PCMD Histology Core Learning Lunch Series

Whole mount staining and confocal imaging

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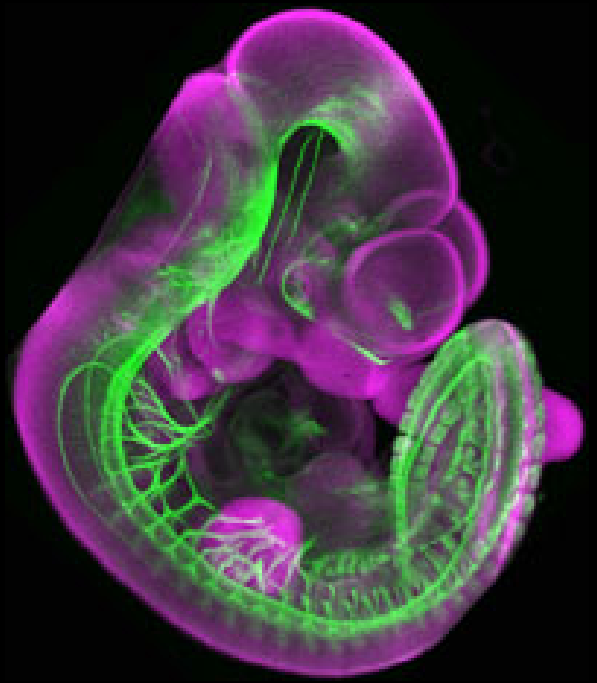
Outline

- Introduction
- Sample preparation
- Sample sectioning
- Whole mount staining
- Confocal imaging and analysis

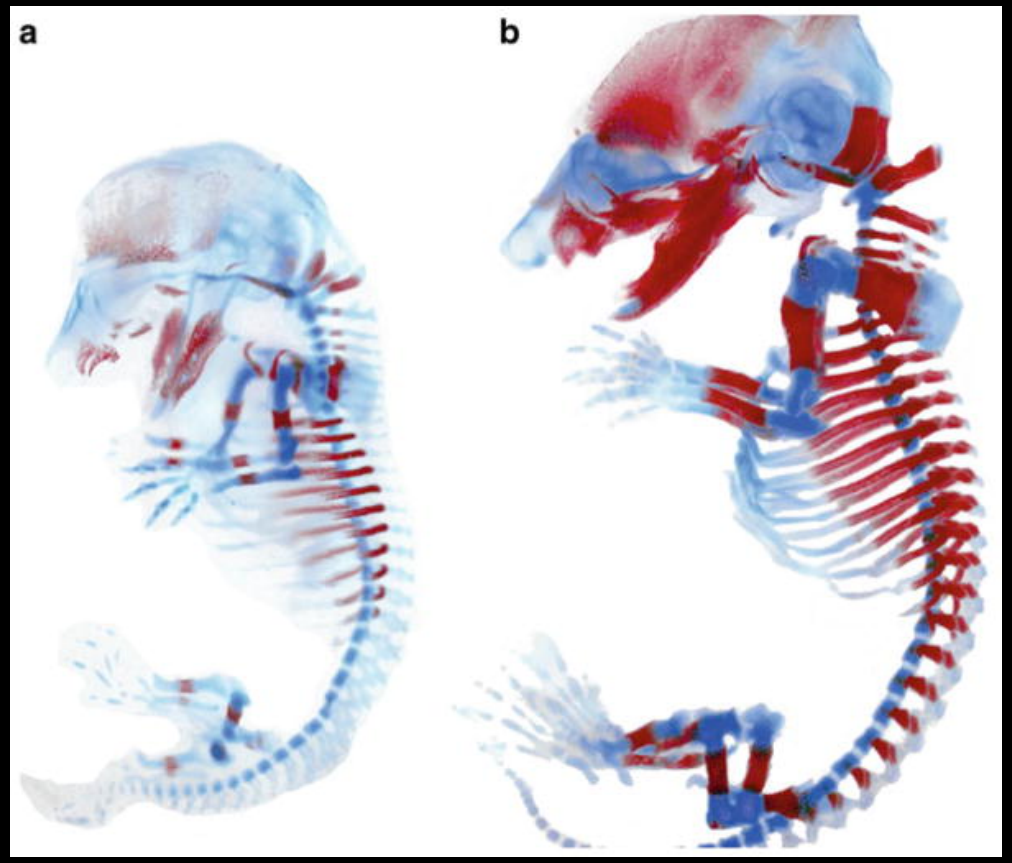
Whole mount staining

First introduced in 1897.

Whole mount staining is the staining of small pieces of tissue – usually embryos – without sectioning. Whole mount staining is very similar to immunocytochemistry (ICC) or staining of cryosections. The difference is that the sample being stained is much larger and thicker than a normal section on a slide.



Whole mount skeletal staining



Whole-Mount Skeletal Staining. Diana Rigueur and Karen M. Lyons. Methods Mol Biol. 2014.

The advantage of whole mount method

- Maintains tissue integrity as well as structure and cell localization.
- Provide high-definition fine three dimensional information about the location of proteins or cells.
- Especially useful for difficult-to detect rare cells that are localized within the deepest, most centrally located tissues of the whole organ, such as cells in bone marrow.
- Without serial sectioning, less tissue structure damage and better preservation of tissue morphology.
- Save time compared to serial sectioning.

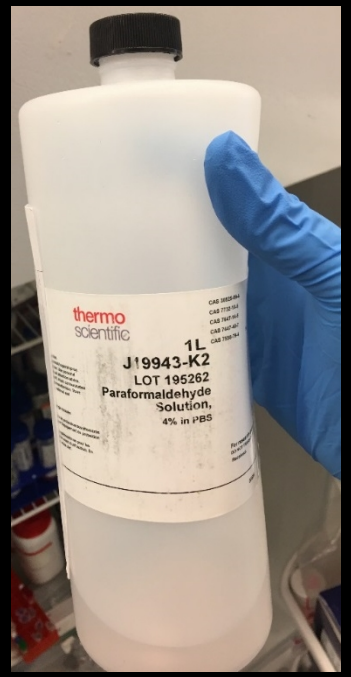
The disadvantage of whole mount method

- Need confocal microscopy to image sample
- Use confocal microscopy is expensive
- There is a depth limitation for antibody penetration (<150 μm), 300 μm for immunostaining.

