

CryoEM 的原理及應用

Cryo-EM Principle and Application
For
JEM-2100Plus Transmission Electron Microscope

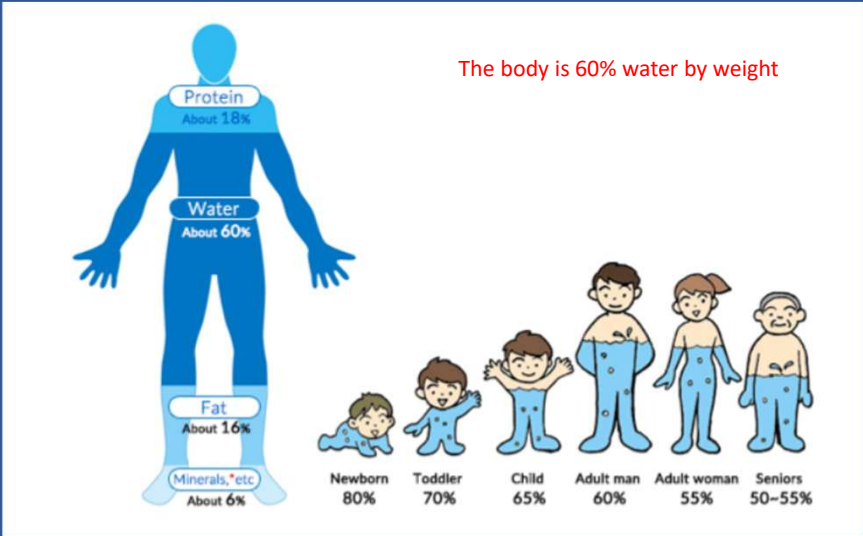
Speaker: Yieng-Chieh Hung
洪英傑

JieDong Co., Ltd
捷東公司

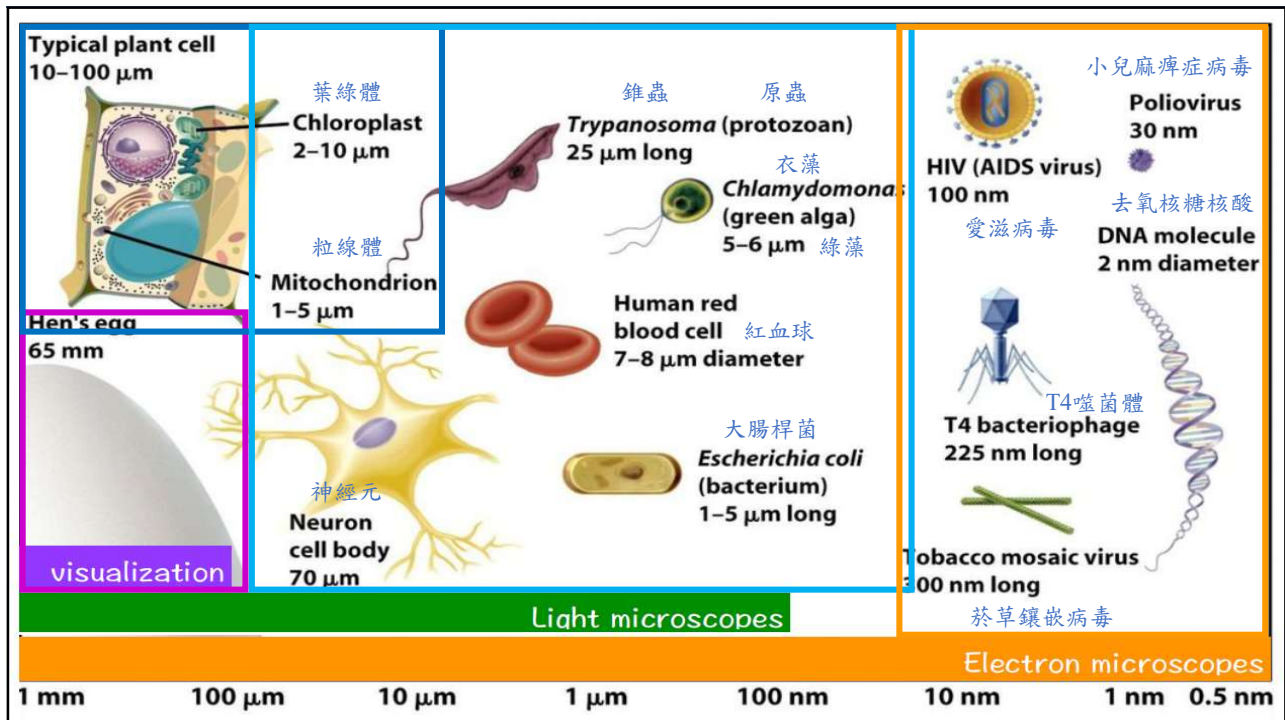


1

Water % in our body



2



3

Sample Transfer in Cryo EM



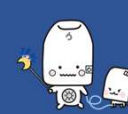
Virus sample (liquid)

How to transfer?





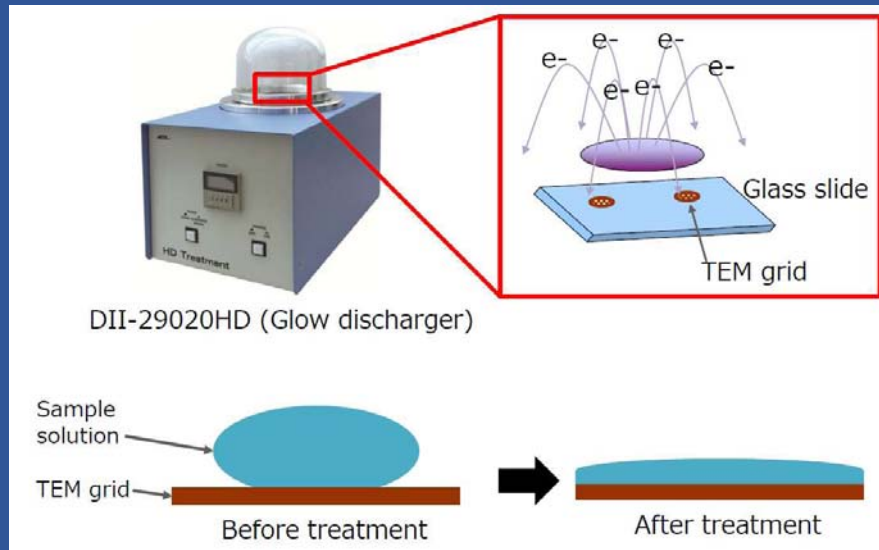
TEM (Vacuum)




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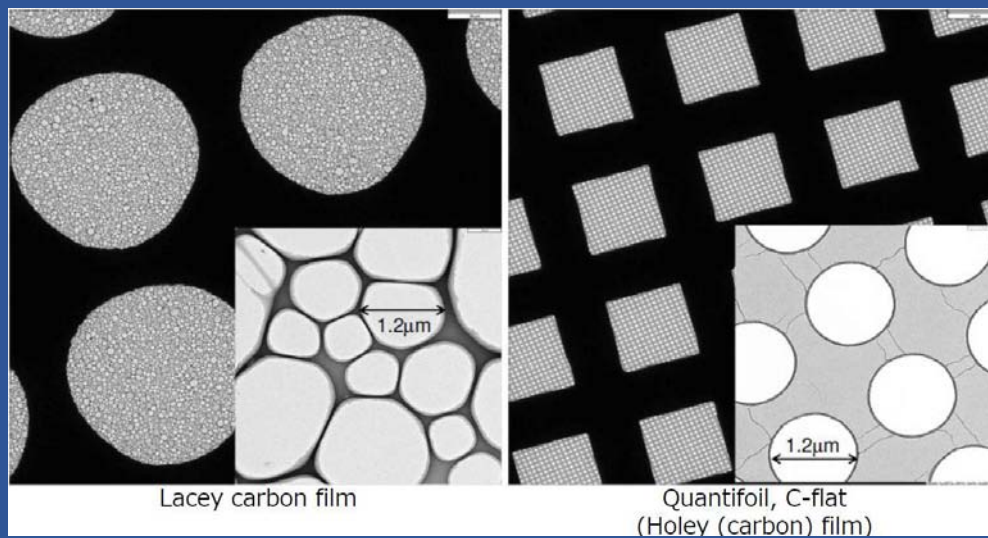
Hydrophilization for TEM grid and support film 親水性處理



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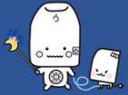
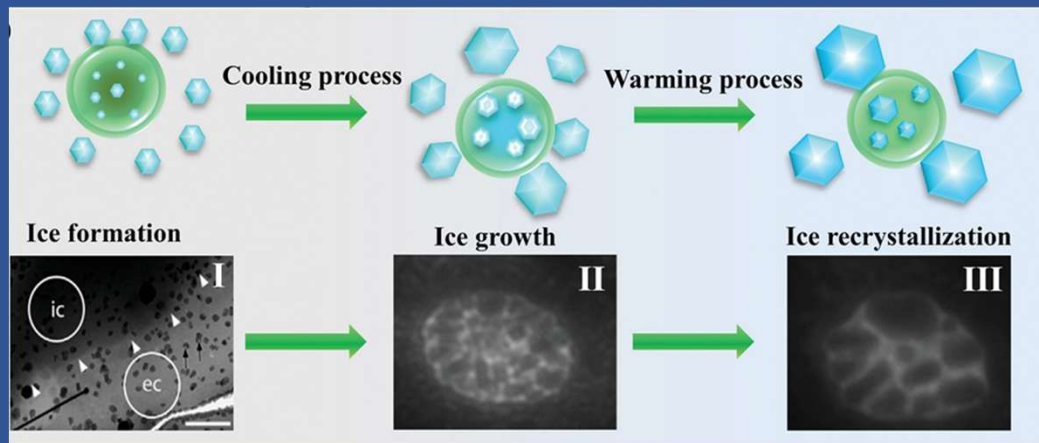
Carbon support film for cryoTEM



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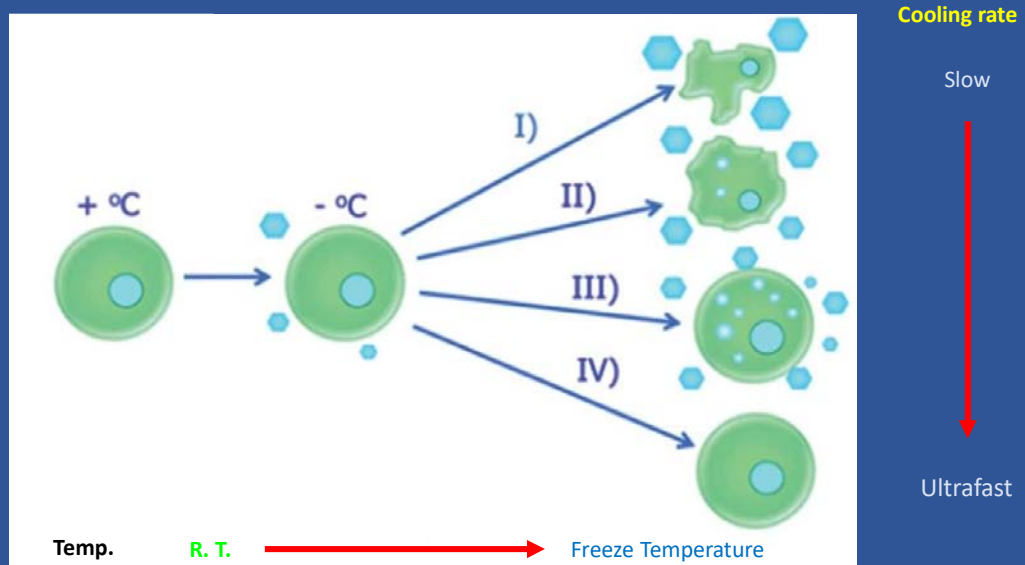
Ice Crystal



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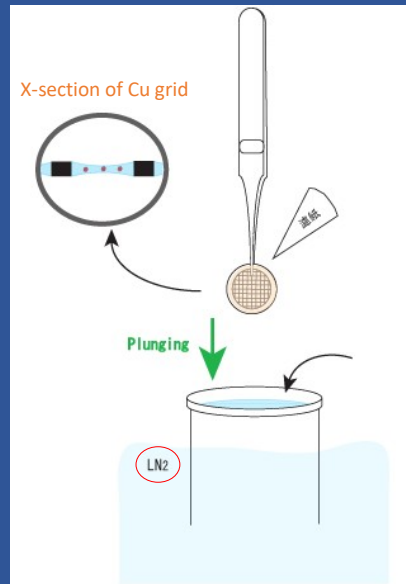
Ice crystal formation during different cooling rate



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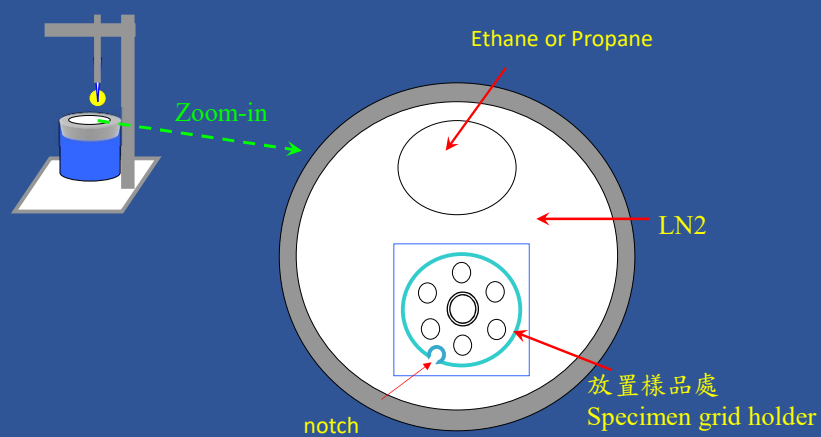
Cryo Plunge(Ice Embedding)



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Ice Embedding (Cryoplunge)

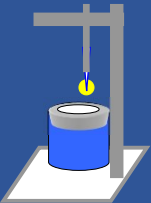


- 1: 將所需要工具 (如丙烷, grid holder, 夾子等) 先浸泡在液態氮預冷.
- 2: 待丙烷凝固後, 將樣品迅速置入丙烷中浸泡 (丙烷可預防冰晶).
- 3: 將樣品移至 grid holder 中.

