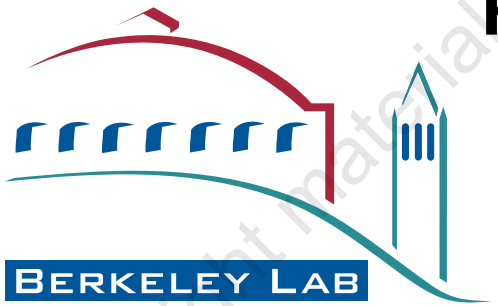
IN-FOCUS PHASE CONTRAST FOR EM

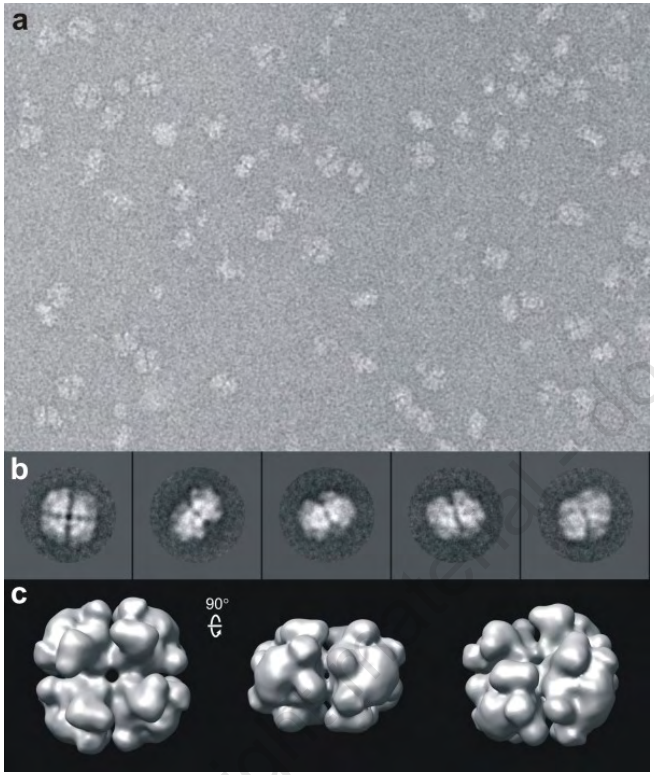
- **Why It Will Bring Electron Microscopy Of Multiprotein Complexes To A New Level**
- **And what It Will Take To Do So**