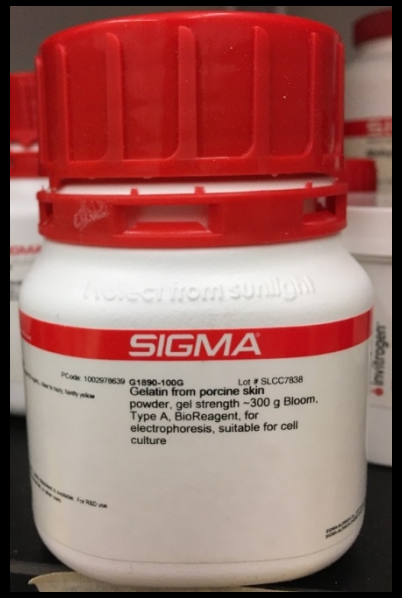
Sample preparation

Chemicals:

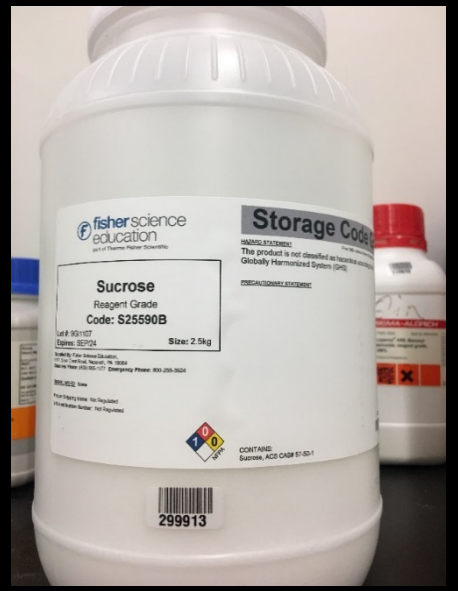
4% paraformaldehyde (PFA)



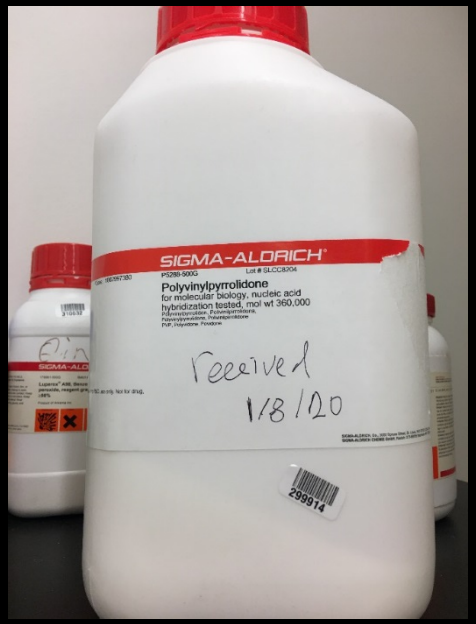
Gelatin



Sucrose



Polyvinylpyrrolidone (PVP)



Sample (soft tissue) preparation

Fixation: Freshly dissected soft tissues (fat, liver, spleen, brain, lung, heart, et al) are immediately fixed in ice-cold 4% PFA solution for 4-6 hr.

Dehydration: Fixed tissue are washed in PBS and then immersed into 20% sucrose and 2% PVP solution for 24 hr.

Embedding: The tissues are embedded in 8% gelatin (porcine) with 20% sucrose and 2% PVP.

Preparation time: 3 days

Sample (bone tissue) preparation

Fixation: Freshly dissected bone tissues are immediately fixed in ice-cold 4% PFA solution for 4-24 hr.

Decalcification: After fixation, wash sample in ddH_2O. Decalcification are carried out with 10% EDTA for 1 week at 4°C with constant shaking.

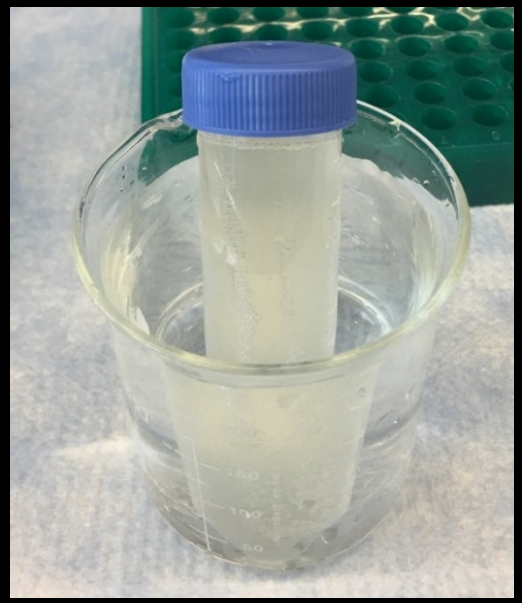
Dehydration: Decalcified bones are washed in PBS and then immersed into 20% sucrose and 2% PVP solution for 24 hr.

Embedding: Finally, the tissues are embedded in 8% gelatin (porcine) with 20% sucrose and 2% PVP.

