# **Documentation: trainsegmentation v 0.1.8**

# Importing training data

Training data should be 2d-numpy arrays. Labeled images should be the same size as the corresponding training image and can be imported from binary masks with the filenames from a separate directory using import\_training\_data.

**import\_training\_data**(imgdir,maskdir,ext = '.tif'): imports images and labels from different directories to use for generating feature sets and training data.

#### Parameters:

imadir: str

path to the image directory as string

maskdir: list of str

list of strings indicating paths containing labeled images with matching filenames to the images in the image directory to ensure corresponding images and labels.

ext: str

extension of image filenames. Default is '.tif'

## Returns:

IMG: list of ndarray

list containing images (ndarrays) imported from imgdir

LABELS: list of ndarray

list containing labeled images corresponding to IMG. Label numbers match the order of paths listed in maskdir (e.g. 1, 2, 3, etc.)

# Generating feature sets for training data

Once training data is imported, feature sets are generated to create the input needed for pixel classification.

**get\_training\_data**(IMG,LABELS,featureselect,loaddatafile = None, savedatafile = None): takes lists of images, corresponding labels and selected features (see Defining Features) to generate feature sets then output training data to use with a classifier..

#### Parameters:

IMG: list of ndarray

list containing images (ndarrays) imported from imgdir

LABELS: list of ndarray

list containing labeled images corresponding to IMG. Label numbers match the order of paths listed in maskdir (e.g. 1, 2, 3, etc.)

featureselect: list of str

list of strings indicating features (see Defining Features)

## Returns:

traininglabels: ndarray

a flattened (1d) ndarray contining the training labels

trainingfeatures: *ndarray* 

a flattened (2d) ndarray containing the feature sets, wherein len(axis = 1) is equal to the

number of features featureselect: *list of str* 

list of strings indicating features (see Defining Features)

loaddatafile: str

string with path to a previously generated data file which will be appended to the data

generated from the input images and labels

savedatafile: str

string with path to save data file as a pickle file

# Training a classifier

The training data is then used to train an sklearn classifier.

**train\_classifier**(traininglabels, trainingfeatures, featureselect, saveclftofile = None, clf = None). Trains a classifier using labels and features generated by get\_training\_data.

#### **Parameters:**

traininglabels: ndarray

a flattened (1d) ndarray contining the training labels

trainingfeatures: ndarray

a flattened (2d) ndarray containing the feature sets, wherein len(axis = 1) is equal to the

number of features featureselect: *list of str* 

list of strings indicating features (see Defining Features)

saveclftofile: str

string with path to classifier as a pickle file

clf: sklearn classifier

A sklearn classifier, default (None) is clf =

sklearn.ensemble.RandomForestClassifier(n\_estimators=50, n\_jobs=-1,max\_depth=10,

max\_samples=0.05)

# Returns:

clf: sklearn classifier trained sklearn classifier

# Applying a classifier

Once trained, classifiers can be applied to similar images to generate probability or binary masks.

load\_classifier(clffile): loads a classifier (pickle file) saved by train\_classifier

#### Parameters:

clffile: str

string with path to pickle file containing classifier

#### Returns:

clf: sklearn classifier trained sklearn classifier

**classify\_image**(img,clf,featureselect): generates feature set for an input image and outputs predicted classification.

#### Parameters:

img: ndarray input image clf: sklearn classifier

classifier trained by train\_classifier

featureselect: list of str

list of strings indicating features (see Defining Features) that <u>must match those used when</u> training the classifier

#### Returns:

result: ndarray

labeled ndarray with shape img.shape

**classify\_image\_probability**(img,clf,featureselect): generates feature set for an input image and outputs probability of classification.

# Parameters:

img: ndarray input image clf: sklearn classifier

classifier trained by train classifier

featureselect: list of str

list of strings indicating features (see Defining Features) that <u>must match those used when</u> training the classifier

#### Returns:

result: ndarray

labeled ndarray with shape (img.shape[0], img.shape[1], # of labels)

**classify\_image\_label**(img, clf, featureselect, selectlabel = 1): generates feature set for an input image and outputs predicted classification for the indicated label.

