# The Nematode Caenorhabditis elegans A Model Animal "Made for Microscopy"

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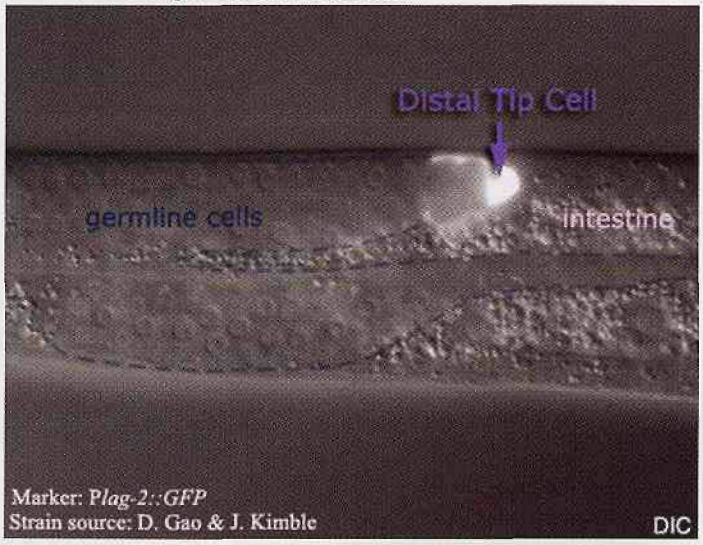
The small unassuming nematode, *Caenorhabditis elegans* is only one millimeter long and lives in the soil munching on bacteria. While many nematode (roundworm) species are parasites with medical or agricultural importance, *C. elegans* seems to harm no one. Yet, this animal has attained a status in medical science that compares to more complex organisms such as the mouse or fruit fly in its utility for scientific discovery. It has been the subject of thousands of studies dealing with topics as diverse as nutrition, aging, and nervous system development. About 5000 scientists are now pursuing this single species in hundreds of laboratories worldwide. In 2002, the Nobel Prize in Medicine was awarded to three of the pioneers in establishing *C. elegans* as a "model organism": Sydney Brenner, John Sulston, and H. Robert Horvitz. Why study worms?

Sydney Brenner first turned his attention to *C. elegans* in the 1960's. Working at the Medical Research Council in England, he was looking for a small animal with inexpensive tastes that could be easily cultured in the laboratory. In particular, he wanted an animal with simple anatomy amenable to microscopic studies of identified cells. He also wanted a species well suited to genetic analysis of development and behavior[1]. Brenner had previously worked in conjunction with Francis Crick to analyze how DNA sequences were translated from messenger RNAs into amino acid sequences, work that led to the discovery of the triplet DNA code. Brenner was now looking for the next big frontier in biology. He imagined that by comprehensive study of mutants that showed altered behavior, he could discover the genetic rulebook, the internal programs governing the development and operation of the animal's nervous system. This topic remains today as one of the great unanswered puzzles in biology, but is finally beginning to yield up its secrets.

*C. elegans* presented several attractive features as a model organism. Its small transparent body seemed ideal for microscopy, and its cylindrical bodyplan seemed to favor serial section reconstruction from transverse EM thin sections. Transparency also permitted tissues or cell positions to be observed by light microscopy within the living animal. Nomarski optics permitted one to visualize cell nuclei and sometimes smaller organelles and cell borders. Intracellular markers such as green fluorescent protein (GFP) now provide good views of single cells inside the live animal. *C. elegans* develops quickly, going from single cell embryo through four larval stages to adulthood in about 3 days. The mature adult has only 959 somatic cells, and lives as a self-fertilizing hermaphrodite - an animal that makes both eggs and sperm. John Sulston showed that every single cell in a given animal could be accounted for and its final cell fate determined by a mixture of LM and EM studies[2,3,20]. In fact, the exact lineage of the animal proved to be invariant, so that each adult contains the same complement of differentiated cells. Most cell fates in the nematode are determined principally by autonomous decisions within a cell's nucleus. Furthermore, there is virtually no replacement of cells when one damages an early blastomere or a later differentiating cell. One can kill selected cells at will in the embryo, the larva or adult using a laserbeam guided under the light microscope, and then assess the resulting developmental and behavioral changes hours or days later.

