I-SHG treat

2012 : Originally written by M. Rivard "I_SHG.m", CPU-intense code; Matlab R2012a

2013 : CA Couture "fit_I_SHG.m", improvements, fast code

2014 : S. Bancelin : other improvements of I-SHG.m; Matlab R2012a

2015, 2016, 2017 :GUI + every scripts in M-files (inspired from old, modified) ; M. Pinsard

including, but not restricted to : "I_SHG_GUI.m", 3-phases algos

see each M-file to see the authors

tested in Matlab R2016a then R2017b

"I_SHG_2.m" by M. Pinsard with new features, discontinued since 2016, minor changes required to make it work again

I_SHG_GUI.m that uses a lot of files (see inside the files for some comments)

The GUI is ~5000 lines of code, including 2000 lines for the GUI/
I_SHG_2 was 134+40 ~ 170 code lines

Main features :

- Choice of what we want to calculate / plot
  - Colormap change, saturation level
  - Easy setting of parameters
  - Choose language figures
  - Choice of calculation options (filter, weighted filter ...)
  - Possibility to plot on separate figures
- Act on phase map : unwrap, untilt, corr by ref
- Batch processing
- Miji export
- Simulation of perturbation

I-SHG treat (2)

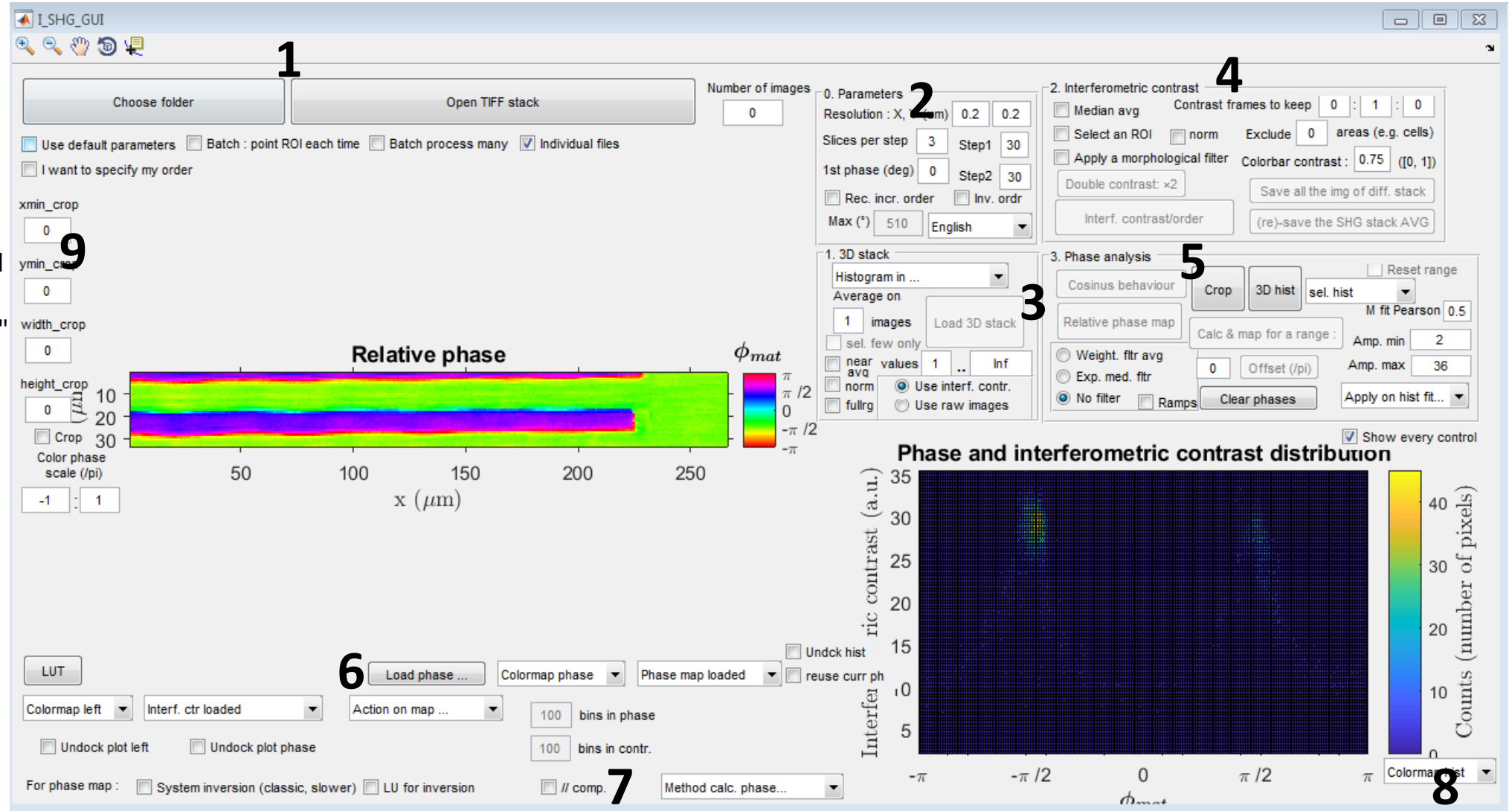
Load the file by browsing:

1

(you can select the folder, first if wou want)
Choose "individual files" if the files each contain one slice, or uncheck if you load a stack.
"I want to specify my order" is for special order of p-s only, usually not used.

Input parameters: **2**

XY resolution is mostly cosmectics, and for Miji
Step per ps is the number of phase-shifts (ps) that a step contains. 3 means 0, 180, 360° e.g.
There are step1 and step2, to indicate if the main step is not regular (usually it is).



I-SHG treat (3)

Classic parameters :

- ISHG gp slow : stack (labview) or indiv. files (python), 3 slices per step, step1 = step2 = 30° (or 15°), 18 ps (or 36), no increasing order. Interf. contrast x2.
- ISHG fast EOM: indiv. files (python), 5 slices per step, step1 = step2 = 30° , 30 ps, recogn. increasing order. Interf. contrast x8.

Other parameters: **3**. Choose if you work in the raw frames, or if you want to do a 2by2 subtraction to have "interf. contrast" frames. If so, you need to have 180° spaced frames in your stack. “sel. few only” allows you to use “value” frames in the whole stack, only (instead of having to reload only the used frames). You may “avg frames”, i.e. average many images together if you acquired your stack with avg. You can also avg in each frames the nearest neighbors, by avg or median, with weight or not. Also, you can “normalize” each frames to the same values, or use the “full range” of the data type (0 2^8 or 2^16 or other).

Contrast treat: **4**. For contrast treat, choose the number of times you want to increase it (x2, 4, 8 …). You can also do a nearest neighbors averaging on the interf. contrast frames, or select a ROI. “norm” is used to test the robustness, by dividing the interf. contrast by the avg of the ctr frames. Possibility to keep certain ctr frames, crop in beginning and/or end, or 1 frame every X. If there are recurrent areas to remove (by putting it to NaN, for low signal zones). “Morphological filter” was used by Stef, for filtering the shapes inside image. Possibility to save the ctr frames, or to save a global average.

Phase map: **5**. “weight fltr” or “exp fltr” allows you to do the nearest neighbors avg on ϕ-map. Once calculated, it is possible to plot the “3D hist.”, or the 2D hist with the popup menu (also, polar hist if needed). “Crop” can be used to select an ROI in the ϕ-map. Treating of the phase: you can select a certain range of contrast (if selected in 2, otherwise of SHG counts) to plot the phase. The rest will be put to white or black. It is useful for removing the baseline on 2D plots, and being able to perform a good fit (choices: Gaussian, Lorentzian, product of both, Pearson …). You can add an offset (in fraction of pi) to the phase, to be able to fit correctly (e.g. if the peak is a the edges). It is ok because the calculated phase is relative. You may also clear all the phases, when too much are in memory.

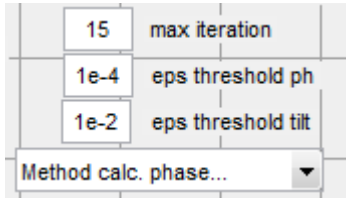
I-SHG treat (4)

The phase and constrast will be plotted on the left axes, the hist 3D on the right.

Plots: **6**. “Action on phase” : allows you to save the phase (or any plot on the left) with Miji, that keeps the LUT in tiff. Also possible to remove the image 2D tilt by various methods, to unwrap the phase 2D or 1D, to do a neighbor average on the map, to plot the profile along a line that you draw. “undock” is when you want to plot outside the GUI. The phase and others that you plot are stored in the popup menus, you can choose to display them later on if they are still in memory. You can change the colormap of display, if you don’t like the default ones. Also, possibility to change the number of bins of the histograms.

