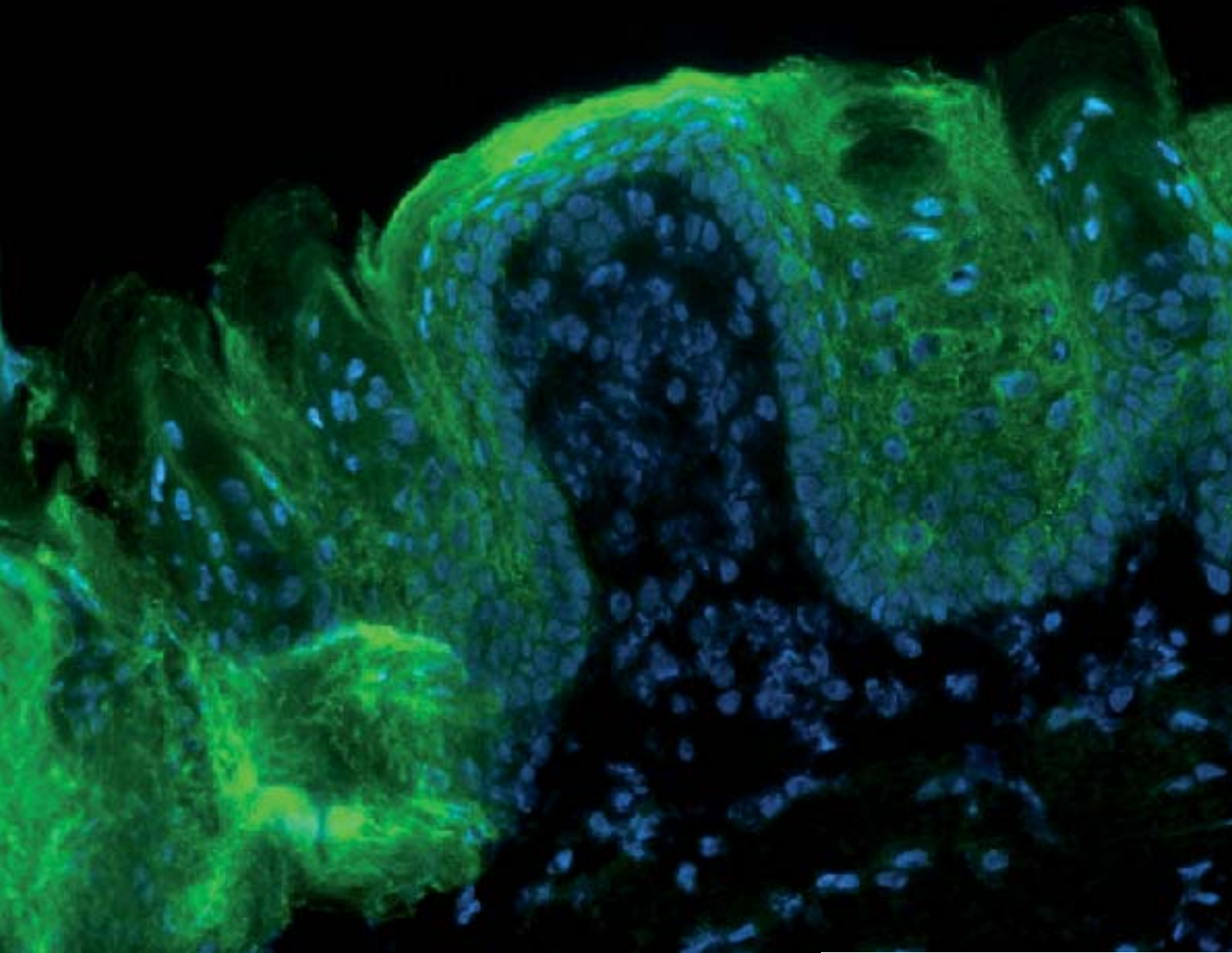


# PALM User Protocols

## Immunofluorescence on frozen sections



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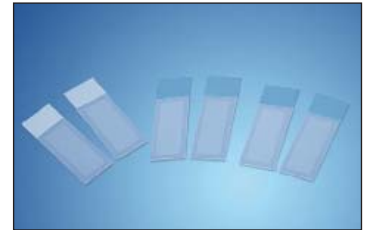
We make it visible.

## Immunofluorescence

### Some helpful tips before starting:

To prepare sections for non-contact laser capture microdissection (LCM) we generally recommend **MembraneSlides**. If weak fluorescence or small objects must be detected the MembraneSlide 1.0 PET (Order No. 415190-9051-000) is recommendable.

