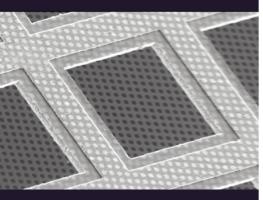


# **C-flat™ Holey Carbon Grids for Cryo-TEM**

The premier holey carbon grid for cryo-transmission electron microscopy

#### **Overview**

C-flat<sup>™</sup> is an ultra-flat, holey carbon-coated TEM support grid for transmission electron microscopy (TEM). Unlike competing holey carbon films, C-flat<sup>™</sup> is manufactured without plastics, so it is clean upon arrival and the user has no residue to contend with.



#### **Articles**

Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Daniel Wrapp, Nianshuang Wang, Kizzmekia S. Corbett, Jory A. Goldsmith, Ching-Lin Hsieh, Olubukola Abiona, Barney S. Graham, Jason S. McLellan, 2020. Science, 13 Mar 2020: Vol. 367, Issue 6483, pp. 1260-1263, DOI: 10.1126/science.abb2507

An improved holey carbon film for cryo-electron microscopy. Quispe J, Damiano J, Mick SE, Nackashi DP, Fellmann D, Ajero TG, Carragher B, Potter CS, (2007). Microscopy and microanalysis, 13(5), 365-371.

Improving the technique of vitreous cryo-sectioning for cryo-electron tomography: electrostatic charging for section attachment and implementation of an anti-contamination glove box. Pierson J, Fernández JJ, Bos E, Amini S, Gnaegi H, Vos M, Bel B, Adolfsen F, Carrascosa JL, Peters PJ., J Struct Biol. 2010 Feb;169(2): 219-25. Epub 2009 Oct 12.

C-flat<sup>™</sup>, CD-Flat<sup>™</sup>, and Au-Flat<sup>™</sup>, are trademarks of Protochips, Inc. All rights reserved.



### The C-flat™ Advantage

#### C-flat™ leads to better data sets.

Made with patented technology, C-flat™ provides an ultra-flat surface that results in better particle dispersion and more uniform ice thickness. Patterning is done using deep-UV projection lithography, ensuring the most accurate and consistent hole shapes and sizes down to submicron features. The precise methods by which C-flat™ is manufactured eliminate artifacts such as excess carbon and edges around holes.

#### C-flat™ is affordable

C-flat<sup>™</sup> is available in 25, 50, and 100 packs at a per-grid price less than competing products. With 54 varieties, there is always a C-flat<sup>™</sup> product optimized for your needs.

#### **Applications**

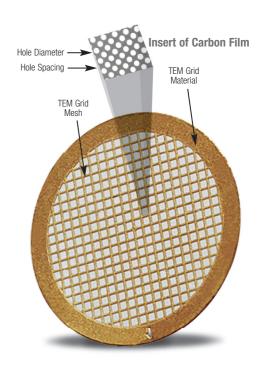
C-flat<sup>™</sup> holey carbon grids provide the ideal specimen support to achieve high resolution data in cryo-TEM making them an ideal choice for single particle analysis, cryo electron tomography and automated TEM analysis.

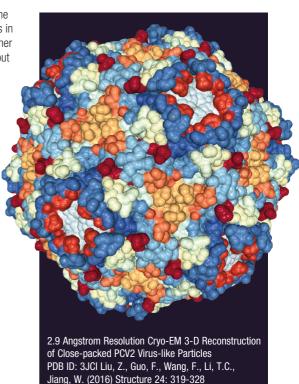
## Cryo-electron tomography (cryoET) and Single Particle Analysis (SPA):

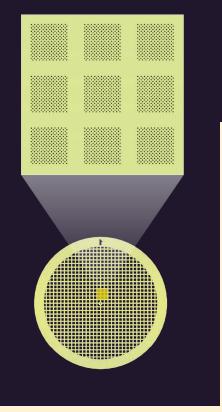
Numerous researchers have reported that the ultra-flat surface of C-flat™ leads to even ice thickness and uniform particle distribution within the hole areas. This optimal particle distribution results in superior data being collected as compared with other holey support films. 2µm hole sizes are standard but custom hole sizes are available so C-flat™ can accommodate the common magnifications used for quantitative TEM analysis.