Method of calculation for the phase: **7**. You can speedup the calc. by using parallel computing (//), and change the method of fit, matrix division (they are inhenrently similar though). Also, you can use the three-steps algorithm developed Liu&Wang (most recent “Modified three-step iterative algorithm for phase-shifting interferometry in the presence of vibration”): it will normally converge to a more precise solution after few iterations. You can choose to correct for tilt, or vibrations in the frames, or not.

If also 3steps is chosen, you can change the number of maximum iteration, to limit the algorithm to a reasonable calculation time, even if the required precision is not reached.
You can also change the threshold of the difference ε on the obtained phases at Nth and (N-1) steps. Usually 1e-4. The smaller, the longer the algo will try to converge. Also, if you correct the tilt (advanced algorithm), you can set the ε of the error on the tilt.



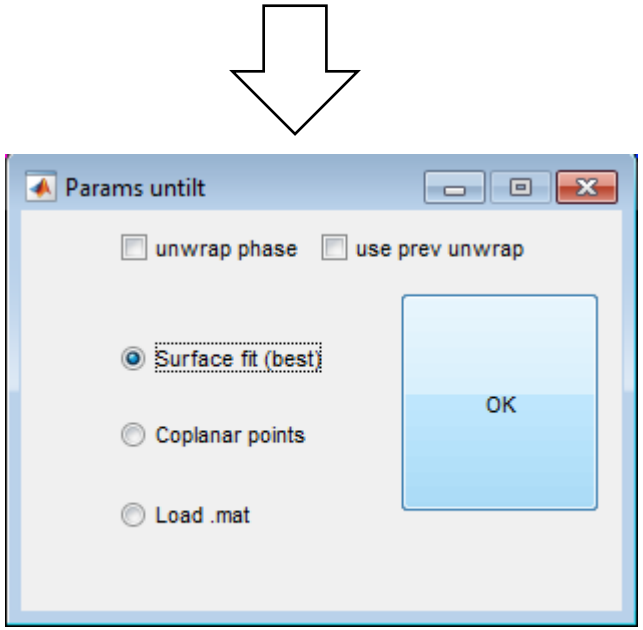
Hist: **8**. the 3D hist, colormap

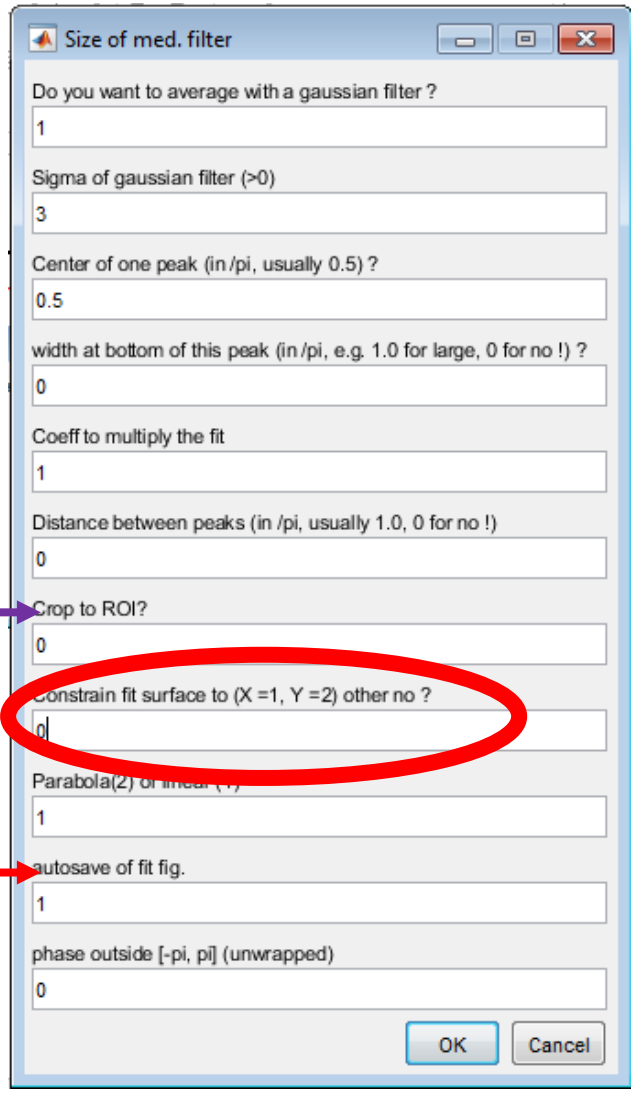
Batch: **9**. You can treat many frames automatically, with same parameters (batch). Just open many folders (or stacks), having selecting the batch options. There is a possibility to select each time the ROI, by user’s pointing. Also, there are boxes to put a pre-defined crop that you’ve measured before.

I-SHG treat (5) – Advanced action (I): saving the matrix of tilt for correction by reference

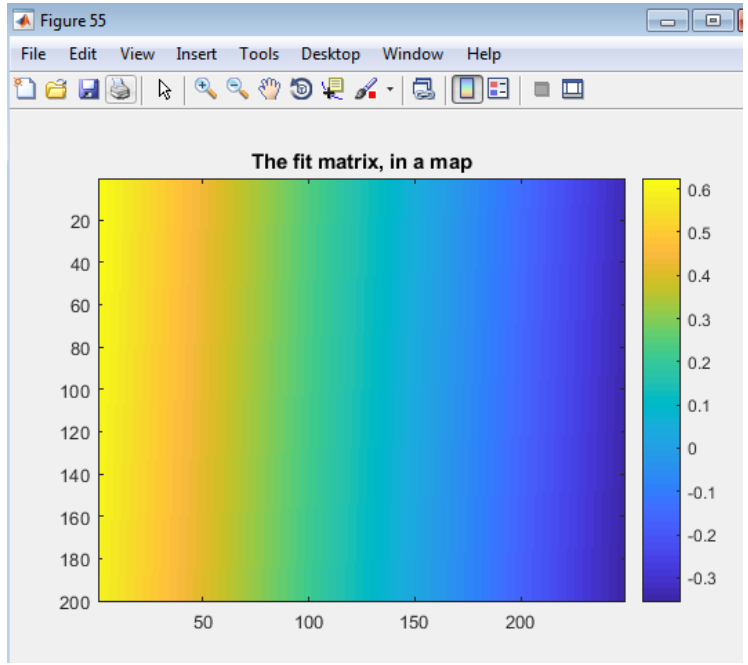
'Action on map…' menu

→ 'Correct phase tilt'





The result, in .fig



'Action on map…' menu with your sample image

→ 'Correct phase by ref'

Choose the .fig to load the map

→ Choose if the tilt is calculated only on a ROI of the image

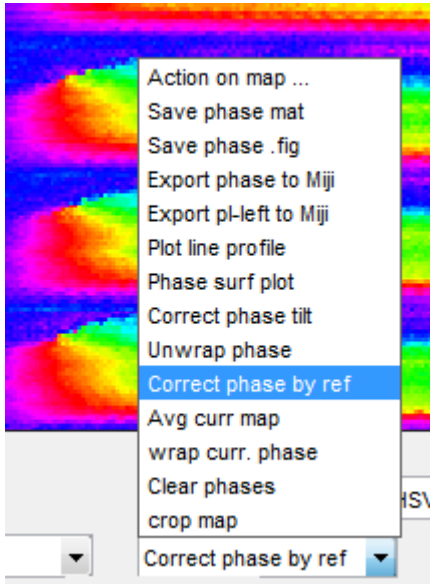
→ The fig of the fit must be saved, you can save it automatically to the folder

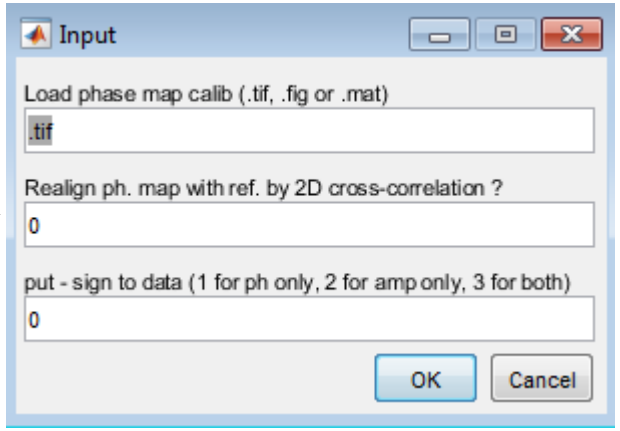
Also you choose if you want the fit to constrain to one direction (e.g. X only for corr. of stage ishgFAST)

