Advanced Cell Biology

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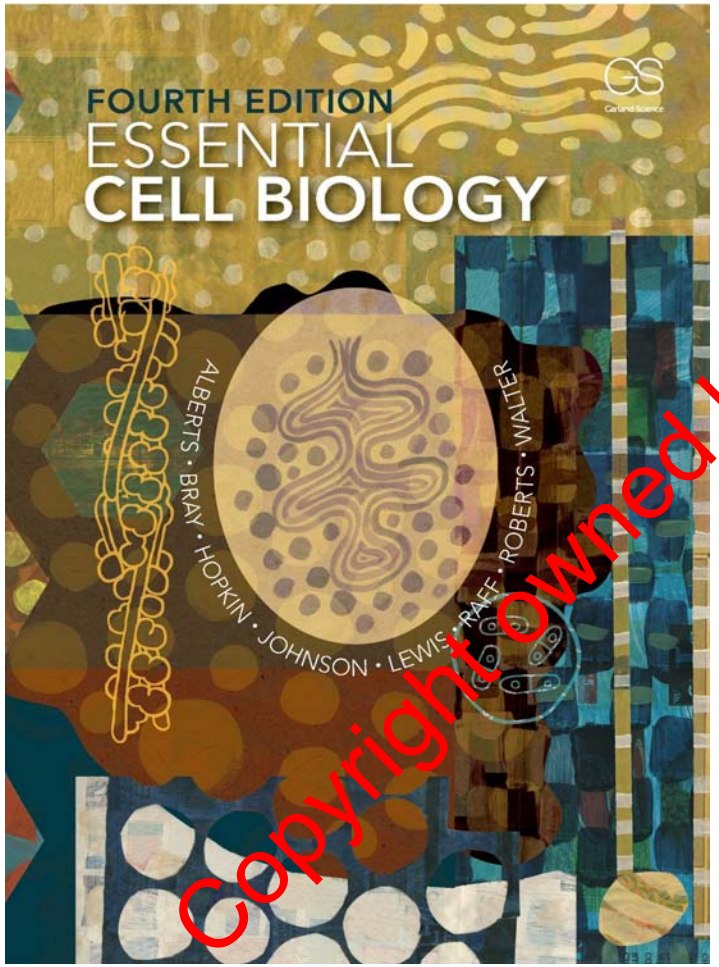
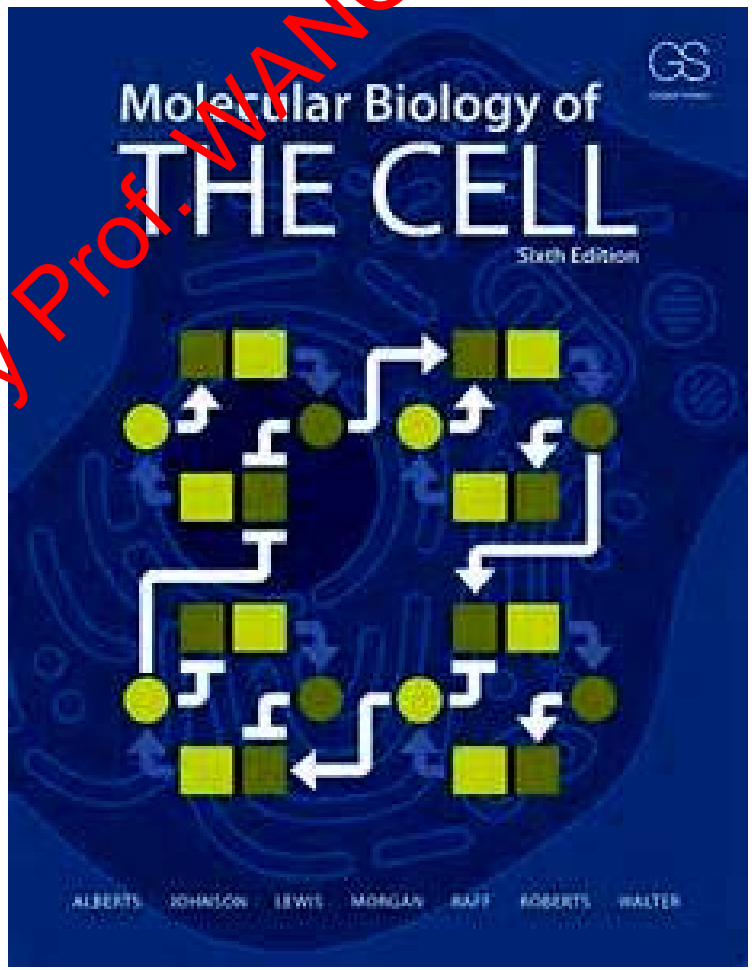
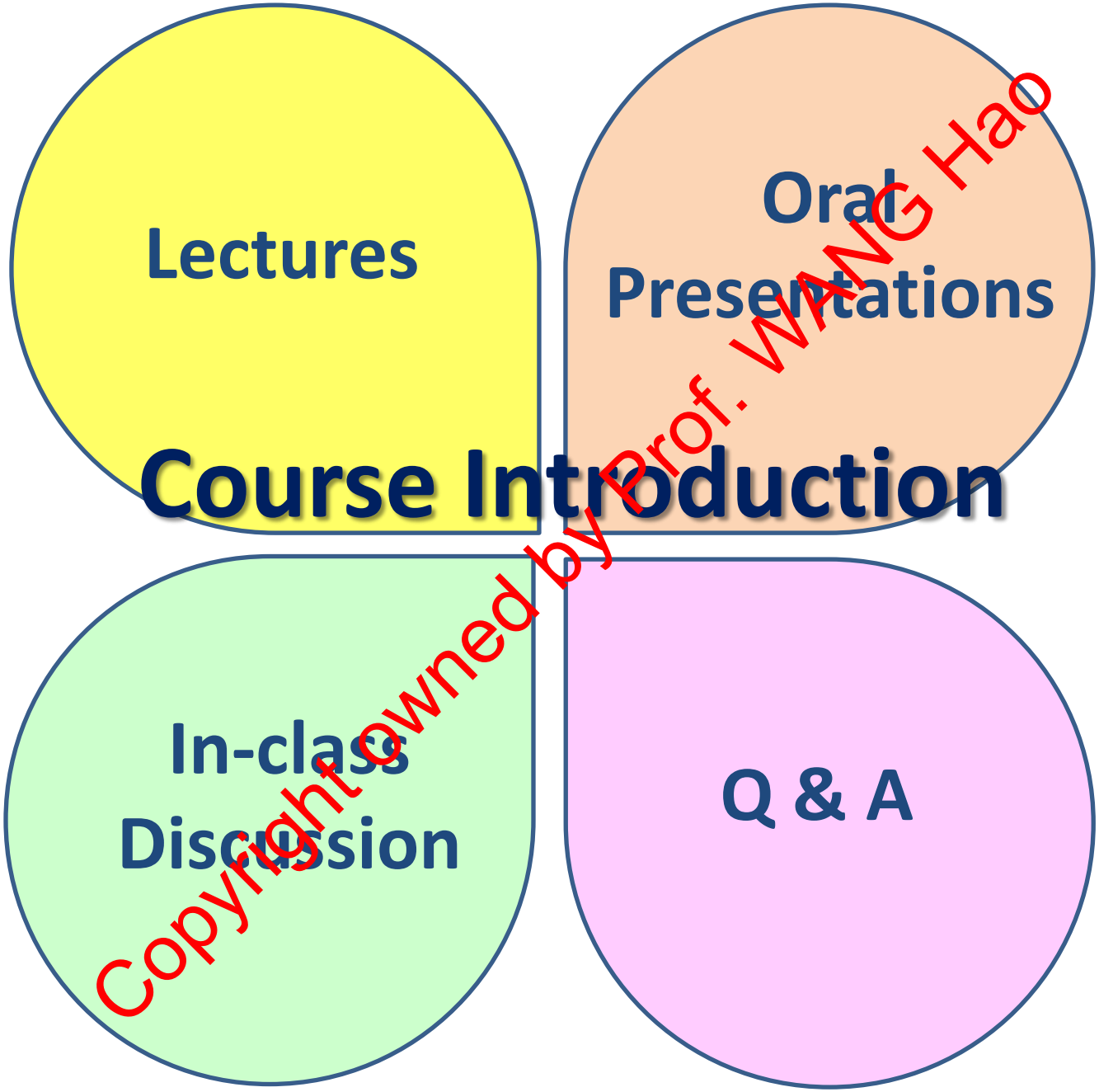
In-class language: English

Reference Books

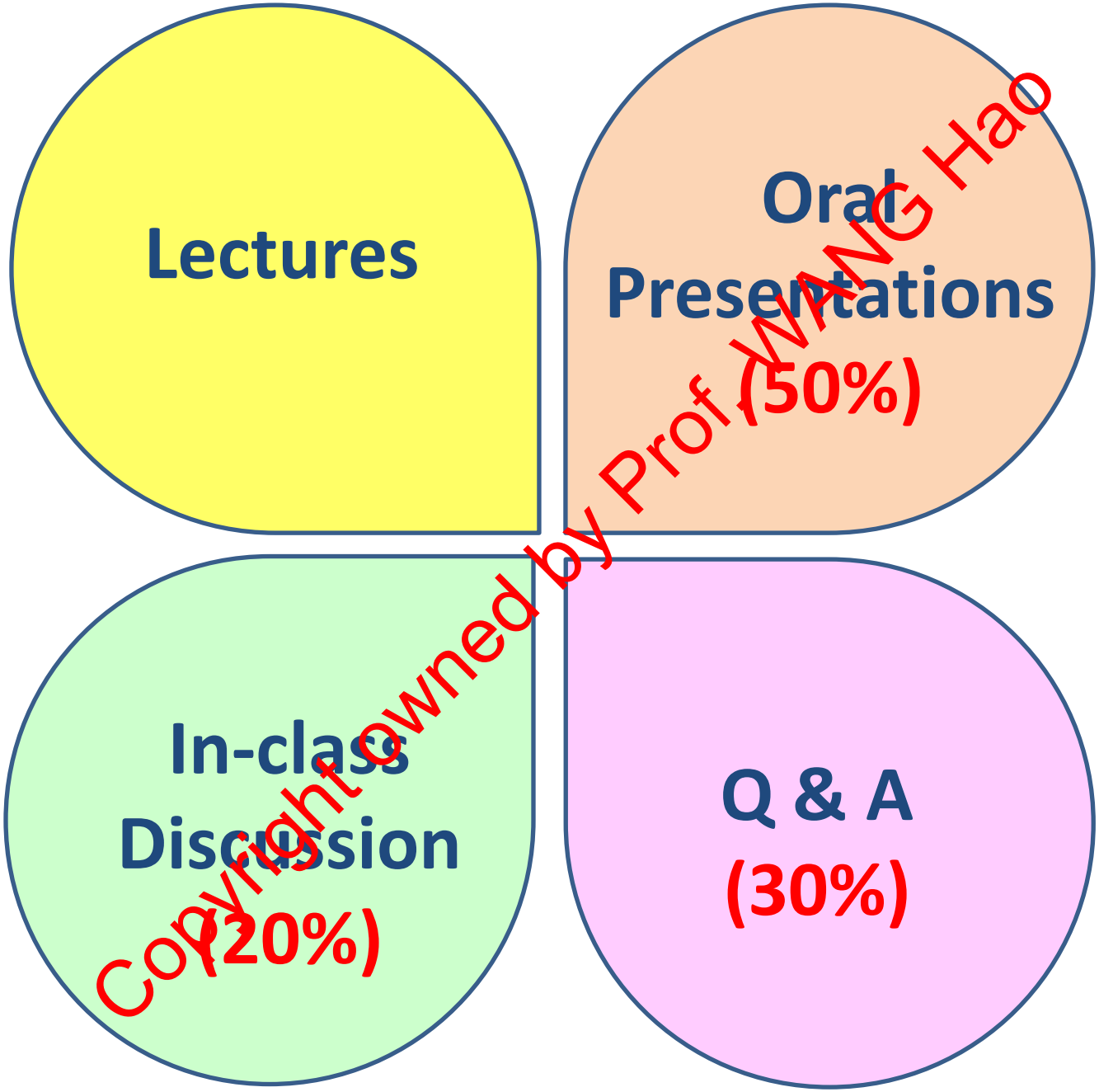






Evaluations

No paper-based examination!



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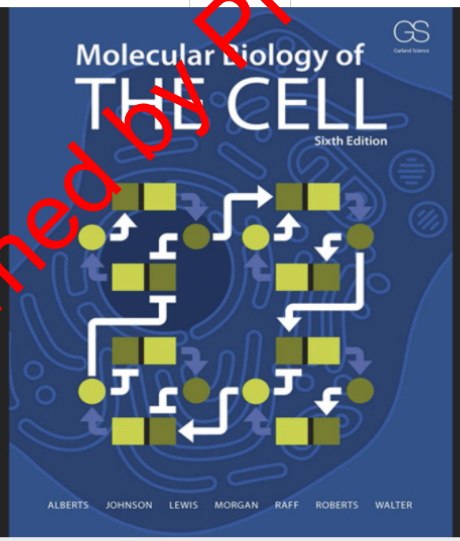
Advanced Cell Biology

About the Course | Contents | Learning Recourses | Questions & Answers | Photo Albums

Reference Book 1



News +MORE


关于2020年华南农业大学研究生教育创新计划项目拟立项名单的公示

Reading Recommendations +MORE

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

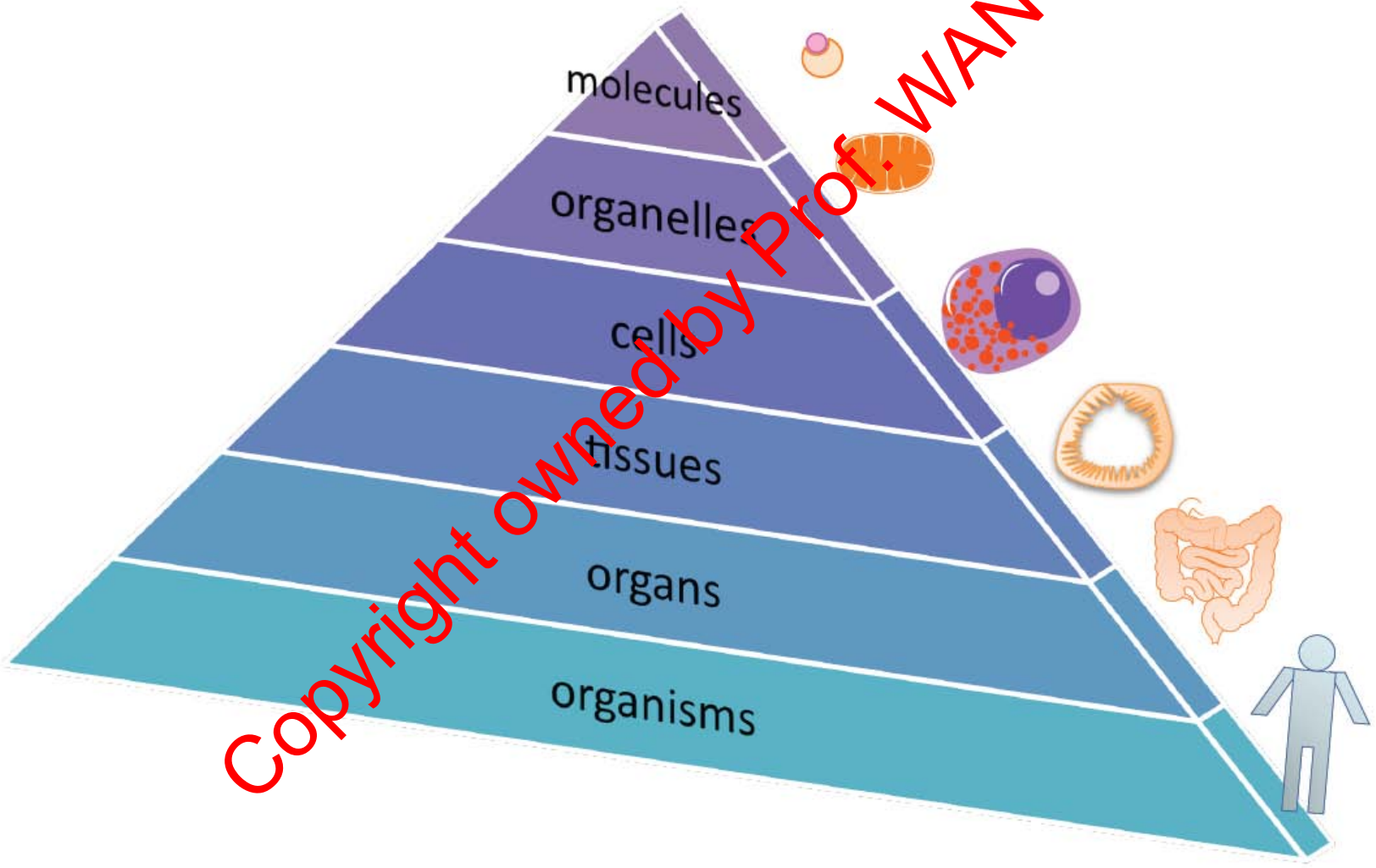
21 Jul 2020

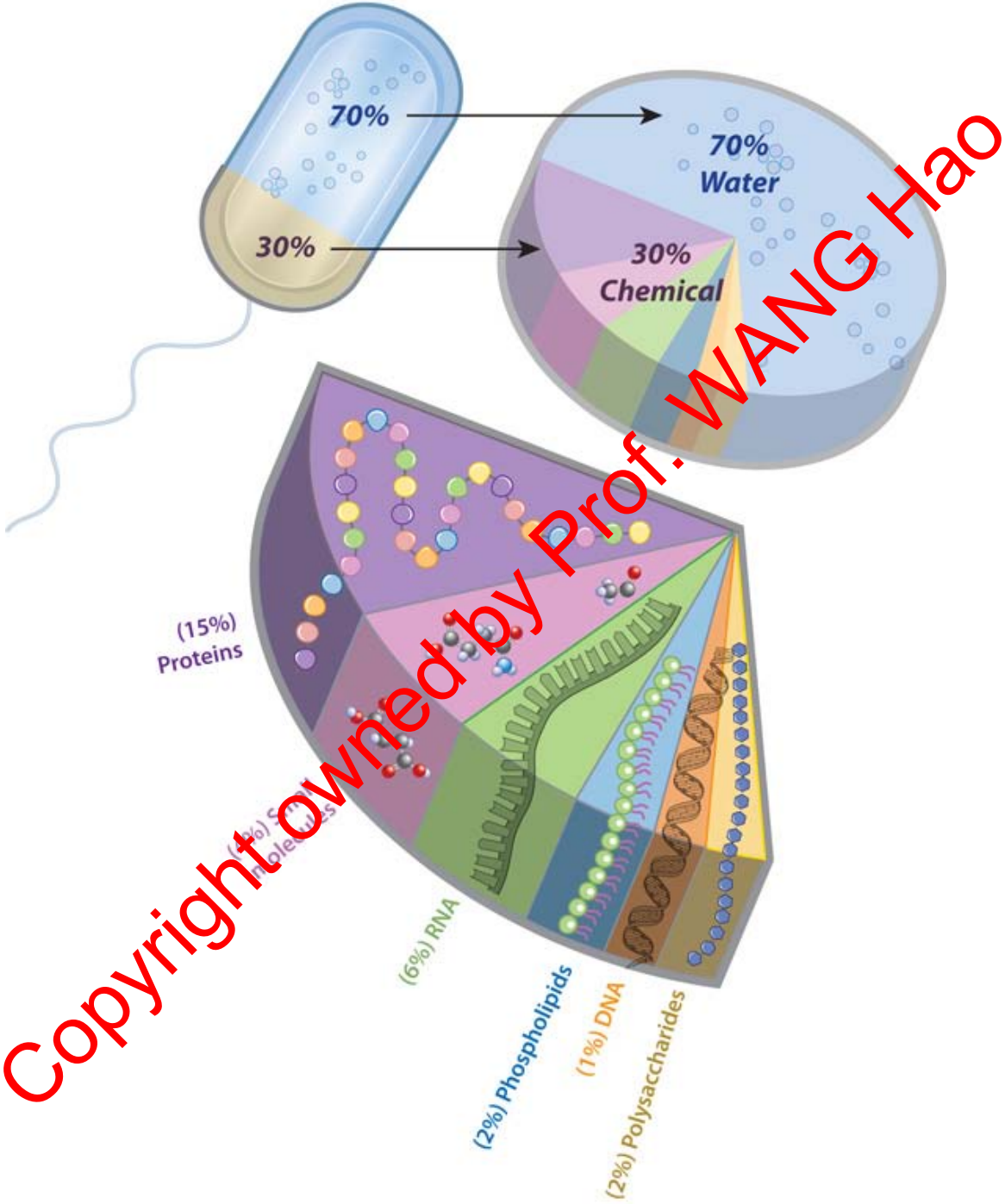
5. Communication by Extracellular Vesicles:Where We Are and Where We Need to Go (Cell)

I. General Introduction of Cell Biology

What is a cell?

Cell: The fundamental functional element of life





Where do cells coming from?

