ImageBinner

1 Getting started

ImageBinner[A] helps to create binned patches from experimental measurements (ImageBinner notebook) and visualizes DeepSTORM prediction results to support parameter adjustments (VisualizeDeepSTORM2DLocs notebook). Some smaller AddOns are collected that have been proven to be helpful for the workflow (DeepSTORM2DAddOns notebook).
DeepSTORM2D is used as Colab notebook in the ZeroCostDL4Mic framework.[1][2]

Execute the code listing in an anaconda prompt to:

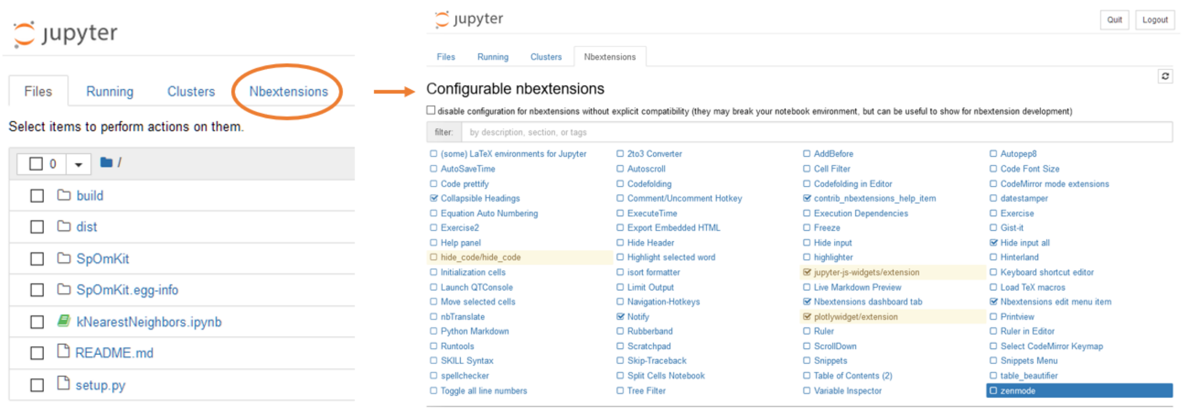
- create a virtual environment in anaconda and activate it
- change the directory to the *.whl file and install it
- additionally install jupyter nbextensions and activate them
- run the notebook process

Configure the notebook settings on the first run by clicking on the Nbextensions menu and selection „Collapsible Headings“ and „Hide input all“ (Fig. 1).

Listing 1: Commands to install *ImageBinner* and jupyter extensions in the anaconda prompt.

```
conda create --name ImageBinner
conda activate ImageBinner
cd path_to_file
conda install pip
pip install jupyterlab
pip install ImageBinner-XXXXX-py3-none-any.whl
pip install jupyter_contrib_nbextensions
jupyter notebook
```

[A] https://github.com/JohannaRahm/ImageBinner



☐ disable configuration for nbextensions without explicit compatibility (they may break your notebook environment, but can be useful to show for nbextension development)

☐ (some) LaTeX environments for Jupyter	☐ 2to3 Converter	☐ AddBefore	☐ Autopep8
☐ AutoSaveTime	☐ Autoscroll	☐ Cell Filter	☐ Code Font Size
☐ Code prettify	☐ Codefolding	☐ Codefolding in Editor	☐ CodeMirror mode extensions
✓ Collapsible Headings	☐ Comment/Uncomment Hotkey	✓ contrib_nbextensions_help_item	☐ datestamper
☐ Equation Auto Numbering	☐ ExecuteTime	☐ Execution Dependencies	☐ Exercise
☐ Exercise2	☐ Export Embedded HTML	☐ Freeze	☐ Gist-it
☐ Help panel	☐ Hide Header	☐ Hide input	✓ Hide input all
☐ hide_code/hide_code	☐ Highlight selected word	☐ highlighter	☐ Hinterland
☐ Initialization cells	☐ isort formatter	✓ jupyter-js-widgets/extension	☐ Keyboard shortcut editor
☐ Launch QTConsole	☐ Limit Output	☐ Live Markdown Preview	☐ Load TeX macros
☐ Move selected cells	☐ Navigation-Hotkeys	✓ Nbextensions dashboard tab	✓ Nbextensions edit menu item
☐ nbTranslate	✓ Notify	✓ plotlywidget/extension	☐ Printview
☐ Python Markdown	☐ Rubberband	☐ Ruler	☐ Ruler in Editor
☐ Runtools	☐ Scratchpad	☐ ScrollDown	☐ Select CodeMirror Keymap
☐ SKILL Syntax	☐ Skip-Traceback	☐ Snippets	☐ Snippets Menu
☐ spellchecker	☐ Split Cells Notebook	☐ Table of Contents (2)	☐ table_beautifier
☐ Toggle all line numbers	☐ Tree Filter	☐ Variable Inspector	☐ zenmode

Fig. 1: Configure jupyter notebook settings.

2 General information

2.1 How to start and run a notebook

Change the directory to the notebooks and start them by typing „jupyter notebook“ in the anaconda prompt. The process is opened in a browser. Select a notebook, click on restart the kernel to load the widgets (1), click on show codecell inputs to hide code (2), collapse headings if needed (3) (Fig. 2). Each notebook defines the needed input files, parameters can be adjusted, the process executed and results saved, all via userfriendly widgets.