# Parameters:

img: ndarray input image clf: sklearn classifier

classifier trained by train\_classifier

featureselect: list of str

list of strings indicating features (see Defining Features) that <u>must match those used when training the classifier</u>

selectlabel: int

integer indicating the label to output classification

#### Returns:

result: ndarray

labeled ndarray with shape img.shape

**classify\_image\_label\_probability**(img,clf,featureselect,selectlabel = 1): generates feature set for an input image and outputs probability of classification for the indicated label.

# Parameters:

img: ndarray input image clf: sklearn classifier

classifier trained by train\_classifier

featureselect: list of str

list of strings indicating features (see Defining Features) that must match those used when

training the classifier

selectlabel: int

integer indicating the label to output classification

#### Returns:

label: ndarray

labeled ndarray with shape img.shape

**threshold\_mask**(img, threshmethod = sklearn.filters.threshold\_minimum): generates a mask by thresholding the input image with the given threshold method.

# Parameters:

img: *ndarray* input image

threshmethod: *function* a thresholding function

# Returns:

mask: *ndarray* 

labeled ndarray with shape img.shape; outputs a binary mask 0 or 255.

# **Defining Features**

The following functions are used to generate image features. The function names are passed into training and classification functions as strings in a list (e.g. ['Neighbors','Mean']).

**Difference\_of\_Gaussians**(img,minSigma = 1,maxSigma = 16)\*\*: performs convolutions with Gaussian kernels with the normal n variations of  $\sigma$  and subtracts the previous iteration to obtain the feature image, with the first image to be subtracted being the original image.

# Parameters:

img: ndarray
input image
minSigma: int
minimum value of σ
maxSigma: int
maximum value of σ

#### Returns:

meta: list of str

Meta data for *features*, wherein strings indicate feature of each ndarray in *features*. e.g. 'Difference\_of\_Gaussian\_1\_0' wherein the integer indicates  $\sigma$  for initial image and  $\sigma$  for the subtracted image respectively (the original image is 0).

features: list of ndarray

List containing feature images each of shape(img)

**Guassian\_blur**(img,minSigma = 1,maxSigma = 16)\*\*: performs n individual convolutions with Gaussian kernels with the normal n variations of  $\sigma$ . The larger the radius the more blurred the image becomes until the pixels are homogeneous

#### **Parameters:**

img: ndarray
input image
minSigma: int
minimum value of σ
maxSigma: int
maximum value of σ

#### Returns:

meta: list of str

Meta data for *features*, wherein strings indicate feature of each ndarray in *features*. e.g. 'Gaussian blur 1' wherein the integer indicates  $\sigma$ .

features: list of ndarray

List containing feature images each of shape(img)

**Hessian**(img, minSigma = 1, maxSigma = 16)\*\*: runs a Hessian filter (sklearn.filters.hessian). Gaussian blurs with  $\sigma$  varying as usual are performed prior to the filter.

#### **Parameters:**

img: *ndarray*input image
minSigma: *int*minimum value of σ

minimum value of d

maxSigma: int

maximum value of  $\sigma$ 

#### **Returns:**

meta: list of str

Meta data for features, wherein strings indicate feature of each ndarray in features. e.g.

'Hessian\_1.0' wherein the integer indicates  $\sigma$ .

features: list of ndarray

List containing feature images each of shape(img)

**Laplace**(img, minsigma = 1, maxsigma = 16): applies skimage.filters.laplace() to each image after a Gaussian blur with  $\sigma$  varying as usual performed prior to the filter.

# Parameters:

img: *ndarray* input image minSigma: *int* 

minimum value of  $\sigma$ 

maxSigma: int

maximum value of  $\sigma$ 

#### Returns:

meta: list of str

Meta data for features, wherein strings indicate feature of each ndarray in features. e.g.

