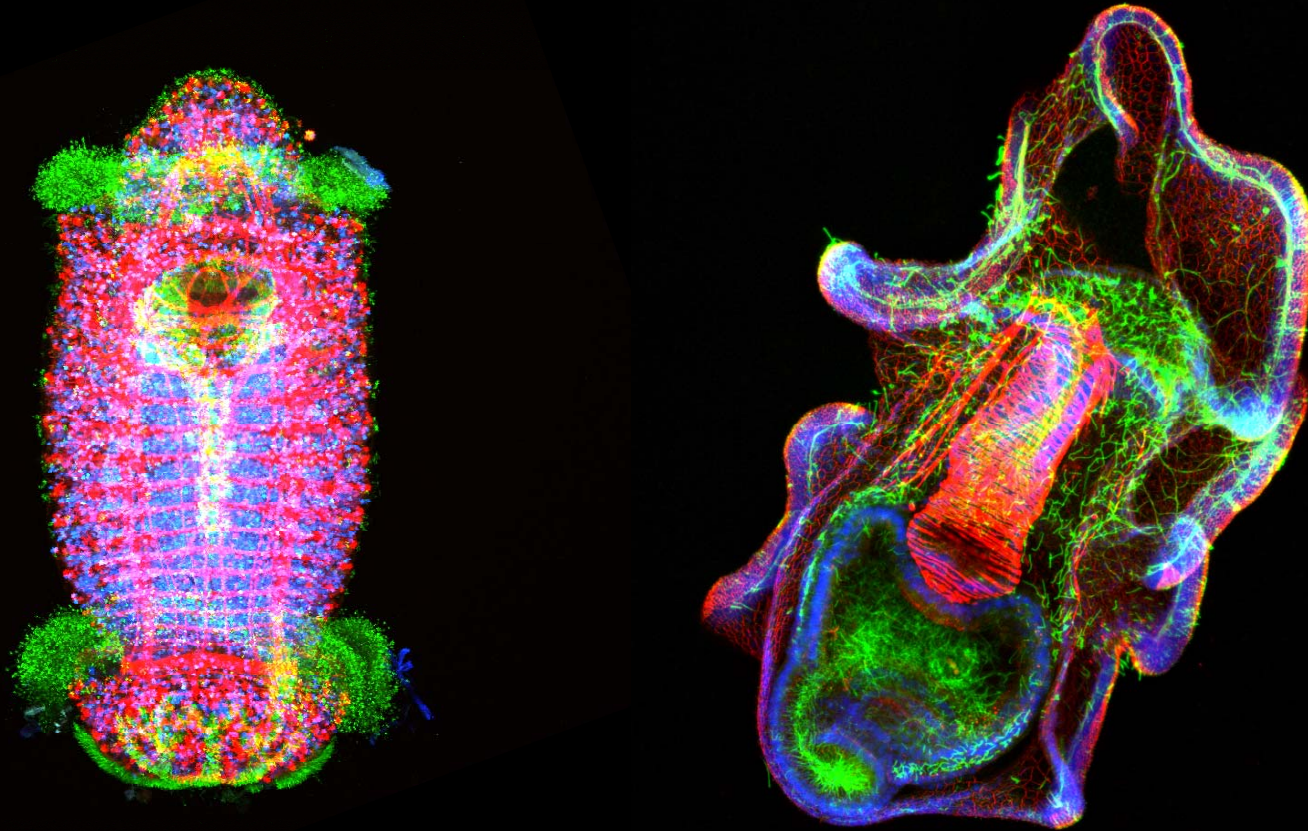


Immunocytochemistry Staining with WSBS Embryology Course

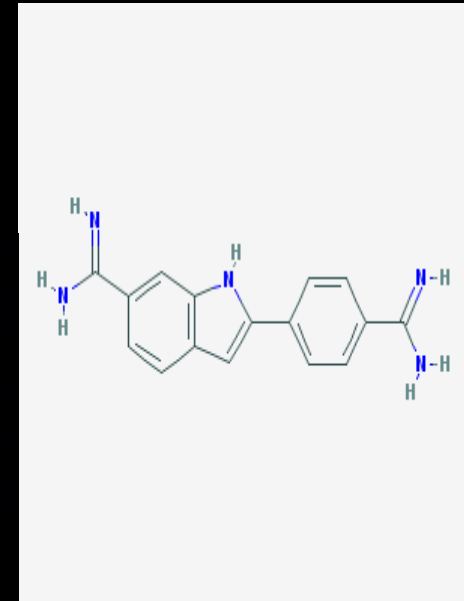
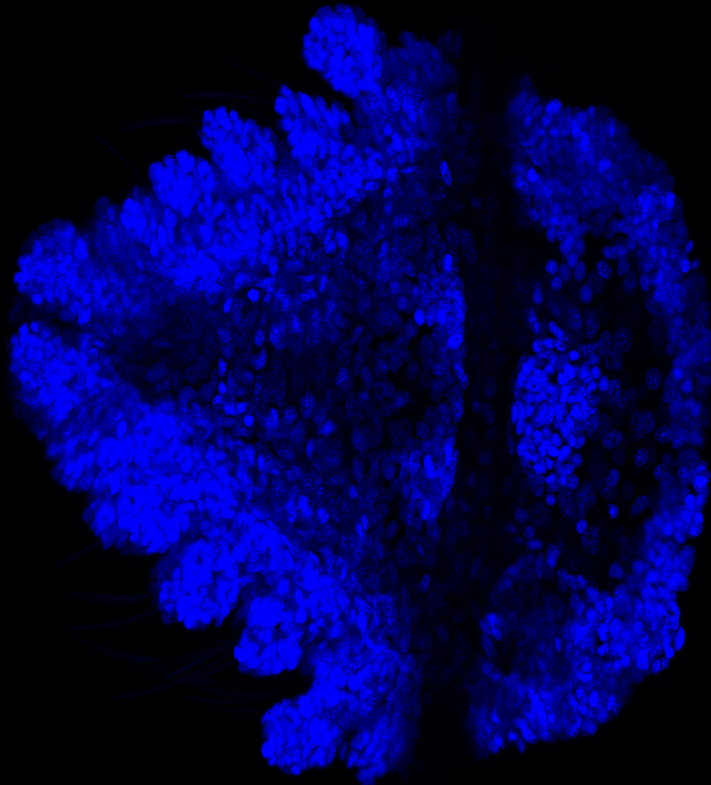


ICC Staining Methods

1. Relaxation
 - MgCl_2
2. Fixation
 - PFA
3. Washing
 - PBS
4. Blocking
 - NGS
5. Antibody 1
 - A-tubulin
6. Antibody 2
 - Alexa Fluor 488
7. Phalloidin
 - Direct
8. Embed
 - Contains DAPI

