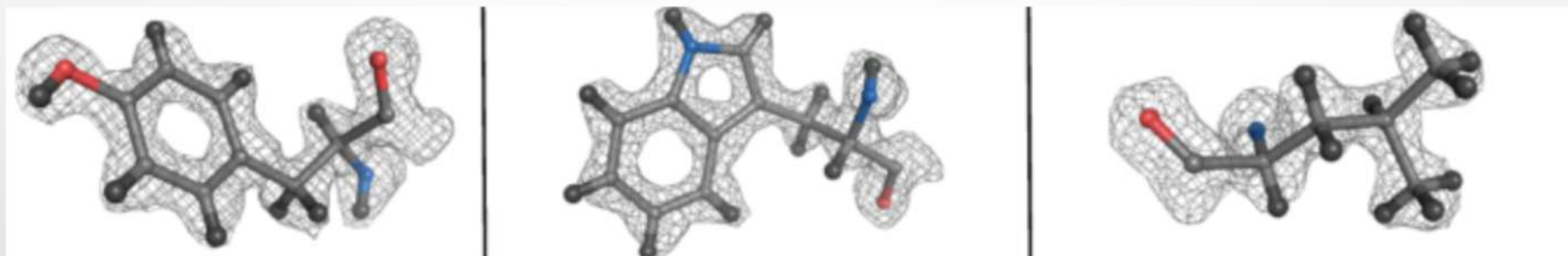


КРИО-ЭЛЕКТРОННАЯ МИКРОСКОПИЯ

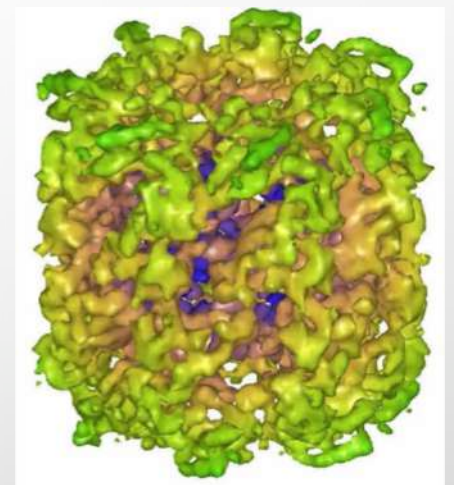
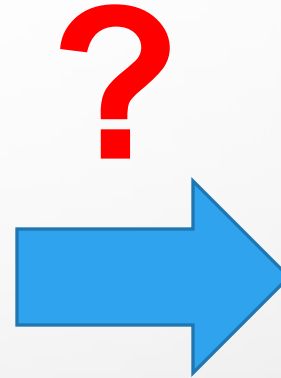
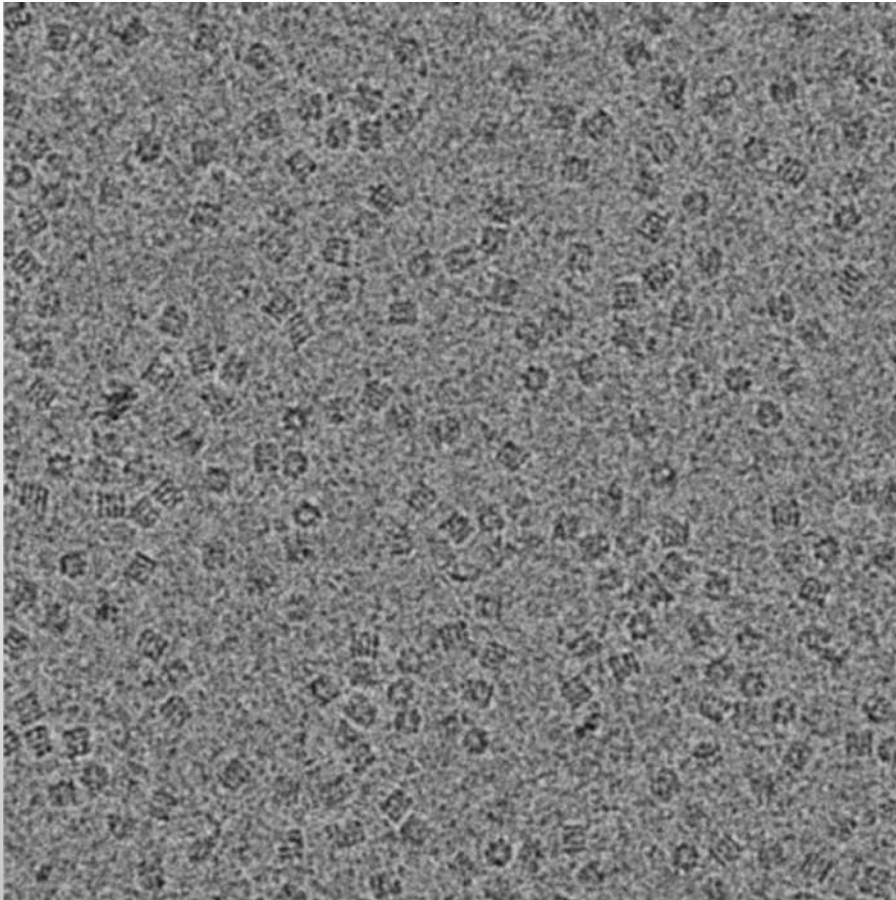
ВВЕДЕНИЕ



ОЛЬГА СЕРГЕЕВНА СОКОЛОВА
Д.Б.Н., ПРОФЕССОР РАН
SOKOLOVA184@GMAIL.COM

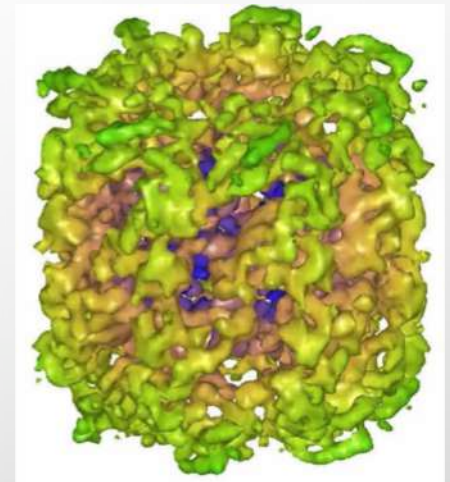
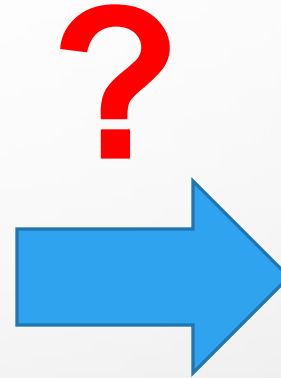
4. ПОЛУЧЕНИЕ РЕКОНСТРУКЦИЙ

ПОЛУЧЕНИЕ РЕКОНСТРУКЦИИ



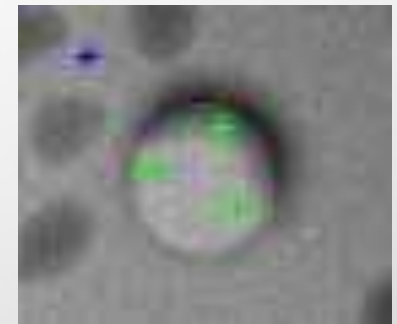
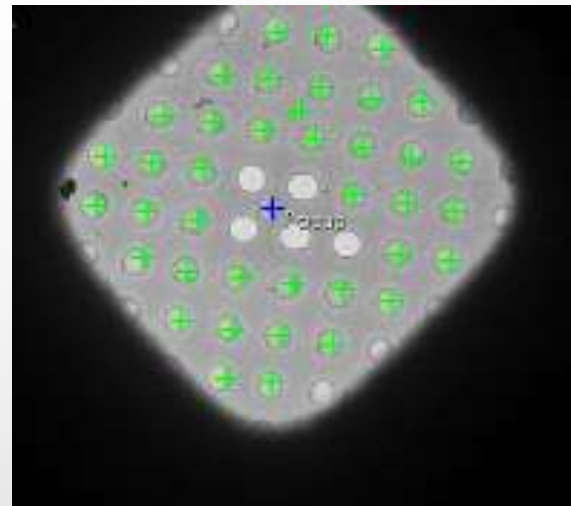
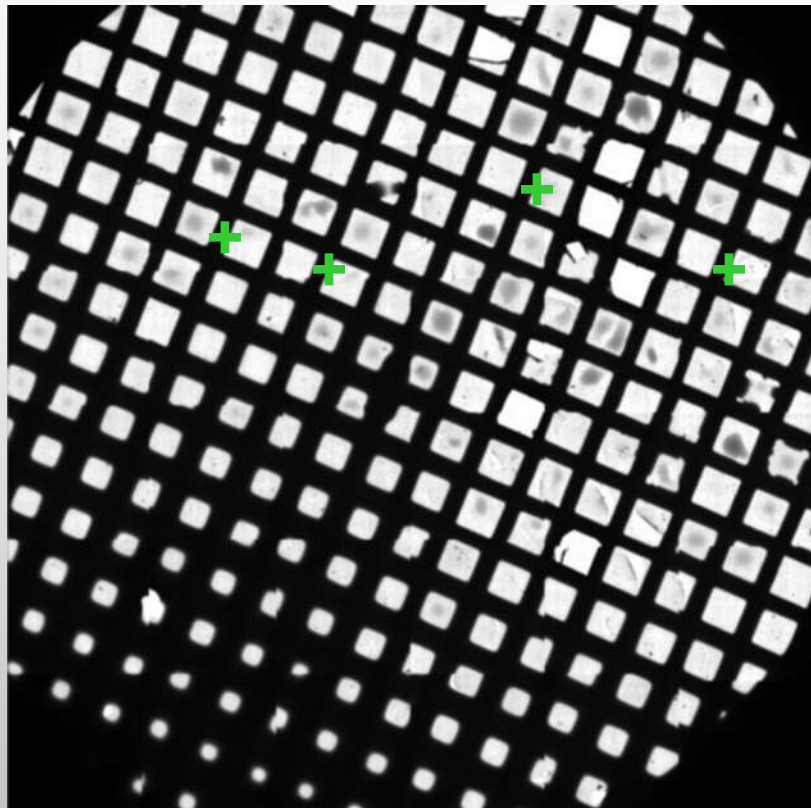
ПОЛУЧЕНИЕ РЕКОНСТРУКЦИИ

- Запись или получение изображений образца в ПЭМ называется сбором данных (или получением изображений);
- Для SPA высокого разрешения* требуется несколько тысяч (несколько миллионов) изображений;
- Автоматизированный сбор данных позволяет получать изображения большого количества частиц.

