



A DOREMI Short course entitled:

CELOD: cellular effects of low doses and low dose-rates with focus on DNA damage and stress response

Stockholm University, Sweden
April 16th - April 27th 2012

Aim of the course

The aim of the course is to acquaint students with the state of the art of cellular effects of low doses and low dose-rates and methods used to detect such phenomena as adaptive response and bystander effect. The course will contain both lectures and practical laboratory work. The lectures will focus on the mechanisms of cellular responses to low dose and low dose-rate effects as well as on techniques to detect them using cytogenetic, immunogenetic and stress response markers. The practical part will focus on teaching laboratory techniques used to study such phenomena as adaptive response and bystander effect in cells exposed to low doses and low dose-rates of radiation.

The course will last 2 weeks and will be nested in a 10 week “introduction to radiobiology” course held at the Stockholm University (SU). The regular SU course is held for a maximum of 12 students. CELOD will harbour a maximum of additional 12 students, bringing the total number of participants to 24.

Course description

To facilitate work in the lab the students will be divided into 4 groups with 6 people per group. Each group will carry out one experiment with different endpoints. At the end of the course students will present and discuss their results.

The course lectures will be held in the morning hours during week one. Practical work will be carried out in the afternoon hours of week 1 and during the whole days of week 2. The practical work will be divided into a “lab-teaching” part and a “results-analysis” part. Each group will learn 4 techniques (see below). Hence, each student will spend 4 time-blocks in the lab, where she/he will carry out the steps associated with a technique. The rest of the practical time will be devoted to learning how to analyse the results on microscopic slides/images. The rationale for dividing the students into small groups is that it will allow the student to really perform the work and not only watch a demonstration. Each group will be supervised by an experienced employee of GMT.

Each group will also learn how to irradiate cells using the exposure facilities at GMT: low-dose rate ¹³⁷Cs exposure facility, high dose-rate ¹³⁷Cs exposure facility, X-ray facility and ²⁴¹Am alpha-exposure facility.

Although students will learn how to generate microscopic slides/images that can be used for analysing the results, the scoring part of the course will be carried out using slides/images prepared beforehand by the GMT employees. This strategy will guarantee high quality slides/images for scoring. Students will score dose-response curves. At the end of the course the scoring results will be collated and the quality of the dose-response curves assessed and discussed.

Students will learn 4 techniques:

- 1: Chromosomal aberrations
2. Micronuclei
- 3: Gamma H2AX foci
4. Level of superoxide dismutases (SOD) and the 8-oxo-dGTPase hMTH1 (Western blot)

Experiments will be carried out with TK6 cells (aberrations and micronuclei) and with VH10 cells (gamma H2AX and Western). The cell lines/techniques are established and currently used in our laboratory.

A detailed description of the course is given on the next page. Leading national and international scientists will be invited to lecture.

Monday April 16 - day 1

Morning lecture: DNA damage and repair following irradiation of cells (1.5 h)

Morning lecture: Oxidative stress induced by low doses of radiation (1.5 h)

Afternoon: Splitting of students into groups, introduction to practical work, distribution of literature for self-studies (5h)

Tuesday April 17 – day 2

Morning lecture: Radiation-induced chromosomal aberrations (1.5 h)

Morning lecture: Radiation-induced micronuclei (1.5 h)

Afternoon: Group 1 learns the micronucleus assay, Group 2 learns Western blotting
Groups 3 and 4: analysis of experimental results

Wednesday April 18 – day 3

Morning lecture: Factors influencing the radiosensitivity of cells (1.5 h)

Morning lecture: Adaptive response/hormesis (1.5 h)

Afternoon: Group 3 learns the gammaH2AX-assay, Group 4 learns the chromosomal aberration assay
Groups 1 and 2: analysis of experimental results

Thursday April 19 – day 4

Morning lecture: Genomic instability (1.5 h)

Morning lecture: Bystander effects (1.5 h)

Afternoon: Group 1 learns the chromosomal aberration assay, Group 2 learns the gammaH2AX-assay
Groups 3 and 4: analysis of experimental results

Friday April 20 – day 5

Morning lecture: Introduction to proteomics (1.5 h)

Morning lecture: GammaH2AX foci assay (1.5 h)

Afternoon: Group 3 learns the micronucleus assay, Group 4 learns Western blotting
Groups 1 and 2: analysis of experimental results

Sunday April 22 a one day trip with a visit to a museum and an evening reception will be organised

Monday April 23 – day 6

Morning: (3h) Group 1 learns the gamma-H2AX assay, group 2 learns the aberration assay. Other groups learn to score chromosomal aberrations/micronuclei/gamma-H2AX foci/Western blot.

Afternoon: All groups learn to score chromosomal aberrations/micronuclei/gamma-H2AX fo-ci/Western blot (5h).

Tuesday April 24 – day 7

Morning: Group 3 learns the aberration assay, group 4 learns the gamma-H2AX assay (3h). Other groups learn to score chromosomal aberrations/micronuclei/gamma-H2AX foci/Western blot.

Afternoon: All groups learn to score chromosomal aberrations/micronuclei/gamma-H2AX foci/Western blot (5h).

Wednesday April 25 – day 8

Morning: Group 1 learns the Western assay, group 2 learns the micronucleus assay (3h). Other groups learn to score chromosomal aberrations/micronuclei/gamma-H2AX foci/Western blot.

Afternoon: All groups learn to score chromosomal aberrations/micronuclei/gamma-H2AX foci/Western blot (5h).

Thursday April 26 – day 9

Morning: Group 3 learns the Western assay, group 4 learns the micronucleus assay (3h). Other groups learn to score chromosomal aberrations/micronuclei/gamma-H2AX foci/Western blot.

Afternoon: All groups learn to score chromosomal aberrations/micronuclei/gamma-H2AX foci/Western blot (5h).

Friday April 27 – day 10

Whole day: presentation of results, general discussion (8h)

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A diagram illustrating the timing of CELOD components is shown below. Blue: lectures/seminars, yellow: experimental work in the lab, green: scoring of slides/images.

		Week 1					Week 2				
		Monday	Tuesday	Wednesday	Thursday	Friday	Monday	Tuesday	Wednesday	Thursday	Friday
Morning		LECTURES	LECTURES	LECTURES	LECTURES	LECTURES	gamma-H2AX	Scoring Mn and Aberrations slides from experiment	Western	Scoring Mn and Aberrations slides from experiment	Reporting, end discussion
							Aberrations		Mn		
Afternoon	ORGANISATIONAL remarks, getting to know the infrastructure, getting to know each other	Scoring Mn slides from experiment	Mn	Scoring Mn slides from experiment	Aberrations	Scoring Aberration slides from experiment	Scoring Mn and Aberrations slides from experiment	Aberrations	Scoring Mn and Aberrations slides from experiment	Western	
			Western							gamma-H2AX	Mn
			gamma-H2AX	Scoring Aberration slides from experiment	Mn	Scoring gamma H2AX	Analysing Western results	Analysis of results	Analysis of results	Reporting, end discussion	
		Aberrations	Western								