User manual for

DIGITAL LENSLESS HOLOGRAPHIC MICROSCOPY (DLHM)

DIGITAL LENSLESS HOLOGRAPHIC MICROSCOPY (DLHM)– Version 1.0

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1 Getting started

1.1 Introduction

DIGITAL LENSLESS HOLOGRAPHIC MICROSCOPY (DLHM) is a plugin developed to work on the well-known software for image processing ImageJ. This plugin enables the numerical reconstruction and simulation of digitally recorded holograms in the architecture of digital lensless holographic microscopy (DLHM). The plugin can be used for teaching and research purposes.

1.2 Installation

The installation of the plugin can be done, following these steps:

1. Make sure you are running ImageJ 1.48s or superior. The installation procedure for ImageJ can be found at http://imagej.nih.gov/ij/docs/install/.
2. Download the *.zip* file, which includes all the needed libraries, available on the *Downloads* section at https://unal-optodigital.github.io/DLHM/.
3. Extract the contents of the *zip* file in the *imagej/plugins* folder.

1.3 Changes

Version 1.0

- Initial release.

1.4 Support

If you are using DIGITAL LENSLESS HOLOGRAPHIC MICROSCOPY (DLHM) and find a bug, please contact us at jppiedrahitaq@unal.edu.co or catrujila@unal.edu.co.

1.5 Credits

DIGITAL LENSLESS HOLOGRAPHIC MICROSCOPY (DLHM) uses the fast and reliable FFT routines of JTransforms (https://sites.google.com/site/piotrwendykier/software/jtransforms/) and the icons provided in the Silk icon set (http://www.famfamfam.com/lab/icons/silk/).

2 Running the plugin

To start working with the DIGITAL LENSLESS HOLOGRAPHIC MICROSCOPY (DLHM) plugin, ImageJ must be running, if you don't know how to do so, please refer to the ImageJ user guide. If the DLHM plugin is installed, a new menu entry (*OD*) should appear on the ImageJ's main window, as shown in Figure 1. To run the plugin, just click the *Reconstruction* or *Simulation* entries and the respective window should be displayed.

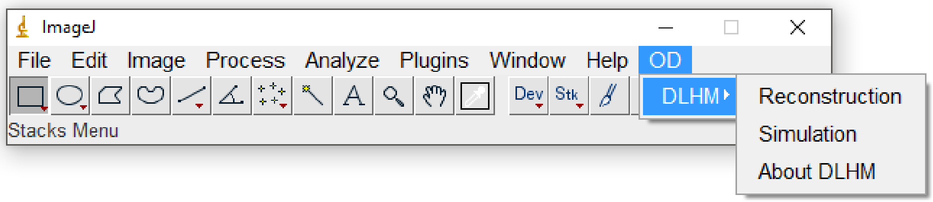

Figure 1. *ImageJ main menu. See the OD label under which is the DLHM plugin with the "Reconstruction" and "Simulation" options.*

2.1 Reconstruction window

The reconstruction window of the plugin is shown in Figure 2. The graphical interface can be divided in four panels: parameters (1), log (2), outputs (3) and buttons (4).