The First Cell – Hypothesis

Early Atmosphere

H_2 H_2O NH_3 CH_4 +

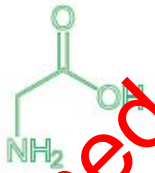
Electric Storm

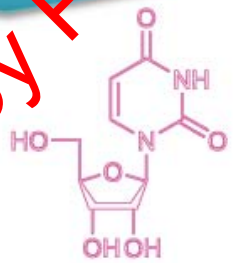
UV

HCN

Chemical Reactions

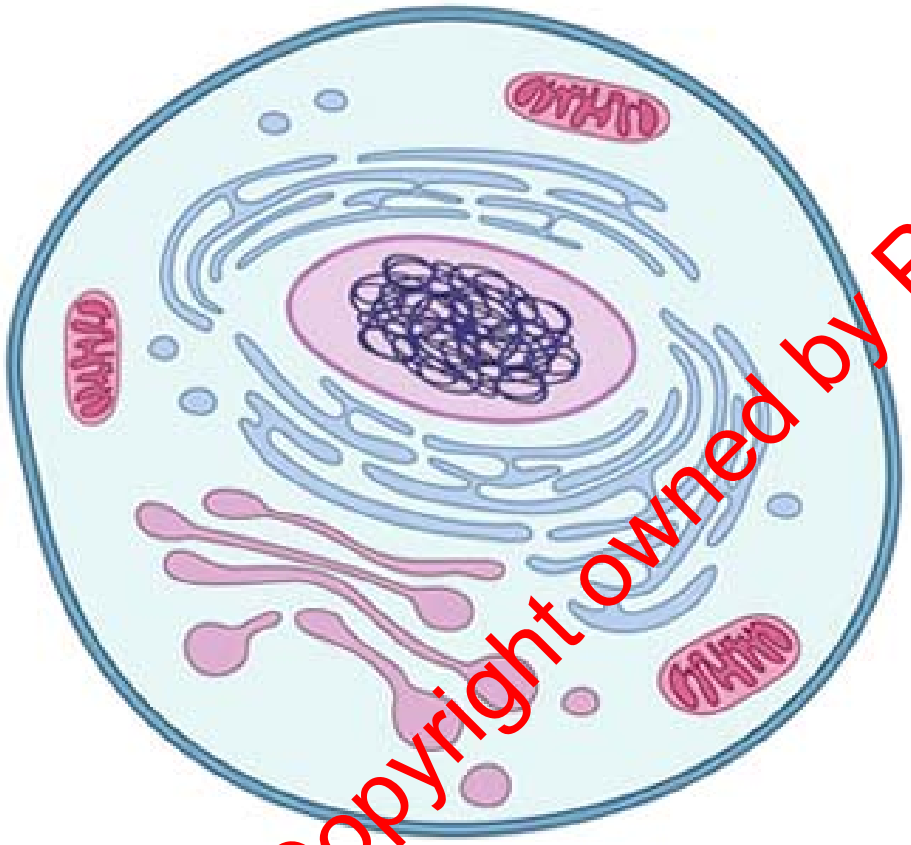
Amino Acid



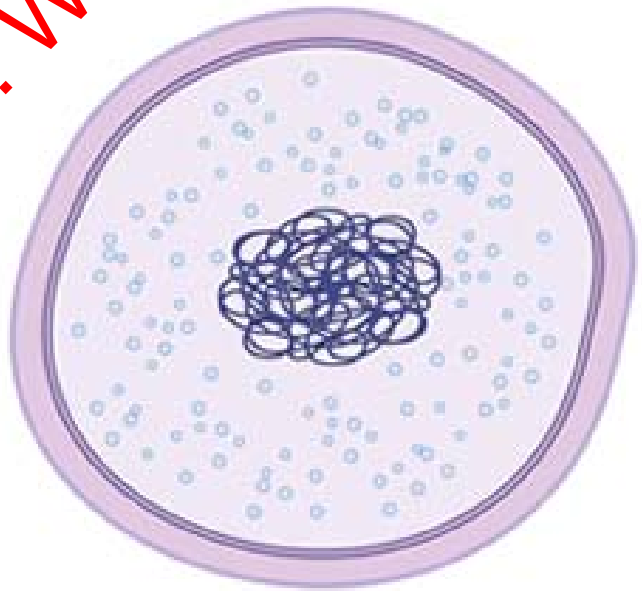


Nucleoside

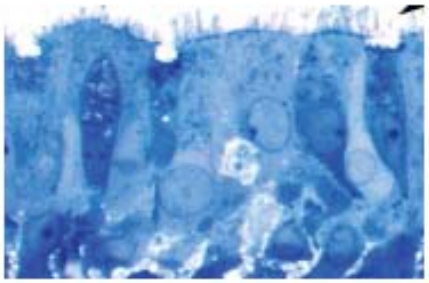
Eukaryotic Cell



Prokaryotic Cell

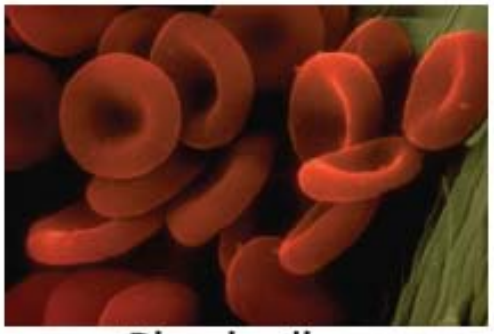


Cell specialization/ differentiation

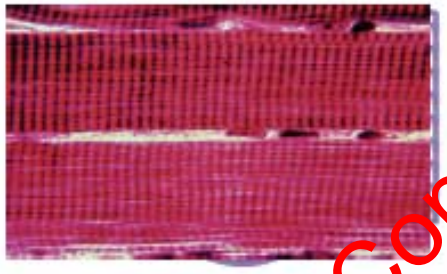


Epithelial cells

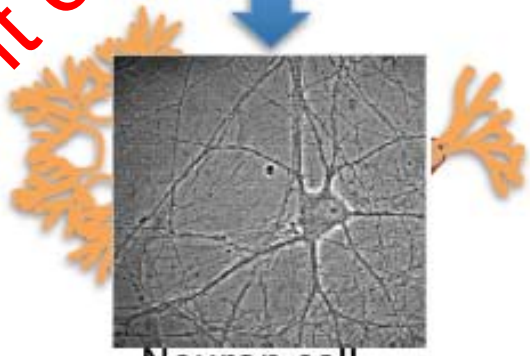
Fertilized egg



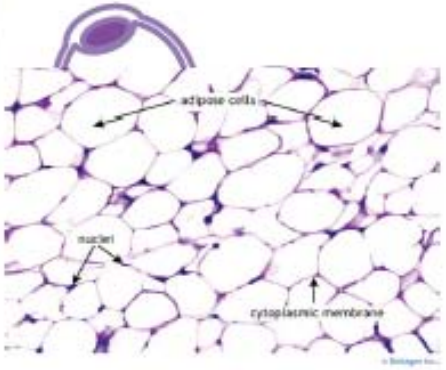
Blood cells



Muscle cells



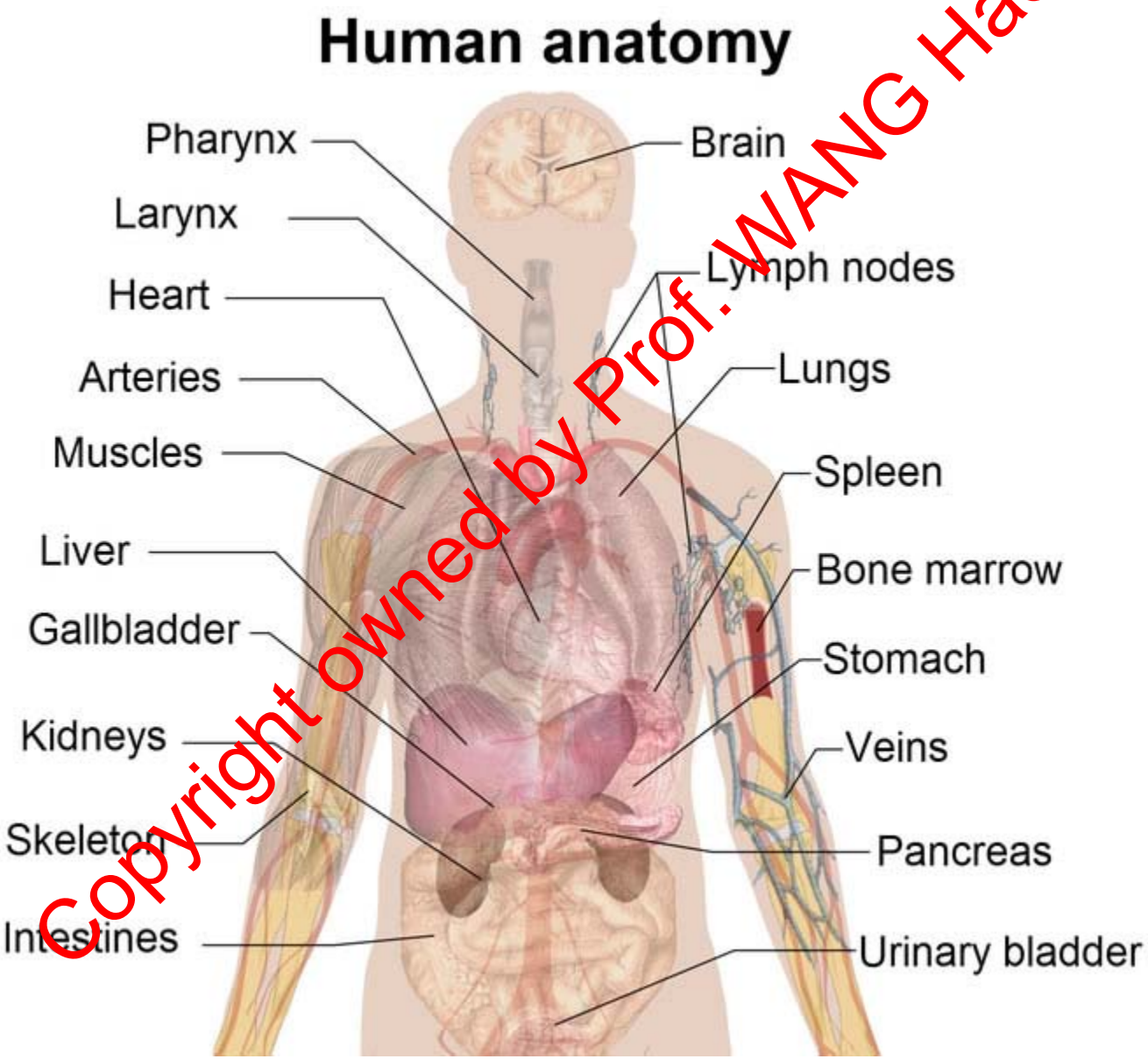
Neuron cell



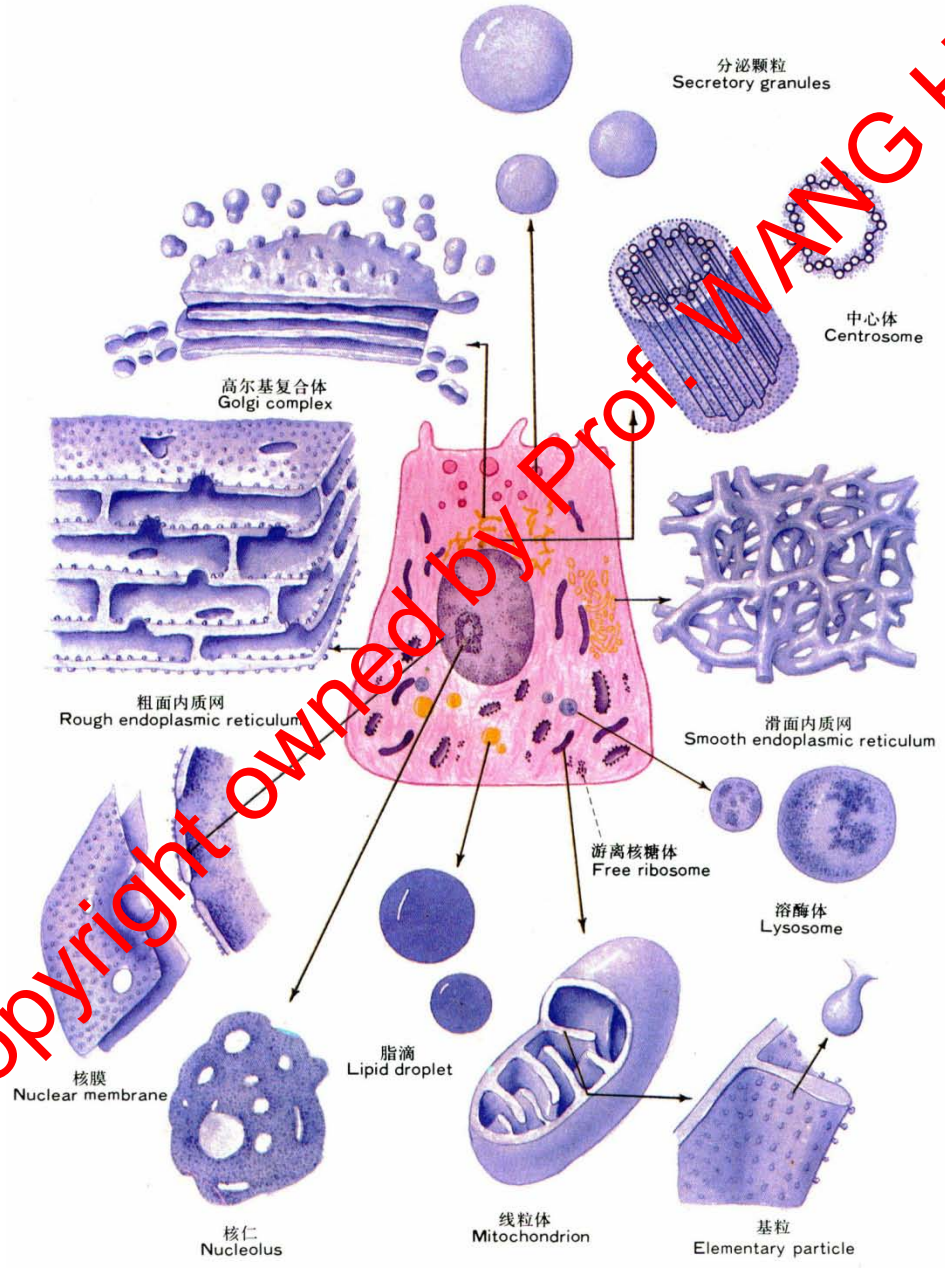
Adipose cells

How do cells work?

Organs in Human

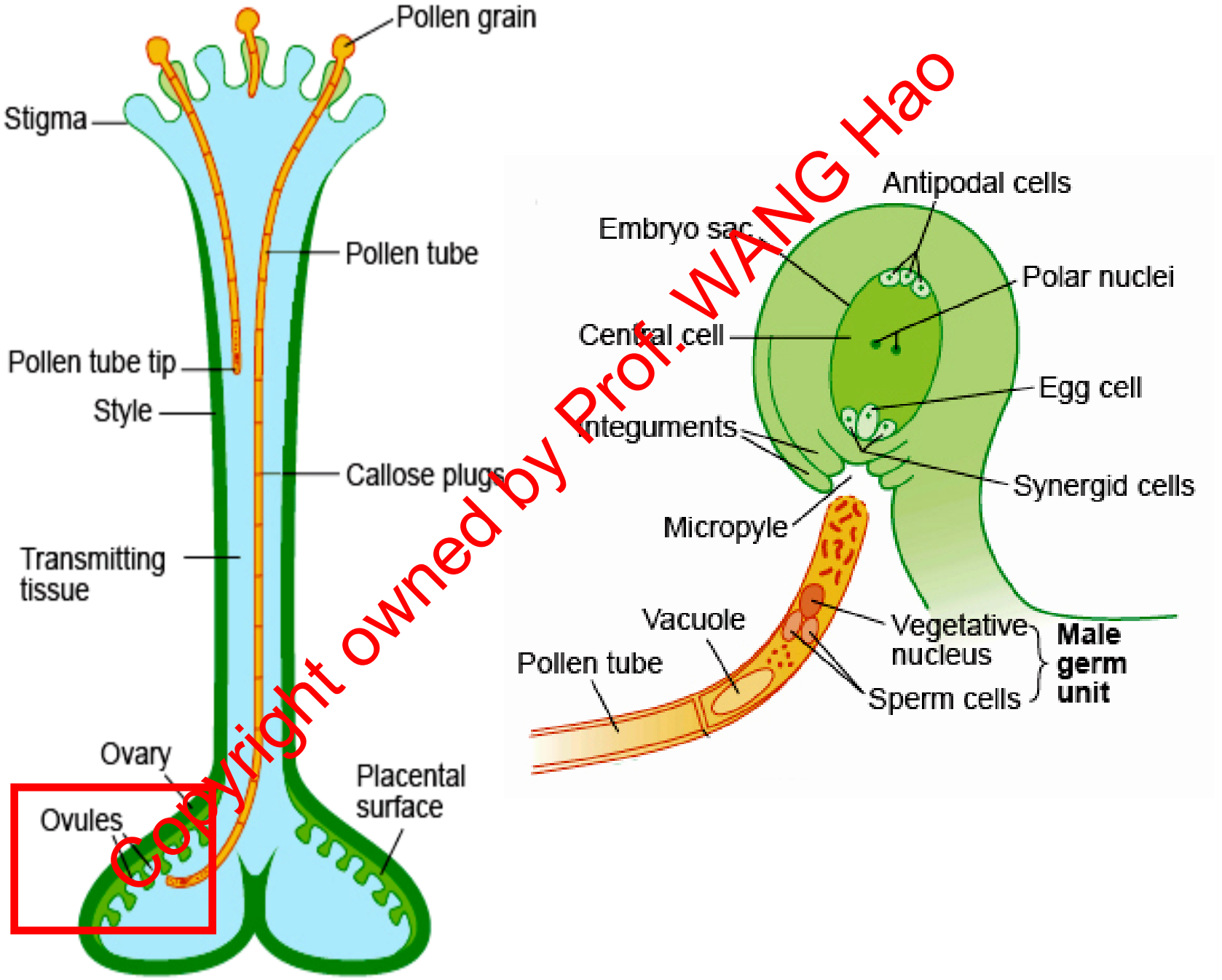


Organelles in a cell



What is cell biology about?

A rapid growing lily pollen tube



Pollen and pollen tube

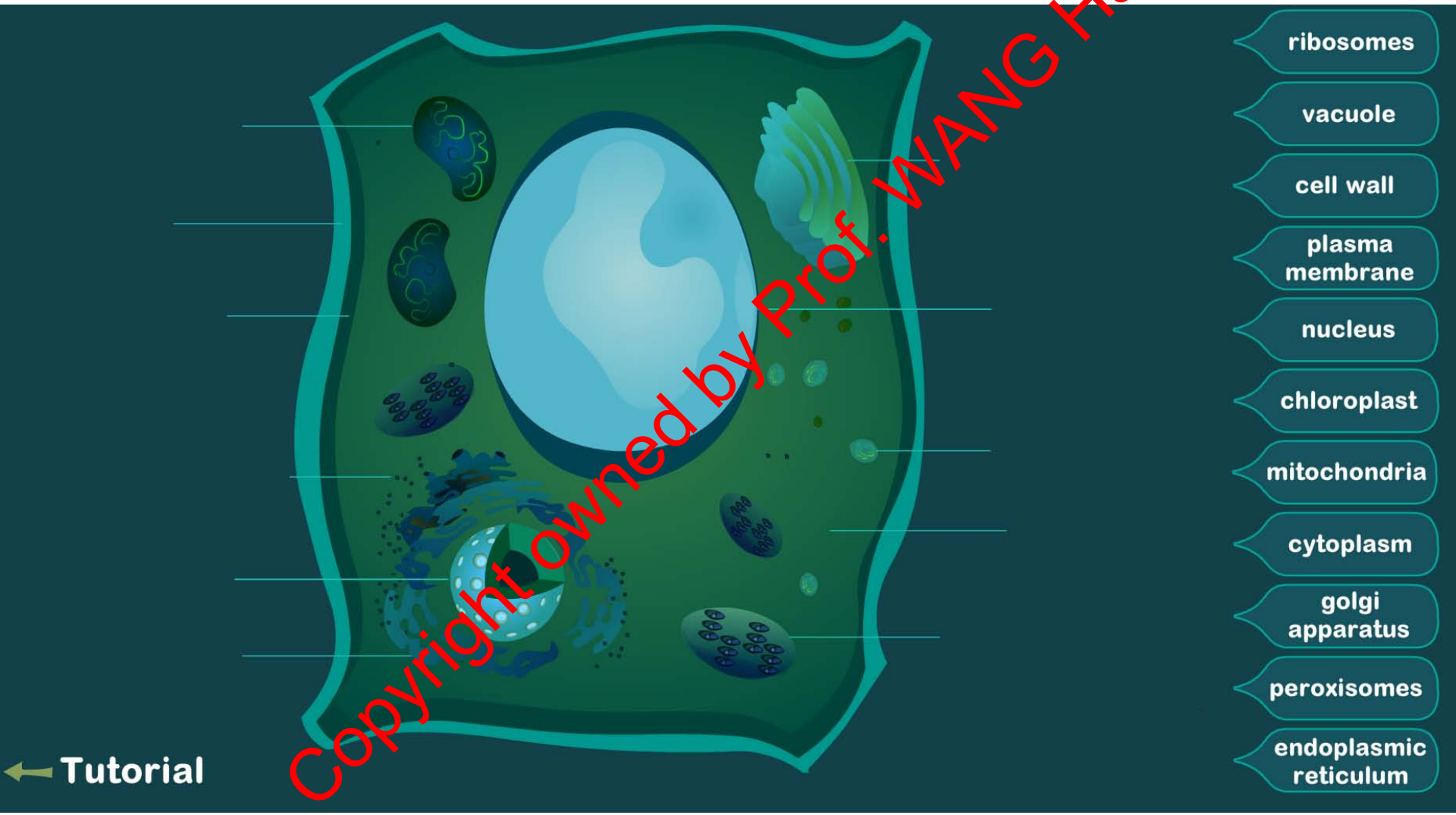
Cell cytokinesis

Molecular Mechanism of Synaptic Function

New Limb Regeneration

What is cell biology about?

Organelle Identification in Plant Cell



II. Visualizing Cells (Bioimaging)

Visualizing Cells (Bioimaging)

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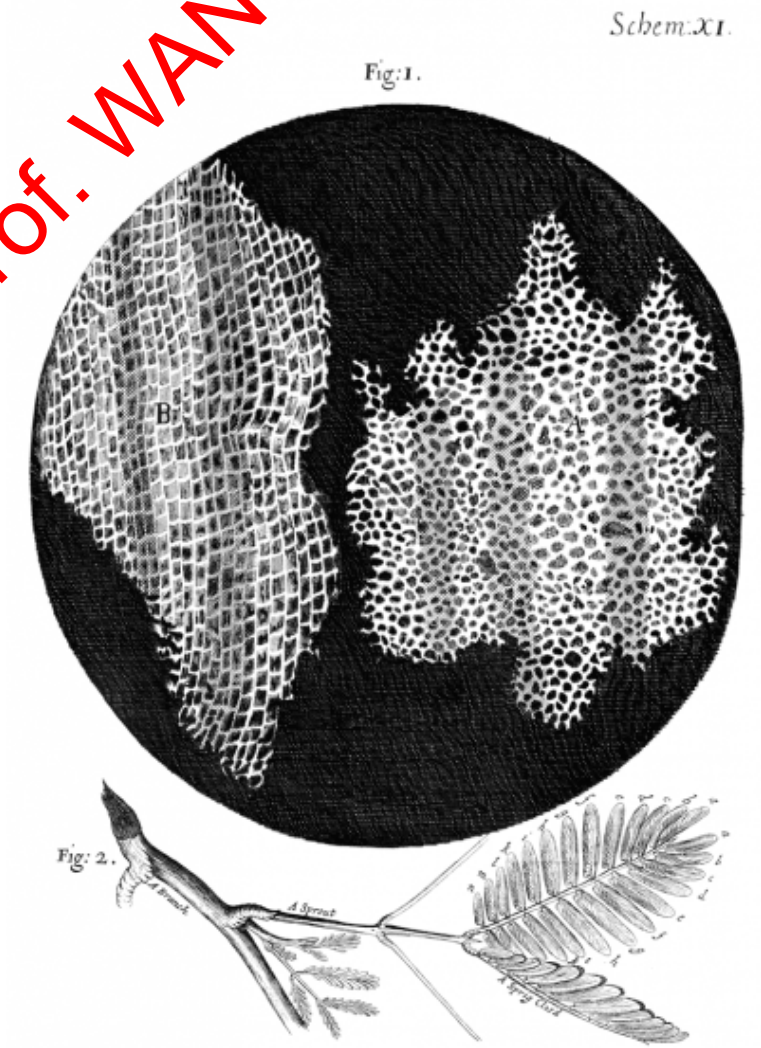
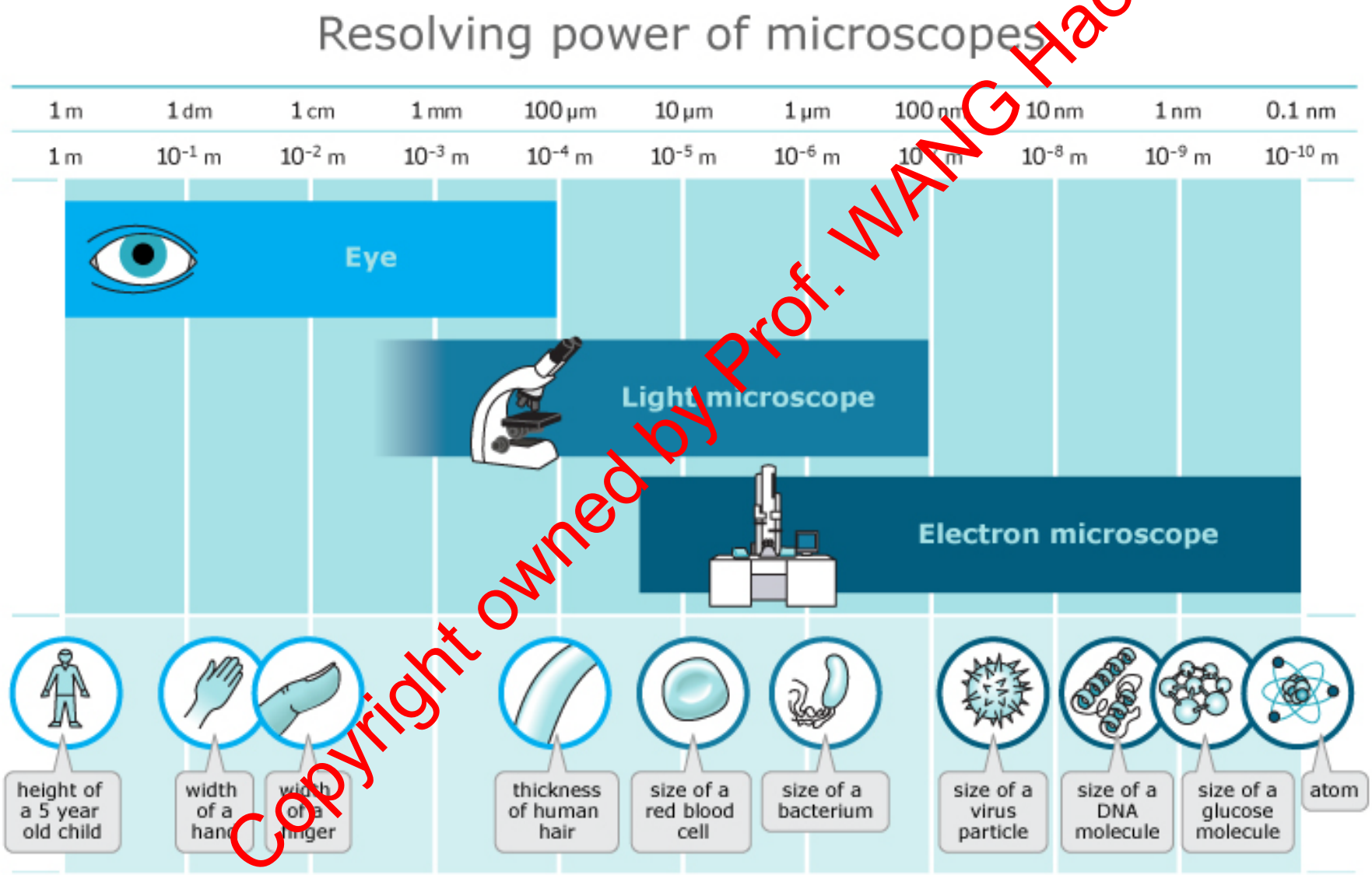
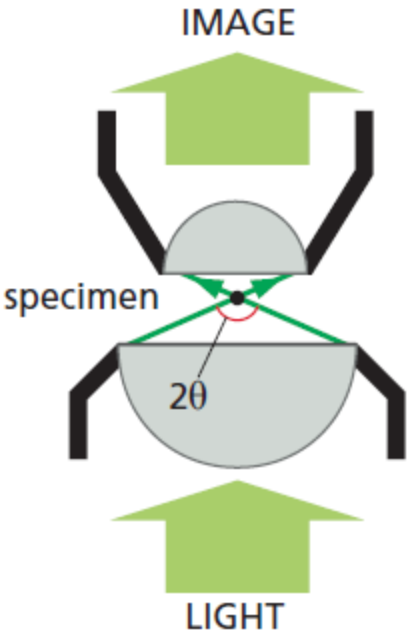


