

Advanced Cell Biology

Prof. WANG Hao

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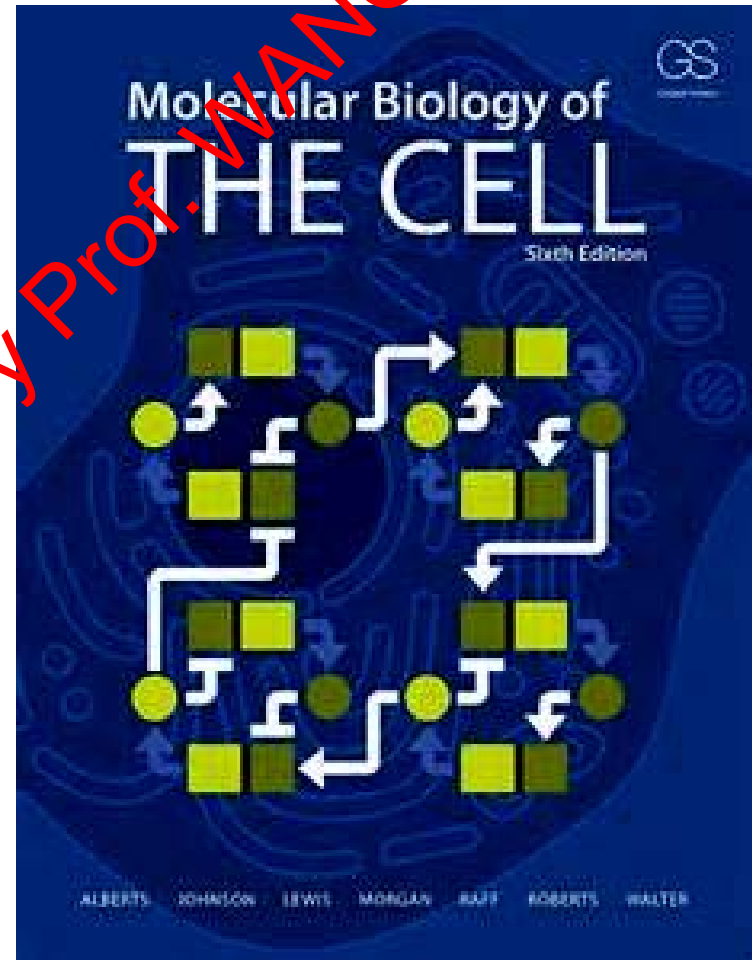
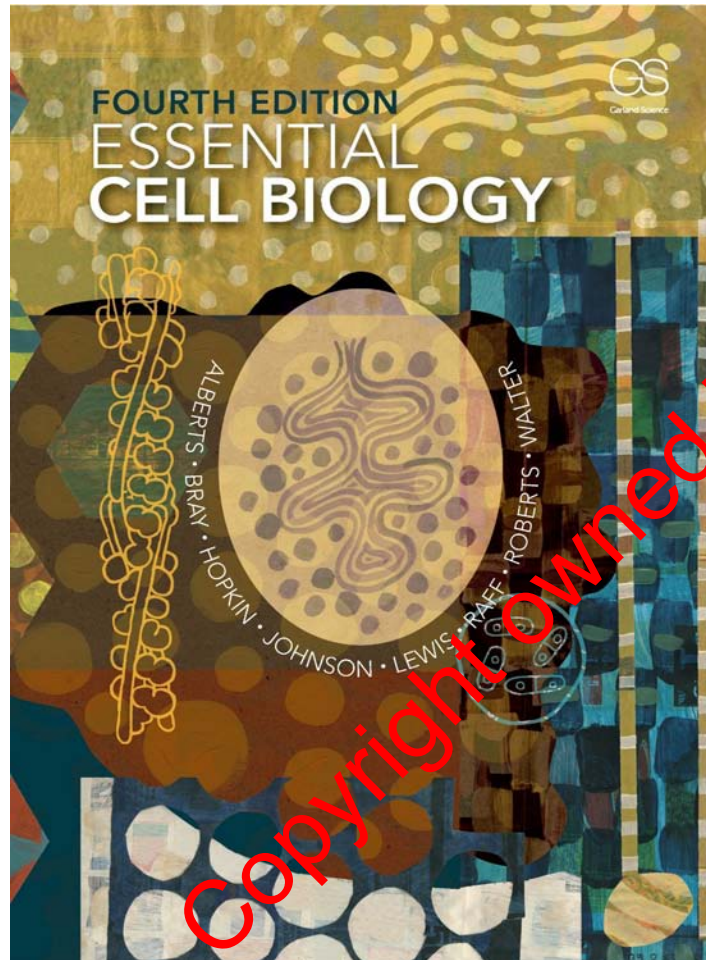
Address: Room 617, North Block, College of Life Sciences

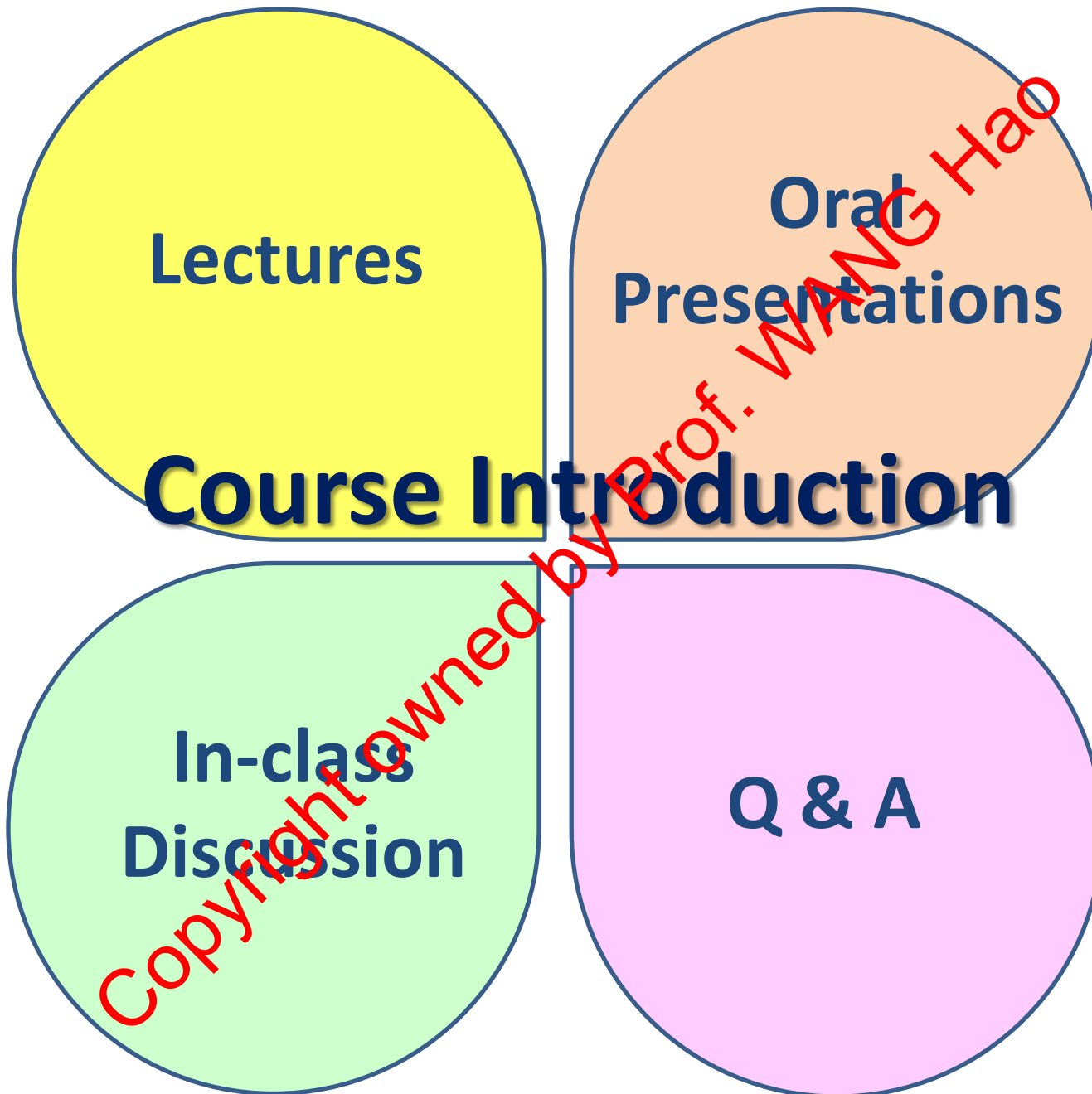
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In-class language: English

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Reference Books





Evaluations

No paper-based examination!

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Lectures

**Oral
Presentations
(50%)**

**In-class
Discussion
(20%)**

**Q & A
(30%)**

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Advanced Cell Biology

About the Course

Contents

Learning Recourses

Questions & Answers

Photo Albums

Reference Book 1



News



+ MORE

Reading Recommendations

+ MORE

6. Atomistic Autophagy: The Structures of Cellular Self-Digestion (Cell)

21 Jul 2020

5. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go (Cell)

I. General Introduction of Cell Biology

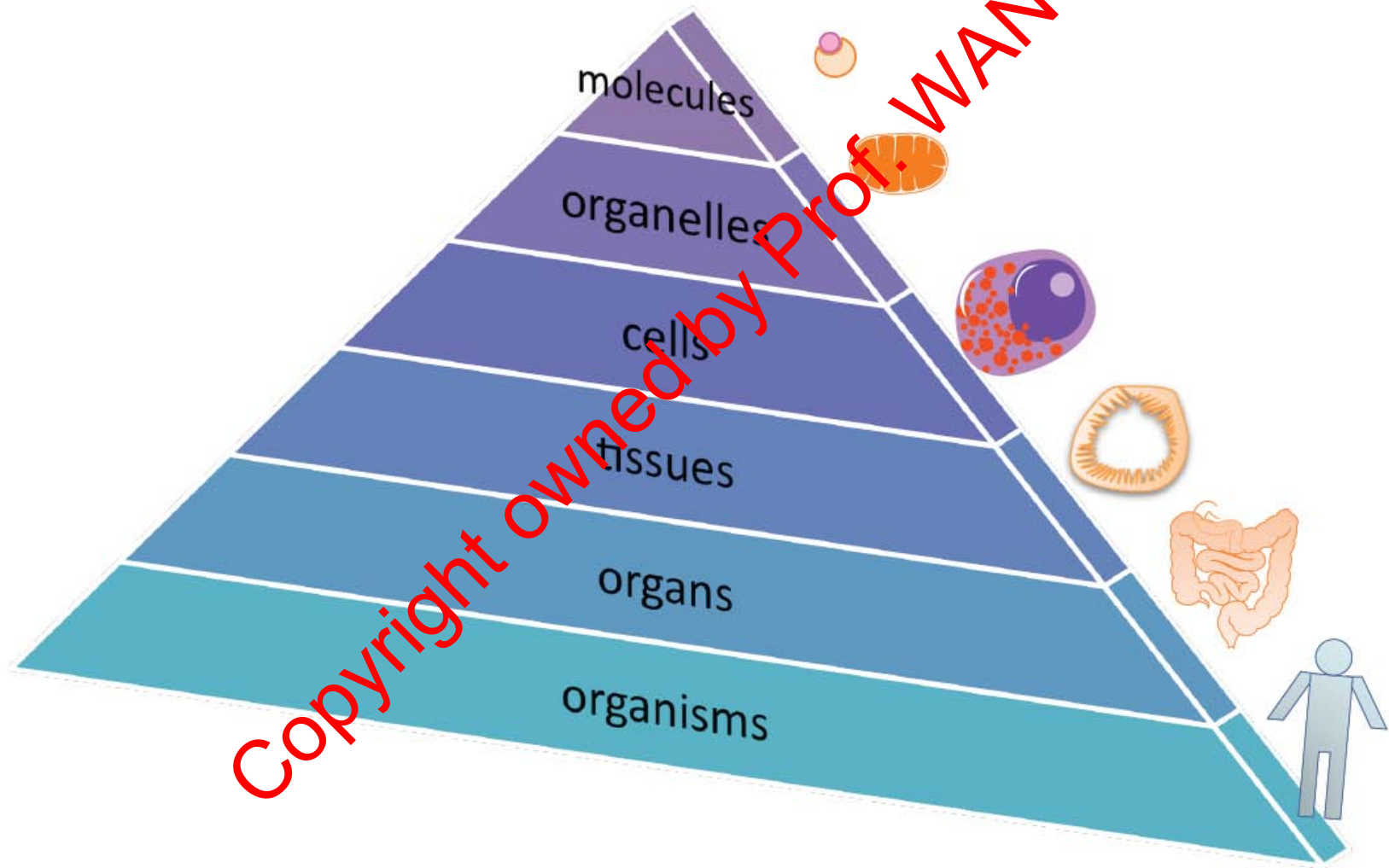
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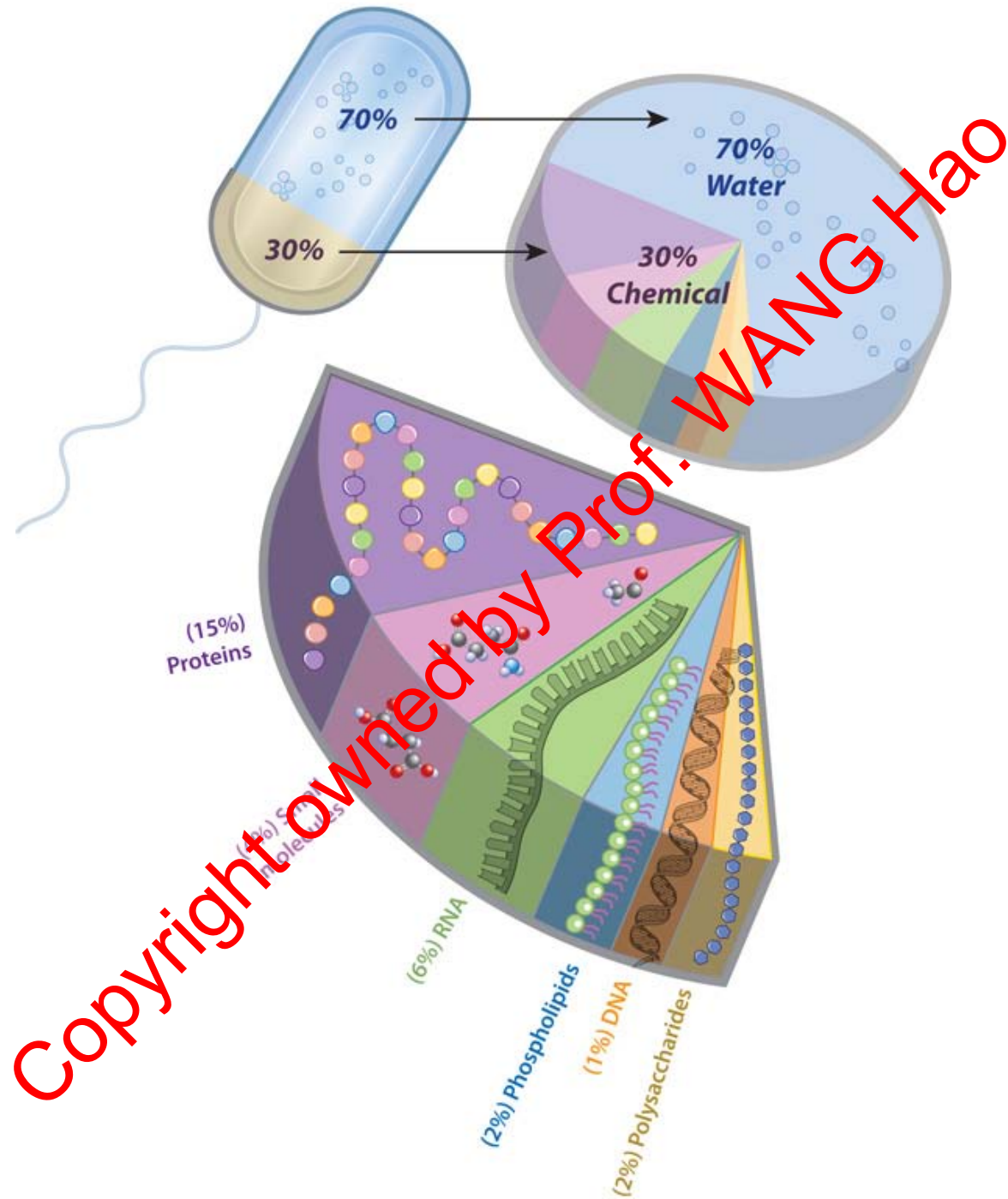


What is a cell?

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Cell: The fundamental functional element of life







Where do cells coming from?

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The First Cell – Hypothesis

Early Atmosphere

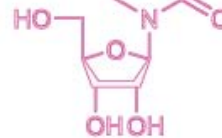
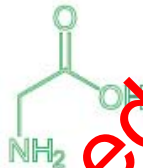


Electric storm

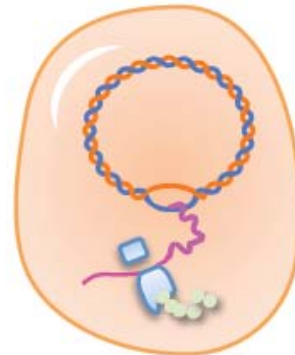
UV

Chemical Reactions

Amino Acid

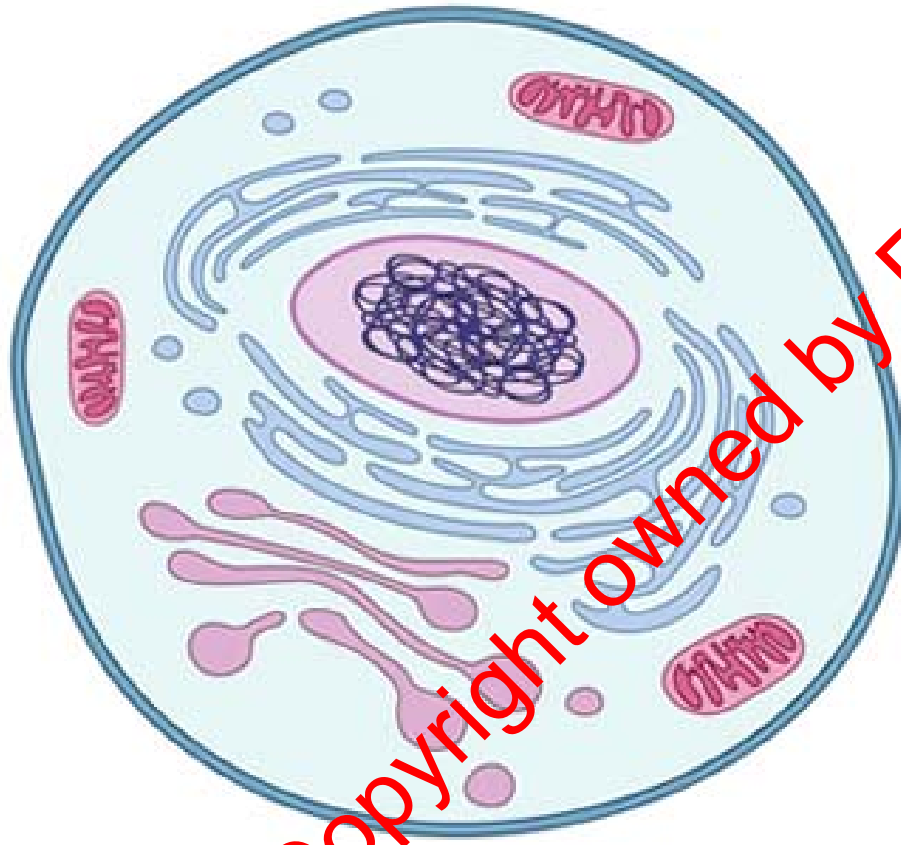


Nucleoside

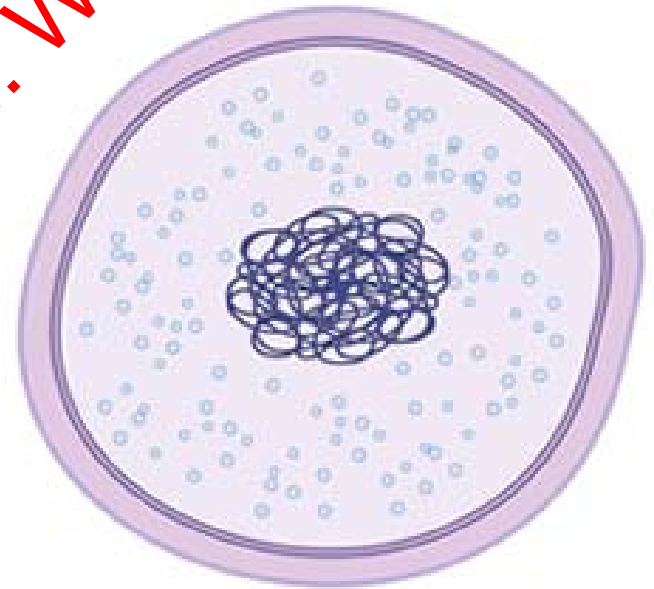


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Eukaryotic Cell



Prokaryotic Cell



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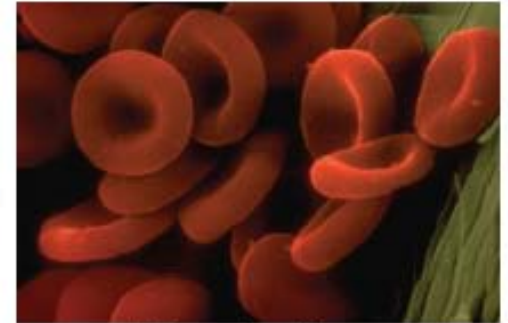
Cell specialization/ differentiation



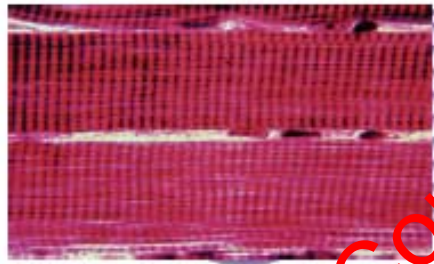
Epithelial cells



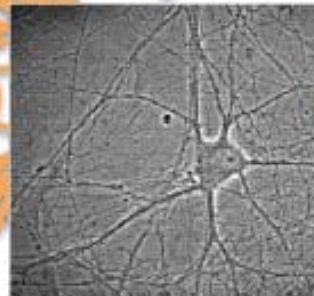
Fertilized egg



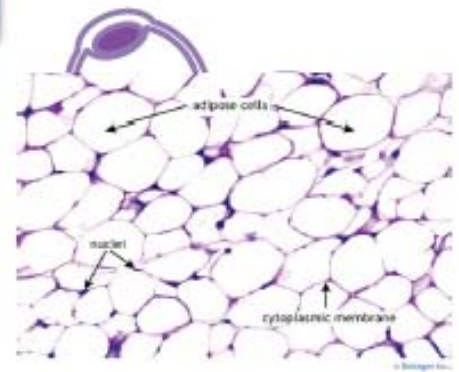
Blood cells



Muscle cells



Neuron cell



Adipose cells

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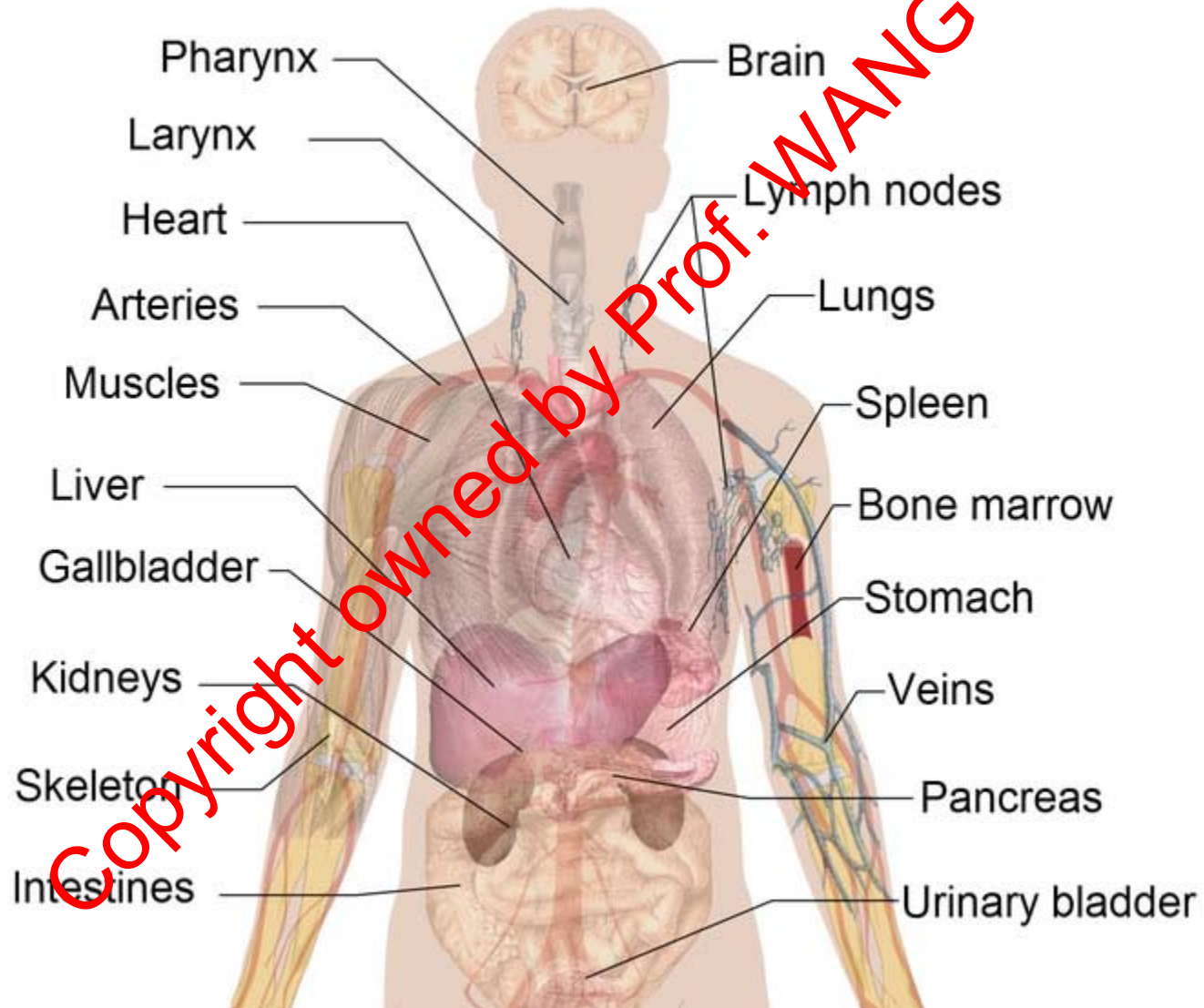


How do cells work?

