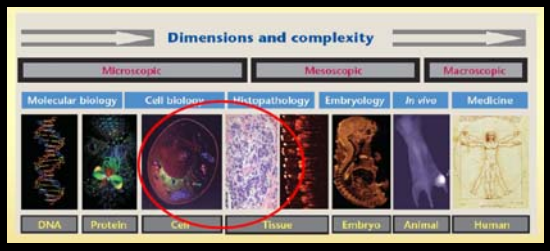
Neuro-imaging: looking with lasers in the brain

Marloes Groot
Vrije Universiteit Amsterdam
Faculteit Exacte Wetenschappen-Natuurkunde
Biophotonics group

LaserLab Amsterdam — vrije Universiteit amsterdam — Neuroscience Campus Amsterdam

Aim: *To image life cells, label-free, with cellular resolution in deep-tissue*



Neuroscience Campus Amsterdam

Confocal and non-linear microscopy

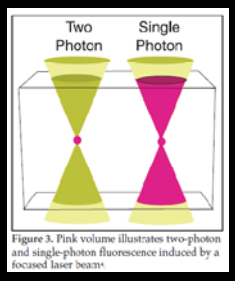


Figure 3. Pink volume illustrates two-photon and single-photon fluorescence induced by a focused laser beam[4]

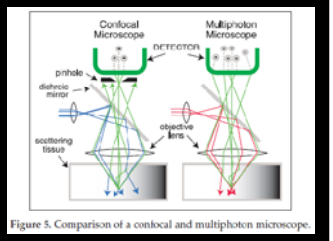
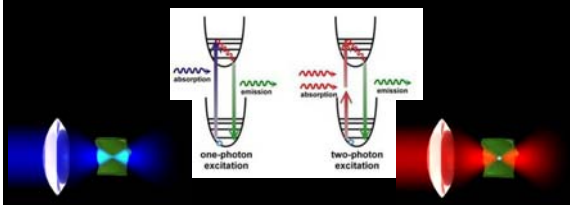


Figure 5. Comparison of a confocal and multiphoton microscope.

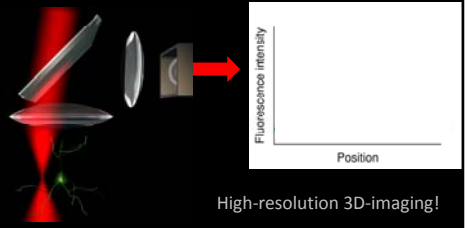
Nonlinear microscopy with fluorescent labels

- Laser-induced nonlinear process provides contrast.
- Localized to the laser focus, since excitation ~$I^{2\text{-}3}$.
- 3D-imaging by scanning the focus through the sample.



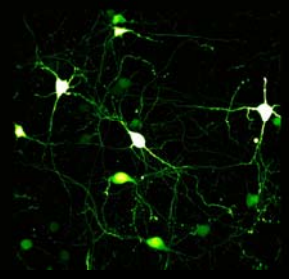
Two-photon fluorescence microscopy

- Laser excitation of a nonlinear process.
- Localized to the focal spot.
- Scan the laser beam and map fluorescence vs. position.



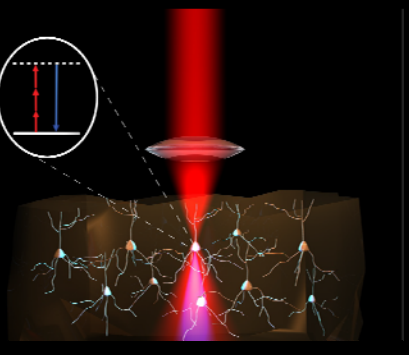
High-resolution 3D-imaging!

Two-photon fluorescence microscopy



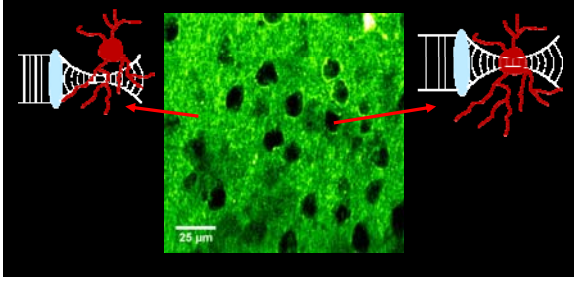
- Interneurons containing Green Fluorescent Protein.
- 2-photon excitation at 970 nm.
- Requires a dye or other fluorescent probe.

