HexAuFoil

Improving every image:

HexAuFoil® ultra-small hole sample supports for CryoEM reconstructions

quantifoil

part of sptlabtech

ABSTRACT

Over the last decade, cryo-electron microscopy (cryoEM) has undergone a “resolution revolution” and become a significant source of high-quality 3D-structural information for the biological community. In fact, for some important drug targets, such as GPCRs and channels, it has become the primary source of near-atomic resolution macromolecular structural data. The resulting increasing use of cryoEM in drug discovery and mechanistic studies has driven a demand for ever higher quality and higher resolution data.

One continuing major source of information loss, and therefore map quality reduction, is particle movement during imaging. While data processing can reduce the effect of this issue, novel sample preparation techniques are required to eliminate it. In this presentation, we are excited to describe one such novel sample preparation device, developed at MRC Laboratory of Molecular Biology, Cambridge [1, 2], and currently being commercialised by Quantifoil, part of SPT Labtech. HexAuFoil® ultra-small hole gold supports reduce particle movement to sub-Ångstrom levels: the grids small hole size provides extra support to the thin ice layer that supports biological specimens and thus reduces or eliminates ice buckling caused by the stress of cryo-cooling. The resultant improvement in particle localisation enables maps to be extrapolated to zero electron loss, before radiation damage. At the same time as reducing particle movement, consideration has been given to increasing automated cryoEM throughput at no cost to map quality with features such as maximal hole density.

Taken together, these developments mean that HexAuFoil® sample supports will improve every image.

References

[1] K Naydenova et al. Science 370: 223-226 (2020)
[2] K Naydenova and C J Russo. Ultramicroscopy [online] 232: 113396 (2022)

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quantifoil.com

+44 (7790) 307052

marketing@quantifoil.com

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INTRODUCTION

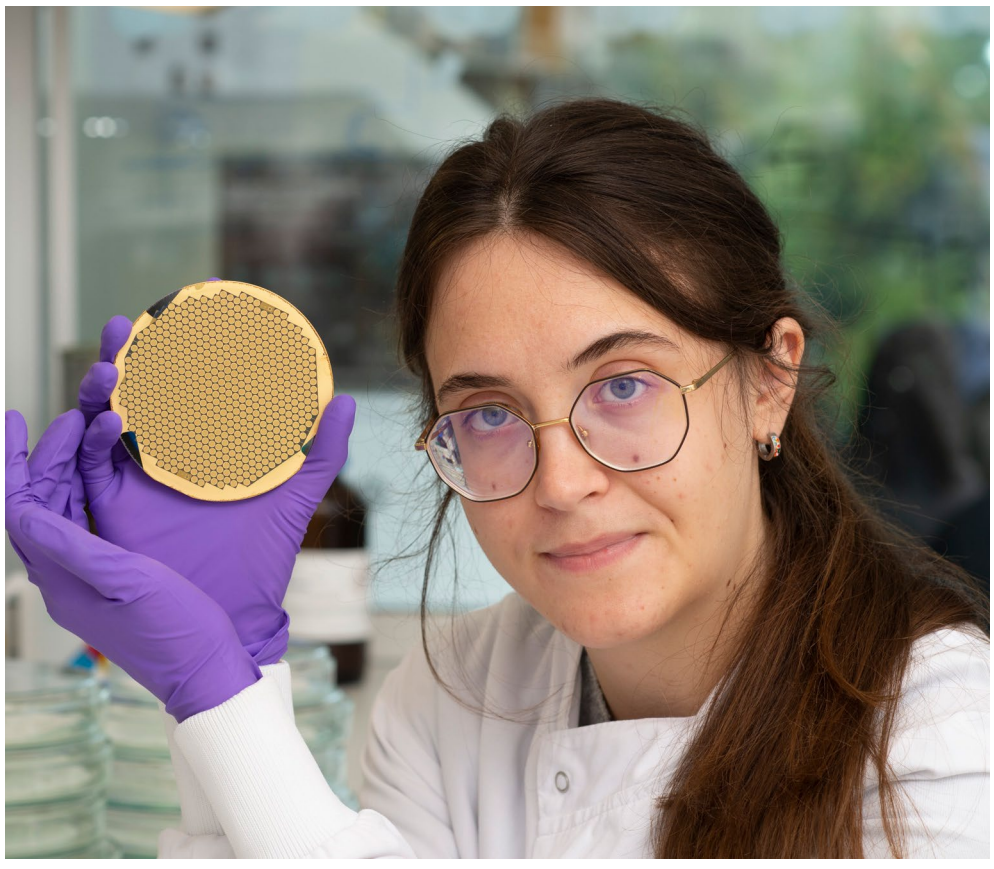
WHY DO WE NEED A NEW GRID?