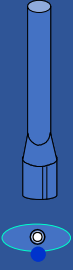
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Cryo sample transfer



Cryo Plunge sample



Lock and quickly transfer to tube with LN2



Storage in LN2 Tank

Note: to Prevent Frost, keep sample in LN2 full time




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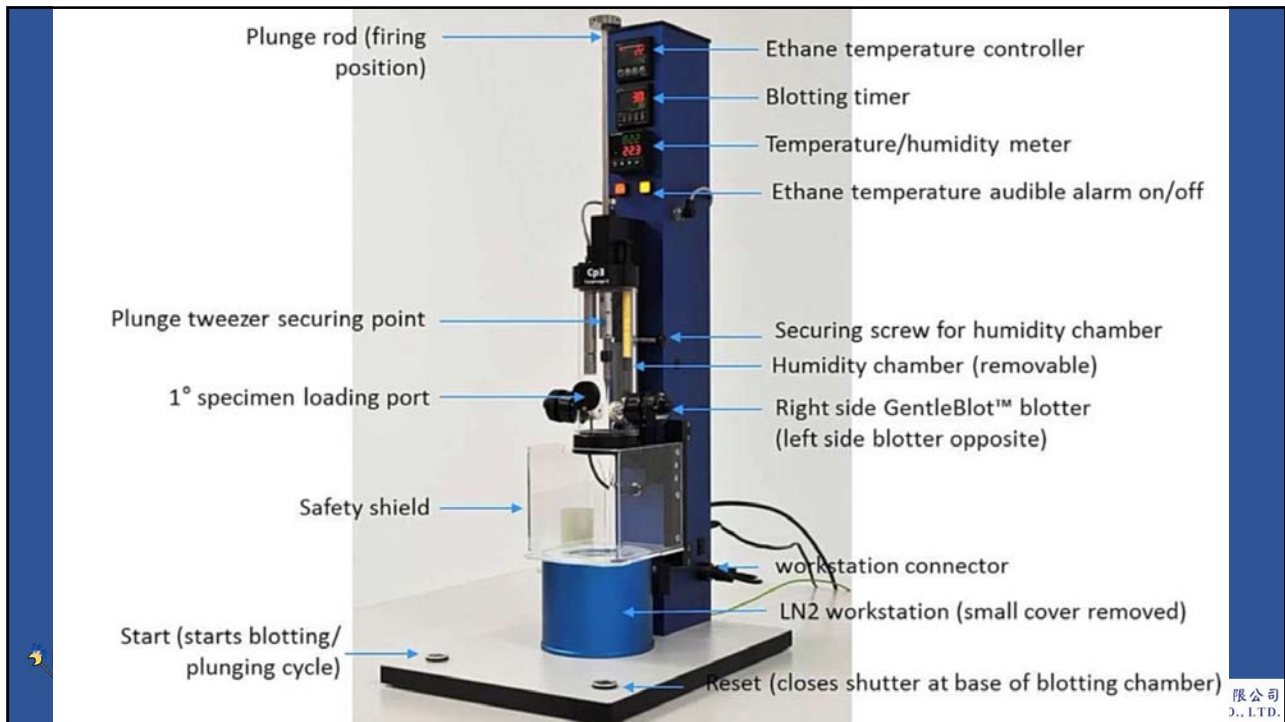
Cryoplunge 冷凍衝頭裝置



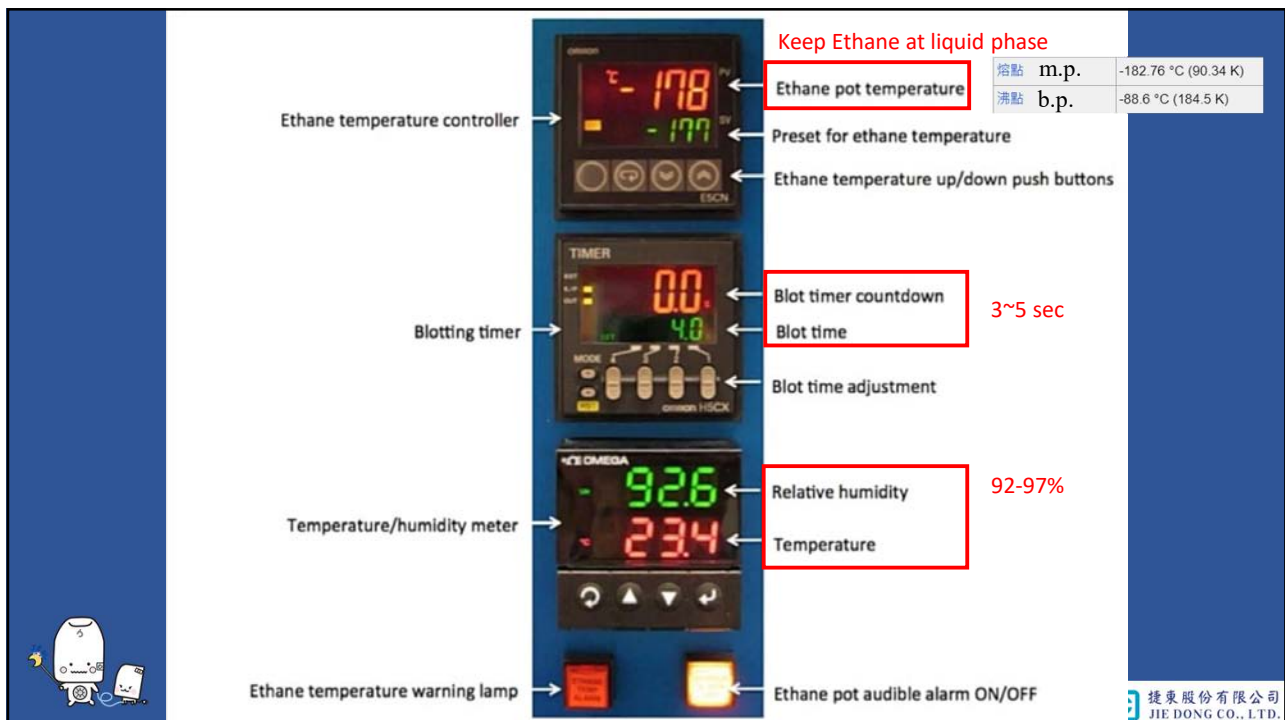



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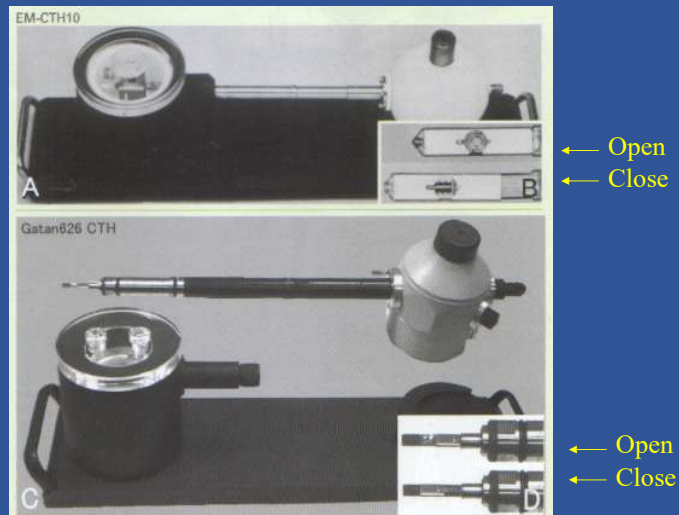


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Cryo Transfer holder

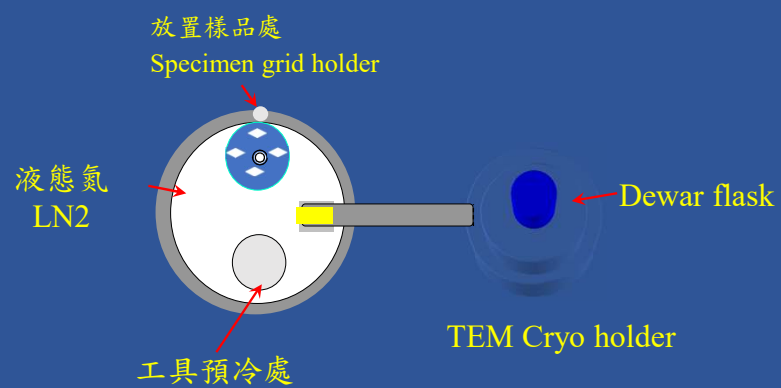


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Cryo Holder into workstation

1. Cooling Workstation → cooling holder
2. Connect Temp. controller → make sure down to -170°C
3. Tools Pre-cooling → Transfer sample to holder



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914 High tilt Cryo Transfer holder (x2)



Figure 2. Model 914 cryo-transfer holder in the workstation.



Figure 3. Inserting the specimen grid into the Quickload specimen securing mechanism.



Specifications

Drift rate at 0° tilt (nm/min)	1.5
Resolution at 0° tilt (nm)	0.34
Observable area at 0° tilt (mm ²)	3.24
Diameter (mm)	2.03
Observable area at 80° tilt (mm ²)	0.23
Standard specimen cup/holder tip material	Beryllium copper
Capacity	
Number of grids	1
Diameter (mm)	3
Max. grid thickness (μm)	25
Cryogen	Liquid nitrogen
Min. operating temperature (°C)	Less than -170
Time to reach min. operating temperature (min)	~30
Dewar capacity (mL)	175
Hold time at min. operating temperature (h)	3.5 – 4



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910 Multi-Specimen Single Tilt Cryo-Transfer Holder



Figure 1. Workstation with model 910 holder inserted.

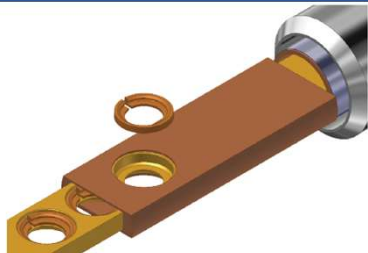
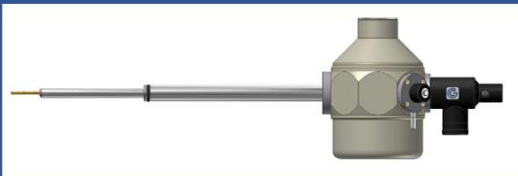


Figure 2. Tip of model 910 holder showing Splitring specimen securing mechanism.



Specifications

Drift rate at 0° tilt (nm/min)	1.5
Resolution at 0° tilt (nm)	0.34
Observable area at 0° tilt (mm ²)	4.10
Diameter (mm)	2.3
Observable area at 60° α-tilt ¹ (mm ²)	0.69
Specimen carrier material	Beryllium copper
Capacity	
Number of grids	2 or 3
Diameter (mm)	3
Max. grid thickness (μm)	100
Cryogen	Liquid nitrogen
Min. operating temperature (°C)	Less than -170
Time to reach min. operating temperature (min)	~30
Dewar capacity (mL)	175
Hold time at min. operating temperature (h)	3.5 – 4

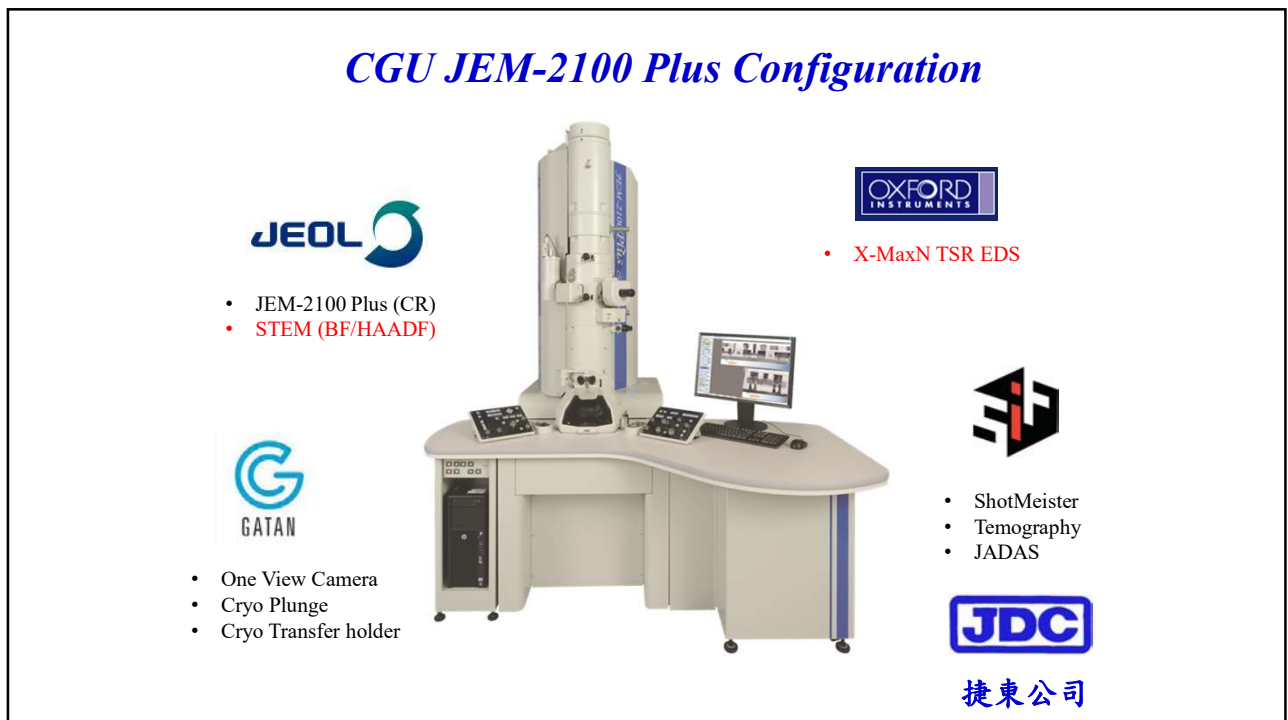


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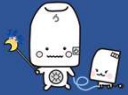
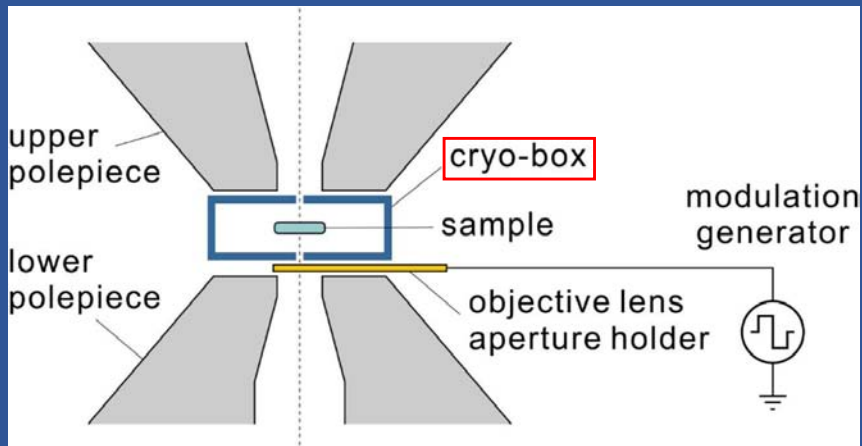


