

## **Training Course in Organotypic Brain Slice Culturing, 17-19 June 2009**

Slices of brain tissue, from the still developing brain, can by standardized technology be grown for several weeks, maturing into organotypically organized pieces of brain with a basic cellular content and synaptic network characteristic of the brain areas *in vivo*. Since brain slice cultures are easily inspected by microscopy and easily manipulated through additions to the culture medium or physical exposures, they are much used in studies of normal and pharmacologically or physically manipulated interactions between neurons and neurons and glia. Organotypic hippocampal slice cultures have thus successfully been used to model a broad range of neuronal diseases and for screening of drug candidates and neurotoxic and neurotrophic compounds. Due to the presence of neural progenitor cells in the dentate gyrus, hippocampal slice cultures are also used for experimental studies of neurogenesis.

See also: [www.scitopics.com/Organotypic\\_Brain\\_Slice\\_Cultures.html](http://www.scitopics.com/Organotypic_Brain_Slice_Cultures.html)

**Aim:** The training course in brain slice culturing aims to provide the participants with both practical and theoretical experience with the technique as well as examples of its experimental use.

**Number of participants:** Max 8-10

**Course organizers:** prof. Jens Zimmer and assoc. prof. Jens Noraberg

**Teachers:** Jens Zimmer (JZ), Oliver Raineteau (OR), Jens Noraberg (JN), Jan Bert Gramsbergen (JBG), Maria Montero (MaM), Randi Godskesen (RG), Jeanne Sindberg (JS)

**Secretary:** Bodil Theilade (btheilade@health.sdu.dk)

**Location:** Laboratories of Anatomy and Neurobiology, Institute of Medical Biology, Winslowparken 21, Denmark, DK-5000, Odense C and class rooms next door in Winslowparken 19, (rooms 19.04 and 19.15).

**Telephone:** (+045) 6550 3800 (secretary); (+045) 6550 3810 (laboratory)

### **Wednesday, 17 June (Winslowparken 19, room 19.04)**

- 08.45-09.00** Welcome and Introduction of course participants and teachers
- 09.00-09.30** Introduction to organotypic brain slice cultures (JZ)
- 09.30-10.30** Are organotypic slice cultures more than an inert piece of neonatal tissue? Experimental and technical considerations onto the normal maturation of organotypic slice cultures (OR)
- 10.30-10.45** Break
- 10.45-11.45** Hippocampal slice cultures for studies of neurodegeneration and neuroprotection (JN)
- 11.45-12.45** Lunch
- 12.45-16.00** Practical Sessions, incl. break. Winslowparken 21, ground floor
  - Exercise 1* Practical introduction to and set up of hippocampal slice cultures.
  - Exercise 2* Practical introduction to and set up of dopaminergic ventral mesencephalic slice cultures.
  - Exercise 3* Preparing for excitotoxic NMDA exposure and cellular uptake of Propidium Iodide (PI) uptake as a marker of cell death

## **Thursday, 18 June (Winslowparken 19, room 19.15)**

- 9.00-10.00** Dopaminergic, ventral mesencephalic slice cultures and nigrostriatal co-cultures for experimental modeling of Parkinson disease (JBG)
- 10.00-10.15** Break
- 10.15-11.00** Neurodegeneration and microglia in hippocampal slice cultures (MaM)
- 11.00-11.45** *Exercise 4* Practical microscopy and discussion of CNS slice cultures
- 11.45-12.45** Lunch
- 12.45-15.45** **Practical Sessions, Winslowparken 21, ground floor.**  
*Exercise 5* Recording of PI uptake as a marker of cell death in NMDA-exposed cultures  
*Exercise 6* Monitoring of cell proliferation in slice cultures by BrdU
- 15.45-17.00** **Discussion of use of brain slice cultures in own research projects**

## **Friday, 19 June (Winslowparken 19, room 19.15)**

- 09.00-10.00** Culturing slices of whole fetal brain for studies of neuronal development (JN + video fra JOVE)
- 10.00-10.30** Forebrain slice cultures with rostral migratory stream cell migration (JZ)
- 10.30-11.00** Break
- 11.00-11.45** Organotypic brain slice cultures from mice expressing transgenic fluorescent neuronal or glial proteins (JN, MaM)
- 11.45-12.45** Lunch
- 12.45-15.15** **Practical Session. Winslowparken 21, ground floor.**  
*Exercise 7* Quantifying cell death by densitometric analysis of PI uptake  
*Exercise 8* Imaging of live and fixed cultures.
- 15.15-16.00** **Round-up and closing of course**

## PhD-course Flow Cytometry and Cell Sorting 17-19 June, 2009

**General objectives:** Objective: This course is designed to equip those having only limited or no prior experience of flow cytometry with a basic grounding in the principles of the technique and working knowledge of its practice with special emphasis on the application of flow cytometry to cell sorting.

