CRYOEM 001 :
INTRO TO CRYOEM

NCCAT Embedded Training – Master Class series

August 20, 2020

NATIONAL CENTER FOR
CRYOEM ACCESS & TRAINING

New York Structural
Biology Center

SIMONS ELECTRON
MICROSCOPY CENTER

NCCAT CROSS-TRAINING

knowledge

capability

capacity

productivity

performance

Training is teaching, or developing in oneself or others, any skills and knowledge that relate to specific useful competencies. Training has specific goals of improving one's capability, capacity, productivity and performance.

https://en.wikipedia.org/wiki/Training

THE STARTING POINT – WHAT IS YOUR LEVEL?

Core EM knowledge

Beginner
Intermediate
Advanced

Sample preparation

Negative stain

Beginner
Intermediate
Advanced

CryoEM

Beginner
Intermediate
Advanced

Microscope operations

Screeners

Beginner
Intermediate
Advanced

High-end

Beginner
Intermediate
Advanced

Data Collection

Beginner
Intermediate
Advanced

Data Processing/Analysis

Quality assessment

Beginner
Intermediate
Advanced

Structural biology

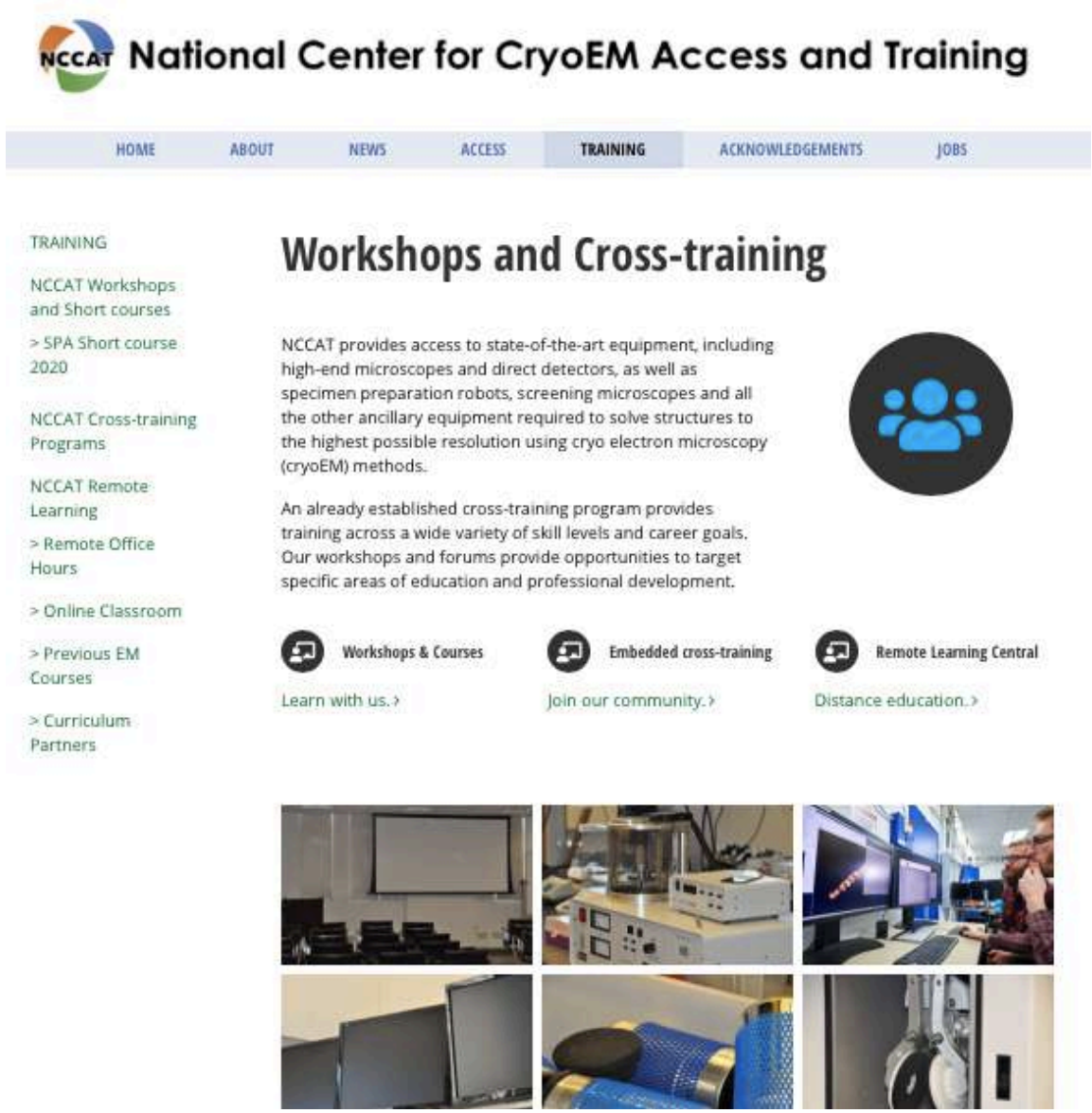
Beginner
Intermediate
Advanced

NCCAT TRAINING DOCUMENTATION

https://nccat.nysbc.org/activities/nccat-cross-training/

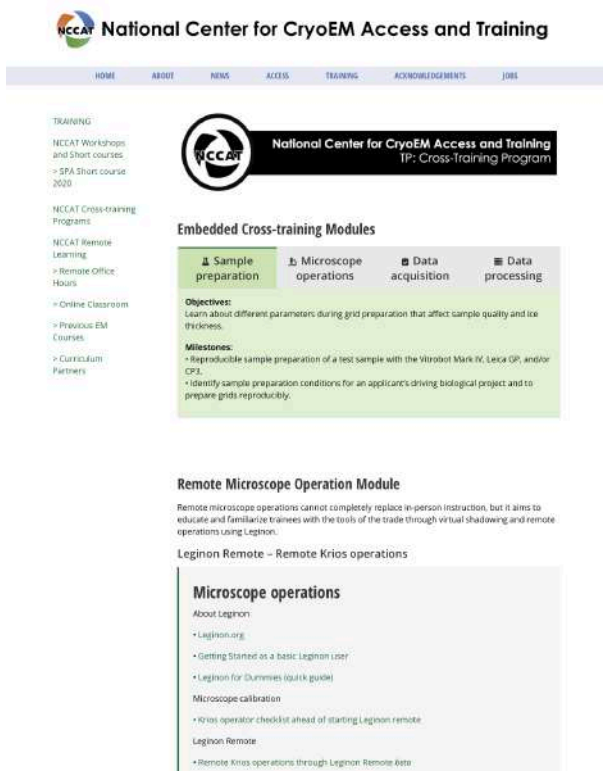
https://nccat.nysbc.org/activities/nccat-remote-learning/

https://nccat.nysbc.org/activities/nccat-cross-training/remote-embedded-cross-training/

