Deep tissue two-photon microscopy

How it works, limits and applications

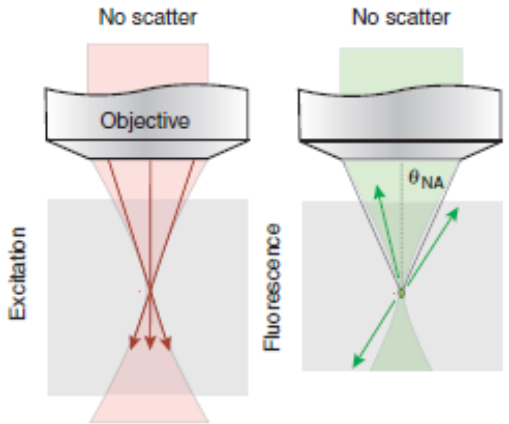
Vincent Adam

Gatsby Tea Talk, 16/05/2013

How it works

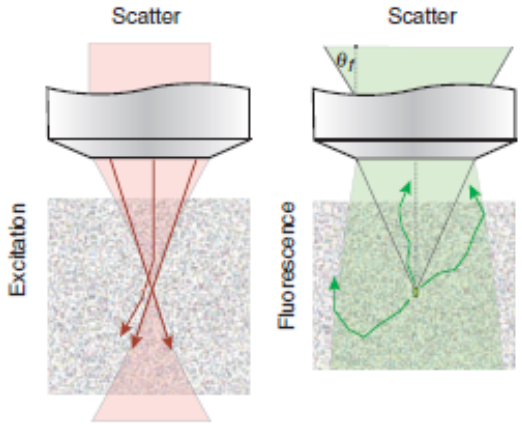
Goal: Imaging specific elements in deep tissue
How: Use absorption processes for contrast generation at precise location (focus a light beam)

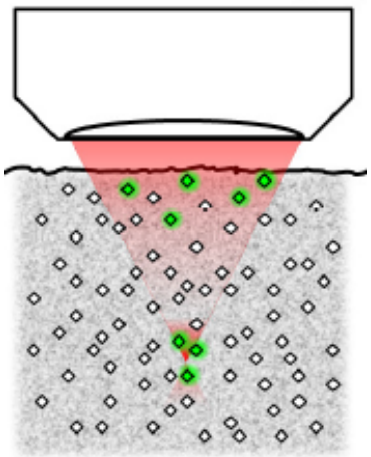
- Excitation: Send light on tissue
- Detection: Detect fluorescence response



Main Problems

1. Biological tissues strongly scatter light (very little absorption)
   $\rightarrow$ Difficult to light desired target = need more power
2. Light has to go through layers of tissue to reach deep targets
   $\rightarrow$ Potentially activates fluorescence at undesired locations = reduce contrast



