***C. elegans* anatomy and live analysis**

(the joys of Nomarski live analysis of *C. elegans*)

A. Mounting worms for live analysis
B. Theory and practice of Nomarski DIC
C. Some anatomy basics
D. Dye filling of exposed sensory neurons
E. Anesthetics in *C. elegans*

13A. The basics

I. Preparing slides for Nomarski DIC microscopy

(Adapted from Sulston and Hodgkin*, C. elegans* I)

Worms will be crushed if simply mounted between cover slip and slide. We use pads of agar to give the worms a bit of support. First make up 4% agar. (If in a hurry, simply melt NGM agar from a worm plate). I prefer to make up ~100 ml at a time, aliquot into 5 ml disposable tubes. Melt a tube as needed and keep the agar molten in a 65°C hot block. To prepare an agar pad:

1. Place a slide between two spacer slides onto which you have put two layers of lab tape.
2. Using a Pasteur pipet put a few drops of hot agar on the slide, avoiding bubbles in the agar.
3. Rapidly cover the hot agar with another slide and press down gently (Figure 1).
4. Let the agar solidify (30 seconds). Slide the top slide off the pad. Don't try to lift it off, slide it off to one side.

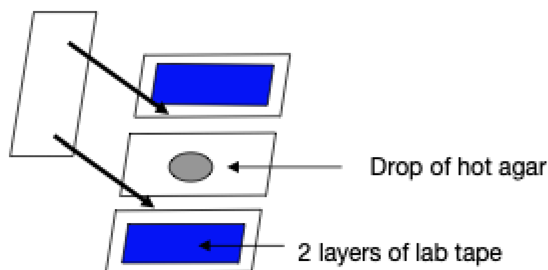

Figure 1. How to make an agar pad.

NOTE: keep a tube of water to rinse the pipet in so it does not clog with solidified agar. The molten agar is good for a couple of days, although if you are doing some exacting lineage analysis you should melt it fresh every day.

II. Mounting Worms

The exact technique will vary depending on the purpose of your slide, but the general rules are the same.

Quick Mount

Put a small (~4 µl) drop of M9 or S Basal onto the middle of the slide. Transfer worms to the drop from the plate using your pick. Let the worms off by gently moving your pick back and forth in the drop. Be careful not to cut the agar pad. Lower a cover-slip onto the slide, trying to avoid bubbles; a pair of jeweler's forceps can be useful here. 18 x18 mm or 22 x 22 mm cover slips are fine for most applications; some people use 12 x 12 mm coverslips for lineaging, but these can be hard to find.

Anesthetic Mount

Used for some anatomical studies, photos, and laser ablations. Same as Quick Mount, but use a 30 mM solution of sodium azide (NaN_3) in M9 or S basal for the drop. Let the worms swim in it for a short while before lowering the coverslip.

Worms anesthetized with NaN_3 can be recovered from the slide, but success of recovery will drop precipitously after 20 – 30 minutes. Other anesthetics are in use in the worm field (see below) and each has its own uses.

Long term mount

Used for lineage analysis and other prolonged observations of larvae and adults. Same as quick mount, with the following addition: Using your pick, add some bacteria to the middle of the coverslip, face down. Bacteria are needed for continued development, to confine the worms and to encourage browsing rather than rapid locomotion. Once you have lowered the coverslip, cut off any agar that projects beyond the coverslip with a razor blade. Seal off all four sides with vaseline to prevent evaporation and drying of the agar pad. This is easiest done by having by painting some liquid vaseline that is kept warm in a temp block. Do not let the microscope objective touch the vaseline, as this will ruin the optics. (NOTE: for most purposes we now use immersion oil instead of Vaseline; this can caused problems in the past as some formulations of immersion oil can be toxic to worms. If you run into these problems, use Vaseline and be careful.)

Multi-Mount

If you have to look at many separate samples, this is for you. Cut the agar pad into several (4 to 16, depending on the number of samples and how ambitious you are) smaller squares with a razor blade. Put correspondingly smaller drops on each of the minipads (3 µl for 4 pads, 1 µl for 16). Then proceed as before.

III. Microscopy

Once prepared, the worm slide is viewed using Nomarski DIC optics. See section B below for the details.

IV. Looking at worms through DIC optics

Look at the Appendix of the *C. elegans* Book I to get a general idea of worm gross anatomy. Spend some time identifying the developmental stage of your worm, and the various organs. The online WormAtlas may also help.

Nomarski optics usually do not usually allow you to identify cell boundaries, but cell nuclei should be readily visible. Hypodermal cell nuclei are rather smooth and look like eggs sunny side up, neuronal cell nuclei are smaller and grainier, resembling slices of pepperoni (see chapter by Sulston in *C. elegans* I).