Preparation time: 10 days

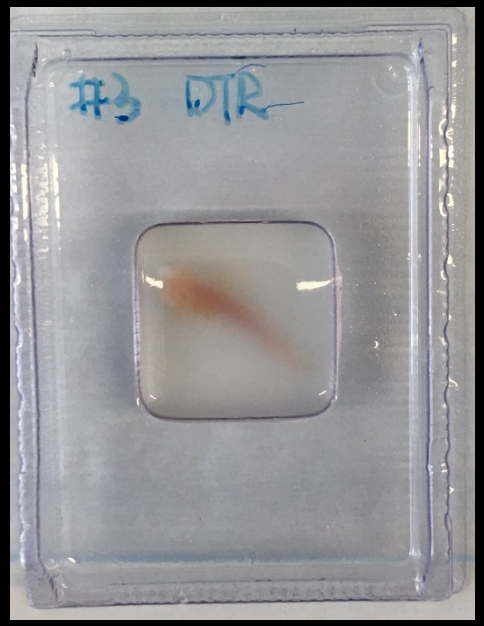
Tips for embedding

1. Pre-warm 8% gelatin embedding medium at 60°C in oven for 30 min.
2. Or microwave a cup of water to 60°C and place gelatin inside for 30 min.
3. Place the sample at the right direction in the base mold and pipette 1 ml embedding medium into the mold. Let sample solidify at RT for 10-30 min.
4. Store samples at -80°C.
5. Don’t keep un-sectioned block at -20°C because samples tend to shrink over time.



Embedding at RT for 10-30 min

10 min later



Sectioning

Leica Cryostate I in PCMD histology core

Superfrost Plus Slide

Section thickness: 30 µm to half bone

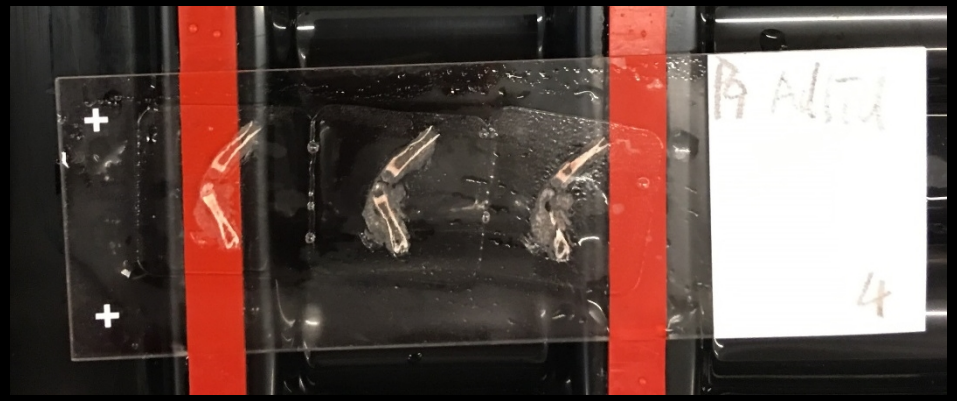
Section should be stored at -80℃

Staining

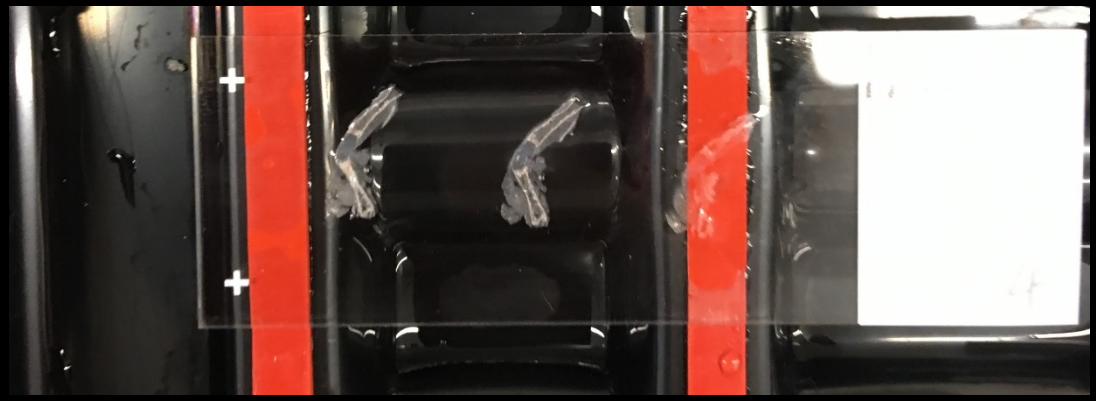
1. Air dry frozen section >20 min.
2. Rehydration: Heat PBS in a jar to 40°C.
3. Immerse slides in warmed PBS for 5-7 min.
4. Permeabilize for 30 min in 0.5% Triton X-100 (for intracellular protein staining).
5. Block in 3% BSA at RT for 30-60 min.
6. Probe with primary antibody in 3% BSA for O/N in cold room.
7. The second day, washed 3x in PBS, 10 min/time.
8. Probe with secondary antibody in 3% BSA for 1 hr at RT.
9. Rinse 3x in PBS, 10 min/time.
10. Mount slides with mounting medium containing DAPI.

Gelatin clearance before staining is important.

Before

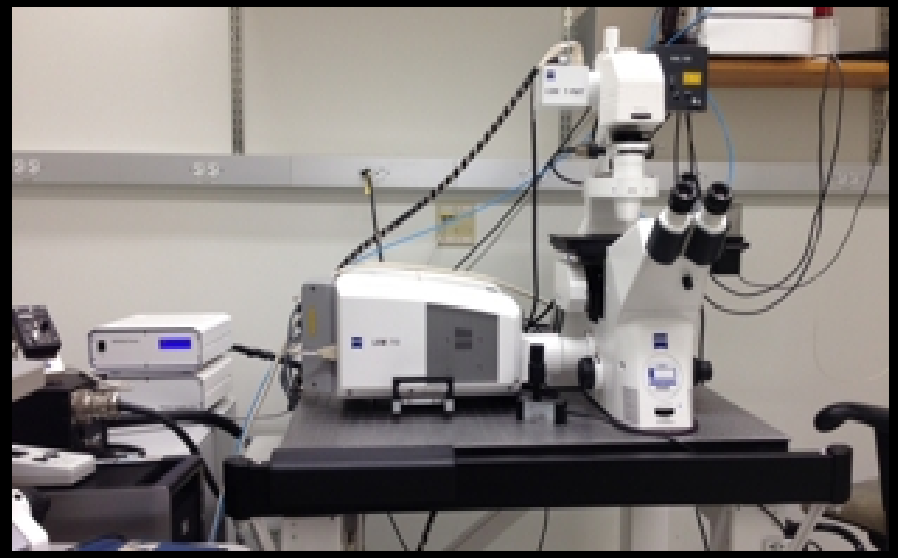


After



Imaging

Zeiss LSM 710 Confocal in CDB microscopy core



Excitation laser lines: 405, 458, 488, 514, 561, 594, 633 nm

Z-stack: by recording images at different focal planes the entire sample volume can be rendered and visualized

