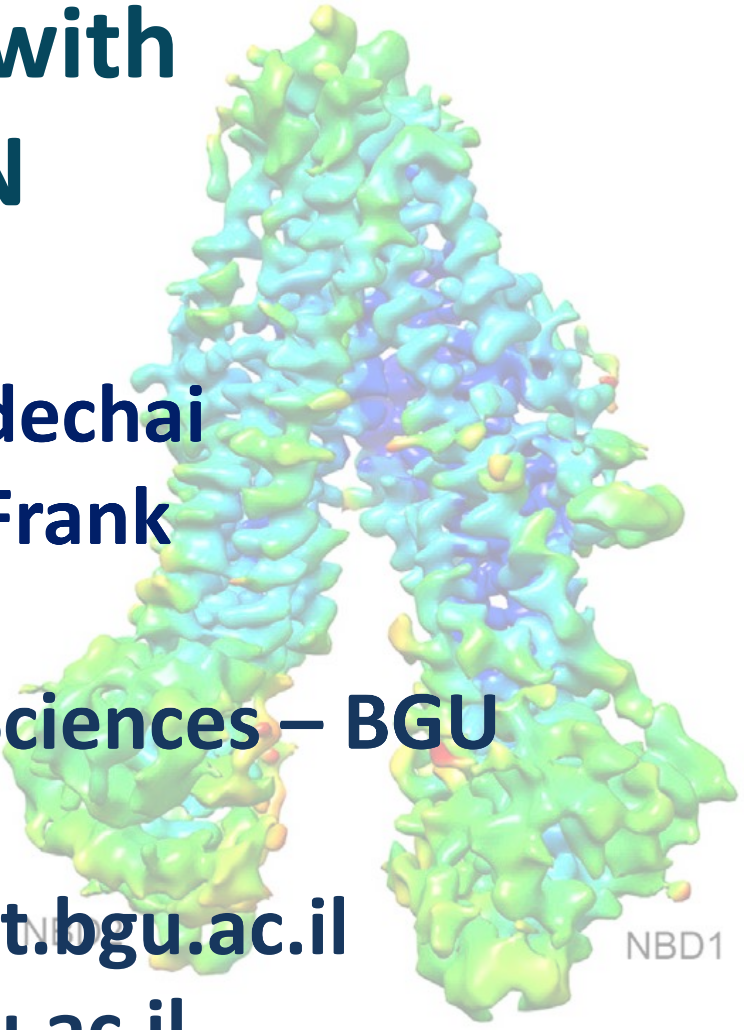
2D classification and initial model building with EMAN