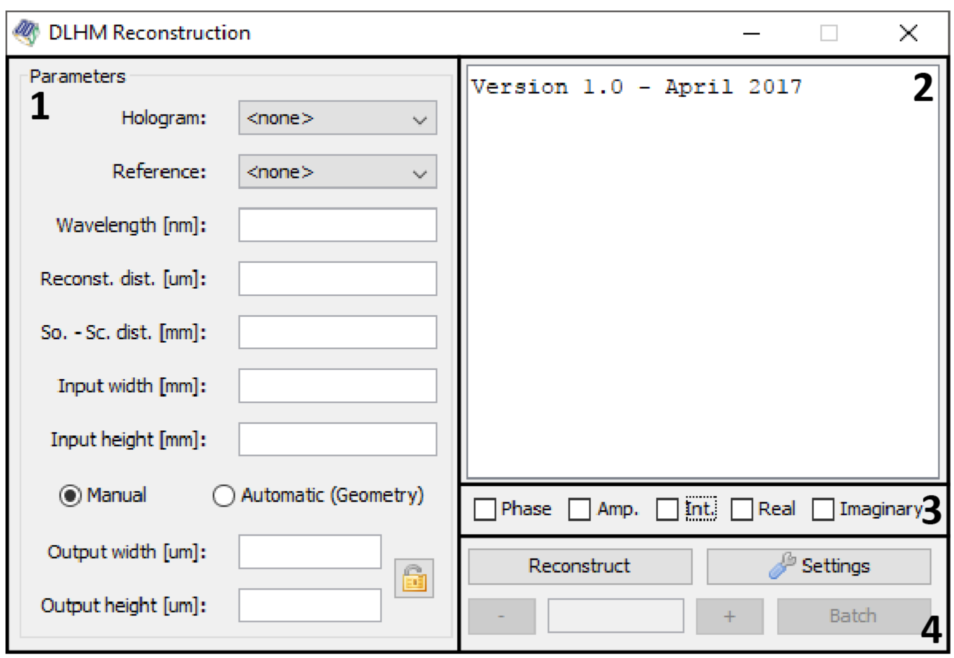

Figure 2. *Reconstruction window.*

2.1.1 Parameters

Hologram: The digitally recorded hologram (image) in a DLHM setup.

Reference (Optional): The digitally recorded hologram (image) with no sample present in a (DLHM) setup. Used to compute the contrast hologram.

Wavelength: The illumination wavelength used to record the hologram. The wavelength must be a positive number and different from zero.

Reconst. dist. (Reconstruction distance): Axial distance between the position of the point source illumination and the position of the reconstruction plane.

So. – Sc. dist. (Source to screen distance): Axial distance between the position of the point source illumination and the position of the screen (camera). Source to screen distance must be a positive number and different from zero.

Input width/height: Width/height of the input plane, i. e., the camera dimensions. The input dimensions must be positive numbers and different from zero.

Manual/Automatic (Geometry): Determines if the output window dimensions are selected manually by the user or calculated automatically using triangle relations with the illumination cone.

Output width/height (Manual mode only): Width/height of the output plane. The output dimensions must be positive numbers and different from zero.

Padlock button (Manual mode only): If the padlock is closed (by pressing the button), the aspect ratio of the input plane is kept for the output plane.

2.1.2 Log

Recent history of the plugin usage. When a numerical reconstruction operation is performed, the parameters (input images, dimensions, wavelength, etc.) are printed in this text area. The log can be cleared by right-clicking the area and selecting the *Clear* option.

2.1.3 Outputs

Possible representations of the numerical reconstruction. When the hologram is reconstructed, each one of the selected representations is shown in a separated window.

2.1.4 Buttons

Reconstruct: Performs the numerical reconstruction over the hologram according to the selected parameters and settings.

Settings: Shows the settings window.

- / +: Performs the numerical reconstruction at a reconstruction distance equals to the last used minus or plus the step given in the text field between the buttons.

Batch: Shows the batch reconstruction window.

2.2 Simulation window

The simulation window of the plugin is shown in Figure 3. As the reconstruction window, the graphical interface can be divided again in four panels: parameters (1), log (2), outputs (3) and buttons (4).

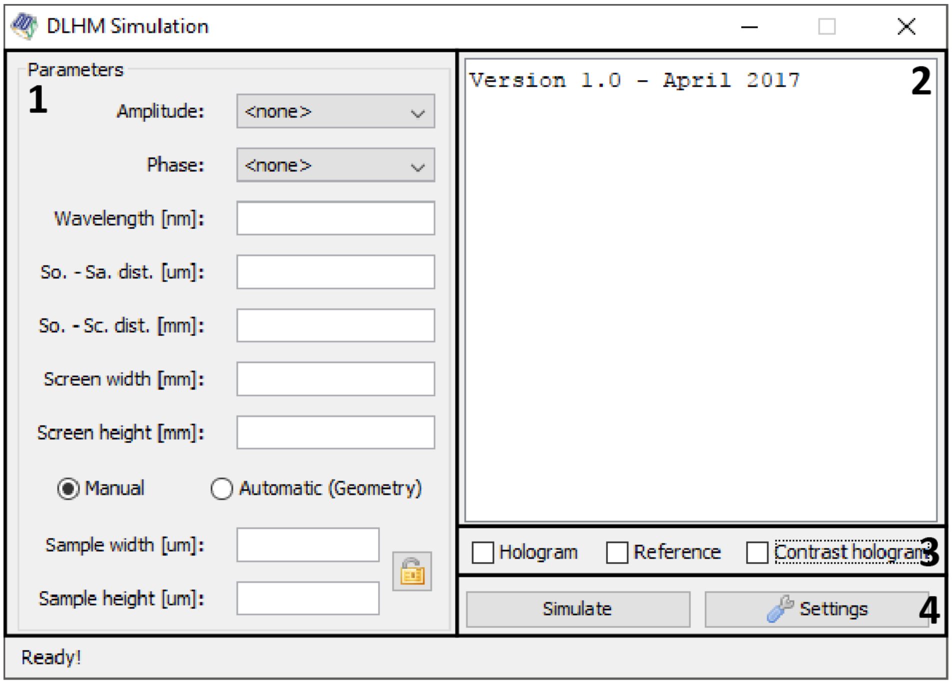

__Figure 3.__ Simulation window.

2.2.1 Parameters

Amplitude (Optional): The amplitude distribution of the simulated sample. It is recommended to be a floating-point image having values within the 0 - 1 range. If the selected image has values outside of the said range, it is rescaled to the range 0 - 1. If only the amplitude image is selected, the phase distribution is assumed to be zero at every point.

