ISM Decay Reconstruction BrightEyes-TTM v1 opensource

October 7, 2021

1 ISM reconstruction from BrightEyes-TTM time-tag data

In this notebooks I outline how you can use MIPLIB library functions to reconstruct 4D array detector data (xytc).

```
[177]: import h5py
       import os
       import numpy as np
       import cython
       import usb
       import struct
       import time
       import signal
       import sys
       import tqdm #This package is usefull for have nice progress bar
       from scipy.optimize import curve_fit
       from ipywidgets import interact, interactive, fixed, interact_manual
       import ipywidgets as widgets
       import datetime
       import matplotlib.gridspec as gridspec
       import subprocess
       import pandas as pd
       import numpy as np
       from matplotlib.colors import LogNorm
       from matplotlib import pyplot as plt
       from ipyfilechooser import FileChooser
       from pathlib import Path
       from miplib.ui.plots import image as implots
       from miplib.data.adapters import array_detector_data as adapters
       #from miplib.data.containers.array detector data import ArrayDetectorData
       import miplib.ui.cli.miplib_entry_point_options as options
       import miplib.processing.ism.reconstruction as ismrec
       import miplib.processing.ism.helpers as ismhelps
       from miplib.ui.plots import scatter
       from miplib.processing import itk as itkutils
```

```
[178]: %pylab inline
      #%% Plot Config
      SMALL_SIZE = 14
      MEDIUM_SIZE = 18
      BIG SIZE = 22
      BIGGER_SIZE = 28
      plt.rc('font', size=SMALL_SIZE)
                                                # controls default text sizes
      plt.rc('axes', titlesize=BIG_SIZE)
                                                # fontsize of the axes title
                                                # fontsize of the x and y labels
      plt.rc('axes', labelsize=BIG_SIZE)
      plt.rc('xtick', labelsize=MEDIUM_SIZE)
                                                # fontsize of the tick labels
      plt.rc('ytick', labelsize=MEDIUM_SIZE)
                                                # fontsize of the tick labels
      plt.rc('legend', fontsize=MEDIUM_SIZE)
                                                # legend fontsize
      plt.rc('figure', titlesize=BIGGER_SIZE)
                                                # fontsize of the figure title
      plt.rc('figure', titlesize=BIGGER_SIZE)
                                                # fontsize of the figure title
```

Populating the interactive namespace from numpy and matplotlib

2 Imported Data Settings

3 4D file selection

Load 4D data x-y-t-ch for ISM analysis

```
[180]: fc = FileChooser()
   fc.default_path='/home/labuser/myDev/timetaggingplatform/pythonNotebooks'
   fc.use_dir_icons = True
   display(fc)
```

FileChooser(path='/home/labuser/myDev/timetaggingplatform/pythonNotebooks', ofilename='', title='HTML(value='', ...

```
[181]: filename = fc.selected #<--- 4D file selection (NB-time decays should be

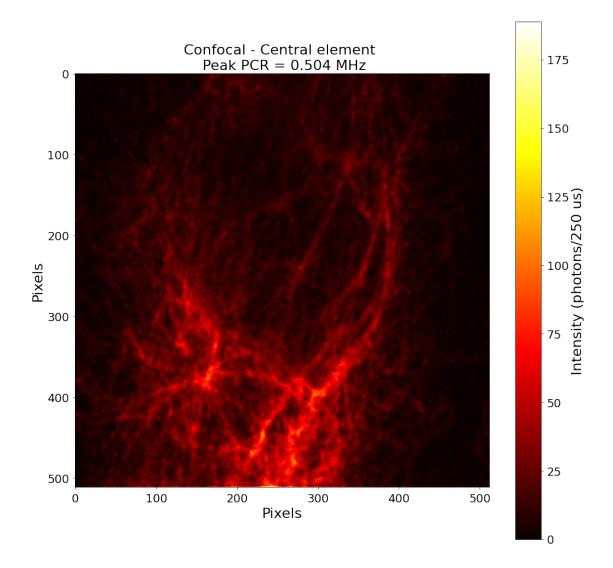
→referenced already for phasor plot analysis)

data_filename=os.path.basename(filename)

data = h5py.File(filename, 'r+')
```

