bright field pheromone imaging

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Working

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ABSTRACT

For imaging aggregation behaviour of 40 young adult C. elegans on agar using the Phoenix multi-worm tracker system. Worms are synchronised by bleaching and refeeding for 72 hours, and then 40 young adult hermaphrodites are transferred by glass pipette onto a 35 mm regular NGM plate for imaging for 1 hour at 25 fps.

EXTERNAL LINK

https://www.biorxiv.org/content/early/2018/11/01/398370

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

SAFETY WARNINGS

BEFORE STARTING

Prior to collecting the full dataset, a single batch of OP50 was grown overnight, diluted to OD600 = 0.75, aliquoted for use on each imaging day, and stored at 4°C until use.

Imaging plate preparation (Day -7)

1 A separate batch of imaging plates is poured exactly seven days before each imaging day and stored at 4°C.

Imaging plates are 35 mm Petri dishes containing 3.5 mL low peptone (0.013% Difco Bacto) NGM agar (2% Bio/Agar, BioGene) to limit bacteria growth.

Bleach synchronising worms (Day -7 to -4)

2 Bleach synchronise gravid hermaphrodites. Leave on rotator at 20 °C until use.

Re-feed worms (Day -3, PM)

3 Re-feed starved L1 worms onto 3-4 plates using a glass pipette. Incubate at 20 °C.

Culture plates are 55 mm Petri dishes containing 15 mL low peptone 0.013% Difco Bacto) NGM agar (2% Bio/Agar, BioGene), and seeded with OP50.

Imaging plate preparation (Day -1)

4 Imaging plates are dried at 37°C overnight with the agar side down.

Seeding imaging plate (Day 0, AM)

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5 The center of an imaging plate is seeded with a single 20 µL spot of cold diluted OP50 (OD=0.75) one to three hours before imaging.

The overnight plate drying step allowed the bacteria to quickly dry atop the media in order to achieve a more uniform lawn by minimizing the “coffee ring” effect that would thicken the circular edge of the bacteria lawn.

Imaging (Day 0)

6 Wash animals off of culture plates with M9 and collect in a 15 mL Falcon tube, wash in M9 twice, and aspire as much supernatent as possible after the last wash.

7 Forty animals are transferred by a glass pipette onto the imaging plate in a small drop of M9, away from the bacteria lawn.

8 After M9 is absorbed into the media following worm transfer in liquid, imaging plates containing the animals are subjected to a gentle vibration at 600 rpm for 10 s on a Vortex Genie 2 shaker (Scientific Industries) to disburse animals and synchronize aggregation start across replicates.

9 Imaging commences 20 s after the vibration finish, on a custom-built six-camera rig (the Phoenix) equipped with Dalsa Genie cameras (G2-GM10-T2041). One-hour recordings with red illumination (630 nm LED illumination, CCS Inc.) are taken at 25 Hz using Gecko software (v2.0.3.1), whilst the rig maintain the imaging plates at 20 °C throughout the recording durations.

Image data processing

10 Images are segmented in real time by the Gecko software.

11 Automated animal tracking is performed post-acquisition using Tierpsy Tracker software.