

# Микроскопия супер- разрешения

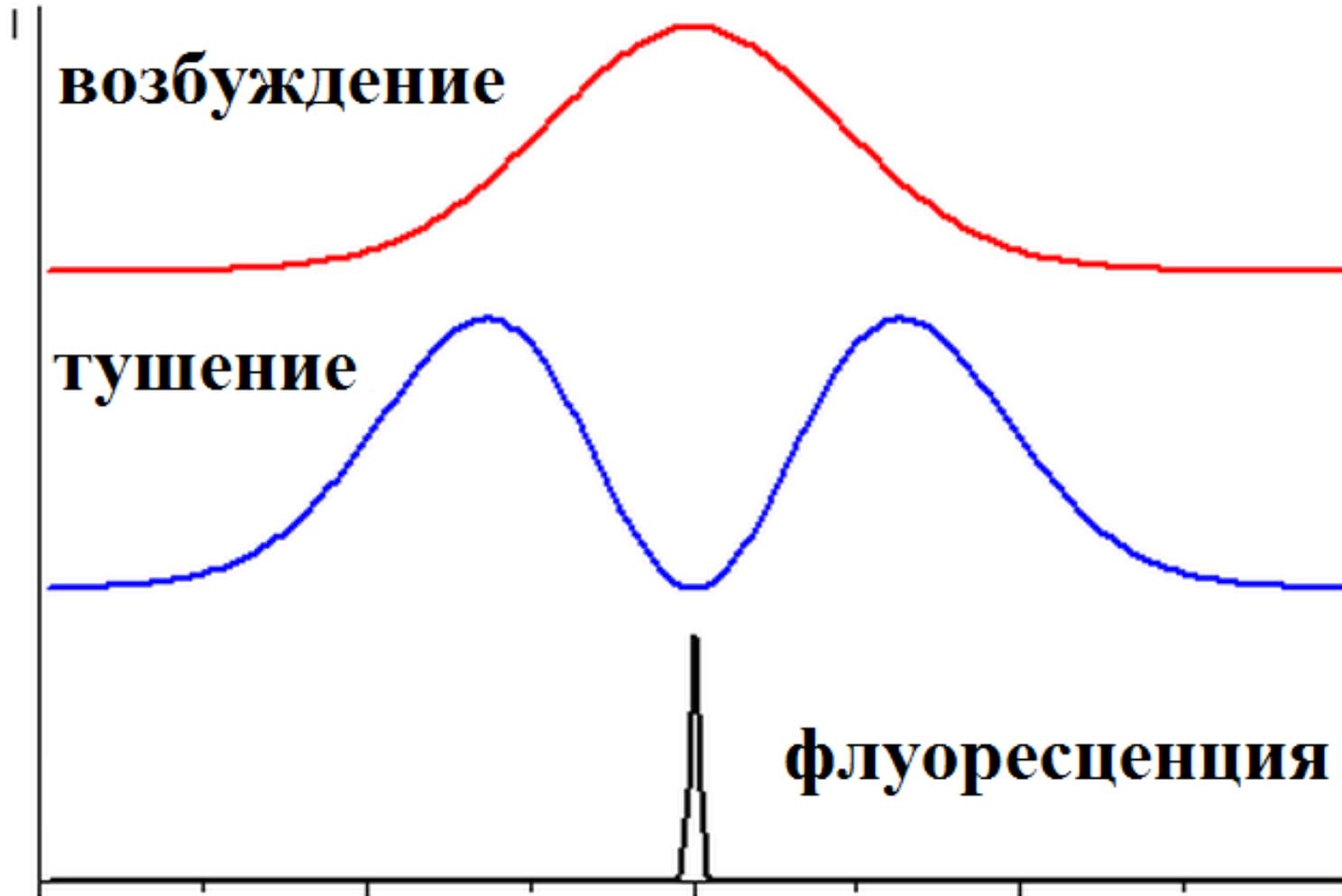
STED, PALM и STORM, SIM, MINIFLUX

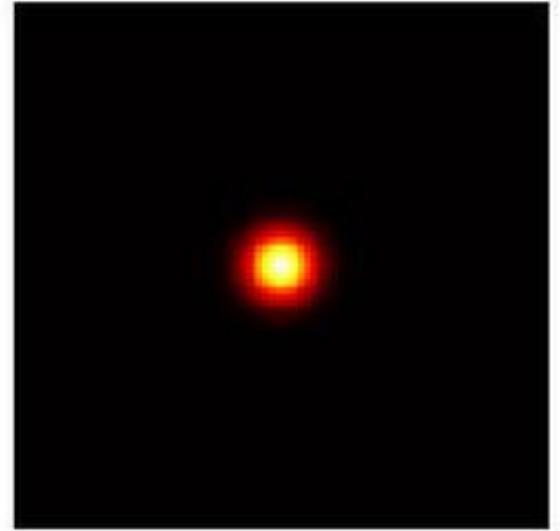
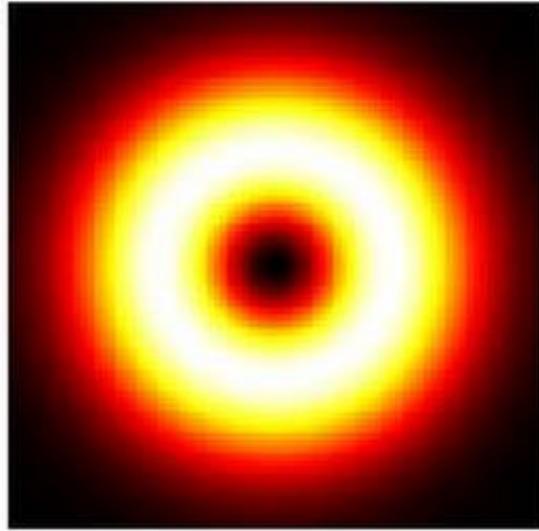
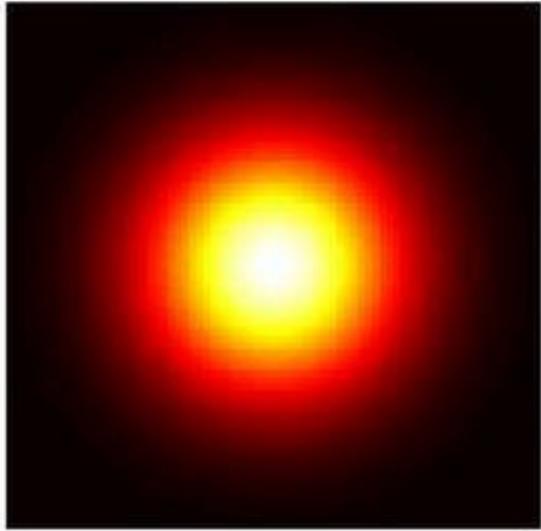
# Дифракционный предел в классической микроскопии

$$(\delta x_{\min}, \delta y_{\min}) = \Delta_{\min} = \frac{\lambda}{2n \sin \alpha},$$

$$\delta z_{\min} \approx \frac{\lambda}{2n (\sin \alpha)^2}$$

# STED: **S**timulated **E**mission **D**epletion



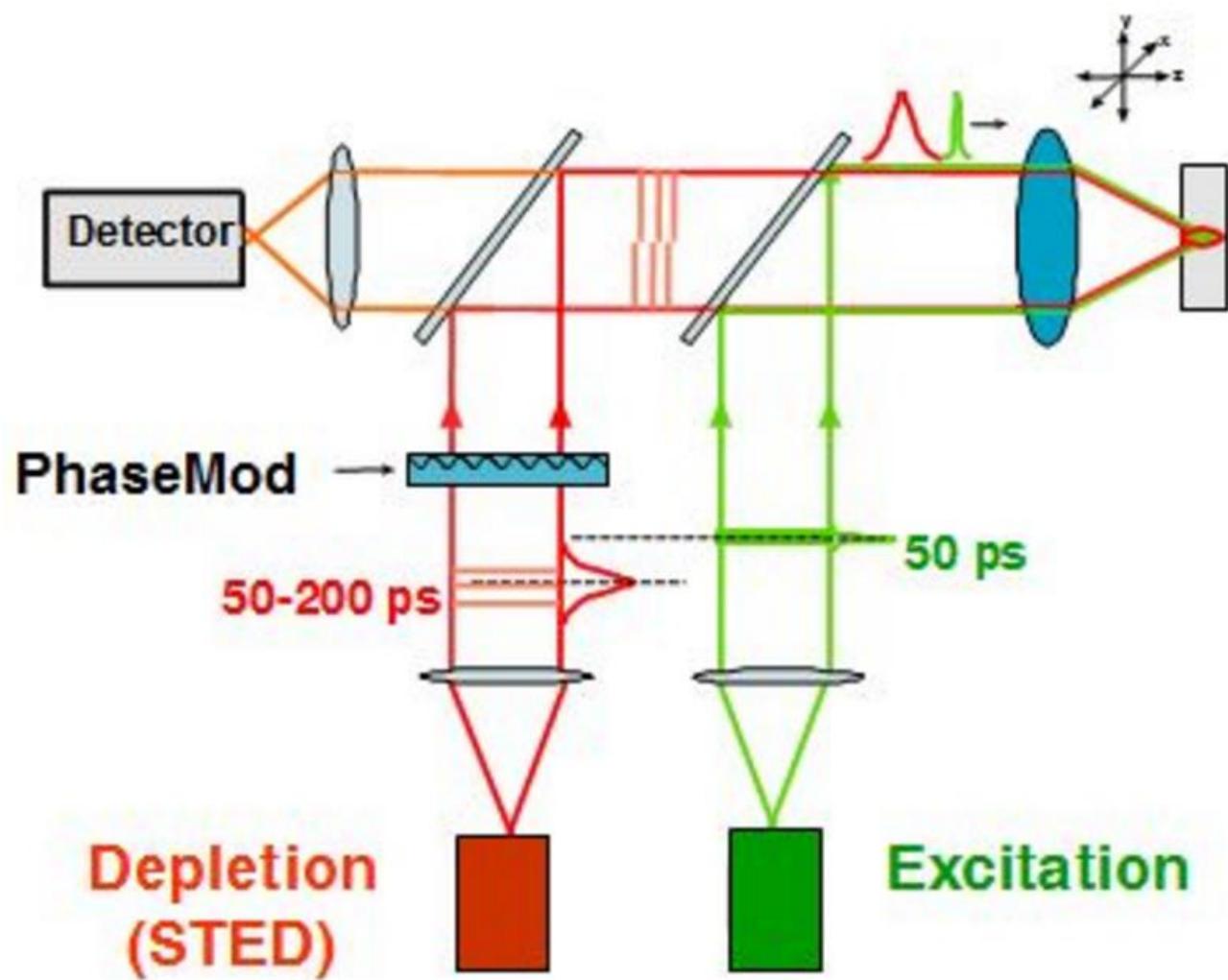


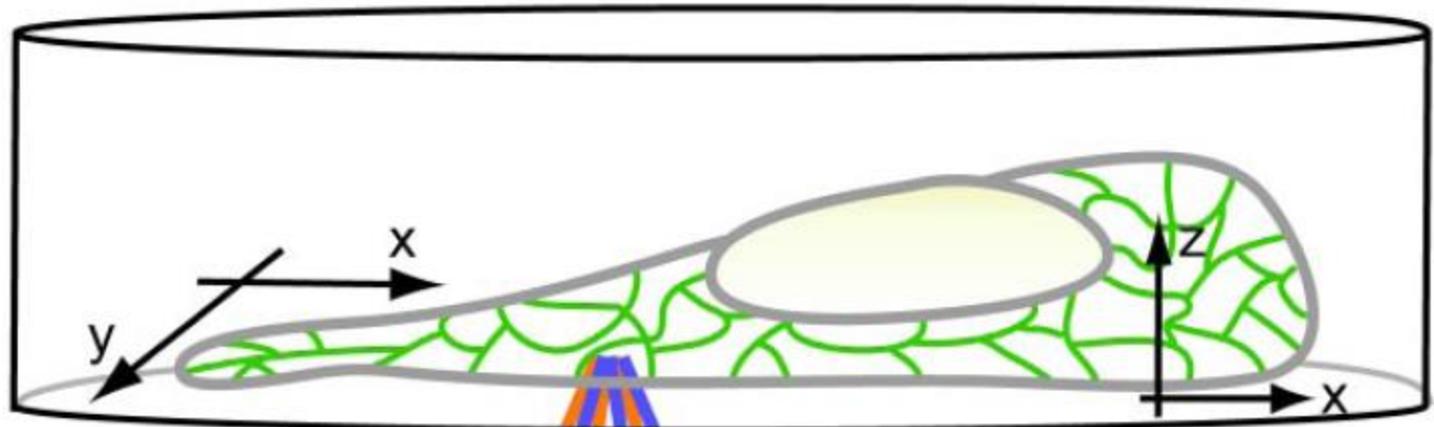
Doughnut-shaped mode

# Преодоление дифракционного барьера

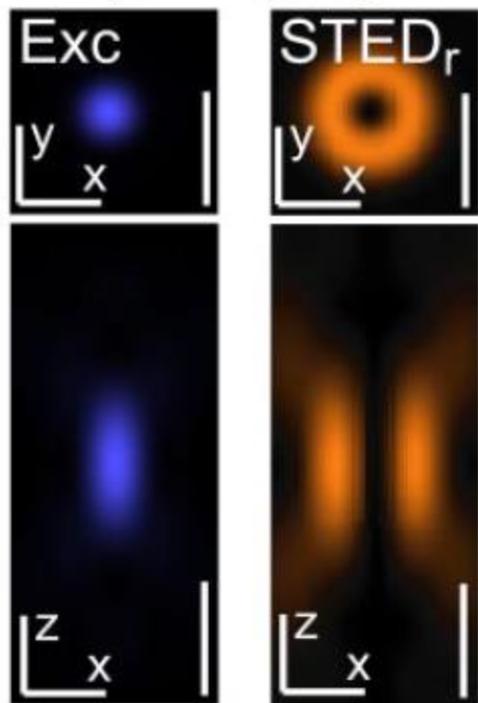
$$\Delta r \cong \lambda / 2NA \sqrt{1 + I / I_s}.$$

NA=Numerical Aperture

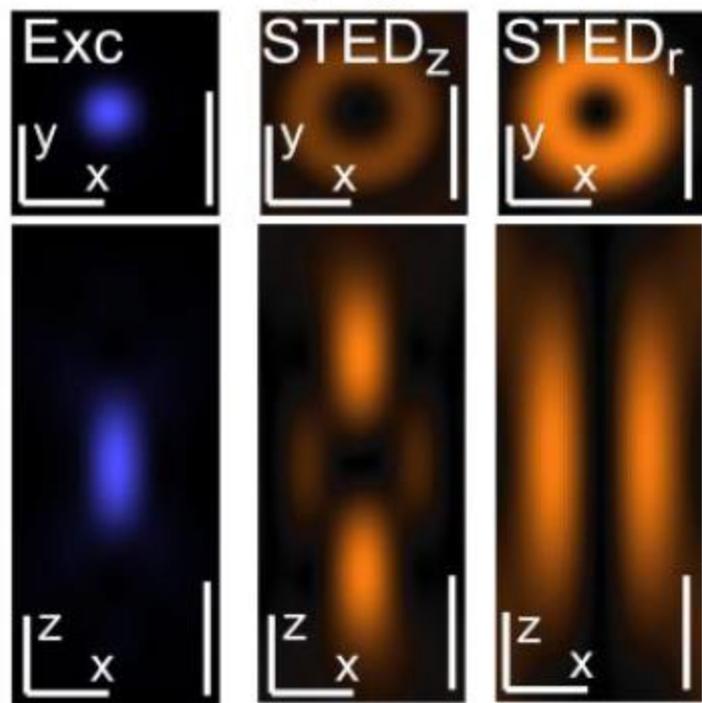


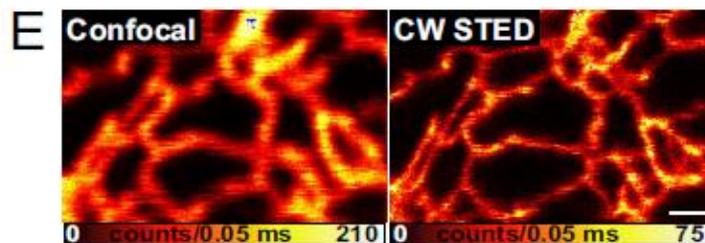
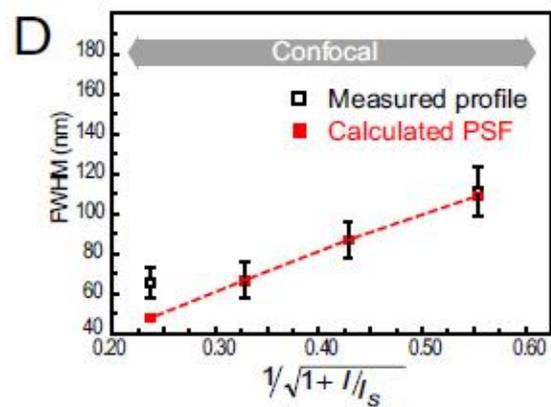
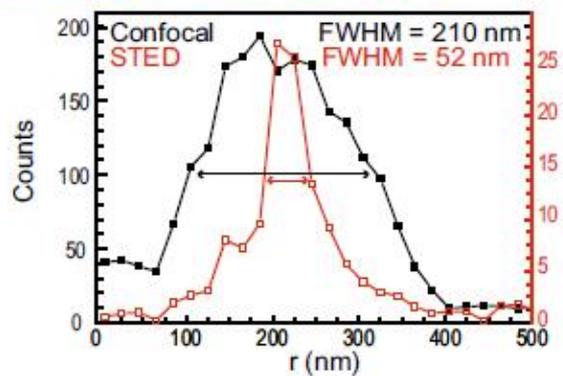
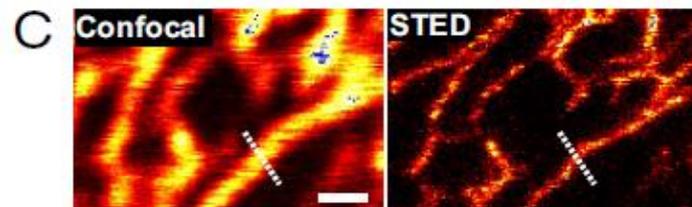
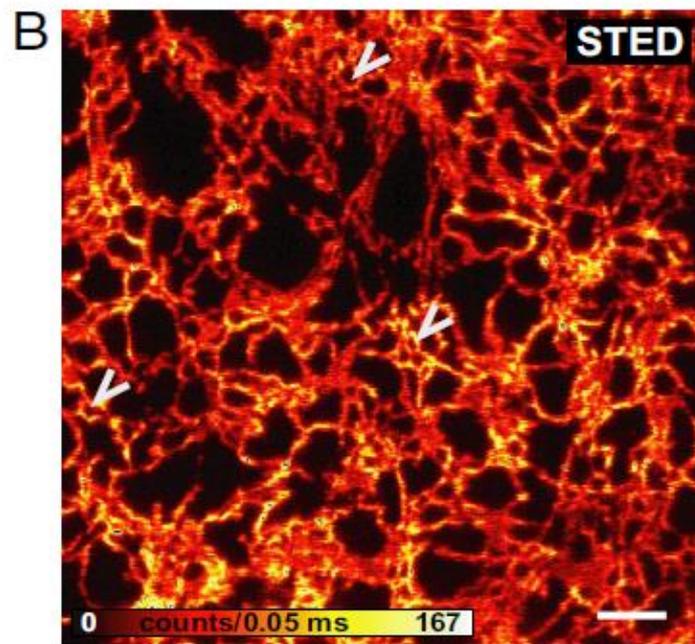
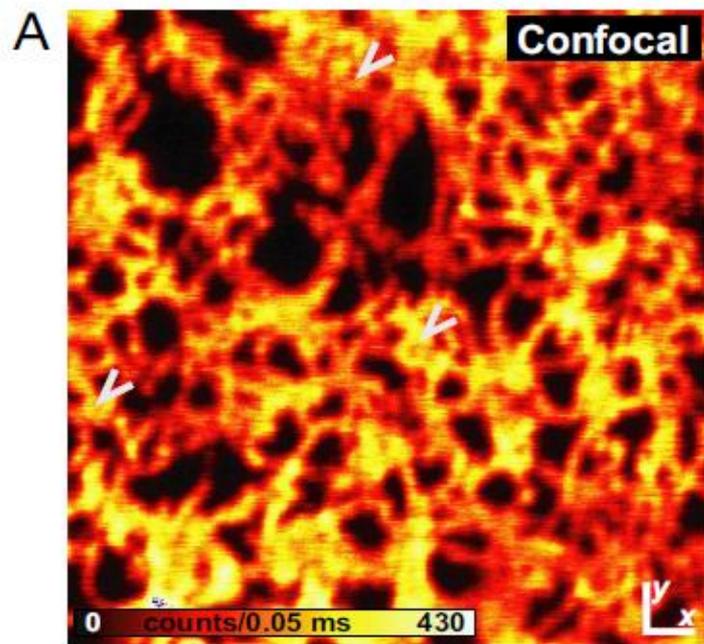


