Hannover Medical School

Research Core Unit for Laser Microscopy (ReCoLa)

"Hands on" laboratory course in immunofluorescence microscopy

March 17-18th, 2020

Laboratory room 1030 ("Labor 23"), level H, building J1

This lecture and "hands on" laboratory course will focus on the preparation of cultured cells for fluorescence microscopy.
This is a basic course suited for doctoral students, postdocs, and technicians with no or little experience in immunofluorescence techniques.
On the first day, we will present background information on all steps of the preparation and staining procedure. We will prepare whole mount immunofluorescence preparations of adherent and non adherent cultured cells.
On day 2 we will analyse the samples with confocal laser scanning microscopy in small groups. Controls, artefacts, analysis of colocalization and presentation of fluorescence microscopy data will be discussed.

The course is free of charge and will be in English.

For more information, call Wolfgang Posselt: -8458.

Program:

Lecture	Practical
Day 1: Tuesday March 17th 2020	
Start 10:00	
L1. Cell culture for immunofluorescence	
L2. Chemical fixation and permeabilization	
	P1. Fixation of adherent cells
L3. Selection and handling of primary antibodies	
L4. Discussing controls	
	P2. Incubation with primary antibodies
L5. Selection of secondary antibodies and fluorescent dyes	
	P3. Incubation with secondary antibodies
Lunch break	**13:00-14:00**
L6. Mounting procedures	
	P4. Sample embedding
L7. Epi and confocal fluorescence microscopy	
L8. Analysing colocalization	
L9. Presenting microscopic data	
End	**16:00**
Day 2: Wednesday March 18th 2020	
Start at 9, 11, 13, or 15:00	**4 groups, 3 participants each**
	P5. Microscopy (2 hrs for each group)

Lecture content in keywords

L1. Cell culture for immunofluorescence:
Culture dishes, preparation of glass surfaces, culture media and serum, cell density, fluorescent proteins

L2. Chemical fixation and permeabilization:
Aldehyde fixation, organic solvents, buffers, pH, temperature, time, detergents

L3. Selection and handling of primary antibodies:
Antibody structure, Mono and poly clonal antibodies, selection, purification, storage, concentration and titration, reducing unspecific binding

L4. Discussing controls:
Controls, controls, controls

L5. Selection of secondary antibodies and fluorescent dyes:
Why indirect immunofluorescence? Species cross absorbance, double immunofluorescence, choosing dyes for epi and laser microscopy, fluorescent proteins, direct staining methods: nucleus, mitochondria, lysosomes, microfilaments

L6. Mounting procedures:
Refractive index, anti fade, Moviol, commercial reagents

L7. Epi and confocal fluorescence microscopy:
Physical and digital resolution, contrast, fluorescence, 3D

L8. Analysing colocalization:
Cross talk, quantifying fluorescence, quantifying colocalization,

L9. Presenting microscopic data:
Sampling, image processing, black and white or colour, overlays, data file formats, software, digital imaging ethics