# PyHoLo Manual (v. 3.0)

<u>Note 1</u>: Some of the screenshots shown in this manual come from the old version of program which had much less user-friendly interface. I will update those images in the next version of PyHoLo manual.

## A) Tools required for running PyHoLo software

In order to run PyHoLo you will require:

- Windows Vista, 7, 8, 10 (≥ Vista),
- Python language interpreter version 3.6 or later (e.g. Anaconda3),
- (optionally) external Python libraries, such as PyQt5.
- 1. Download the Anaconda environment from <a href="https://www.anaconda.com/distribution/">https://www.anaconda.com/distribution/</a>. Choose a version compatible with Python 3.6 or later.
- 2. Install the Anaconda environment by running the installation file and following the instructions on the screen. We recommend you to install Anaconda in the default location suggested by the installator. In Windows it will be 'C:\Users\user\_name\Anaconda3'.
- 3. After installation open Anaconda Prompt. In Windows 7 it can be found under Start menu, in All Programs → Anaconda3 → Anaconda Prompt.
- 4. New version of Anaconda (3.7) comes with a number of libraries which are required for PyHoLo to work. However, older Anaconda versions may be missing newer distributions of those libraries, e.g. PyQt5, scikit-image. To install those packages (i.e. if you stumble upon some error during running PyHoLo), type the following command in the Anaconda Prompt:

```
conda install <package_name>
(e.g. conda install pyqt)
```

After each command you will be asked for confirmation of the library name which you want to install. You can confirm it by typing 'y' into the Anaconda Prompt and pressing Enter. If the installation of all libraries is successful, you can close the Anaconda Prompt.

## B) Running and using PyHoLo software

1. Download PyHoLo software from the following site:

#### https://github.com/morawatur/PyHoLo

- 2. Extract files from zip archive to a directory on local disk (e.g. C:\python programs\PyHoLo).
- 3. Open the Anaconda Prompt. In Windows 7 it can be found under Start menu, in All Programs  $\rightarrow$  Anaconda Prompt.
- 4. From the Anaconda Prompt level go to the directory with extracted files. You can do it by entering the following command:

5. Run the PyHoLo.py script by entering the following command:

A dialog window should appear, asking you to point to a directory with series of images.

<u>Note 2</u>: Images must be in dm3 format (internal file format of DigitalMicrograph software). In order to read series of images you need to mark first image and press Open.

<u>Note 3</u>: Image names must have a common set of characters which denote series name and they must be numbered according to the following convention:

holo01.dm3, holo02.dm3, holo03.dm3 etc.

where 'holo' is a name of series (digits smaller than 10 must be preceded with '0', eg. 'holo03.dm3').

Note 4: Exemplary series of holograms is located in the folder 'input' in a directory with PyHoLo files.

6. Information about the progress of reading images will be displayed in the interpreter window. After all images are loaded the PyHoLo window should appear on the screen (see Fig. 1).

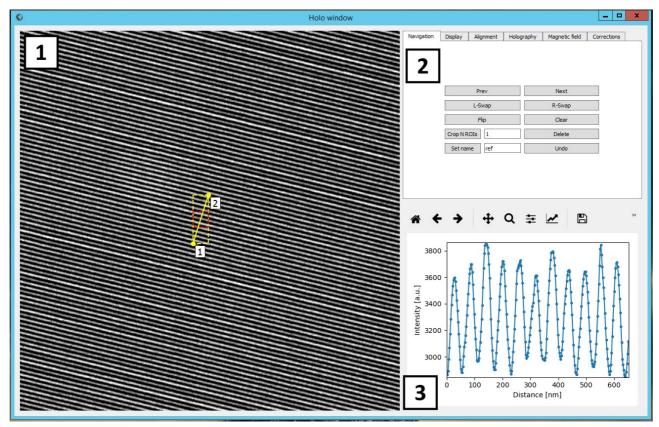


Fig. 1. Graphical user interface of PyHoLo program.

The GUI of PyHoLo software consists of a window for displaying images, control panel with 6 different tabs and a plotting area. Below you can find the description of all program functions.

## 1. Display for images

This window displays images from the current image queue, including holograms, electric and magnetic phase images and the results of other operations, such as FFT, sum, difference and gradient calculation.

## 2. Control panel

Control panel is divided between 6 tabs: Navigation, Display, Alignment, Holography, Magnetic field and Corrections.

The list of features (and their functions) included in individual tabs are listed below:

#### 2.1. Navigation tab

Prev, Next – go to the previous/next image in series (queue).

L-Swap, R-Swap – swap currently displayed image with the prevoius/the next one.

Flip – replace current image (its amplitude as well as its phase) with the horizontally reversed image (mirror image).

