

Using HexAuFoil Grids at eBIC

Revision: 4.1

EPU version: 3.11

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Revision summary: 1) Updated to use 'change detector area'.

Comments to: Peter Harrison

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Introduction

This document is intended as a guide for users to enable them to collect data on HexAuFoil grids.

Specific settings will vary for the individual microscope, depending on set-up. This guide was prepared with Krios IV and Glacios II (Research Complex at Harwell) at eBIC, Diamond Light Source. Krios IV is equipped with a K3 direct electron detector and a BioQuantum energy filter and Glacios II is equipped with a Falcon 4 detector. Both instruments were using EPU version 3.11.

For any questions, or if you are unsure, please get in touch with your local contact.



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1 Session Set-Up

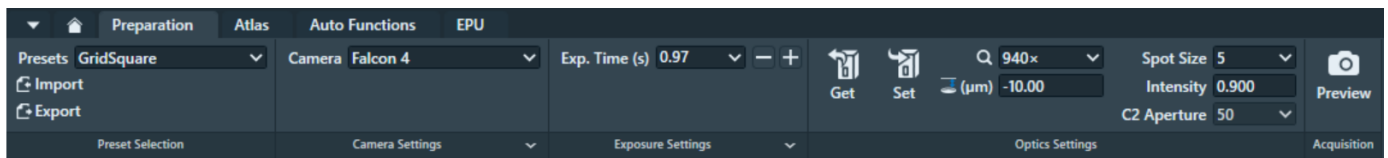
1.1 Atlas Magnification

No changes are required at Atlas magnification for using HexAuFoil grids.

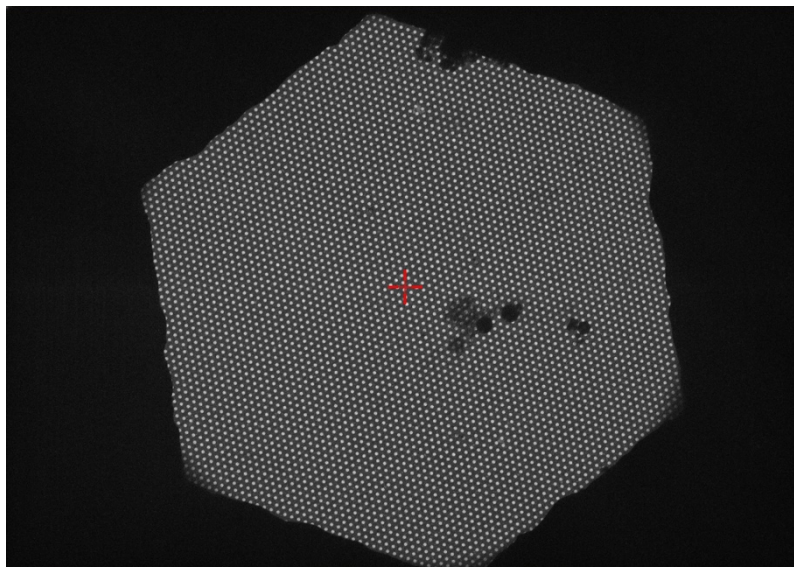
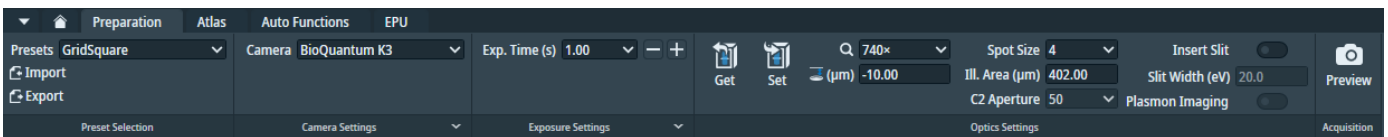
1.2 GridSquare Magnification

As with all grids, the field of view at the GridSquare magnification should contain just one square/hexagon.

The magnification may vary slightly between instruments. A magnification of $\sim 740\times$ (300 keV) or $\sim 940\times$ (200 keV) with a defocus of $-10\ \mu\text{m}$ should be sufficient for the GridSquare preset.



If you are operating a microscope with an energy filter, plasmon imaging can be used to give a better idea of the ice thickness within the holes.