*1, The male adult nematode (SEM by Carolyn Marks; false color by Zeynep Altun). Image reprinted from WormAtlas.org*




*2. The larval hermaphrodite gonad, in which the distal tip cell is expressing GFP. Lateral view. The distal tip cell wraps tightly onto the germline as it crawls towards the right along the dorsal bodywall. Transgenic Plag-2::GFP strain is courtesy of D. Gao and J. Kimble (Nomarski image and layout by Robyn Lints). Image reprinted from WormAtlas.org*

Cell death proved to be a prominent feature in the development of the normal animal. Bob Horvitz established that a series of intracellular decisions could lead to the programmed cell death of a cell during embryogenesis, and that the same set of cells followed this genetic pathway in each animal[4]. Programmed cell death is a specific cell fate, and about 15% of all early cells in the nematode embryo choose to die rather than to live. As Horvitz and his colleagues worked out the set of signals and decisions in the "cell death pathway," they also recognized that this same type of death, or "apoptosis," occurs in higher animals. Apoptosis is especially important in human brain development, where almost half of a child's neurons are destined to die by apoptosis after they are tested and found to be dispensable. The remarkable similarities between the cell death pathway in nematode and man caught the attention of the scientific community. Since 1990, many developmental studies began to include *C. elegans* as a key reference point.

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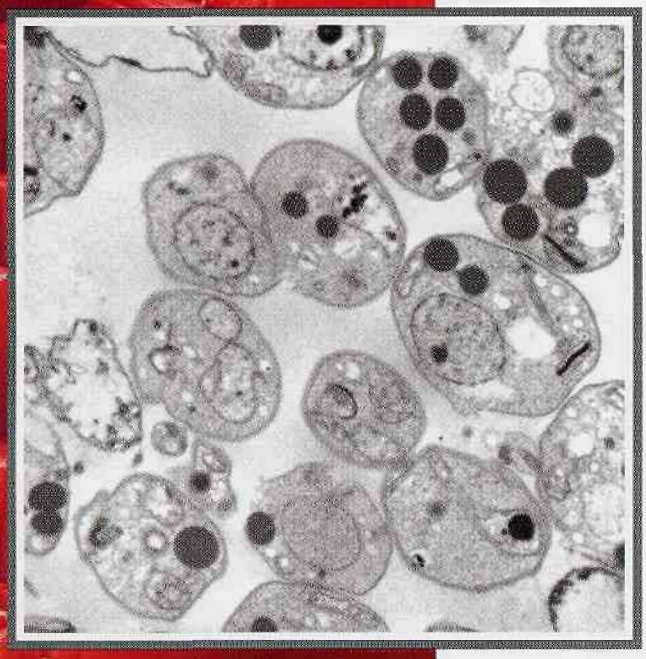

