様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Adaptive optics approach for high-throughput, high-resolution cryo-electron microscopy of biological macromolecules and complexes

研究課題名（英文）Adaptive optics approach for high-throughput, high-resolution cryo-electron microscopy of biological macromolecules and complexes

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研究成果の概要（和文）：低温電子顕微鏡法は、タンパク質の三次元構造を高解像度で決定できる強力な手法です。 この手法はサンプルから何千もの画像を取得する必要があります。以前は、高品質の結果を得るために数日間のデータ収集が必要でした。 毎月数人の研究者しか高価な電子顕微鏡を使用できなかったため、あまり効率的ではありませんでした。 本プロジェクトでは、画像取得速度を5倍以上向上させる手法を開発し、実用化しました。 以前は、1日に約1000枚の画像しか収集できませんでした。現在では、平均して毎日約5500枚の画像を収集しています。 これにより実験の効率が大幅に向上し、より多くの研究者が顕微鏡を使用できるようになりました。

研究成果の学術的意義や社会的意義
低温電子顕微鏡における画像取得の大幅な速度改善により、タンパク質の構造研究の加速が期待されます。 生体分子の機能を理解するには、構造を知ることが不可欠です。したがって、 今回の研究の成果である新しい方法は、生物の基本的なメカニズムに関する知見が得られると期待されます。 さらに、分子の生物学的役割と機能についての深い知識は、さまざまな病気の原因と影響を理解するのに役立ちます。 高解像度の構造情報は、慢性および感染症の治療薬の開発に不可欠です。この研究は、私たちの基本的な生物学的知識を拡げ、新しい病気の治療法の開発を支援することにより、社会の幸福と生活の質の向上に貢献します。

研究成果の概要（英文）：Cryo-electron microscopy is a powerful technique that can determine the three-dimensional structure of proteins with high-resolution. It requires the acquisition of thousands of images from the sample. Previously, several days of data collection were required to produce a high-quality result. This was not very efficient because only a few researchers could use the expensive electron microscope every month. In this project, we developed and applied in practice a method that improved the image acquisition speed more than five times. Before, we could collect only about 1000 images per day. Now, we are collecting on average about 5500 images every day. This greatly improved the efficiency of the experiments and allowed more researchers to use the microscope.

研究分野：構造生物学

キーワード：cryo-electron microscopy　molecular structure

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景（Research background）

In the last ten years, cryo-electron microscopy (cryo-EM) experienced a “resolution revolution”. The performance of the technique was improved drastically by the introduction of direct electron detectors. These new cameras provide much better signal-to-noise ratio by enabling detection and counting of single electron and allow the acquisition of multi-frame “movies” instead of single shot images. The jump in detector performance and new data processing methods led to vastly higher resolution in the reconstructed 3D maps biological macromolecules. The resolution improved from ~10 Å to ~3 Å, which allows building of atomic models of the investigated proteins. The new performance level of cryo-EM greatly expanded its scope of applications and increased its popularity. Many researchers from other structural biology fields, such as x-ray crystallography, became interested in using cryo-EM in their studies. The high cost and limited availability of high-end cryo-microscopes created a congestion at microscope facilities. A typical dataset for a high-resolution 3D reconstruction of a molecule consists of ~5000 images. The automated data acquisition speed was typically ~1000 images/day. This meant that each dataset required several days of data acquisition. Consequently, each microscope could be used for less than ten projects per month.

２．研究の目的（Purpose of the research）

With this research we aimed to resolve the experimental throughput limitation of high-end electron microscopes by increasing the acquisition speed several-fold. This will improve the efficiency of microscope usage and allow more researchers and projects to be accommodated in the same amount of time. The increased throughput also means that researchers will be able to get faster results and/or results from more samples. And finally, by improving the productivity of high-cost electron microscopes, the use of public funds in the form of research budgets will be much more efficient.

３．研究の方法（Method of research）

To improve the efficiency and speed of cryo-EM data acquisition, we will develop and implement an “adaptive optics” method. One of the most time-wasting steps in cryo-EM data collection is the wait of ~20 s after each sample stage movement for the mechanical drift to settle. If we can eliminate this step, the image acquisition speed will be drastically improved. The new optical approach involves using the beam deflectors of the microscope to shift the beam optically to nearby areas on the sample instead of moving there with the specimen stage. This will eliminate the mechanical drift associated with stage moves and will significantly reduce the required instances of drift-settling pauses. To achieve our goal, we followed the following research plan:

(1) Accurately calibrate the beam deflector and investigate its linearity and range.
(2) Develop acquisition routines for multi-shot “adaptive optics” data collection and implement them using the scripting language of the data acquisition automation software package SerialEM.
(3) Test the adaptive optics routines with test samples to determine their efficiency and to incorporate possible improvements.
(4) Use the new method for data acquisition of actual research project samples and further refine the routines if necessary.