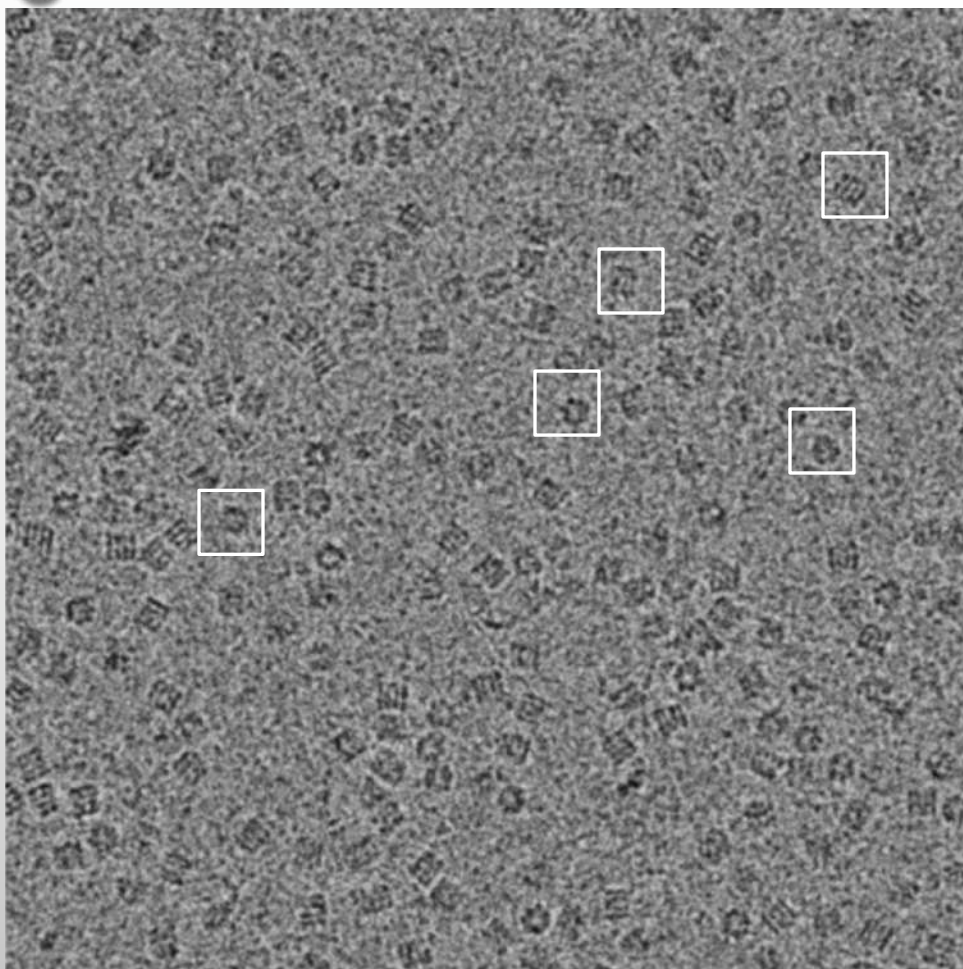


*SPA – Single Particle Analysis

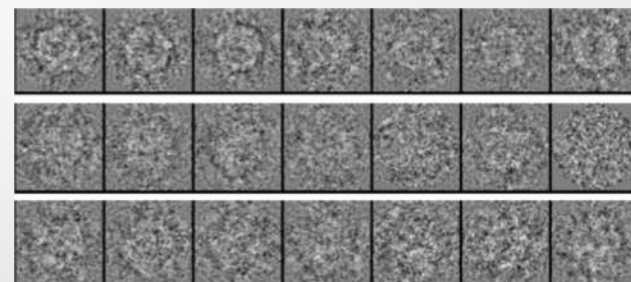
АВТОМАТИЧЕСКИЙ СБОР ДАННЫХ



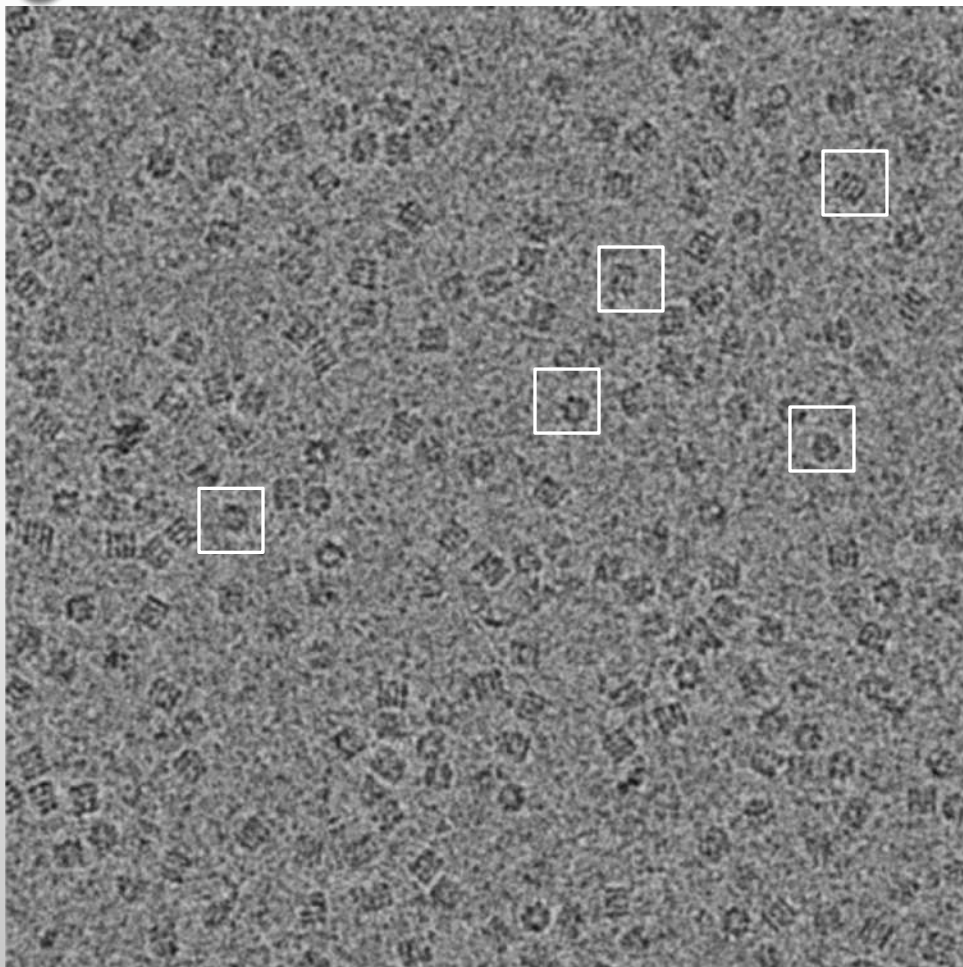
СБОР ЧАСТИЦ



- Ручной сбор
- Полуавтоматический
- Автоматический



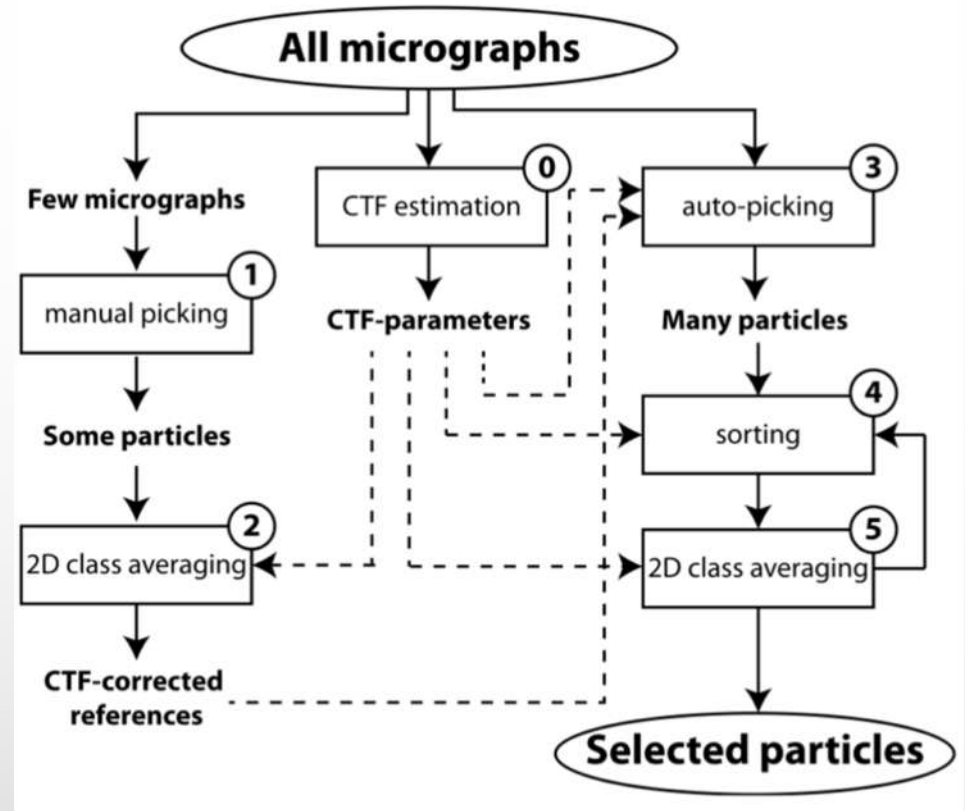
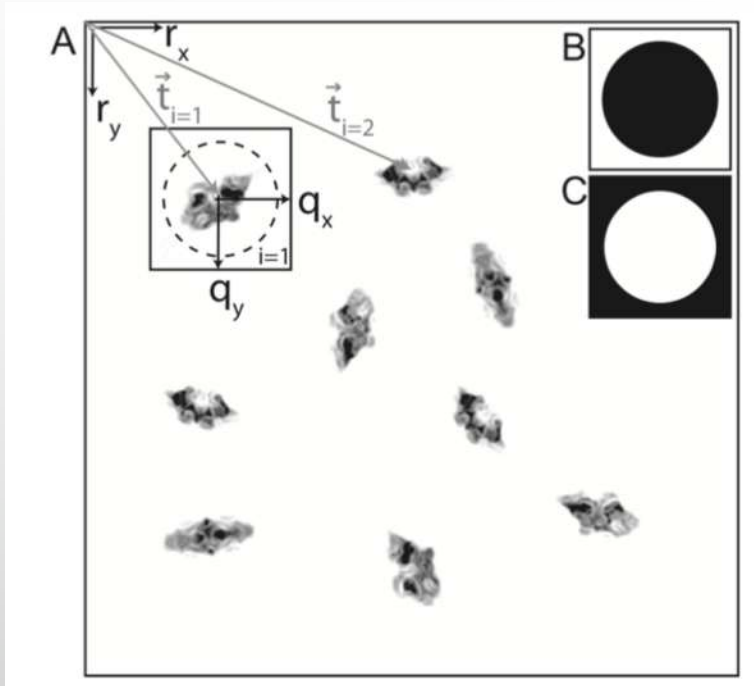
СБОР ЧАСТИЦ



- Ручной сбор
- **Полуавтоматический**
- Автоматический

СБОР ЧАСТИЦ

- **полуавтоматический**

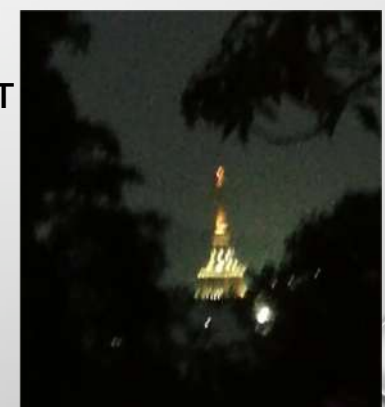
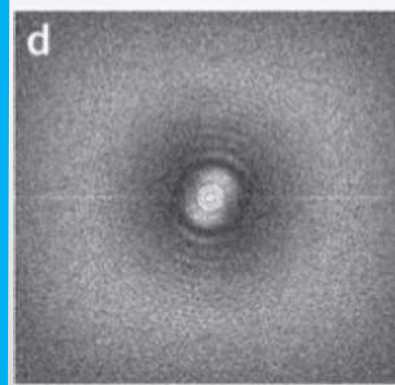
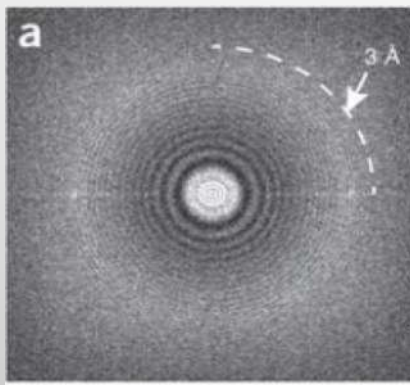
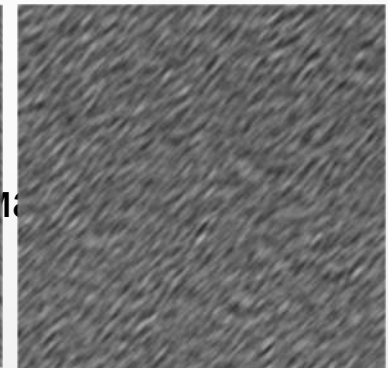
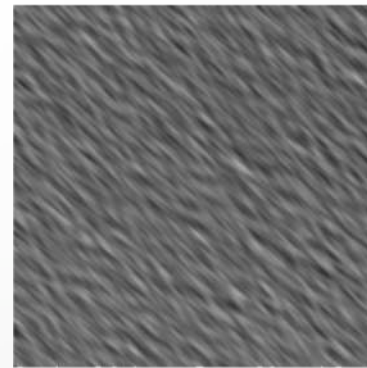
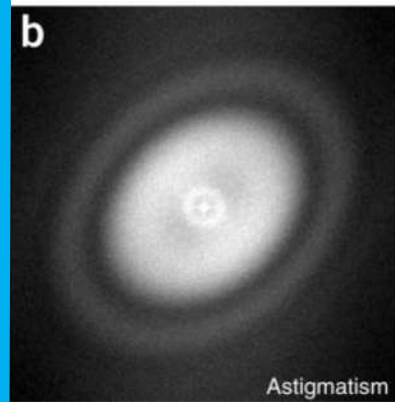
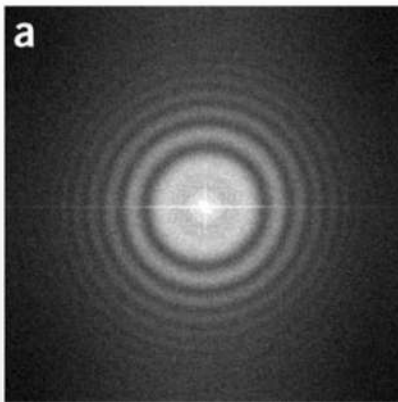


workflow for semi-automated particle selection in RELION

АНАЛИЗ ИЗОБРАЖЕНИЙ: КАЧЕСТВО

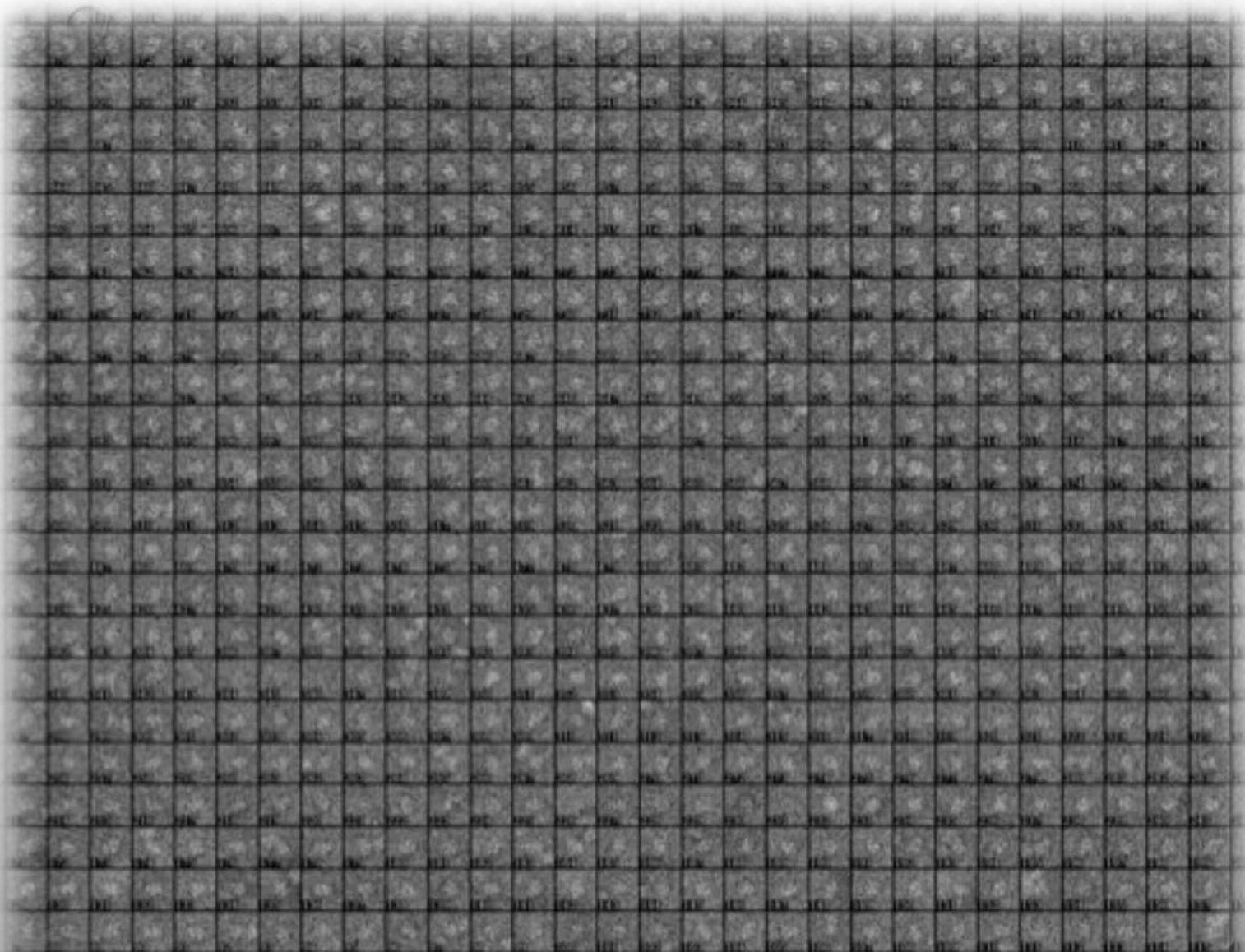
хорошее

плохое

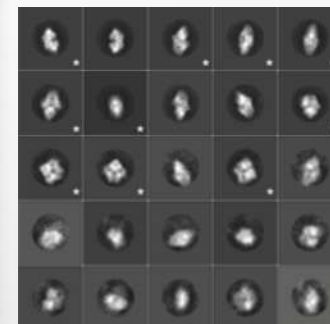


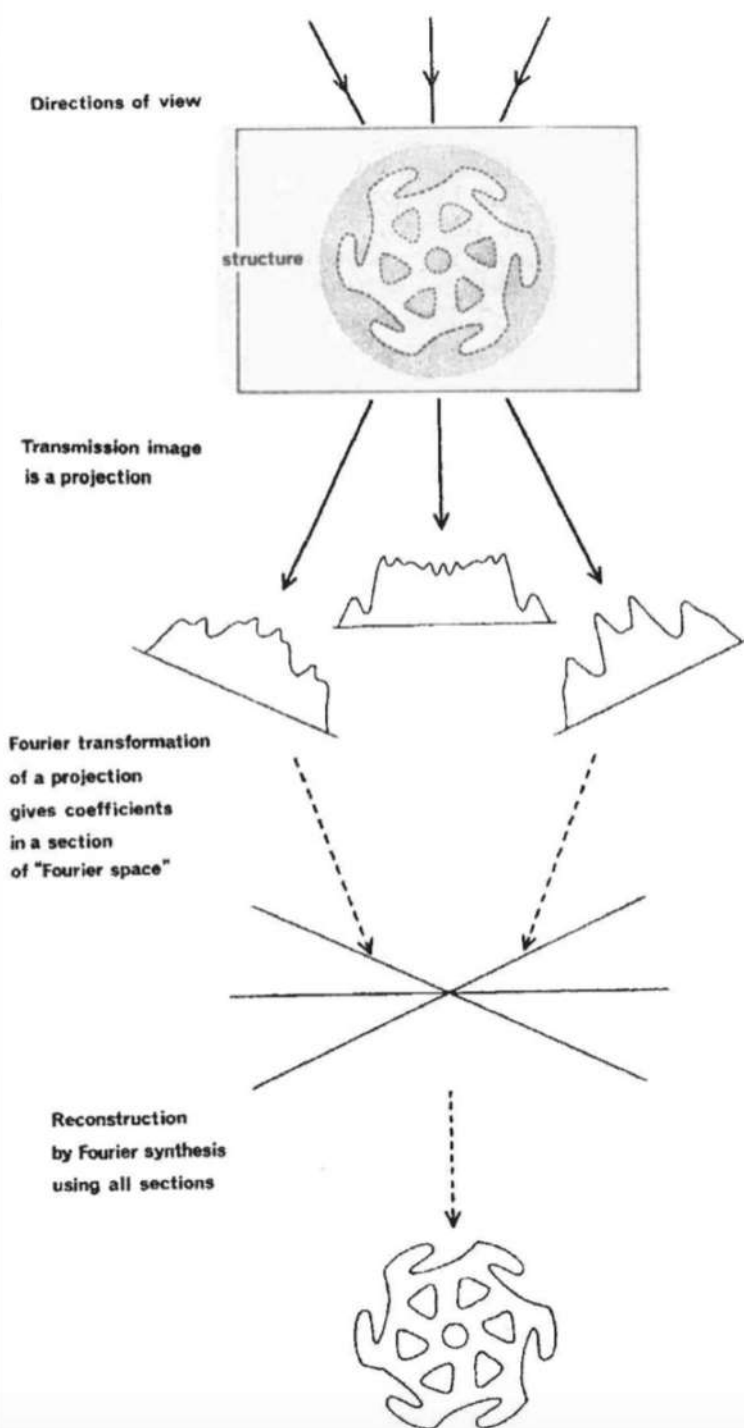
АНАЛИЗ ИЗОБРАЖЕНИЙ: КЛАССИФИКАЦИЯ

25000 частиц



25 классов





1. Изображение в электронном микроскопе представляет собой проекцию трехмерного распределения плотности объекта перпендикулярно направлению обзора.