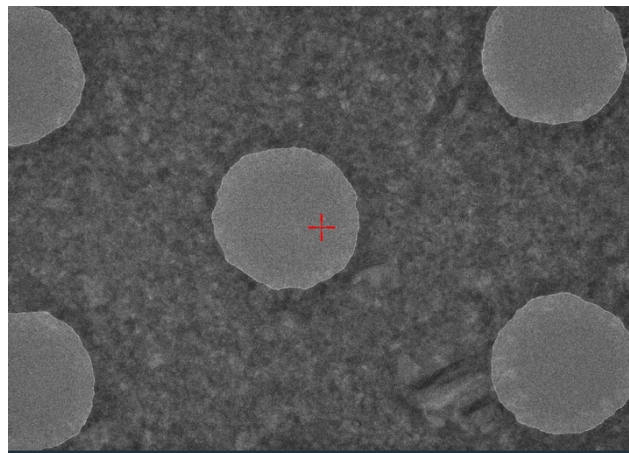
1.3 Hole/Eucentric Magnification

Imaging a HexAuFoil at a 'normal' Hole/Eucentric magnification (~13, 500 x on a 300 keV system) results in a wide field of view, pre-exposing a large area of the grid. Ideally, the Hole/Eucentric preset magnification should have a single hole in the field of view, with partial adjacent holes.

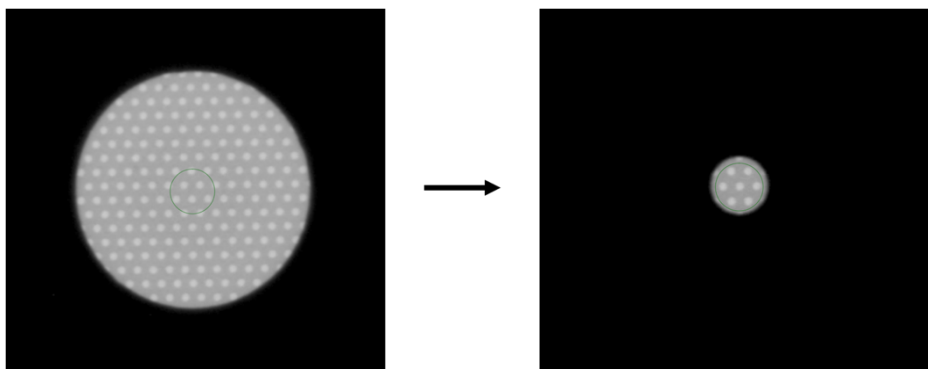
To ensure only one hole is in the field of view, one of two approaches can be taken; either adjust the magnification or change the detector readout area.

1.3.1 Adjust the Hole/Eucentric Magnification: 300 keV

Increasing the magnification to ~42, 000 x will give roughly one hole in the field of view. Ideally, one should decrease the size of the beam to avoid pre-exposing too many adjacent holes.

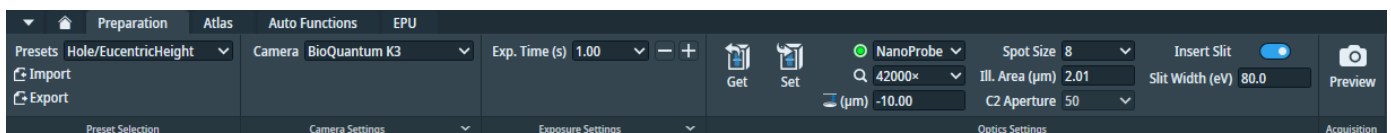


With an energy filter, aim for the size of the beam to be slightly bigger than the energy filter. The beam should be sufficiently larger than the detector area such that when the beam is tilted, the edge of the beam does not come into view.



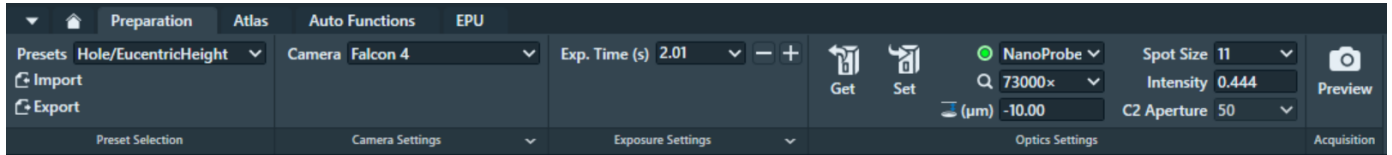
However, care must be taken when decreasing the size of the beam at a higher magnification as this will result in a high dose over a small area. Adjust the spot size and exposure time to ensure that the dose is around $0.1 \text{ e}^- \text{ \AA}^{-2}$ ($10 \text{ e}^- \text{ nm}^{-2}$).

