

# **Excitation microscopy** Anna Ayyazyan



# Outline



Confocal vs Two-photon microscopy Application of 2P microscopy Sutter MOM fluorescence microscope at DELTA Examples 3D imaging



# Two-photon excitation microscopy applications

- Bio-medicine
- Bio-chemistry
- **Bio-physics**
- Bio-technology
- Microbiology

Histology
Environmental sciences
Material sciences
Life sciences
Drug design

## **Two-photon excitation**



specimen at the microscope focal plane.

CANDLE



**Difference between single-photon** and two-photon.





The rhodamine-stained polymer film specimen presented in this figure is itself non-scattering (a) - the intensity exhibits a steady decrease with penetration depth (b) - the intensity is relatively constant with penetration depth

**Intensities of TPEF versus** pulse duration obtained with a 10 and b 40 objectives. The squares are for TPEF signals from dilute fluorescein solution.

> University of California, Irvine Beckman Laser Institute, Laser Micro beam and Medical Program, and Department of **Biomedical Engineering** 5/20

#### Wide field confocal 2 photon





Two-photon fluorescent imaging of pollen grain.

Imaging Quality in Confocal and Multiphoton Microscopy





These images were collected at 80micrometers below the specimen surface, which is the maximal depth allowing sufficient image contrast from this specimen utilizing confocal microscopy. It uses 1047 nm wavelength.

## **Application of 2P microscopy**



#### In vivo tissue imaging.

n vivo two-photon microscopy to 1.6-mm depth in mouse



Two photon fluorescence images of cortical vasculature in a mouse brain. (a) 235 x-y frames.3D reconstruction is made in ImageJ software using the volume viewer plug-in. Expanded 3D stacks are shown for the deepest sections (> 1130  $\mu$ m).

## Fluorescence intensity as a function of imaging depth for the stack .

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**Cornell University, School of Applied and Engineering Physics, New York** 

#### **Compound-selective labeling** Environmental sciences, Bio-medicine, Food control, etc.

Mapping of Heavy Metal Ion Sorption by Using Metal-Selective Fluorescent Probes and Confocal Laser Scanning Microscopy.



Applicability of highly selective metal fluorescent probes for environmental research. Cultures were analyzed, containing Cu2+, Zn2+, and Hg2+.

Likai Hao et al, Appl. Environ. Microbiol. 2013, 79(21):6524.



Fluorescence Microscopy Procedure for Quantitation of Yeasts in Beverages. HERBERT A. et all. Division of Microbiology, Food and Drug Administration, Washington.



Discrimination of (A) dead and (B) living yeast cells . Magnification, x 1,000.

#### Time-lapse imaging



#### Drug design Environmental sciences Histology, etc



(A) Transmission image of the biofilm before treatment. (B) Staining observed in the biofilm immediately prior to the introduction of a 0.12% chlorhexidine solution.(C to G) Progressive loss of fluorescence as the chlorhexidine penetrates the biofilm (for time points 90, 180, 270, 360, and 450 s, respectively). (H) Transmission image of the biofilm after treatment. Scale bar, 30  $\mu$ m.

#### Center for Biofilm Engineering, Montana State University, New Jersey.

### Sutter MOM Instruments 2P Microscope at DELTA laboratory





## Excitation and fluorescence spectra CANDLE of some fluorofores.









600 700 **11/20** Wavelength (nm)









very important to receive quality image without any sample damages Images were obtained on Sutter MOM Instruments 2P-laser scanning microscope in the AREAL



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Rat chromosomes (1) and cell nuclei (2) after tissue irradiation by electron beam on AREAL linac



Bottom surface of the kalanchoe leaf with stomas.



# Institute of Molecular Biology (NAS RA).The influence of oxidative stress protectors on the organism.



The images were obtained using a Reactive Oxygen Species (ROS)-sensitive fluorescent probe – chloromethyl-2'7'-dichlorofluorescein diacetate (CM-H2DCFDA)-AM

**Institute of Physics Research ZnO based semiconductor surfaces with different** impurities. **Er-doped** 



**Li-doped** 



Institute of Biotechnology interaction of the different cultures during cooperative fermentation for production of the biologically active compounds.





# **3D imaging**





The number of cross sections of pollen grain .

The 3D Image of erythrocytes made by ImageJ software





20 µm









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