Using NeuroPAL for Neural ID

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Introduction

PLEASE READ ME FIRST

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 & follow ALL STEPS outlined in the section "Getting Started".

Getting Started

Please follow these steps to get started using the NeuroPAL:

1. **Freeze the NeuroPAL strains** into your collection.
2. **Configure your microscope** for NeuroPAL imaging:

   See the manual titled "**Configuring Your Microscope for NeuroPAL**".
3. **Plan a week to focus exclusively on learning the NeuroPAL** (see page 8):
   A. This manual IDs multiple volumes. These volumes are included & can be viewed in Zeiss's Zen Lite Blue Edition. Please use these volumes, in conjunction with this manual, to learn to perform neural ID with NeuroPAL.
   B. After reading this manual:
      i. Take ~10 images of the tail with your microscope & ID as many neurons as you can in your image volumes.
      ii. Take ~20 images of the head with your microscope & ID as many neurons as you can in your image volumes.
4. You will now be proficient in using the NeuroPAL.

Learning to ID with NeuroPAL ☺

- **<u>Be patient. It will take only 1 week of your dedication to be proficient.</u>**

- Those who have performed extensive neural ID know that, in the past, this process could take months & up to a year of guesses, crosses, & unfortunate discoveries, involving extensive errors in the literature, that confound the process of neural ID. Frequent Wormbase annotations like “some head/tail neurons”, attest to the difficulty of this task.

- **The NeuroPAL requires a single cross & 15-30 good pictures, totaling far less than 2 weeks of work, a considerable time savings. <u>Please consider how much time you’re saving & invest the 1 week it takes to learn to ID with the NeuroPAL.</u>**

Viewing the Accompanying NeuroPAL Volumes

Viewing the Accompanying NeuroPAL Volumes (.CZI Files) with Zeiss's Zen Lite (Blue Edition) Windows Users

Installing Zeiss's Zen Lite (Blue Edition)

PLEASE DO THE FOLLOWING:

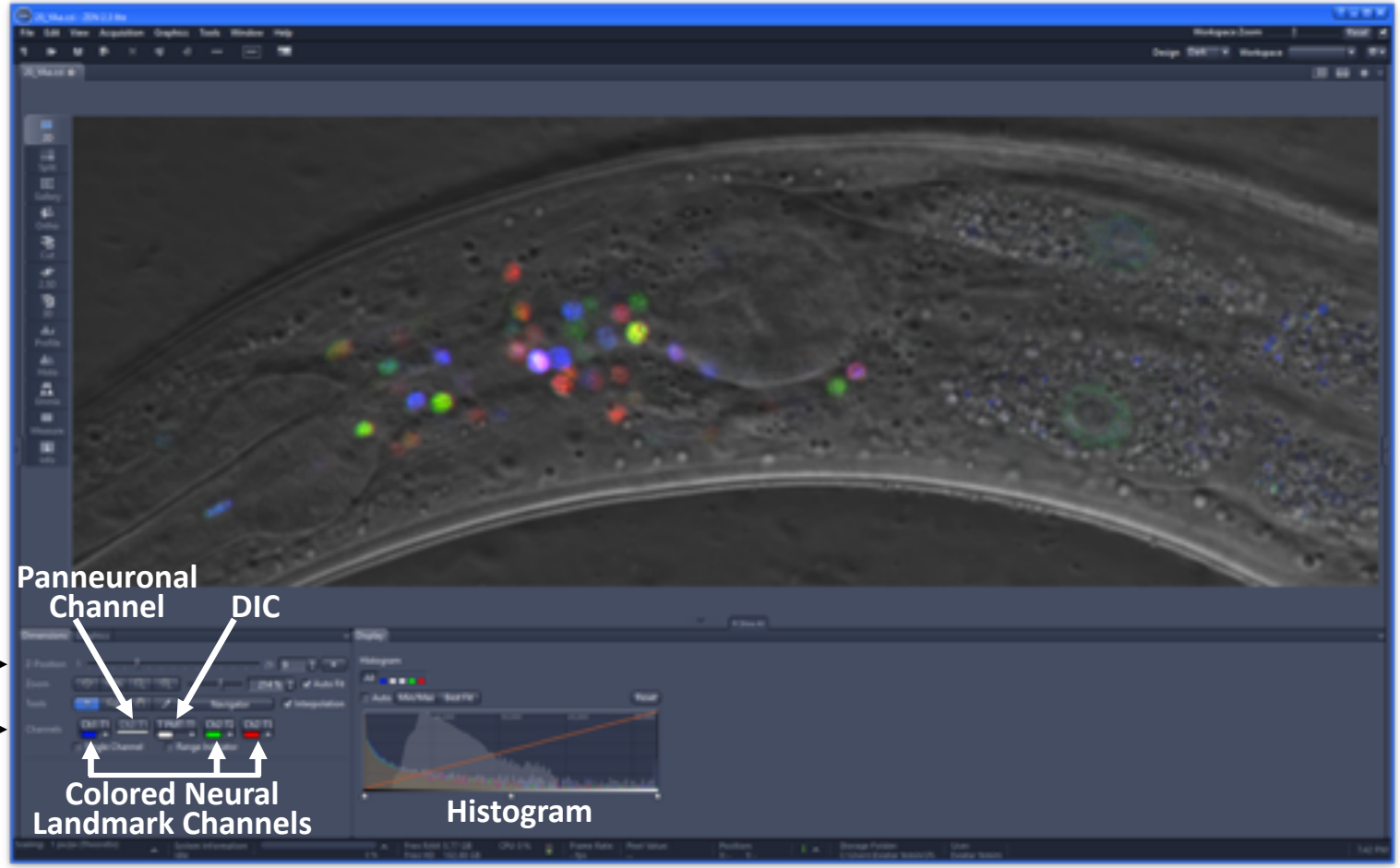
- You will be installing Zeiss's Zen Lite. Presently, it only runs on Windows.
- **If you're not running Windows, please use a virtual machine (VM) to run Zen or use Fiji instead.**
  - VirtualBox is a good free VM:
    https://www.virtualbox.org
  - Windows is available here (Zeiss's Zen Lite requires Windows version 7 or later):
    https://www.microsoft.com/en-us/software-download

- **Download & install Zeiss's Zen Lite (Blue Edition)**:
  https://www.zeiss.com/microscopy/int/products/microscope-software/zen-lite.html
- All image projections in this manual are included as Zen .czi files. These will be the first images you will learn to ID. The answers are in this manual, cheat without remorse.
- Start with the tail, it has very few neurons, life will be simple.

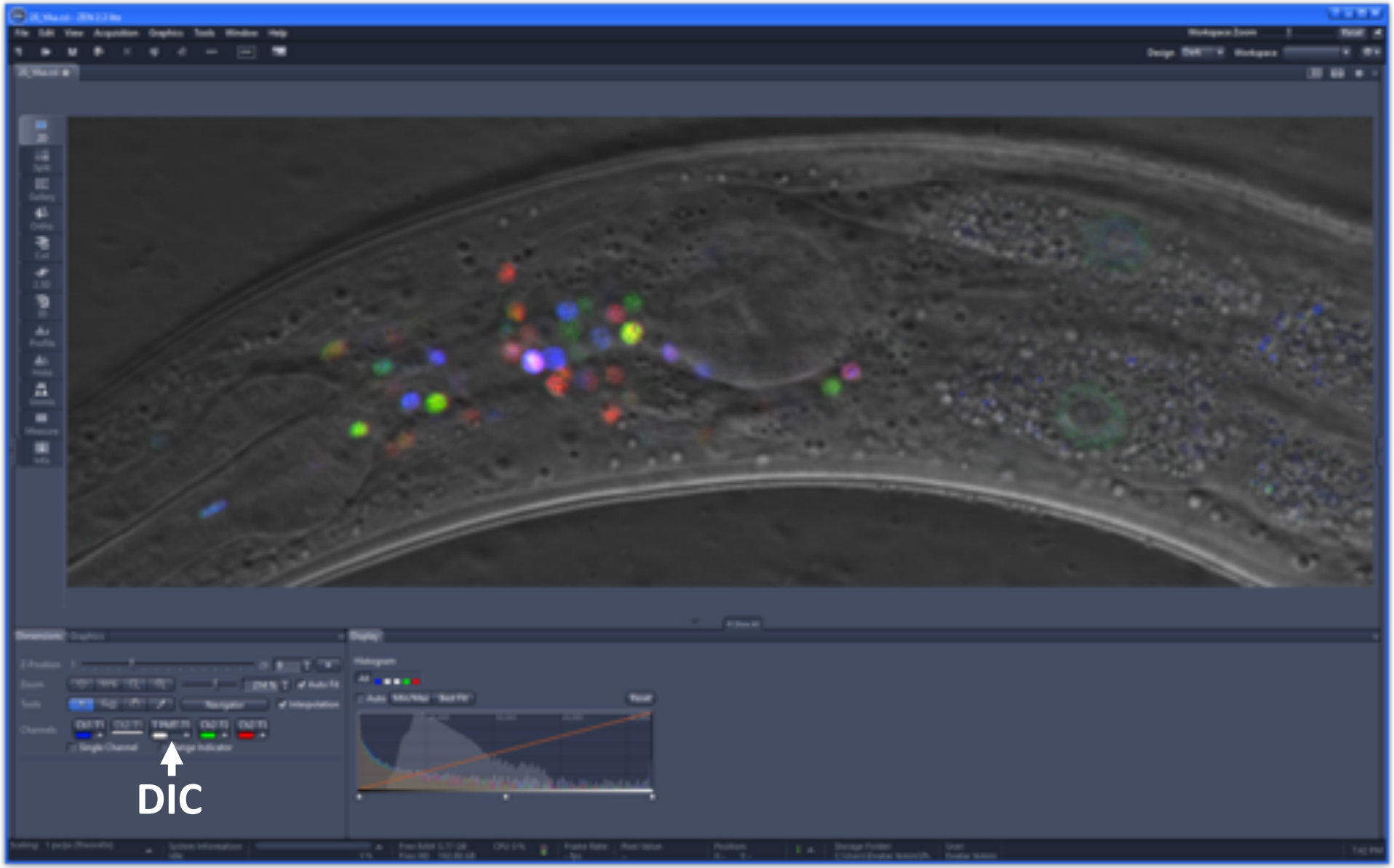
- **PLEASE READ THE FOLLOWING SECTIONS & FOLLOW THEIR INSTRUCTIONS.**

Using Zeiss's Zen Lite Blue Edition

- All images in this manual are included as Zen .czi files in the directory "NeuroPAL Volumes"
- For each image in this manual, open it's respective NeuroPAL volume in the "NeuroPAL Volumes" directory:
  - Adjust the "Z-Position" to navigate through the images Z slices.
  - Adjust the "Channels" to visualize the DIC, panneuronal marker, and/or colored landmarks.

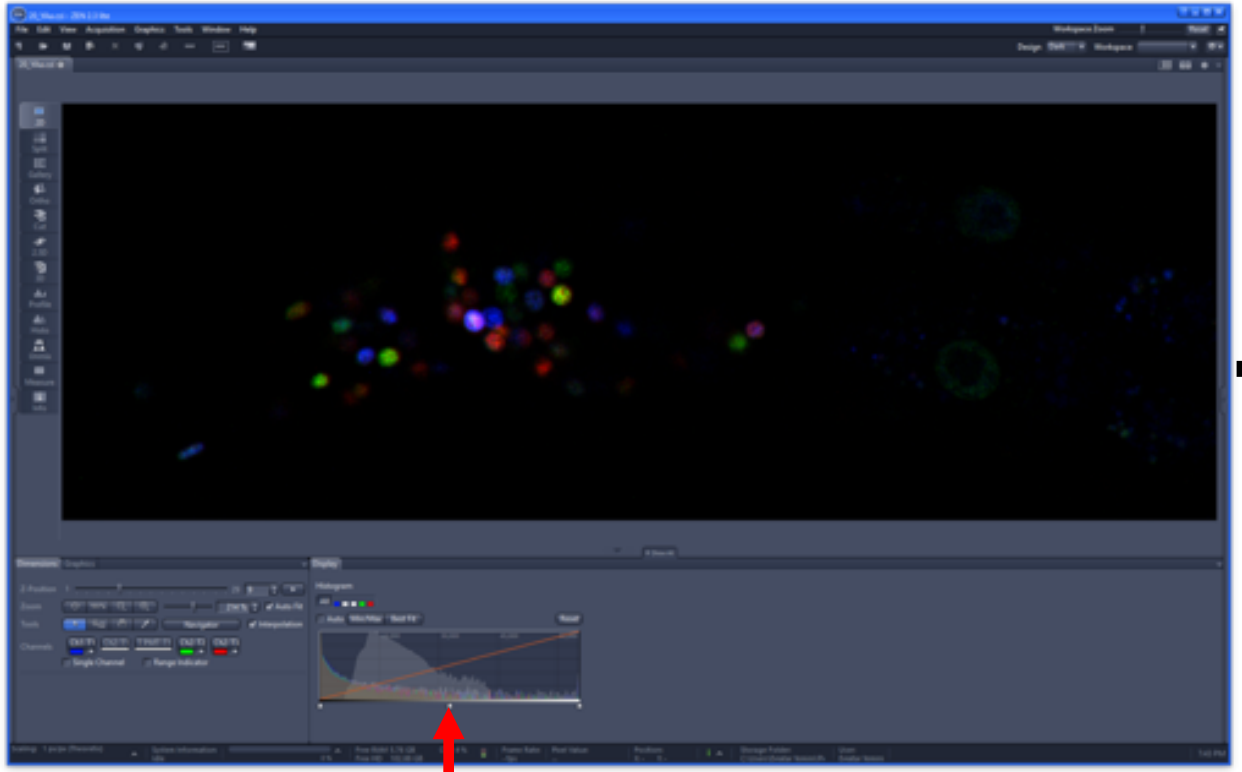


Zeiss's Zen: Adding the DIC Channel Brightens the NeuroPAL Colors & Displays the Worm's Anatomy

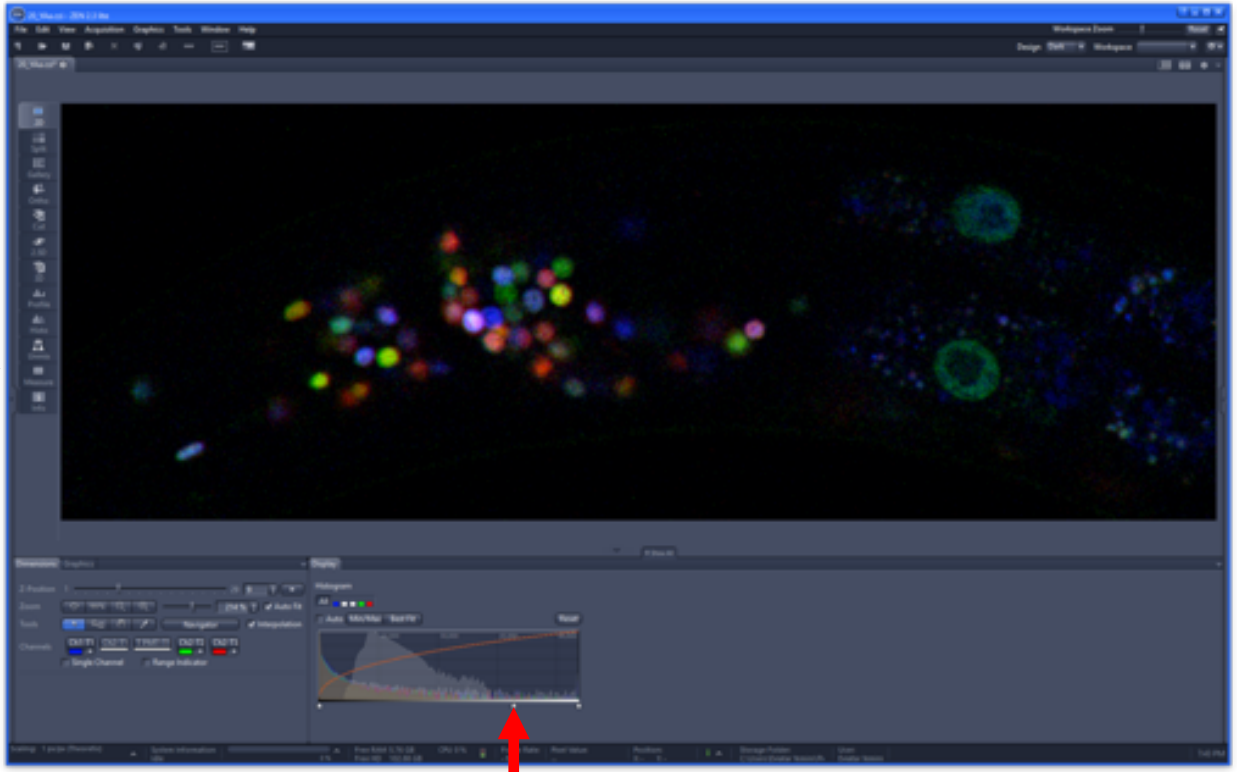


Zeiss's Zen: Adjusting the Gamma is Better than Adjusting the Histogram's Min/Max

If you turn off the DIC, you can improve the colors by increasing the gamma.
To do this in Zen, slide the **gamma** adjustment (**red arrow**) to increase the coloring.
Avoid going too far as this will increase the background noise & confuse you.



Gamma (Neutral)



Gamma (+ ~50%)

Viewing the Accompanying NeuroPAL Volumes (.CZI Files) with Fiji (ImageJ)- Mac/Linux/Windows Users

Installing Fiji (ImageJ)

PLEASE DO THE FOLLOWING:

- You will be installing Fiji. Fiji runs on Mac, Linux, and Windows operating systems.
- **Download & install Fiji:**

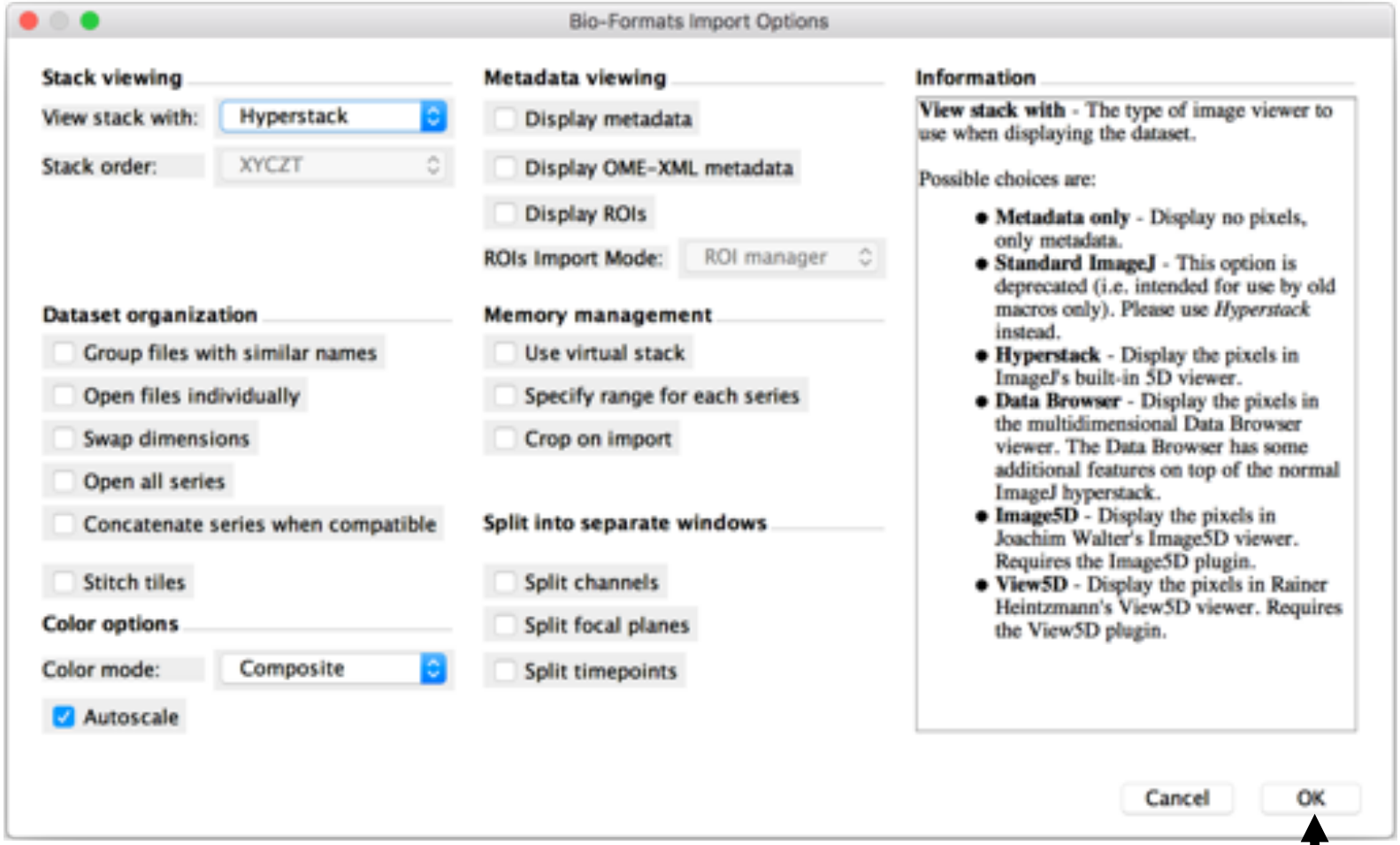
  https://imagej.net/Fiji/Downloads

- All image projections in this manual are included as Zen .czi files. These will be the first images you will learn to ID. The answers are in this manual, cheat without remorse.
- Start with the tail, it has very few neurons, life will be simple.

- **PLEASE READ THE FOLLOWING SECTIONS & FOLLOW THEIR INSTRUCTIONS.**

Opening .CZI Files in Fiji (ImageJ)

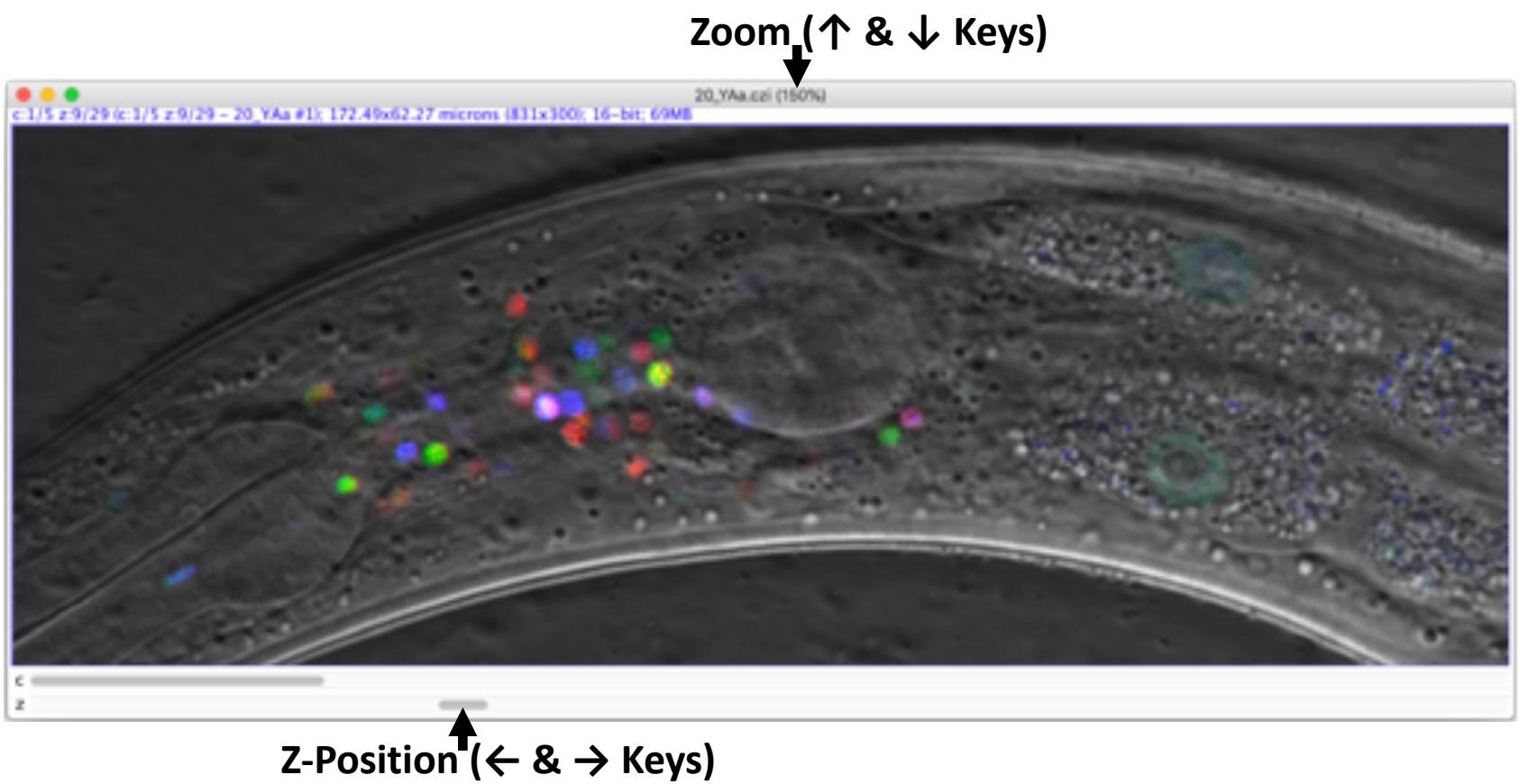
When opening a .czi file, you will be greeted with the following window.
Keep the default settings. **Just click "OK".**

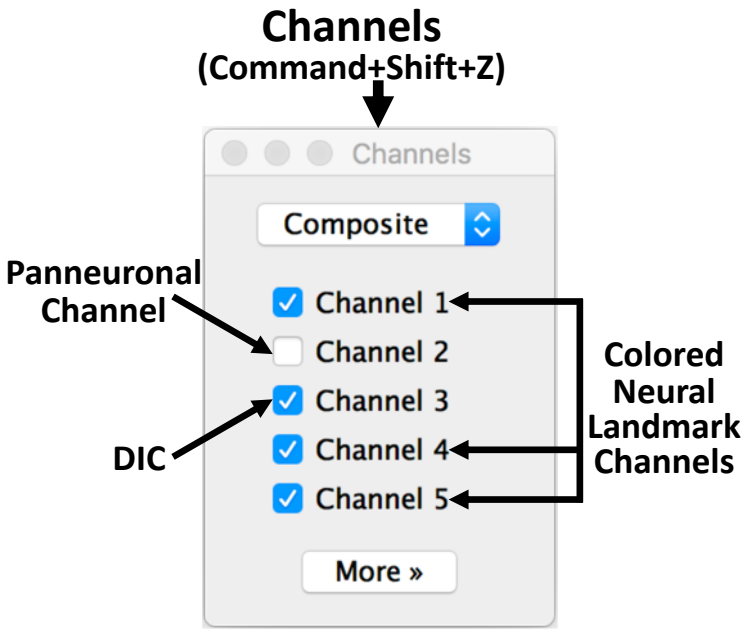


Don't change anything, just click here.

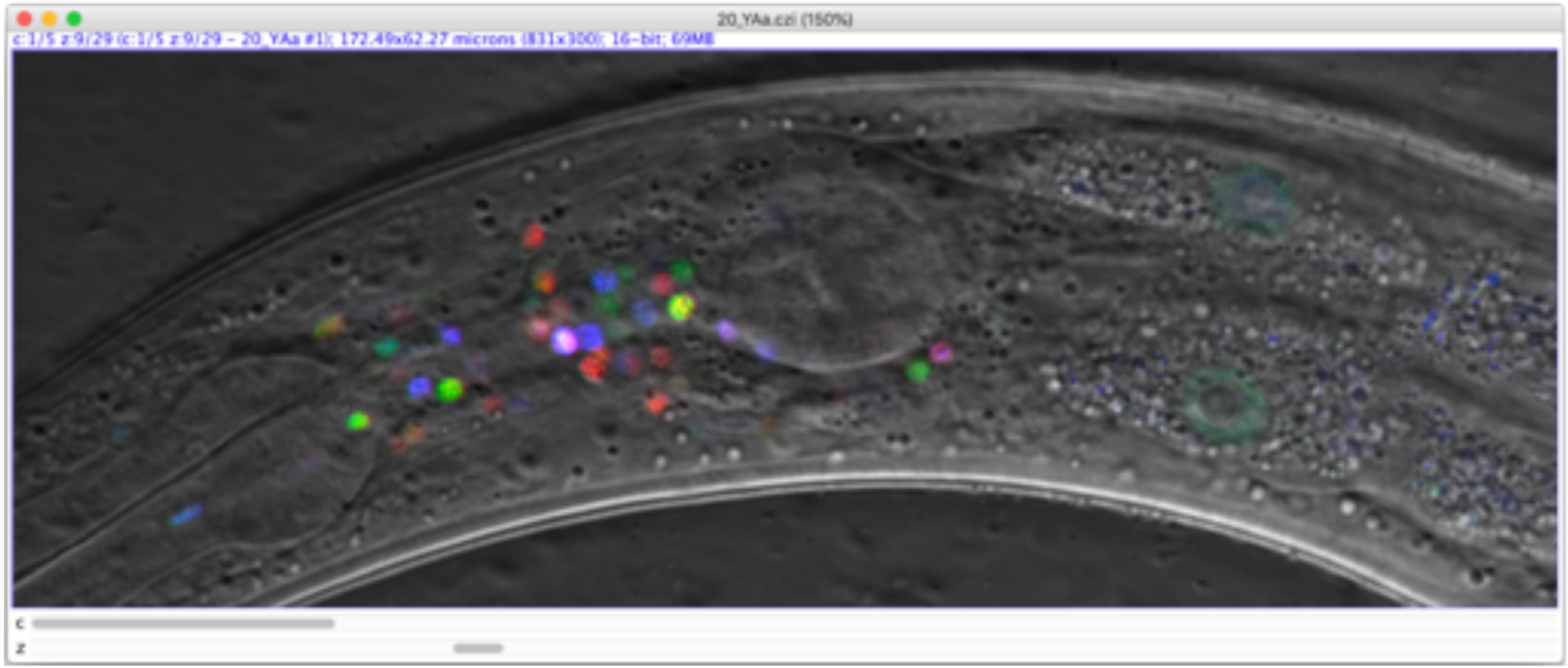
Using Fiji (ImageJ)

- All images in this manual are included as Zen .czi files in the directory “NeuroPAL Volumes”
- For each image in this manual, open it’s respective NeuroPAL volume in the “NeuroPAL Volumes” directory:
  - Adjust the **Z-Position (← & → key shortcuts)** to navigate through the images Z slices.
  - Adjust the **Channels (Command+Shift+Z shortcut)** to visualize the DIC, panneuronal marker, and/or colored landmarks.
  - Adjust the **Zoom (↑ & ↓ key shortcuts)** to make the image bigger.