The defocus must be kept as low as possible to limit defocus induced rotation and improve AFIS accuracy. A defocus of -10 \mu m should give sufficient contrast with gold foils.



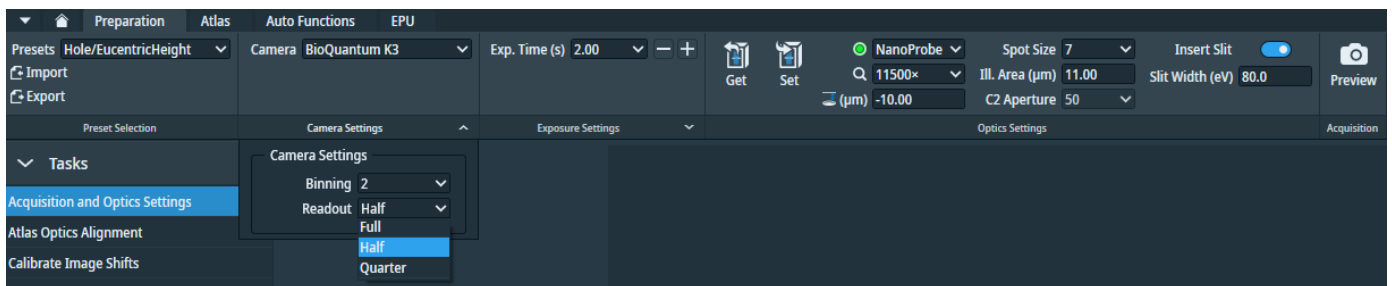
1.3.2 Adjust the Hole/Eucentric Magnification: 200 keV

On a 200 keV system, similarly a larger magnification is required for the Hole/eucentric magnification. A magnification of $\sim 73,000 \times$ should be sufficient. In order to reduce the size of the beam to avoid pre-exposing too much of the sample, use nanoprobe and set the beam to parallel. Adjust the spot size and exposure time to drop the dose to around $0.1 \text{ e}^- \text{ A}^{-2}$ ($10 \text{ e}^- \text{ nm}^{-2}$).

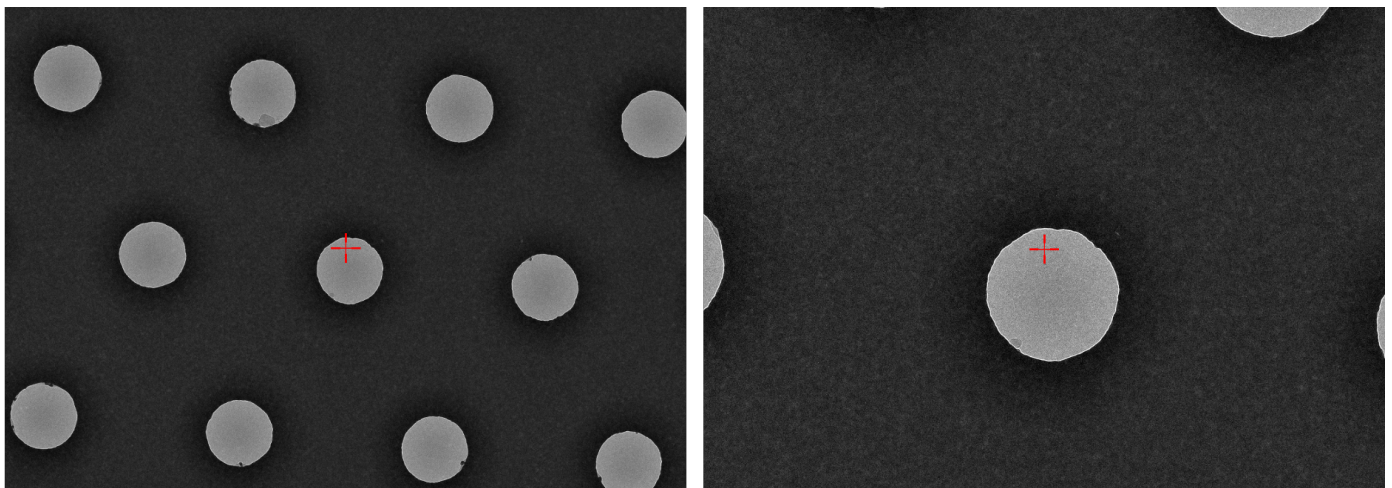


1.3.3 Change the Detector Readout Area

This protocol applies to both 200 and 300 keV systems. In the drop down menu of 'Camera Settings', the readout of the camera can be changed from 'full' to either 'half' or 'quarter'. This will decrease the area of the camera that is readout, without requiring adjustment of the magnification.