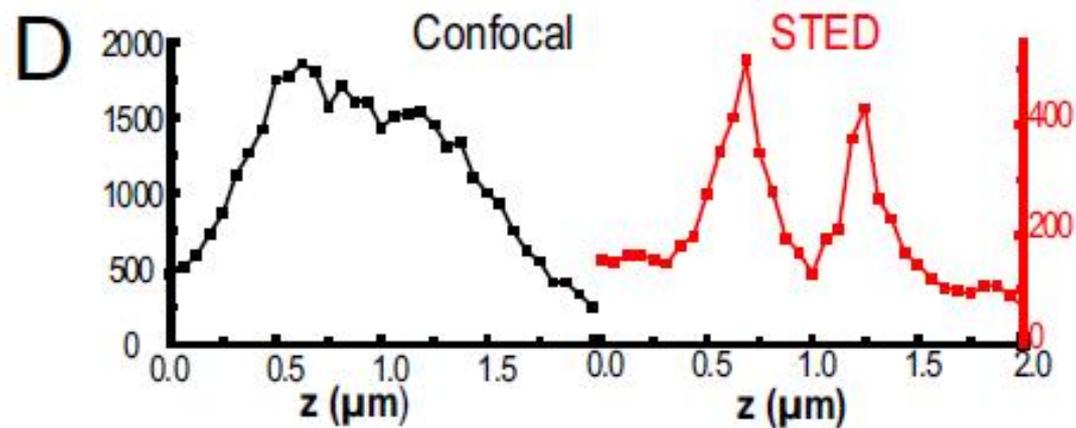
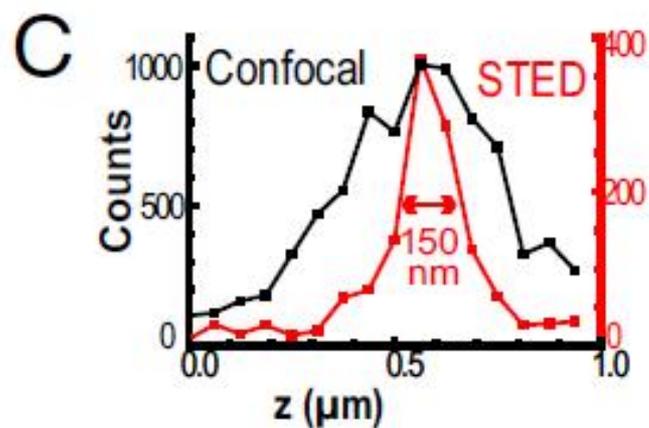
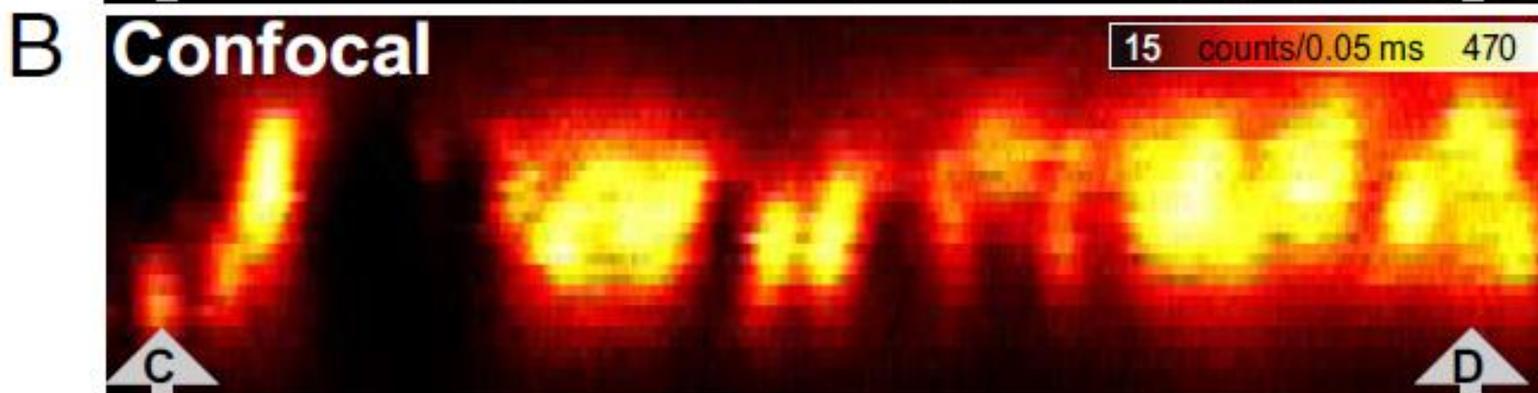
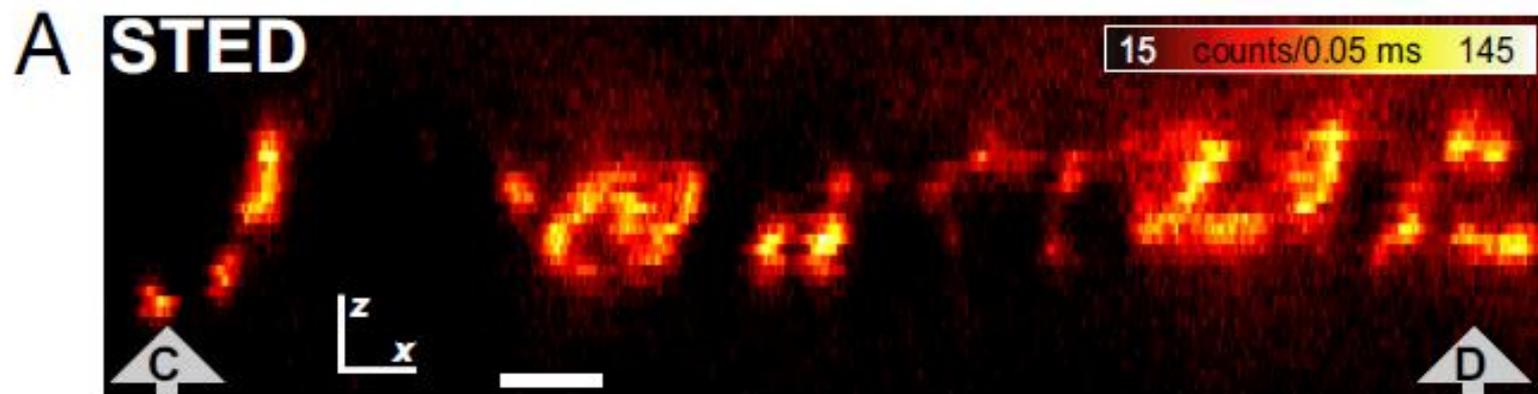
xy imaging



xz imaging



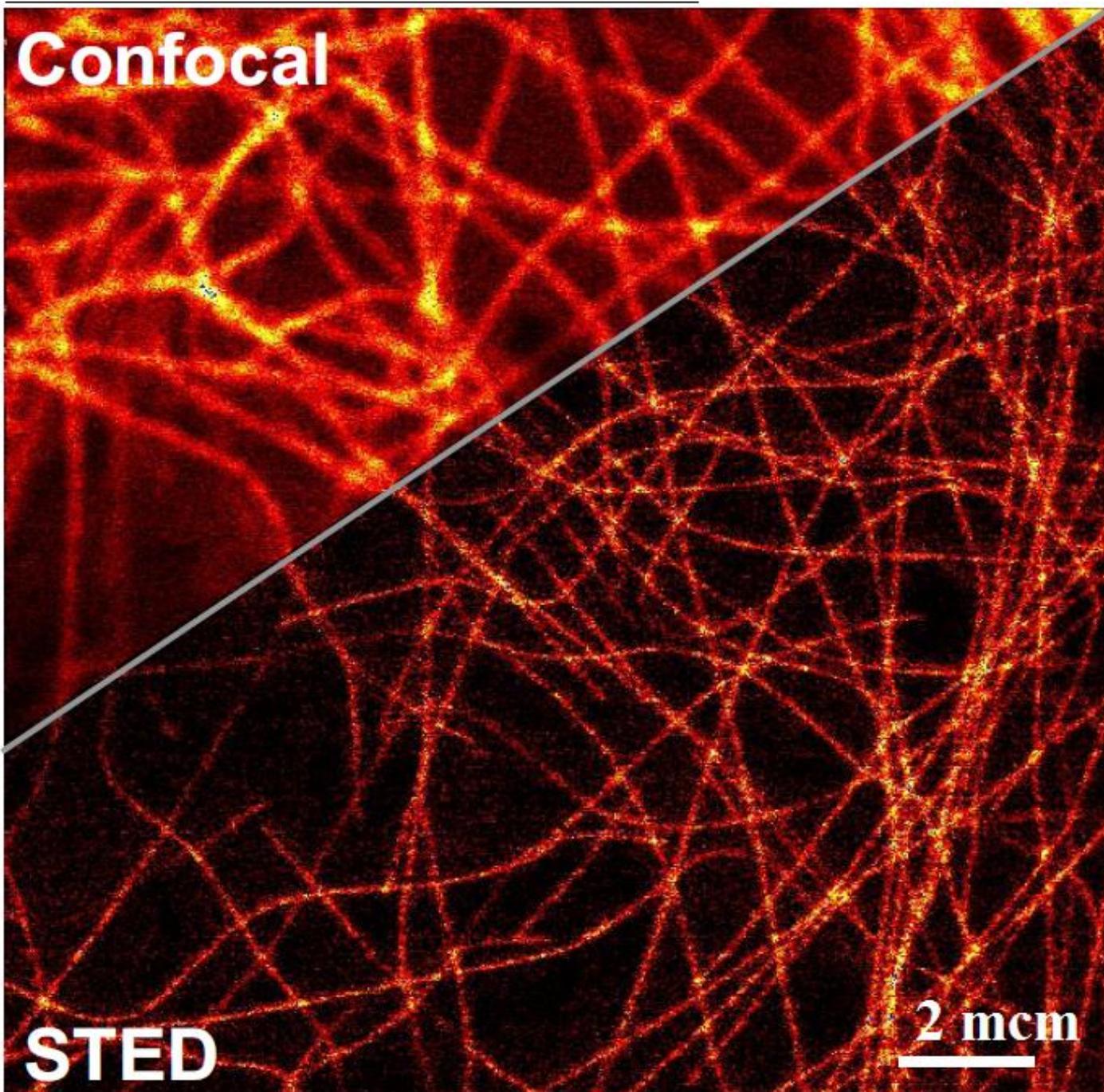




**Confocal**

**STED**

**2  $\mu$ m**



# PALM and STORM

- PALM=

Photoactivated Localization Microscopy

- STORM=

Stochastic Optical Reconstruction Microscopy

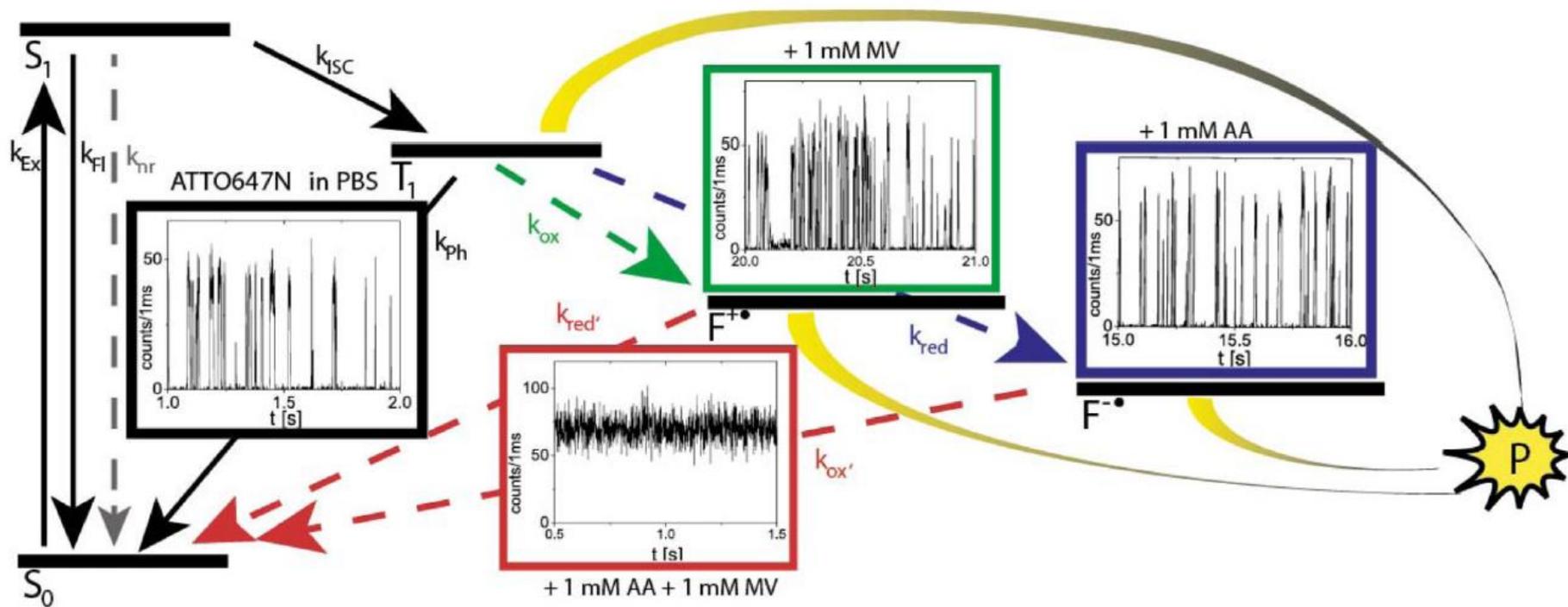
# Основная идея

- Чередование импульсов активации и деактивации флюорофоров
- Статистический анализ распределения числа фотонов для локализации отдельных флюорофоров

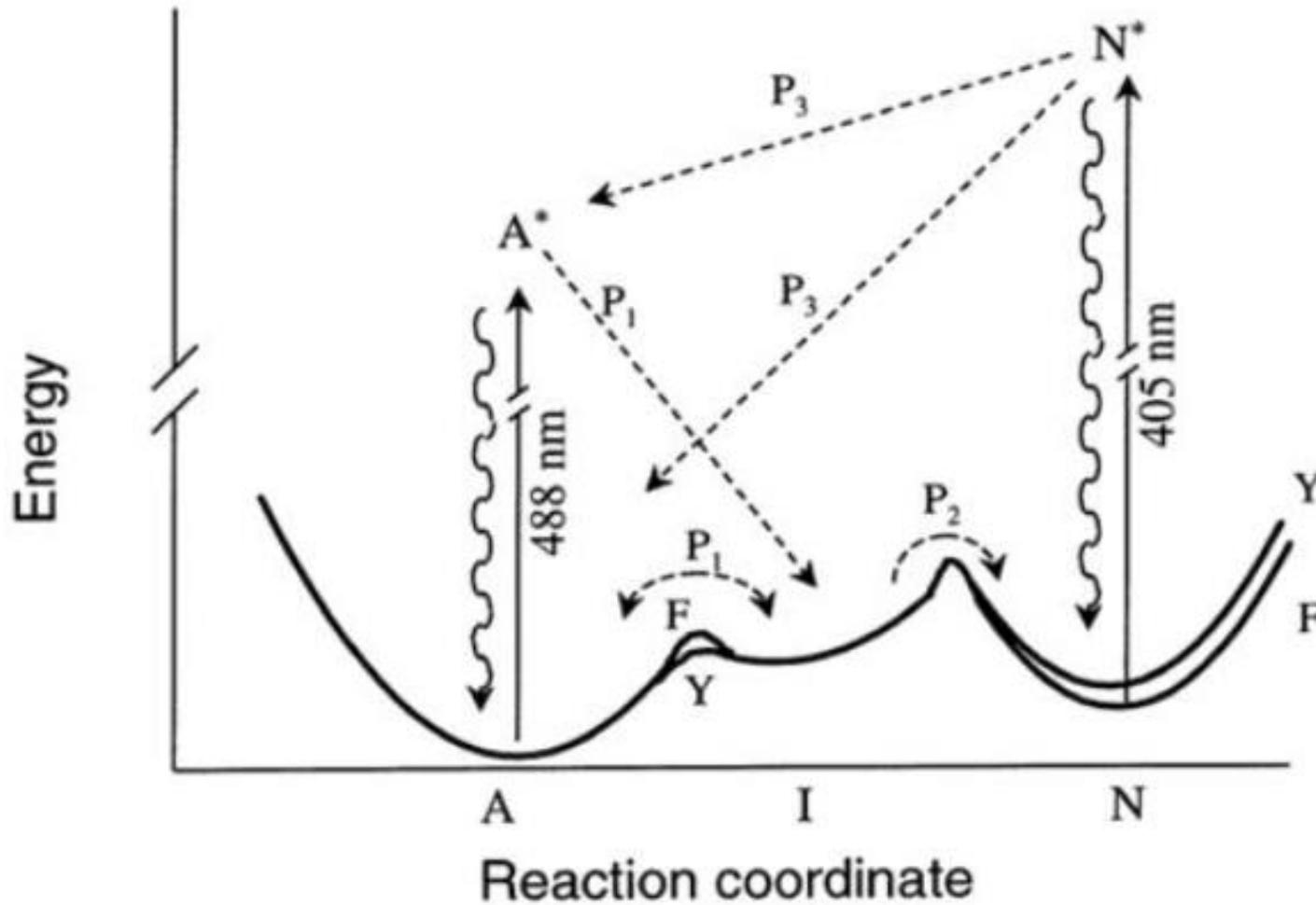
# Основные причины «мигания» флуоресцентных молекул