Visualizing Cells (Bioimaging)



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 μm 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

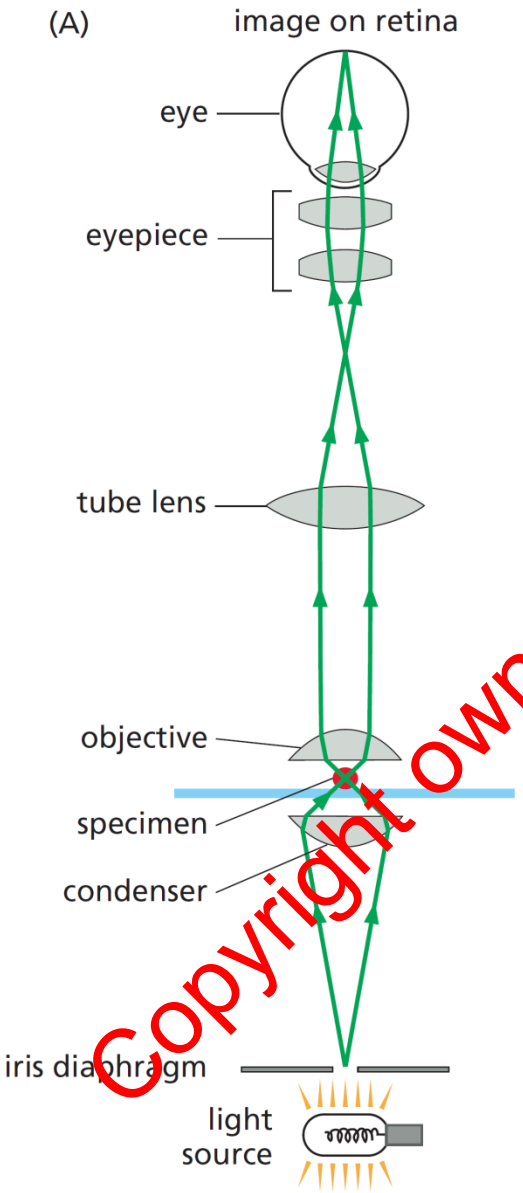
Resolution allows us to see

objects as separate

from one another

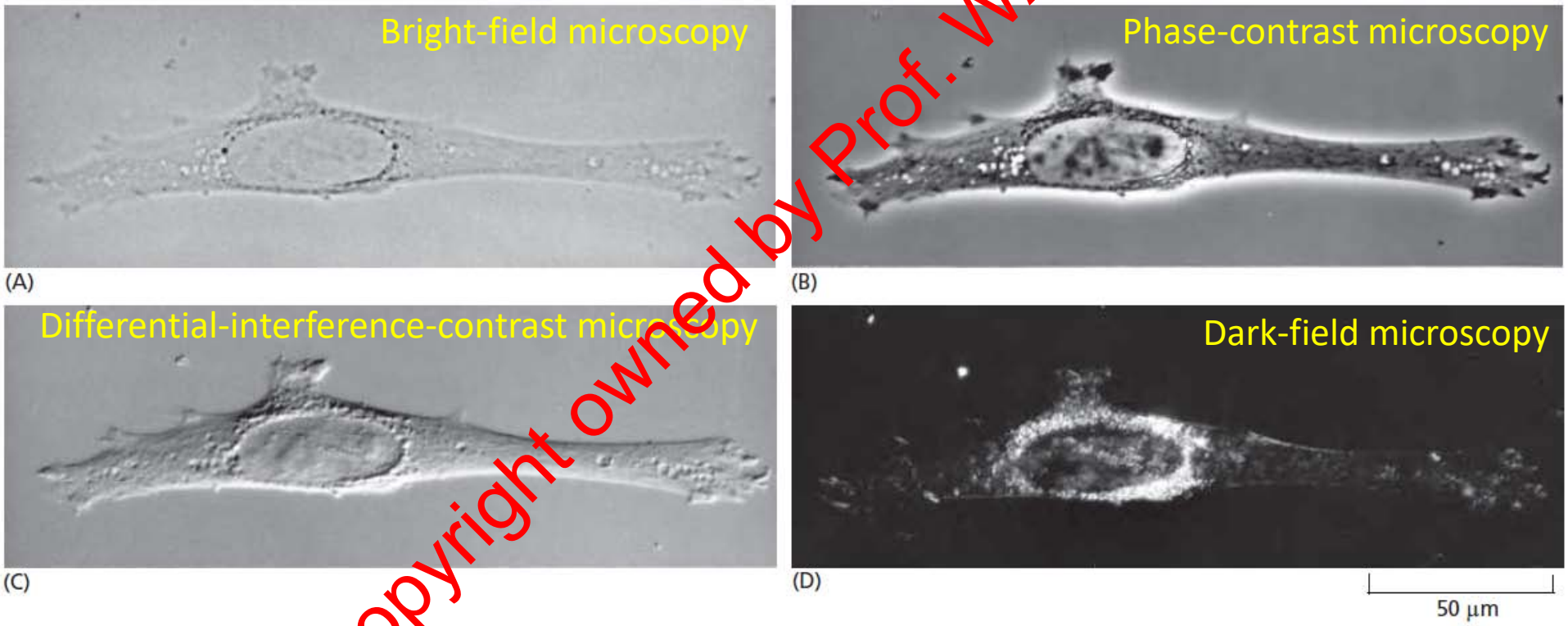
Image resolution

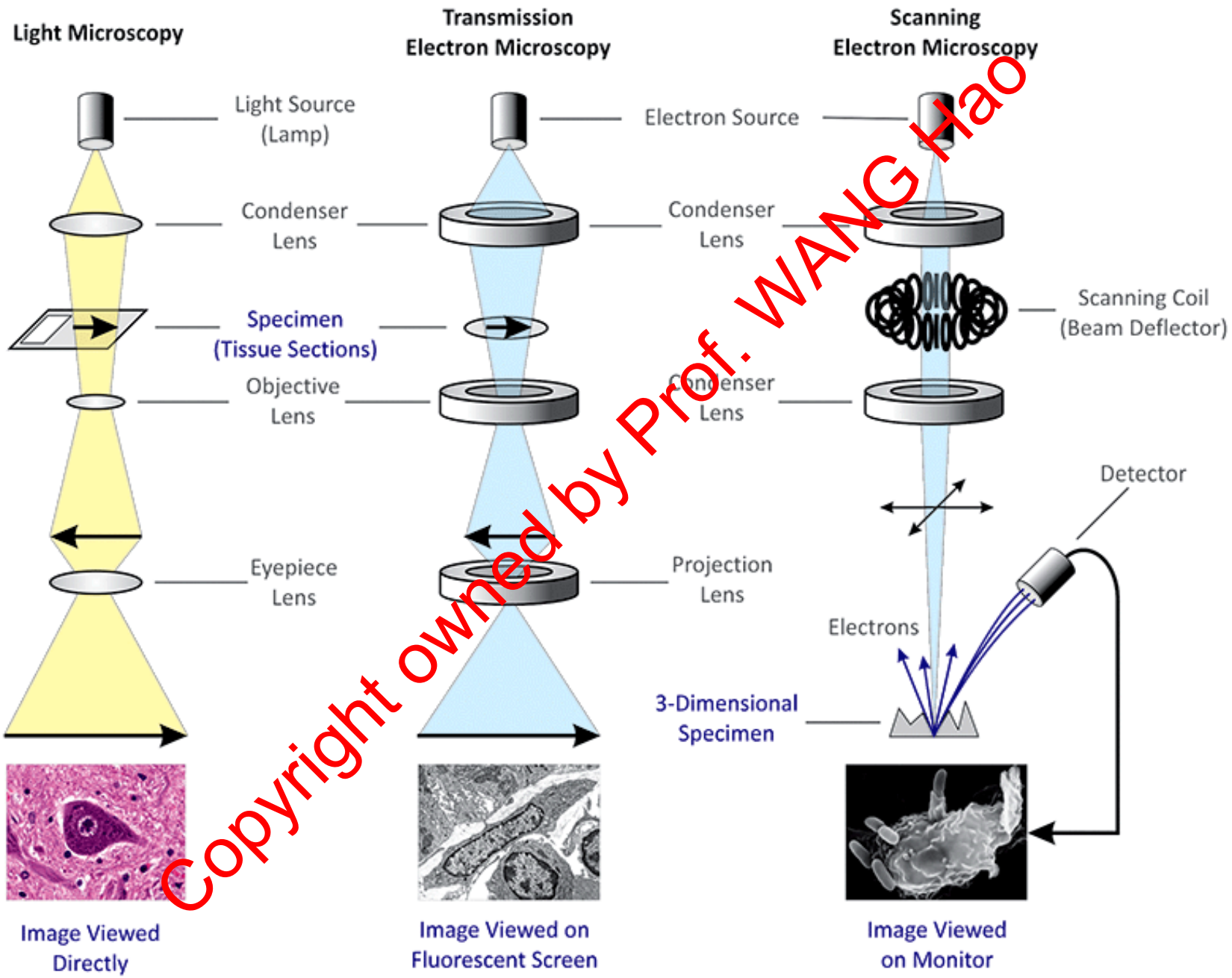
Visualizing Cells-Light Microscopy



Visualizing Cells-Light Microscopy

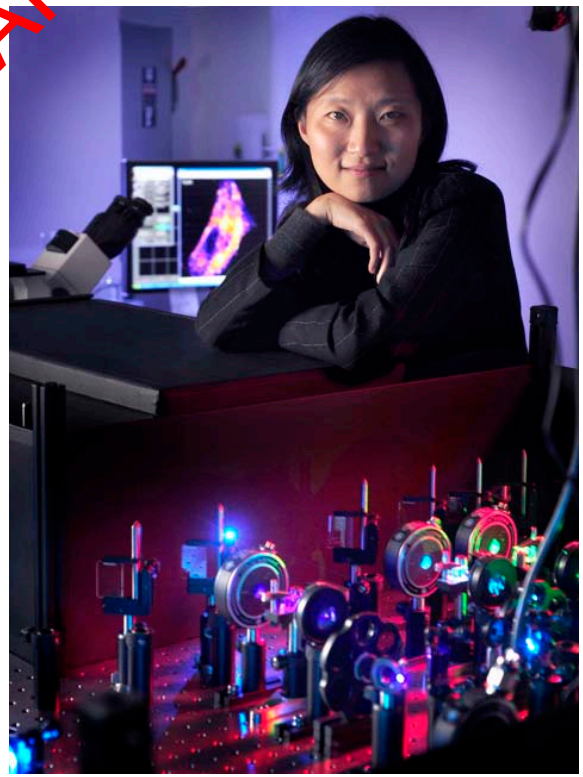
Four types of light microscopy





WHICH MICROSCOPE?

Visualizing Cells Using Laser



The Nobel Prize in Chemistry 2008

Osamu Shimomura, Martin Chalfie, Roger Y. Tsien

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The Nobel Prize in Chemistry 2008

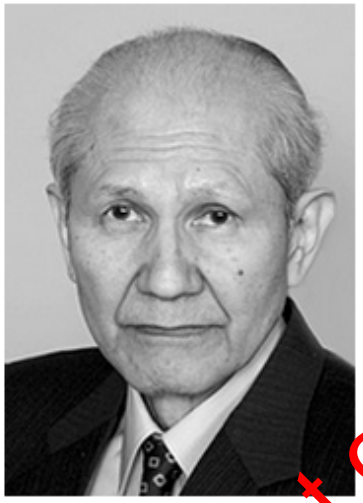


Photo: U. Montan

Osamu Shimomura

Prize share: 1/3

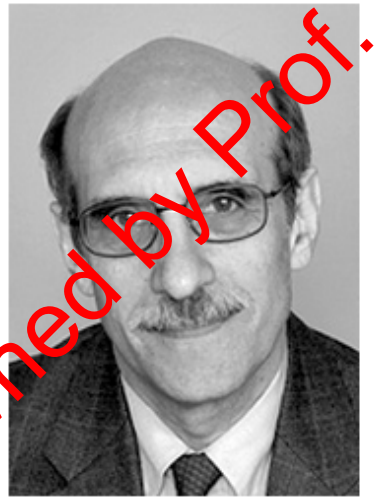


Photo: U. Montan

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"*.

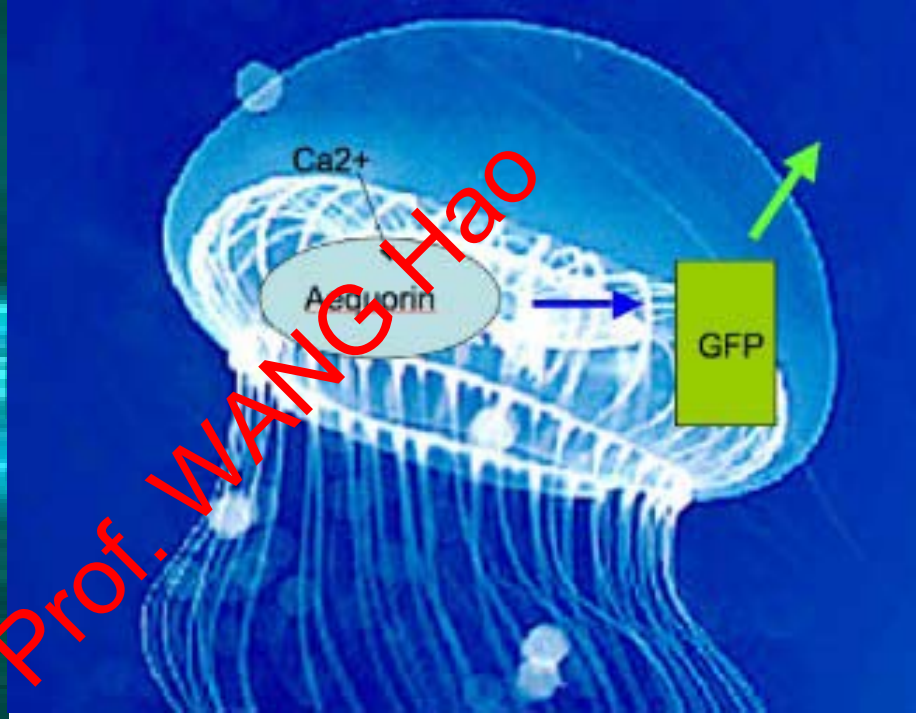
Roger Tsien

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.

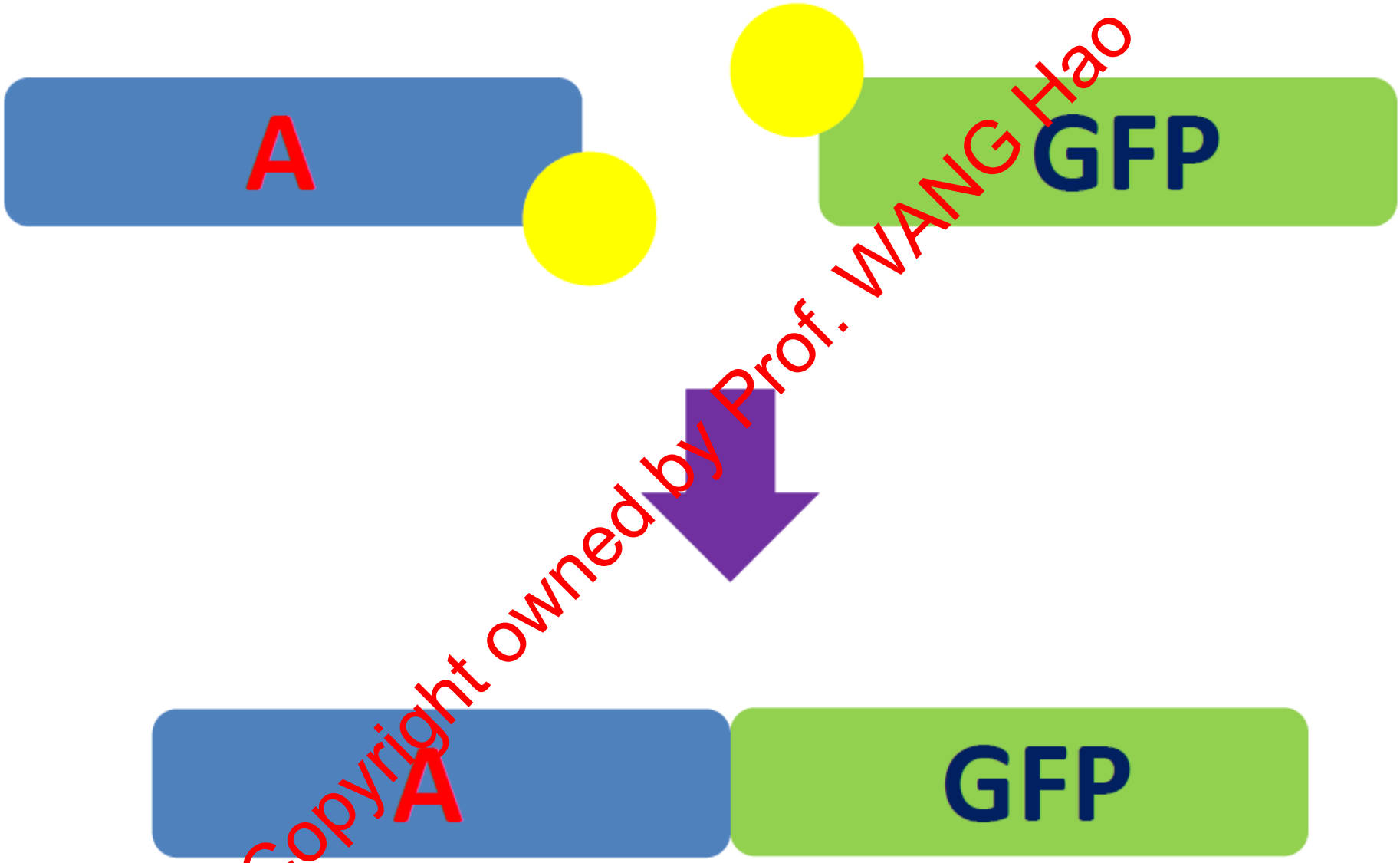
"I've always been attracted to colors," Tsien told the San Diego Union-Tribune in 2008. "Color helps make the work more interesting and endurable. It helps when things aren't going well. If I had been born colorblind, I probably never would have gone into this."

