

Foerster Resonance Energy Transfer (FRET) microscopy as a tool for functional imaging and quantitative analysis (26-28 September,2012)

1.- Summary (up to one page)

The workshop was conducted successfully on the proposed dates of the 26th to the 28th of September 2012. In addition to the funding provided by the ESF, the workshop profited from the substantial support of Leica Microsystems, who installed their latest confocal microscope model Leica TCS SP8 (released in May 2012) for the first time in Spain for this event. They additionally provided two application specialists from the international and the national unit to help with the teaching in the event.

34 applicants from eight countries applied for the course. All seven applicants from abroad (one had to decline due to scheduling issues) as well as 18 from Spain were selected for the 25 open slots. Six Spanish participants came from five other locations, 11 participants from five other institutes in the Barcelona area and one participant came from a CRG research group.

Among the Spanish participants priority was given to applicants from outside Barcelona and inside Barcelona to people working in imaging facilities that could act as multipliers. The remaining positions were assigned to applicants from different research groups.

Only one person (an Indian PhD student) working in Bonn, Germany did not show up at the beginning of the course and was replaced on short notice by a CRG member that will require FRET expertise for her work and that was already involved by providing samples for the hands-on sessions.

The workshop was split between lectures by experts in different aspects of the field and hands-on sessions on different topics on advanced instruments. This was supplemented by a demonstration of Selective Plane Illumination Microscopy for tissue imaging on an instrument in the laboratory of James Sharpe and by the opportunity for all five subgroups (consisting of five participants) to sit down for an in-depth discussion with the external lecturers.

2.- Description of the scientific content of and discussions at the event (up to four pages)

Foerster Resonance Energy Transfer (FRET) is a nonradiative energy exchange between a donor and an acceptor molecule that can only take place over distances up to 100 Angstrom. In the case of fluorescent molecules as donor and/or acceptor molecules this process is therefore ideally suited to observe processes at the molecular level on a fluorescence microscope.

The microscopic detection methods (and the subsequent analysis) as well as the development of increasingly sensitive and specific biosensors have made enormous progress in the last years and continue to be areas of highly active development. Especially the use of fluorescent proteins as genetically encodable reporters for molecular interactions and intramolecular conformation changes allows the study of molecular events in real time in living cells, tissues and animals.

Almost any development in fluorescence microscopy allows the improvement of already established FRET detection methods. This can be by faster or more sensitive imaging or by the application of new imaging architectures like Single Plane Illumination Microscopy (SPIM). Lifetime imaging methods that are very well suited for FRET detection are becoming more widely available and offer the potential of fully quantitative FRET measurements that provide full information on the interacting population of molecules.

This workshop aims to give insights into the following aspects:

- probe design
- biological applications examples
- microscope detection methods
- data analysis

The considerations for designing FRET based biosensors will be explained in talks by specialists in this field. The potential for biological experiments will be covered by scientific talks and partially in practical demonstrations and sessions on microscopes on site. An overview of the existing FRET detection methods will be given in seminars and these will be applied in the practical sessions. The analysis of FRET experimental data will be explained theoretically and will be executed in hands-on sessions on analysis workstations under expert supervision.

The limited number of participants in the workshop will allow to provide practical training for each participant in addition to the lectures. For the practical part the whole range of currently commercially available microscope systems will be made available right up to Fluorescence Lifetime Imaging Microscopy (FLIM) by time-correlated

single photon counting (TCSPC).

A special focus will be put on the analysis of FRET data which will comprise almost one third of the workshop. The participants should therefore come back from this workshop with an understanding of sensor design, the basic ability to perform FRET measurements and the confidence to be able to properly analyze their data, understanding when results are qualitative, when semi-quantitative and when they can be used for global analysis and modeling.

The local organizer and the unit staff have many years of working experience in FRET imaging from basic approaches like acceptor photobleaching and ratiometric Calcium measurements up to the complex analysis required for FLIM experiments. They will be supplemented by a team of expert speakers who are renowned specialists in the fields of probe design, imaging and analysis of FRET data.

3.- Assessment of the results and impact of the event on the future directions of the field (up to two pages)

Feedback

The anonymous feedback for the event was collected at the end of the workshop and was very positive (18 feedback forms collected).

