LOOKING AT SLOW AXONAL TRANSPORT

Stephen W. Carmichael and W. Stephen Brimijoin,[1] Mayo Clinic

Neurons are about as polarized as cells ever get. Their axonal process can extend a distance that is up to a million times the diameter of the nerve cell body. Axons have none of the ribosomal machinery responsible for protein synthesis, so all neuronal proteins and peptides must be manufactured near the nucleus and carried out to the periphery. This distribution involves at least two distinct mechanisms, fast axonal transport, moving at almost 500 mm per day, and slow axonal transport, moving only 0.1 to 3 mm per day. It turns out that proteins of the neuronal cytoskeleton, along with many soluble cytosolic proteins, are transported exclusively by the slower process. A long-standing unresolved question concerns the physical state in which cytoskeletal proteins are transported. Until recently it has not been known whether the axonal neurofilaments are assembled in the cell body and transported as intact structures, or whether their simple protein subunits are carried down the axon for assembly at or near the final destinations. Now a report[1] by Sumio Terada, Takao Nakata, Alan Peterson and Nobutaka Hirokawa, from the University of Tokyo and McGill University, definitively answers one part of this interesting question.

Clever methods and microscopic techniques provided the key to the answer. First, Terada and colleagues constructed a recombinant adenoviral vector encoding a specific, midsize neurofilament protein (NFM) tagged with a c-myc epitope. The selected protein could not form polymers by itself, so any transport along axons that lacked native neurofilaments would prove that subunits could be transported as such. NFM viral vector was used to infect sensory neurons, without damage, in dorsal root ganglia of transgenic mice. Most axons in these mice were completely free of neurofilaments, which precipitated in the cell body before exiting to the periphery. Epifluorescent microscopy showed that the exogenous NFM was incorporated into a characteristic neurofilamentous array in the sensory ganglia, as in normal mice. Furthermore, immunoelectron microscopy demonstrated that the protein co-assembled with endogenous neurofilaments in the nerve cell bodies. These results showed that the recombinant protein was functionally similar to native NFM. The question was whether excess subunits of recombinant NFM would move into sensory axons.

To answer that question, Terada *et al.* examined peripheral nerve about one week after ganglionic infection. Recombinant NFM protein was revealed by immunocytochemistry with a monoclonal antibody to the c-myc epitope, using a confocal laser scanning microscope. As expected, the protein could still be localized in the infected cell bodies, but some of it had also been transported into the peripheral axons. The front tip of transported NFM appeared to be displaced distally at about 1 mm/day, squarely in the range for "slow axonal transport." Interestingly, there were signs of synchronous NFM transport in different axons. These and other results were consistent with an active transport mechanism, rather than mere passive diffusion. The evidence is thus convincing that at least one neurofilament protein can be transported as monomers or small oligomers, even in the absence of neurofilaments.

The study of Terada *et al.* represents a breakthrough in neurofilament biology. Considered with other studies, the results support the theory that neurofilament components can be propelled by members of the kinesin superfamily, acting as "motors", along microtubules, acting as "rails". Terada *et al.* consider the slow axonal transport system to be a kind of default pathway. Though it remains to be explained why the average speed in this pathway is so slow, the new findings are a major step forward in addressing one of the general problems of cell biology: How do cytosolic proteins and components of the neuronal cytoskeleton reach their appropriate destinations?

1 The authors gratefully acknowledge Professor Nobutaka Hirokawa for reviewing this article.

2. Terada, S., T. Nakata, A.C. Peterson, and N. Hirokawa, Visualilzation of slow axonal transport in viro, *Science* 273;784-788, 1996.

Front Page Image

ULTRA HIGH RESOLUTION STEM IMAGE OF SI BOUNDARY

Z-contrast image of a 39° symmetric tilt boundary in silicon (S = 9 {221} < 110 > Si) as viewed along the [110] direction, showing the periodic array of perfect edge dislocations. Images taken with the VG Microscope HB603U, 300 kV STEM at Oak Ridge National Laboratoary (courtesy of M.F. Chesholm and S.J. Pennycook). For further information on this subject, refer to the article "The Analytical Limits: HADF (High Angle Dark Field Imaging)" by Dr. Michael Kersker of JEOL USA on page 14 of this publication.