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

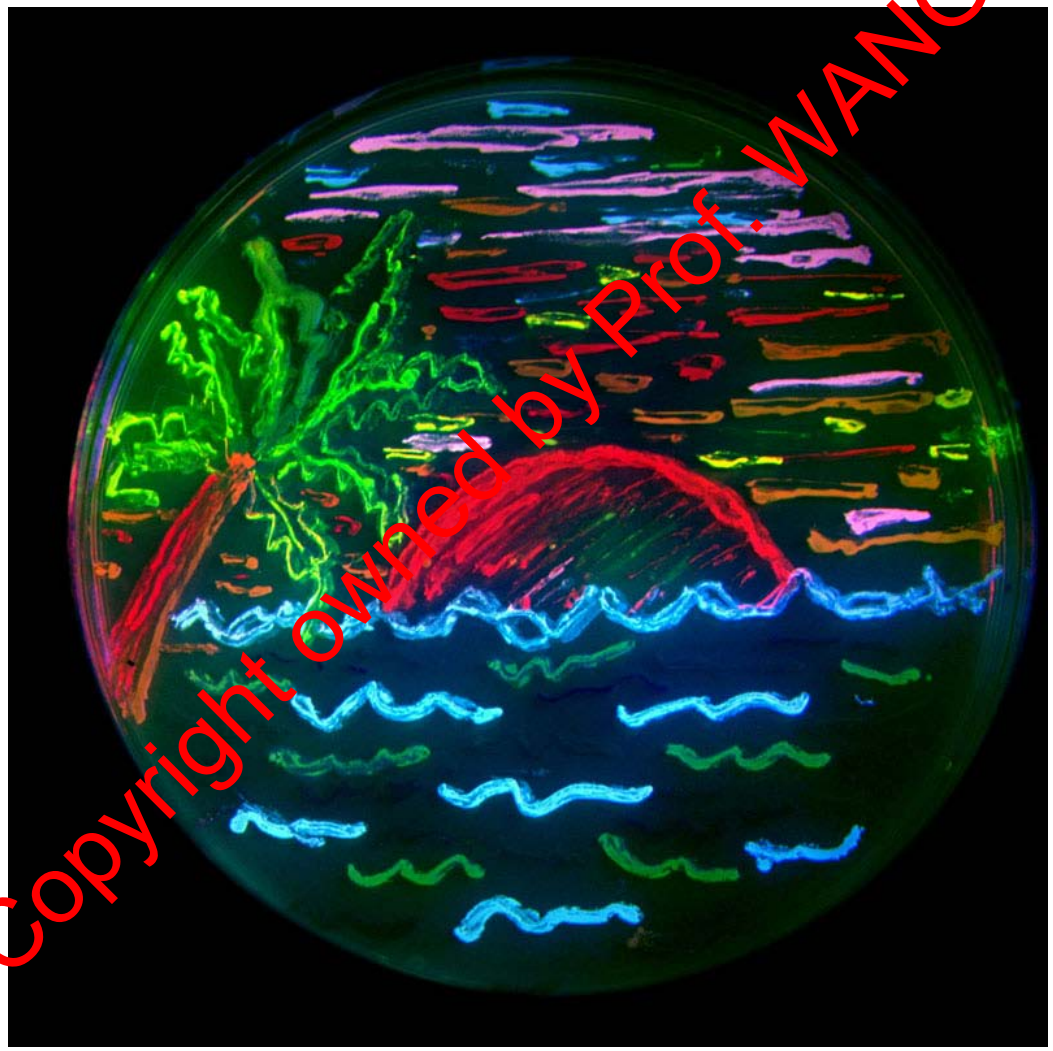


GFP＝Green Fluorescence Protein

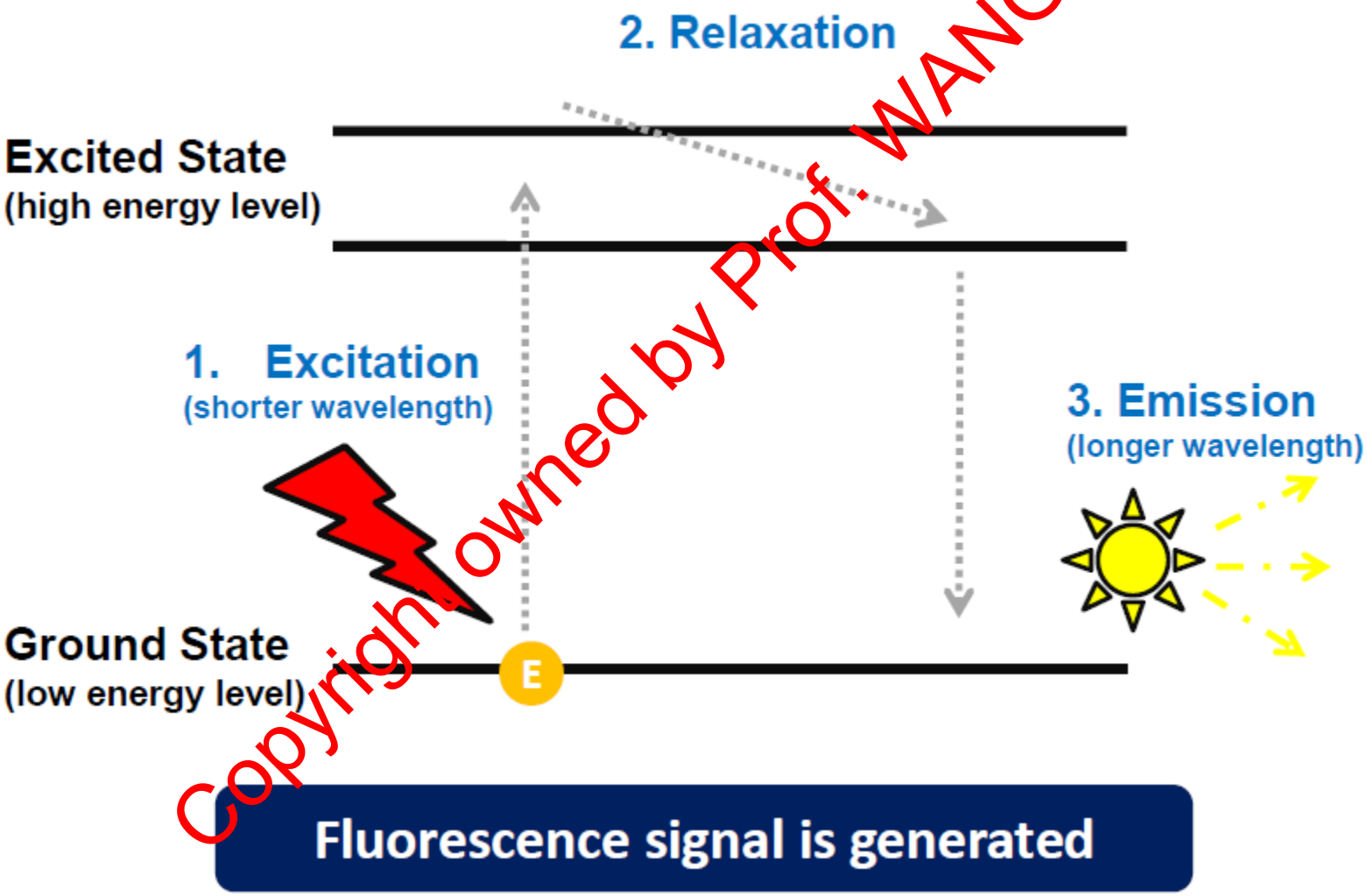
Green fluorescent proteins (GFP) is found and derived from jelly fish first



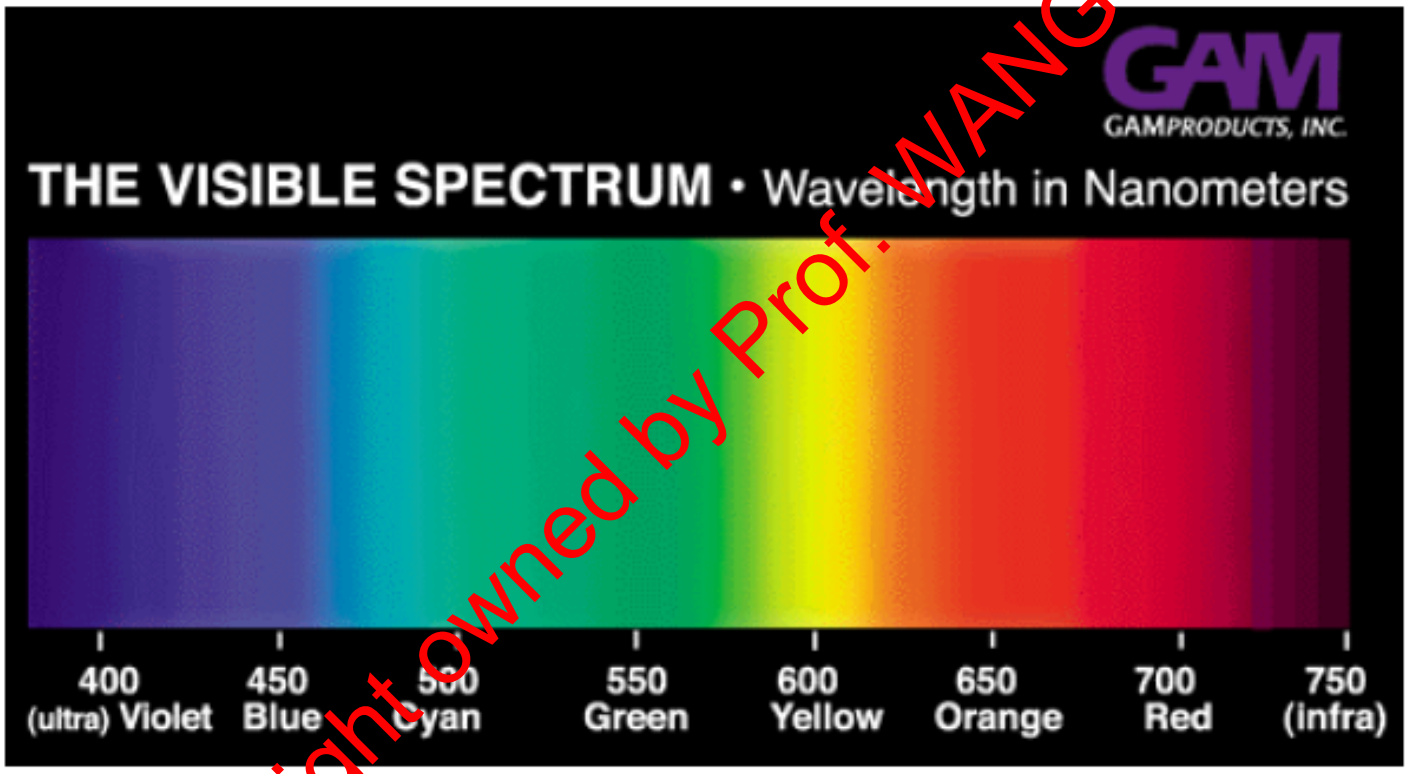
What is fluorescence?



What is fluorescence?

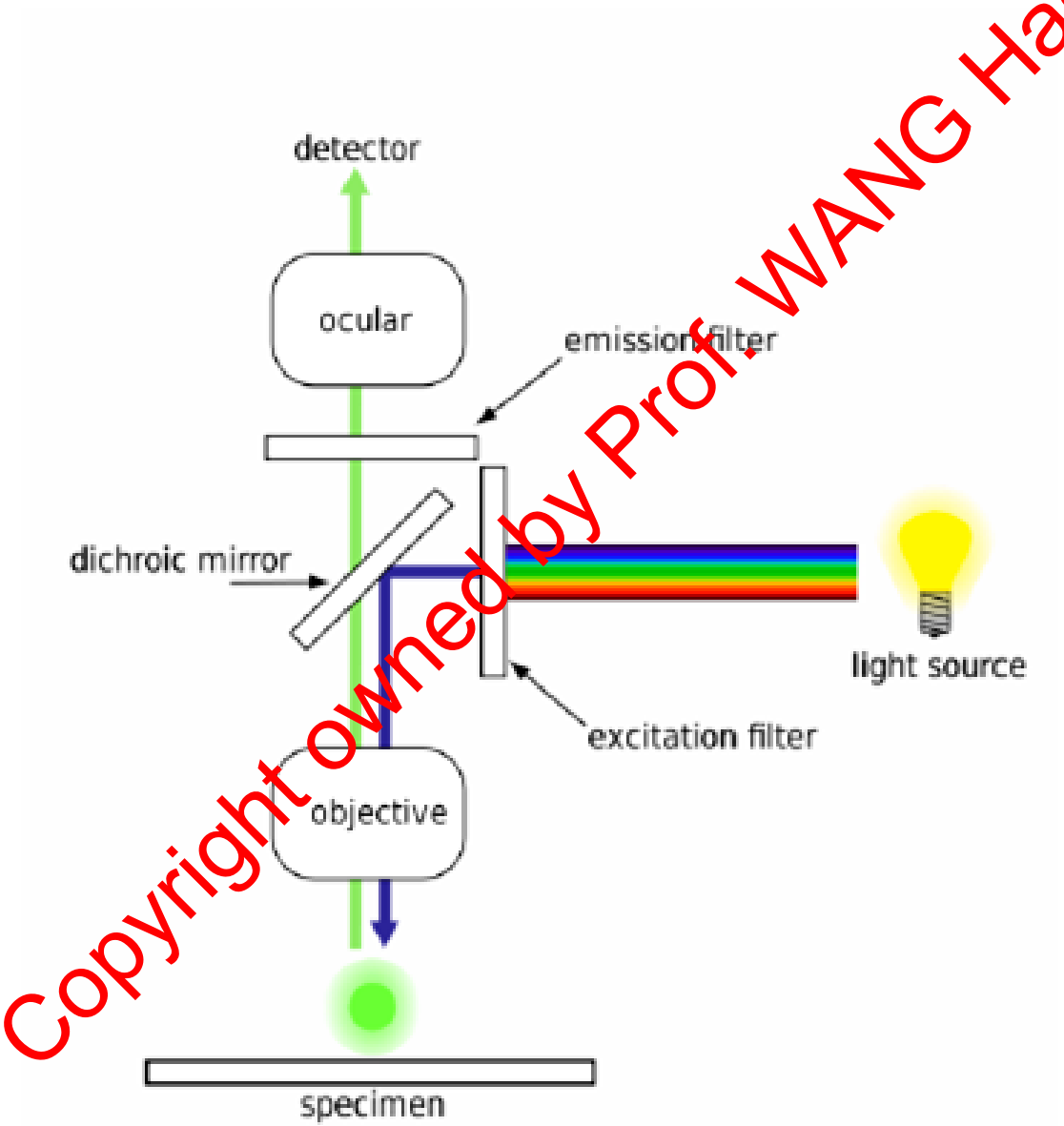


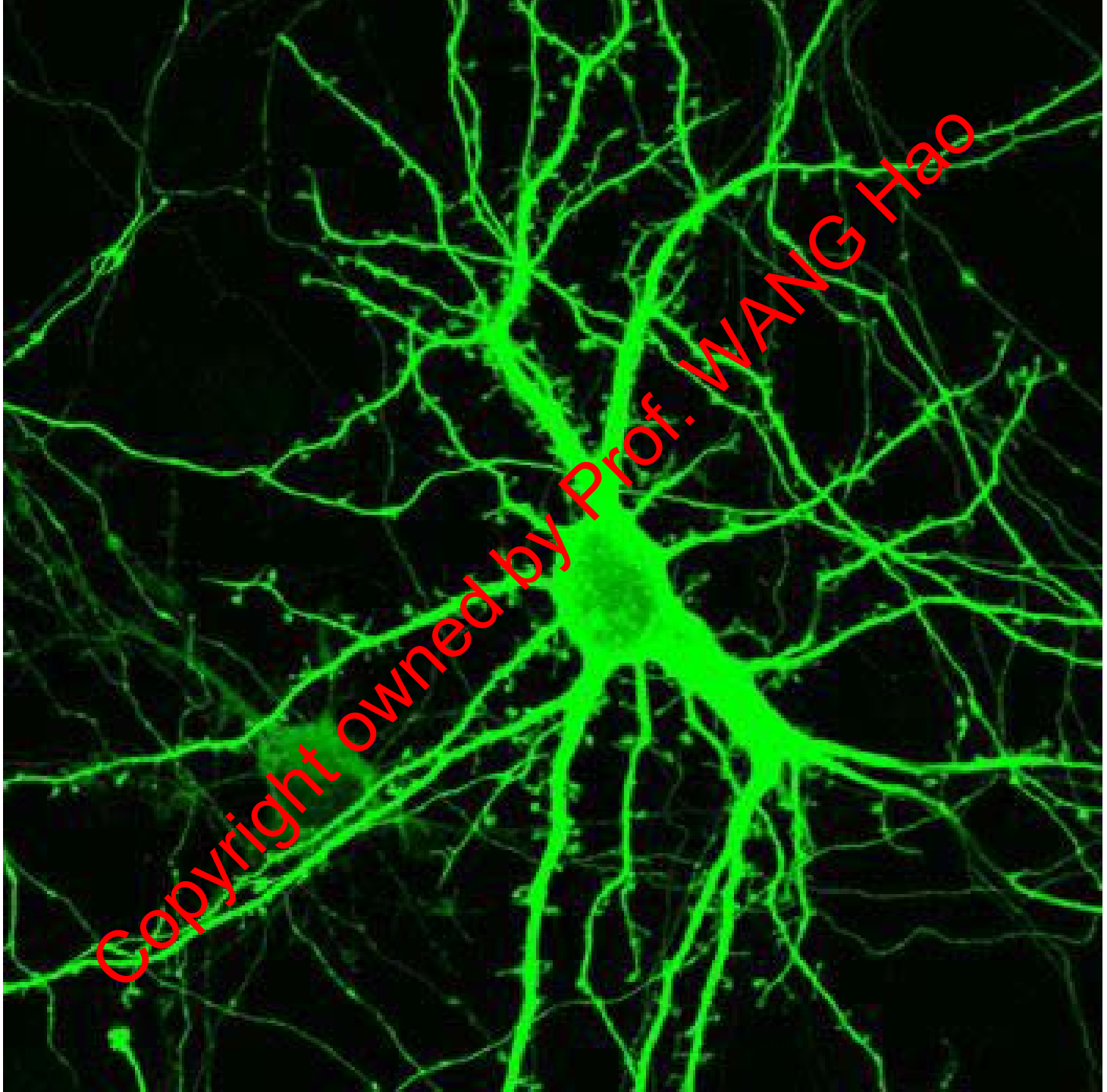
Fluorescence Excitation and Emission

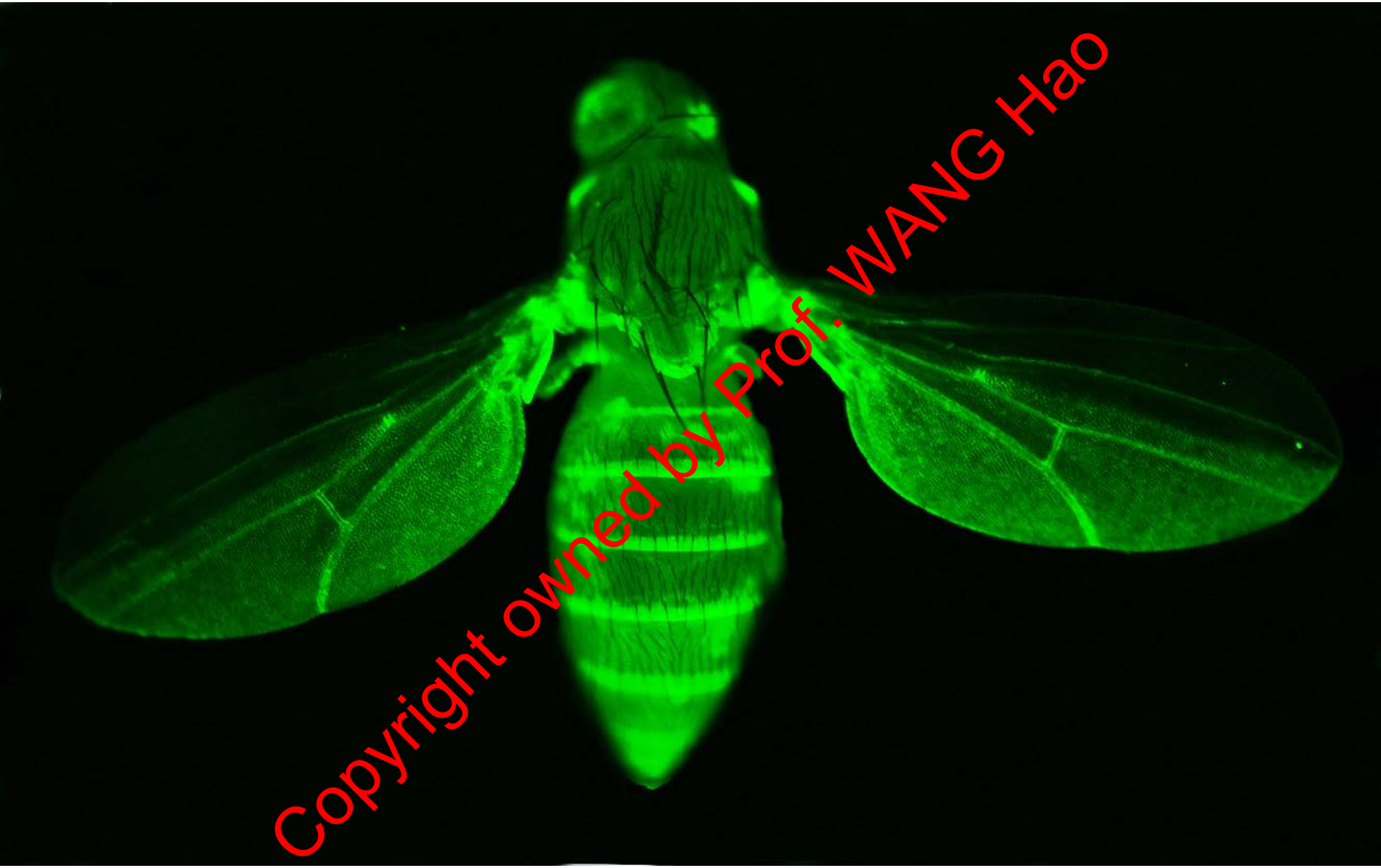


Color can be seen if the wavelength of the emission photon falls into the visible spectrum.

How to see fluorescence signals ?



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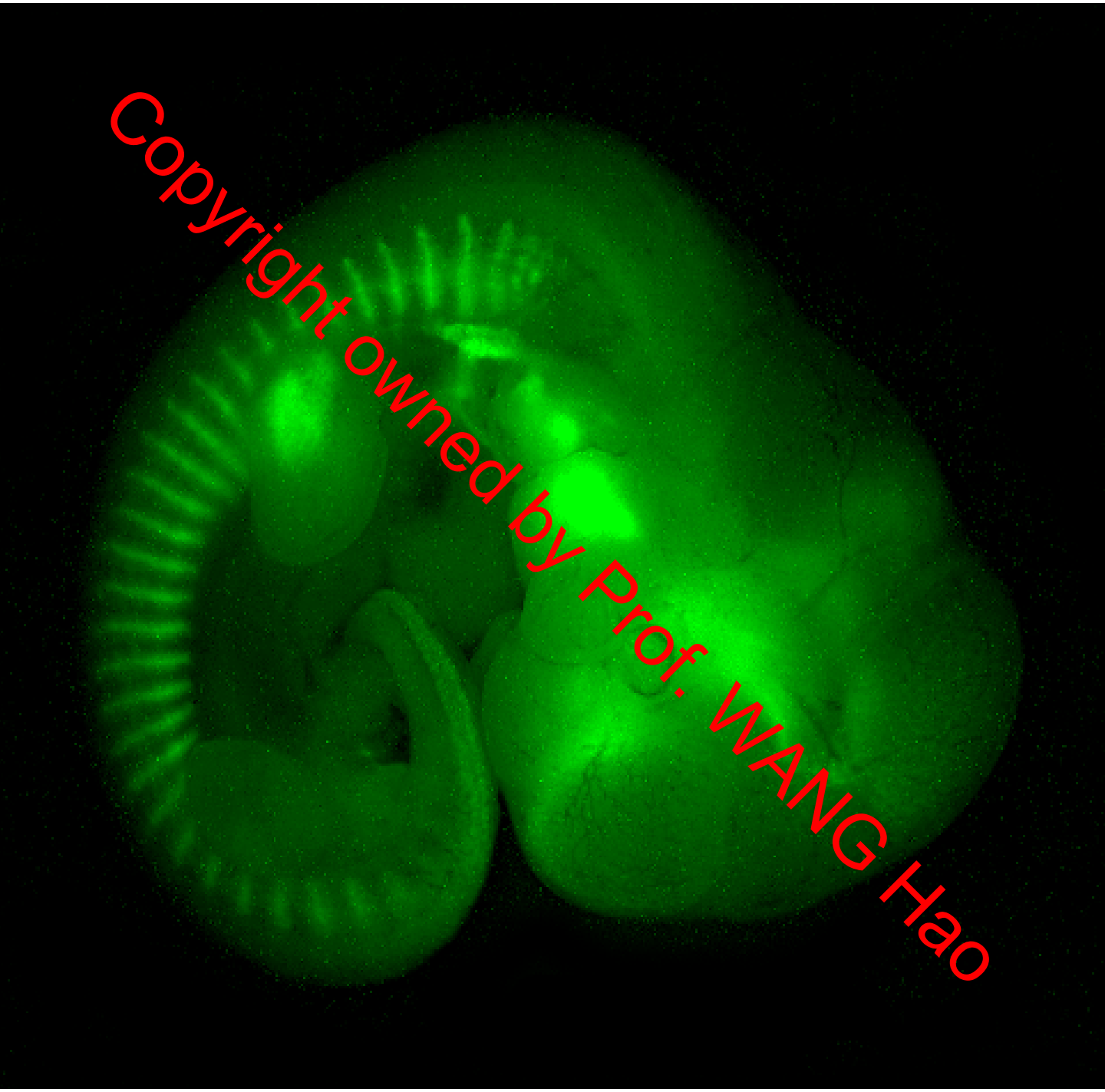
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Tools for observing fluorescence signals in cells