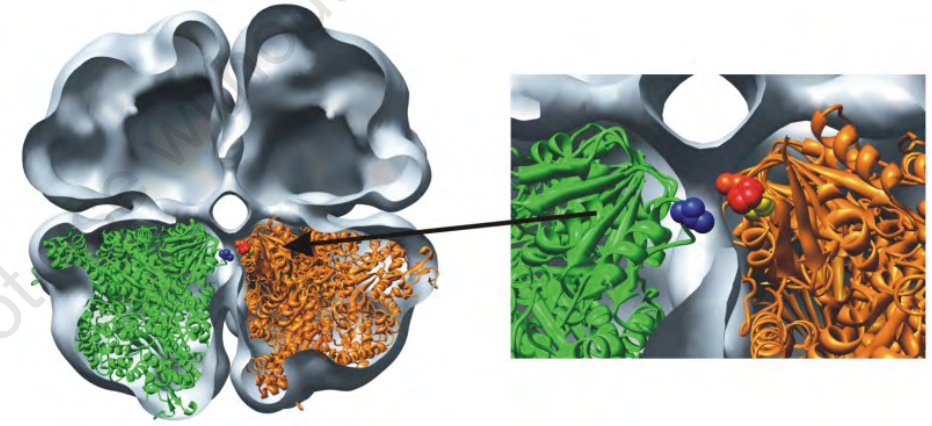
Robert M. Glaeser



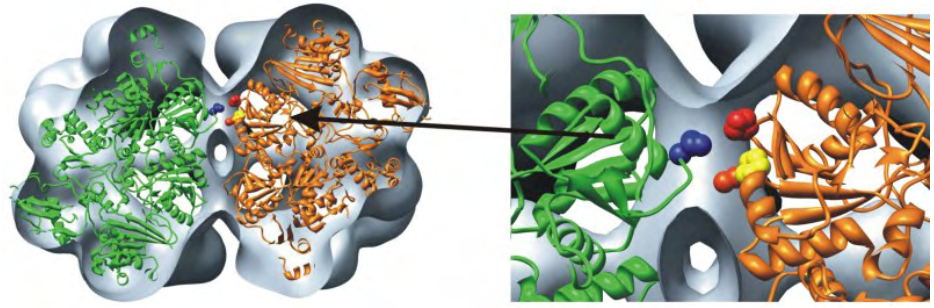
HYBRID IMAGING (EM + Crystallography) PROVIDES A POWERFUL METHOD TO CHARACTERIZE LARGE MULTIPROTEIN COMPLEXES

Example from the Genomics:GTL program at LBNL: A single valine-residue insertion causes this redox enzyme to assemble into a 1 MDa octomer in a microbe of interest to DOE's program in bioremediation