#### **Automated TEM:**

C-flat™ provides a regular array of analysis sites compatible with automated data collection software such as Leginon. This compatibility, in combination with the more uniform ice thickness and particle distribution reported by numerous researchers, results in more high-quality target sites per grid.











# NEW Au-flat™

**Holey Carbon Grids for High Resolution Imaging** 

Improve your image quality and resolution with the gold alloy film that reduces particle movement during imaging

#### What is Au-Flat?

Au-Flat is an ultrastable Cryo-EM sample support with a 45nm holey Gold alloy film on 3 mm gold mesh grids. Au-Flat is a derivative of our patented C-Flat product.

## What varieties of Au-Flat are available?

Au-Flat is offered in two configurations:

1.2 µm/1.3 µm hole pattern on 300 mesh Gold grids 2µm/2µm Hole Pattern on 200 mesh Gold grids

# What are the benefits of Au-Flat over Holey Carbon Supports like C-Flat?

#### Better reconstructions with less data

Au-Flat significantly reduces beam-induced motion during imaging compared to carbon films, improving image quality and resolution.

#### **Biocompatible**

Au-Flat features a holey Au/Pd film on a gold mesh grid, so it's chemically inert and biologically compatible.

#### Durable

Au-Flat's film is significantly stronger than carbon films and is more capable of surviving the Cryo-EM workflow including tweezer handling, glow discharge, blotting, auto-grid loading and plunge freezing.

## What are the benefits of Au-Flat over other Gold Foil TEM Grids?

#### **Fewer Mistakes**

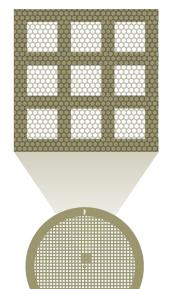
The lighter color of the Au/Pd alloy film compared to the underlying gold mesh grid makes it easy to identify the "holey" side of the grid during sample prep. This ensures the sample is deposited on the correct side and that the grid is loaded and imaged in the correct orientation.

#### Stronger

Au-Flat is produced with gold mesh grids that are about 6 microns thicker than typical Cryo-EM grids. This makes the grids stiffer and less likely to bend or deform. The increased thickness is fully compatible with side-entry holders as well as auto-loaders.

### **Ordering Information**

Cat. #	Hole Size	Hole Spacing	TEM Mesh	TEM Grid	Qty.
AUFT313-50	1.2 µm	1.3 µm	300	Au	50/pk
AUFT313-05	1.2 µm	1.3 µm	300	Au	5/pk
AUFT222-50	2 µm	2 µm	200	Au	50/pk
	AUFT313-50 AUFT313-05	Cat. #         Size           AUFT313-50         1.2 μm           AUFT313-05         1.2 μm	Cat. #         Size         Spacing           AUFT313-50         1.2 μm         1.3 μm           AUFT313-05         1.2 μm         1.3 μm	Cat. #         Size         Spacing         Mesh           AUFT313-50         1.2 μm         1.3 μm         300           AUFT313-05         1.2 μm         1.3 μm         300	Cat. #         Size         Spacing         Mesh         Grid           AUFT313-50         1.2 μm         1.3 μm         300         Au           AUFT313-05         1.2 μm         1.3 μm         300         Au



# **NEW CD-flat™**

#### **Holey Carbon Grids for Automated S/TEM Imaging and Metrology**

The C-flat™ advantage in a new pattern

CD-flat<sup>TM</sup> — the premier ultra-flat holey carbon grid is ready to use with no additional cleaning or handling steps, and is now available, featuring a **NEW** 8/2 hole pattern. CD-flat is designed for CD-TEM metrology of large specimens, like 3D NAND FIB lamella.