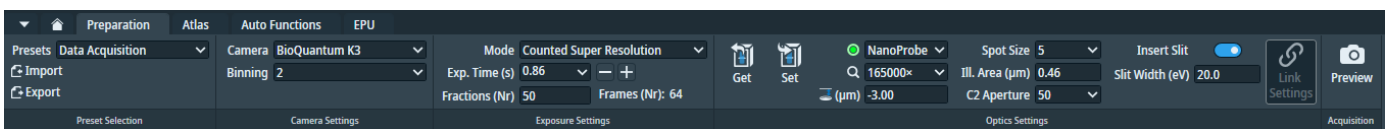
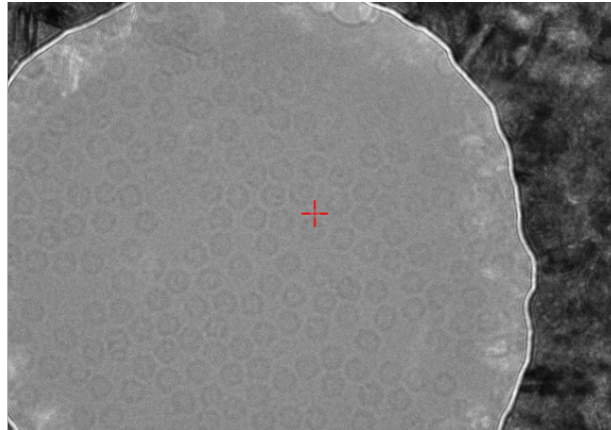
For example, in the images below, the image on the left was taken at 11,500x with full camera readout, whilst the image on the right was taken at the same magnification with half camera readout. Moreover, the beam size will still be large (11 μm in this instance) so care must still be taken to ensure the dose is around $0.1 \text{ e}^- \text{ A}^{-2}$ ($10 \text{ e}^- \text{ nm}^{-2}$) to prevent excessive pre-exposure of the grid.



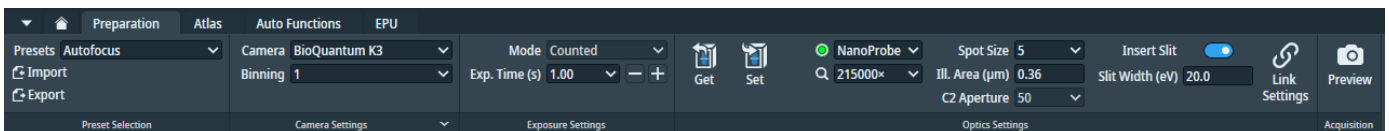
1.4 Data Acquisition & Autofocus Magnification

1.4.1 300 keV

HexAuFoil grids require equal illumination over the entire hole for optimal performance (as this helps to reduce beam-induced motion). As such, a magnification should be selected that images as much of the hole as possible, whilst ensuring the beam size is > 300 nm. On a 300 keV system, a magnification of $\sim 165,000$ x will achieve this. Higher magnifications can be used, but the target should always be centred on the hole to produce equal illumination (as much as possible).

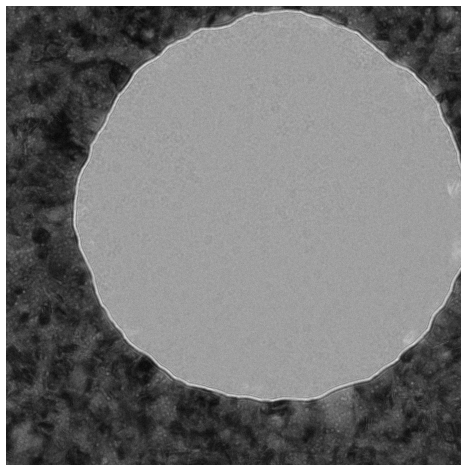


A higher magnification with a smaller beam size should be used for the Autofocus preset ($\sim 215,000$ x), to ensure the beam does not pre-expose a hole when autofocusing. To achieve this, unlink the Autofocus preset from Data Acquisition and set the magnification and beam size.