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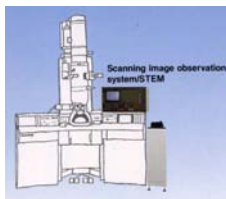
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Cryo Pole Piece



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Attachment for TEM



STEM system



JEOL 2100 Plus
(0.27 nm for CR)



EDS



Digital image system

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Advanced Photo Control software



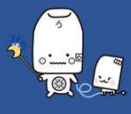
- Anti Drift System(ADS)
抗飄移
- Ultimate Dose Control(UDC)
低劑量
- Limitless Panorama (LLP)
無限拼接
- Sequential Acquisition (SQA)
序列對焦



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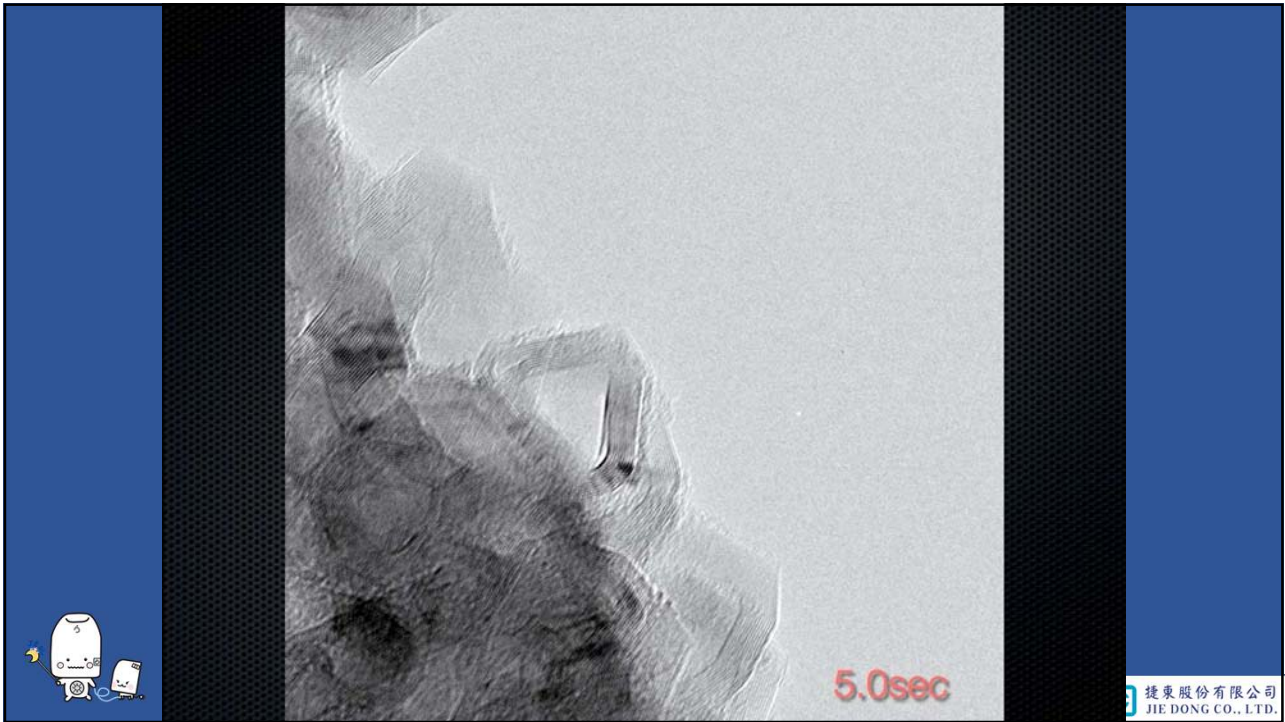
Anti Drift System(ADS) 抗飄移

Sample #1: Graphite
Mag: **250K** JEM 2100F

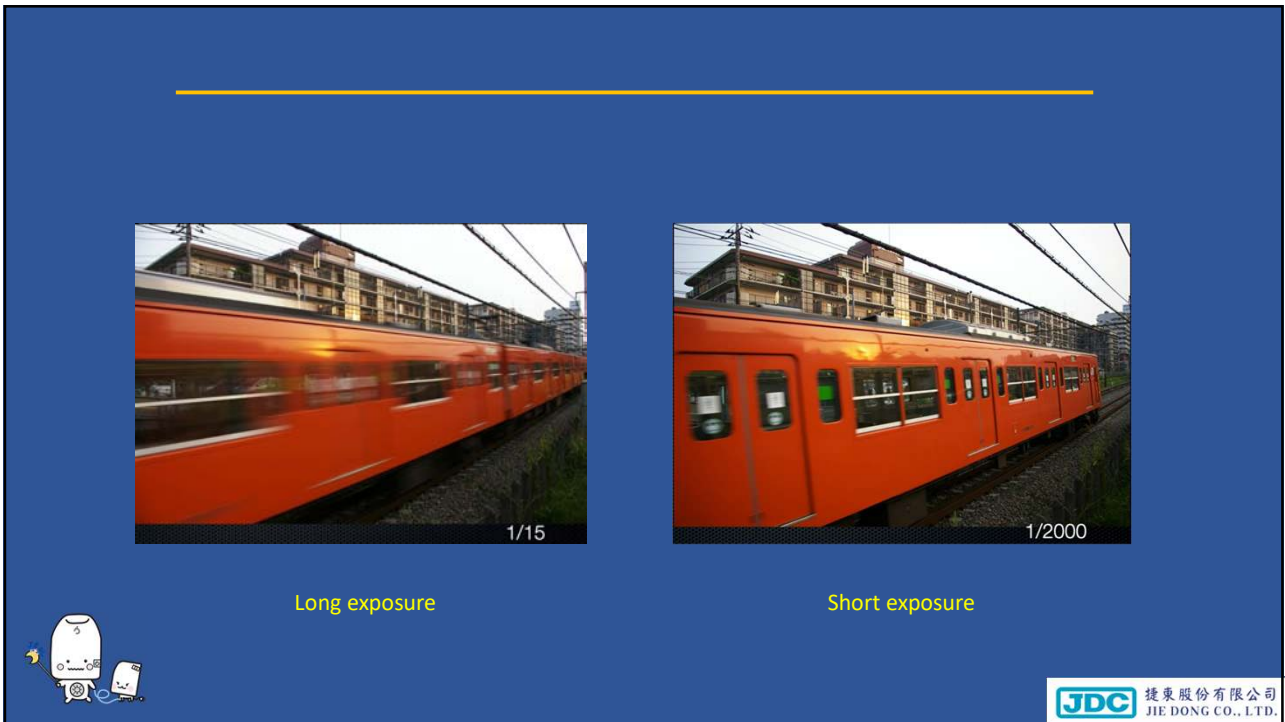


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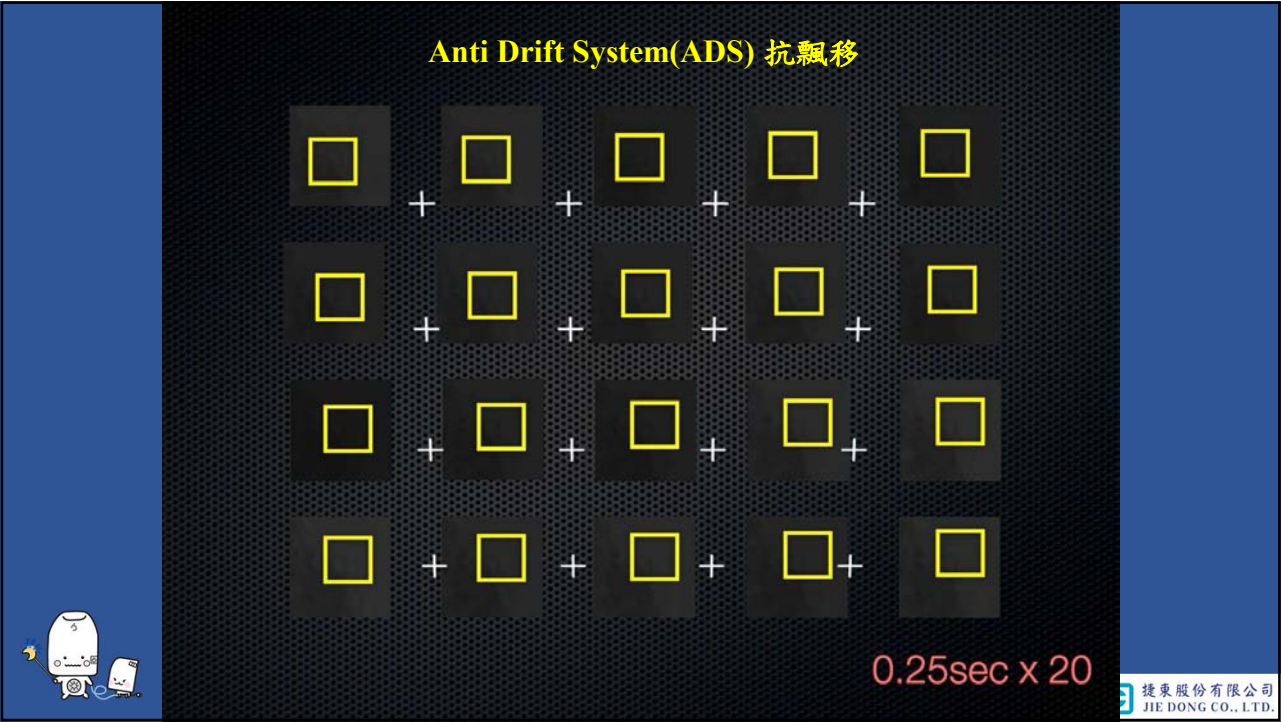
28



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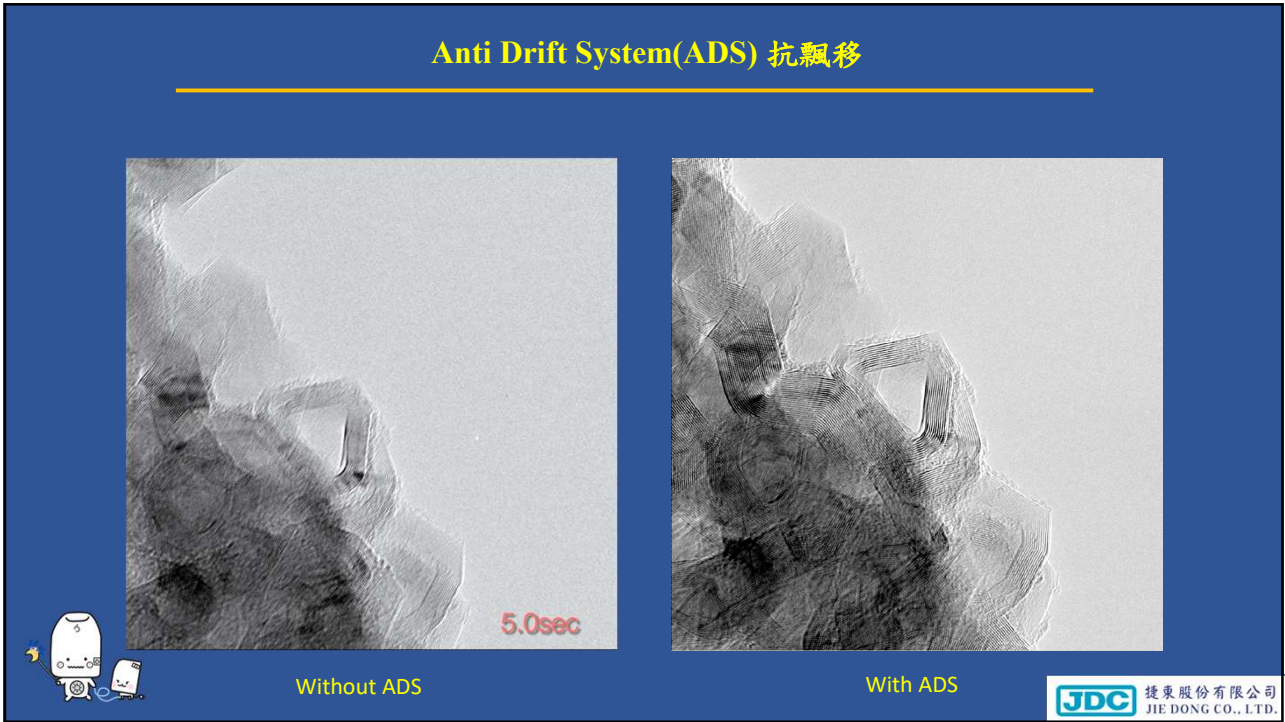
31