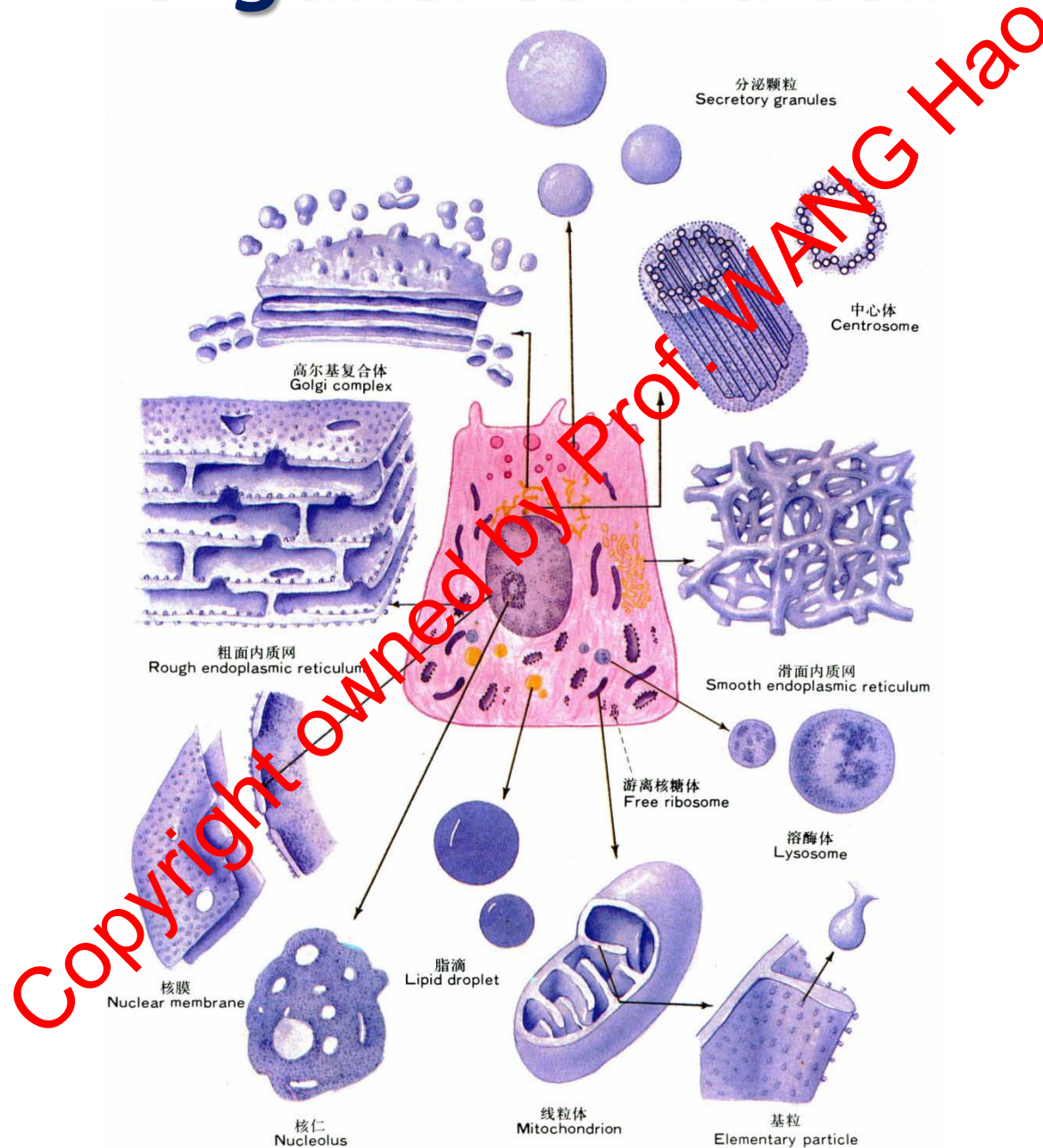
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Organs in Human

Human anatomy



Organelles in a cell





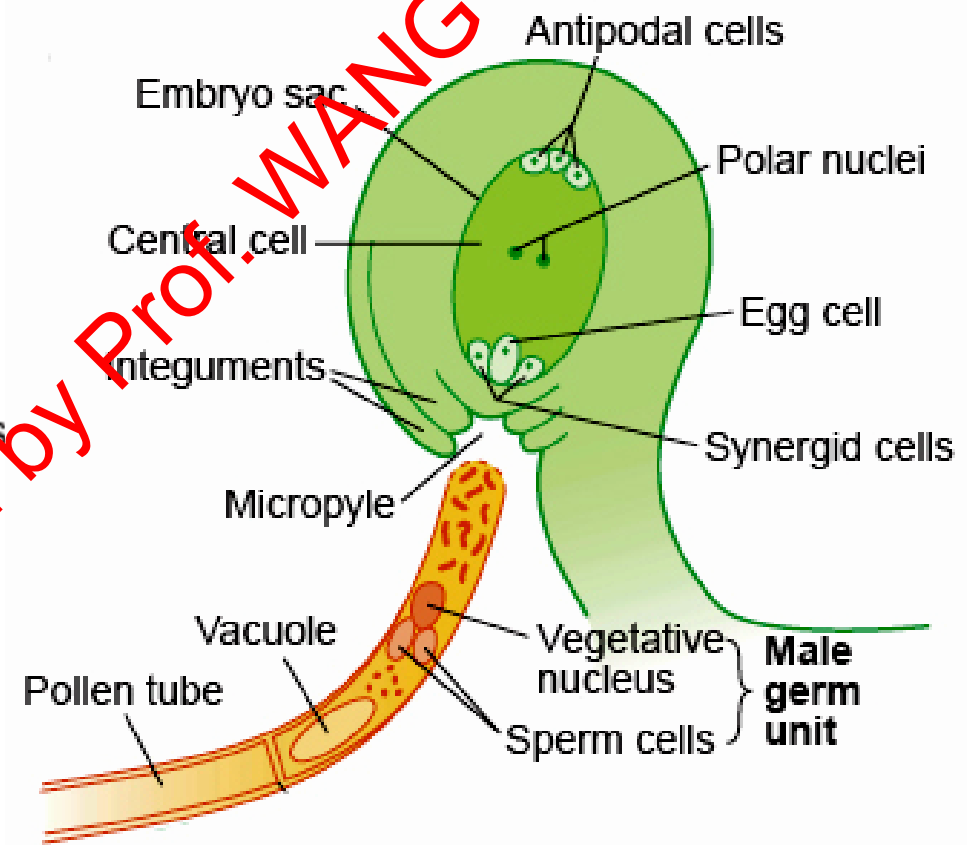
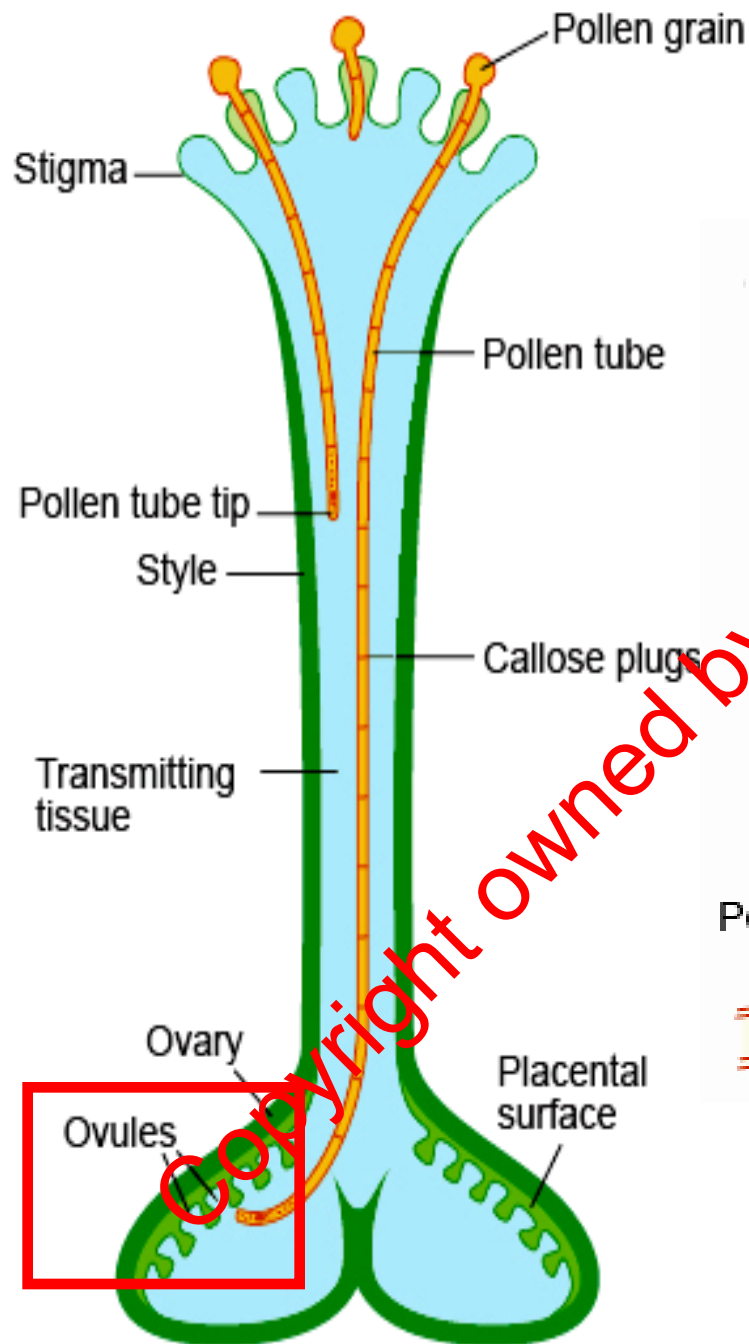
What is cell biology about?

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A rapid growing lily pollen tube



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Pollen and pollen tube

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Cell cytokinesis

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Molecular Mechanism of Synaptic Function



New Limb Regeneration





What is cell biology about?

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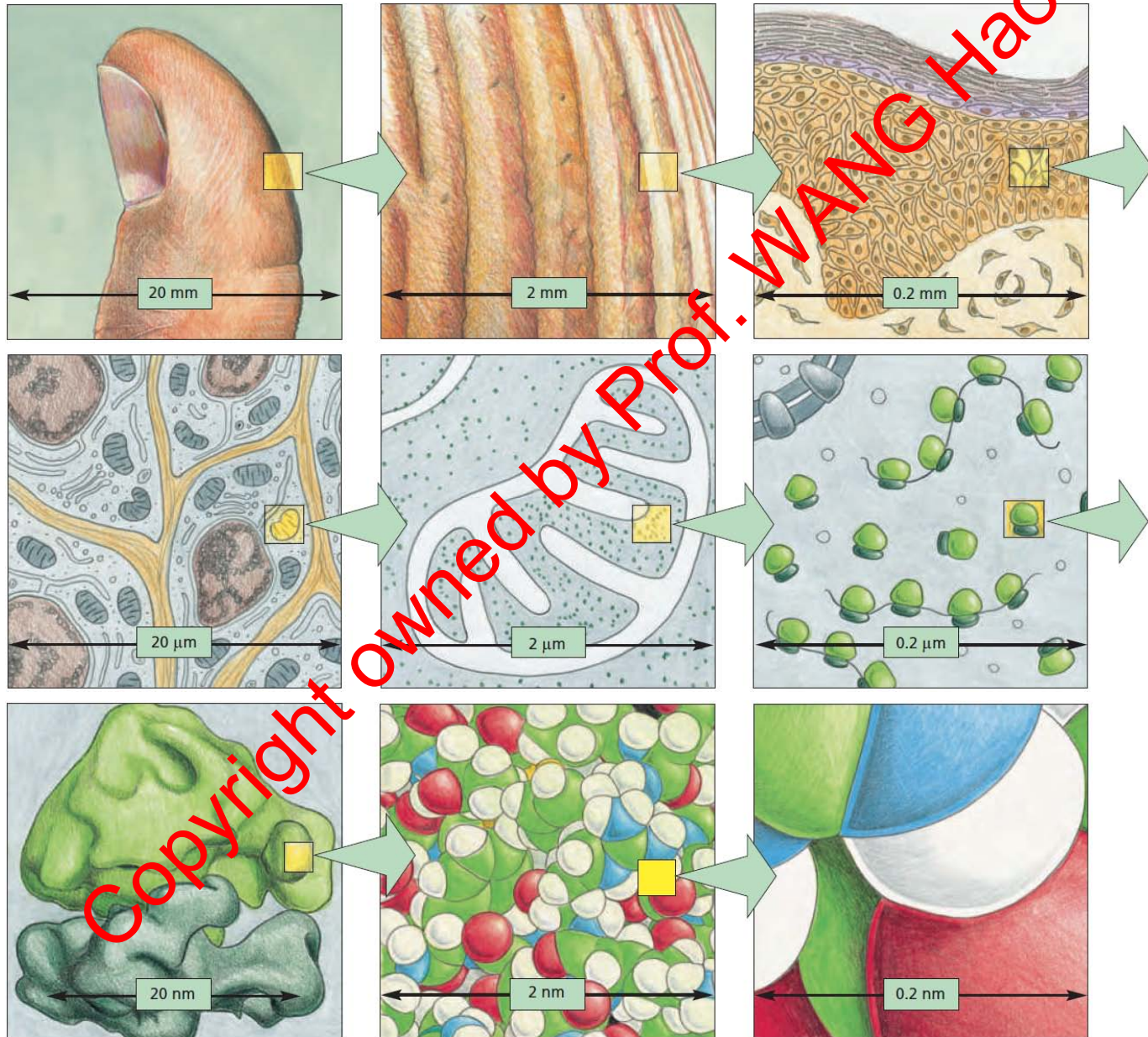
Organelle Identification in Plant Cell



II. Visualizing Cells (Bioimaging)

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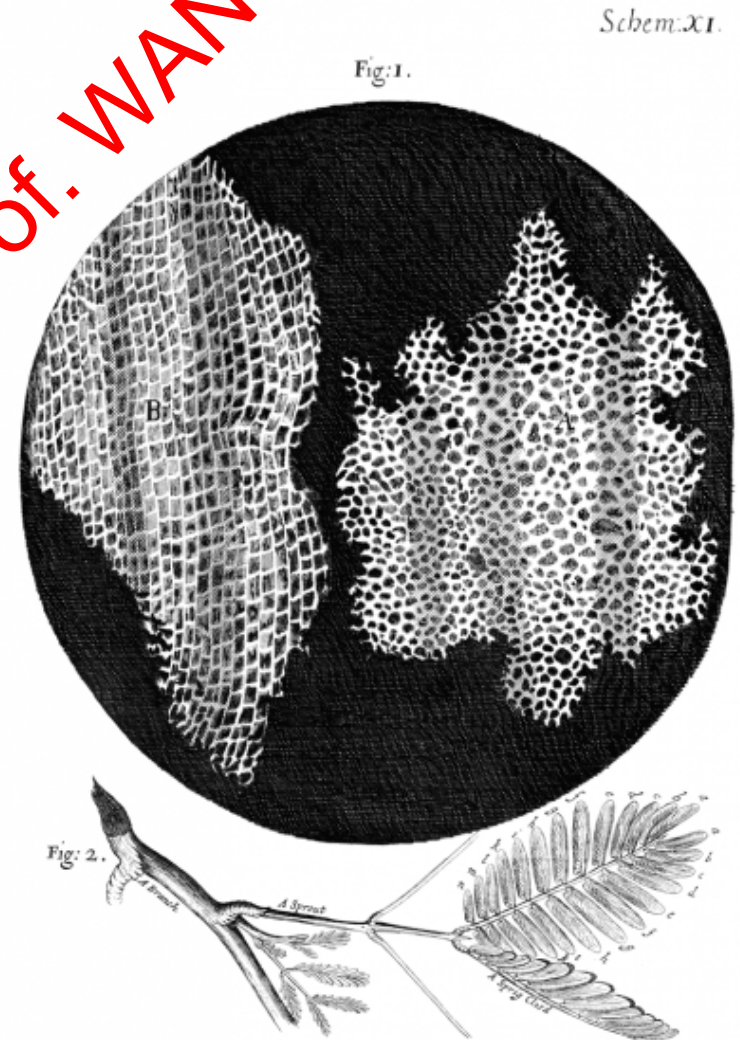
Visualizing Cells (Bioimaging)



Visualizing Cells (Bioimaging)

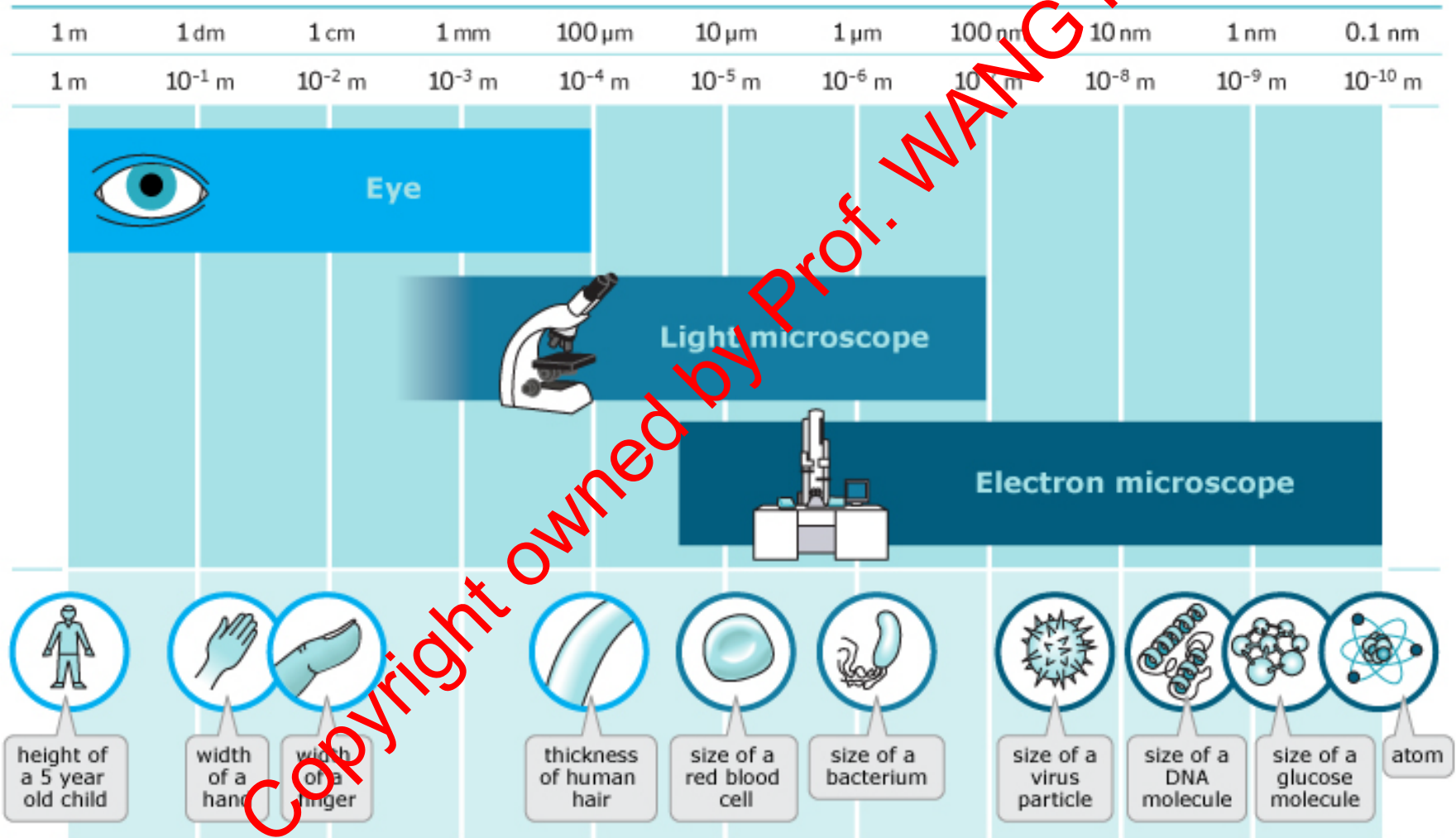


Hooke Microscope
(circa 1670)



Visualizing Cells (Bioimaging)

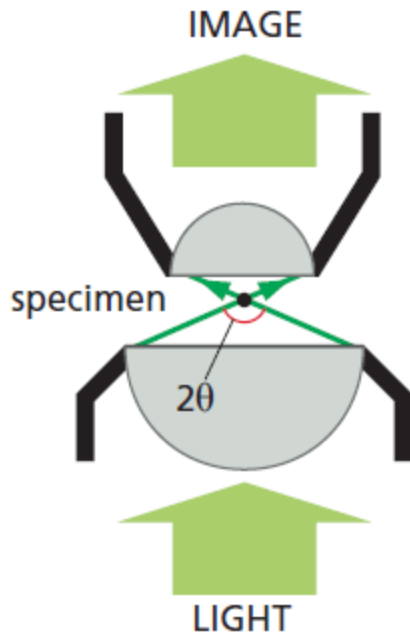
Resolving power of microscopes





What is resolution?