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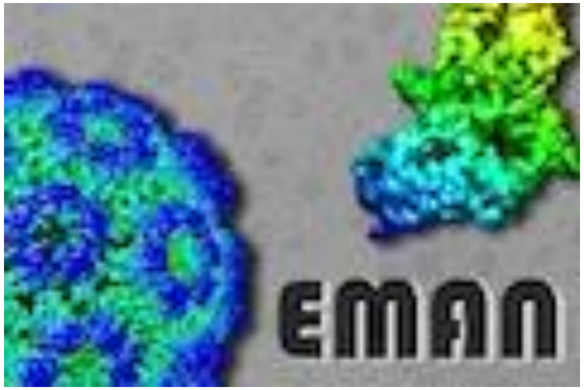
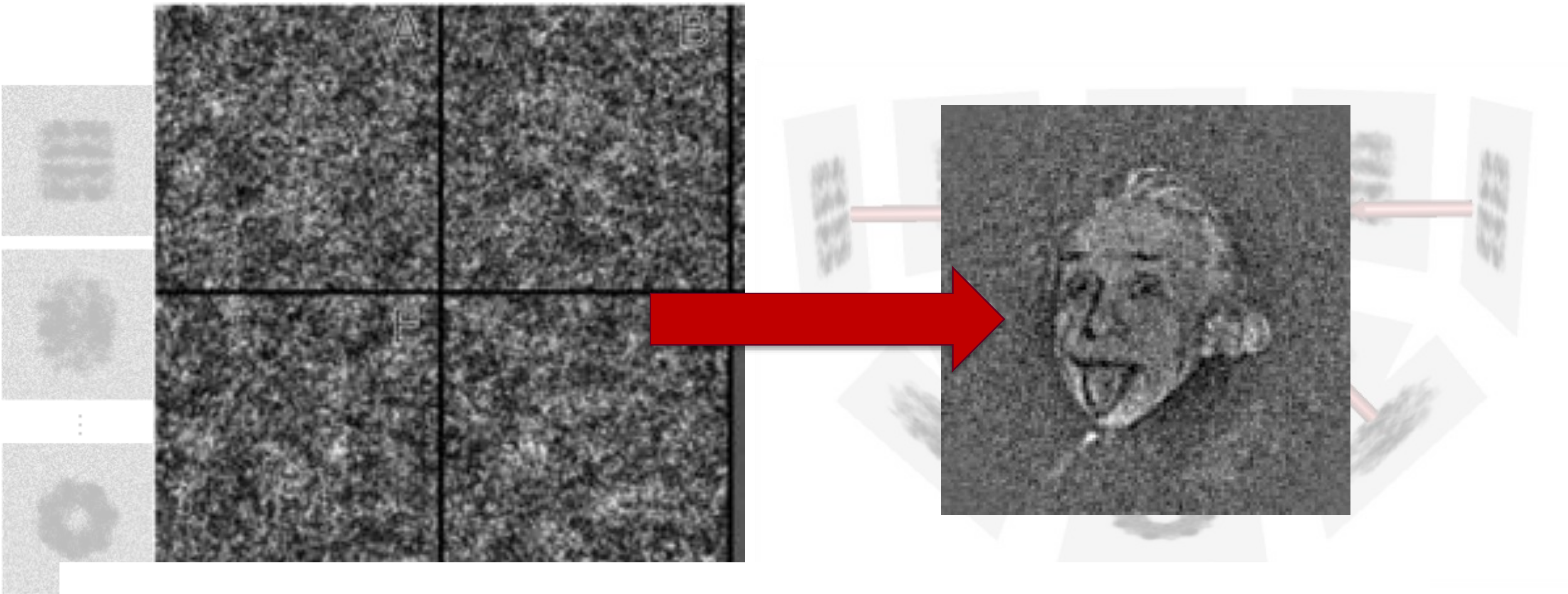
EMAN2

- **EMAN2 is a group of programs, utilities and a pipeline for EM image processing**
- **EMAN excels in several topics:**
    - **Quick computation on small computers**
    - **Tweakable 3D initial model building**
    - **Template based and AI based particle picking**



Maintained by S. Ludtke
Baylor College of Medicine
(https://blake.bcm.edu/emanwiki/EMAN2)

