JAMES RENNIE BEQUEST

REPORT ON EXPEDITION/PROJECT/CONFERENCE

Expedition/Project/Conference Title: Immunocytochemistry, In-Situ Hybridization & Live Cell Imaging Course

Travel Dates: 3rd - 15th November 2009

Location: Cold Spring Harbor Laboratory, NY, USA

Group Member(s): Jan H. Bergmann

Aims: to broaden and further the skills in and knowledge of advanced microscopy protocols and operation

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OUTCOME (not less than 300 words):-

Microscopy has revolutionized the way scientists across various disciplines are able to approach questions relating to their objects of interest. Variations of increasingly sophisticated microscope components in combination with elaborate technical approaches render light microscopy today one of the most versatile tools available to address an ever increasing range of problems. In particular in the field of the biological sciences, modern light microscopy has opened the path to study, in unprecedented detail, a wide range of aspects of cell and molecular biology in fixed tissues and cells, or even living specimens. These include both structurally more rigid as well as highly dynamic cellular architecture and processes, respectively. Improvements to microscope hardware, camera technology, analytical software and labelling methods have led to a drastic increase in the number of available advanced microscopic approaches. Combined with biophysical and computational power, light microscopy now even allows to image structures beyond the classical Abbe resolution limit of light. In order to obtain and be able to critically interpret high-quality microscopic data in the context of increasingly complex hard- and software, it is not only of paramount importance to thoroughly understand the principles of different microscopic systems and applications, but also critically depends on the initial preparation and control of the samples to be analyzed.

The "Immunocytochemistry, *In-Situ* Hybridization and Live Cell Imaging" course (dubbed "Live-FISH") provides an excellent platform to learn from and discuss with some of the world's leading experts in the application of microscopy, various aspects and tweaks of sample preparation and operation of high-end microscopes. All this was taking place in the inspiring backdrop of Cold Spring Harbor Laboratory (CSHL), one of the leading biological research institutions in the United States. CSHL is remotely located on the Northern shore of Long Island, bordering a vast forest region, allowing to take in the breathtaking beauty of the Autumn colors around the harbor area under a sunny blue sky. While this has not even been my first visit to CSHL, the atmosphere of this place did not cease to fascinate me throughout my two weeks stay, and I can only recommend anyone travelling in the area to pay at least a short visit to this remarkably unique place.

This year's course directors where John Murray (University of Pennsylvania, USA), Ke Hu (University of Indiana, USA) and Viki Allan (University of Manchester, UK). Commercial Faculty was represented by experts from Olympus, Nikon, Carl Zeiss, Andor, Applied Precision and Perkin-Elmer, who besides their support and knowledge also provided the state-of-the-art microscope systems used during the course, ranging from standard transmitted light and epifluorescence scopes through spot-scanning or spinning-disk confocal systems up to the impressive OMX set-up by Applied Precision. Mark Bates (Harvard University, USA) , Hari Shroff (University of Berkeley, USA) and Paul Goodwin (Applied Precision) where invited to represent the super-resolution microscopy section, which was a new addition to

the course this year, and besides theoretical lectures included STORM (Stochastical Optical Reconstruction Microscopy), PALM (Photoactivated Localization Microscopy) and Structured Illumination setups with discussion of associated staining and application protocols.

The course was running for 13 full days straight, including one day off about half-way through to be used for either leisure activities or additional experiments in the lab. It started off with a cheese-and-wine reception in a relaxed atmosphere to introduce all of the instructors and the commercial faculty as well as the total of 16 students on the course, whose background was generally in biomedical research and was ranging from PhD students through PostDocs to starting-up Principle Investigators. This setting was ideal to help to break the ice and to get to know the people one would be spending most hours of the subsequent two weeks with. The general lay-out of a course day started after breakfast, with an informal get-together to present the data acquired in the lab sessions the previous day, to discuss the protocols used and to troubleshoot problems. This was followed by a day's (and open-ended night's) worth of lab work, pursuing either a vast set of provided staining or imaging protocols in teams of two, or to follow one's individual research interests using the materials provided, or reagents brought from the home institution. Daily seminars in the afternoon held by the instructors, commercial faculty or special invited guest speakers covered topics related to the course agenda. A personal, special highlight worth mentioning was a talk given by David Spector (Cold Spring Harbor Laboratory, USA), one of the founding fathers of the course and leading expert in employing live cell imaging approaches to study dynamics of nuclear processes. He presented some of his lab's groundbreaking and truly inspiring work on visualizing and measuring the processes occurring during the induction and repression of gene expression.

