

Quick Start Guide to NeuroPAL

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The NeuroPAL

1. The NeuroPAL is good for:
 - A) ID'ing reporter expression in neurons (using GFP, YFP, or CFP reporters).
 - B) Analyzing mutants by noting changes to the NeuroPAL colors in the mutant background.
 - C) Analyzing neuronal positioning under a variety of experimental manipulations.
2. But, like any tool, it has a learning curve.
3. Multiple people have learned to use this tool.
4. Please be patient when learning this new method.

Getting Started

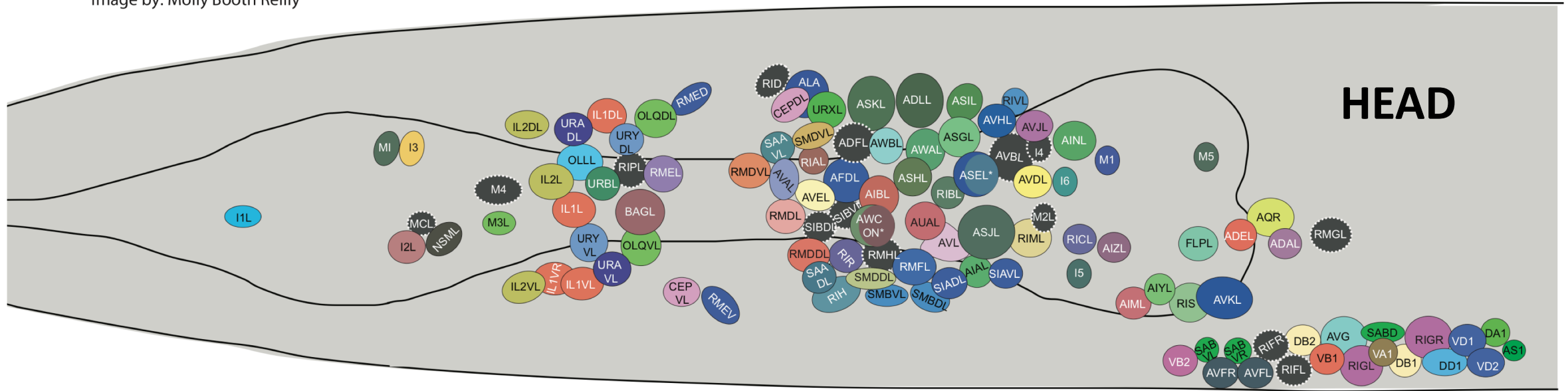
- **Freeze the NeuroPAL strains** into your collection.
- **There are 4 manuals available in multiple formats:**
 - Configuring Your Microscope for NeuroPAL
 - Using NeuroPAL for ID
 - NeuroPAL Reference Manual
 - OH15500 ID Reference Manual
- ***** If you have the time, I suggest the following:**
 1. **Configure your microscope** using the manual titled “**Configuring Your Microscope for NeuroPAL**”.
 2. **Learn to ID with NeuroPAL** using the manual titled “**Using NeuroPAL for ID**”.