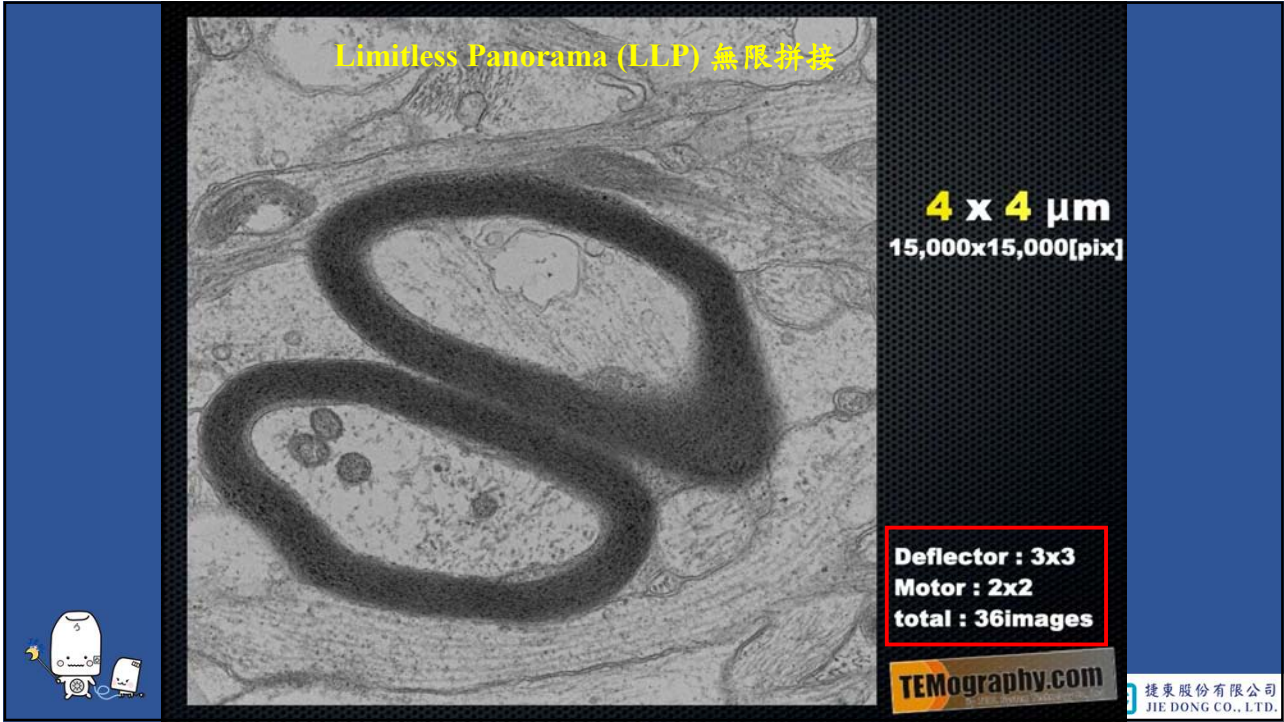
32



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34

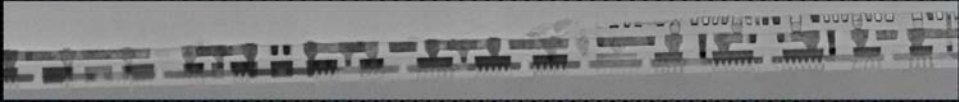


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Limitless Panorama (LLP) 無限拼接



9 x 0.9 μm
25000x2600 [pix]

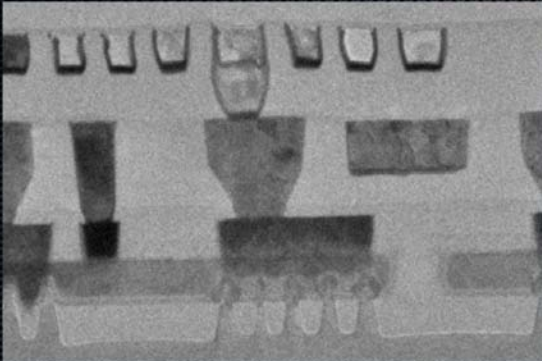
Deflector : 5x1
Motor : 2x1
total : 10 images

TEMography.com

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Limitless Panorama (LLP) 無限拼接

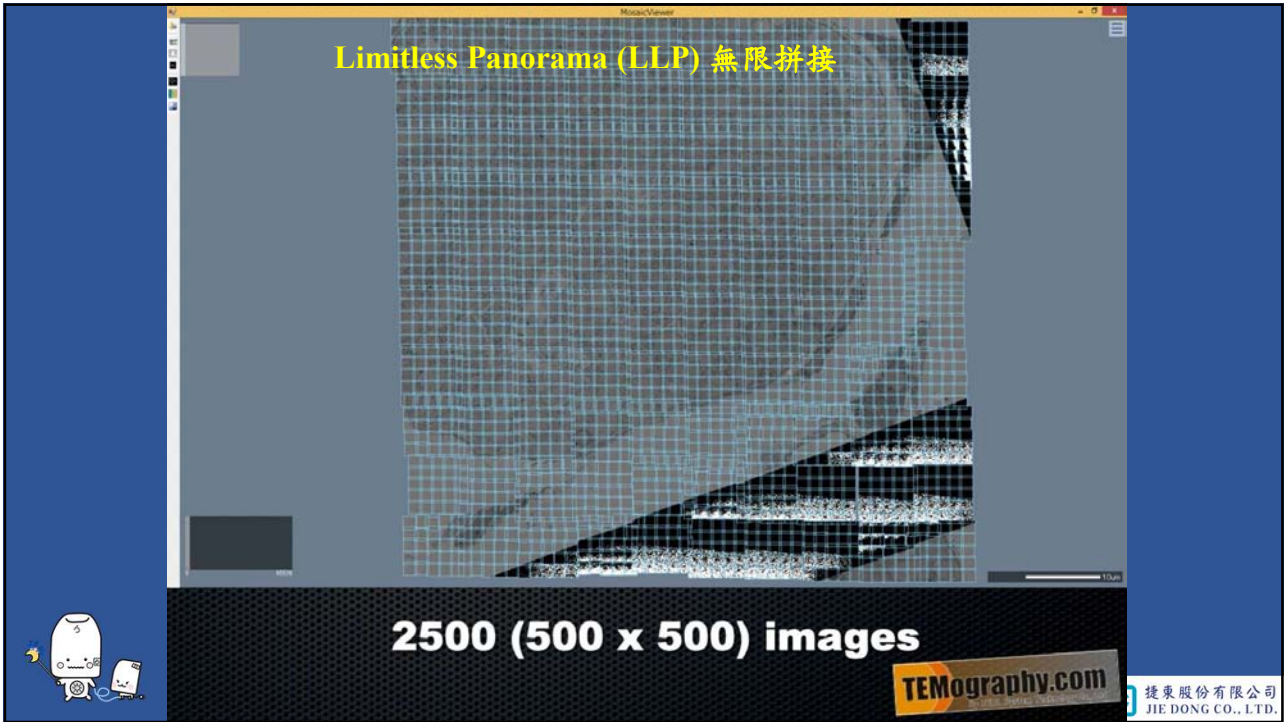


TEM :
JEM-2100LaB6(HRP)
Camera :
Gatan Orius SC1000A
Acc Volt :
200kV
original Mag :
x12k
Specimen courtesy
JEOL LTD.

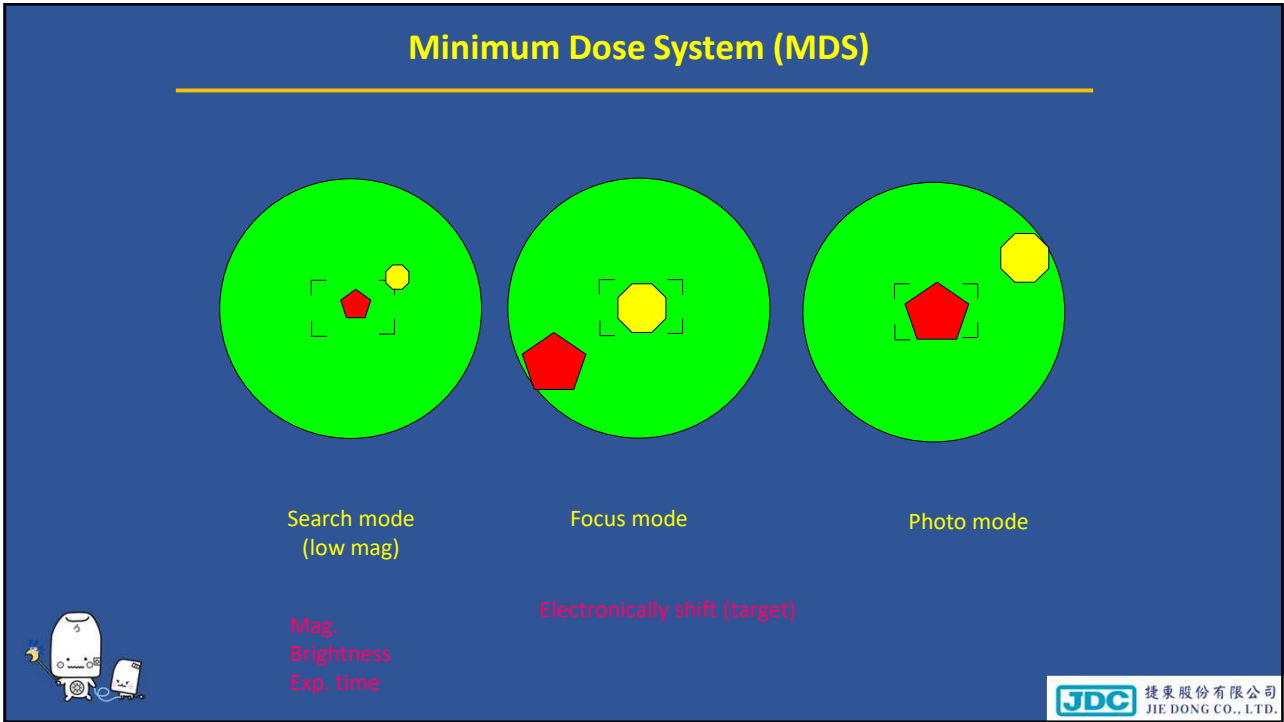
TEMography.com

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Minimum Dose System (MDS)

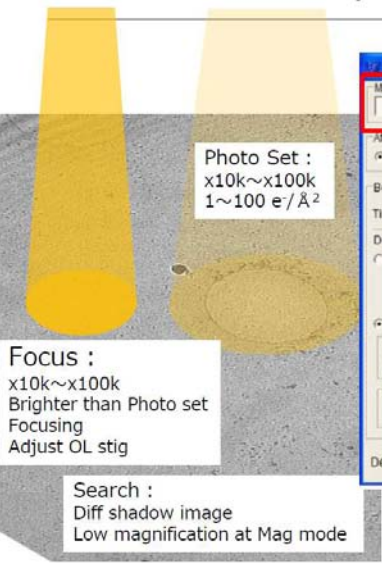


Photo Set :
x10k~x100k
1~100 e-/Å²

Focus :
x10k~x100k
Brighter than Photo set
Focusing
Adjust OL stig

Search :
Diff shadow image
Low magnification at Mag mode

MDS

Mode: OFF Search Focus Photo Set

After Photo Mode: Search Focus Photo

Beam Blanking: Time: 0 sec

Deflector: Hard Ware

Soft Ware: Coil Gun Align1 Beam Shift SPOT Align Gun Align2 Beam Tilt

Amp: X: 0000 Y: 0000 Set

Delay Time: 0 sec (0-5.0)



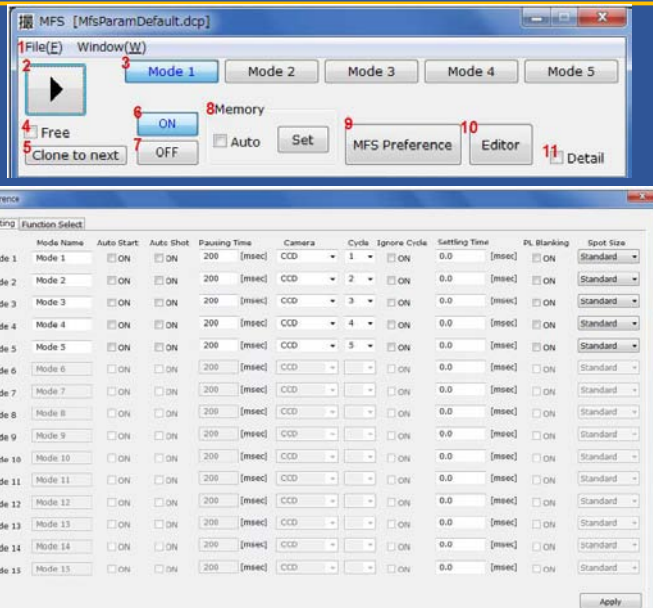
MDS can assign for multi function keys of control panel.



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MFS (Multi Function System)-UDC



The screenshot shows the MFS software interface with the following components:

- Top Bar:** File(E) Window(W) Mode 1 Mode 2 Mode 3 Mode 4 Mode 5
- Left Panel:** 1 Play button, 2 Stop button, 3 Memory, 4 Free, 5 Clone to next, 6 ON, 7 OFF, 8 Auto, 9 Set, 10 MFS Preference, 11 Editor, 12 Detail.
- Main Window:** MFS Preference dialog box with a table of mode settings.