It takes practice to be able to reliably identify specific cells. Practice by drawing what you see over and over again.

Early embryonic lineage

Early *C. elegans* embryonic development is a true pleasure to lineage, as cell divisions are rapid, but easily followed because the cells are large and few. Because young embryos usually are retained in the mother's uterus for a significant amount of time, we will need to cut open adults to get young embryos.

1. Transfer some gravid (embryo-bearing) adult hermaphrodites to a watch glass.
2. Use a curved #21 scalpel to cut open the adult worms in the mid-body region. Embryos inside the uterus will be released into the surrounding liquid.
3. Use your mouth pipets to transfer one or a few early embryos (try to get a 2-4 cell stage embryo if possible) to a agar pad on a slide. You can use the "quick mount" protocol for this application.

4. Draw the position of cell nuclei (and cell borders as long as you can) at frequent intervals (every few minutes).
5. As development proceeds, you will get cell nuclei at multiple focal planes. Use color to help you out (e.g. cells in the middle of the embryo are black, cells on the top are red, cells on the bottom are green). Try to keep track of who divides to generate whom.

Another good place to practice your lineage analysis skills is the hermaphrodite vulva, which develops over a few hours and which contains a small number of large cells.

V. RNAi phenotypic analysis

Once you have some experience looking at the wild type, take a look at your RNAi embryos and adults. Try to spot any differences. You will quickly learn one of the truisms of *C. elegans* biology: it's hard to identify a mutant if you don't know what a wild-type looks like. Learn to know and love your wild types!

13B. Theory and Practice of Nomarski DIC

B1. Introduction
To see things in living worms we use Nomarski Differential Interference Contrast (DIC) optics. optics allow you to see an 'optical section' with *differences* in refractive index generating the contrast. This gives a nice '3-D' effect, but only between regions of different refractive index (nucleus and cytoplasm). Adjacent regions of the same index will appear uniform, that's why cell boundaries can't be seen in larval worms. The '3-D' effect is an artefact of the optics and doesn't correspond to any true 3D structure. Nomarski DIC is easy to use even if you don't really understand how it works (few of us do).

B2. Know your microscope
The parts you will come into contact with are, in order of optical path from lamp to eye:

1. Halogen lamp

2. Field iris (= field diaphragm)
Adjust to regulate amount of light entering condenser. Use the condenser centering screws to center the diaphragm outline.

3. Bottom polarizer
Sits in rotatable holder below the condenser. Should always be in 0° position but can get knocked out.

4. Substage condenser
The group of lenses directly under the slide, focuses light on the specimen, contains diaphragm that can be opened or closed to match objective NA and to vary depth of field.

Some DIC condensers are simple, with a single lens and a Wollaston prism. These cannot be used for polarized light or phase contrast optics. Others have multiple lenses for DIC, phase, dark field and so on; rotate the condenser to get the right lens for the relevant objective.

Condensers have internal diaphragms that you adjust according to the NA of the objective.

5. Slide, specimen, and coverslip.

6. Objective lenses

Mounted on the rotating turret above stage.
Low magnification 4x, 10x, 20x are usually dry lenses
High magnification: 40x (can be oil or dry); 63x, 100x are always oil-immersion.

What do those numbers on the sides of the objectives mean?

e.g. "**Plan-Neofluar 100x/1,3; ∞/0,17**" means 100x magnification, Numerical Aperture 1.3, infinity corrected optics, for use with cover slips of 0.17 mm thickness ("No. 1 1/2" thickness is supposed to be 0.17 mm +/- 0.02 mm; Corning cover slips are supposedly the closest to the standard.)

'Plan' means flat-field, i.e., the entire field of view is in focus

'Neofluar' is a Zeiss name for the type of glass in the lens; Neofluars are semi-apochromatic—that is, they are corrected for chromatic aberration at at least 3 wavelengths. Planapo means fully apochromatic Plan lens, corrected for chromatic aberrations at >4 wavelengths. See B8 below for more on this.

Oil immersion objectives are designated "Oil" on the side—*if it doesn't say this then it is a dry lens and should not be used with oil.*

Zeiss objectives that are strain-free (i.e., better) are labeled in red, not black.

DIC objectives have their own Wollaston prisms for Nomarski mounted in slots on the turret above each objective. Prisms are matched to their objectives: a 40x prism will not work on a 63x objective; also a 63x Plan Neofluar prism will not work well on a 63x Planapo objective, etc. If the Nomarski looks bad make sure the prisms are installed correctly.

[Definitions (in case you had forgotten).

Numerical Aperture (NA) = *n*sinq
Where *n* = refractive index of the medium (maximum = 1.0 for air, up to 1.5 for oil)
and q = half the angle of the light cone entering the objective

Resolution D = 0.61l/N.A., where l = wavelength of light.
Thus higher refractive index means higher NA means higher resolution.]