I-SHG treat (6) – Advanced action (II): correction of phasemap by reference

→ Used to correct the map of galvos mode, also correct the scan effect if using ishg FAST

'Action on map…' menu



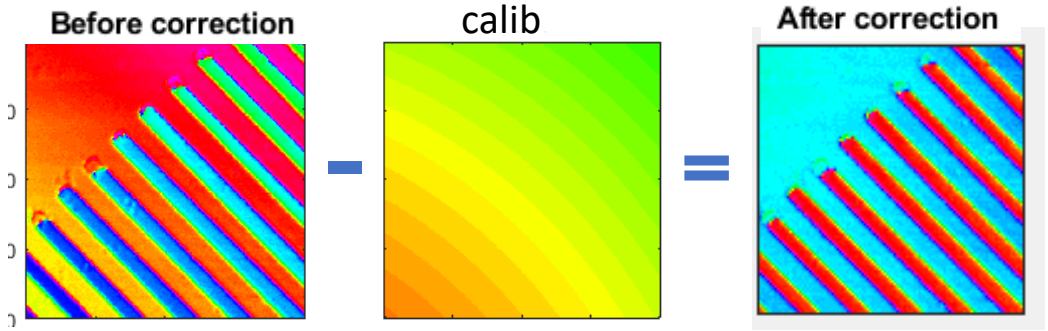


→Choose format to load calib file

You can put a – sign to the amp map that you will load, if it is inverted (because the sign is important for amplitude).

Choose here if you are able to load a different map for amplitude (interf. Contrast), or if you use the same as previous (or with a minus sign). For no corr. of amp, just exit this window.

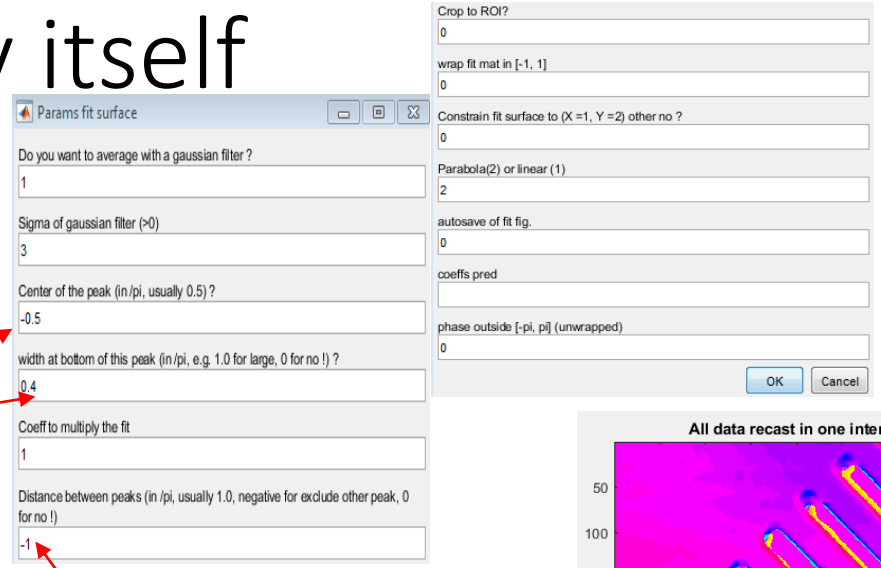
result



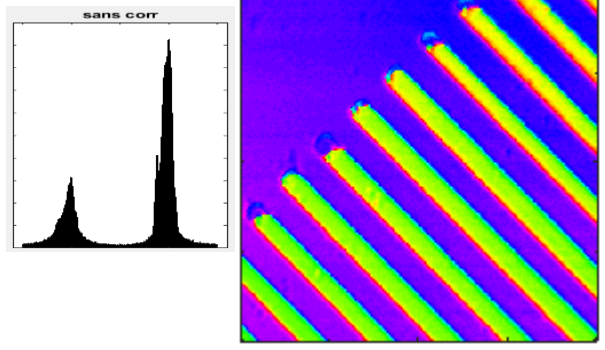
The corrected phasemap will be docked on the GUI as a new map, which you can save after

I-SHG treat (7) – Advanced action (III): correction of phasemap by itself

'Action on map…' menu
→ 'Correct phase tilt'

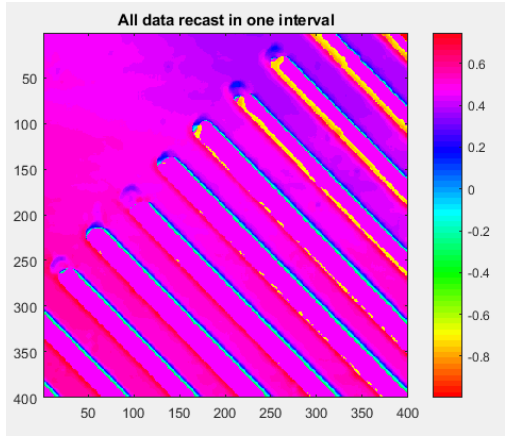


Raw with a background at +0.5 and areas at -0.5+-0.2

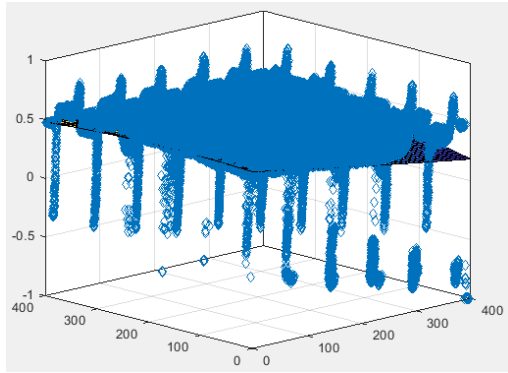


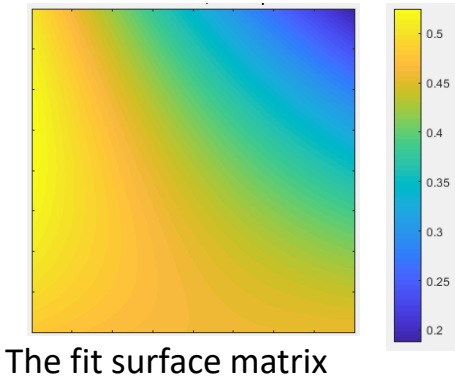
Negative value to exclude peak selected (otherwise it recast values of other peak in this one)

We can take the values in -0.5+-0.2 and put them to the avg of background



It allows to do a surface fit that is approximately good

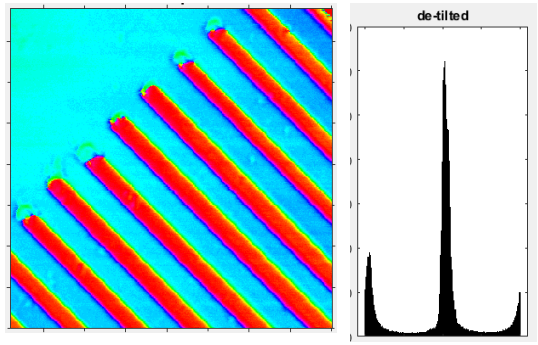




The fit surface matrix

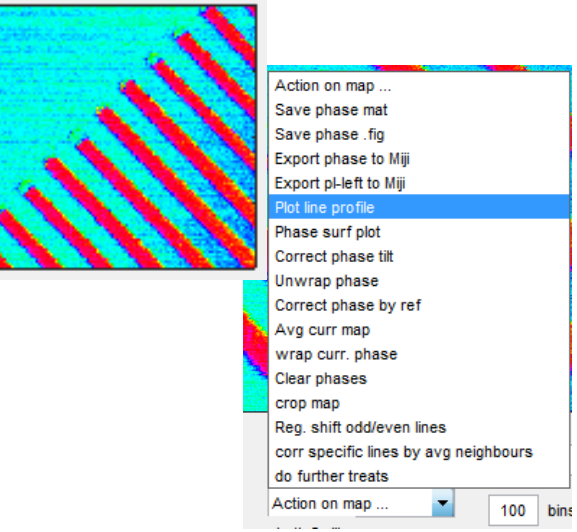
subtraction

Result, de-tilted

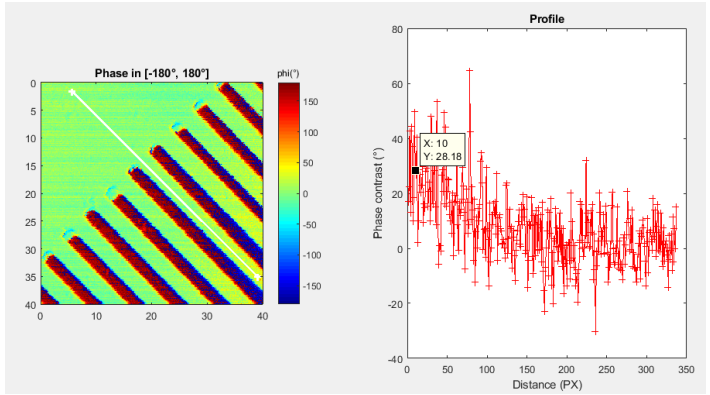


I-SHG treat (8) – Advanced action (IV): correction of tilt manually

→ You see that there is a tilt in one direction, but the soft cannot correct it itself: trace a line profile to verify