LENSES



the **objective** lens collects a cone of light rays to create an image

the **condenser** lens focuses a cone of light rays onto each point of the specimen

RESOLUTION: the resolving power of the microscope depends on the width of the cone of illumination and therefore on both the condenser and the objective lens. It is calculated using the formula

$$\text{resolution} = \frac{0.61 \lambda}{n \sin \theta}$$

where:

θ = half the angular width of the cone of rays collected by the objective lens from a typical point in the central region of the specimen (since the maximum width is 180° , $\sin \theta$ has a maximum value of 1)

n = the refractive index of the medium (usually air or oil) separating the specimen from the objective and condenser lenses

λ = the wavelength of light used (for white light a figure of $0.53 \mu\text{m}$ is commonly assumed)

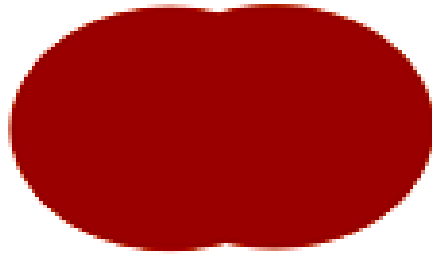
Image resolution

Resolution is the ability to distinguish closely spaced points as separate.

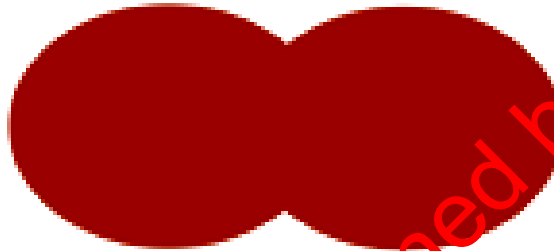
Resolution can also be understood as the least distance between two closely opposed points, at which they may be recognized as two separate entities.

The smallest distance we can see between points in a light microscope (LM) is about 200 nm [There are 1000000 nm (= nanometers) in 1 mm] whereas a typical scanning electron microscope (SEM) can distinguish gaps smaller than 10 nm.

Image resolution



Resolution allows us to see



objects as separate



from one another

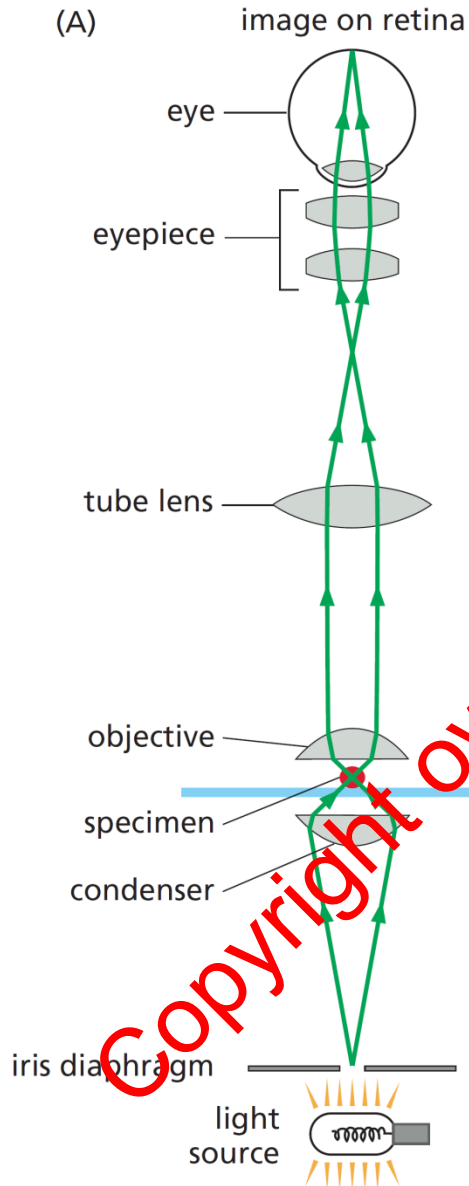
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Image resolution



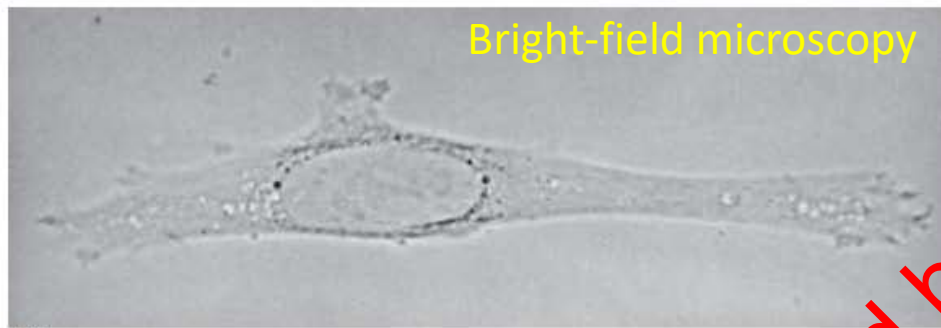
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Visualizing Cells-Light Microscopy

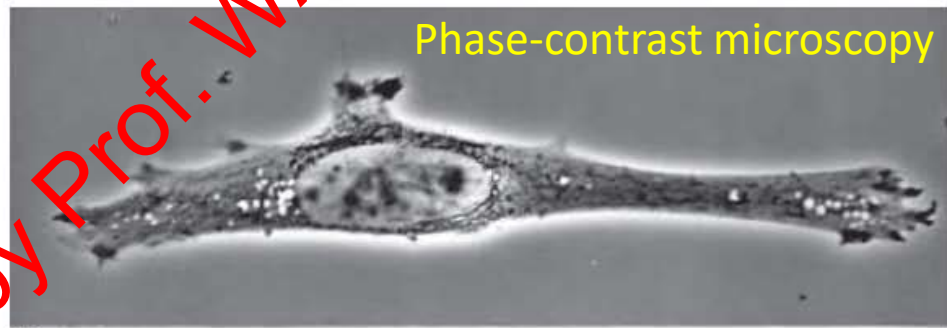


Visualizing Cells-Light Microscopy

Four types of light microscopy



(A)



(B)



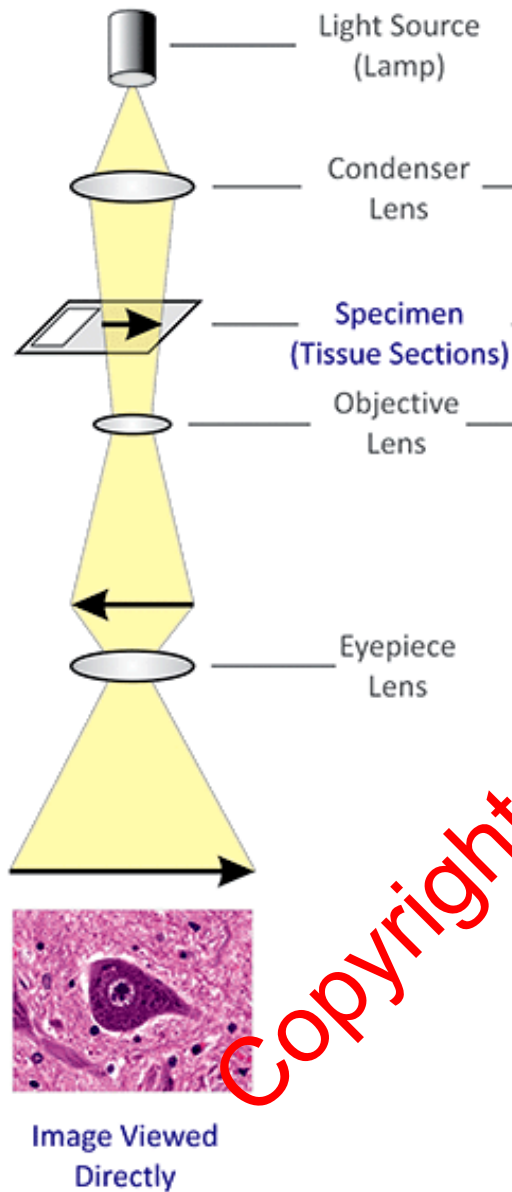
(C)



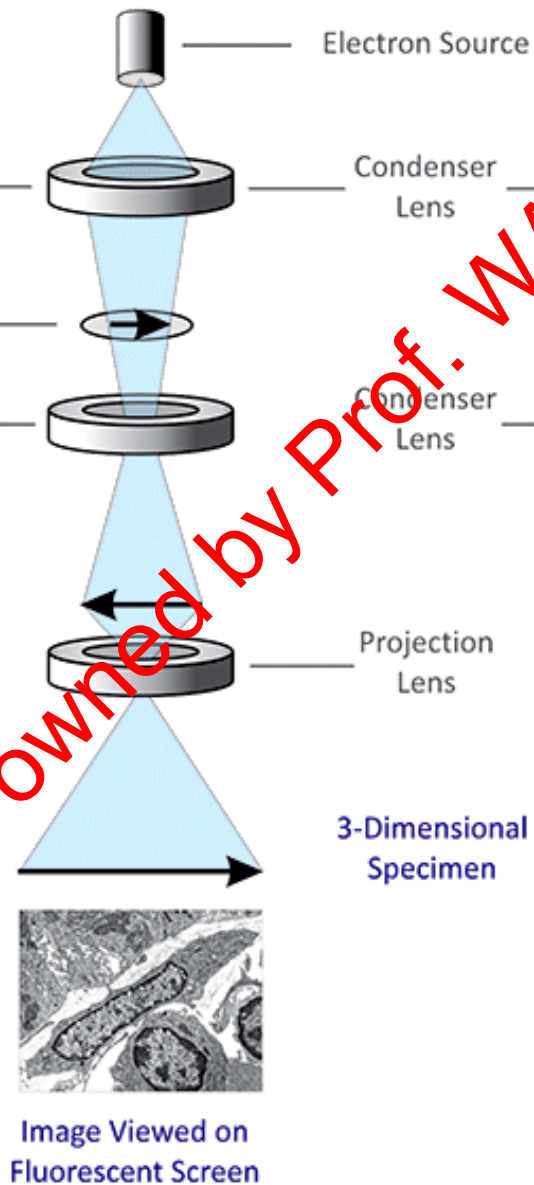
(D)

50 μm

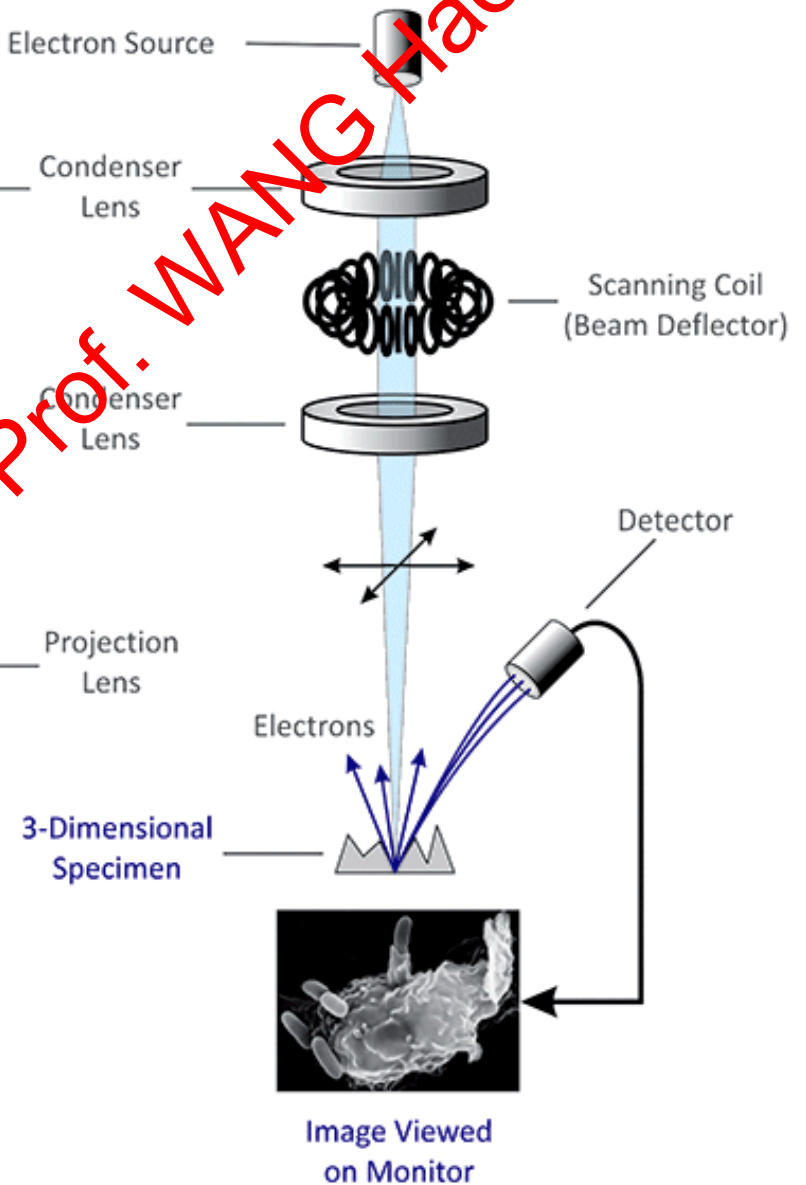
Light Microscopy



Transmission Electron Microscopy



Scanning Electron Microscopy



WHICH MICROSCOPE?



Stereomicroscope (light)

Transmission electron microscope (TEM)

I want to look at the surface of a sample at high resolution

Yes

No

Compound microscope (light)

Confocal laser scanning microscope

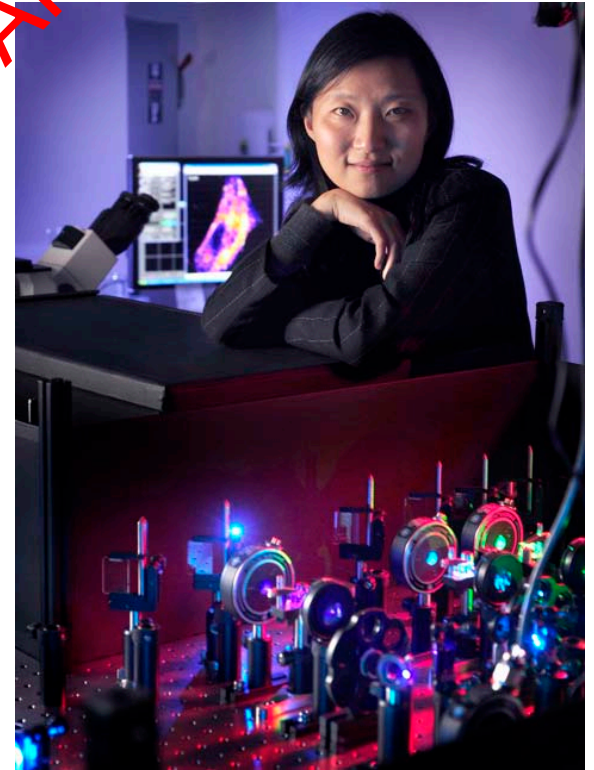
Scanning electron microscope (SEM)

CryoSEM

Electron tomography

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Visualizing Cells Using Laser



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The Nobel Prize in Chemistry 2008

Osamu Shimomura, Martin Chalfie, Roger Y. Tsien

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The Nobel Prize in Chemistry 2008

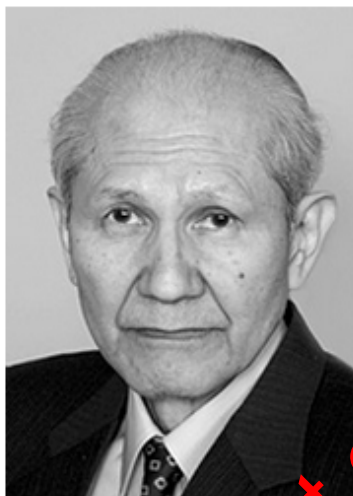


Photo: U. Montan

Osamu Shimomura

Prize share: 1/3



Photo: U. Montan

Martin Chalfie

Prize share: 1/3



Photo: U. Montan

Roger Y. Tsien

Prize share: 1/3

The Nobel Prize in Chemistry 2008 was awarded jointly to Osamu Shimomura, Martin Chalfie and Roger Y. Tsien *"for the discovery and development of the green fluorescent protein, GFP"*.



Roger Tsien

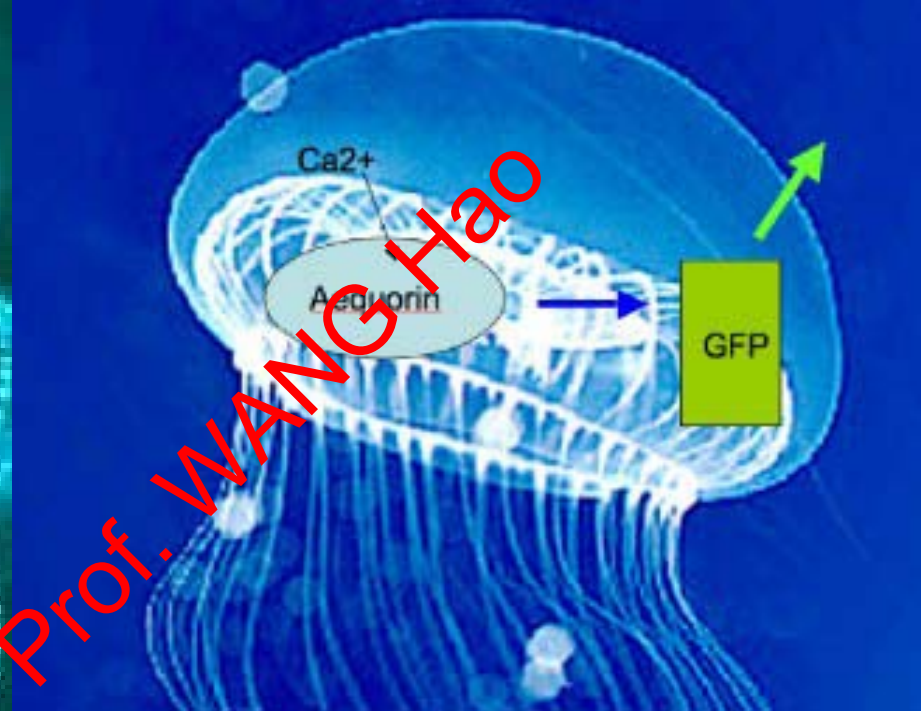
Roger Tsien (February 1, 1952-August 24, 2016), a professor of pharmacology, chemistry and biochemistry at University of California, San Diego, shared the 2008 Nobel Prize in chemistry for helping develop fluorescent markers that could tag cancer cells or track the advance of Alzheimer's disease in the brain.

He helped turn green fluorescent protein from a jellyfish into a research tool - markers that, under ultraviolet light, glow in a wide variety of colors. Researchers use the markers to track cellular processes in everything from brain cells to bacteria.

"I've always been attracted to colors," Tsien told the San Diego Union-Tribune in 2008. "Color helps make the work more interesting and enduring. It helps when things aren't going well. If I had been born colorblind, I probably never would have gone into this."

CREDIT: SAM YEH/AFP/Getty Images; Getty; University of California, San Diego

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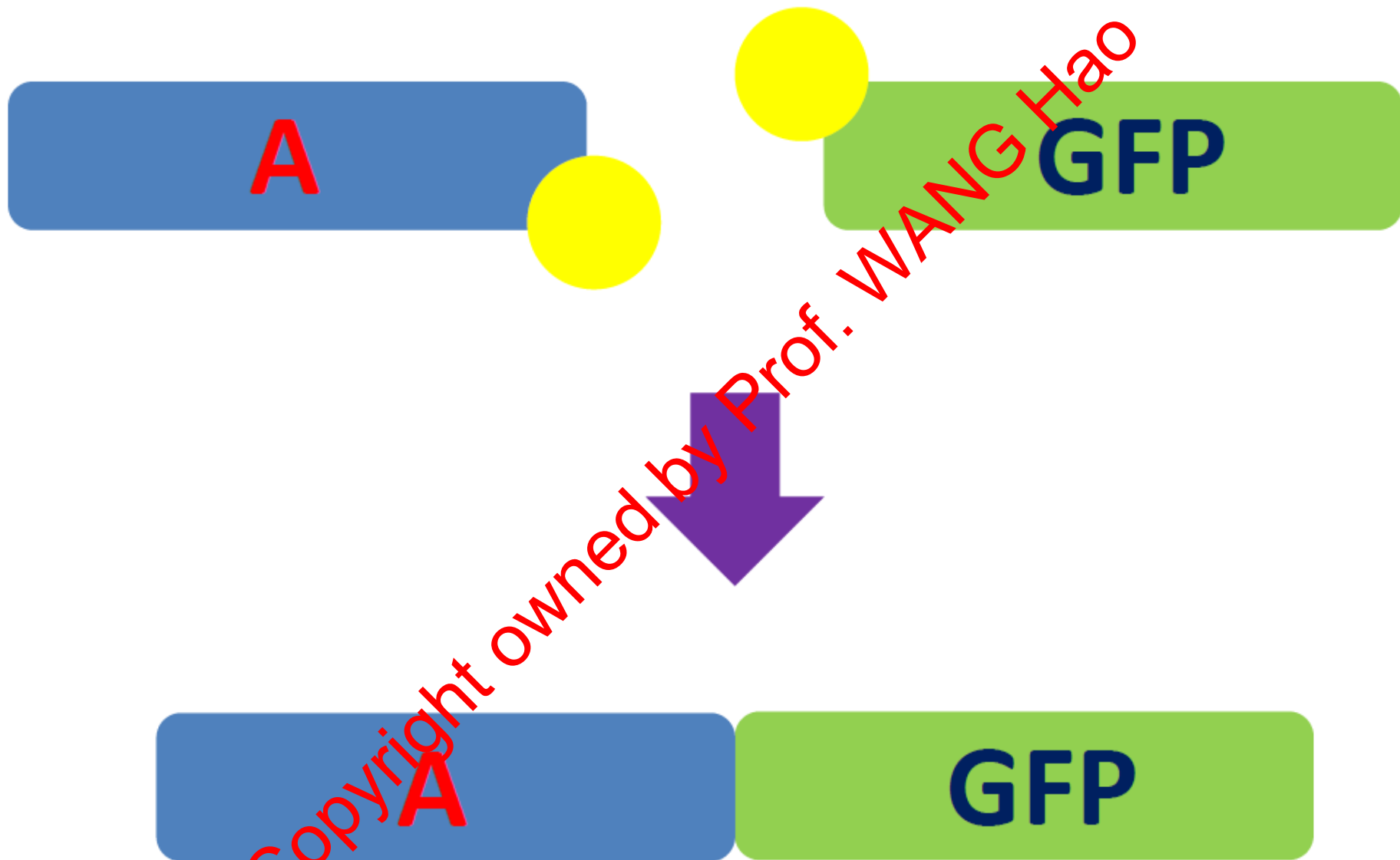