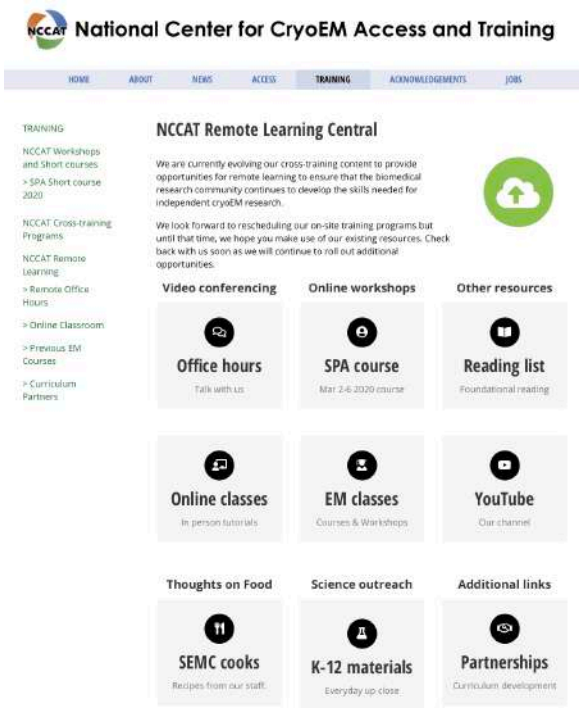


NCCAT CROSS-TRAINING RESOURCES

Remote Cross-training Program



Remote Learning Central



SEMC meetings

Lab meetings

Project meetings

Office hours

NCCAT workshops

Roundtables

Appion

Remote learning modules

Core knowledge

Sample preparation

Microscope operations

Data collection

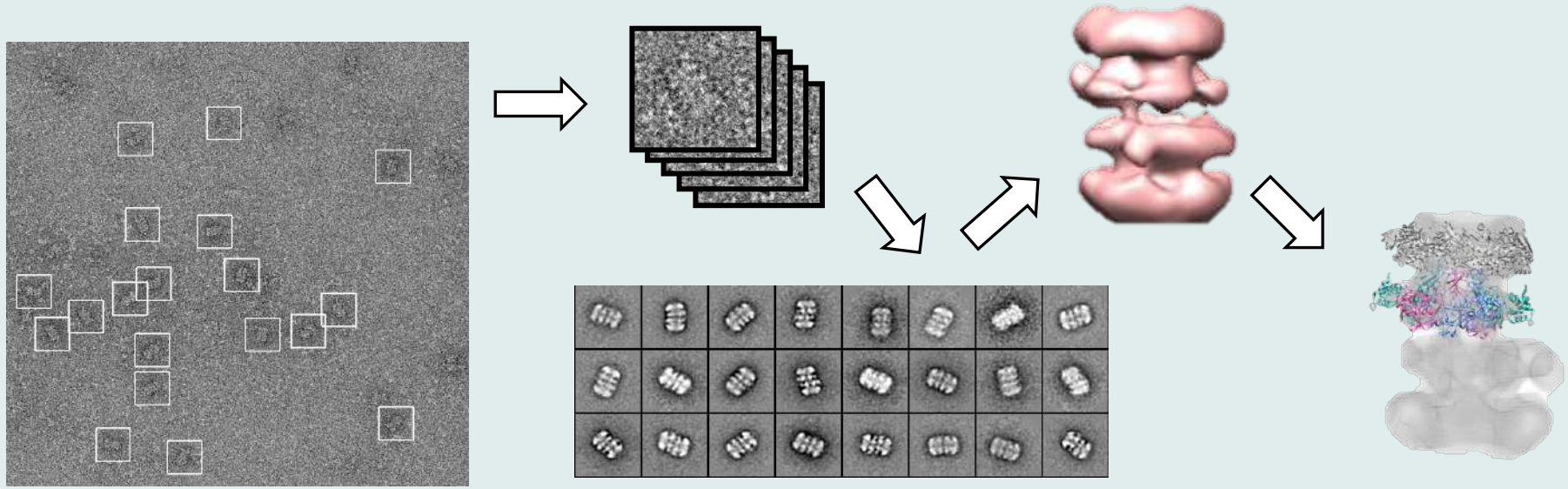
Data processing

APPION WORKSHOPS – AN OVERVIEW

Single particle

Tomography

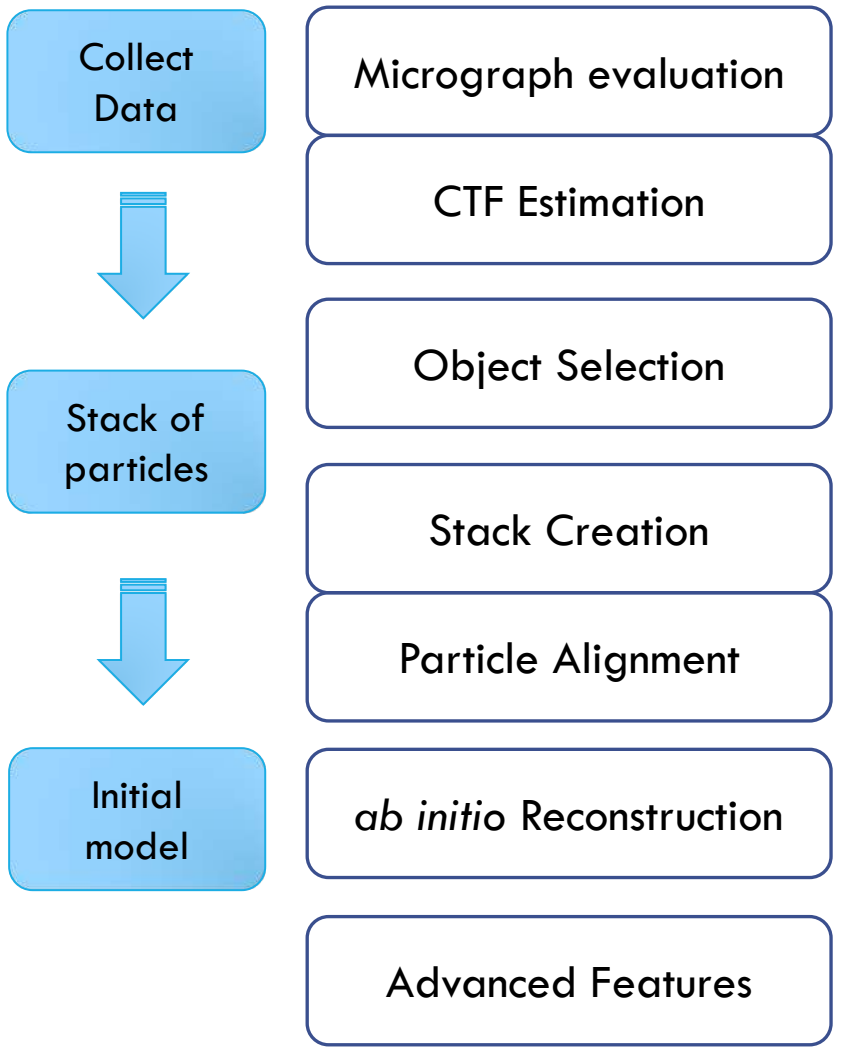
Part I	Part II	Part III
Tools to generate a particle stack from micrographs and obtain an initial assessment of the sample.	**3D reconstruction, refinement and validation.**	Tomography tools.

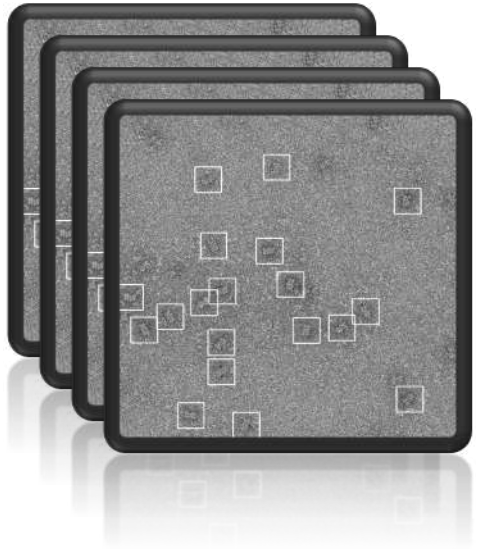


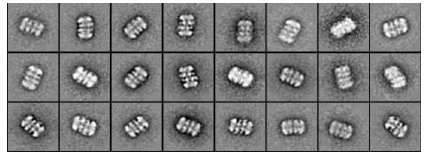
DEBRIEF ON APPIONI

JULY 29-30, 2020 2PM-4PM

PRACTICAL WORKSHOP WHERE STUDENTS WILL WORK IN PAIRS ON A TEST DATASET. AFTER EACH LECTURER GIVES AN OVERVIEW OF THE TOPIC THE ATTENDEES WILL HAVE HANDS-ON TIME TO USE THE INTERFACE.