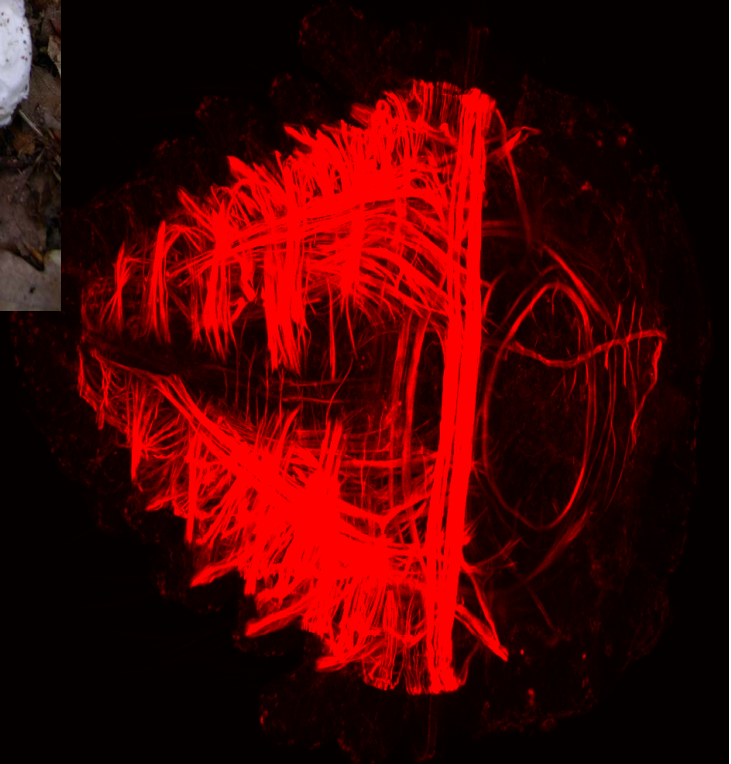
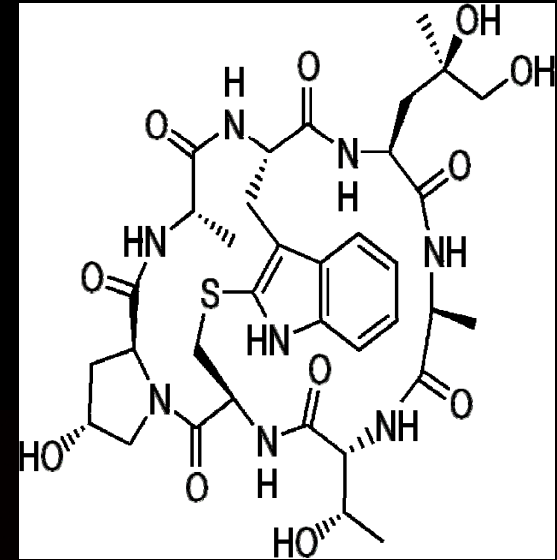
DAPI Staining

- Direct Stain
- Binds: A-T in DNA
- Small
- Excites: 358 nm
- Emits: max. 461 nm
- Detected thru blue/cyan filt
- Viewing: DNA/nucleus



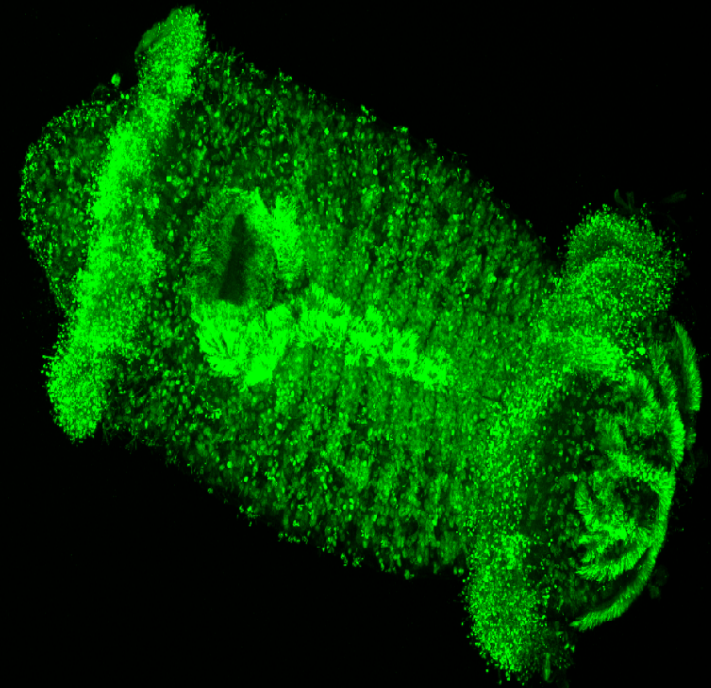
Phalloidin

- Direct stain
- Comes from death mushroom (*Amanita phalloides*)
- Bicyclic heptapeptide
- Binds: F-actin
- What is actin?
- Frequently conjugated with eosin
- Excites: 540 nm
- Emits: 565 nm
- View: microfilaments (e.g. Musculature systems)



α -tubulin & Alexa Fluor 488

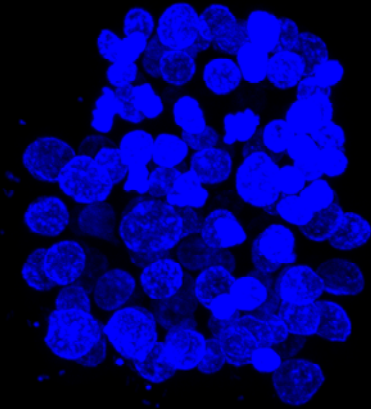
- Synthetic peptide with indirect staining
- Binds: GTP within tubulin
- Primary-binds to tubulin
- Secondary (Alexa Fluor 488) binds to primary
- Excites: 652 nm
- Emits: 668 nm
- View: cilia, flagella



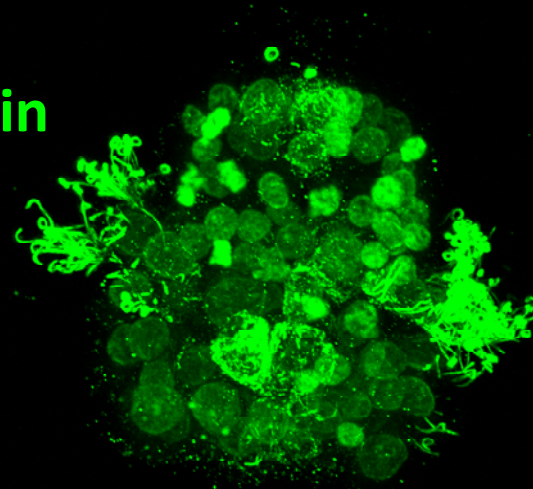
100 μ m

Ophelia limacina (early trochophore)