After an introductory lecture covering various aspects of microscope basics such as resolution and contrast, introduction to the point spread function, signal and noise and other biophysical aspects of light microscopy, the course was essentially divided into two parts, starting with fixed sample microscopy including cell and tissue specimen, followed by intensive hands-on work with living cellular specimen, exploring the different high-end microscope systems. The initial days where dealing with various aspects of immunofluorescence staining options for tissue and cultured cells. Besides the possibility of for example optimizing own antibodies, the lab provided a vast range of antibodies, probes and dyes to either follow pre-designed experimental protocols or individually designed experimental questions. Pre-designed exercises in this section dealt with different fixation protocols and their effects on the preservation of different cellular compartments and antigenicity, blocking steps during antibody staining, and the application of cell-permeant or cell-impermeant non-immunological labelling reagents. Other exercises included protocols for staining of thick tissue sections, covering theoretical and practical aspects of fixation, antigen-retrieval, signal enhancement and reduction of autofluorescence.

Subsequently, the course allowed to choose between either performing Fluorescence *In Situ* Hybridization experiments, or to prepare and analyze samples using the super-resolution microscope systems mentioned above. For the latter part, transiently and stably transfected cell lines expressing for example Dronpa-tagged cell adhesion components where made available to the preference of the student, and allowed hands-on experience employing these next-generation microscope systems to gain insight into the power, but also shortfalls, of these approaches to increase the optical resolution by means of immense data sets and statistical or physical calculating power.

The remainder of the course was covering practical live cell imaging, using again a vast range of culture cells transiently or stably expressing a variety of fluorescence fusion constructs including photo-activatable or photo-switchable fluorophores. Accompanying seminars where covering general points to consider when performing live cell imaging, ranging from temperature and pH control to the different microscope systems and parameters used for short- or long-term four-dimensional imaging. Various established and "new-generation" fluorescent proteins where discussed, as was the importance for adequate controls in light of photo-bleaching and photo-toxicity, but also artefactual data stemming from for example overexpression of the fusion protein of interest or interference with protein function due to the attached tag. Again, a wide variety of pre-designed experiments were provided, with the option of employing the available cell lines for questions addressing personal interests. Microscope booking sheets, teams of not more than two students per scope and the direct availability of commercial faculty were designed to allow the best-possible use of time for direct hands-on work with the impressive range of different microscope systems to perform the given experiments and to assess differences of individual systems.

The course officially concluded with a talk from the distinguished guest speaker, Xiaowei Zhung (Harvard University, USA), the “inventor” of STORM imaging. Xiaowei provided an intriguing and visionary perspective on the future of super-resolution microscopy in both, three-dimensional localization and live cell imaging. The talk was followed by a visit to the infamous CSHL bar (for those who might have missed this venue the previous nights) and a splendid lobster banquet, and rounded up with a late-night gathering with music, champagne, wine and beer (there might have been some water as well) to bid farewell to colleagues and newly-acquired friends.

The Live-FISH course was one of the most inspiring and enjoyable scientific events I have been fortunate to attend to date. An excellent complement of in-depth lectures and discussions, accompanied by intensive practical training sessions under instruction form experts in the field using state of the art equipment conferred an immense amount of theoretical and technical awareness and knowledge. Besides imparting a vast range of background on current labelling strategies, microscopy techniques and principles, another important result of these days was a drastic increase in the confidence of operating both basic as well as elaborate high-end microscopy systems, allowing me to have increased confidence in the quality of data I am producing. In addition, the course has strongly enhanced my passion for microscopy as well as my wish to make microscopic techniques a central component of my future career path.

Apart from the technical and scientific know-how, I also greatly benefited from attending this course on a personal level. Despite its intense schedule, the course was extremely invigorating and I enjoyed immensely the company of the staff as well as my fellow students. The over-all atmosphere throughout was very good, and everyone got on extremely well with one another. I expect not only to profit from a professional relationship such as collaborative research efforts and the exchange of ideas and materials in the future; I also established a level of friendship with some of my colleagues which I hope to maintain and build on over the years to come. Interaction with a high number of US residents further allowed my to gather interesting information on various aspect of live, leisure and work in this country, and has been extremely useful in making up my mind regarding places to apply for in the next stages of my career.

I am extremely grateful to the James Rennie Bequest, without the funding of which I would not have been able to benefit from the experience gained during this visit, and that has allowed me to further mature on so many different levels.