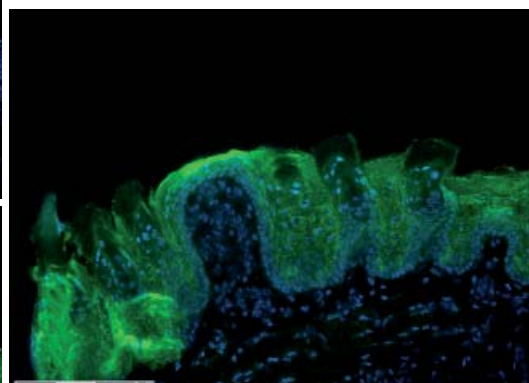
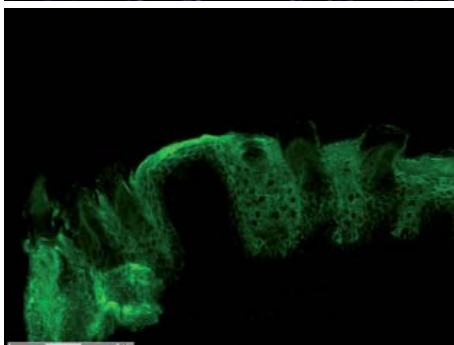
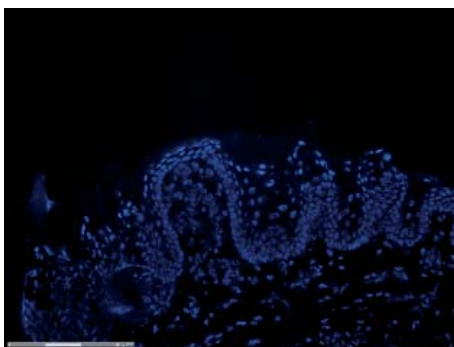
(PEN membrane on Slides can lead to more background in some fluorescent filters due to its structure and stronger autofluorescent effects)



**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 **AdhesiveCaps**:

AdhesiveCap 500 opaque (Order No. 415190-9201-000) or AdhesiveCap 500 clear (Order No. 415190-9211-000).



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

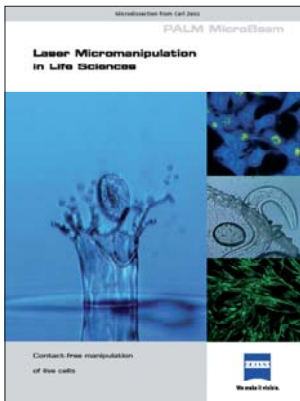
Dilute labelled **antibody** (Ab) solution in **PBS** containing **0.5 µg/ml DAPI** shortly before use (e.g., mouse monoclonal PCK-26, panCytokeratin-FITC; #ab112114, abcam; e.g., 1:25 - 1:100). The optimal dilution has to be found empirically for any tissue. Keep this solution in the dark till use and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