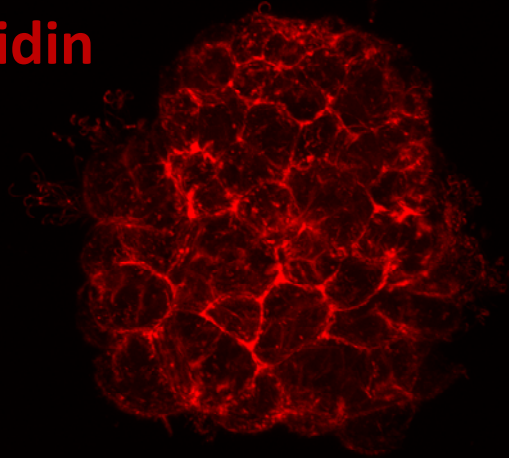
DAPI



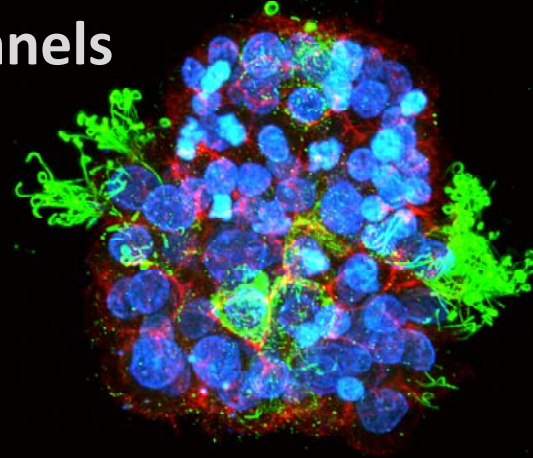
α -Tubulin



Phalloidin

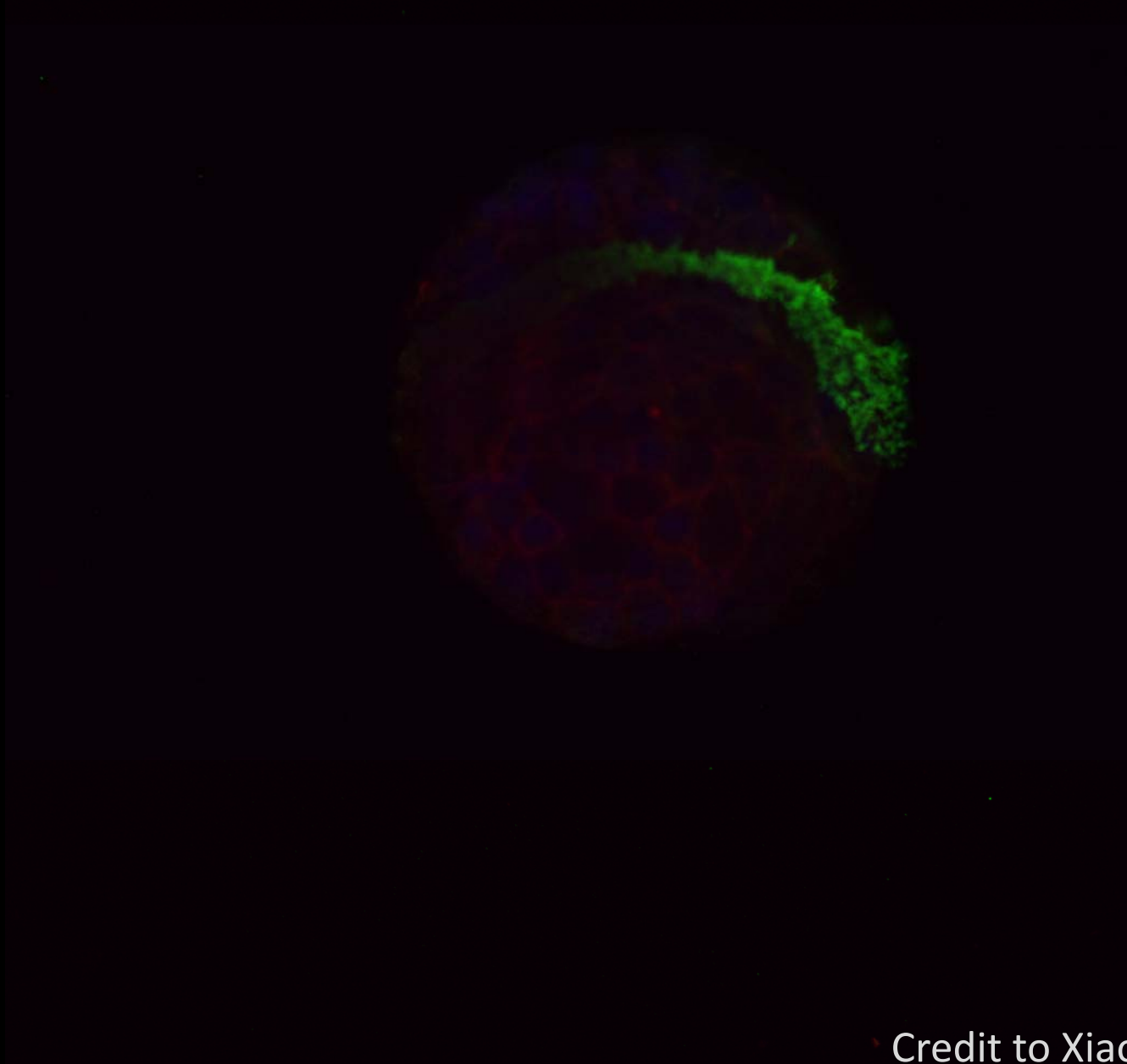


All channels



Credit to Renata & Olga

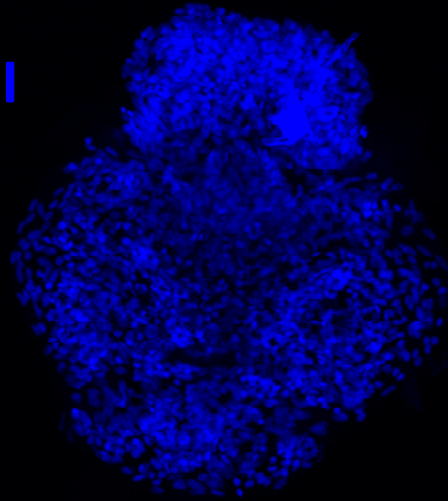
Spirobis armoricana (early trochophore)



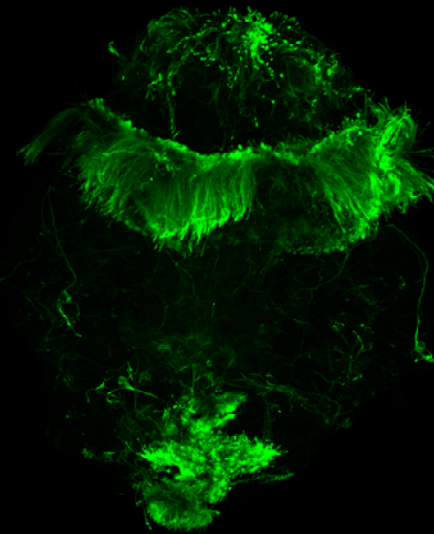
Credit to Xiaoya & Ogla

Spirobis armoricana (metatrochophore?)