Listing 2: Commands to run *ImageBinner* in the anaconda prompt.

```
conda activate ImageBinner
cd path_to_notebooks
jupyter notebook
```

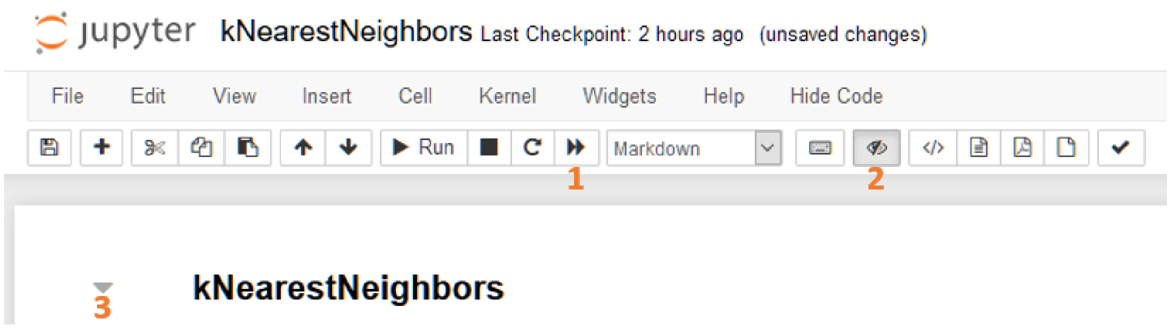
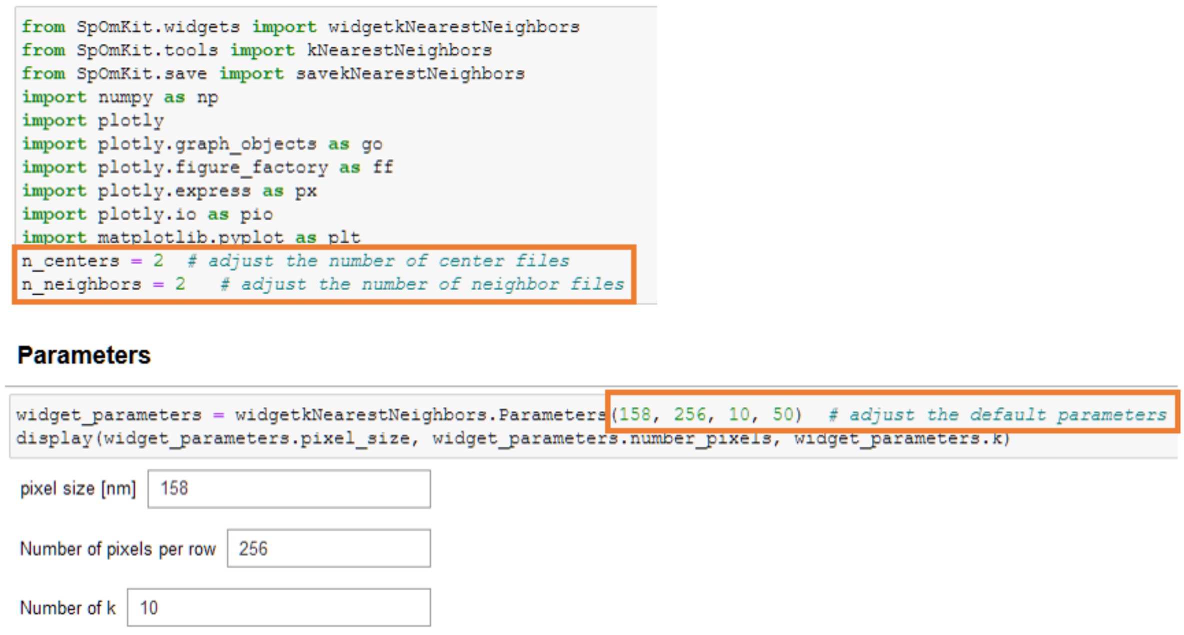


Fig. 2: Start and run a notebook.

2.2 Adjust default parameters

To adjust the default parameters *„Hide input all“* has to be deactivated to see the code. All adjustable settings are highlighted as comments, just change the values to your needs (Fig. 3).



```
from SpOmKit.widgets import widgetkNearestNeighbors
from SpOmKit.tools import kNearestNeighbors
from SpOmKit.save import savekNearestNeighbors
import numpy as np
import plotly
import plotly.graph_objects as go
import plotly.figure_factory as ff
import plotly.express as px
import plotly.io as pio
import matplotlib.pyplot as plt
n_centers = 2  # adjust the number of center files
n_neighbors = 2   # adjust the number of neighbor files
```

Parameters

```
widget_parameters = widgetkNearestNeighbors.Parameters(158, 256, 10, 50)   # adjust the default parameters
display(widget_parameters.pixel_size, widget_parameters.number_pixels, widget_parameters.k)
```