#### **Specifications**

Grid Material	Copper
Mesh Size	300 Mesh
0.D.	3.05mm
Film Material	Holey Carbon
Film Thickness	40nm
Hole Pattern	8/2 (8 um diameter,
	2 μ spacing between holes)

### **Ordering Information**

Product Code	Cat. #	Hole Size	Hole Spacing	TEM Mesh	TEM Grid	Qty.
CDF-8/2-3Cu-T-50	CDFT823-50	8.0 µm	2.0 µm	300	Cu	50/pk

### **Ordering Guide**

#### **Product Line**

C-flat<sup>™</sup> is a holey carbon film supported by a standard TEM grid. C-flat<sup>™</sup> products are fully specified by 4 parameters: the hole diameter and pitch of the holey carbon film and the material type and mesh size of the TEM grid. The image below illustrates these parameters.

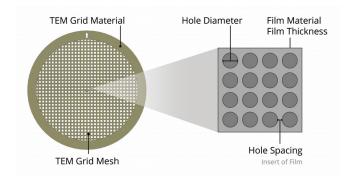
#### Standard Products

The breadth of applications in cryoTEM necessitate a wide range of holey carbon film patterns. And now, with the recent expansion of the product line, a C-flat™ holey carbon film is available for almost any application. Whether 600nm holes are needed for very high magnifications with ultra-high resolution cameras or large open areas are needed for larger specimens, C-flat™ is the perfect holey carbon grid.

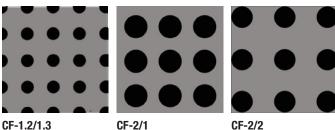
C-flat™ is immediately available in several standard array patterns including hole diameters/hole spacings of 1.2/1.3, 2/1, 2/2, 2/4, 4/2, and a multihole pattern. C-flat™ is supported by your choice of a 200 mesh 300 mesh, or 400 mesh copper TEM grid and sold in quantities of 25, 50, or 100.

#### **Thick Products**

C-Flat™ is available in a thick option that doubles the carbon thickness from approximately 20nm to 40nm. Thick C-flat product numbers end in -T, catalog numbers contain "CFT". Available in quantities of 50 and 100 per pack.



### **Standard Array Patterns**



2.0µm hole, 1.0µm space

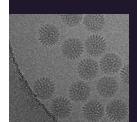
1.2µm hole, 1.3µm space

**CF-2/4**2.0μm hole, 4.0μm space
4.0μm hole, 2.0μm space



2.0µm hole, 2.0µm space

CF-MH Multihole



Consistent: Researchers around the world have reported that the ultra-flat surface of C-flat™ leads to even ice thickness and uniform particle distribution, allowing for superior 3-D reconstructions.

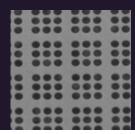
Frozen-hydrated Bacteriophage Capsid (data acquired on CF-1.2/1.4-4C).