Phase (Optional): The phase distribution of the simulated sample. It is recommended to be a floating-point image. If only the phase image is selected, the amplitude distribution is assumed to be one at every point.

At least one input image must be selected, either amplitude or phase in order to perform the simulation process.

Wavelength: The illumination wavelength used to simulate the hologram. The wavelength must be a positive number and different from zero.

So. - Sa. dist. (Source to sample distance)**:** Axial distance between the position of the point source illumination and the position of the sample plane. Source to sample distance must be a positive number and different from zero, also it must be lower than the *source to screen distance*.

So. – Sc. dist. (Source to screen distance): Axial distance between the position of the point source and the position of the screen (camera). Source to screen distance must be a positive number and different from zero, also it must be greater than the *source to sample distance*.

Screen width/height: Width/height of the screen plane, i. e., the camera dimensions. The screen dimensions must be positive numbers and different from zero.

Manual/Automatic (Geometry): Determines if the sample window dimensions are selected manually by the user or calculated automatically using triangle relations with the illumination cone.

Sample width/height (Manual mode only): Width/height of the sample plane. The smaple dimensions must be positive numbers and different from zero.

Padlock button (Manual mode only): If the padlock is closed (by pressing the button), the aspect ratio of the screen plane is kept for the sample plane.

2.2.2 Log

Recent history of the plugin usage. When a numerical simulation operation is performed, the parameters (input images, dimensions, wavelength, etc.) are printed in this text area. The log can be cleared by right-clicking the area and selecting the *Clear* option.

2.2.3 Outputs

Possible representations of the numerical reconstruction. When the hologram is simulated, each one of the selected representations is shown in a separated window.

2.2.4 Buttons

Simulate: Performs the numerical reconstruction over the hologram according to the selected parameters and settings.

Settings: Shows the settings window.

3 Numerical reconstruction of holograms in DLHM (Working with the plugin)

The plugin allows the calculation of the numerical reconstruction through one process that can be summarized in three simple steps: i) *Input:* reading of the input parameters and building of the contrast hologram, ii) *Reconstruction:* numerical reconstruction of the contrast hologram and iii) *Output:* display of the selected representations for the numerically reconstructed hologram.

3.1 Method

The plugin performs the numerical reconstruction by propagating the selected input hologram through a modification of the Kirchhoff-Helmholtz diffraction formula, working for high numerical apertures in discrete coordinates. The resulting reconstructed hologram can be expressed by

$$A_{\text{out}}(\boldsymbol{r}')=-\frac{iA_R}{2\lambda}\iint_{\text{Sensor}}\tilde{H}(\boldsymbol{r})\exp\left[-ik\frac{\boldsymbol{r}\cdot\boldsymbol{r}'-(r'^2/2)}{r}\right]\left(\frac{1}{r^2}\right)(1+\cos\chi)\mathrm{d}\boldsymbol{r} \tag{1}$$

where $A_{\text{out}}(\boldsymbol{r}')$ is the reconstructed hologram with $\boldsymbol{r}'=(\xi,\eta,z)$ the coordinate vector of every point composing the output field; and $\tilde{H}(\boldsymbol{r})$ is the contrast hologram with $\boldsymbol{r}=(x,y,L)$ the coordinate vector of every point composing the input contrast hologram. z and L represent the distance from the point source to the plane of reconstruction and recording, respectively; $k=2\pi/\lambda$ stands for the angular wavenumber with λ the illuminating wavelength; and $i=\sqrt{-1}$. After a coordinate change given by

$$\begin{aligned} x&=XL/R\\ y&=YL/R\\ R&=\left(L^2-X^2-Y^2\right)^{1/2}\end{aligned} \tag{2}$$

and the discretization of the resulting integral, the reconstructed field can be numerically expressed by

$$A_{\text{out}}[p,q]=\Delta X\Delta Y\exp\left[-\frac{i\pi}{\lambda L}\left(p^2\Delta X\Delta\xi+q^2\Delta Y\Delta\eta\right)\right]\text{FFT}^{-1}\{F_1F_2\}\ . \tag{3}$$

