

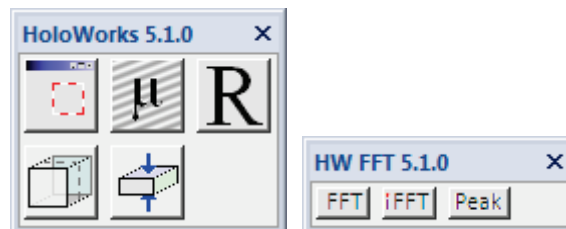
HoloWorks v.5.1

**A Fourier-Optics
Off-Axis Hologram &
General Data
Processing & Simulation
Software Package¹**

**A plug-in for Gatan's Software
DigitalMicrograph[®]**

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¹ HoloWorks v5 shall not be used for the purposes of light-optical semiconductor or reticle defect detection or inspection.

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1 Preamble

Off-axis holography is a technology that requires the ultimate in instrumentation. The hardware exceeds standard instrument requirements, the data acquisition unit needs to be excellent and the image processing requirements for live processing still surpass the fastest desktop computers. In 1993, when the development of HoloWorks started, many of today's features were impossible to create. Back then, to simply display a Fourier transform of a 1k by 1k image required several seconds and the time for reconstructing a hologram was just short of a minute. Today, data acquisition with modern CCD cameras has improved drastically and the computers have become faster. Yet, they still can limit the performance of off-axis holography as an on-line tool. Thus, holographic software will continue to be improved and additional features and new capabilities will be added.

Today, HoloWorks has grown beyond its initial purpose and is on its way to a general-purpose data processing utility. Because data processing applied and designed for images of type complex can easily be applied to images of type real or even rgb.

The key structure of HoloWorks allows seamless data processing and has as of version 5 strongly supported the concept of creating data- acquisition/processing workflows without writing a single line of code. This feature is very unique to HoloWorks and will continue to be strengthened with additional features in the near future.

The development of HoloWorks v5 steps forward significantly beyond its predecessors. Its development took over 2 years of intensive work, requiring a rewrite of practically every function and every detail. In version 5, not only has the GUI (graphical user interface) seen a major lift, but the underlying algorithms and code have changed even more. Many additional features have been embedded, making HoloWorks v5 the most attractive package for off-axis holography today. The result is a simple but powerful interface for the novice and a wide variety of possibilities for the expert for applying off-axis holography technology for his or her research purposes. Last but not least, the software has been developed with a strong potential for teaching Fourier optics with hands-on use in the classroom, as it provides most features found on a modern microscope starting from changing the focus, applying different numerical apertures or experimenting with dark field imaging.

New Seamless Integration

In previous versions, the simultaneous use of DigitalMicrograph[®] standard tools, like the linescan tool, with any function of HoloWorks was not possible. Now, for the first time, all HoloWorks functions have been designed as “threads.” This means, the HoloWorks tool suite and the Gatan tool suite work together seamlessly.

Menu Items Become Alive

A further important step has been added to the HoloWorks v5 software. When selecting a HoloWorks menu item while holding down the Shift key, many menu items become *live menu items* providing additional features. In most cases this means that the same operation continues to be applied to the image whenever one of two things happens:

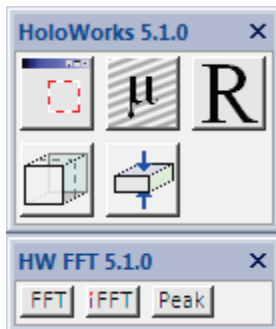
1. The image is being updated (or changed)
2. An existing selection in the image was moved

Build your own holographic script

As electron holography continues to require additional functionality, the HoloWorks v5 software supports the user in building their own functions using the reliable core concepts of HoloWorks. Instead of rewriting hundreds or thousands of lines of code, many complex activities can now be embedded with a single function call. For example, a fully interactive reconstruction process, including image process tracking, can now be called by 12 lines of code, representing over ten thousand lines of original code.

Additional Functionalities

The full list of additional features is too long to be listed here. However, features like a new sidebar element that contains the most-used features is available from the Windows/Floating Windows menu



of DigitalMicrograph (see left figure), aberration correction of live images, live fringe contrast and fringe movement measurements or thickness determination that includes a small data listing many of the mean inner potential values found in the book “Introduction to Electron Holography,” base for reference, are amongst the highlights of this software release. Note especially that all functions are modular and can be built on top of each other. Specifically, the new in HW5 function of collecting data into a 3D bin, both for 2D type images (both for real and complex images) and 1D type images usually displayed as line scan. Furthermore, complimented by the “average”

button allowing alignment of 2D images laterally, track the alignment parameters, and when averaging complex type images, includes the necessary phase adjustments.

Fourier Optics

The Fourier transform in HoloWorks handles images of arbitrary size. Because of the slightly different implementation of the Fourier algorithm for non-power-of-two and non-square images, the Fourier space looks as expected from the experimentally oriented user: it looks like the back focal plane in the microscope. There is no skewing because of non-square image sizes or selections. This allows observation of symmetry features without further ado. Also, the Fourier transform as implemented in HoloWorks always takes the full image into account - and not a square sub-area for rectangular- non-square images.

Scale Independence

Aberrations can be corrected for a large range from meters (m) to Pico meters (pm). The only conditions being: coherent imaging conditions and Fourier optics applicability.

1.1 Computer Requirements

HoloWorks v5 is developed for the Windows 7 – Windows 10 operating systems. Although the speed of most late computer models is acceptable, only computers with multi-core processors and >3GHz can be recommended. This is especially true for the live features of HoloWorks. A basic requirement is also the amount of RAM. 8GB of RAM should be the minimum configuration when working with basic images up to 4k by 4k. When working with data stacks of any size, 16GB of RAM or more is highly recommended.

In the case of DigitalMicrograph[®] coming back with the message "not enough memory", it should be considered that large numbers of displayed image (especially 2k by 2k and above images) take up significant amounts of memory very quickly. The actual amount used can be checked under the "Window" –menu of DigitalMicrograph[®]. Windows 7 or 10, 64bit is highly recommended to avoid such situations.

For HoloWorks v5 DigitalMicrograph[®] (GMS 2.3.0 or higher) from Gatan is strongly recommended. Backwards compatibility has been tested down to GMS 1.6 and no problems are known at this point. Contact your nearest Gatan office or check on <http://www.Gatan.com/> for latest information. Also look for updates under <http://www.holowerk.com>. HoloWorks v5 is available for the Windows platform only.

Because many of the HoloWorks menus will result in the displays of several windows, a screen size of 1200 by 1600 pixels or larger is recommended. New laptops do support external 4k monitors and they are an excellent choice.

The minimum requirement is a Windows 7(64bit) computer with >2.6GHz and quad-core or higher processing capabilities. Windows XP computers are no longer recommended.

A minimum of 8GB RAM for Windows 7-10 (64bit) is highly recommended.

For workflows, display monitor should exceed 1200 by 1600 pixel resolution whenever possible.

1.2 Data Reproducibility

One of the most important steps often overlooked in image processing is the reproducibility of data. Currently, several Journals are reconsidering their requirements for paper submission and are planning to not only require the submission of the original data, but also the detailed steps of data processing involved so the results can be reproduced.

Reproducibility of data is a corner stone in science and certainly should be an active part of image processing, as has been intrinsic to HoloWorks from the beginning: As hologram processing encompasses a significant amount of free parameters, care has been taken to make images “reproducible.” HoloWorks v5, as its predecessors, saves the essential image processing data and parameters with each image it creates. Image processing details are stored with the image under:

Image Display Info / Image / Tags / History.

Right-click on the image for an image-processing-information review. This opens the “Image Display Info” window for that image.² Selecting Tags under the Image listing (marked blue in Figure 1), allows to open the “History” menu on the right-hand side of the Image Display Info window. As in the example of Figure 1, the name of the source image (hologram) was “Nabraj_Au-Pd_oct8_0031” with all necessary details to reproduce the data, like the radius of the reconstruction aperture of 113 pixels. Image processing information is also displayed in DigitalMicrograph’s[®] “Output” window, see Figure 2, where the image processing parameters are continuously updated as the image processing proceeds.

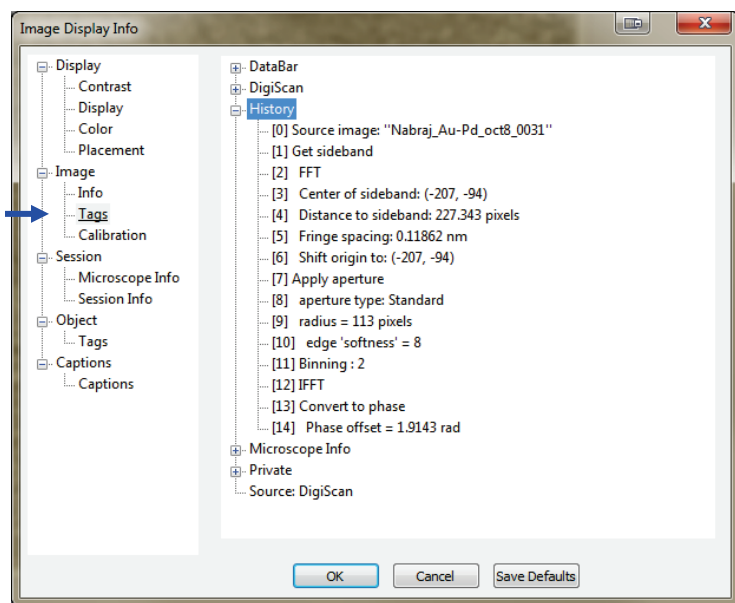


Figure 1 The essential image processing parameters are stored with each image. This enables a review of the executed image processing steps and allows reproducibility of data even after significant amounts of image processing as required for off-axis holography.

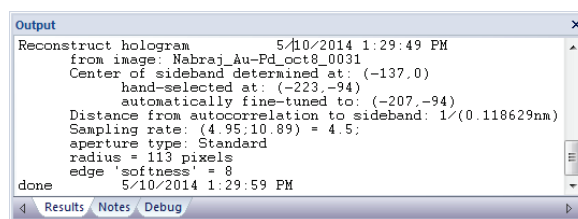


Figure 2 Essential reconstruction parameters as e.g., the size and order of the applied filters are listed in the DigitalMicrograph’s[®] Results window together with a time stamp.

² This window can also be opened through DigitalMicrograph’s[®] “Object” menu, selecting the Image Display ... sub-menu.

2 A Short History of ‘Off-axis’ Type Holography

Any introduction to the technique of holography should start with Denis Gabor and a reference to his paper in *Nature* in 1948.³ There, Gabor describes the idea he had around Easter 1947, on how to correct the spherical aberration of an electron-optical objective lens – on less than 2 pages. Many details about the historical background can be found directly on the Internet,⁴ but it should be mentioned that Gabor’s idea initially focused on in-line holography.⁵



Figure 3 The first biprism assembly for electron off-axis holography around 1952 (from: *Introduction to Electron Holography*, Plenum Publishers, 1999).

The theory and technique being used in today’s laboratories however is mainly off-axis holography and is built on the invention of the biprism by Möllenstedt in 1950 and the works of Emmett Leith and Juris Upatnieks in 1962.^{6,7} The origin of the theory of off-axis holography should probably be dated significantly earlier than 1962, as it was classified back then for its use in military applications. It is also worthwhile to notice that the theory of off-axis holography is based actually on communication theory and encompasses a much broader area than optics. The first off-axis holography assembly⁷ designed for electrons is shown in Figure 3, while two key sketches borrowed from Leith’s original paper⁶ are shown in Figure 4.

Because most everything in holography is about the access to the image phase, another name should be mentioned here as well: Frits Zernike in 1930 discovered the Zernike phase contrast for light optics and received a Noble Prize in 1953 for his invention.⁸ Interestingly, Leith’s and Upatnieks’ paper refers to light optics in 1962, whereas Gabor’s original paper, 1949, refers directly to electron optics and is followed by Möllenstedt’s first electron optical off-axis holography type images around 1952.

Obviously, there is a strong interaction between light optics and electron optics which can be surprising when considering the nature of the particles involved: electrons are Fermions and light is represented by Bosons. This fundamental difference between the particles (or waves) can be ignored

³ D. Gabor, *Nature*, *A New Microscopic Principle*, No. 4098 May 15, 1948

⁴ <http://www.holophile.com/history.htm>, or: http://nobelprize.org/nobel_prizes/physics/laureates/1971/gabor-lecture.pdf

⁵ D. Gabor, *The Principle of Electron Microscopy by Reconstructed Wavefronts*, Proc. Royal Society, A, 197, 454, 1949

⁶ Emmett N Leith and Juris Upatnieks, *Reconstructed Wavefronts and Communication Theory*, Journal of the Optical Society of America Vol 52, Number 10, October 1962.

⁷ *Introduction to Electron Holography*, Eds., E. Voelkl, L.F. Allard and D.C. Joy, 1999 Kluwer Academic / Plenum Publishers, New York, Möllenstedt biprism, see Chapter 1

⁸ http://nobelprize.org/nobel_prizes/physics/laureates/1953/zernike-bio.html

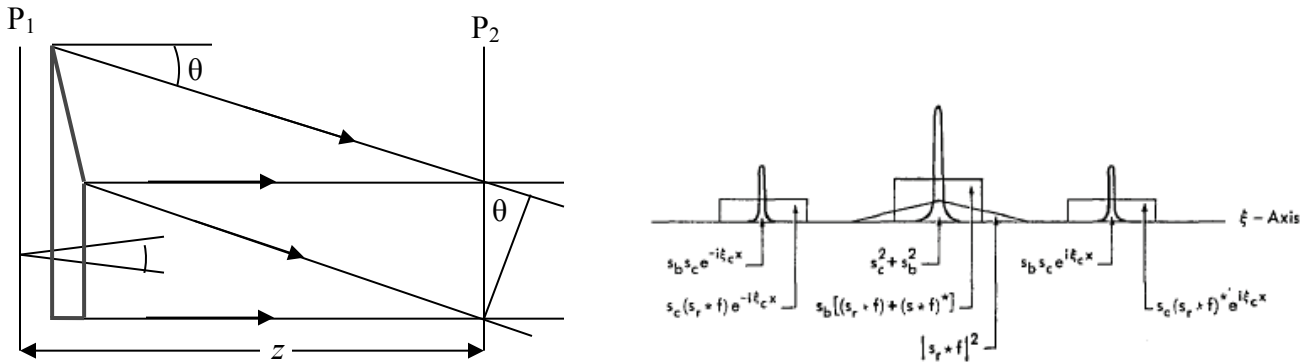


Figure 4 Left: basic ray path for off-axis holography. The beam traveling from the left is split by an optical wedge. The upper part of the wedge deflects part of the beam such that two beams overlap in the plane P_2 with the angle θ . The plane P_1 contains an object point indicated by two emerging diffracted beams. The actual hologram is recorded in plane P_2 . Right: Fourier transform of an off-axis hologram containing the 2 sidebands and autocorrelation. Bandwidth of the image wave is limited as indicated by rectangles. The center also shows higher orders of the object caused by recording of the intensity of the object; these higher orders do not show in the sidebands.⁶

and the same equations (with different constants) can be used: but only under the explicit condition that there is only one Fermion (electron) in the set-up at a time (which means there is no interaction between electrons involved). Otherwise the interaction between the Fermions must be included in the Schrödinger equation which generally results in the destruction of the typical off-axis interference fringes.

Today, the reason for the invention of holography, to compensate the spherical aberration of an electron lens, has passed away as the spherical aberration together with over 20 additional aberrations are corrected with compensating (non-round) lenses.

The main reason why holography is still a viable technique today was already laid out by Frits Zernike in 1930 with his discovery of phase contrast for which he received the Nobel prize in 1953.⁹ Today we are able to not only obtain phase contrast in an ideal way via holography, but the data are also highly quantitative due to CCD cameras and digital data processing.

Off-axis electron holography is not an easy technique. It continues to demand extraordinary instrumental specifications and great care on the instrumental as well as on the data acquisition and processing side. The software HoloWorks 5 is based on Fourier optics and designed aid in the acquisition of data sets, to optimize data processing and provide the means to simulate an optical bench according to Fourier optics. Therefore, HoloWorks 5 was designed and can be used for hands-on experience for not only teaching Fourier optics but also for teaching the pitfalls of discrete Fourier optics.

HoloWorks 5 also encompasses new developments on the data processing side. Complex Fourier-optical and/or data processing elements can be built and applied live as most operations are available

⁹ http://nobelprize.org/nobel_prizes/physics/laureates/1953/zernike-bio.html

as independent threads: the output of one thread can be used as the input for another thread. Therefore complex imaging systems and/or complex data processing schemes can be generated and tested.

HoloWorks 5 is also consistent with the hologram processing routines as laid out in the book “Introduction to Electron Holography”.¹⁰ This book is highly recommended as a reference guide. It provides an excellent overview over the developments in the area of electron holography as well as a detailed introduction to the field. Specifically in the area of electron holography, many new developments have occurred over the last years. To name only two:

1. Avoiding Fresnel fringes in electron off-axis holography by using 2 independent biprisms. This invention leads to the removal of potential complications in the reconstruction process.¹¹ Although not mentioned in that paper,¹¹ it should be pointed out that this method also helps suppressing vignetting effects around the Möllenstedt biprism.
2. Hardware stabilization of electron optical elements. This stabilization is mainly found in the latest generation of electron microscopes that have been designed to house Cs correctors. As a side-effect of the tightened requirements for these instruments, the phase of the illumination wave front has increased stability by over an order of magnitude.¹² This allows useful acquisition of large sets of holograms and their successful averaging.^{13, 14, 15}

HoloWorks (version 4 and 5) already provide the features necessary to take full advantage of these improvements and enable extremely high phase resolution. As a consequence, many new applications can be expected in the area of electron holography.

At this point it is assumed that the reported increased wave front stability is caused by the increased stability requirements for electron microscopes that house or can house a Cs corrector. Routine phase resolution in excess of $2\pi/1000$ can be expected at this point for electron holography and exciting new developments are expected therefore in the applications fields.¹⁶

Last but not least a potentially significant difference between light optical and electron optical holography should be pointed out. In light optics, all optical elements have a surface from which light is being reflected and unwanted reflected beams will contribute to the hologram, sometimes in form of ring structures. Also, each light-optical element will contribute to diffuse scattering processes. Therefore, the quality of light-optical holograms is often deteriorated by the lens quality (and specifically its coating), whereas electron optical lenses operate in vacuum and provide no surfaces from which back-reflected electrons could be generated and affect hologram quality.

¹⁰ *Introduction to Electron Holography*, Eds., E. Voelkl, L.F. Allard and D.C. Joy, 1999 Kluwer Academic / Plenum Publishers, New York.

¹¹ K Harada et al., *Optical System for Double-biprism electron holography*, Journal of Japanese Society of Microscopy, 54(1): 19-27 (2005)

¹² D Cooper, et al., *Medium resolution off-axis electron holography with mV sensitivity*, Applied Physics Letter

¹³ E. Voelkl, L.F. Allard and B.G. Frost, "Electron Holography: Recent Developments and Applications," Pfefferkorn Conference, 1996, Silverbay

¹⁴ E. Voelkl, L.F. Allard and B.G. Frost, "Electron Holography: Recent Developments," Scanning Microscopy, Vol 11 (1997) p407-416

¹⁵ E. Voelkl, Routine Phase Resolution of $2\pi/100$ and Better in Off-Axis (Electron) Holography, Proceedings of Microscopy and Microanalysis 2008, Vol. 14, Supplement 2, p840-841.

¹⁶ E. Voelkl and D. Tang, Approaching routine $2\pi/1000$ phase resolution for off-axis type holography, Ultramicroscopy 110 (2010) 447-459

Therefore, the ultimate quality obtainable by either electron off-axis holography or light-optical holography will be determined by rather different parameters and work still needs to be done to determine the ultimate resolution capabilities for off-axis holography in either field.

A short overview over the key technical developments of holography is given in the following list.

- 1930** Invention of Phase contrast, F Zernike; Nobel Award 1953
- 1948** Invention of (in-line) holography, D Gabor; Nobel Award 1971
- 1962** Key paper on theory of off-axis type hologram reconstruction, E Leith and J Upatnieks
- 1986** Atomic resolution holograms, H Lichte
- 1991** Successful aberration correction (reason for invention of holography), H. Lichte
- 1991** Digital hologram acquisition and processing, W Rau (see also T Hirayama, 1994)¹⁷
- 2005** Dual biprism for avoiding Fresnel fringes in electron holography, K Harada
- 2006** Report on wave front stability allowing long acquisition times and high phase resolution, D Cooper
- 2011** Phase resolution in excess of $2\pi/1000$ by hologram averaging, E Voelkl
- 2011** Correct computation of phase noise including the reconstruction aperture, E Voelkl¹⁸

¹⁷ T. Hirayama, J. Chen, T. Tanji and A. Tonomura, Dynamic observation of magnetic domains by on-line real-time electron holography: Ultramicroscopy 54, 9 (1994).

¹⁸ E. Voelkl, Noise in off-axis type holograms including reconstruction and CCD camera parameters, Ultramicroscopy 110 (2010) 199-210.

3 Basic Theory: Reconstruction and Processing Of Holograms and Hologram Sets

3.1 Single Holograms: Reconstruction

The hologram is the result of a coherent superposition of two waves: The object wave and the reference wave. It must be a coherent superposition, otherwise no hologram is obtained and no interference fringes are visible. In reality, the object and reference wave are hardly ever perfectly coherent for a variety of reasons and thus the contrast of the interference fringes, measured as $(I_{max} - I_{min}) / (I_{max} + I_{min})$ is less than 1 or less than 100%.¹⁹ In the case of electron holography, this means that a field emission electron microscope is a necessity. In light optics, the choice of a laser seems ideal, however the long coherence length of the laser can be a rather significant disadvantage.²⁰ Examples of electron- and light- holograms are shown in Figure 5.

In general, the basic contributions to the hologram can be written as:

$$(1) \quad I(\mathbf{r}) = I_{\text{tar}}(\mathbf{r}) + I_{\text{ref}}(\mathbf{r}) + I_{\text{inel}}(\mathbf{r}) + 2\mu a(\mathbf{r}) b(\mathbf{r}) \cos[2\pi \Delta\mathbf{k} \cdot \mathbf{r} + \Delta\varphi(\mathbf{r}) + \varphi_0(\mathbf{r})];$$

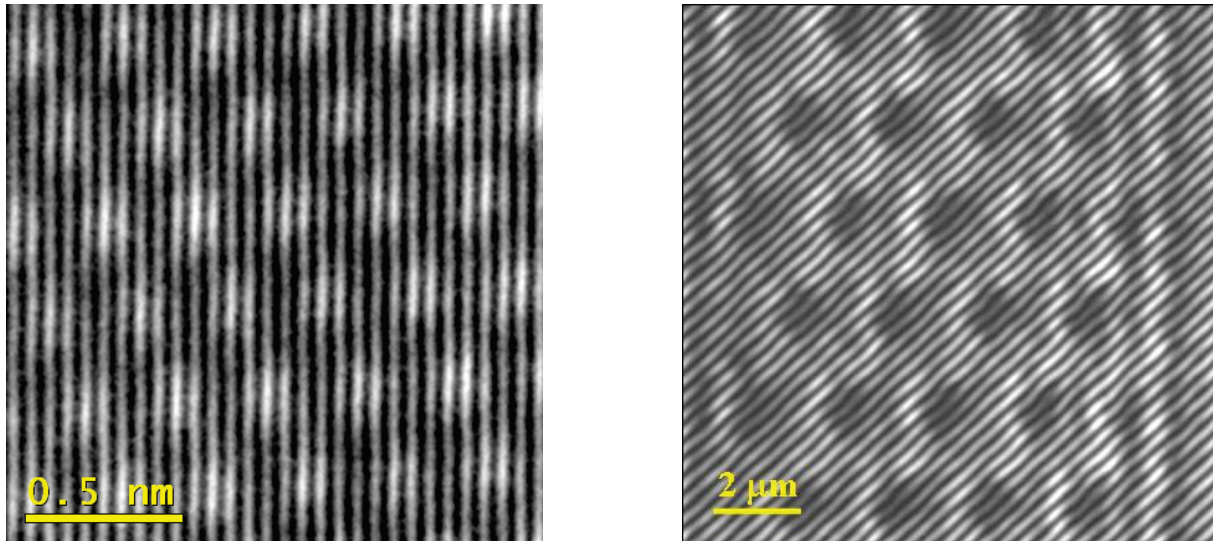


Figure 5 Left: electron hologram of a selected area of a rather thin CeO_2 particle in a 110 direction. Right: optical light hologram of a periodic structure in reflection mode. The fringes in these examples show only a slight bending; the bending however contains the phase information lost in conventional imaging.

¹⁹ The contrast is measured in transmission mode versus reflection mode: in transmission mode, the object is removed to avoid potential impact by the object on the measurement, whereas in reflection mode a “perfectly” flat sample with homogeneous reflectivity is used.

²⁰ N. Warnasooriya and M.K. Kim, *LED-based multi-wavelength phase imaging interference microscopy*, Department of Physics, University of South Florida, Tampa, FL 33620, 23 July 2007 / Vol. 15, No. 15 / OPTICS EXPRESS 9247

where $I(\mathbf{r})$ is the intensity distribution in the image plane as recorded e.g., from the CCD camera. $I_{\text{tar}}(\mathbf{r})$ is the intensity of the target wave $\psi_{\text{tar}}(\mathbf{r}) = a_{\text{tar}}(\mathbf{r}) \exp(i \varphi_{\text{tar}}(\mathbf{r}))$, the so-called target- or object-wave. $I_{\text{ref}}(\mathbf{r})$ is the intensity of the reference wave $\psi_{\text{ref}}(\mathbf{r}) = a_{\text{ref}}(\mathbf{r}) \exp(i \varphi_{\text{ref}}(\mathbf{r}))$, the so-called reference wave. $\Delta\varphi(\mathbf{r})$ represents the phase-difference between the image wave and the reference wave.²¹ $\varphi_0(\mathbf{r})$ represents a phase term that describes the position of the fringe pattern with respect to the CCD camera (also known as lateral phase) where the dependence of \mathbf{r} can e.g., describe distortions from the CCD camera (i.e., the fiber optic bundles for electron microscopy type cameras). I_{inel} represents incoherent contributions, e.g., inelastically scattered electrons or stray light from a different source to the hologram and μ is the contrast of the interference fringes, preferably measured without an object for transmission type holography and with a perfect mirror for reflective holography.

If it can be assumed that a modulation of the phase of the wave front illuminating the object contributes only an additive term to the phase of the wave front leaving the object, then the above equation truly allows separation of the instrumental phase modulation from the modulations by the object. It also means that by taking another hologram after removing the object (the reference hologram), the instrumental phase modulations can be removed successfully and only the response of the object to a plane wave remains. It may sound trivial, but it should be remembered that an illumination with a truly flat wave front is unusual. This furthermore implies that when interpreting the data after using a reference hologram the following is implicitly assumed: the modulation of the illumination by the object is identical for both an ideally flat illumination and the given, real illumination. **Only after this warning can the use of a reference hologram be recommended** and instrumental phase variations can be eliminated via a reference hologram.

Under these assumption, equation (1) can be written in a simpler form by assuming the reference wave to be a plane wave as:

$$(2) \quad I(\mathbf{r}) = I_{\text{tar}}(\mathbf{r}) + 1 + I_{\text{inel}}(\mathbf{r}) + 2\mu a(\mathbf{r}) \cos[2\pi \Delta\mathbf{k} \cdot \mathbf{r} + \varphi(\mathbf{r}) + \varphi_0(\mathbf{r})];$$

by assuming a plane wave of amplitude 1 for the reference wave.

Unfortunately, the instrumental phase variations $\varphi_0(\mathbf{r})$ are often time dependent. In their simplest form, they manifest themselves as a time dependent drift of the overall fringe position with respect to the CCD camera, i.e. a lateral drift of the fringes. This of course limits the maximum acceptable exposure time and therefore the achievable phase resolution (limited by shot noise). It appears that, specifically for the latest generation of electron microscopes that supports spherical aberration correction, there are significant improvements (order of magnitude or better) for the overall instrumental stabilities. The consequences are discussed in the next chapter.

In most cases, it is the image-phase $\varphi(\mathbf{r})$ which contains the most interesting information and since this information is hidden in the cos- term of the equation (2), i.e., in the deformation of the interference fringes of the hologram, the phase information has to be retrieved from the hologram to make it visible. This process is generally referred to as “reconstruction” for any type of off-axis holograms.

²¹ As a reminder, the actual phase of a wave remains inaccessible and is destroyed by the recording process. However, with the assumption that the reference wave is a plane wave, $b(\mathbf{r})$ conventionally represents the phase of the image wave.

The basic reconstruction routine consists of several steps:

1. The first step is to perform a Fourier transform of the hologram (2 examples in Figure 6). The central area is known as the autocorrelation, which corresponds directly to the Fourier transform of a conventional (non-holographic) image. The sidebands are arranged like “ears” around the autocorrelation. A closer look reveals that there is no centro-symmetry around the center of the sideband: Opposite beams (with respect to the center of the sideband) display different intensities, whereas the autocorrelation is centro-symmetric.²²

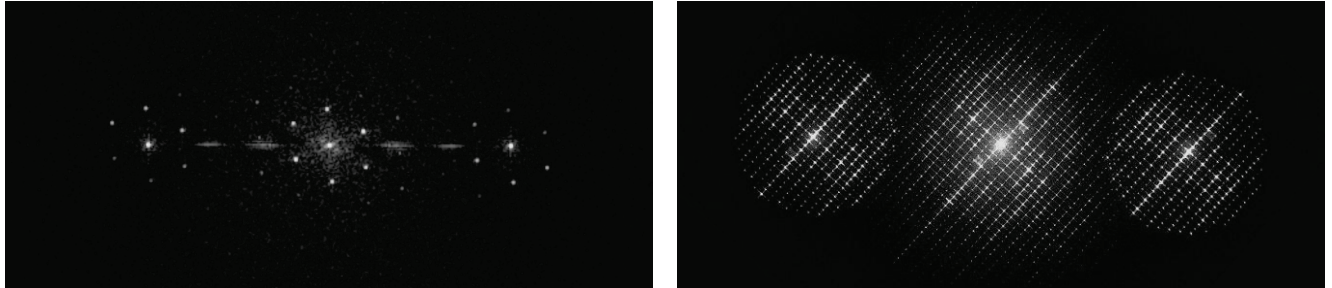


Figure 6 Left: sub-area of the Fourier transform of the electron hologram displayed partially in Figure 5, left. Sidebands are well separated from the autocorrelation in the center. Right: sub-area of the Fourier transform of the light-optical hologram displayed partially in Figure 5, right. The outline of the numerical aperture is clearly visible; the sidebands slightly overlap with the autocorrelation.

2. The second step is to center and then isolate one of the two sidebands by applying a filter thus isolating one of the sidebands from all other data. Since both sidebands carry the identical information (one is the conjugate complex of the other), there is usually no need to distinguish between them.

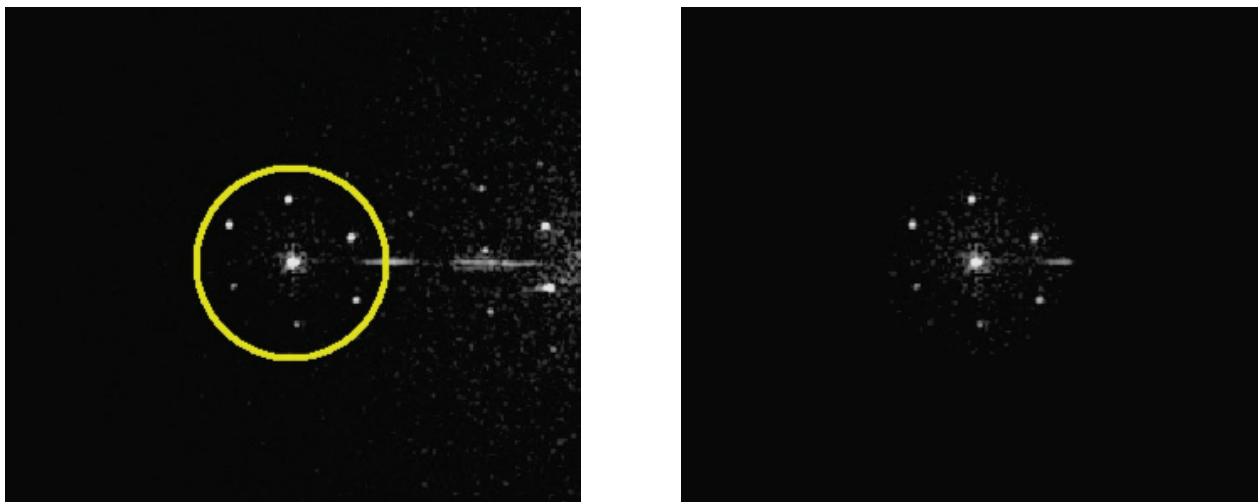


Figure 7 The sideband (taken from Figure 6, left), outlined in yellow is copied into a new image and a filter rejects information not part of the sideband (see details in Chapter 5.1.3).

²² It can be shown mathematically that the Fourier transform of a non-complex image show centro-symmetry.

3. As a third step, the new image is then inverse Fourier transformed to yield the complex image wave: $a(\mathbf{r}) \exp(i(\varphi(\mathbf{r}) + \varphi_0))$. From the complex image, the images amplitude $a(\mathbf{r})$ and phase $\varphi(\mathbf{r})$ can be extracted (see Figure 8) easily.

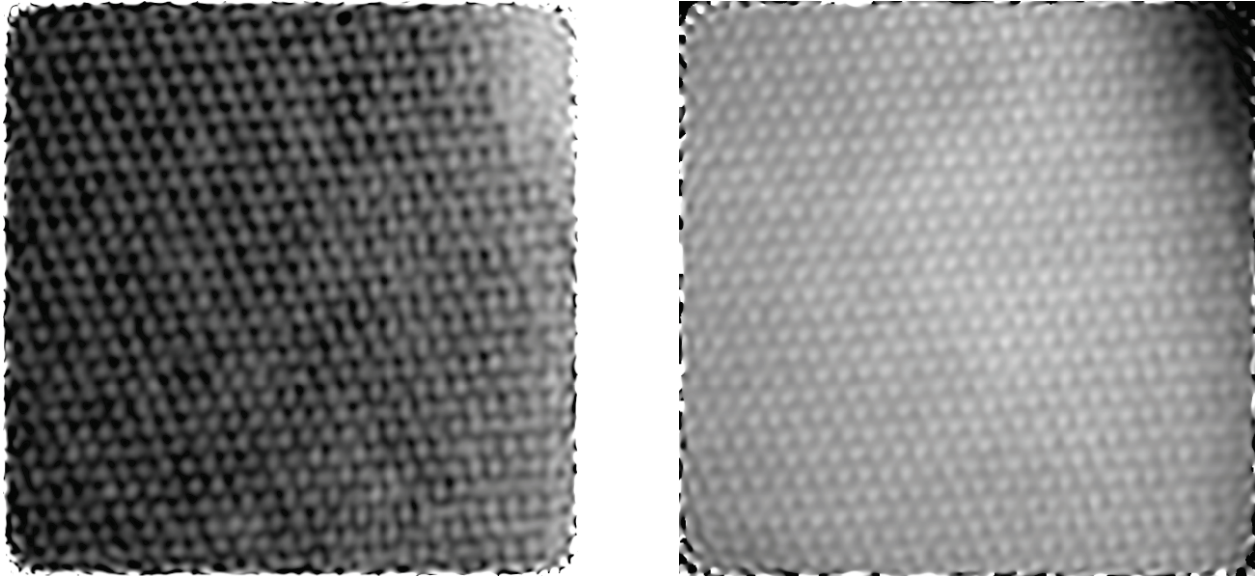


Figure 8 The amplitude and phase of the complex image as obtained after an inverse Fourier transform of the filtered sideband of Figure 7. Note the “frame” around both images. It is an indication that an Apodization window has been used during the reconstruction process (for details on Apodization windows, see Chapter 5.15).

The very last point of this chapter is the development of data averaging which is especially important for electron holography, as there are and will be no electron lasers (they are Fermions) and thus the coherence of electron sources will always be significantly inferior to light sources. The first works appear to have been reported at the Pfefferkorn conference at Silverbay in 1995,¹³ where 7 holograms of latex spheres were recorded, reconstructed and their wave fronts averaged. However at that time, the process was rather slow and the illuminating wave front was found to not be sufficiently stable for averaging purposes of larger numbers.¹⁴ Fortunately, electron microscopes and specifically those who have been designed to house a C_s corrector have improved their stability and several reports have been published about a wave front stability exceeding several minutes with a phase gradient stability of significantly less than 1mrad per pixel.^{12, 15}

This concludes the rather brief introduction but should help in understanding the basic terms and expressions throughout this software and the very basics of the reconstruction process. In the following it will be demonstrated how HoloWorks not only helps to reconstruct single holograms or hologram pairs (a hologram and reference hologram combination) but also helps in the acquisition of sets of holograms that can consist of >100 holograms per set. These data sets have to be reconstructed and processed as well but require large amounts of RAM and fast processors.

3.2 Hologram Sets: Acquisition, Reconstruction and Averaging

A good signal/noise ratio is always welcome in the reconstructed image phase. There are several basic limitations to consider:

- i. dynamic range of the CCD camera
- ii. illumination intensity (shot noise)
- iii. fringe contrast (in terms of lateral coherence of the beam)
- iv. instrumental stability over time (e.g., wave front stability or electron-biprism stability)
- v. sample stability over time (e.g., sample drift)

Items i. and ii. define together the maximum exposure time T_{\max} for the hologram before saturation of the CCD chip occurs. Items iv. and v. play a significant role as they can be sufficiently strong to reduce T_{\max} to a much shorter exposure time T_{acc} e.g., because of fringe contrast reduction.²³

Therefore, acquiring large sets of holograms with short exposure times typically provides better results because the limitation by the dynamic range of the CCD camera is effectively expanded and the effects of sample drift and fringe drift can often be compensated via computer from large data sets with short exposure time – but not from single long exposures.

Key literature:

Approaching routine $2\pi/1000$ phase resolution for off-axis type holography, E. Voelkl and D. Tang, *Ultramicroscopy* 110 (2010) 447–459

Noise in off-axis type holograms including reconstruction and CCD camera parameters, E. Voelkl, *Ultramicroscopy* 110 (2010) 199–210

²³ Specifically for electron holography, items iv. and v. play a significant role as T_{\max} easily exceeds 30s on most instruments.

4 Getting Started: Quick Manual

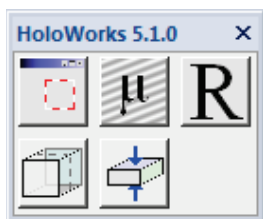


Figure 9 The dockable UI of HW5.

The HoloWorks 5.0.x user interface (UI) can be found under the Digital Micrograph's menu: Window/Floating Windows/HoloWorks 5.0. The UI consists of 5 main buttons: 3 in the upper row and 2 in the lower row. The buttons in the upper row work for both 2D and 3D data sets and provide additional features when activated with the Shift key held down. The buttons in the lower row are reserved for 3D data stacks containing 2 or more slices. For details on 3D data stacks and slices, please refer to the DigitalMicrograph manual.



The “Sub-image” button cuts a selected area from a given image, or Line plot, and creates a new image from the selected area. This feature is similar to earlier HoloWorks versions. For a 3D data set however, the number of slices to be copied can be selected in a similar manner as in a standard printing dialog.



The “Fringe contrast” button computes the fringe contrast – preferably – from a selected area (size: power-of-two). Using the Shift key on the keyboard together with this button starts a line plot type display and tracks 3 parameters:

1. fringe contrast
2. fringe drift
3. Q-factor

This is a handy tool for optimizing e.g., a live hologram. For 3D data sets, the full stack is evaluated and the parameters are plotted for all slices in the stack.



The “Reconstruct” button reconstructs the selected image or the selected area in that image. It creates an additional window where a selection between automatic or manual parameter adjustments is offered. The button works for 2D and 3D data sets. Activating with the Shift key held down provides a live reconstruction environment where the reconstructed images are updated as soon as a new image appears in the selected image.



The “Acquire 3D data set” button allows setting the number of slices of the generated 3D data set. The 3D data set is then filled with the contents of the selected image (continuous acquisition mode) such that whenever a new image appears in the selected image, its content is copied into the 3D stack. The last image copied is the top image in the stack.



The “Compress 3D data set” button compresses 3D data stacks. Both real/integer data and complex data are supported, but the resulting user interface offers different options depending on the data type found.

The function to unwrap a phase image is found in “HW5.0/Unwrap phase”

The function to remove a phase wedge is found in “HW5.0/Phase games/Phase wedge...”



Figure 10 The FFT items including peak function, as of HW v5.0.2

As of HW v5.0.2, an additional floating window is available. It allows easy access to frequently used functions like FFT, iFFT (inverse FFT) and the peak function.

As always, keeping the “Shift” key (on the keyboard) down when pressing any of these buttons will start a continuous thread.²⁴ These threads can be combined to create a workflow. Such a workflow can represent a rather complex data processing workflow – “without writing a single line of code.”

The FFT of HoloWorks works as expected, but it can also be applied to sections of images that are rectangular, non-square and not of the power of two.

Different from Gatan’s FFT, the scaling in Fourier space remains symmetric while allowing for rectangular source images; meaning diffraction patterns continue to appear correctly and not skewed. In more recent versions, Gatan’s FFT performs an FFT of the largest square area of an arbitrary-sized image. In such a case, areas of the image outside of the square area do not contribute to the FFT. In HoloWorks, the full image area contributes to the FFT while maintaining scaling in the Fourier space to be equal in *x*- and *y*- directions.

This is explained in detail on page 61 ff.

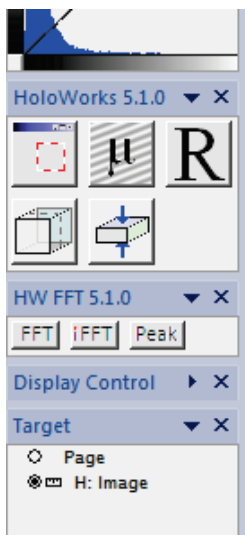


Figure 11 The two floating windows of HoloWorks, integrated with DM’s floating windows. The floating windows can be added or removed via the DM’s menu item: Window/Floating Windows/...

²⁴ Note: all threads can be “killed” using the key combination CTRL/BREAK.

4.1 General Description of the HoloWorks 5 Menu Items

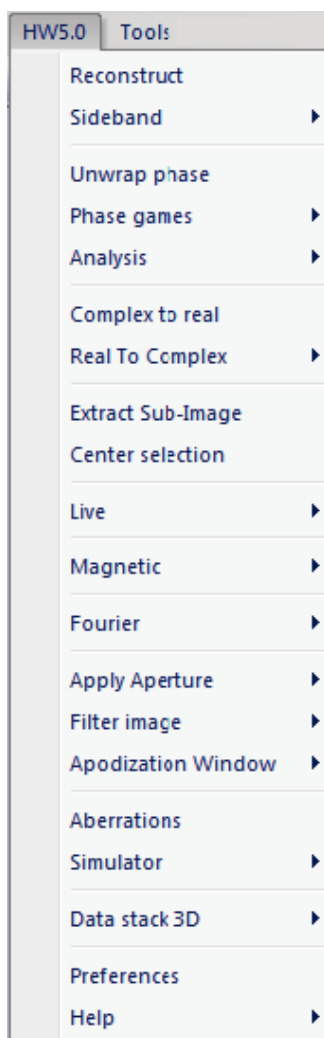


Figure 12 Main menu of HoloWorks v5.

The menu items of HoloWorks v5 appear under the menu “HW5.0” of DigitalMicrograph[®]. For most items, there are two modes of activation:

1. Selection of the sub-menu item via mouse
2. Selection of the sub-menu item via mouse **while “Shift” key is held down**

While all items in any activation mode are thread based, the activation using the Shift key specifically requires a user to terminate the activity.

The menu “Extract Sub-Image” (left) is a typical example. Selecting this menu item (as shown in Figure 12) with an arbitrary selection in an image will return a new image of the size of the selection. Once the new image appears, the function is finished and no further action occurs.

Selecting the sub-menu “Extract Sub-Image” while holding down the “Shift” key, causes the selection (ROI) to turn a solid red, the general indication for an active thread. The new image appears as before however, the function remains active in this case, as indicated by the red selection (or ROI). As long as the function “Extract Sub-Image” remains active, changes of the selection, e.g., moving the selection (or changing its size²⁵) causes changes the in sub-image accordingly. It also means that if the content of the original image is modified (e.g., image update by the CCD camera), the sub-image is updated as well.

Threads, as indicated by red ROIs, must be terminated by one of the following:

- **delete the image containing the red ROI**
- **delete the red ROI**
- **delete the user interface (UI) window if present**

Once the threads are terminated, the red selection returns to its original states (with the red and white outline, i.e., becomes volatile (see Gatan manual)), thus providing a visual feedback to the user that the thread has been terminated correctly.

²⁵ A change of size of the ROI may not be available for some functions.