GFP = Green
Fluorescence
Protein

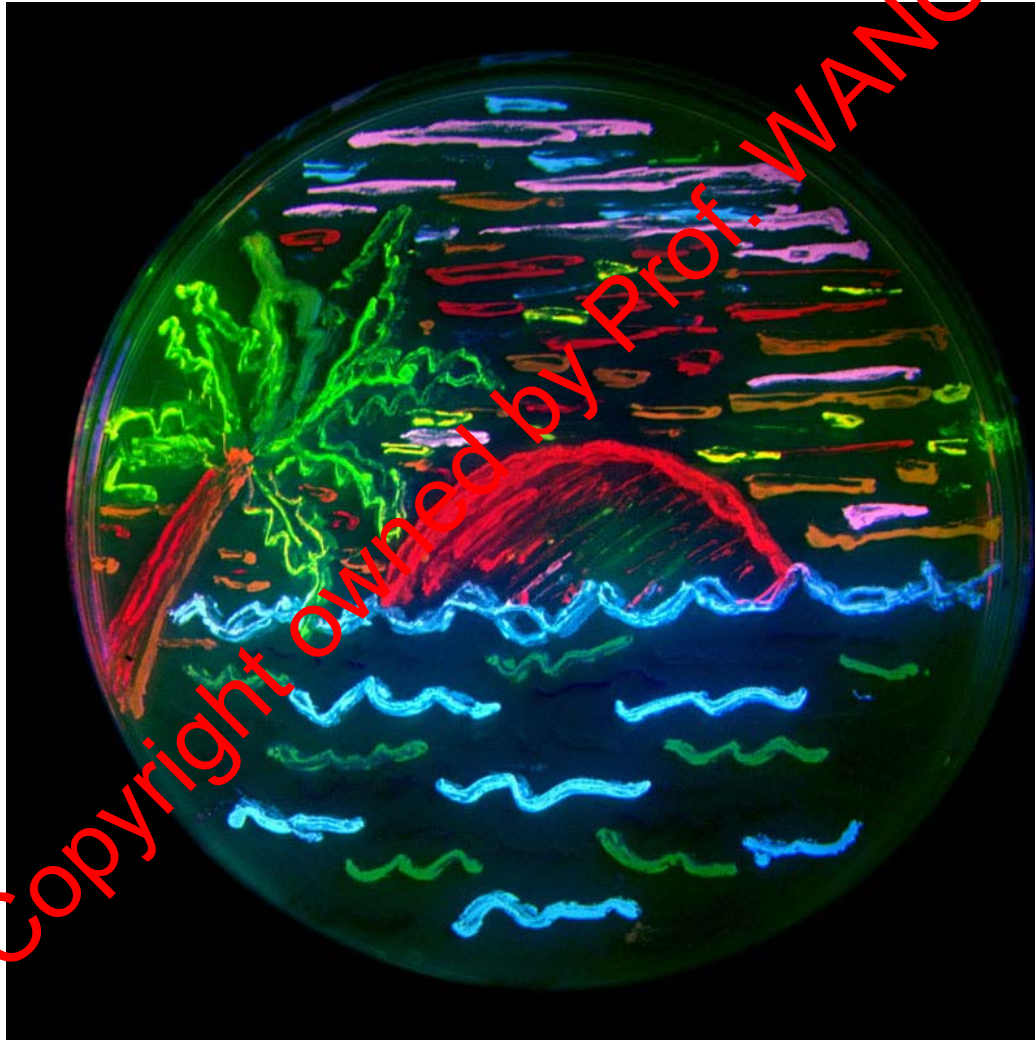


*Green fluorescent proteins
(GFP) is found and derived
from jellyfish first*

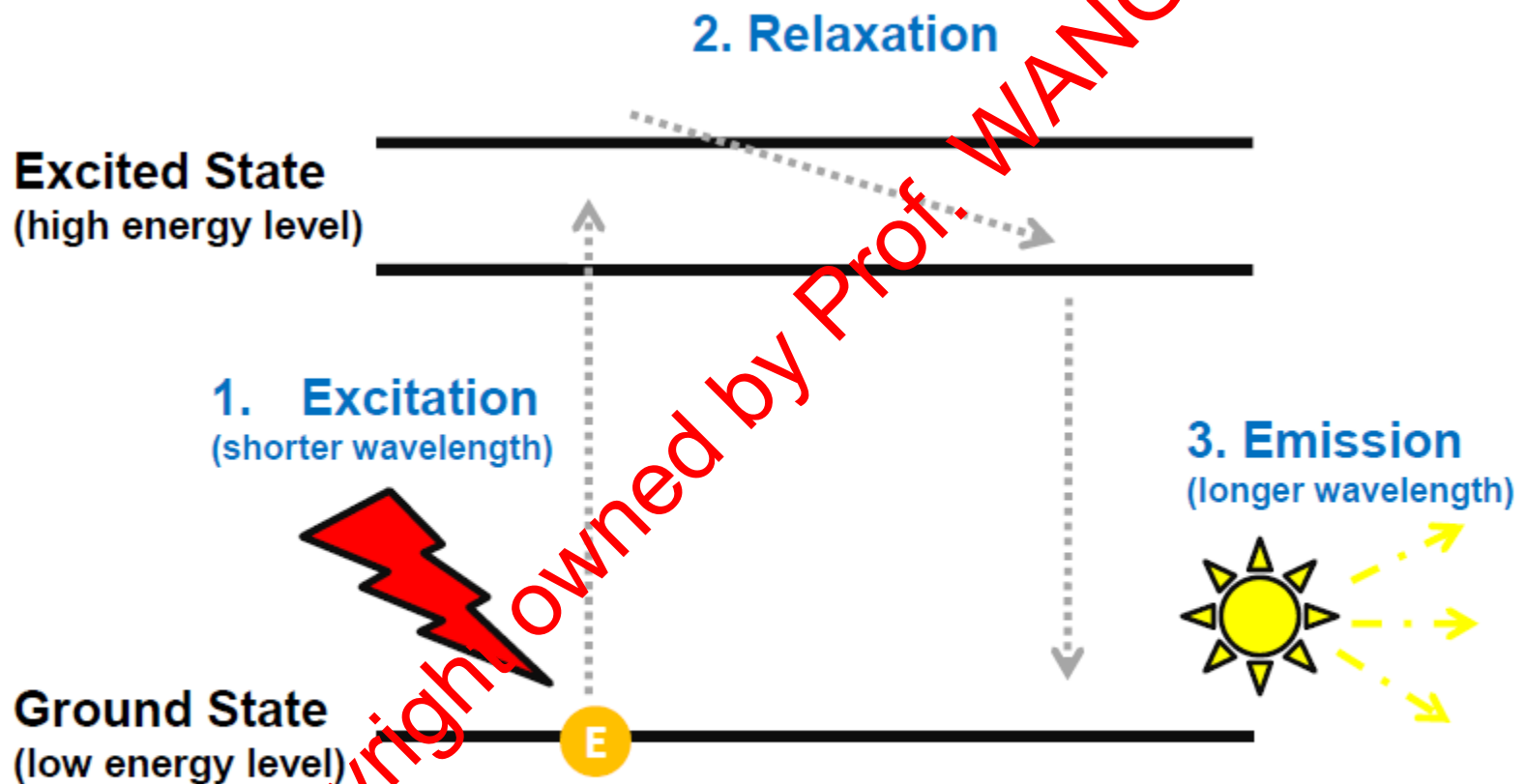




What is fluorescence?

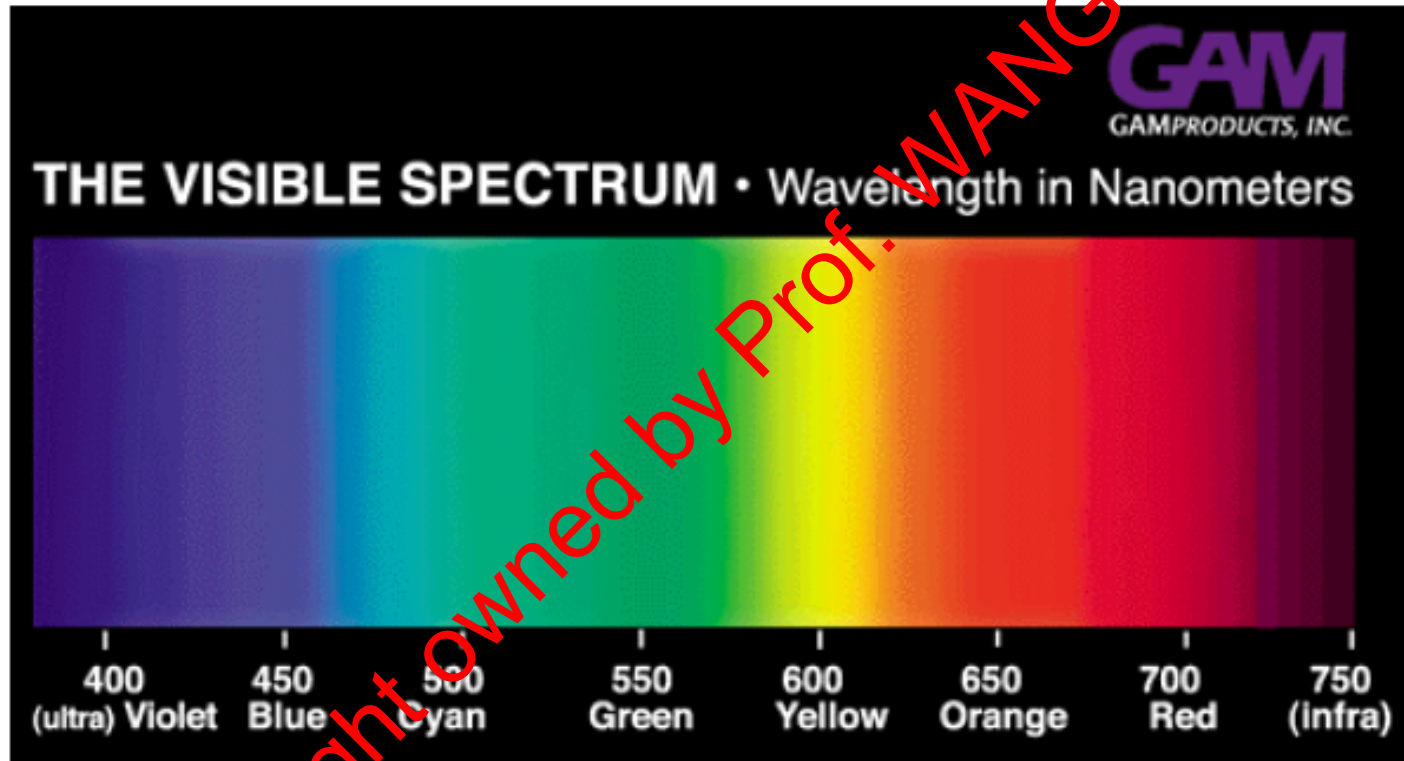


What is fluorescence?



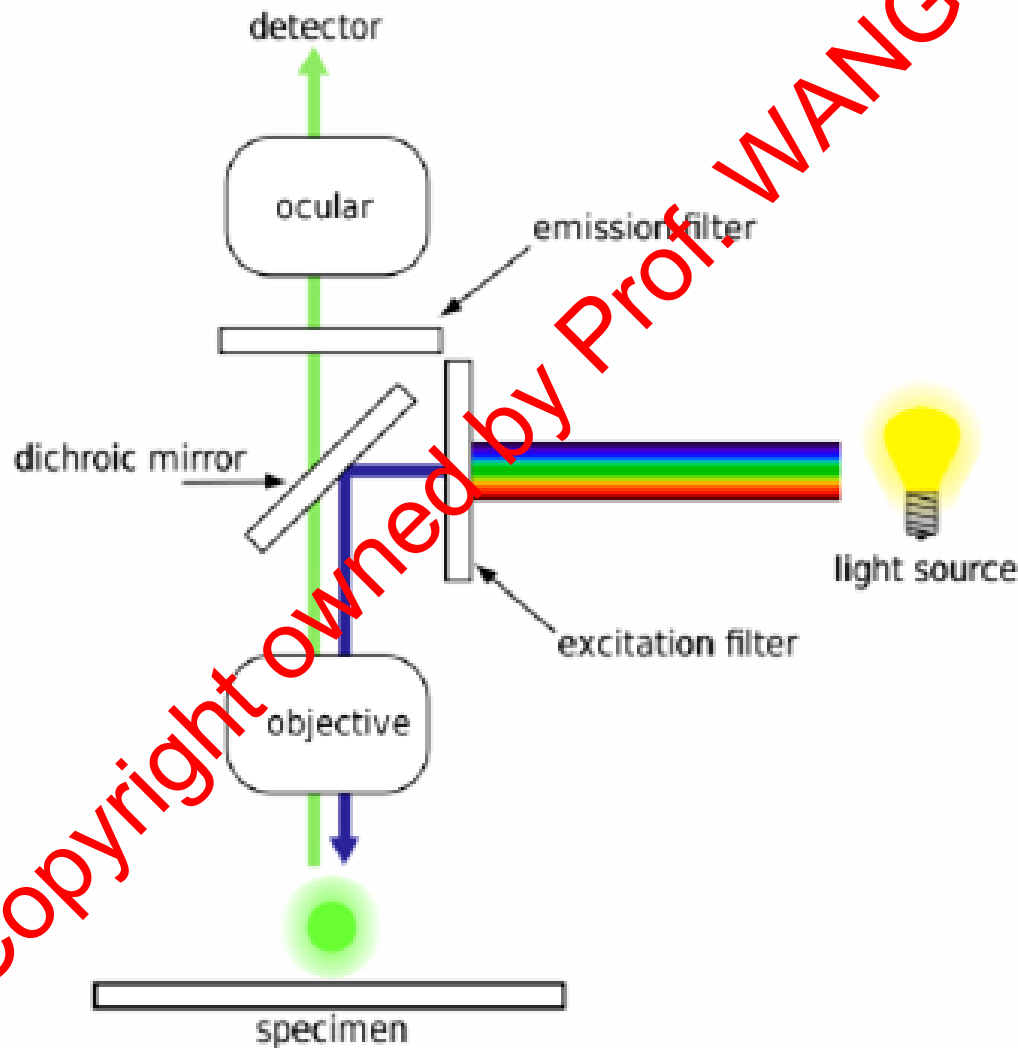
Fluorescence signal is generated

Fluorescence Excitation and Emission



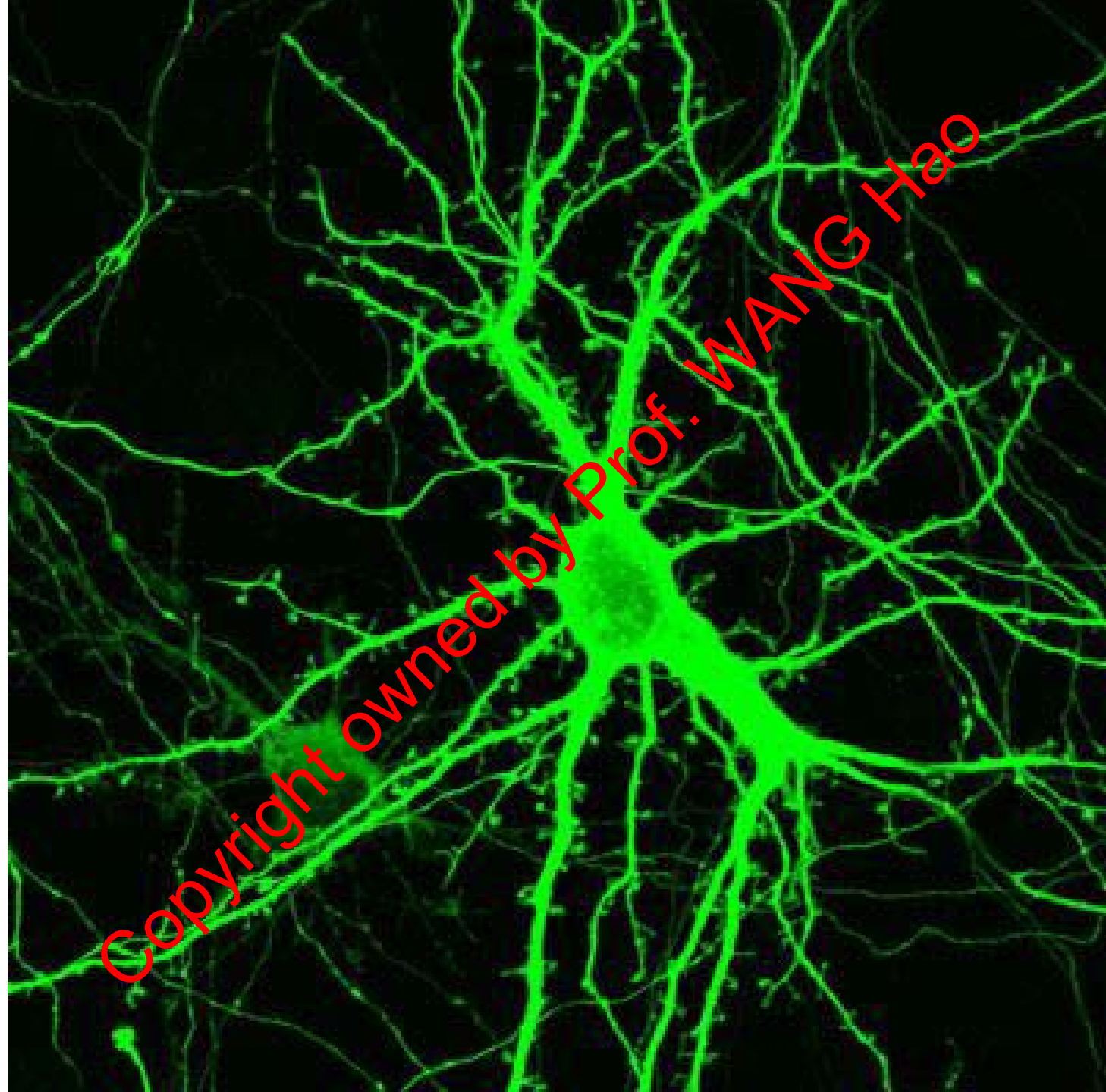
Colors can be seen if the wavelength of the emission photon falls into the visible spectrum.

How to see fluorescence signals ?



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Tools for observing fluorescence signals in cells



Conventional
fluorescence microscope



Laser Scanning Confocal
microscope



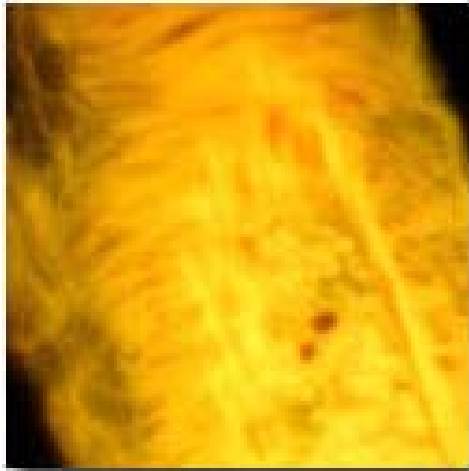
Spinning-disk confocal microscope



Super-resolution fluorescence microscope

Confocal laser scanning microscopy

Confocal and Widefield Fluorescence Microscopy



(a)



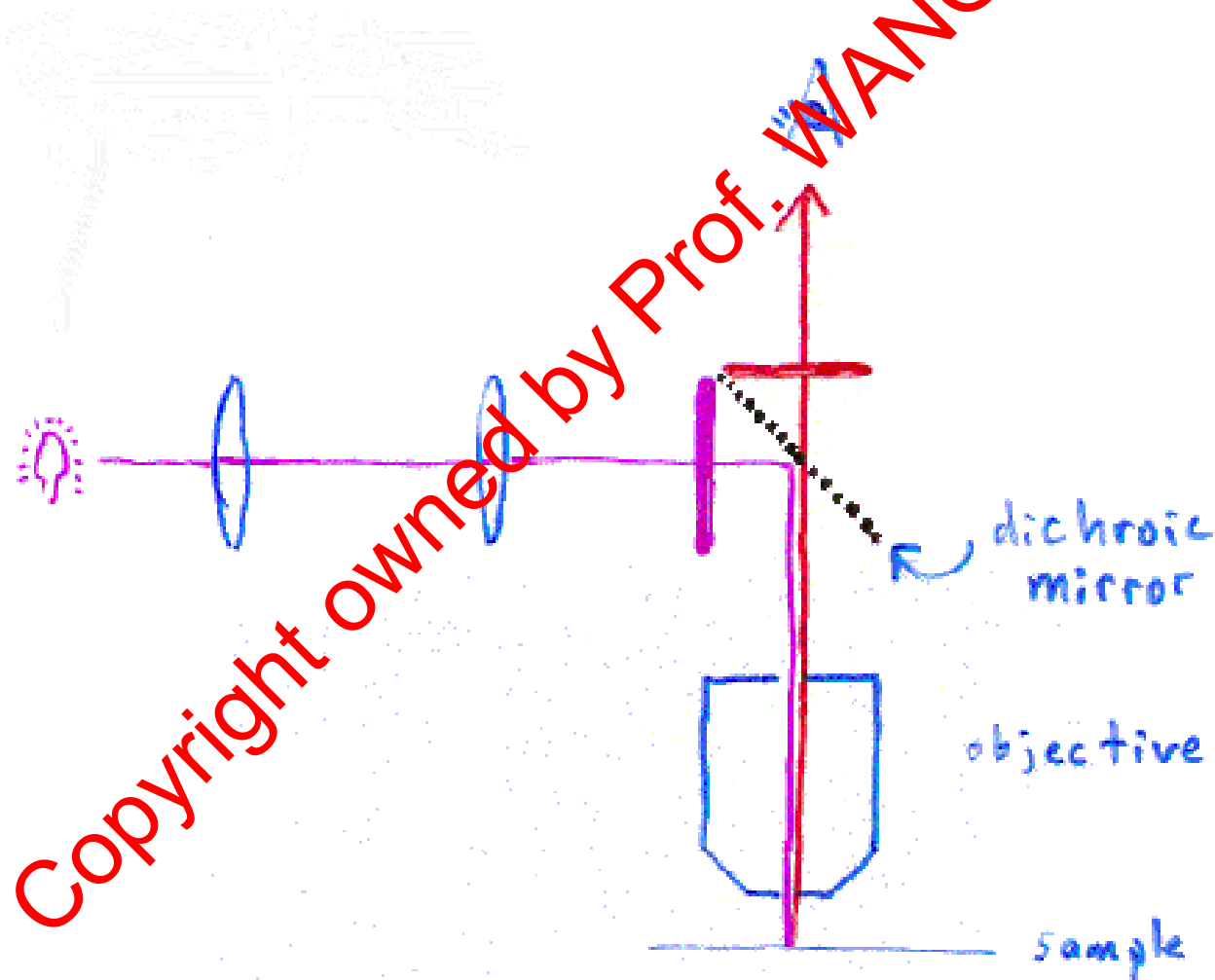
(b)



(c)

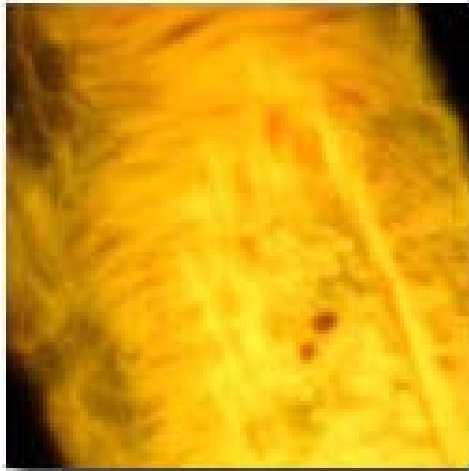
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How does a fluorescence microscope work?



