Preparation of fluorescent beads under *C. elegans* for fluorescence microscopy with adaptive optics

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Method Article

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Abstract

We describe a protocol for the preparation of a sample of fluorescent beads under *C. elegans*. This type of sample is useful for research into the use of adaptive optics to correct sample-induced optical aberrations in fluorescence microscopy.

Introduction

Adaptive optics can correct optical aberrations and restore the image quality in a variety of fluorescence microscopy methods[1]. By applying adaptive optics in fluorescence microscopy, we can significantly improve imaging capability and fidelity, especially when imaging through a thick multicellular samples[2]. In research into combining fluorescence microscopy with adaptive optics, a standard sample (e.g., fluorescent beads with the diameter smaller than the diffraction limit) is needed as a measure to evaluate the effect of aberration corrections, as well as the imaging capability of the imaging system. A suitable sample is fluorescent beads under a *C. elegans* adult hermaphrodite, where the *C. elegans* tissues induce optical aberrations and the fluorescent beads are the imaging metric. Here we provide a detailed description of preparing a sample of fluorescent beads under *C. elegans* to be used to evaluate the performance of the imaging system combining super-resolution fluorescence microscopy with adaptive optics.

Reagents

1. 100-nm fluorescent beads (T7279, Life Technologies, TetraSpeck™ Microspheres, 0.1 µm, fluorescent blue/green/orange/dark red)
2. Tetramisole hydrochloride (T1512, Sigma-Aldrich)
3. Glycerol (CAS # 56-81-5, Sigma-Aldrich)
4. Wild type adult *C. elegans* growing on agar

Equipment

1. Charged slides (9951APLUS-006, Aqua ColorFrost Plus, Erie Scientific)
2. Cover slips (Gold Seal 22 mm × 22 mm, #1.5)
3. Nail polish (Sally Hansen's Hard as Nails or similar)
4. 1.5 mL Centrifuge tubes (Eppendorf 022364111)
5. Hot plate (VWR VMS-C7)

6. Vortex Mixer (VWR 10153-838)

7. Manual Pipettes (VWR 89079-964, 89079-970, 89079-974): 2 – 20 µL, 20 – 200 µL, and 100 – 1000 µL

8. Pipette tips (VWR 1041-960-306 and 1041-800-300): 200 µL and 1000 µL.

Procedure

1. Dilute the original 100-nm fluorescent bead solution by a factor of 100 (v/v) with distilled water in an Eppendorf tube. Mix with the Vortex Mixer..

2. Drop 10 µL of the diluted bead solution on a charged slide and let it dry for 10 minutes on a hot plate set to 35° C.

3. Place a wild-type *C. elegans* adult hermaphrodite on a clean coverslip with 10 µL of a 50 mM solution of phosphatase inhibitor Tetramisole hydrochloride to inhibit worm movement[3].

4. Before the Tetramisole solution dries completely, drop 10 µL of glycerol on the slide prepared in step 2.

5. Mount the coverslip prepared in step 3 onto the slide prepared in step 1 quickly and fix it carefully using nail polish.

Troubleshooting

1. If the fluorescent beads are not attached onto the slide surface and floating in the glycerol:

Let the fluorescent beads dry for a longer time on the charged slide.

2. If the worm body is not intact, use more glycerol when mounting the coverslip, and then use a Kim-wipe to wick away the excess solution.

3. If the concentration of beads is too dense when imaging, dilute the bead solution by an additional 10x.

Time Taken

The time taken to prepare the sample of fluorescent beads under the *C. elegans* is typically about 15 min. The timing probably depends on the humidity in the lab, which may affect the time needed for the solutions to dry.

Anticipated Results

You should see a layer of fluorescent beads laying under the *C. elegans* in the fluorescence microscope. The image of the fluorescent beads below the *C. elegans* should be distorted due to the optical aberrations caused by the worm body.

References

1. Ji N. Adaptive optical fluorescence microscopy. *Nature Methods* **14**, 374 (2017).

2. Thomas B., Wolstenholme A., Chaudhari S. N., Kipreos E. T., Kner P. Enhanced resolution through thick tissue with structured illumination and adaptive optics. *J Biomed Opt* **20**, 026006 (2015).

3. Dong L*., et al.* Reversible and long-term immobilization in a hydrogel-microbead matrix for high-resolution imaging of Caenorhabditis elegans and other small organisms. *PLOS ONE* **13**, e0193989 (2018).