7. Top polarizer ('Analyzer')

Used always to be slider above the turret, always stays IN for Nomarski, remember to pull OUT of light path for low-intensity fluorescence. Supposedly it is bad for the analyzer to be exposed to too much fluorescence, and it also wastes your fluorescent signal. The analyzer can also be installed in the same turret at the filter cubes, as in the scopes we have set up here. This works fine but means you cannot do simultaneous fluorescence and DIC.

8. Fluorescence sliders, turrets or 'Optivar' slider (contains extra 'empty' magnification lenses—make image bigger but have no extra resolving power)

9. Eyepieces

Good ones are 10X, 23 mm field number. The larger the field number the bigger the field of view. Usually one eyepiece can be focused.

B3. Setup for Nomarski DIC

1. Place a small drop of immersion oil on the condenser or on the underside of the slide.

(The Nomarski is passable without oil on the condenser but oil will give best results because you are maximizing the refractive index of the medium. Place the slide on the stage and raise the condenser so that the oil contacts the slide. If the drop is too small or if there are bubbles you will get horrible optics due to oil/air interfaces, so use enough oil to get a decent film between condenser top lens and slide.)

If using Zeiss scopes, use Zeiss immersion oil. The lenses are designed to work with oil of a specific refractive index. Zeiss makes several immersion oils that are immiscible: if you mix them you will experience psychedelic visions while trying to see your worms. We have experienced bad batches of immersion oil that contain crystalline gunk; this is not fatal but is grounds to request free replacement oil from your Zeiss guy.

2. Turn on the halogen lamp. Keep the lamp intensity low so as not to fry the specimen under long-term observation. Using a low power (10x or 20x) objective, focus on the edge of the cover-slip then find the specimen.

3. Set up Köhler illumination (highest resolution, uniform illumination)

i. close down the field diaphragm and focus the condenser so that the edge of the diaphragm is in focus (same conjugate focal plane as specimen).

ii. Open up the diaphragm so that it is just outside the field. Center the condenser if necessary.

[Under Köhler illumination the following are in conjugate focal planes (field planes): field diaphragm, specimen, ocular field stop, retina.]

4. Place a drop of oil on top of the coverslip. Refocus. Close down field iris to smallest aperture.

5. Be sure that the specimen is in the center of the field. Back off the focus a half or quarter turn, rotate in high power objective (hold the turret, not the objective). SLOWLY focus back up using the field iris as a guide for the focal plane. The depth of focus with the high power objectives is so shallow that it's easy to focus through the specimen and not notice it, but it's difficult to miss the field iris.

The objectives are set up to be parfocal—ie if you are in focus at 20x you should stay in focus at 100x when you rotate the turret. So why should you bother to focus back and forth every time you change magnification? The reason is that the cover slips are floating on buffer, so rotating an oiled objective in or out of position will move the coverslip and you can lose the specimen.

Note: Some 63X and 100X objectives are spring-loaded so that if you hit the slide they will retract. The 100X locks up (by turning the lower part of the objective) and should be kept in that position when not in use so that you can rotate through without hitting the slide or the stage.

Note: *If you cannot find the specimen and have lost the focus, don't drive the objective into the slide trying to randomly find the plane of focus*. If you are using a dry objective, focus on the edge of the coverslip and move back to your sample. At high magnification or when using an oil objective, close the field diaphragm and focus on it; if you are still in Köhler illumination then the specimen should be in focus as well. <u>If you still can't find the specimen, close down the condenser aperture: this increases depth of field so that even an object far from the plane of focus creates a shadow.</u>

6. For best Nomarski optics you try to match the NA of the condenser to the NA of the objective. In practice with high-N.A. objectives the condenser iris should be wide open. For low power objectives close down the condenser until it looks optimal (in theory when the condenser aperture is closed to ~70% of the field.)

7. Be sure the bottom polarizer is at 0°. The polarizer can be rotated out or simply dialed to 75° to remove. The polarizer should be at the position that gives the darkest image when the Wollaston prism is at the null point (no shear).

8. Adjust the shear (to personal taste) by rotating the knob on the Wollaston prism.
The offset introduced by the prism determines how contrasty the Nomarski image is. At the middle of the prism's travel there is no shear and the image should be darkest (crossed polarizers). As you twiddle the knob on the prism the shear will make the image progressively brighter.

B4. Simple trouble shooting: what to check first if the Nomarski looks bad (fuzzy, out of focus):

The most common error is that the condenser is not focused and you do not have Kohler illumination, but if that looks OK, check the following:

1. Is there enough oil (above or below slide)? (If you put only a tiny drop on there will be aberration at the edge of the drop).