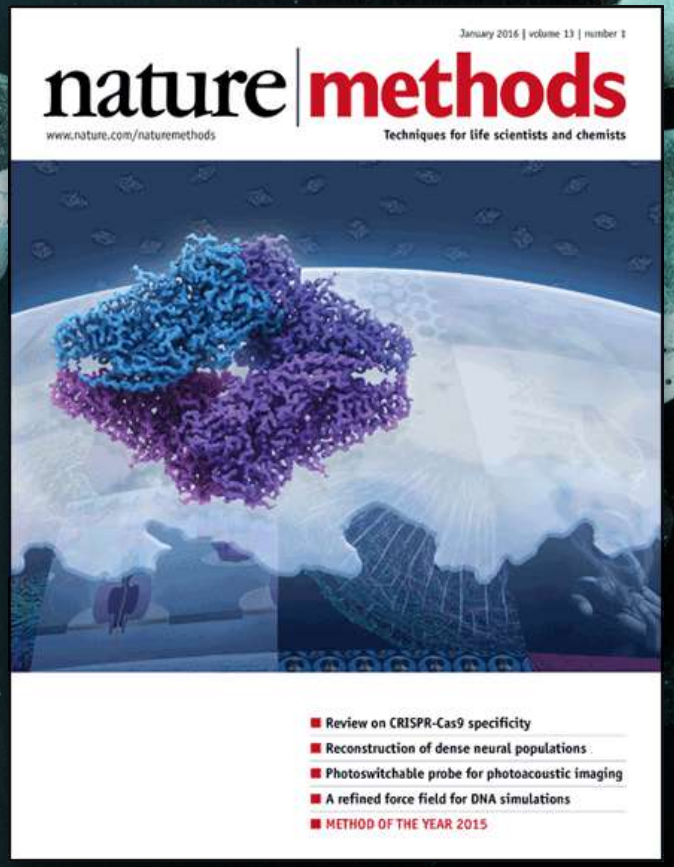




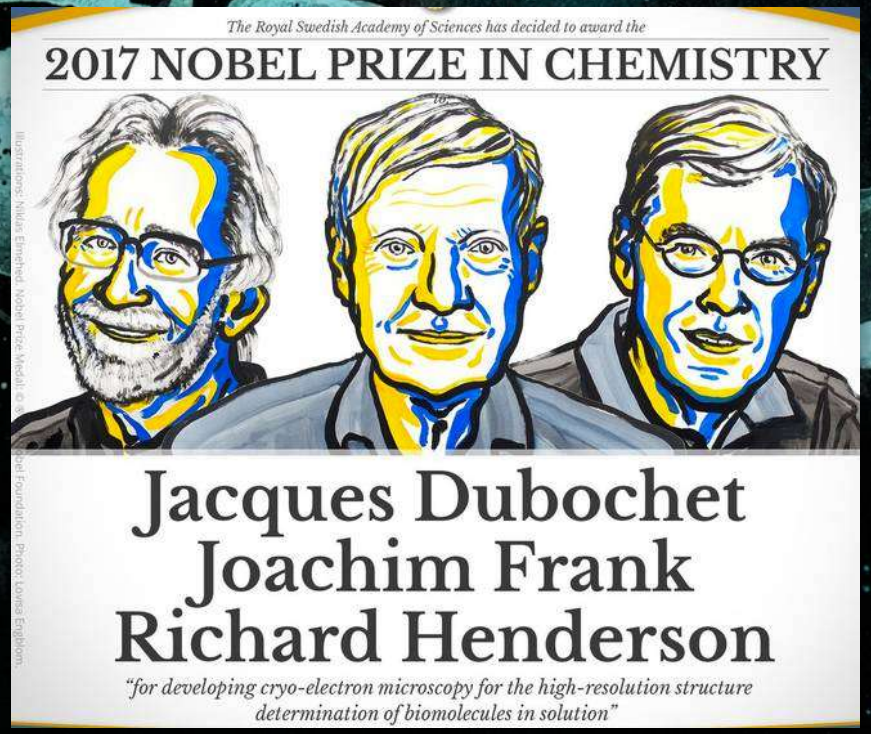

001
CRYOEM ~~101~~
START
HERE..

CRYOEM: TECHNOLOGY ON THE RISE

Single-particle cryo-electron microscopy (cryo-EM) is the Method of the Year 2015



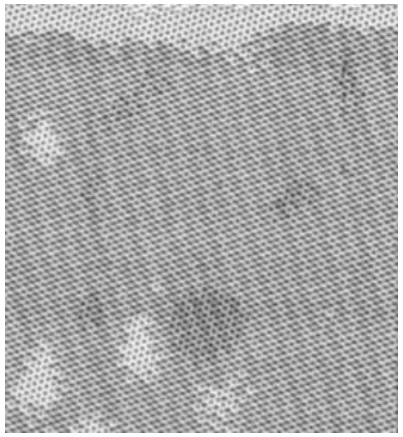
Chemistry Nobel prize 2017

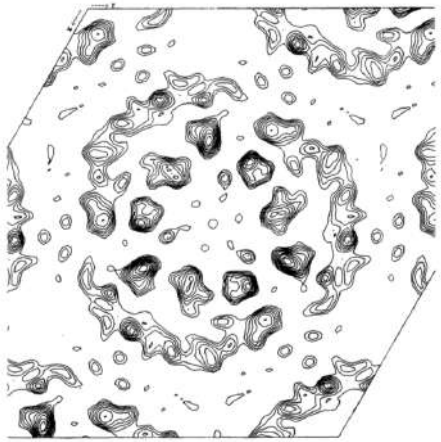


microED
Science breakthrough of the year runner-up 2018

CRYOEM: TECHNOLOGY ON THE RISE

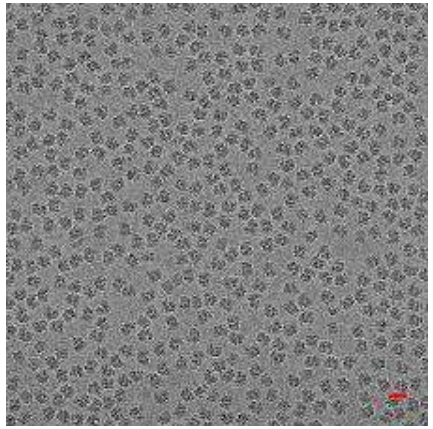
1986

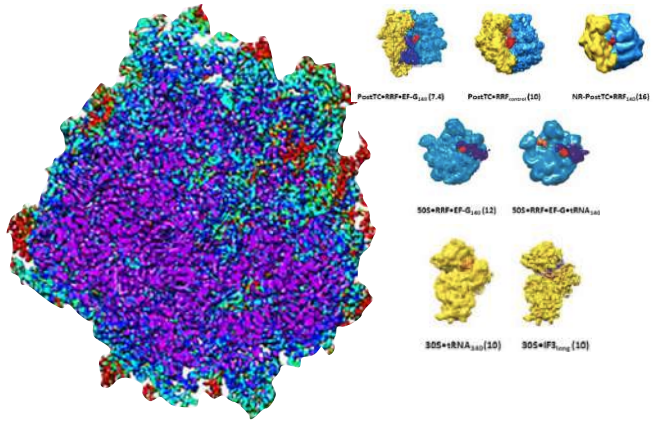




Henderson, et al. (1986)

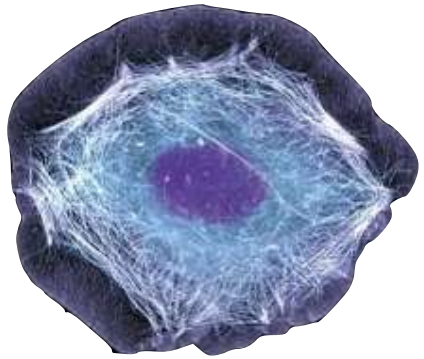
2017

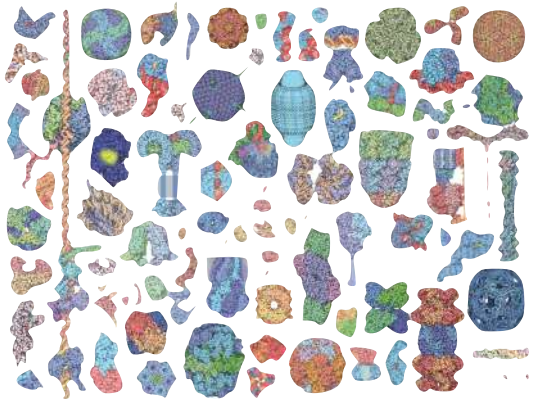




Frank (2017)

????