pixel size [nm] 158

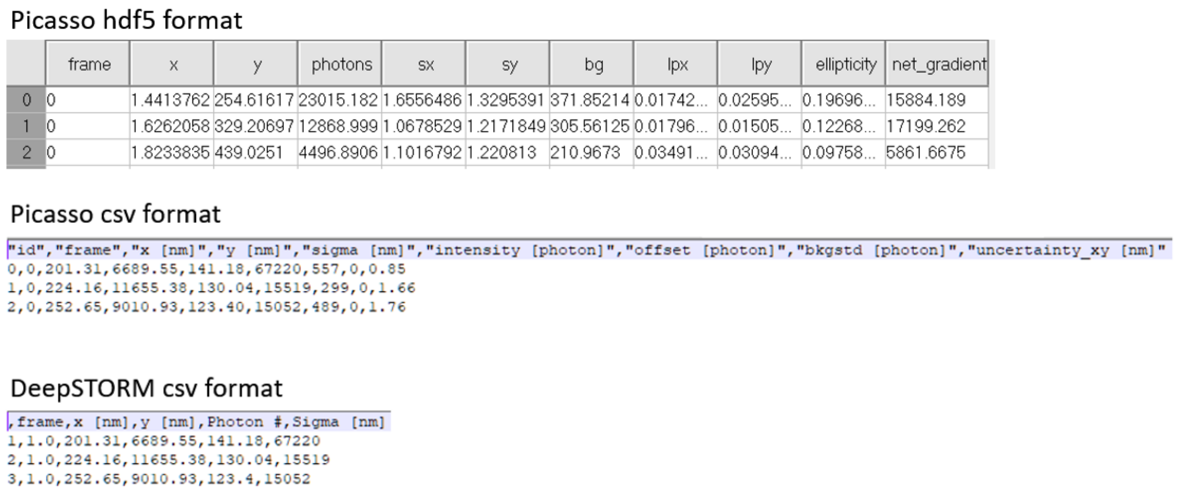
Number of pixels per row 256

Number of k 10

Fig. 3: Two examples of where to adjust the default parameters in the code. The places are marked with # comments.

3 Input filetypes

The notebooks require *.tif files that contain multiple frames of a measurement and corresponding localization files. We use Picasso localization files as ground truth. The files have to be converted to be used in DeepSTORM2D ZeroCostDL4Mic (=DeepSTORM file format) (fig. 4).



Picasso hdf5 format

	frame	x	y	photons	sx	sy	bg	lpx	lpy	ellipticity	net_gradient
0	0	1.4413762	254.61617	23015.182	1.6556486	1.3295391	371.85214	0.01742...	0.02595...	0.19696...	15884.189
1	0	1.6262058	329.20697	12868.999	1.0678529	1.2171849	305.56125	0.01796...	0.01505...	0.12268...	17199.262
2	0	1.8233835	439.0251	4496.8906	1.1016792	1.220813	210.9673	0.03491...	0.03094...	0.09758...	5861.6675

Picasso csv format

```
"id","frame","x [nm]","y [nm]","sigma [nm]","intensity [photon]","offset [photon]","bkgstd [photon]","uncertainty_xy [nm]"
0,0,201.31,6689.55,141.18,67220,557,0,0.85
1,0,224.16,11655.38,130.04,15519,299,0,1.66
2,0,252.65,9010.93,123.40,15052,489,0,1.76
```

DeepSTORM csv format

```
,frame,x [nm],y [nm],Photon #,Sigma [nm]
1,1.0,201.31,6689.55,141.18,67220
2,1.0,224.16,11655.38,130.04,15519
3,1.0,252.65,9010.93,123.4,15052
```

Fig. 4: Example of hdf5 file format of Picasso (top) csv file format of Picasso (middle) csv file format of DeepSTORM (bottom).

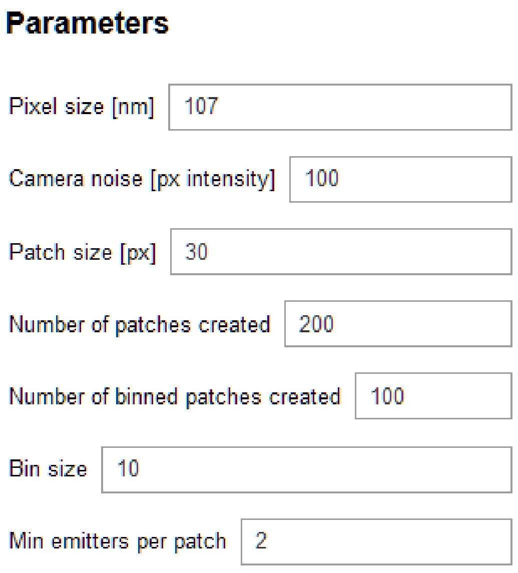
4 Notebooks

4.1 ImageBinner

This notebook takes movies and localization files of multiple measurements, cuts patches and randomly bins them. It is compatible with DeepSTORM and Picasso file formats.

4.1.1 Parameters

Following parameters have to be defined. The pixel size in nm. The camera noise in px intensitiy (average px intensity with closed shutter), as the noise is only regarded once and is subtracted bin size - n times from the final binned image. The final patch size in px, the DeepSTORM notebook handles input images with a maximum size of final patch size - 1, if a patch size of 26x26 px^2 should be the input in DeepSTORM notebook, the patch size has to be set to 27 in the ImageBinner notebook. The number of patches created defines the number of randomly cropped patches from the input measurement *.tif files. Number of binned patches created defines the final number of binned patches saved as *.tif movies, where each patch is a frame and corresponding localizations are saved as csv in DeepSTORM format, the patches for binning are drawn from the created patches and only used once per binned patch. Bin size determines the number of patches binned together. Min emitters per patch defines the minimum number of emitters a patch has to contain (before binning) to be used, because empty patches do not contain information for the network.