A Quick Tutorial for ID'ing With NeuroPAL

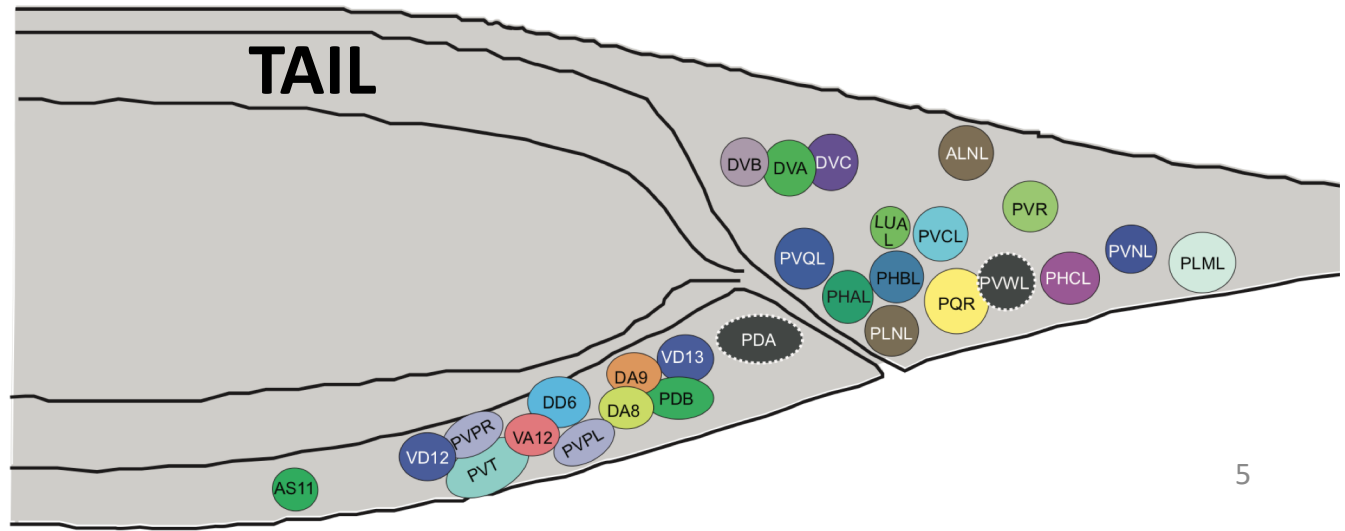
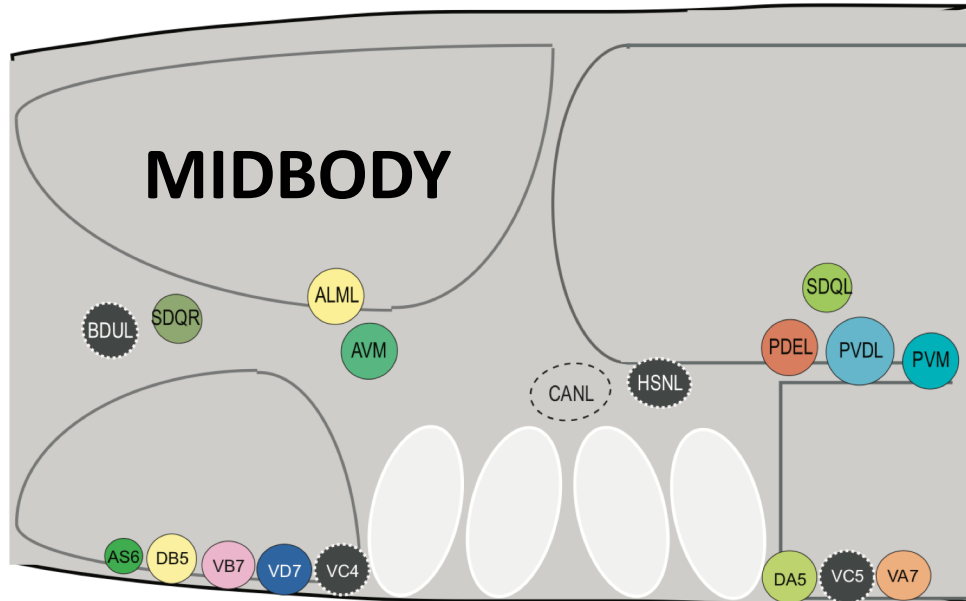
Please Read
“Using NeuroPAL for ID”
For a More In-Depth Guide

NeuroPAL Cartoon (Credit: Molly Booth Reilly)

Image by: Molly Booth Reilly



*AWC and ASE express different colors on left and right side of the worm. Both colors are shown overlapping.
 Neurons outlined by dotted white line are visible only with panneuronal marker on.
 CAN is not hit by neuroPAL or panneuronal.