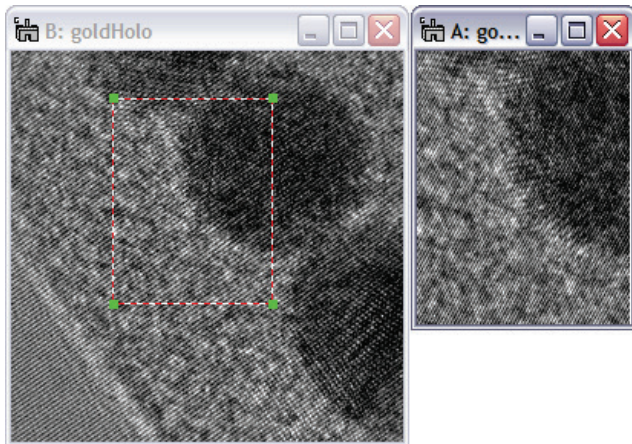


Figure 13 Menu item “Extract Sub-Image” creates a new image from the selected region, then quits. The created image is referred to as sub-image and is of the same data type as the original image.

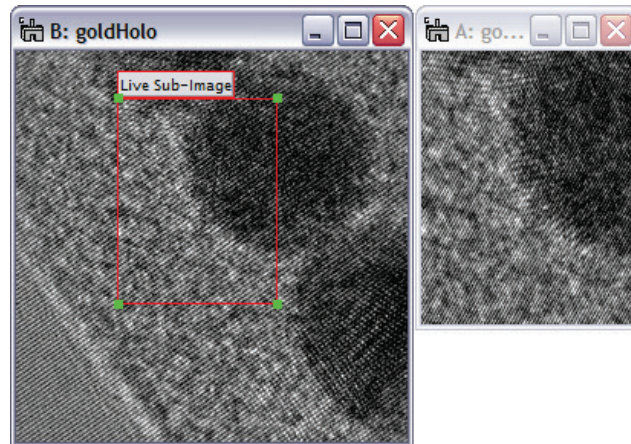


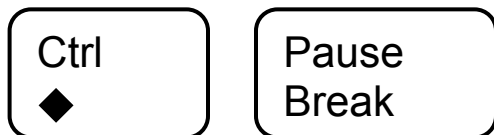
Figure 14 Using the “Shift” key when selecting the menu item “Extract Sub-Image” creates a live connection between the ROI and the sub-image until the thread is ended by the user (ROI deleted).



Alternatively, instead of selecting the menu item “Extract Sub-Image”, simply press this button with / without holding the Shift key (on the keyboard) down.

To terminate all active threads at any time:

Press the two keys “Ctrl” and “Break” on the keyboard simultaneously:



5 Detailed Description of the HoloWorks 5 Menu Items

5.1 *Reconstruct*

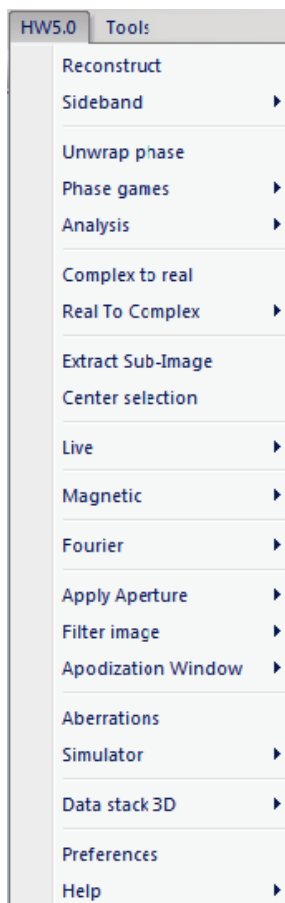


Figure 15 With HWS all earlier reconstruct - features are merged into a single menu item.

DigitalMicrograph[®] is busy exposing and acquiring the next image, the processor is no longer idle but processes and displays the most recent hologram.²⁷ This capability is especially significant for multi-core processor systems.

The reconstruction engine of HoloWorks v5 is very different from earlier versions of HoloWorks:

1. All reconstruction features are unified and appear as single menu item
2. The reconstruction function works on real as well as complex data sets
3. The size of the hologram is arbitrary and no longer limited to image areas either square or of the power of two.²⁶
4. During the reconstruction process, all standard tools of DigitalMicrograph[®] remain accessible.

The reconstruction process takes advantage of the features discussed in chapter 5.12.1 where the dimensional scaling in Fourier space remains independent of the aspect ratio of the image area in real space. From the perspective of Fourier optics, the Fourier space looks as expected in e.g., an electron microscope.

This single, unified menu item allows reconstruction of single hologram or hologram pairs, both for 2D data sets as well as 3D data sets, at selectable levels of interaction with an easy to use interface.

It also replaces the “Holo Stick” of HoloWorks version 3. This was both possible and desirable for several reasons. Contrary to earlier versions of HoloWorks, image acquisition in version 4 and later is left entirely to DigitalMicrograph[®]. This leads to a vastly improved overall experience for the user, because there is only one interface for the camera control and because throughput, i.e., the number of frames per second, is increased for live holography. The latter is possible because the image acquisition threads and image processing threads are independent. In simple words: while

As in earlier versions of HoloWorks, many of the reconstruction parameters are predetermined in the **Preferences** menu. Both the **Preferences** menu and the **Reconstruction** menu allow a maximum in user friendliness and an increased flexibility for the evaluation of holographic data.

²⁶ This allows some camera types with an image size that is rectangular and not of the power-of-two to be used to their full extent.

²⁷ Furthermore, this allows the user to write a plug-in for DigitalMicrograph[®] and use an arbitrary camera for acquisition.

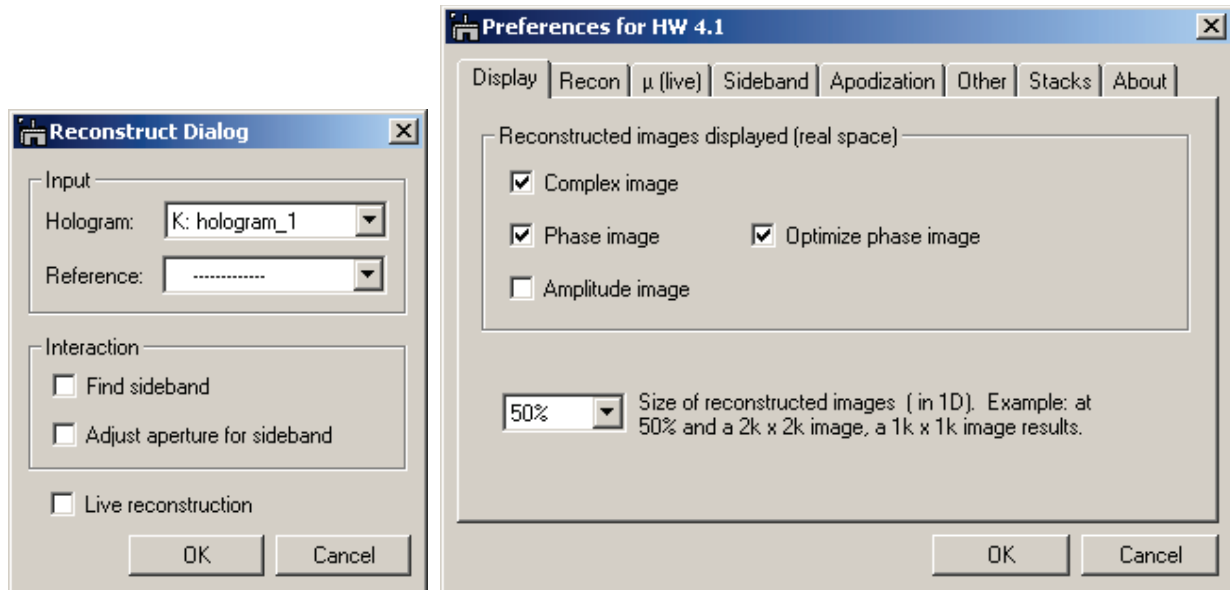


Figure 16 The Reconstruction menu and the Preferences menu working together. The details that govern the reconstruction mode are dealt with in the Reconstruction dialog, while details of the reconstruction are chosen through the Preferences menu.

As shown in Figure 16, left, on activation of the reconstruction menu, the top - most image, or hologram, appears in the “Hologram” popup. The popup menu allows selecting among the displayed images. The “Reference” popup initially shows no selection, but allows picking any of the displayed images as reference hologram. If desired, hologram and reference hologram can be selected identical, but the result will be a complex image of amplitude 1 and phase π . The checkboxes in the “Interaction” box allow detailing of the reconstruction process from fully automatic, when none of the checkboxes is checked, to fully interactive, when both checkboxes are activated.

Checking the “Live” checkbox enables the live reconstruction process where any change to the displayed image activates the full reconstruction procedure until the process is stopped by the user. This is the mode that replaces the HoloStick of HoloWorks, version 3. The checkbox “Live” is automatically checked, when activating the menu item while holding down the “Shift” key.

Details of all the different processing possibilities are discussed on the following pages. Details of the reconstruction as e.g., which reconstructed images are displayed and parameters that e.g., control the final image size are determined in the **Preferences** menu and discussed in chapter 5.19.

5.1.1 **Fully automatic reconstruction of a single hologram**

By deselecting both checkboxes in the “Automation” box of Figure 16, the reconstruction is performed without further user interaction. All reconstruction parameters are determined in this case according to the selections in the **Preferences** menu, and are detailed in Chapter 3.17. Note that the checkboxes in the “Automation” box appear checked or un-checked as defined in the **Preferences** menu as well.

5.1.2 Interactive determination of sideband position

By selecting the checkbox “Find sideband” in the “Interaction” box in Figure 16, a display of the Fourier transform of the selected hologram appears. This is demonstrated in Figure 17 for the example hologram “gold.dm3.”

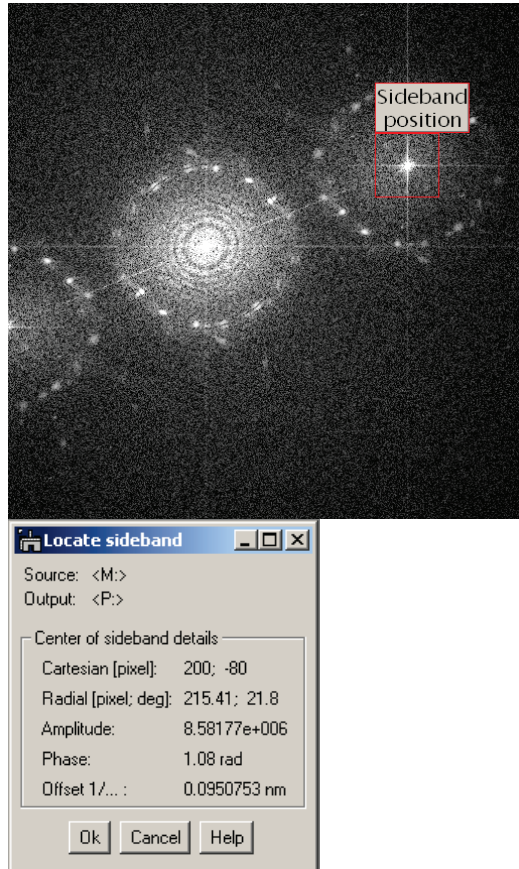


Figure 17 Left: The automatically detected sideband is framed by the red ROI. Moving the ROI allows selecting a different center for the sideband. Right: Details for the center of the sideband are updated in the window to the right. Deleting the ROI continues the reconstruction process.

The sideband position is detected with high reliability through HoloWorks. However, the sideband can be hand-selected by moving and/or resizing the ROI.

Details of the sideband position are given in a window next to the Fourier transform and the data in the window are updated as the ROI is changed. Note that the display of the Fourier transform can be adjusted using the standard DigitalMicrograph® tools.

Note that adjusting the red ROI to the size of power-of-two activates the display of the phase image as reconstructed directly from the red ROI. An example is displayed in Figure 18 left and right. The center of the sideband is found correctly for the left phase image, whereas the center of the sideband is off by 2 pixels in both x- and y- direction. This can be rather helpful in determining the correct sideband position visually.

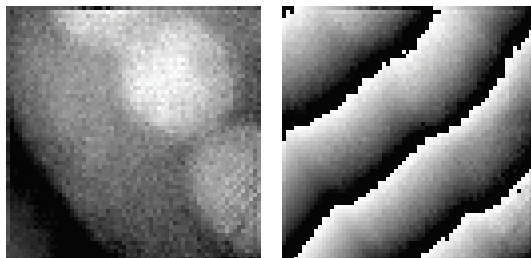


Figure 18 Example phase images: available as the ROI in Figure 17 is of the power-of-two. Left: ROI centered correctly. Right: ROI was moved 2 pixels in each direction.

As the ROI is deleted, the reconstruction process is continued and the attached window as well as the additional phase image disappears. Alternatively, the **Ok** or **Cancel** button in the attached window can be pressed to continue or abort the reconstruction process. The **Help** button activates a window with a reminder about the details of the process.

5.1.3 Interactively filtering the sideband

By selecting the checkbox “Adjust filter” in the “Automation” box (Figure 16), a display of the Fourier transform of the selected hologram appears, but with the sideband now shifted to the center of the Fourier transform. This is demonstrated in Figure 19 for the hologram “gold.dm3.” Note that at this point the position of the sideband is fixed and cannot be changed.

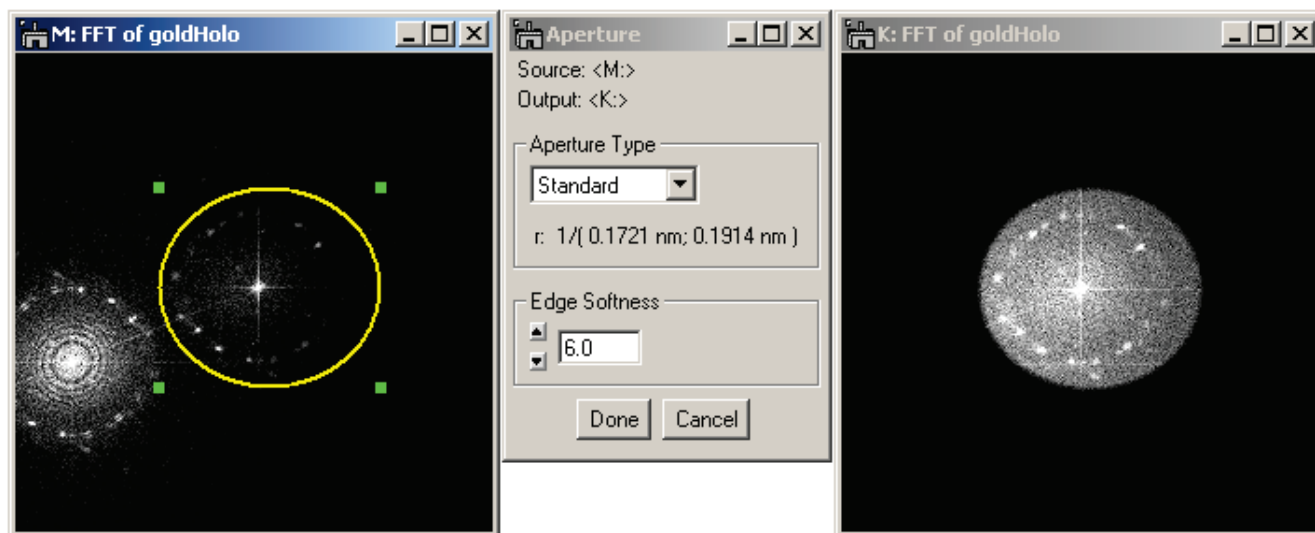


Figure 19 HoloWorks determines and suggests an aperture to isolate the sideband according to the selections made in the Preferences menu. This aperture can be adjusted manually from round to elliptic or can be positioned anywhere in the image. The effect of the aperture is displayed in a separate window (right) while details of the filter can be adjusted in the window in the middle.

The flexibility for applying an aperture to isolate the sideband is an essential feature in optimizing the reconstructed image quality. The aperture in HoloWorks can be changed in size, can be round or oval and can be positioned anywhere in the image. Three different aperture types can be selected: a standard aperture, a Butterworth filter and a filter with a hard edge. Setting the order for any aperture type to zero provides a hard aperture with no soft edges.

As a reminder, apertures with hard edges have a tendency to cause artifacts in the reconstruction and should be avoided if possible. A filter with a soft edge, like the Standard or Butterworth filter is preferable. The softness of the edge can be adjusted for each aperture types by use of the up and down arrow buttons or simply by typing a number in the corresponding field followed by the use of the **Enter** key on the keyboard.

Because of the modularity of HoloWorks, an additional thread like the inverse Fourier transform discussed in chapter 5.12.2 can be attached, to which a conversion to image phase can be attached as well. Thus the effect of an aperture change on the reconstructed image phase can be observed directly. This feature is discussed in detail in chapter 0.

As the “Oval annotation” representing the aperture is deleted, the reconstruction process is continued and the attached window as well as the additional resulting image disappears. Alternatively, the **Ok** or **Cancel** button in the attached window can be pressed to continue or abort the reconstruction process.

5.1.4 Reconstruction with a reference hologram

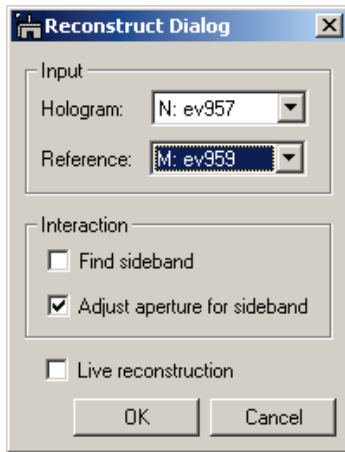


Figure 20 Reconstruction with a reference hologram.

To reconstruct with a reference hologram, select the reference hologram through the popup list marked “Reference:”.

The sideband position in this case will be determined from the reference hologram and thus usually does not require user interaction. However, if selected, any adjustment of the sideband position will be available only through the reference hologram.

The adjustment of the filter will be performed on the actual hologram and is described on the previous page.

Either click on the **OK** button or press **Enter** on the keyboard to continue. The **Cancel** button allows abortion of the reconstruction process.

5.1.5 Live reconstruction



Figure 21 Live reconstruction setting.

To start the Live reconstruction process, no reference hologram is needed. First, the reconstruction parameters are established, either automatically or interactively. In the example in Figure 21, the sideband will be selected automatically and interaction for the adjustment of the filter is requested. Also, the “**Live reconstruction**” checkbox at the bottom of the window is checked.

Upon pressing the **OK** button, the reconstruction process is started and the resulting images are displayed (see Figure 24) according to the presets in the **Preferences** menu.

Every time the input image is updated via, e.g., an active camera, the reconstruction process is performed again with the reconstruction parameters established in the initial run. If the image is not updated because e.g., the camera acquisition was stopped, the reconstruction thread turns idle thus minimizing the processor load.

The reconstruction thread is indicated in the original image by a red ROI with the label “**Live reconstruction.**” Deleting the image or ROI ends the thread.

In addition to the ROI, the live reconstruction thread is also controlled by the **Live rec** window, as displayed in Figure 22.

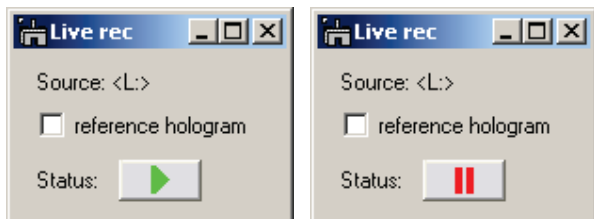


Figure 22 Same window but different status: Green arrow indicates process is active, red indicates process is paused. Checking check box “reference hologram” opens the window in Figure 23.

By clicking on the green arrow, the “Live rec” window allows to temporarily pause the reconstruction process indicated in red. Pressing the red button reverts the status to green and resumes the reconstruction process.

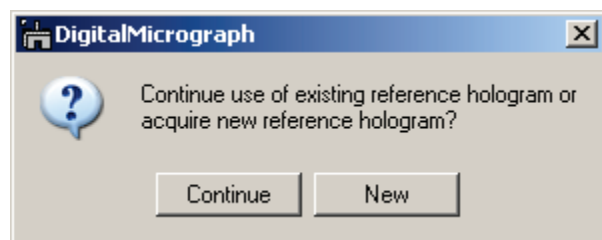


Figure 23 For the live reconstruction process the ability to update the reference hologram is essential.

Selecting the checkbox “reference hologram” in the Live rec window activates the use of a reference hologram to compensate for Fresnel fringes, phase distortions and camera defects.

When selecting the checkbox, the first time, it is assumed, that no object is present in the hologram at that time. Therefore, it is necessary to move to a specimen-free area before activating the reference hologram. This hologram then serves for all consecutive holograms as reference.

When selecting the checkbox again, a user-dialog appears offering to continue the use of the already existing reference hologram, or to acquire a new reference hologram.

De-selecting the checkbox drops the use of the reference hologram without deleting the reference hologram. Thus, the results of reconstruction with and without a reference hologram can be compared easily.

Deleting the “Live rec” window ends the live reconstruction process and the ROI in the source image is removed.



Figure 24 The area within the ROI is processed each time the image (left) is updated or the selection is moved. Consequently, the complex (middle) and phase image (right) are updated as well. Additional processing steps can be spawned off each image as discussed in detail in Chapter 0.

5.2 Sideband

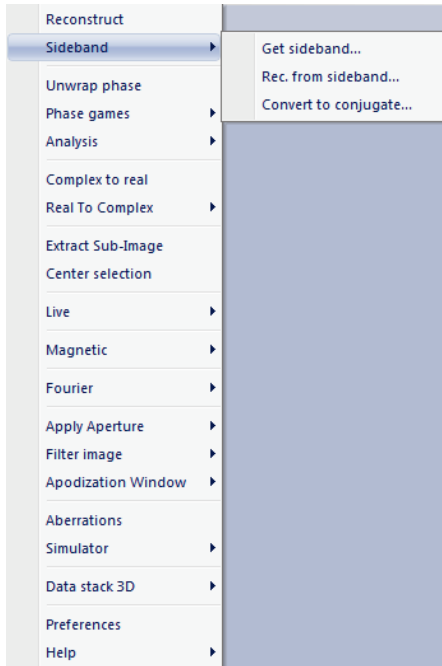


Figure 25 Sub-menus in ‘Sideband.’

The sub-menus of the menu sideband allow separation of the reconstruction processes.

The **Get sideband** sub-menu interactively searches for the sideband position and re-centers the Fourier transform so the sideband appears centered.

The **Rec. from sideband** sub-menu performs an inverse Fourier transform and displays the resulting complex, phase and amplitude images as defined in the **Preferences** menu.

The **Convert to conjugate** sub-menu basically swaps the reference wave of the hologram with the target wave. Although mathematically irrelevant, its obvious effect is to change the sign in the reconstructed phase. Less obviously, it also changes the sign for the aberration parameters discussed in chapter 3.15.

5.2.1 Get sideband

This sub-menu item provides direct access to the sideband, centered within the coordinate system of the Fourier transform. The sideband is initially positioned by the software at the center of the sideband.

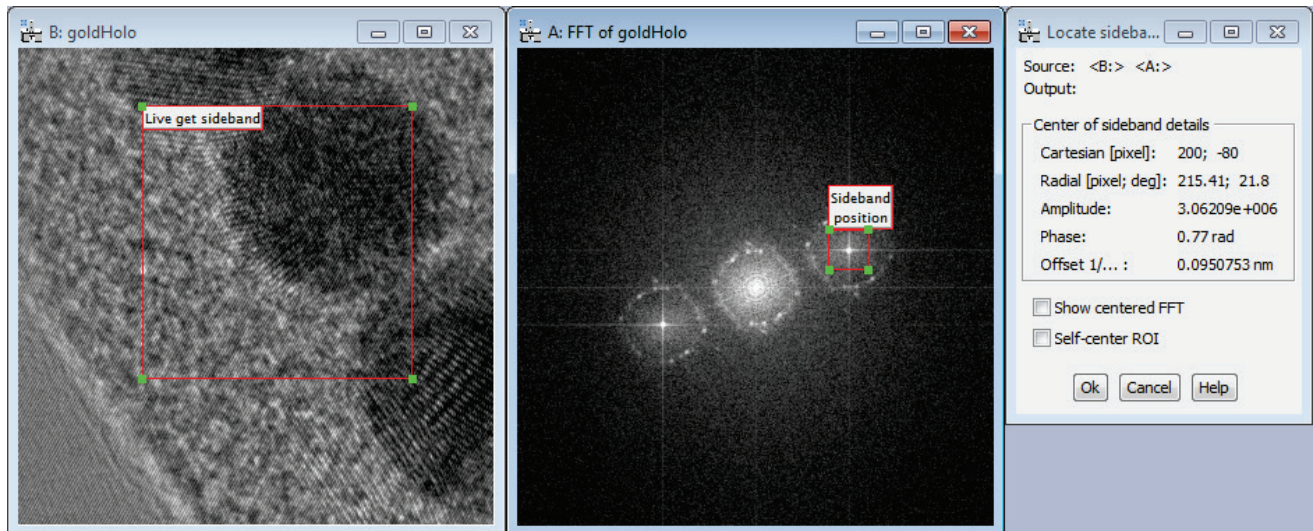


Figure 26 “Get sideband interactively” provides many details to study the effects of different sideband positions. The ROI “Live get sideband” is moveable and the ROI “Sideband position” is resizable and can “self-center.” Also, the centered sideband can be displayed as shown in Figure 27 and used as input for additional processing functions.

This menu item reacts to changes to the source image e.g., a live image (or for 3D data stacks: a change in the slice), and the selected area is moveable. In addition, and as shown in Figure 27, the centered sideband can be displayed as well and serve as input for larger workflows. Working with an automatically centered and updated sideband can be especially useful - when the reconstruction process is to be split up in its parts to study in detail the effect of each reconstruction parameter on the reconstructed images.

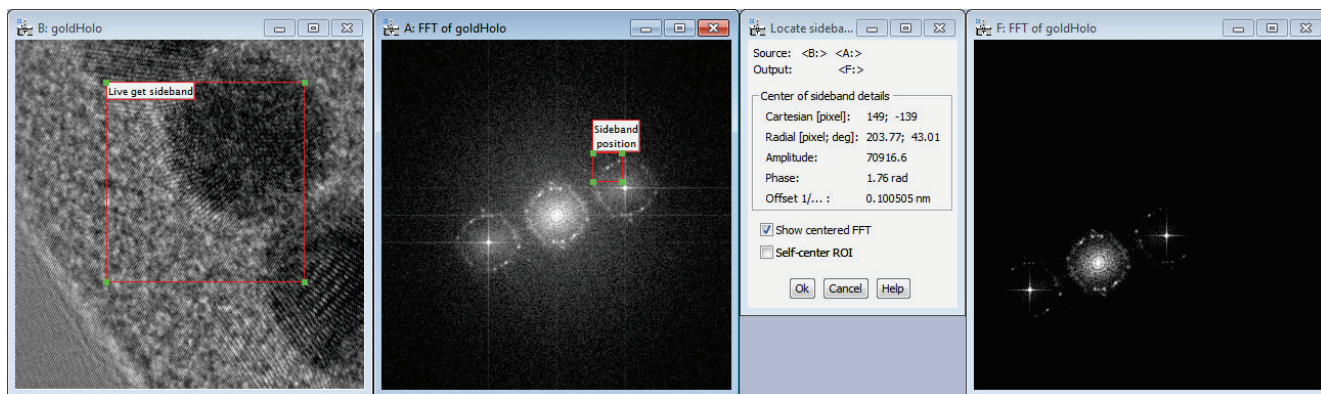


Figure 27 Checking the check-box “Show centered FFT” provides a live and shifted FFT of the source image.

Furthermore, data processing besides holography can be built, for example by selecting a diffracted peak and reviewing the reconstructed phase image from that peak. This can give quick insights into strain or simply the localization of the crystal in real space at high resolution.

Deleting the ROI ends the dependency between the input and output images. The use of the Shift key when evoking this menu item has no effect in this case. The Cancel button deletes all images created via this menu item.

5.2.2 Rec. from sideband

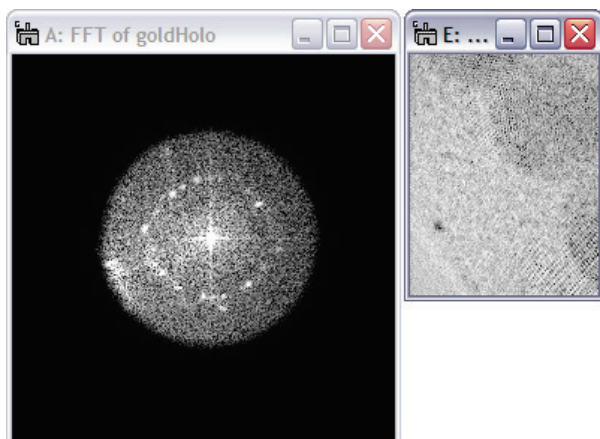


Figure 28 This function is effectively an inverse Fourier transform but the resulting image may be neither square or of the power-of-two.

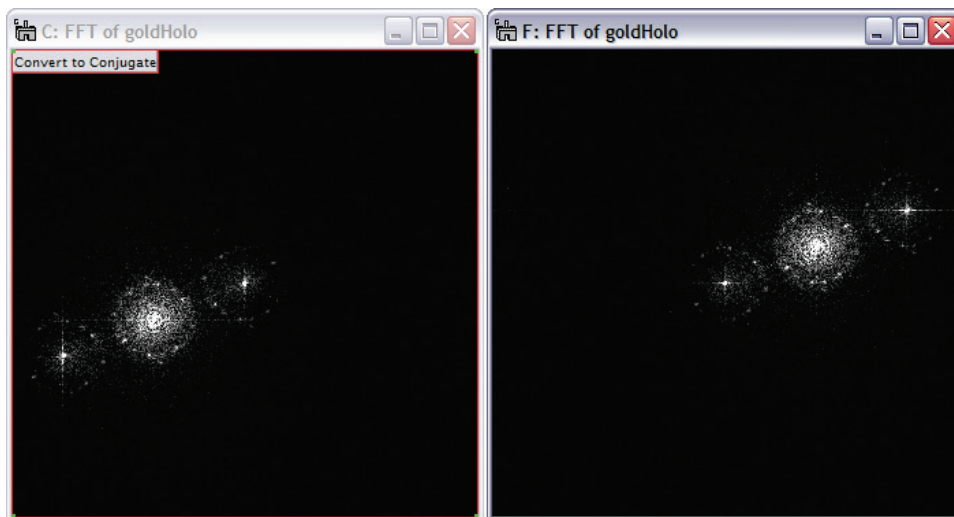
This sub-menu item provides reconstruction means from the sideband directly. Its functionality is an inverse Fourier transform, but is Fourier optics oriented. This means that if the original, real-space image (or image selection) was rectangular before the Fourier transform (the Fourier transform is always square) the image generated by the inverse Fourier transform will be rectangular. This function ignores selections in the source image!

Mode 1: The complex image wave is computed by using an inverse Fourier transform and displayed next to the sideband. Amplitude and phase images are computed and displayed as well according to the settings in the **Preferences** menu.

Mode 2: Activating the sub-menu with the **Shift** key down creates the complex image wave as well as the amplitude and phase images as specified in the settings for the **Preferences** menu. The sideband then displays an ROI with the label “**Get image wave.**” Anytime the sideband is modified or updated, all images are recomputed accordingly. Deleting the sideband or the ROI ends the dependency between input and output images.

5.2.3 **Convert to conjugate**

This sub-menu converts a sideband into its conjugate. It provides an alternative means to switch between the two sidebands. Since two sidebands carry identical information, this function can be useful as an intermediate step for live image processing.



Mode 1: The sideband is reproduced, but as the conjugate sideband.

Mode 2: The sideband is reproduced, but in its conjugate form as long as the ROI “**Convert to Conjugate**” is valid.

This function ignores ROIs in the source image (C:) !

Figure 29 Converting the sideband to its complex conjugate. A direct consequence of this procedure is an inverted phase contrast in the reconstructed phase image.

5.2.4 **Reconstruction as a detailed workflow**

On the next page, in Figure 30, the capability of creating a workflow is demonstrated for the reconstruction process. In this example workflow, the entire reconstruction process is laid out in detail where every workflow parameter can be adjusted while directly observing its effects all the way to the final data. Such a workflow can be especially useful for

- teaching purposes
- complicated data sets where the interpretation of the results may require a detailed review.

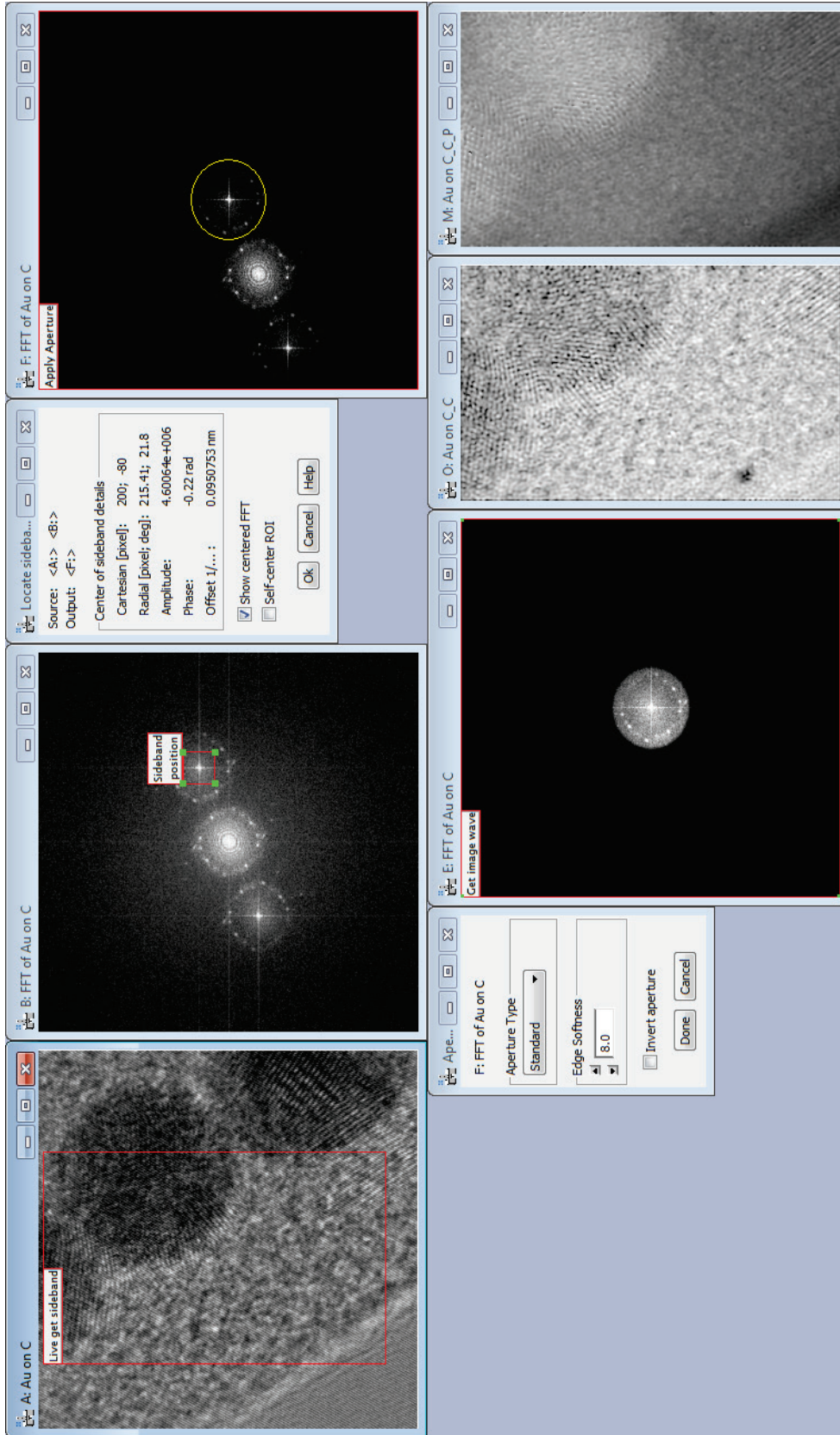


Figure 30 Example of a workflow enabled by HoloWorks. The reconstruction process is split up in its elements and each reconstruction parameter can be adjusted separately. The effect of any adjustments is directly observable. Note that the source image (here: A: Au on C) can be a live image or a 3D data cube: Any change in the source is processed through this workflow change immediately. The resulting image at the lower right corner contains all the parameters of the data processing. Just right-click the image, select “Image display info,” then “Tags” under “Image” and open the “History” entry.

5.3 Unwrap phase

The function is fully automated and returns a phase unwrapped image. 2D and 3D data sets are supported. The un-wrap algorithm is proprietary, very fast and provides excellent results in almost all cases. The input image is required to be of type real with a dynamic range to not exceed 2π .



Figure 31 The Unwrap phase menu.

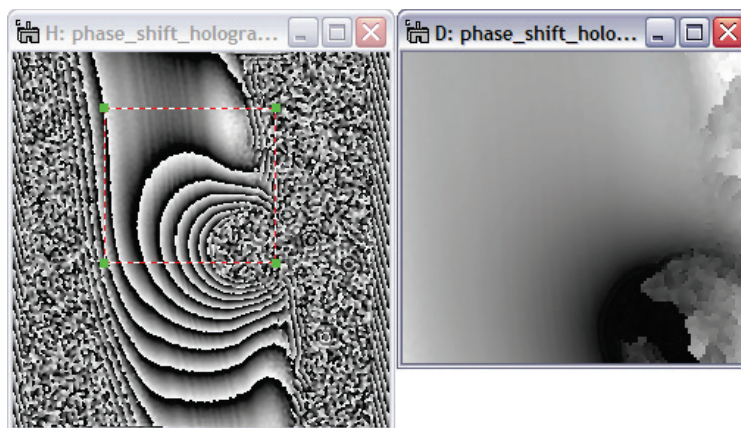


Figure 32 Removing phase jumps (from selected areas) is key in measuring e.g., the electrostatic potential around a Latex sphere.

Mode 1: No Shift key used.

A new image is generated from the original image or from its selection. For 3D data sets, a user interface displays the options to unwrap the (selection in the) displayed slice or to unwrap all slices and create another 3D data set. All tag information including image calibration and the history tree are transferred into the new phase – unwrapped image.

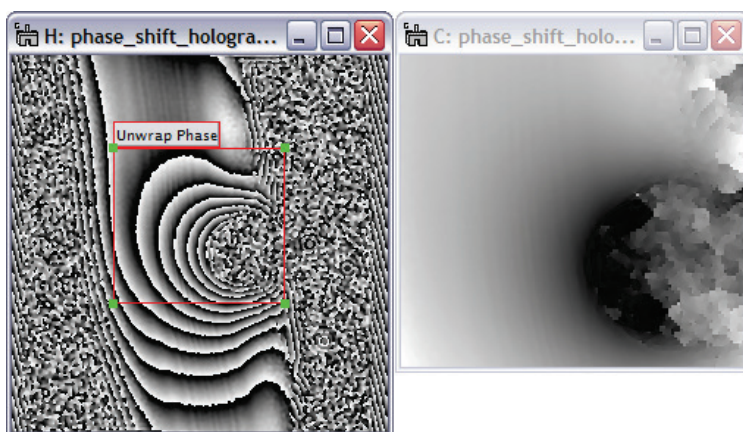


Figure 33 The selected area can be moved and resized while the image on the right is immediately phase unwrapped.

Mode 2: Shift key used.

A solid red ROI indicates that the image on the right-hand side is updated when:

- i. Selection is moved
- ii. Selection is resized
- iii. Input image is updated
- iv. Slice is changed (3D data)

All Tag information including image calibration and the history tree are transferred into the image. To terminate the interaction, the ROI must be deleted.

At any time, access to the standard tools of DigitalMicrograph® is maintained.

5.4 Phase games

The functionalities of the sub-menus of “Phase games” are tailored towards cleaning up the phase image from unwanted artifacts. In general, the clean-up activities relate either to a phase ambiguity of 2π , an arbitrary phase-offset constant, or a phase wedge i.e., the position of the sideband not coinciding with a pixel in Fourier space.

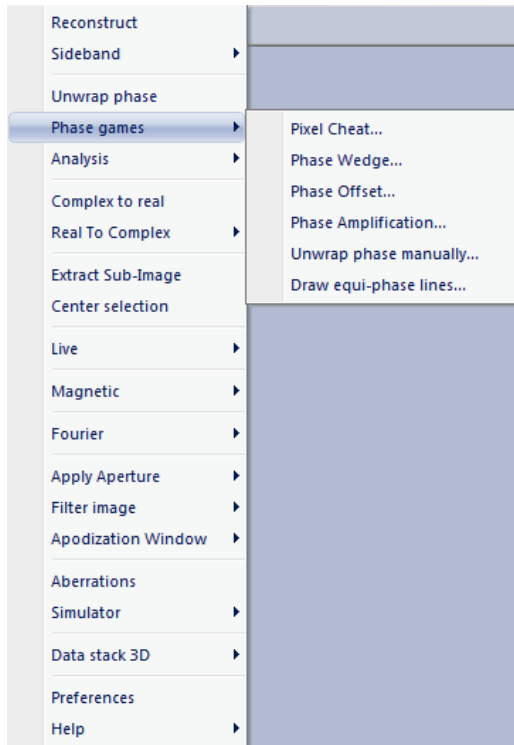


Figure 34 The Phase games menu item with its six sub-menu items.

The following six sub-menus assist in cleaning up or modifying phase images:

- i. Phase ambiguities on a pixel by pixel event
- ii. Inaccurate centering of the sideband (also 3D data!)
- iii. The arbitrary phase offsets
- iv. Phase amplification (creates more phase jumps)
- v. Phase ambiguities on a larger scale than a few pixels
- vi. Drawing equi-phase lines

All sub-menus create new images and maintain image calibration, image tags and the history tree.

The sub-menu “Phase Wedge...” is the only sub-menu under “Phase games” that is designed to deal with 3D data sets. The specific application is to 3D data sets of phase images or complex images waves from 3D reference hologram data sets.

All other sub-menus focus on the actual displayed images only, as their functionalities usually don’t lend themselves to automation and therefore are specific to only one image.

5.4.1 Pixel Cheat ...

The function is designed to adjust single pixels values or small sets, areas, of pixel values. Since the phase is defined modulo 2π only, adding or subtracting 2π from a pixel does not change the available information from an image in the mathematical sense, however may help to better visualize its information content. Offsets with multiples of 2π are possible as well as can be seen from the window in the middle of Figure 35. Use of the “**Help**” button opens an additional help window with indications for keyboard shortcuts. This sub-menu requires an image of type real.

Instructions: Evoke the “Pixel Cheat” sub-menu on a selection in the image or the full image. A new image and a new window appear as seen in Figure 35. The new image contains a red ROI with a tag indicating the value by which the pixels within the ROI will be changed. Use the magnifier tool of DigitalMicrograph to adjust the magnification such that pixels are easily recognized. Whenever using the “a” and “s” keys on the keyboard, the pixel values within the ROI increase or decrease by e.g., 2π . Click “Done,” or delete the ROI when finished.

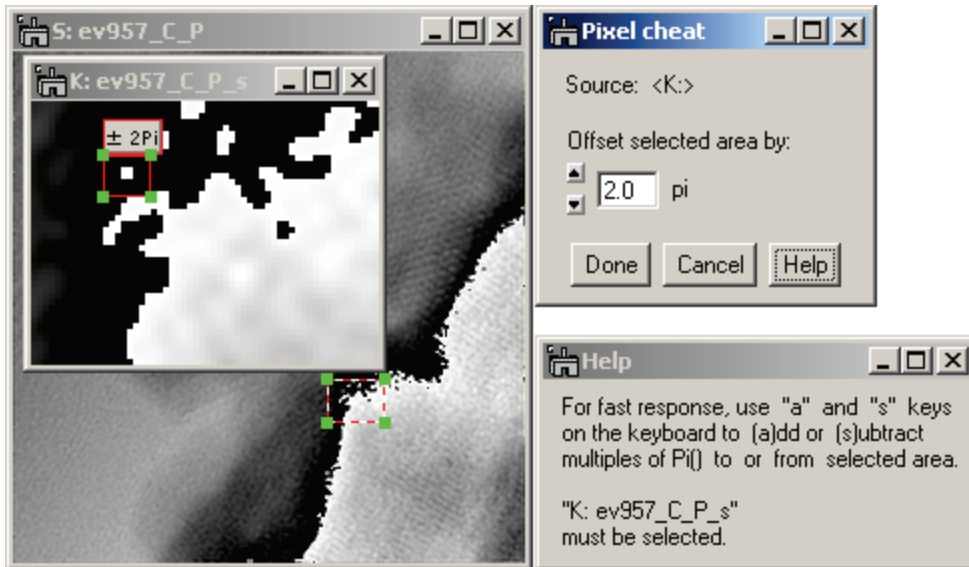


Figure 35 Changing the phase value by an integer multiple of 2π does not change the information content of the image, but may help to better visualize the data. Non-integer multiples of 2π change the information content and should be used with care.