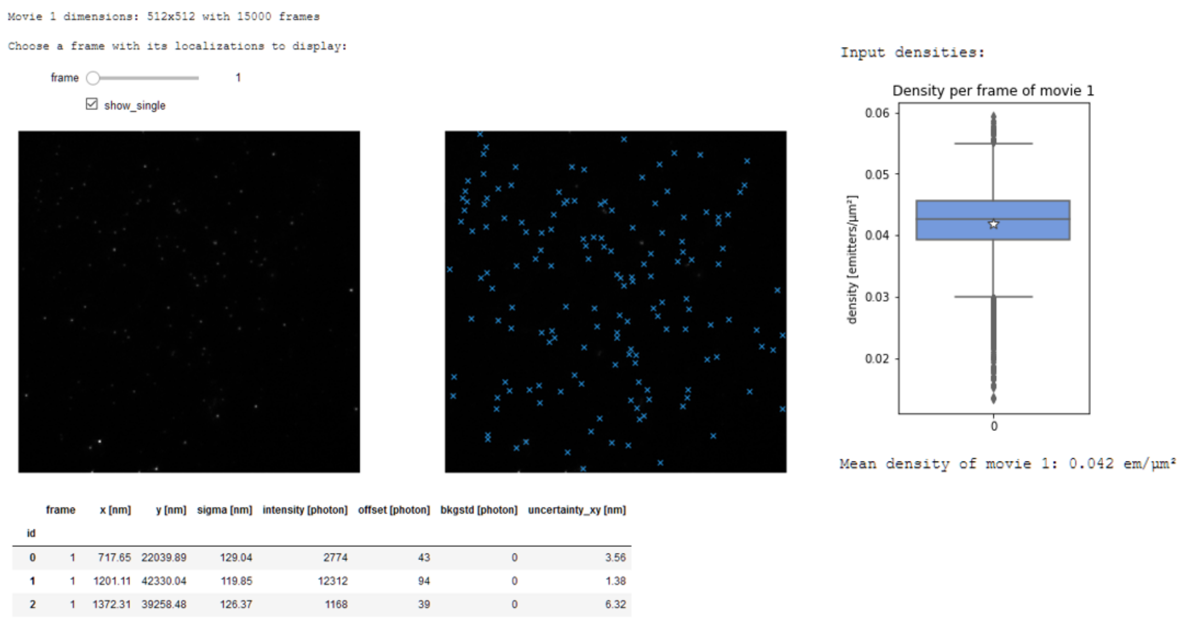

Parameters

Pixel size [nm]	107
Camera noise [px intensity]	100
Patch size [px]	30
Number of patches created	200
Number of binned patches created	100
Bin size	10
Min emitters per patch	2

Fig. 5: Parameters of ImageBinner Notebook.

4.1.2 Input

Visualization of the measurement inputs (fig. 6). A chosen measurement is visualized framewise and localizations are marked with crosses. The mean density per frame is calculated to help setting the bin size parameter.


```
Movie 1 dimensions: 512x512 with 15000 frames

Choose a frame with its localizations to display:
```

id	frame	x [nm]	y [nm]	sigma [nm]	intensity [photon]	offset [photon]	bkgstd [photon]	uncertainty_xy [nm]
0	1	717.65	22039.89	129.04	2774	43	0	3.56
1	1	1201.11	42330.04	119.85	12312	94	0	1.38
2	1	1372.31	39258.48	126.37	1168	39	0	6.32

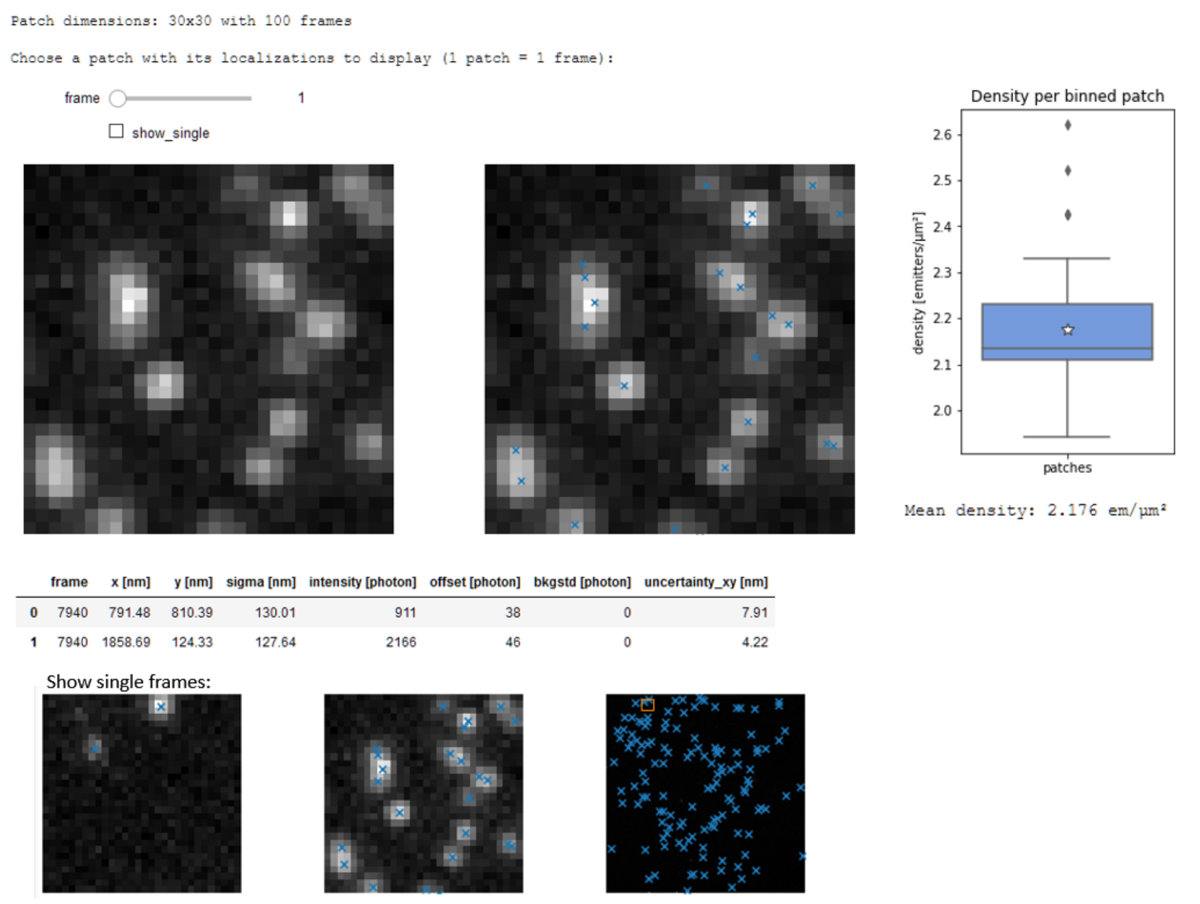
```
Input densities:
```

```
Mean density of movie 1: 0.042 em/µm²
```

