分子イメージングセミナー
RIKEN Molecular Imaging Seminar

生物試料超薄切の課題および電子顕微鏡・ウルトラミクロトームの関連技術紹介

日 時　　2018 年 4 月 19 日（木）13:00～17:30
場 所　　理化学研究所　生命機能科学研究センター
　　大会議室　（神戸 MI R&D センター 2F）
　　神戸市中央区港島南町 6-7-3
アクセス　　http://www.clst.riken.jp/access.html
言 語　　日本語、英語

スケジュール
13:00-13:15　　生物試料超薄切の課題
　　講演者: 久米 慧嗣 (理研 BDR、理研 RC)
13:15-13:25　　電子顕微鏡関連製品の紹介
　　講演者: 工藤 大樹 (日新イーエム株式会社)
13:25-14:15　　バイオ分野における超薄切片法
　　講演者: Helmut Gnaegi (DiATOME)
14:15-14:30　　ダイヤモンド単結晶の製造技術とその応用
　　講演者: 藤森直治（株式会社イーディーピー）
14:30-15:00　　ブレーク ＆ ディスカッション
15:00-17:30　　ウルトラミクロトーム実技講習 (DiATOME) ※
※希望者５名程度で実施予定。希望者多数の場合には事務局で選考を行います。

主 催　　理化学研究所 生命機能科学研究センター
共催　　理研エンジニアリングネットワーク、健康“生き活き”羅針盤リサーチコンプレックス

セミナー参加の事前申込が必要です。以下の参加登録フォームよりご登録ください。
https://goo.gl/jyPkz7

お問合わせ：理化学研究所 生命機能科学研究センター
　　細胞機能評価研究チーム
　　(TEL:078-304-7160, E-mail: riken-cfi-en-seminar@ml.riken.jp)

分子イメージングセミナー
RIKEN Molecular Imaging Seminar

Issues of Ultra-Thin Sectioning for Biological Samples and Introduction of Technologies and Products Related to Electron Microscope and Ultramicrotome

Date&Time	13:00～17:30 on 19th April 2018 (Thu.)
Venue	Conference Room, RIKEN Center for Biosystems Dynamics Research 6-7-3 Minatojima-minamimachi, Chuo-ku, Kobe
Access	http://www.clst.riken.jp/en/about/access/
Language	Japanese, English

Schedule

13:00-13:15	Issues of ultra-thin sectioning for biological samples Speaker : Satoshi Kume (Research scientist, RIKEN BDR, RIKEN RC)
13:15-13:25	Introduction of products related to electron microscope Speaker : Hiroki Kudo (Nisshin EM Co., Ltd.)
13:25-14:15	**Ultramicrotomy in biology (including serial sectioning)** **Speaker : Helmut Gnaegi (Managing Director, DiATOME)**
14:15-14:30	Manufacturing of artificial single crystal diamond and its application Speaker : Naoji Fujimori (CEO, EDP corp.)
14:30-15:00	Break & Discussion
15:00-17:30	Practical training course for Ultramicrotome (DiATOME) ※

※We plan to implement it with about 5 participants. In the case of a large number of applicants, we will make a selection at the secretariat.

Organizer	RIKEN Center for Biosystems Dynamics Research
Co-organizer	RIKEN Engineering Network Project, The “Compass to Healthy Life” Research Complex program

Advance application for seminar participation is necessary. Please register from the below form.
https://goo.gl/jyPkz7

Inquiry Laboratory for Cellular Function Imaging,
RIKEN Center for Biosystems Dynamics Research
(TEL:078-304-7160, E-mail: riken-cfi-en-seminar@ml.riken.jp)

Special lecturer

Helmut Gnaegi (Managing Director, DiATOME)

Ultramicrotomy in Biology

Abstract

Serial ultrathin sectioning for 3D reconstruction gains increasing interest in the life science community. Sample trimming, sectioning, pick-up on TEM grids, glass slides or silicon wafers is presented. Sections as thin as 30nm are requested to get better resolution in TEM or SEM. The compression is a major obstacle to achieve such thin sections. The thinner the sections, the higher the compression. A nominal setting of 30nm at the ultramicrotome results in sections with a thickness of approx. 50-60nm. The compression factor also depends on the resin type. We discuss the compression and how to reduce or avoid.

Cryo-ultramicrotomy is used to obtain ultrathin cryo-sections from cryo-fixed or aldehyde-fixed cryo-protected samples. Samples which were soaked in 2.3M sucrose over night are removed from the sucrose, cut into small pieces and placed on the aluminium pins of the UC7/FC7 cryochamber. The pins with the samples are immersed in liquid nitrogen, then transferred to the cryochamber. Trimming is performed preferably with the socalled trim diamond blades. The size of the sample block depends on the desired section thickness. Good trimming results in long section ribbons. Section ribbons are cut with a cryo 35° diamond knife. For both the trimming and the sectioning the use of an antistatic device is mandatory. The ribbons are picked up with the aid of a loop and a sucrose/methyl cellulose droplet, then placed onto the grids at room temperature. The use of sucrose/methyl-cellulose led to a better structural preservation (W. Liou et al., Histochem. Cell Biol. 1996). Excellent structure preservation is mandatory for precise localisation of proteins.

Cryo-ultramicrotomy of frozen hydrated samples is performed at 120K. The sample preparation by high pressure freezing, cryoultramicrotomy, cryo-transfer and cryo-TEM preserve living matter close to the native state (A. Al-Amoudi et al., EMBO Journal 2004). Ultrathin cryosections serve for high resolution imaging and 3D reconstruction (C. E, Hsieh et al., Joumal of Structural Biology 2006. A. Al-Amoudi et al., Nature 2007). Special 25° and 35° cryo diamond knives allow the cutting of cryo-sections with reduced compression. A crucial part in cryo-sectioning is the section pick-up. An ionizer/charger eliminates electrostatic charging in the cryo-chamber and improves the gliding of the cryo-sections (J. Pierson et al., Journal of Structural Biology 2010). In addition the ionizer/charger electrostatically fixes cryo-sections on the carbon film of the grid. A crucial part is the section pick-up. A micromanipulator system composed by two parts was developed (Studer et al., Journal of Structural Biology 2014) for pulling the section ribbons and holding the grid. A holy carbon film keeps the sections flat during tilting and imaging (J. Quispe et al., Microscopy & Microanalysis 2007). An obstacle for successful electron tomography of vitreous sections (TOVIS) is the compression and the crevasses. Possible scenarios for reducing these artifacts are discussed.