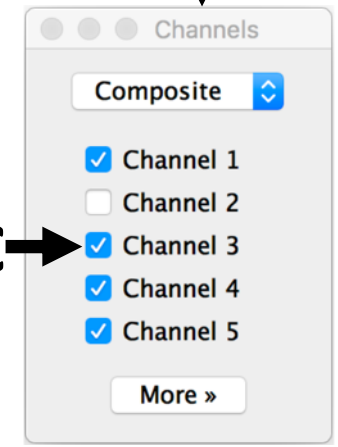




Fiji (ImageJ): Adding the DIC Channel Brightens the NeuroPAL Colors & Displays the Worm's Anatomy



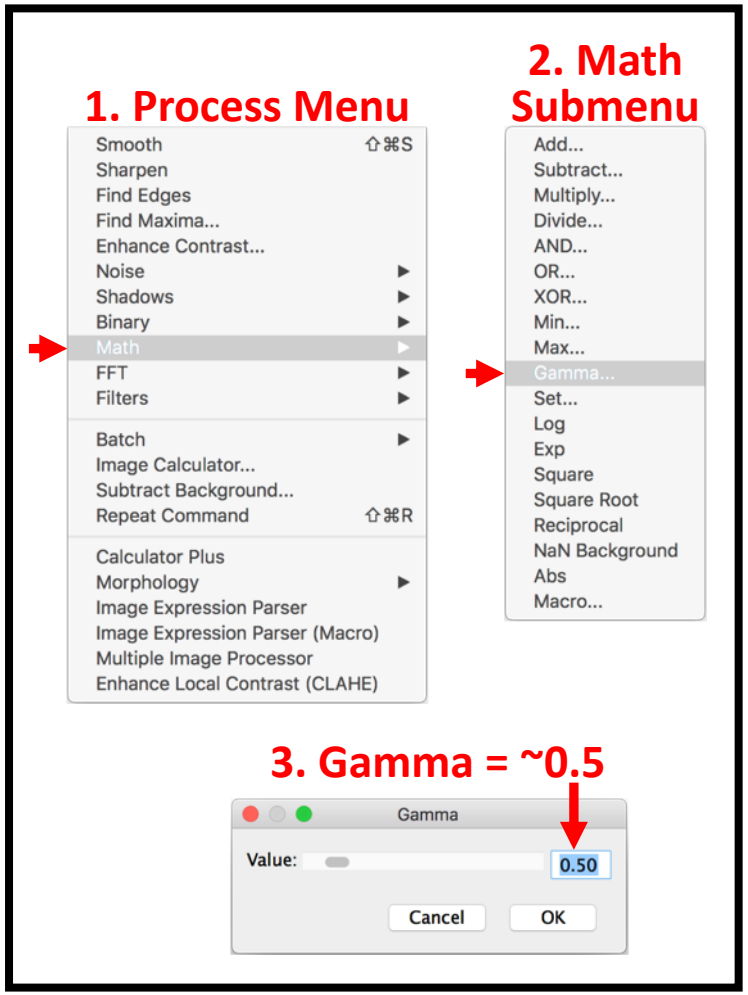
Channels
(Command+Shift+Z)

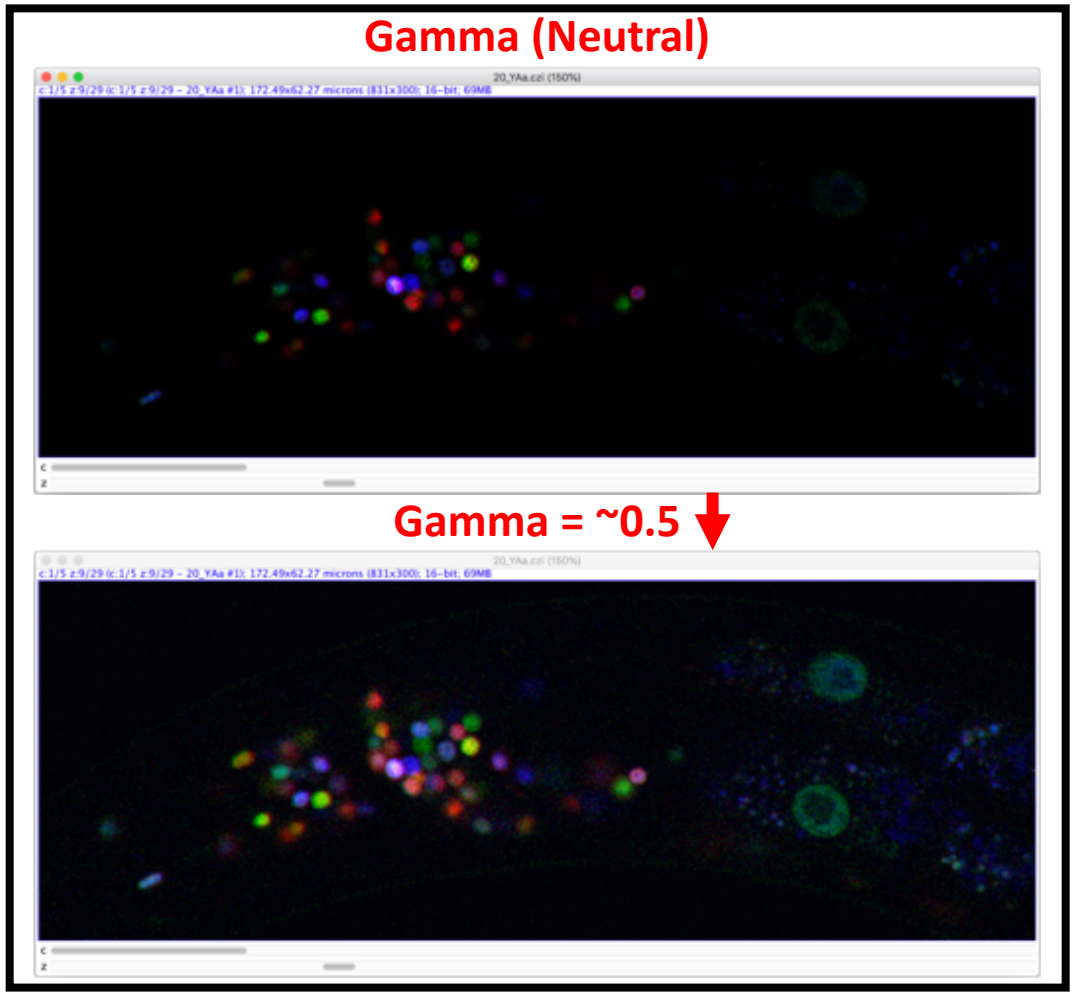


DIC

Fiji (ImageJ): Adjusting the Gamma is Better than Adjusting the Histogram's Min/Max

If you turn off the DIC, you can improve the colors by increasing the gamma.
To do this in Fiji, select the "Process|Math|Gamma…" submenus (**red arrows**) to increase the coloring.
Avoid going too far as this will increase the background noise & confuse you.





LEARNING TO ID WITH NEUROPAL

Learning to ID with NeuroPAL: Step 1, Orienting Your Worm

Orienting Your Worm: Conventions for Publication
PLEASE READ & FOLLOW THESE INSTRUCTIONS

- Worm publications follow strict conventions for worm orientation:

1. **ALWAYS orient your worm so that:**
   ANTERIOR is LEFT (WEST) & POSTERIOR is RIGHT (EAST).

2. **For lateral views (sagittal plane), ALWAYS orient your worm so that:**
   DORSAL is UP (NORTH) & VENTRAL is DOWN (SOUTH).
   *** Note: most neurons are easiest to ID using a lateral view.**

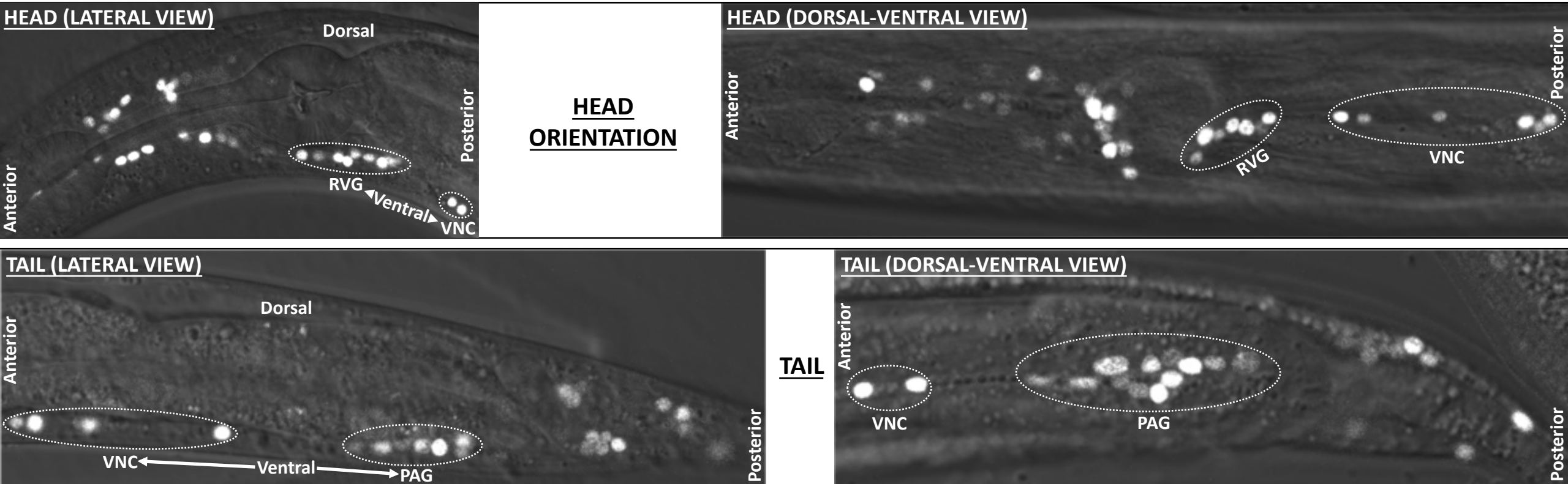
3. **For dorsal-ventral views (coronal plane), I'm not aware of any convention but I use:**
   Left-side up (north) & Right-side down (south).
   *** Note: the ventral, retro-vesicular, & pre-anal ganglia can ONLY be ID'd using a dorsal-ventral view.**

- ***** IF YOUR WORM IS ORIENTED INCORRECTLY, YOU WILL NOT BE ABLE TO ID IT.**
- ***** DON'T USE TWISTED WORMS, YOU WILL CONFUSE EVERYONE, INCLUDING YOURSELF ☺**

Orienting Your Worm

- **<u>Use the taper at the head & tail to orient your worm anterior on left (west), posterior on right (east):</u>**
  - At the head, the taper is oriented so that the head narrows as you progress anterior.
  - At the tail, the taper is oriented so that the tail narrows as you progress posterior.
- **<u>For lateral views, use the ventral nerve cord (VNC), its head extension the retro-vesicular ganglion (RVG), & its tail extension the pre-anal ganglion (PAG), to orient your worm dorsal up (north), ventral down (south):</u>**
  - Use the **<u>panneuronal (TagRFP-T) channel</u>**: the neurons in the **<u>VNC, RVG, & PAG are down (south)</u>**, the empty-ish space is up (north).
- For dorsal-ventral views, you can use several neurons to orient the left & right sides of your worm:
  - *** This task will be much easier after you've learned to ID with NeuroPAL, for now, you can ignore it.

<u>Head</u> neurons: <u>Left=I6,M5</u> vs. <u>Right=AQR, M1</u> <u>Tail</u> neurons: <u>Left=PQR</u> vs. <u>Right=PVR</u>



Learning to ID with NeuroPAL: Step 2, Orienting Yourself Inside the Worm

Orienting Yourself Inside the Worm

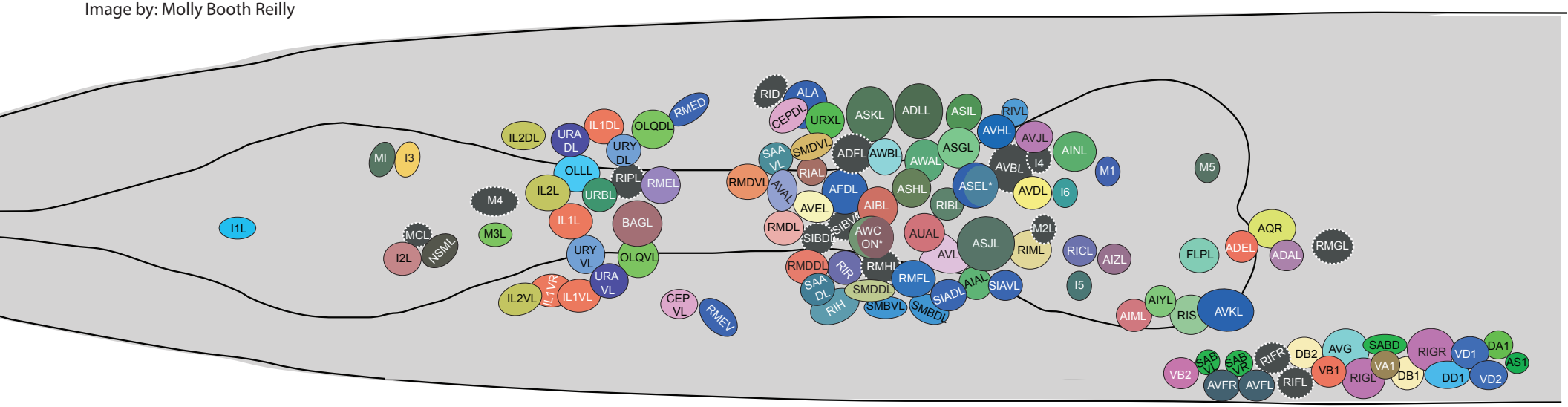
- **To ID, you must <u>orient yourself inside the worm</u>. You must know:**
  - **<u>Which ganglion are you in?</u>**
  - **<u>Where in the ganglion are you (dorsal, ventral, anterior, posterior, left, right, center, and/or edge)?</u>**
  - This may seem daunting but, if your worm is correctly oriented, it will be easy.

- The worm has a lot of visual cues to help you orient yourself for ID. The following visual orientation cues will be highlighted in the ID volumes presented later in this manual:
  - Anatomical landmarks (e.g., pharyngeal bulbs, nerve ring, gut, rectum, ...).
  - Neural clusters with easily recognizable color schemes.

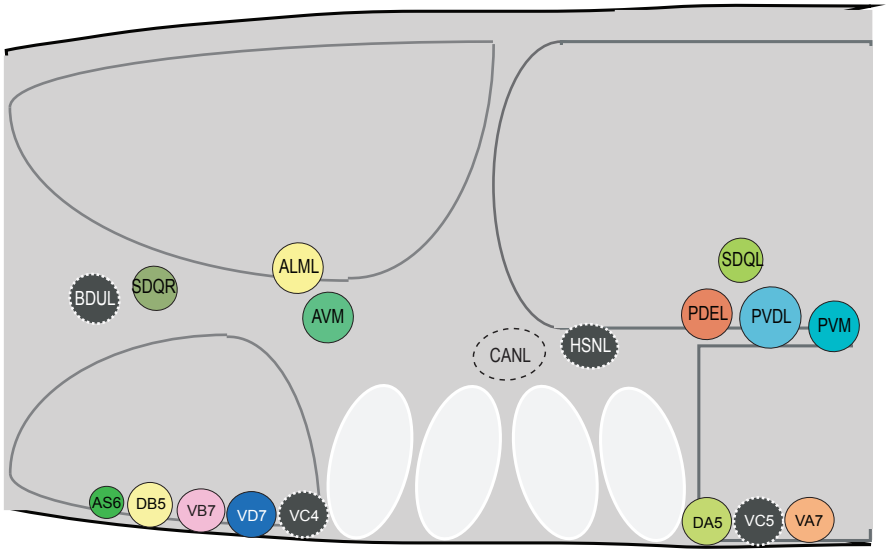
- **The neurons themselves have several, <u>redundant cues to help you ID</u> them:**
  - **<u>A combination of colored landmarks</u>** (e.g. AVH is blue & its neighbor AVJ is purple).
  - **<u>Brightness</u>** (e.g., in addition to shape cues, AS1 is weakly colored & its neighbor SABD is brightly colored).
  - **<u>Position</u>** (e.g., in addition to size & color cues, ASG is always dorsal-posterior to AWB).
  - **<u>Size</u>** (e.g., LUA has a small nucleus & its neighbors all have much larger nuclei).
  - **<u>Shape</u>** (e.g., in addition to coloring cues, SABD is oblong & its neighbors are round).
  - **<u>Nuclear Stippling</u>** (e.g., in addition to shape & color cues, AVG is stippled & its neighbor DD1 is solid in coloring).

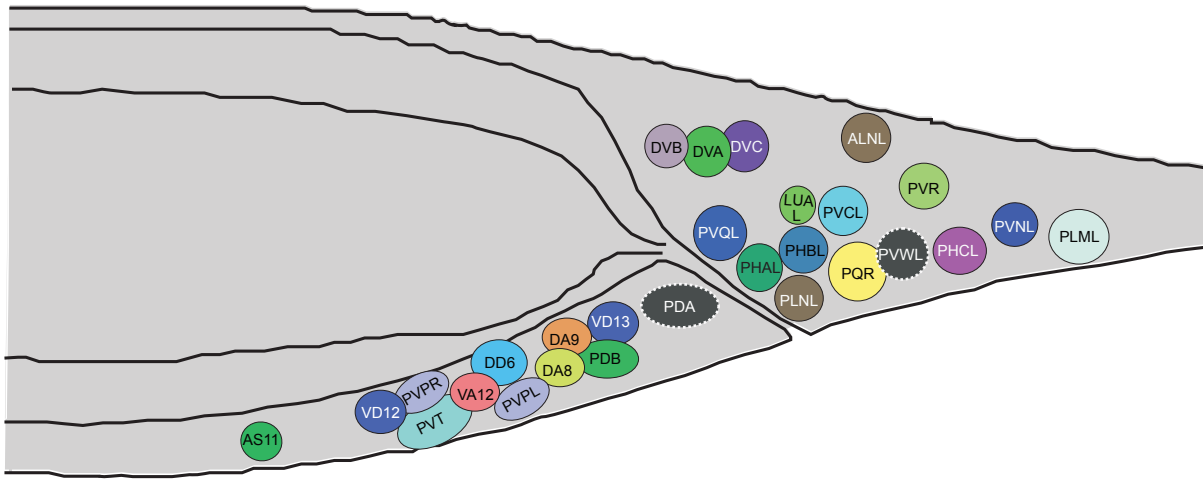
NeuroPAL cartoon (Credit Molly Booth Reilly)

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.





Which Neurons Move Around?

- **Short answer, all of them. That's why we developed the NeuroPAL ☺**
- **Medium answer, <u>don't worry too much about this</u>**:

1. When developing the NeuroPAL, I noted which neurons stray far from their canonical position. These neurons were deliberately colored so as to avoid confusion with the various neighbors whom they might end up next to.
2. The majority neurons stay close to the canonical positions represented in this manual. If you have 10 or more pictures, you will find that they almost always match the neural configurations pictured in the manual.

- **Long answer (if you care …)**:

1. Pharyngeal neurons display extreme stereotypy. You will find them exactly where expected.
2. Neurons in dense, gangliar regions don't move very far. They're usually found within 1-2 neurons distance from their canonical position (e.g. AWA & AWB usually just switch their dorsal & ventral configuration). This holds true even for the VNC, although, neurons at the vulva occasionally cross to the opposing side.
3. Neurons at sparse, edges of ganglia have much more leeway, especially when there is no major physical barrier in their way (e.g., AIN can move markedly posterior & PVQ can move markedly anterior).
4. Physical barriers like the nerve ring and, especially, the excretory duct & canal, don't present a strong challenge to cross. If a neuron looks like it crossed the nerve ring from the anterior to the lateral or ventral ganglia (and vice versa) it may well have done so (e.g. RIH is often found at the edge of the anterior ganglion). Similarly, the ventral ganglion's posterior edge & RVG's anterior edge is a murky boundary (e.g., AIA, SIAV, SMBV, AIM, AIY, AVK, RIS, & VB2 occasionally congregate near each other, creating some confusion).
5. The ventral ganglion is a messy place, densely packed with neurons & squashed under the posterior pharynx. ID'ing neurons herein can often be confusing. If need be, take many more pictures to ID this ganglion.

Learning to ID with NeuroPAL: Step 3, Distinguishing Colors

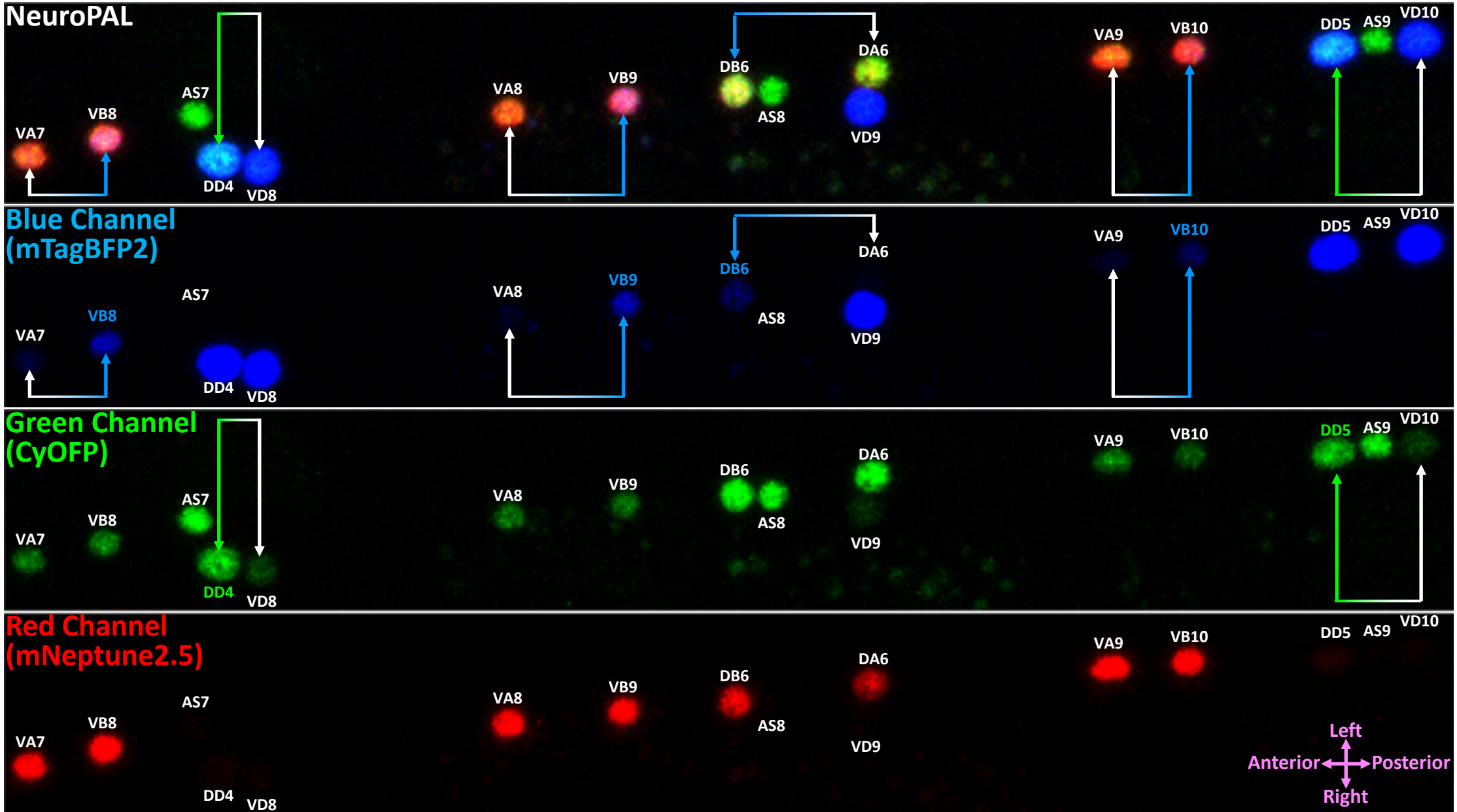
Distinguishing Colors

- The **NeuroPAL coloring scheme** was chosen, as best possible, to accentuate color differences between nearby neurons, thereby making them easy to distinguish from each other & ID.
- **The idea is simple: neighboring neurons should look as dissimilar as possible.**
- For the majority of the NeuroPAL's neurons, color permits easy identification (alongside redundant cues from nuclear morphology, position, & texture).
- On occasion, **you may need to inspect the individual color channels to help you distinguish a neuron from its similarly colored neighbor.**

- The following page shows an example from the ventral nerve cord (VNC).
- The VNC contains 8 classes of motor neurons. These classes have distinguishable coloring ***BUT***:
  - DD (blue+green) may express weak green &, therefore, look similar to VD (blue).
    DB (blue+green+red) may express weak blue &, therefore, look similar to DA (green+red).
  - VB (blue+red) may express weak blue &, therefore, look similar to VA (red).
- The following page will illustrate how to inspect the individual color channels to help you distinguish between similar looking motor neurons in the VNC.

Please Open: “NeuroPAL Manual Image Volumes/ Midbody/ Dorsal-Ventral Midbody/22_YAmV_otIs669_x_him-5.czi”

Turn the landmark color channels on & off to see that: 1) The DDs are greener than the VDs. 2) The DBs are bluer than the DAs. 3) The VBs are bluer than the VAs.



Learning to ID with NeuroPAL: ID Maps

Manual ID Conventions

PLEASE READ & PRINT THIS FOR YOUR REFERENCE!

- **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.