### **C-flat™ Holey Carbon Grids for TEM**

#### **Copper Only**

		llala	llele	TEM	TEM	
Product Code	Cat. #	Hole Size	Hole Spacing	TEM Mesh	TEM Grid	Qty.
CF-1.2/1.3-2C	CF213-25	1.2 µm	1.3 µm	200	Cu	25/pk.
	CF213-50	1.2 µm	1.3 µm	200	Cu	50/pk.
	CF213-100	1.2 µm	1.3 µm	200	Cu	100/pk.
CF-1.2/1.3-3C	CF313-25	1.2 µm	1.3 µm	300	Cu	25/pk.
	CF313-50	1.2 µm	1.3 µm	300	Cu	50/pk.
	CF313-100	1.2 µm	1.3 µm	300	Cu	100/pk.
CF-1.2/1.3-4C	CF413-25	1.2 µm	1.3 µm	400	Cu	25/pk.
	CF413-50	1.2 µm	1.3 µm	400	Cu	50/pk.
	CF413-100	1.2 µm	1.3 µm	400	Cu	100/pk.
CF-2/1-2C	CF212-25	2.0 µm	1.0 µm	200	Cu	25/pk.
	CF212-50	2.0 µm	1.0 µm	200	Cu	50/pk.
	CF212-100	2.0 µm	1.0 µm	200	Cu	100/pk.
CF-2/1-3C	CF312-25	2.0 µm	1.0 µm	300	Cu	25/pk.
	CF312-50	2.0 µm	1.0 µm	300	Cu	50/pk.
	CF312-100	2.0 µm	1.0 µm	300	Cu	100/pk.
CF-2/1-4C	CF412-25	2.0 µm	1.0 µm	400	Cu	25/pk.
	CF412-50	2.0 µm	1.0 µm	400	Cu	50/pk.
	CF412-100	2.0 µm	1.0 µm	400	Cu	100/pk.
CF-2/2-2C	CF-222C-25	2.0 µm	2.0 µm	200	Cu	25/pk.
	CF-222C-50	2.0 µm	2.0 µm	200	Cu	50/pk.
	CF-222C-100	2.0 µm	2.0 µm	200	Cu	100/pk.
CF-2/2-3C	CF-322C-25	2.0 µm	2.0 µm	300	Cu	25/pk.
	CF-322C-50	2.0 µm	2.0 µm	300	Cu	50/pk.
	CF-322C-100	2.0 µm	2.0 μm	300	Cu	100/pk.
CF-2/2-4C	CF-224C-50	2.0 µm	2.0 µm	400	Cu	50/pk.
	CF-224C-100	2.0 µm	2.0 µm	400	Cu	100/pk.
CF-2/4-2C	CF242-50	2.0 µm	4.0 μm	200	Cu	50/pk.
0. 2, . 20	CF242-100	2.0 µm	4.0 μm	200	Cu	100/pk.
CF-2/4-3C	CF342-25	2.0 µm	4.0 μm	300	Cu	25/pk.
0. 2, . 00	CF342-50	2.0 µm	4.0 µm	300	Cu	50/pk.
	CF342-100	2.0 µm	4.0 μm	300	Cu	100/pk.
CF-2/4-4C	CF442-25	2.0 µm	4.0 µm	400	Cu	25/pk.
	CF442-50	2.0 µm	4.0 μm	400	Cu	50/pk.
	CF442-100	2.0 µm	4.0 μm	400	Cu	100/pk.
CF-4/2-2C	CF422-25	4.0 µm	2.0 µm	200	Cu	25/pk.
	CF422-50	4.0 μm	2.0 μm	200	Cu	50/pk.
	CF422-100	4.0 µm	2.0 µm	200	Cu	100/pk.
CF-4/2-3C	CF423-50	4.0 µm	2.0 µm	300	Cu	50/pk.
	CF423-100	4.0 µm	2.0 µm	300	Cu	100/pk.
CF-4/2-4C	CF424-50	4.0 µm	2.0 µm	400	Cu	50/pk.
	CF424-100	4.0 µm	2.0 µm	400	Cu	100/pk.
CF-MH-2C	CF2MH-50		tihole*	200	Cu	50/pk.
J. IIII 20	CF2MH-100	Multihole*		200	Cu	100/pk.
CF-MH-3C	CF3MH-25		tihole*	300	Cu	25/pk.
S. IIII 00	CF3MH-50	Multihole*		300	Cu	50/pk.
	CF3MH-100		tihole*	300	Cu	100/pk.
CF-MH-4C	CF4MH-25		tihole*	400	Cu	25/pk.
J. 11111 40	CF4MH-50		tihole*	400	Cu	50/pk.
	CF4MH-100		tihole*	400	Cu	100/pk
	J 100	iiiui		.50	- u	. 0 0, pit

The Multihole device has a staggered pattern of six features consisting of three circle patterns of 1 micron, 1.4 micron and 2 micron diameter and three ellipse patterns of 1 x 4 microns,  $1.4 \times 5.6$  microns and  $2 \times 8$  microns.