Over the last decade, the use of cryo-EM to study biomolecules has increased exponentially, and it can now produce truly atomic resolution structures. However, sample preparation is widely recognised as a significant problem slowing the routine use of the technique for drug discovery and basic research. Plunge-freezing of biological samples is required for cryo-EM analysis, but results in the build up of compressive strain in the ice. Release of this strain due to electron-beam induced local heating causes the image to blur, and reducing the quality and achievevable resolution.

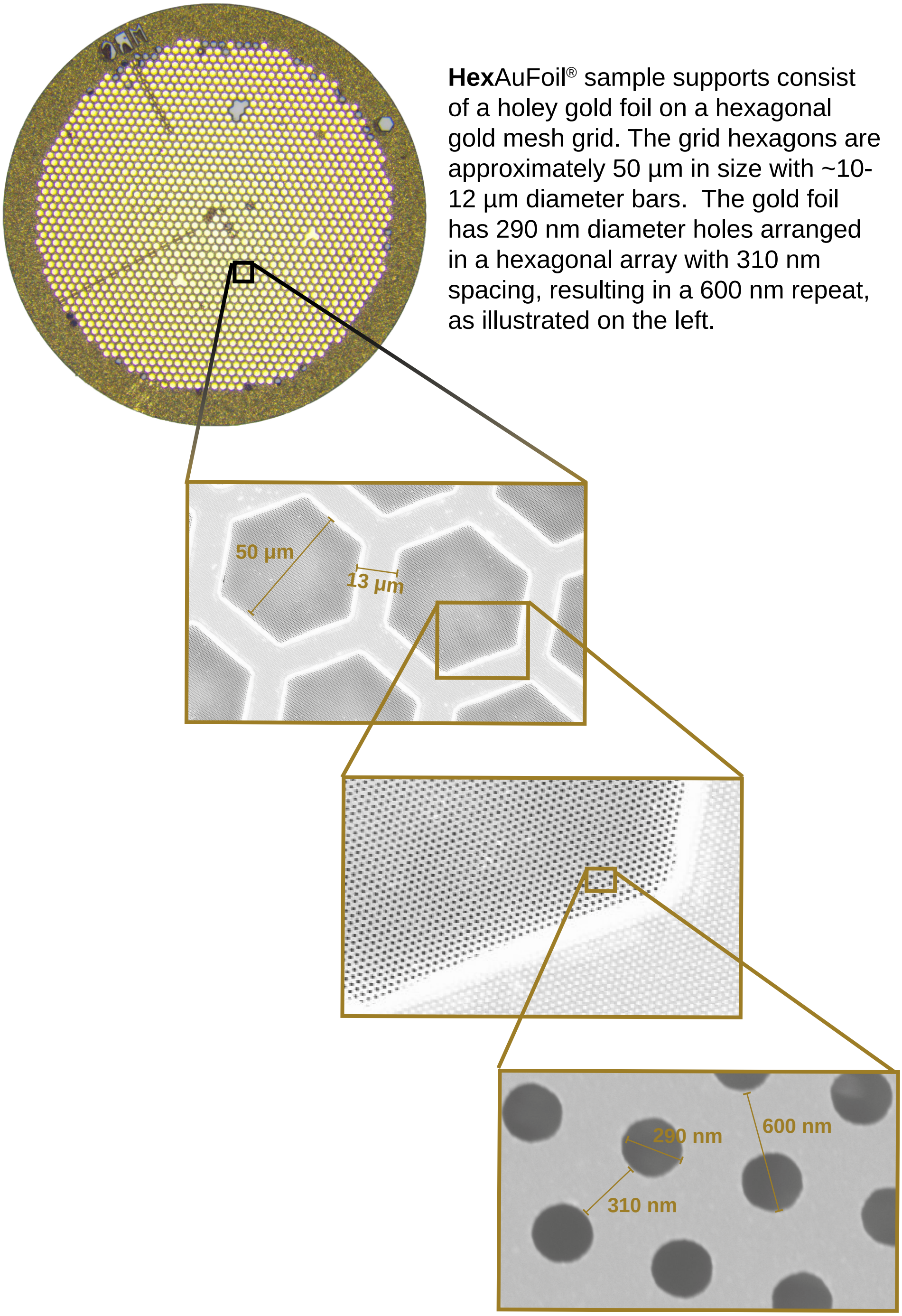
SO WHAT ARE HEXAUFOIL AND WHO DEVELOPED THEM?