Clear – remove all points marked on the current image. Crop N ROIs (previously called Zoom N images) – crop square fragment defined by two points marked on the current image. First user must mark the top-left corner of an area to crop and then its bottom-right corner (see Fig. 3). Created rectangle will be automatically transformed to a square with side length equal to the shorter side of a rectangle. In the textbox on the right side of the Crop N ROIs button user can enter the number of images from which the desired area is to be

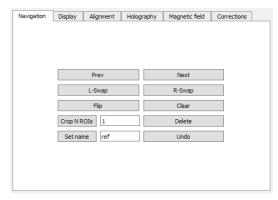


Fig. 2. Navigation tab

cropped. Magnified fragments will be placed at the end of image queue.

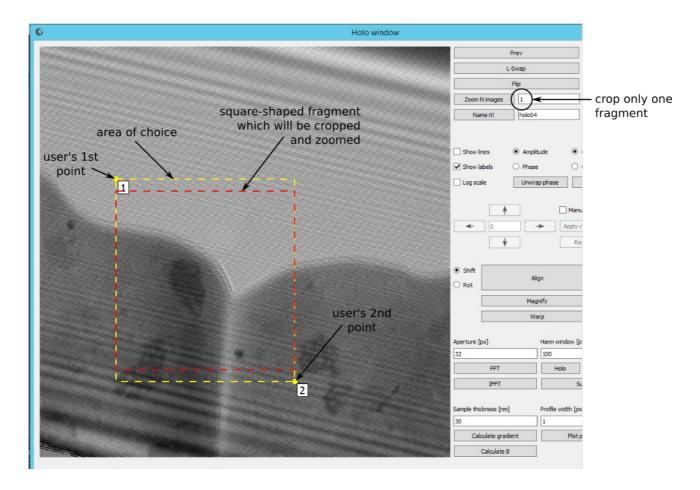


Fig. 3. The way of selecting a fragment to crop from N next images in queue (including the currently displayed image). Yellow dashed line shows the rectangle created by selection of two points (1 – top-left, 2 – bottom-right). Red dashed line on the other hand shows the actual fragment which will be cropped from images. In the above example only one fragment will be cropped, because value in the textbox marked with black circle is N=1.

Delete – remove the current image from the image queue (this operation cannot be undone). Set name (previously called Name it!) – name the current image in order to make it easier to navigate across image queue. Enter image name in the textbox on the right side of the button. The name will be saved after clicking the Set name button. If user decides to export the image to a file

then this name will also be used by default as the file name.

Undo – remove last point from the set of points marked on the current image.

## 2.2 Display tab

#### 2.2.a) Display panel

Show lines – if this box is checked then lines between selected points will be displayed on image.

Show labels – if this box is checked then numbering labels will be displayed next to selected points.

Log scale – if this box is checked then the image will be displayed in logarithmic scale. Unchecking this box will cause the image to be displayed in linear scale.

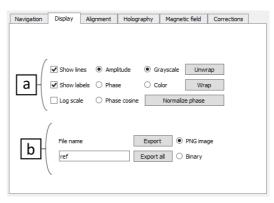


Fig. 4. Display tab

Amplitude/Phase/Phase cosine – switches between amplitude, phase and phase cosine of the current image.

Grayscale/Color – switches between grayscale and RGB color maps.

Unwrap phase – unwraps the image phase so that the phase is not limited to the  $(-\pi, +\pi)$  range, i.e. discrete changes (jumps and drops) in phase will become continuous.

Wrap phase – wraps the image phase, i.e. performs modulo operation which transforms  $+\pi+\Delta\pi$  values (where  $0 < \Delta\pi < 2\pi$ ) into  $-\pi+\Delta\pi$  values.

Normalize phase – normalizes phases of all images in a way that the selected pixel has the same phase value (0) on every image. Before using this function user must mark one point on the current image so that all other phase values will be shifted in relation to this point's phase value.

## 2.2.b) Export panel

File name – in this textbox you can enter path and name of the exported file. You don't have to enter file extension. In the case of images saved to PNG format the extension .png will be added automatically. Binary files don't need an extension. The default path for exporting data is the directory which contains source files of PyHoLo. For example, typing 'results/img' in File name textbox will cause the image to be saved in the 'results' catalogue which should be located in the PyHoLo directory, under the name 'img.png' (or just 'img' if it is written to binary file).

Export – save current image to the given file.

Export all – save all images to the default directory with the use of names assigned to each image. All images will be saved as amplitudes or phases depending on the currently selected Amplitude/Phase option. The same applies to the selection of Grayscale/Color and PNG/Binary options.

### 2.3. Alignment tab

### 2.3.a) Manual alignment panel

After checking the Manual mode checkbox the buttons for manual alignment of images (both amplitude and phase) become active, including buttons for shifting image horizontally ( $\leftarrow \rightarrow$ ), vertically ( $\uparrow \downarrow$ ) and rotating image clockwise and counter-clockwise ( $\uparrow \sim$ ). In the textbox between shifting buttons you can enter the number of pixels

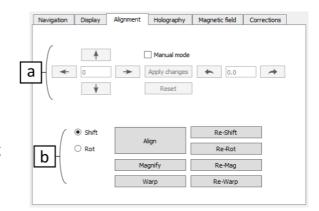


Fig. 5. Alignment tab

by which the image is to be shifted in the chosen direction. Similarly you can define the angle of rotation (in degrees) in the textbox between rotation buttons.