In equation (3) $A_{out}[p,q]$ is the numerically reconstructed DLHM hologram with p and q whole integers that represent the coordinates of every pixel composing the output image; ΔX and ΔY are the sampling pitches associated to the new coordinate system given by equation (2); $\Delta\xi$ and $\Delta\eta$ are the output sampling pitches; the operator $FFT^{-1}\{\ \}$ stands for the inverse fast Fourier transform of the pixel-wise multiplication of

$$\begin{aligned} F_1&=FFT\left\{\tilde{H}'[m,n]\exp\left[-\frac{i\pi}{\lambda L}\left(m^2\Delta X\Delta\xi+n^2\Delta Y\Delta\eta\right)\right]\right\}\\ F_2&=FFT\left\{\exp\left[\frac{i\pi}{\lambda L}\left(m^2\Delta X\Delta\xi+n^2\Delta Y\Delta\eta\right)\right]\right\}\end{aligned}\ . \tag{4}$$

where $FFT\{\ \}$ is the operator of the fast Fourier transform and $\tilde{H}'[m,n]$ is the prepared hologram with m and n whole integers; that again represent the coordinates of every pixel that composes the contrast hologram. The prepared hologram in turn can be expressed by

$$\tilde{H}'[m,n] = -\frac{iA_R}{2\lambda}\tilde{H}[m,n]\exp\left[-\frac{ikR}{L}\left(z-\frac{r'^2}{2}\right)\right]\left(\frac{1}{R^2}\right)\left(1+\frac{R}{L}\right). \tag{5}$$

The contrast hologram $\tilde{H}[m,n]$, with coordinates for every pixel m and n, can be built in four different ways (see Contrast hologram calculation):

i) As the result of the pixel-wise subtraction of the hologram minus the reference image (only if a reference image is previously loaded).

ii) As the result of the pixel-wise subtraction of the hologram and a modeled spherical wave, whose radius is the distance from the point source to the screen.

iii) As the pixel-wise subtraction of the hologram and an average value calculated in different regions of the hologram.

iv) As the input hologram with no modification.

Further information on the analytical development of the model for the reconstruction and its numerical implementation can be found in the patent "Microscopio holográfico digital sin lentes (MHDSL) y método para visualizar muestras" submitted by J. Garcia-Sucerquia, C. Trujillo, and J. Restrepo Agudelo.

3.2 Single reconstruction

A single numerical reconstruction is performed when the *Reconstruct* button is clicked, then the plugin reads the input parameters from the text fields on the reconstruction window and performs the calculations over the hologram. If the reference image is selected to build the contrast hologram, then this image is initially pixel-wise subtracted from the hologram.

3.3 Step reconstruction

Step reconstruction can be done through the - and + buttons present on the reconstruction window. This type of reconstruction is only available after a single reconstruction is performed since it propagates the interpolated version of the previously selected hologram. The hologram is numerically reconstructed using all the lastly used parameters with exception of the *Reconstruction distance* and the *Output width/height*. The new distance is equal to the last used minus or plus the step given in the text field between the buttons. The given step must be a positive number and different from zero. The new *Output width/height* is given by the parameters introduced in the respective text fields when the Step reconstruction is performed. If the *Automatic* option is selected the new *Output width/height* is calculated using triangle relations for the new *Reconstruction distance*.

To perform a step reconstruction, introduce the step value in the text field using the same units as the reconstruction distance parameter, then click the - or + button.

3.4 Batch reconstruction

Batch reconstruction is available through the *Batch* button present in the reconstruction window. As the step reconstruction, it propagates the interpolated version of the previously selected hologram and is only available after a single reconstruction is performed. Batch reconstruction consist in a distance sweep. The hologram is numerically reconstructed using all the lastly used parameters with exception of the *Reconstruction distance* and the *Output width/height*. The reconstruction distances are given by starting and ending reconstruction distances (*From* and *To*), and the number of planes (*Planes*) or the step distance (*Step*) as shown in Figure 4. Both windows can be accessed with the *Batch* button when the respective option in the settings window is set.

