HIGH-RESOLUTION ELECTRON MICROSCOPY WITH SUPERCONDUCTING LENSES AT LIQUID HELIUM TEMPERATURES*

By Humberto Fernández-Morán

DEPARTMENT OF BIOPHYSICS, UNIVERSITY OF CHICAGO

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Following our first successful imaging experiments[1-3] with high-field superconducting solenoid lenses, numerous methodological and conceptual problems remained to be solved before the specific advantages of this approach could be adequately established for high-resolution electron microscopy. These problems included: (*a*) incorporation of superconducting solenoid lenses and associated cryogenic components into high-performance electron microscope systems; (*b*) precise control and reproducible current setability for focusing superconducting solenoid lenses; (*c*) satisfactory specimen mounting to prevent temperature drift and achieve high degree of stability during irradiation; (*d*) stabilization of lens excitation currents and accelerating voltage, and improved electron source characteristics for low-temperature microscopy; (*e*) reduction of magnetic, electrical, and mechanical perturbations under carefully controlled conditions in the requisite cryogenic environment; (*f*) adequate continuous recording of images without breaking high vacuum under cryogenic conditions.

Meeting these requirements has involved a major development and research effort in this laboratory during the past few years. Pursuing a comprehensive research program with different types of cryo-electron microscopes, our earlier work[1-6] has been extended, confirming the exceptional stability of high-quality images (50–100-Å resolution) recorded exclusively with superconducting solenoid lenses at 4–32 kilogauss and 4–50 kv accelerating potential. Using a superconducting niobium-zirconium objective lens, operating in a specially designed liquid helium cryostat with superconducting stigmators and regulating circuitry, it has now been possible to record, for the first time, electron micrographs of biological specimens at 4.2°K, reproducibly attaining resolutions of 10–20 Å with minimized specimen modifications. In addition, the unique combination of high magnetic fields, liquid helium temperature, and high electron optical magnifications has enabled us to make preliminary observations on characteristic electron optical phenomena associated with trapped fluxes in thin superconducting films. Salient aspects of this work are described in the present report.

Experimental.—All of the equipment used was specially designed, developed, and tested in our laboratories, making use, wherever possible, of commercially available electronic and cryogenic components. In view of the stringent requirements, even special superconducting equipment which was built to our specifications by commercial firms had to be extensively modified and adapted in our workshops. In general, our work has centered on two approaches:

(1) *Cryo-electron microscope optical bench system* (Fig. 1) comprises a modified air-core liquid helium Dewar with different types of niobium-zirconium solenoid lenses[1-3] operating at 4–32 kilogauss without pole pieces, and with modified objective, and objective-projector pole pieces. The objective pole pieces were essentially of conventional design with focal lengths of 1.6–2.6 mm. Available 25 amp regulated power supplies with storage batteries had to be considerably improved and used in conjunction with additional current vernier control circuits. Under suitable conditions this system permits adjustable current changes of 10^{-9} for achieving reproducible "super-

fine" focusing, which is orders of magnitude better than conventional systems. The current vernier control specially developed for our lenses by Westinghouse Cryogenics Division consists essentially of a low-field, two-winding, superconducting toroid with its secondary connected in series with the main solenoid. After achieving optimum focusing adjustment, transfer of toroid primary to persistent mode places the entire system in a lossless, completely stable condition. Improved point cathode sources[7, 8] which give stable coherent microbeam illumination of small spot size, (0.4–1 μ diameter), high specific brightness, and low energy spread were used with highly stabilized power supplies (mainly 50 kv) connected to a central regulated motor generator set with solid-state regulators giving better than 0.1% main line voltage stability and very low harmonic distortion. Special precautions were taken throughout to minimize mechanical, electrical, and particularly magnetic field perturbations which require extensive moly-permalloy 4-79 shielding. Various types of specimen holders were specially developed and tested to give optimum stability in cryogenic environment, including provisions for multiple sample holders and piezoelectric specimen manipulation within the Dewar described in detail elsewhere.[4]