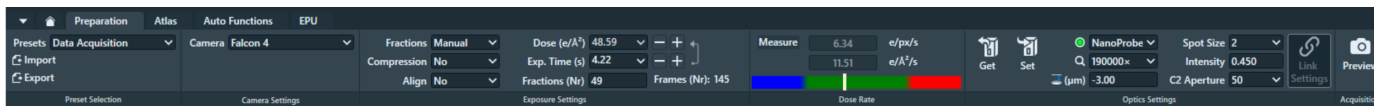


1.4.2 200 keV

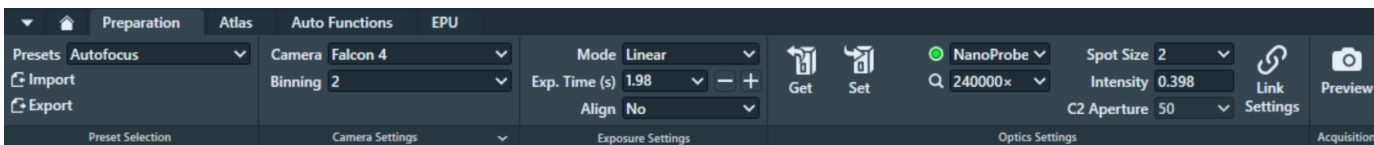
On a 200 keV system, the same principle of equal illumination over the hole still applies.



However, the beam size on a 200 keV system will be significantly larger than that of a 300 keV system. As such, the beam size will be dictated by making the beam parallel. All other Data Acquisition presets can be set to achieve the desired dose so long as the beam is parallel.



For the Autofocus preset, again, this can be unlinked from Data Acquisition and manually changed to a higher magnification and smaller beam size (ensuring the size of the beam is bigger than the area of the detector). The beam at Autofocus does not need to be parallel.





2 Summary of Settings

2.1 300 keV

	Atlas	Gridsquare	Hole/eucentric		Data Acquisition	Autofocus
			Change Mag	Change Detector Readout Area		
Magnification (x)	135	740	42,000	11,500	165,000	215,000
Defocus (μm)	-1000	-10	-10		Experiment dependent	N/A
Spot Size	8	8	4	7	5	5
Illuminated Area (μm)	900	402	2.01	11	0.460	0.360
C2	50	50	50		50	50
Slit?	No	No	No		Yes (20 eV)	Yes (20 eV)
Exposure Time (s)	1	1	1	2	Experiment dependent	1
Detector Readout Area	Full	Full	Full	Half or Quarter	Full	Full

2.2 200 keV

	Atlas	Gridsquare	Hole/eucentric		Data Acquisition	Autofocus
			Change Mag	Change Detector Readout Area		
Magnification (x)	115	940	73,000	11,000	190,000	240,000
Defocus (µm)	-3000	-10	-10		Experiment dependent	N/A
Spot Size	7	5	11	7	2	2
Intensity	1.100	0.900	0.444	0.595	0.450	0.398
C2	150	50	50		50	50
Micro- or Nanoprobe	Microprobe	Microprobe	Nanoprobe	Microprobe	Nanoprobe	Nanoprobe
Exposure Time (s)	1	1	1	2	Experiment dependent	2
Detector Readout Area	Full	Full	Full	Half or Quarter	Full	Full

3 Session Set Up

3.1 Image Shift Calibrations

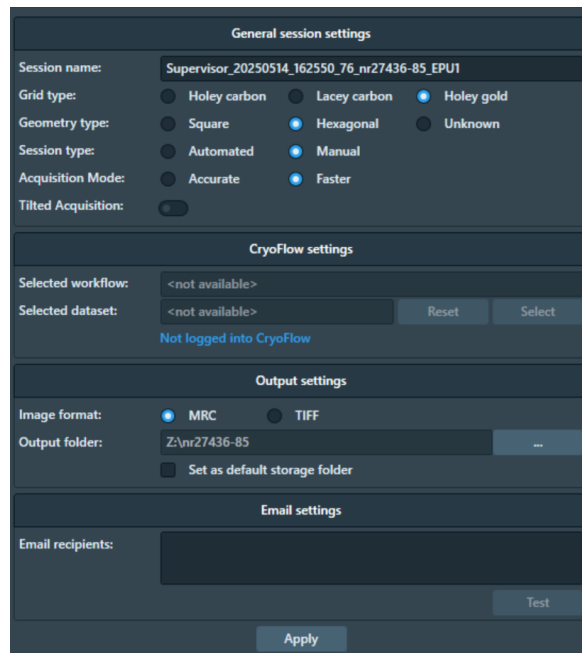
As less defocus is used when working with HexAuFoil grids, it can be difficult to identify features, such as contamination, required for doing image shift calibrations. As such, it may be necessary to temporarily add more defocus or change the magnification to aid isolation of a feature. However, ensure there is no additional defocus applied and that you are at the correct magnification when performing the image shift calibration routine.

