cellpose computes the flow field representation for each mask image (dynamics. labels\_to\_flows).

The cellpose pretrained models are trained using resized images so that the cells have the same median diameter across all images. If you choose to use a pretrained model, then this fixed median diameter is used.

If you choose to train from scratch, you can set the median diameter you want to use for rescaling with the --diam\_mean flag. We trained all model zoo models with a diameter of 30.0 pixels, except the *nuclei* model which used a diameter of 17 pixels, so if you want to start with a pretrained model, it will default to those values.

The models will be saved in the image directory (--dir) in a folder called models/.

The same channel settings apply for training models.

Note Cellpose expects the labelled masks (0=no mask, 1,2...=masks) in a separate file, e.g:

wells\_000.tif
wells\_000\_masks.tif

You can use a different ending from \_masks with the --mask\_filter option, e.g. --mask\_filter \_masks\_2022.

Also, you can train a model using the labels from the GUI (\_seg.npy) by using the following option --mask\_filter \_seg.npy.

If you use the *-*img\_filter option (--img\_filter \_img in this case):

wells\_000\_img.tif
wells\_000\_masks.tif

Warning: The path given to --dir and --test\_dir should be an absolute path.

To train on cytoplasmic images (green cyto and red nuclei) starting with a pretrained model from cellpose (one of the model zoo models), we also have included the recommended training parameters in the command below:

You can train from scratch as well:

```
python -m cellpose --train --dir ~/images_nuclei/train/ --pretrained_model None
```

To train the cyto model from scratch using the same parameters we did, download the dataset and run

You can also specify the full path to a pretrained model to use:

```
python -m cellpose --dir ~/images_cyto/test/ --pretrained_model ~/images_cyto/test/model/

$\to$cellpose_35_0 --save_png$
```

Training arguments

| train                           | train network using images <b>in</b> dir                   |  |  |  |  |  |
|---------------------------------|--|--|--|--|--|--|
| train_size                      | train size network at end of training                      |  |  |  |  |  |
| test_dir TEST_DIR               | folder containing test data (optional)                     |  |  |  |  |  |
| mask_filter MASK_FILTER         |  |  |  |  |  |  |
|                                 | end string <b>for</b> masks to run on. Default: _masks     |  |  |  |  |  |
| diam_mean DIAM_MEAN             |  |  |  |  |  |  |
|                                 | mean diameter to resize cells to during training <b>if</b> |  |  |  |  |  |
|                                 | starting from pretrained models it cannot be changed       |  |  |  |  |  |
|                                 | <b>from</b> 30.0   |  |  |  |  |  |
| learning_rate LEARNING_RATE     |  |  |  |  |  |  |
|                                 | learning rate. Default: 0.2                                |  |  |  |  |  |
| weight_decay WEIGHT_DECAY       |  |  |  |  |  |  |
|                                 | weight decay. Default: 1e-05                               |  |  |  |  |  |
| n_epochs N_EPOCHS               | number of epochs. Default: 500                             |  |  |  |  |  |
| batch_size BATCH_SI             | ZE   |  |  |  |  |  |
|                                 | batch size. Default: 8                                     |  |  |  |  |  |
| min_train_masks MIN_TRAIN_MASKS |  |  |  |  |  |  |
|                                 | minimum number of masks a training image must have to      |  |  |  |  |  |
|                                 | be used. Default: 5  |  |  |  |  |  |
| residual_on RESIDUA             | L_ON   |  |  |  |  |  |
|                                 | use residual connections                                   |  |  |  |  |  |
| <pre>style_on STYLE_ON</pre>    | use style vector   |  |  |  |  |  |
| concatenation CONCA             | concatenation CONCATENATION                                |  |  |  |  |  |
|                                 | concatenate downsampled layers with upsampled layers       |  |  |  |  |  |
|                                 | (off by default which means they are added)                |  |  |  |  |  |
| save_every SAVE_EVERY           |  |  |  |  |  |  |
|                                 | number of epochs to skip between saves. Default: 100       |  |  |  |  |  |
| save_each                       | save the model under a different filename per              |  |  |  |  |  |
|                                 | save_every epoch <b>for</b> later comparsion               |  |  |  |  |  |

#### EIGHT

### **IN A NOTEBOOK**

See settings for more information on run settings.

```
import numpy as np
import matplotlib.pyplot as plt
from cellpose import models
from cellpose.io import imread
# model_type='cyto' or 'nuclei' or 'cyto2'
model = models.Cellpose(model_type='cyto')
# list of files
# PUT PATH TO YOUR FILES HERE!
files = ['/media/carsen/DATA1/TIFFS/onechan.tif']
imgs = [imread(f) for f in files]
nimg = len(imgs)
# define CHANNELS to run segementation on
# grayscale=0, R=1, G=2, B=3
# channels = [cytoplasm, nucleus]
# if NUCLEUS channel does not exist, set the second channel to 0
channels = [[0,0]]
# IF ALL YOUR IMAGES ARE THE SAME TYPE, you can give a list with 2 elements
# channels = [0,0] # IF YOU HAVE GRAYSCALE
# channels = [2,3] # IF YOU HAVE G=cytoplasm and B=nucleus
# channels = [2,1] # IF YOU HAVE G=cytoplasm and R=nucleus
# if diameter is set to None, the size of the cells is estimated on a per image basis
# you can set the average cell `diameter` in pixels yourself (recommended)
# diameter can be a list or a single number for all images
masks, flows, styles, diams = model.eval(imgs, diameter=None, channels=channels)
```

See full notebook at run\_cellpose.ipynb.

### NINE

### **COMMAND LINE**

### 9.1 Input settings

- dir: (string) directory of images
- img\_filter: (string) (optional) ending of filenames (excluding extension) for processing

### 9.2 Run settings

These are the same settings, but set up for the command line, e.g. *channels* = [*chan, chan2*].