Chris Russo and Katerina Naydenova (pictured below) of the MRC Laboratory of Molecular Biology (LMB) in Cambridge, UK, created HexAuFoil® gold supports to overcome this issue. The <1 µm holes in the supports maintain a ratio of hole size to ice thickness that prevents strain building to a high enough level for these effects to occur. As a result, particles remain stationary and structures can be extrapolated to zero dose before the biomolecules are damaged by exposure to the electron beam. In Chris Russo's own words, "By using these grids, your data is always better: bringing the movement to zero improves every image you take."

An additional benefit is that the hexagonal hole arrangement has a high-packing density that leads to higher data collection rates and therefore increases the throughput of resolved structures which is important for rapid lead optimization.



WHAT IS A HEXAUFOIL® GRID?

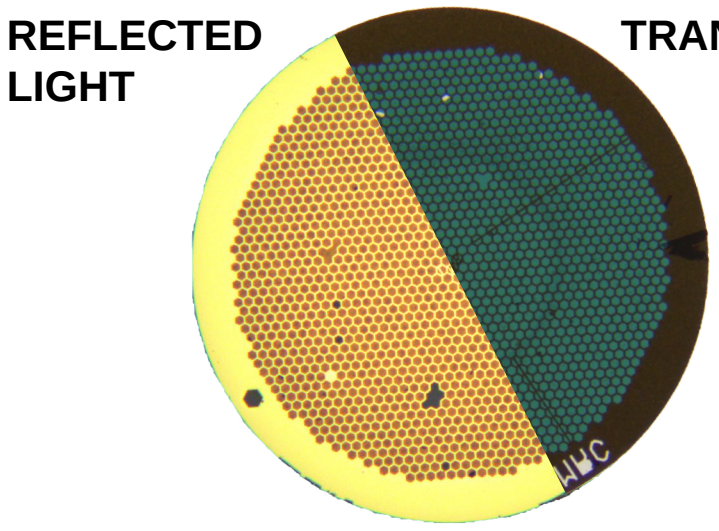


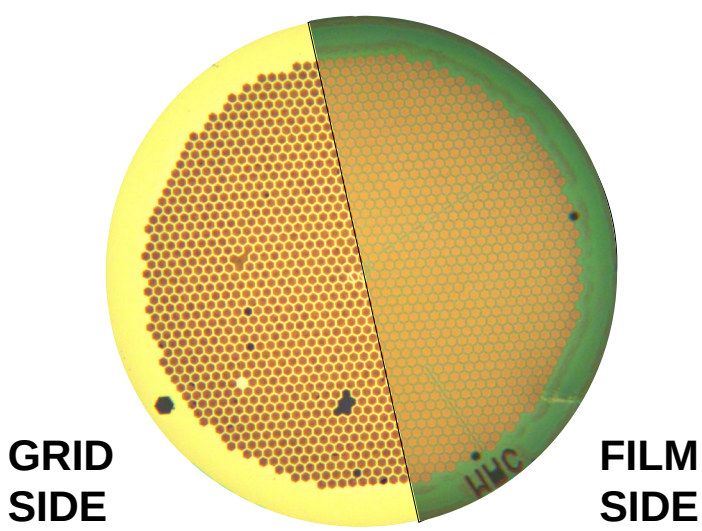
HexAuFoil® sample supports consist of a holey gold foil on a hexagonal gold mesh grid. The grid hexagons are approximately 50 µm in size with ~10-12 µm diameter bars. The gold foil has 290 nm diameter holes arranged in a hexagonal array with 310 nm spacing, resulting in a 600 nm repeat, as illustrated on the left.

WHAT DO HEXAUFOIL® GRIDS LOOK LIKE?

UNDER A LIGHT MICROSCOPE

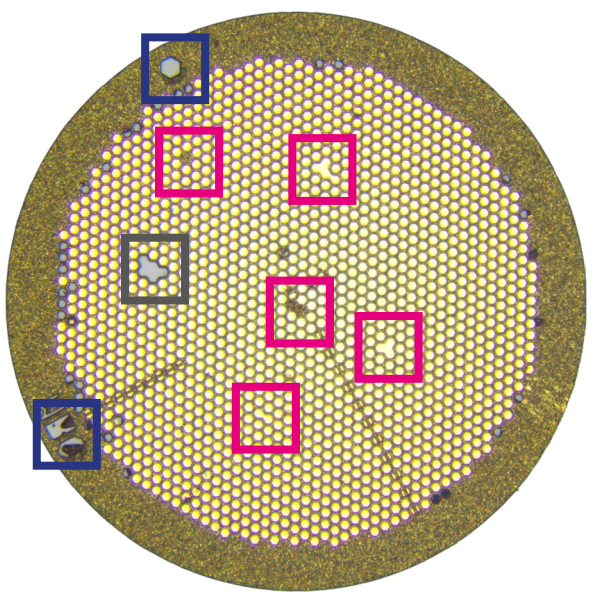
When inspected by eye, HexAuFoil® sample supports should appear a shiny gold, similar to UltrAuFoil® grids. However, when using a microscope to check for foil integrity, the appearance will change depending on the type of illumination selected. Under reflected light, the grid bar side will be light yellow, and the foil side will be shiny with a brown tint (LHS in left image below). In contrast, if HexAuFoil® are viewed using transmitted light, plasmon resonance from the regularly-arranged small holes will make the foil appear blue (RHS in left image below). The same effect can make parts of the foil in contact with grid appear green-blue in reflection (RHS in right image below).