Learning to ID with NeuroPAL: Step 4, ID'ing the Tail

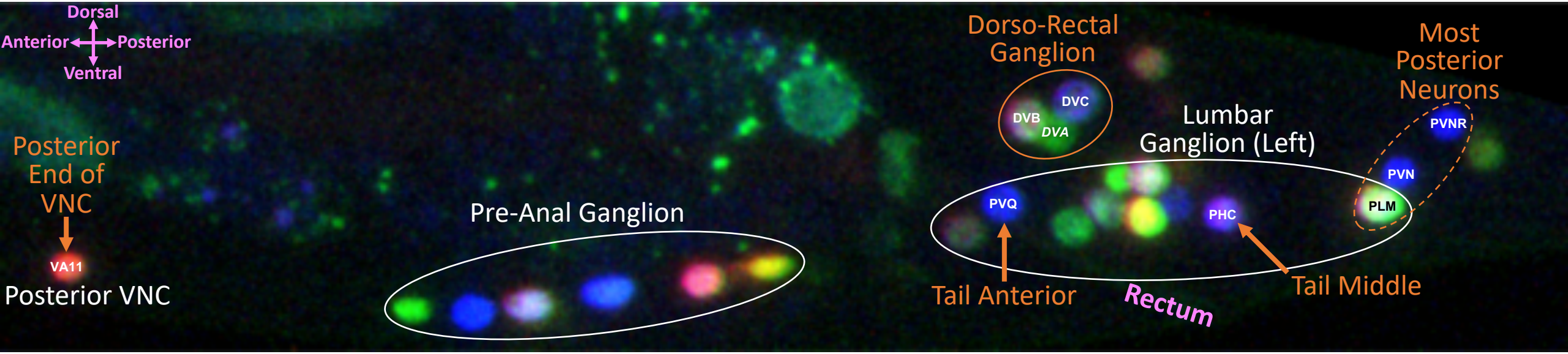
Tail (Lateral Projections)

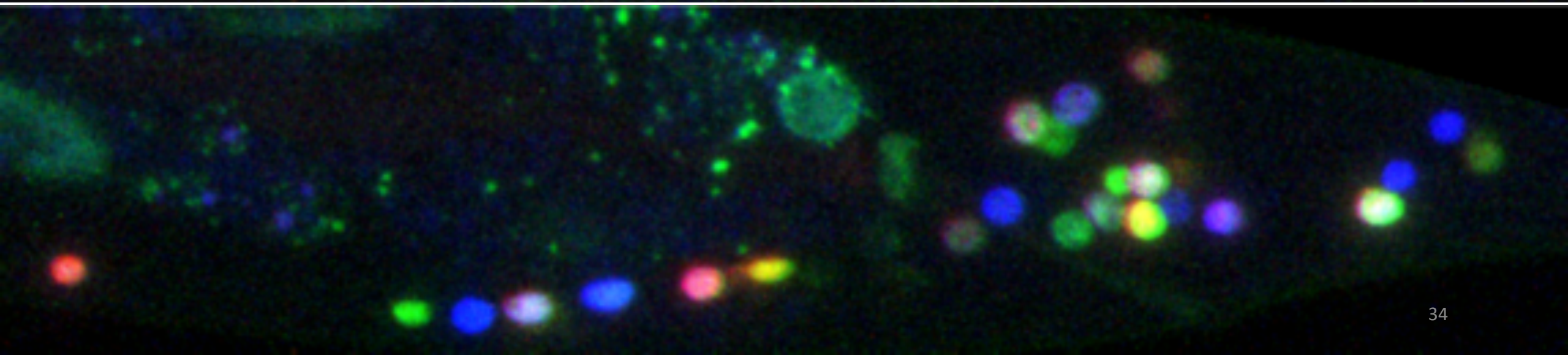
6-1_L4p_otIs669_x_him-5 (Tail Left Side)

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.

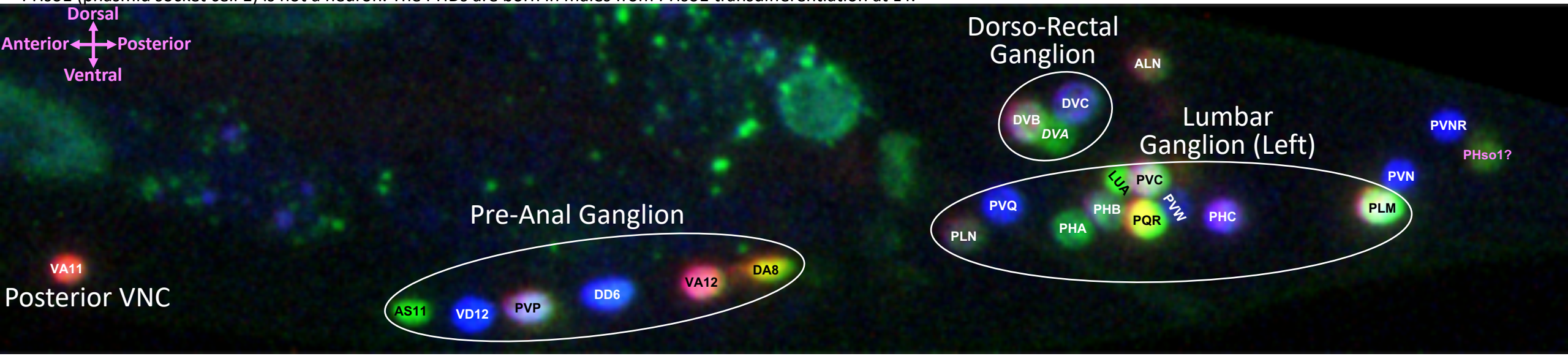


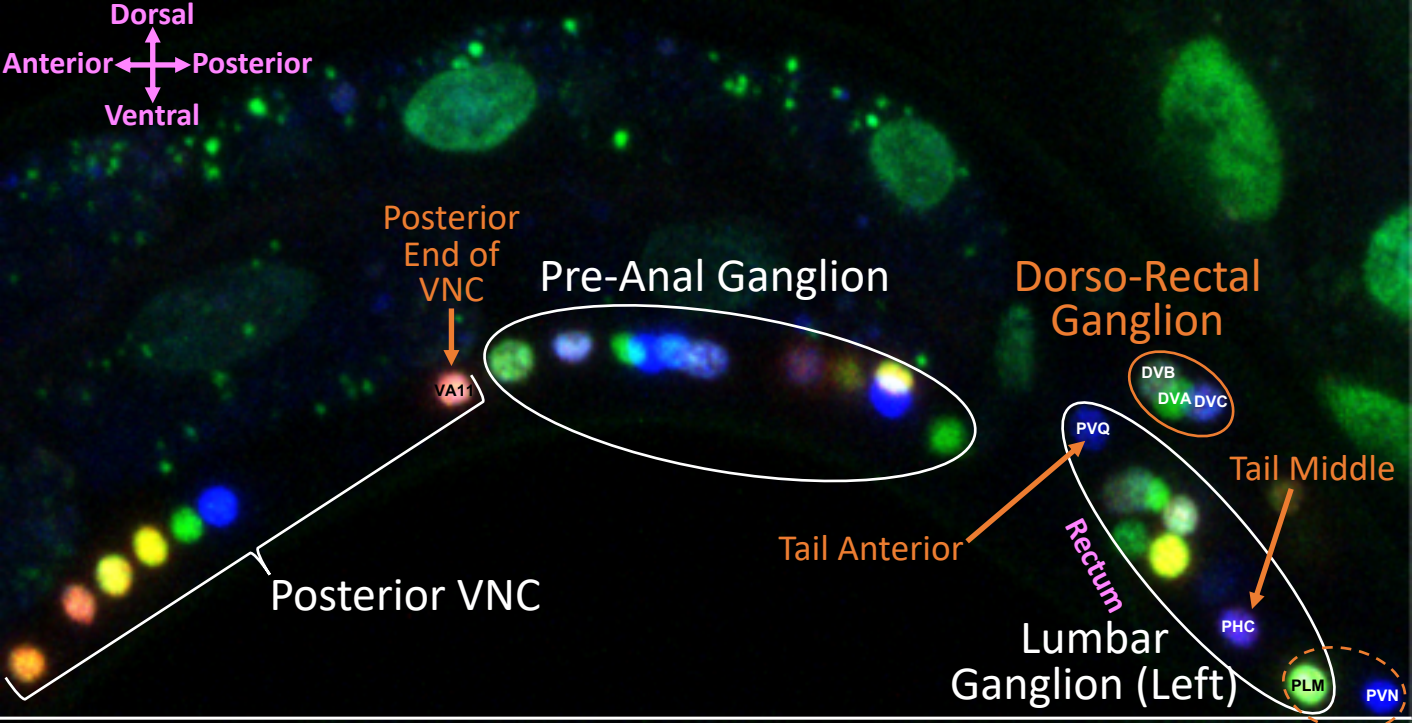


6-1_L4p_otIs669_x_him-5 (Tail Left Side)

Tail Notes:

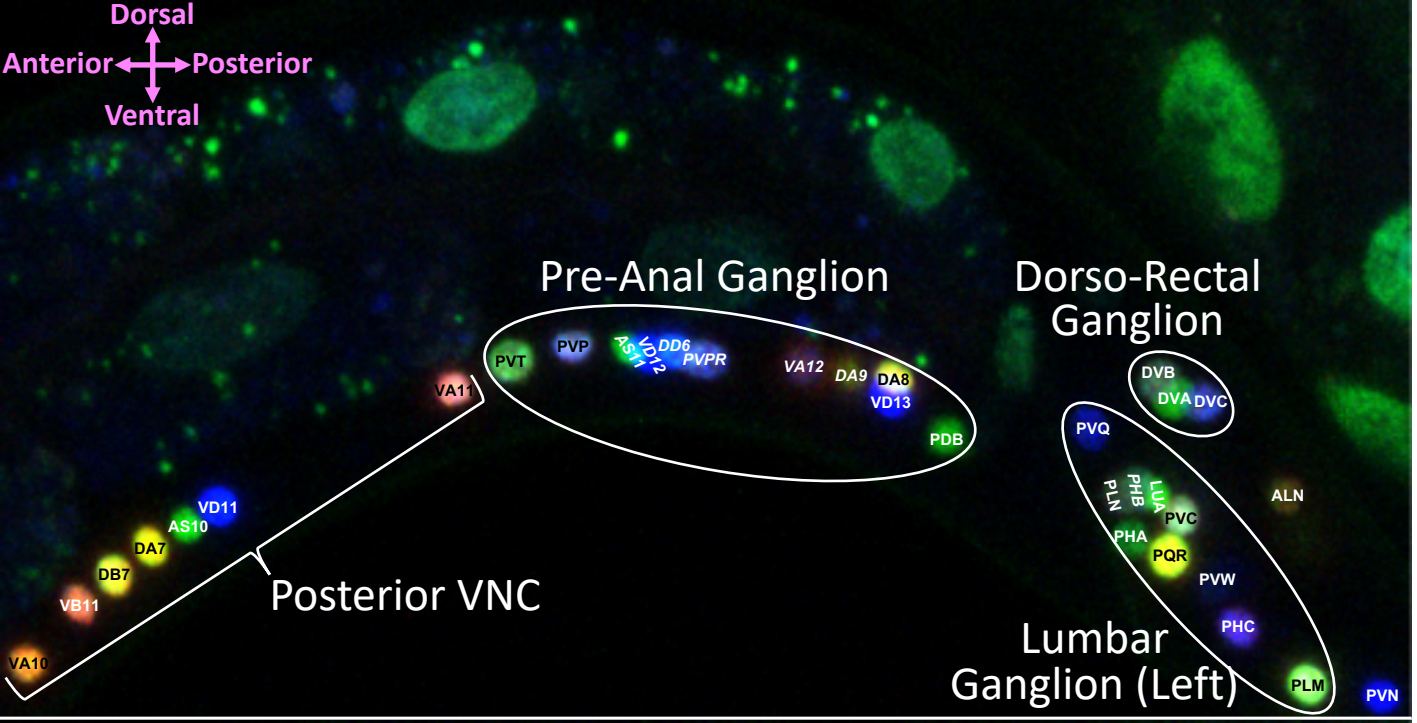
- PVC & PLM both display all 3 landmark colors. PVC is bright & PLM is even brighter.
- PHA is labeled by CyOFP only (green). PHB displays all 3 colors but is dimmer than PVC.
- PVW can range from light blue to solely displaying the panneuronal marker.
- LUA has a small nucleus.
- PHso1 (phasmid socket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

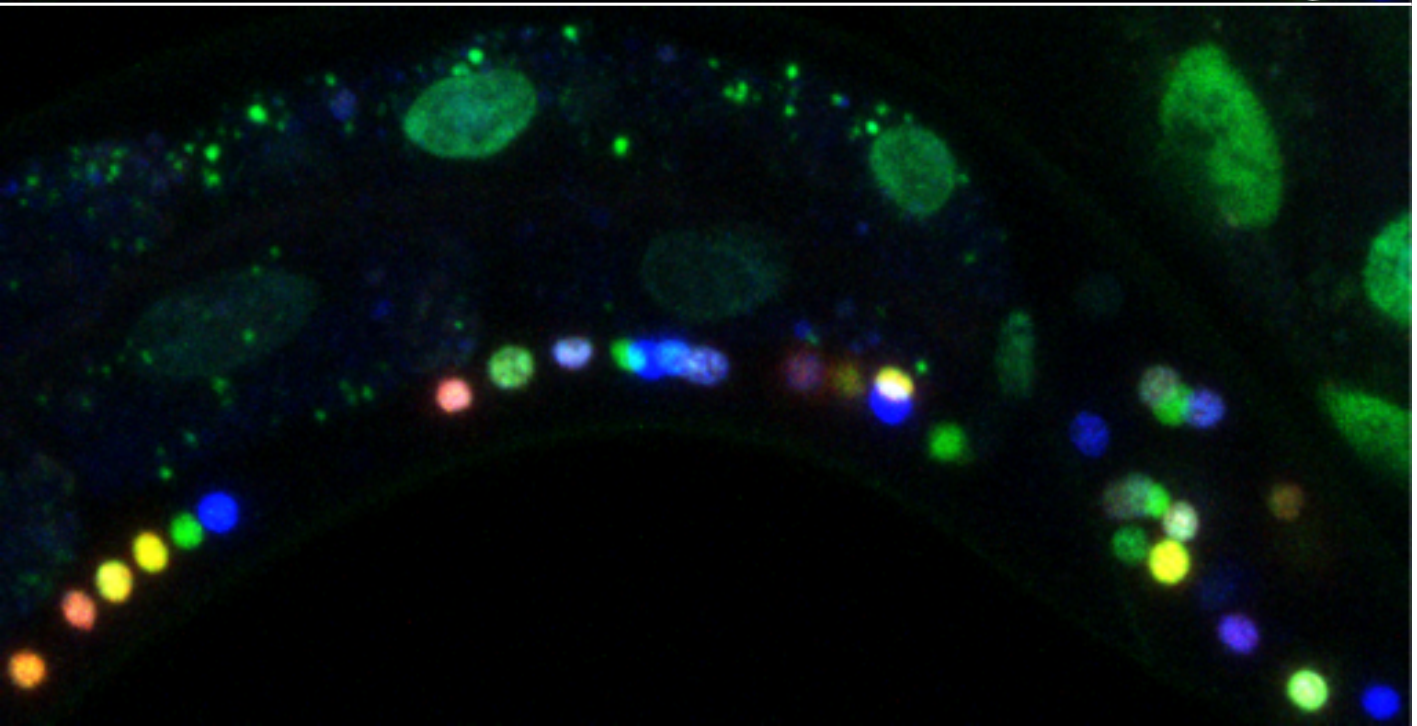




Open
“Tail/Lateral Tail/2_L4p_otIs669_x_him-5”:
1) Orient yourself, on the left side of the tail, using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.

2_L4p_otIs669_x_him-5 (Tail Left Side)





2_L4p_otls669_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.

Pre-Anal Ganglion Notes:

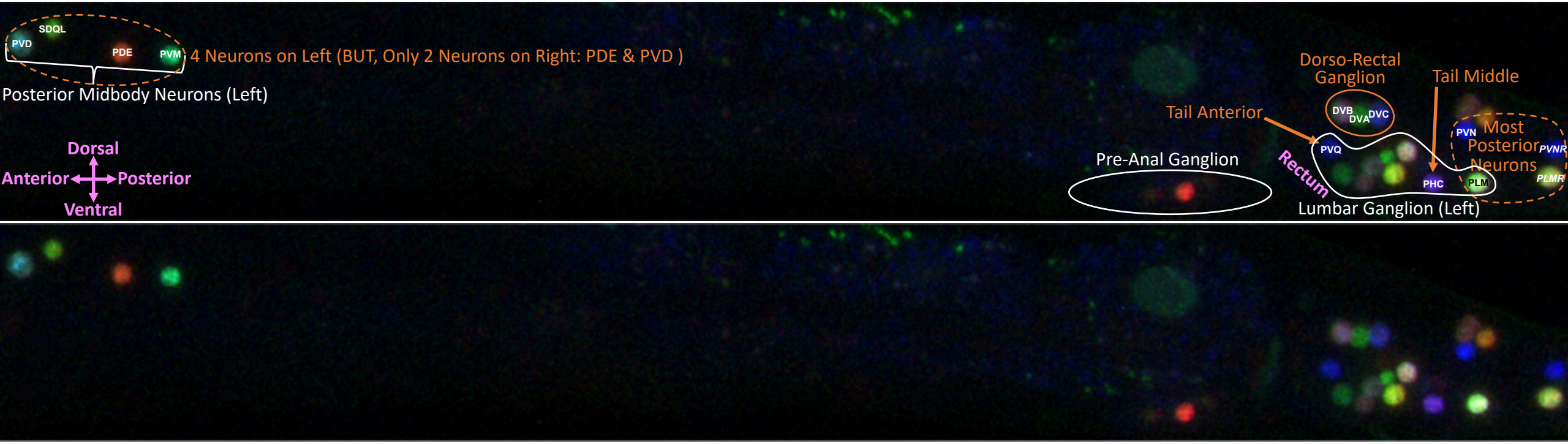
- I assume that VD12 is anterior to VD13. It's a guess.
- While PVP/DD/VD are mostly blue, the PVPs express all 3 colors & the DDs express more green than the VDs.
- DA8 expresses more green than DA9. And, DA8 expresses more red than PDB.
- In the VNC, VA/VB are mostly red, VAs express some green & VBs express some blue. **BUT,** at the PAG, VA11/12 express blue too (thankfully, there are no VBs nearby to confuse with these VAs.).

VNC Notes:

- AS/DA/DB are overtly green. AS has a small nucleus. DA/DB express red. DB 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 VA10 & VB11 in the left image).

6-2_L4p_otIs669 (Tail Left + ~Right Sides)

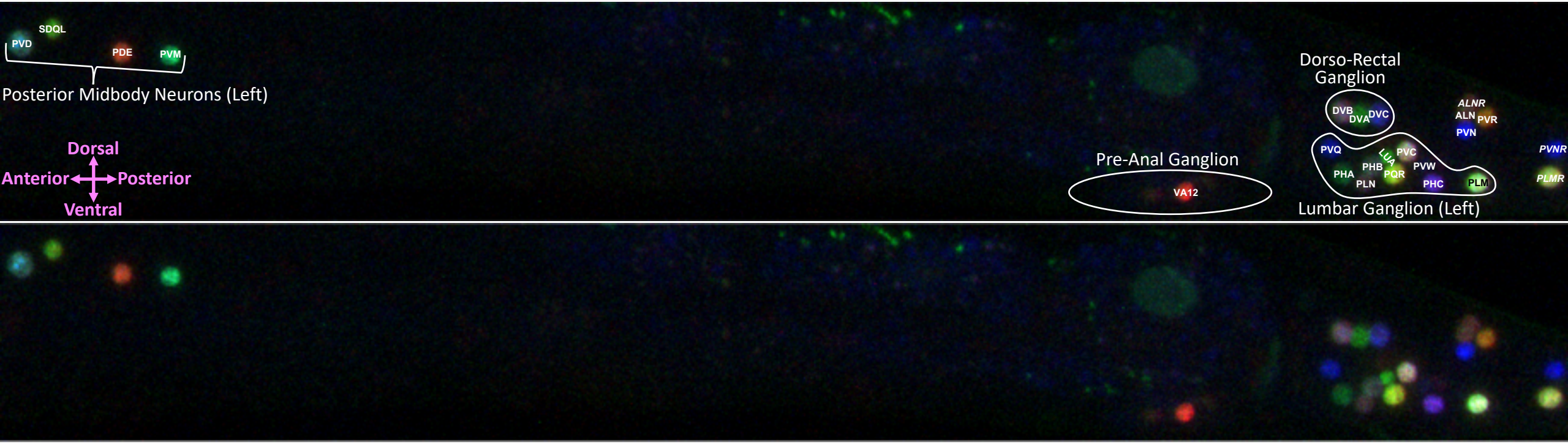
Open "Tail/Lateral Tail/6-2_L4p_otIs669.czi":
1) Orient yourself, on the left side of the tail, using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.



6-2_L4p_otIs669 (Tail Left + ~Right Sides)

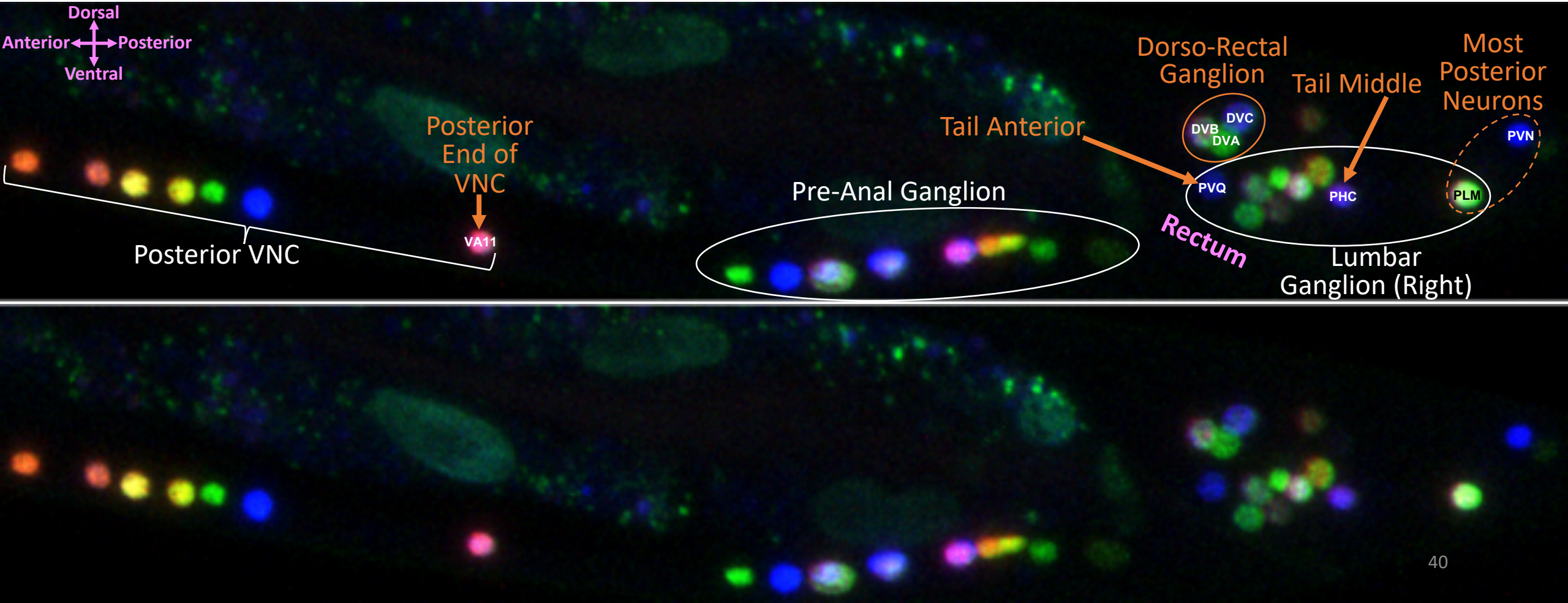
Tail Notes:
- PQR (left side of tail) is brighter & more anterior than PVR (right side of tail).
- PVC & PLM both display all 3 landmark colors. PVC is bright & PLM is even brighter.
- PHA is labeled by CyOFP only (green). PHB displays all 3 colors but is dimmer than PVC.
- PVW can range from light blue to solely displaying the panneuronal marker.
- LUA has a small nucleus.

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).



6-1_L4p_otIs669_x_him-5 (Tail Right Side)

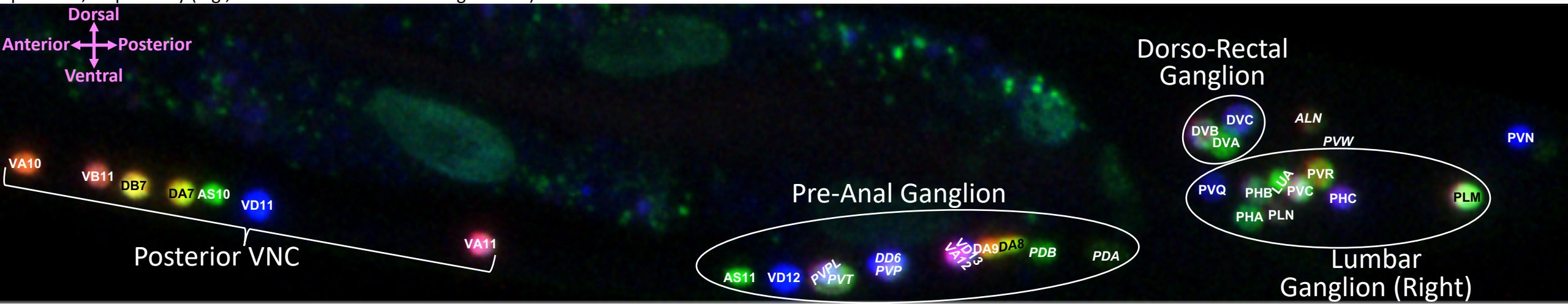
Open "Tail/Lateral Tail/6-1_L4p_otIs669_x_him-5.czi":
1) Orient yourself, on the left side of the tail, using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.
3) The left side of this tail is ID'd on pages 27-28.



6-1_L4p_otIs669_x_him-5 (Tail Right Side)

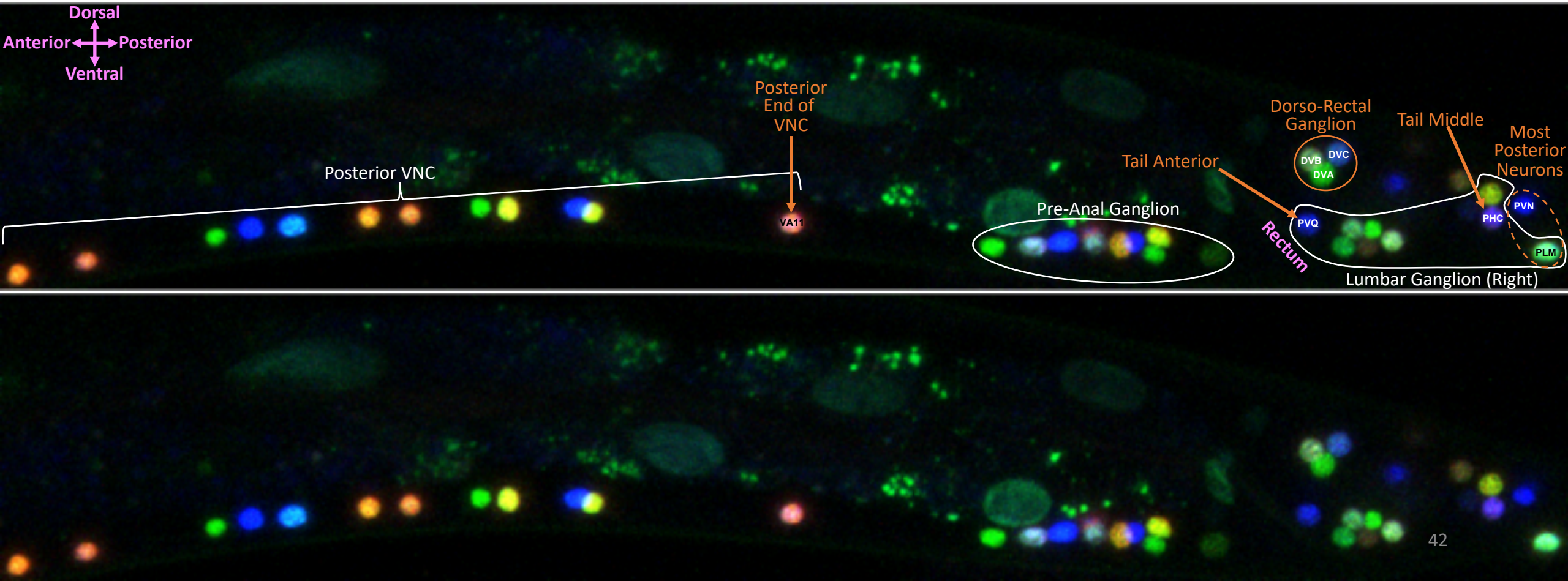
Tail Notes:
- PVC & PLM both display all 3 landmark colors. PVC is bright & PLM is even brighter.
- PHA is labeled by CyOFP only (green). PHB displays all 3 colors but is dimmer than PVC.
- PVW can range from light blue to solely displaying the panneuronal marker.
- LUA has a small nucleus.

VNC Notes:
- AS/DA/DB are overtly green. AS has a small nucleus. DA/DB express red. DB expresses light blue.
- In the VNC, VA/VB are mostly red, VAs express some green & VBs express some blue. **BUT,** at the PAG, VA11/12 express blue too (there are no VBs nearby to confuse with these VAs.).
- 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 VA10 & VB11 in the image below).



5_L4p_otIs669_x_him-5 (Tail Right Side)

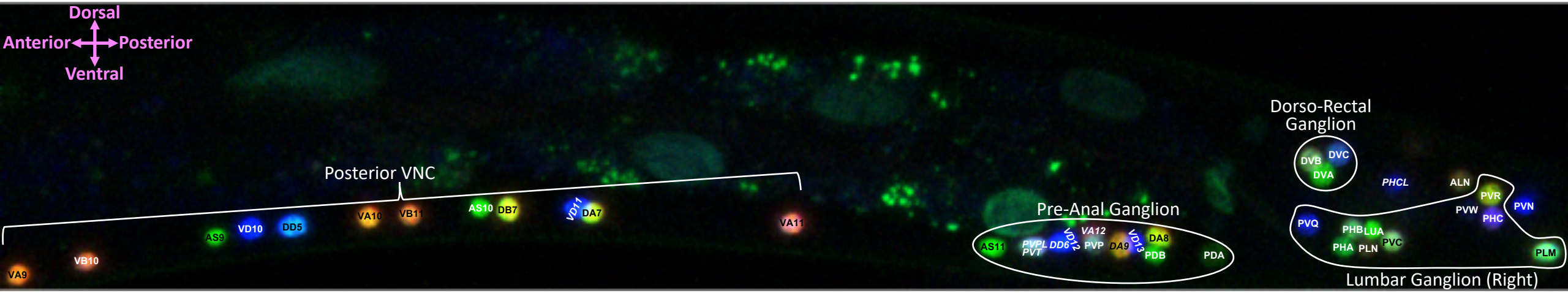
Open "Tail/Lateral Tail/5_L4p_otIs669_x_him-5.czi":
1) Orient yourself, on the left side of the tail, using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.



