CRYOEM 001 : BUILDING AN EM TOOLKIT

NCCAT Embedded Training — Master Class series

September 9, 2020

New York Structural Biology Center



SIMONS ELECTRON MICROSCOPY CENTER



S C

CRYOEM 001 : SINGLE PARTICLE MASTERCLASS

Introduction to cryoEM: SPA Building a cryoEM toolkit EM compatible samples EM support films and grids Sample preparation Tools of the trade: microscopes and detectors

Microscope operations Data collection strategies Data assessment & QC Data processing: cryoEM IT infrastructure On-the-fly feedback **3D** Reconstruction Visualization and validation

ED'S EM TOOLKIT



Circles 90mm Ø Cat No 1004 090

L'C

Qualitative



atman

Whatman

Type CF 100 Strips

pH 0-14

DH INDICATOR PAPER

A

Mhatman

A DESCRIPTION OF THE PARTY OF T

Cat # 80055 Grating Replica on Latex Spheres Electron Microscopy Sciences WHAT IS INSIDE?

Shanhia

Nº099186

EACH

If EM is a major part of your project or you become a regular EM user, you should have your own EM supplies and build an EM toolbox.

TE MICA, V-1 QUALITY

Lot 14021

NEGATIVE STAIN

Suggested core equipment:

1 pair of DUMONT anti-capillary reverse (self-closing) tweezers

pair of DUMONT anti-capillary tweezers
EM grids
grid boxes

Whatman 1 or 4 filter paper aluminum foil microfuge tubes 0.22 um syringe filters 1mL-5mL syringes Petri dish Glass slides Parafilm Pipetman / pipette tips

CRYO

Suggested core equipment:

Plunge freezer tweezers

1 pair of DUMONT anti-capillary reverse (self-closing) tweezers

2 pairs of DUMONT anti-capillary tweezers EM grids / Quantifoil grids

Cryo grid boxes

Cryo box screwdriver/opener

Short and long Dissecting forceps to hold grid boxes under LN2 Whatman 1 or 4 filter paper aluminum foil microfuge tubes 0.22 um syringe filters 1mL-5mL syringes Petri dish **Glass slides** Parafilm Pipetman / pipette tips **Conical tubes** Dewars Magnifying glass Flashlight

WHAT IS IN AN EM TOOLKIT?

grids grid boxes forceps pucks cryogenic dewars and shippers negative stain accessories vitrification accessories grid clipping accessories grid inspection accessories





Common Materials Copper Nickel Gold Aluminum Molybdenum Titanium **Stainless Steel**

https://www.tedpella.com/grids_html/

GRIDS: STATS

Rough grid parameters

Rim Width: 350-400µm.

Thickness: PELCO® Grids are approximately 25µm thick.

Diameter: 3.0 to 3.05mm

Finish: Copper, Nickel and Gold grids have a matter finish on one side and a shiny finish on the other side.

Pitch: Is 1"/mesh or 25.4mm/mesh

Example 200 mesh pitch = $25.4/200 = 127 \mu m$



500

51

28

23

30

GRID BOXES











FORCEPS







Alloys and Materials

DS DUMOSTAR™

An alloy blend of steel, chromium, nickel, and cobalt, elastic in nature for "flexible fatigue resistant tips", resists temperatures to 550°C, and is nonmagnetic and anti-corrosive. The best overall material for tweezers. Rockwell 63-64.

SS Stainless Steel

High tensile strength chromium/carbon steel alloy (various compositions) provides for good corrosion and stain resistance, second in hardness only to DUMOSTAR[™] Note: stainless steel can rust under some circumstances.

NM-SS Non-magnetic Stainless Steel

Same properties of stainless steel with additional nickel to create the nonmagnetic characteristic.

NM Non-magnetic

A standard alloy composed of C, Cr, Ni and Mo. It offers a good resistance to corrosion thanks to its high concentration of Mo and is 80% non-magnetic, but it is not as hard as NM-stainless steel. NM is resistant up to temperatures of around 400°C (DIN 50 914) and is suitable for autoclave sterilization at 270°C. **Ti Titanium**

Flexible tips, non-magnetic, light weight (33% less than stainless steel alloys), durable, low particle shedding compared to stainless steel, extremely acid and stain resistant, hypoallergenic. Good for use around sensitive electronics or high magnetic fields. Will resist temperatures in excess of 400°C. Rockwell 36.

CS Carbon Steel

Very durable, hard material that is less flexible and more brittle than DS, SS and Ti. Highly magnetic, will stain and rust easily. Rockwell 60.

DUMOXEL SS Stainless Steel Alloy

Highly corrosive resistant and provides soft, flexible tips. More stain resistant than stainless steel, Rockwell 63 hardness. 95% anti-magnetic, resistant to temperatures above 400°C. Can be autoclaved.

INOX SS Stainless Steel Alloy

Composed of a special mix of Carbon Steel and Chromium. Provides soft, flexible tips, Rockwell 55.

FORCEPS

Repair kit to care for you main tool



To straighten tweezers tips that are bent outwards or sideways, press the bent tip against the steel block or in the V-groove until straight. Fine adjustments can be made by bending between the dual pins.



Tweezer Repair Kit

Salvage damaged tweezers with this kit. Use for straightening, sharpening and deburring dull or damaged tweezers and other small precision tools. To straighten tweezers tips that are bent inwards or hooked, press or pull them against the pin or bend against one pin while using the second pin as leverage to straighten the tip.





https://www.tedpella.com/twzr-mis_html/twzr-acc.htm.aspx#54528

PUCKS – HIGH DENSITY STORAGE



CRYOGENIC DEWARS AND SHIPPERS









ADDITIONAL ACCESSORIES

negative stain accessories vitrification accessories grid clipping accessories grid inspection accessories



ADDITIONAL ACCESSORIES

negative stain accessories vitrification accessories grid clipping accessories grid inspection accessories



EM VENDORS

There are several vendors where you may obtain the necessary equipment. A few of them that we use often are:

Electron Microscopy Sciences - http://www.emsdiasum.com/microscopy/

TedPella - <u>http://www.tedpella.com/</u>

SPI - <u>http://www.microscopy.cc</u>

SubAngstrom - https://subangstrom.com/Subangstrom

Protochips - <u>https://www.protochips.com</u>

Quantifoil - <u>https://www.quantifoil.com</u>

Nanosoft - <u>https://www.nanosoftmaterials.com</u>

WHAT NEXT?

1 Barles at 3+ 23 () + 1

cryoEM 001 : Single Particle Masterclass

- 1. Building a cryoEM toolkit
- 2. EM compatible samples
- 3. EM support films and grids
- 4. Sample preparation
- 5. Tools of the trade: microscopes and detectors
- 6. Microscope operations
- 7. Data collection strategies
- 8. Data assessment & QC
- 9. Data processing:
 - cryoEM IT infrastructure
 - On-the-fly feedback
 - 3D Reconstruction
- 10. Visualization and validation