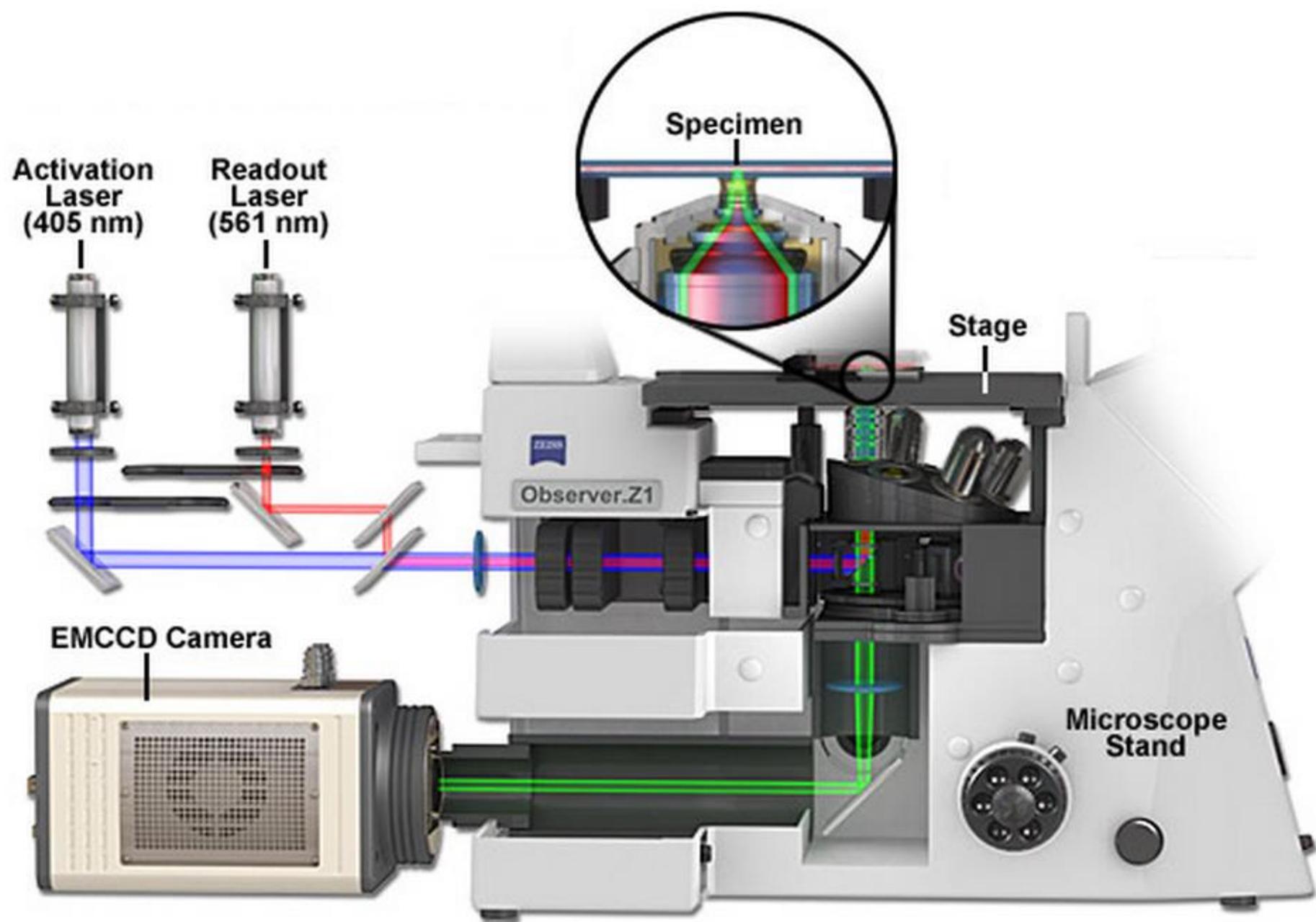
- 1) Переход в триплетные состояния
- 2) Обратимые переходы в альтернативные химические состояния (фотоиндуцированный перенос электрона -> формирование радикал-аниона или радикал-катиона)
- 3) Фотохромный эффект: обратимые химические модификации, изомеризация.

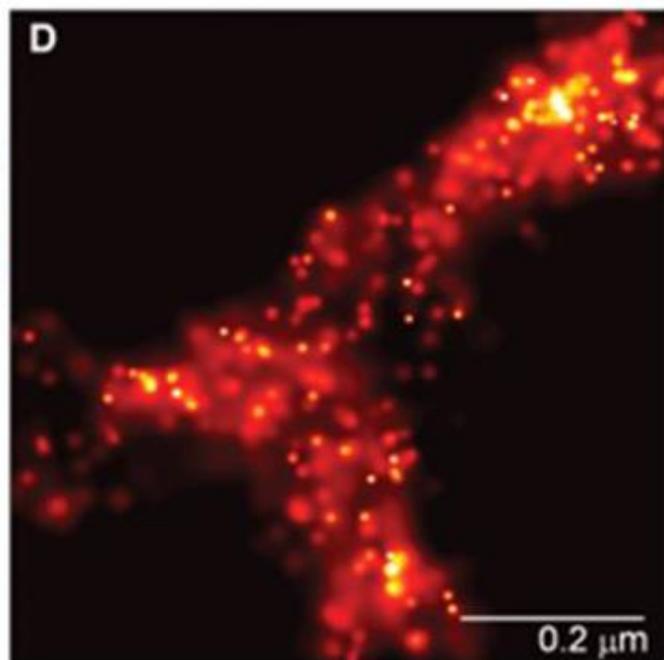
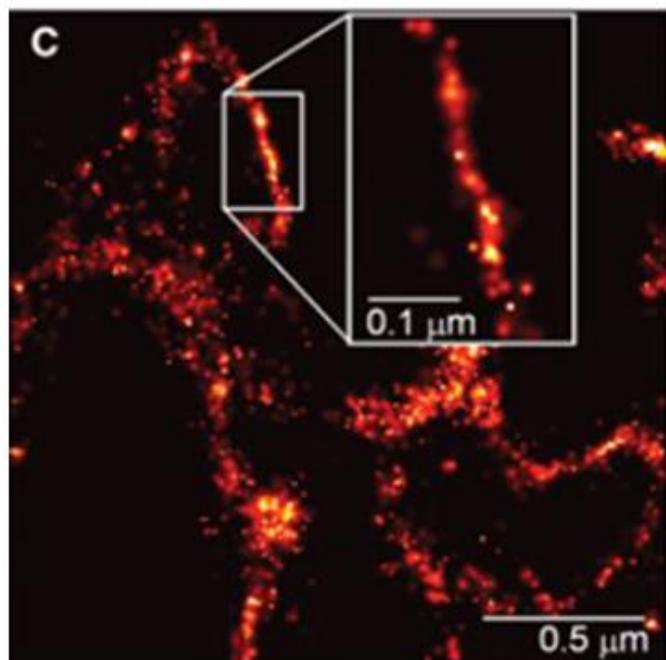
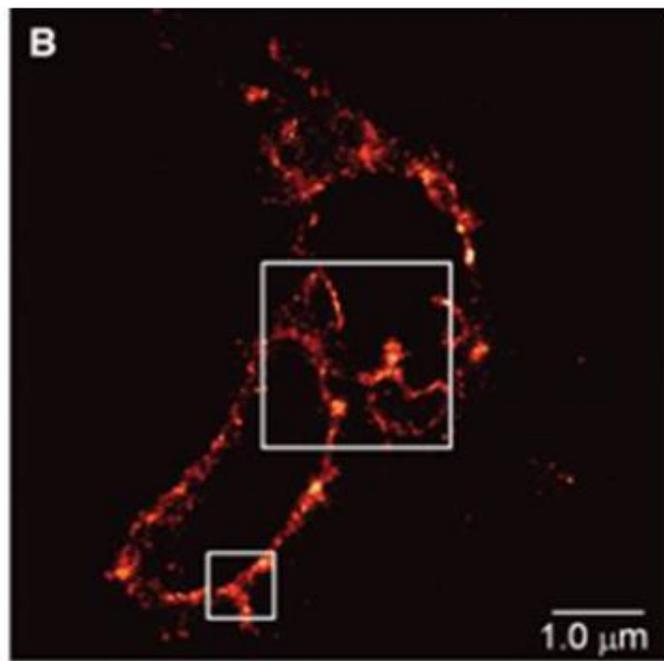
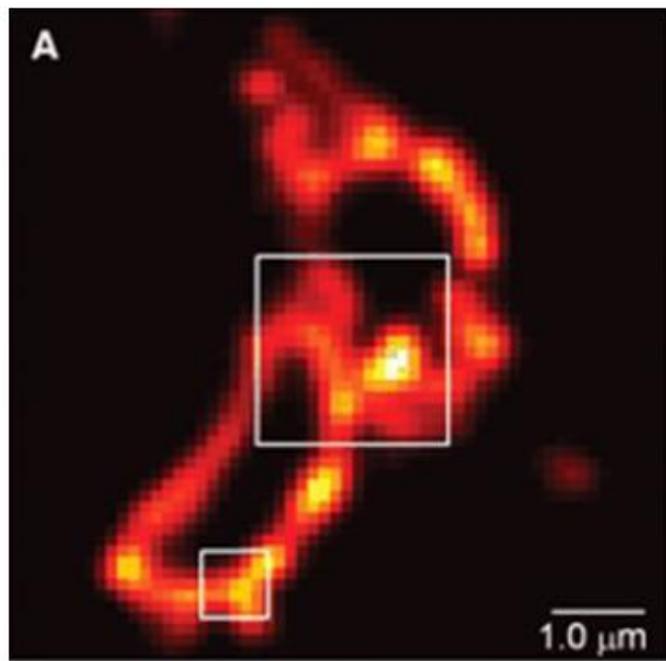
# Нестабильная флуоресценция



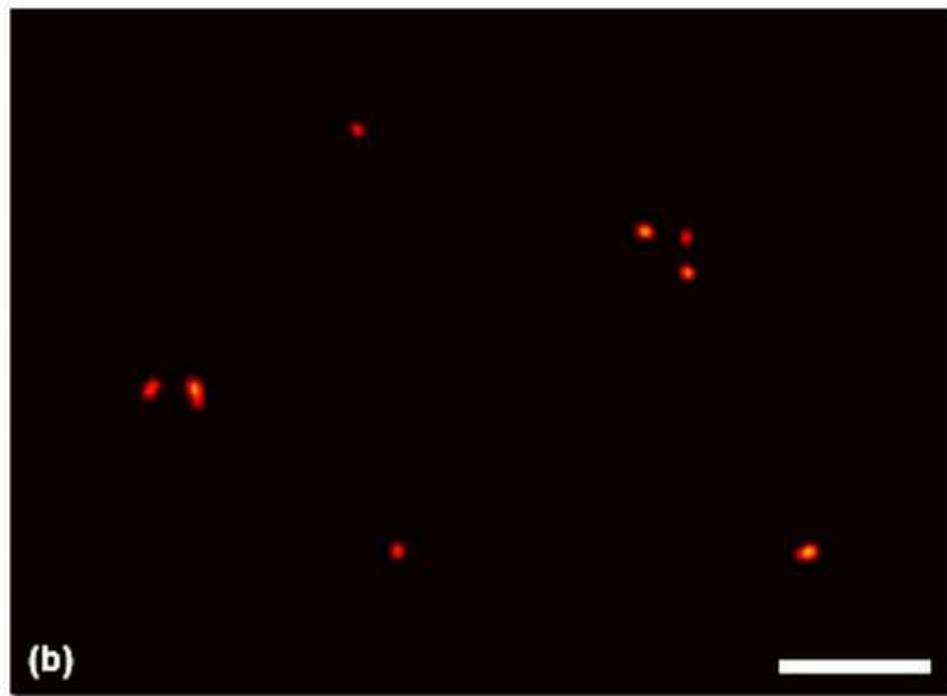
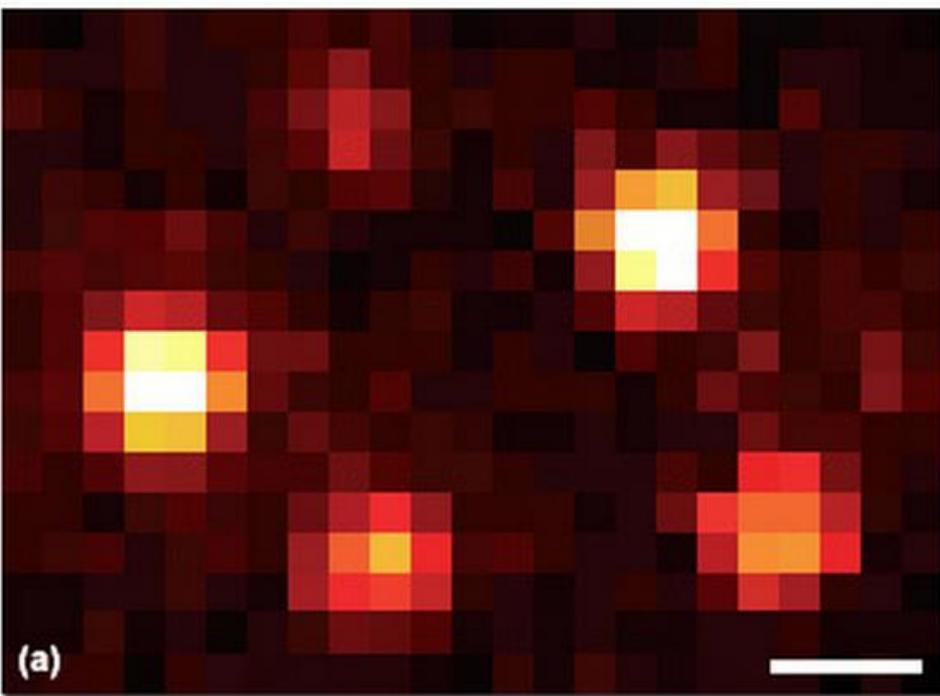
# Поведение mutGFP

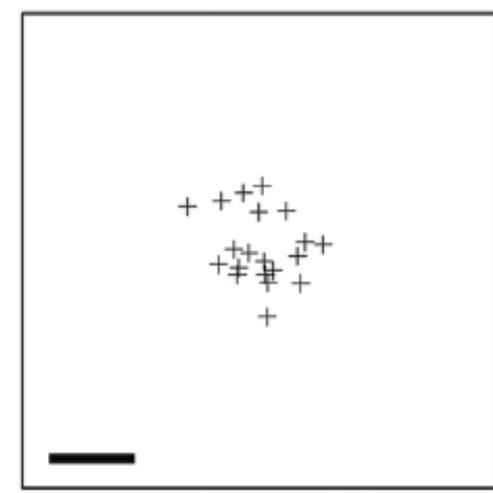
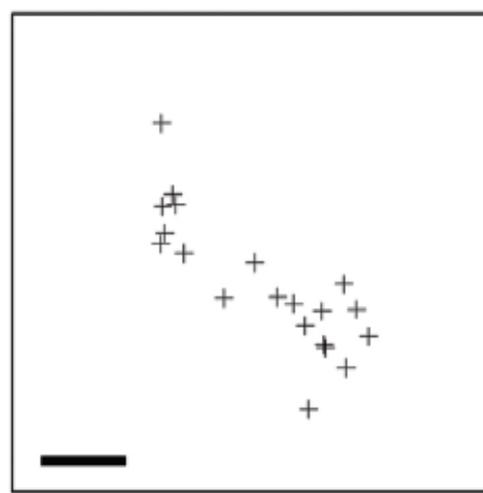
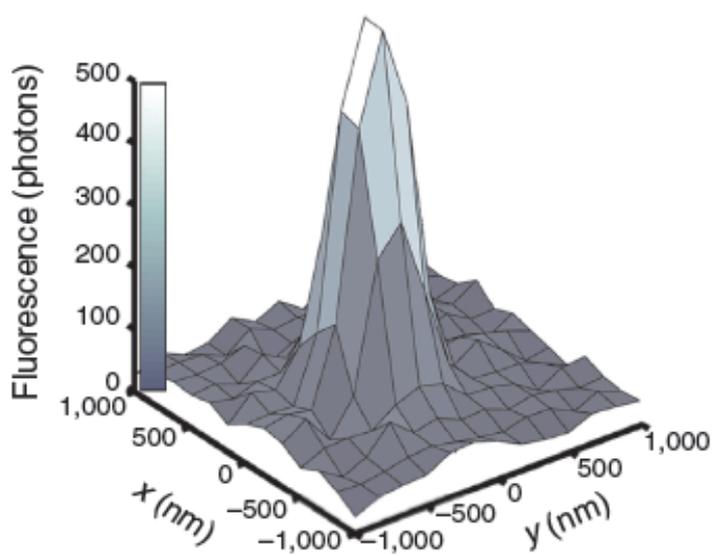
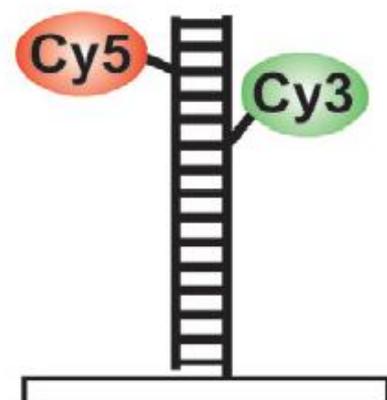
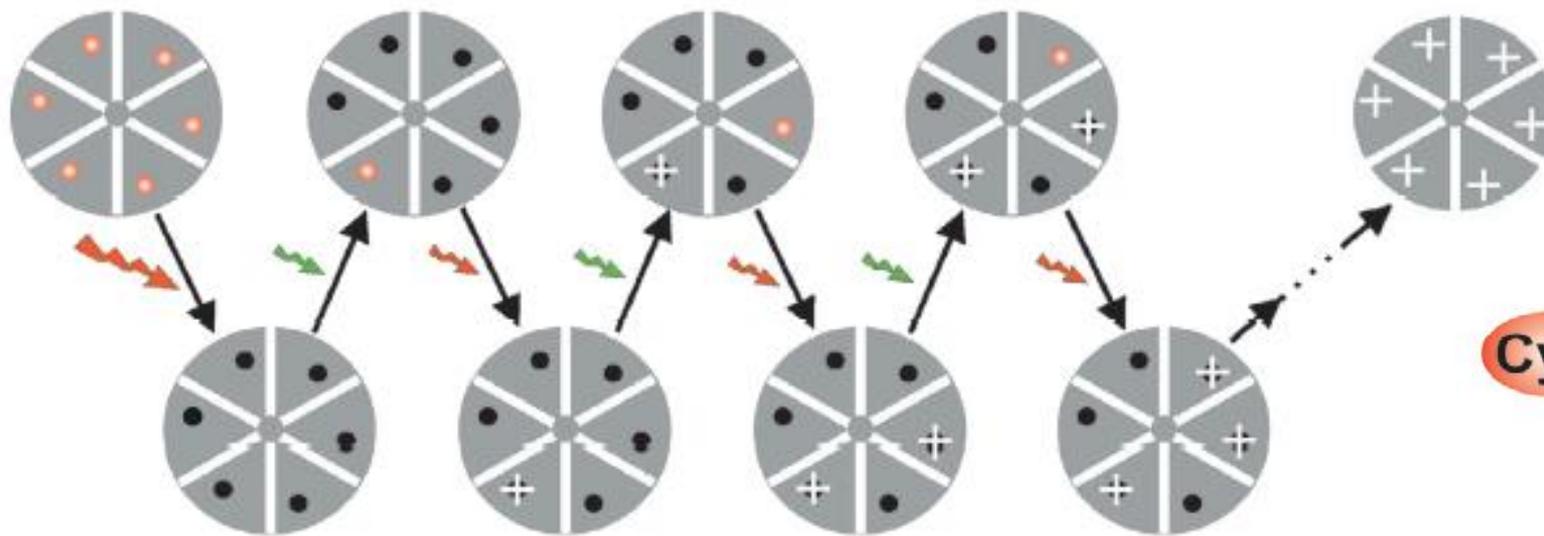