2. Is the condenser iris fully open? (it should be, for 40x and higher)

3. Is the correct condenser in place? Check that the condenser NA matches the objective.

4. Are the polarizers fully crossed? Twiddle the upper Wollaston prism to the null point (darkest) and the rotate the bottom polarizer to check that it is as dark as it can get. The bottom pol should always be at 0° but can get knocked out.

If none of the above helps, the objectives or condenser may need cleaning. Find someone who knows how to do this properly.

B5. Setup for Epifluorescence

Optics

Since you are illuminating through the objective, the condenser and field iris have no effect on the image. If you are switching between Nomarski and fluorescence you can leave the condenser in the position for Nomarski. Slide the top polarizer (analyzer) out of the light path. Shining UV light through the analyzer is bad for it.

Care of fluorescence burners

Mercury burners are expensive. Their life can be maximized if they are taken care of:

1. Whenever the burner is switched on it should be left on for *at least 30 minutes*.

2. After the burner has been switched off it **must** be left to cool down for at least 30 minutes, preferably 1 hour. Turning on the burner when it's still warm causes the burner to cloud up, thereby shortening burner life and reducing light output—so don't do it!!

3. Minimize the number of times the bulb is turned on.

4. Modern power supplies allow you to vary burner intensity. Only use at 100% power when you need it. Switch to low power when not actively observing. New bulbs can be used at 10-20%, older bulbs cannot be turned down so far (if the bulb flickers it needs more power). A burner should be usable for at least 300 hours; its output slowly decreases with age. Don't try to install a new burner unless you know what you are doing; follow the detailed instructions provided with the microscope.

The collector lens (between the burner housing and the rest of the scope) can be adjusted to give a smaller more intense beam or a larger more diffuse beam. Twiddle the knob at the side.

Use of filter sliders

Be especially careful not to touch the filters with your fingers—they are difficult to clean. Keep in dust-free box when not in use. Never leave them sitting on the table.

B6. Taking care of a compound microscope

General rules

1. All microscopes should be covered when not in use. This stops dust getting on optical surfaces.

2. Never ever touch optical surfaces with your greasy fingers.

1. Objectives

Daily use: draw lens paper across the bottom of the objective to remove excess oil. Do not touch the objective with your finger, do not rub it hard with the lens paper. Having some oil on the objective does not damage it. Use absorbent lens paper which is not siliconized. (Kimwipes are fine, contrary to popular belief).

Rarely: If objective is very dirty (or if oil got on a dry lens), it needs to be cleaned with solvent. Don't try this unless you know what you are doing. Wet a cotton swab with acetone or ether and rotate over the lens. I use a dissecting scope to inspect the objective while doing this. Your microscope rep should know how to do this.

DO NOT DROP OR KNOCK OBJECTIVES. They are delicate precision instruments and horribly expensive (A 63X Planapo costs about $5000!). Even if not broken, vibration introduces strain into the glass that compromises the DIC optics. Internal lenses can also be dislodged from their mounts, in which case the objective has to be sent back to a factory in Germany for repair (takes months and costs hundreds of dollars).

2. Condenser

Routine (all users): Always wipe oil off the condenser front (top) lens (with lens paper) after you are finished. Excess oil drips down the sides of the condenser and can work its way into the interior where it will ruin the lenses. The groove running round the top lens is not an oil tray!

Rare: If there is visible dust inside the condenser it needs to be taken apart and cleaned. Don't try this yourself unless you have been trained.

3. Polarizer. Occasionally: swing out polarizer. Wipe with lens tissue or blow off dust with canned air. Make sure polarizer is at 0 degrees.

4. Eyepieces. The surfaces of the eyepieces are not in the field plane so a little dirt is not fatal. For occasional cleaning use lens paper and breathe on the eyepiece (never use dry lens paper, it cuts through the lens coating on the eyepiece).

For thorough cleaning use cotton swab with acetone or Kodak lens cleaner. Remove lens cleaner residue with acetone or ether, avoid touching the rubber eye guards as they will dissolve in organic solvent and leave residues on the eyepiece.

5. Field Diaphragm. The cover to the field diaphragm is not in the field plane with the specimen so dust here should cause little trouble but it gets pretty dirty and needs to be cleaned occasionally. Use a cotton swabs wetted with acetone, rotate the swab as you wipe.

B7. Theory of Nomarski Optics (for Dummies)

See Chapter 10 of Oldfield (1994) for the clearest non-mathematical explanation, of which the following is a summary:

1. Incoming light is polarized by the bottom polarizer

2. Polarized light hits the bottom Nomarski* prism at 45° to its optical axis. Due to birefringence the prism splits the beam into two rays (ordinary O and extraordinary E) polarized at 90° to each other. The beams are split (sheared) due to different velocities of the O and E rays in the crystal but there is no phase difference.