Fig. 6: Visualization of the measurement input in the ImageBinner notebook. A chosen measurement is visualized framewise and the localizations are marked with blue crosses. Average frame density is calculated and displayed as boxplot.

4.1.3 Patches

Patches are created from frames of measurement files and binned. Visualization of the created binned patches (fig. 7). Binned patches are visualized framewise and localizations are marked with crosses. The mean density per patch is calculated to show the final density. Binned patches and a corresponding localization list are saved and are ready to use in the DeepSTORM notebook as input.


```
Patch dimensions: 30x30 with 100 frames

Choose a patch with its localizations to display (1 patch = 1 frame):
```

```
Mean density: 2.176 em/µm²
```

	frame	x [nm]	y [nm]	sigma [nm]	intensity [photon]	offset [photon]	bkgstd [photon]	uncertainty_xy [nm]
0	7940	791.48	810.39	130.01	911	38	0	7.91
1	7940	1858.69	124.33	127.64	2166	46	0	4.22

Show single frames:

Fig. 7: Visualization of the created patches in the ImageBinner notebook. Binned patches are visualized framewise and the localizations are marked with blue crosses. Average binned patch density is calculated and displayed as boxplot. By enabling show_single, single patches are shown that contribute to the binned image (left) single patch (middle) final binned patch (right) orange region markes the patch on the measurement frame.

4.2 DeepSTORM2DAddOns

DeepSTORM2DAddOns are small add ons that have proven helpful for the workflow.

4.2.1 Convert Picasso csv file to DeepSTORM2D csv format

A Picasso csv file is converted into DeepSTORM csv file format.
Input file: Picasso localization file csv
Output file: DeepSTORM2D file csv

4.2.2 Convert Picasso hdf5 file to DeepSTORM2D csv format

A Picasso hdf5 file is converted into DeepSTORM csv file format.
Input file: Picasso localization file hdf5
Output file: DeepSTORM2D file csv

4.2.3 Split tif movie and corresponding localization file at defined frame

A *.tif movie and corresponding localization file are split into two files at defined frame.
Input file: Tif movie and csv file (Picasso or DeepSTORM2D format).
Parameter split at frame: Number of frames of the first file.
Output file: Two tif movies and csv files, split at defined frame.

4.2.4 Merge multiple movies and localization files

Merge multiple movies and their localization files to one.
Input files: Define multiple paths to movies and their localization files (Picasso or DeepSTORM format).
Output file: Tif movie with frames of movies stacked and localization file with continous frame numbering.

4.2.5 Split into single frames

Split a movie and localization file into single frames.
Input file: Tif movie and csv file (Picasso or DeepSTORM2D format).
Output files: Single frames of tif movies with corresponding csv file saved in defined directory.

4.3 VisualizeDeepSTORM2DLocs

This notebooks visualizes the found localizations after the post-processing in the Deep-STORM notebook and helps to tune post-processing parameters (fig. 8-10).

Prediction directory: Define the directory to test file single frames and localization files (prediciton output of DeepSTORM2D, sec 6.1), optionally this directory contains the ground truth localization file.

Confidence threshold: All localizations below this threshold will be filtered out. If no filtering should be applied, set the value to 0.

Save: The localizations are filtered by the confidence threshold and saved as new csv file.