(2) *Superconducting objective lens in liquid helium Dewar of special design* (Fig. 2) may be used as an integral part of cryo-electron microscopes, or to replace the objective lens in modified high-resolution commercial electron microscopes. This lens, which was designed and built according to our specifications by Westinghouse Co., comprises niobium-zirconium main and vernier coils (32,580 amp-turns) with superconducting stigmators, persistent current switches, and improved current control devices. As described elsewhere,[4] specimens are mounted on microstages maintained at 4.2°K, and can also be inserted within pole pieces of different types, including short focal length (f = 1.5–1.8 mm), single-field condenser-objective pole pieces[9] of iron or dysprosium, and trapped-flux Nb_3Sn miniature lenses. Our cryoelectron microscope IV is mounted on a 10-ton vibration isolated base, and features extensive magnetic shielding, ultrahigh vacuum ion pump system, improved field and T-F emission source, 50 kv–100 kv highly stabilized accelerating potential, and image intensifier with provision for videotape recording. Photographic recording was usually carried out on high-resolution Kodak or Ilford plates and on Kodak High Definition 70-mm films with a thin polyester base. The test specimens included replicas of diffraction gratings (2160 lines/mm), fenestrated carbon films with catalase and asbestos, and evaporated thin-films of niobium, niobium-zirconium, or lead. The results reported here are based on evaluation of more than 1000 plates and films directly recording the electron optical images.

Results.—After systematically overcoming the main experimental difficulties (elimination of specimen drift and of mechanical vibrations due to cryogenic liquid boiling, attainment of precise focusing and stability of image, etc.), it was possible to operate these cryo-electron microscopes, routinely attaining reproducible results when operating at 4–32 kilogauss, preferably in persistent current mode, and 50 kv. After focusing the specimen image on the fluorescent screen with the precision setability circuitry at electron optical magnifications of 200–20,000×, using solely superconducting lenses, the solenoid is switched into persistent current mode. Aside from slight initial shifts in the image, which can be readily corrected while still in the persistent current mode, the images thus maintained without any external current source are of an unprecedented degree of stability and high quality (Fig. 3) with resolutions of 50–100 Å, susceptible of considerable improvement by correction of astigmatism.

By virtue of this exceptional stability, exposures of 30 sec to several minutes are possible when using microbeam illumination of low-intensity and high-resolution plates. As a result of the integrated long-term exposure made possible by the superstability of the lens, fine detail can be observed, in micrographs displaying a very large field recorded with a single lens (Fig. 3*e*), which is normally only discernible in the best high-resolution micrographs at higher magnifications (Fig. 3*g*). The same area can be continuously recorded at 5- to 15-min intervals (Figs. 3*a*–*d*) over periods of 10–20 hr under carefully controlled conditions without detectable