TBD (20??)

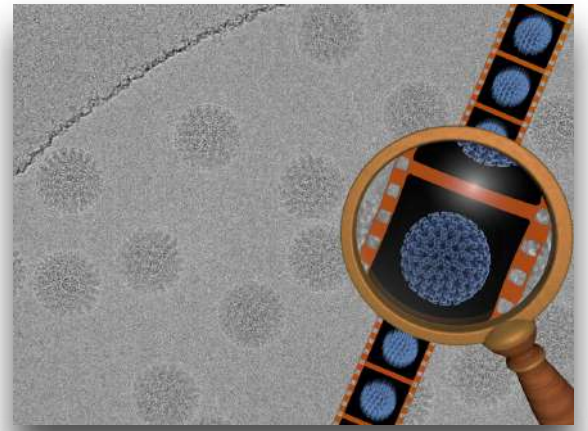
Resolution revolution

(~2012-2014)

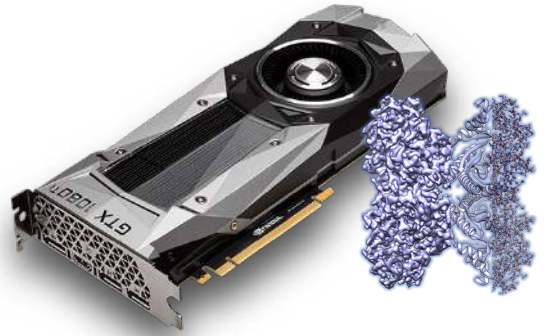
Hardware

Microscopes

Direct Detectors

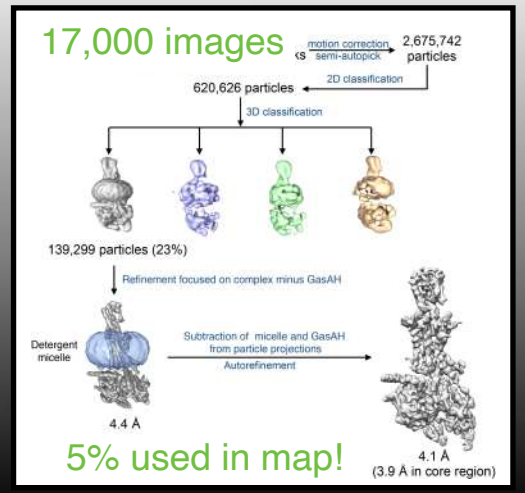


Computers



2012->2017
Cost reduced by 100x

Software

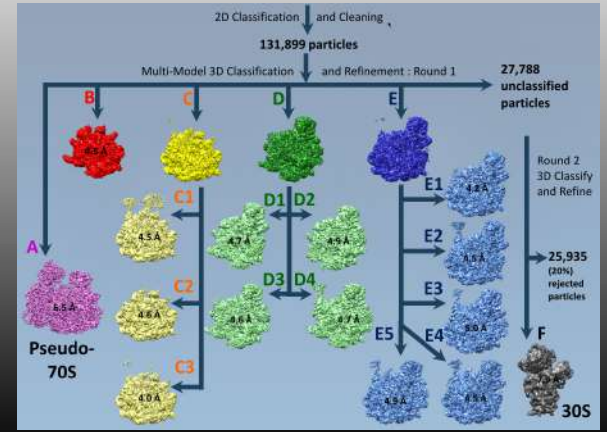


Leginon / SerialEM / EPU, …

MotionCorr2, Unblur, …

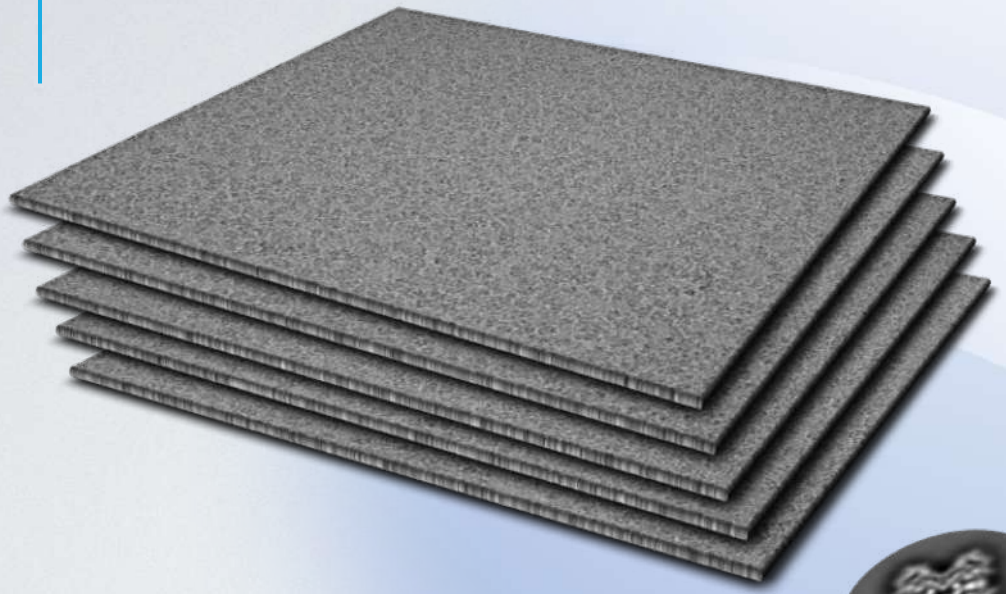
RELION, FREALIGN/cisTEM, cryoSPARC
EMAN, Sparx, SPHIRE, XMIPP, …

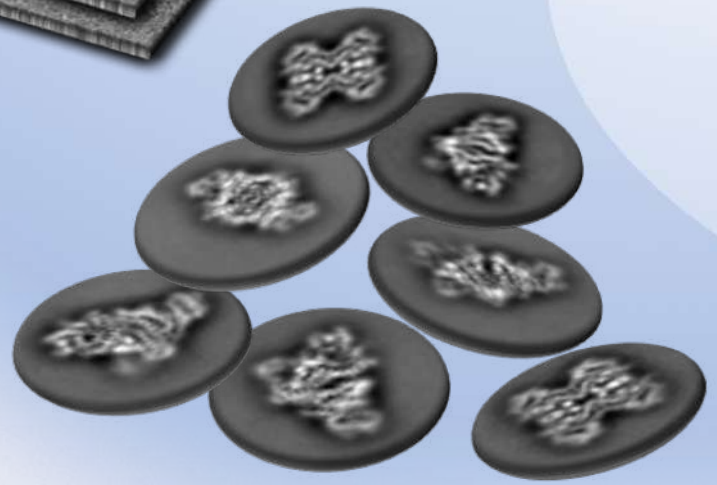
14 independent structures

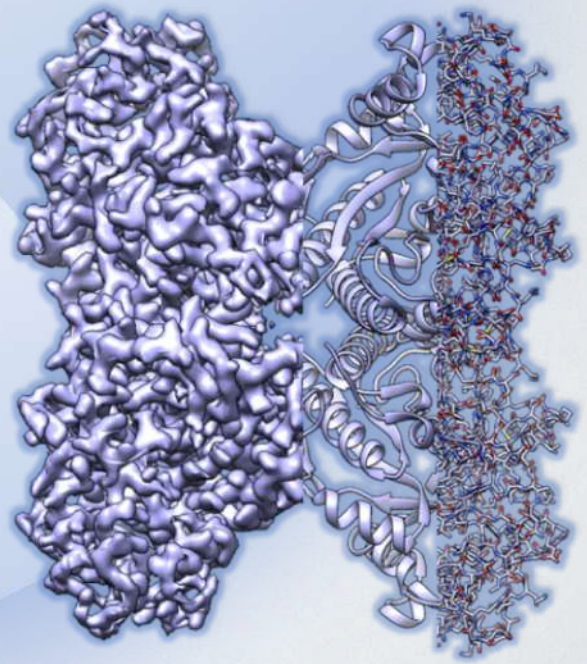


WHAT IS POSSIBLE TODAY?