Third-harmonic generation imaging



THG microscopy on brain tissue

- THG microscopy on mouse brain tissue.
- Neurons are clearly visible as dark shadows.

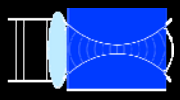


Third-harmonic generation

$$i_{3\omega} = \left(\frac{3\omega}{2n_{3\omega}c}\right)^2 \chi^{(3)} I_\omega^3 \left| \int_{-\infty}^{\infty} \frac{e^{i\Delta kz}}{(1+2iz/b)^2} dz \right|^2 , \text{ where } (\Delta k = k_{3\omega} - 3k_\omega)$$

Isotropic medium, tight focusing:

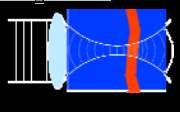
THG generated before and after the focus cancel due to Gouy phase (if Δk≥0).



→ No THG

Discontinuity (in $n_{3\omega}$ or $\chi^{(3)}$):

Asymmetry in phase before and after focus, no THG cancellation.

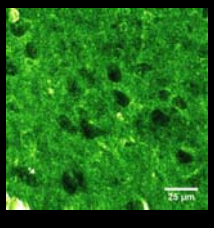


→ THG signal!

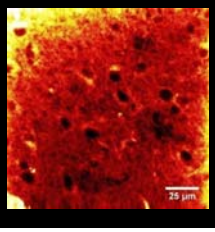
Origin of the THG signal

- The main component providing a high $\chi^{(3)}$ are the lipids in the cell membrane.
- Checked by staining with the lipid-sensitive dye Nile Red:

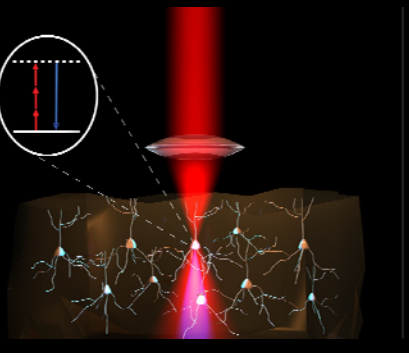
THG signal:



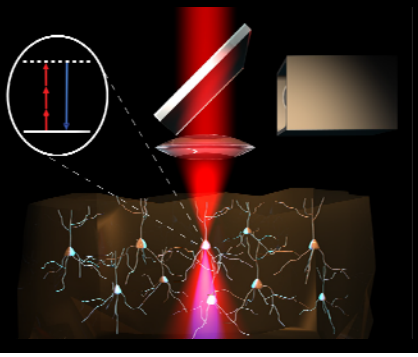
Nile Red fluorescence:



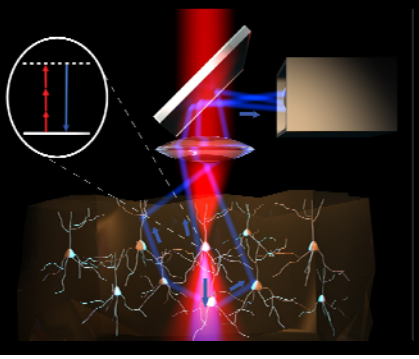
Third-harmonic generation imaging



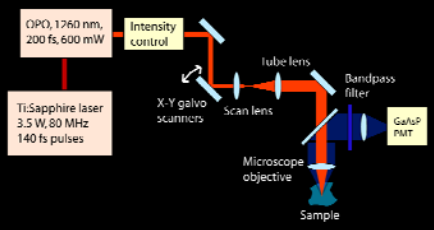
Third-harmonic generation imaging



Third-harmonic generation imaging



THG microscopy setup



- Optimal wavelength range 1200-1350 nm.
  - UV generation at shorter λ
  - Water absorption at longer λ

THG brain imaging

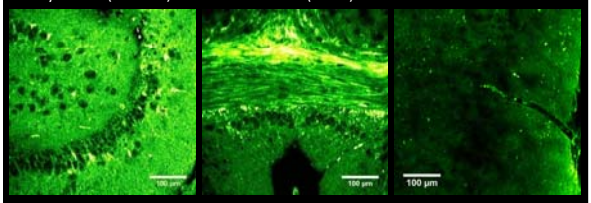
Depth scan through the prefrontal cortex of a mouse:

Image size 500 x 500 µm.
Scanned depth 360 µm.