Jean-Baptiste Joseph Fourier



1768 – 1830

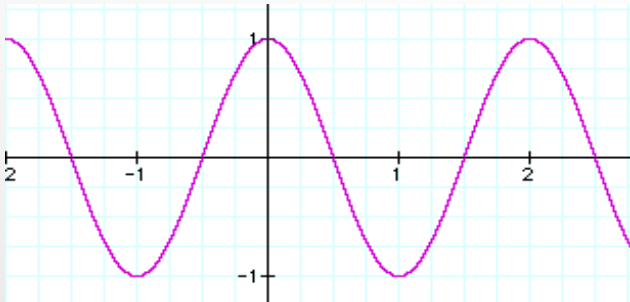
... любая функция от
переменной может быть
представлена серией

синограмм

СВОЙСТВА ПРЕОБРАЗОВАНИЯ ФУРЬЕ

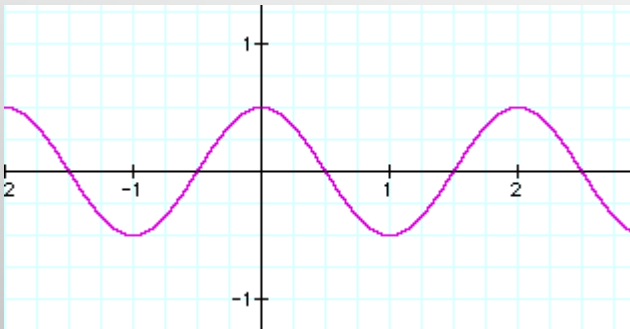
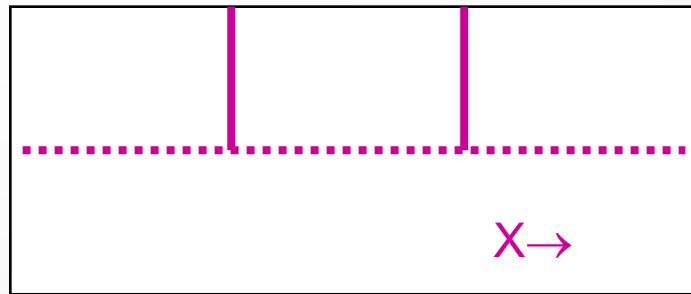
1. Линейность

Fourier transform of a cosine wave.



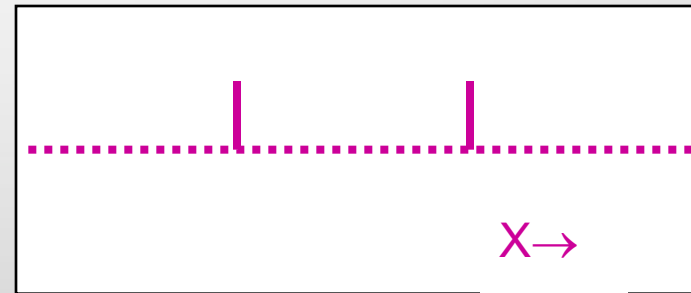
$$y = \cos x$$

FFT
⇒

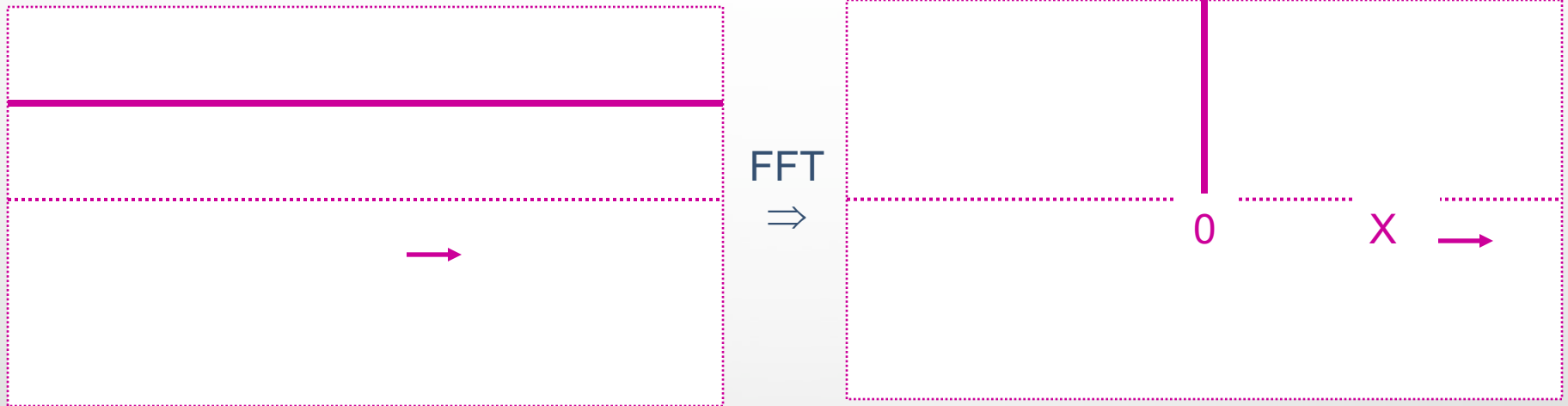


$$y = 1/2 \cos x$$

FFT
⇒



The Fourier Transform of a constant.

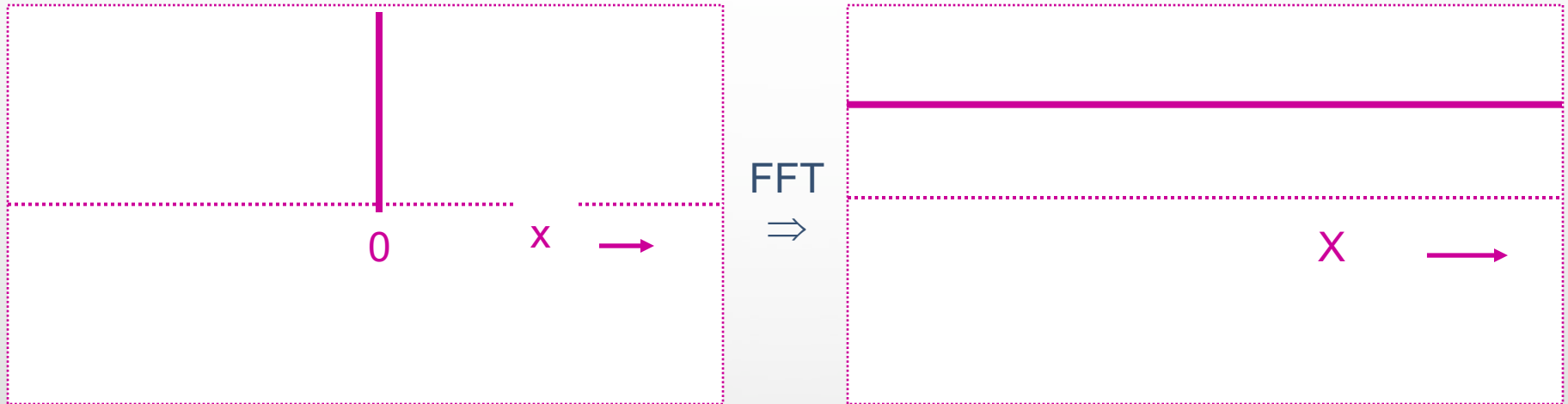


$f(x)=1$

“delta function”

The Fourier transform of a constant is a peak at the origin.

Fourier transform of a delta function

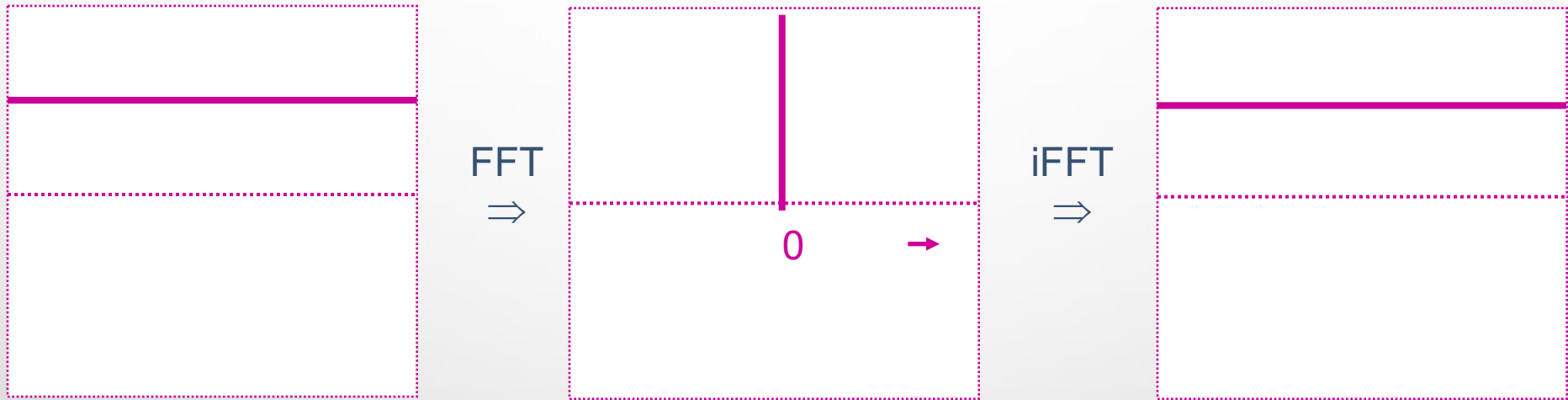


“delta function”


$F(X)=1$

The Fourier transform of a delta function is a constant

ОБРАТНОЕ ПРЕОБРАЗОВАНИЕ ФУРЬЕ



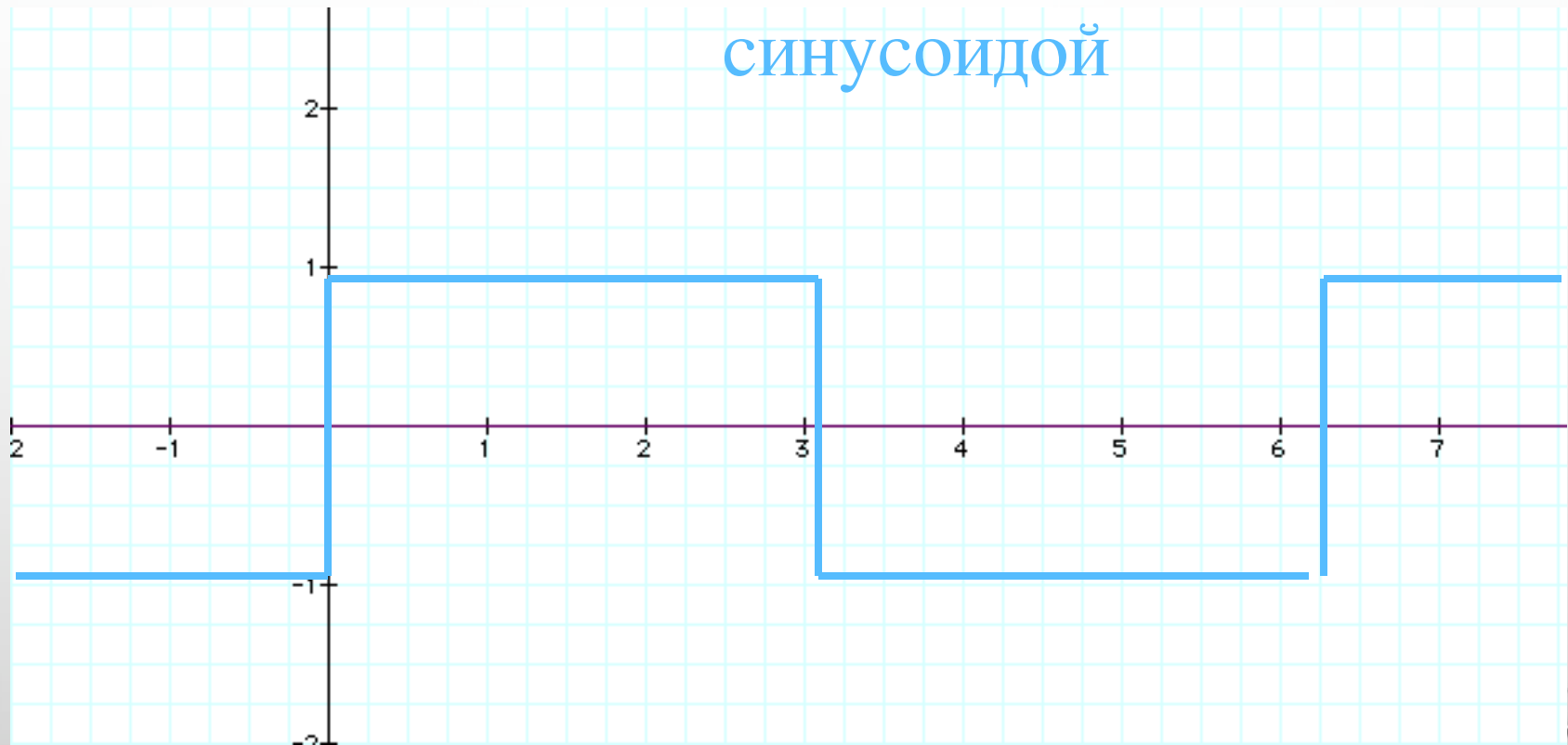
The inverse Fourier transform of the Fourier transform is identical to the original object.



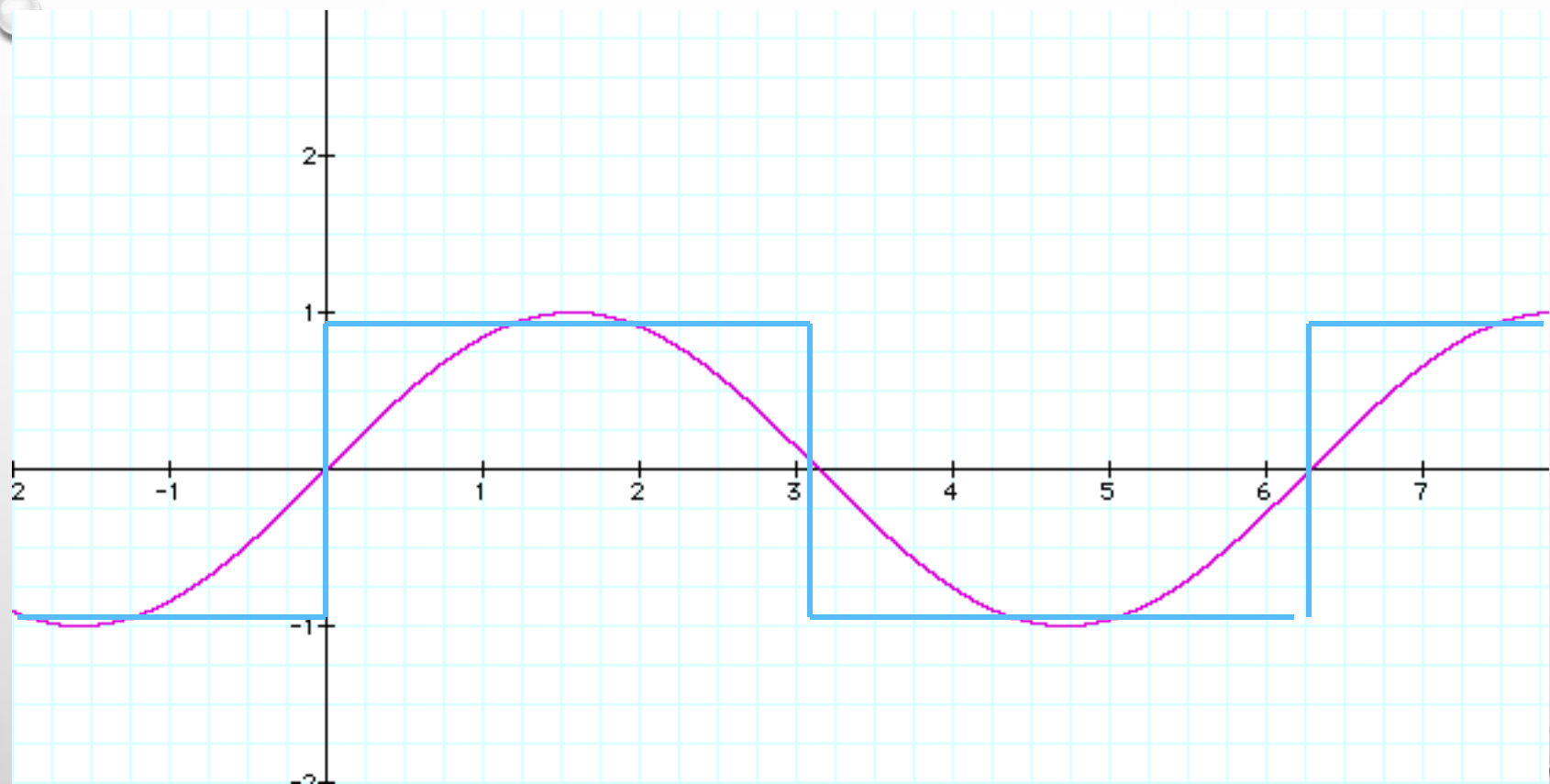
Преобразование Фурье суммы
двух функций равно сумме их
преобразований Фурье.