Re-arranging the dataset

```
[182]: dataset = data['dataset_1']
       spacing = (1, 25/pixel_number, 25/pixel_number)
       dataset.attrs["spacing"] = spacing
[183]: # Central element Phasor plot RAW Dataset
       central_element_raw = dataset[:,:,:,10] #12 identify the central element of the
       central_element_raw_image = np.sum(central_element_raw, axis=2)
       p=linspace(0,nbins-1,nbins)
       cosine=np.cos(2*np.pi*p*h/nbins)
       sine=np.sin(2*np.pi*p*h/nbins)
       cosine_matrix = np.tile(cosine,(pixel_number,pixel_number,1))
       sine_matrix = np.tile(sine,(pixel_number,pixel_number,1))
       A=central_element_raw*cosine_matrix
       g_central_element_raw=np.sum(A, axis=2)
       B=central_element_raw*sine_matrix
       s_central_element_raw=np.sum(B, axis=2)
[184]: #Plotting central element image
       fig= plt.figure(figsize=(12,12))
       plt.imshow(central_element_raw_image, cmap='hot')
       fig=plt.gcf()
       tight_layout()
       cbar=plt.colorbar()
       cbar.ax.set_ylabel('Intensity (photons/'+str(pixel_dwell_time)+' us)')
       plt.title('Confocal - Central element \n Peak PCR =__
       →'+str(central_element_raw_image[0:511,0:511].max()/pixel_dwell_time)+' MHz')
       plt.xlabel('Pixels')
       plt.ylabel('Pixels')
       #Uncomment for saving the image as vector file
       # plt.rcParams['svq.fonttype'] = 'none'
       \# fig.savefig("Central\_Element\_"+data\_filename+".svg", transparent=True, \_
       \rightarrow format='svq', dpi=1200)
       # fig.savefig("Central_Element_"+data_filename+".eps", transparent=True,_
        \rightarrow format='eps', dpi=1200)
[184]: Text(69.5400000000002, 0.5, 'Pixels')
```



```
[185]: # Computing the g & s vector for pixel having >5% and >10% of max intensity_

respectively

threshold_1 = 10 #plotting pixels with intensity >10% of the max
threshold_2 = 5 #plotting pixels with intensity >5% of the max

g_central_element_raw_final = g_central_element_raw[6:506,6:506]/

central_element_raw_image[6:506,6:506]

s_central_element_raw_final = s_central_element_raw[6:506,6:506]/

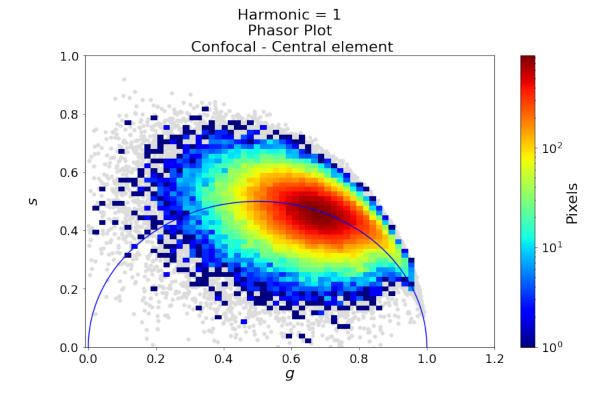
central_element_raw_image[6:506,6:506]

# Specify a threshold for the minimum amout of photons per pixel to analyze
```

```
\rightarrow 506,6:506].max()/100))
       central_threshold_5percent = floor(threshold_2*(central_element_raw_image[6:
        \rightarrow 506,6:506].max()/100))
       central_10percent=central_element_raw_image[6:506,6:
        →506]>central_threshold_10percent
       central_5percent=central_element_raw_image[6:506,6:
       →506]>central_threshold_5percent
       s_final_10percent = s_central_element_raw_final*central_10percent
       g_final_10percent = g_central_element_raw_final*central_10percent
       s_final_5percent = s_central_element_raw_final*central_5percent
       g_final_5percent = g_central_element_raw_final*central_5percent
       g_where_are_NaNs_10percent = np.isnan(g_final_10percent)
       s_where_are_NaNs_10percent = np.isnan(s_final_10percent)
       g where are NaNs 5percent = np.isnan(g final 5percent)
       s_where_are_NaNs_5percent = np.isnan(s_final_5percent)
       g_final_nan_10percent=g_final_10percent[g_where_are_NaNs_10percent==False]
       s_final_nan_10percent=s_final_10percent[s_where_are_NaNs_10percent==False]
       g_final_nan_5percent=g_final_5percent[g_where_are_NaNs_5percent==False]
       s final nan 5percent=s final 5percent[s where are NaNs 5percent==False]
      <ipython-input-185-15d3e579ad9d>:6: RuntimeWarning: invalid value encountered in
      true_divide
        g_central_element_raw_final =
      g_central_element_raw[6:506,6:506]/central_element_raw_image[6:506,6:506]
      <ipython-input-185-15d3e579ad9d>:7: RuntimeWarning: invalid value encountered in
      true_divide
        s_central_element_raw_final =
      s_central_element_raw[6:506,6:506]/central_element_raw_image[6:506,6:506]
[186]: fig= plt.figure(figsize=(12,8))
       plt.scatter(g_final_nan_5percent[g_final_nan_5percent>0],__
        →s_final_nan_5percent[g_final_nan_5percent>0],c='gainsboro',alpha=1,rasterized=True)
       plt.hist2d(g_final_nan_10percent[g_final_nan_10percent>0],__
        ⇒s_final_nan_10percent[g_final_nan_10percent>0], bins=50, cmap =
       →'jet',norm=LogNorm())
       cbar=plt.colorbar()
       cbar.ax.set ylabel('Pixels')
       plt.title('Harmonic = '+str(h)+'\n Phasor Plot \n Confocal - Central element')
```