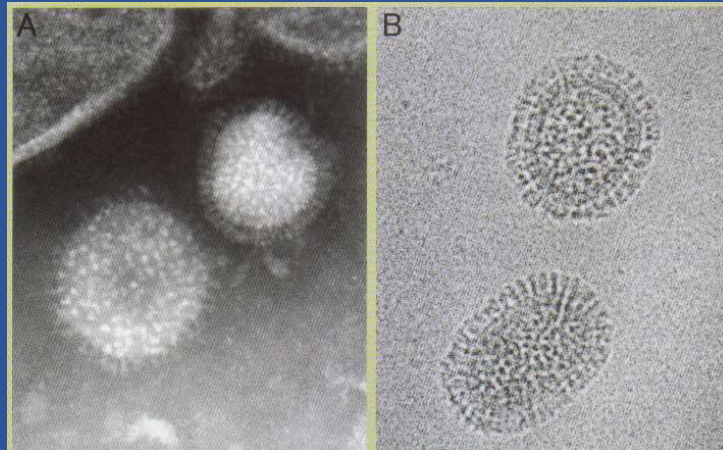
Mode Name	Auto Start	Auto Shot	Pausing Time	Camera	Cycle	Ignore Cycle	Settling Time	PL Blanking	Spot Size
Mode 1	ON	ON	200 [msec]	CCD	1	ON	0.0 [msec]	ON	Standard
Mode 2	ON	ON	200 [msec]	CCD	2	ON	0.0 [msec]	ON	Standard
Mode 3	ON	ON	200 [msec]	CCD	3	ON	0.0 [msec]	ON	Standard
Mode 4	ON	ON	200 [msec]	CCD	4	ON	0.0 [msec]	ON	Standard
Mode 5	ON	ON	200 [msec]	CCD	5	ON	0.0 [msec]	ON	Standard
Mode 6	ON	ON	200 [msec]	CCD		ON	0.0 [msec]	ON	Standard
Mode 7	ON	ON	200 [msec]	CCD		ON	0.0 [msec]	ON	Standard
Mode 8	ON	ON	200 [msec]	CCD		ON	0.0 [msec]	ON	Standard
Mode 9	ON	ON	200 [msec]	CCD		ON	0.0 [msec]	ON	Standard
Mode 10	ON	ON	200 [msec]	CCD		ON	0.0 [msec]	ON	Standard
Mode 11	ON	ON	200 [msec]	CCD		ON	0.0 [msec]	ON	Standard
Mode 12	ON	ON	200 [msec]	CCD		ON	0.0 [msec]	ON	Standard
Mode 13	ON	ON	200 [msec]	CCD		ON	0.0 [msec]	ON	Standard
Mode 14	ON	ON	200 [msec]	CCD		ON	0.0 [msec]	ON	Standard
Mode 15	ON	ON	200 [msec]	CCD		ON	0.0 [msec]	ON	Standard



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Influenza virus (流感病毒)



Negative staining

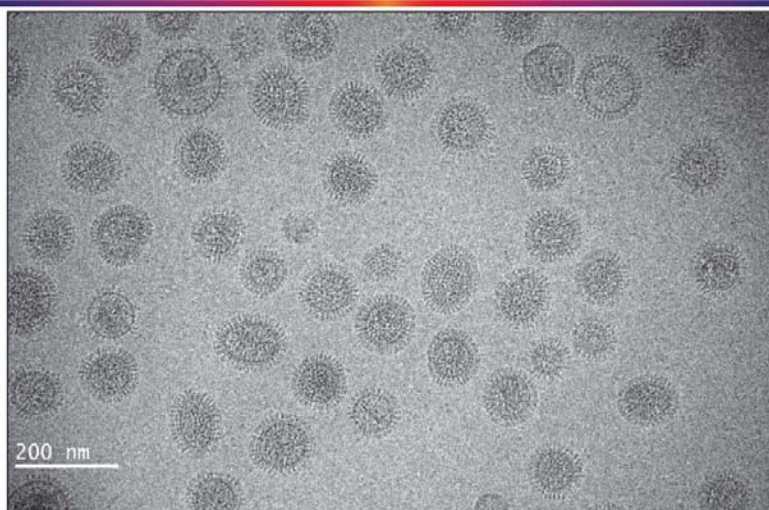
Ice embedding

(by Uranyl acetate, $\text{UO}_2(\text{C}_2\text{H}_3\text{O}_2)_2$)



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Cryo-EM of Influenza Vaccine strain

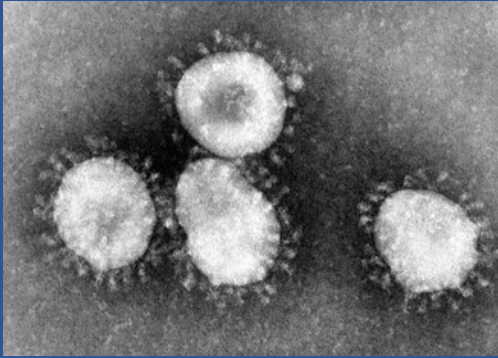


20070927 NIBSC JEM-2100/895

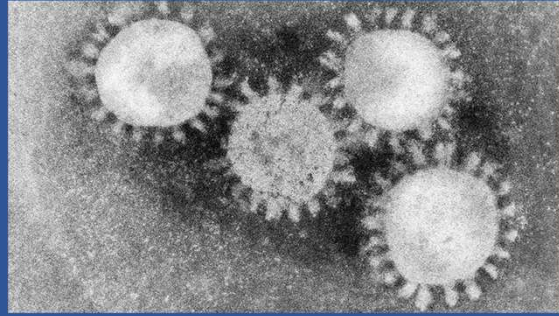


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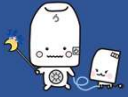
TEM image of COVID-19



COVID-19
March 2020



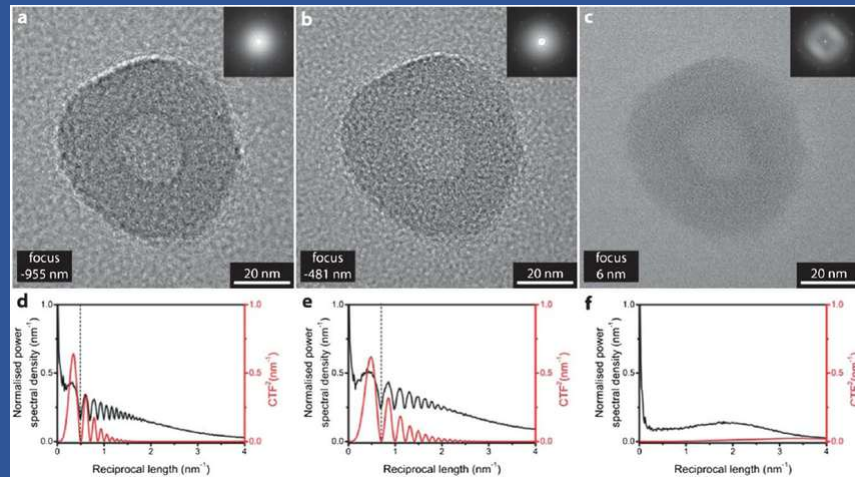
COVID-19
Feb 2021
(U. K. Variant)



Ebola Virus



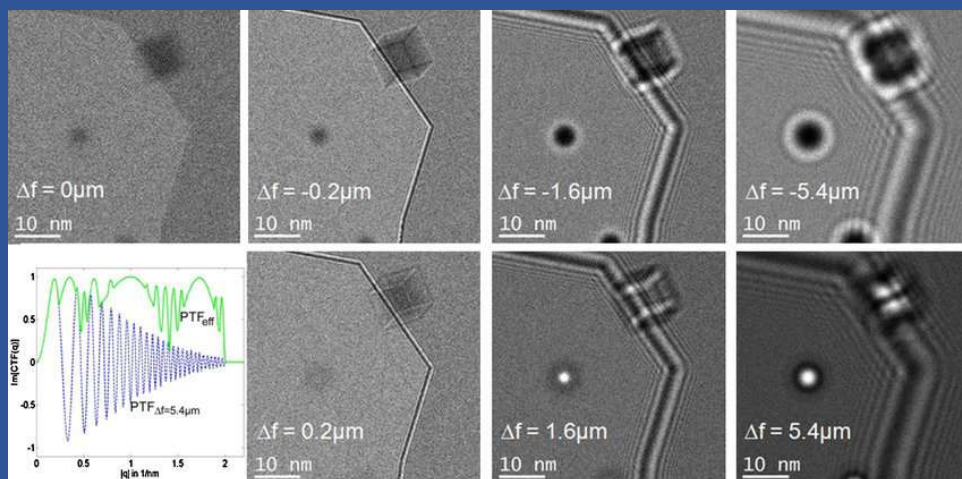
Sequential Acquisition (SQA) 序列對焦



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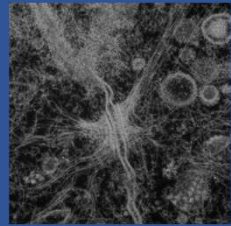
Sequential Acquisition (SQA) 序列對焦



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3D Tomography



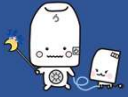
TEM

+

CT

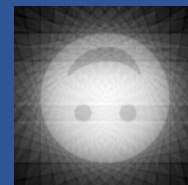
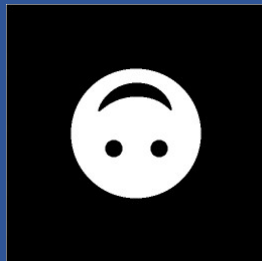
Transmission Electron Microtomography

CT stands for "Computerized Tomography".



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Principle of CT Method



Specimen Tilting

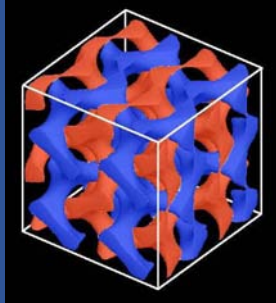
Projection

Back-Projection



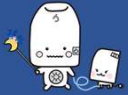
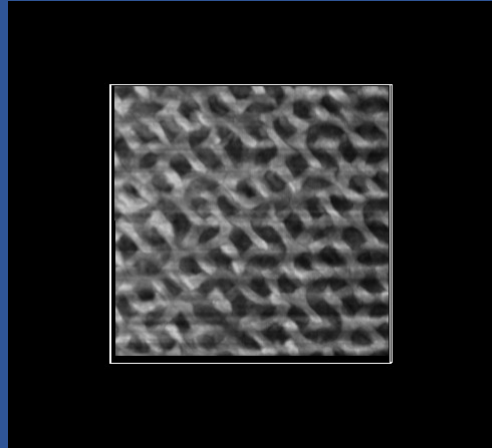
50

Gyroid



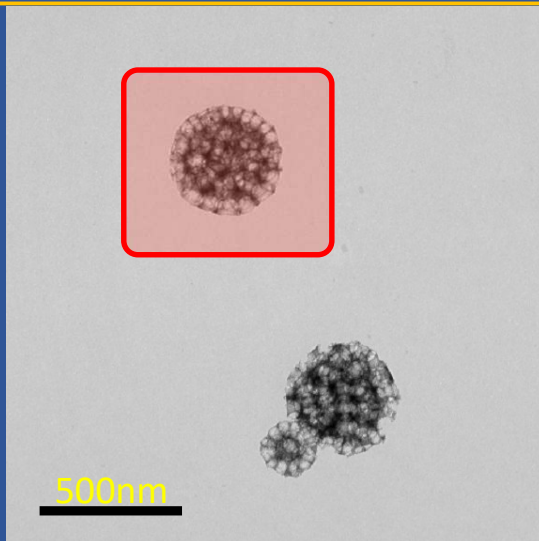
Tilting angle :
+60 - -60 deg.
(2.5 deg.step)

Magnification :
× 20k



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Porus Particle (Sirica)