5_L4p_otIs669_x_him-5 (Tail Right 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.

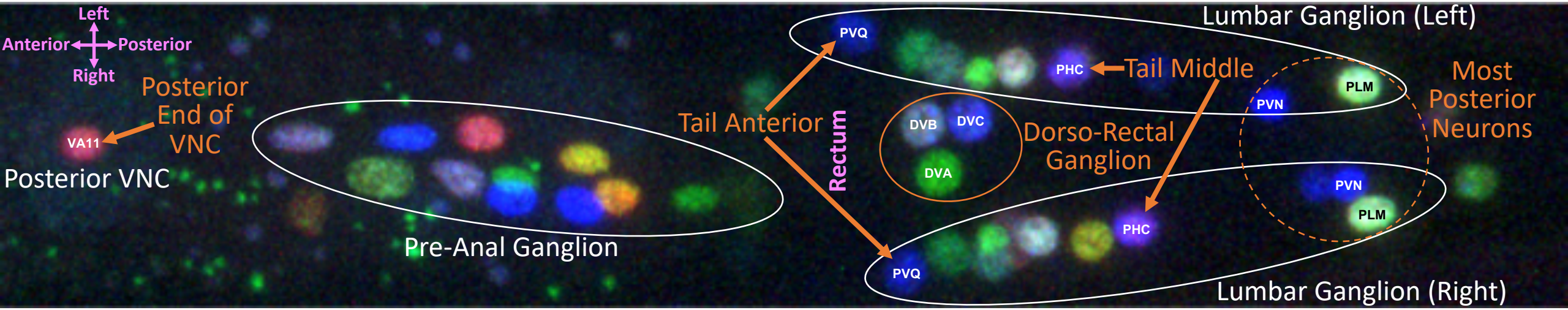
VNC Notes:
- AS/DA/DB are overtly green. AS has a small nucleus. DA/DB express red. DB expresses light blue.
- In the VNC, VA/VB are mostly red, VAs express some green & VBs express some blue. **BUT,** at the PAG, VA11/12 express blue too (there are no VBs nearby to confuse with these VAs.).
- Neighboring 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 VA10 & VB11 in the image below).



Tail (Dorsal-Ventral Projections)

11_YApV_otIs669_x_him-5 (Tail Dorsal-Ventral View)

Open "Tail/Dorsal-Ventral Tail/11_YApV_otIs669_x_him-5.czi":
1) Orient yourself using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.



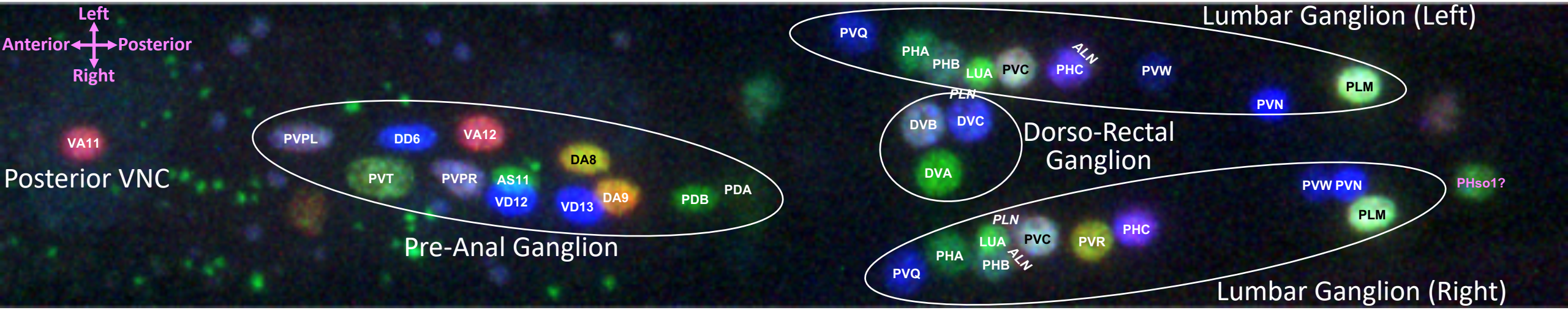
11_YApV_otIs669_x_him-5 (Tail Dorsal-Ventral View)

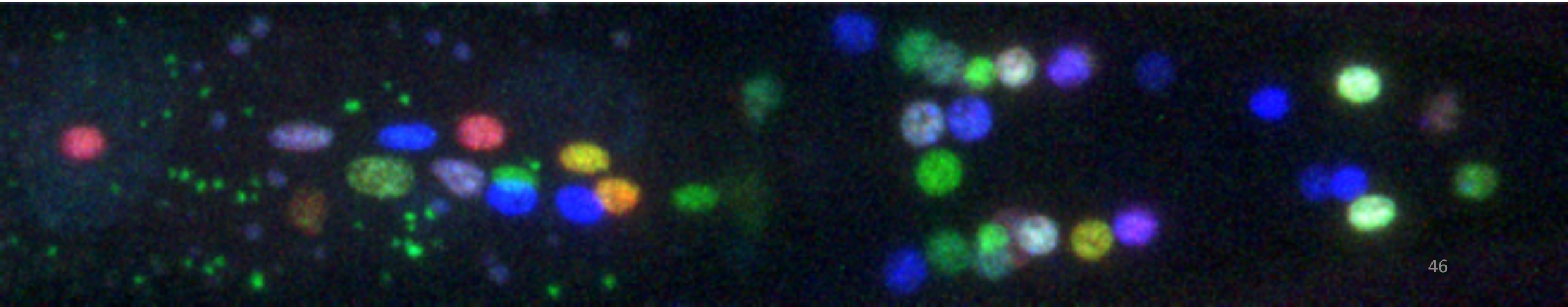
Tail Notes:

- *In this worm, PQR failed to migrate into position (extremely rare), it's located anterior to VA11.*
- 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.

Pre-Anal Ganglion:

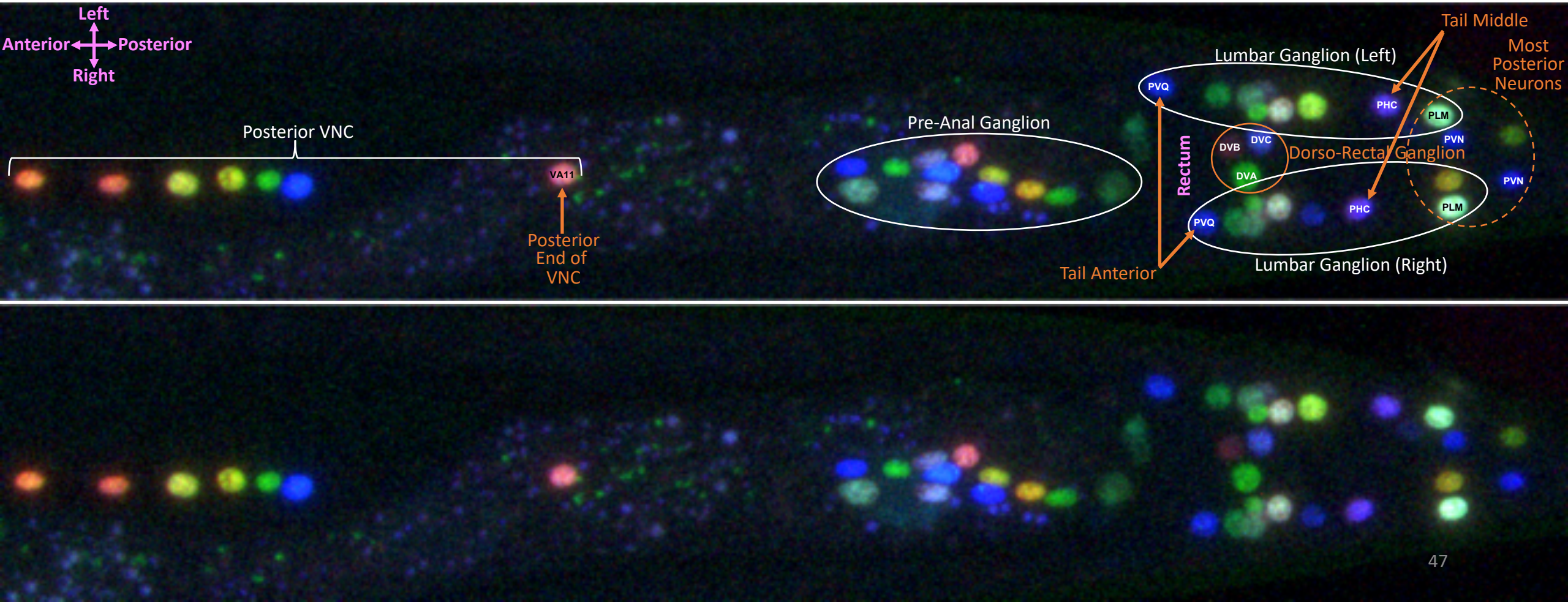
- I assume that VD12 is anterior to VD13. It's a guess.
- While PVP/DD/VD are mostly blue, the PVPs express all 3 colors & the DDs express more green than the VDs.
- DA8 expresses more green than DA9. And, DA8 expresses more red than PDB.
- In the VNC, VA/VB are mostly red, VAs express some green & VBs express some blue. **BUT,** at the PAG, VA11/12 express blue too (there are no VBs nearby to confuse with these VAs.).
- PDA is visualized via the panneuronal marker.





2_YApV_otIs669 (Tail Dorsal-Ventral View)

Open "Tail/Dorsal-Ventral Tail/2_YApV_otIs669.czi":
1) Orient yourself using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.



2_YApV_otIs669 (Tail Dorsal-Ventral View)

Tail Notes:

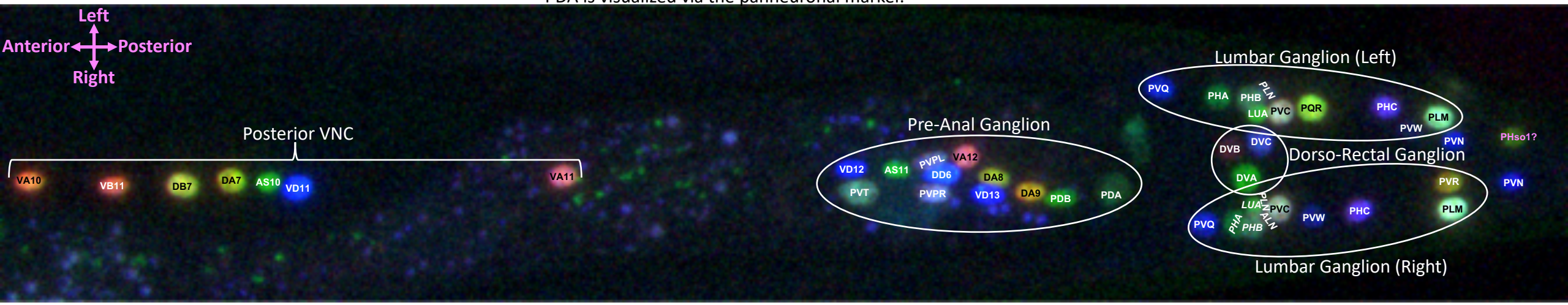
- PVC & PLM both display all 3 landmark colors. PVC is bright & PLM is even brighter.
- PHA is labeled by CyOFP only (green). PHB displays all 3 colors but is dimmer than PVC.
- PVW can range from light blue to solely displaying the panneuronal marker.
- LUA has a small nucleus.

Pre-Anal Ganglion:

- I assume that VD12 is anterior to VD13. It's a guess.
- While PVP/DD/VD are mostly blue, the PVPs express all 3 colors & the DDs express more green than the VDs.
- DA8 expresses more green than DA9. And, DA8 expresses more red than PDB.
- In the VNC, VA/VB are mostly red, VAs express some green & VBs express some blue. **BUT,** at the PAG, VA11/12 express blue too (there are no VBs nearby to confuse with these VAs.).
- PDA is visualized via the panneuronal marker.

VNC Notes:

- AS/DA/DB are overtly green. AS has a small nucleus. DA/DB express red. DB 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 VA10 & VB11 in the left image).



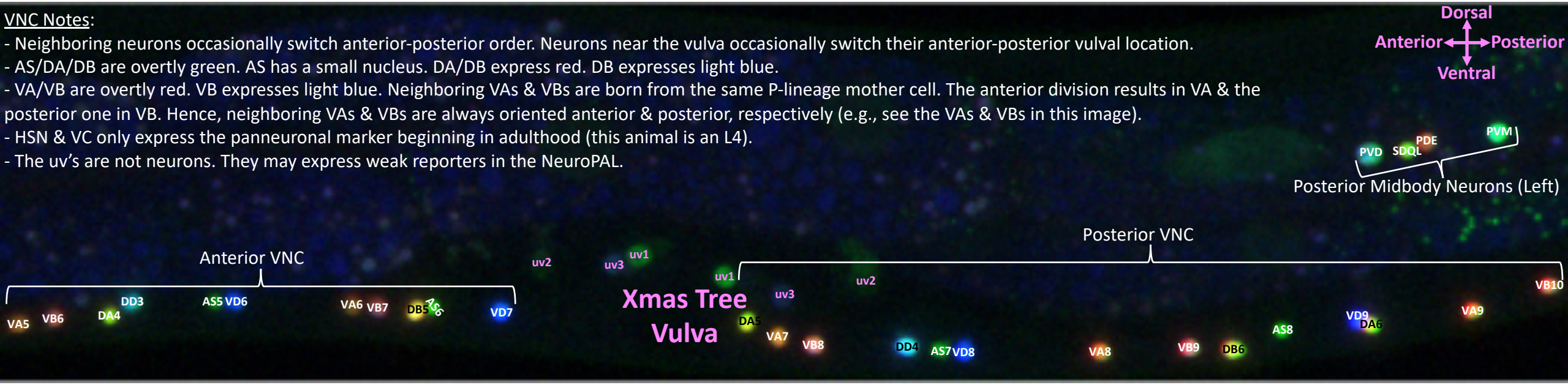
Learning to ID with NeuroPAL: Step 5, ID'ing the Midbody

Midbody (Lateral Projections)

6-1_L4m_otIs669_x_him-5 (Midbody Left Side)

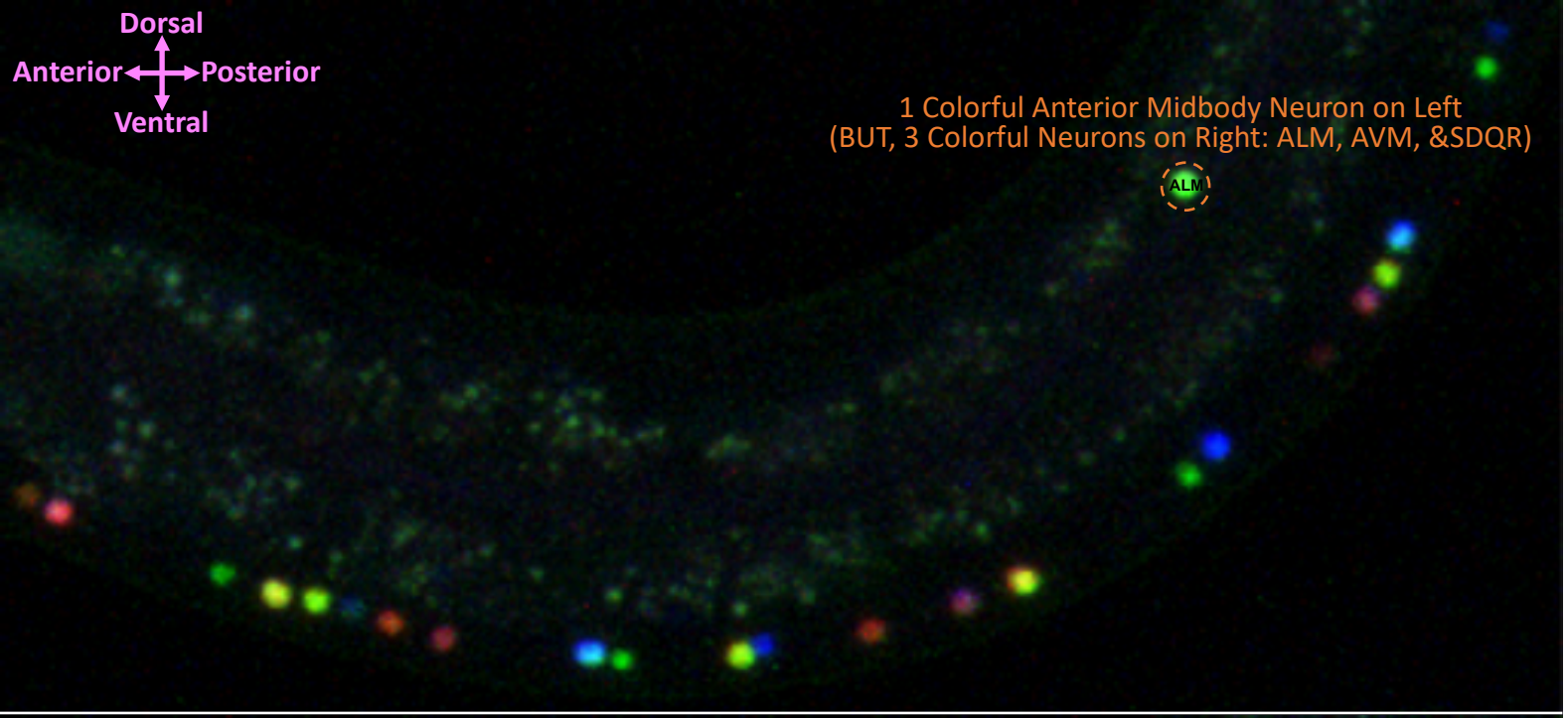
Open "Midbody/Lateral Midbody/6-1_L4m_otIs669_x_him-5.czi":
1) Orient yourself, on the left side of the midbody, using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.
3) The tail of this worm is ID'd on pages 27-28 & 33-34.

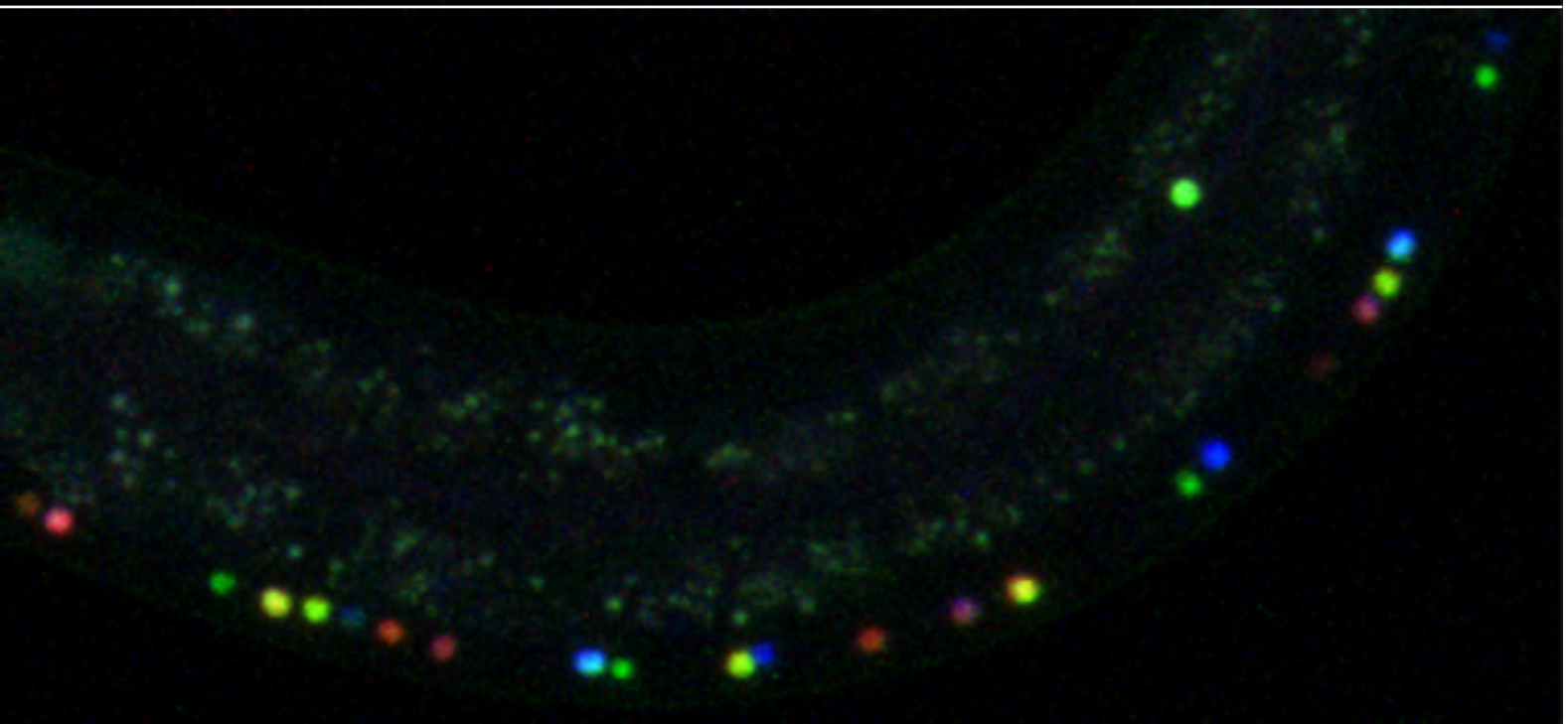
6-1_L4m_otIs669_x_him-5 (Midbody Left Side)



Posterior Midbody Notes:

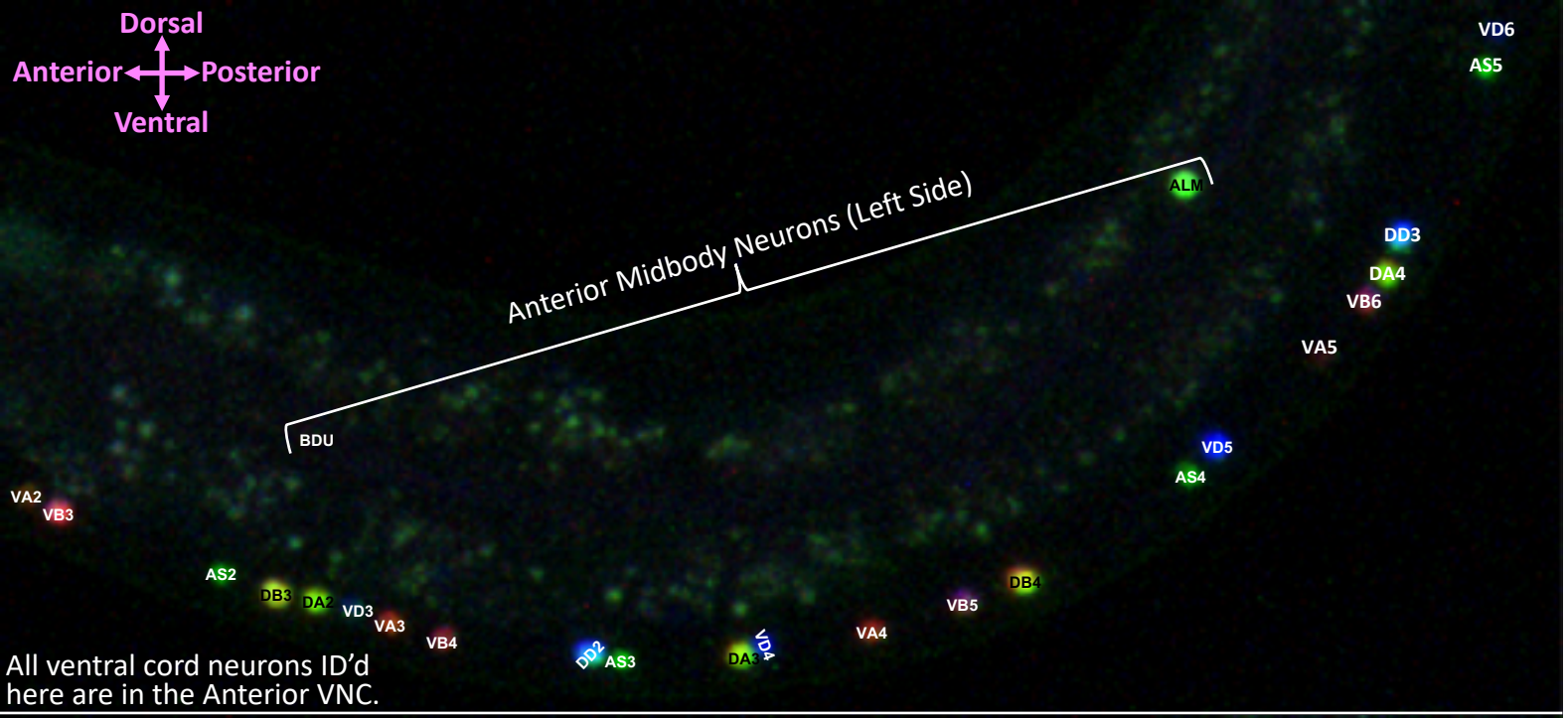
- PDE & PVD are also present on the right side & look identical to their left counterparts.
- SDQL (left side, posterior to the vulva) is less brighter than SDQR (right side, near the head).
- PVM (left side, posterior to the vulva) shares similar coloring to AVM (right side, near the head).



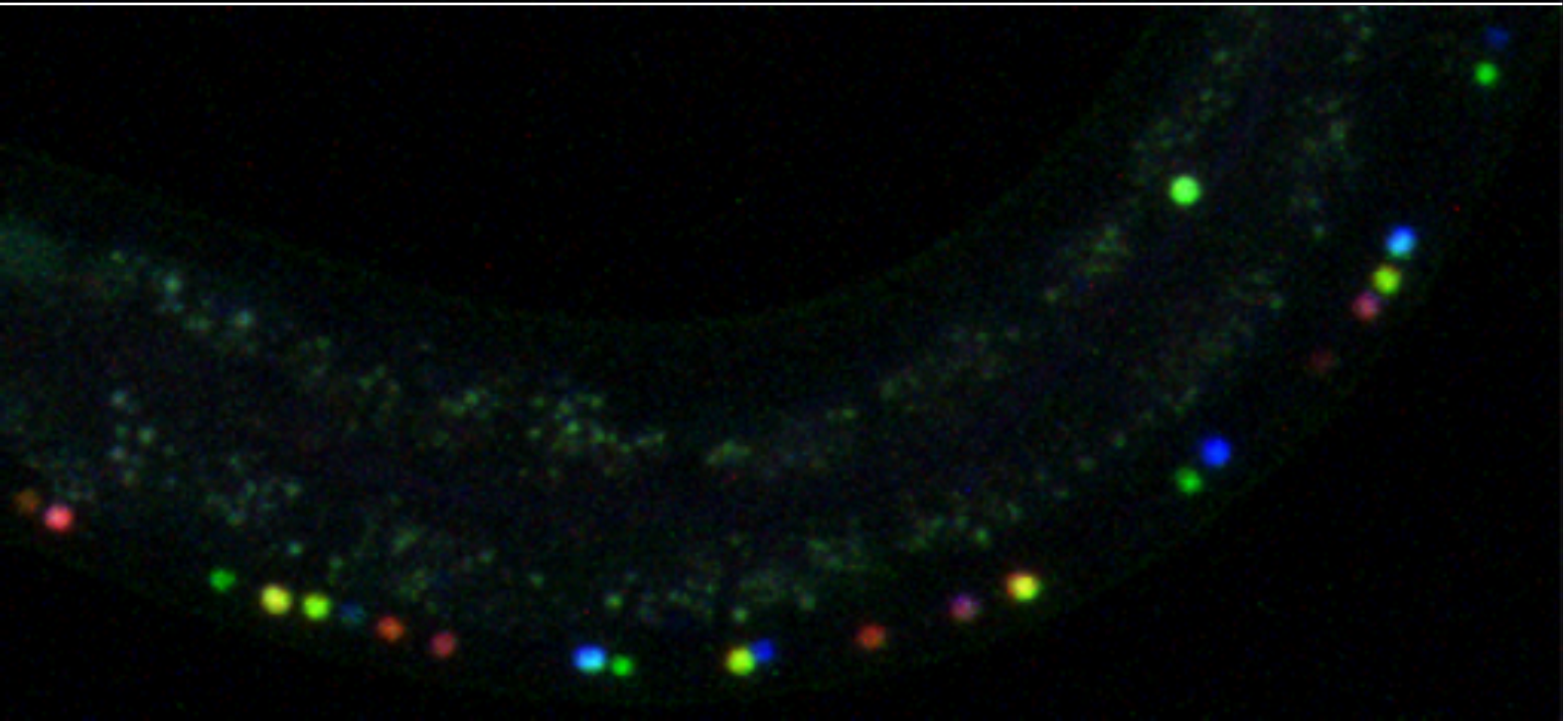


Open "Midbody/Lateral Midbody/ 18_L4w_otIs669_20xObjective.czi":
1) Orient yourself, on the left side of the anterior midbody, using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.

