

TransMedRI

**ENDOCYTOSIS – INTERCELLULAR TRAFFICKING
WORKSHOP**

**Medical Faculty of Rijeka
September 19th – 21st 2011**



MAIN SCHEDULE

Date	Type of activity	Head	Title	Schedule
19.09.2011.	Lecture 1	Prof. dr. sc. Zlatko Trobonjača	Flow citometry	9.00-11.00
	Practical 1	Doc. dr. sc. Hana Mahmutefendić	Flow citometry	11.15-12.00 13.00-16.45
20.09.2011.	Lecture 2	Doc. dr. sc. Hana Mahmutefendić	Immunofluorescence and confocal microscopy	9.00-9.45 11.00-11.45
	Practical 2a	Doc. dr. sc. Hana Mahmutefendić	Preparation of the samples for the confocal microscopy Analysis in ImageJ program	10.00-10.45 11.45-14.00
21.09.2011.	Practical 2b	Doc. dr. sc. Hana Mahmutefendić	Analysis of the samples on the confocal microscope	11.30-13.00

METHODS

Every method is divided in the theoretical part (2h) and the practical part*, as shown below:

* 1h = 45'

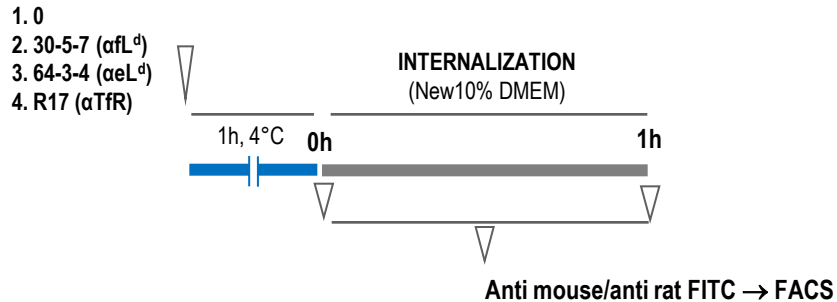
METHOD	Duration	Review of the field
<p style="text-align: center;">FLOW CITOMETRY (FACS)</p>	<p>1 day (2 h lecture and 6h practical)</p>	<p>1. LECTURE – Flow cytometry (<i>Mon. 19. 09. 2011. (9.00-11.00)</i>)</p>
		<p>1. PRACTICAL (<i>Mon. 19. 09. 2011. (11.15-12.00 and 13.00-16,45)</i>)</p> <ul style="list-style-type: none"> - Laboratory work: Internalization (1h) of MHC-I molecules and TfR on the L-L^d cell line - Analysis of the test results of the immunophenotypisation on the human peripheral blood lymphocytes - Analysis in the WinMDI program (the results obtained after internalization protocol)
<p style="text-align: center;">IF & CONFOCAL MICROSCOPY</p>	<p style="text-align: center;">2 days</p> <p><u>Day 1</u> <i>Preparation of the samples for the IF microscopy (2h lecture**+4h practical)</i></p> <p><u>Day 2</u> <i>Confocal microscopy (2h practical)</i></p> <p><small>**1. day lecture covers both fields</small></p>	<p>2. LECTURE – Immunofluorescent and confocal microscopy (<i>Tue. 20. 09. 2011. (9.00-9.45 and 11.00-11.45)</i>)</p>
		<p>2a. PRACTICAL (<i>Tue. 20. 09. 2011. (10.00-10.45 and 11.45-14.00)</i>)</p> <p>- Laboratory work– preparation of the samples for confocal microscopy:</p> <ul style="list-style-type: none"> a) Cointernalization (1h) of MHC-I molecules and Tf on HeLa and Balb3T3 cell lines b) Cointernalization (1h) of CTxB and Tf on Balb3T3 cell line <p>- Image processing in the ImageJ program (quantification of colocalization and 3d rendering)</p>
		<p>2b. PRACTICAL (<i>Wed. 21. 09. 2011. (11.30-13.00)</i>)</p> <p>Laboratory work - work on confocal microscope (LSCM) – analysis of the samples from the day before.</p>

	MONDAY (19.09.2011)	TUESDAY (20.09.2011)	WEDNESDAY (21.09.2011)	
9.00-10.00	LECTURE (ZT) Flow cytometry	LECTURE (HM) Immunofluorescent and confocal microscopy (1)		
10.00-11.00		IF microscopy - preparation of samples Laboratory work(HM) (preparation of samples(1) - binding of the 1°Abs on the cells)		
11.00-12.00	FACS Laboratory work (HM) (Cell trypsinization and adding of 1°Ab)	LECTURE (HM) Immunofluorescent and confocal microscopy (2)		Confocal microscopy Laboratory work (HM) Analysys of the samples on the confocal microscope
12.00-13.00	Lunch break	IF microscopy Laboratory work (HM) (preparation of samples (2) -Fixation and permeabilization of the cells, 2°Ab binding)		
13.00-14.00	FACS Laboratory work (HM) (binding of the 2°Ab in the zero time, and 1h following internalization, and analysis of the samples at the flow cytometar (FACS)) Analysis of the test results (HM) 1. Immunophenotypization of the peripheral blood lymphocytes (patient's test results) 2. Analysis of the results of internalization in WinMDI program	Image processing in the ImageJ program (HM) 1. Quantification of colocalization 2. 3d rendering		
14.00-15.00				
15.00-16.00				Lecture
15.00-16.45				Practical

PROTOCOLS OF THE PRACTICAL WORK TO BE PERFORMED

1. Flow cytometry

Internalization of MHC-I molecules and TfR at the L-L^d cell line (\pm IFN γ)



2. Preparation of the samples for the confocal microscopy

- a) Cointernalization of the MHC-I molecules and the TfR at the Balb3T3 and HeLa cell lines
- b) Cointernalization of the CTxB and transferrin at the Balb3T3 cell line