equipment

JEM-2200FS
with GATAN MSC

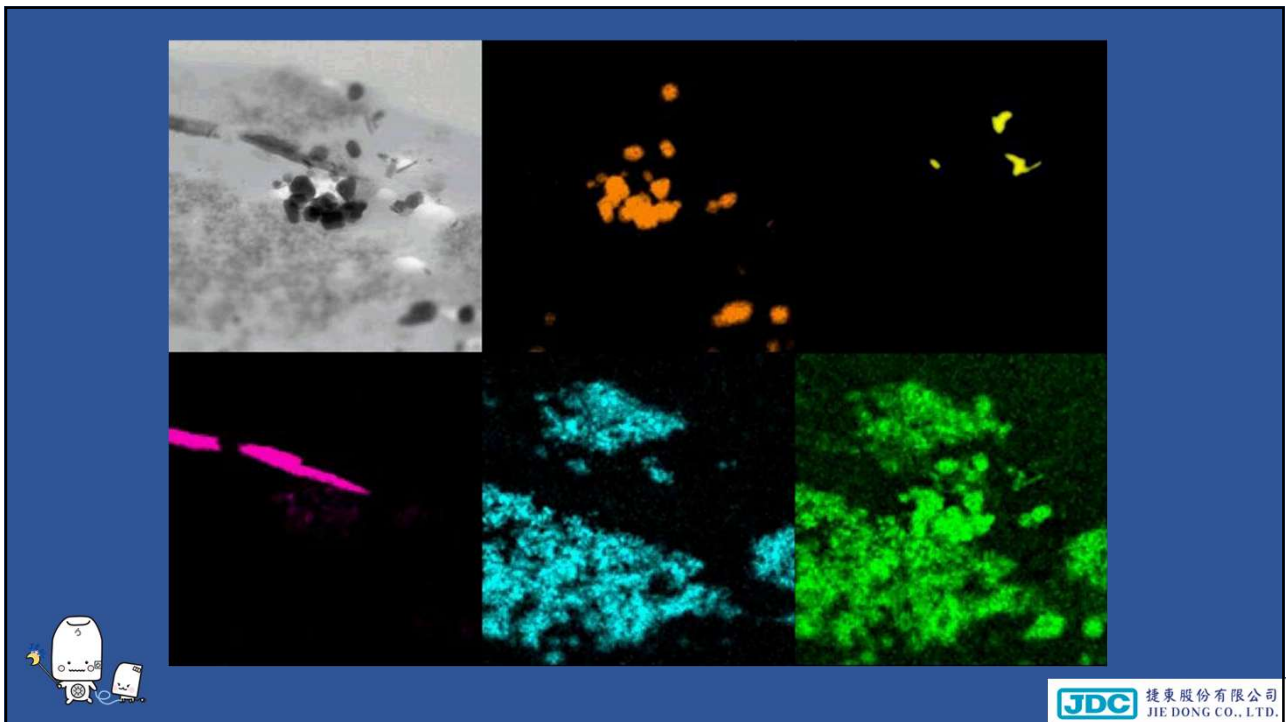
condition

-60deg. >> +60deg.
1deg. step
Zero loss image



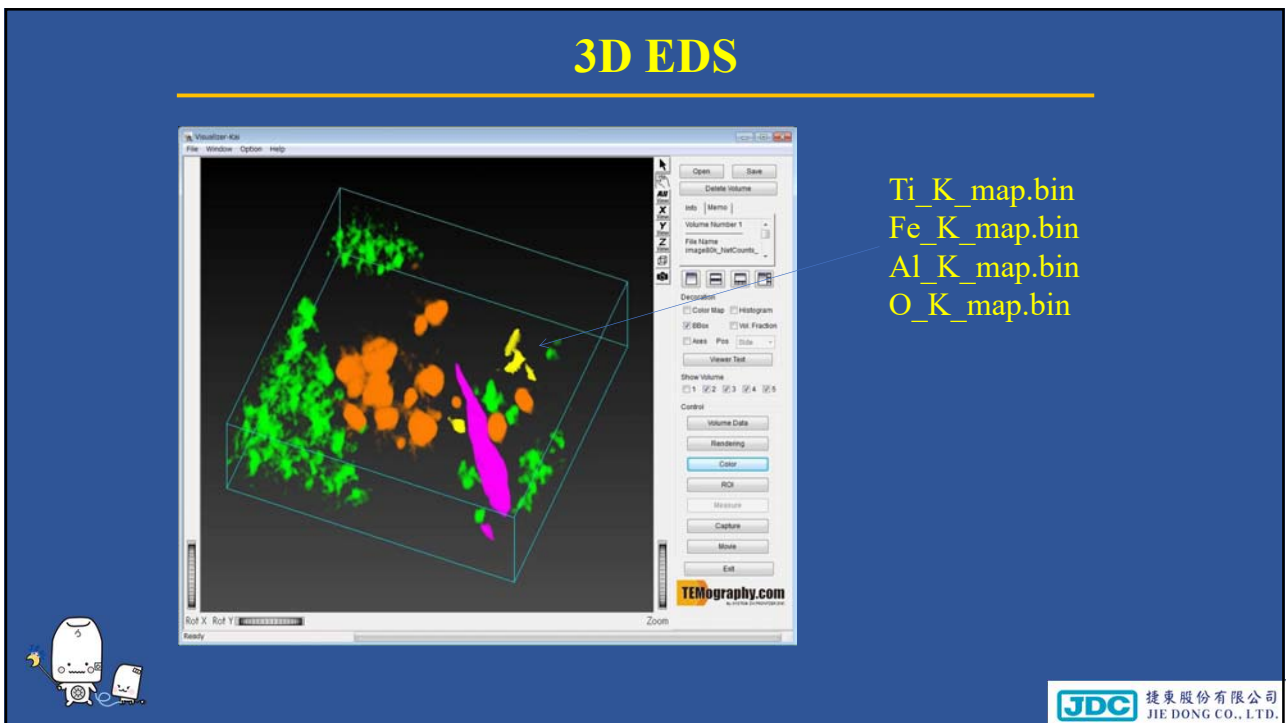
Data courtesy of the NEDO

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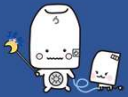
3D EDS



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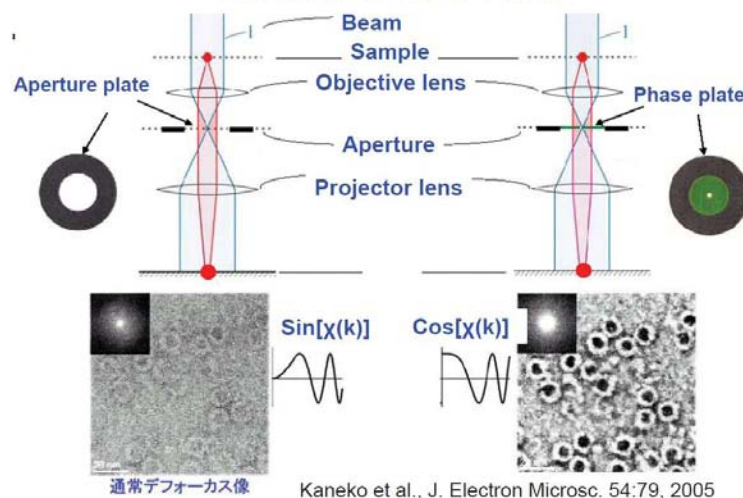
Other Advanced Technology for Bio

- Phase plate (improve contrast)
- EELS (energy filter & Improve Contrast)
- Direct Detection camera (high sensitivity camera)
- Cryo Auto TEM (Cryo sample auto-exchange)
- and more



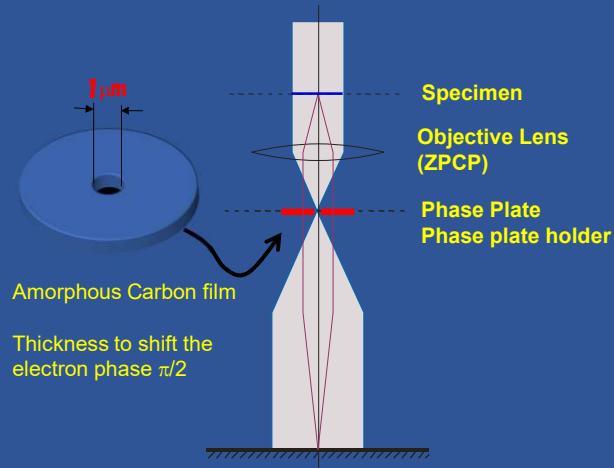
55

Zernike Phase Plate



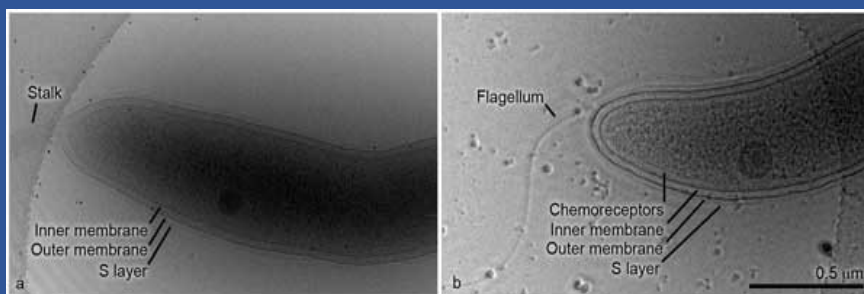
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Composition of the PTEM (phase contrast TEM)



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Caulobacter (柄桿菌)



Caulobacter Cb13b1a cells collected on a JEOL 2200FS 200kV energy-filtered FEG-TEM equipped with the ZPC system. Cells imaged under low-dose conditions and a defocus of 6 micrometers, without (a) and with ZPC (b). Data was collected in Dr Prof. Kuniaki Nagayama's lab at the Okazaki Institute for Integrative Bioscience in Japan.



JEOL 2200FS with phase plate

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Contrast improvement by phase-plate imaging

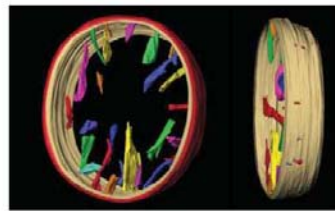
230-nm-thick frozen-hydrated liver section



PP, in focus



No PP, 20 μm underfocus



Tomographic reconstruction

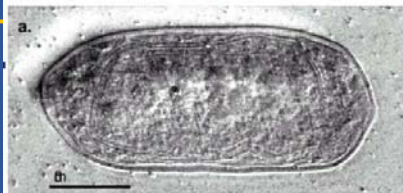
Marko et al. (2006) Proc. IMC-16, Sapporo 1:100



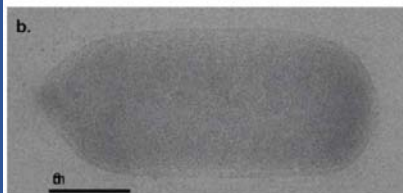
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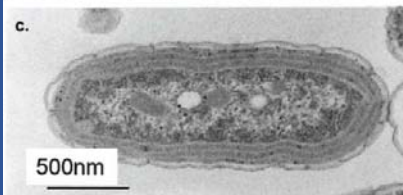
Cyanobacteria in CTEM and PTEM image



A . Hilbert phase contrast image
at 300kV
NO fix, No stain, Ice embedded



B . CTEM image at 300kV
NO fix, No stain, Ice embedded



C . CTEM image at 100kV
(Fixed by Osmium, Epoxy
embedded, thickness ~
100nm)

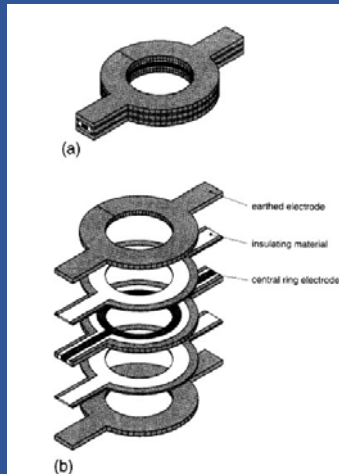
Kaneko et al., J. Electron Microsc. 54



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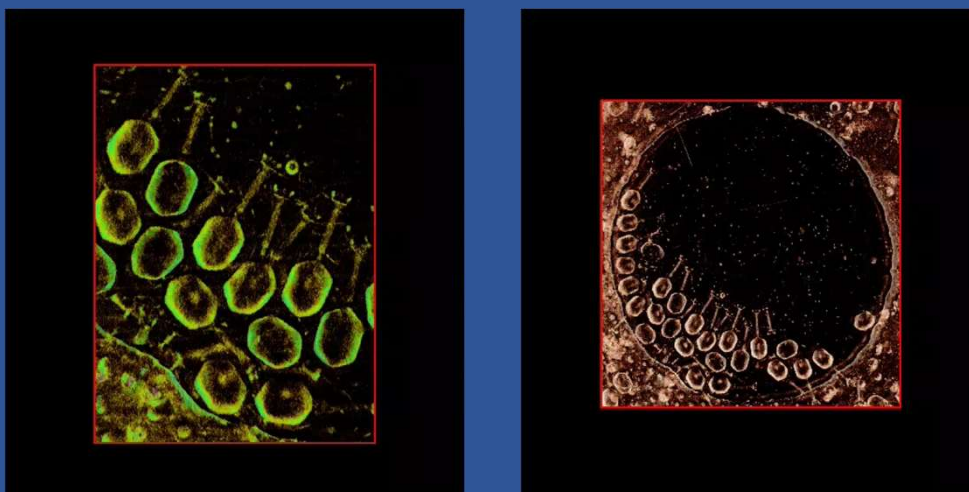
靜電式相位板



Matsumoto 提出之靜電式相位板結構圖

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T4 virus(JEM-2200FS with phase plate)



Courtesy of Radostin Danev (Okazaki Bioscience center)

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OneView Camera

No compromises



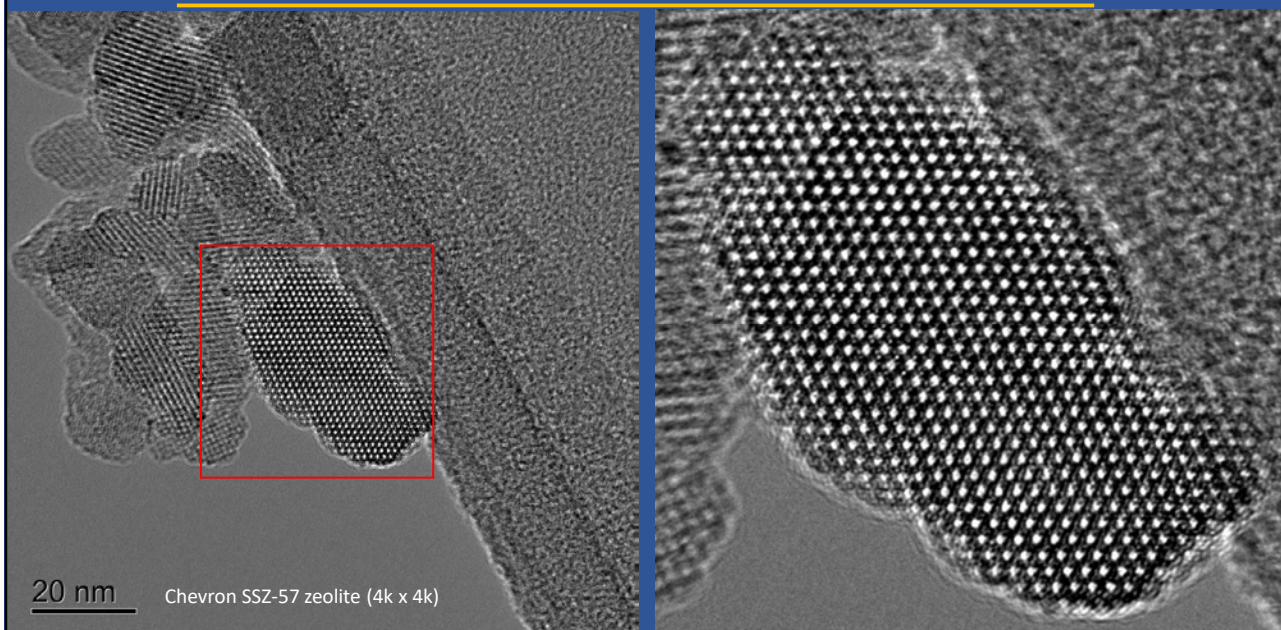
25fps @4k*4k
300fps @512*512

High-Speed + Full Resolution
...delivered *at the same time, all the time.*



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Image of gatan OneView



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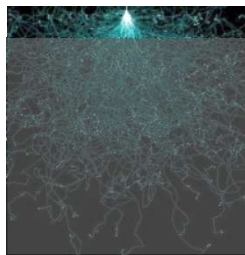
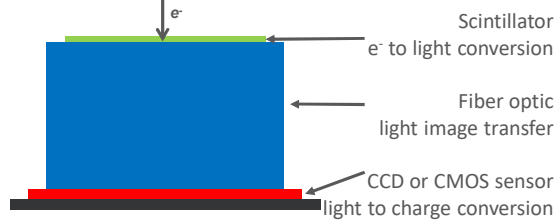
67



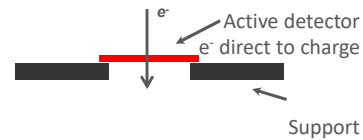
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A Comparison of Detectors

Traditional Fiber-Coupled Camera (CCD or CMOS)



Direct Detection Camera



Limiting factors:

- Electron scattering in ~~high-Z~~ ^{low-Z} scintillator
- ~~Electron back scattering from fiber optic~~
- ~~Scattering of light in fiber optic~~
- ~~Distortions from fiber optic~~
- Electronic read noise



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...Until Direct Detection and Electron Counting!