Single particle reconstruction

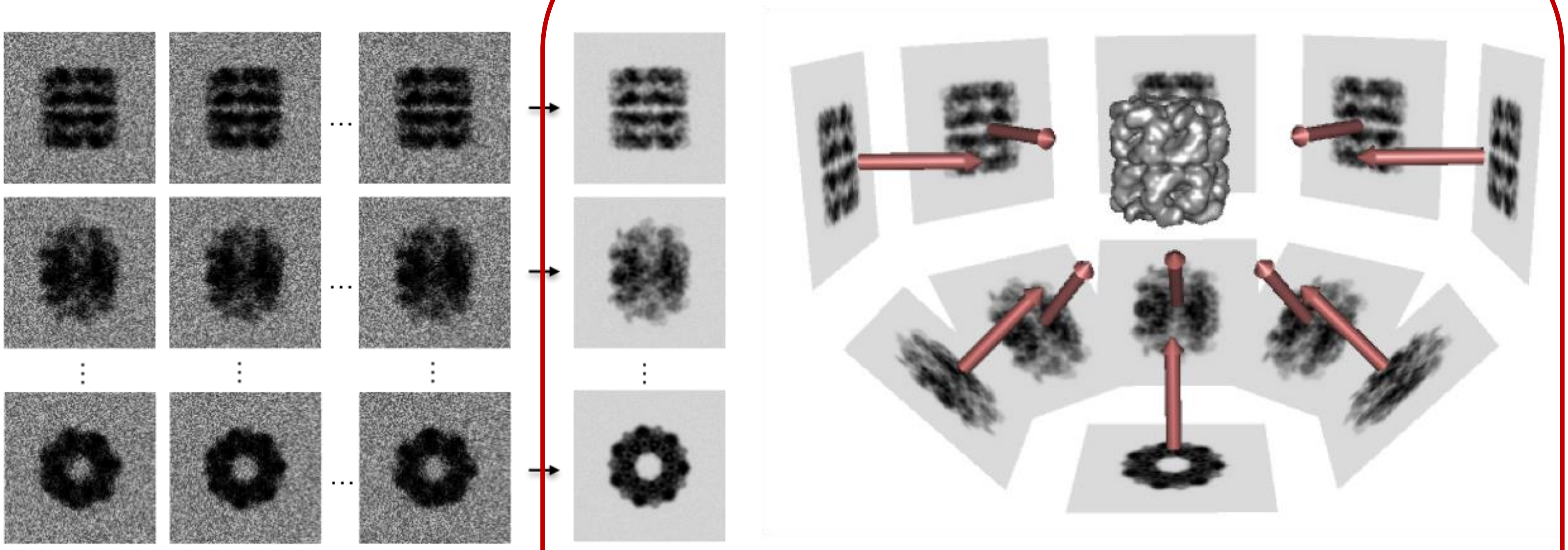


Reconstruction is a type of a fit!!!

- We determine the shifts and rotation parameters of each particle
- Very high noise

Clear need for a good initial guess for expectation maximization to succeed

Single particle reconstruction



Particle peaking

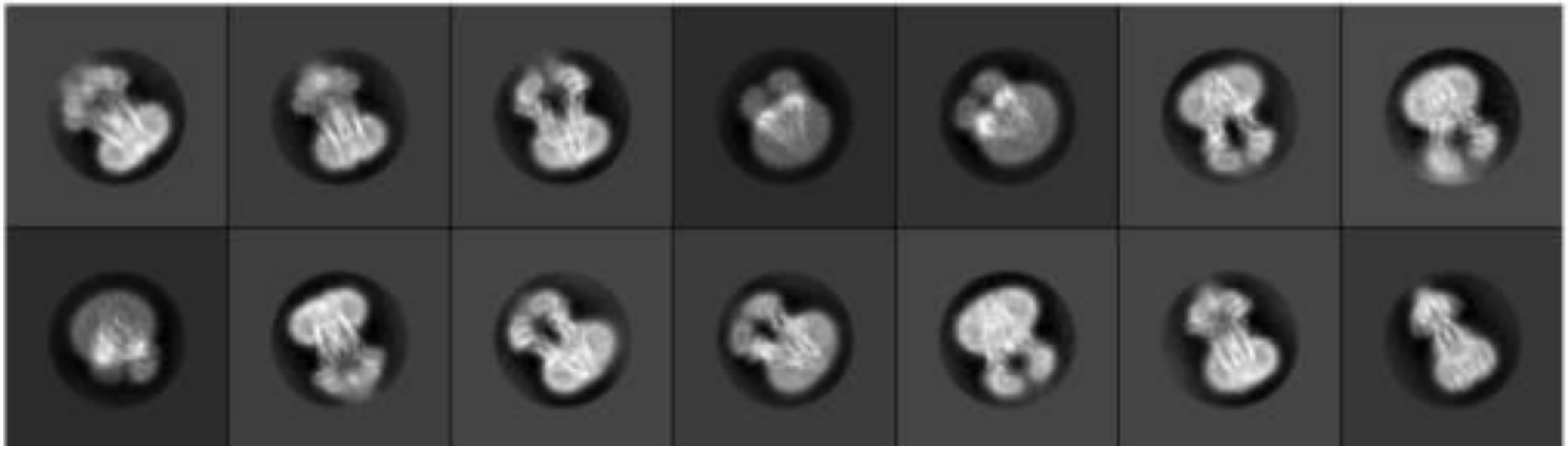
Class averages

Reconstruction

EMANS strategy: 2D Classes averages are less noisy
Used them for generating the initial model