**Organizer:** Assoc. prof. Graham Leslie, Immunology and Microbiology, University of Southern Denmark, Odense.

**Duration:** 3 days

**ECTS points:** 2

**Number of participants:** Max. 8

**Teachers:** Henrik Holst (HH), BD Biosciences; Ulrik Sprogøe (US), Dept. Clinical Immunology, OUH; Claus H. Nielsen (CHN), Copenhagen University Hospital; Graham Leslie (GL), Charlotte Harken Jensen (CHJ), Inger Andersen (IA), Anette Kliem (AK), Morten Løbner (ML) University of Southern Denmark

**Location:** Winsløwparken 19 and 21, University of Southern Denmark, Odense

### Programme

#### Wednesday, 17 June

(Winsløwparken 19, room 19.01 ground floor)

##### *Theoretical Session*

9.00 – 09.15 Introduction - **GL**

9.15 – 10.45 **Session 1. The basic principles – GL**

- The flow system
- Laser excitation
- Light scatter and fluorescence
- Multiple fluorochrome compensation
- Autofluorescence
- Data analysis
- Quality control

10.45 – 11.00 *Coffee*

11.00 – 12.00 **Session 2. The basic practicalities – US**

- The cell preparation
- The parameters for investigation (membrane glycoproteins, surface lipids, intracellular proteins, DNA, free Ca<sup>2+</sup>, oxidative metabolites etc.)
- Labelling with fluorochromes (incl. choosing the right marker-fluorochrome combination)
- Data acquisition – the software
- Gating
- Positive and negative controls

12.00 – 12.30 **Session 2a. Flow Cytometry on cells from tissues – CHJ**

12.30 – 13.15 *Lunch*

##### *Practical Session*

(Winsløwparken 21, 1<sup>st</sup> floor, flow cyto lab.)

13.00 – ca. 16.00

Exercise 1 The basics – **(AK, GL, IA, US)**

- Starting up
- Instrument settings
- Calibration with standard beads
- Compensation
- Maintenance

Exercise 2 Identification of blood leukocyte sub-populations – **(AK, GL, IA, US)**

- Morphological discrimination
- Identification with the help of surface markers (CD3, CD19 etc.)

## Thursday, 18 June

(Winsløwparken 19, Room 19.11, 1<sup>st</sup> floor)

### *Theoretical Session*

#### 9.00 -10.00 **Session 3. Antibodies as probes - GL**

- The antibody-antigen interaction
- Monoclonal vs. polyclonal antibodies
- Direct vs. indirect labelling
- The biotin-streptavidin connection
- Extracellular vs. intracellular probing (fixation and permeabilisation)

10.00 - 10.15 *Coffee*

#### 10.15 - 11.15 **Session 4. Other probes – CHN**

- DNA staining
- Staining for detection of cell division
- Staining for markers of apoptosis
- Free calcium staining
- Staining for oxidative metabolites

11.15 – 12.15 Discussion of results – Exercises 1 and 2.

12.15 -13.00 *Lunch*

### *Practical Session*

(Winsløwparken 21, 1<sup>st</sup> floor, flow cyto lab)

13.00 – ca. 16.00

Exercise 3 Viability assessment – **(AK, ML, GL, IA)**

- Apoptosis vs. necrosis (PI + Annexin V)

Exercise 4 Assessment of cell proliferation – **(AK, ML, GL, IA)**

- CFSE approach

Exercise 5 Quantitation of a cell-surface receptor (work-station exercise).

## Friday, 19 June

(Winsløwparken 19, Room 19.11, 1<sup>st</sup> floor)

#### 09.00 – 10.00 **Session 5. Multicolor flowcytometry – HH**

#### 10.00 – 10.45 **Session 6. Cell Sorting – HH**

- Basic considerations
- Important Factors for Successful Cell Sorting
  - Quality of the cell preparation
  - Cell density
  - Sorting rate v. viability
  - Purity versus yield
  - “Invisible” impurities
  - Sorting strategies
- Alternatives to phenotype sorting – side population sorting. reporter genes.
- Practicalities
  - The time factor
- Recovery – the harsh reality

10.45 – 11.00 *Coffee break*

#### 11.00 – 12.00 **Session 7. Quantitative flow cytometry - US**

- Quantitation cell sub-populations
- Quantitation of surface markers
- Cell-cycle analysis

12.00 – 12.30 Discussion of results – Exercises 3 and 4.

