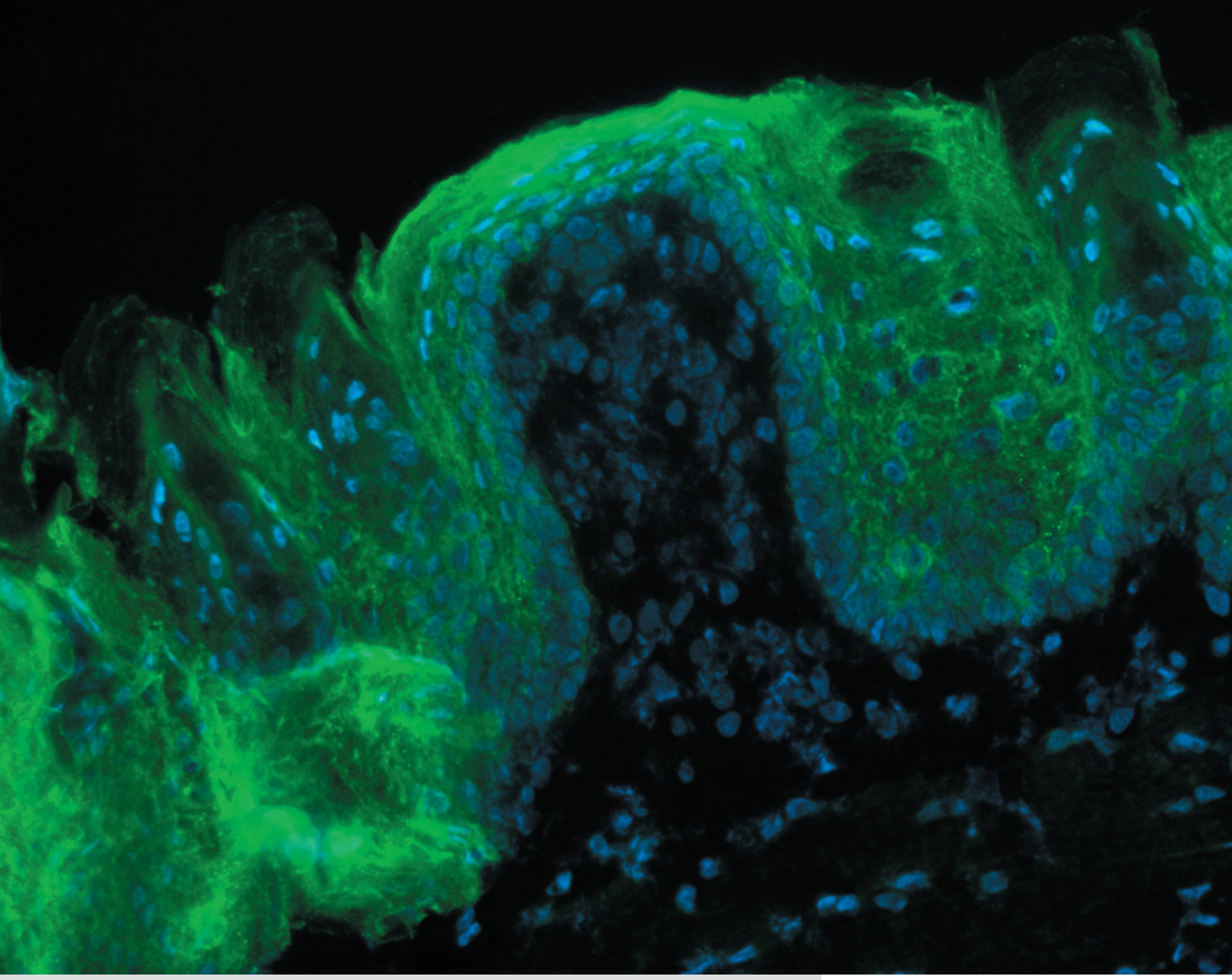
PALM User Protocols

Immunofluorescence on frozen sections



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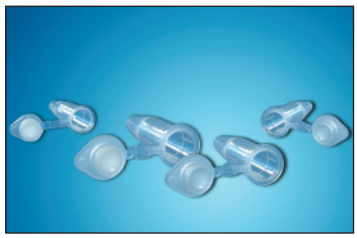
Immunofluorescence

