No. 25

Integration of Imagings Using Electrons and Photons

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1) Introduction

As a visualization method for biological systems, currently, fluorescence microscopy, together with genetical labeling of fluorescent proteins has been an indispensable and profoundly popularized tool. On the contrary, biological electron microscopy (EM) is not any more a champion of microscopes as resolution-wise various probe microscopes can already match with that of EM and moreover internal drawbacks of EM such as tedious sample preparation or electron radiation damages have been negatively high-lighted. To overcome this long-term pitfall, what our electron microscopists have to do is innovations in EM methodology. One example of innovations recently developed in my laboratory is a phase contrast EM using phase plates and the other is a hybrid microscope capable to visualize the same field simultaneously with electrons and photons. Toward an integration of the two microscopic fields, I will report my recent experience with the two innovations.

2) Development and Applications of Phase Plate Electron Microscopy

The tedious sample preparation in EM has been recently replaced by cryo-technology including cryo-fixation and cryo-electron microscope. Due to the artifacts inevitably implemented in the traditional sample preparation, almost no novel kind of discoveries in biological structure has been reported with EM for last twenty years. Starting from 1980s and established in 1990s, the cryo-technique using nitrogen liquid, quick or rapid freezing, has settled at once the issues of fixation and embedding otherwise lengthy processes in the traditional preparation. As shown in Fig.1, however, EM researchers have been suffering from a new kind of drawback; a very weak imaging contrast.

Fig. 1 Fine Cryo-Fixation but No Contrast

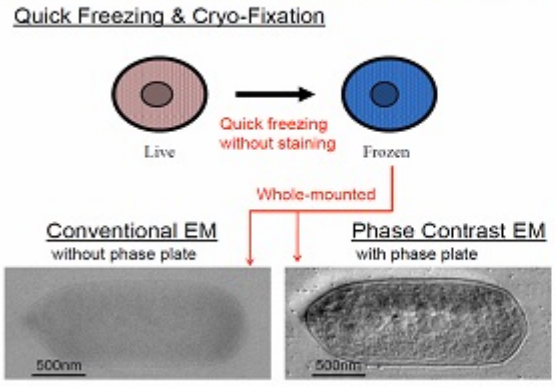
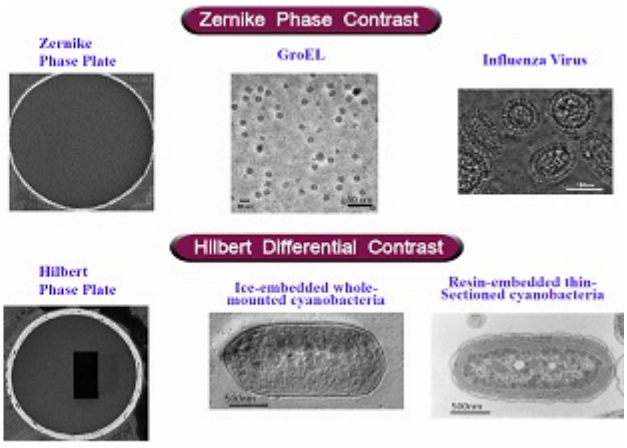


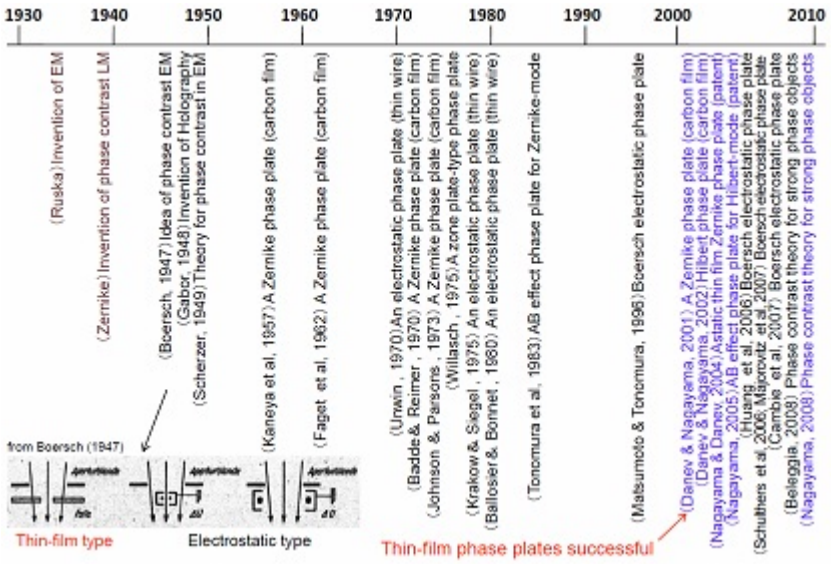
Fig. 2 Phase-plate Electron Microscopy



Zernike phase contrast EM using a phase plate introduced in 2001 [1] and Hilbert differential contrast EM using another type of phase plate [2] (refer to Fig. 2) have completely smashed over the difficulty of low contrast associated with conventional EM. This innovation born in Okazaki has been developed also to the practical biological applications.