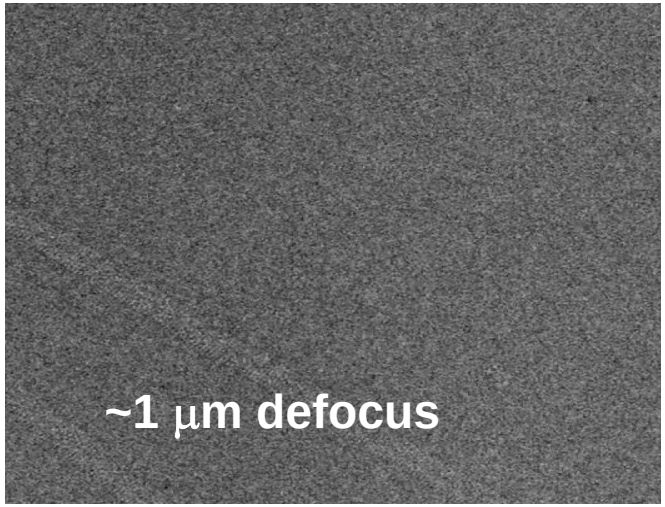


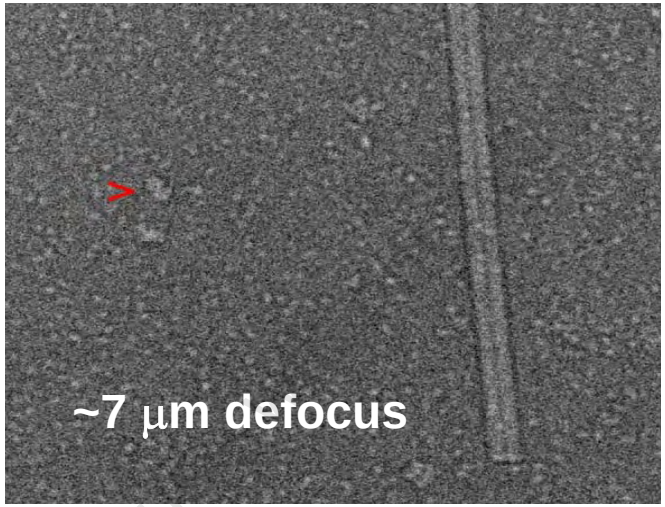


Garczarek et al. (2007) In Press



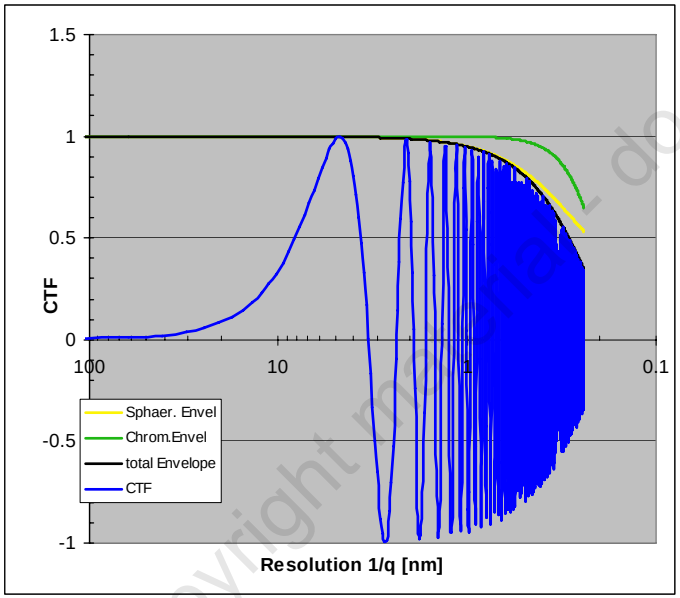
MOST APPLICATIONS INVOLVE SPECIMENS THAT ARE WEAK PHASE OBJECTS

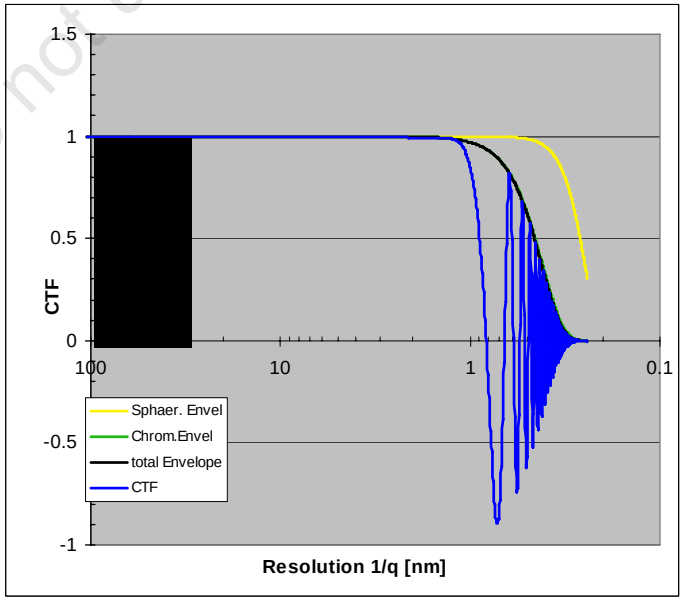




Dimeric protein,
MW = 250 kDa
plus TMV "marker"
Garczarek et al.
(2007) J. Struct. Biol.

Contrast *vs* Resolution





Defocused phase contrast (left)
vs
PROPOSED
in-focus phase contrast (right)

PHASE CONTRAST REALLY WORKS AS EXPECTED !

