Second Harmonic Generation Microscopy : A way to study neuronal potentials

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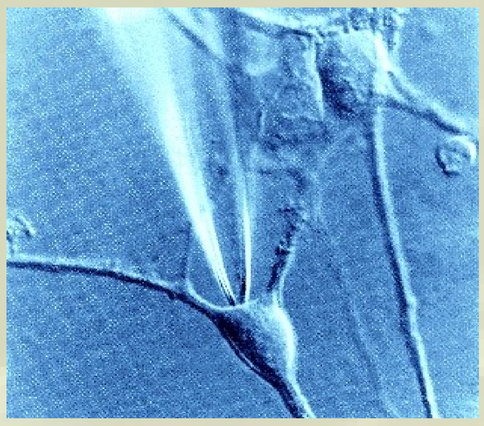
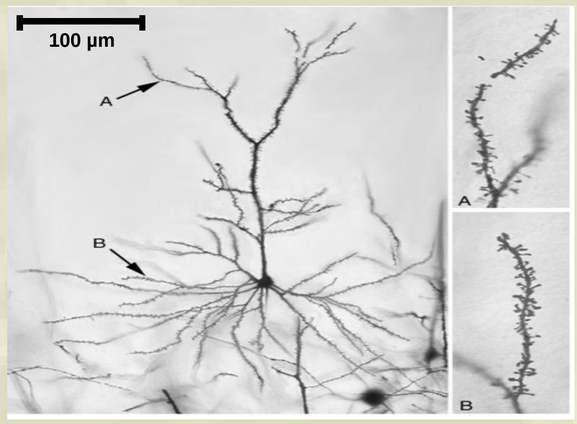
Challenges for optical electrophysiology of subthreshold neural potentials

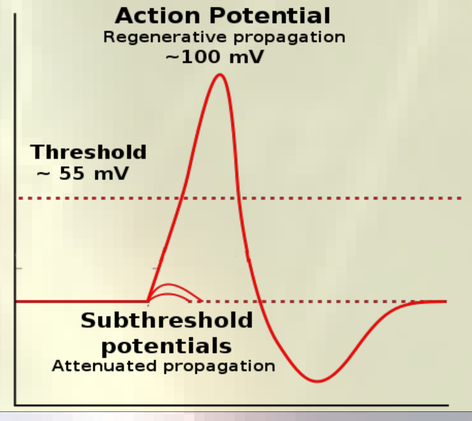
Access to properties of dendrititic tree and spine

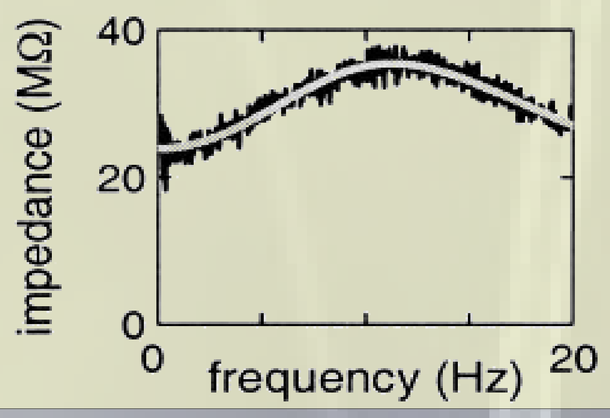




[1]

Subthreshold signals and their frequency dependance

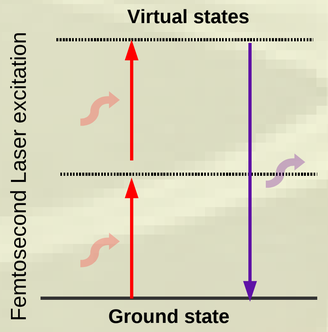




[2]