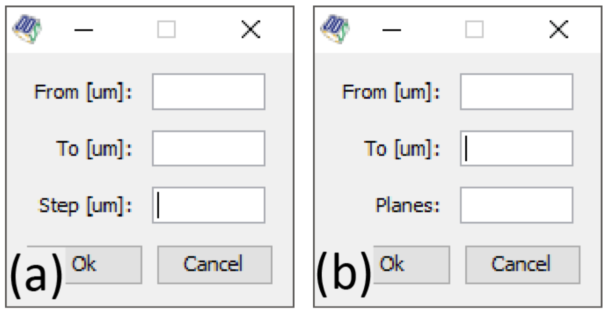

Figure 4. *Batch window.*

The *Output widths/heights* for the resulting reconstructions are given by the parameters introduced in the respective text fields when the *Batch* button is clicked, this way all the resulting reconstructions will have the same *Output width/height*. If the *Automatic* option is selected the new *Output widths/heights* are calculated using triangle relations for the corresponding *Reconstruction distance* for each plane.

While the Batch operation is being performed, in the ImageJ main window two indicators are shown, a progress bar and a status label. Finally, the output images are represented as ImageJ stacks and for a correct visualization the amplitude and intensity images are represented as 8 bit images and for the phase information it is assumed for the scaling that the minimum and maximum values are $-\pi$ and π, respectively.

4 Numerical simulation of holograms in DLHM (Working with the plugin II)

The plugin allows the calculation of the numerical simulation through one process that, as the reconstruction process, can be summarized in three simple steps: i) *Input:* reading of the input parameters and building of the contrast hologram, ii) *Simulation:* numerical simulation of the sample field and iii) *Output:* display of the selected representations for the numerically simulated hologram.

4.1 Method

The plugin performs the numerical simulation by propagating the selected sample field through a modification of the Rayleigh–Sommerfeld diffraction formula, working for high numerical apertures in discrete coordinates. The resulting simulated complex field in the camera plane can be expressed in its discrete form by

$$A_{\text{out}}\left[p,q\right]=\Delta X\Delta Y\frac{1}{i\lambda R}\exp\left[ikR\right]\exp\left[-\frac{ik}{2R}\left(\Delta\xi\Delta Xp^2+\Delta\eta\Delta Yq^2\right)\right]\text{FFT}^{-1}\left\{F_1F_2\right\} \tag{6}$$

where p, q, m and n whole integers that represent the positions of every pixel in the camera and sample planes, respectively; $A_{\text{out}}\left[p,q\right]$ is the simulated complex field in the camera plane; $k=2\pi/\lambda$ is the angular wavenumber with λ the illuminating wavelength; $\Delta\xi$ and $\Delta\eta$ represent the sampling pitches at the sample plane ; $R=\left[\left(L-z\right)^2+\left(m\Delta X\right)^2+\left(n\Delta Y\right)^2\right]^{1/2}$ with z and L representing the distances from the point source to the plane of the sample and camera, respectively; and $i=\sqrt{-1}$. The operator $FFT^{-1}\left\{\ \right\}$ stands for the inverse fast Fourier transform of the pixel-wise multiplication of

$$\begin{aligned} F_1&=FFT\left\{A_{\text{in}}\left[m,n\right]\exp\left\{\frac{ik}{2R}\left[\Delta\xi\left(\Delta\xi-\Delta X\right)m^2+\Delta\eta\left(\Delta\eta-\Delta Y\right)n^2\right]\right\}\right\}\\ F_2&=FFT\left\{\exp\left\{\frac{ik}{2R}\left[\Delta\xi\Delta X\left(m-p\right)^2+\Delta\eta\Delta Y\left(n-q\right)^2\right]\right\}\right\}\end{aligned} \tag{7}$$

where $FFT\left\{\ \right\}$ is the operator of the fast Fourier transform and $A_{\text{in}}\left[m,n\right]$ is the complex field that represents the sample with m and n whole integers; that again represent the coordinates of every pixel that composes the sample field. ΔX and ΔY represent the sampling pitches at the screen plane and are related with the actual camera pitches Δx and Δy by

$$\begin{aligned} m\Delta X&=s\Delta x\left[1-\left(\frac{s\Delta x}{z}\right)^2+\left(\frac{t\Delta y}{z}\right)^2\right]^{-1/2}\\ n\Delta Y&=t\Delta y\left[1-\left(\frac{s\Delta x}{z}\right)^2+\left(\frac{t\Delta y}{z}\right)^2\right]^{-1/2}\end{aligned}. \tag{8}$$

The relation in equation (8) implies that the output field obtained with equation (6) is represented in a non-uniform grid. To restore the output field into an evenly spaced grid it has to be interpolated into a new grid with coordinates given by $s\Delta x$ and $t\Delta y$. After this the hologram can be extracted as the squared modulus of the new interpolated field.