Confocal laser scanning microscopy

Confocal and Widefield Fluorescence Microscopy



(a)



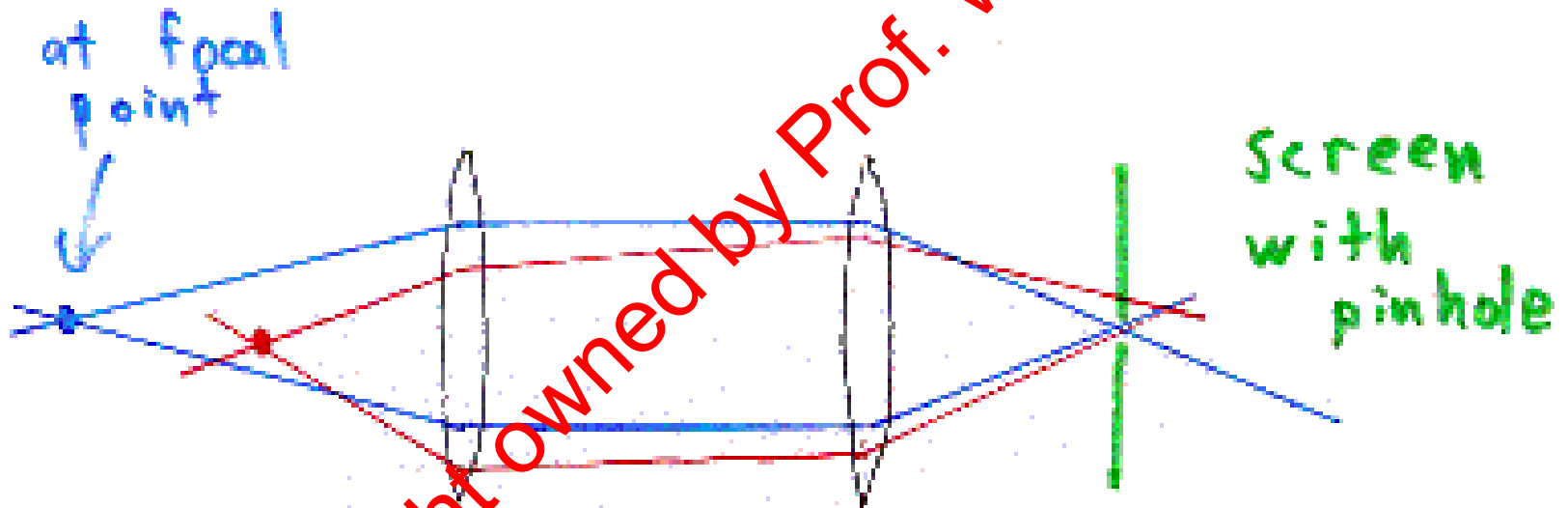
(b)



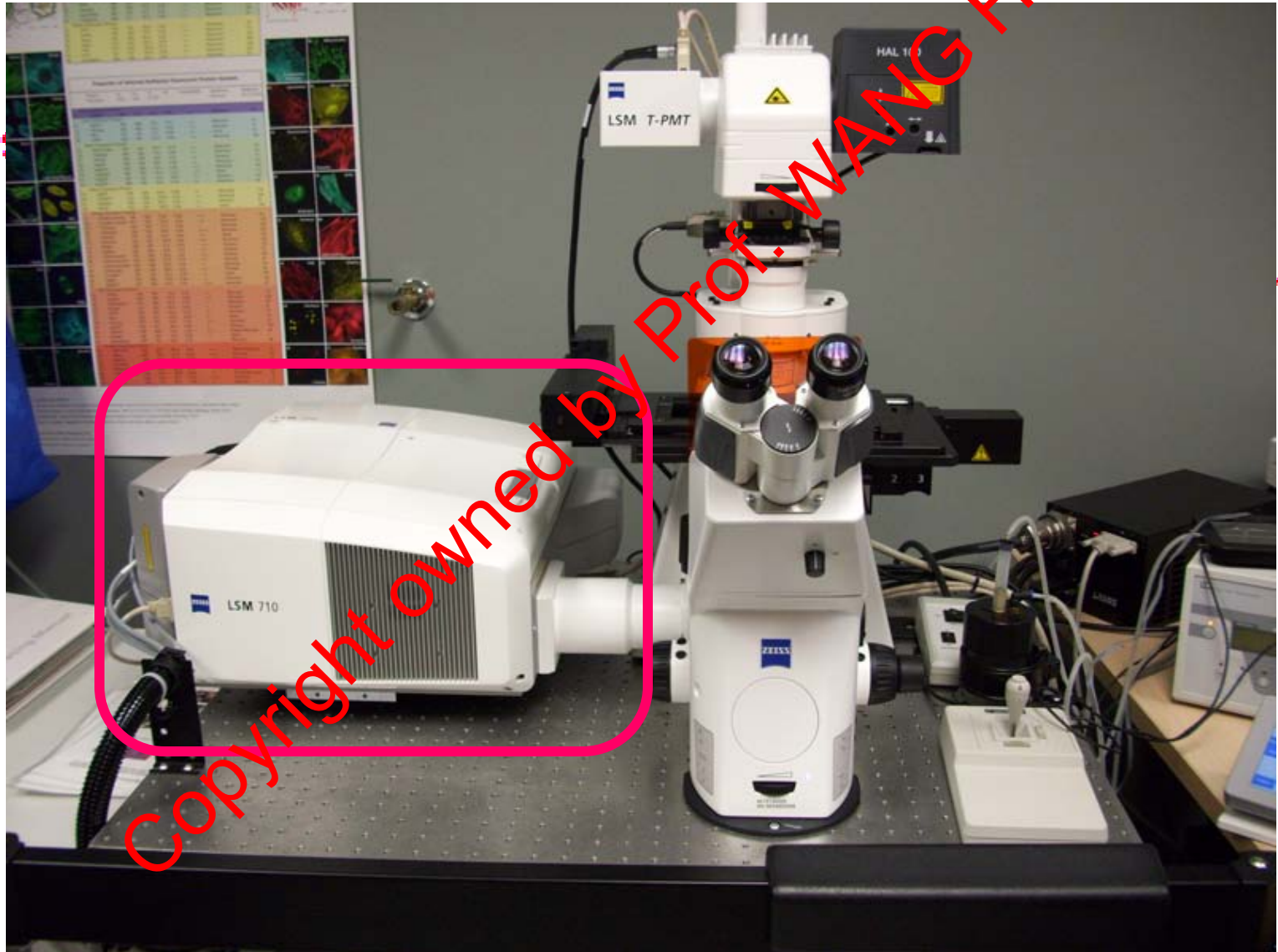
(c)

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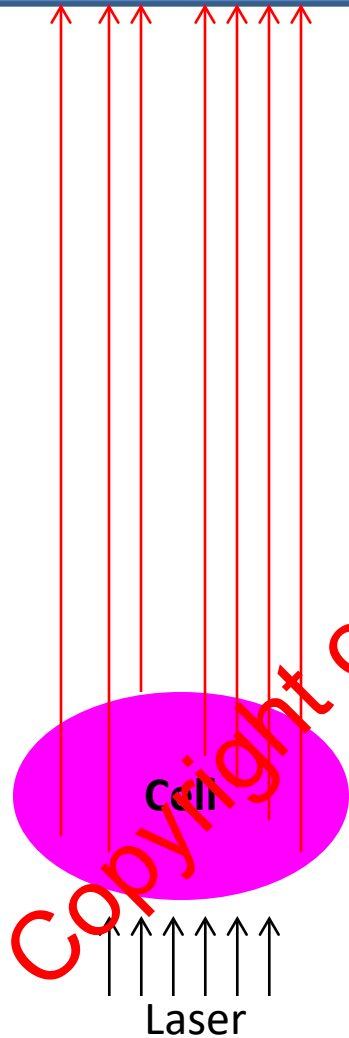
What's this got to do with confocal microscopy?



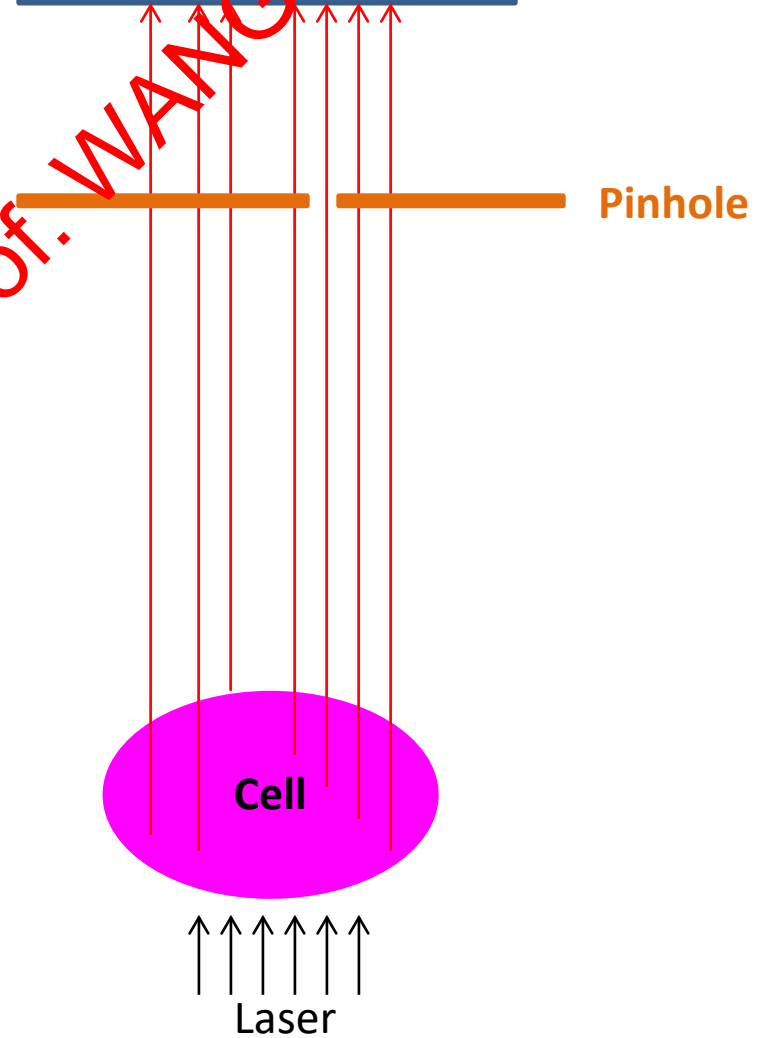
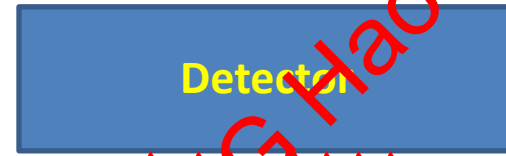
How does a confocal microscope work?



Wide Fluorescence Microscopy

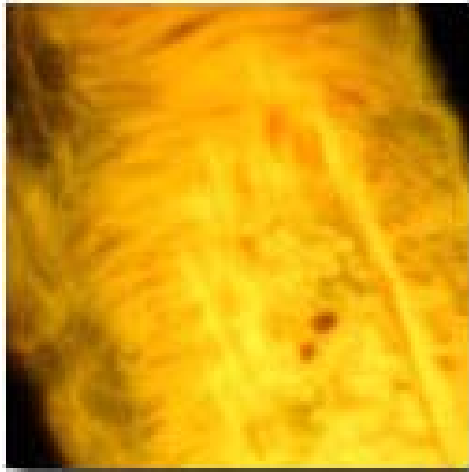


Confocal Microscopy



Confocal laser scanning microscopy

Confocal and Widefield Fluorescence Microscopy



(a)



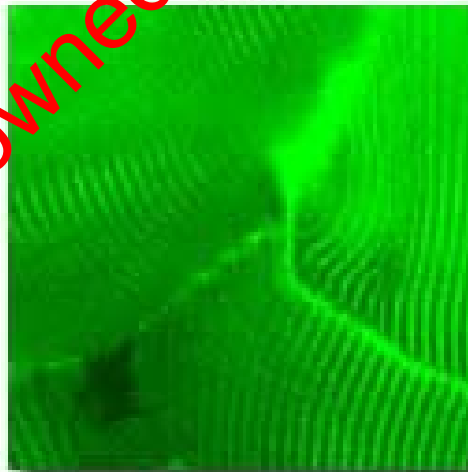
(b)



(c)



(d)



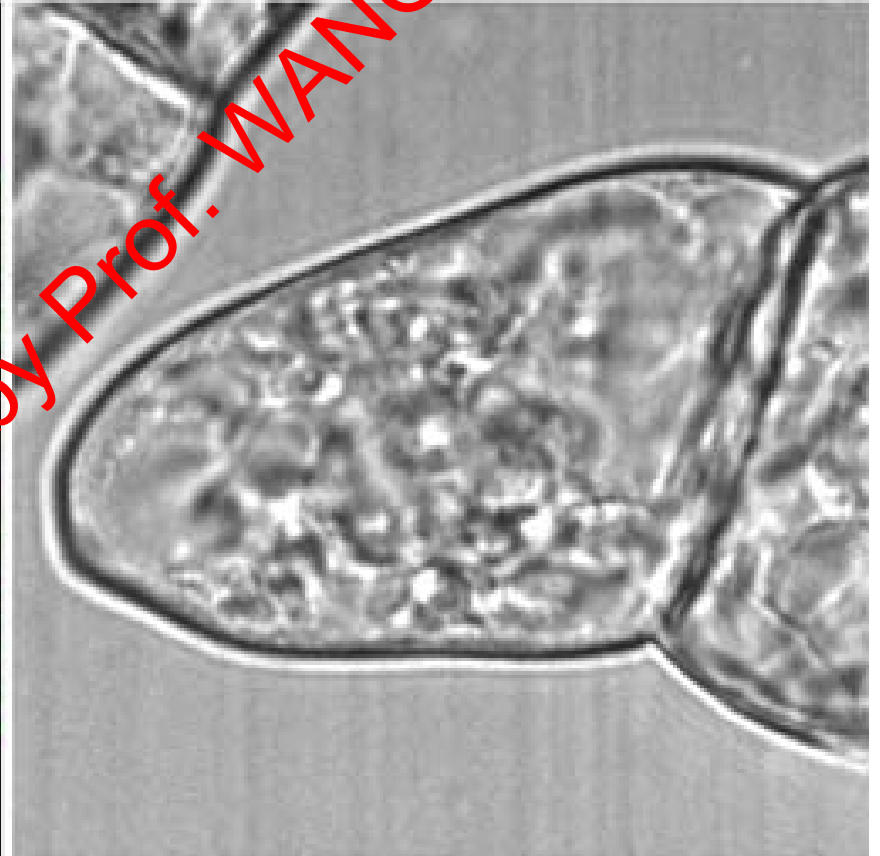
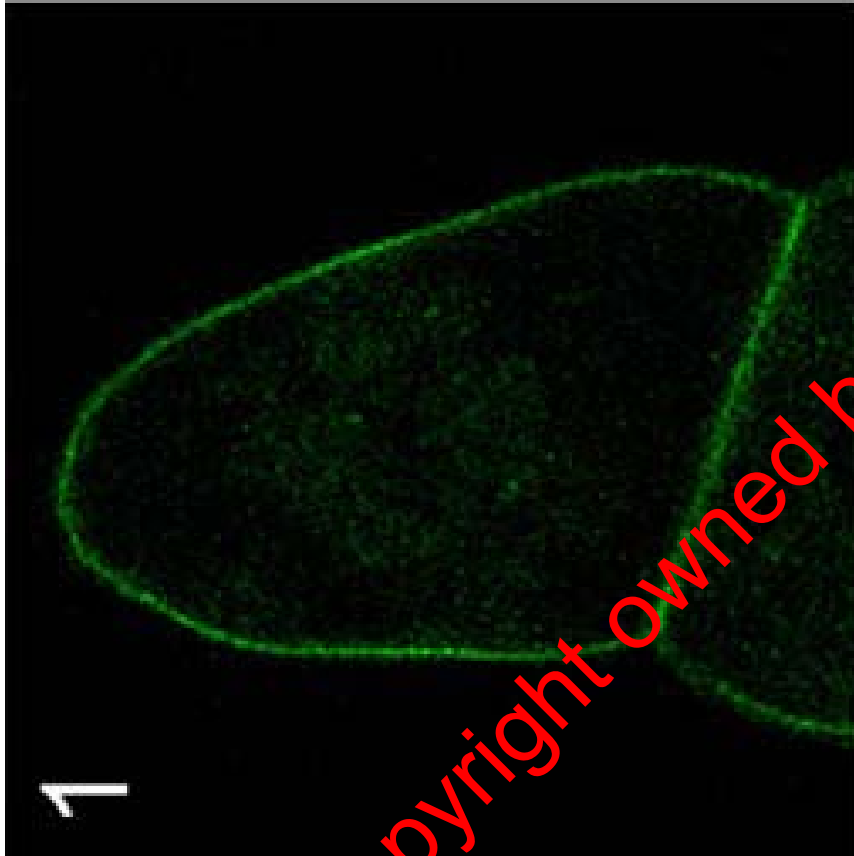
(e)



(f)

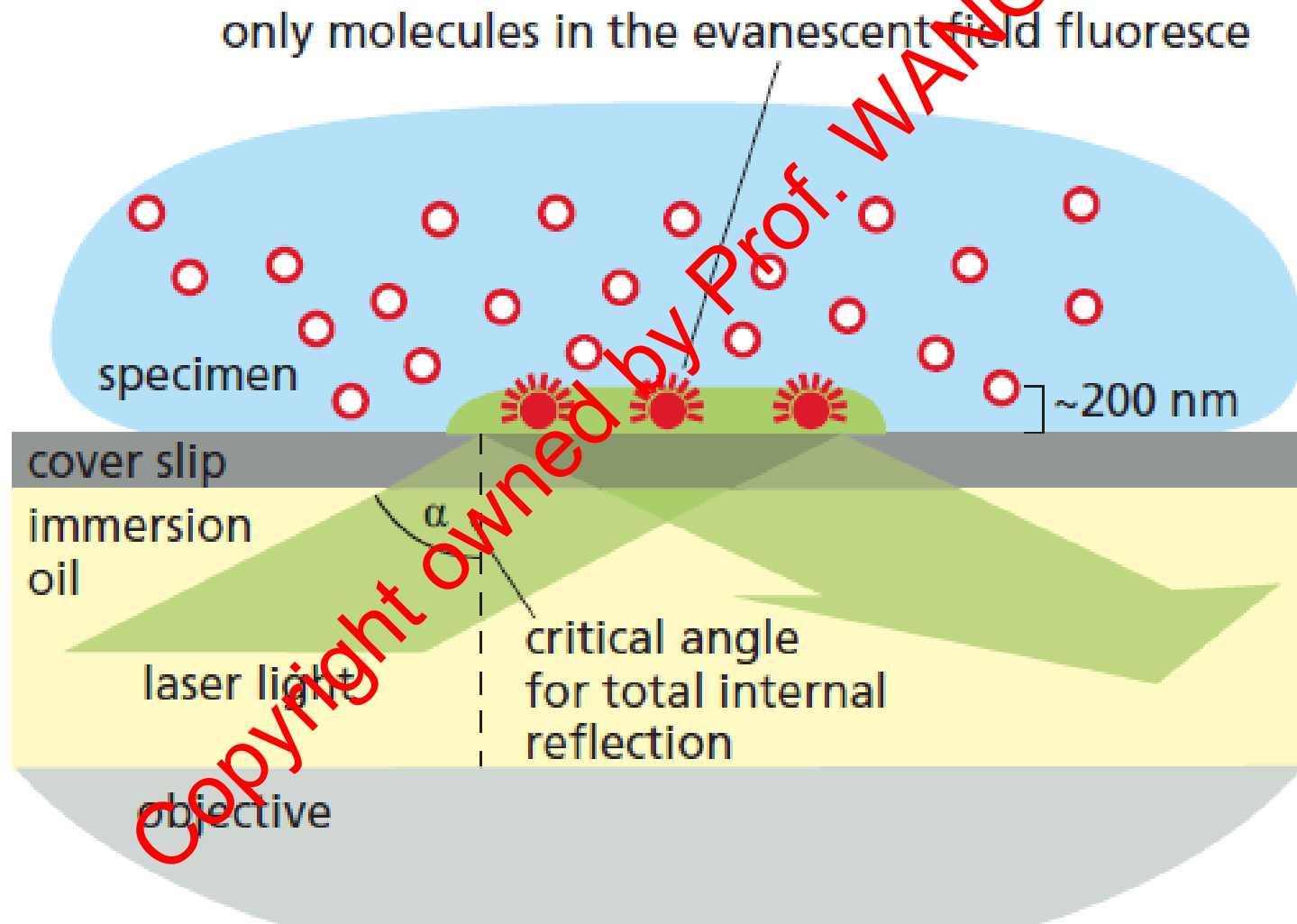
Figure 1

Visualizing Cell-TIRF Microscopy

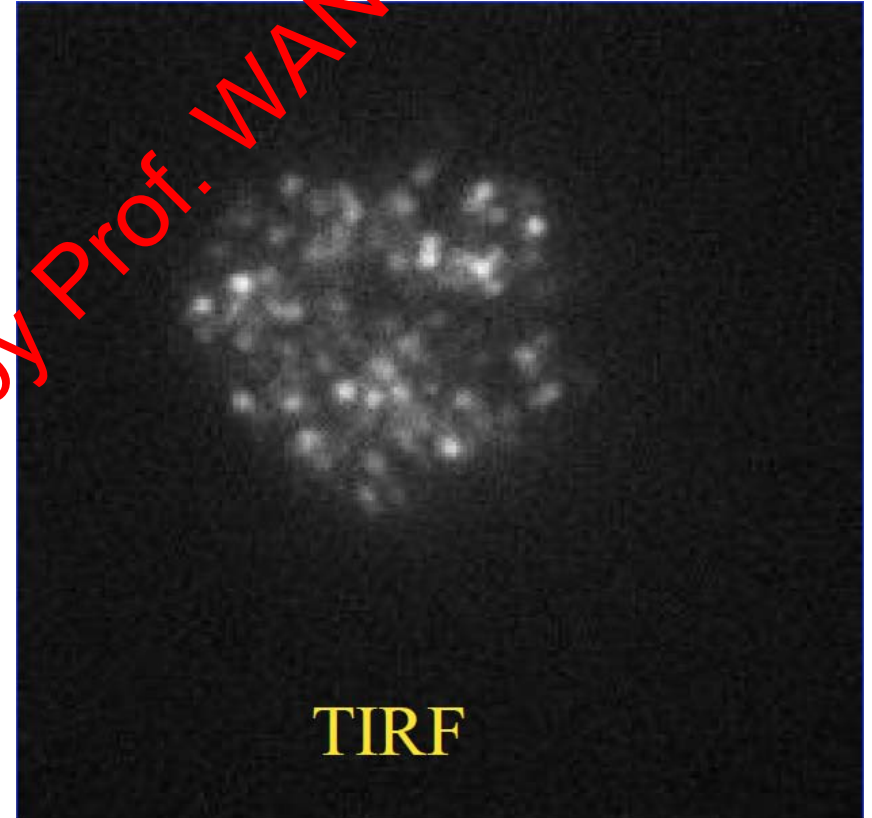
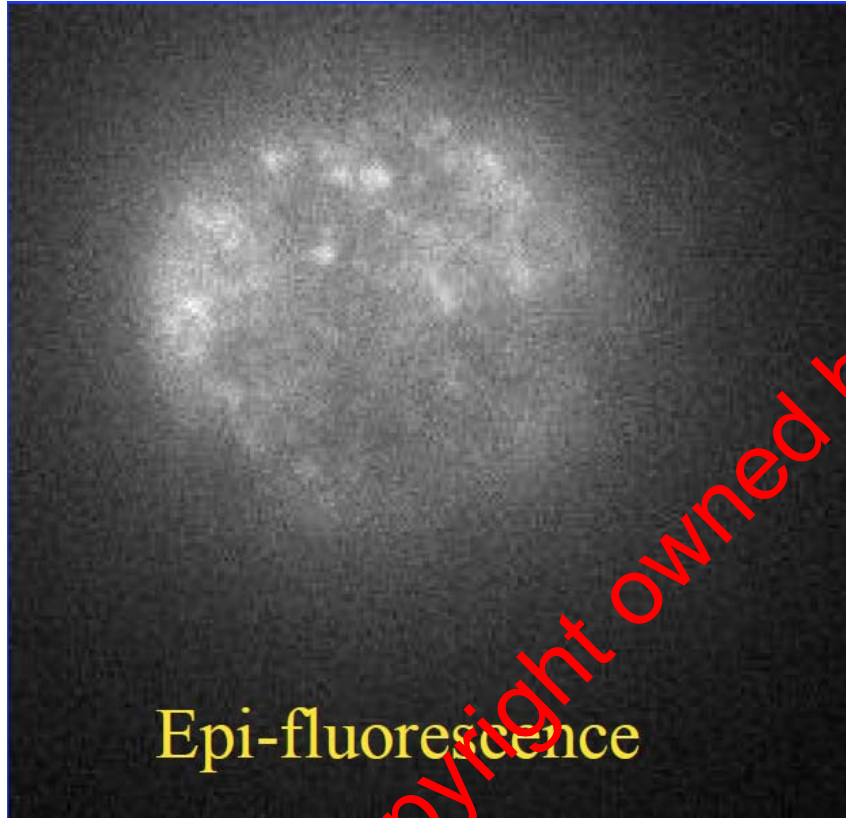