- **Comparison on the left is courtesy**

  K. Nagayama & R. Danev,

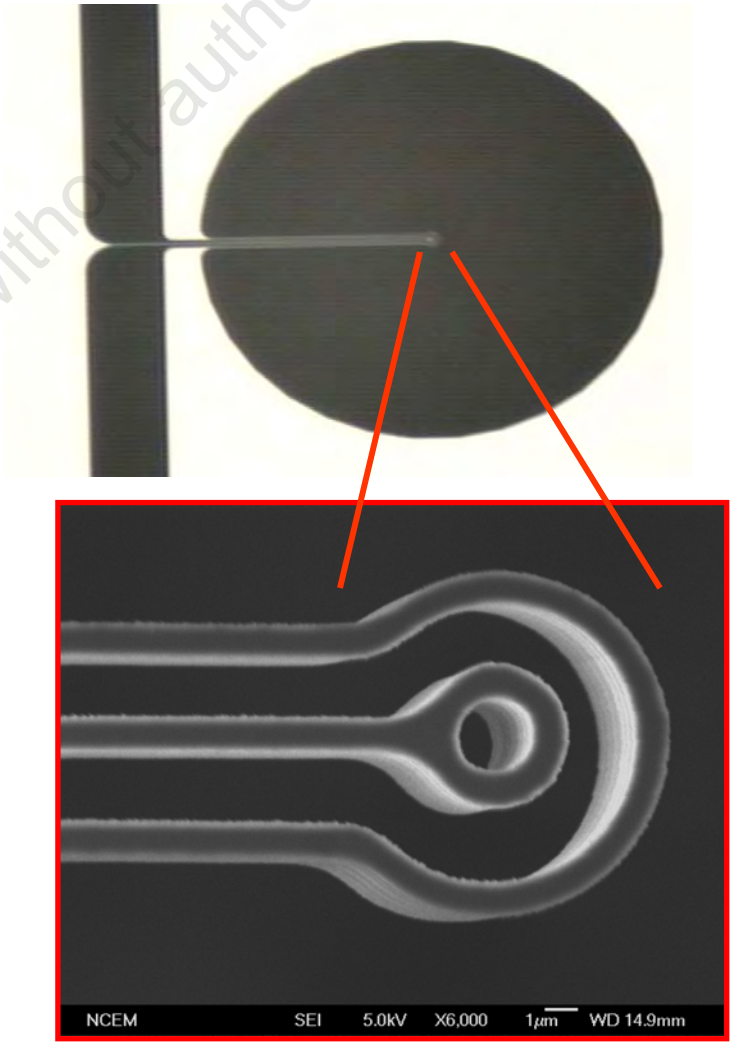
  Okazaki Center for Integrative Bioscience

  - **GroEL, unpublished**
  - **Objective aperture covered with a thin carbon film with an 0.5 μm central hole**

- **Particles as small as ¼ the size of GroEL should be easy to identify and "box"**
  - **i.e. M_r ~200 k**
  - **Without the limitations of CTF oscillation**

DEVELOPMENT OF IN-FOCUS PHASE CONTRAST IS UNDER WAY WITHIN THE LIFE SCIENCES DIVISION AT LBNL

- **Based on Jian Jin's design for a shielded "drift tube"**
- **Easily fabricated on the μm scale (Rossana Cambie)**
- **Electrostatic calculations and an experimental proof of concept have just been published: Cambie et al. (2007) Ultramicroscopy 107:329-339**
- **The full system is currently awaiting review**