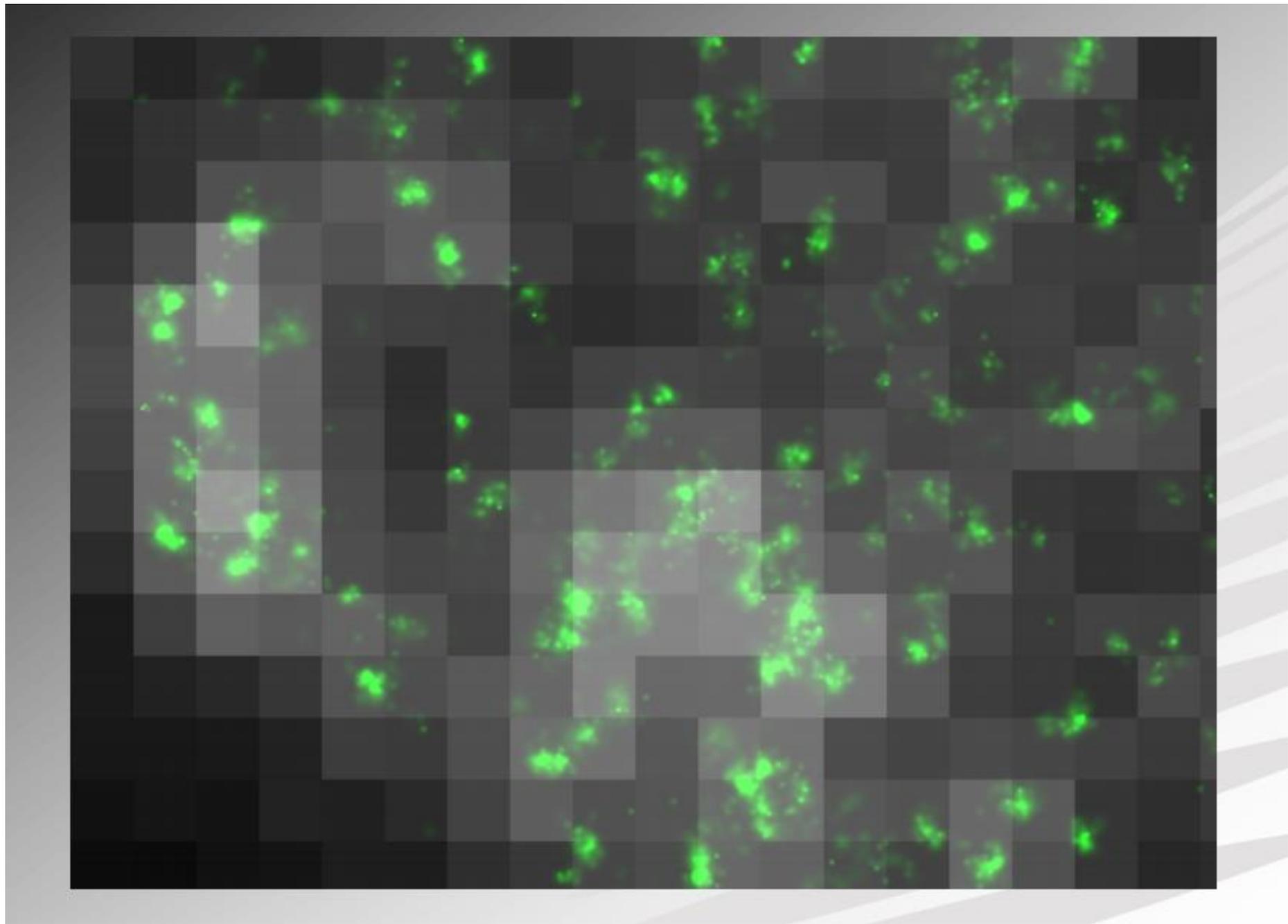


# Оптическая локализация традиционно неразрешимых молекул



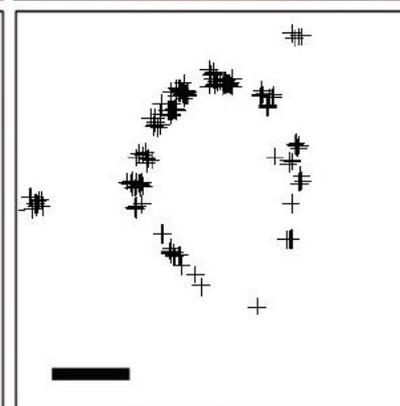
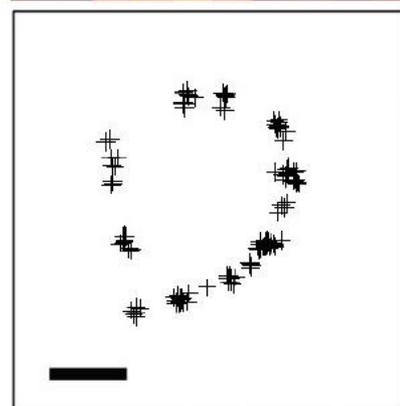
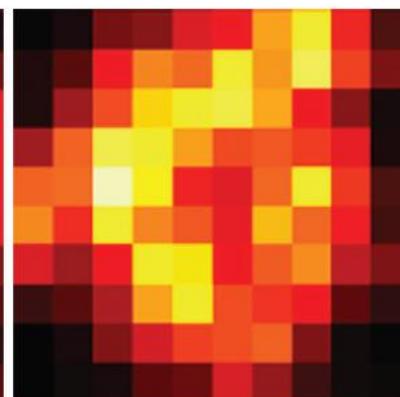
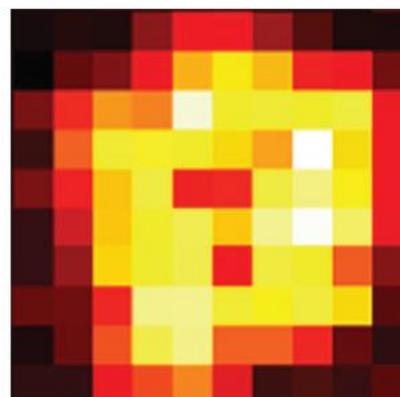
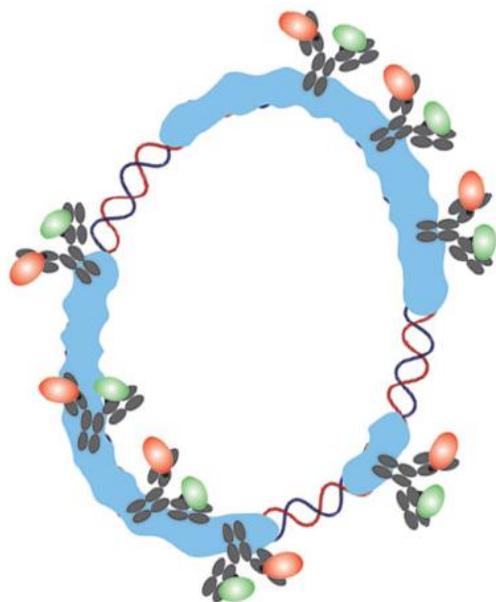
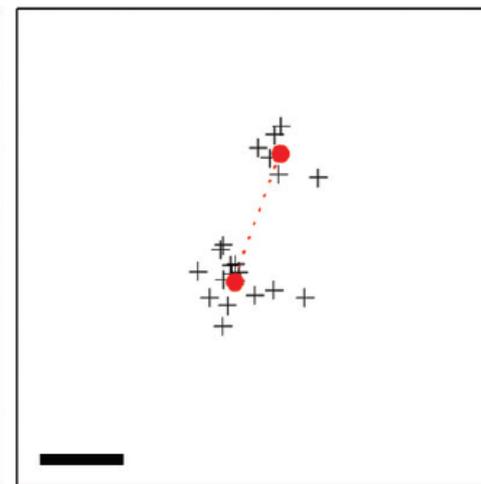
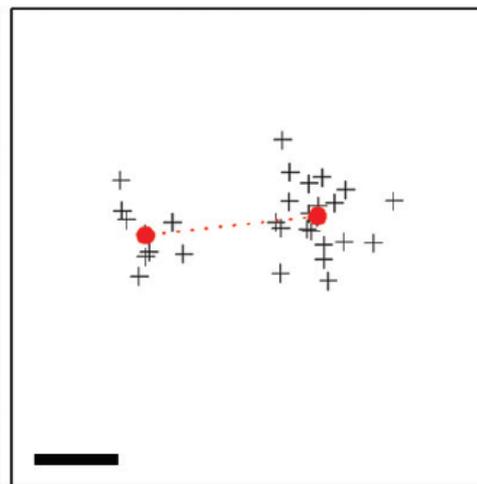
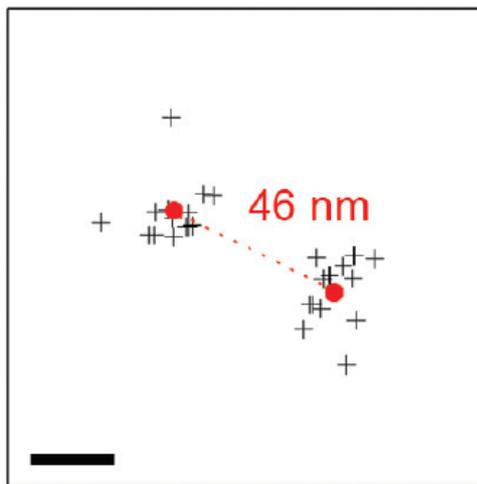
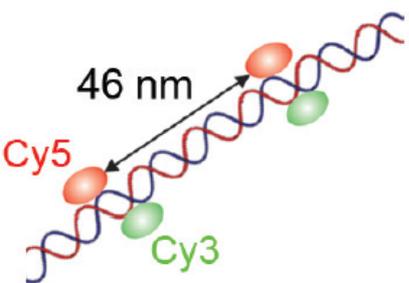


масштаб = 20 нм!



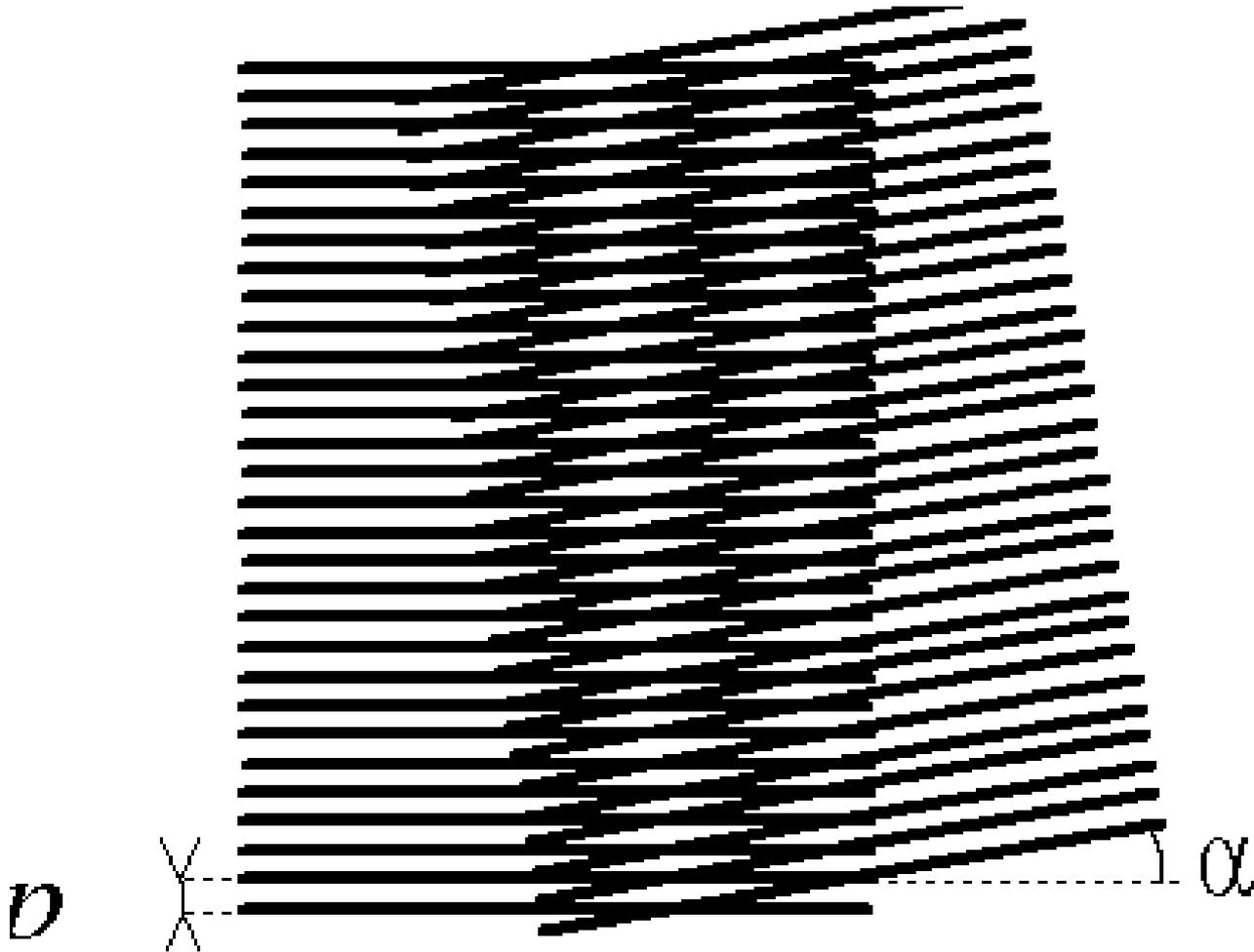
Ошибка определения положения флуорофора после устранения дрейфа

$$\sigma = \sqrt{\left(\frac{s_i^2 + \frac{a^2}{12}}{N}\right) \cdot \left(\frac{16}{9} + \frac{8\pi s_i^2 b^2}{a^2 N}\right)}$$

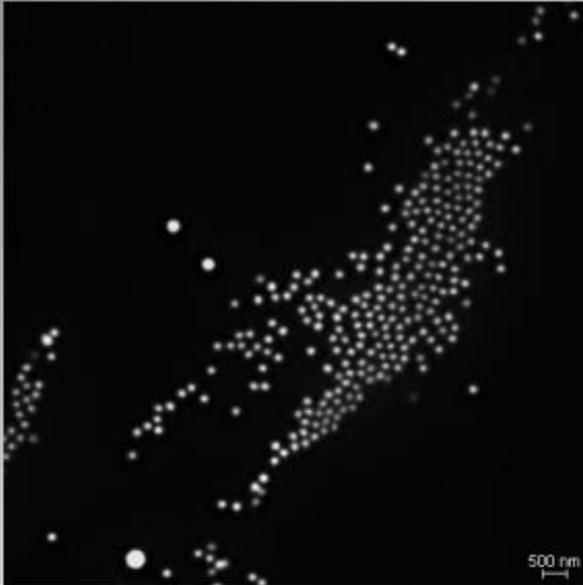
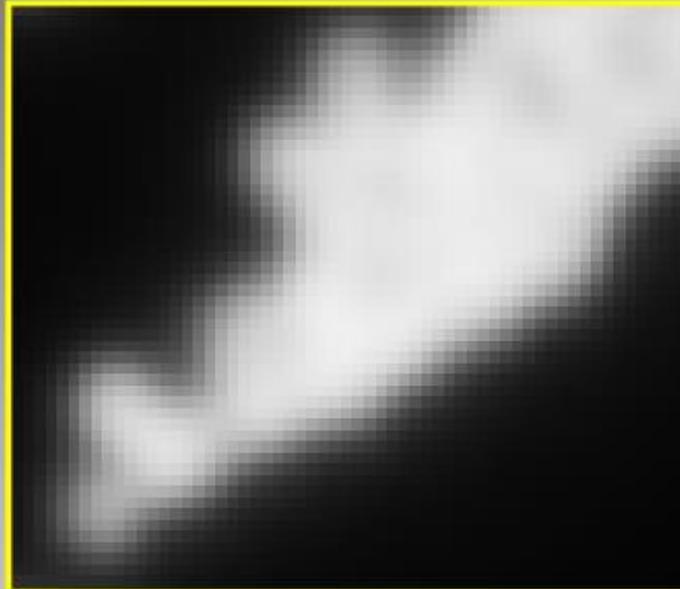
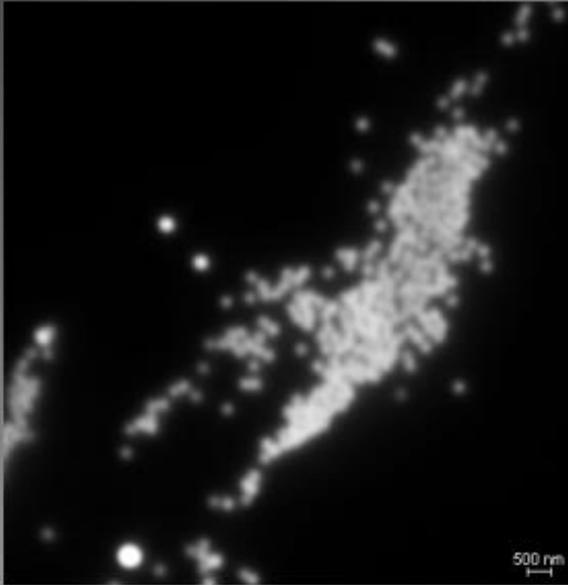