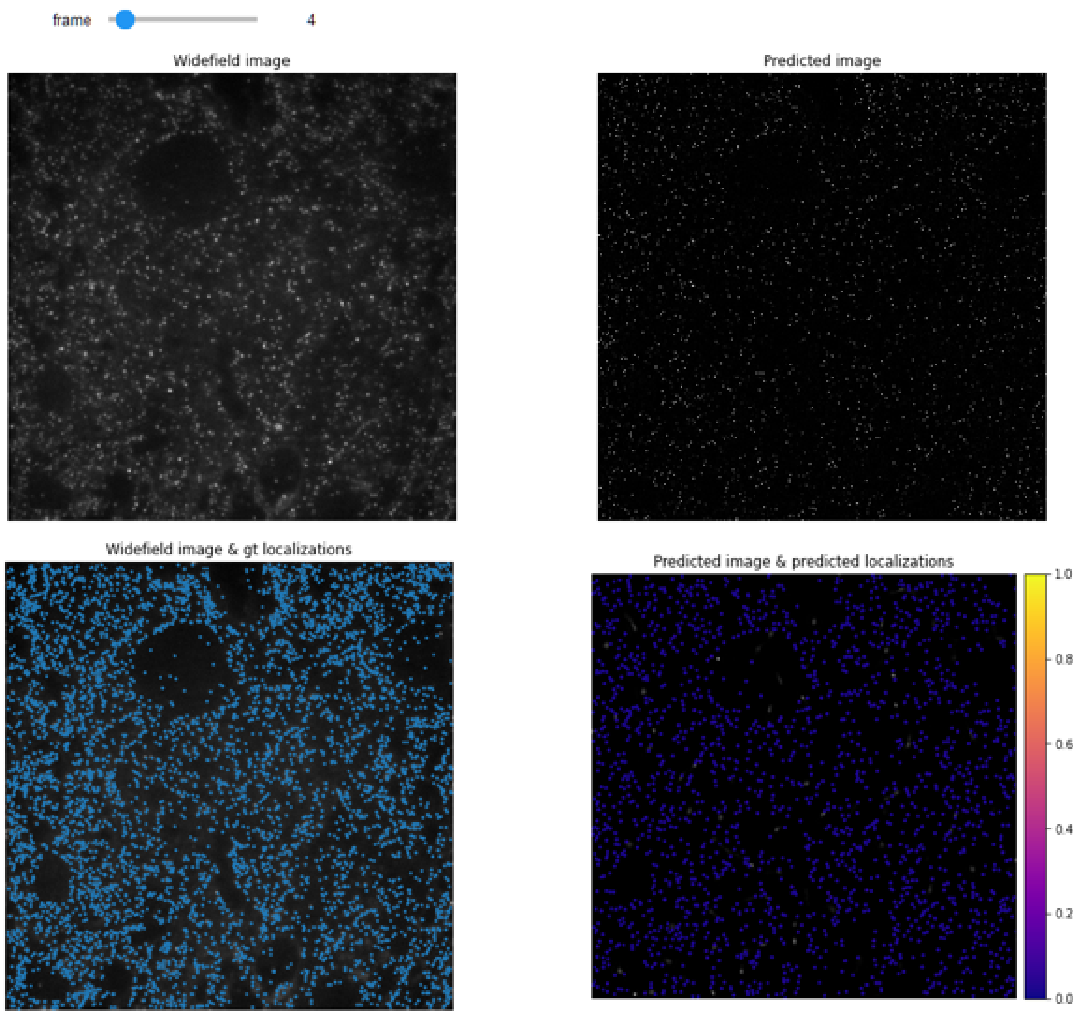

frame
4
Widefield image
Predicted image
Widefield image & gt localizations
Predicted image & predicted localizations
1.0
0.8
0.6
0.4
0.2
0.0

Fig. 8: Framewise visualization of recorded widefield image, predicted image, widefield image and ground truth localizations (if available) as blue crosses and predicted image and predicted localizations as crosses color coded by their confidence.

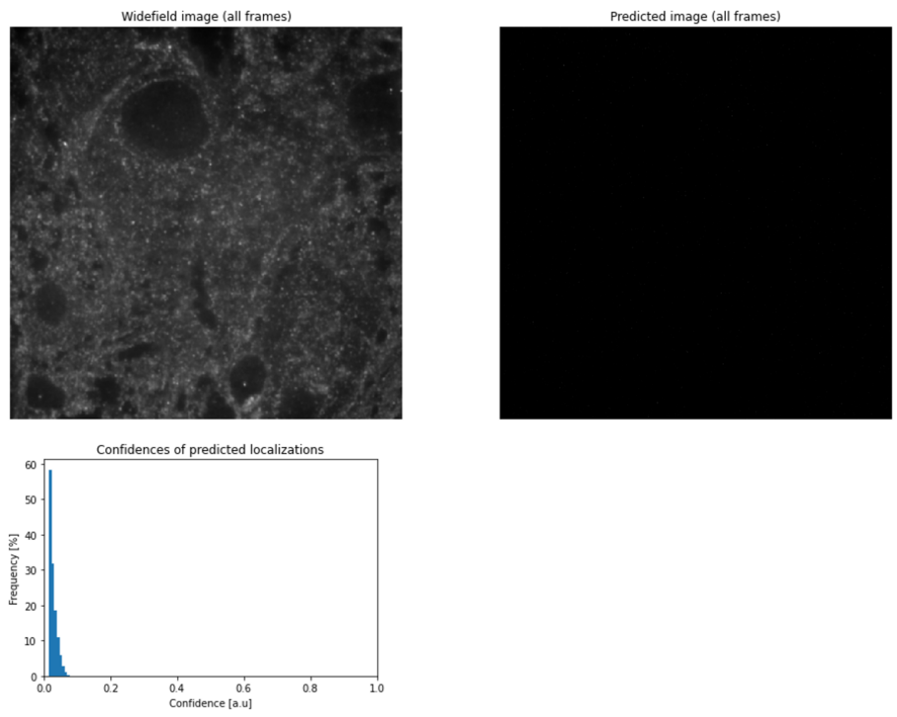
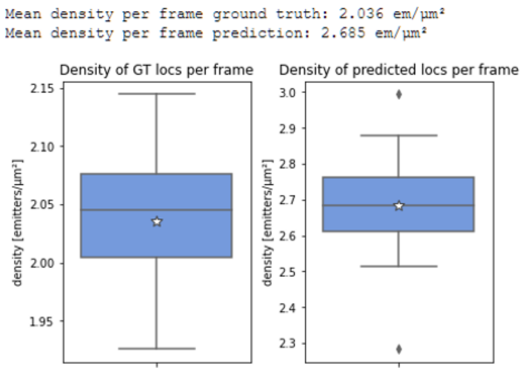


Fig. 9: Visualization of the merged widefield image (all frames are displayed as once) and the merged prediction image. Histogram of confidences of found localizations.