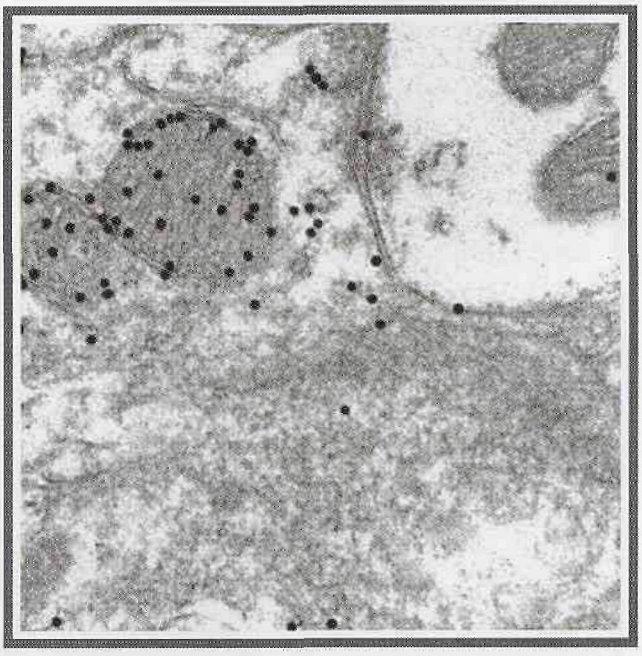

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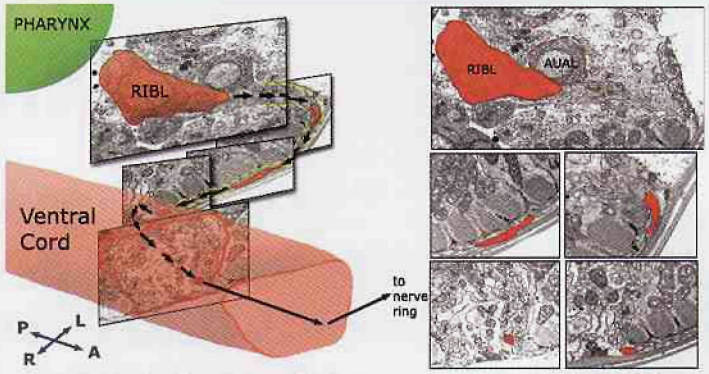
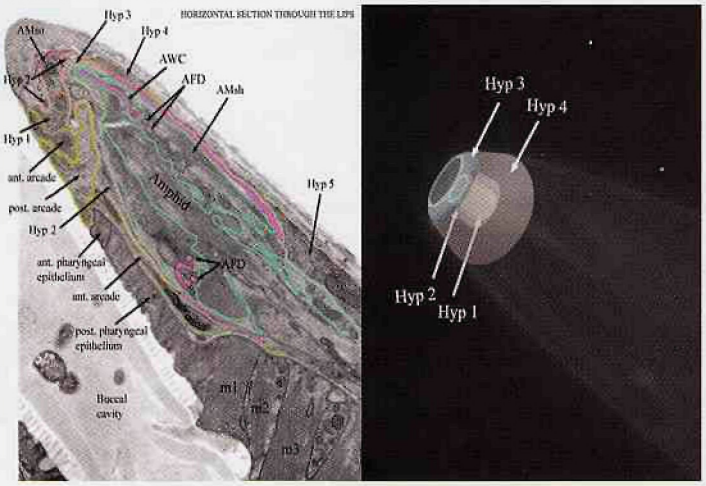



*3. Neurites from RIBL and AUAL enter a commissure underneath the bodywall muscle from their cell bodies in the lateral ganglion. (TEM by David Hall; cartoon and layout by Zeynep Altun). Image reprinted from WormAtlas.org*

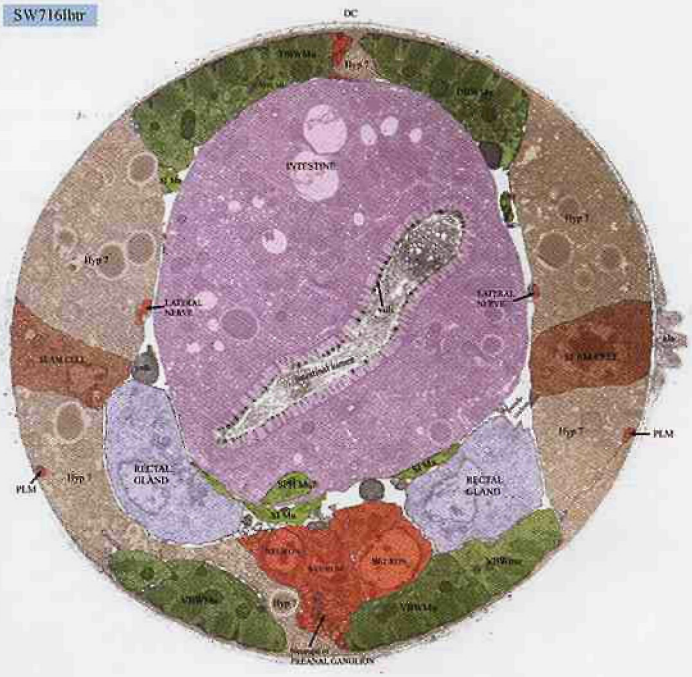
John White, also working with Brenner, succeeded in establishing the complete synaptic pattern or "wiring diagram" of the 302 neurons in the adult worm from serial EM sections[5]. Some normal neurons proved to change their synaptic patterns in specific ways between early larval life and adulthood. White also showed that some uncoordinated mutants had very specific errors in wiring between identified cells. Recent studies have shown that the genetic control of synapse formation is strongly conserved between nematode, fruit fly and man[6]. Physiological recordings of *C. elegans* neuronal or muscle activity *in situ* are made difficult by the animal's small size and thick cuticle, but are now beginning in earnest due to new technical advances[7].

Prior to efforts to determine the human genome sequence, John Sulston and Robert Waterston led an international effort to fully sequence the genome of the nematode, completing this task in 1998[8]. Information from the worm genome has helped in interpreting those of more complex animals, in large part because in the worm, gene function can be related to well known cell fates and developmental histories. Unexpectedly, conservation of gene organization and function is proving to be far higher than anyone dared to imagine across vast evolutionary times. Thus, studies of gene function in the worm often hold direct lessons for corresponding genes in higher animals, even in vertebrates and man. This conservation of gene function is helping to raise the profile of *C. elegans* as a model animal.




