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Imaging Platform

DRESDEN
concept
Exzellenz aus
Wissenschaft
und Kultur

Introduction to Multiphoton Microscopy

Sebastian Bundschuh, MPI-CBG

TECHNISCHE UNIVERSITÄT DRESDEN
biotec Biotechnology Center TU Dresden
CRTD Center for Regenerative Therapies TU Dresden
DZNE Deutsches Zentrum für Neurodegenerative Erkrankungen in der Helmholtz-Gemeinschaft
CBG Max Planck Institute of Molecular Cell Biology and Genetics
Carl Gustav Carus 1789 – 1869
DFG Deutsche Forschungsgemeinschaft

Conventional Single-Photon Microscopy

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- **Conventional light microscopy limited to tissue surface**
  - ➢ up to ~100 µm into the tissue

  ➔ **Absorption of light**

  ➔ **Scattering of light**

Multi-Photon Excitation

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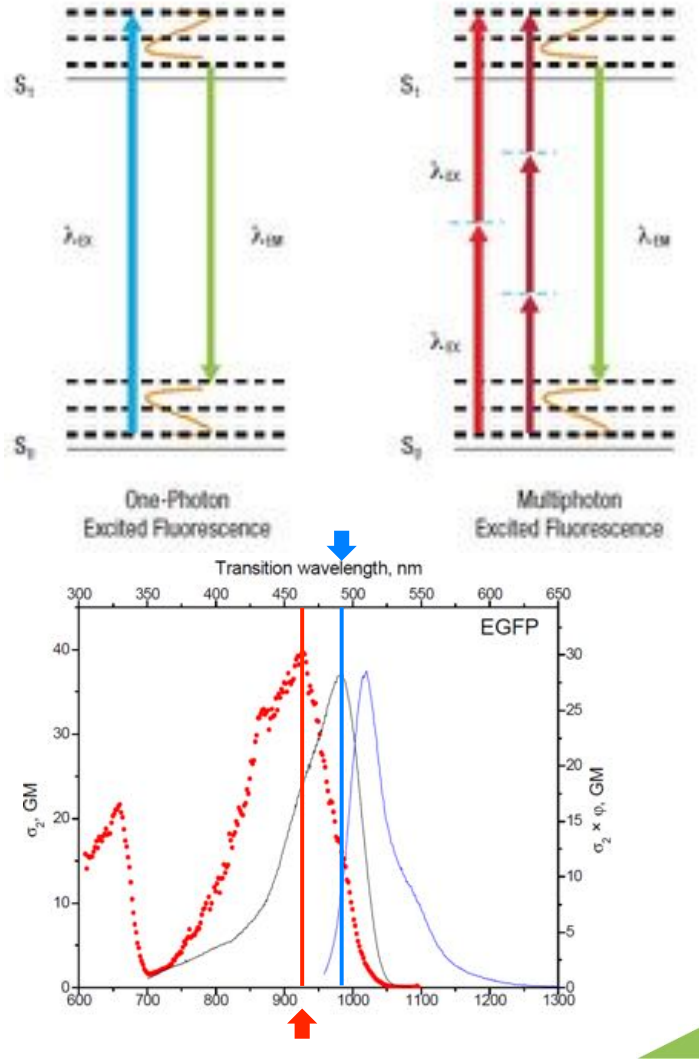
- **Excitation with more than one photon**
  - Photons with lower energy required
  - Longer wavelength excitation in the Near Infra-Red (NIR) range
- **Very low probability**
  - Absorption of two photons within a time window of 10^{-18}s
- **Requires very high density of photons**
  - Pulsed femtosecond laser
  - Peak power 10 million times higher