THG brain imaging

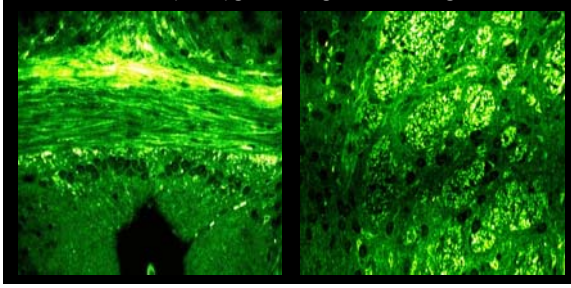
Various brain structures can be imaged simultaneously:

Grey matter (neurons): White matter (axons): Blood vessels:



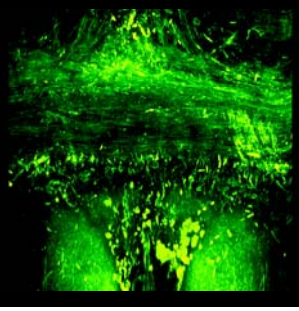
THG brain imaging

- White matter structures can be visualized as well.
- Fiber bundles (axons) generate significant THG signals.



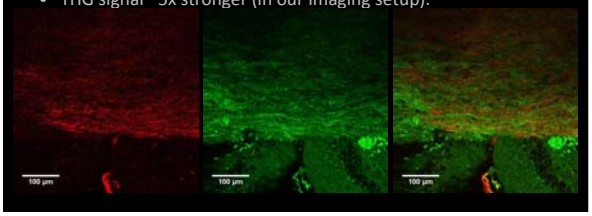
THG brain imaging

3D-reconstruction of the Corpus Callosum from a mouse brain:



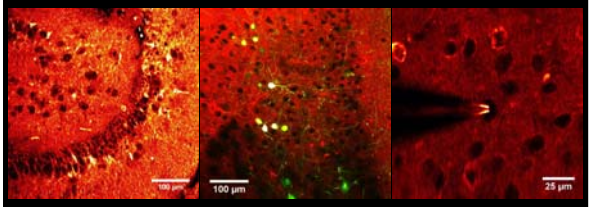
SHG versus THG imaging

- Webb et al. (PNAS): uniform polarity microtubule ensembles (axons) produce SHG.
- THG shows both myelin sheaths (lipids) and grey matter.
- SHG is polarization dependent.
- THG signal ~5x stronger (in our imaging setup).



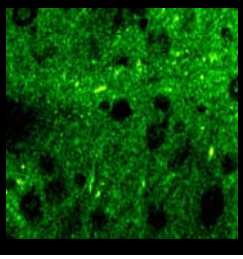
THG brain imaging

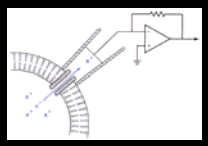
- Versatile and high-resolution brain imaging.
- Combined THG and 2PF well possible.
- We have performed THG-guided patch clamping on neurons.



THG guided patch clamping

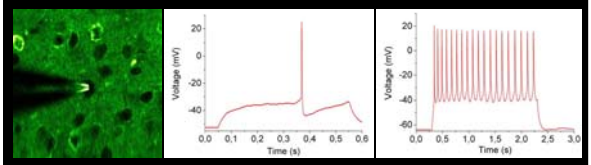
- THG imaging allows us to guide manipulation tools into tissue.
- Example: a patch-clamp micro-pipette inserted into a designated neuron.





THG guided patch clamping

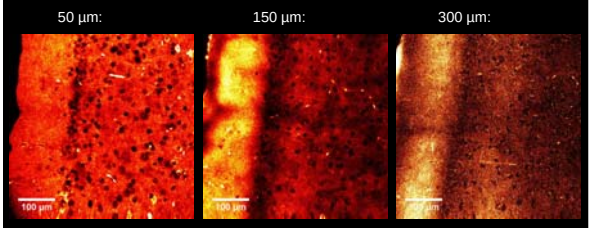
- By placing an electrode inside the pipette, we can monitor and control the neuron's electrical activity.
- Action potential firing in response to current injection:



- Demonstrates our ability to manipulate living tissue with sub-cellular precision.