Apply changes – save changes introduced to the image by manual alignment. Reset – reset introduced changes.

#### 2.3.b) Automatic alignment panel

This panel allows for alignment of two images, current one and the one after it, semiautomatically.

Shift/Rot – alignment mode. If it is enough to shift one image towards the other without the need to rotate one of them, then you should select Shift option. Otherwise, if both shift and rotation are necessery, you should select Rot option. In both cases you must mark two sets of points indicating the same characteristic features on both images (the current one and the next one).

<u>Note 5</u>: Both sets of points must contain the same number of points, and the points must be marked in the same order on both images.

In the Shift mode clicking Align button will cause the average shift between images to be calculated based on the shifts between individual pairs of points. After that shifted image (second image shifted to the first-reference image will be added to the queue. Similarly, if you click Align with Rot option selected, then the average position of rotation center and the rotation angle will be calculated. Next, two images will be added to the queue: 1 - a copy of first image, shifted in such a way that the rotation center overlaps with the center of display window, and 2 - a copy of second image, shifted in the same way as the first image and then rotated by calculated angle. Shifting (as well as rotating) an image will cause a loss of data on sides of that image. At the same time, areas near opposite sides will have to be filled with zero values, i.e. shifting an image by [+10, +10] pixels will produce black stripes on upper and left sides of an image. In such case the Crop N(=2) ROIs button may become handy to cut off those black areas (see Fig. 7).

Re-shift – after each shift-operation last shift value is saved in a program's memory. When Re-shift button is clicked then the current image gets shifted in the same way as was the last shifted image. Re-rot – similarly, after each rot-operation last value of rotation angle is saved. When Re-rot button

is clicked then the current image gets rotated by the same angle as was the last rotated image.

Magnify – use sets of points marked on two images to determine difference in scale between those images. Then rescale one of them to make both images scaled equally (i.e. clicking Magnify button will add two images to the queue: copy of the first image and rescaled copy of the second image).

Re-mag – after each rescale operation the scaling factor is saved so it can be used to rescale any other image in the same way.

Warp – transform one of the images geometrically to make one set of points (on the first image) correspond exactly to the other set of points (on the second image), i.e. after warping transformation points with the same numbers on both images will overlap with each other (clicking Warp button will add a warped copy of the second image to the image queue).

Re-warp – repeat last warp transformation on the current image.

<u>Note 6:</u> In all cases which involve selecting two sets of points on two images the function button (Align, Magnify, Warp) must be clicked when the second image is selected (displayed). Then the software will know to use the previous image as the reference image and to modify (align, magnify, warp) the current image.

<u>Note 7</u>: For the warping effect to be satisfactory, you should mark a significantly large number (i.e. above 4) of points on both image.

## 2.4. Holography tab

Aperture – radius (in pixels) of numeric aperture which is used to mask one of the sidebands on the Fourier transform of the hologram (i.e. filter out everything except that sideband). Size of the aperture determines the range of spatial frequencies which will be considered in the phase reconstructed from hologram – smaller aperture means that only lower frequencies will be taken into account causing the



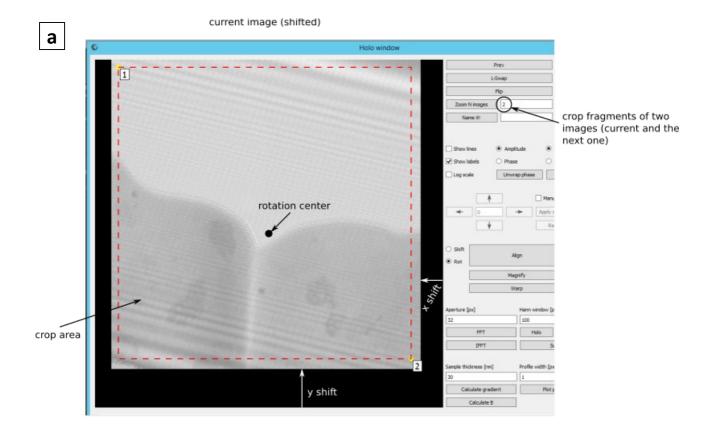
Fig. 6. Holography tab

phase image to be less noisy. On the other hand significantly large aperture is needed in order to observe faster changes of phase, i.e. better resolution of phase image. The default diameter of numeric aperture is set to 32 pixels.

Hann window – the side length (in pixels) of square Hanning window which becomes imposed on the image of the (cropped and centered) sideband in order to smooth out the sudden drops of intensity on the edges of aperture. The default width (and height) of Hanning window is set to 100 pixels.

FFT – calculate and display Fourier transform (FFT) of the current image.

<u>Note 8</u>: You will have to switch to the Log scale display mode in order to see all features on the calculated Fourier transform image.