*4. Sample cross section of the lips to display epithelial compartments. (TEM by David Hall; color overlay and cartoon by Zeynep Altun). Image reprinted from WormAtlas.org*

While the worm has a relatively simple bodyplan and few cell types, the cell fates and developmental histories for fly, mouse or man are much more difficult to analyze because of higher complexity in tissue organization. Currently, one overriding concern in the *C. elegans* community is to explore which genes are expressed in the nematode's various cell types during its development. The goal is to uncover networks of genes that are essential for each developmental program. Microscopy remains a key tool for both normal and mutant animals, in visualizing gene expression patterns, and in exploring mutant pathologies. The Center for *C. elegans* Anatomy is an NIH-funded group devoted to providing comprehensive anatomical information regarding this nematode. Starting from Sulston's comprehensive list of the cells that comprise each tissue in the worm, we are compiling the subcellular details that distinguish them according to their separate cell fates. In coordination with expert anatomists for other model animals, we hope to draw parallels between specific nematode cell fates and the cell fates known for similar tissues in higher animals[18]. By drawing up parallel "ontologies" between animal species for all cells, genes, metabolic processes, and developmental events, we hope to discover deeper truths linking all animal life.




*5. Slideable Worm image showing the layout of tissues in the hermaphrodite tail (TEM by David Hall; artwork by Zeynep Altun). Image reprinted from WormAtlas.org*

The Center provides a variety of EM services to collaborating laboratories around the world. Our staff members have trained many students and improved the methods for TEM preparation of *C. elegans*, including fixation aided by microwaves or by high pressure freezing, and application of gold-tagged antibodies onto thin sections to localize specific proteins in identified cells at the EM level. The Center is currently testing new computerized methods for 3D reconstruction of exact cell shapes from EM micrographs. Working collaboratively, our staff members have conducted many pathological studies of mutant strains by TEM and SEM to look for changes in cell development related to brain and behavior[9], aging[10,11], germline development[12], and kidney function[13], among others. On a longer term basis, the Center's staff is also cooperating in several

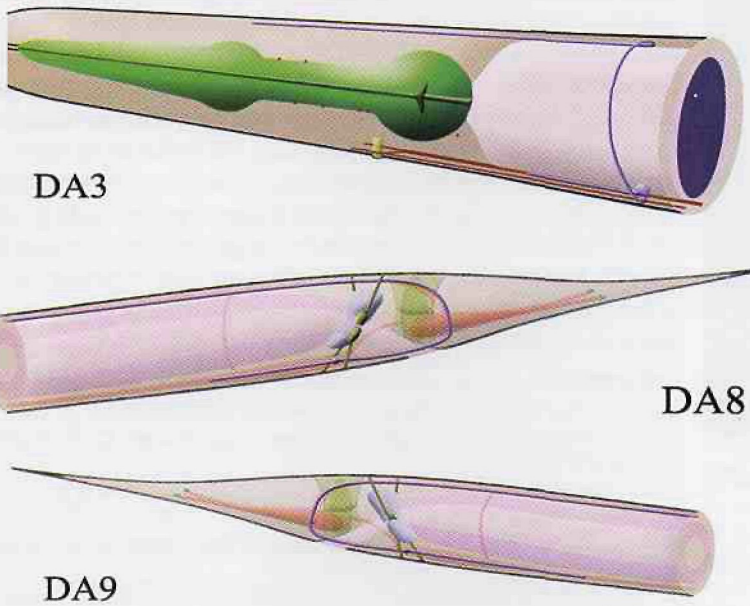
| CELL | TYPE | LINEAGE | LOCATION | NEUROTRANSMITTER | ASSOCIATED GLIA |
|---|---|---|---|---|---|
| DA1<br>DA2<br>DA3<br>DA4<br>DA5<br>DA6<br>DA7<br>DA8<br>DA9 | Motor neuron | ABprppapaap<br>ABplppapapa<br>ABprppapapa<br>ABplppapapp<br>ABprppapapp<br>ABplpppaaap<br>ABprpppaaap<br>ABprpapappp<br>ABplpppaaaa | Body; VC | ACh<br><br>(C. elegans book II CSHL Press, 1997.Rand J.B. and Nonet M.L. Appendix II: Neurotransmitter assignments for specific neurons) | None |