Пример: как можно представить прямоугольную волну

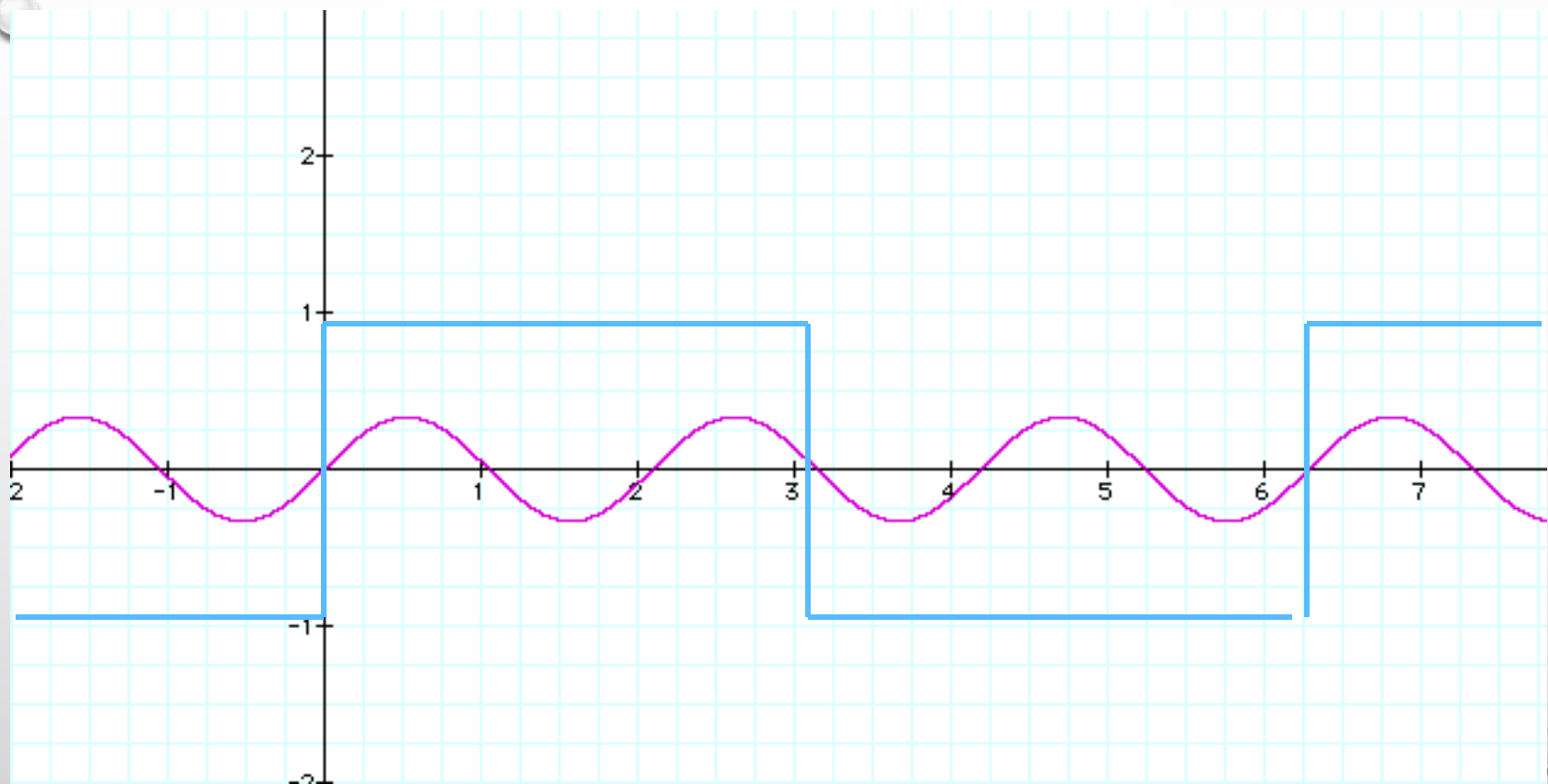
синусоидой



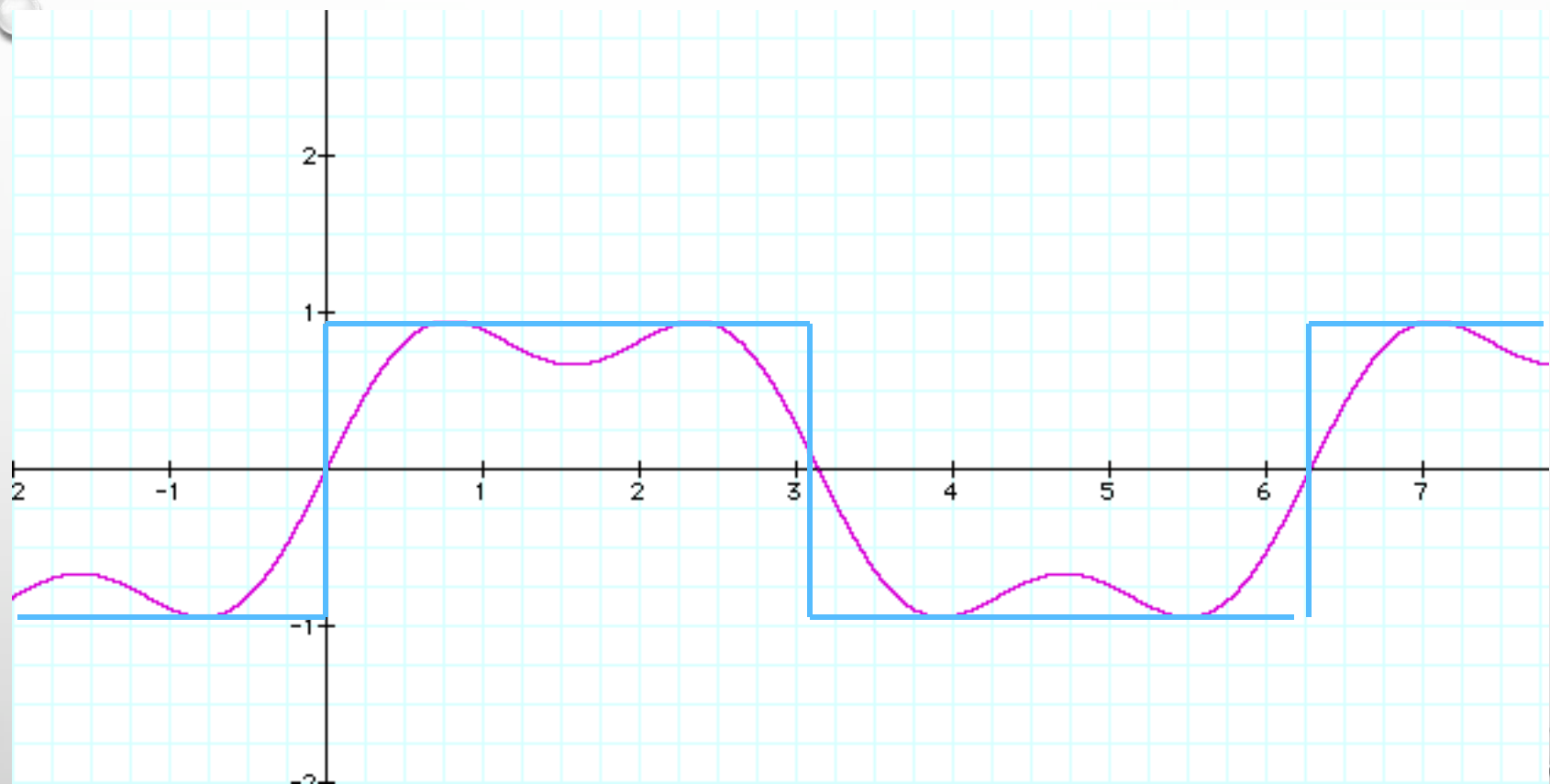
■ $y = \sin x$



$$\blacksquare y = \frac{1}{3} \sin 3x$$



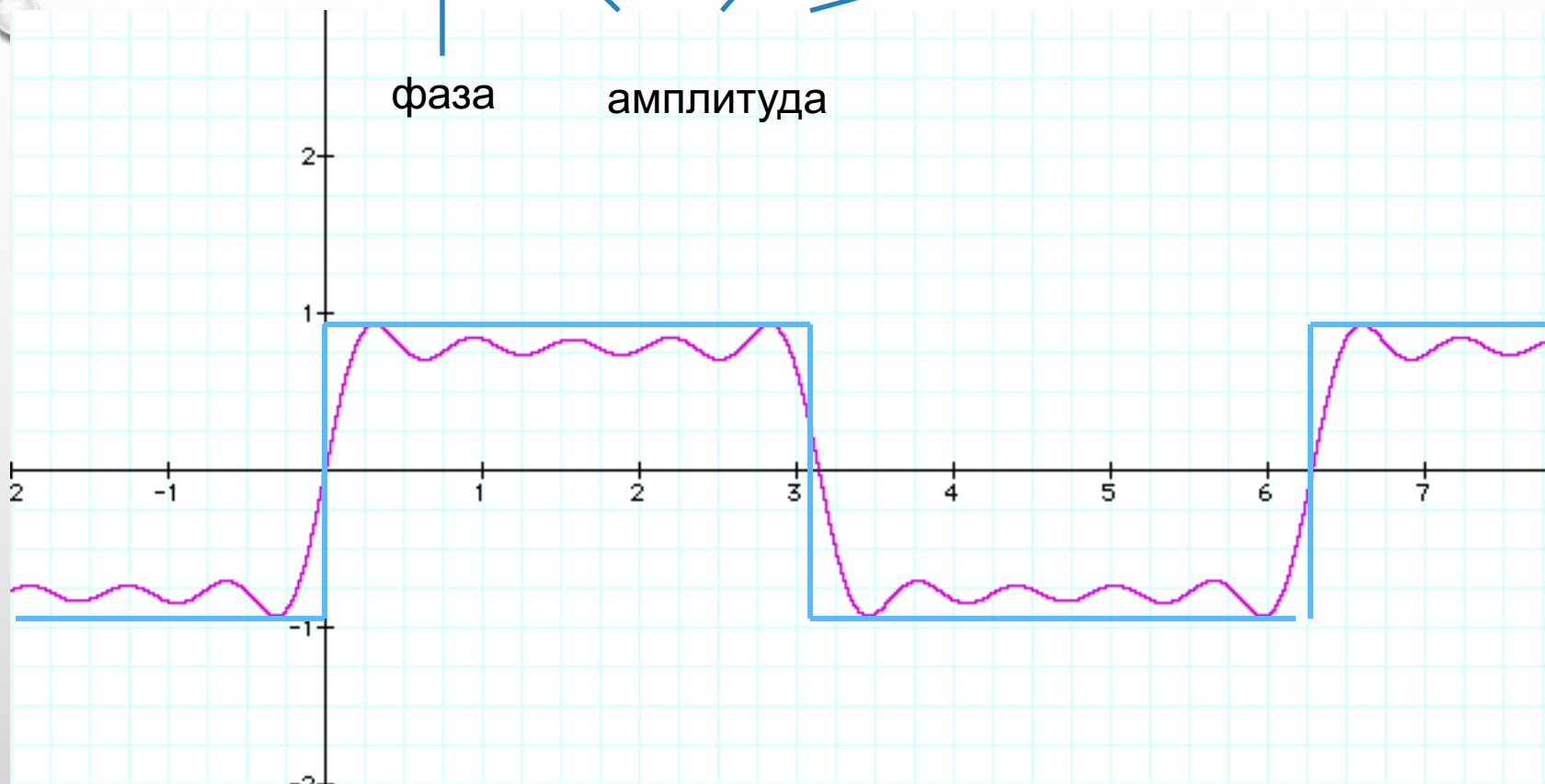
$$\blacksquare y = \sin x + \frac{1}{3} \sin 3x$$



$$y = \sin(x) + \frac{1}{3} \sin 3x + \frac{1}{5} \sin 5x + \frac{1}{7} \sin 7x + \frac{1}{9} \sin 9x$$

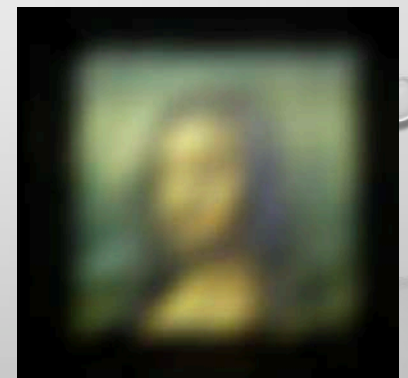
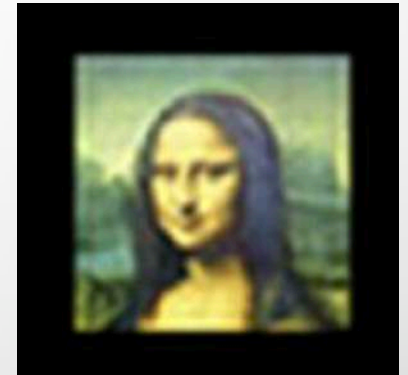
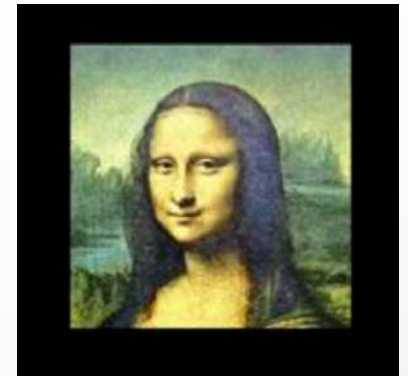
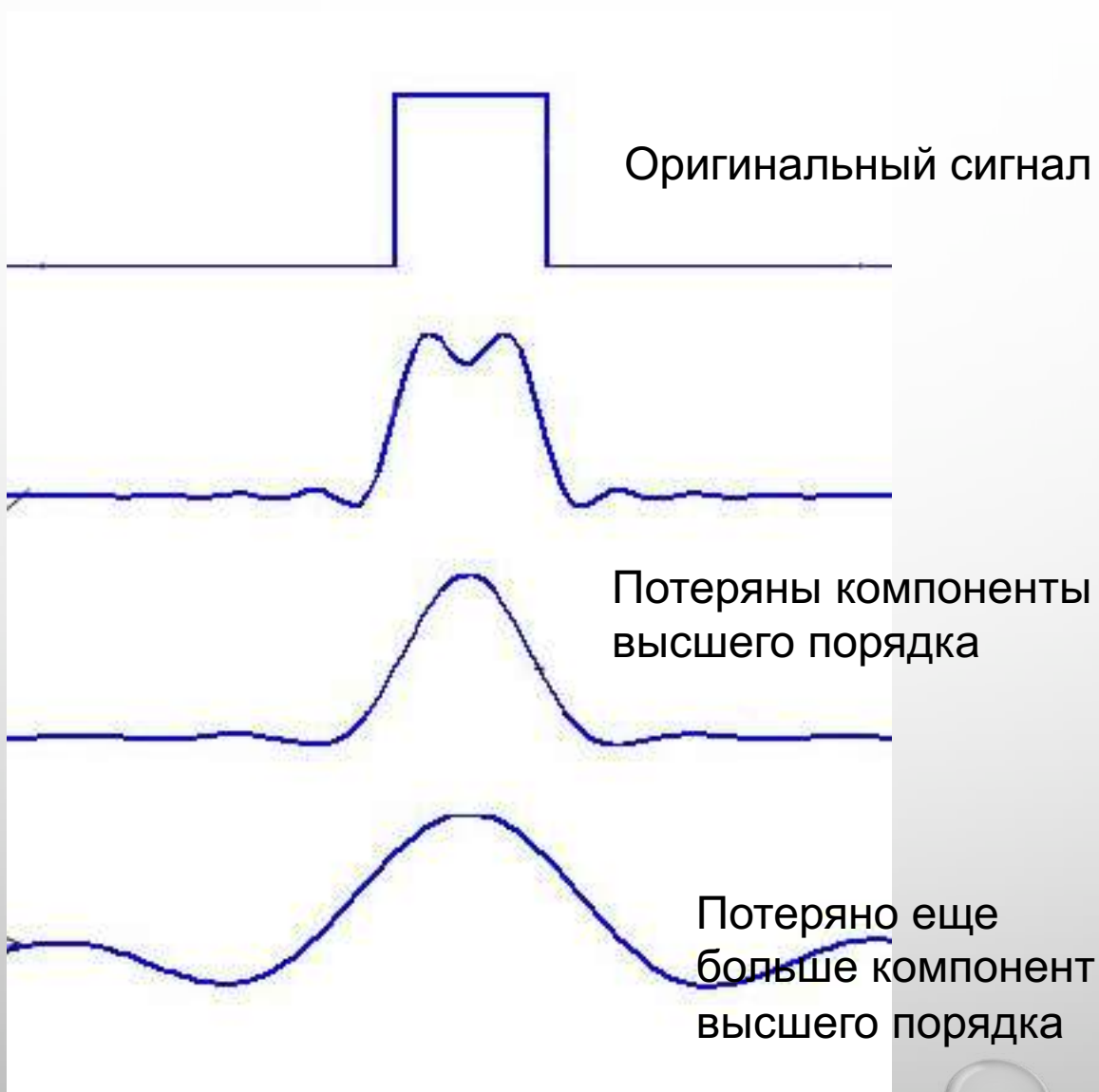
фаза

