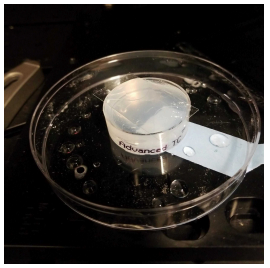
Jun 13, 2025 Version 2

Thick 3D Tetraspeck Beads sample preparation V.2



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Protocol status: Working
We use this protocol and it's working

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Abstract

Create a thick sample of agar and fluorescent beads in a well plate, to use for microscopy troubleshooting (e.g. to determine the point spread function of your system).

Materials

- **Tetraspeck beads, 0.5 um, ThermoFisher Scientific cat. no: T7281. TetraSpeck™ Microspheres, 0.5 µm, fluorescent blue/green/orange/dark red**
- **Agarose HOEFER | SKU: SKU:GR140-25**
- **P1000 and P200 micropipettes + tips**
- **vortexer (any)**
- **rice cooker or microwave**
- **Thermometer**
- **precision scale**
- **15 mL falcon tubes**
- **glass bottom well plate, e.g. CLS-1811 CLS-1811 - GLASS BOTTOM CULTURE DISHES, TC TREATED, NEST-Chemglass Life Sciences**

Prep 1% [w/v] Agarose solution

1 Heat DPBS to approximately 100 °C, using a rice cooker, pressure cooker, or microwave

use a thermometer to verify temperature

2 Measure out 104 mg agar to 10.4 mL hot DPBS and mix vigorously with vortexer. If using a larger well plate, you can adjust these amounts accordingly. Verify concentration made using this website **Percent (%) Solutions Calculator - PhysiologyWeb** .

Mix in the beads

3 Let Agar solution cool to 40-50 °C

Meanwhile, vortex the stock tetraspeck vial

Equipment	
Vortex mixer	NAME
Any	BRAND
xx	SKU

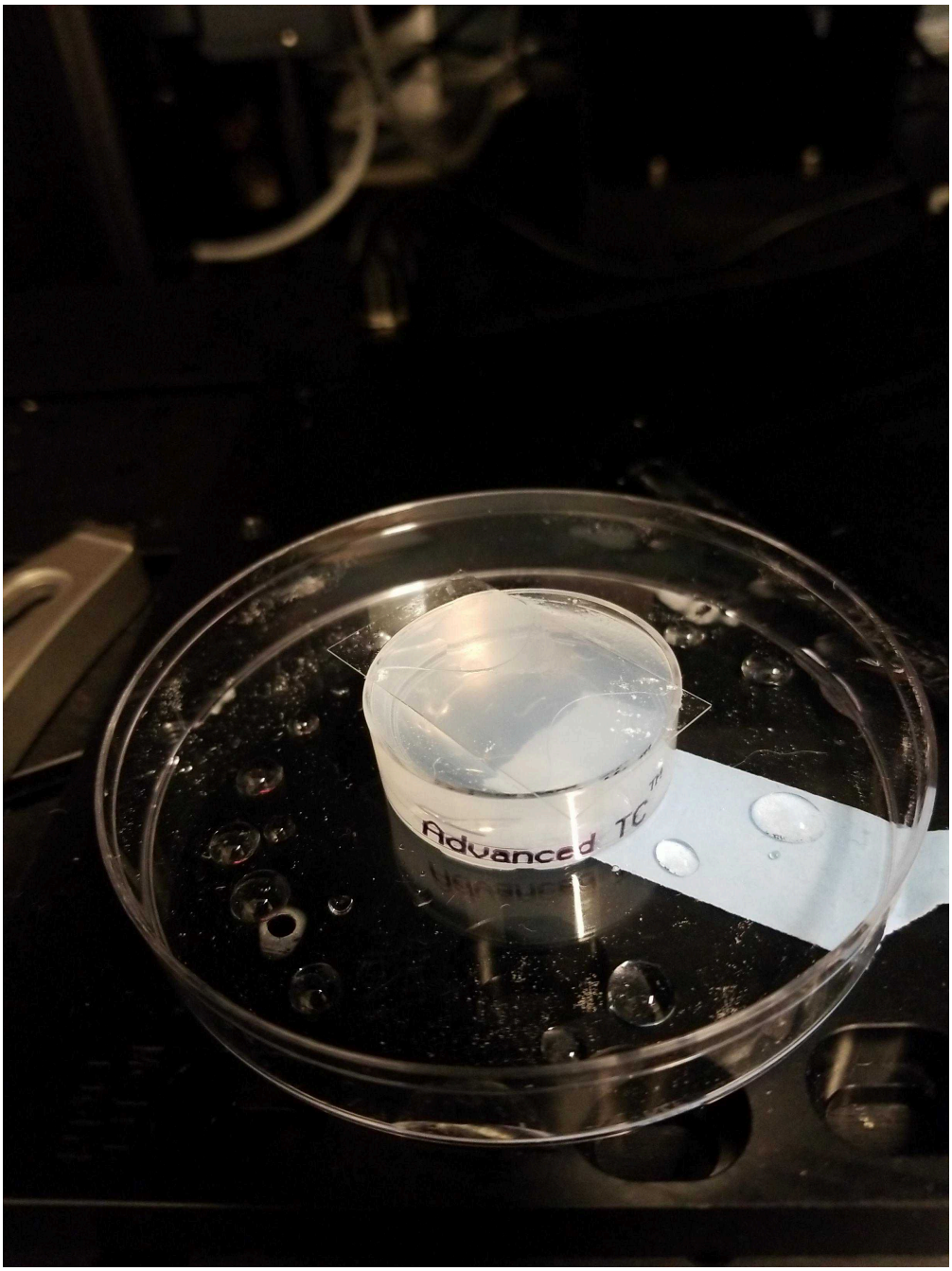
3.1 When DPBS-Agar mix has cooled to 40-50 °C, use a P200 micropipette to add 60 µL of the stock beads to the 10 mL of hot Agar-DPBS. For a sparser population of beads, add 30 microliters instead.

Vortex the resulting mixture vigorously.

Well plate

4 Use a simple transfer pipette to transfer the beads-agarose solution to a glassbottom well plate. Fill the plate to the very top, avoiding the formation of bubbles. Before completely filling the plate, tap the bottom of the plate gently to release any bubbles.

5 Carefully place a coverslip on top of the well plate, and let the agarose cool and harden. Shield the sample from light.



Agarose-Beads suspension in a thick well plate, with a glass coverslip on top. This is then stored in a larger well plate dish.

> Store the sample in a larger well plate or similar box to keep the glass from being knocked off. Add tape.

Protocol references

Method adapted from **GitHub - amsikking/fluorescent_beads_in_agarose: How to make a 3D sample of beads for PSF measurements with ~water refractive index (1.33)**

Above was adapted from:
Yiming Li, Yu-Le Wu, Philipp Hoess, Markus Mund, and Jonas Ries, "Depth-dependent PSF calibration and aberration correction for 3D single-molecule localization," Biomed. Opt. Express **10**, 2708-2718 (2019)

Acknowledgements

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