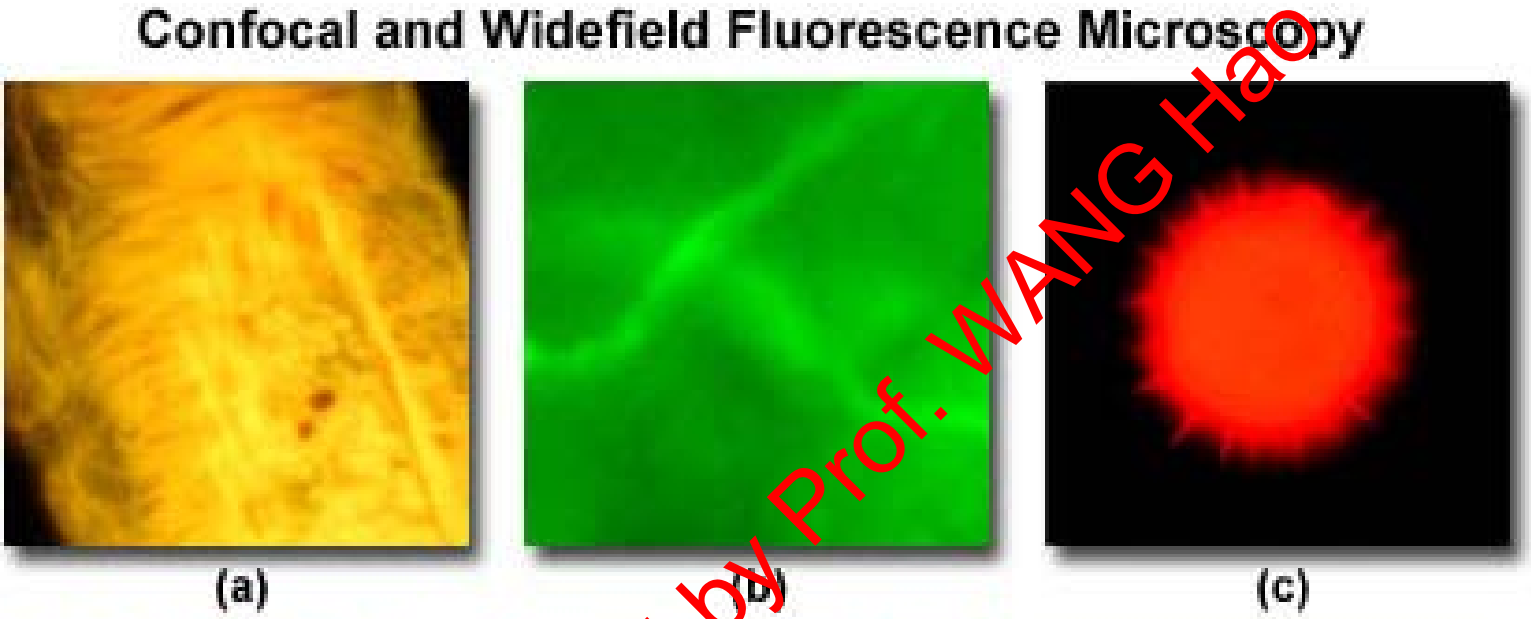
Conventional fluorescence microscope

Laser Scanning Confocal microscope

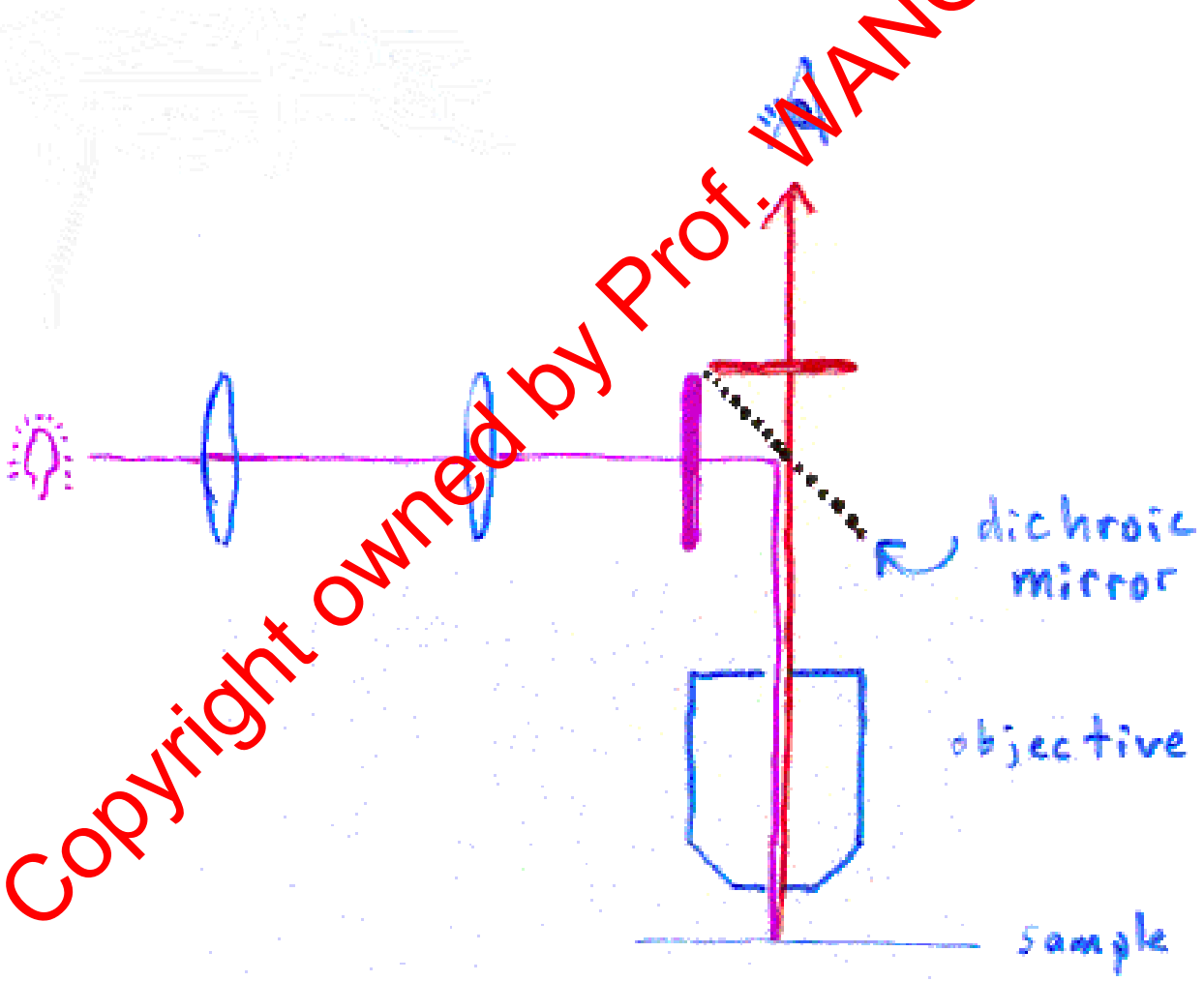
Spinning-disk confocal microscope

Super-resolution fluorescence microscope

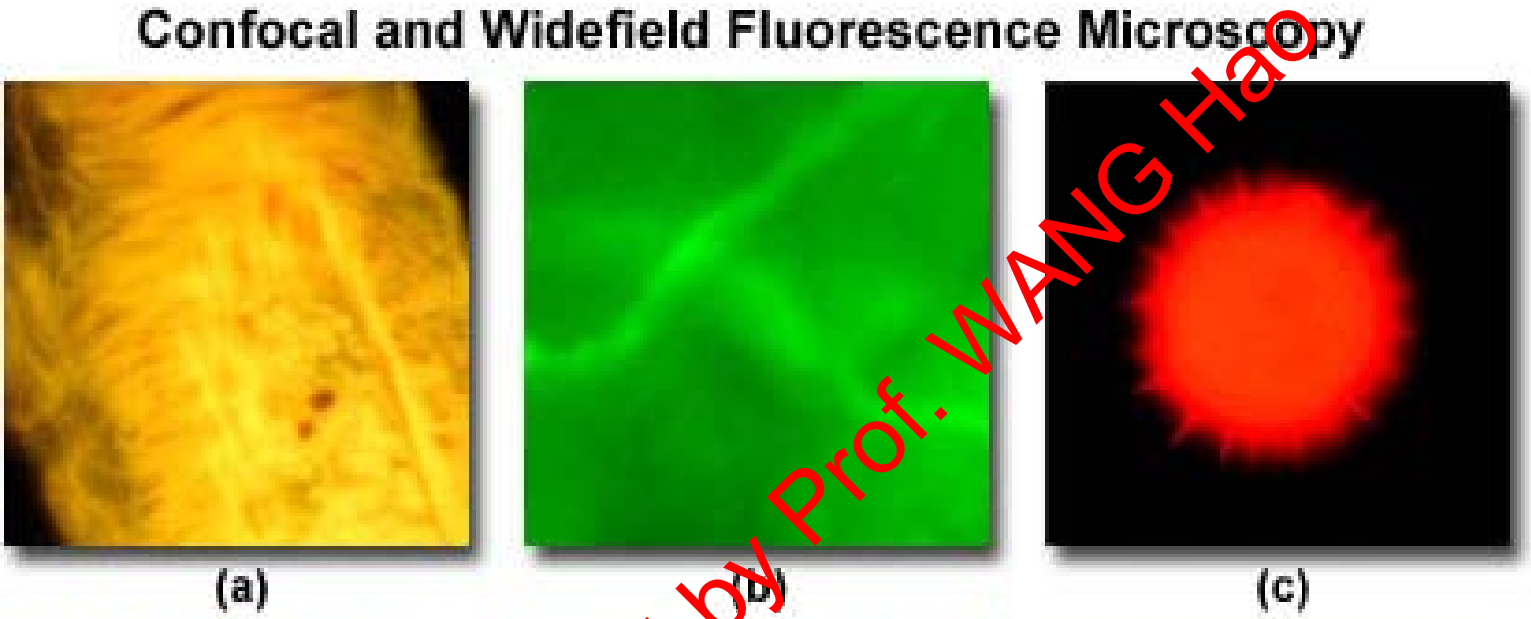
Confocal laser scanning microscopy



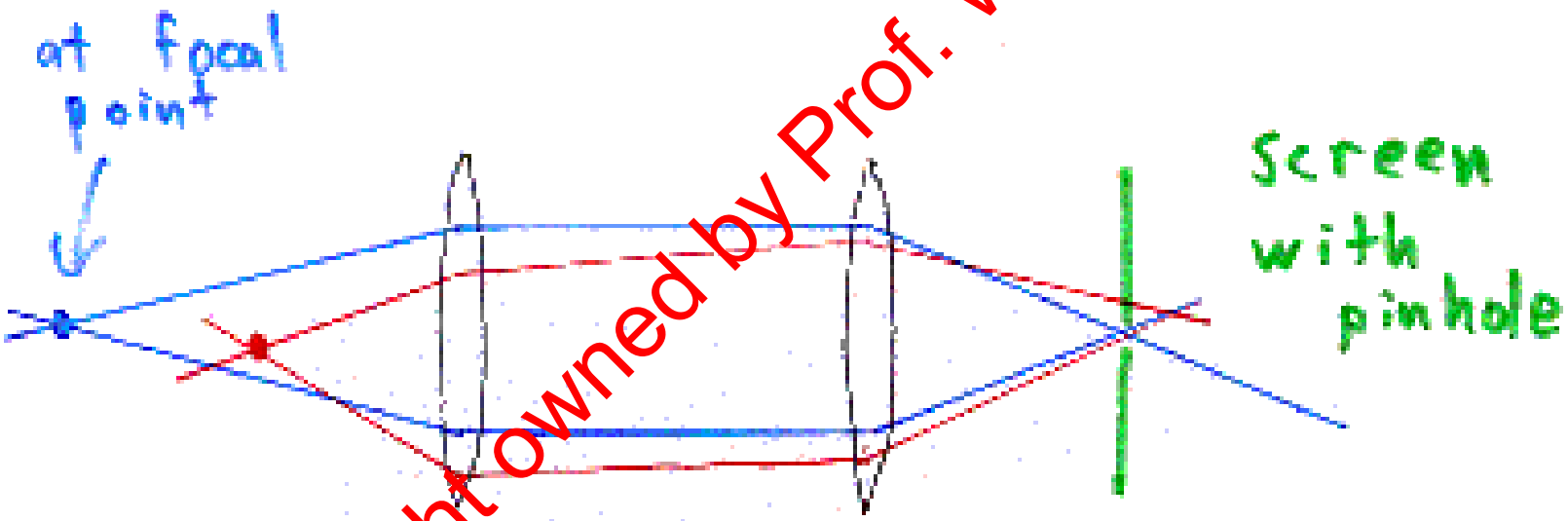
How does a fluorescence microscope work?



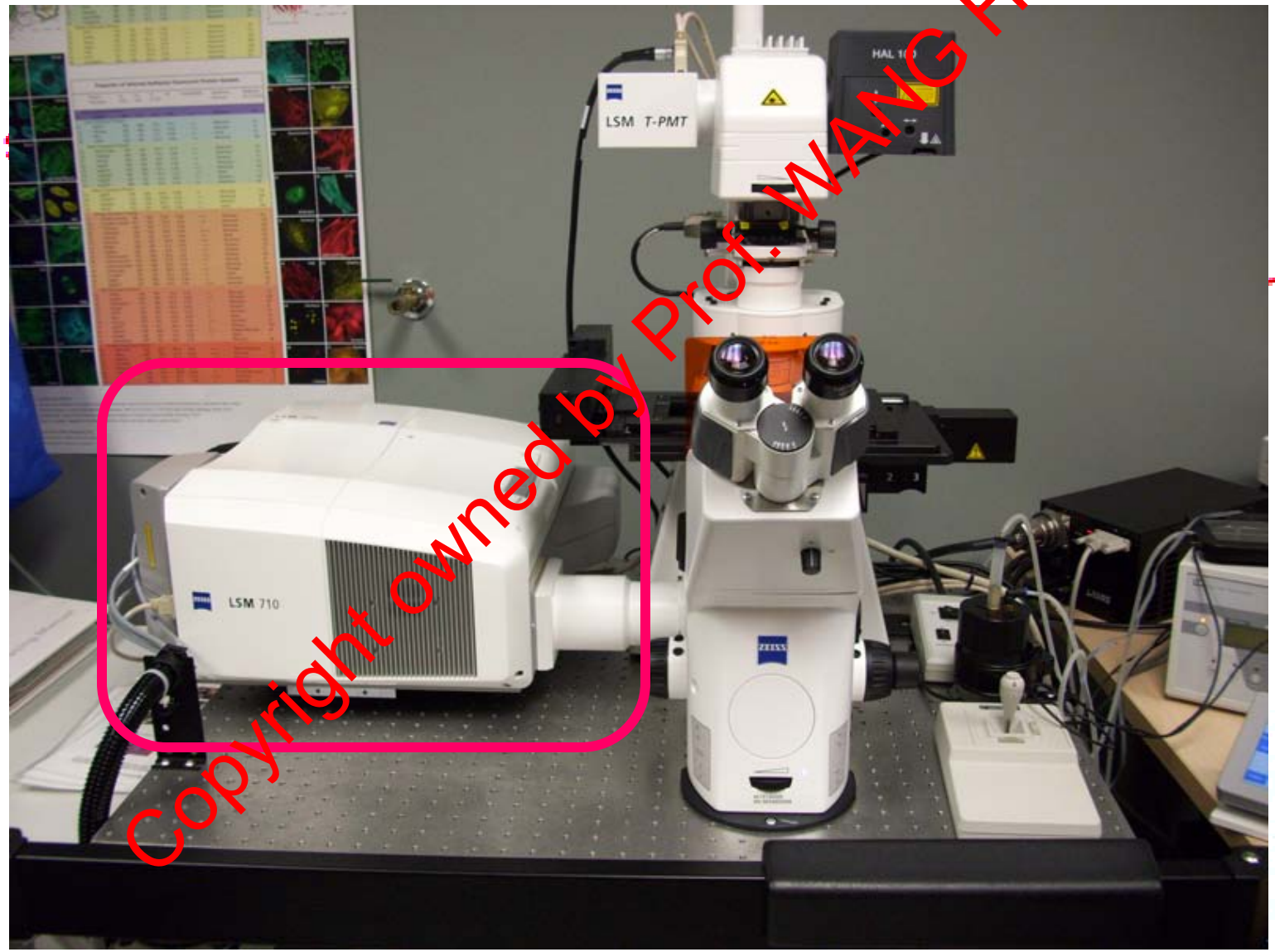
Confocal laser scanning microscopy

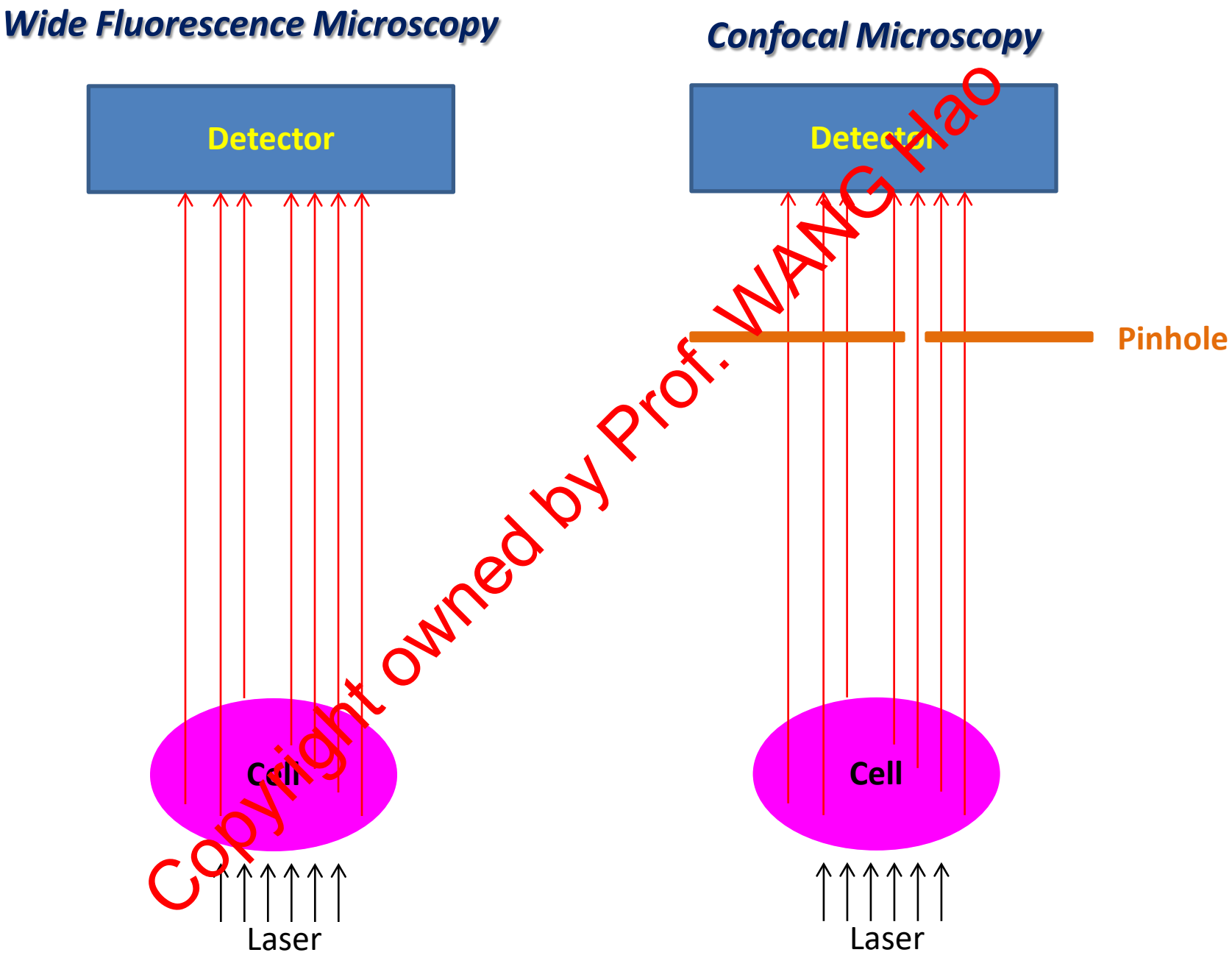


What's this got to do with confocal microscopy?



How does a confocal microscope work?





Confocal laser scanning microscopy

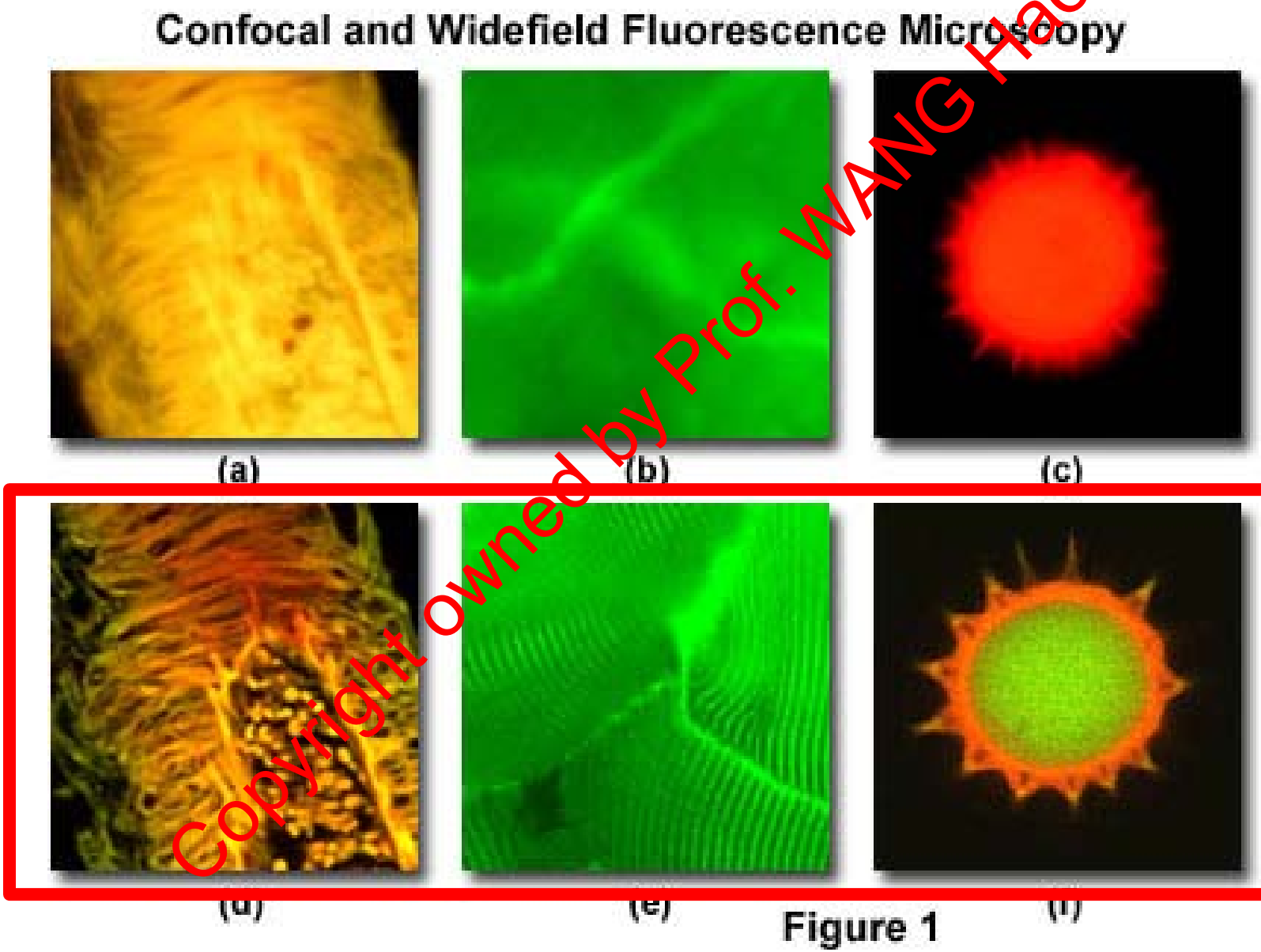
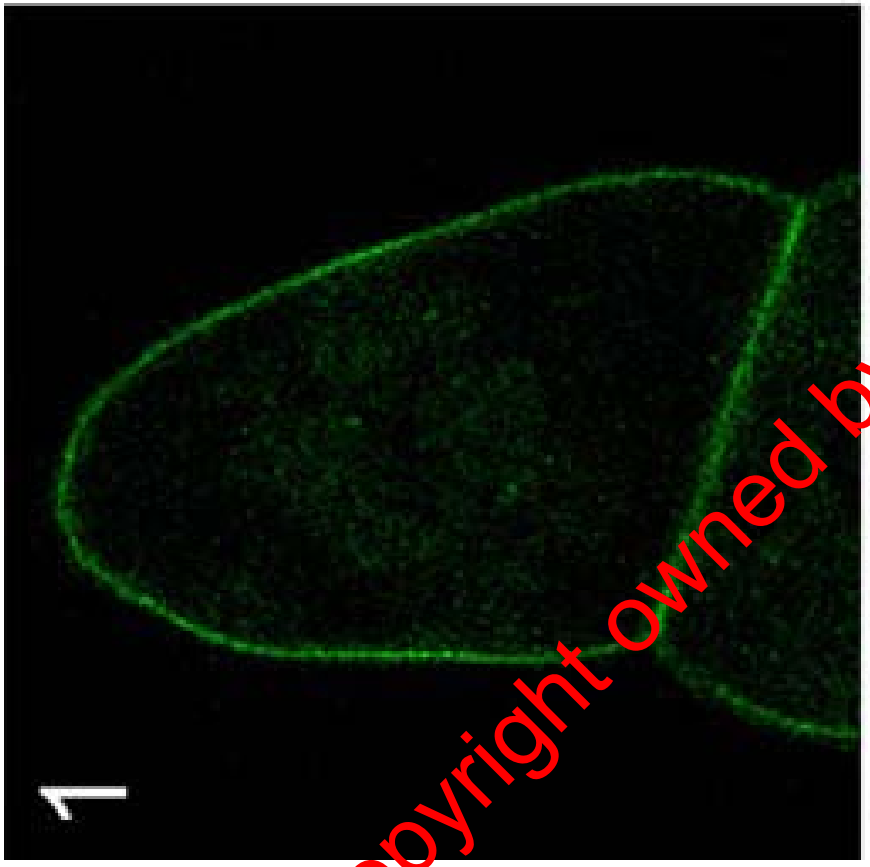
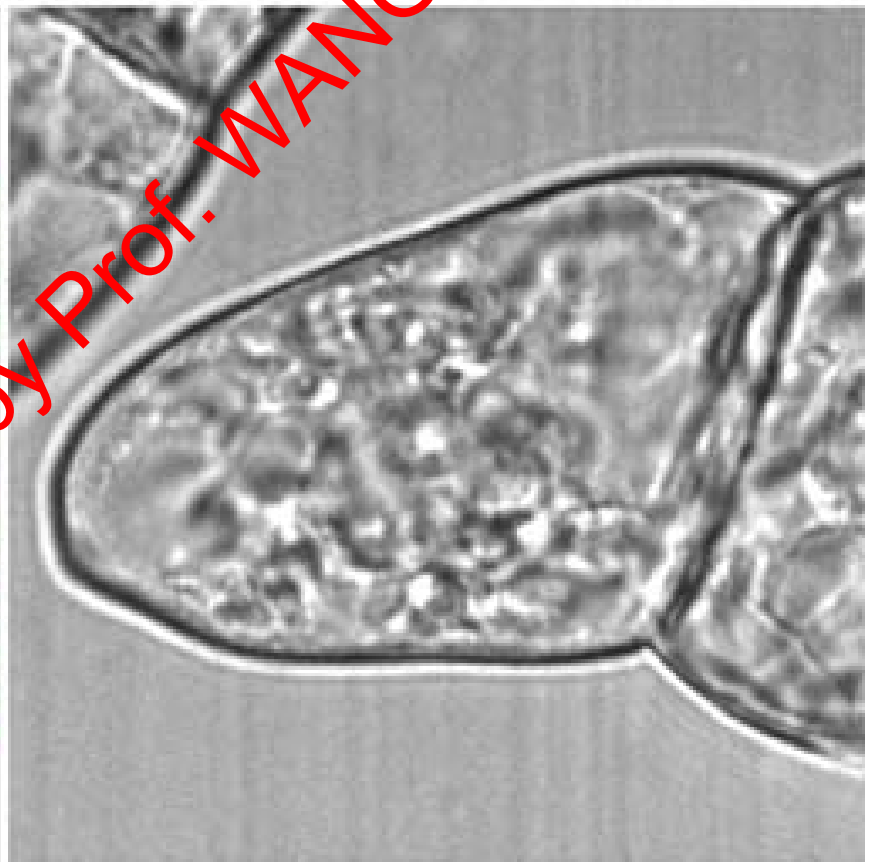