Manual ID Conventions

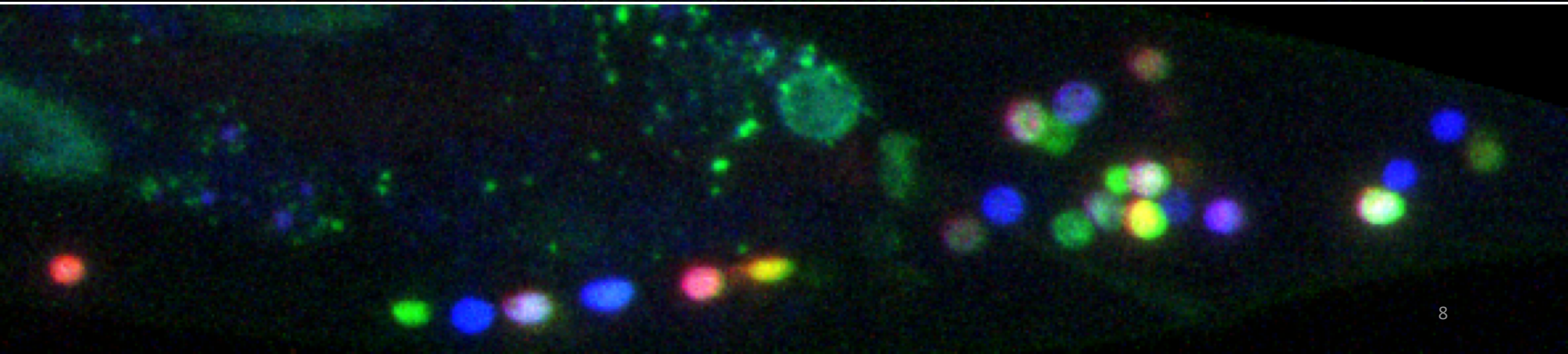
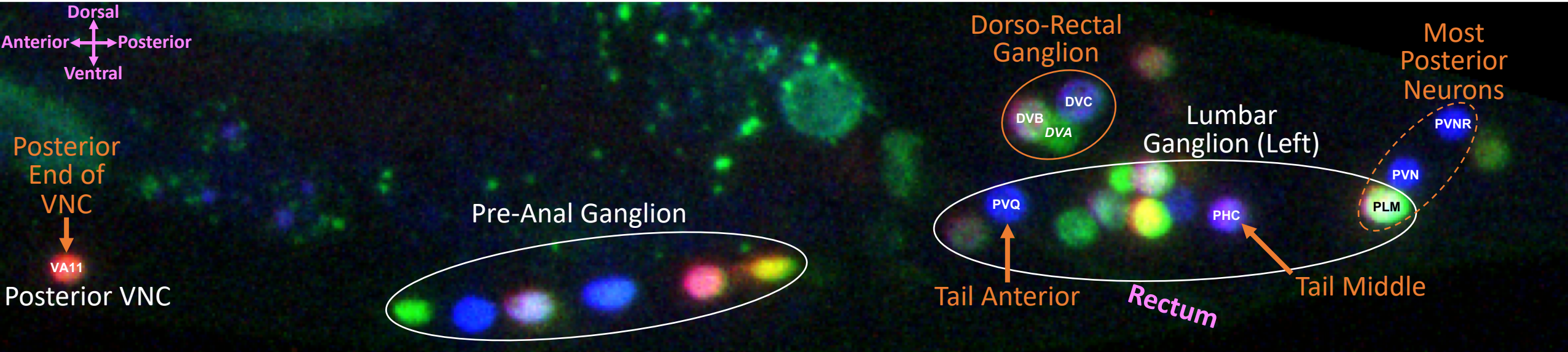
- This tutorial's example image volumes can be viewed using Zeiss's Zen Lite or Fiji (ImageJ).
- I significantly increased the gamma so as to increase cell visibility. The actual volumes may have fainter coloring.
- I use several conventions to help you learn to ID & use the NeuroPAL maps:
- The pages are ordered consecutively as follows:
 - Every 2 consecutive pages have identical images:
 1. Page 1 = anatomical landmarks & neural clusters with recognizable color schemes to help orient you.
 2. Page 2 = neural IDs & gangliar boundaries.
 - Every page has the same image, duplicated on top & on bottom:
 1. Top image = annotations.
 2. Bottom image = NO annotations, just a clear picture so you can see everything unobscured.
- *Italics* = the neuron's color is obscured due to being out of plane and/or another overlapping cell.
- * = the neuron is in a non-canonical location (it's usually found elsewhere).
- Black & White Text/Shapes/Lines = neurons & ganglia (I use black or white, depending on which is more visible).
- Lines: solid = ganglion or neural region, dotted = pharyngeal bulb, dashed = neural group.
- **Orange Text** = a good neuro-anatomical landmark to help orient yourself in the worm.
- **Pink Text** = not a neuron.