WHAT FEATURES DO THEY HAVE?

The grids include a number of fiducial marks to aid with orientation. There are 2 fiducial marks on the rim of the grid which are visible by the naked eye (differing slightly from those illustrated here). In addition, there are a further 6 on grid fiducial marks to aid with orientation. The largest on grid mark, bottom left in the illustration, is free of foil to facilitate microscope alignments and flux measurements.



FIDUCIAL VISIBLE BY EYE

ADDITIONAL FIDUCIAL TO AID ORIENTATION IN MICROSCOPE

FOIL-FREE FIDUCIAL FOR SCOP ADJUSTMENT AND FLUX MEASUREMENT

DO YOU NEED TO MAKE CHANGES TO YOUR SAMPLE PREPARATION PROTOCOL?

YES!

1. PLASMA CLEANING

HexAuFoil® grids should be plasma cleaned or glow discharged to increase their wettability prior to use. In general, for best results, they will need glow discharging for significantly longer than other sample supports, such as UltrAuFoil® holey gold supports. However, the gold foil is not volatile when glow discharged or plasma treated, so the grids may be subjected to more extensive plasma treatments than standard carbon foils, without any risk of degrading the surface. A good rule of thumb is to approximately double the plasma cleaning/glow discharge times compared to UltrAuFoil® grids. A table of suggested settings is provided below for some common glow discharge instruments (as originally published in Naydenova and Russo, 2022).

SUGGESTED PLASMA CLEANING SETTINGS

PARAMETER	FISCHIONE 1070	EDWARDS S150B	TEDPELLA EASIGLOW
ATMOSPHERE	9:1 Ar:O_2	Residual air	Residual air
PROCESS PRESSURE	21 mTorr	150 mTorr	0.39 mBar (290 mTorr)
POWER/ CURRENT	40 W	30 mA	25 mA
EXPOSURE TIME	120-180 s	60 s	90 s

2. VITRIFICATION SETTINGS

In general, HexAuFoil® grids can be directly substituted into your vitrification protocol, with the exception that blot times should be extended compared to similar samples on a standard larger hole grid. For example, if you currently blot for 10 seconds, with HexAuFoil® grids, you should initially try a 15 second blot. We recommend applying sample to the foil side of the grid and blotting from the same or both sides, but this is sample dependent, and any variation can be used.

Typical settings for vitrification with a HexAuFoil® grid on some common plunge freezing instruments are given below by way of example (taken from Naydenova and Russo, 2022).