'Median 1' wherein the integer indicates  $\sigma$ .

features: list of ndarray

List containing feature images each of shape(img)

**Neighbors**(img, minSigma = 1, maxSigma = 16)\*\*: shifts the image in 8 directions by a certain number of pixels,  $\sigma$ . Creates 8n feature images where  $(2^{n-1})$ \*minSigma  $\leq$  maxSigma.

# Parameters:

img: *ndarray* input image minSigma: *int* 

minimum value of  $\sigma$ 

maxSigma: int

maximum value of σ

#### Returns:

meta: list of str

Meta data for features, wherein strings indicate feature of each ndarray in features. e.g.

'Neighbors 1 0' wherein the integers indicate  $\sigma$  and direction respectively.

features: list of ndarray

List containing feature images each of shape(img)

**Maximum**(img, minsigma = 1, maxsigma = 16): gets the maximum of each pixel and surrounding pixels distance  $\sigma$  with the normal n variations of  $\sigma$ .

# Parameters:

img: *ndarray* input image minSigma: *int* 

minimum value of  $\sigma$ 

maxSigma: int

maximum value of  $\sigma$ 

#### Returns:

meta: list of str

Meta data for features, wherein strings indicate feature of each ndarray in features. e.g.

'Maximum 1' wherein the integer indicates  $\sigma$ .

features: list of ndarray

List containing feature images each of shape(img)

**Mean**(img, minsigma = 1, maxsigma = 16): gets the mean of each pixel and surrounding pixels distance  $\sigma$  with the normal n variations of  $\sigma$ .

#### Parameters:

img: *ndarray* input image minSigma: *int* 

minimum value of σ

maxSigma: int

maximum value of  $\sigma$ 

#### Returns:

meta: list of str

Meta data for features, wherein strings indicate feature of each ndarray in features. e.g.

'Mean 1' wherein the integer indicates  $\sigma$ .

features: list of ndarray

List containing feature images each of shape(img)

**Median**(img, minsigma = 1, maxsigma = 16): gets the median of each pixel and surrounding pixels distance  $\sigma$  with the normal n variations of  $\sigma$ .

#### Parameters:

img: ndarray input image minSigma: int

minimum value of  $\sigma$ 

maxSigma: int

maximum value of  $\sigma$ 

# Returns:

meta: list of str

Meta data for *features*, wherein strings indicate feature of each ndarray in *features*. e.g. 'Median 1' wherein the integer indicates  $\sigma$ .

features: list of ndarray

List containing feature images each of shape(img)

**Median\_blur**(img, minsigma = 1, maxsigma = 16): applies skimage.filters.median() to each image after a Gaussian blur with  $\sigma$  varying as usual performed prior to the filter.

#### Parameters:

img: *ndarray* input image minSigma: *int* 

minimum value of  $\sigma$ 

maxSigma: int

maximum value of  $\sigma$ 

# Returns:

meta: list of str

Meta data for *features*, wherein strings indicate feature of each ndarray in *features*. e.g. 'Median 1' wherein the integer indicates  $\sigma$ .

features: list of ndarray

List containing feature images each of shape(img)

**Meijering\_filter**(img, minSigma = 1, maxSigma = 16): applies Meijering neuriteness filter on n images, with Gaussian blurs with  $\sigma$  varying as usual are performed prior to the filter.

#### Parameters:

img: *ndarray* input image minSigma: *int* 

minimum value of σ

maxSigma: int

#### maximum value of σ

#### Returns:

meta: list of str

Meta data for features, wherein strings indicate feature of each ndarray in features. e.g.