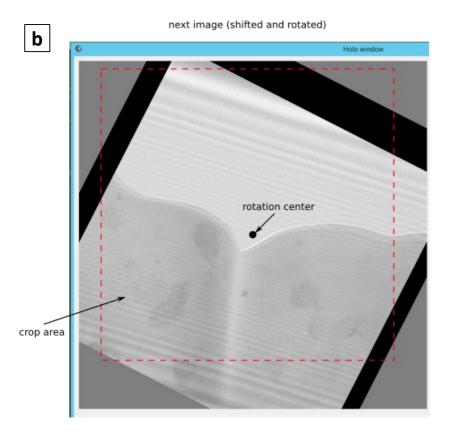


Fig. 7. The result of rot-alignment of images (a) and (b) which were shifted and rotated with respect to each other. The center of rotation was marked, which does not overlap with the center of original holograms. Red dashed line shows the square area which will be cropped from both images in order to get rid of areas which do not carry any information (uniform black and gray areas).

IFFT – calculate and display inverse Fourier transform of the current FFT image.

Holo – filter the sideband selected for hologram reconstruction. Before using this function you must calculate FFT of the hologram (FFT button) and mark the area around the sideband chosen for hologram reconstruction by selecting two points: top-left and bottom-right (see Fig. 8). When you click the Holo button the program will find center of the sideband (i.e. position of maximum intensity) inside of the marked area. Then it will crop the sideband with the circular aperture of a given radius (i.e. rest of the FFT will be masked) and shift the sideband to the center of FFT image. Moreover this modified FFT image will be multiplied by a Hanning window of a given width (= height) in order to smooth out edges of the aperture. This sideband image will be added to the queue. In order to see the result of hologram reconstruction you need to calculate the IFFT of the sideband image (IFFT button).

Holo+Ref – this function works in a similar way as Holo, but it uses also the reference hologram (i.e. equivalent sidebands are filtered from specimen hologram and from reference hologram). In this case you must calculate the FFT of reference hologram first, before clicking Holo+Ref button. Also, both holograms must be ordered in the appropriate way – first the reference hologram and then the specimen hologram. After FFT calculation you have to mark one of the sidebands and click Holo+Ref button. This will cause the program to locate center of the sideband and to crop it with the aperture from reference FFT image as well as from specimen FFT image.

<u>Note 9</u>: You don't have to calculate the FFT of specimen hologram yourself. The program will do it for you after you click Holo+Ref button.

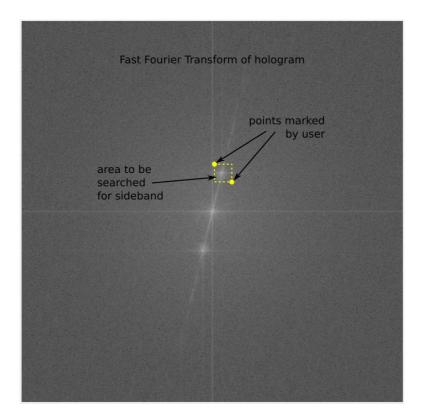


Fig. 8. The way of locating sideband on Fourier transform of typical hologram. Program searches for center of the sideband (i.e. pixel of maximum intensity) inside of the square area marked by user.

Two images with centered sidebands will be added to the queue. Then you can calculate IFFT from both of them in order to observe the reference phase shift as well as the total phase shift introduced to wave function by internal fields of a sample. Reference holograms are used to remove the artifacts related to the specific characteristic (information transfer) of CCD camera used for hologram registration. You can remove those artifacts by calculating the difference between total phase and reference phase. You can accomplish this by using Diff function which will subtract phase of the previous image (reference phase) from phase of the currently displayed image (total phase).

Sum – calculate the sum of two phases: 1 – phase of currently displayed image and 2 – phase of the previous image. Resulting image will be added to the queue after both images.

Diff – calculate the difference between two phases: 1 – phase of currently displayed image and 2 – phase of the previous image.

<u>Note 10</u>: When you apply Sum or Diff function the resulting image will have its amplitude equal to the product of amplitudes of two previous images.

Amp. factor – amplification factor  $\alpha$  used to amplify the phase image.

Amplify – amplify the phase image  $\varphi$  according to the following formula  $\varphi' = \sin(\alpha \cdot \varphi)$ , where amplification factor  $\alpha$  can be specified in the Amp. factor textbox.

### 2.5. Magnetic field tab

Plot profile – display the profile of intensity corresponding to the line marked on the image by user. Profile intensity is displayed in the Plotting area of PyHoLo window (see section 3).

Profile width – width of intensity profile given in pixels (see Fig. 9). If its value is set to be > 1 then each point of the intensity profile will be calculated as a sum of pixels placed on a line perpendicular to

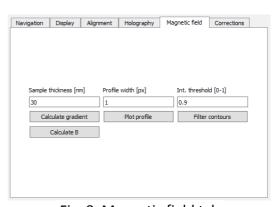


Fig. 8. Magnetic field tab

the marked profile at a distance from the range (-d/2, +d/2) from that profile (d is a given profile width).

Filter contours – filter image features with intensity greater than the given fraction (Int. threshold) of maximum intensity.