Tail

6-1_L4p_otIs669_x_him-5 (Tail Left Side)

Open "Tail/Lateral Tail/6-1_L4p_otIs669_x_him-5.czi":

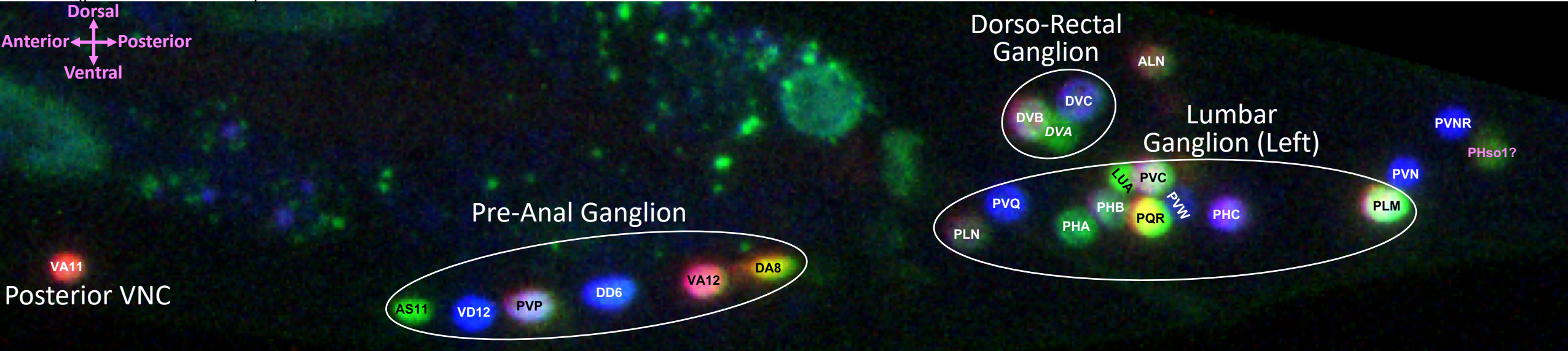
- 1) Orient yourself, on the left side of the tail, using the landmarks shown here.
- 2) Use the map on the next page to locate & ID each neuron.



6-1_L4p_otIs669_x_him-5 (Tail Left Side)

Tail Notes:

- PVC & PLM both display all 3 landmark colors. PVC is bright & PLM is even brighter.
- PHA is labeled by CyOFP only (green). PHB displays all 3 colors but is dimmer than PVC.
- PVW can range from light blue to solely displaying the panneuronal marker.
- LUA has a small nucleus.
- PHso1 (phasmid socket cell 1) is not a neuron. The PHDs are born in males from PHso1 transdifferentiation at L4.