Compatible: C-flat™ provides a regular array of analysis sites compatible with automated data collection software such as Leginon. This compatibility, in combination with the more uniform ice thickness and particle distribution reported by numerous researchers, results in more high-quality target sites per grid.



Clean: C-Flat™ uses no plastics or polymers in its production. This means C-Flat™ is shipped clean, so it's ready to use out of the box and requires no solvent washing steps prior to use, leading to less breakage of the holey carbon film.

### **C-flat™ Holey Carbon Grids for TEM**

**Thick, Copper Only** 

Product Code	Cat. #	Hole Size	Hole Spacing	TEM Mesh		Qty.
CF-1.2/1.3-2C-T	CFT213-50	1.2 µm	1.3 µm	200	Cu	50/pk.
	CFT213-100	1.2 µm	1.3 µm	200	Cu	100/pk.
CF-1.2/1.3-3C-T	CFT313-50	1.2 µm	1.3 µm	300	Cu	50/pk.
	CFT313-100	1.2 µm	1.3 µm	300	Cu	100/pk.
CF-1.2/1.3-4C-T	CFT413-50	1.2 µm	1.3 µm	400	Cu	50/pk.
	CFT413-100	1.2 µm	1.3 µm	400	Cu	100/pk.
CF-2/1-2C-T	CFT212-50	2.0 µm	1.0 µm	200	Cu	50/pk.
	CFT212-100	2.0 µm	1.0 µm	200	Cu	100/pk.
CF-2/1-3C-T	CFT312-50	2.0 µm	1.0 µm	300	Cu	50/pk.
	CFT312-100	2.0 µm	1.0 µm	300	Cu	100/pk.
CF-2/1-4C-T	CFT412-50	2.0 µm	1.0 µm	400	Cu	50/pk.
	CFT412-100	2.0 µm	1.0 µm	400	Cu	100/pk.
CF-2/2-2C-T	CFT-222C-50	2.0 µm	2.0 µm	200	Cu	50/pk.
	CFT-222C-100	2.0 µm	2.0 µm	200	Cu	100/pk.
CF-2/2-3C-T	CFT-223C-50	2.0 µm	2.0 µm	300	Cu	50/pk.
	CFT-223C-100	2.0 µm	2.0 µm	300	Cu	100/pk.
CF-2/2-4C-T	CFT-224C-50	2.0 µm	2.0 µm	400	Cu	50/pk.
	CFT-224C-100	2.0 µm	2.0 µm	400	Cu	100/pk.
CF-2/4-2C-T	CFT242-50	2.0 µm	4.0 µm	200	Cu	50/pk.
	CFT242-100	2.0 µm	4.0 µm	200	Cu	100/pk.
CF-2/4-3C-T	CFT342-50	2.0 µm	4.0 µm	300	Cu	50/pk.
	CFT342-100	2.0 µm	4.0 µm	300	Cu	100/pk.
CF-2/4-4C-T	CFT442-50	2.0 µm	4.0 µm	400	Cu	50/pk.
	CFT442-100	2.0 µm	4.0 µm	400	Cu	100/pk.
CF-4/1-2C-T	CFT241-100	4.0 µm	1.0 µm	200	Cu	100/pk
CF-4/2-2C-T	CFT422-50	4.0 µm	2.0 µm	200	Cu	50/pk.
	CFT422-100	4.0 µm	2.0 µm	200	Cu	100/pk.
CF-4/2-3C-T	CFT423-50	4.0 µm	2.0 µm	300	Cu	50/pk.
	CFT423-100	4.0 µm	2.0 µm	300	Cu	100/pk.
CF-4/2-4C-T	CFT424-50	4.0 µm	2.0 µm	400	Cu	50/pk.
	CFT424-100	4.0 µm	2.0 µm	400	Cu	100/pk.
CF-MH-2C-T	CFT2MH-50	Mul	tihole*	200	Cu	50/pk.
	CFT2MH-100		tihole*	200	Cu	100/pk.
CF-MH-3C-T	CFT3MH-50		tihole*	300	Cu	50/pk.
	CFT3MH-100		tihole*	300	Cu	100/pk.
CF-MH-4C-T	CFT4MH-50		tihole*	400	Cu	50/pk.
	CFT4MH-100		tihole*	400	Cu	100/pk
CF-1/1-3C-T	CFT31-100	1.0 µm	1.0 µm	300	Cu	100/pk.
CF-1/1-4C-T	CFT41-100	1.0 µm	1.0 µm	400	Cu	100/pk.