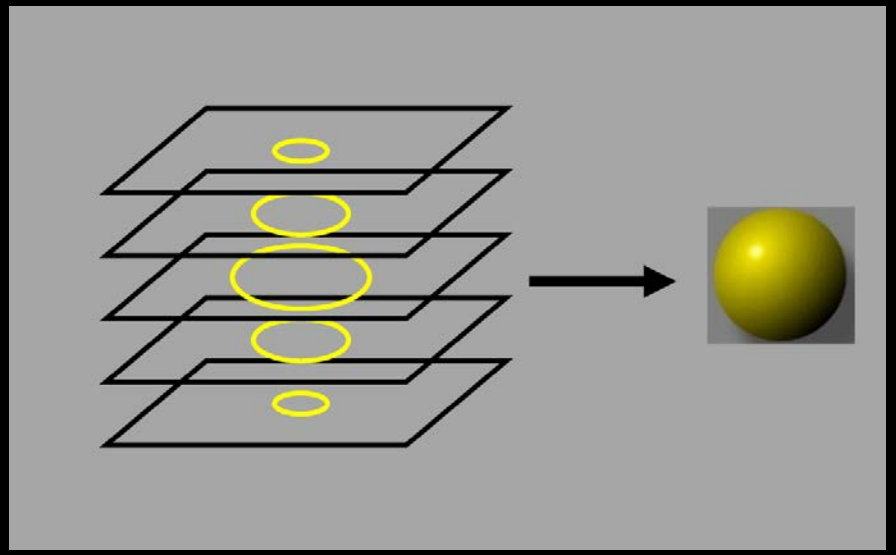


Image depth

Objective	Image depth
10x	4 mm
40x	200 µm
63x	200 µm

Image analysis

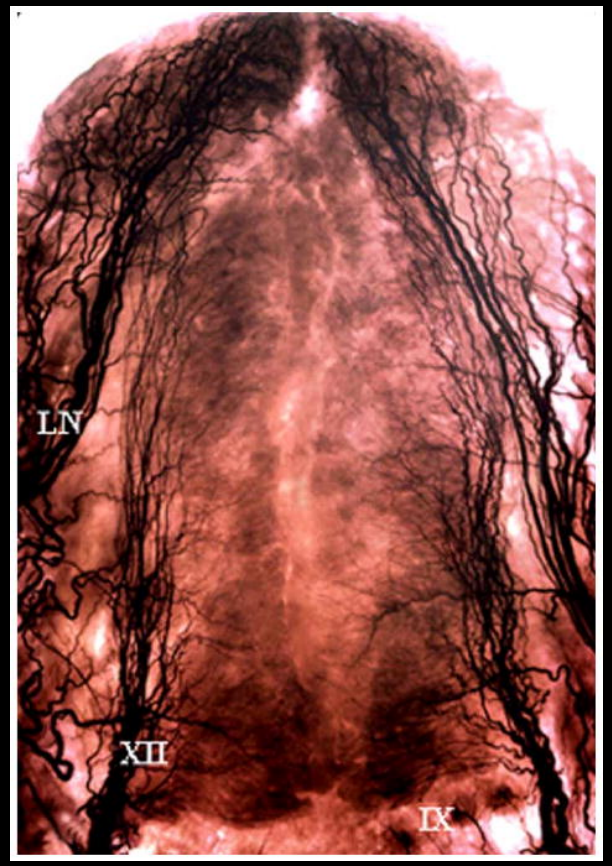
Use Imaris software in CDB core for creating 3D reconstruction and movies.

Image J

Image → Stacks → Z-project (Max intensity)

