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THE RECENT ELECTRON MICROSCOPY APPLICATIONS (ELECTRON TOMOGRAPHY)

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ABSTRACT

Electron tomography is a new technique for obtaining detailed 3D structures of both biological and non-biological samples relevant to the life and biomedical sciences. Electron tomography is an extension of the traditional transmission electron microscopy. Different protocols concerning the 3D electron tomography, Conventional TEM, Immuno-electron microscopy and cryoEM will be covered. With these methods, relatively large volumes of resin-embedded biological structures can be analyzed at resolutions of a few nm within a reasonable expenditure of time. Therefore, this work could support the collaborative research activities of many investigators from various departments in the faculty of veterinary medicine whose work has already had a major impact in the areas of biological, life and physical sciences.

INTRODUCTION

Electron tomography is a technique for obtaining detailed 3D structures of both biological and non-biological samples relevant to the life and biomedical sciences. Electron tomography is an extension of traditional transmission electron microscopy . In electron microscopy, where the beam direction is fixed, the specimen holder is tilted around a single axis. However; the usage of electron tomography has recently become more liberal, encompassing arbitrary geometries, provided that the specimen is actively tilted into multiple angles. Also, the modern scanning electron microscopes (SEM) enable sample imaging at magnifications up to x1, 000,000. Imaging coupled with analytical capability for chemical and biological evaluation makes SEM a powerful tool in anatomy and developmental biology applications. Different facilities for Electron Microscopy Research such as the 3D electron tomography, Conventional TEM, Immuno-electron microscopy and cryoEM are used. This work could support and foster the collaborative and multidisciplinary research activities of many investigators from various departments in the faculty of medicine whose work has already had a major impact in the areas of biological, life, materials, and physical sciences.

Objectives

- Comprehensive study of the electron microscopy tomography which include theory, principles and practical applications of imaging. Beside analysis and advanced sample preparation relevant to the biological materials.
- The proposed course will provide the opportunity to acquire advanced knowledge in the field of the electron microscopy tomography.

- Utilize all the gained knowledge and understanding to improve the biomedical research

Different electron microscopy protocols

1. Conventional TEM

Aims: To gain hands on experience from all steps of conventional TEM, including fixation and preparation of specimens, operation of a microscope and basic image analysis.

Content: Preparation of tissues as well as cultured mammalian cells for TEM analysis. The protocols for embedding tissue blocks and cultured cells, the use of ultramicrotomes and in addition, image analysis and processing will be covered.

2. Basics of SEM

Aims: To gain hands on experience from all steps of conventional SEM, including specimen preparation and microscope operation.

Contents: Preparation of different types of specimens: cultured mammalian cells and multicellular organism, and learn examination by SEM.

3. Immuno-electron microscopy

Aims: To get familiar with pre-embedding immunolabelling and flat embedding techniques for cultured mammalian cells, and an immunolabelling of acrylic sections from tissue block.

Content: Cultured mammalian cells will be subjected to pre-embedding immunolabelling using nano-gold conjugated secondary antibodies followed by silver enhancement and gold toning prior flat embedding. The other part consists of immunolabelling of pre-prepared sections from plastic-embedded tissue. The trainee will get hands on ultra-thin sectioning, image analysis and processing required for obtaining high quality images and quantitative data will be covered.

4. Electron tomography

Aims: To practice the whole process of 3D electron tomography of plastic embedded specimen.

Content: hands-on training on how to acquire dual axis tilt series, make a tomogram and model an organelle by using computer software Serial EM, IMOD, Microscopy Image Browser.

5. Correlative light electron microscopy

Aims: To guide through the steps from live cell imaging to TEM analysis of the same cell and its subcellular structures with both techniques. These steps include cell culture and imaging on glass bottom dish, fixation and flat embedding, selecting the area of interest for ultra-thin sectioning, and identification of the cell of interest.

Content: Cultured mammalian cells will be transfected with fusion constructs, and analyze the cells first by using live cell imaging, perform photo-oxidation reaction and then process cells for TEM. The ultra-thin sectioning will be demonstrated.

6. CryoEM

Aims: The aim of this advanced module is to experience the process of cryoEM and three-dimensional image processing. The trainee will gain hands on experience in specimen preparation (verification), low-dose TEM imaging, and three-dimensional image analysis.

Content: One week will be spent on specimen preparation and data collection, two weeks will be spent making and analyzing a three-dimensional reconstruction of a macromolecular complex using the software EMAN and CHIMERA.

Expected outcome:

- Improve the practical applications of imaging, analysis and advanced sample preparation relevant to anatomy and developmental biology applications
- Acquire advanced knowledge in electron microscopy field to improve the biomedical research .

REFERENCES

Villinger C1, Schauflinger M, Gregorius H, Kranz C, Höhn K, Nafeey S, Walther P. : **Three-Dimensional Imaging of Adherent Cells using FIB/SEM and STEM. Methods Mol Biol. 2014; 1117:617-38.**

Scott, M. C.; Chen, C. C.; Mecklenburg, M.; Zhu, C.; Xu, R.; Ercius, P.; Dahmen, U.; Regan, B. C.; Miao, J. (2012). **"Electron tomography at 2.4-ångström resolution".** ***Nature*** **483 (7390): 444. doi:10.1038/nature10934**.

Kremer , Lippens , Bartunkova S, Asselbergh B, Blanpain C, Fendrych M, Goossens A, Holt M, Janssens S, Krols M, Larsimont JC, Mc Guire C, Nowack MK, Saelens X, Schertel A, Schepens B, Slezak M, Timmerman V, Theunis C, VAN Brempt R, Visser Y, Guérin CJ. (2015) **Developing 3D SEM in abroad biological context. J Microsc. 2015 Jan 26. doi: 10.1111/jmi.12211. [Epub ahead of print].**

الملخص العربي

التصوير الالكتروني المقطعي وتطبيقاته الحيوية

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التصوير الإلكتروني المقطعي هو أسلوب حديث للحصول على صور ثلاثيه الابعاد مفصلة من كل من العينات البيولوجية وغير البيولوجية ذات الصلة إلى الحياة والعلوم الطبية الحيوية. التصوير الإلكتروني المقطعي هو امتداد للمجهر الإلكتروني النافذ. فيما يتعلق بالتصوير المقطعي الإلكتروني 3D، Conventional TEM, Immuno-electron microscopy cryo EM. مع هذه الأساليب، كميات كبيرة نسبيا من العينات البيولوجية يمكن تحليلها بدقة الي عدد قليل ممن النانومتر ضمن فتره معقولة من الزمن. لذلك، يمكن لهذا العمل دعم أنشطة البحوث التعاونية لكثير من المحققين من مختلف الإدارات في كليات الطب البيطري الذي لها تأثير كبير في مجالات البيولوجية والحياة والعلوم الفيزيائية.