*Usually called a Wollaston prism; a Nomarski prism is (roughly) a modified Wollaston.

3. The O and E rays pass through adjacent regions of the specimen and are advanced or retarded in phase depending on the refractive index.

4. O and E rays are combined in the upper Nomarski prism, in mirror image of below. Since rays are still polarized at 90°, there is no interference yet.

5. O and E rays are aligned into the same direction by the analyzer and now interfere.

If there is no shear introduced in the upper Wollaston prism, then O and E rays are recombined on top of each other. If the path difference is zero (no specimen) then the recombined beam will be completely polarized in the same direction as when it started and nothing will get through the analyzer (extinction—i.e., crossed polarizers). If there is a path difference (specimen) the rays will be slightly out of phase resulting in an image with no "3-D relief".

If the upper prism is moved so that an additional offset (shear) is introduced, the rays will undergo interference with adjacent rays along the optical axis of the prism. The offset between the O and E wavefronts will give a 3-D effect in which the sides of the specimen perpendicular to the prism axis will appear to be illuminated and shadowed (the 'direction' of shadowing can be reversed depending on the direction of prism offset).

"How much should I offset the prism?"
You usually want to get the maximum contrast out of your sample. Start at the extinction point (middle of the prism's travel). Move the prism until you get enough light through to see things but the 'shadowed' parts are still very dark.
Conventionally, for publication quality pictures, the worm should appear as if it is being illuminated from the dorsal side, or from the top of the figure.

Historical note: *Why is it called 'Nomarski' optics?*

Many types of interference contrast microscopes were developed in the 1940s and 50s, including those by Francis Smith and Maurice Françon. Smith-type DIC was the first to use Wollaston prisms as beam splitters/combiners. A major problem with using a conventional Wollaston prism as the beam combiner was that the prism had to be in the back focal plane of the objective, which was usually inside the objective.

Georges Nomarski figured out how to modify the basic Wollaston prism so that it could be easily placed outside the objective. In a Nomarski-modified Wollaston prism the crystals are cut obliquely to the optical axis; somehow this allows the 'plane of fringe localization' to be outside the prism, and thus in the back focal plane of the objective. Now the combining prism can be placed in the objective turret and the setup for Nomarski DIC is simple and cheap. Microscopes with such Nomarski-type DIC optics were first introduced in the late 1960s by Zeiss.

References
Oldfield, R.J. (1994) Light Microsopy: an illustrated guide. Wolfe.
Inoue, S. and Spring, K.R. (1997). Video Microscopy: the fundamentals (2nd Edition). Plenum Press.
Francon, M. and Mallick, S. (1971). Polarization Interferometers. Wiley. QH212.I5F7
Ross, K.F.A. (1967). Phase Contrast and Interference Microscopy for Cell Biologists. St. Martin's Press. QH212.I5R6. (Chapter 5 covers interference contrast including Nomarski DIC.)

B8. Consumers guide to Zeiss scopes and parts

The CSHL Worm course mostly uses Zeiss compound scopes. Many worm people prefer the Zeiss Nomarski optics, but that may be simply habit. If you are in the market, there are only four major companies (Zeiss, Leica, Olympus, Nikon), so it is simple to them all out and go with what you prefer (or can get the best deal on).

1. Objectives.

Terminology:
Plan = corrected so that entire field of view is in focus.
Plan-Neofluar: good all purpose objective, preferred objective for DIC, also has high fluorescence (UV) transmission. Corrected for spherical and chromatic aberration at blue, green and red. Zeiss hand sorts them, the ones that are sufficiently strain free are designated 'Pol' and marked up accordingly.

Planapo (Plan-apochromat) = also corrected for aberration at dark blue, mostly used for brightfield color photography. Such lenses usually not used for DIC because there are many elements (up to 15 lenses) in these objectives and they introduce strain which will depolarize light. The exception is the Zeiss 63X Planapo, which is hand-picked to be strain free (that's why it's expensive).
LD Achroplan - long working distance, can see through petri plates. Plastic will depolarize light so petri dishes eliminate Nomarski. Poor DIC, fluorescence OK with high NA to get better light throughput.

Objectives:
Low power scanning objectives. The Axioskop will only fill the field of view of a 10X objective with the NA1.4 condenser, so there is no good reason to get lower power. We also have a 20X NA 0.5 for occasional low power use.
High power objectives:
40X 1.3 Plan-Neofluar Oil. Good DIC.
63X Planapo NA1.4 Oil. A great objective: high NA means excellent fluorescence and better resolution than the 100x (NA 1.3). Disadvantage: poor UV transmission, since it has the color corrected lens in it.
100X Plan-Neofluar NA 1.3 Oil. Best for Nomarski DIC, OK for fluorescence.

