bright field standard swarming imaging

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Working

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ABSTRACT

For imaging swarming 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 7 hours 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 Imaging commences immediately following animal transfer in a liquid drop, on a custom-built six-camera rig (the Phoenix) equipped with Dalsa Genie cameras (G2-GM10-T2041). Seven-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

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

10 The recordings are manually truncated post-acquisition to retain aggregation and swarming dynamics only.

The start time is defined as the moment when the liquid dries and the all the worms crawl out from the initial location of the drop, and the end time is when the food is depleted and worms disperse with increased crawling speed.