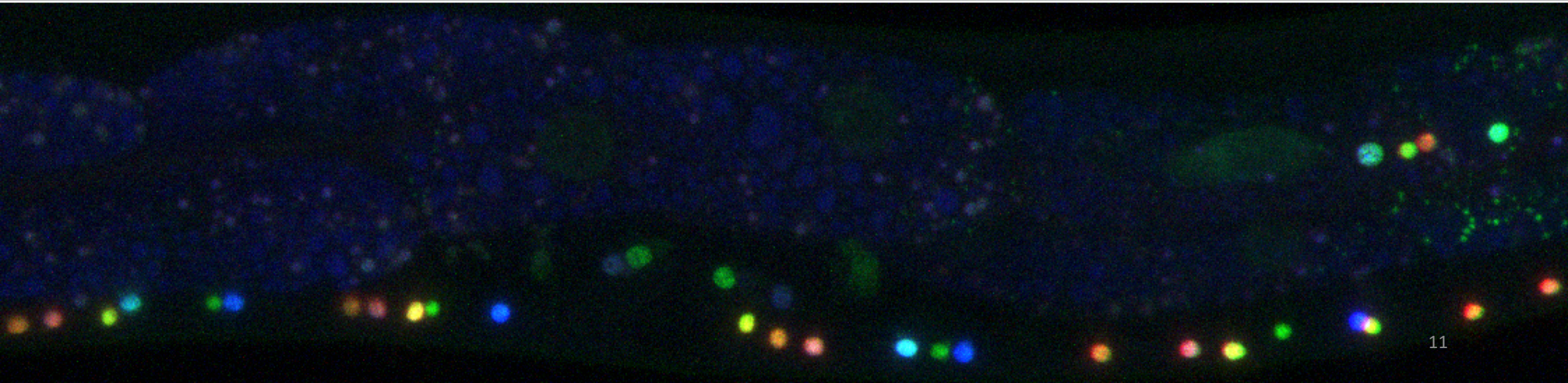
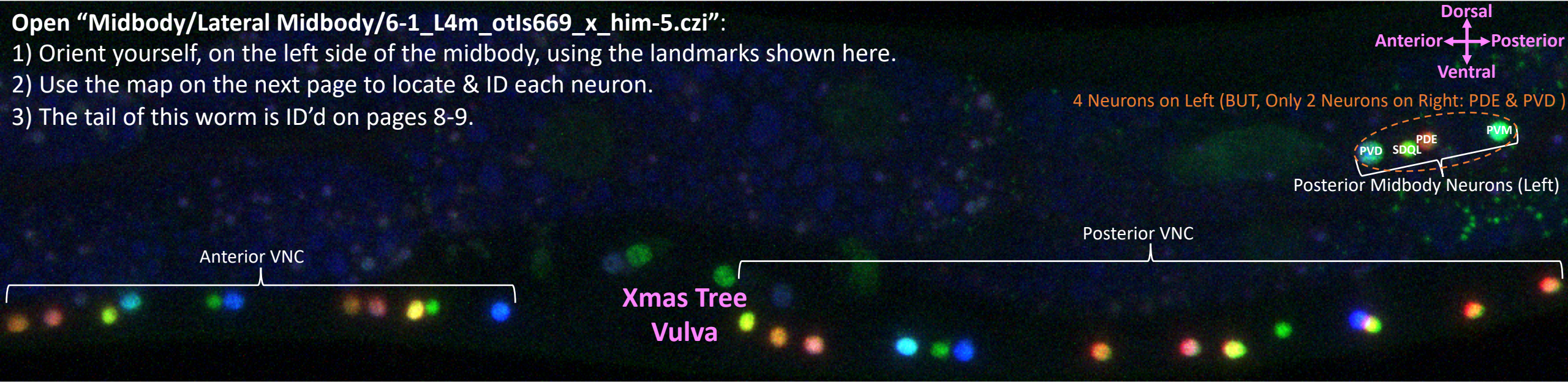


Midbody

6-1_L4m_otIs669_x_him-5 (Midbody Left Side)

Open "Midbody/Lateral Midbody/6-1_L4m_otIs669_x_him-5.czi":

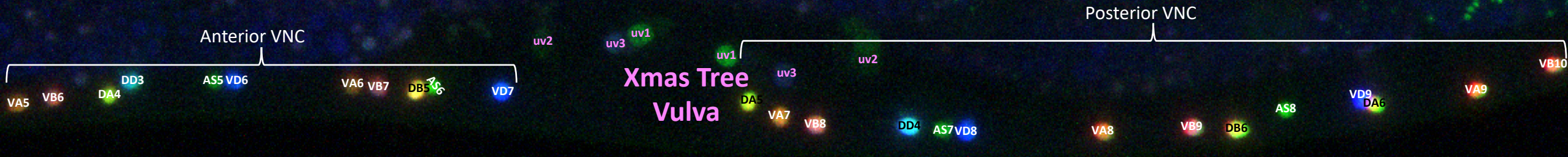
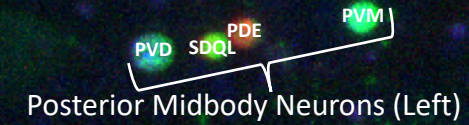
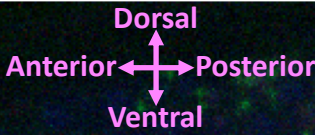
- 1) Orient yourself, on the left side of the midbody, using the landmarks shown here.
- 2) Use the map on the next page to locate & ID each neuron.
- 3) The tail of this worm is ID'd on pages 8-9.



6-1_L4m_otIs669_x_him-5 (Midbody Left Side)

VNC Notes:

- Neighboring neurons occasionally switch anterior-posterior order. Neurons near the vulva occasionally switch their anterior-posterior vulval location.
- AS/DA/DB are overtly green. AS has a small nucleus. DA/DB express red. DB expresses light blue.
- VA/VB are overtly red. VB expresses light blue. Neighboring VAs & VBs are born from the same P-lineage mother cell. The anterior division results in VA & the posterior one in VB. Hence, neighboring VAs & VBs are always oriented anterior & posterior, respectively (e.g., see the VAs & VBs in this image).
- HSN & VC only express the panneuronal marker beginning in adulthood (this animal is an L4).
- The uv's are not neurons. They may express weak reporters in the NeuroPAL.