Point the two positions

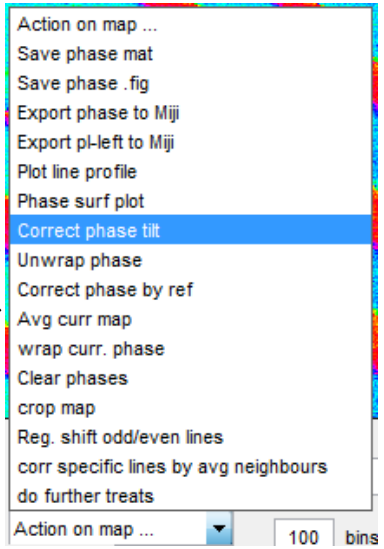


There is indeed 30° variation over 350 pixels

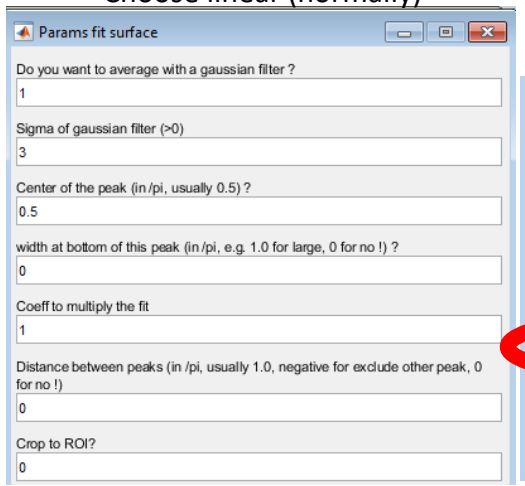
coeffX = coeffY = 30/180/350/2
=2.3810e-04 in fraction of pi
Because here the angle is 45°

Otherwise multiply by cos^2(a) X and sin(a) ^2 Y

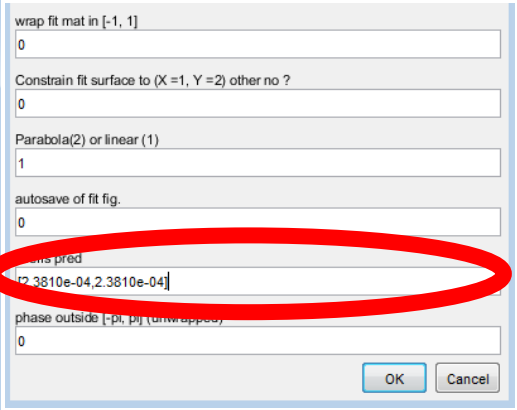
Choose surf fit



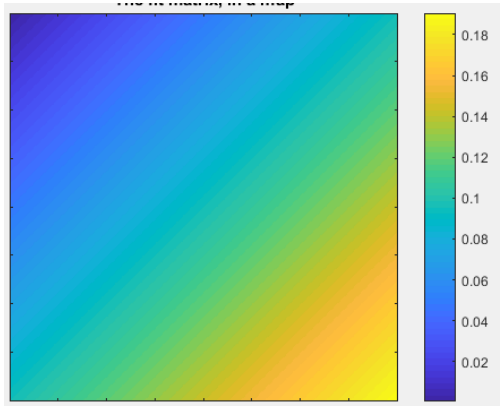
Choose linear (normally)



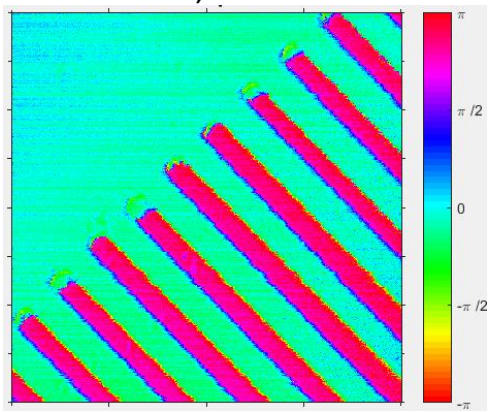
Put the coeff you found



Verify the fit surface matrix

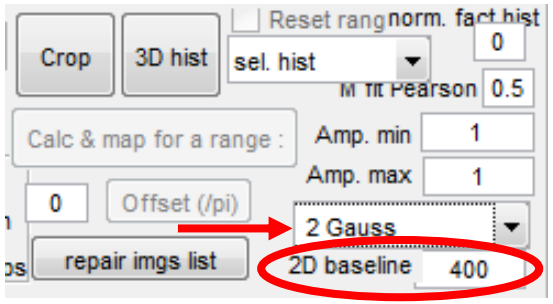


Result, de-tilted



I-SHG treat (9) – Advanced action (V): fit of hist2D

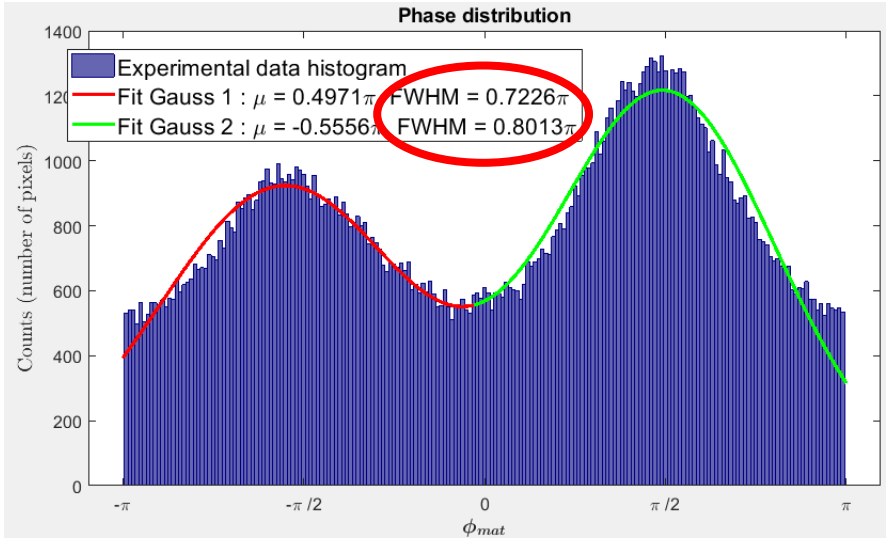
Fit : you can choose which one (manual, 2 curves) with the type of fit.
Also, put the baseline level you see on 2D hist for better fit



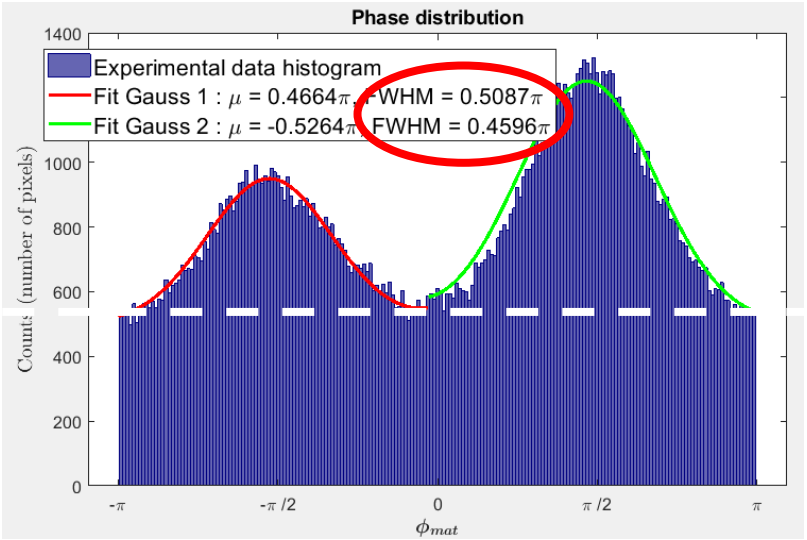
→ Without baseline, the FWHM is over-estimated and too high, because the curve goes until the bottom. It's a bit less worse with manual selection.