- 2Å within a day

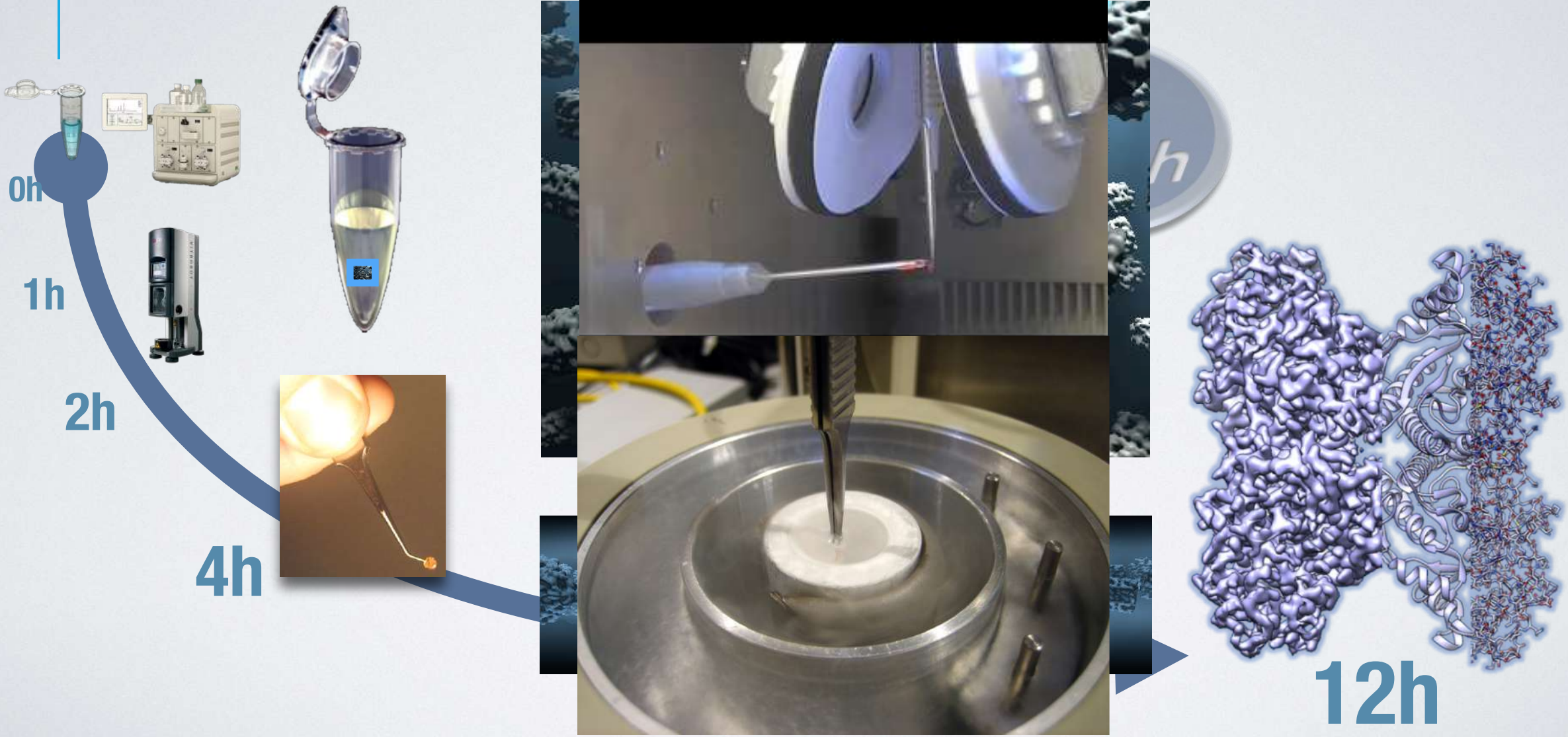






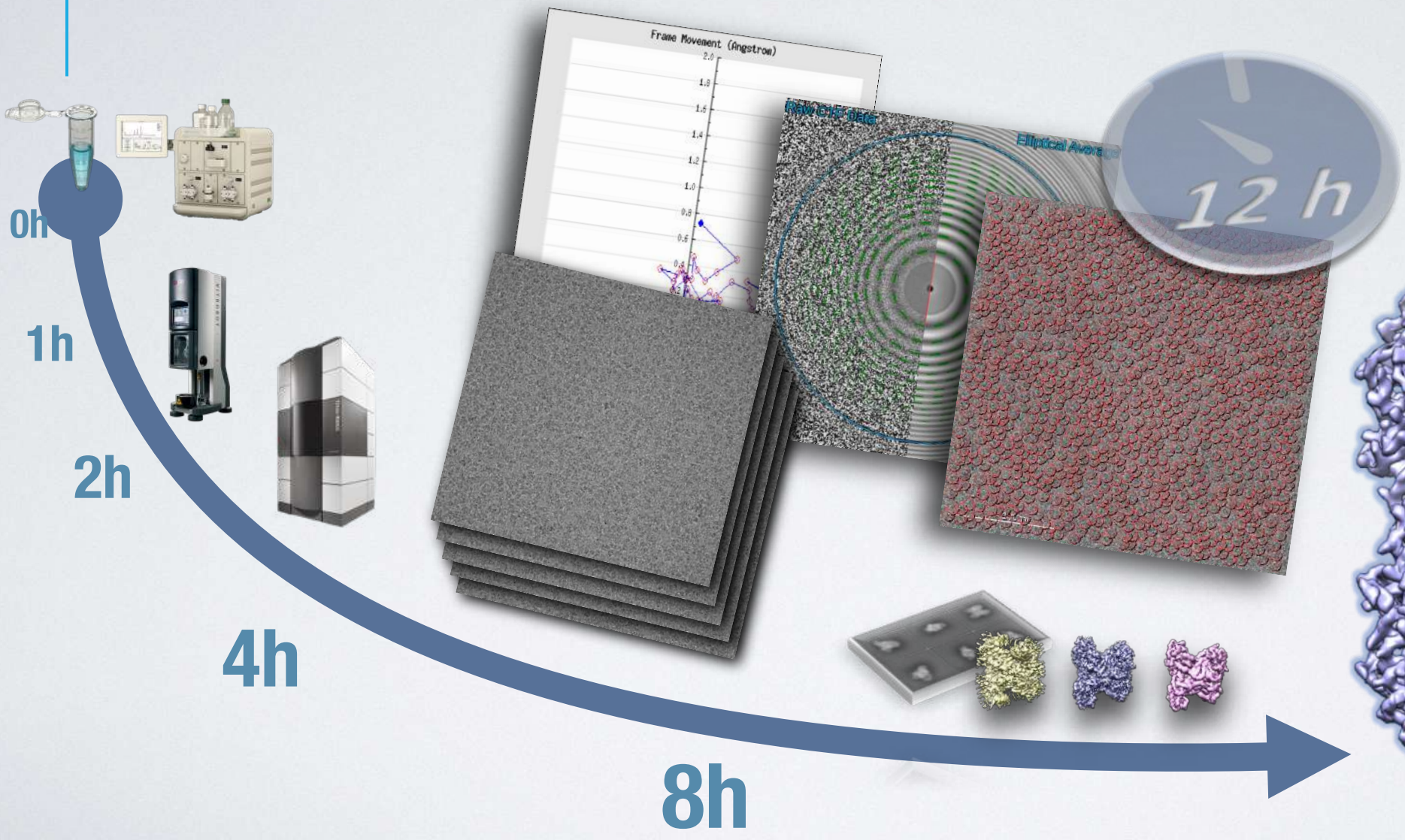
WHAT IS POSSIBLE TODAY?