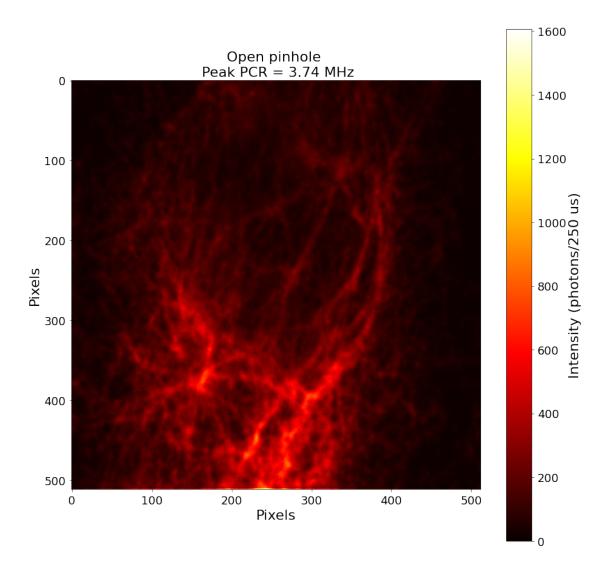
central_threshold_10percent =floor(threshold_1*(central_element_raw_image[6:

```
plt.xlabel('$g$')
plt.ylabel('$s$\n')
xx=np.linspace(-2,2,400)
yy=np.linspace(-2,2,400)
[X,Y]=np.meshgrid(xx,yy)
plt.xlim((-0.01,1.2)) # set the xlim to left, right
plt.ylim((0, 1))
                 # set the xlim to left, right
Z=X**2-X+Y**2
plt.contour(X,Y,Z,[0],colors='b')
fig=plt.gcf()
tight_layout()
#Uncomment for saving the image as vector file
# plt.rcParams['svq.fonttype'] = 'none'
# fig.savefig("Central_Element_PhasorPlot"+data_filename+".svg",__
\rightarrow transparent=True, format='svg', dpi=1200)
#fig.savefig("Central_Element_PhasorPlot"+data_filename+".eps",__
→ transparent=True, format='eps', dpi=1200)
```



```
[187]: all_elements_raw = np.sum(dataset, axis=3)
       all_elements_raw_image = np.sum(all_elements_raw, axis=2)
       fig= plt.figure(figsize=(12,12))
       plt.imshow(all_elements_raw_image, cmap='hot')
       fig=plt.gcf()
       tight_layout()
       cbar=plt.colorbar()
       cbar.ax.set_ylabel('Intensity (photons/'+str(pixel_dwell_time)+' us)')
       plt.title('Open pinhole \n Peak PCR = '+str(all_elements_raw_image[0:511,0:511].
       →max()/pixel_dwell_time)+' MHz')
       plt.xlabel('Pixels')
       plt.ylabel('Pixels')
       # Uncomment to save images in vector format
       # plt.rcParams['svg.fonttype'] = 'none'
       # fig.savefig("SUM_"+data_filename+".svg", transparent=True, format='svg',
       \rightarrow dp i=1200)
       # fig.savefig("SUM_"+data_filename+".eps", transparent=True, format='eps',
        \rightarrow dp i=1200)
```