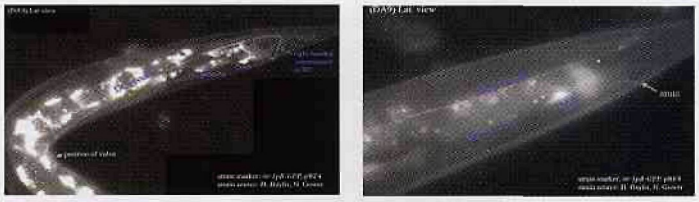
**DESCRIPTION**

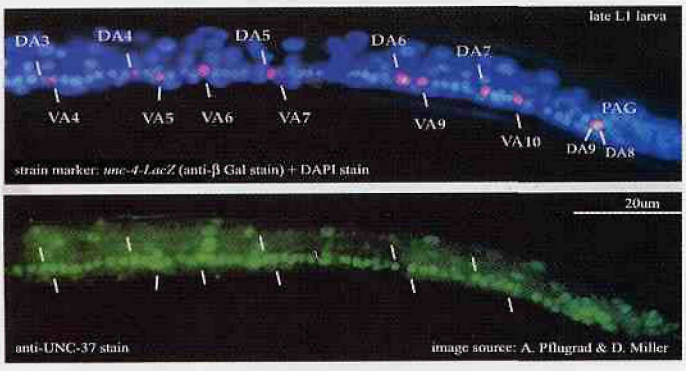
Ventral cord "dorsal A" motor neurons, innervate dorsal muscles.

**Functions:** Backward locomotion. Receive input from the driver interneuron AVA, modulator interneuron AVD and AVE. Send output to VD neurons. See Locomotory circuit.

**Receptor expression:** DA9 expresses a splice variant of the tyramine receptor SER-2 (Hobert O., pers. comm.).





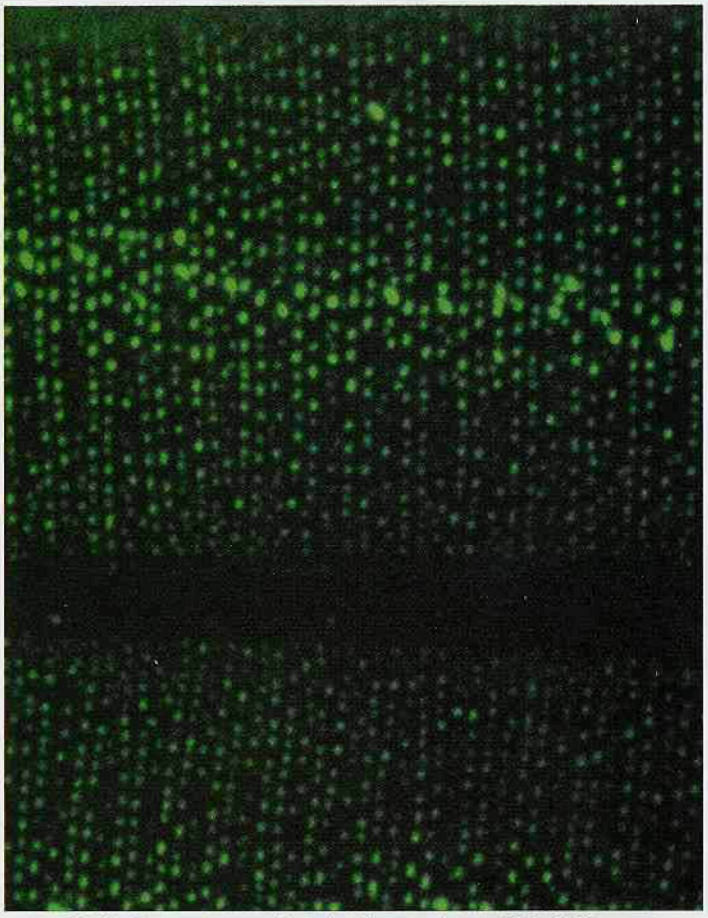

*6. Sample Neuron page for the DA motoneurons. Text shown in blue represent links to other website pages (layout and art by Zeynep Altun). Image reprinted from WormAtlas.org*