Near Infra-Red Excitation

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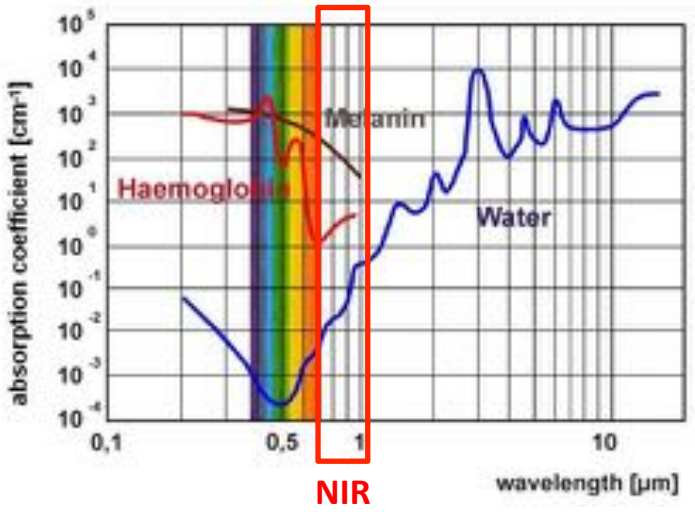
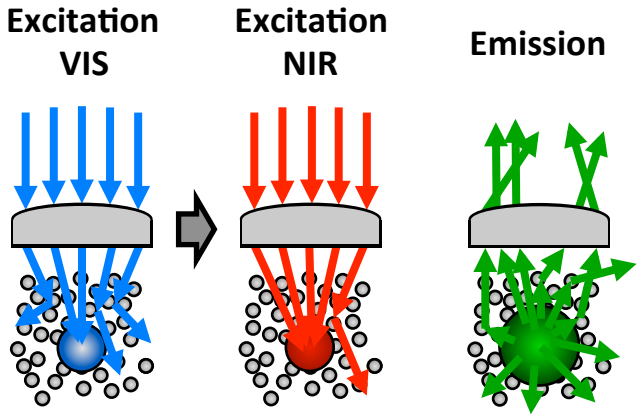
- **Absorption within tissue**
  - Water: Minimal in visual range
  - Haemoglobin/Melanin: better in near infra-red range

- **Scattering**
  - Excitation light scattered
    → Reduced focality
  - Emission light scattered
    → Image bluring
  - Wavelength dependent (scales with $1/\lambda^4$)

Improved tissue penetration





Optical Sectioning

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Single-photon excitation | Multi-photon excitation

- **Single-Photon**
  Excitation along the entire illumination path
- **Multi-Photon**
  Excitation only within the focus

Optical Sectioning

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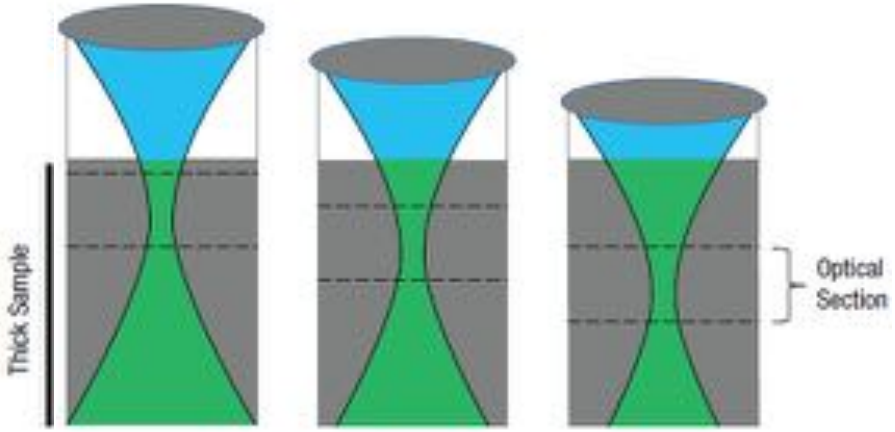
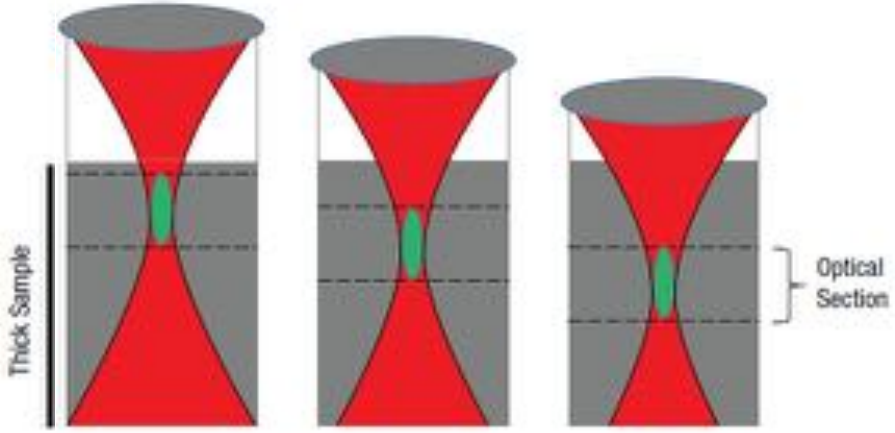
Single-photon excitation