Figure 1

Visualizing Cell-TIRF Microscopy





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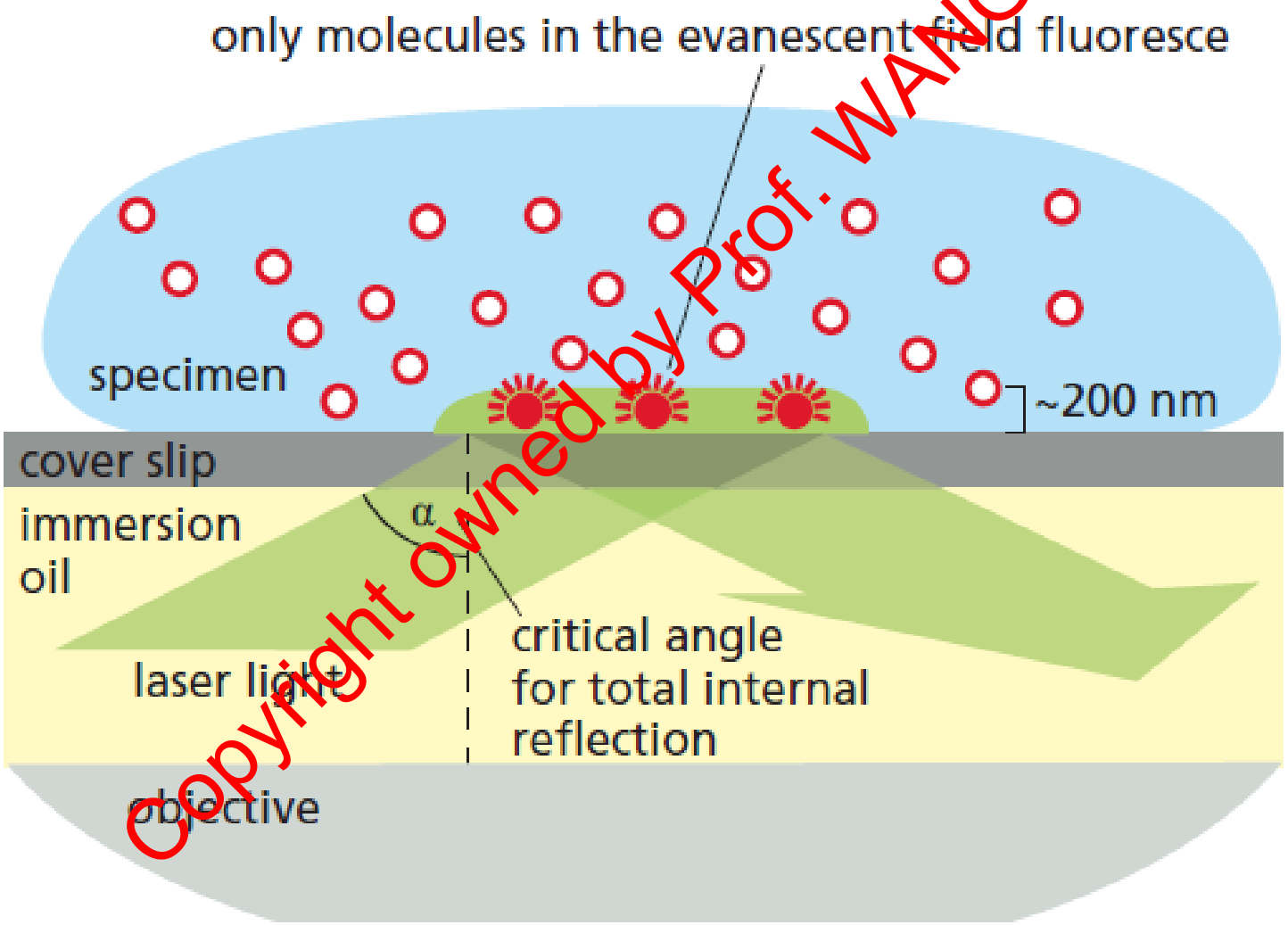

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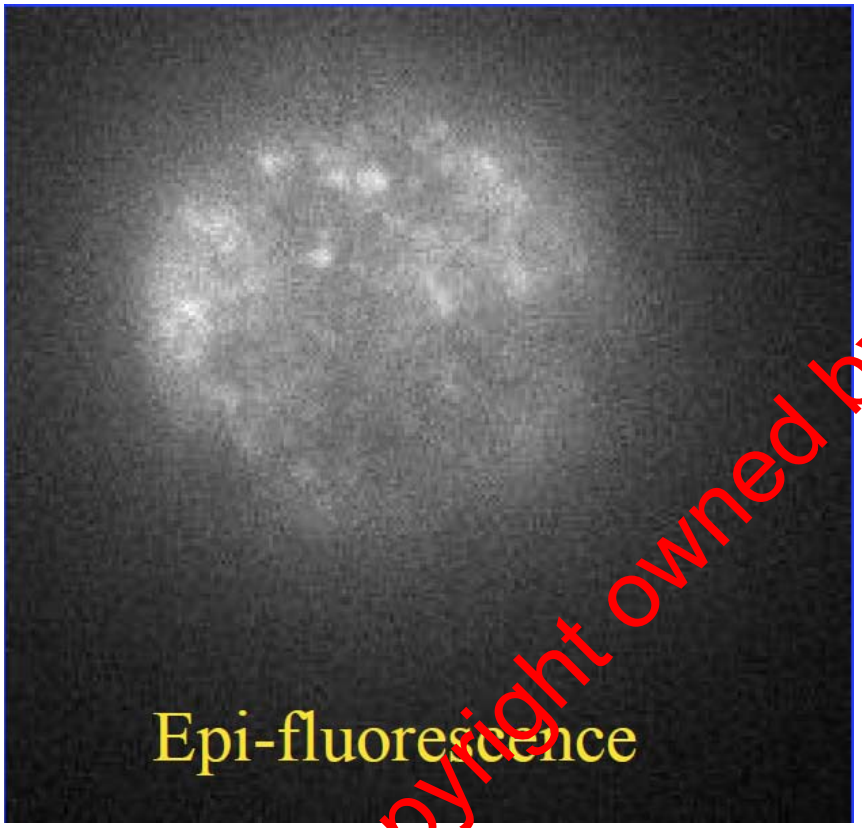
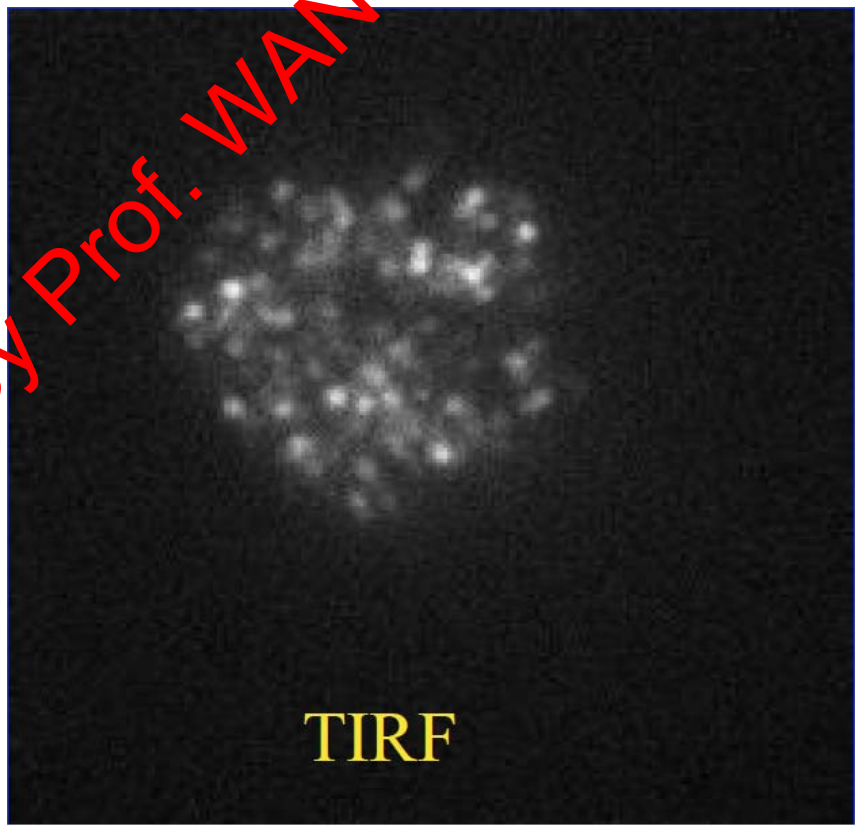
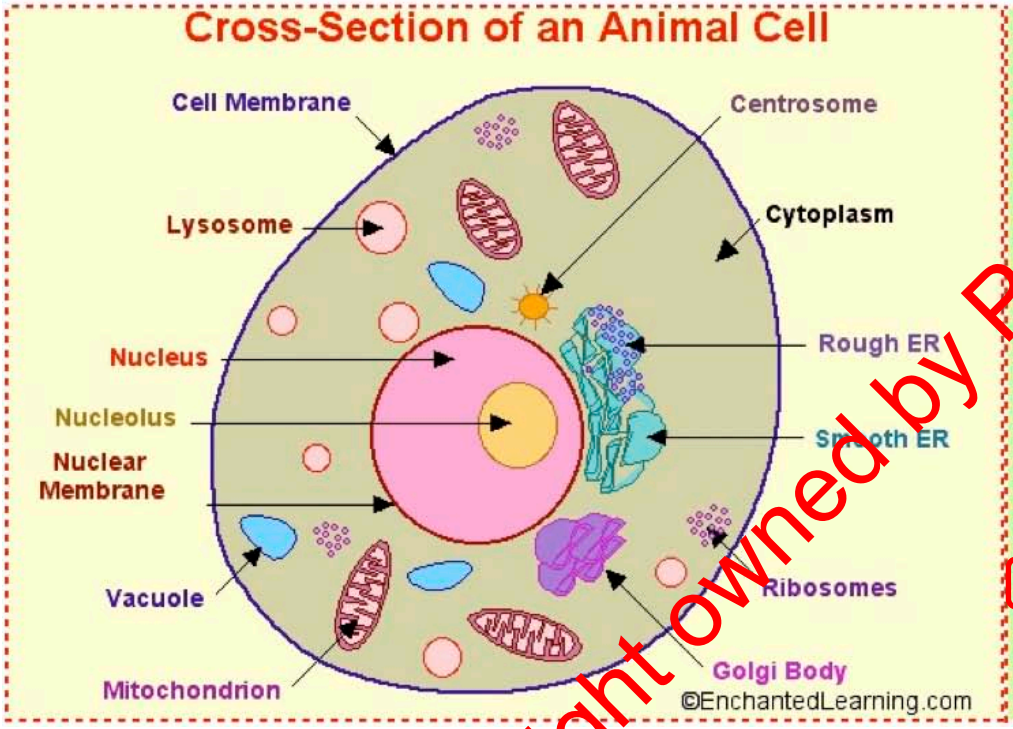
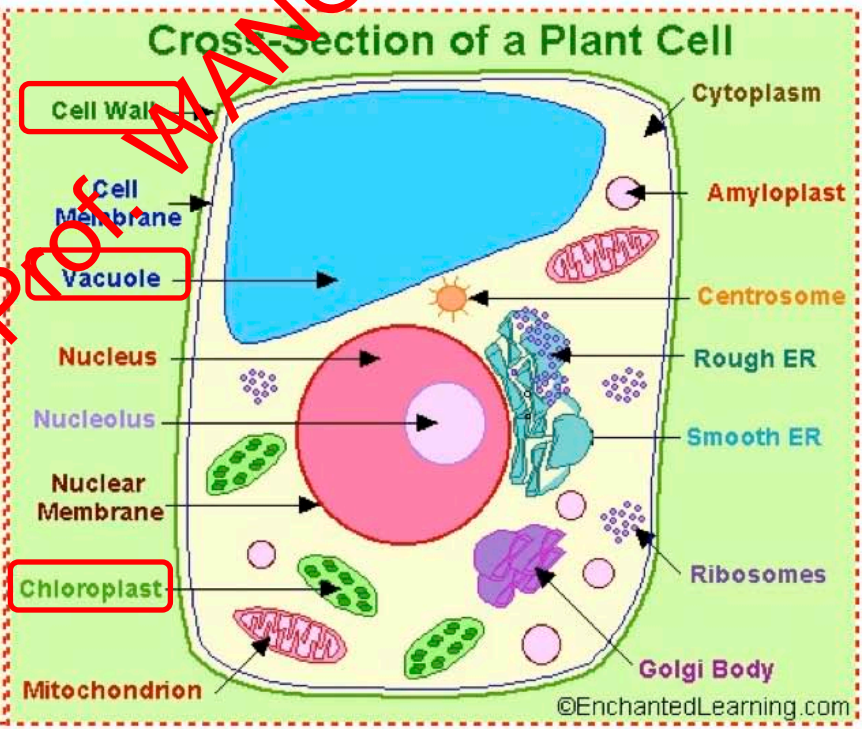