→ With baseline, the FWHM is correct.

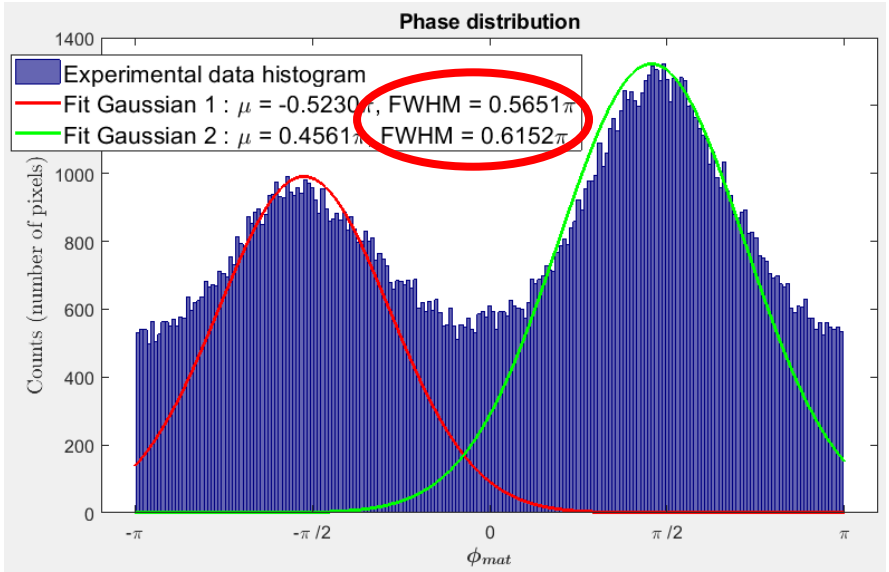
Gaussian no baseline



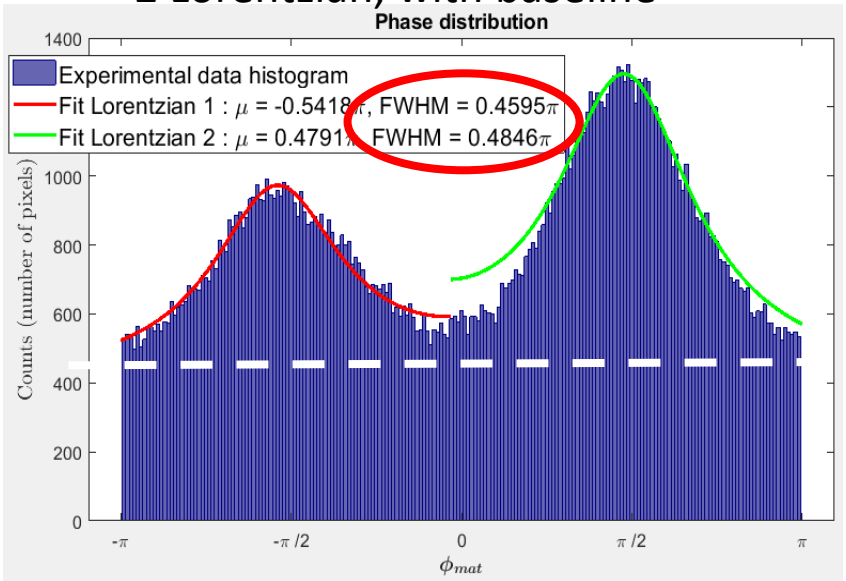
Gaussian with baseline



2 Gaussian manual, no baseline



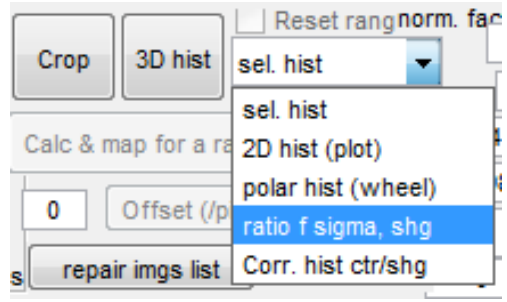
2 Lorentzian, with baseline

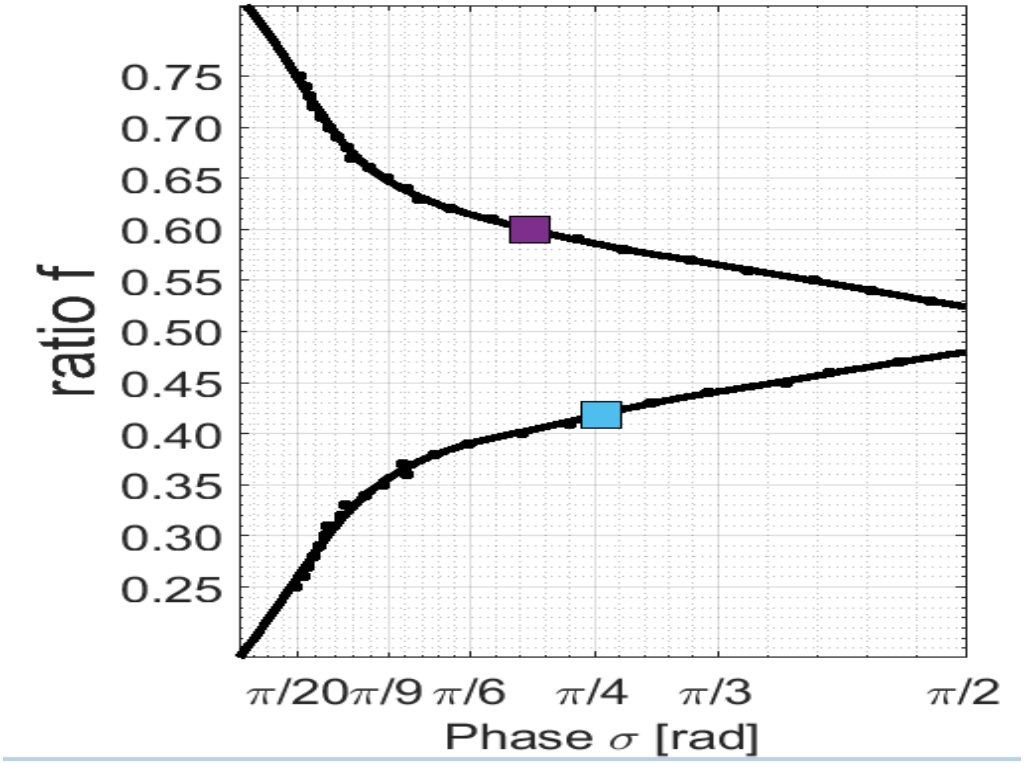


I-SHG treat (10) – Advanced action (VI): ratio f

See “The Impact of Collagen Fibril Polarity on Second Harmonic Generation Microscopy", or Rivard 2015 Biph J.

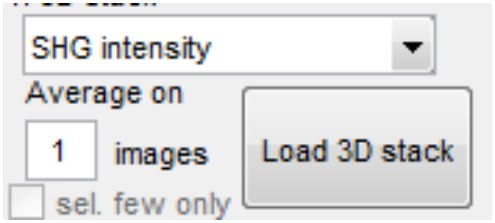
Once you have fitted the 2D histogram, the sigma widths of two peaks are available.
→ You can plot the ratio f curves: you’ll obtain the plot below if you chose « interf. Contrast » for 3D histograms (if shg, see next)





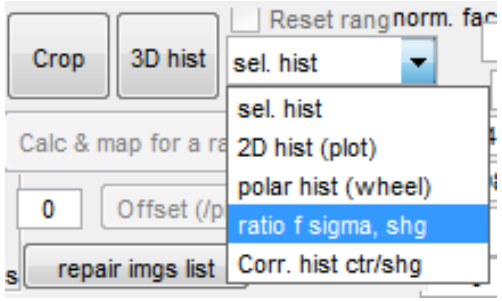
$$f = \frac{N(+\chi^{(2)})}{N(+\chi^{(2)}) + N(-\chi^{(2)})}$$

I-SHG treat (10) – Advanced action (VI): load SHG img

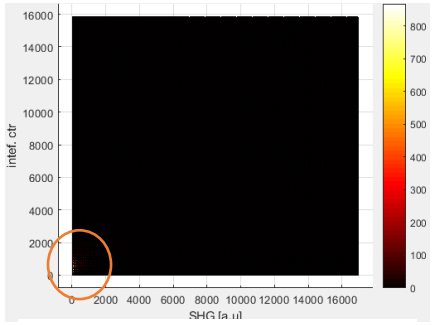


After having chosen SHG intensity for loading, it will ask you to load the SHG image (if stack, take 1st image). If already treated, right-click on the load button not to save the results.