It may be necessary to repeat the Image Shift Calibrations an additional time to ensure that they are as accurate as possible. It may also be necessary to re-collect the Atlases after performing the Image Shift Calibrations to ensure optimal alignment.

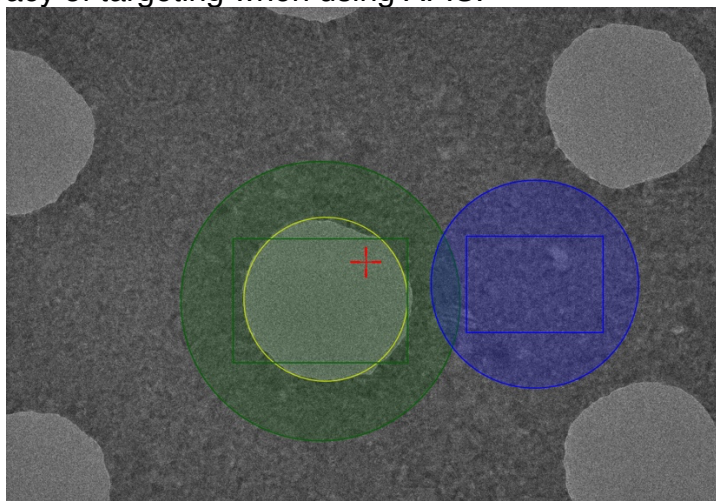
3.2 EPU

3.2.1 300 keV

When creating a new session in EPU, under 'Grid type' select 'Holey gold' and under 'Geometry type' select 'Hexagonal'. The rest of the session set up for HexAuFoil grids is no different to any other grid type in EPU.

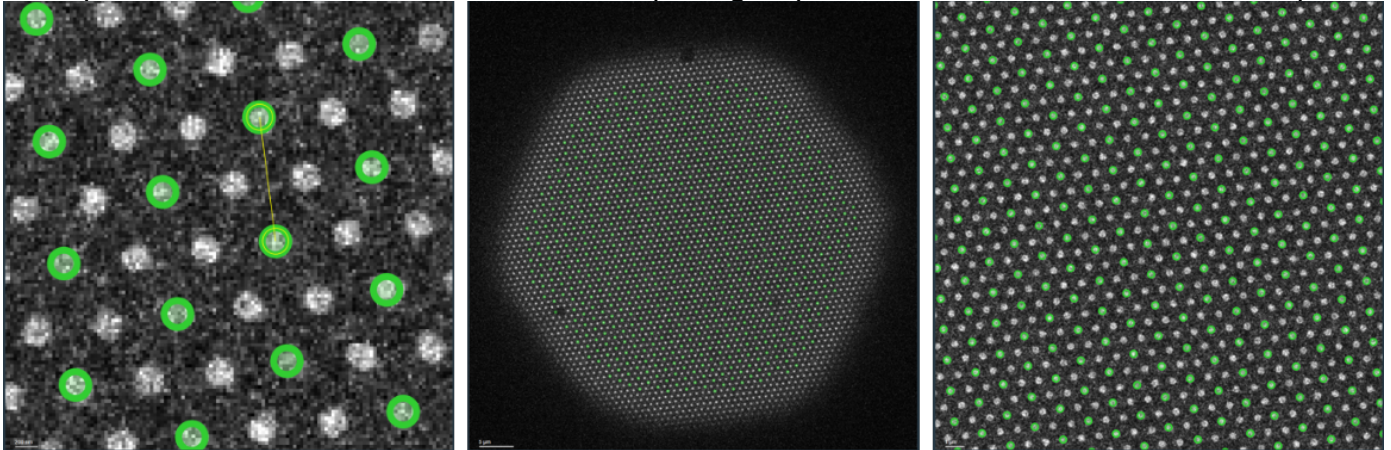


When performing hole finding, make sure the yellow circle accurately matches the hole, as this can directly affect the accuracy of targeting when using AFIS.