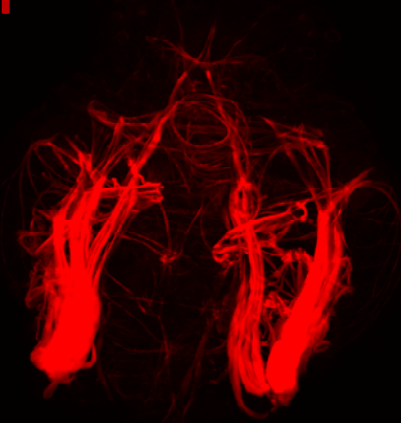
DAPI



α -Tubulin



Phalloidin



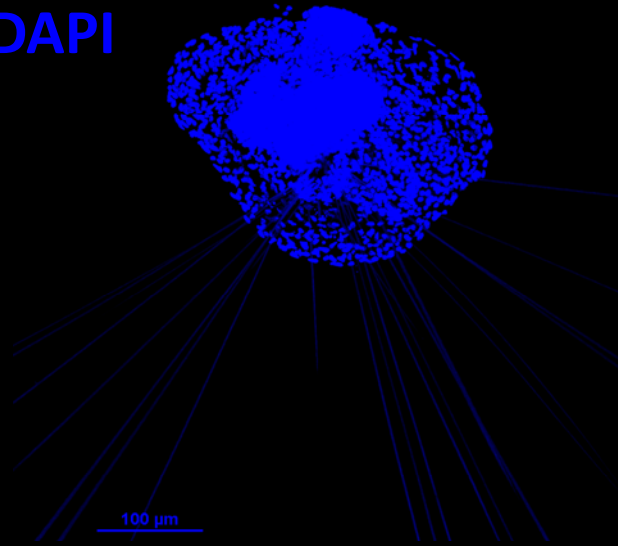
All channels



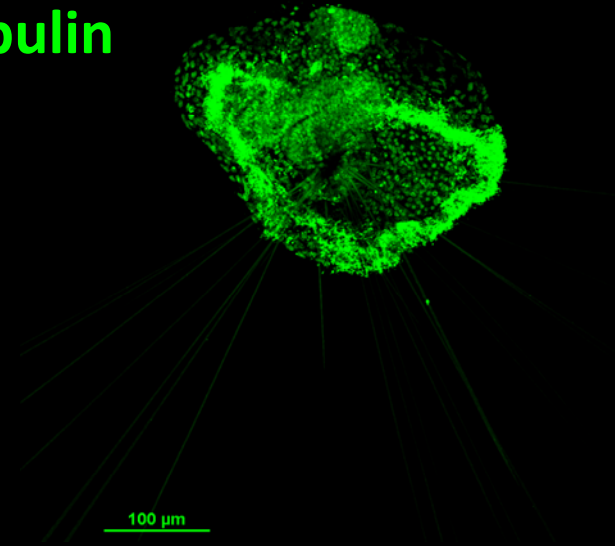
Credit to Xiaoya & Ogla

Oweniidae (mitraria)

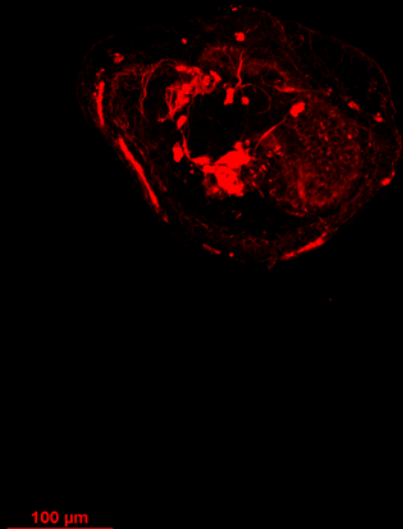
DAPI



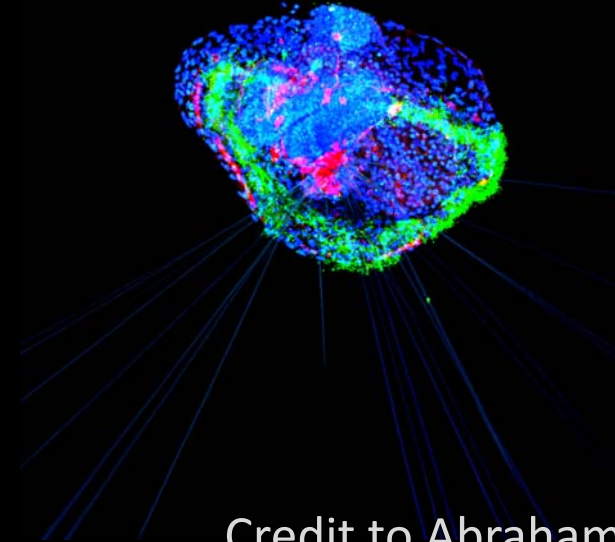
α -Tubulin



Phalloidin



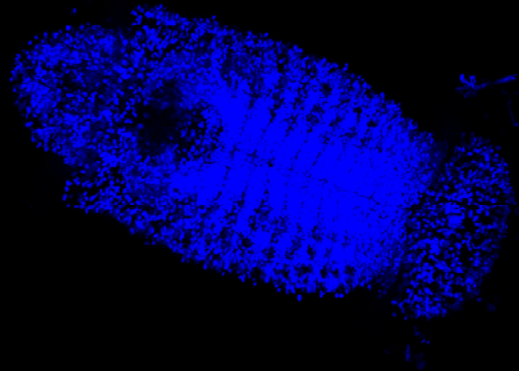
All channels



Credit to Abraham & Sophie

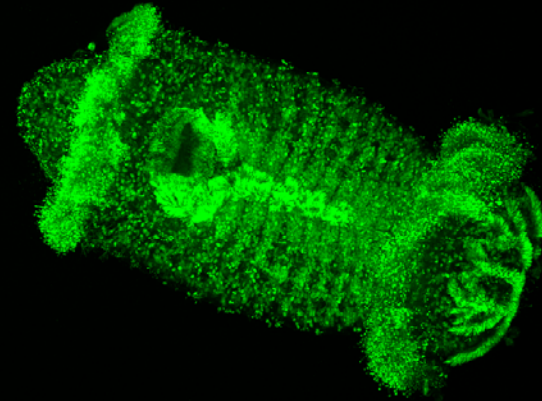
Capitella (metatrochophore)