амплитуда

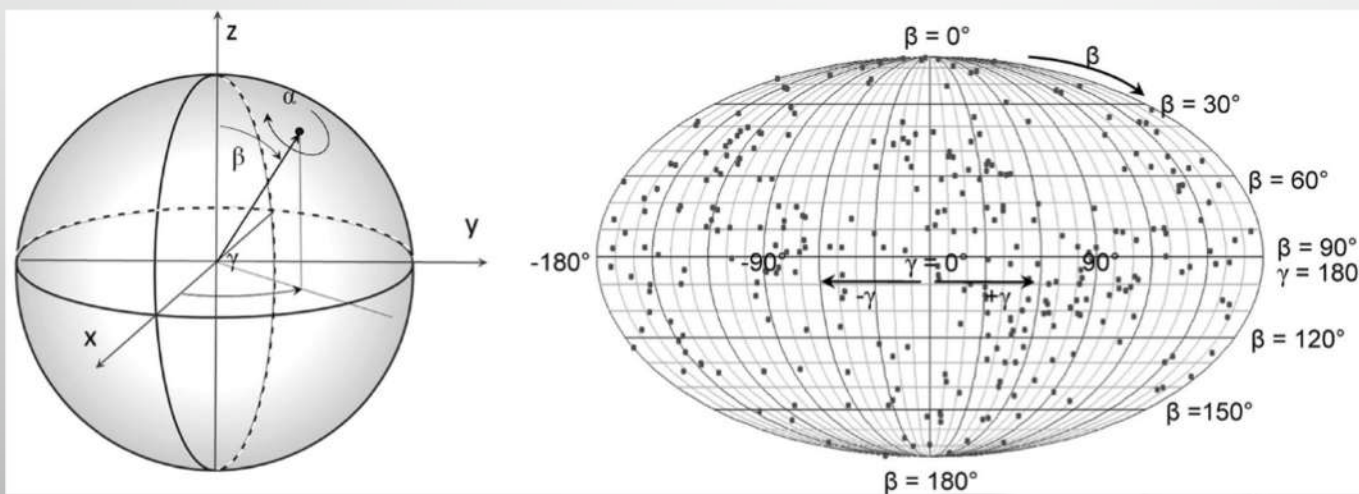
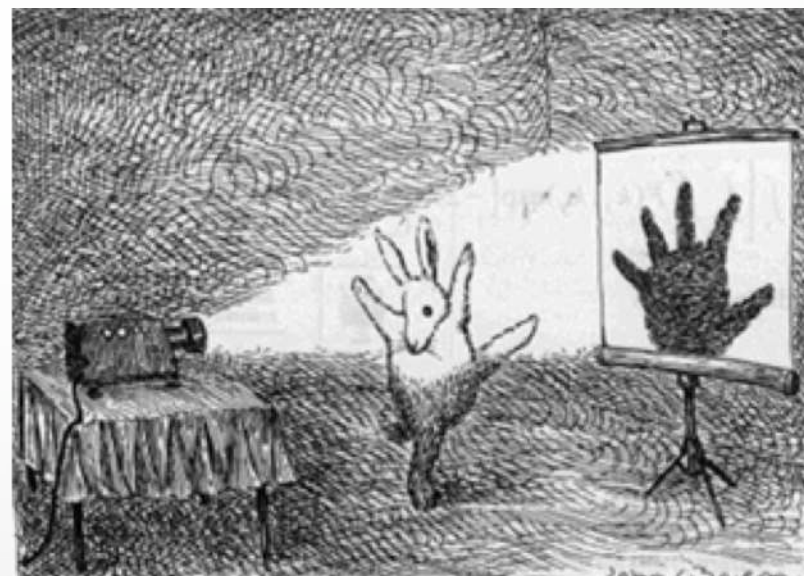
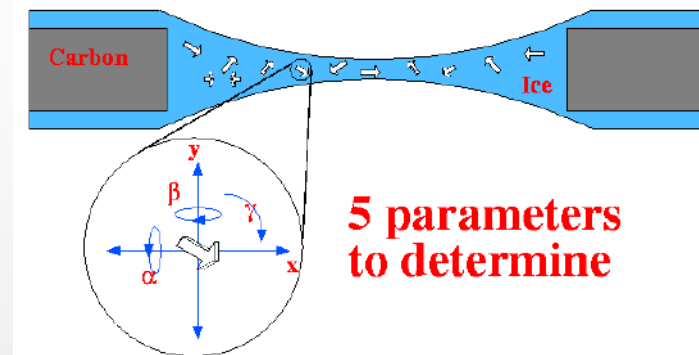


Добавление более высокого
порядка к преобразованию Фурье
увеличивает разрешение

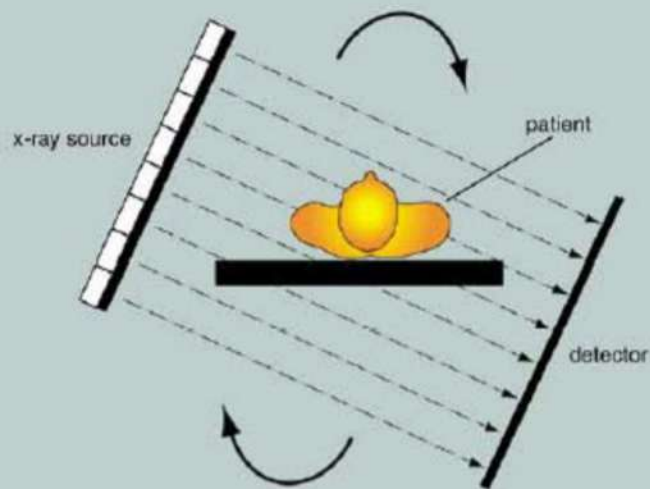
РАЗРЕШЕНИЕ



ЧТО НУЖНО ДЛЯ РЕКОНСТРУКЦИИ

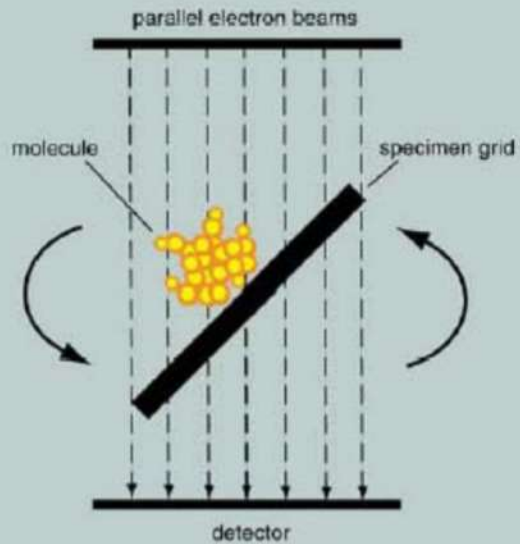


КАК ПОЛУЧИТЬ РАВНОМЕРНОЕ ПОКРЫТИЕ УГЛОВ?



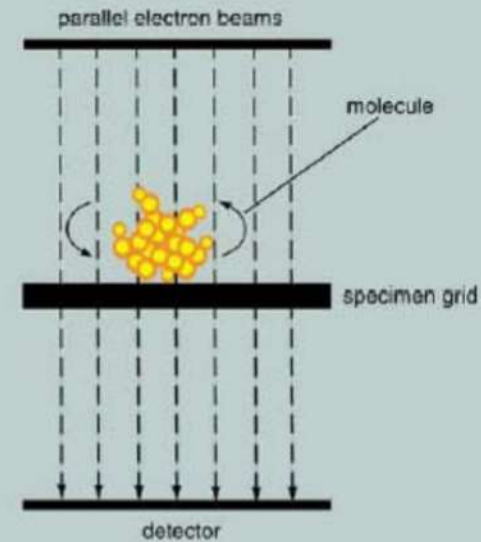
CAT - scan

- beam rotating
- patient stationary



Electron Tomography

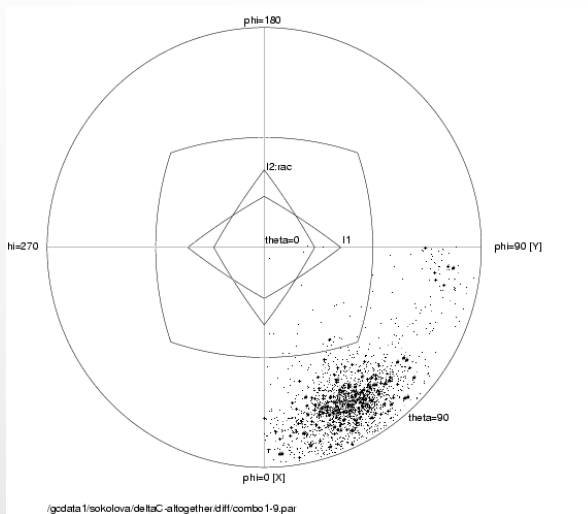
- molecule rotating
- beam stationary



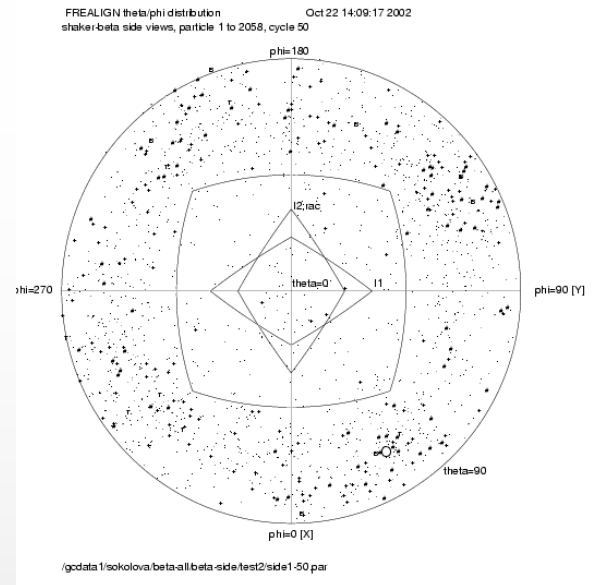
Single particle reconstruction

- molecule "rotating"
- beam stationary

3D УТОЧНЕНИЕ: ИТЕРАЦИИ

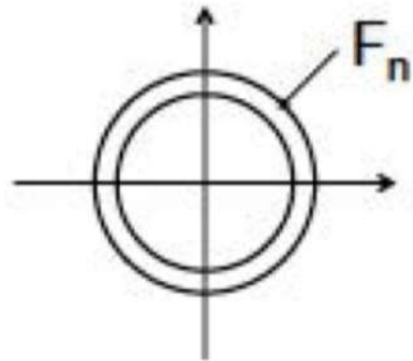


2 итерации

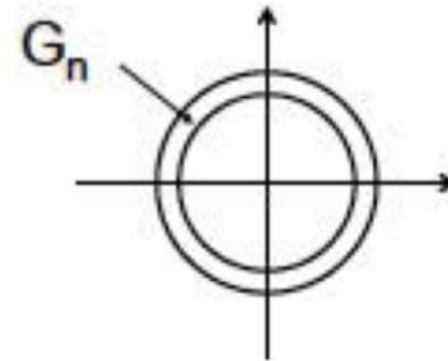


20 итераций

РАЗРЕШЕНИЕ

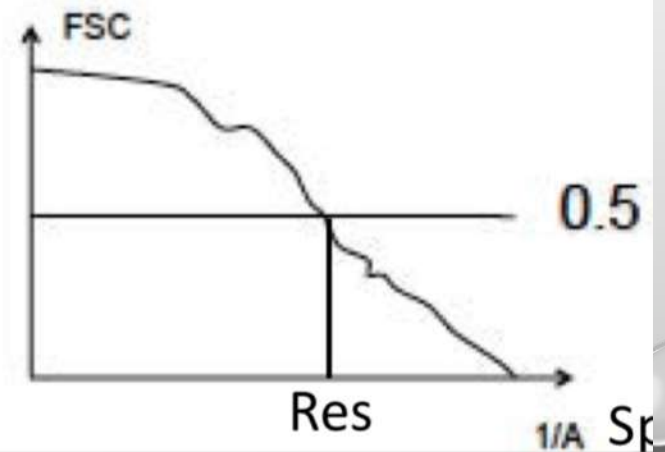


Reconstruction 1



Reconstruction 2

$$FSC(R) = \frac{\sum_{n \in R} F_n G_n}{\left\{ \sum_{n \in R} |F_n|^2 \sum_{n \in R} |G_n|^2 \right\}^{1/2}}$$



ПОЧЕМУ РАЗРЕШЕНИЕ НЕ ИДЕАЛЬНО?

- Instrumental: partial coherence
- Particle with different height all considered having the same defocus
- Numerical: interpolations, inaccuracies
- Failure to exhaust existing information
- Conformational diversity

5. НА ПУТИ К АТОМНОМУ РАЗРЕШЕНИЮ

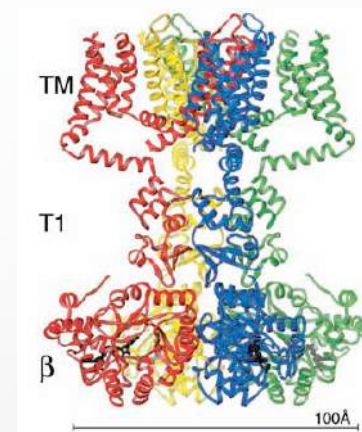
СРАВНЕНИЕ СТРУКТУРНЫХ МЕТОДОВ

- РСА

АТОМНОЕ РАЗРЕШЕНИЕ

3D КРИСТАЛЛЫ, ОГРАНИЧЕНИЯ ПО РАЗМЕРУ

ПРОБЛЕМЫ С КОНФОРМАЦИЕЙ



- ЯМР

АТОМНОЕ РАЗРЕШЕНИЕ

ДИНАМИЧЕСКИЕ ПРОЦЕССЫ

ОГРАНИЧЕНИЯ ПО РАЗМЕРУ

- ПЭМ

НЕТ КРИСТАЛЛОВ И ВЕРХНИХ ОГРАНИЧЕНИЙ ПО РАЗМЕРУ

МАЛАЯ КОНЦЕНТРАЦИЯ БЕЛКА

АТОМНОЕ РАЗРЕШЕНИЕ



СОВРЕМЕННЫЕ КРИОЭЛЕКТРОННЫЕ МИКРОСКОПЫ



ЭЛЕКТРОННАЯ МИКРОСКОПИЯ В БИОЛОГИИ – СОВРЕМЕННАЯ СХЕМА



Очистка белка



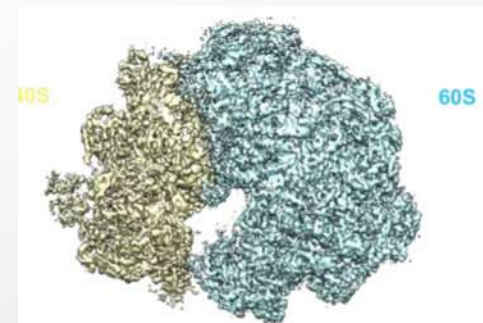
замораживание



Получение изображений



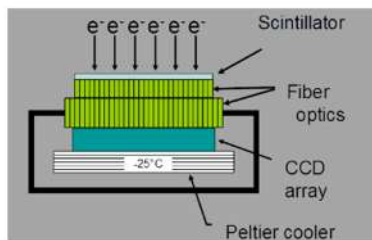
Image processing



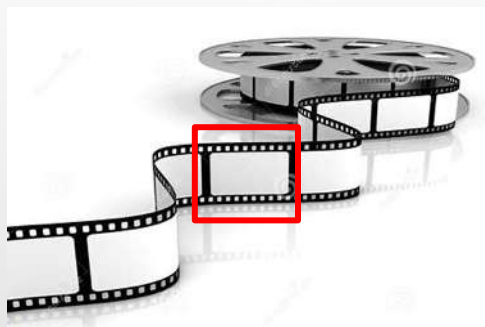
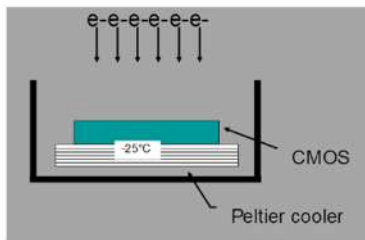
Структура белка
с высоким
разрешением