４．研究成果（Research results）

(1) We performed a careful and accurate calibration of the beam-shift deflector coils. This included measurement of the absolute amount of the beam shift and the perpendicularity of the X and Y shifts. Furthermore, beam shift alone is not sufficient for optically shifting the image area. It must be combined with image-shift deflector coils in order to bring the shifted beam image back on the detector. We carefully calibrated the beam-image shift compensation to achieve accurate compensation and stationary beam position on the camera independent of the applied beam-image shift. After completing the calibrations, we measured the usable range of the beam-image shift and determined that at least 10 µm of shift can be used in all directions. This completed the microscope preparations for starting the development of the software components for the adaptive optics approach.

(2) We selected the free microscope control and automation software package SerialEM as the platform on which to build the adaptive optics routines. It provides almost full remote control of the microscope hardware, has many built-in functions and offers comprehensive scripting support. We started development and testing of the routines with the simplest target acquisition pattern comprising just four support film holes around the stage position in the center. After confirming its feasibility, we collected a test dataset which also showed the beam-image shift routine is working as intended. We then moved on to developing a routine that is capable of collecting images in nine holes around the stage position. The development was successful, and the new routine became the standard data acquisition method at the cryo-EM facility.

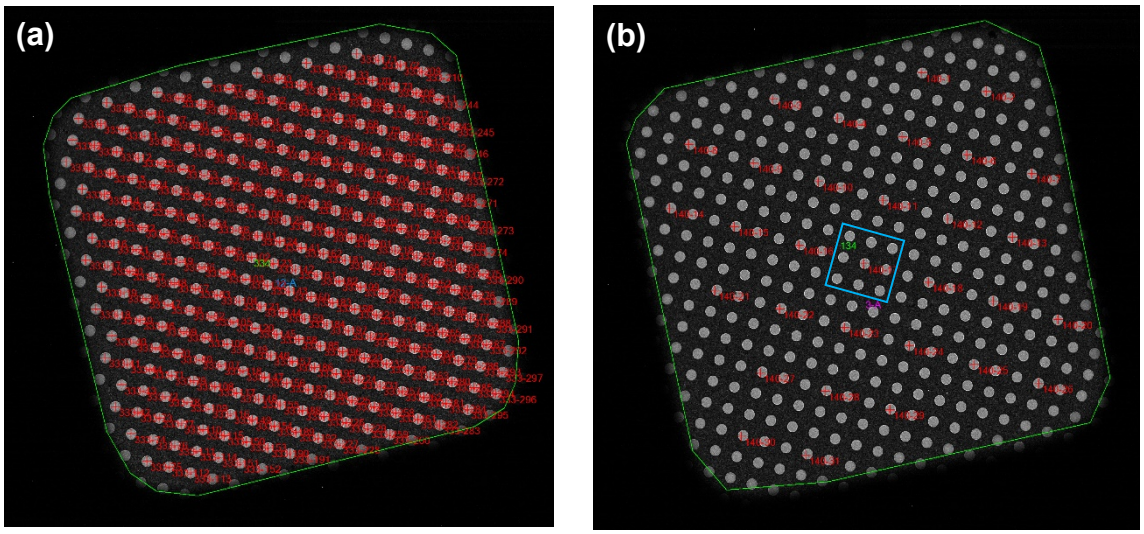

Figure 1. Illustration of the stage positions (red crosses) before (a) and after (b) the implementation of the adaptive optics approach. The blue square in (b) shows a nine-hole area around a stage position that will be acquired with beam-image shift.

Fig. 1 shows a comparison between the stage movement positions (red crosses) without (a) and with (b) the adaptive optics approach. The new approach reduces the stage moves by almost ten times and saves the according amount of drift-settling time.

(3) We tested the new data acquisition method with an apoferritin test sample and it performed according to the desired specifications. We added additional options for automated centering of the zero-loss peak of the energy filter and camera dark reference acquisition every few hours, which improved stability of the system and the quality of the data. At this stage of the development we hadn't yet incorporated a beam-tilt compensation algorithm, but because coma-correction could be performed in the data processing software we started using the new acquisition technique in actual biological project applications.

(4) After the optical shift data acquisition approach started working reliably in tests, we immediately started to use it for actual data acquisition. From the beginning, the method worked very well and we were able to acquire data much faster than with the old stage-move technique. On average, the new method achieved image acquisition throughput of 260 images/hour, which translates to 5,500 ~ 6,000 images/day. The variations in daily throughput are due to the extra offline time required to load and set up the acquisition for a new sample. This was a dramatic improvement and allowed to acquire a complete dataset in 24 hours instead of several days. After using the method in several projects and correcting the off-line coma during software processing, we incorporated the final component of the approach - on-the-fly beam-tilt compensation. This reduced the off-axis coma in the data to almost negligible levels and now provides a much better starting point for achieving high-resolution reconstructions. With the use of the new data acquisition method, we were able to achieve several groundbreaking results in determining protein structures at unprecedented resolutions. This includes several G-protein coupled receptor complexes, that are traditionally a difficult target for cryo-EM, at resolutions better than 2.5 Å (see publications list). We will continue to develop and improve the adaptive optics approach in the future in our constant pursuit of excellence in our scientific research.