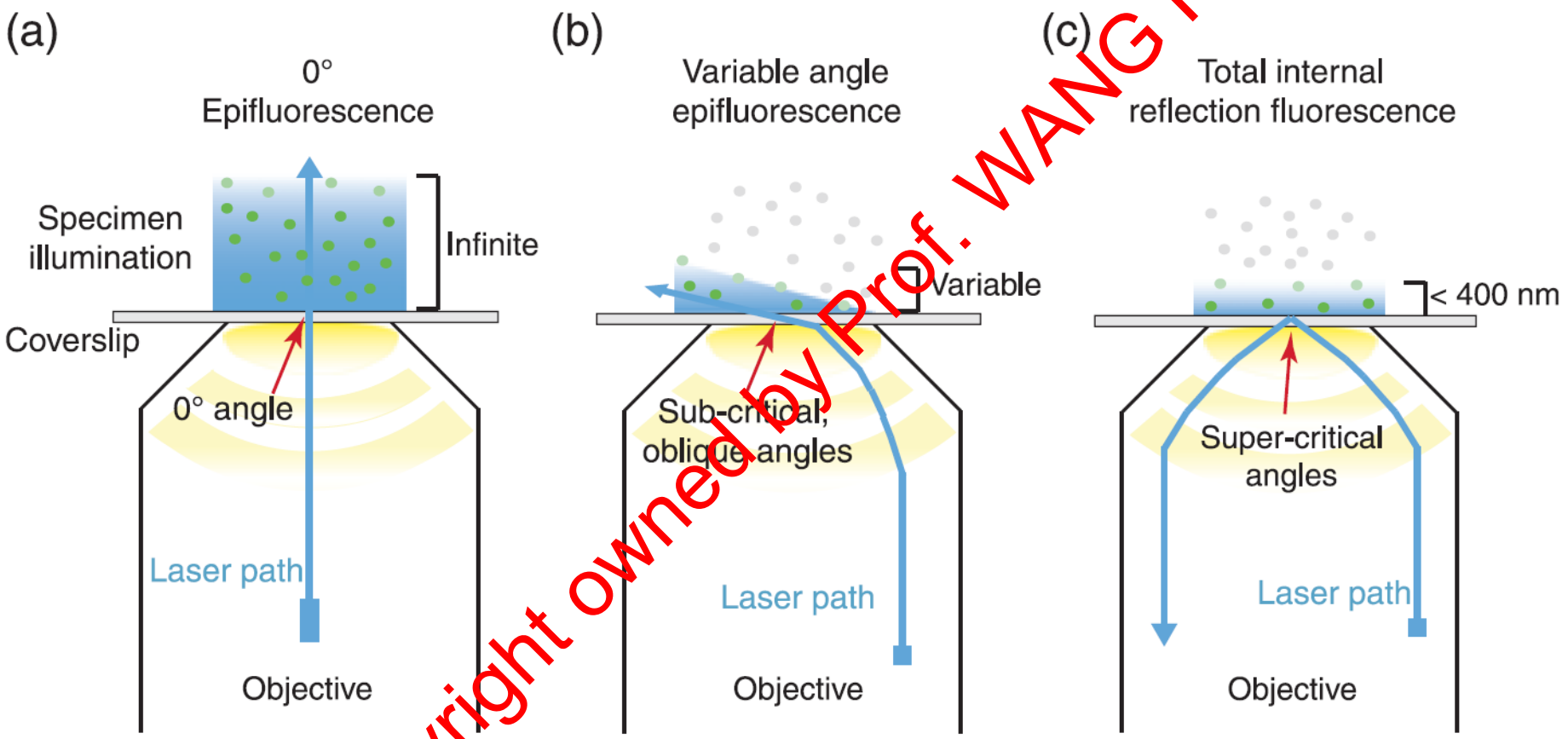


Visualizing Cell-TIRF Microscopy

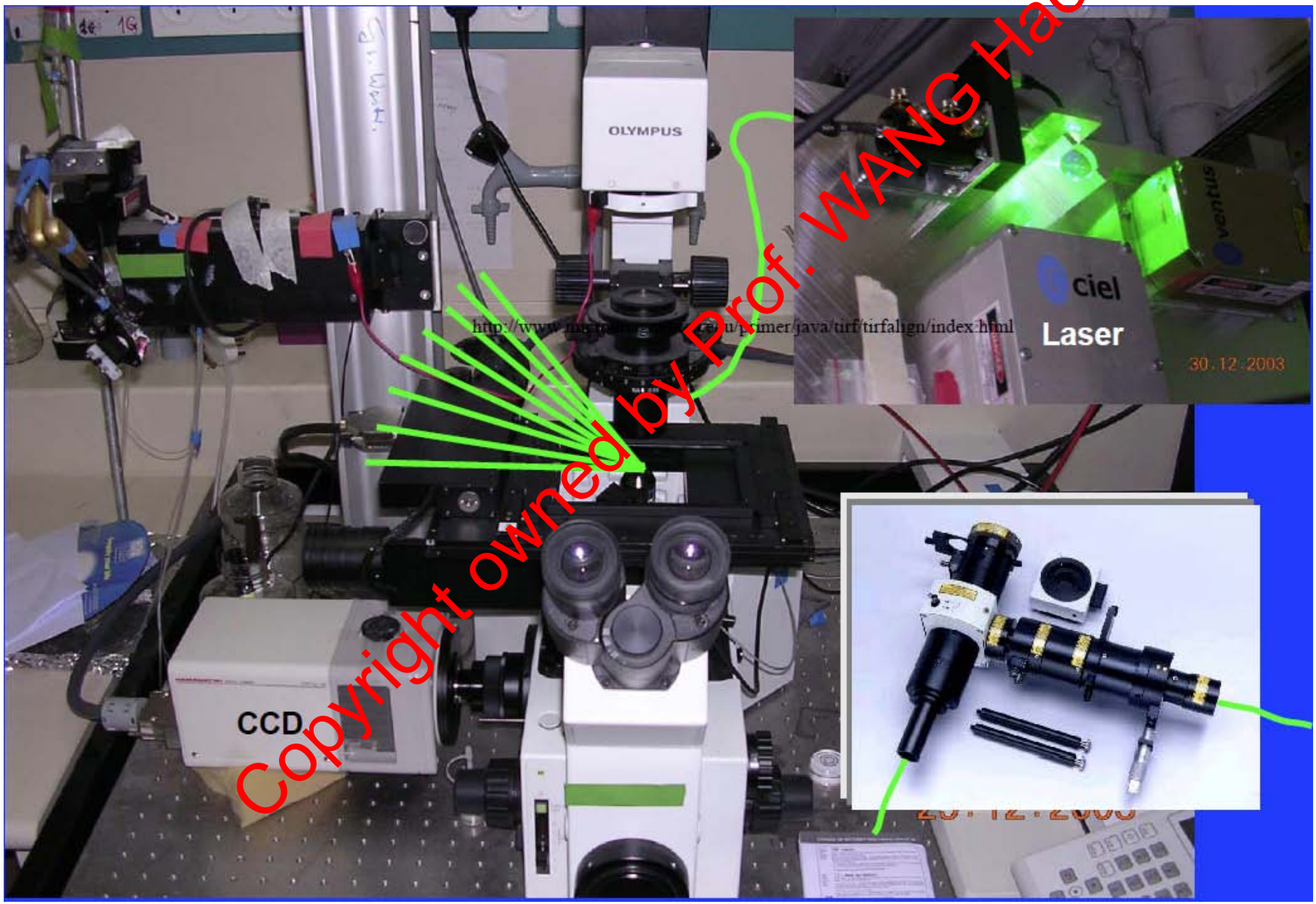




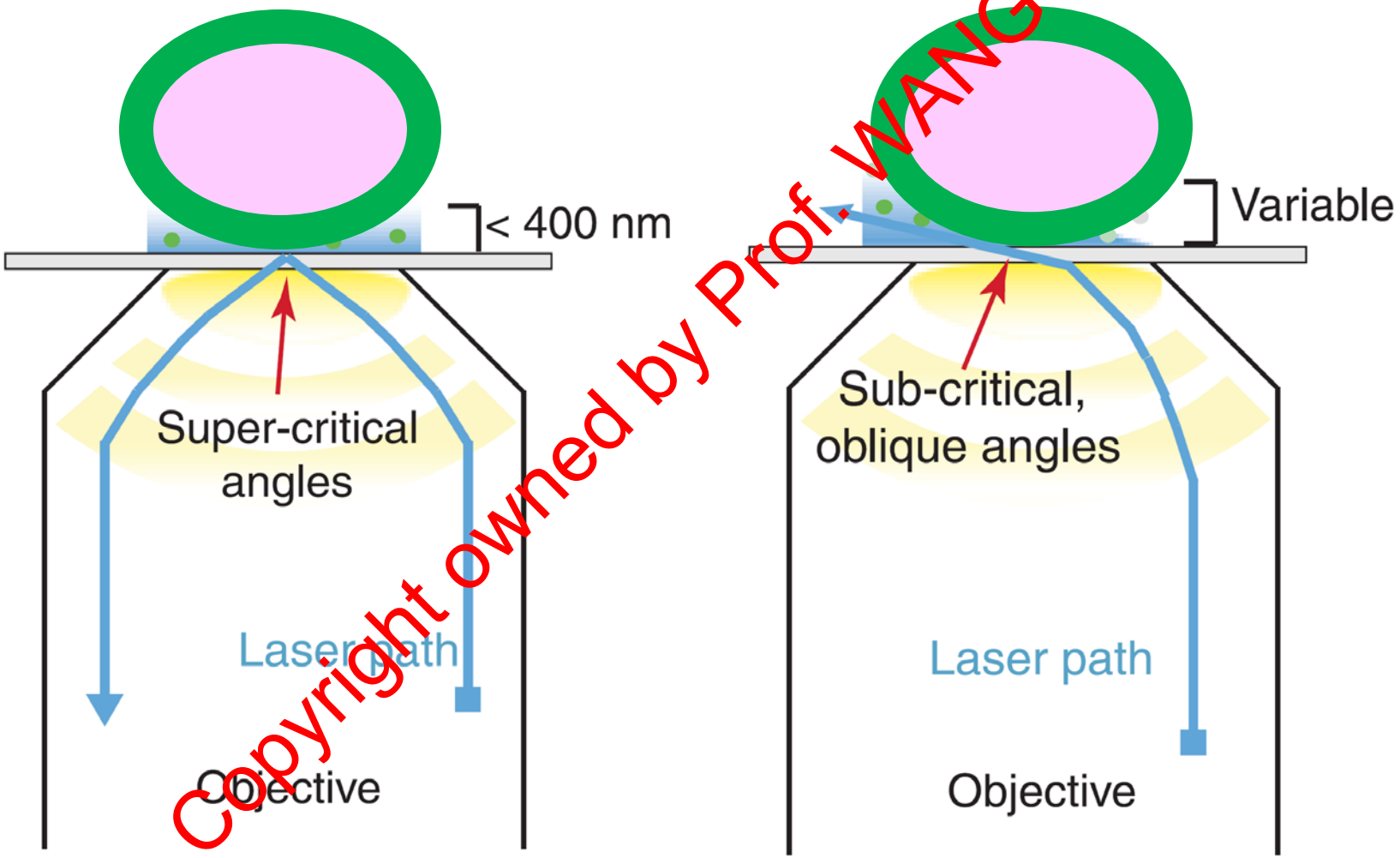
Visualizing Cell-TIRF Microscopy



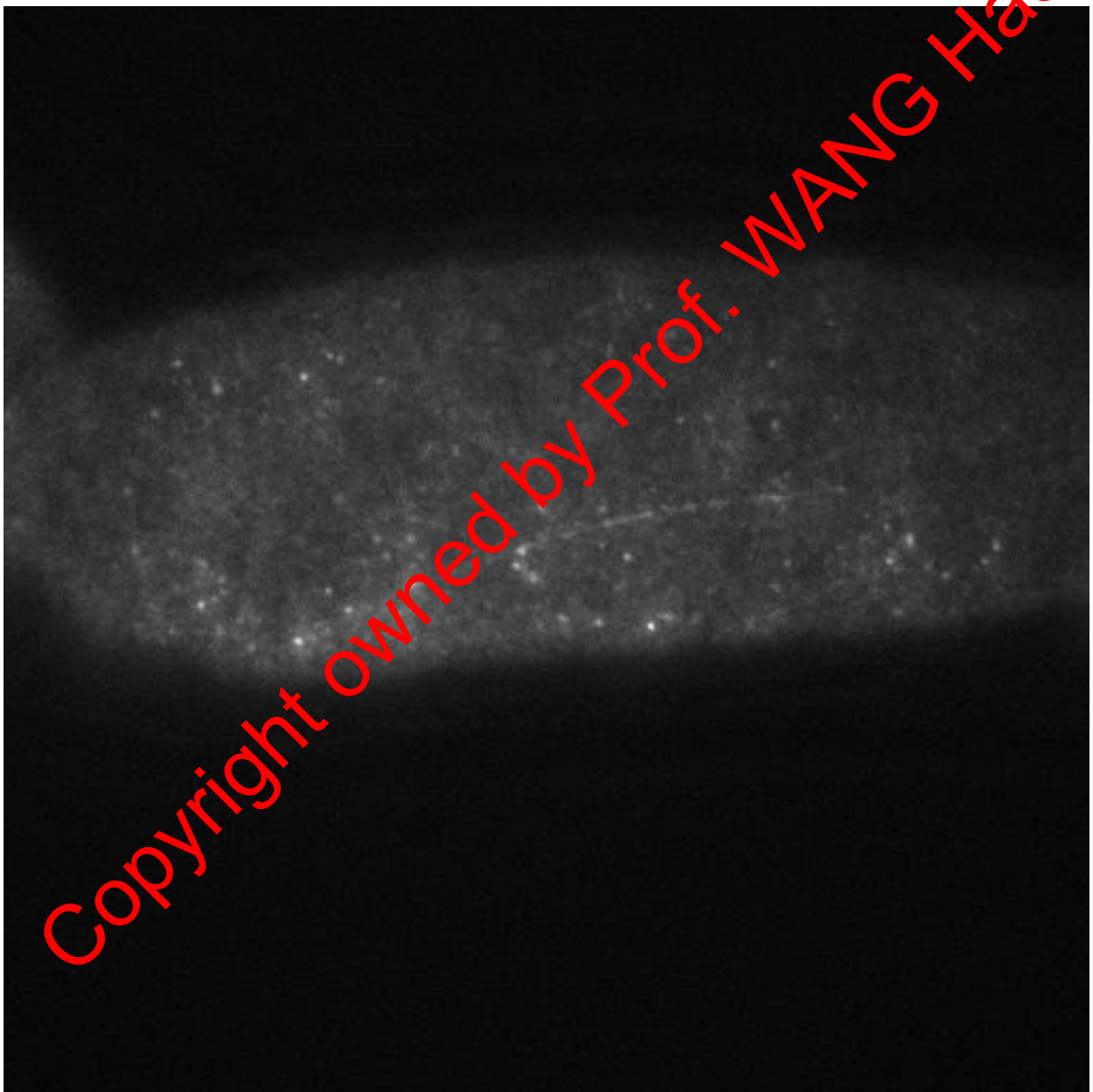
Visualizing Cell-TIRF Microscopy



Visualizing Cell-VAEM



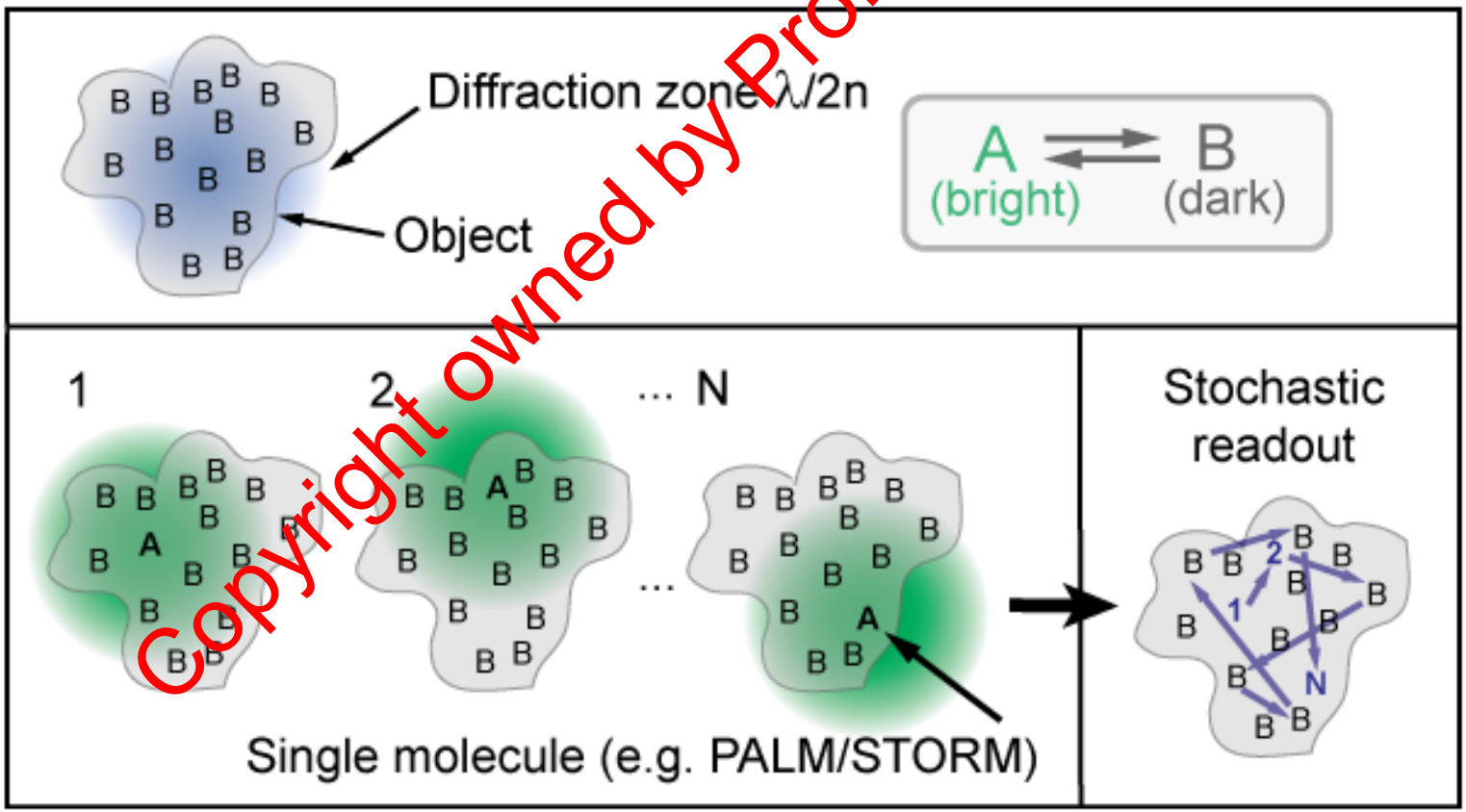
Visualizing Cell-VAEM

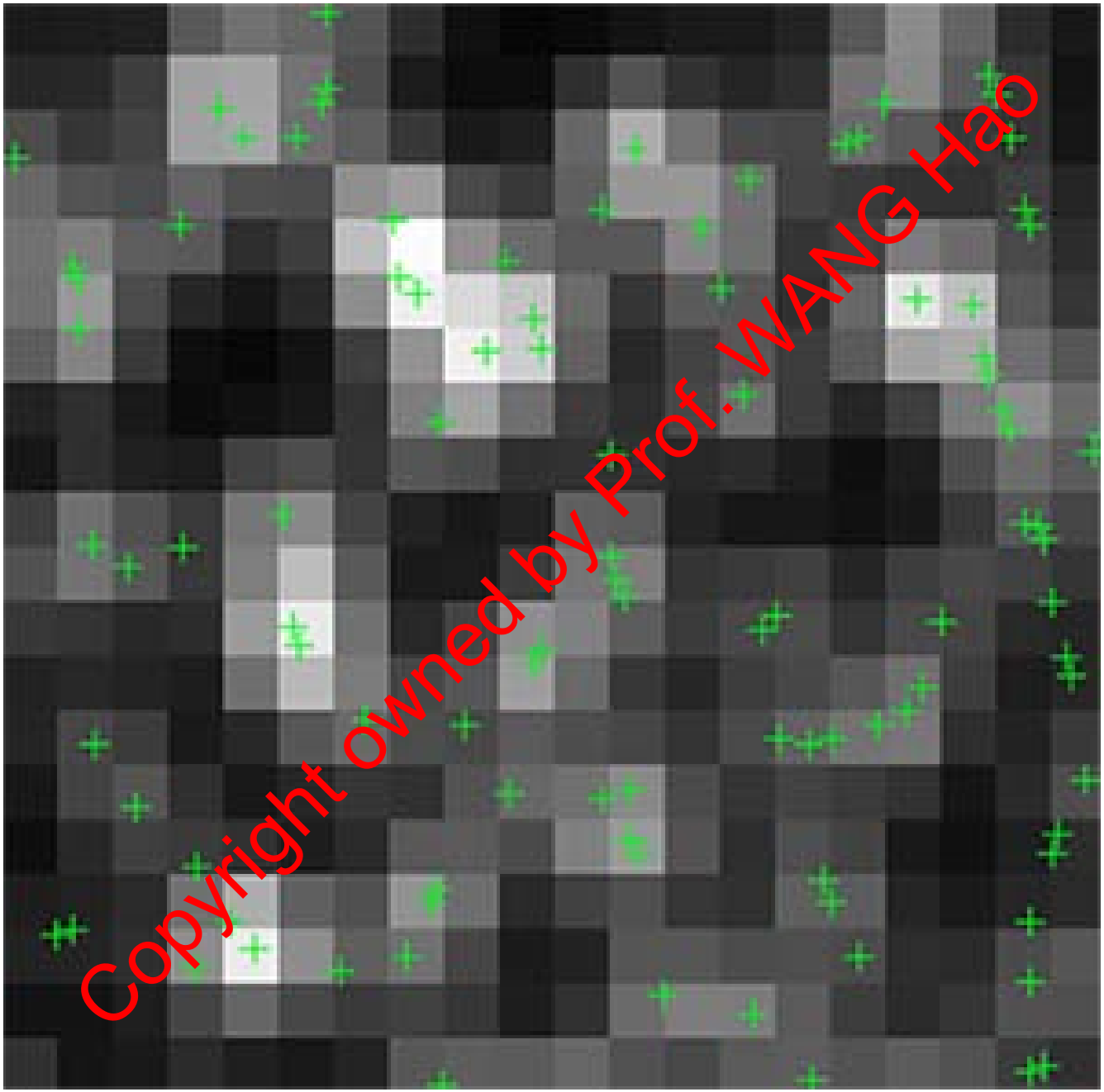


Super-resolution Microscopy: STROM

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.

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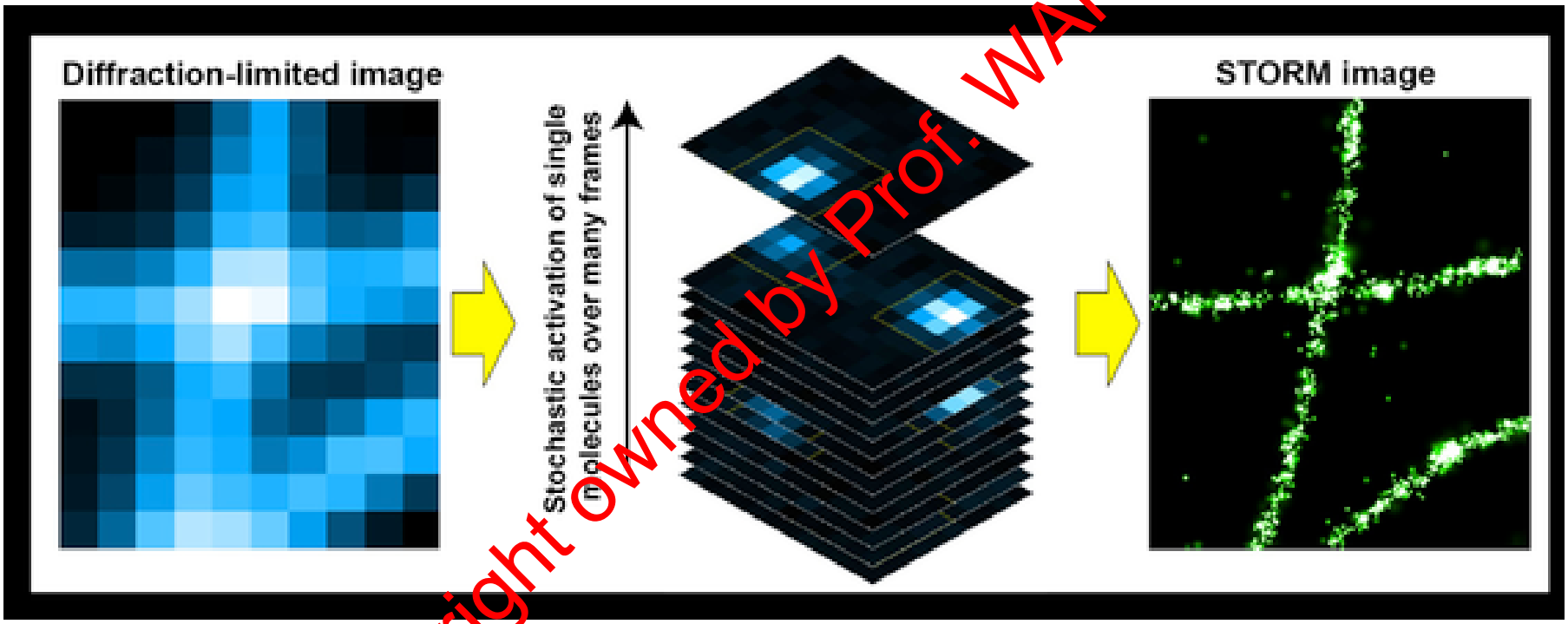


Basic Principle of STORM Superresolution Imaging

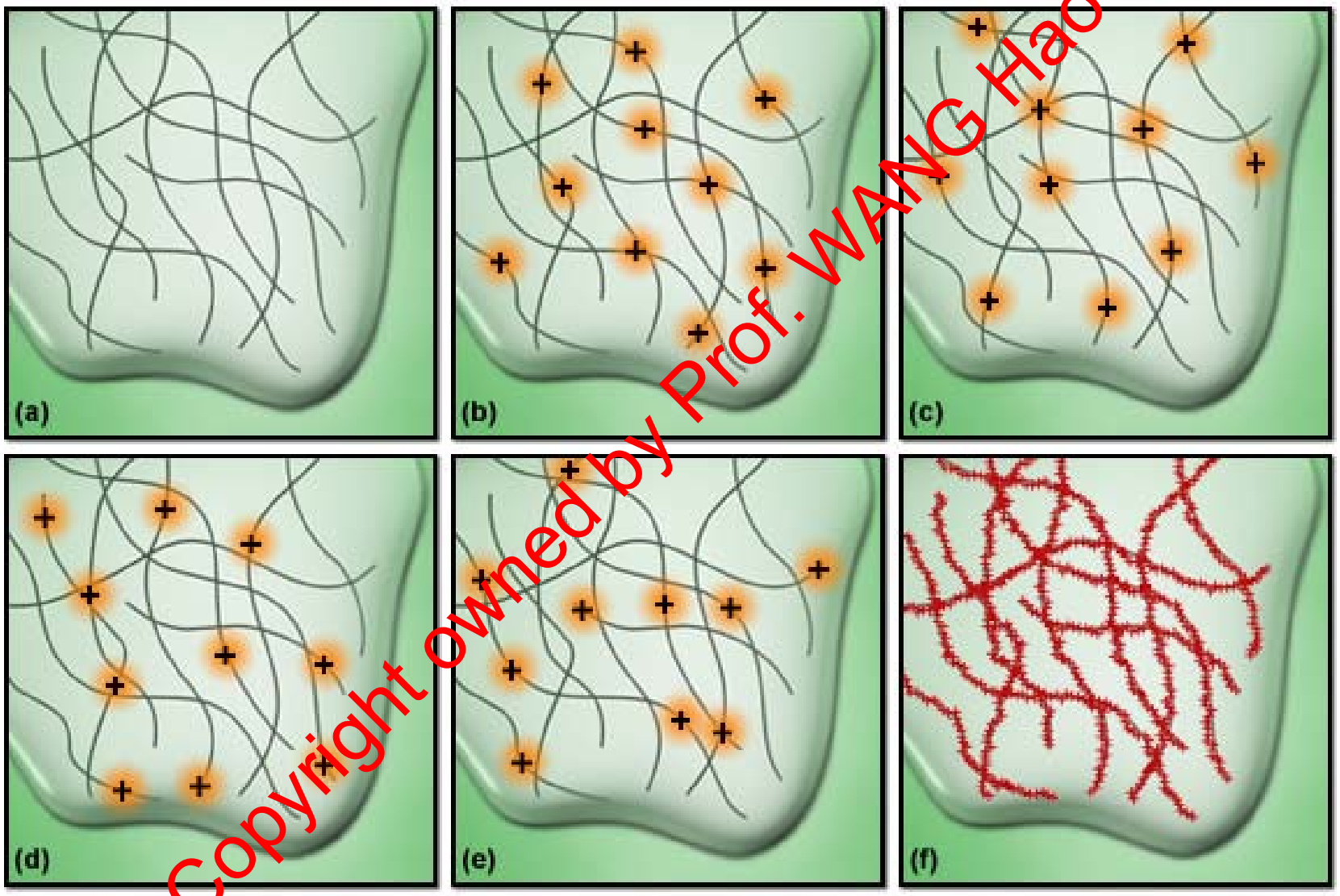
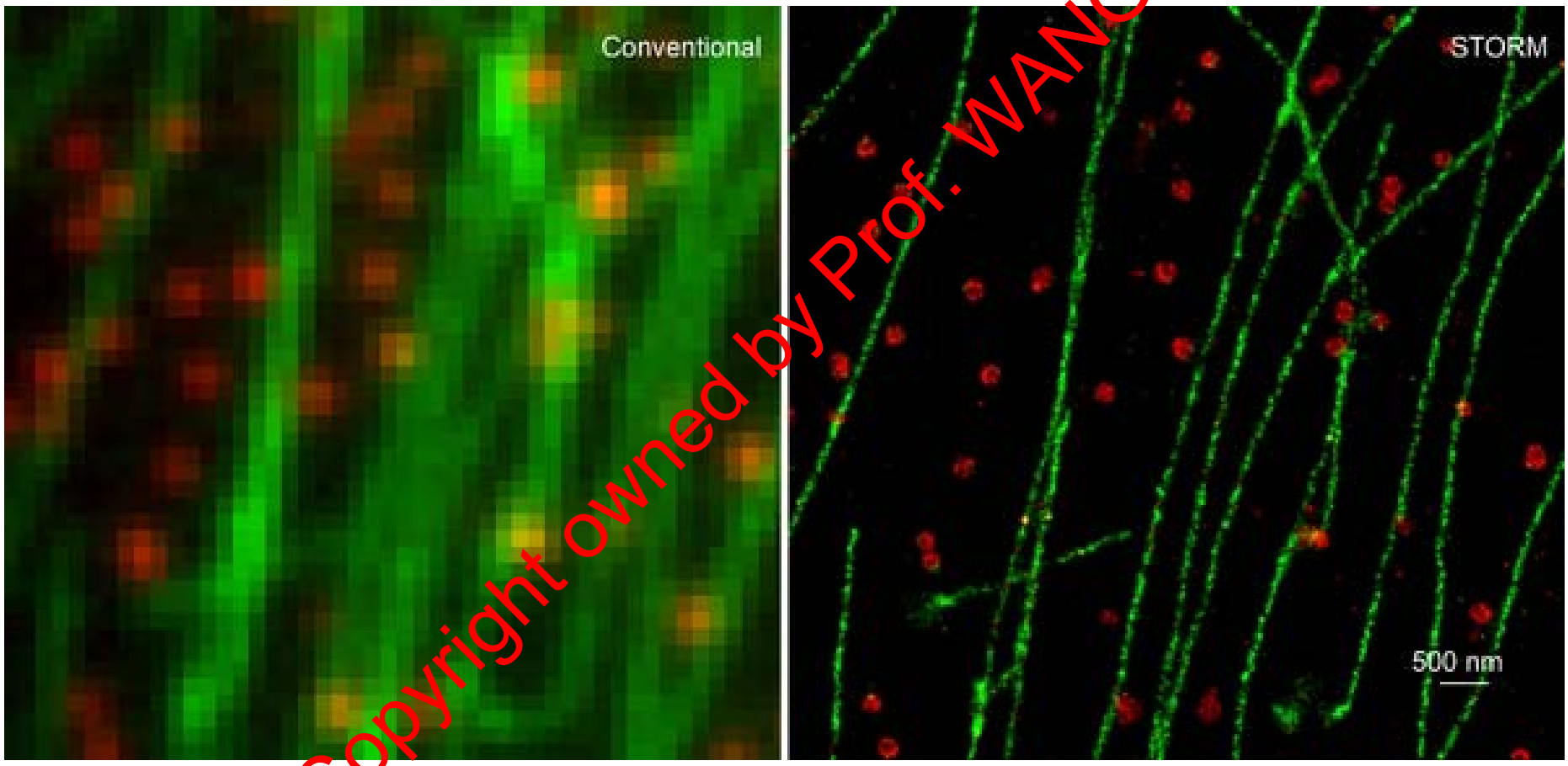
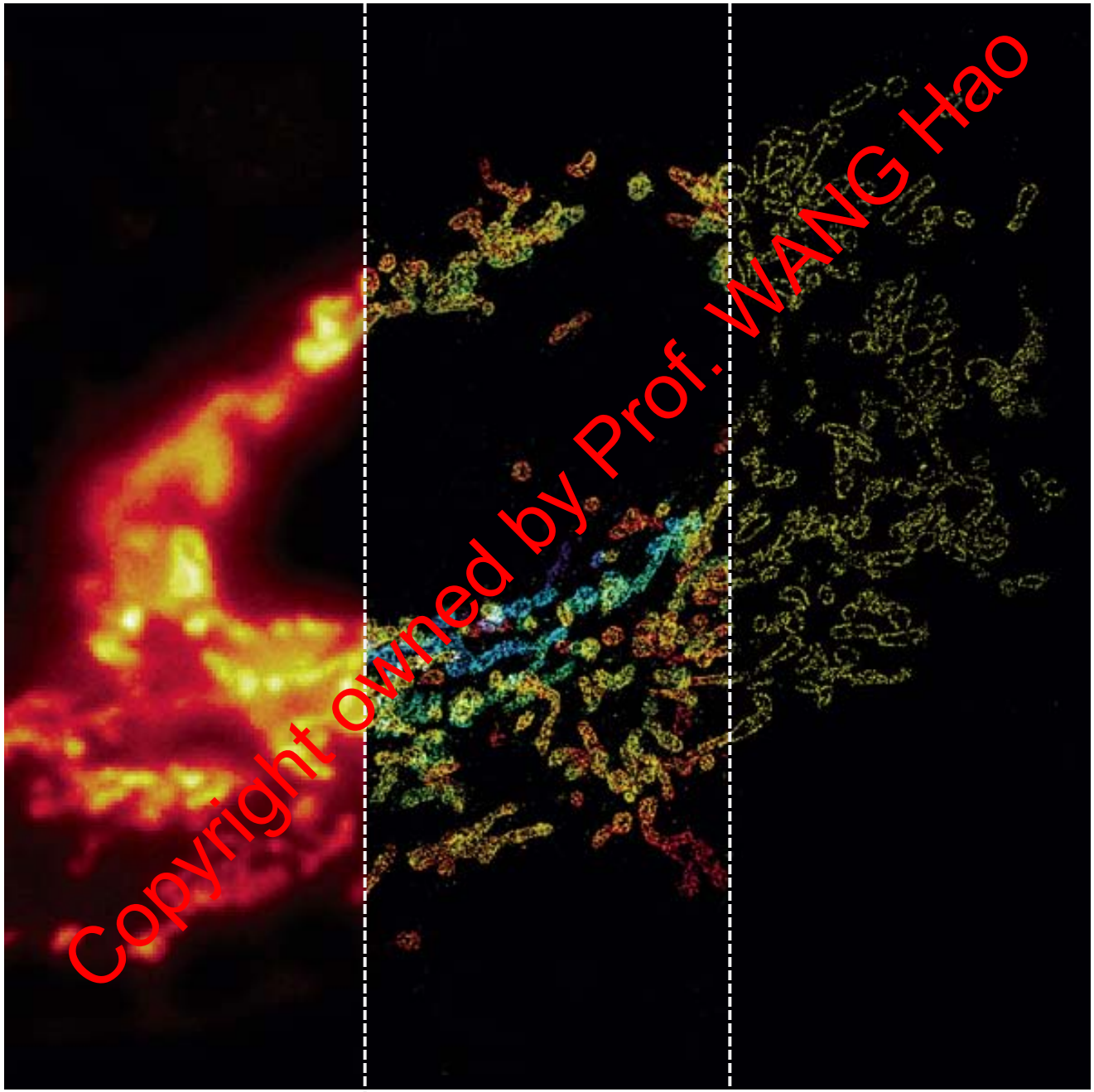


