# Two-photon calcium imaging with genetically encoded indicators in mouse primary visual cortex (V1) *in vivo*

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Neural system is a highly complex and a dynamically changing biological system in which different measurement tools has been developed according to the spatial and temporal scale of the process we would like to study and understand. This scale can be highly various, from the spatial ranges of neural synapses (100-500 nm) to large-spread neural circuits (1-30 mm), and from time scales of different ionotropic receptors (<ms) to long term plasticity (few days). Two-photon absorption based microscopy allows us to handle most of the aforementioned challenges with an optimal indicator and scanning method.

Understanding the neural code and processing of visual information is a field of research developing faster year by year from the 60's, partly due to the numerous prosperous properties of two-photon microscopy. Using this tool does not simply allow us to measure the activity of V1 (*primary visual cortex*) cortical cells and cell compartments, but to measure population activity of cells in relatively large volume of neural tissue, in vivo.

The purpose of our measurements was on one hand, to study mouse V1 cell responses given to visual stimuli which are not well-described in the literature, using genetically encoded calcium indicators (*GCaMP6*) [1]; on the other hand to map the possible changes in orientation- and direction-selective cell activity after visual behavioral learning tasks, using behavioral systems developed by our group.

We acquired our measurements with 2D galvanometric, resonant and with ultrafast 3D acusto-optical (AO) two-photon microscopes [2] to map the visual sensitivity of the cellular compartments of a given neuron and to study activity of large cell population as well. According to the used architecture we have been able to image hundreds of cells in relatively huge 3D volumes simultaneously, or measure cell activity in deep cortical 2D imaging regions (800 μm), both with the possibility of motion correction.

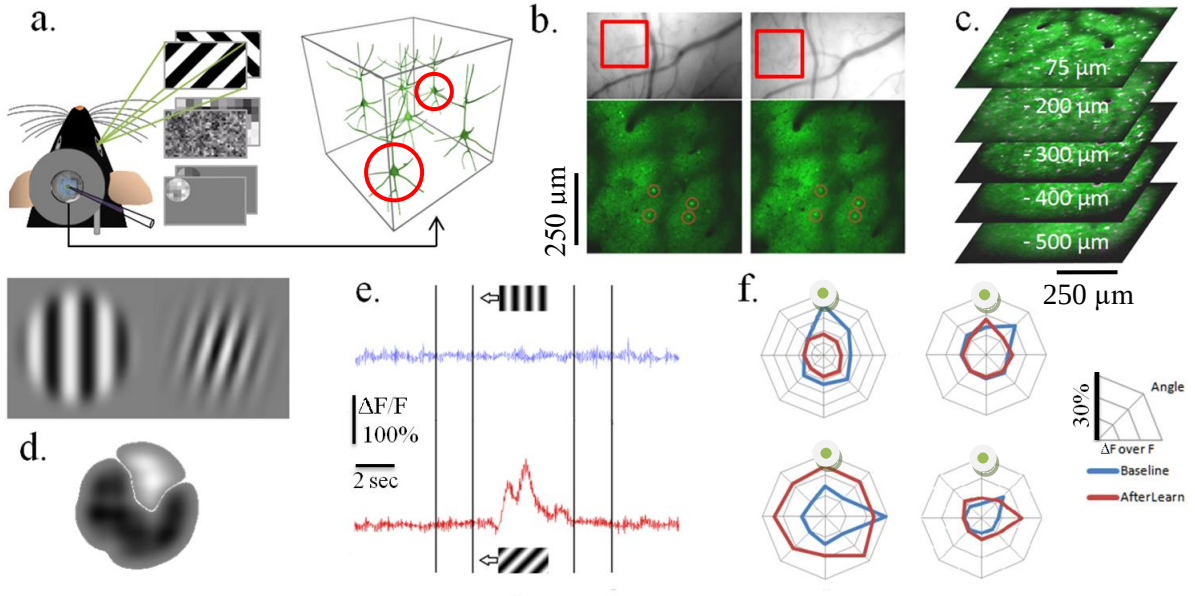


**Figure 1. a.** *Schematic model of two-photon $Ca^{2+}$ imaging during visual stimulation* **b.** *The same imaging site after 5 days in mouse V1* **c.** *Partial view of a Z-stack, made from two-photon microscopic images in different depths of the cortex* **d.** *Model and real receptive field of a neuron as revealed by our measurements* **e.** *$Ca^{2+}$ curves of an orientation- and direction-selective cell for different drifting grating stimuli* **f.** *Orientation- and directionselectivity graphs given to different drifting grating stimuli (from 0°(green spot) to 315° with a 45° step) of V1 cortical cells (n=4) before (blue ) and after (red) visual behavioral learning task*


[1] T. Chen, T. Wardill, Y. Sun, S. Pulver, S. Renninger, A. Baohan, E. Schreiter, R. Kerr, M. Orger, V. Jayaraman, L. Looger, K. Svoboda and D. Kim, 'Ultrasensitive fluorescent proteins for imaging neuronal activity', *Nature*, vol. 499, no. 7458, pp. 295-300, 2013.

[2] G. Katona, G. Szalay, P. Maák, A. Kaszás, M. Veress, D. Hillier, B. Chiovini, E. Vizi, B. Roska and B. Rózsa, 'Fast two-photon in vivo imaging with three-dimensional random-access scanning in large tissue volumes', *Nature Methods*, vol. 9, no. 2, pp. 201-208, 2012.