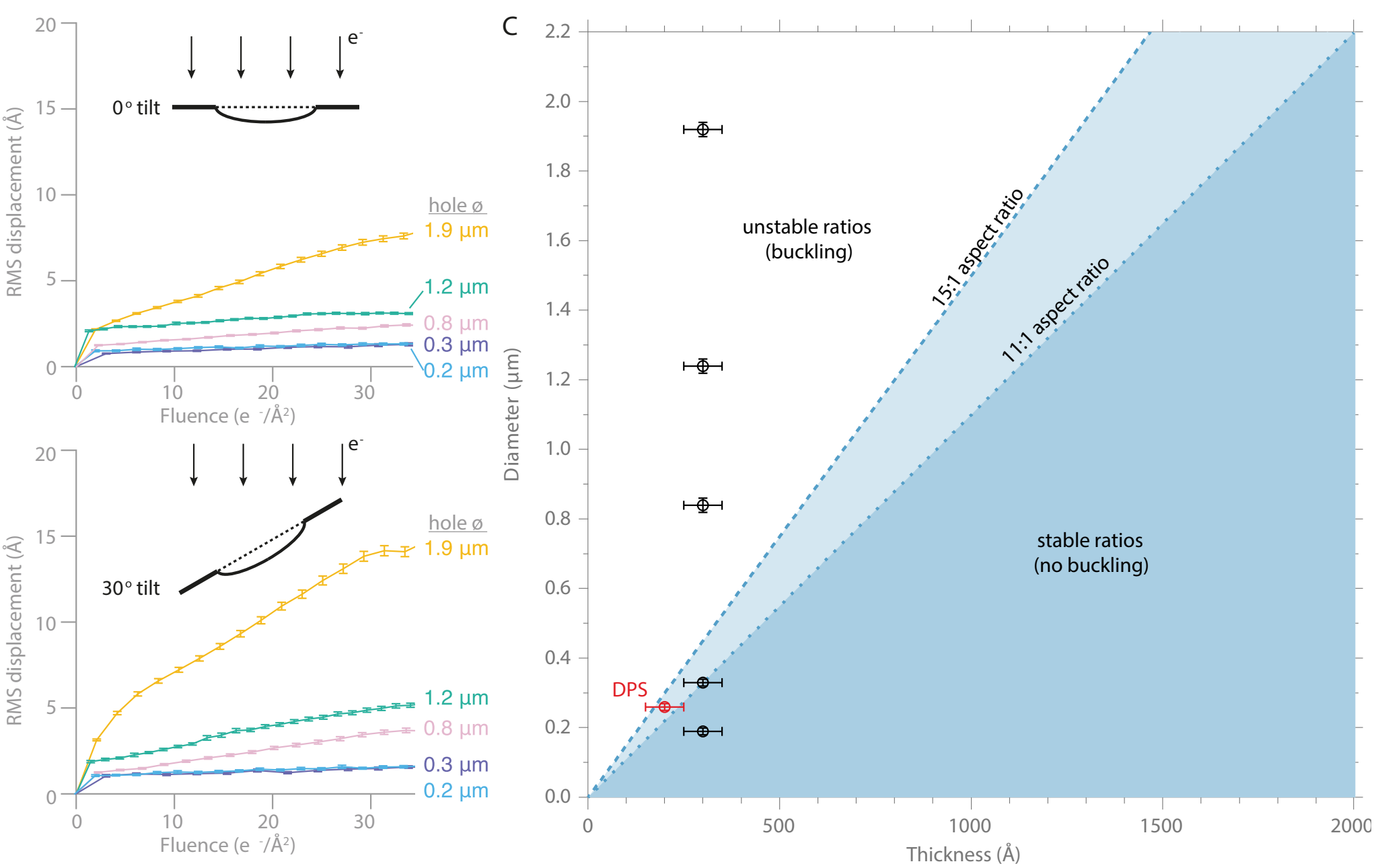
SUGGESTED VITRIFICATION SETTINGS

PARAMETER	MANUAL PLUNGER	VITROBOT MKIV	LEICA GP2
TEMP	4°C	4°C	4°C
RELATIVE HUMIDITY	100 %	100 %	100 %
WAIT TIME	0 s	0 s	0 s
FORCE SETTING	n/a	10	n/a
BLOT TIME	15 s	5 s	5 s
DRAIN TIME	0 s	0 s	0 s
VITRIFICATION MEDIA	Ethane	Ethane	Ethane
VITRIFICATION TEMP	93 K	93 K	93 K

HOW DO HEXAUFOIL® SUPPORTS IMPROVE YOUR IMAGES?

1. SMALLER HOLES, THINNER, STABLE ICE

The movement of thousands of gold nanoparticles vitrified on grids with varying hole size was tracked. With smaller hole-sizes, the compressive strain on the ice caused by local heating from the electron beam was reduced. This in turn led to less buckling and therefore lower particle movement. With small enough hole size, for a given ice thickness, buckling is eliminated and particle movement reduces to less than an Ångstrom.



By measuring the observed particle movement parallel to the beam, it is possible to determine a ratio between ice thickness and hole size that determines whether buckling can occur. Ice buckling at hole diameter:ice thickness ratios of 11:1 or greater. For biological specimens, where the ice needs to be at maximum 3-400 Å thick, this means that hole diameters should be no more than 300 nm, as with HexAuFoil® ultra-small hole gold sample supports.

2. STABLE ICE GIVES HIGHER QUALITY, MORE CONSISTENT DATA

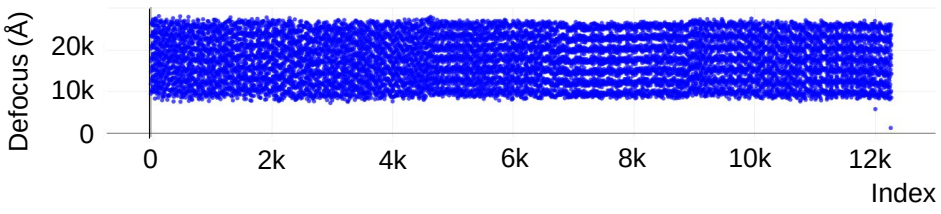
The combination of 300 nm holes in a gold film eliminates both buckling and in plane beam-induced motion. Overall, particle motion is reduced to sub-Ångstrom levels that can be attributed to Brownian motion-type movement.