The Multihole device has a staggered pattern of six features consisting of three circle patterns of 1 micron, 1.4 micron and 2 micron diameter and three ellipse patterns of 1 x 4 microns, 1.4 x 5.6 microns and 2 x 8 microns.

### **C-flat™ Holey Carbon Grids for TEM**

**Gold Only** 

Draduat Cada	Cot #	Hole	Hole	TEM		Otre
Product Code	Cat. #	Size	Spacing	Mesh		Qty.
CF-1.2/1.3-2Au	CF213-50-Au	1.2 µm	1.3 µm	200	Au	50/pk
OF 4 0/4 0 0A.	CF213-100-Au	1.2 µm	1.3 µm	200	Au	100/pk
CF-1.2/1.3-3Au	CF313-50-Au	1.2 µm	1.3 µm	300	Au	50/pk
05 1 0/1 0 44	CF313-100-Au	1.2 µm	1.3 µm	300	Au	100/pk
CF-1.2/1.3-4Au	CF413-50-Au	1.2 µm	1.3 µm	400	Au	50/pk
	CF413-100-Au	1.2 µm	1.3 µm	400	Au	100/pk
CF-2/1-2Au	CF212-50-Au	2 µm	1 µm	200	Au	50/pk
	CF212-100-Au	2 µm	1 μm	200	Au	100/pk
CF-2/1-3Au	CF312-50-Au	2 µm	1 µm	300	Au	50/pk
	CF312-100-Au	2 μm	1 μm	300	Au	100/pk
CF-2/1-4Au	CF412-50-Au	2 µm	1 µm	400	Au	50/pk
	CF412-100-Au	2 µm	1 μm	400	Au	100/pk
CF-2/2-2Au	CF222C-50-Au	2 μm	2 µm	200	Au	50/pk
	CF222C-100-Au	2 µm	2 μm	200	Au	100/pk
CF-2/2-3Au	CF223C-50-Au	2 µm	2 μm	300	Au	50/pk
	CF223C-100-Au	2 µm	2 μm	300	Au	100/pk
CF-2/2-4Au	CF224C-50-Au	2 µm	2 μm	400	Au	50/pk
	CF224C-100-Au	2 µm	2 µm	400	Au	100/pk
CF-2/4-2Au	CF242-50-Au	2 μm	4 μm	200	Au	50/pk
	CF242-100-Au	2 µm	4 µm	200	Au	100/pk
CF-2/4-3Au	CF342-50-Au	2 µm	4 μm	300	Au	50/pk
	CF342-100-Au	2 µm	4 μm	300	Au	100/pk
CF-2/4-4Au	CF442-50-Au	2 µm	4 μm	400	Au	50/pk
	CF442-100-Au	2 µm	4 µm	400	Au	100/pk
CF-4/1-4Au	CF441-100-Au	4 μm	1 μm	400	Au	100/pk
CF-4/2-2Au	CF422-50-Au	4 μm	2 µm	200	Au	50/pk
	CF422-100-Au	4 μm	2 μm	200	Au	100/pk
CF-4/2-3Au	CF423-50-Au	4 μm	2 µm	300	Au	50/pk
	CF423-100-Au	4 μm	2 µm	300	Au	100/pk
CF-4/2-4Au	CF424-50-Au	4 μm	2 µm	400	Au	50/pk
	CF424-100-Au	4 μm	2 µm	400	Au	100/pk
CF-MH-2Au	CF2MH-50-Au	Multi	hole*	200	Au	50/pk
	CF2MH-100-Au	Multi	hole*	200	Au	100/pk
CF-MH-3Au	CF3MH-50-Au	Multi	hole*	300	Au	50/pk
	CF3MH-100-Au		hole*	300	Au	100/pk
CF-MH-4Au	CF4MH-50-Au		hole*	400	Au	50/pk
	CF4MH-100-Au		hole*	400	Au	100/pk