Int. threshold – fraction from the range 0-1 (where maximum intensity on the image corresponds to 1.0), below which all pixels will be blackened when you use Filter contours function.

Calculate gradient – calculate directional x,y-gradients and total gradient of the image and add results to the image queue.

Sample thickness – input for sample thickness (in nm) in the area of interest. Its value is necessary to calculate magnetic field B in this area.

Calculate B – calculate magnetic field B based on phase profile plotted in panel (9) and the information about sample thickness in the area of interest. The calculation of B follows the formula:

(Eq. 1) 
$$|\vec{B}_{\perp}| = \frac{\hbar}{et} \frac{\partial \varphi_m}{\partial x} \approx \frac{\hbar}{e} \frac{\Delta \varphi_m}{t\Delta x} = \frac{\hbar}{e} \frac{(\varphi_2 - \varphi_1)}{t\Delta x}$$

where t is a sample thickness and  $\frac{\partial \varphi_m}{\partial x}$  is a gradient of magnetic phase.

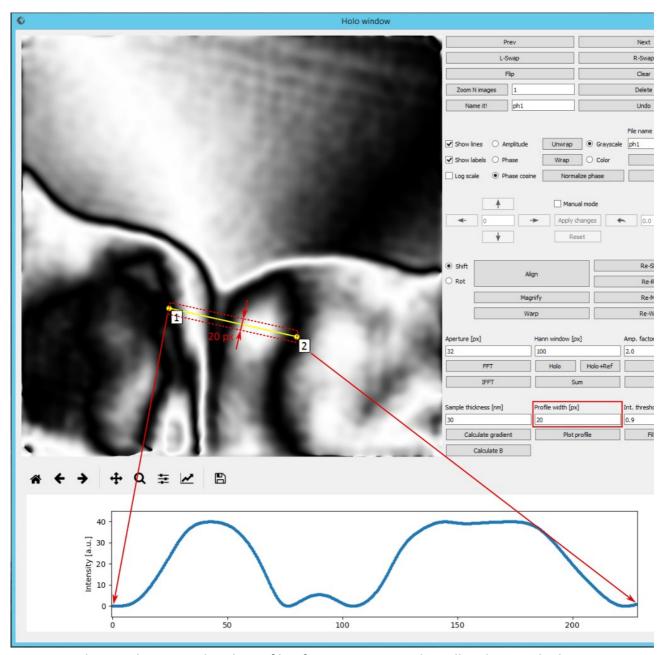


Fig. 9. Plotting the 20-pixel-wide profile of intensity across the yellow line marked on image.

To determine magnetic field B you will need to mark a straight line on the magnetic phase image (the procedure of getting such image will be described later in this manual) and plot the phase shift profile across this line. Next, you will have to select two points on the phase profile by clicking on a graph with your mouse (after each click a red circle should appear on the plot). Those two

points will determine a section of phase profile on which the phase gradient is to be calculated. Thus you should select a section where phase change is roughly constant (i.e. the phase gradient is constant). Only x-coordinates of selected points are used by program (y-values are derived from the phase profile automatically) so the points don't necessarily have to coincide with the plot. After you do all of the above steps and click the Calculate B button the magnetic field value (in T units) will be displayed in the interpreter window.

Note 11: You will be able to select points on a phase profile only after clicking on the +-button in the Plotting area (see section 3).

## 2.6. Corrections tab

Here you can change brightness, contrast and gamma of the currently displayed image by adjusting the corresponding sliders or entering values into the corresponding textboxes.

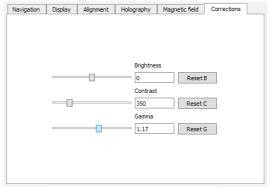


Fig. 10. Corrections tab

#### 3. Plotting area

For more information about plotting profiles see the descriptions of Plot profile button and Profile width input in the section regarding Magnetic calculations panel (8).

The most useful functions for operating on plots are listed below:

- reset the plot to the original state.
- move the plot across the graph area with your mouse. Clicking this button will enable the possibility to mark points (red circles) in the graph area (which is necessary to calculate magnetic field B).
- **Q** zoom a section of the plot.
- modify axis parameters and labels, choose color and style of the plot.
- 🖺 save the plot on a disk.

## C) The procedure of reconstruction of magnetic phase image

Below you can find an example of a reconstruction procedure which is based on two electron holograms showing the same area of a sample, but acquired for its two different orientations: upside and downside. For each one of those two holograms also the reference hologram from a vacuum area was acquired. DM3 files with those four holograms can be found in the input folder:

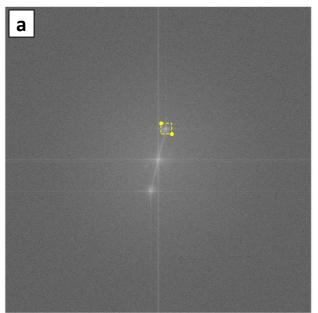
'holo01.dm3' – reference hologram for an "upside" orientation of a sample