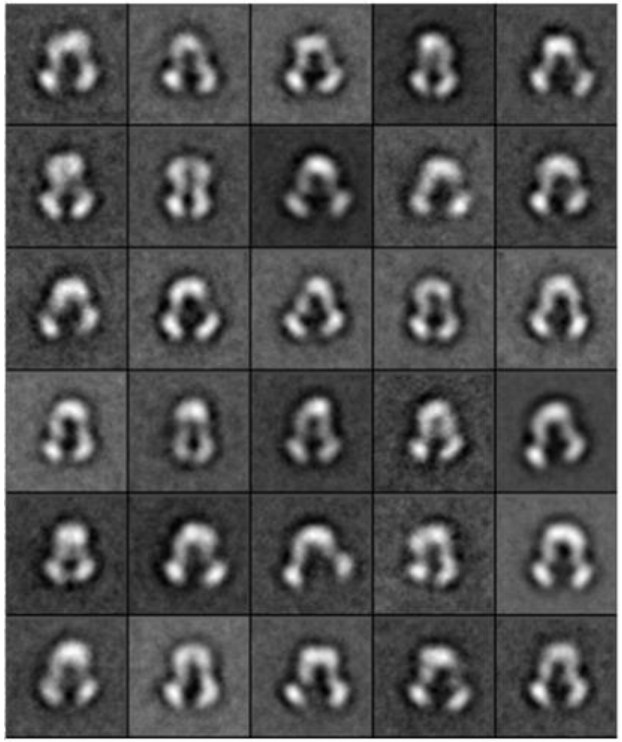
We can learn from 2D class averages

2D class averages is the last unbiased look you have on your data!



Data quality:
Look for secondary stricture elements

Flexibility – DDM vs nanodiscs

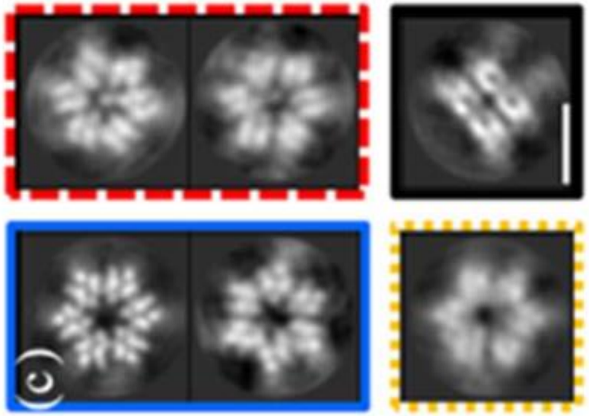


DDM

POPG nanodiscs (MSP1D1)

Quality of the biochemistry: (negative stain in this case)

- Preferred orientation
- Integrity of molecular complexes
- Symmetry and number of units
- Class average which are mirror image of each other – A good sign
- Compositional and conformational variably



