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2. Sample Preparation - An Easy-to-Prepare Sample Slide

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Abstract

This protocol describes how to create a high dynamic range fluorescent sample using basic office and microscopy lab tools. The protocol is based on the usage of the ink of a fluorescent text marker to create a thin fluorescent layer on a microscope cover glass (Olevsko et al., 2021). The result is a carrier with a thin, homogeneous fluorescent film with abrupt edges and a thickness in the order of a few micrometers. This type of sample can be used to make images with a smooth gradient going from maximum intensity to background, as described in the protocols for determination of a detector's photon conversion factor.

Guidelines

Please refer to the introduction protocol for a more detailed description of a detection system, its parameters, and various aims for performance monitoring.

This protocol is part of a collection of protocols developed by QUAREP-LiMi WG2 for characterizing light microscopy detection system performance.

Materials

Yellow fluorescent marker (for instance: Stabilo Boss Original, Art.No70/24, Heroldsberg, Germany)

Note

The marker is a critical component. Different brands have different formulas for the ink, resulting in different layer characteristics after drying of the ink. The Stabilo marker resulted in a 5 µm thin and homogeneous coverage of the glass surface without cracks and granules. Yellow, for excitation within the range of 488 nm.

- Borosilicate microscope cover glass (e.g., 18 x 18 mm^2, thickness #1.5, Eur. Cat. No. 631-0121, Avantor delivered by VWR, Radnor, Pennsylvania, United States)
- Standard microscope slides (e.g., ground edges frosted, Eur. Cat. No. 631-1553, Avantor delivered by VWR or Microscope slides,CC frosted, Epredia)
- Picodent Twinsil22 (Picodent twinsil®, picodent, Dental Produktions- und Vertriebs GmbH) or Polysil 3481 silicone (Polysil Ltd), for sealing.
- Gloves (vinyl lab gloves)
- EtOH 70% for cleaning microscope cover glass
- Lens paper for cleaning microscope cover glass (e.g., Whatman® lens cleaning tissue, Grade 105, Merck or Linsenpapier, Assistent)
- Permanent marker pen or pencil to write on the microscope slide
- Nail polish

Safety warnings

Ensure you follow general lab safety guidelines for radiation sources and chemicals as outlined within your organisation.

Before start

Collect **all** the materials (see list in Materials tab) and put on the gloves (to avoid contamination of the sample).

Sample preparation

1 Fold a sheet of lens paper, put a microscope cover glass in between the folded sheet, wet with ethanol (EtOH, see Materials), and rub to remove contaminations and oily substances.

Note

In case EtOH is not enough to create a clean surface, rub the glass between the fingers with detergent and rinse it with tap water, before cleaning with EtOH again.

Wear gloves when cleaning the coverglass with ethanol and lens paper

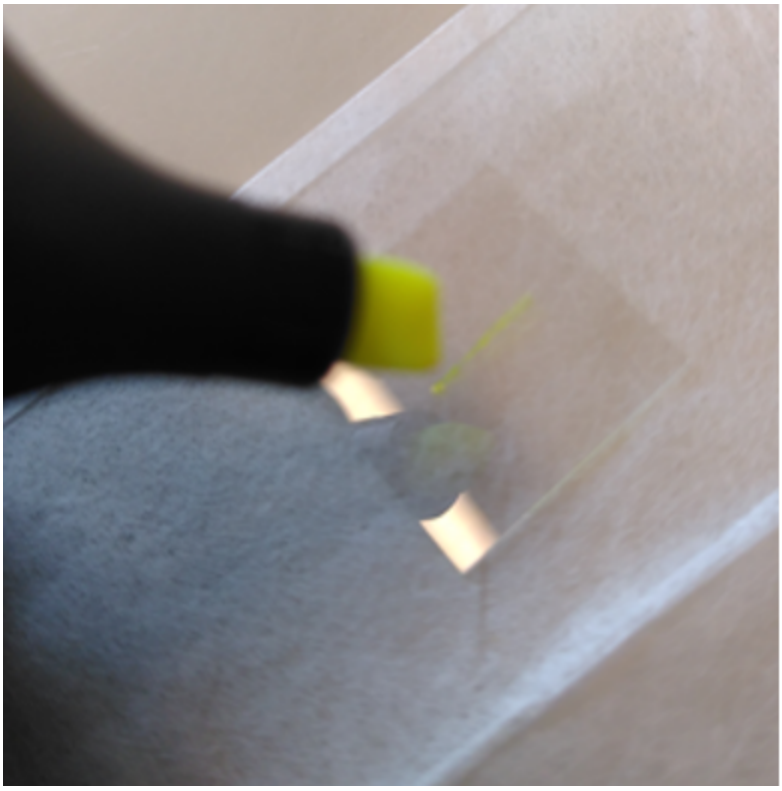
2 Dry the cover glass with a fresh sheet of lens paper.