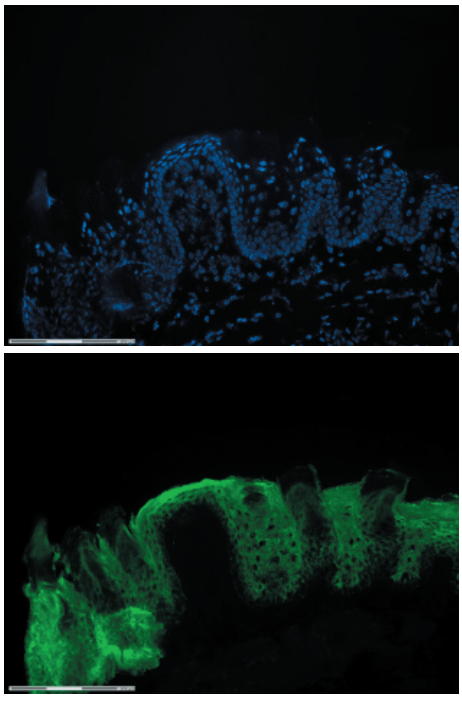
Some helpful tips before starting:

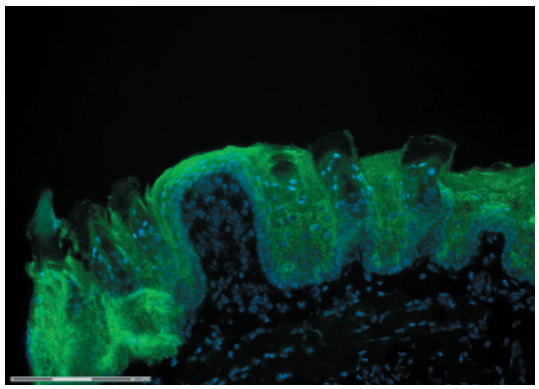
To prepare sections for non-contact laser capture microdissection (LCM) we generally recommend **PALM MembraneSlides**. If weak fluorescence or small objects must be detected the MembraneSlide 1.0 PET (Order No. 415101-4401-050) is recommendable.
(PEN membrane on PALM MembraneSlides can lead to more background in some fluorescent filters due to its structure and stronger autofluorescent effects)

Note: *To allow subsequent cutting and lifting a **coverslip** and standard mounting medium **must not be applied!** Freezing media like OCT or similar may be used for sectioning but should be kept to a minimum and have to be removed before laser cutting.*



For collecting microdissected samples we recommend the special **PALM AdhesiveCaps**:
AdhesiveCap 500 opaque (Order No. 415101-4400-250) or
AdhesiveCap 500 clear (Order No. 415101-4400-255).





Tongue epithelium
(Rat, Anti-Cytokeratin-FITC, DAPI)

Immunofluorescence

Microtome cutting and antibody staining

- Tissue (e.g., frozen rat tongue) is cut as usual (8-10 µm).
- The frozen section is transferred from the blade to the warmer slide by cautious touching.
- **Dry** in the cryostat for **1 minute** at about **-20°C**.

Note: *Few minutes of longer drying or previous poly-L-lysine coating of the slide may improve the adhesion of sections during the following staining steps.*

- Subsequently **fix** and dehydrate the section in **ice-cold** pure **acetone** for **30 seconds**.
- Finally **air-dry** at room temperature for **2-3 minutes**. Keep refrigerated till further use.
- To reduce the necessary incubation volumes draw a hydrophobic line around the sections with a special pen (e.g., Dako Pen #S2002).
- **Rehydration** and **blocking** of unspecific binding is done by covering the section with a drop of ready-to-use Protein Block Serum-Free (DakoCytomation #X0909) for **15 minutes** at room temperature.
- Pour off protein block and dip once into **PBS** for short **washing**.
- Remove excess liquid from the slide by tapping on an absorbant surface.
- Incubate with **Ab/DAPI-solution** for **1 hour** at room temperature in a dark wet chamber.
- Pour off Ab/DAPI-solution and dip once into **PBS** for short **washing**.
- Remove excess liquid from the slide by tapping on an absorbant surface.
- Sections can now be viewed on the microscope and used for LCM. To stabilize the fluorescent signals a drop of PBS or VECTASHIELD may be added as cover but microdissection will only be possible without too much liquid.

Note: *FITC-fluorescence is very sensible to bleaching without protection (often not more than 30 seconds) and therefore fluorescent illumination time should be kept to a minimum. The „Freeze Mode"-function of the PALM RoboSoftware will be very helpful in this context.*

Preparation of Ab/DAPI-staining solution

Dilute labelled **antibody** (Ab) solution in **PBS** containing **0.5 µg/ml DAPI** shortly before use (e.g., mouse monoclonal PCK-26, panCytokeratin-FITC; #ab112114, abcam; e.g.,1:25 - 1:100). The optimal dilution has to be found empirically for any tissue. Keep this solution in the dark till use and during the incubation.

Carl Zeiss MicroImaging GmbH
Location Bernried

Am Neuland 9+12
82347 Bernried, Germany
Phone: +49 (0) 8158-9971-300
E-Mail: palm-labszeiss.de
Web: www.zeiss.de/microdissection