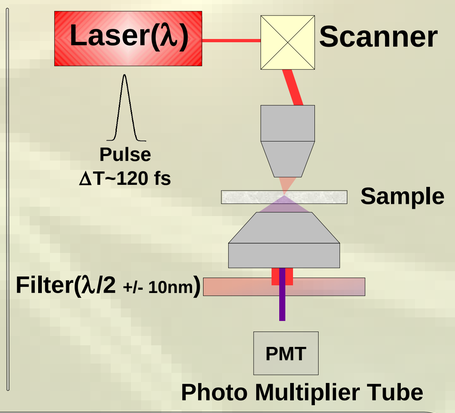
SGH membrane imaging principle

SHG : a non linear phenomenon

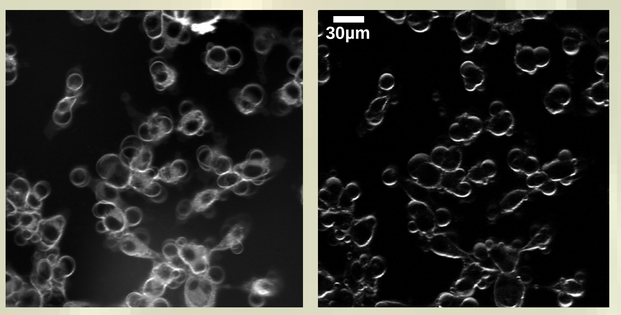


Intrinsic high resolution (µm^3)

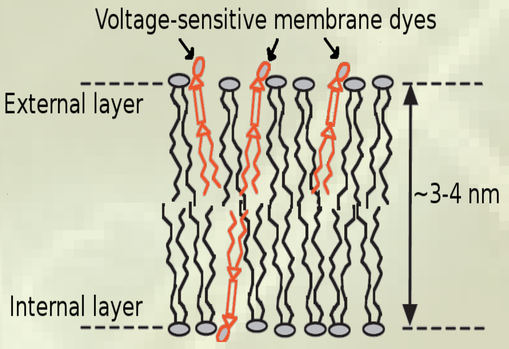
Experimental setup



SHG reveals non-centrosymmetric assemblies



2ph Fluorescence SHG

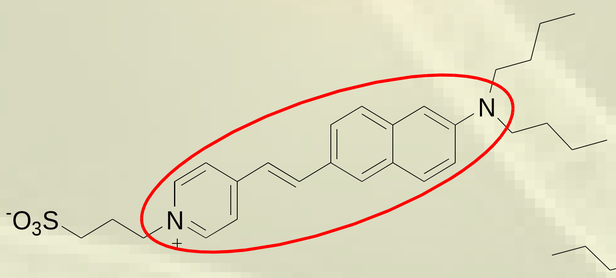


Suppression of background signal and increasing of S/N

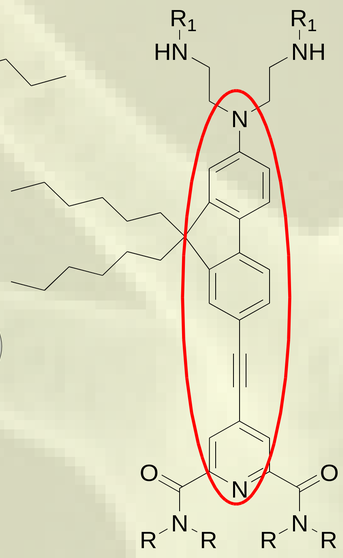
[3]