'holo02.dm3' – hologram showing fragment of the sample in "upside" orientation

'holo03.dm3' – reference hologram for a "downside" orientation of a sample

'holo04.dm3' – hologram showing the same fragment of sample in "downside" orientation

- 1. After you run PyHoLo program point to the input folder were the holograms are stored, select 'holo.01.dm3' and click Open. The information about next images being loaded will be displayed in the interpreter window. After last image is loaded the main program window should appear (see Fig. 1). By clicking on Next and Prev buttons you can look through all holograms. By default the dm3 files are treated as amplitude images (i.e. intensity values of image pixels are treated as amplitude values of an electron wave passing through specimen). Thus the phase of electron wave is set to be zero, and if you switch to the Phase mode of displaying image you will se an empty (dark) image.
- 2. It's a good idea to name each hologram in order to simplify the navigation between them. First and third images are reference holograms so you can name them 'ref1' and 'ref2' respectively (Display tab  $\rightarrow$  Set name). Similarly you can name second and fourth holograms as 'holo1' and 'holo2'.
- 3. You can notice that holo1 and holo2 show the same area of sample but they are mirrored with respect to each other. Thus in order to align them together we need to flip one of them (and its reference) horizontally. Let's flip the second pair of hologram: ref2 and holo2 (Navigation tab  $\rightarrow$  Flip).
- 4. Due to the fact that we have reference holograms for our disposal we will use the Holo+Ref function. Let's select ref1 and calculate its Fourier transform (Holography tab  $\rightarrow$  FFT). Then switch to the Log scale mode (Display tab  $\rightarrow$  Log scale) in order to make the sidebands visible.
- 5. Mark one of the sidebands by selecting two points and thus surrounding the sideband with a rectangle (see Fig. 11). You should be consistent in selecting sidebands of all holograms. In this example we will always mark the sideband which is located "on the vacuum side" of the specimen (as in Fig. 11).



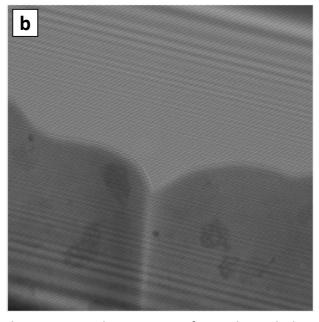


Fig. 11. Selection of a sideband on ref1 FFT (a) with respect to the position of sample on holo1 hologram (b).

6. Let's leave default values of aperture radius and width of Hanning window (Holography tab  $\rightarrow$  Aperture, Hann window), i.e. 32 and 100 pixels, respectively.

Note 12: Width of Hanning window should be larger than diameter of numeric aperture (in this example we have 2.32 = 64 < 100).

Now you can click the Holo+Ref button. When you do that you will notice that two images have been added to the queue just after holo1. First one shows the centered sideband of ref1 FFT and second one shows the centered sideband of holo1 FFT. Thanks to this the same ranges of spatial frequencies will be taken into account during reconstruction.

- 7. Go to the sideband image of ref1 and click IFFT button. You will get the vacuum image without holography fringes, restored by considering only the low-angle spatial frequencies around the centered sideband. Obtained image contains amplitude data as well as phase data which you can review by switching between Amplitude and Phase modes in the Display tab.
- 8. Now you can go to the sideband image of holo1 and again click IFFT button. This time you will get the restored image of sample, i.e. amplitude and total phase of electron wave which propagated through specimen. Now may be a good time to clean up the image queue by deleting FFT images and sideband images (Navigation tab  $\rightarrow$  Delete). We won't use them any more. Let's also name two of the restored images as res1 and res2 respectively.
- 9. You can notice that phases of res1 and res2 are wrapped and contain characteristic phase contours in the areas of sudden phase drops from  $+\pi$  to  $-\pi$ . We can remove those contours by unwrapping phase images, i.e. making sure that phase values are not limited to  $(-\pi, +\pi)$  range. In order to do that switch to the Phase mode (if you haven't done that already) and click Unwrap phase button (Display tab  $\rightarrow$  Unwrap phase). After a while phase image will be replaced with the new one that doesn't have any wrappings. If the new image looks the same as the old one it means that original phase values were already contained within  $(-\pi, +\pi)$  range. Repeat unwrapping procedure for res1 and res2.
- 10. Now you can use the reference phase (phase of res1) to remove the background data (effects of camera) from the total phase image (phase of res2). In order to do that make sure that res2 is right after res1 in the image queue, switch to res2 and click Diff button (Holography tab  $\rightarrow$  Diff). You will get a new image whose phase  $\varphi'$  is equal to the difference of res2 and res1 phases:  $\varphi' = \varphi_{tot} \varphi_{ref}$ , and its amplitude is equal to the product of amplitudes:  $A' = A_{tot} \cdot A_{ref}$ . Let's name the new image as phase1 having in mind that it contains data about total phase of electron wave restored from an "upside" pair of holograms (i.e. total phase shift introduced to the electron wave function, passing through a sample, by internal electric and magnetic fields of a sample, while it was in an "upside" orientation towards the incident electron beam).
- 11. Repeat the steps 5-10 for the "downside" pair of holograms (ref2 and holo2) and name the resulting image as phase2 (i.e. total phase of electron wave, restored from a "downside" pair of

holograms).