DAPI



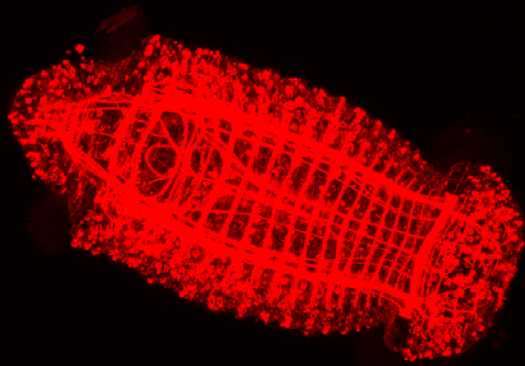
100 μm

α -Tubulin



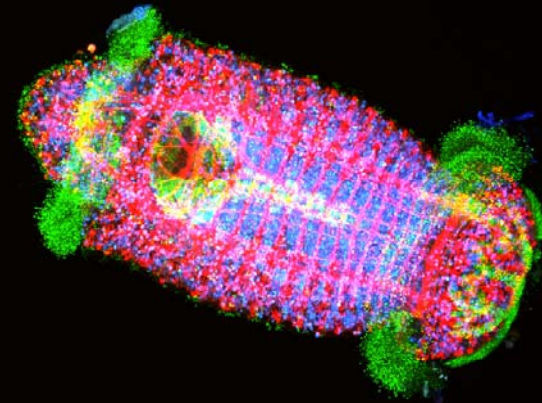
100 μm

Phalloidin



100 μm

All channels

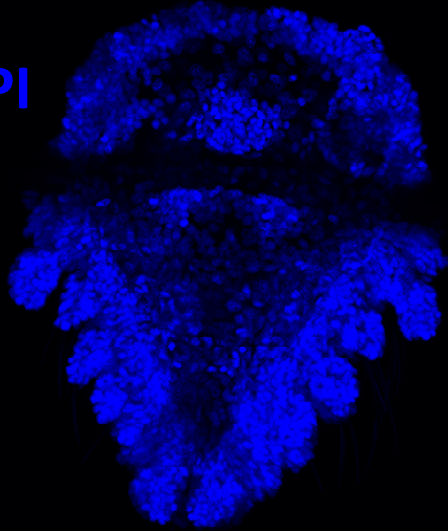


100 μm

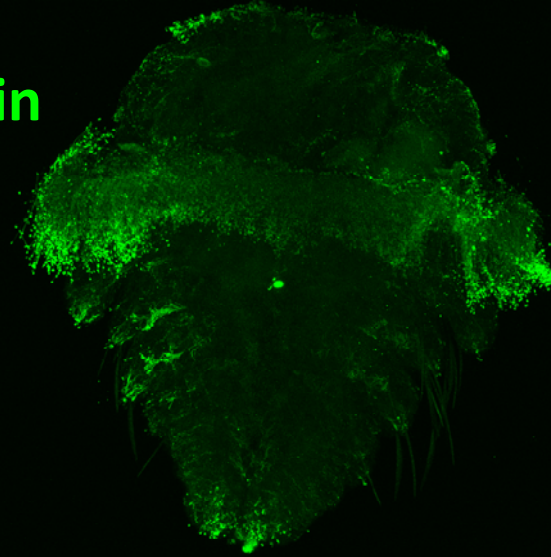
Credit to Abraham & Sophie

Phyllodocidae? metatrochophore

DAPI



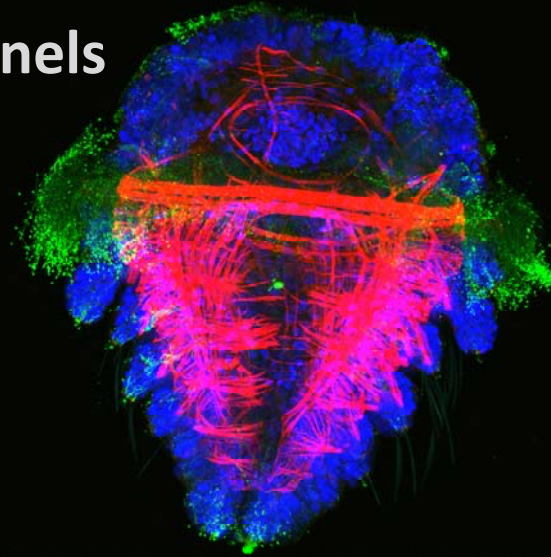
α -Tubulin



Phalloidin



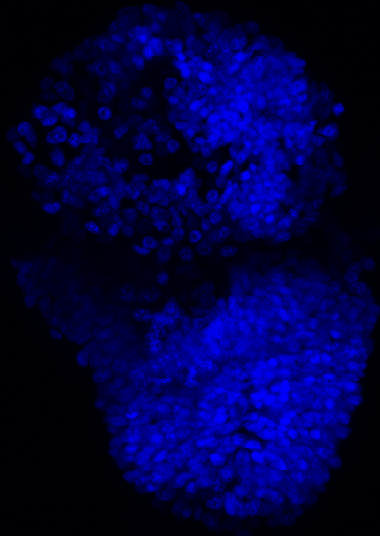
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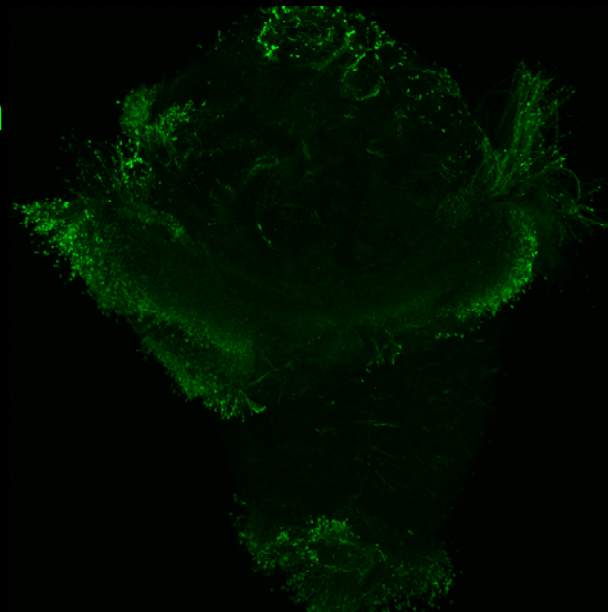
Credit to Abraham & Sophie