# SIM: structural illumination microscopy (микроскопия структурированного освещения)



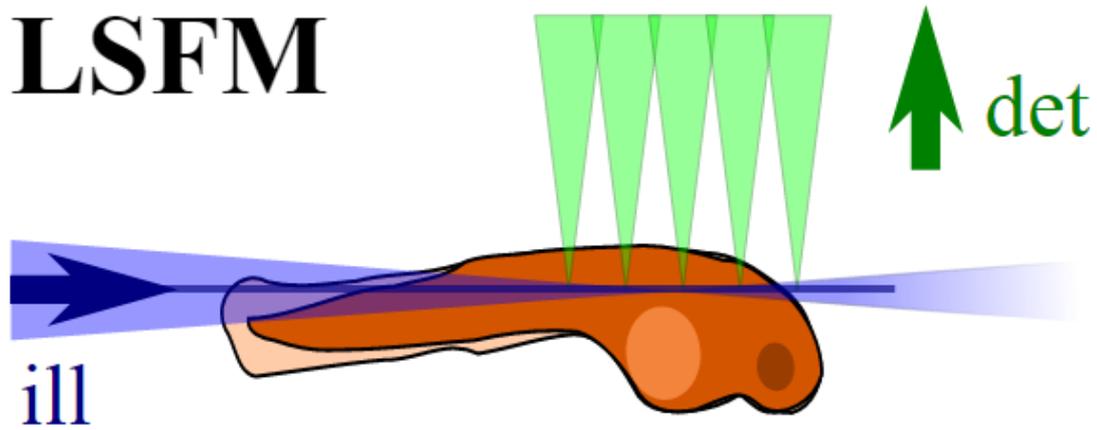
# 200 nm green/yellow beads



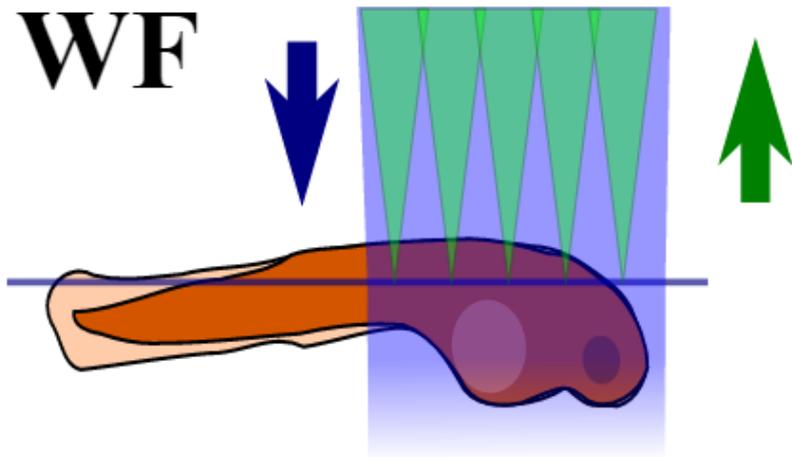
# Микроскопия световых «листов»



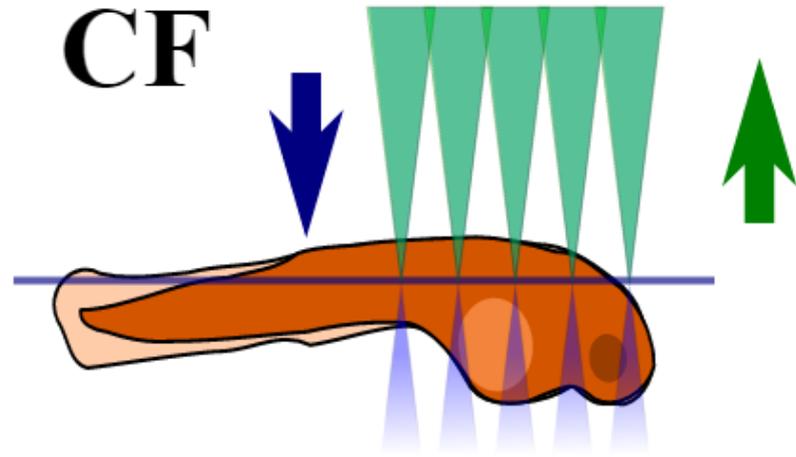
**LSEFM**

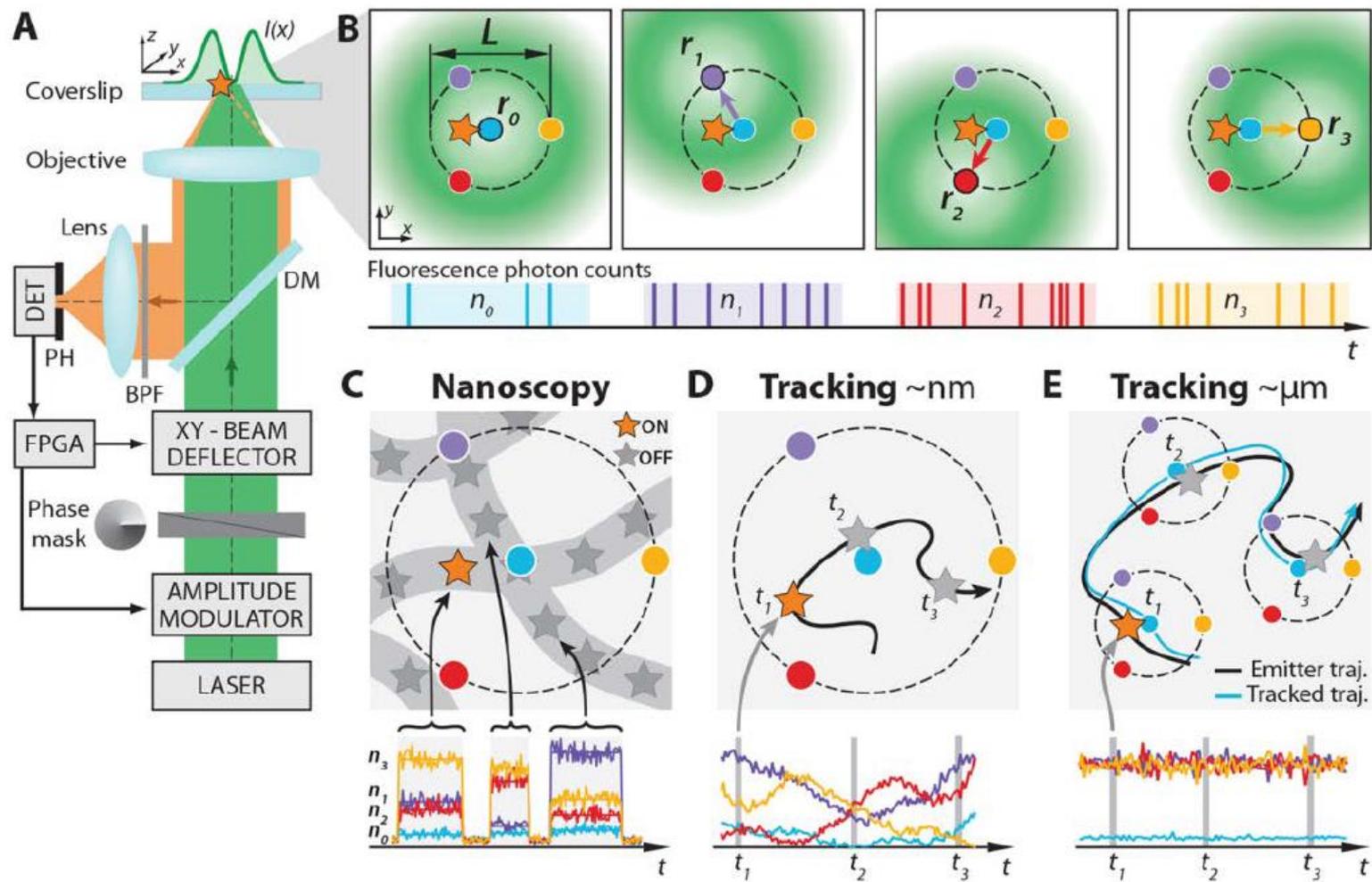


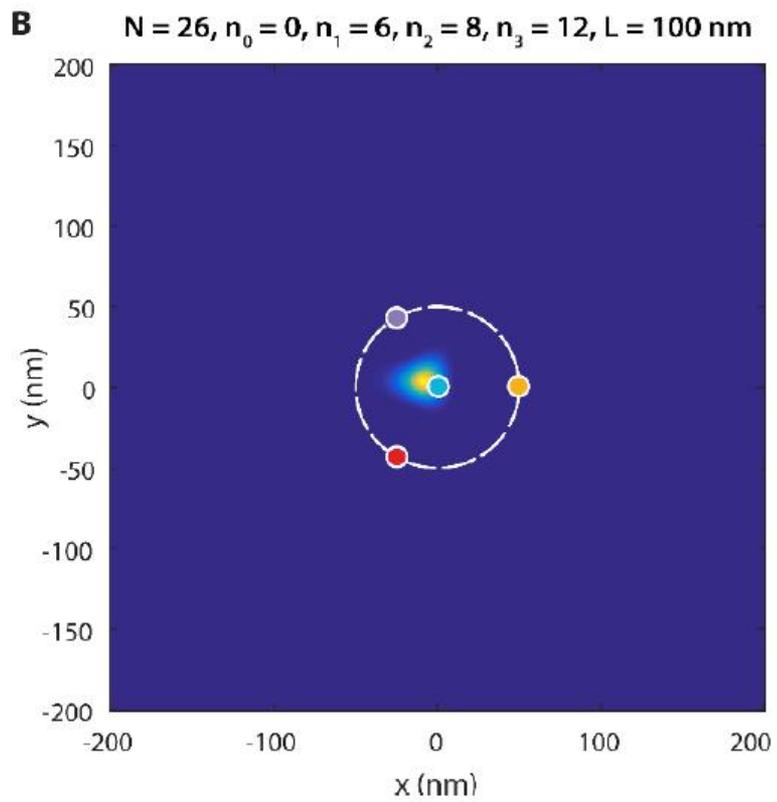
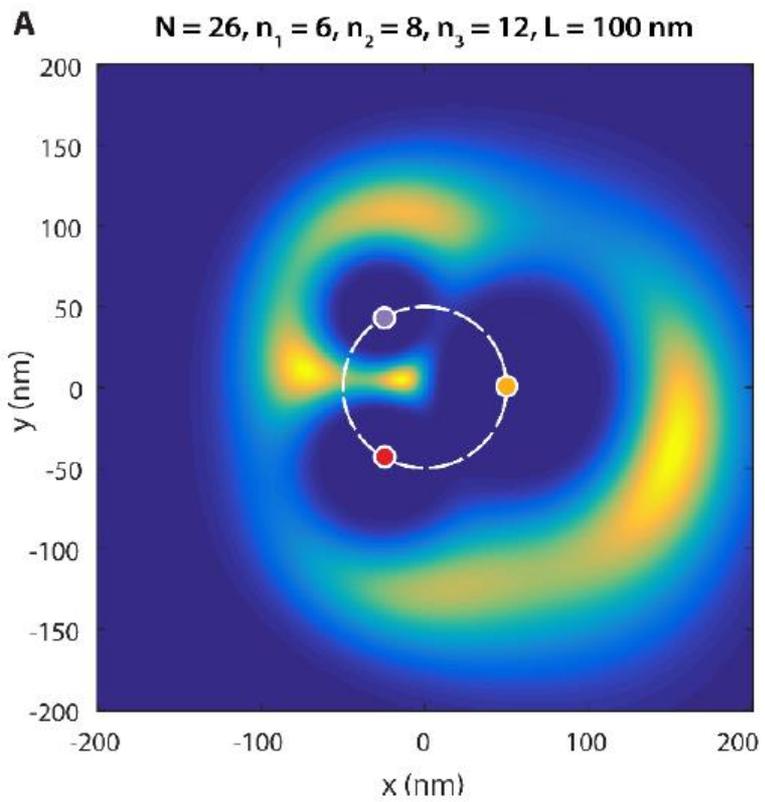
**WF**

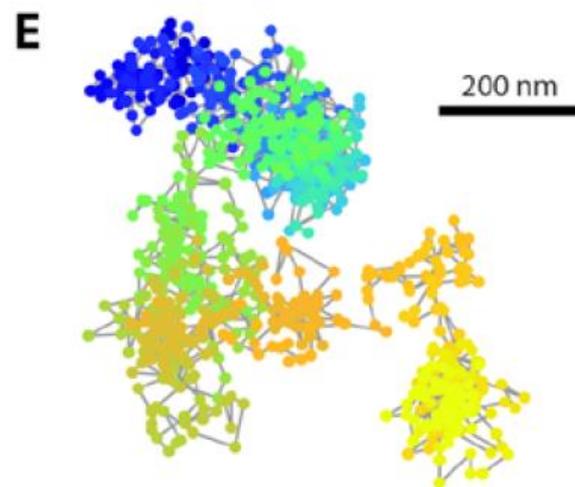
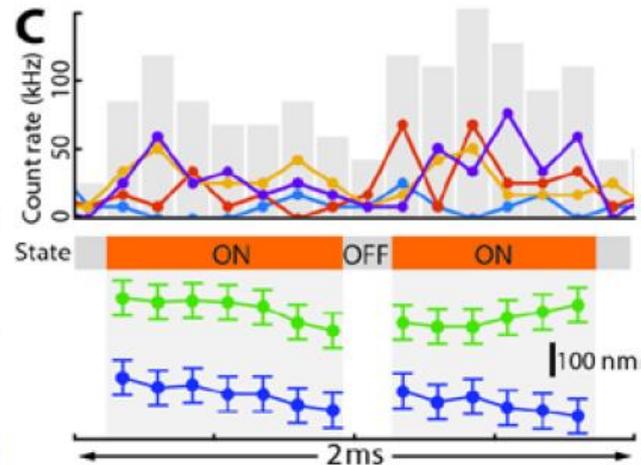
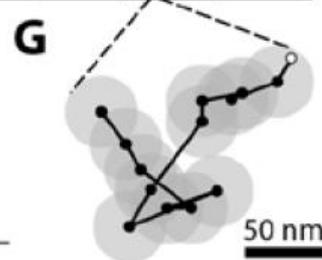
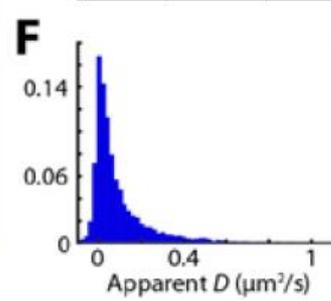
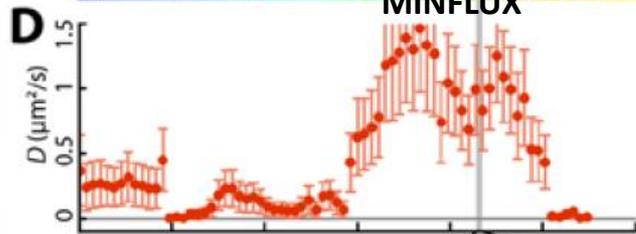
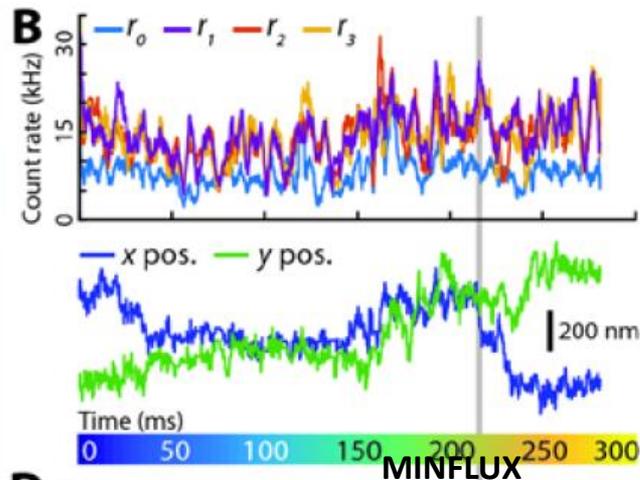
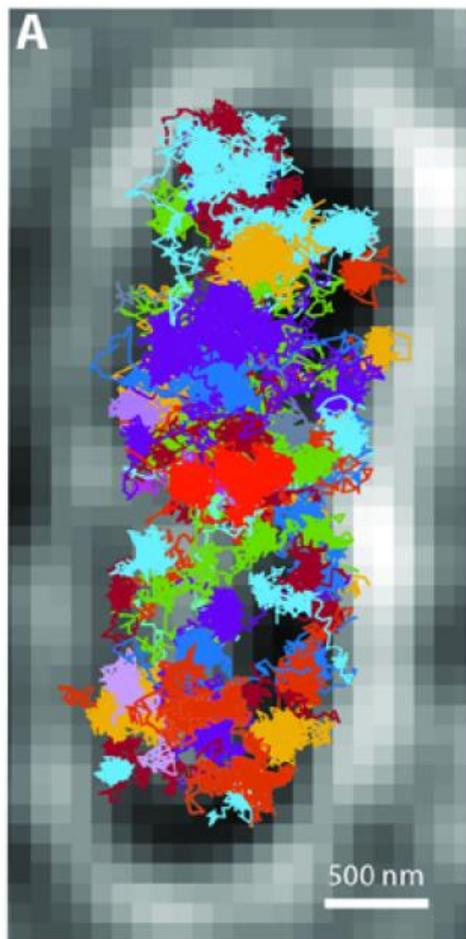