[187]: Text(69.5400000000002, 0.5, 'Pixels')



```
[188]: # All elements Phasor plot RAW Dataset
all_elements_raw = np.sum(dataset, axis=3)
all_elements_raw_image = np.sum(all_elements_raw, axis=2)

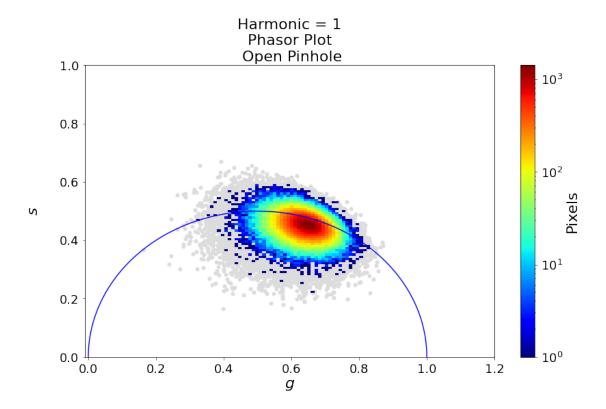
g_all_elements_raw=np.zeros((pixel_number,pixel_number))
s_all_elements_raw=np.zeros((pixel_number,pixel_number))

A=all_elements_raw*cosine_matrix
g_all_elements_raw*sine_matrix
s_all_elements_raw*sine_matrix
s_all_elements_raw=np.sum(B, axis=2)
```

```
[189]: # Computing the q & s vector for pixel having >5% and >10% of max intensity.
        \rightarrow respectively
       threshold_1 = 10 #plotting pixels with intensity >10% of the max
       threshold_2 = 5 #plotting pixels with intensity >5% of the max
       g_all_elements_raw_final = g_all_elements_raw[6:506,6:506]/
        \rightarrowall_elements_raw_image[6:506,6:506]
       s_all_elements_raw_final = s_all_elements_raw[6:506,6:506]/
       \rightarrowall_elements_raw_image[6:506,6:506]
       # Specify a threshold for the minimum amout of photons per pixel to analyze
       sum_threshold_10percent = floor(threshold_1*(all_elements_raw_image[6:506,6:
        \hookrightarrow 506].max()/100))
       sum_threshold_5percent = floor(threshold_2*(all_elements_raw_image[6:506,6:506].
        \rightarrowmax()/100))
       sum_10percent=all_elements_raw_image[6:506,6:506]>sum_threshold_10percent
       sum_5percent=all_elements_raw_image[6:506,6:506]>sum_threshold_5percent
       s_final_10percent = s_all_elements_raw_final*sum_10percent
       g_final_10percent = g_all_elements_raw_final*sum_10percent
       s_final_5percent = s_all_elements_raw_final*sum_5percent
       g_final_5percent = g_all_elements_raw_final*sum_5percent
       g_where_are_NaNs_10percent = np.isnan(g_final_10percent)
       s_where_are_NaNs_10percent = np.isnan(s_final_10percent)
       g_where_are_NaNs_5percent = np.isnan(g_final_5percent)
       s_where_are_NaNs_5percent = np.isnan(s_final_5percent)
       g final nan 10percent=g final 10percent[g where are NaNs 10percent==False]
       s final nan 10percent=s final 10percent[s where are NaNs 10percent==False]
       g_final_nan_5percent=g_final_5percent[g_where_are_NaNs_5percent==False]
       s final nan 5percent=s final 5percent[s where are NaNs 5percent==False]
      <ipython-input-189-b3f24b8475c5>:7: RuntimeWarning: invalid value encountered in
      true_divide
        g_all_elements_raw_final =
      g_all_elements_raw[6:506,6:506]/all_elements_raw_image[6:506,6:506]
      <ipython-input-189-b3f24b8475c5>:8: RuntimeWarning: invalid value encountered in
      true_divide
        s_all_elements_raw_final =
```