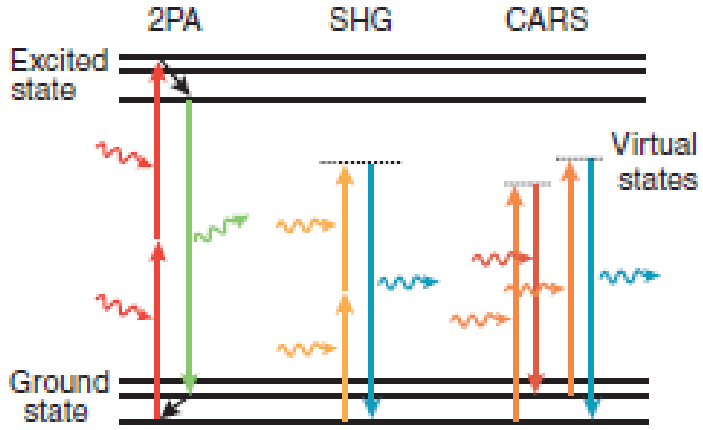


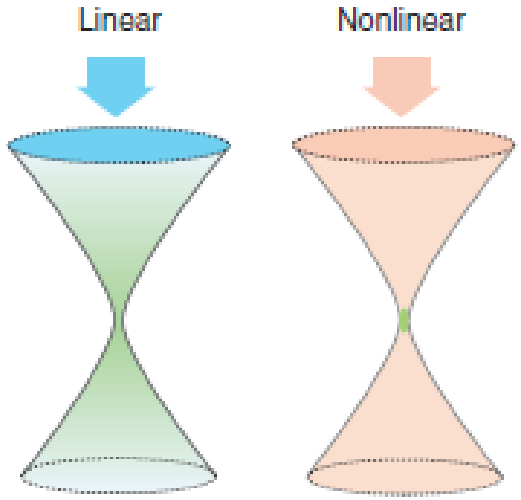
Problem: Illuminating the desired target

Solution:
Use non-linear absorption process (focus on multiphoton absorption)

Main Advantages:

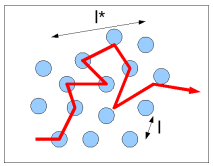
- Better spatial concentration of excitation
- photons combine their quantum energy
  - Low energy excitation (less damage, less scatter)
    Typically near-infrared 700-1000
  - High energy emission (visible range)
- Reduce early layer excitation problem





Problems: Scattering

- Strengh of scattering is described by the **mean free path** l_s: average distance between scattering events.



  Mean free path depends on
  - wavelength (the biggest the more)
  - tissue composition

  For brain grey matter: $l_s = 50 - 100\mu m$ at $630nm$ (red)
- Illumination power in tissue (of non scattered -or ballistic- photons)

$$P_{ball} = P_{surface} e^{-z/l_s}$$

- Fluorescence signal power (assuming same scattering)

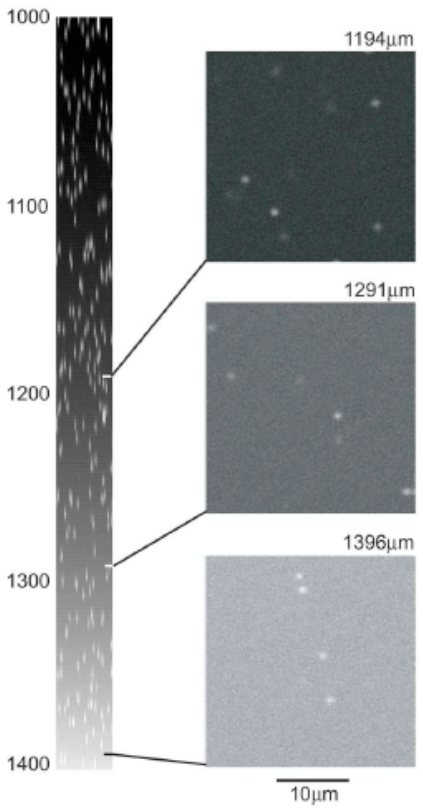
$$P_{ball} = P_{surface} (e^{-z/l_s})^2 = P_{surface} e^{-2z/l_s}$$

Problems: A fundamental limit

There is a fundemental limit in terms of depth.