On injection scope:
10X Plan-neofluar scanning objective (4x would work fine also because the condenser is low NA)
40X 0.75 Plan-neofluar dry. Good for polarized or DIC.

2. Condensers.

Fancy microscopes have multi-purpose turret condensers so that one can rotate in phase rings, polarized light, or DIC prisms for both low and high magnification. If you only use DIC and only use low magnification bright field to scan the slide, it is cheaper to get a dedicated high-powered DIC condenser (top lens = NA1.4 oil/0.9 air). (You can't use the DIC condenser for polarizing microscopy because the DIC prism is mounted in the condenser and it will depolarize the light; if you need polarized light, get a "cheap" flip-top condenser.)

For injections a NA 0.55 condenser is fine. Higher NA condensers would give better Nomarski but the small working distances are awkward. If budgets are very tight it is just about possible to inject on an upright scope or do Nomarski on inverted scope.

3. Stages

For following cell lineages in moving worms it's useful (almost essential) to have a fully rotating stage, so you can orient the worm properly no matter where it goes. Current Zeiss stages cannot be rotated more than 240° because they are so big that they hit the scope. Zeiss does make a fully rotating stage (for polarizing microscopy) but it is very expensive and hard to control. There are old Zeiss stages that work better for this purpose but they are getting hard to find so I will not bore you with the details.

For injections use a gliding stage, allows very fine control in all directions.

13C. Some worm anatomy basics

Topology

Worm body is a tube of “ectoderm” (epidermis, neurons) enclosing “endodermal” tube of the gut. Between the two tubes are mesodermal tissues (muscle, gonads, pharynx).

Ventral is defined as the side that the major nerve cord runs along.

The major tissues and organs:

Epidermis (called ‘hypodermis’ by old-timers, although this is a misnomer)

Simple epithelium

Secretes cuticle apically, basement membrane basally

Comprised of 11 cells in adult named hyp1 through hyp11—syncytia formed by fusion of >100 cells.

Very thin over the muscles; thicker ‘ridges’ at ventral and dorsal midline.

Lateral epidermal cells called “seam” cells; secrete cuticle with alae (longitudinal ridges) (L1s and dauers have their own distinctive alae).

Large orifices (mouth, rectum, vulva) are formed by stacked toroids of cells. Smaller orifices (sensilla) are channels within single cells (socket cells).

EM of vulva—paper by White et al 1996

<u>Nervous system</u>

302 cells, 118 morphological types.

Sensory cells in head and tail. Motor neuron cell bodies in ventral cord along body. Interneurons mostly in head ganglia

Process tracts: nerve ring (~100 processes), ventral cord, dorsal cord, sublateral cords. Commissural processes from ventral to dorsal nerve cords.

Ventral cord is asymmetrical: most processes run on the right hand side of an epidermal ridge; only 4 on the left.

Durbin EM: AVG pioneers right tract, others follow. Basis for LR asymmetry not known.

See the Mind of the Worm (White et al 1986) for ultrastructure (online at WormAtlas).

Glial-like ‘sheath’ and ‘socket’ cells are accessory cells in sensilla; socket cells form the interface with the epidermis.

Small number of lateral neurons (CAN, HSN, touch cells) are easily scored and have been used in many cell migration screens.

Neurons usually simple monopolar or bipolar; synapses formed *en passant*; some have defined axon-dendritic polarity, but in others a single process may have both pre and post synaptic zones. Some neurons appear to have mixed functions e.g. M3 is

sensory and motor neuron in pharynx, may form a single-cell proprioceptive feedback loop.

Body muscle

Arranged in 4 quadrants: left and right/dorsal and ventral.

Attached to cuticle via basement membrane and IF-containing trans-epidermal attachment structures.

Muscles send processes ('muscle arms') to presynaptic regions of neurons—neurons do not send processes to axons.

Neurons innervate ventral and dorsal quadrants in opposition, so all locomotion due to dorsoventral bending of body. Head muscles are innervated separately, allowing left-right flexures in foraging.

Hermaphrodite uterus/vulva and male tail have specialized 'sex muscles', these develop in L3 and L4 stages.

Gonads

Somatic gonad is tube enclosing germline. Gonad has twofold rotational symmetry. Develops from 4-cell primordium from L1 to L4. Anterior arm is righ hand side, posterior is left.

Somatic distal tip cell (DTC) and various sheath cells (smooth muscle like) required for germline development/function.

Hermaphrodite gonad two-armed; uterus in midbody; uterine cell known as 'anchor cell' induces adjacent epidermis to form vulva; later degenerates to form the opening between uterus and vulva.

sperm reside in spermatheca; oocytes fertilized as they pass thru spermatheca into uterus; undergo first few divisions in uterus then laid.