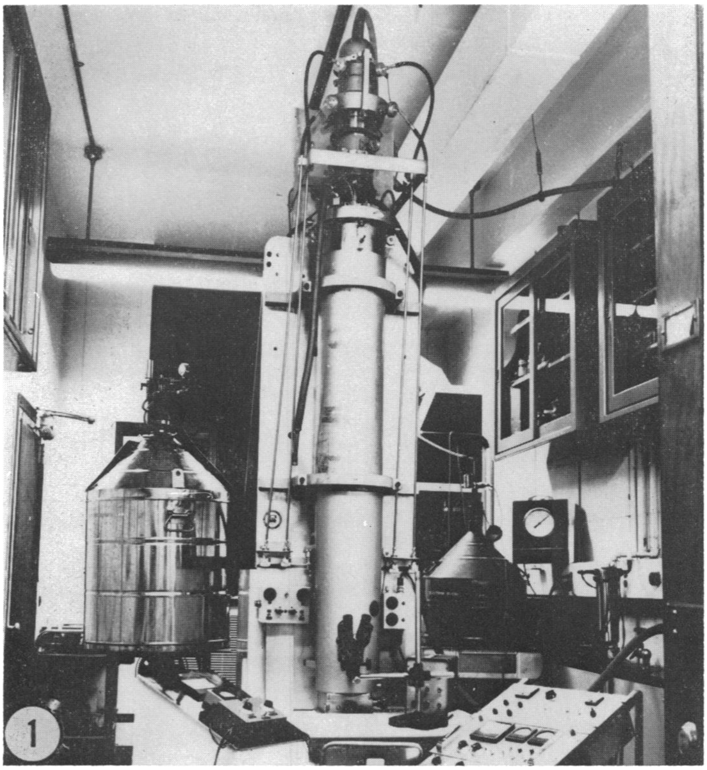
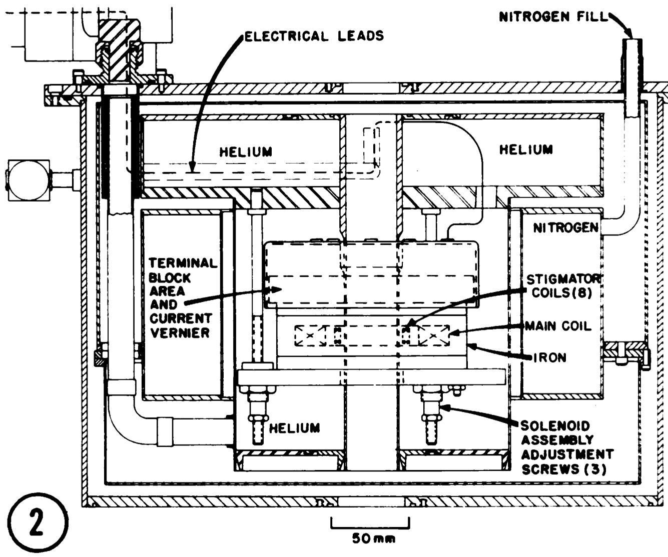


FIG. 1.—Cryo-electron microscope with high-field Nb-Zr superconducting solenoid lenses, regulating circuitry, and power supply controls.



SUPERCONDUCTING OBJECTIVE LENS AND LIQUID HELIUM COLD STAGE ASSEMBLY FOR CRYO-ELECTRON MICROSCOPE

FIG. 2.—Sketch of superconducting objective lens with stigmators and vernier coils of special design.