5.4.2 Phase Wedge ...

This sub-menu creates a new image on the right hand side of the interactive window as displayed in Figure 36. The sub-menu works on real / complex type images and returns real / complex type images as well as for 2D images and 3D data stacks. The size of the image or selected area can be arbitrary, the ROI can be moved and for 3D data changes in the slice are recognized.

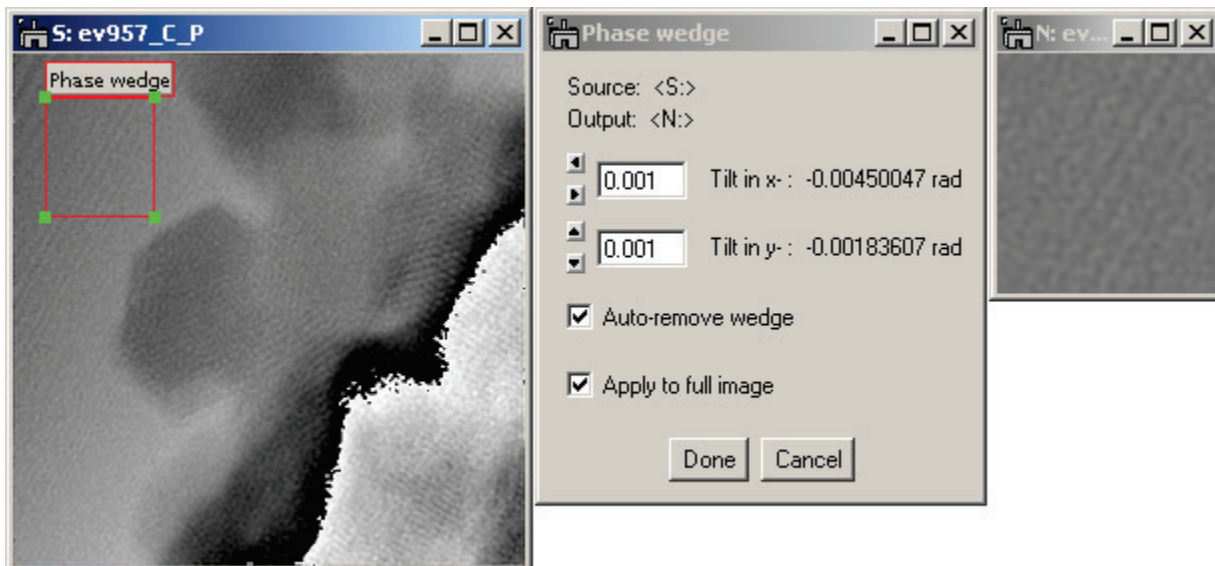


Figure 36 The interactive window in the middle allows to select tilt angles in the x- and y- direction for the image, given on the left. The resulting image is displayed on the right. Note the capability for auto-remove and the capability to apply the wedge from the selected area to the full image (see Figure 37).

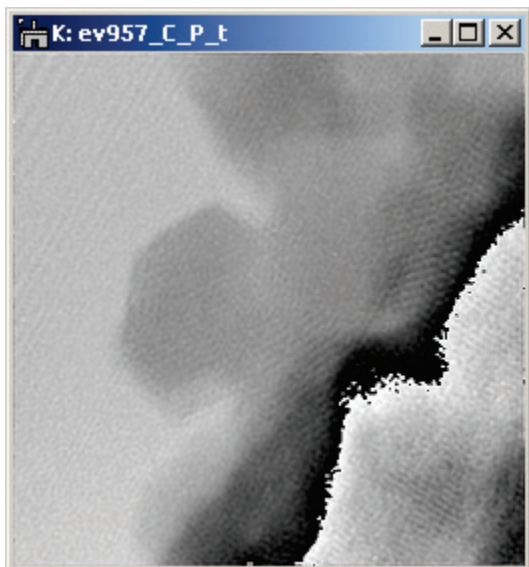


Figure 37 When activating “Apply to full image” as in Figure 36, the phase wedge determined via the selected area is applied to the full image as well. This can be especially convenient for 3D data stacks.

When activating the option “Apply to full image”, another image is created where the wedge determined from the selected area is applied to the full image.

This is a rather important function for holography, as in almost all cases the position of the sideband does not coincide exactly with the discrete pixel structure of the Fourier space. In such a case, the reconstructed phase image will carry an artificial phase wedge. To remove, select an area known to be flat, compute the required wedge and apply to the full image.²⁸

The effect of the sub-menu is different for real type and complex type images:

is determined at the image edges.

In addition, if a sub-area is selected in the source image, the wedge can also be applied to the full image, thus creating two images simultaneously. This can be of advantage especially when the auto-remove feature is activated.

- For images of type real, a wedge is added to the image whose parameters are determined in the interactive window. The wedge tilts around the center point in the image and the tilt amount that appears in the UI window
- For images of type complex, a wedge is added by multiplication of the original image with a phase $\exp(i\theta(\mathbf{r}))$, where $\theta(\mathbf{r})$ is a real function and describes the wedge as in the case for real images. The phase wedge on complex images has a similar effect in real space as an offset of the sideband position during reconstruction. This means, as the phase wedge is applied, phase jumps may disappear or occur in the phase image as a consequence.²⁹

The selection in the source image can be moved freely and any repositioning of the selection automatically updates the resulting image. The update also occurs when the source image is updated or modified. The red selection indicates that a thread is attached to the source image. Deleting the selection ends automatically the thread, as does the deletion of source image, the interactive window, the resulting image or by pressing the “Done” button. Pressing the “Cancel” button deletes the resulting image as well.

This function works for 2D and 3D data sets.

²⁸ Ideally, the phase wedge is already taken care of by using a reference hologram. However, instrument instabilities may still require the use of this function, especially when working with 3D data sets.

²⁹ Note that any complex image can be displayed in its window as phase by right-click into the image and selecting “ImageDisplay...” from the popup menu. Selecting “complex” in the resulting popup menu allows selection of how the complex image is displayed.

5.4.3 Phase Offset ...

This sub-menu creates a new image on the right hand side of the interactive window. The sub-menu works on real / complex type images and returns real / complex type images. The size of the image or selected area can be arbitrary.



Figure 38 Phase offset allows an offset of the phase of real or complex images by a constant. If the dynamic range of the image is $< 2\pi$, the resulting phase will wrap automatically.

The effect of the sub-menu is different for real type and complex type images:

- For images of type real and a phase dynamic within $[0, 2\pi]$, the phase offset value is added or subtracted from the image. Consequently, there can be pixels whose value falls outside the $[0, 2\pi]$ range. The value of these pixels is then modified automatically to remain within $[0, 2\pi]$. This is called wrapping the phase (see Figure 38).
- For images of type real with a phase dynamic within $> 2\pi$, the phase offset value is simply added to the image, as it is assumed that no phase wrapping is wanted in this case.
- For images of type complex, the offset value is multiplied as a complex phase value with the complex image.³⁰

The selection in the source image can be moved freely and repositioning the selection automatically updates the resulting image. The update occurs as well whenever the source image is updated itself. The red selection indicates that a thread is attached to the source image. Deleting the selection ends automatically the thread, as does the deletion of source image, the interactive window, the resulting image or by pressing the “Done” button. Pressing the “Cancel” button also deletes the resulting image.

“Phase Offset” presently not supports 3D data stacks.

³⁰ Note that the complex image can be displayed as its phase by right-click into the image and selecting “ImageDisplay...” from the popup menu. Selecting “complex” in the resulting popup menu allows selection between several modes of how to display the complex image (e.g., as phase).

5.4.4 Phase Amplification...

This sub-menu creates a new image on top of the original image. The sub-menu works on real (i.e., phase images) and complex type images. The size of the image or selected area can be arbitrary. This sub-menu supports visualization of lines of equal phase (similar to changing the wave length of the electron beam).

For phase images that contain phase jumps, a phase-unwrap is recommended. This is important, because otherwise artifacts may occur. This is also true when starting from a complex image.

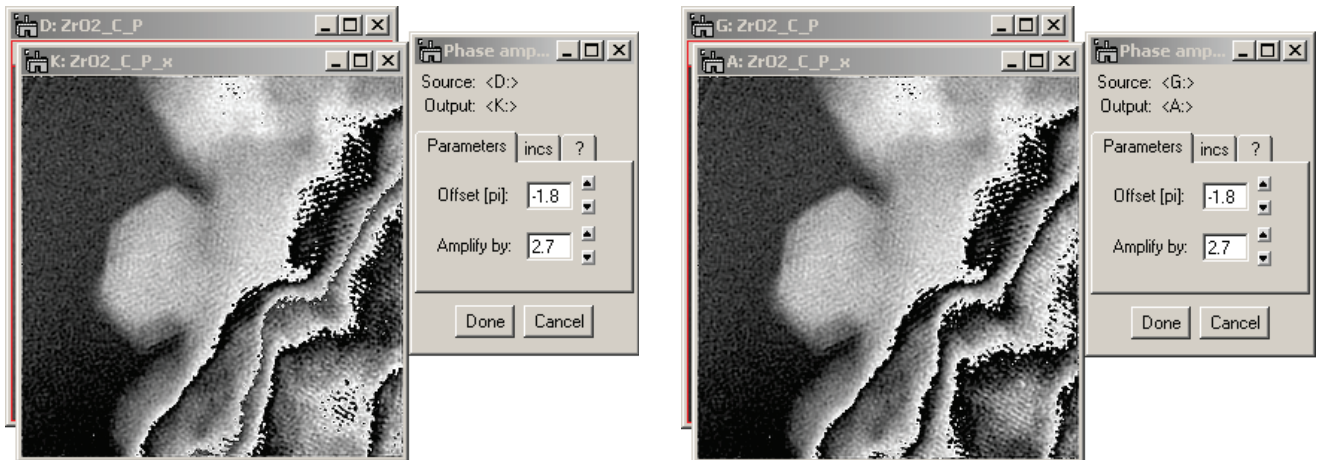


Figure 39 Left: original phase image was not un-wrapped. Note the resulting “grayish” 2nd phase wrap. Right: the original phase image was phase-unwrapped before applying the amplification. No artifacts occur.

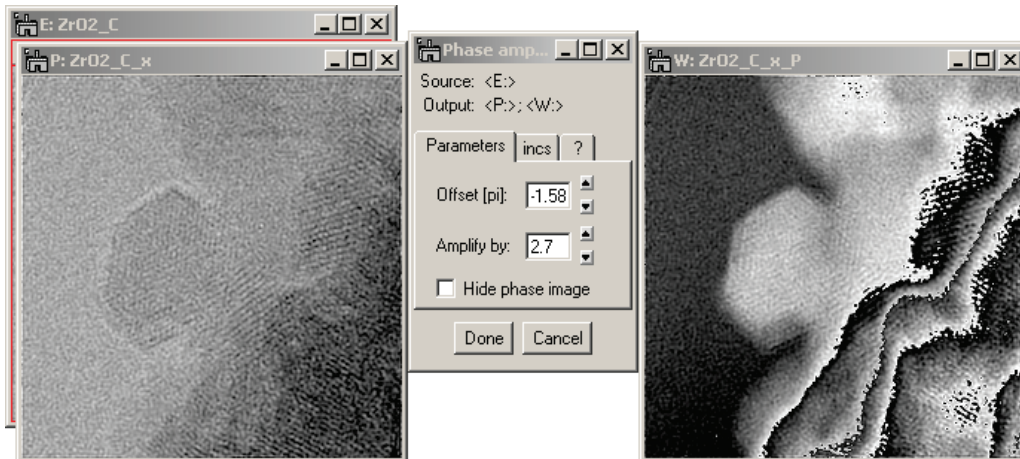


Figure 40 Working from a complex image also shows the phase image. The resulting phase image however has the same issue as the not un-wrapped image (Figure 39, left).

Deleting the red ROI or pressing either the Done or Cancel button ends the thread. Using the Cancel button ends the thread, undoes all image changes and deletes the output image(s).

5.4.5 **Unwrap Phase Manually ...**

This sub-menu is designed to assist in cases where automated phase unwrapping does not produce a satisfactory result. It provides the means to manually adjust the phase range and assist in manually unwrapping the image phase. This sub-menu works on real type images only. The size of the selected area can be arbitrary but should be small for most applications.

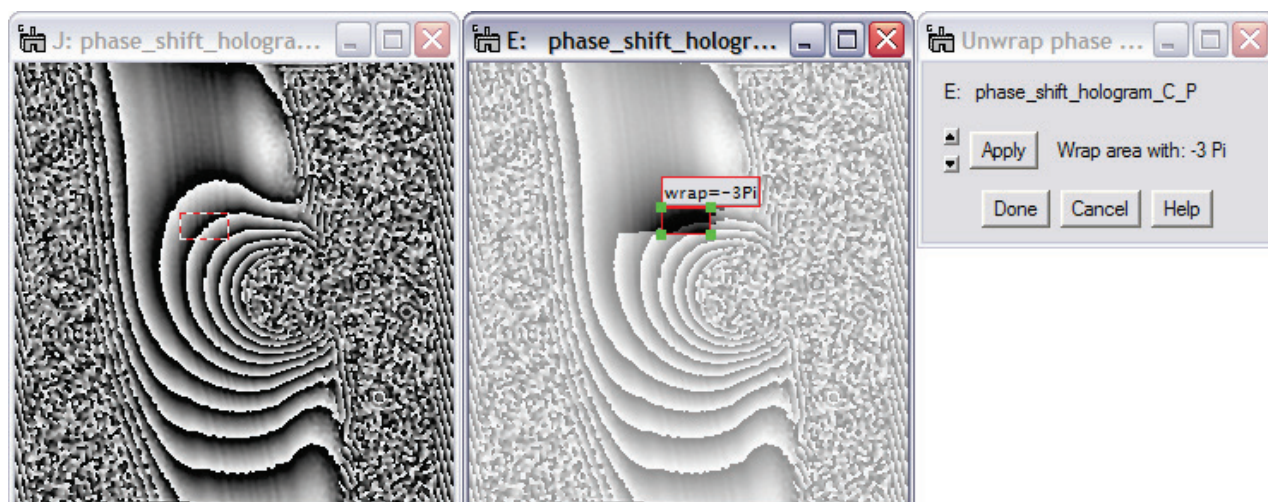


Figure 41 The phase image of a charged Latex particle (left) exhibits many phase jumps due to the strong electric field around the particle causing phase shifts $\gg 2\pi$. A manual phase unwrap (middle and right) is time consuming.

The best approach to manual phase unwrapping is to consecutively work from simple areas into complex areas. Start by picking one line of phase wrapping and eliminate it via the following procedure:

1. Select an area (ROI) small enough to contain a maximum of one phase jump.
2. Pressing the up or down buttons in the UI window, until the phase jump within the ROI is eliminated.
3. Move the ROI along the phase jump line and press the “**Apply**” button.
4. Continue until the phase wrap has disappeared.
5. Pick the next phase wrap line and start over.

In order to speed up the process, the image (Figure 38, middle) is also sensitive to certain keyboard actions. Pressing the “a” key corresponds to the up arrow in the UI window. The “s” key corresponds to the down arrow in the UI window and the **Tab** key corresponds to the **Apply** button.

Deleting the selection ends automatically the thread, as does the deletion of source image, the interactive window, the resulting image or by pressing the “**Done**” button. Pressing the “**Cancel**” button deletes the resulting image as well.

5.4.6 Draw equi-Phase Lines

This sub-menu is designed to visualize lines of equal phase value. This can provide a better understanding of the thickness distribution of the specimen, or indicate the distribution of fields either electrostatic or magneto static in nature.

Below, an example is given on how to use this sub-menu. In all cases, it is important to define the zero phase first by offsetting the phase of the image to move the zero phase value e.g., at the interface between vacuum and material. This is important, because the zero phase line remains independent of the delta between equi-phase lines. Please note that phase wraps will lead to artifacts and a successful phase unwrapping is strongly recommended.

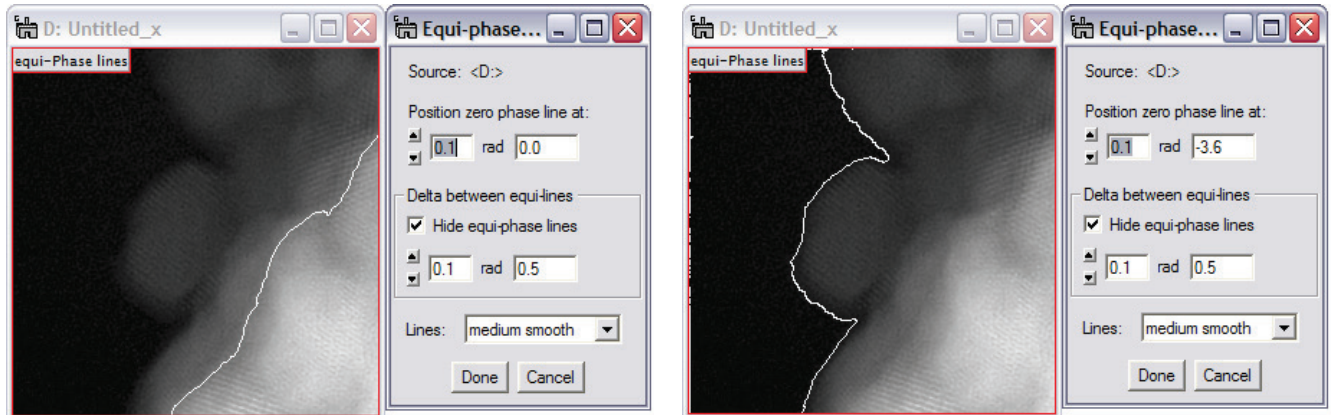


Figure 42 To draw equi-phase lines correctly, the first step is to pick the zero phase line, e.g., at the interface to vacuum or, in this case, by adjusting the zero phase line from zero to -3.6rad . Note that a medium smooth was used for a better display. Also, because of the dynamic range of this phase image ($\sim 4\pi$), the delta for the equi-phase lines has been selected as 0.5rad .

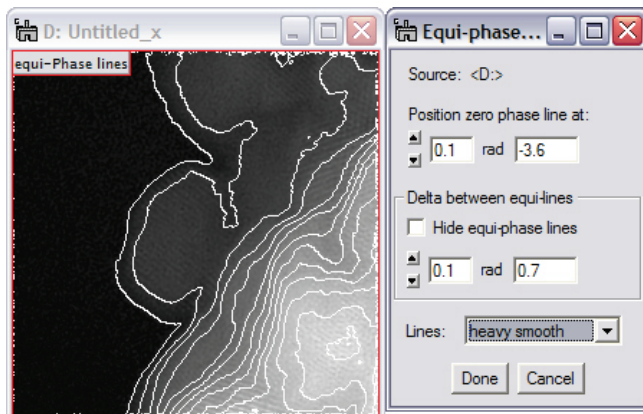


Figure 43 Un-hiding the equi-phase lines provides a good visual indication of the thickness distribution in this sample of ZrO_2 particles. The equi-phase lines are at a distance of 0.7rad and heavy smoothing has been selected for better display.

The equi-phase lines are indicated as white lines. Note that during the process of adjusting the phase lines, all DigitalMicrograph tools and features remain accessible. For example, adjusting the contrast of the image, or turning the survey mode off might be a useful assist.

Deleting the red ROI or pressing either the Done or Cancel button ends the thread, while using the Cancel button ends the thread and undoes all image changes.

5.5 Analysis

Extracts materials' analysis information from either complex or real type images. If a selection is present, the area of the selection will be used.

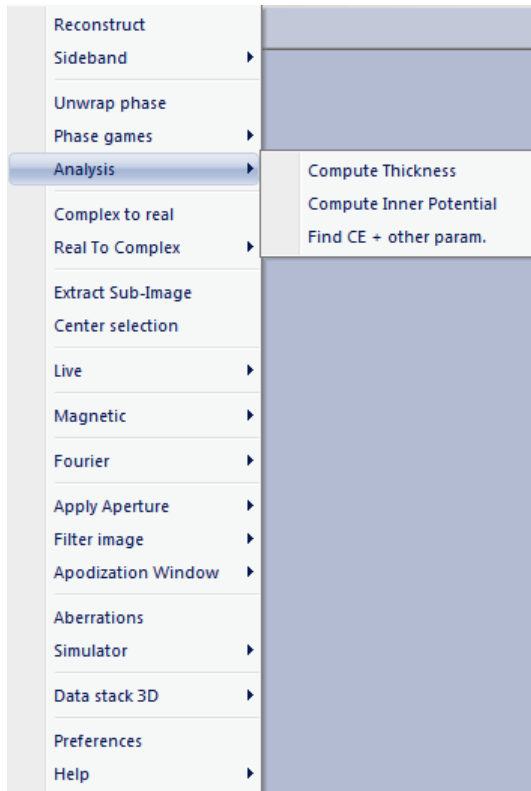


Figure 44 The analysis menu provides basic materials analysis capabilities.

As input data, holographic reconstructed data as either the complex image, amplitude or phase images are expected. The images are then processed according to the textbook “Introduction to Electron Holography.” In all cases, a reference area is required which -- in the absence of electrostatic or magnetostatic fields outside the specimen – is represented by an area in the vacuum. There, the phase φ serves as reference phase with value 0, while the amplitude serves as reference value 1. As relation between phase and thickness, the following expression is used:

$$\varphi(x,y) = C_E |V_0| t(x,y)$$

with C_E an energy dependent constant, V_0 the mean inner potential and $t(x,y)$ the local thickness.

All new images contain the history tree as well as scaling parameters if found in the original image. For both sub-menus an active thread is evoked that requires manual termination. All DigitalMicrograph[®] standard tools remain directly accessible at all times.

5.5.1 Compute Thickness

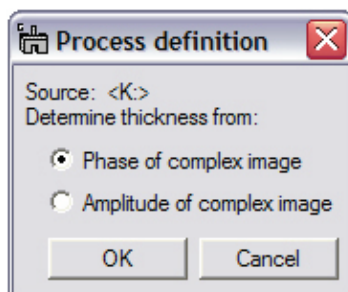


Figure 45 Determining the type of input data: amplitude or phase?

The first step when computing the thickness of the specimen is to choose what method to use. The phase information usually contains the highest quality data with the least noise in the results and provides a thickness value in nm directly.

The amplitude image often displays high noise levels, only the relative thickness is computed just like the relative thickness as obtained by EELS.

If the input data represent the complex image wave, or if the input data cannot be recognized as amplitude or phase data, the window on the left appears. If the input data are recognized by the program as phase or amplitude data, the dialog is omitted.

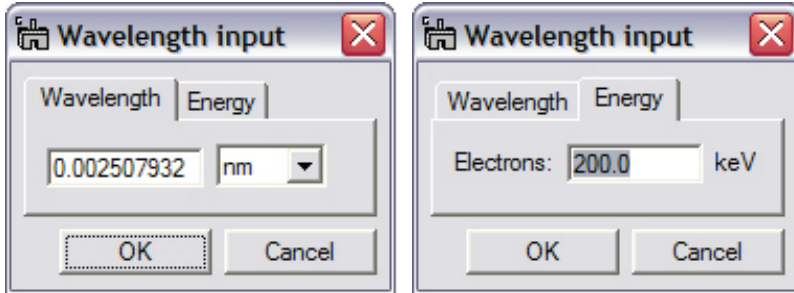


Figure 46 The input windows allow either direct wavelength input or indirect wavelength input via the acceleration energy for electrons.

In order to obtain the thickness of the sample from the phase image, the wavelength must be known. The wavelength information may already be part of the general information stored with the image, however, the correct input is crucial to obtain a correct thickness interpretation.

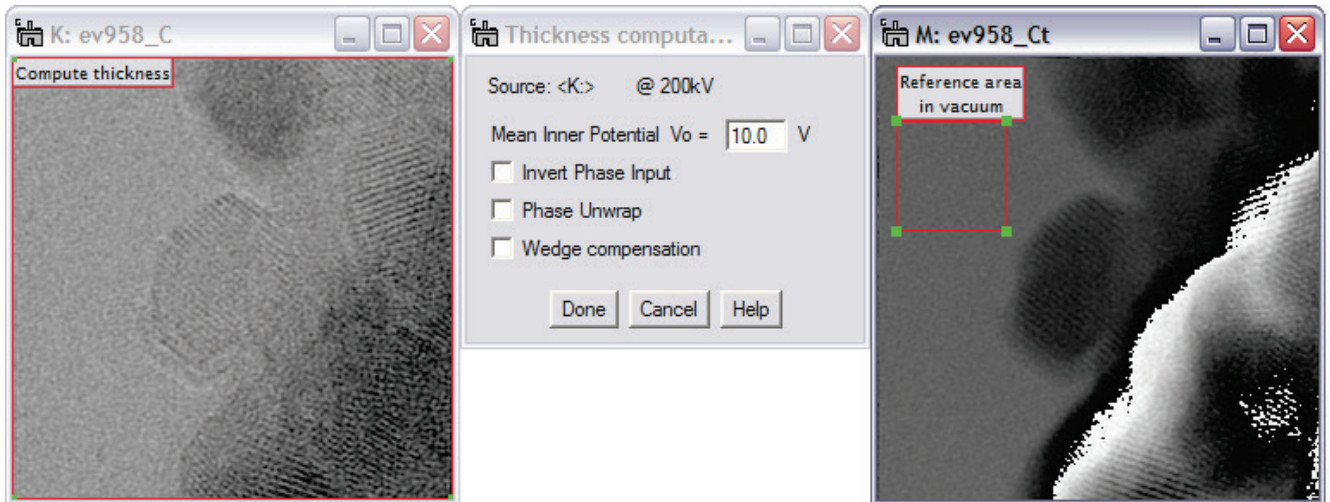


Figure 47 In order to obtain reliable readings from the phase information, the phase value in vacuum should be set to zero. This is necessary, as it corresponds to a sample thickness of 0nm.

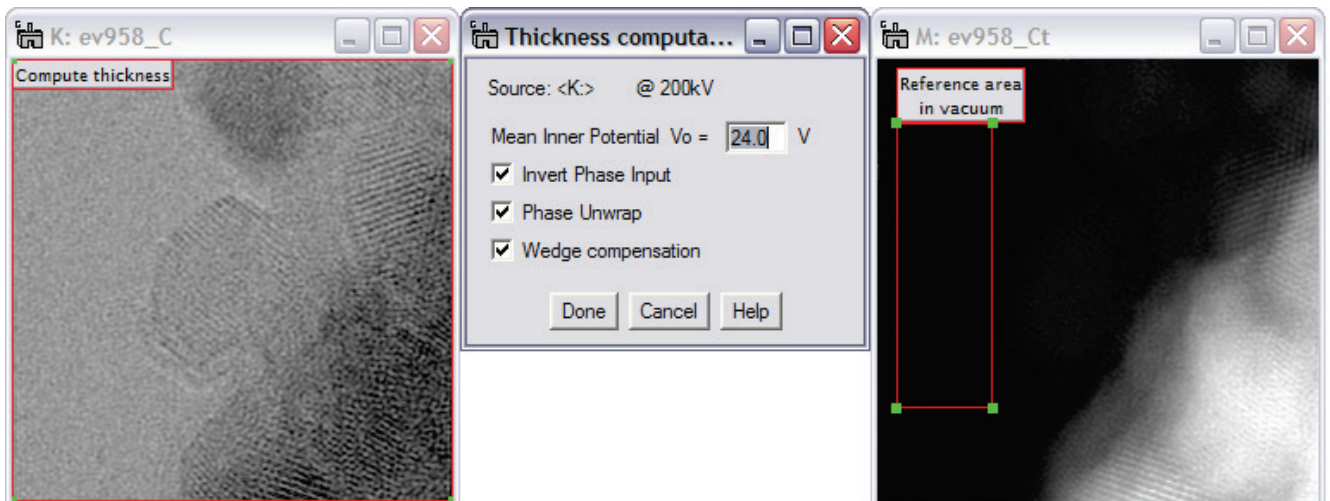


Figure 48 to obtain positive numbers for sample thickness, the phase information may have to be inverted. Also, phase wraps should be eliminated and the selected reference area in vacuum maximized. Even when working with reference holograms, the phase in vacuum may still exhibit a “wedge,” which can be compensated automatically.

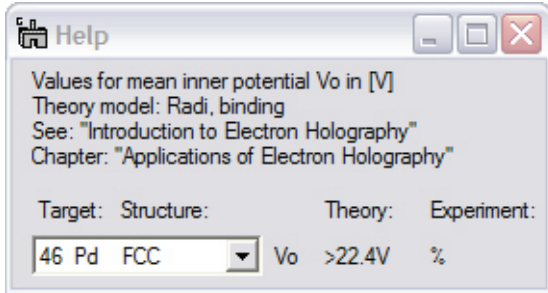


Figure 49 Help is available for selecting a reasonable mean inner potential value.

For many elements and compounds, a theoretical estimate is known, but for most cases, experimental values are lacking. Please be also aware that the mean inner potential values given may vary significantly for real samples. This is true especially when crystals are oriented along major zone axes. This condition should generally be avoided if a good thickness reading is desired. **Note that the thickness values appear in the “Image Status” window.**

The last, but also very important step in determining a correct thickness reading for the specimen is to select the correct mean inner potential. Unfortunately, the present data base is limited, but HoloWorks provides the values listed in “Introduction to Electron Holography.”

Processing amplitude data:

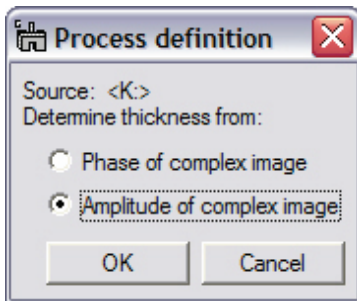


Figure 50 Selecting the amplitude for thickness determination.

When selecting thickness determination from amplitude data, the values are given only as relative values. The following considerations lead to the relative thickness determination:

- $a_0 \dots$ beam intensity with no sample (vacuum area)
- $a(x,y) \dots$ beam intensity with no inelastically scattered electrons in sample area
- $t_{rel} \dots$ relative thickness

$$t_{rel} = a_0 / (a_0 - a(x,y))$$

yielding values close to the relative thickness as obtained from EELS.

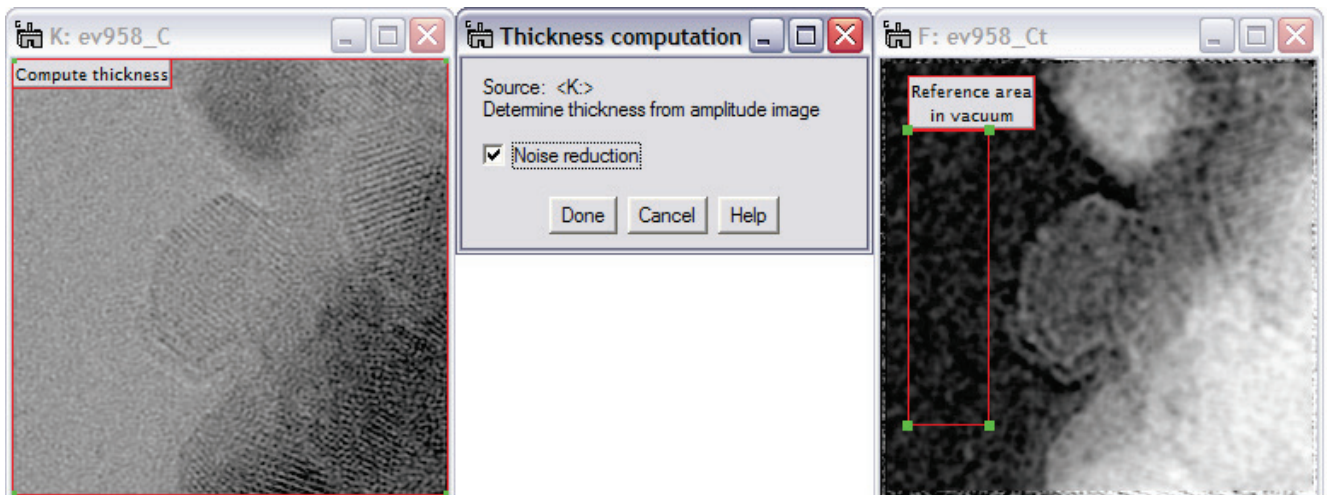


Figure 51 Obtaining a relative thickness value from the amplitude information from an hologram. Note the selection of the reference area in the vacuum and the selection of the “Noise reduction” checkbox do to the generally high noise in amplitude images.

5.5.2 Compute Inner Potential

The compute mean inner potential sub-menu item is structured in direct correspondence with the chapter of M. Gajdardziska-Josifovska and A. Carim in the standard text book “Introduction to Electron Holography.” To summarize, the following equations are being used:

$$\varphi(x, y) = C_E |V_0(x, y)| t(x, y)$$

$$A_n(x, y) = \exp(-t(x, y) / (2\lambda_i))$$

where $A_n(x, y)$ is the normalized amplitude (i.e., amplitude / amplitude in vacuum area) and λ_i is the mean free path length of the electrons in the specimen. The equations are combined to eliminate the often unknown thickness $t(x, y)$:

$$\varphi(x, y) / (-2 C_E \ln A_n(x, y)) = V_0(x, y) \lambda_i(x, y); \quad \text{or :}$$

$$V_0(x, y) = \varphi(x, y) / (-2 C_E \lambda_i(x, y) \ln A_n(x, y))$$

Please note that due to the nature of the expression $\ln A_n(x, y)$, vacuum areas and thin areas are highly noise driven, as in this case $A_n(x, y) \approx 1$ and thus $\ln A_n(x, y) \approx 0$. Therefore noise reduction is offered to help alleviate the problem to some degree. It should also be noted that the equation holds only in the absence of dynamic diffraction contrast, which is not necessarily fulfilled in the example below.

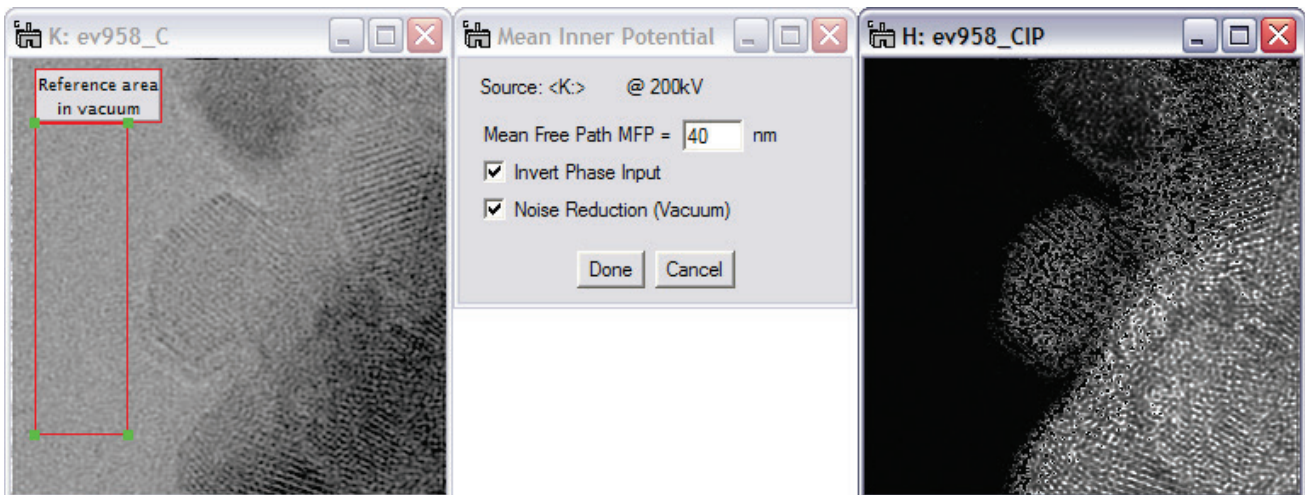


Figure 52 When computing the mean inner potential independent of specimen thickness, the mean free path for electrons in the specimen must be known.

5.5.3 Finding C_E

The parameter C_E in the above equations has been given incorrectly in some publications. This menu item computes its value and prints it to the Results window, including parameters like electron speed (relativistic and non-relativistic) and wavelength.

5.6 Complex To Real

The menu item “Complex to real” converts any complex data, 1D, 2D or 3D into real data. It handles data displayed in ‘Raster’- and ‘Line Plot’-style. Volatile ROIs are always recognized and the ‘selected’ area is processed. The original data set is never overwritten and the new data set maintains all image tags and calibrations of the source image; in addition, data processing details are listed under the ‘History’ tag assuring reproducibility (including information about the ROI (if present)).

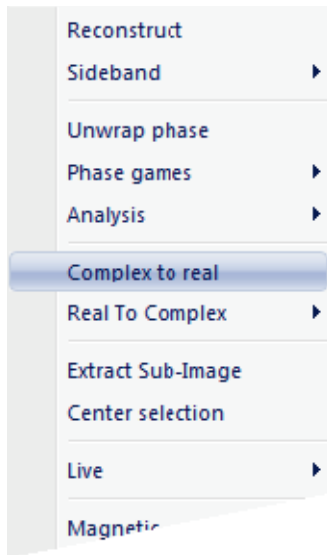


Figure 53 Converting complex images made easy.

If a selection (volatile ROI) is present, the area of the selection will be converted into one or several images of type real.

There are 5 basic options for converting a complex image represented by $a(\mathbf{r}) \exp(ib(\mathbf{r}) + id_0)$. They are defined as:

- Real $a(\mathbf{r}) \cos(b(\mathbf{r}) + d_0)$
- Imaginary $a(\mathbf{r}) \sin(b(\mathbf{r}) + d_0)$
- Intensity $| a(\mathbf{r}) \exp(ib(\mathbf{r}) + id_0) |^2 = a^2(\mathbf{r}) = I(\mathbf{r})$
- Amplitude $a(\mathbf{r})$
- Phase $b(\mathbf{r}) + d_0$; modulus 2π ; i.e., $\in [0, 2\pi[$;

where d_0 is an arbitrary phase constant with no physical meaning (it can however lead to inconvenient ‘phase-jumps’ in the visualization/image).

If ‘optimize phase’ is selected, the constant d_0 will be optimized, minimizing these ‘phase jumps.’

‘Live thread’- mode: whenever the source image updates, the resulting image(s) are updated as well. Currently, this detail is not available for ‘Line Plot’ style images.

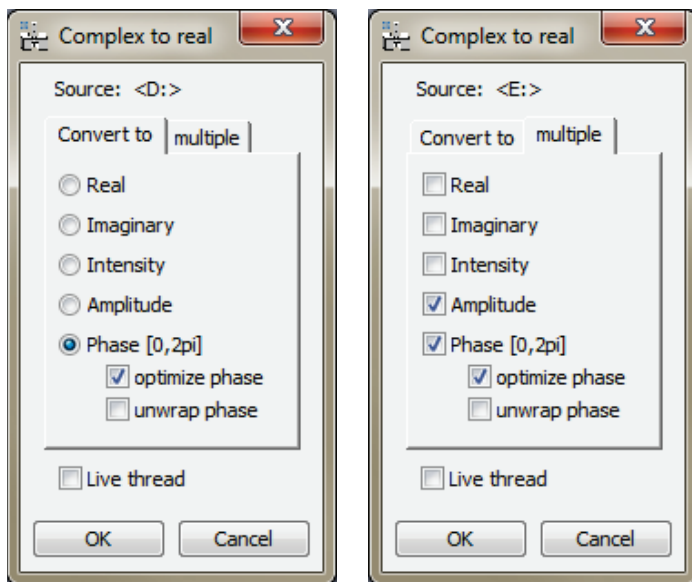


Figure 54 Left: ‘Complex to real’ results in one image. Right: ‘Complex to real’ results in one or several images. ‘Live thread’ makes this function workflow capable.

In most cases, the conversion of an image of type complex is a simple event and thus the UI can be straight forward. As a simple option, several images can be generated from one source.

Thus this function can be used for workflows off 1D and 2D data at the microscope, or even when building a workflow off a 3D data set. Both actions are similar in that whenever the source data, the output data are recomputed.

For a 3D data set however, it is not expected that the total data set is updated. Instead, the data source is considered changed, if the slice of the data set is changed.

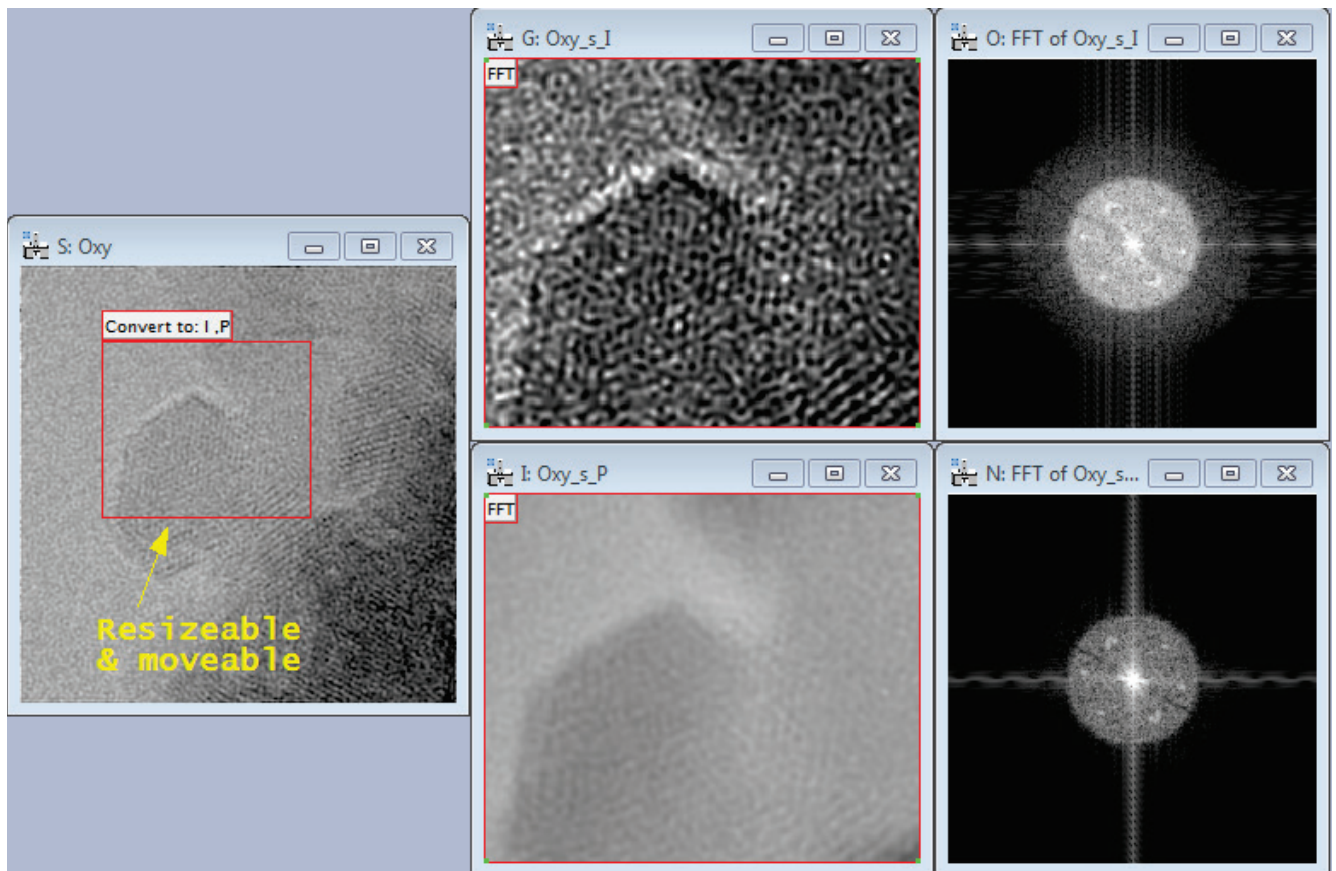


Figure 55 Example of a workflow creation. The Source image appears on the left, where a volatile ROI had been selected. The ‘Complex to real’ function is used to create an intensity- and phase image as shown in the middle. The selection in the source image can be re-adjusted in size and position, and the images in the middle update accordingly. The also update when the source image data change, for example during a live hologram acquisition. To drive the workflow further, live Fourier transforms can be attached. Note that the Fourier transforms maintain the correct aspect ratio.

A note on 3D data sets:

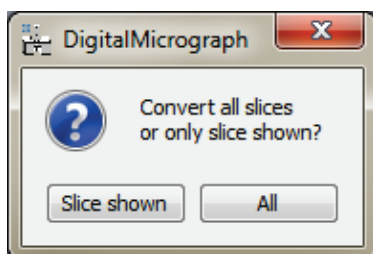


Figure 56 Additional control window for data sets with a z-component (3D data).

3D data sets represent an ambiguity: as to whether a user wants to apply the function to each and every slice, or prefers to work on the currently displayed slice only. Therefore, after the choices have been made as in Figure 54, another window (Figure 56) will appear to offer the processing of either ‘All’ data or ‘Slice shown.’

5.7 Real to Complex

Converts one or two images of type real into an image of type complex. All sub-menu items of “Real To Complex” can be evoked in two ways: With no Shift key down and with the Shift key down.

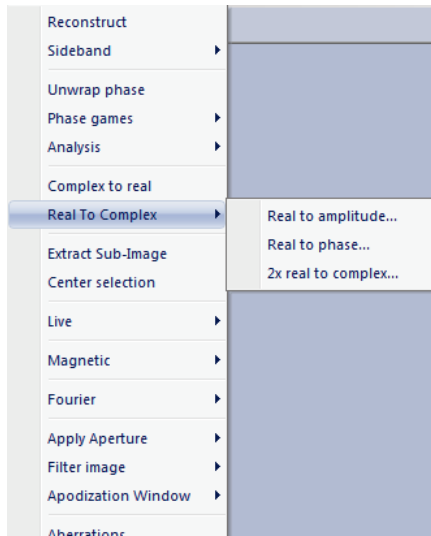


Figure 57 The sub-menu items of “Real To Complex.”

There are several options that convert the real image $g_1(x,y)$ or $g_2(x,y)$ into a complex image $a(r) \exp(ib(r) + id_0)$ which are defined mathematically as:

- i. Real to amplitude $\Rightarrow g_1(x,y) \exp(id_0)$
- ii. Real to phase $\Rightarrow a_0 \exp(ig_1(x,y) + id_0)$
- iii. 2x real to complex $\Rightarrow g_1(x,y) \exp(ig_2(x,y) + id_0)$

where d_0 is an arbitrary phase constant with little physical meaning (e.g., fringe position of a hologram with respect to the CCD camera). The value a_0 represents a constant amplitude value.

Use of the Shift key for all sub-menus evokes an interactive thread that allows adjustment of the parameters d_0 and a_0 . The resulting image contains information about the original image, scaling and conversion parameters.

5.7.1 Real to amplitude

This sub-menu item creates a complex image from one input image of type real as described mathematically in Chapter 5.6. Activating the sub-menu with no Shift key simply returns a complex type image with no further interaction. Activating the sub-menu while the Shift key is held down allows adjustment of the phase constant φ_0 interactively.

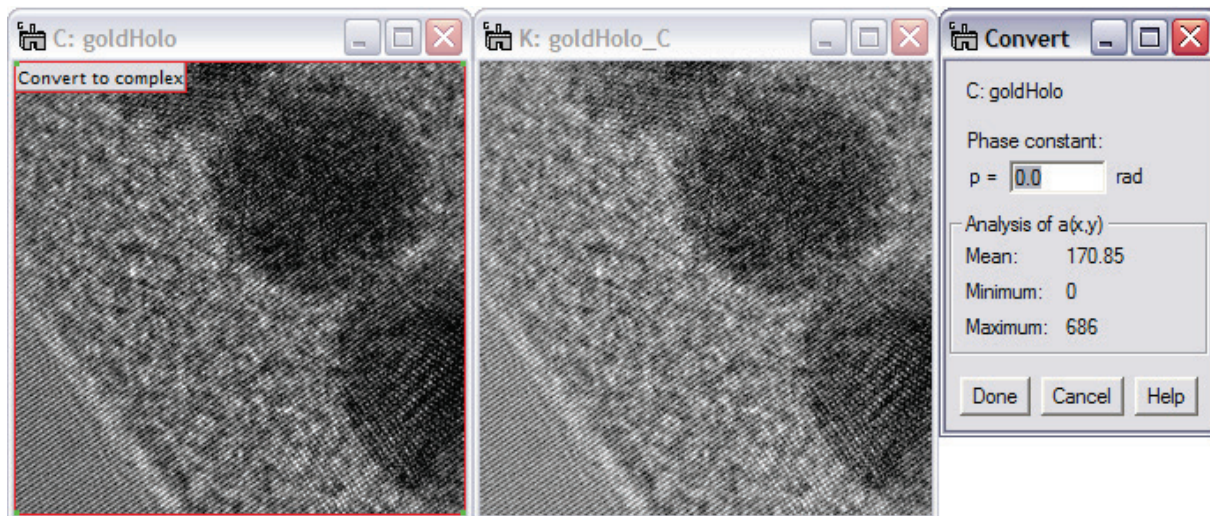


Figure 58 With the shift key down (“mode 2”), the phase constant can be adjusted interactively. With no Shift key down (“mode 1”), the phase constant is always zero. For mathematical details: press Help button.

5.7.2 Real to phase

This sub-menu item creates a complex image from one input image of type real as described mathematically in 3.6. Mode 1: Activating the sub-menu with no Shift key down simply creates from the selected area or the whole image a new, complex image without further interaction. The phase constant φ_0 is set to zero in this case. Mode 2: Activating the sub-menu while the Shift key is held down allows to interactively adjust the amplitude and phase constants a_0 and φ_0 .

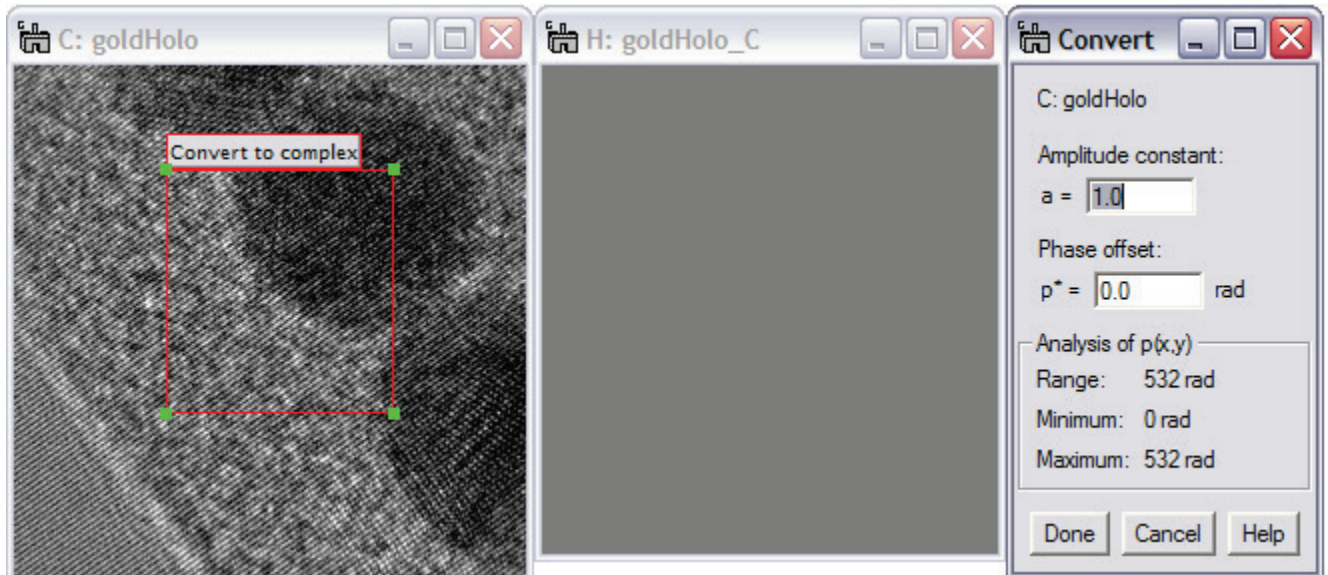


Figure 59 With the shift key down (“mode 2”), the amplitude and phase constants can be adjusted interactively. With no Shift key down (“mode 1”), the phase constant is always zero and the amplitude is 1. Note that the resulting complex image usually looks “empty,” as the display is preset to show the log of the amplitude. For mathematical details: press Help button.

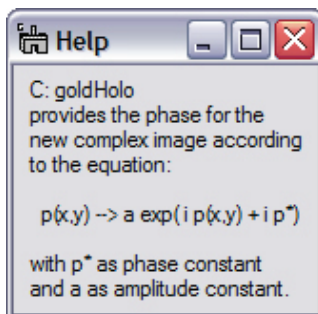


Figure 60 The ‘Help’ window provides mathematical details.

Furthermore, in this mode adjustments made to the input image are translated directly into the output image.

When pressing the help button in the UI window, the mathematical detail is explained on how the conversion is performed.

5.7.3 2x real to complex

This script creates a complex image from two input images of type real. The input images need to be of the same size and the same image can be chosen for both amplitude and phase.

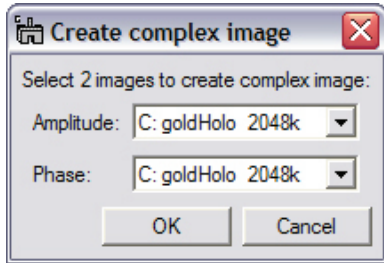


Figure 61 The initial UI window for selecting 2 input images.

Mode 1: Two images are selected through a UI window. The images can be identical but always need to be of the same size. Upon pressing the OK button, the complex image is created from the input images as described in Chapter 5.6, with the phase constant φ_0 as 0. All tag information, scaling and HoloWorks tree information is taken from the amplitude image. Also, the name of the image selected as phase image is transferred to the new image.

Mode 2: Activating the sub-menu with the shift key down, presents the same UI window as in Mode 1, however, the phase constant φ_0 is adjustable and important parameters for creating the complex image are tracked. This allows a change of the input images via e.g., another script and track the changes in important parameters as displayed in Figure 62, left. The changes to the input image are transferred directly to the output image. Note that if the phase dynamic is $> 2\pi$, phase ambiguities will arise when converting the complex image back to a real phase image. Attention should be paid to assure that the amplitude does not become negative. Negative values for an amplitude are mathematically incorrect and will lead to serious artifacts.

Pressing the **Done** button quits the thread, whereas the use of the **Cancel** button deletes the complex image and the UI window. The **Help** button provides the mathematical background to the operation performed with the input image.

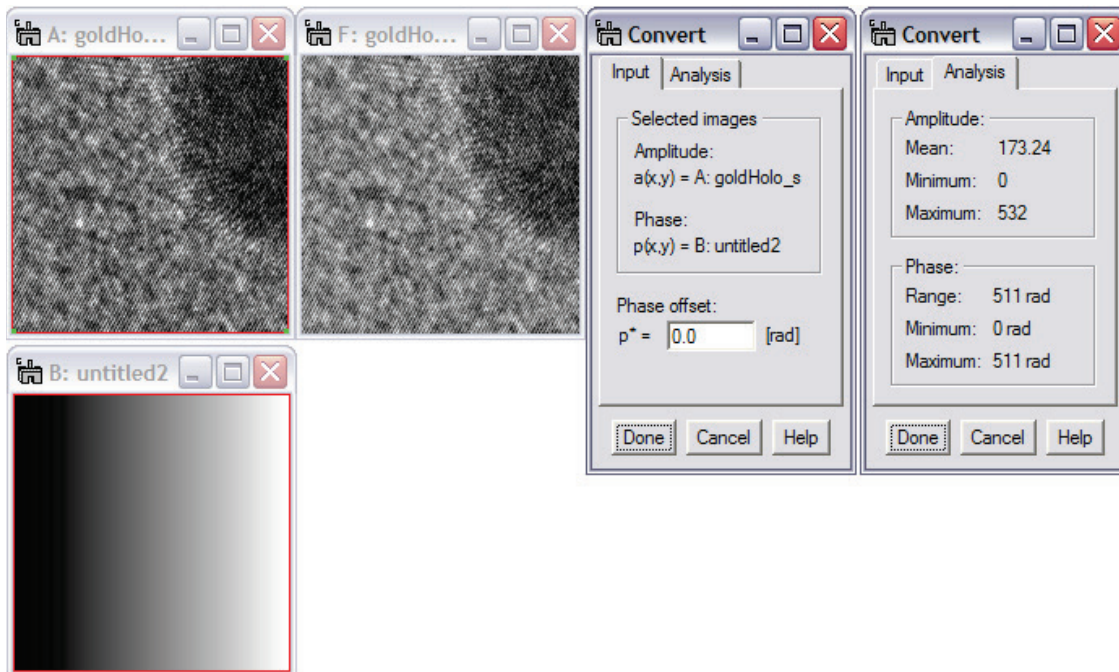


Figure 62 Left: Using the interactive mode with the Shift key down converts two real-type images (goldHolo_s and untitled2) into a complex image. The phase offset is entered in [rad] in the UI window. The input images can be modified while their characteristic values are tracked and the resulting complex image is updated on-the-fly.

5.8 Extract sub-image(s)



This button and its corresponding menu item allows to 1) duplicate an image, 2) extract a selection from an image or 3) extract a sub-set from a 3D data set containing 2 or more slices. The type of the image, e.g., real, RGB or integer, is maintained. The new image maintains scaling parameters and contains all tags from the source image for tracking purposes and to maintain reproducibility. There are two modes to activate the menu item: with no Shift key or with the Shift key down. The menu item works on data of type “image” and “Line plot”.

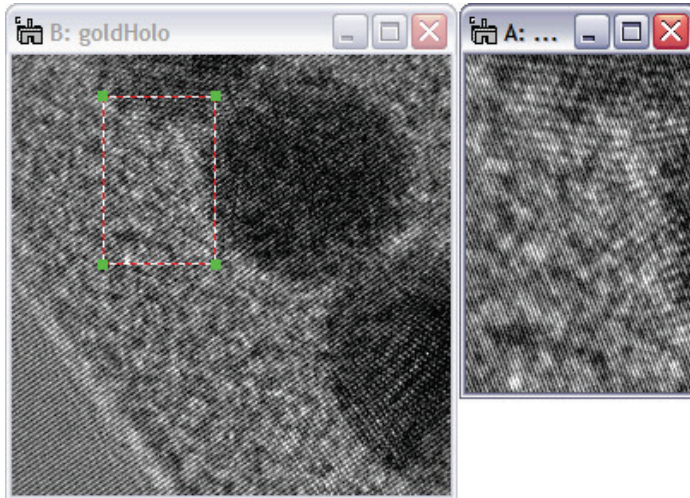


Figure 63 Extract sub-image creates an instant clone of the selected image or a sub-area as indicated by the ROI.

Mode 1: Activating this menu item with no Shift key down, presents a copy of the image or a copy of the selected area next to it. The created image contains all tag information and calibration data of the original image as well as information about the selected area to assert reproducibility of the image data. The HoloWorks history tree is maintained

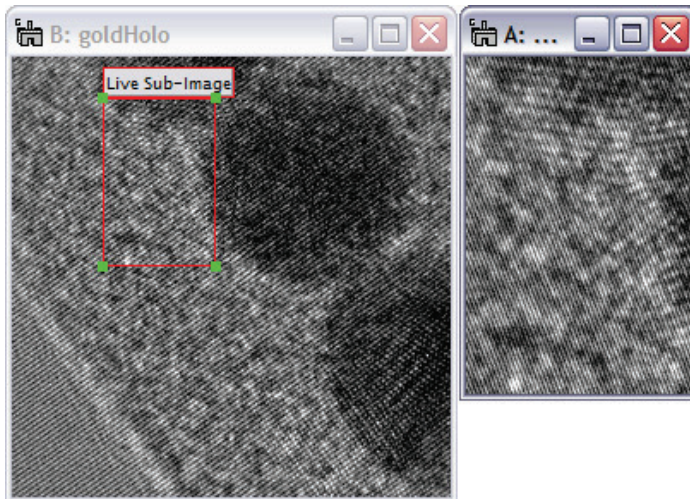


Figure 64 Holding down the Shift key while selecting the menu item provides live update.

Mode 2: Live. Activating this menu item with the Shift key down, provides the same image as in mode 1, however, the ROI is converted to show a solid red outline. The solid outline is an indication that the selected area can be

- i. resized
- ii. moved

while the image on the right-hand side (Figure 64) is updated in real-time. Furthermore, if the image (in this case “goldHolo”) or the area indicated by the ROI is updated, the image on the right-hand-side is updated as well. At any time access to the

standard tools of DigitalMicrograph® is maintained. Deleting the ROI marked “Live Sub-image” ends the interaction between the two images and re-creates the standard ROI seen in Figure 63, left.

In case the input data set is a 3D data set with 2 or more slices, additional options become available for each mode:

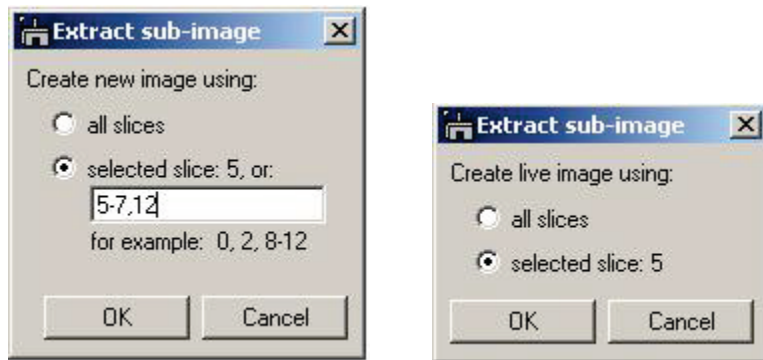


Figure 65 For 3D data sets, slices can be selected that are used to create the new data set. The procedure is logically similar to selecting pages for printing purposes. Right: in case the “Live” option has been activated for 3D data sets, either the presently displayed slice or all slices can be used for creating a new data set.

As illustrated in Figure 65, left, selected slices can be used to create a new data set. In this example, the new data set will contain the following four slices: 5,6,7,12 in exactly this order.

For the “Live” case, the options are somewhat reduced, allowing to choose between the selected slice or all slices. The case of just one selected slice can be used to isolate a slice from the data set for additional processing. By varying the selected slice, each slice can be processed independently.

5.9 Center selection

Centers a ROI on the highest value within the selected area. As shown in Figure 66, an ROI is drawn onto a diffraction pattern. The menu item Center Selection then determines the highest pixel value within the ROI and move the ROI such that it is now centered with respect to the pixel with the highest value. For complex type images, the highest value is determined from the absolute value of each pixel.

This menu item works on real, integer and complex value images.

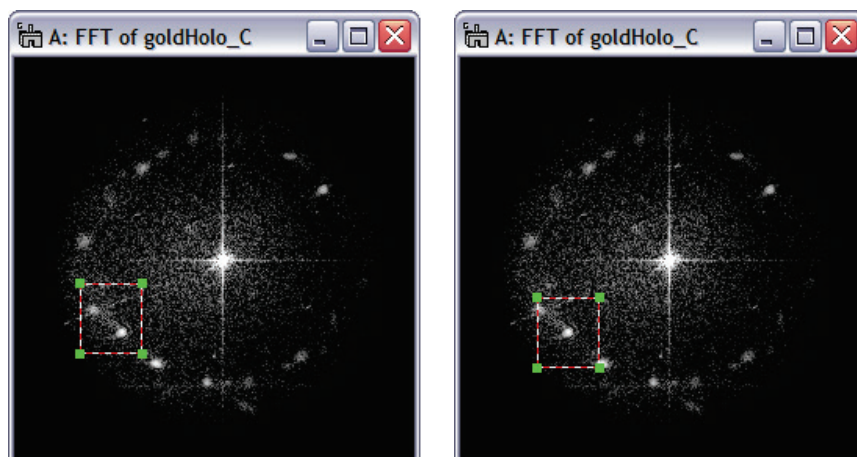


Figure 66 The center selection menu simple centers a ROI around the highest value within the selected area.

5.10 Live

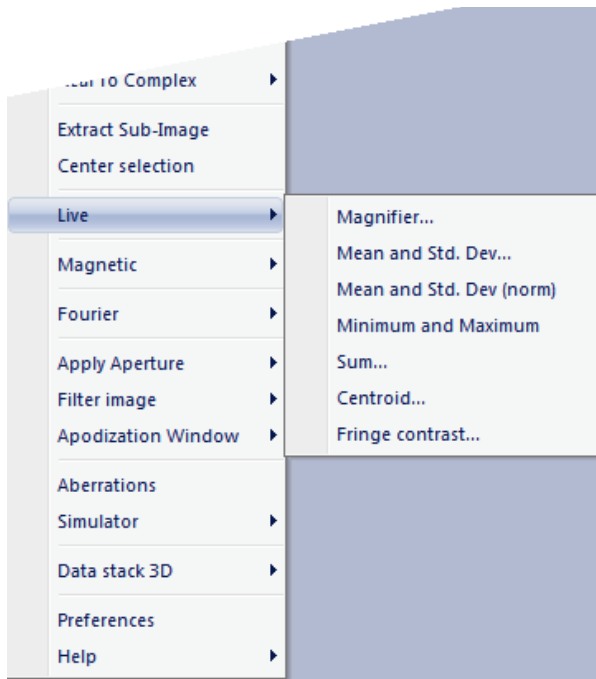


Figure 67 The “Live” menu item with 7 sub-menus. All of them invoke a thread that must be cancelled by the user.

Live provides 7 separate sub-menus:

- 1) Magnifier that renders magnifications without pixilation
- 2) Mean and standard deviation, e.g., for images of type phase-image
- 3) Mean and *normalized* standard deviation, e.g., for amplitude type images (standard deviation is computed from image normalized to ‘1’)
- 4) Minimum and Maximum
- 5) Sum
- 6) Centroid, providing the centroid of a selected area,
- 7) Fringe contrast in sub-areas of the hologram, e.g., for fine-tuning fringe contrast interactively at the microscope (also available via button). Works also to measure peaks from a high resolution image.

All 7 sub-menus evoke a live thread that requires termination by the user (by deleting the ROI). Using the “**Shift**” key while evoking any of these sub-menus activates additional features described below.

5.10.1 *Magnifier...*

provides the means for magnifying small image areas with sub-pixel “resolution.” While ordinary magnifiers will cause details to become pixelated quickly, this magnifier uses a Fourier based interpolation technique to provide a crisp magnification without pixilation.³¹ This Fourier based approach is ideal as long as the image does not contain components that exceed the Nyquist limit.³²

The preset magnification factor is 4 and cannot be changed currently. The selected area must be of the power of two but can be moved arbitrarily while the magnified image (Figure 68) is updated correspondingly. The selected area cannot be resized. For resizing, simply delete the ROI, which ends the thread and changes the ROI from red to red/white. Resize the red/white ROI to the desired size and re-activate the Magnifier.

³¹ The quality of any interpolation can be observed best in Fourier space, as the Fourier transform is especially sensitive to artifacts. The Fourier transform for this magnifier should show no artifacts as long as the basic requirements of digital Fourier processing is not violated.

³² A typical example that violates the Nyquist limit for an image is an image with a sharp jump. It can be generated easily in DigitalMicrograph by creating a new image with a constant value, e.g., 1. A small selection in the image is then changed to a different value, e.g., 10. The Fourier transform of such an image will exhibit streaks that go right to the edge of the image. This is a strong indication that the image is sub-sampled in terms of its Fourier components.

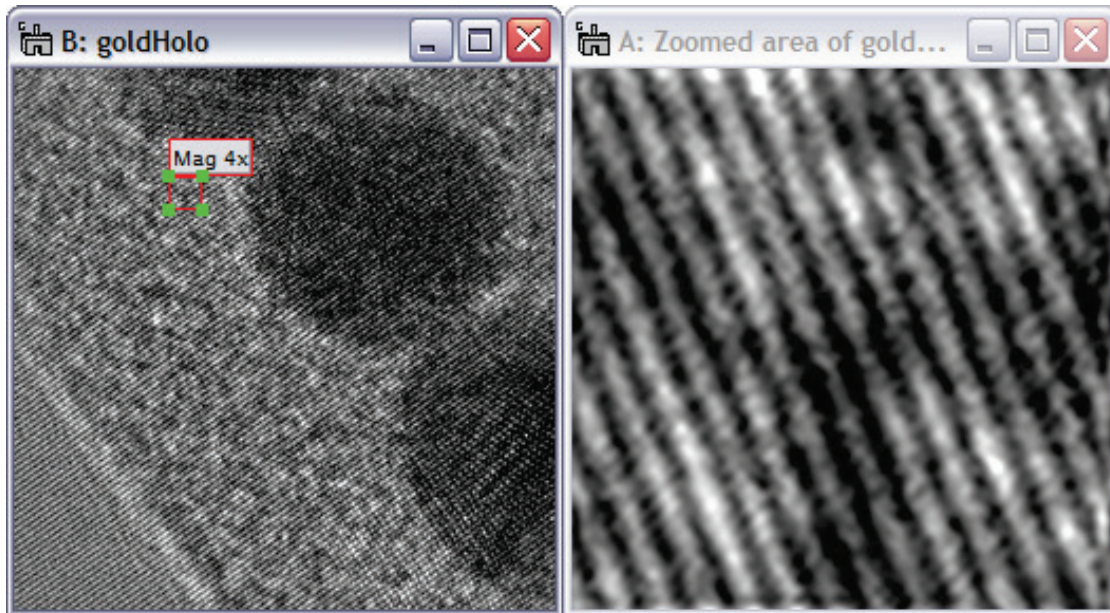


Figure 68 Hologram of small Au particles on amorphous carbon foil. Activating the sub-menu **Magnifier** on a small (power-of-two) selection, or ROI, provides a display of the selected area magnified by 4 using interpolation techniques. The ROI is moveable, but not resizable and the magnification is fixed.

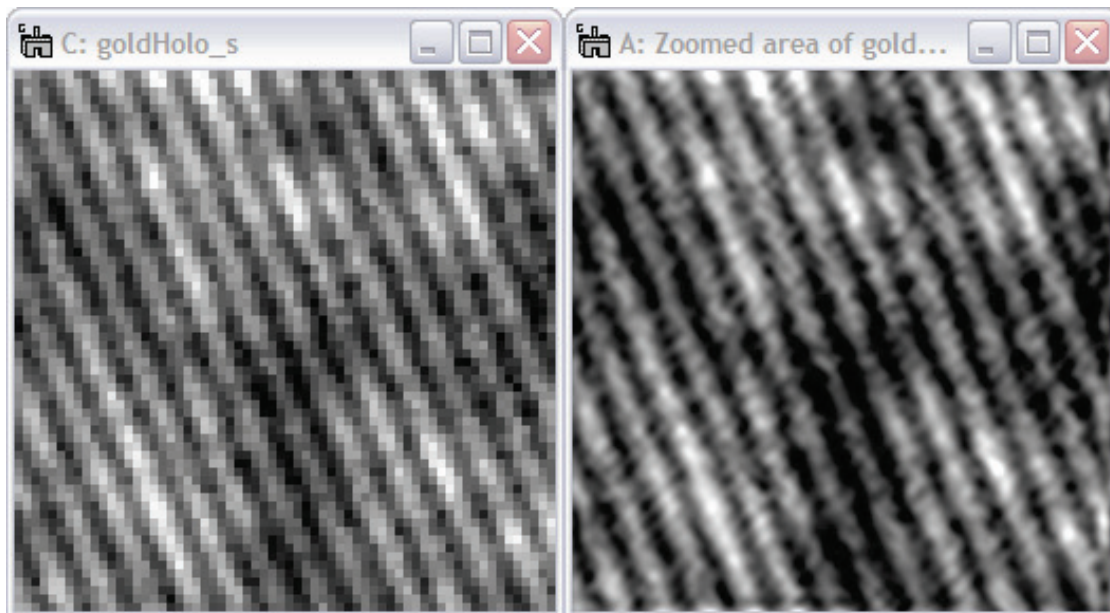


Figure 69 A comparison of images obtained from the same area. Left: using the DM standard magnification tool (left) that displays the raw data. Right: using the Magnifier tool that uses interpolation techniques and suppresses pixilation when magnifying.

The selected area needs to be of the power of two, but does not need to be square. Deleting the ROI or the resulting image ends the live thread characterized by the solid red ROI and redraws the ROI in its standard red/white outline as before. The magnified image using the HoloWorks magnifier contains the information about the original image and the selected area it represents.

5.10.2 Mean and Std. Dev....

provides the mean value as well as the standard deviation value of the pixel values of the selected area. The selected area can be resized at any time and the values are recomputed on-the-fly. The mean value and standard deviation value are also updated when the selected area is moved or the image is updated (e.g., a live image from the CCD camera) or, for 3D data sets, when the slice is changed.

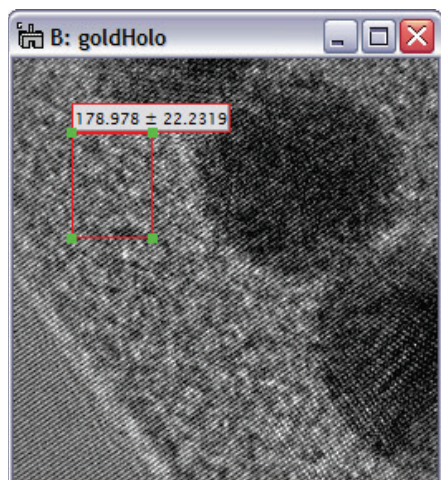


Figure 70 Mode 1: The mean and standard deviation values are updated “live.”

Mode 1: Menu item with no Shift key used computes on-the-fly the mean and standard deviation values of all pixel values within the selected area. These results, as well as the position of the selected area, are also listed in the Results window.

The ROI must be deleted manually.

In all modes, access to DigitalMicrograph[®] standard tools remains available. The results of the Mean and Std. Dev. tool are also displayed in the results window, together with the location of the selection.

Note: when determining the standard deviation for amplitude images, the normalized standard deviation is advised. This simplifies the comparison of noise levels for images with different mean values.

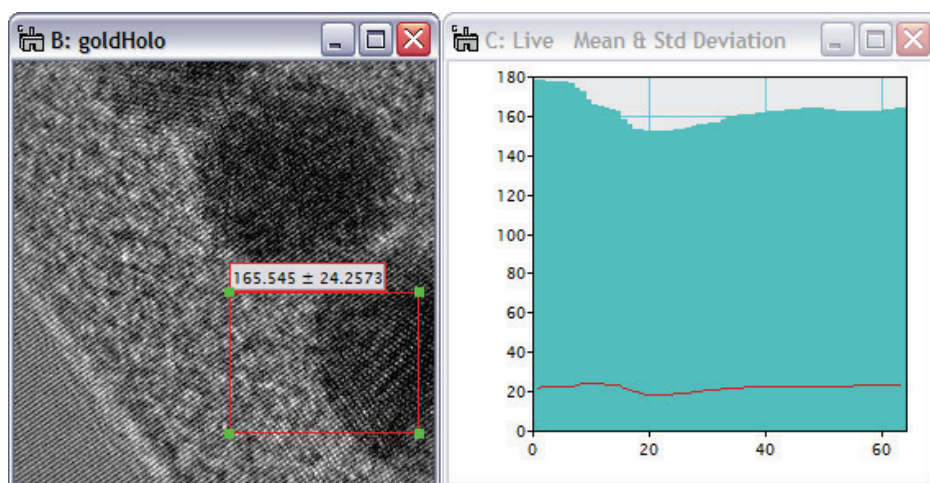


Figure 71 Mode 2: Activating the sub-menu Mean and Std. Dev. with the “Shift” key held down also starts a line scan that tracks both the mean (in “teal”) and the standard deviation (in red) values as changes occur.

Mode 2: Activating this script while the “Shift” key is held down activates a line-scan (see Figure 71) that tracks the value of the mean and the standard deviation with e.g., time. The mean is displayed in solid bars (fill mode) in teal, while the standard deviation is tracked in “line mode” in red. Color and line styles are adjustable by right-click on the line-scan.

Left: tracing the mean and standard deviation values during a live, continuous image acquisition.

5.10.3 *Minimum and Maximum*

provides the minimum and maximum values of all pixels either from the full image or from within the selected area. The image can be updated, the selected area can be moved (if smaller than the actual image) and it can be resized at any time (for 3D data stacks, the slices can be changed as well). The value of the sum is displayed with the ROI as well as in the “Results” window. There are two modes to activate the menu item: with no Shift and with the Shift key down.

Mode 1 (no ‘Shift’ key):

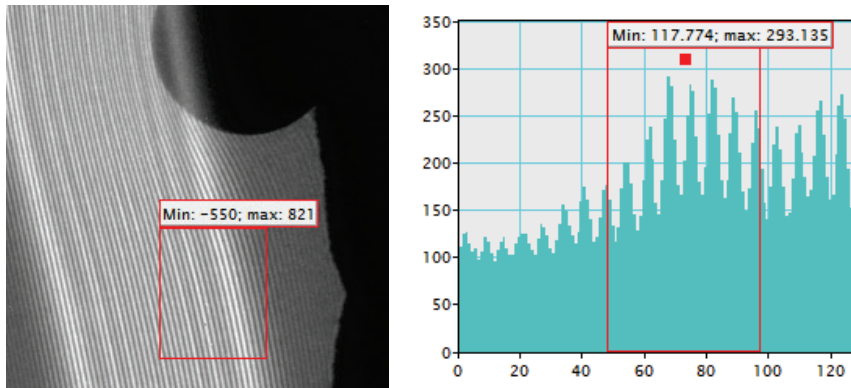


Figure 72 Live computation of the minimum and maximum values in a given region (ROI). Can be used on ‘Raster’ and ‘Line Plot’ style images.

Computes the minimum and maximum within the ROI. The ROI can be moved and resized and the function can be used on live images as well as when sorting through the slices of a 3D data stack.

Manual deletion of the ROI or image ends the thread.

Mode 2 (holding down the ‘Shift’ key when clicking menu item):

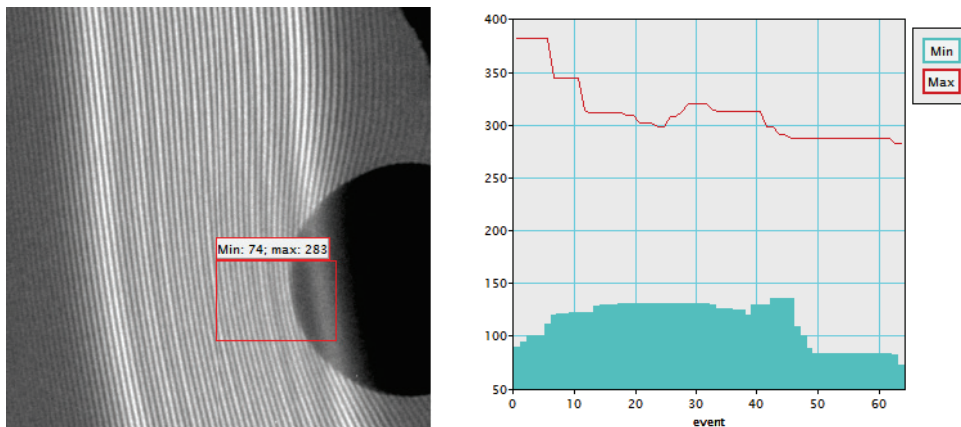


Figure 73 Simultaneous use of ‘Shift’ key when selecting the menu item starts plotting the minimum and maximum values in the given region.

This feature can be used for tracing the minima and maxima, e.g., for a continuous live image acquisition,

Manual deletion of the ROI or image ends the thread.

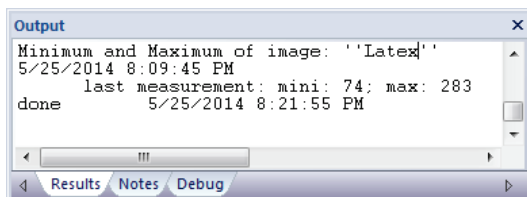


Figure 74 Output generated upon thread exit

When exiting the thread, the last measurement is listed in the Results window.

5.10.4 Sum....

provides the value of the sum of all pixels either from the full image or from within the selected area. The image can be updated, the selected area can be moved (if smaller than the actual image) and it can be resized at any time (for 3D data stacks, the slices can be changed as well). The value of the sum is displayed with the ROI as well as in the “Results” window. There are two modes to activate the menu item: with no Shift and with the Shift key down.

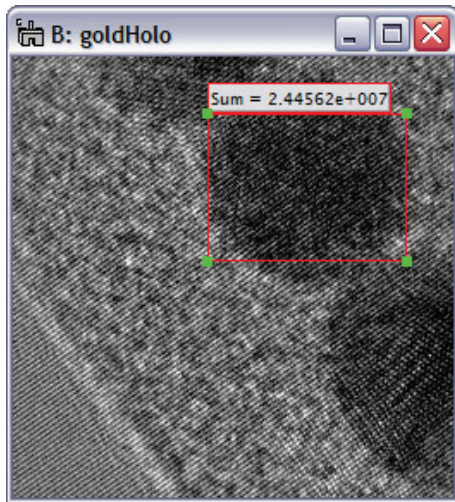


Figure 75 The sum of all pixel values within the selection is updated “live”.

Mode 1: Menu item with no Shift key used computes on-the-fly the sum of all pixel values within the selected area. The sum, as well as the position of the selected area, is also listed in the Results window.

The ROI must be deleted manually to end the thread.

In all modes, access to DigitalMicrograph® standard tools remains available. The results of the Mean and Std. Dev. tool are also displayed in the results window, together with the location of the selection.

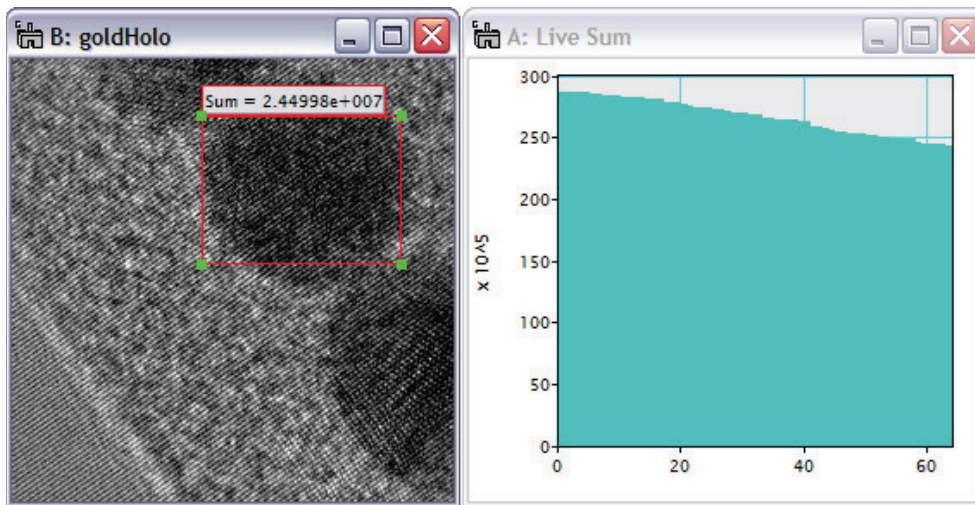


Figure 76 Mode 2: Using the Shift key allows observation of the sum of all pixel values in the ROI. Changes can be caused e.g., during live, continuous image acquisition and by changing the illumination.

Mode 2: Activating the sub-menu Sum with the “Shift” key held down also starts a line-scan that tracks the Sum of the pixels in the ROI (in “teal”) as changes occur. The sum is displayed in solid bars (fill mode) in teal and color and line styles are adjustable by right-click on the line-scan. The results of ‘Sum’ are also displayed in the results window, together with the location of the selection.

5.10.5 Centroid...

... provides the location of the centroid from within an ROI.³³ The image can be updated; the red ROI can be moved and resized. The location of its centroid is displayed as its label - as well as in the “Results” window. The Origin of the coordinate system is indicated with a yellow ROI, centered on the Origin. The yellow ROI can be moved to establish a new origin. The new Origin is reflected in the red ROI automatically. Deleting the yellow ROI avoids accidental movement.

Deleting the red ROI ends the script.

There are two modes to activate the menu item: with no Shift and with Shift key down.

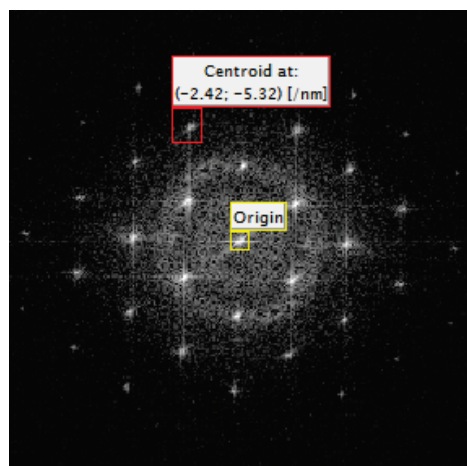


Figure 77 The centroid of the selected area is updated “live”.

Mode 1: Menu item with no Shift key computes on-the-fly the location of the centroid of all pixel values within the selected area. The location of the centroid is also listed in the Results window.

The ROI must be deleted manually to end the thread.

In all modes, access to DigitalMicrograph® standard tools remains available. The results of the Mean and Std. Dev. tool are also displayed in the results window, together with the location of the selection.

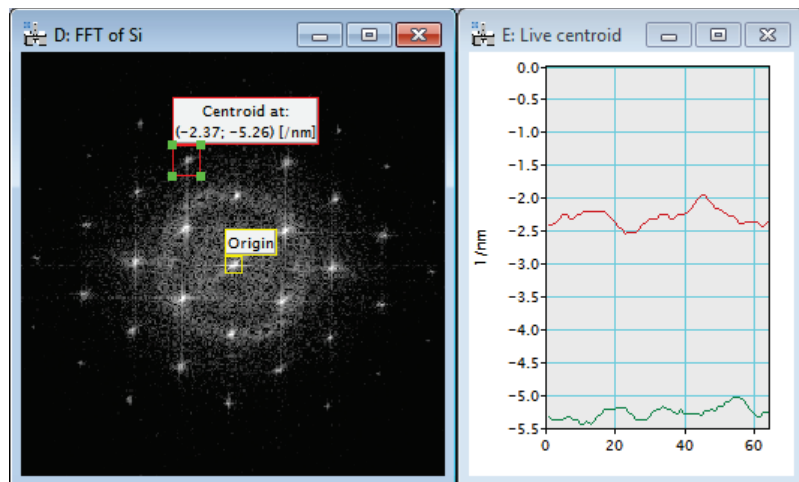


Figure 78 Mode 2: Using the Shift key allows observation of the centroid location for the ROI. Changes can be caused e.g., by moving the object in the image.

Mode 2: Activating the sub-menu Centroid... with the “Shift” key held down also starts a line-scan that tracks the centroid location for the ROI as changes occur. The x-direction is displayed in red, while the y-direction is displayed in green. Color and line styles are adjustable by right-click on the line-scan. The results of ‘Centroid...’ are also displayed in the results window. The location of the centroid within the ROI is given with respect to the Origin of the image as defined in the Image Display Info under Calibration.

³³ For a definition of centroid, see e.g., <http://mathworld.wolfram.com/GeometricCentroid.html>

5.10.6 Fringe contrast....

provides the a) fringe contrast, b) fringe drift and c) hologram quality ($\mu\sqrt{N}$) either from the full image or from within a selected area for both 2D and 3D data sets. The selected area must be of the power-of-two, but may be square or rectangular. There are two modes to activate the menu item: with no Shift key or with the Shift key down. For 2D and 3D images, different options are available:

- 2D images, mode 1: Fourier-based, real-time computation of all three parameters. The parameter fringe contrast (Figure 79) is updated on-the-fly whenever the image is updated (e.g., a live image from the CCD camera) or whenever the red ROI is moved.

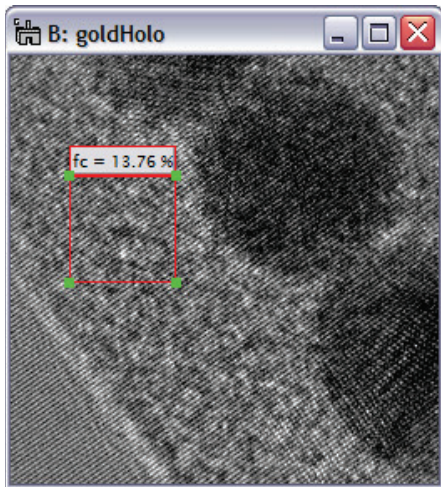


Figure 79 Fringe contrast measurements allow determination of hologram quality.

- 2D images, mode 2: Activation with the Shift key down: The fringe contrast, fringe drift and hologram quality values are now plotted in a separate window (Figure 80). The fringe drift is tracked modulo 2π . In addition, the sampling rate is provided as removable text annotation. This mode exists also in the HW5.0 user interface and activated via the μ button. This mode can help to determine the maximum acceptable exposure time (based on the fringe drift), maximize hologram quality or fine-tune fringe contrast (electrons: astigmatic illumination).

- 3D images: Fourier-based, real-time computation of all 3 parameters within the ROI either for the shown slice or all slices as displayed in Figure 81. Selecting all slices plots a fringe contrast, fringe drift and hologram quality in a separate window as shown in Figure 82.

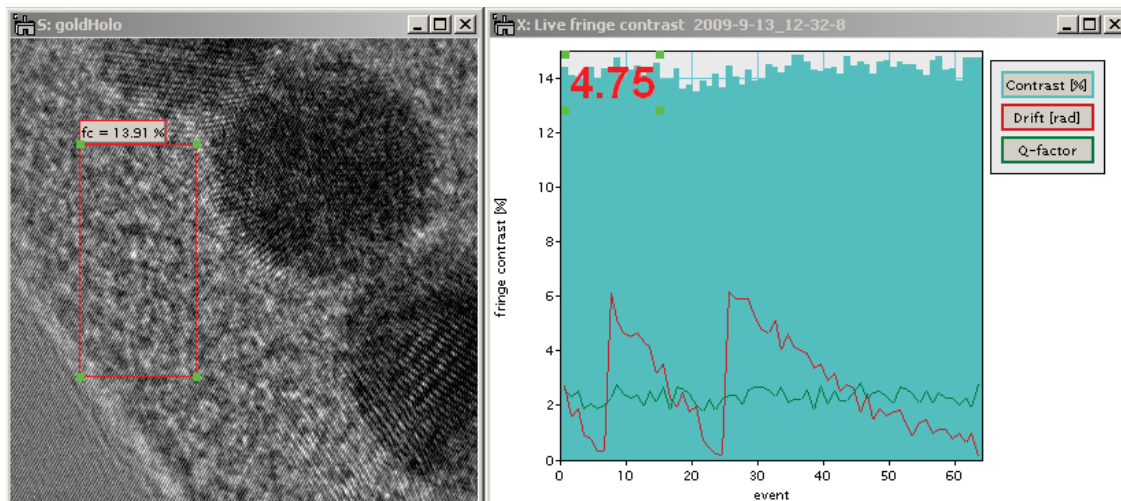


Figure 80 Using the 'Shift' key when starting the sub-menu allows observing live i) fringe contrast, ii) fringe drift and iii) hologram quality. Values are updated by moving the ROI, updating the image (for example via the CCD camera) or flipping through a 3D data stack.



Figure 81 Computation of the fringe contrast from 3D data sets. Choose between evaluation within the selected slice or evaluate the full data set.

As can be seen from Figure 82, the fringe contrast is stable and the drift of the fringes (with respect to the CCD camera) was relatively small as it appears from the red trace. Given for example an exposure time of ~2 seconds for each image, and 1 second for readout time, an exposure time of up to 30 seconds would likely yield little reduction in fringe contrast as the expected fringe drift would be $<2\pi/6$ for most holograms.

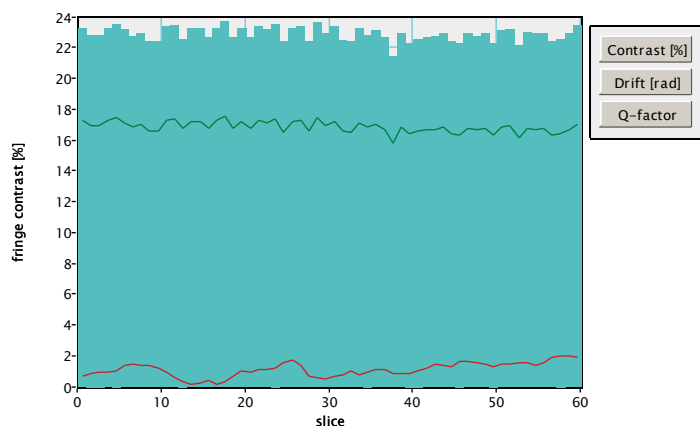


Figure 82 Plot as obtained from a 3D data set. Fringe contrast is slightly above 20% and the fringe movement over 60 exposures is relatively small (< 1 fringe).

- 3D images, mode 1: selecting “evaluation in displayed slice” (upper radio button in Figure 81). The behavior corresponds directly to Mode 1 of 2D images, with the exception that there the image update is replaced by the slice selection. The selection of different slices is facilitated by DM’s floating window “Slice” an example of which is displayed in Figure 83.

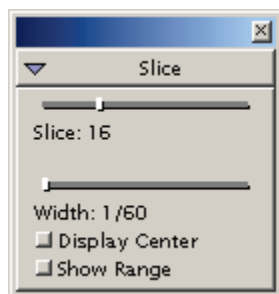


Figure 83 This DM window allows selection of a certain slice from a 3D data set.

- 3D images, mode 2 with the Shift key down, corresponds again to the 2D images, mode 2, but the response to updated images is replaced by the slice selection. The selection of different slices is facilitated by DM’s floating window “Slice” an example of which is displayed in Figure 83 as already mentioned.

In addition to displaying the fringe contrast directly with the ROI, the fringe contrast is also tracked in the Results window. There, the fringe contrast is tracked together with the position of the selection as well as the position of the sideband.

Caveat: It should be noted that the algorithm used for computing the fringe contrast requires that the fringes extend throughout the selected area. If the fringes extend only through part of the selected area, the accuracy of the results will decrease.

5.11 Magnetic / Induction / Contour

This new function was added to HoloWorks version 5.0.7 as a direct consequence of a user request.

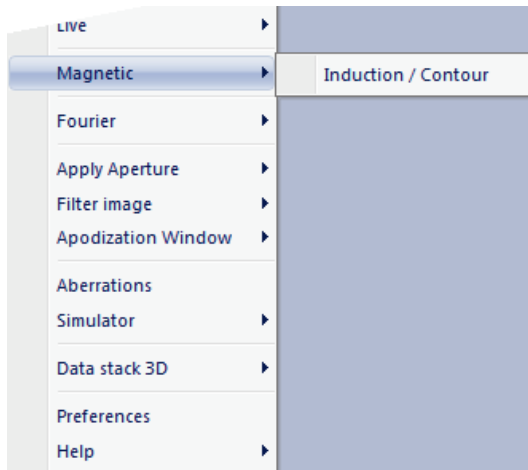


Figure 84 New in HW 5.0.7.

This function is a specific extension for holographic work on magnetic samples. It requires a phase image as input, preferably phase-unwrapped. Two colorized images are computed from the phase image: the magnetic induction map and the magnetic contour map. The complete workflow is displayed in Figure 85.

This function can be used at the microscope as a direct extension of the hologram reconstruction workflow. Thus, as the live holograms are acquired and the live phase images are computed, the contour maps are updated as well. Basically, the sample can be moved and the induction and contour images are updated at frame rates > 2 fps. This function may work in a limited manner in GMS versions < 2.0 . Significant CPU power is required.

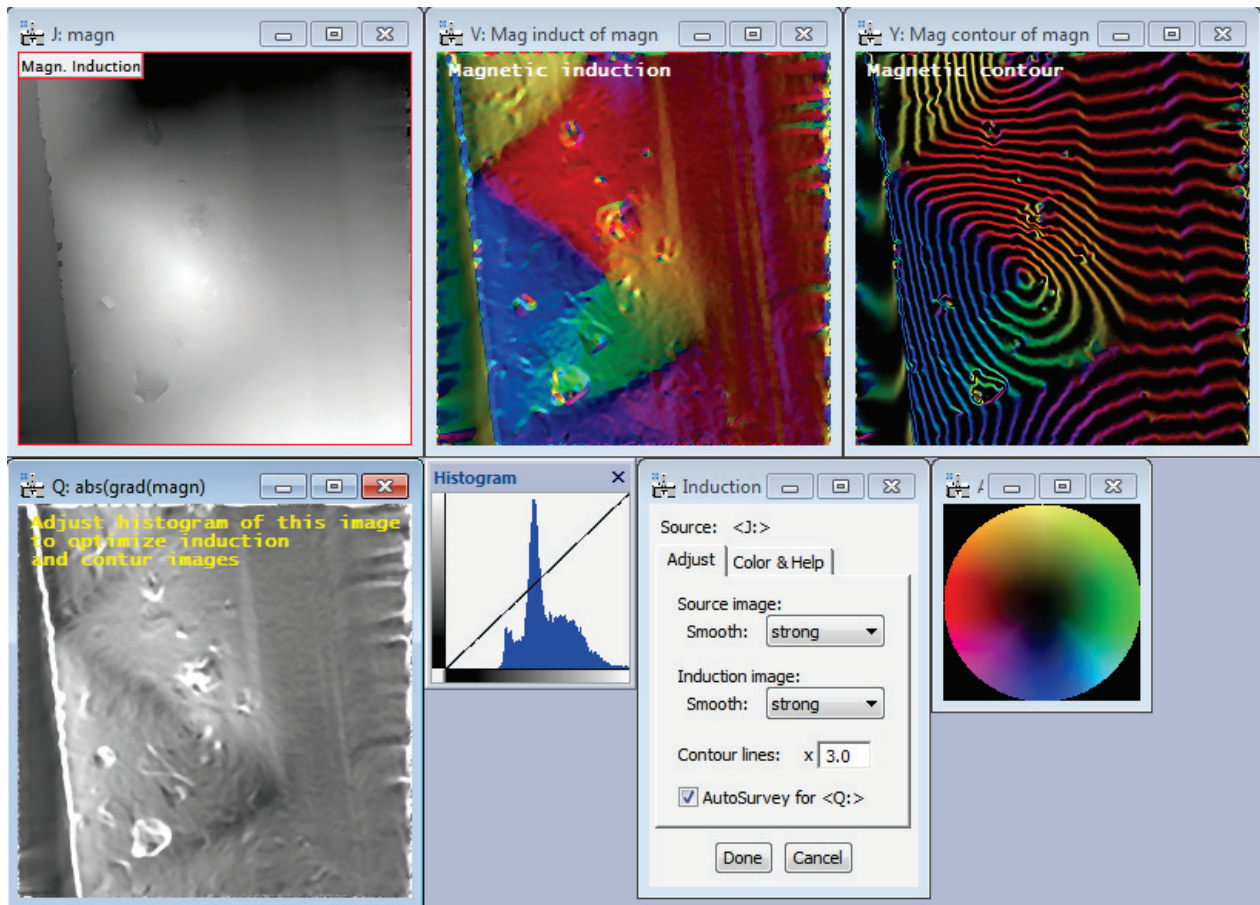


Figure 85 Workflow, processing a phase image of a strongly magnetic sample, courtesy of Dr. Suk Chung. When adding this workflow to a live reconstruction process, the computational requirements are high.

5.11.1 **Detailed description**

As already indicated, this colorized visualization of the magnetic induction and contours requires a phase image – phase unwrapped as input. The process can be used off-line on existing data, but it may be more interesting to use this workflow at the microscope.

Basically, the gradient ('Nabla' operator) of the existing phase image computed, which results in a two-dimensional vector. From this vector, its direction (angle) and amplitude can be determined for each point in the image (but the outermost pixels). Using a color scheme for the angle from that vector, weighted with the computed amplitude of the vector (high amplitudes result in brighter colors), the color image for the magnetic induction is obtained.

At the same time, the phase image can be phase amplified and the phase-contour-lines computed. The contour lines are then superimposed on the on the magnetic induction image and the magnetic contour image is obtained.

Further details:

Gradients from noisy electron images have the tendency to overemphasize the noise in these images. Therefore, filtering is almost always essential. Filtering these images requires significant CPU capacities, but should be no problem for off-line processing.

Two filter levels are employed: filtering of the source image and filtering after the gradient. Both filters have different effects and the best recommendation is to just go ahead and try.

An important detail is the display of the absolute value of the gradient. By adjusting its intensity range via the histogram (the gray scale image), the histogram (from GMS) can be used to improve visualization. From experience, it is best to leave some space in the histogram on the left side. If the 'AutoSurvey' checkbox is checked, this will happen automatically.

Using this capability for on-line processing is a fantastic way of getting results from the microscope. Start the live image acquisition via Gatans UI, add the reconstruction process from HoloWorks, add the phase live unwrap and add computation / visualization of the magnetic induction and contour images.

Last, the color distribution can be adjusted by rotating the color wheel. This is possible via the second tab in the control window (Color & Help).

A fast quad core processor on a 64bit system is definitely recommended.

5.12 Fourier

The first two sub-menu items provide a similar feature as the Fourier and its inverse transform provided by Gatan under the Process menu. However, with these sub-menus, the history tree of the original image is preserved. All four submenus can be evoked in two modes: with the Shift key pressed or with the Shift key.

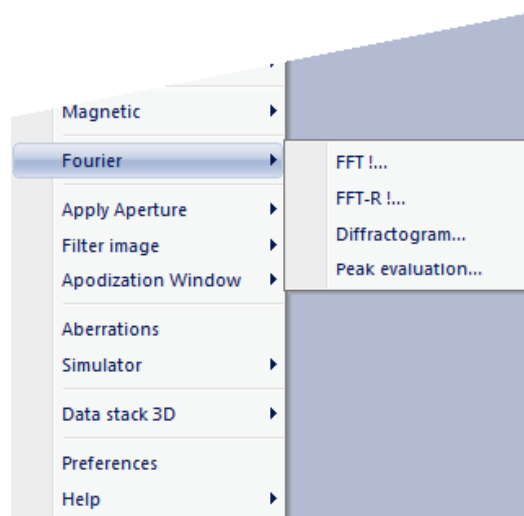


Figure 86 The sub-menu items of the Fourier menu.

Mode 1: With no Shift key pressed, the sub-menu items are executed and all Tags, scaling parameters and the history tree are maintained.

Mode 2: With the Shift key pressed, all sub-menus become interactive. The Fourier transform corresponds directly to the Process/Live/FFT sub-menu of DigitalMicrograph[®]. The sub-menus

- i. FFT
- ii. FFT-R!
- iii. Diffractiongram
- iv. Peak evaluation

follow the rules established by the DigitalMicrograph[®] routines. Their functionalities are detailed in the following.

5.12.1 FFT !

This fast Fourier transform is accepting 1D, 2D and 3D images of any size. The resulting Fourier transform is two-dimensional³⁴ and is always of the power-of-two. E.g., an image of size 468×812 pixels will be transformed into an image of size 1024×1024 pixels, as displayed in Figure 87.

Although surprising initially, it is justifiable from the perspective of Fourier optics: if a rectangular aperture is applied e.g., in the selected area aperture), the back focal plane³⁵ would not be skewed.

So this is how the **FFT!** is understood: images with a pixel number not equal to a power-of-two value are considered as actual square and power-of-two images but limited by a selected area aperture. This preserves the calibration of the scaling parameters in Fourier space like in a real microscope, while the standard implementation of Fourier transforms in image processing sacrifices the resemblance to real world Fourier optics for computational speed, as explained in Figure 88 - Figure 89.

The sub-menu can be activated in two modes. In Mode 1, a complex image is created from the original image, or of its selected sub-area. All Tags, scaling parameters and the history tree are maintained in the new image. In Mode 2, when evoking the sub-menu while holding down the shift key, the selection turns into an ROI which can be moved and resized, while the resulting FFT is updated on the fly. Changes of the original image are recognized as well and appear in the FFT. Deleting the ROI ends the thread.

³⁴ No three-dimensional Fourier transform is provided.

³⁵ i.e., the Fourier transform

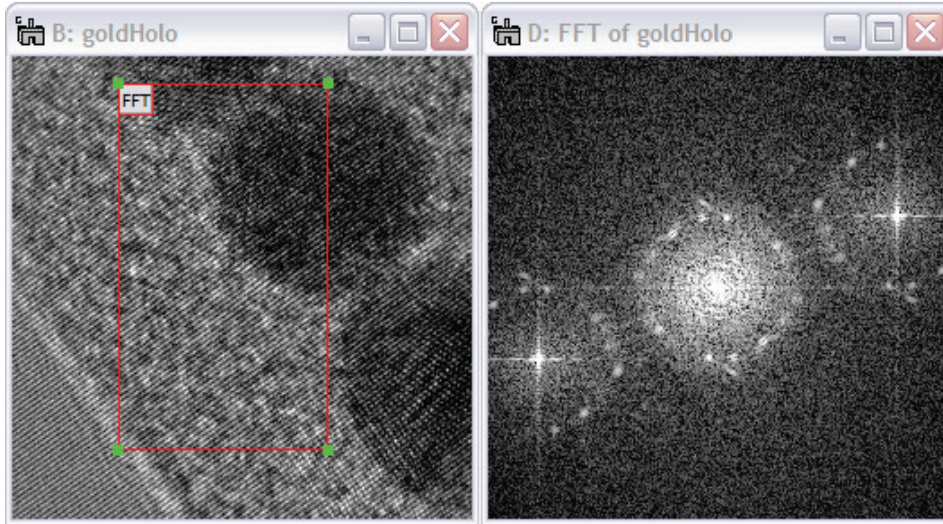


Figure 87 The HoloWorks Fourier transform allows to display the Fourier transform of any size area at the expense of minor streaking. The selected area, indicated in red, is 468 by 812 pixels.

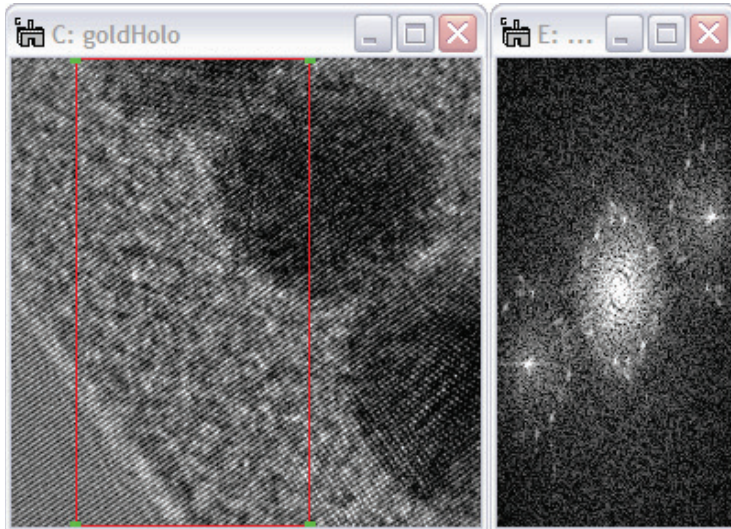


Figure 88 The standard Fourier transform from a non-square area outlined in red appears skewed due to the standard algorithms in image processing which are designed to compute the minimum required number of pixels in Fourier space.

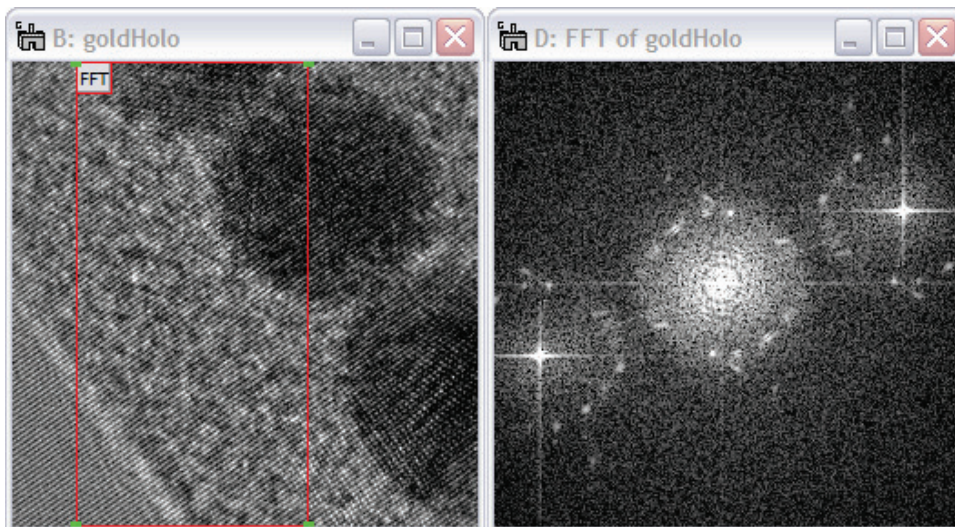


Figure 89 The Fourier transform of HoloWorks preserves the aspect ratio in the Fourier plane independent of the area selected. This is the behavior experienced at the microscope.

5.12.2 *Inverse FFT*

This fast inverse Fourier transform is accepting only square 2D images of the power-of-two. The application of filters is highly recommended over the use of an inverse Fourier transform on non-square images.

This sub-menu can be activated in two modes. In Mode 1, a complex image is created from the original image, or of its selected square and power-of-the-two sub-area. All Tags, scaling parameters and the history tree are maintained in the new image. In Mode 2, when evoking the sub-menu while holding down the shift key, the selection turns into an ROI which can be moved but not resized, while the resulting inverse FFT image is updated on the fly. Changes of the original image are recognized as well and appear in the inverse FFT image. Deleting the ROI ends the thread.

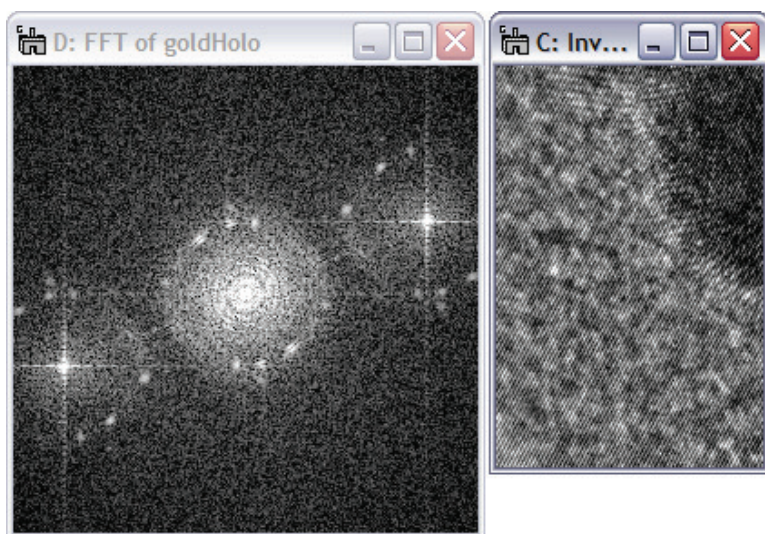


Figure 90 The inverse Fourier transform reinstates the aspect ratio of the original image.

5.12.3 *Diffraction*

The diffractogram is similar to the FFT, but heavily suppresses artificial streaks in the resulting Fourier transform. It accepts 1D, 2D and 3D data sets. The streak reduction is achieved by applying a two-dimensional Hanning window [W.J. de Ruijter] to the image before the Fourier transform is applied (this is called Apodization). Instead of the Fourier transform of a rectangular or square window being convoluted with each spot in the Fourier space, the Fourier transform of the Hanning window is convoluted instead. Since the Hanning window is a smooth function, the streaks become suppressed. In addition, the application of a two-dimensional Hanning window allows determination of peak amplitude and position with sub-pixel accuracy. Therefore, the use of the Diffractogram should replace the Fourier transform (FFT) when it is intended to determine e.g., lattice spacings and their amplitudes in Fourier space. Note that the application of the Hanning window suppresses image data close to the edge of the image or ROI. Assure that the essential image information is in the center of the image and not at its edge. The sub-menu Diffractogram can be activated in two modes:

Mode 1: a complex image is created from either the original image, or of its selected sub-area. All Tags, scaling parameters and the history tree are transferred to the new image.

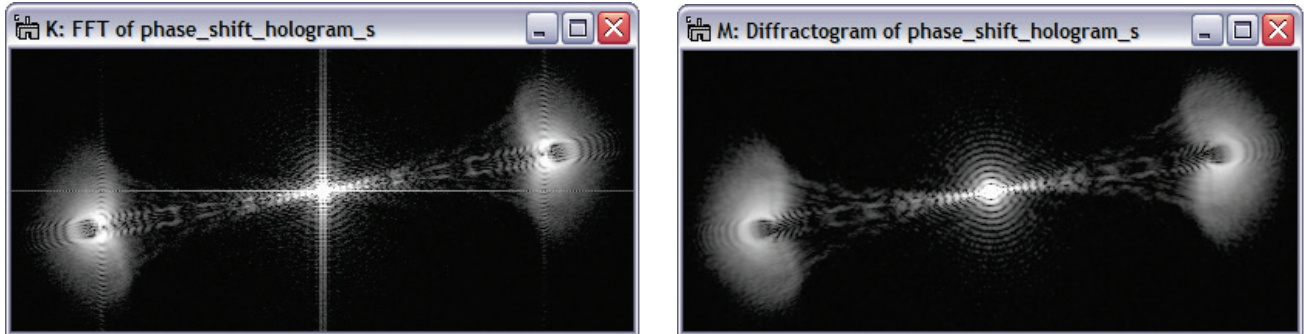


Figure 91 Comparison of a Fourier transform (left) versus a diffractogram (right). Due to Apodization in real space, the streaks have disappeared in the image on the right hand side. Also due to the Apodization, some information close to the edges of the image is suppressed. Both images have been cropped for better display.

Mode 2: Activating this menu item with the Shift key down, provides the same image as in mode 1, however, the selected area is converted to a solid red ROI. The solid outline is an indication that the selected area can be:

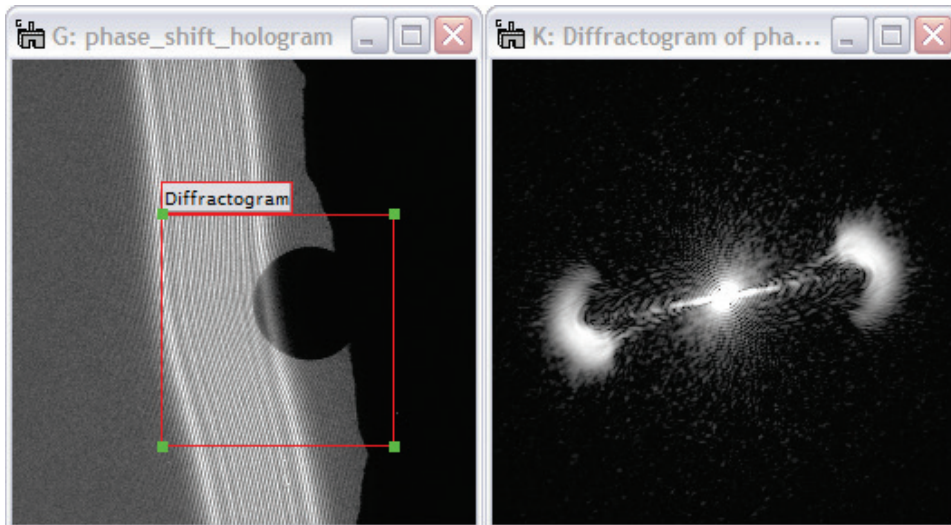


Figure 92 Activation of the sub-menu Diffractogram with the Shift key down allows to observe the Fourier space while moving the ROI or changing the contents of the image itself.

- i. resized
- ii. moved

while the image on the right-hand side (Figure 92) is updated in real-time. Furthermore, if the image or the area indicated by the ROI is updated, the image on the right-hand-side is updated as well. At any time access to the standard tools of DigitalMicrograph® is maintained. Deleting the ROI marked

“Diffractogram” ends the interaction between the two images and re-creates the standard ROI with its red/white outline.

5.12.4 Peak analysis

From a small selected area, the maximum pixel value is computed and displayed as label to the ROI. This sub-menu is designed to work on diffractograms (see 3.11.3) but also can work on regular Fourier transforms (as of HW version 5.0.1).

The main difference between using a regular FFT and a Diffractogram is that peak position (and amplitude) determination is more accurate from the latter if the lattice extends throughout the investigated area in real space. “Lattice fringes throughout” is important because then the peaks are convoluted with a Hanning window and be evaluated using the assumption that the Hanning window shapes the actual peak (there exists a mathematical solution for this case).

If however the lattice does not extend throughout the image area from which the Diffractogram was taken, the peak in Fourier space is convoluted with a “shape” function of the e.g., crystal, and the key assumption that a Hanning window shapes the form of the peak becomes void.

HoloWorks does distinguish between simple Fourier transforms and Diffractograms. If a simple Fourier transform is used for peak evaluation, then a second order interpolation will be used instead.

In short, for the highest accuracy for peak- position and amplitude, it is recommended to create a Diffractogram from the area that contains the fringes of interest throughout and **not** just partially. If a sub-pixel accuracy of about 1/3rd to one 1/5th is sufficient, then using a regular FFT and the Peak function is sufficient and the lateral extent of the lattice in real space can be ignored – mostly.

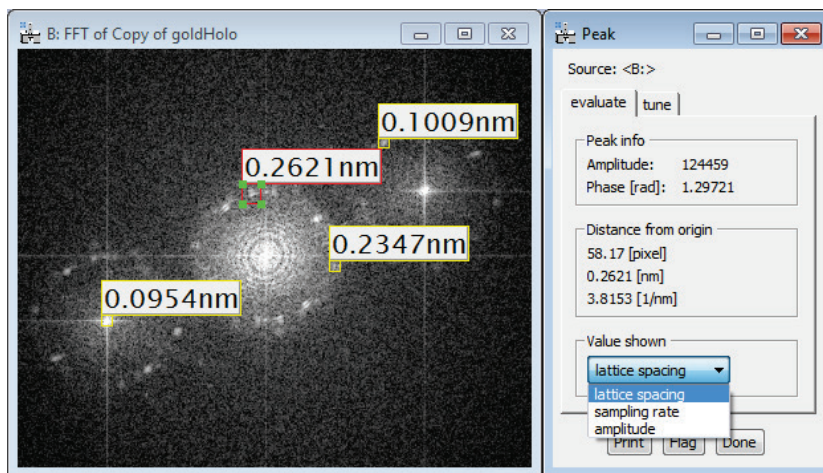


Figure 93 Determining peak positions and peak amplitudes. Peak positions are estimated with a better than single-pixel accuracy. The red ROI indicates the live thread and the yellow flags have been generated by pressing the “Flag” button.

The UI window appears next to the image and displays many details of the peak evaluation. If the image is calibrated, the position of the peak (within the red ROI) is estimated and given in number-of-pixels as well as in spacings and reciprocal spacings as indicated in Figure 93. In addition, the estimated amplitude and phase (if image is complex) for the actual peak position is displayed. This is important for almost all cases, as the actual peak position generally falls in between pixels and a direct reading of the amplitude of the pixel with the highest value can be rather inaccurate.

- Using the “Print” button in the UI prints the details of the peak into the results window with successive numbering.
- Using the “Flag” button draws a yellow ROI centered on the peak displaying a tag indicating the corresponding spacing or amplitude, as selected.

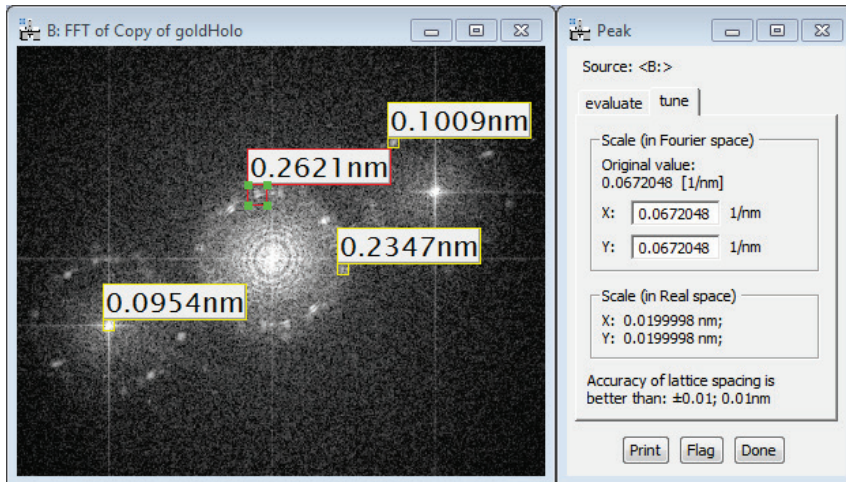


Figure 94 The Peak function supports re-scaling of images to determine accurate scale parameters. Real space values are given accordingly. The accuracy should be significantly better than $\pm 1/2$ pixel, i.e., here: ± 0.01 nm.

It is also possible to use the Peak function for scaling an image with high accuracy. In this case, select the “tune” tab. The scale of the Fourier transform can be adjusted until the red ROI shows the true value. The yellow flags are not updated at this point, but this may be an additional option for a later version of HoloWorks.

Note that the accuracy for the scaling is indicated as better than single pixel accuracy, which in this case corresponds to better than ± 0.01 nm.

For 3D data sets, or when a live diffractogram (or FFT) has been initiated, the peak data are sensitive to changed input. As an example, such a set-up is shown in Figure 95. From a hologram (on the left), a live diffractogram is generated (middle) that contains an active ROI for peak evaluation. The UI on the right-hand side provides the information about the peak. Reposition of the live ROI in the left image updates the Diffractogram in the middle and thus the peak parameters which are updated immediately in the UI. The same chain reaction occurs if the hologram would be updated (e.g., live at the microscope) or if different slices of a 3D data stack would be selected.

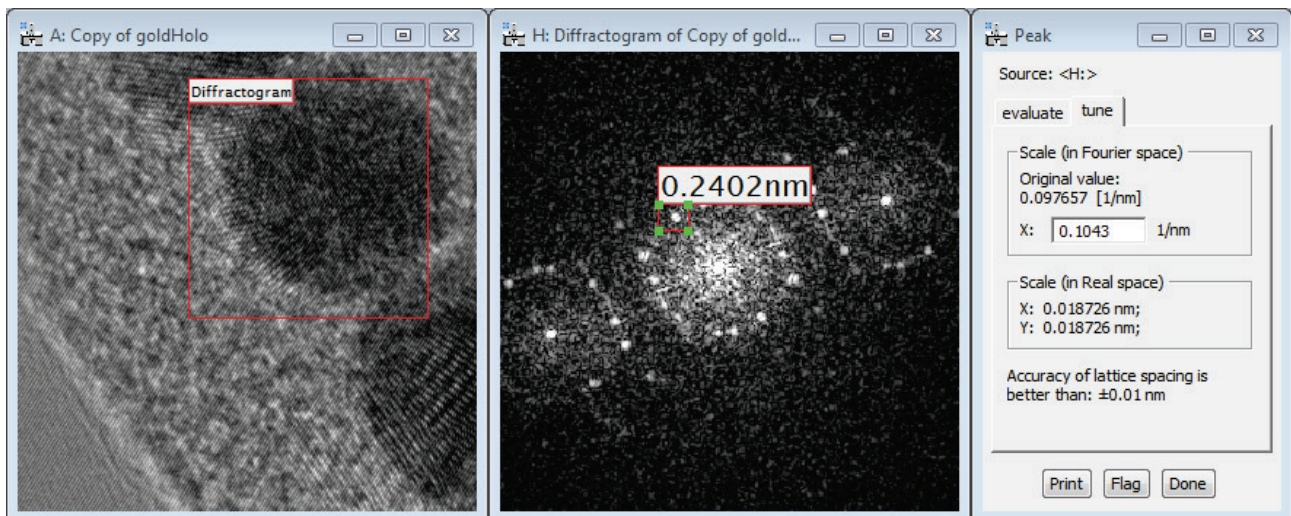


Figure 95 The sub-menus Diffraction and Peak working together: upon moving the ROI in the left image, or upon updating the image (if it is a live image acquisition), the ROI in the middle image and the UI on the right are updated.

5.12.5 Add sampling rate circle

This is a simple way of setting up live holography. It allows to adjust the current sampling rate via the biprism voltage accurately. It requires a live Fourier transform of the live hologram. When called, this function will ask for the desired sampling rate and draw a bright yellow circle onto the live FFT. Since live holograms for preview typically run at several frames per second, the voltage of the biprism can be adjusted quickly and monitored easily.

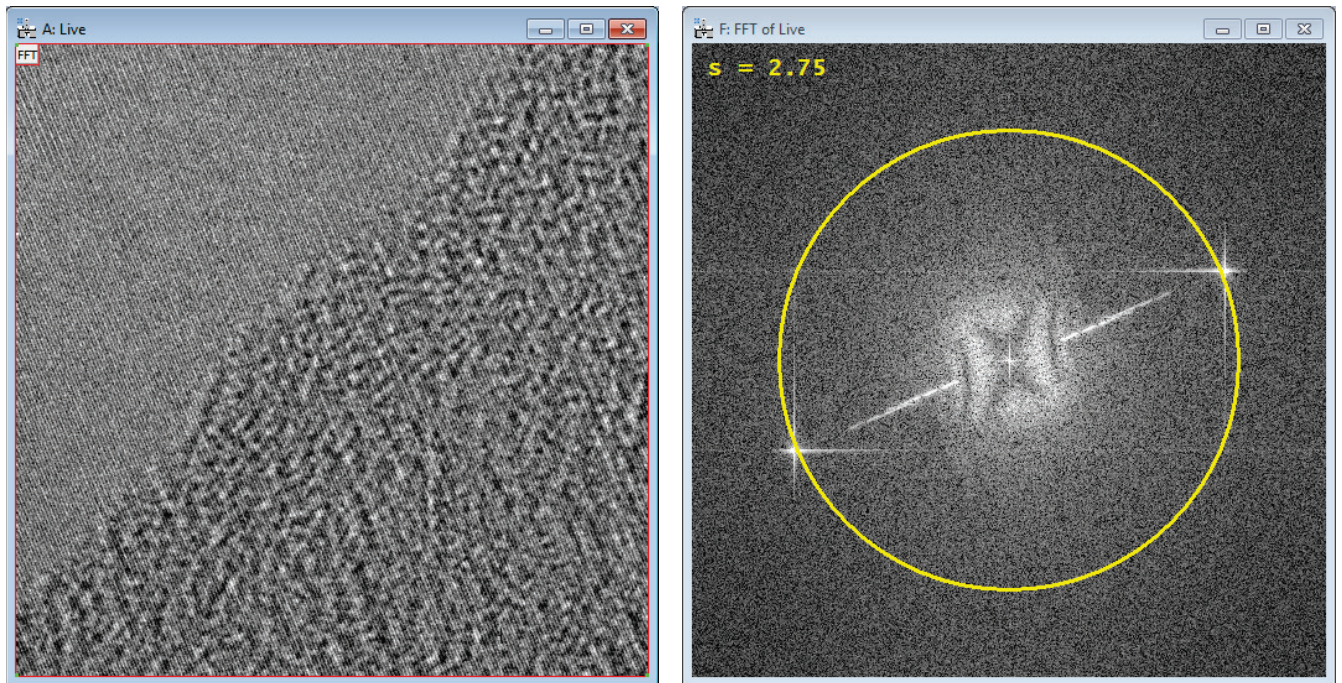


Figure 96 Left: the hologram in preview mode, typically at a sampling rate $s \ll 4$. Right: the position of the sideband is easily recognized in the live FFT and can be made to coincide with a given sampling rate by matching the sideband position with the yellow circle via the biprism voltage.

The acquisition rate in the above example was 5fps for a 2k camera acquired with a binning factor of 4. Under these circumstances, there are sufficient electrons per pixel available (x16 as compared to the acquisition with a binning factor of 1) to easily allow frame rates of 5fps or more.

Please note, these images should not establish the final images. These are preview images for preparing the final acquisition only. In this case, the final acquisition was performed with full frame, binning factor 1 and an exposure time of 2s.

5.13 Apertures

In general, apertures in a microscope work in preferred plans, and are positioned either in one of the back focal planes or an image plane. Their effect is to modify the incident wave by blocking certain parts of the wave and transmitting other parts. Therefore, there is an incident wave onto the aperture and an exit wave. The incident wave is represented by the image upon which the sub-menu is called, which then produces an exit wave according to parameters specified through the menu item.

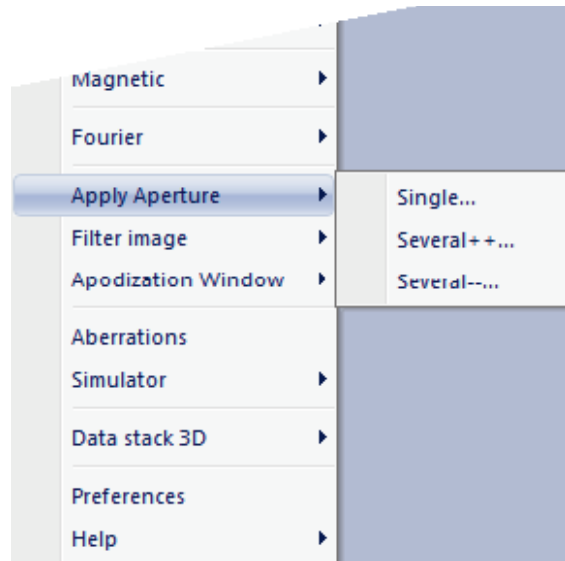


Figure 97 The Apply Aperture menu containing 3 sub-menus.

There are three options for the menu item Apply Aperture:

- i. Single
- ii. Several++
- iii. Several--

The aperture found in most microscopes is an aperture with a single opening, as in the sub-menu item Single. Because of the discrete nature of digital images, artifacts are encountered for aperture with a “hard” edge. Therefore, the sub-menu Single provides several aperture types to choose from with soft edges. The sub-menus Several++ and Several-- are more specialized and allow the application of several overlapping or disjoint apertures onto the same image, e.g., like an aperture with several openings, or a transparent aperture with several disjoint opaque areas.

Apertures can be applied to both real and complex type images,³⁶ but not to RGB type images. From all sub-menus, a new image is generated and the process of applying an aperture is interactive.

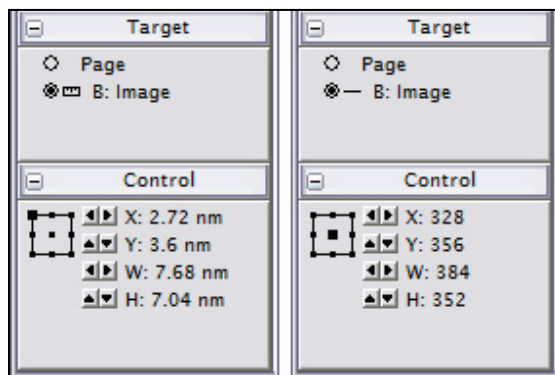


Figure 98 Fine control of apertures can be achieved through the DigitalMicrograph® standard tools. Left: scaling turned on. Right: scaling turned off.

The use of all standard DM tools is maintained and any update of the input image results in an update of the resulting image (or the exit wave). DigitalMicrograph® standard tools are used to control scaling and positioning of apertures as displayed in Figure 97. Note that the position of the aperture can be fine-tuned through “Control”, and the size of the aperture is scaled in the “Target” window when the image is scaled. The scaling can be turned off by a mouse-click on the measurement tool in the Target window.

Apertures are presently not supporting 3D data sets.

³⁶ For complex images, the aperture is applied to both the real and imaginary part of the image.

5.13.1 *Single*

Because of the nature of discrete images, hard apertures have a tendency to create artifacts when used in context with discrete Fourier transforms. Therefore, several aperture types are offered that help minimizing potential artifacts and help emulating a real aperture in a microscope as closely as possible.

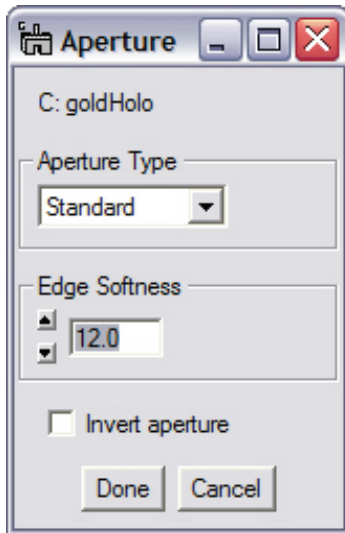


Figure 99 One of three options for a filter type.

A selection of three aperture types is available:

- i. Standard
- ii. Butterworth
- iii. Hard

For the Standard aperture, an Edge Softness factor can be chosen that literally softens the edge of the aperture. A large factor for edge softness creates a very soft edge, whereas a small factor creates a more well-defined edge. An edge softness factor of “0” creates a hard aperture. The softness of the Standard aperture is defined for positive integer numbers, although positive real numbers can be used as well.

For the Butterworth³⁷ aperture, small order numbers contribute to a soft aperture appearance, whereas large numbers approach the hard aperture. By choosing “0” as order, a hard aperture is created. The order for the Butterworth filter is defined for positive integer numbers, although positive real numbers can be used as well (see also Chapter 5.14.1).

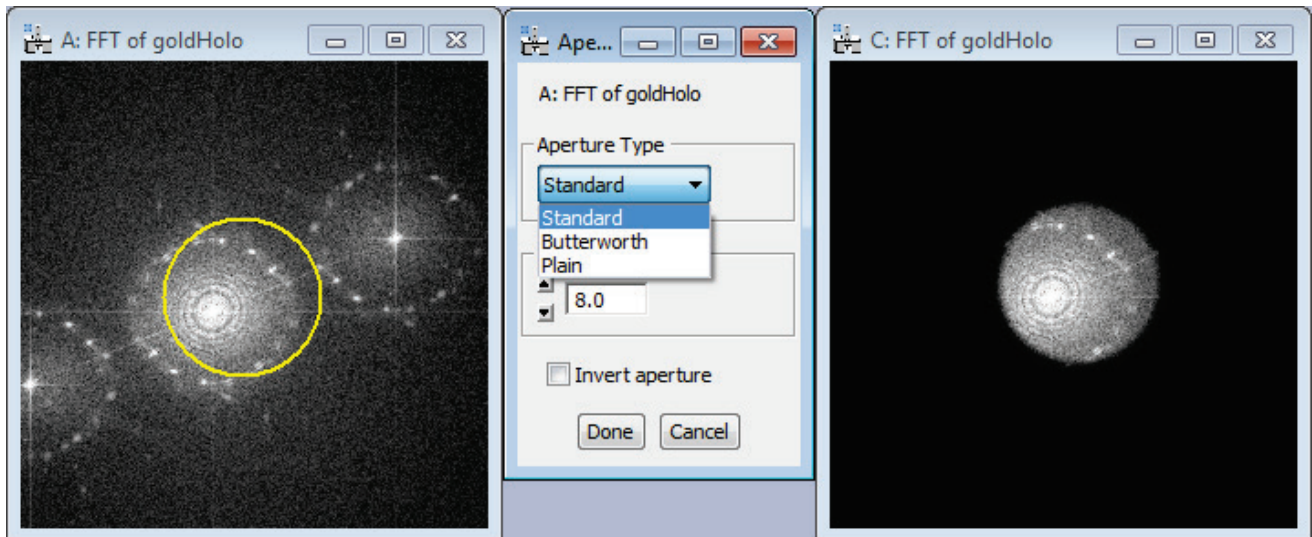


Figure 100 Left: Image data representing the “incident wave” onto the aperture. Center: the GUI. Right: Image data representing the “exit wave” from the aperture.

³⁷ The Image Processing Handbook, J.C. Russ, CRC Press, 1992, ISBN 0-8493-4233-3

5.13.2 Several++

Apertures can be used to determine the contribution of diffraction spots to an image. For complex images, the incident wave would correspond to the diffraction pattern and the aperture corresponds to an aperture in the microscope with arbitrary diameters. This allows, e.g., determination of atomic structures with inclusions with highly complex diffraction patterns.

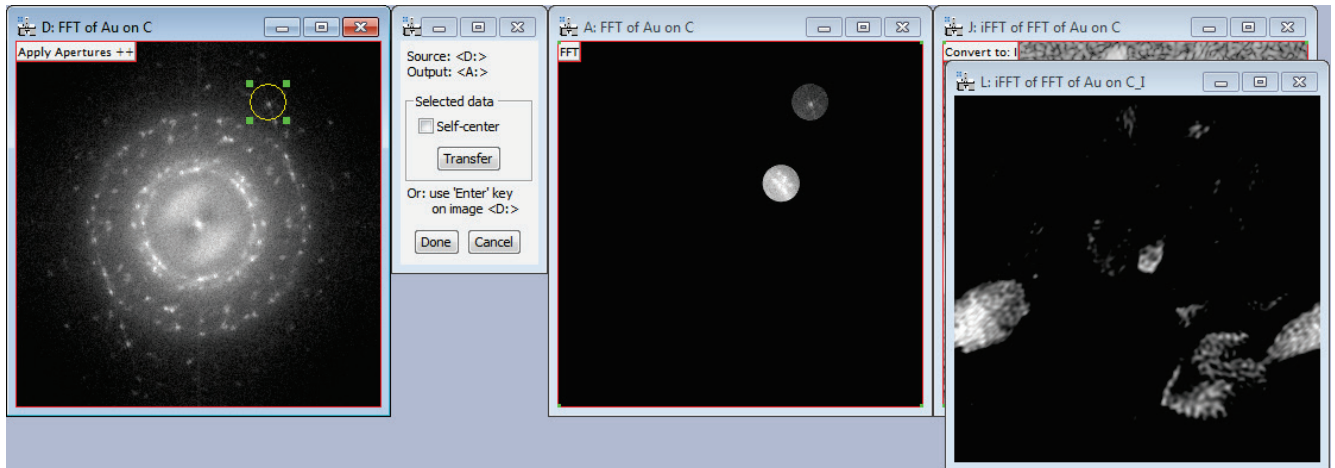


Figure 101 Left two images: Apertures are being applied to a FFT (here: 2 diffraction areas selected). By adding a live inverse FFT and a ‘Complex to real’ conversion to image intensity a workflow is created where areas in the original image can be identified that contribute to a certain diffraction peak.

The sub-menu Several++ can be applied to real- and complex-type images. The oval annotation can be adjusted arbitrarily and may extend beyond the image. Upon pressing the button Transfer in the UI window, the image data are transferred from the original image, or incident wave, to the resulting image, or the exit wave.

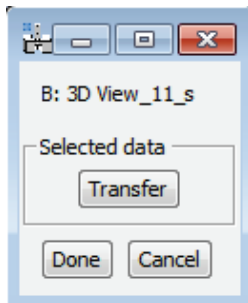


Figure 102 For images of type real the ‘Self-center’ feature is not provided.

For complex images, the option “Self-center” positions the aperture centered on the highest value (absolute value for complex numbers). The “Self-center” feature activates as soon as the Transfer button is pressed; the oval annotation moves accordingly for visual feedback. A Dialog requests confirmation to assure that the oval annotation indeed should be transferred.

The self-centering option is not available for images of type ‘real’; the DigitalMicrograph® standard control window “Control” can be used instead to center on the pixel with the highest value.

As all standard DigitalMicrograph® tools remain available, an inverse Fourier transform could be attached to the exit wave image (image on the right-hand side of Figure 100) and the contribution of each diffracted beam observed in a corresponding image plane.

As a shortcut, pressing the Enter key on the keyboard, while the input image is selected, is equivalent to pressing the Transfer button in the user interface window.

5.13.3 **Several --**

Apertures can be used to remove certain contributions of e.g., diffraction spots to an image. This supports, e.g., the determination of, e.g., atomic structures with inclusions with highly complex diffraction patterns.

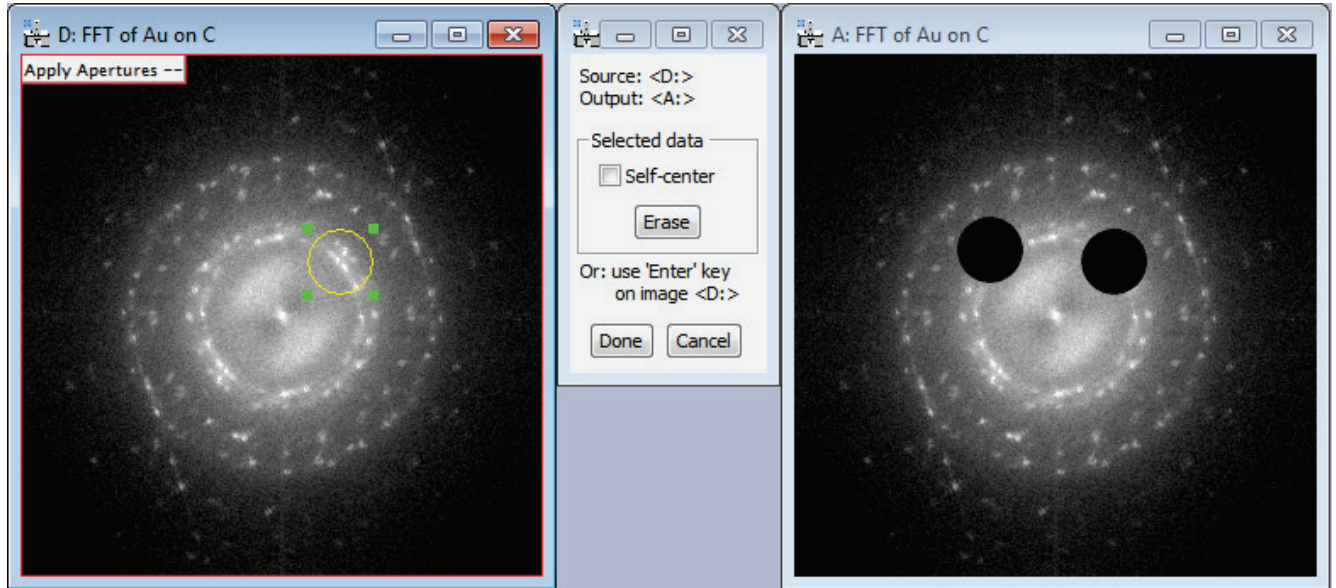


Figure 103 Applying several opaque areas to an image allows to take out e.g., certain diffracted spots in a diffraction pattern. The opaque areas remain opaque if the input image on the left changes.

The sub-menu **Several++** can be applied to real- and complex-type images. The oval annotation can be adjusted arbitrarily and may extend beyond the image. Upon pressing the button **Erase** in the UI window, the image data are removed from the resulting image, i.e., the exit wave.

For complex images, the option “**Self-center**” positions the aperture symmetrically around the highest value (of the absolute value of the complex numbers). The “**Self-center**” feature activates as soon as the **Transfer** button is pressed and the oval annotation moves accordingly. A Dialog is then presented to confirm that the centering was successful and the area of the oval annotation indeed should be removed from the resulting image. This option is not available for real-type images, as the DigitalMicrograph[®] standard tool “**Control**” provides the value of the pixel the aperture is centered on.

As all standard DigitalMicrograph[®] tools remain available, an inverse Fourier transform could be attached to the exit wave image (image on the right-hand side of Figure 102) and the contribution of each diffracted beam observed in a corresponding image plane.

Note that when the option **Self-center** is selected, the maximum pixel is actually obtained from the original image (image **D**: in the example in Figure 102).

As a shortcut, pressing the **Enter** key on the keyboard, while the input image is selected, is equivalent to pressing the **Erase** button in the user interface window.

5.14 Filter image

The Butterworth and Gauss-line filters are designed to filter images of both, real and complex data type. In contrast to the menu item “Apertures”, “Filter image” works close to a filter as defined for general image processing. The input image is Fourier transformed, an aperture is applied to the Fourier transform and an inverse Fourier transform applied to the resulting image. The optical equivalent would correspond to imaging a target through a lens with an adjustable aperture in the back focal plane of the lens.

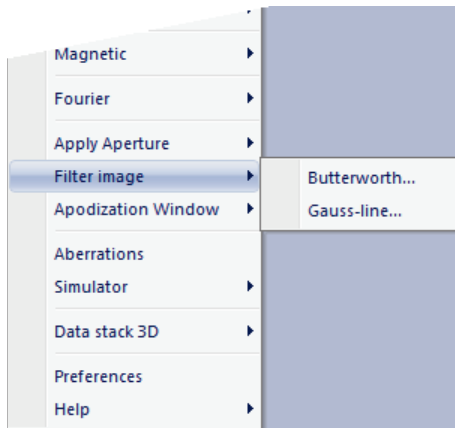


Figure 104 The “Filter image” menu item with two options.

Providing a complex image as input, is equivalent to working with a microscope. The complex wave corresponds to an image wave in the microscope and aperture functions can be applied just as apertures can be applied in a microscope. This allows for example dark field imaging or transmitting only certain diffracted beams. Furthermore, as the filter size can assume almost any size or shape in data processing, there is more flexibility to filtering in simulations than there is in the microscope.

As an interactive UI window is always required, the “Filter image” sub-menus do not provide additional features by using the Shift key. All sub-menus allow interactive filter adjustments and provide flexibility on the selected area. Image updates are recognized and will cause the new image to be processed with the active parameters.

All “Filter image” functions work on 2D and 3D data sets.

5.14.1 Butterworth

The Butterworth filter is designed according to e.g., [J.C.Russ] as:

$$\text{Butterworth filter} = 1 / [1 + (R/R_0)^{2n}]$$

where R is the distance from the center of the filter and R_0 is the nominal filter cut-off value. The cut-off value is defined at that point where the magnitude of the filter drops to a 50% value. The integer n is the order of the filter. For holographic applications, its order is usually >10 to ensure a good separation between the autocorrelation and the sideband. An example of the application of a Butterworth filter to a hologram is given in Figure 104. For a large filter radius the interference fringes, in the image resulting from the application of the filter, remain. Decreasing the filter radius at some point eliminates the interference fringes in the resulting image.

The Butterworth filter is integrated seamlessly with the other functions of DigitalMicrograph[®]. This means, that while the Butterworth filter remains active, all other DigitalMicrograph[®] tools remain available. For example, the linescan tool can be used to observe the edge profile of the filter, as demonstrated in Figure 104. In this way it can be observed that as the radius for the Butterworth filter increases, so does its softness at the edge.

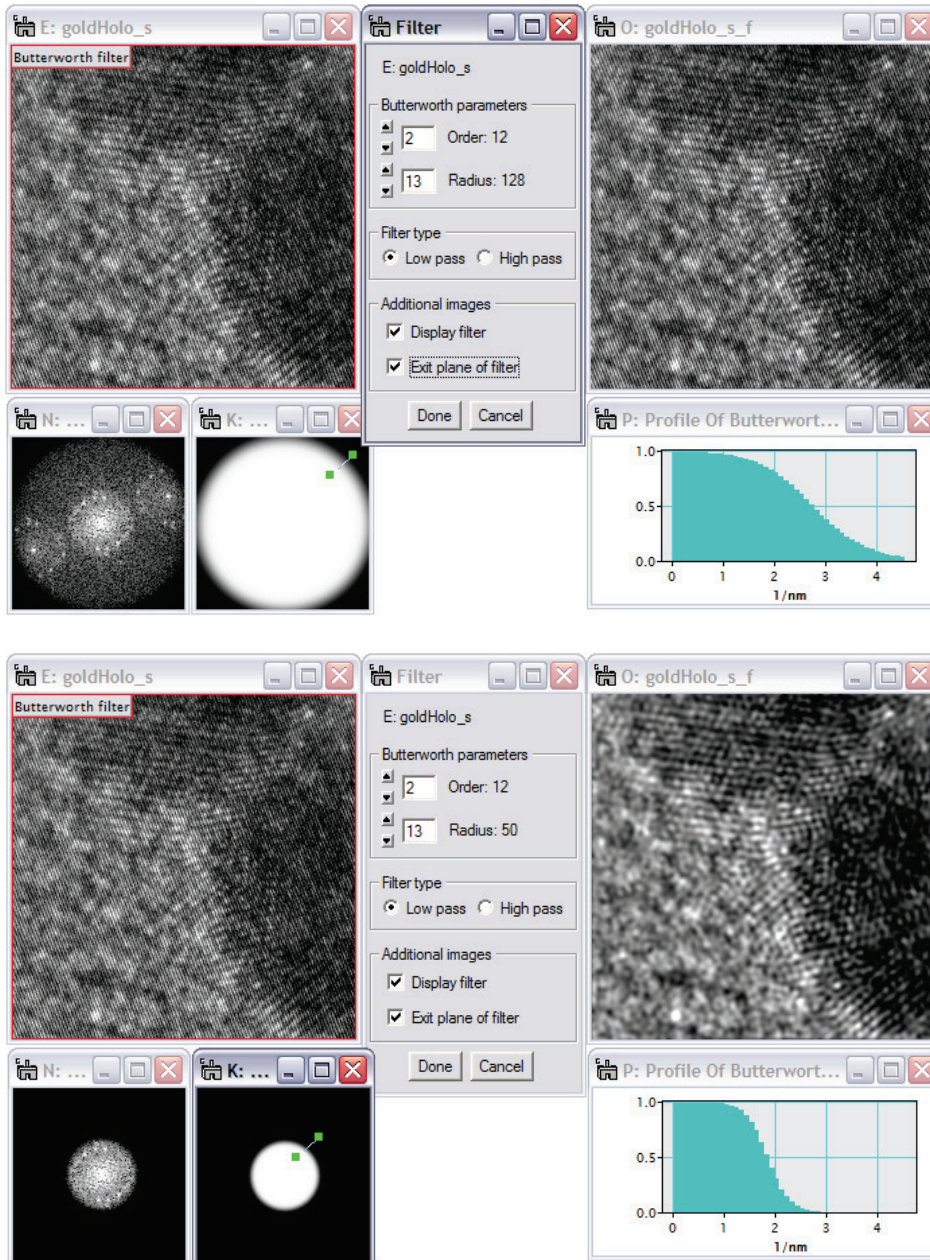


Figure 105 The interactive Butterworth filter allows adjusting the order of the filter as well as its diameter. The display of the filter and filtered FFT provide assistance in selecting the optimum filter parameters. The resulting image can be observed and evaluated at any time during the process. In this example, the reduction of the filter radius from 58 (upper figure) to 28 pixels (lower figure) effectively removes the interference fringes from the hologram.

The Butterworth filter recognizes selected areas in the selected image. The selected area can be moved freely within the image and all dependent images are updated immediately. For small movements of the selected area, both the DigitalMicrograph's® 'Control' -window or the arrow keys on the keyboard can be used. Changes to the input image, e.g., by continuous image acquisition, are recognized and the resulting image is updated. The selected area or the full image must can be of any size as of HoloWorks 5.0.8. The filter works on all images of type complex, integer or real. Note that the effect of the filter is different for complex and non-complex images.

5.14.2 *Gauss-line*

The Gauss-line filter is design to eliminate lines or streaks in Fourier space for 2D images, 3D data stacks both of real and complex data type. Its primary use is to remove Fresnel fringes from electron holograms, specifically if they cannot be removed through a reference hologram, but lines in Fourier space can show up in other applications as well. Specifically, for electron holograms, the lateral coherence is severely limited and thus the field of view often contains Fresnel fringes caused by the Möllenstedt type electron biprism as illustrated in Figure 105.



Figure 106 Left: Electron hologram exhibiting Fresnel fringes caused by the Möllenstedt biprism. Middle: The sideband exhibiting the trace of the Fresnel fringes as an artificial line. Right: Fresnel fringes appear in the reconstructed image amplitude as well.

Through the use of the “Gauss-line filter” Fresnel fringes can be removed without the use of a reference hologram. Also, when the electron biprism is unstable, the biprism may have moved sufficiently to fail in its capability to remove all Fresnel fringes.

In the specific case of electron hologram stacks that take several minutes to acquire, the biprism drift is almost impossible to avoid. In such a scenario, removing Fresnel fringes despite the existence of reference holograms is a must. As already indicated, this linescan filter works basically on all data sets with the exception of 1D data sets.

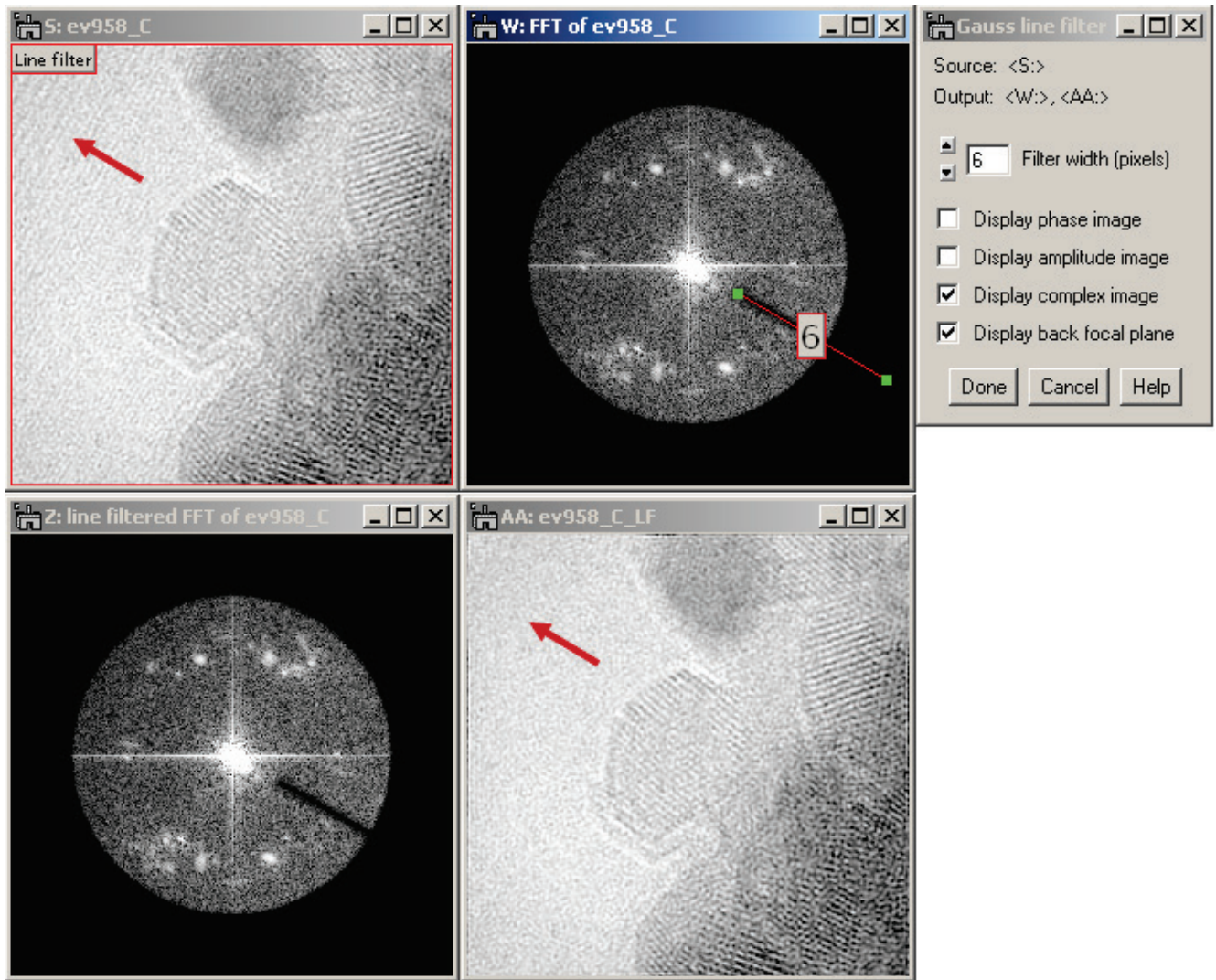


Figure 107 Top left: Starting from the reconstructed complex image in Figure 105, right, the Gauss-line filter, indicated by the red ROI in the top-center image, can surgically remove lines from Fourier space. Top right: This window controls the Gauss-line filter. Bottom left: The Fourier transform after the line filter is applied (i.e., the back focal plane). Bottom right: The Fresnel fringes have disappeared. Note: all images are accessible at all times.

The Gauss-line filter minimizes the impact of filtering Fresnel fringes especially as it allows fine-tuning of the filter parameter “Filter width” while observing the effect live in both the back focal plane and the reconstructed images as indicated in Figure 106.

The Gauss-line filter also works for 3D data stacks by using the displayed slice to determine the desired parameters. Once the parameters are confirmed, the filter is applied to each slice in the 3D data stack, providing a new Gauss-line filtered 3D data stack.

For real images, the Gauss-line filter can be applied as well, but in that case the Fourier transform is point-symmetric and therefore the Gauss-line filter is applied twice as demonstrated in Figure 107.

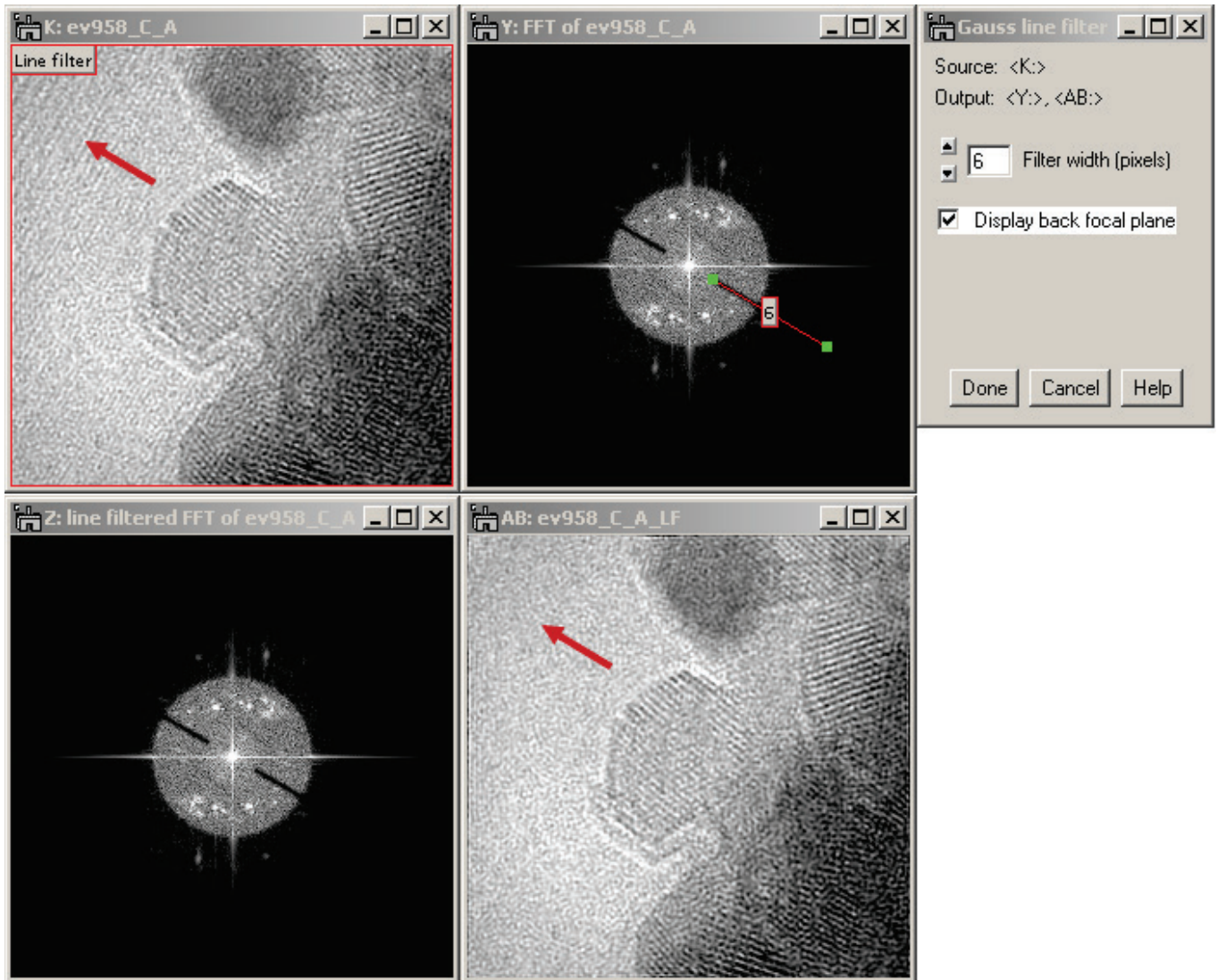


Figure 108 A similar situation is in Figure 106, but the source is not a complex data type. Therefore, the Fourier transform is point-symmetric (a mathematical necessity) and thus the filter is point symmetric too. Also, because the source data are not complex, the options to display amplitude and phase images are not given.

The Gauss-line filter works with any-size images as of HoloWorks 5.0.8. It also works for 2D and 3D data sets. This filter is computationally intensive.

5.15 Apodization Window

Window functions can be essential for obtaining highest quality phase and amplitude images. This is simply due to the fact that Fourier transforms are designed for band limited data sets as discussed in detail, e.g., in *Digital Processing of High Resolution Electron Holograms*, by E. Voelkl and L.F. Allard, MSA Bulletin, Vol. 24, No. 1: pp. 466-471 (1994). Instead of the inherent rectangular window function of the CCD camera, a *tapering* window or *apodization function* can be used to smoothly bring down the edges of the image to zero. A list of apodization functions can be found on the Internet e.g., at <http://mathworld.wolfram.com/ApodizationFunction.html>, (Weisstein, Eric W. "Apodization Function." From *MathWorld*--A Wolfram Web Resource.).

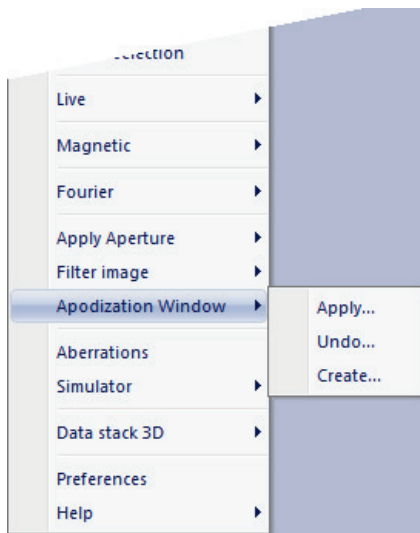


Figure 109 The window menu item with 3 sub-menu items.

HoloWorks presently offers 2 apodization functions. The following menus assist in applying, removing and creating one of the following window functions:

- Hanning window of variable order n
- Gauss window of variable order n

where the Hanning window is defined (in one direction) by:

$$H_m^n = N/(\pi n) \sum (-1)^j / j \sin(\pi j (N-1)/N) [\cos(2\pi j m/N) - 1]$$

summing over j with $1 < j \leq n$, if $n > 1$ and:

$$H_m = 1 - \cos(2\pi m / N);$$

for $j = n = 1$

m refers to the m^{th} pixel. Note the factor $N/(\pi n)$ that replaces the originally published value $2/\pi$ to avoid an intensity scaling of the window as a function of window size.

The (apodized) Gauss window is defined by:

$$\text{Gauss} = \exp[-(x^2 + y^2)], \quad \text{with } -n \leq x < n$$

where n is referred to as “order”, describing the allowed range for x across the image.

The windows can be applied to an image, removed from an image or created as an image. In all cases, a new window is generated. Window functions always work on full size images and are centered within the image, which is different from the aperture functions, as they serve a different purpose.

Holding down the Shift-key when evoking the menu item, starts a thread that is sensitive to changes in the source image. Once a change in the source image is detected, the output image is updated.

Apodization windows work on 2D and 3D data sets

5.15.1 Create window

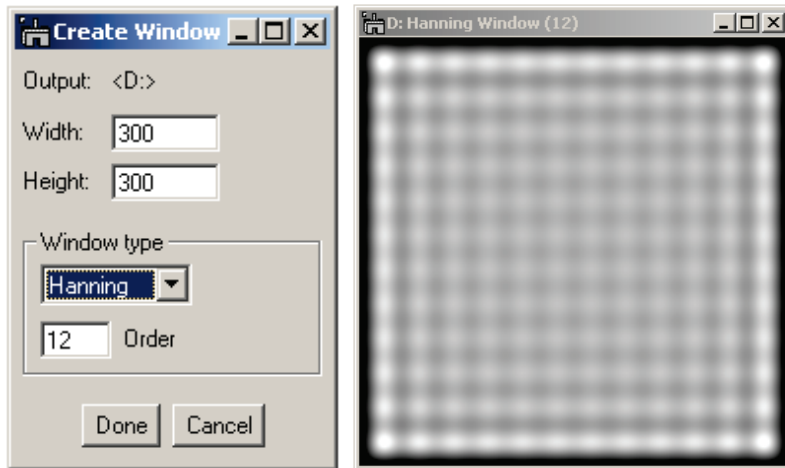


Figure 110 The Create window dialog with the live-generated image next to it. Changing any parameter changes the image on the right-hand side, while all standard DM functions remain accessible.

The “Create window” dialog is fully interactive and allows changing the window type, the order or “sigma” value or even the size of the generated window. Both 1-D and 2-D images can be generated where 1-D images are displayed in the linescan style. The window type provides a selection between a Hanning or Gauss window. For the Hanning window, the order of the window can be changed. For a Gauss-type window, the order is given in the more general notation of “sigma”. On changing the window type, the last value used for that

window is restored. This allows a comparison between similar windows.

Note that specifically the first order Hanning window is widely used to reduce streaking in Fourier space. Furthermore, it is heavily used for the determination of peaks in Fourier space for sub-pixel accurate position and intensity.

The “Create window” function is already a live thread (because there is no input image) and the use of the “Shift” key on the keyboard has not effect on functionality in this case. The created image carries the definition of its parameters in its History tree which can be reviewed at any time.

5.15.2 Apply window

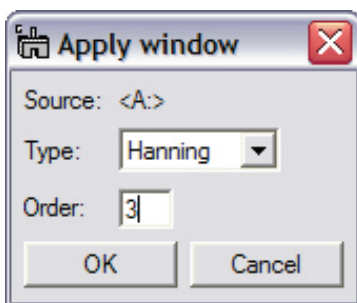


Figure 111 The Apply window dialog keeps track of the source image.

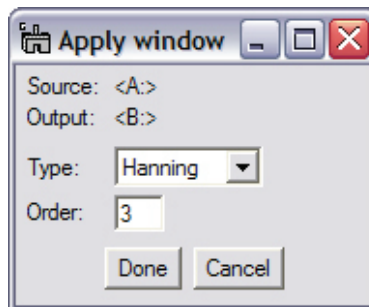
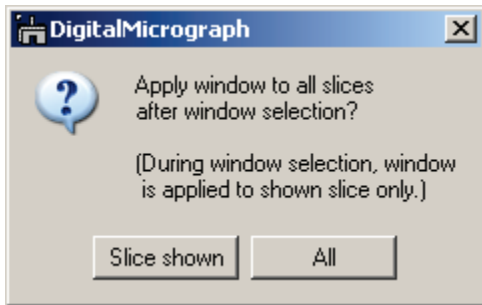


Figure 112 The Apply window dialog evoked as thread tracks the output image.

The “Apply window” dialog applies the selected window to a2D or 3D image or a selection within the image. In all cases, a new image is generated. Holding down the Shift-key when evoking the menu item, starts a thread that is sensitive to changes in the source image. Once a change in the source image is detected, the output image is updated.



For 3D data sets there are two options: Apply (or remove) the window to just the displayed slice or apply (remove) the window to (from) all slices.

Figure 113 For 3D data sets, a window appears that allows to just select the displayed slice or to work on all slices.

5.15.3 *Undo window*

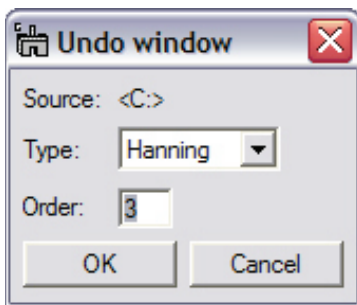


Figure 114 The Undo window removes a window function that was applied earlier

The “Undo window” dialog removes the selected window function from a 2D or 3D image or a selection within the image. In all cases, a new image is generated. Before posing the dialog, the history tree in the source image is checked for information on a window applied earlier. If the information is found, the window will present the window most likely to be undone.

For 3D data sets, the same window as in Figure 112 occurs.

Holding down the Shift-key when evoking the menu item, starts a thread that is sensitive to changes in the source image. Once a change in the source image is detected, the output image is updated.

5.16 Aberrations

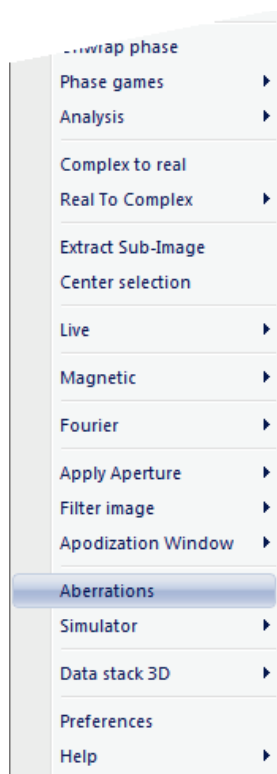


Figure 115 The menu item “Aberrations”

The menu item **Aberrations** is designed to either introduce aberrations into or to remove aberrations from an image. The input image must be from real space and not from Fourier space. Presently available are the basic aberrations for electron microscopy: focus, two-fold astigmatism and spherical aberration.

In order to introduce or remove aberrations, the parameter for the wave length (electrons, photons, neutrons, phonons, etc.) and the image size must be known. The menu item **Aberrations** covers a large range of wavelength and image sizes and is thus designed for a large variety of particles: specifically particles for which Fourier optics can be used to describe their respective imaging systems.

Therefore, when evoking the menu item, the image is checked for calibration. If it is not found, the function quits.

If the image is found to be calibrated, the wavelength can be selected interactively between pm to m. As electron microscopists are used to rather think in acceleration voltage, this option has been implemented as well and can be accessed through the Energy tab, as displayed in Figure 115.

At this point, the wave length and image size are established and the aberrations can be applied.

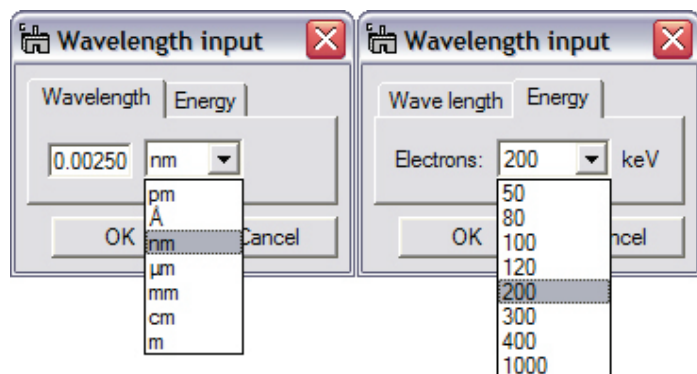


Figure 116 For convenience to the electron microscopist, the wavelength can be entered either as wavelength or as energy.

aberration (C_3) are provided. Selecting any of the aberrations changes the corresponding aberration parameters. Focus and spherical aberration each have one aberration parameter, whereas (two-fold) astigmatism has two parameters. Control over the parameters in the Aberration Tab is provided via the up/down arrow buttons, followed by a field containing the incremental value for a parameter change. The incremental value can be changed by typing the new value in the field follow by use of the **Enter** key (use of the Enter key indicates to the computer to

By pressing the OK button in the Wavelength input window, the “Aberrations” window appears which is displayed in Figure 116 - Figure 118. The Aberrations window contains 3 Tabs providing the functionalities Aberration, Constants and Image handling discussed in the following.

The **Aberrations Tab** allows selecting between the aberrations via radio buttons. Focus, (two-fold) astigmatism and spherical

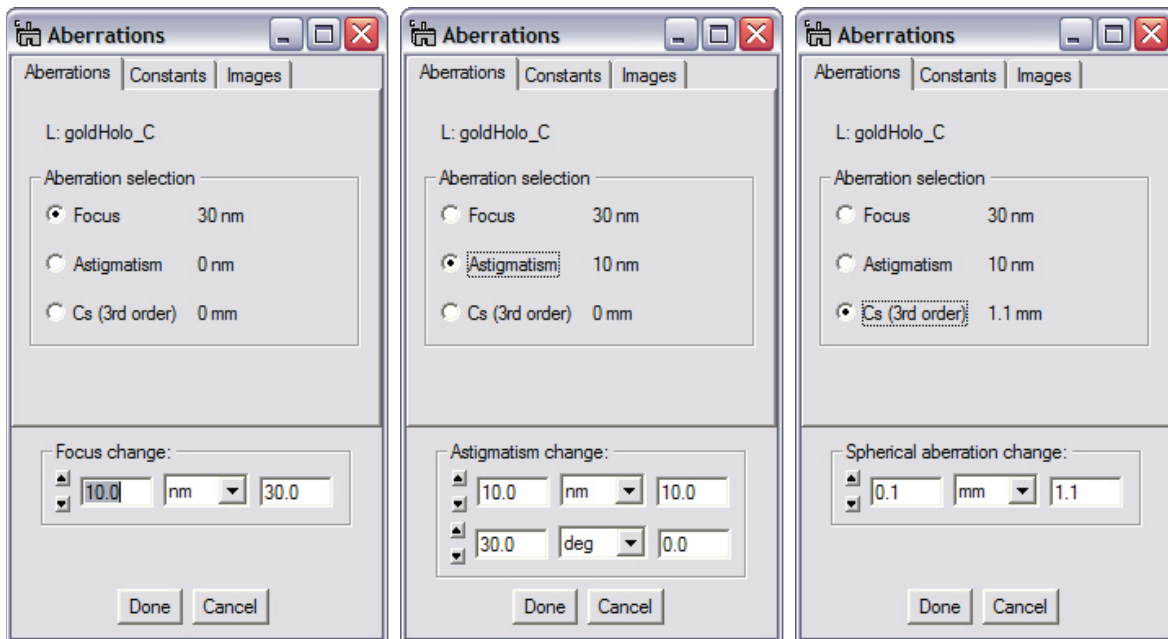


Figure 117 From left to right: The available aberration controls for Focus, Astigmatism and Spherical Aberration. Each parameter can be changed via incremental values using the up/down buttons, or by direct input of the desired values. Values can be entered from pico-meter to meters or in rad or degrees.

accept the value). All aberration parameters are characterized either by length-scale or angle, and are controlled via the popup menu after the incremental field value. The field on the right hand side provides the total aberration value and allows directly setting a desired value. The total aberration value also appears in the “Aberration selection” box to maintain an overview over the different aberration states, but cannot be modified there.

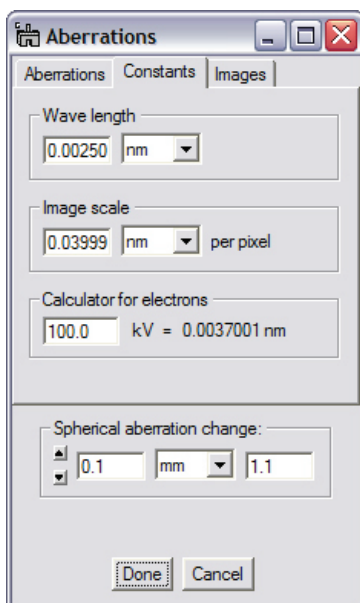


Figure 118 Fine-tuning image scale or wavelength parameters

The **Constants Tab**, as displayed in Figure 117 allows adjusting additional parameters as the wavelength, the image scaling and provides a tiny calculator to convert acceleration voltage for electrons into electron wavelength.

The aberrations window allows changing at least one aberration parameter independent of the selected Tab.

It should be noted that any change of wavelength or image scaling affects the aberrations directly and even small changes in wave length or image scale have a significant impact on the aberration effects as can be seen directly in the images.

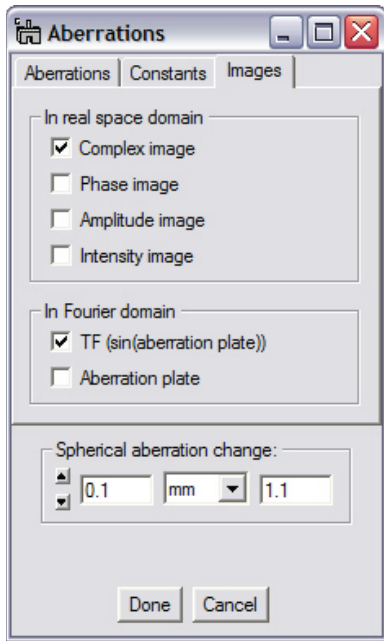


Figure 119 The Images tab provides control over images displayed or hidden.

The **Images Tab**, as displayed in Figure 118 allows control over the number of images displayed at any time. The following images can be displayed or hidden by selecting or deselecting their appropriate checkbox:

- i. Complex image
- ii. Phase image
- iii. Amplitude image
- iv. Intensity image
- v. Transfer function (in 2D)
- vi. Phase plate image (complex)

Hiding images also discontinues the update of these images, which can be an advantage on slow computer systems. Activating hidden images updates images only at that point.

It is also possible to spawn other images from the complex image through e.g., the “Complex to Real / Select” sub-menu of HoloWorks.

Figure 119 provides an overview over a typical layout for the aberration application. The original image displays the ROI **Live aberrations** to indicate that a thread is spawned off that image. The resulting complex, amplitude and phase images are displayed as well as 2-dimensional phase plate. As with all other HoloWorks functions, the standard DigitalMicrograph[®] tools remain fully accessible.

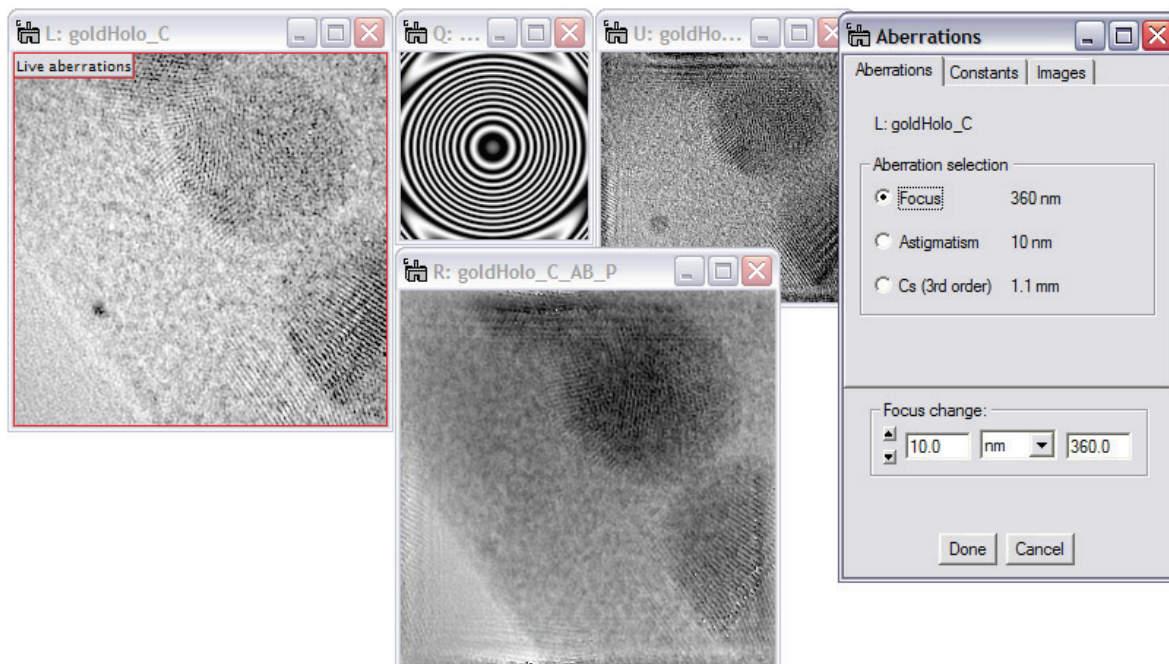


Figure 120 Example layout for correcting aberrations for a reconstructed wave function from a hologram. The original hologram was far out of focus. With the aberrations menu still active, the phase contrast was inverted to display the stronger phase shift of the Au particles as darker than its surrounding.

5.17 Simulator

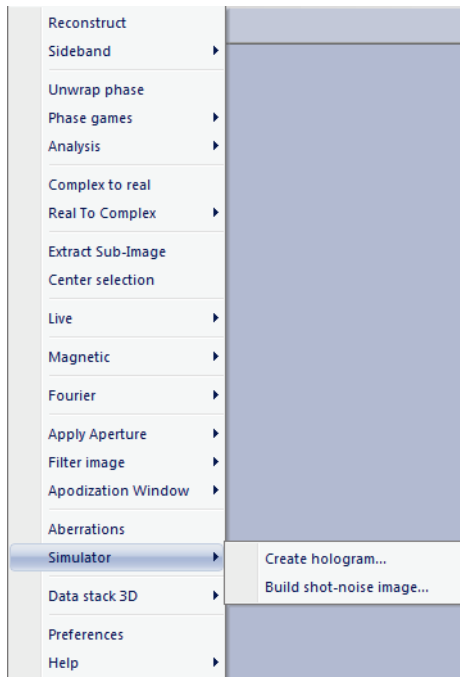


Figure 121 The submenus in the simulator.

The simulation tools were designed to provide additional insight into the capabilities of holography and to some degree as a tool for teaching Fourier optics and experimenting with imaging features inside a microscope.

Whether starting with a hologram or a complex wave anywhere in the microscope the processing of the complex waves always leads to the same behavior that is essential for the understanding and application of Fourier optics. The software HoloWorks intends to support the understanding of the basic processes for microscopy and tries to “playfully” build expertise in this area.

At the same time, HoloWorks provides support in building an understanding of the limitations imposed by discrete imaging and discrete Fourier optics, as the limitations of digital data processing and the ways to deal with them will become a much more significant part of evaluating data from any microscopy related technique in the future.

5.17.1 Create Hologram

This menu item creates an off-axis-type hologram from one or two images. It can be selected with or without the **Shift** button pressed. The required input parameters are: amplitude and phase images for the target wave, the amplitude and phase constants for the (ideal) reference wave, and the angle between the target and reference wave and the direction of the resulting interference fringes in the image. Both the angle and the direction are described by the unit-free *sampling rate*, which is requested for the x- and y- direction separately. The name of the resulting image defaults to “Hologram.dm3.”

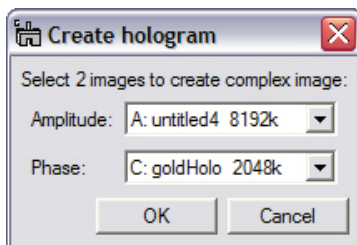


Figure 122 Selecting the images representing the amplitude and phase of the target wave.

Upon selecting the submenu “Create hologram” in the menu “Real to Complex,” a dialog box appears allowing to select two images. It is possible to select the same image in both popup items, but then the amplitude and the phase in the hologram will be identical, which is typically not the case in reality. Image 0 represents the input for the amplitude and Image 1 represents the phase of the target wave. Note that the selected images cannot be of type complex or RGB.

Make sure, the image selected to represent the target amplitude does not contain any negative values for strict mathematical reasons: the amplitude of a complex number $u+iv$ is defined as $\sqrt{|u+iv|^2}$ and thus definitely positive. Negative values in the amplitude image can cause undesirable artifacts in the

resulting hologram. Therefore, care should be taken to avoid negative numbers in the image selected for the target amplitude.

The scaling for the hologram is taken from the scaling for the amplitude image. If this fails, scaling values will be extracted from the phase image. If that fails too, the resulting hologram will not be scaled.

Once the images representing amplitude and phase for the target wave are selected, the “Create Hologram” window appears (Figure 122, left).

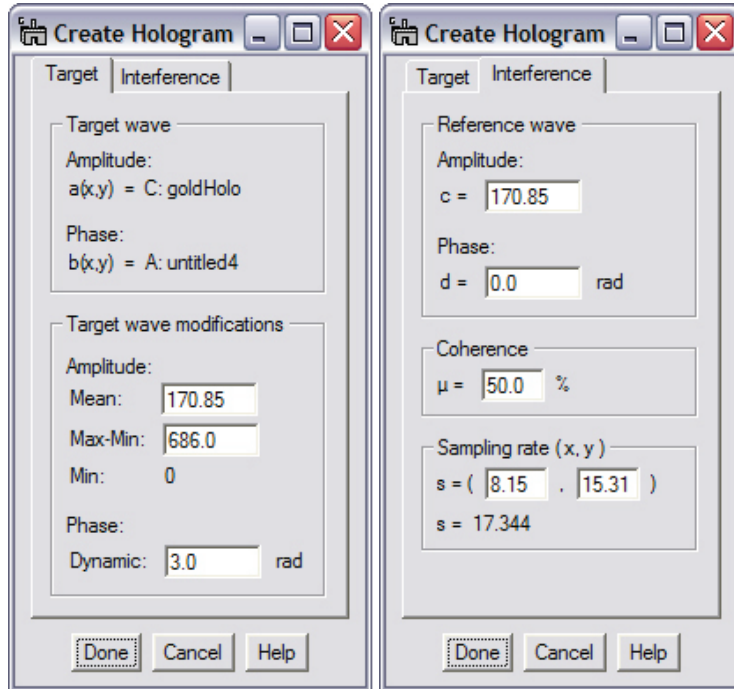


Figure 123 Left: Adjusting the parameters of the reference wave. Center: Determining the coherence and superposition parameters for the target and reference wave. Right: Fine tuning of the target wave parameters (does not change the input images).

Initially, the tab “Target” is selected providing the means to adjust the mean and dynamic range for the target wave as well as its phase dynamic, without changing the input image.

Selecting the tab “Interference,” allows adjusting the parameters for the (ideal) reference wave: the amplitude value “c” corresponds to the average value of the target amplitude; the phase value “d” is set to zero. Any change of the phase value only shifts the interference fringes of the hologram and carries little relevance. Also, the parameters for superimposing the target and reference waves can be adjusted here. The initial value “μ” for the coherence between the target and reference wave is recalled from the last time the menu item was used. It refers to the nominal fringe contrast as measured under “ideal” conditions, i.e., with a constant

amplitude and phase in the target wave. For electron holograms, values below 50% are typical, for light optical holograms typical values are above 80%. The values for the sampling rate are recalled from the last time the menu item was used. They describe how many pixels are necessary to cover one fringe (or 2π) of the interference fringes in both the x- and y- direction. Note that setting the sampling rate in x- direction to “0” will cause the interference fringes to run parallel to the y- direction and vice versa.

The appearance of the elements in “Target” depends on whether the menu was called with the Shift key pressed or not. The following table lists the resulting feature differences:

Feature	Shift key pressed	No Shift key
Target wave modifications	Grayed out, target wave cannot be changed through this tab	Active parameter change of target wave
Sub-area in source images	Moveable	Fixed
Input images	Change affects hologram	Change does not affect hologram

Table 1 Different features are evoked depending on how the menu item “Create hologram” was selected.

Note: This sub-menu follows the standard guidelines of responding to a change of the input images – if the sub-menu is selected with the Shift key held down. This can be observed directly by applying the Process/Live/FFT submenu to the hologram.

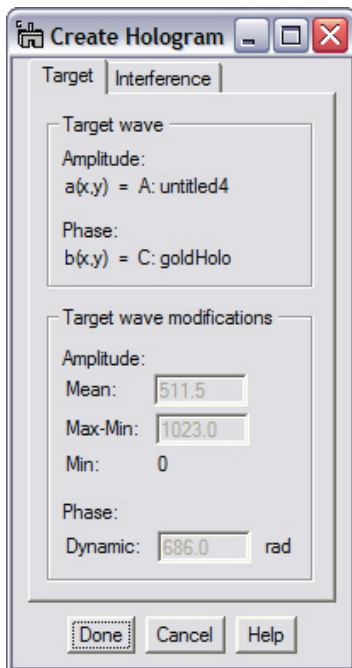


Figure 124 With the Shift key pressed when selecting the menu, the hologram is modified by changing the input images.

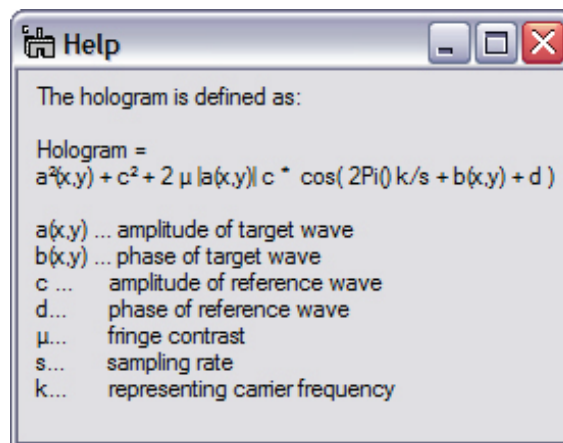


Figure 125 The help button displays basic information for how the hologram is created and provides the nomenclature as used in this context.

5.17.2 Single electron simulator

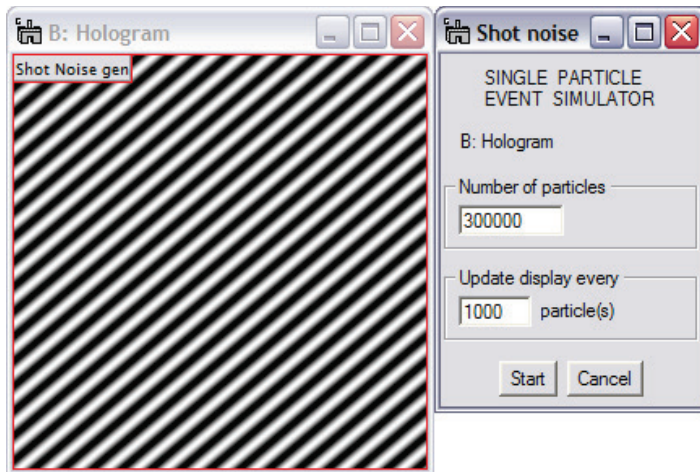


Figure 126 For a given area, the corresponding shot noise image will be generated one particle at a time.

image is updated. Since each image update requires time, it is important for large numbers of particles to minimize the overall time required for simulating all particle incidents.

The single electron simulator simulates shot noise for a given probability distribution. The probability distribution corresponds to the image intensity which, by definition, cannot be < 0 . Therefore, the input image, Figure 125, left, must be real and each pixel value must be 0 or positive. Selections in the input image are recognized and can be adjusted before the simulation process is started.

The interactive window allows setting the number of particles that will be collected by the CCD camera and also allows setting after how many electrons the resulting

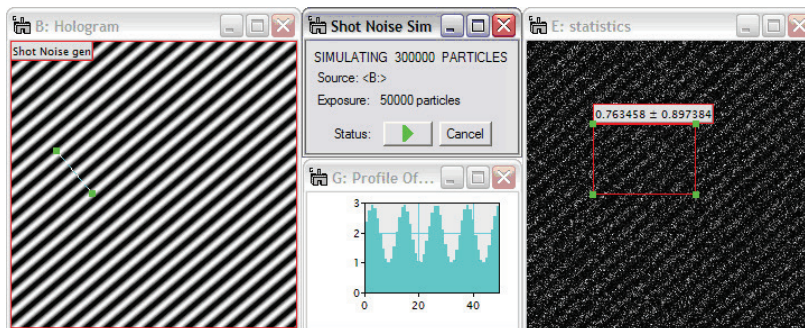


Figure 127 The shot noise generation can be paused or stopped at any time. The particle exposure box indicates how many particles have been recorded by the CCD camera at any given time.

keeps the log about the number of particles already generated as well as the total number of particles to be generated.

Once the simulation process has been started, the ROI can no longer be moved or readjusted in its size. The simulation process however can be paused, continued or cancelled at any time. The pause/run button changes appearance according to the actual status. The appearance of the “Pause” sign (II) means the process is paused.

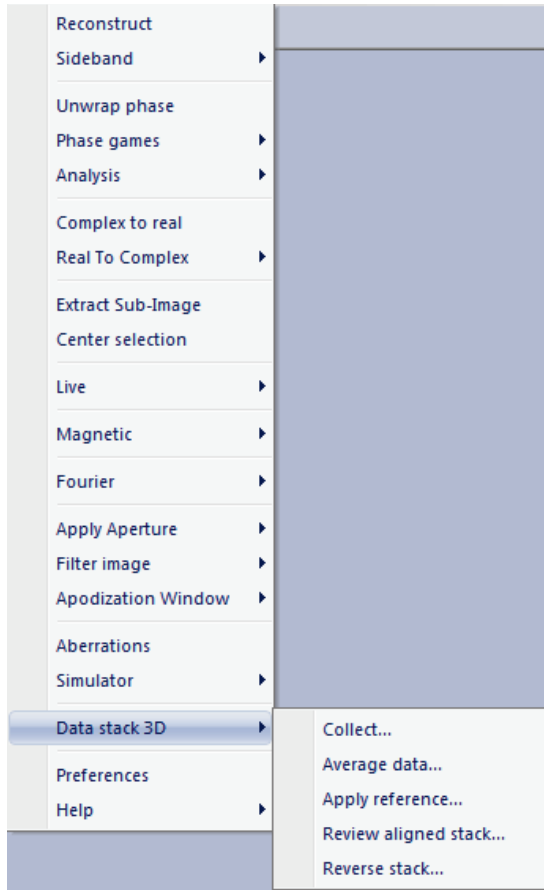
Note that by starting the process, an entry is made into the DigitalMicrograph[®] results window with a time stamp. This allows an estimation of the remaining time for the simulation process.

The window in Figure 126, center,

The final image Figure 126, right, simulates what the image on the left might look like after 50000 particles with an ideal CCD camera. Note that by repeating the process, the simulated particle distribution on the CCD camera will be different, as would the actual experiment if it was performed e.g., with electrons.

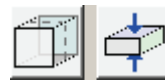
As with all other processes, the standard tools of DigitalMicrograph[®] can be used freely. In this case, a line scan and the Live / Mean and Std. Dev ... submenu were used.

5.18 Data stack 3D



The data stack 3D sub-menus provide the capabilities to acquire and process data stacks that can contain large numbers of images. Collecting images in a stack has the advantage that the “real estate” on the screen is treated carefully, as images are stacked in a single container and not spread all over the screen.

In most cases, the goal of using 3D data stacks is to acquire and process data sets so averaged images at a significantly improved signal/noise ratio can be obtained.



The first two sub-menus of “Data stack 3D,” i.e., “Collect...” and “Average data...” can be easily

accessed via the HoloWorks UI as detailed in Chapter 4, page 18.

In order to provide smooth integration with the features of DigitalMicrograph[®], there is no interference with DigitalMicrograph’s standard data acquisition.

Figure 128 The main goal for data stack acquisition and processing is noise reduction.

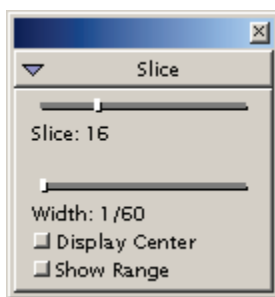


Figure 129 Standard UI window of DM for handling 3D stacks.

The standard user interface (UI) of DigitalMicrograph[®] to deal with 3D data stacks can be found under the menu Window/Floating Window/Slice. The ruler allows picking a certain slice for display. A single click with the mouse to either the left or the right moves the slice backward or forward by one. Clicking fast several times moves the slider to the approximate location of the mouse clicks.

The “Width” slider is used to average images on the fly but without e.g., lateral drift correction.

Thus the tools of DigitalMicrograph[®] and HoloWorks are complementary. Details of the “Data stack 3D” sub-menus are given in the following.

5.18.1 Collect

A 3D data stack is acquired by attaching a thread to, e.g., a continuous camera acquisition. Whenever the selected “live” image updates, the data are copied into the data stack. Before this can be done, the depth of the data stack must be defined.

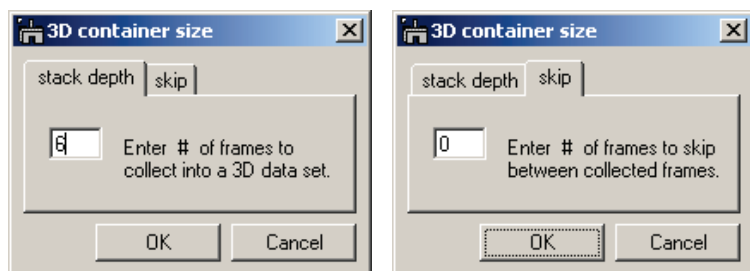


Figure 130 When acquiring a data stack, the depth of the data stack must be known. Skipping images is supported as well.

frames as well as how many frames are skipped between copy events. The limit for the depth is defined partially by the amount of RAM memory available, but will be different for almost any system.



The data stack acquisition is started by either clicking on the button to the left in the HoloWorks UI, or by selecting the menu item “Holo5.0/Data stack 3D/Collect...”

At this point the window in Figure 129 appears where the user can defined the actual depth of the stack, i.e., number of

Once the OK button is pressed, a thread is attached to the live image and each new image is copied into the data stack. Once the data stack is full, the thread stops, unless the user has unchecked the checkbox in Figure 130. In this case, the oldest image in the stack is discarded and the new image is added. This process continues until the thread is ended by either deleting the interactive window or by re-checking the checkbox.

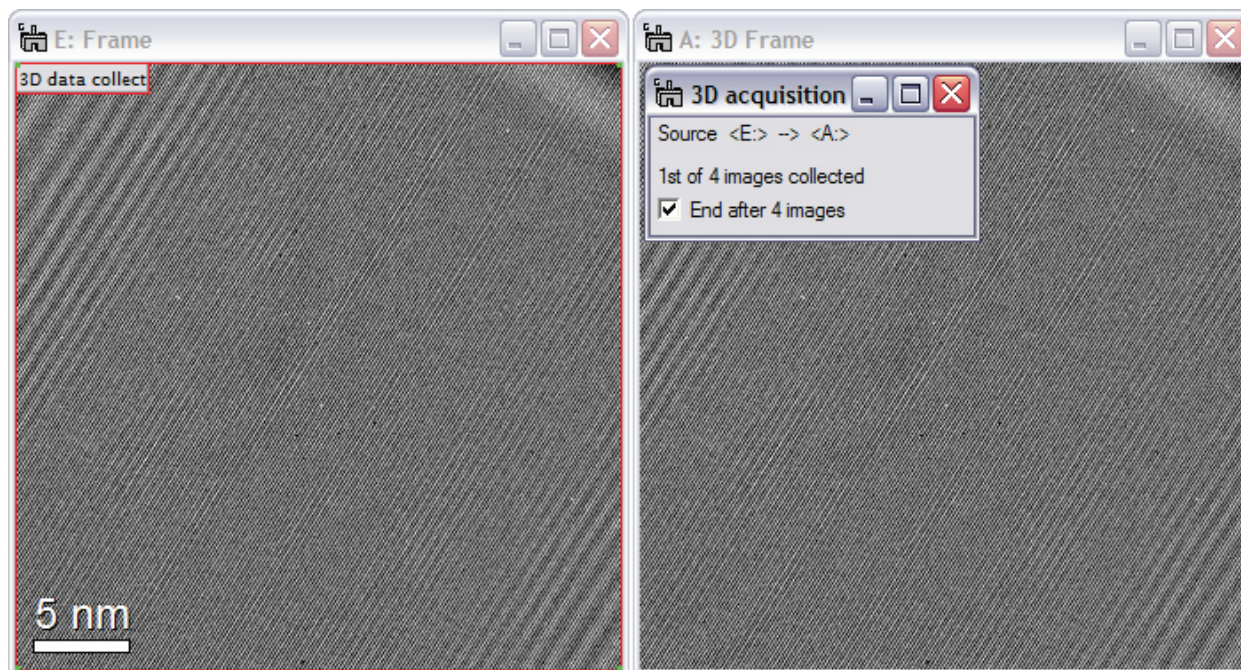


Figure 131 Acquiring images (left) into a data stack (right). As the image on the left is updated (continuous acquisition mode for the CCD camera), it is also copied into the data stack on the right. Once the acquisition has ended, the data stack is ready for processing.

5.18.2 Average data ...

The last step in processing images or holograms in a data stack is to average the images. This process is fully automated and only limited user interaction is required.



The data averaging process can be started by either clicking on the button to the left in the HoloWorks UI, or by selecting the menu item “Holo5.0/Data stack 3D/Average data...”

Once the data averaging process is started, some options are available:

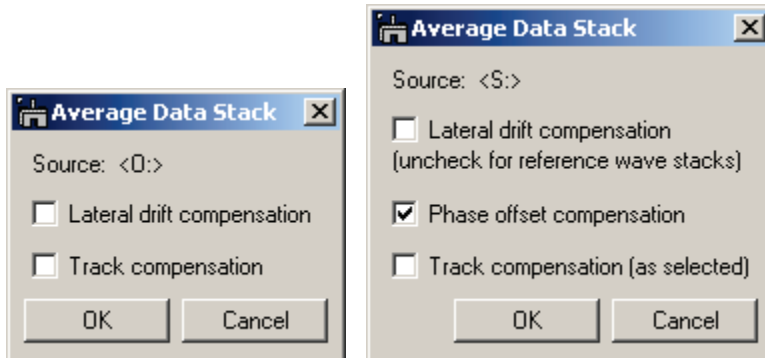


Figure 132 The dialogs for averaging 3D data stacks. Left: the dialog for real type data sets, Right: the dialog for complex-type data sets.

The lateral drift compensation uses a standard cross-correlation algorithm to determine the offset between the images. The accuracy of the detected offset is, in most cases, around $1/10^{\text{th}}$ of a pixel. The algorithm used for the sub-pixel image offset is mathematically accurate under the condition that all data in the image is bandwidth limited and does not exceed the Nyquist limit. In practice, and e.g., given Poisson noise in every image, the algorithm usually is reliable to

$1/100^{\text{th}}$ of a pixel and thus exceeds almost all requirements on accuracy.

Depending on the type of the input data, a different UI appears. For both real and complex data types, lateral drift compensation is offered. Also, there is an option to trace the compensation parameters via a linescan resulting in a linescan as displayed in Figure 132.

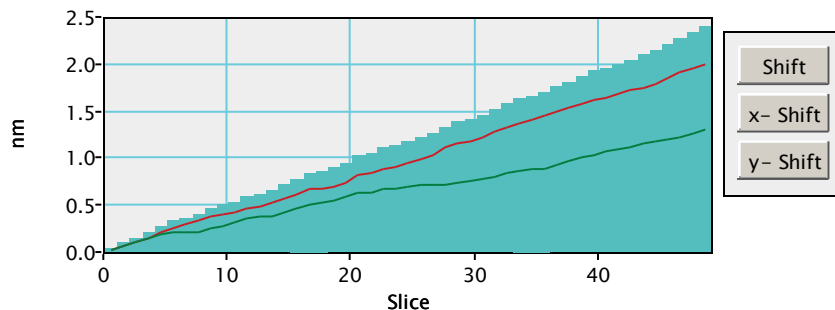


Figure 133 Example of a trace over 50 images showing an almost linear sample drift. The drift is most typical for a drift pattern caused by a temperature difference between the sample and the microscope.

It is important to note that the drift compensation can also be accomplished by providing an external data set. Before the lateral drift determination is started, there is a possibility for the user to select an external data set as a reference.

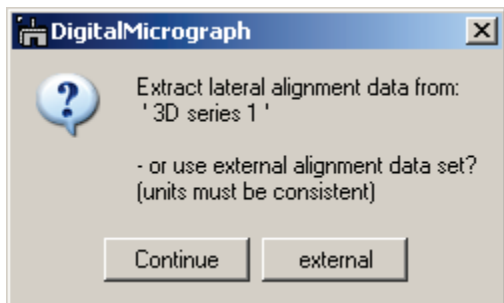


Figure 134 Drift correction via external data is supported.

Accurate drift determination can be challenging for some data sets and HoloWerk does not claim it provides the best solution under all circumstances. In some cases it might be necessary to filter the data set before the lateral alignment takes place.

If for the lateral drift correction of a filtered data set the “Track compensation” option was selected, the resulting track data can be used as input for aligning the un-filtered data set providing a much improved averaged image.

For data sets of type complex, phase offset compensation is an option as well. It takes into account that the phase is determined only modulo a constant phase value. A drift of interference fringes for example will cause a variable phase offset in consecutive images. Before complex images can be averaged, this variable phase offset must be compensated. By checking the corresponding box, HoloWorks applies an algorithm to determine the phase offset and then compensates for the offset before averaging the images.

In the specific case of averaging reference images, lateral drift compensation is usually not required. It is recommended to uncheck the checkbox in this case (see Figure 131, right, top check box).

5.18.3 *Apply reference ...*

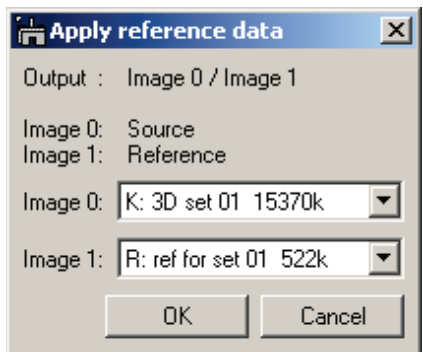


Figure 135 Applying a single image to a data stack.

As a simple convenience, this sub-menu item allows to apply a 2D reference image to each slice of a 3D stack. Both complex and real type images are supported.

Thus for any data stack it is possible to use an arbitrarily generated image as a reference.

5.18.4 **Review image alignment**

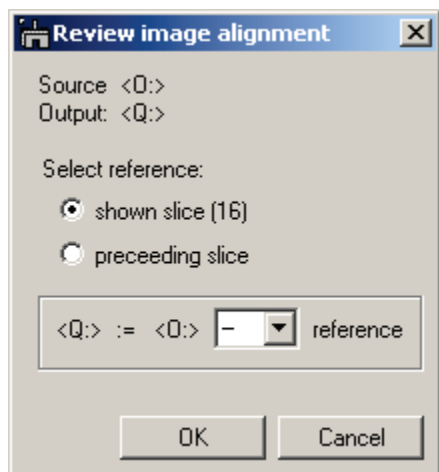


Figure 136 Testing the self-consistency of an aligned data stack.

As a further convenience, this submenu allows to check the quality of an aligned data stack. By selecting one slice of the stack and then evoking this sub-menu, either:

- all slices of the stack are divided by the selected slice or
- the selected slice is subtracted from each slice of the stack.

The outcome ideally is a new stack of images with value 1 or 0, depending on the method selected.

5.18.5 **Reverse stack...**

When 3D data sets are acquired, the last acquired image appears on top of the stack. However, sometimes it is preferred to have the first acquired image to be the first image in the stack (e.g., for preparing a movie from such a stack). The “Reverse stack...” sub-menu allows reversion of the order of the images in a stack. This is done rapidly and such an event is stored in the History tag of the data set.

5.19 Preferences



Figure 137 The Preferences menu

“Preferences” is the second-last entry in the HoloWorks v5 menu. As with any other professional software, parameters can be modified to allow customizing basic features of HoloWorks and make the overall experience more pleasant.

Since there are many values to be determined, the preference window was split up in 8 tabs. Each tab refers to certain activities and the preset values most likely to be customized by the user have been put on the 1st tab.

In the following, details for each of the 8 tabs are given.

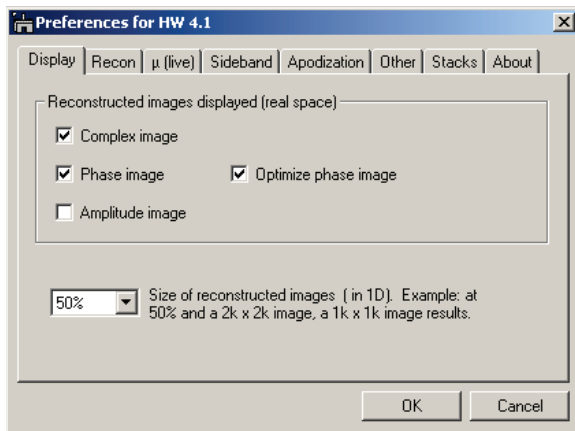


Figure 138 Default settings in the “Display” tab.

The Display tab

is used to select the images that are displayed after the reconstruction process by default. In the example, Figure 137, the complex image and the phase image will be displayed, but not the amplitude image.

Also selected is the Optimize phase offset checkbox. This selection optimizes the phase display such that the number of occurring phase jumps is minimized without performing an actual “phase unwrap.” A phase optimized image has a phase dynamic of 2π . The “optimized phase image” feature is especially important in the live reconstruction process, as the effects of a drift of the interference fringes will be minimized.

The size of the resulting images has been selected at 50%, i.e., a hologram of size 1024 by size 1024 pixels will yield images of size 512 by 512.

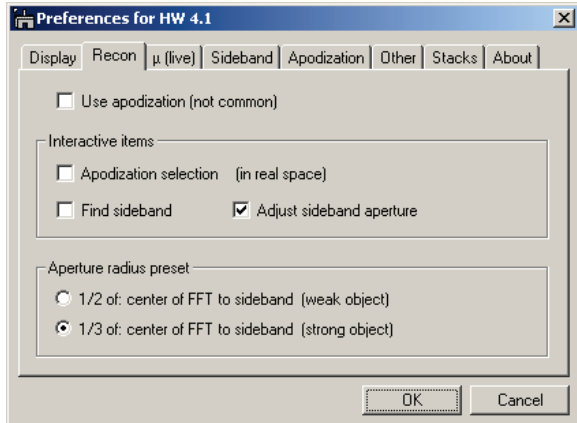


Figure 139 Defaults settings in the “reconstruction” tab.

objects because of a large autocorrelation radius. In general, an interactive adjustment of the sideband filter is preferable to automated filters.

The Reconstruction tab

is used to preset the suggested level of interaction when reconstructing a hologram. In this example, Figure 138, the search of the sideband position is automated, while the filter for the sideband is interactive. Therefore, when activating the reconstruction process either via the user-interface Reconstruct button (page 18) or via the HoloWorks menu item “Reconstruct,” the options (Figure 16, left) are preset accordingly.

The radio buttons are used to propose the radius for the filter for the sideband. Depending on the sample, theory requests a smaller aperture radius for strong

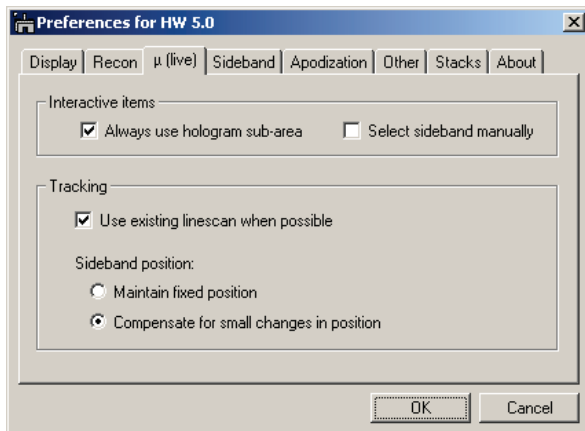


Figure 140 Default settings in the “Fringe contrast μ (live)” tab.

Checking the ‘Use existing linescan when possible’ allows continued data acquisition via the previously used linescan, instead of creating a new linescan each time.

The live Fringe Contrast tab

In this tab the position of the sideband can be set to be determined either interactively or automatically. The latter is usually more practical because this function is mainly used under circumstances where the fringe contrast is good.

For convenience at the microscope, there is an option to ‘Always use hologram subarea.’ This means that if the user has not drawn a volatile ROI on the image already, the software will create the ROI – if the Shift key was used when pressing the μ button (page 18) for continuous fringe contrast tracking.

Furthermore, the option exists to allow a small, automatic correction of the sideband position in case e.g., the biprism voltage needs a slight adjustment. Under high level noise situation, the option “maintain fixed position” might be a better choice, although HoloWorks suspends automatic correction if the mean pixel value in the hologram drops below the default value of 20 (adjustable via File/Global Info.../Tags/Holography/Fringe contrast/Live/no auto track for <P> < >).

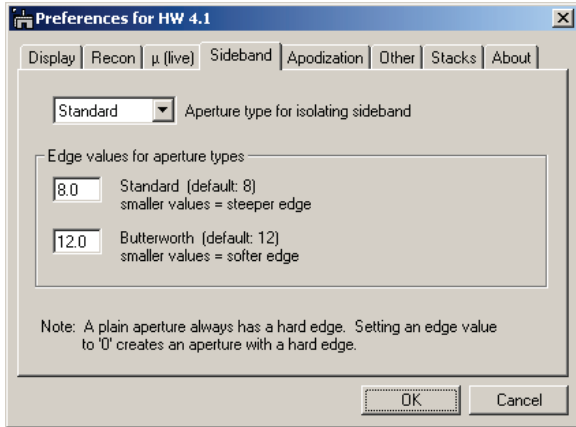


Figure 141 Default settings in the “Sideband” tab.

The Sideband tab

HoloWorks offers 3 different apertures for isolating the sideband from all other contributions. The Standard aperture, as provided by Gatan, the Butterworth filter and a hard aperture.

Hard apertures in Fourier space have a strong tendency to cause artifacts in the reconstruction. Therefore, soft edges are a preferable selection. Please note that a smaller value for the Standard aperture causes a sharper edge, while a smaller value for the Butterworth filter edge causes the edge to be softer.

Setting the edge values to zero will cause a hard edge in all cases.

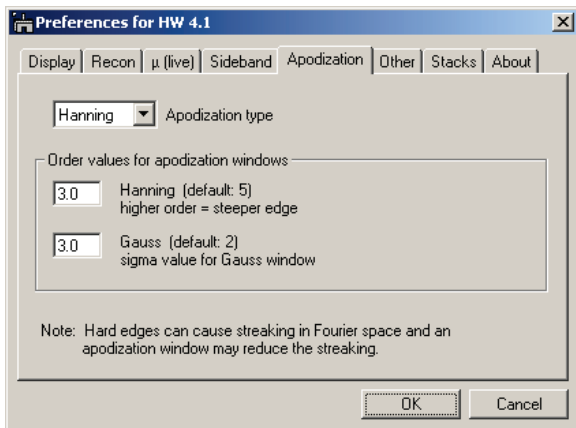


Figure 142 Default settings in the “Apodization” tab.

The Apodization tab

Digital holograms and Fourier transforms have one clear disadvantage compared to waves in a microscope: the Nyquist limit and the hard edges of the CCD camera. To minimize artifacts, Apodization windows can be used (see also Chapter 5.15).

The popup box allows pre-selection of the filter used for isolating the sideband, in this case a Hanning window.

Typical orders for the Hanning window and the Gauss window are provided, but due to the mathematical expression for each of the Apodization windows,

higher order Hanning windows have a steeper edge but retain more of the image itself, whereas higher order Gauss windows retain less and less of the image.

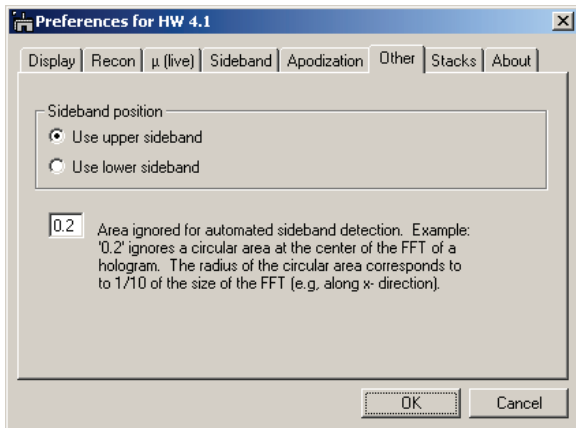


Figure 143 Default settings in the “Other” tab.
increase of its value will alleviate the problem.

As default sideband position, either the upper or the lower sideband must be picked. Since the information is identical in both sidebands (apart from signs) either sideband can be picked. By default, the upper sideband (located in the upper have of the FFT) is picked over the lower sideband position.

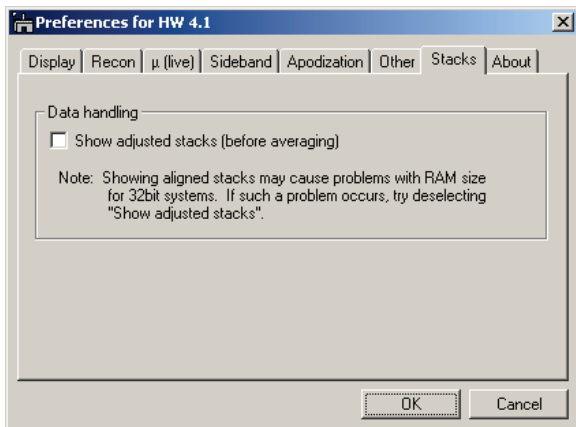


Figure 144 Default settings in the “Stacks” tab.

Hopefully this may be an issue of the past once 64bit systems are introduced.

For these reasons, the generally recommended setting for this tab is to leave the checkbox unchecked.

The Other tab

This tab provides access to values that are rarely changed. The “area ignored” value is used in the process of finding the position of the sideband. Its allowed values are between 0 and 1. If it is 1, then all pixels within the radius of half the Fourier image size will be ignored. If set to 0, no pixel will be ignored and the algorithm will find the autocorrelation peak instead of the sideband. In practice, if the sideband is located very close to the autocorrelation, it might be necessary to decrease this value slightly. If the algorithm for finding the sideband ends up picking a value within the autocorrelation area instead, a slight

The Stacks tab

When working with stacks, it can be useful to review if the stack was properly aligned with respect to lateral alignments or the alignments of phase offsets before averaging.

Working with stacks in general can put serious demands on memory. A stack with 100 2k×2k images exceeds 1.6GB. Adding processing on top easily exceeds the memory capabilities of 32 bit systems. Keeping stacks around that are no longer needed for processing – but are wanted for review purposes – can prevent the data processing to finish.

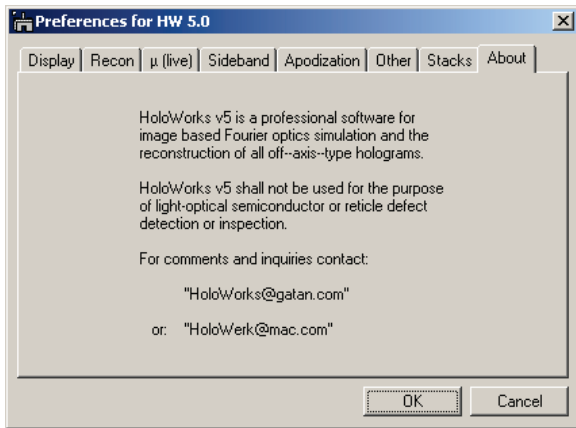
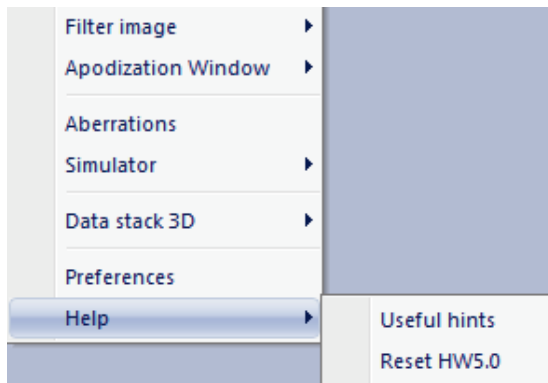


Figure 145 The 'About' tab

The About tab

provides some basic information how to get technical support. Also, bug reports and requests for additional features are welcome.

5.20 Help



The help menu item contains some useful general hint for the use of HoloWorks and provides a Reset button that restores the original parameters of HoloWorks.

Figure 146 In useful hints, essential features for HoloWorks 5 are summarized.

5.20.1 Useful hints

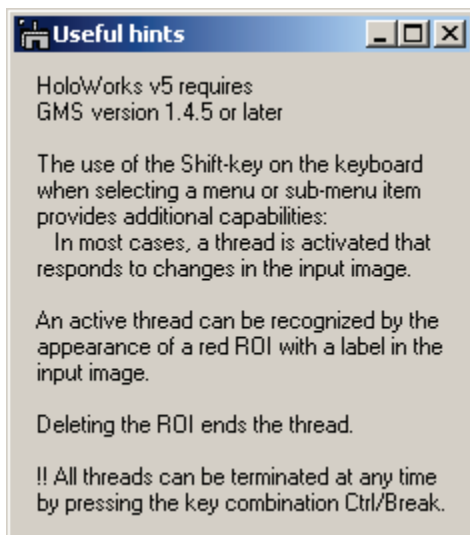


Figure 147 Some useful, very short hints for using HoloWorks.

5.20.2 Reset HW5

Resets HoloWorks parameters to their standard, predefined values.

6 Examples for workflow-based data processing / simulation:

In the following an extensive example of the power of the new HoloWorks is given. It is recommended to review this example as it demonstrates the concepts on which the new HoloWorks version 5 has been built.

In the second part, a short chapter on advanced scripting is provided. For all scripting capabilities, please refer to the extensive Gatan manual under the Help/Search menu. The short chapter on advanced scripting provided here can only give a glimpse at what can be done. Nonetheless, if there is an interest in customizing or creating your own processing capabilities, this is how its done.

6.1 *Project:*

In this project, a target wave, representing a weak phase object (like a real thin amorphous carbon film in transmission electron microscopy) is created with a realistic structure, a hole, in it. This structure is then imaged by introducing aberrations. The effect of transfer functions is reviewed in both real and Fourier space.

6.1.1 *Creating an image that approximates an amorphous thin film*

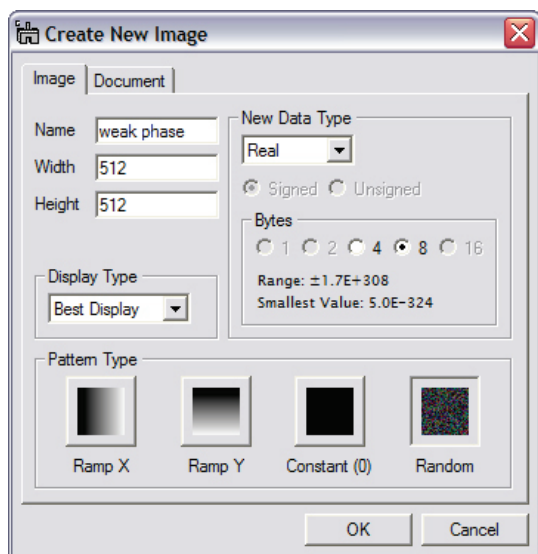


Figure 148 Using DigitalMicrograph's® File/New menu item to create an image that resembles amorphous thin film.

Here, an image is created that represents the simplified weak phase of the object wave as it leaves the object.

Using the DigitalMicrograph® File/New menu, provides the dialog displayed in Figure 147. Note that the “Random” Button is selected, the image size is 512 pixels and the data type is “Real.” The image is named “weak phase.” Click the OK button to create this new image.³⁸ The resulting image “weak phase” has a dynamic range of [0, 1].

To limit the contrast of the image, the value of 0.5 is added to the image by using the “Process/Simple Math...” menu of DigitalMicrograph®.

³⁸ The spectrum of the image created is white. This means a Fourier transform of the image provides an even spectrum, which is not a very good approximation of the scattering amplitude of a thin amorphous film. However, for reasons of simplicity, this will not be investigated further.

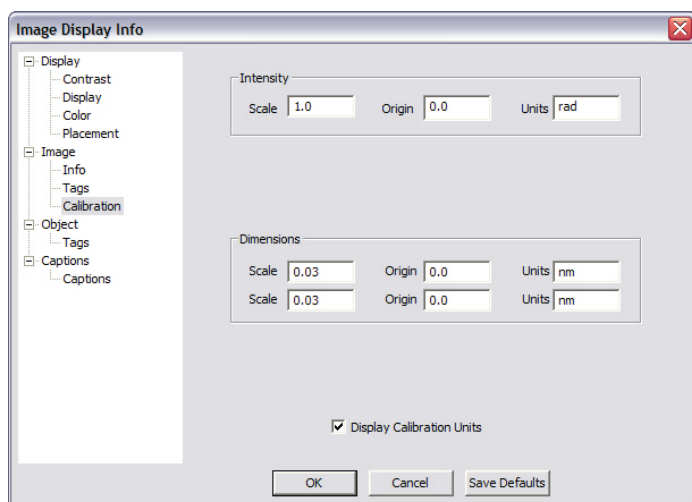


Figure 149 Scaling the dimensions of an image using the DigitalMicrograph® tools.

To determine the physical dimensions of the image a standard 200kV FEG transmission electron microscope is assumed, with e.g., a cut-off frequency (an aperture) at 0.184nm. The effective pixel size of the image should be $\leq 0.184\text{nm}/6$, e.g., 0.023nm. With a $\sim 23\mu\text{m}$ pixel size of the camera, this represents a magnification of $\sim 1,000,000$.

By right-clicking the imaging and selecting the “ImageDisplay...” submenu, the “Image Display Info” Dialog window appears and allows entering the scale values given in Figure 148.

To make the simulated amorphous thin film a bit more realistic, a hole is generated as a next step. For this, the HW5.0/Apply Aperture/Single menu item allows just that. Adding a linescan to the resulting image provides guidance in determining good parameters for the applied aperture.

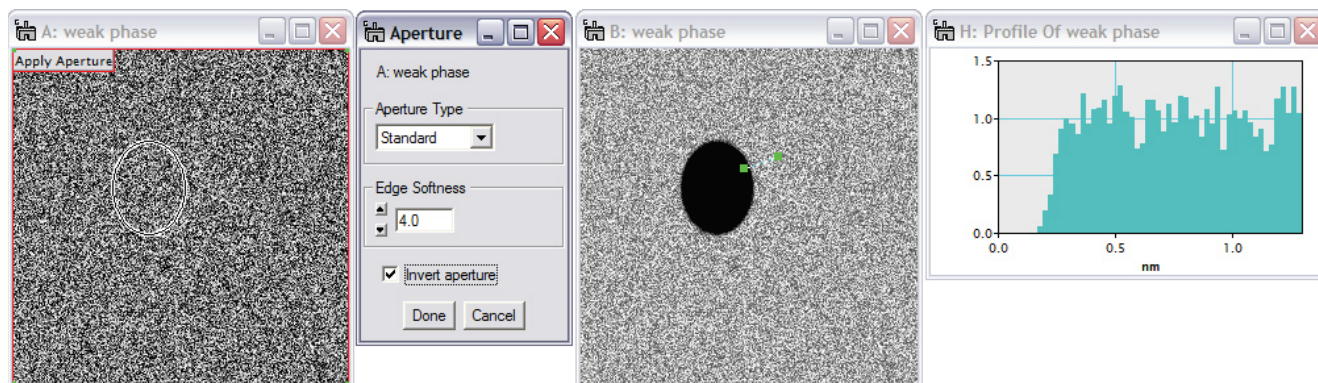


Figure 150 Adding a hole to the simulated amorphous thin film. By using the DigitalMicrograph® linescan, the edge of the aperture is adapted to provide an edge at the hole that is neither too sharp nor too smooth.

The next step is to limit the spatial frequencies of the present holey carbon film. It is not necessary to press the “Done” button in the “Apply Aperture” window. By leaving the window open, the aperture parameters can be fine-tuned later.

6.1.2 Limiting the spatial frequency range

Without ending the thread “Apply Apertures --“, it is now time to filter the phase image and limits its spatial frequency range. Given a pixel size of 0.023nm and an image of size 512, the corresponding pixel size in Fourier space is $1/(512 * 0.023)\text{nm}$. Thus the number of pixels for the radius of the filter that limits the spatial resolution in the simulated image computes as: $1/(0.184\text{nm}) = n * 1/(512 * 0.023\text{nm})$ with $n = 64$ pixels.

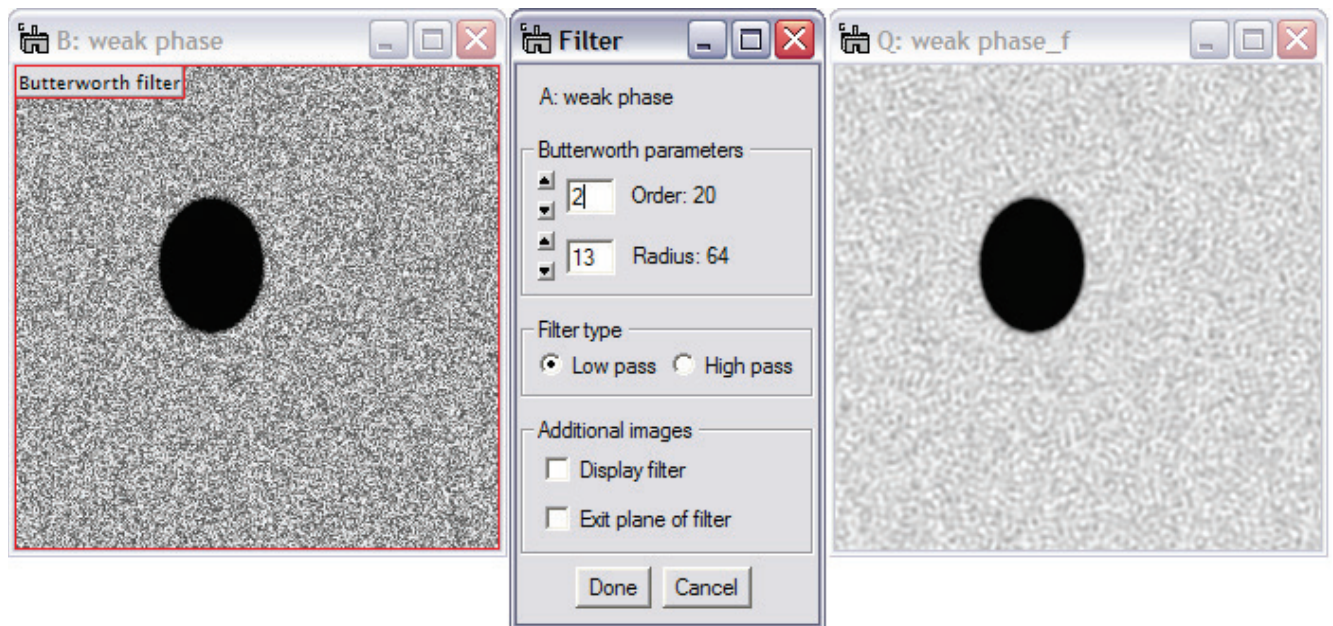


Figure 151 Filtering the image on the right-hand side of Figure 149, creates the desired phase image with a spatial frequency limited to 64 pixels or $1/(0.184\text{nm})$ in this case.

Note: as long as the “Apply Aperture” window remains open, the aperture can be moved and resized (in the image on the left-hand side of Figure 149). As a result, the aperture in the filtered image (Figure 150, right) will be moved as well.

6.1.3 Creating the complex object wave

The next step is to create the amplitude image. Again, the File/New menu, opens the dialog displayed in Figure 147, but this time the “Constant (0)” Button is selected instead of the “Random Button.” The image is named “amplitude” in this case. Set its content to 1 via e.g., the “Process/Simple Math...” menu of DigitalMicrograph[®]. Since the phase image is already calibrated, the calibration step is not necessary for the amplitude image.

Now 2 images of the size 512 by 512 pixels are present, one is representing the amplitude of the object wave, the other one (after filtering, named “weak phase_f”) represents the phase of the object wave.

The menu item “HW5.0/Real To Complex/2x real to complex” allows to merge the two images. Use the menu item with the Shift key down maintaining the string of dependent images.

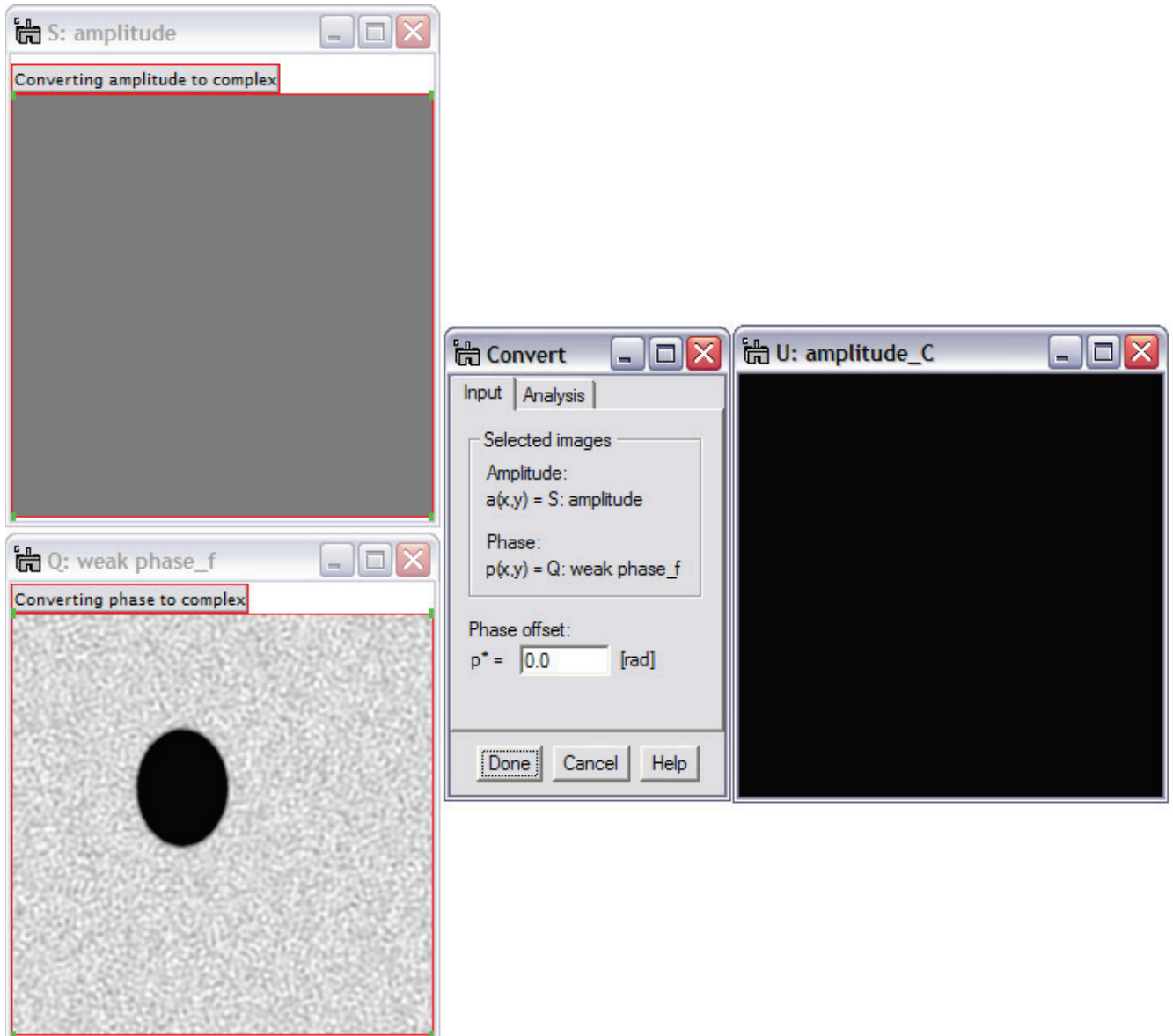


Figure 152 Using the menu item “Holo5.0/Real To Complex/2x real to complex,” the amplitude and phase images are merged to form the complex object wave that carries the name of the amplitude input image with an _C added.

The complex image resulting from merging the amplitude and phase image is seen on the right-hand side of Figure 151. Since all information is in its phase, the image originally appears featureless. Introducing aberrations common in electron microscopes is the task for the next chapter.

6.1.4 *Introducing aberrations*

The menu item “HW5.0/Aberrations” provides the capability to either correct for aberrations or apply aberrations to an image and thus, in the latter case, simulate the effects of imaging. These capabilities will be used on the simulated wave of a thin amorphous carbon foil, the image “amplitude_C.”

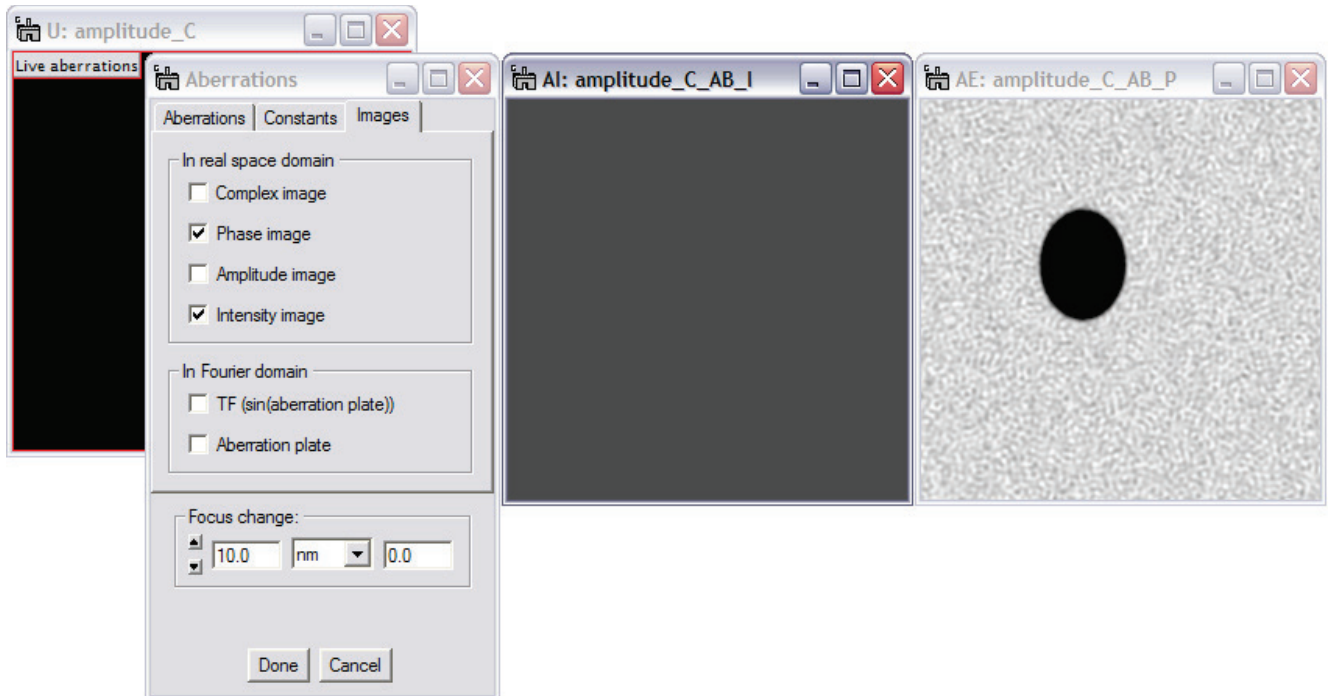


Figure 153 After starting the menu “Holo5.0/Aberrations” the Images tab was selected and the intensity image and phase image were selected for display. The intensity image (middle with the _I at the end of its name) represents what would be recorded in a microscope with no aberrations. The phase image, obtainable through holography, is displayed on the right-hand side.

First, the “Images tab” in the Aberrations window was used to pick the images for display. In this case we were interested in seeing the image as it would be recorded in the microscope. Since originally no aberrations were selected, the image intensity, Figure 152, middle, is flat as phase information only was used to create the object wave. Thus the intensity is flat apart from computational artifacts. By adjusting the display range of the intensity image to reasonable values, the artifacts disappear from display. All information is contained in the phase image on the right-hand side of Figure 152.

Aberrations were introduced using the “Aberrations” tab in the Aberrations window. A spherical aberration value of 1.2mm is an average value for a 200kV microscope. Scherzer focus is defined at 65nm in this case and the focus value was set accordingly.

The filter radius of the Butterworth filter was also changed to a smaller value to suppress some artifacts in the hole of the carbon film. A value of 1.2 was subtracted from the original noise image to improve the phase image display, as the mean inner potential is expected to shift the phase value and a retardation of the phase was opted to be appear darker in the phase than the average phase value in the hole.

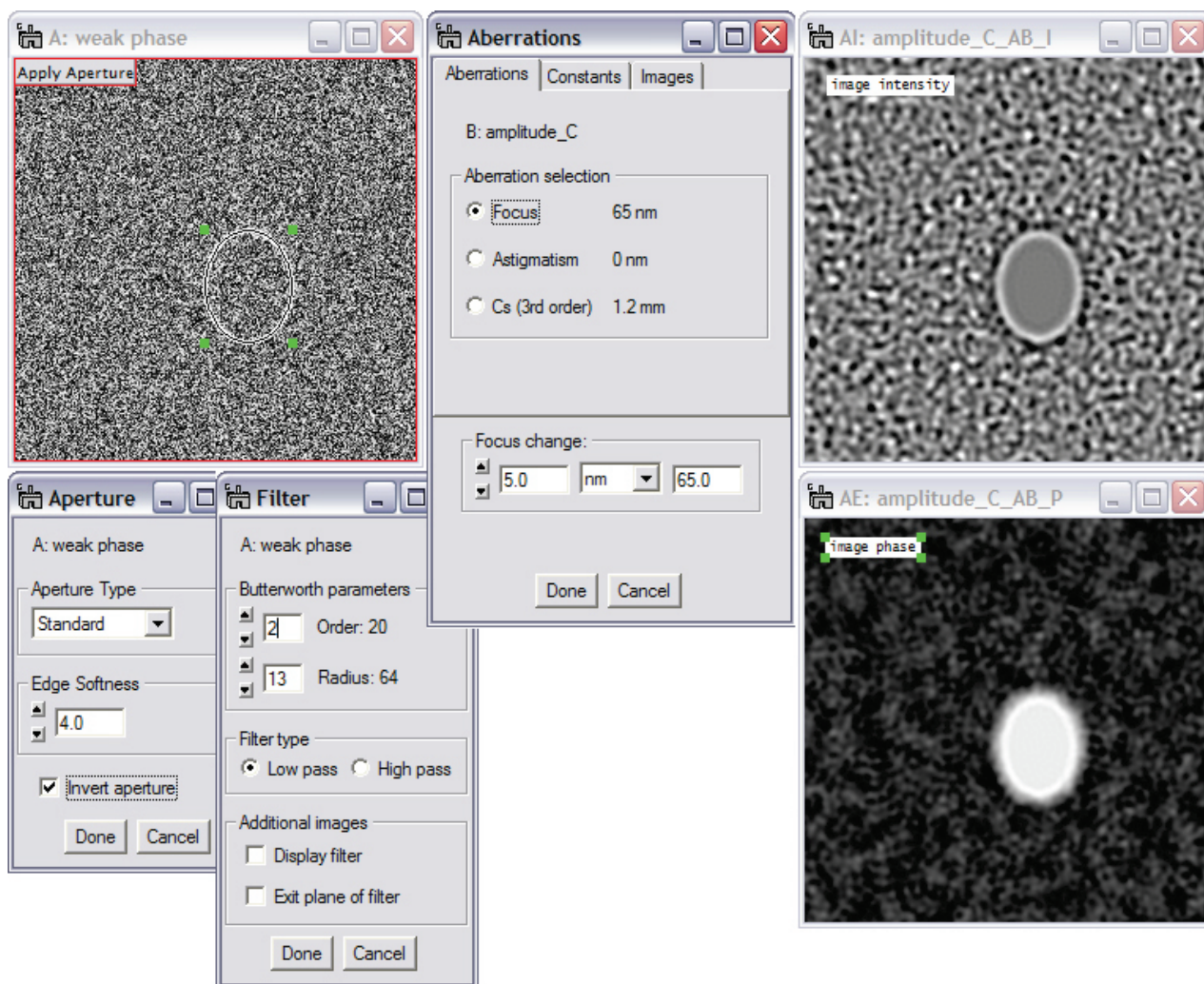


Figure 154 Set-up for microscope simulation. The input images can be changed, for example by shifting the “hole” or by modifying the weak phase or amplitude image (the amplitude image is hidden for a better overview). Whenever a change occurs, its effect is directly observed in the image intensity as recorded in a TEM.

7 Advanced scripting capabilities

One of the goals of HoloWorks v5 is to provide the user with all possible means to get the job done. This means, the software aims at a high flexibility of its applications and aims to provide means for the user to create specialized applications themselves with little effort. Therefore, access to a large number of scripting functions is provided that complement and augment the library of function calls already supported by DigitalMicrograph[®].

Two different types of scripting functions are provided:

1. Standard additional functional calls structured as the standard DigitalMicrograph[®] scripting calls
2. Type 2 function calls that require a class environment

The standard function calls can be used in any script.³⁹ Assuming familiarity with the basics of the DigitalMicrograph[®] scripting capabilities, a short introduction to the type 2 function calls will be given:

```
class reconstruct_ : thread
{
    object init(object self)
        return self
    void runThread(object self)
    {
        number maxX, maxY, rX, rY, order, dX, dY
        string filter_Type

        image front := GetFrontImage();

        result("\n\nReconstruct hologram\t\t" + DateStamp() + "\n");

        complexImage frontF      := HW_Live_GetCoordinatesOfSideband( front, maxX, maxY, 0 );
        complexImage hologramFS := HW_ShiftFFT( frontF, maxX, maxY );
        complexImage sideband    := HW_Live_FilterSideband( hologramFS, filter_Type, rX, rY, order, dX, dY, 0);

        complexImage imageWave := HW_ConvertFullSidebandToImageWave( sideband );
        HW_displayNewImageNextToOriginalImage( imageWave, front, 1 );

        image phaseImage := HW_computePhase( imageWave, 1 );
        HW_displayNewImageNextToOriginalImage( phaseImage, front, 1 );

        image amplitudeImage := HW_ComputeAmplitude( imageWave );
        HW_displayNewImageNextToOriginalImage( amplitudeImage, phaseImage, 1 );

        result("done\t\t" + dateStamp() + "\n\n");
    }
}
alloc(reconstruct_).StartThread()
```

The **blue, bold-face lines** establish the class “reconstruct_”. The commands in **red** provide fully interactive-capable functions that work only within the blue framework. **ALL red commands work only within the blue environment.**

³⁹ See scripting language manual under the Help/Search... menu of DigitalMicrograph.

8 HoloWorks specific script calls

Note the distinction between number variables and numbers: a number variable cannot be substituted by a number.

Function calls that need to be run from within a thread are listed in red.

image	HW_unwrapPhase(image source, number updateDetails) image source is expected to be a phase image (phase of an image of type complex); updateDetails is a binary; if updateDetails = 1, scaling, tags and image name are transferred. If updateDetails = 0, these details are <i>not</i> transferred.
image	HW_unwrapPhase(image source, number updateDetails, number top, number left, number bottom, number right) image source is expected to be a phase image (phase of an image of type complex); updateDetails is a binary; if updateDetails = 1, scaling, tags and image name are transferred. If updateDetails = 0, these details are <i>not</i> transferred.
complexImage	HW_FFT(image front) Creates a FFT of real or complex images (or selections therein) of arbitrarily sized images or line plots. This call also adds to the History tree in the resulting window.
complexImage	HW_ShiftFFT(complexImage frontC, number dX, number dY) Creates a complex type image that is identical to the input image, including scaling parameters, but shifted by dX in x- direction and by dY in y- direction. Both dX and dY are integer numbers. Also, a noteLabel is created with the new image that contains the values of dX and dY as a complex number.
void	HW_ShiftFFT(complexImage frontC, complexImage shiftedFFT, number dX, number dY) Overwrites the image "shiftedFFT" with a shifted version of the image "frontC." Both dX and dY are integer numbers. Also, a noteLabel is created or updated for the image shiftedFFT that contains the values of dX and dY as a complex number.
number	HW_IsComplexImage(image front) Checks if a given image is complex or not. Returns "1" if image is complex, returns "0" if image is of different type. This call recognizes all DM supported images of type complex, i.e., packed-complex image data, as well as complex 8- and 16- Byte data.
number	HW_ValidHistoryEntries(image front) Determines the number of entries in the <i>Image Display Info / image / Tags / History</i> tree.
string	HW_FindLastHistoryEntryContainingString(string searchstring, image front) Searches for the last string in <i>Image Display Info / image / Tags / History</i> tree that contains the search string and returns the complete History entry string.
string	HW_FindLastHistoryEntryContainingString(string searchstring_1, string searchString_2, image front) Searches for the last string in <i>Image Display Info / image / Tags / History</i> tree that contains both search strings and returns the complete History entry string.
string	HW_FindLastHistoryEntryContainingString(string searchstring, image front, number variable entryValue) Searches for the last string in <i>Image Display Info / image / Tags / History</i> tree that contains the search string and returns the complete History entry string, as well as the number for the entry.
complexNumber	HW_ExtractCoordinatesFromString(string searchString) Returns the coordinates of a string from the History tree containing coordinates as a string.

void	<p>HW_ExtractFilterCharacteristics(image front, string filter_type, number variable filter_radius, number variable filter_order)</p> <p>Extracts filter characteristics from the history tree of an image. The filter_type can be "Standard" or "Butterworth"</p>
image	<p>HW_CreateCenterCutImage(complexImage hologramF, number centercut)</p> <p>Creates an image with a hard aperture where the center area is 0, the outer area is 1 and the number centercut determines the radius of the aperture. Acceptable values for the radius are [0,1] with 1 meaning a circle fully inscribed into the image. The complex image hologramF provides the size for the aperture and remains unchanged.</p>
void	<p>HW_positionDialogWindowNextToImage(DocumentWindow dialog_win, image display)</p> <p>Positions a dialog window next to a standard image. First, it attempts to position the dialog on the right hand side of the image. If the right hand side of the image is less than 140 pixels away from the screen end then the document window is positioned on top of the image, but offset by 22 pixels to the right and 18 pixels in y-direction. It is assumed that the dialog window is already displayed.</p>
void	<p>HW_displayNewImageNextToOriginalImage(image new, image original)</p> <p>Displays a new image next to a selected image. First, it attempts to position the new image on the right hand side of the original image. If the right hand side of the image is less than 140 pixels away from the right side of the screen then the new image is positioned on top of the image, but offset by 22 pixels to the right and 18 pixels in y-direction. The original image must be displayed at this point.</p>
void	<p>HW_displayImageNextToDialogWindow(DocumentWindow dialog_win, image display)</p> <p>Displays a new image next to a dialog window. First, it attempts to position the new image on the right hand side of the dialog window. If the right hand side of the dialog window is less than 140 pixels away from the screen end then the new image is positioned on top of the dialog window, but offset by 22 pixels to the right and 18 pixels in y-direction. It is assumed that the dialog window is already displayed.</p>
void	<p>HW_resizeImageBToMatchImageA(image imageB, image imageA)</p> <p>Resizes the image "imageB" to be of equal or smaller size than image "imageA." Seeks the adjustment in x- direction only. For display type "Line Plot" data, the window size is adjusted so image A and B have an identical window size.</p>
void	<p>HW_displayNewImageNextToOriginalImage(image new, image original, number resize)</p> <p>Displays a new image next to a selected image. First, it attempts to position the new image on the right hand side of the original image. If the right hand side of the image is less than 140 pixels away from the right side of the screen then the new image is positioned on top of the image, but offset by 22 pixels to the right and 18 pixels in y-direction. The original image must be displayed at this point. The number resize = 1 assures that the new image won't be displayed in a larger window (x- direction only) than the original image.</p>
complexImage	<p>HW_IFFT(complexImage sideband)</p> <p>Creates a complex image that is the inverse Fourier transform of a complex input image. It copies the history tree but not the scaling parameters and sets a name for the new image that consists of a modified version of the input image and adds a "_C" to the image name.</p>
image	<p>HW_computeAmplitude(complexImage imageWave)</p> <p>Creates a real type image that is the amplitude or abs() value of a complex input image. It copies the history tree and the scaling parameters and sets a name for the new image that consists of the input image name and adds a "_A" to the image name.</p>
image	<p>HW_ComputeAndDisplayAmplitude(complexImage waveImage, number mode)</p> <p>Creates a real type image that is the amplitude or abs() value of a complex input image. It copies the history tree and the scaling parameters and sets a name for the new image that consists of the input image name and adds a "_A" to the image name. According to the mode value (0,1,2), the image is either only computed but not displayed (0); computed and displayed in an arbitrary position (1).</p>
image	<p>HW_computePhase(complexImage imageWave, number mode)</p> <p>Creates a real type image that is the phase or phase() + π value of a complex input image. It copies the history tree and the scaling parameters and sets a name for the new image that consists of the input image name and adds a "_P" to the image name. According to the mode value (0,1,2), the image is either computed with NO phase offset correction (0); computed with phase-offset-correction (1). The intensity units are set to "rad."</p>
complexImage	<p>HW_ConvertFullSidebandToImageWave(complexImage sideband)</p> <p>Converts filtered and centered sideband to the complex image wave.</p>

image	<p>HW_CreateButterworthFilter(number sizeX, number sizeY, number rX, number rY, number order)</p> <p>Creates an image representing a Butterworth filter. The numbers sizeX and sizeY are expected to be integers. rX and rY provide the nominal radius in x- and y- direction, order refers to the order of the Butterworth filter.</p>
image	<p>HW_CreateShiftedButterworthFilter(number sizeX, number sizeY, number apertureOffsetX, number apertureOffsetY, number rX, number rY, number order)</p> <p>Creates an image representing a Butterworth filter. The numbers sizeX and sizeY are expected to be integers. rX and rY provide the nominal radius in x- and y- direction, order refers to the order of the Butterworth filter. apertureOffsetX and apertureOffsetY describe the offset of the filter in x- and y- direction.</p>
void	<p>HW_UpdateShiftedButterworthFilter(image filter, number apertureOffsetX, number apertureOffsetY, number rX, number rY, number order)</p> <p>Requires an existing image as input, which will be modified to become the updated filter. The apertureOffset values are in pixels as are the radii for the aperture. The order is the order of the Butterworth filter and "0" refers to a hard edge.</p>
void	<p>HW_CopyAllTagsFromTo(image source, image new)</p> <p>Copies all Tags from image <i>source</i> to image <i>new</i>. If image <i>source</i> does not have a history tree, then a new tree is started with the image <i>new</i> listing the name of the image <i>source</i> as the original image.</p>
void	<p>HW_CopyAllTagsAndCalibrationFromTo(image source, image new)</p> <p>Copies all Tags and calibration from image <i>source</i> to image <i>new</i>. If image <i>source</i> does not have a history tree, then a new tree is started with the image <i>new</i> listing the name of the image <i>source</i> as the original image.</p>
complexImage	<p>HW_CreateConjugateSideband(complexImage frontC)</p> <p>Creates a new image that is the conjugate sideband of the input file.</p>
void	<p>HW_ConvertSidebandToConjugate(complexImage frontC)</p> <p>Converts the given sideband into its conjugate sideband.</p>
number	<p>HW_IsPowerOfTwo(number top, number left, number bottom, number right)</p> <p>Checks if the selected area as defined by the numbers top, left, bottom, right is of the power of 2. Returns "1" if is power of 2 and 0 if not.</p>
image	<p>HW_CreateHanningWindow(number sizeX, number sizeY)</p> <p>Creates a basic Hanning window according to $1-\cos(2\pi m/M)$ with m in [0, M-1] and M as the number of pixels.</p>
image	<p>HW_CreateHanningWindow(number sizeX, number sizeY, number order)</p> <p>Creates a modified Hanning window according to the publication in MSA Bulletin, (1994) Vol 24, No 1: pp 466-471. The difference between the basic and modified Hanning window is such that for order \rightarrow infinity, the window becomes a rectangle window. For order=1, the exact standard Hanning window is created. For larger values, a slightly different constant is being used to satisfy the condition order \rightarrow infinity \Rightarrow rectangular, periodic window.</p>
image	<p>HW_CreateInverseHanningWindow(number sizeX, number sizeY, number order)</p> <p>Creates an inverse modified Hanning window by first creating a Hanning window using the function call HW_CreateHanningWindow() then after avoiding zeros in the window returning 1/window.</p>
image	<p>HW_CreateApodizedGaussWindow (number sizeX, number sizeY, number order)</p> <p>Creates an apodized Gauss window. The shape of the Gauss function throughout the image is image size independent. The function is defined as $f(x) = \exp(-(x^2+y^2))$. The range for x is defined by the value of the parameter order, so for order = 1, the range for x is $-1 \leq x < 1$. This function is then converted to discrete values as defined by the image-size parameters sizeX, sizeY.</p>
image	<p>HW_CreateInverseApodizedGaussWindow (number sizeX, number sizeY, number order)</p> <p>Creates an inverse apodized Gauss window by first creating a Gauss window using the function call HW_CreateApodizedGaussWindow () then replacing all values $< 1e-8$ by $1e-8$ returning 1/window.</p>
number	<p>HW_determinePhaseOffset(image reference)</p> <p>determines a value for a potential phase-offset that can minimize the number of phase-jumps in a given image.</p>

image `HW_AddNoiseToSimulatedImage(image sim)`
initially creates a “noise” image of same size as image sim that contains white noise with an average of 0 and a standard deviation of ± 1 . The “noise” image is then multiplied with the square root of the input image. The latter image is then added to the input image and the resulting image is returned.
Input requirements: All values of the input image must be positive and correspond to an image intensity distribution.
Note: the maximum and minimum values in the noise image are $\pm\sqrt{12}/2$. Thus the simulated intensity image should not contain values below $\sqrt{12}/2$, as negative values would result in the output image. Since this is physically not acceptable, negative values are reset to zero by this function call. The reset however alters the noise distribution and should be treated with care.

complexNumber `HW_Centroid(image front, number top, number left, number bottom, number right)`
determines the centroid of a given selection or of the full image. The real part of the returned number is the centroid position on x- direction, while the imaginary part of the complex number represents the y- component of the centroid. The definition of the centroid is $\text{Sum}(\text{pixel location} * \text{pixel values}) / \text{Sum}(\text{pixel values})$, as e.g. defined in <http://mathworld.wolfram.com/GeometricCentroid.html>.

image `HW_Correlate(Image img_1, Image img_2, number useRealSpaceFilter, number useFourierSpaceFilter)`
creates an image that represents the crosscorrelation between two real or two complex images. The numbers “useRealSpaceFilter” and “useFourierSpaceFilter” can have the values “0” or “1”, meaning no use or use of the filters. In general, for the cross-correlation of two real images “useFourierSpaceFilter” is set to 0, while for complex images, the value is often set to 1. The function call works also on image that are neither square nor of the-power-of-two.

complexNumber `HW_computeSubPixelOffset(image CrossCorrelationImage, number digits)`
returns a complex number that represents the offset between images in pixels. The real part refers to the pixel offset in x- direction, the imaginary part refers to the offset in y- direction. The input image is expected to be a real type image that is obtained from the `HW_Correlate()` function call. The number “digits” defines the number of digits giving the fraction of a pixel. “0” means only integer values are returned. “1” means, offset is returned within a tenth of a fraction of a pixel. “2” means, offset is returned within a hundreds of a fraction of a pixel. “3” means, offset is returned within 1/10000 of a fraction of a pixel, which of course is not realistic. Special care for the image calibration must be taken if the images used for the `HW_Correlate()` call were not of the power-of-two!

number `HW_CCD_contrast_factor(number samplingX, number samplingY)`
returns a number between 0 and 1 for sampling rates 2 and higher. It is assumed that the active area of the CCD element is 100%, which is usually true for cameras for electron microscopes, but often not true for cameras designed for the light optical field. The numbers `samplingX` and `samplingY` refer to the sampling frequency for a given spatial frequency.

number `HW_CCD_contrast_factor(number samplingX, number samplingY, number efficiencyX, number efficiencyY)`
returns a number between 0 and 1 for sampling rates 2 and higher. It is assumed that the active area of the CCD element is 100%, which is usually true for cameras for electron microscopes, but often not true for cameras designed for the light optical field. The numbers `samplingX` and `samplingY` refer to the sampling frequency for a given spatial frequency. The numbers `efficiencyX` and `efficiencyY` refer to efficiency of each camera pixel. An active pixel could cover e.g., 80% of the available area in x- direction and 75% in y-direction, and thus the corresponding input values would be 0.8 and 0.75 respectively, providing an active area of 60%.

number `HW_GetVolatileRectangularROIcoordinatesAndID(ImageDisplay frontDisp, numbervariable top, numbervariable left, numbervariable bottom, numbervariable right)`
returns the ID of a rectangle, volatile ROI. If no ROI is found, 0 is returned. The numbervariables return the coordinates of the selection or the size of the image (if no rectangular, volatile ROI found). Works for 1D – 3D data sets.

number `HW_BuildListOfImageNamesAndIDs(TagGroup nameList, TagGroup idList)`
returns the number of list elements. The tag groups contain the lists with the names of displayed images.

number `HW_BuildListOfImageNamesAndIDs_ofLinePlots(TagGroup nameList, TagGroup idList)`
returns the number of list elements. The tag groups contain the lists with the names of displayed images, but displayed in linescan mode.

number `HW_SelectedROISendToBack(image front)`
sends the selected ROI to the back and returns the component ID of the ROI (not the ROI ID).

number `HW_SelectedROIBringToFront(image front)`
brings the selected ROI to the front and returns the component ID of the ROI (not the ROI ID).

number	<p>HW_getSize(image front, numbervariable sizeX, numbervariable sizeY, numbervariable sizeZ, numbervariable slice_start, numbervariable slice_end)</p> <p>determines the size of an image. Image can be 1D - 3D or a line profile. If 3D, then the shown slice (start and end) is given as well. The return value returns the sizeZ value. All parameters are number variables! A 1D image has sizeY = sizeY = 1 and sizeZ = 1 (this allows to set selection[] parameters to size values by default).</p>
number	<p>HW_getSize(image front, numbervariable sizeX, numbervariable sizeY, numbervariable sizeZ)</p> <p>determines the size of an image. Image can be 1D - 3D or a line profile. The return value returns the sizeZ value. All parameters are number variables! A 1D image has sizeY = sizeY = 1 and sizeZ = 1 (this allows to set selection[] parameters to size values by default).</p>
void	<p>HW_getScale(image front, numbervariable scaleX, numbervariable scaleY, numbervariable scaleZ)</p> <p>determines the scale parameters of an image. Image can be 1D - 3D or a line profile. value. All parameters are number variables! If number variables don't exist, their value is returned as zero.</p>
void	<p>HW_getScale(image front, numbervariable scaleX, numbervariable scaleY)</p> <p>determines the scale parameters of an image. Image can be 1D - 3D or a line profile. value. All parameters are number variables! If number variables don't exist, their value is returned as zero.</p>
number	<p>HW_compute2DsamplingRate(number s_x, number s_y, number digitsAfterDecimalPoint)</p> <p>Computes the 2D sampling rate from the 1D sampling rate values. Either s_x or s_y or both can be infinite. If both s_x and s_y are infinite, a zero (instead of infinity) is returned, indicating the zero-frequency sampling rate. The number digitsAfterDecimalPoint does what the variable name suggests. Values of 0,1,2,3,4,5 are permissible; otherwise no rounding occurs.</p>
number	<p>HW_compute2DsamplingRate(number s_x, number s_y)</p> <p>Computes the 2D sampling rate from the 1D sampling rate values. Either s_x or s_y or both can be infinite. If both s_x and s_y are infinite, a zero (instead of infinity) is returned, indicating the zero-frequency sampling rate.</p>

Function calls that can be used only within a thread:

complexImage	<p>HW_Live_GetCoordinatesOfSideband(image hologram, numbervariable maxX, numbervariable maxY, number mode)</p> <p>Allows to interactively or manually determine the sideband position. Mode = 0 means fully automated, mode 1 means interactively. This call works for 2D and 3D data sets. For 3D data sets, the displayed slice is picked as the image to determine the sideband coordinates from. This call must be embedded in a class environment.</p>
void	<p>HW_Live_FilterSideband(complexImage live, numbervariable filterRadiusX, numbervariable filterRadiusY, numbervariable order, numbervariable apertureOffsetX, numbervariable apertureOffsetY, number mode)</p> <p>Allows to interactively or manually isolate the sideband. Mode = 0 means fully automated, mode 1 means interactively. This call must be embedded in a class environment.</p>
Image	<p>HW_ApplyApodizationWindow(image front, number mode, numbervariable apodizationType, numbervariable apodizationOrder)</p> <p>Allows to interactively or automatically apply an Apodization window to a real or complex type image by reproducing a new image that is either complex or real depending on the input. The parameters for the Apodization type and order are read from the persistent number notes and set via the preferences. The number variables are ignored in the input, but contain the information of the window type applied in the output. Note that the output can be a complex image (!) even though the function call is defined as a real type image. Images can be of 1D, 2D or 3D. Mode = 0 means fully automated, mode 1 means interactively. Modes 2 and 3 correspond to modes 0 and 1, but the Shift-key sensitivity is blocked as is the application to all slices of a 3D data set. For 3D images, the window is initially applied to the selected slice but offers the option to apply to all slices as well (in modes 0 and 1). This call must be embedded in a class environment.</p>

Image

HW_UndoApodizationWindow(image front, number mode)

Allows to interactively or manually remove an Apodization window from a real or complex type image by producing a new image that is either complex or real depending on the input. This feature may cause some complications. Images can be of 1D, 2D or 3D. Mode = 0 means fully automated, mode = 1 means interactively. For 3D images, the window is initially applied to the selected slice but offers the option to apply to all slices as well. This call **must** be embedded in a class environment.

Image

HW_averageDataStack(image front, number mode)

Averages a complex or real or integer type data stack and returns the averaged image. Mode = 0 means fully automated with no user interaction. Mode = 1 means a user interface will show. Both modes distinguish complex and non-complex data type. For complex data types and mode = 0, no lateral drift correction is applied; instead phase compensation is performed. For non-complex data types and mode = 0, the lateral drift compensation is enabled, but there is no phase compensation. Usually, this call with mode 0 would be found in a reconstruction procedure involving 3D data sets. This call **must** be embedded in a class environment.

9 Table of “endings”

_a	One or several hard apertures applied to a complex image
_A	Modulus (amplitude) of a complex image
_C	Complex image has been created from one or several real images
_diff	Diffraction pattern of an image
_F	Fourier transform of an image
_f	Image was filtered
_t	image was tilted
_w	Apodization window applied (removed) to (from) an image
_Im	Imaginary part of a complex image
_P	Phase of a complex image
_R	Inverse Fourier transform of an image
_Re	Real part of a complex image
_I	Intensity of a complex image
_s	Subarea (ROI) of an image
_AB	Aberrations were introduced / removed

Example: The ending

_s_F_s_R_A

Has the following meaning:

The sub-image function has been applied to the original image, then a Fourier transform was performed, from which a sub-image has been taken, which has been inverse Fourier transformed, from which the modulus (amplitude) has been extracted.

10 References:

HoloWorks can be referenced! The following paper describes HoloWorks version 1:

A software package for the processing and reconstruction of electron holograms, E. Voelkl, L.F. Allard and B. Frost, *Journal of Microscopy*, Vol. 180, Pt 1, October 1995, pp. 39-50.

Essential literature:

Electron Holography, A. Tonomura, Springer series in optical sciences, Springer-Verlag, Berlin Heidelberg, New York, 1999.

Introduction to Electron Holography, E. Voelkl, L.F. Allard and D.C. Joy, Ed. (1999), Kluwer Academic / Plenum Publishers, New York.

Advances in Imaging and Electron Physics: Electron Microscopy and Holography II. Author: Peter W. Hawkes, Academic Press, 2002, ISBN/UPC: 0120147645.

The Image Processing Handbook, J.C. Russ, CRC Press, 1992, ISBN 0-8493-4233-3

Approaching routine $2\pi/1000$ phase resolution for off-axis type holography, E. Voelkl, D. Tang, *Ultramicroscopy* 110 (2010) 447–459.

Noise in off-axis type holograms including reconstruction and CCD camera parameters, E. Voelkl, *Ultramicroscopy* 110 (2010) 199–210.

For off-axis holography in light optics, consider the following reference:

Direct to digital holography for semiconductor wafer defect detection and review, C. E. Thomas, Jr., T. M. Bahm, L. R. Baylor, P. R. Bingham, S. W. Burns, M. Chidley, L. Dai, R. J. Delahanty, C. J. Doti, A. El-Khashab, R. L. Fisher, J. M. Gilbert, J. S. Goddard, Jr., G. R. Hanson, J. D. Hickson, M. A. Hunt, K. W. Hylton, G. C. John, M. L. Jones, K. R. Macdonald, M. W. Mayo, I. McMackin, D. R. Patek, J. H. Price, D. A. Rasmussen, L. J. Schaefer, T. R. Scheidt, M. A. Schulze, P. D. Schumaker, B. Shen, R. G. Smith, A. N. Su, K. W. Tobin, Jr., W. R. Usry, E. Voelkl, K. S. Weber, P. G. Jones, and R. W. Owen; *Proc. SPIE* 4692, 180 (2002).

Proof that aberration correction on Fourier optics principles can be obtained beyond a numerical aperture of 0.5NA can be found here:

Using diffractograms to evaluate optical systems with coherent illumination, *Optics Letters*, Vol. 28, No. 23, December 1, 2003, pages 2318-2320.

11 Technical Support

Questions or comments can be sent by email to:

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Updates:

www.HoloWerk.com

Acknowledgements:

Thanks are expressed to the company nLine and its employees for the experimental verification of the fact that mathematically and from the viewpoint of data processing there is no difference between light and electron holography despite the much better fringe contrast and absence of the biprism-Fresnel fringe nuisance that plagues electron optics. On the other hand, electron holography is not plagued by back reflections and diffuse scattering as its optical elements are not made from matter. There is little doubt left that off-axis holography works for any particles or waves, as long as the involved particles, or waves, are coherent.

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12 Terms of use:

HoloWorks v5 shall not be used for the purposes of light-optical semiconductor or reticle defect detection or inspection.

Dr E. Voelkl does not make any warranty, express or implied, or assumes any legal liability or responsibility for the use of this software.

The purchase of 1 (one) HoloWorks software package (i.e., license) permits installation on 1 (one) microscope computer and on up to 3 (three) off-line computers.