s_all_elements_raw[6:506,6:506]/all_elements_raw_image[6:506,6:506]

```
[190]: fig= plt.figure(figsize=(12,8))
       plt.scatter(g_final_nan_5percent[g_final_nan_5percent>0],__
       →s_final_nan_5percent[g_final_nan_5percent>0],c='gainsboro',alpha=1,rasterized=True)
       plt.hist2d(g_final_nan_10percent[g_final_nan_10percent>0],__
       →s_final_nan_10percent[g_final_nan_10percent>0], bins=50, cmap =
       cbar=plt.colorbar()
       cbar.ax.set ylabel('Pixels')
       plt.title('Harmonic = '+str(h)+'\n Phasor Plot \n Open Pinhole')
       plt.xlabel('$g$')
       plt.ylabel('$s$\n')
       xx=np.linspace(-2,2,400)
       yy=np.linspace(-2,2,400)
       [X,Y]=np.meshgrid(xx,yy)
       plt.xlim((-0.01,1.2)) # set the xlim to left, right
       plt.ylim((0, 1)) # set the xlim to left, right
       Z=X**2-X+Y**2
       plt.contour(X,Y,Z,[0],colors='b')
       fig=plt.gcf()
       tight_layout()
       # plt.rcParams['svq.fonttype'] = 'none'
       \# fig.savefig("SUM\_PhasorPlot\_"+data\_filename+".svg", transparent=True, \_
       \rightarrow format='svg', dpi=1200)
       \#fig.savefig("SUM\_PhasorPlot\_"+data\_filename+".eps", transparent=True, \_
       \rightarrow format='eps', dpi=1200)
```



For reconstruction purposes it makes more sense to reorder the axes a little bit.

3.1 Calculating the ISM shift vectors

MIPLIB uses an internal data structure called ArrayDetectorData to contain ISM data and results. Here instead of doing a real conversion, I just wrap an array into an adapter, that makes the data act like an ArrayDetectorData object. In order to have the data in a correct shape, I sum over the time bins, and add a new empty axis for a photosensor. We use the photosensor in the traditional ISM to split a pixel dwell time into several pieces. It of course has no other purpose in here than having the data in a shape that is compatible with the library functions.

```
[192]: ad_data_2D = adapters.ArrayAdapter(np.expand_dims(np.sum(data, axis=1), axis=0), dataset.attrs["spacing"][1:])
```

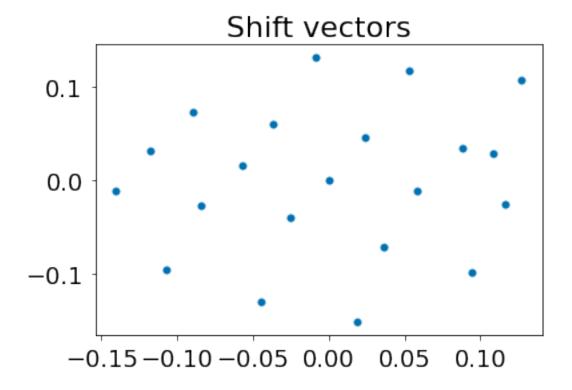
Then it's time to run the registration. There are several parameters that you can tune, but the defaults should be fine in 99.9% of cases. Please adjust the *fixed_idx* to match the index of the central detector. It's normally 12, but 10 here, because the corner pixels are missing. Actually the registration will in principle work with any of the images as reference, but probably not very robustly.

Below I plot the detected xy offsets. The detector seems to be somewhat misaligned. In any case the ISM should work just fine, as there are clear offsets between the channels.

```
[194]: ys = np.transpose(offsets)[0]
xs = np.transpose(offsets)[1]

plt.title('Shift vectors')
plt.scatter(xs,ys)
```