Pectinariidae/ Opheliidae ? trochophore

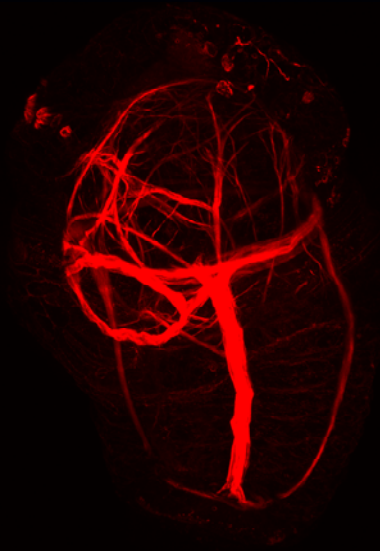
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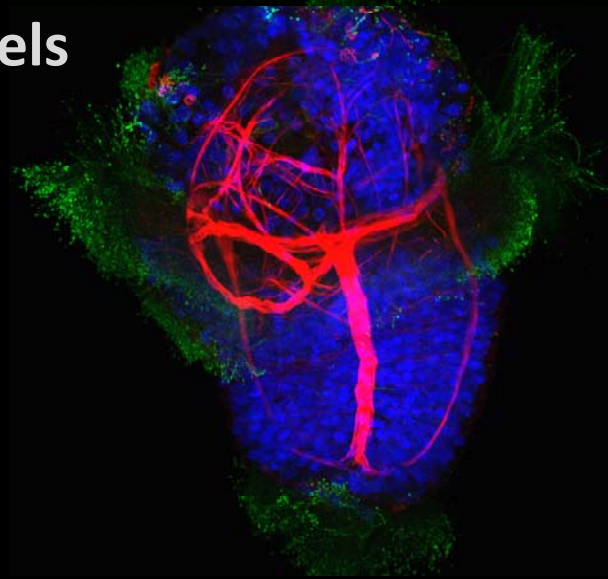
α -Tubulin



Phalloidin

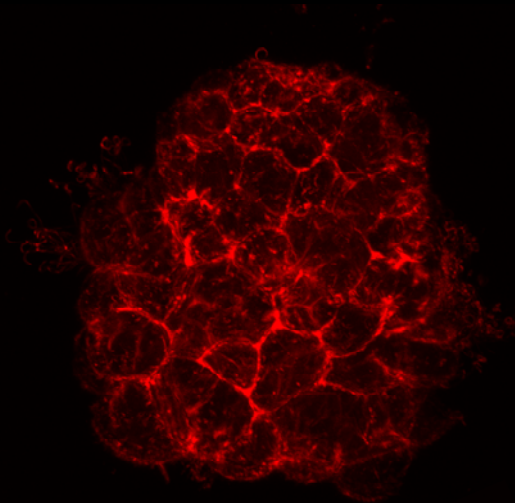


All channels

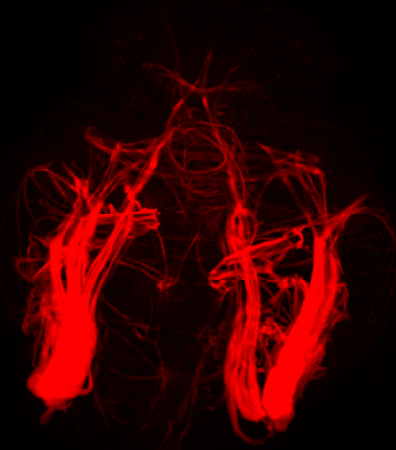


Credit to Abraham & Sophie

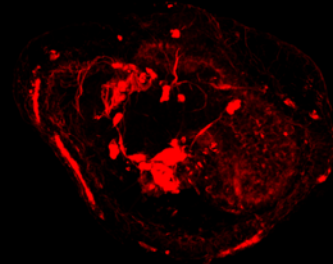
Phalloidin stainings



Ophelia

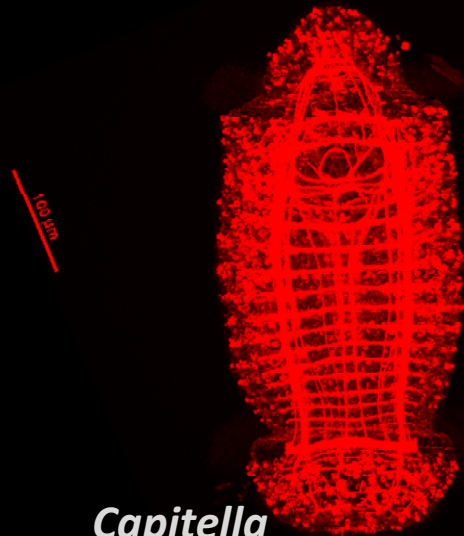


Spirobis



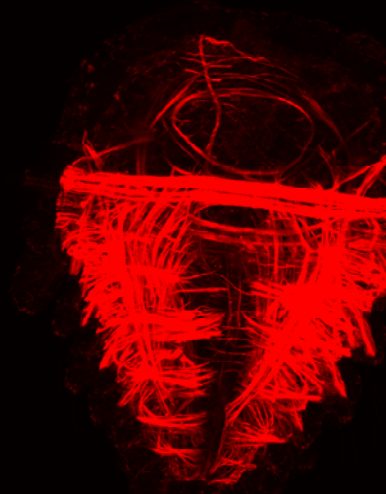
Oweniidae

100 μm

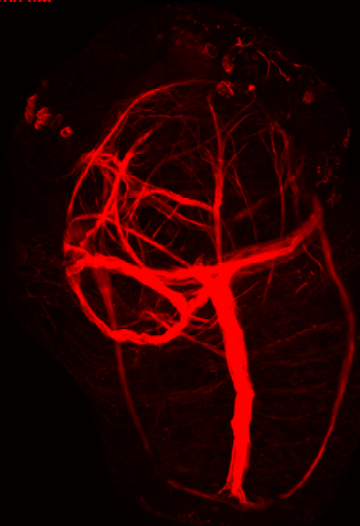


100 μm

Capitella



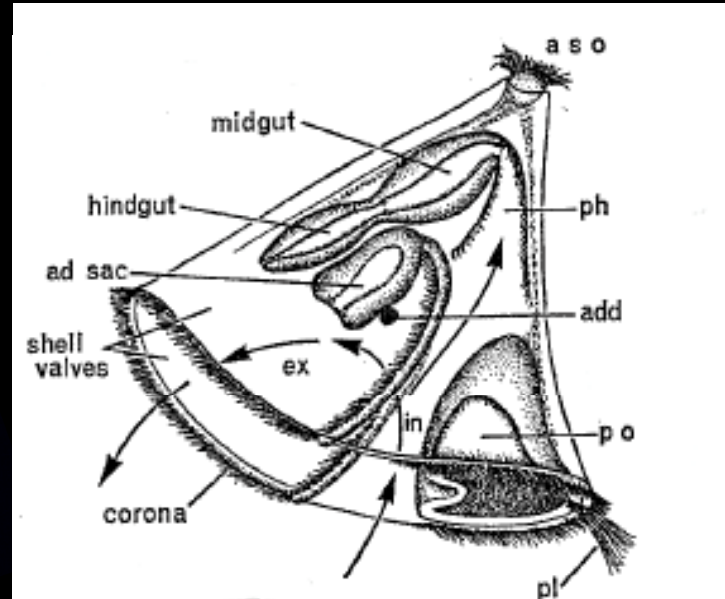
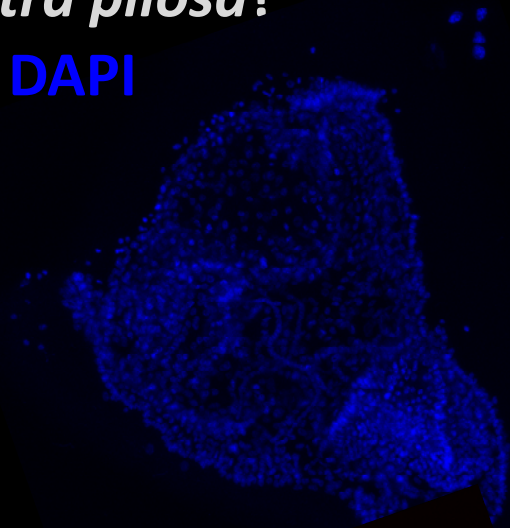
Phyllodocidae?