- chan: (int) channel to segment, ones-based because zero is gray (average of all channels) 0 = grayscale; 1
   = red; 2 = green; 3 = blue
- chan2: (int) nuclear or other channel, ones-based because zero means set to all zeros (optional); 0 = None (will be set to zero); 1 = red; 2 = green; 3 = blue
- **pretrained\_model:** (string) cyto = cellpose cytoplasm model; nuclei = cellpose nucleus model; can also specify absolute path to model file
- diameter: (float) average diameter of objects in image, if 0 cellpose will estimate for each image, default is 30
- use\_gpu: (bool) run network on GPU
- save\_outlines: FLAG save outlines as text file for ImageJ
- save\_png: FLAG save masks as png
- save\_tif: FLAG save masks as tif
- no\_npy: FLAG turn off saving of \_seg.npy file
- batch\_size: (int, optional 8) batch size to run tiles of size 224 x 224

### 9.3 Command line examples

Run python -m cellpose and specify parameters as below. For instance to run on a folder with images where cytoplasm is green and nucleus is blue and save the output as a png (using default diameter 30):

You can specify the diameter for all the images or set to 0 if you want the algorithm to estimate it on an image by image basis. Here is how to run on nuclear data (grayscale) where the diameter is automatically estimated:

```
python -m cellpose --dir ~/images_nuclei/test/ --pretrained_model nuclei --diameter 0. --

→ save_png
```

**Warning:** The path given to --dir must be an absolute path.

#### 9.4 Options

You can run the help string and see all the options:

```
usage: __main__.py [-h] [--use_gpu] [--check_mkl] [--dir DIR]
               [--look_one_level_down] [--img_filter IMG_FILTER]
               [--channel_axis CHANNEL_AXIS] [--z_axis Z_AXIS]
               [--chan CHAN] [--chan2 CHAN2] [--invert] [--all_channels]
               [--pretrained_model PRETRAINED_MODEL] [--unet]
               [--nclasses NCLASSES] [--no_resample] [--net_avg]
               [--no_interp] [--do_3D] [--diameter DIAMETER]
               [--stitch_threshold STITCH_THRESHOLD] [--fast_mode]
               [--flow_threshold FLOW_THRESHOLD]
               [--cellprob_threshold CELLPROB_THRESHOLD]
               [--anisotropy ANISOTROPY] [--exclude_on_edges] [--save_png]
               [--save_tif] [--no_npy] [--savedir SAVEDIR] [--dir_above]
               [--in_folders] [--save_flows] [--save_outlines]
               [--save_ncolor] [--save_txt] [--train] [--train_size]
               [--test_dir TEST_DIR] [--mask_filter MASK_FILTER]
               [--diam_mean DIAM_MEAN] [--learning_rate LEARNING_RATE]
               [--weight_decay WEIGHT_DECAY] [--n_epochs N_EPOCHS]
               [--batch_size BATCH_SIZE]
               [--min_train_masks MIN_TRAIN_MASKS]
               [--residual_on RESIDUAL_ON] [--style_on STYLE_ON]
               [--concatenation CONCATENATION] [--save_every SAVE_EVERY]
               [--save_each] [--verbose]
cellpose parameters
optional arguments:
-h, --help
                      show this help message and exit
--verbose
                      show information about running and settings and save
                        to log
hardware arguments:
--use_gpu
                      use gpu if torch with cuda installed
                      check if mkl working
--check_mkl
input image arguments:
--dir DIR
                      folder containing data to run or train on.
--look_one_level_down
                        run processing on all subdirectories of current folder
```

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```
--img_filter IMG_FILTER
                        end string for images to run on
--channel_axis CHANNEL_AXIS
                        axis of image which corresponds to image channels
--z_axis Z_AXIS
                      axis of image which corresponds to Z dimension
--chan CHAN
                      channel to segment; 0: GRAY, 1: RED, 2: GREEN, 3:
                        BLUE. Default: 0
--chan2 CHAN2
                      nuclear channel (if cyto, optional); 0: NONE, 1: RED,
                        2: GREEN, 3: BLUE. Default: 0
--invert
                      invert grayscale channel
--all_channels
                      use all channels in image if using own model and
                        images with special channels
model arguments:
--pretrained_model PRETRAINED_MODEL
                        model to use for running or starting training
--unet
                      run standard unet instead of cellpose flow output
--nclasses NCLASSES
                      if running unet, choose 2 or 3; cellpose always uses 3
algorithm arguments:
--no_resample
                      disable dynamics on full image (makes algorithm faster
                        for images with large diameters)
--net_avg
                      run 4 networks instead of 1 and average results
                      do not interpolate when running dynamics (was default)
--no_interp
--do_3D
                      process images as 3D stacks of images (nplanes x nchan
                        x Ly x Lx
--diameter DIAMETER
                      cell diameter, if 0 will use the diameter of the
                        training labels used in the model, or with built-in
                        model will estimate diameter for each image
--stitch_threshold STITCH_THRESHOLD
                        compute masks in 2D then stitch together masks with
                        IoU>0.9 across planes
--fast mode
                      now equivalent to --no_resample; make code run faster
                        by turning off resampling
--flow_threshold FLOW_THRESHOLD
                        flow error threshold, 0 turns off this optional QC
                        step. Default: 0.4
--cellprob_threshold CELLPROB_THRESHOLD
                        cellprob threshold, default is 0, decrease to find
                        more and larger masks
--anisotropy ANISOTROPY
                        anisotropy of volume in 3D
--exclude_on_edges
                      discard masks which touch edges of image
output arguments:
--save_png
                      save masks as png and outlines as text file for ImageJ
                      save masks as tif and outlines as text file for ImageJ
--save_tif
                      suppress saving of npy
--no_npy
--savedir SAVEDIR
                      folder to which segmentation results will be saved
                        (defaults to input image directory)
--dir_above
                      save output folders adjacent to image folder instead
                        of inside it (off by default)
```

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```
--in folders
                      flag to save output in folders (off by default)
                      whether or not to save RGB images of flows when masks
--save_flows
                        are saved (disabled by default)
                      whether or not to save RGB outline images when masks
--save_outlines
                        are saved (disabled by default)
--save_ncolor
                      whether or not to save minimal "n-color" masks
                        (disabled by default
                      flag to enable txt outlines for ImageJ (disabled by
--save_txt
                        default)
training arguments:
--train
                      train network using images in dir
--train_size
                      train size network at end of training
--test_dir TEST_DIR
                      folder containing test data (optional)
--mask_filter MASK_FILTER
                        end string for masks to run on. Default: _masks
--diam_mean DIAM_MEAN
                        mean diameter to resize cells to during training -- if
                        starting from pretrained models it cannot be changed
                        from 30.0
--learning_rate LEARNING_RATE
                        learning rate. Default: 0.2
--weight_decay WEIGHT_DECAY
                        weight decay. Default: 1e-05
--n_epochs N_EPOCHS
                      number of epochs. Default: 500
--batch_size BATCH_SIZE
                        batch size. Default: 8
--min_train_masks MIN_TRAIN_MASKS
                        minimum number of masks a training image must have to
                        be used. Default: 5
--residual_on RESIDUAL_ON
                        use residual connections
--stvle on STYLE ON
                      use style vector
--concatenation CONCATENATION
                        concatenate downsampled layers with upsampled layers
                        (off by default which means they are added)
--save_every SAVE_EVERY
                        number of epochs to skip between saves. Default: 100
                      save the model under a different filename per
--save_each
                        --save_every epoch for later comparsion
```