СПОСОБЫ РЕГИСТРАЦИИ ИЗОБРАЖЕНИЯ

CCD

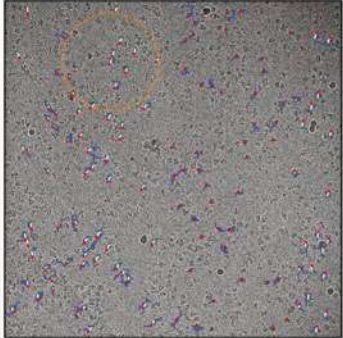


Direct detector

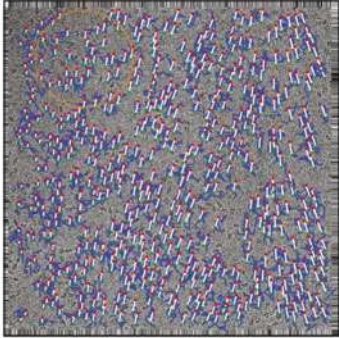


BEAM-INDUCED MOTION

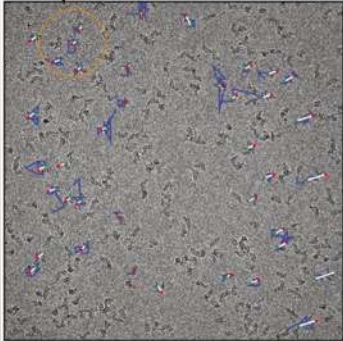
γ -secretase



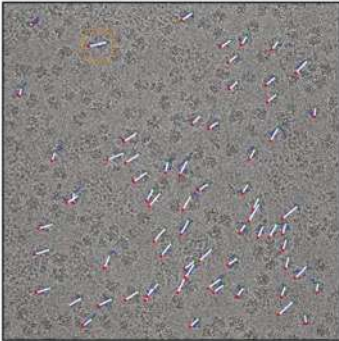
β -galactosidase



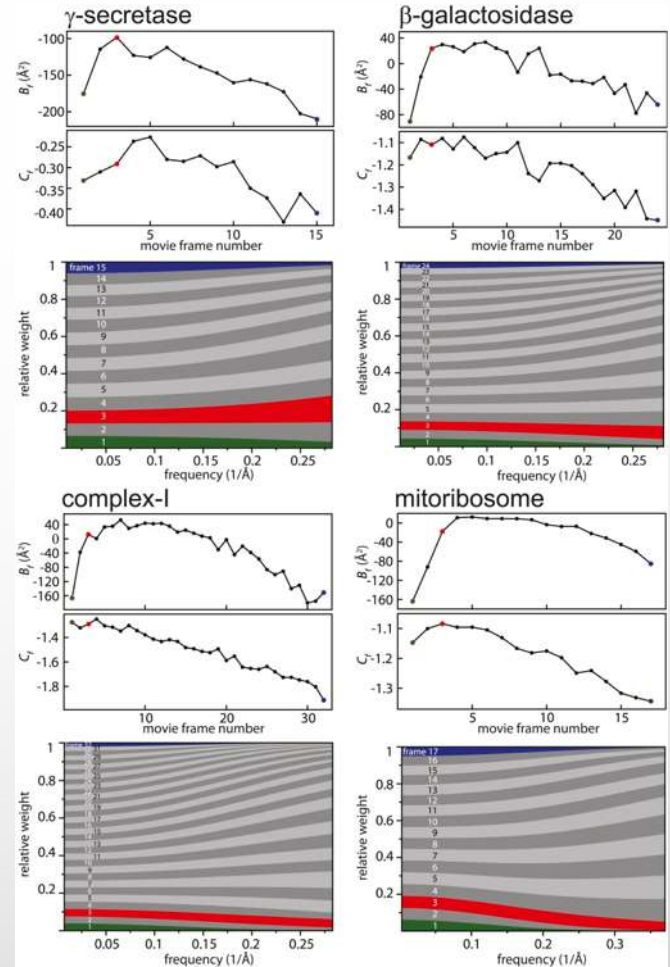
complex-I



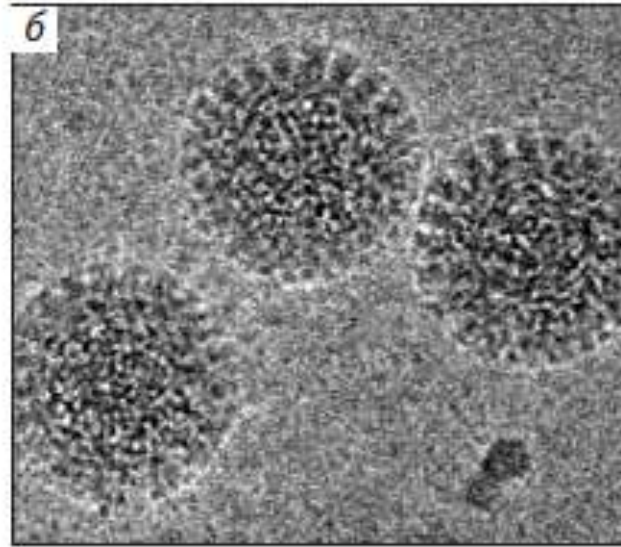
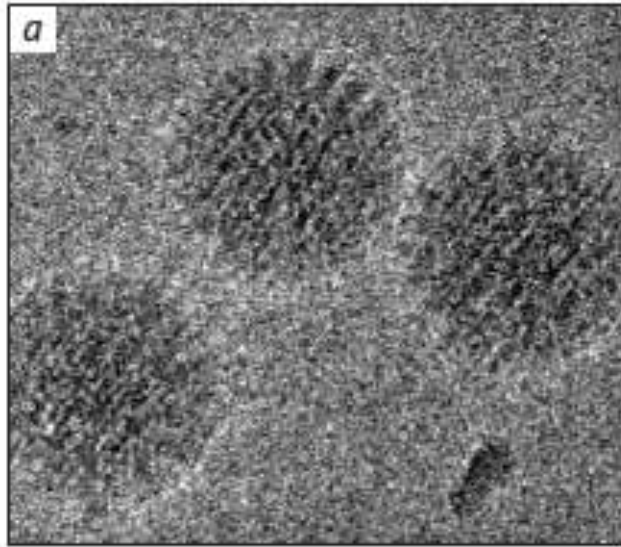
mitoribosome



[SHW Scheres, 2014](#)

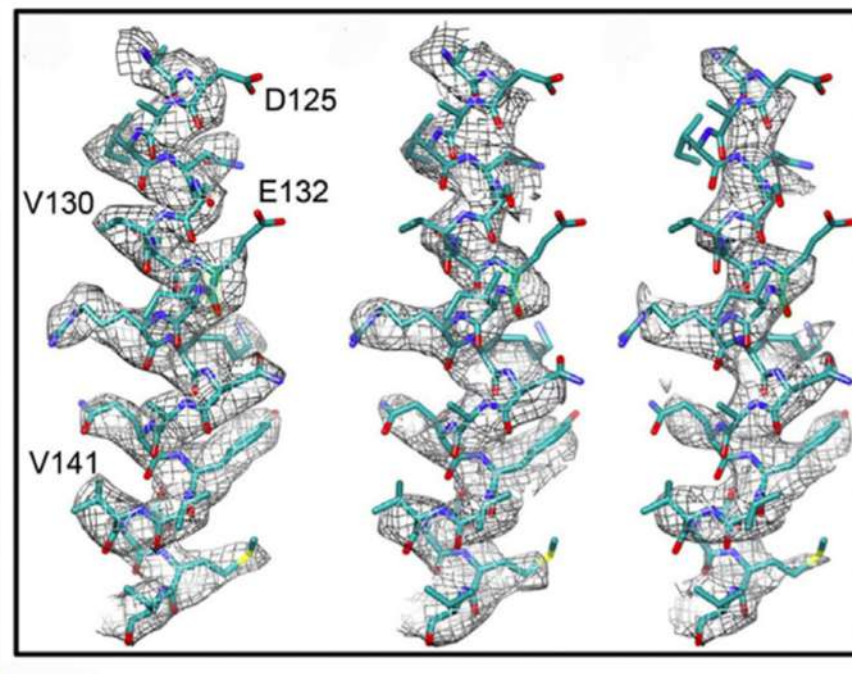


MOTION CORRECTION



N.Grignorieff ©

ОПЯТЬ РАДИАЦИОННОЕ ПОВРЕЖДЕНИЕ



Alegretti et al, 2014

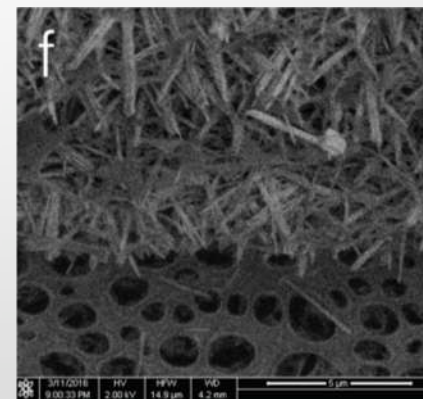
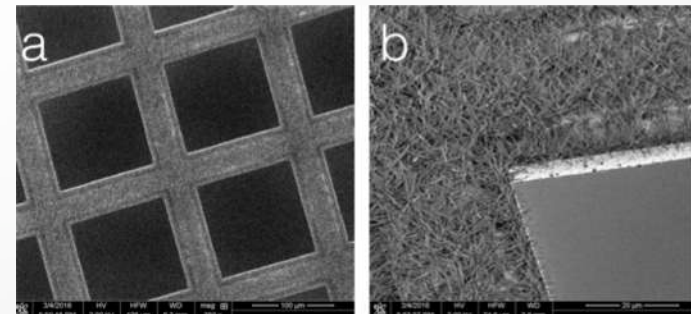
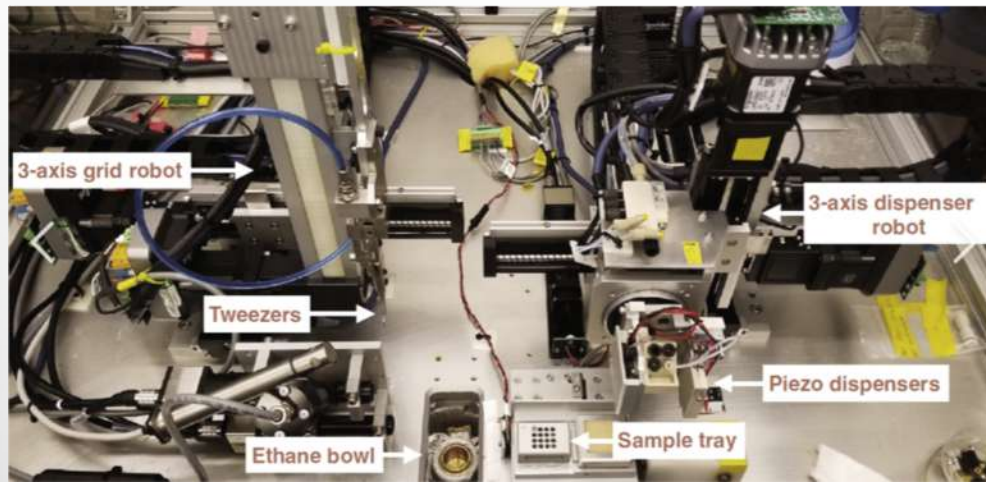
$10 \text{ e}/\text{\AA}^2$ (3.36 Å), $\approx 24 \text{ e}/\text{\AA}^2$ (3.94 Å), $\approx 49 \text{ e}/\text{\AA}^2$ (4.16 Å)

НОВЫЕ МЕТОДЫ ВИТРИФИКАЦИИ

Spotiton

“Self-blotting” TEM grids

Nano-wires



A new method for vitrifying samples for cryoEM

Ivan Razinkov, Venkat Dandey, Hui Wei, Zhening Zhang, David Melnekoff, William J. Rice, Christoph Wigge, Clinton S. Potter, Bridget Carragher

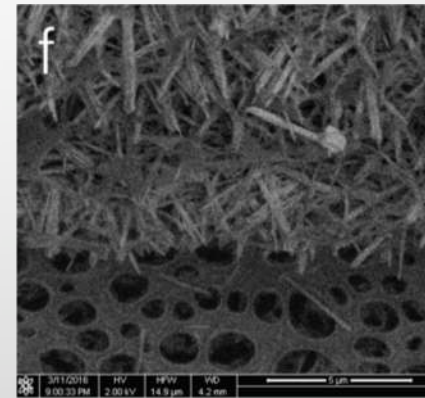
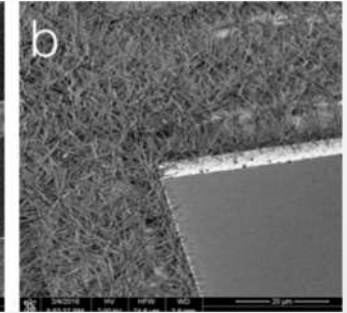
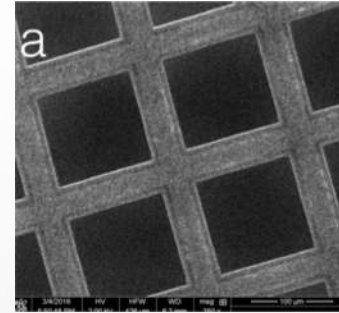
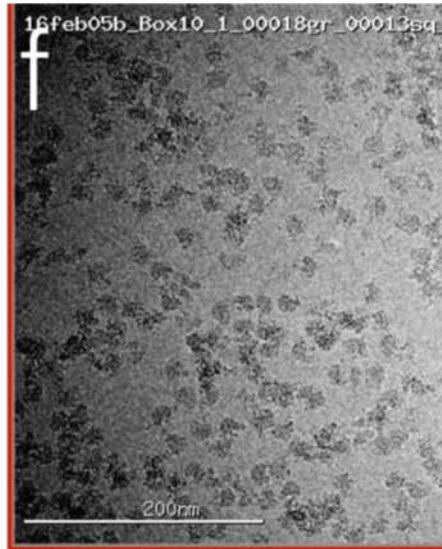
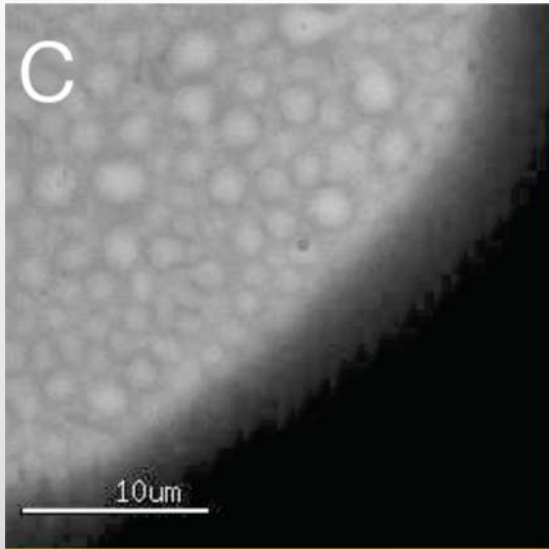
PII: S1047-8477(16)30107-1

DOI: <http://dx.doi.org/10.1016/j.jsb.2016.06.001>

НОВЫЕ МЕТОДЫ ВИТРИФИКАЦИИ

“Self-blotting” TEM grids

Nano-wires

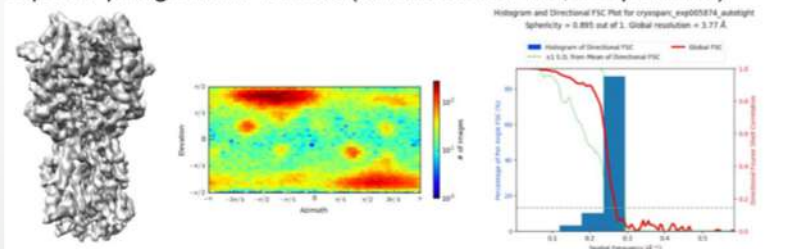


Reducing effects of particle adsorption to the air-water interface in cryoEM

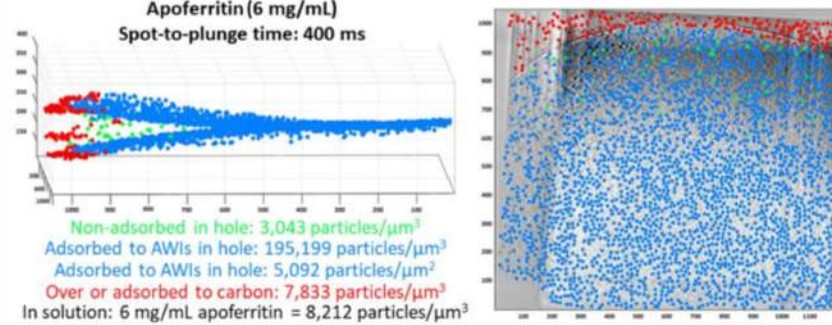
Alex J. Noble, Hui Wei, Venkata P. Dandey, Zhening Zhang, Yong Zi Tan, Clinton S. Potter, Bridget Carragher

doi: <https://doi.org/10.1101/288340>

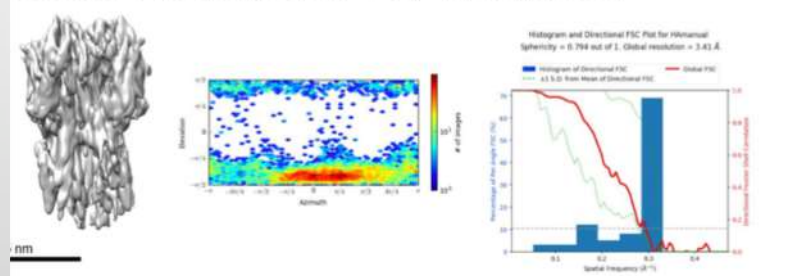
Spot-to-plunge time: 100 ms (full dataset of 130,000 particles)