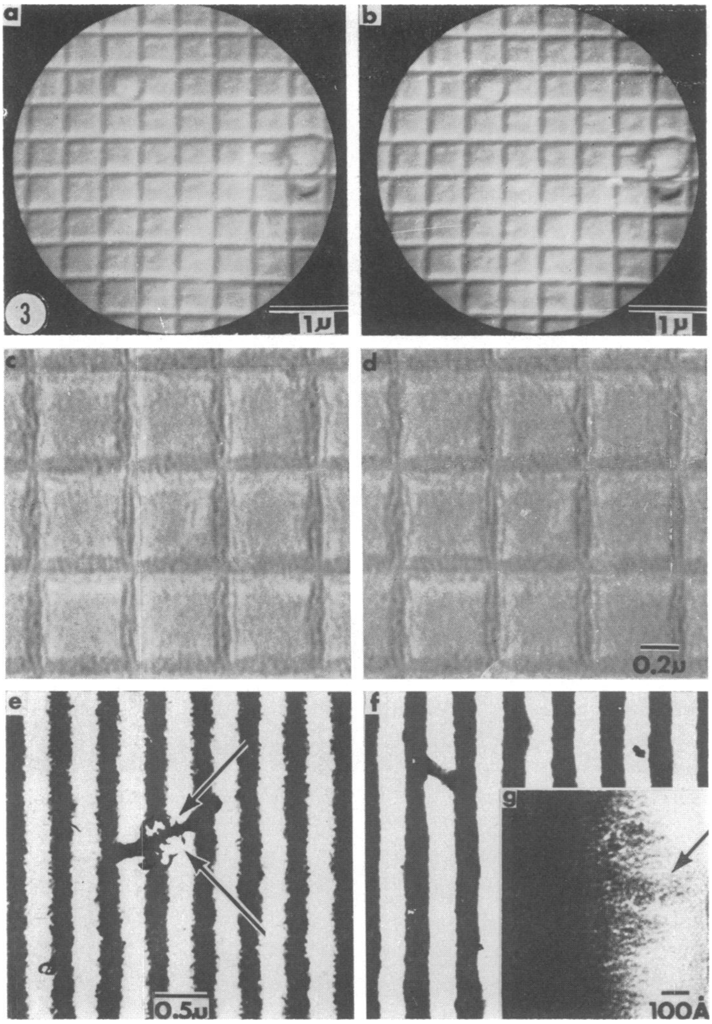


FIG. 3.—Electron micrographs of 2160 lines/mm diffraction grating replicas recorded at 50 kv (10–20,000 × electr. opt.) with superconducting lens in persistent current mode continuously over 10-hr period. Unaltered images of first (*a,c*) and final (*b,d*) series demonstrate lens superstability. Fine detail discernible in (*e*) resulting from long-term exposure is only found in control standard micrographs (*f*) at higher magnification (*g*).

image changes. Demonstration of this typical long-term superstability has, in fact, led to a critical survey and detection of other sources of mechanical, magnetic, and electrical perturbations which had previously been masked by lens fluctuations and ripple. In conjunction with the improved (cryogenic) vacuum and reduced specimen damage, this unique lens stability already represents a major advantage of the superconducting lens system. Preliminary experiments with high-resolution electron microscopy and diffraction, combining the exceptional stability of superconducting lenses with coherent microbeam illumination, appear promising for practical realization of Gabor's wave-front reconstruction microscopy.[16]

Electron microscopy and diffraction of specimens at liquid helium temperatures:

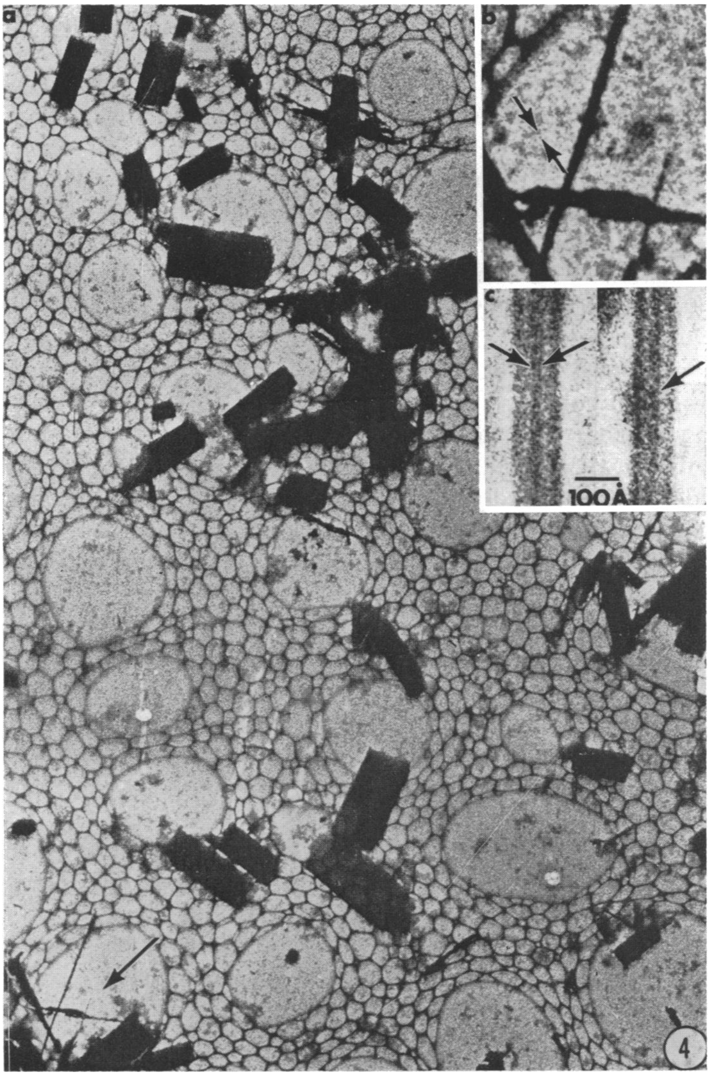


FIG. 4.—Electron micrographs of stained catalase asbestos specimen on carbon film recorded at 4.2°K using superconducting objective lens without pole piece in persistent current mode at 75 kv, demonstrating 10–20-Å resolution in asbestos filaments (*c*). (*a*) 18,000×; (*b*) 55,000×; (*c*) 700,000×.

With improved objective lens and cold stage assemblies, it has recently been possible to examine biological and other types of specimens which are kept at 4.2°K. The major problem of specimen drift previously encountered has been largely solved by combined use of adequate cryogenic shielding, stable specimen mounting, and microbeam illumination of low intensity and small spot size. Moreover, without using pole pieces, it is possible to compensate our superconducting objective lens, operating in the persistent current mode at 4–8 kilogauss, by using the special superconducting stigmators and shim coils. Under optimum conditions, excellent electron optical images can be recorded at magnifications of 200–40,000× using the superconducting lens in conjunction with standard projector and intermediate lenses. As shown in Figures 4*a*–*c*, test specimens of catalase with asbestos fibers (of *ca.* 100–200 Å diameter and central channel of 20–50 Å) stained with uranyl-acetate, exhibit clearly resolvable details in the size range of 10–20 Å (point resolution). Although no objective apertures were used, the image contrast is good, and the 80–90 Å periodic structure of the catalase crystals can be detected in certain areas. The transparency and clarity of the images are noteworthy, particularly the lack of contamination after prolonged exposure. In specimens shadowed with lead, the characteristic electron transparency at low temperatures, first described by Boersch,[10] can be detected by electron microscopy and diffraction.

Trapped flux experiments: Thin films (*ca.* 200–500 Å) of niobium or niobium-zirconium evaporated on fenestrated thin-film carbon substrates, exhibit interesting phenomena when examined at low temperatures (4.2°K) and relatively high fields (4–8 kilogauss without pole pieces and up to 20 kilogauss with pole pieces). As shown in Figure 5*a*, the control micrograph recorded at liquid nitrogen temperature and low fields differs markedly from micrographs of the same area examined at 4.2°K and higher fields of about 8 kilogauss (Fig. 5*b*). A series of characteristic fringe patterns is observed under the latter conditions, which vary in configuration and dimensions according to the applied field strength. This reproducible effect is tentatively assumed to be associated with the Lorentz deflections induced by the trapped magnetic flux patterns, resembling the imaging of magnetic domains in conventional microscopy at normal temperatures.[11] Preliminary observations made under conditions of higher beam intensity, and close to critical transition temperatures, reveal unexpected dynamic and complex phenomena displaying a constant state of flux of vortexlike regions resembling the "bee swarm" effect[11] described in thin-carbon and Permalloy films. These interesting observations, which can now be readily made over a wide range of temperatures and electron optical conditions, are being continued and will be reported in detail elsewhere.

Discussion.—The unique stability and relatively high quality of the images recorded at low temperatures with superconducting solenoid lenses is revealing and encouraging, considering the advanced state of development of conventional high-resolution electron microscopy. It clearly indicates the potentialities of a highly promising experimental approach which has opened up with recent advances in superconducting technology[12–14] but which is only now emerging from the confines of timid or skeptical speculation. Our own experimental approach is primarily designed to provide essential data for the development of high-resolution electron microscopy of biological specimens, examined in the frozen hydrated state under the ideal low-temperature conditions of minimized specimen contamination, radiation damage, and thermal noise.