**CF**









Изучение динамики отдельных  
молекул при помощи FRET/TIRF  
микроскопии

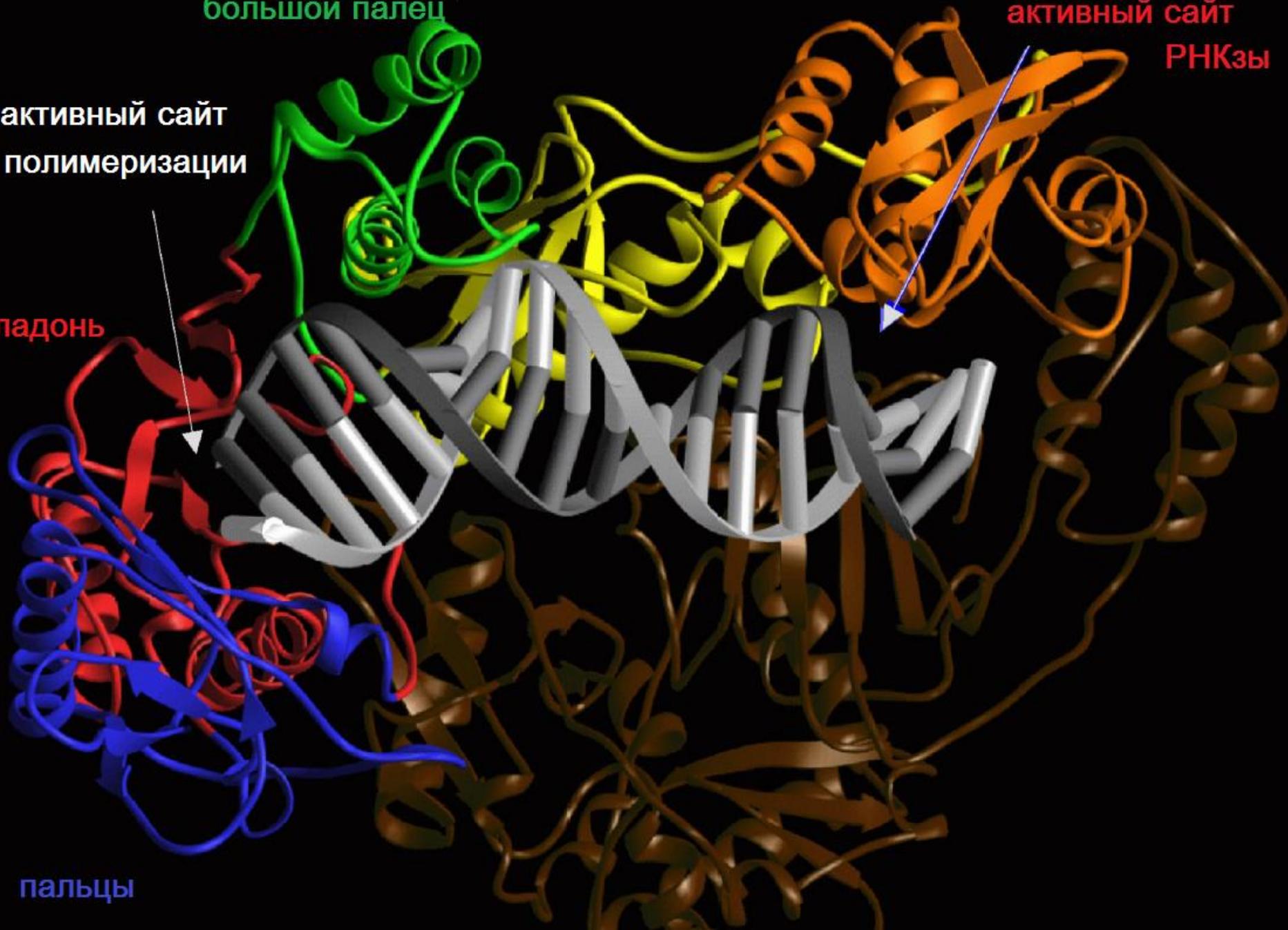
большой палец

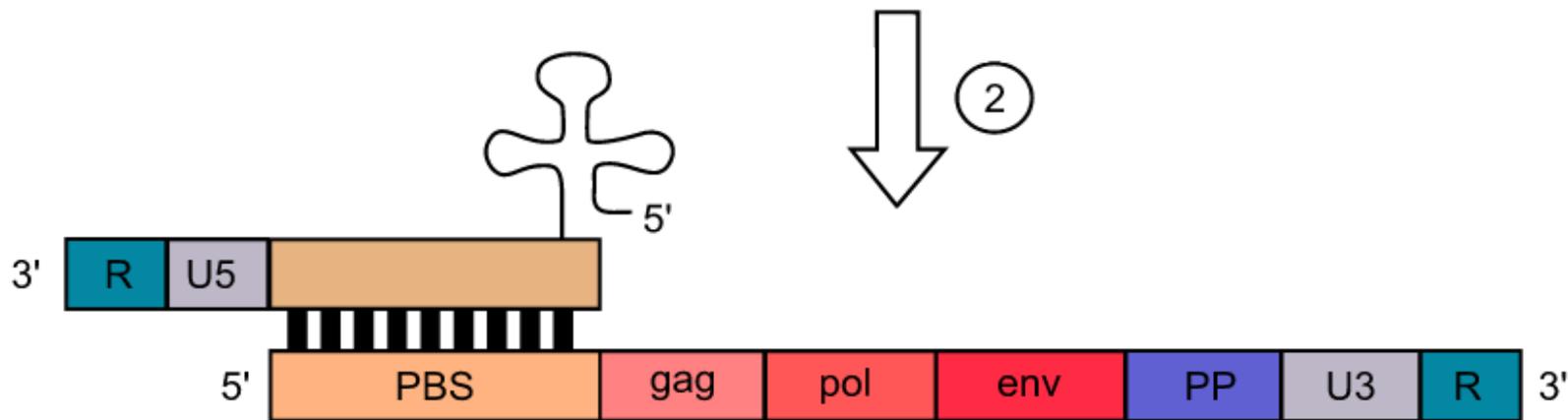
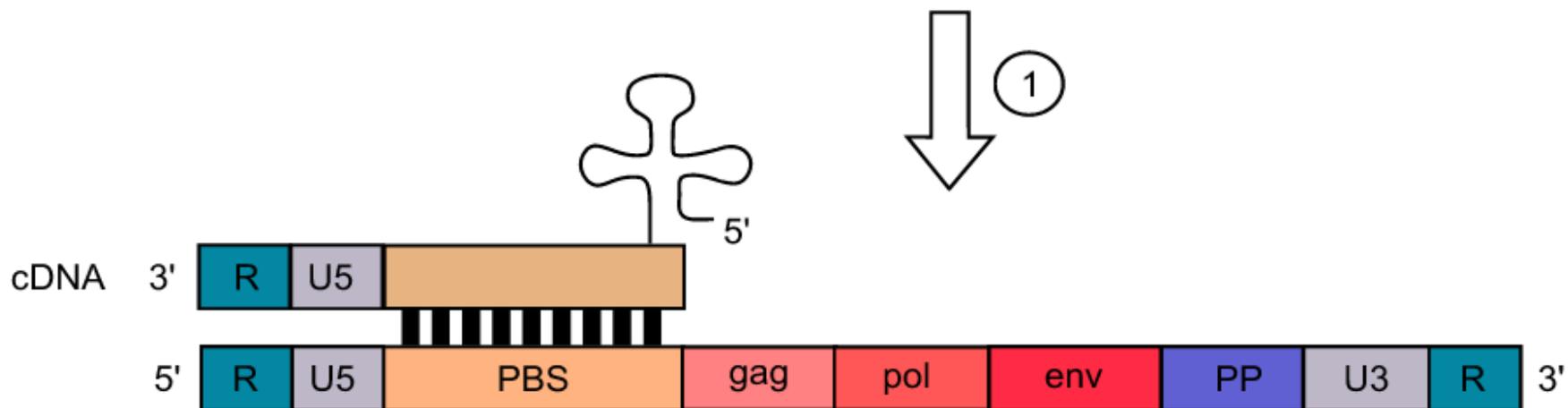
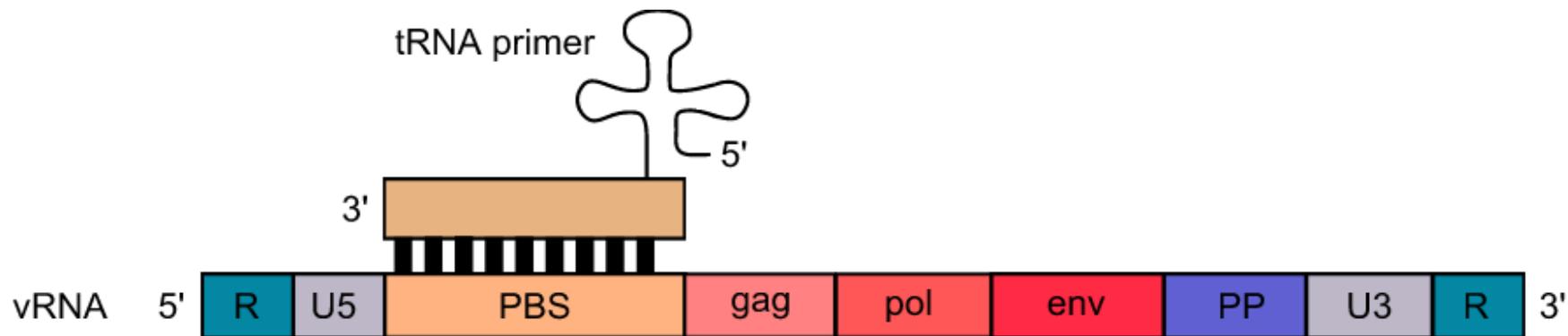
активный сайт  
РНКзы

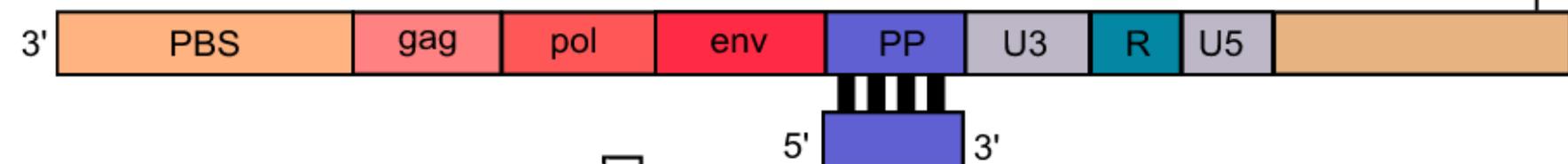
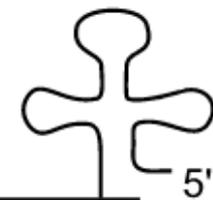
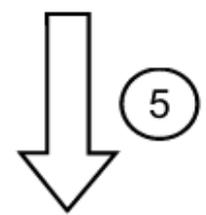
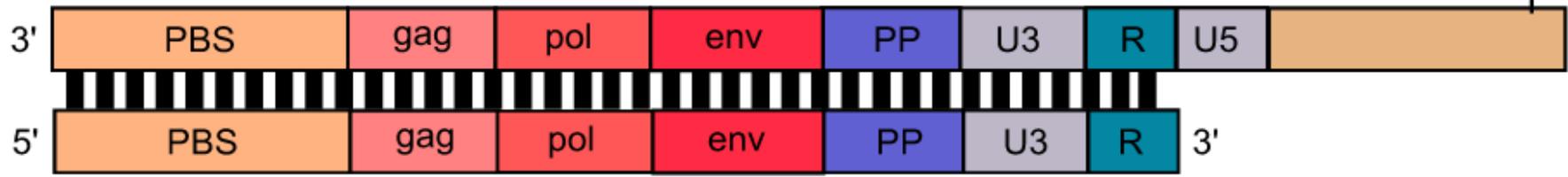
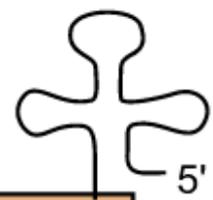
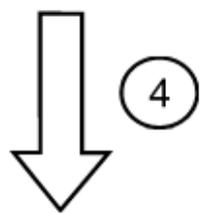
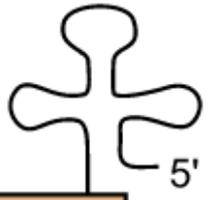
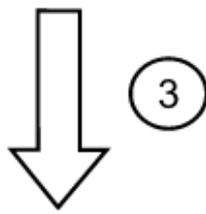
активный сайт  
полимеризации