❋2Å within a day

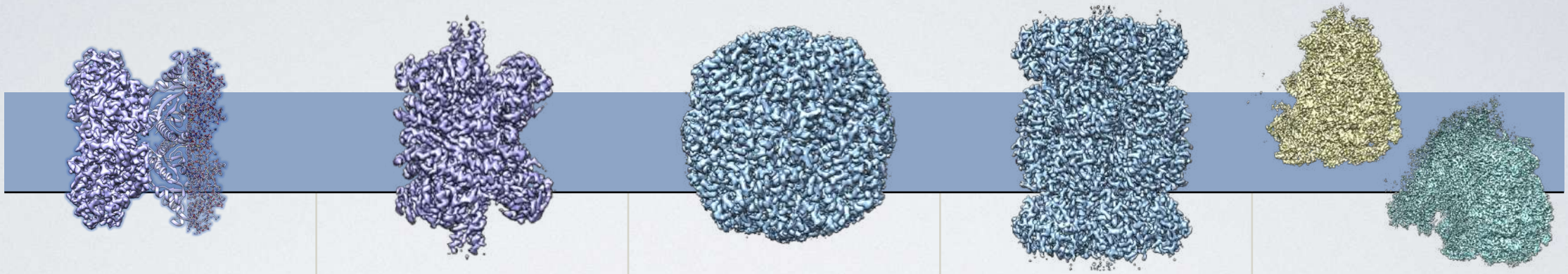


WHAT IS POSSIBLE TODAY?

❋2Å within a day



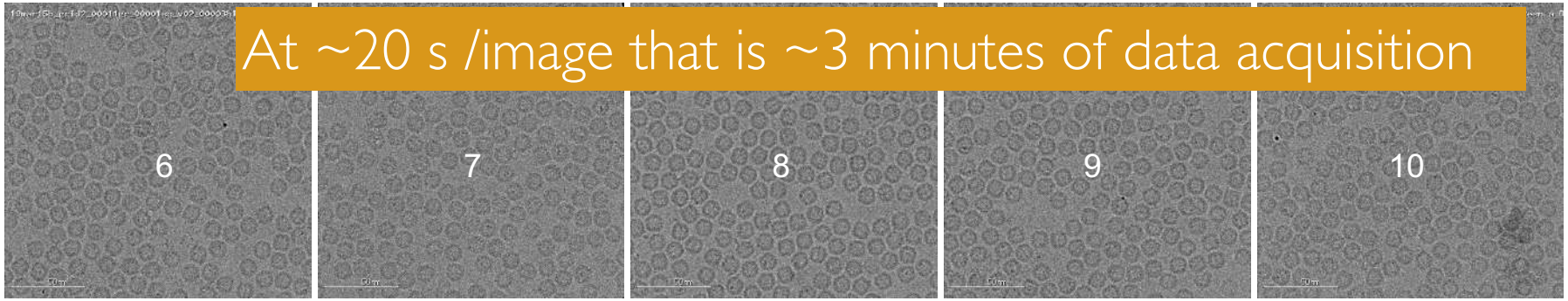
IS THIS ROUTINELY DONE?



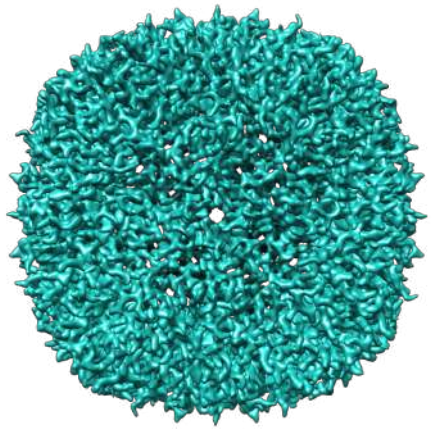
Aldolase	Glutamate dehydrogenase	Apoferritin	20S proteasome	60S/80S ribosome
D2	D3	O	D7	C1
~150kDa	334kDa	443kDa	750kDa	~2-4MDa
rabbit muscle	cow liver	horse spleen	Thermoplasma or Mycoplasma	human

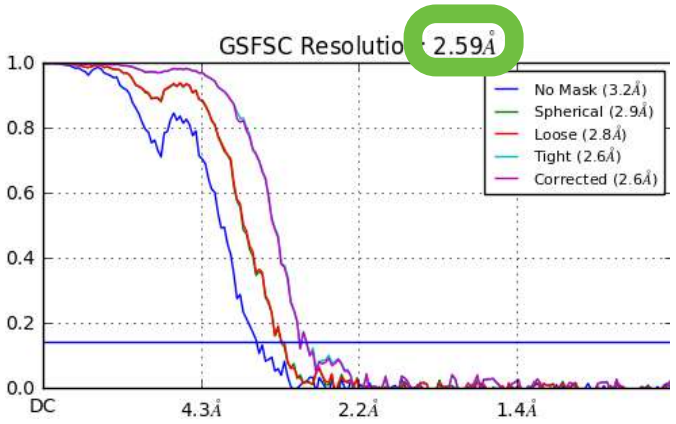
HOW MANY IMAGES DO WE NEED?

For a "good" sample, 10 images is sufficient for a sub 3 Å map



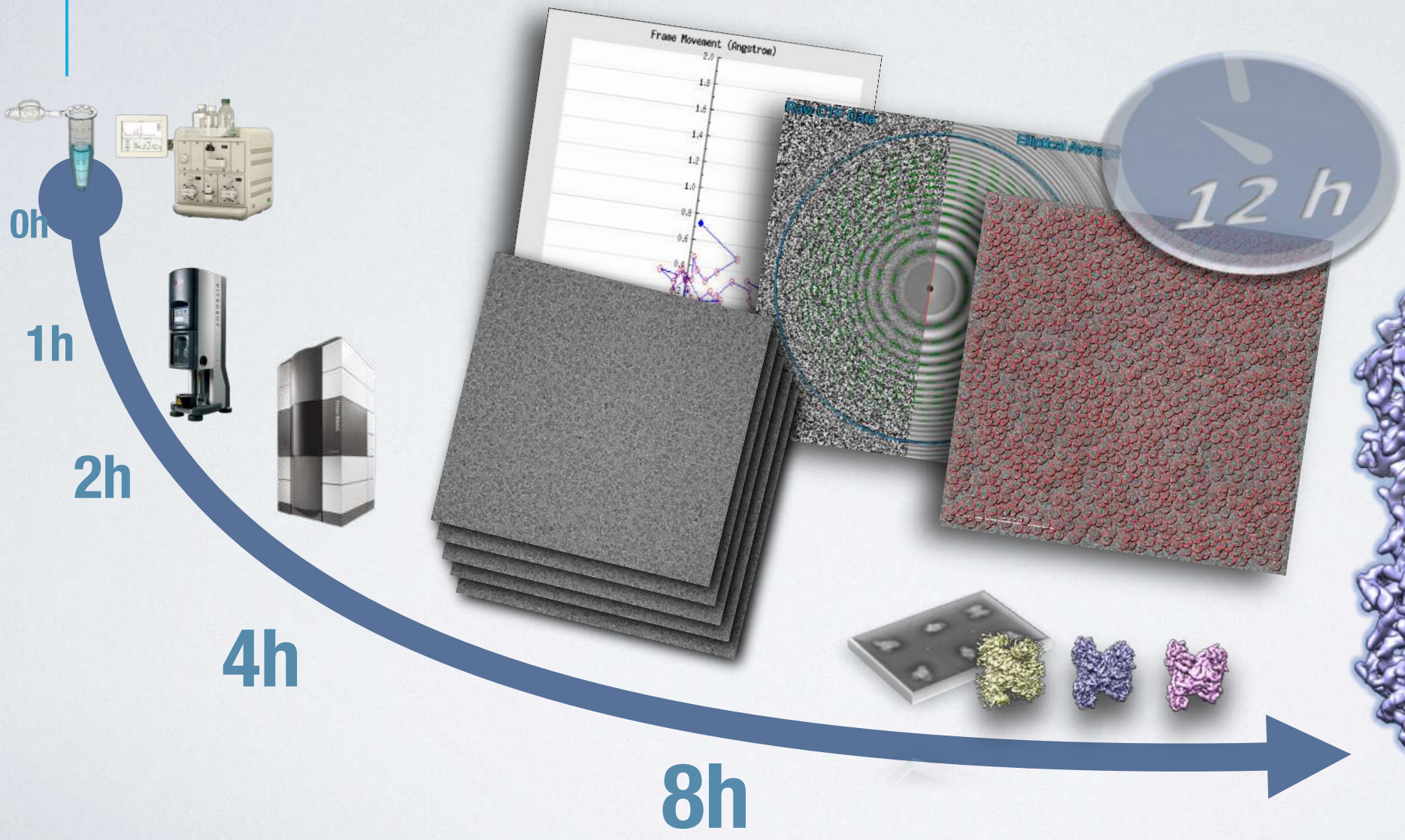
At ~20 s /image that is ~3 minutes of data acquisition