## **CELLPOSE API GUIDE**

### 10.1 Cellpose class

**class** cellpose.models.**Cellpose**(*gpu=False*, *model\_type='cyto'*, *net\_avg=False*, *device=None*) main model which combines SizeModel and CellposeModel

#### Parameters

- **gpu** (*bool* (*optional*, *default False*)) whether or not to use GPU, will check if GPU available
- model\_type (*str* (*optional*, *default* '*cyto*')) 'cyto'=cytoplasm model; 'nuclei'=nucleus model; 'cyto2'=cytoplasm model with additional user images
- **net\_avg** (*bool* (*optional*, *default False*)) loads the 4 built-in networks and averages them if True, loads one network if False
- **device** (*torch device* (*optional*, *default None*)) device used for model running / training (torch.device('cuda') or torch.device('cpu')), overrides gpu input, recommended if you want to use a specific GPU (e.g. torch.device('cuda:1'))

eval (x, batch\_size=8, channels=None, channel\_axis=None, z\_axis=None, invert=False, normalize=True, diameter=30.0, do\_3D=False, anisotropy=None, net\_avg=False, augment=False, tile=True, tile\_overlap=0.1, resample=True, interp=True, flow\_threshold=0.4, cellprob\_threshold=0.0, min\_size=15, stitch\_threshold=0.0, rescale=None, progress=None, model\_loaded=False)

run cellpose and get masks

#### Parameters

- **x** (*list or array of images*) can be list of 2D/3D images, or array of 2D/3D images, or 4D image array
- **batch\_size** (*int* (*optional*, *default* 8)) number of 224x224 patches to run simultaneously on the GPU (can make smaller or bigger depending on GPU memory usage)
- **channels** (*list (optional, default None*)) list of channels, either of length 2 or of length number of images by 2. First element of list is the channel to segment (0=grayscale, 1=red, 2=green, 3=blue). Second element of list is the optional nuclear channel (0=none, 1=red, 2=green, 3=blue). For instance, to segment grayscale images, input [0,0]. To segment images with cells in green and nuclei in blue, input [2,3]. To segment one grayscale image and one image with cells in green and nuclei in blue, input [[0,0], [2,3]].
- **channel\_axis** (*int* (*optional*, *default None*)) if None, channels dimension is attempted to be automatically determined

- **z\_axis** (*int* (*optional*, *default None*)) if None, z dimension is attempted to be automatically determined
- **invert** (*bool* (*optional*, *default False*)) invert image pixel intensity before running network (if True, image is also normalized)
- **normalize** (bool (optional, default True)) normalize data so 0.0=1st percentile and 1.0=99th percentile of image intensities in each channel
- **diameter** (*float* (*optional*, *default* 30.)) if set to None, then diameter is automatically estimated if size model is loaded
- **do\_3D** (*bool* (*optional*, *default False*)) set to True to run 3D segmentation on 4D image input
- **anisotropy** (*float* (*optional*, *default* None)) for 3D segmentation, optional rescaling factor (e.g. set to 2.0 if Z is sampled half as dense as X or Y)
- **net\_avg** (bool (optional, default False)) runs the 4 built-in networks and averages them if True, runs one network if False
- **augment** (bool (optional, default False)) tiles image with overlapping tiles and flips overlapped regions to augment
- **tile** (*bool* (*optional*, *default True*)) tiles image to ensure GPU/CPU memory usage limited (recommended)
- **tile\_overlap**(*float* (*optional*, *default* 0.1)) fraction of overlap of tiles when computing flows
- **resample** (*bool* (*optional*, *default True*)) run dynamics at original image size (will be slower but create more accurate boundaries)
- **interp** (*bool* (*optional*, *default True*)) interpolate during 2D dynamics (not available in 3D) (in previous versions it was False)
- **flow\_threshold**(*float* (*optional*, *default* 0.4)) flow error threshold(all cells with errors below threshold are kept) (not used for 3D)
- **cellprob\_threshold** (*float* (*optional*, *default* 0.0)) all pixels with value above threshold kept for masks, decrease to find more and larger masks
- **min\_size** (*int* (*optional*, *default* 15)) minimum number of pixels per mask, can turn off with -1
- **stitch\_threshold** (*float* (*optional*, *default* 0.0)) if stitch\_threshold>0.0 and not do\_3D and equal image sizes, masks are stitched in 3D to return volume segmentation
- **rescale**(*float* (*optional*, *default None*)) if diameter is set to None, and rescale is not None, then rescale is used instead of diameter for resizing image
- **progress** (*pyqt progress bar* (*optional*, *default None*)) to return progress bar status to GUI
- model\_loaded(bool (optional, default False)) internal variable for determining if model has been loaded, used in \_\_main\_\_.py

#### Returns

• **masks** (*list of 2D arrays, or single 3D array (if do\_3D=True)*) – labelled image, where 0=no masks; 1,2,...=mask labels

- flows (*list of lists 2D arrays, or list of 3D arrays (if do\_3D=True)*) flows[k][0] = XY flow in HSV 0-255 flows[k][1] = XY flows at each pixel flows[k][2] = cell probability (if > cellprob\_threshold, pixel used for dynamics) flows[k][3] = final pixel locations after Euler integration
- **styles** (*list of 1D arrays of length 256, or single 1D array (if do\_3D=True)*) style vector summarizing each image, also used to estimate size of objects in image
- **diams** (*list of diameters, or float* (*if do\_3D=True*))

