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Pollen Preparation for a Callibration Standard V.1

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We use this protocol and it's working

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Abstract

This process is meant to establish three different sample variants based on the media encapsulating the pollen grains. This is used to compare the limitations and capabilities of the mAO-MPFM system.

Attachments

PDF

CyGEL-protocol-bookl...

666KB

Guidelines

Further options and troubleshooting for CyGEL can be found in the attached document. Steps for the CyGEL preparation and mounting were adapted from the guide attached.
abcam. (2023). CyGEL Protocol.

Materials

Materials:

1. Fresh pollen or flowers/grass to gather pollen, various types (Greenhouse Laboratory, The University of Kansas), stored at room temperature in a sealed container
2. Single Cavity microscope slides (Globe Scientific Inc., Item: 1341)
3. Plain microscope slides (Fisher Scientific, Cat. No.: 125493, Lot: 23326)
4. Microscope cover glass (Fisher Scientific, Cat. No.: J2-548, thickness: 1, size: 22 mm circle)
5. Microscope cover glass (Fisher Scientific, Cat. No: 12541B, Lot: 20850, thickness: 1.5, size: 22×22)
6. Agarose, low-gelling temperature (Sigma, Cat. No: A9414-10G, Lot: SLCCG4128), stored at room temperature
7. Vectashield Plus (Vector Labs, Cat. No: H-1900, Lot: ZJ0909), stored at 4°C
8. Vectashield HardSet (Vector Labs, Cat. No: H-1400, Lot: ZE0214), stored at 4°C
9. CyGel kit (abcam, Cat.No: ab109204), stored at 4°C
10. Kimwipe
11. Ice pack, with flat surface
12. Deionized water

Equipment:

1. Laboratory flammable refrigerator and freezer (Fisher Scientific, Model: 10FCEEFSA catalog number: 13986111A,)
2. Certified Biological Safety Cabinet (BSC) Class II Type A2 (Labconco, Logic series, catalog number: 343000110815)
3. 0.5-10 µL, 20-200 µL pippettes (Midsci, Alphα Pette)
4. 0.1-10 µL pipette tips (Fisher Scientific, Fisherbrand, catalog number: 21-277-2A)
5. 200 µL pipette tips (Fisher Scientific, Fisherbrand, catalog number: 02-707-500)
6. 200 µL large orifice tips (USA Scientific, Item Number: 1011-8000)
7. 1.5 mL microcentrifuge tube (Fisher Scientific, Fisherbrand, catalog number: 05-408129)
8. Dissecting microscope with light
9. Tweezers
10. Paint brush
11. Toothpick, cotton swab, or small wooden dowel
12. Clear nail polish
13. Disposable Pasteur pipette
14. mAO-MPFM imaging system (3i/Zeiss Upright Two-Photon, The University of Kansas at Lawrence)

Safety warnings

Temperature is crucial for handling CyGEL, move swiftly and carefully once the CyGEL is activated.

2D Preparation: Vectashield Plus and HardSet

20m

1 Remove one plain slide or one single cavity slide from the slide box.

1.1 Use the plain slide with the HardSet Vectashield or the single cavity slide with the softset Vectashield Plus.

2 Clean plain slide or cavity slide with 70% ethanol.

3 Allow slide to dry and place on kimwipe.

4 Remove one square cover glass for the plain slide or one circular glass cover for the single cavity slide.

4.1 Use square glass with the plain slide and circular glass with the cavity slide .

5 Clean square or circular cover glass with 70% ethanol.

6 Allow cover glass to dry and place on kimwipe.

7

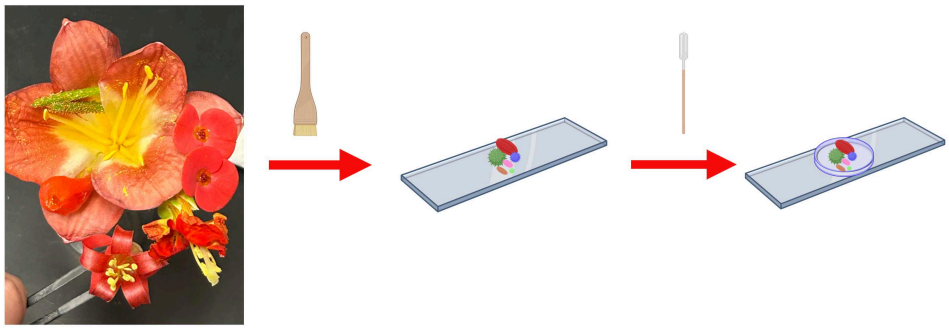

Figure 1. Preparation of slide using pollen and Vectashield (created with BioRender)