Multi-photon excitation



- **No out-of-focus excitation**
  - ➢ Less bleaching
  - ➢ Less photo-toxicity
- **All signal originates from focus**
  - ➢ No pinhole required
  - ➢ Signal can be detected from anywhere

Basic Properties

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- **"Point" illumination technique**
- **Detector: PMT**
- **Relatively low temporal resolution**
  - Typically seconds per image with a standard FOV of 512x512 pixels
  - Can be quite fast for low pixel numbers

Non-Descanned Detection

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- **Detectors close to objective**
  - ➢ Higher sensitivity
- **Sensitive to scattered light**
- **Detection of confocal signal in epi- and trans-direction**

Up to 1 mm deep into tissue

Advantages

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- **Deep Tissue Penetration**
- **No bleaching outside the focal plane**
- **Less photo-toxicity**
- **Intrinsic signals from various structures (SHG/THG)**

Deep Tissue Imaging

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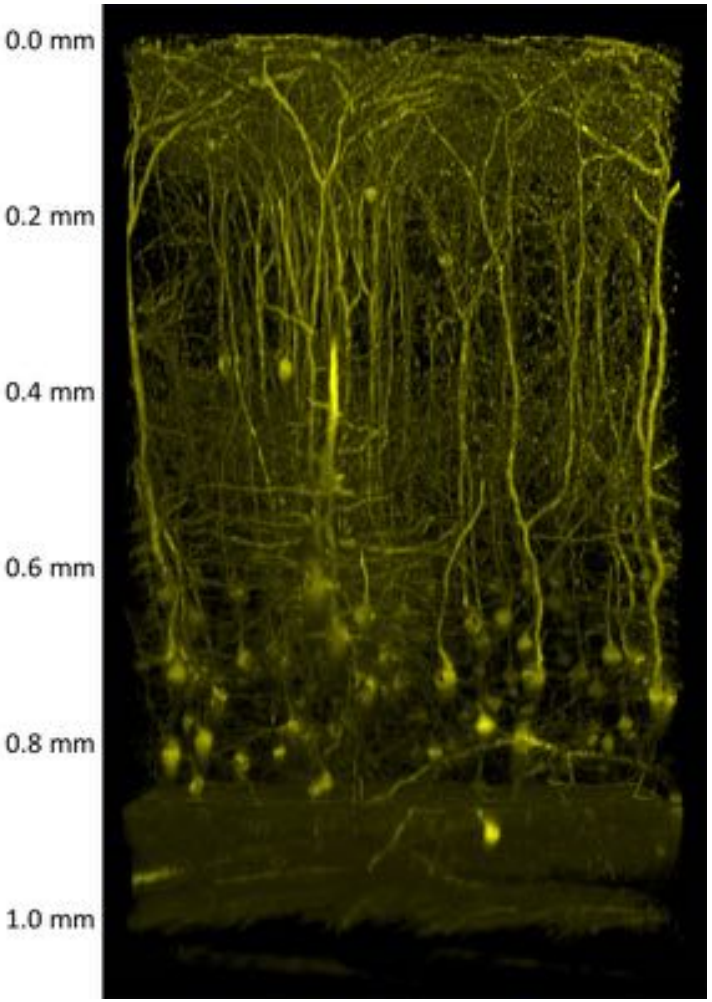