Fiber optical coupled CCD

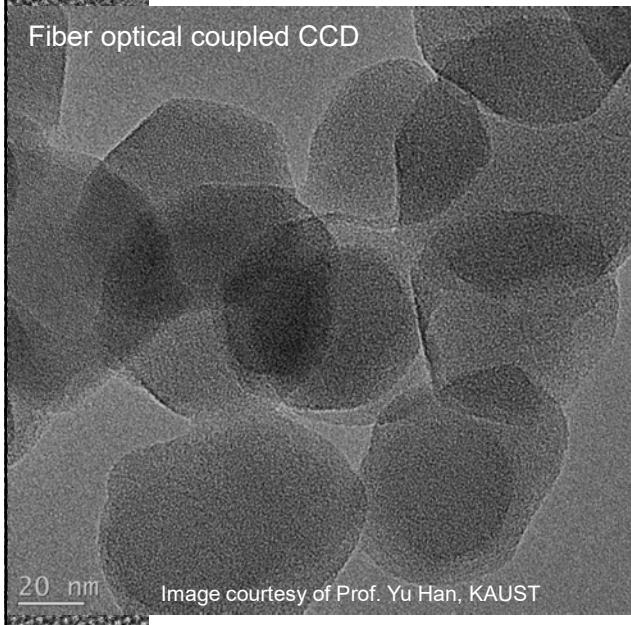
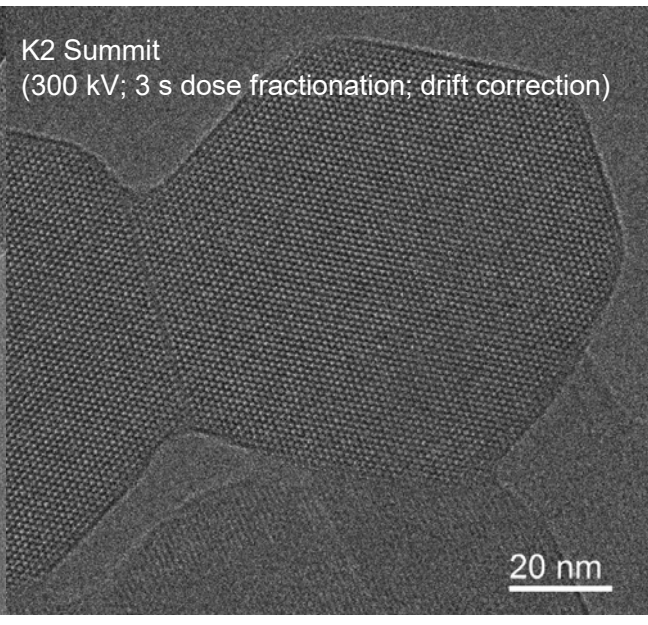


Image courtesy of Prof. Yu Han, KAUST

K2 Summit
(300 kV; 3 s dose fractionation; drift correction)



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CRYO ARM 200/300

Accelerating voltage: 200 & 300 kV

Electron gun: Cold FEG

Cryo pole piece

Omega filter

Hole-free phase plate

Camera: GATAN K3

Auto acquisition software: JADAS



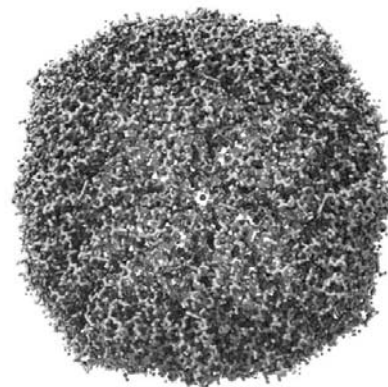
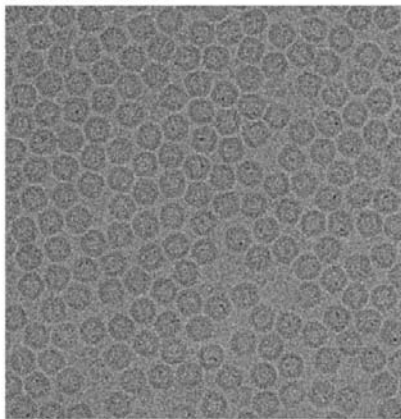
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Cryo ARM 300 data

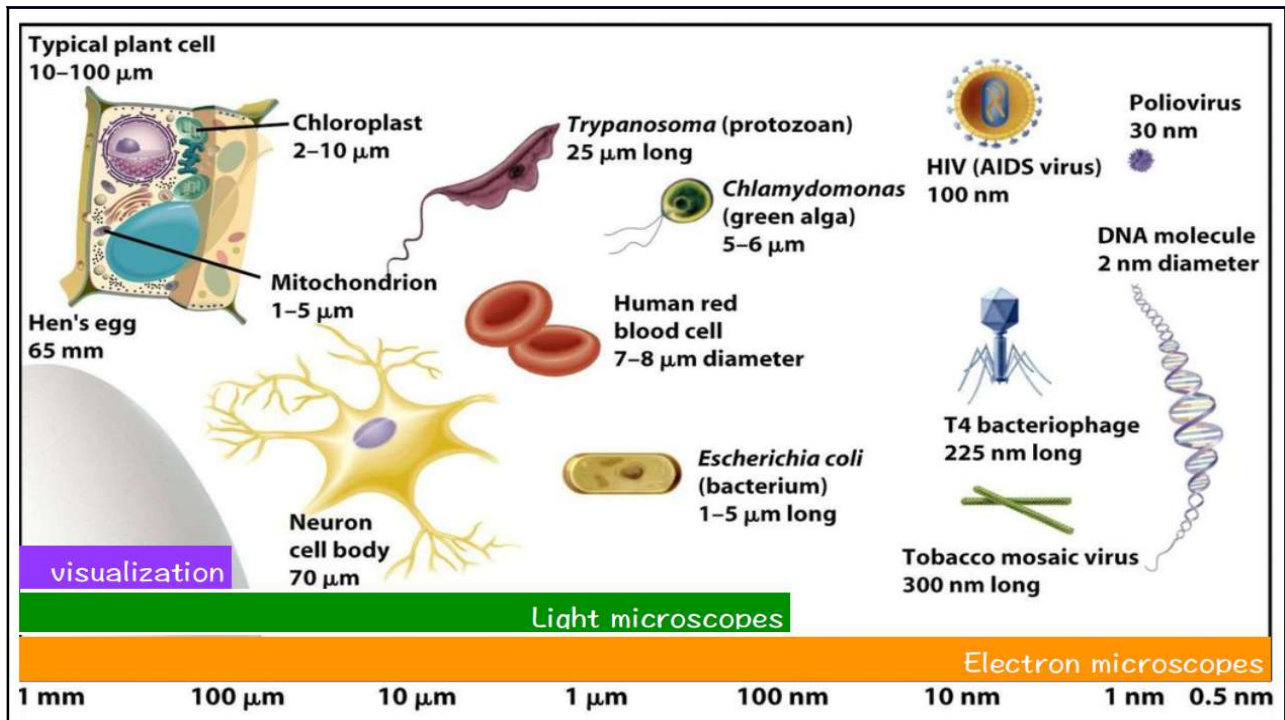
The highest resolution 1.53 Å achieved by cryoEM 2019.02

mouse apoferritin

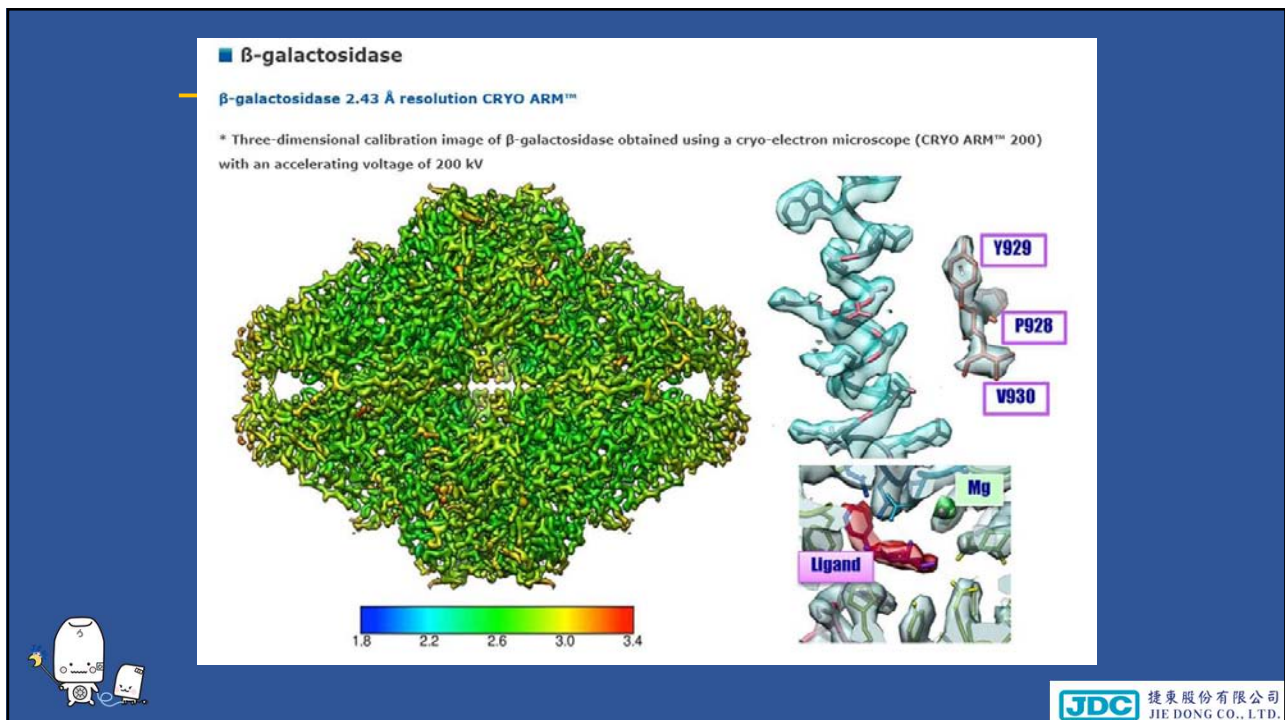


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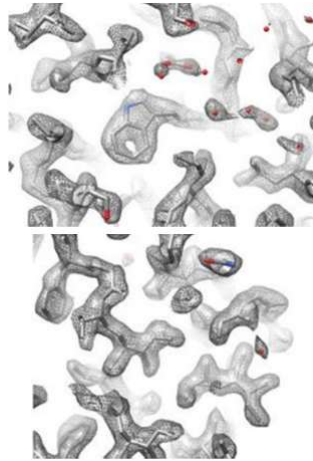
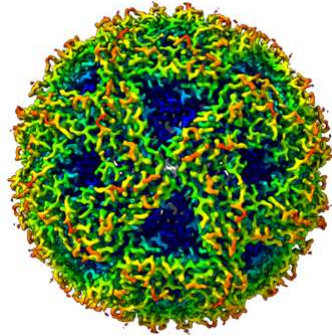
Glasgow

Lumazine synthase by Glasgow CRYOARM 300

SCMI
@SCMICryoEM

フォローする

2.4 angstrom structure of Lumazine Synthase, solved from 22,000 particles imaged on the SCMI CryoARM 300 with the DE64 operated in bin x2 counting.



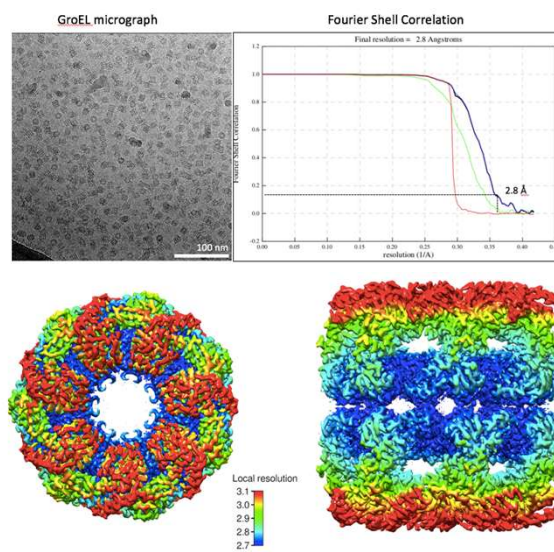
Data courtesy of Prof. David Bhella (SCMI)

Solutions for Innovation JEOL

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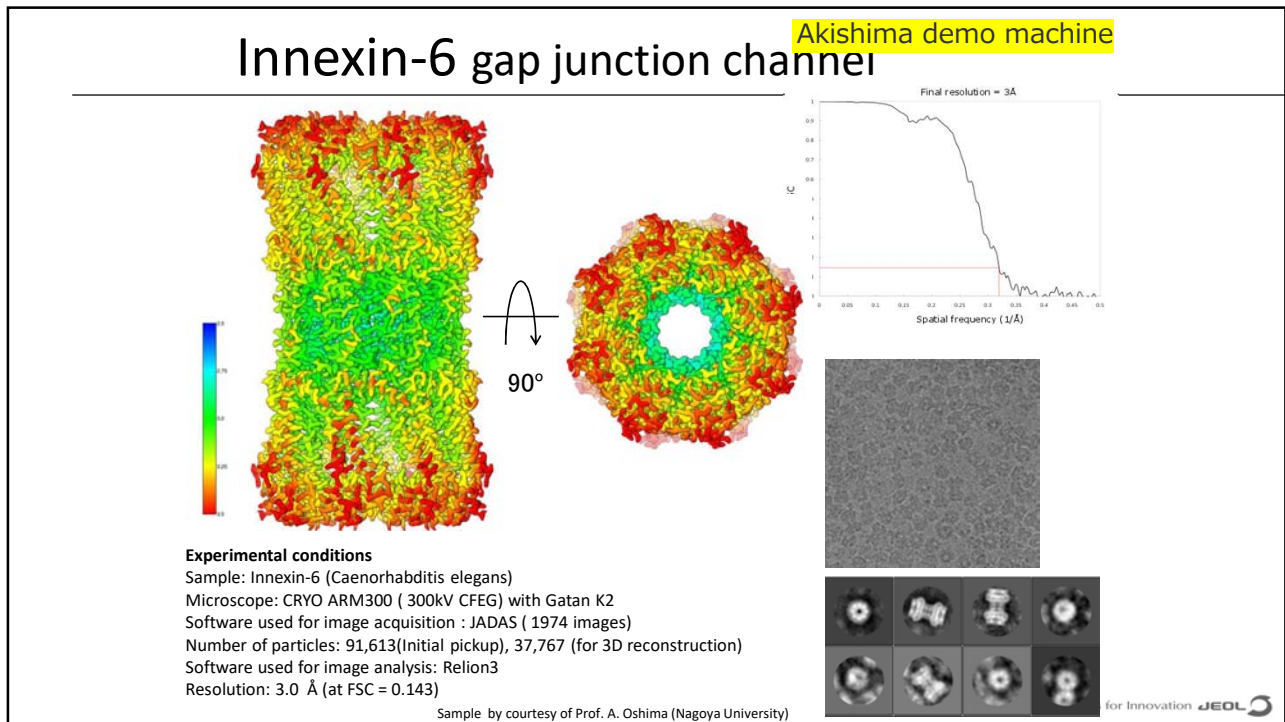
VIB

GroEL by VIB CRYOARM 300



Solutions for Innovation JEOL

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PyJEM

a Python based Scripting system in order to control JEOL TEM automatically.