18_L4w_otIs669_20xObjective (Anterior Midbody Left Side)



All ventral cord neurons ID'd here are in the Anterior VNC.



Anterior Midbody Notes:

- The left anterior midbody has only 2 non-VNC neurons, BDU & ALM. The right side has BDU & ALM and adds the AVM & SDQR.

- BDU only expresses the panneuronal marker.

VNC Notes:

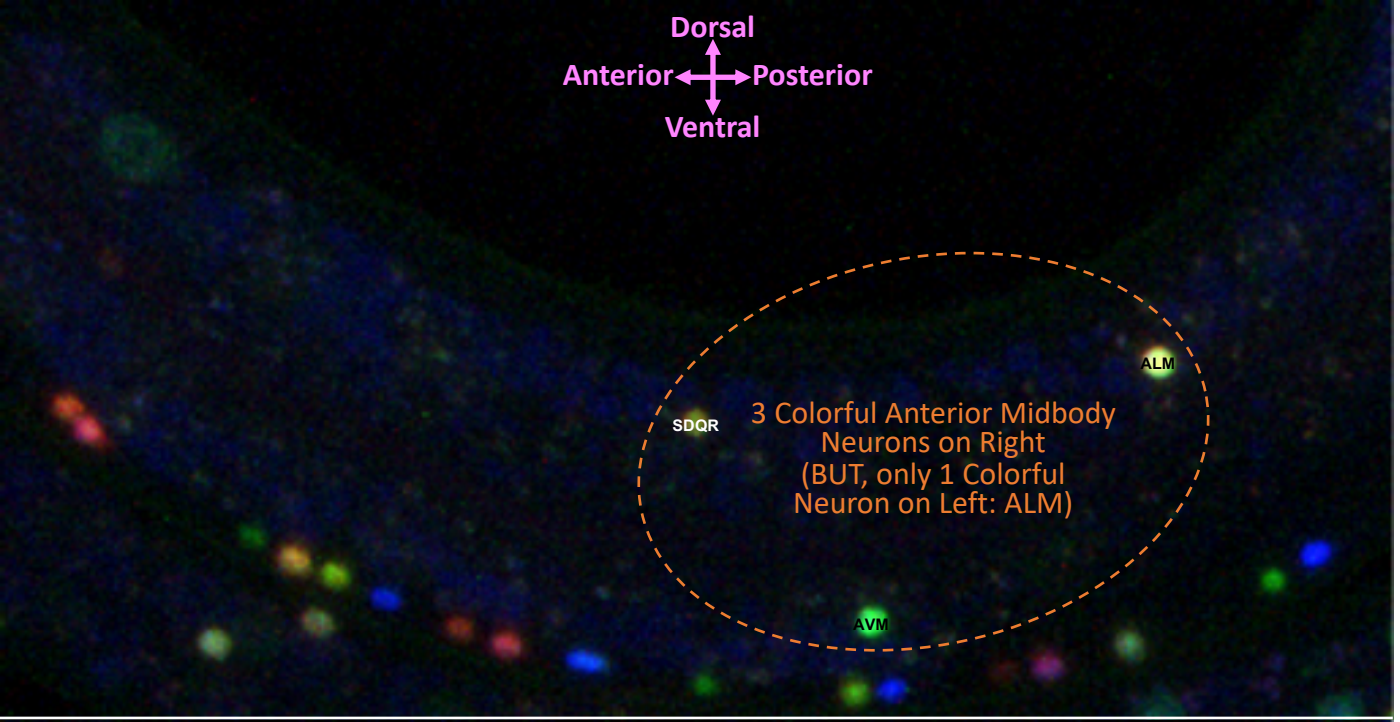
- Neighboring neurons occasionally switch anterior-posterior order. Neurons near the vulva occasionally switch their anterior-posterior vulval location.

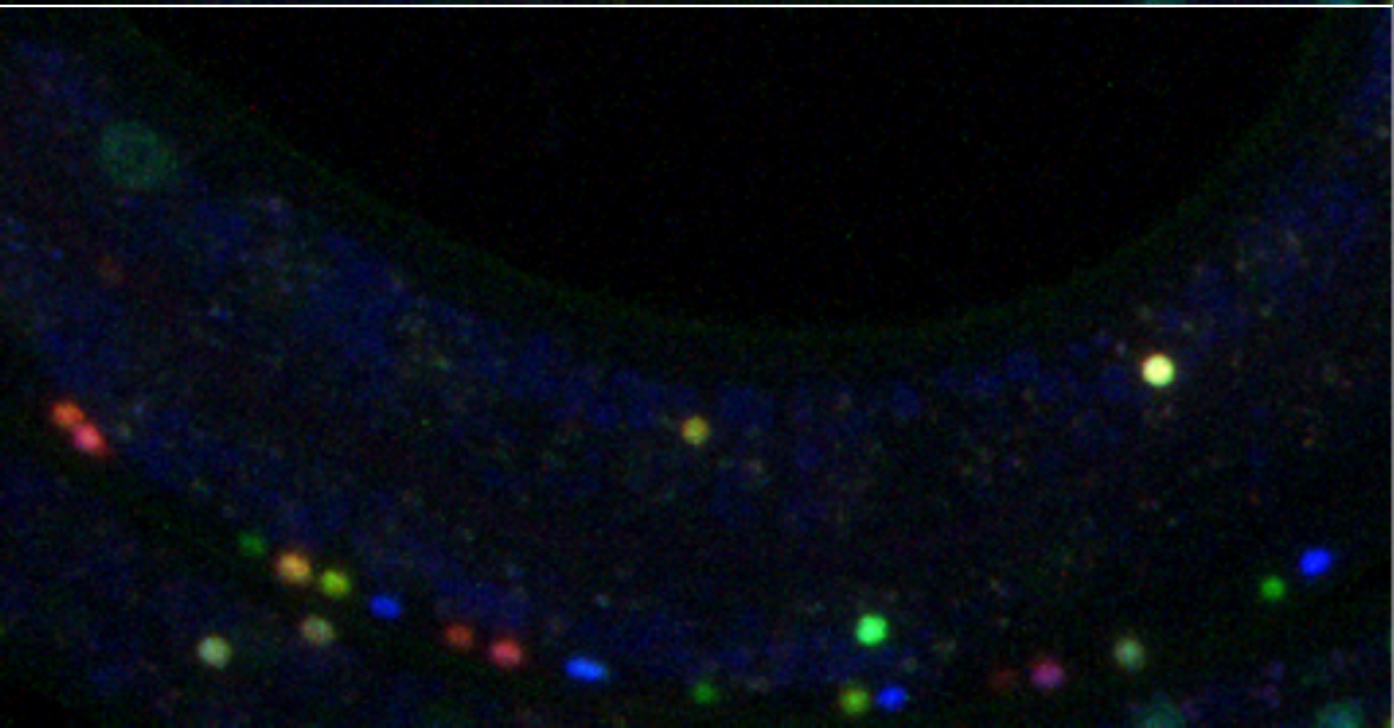
- AS/DA/DB are overtly green. AS has a small nucleus. DA/DB express red. DB expresses light blue.

- 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).

18_L4w_otls669_20xObjective (Anterior Midbody Left Side)



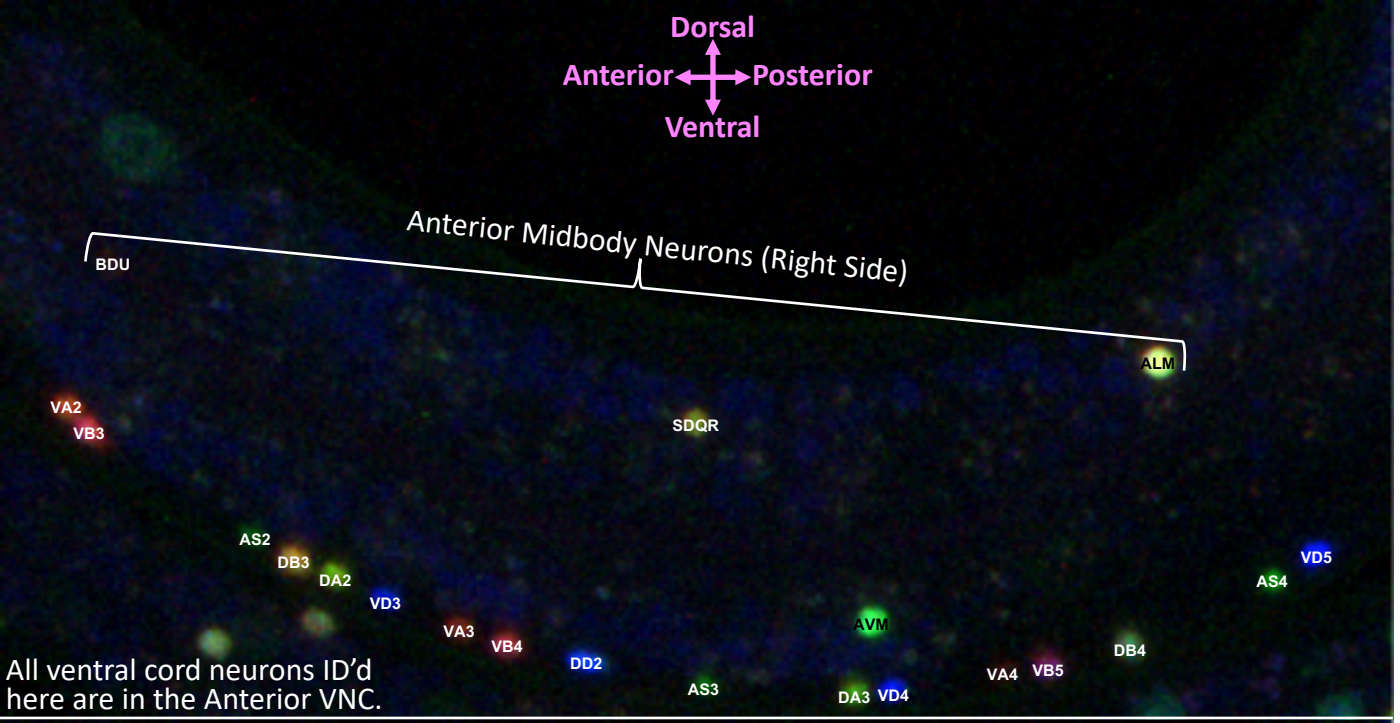


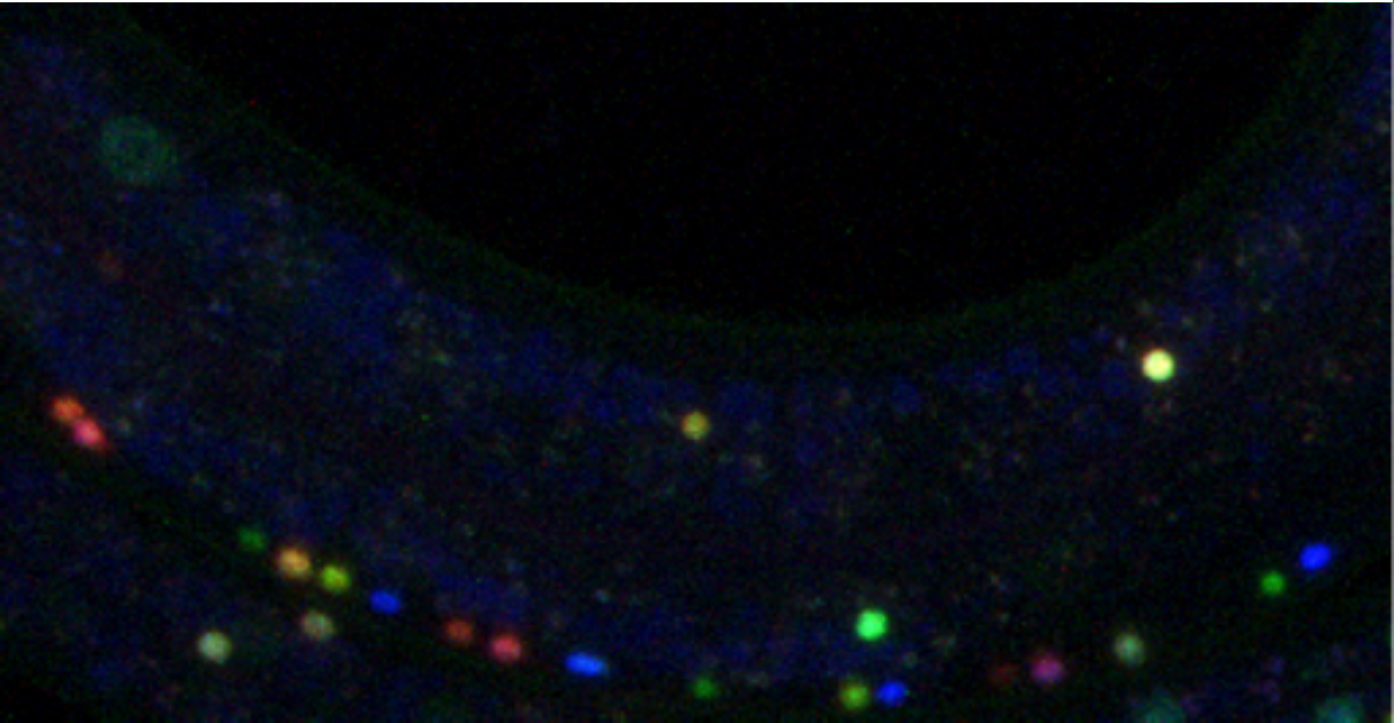
Open "Midbody /Lateral Midbody/ 17_L4w_L1w_otIs669.czi":

1) Orient yourself, on the right side of the anterior midbody, using the landmarks shown here.

2) Use the map on the next page to locate & ID each neuron.

17_L4w_L1w_otIs669 (Anterior Midbody Right Side)





Anterior Midbody Notes:

- The right anterior midbody has 2 more non-VNC neurons, than the left side, AVM & SDQR.

- SDQR expresses light green+red and is usually dorsal, anterior to both AVM & ALM.

- AVM expresses bright green and is usually ventral to & centered between the dorsal SDQR & ALMR.

- ALM expresses all 3 colors brightly and is usually located dorsal, posterior to both AVM & SDQR.

- Because the Q lineage neurons, AVM & SDQR, are born of an L1 migration, whereas the ALM is born embryonically, I prefer to use color instead of position to ID them.

- BDU only expresses the panneuronal marker.

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.

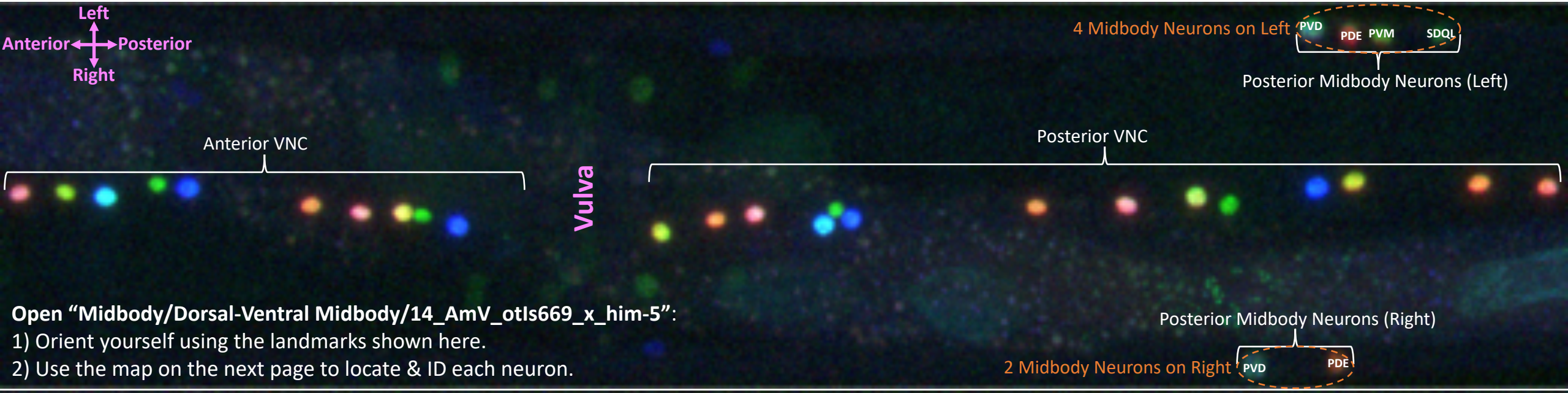
- VA/VB are overtly red. VB expresses light blue. Neighboring VAs & VBs are born from the same P-lineage mother cell. The anterior division results in VA & the posterior one in VB. Hence, neighboring VAs & VBs are always oriented anterior & posterior, respectively (e.g., see the VAs & VBs in this image).

- HSN & VC only express the panneuronal marker beginning in adulthood (this animal is an L4).

17_L4w_L1w_otIs669 (Anterior Midbody Right Side)

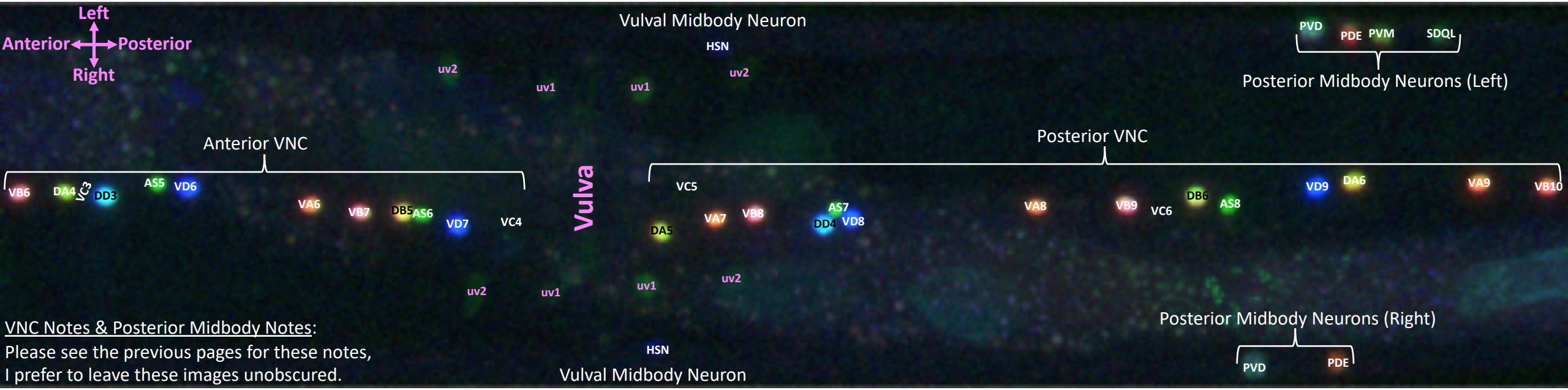
Midbody (Dorsal-Ventral Projections)

14_AmV_otIs669_x_him-5 (Midbody Dorsal-Ventral View)



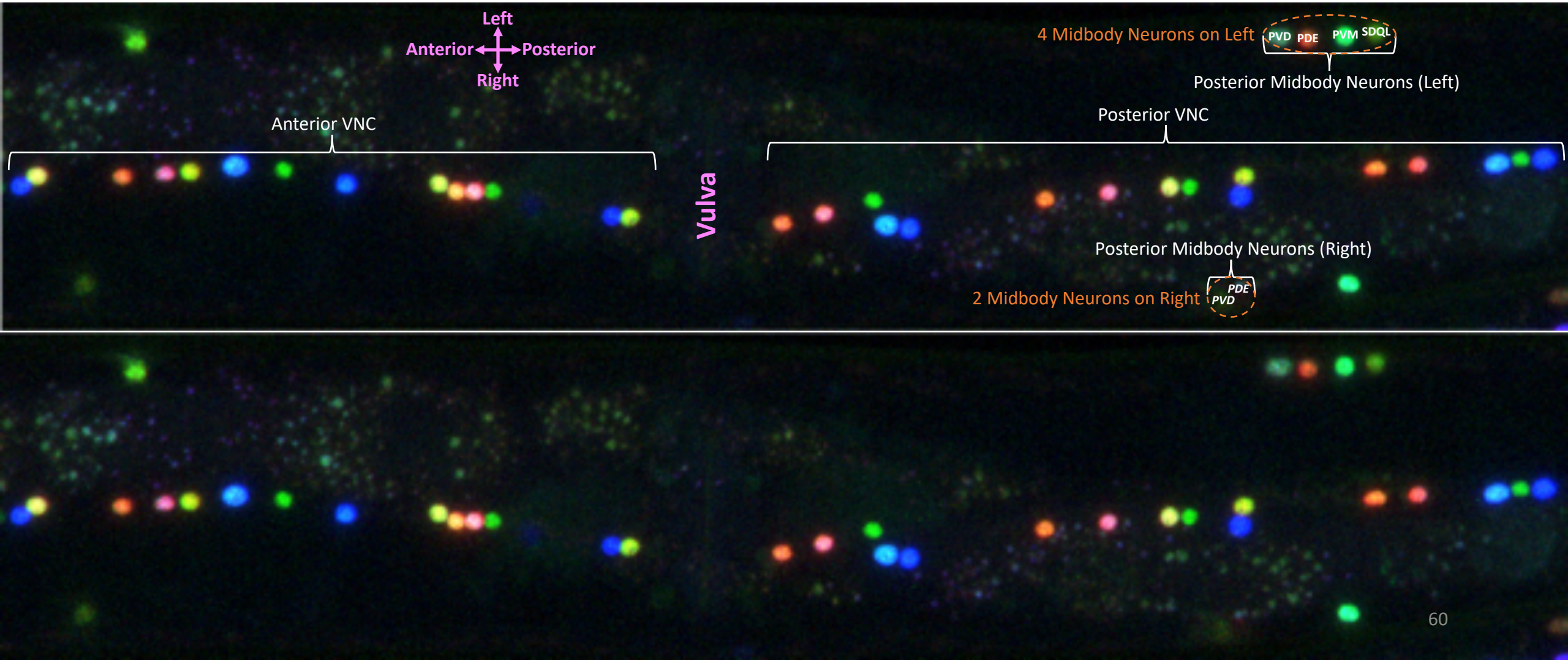
Open "Midbody/Dorsal-Ventral Midbody/14_AmV_otIs669_x_him-5":
1) Orient yourself using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.

14_AmV_otIs669_x_him-5 (Midbody Dorsal-Ventral View)



22_YAmV_otIs669_x_him-5 (Midbody Dorsal-Ventral View)

Open "Midbody/Dorsal-Ventral Midbody/22_YAmV_otIs669_x_him-5":
1) Orient yourself using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.



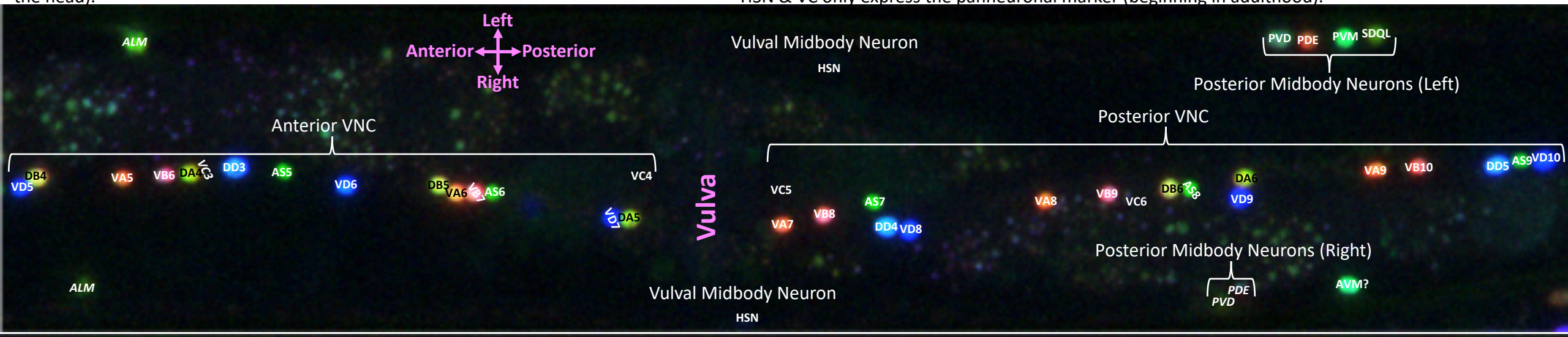
22_YAmV_otIs669_x_him-5 (Midbody Dorsal-Ventral View)

Midbody Notes:

- This worm appears to have experienced starvation.
- *AVM appears to have migrated too far posterior (extremely rare).*
- 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).

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 the image below).
- HSN & VC only express the panneuronal marker (beginning in adulthood).



Learning to ID with NeuroPAL: Step 6, ID'ing the Head

ID'ing in the Head
PLEASE READ THIS!

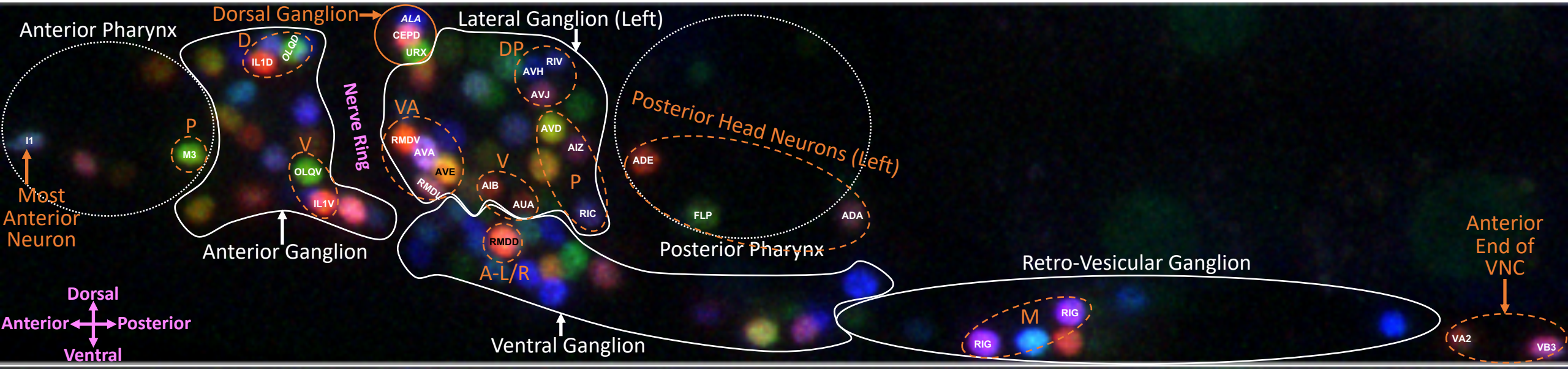
- **The worm's head contains the majority of its neurons.**
- The Anterior, Dorsal, Lateral, Ventral, Retro-Vesicular ganglia, 2 pharyngeal bulbs, & several unbound neurons all compete for space in the head. Physically separating boundaries are often weak (e.g., the nerve ring) or missing (e.g., ADA, 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.***

Head (Left Side Projections)

18_YAa_otIs669 (Head Left Side)

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

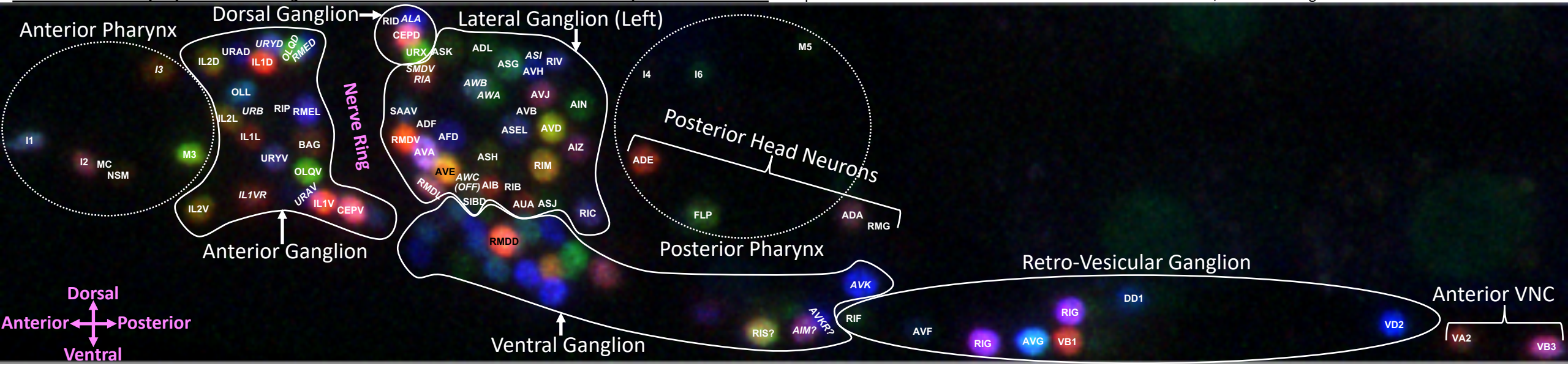
1) Orient yourself, on the left side of the head, using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.
3) On the right, the Posterior Head Neurons include 1 more neuron, AQR.