Posterior Midbody Notes:

- PDE & PVD are also present on the right side & look identical to their left counterparts.
- SDQL (left side, posterior to the vulva) is less brighter than SDQR (right side, near the head).
- PVM (left side, posterior to the vulva) shares similar coloring to AVM (right side, near the head).

Head

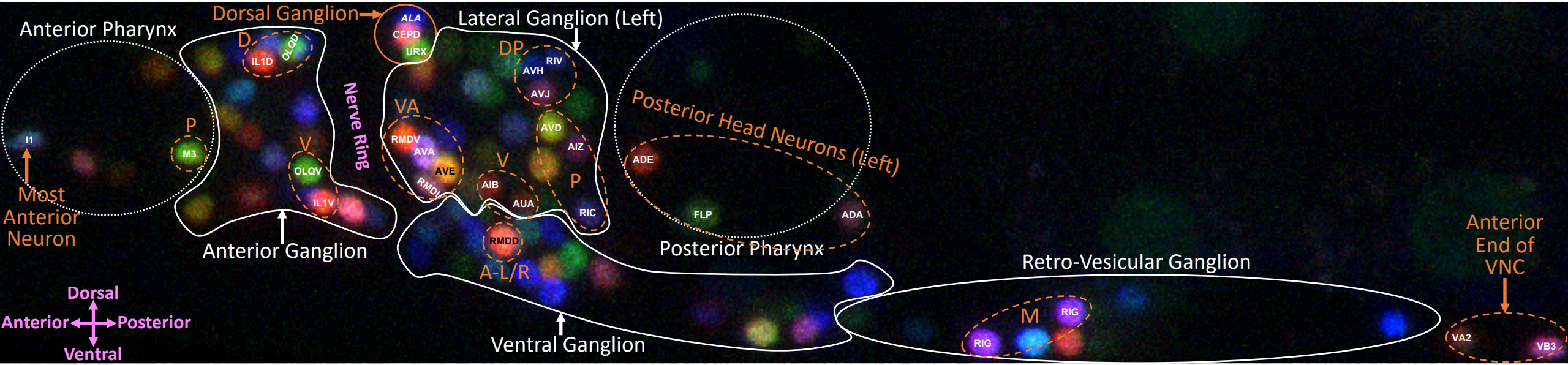
ID'ing in the Head

- **The worm's head contains the majority of its neurons.**
- The Anterior, Dorsal, Lateral, Ventral, Retro-Vesicular ganglia, 2 pharyngeal bulbs, & several unbound neurons all compete for space in the head. Physically separating boundaries are often weak (e.g., the nerve ring) or missing (e.g., ADA, ADE, AQR, FLP, & RMG are not really restricted). It's not easy to see where one ganglion ends & another begins.
- Due to the density of neurons & the lack of clear boundaries, **I highlighted clusters of neural landmarks that help delineate gangliar borders. These clusters are nearly always found at their stated border positions.** Some examples:
 - The I1s are the most anterior neurons in the worm, anything found more anterior is not a neuron (except in males).
 - The dorsal/ventral IL1s & OLQs delineate the posterior corners of the Anterior Ganglion.
 - The dorsal ganglion is distinguishable by its trio of blue, green, & reddish neurons (ALA, URX, & CEPD).
 - The boundaries of the lateral ganglion are delineated by the following clusters of landmarks:
 - Ventral Anterior corner = AVA, AVE, RMDs.
 - Dorsal Posterior corner = AVH, AVJ, RIV.
 - Posterior border = AIZ, AVD, RIC.
 - Ventral border = AIB, AUA.
 - The 2 RMDDs delineate the anterior left & right corners of the ventral ganglion.
 - The 2 RIGs are positioned near the middle of the Retro-Vesicular Ganglion.
 - VA2 & VB3 delineate the start of the Ventral Nerve Cord (always ordered anterior-posterior due to their P lineage divisions).
- **The gangliar landmark position glossary is: A=Anterior, P=Posterior, D=Dorsal, V=Ventral, L=Left, R=Right, M=Middle**
- **PLEASE LEARN TO SPOT & USE THESE CLUSTERS OF NEURAL LANDMARKS TO POSITION YOURSELF IN THE HEAD.**