Pharynx

Tubular muscular organ; ~80 cells; threefold rotational symmetry.

Ectodermal cells secrete a special cuticle that lines the lumen

EM anatomy by Albertson + Thomson 1976.

Some muscles are syncytial, formed by fusion.

Has its own nervous system that modulates a myogenic pumping rhythm.

Pharyngeal gland cells—unknown function.

Connected to gut by pharyngeo-intestinal valve.

Gut

Simple epithelium. Calcium oscillations in gut cells are the clock that keeps worms regular. Has two associated muscles in the posterior, for defecation. rectum composed of 3 cell types; has cuticle.

Body cavity

Fluid-filled space between the basement membranes of the epidermis and the gut or gonad. Also called ‘pseudocoelom’.

Excretory system

4 cells; excretory cell is huge H-shaped cell with processes all down the worm; interior canals of cell visible under Nomarski. Essential functions in osmoregulation.

Coelomocytes

4 or 5 cells; MS-derived; phagocytose GFPs secreted into body cavity; true function not defined.

Name in Wormbase: cc

male tail

Super-complex structure with dozens of male-specific neurons, muscles, sensilla. Spicules are intromittent organs that hold vulva open during sperm transfer. See Albertson et al. EM paper for ultrastructure.

Online Anatomy resources: go to www.wormatlas.org. There you will find:

(1) an online **handbook of worm anatomy**. This is currently under construction but promises to be very useful.
(2) a practical **guide to cell identification** using Nomarski DIC. Still under construction, but useful.
(3) HTML versions of the classic EM anatomical studies.

Also see the ‘cells’ section of Leon Avery’s web site. Contains a digital version of the worm lineage drawing and scans of some electron micrographs used in the Mind of the Worm reconstruction (labeled ‘N2U serial electron micrographs’).

To quickly find the name and lineage origin of a cell, look in the parts list (Appendix 1 of the first worm book).

Wormbase has a **pedigree browser** in which you can find the position of a cell or group of cells in the lineage. Also you can search Wormbase for cells by name and get a **cell report** detailing ancestry, putative function, and lists of genes expressed in that cell or similar cells.

13D. Dye Filling of sensory neurons

This is a very simple way to assay neuron morphology in living animals; although to some extent superseded by GFPs, it is convenient in that you do not need to do crosses to introduce transgenes.

1. Dilute DiO stock 1:200 in M9, to final concentration 10 g/ml. Some dye will precipitate when you do this; don't worry about it.
2. Put ~150 µl stain in a microtiter well and use a worm pick to put transfer some worms into the dye. Incubate 2-3 hours at room temperature. 24-well plates are easier to pick worms into than 96-well plates, but anything will do.
(This is for staining small batches of worms. If you want to stain lost of worms do it in a 15 ml tube on a roller drum. At 2 hr the 6 amphid neurons will be labeled. At 4 hours the amphid sheath cell will also become labeled. Overnight incubation will label the ILs and the socket cells.)
3. Use a mouth pipette to transfer the worms to a fresh plate, and let them crawl on a bacterial lawn for about 1 hour to destain. (Or wash the worms 3X in M9).
4. Put worms on azide agar pads and use FITC filters.

Positions of cell bodies.

Notes:

DiO is 3,3'-dioctadecyloxacarbocyanine perchlorate, m.w. 880 from Molecular Probes, catalog # D-275. Stock solution is 2 mg/ml in dimethylformamide (DMF), stored at -20° in a foil wrapped tube. DiO fills: ADL, ASK, ASI, ASH, ASJ, ADF, PHA, PHB.

1. **DiI** (DiIC18(3) 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) (Molecular Probes # D-282) fills the same cells as DiO except that ADF doesn't stain and AWB does (?); also fills sheath better. Same protocol, use Texas Red filters.

2. If worms are left in dye for longer than 4 hours or are starved, DiI can variably stain a lot of other neurons, including the amphid sheaths. Some people (e.g. Bargmann lab) stain for 18-24 hours; this slows down photobleaching significantly but the intestine can stain strongly.

3. **FITC filling** (after Hedgecock *et al.*, 1985). Largely superseded by DiO. FITC loads ADF, ASH, ASI, ASJ, ASK and ADL (6/8 exposed amphid neurons). Stock solution is 20 mg/ml in DMF, dilute 1:4 in M9 and apply to seeded plate. After allowing dye to diffuse for 2 hr, pick worms onto plate and allow to stain for 2 hr/over night. Then transfer to plate without dye for at least 10 minutes to remove dye from intestine. For staining phasmids or PDEs, stain in ice-cold buffer with 0.4 mg/ml FITC, wash in cold buffer then transfer back to plate (the animals don't feed, so no FITC gets into the intestine).