The complex sample field $A_{\text{in}}[m,n]$ can be expressed constructed as

$$A_{\text{in}}[m,n] = A[m,n]\exp\{i\varphi[m,n]\}A_{\text{illu}}[m,n]. \tag{9}$$

where $A[m,n]$ and $\varphi[m,n]$ represent the amplitude and phase distributions of the sample, respectively. $A_{\text{illu}}[m,n]$ represents the spherical illumination in the sample plane. The single hologram is obtained using $A_{\text{in}}[m,n]$ as it is presented in equation (9). To obtain the contrast hologram, the reference intensity must be calculated and subtracted from the hologram. The reference intensity is computed using $A_{\text{in}}[m,n] = A_{\text{illu}}[m,n]$.

Further information on the analytical development of the model for the simulation and its numerical implementation can be found in the paper "Diffraction-based modeling of high-numerical-aperture in-line lensless holograms" published by J. F. Restrepo and J. Garcia-Sucerquia.

4.2 Simulation

The numerical simulation is performed when the *Simulate* button is clicked, then the plugin reads the input parameters from the text fields on the simulation window and performs the calculations over the hologram. There must be at least one input image selected, amplitude or phase. If only the amplitude image is selected, the phase distribution is assumed to be zero at every point. Otherwise, if only the phase image is selected, the amplitude distribution is assumed to be one at every point. Finally, the complete sample field is computed using equation (9).

5 Settings

5.1 Reconstruction settings

The plugin offers a *Settings* window to customize the reconstruction operation, this window is accessible through the *Settings* button in the reconstruction window. As can be seen in Figure 5, this window has three tabs (Units, Reconstruction and Scaling).

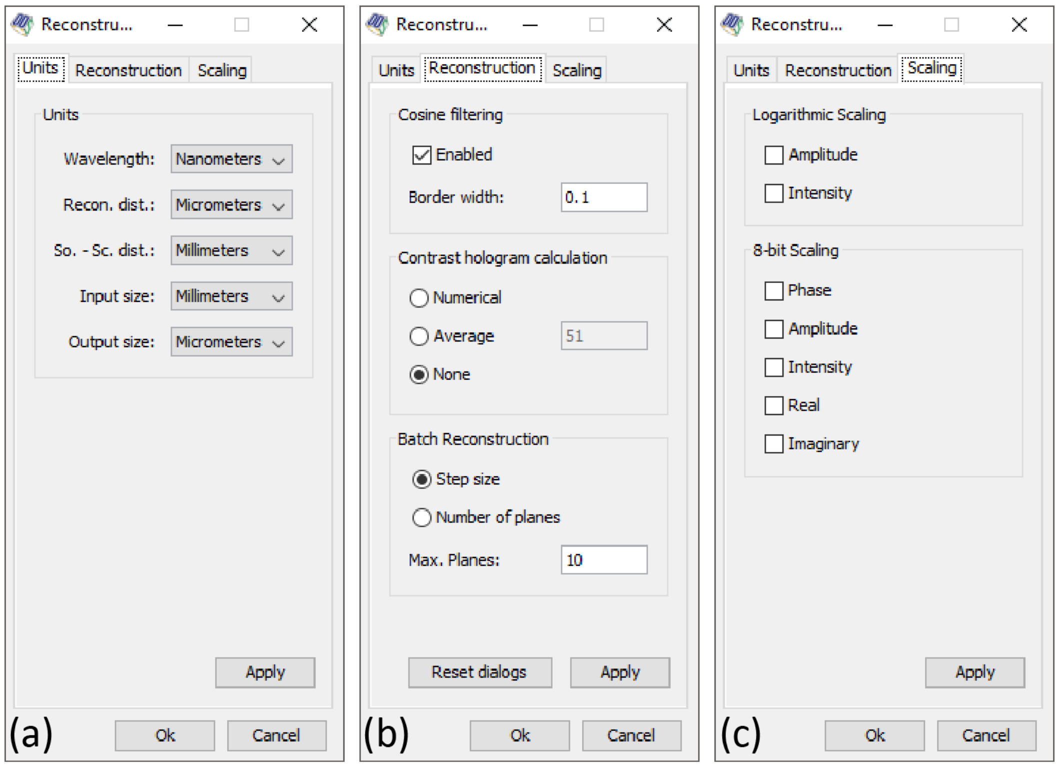

Figure 5. *Reconstruction settings window. (a)* ***Units*** *tab. (b)* ***Reconstruction*** *tab. (c)* ***Scaling*** *tab.*

5.1.1 Units

In the *Units* tab, the units of the parameters can be changed. The options offered are nanometers (nm), micrometers (µm), millimeters (mm), centimeters (cm) and meters (m).