Contrasts degrades as

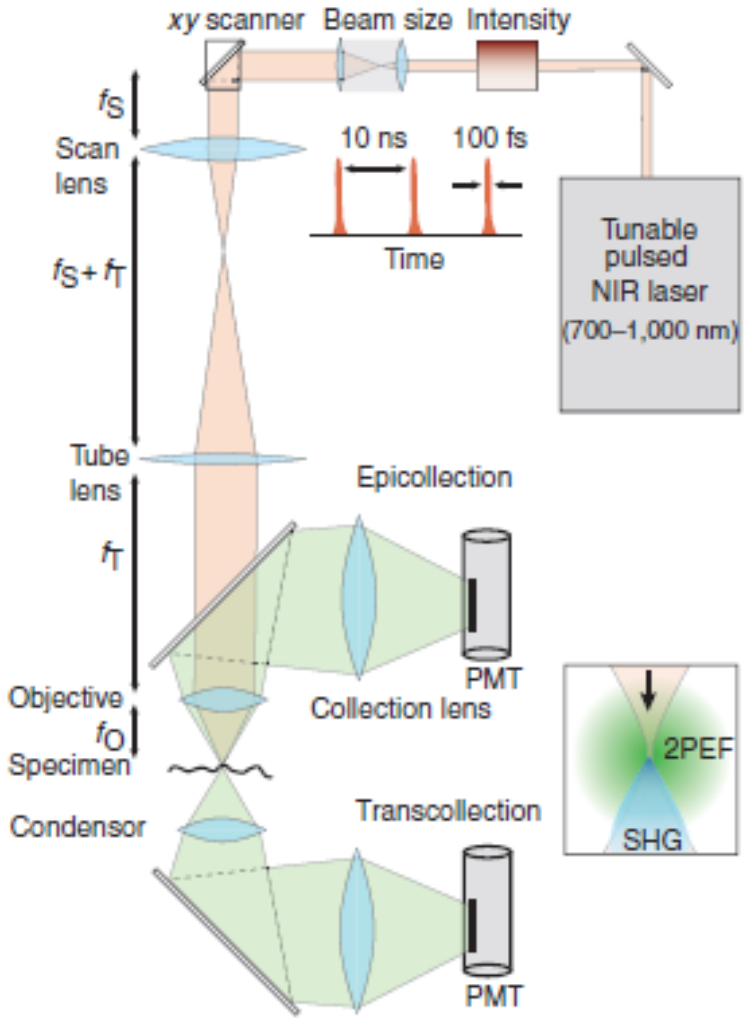
- more power is needed to reach deeper target
- this power excites early layers



A classical set-up

Classical set up

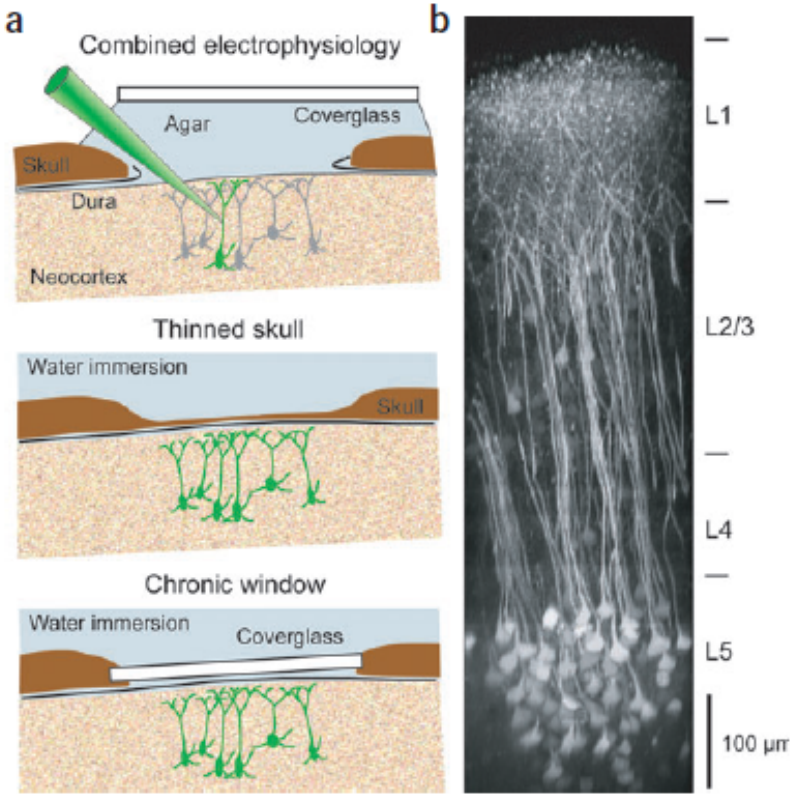
- Pulsed Laser source
- Intensity modulator
- Beam expander
- Deflection module (for scanning)
- High NA objective
- Detection module



Applications

Applications to neuroscience

- Monitoring cell structure and function (after in-vivo labelling)
- Imaging cellular network dynamics
  (e.g. calcium indicators)



Thank you for your attention