Under the dissecting microscope use a paint brush to dust pollen from the flower/grass (source) onto the slide.

7.1 Keep the pollen in the center of the cavity or center of the slide.

7.2 Use the paint brush to push the pollen towards the center if needed.

8 If pollen sticks to the paint brush, use a toothpick or a small wooden dowel (like the end of a cotton swab) to remove the pollen and place it on the slide.

9 Once enough pollen has been deposited (should be visible to naked eye), 150-200 µL of Vectashield can be slowly dropped onto the slide.

Note

Use the large orifice tips for this step.

10 Use tweezers to place the cover glass on top of the Vectashield and pollen.

10.1 For the HardSet wait ~10-15 minutes for it to set. 15m

10.2 For the Plus, wait ~5 minutes before sealing. 5m

11 Gently paint the edges of the cover glass with clear nail polish to seal the slide.

3D Preparation: 2% Agarose

12 Note: work quickly with the agarose solution. Once it is made, it sets quickly while working with pollen.

13 Remove one single cavity slide from the slide box.

14 Clean the slide with 70% ethanol.

15 Allow slide to dry and place on kimwipe.

16 Remove one cover glass (circle) from the box.

17 Clean cover glass with 70% ethanol.

18 Allow cover glass to dry and place on kimwipe.

19 2% Agarose preparation should begin only after sample layout (see 20 and Note below) has been chosen.

19.1 In a 1.5 mL microcentrifuge tube add 1 mL of deionized water.

19.2 Weigh 0.02 g of agarose.

19.3 Add the agarose to the deionized water and mix well.

20 The following note describes the order of the procedures to be used according to which of three different layouts (pollen underneath Agarose, pollen layered in Agarose, pollen on top of Agarose) is to be used as well as when the Agarose should be made for each layout.

Note

Step 19 provides directions for preparing Agarose.
Steps 21 and 22 can be interchanged, depending on the sample layout to be used.

If the pollen is to be placed at the bottom of the agarose, use procedures in the following order: 21,19, 22.

If the pollen is to be used for a one layer sample, use procedures in the following order: 19, 21, 22. If the pollen is to be used for a two layer sample, use 19 once, repeat 21 and 22 twice.

If the pollen is to be placed on the top of the agarose, use procedures in the following order: 19, 22, 21

21 Steps to layer the pollen under the agarose are as follows:

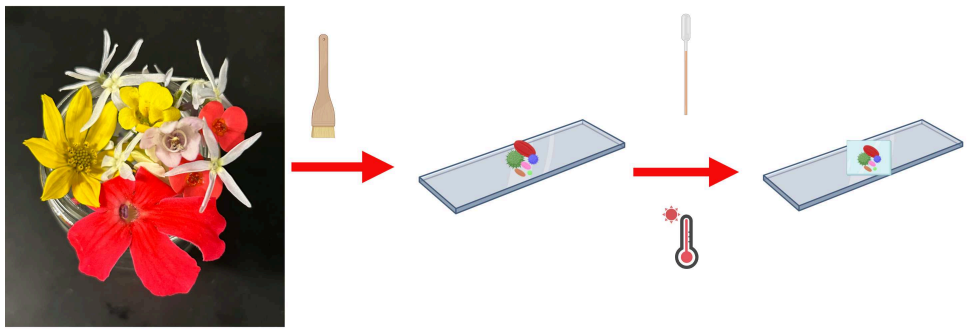

Figure 2. Preparation of slide using pollen and 2% agarose (created with BioRender).

Under the dissecting microscope, use a paint brush to dust pollen from the flower/grass (source) onto the slide.