5.1.2 Reconstruction

On the *Reconstruction* tab, a cosine filtering can be enabled or disabled via a checkbox. The other options correspond to the contrast hologram calculation and the batch reconstruction.

Cosine filtering

When enabled, the user can control the border width of the cosine filter applied to the contrast hologram. This means the user can adjust the percentage (between 0 and 0.5, since the hologram is symmetrically filtered with respect its center) of the total area in the contrast hologram that is affected by the cosine filtering.

Contrast hologram calculation

If a reference image is previously loaded, this section is ignored during the reconstruction process; in this case, the contrast hologram is the pixel-wise subtraction between the hologram and the reference image. When no reference image is loaded, there are three different options to build the contrast hologram:

1. When the *Numerical* option is selected, the result of the pixel-wise subtraction of the hologram and a modeled spherical wave, whose radius is the distance from the point source to the screen, is the contrast hologram.
2. When the *Average* option is selected, the pixel-wise subtraction between the hologram and average values calculated for different regions of the hologram compose the contrast hologram; these regions are squares whose side size is determined by the user in the text box related to this option. If the number inserted in the text box is equal to 1 or greater than the dimensions of the hologram, the average is calculated over the entire hologram.
3. When the *None* option is selected, the contrast hologram is the hologram loaded in the parameters with no modification.

Batch Reconstruction

In this panel, the parameters for the batch reconstruction can be selected. The user can implement the batch reconstruction tool by means of the step size or the number of planes, just by selecting the corresponding radio button.

The *Max. Planes* option configures the number of planes from which the plugin shows a warning message. This option is useful to avoid the reconstruction of an excessively quantity of planes by mistake, which can cause the freezing of ImageJ.

5.1.3 Scaling

In this tab, the options related to the scaling of the image representations can be found. It offers the option to display the images in logarithmic and 8-bit scaling (0 - 255).

5.2 Simulation settings

The plugin also offers a *Settings* window to customize the simulation operation, this window is accessible through the *Settings* button in the simulation window. As can be seen in Figure 6, unlike the reconstruction settings this window only has options for the units

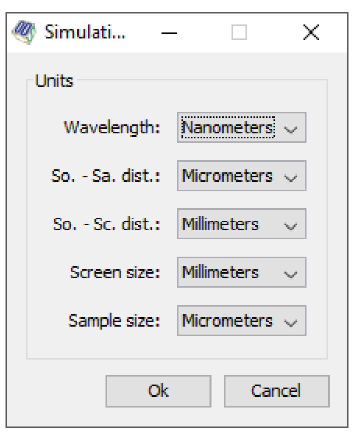

Figure 6. *Simulation settings window.*

5.2.1 Units

In the *Units* tab, the units of the parameters can be changed. The options offered are nanometers (nm), micrometers (µm), millimeters (mm), centimeters (cm) and meters (m).

6 Usage examples

6.1 Reconstruction of a DLHM hologram

A step-by-step guide to reconstruct a DLHM hologram is described below. In Figure 7, an experimental DLHM hologram, a reference image, its amplitude reconstruction and its intensity reconstruction are shown.

1. Open the digital hologram image file.
2. Select the opened image in the *Hologram* input combo box.
3. Open the digital reference image file (optional).
4. Select the opened image in the *Reference* input combo box (optional).
5. Select the mode of the contrast hologram calculation (optional) and enabled/disable the cosine filtering by pressing the *settings* button.
6. Introduce the parameters *Wavelength*, *Reconstruction distance*, *Source to Screen distance*, *Input width/height*, in the corresponding text fields. In the case that the manual radio button is selected, also introduce the *Output width/height*.
7. Select the representations of the reconstructed hologram.
8. Click on the *Reconstruct* button.