[194]: <matplotlib.collections.PathCollection at 0x7f5b6b146340>

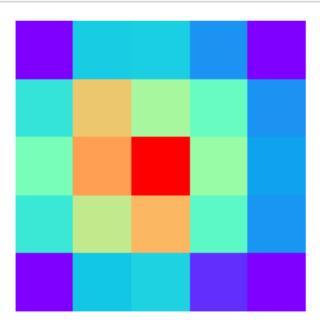


Just to take a closer look at the alignment situation, below you can see the fingerprint image. Indeed it seems that the PSF is not really in the center of the detector, but rather close to the bottom.

```
[195]: fingerprint = np.sum(data, axis=(1,2,3))

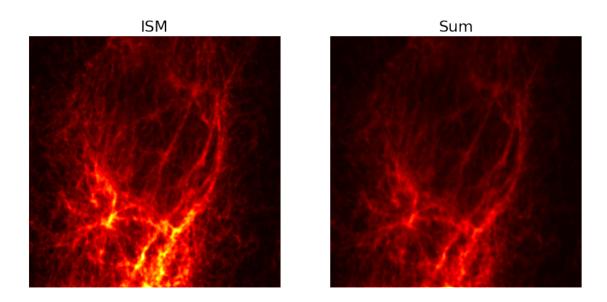
missing = (0,4,20,24)
for idx in missing:
    fingerprint = np.insert(fingerprint, idx, 0)

implots.display_2d_image(fingerprint.reshape(5,5))
```



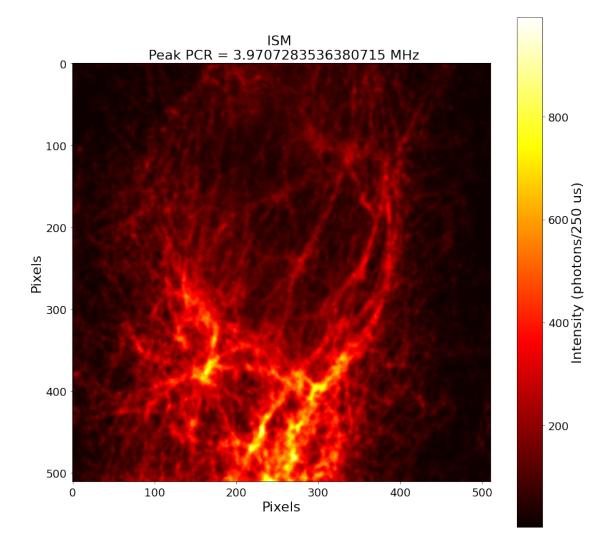
3.2 ISM pixel reassignment

Now that we have the 2D offsets, we can calculate the ISM results. First I will start with the 2D (time-projected) dataset that was used above, just to make sure that everything works properly. The result_sum is in practice the regular confocal with a pinhole matching the detector FOV, and result_ism is the reassignment result. Nothing extraordinary here, but the reassignment does increase the image quality clearly.



```
[198]: fig= plt.figure(figsize=(15,15))
    plt.imshow(result_ism[0:511,0:511], cmap='hot')
    cbar=plt.colorbar()
    cbar.ax.set_ylabel('Intensity (photons/'+str(pixel_dwell_time)+' us)')
    plt.title('ISM \n Peak PCR = '+str(result_ism.max()/pixel_dwell_time)+' MHz')
    plt.xlabel('Pixels')
    plt.ylabel('Pixels')
```

[198]: Text(0, 0.5, 'Pixels')



In order to process the full dataset, we just basically have to do the same reassignment on each of the time bins. Here in order to speed up things a little bit I construct 3D spatial transforms with z (== time) offset set to zero. That allows me to use 3D resampling functions for the reassignment, which is quite a bit faster than looping over the time bins one-by-one. Please note that I use exatly the same library functions as above.

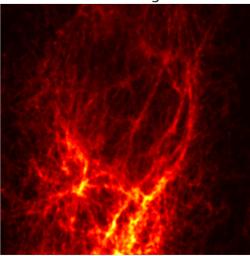
Here I compare the two ISM results: one obtained by summing of time first and then calculating

the reassignment, and the other by doing the reassignment first and then summing over time. Of course the difference is that the 3D result, maintains the time information, so you can do lifetime fitting etc if you like.

```
[201]: implots.display_2d_images(result_ism, result_ism_3d_tproject, image1_title='ISM - summed over \n t before \to re-assignment', image2_title='ISM - summed over \n t after \to re-assignment')
```

ISM - summed over t before re-assignment

ISM - summed over t after re-assignment



Just to make sure that alls is as it should, here's the difference between the above two images. The noise distribution seems to be a bit different, but that is to be expected.

