

Picasso IMS Integration

Last change: 01-July-2022 MTS - Picasso Version 0.4.2

[Installation](#)

[Localizing Data](#)

[Using z-stacks for z-calibration](#)

[Fitting data](#)

[Processing multiple files](#)

[Exporting Data for Imaris \(*.ims\)](#)

[Image Dimensions](#)


Requirements: Windows

Installation

⚠ The picasso release is not compatible with previously created hdf files, as additional metadata is exported. Please re-reconstruct the data.

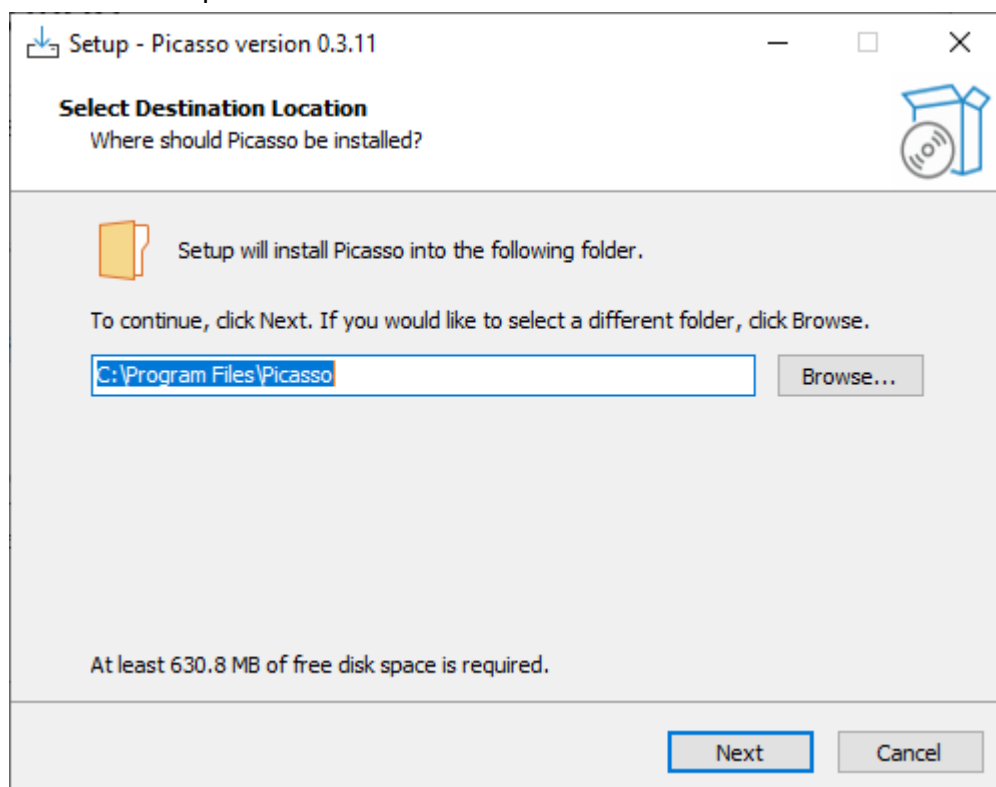
⚠ Please uninstall any previous Picasso version and delete the Picasso program folder.

1. Install Picasso via the one-click installer.
 - a. Double click on the Picasso-Windows-64bit-0.3.11_ims.exe

 Picasso-Windows-64bit-0.3.11_ims.exe	3/14/2022 9:46 PM	Application	193,985 KB
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- c. (If a warning is displayed about Picasso being from an unknown Publisher, this can be ignored)