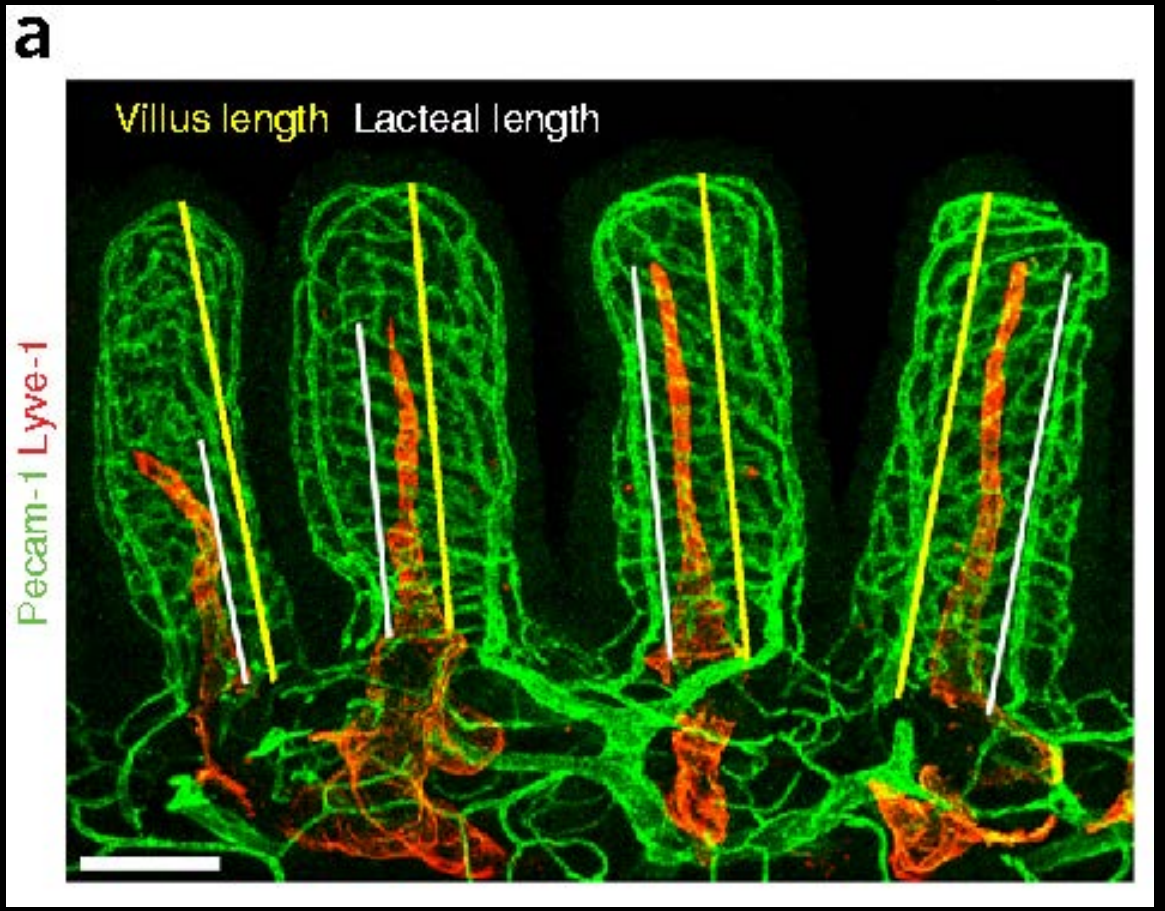
Whole mount staining on soft tissue

Adult human tongue (never staining)



Sihler's whole mount nerve staining technique: a review
L Mu and I Sanders. Biotech Histochem. 2010