Figure 1

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

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. Stelzer[1]

Nov, 2008, Science

In order to get a three-dimensional resolution image:

Usual confocal microscopy + multiphoton microscopy

Disadvantages:

1.Low speed image

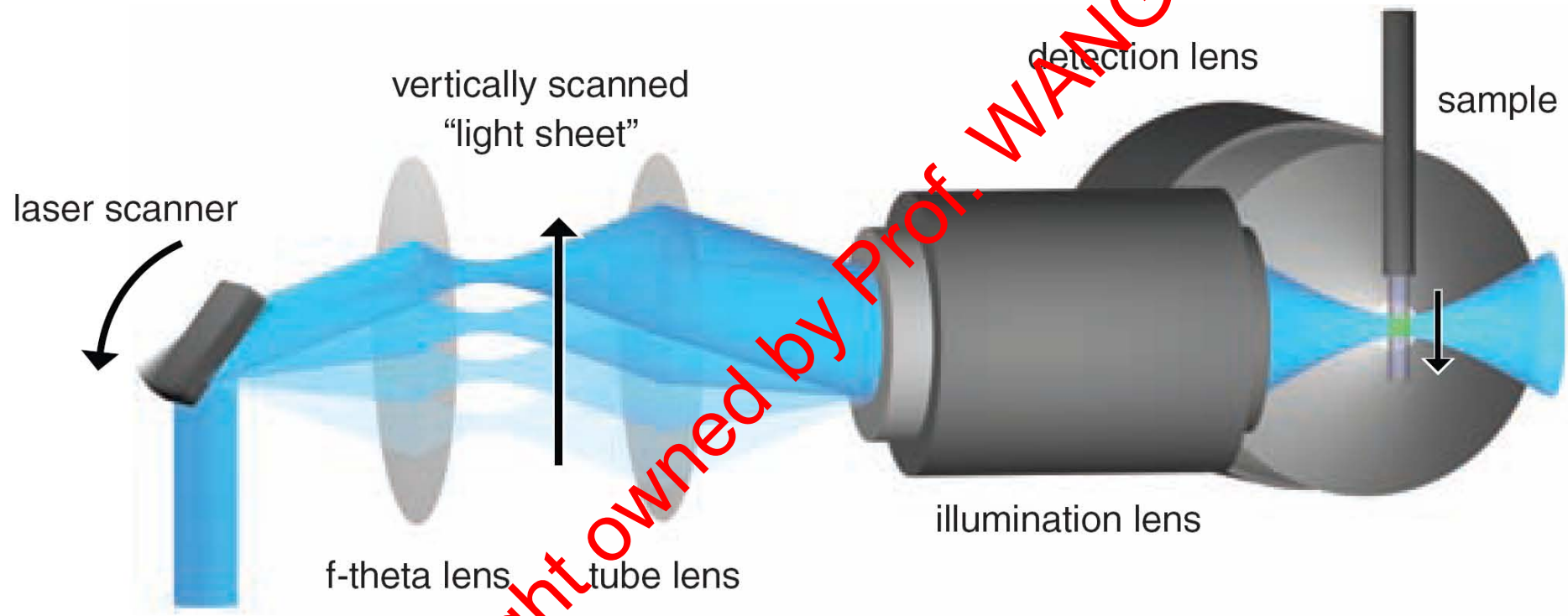
2.High phototoxicity

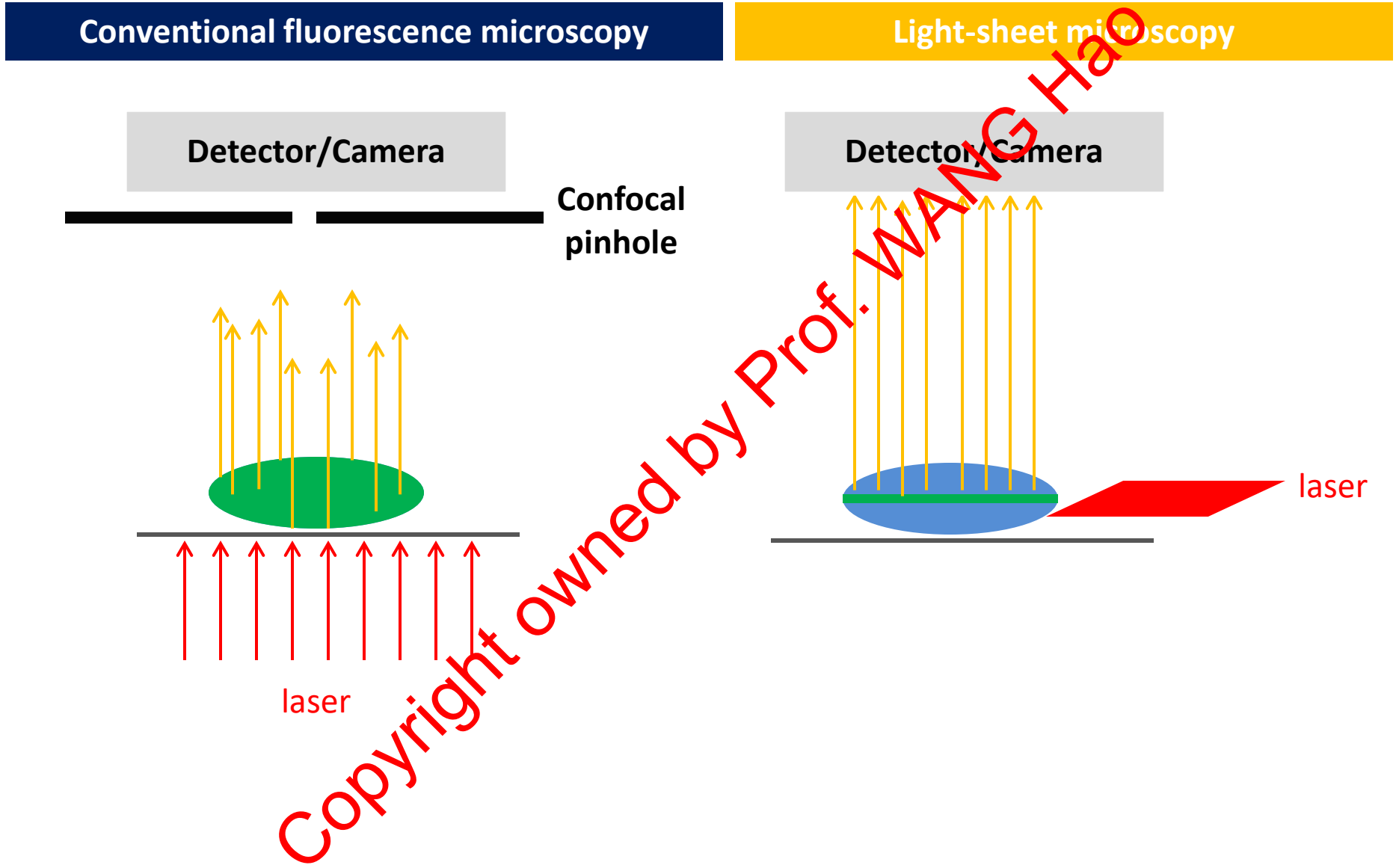
DSLM combines:

(i) an imaging speed of 63 million voxels per second,

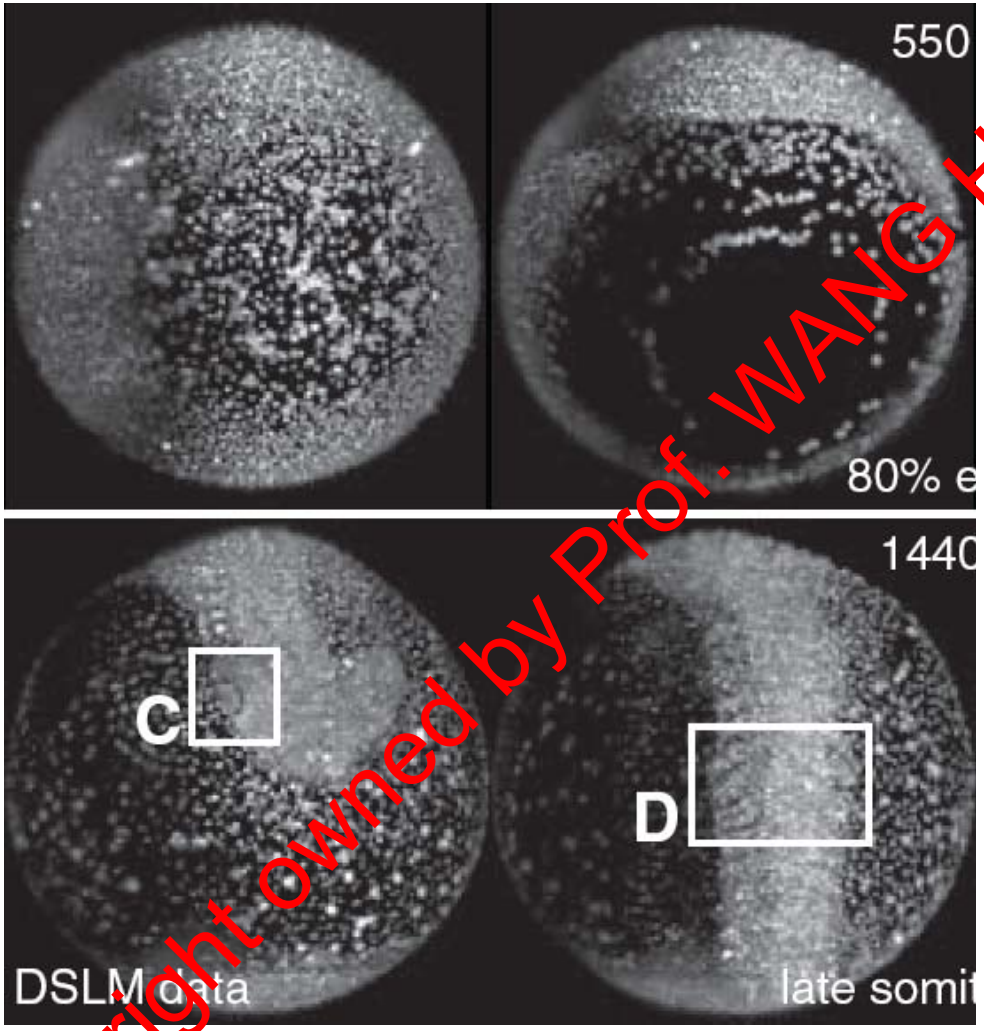
(ii) A signal-to-noise ratio of 1000:1 at a lateral and axial resolution of 300 and 1000 nm, respectively,

(iii) ultralow excitation energies confined to a single plane (1.7 mJ at 488 nm passing each plane in the zebrafish experiments)





Live cell migration during embryo development



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.

light-sheet microscopy

Advantages:

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

Disadvantages:

1. Resolution @ cellular level

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 Böhme,[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||]

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The Nobel Prize in Chemistry 2014

Photo: Matt Staley/HHMI

Eric Betzig

Prize share: 1/3

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Stefan W. Hell

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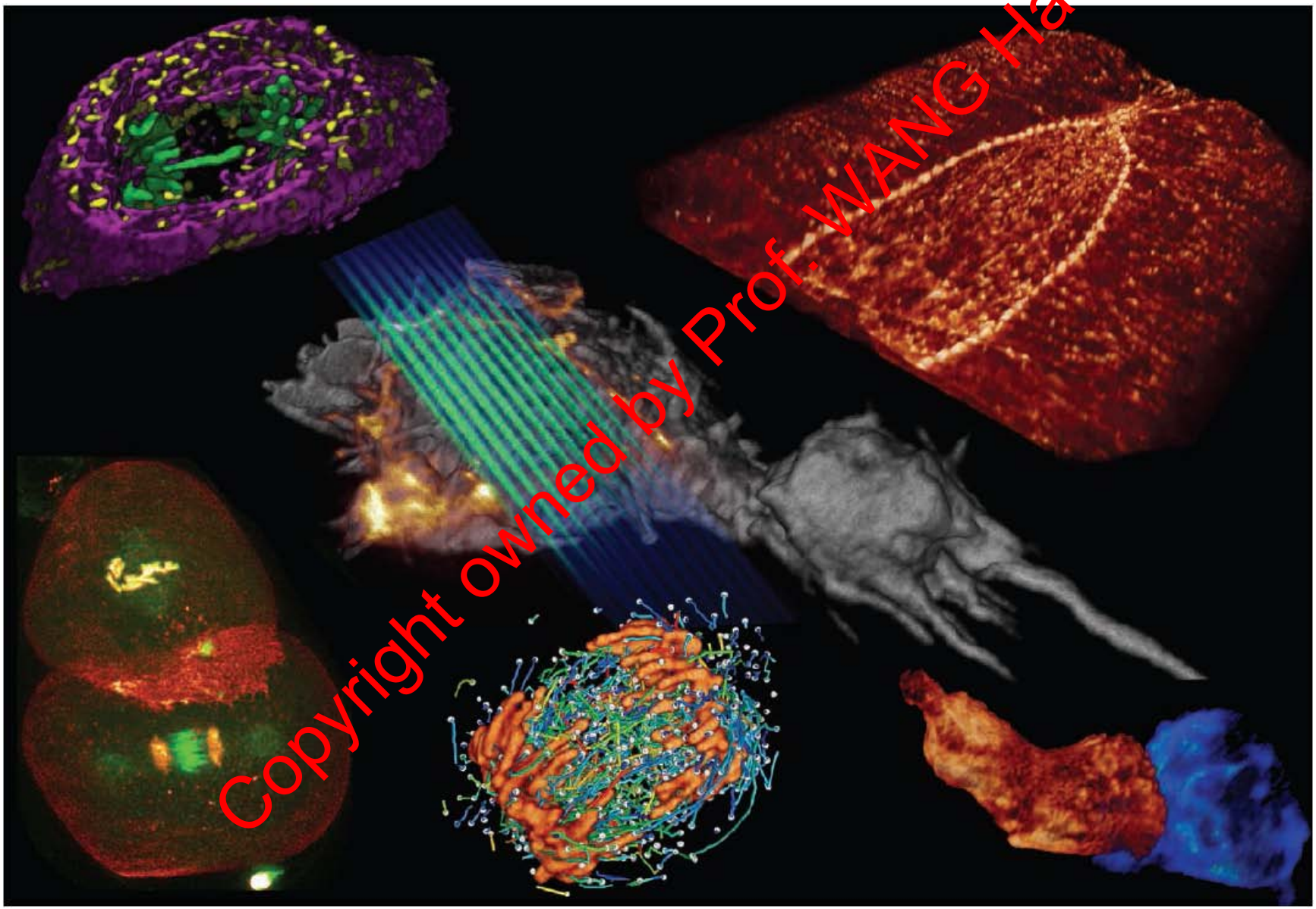
Photo: K. Lowder via Wikimedia Commons, CC-BY-SA-3.0

William E. Moerner

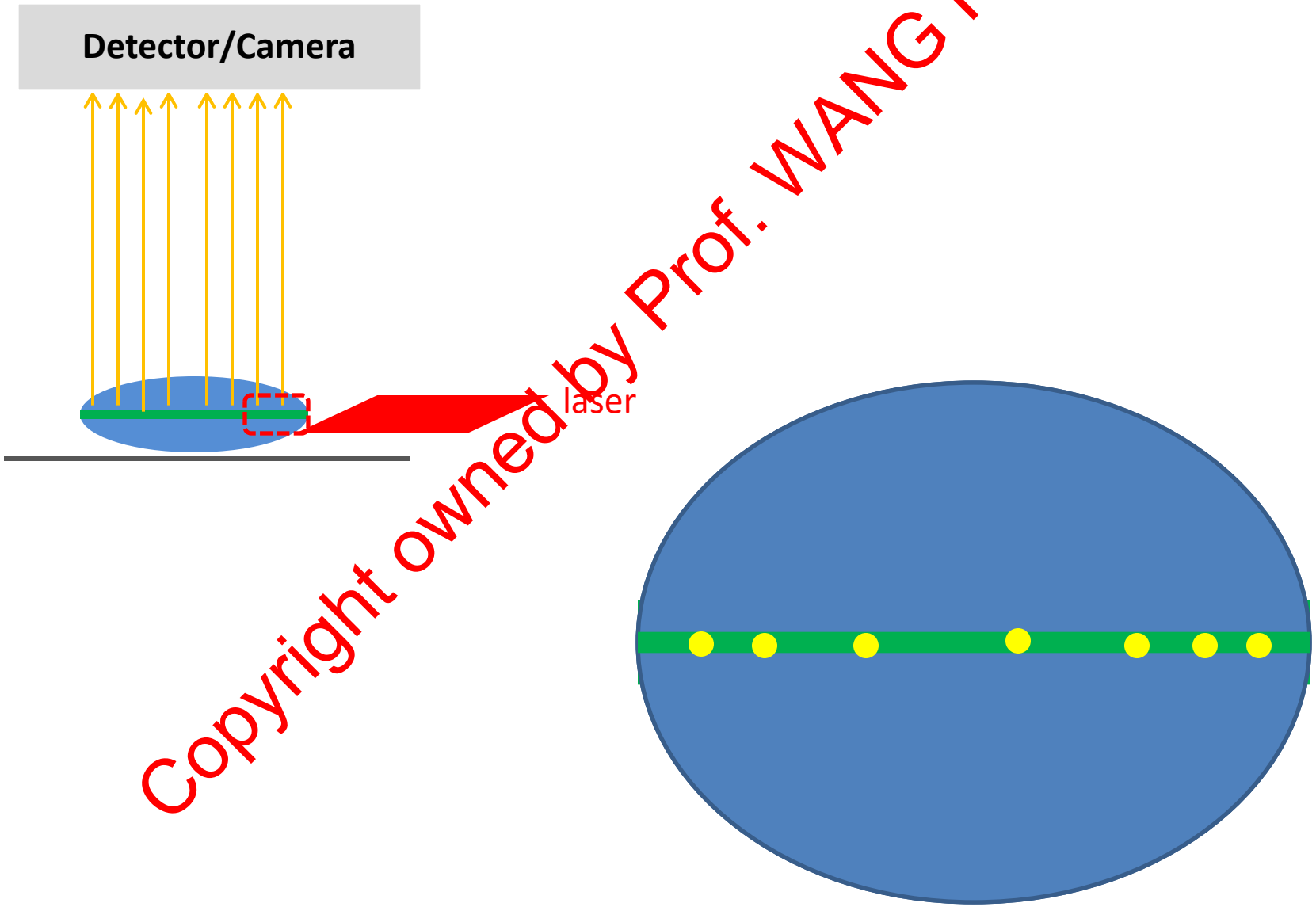
Prize share: 1/3

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



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!

Watching mitosis

Lattice Light-sheet Microscopy

Lattice Light-sheet Microscopy