### **C-flat™ Holey Carbon Grids for TEM**

**Thick Gold Only** 

Product Code	Cat. #	Hole Size	Hole Spacing	TEM Mesh		Qty.
CF-2/4-3Au-T	CFT342-50-Au	2.0 µm	4.0 µm	300	Au	50/pk
	CFT342-100-Au	2.0 µm	4.0 µm	300	Au	100/pk
CF-4/2-3Au-T	CFT423-50-Au	4.0 µm	2.0 µm	300	Au	50/pk
	CFT423-100-Au	4.0 µm	2.0 µm	300	Au	100/pk
CF-MH-3Au-T	CFT3MH-50-Au	Multil	nole*	300	Au	50/pk
	CFT3MH-100-Au	Multil	nole*	300	Au	100/pk

The Multihole device has a staggered pattern of six features consisting of three circle patterns of 1 micron, 1.4 micron and 2 micron diameter and three ellipse patterns of 1 x 4 microns, 1.4 x 5.6 microns and  $2 \times 8$  microns.

# **C-flat**™

**Holey Carbon Grids for Cryo-TEM** 

### **Cryo Preparation Using C-flat™**



Frozen-Hydrated Bacteriophage Capsid (data acquired on CF-1.2/1.3-4C)

#### **Overview**

C-flat™ is a holey carbon support film, manufactured using a patent pending semiconductor-based technology without plastics, resists or other soft materials. As a result, the carbon films are flat, uniform and free of residues or plastics. C-flat™ is designed to be an "out of the box" solution, and should require minimal sample preparation. Extensive plasma cleaning is not needed, and could potentially thin the carbon, making it too fragile for blotting or freezing.

#### **Plasma Preparation**

If you are using C-flat<sup>™</sup> for the first time, it is recommended that no plasma preparation be used initially. As with any carbon film, plasma preparation is sometimes necessary to make the surface more hydrophilic. If your initial results dictate making the films more hydrophilic, below are some guidelines for preparation using several common systems.

#### **Fischione Model 1020**

- 25% Oxygen/75% Argon
- Use 5 grid holder and dampening shield
- Plasma clean grids for 10-30 seconds

Note: It is recommended that the dampening shield be used when cleaning C-flat<sup>™</sup> using the Fischione Model 1020 plasma cleaner. The shield will dampen the effect of the plasma, reducing the erosion rate of the carbon while allowing the film to become more hydrophilic.

#### Gatan Solarus™

- 25% Oxygen/75% Argon
- Place grids on a support (e.g. glass slide)
- Set slide in the bottom of the chamber
- Set RF power to 25 watts
- Plasma clean grids for 10-20 seconds

#### **Glow Discharge**

These systems vary widely depending on the manufacturer. Typically, keep the glow from the plasma dim and the clean time approximately 10-30 seconds.

#### **Plunge Freezing**

Recommended settings for plunge freezing with the  $Vitrobot^{\mathsf{TM}}$ 

• Temperature: 4°C

• **Humidity:** 100% (can vary between 90–100%)

Blot Time: 3-5 seconds
 Volume on Grid: 3µL (can vary)

• Drain Time: 0 seconds

• Offset: 0 for regular samples, -1 for viscous

When using the Vitrobot™, it is recommended that the filter paper be changed regularly (generally after freezing 4-5 grids or 10 minutes, whichever comes first.) The filter paper can become saturated in the high humidity environment of the chamber.