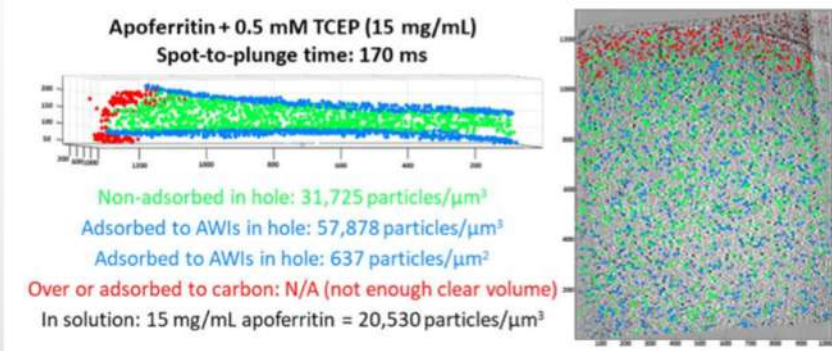
Apoferritin (6 mg/mL)
Spot-to-plunge time: 400 ms



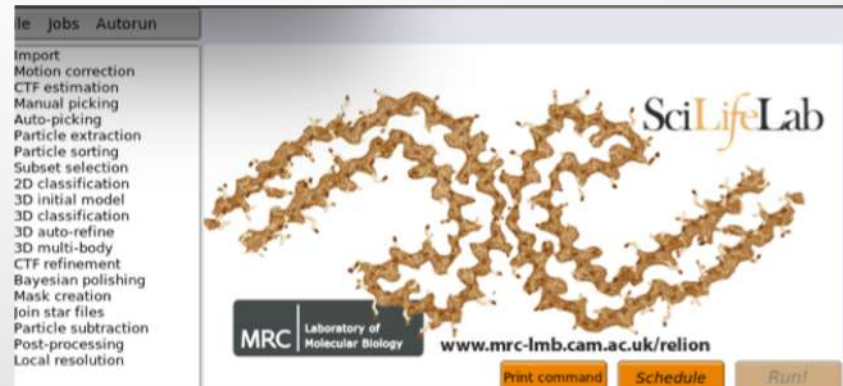
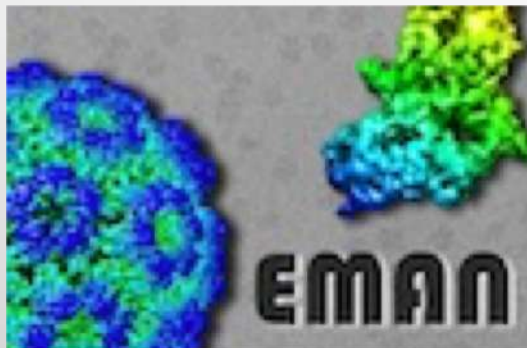
Gatan CP3 with plunge time: ~1 s (~15,000 particles)



Apoferritin + 0.5 mM TCEP (15 mg/mL)
Spot-to-plunge time: 170 ms



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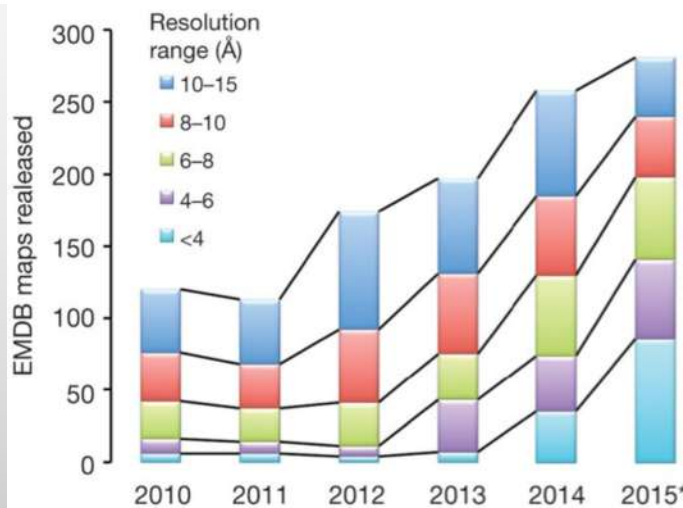
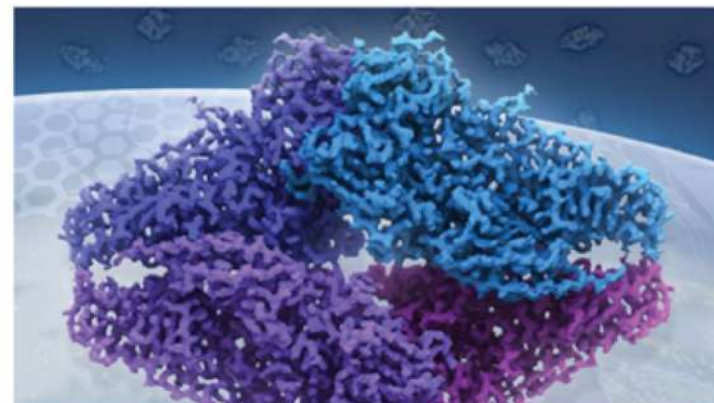


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FOCUS | 30 DECEMBER 2015

Method of the Year 2015

Our choice for Method of the Year 2015 is single-particle cryo-electron microscopy. A collection of articles discusses how recent technical advances, especially the development of direct-detection cameras, have enabled this structural biology technique to make... [show more](#)



НОБЕЛЕВСКАЯ ПРЕМИЯ 2017 ПО ХИМИИ



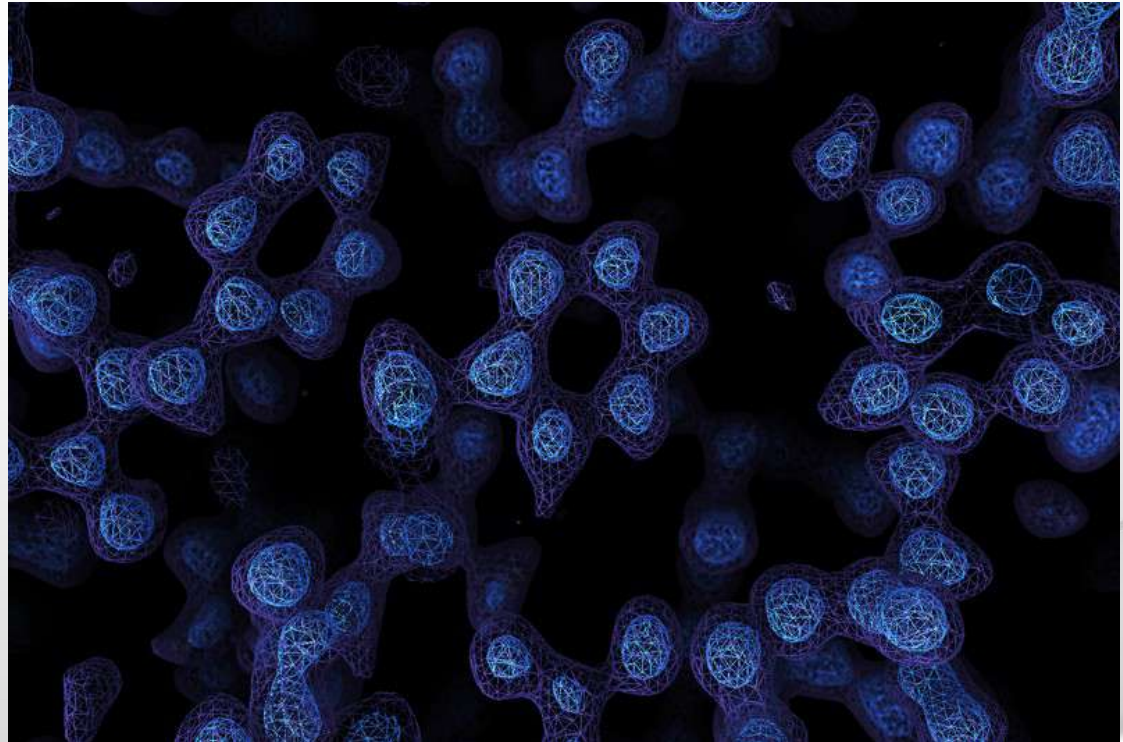
NEWS • 03 JUNE 2020

nature

'It opens up a whole new universe': Revolutionary microscopy technique sees individual atoms for first time

Cryo-electron microscopy breaks a key barrier that will allow the workings of proteins to be probed in unprecedented detail.

<https://www.nature.com/articles/d41586-020-01658-1>



FIRST CRYO-EM STRUCTURES WITH TRUE ATOMIC RESOLUTION

Article | Published: 21 October 2020

Single-particle cryo-EM at atomic resolution

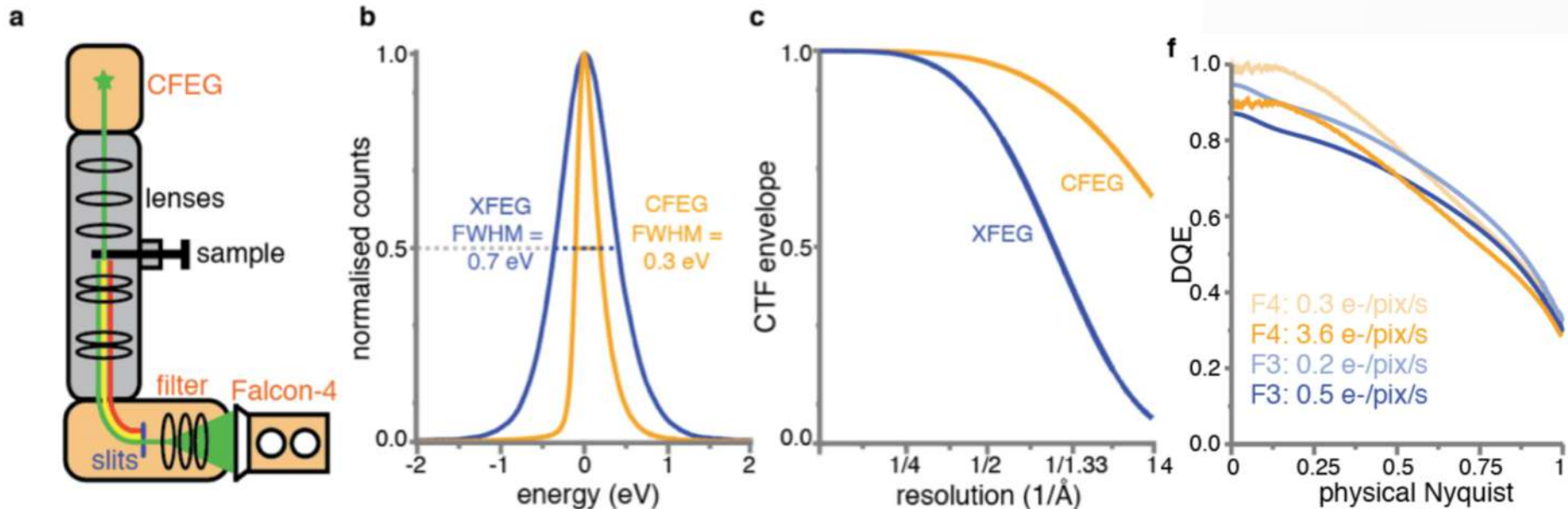
Takanori Nakane, Abhay Kotecha, Andrija Sente, Greg McMullan, Simonas Masiulis, Patricia M. G. E. Brown, Ioana T. Grigoras, Lina Malinauskaite, Tomas Malinauskas, Jonas Miehling, Tomasz Uchański, Lingbo Yu, Dimple Karia, Evgeniya V. Pechnikova, Erwin de Jong, Jeroen Keizer, Maarten Bischoff, Jamie McCormack, Peter Tiemeijer, Steven W. Hardwick, Dimitri Y. Chirgadze, Garib Murshudov, A. Radu Aricescu ✉ & Sjors H. W. Scheres ✉

Nature (2020) | [Cite this article](#)

Abstract

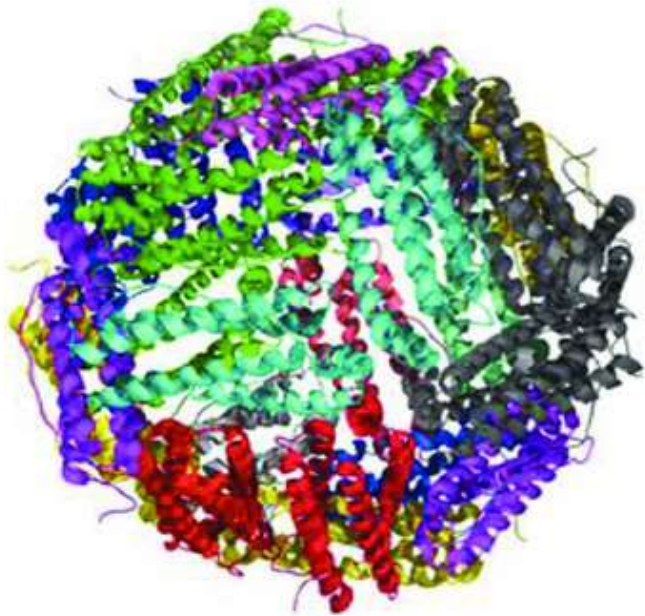
The three-dimensional positions of atoms in protein molecules define their structure and provide mechanistic insights into the roles they perform in complex biological processes. The more precisely atomic coordinates are determined, the more chemical information can be derived and the more knowledge about protein function may be inferred. With breakthroughs in electron detection and image processing technology, electron cryo-microscopy (cryo-EM) single-particle analysis has yielded protein structures with increasing levels of detail in recent years^{1,2}. However, obtaining cryo-EM reconstructions with sufficient resolution to visualise individual atoms in proteins has thus far been elusive. Here, we show that using a new electron source, energy filter and camera, we obtained a 1.2 Å reconstruction that, for the first time, offers a genuine atomic resolution view of a protein molecule using single particle cryo-EM. Moreover, the scattering potential from many hydrogen atoms can be visualised in difference maps, allowing a direct analysis of hydrogen bonding networks. Combination of the technological advances described here with further approaches to accelerate data acquisition and improve sample quality provide a route towards routine application of cryo-EM in high-throughput screening of small molecule modulators and structure-based drug discovery.

КАТОД + ЭНЕРГЕТИЧЕСКИЕ ФИЛЬТРЫ + FALCON 4 = АТОМНОЕ РАЗРЕШЕНИЕ



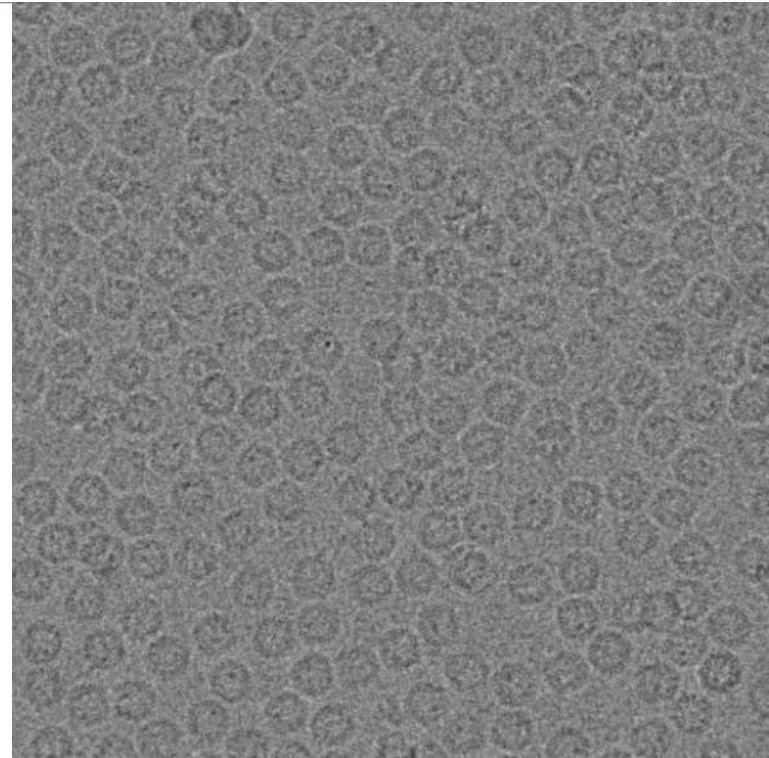
Single-particle cryo-EM at atomic resolution Nakane et al. ,2020

APO-FERRITIN

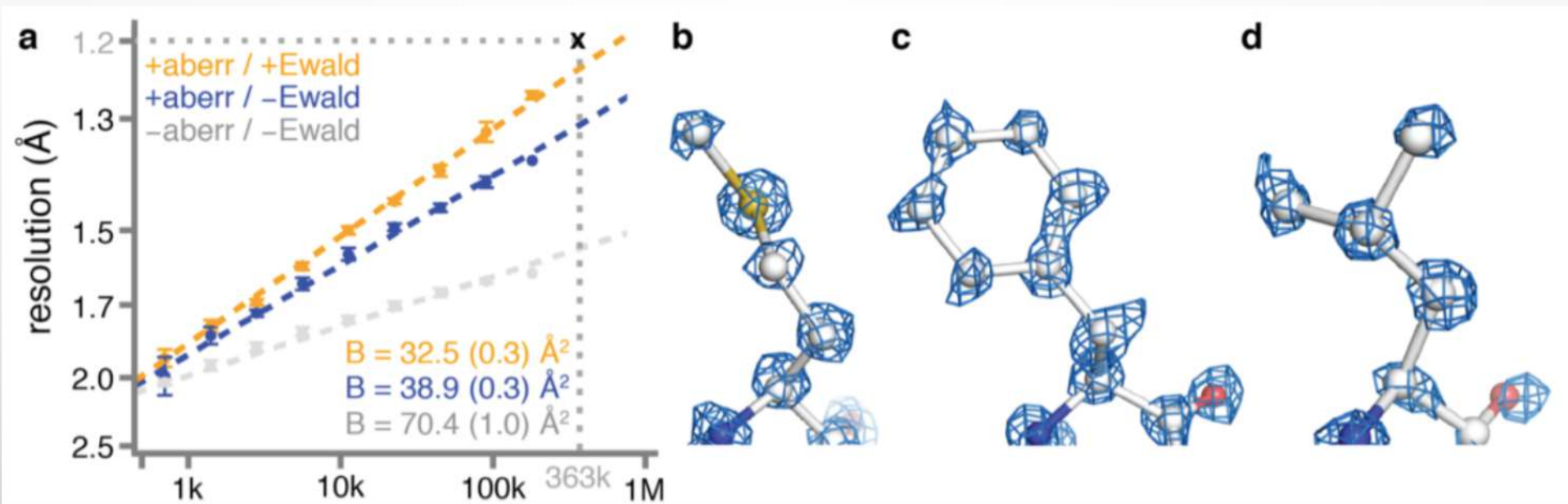


120 Å

24 peptide chain, hollow,
quaternary structure of
apoferritin
Internal diameter = 80 Å



APO-FERRITIN RECONSTRUCTION AT ATOMIC RESOLUTION



DOES HARDWARE CORRECTION OF OPTICAL ABERRATIONS WORK FOR CRYO-EM?