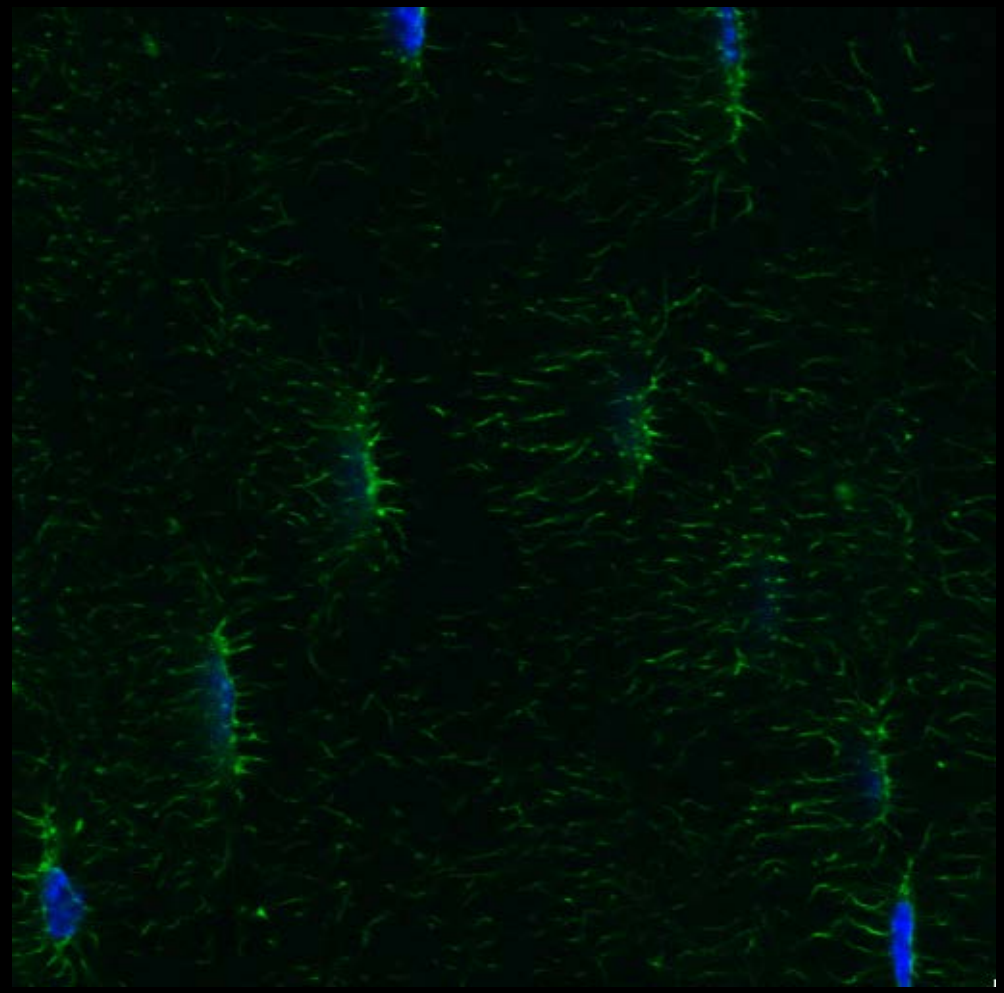
Intestinal whole-mount image



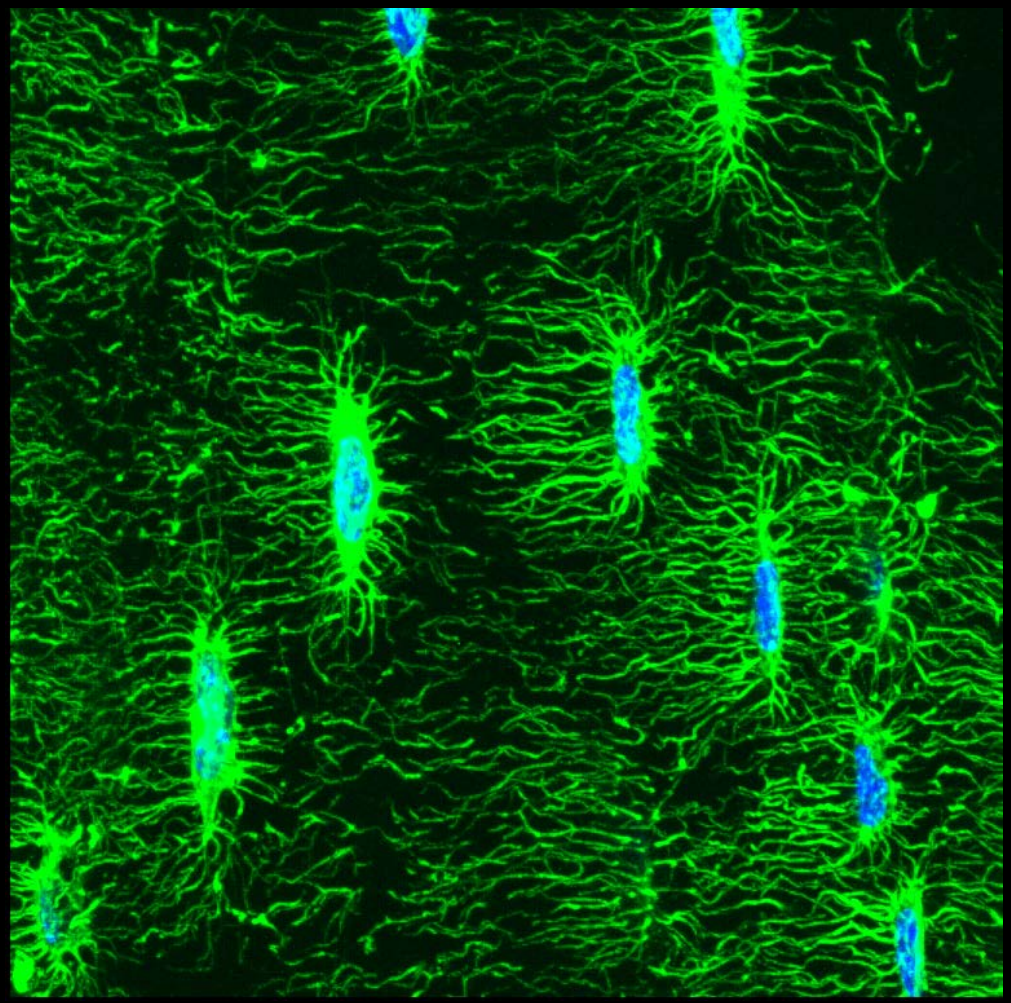
High-resolution 3D analysis of mouse small-intestinal stroma. Jeremiah Bernier-Latmani et al. Nat Protoc. 2016.