５．主な発表論文等

〔雑誌論文〕　計6件（うち査読付論文　6件／うち国際共著　6件／うちオープンアクセス　1件）

１．著者名 Danev Radostin、Yanagisawa Haruaki、Kikkawa Masahide	４．巻 44
２．論文標題 Cryo-Electron Microscopy Methodology: Current Aspects and Future Directions	５．発行年 2019年
３．雑誌名 Trends in Biochemical Sciences	６．最初と最後の頁 837～848
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.tibs.2019.04.008	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Eisenstein Fabian、Danev Radostin、Pilhofer Martin	４．巻 208
２．論文標題 Improved applicability and robustness of fast cryo-electron tomography data acquisition	５．発行年 2019年
３．雑誌名 Journal of Structural Biology	６．最初と最後の頁 107～114
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jsb.2019.08.006	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Zhao Peishen、Liang Yi-Lynn、Belousoff Matthew J.、[14 co-authors]、Danev Radostin、Sexton Patrick M.、Wootten Denise	４．巻 577
２．論文標題 Activation of the GLP-1 receptor by a non-peptidic agonist	５．発行年 2020年
３．雑誌名 Nature	６．最初と最後の頁 432～436
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41586-019-1902-z	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Liang Yi-Lynn、Belousoff Matthew J.、Zhao Peishen、Koole Cassandra、Fletcher Madeleine M.、Truong Tin T.、Julita Villy、Christopoulos George、Xu H. Eric、Zhang Yan、Khoshouei Maryam、Christopoulos Arthur、Danev Radostin、Sexton Patrick M.、Wootten Denise	４．巻 77
２．論文標題 Toward a Structural Understanding of Class B GPCR Peptide Binding and Activation	５．発行年 2020年
３．雑誌名 Molecular Cell	６．最初と最後の頁 656～668.e5
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.molcel.2020.01.012	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Liang Yi-Lynn、Belousoff Matthew J.、Fletcher Madeleine M.、Zhang Xin、Khoshouei Maryam、Deganutti Giuseppe、Koole Cassandra、Furness Sebastian G. B.、Miller Laurence J.、Hay Debbie L.、Christopoulos Arthur、Reynolds Christopher A.、Danev Radostin、Wootten Denise、Sexton Patrick M.	4．巻 3
2．論文標題 Structure and Dynamics of Adrenomedullin Receptors AM1 and AM2 Reveal Key Mechanisms in the Control of Receptor Phenotype by Receptor Activity-Modifying Proteins	5．発行年 2020年
3．雑誌名 ACS Pharmacology & Translational Science	6．最初と最後の頁 263～284
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acsptsci.9b00080	査読の有無 有
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1．著者名 Danev Radostin、Iijima Hirofumi、Matsuzaki Mizuki、Motoki Sohei	4．巻 7
2．論文標題 Fast and accurate defocus modulation for improved tunability of cryo-EM experiments	5．発行年 2020年
3．雑誌名 IUCrJ	6．最初と最後の頁 566～574
掲載論文のDOI（デジタルオブジェクト識別子） 10.1107/S205225252000408X	査読の有無 有
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〔学会発表〕　計10件（うち招待講演　9件／うち国際学会　3件）

1．発表者名 Radostin Danev
2．発表標題 Cryo-EM with and without phase plates
3．学会等名 Japanese Society of Microscopy Soft Materials Branch Meeting（招待講演）
4．発表年 2018年

1．発表者名 Radostin Danev
2．発表標題 What makes a great cryo-EM?
3．学会等名 Future of Cryo-EM Developments Meeting（招待講演）
4．発表年 2019年

１．発表者名
Radostin Danev

２．発表標題
Phase Plates in Cryo-EM

３．学会等名
EMBL Cryo-EM in Industry and Academia Workshop（招待講演）（国際学会）

４．発表年
2019年

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Radostin Danev

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３．学会等名
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４．発表年
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１．発表者名
Radostin Danev

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Recent Cryo-EM Advancements

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Radostin Danev

２．発表標題
Practical Performance of the Volta Phase Plate

３．学会等名
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１．発表者名
Radostin Danev

２．発表標題
Cryo-EM of Membrane Proteins With and Without Phase Plates

３．学会等名
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１．発表者名
Radostin Danev

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３．学会等名
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１．発表者名
Radostin Danev

２．発表標題
Performance Contributions of Energy Filtering and Phase Plates for Single Particle Analysis of Membrane Proteins

３．学会等名
NIPS EM Workshop 2019（招待講演）

４．発表年
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１．発表者名
Radostin Danev

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Performance Contributions of Phase Plates and Energy Filtering for Membrane Protein Structure Determination

３．学会等名
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４．発表年
2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考