### 10.2 CellposeModel

#### Parameters

- **gpu** (*bool* (*optional*, *default False*)) whether or not to save model to GPU, will check if GPU available
- **pretrained\_model** (*str or list of strings* (*optional, default False*)) path to pretrained cellpose model(s), if None or False, no model loaded
- **model\_type** (*str* (*optional*, *default None*)) 'cyto'=cytoplasm model; 'nuclei'=nucleus model; if None, pretrained\_model used
- **net\_avg** (*bool* (*optional*, *default False*)) loads the 4 built-in networks and averages them if True, loads one network if False
- **diam\_mean** (*float* (*optional*, *default* 30.)) mean 'diameter', 30. is built in value for 'cyto' model; 17. is built in value for 'nuclei' model; if saved in custom model file (cellpose>=2.0) then it will be loaded automatically and overwrite this value
- **device** (torch device (optional, default None)) device used for model running / training (torch.device('cuda') or torch.device('cpu')), overrides gpu input, recommended if you want to use a specific GPU (e.g. torch.device('cuda:1'))
- **residual\_on** (*bool* (*optional*, *default True*)) use 4 conv blocks with skip connections per layer instead of 2 conv blocks like conventional u-nets
- **style\_on** (*bool* (*optional*, *default True*)) use skip connections from style vector to all upsampling layers
- **concatenation** (*bool* (*optional*, *default False*)) if True, concatentate down-sampling block outputs with upsampling block inputs; default is to add
- **nchan** (*int* (*optional*, *default* 2)) number of channels to use as input to network, default is 2 (cyto + nuclei) or (nuclei + zeros)
- eval (x, batch\_size=8, channels=None, channel\_axis=None, z\_axis=None, normalize=True, invert=False, rescale=None, diameter=None, do\_3D=False, anisotropy=None, net\_avg=False, augment=False, tile=True, tile\_overlap=0.1, resample=True, interp=True, flow\_threshold=0.4, cellprob\_threshold=0.0, compute\_masks=True, min\_size=15, stitch\_threshold=0.0, progress=None, loop\_run=False, model\_loaded=False)

segment list of images x, or 4D array - Z x nchan x Y x X

Parameters

- **x** (*list or array of images*) can be list of 2D/3D/4D images, or array of 2D/3D/4D images
- **batch\_size** (*int* (*optional*, *default* 8)) number of 224x224 patches to run simultaneously on the GPU (can make smaller or bigger depending on GPU memory usage)
- **channels** (*list (optional, default None*)) list of channels, either of length 2 or of length number of images by 2. First element of list is the channel to segment (0=grayscale, 1=red, 2=green, 3=blue). Second element of list is the optional nuclear channel (0=none, 1=red, 2=green, 3=blue). For instance, to segment grayscale images, input [0,0]. To segment images with cells in green and nuclei in blue, input [2,3]. To segment one grayscale image and one image with cells in green and nuclei in blue, input [[0,0], [2,3]].
- **channel\_axis** (*int* (*optional*, *default None*)) if None, channels dimension is attempted to be automatically determined
- **z\_axis** (*int* (*optional*, *default None*)) if None, z dimension is attempted to be automatically determined
- **normalize** (*bool* (*default*, *True*)) normalize data so 0.0=1st percentile and 1.0=99th percentile of image intensities in each channel
- **invert** (*bool* (*optional*, *default False*)) invert image pixel intensity before running network
- **diameter**(*float* (*optional*, *default* None))-diameter for each image, if diameter is None, set to diam\_mean or diam\_train if available
- **rescale**(*float* (*optional*, *default* None)) resize factor for each image, if None, set to 1.0; (only used if diameter is None)
- **do\_3D** (*bool* (*optional*, *default False*)) set to True to run 3D segmentation on 4D image input
- **anisotropy** (*float* (*optional*, *default* None)) for 3D segmentation, optional rescaling factor (e.g. set to 2.0 if Z is sampled half as dense as X or Y)
- **net\_avg** (*bool* (*optional*, *default False*)) runs the 4 built-in networks and averages them if True, runs one network if False
- **augment** (*bool* (*optional*, *default False*)) tiles image with overlapping tiles and flips overlapped regions to augment
- **tile** (*bool* (*optional*, *default True*)) tiles image to ensure GPU/CPU memory usage limited (recommended)
- **tile\_overlap**(*float* (*optional*, *default* 0.1)) fraction of overlap of tiles when computing flows
- **resample** (*bool* (*optional*, *default True*)) run dynamics at original image size (will be slower but create more accurate boundaries)
- **interp** (*bool* (*optional*, *default True*)) interpolate during 2D dynamics (not available in 3D) (in previous versions it was False)
- **flow\_threshold**(*float* (*optional*, *default* 0.4)) flow error threshold(all cells with errors below threshold are kept) (not used for 3D)
- **cellprob\_threshold** (*float* (*optional*, *default* 0.0)) all pixels with value above threshold kept for masks, decrease to find more and larger masks

- **compute\_masks** (*bool* (*optional*, *default True*)) Whether or not to compute dynamics and return masks. This is set to False when retrieving the styles for the size model.
- **min\_size** (*int* (*optional*, *default* 15)) minimum number of pixels per mask, can turn off with -1
- **stitch\_threshold** (*float* (*optional*, *default* 0.0)) if stitch\_threshold>0.0 and not do\_3D, masks are stitched in 3D to return volume segmentation
- **progress** (*pyqt progress bar* (*optional*, *default None*)) to return progress bar status to GUI
- **loop\_run** (*bool* (*optional*, *default False*)) internal variable for determining if model has been loaded, stops model loading in loop over images
- model\_loaded (bool (optional, default False)) internal variable for determining if model has been loaded, used in \_\_main\_\_.py

#### Returns

- **masks** (*list of 2D arrays, or single 3D array (if do\_3D=True)*) labelled image, where 0=no masks; 1,2,...=mask labels
- flows (list of lists 2D arrays, or list of 3D arrays (if do\_3D=True)) flows[k][0] = XY flow in HSV 0-255 flows[k][1] = XY flows at each pixel flows[k][2] = cell probability (if > cellprob\_threshold, pixel used for dynamics) flows[k][3] = final pixel locations after Euler integration
- **styles** (*list of 1D arrays of length 64, or single 1D array (if do\_3D=True)*) style vector summarizing each image, also used to estimate size of objects in image

#### loss\_fn(lbl, y)

loss function between true labels lbl and prediction y

train(train\_data, train\_labels, train\_files=None, test\_data=None, test\_labels=None, test\_files=None, channels=None, normalize=True, save\_path=None, save\_every=100, save\_each=False, learning\_rate=0.2, n\_epochs=500, momentum=0.9, SGD=True, weight\_decay=1e-05, batch\_size=8, nimg\_per\_epoch=None, rescale=True, min\_train\_masks=5, model\_name=None)

train network with images train\_data

- train\_data (list of arrays (2D or 3D)) images for training
- train\_labels (list of arrays (2D or 3D)) labels for train\_data, where 0=no masks; 1,2,...=mask labels can include flows as additional images
- **train\_files** (*list of strings*) file names for images in train\_data (to save flows for future runs)
- test\_data (list of arrays (2D or 3D)) images for testing
- **test\_labels** (*list of arrays (2D or 3D*)) labels for test\_data, where 0=no masks; 1,2,...=mask labels; can include flows as additional images
- **test\_files** (*list of strings*) file names for images in test\_data (to save flows for future runs)
- channels (list of ints (default, None)) channels to use for training
- **normalize** (*bool* (*default*, *True*)) normalize data so 0.0=1st percentile and 1.0=99th percentile of image intensities in each channel