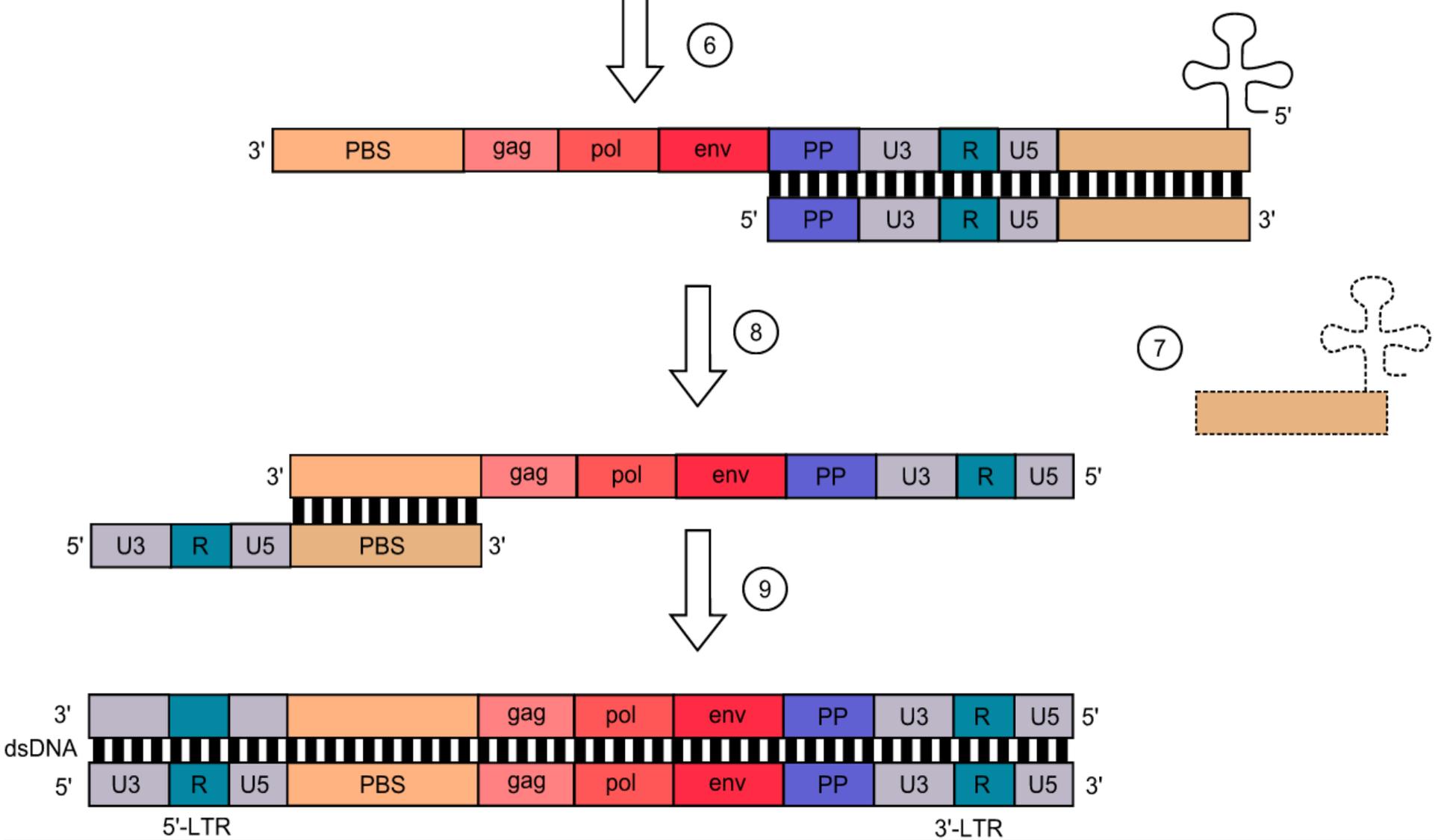
ладонь

пальцы

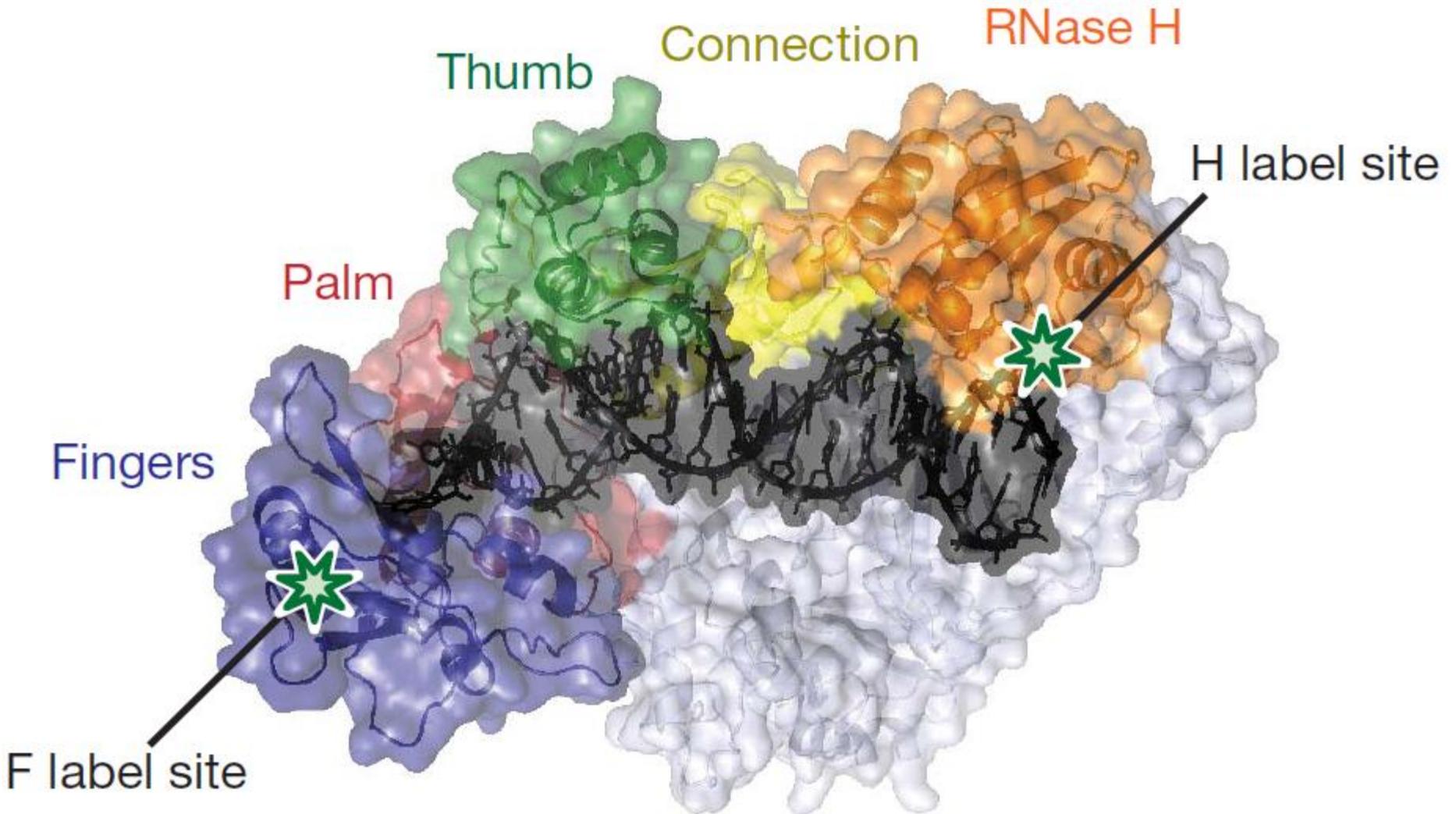




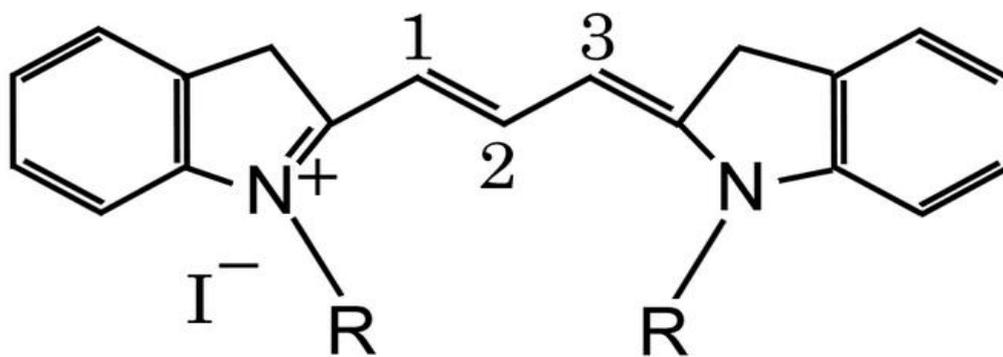




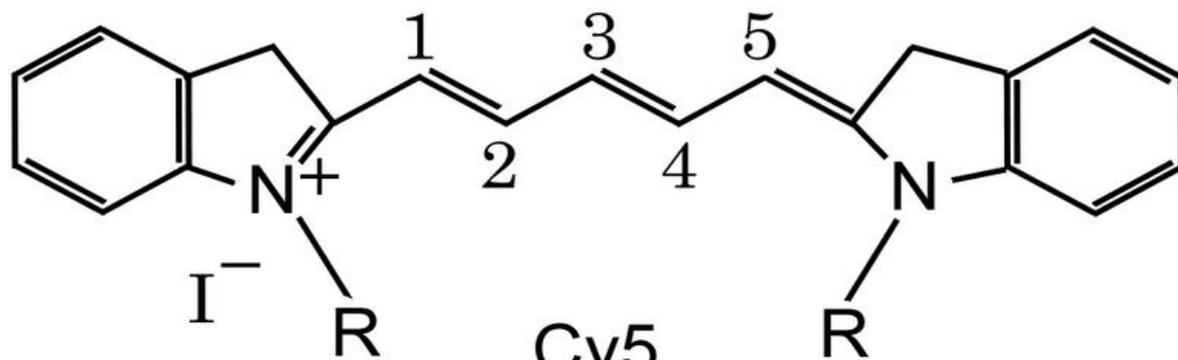
# TIRF + FRET: Опыты с ОТ ВИЧ



# Цианины

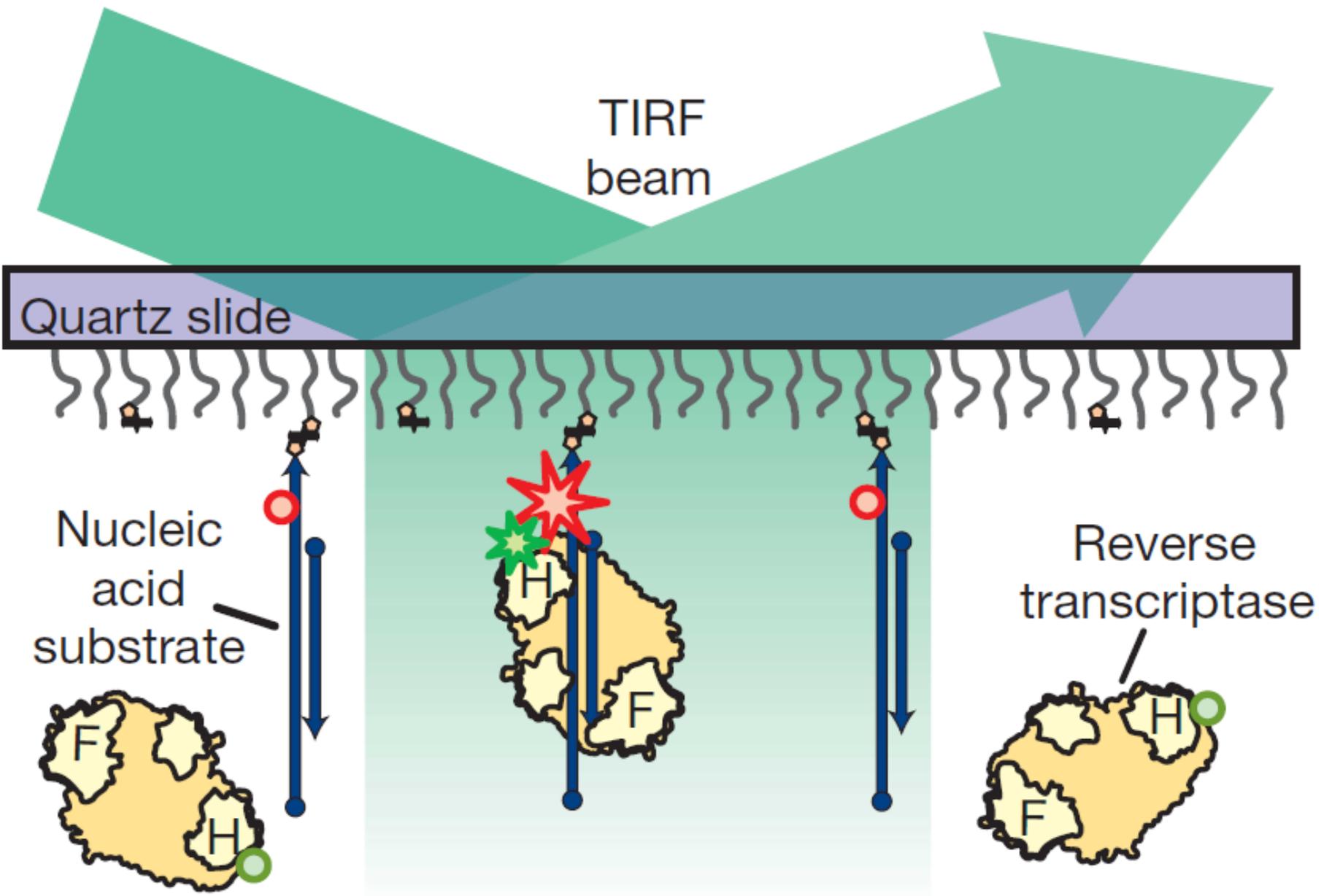


Cy3



Cy5

Dye	Absorbance Max	Emission Max	Quantum yield in PBS buffer	Molecular weight (Da)
Cy3	550 nm	570 nm	0.04 <sup>[5]</sup>	766
Cy5	649 nm	670 nm	0.28	792

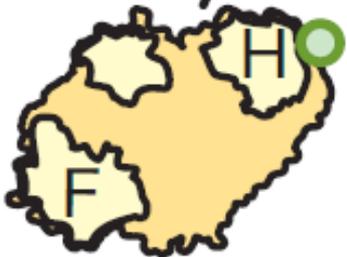
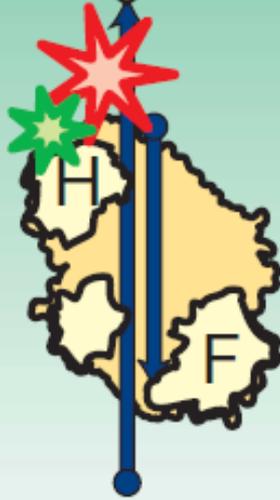
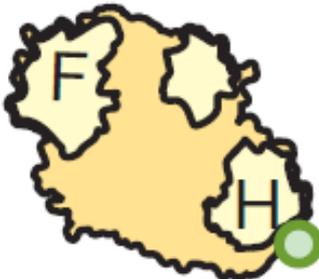


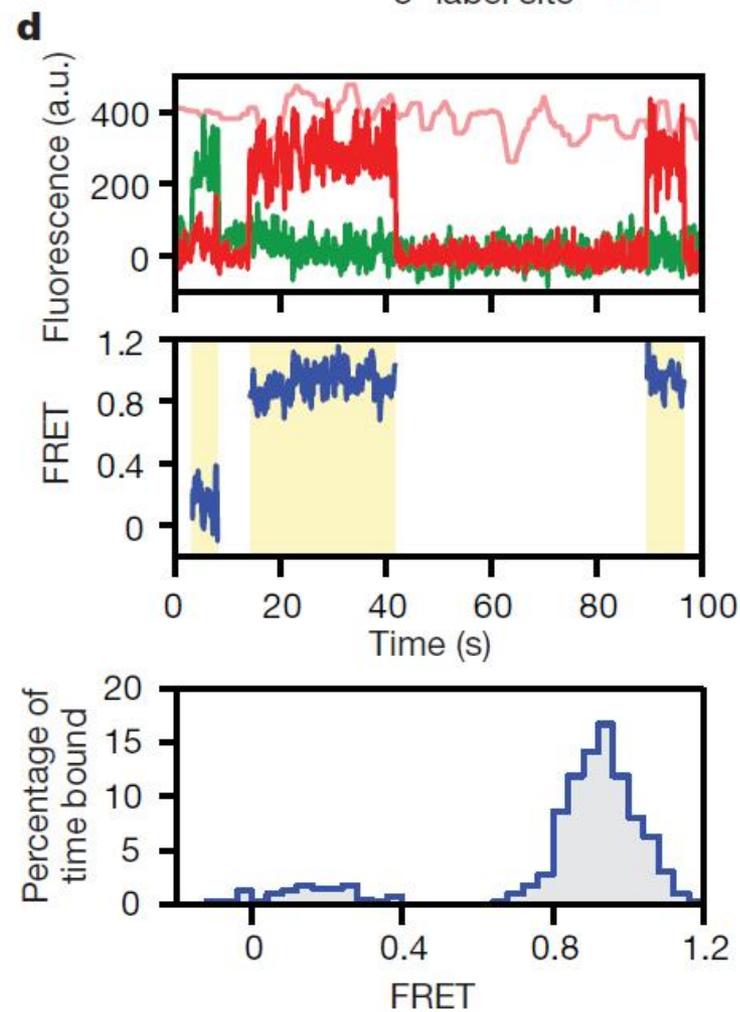
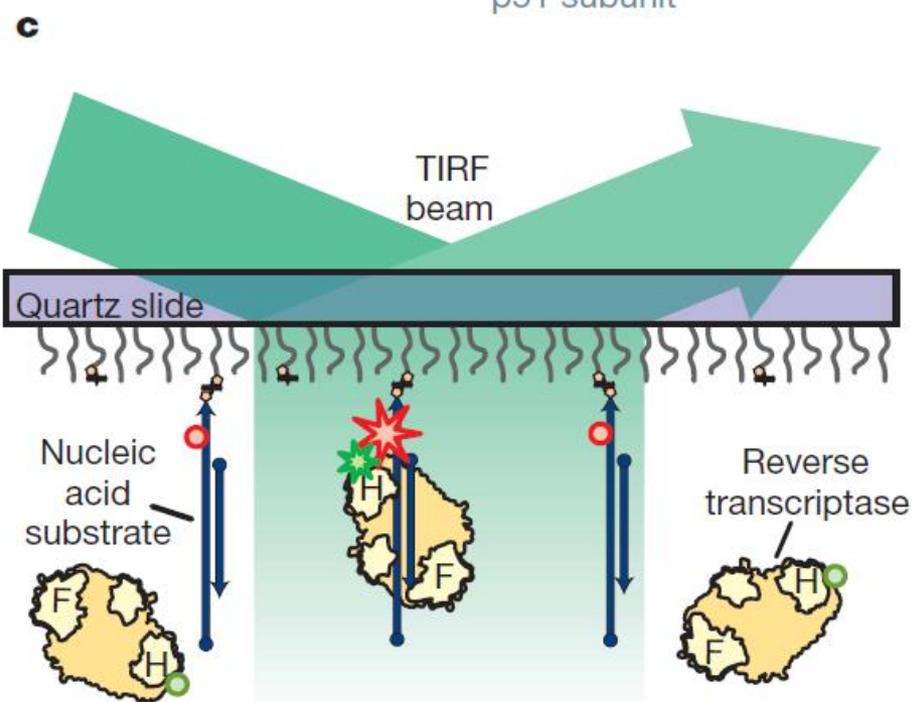
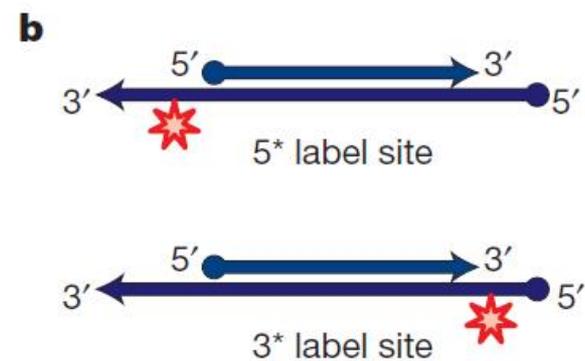
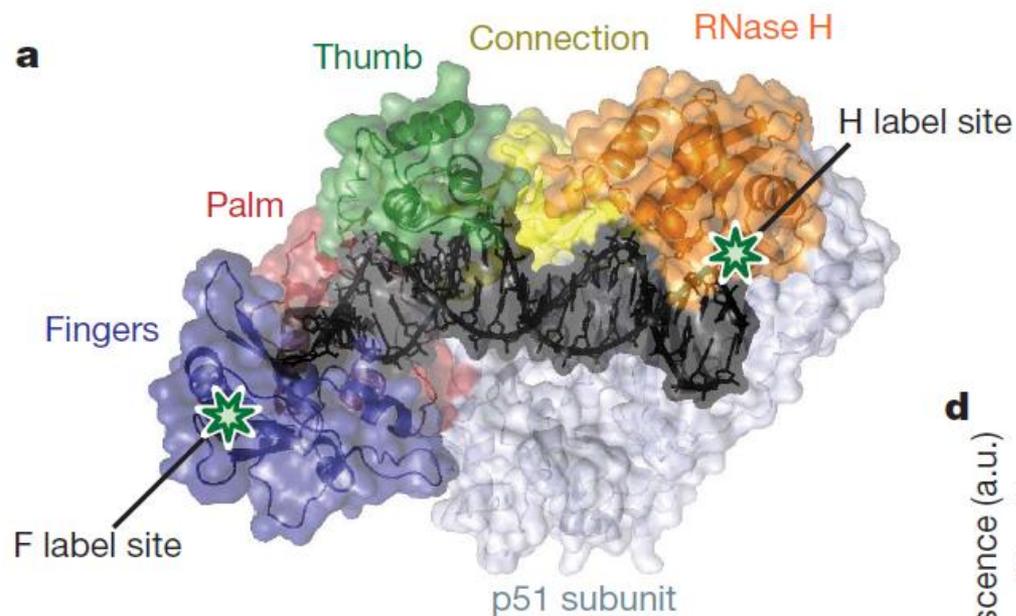
TIRF  
beam

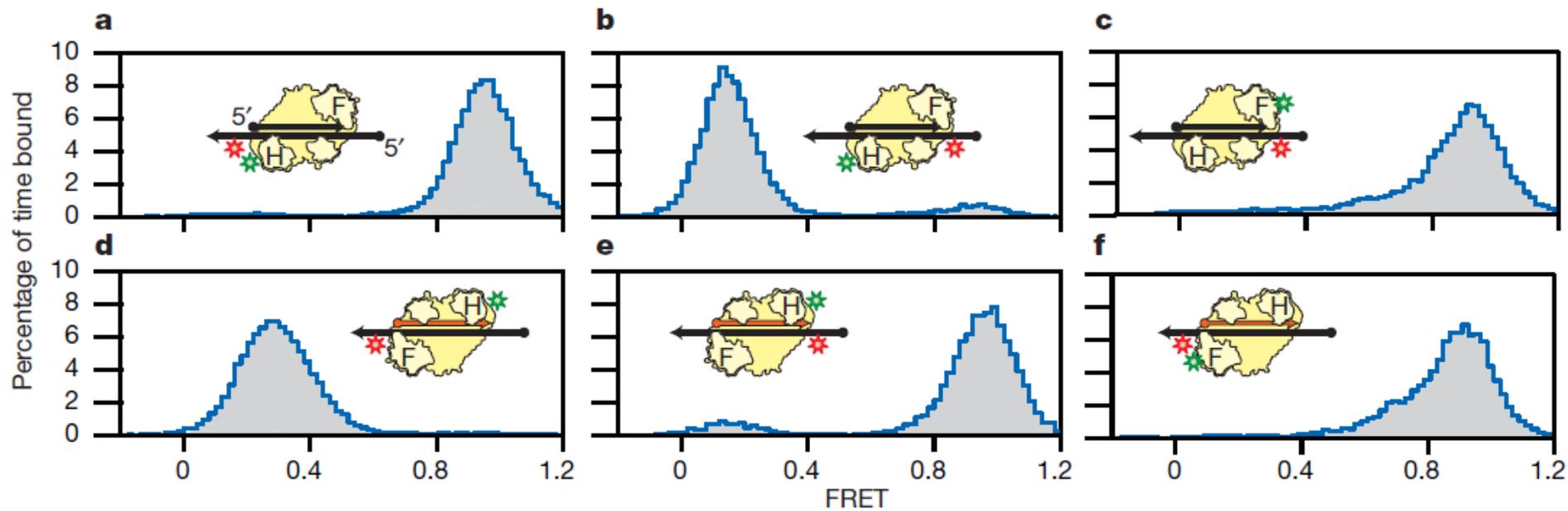
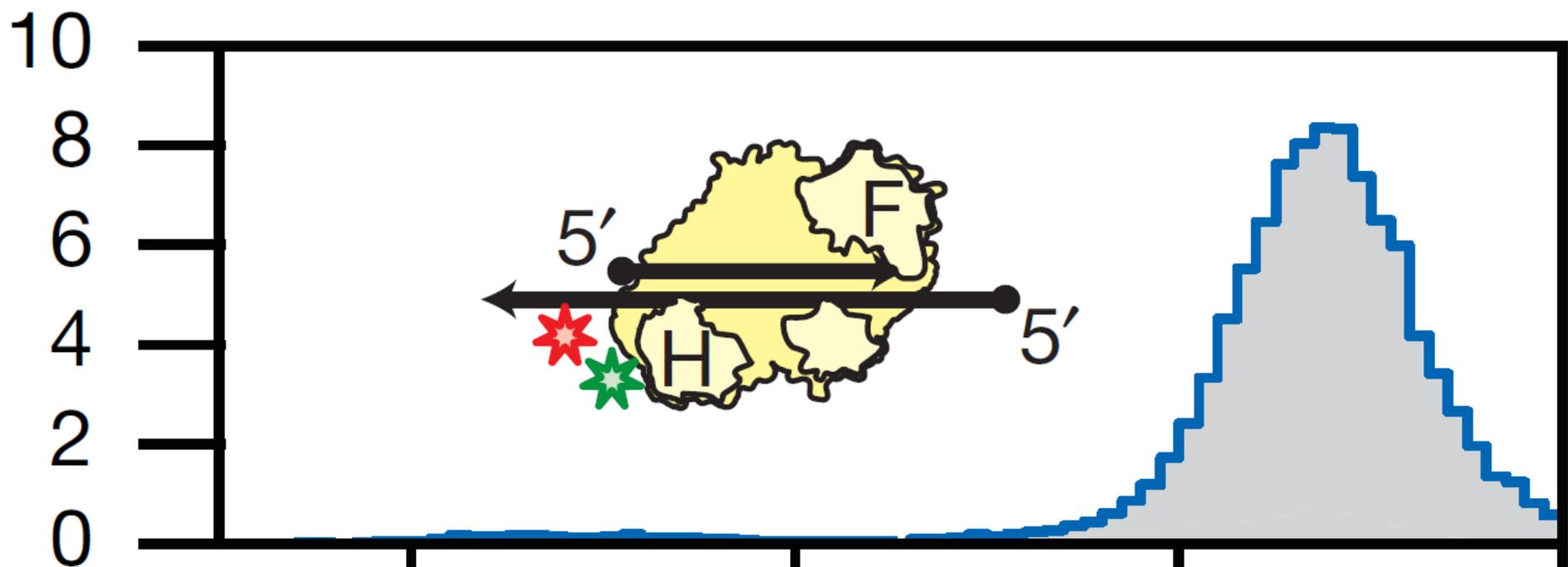
Quartz slide