efforts to extend our knowledge of the nematode's nervous system development and final wiring. In a collaborative project with Scott Emmons at AECOM, the serial reconstruction of the adult male nervous system is still in progress. The male animal has many specialized sex muscles and almost 90 extra neurons devoted to controlling specialized mating behavior[20]. These male-specific neurons are more complex in geometry and present opportunities to study sex-specific genetic programs required for their development. We obtained an excellent set of serial prints from the MRC (courtesy of John Sulston and Donna Albertson) and are completing their reconstruction of the tail ganglia circuitry[20]. Working with Edward Hedgecock and Carolyn Norris at Johns Hopkins University, Hall has provided serial thin sections to explore key stages in embryonic development of the nervous system. Center staff continue to collect much new TEM and SEM data for further documentation of the wild type anatomy in both sexes and intermediate stages of development.

One of the principal current activities of the Center's scientific staff (Hall, Altun and Lints) is the completion of an atlas of microscopic anatomy for every tissue and cell in the adult nematode. This information is provided to the community on the WormAtlas website at no charge[14]. The website provides comprehensive information derived from EM and LM studies of the normal animal, and GFP studies of live transgenic worms. Much of the data comes from other cooperating scientists, who also provide peer review for the final product. The Center is also the repository for the original TEM data from Brenner's laboratory at the MRC, including the EM work of Sulston and White[2,3,5,20]. These shared resources are an invaluable foundation for the creation of a complete Atlas.

The online Atlas is still in development, but already features several interesting applications. Figures 1-7 offer examples of some of the types of data that are being developed for the website.

1. A Handbook of Anatomy is reaching completion, and illustrates each cell type in the adult hermaphrodite from a variety of aspects, using a combination of data types including annotated TEM or SEM images (Figure 1), light micrographs, GFP-labelled cells shown in situ within the living animal (Figure 2), and in some cases, a summary of the developmental events and genetic interactions known to be required for the morphogenesis of a given cell type. Working from our collection of serial thin section data, new hand-drawn illustrations are often used to depict the 3-D relations between various tissues (Figures 3 and 4). The Handbook is heavily annotated with current literature references, and with links to other websites offering related information regarding molecular expression patterns, gene sequence data, and so forth. Separate chapters of the Handbook also cover non-cellular aspects, including the nature of the pseudocoelomic spaces between tissues, the cuticle, the eggshell, the basal lamina, and the variety of intercellular junctions utilized by various tissues.
2. The "Slideable Worm" applet was designed using JAVA code borrowed with permission from the "Visible Human" project[19]. The Slideable Worm eventually will offer some 800 selected EM cross-sections in three formats, as raw micrographs, or including transparent colors overlying each tissue



*7. C. elegans transgenic animal expressing a bli-1::GFP reporter gene. bli-1 encodes a cuticle protein that associates with struts, pillar-like structures found in the middle layer of the cuticle that are organized in a regular array. bli-1::gfp transgenic strain provided by J. Crew and J. Kramer (Northwestern University Medical School, Chicago, IL). (LM image by Robyn Lints) Image reprinted from WormAtlas.org*

(Figure 5), or showing the color overlays alone. We have assigned every tissue type in the animal a separate color so that virtually all images on the website can display any given cell type according to the same schema.

3. A Glossary of all anatomical terms used in the scientific literature regarding *C. elegans* cells and tissues is presented, again including many references to key papers.
4. Current protocols for common anatomical procedures are available online, including the most common methods for TEM, SEM and antibody staining. A new section is providing a forum for comparing and discussing results of anatomical protocols under current testing from any laboratory wishing to participate.
5. User guides are offered that help to orient the website users in regards to the color code, common perspectives used in the illustrations, nomenclature and common abbreviations, conventions used in linking to other data sets, etc.
6. Some of the most commonly addressed data listings are available from the front page of the site as hot buttons. One button links to the known lineages for all cells, from the work of Sulston and Horvitz. Another button jumps to a complete list of all neurons, from which one can jump to individual "neuron pages". There is a page for each neuron cell type (example in Figure 6), showing the cells in cartoon form, with information on their known lineages, synaptic relationships, neurotransmitter(s), and with links to other websites offering the molecular expression patterns for those cells. Where possible, the same cell is also depicted *in situ* from a transgenic strain in which the cell has been well marked by GFP or an antibody, so that the reader can compare a "live" image from a real animal to the cartoon version.
7. One of the most popular features accessed from page one of the Handbook section is a "bird's eye view" of *C. elegans*, taken by stitching 165 SEM images to build a Quicktime® movie to show the reader the adult animal from head to tail, up close and personal.
8. Beginning later this year, the Handbook will be expanded to cover the anatomy of tissues in the male adult nematode, and eventually we plan to extend this work to illustrate several stages in embryonic and larval development.
9. A printed version of the Atlas of the hermaphrodite is in preparation in conjunction with the Cold Spring Harbor Laboratory Press.