- **save\_path** (*string* (*default*, *None*)) where to save trained model, if None it is not saved
- save\_every (int (default, 100)) save network every [save\_every] epochs
- **learning\_rate** (*float or list/np.ndarray (default, 0.2*)) learning rate for training, if list, must be same length as n\_epochs
- **n\_epochs** (*int* (*default*, 500)) how many times to go through whole training set during training
- weight\_decay(float (default, 0.00001)) -
- SGD (bool (default, True)) use SGD as optimization instead of RAdam
- **batch\_size** (*int* (*optional*, *default* 8)) number of 224x224 patches to run simultaneously on the GPU (can make smaller or bigger depending on GPU memory usage)
- **nimg\_per\_epoch** (*int (optional, default None*)) minimum number of images to train on per epoch, with a small training set (< 8 images) it may help to set to 8
- **rescale** (*bool* (*default*, *True*)) whether or not to rescale images to diam\_mean during training, if True it assumes you will fit a size model after training or resize your images accordingly, if False it will try to train the model to be scale-invariant (works worse)
- min\_train\_masks (*int* (*default*, 5)) minimum number of masks an image must have to use in training set
- **model\_name** (*str* (*default*, *None*)) name of network, otherwise saved with name as params + training start time

### 10.3 SizeModel

#### **class** cellpose.models.**SizeModel**(cp\_model, device=None, pretrained\_size=None, \*\*kwargs)

linear regression model for determining the size of objects in image used to rescale before input to cp\_model uses styles from cp\_model

#### Parameters

- cp\_model (UnetModel or CellposeModel) model from which to get styles
- **device** (*torch device* (*optional*, *default None*)) device used for model running / training (torch.device('cuda') or torch.device('cpu')), overrides gpu input, recommended if you want to use a specific GPU (e.g. torch.device('cuda:1'))
- **pretrained\_size** (*str*) path to pretrained size model
- eval(x, channels=None, channel\_axis=None, normalize=True, invert=False, augment=False, tile=True, batch\_size=8, progress=None, interp=True)

use images x to produce style or use style input to predict size of objects in image

Object size estimation is done in two steps: 1. use a linear regression model to predict size from style in image 2. resize image to predicted size and run CellposeModel to get output masks.

Take the median object size of the predicted masks as the final predicted size.

#### Parameters

• **x**(*list or array of images*) – can be list of 2D/3D images, or array of 2D/3D images

- **channels** (*list (optional, default None*)) list of channels, either of length 2 or of length number of images by 2. First element of list is the channel to segment (0=grayscale, 1=red, 2=green, 3=blue). Second element of list is the optional nuclear channel (0=none, 1=red, 2=green, 3=blue). For instance, to segment grayscale images, input [0,0]. To segment images with cells in green and nuclei in blue, input [2,3]. To segment one grayscale image and one image with cells in green and nuclei in blue, input [[0,0], [2,3]].
- **channel\_axis** (*int* (*optional*, *default None*)) if None, channels dimension is attempted to be automatically determined
- **normalize** (*bool* (*default*, *True*)) normalize data so 0.0=1st percentile and 1.0=99th percentile of image intensities in each channel
- **invert** (bool (optional, default False)) invert image pixel intensity before running network
- **augment** (*bool* (*optional*, *default False*)) tiles image with overlapping tiles and flips overlapped regions to augment
- **tile** (*bool* (*optional*, *default True*)) tiles image to ensure GPU/CPU memory usage limited (recommended)
- **progress** (*pyqt progress bar* (*optional*, *default None*)) to return progress bar status to GUI

#### Returns

- **diam** (*array*, *float*) final estimated diameters from images x or styles style after running both steps
- diam\_style (array, float) estimated diameters from style alone

train size model with images train\_data to estimate linear model from styles to diameters

- train\_data (list of arrays (2D or 3D)) images for training
- train\_labels (list of arrays (2D or 3D)) labels for train\_data, where 0=no masks; 1,2,...=mask labels can include flows as additional images
- channels (list of ints (default, None)) channels to use for training
- **normalize** (*bool* (*default*, *True*)) normalize data so 0.0=1st percentile and 1.0=99th percentile of image intensities in each channel
- **n\_epochs** (*int* (*default*, 10)) how many times to go through whole training set (taking random patches) for styles for diameter estimation
- **12\_regularization** (*float* (*default*, 1.0)) regularize linear model from styles to diameters
- **batch\_size** (*int* (*optional*, *default* 8)) number of 224x224 patches to run simultaneously on the GPU (can make smaller or bigger depending on GPU memory usage)

### **10.4 Metrics**

cellpose.metrics.aggregated\_jaccard\_index(masks\_true, masks\_pred)

AJI = intersection of all matched masks / union of all masks

#### Parameters

- masks\_true (list of ND-arrays (int) or ND-array (int)) where 0=NO masks; 1,2... are mask labels
- masks\_pred (list of ND-arrays (int) or ND-array (int)) ND-array (int) where 0=NO masks; 1,2... are mask labels

#### Returns aji

Return type aggregated jaccard index for each set of masks

cellpose.metrics.average\_precision(masks\_true, masks\_pred, threshold=[0.5, 0.75, 0.9])

average precision estimation: AP = TP / (TP + FP + FN)

This function is based heavily on the *fast* stardist matching functions (https://github.com/mpicbg-csbd/stardist/blob/master/stardist/matching.py)

#### **Parameters**

- masks\_true (list of ND-arrays (int) or ND-array (int)) where 0=NO masks; 1,2... are mask labels
- masks\_pred (list of ND-arrays (int) or ND-array (int)) ND-array (int) where 0=NO masks; 1,2... are mask labels

#### Returns

- **ap** (*array* [*len*(*masks\_true*) *x len*(*threshold*)]) average precision at thresholds
- **tp** (*array* [*len*(*masks\_true*) *x len*(*threshold*)]) number of true positives at thresholds
- **fp** (*array* [*len*(*masks\_true*) *x len*(*threshold*)]) number of false positives at thresholds
- fn (array [len(masks\_true) x len(threshold)]) number of false negatives at thresholds

cellpose.metrics.boundary\_scores(masks\_true, masks\_pred, scales)

boundary precision / recall / Fscore

cellpose.metrics.flow\_error(maski, dP\_net, use\_gpu=False, device=None)

error in flows from predicted masks vs flows predicted by network run on image

This function serves to benchmark the quality of masks, it works as follows 1. The predicted masks are used to create a flow diagram 2. The mask-flows are compared to the flows that the network predicted

If there is a discrepancy between the flows, it suggests that the mask is incorrect. Masks with flow\_errors greater than 0.4 are discarded by default. Setting can be changed in Cellpose.eval or CellposeModel.eval.