Nucleic  
acid  
substrate

Reverse  
transcriptase



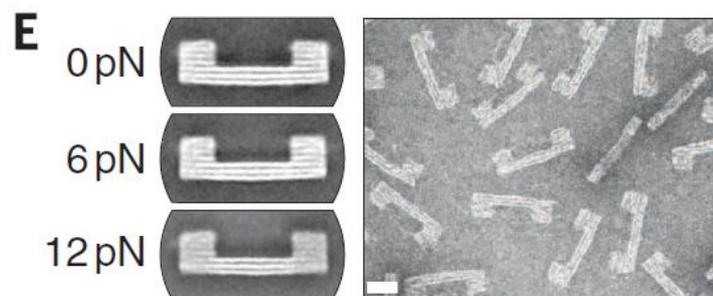
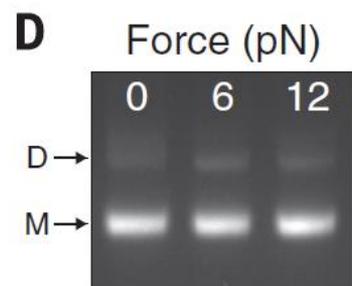
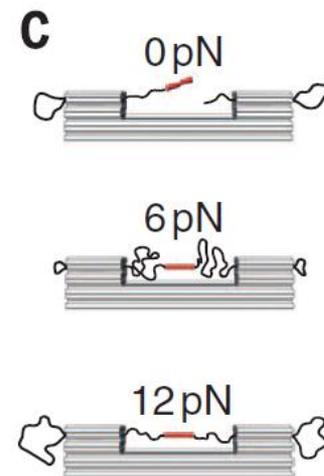
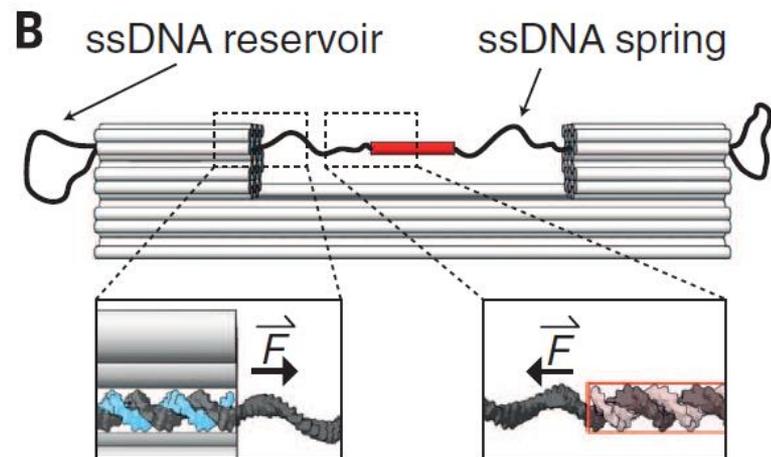
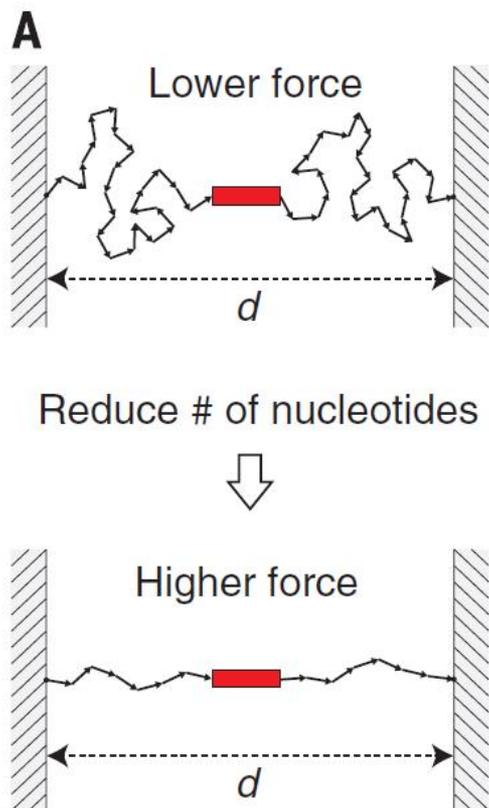


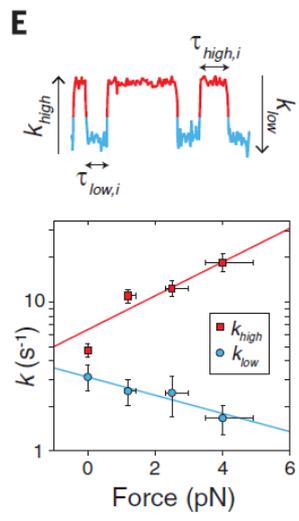
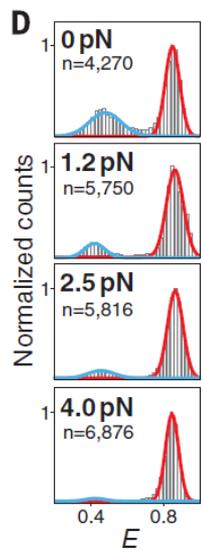
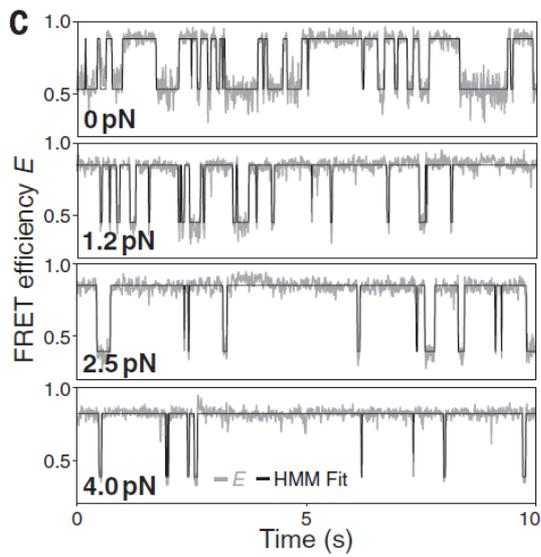
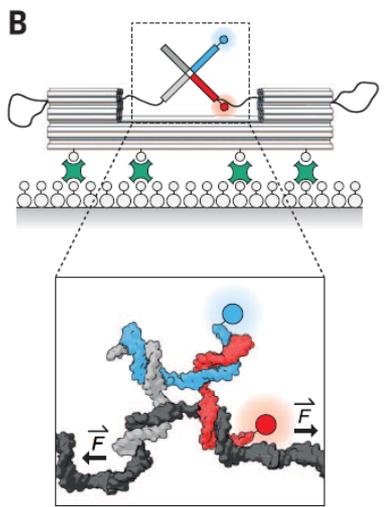
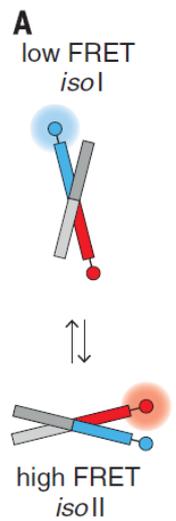


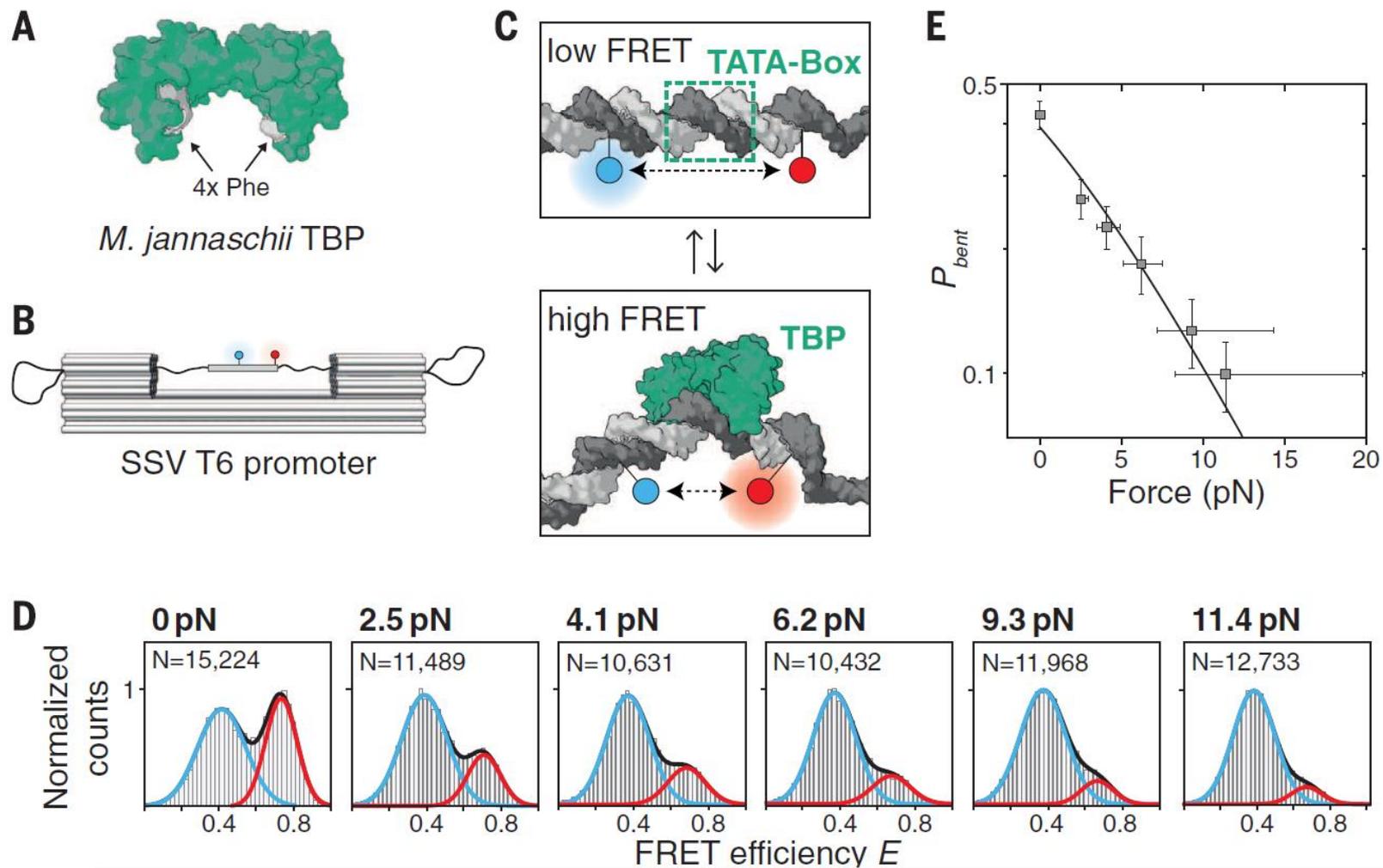
**FORCE SPECTROSCOPY**

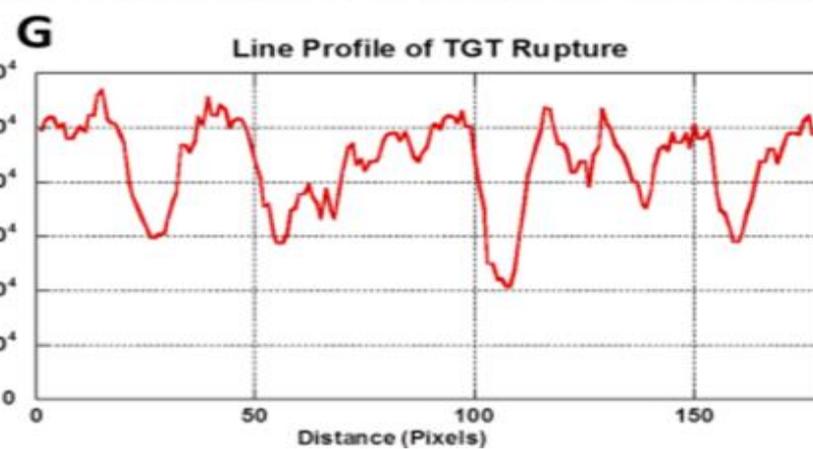
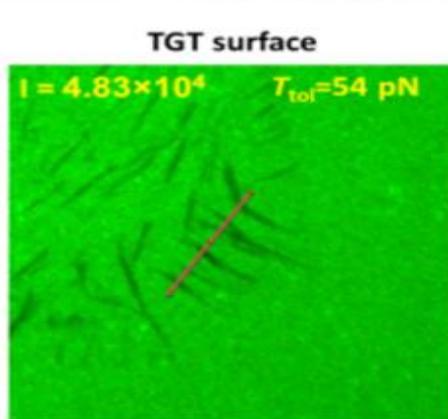
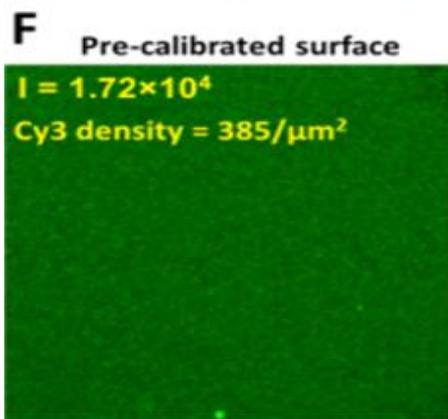
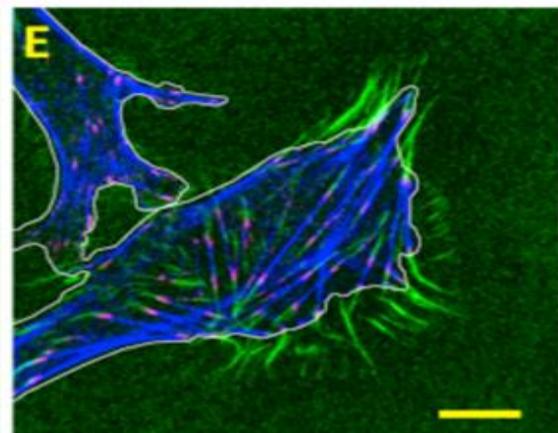
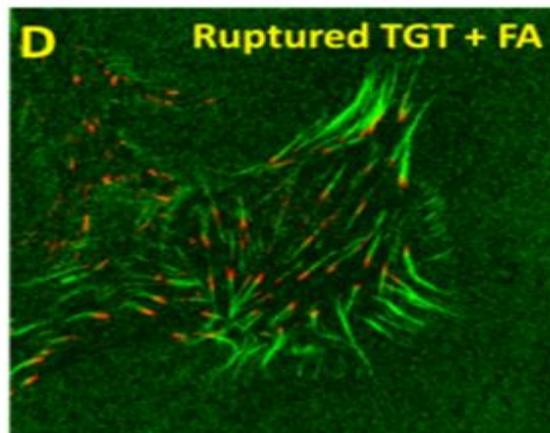
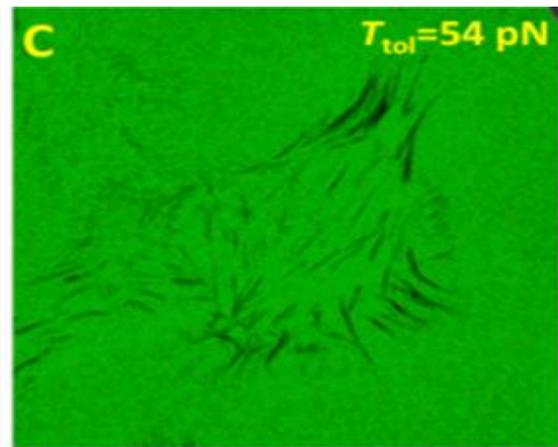
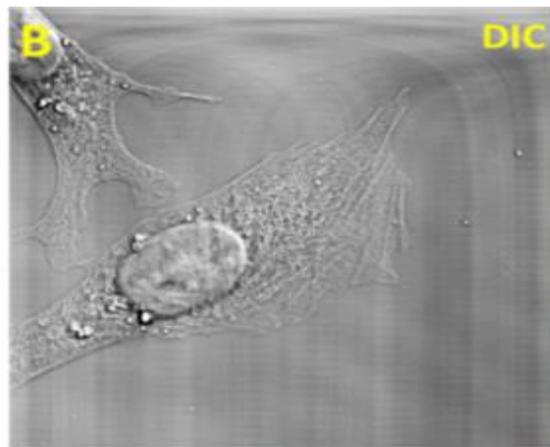
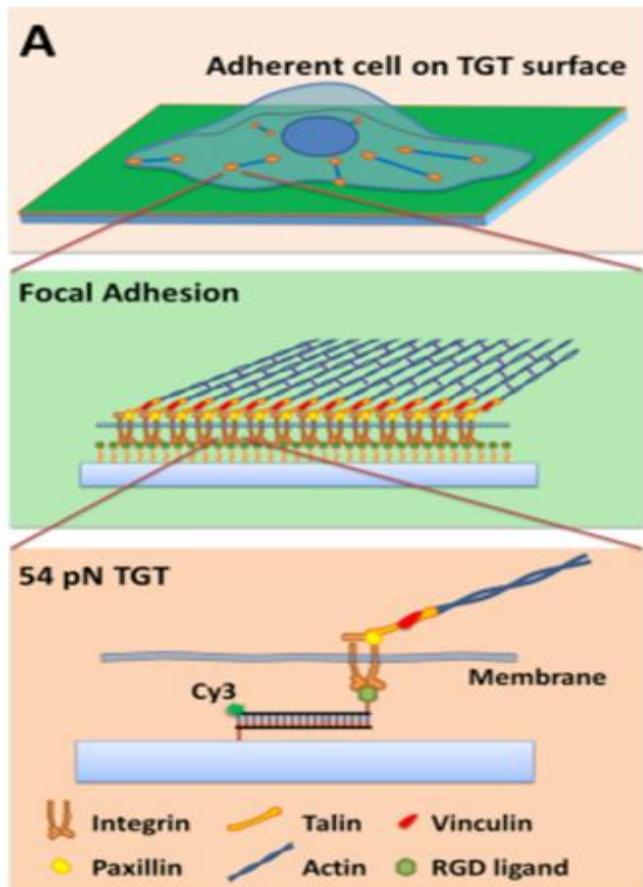
# **Molecular force spectroscopy with a DNA origami-based nanoscopic force clamp**

**Philipp C. Nickels,<sup>1</sup> Bettina Wunsch,<sup>2</sup> Phil Holzmeister,<sup>2\*</sup> Wooli Bae,<sup>1</sup> Luisa M. Kneer,<sup>1</sup>  
Dina Grohmann,<sup>2†</sup> Philip Tinnefeld,<sup>2‡</sup> Tim Liedl<sup>1‡</sup>**









# Силовой ДНК-сенсор