However, now that the advantages of superconducting lenses in electron microscopy have been demonstrated, the way is open for new approaches which are unique to superconductors as perfect diamagnetic materials. Thus, the possibility of using superconductors for correction of spherical aberration as first suggested by Marton[15] becomes susceptible of experimental verification. Moreover, the obvious advantages of high-field superconducting lenses for both high-voltage and low-voltage electron microscopy enter into the realm of practical realization. Cryo-electron microscopes should also prove to be a powerful tool for direct visualization

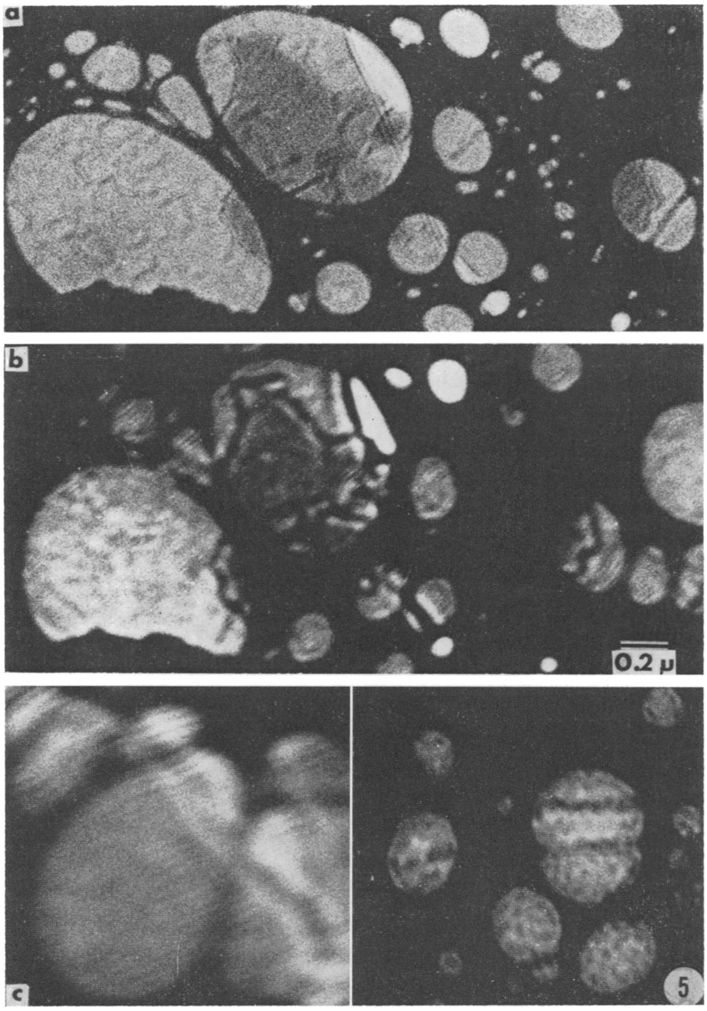


FIG. 5.—Electron micrographs of thin niobium film on fenestrated carbon substrate recorded with superconducting objective lens. Compare control images at low field, 90°K, with corresponding areas (*b,c*) examined at 4.2°K, and high fields, showing characteristic fringe patterns presumably associated with trapped flux. 30,000×.

of fundamental phenomena in thin superconducting films. It is therefore hoped that despite the inherent experimental difficulties and uncertainties, further critical and constructive work will continue in this field.

Summary.—Extending earlier work, results are described which were obtained with different types of cryo-electron microscopes using high-field, niobium-zirconium solenoid lenses with specially designed cryogenic specimen stage assemblies, pole pieces, and superconducting regulating circuitry. Exceptional long-term stability and high quality of electron microscopic images directly recorded at 200–20,000× were demonstrated under carefully controlled conditions, operating in persistent current mode at 4–32 kilogauss and 4–50 kv accelerating potential. Electron micrographs of biological specimens were recorded at liquid helium temperatures (4.2°K)

with a superconducting Nb-Zr objective lens of special design, reproducibly attaining resolutions of 10–20 Å. Preliminary experiments have demonstrated characteristic electron optical phenomena associated with trapped fluxes in thin superconducting films.

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