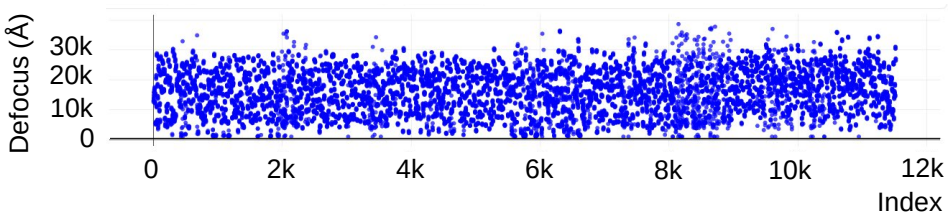
HexAuFoil® grids deliver consistent thin ice across large regions of the grid. This consistency combined with increased particle stability delivers reduced re-focussing and increased consistency in data collection parameters such as defocus and ctf correction.

HexAuFoil

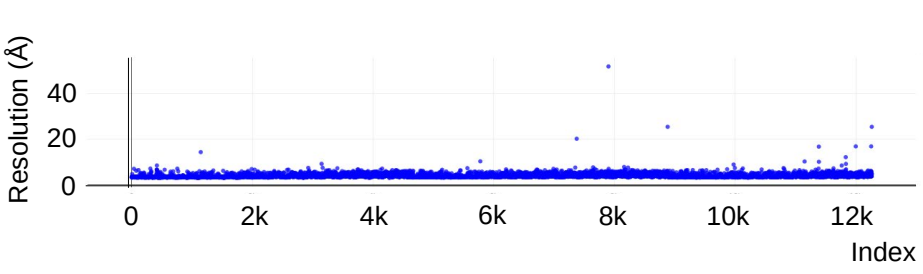
UltrAuFoil®

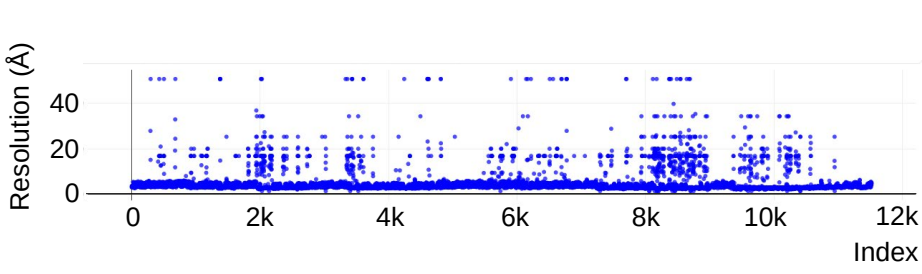
AVERAGE DEFOCUS (Å)



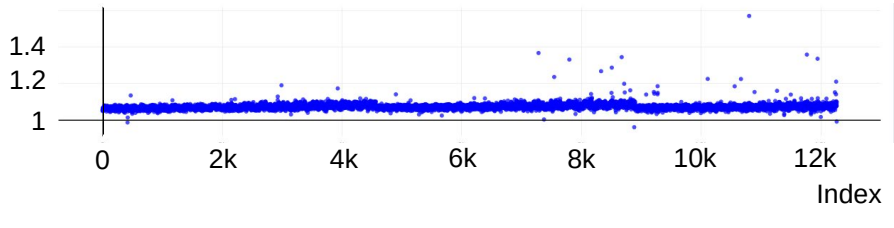


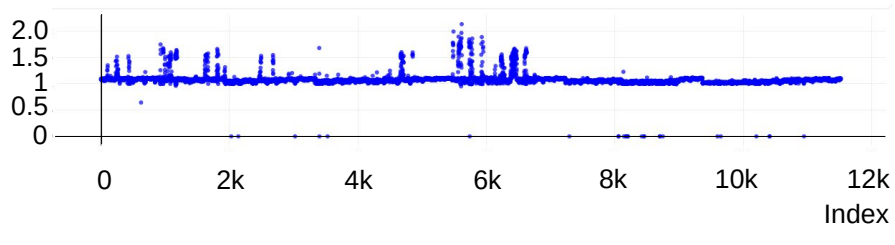
CTF FIT RESOLUTION (Å)