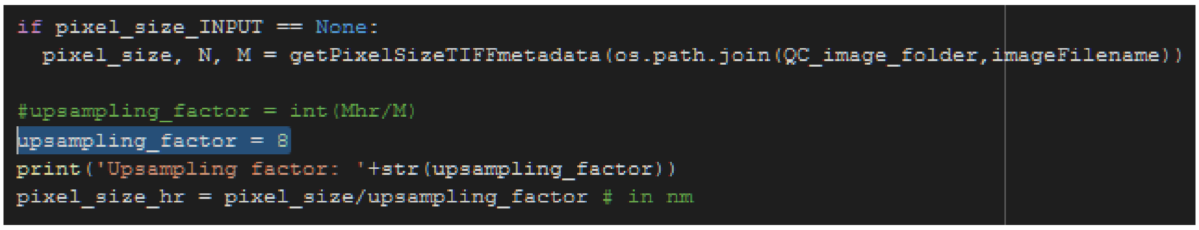
```
Mean density per frame ground truth: 2.036 em/µm²
Mean density per frame prediction: 2.685 em/µm²
```

Fig. 10: Mean density of ground truth localizations and predicted localizations.

5 DeepSTORM2D notebook workflow and code adjustments

In Section 3.2 Generate training patches, patch_size is set to the patch_size created in ImageBinner - 1 (smaller patch sizes are possible as well). To consider each binned patch once, the parameter num_patches_per_frame is set to 1.

In Section 5.2 Error mapping and quality metrics estimation, the upsampling factor has to be hard coded, because image sizes are calculated based on the inputs in Section 3.2 that differ from the test image dimensions.

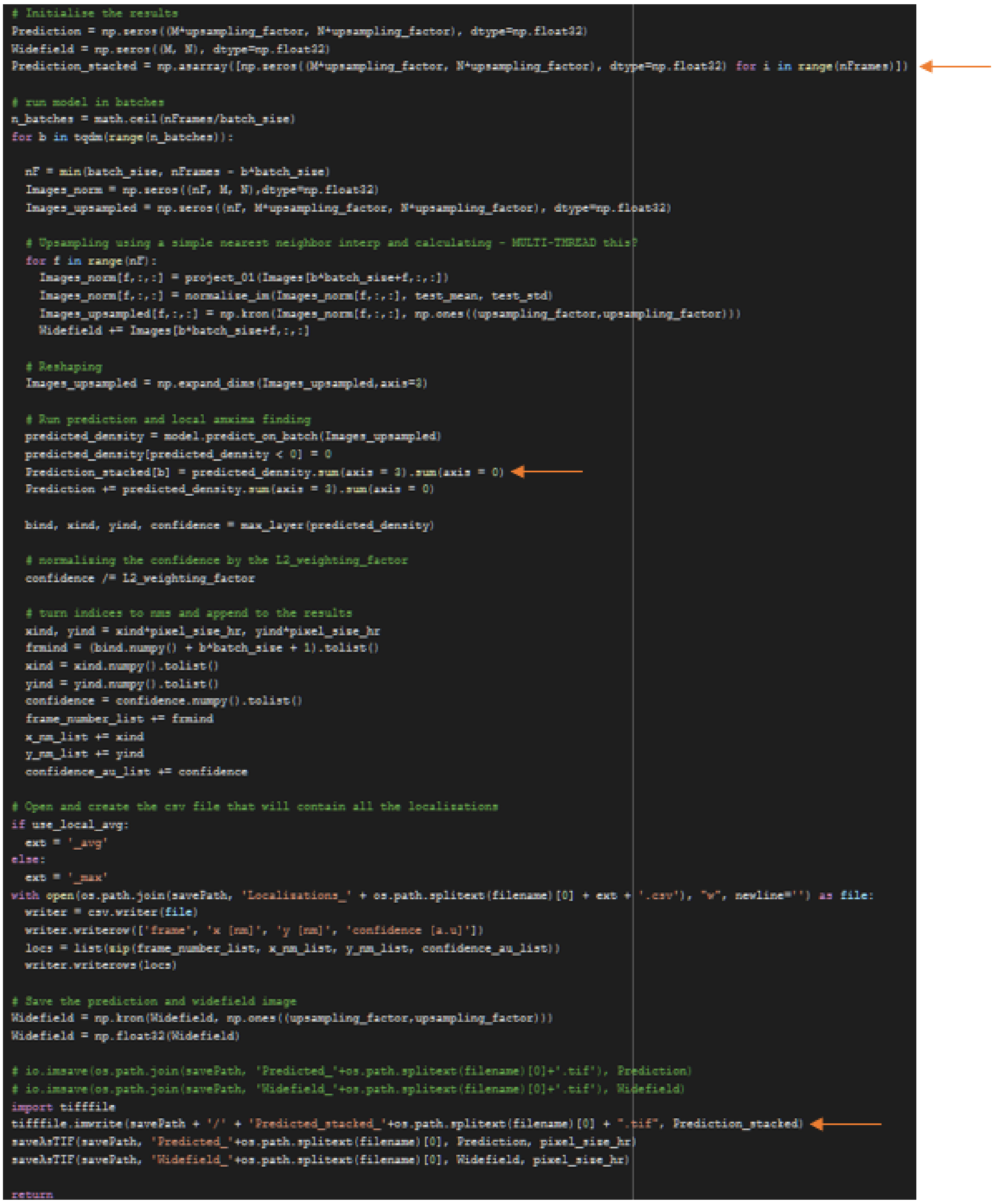


```
if pixel_size_INPUT == None:
  pixel_size, N, M = getPixelSizeTIFFmetadata(os.path.join(QC_image_folder,imageFilename))

#upsampling_factor = int(Mhr/M)
upsampling_factor = 8
print('Upsampling factor: '+str(upsampling_factor))
pixel_size_hr = pixel_size/upsampling_factor # in nm
```