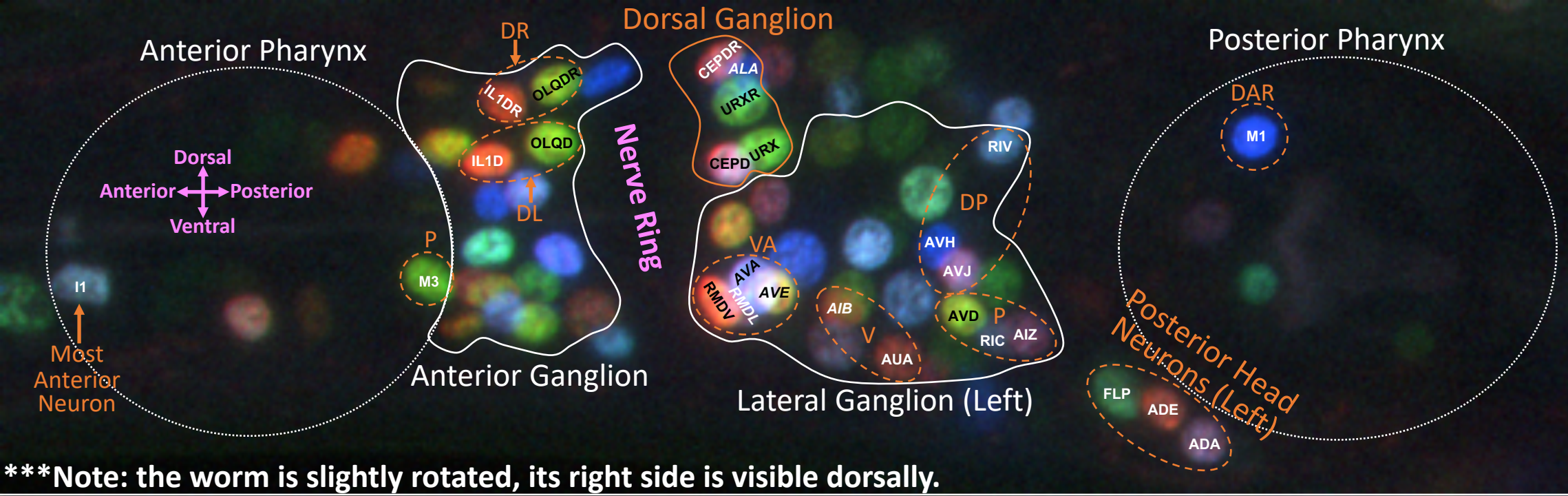


18_YAa_otIs669 (Head Left Side)

Head Notes:

- 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 gangliar 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.

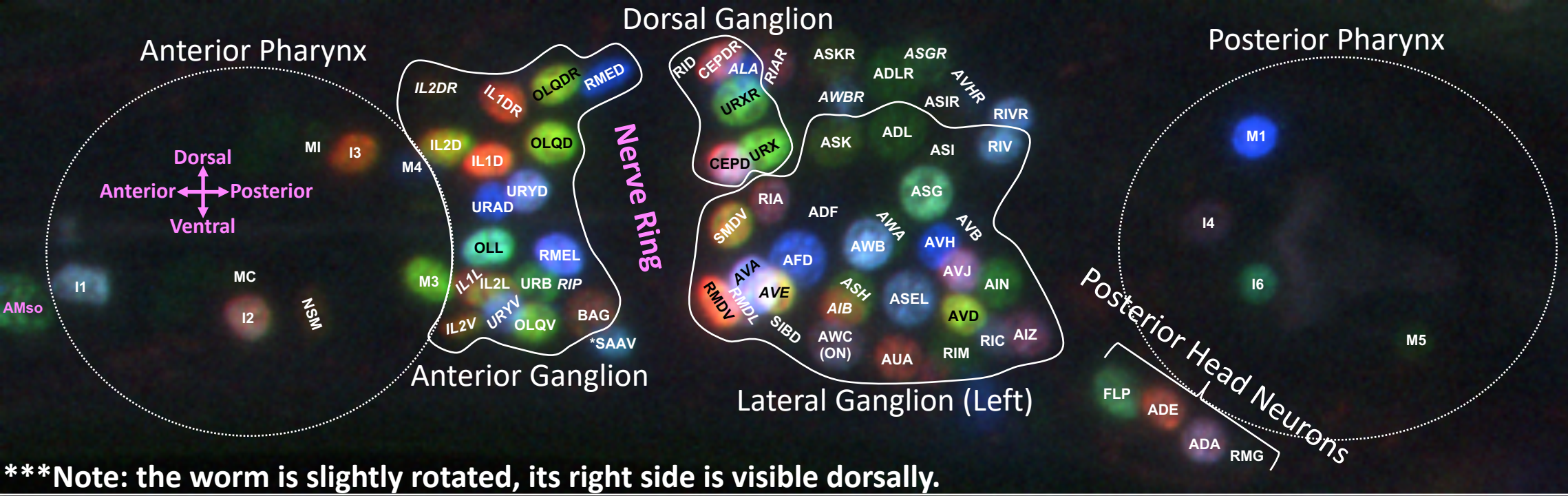




***Note: the worm is slightly rotated, its right side is visible dorsally.

Open "Head/Lateral Head/YAa_otIs669_Airyscan.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.

YA_otIs669_Airyscan (Head Left + ~Right Sides)

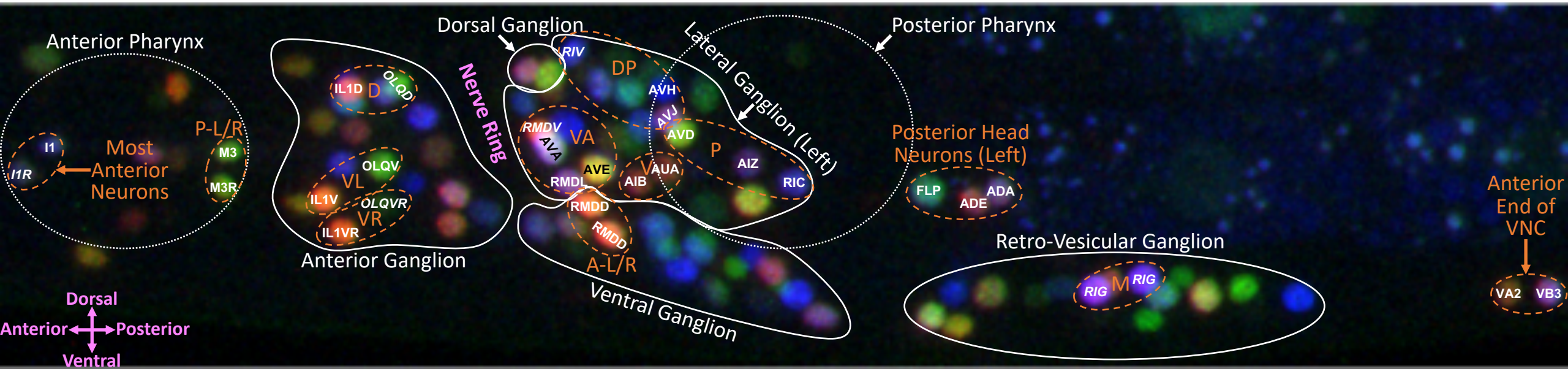


***Note: the worm is slightly rotated, its right side is visible dorsally.

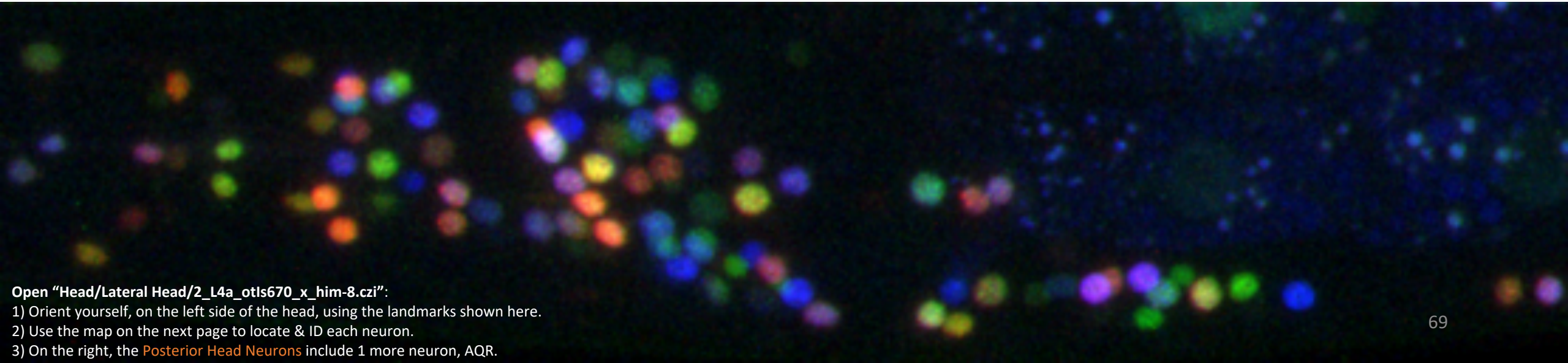
<u>Head Notes</u>:
- The worm's rotation provides a perfect view of the left-right split at the dorsal midline.
- SAAV may have drifted across the nerve ring into the anterior ganglion (could be the perspective).
- MI (panneuronal) is anterior to M4 (light blue) & MC (panneuronal) is ventral to both. I4 is anterior to M5.
- AMso (amphid socket cell) is not a neuron. In males, at L4, AMso divides to yield an MCM neuron & an AMso.
- Please see the previous pages for additional notes (in particular page 59), I prefer to leave these images unobscured.

YA_otIs669_Airyscan (Head Left + ~Right Sides)

2_L4a_otIs670_x_him-8 (Head Left + ~Right Sides)

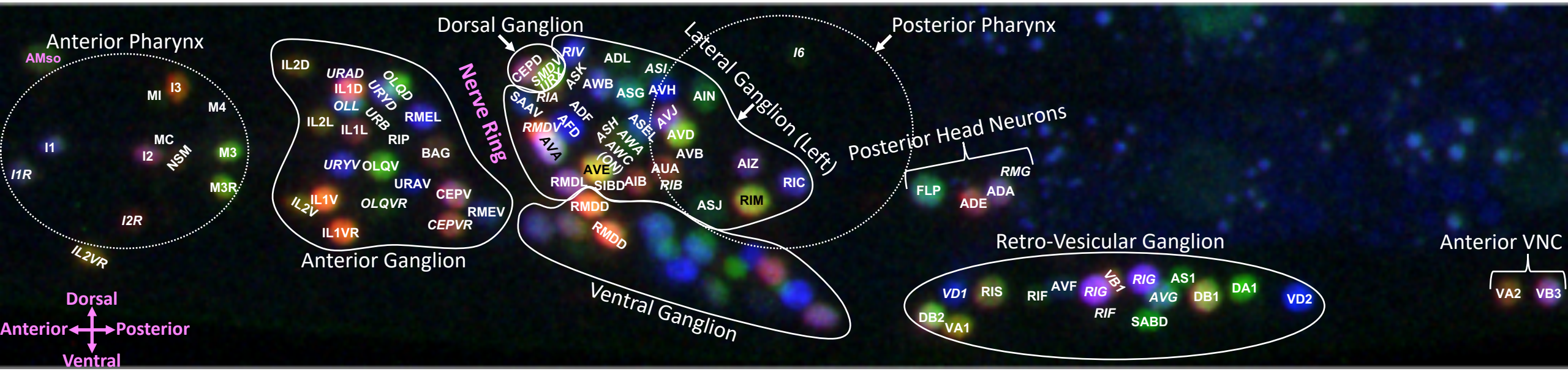


*****Note: the worm is slightly rotated, its right side is visible ventrally.**

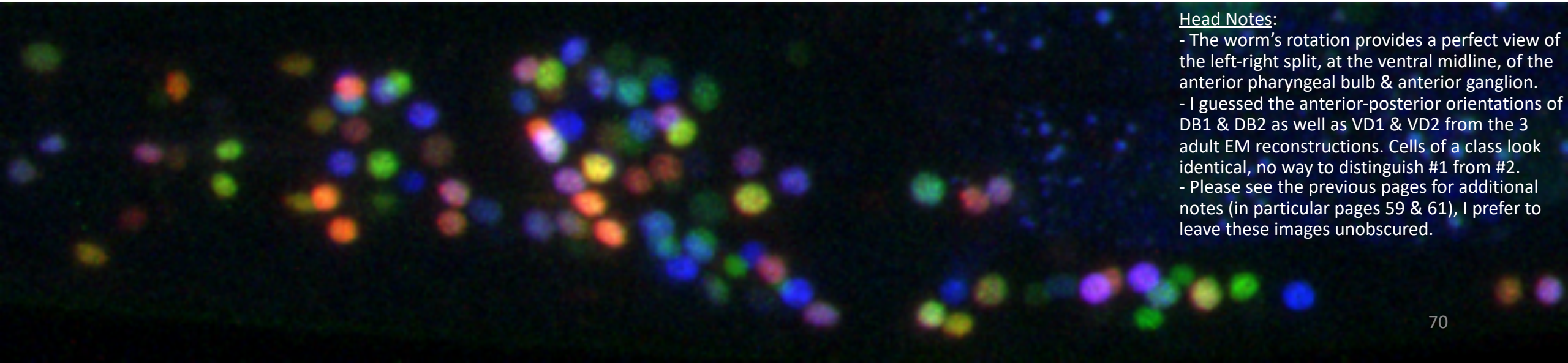


Open "Head/Lateral Head/2_L4a_otIs670_x_him-8.czi":
1) Orient yourself, on the left side of the head, using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.
3) On the right, the Posterior Head Neurons include 1 more neuron, AQR.

2_L4a_otIs670_x_him-8 (Head Left + ~Right Sides)



*****Note: the worm is slightly rotated, its right side is visible ventrally.**

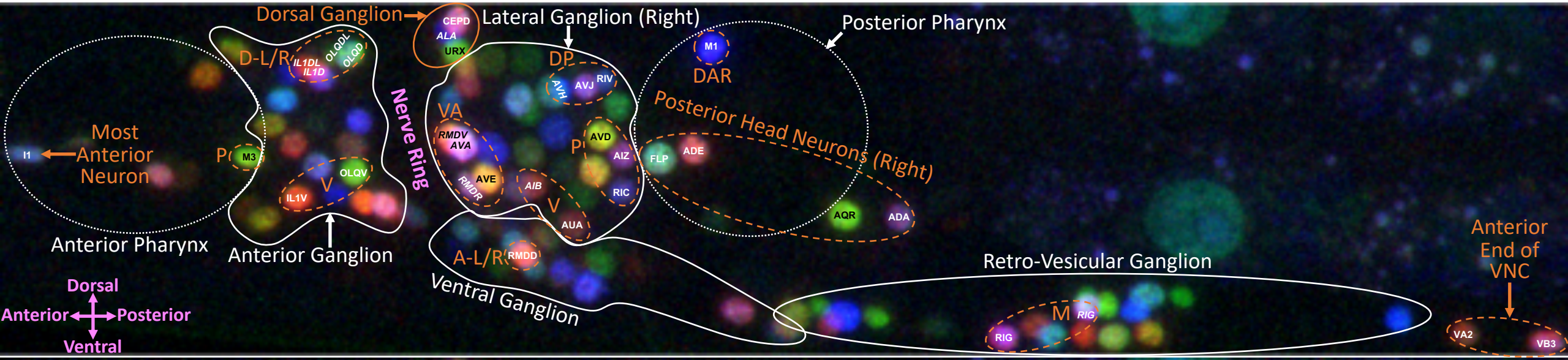


Head Notes:
- The worm's rotation provides a perfect view of the left-right split, at the ventral midline, of the anterior pharyngeal bulb & anterior ganglion.
- I guessed the anterior-posterior orientations of DB1 & DB2 as well as VD1 & VD2 from the 3 adult EM reconstructions. Cells of a class look identical, no way to distinguish #1 from #2.
- Please see the previous pages for additional notes (in particular pages 59 & 61), I prefer to leave these images unobscured.

Head (Right Side Projections)

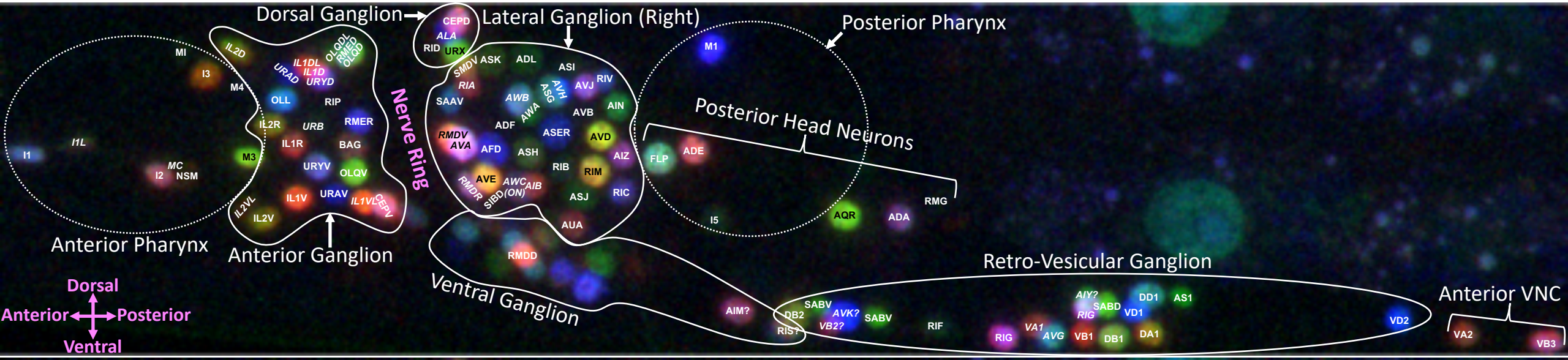
18_YAa_otIs669 (Head Right Side)

Open "Head/Lateral Head/18_YAa_otIs669.czi":
1) Orient yourself, on the right side of the head, using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.
3) The left side of this head is ID'd on pages 58-59.

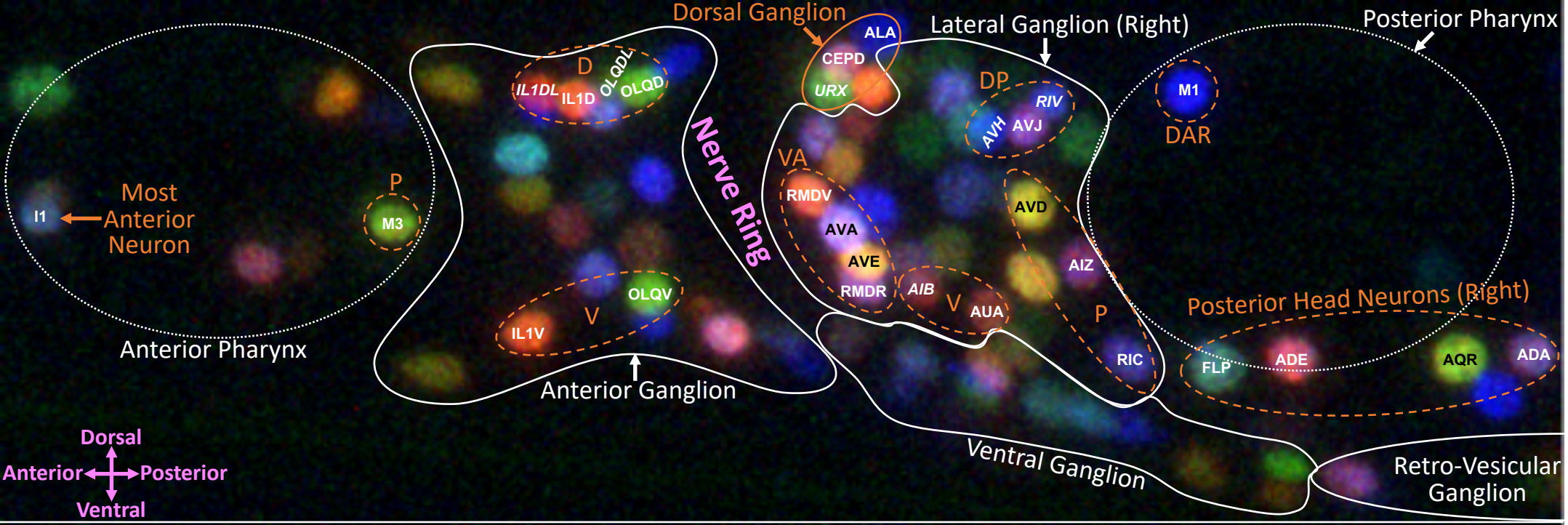


18_YAa_otIs669 (Head Right Side)

Head Notes:
- The left side of this head is ID'd on pages 58-59.
- AQR is only present on the right side of the head.
- Please see the previous pages for additional notes (in particular pages 59 & 61), I prefer to leave these images unobscured.



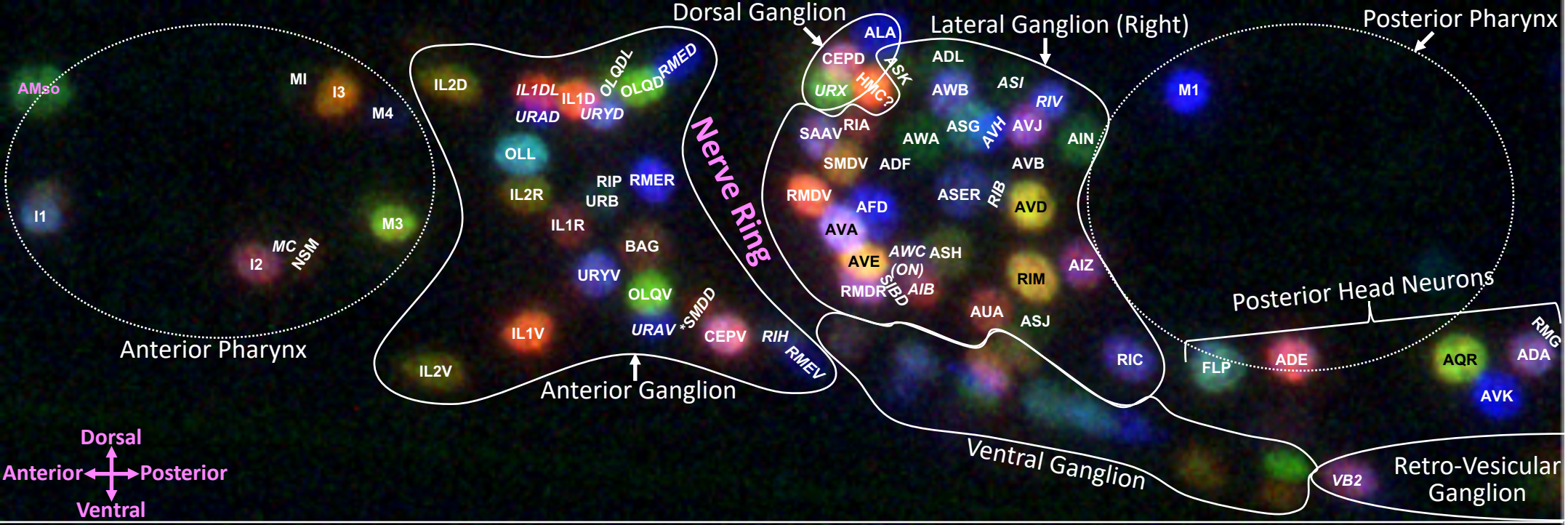
1_YAa_otIs669 (Head Right Side)



Open "Head/Lateral Head/1_YAa_otIs669.czi":

1) Orient yourself, on the right 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 left, the Posterior Head Neurons are missing 1 neuron, AQR.

1_YAa_otls669 (Head Right Side)

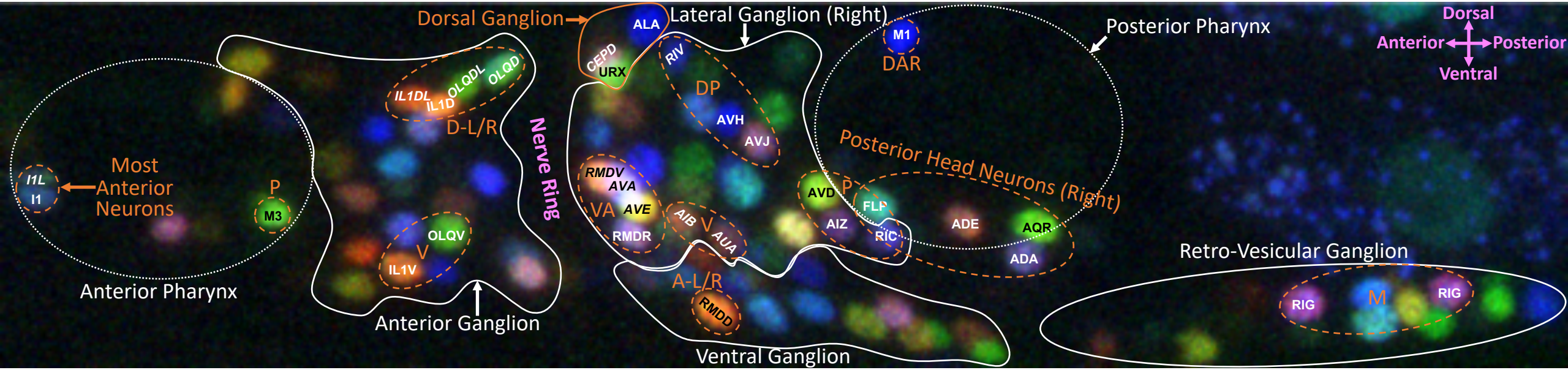


Head Notes:
- SMDD appears to have drifted across the nerve ring into the anterior ganglion.
- HMC is not a neuron, it's usually found dorsal & posterior to the head (much further posterior to the posterior pharyngeal bulb than seen here). It may or may not express color. Since it rarely proceeds this anterior & there are no other similarly colored neurons nearby, it's unlikely to confuse you.
- Please see the previous pages for additional notes (in particular pages 59 & 61), I prefer to leave these images unobscured.