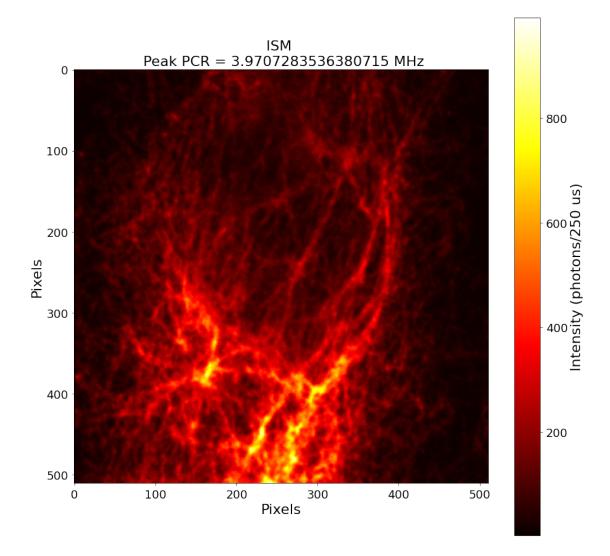
```
[202]: implots.display_2d_image(result_ism_3d_tproject-result_ism)
```



Just to make sure, to conclude, here I plot the time trace thorugh the stack (all pixels summed). Seems to be fine.

```
[203]: shifted_sum_3d = ismrec.shift_and_sum(ad_data, transforms_3d)
[204]: shifted_sum_3d_dataset_image=np.sum(shifted_sum_3d, axis=(0))
[205]: fig= plt.figure(figsize=(12,12))
       plt.imshow(shifted_sum_3d_dataset_image[0:511,0:511], cmap='hot')
       fig=plt.gcf()
       tight_layout()
       cbar=plt.colorbar()
       cbar.ax.set_ylabel('Intensity (photons/'+str(pixel_dwell_time)+' us)')
       plt.title('ISM \n Peak PCR = '+str(result_ism.max()/pixel_dwell_time)+' MHz')
       plt.xlabel('Pixels')
       plt.ylabel('Pixels')
       # Uncomment to save vector image
       # plt.rcParams['svg.fonttype'] = 'none'
       # fig.savefig("ISM_"+data_filename+".svg", transparent=True, format='svg',
        \rightarrow dp i=1200)
       # fig.savefig("ISM "+data filename+".eps", transparent=True, format='eps', ____
        \rightarrow dpi=1200)
```