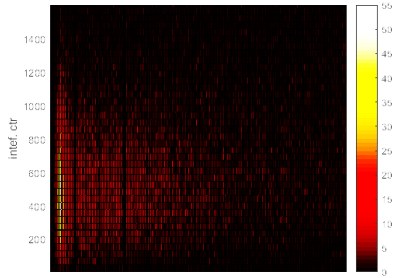
→ The 3D histogram will be plotted with the SHG in ordinates, not contr. !



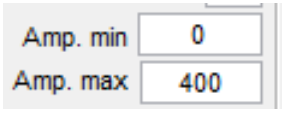
Using these actions, can plot the correlation hist ctr/shg



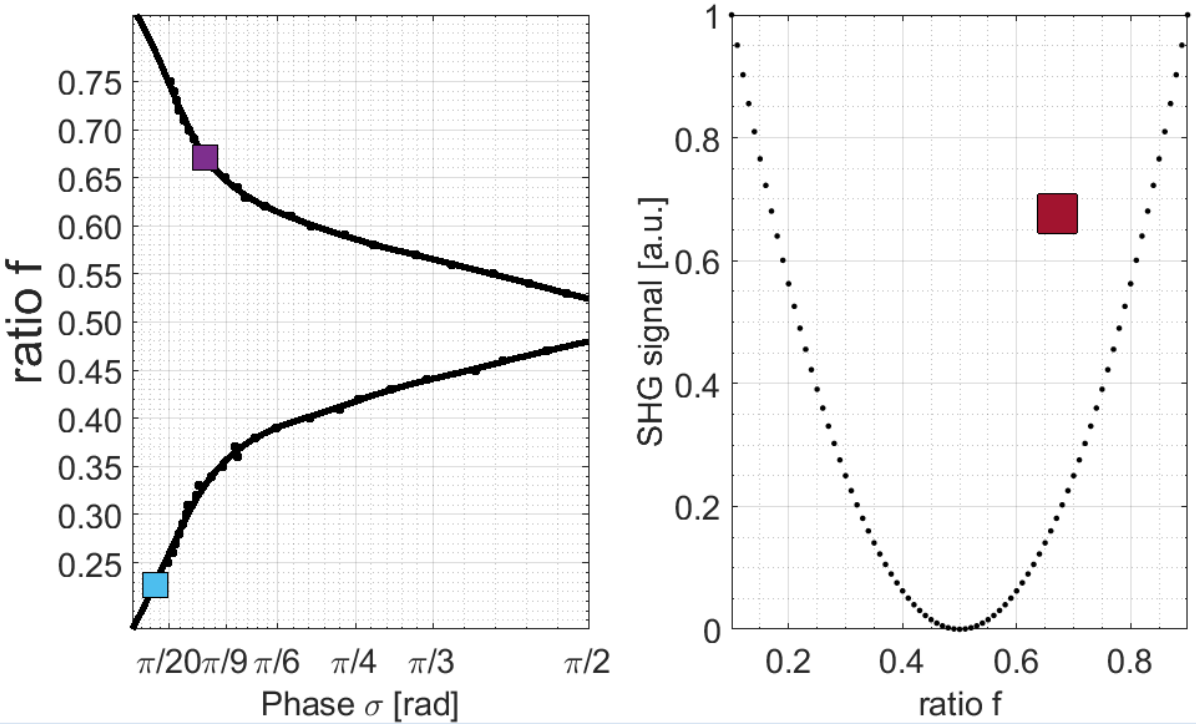
You can zoom or change bin size to have better plot



Also, you might change the range of SHG using the controls amp min&max



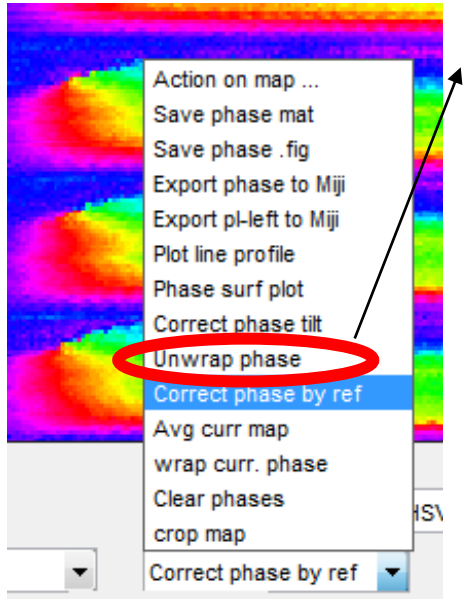
See “The Impact of Collagen Fibril Polarity on Second Harmonic Generation Microscopy", or Rivard 2015 Biph J.



→ If you plot ratio_f, it will plot also the dependence with SHG signal

→ Select consecutively different ROI to make the plot

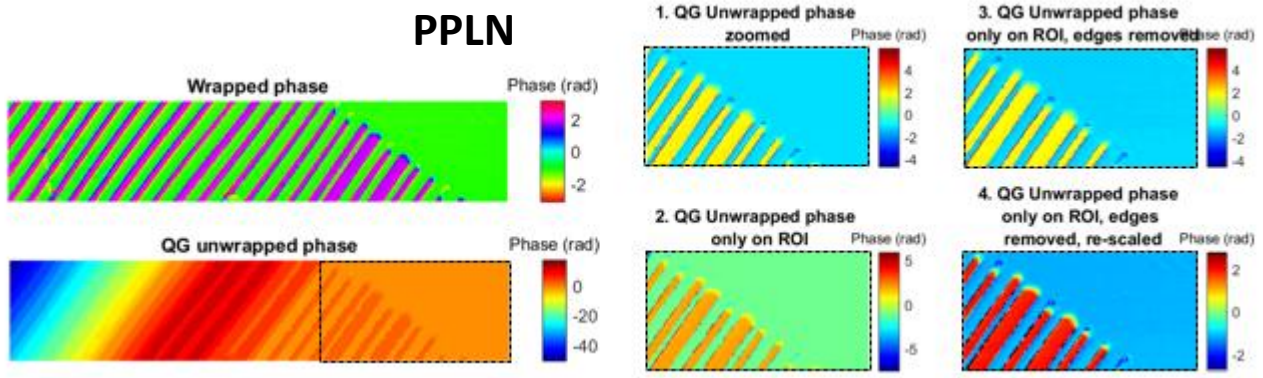
I-SHG treat (11) – 2D phase unwrapping



2D Unwrap using the advanced algorithm of quality-guided unwrapping

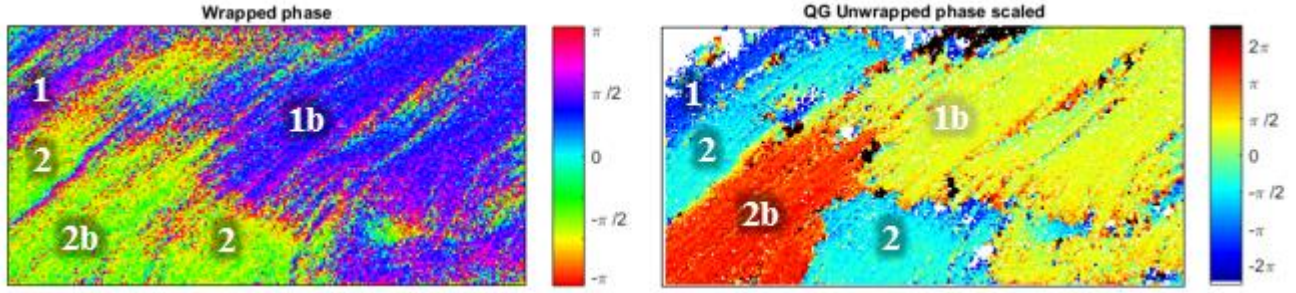
It will ask you how to seed the phase map, choose default parameters if you don't know. Other choices are mostly for the plots.

PPLN



In PPLN, the algorithm seems to fail when the image do not contain the boundaries of the domains, i.e. outisde the black box. Indeed, there is nothing to unwrap in PPLN.

Cornea (en-face)

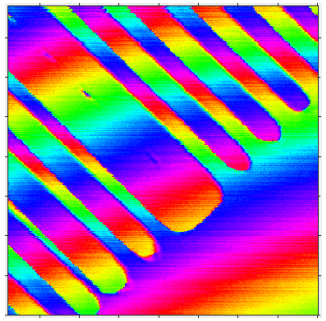


For cornea (biological tissue, with more SNR), it is more interesting as the algorithm seems to reveal two different zones in 1 (violet, left) that are π phase-shifted (1 and 1b). In 2 also : 2 and 2b.