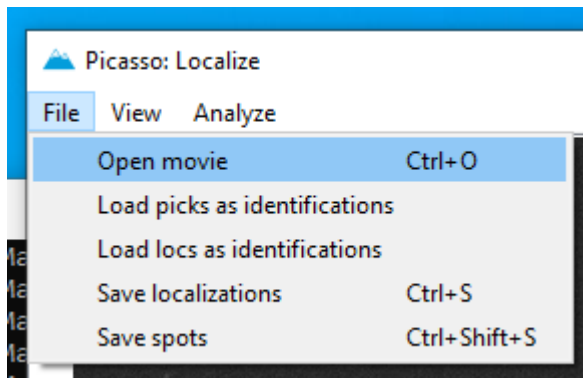
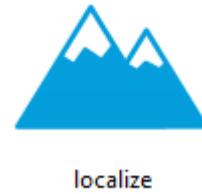
2. Follow the setup instructions.



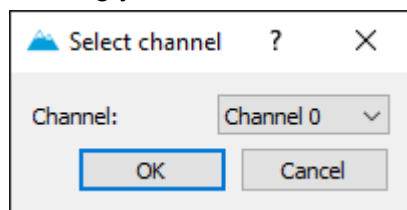
3. After successful installation, you will find several shortcuts on the Desktop to start the different Picasso modules.

Localizing Data

4. Open the Localize Application:
 - a. Via the Windows Start Menu: **Picasso / Localize**
 - b. Via the Windows Desktop Shortcut: **Picasso**
 - c. In the Windows Search bar type **Localize**
5. Drag and Drop ims file in main Window or open via **File / Open movie**



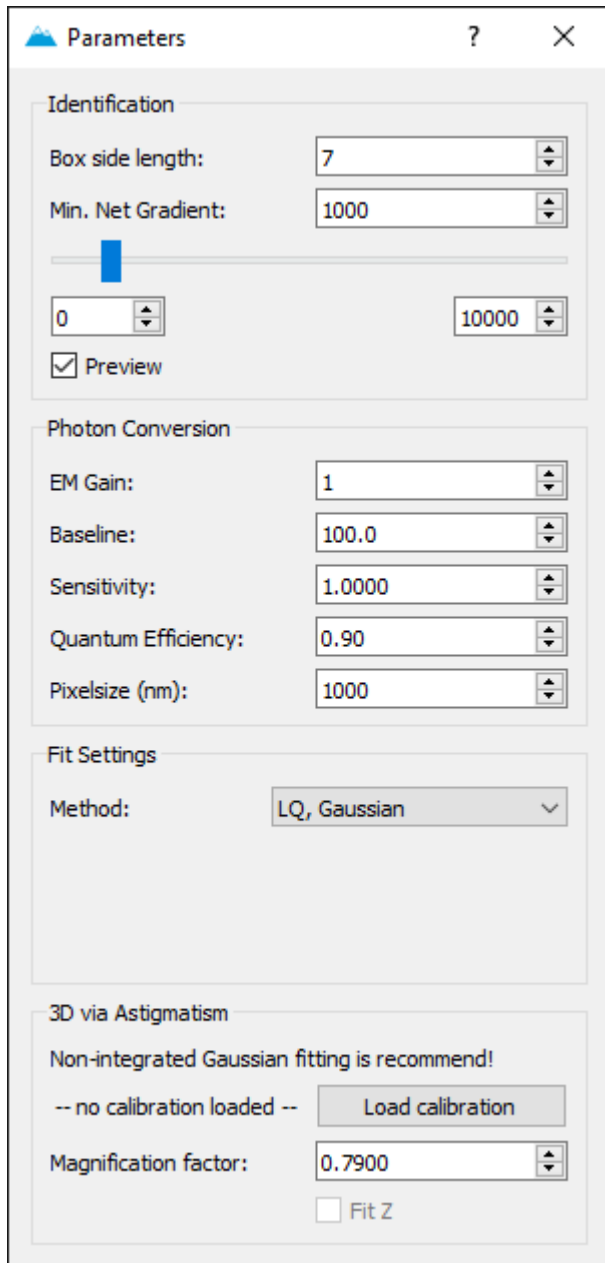
6. When opening an IMS file it should be displayed immediately in the localize window. You can navigate through the file using the arrow keys on your keyboard. The current frame is displayed in the lower right corner.
 - a. When opening an IMS file with multiple channels, a dialog window will appear allowing you to select the channel that should be loaded.



