We at Denton Vacuum then introduced the magnetron sputterer for EM work. It employs lower voltages and produces much higher yield per unit of power which is much better for biological specimens. All EM sputterers now sold are magnetron. We added one further wrinkle, a grid anode which further reduces electron bombardment of the specimen. Other techniques such as cooling the specimen have also been used. Some of these mechanical pumped units are compatible for carbon evaporation with argon bleed.

Another answer without a question for a good many years was freeze drying. In this method, the specimen is quick frozen on the bench using liquid nitrogen in one way or another and placed on a cold surface in a vacuum chamber. The cooling in the chamber is done by multi-stage Peltier cooling.

The specimen must be kept solid to preserve the basic structure while the water sublimes. I believe the first system for this procedure was invented by Pearse in England and built by Edwards. It has a port at the top where a potting resin can be placed and dropped onto the specimen after dehydration. Then the current on the Peltier stage may be reversed to provide heat and set the resin.

For TEM work, the specimen must then be sectioned. As with freeze drying, this procedure did not receive wide use until the scanning electron microscope came along.

Russell Steere was a pioneer in the process known as freeze fracture or freeze etching. He did this work while a student of Robley Williams who had moved to the University of California, Berkeley. Steere spent three years at Berkeley working on this process.

Balzers entered the field and later made the first production units specifically for freeze fracturing. They introduced a cleverly designed microtome for fracturing. Balzers makes most of the freeze etching equipment today.

As the field progressed, the necessity for minimizing specimen contamination was apparent. Steere's system, built by Denton Vacuum, utilized a liquid nitrogen cooled shroud around the specimen which is fractured, shadow cast, and carbon is replicated inside this shroud.

The specimen is shadowed and replicated through two small apertures. This, I believe, was the first really clean freeze fracturing. Another approach is to pump the entire chamber to a very low pressure using cryopumps and liquid nitrogen traps. A Frenchman, Jacques Escaig, made some very sophisticated, commercial, and cryogenic systems using ion and cryo pumps.

All drying and freeze techniques are for specimens containing water. One preparation method used on electrically conducting specimens was called cathodic etching. This was first used in 1948 by Don McCutcheon of Ford. The specimen was made the cathode in a DC discharge. Today I don't think this technique is used since there are now many variations available of ion bombardment, plasma etching, etc. that boggles the mind.

Many hard specimens such as metals, cermet, ceramics, semiconductors are prepared for TEM examination by ion beam thinning. Ion beam thinning is a standard method for TEM specimens in which the specimen is ground very thin, then placed in a vacuum chamber and bombarded with dual ion guns until thin enough for examination. This technique was developed in France by Paulus and Reverchon in 1961 (see following Figure). The first production systems were made in France, I'm told, by Alba, but no one seems to have any photos or literature on them. Several companies now offer ion beam thinning equipment.
To argue the number is a difficult win.
As there is but one angel dancing on the head of a pin
... Compliments of Jean-Paul Revel