Primary somatosensory cortex, 8-week-old mouse expressing thy1-YFP

Dr. Hajime Hirase and Katsuya Ozawa, RIKEN Brain Science Institute, Wako, Japan

Deep Tissue Imaging

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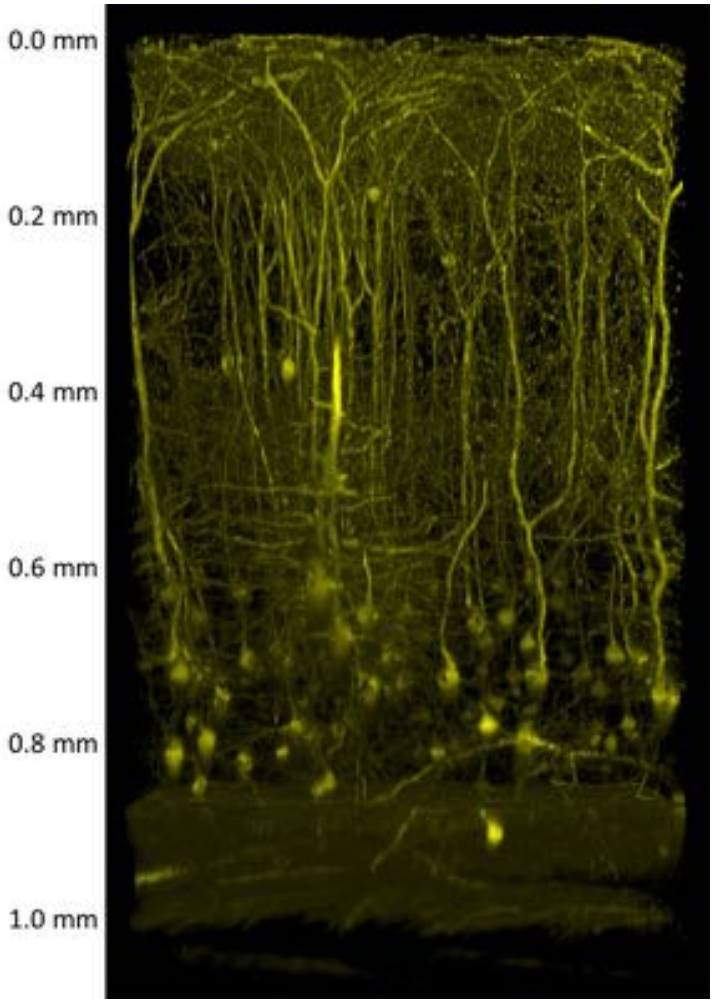

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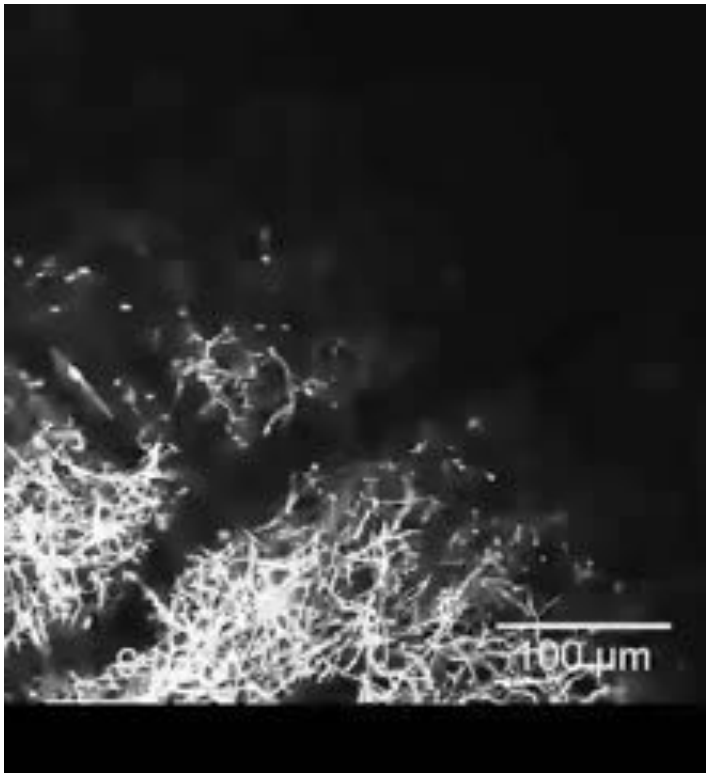