12.30 – 13.15 *Lunch*

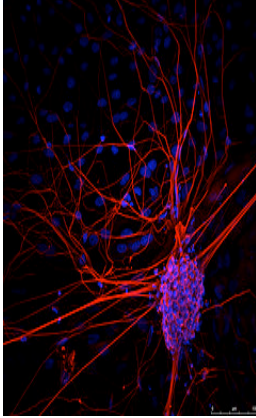
### *Practical Session*

(Winsløwparken 21, 1<sup>st</sup> floor, flow cyto lab)

13.15 – ca.15.00

Exercise 6 (Demonstration) Introduction to the cell sorter – **HH**

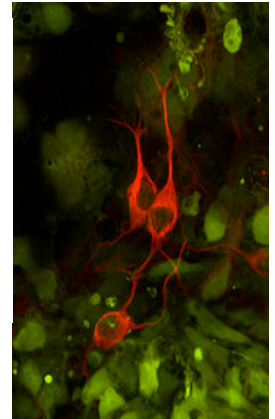
Exercise 7 (Demonstration) A rare event sort – **HH**



## Imaging Techniques

Theory alternating with practical demonstrations

June 17-19th 2009



### Objective:

The overall objective of the course is that the students acquire theoretical and practical knowledge about techniques employed to visualize and study stem cells *in vitro* and in biological tissues

### Place:

Anatomy and Neurobiology, Institute of Medical Biology, University of Southern Denmark, Winsløwparken 21 (J.B. Winsløws Vej), DK-5000 Odense C

### Course leaders:

Professor Poul Hyttel (PH), Department of Basic Animal and Veterinary Sciences, Faculty of Life Sciences, University of Copenhagen & Associate Professor Morten Meyer (MM), Anatomy and Neurobiology, Institute of Medical Biology, University of Southern Denmark

### Contents:

June 17 – Winsløwparken 19, room 19.02 (ground floor) and 21, 3<sup>rd</sup> floor, Seminar room

- 09.15 Course introduction (MM)
- 09.30 MR Imaging: Methodology and features of relevance for visualization of stem cells (HSJ)
- 10.30 Coffee
- 10.45 Transmission and scanning electron microscopy – methodological aspects and stem cell ultrastructure (PH)
- 12.00 Lunch (after lunch, go to Seminar room, WP 21, 3<sup>rd</sup> floor)
- 13.00 The pig as a model of pluripotency - biomedical applications and bioimaging (VH)
- 13.45 Multimodality imaging to track stem cells in the naive and injured brain (RG)
- 15.15 Coffee
- 15.30 Informal student project presentations: Bioimaging in your research (3 min/student – 3 slides; MM)
- 16.45 End of session

June 18 – Winsløwparken 19, room 19.14 (1<sup>st</sup> floor) and Winsløwparken 21, 3<sup>rd</sup> floor

- 09.15 Principles of immunocytochemistry – controls and pitfalls (CHJ)
- 10.00 Coffee (after coffee, go to Seminar room, WP21, 3<sup>rd</sup> floor)
- 10.15 Basic microscopy, phase contrast and interference contrast microscopy, theory and practical demonstration (AH)
- 12.00 Lunch

- 13.00 Camera and software in microscopy - theory and practical demonstration (AH)
- 13.45 Coffee
- 14.00 Fluorescence microscopy and confocal laser scanning microscopy - advantages, limitations and pitfalls (MH)
- 14.45 Principles of digital imaging (MH)
- 15.30 Confocal laser scanning microscopy – practical demonstration (AH/UM)
- 17.15 End of session

June 19 – Winsløwparken 21, 3<sup>rd</sup> floor, Seminar room

- 09.15 Molecular imaging using MALDI mass spectrometry (MA)
- 10.15 Coffee
- 10.30 Non-viral and viral methods for introduction of fluorescence markers in stem cells (NS)
- 11.15 *In vivo* electroporation: A method for transfecting cells (JB)
- 12.00 Cell labeling and practical demonstration of image processing (MB)
- 13.15 Lunch and end of course

**Speakers and instructors:**

- (CHJ) Charlotte Harken Jensen, Immunology and Microbiology, University of Southern Denmark
- (HSJ) Hans Stødkilde-Jørgensen, MR Center, Skejby/Aarhus University Hospital
- (AH) Allan Hemmingsen, Olympus A/S, Denmark
- (MB) Morten Blaabjerg, Dept. Neurology, Odense University Hospital
- (VH) Vanessa Hall, Life Sciences, University of Copenhagen
- (MH) Michael Hansen, Life Sciences, University of Copenhagen
- (NS) Nedime Serakinci, Biopark-Vejle, University of Southern Denmark
- (JB) Jonas B Blom, Medical Biotechnology Center, University of Southern Denmark
- (MA) Malin Andersson, Uppsala University
- (RG) Raphael Guzman, Stanford University
- (UM) Ulla Melchior Hansen, Physiology and Pharmacology, University of Southern Denmark
- (PH) Poul Hyttel, Life Sciences, University of Copenhagen
- (MM) Morten Meyer, Anatomy and Neurobiology, University of Southern Denmark