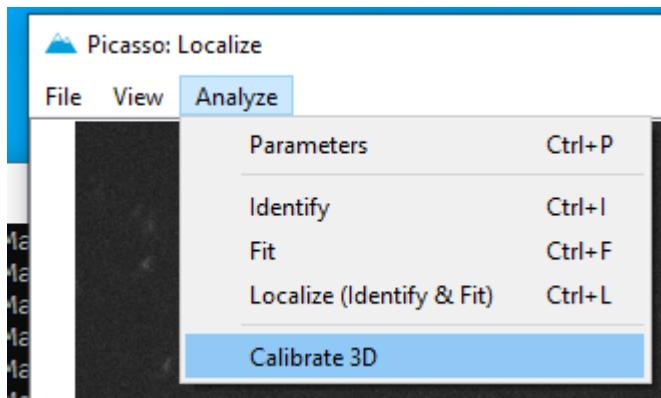


Using z-stacks for z-calibration

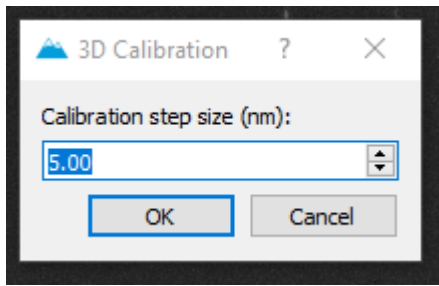
7. After loading a file, adjust the gradient so that you select all spots. Check Preview to see the identifications for the current gradient.



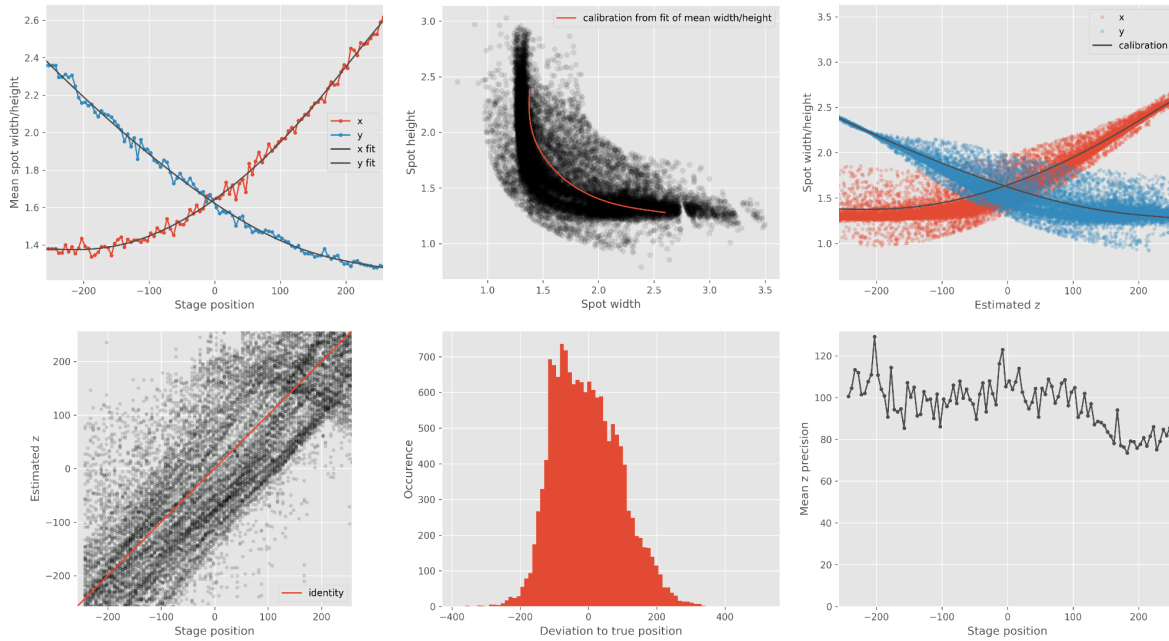
- To generate a 3D calibration, select **Analyze / Calibrate 3D**