The following questions of the feedback form are weighted from “Excellent” (5) to “Very Good” (4) to “Good” (3) to “Fair” (2) to “Poor” (1)

General

- 1) How would you rate this course overall? 3.95
- 2) How well did the workshop meet your expectations? 3.74
- 3) How would you rate this course with respect to other similar courses? 4.0, No answer: 7
- 4) Would you recommend this course? YES: 18 NO: 0
- 5) Please write one sentence that encapsulates your experience in the course:
Individual answers

Scientific Content

- 6) Which topics were most useful/interesting to you? Individual answers
- 7) Which missing topics would you have liked to see covered? Individual answers
- 8) What is your rating on the content of the practical sessions? 3.74
- 9) What is your rating on the support received during the practical sessions?
4.17

Organization and materials

- 10) How would you rate the overall organization of the workshop? 3.9
- 11) How would you rate the venue (including food) of the course? 4.21
- 12) How would you rate the relevance and usefulness of the training materials?
3.72
- 13) Suggestions on how to improve the course: Individual answers

Combined rating of all questions: 3.92, consisting of 32x "Excellent", 65x "Very Good", 41x "Good" and 1x "Fair"

4.- Annexes: programme of the meeting and full list of speakers and participants

FINAL PROGRAM

26th September, 2012

Morning sessions - Ramon y Cajal Room – (PRBB Inner Square)

9:15-10:00 - *Basics of FRET* (Timo Zimmermann)

10:00-10:45 - *FRET imaging methods (Part 1)* (Timo Zimmermann)

10:45-11:15 - Coffee Break (PRBB Inner Square)

11:15-11:45 - *Ratiometric FRET measurements* (Xavier Sanjuan)

11:45-13:00 - *FLIM-FRET measurements* (Anthony Squire)

13:00-14:30 - Lunch Break (PRBB CANTEEN)

Afternoon Session

14:30-16:00 - *Microscopy Hands-on Session Basics 1: 4 parallels*

16:00-16:30 - Coffee Break (5th floor Terrace)

16:30-18:00 - *Microscopy Hands-on Session Basics 2 : 4 parallels*

27th September, 2012

Morning sessions - Ramon y Cajal Room – (PRBB Inner Square)

9:15-10:30 - *Sensor development , in-vivo applications and quantitative analysis* (Olivier Pertz)

10:30-11:00 - Coffee Break (PRBB Inner Square)

11:00-12:15 - *Development of enhanced FRET reporter constructs* (Raik Gruenberg)

12:15-12:45 - *FRET imaging methods (Part 2)* (Timo Zimmermann)

12:45-14:30 - Lunch Break (PRBB CANTEEN)

Afternoon Session

14:30-15:30 - *FLIM practical and SPIM demonstration 1, Hands-on Session Advanced 1*

15:30-16:30 - *FLIM practical and SPIM demonstration 2, Hands-on Session Advanced 2*

16:30-17:00 - Coffee Break (5th floor Terrace)

17:00 – 18:00 - *FLIM practical and SPIM demonstration 3, Hands-on Session Advanced 3*

20.30h - Workshop Dinner at Ca La Nuri Restaurant

Passeig Marítim de la Barceloneta, 55

08005 Barcelona

28th September, 2012

Morning Sessions - Ramon y Cajal Room – (PRBB Inner Square)

9:15-10:15 - *FLIM practical and SPIM demonstration 4, Hands-on Session Advanced 4*

10:15-11:15 - *FLIM practical and SPIM demonstration 5, Hands-on Session Advanced 5*

11:15-11:45 - Coffee Break (PRBB Inner Square)

11:45-13:00 - *FRET methods + FRET analysis (Peter Verveer)*

13:00-14:30 - Lunch Break (PRBB CANTEEN)

Afternoon Session - Ramon y Cajal Room – (PRBB Inner Square)

14:00-16:00 - *FRET data analysis hands-on on computers*

List of speakers/instructors

- **Peter Vermeer**, Max-Planck-Institute of Molecular Physiology, Dortmund, Germany: *FRET analysis methods*
- **Tony Squire**, Imaging Center Essen, Germany: *FLIM measurement techniques*
- **Raik Gruenberg**, Canada: *Development of enhanced FRET reporter constructs*
- **Olivier Pertz**, University of Basel, Switzerland: *Sensor development , in-vivo applications and quantitative analysis*
- **Xavier Sanjuan**, UPF, Barcelona, Spain: *Calcium imaging with FRET sensors*
- **Timo Zimmermann**, CRG, Barcelona, Spain: *FRET imaging methods and analysis*

Details of Venue

Center for Genomic Regulation

C/ Dr. Aiguader, 88, 08003 Barcelona, Spain

Resources available for the workshop

- Lecture rooms (Room Ramón y Cajal, Ground Floor)
- Course room for data analysis session
- Advanced Light Microscopy Unit (ALMU): currently 6 confocal microscopes (1x 2P, 1x 2P/F LIM/FCS, 1x CW-STED, 1 x spinning disk/bleaching/TIRF, 2x standard confocals), 1 fully automated widefield microscope, supporting staff of four imaging specialists
- Additionally available instruments for the course: 1 Leica TCS SP8 demo system, 1 SPIMç (James Sharpe laboratory)

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