Article | Published: 21 October 2020

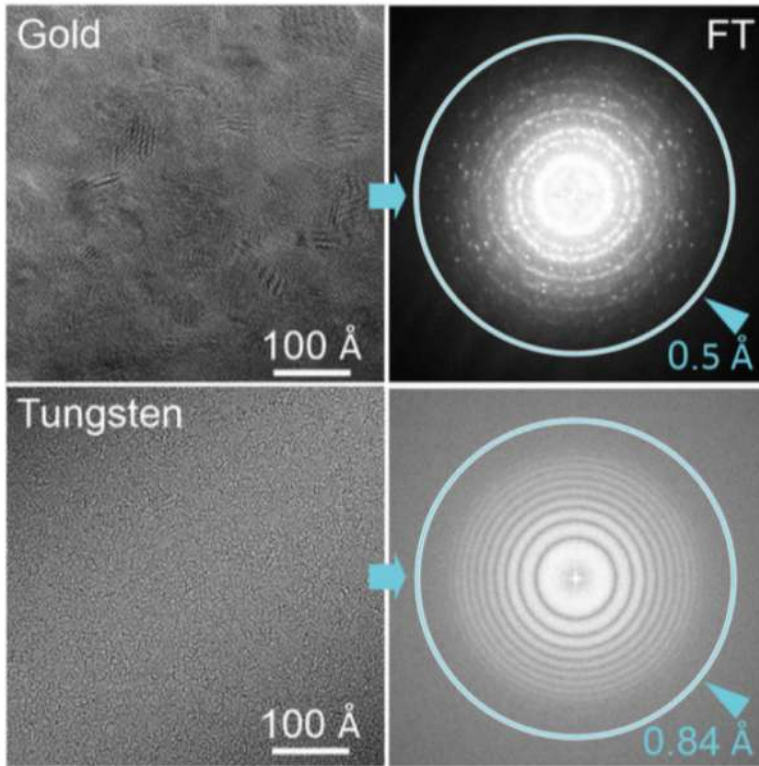
Atomic-resolution protein structure determination by cryo-EM

Ka Man Yip, Niels Fischer, Elham Paknia, Ashwin Chari & Holger Stark 

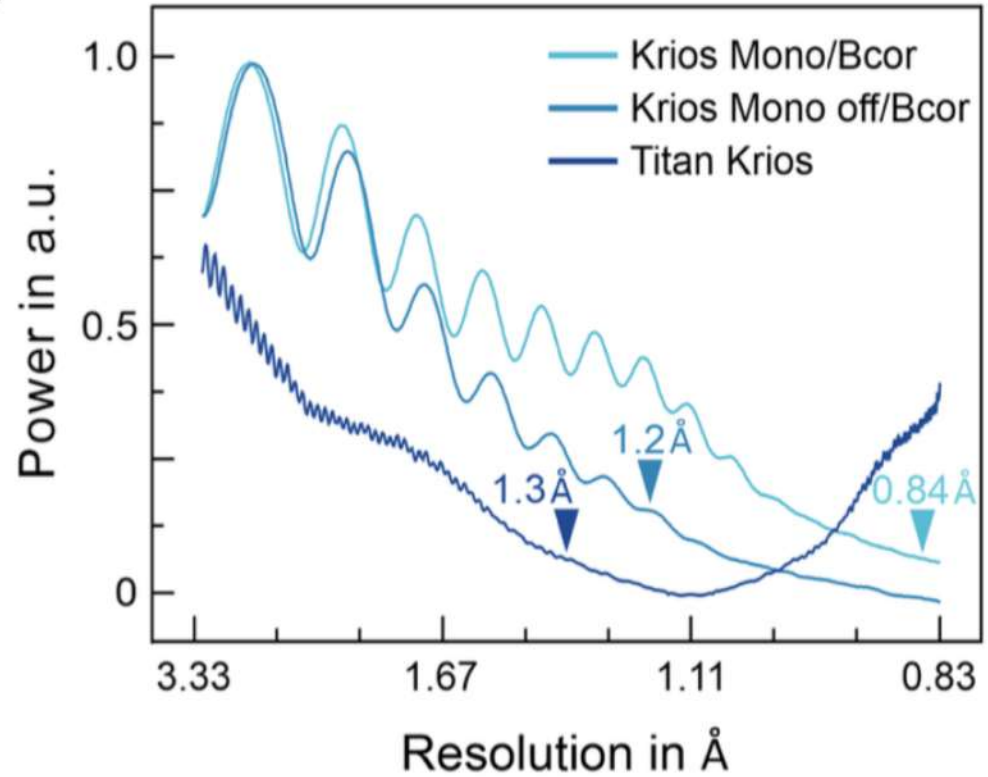
Nature (2020) | [Cite this article](#)

KRIOS MONO/BCOR

a

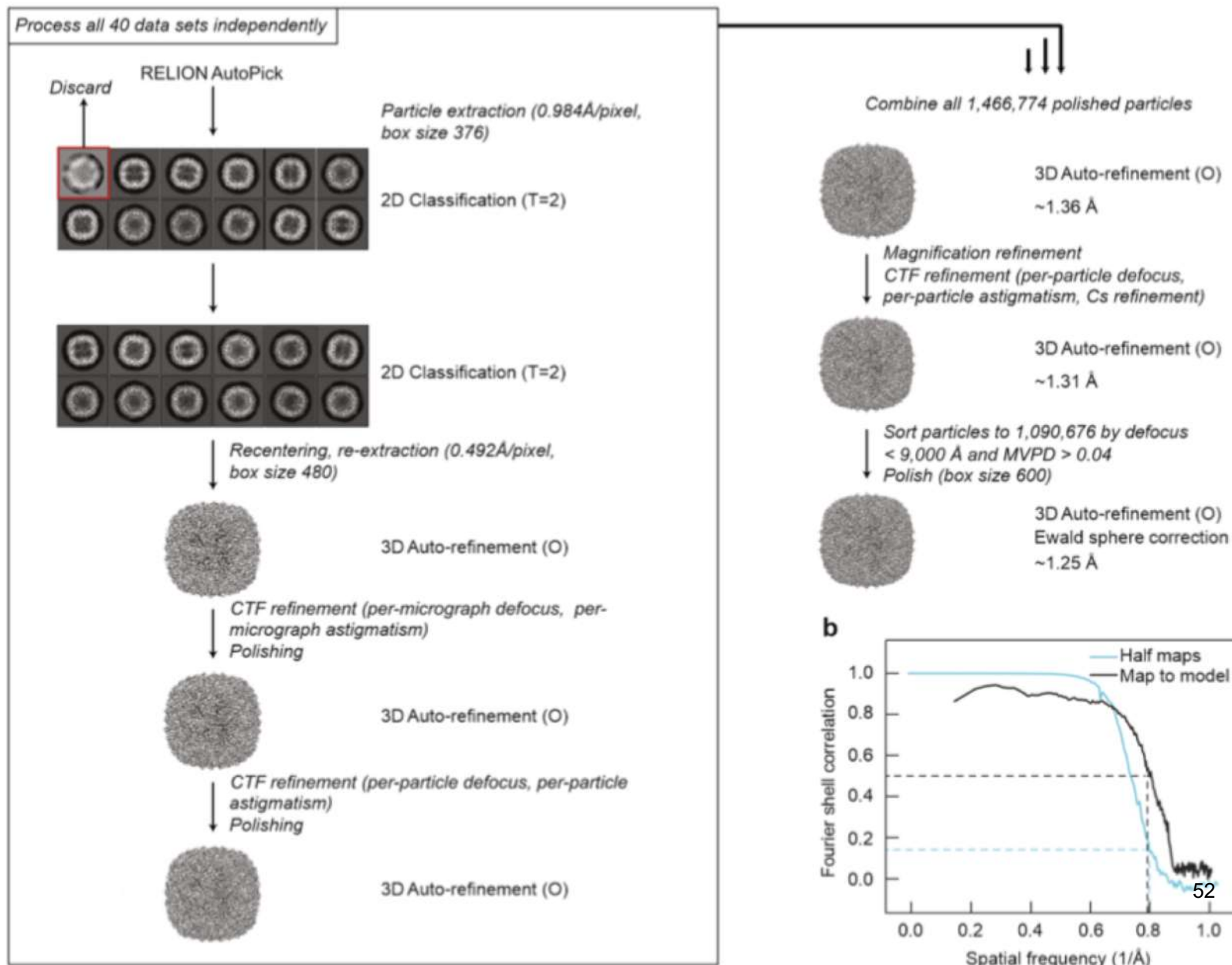


b



Suppl Figure 2

a



ANOTHER APO-FERRITIN RECONSTRUCTION AT ATOMIC RESOLUTION

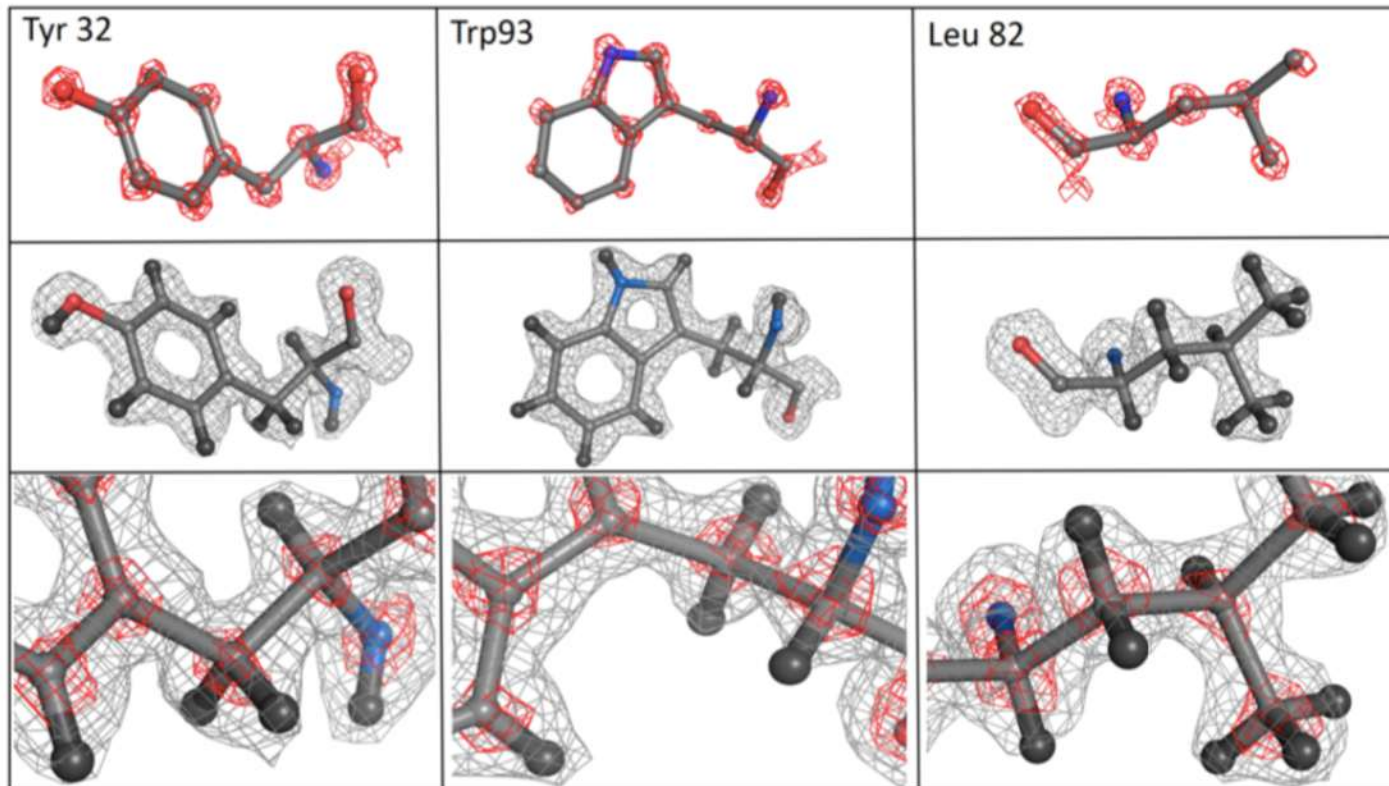


Fig. 3 True atomic resolution: Visualization of individual atoms and hydrogens at 1.25 Å resolution

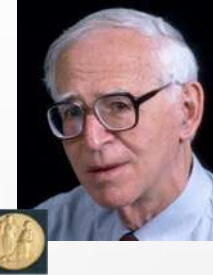
5. ЛЮДИ В КРИО-ЭМ

НОБЕЛЕВСКИЕ ЛАУРЕАТЫ

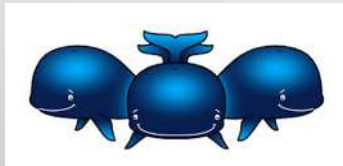
- 1928 M. KNOLLE, E. RUSKA: FIRST MICROSCOPE;
- 1960 A. KLUG: SINGLE PARTICLE;
- 1980 J. DUBOCHET: CRYO;
- R.HENDERSON: ELECTRON CRYSTALLOGRAPHY
- J.FRANK: RIBOSOME



1986 "for his fundamental work in electron optics, and for the design of the first electron microscope"



1982 "for his development of crystallographic electron microscopy and his structural elucidation of biologically important nucleic acid-protein complexes"



2017 "for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"

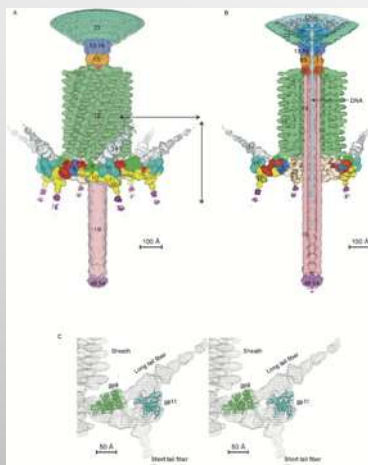
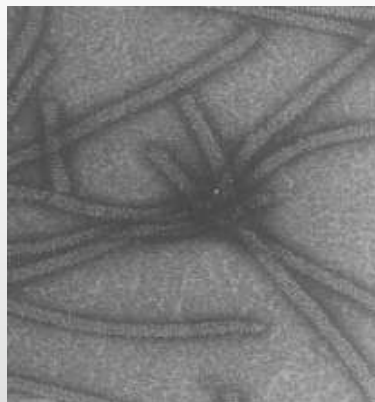
СТРУКТУРНАЯ ПЭМ В СССР



Н.А.Киселев(ИК РАН)

В.В.Месянжинов (ИБХ РАН)

И.Г.Атабеков (МГУ)



Structure of Products of Protein Reassembly and Reconstruction of Potato Virus X

A. S. KAFTANOVA, N. A. KISELEV

Institute of Crystallography of the USSR Academy of Sciences

AND

V. K. NOVIKOV AND J. G. ATABEKOV

Laboratory of Biorganic Chemistry and Department of Virology of Moscow State University, Moscow 117234, USSR

Accepted May 8, 1975

Upon polymerization of potato virus X (PVX) protein in 0.2 M phosphate buffer solutions, pH 7.0-7.5, double-layer disks can be produced. The disks can aggregate into short rodlike stacks. No helically repolymerized structures were revealed. Long filamentous particles can be reassembled *in vitro* on incubation of PVX protein with viral RNA in low