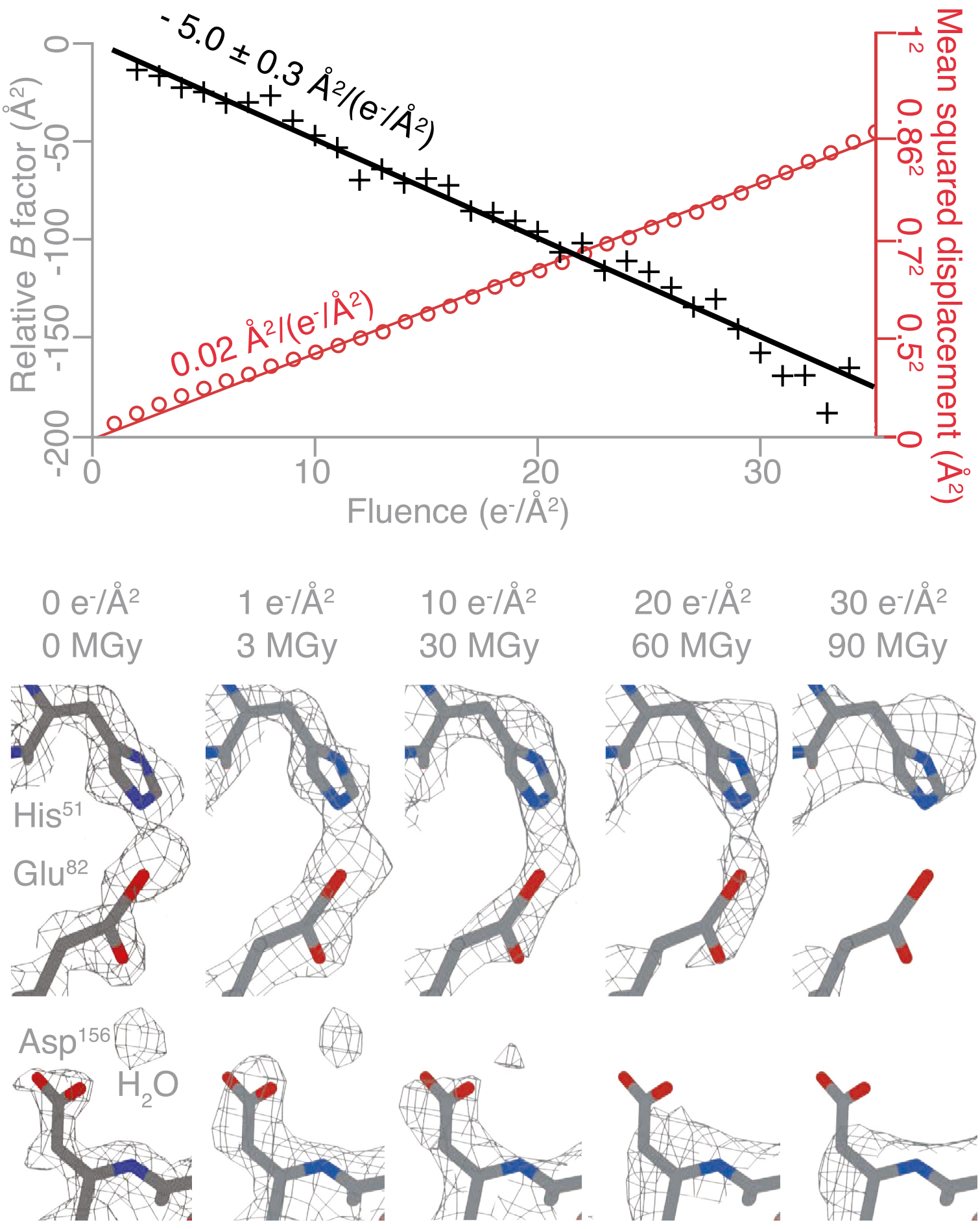
RELATIVE ICE THICKNESS



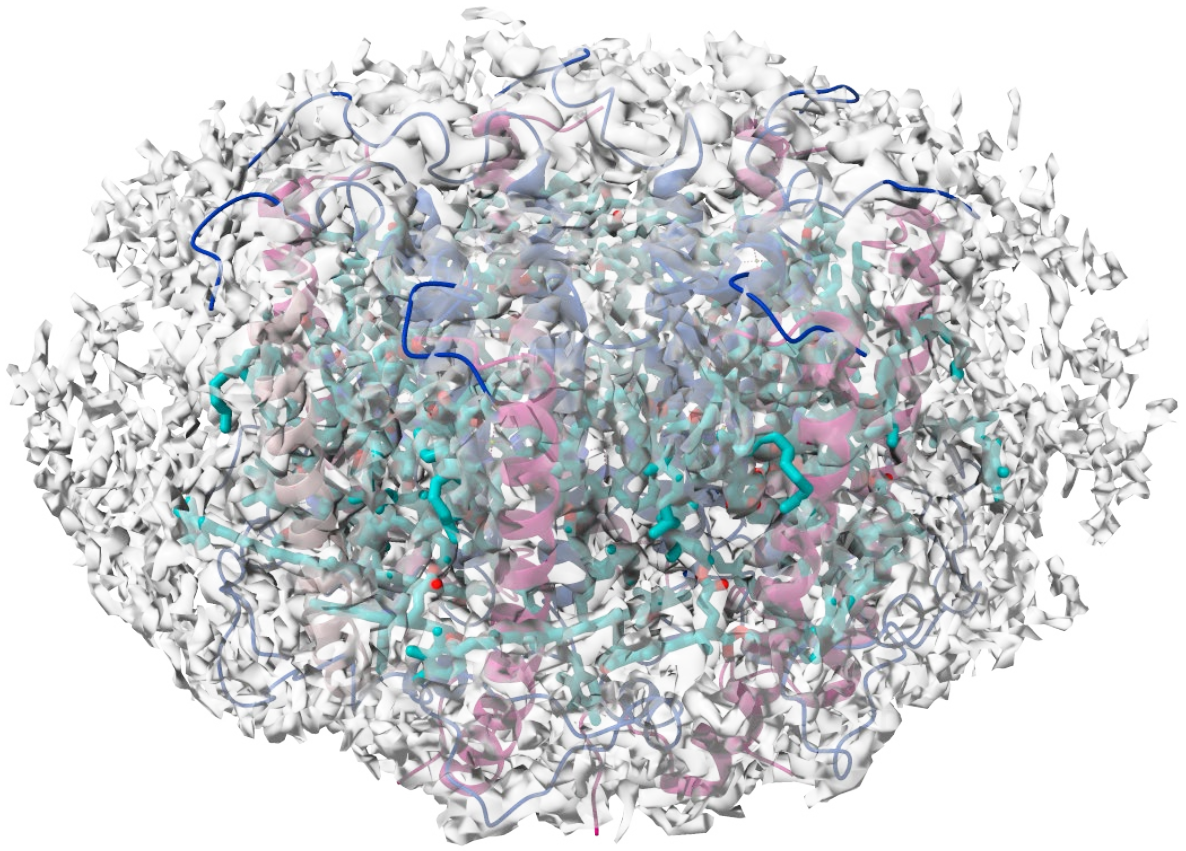


3. EXTRAPOLATE RECONSTRUCTIONS TO ZERO DOSE

With movement at sub-Ångstrom (Brownian motion) levels, B-factors are no longer dominated by beam-induced motion. Well-developed theory relating B-factor to atomic motion and radiation damage can therefore be applied, to allow the calculation of structure factors and subsequently reconstructions at zero dose. The program 0dose (https://www.mrc-lmb.cam.ac.uk/crusso/resources.html) will shortly be included with software packages including CCPEM, allows researchers to apply this correction to their data. Reconstructions at zero dose offer consistency of reconstructions between datasets, facilitating comparisons between lead compounds in drug discovery as well as offering improved reconstructions.



FURTHER INFORMATION ABOUT THE DEVELOPMENT AND USE OF HEXAUFOIL® GRIDS



Light-harvesting 2 complex: PDBID 6ZXA, EMDB entry 11516. Structure solved with data collected using HexAuFoil® grids. As described in Gardiner *et al* (Sci. Adv.,2021)

HEXAUFOIL® DEVELOPMENT AND BEST PRACTICE

More details about the development and use of HexAuFoil grids can be found at:

Naydenova and Russo. Integrated wafer-scale manufacture of electron cryomicroscopy specimen supports. Ultramicroscopy 232: 113396 (2022).

Naydenova, Jia, Russo. Cryo-EM with sub-1 Å specimen movement. Science 370: 223-226 (2020).

STRUCTURES SOLVED USING HEXAUFOIL® GRIDS

Naydenova et al. Structure of the SARS-CoV-2 RNA-dependent RNA polymerase in the presence of favipiravir-RTP. Proc. Natl Acad. Sci 118: e2021946118 (2021).

Gardiner et al. The 2.4 Å cryo-EM structure of a heptameric light-harvesting 2 complex reveals two carotenoid energy transfer pathways. Sci. Adv. 7: e4650 (2021).

Qian et al. 2.4-Å structure of the double-ring Gemmatimonas phototrophica photosystem. Sci. Adv. 8: eabk3139 (2022).

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✉ marketing@quantifoil.com

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