The WormAtlas website also provides classic *C. elegans* anatomy papers, reproduced in HTML format from the older literature. Information on WormAtlas is heavily cross-linked to other key websites. WormBase surveys the worm genome in complete detail, and lists all known mutant strains[15]. The *C. elegans* WWW Server offers excellent coverage of all *C. elegans* literature and the worm community[16]. Other sites offer information on gene expression patterns from microarray studies, and images of mutant embryos from many gene knockouts[17], *etc.* This successful integration of parallel websites reflects the long history of cooperation within the "worm community," as exemplified by its founders. By building links rather than barriers to information exchange, the whole community benefits dramatically. All of this information is increasingly useful to the broader scientific community in the analysis of gene expression and cell development in higher animals. ■

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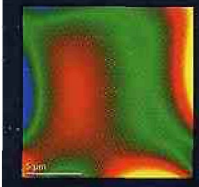

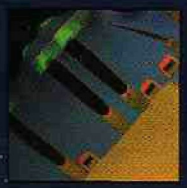

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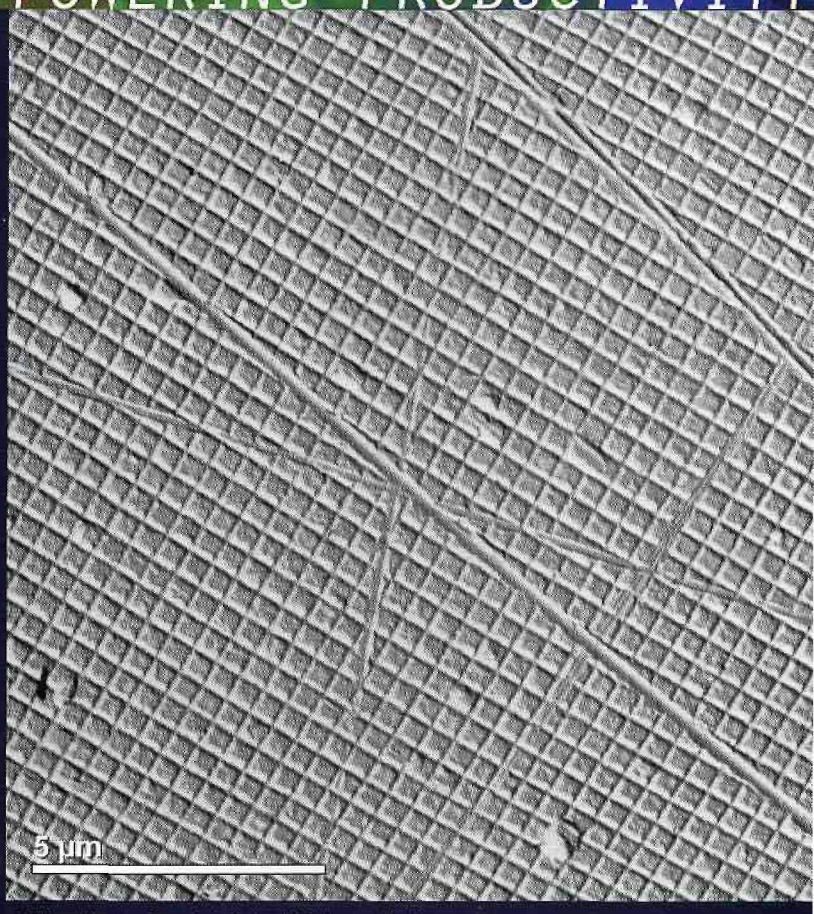


*Left-hand Images (from top to bottom): (1) Energy deviation across image field corrected thru 3rd order to < 2 eV. (2) Unfiltered Bright-field TEM image of semiconductor device structure. (3) Color composite of elemental maps of semiconductor device structure.*

*Above Images: (top) Zero-loss filtered CBED pattern of Si [111] taken at 200 keV. FOLZ ring represents scattering angle of 78 mR. (bottom) Zero-loss image of grating replica showing large field of view for EFTEM. Grating pitch is 463 nm.*

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