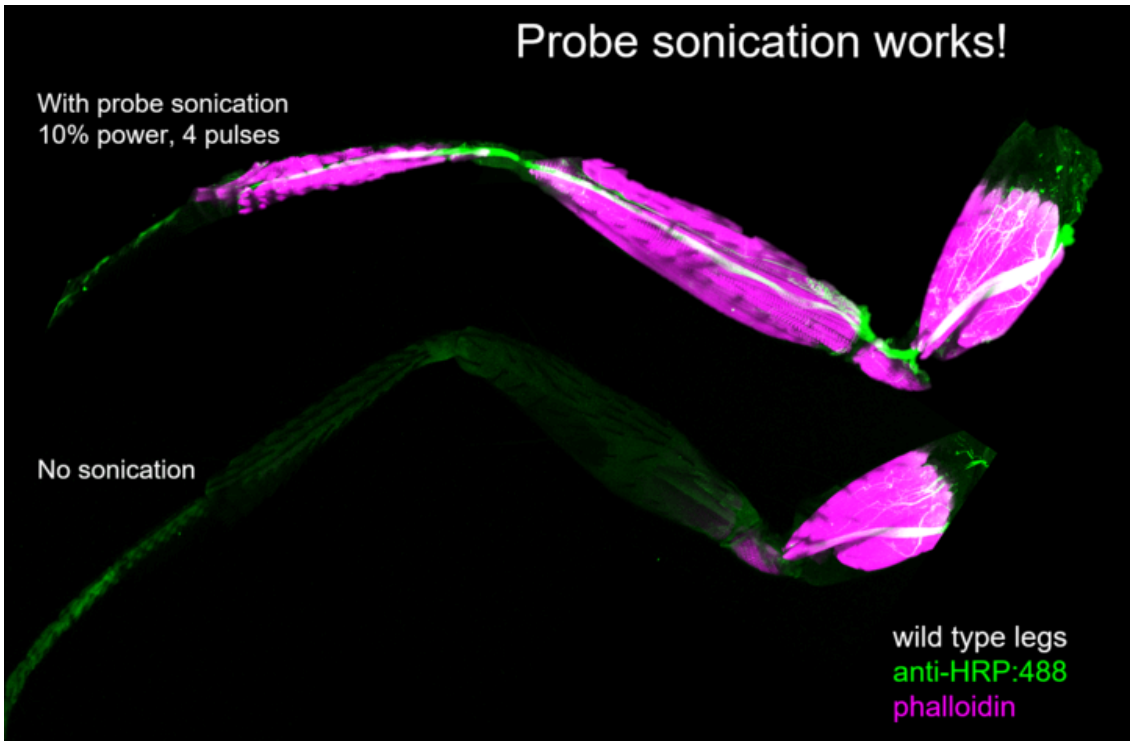
Fly Leg Phalloidin Staining Protocol with Sonication

June 2024 - Anne Sustar - Tuthill Lab

Note:
Sonication makes small tears in the cuticle and allows reagents to penetrate. Typically I work in batches of 20 legs per experiment, and I see a mix of results: about a third of the legs are over-damaged (thus bad morphology), a third are under-damaged (thus, bad staining penetration), and a third are just right.



Dissection and fixing

Dissect legs from an anesthetized fly.
Put legs in a 1.5 mL tube with fixative.
Nutate in fixative for 20 mins.

Fixative

390 µL	PBS
10 µL	PBS-Tx (PBS with 0.2% Triton-X)
48µL	38% aqueous formaldehyde (Macron 5016-02)

Wash

Wash with PBS-Tx (0.2% triton-X) 3x over 30-60 mins at RT, then 2x with PBS.
End with ~500mL volume of PBS.

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Probe sonication

For a *Branson Sonifier 450:*
- Set to 10% output control (higher power will shred the legs)
- Dip the probe into the tube. Assuming legs are all at bottom of the tube, the probe tip should be lowered to ~3mm above the legs.
- Do 4 short (~2 second each) pulses. Place tube on ice between the pulses
- Clean probe with ethanol.

Phalloidin Stain with extra penetrants

Make a big batch, freeze in 0.5mL aliquots **(based on a recipe form Igor Siwanowicz at Janelia)**

phalloidin stain			final concentraion
75	µL	triton X-100 (VWR 0694-1L)	1%
37.5	µL	DMSO (Sigma 154938)	0.5%
0.375	mg	Escin (Sigma E1378)	0.05 mg/mL
225	µL	normal goat serum (Sigma G9023)	3%
150	µL	Alexa Fluor™ 647 Phalloidin (Thermofisher A22287)	2%
1.5	µL	sodium azide (Sigma, S2002)	0.02%
7	mL	PBS	
7.5	mL	TOTAL	

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Incubate legs for ~ 3 days at room temperature in a small tube. Briefly vortex twice per day.

Clear and mount (FocusClear/ Focus Mount)

Wash 3x with PBS-Tx over one day, RT,
Wash 1x in PBS.

Remove PBS and replace with ~100 µL FocusClear (CelExplorer FC-101).
Incubate in FocusClear at RT for about 20-30 minutes.
Flick tube periodically to make sure legs are submerged (Legs tend to float in FocusClear)

Transfer leg pieces to a slide with a small drop of MountClear (CelExplorer MC-301).
Cover with size #0 coverslips with 3M Scotch doublestick tape (1 layer) as spacers.