ADDITIONAL DESIGNS ARE CURRENTLY PROPOSED OR IN DEVELOPMENT WITHIN THE EM-COMMUNITY

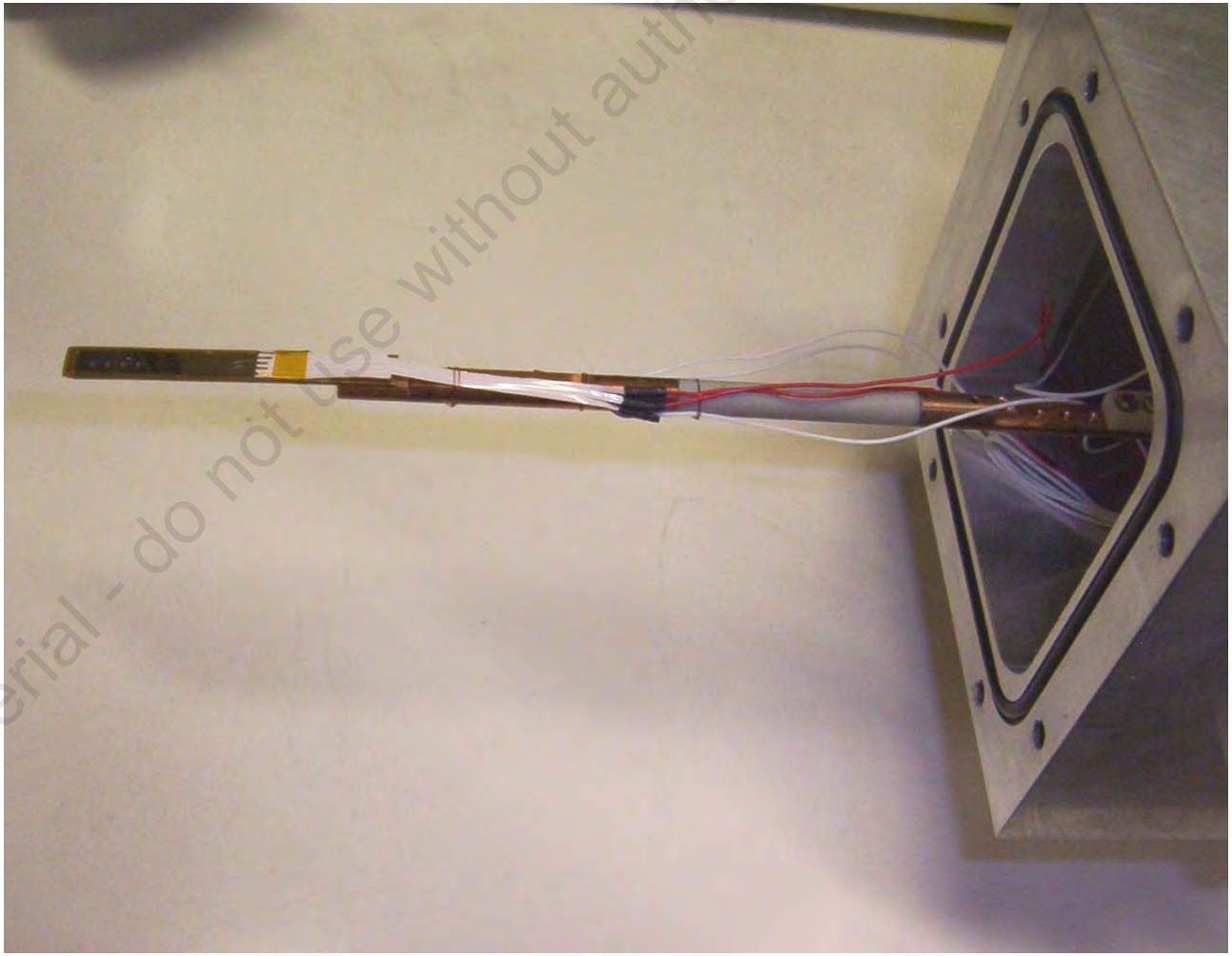
- **Thin-film (quarter-wave, "Zernike") phase plates are currently the leading technology**
  - **Nagayama's lab (Okazaki)**
  - **Mike Marco (Albany)**
- **Electrostatic phase-contrast apertures come in at least two designs**
  - **The 3-electrode, Einzel lens that was first proposed by Boersch in 1947**
    - **Schroeder et al. (Frankfurt)**
    - **Kisielowski et al. (LBNL)**
- **A clever, third alternative that would apply the phase shift to the unscattered beam at a "line" focus with an aberration-correcting lens element**
  - **Schroeder et al. (Frankfurt)**