- 12. Clean up the image queue so that only four images are left: holo1, holo2, phase1 and phase2.
- 13. We want to calculate the sum ("electric phase") and the difference ("magnetic phase") of both total phases (phase1 and phase2). To do this, however, we need to align phase1 and phase2 with each other by shifting and rotating them. Shift and rotation can be derived from amplitudes of phase1 and phase2 or from original holograms holo1 and holo2. In this example it will be easier to use holo1 and holo2. Let's try to align holo1 and holo2 by marking three points on each one of them. Each set of points should mark the same set of characteristic features on a sample, i.e. point "1" on holo1 should correspond to point "1" on holo2 and so on (see Fig. 12). Of course we could mark more than three points, but in this example it should be enough.

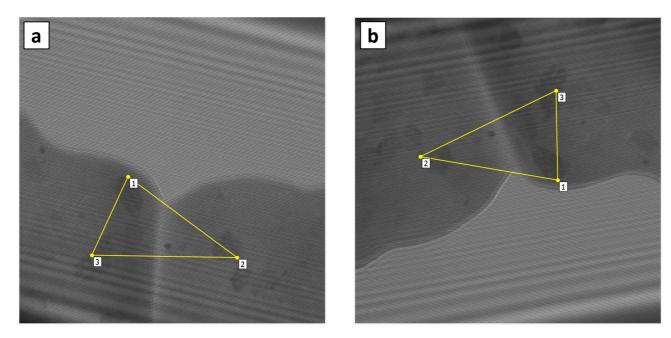


Fig. 12. Determining shift and rotation between holo1 (a) and holo2 (b) based on two sets of user-defined points.

After selecting two sets of points, go to the Alignment tab and switch to the Rot option in order to make sure that both shift and rotation will be applied. Then make sure that holo1 and holo2 are next to each other (holo1 should preceed holo2), switch to holo2 image and click the Align button. After a little while alignment should be finished and two new images should appear after holo2 image: first one being a copy of holo1, shifted so that the rotation center is in the middle of display and second one being a copy of holo2, shifted in the same way as holo1 and rotated accordingly. If you are not satisfied with the result of alignment you can repeat the whole procedure, i.e. delete aligned images, mark new sets of points on holo1 and holo2 and click the Align button.

14. If you are satisfied with the result, delete the original holo1 and holo2 images and name the aligned images as holo1\_new and holo2\_new. Now we can use the information about shift and rotation which is stored in the program's memory and align phase1 and phase2 accordingly. First go to phase1 image and click Re-Shift button to shift it in the same way as holo1 was shifted in the

previous step. Name the new image as phase1\_new and delete the original phase1. Then go to the phase2 image and again click the Re-Shift button. After the shift is done, keep the new image selected, and click the Re-Rot button. This will cause the shifted phase2 image to be rotated in the same way as holo2 was rotated in the previous step. Now you can delete the original phase2 and shifted phase2 images and name the new shifted-and-rotated image as phase2\_new. As a result you should be left with four aligned images: holo1 new, holo2 new, phase1 new, phase2 new.

15. Shift and rotation caused the black empty areas to appear on the edges and near the corners of resulting images. Let's cut out most of them by cropping the same region-of-interest (ROI) from all four images. Go to the first image in queue (holo1\_new) and select the ROI which contains as much information as possible, but doesn't contain any black areas (see Fig. 7). Then go to the Navigation tab, enter the number of images (which is 4) in the textbox on the right side of Crop N ROIs button and click the button. This will cause the same area to be cropped from all four images. Those four fragments will be rescaled to take the whole display area and added to the image queue. Let's now delete the source images (holo1\_new, holo2\_new, phase1\_new and phase2\_new) and name the cropped fragments as h1, h2, ph1, ph2.

16. We still keep h1 and h2 (being the fragments of original, experimental holograms), because they may become handy if we would like to use some other graphics program to superimpose the results of reconstruction (e.g. magnetic phase contours) on the specimen image (shown on both h1 and h2). Now it's a good time to export h1 and h2 to the disk. Let's go to h1, switch to the Display tab, make sure that PNG option is selected and click the Export button. Then do the same with h2. By default, this will cause both images to be saved in the PyHoLo folder as 'h1.png' and 'h2.png'. The proper information about saved images will be displayed in the interpreter window. After export is completed you can remove h1 and h2 from the image queue.

17. Having ph1 and ph2 images we can finally restore the electric phase (sum) and the magnetic phase (difference). To get the electric phase go to ph2 image and click the Sum button under the Holography tab. The new image will appear with its phase equal to sum of ph1 and ph2 phases. To get the magnetic phase go back to ph2 image, but this time click the Diff button. Another image will appear with its phase equal to the difference of ph1 and ph2 phases. Let's name those new images as sum (or el) and diff (or mag).