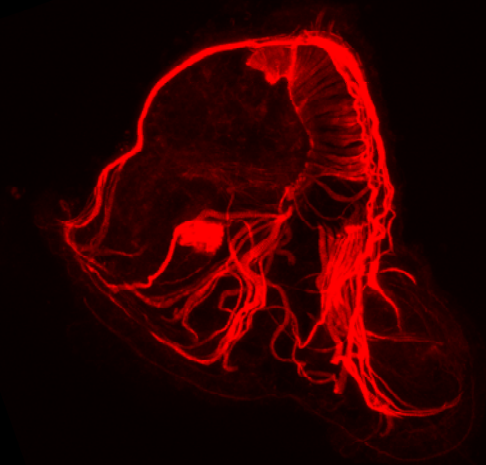
Pectinariidae/
Opheliidae?

Bryozoa (planktonic larva - cyphonaut),
Electra pilosa?

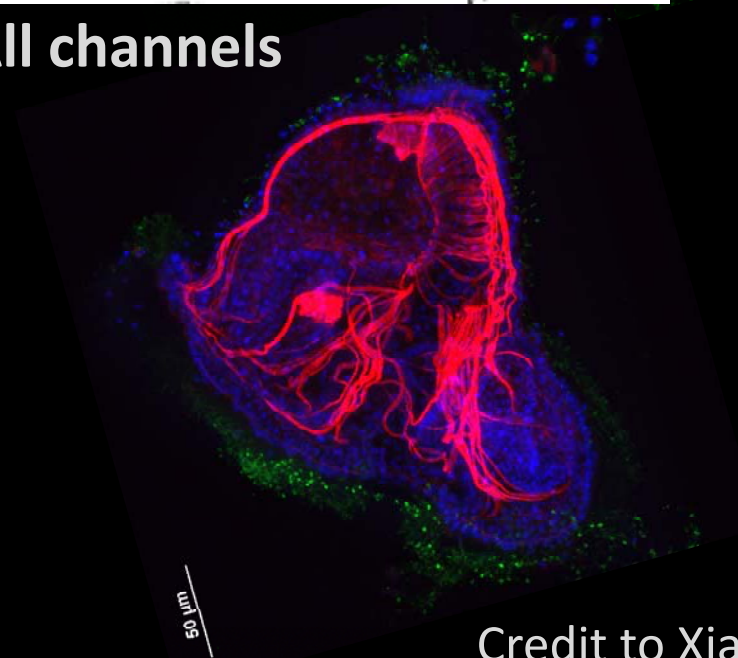
DAPI



Phalloidin



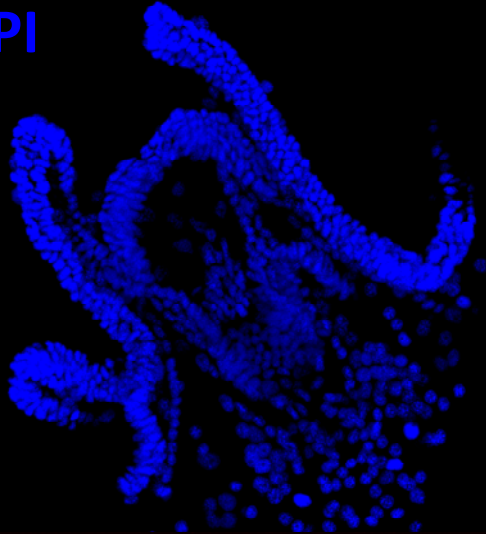
All channels



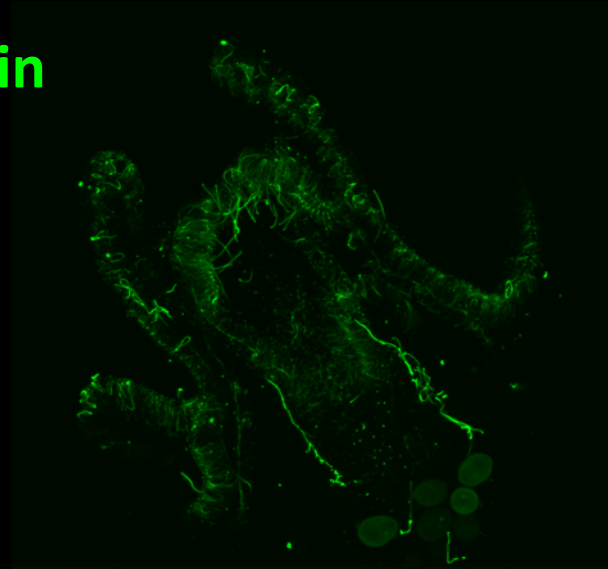
Credit to Xiaoya & Olga

Ophiopluteus, echinoderm larva

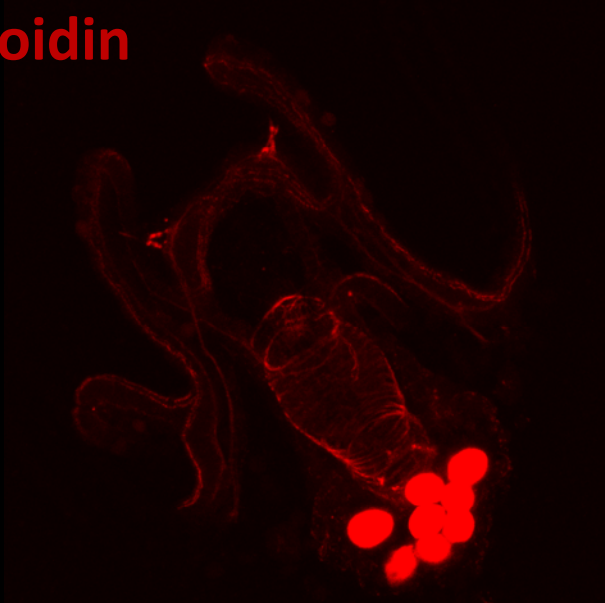
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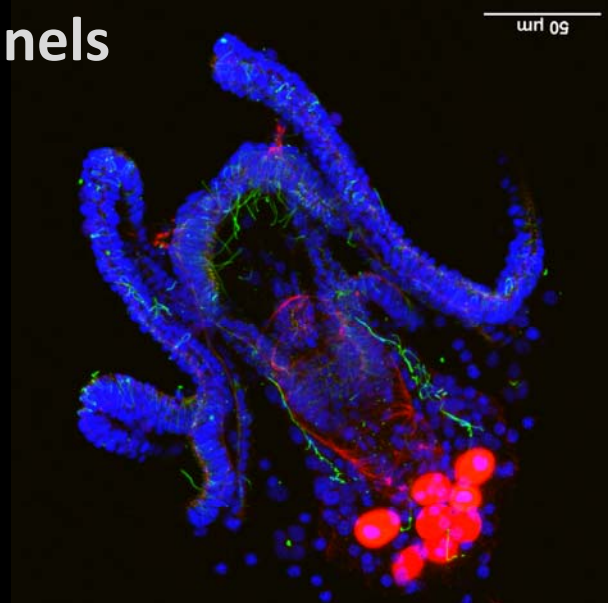
α -Tubulin