Fig. 11: Code change in section 5.3: Hardcode the upsampling factor in this section to run quality control on differently shaped test images.

In Section 2 save the prediciton not only as a merged image over all frames, but also as a stacked *.tif with each frame accessible. This helps to adjust post-processing parameters, but needs a lot of disk space.



```python
# Initialise the results
Prediction = np.zeros((M*upsampling_factor, N*upsampling_factor), dtype=np.float32)
Widefield = np.zeros((M, N), dtype=np.float32)
Prediction_stacked = np.asarray([np.zeros((M*upsampling_factor, N*upsampling_factor), dtype=np.float32) for i in range(nFrames)])

# run model in batches
n_batches = math.ceil(nFrames/batch_size)
for b in tqdm(range(n_batches)):

  nF = min(batch_size, nFrames - b*batch_size)
  Images_norm = np.zeros((nF, M, N),dtype=np.float32)
  Images_upsampled = np.zeros((nF, M*upsampling_factor, N*upsampling_factor), dtype=np.float32)

  # Upsampling using a simple nearest neighbor interp and calculating - MULTI-THREAD this?
  for f in range(nF):
    Images_norm[f,:,:] = project_01(Images[b*batch_size+f,:,:])
    Images_norm[f,:,:] = normalize_im(Images_norm[f,:,:], test_mean, test_std)
    Images_upsampled[f,:,:] = np.kron(Images_norm[f,:,:], np.ones((upsampling_factor,upsampling_factor)))
    Widefield += Images[b*batch_size+f,:,:]

  # Reshaping
  Images_upsampled = np.expand_dims(Images_upsampled,axis=3)

  # Run prediction and local amxima finding
  predicted_density = model.predict_on_batch(Images_upsampled)
  predicted_density[predicted_density < 0] = 0
  Prediction_stacked[b] = predicted_density.sum(axis = 3).sum(axis = 0)
  Prediction += predicted_density.sum(axis = 3).sum(axis = 0)

  bind, xind, yind, confidence = max_layer(predicted_density)

  # normalizing the confidence by the L2_weighting_factor
  confidence /= L2_weighting_factor

  # turn indices to nms and append to the results
  xind, yind = xind*pixel_size_hr, yind*pixel_size_hr
  frmind = (bind.numpy() + b*batch_size + 1).tolist()
  xind = xind.numpy().tolist()
  yind = yind.numpy().tolist()
  confidence = confidence.numpy().tolist()
  frame_number_list += frmind
  x_nm_list += xind
  y_nm_list += yind
  confidence_au_list += confidence

# Open and create the csv file that will contain all the localizations
if use_local_avg:
  ext = '_avg'
else:
  ext = '_max'
with open(os.path.join(savePath, 'Localizations_' + os.path.splitext(filename)[0] + ext + '.csv'), "w", newline='') as file:
  writer = csv.writer(file)
  writer.writerow(['frame', 'x [nm]', 'y [nm]', 'confidence [a.u]'])
  locs = list(zip(frame_number_list, x_nm_list, y_nm_list, confidence_au_list))
  writer.writerows(locs)

# Save the prediction and widefield image
Widefield = np.kron(Widefield, np.ones((upsampling_factor,upsampling_factor)))
Widefield = np.float32(Widefield)

# io.imsave(os.path.join(savePath, 'Predicted_'+os.path.splitext(filename)[0]+'.tif'), Prediction)
# io.imsave(os.path.join(savePath, 'Widefield_'+os.path.splitext(filename)[0]+'.tif'), Widefield)
import tifffile
tifffile.imwrite(savePath + '/' + 'Predicted_stacked_'+os.path.splitext(filename)[0] + ".tif", Prediction_stacked)
saveAsTIF(savePath, 'Predicted_'+os.path.splitext(filename)[0], Prediction, pixel_size_hr)
saveAsTIF(savePath, 'Widefield_'+os.path.splitext(filename)[0], Widefield, pixel_size_hr)

return
```

Fig. 12: Code change in section 2: Save prediciton as stacked tif with single frames additionally to merged prediction output.

6 Literatur

[1] E. Nehme, L. E. Weiss, T. Michaeli, Y. Shechtman, *Optica* **2018**, *5*, 458–464.

[2] L. Chamier, R. F. Laine, J. Jukkala, C. Spahn, D. Krentzel, E. Nehme, M. Lerche, S. Herández-Pérez, P. K. Mattila, E. Karinou, S. Holden, A. C. Solak, A. Krull, T.-O. Buchholz, M. L. Jones, L. Royer, C. Leterrier, Y. Shechtman, F. Jug, M. Heilemann, G. Jacquemet, R. Henriques, *Nat. Comm.* **2021**, 12:2276.