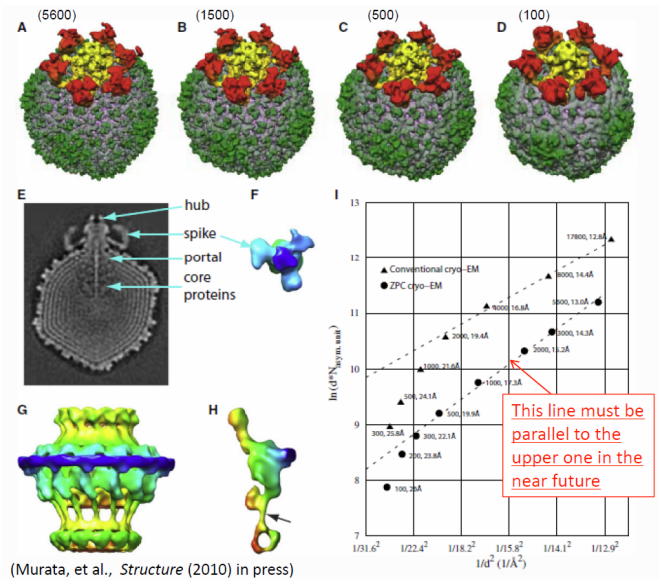
Historically the quest to find a workable phase-plate EM has begun from 1940s immediately after the innovation of EM itself. But due to the enigmatically difficult issue of phase plate charging, its realization belongs to a news within the past ten years.

Fig. 3 60-year Quest of Phase-Plate EM



Actually our papers reported for last ten years as shown in references [3-11] are data collection of successful experimental images, which have happened to be recorded by using with relatively charge-free phase plates. The success rate of charge-free phase plates has always been very low. A difficulty worse than the low success rate is a charge evolution inside the EM column, even with a initially charge-free phase plate, mainly due to the water burst from an ice-embedded specimen itself in the electron irradiation. After insertion of phase plates and a stat of electron irradiation to samples, rather soon, the charge-free one changed to charged one. This is natural as the phase plate even heated at 200℃～300℃ is very close to the object of ice-embedded specimens. Something evaporated from the specimen and ice may be causes of various kinds of contaminations including water.

Fig.4 PP EM Improve the Efficiency for Single Particle Analysis



(Murata, et al., *Structure* (2010) in press)

Recently a completely charge-free phase

plate even under the condition of electron irradiation to specimens has been reported in a meeting held in Portland (M&M 2010) [12].

Figure 4 is showing an example to what extent the phase plate renovated EM in the analysis of single particles. A kind of virus species was studied in the 3D conformation and a phase-plate EM has shown higher efficiency in a 3D structure analysis of a virus compared with conventional one.

Fig.5 Conventional Tomogram of T4 Phage (3.2 μm underfocus)

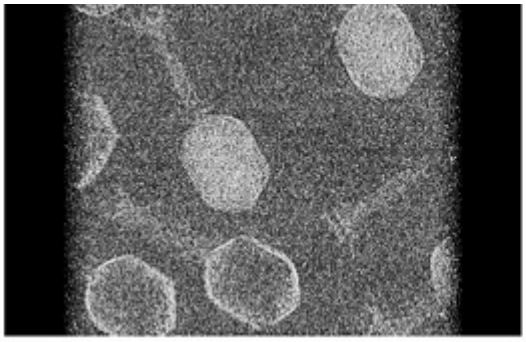
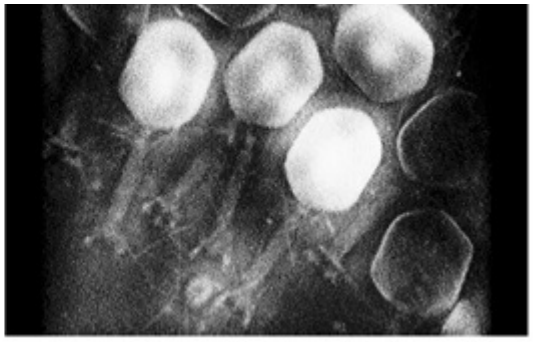


Fig.6 Zernike Phase Contrast Tomogram of T4 Phage (in focus)



Also in cryo-tomography, phase-plate EM is quite useful as shown in Figs.5 and 6. Until now phase contrast cryo-tomography has been applied to various biological systems such as DNA-lipid complexes, protein complexes, viruses and cryo-sectioned brain tissues.

3) Correlative Microscopy with a Electron-Photon Hybrid Microscope

Under the financial support of CREST (2006-2009), our laboratory together with JEOL OB-engineers has been coordinating an integration of two individually developed microscopes, namely transmission EM (TEM) and fluorescent LM (FLM). What is the most important notion in the integration is a recording of TEM and FLM images must be made for the same view field at the same moment. To fulfill this novel instrumental idea, a hybrid microscope managing electrons and photons simultaneously as illumination and detection sources has been designed and manufactured as shown in Figs. 7 and 8.