What to look for in your 3D model

- Features with handedness
- Good fit of the projections to the 2D classes

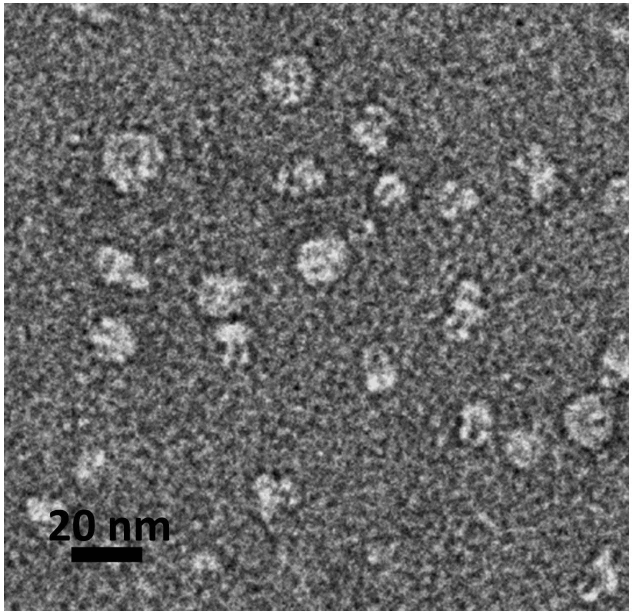
Tricks

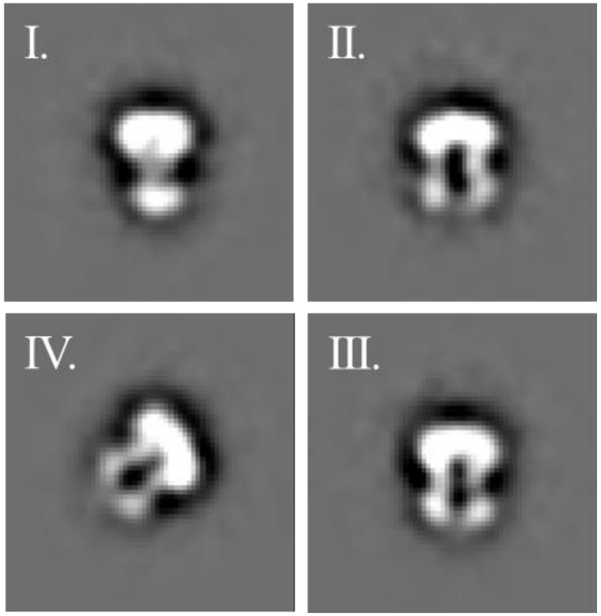
- Automatically deleted classes can be added in a larger number to insure their incorporation
- Class averages can be introduced from any other program
- Reduce box size in case of “long” artificial horns