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Visualizing Cell-TIRF Microscopy



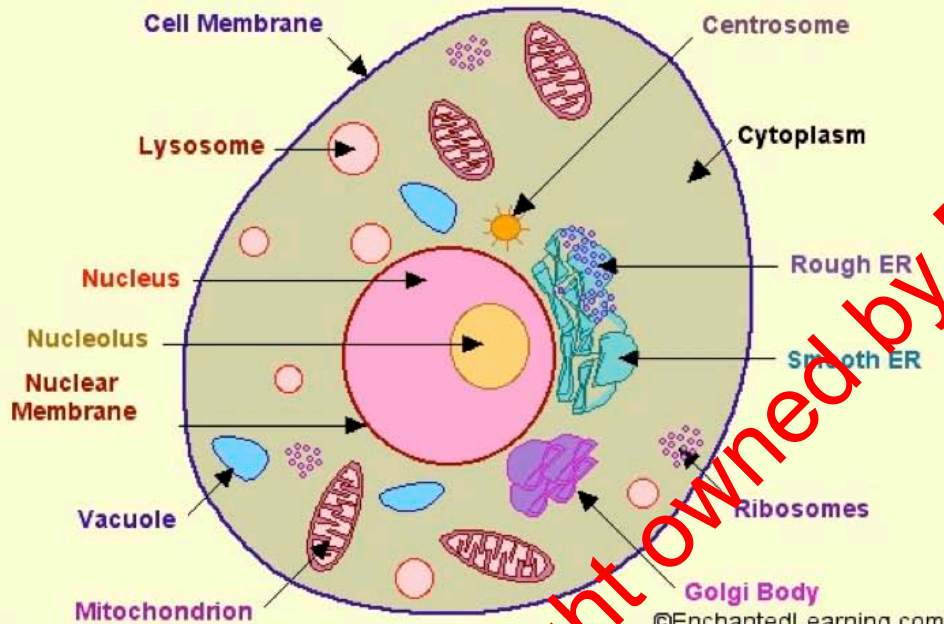
Visualizing Cell-TIRF Microscopy



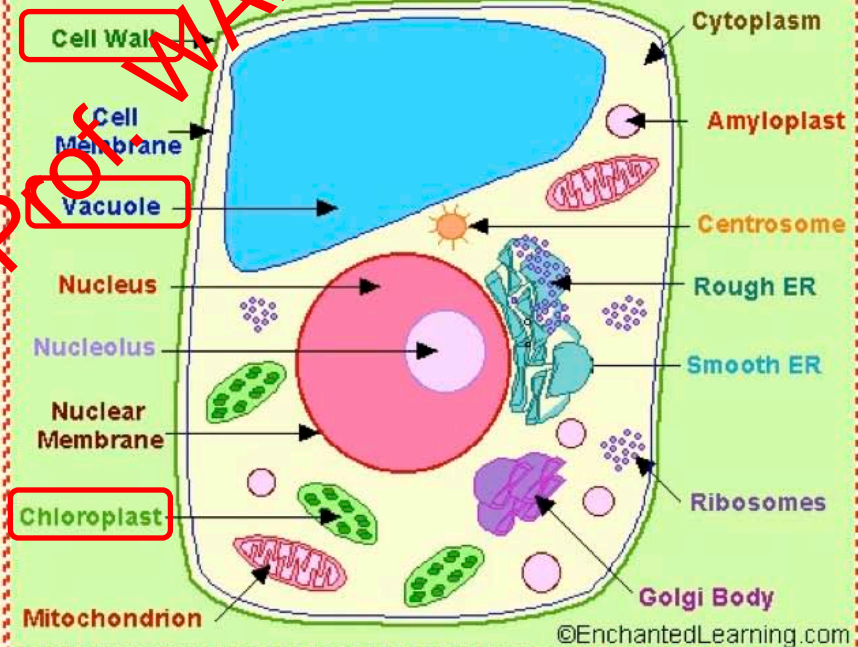
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Visualizing Cell-TIRF Microscopy