- After spot finding (identifying) and localization (fit) the calibration step size needs to be entered:



10. A summary figure will pop up and show the z-calibration.



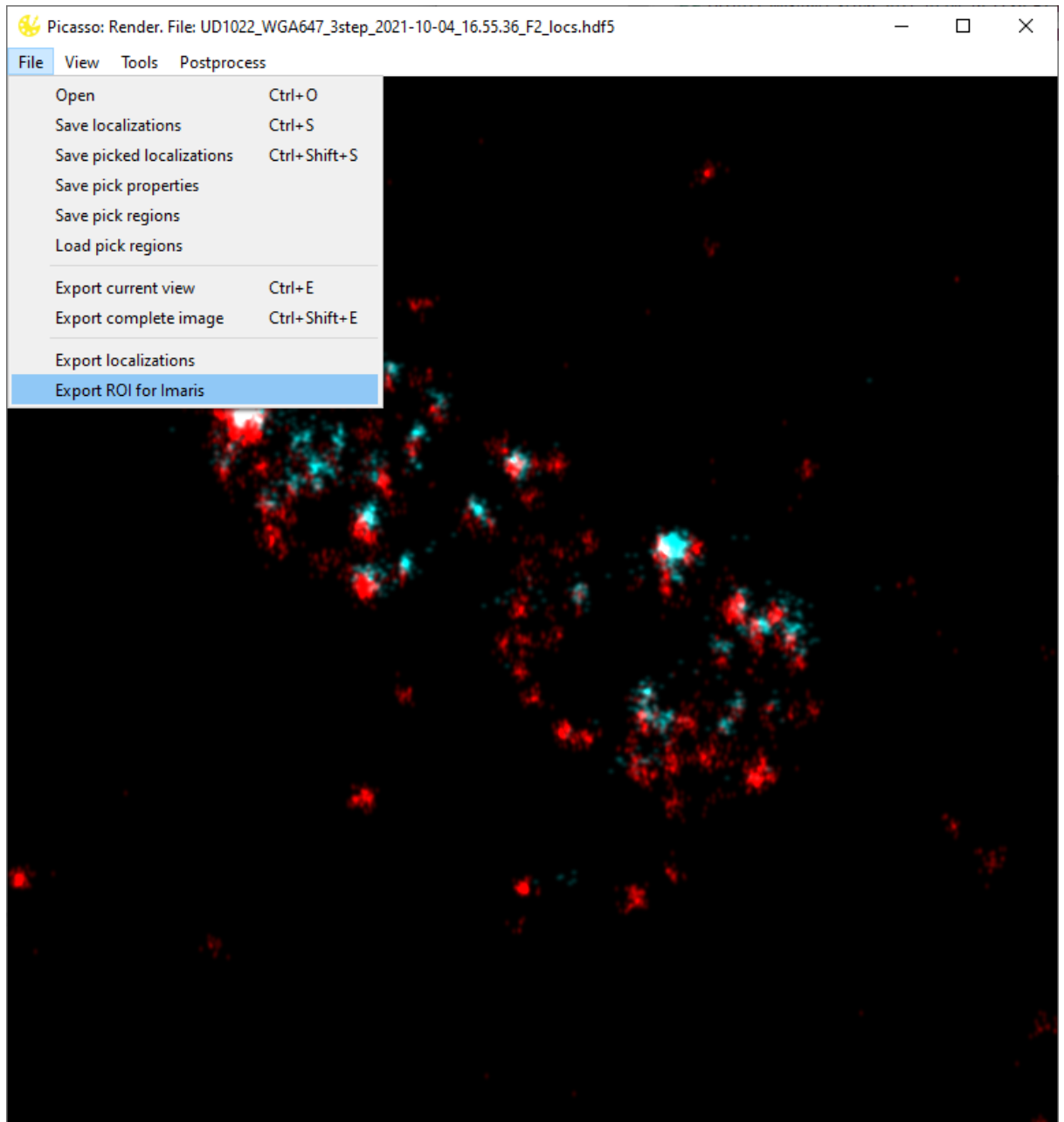
Fitting data

11. Follow the description in the Picasso