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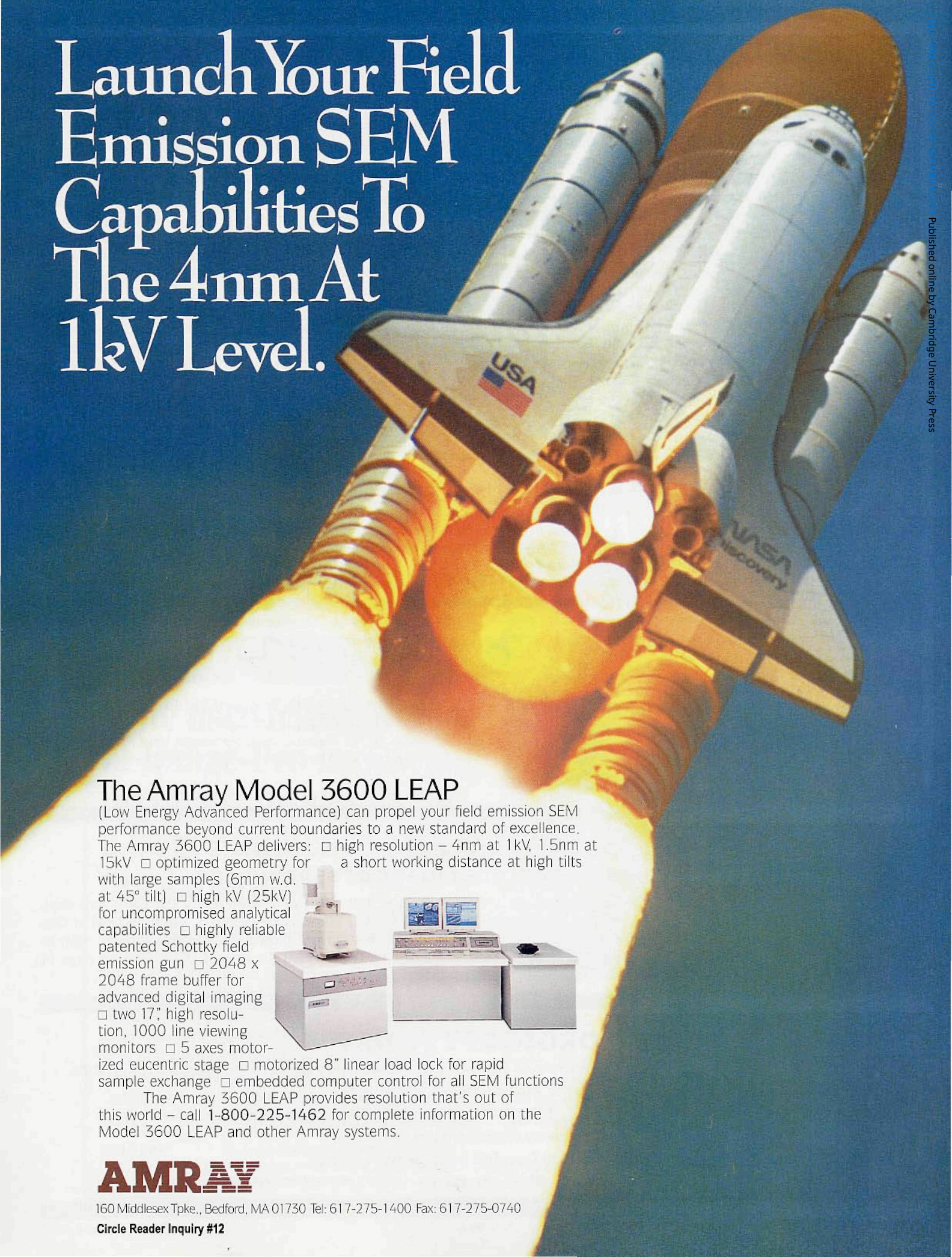

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