Users freely edit a script for their purpose.



Network connection to TEM





The script run on TEM remotely




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Purpose of PyJEM



Researcher

I have developed a **new method** to observe a nano-structure by using TEM. Can your company develop a new software for this method?

My work is almost **routine**. Everyday I observe same kind of samples with same acquisition condition. I want to do this **automatically**.



Industrial Developer



PyJEM is useful to **create a new software!**



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Example of the PyJEM script

Sample script "Tilt 10 degree and Get image"

```

1 # -*- coding: utf-8 -*-
2
3 import os
4 from PyJEM import TEM3
5 from PyJEM import detector
6
7 _Stage = TEM3.Stage3()
8 _detector = detector.Detector("STEM BF IMAGE")
9
10 folderPath = os.getenv("HOMEDRIVE") + os.getenv("HOMEPATH") + "\\Desktop\\PyJEMdata"
11 if not os.path.exists(folderPath): #フォルダが無かったら作成
12     os.makedirs(folderPath)
13
14 writeImageName = folderPath + "\\image"
15
16 _Stage.SetTiltXAngle(10)
17 _detector.snapshot("jpg", True, writeImageName, False)
18
19 """
20 Created on Fri Nov  2 20:37:03 2018
21
22 @author: yaoyama
23 """
  
```

Define macro in this script

Shortcut

Create a folder on Desktop

Define image name

Tilt X = 10 degree
Get image



Python skill is necessary to use PyJEM.
Users have to know these commands.

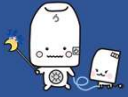


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PyJEM Function

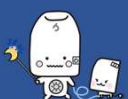
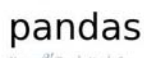
- (1) **Lens control**
Magnification, Brightness, Detector In/Out etc.
- (2) **Stage control**
x, y, z, tilt x, tilt y, piezo control
- (3) **Image capture**
TEM/STEM image capture, Image size, Image format etc.
- (4) **Auto-Function**
Auto-Focus, Auto CB, Auto-Stigmator



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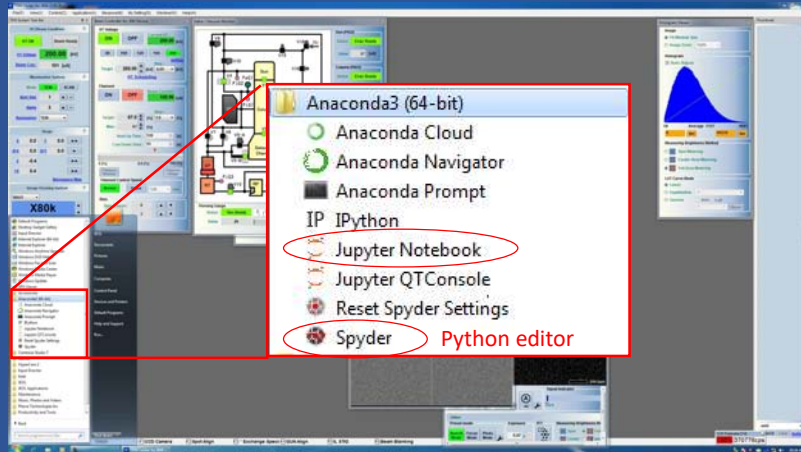
Python Package

Package	Function
Scipy	Scientific calculation
Numpy	High speed calculation
Matplotlib	Graphic
Sympy	Symbolic calculation
Pandas	Time series data processing
PyQT	GUI
OpenCV	Image processing
Scikit-learn	Machine Learning



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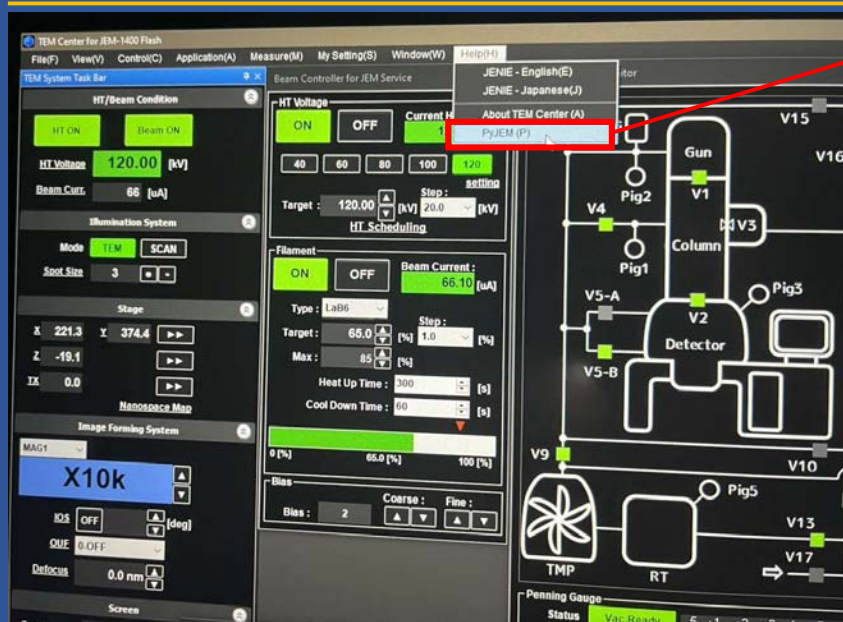
PyJEM system installed in TEMCenter PC



Anaconda3 in Windows menu is corresponding to PyJEM system.

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PyJEM function in JEM-1400HR

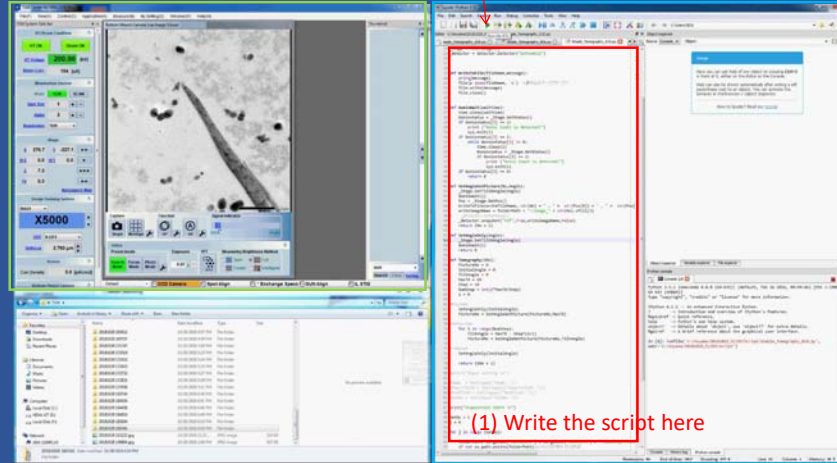


PyJEM function in JEM-1400HR

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Python editor "Spyder"

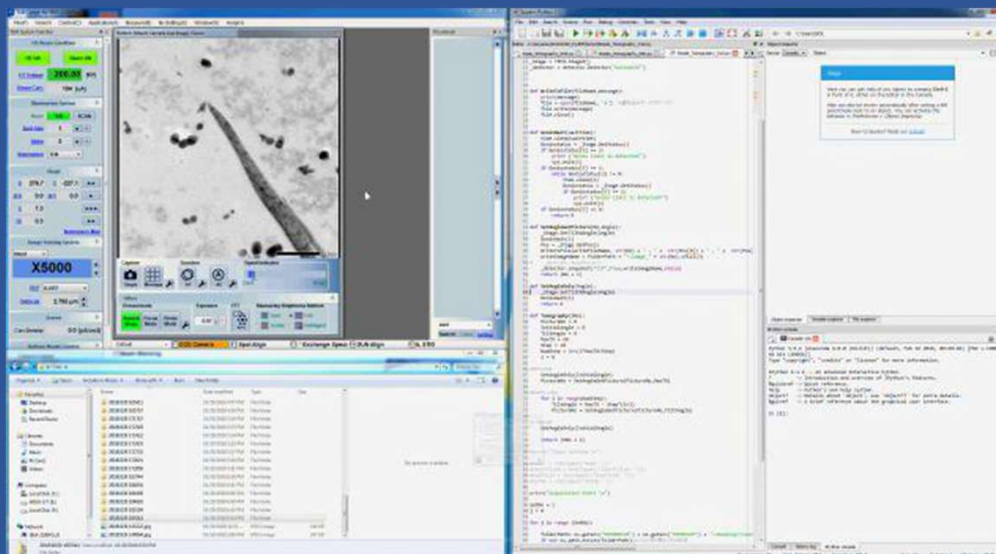
TEMCenter (2) Click here to run the script Spyder



By using Jupyter Notebook and Spyder, Users can edit and the PyJEM script can run.

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Script for Tomography (movie)



Of course, GUI can be made by Python.

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Hardware and Software requests for PyJEM

PC

Windows 7 、 Windows 10 、 Windows 11 (64 bit)

Anaconda 3.4

Python 3.5~3.10

Python library (scipy, numpy, OpenCV, PIL, matplotlib etc.)

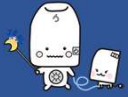
PyJEM

Sample script code

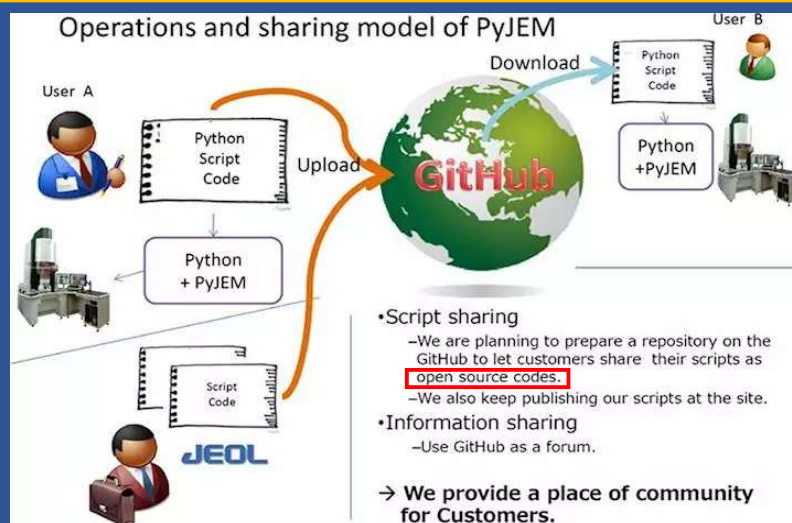
Manual

Offline Package

TEMExt (JEOL TEM External control library)



Virtual TEM for develop & testing (off-line)



GitHub (open source for JEOL PyJEM)

**dmaekawa** Remove document source code 6121b6c · 2 weeks ago 62 Commits

docs	→ User manual	Update manual 1.3.9.3650	last month
vjem	→ Virtual TEM	vjem file (python 3.5- 3.10)	2 years ago
README.md		bug fix: not enough a offline data file and crash the offline i...	last year
requirement.txt		update document	last year



GitHub website <https://github.com/PyJEM/PyJEM?tab=readme-ov-file>

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PyJEM User Manual

PyJEM is a Python package that provides control TEM, capture live image, and so on. This document introduces how to install PyJEM, FAQ, and simple script code.

- Introduction
 - Summary
 - Environment
 - Functions
 - Connection model
 - Forum (Message Board)
- Installation
 - 1.Miniconda
 - 2. vjem (virtual env)
 - 3.PyJEM install
 - 4.PyJEM update
 - Appendix
- Tutorial
 - Getting started
 - Sample code (detector)
 - Sample code (TEM3)
 - Sample code (eds)
 - More customize
- API Reference
 - Subpackages
 - Submodules
 - Module contents
- What is New
 - Version 1.1.0
 - Version 1.1.1
 - Version 1.2.0
 - Version 1.3.0



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Python example in GMS

```

1
2 print( "\n\n\n" )
3 print( "Listing all registered cameras: \n" )
4 print( "-----\n" )
5
6 nCams = DM.GetNumberOfCameras( )
7 print( " # of registered cameras: ", nCams )
8
9 for camID in range(0, nCams):
10     camera = DM.GetCameraByID( camID )
11
12     print( "\t (ID=", camID, ") " )
13     print( "\t Name: ", camera.GetName( ) )
14     print( "\t ( @ Location: ", camera.GetDeviceLocation( ), ") " )
15
16     sx, sy = camera.GetSize( )
17     print( "\t\t\t Size: ", sx, "x", sy, " pixel" )
18
19     psx, psy = camera.GetPixelSize( )
20     print( "\t\t\t Pixel: ", psx, "x", psy, " micron" )
21
22     del camera
23
24 #acquire image from active camera
25 camera = DM.GetActiveCamera( )
26
27 camera.PrepareForAcquire( )
28
29 img = camera.AcquireImage( )
30 img.ShowImage( )
31
32 del camera

```

Both Python & DM script can be edit

Python `print()` function outputs to DM Results window.

Python `input()` function pops up dialog

- Access to acquisition from Camera, DigiScan, EELS...
- Access to all NumPy and SciPy functionality within GMS
- Full exposure of DM object-model to Python allowing for integrated acquisition and analysis

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