documentation: [localize — Picasso 0.2.7 documentation \(picassosr.readthedocs.io\)](https://picassosr.readthedocs.io)
12. Be sure to load the calibration file when reconstructing 3D datasets.

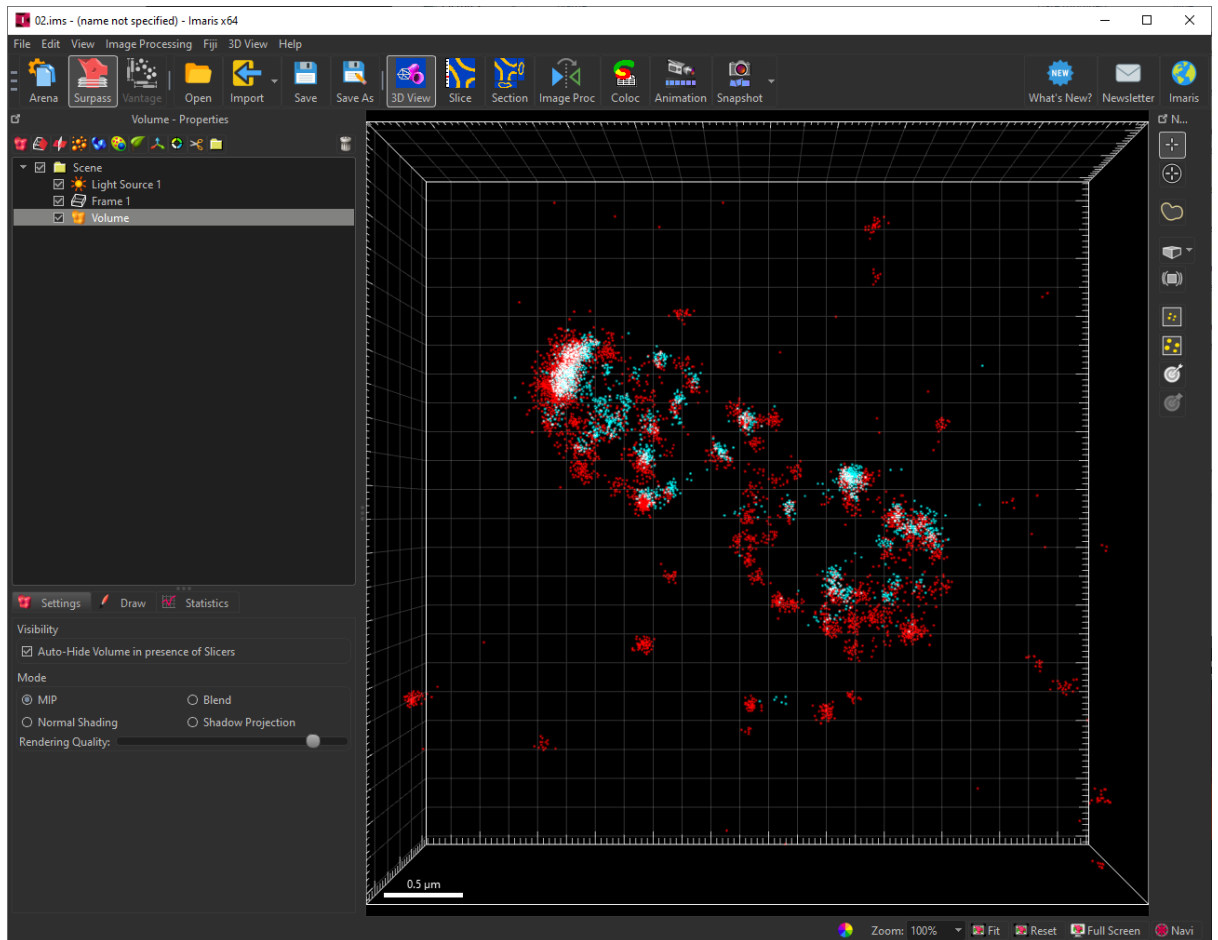
Exporting Data for Imaris (*.ims)