Functional Calcium Imaging *in vivo*

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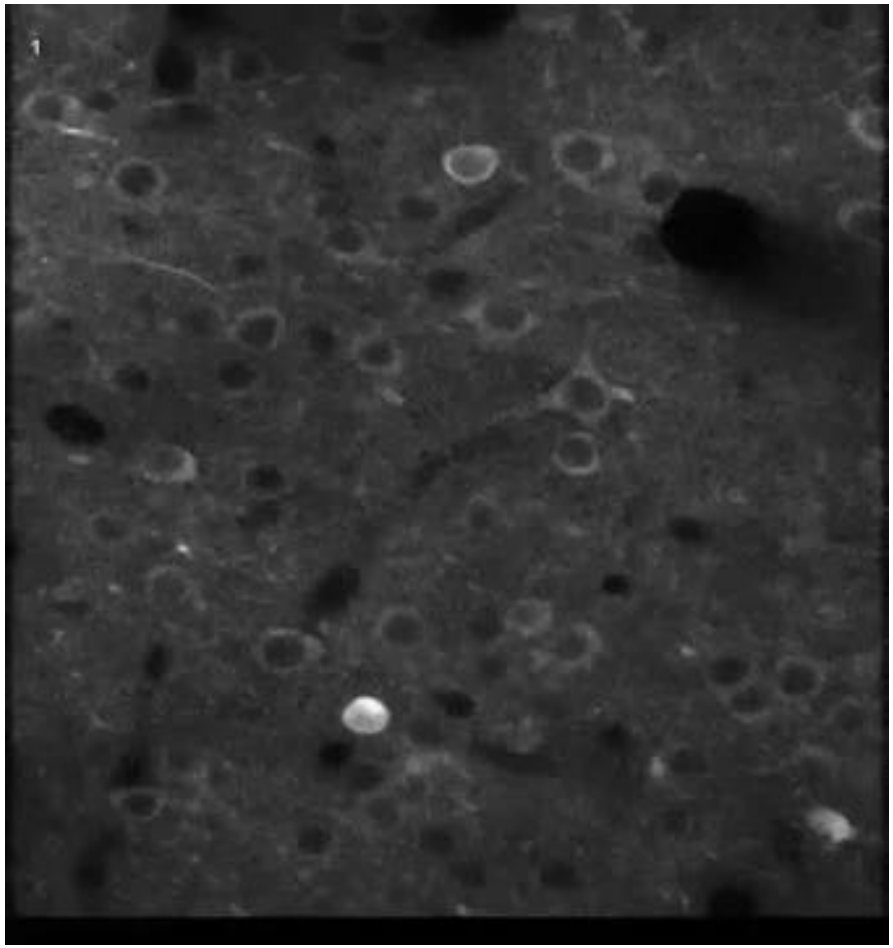
Layer 2/3 neurons in visual cortex

Awake mouse expressing the calcium indicator GCaMP5G. Calcium signals in response to a moving grating stimulus. Images recorded at 28 fps (sped up for presentation purposes)



Dr. Tobias Rose, Max Planck Institute for Neurobiology, Munich, Germany

Applications

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- **Imaging of thick specimen**
- **Long-Term Imaging**
  - Development
  - Functional imaging
- **Intravital / *in vivo* Imaging**
  - Entire tissues in the living animal
- **Imaging of Light-Sensitive Structures**
  - Retina

Limitations

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- **Lower resolution than single photon point-scanners**
- **Multicolor imaging more difficult**
  - ➢ Broader excitation cross-section of fluorophores
  - ➢ Usually only a single laser line
- **Many dyes not well characterized**
  - ➢ Excitation Spectra also depend on laser properties
- **Fine line between imaging and cutting**
- **Expensive**

Recent Development

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- **Wavelength range of lasers extended to 1400 nm**
  - Better excitation of far-red dyes
  - Three-photon / THG imaging
- **Simultaneous imaging with multiple laserlines**
  - Improved multicolor imaging
- **Transfer of multi-photon excitation to other imaging modalities**
  - E.g. Lightsheet microscopy

Inventor

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Winfried Denk

Max Planck Institute of Neurobiology, Germany