Phalloidin

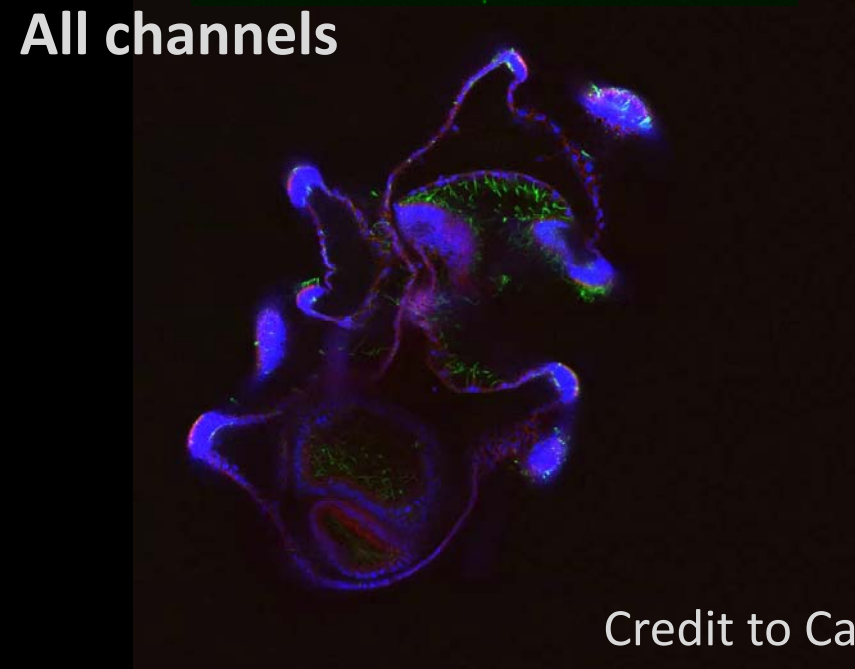
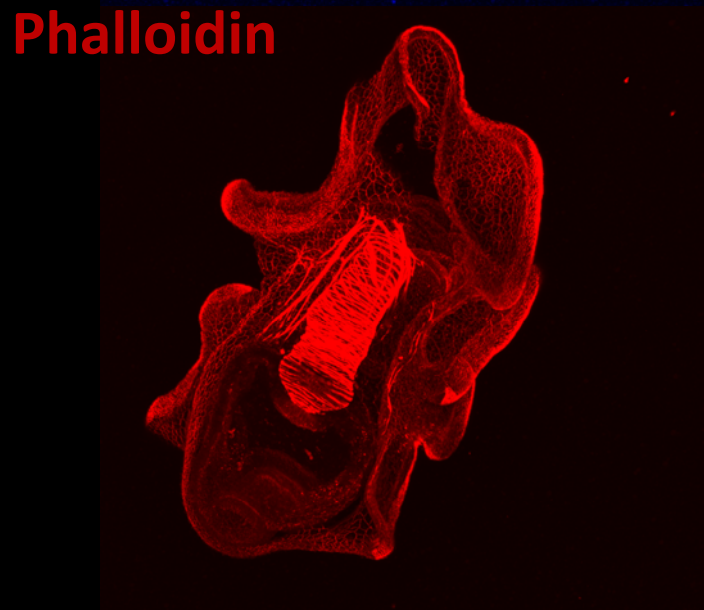
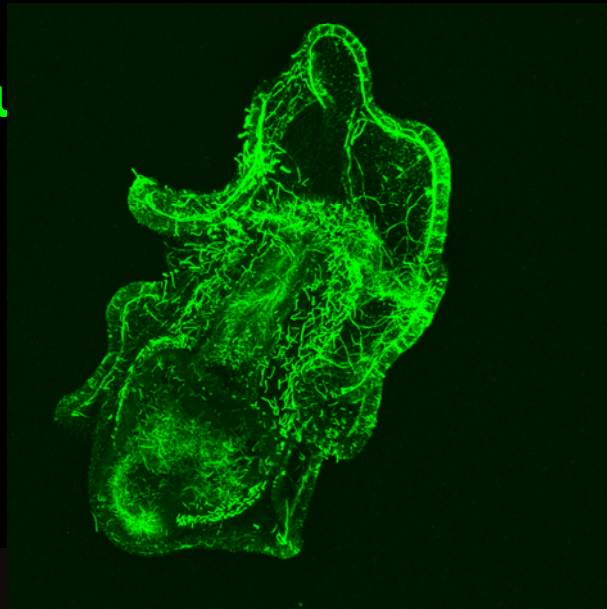
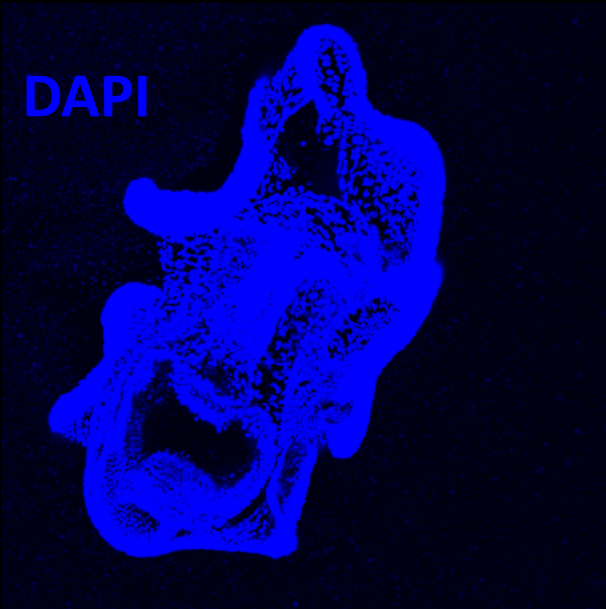


All channels



Credit to Carmen

Bipinnaria, echinoderm larva (*Asterias rubens*)



Credit to Carmen

Thank you!



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