1. Load datasets by opening via **File/Open** (Shortcut **CTRL+O**) or drag-and-drop in the Render window.
2. Use the navigation area to find a region of interest (ROI).

3. Click on **File / Export ROI for imaris** and enter a filename for export.



4. Picasso will export the current region of interest with the current oversampling settings. If multiple channels are loaded it will export the channels with the same colors as set in Picasso (Shortcut **CTRL+F** or **View / Files** to change.) Depending on the size of the ROI, the export will take a couple of seconds. Once exporting is finished, the file will be saved at the set location.
5. The resulting file can be opened e.g. with ImarisViewer or Imaris.



Note that the orientation should be the same as in Picasso.

Technical Notes

Image Dimensions

When loading an IMS file, Picasso will attempt to extract the pixel size from the IMS metadata. It will do so by accessing the **ExtMin0 / ExtMin1** and **ExtMax0 / ExtMax1** attribute from **DataSetInfo/Image**. By subtracting the respective minima and maxima, the image extend is calculated. By further reading the number of pixels **X** and **Y** from **DataSetInfo/Image**, the pixel size is calculated. Here, Picasso assumes squared pixels and takes the arithmetic mean of the pixel size in x and y.

The values are saved as GlobalExt in the yaml file:

```
UD1022_WGA647_3step_2021-10-04_16.55.36_F1_locs.yaml - Notepad
File Edit Format View Help
Channel: Channel 0
Frames: 7000
Generated by: IMS Metadata
GlobalExtMax0: 62783.3
GlobalExtMax1: 83784.5
GlobalExtMax2: 0.001
GlobalExtMin0: 62749.1
GlobalExtMin1: 83753.7
GlobalExtMin2: 0.0
Height: 358
Pixelsize: 95.59145008502398
Width: 322
---
Box Size: 7
Generated by: Picasso Localize
Min. Net Gradient: 2000
Pixelsize: 95
ROI: null
Z Calibration:
  X Coefficients:
  - 3.2124851372832934e-17
  - -9.97623537022415e-15
  - -2.9000994984279997e-11
  - -2.176456365402788e-09
  - 8.120790296875056e-06
  - 0.0025892945163337633
  - 1.4099536446774683
  Y Coefficients:
  - -1.8443348052000283e-17
  - -8.498138285746225e-15
  - 4.73171079369886e-12
  - 6.304198517558227e-09
  - 1.328287521223895e-06
  - -0.0025578416873631923
  - 1.7987348652580155
Z Calibration Path: C:/Users/Maximilian/Desktop/ALL IMS/docs/568nm beads 20nm_3d_calib.yaml
Ln 1, Col 1 100% Windows (CRLF) UTF-8
```

When exporting a voxelized image as ims, all pixels will have the same pixel size.
When displaying 2D-data, the number of z-pixels is set to one. Within Imaris Viewer, the Voxel Size can be displayed in **Edit/Image Properties (CTRL + I)**.
When exporting, the global offset of an image will be set to 0 (ExtMin = 0).

Export from multiple Regions from one image is consistent:

