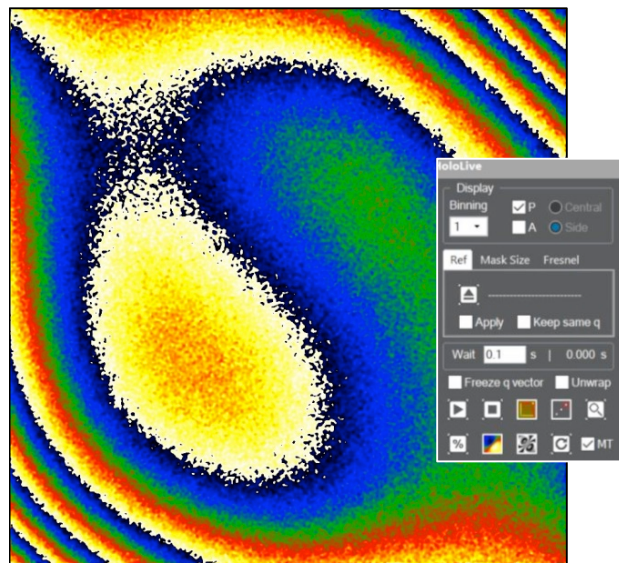# *HoloLive!*
for DigitalMicrograph

Live phase images for
off-axis electron holography

HoloLive Manual 1.0

HREM Research Inc

Conventions

The typographic conventions used in this help are described below.

Convention	Description
Bold	Used to denote components of the user interface such as buttons, field names, menus, and menu options.
	For example, the **New** button.
Menu...MenuOption	Select the menu from the menu bar then select the menu option from the menu.
	For example, **File...Open** would mean to select the **File** menu and then the **Open** option.
CAPS	Used to denote the name of a key on the keyboard.
	For example, the ENTER key.
Italics	Used to denote emphasis, captions and the result of an action in a procedure.

Contact Us

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Copyright Statements

Introduction to HoloLive!

Welcome to the HoloLive! plug-in for DigitalMicrograph (Gatan Inc.). This software is designed to provide live phase images during off-axis electron holography experiments, hence the exclamation mark. The software was developed because electron holograms are almost impossible to interpret directly by the human eye: the important information is contained in the phase, which requires reconstruction. We have found that being able to see the phase during holography experiments changes the experience completely, particularly for in-situ work.

The main requirement is that the live holograms at the microscope are displayed within DigitalMicrograph, in the usual viewing mode for Gatan cameras. As the hologram changes over time, HoloLive! automatically recalculates and displays the corresponding phase image. Using current computing power, a speed of 4 phase images per second can be obtained for a full 4K by 4K digital hologram, with even higher speeds for smaller-sized holograms. Live features include:

- Automatic detection and tracking of side band (via live FFT)
- Carrier frequency refinement (via user-defined reference area)
- Distortion correction (via a reference hologram)
- Phase unwrapping
- Fresnel fringe filtering
- Amplitude and Cosine visualisation

HoloLive! has been briefly described in the following publication:

[1]   C. Gatel, J. Dupuy, F. Houdellier, M.J. Hÿtch, **Appl. Phys. Lett.** 113, 133102 (2018). *Unlimited acquisition time in electron holography by automated feedback control of transmission electron microscope. 10.1063/1.5050906*

Development versions of HoloLive! were used extensively in the following in-situ electron holography work:

[2]   C. Gatel, B. Warot-Fonrose, N. Bizière, L. A. Rodriguez, D. Reyes, R. Cours, M. Castiella, M.-J. Casanove, **Nature Communications** 8, 15703 (2017). *Inhomogeneous spatial distribution of the magnetic transition in an iron-rhodium thin film. 10.1038/ncomms15703*

[3]   C. Gatel, X. Fu, V. Serin, M. Eddrief, V. Etgens, B. Warot-Fonrose, **Nano Letters** 17, 2460−2466 (2017). *In Depth Spatially Inhomogeneous Phase Transition in Epitaxial MnAs Film on GaAs(001). 10.1021/acs.nanolett.7b00144*

Without phase visualisation during experiments, how can you tell if the data being collected is valuable? And during in-situ work, how could you tell if the applied stimuli are indeed changing the fields? HoloLive! has been designed to help you during experiments, to make them more productive and more fun.

In this manual, we will dive straight into the use of the HoloLive! package with a prepared example. Indeed, we have provided a stack of holograms, acquired experimentally, to simulate work at the microscope. A simple script refreshes the hologram in a viewing window by cycling through the data stack.

But before starting, there are a few important points to remember:

1. *HoloLive! is a plug-in for DigitalMicrograph (Gatan Inc.)*. This means that results are fully compatible with the other functions present in DM. For example, profiles drawn across the live phase images using the **DM line profile tool** will be automatically updated. Similarly, any calibration of the images within DM will be transferred to the phase images.

2. *The complete list of commands related to HoloLive! are located in the menu:*
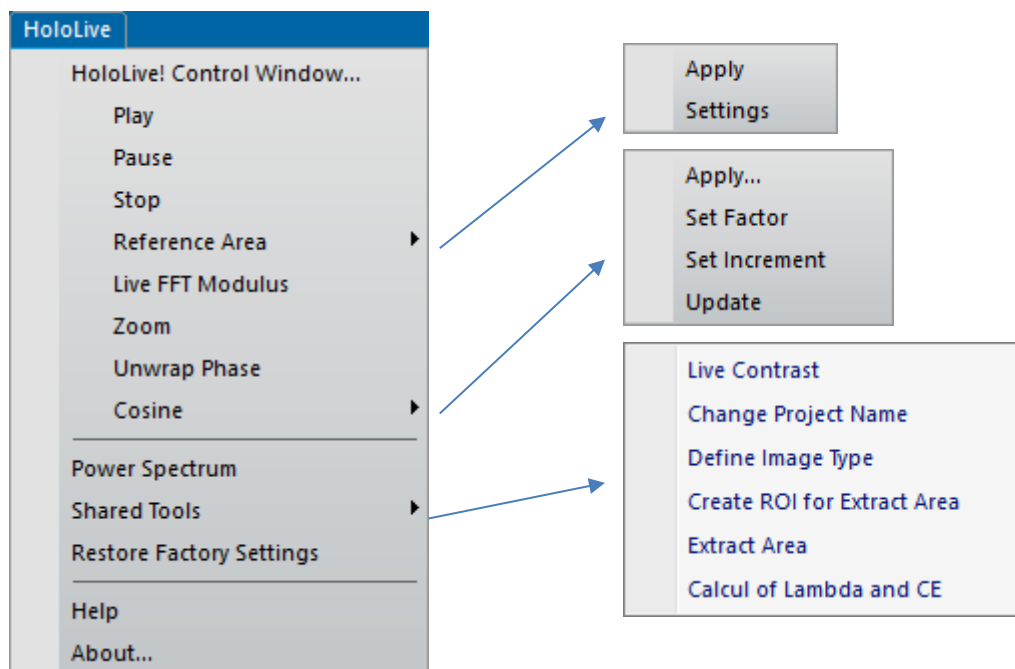
3. *However, it is more fun to access command using the HoloLive! control window.* The following window can be opened from the first menu of the HoloLive!:
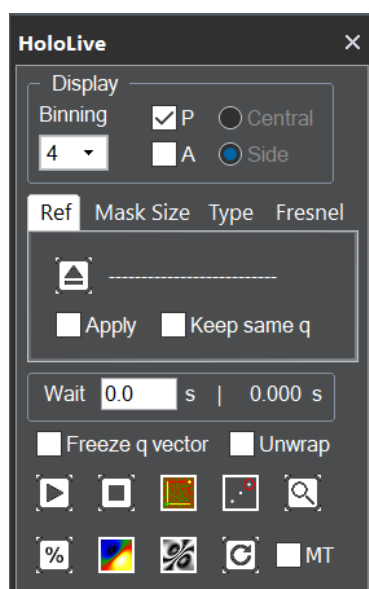
In the following, we will assume use of the HoloLive control window, but all commands can be accessed via **HoloLive** menu.

Like a TV remote controller, check out the overall features on the next page.
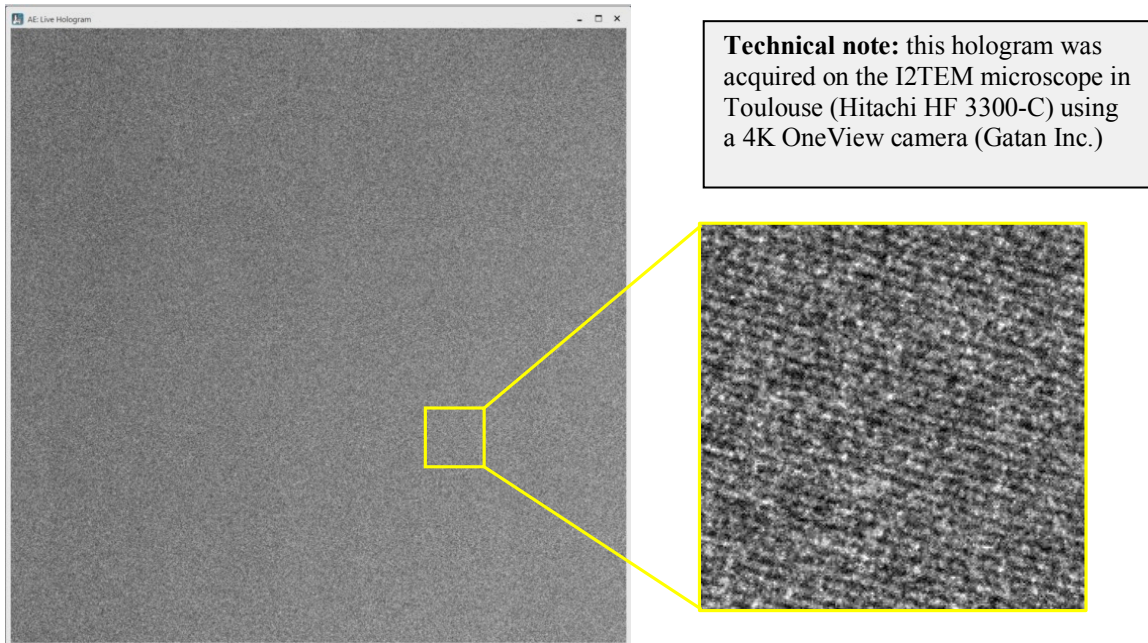
HoloLive! Control Window
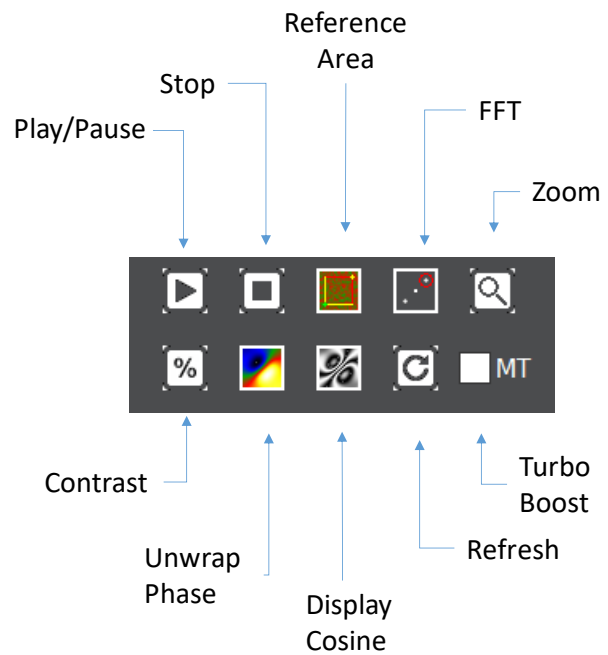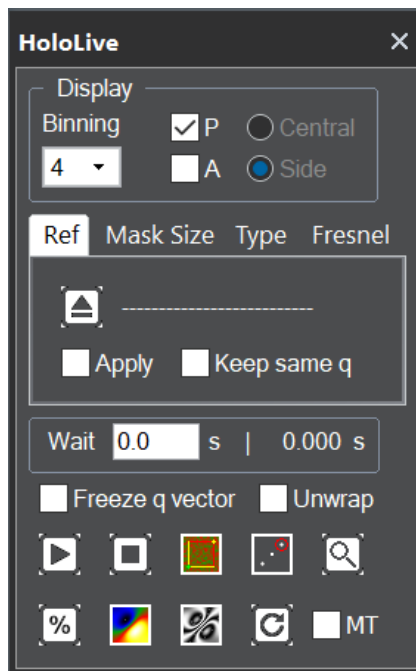
Note : CTRL and clicking on a button will reveal the Settings (if present)

HoloLive! Tutorial

Getting Started

Open the image "Live Hologram" using the DM command **File…Open** from the HoloLive Manual folder:

Technical note: this hologram was acquired on the I2TEM microscope in Toulouse (Hitachi HF 3300-C) using a 4K OneView camera (Gatan Inc.)

It does not look like much for the moment but it is a real hologram. Use the **DM magnifying glass tool** to zoom in to see the narrow hologram fringes. Hint: if you are new to GMS 3, remember you need to right-click on the image for the tools to appear.

Now let's use HoloLive! to calculate the phase image from this static hologram. Later on, we will make this image move to simulate a microscope session. Select the "Live Hologram" image by clicking on it somewhere (as usual in DM) and then just click on the **Play** button in the **HoloLive! control window**:
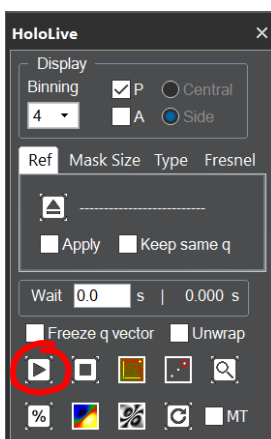
Hint: if someone else has been playing the HoloLive! before you, check that the options in the control window look like this and change values if necessary.

Since this is the first time **HoloLive…Play** operates on this particular image window, the power spectrum of the hologram will be displayed along with the all important dialog window. It should look something like this:
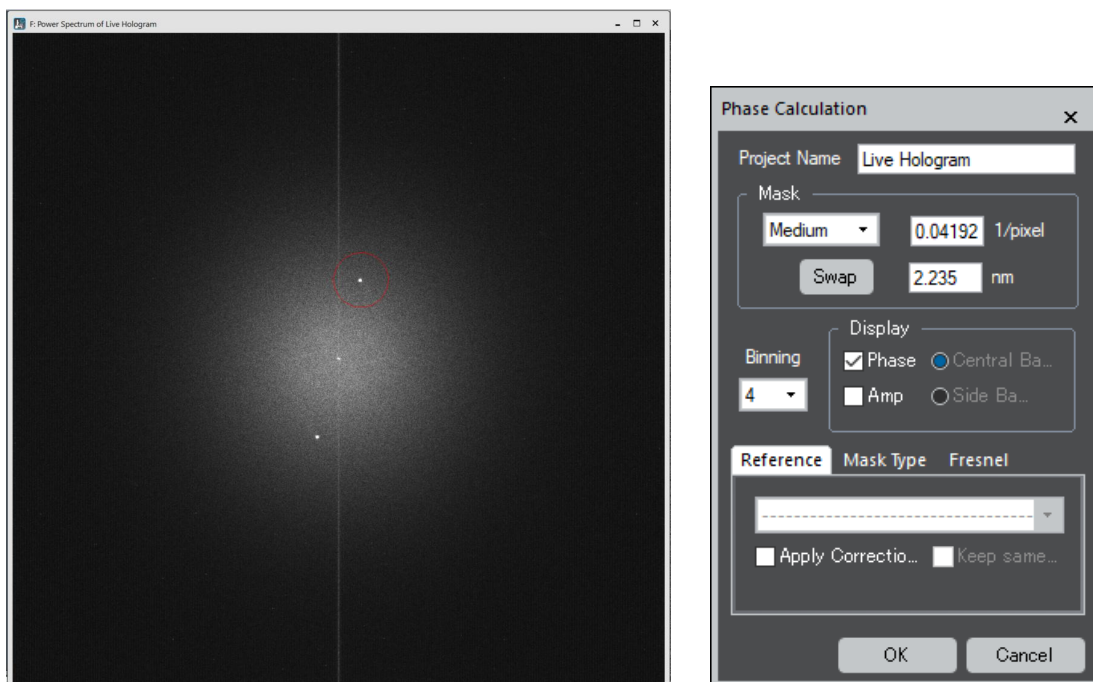
> **Technical note:** the power spectrum is the modulus squared of the Fourier transform of the image. To avoid streaking from edge effects, the image was prefiltered. Any remaining vertical streaking is due to the camera (in this case) or the finite width of the hologram.

Notice that the side band (at carrier frequency **q**) has been automatically detected and a red circle has been placed around it with a radius indicative of the mask size. If for some inexplicable reason (this is a worked example!) there is no circle or the circle is in the wrong place, you can use the **DM rectangular ROI tool** to tell HoloLive! were the side band is and continue. Just make sure the ROI encloses the side band…

We will look at the other features later, but for now just click on **OK**, and you should see you first phase image, called "Live Hologram P":
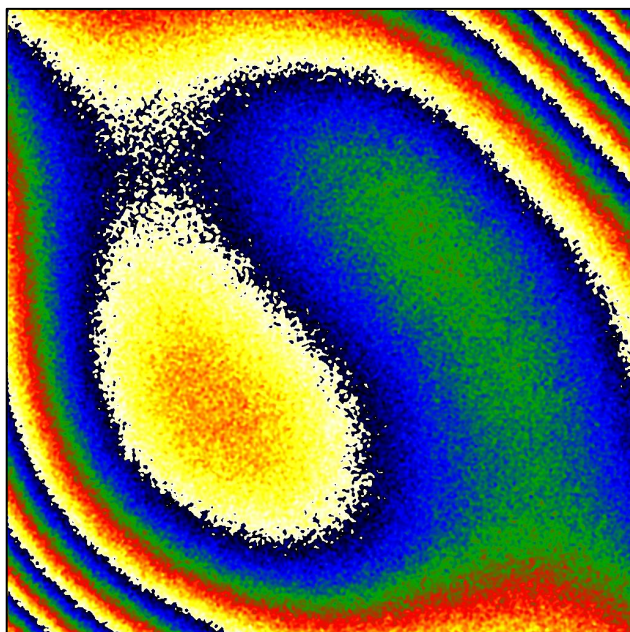

> **Technical note:** we like displaying phase images using the "Temperature" colour scale in DM but you can use grey scales if you really want, just use the DM menu **Object…Image Display…Color**. The actual values in the phase image will not be changed, only its appearance.

> **Technical note:** in HoloLive!, every image has a "type", used in the titles and in the image tags. For example, a phase image is type P.

You may have noticed that the **Play** button has moved to **Pause**:
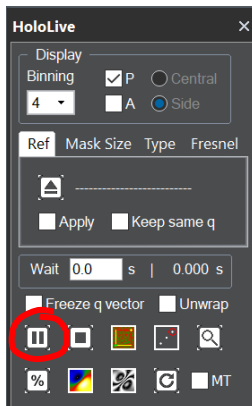
Hint: if ever your close the phase image, by mistake or by design, you may notice that the **P** box becomes unticked. HoloLive will continue working in the background. To make the phase image reappear, just click the **P** box.
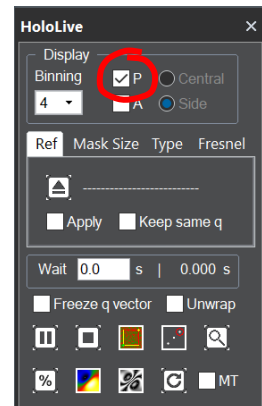
This is normal, indicating that HoloLive! is running. The phase image is not changing though because the hologram is static. We will see moving phase images and the use of this **Play/Pause** button later.
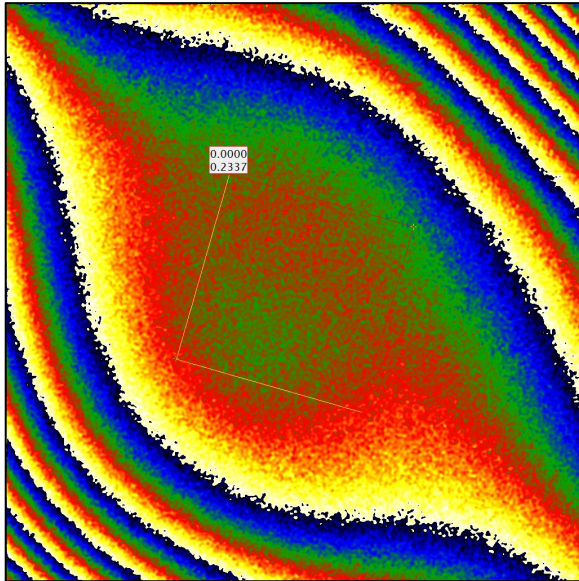
HoloLive…Reference Area

It is customary when analysing phase images to define a reference area. Usually, a region of vacuum is chosen where the hologram is undisturbed by any stray fields. In this example, the whole hologram was taken in empty vacuum so you can choose anywhere using the **DM rectangular ROI tool**. Alternatively, you can use the default setting of HoloLive! and just click on the **Reference Area** button:
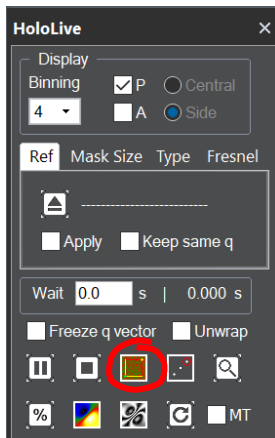

Technical note: the default for HoloLive! is a reference area at the centre of the image, rotated parallel to the hologram fringes. This can be modified in the **HoloLive…Reference Area…Settings.** These settings can be accessed via the HoloLive! menu or by pressing CTRL while clicking on the **Reference Area** button.
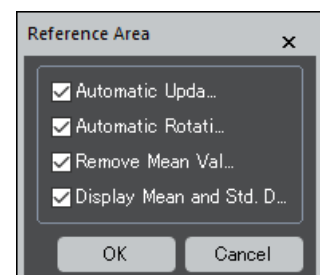
HoloLive! will determine the average phase gradient in the reference area and subtract the corresponding phase ramp from the whole image. This is equivalent to ensuring that the carrier frequency **q** corresponds exactly to that of the chosen reference area of the hologram. The result should look like this:
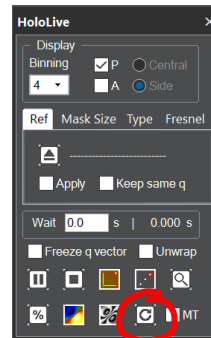
The mean phase and standard deviation of the phase in the reference area are displayed on the top left of the reference ROI. Feel free to move the ROI around and you will see the phase image automatically updated (if not, check that the Automatic Update box is ticked in the **HoloLive…Reference Area…Settings**. Remember that CTRL plus clicking on the **Reference Area** button will display the settings.) You can also change the size, shape and orientation of the ROI by grapping and dragging the different corners. Have a play.

Live Holograms

We could continue explaining the other features using a static hologram but it is more fun to use a live one, just like at the microscope.

To simulate an on-line holography session off-line so to speak, we have created for you a stack of 50 holograms acquired on the microscope. Open the image stack "HoloLive stack". It is a stack of 4K by 4K holograms weighing a total of 3Gb so there might be a pause. Scroll through the holograms if you like with the **Slice** tab of the DM **Display** window (if absent use **DM Window…Floating Window…Display**). Now open the script "HoloLive stack simulator". Check that the "Live Hologram" image is still open (and preferably the frontmost image) and run the script. You should see the following dialog:
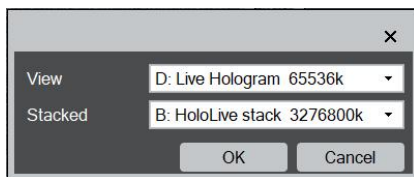

Choose as shown the "Live Hologram" to be the equivalent of the viewing screen (View) and "HoloLive stack" to provide the images. On **OK** the script will replace the hologram in the Live Hologram window with a hologram from the stack. The frequency is determined by the number variable "wait" in the script (in seconds). Change it if you like. To halt the movie, press down CTRL-SHIFT.

As if by magic, you should see the phase image changing as if it were live. The delay between phase images is displayed on the HoloLive! control window:
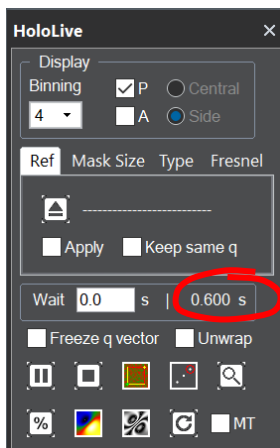
Hint: make sure you are on **Binning** 4 (or higher) unless you want your computer to have a headache. See later for more details.
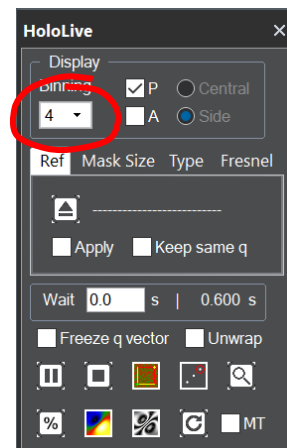
On the laptop used for writing this tutorial, the time is about 0.6s and varies depending what else is going on the computer. To speed things up use the **Turbo Boost**:
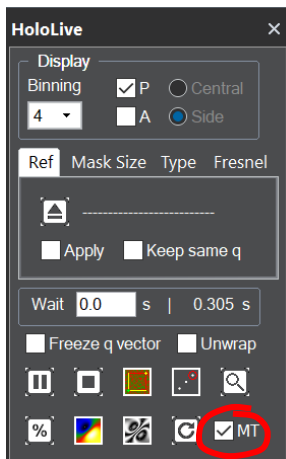
Technical note: In fact, MT stands for "Multi-Threading". By programming in parallel certain operations in the sequence (FFT, masks, reference) the delay between successive phase images is reduced. However, the time to create the first phase image in a sequence is always the same and corresponds to the delay time without **Turbo Boost**.

Use the **Play/Pause** button to stop and start. Note that there is a delay between stopping and starting. This is because it takes a certain time to create the first phase image in a sequence which is longer than the delay between successive phase images using the **Turbo Boost**.

Please be kind to HoloLive!
Your computer is having to do some pretty heavy calculations, especially with a stack of 4K holograms. Everything has been done to optimise the algorithms in order to reach these speeds but the computer is working flat out. There will always be slight delays after clicking on a buttons. Please be patient before clicking again.

To assuage your computer, you can always include a "Wait" time:
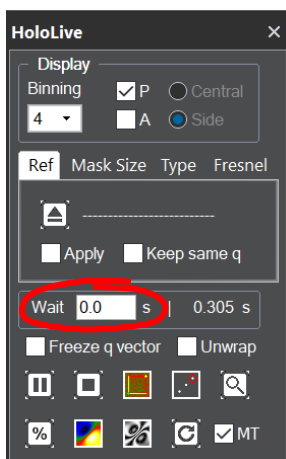
Hint: after putting say 1 into the **Wait** box, click somewhere else for it to be taken into account. You should see the 1 become 1.0 as HoloLive! recognises a number

You may hear your laptop less overheating. There are, however, better ways to help your computer by using Binning and ROIs, as we will see later.

HoloLive… Live FFT Modulus

By default, the FFT of the hologram is shown live alongside the phase image. If it is not, click on the **FFT** button in the **HoloLive Control Window**:
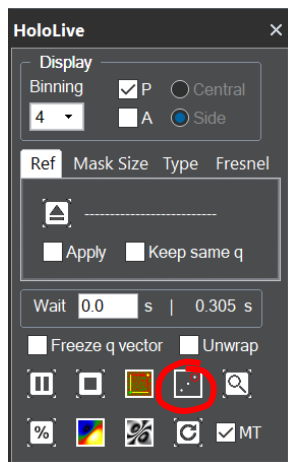

> **Technical note:** it is too time consuming for HoloLive! to calculate a live Power Spectrum (which includes masking in real-space). The **FFT** button shows the modulus of the FFT of the hologram (which had to be calculated anyway for the phase image). You will therefore notice streaking of the spots. Purists can see the *static* Power Spectrum using **HoloLive…Power Spectrum**.

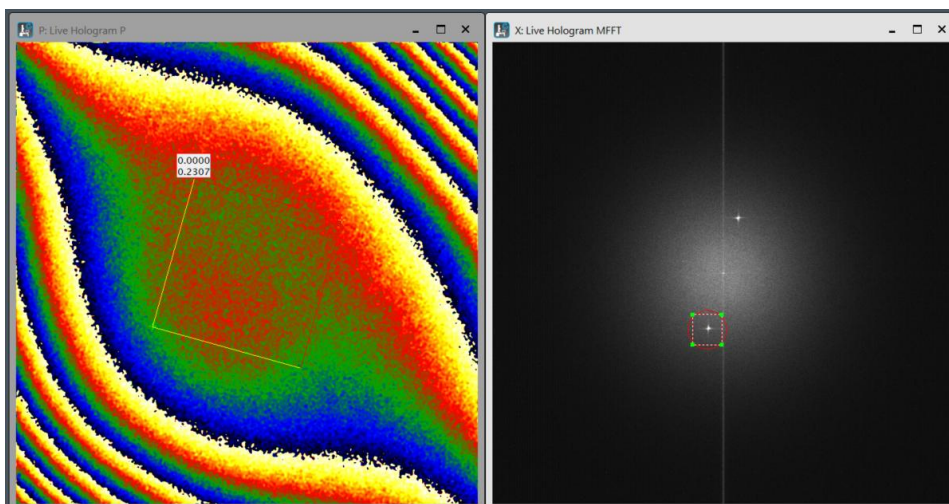Now place the Live FFT next to the phase image:
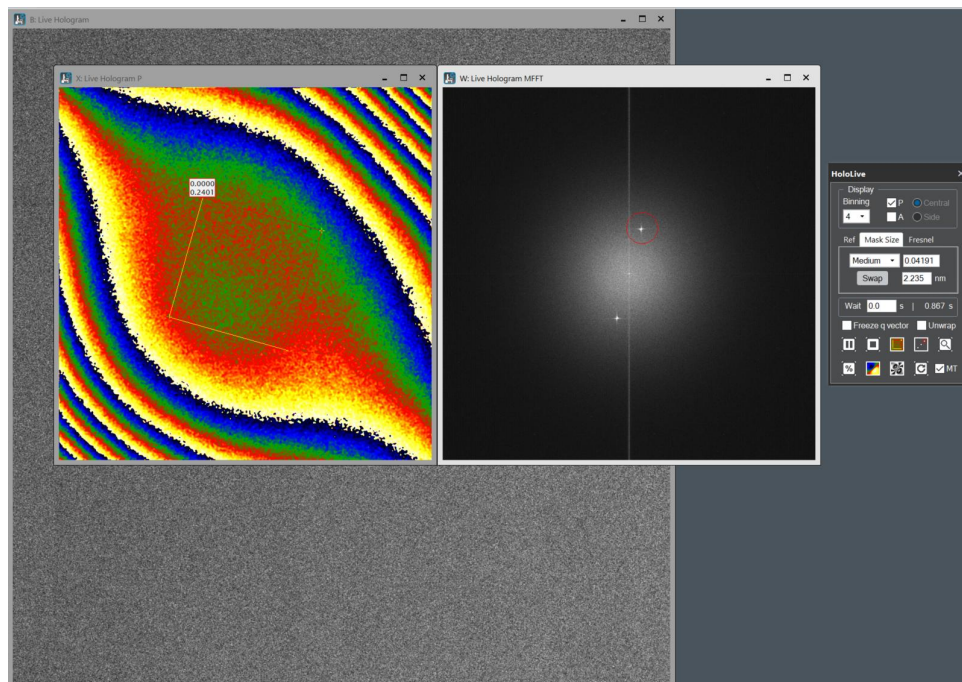
The **FFT** button does more than just displaying the FFT. It can be used to control the choice of sideband. Place a **DM rectangular ROI** around the other sideband like so:
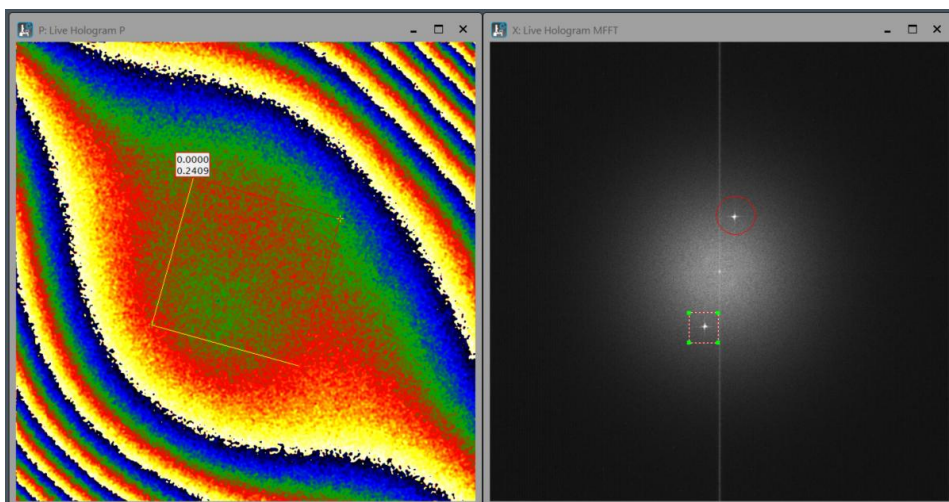
Now click on the **FFT** button. This tells HoloLive! to hunt for the sideband within the ROI. You should see the red circle denoting the mask swap to the new position:


Notice that the phase has changed sign, as it should do since **q** has changed to –**q**. This is such a useful operation, that we have included a special **Swap** button on **Mask Size** tab of the **HoloLive Control Window** to do this:
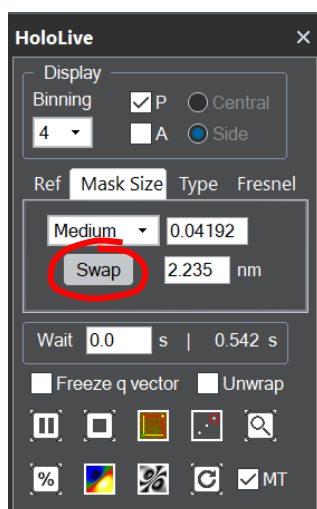
Technical note: the choice of sideband is important for subsequent quantification. Our convention is that the sideband pointing towards the reference wave (usually the vacuum) should be chosen. In that case, the phase is positively proportional to the mean-inner potential of the material. A positive phase gradient means increasing sample thickness.

Have a go but remember the delay.

The main reason for including the ROI option on the FFT was for cases where HoloLive! does not find the sideband. A classic example is when a hologram becomes too noisy (for example, after jolting the microscope or biprism) and loses all contrast. The ROI guides HoloLive! to the right place. Another way to avoid such mishaps is to use **Freeze q vector**:
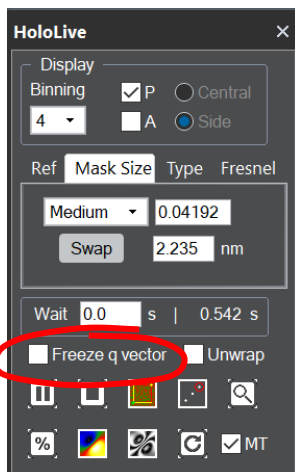

By choosing this option, HoloLive! no longer hunts for the sideband in the live FFT, it just continues to use the same carrier frequency **q**. There is a slight increase in speed. Unticking the option just lets HoloLive! go back to automatically detecting the sideband.

A typical experimental procedure is to change the biprism voltage and the carrier frequency changes. Untick the **Freeze q vector** and HoloLive! tracks the carrier frequency. Once the biprism voltage is stable again, **Freeze q vector** can be reselected.

Another use of freezing **q** is to detect electric fields during in-situ biasing experiments. The q vector can be frozen in the vacuum area, when a bias is applied, any gradient in the phase image indicates the presence of an electric field. With automatic tracking, the phase gradient would be automatically removed!

HoloLive…Mask Size

Since we are on the **Mask Size** tab, let us look at the effect of changing the mask size for the phase reconstruction.
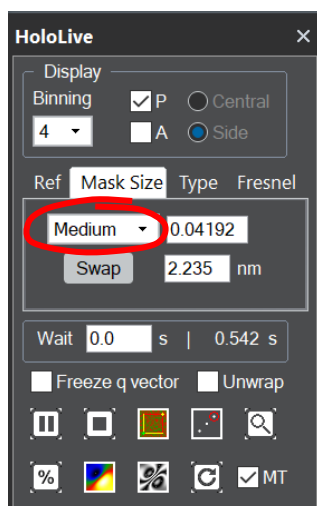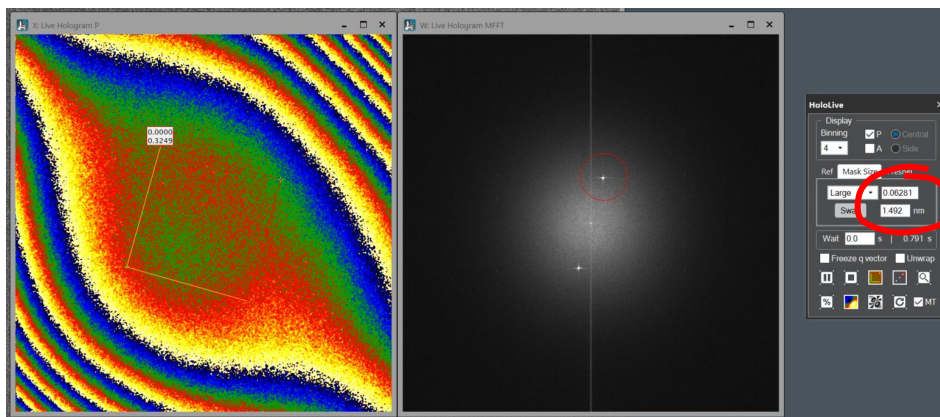

> **Technical note:** the default mask radii are the following: Small q/4, Medium q/3 and Large q/2.
>
> Technically, a mask size larger than Medium lets information from the centre band leak into the masked region. Holographers argue about whether Large is acceptable or not.

Use the scroll-down menu to choose a "Large" mask size. You should see the following result:

The red circle has a larger radius on the live FFT and the phase image should look noisier but finer grained. The standard deviation of the phase is indeed larger (see the information on the **Reference Area** ROI). The spatial resolution of the phase map is now higher (see the information on the **HoloLive Control Window**.) This is the price to pay for higher spatial resolution: a lower signal-to-noise ratio for the phase.

You can choose any mask size by scrolling down to Custom and filling in the spatial resolution you want into the boxes (either in pixels or in nm). Choose 5 nm spatial resolution for the phase reconstruction:
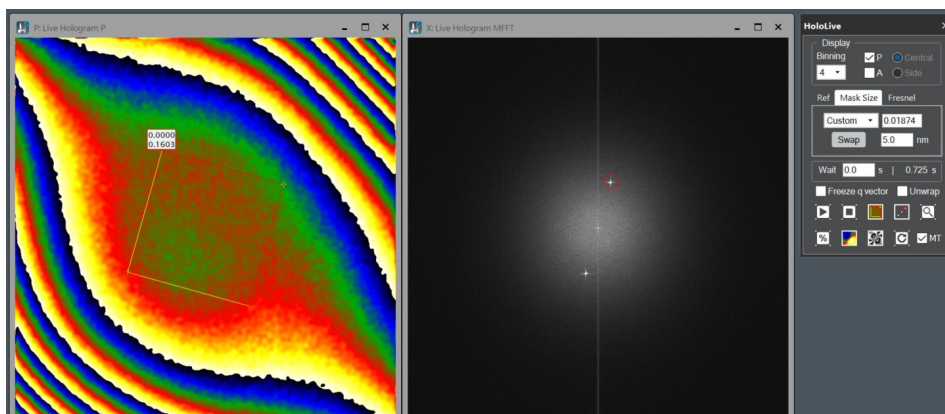
This is indeed a better way to define the mask size as it does not depend on the carrier frequency, which might change during the experiment.

Now that we have seen the FFT, let us be nice to the computer and close the FFT window. Just close is as usual in DM. If you need it again, just click on the HoloLive **FFT** button.
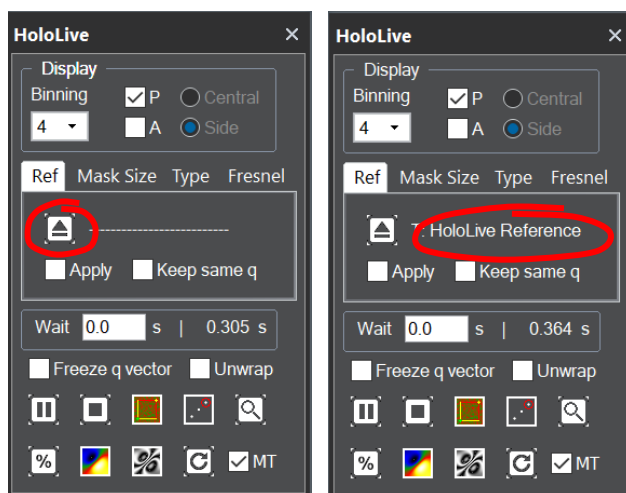
HoloLive…Reference Hologram

The large phase variations seen in this example are due to optical distortions of the microscope, the projector lens distortions to be exact. To remove them, the conventional procedure is to acquire a reference hologram in empty vacuum. Put HoloLive! on **Pause** and open the reference hologram that we have supplied "HoloLive Reference":
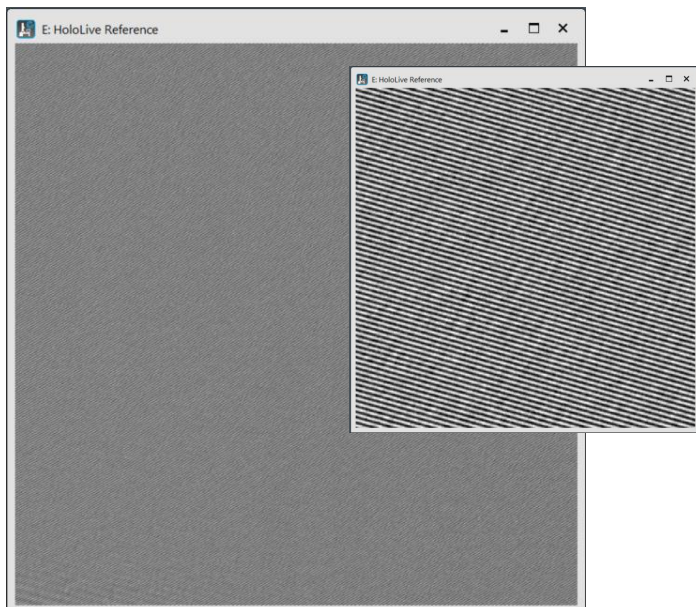
Technical note: this reference hologram has some pretty sensational contrast because we used the drift compensation, exposure time of 4 mins and π-shift reconstruction (see Gatel et al. APL 2018)

Hint: use the **DM magnifying glass** tool 🔍 to zoom in.

With the "HoloLive Reference" as the frontmost image, click on the **HoloLive…Reference…Load** button:


Technical note: as usual there will be a pause as HoloLive! calculates and stores the phase reconstructed from the Reference Hologram.

When it is over, the title of the image will appear. It will be truncated to fit into the box.

HoloLive! uses exactly the same parameters (notably the mask size and binning) to reconstruct the phase of the reference hologram. It stores the result ready for use.

Set the live phase image off again by clicking on the **Play** button and click on the **Apply** box to see the correction work:
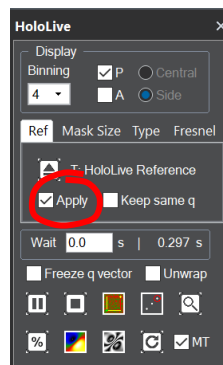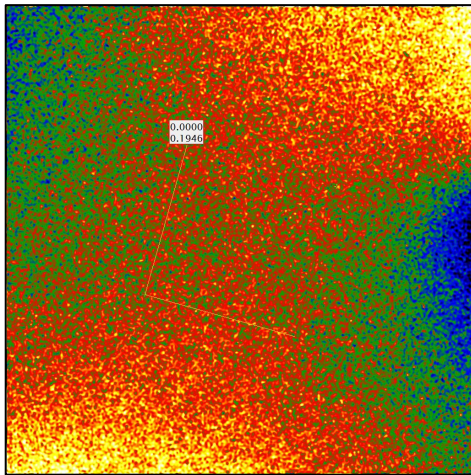
The phase variation across the field of view is much smaller. Click and unclick **Apply** to see the improvement. Ideally, there should be no phase variation on correction (apart from the noise from the counting statistics). As is often the case, the reference image was acquired a bit later in the day when the imaging conditions had changed slightly.

Reference hologram…Keep same q

The reference phase was reconstructed using the same parameters as the live phase image, but not necessary the same carrier frequency **q**. Indeed, HoloLive! hunts for the sideband automatically and determines the peak position itself. This can be useful because the biprism voltage can change slightly, or wilfully, between the current conditions and the reference hologram conditions. Purists, who want exactly the same carrier frequency to be used for the current hologram and reference hologram, can use the **Keep same q** option:
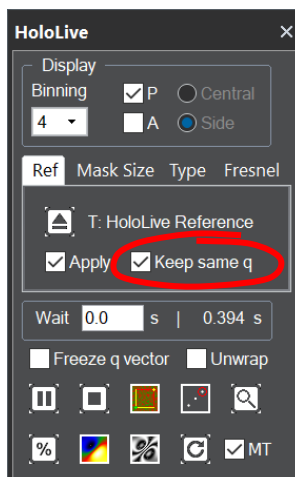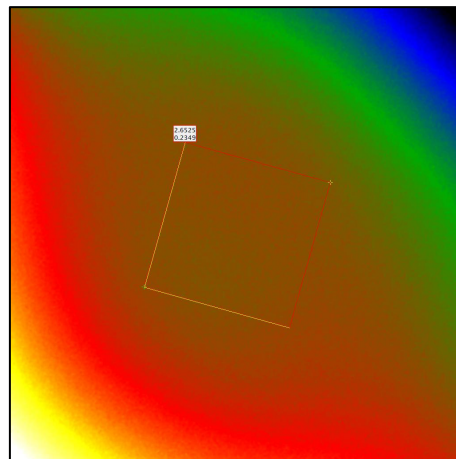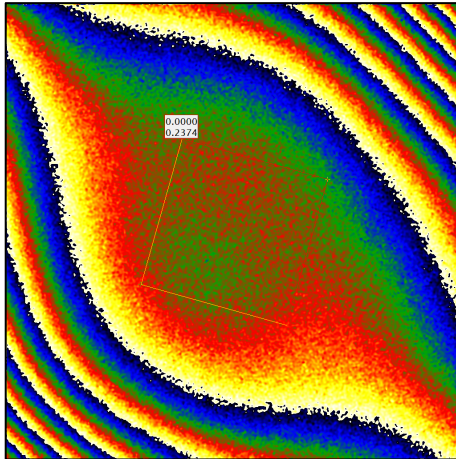

HoloLive…Unwrap Phase

As is well known, the phase is well defined in a range of 2π, and in our convention between $-\pi$ and π. Holographers sometimes like to "unwrap" the phase. This means extending the phase continuously beyond the 2π range. For example, if we are looking at a wedge-shaped sample, we would like to see the phase from the mean-inner potential increase continuously from the edge of the sample. We have therefore included an unwrapping capability.

To see the full effect, remove the Reference Hologram correction (untick **Apply**) so that the phase has many phase jumps. Now click on **Unwrap Phase** to see the transformation:

Technical note: there is no unique solution to phase unwrapping in 2D, so it can fail or look weird.

With the **Turbo** on there is little loss of speed thanks to many hours of optimising the code…

HoloLive…Zoom

Sometimes, you do not want to see the phase image corresponding to the whole field of view. HoloLive allows you to see the phase in a smaller region.

First, put HoloLive on **Pause** (this is not absolutely necessary but is politer to the computer) and select the Live Hologram window by clicking on it. Then draw a region with the **DM rectangular ROI** tool:
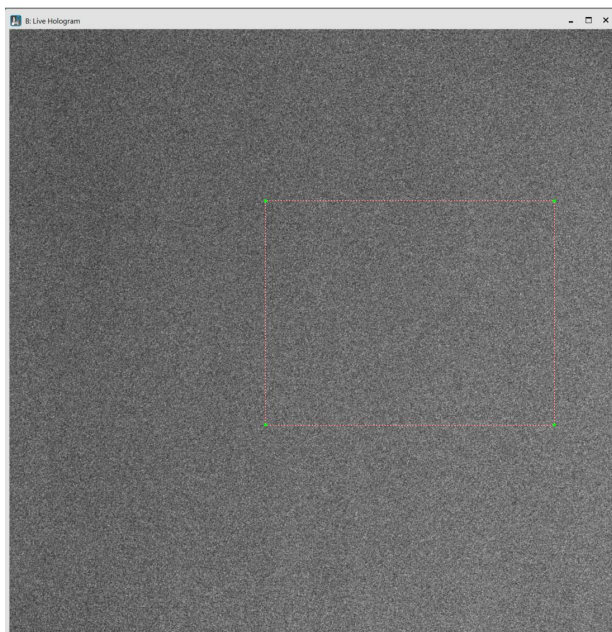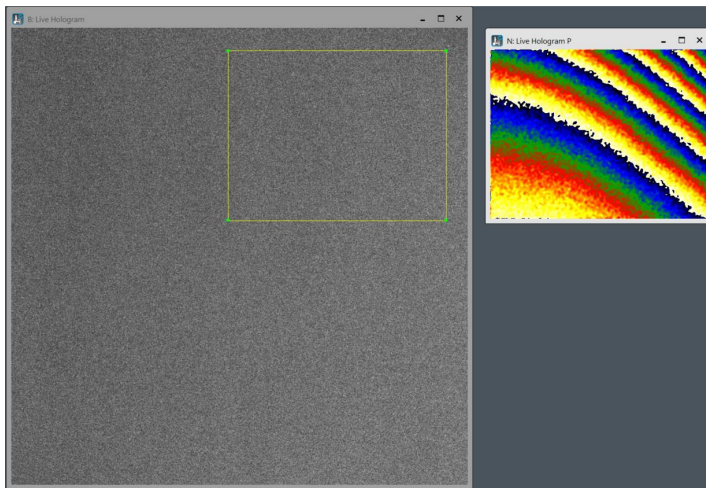

Hint: if you hold down SHIFT, you force a square region, and ALT-SHIFT forces sizes of powers of 2. Do not worry though, HoloLive can deal with any size or proportion.

Now click on the **HoloLive…Zoom** or Magnifying Glass button:
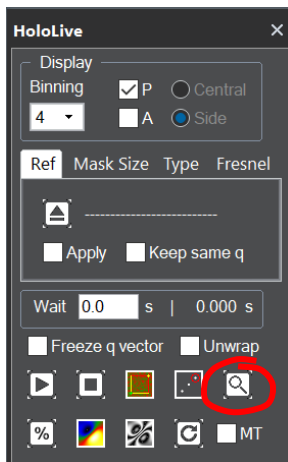
HoloLive will calculate and display the phase image corresponding to only this small region of the hologram:

Technical note: the phase image is calculated using your current settings except **Reference Area**.

Notice that the ROI has become yellow, which indicates that it is active in HoloLive.

You can even move the ROI around (use the **DM select/arrow** tool to click inside the ROI, then holding the left-click down, move the mouse) and the phase image is updated automatically. You can maybe see this more clearly by not applying a reference hologram correction and freezing the q vector.

The size and shape of the ROI can also be changed. However, be careful not to grab one of the corners and change the shape too rapidly! HoloLive has to recalculate from scratch each time the size changes and performance suffers. Be kind to the computer, shape changing is best done gently (even maybe on **Pause**).

HoloLive…Cosine

While we are in the **Zoom** mode, let us look at the **Cosine** button and click:
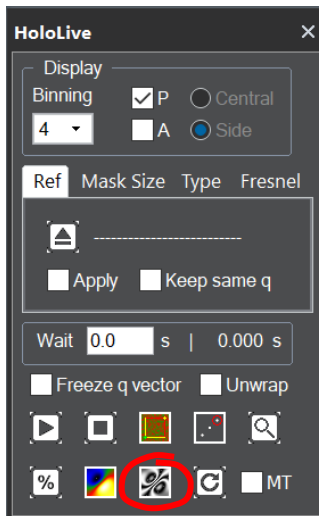
Technical note:
the **amplification Factor** determines the cosine phase magnification. Displayed will be the cosine of the phase times the
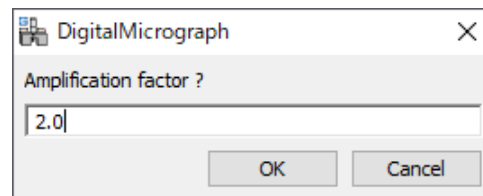
With an amplification factor, α, HoloLive! will display $\cos(\alpha\phi)$ in a new window, where ϕ is the phase... For the default factor of two, the result will look something like:
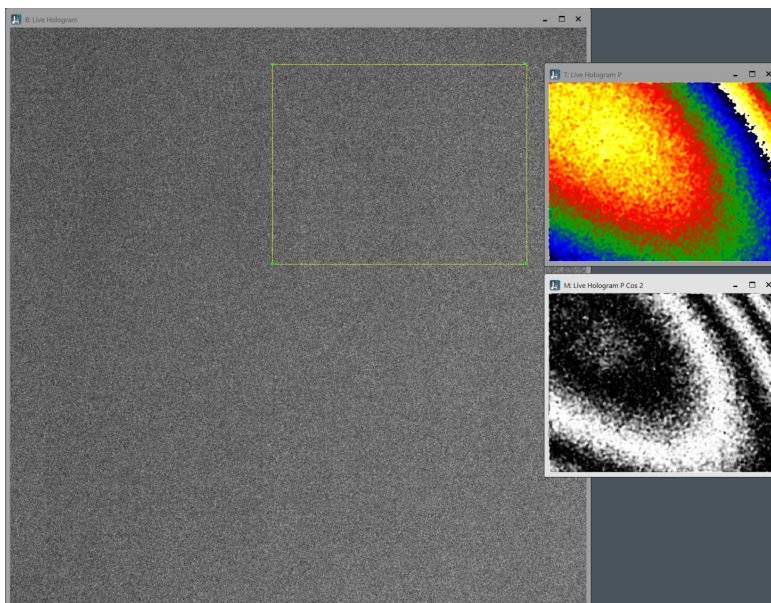

Hint: to change the cosine factor *en route*, CTRL click on the **Cosine** button to modify the setting.

Holographers sometimes finds this useful to accentuate the phase variations. In fact, the cosine function harks back to the original way of phase visualisation in the analogue days pre-digital.

HoloLive…Contrast

Another useful feature linked to a selected area in the hologram image is the **Live Contrast** function. Make sure that the Live Hologram is the frontmost image, and click on the **Live Contrast** button:
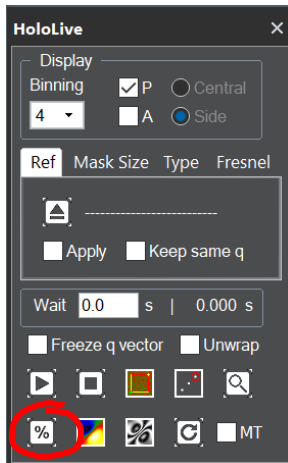
> **Technical note:** you do not even have to have phase images running for this function to work.

If an ROI was present, HoloLive! will use it, otherwise a default ROI will be created:
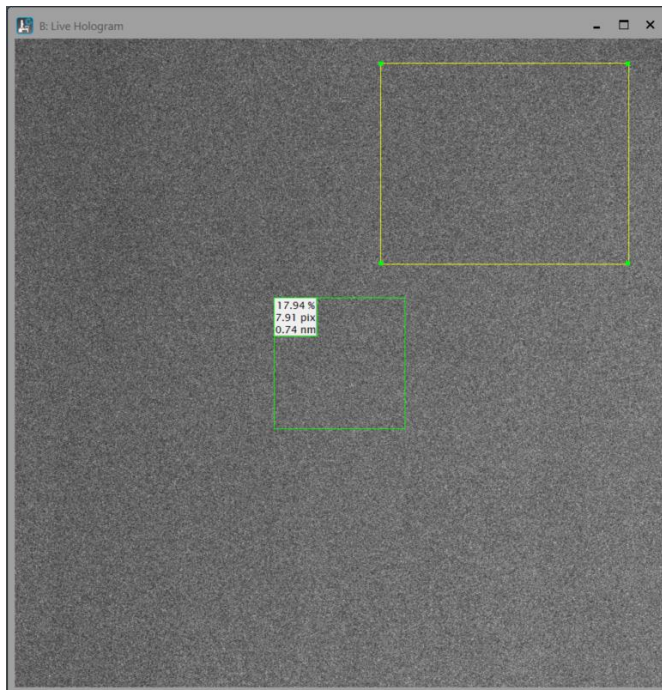
> **Hint:** feel free to move the ROI around or to change its shape or size.

The information provided from top to bottom is the following: hologram fringe contrast, fringe spacing in pixels, and the corresponding spacing in nm if the image has been calibrated.

The contrast uses the same definition as fringe visibility:

$$V = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$

In the presence of noise, we should isolate the fringes from the background and measure their average amplitude. However, for speed, we use an approximation based on the ratio of the standard deviation of the intensity to the mean intensity:

$$V = \frac{1}{\sqrt{2}} \frac{\sigma(I)}{\langle I \rangle}$$

For pure cosine fringes, the result would be correct. However, the method breaks down for a noisy image with weak fringes. In the extreme case, in the absence of hologram fringes, we would be measuring the noise level rather than the fringe contrast. Bare this in mind.

HoloLive…Stop

All this time you may have been wondering what the **Stop** button does and the difference with **Pause**. Give it a go:
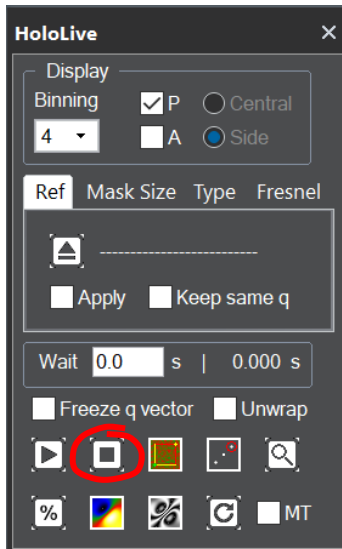
You may not have seen everything but it is enough for now.
This is the end of the tutorial for HoloLive!