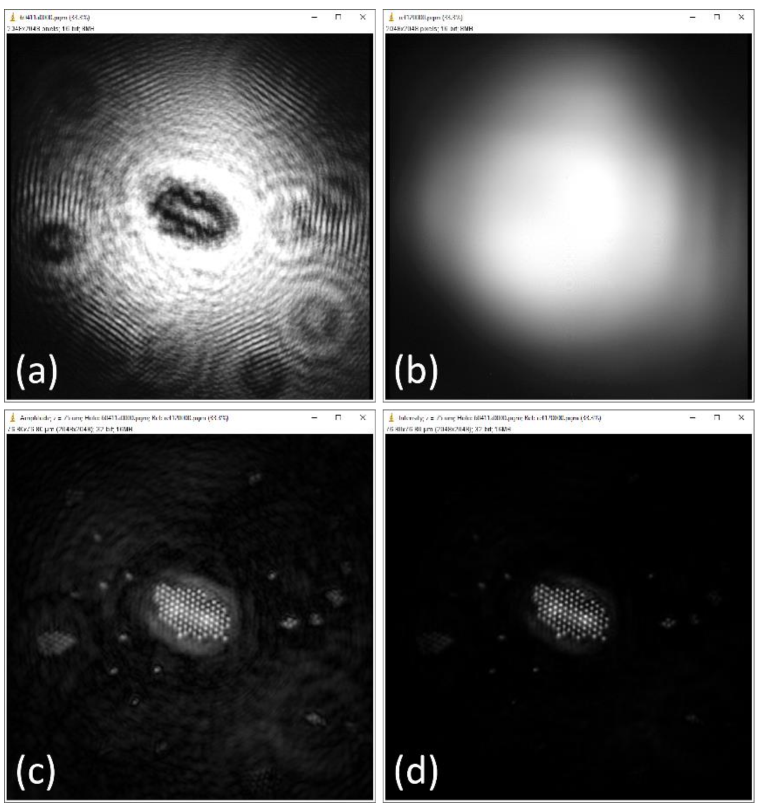

Figure 7. *Reconstruction of a DLHM hologram. (a) Experimental DLHM hologram. (b) Experimental DLHM reference. (c) Amplitude representation of the reconstructed field. (d) Intensity representation of the reconstructed field.*

6.2 Simulation of a DLHM hologram

A step-by-step guide to simulate a DLHM hologram is described below. In Figure 8, the amplitude and phase distributions for the sample are shown. In the same figure its simulated hologram and the contrast hologram are also shown.

1. Open the digital amplitude image file.
2. Select the opened image in the *Amplitude* input combo box.
3. Open the digital phase image file.
4. Select the opened image in the *Phase* input combo box.
5. Introduce the parameters *Wavelength*, *Source to sample distance*, *Source to Screen distance*, *Screen width/height*, in the corresponding text fields. In the case that the manual radio button is selected, also introduce the *Sample width/height*.
6. Select the representations of the simulated field.
7. Click on the *Simulate* button.

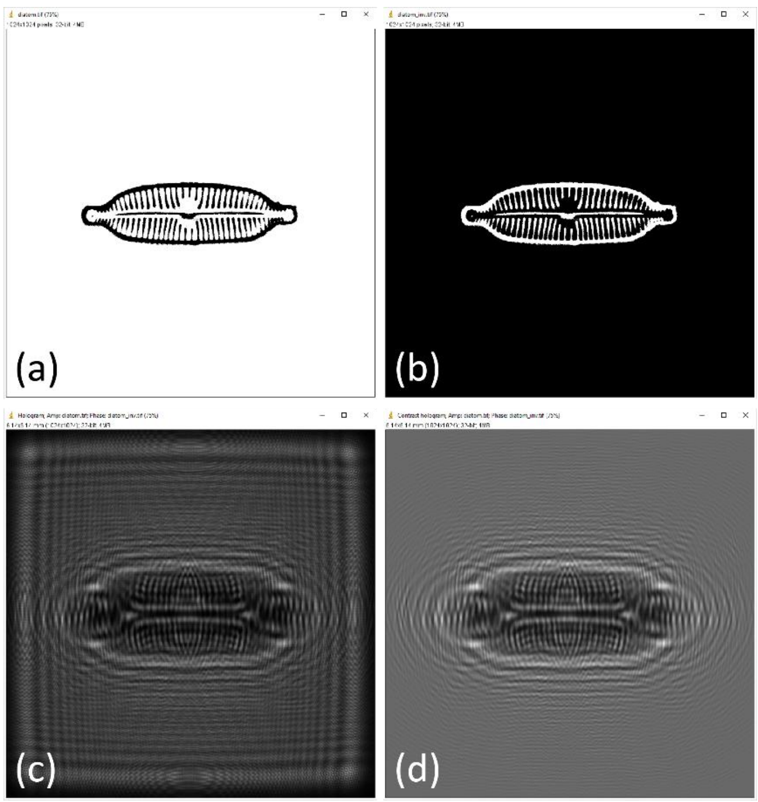

Figure 8. *Simulation of a DLHM hologram. (a) Amplitude distribution of the sample. (b) Phase distribution of the sample. (c) Simulated single hologram. (d) Simulated contrast hologram.*