I-SHG treat (12) – phase correction with unwrapping

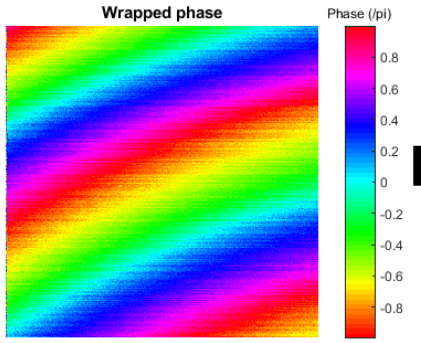
The phase calibration (quartz) goes outside of [-pi,pi]. You could correct directly the sample phase with it, but you want t ouse a surface to avoid noise.

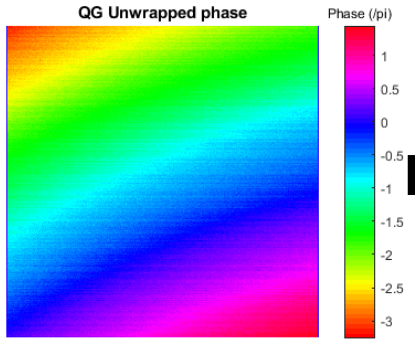
Original PPLN image



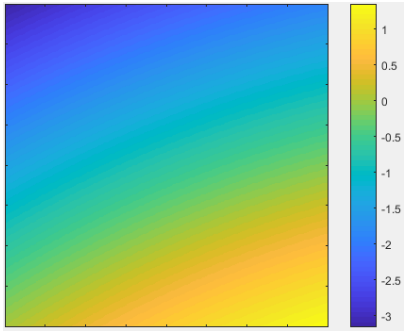
Instead, unwrap the calibration, do a surface fit.
Then you can wrap the surface fit and use it to correct the phase map

Quartz calibration

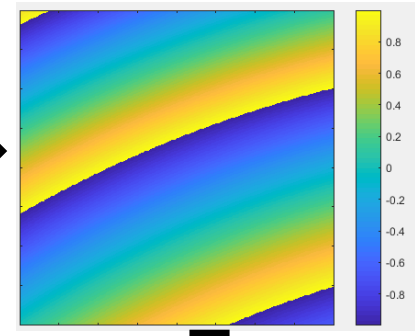




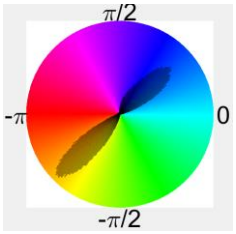
Surface fit of unwrapped

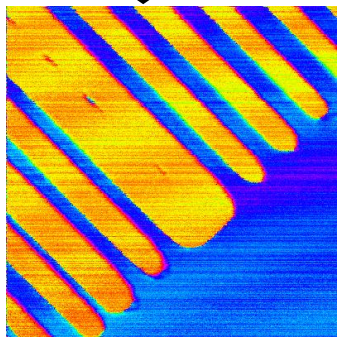


Wrapped fit

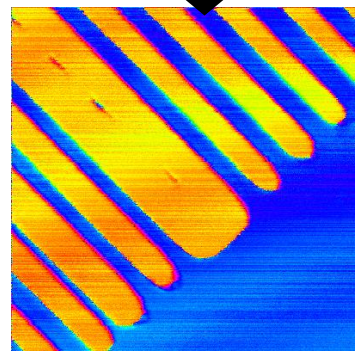


PPLN (more noisy)

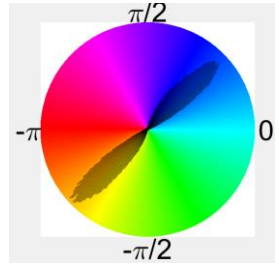




training

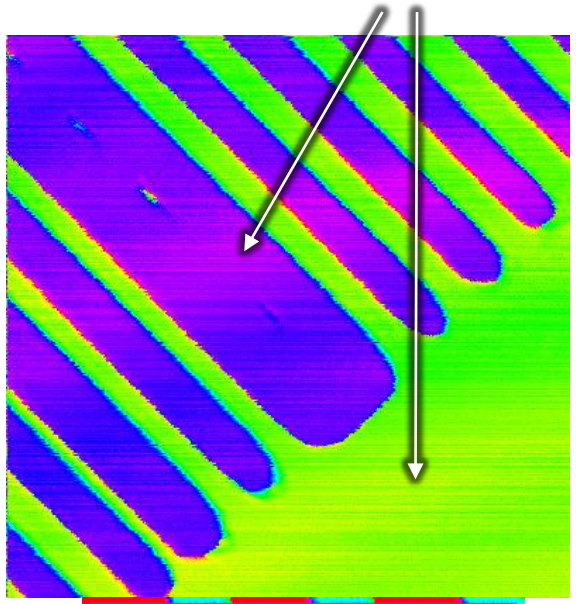


PPLN (less noisy)



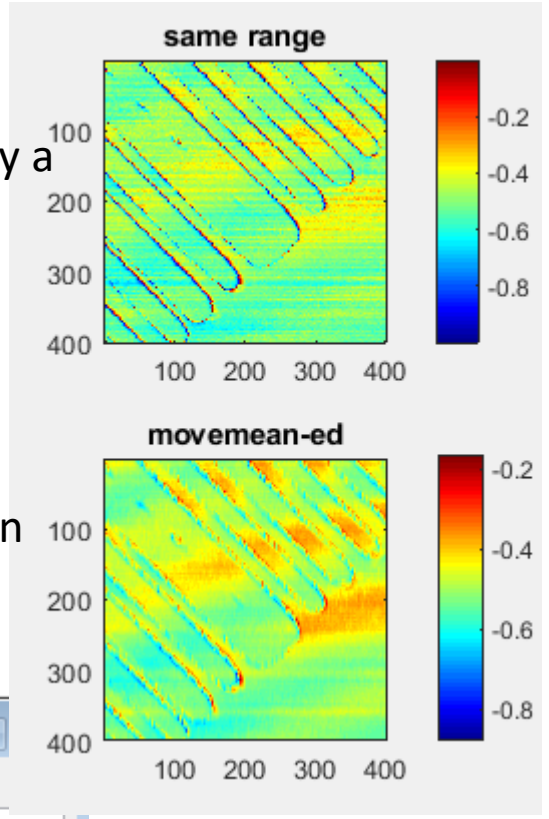
I-SHG treat (13) – correction of oscillations

Flutuations due to imperfect correction

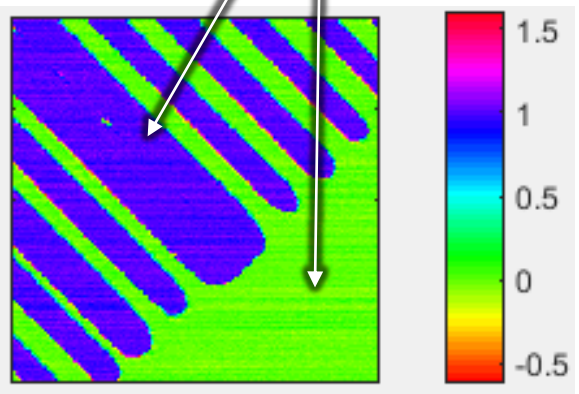


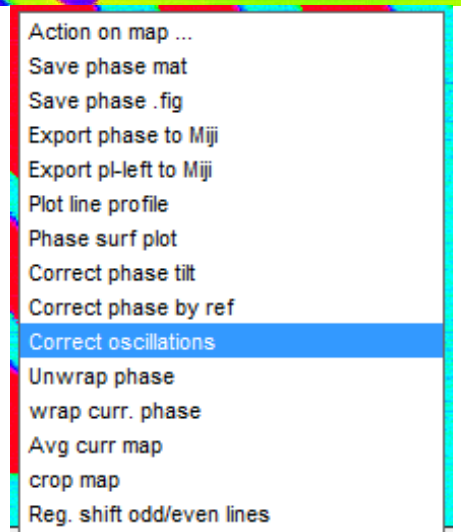
Pi phase-shift is removed. To apply a fit, or smooth, or interp…