<u>Note 13</u>: Electric phase corresponds to phase shift introduced to electron wave by electric field in the sample. Similarly, magnetic phase corresponds to phase shift introduced to electron wave by magnetic field in the sample.

18. It is a known practice to present the electric and magnetic phases by their cosinus functions. Then the phase cosine can easily be amplified with the use of the following formula:

(Eq. 2) 
$$\Phi = \cos(\alpha \cdot \phi)$$

where  $\alpha$  is the amplification factor.

The amplification of magnetic phase is used to obtain so-called phase contours which in theory are parallel to the direction of magnetic field vector projected on the investigated plane of sample. In order to amplify sum and diff image select one of them, go to the Holography tab, enter the amplification factor of your choice in the proper textbox and click the Amplify button to see the result.

<u>Note 14</u>: When you amplify phase image then program automatically switches to the Phase cosine mode of displaying images.

If you use  $\alpha = 4$  for both sum and diff then the results should resemble images shown in Fig. 13.

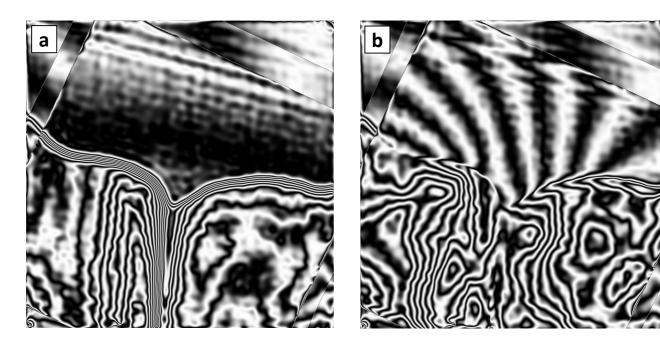
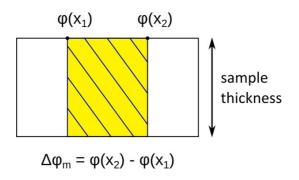


Fig. 13. Electric phase contours (a) and magnetic phase contours (b) obtained with PyHoLo. Edges visible in top-left and top-right corners of images are the effects of the applied alignment procedure. Areas outside of the edges can be removed by cropping only the middle parts of images or they can be covered with an uniform color in another graphics program (e.g. Inkscape).

19. Last but not least we can try to calculate magnetic field confined in a selected area of the sample. Below you can see a very schematic side view of our sample.



Let's assume that we want to calculate the magnetic field confined in the yellow area. This area is defined by two points with different magnetic phases and the thickness of sample. According to the Eq. 1 the value of magnetic field is proportional to the difference of those magnetic phases and inversely proportional to the area of the rectangle. Let's go to the diff image and switch to the Phase mode. Then mark two points corresponding to different phase values. Have in mind that the change of phase between those points should be approximately linear. Then go to the Magnetic field tab, make sure that the Profile width is set to 1 pixel and click the Plot profile button. The phase profile corresponding to the section between two selected points should appear in the Plotting area. To calculate the slope factor of the phase profile (i.e. phase gradient) you have to select two points on the profile: first one at the beginning of a section where phase changes linearly and second one at the end of this section (see two red markers in Fig. 14 for an example on how to do that). You can enable the possibility to mark points on the profile by clicking on a cross button (see blue square in Fig. 14). After selecting both points you also need to specify the sample thickness in the proper textbox under Magnetic field tab (see red rectangle in Fig. 14). In this example we have used the thickness of 50 nm. Finally click the Calculate B button. You should see the calculated value of magnetic field in the interpreter window.

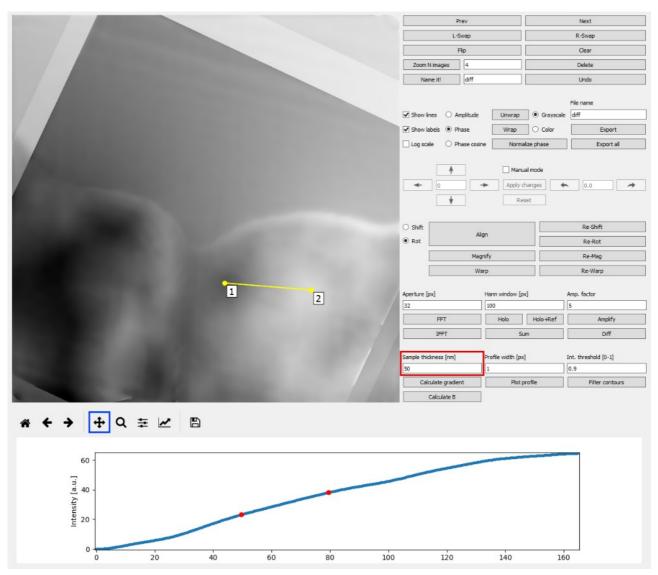


Fig. 14. The way of determining magnetic field value from the magnetic phase profile.

20. Finally you can export the results. Select the image which you want to save as file (remeber to specify the type of data by selecting Amplitude, Phase or Phase cosine option and the file format by choosing PNG or Binary option) and click the Export button.