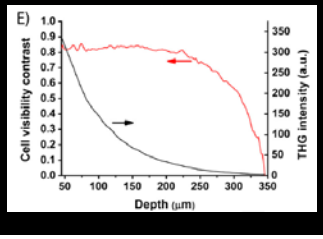
Cell visibility

- Detectable signal down to a depth of 350 µm.
- Cell contrast still good, limited by signal strength.



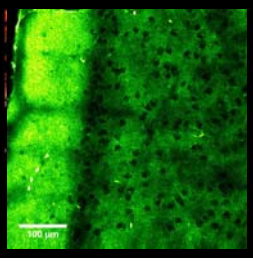
Cell visibility

- Define cell contrast as $C = (I_{outside} - I_{cell}) / I_{outside}$
- Cell visibility limited by uncertainty, not decreasing contrast
- Main limiting factor: $I_{outside} \rightarrow 0$



Reconstructing the neurons

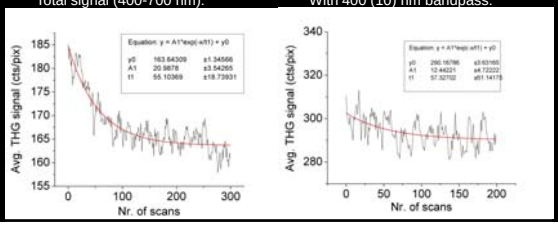
- How to get the structure of the brain?
- Automatic cell detection algorithm reconstructs the neurons.
- Allows selective visualization of all neurons in a piece of tissue.



Bleaching and signal decay

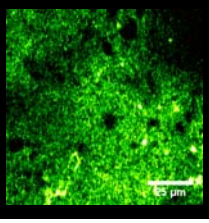
- Some signal decay is observed after extended scanning.
- Fits well to a single exponential plus a constant signal.
  → Auto-fluorescence background on top of the THG signal?

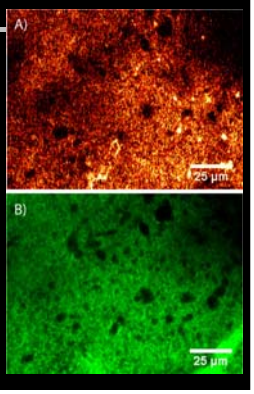
Total signal (400-700 nm): With 400 (10) nm bandpass:



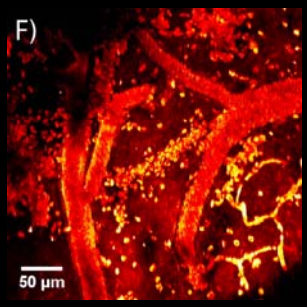
In-vivo THG microscopy

- Imaging the brain of a live, anesthetised mouse.
- Neurons clearly visible at 200 µm depth in the cortex.



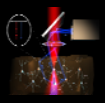


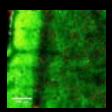
Blood cells..



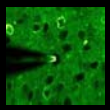
Conclusions

- THG microscopy enables dye-free 3D brain imaging at high resolution.





- Allows high quality reconstruction of various brain structures.
- Allows guiding of diagnostic (surgical?) tools with sub-cellular precision.

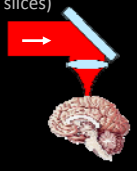


- In-vivo imaging demonstrated

Label-free live brain imaging and targeted patching with third-harmonic generation microscopy
Witte et al, **PNAS** 108, 15, 2011

Perspectives

- Brain tumors: essential to remove only malignant tissue, develop THG for rapid non-invasive “optical biopsy”.
- Apply THG etc in neuromedical research: Image neurodegeneration (Alzheimer) in-vivo (brain slices)
- Super-resolution, down to 20 nm, for neurbiology



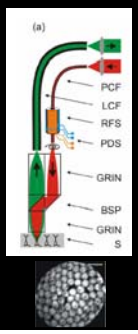
Fiberscope

Label-free cellular resolution during tumor surgery

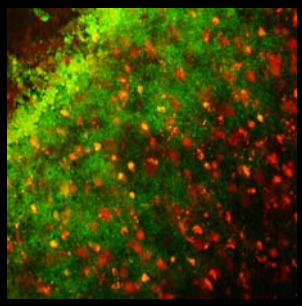
→ Construct fiber-endoscope
- Spatial temporal phase shaping at in coupling
- Validate on mouse models, illumination dose
- Combine with surgery