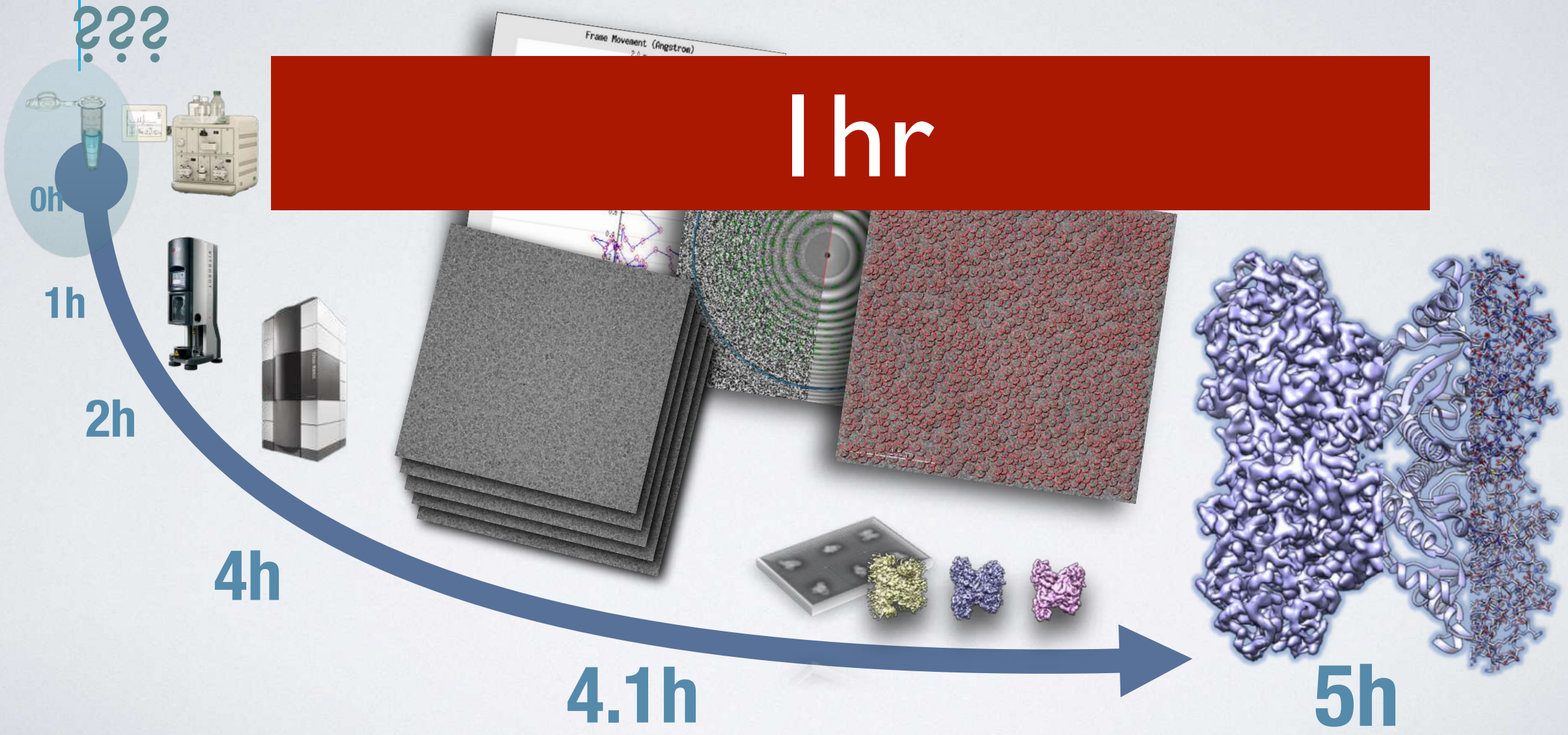
WHAT IS POSSIBLE TODAY?

❋2Å within a day



WHAT IS POSSIBLE TODAY?

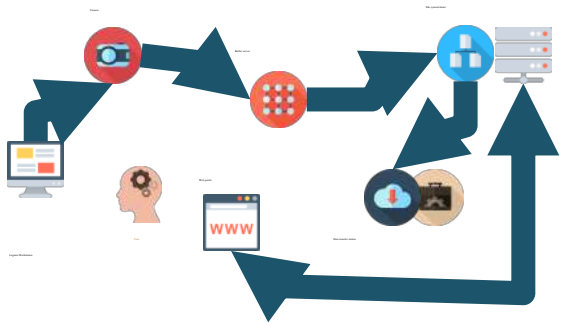
❋2Å within hours



WHERE ARE THE BOTTLENECKS?

NRAMM

technology aimed towards completely automating the processes involved in solving macromolecular structure using cryo-electron microscopy (cryoEM)