'Meijering filter 1' wherein the integer indicates  $\sigma$ .

features: list of ndarray

List containing feature images each of shape(img)

**Membrane\_projections**(img,nAngles = 30, patchSize = 19,membraneSize = 1)\*\*: enhances membrane-like structures of the image through directional filtering. The initial kernel for this operation is patchsize<sup>2</sup> zero matrix with the membraneSize number of middle column entries set to 1. Multiple kernels are created by rotating the original kernel 180 degrees/nAngles for a number of kernels = nAngles. Each kernel is convolved with the image and then the set of images are Z-projected into a single image via 6 methods:

- sum of the pixels in each image
- mean of the pixels in each image
- standard deviation of the pixels in each image
- median of the pixels in each image
- maximum of the pixels in each image
- minimum of the pixels in each image

Each of the 6 resulting images is a feature. Hence pixels in lines of similarly valued pixels in the image that are different from the average image intensity will stand out in the Z-projections.

#### Parameters:

img: *ndarray* input image nAngles: *int* 

number of kernels

patchSize: int

size of matrix (patchSize x patchSize) to convolve image

membraneSize: int

number of columns in middle of patch to set to one before rotating and convolving

#### Returns:

meta: list of str

Meta data for *features*, wherein strings indicate feature of each ndarray in *features*. e.g. 'Membrane projections 0' wherein the integer indicates the method.

features: list of ndarray

List containing feature images each of shape(img)

**Minimum**(img, minsigma = 1, maxsigma = 16): gets the minimum of each pixel and surrounding pixels distance  $\sigma$  with the normal n variations of  $\sigma$ .

#### Parameters:

img: *ndarray* input image minSigma: *int* 

minimum value of  $\sigma$ 

maxSigma: int

maximum value of  $\sigma$ 

#### Returns:

meta: list of str

Meta data for features, wherein strings indicate feature of each ndarray in features. e.g.

'Minimum\_1' wherein the integer indicates  $\sigma$ .

features: list of ndarray

List containing feature images each of shape(img)

**Sklearn\_basic**(img): runs sklearn. feature.multiscale\_basic\_features(img) to image and returns results.

# Parameters:

img: *ndarray* input image

#### **Returns:**

meta: list of str

Meta data for features. Repeating list of strings 'Sklearn basic' with length of features

features: list of ndarray

List containing feature images each of shape(img)

**Sobel\_filter**(img, minSigma = 1, maxSigma = 16)\*\*: calculates an approximation of the gradient of the image intensity at each pixel using ndimage.sobel. Gaussian blurs with  $\sigma$  varying as usual are performed prior to the filter.

# Parameters:

img: *ndarray* input image minSigma: *int* 

minimum value of  $\sigma$ 

maxSigma: int

maximum value of σ

# Returns:

meta: list of str

Meta data for *features*, wherein strings indicate feature of each ndarray in *features*. e.g.

'Sobel\_filter\_1' wherein the integer indicates σ.

features: list of ndarray

List containing feature images each of shape(img)

**Watershed\_distance**(img, threshmethod = filters.threshold\_yen): thresholds the image with the indicated threshold method and transforms the image based on distances between objects (see Distances output in example: <a href="https://scikit-image.org/docs/stable/auto\_examples/segmentation/plot\_watershed.html">https://scikit-image.org/docs/stable/auto\_examples/segmentation/plot\_watershed.html</a>). Default threshmethod is skimage.filters.threshold\_yen. Other threshold methods can be applied from skimage.filters

### **Parameters:**

img: *ndarray* input image

threshmethod: function

select threshold method from skimage.filters. Default is skimage.filters.threshold\_yen.

#### Returns:

meta: list of str

Meta data for features, ['Watershed distance']

features: list of ndarray

List containing feature image of shape(img)

**Variance**(img, minsigma = 1, maxsigma = 16): gets the variance of each pixel and surrounding pixels distance  $\sigma$  with the normal n variations of  $\sigma$ .

# Parameters:

img: *ndarray* input image minSigma: *int* 

minimum value of  $\sigma$ 

maxSigma: int

maximum value of σ

#### **Returns:**

meta: list of str

Meta data for features, wherein strings indicate feature of each ndarray in features. e.g.

'Variance\_1' wherein the integer indicates  $\sigma$ .

features: list of ndarray

List containing feature images each of shape(img)