Fig.7 An Electron-Photon Hybrid Microscope

(Iijima et al, unpublished)

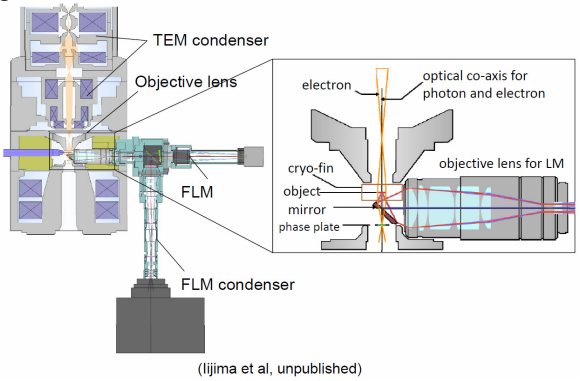
TEM

Body	JEM-2010
Emitter	LaB6
Objective lens	f=10.9mm r=0.42nm Cs=10.5mm Cc=8.4mm
CCD camera	Watec WAT-120N+ Pixels: 640x480 Cell size: 8.4μmx9.8μm

FLM

Objective lens	Mitsutoyo PlanApoSL 50x NA=0.42, f=4mm, WD=19.0mm, r=0.7μm
CCD camera	Watec TGv-M Pixels: 640x480 Cell size 8.4μmx9.8μm

Fig.8 Schematics of a Simultaneous Correlation



(Iijima et al, unpublished)

Fig. 9 EM Finds What is There but Not What We Want to Find

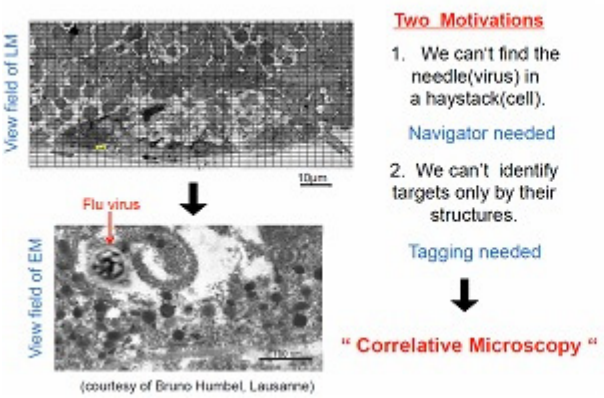
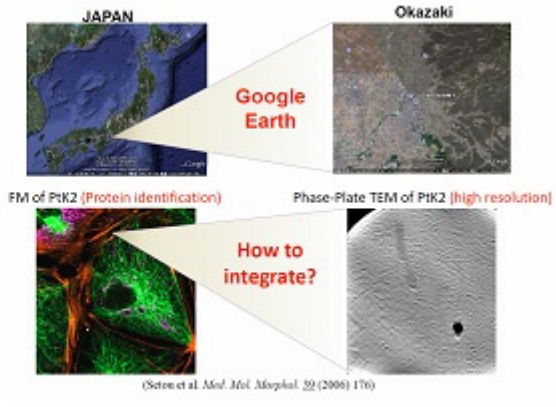


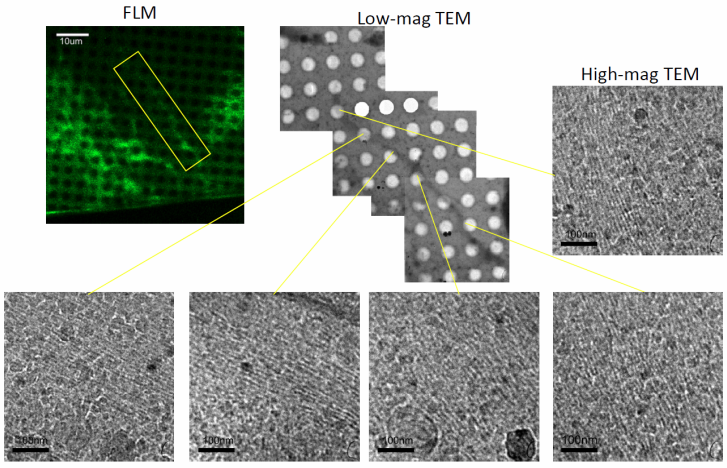
Fig. 10 How to Integrate Two Microscopic Modalities



The motivation behind the development was double. One is to fix the weak point of TEM that it is not easy to observe a target what observers want to find. This is schematically shown in Fig. 9 as TEM is not good for target specific labeling and the TEM view-field is too small for a large are to be surveyed to find the target. The other is that it is desirable as like Google Earth to survey a wider field, search the target and close it up at a higher magnification (refer to Fig. 10).

One of application examples of the developed electron-photon hybrid microscope is shown in Fig.11, where you can recognize bundles of actin filaments with FLM and TEM simultaneously. The connection between very differently magnified two images of FLM and TEM was made by using a low-magnified TEM image as shown in Fig. 11. The fine fiber bundles observed with TEM has been to date interpreted as actin one and this simultaneous observation for the target has now been experimentally proving it.

Fig. 11 Real-time CM Images for a PtK2 Whole Cell Expressing YFP-βactin



4) Conclusions

The way for us to show with two kinds of TEM innovations is only a first step toward integrated correlative microscopy.

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