#### **Parameters**

- **maski** (*ND-array* (*int*)) masks produced from running dynamics on dP\_net, where 0=NO masks; 1,2... are mask labels
- **dP\_net** (*ND-array* (*float*)) ND flows where dP\_net.shape[1:] = maski.shape

#### Returns

• **flow\_errors** (*float array with length maski.max()*) – mean squared error between predicted flows and flows from masks

• dP\_masks (ND-array (float)) – ND flows produced from the predicted masks

cellpose.metrics.mask\_ious(masks\_true, masks\_pred)

return best-matched masks

### 10.5 Flows to masks

cellpose.dynamics.compute\_masks(dP, cellprob, p=None, niter=200, cellprob\_threshold=0.0, flow\_threshold=0.4, interp=True, do\_3D=False, min\_size=15, resize=None, use\_gpu=False, device=None)

compute masks using dynamics from dP, cellprob, and boundary

cellpose.dynamics.follow\_flows(dP, mask=None, niter=200, interp=True, use\_gpu=True, device=None) define pixels and run dynamics to recover masks in 2D

Pixels are meshgrid. Only pixels with non-zero cell-probability are used (as defined by inds)

#### **Parameters**

- dP (float 32, 3D or 4D array) flows [axis x Ly x Lx] or [axis x Lz x Ly x Lx]
- **mask**((optional, default None)) pixel mask to seed masks. Useful when flows have low magnitudes.
- niter (int (optional, default 200)) number of iterations of dynamics to run
- **interp** (*bool* (*optional*, *default True*)) interpolate during 2D dynamics (not available in 3D) (in previous versions + paper it was False)
- **use\_gpu** (bool (optional, default False)) use GPU to run interpolated dynamics (faster than CPU)

#### Returns

- **p** (*float32, 3D or 4D array*) final locations of each pixel after dynamics; [axis x Ly x Lx] or [axis x Lz x Ly x Lx]
- inds (*int32, 3D or 4D array*) indices of pixels used for dynamics; [axis x Ly x Lx] or [axis x Lz x Ly x Lx]

#### cellpose.dynamics.get\_masks(p, iscell=None, rpad=20)

create masks using pixel convergence after running dynamics

Makes a histogram of final pixel locations p, initializes masks at peaks of histogram and extends the masks from the peaks so that they include all pixels with more than 2 final pixels p. Discards masks with flow errors greater than the threshold. :param p: final locations of each pixel after dynamics,

size [axis x Ly x Lx] or [axis x Lz x Ly x Lx].

- **iscell** (*bool*, 2D or 3D array) if iscell is not None, set pixels that are iscell False to stay in their original location.
- **rpad** (*int* (*optional*, *default* 20)) histogram edge padding
- **threshold** (*float* (*optional*, *default* 0.4)) masks with flow error greater than threshold are discarded (if flows is not None)

- **flows** (float, 3D or 4D array (optional, default None)) flows [axis x Ly x Lx] or [axis x Lz x Ly x Lx]. If flows is not None, then masks with inconsistent flows are removed using *remove\_bad\_flow\_masks*.
- **Returns** M0 masks with inconsistent flow masks removed, 0=NO masks; 1,2,...=mask labels, size [Ly x Lx] or [Lz x Ly x Lx]

Return type int, 2D or 3D array

```
cellpose.dynamics.labels_to_flows(labels, files=None, use_gpu=False, device=None, redo_flows=False) convert labels (list of masks or flows) to flows for training model
```

if files is not None, flows are saved to files to be reused

- **Parameters labels** (*list of ND-arrays*) labels[k] can be 2D or 3D, if [3 x Ly x Lx] then it is assumed that flows were precomputed. Otherwise labels[k][0] or labels[k] (if 2D) is used to create flows and cell probabilities.
- **Returns flows** flows[k][0] is labels[k], flows[k][1] is cell distance transform, flows[k][2] is Y flow, flows[k][3] is X flow, and flows[k][4] is heat distribution

**Return type** list of [4 x Ly x Lx] arrays

#### cellpose.dynamics.map\_coordinates(I, yc, xc, Y)

bilinear interpolation of image 'I' in-place with ycoordinates yc and xcoordinates xc to Y

#### Parameters

- **I** (C x Ly x Lx) -
- **yc** (*ni*) new y coordinates
- **xc** (*ni*) new x coordinates
- $\mathbf{Y}(C \times ni) I$  sampled at (yc,xc)

cellpose.dynamics.masks\_to\_flows(masks, use\_gpu=False, device=None)

convert masks to flows using diffusion from center pixel

Center of masks where diffusion starts is defined to be the closest pixel to the median of all pixels that is inside the mask. Result of diffusion is converted into flows by computing the gradients of the diffusion density map.

Parameters masks (int, 2D or 3D array) – labelled masks 0=NO masks; 1,2,...=mask labels

Returns

- **mu** (*float, 3D or 4D array*) flows in Y = mu[-2], flows in X = mu[-1]. if masks are 3D, flows in Z = mu[0].
- **mu\_c** (*float, 2D or 3D array*) for each pixel, the distance to the center of the mask in which it resides

#### cellpose.dynamics.masks\_to\_flows\_cpu(masks, device=None)

convert masks to flows using diffusion from center pixel Center of masks where diffusion starts is defined to be the closest pixel to the median of all pixels that is inside the mask. Result of diffusion is converted into flows by computing the gradients of the diffusion density map. :param masks: labelled masks 0=NO masks; 1,2,...=mask labels :type masks: int, 2D array

#### Returns

• **mu** (*float, 3D array*) – flows in Y = mu[-2], flows in X = mu[-1]. if masks are 3D, flows in Z = mu[0].