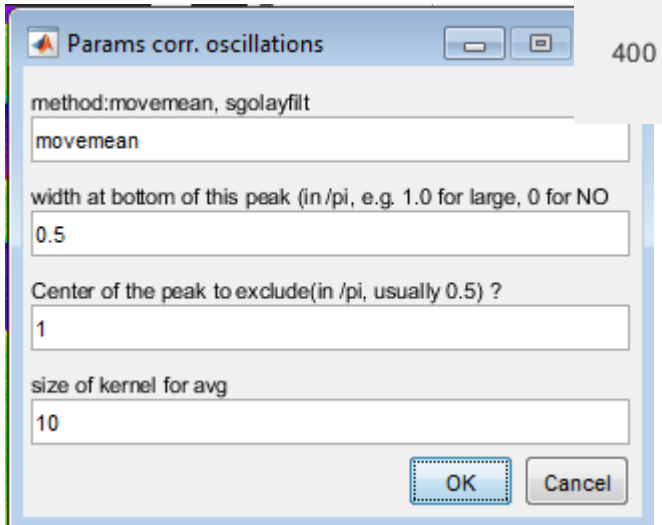
Map is smoothed in 2D



No oscillations anymore



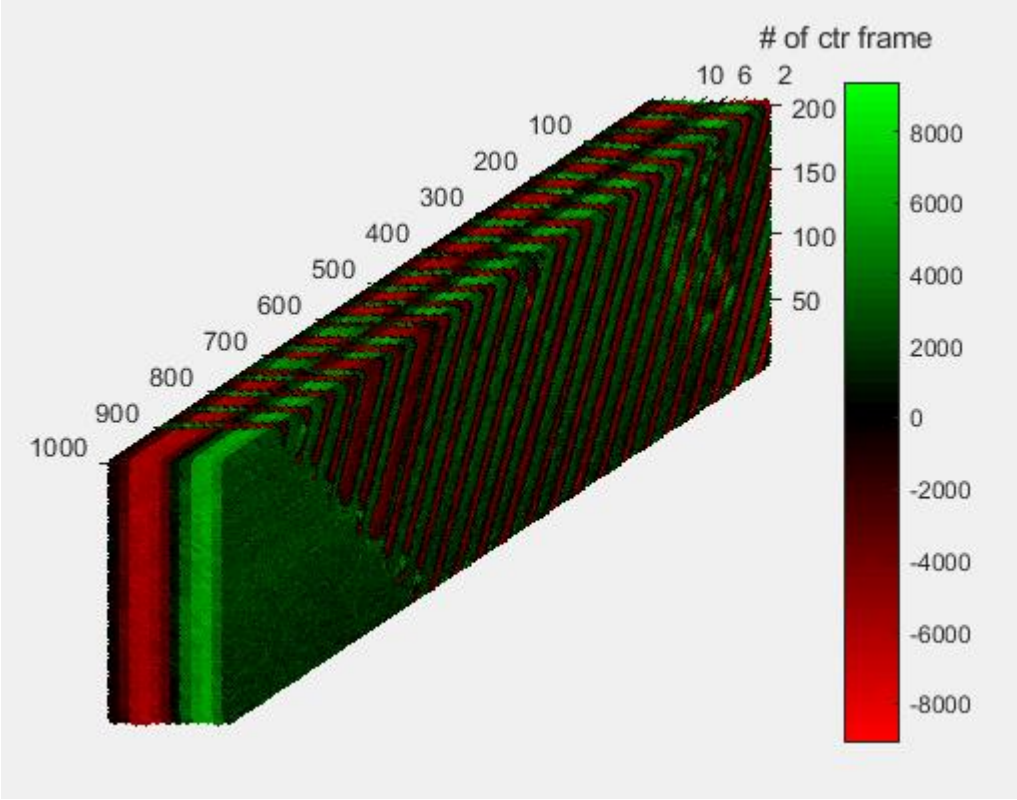


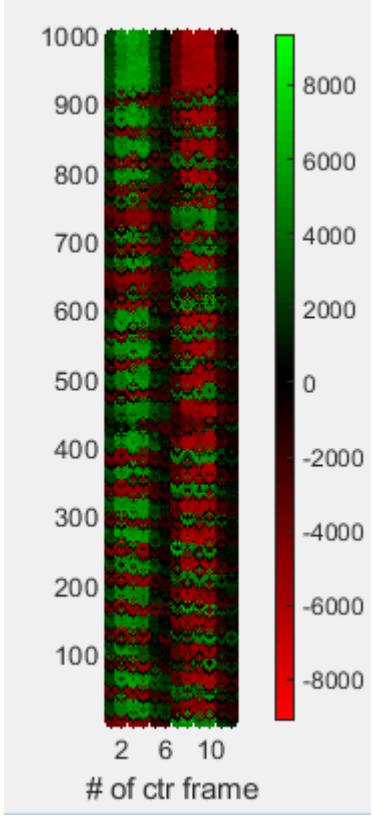


training

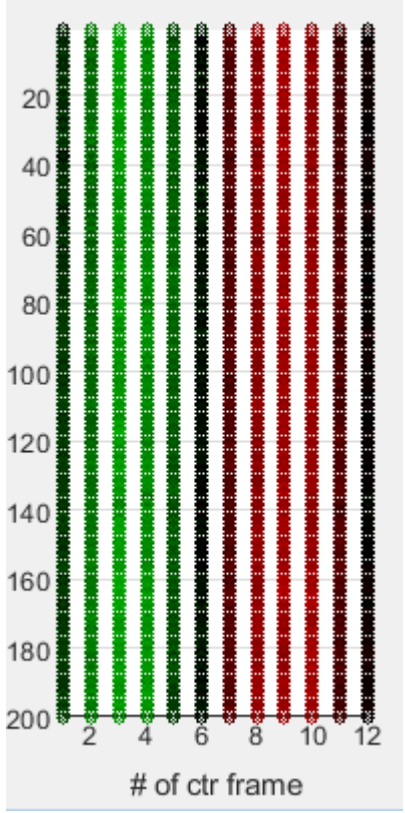
I-SHG treat (14) – 3D contrast frames

If you choose ‘analyze_cos_behavior’ and then ‘whole_3Dimg’, you can plot the 3D contrast frames





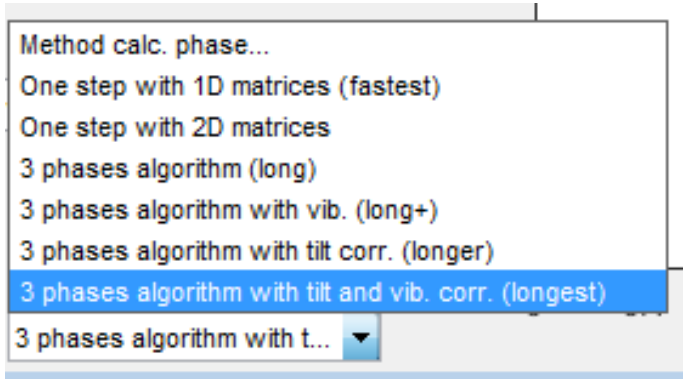
view(0,90)



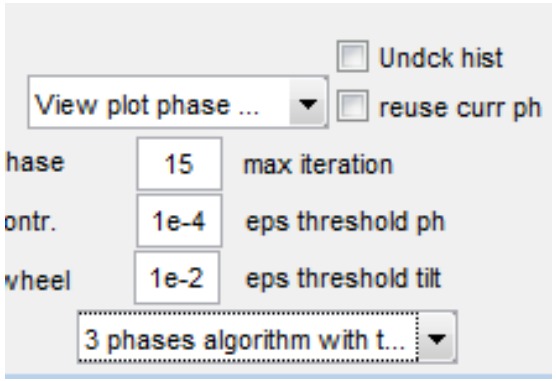
view(0,180)

```
set(gca,'DataAspectRatio', [1,
min(8,size(contr,2)/size(contr,3)),
min(8,size(contr,1)/size(contr,3))]);
set(gca,'PlotBoxAspectRatio',
```

I-SHG treat (14) – 3phases 3steps algorithm(s)



You can choose the algorithm to do



Set the parameters (max iteration, residual errors epsilon)

→ Problematic with low SNR samples

→ Converge in PPLN, but sometimes the vibration only or tilt only version have problems

Papers:

Q. Liu, Y. Wang, J. He, and F. Ji,
"Modified three-step iterative algorithm for phase-shifting interferometry in the presence of vibration,"
Appl. Opt. **54**, 5833–5841 (2015).

Q. Liu, Y. Wang, J. He, and F. Ji,
"Phase shift extraction and wavefront retrieval from interferograms with background and contrast fluctuations,“
J. Opt. **17**, 025704 (2015).

Q. Liu, Y. Wang, F. Ji, and J. He, "A three-step least-squares iterative method for tilt phase-shift interferometry,"
Opt. Express **21**, 29505 (2013).

Z. Wang and B. Han, "Advanced iterative algorithm for phase extraction of randomly phase-shifted interferograms,“
Opt. Lett. **29**, 1671 (2004).