Improving staining efficiency with new voltage-sensitive dyes

Use new chromophores to enhance SHG and voltage-sensitivity

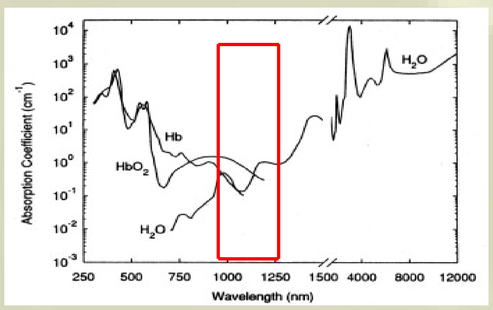


A comercial styril dye : di-4-ANEPPS



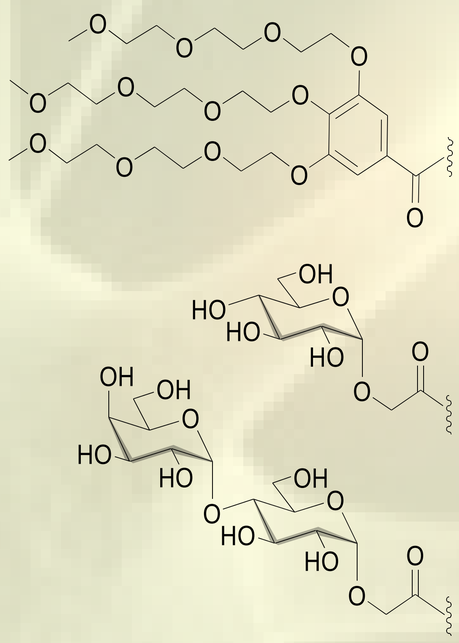
Pyridinedicarboxamide chromophore

Decrease photodamage



Transparency window of biological tissue

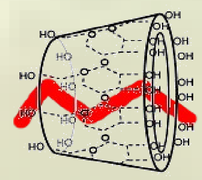
Improve membrane specificity



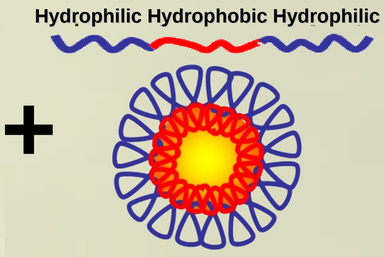
New functional groups tested

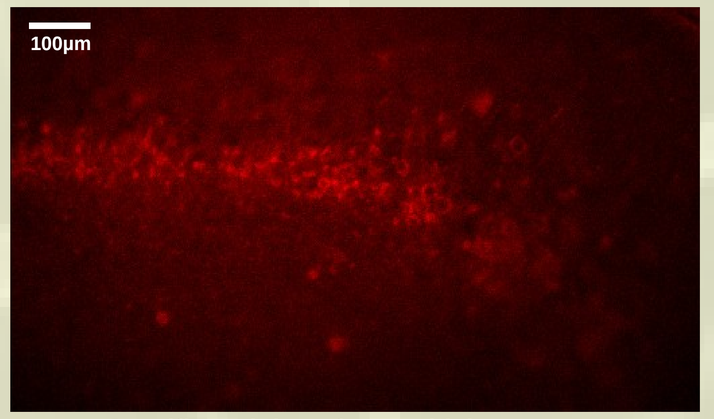
Optimize solubilisation protocols

cyclodextrin



pluronic





Specific staining of pyramidal neurons in acute rat slice

[4]

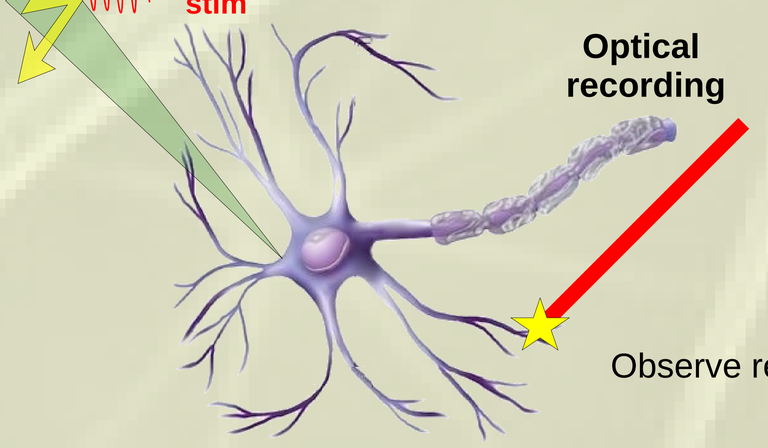
Shed light on neural transfer function

Electrical stimulation

$I_{SHG} = f(\nu_{stim})$

ν_{stim}

Optical recording



Future developements

Increase sensitivity of membrane dyes

Perform innovative solubilisation and transport protocols

Implement targeted path scaning setup

Grow neurons with contolled geometry of dendrite

Enhance S/N with repetitive excitation and acquisition

Observe real-time propagation of subthreshold signal along dendrite

References

[1] Araya et al. *The spine neck filters membrane potentials.* PNAS, 103, 17961-17966. 2006

[2] Schreiberet al. *Subthreshold resonance explains the frequency-dependent integration of periodic as well as random stimuli in the entorhinal cortex.* J Neurophysiol, 92,408-415. 2004

[3] Sacconi et al. *Optical recording of electrical activity in intact neuronal networks with random access second-harmonic generation microscopy.* Opt Express, 16, 14910-14921. 2008

[4] Barsu et al. *Molecular Engineering of Neutral Push-Pull Chromophores for Nonlinear Optical Imaging of Cell Membrane.* submitted. 2009

Acknowledgments

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