HURDLE #1

PHASE-CONTRAST APERTURES ARE *EXTREMELY* SENSITIVE TO CHARGING THAT OCCURS IF THERE IS ANY CONTAMINATION

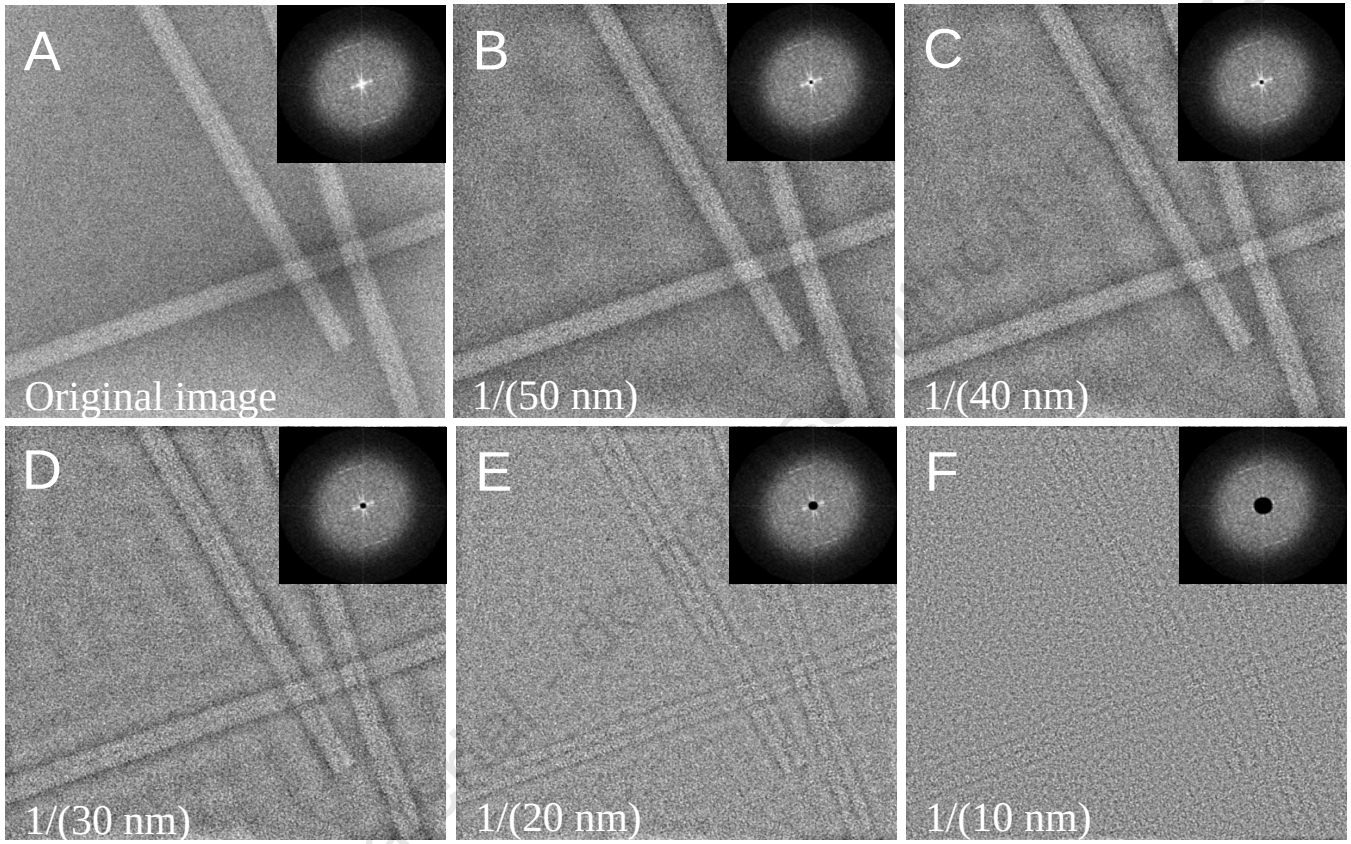
LBNL design allows heating the aperture strip to 200 or 300 C during operation, in order to avoid beam-induced contamination.

Piezo-positioner provides for feedback-recentering during operation



HURDLE #2

THE ELECTRODE-DEVICE MUST BE EXTREMELY SMALL IN ORDER TO PASS THE LOW SPATIAL FREQUENCIES NEEDED TO SEE SPECIMENS



Simulation completed by Dieter Typke

POTENTIAL SOLUTIONS ARE:

- **Magnify the electron diffraction pattern at the plane of the aperture**
- **Further reduce the scale of the electrodes in the aperture**

ACKNOWLEDGEMENTS

- **Design and Engineering**
  - **Jian Jin**
  - **Rossana Cambie**
- **Preliminary expts. and conceptual simulations**
  - **Dieter Typke**
  - **Jian Jin**
  - **Rossana Cambie**
  - **Ken Downing**
- **Single-particle EM**
  - **Eva Nogales; Florian Garczarek;**
  - **Colleagues within the Protein Complex Analysis Project (PCAP) at LBNL**
- **Research Support**
  - **DOE Genomics:GTL**
    - **Sub-project on electron microscopy of multiprotein complexes**
  - **LDRD within LBNL**
  - **CRADA with Gatan, Inc**