18_YAa_otIs669 (Head Left Side)

Open "Head/Lateral Head/18_YAa_otIs669.czi":

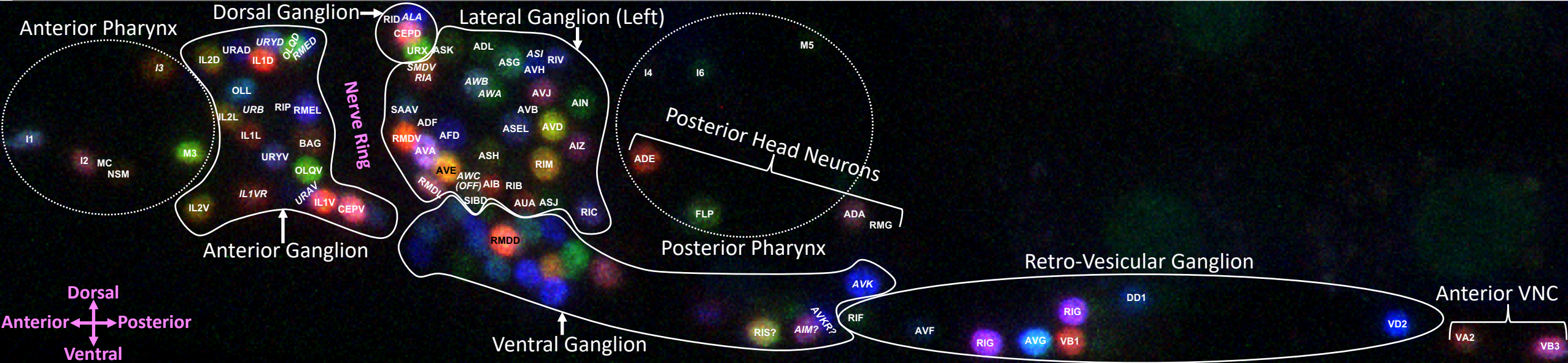
- 1) Orient yourself, on the left side of the head, using the landmarks shown here.
- 2) Use the map on the next page to locate & ID each neuron.
- 3) On the right, the **Posterior Head Neurons** include 1 more neuron, AQR.



Head Notes: 18_YAa_otIs669 (Head Left Side)

- MC, RIP, RID, ADF, ASI, AVB, I4, & RMG only express the panneuronal marker.
- IL2D/V & OLL lack positional stereotypy in the anterior-posterior plane.
- URA & URY are overtly blue. URY expresses all 3 colors. RMEL/R is mostly blue+red & is located at the D-V midline.
- ASK & ADL are overtly green. ASK expresses red as well, is anterior to ADL, & has a smaller nucleus than ADL.
- ASG, AWB, & AWA are overtly green. ASG expresses blue as well & is posterior to AWB. AWB expresses all 3 colors.
- AWA is green. ASH expresses red+green & is ventral to AWA. RIB expresses faint blue+green & is posterior to ASH.
- AVH & RIV are overtly blue. RIV expresses all 3 colors & has a small nucleus. AVJ is dorsal to AIZ.
- **AWC is stochastically asymmetric. OFF is greener than ON. AWC OFF is external to ASH & expresses more colors.**

- AIB & AUA are overtly red. AIB is greener, anterior & more internally positioned than AUA.
- ASE is deterministically asymmetric. ASEL expresses a green landmark, ASER does not.
- SAAV has a small nucleus & is anterior to AWB. AWB is anterior to ASE.
- AVD is dorsal to & has a smaller nucleus than RIM. RIM can move further ventrally & may appear weaker or brighter.
- AIZ & RIC lack positional stereotypy at their ganglionic location. AIZ expresses more red than RIC.
- SIBD, RIB, & ASJ are overtly green. SIBD has a very small nucleus & is anterior to RIB. RIB expresses blue & is positioned between SIBD & ASJ in the A-P axis. Relative to ASJ, RIB is fainter green & has a smaller nucleus.



Please Read “Using NeuroPAL for ID”
For More Details