#### **Working with Viscous Samples**

Generally, lowering the volume of solution on the grid can help to eliminate the need for multiple blots, which can damage the carbon film. As little as  $1\mu L$  of solution can cover a 3mm grid area if the pipette tip is used to spread the drop, but reducing the volume to 1.5 or 2.0 $\mu L$  will help as well. Once the sample is on the grid, it should be blotted within a few minutes before further evaporation occurs. If a Vitrobot  $^{TM}$  is used, changing the offset from 0mm to  $^{-1}$  or  $^{-2}$ mm can also help.

#### Hydrophilicity/Hydrophobicity

Increasing the hydrophilicity of the carbon film will help a droplet spread evenly over the carbon, rather than pool on the surface. The most common method for achieving this is by plasma or glow discharge; recommended settings for various equipment are given. Keep in mind that C-flat is manufactured without any plastics or soft materials in the process, therefore plasma or glow discharge steps are only needed to make the surface more hydrophilic, not to clean. For this reason, a lower power and time is generally used.

#### Adding Carbon to C-flat™

Many C-flat<sup>™</sup> parts are now offered in both the standard as well as a thicker carbon film, designed to give each lab the option to choose not only the most appropriate hole geometry and size, but also the ideal carbon thickness for their application. In addition, carbon can be added to C-flat<sup>™</sup> either to thicken the existing hole pattern, or as a thin continuous overlay across the hole pattern. Overlays are often used when particles have a strong affinity towards the carbon material.

#### **Keeping the Carbon Intact**

C-flat™ is designed to be an "out of the box" solution. Extensive sample preparation steps are generally not required, and often carbon that is torn or broken is a sign of plasma cleaning that is too long and/or at too high a power setting. Please refer to the suggestions on plasma cleaner settings, as well as on working with viscous samples.

#### Publications using C-flat™:

Near-atomic resolution using electron cryomicroscopy and single-particle reconstruction. Proceedings of the National Academy of Sciences, Volume 105, Number 6, pp. 1867-1872, 2008. X. Zhang, E. Settembre, C. Xu, P. R. Dormitzer, R. Bellamy, S. C. Harrison, and N. Grigorieff

Preparation of macromolecular complexes for cryo-electron microscopy. Nature Protocols, Volume 2, pp. 3239 - 3246, 2007. R. A. Grassucci, D. J. Taylor, and J. Frank

Segrosome structure revealed by a complex of ParR with centromere DNA. Nature, Volume 450, pp. 1268-1271, 2007. M. A. Schumacher, T. C. Glover, A. J. Brzoska, S. O. Jensen, T. D. Dunham, R. A. Skurray and N. Firth

Automation of random canonical tilt and orthogonal tilt data collection using feature-based correlation. Journal of Structural Biology, Volume 159, Issue 3, pp. 335-346, September 2007. C. Yoshioka, J. Pulokas, D. Fellmann, C. S. Potter, R. A. Milligan and B. Carragher

Automated cryoEM data acquisition and analysis of 284 742 particles of GroEL. Journal of Structural Biology, Volume 155, Issue 3, pp. 470-481, September 2006. S. M. Stagg, G. C. Lander, J. Pulokas, D.s Fellmann, A. Cheng, J. D. Quispe, S. P. Mallick, R. M. Avila, B. Carragher and C. S. Potter

Contamination buildup limit throughput for automated cryoEM? Journal of Structural Biology, Volume 154, Issue 3, pp. 303-311, June 2006. A. Cheng, D. Fellmann, J. Pulokas, C. S. Potter and B. Carragher



# Plunging Tweezers for the CP3 (Cryoplunge™3)

Custom tweezers specifically made to fit the Gatan Cryoplunge™ an instrument used in the preparation of frozen hydrated specimens for cryoEM.



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