[205]: Text(69.5400000000002, 0.5, 'Pixels')



```
[206]: # Phasor plot calculation for ISM data

p=linspace(0,nbins-1,nbins)
    cosine=np.cos(2*np.pi*p*h/nbins)
    sine=np.sin(2*np.pi*p*h/nbins)

cosine_matrix = np.tile(cosine,(pixel_number,pixel_number,1))
    cosine_matrix.shape

sine_matrix = np.tile(sine,(pixel_number,pixel_number,1))

cosine_matrix_sw=np.swapaxes(np.swapaxes(cosine_matrix,0,2),1,2)
    sine_matrix_sw=np.swapaxes(np.swapaxes(sine_matrix,0,2),1,2)

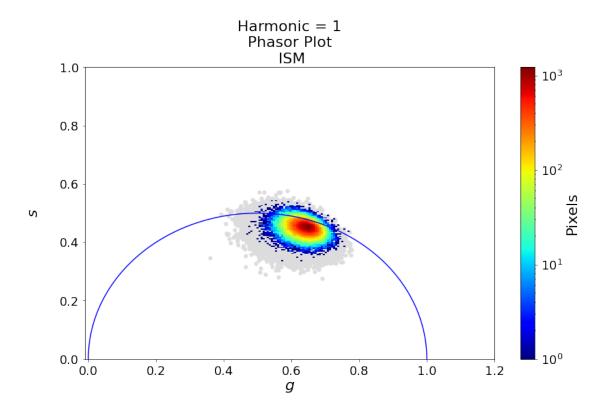
A=shifted_sum_3d*cosine_matrix_sw
```

```
B=shifted_sum_3d*sine_matrix_sw
       s=np.sum(B, axis=0)
[207]: # Computing the g & s vector for pixel having >5% and >10% of max intensity.
       \rightarrow respectively
       threshold_1 = 10 #plotting pixels with intensity >10% of the max
       threshold_2 = 5 #plotting pixels with intensity >5% of the max
       g_final = g[6:506,6:506]/shifted_sum_3d_dataset_image[6:506,6:506]
       s_final = s[6:506,6:506]/shifted_sum_3d_dataset_image[6:506,6:506]
       # Specify a threshold for the minimum amout of photons per pixel to analyze
       sum_shift_threshold_10percent =_
       -floor(threshold_1*(shifted_sum_3d_dataset_image[6:506,6:506].max()/100))
       sum_shift_threshold_5percent =_
       →floor(threshold_2*(shifted_sum_3d_dataset_image[6:506,6:506].max()/100))
       mask_sum_shift_10percent=shifted_sum_3d_dataset_image[6:506,6:
       →506]>sum_shift_threshold_10percent
       mask_sum_shift_5percent=shifted_sum_3d_dataset_image[6:506,6:
       →506]>sum_shift_threshold_5percent
       s_final_10percent = s_final*mask_sum_shift_10percent
       g_final_10percent = g_final*mask_sum_shift_10percent
       s_final_5percent = s_final*mask_sum_shift_5percent
       g_final_5percent = g_final*mask_sum_shift_5percent
       g_where_are_NaNs_10percent = np.isnan(g_final_10percent)
       s_where_are_NaNs_10percent = np.isnan(s_final_10percent)
       g_where_are_NaNs_5percent = np.isnan(g_final_5percent)
       s_where_are_NaNs_5percent = np.isnan(s_final_5percent)
       g_final_nan_10percent=g_final_10percent[g_where_are_NaNs_10percent==False]
       s_final_nan_10percent=s_final_10percent[s_where_are_NaNs_10percent==False]
       g_final_nan_5percent=g_final_5percent[g_where_are_NaNs_5percent==False]
       s_final_nan_5percent=s_final_5percent[s_where_are_NaNs_5percent==False]
```

g=np.sum(A, axis=0)

[208]: fig= plt.figure(figsize=(12,8))

```
plt.scatter(g_final_nan_5percent[g_final_nan_5percent>0],__
→s_final_nan_5percent[g_final_nan_5percent>0],c='gainsboro',alpha=1,rasterized=True)
plt.hist2d(g_final_nan_10percent[g_final_nan_10percent>0],__
→s_final_nan_10percent[g_final_nan_10percent>0], bins=50, cmap =
cbar=plt.colorbar()
cbar.ax.set_ylabel('Pixels')
plt.title('Harmonic = '+str(h)+'\n Phasor Plot \n ISM')
plt.xlabel('$g$')
plt.ylabel('$s$\n')
xx=np.linspace(-2,2,400)
yy=np.linspace(-2,2,400)
[X,Y]=np.meshgrid(xx,yy)
plt.xlim((-0.01,1.2)) # set the xlim to left, right
plt.ylim((0, 1)) # set the xlim to left, right
Z=X**2-X+Y**2
plt.contour(X,Y,Z,[0],colors='b')
fig=plt.gcf()
tight_layout()
# Uncomment for saving vector image
# plt.rcParams['svg.fonttype'] = 'none'
# fig.savefig("ISM Phasor Plot "+data filename+".svq", transparent=True,
\rightarrow format='svg', dpi=1200)
#fig.savefig("ISM_Phasor_Plot"+data_filename+".eps", transparent=True,
\rightarrow format='eps', dpi=1200)
```



4 Data export

```
#Save ISM data in a 3D file with txy
import h5py
h5f = h5py.File('ISM'+data_filename,'w')
h5f.create_dataset('3dISM', data=shifted_sum_3d)
h5f.close()

#Save Confocal_Open_ISM images in h5 with 3 datasets
import h5py
h5f = h5py.File('Confocal_Open_ISM'+data_filename,'w')
h5f.create_dataset('Confocal', data=central_element_raw_image, dtype='f8')
h5f.create_dataset('Open', data=all_elements_raw_image, dtype='f8')
h5f.create_dataset('ISM', data=shifted_sum_3d_dataset_image, dtype='f8')
h5f.close()
```