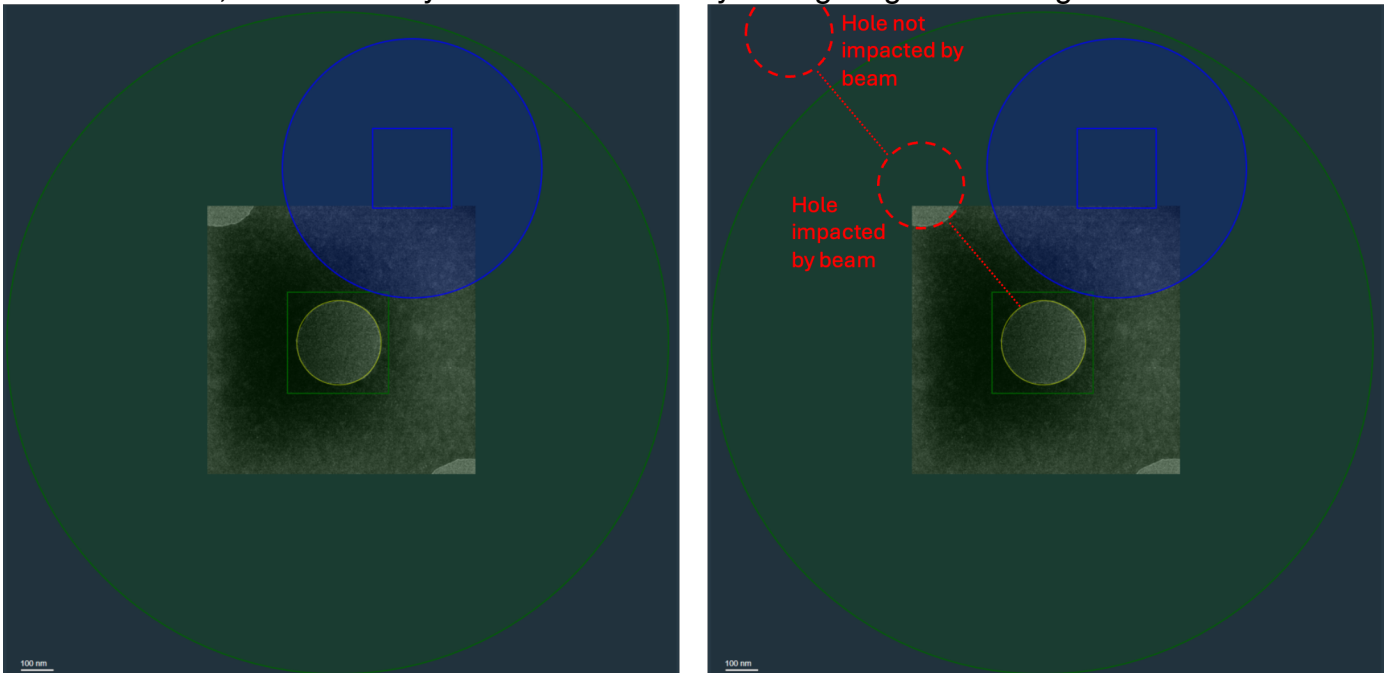


3.2.2 200 keV

Due to the larger size of the beam on a 200 keV instrument, it is not possible to collect on all holes on a HexAuFoil grid without pre-exposing the holes. As such, to prevent collecting on holes pre-exposed, the 'measure hole size' tool must be adjusted to prevent the collection of adjacent holes. The specific size of the beam will dictate the spacing required at the 'measure hole size' step.



It is important to ensure that the size of the yellow circle for hole finding accurately matches the size of the hole, as this directly affects the accuracy of targeting when using AFIS.



Make sure that the acquisition area and the autofocus area do not impinge upon any holes you wish to image. You may need to zoom out when setting up the Template definition to see the size of the beam and check its positioning with regards to nearby holes (you may need to estimate the spacing (as above) depending on your Hole/eucentric magnification).

3.3 Tilted Data Collection

It is possible to collect tilted data on HexAuFoil grids. Tilted collection may function better with a decreased maximum image beamshift range. To facilitate this, you will need to ask your Local Contact or microscope manager. Once the setting has been changed, there is no difference in set up to any other tilted data collection.