Axotomy using laser

All attempts to standardize axotomy procedures, the extent of axon regrowth varies considerably from animal to animal, with standard deviations of 1/3 of the average regrowth. It is not clear whether this stochastic variation reflects unavoidable experimental variability in axotomy or inherent biological variation in regrowth.

Almost all C. elegans neurons survive axotomy, but the regrowth response depends greatly on the lsion location. Most axotomy paradigms use lesions distal from the cell body (>50uM away in C. elegans), after which the severed proximal stump reforms a growth cone. When axons are severed closer (<30uM) to the cell body, the cell responds by sprouting new processes from the soma. (Axon regeneration mechanisms: insights from C. elegans, cell). And they don’t regrow.

Axotomy Protocol

1. Mount 3 L4s on one slide with 0.05% tetramisole
2. Test minimum laser power to cut coverslip at 100x
3. Align the 50uM mark on the eye piece to the cut
4. Use 2 strengths below the min. power to cut axons
5. Measure 50uM from the cell body using the eye piece
6. Cut at the 50uM mark at ~20+ pulses until there is a gap
7. Go through all worms again to make sure that the axons are cut
8. Take note of the orientation that the worm is lying and put them in separate dishes according to orientation (Left or Right)
9. Score them 24h later

Example of Axotomy Conditions

Temperature: RT

Developmental stage: L4

Power: 2 power lower than the min. strength that cuts the coverslip

**Scoring**

1. Mount worms according to the orientation that they are lying in
2. Make sure that the worms are far apart from each other so that you can identify each worm
3. Take pictures at 20x and 20x Z-stacks for each worm
   1. Ensure that entire PLM can be seen
4. Take pictures at 63x and 63x Z-stacks for each worm
   1. Ensure that entire regrowth is in focus
5. Exposure at 20x: 600ms, Exposure at 63x: 100ms (usually)

Decision to score again at 48h

1. Reconnection is unclear
   1. Distal and proximal is very near
2. Fusion in unclear
   1. Distal fragment is healthy even though not as healthy as proximal fragment

**Length of Regrowth**

Measure regrowth only in reconnected but not fused animals at 24 hours

Methods to measure

1. **From cut site (when axon starts to deviate) to end of regrowth**
2. Length of proximal fragment that pass the distal fragment

FIJI set scale: 20x: 3.08 pixels/uM

63x: 9.84 pixels/uM