Whole mount staining on bone tissue

Single slice



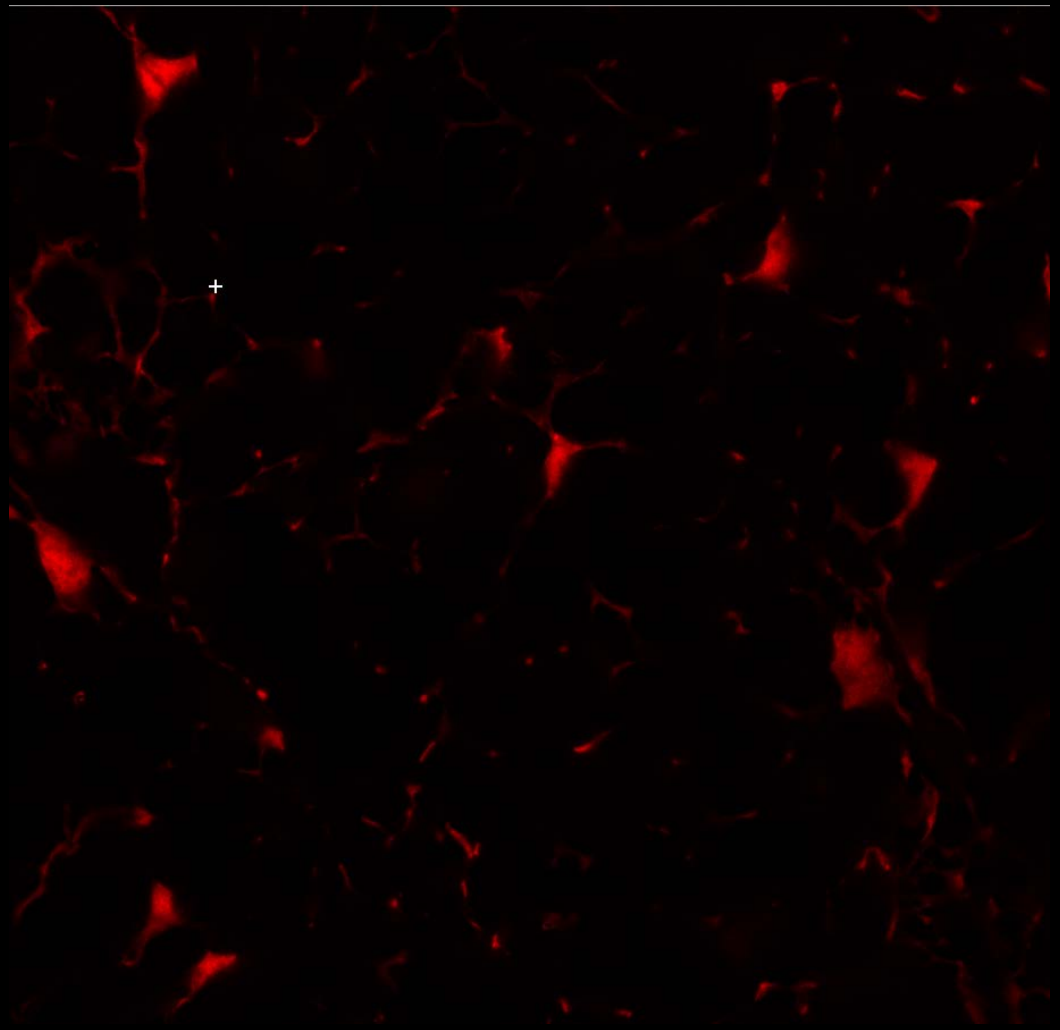
Z-stack

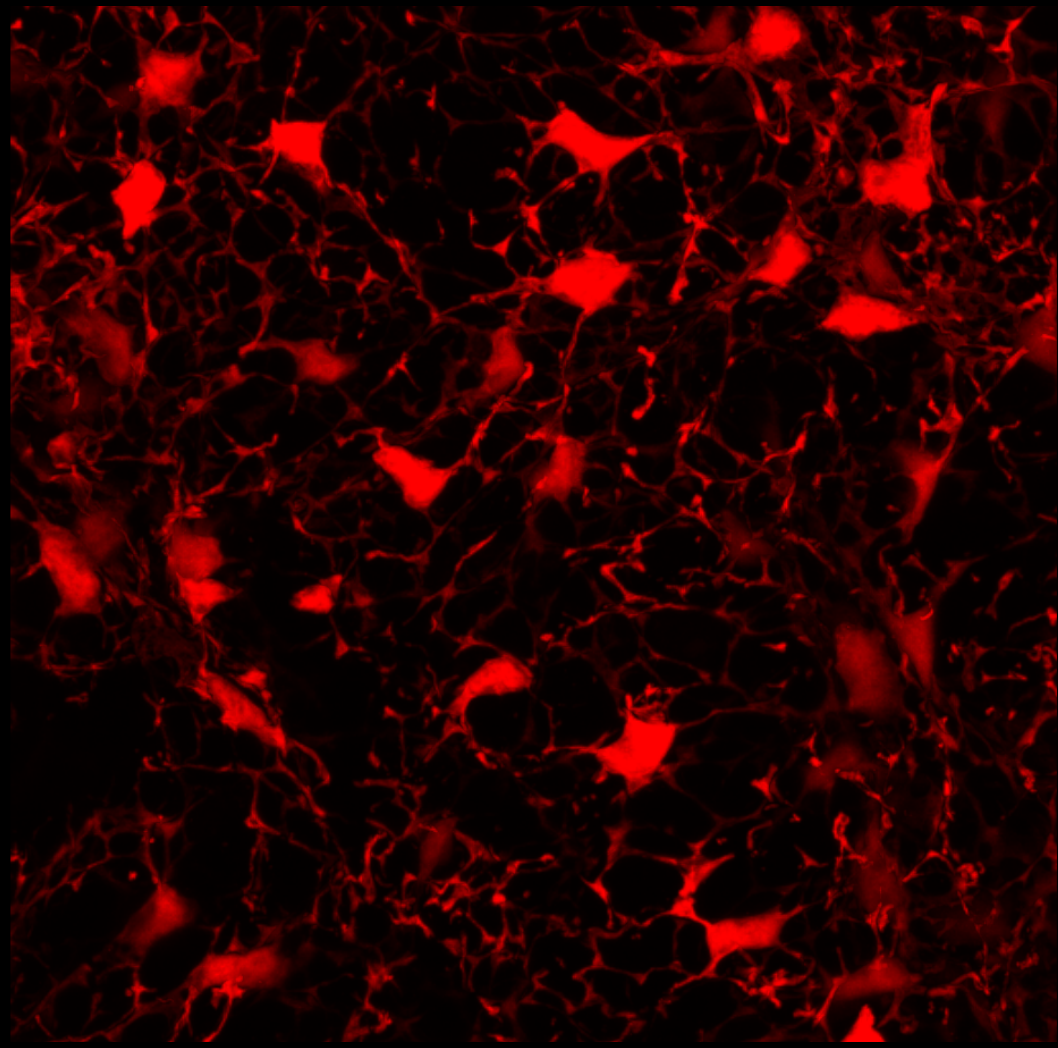


F-actin staining of osteocyte lacunae and canaliculi network

Single slice

Z-stack



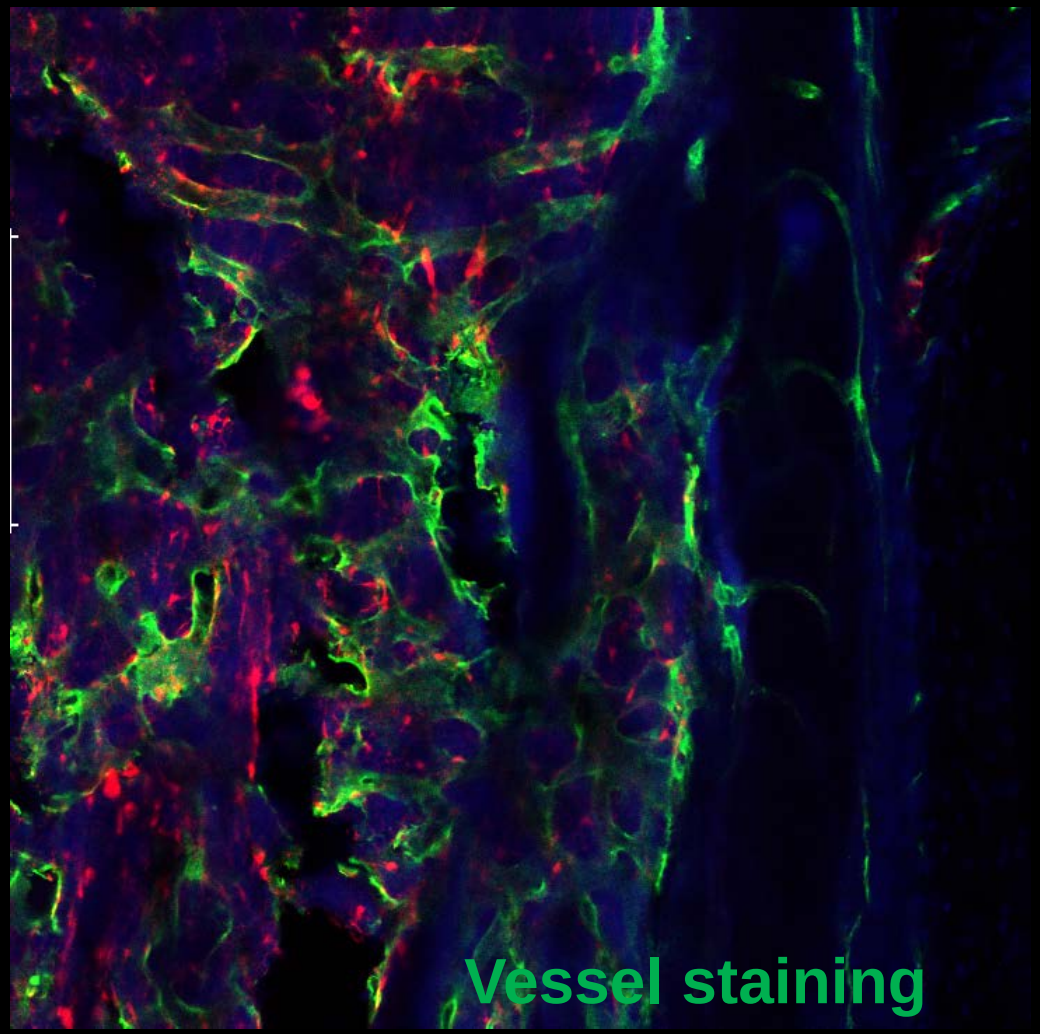


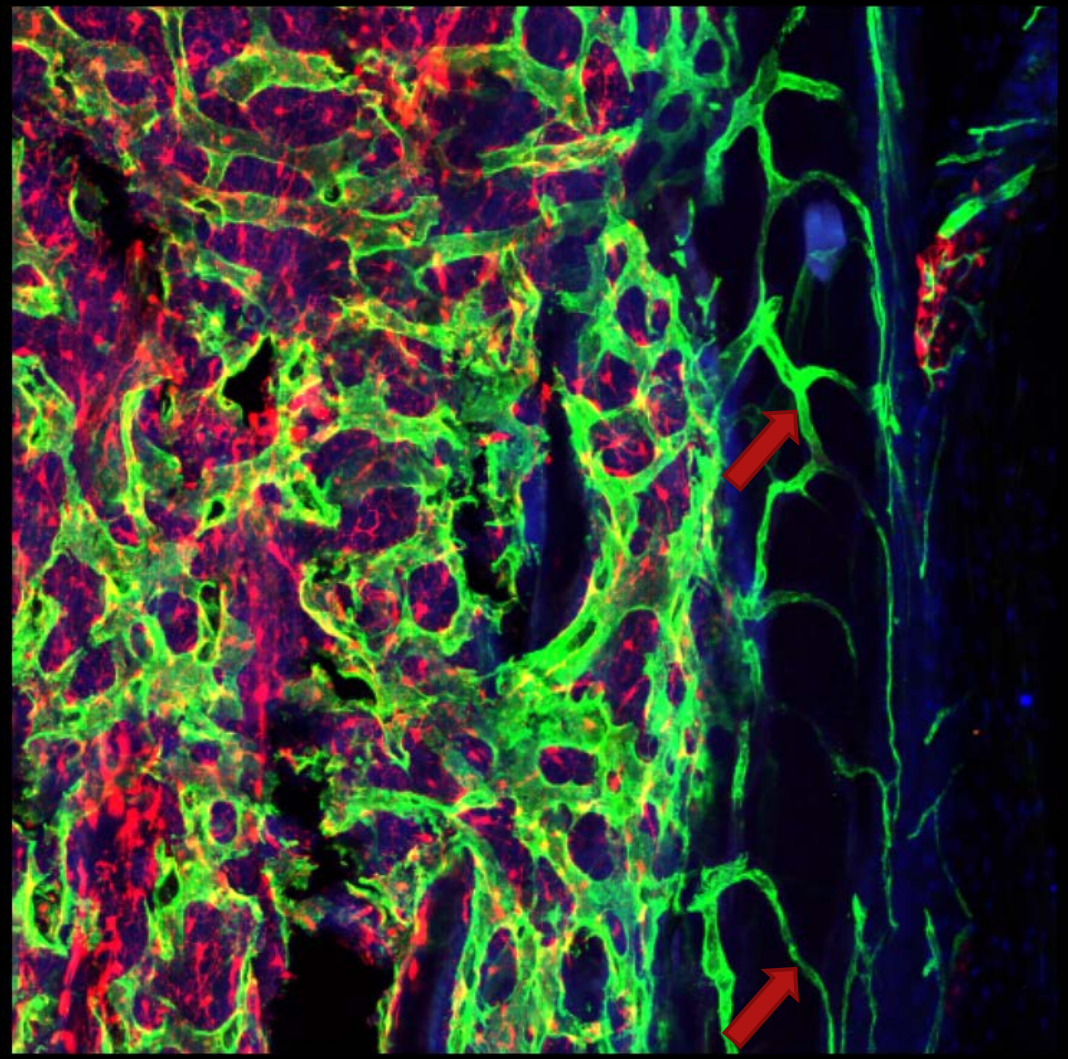
Cell processes from MERA

3D movie

Single slice

Z-stack





Trans-cortical vessel

Recommended Reading:

Whole-mount bone and cartilage staining of chick embryos with minimal decalcification. Yamazaki Y1 et al. Biotech Histochem. 2011 Oct;86(5):351-8.

Whole-mount three-dimensional imaging of internally localized immunostained cells within mouse embryos. Tomomasa Yokomizo et al. Nat Protoc. 2012 Feb 9; 7(3): 421–431.

Whole-Mount Skeletal Staining. Diana Rigueur et al. Methods Mol Biol. 2014; 1130: 113–121.