21.1 Keep the pollen in the center of the cavity.

21.2 Use the paint brush to push the pollen towards the cavity if needed.

22 Using a disposable Pasteur pipette, add enough agarose to cover the pollen.

23 Using tweezers, place a cover glass on top of the sample.

23.1 The cover glass will keep dust off the agarose, maintain its integrity, and assist in shaping it.

3D Preparation: CyGEL

13m

24 Remove a flat surfaced ice pack from -20°C storage.

25 Remove one single cavity slide from the slide box.

26 Clean the slide with 70% ethanol.

27 Allow slide to dry and place on kimwipe.

28 Remove one cover glass (circle) from the box.

29 Clean cover glass with 70% ethanol.

30 Allow cover glass to dry and place on kimwipe.

31 Once the slide is dry, place this on top of the flat ice pack.

31.1 Keep on ice pack until the CyGEL has been activated (mixed with buffer).

32 Remove CyGel kit from 4°C storage.

32.1 Remove one CyGel vial and the 40X PBS vial.

32.2 Return remaining vials to 4°C storage.

33 In the biohood, remove 12.8 µL of 40x PBS and add it to the CyGEL vial.

33.1 Add slowly to avoid creating bubbles.

33.2 Slowly mix with pippette to avoid creating bubbles (2-3 times).

33.3 Close both the PBS and now activated CyGEL.

34 Remove both vials from the hood.

35 Return both vials to 4°C storage.

35.1 Returning the activated CyGEL vial to storage will remove any bubbles created from the mixing of the buffer and CyGEL.

> Note
>
> This is not necessary if the slide is already prepared with the sample, or the sample is being placed on top of the set gel.

36 Remove the slide from the ice pack and use a kimwipe to remove condensation from the back of the slide.

36.1 Wipe down the sample-side (top) of the slide, except for a square around the cavity.

37 Set the cooled slide underneath the dissecting microscope.

38

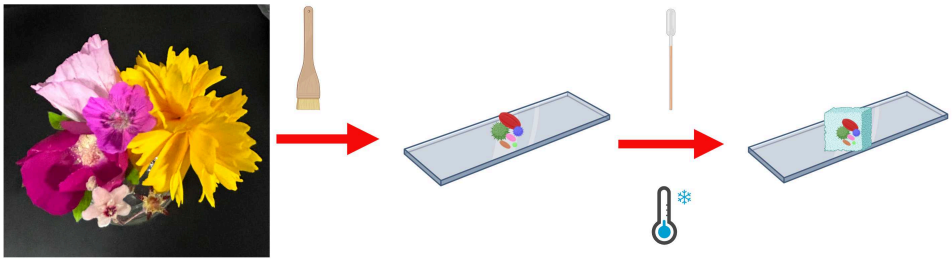

Figure 3. Preparation of slide using pollen and cooled GyGEL, created with Biorender.

Using a paint brush, dust pollen from the flower/grass (source) onto the slide, while observing the transfer of pollen.

38.1 Aim to keep the pollen in the center of the cavity.

38.2 Use the paint brush to push the pollen towards the cavity if needed.

39 If pollen is sticking to the paint brush, use a toothpick or a small wooden dowel (like the end of a cotton swab) to remove the pollen and place it on the slide.

40 Once enough pollen has been gathered (should be visible to naked eye), the CyGEL can be removed from storage.

41 Under the dissecting microscope, slowly drop 150 µL of the activated CyGEL onto the pollen filled slide. 3m

42 Allow the CyGEL to softly set (~1 minute) and add the coverslip using tweezers under the dissecting microscope.

42.1 Can also allow the gel to completely set before adding coverslip if not intending to reuse the slide for multiple sessions.

43 The slide should be completely set in ~10 minutes. 10m

44 For protection and ease of reuse, the edges of the coverslip can be painted with clear nail polish to limit dust on exposed gel and keep the coverslip in place.

Protocol references

Simar, L. & Rosa-Molinar, E. (2025). "Benchmarking Assessment and Implementation of an Imaging Phantom in Nonlinear Optical Microscopy." Unpublished manuscript, *Current Protocols*.

Acknowledgements

The University of Kansas
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