For sensitive applications: brain, nerves

2-photon fluorescence group of Helmchen, Optics Express 2008

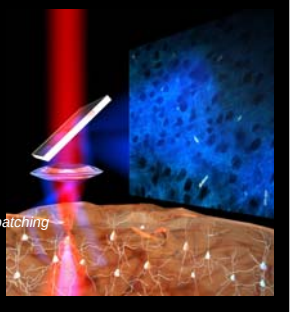


THG (green) SHG (red) of AD patient, PM



People involved:

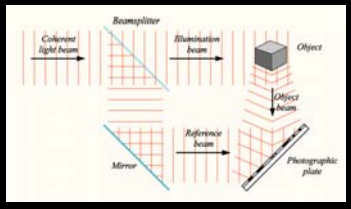
Stefan Witte
Adrian Negrean
Johannes C. Lodder
Christiaan P. J. de Kock
Guilherme Testa Silva
Huibert D. Mansvelder



Label-free live brain imaging and targeted patching with third-harmonic generation microscopy
Witte et al, **PNAS** 108, 15, 2011

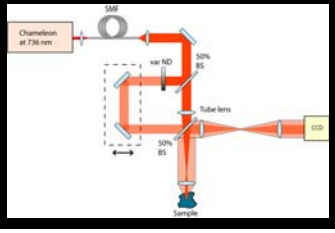
Holography

- Interference between scattered light and a reference beam .
- Intrinsically a 3D technique.
- CCD camera replaces photographic plate → digital holography.



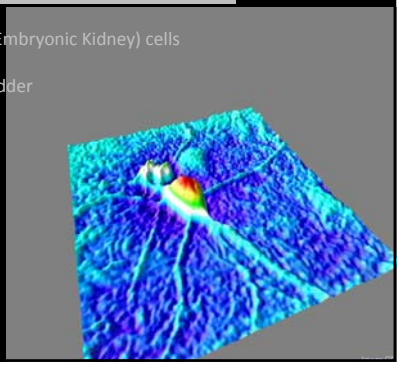
Holographic imaging

- Microscope extended with a reference arm.
- Camera placed in the Fourier plane instead of the image plane.
- Hologram recorded from a slice one coherence length thick.
- Scanning the length of one arm builds a 3D image.



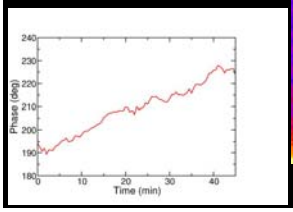
Holographic imaging

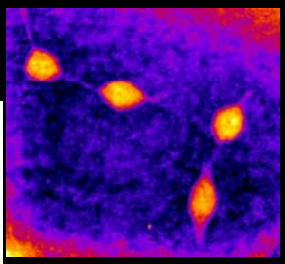
- HEK293 (Human Embryonic Kidney) cells
- With Margreet Ridder



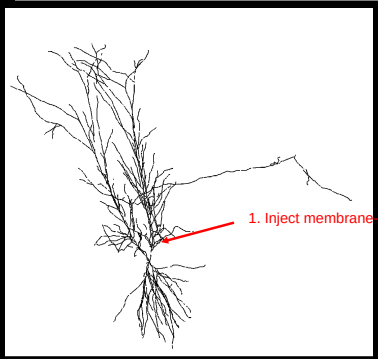
Holographic imaging

- HEK293 cells
- after osmotic shock
- recorded during 45 mins
- With Margreet Ridder

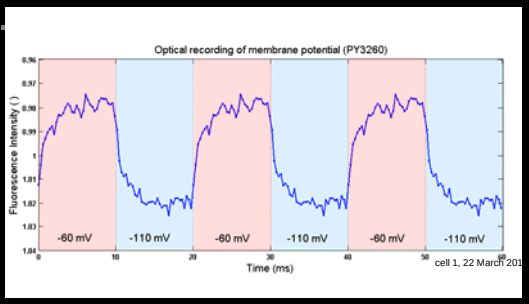




Voltage sensitive dyes, 2PF or SHG



Optical recording of membrane potential steps



- 3500 Hz sampling of 10 ms steps from -110 mV to +60 mV
- In this example the dye sensitivity to Vm is 8.19 % / 100 mV
- average of almost 1200 steps during a 60 s experiment