• **mu\_c** (*float*, 2D array) – for each pixel, the distance to the center of the mask in which it resides

#### cellpose.dynamics.masks\_to\_flows\_gpu(masks, device=None)

convert masks to flows using diffusion from center pixel Center of masks where diffusion starts is defined using COM :param masks: labelled masks 0=NO masks; 1,2,...=mask labels :type masks: int, 2D or 3D array

#### Returns

- **mu** (*float, 3D or 4D array*) flows in Y = mu[-2], flows in X = mu[-1]. if masks are 3D, flows in Z = mu[0].
- **mu\_c** (*float*, 2D or 3D array) for each pixel, the distance to the center of the mask in which it resides

cellpose.dynamics.remove\_bad\_flow\_masks(masks, flows, threshold=0.4, use\_gpu=False, device=None)

remove masks which have inconsistent flows

Uses metrics.flow\_error to compute flows from predicted masks and compare flows to predicted flows from network. Discards masks with flow errors greater than the threshold.

#### **Parameters**

- masks (*int*, 2D or 3D array) labelled masks, 0=NO masks; 1,2,...=mask labels, size [Ly x Lx] or [Lz x Ly x Lx]
- flows (float, 3D or 4D array) flows [axis x Ly x Lx] or [axis x Lz x Ly x Lx]
- **threshold** (*float* (*optional*, *default* 0.4)) masks with flow error greater than threshold are discarded.

**Returns masks** – masks with inconsistent flow masks removed, 0=NO masks; 1,2,...=mask labels, size [Ly x Lx] or [Lz x Ly x Lx]

Return type int, 2D or 3D array

cellpose.dynamics.**steps2D**(*p*, *dP*, *inds*, *niter*)

run dynamics of pixels to recover masks in 2D

Euler integration of dynamics dP for niter steps

#### Parameters

- p(float32, 3D array) pixel locations [axis x Ly x Lx] (start at initial meshgrid)
- **dP** (*float32*, *3D array*) flows [axis x Ly x Lx]
- inds (int 32, 2D array) non-zero pixels to run dynamics on [npixels x 2]
- **niter** (*int32*) number of iterations of dynamics to run

**Returns**  $\mathbf{p}$  – final locations of each pixel after dynamics

#### Return type float32, 3D array

cellpose.dynamics.steps3D(p, dP, inds, niter)

run dynamics of pixels to recover masks in 3D

Euler integration of dynamics dP for niter steps

- p (float 32, 4D array) pixel locations [axis x Lz x Ly x Lx] (start at initial meshgrid)
- **dP** (*float32*, *4D array*) flows [axis x Lz x Ly x Lx]

- inds (int 32, 2D array) non-zero pixels to run dynamics on [npixels x 3]
- **niter** (*int32*) number of iterations of dynamics to run

Returns p – final locations of each pixel after dynamics

Return type float32, 4D array

### 10.6 Image transforms

cellpose.transforms.average\_tiles(y, ysub, xsub, Ly, Lx)

average results of network over tiles

#### Parameters

- **y**(float, [ntiles x nclasses x bsize x bsize]) output of cellpose network for each tile
- ysub (list) list of arrays with start and end of tiles in Y of length ntiles
- xsub (list) list of arrays with start and end of tiles in X of length ntiles
- Ly (*int*) size of pre-tiled image in Y (may be larger than original image if image size is less than bsize)
- Lx (*int*) size of pre-tiled image in X (may be larger than original image if image size is less than bsize)

Returns yf – network output averaged over tiles

**Return type** float32, [nclasses x Ly x Lx]

return image with z first, channels last and normalized intensities

cellpose.transforms.make\_tiles(imgi, bsize=224, augment=False, tile\_overlap=0.1)

make tiles of image to run at test-time

#### if augmented, tiles are flipped and tile\_overlap=2.

- original
- · flipped vertically
- · flipped horizontally
- flipped vertically and horizontally

#### Parameters

- imgi (float32) array that's nchan x Ly x Lx
- bsize (float (optional, default 224)) size of tiles
- augment (bool (optional, default False)) flip tiles and set tile\_overlap=2.
- tile\_overlap (float (optional, default 0.1)) fraction of overlap of tiles

#### Returns

- IMG (*float32*) array that's ntiles x nchan x bsize x bsize
- ysub (list) list of arrays with start and end of tiles in Y of length ntiles

• xsub (list) – list of arrays with start and end of tiles in X of length ntiles

- cellpose.transforms.move\_axis(img, m\_axis=- 1, first=True)
  move axis m\_axis to first or last position
- cellpose.transforms.move\_min\_dim(img, force=False)

move minimum dimension last as channels if < 10, or force==True

cellpose.transforms.normalize99(Y, lower=1, upper=99)

normalize image so 0.0 is 1st percentile and 1.0 is 99th percentile

```
cellpose.transforms.normalize_img(img, axis=-1, invert=False)
```

normalize each channel of the image so that so that 0.0=1st percentile and 1.0=99th percentile of image intensities

optional inversion

**Parameters** 

- img(ND-array (at least 3 dimensions)) -
- axis (channel axis to loop over for normalization) -
- invert (invert image (useful if cells are dark instead of bright)) -

Returns img - normalized image of same size

Return type ND-array, float32

cellpose.transforms.pad\_image\_ND(img0, div=16, extra=1)

pad image for test-time so that its dimensions are a multiple of 16 (2D or 3D)

#### Parameters

- **img0** (*ND-array*) image of size [nchan (x Lz) x Ly x Lx]
- div(int (optional, default 16)) -

#### Returns

- I (*ND-array*) padded image
- ysub (array, int) yrange of pixels in I corresponding to img0
- xsub (array, int) xrange of pixels in I corresponding to img0

cellpose.transforms.**random\_rotate\_and\_resize**(X, Y=None, scale\_range=1.0, xy=(224, 224), do flip=True, rescale=None, unet=False,

random\_per\_image=True)

augmentation by random rotation and resizing X and Y are lists or arrays of length nimg, with dims channels x Ly x Lx (channels optional) :param X: list of image arrays of size [nchan x Ly x Lx] or [Ly x Lx] :type X: LIST of ND-arrays, float :param Y: list of image labels of size [nlabels x Ly x Lx] or [Ly x Lx]. The 1st channel

of Y is always nearest-neighbor interpolated (assumed to be masks or 0-1 representation). If Y.shape[0]==3 and not unet, then the labels are assumed to be [cell probability, Y flow, X flow]. If unet, second channel is dist\_to\_bound.