Sample preparation

Data collection

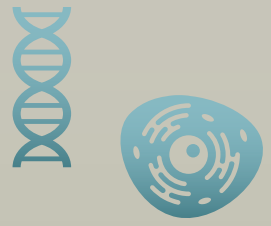
(pre-)Processing

Spotiton

Appion

NCCAT CROSS-TRAINING FOCUS ON 4 AREAS

Core knowledge

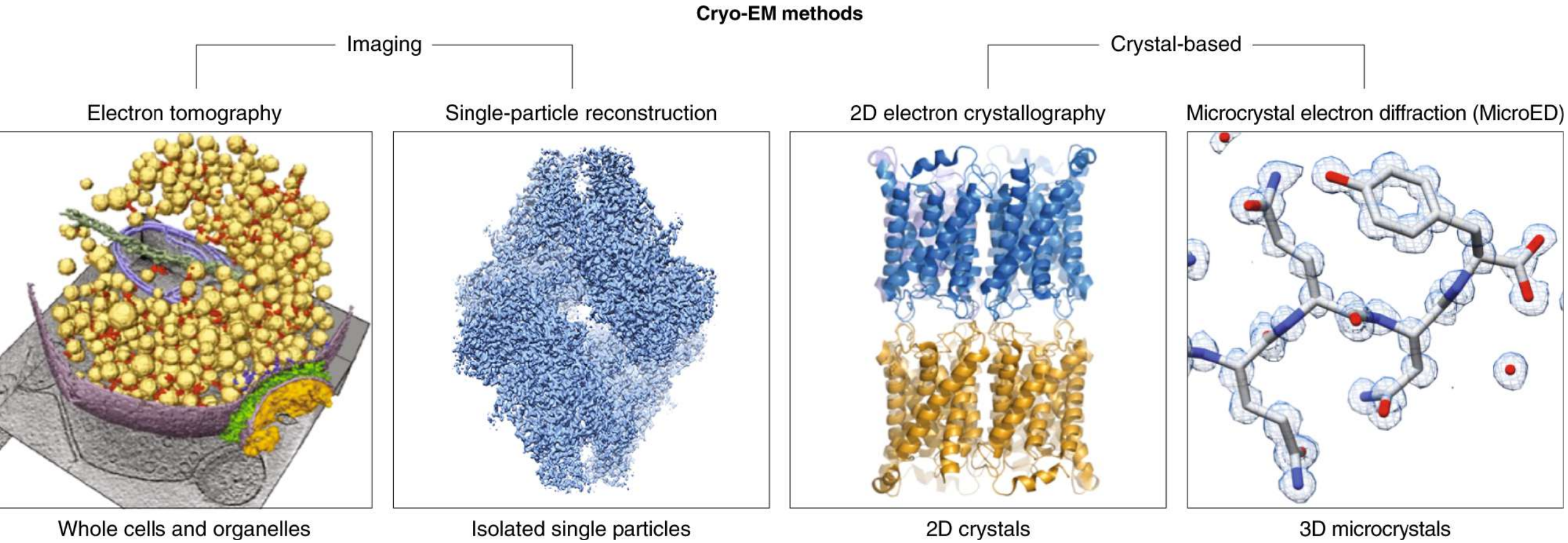


Sample preparation

Microscope operations & Data collection

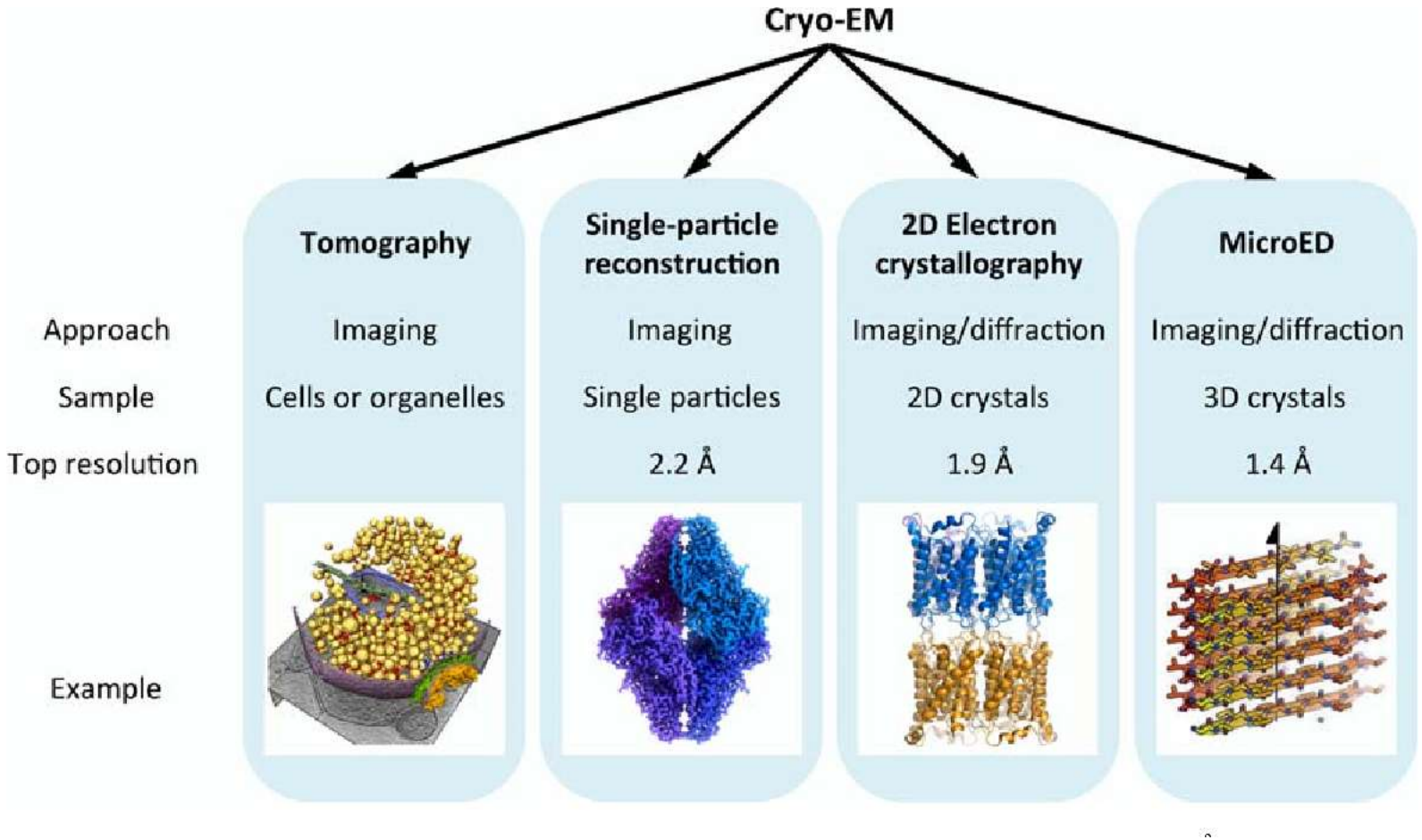
Processing & Data analysis

NCCAT CROSS-TRAINING FOCUS ON 4 AREAS



https://doi.org/10.1038/s41592-019-0395-x

THE FIELD IS CHANGING



https://doi.org/10.1002/pro.2989

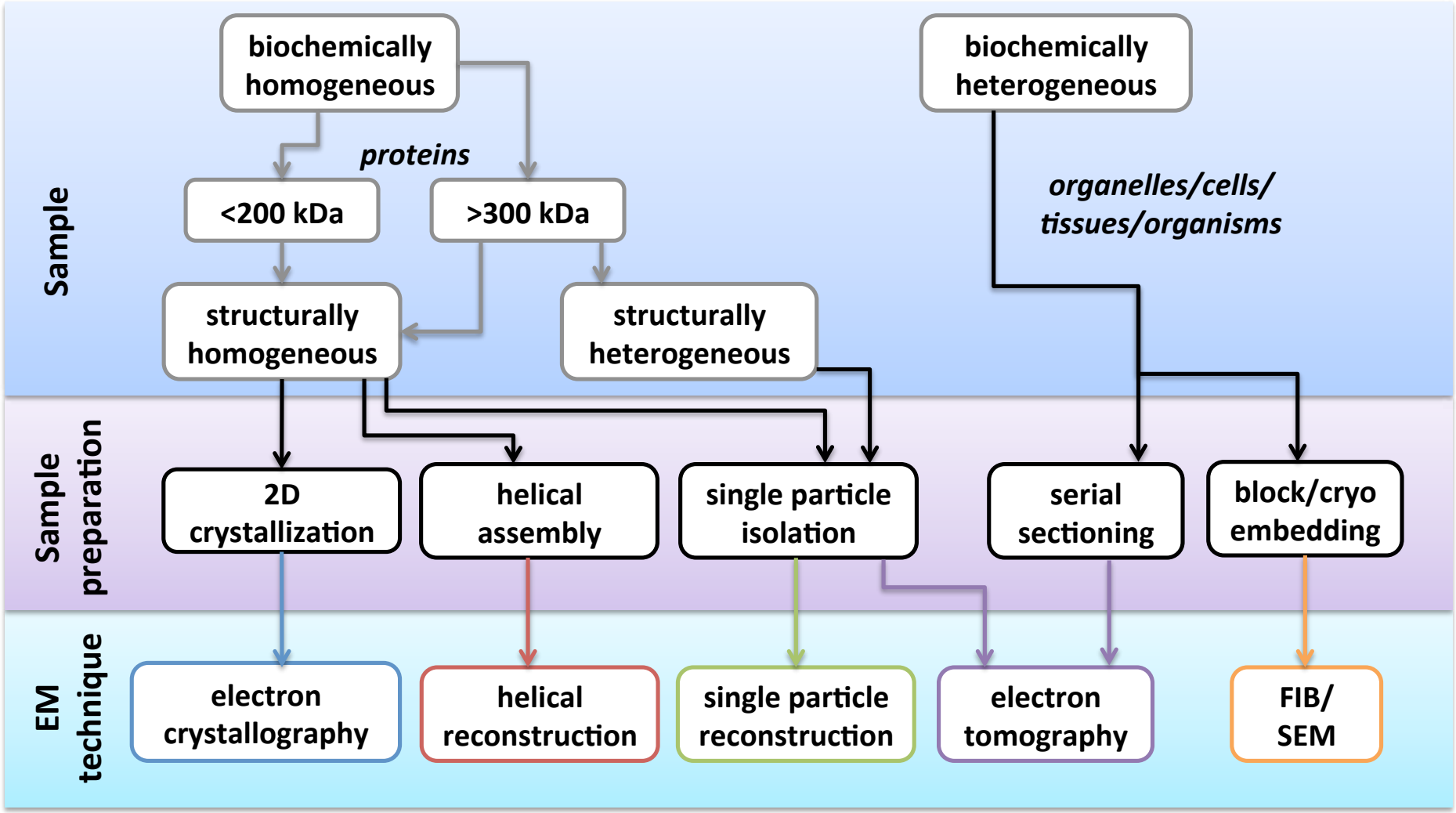
~3.4Å

~1.2Å

~1.9Å

~0.7Å

BUT STILL HAS BOTTLENECKS




WHAT NEXT?