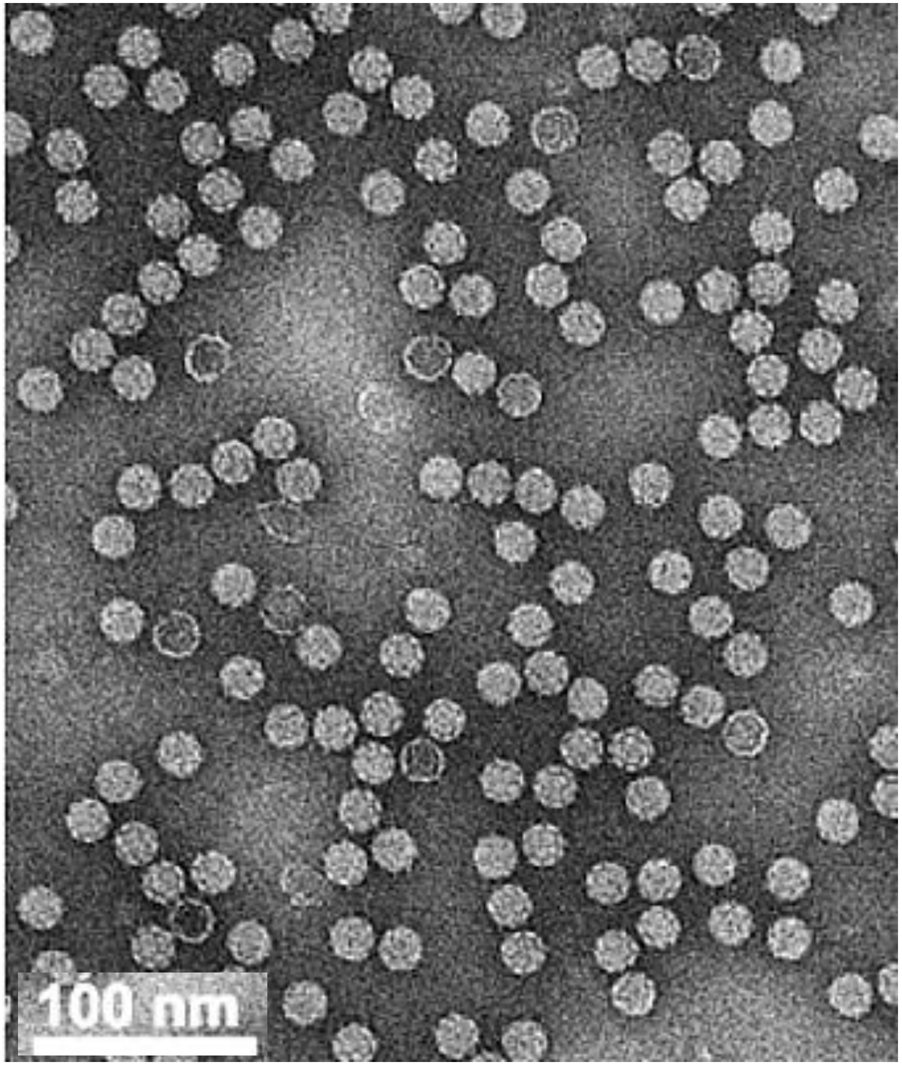
Thanks

Yehuda Baruch – BGU
Mario J. Borgnia - NIH

Negative staining microscopy

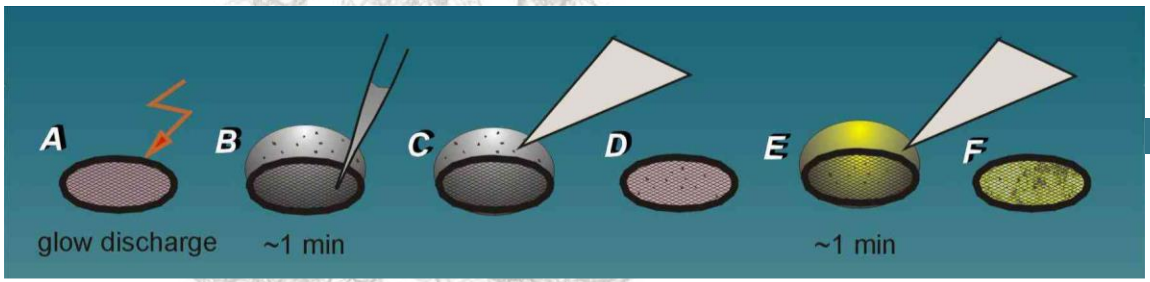


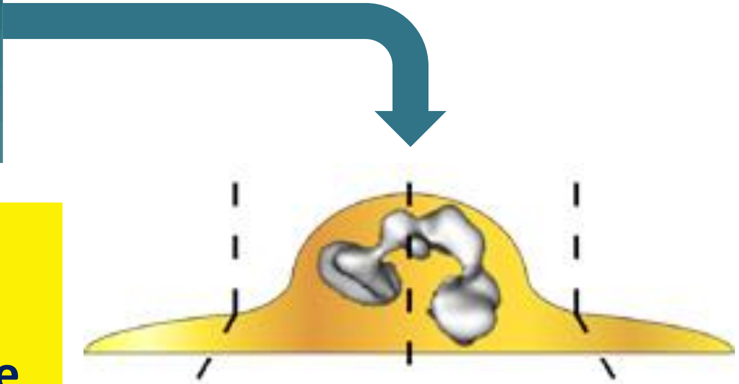




Negative staining microscopy - protocl

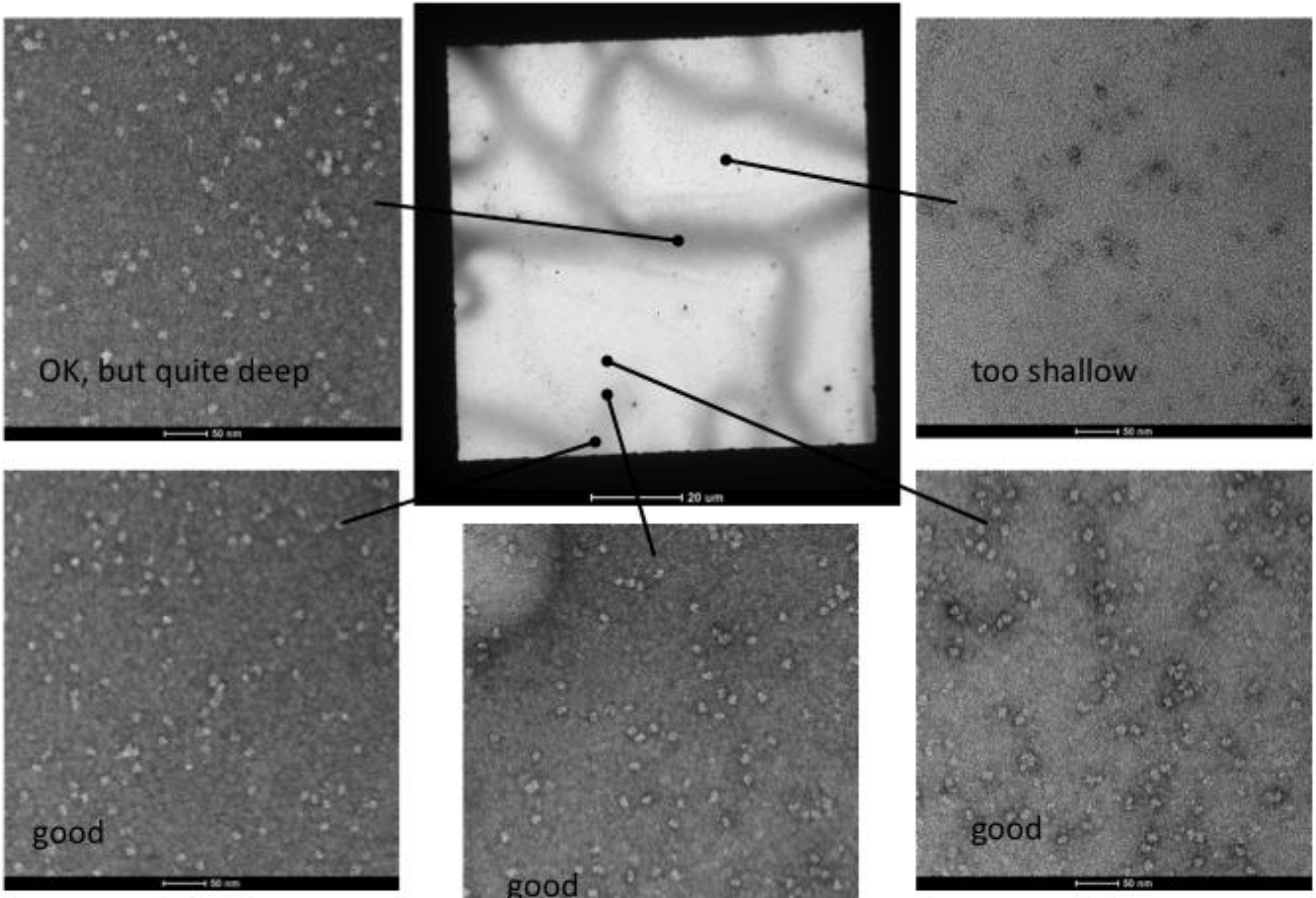
- A sample deposited on a carbon coated grid
- Most of the sample is wicked off
- A drop of staining solution is placed on top of the sample
- Most of the staining solution is wicked off – the rest is allowed to dry
- Staining encloses the structure of interest in a metal salt





- Uranyl Acetate or Formate pH 4-5
- Ammonium Molybdate pH ~7
- Methylamine Vanadate, Methylamine Tungstate

Negative staining microscopy – in the TEM



Negative staining microscopy

- Quick and affordable
- Easy to do and analyze
- Very good for quality control and testing homogeny of the samples
- Quick low resolution epitope mapping
- Only ~20-25 Å
- Artifacts: Effects of pH, interaction with the carbon, samples are "squashed"