# PALM User Protocols

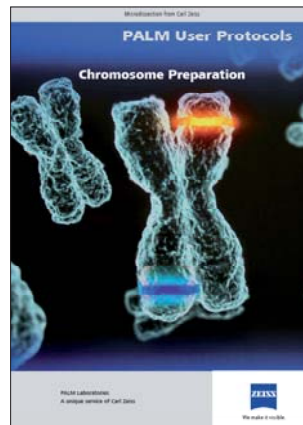
## Immunofluorescence

### Brochures and protocols

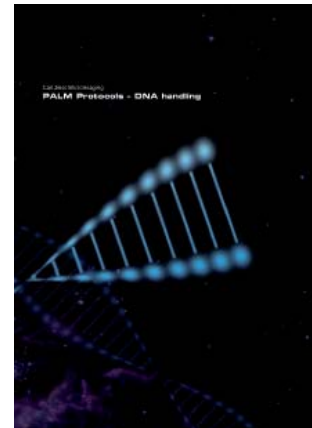
#### Live cells



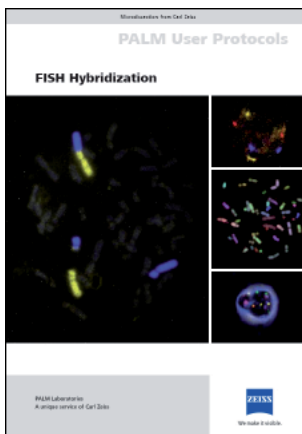
#### Chromosomes



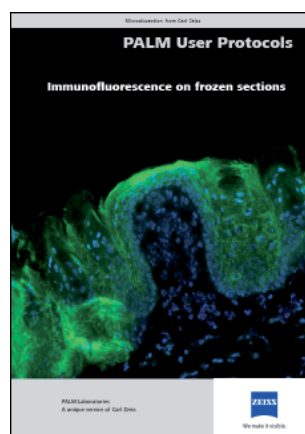
#### DNA



#### FISH



#### Immunofluorescence



#### RNA

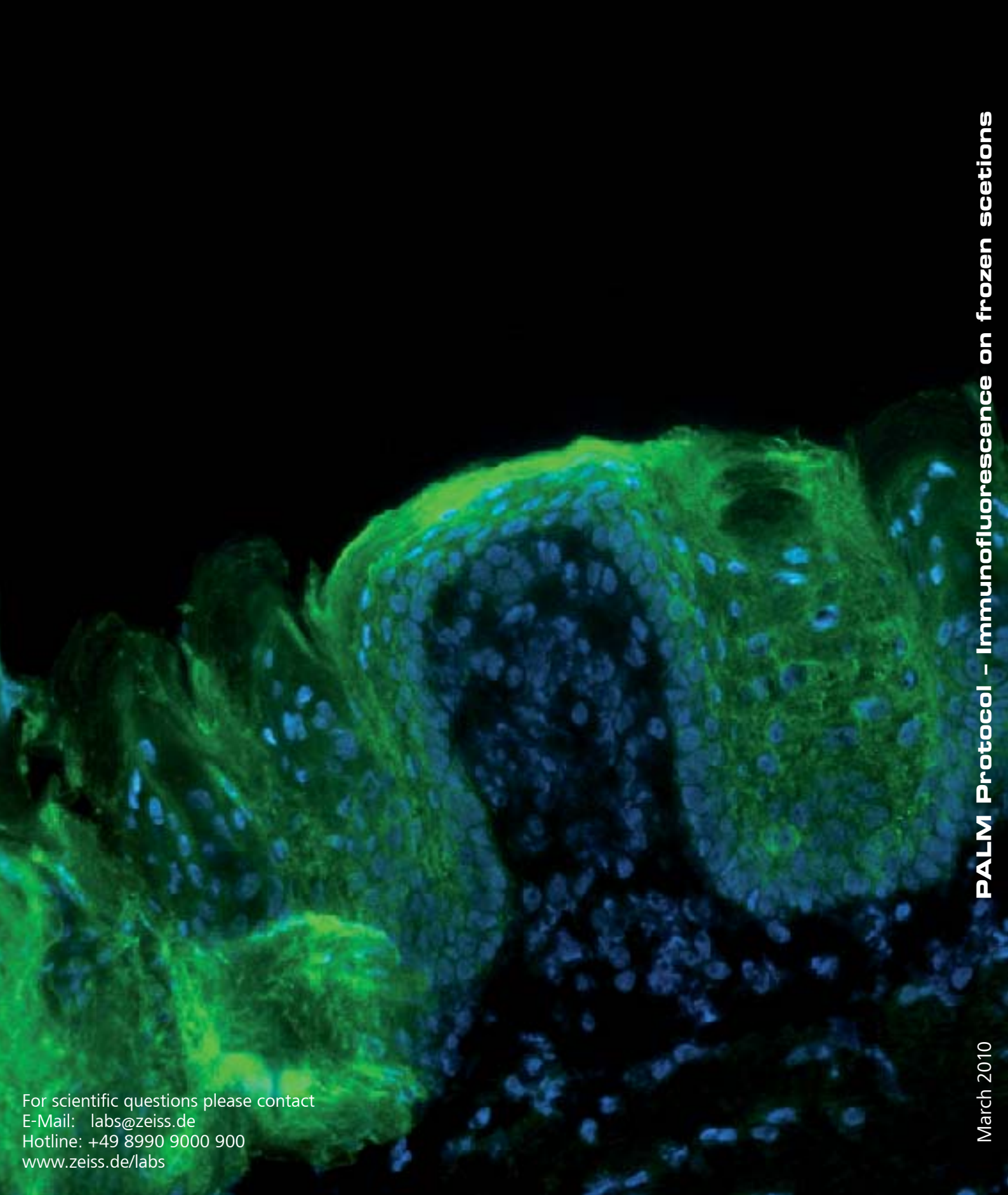


For questions, comments or protocol requests please contact:

#### ZEISS Labs

E-Mail: [labs@zeiss.de](mailto:labs@zeiss.de)

Hotline: +49 8990 9000 900



For scientific questions please contact  
E-Mail: [labs@zeiss.de](mailto:labs@zeiss.de)  
Hotline: +49 8990 9000 900  
[www.zeiss.de/labs](http://www.zeiss.de/labs)

**Carl Zeiss MicroImaging GmbH**  
07740 Jena, Germany

BioSciences I Location Munich  
Phone : +49 8990 9000 800  
Telefax: +49 8990 9000 820  
E-Mail : [palm-info@zeiss.de](mailto:palm-info@zeiss.de)

[www.zeiss.de/microdissection](http://www.zeiss.de/microdissection)



We make it visible.