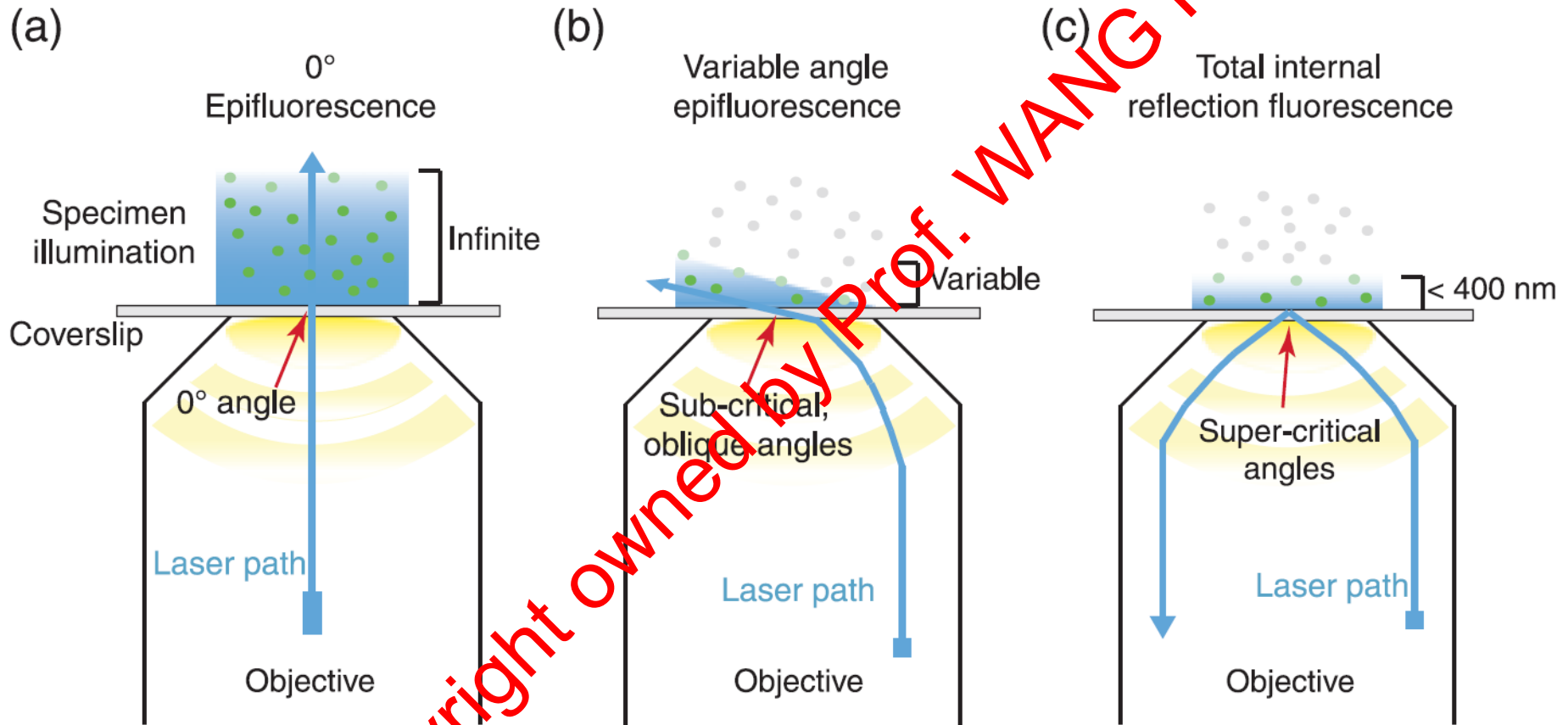
Cross-Section of an Animal Cell



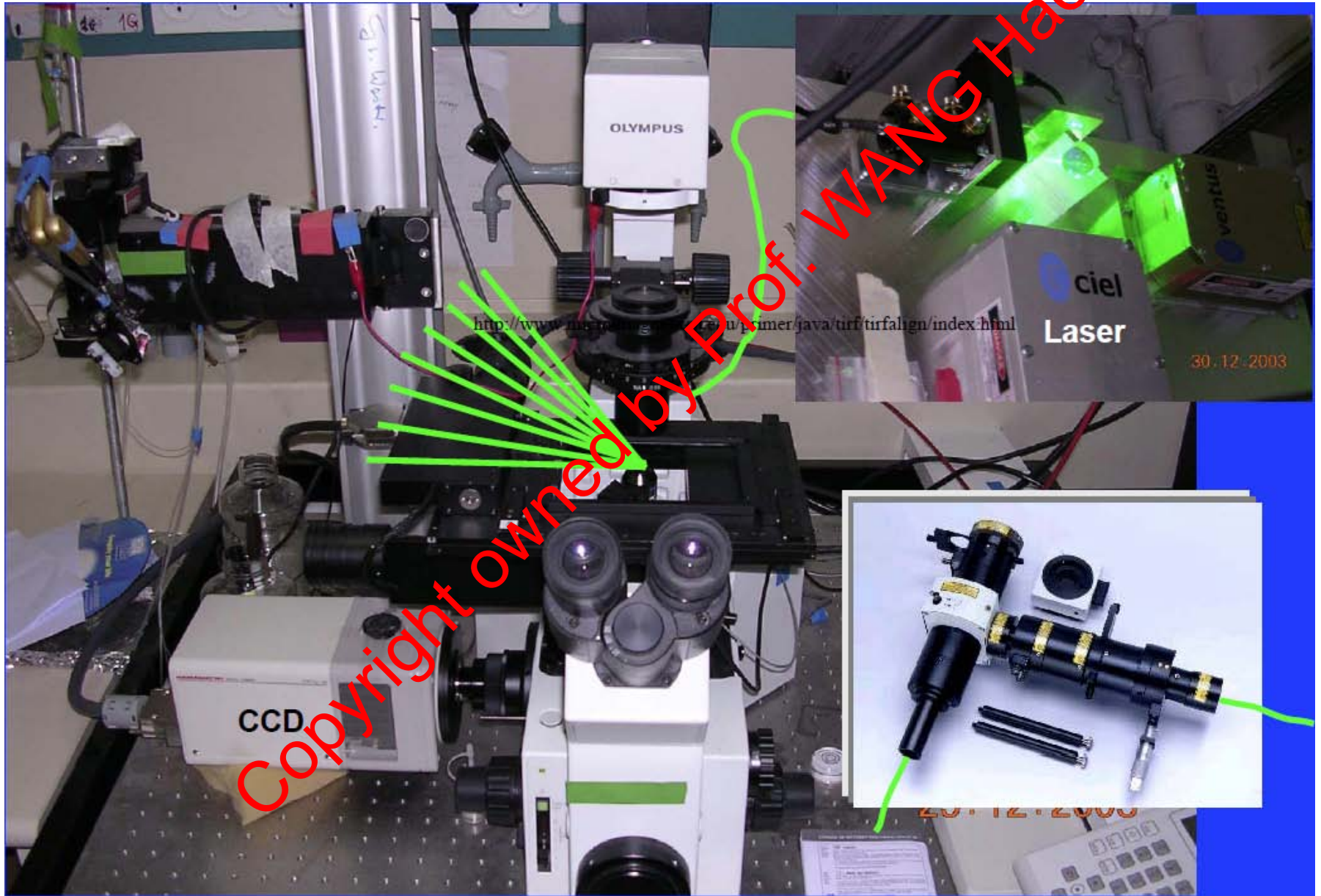
Cross-Section of a Plant Cell



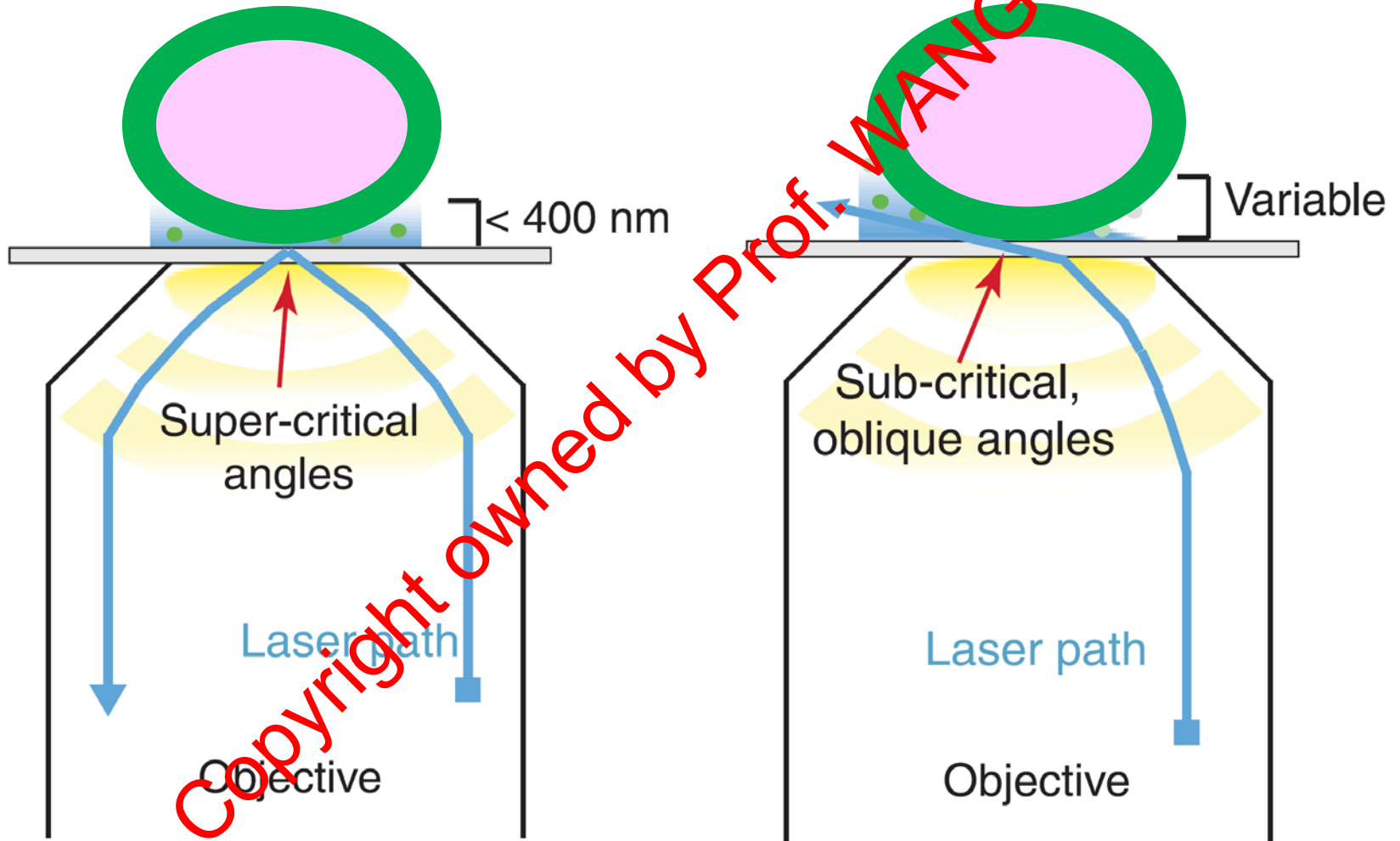
Visualizing Cell-TIRF Microscopy



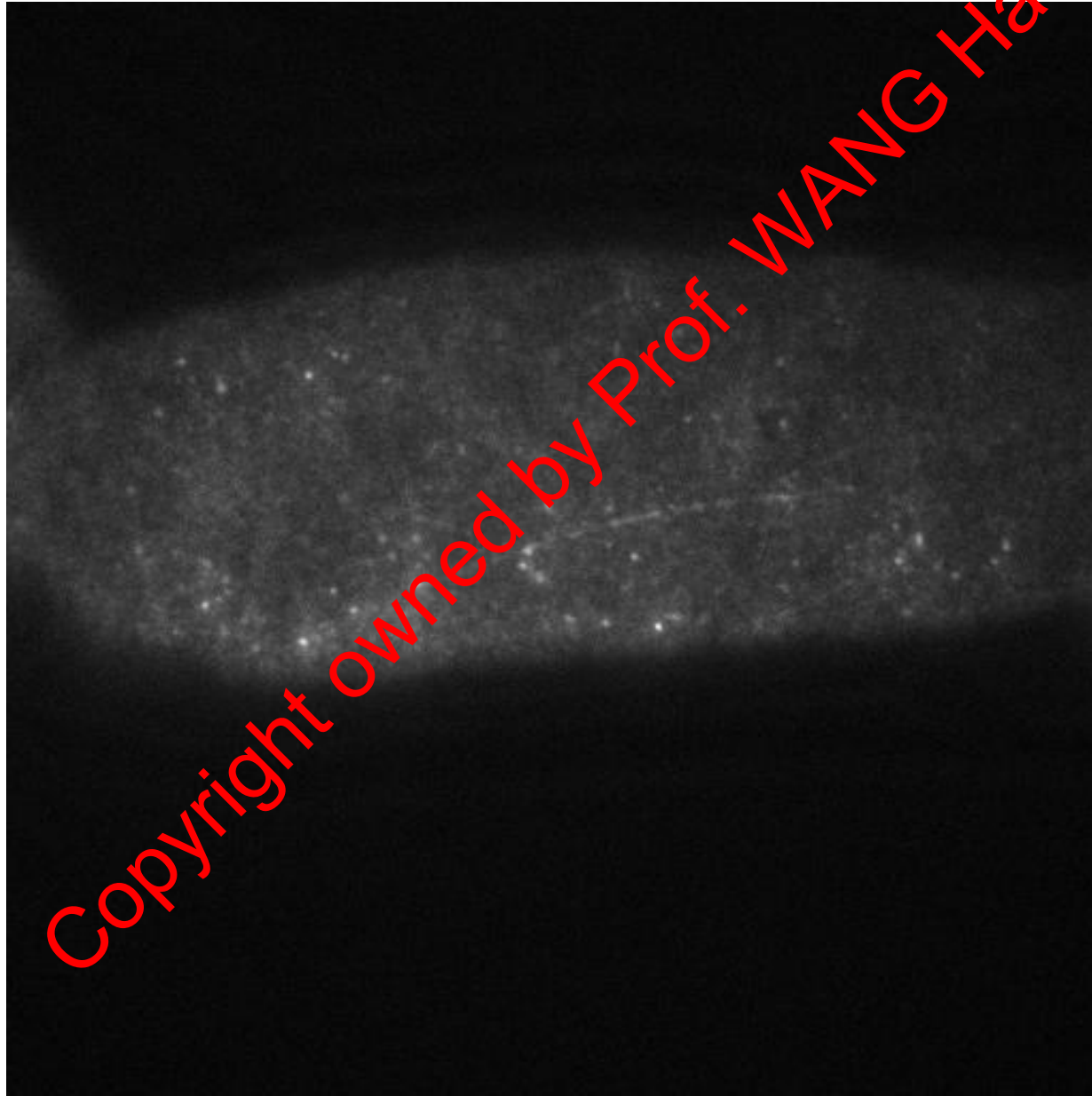
Visualizing Cell-TIRF Microscopy



Visualizing Cell-VAEM



Visualizing Cell-VAEM

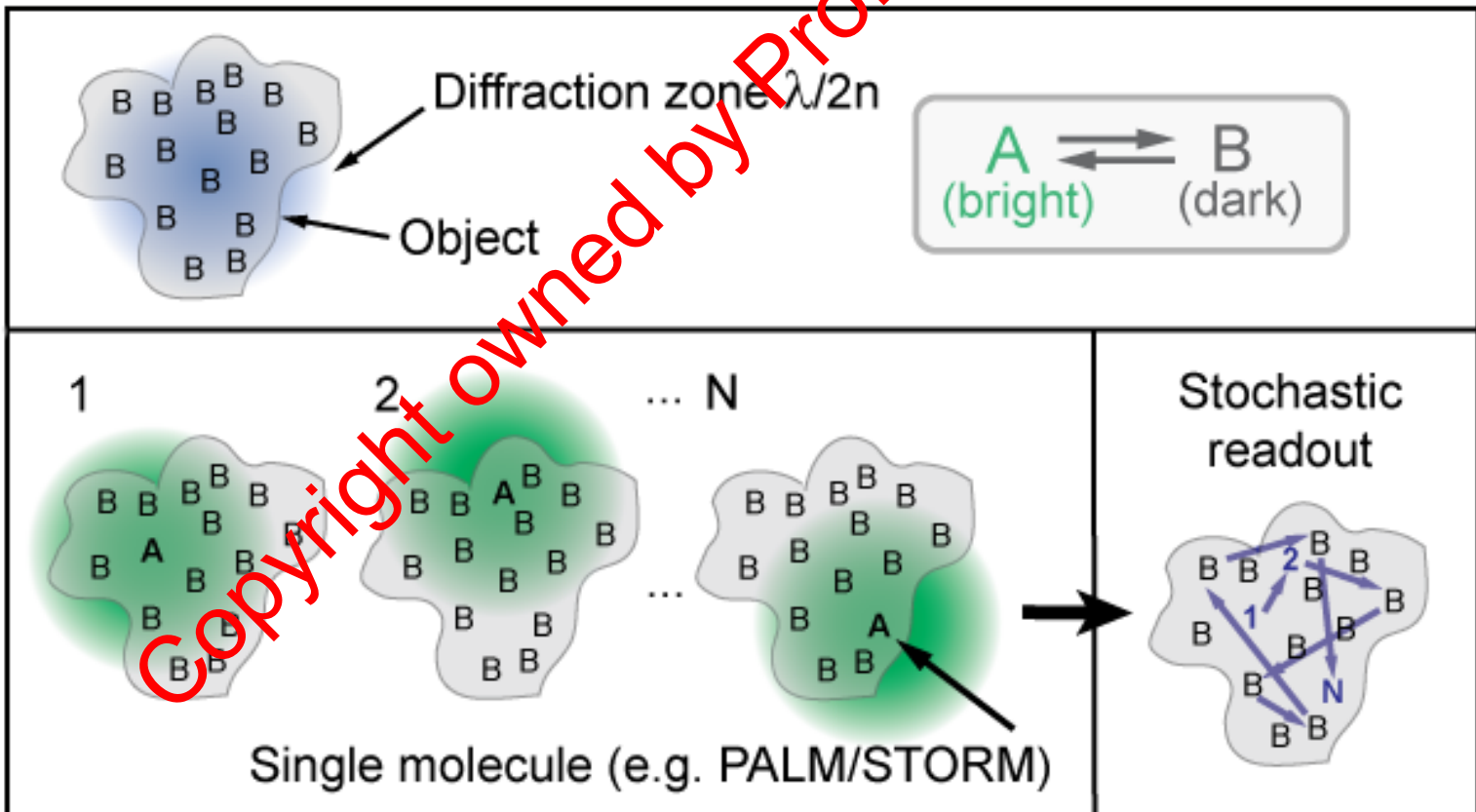


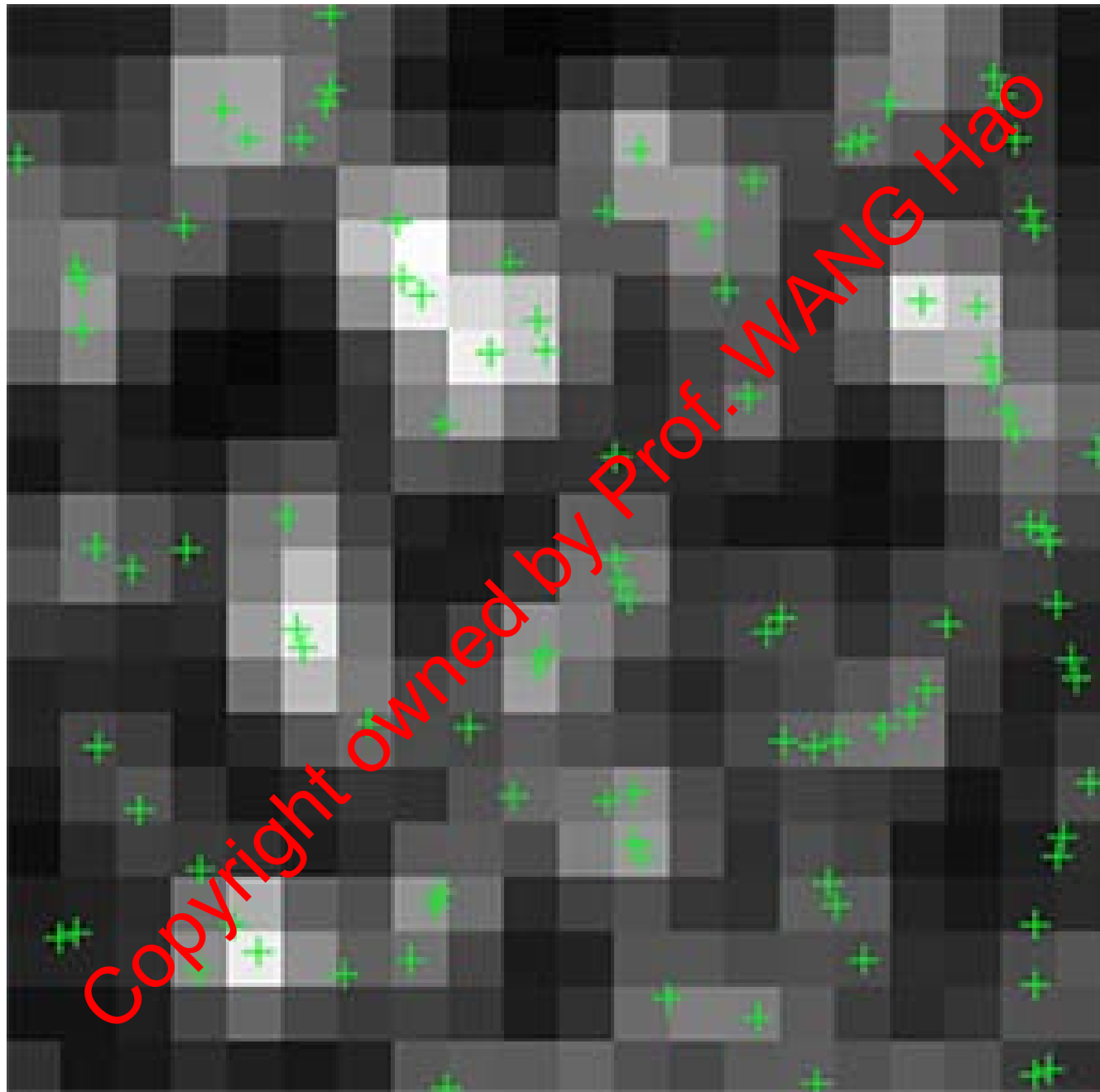
Super-resolution Microscopy: STROM

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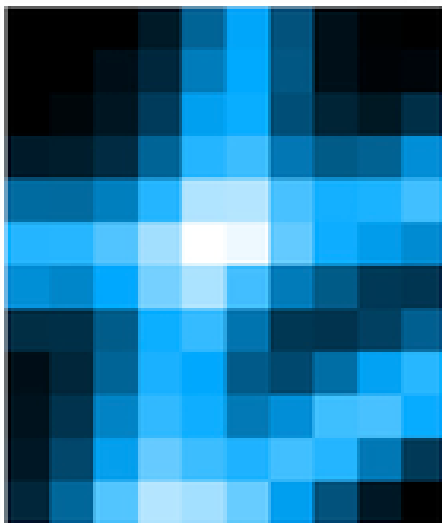
Stochastic Optical Reconstruction Microscopy (STORM)

The ability of certain molecules to switch between a bright and a dark state is the very basic molecular feature underlying all sub-diffraction imaging techniques.

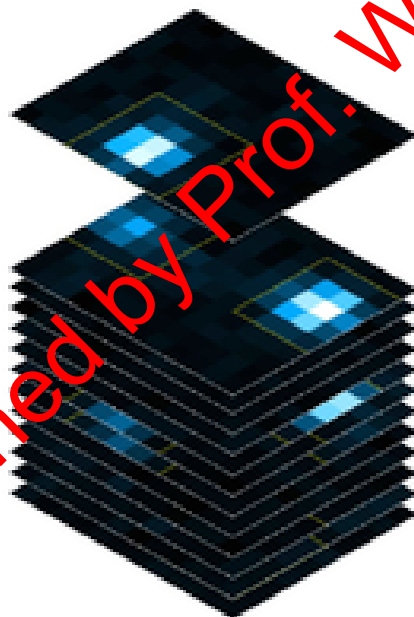
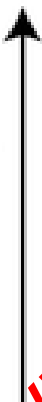




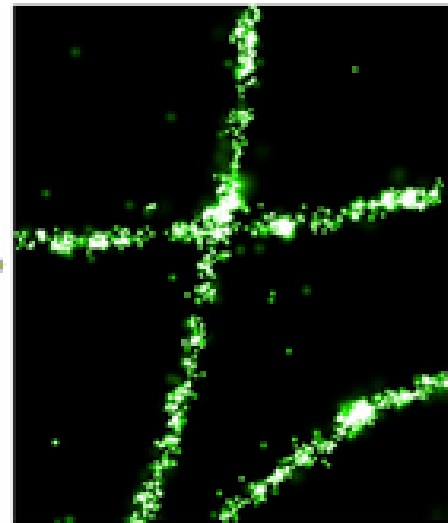
Diffraction-limited image



Stochastic activation of single molecules over many frames



STORM image



Basic Principle of STORM Superresolution Imaging

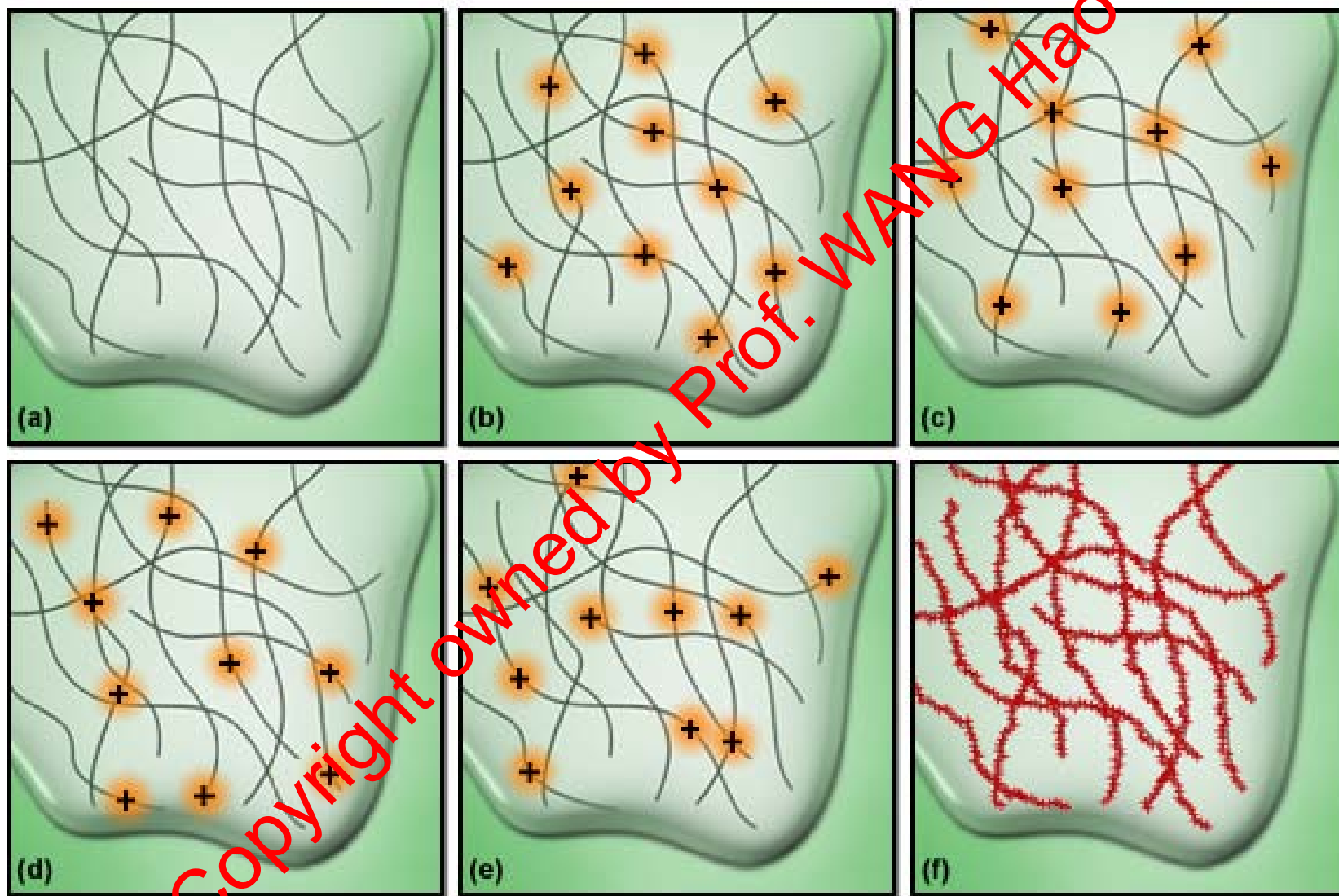
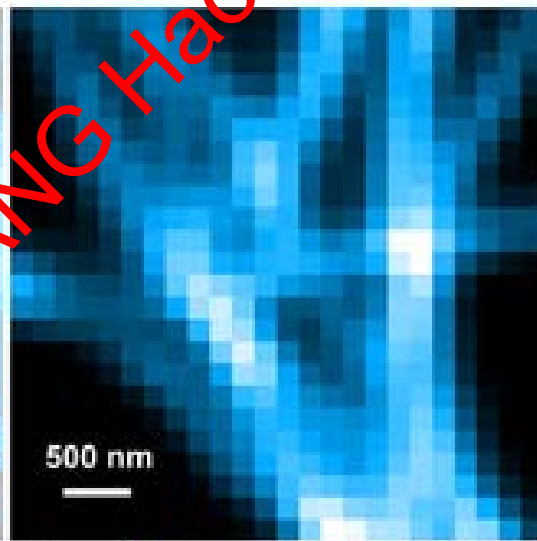
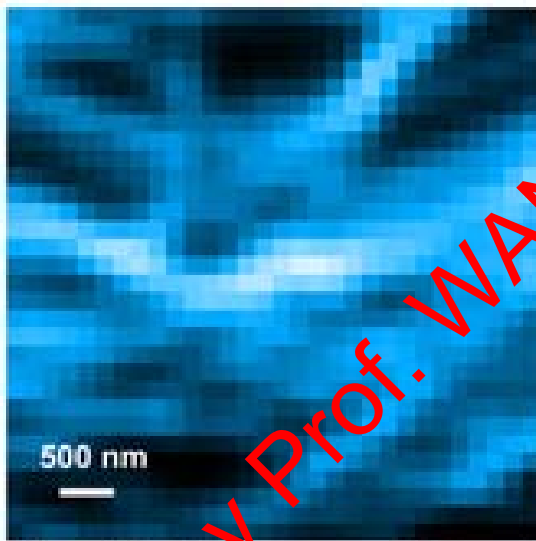
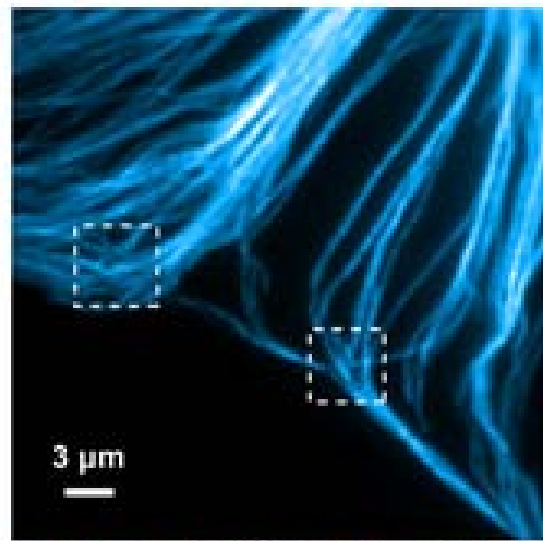
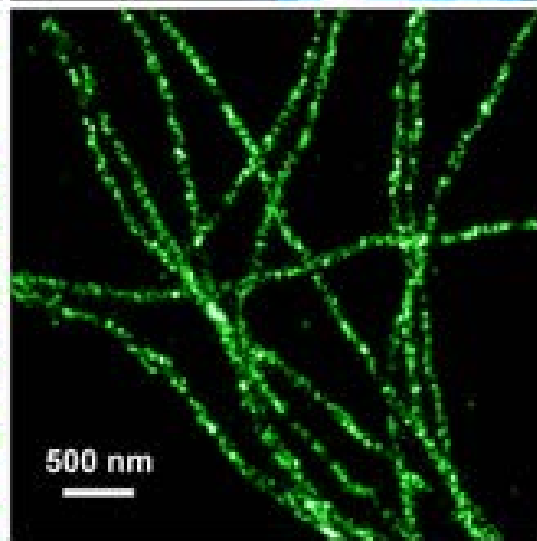
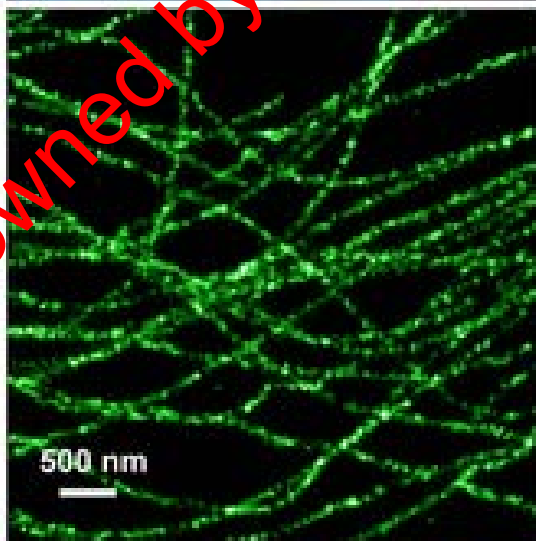
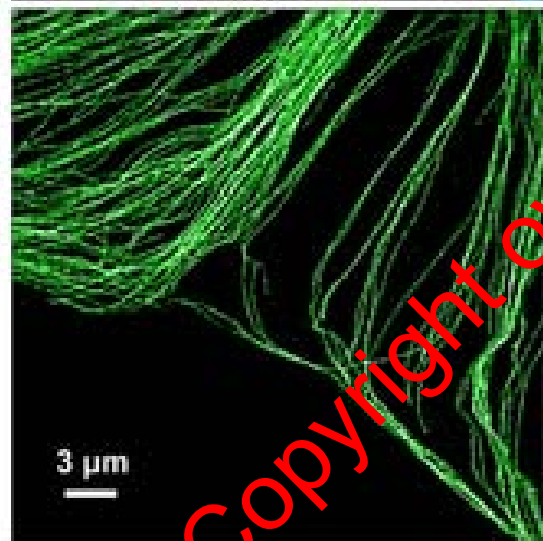


Figure 1

Conventional

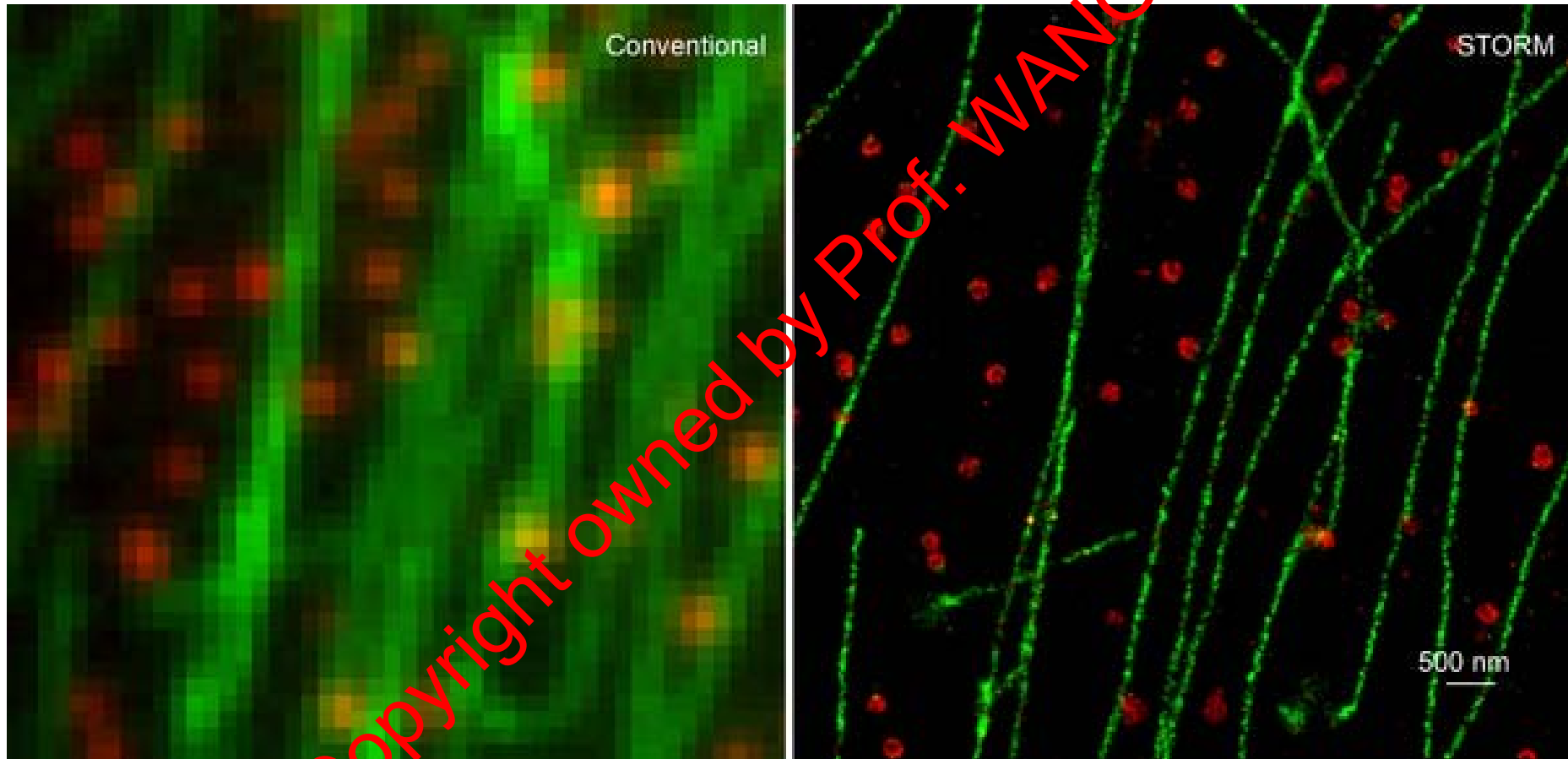


STORM

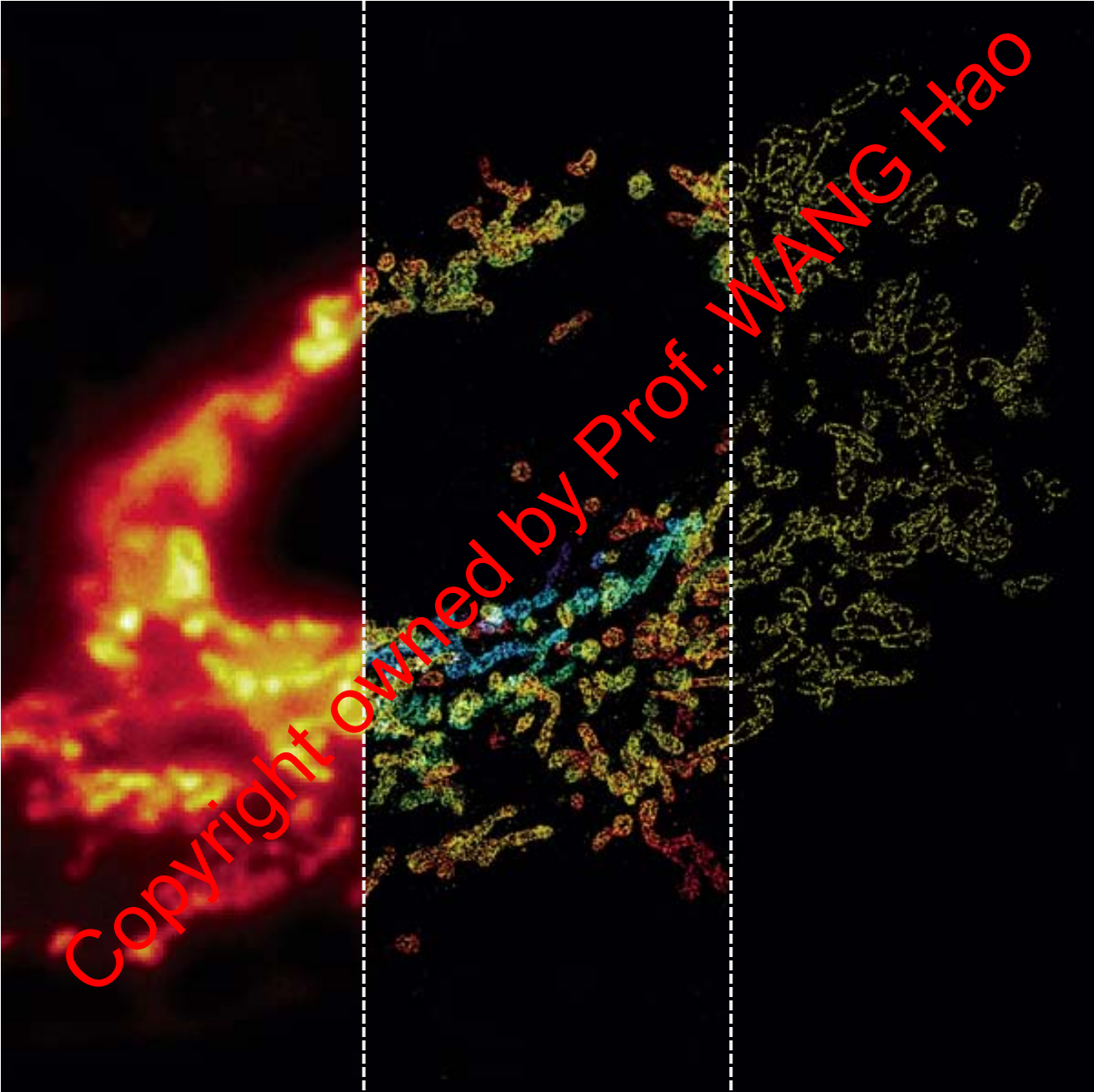


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Example 1: Two-color conventional (Left) and STORM (right) image of microtubules (green) and clathrin-coated pits (red) in a cell