4. The PDEs can fill (at low frequency; 2%?) in *cat-6* or *che-14* mutants.

5. **Di4ANEPPS** (green) stains the amphid sheaths (strongest) and sockets, the IL sheaths and sockets and (weakly) the IL2s (and maybe the OLso, sh?). Make a stock solution 2 mg/ml in DMF and keep in the

dark. Stain at 4-20 g/ml in M9. Stain 2-3 h then wash twice and transfer to plate for 30 min. Keep in the dark at all times; it bleaches really fast.

6. **RH421**. Reddish dye, staining similar to Di4ANEPPS. Use at 20 g/ml in M9.

7. **DiO in Calcium.** Stain in 20-50 mM CaAcetate in water (not M9 or S basal). The IL2s stain strongly, IL sheaths and other cells (amphid) stain variably (?). The IL2s can fill with DiO in some other situations where the amphid neurons don't take up dye, for example in an *osm-3* mutant or when the amphid sheaths are killed (--AC).

8. **Other dyes** (see the great Molecular Probes catalog). DiA fills the same cells as DiO/DiI. Fast DiO and Fast DiI also stain the same (--CB). Smaller dyes (DiOC5, DiOC6) stain everything. DiD stains like DiO except also stains ADF (--KK); red stain so can be done in GFP worms.

Many dyes remain untested—we checked out DiIC$_{12}$(3) and D^{9}-DiI.

Summary of staining patterns

	Exposed								Unexposed				Tail	
Dye	ASE	ADF	ASG	ASH	ASI	ASJ	ASK	ADL	AWA	AWB	AWC	AFD	PHA	PHB
DiO or DiI	-	-	-	+	faint	+	+	+	-	faint	-	-	+	+
FITC	-	+	-	+	+	+	+	+	-	-	-	-	+	+
DiD	-	+	-	+	faint	+	+	+	-	faint	-	-	+	+

References

Hedgecock, E. Staining sensory neurons with carbocyanine dyes. Worm Breeder's Gazette *11*, 56.
Perkins et al. (1986). Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev Biol *117*, 456-487

E. Some notes on worm anaesthetics

There are several ways to keep worms still (but alive) for photography, movies, or laser ablation experiments.

1. Phenoxypropanol (1-phenoxy-2-propanol, $C_9H_{12}O_2$, m.w. 152.19. Old name = propylene phenoxetol.)

The main advantage of phenoxypropanol for worm use is that unlike azide it does not enhance GFP photobleaching. It is also useful for taking photos of male tails, as these curl up under azide.

Propylene phenoxetol was used as a bacteriocide. The earliest reference to its use as a narcotic is Owen (Nature **175** p434, 1955) who used it on molluscs. Ellenby and Smith (Nematologica **10,** p 342, 1964) described its use on nematodes. Why it stops worms moving is uncertain. (Fact: phenoxypropanol is the active component of some teenage anti-zit creams)

Phenoxypropanol is available from Pfaltz & Bauer, 172 E. Aurora St, Waterbury, CT 06708, (203) 574-0075, cat. # P07630 (for a solution of unknown molarity; I don’t know the details.)

Phenoxypropanol is sort of viscous but fairly soluble in water. It seems to become less effective over time after dilution, so we add it direct to the agar just before use. According to Sulston and Horvitz (1977) the concentrations of phenoxypropanol in agar optimal for certain stages are: 0.2% (v/v) for L1s, 0.3% for L2s, L3s and 0.5% for L4s and adults. 0.5% is about 33 mM.

According to Vanfleteren and Ward (WBG 4.1 p17) phenoxypropanol renders worms permeable to lipophilic dyes such as Hoechst 33258 or ethidium bromide.

2. Sodium azide NaN_3, m.w. 65.02

This is the most useful general purpose anesthetic except for GFP.

Original reference: FK Nelson (WBG 7.1 p96), who claims that azide anaesthesia does not make the Nomarski as bad as phenoxypropanol does.

Acts by inhibition of cytochrome oxidase, extremely toxic (to people). Worms are viable after at least 2 hours in azide, although after 30 minutes they look like hell.

We make up a 1 M solution in water and store at 4°C. Sodium azide is heat-labile, so must be added to agar after it has been melted, to a final concentration of 10 mM.

3. Tricaine-levamisole

The combination of tricaine and levamisole (tetramisole) is popular for timelapse movies of oogenesis in adults, as the contractions of the somatic gonad sheath cells are unaffected. Adults are immobilized by incubation for 15-20 min in 0.1% tricaine + 0.01% levamisole in water, then mounted on standard agar pads.