Wide field, label-free, low fluence multiphoton imaging of neurons with high throughput

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The human brain consists of approximately a hundred billion of neurons that communicate through a unique series of biochemical and electrical processes. When neuronal networks are solicited, electrochemical processes happen involving communication between millions of cells within a complex network. The morphology of the neuronal cytoskeleton is fundamental in the establishment of these complex neuronal networks and crucial for the functional integrity of electrical signaling. Traditionally, the neuronal morphology and electrical signals are measured with invasive optical probes, exogenous dyes, or by exogenous electrical recordings. To fully understand the underlying mechanisms involved in neuronal activity (morphological and electrical) and for eventual clinical applications, a direct label-free non-invasive optical probe is of great significance. For many years second harmonic (SH) imaging has been a promise for delivering direct label-free neuronal membrane potential information. However, to date this promise has not been fully delivered, owing to the intrinsic low sensitivity of the method.

We developed a high throughput wide-field second harmonic (SH) imaging system that increases the SH imaging throughput by several orders of magnitude. The increase in throughput was achieved with a wide-field geometry and medium repetition rate laser source in combination with a gated detection. In addition to the enhanced throughput, dynamic and ultrafast measurements can be performed readily with different possible polarization configurations of the excitation and detection. With this technique, we show the possibility of label-free imaging of neuronal structures and electrical neuronal activity employing the unique intrinsic sensitivity of nonlinear optical techniques.

To demonstrate the concept, we perform a side-by-side patch-clamp and second harmonic imaging comparison to demonstrate the theoretically predicted linear correlation between whole neuron membrane potential changes and the square root of the second harmonic intensity. We assign the ion induced changes in the second harmonic intensity to changes in the orientation of membrane interfacial water, which is used to image spatiotemporal changes in the membrane potential and K+ ion flux. We observe a non-uniform spatial distribution and temporal activity of ion channels in cultured neurons. These results demonstrated the possibilities to image in time, label-free and with low fluence, electrical neuronal activity employing the unique intrinsic sensitivity of nonlinear phenomena.