9_YAa_otIs670_x_him-8 (Head Right Side)

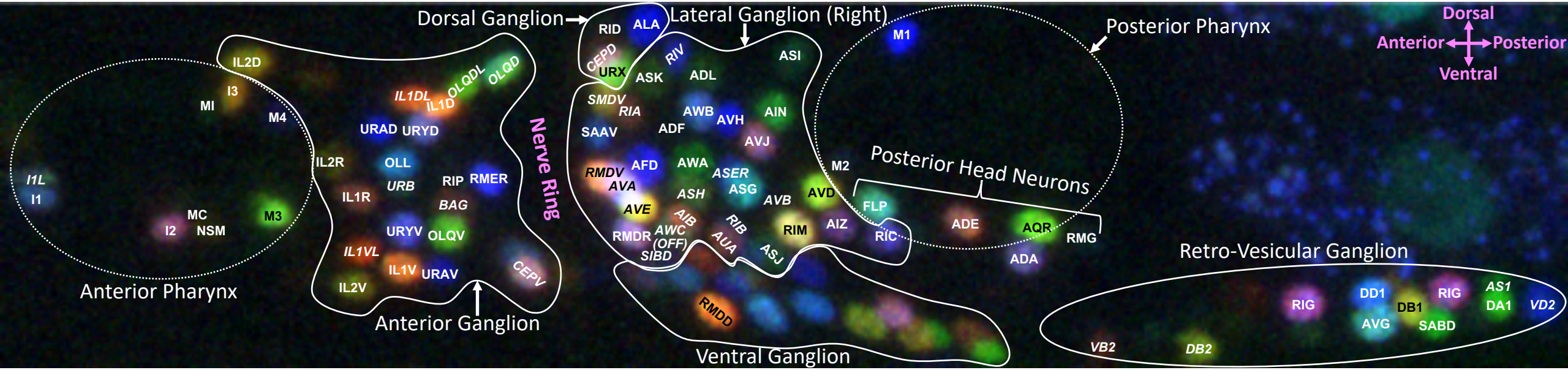
Open "Head/Lateral Head/9_YAa_otIs670_x_him-8.czi":

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

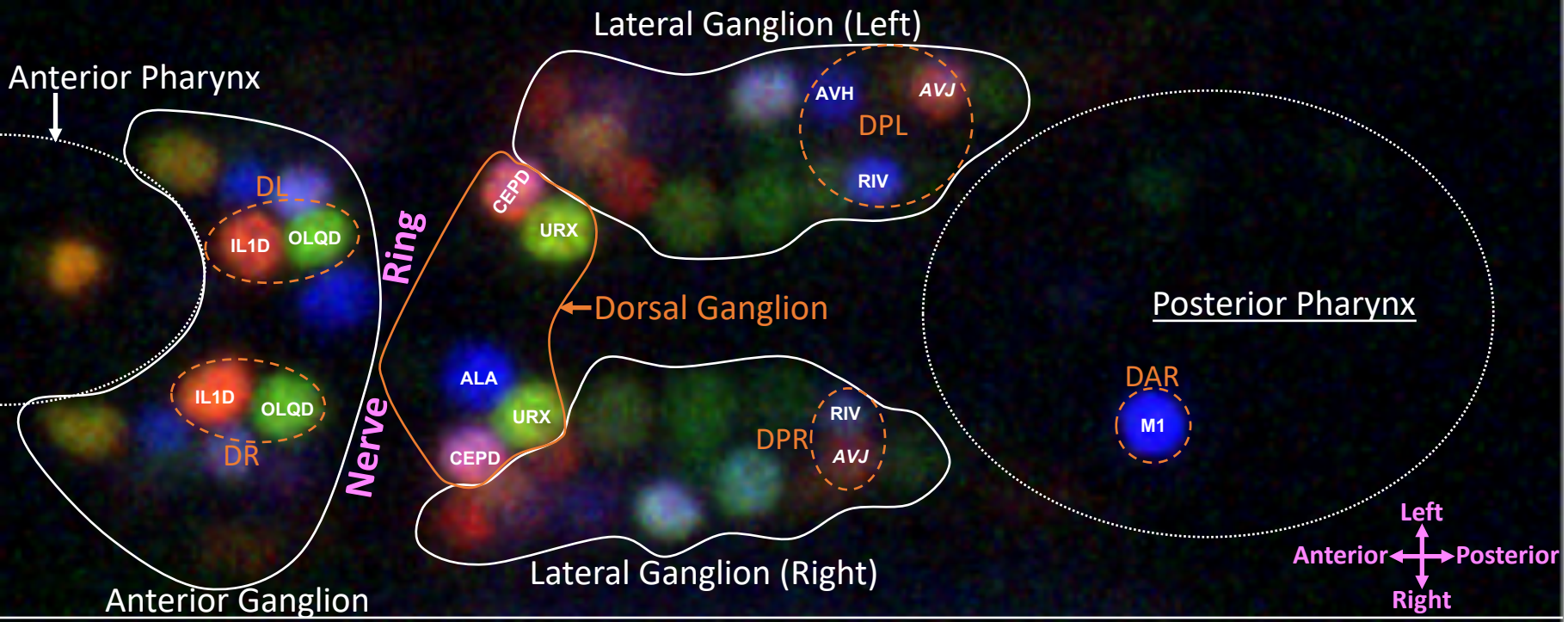


9_YAa_otIs670_x_him-8 (Head Right Side)

Head Notes:
- Please see the previous pages for notes (in particular pages 59 & 61), I prefer to leave these images unobscured.

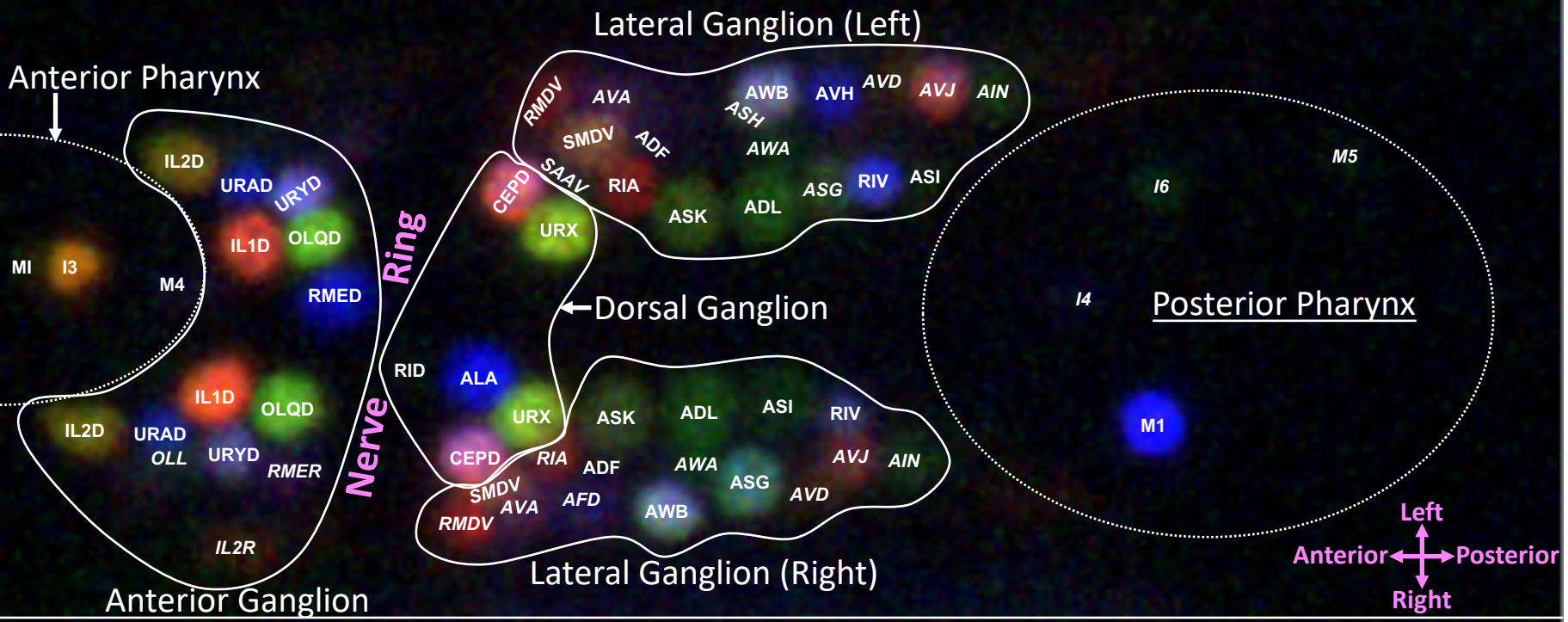


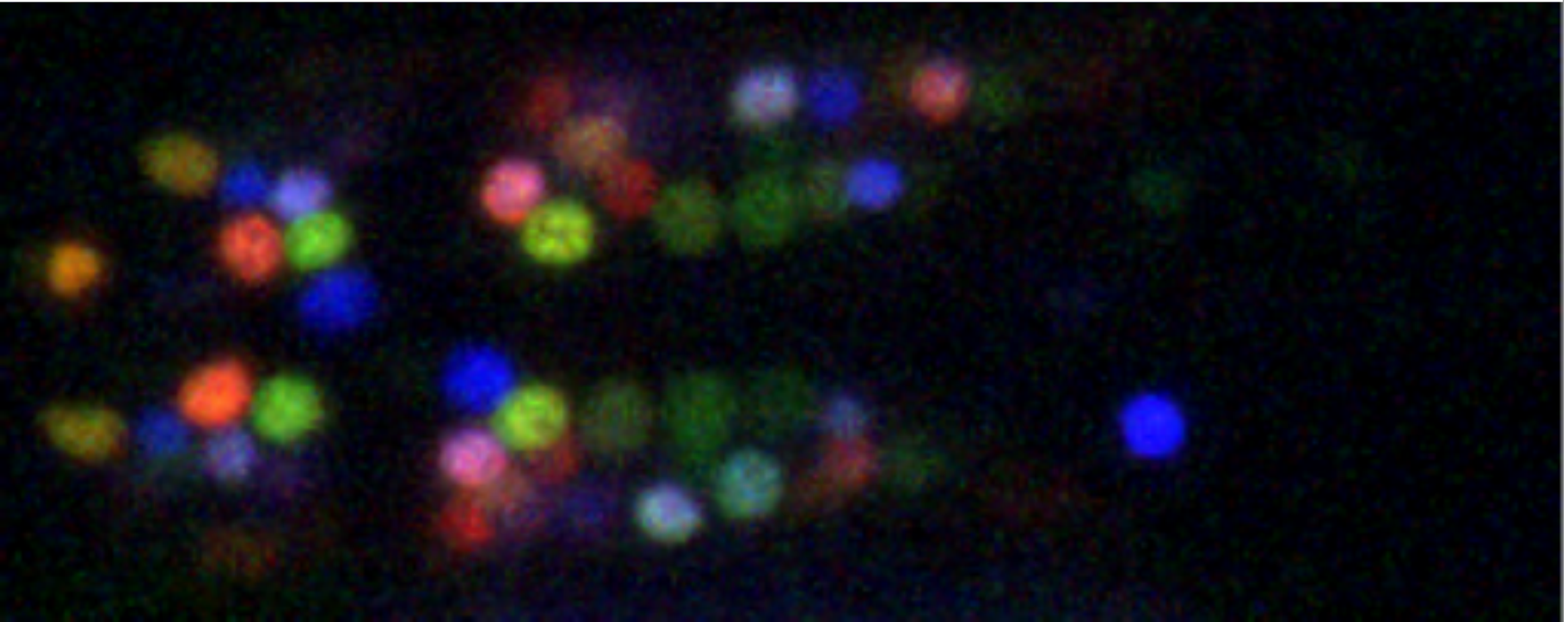
Head (Dorsal Projections)



Open "Head/Dorsal-Ventral Head/34_L3aV_otIs669.czi":
1) Orient yourself using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.

34_L3aV_otIs669 (Head Dorsal View)

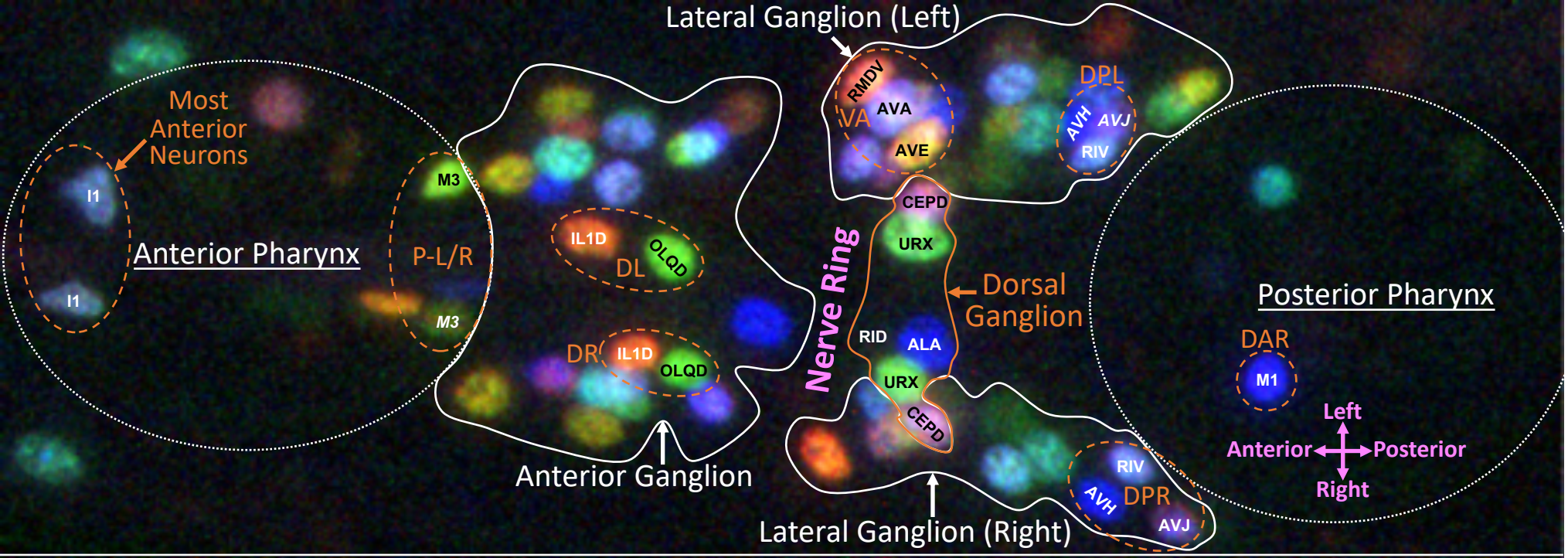


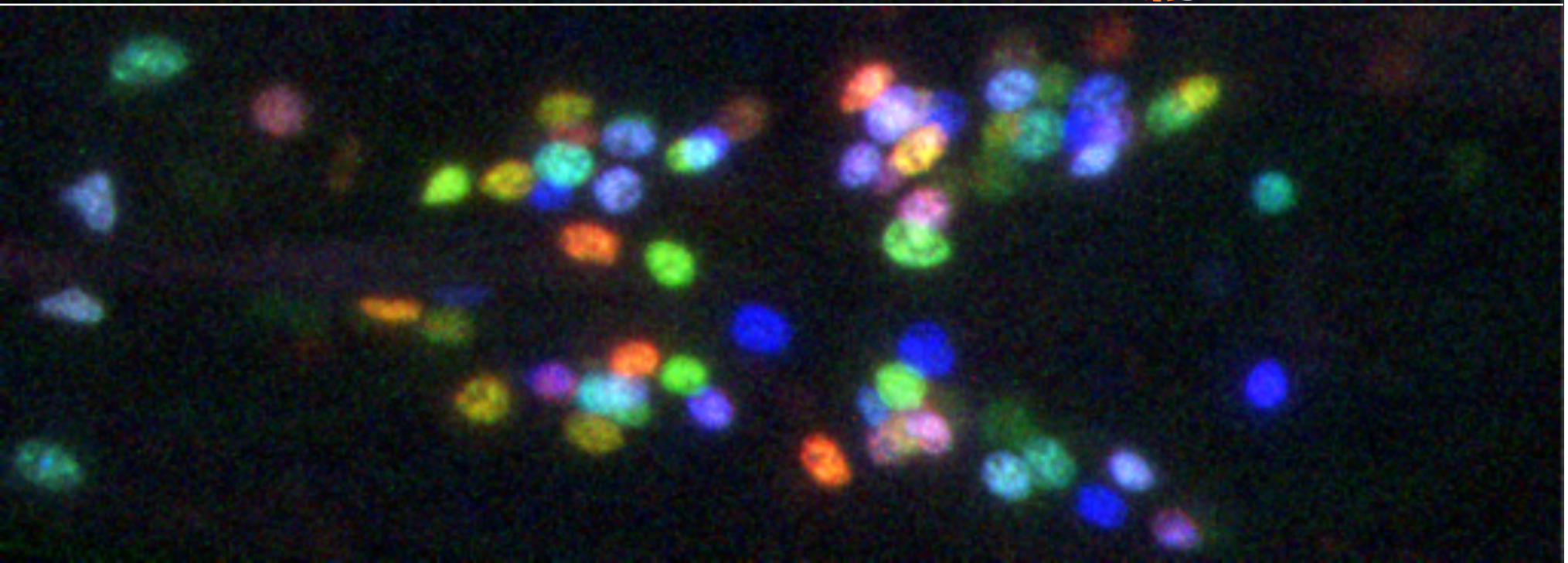


Head Notes:

- M4 & I4 only express the panneuronal marker but they, and the other pharyngeal neurons in general, have nearly unwavering positional stereotypy.
- RID & ADF only express the panneuronal marker. They're in separate ganglia.
- IL2D/V lack positional stereotypy in the anterior-posterior plane.
- URAD/V & RMED/V are blue. URAD/V are found anterior to & have smaller, rounder nuclei than RMED/V. RMED/V have oblong shaped nuclei.
- ALA is posterior to RMED, in a separate ganglion. They never approach each other.
- ASK & ADL are overtly green. ASK expresses red as well, is anterior to ADL, & has a smaller nucleus than ADL. Most of the time, ASI expresses only the panneuonal marker but it can appear very faint green. It's posterior to and has a smaller nucleus than ASK & ADL.
- ASG, AWB, & AWA are overtly green. ASG expresses blue as well & is posterior to AWB. AWB expresses all 3 colors.
- AVH & RIV are overtly blue. RIV has a small nucleus & expresses all 3 colors.

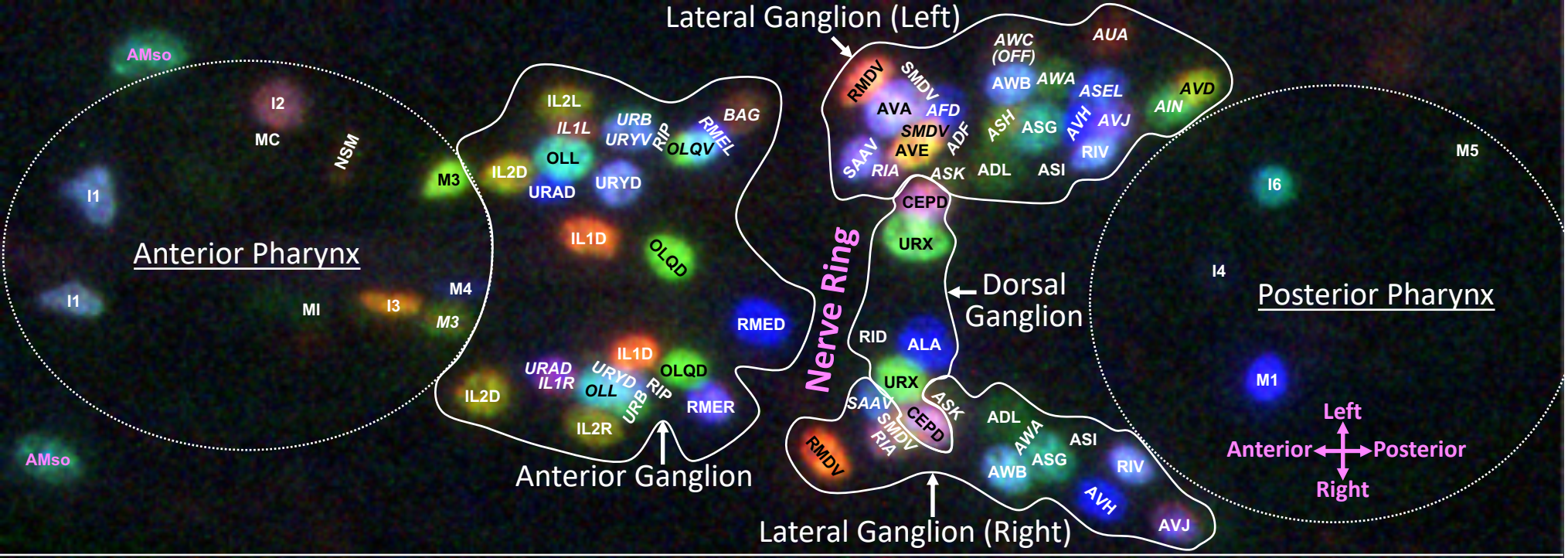
34_L3aV_otls669 (Head Dorsal View)

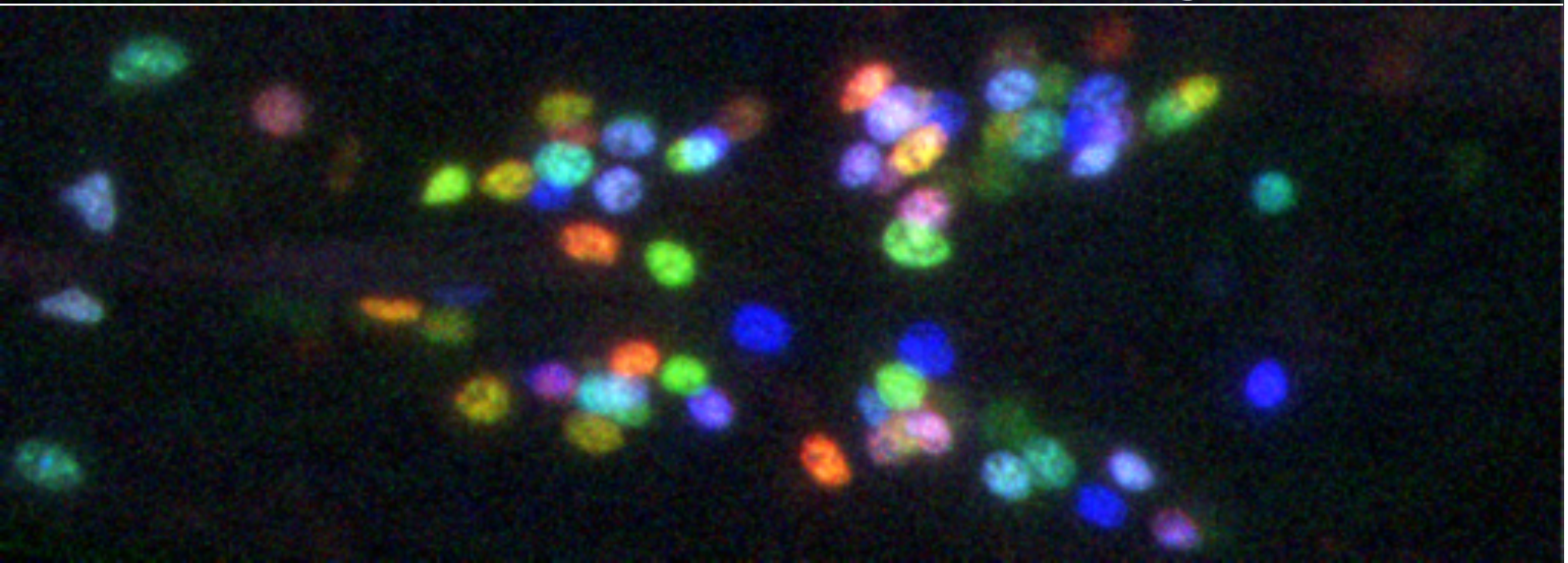




Open "Head/Dorsal-Ventral Head/20_YAaV_otIs669_x_him-5.czi":
1) Orient yourself using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.

20_YAaV_otIs669_x_him-5 (Head Dorsal View)





Head Notes:

- M4 & I4 only express the panneuronal marker but they, and the other pharyngeal neurons in general, have nearly unwavering positional stereotypy.
- RIP, RID, & ADF only express the panneuronal marker. They're in separate ganglia.
- IL2D/V & OLL lack positional stereotypy in the anterior-posterior plane.
- URAD/V & RMED/V are blue. URAD/V are found anterior to & have smaller, rounder nuclei than RMED/V. RMED/V have oblong shaped nuclei.
- ALA is posterior to RMED, in a separate ganglion. They never approach each other.
- ASK & ADL are overtly green. ASK expresses red as well, is anterior to ADL, & has a smaller nucleus than ADL. Most of the time, ASI expresses only the panneuonal marker but it can appear very faint green. It's posterior to and has a smaller nucleus than ASK & ADL.
- ASG, AWB, & AWA are overtly green. ASG expresses blue as well & is posterior to AWB. AWB expresses all 3 colors.
- AVH & RIV are overtly blue. RIV has a small nucleus & expresses all 3 colors.
- RIV is dorsal-posterior-internal to & has a smaller nucleus than AWB.

20_YAaV_otls669_x_him-5 (Head Dorsal View)

Head (Ventral Projections)

A Few Words About the Ventral Ganglion …

- Everything's been child's play up till now. The Ventral Ganglion changes all of that.
- *"WELCOME TO THE JUNGLE!!! Watch it bring you to your knnn knne knees, knees!"*
- **This ganglion's broken me a few times & here's why:**
  - More than 30 neurons are squashed into a tiny little bag. It's total chaos as they jostle for space.
  - This tiny little bag of neurons is being squeezed & stretched out under a hard, massive pharyngeal bulb. In this dense mess, neurons pile on top of each other & push to the extremities, every neuron fends for space.
  - The anterior portion of the Retro-Vesicular Ganglion (RVG) has little respect for the Ventral Ganglion's (VG) boundaries & the lack of space therein. Like a nasty roommate, it often expands right into the posterior of the VG, encroaching wherever it can push its way into. VB2, AVF, & SABV often wander where they don't belong.
  - The reporters available for this ganglion were subpar. I had to screen a ton & build my own personal stash. And, as it turns out, some of these reporters have variable brightness (AIA, AVL, & RIS).
  - All totaled, this is why the ventral ganglion is the only place that still strikes fear in my heart.
- **<u>Now that that's off my chest, don't worry too much, we have a nice solution:</u>**
  1. **TAKE LOTS OF PICTURES!!!** With 5-10 good ventral views, I feel entirely confident in my VG IDs.
  2. **Cheat by using the cell classes.** I've found that if a reporter is expressed in the Lateral Ganglion's RMDs, SAAs, SIBs, and/or SMDs, you will find the reporter also expresses in the Ventral Ganglion counterpart.
- I apologize, I wish I could've done better here but I needed more colors & better landmarks.

Tips & Tricks to ID the Ventral Ganglion

The Ventral Ganglion may seem like a lawless place but I have a few tips & tricks to ID it:

1) ONLY ID CLEAR, VENTRAL VIEWS!

2) Define your boundaries:

A) Delineate the posterior end of the Anterior Ganglion by locating the 2 CEPVs & RMEV.

B) Delineate the ventral ends of the left & right Lateral Ganglia by locating AVE, AUA, AIB, RIM, ASJ, RIC, & AIZ.

C) Delineate the anterior end of the RVG by locating VB2, the AVFs, & the SABVs (just in case).

D) Now you have the Ventral Ganglion walled off.

3) ID the easy neurons:

A) RIH expresses all 3 colors & has a giant nucleus at the anterior midline.

B) The giant RIH is flanked, on either side, by 2 small SAAD nuclei which express all 3 colors.

C) The SAAD nuclei are flanked, on either side by the bright red RMDDs.

D) The SMBs are a pair of bright blue+green neurons on either side of the ganglion.

E) The SIAs are a pair of bright blue neurons on either side of the ganglion. Relative to the AVKs, they're less bright, have smaller nuclei, and are located more anterior.

F) The EM shows anterior-posterior orientations for both SMBD vs. SMBV & SIAD vs. SIAV. Since the dorsal vs. ventral neurons are indistinguishable, I use the EM to make an educated guess.

G) The AIMs are bright blue+red+light-green at the posterior end of the ganglion. Find VB2 (in the RVG) first to avoid confusing the two. The VB2 expresses more green than the AIMs.

H) The AIYs are bright green, at the posterior end of the ganglion, often near the AIMs. Find the SABVs (in RVG) first to avoid confusing the two. The SABVs have smaller nuclei & are posterior.

I) The AVKs are very bright blue with large nuclei. They're usually found extremely posterior, well beyond the other ventral ganglion neurons, by the RVG.

4) ID the medium neurons:

A) The RIR is faint blue+red, located next to the RIH. RIH has a much larger nucleus than RIR.

B) The RMFs express all 3 colors & are located just posterior to RIH at the lateral midline.

C) The RMHs are panneuronal colored, located just posterior to RIH at the lateral midline.

D) The SIBVs are faint green, have a small nucleus, and are located at the extreme left & right sides.

5) ID the hard neurons:

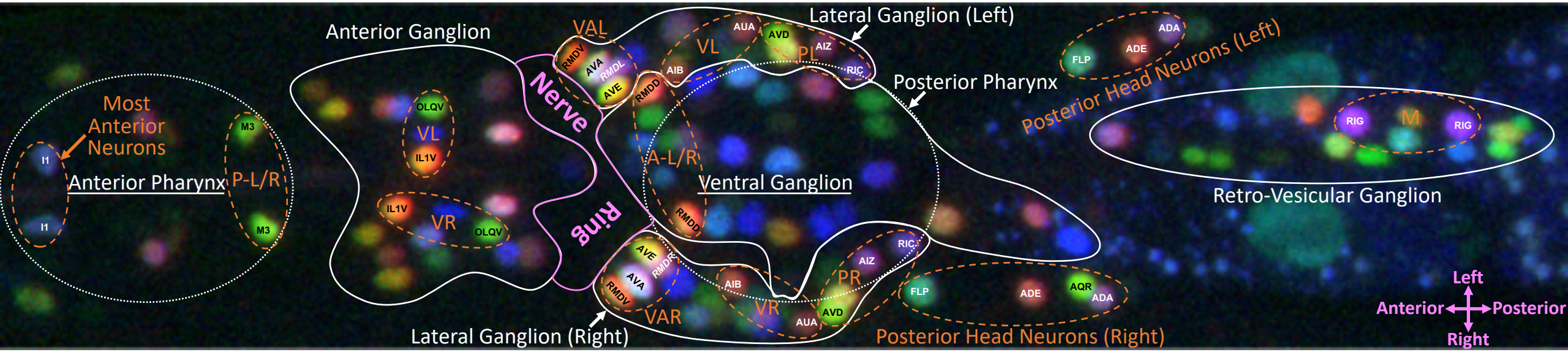
A) The AIAs are located mildly posterior, at the lateral midline, between the posterior SMBs & SIAs. They are red+green but can vary in brightness & appear quite faint.

B) The SMDDs are red+green (like the SMDVs) with slightly oblong nuclei. They're located at the extreme left & right sides, usually anterior, but they often drift as posterior as they feel like.

C) AVL & RIS express all 3 colors but vary in brightness. When faint, they express far more green than blue (giving them a greenish-yellow appearance). AVL & RIS share a reporter & therefore, within each animal, they will have very similar coloring to each other. AVL has a triangular nucleus & RIS has a large round nucleus. AVL is anterior to RIS. AVL is usually located on the far right side, at the center of the anterior-posterior axis of the ganglion. RIS is located on the far right side, at the posterior of the ganglion, near AIMR & AIYR and it has a larger nucleus than these cells.