Note

Do this step immediately while the cover glass is still wet. This will ensure that the dissolved contamination is removed. Do not let the contaminants dry onto the cover glass surface again.

3 Place the clean cover glass on a fresh sheet of lens paper.

4 Draw a thin line on the cover glass with the fluorescent marker using its thin tip.



Drawing a line on cover glass with a marker

5 Let the ink on the cover glass dry for 2 minutes.

6 Put the cover glass upside down on a microscope slide, with the ink towards the slide.

7 Fix the corners of the cover glass on the microscope slide the usual way.

Note

It is not important, what is used to fix the cover slip. Fluorescent marker is not sensitive to nail polish. However, the front lens of high-NA lenses is glued: if nail polish gets on this lens, acetone can seriously damage it. We therefore recommend to use twinsil-type dental silicone to seal the slides.
Generally, use only small amounts of the adhesive substance and let it dry long enough.

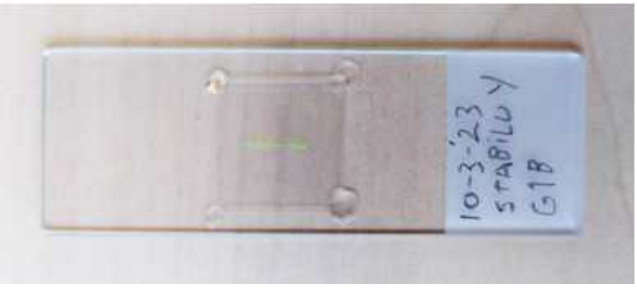
Note

Do not use any embedding medium. It is not necessary for the measurements and might dissolve the marker pen line.

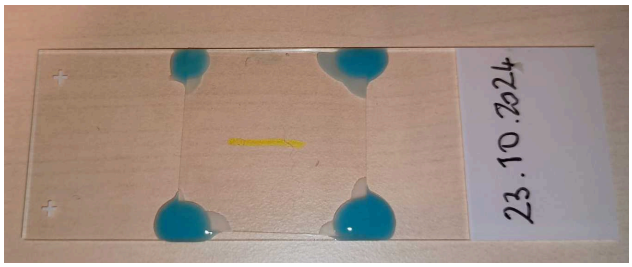
8 Write down the date on the microscope slide.

Note

The marker pen ink will continue to dry out, which might cause cracks and inhomogeneities within part of the ink layer. The sample maybe good to be used for several months, as long as it fullfils the requirements described in the analysis protocol.



End result using nail polish



End result using Twinsil

Protocol references

Olevsko I, Szederkenyi K, Corridon J, Au A, Delhomme B, Bastien T, Fernandes J, Yip C, Oheim M, Salomon A. 2021. A simple, inexpensive and multi-scale 3-D fluorescent test sample for optical sectioning microscopies. *Microscopy Res & Technique* **84**:2625–2635. doi:10.1002/jemt.23813

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