Example 2: Comparison of conventional and 3D STORM image of mitochondria in a cell



Lattice light-sheet microscopy

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Reconstruction of Zebrafish Early Embryonic Development by Scanned Light Sheet Microscopy

Philipp J. Keller,^{1,2*} Annette D. Schmidt,² Joachim Wittbrodt,^{1,2,3,4*} Ernst H.K. Stelzer¹

Nov, 2008, Science



In order to get a three-dimensional resolution image:

Usual confocal microscopy + multiphoton microscopy

Disadvantages:

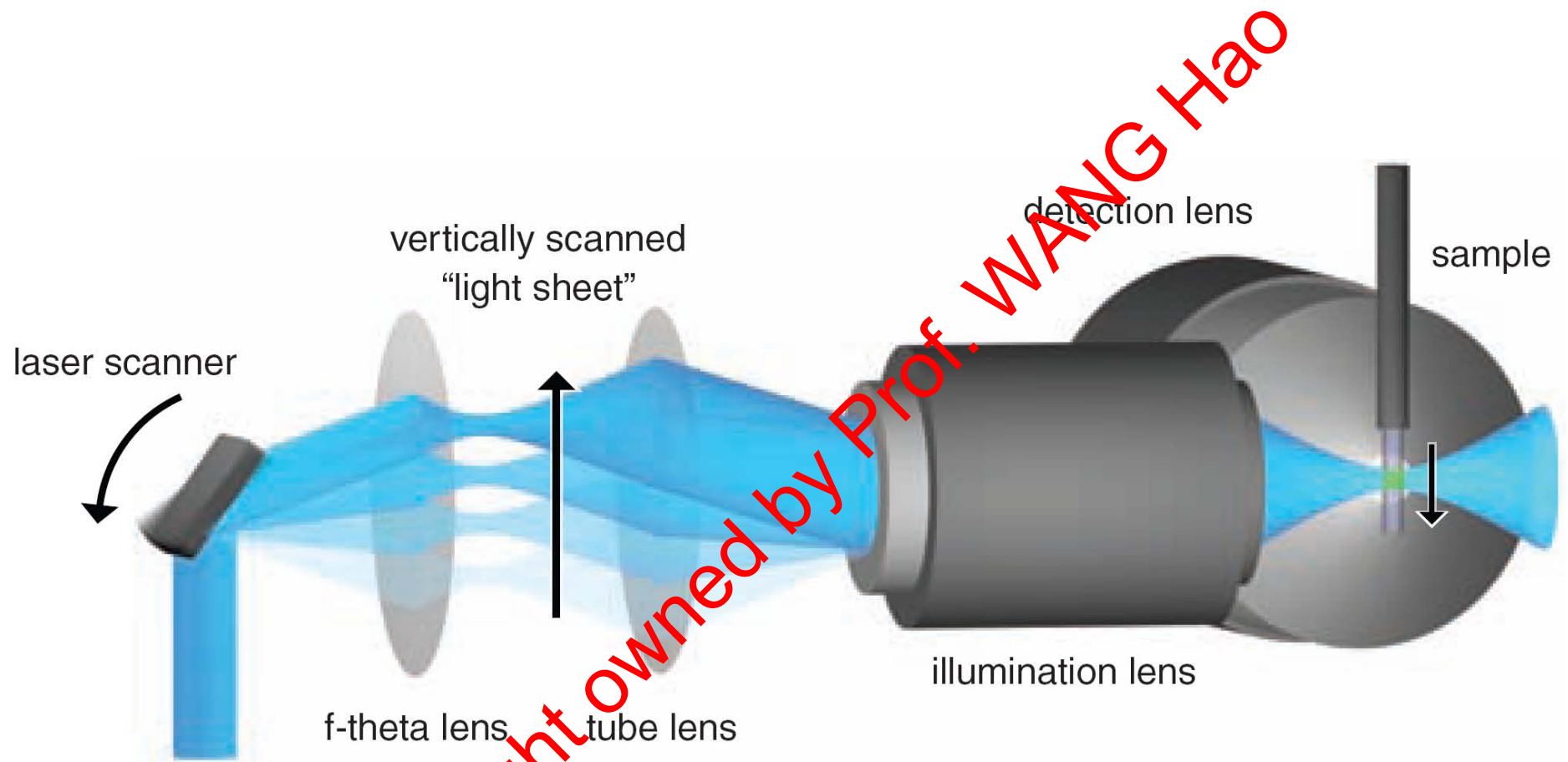
1. Low speed image

2. High phototoxicity

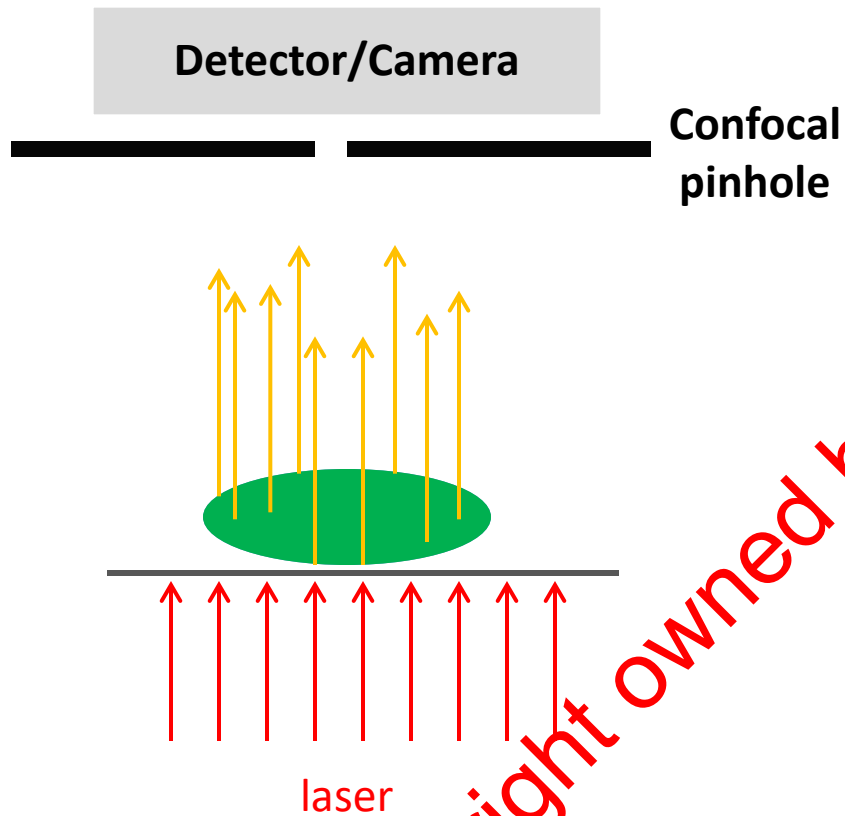
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DSLM combines:

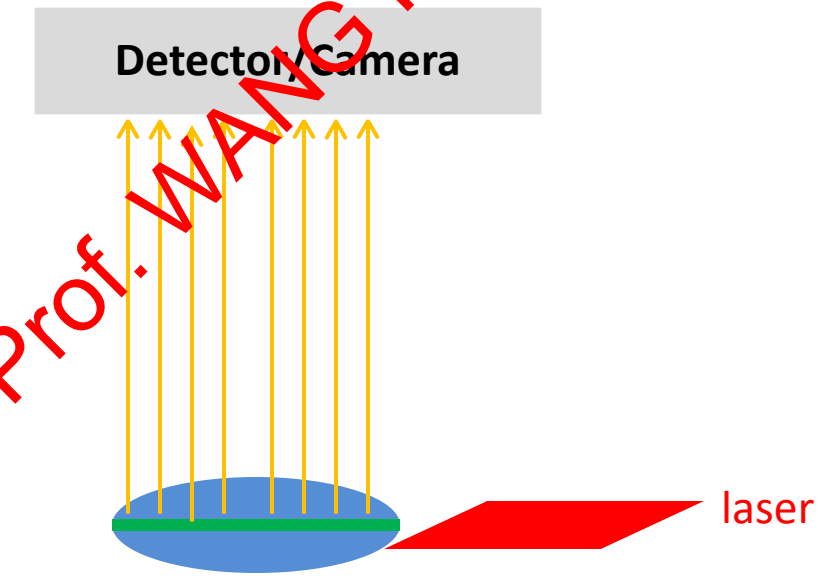
- (i) an imaging speed of 63 million voxels per second,
- (ii) A signal-to-noise ratio of 1000:1 at a lateral and axial resolution of 300 and 1000 nm, respectively,
- (iii) ultralow excitation energies confined to a single plane (1.7 mJ at 488 nm passing each plane in the zebrafish experiments)



Conventional fluorescence microscopy



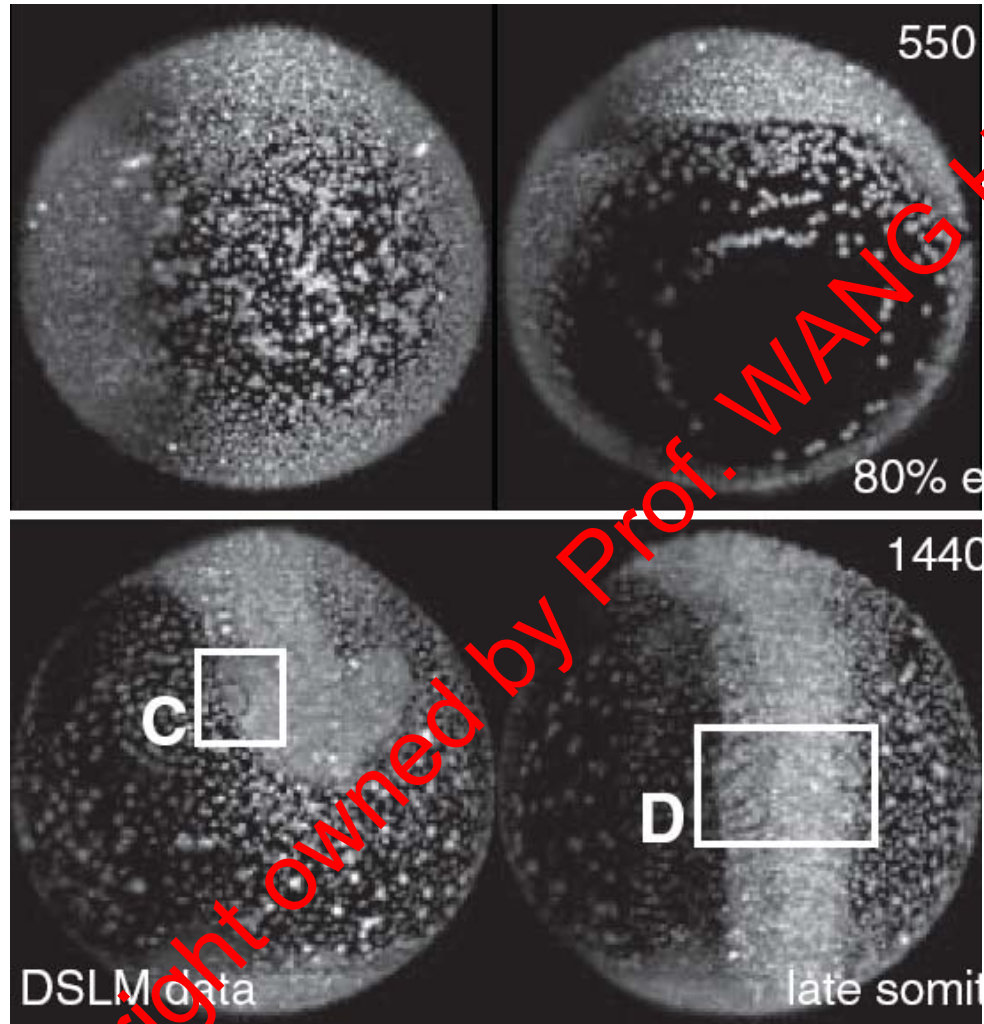
Light-sheet microscopy



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Live cell migration during embryo development

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The movie shows maximum-intensity projections of a DSLM time-lapse multi-view recoding of zebrafish embryonic development, with a view on both the animal and vegetal hemispheres. The wild-type zebrafish embryo was injected with H2B-eGFP mRNA at the one cell stage.

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light-sheet microscopy

Advantages:

1. Low phototoxicity and photobleaching
2. High scanning speed@4D (different angle)
3. Great increasing of Z resolution
4. Good for live cell imaging

Disadvantages:

1. Resolution @ cellular level

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RESEARCH ARTICLE

ADVANCED IMAGING

Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution

Bi-Chang Chen,^{1*†} Wesley R. Legant,^{1*} Kai Wang,^{1*} Lin Shao,¹ Daniel E. Milkie,² Michael W. Davidson,³ Chris Janetopoulos,⁴ Xufeng S. Wu,⁵ John A. Hammer III,⁵ Zhe Liu,¹ Brian P. English,¹ Yuko Mimori-Kiyosue,⁶ Daniel P. Romero,⁷ Alex T. Ritter,^{8,9} Jennifer Lippincott-Schwartz,⁸ Lillian Fritz-Laylin,¹⁰ R. Dyche Mullins,¹⁰ Diana M. Mitchell,^{11‡} Joshua N. Bembenek,¹¹ Anne-Cecile Reymann,^{12,13§} Ralph Böhm,^{12,13} Stephan W. Grill,^{12,13§} Jennifer T. Wang,¹⁴ Geraldine Seydoux,¹⁴ U. Serdar Tulu,¹⁵ Daniel P. Kiehart,¹⁵ Eric Betzig^{1||}



The Nobel Prize in Chemistry 2014

Eric Betzig, Stefan W. Hell, William E. Moerner

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The Nobel Prize in Chemistry 2014



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Eric Betzig

Prize share: 1/3



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Stefan W. Hell

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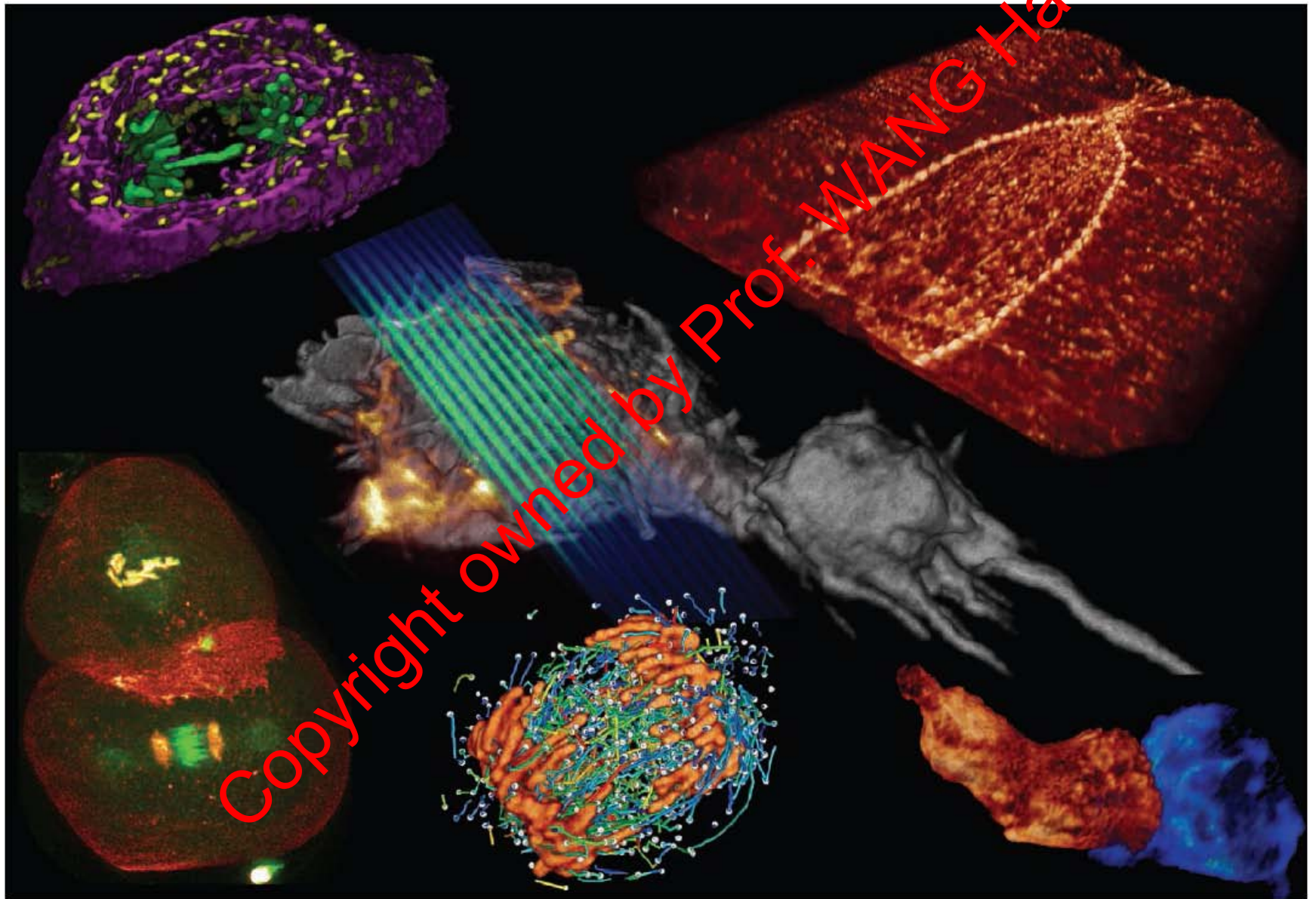
Photo: K. Lowder via
Wikimedia Commons, CC-
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William E. Moerner

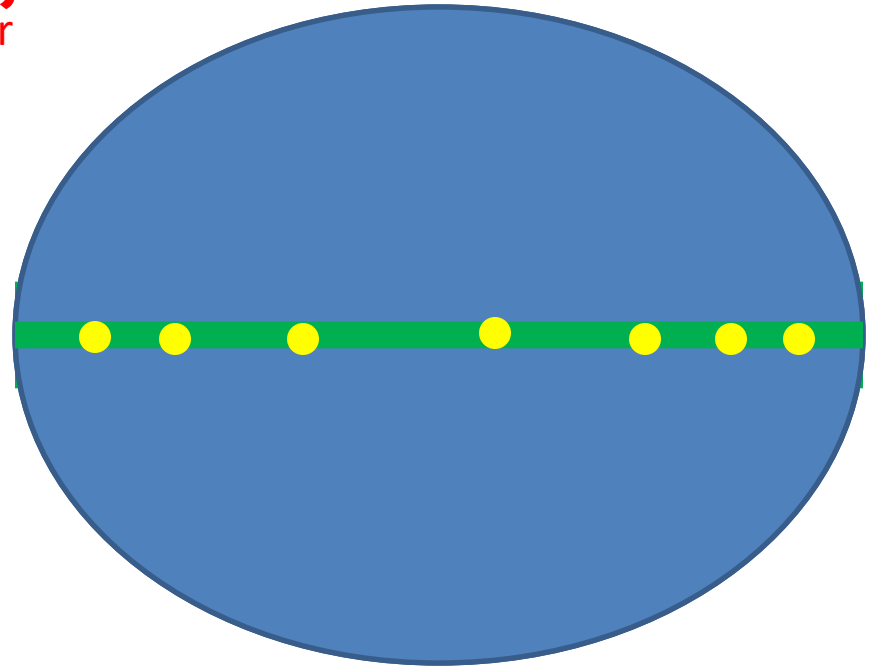
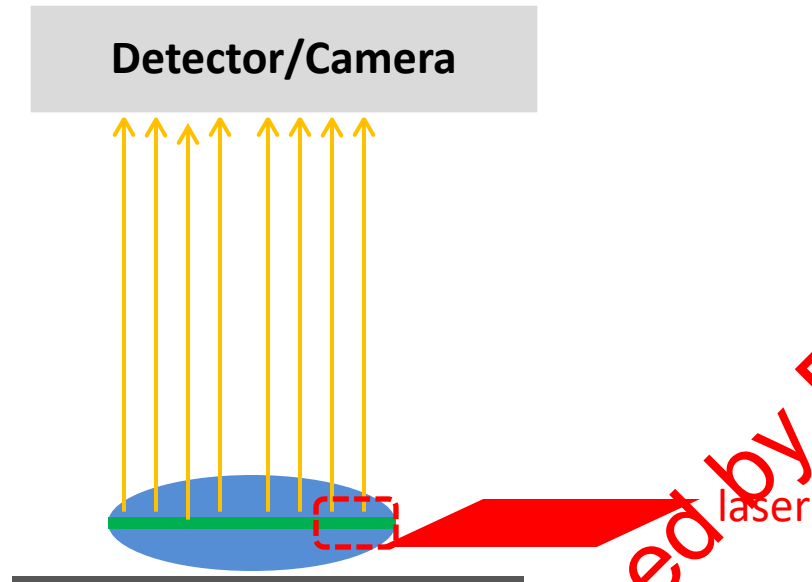
Prize share: 1/3

The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner *"for the development of super-resolved fluorescence microscopy"*.

Lattice Light-sheet Microscopy



Light-sheet microscopy



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Lattice Light-sheet Microscopy Advantages:

1. Tremendous high 4D resolution (nanoscale subcellular level)
2. Super fast imaging speed
3. Ultralow phototoxicity and photobleaching
4. Coverslide free!

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Watching mitosis

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Lattice Light-sheet Microscopy



Lattice Light-sheet Microscopy