1_AaV_otIs670_x_him-8 (Head Ventral View)

Open "Head/Dorsal-Ventral Head/1_AaV_otIs670_x_him-8.czi":
1) Orient yourself using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.

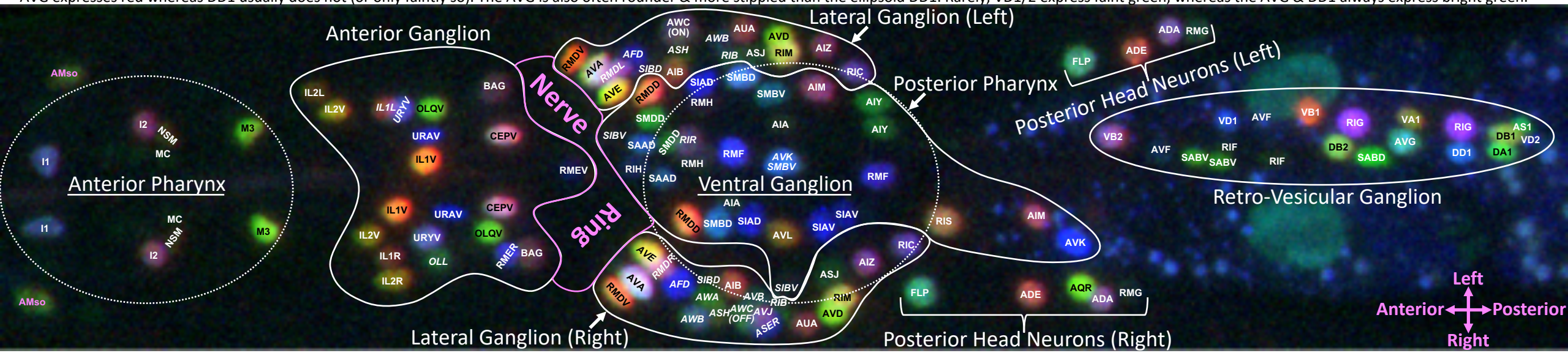


1_AaV_otIs670_x_him-8 (Head Ventral View)

Head Notes:

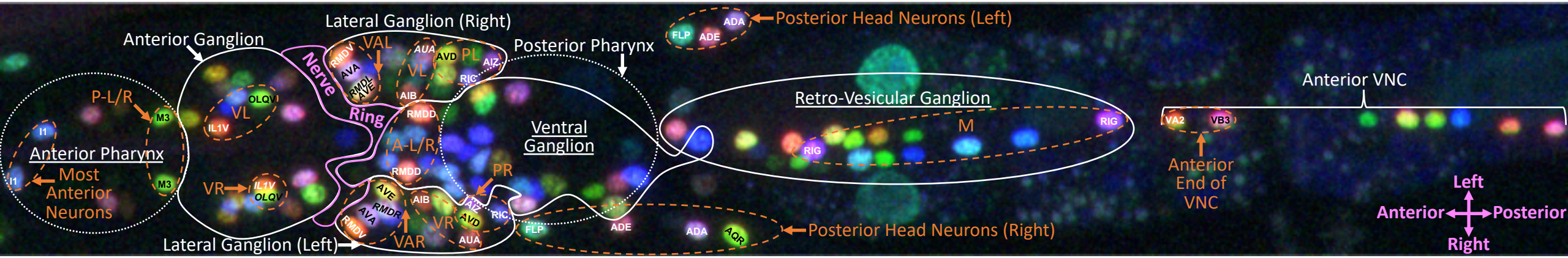
Pages 58-70 explain how to ID the pharyngeal bulbs, anterior + lateral ganglia, & the posterior head neurons. Page 78 explains how to ID the ventral ganglion.

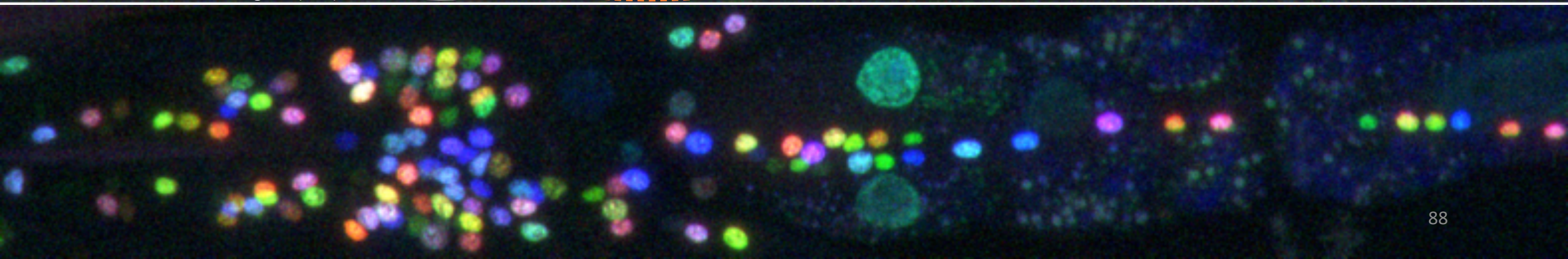
- VB2 expresses all 3 colors, appearing purple-red, and has more green than the AIMs.
- Contrary to their VNC appearance: VB1 is bright red, without any blue, & VA1 is light red+green.
- The AVFs are faint blue & anterior to the faint green RIFs.
- The SABVs are small & anterior to the RVG's anterior-posterior midline while SABD, DA1, & AS1 are posterior to the anterior-posterior midline. SABD is usually anterior to AS1 & DA1.
- SABD is larger than AS1 but both are green. SABD often appears more oblong than DA1. DA1 is green+red. The DBs have all 3 colored landmarks.
- I guessed the anterior-posterior orientations of DB1 & DB2 as well as VD1 & VD2 from the 3 adult EM reconstructions. Cells of a class look identical, no way to distinguish #1 from #2.
- AVG expresses red whereas DD1 usually does not (or only faintly so). The AVG is also often rounder & more stippled than the ellipsoid DD1. Rarely, VD1/2 express faint green, whereas the AVG & DD1 always express bright green.



14_AaV_otIs669_x_him-5 (Head Ventral View)

Open "Head/Dorsal-Ventral Head/14_AaV_otIs669_x_him-5.czi":
1) Orient yourself using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.
3) The midbody of this worm is ID'd on pages 51-52.





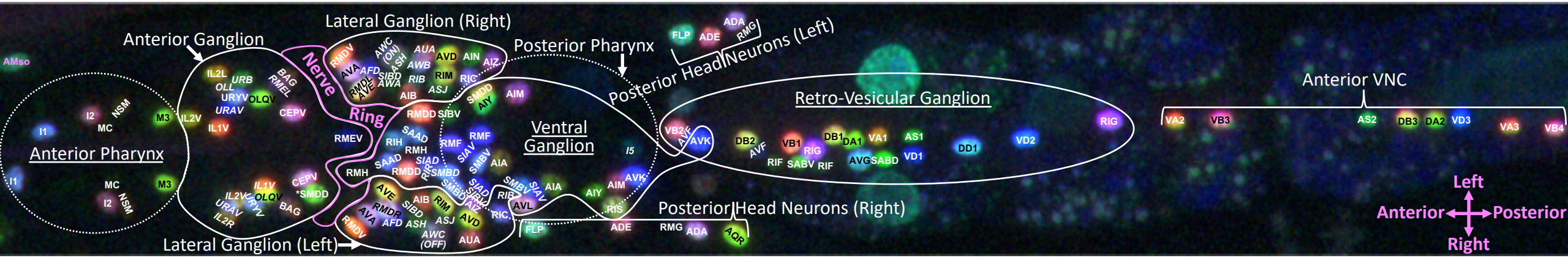
14_AaV_otIs669_x_him-5 (Head Ventral View)

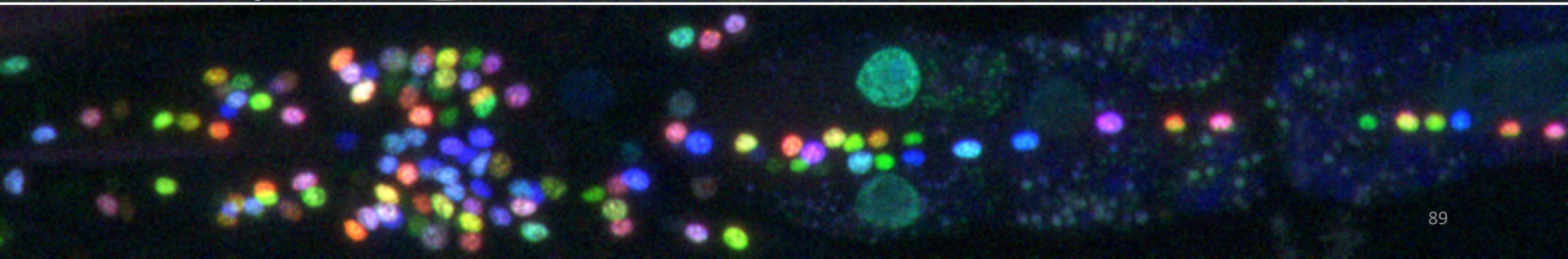
Head Notes:

Pages 58-70 explain how to ID the pharyngeal bulbs, anterior + lateral ganglia, & the posterior head neurons.

Page 78 explains how to ID the ventral ganglion.

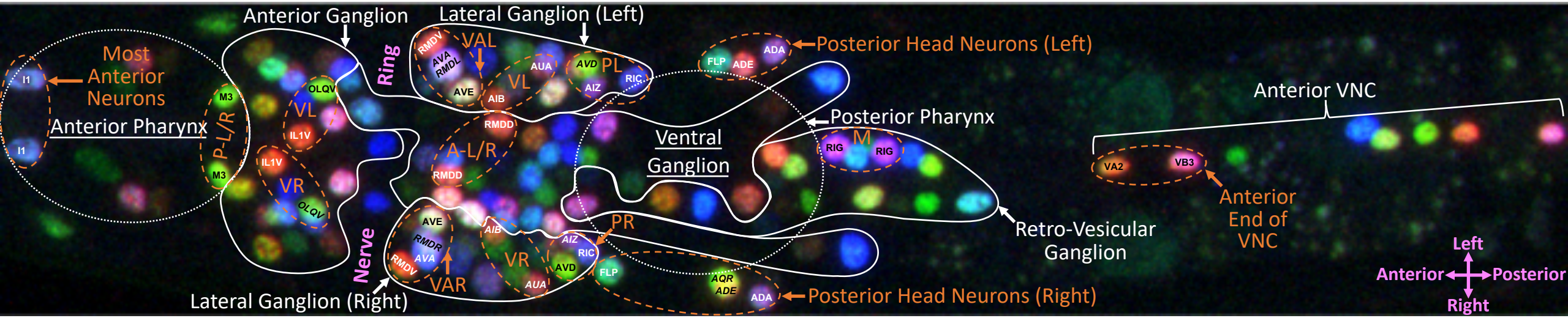
- SMDD appears to have drifted across the nerve ring into the anterior ganglion.
- VB2 expresses all 3 colors, appearing purple-red, and has more green than the AIMs.
- Contrary to their VNC appearance: VB1 is bright red, without any blue, & VA1 is light red+green.
- The AVFs are faint blue & anterior to the faint green RIFs.
- The SABVs are small & anterior to the RVG's anterior-posterior midline while SABD, DA1, & AS1 are posterior to the anterior-posterior midline. SABD is usually anterior to AS1 & DA1.
- SABD is larger than AS1 but both are green. SABD often appears more oblong than DA1. DA1 is green+red. The DBs have all 3 colored landmarks.
- I guessed the anterior-posterior orientations of DB1 & DB2 as well as VD1 & VD2 from the 3 adult EM reconstructions. Cells of a class look identical, no way to distinguish #1 from #2.
- AVG expresses red whereas DD1 usually does not (or only faintly so). The AVG is also often rounder & more stippled than the ellipsoid DD1. Rarely, VD1/2 express faint green, whereas the AVG & DD1 always express bright green.





23_YAaV_otIs669_x_him-5 (Head Ventral View)

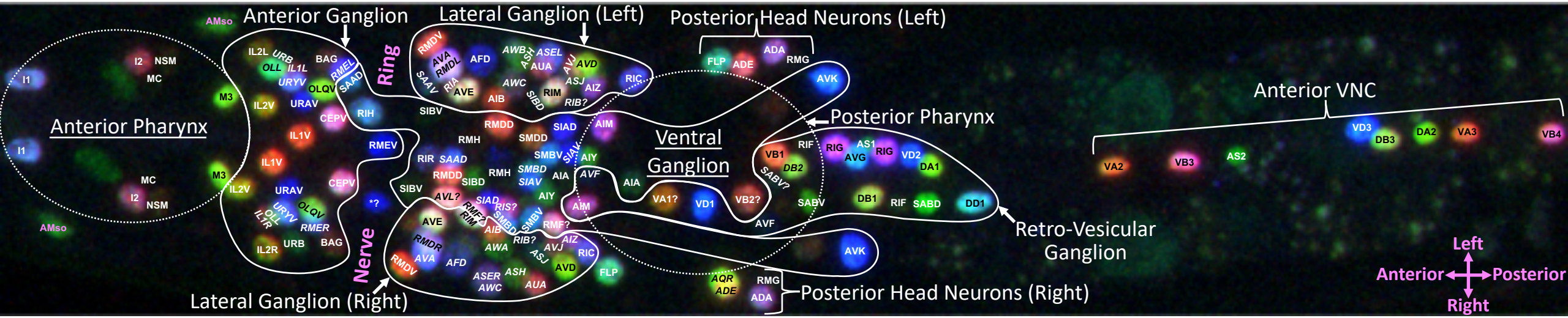
Open "Head/Dorsal-Ventral Head/23_YAaV_otIs669_x_him-5.czi":
1) Orient yourself using the landmarks shown here.
2) Use the map on the next page to locate & ID each neuron.

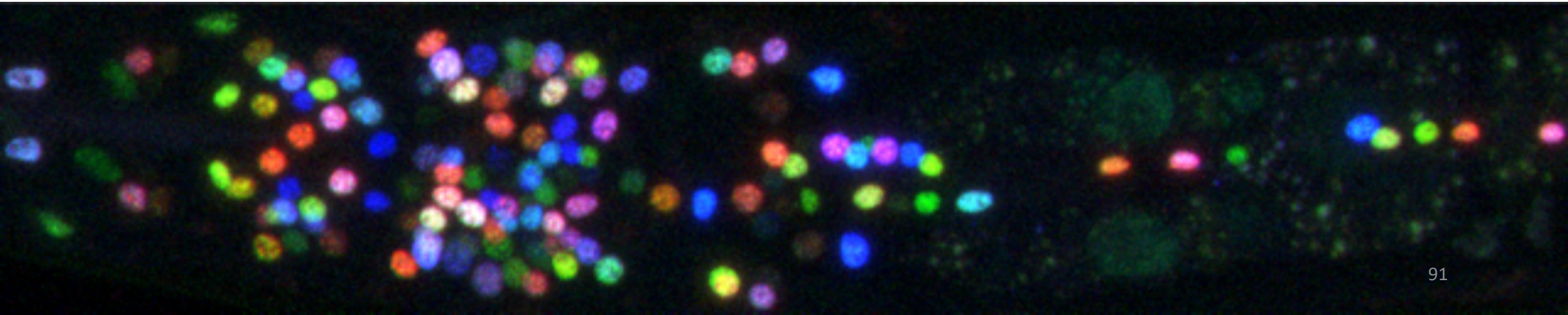


23_YAaV_otIs669_x_him-5 (Head Ventral View)

Head Notes:

- The AVKs are positioned so far posterior, that they've reached the RVG's anterior-posterior midline. This happens often enough that you should be aware of it.
- RIM and what I presume to be AVL & RIS, are much brighter than usual. This happens often enough that you should be aware of it. I suspect that this is some signifier of the worm's health and/or its environmental conditions. Perhaps some kind soul can solve this puzzle?
- There's a blue cell in the nerve ring that was unidentifiable, thankfully this is a rare occurrence.
- Please see the previous pages for additional notes, I prefer to leave these images unobscured.



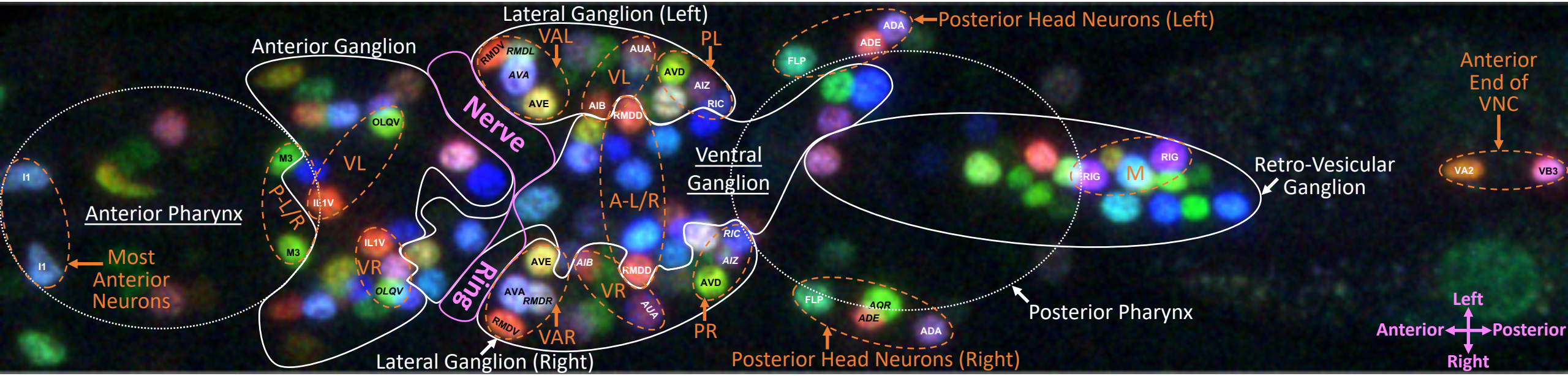


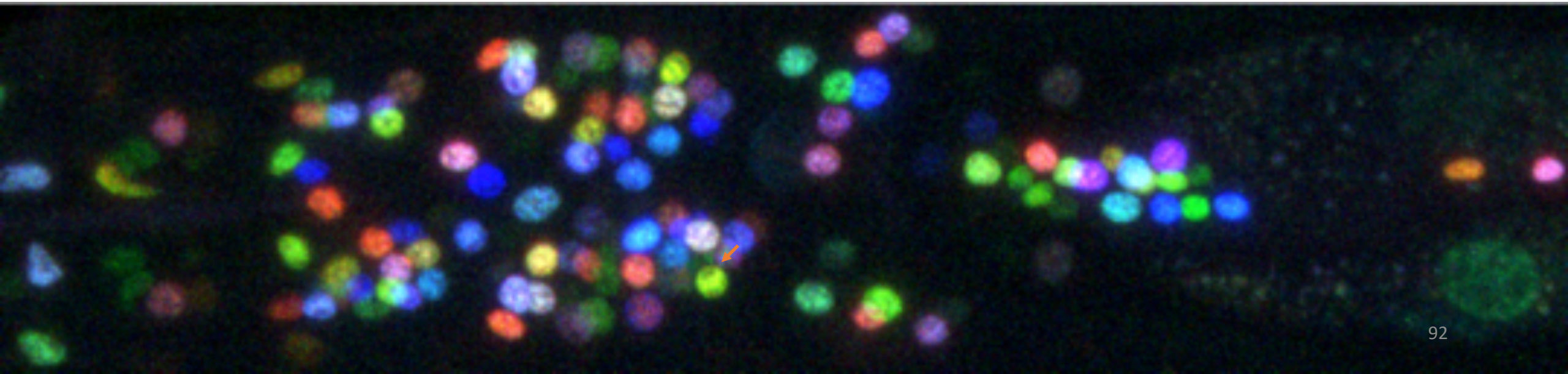
25_AaV_otIs669_x_him-5 (Head Ventral View)

Open "Head/Dorsal-Ventral Head/ 25_AaV_otIs669_x_him-5.czi":

1) Orient yourself using the landmarks shown here.

2) Use the map on the next page to locate & ID each neuron.

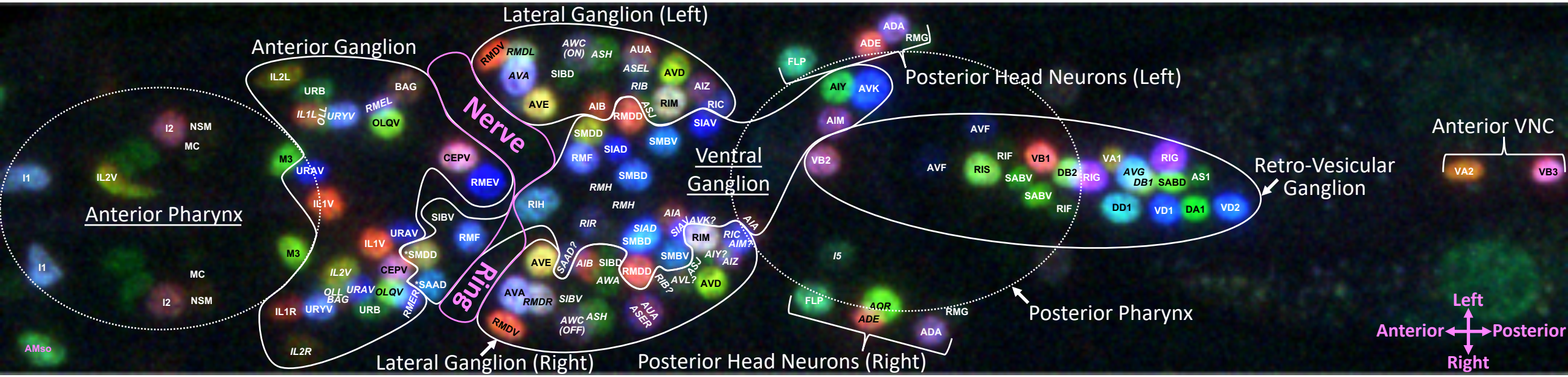




25_AaV_otIs669_x_him-5 (Head Ventral View)

Head Notes:

- This is why you don't ID twisted worms ☺ See all the question marks? Notice how SMDD & SAAD look like they've crossed the nerve ring and, how AVKR is likely sitting on top of RIMR?
- Nonetheless, with several more dorsal-ventral pictures, you can use a larger sample size to correct for the low confidence IDs present here.
- Please see the previous pages for additional notes, I prefer to leave these images unobscured.



Learning to ID with NeuroPAL:
Imaging NeuroPAL
Crosses & Injections

Picking the Right NeuroPAL for your GFP/CFP/YFP Reporter

- There are **3 NeuroPALs: Bright (otIs669 V), Weak (otIs670 V), Weak (otIs696, not on V)**.
- If your reporter is on chromosome V, I would advise crossing to otIs696.
- Otherwise, due to bleed through at the extreme ranges of bright & weak reporters, the following advice may be helpful:

  - **If your reporter is brightly visible** under a dissecting scope, it may bleed into the green CyOFP landmark. To minimize this bleed through, **I would advise using the bright NeuroPAL integrant otIs669**.

  ***** Note: in practice, this is unlikely to confuse you since the bleed through is obvious & there are plenty of nearby neurons in NeuroPAL to help orient you.**

  - **If your reporter is very weak**, you may wish to turn up the gain to the point at which the very faint tail of the green CyOFP landmark may bleed into your reporter channel. To minimize this bleed through, **I would advise using the weaker NeuroPAL integrants otIs670 or otIs696**.

  ***** Note 1: in practice, this only occurs with extremely weak nuclear reporters that, even without the presence of NeuroPAL, are hard to definitively state as present or absent within a neuron. If you encounter this issue, please consider using a cytoplasmic reporter (which is easily distinguishable from NeuroPAL's nuclear landmarks) and/or a brighter reporter line.**

  ***** Note 2: If you suspect the CyOFP landmark has bled into your reporter, you can check the bright green neurons (at the head: M3, OLQ, URX, & AQR – at the midbody: AS, DA, DB – at the tail: PQR & PLM) to find the threshold at which histogram/gamma adjustments, bleed through into your reporter.**

Preparing Your NeuroPAL + Reporter/Mutant Crosses

- Image your reporter **WITHOUT** NeuroPAL.
  - ***** Is your line barely visible without NeuroPAL?**
    If so, get a better line, NeuroPAL can't work a miracle ☺
  - Crossing to NeuroPAL may alter your reporter expression.
    Be sure you know what the groundtruth looks like, count the cells expressing your reporter.

- Nearly 100 lines have now been crossed to NeuroPAL without issue (including mutants with high larval lethality, sick/slow-growth strains, & extrachromosomal arrays).

***** Note: If you experience issues when crossing, please try using more males & hermaphrodites, waiting an extra day to pick cross progeny (in case you've experienced a sick/slow-growth phenotype), and/or using a different NeuroPAL integrant**.

- Your NeuroPAL crosses **MUST** be **homozygous for NeuroPAL**.

***** Note: if the NeuroPAL is not homozygous, many of the colors will be too faint, the maps will not match your images, & you will be confused. Don't do this!**

- **Before imaging your NeuroPAL crosses, adjust the GFP/CFP/YFP channel on your scope to ensure your reporter is clearly visible.** If the NeuroPAL appears dim, ensure your strain is homozygous &, if so, readjust your scope to improve NeuroPAL brightness.

***** See the manual titled "Configuring Your Microscope for NeuroPAL".**

Imaging Your NeuroPAL + Reporter/Mutant Crosses

***PLEASE READ ALL OF THIS!

- **Grow enough worms, you will be taking a lot of pictures!**
- Choose your favorite anesthetic/paralytic & prepare a slide with ~100 worms.
- **The NeuroPAL maps are appropriate for hermaphrodites, from L3 to adults**. In the future, we will release another publication with maps for earlier larval stages, dauers, & males.
- **Use transmitted light and/or the panneuronal TagRFP-T channel to find a hermaphrodite (L4s & young adults are best).**

*** **Note: DON'T use the short wavelength light channels 300-500nm (mTagBFP2, CyOFP, & GFP/CFP/YFP) until taking your picture. YOU WILL BLEACH THE LANDMARK FLUOROPHORES**.

- **DON'T image twisted worms, ID'ing these is very hard.** Ensure your worm is well oriented (lateral or ventral).
- Image the worm using all 3-5 tracks/channels (if you're not using a reporter, you can ignore GFP/CFP/YFP).
- If your reporter or NeuroPAL look weaker than usual or the worm is sick/damaged, discard the image.
- **Take 15-30 images of the head & 10-15 of the tail.** This will give you plenty of images to ID with. Later, you will find some of these images easier to work with than others & appreciate the variety of choices you have.
- If your reporter/mutant affects midbody neurons, take several images of these areas as well.
- **Non-integrant reporters & variable lines will require more images to perform confident ID's.**

Tricks to Orient Your Worms on the Microscope Slide

- **Always use a anesthetic/paralytic to immobilize your worms!**

- I use **50mM sodium azide in M9**. This combination allows me to image a single slide, full of worms, for **~1 hour without issues.**

- **Lateral orientations** are easy & abundant but, if you're having trouble, apply your coverslip early, well before the worms have been immobilized. The worms will swim/thrash under the coverslip and thereby assume a nice lateral orientation.

- **Dorsal-ventral orientations** are easy to obtain using the following techniques:

A) For adults, place your worms in the anesthetic/paralytic, wait ~5 minutes till they've stopped moving, then apply the coverslip (credit: Paschalis Kratsios).

B) For earlier larval stages, place plenty of mixed-staged worms on plate in the anesthetic/paralytic. Use your pick to roughly gather the worms into a dense mass. Now, quickly drop your coverslip on top. You will find that when worms are in a crossed position, lying on top of each other, they often assume a nice dorsal-ventral orientation. This is particularly true for early larval stages stretched/trapped across an adult. I have no idea why this works.

C) Some people say you can slide your coverslip to roll worms into a dorsal-ventral orientation. This rarely works for me but, to each their own.

Using the NeuroPAL Maps

- **Identify where you are & orient the image of your worm correctly (for lateral views: dorsal=north, ventral=south, anterior=west, posterior=east):**
  - Are you looking at the left, right, dorsal, or ventral worm quadrant?
  - Which ganglia are visible & which one are you looking at?
  - Which position are you looking at in this ganglion (dorsal/ventral/anterior/posterior)?

- **Do you see all the cells from your reporter? Do the NeuroPAL colors match the map?**
  - If not, you may have crosstalk. Mis-colored cells are potentially sites of reporter expression (even if they're missing the reporter). Return to your uncrossed reporter images to check the groundtruth.
  - Neuronal colored landmarks might be saturated & out of plane. Look at the volume, not just 1 slice.
  - Even minor twists in the worm can considerably displace cell orientation in the 2D map.
  - Neurons often move from their canonical position. Take enough pictures to avoid confusion!

- **Is your reporter expressed in a neuron?**
  - Neurons are marked by the white panneuronal TagRFP-T landmark and, with a few exceptions, they usually express 1 or more of the other 3 colored landmarks (blue, green, & red).