- scale\_range (float (optional, default 1.0)) Range of resizing of images for augmentation. Images are resized by (1-scale\_range/2) + scale\_range \* np.random.rand()
- **xy** (tuple, int (optional, default (224,224))) size of transformed images to return

- **do\_flip** (*bool* (*optional*, *default True*)) whether or not to flip images horizontally
- **rescale** (*array*, *float* (*optional*, *default None*)) how much to resize images by before performing augmentations
- unet (bool (optional, default False)) -
- **random\_per\_image** (*bool* (*optional*, *default True*)) different random rotate and resize per image

#### Returns

- imgi (ND-array, float) transformed images in array [nimg x nchan x xy[0] x xy[1]]
- **lbl** (*ND-array, float*) transformed labels in array [nimg x nchan x xy[0] x xy[1]]
- scale (array, float) amount each image was resized by

cellpose.transforms.reshape(data, channels=[0, 0], chan\_first=False)

reshape data using channels

#### Parameters

- data (numpy array that's (Z x ) Ly x Lx x nchan) if data.ndim==8 and data.shape[0]<8, assumed to be nchan x Ly x Lx
- channels (list of int of length 2 (optional, default [0,0])) First element of list is the channel to segment (0=grayscale, 1=red, 2=green, 3=blue). Second element of list is the optional nuclear channel (0=none, 1=red, 2=green, 3=blue). For instance, to train on grayscale images, input [0,0]. To train on images with cells in green and nuclei in blue, input [2,3].
- **invert** (*bool*) invert intensities

#### **Returns data**

**Return type** numpy array that's (Z x ) Ly x Lx x nchan (if chan\_first==False)

cellpose.transforms.reshape\_and\_normalize\_data(*train\_data*, *test\_data=None*, *channels=None*, *normalize=True*)

inputs converted to correct shapes for *training* and rescaled so that 0.0=1st percentile and 1.0=99th percentile of image intensities in each channel

#### Parameters

- train\_data (list of ND-arrays, float) list of training images of size [Ly x Lx], [nchan x Ly x Lx], or [Ly x Lx x nchan]
- test\_data(list of ND-arrays, float (optional, default None))-list of testing images of size [Ly x Lx], [nchan x Ly x Lx], or [Ly x Lx x nchan]
- **channels** (*list of int of length 2 (optional, default None*)) First element of list is the channel to segment (0=grayscale, 1=red, 2=green, 3=blue). Second element of list is the optional nuclear channel (0=none, 1=red, 2=green, 3=blue). For instance, to train on grayscale images, input [0,0]. To train on images with cells in green and nuclei in blue, input [2,3].
- **normalize** (*bool* (*optional*, *True*)) normalize data so 0.0=1st percentile and 1.0=99th percentile of image intensities in each channel

#### Returns

• train\_data (list of ND-arrays, float) – list of training images of size [2 x Ly x Lx]

- test\_data (*list of ND-arrays, float (optional, default None*)) list of testing images of size [2 x Ly x Lx]
- run\_test (bool) whether or not test\_data was correct size and is useable during training

check sizes and reshape train and test data for training

resize image for computing flows / unresize for computing dynamics

#### Parameters

- img0 (ND-array) image of size [Y x X x nchan] or [Lz x Y x X x nchan] or [Lz x Y x X]
- Ly (int, optional) -
- Lx (int, optional) -
- rsz (float, optional) resize coefficient(s) for image; if Ly is None then rsz is used
- interpolation (cv2 interp method (optional, default cv2.INTER\_LINEAR))

Returns imgs – image of size [Ly x Lx x nchan] or [Lz x Ly x Lx x nchan]

Return type ND-array

```
cellpose.transforms.unaugment_tiles(y, unet=False)
```

reverse test-time augmentations for averaging

#### Parameters

- **y** (*float32*) array that's ntiles\_y x ntiles\_x x chan x Ly x Lx where chan = (dY, dX, cell prob)
- unet (bool (optional, False)) whether or not unet output or cellpose output

Returns y

Return type float32

### **10.7 Plot functions**

```
cellpose.plot.disk(med, r, Ly, Lx)
```

returns pixels of disk with radius r and center med

cellpose.plot.dx\_to\_circ(dP, transparency=False, mask=None)

dP is  $2 \times Y \times X =>$  'optic' flow representation

- **dP** (2xLyxLx array) Flow field components [dy,dx]
- **transparency** (*bool*, *default False*) magnitude of flow controls opacity, not lightness (clear background)
- mask (2D array) Multiplies each RGB component to suppress noise

cellpose.plot.image\_to\_rgb(img0, channels=[0, 0])

image is 2 x Ly x Lx or Ly x Lx x 2 - change to RGB Ly x Lx x 3

#### cellpose.plot.interesting\_patch(mask, bsize=130)

get patch of size bsize x bsize with most masks

#### cellpose.plot.mask\_overlay(img, masks, colors=None)

overlay masks on image (set image to grayscale)

#### Parameters

- img (int or float, 2D or 3D array) img is of size [Ly x Lx (x nchan)]
- masks (int, 2D array) masks where 0=NO masks; 1,2,...=mask labels
- **colors** (*int*, 2D array (*optional*, *default* None)) size [nmasks x 3], each entry is a color in 0-255 range

Returns RGB – array of masks overlaid on grayscale image

Return type uint8, 3D array

cellpose.plot.mask\_rgb(masks, colors=None)

masks in random rgb colors

#### Parameters

- masks (int, 2D array) masks where 0=NO masks; 1,2,...=mask labels
- **colors** (*int*, 2D array (*optional*, *default* None)) size [nmasks x 3], each entry is a color in 0-255 range

Returns RGB - array of masks overlaid on grayscale image

Return type uint8, 3D array

cellpose.plot.outline\_view(img0, maski, color=[1, 0, 0], mode='inner')

Generates a red outline overlay onto image.

cellpose.plot.show\_segmentation(fig, img, maski, flowi, channels=[0, 0], file\_name=None)

plot segmentation results (like on website)

Can save each panel of figure with file\_name option. Use channels option if img input is not an RGB image with 3 channels.

- fig (matplotlib.pyplot.figure) figure in which to make plot
- img (2D or 3D array) image input into cellpose
- **maski** (*int*, 2D array) for image k, masks[k] output from Cellpose.eval, where 0=NO masks; 1,2,...=mask labels
- **flowi** (*int*, 2D array) for image k, flows[k][0] output from Cellpose.eval (RGB of flows)
- channels (list of int (optional, default [0,0])) channels used to run Cellpose, no need to use if image is RGB
- **file\_name** (*str* (*optional*, *default* None)) file name of image, if file\_name is not None, figure panels are saved
- seg\_norm (bool (optional, default False)) improve cell visibility under labels

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