

**A new 3D imaging approach reveals the secrets
of cell-cell communication in the brain.**

Brain activity is studied in humans at a macroscopic (large-scale) level with brain imaging techniques like functional magnetic resonance imaging (fMRI) to discover the areas activated by specific tasks; it is also studied more microscopically in animal models, through cellular imaging techniques, to understand how the brain works at an elementary level. High-resolution two-photon microscopy is the state-of-art approach to monitor computations at neuronal synapses. Despite its enormous power, two-photon microscopy has also a major limitation: it can monitor just a single bi-dimensional slice of cell (the focal plane) at a time. While this can still correctly report neuronal firing activity (the action potentials) and synaptic communications, it is inadequate to study communications that involve also other brain cells, like glial or vascular cells, which mostly occur three-dimensionally.

Why studying neuron-glia-vascular communications is becoming so important? Because the inner workings of our brains, such as those underlying our learning and memory capabilities, are increasingly appreciated to recruit cells additional to neurons, "in primis" astrocytes. Astrocytes are the major glial cell population, comprising about half of all our brain cells. These highly complex, star-shaped cells connect with both neurons, vascular elements and other glial cells, appearing more and more as "crossroads" elements of the brain cell-cell communication network. Their function, however, remains quite mysterious. Historically, astrocytes were long neglected because, unlike neurons, they do not communicate via electrical signals. However, the advent of fluorescence microscopy and calcium ion imaging in the 1990s brought about a real revolution in our perception of their relevance, by progressively revealing the presence in astrocytes of a sophisticated signaling code based on calcium ion concentration changes.

Today an increasing number of neuroscientists think that, through this "calcium code", astrocytes can process information and send rapid signals to modulate synaptic functions and cerebral blood flow. Malfunction of astrocyte communications in pathological states could be among the mechanisms contributing to the pathogenesis of neuropsychiatric disorders. Therefore, decoding astrocyte calcium signals holds the key to revealing the exact role of these cells in brain function and dysfunction. However, the code of astrocyte communication remains elusive as these cells talk a different language than neurons. In the absence of a Rosetta Stone, scientists find it difficult to understand them.

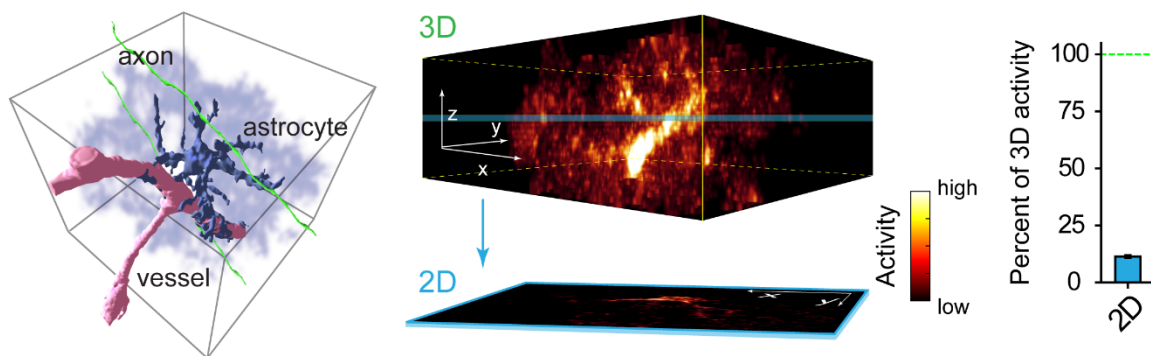
Moreover, while astrocytes are prominently three-dimensional, their activity is studied via bi-dimensional two-photon imaging. This is a critical limitation, as this method monitors at most 10% of the astrocyte structure and activity. By missing the other 90%, it fosters mistakes and contradictions that hinder the deciphering.

Now, the group of Prof. Andrea Volterra at the University of Lausanne has pioneered a new approach allowing correct study of astrocyte-synapse-blood vessel interactions: 3D volumetric two-photon imaging. This method captures the activity ongoing simultaneously

throughout entire 3D astrocytes and connected structures. The breakthrough has required several technological developments, such as use of fast scanning devices, sensitive photon detectors, new calcium sensors transgenically expressed in murine neurons and astrocytes, and appropriate computing infrastructures to analyze the “big data” produced in the 3D imaging experiments. All of this was made possible by grants from the European Research Council and Swiss National Science Foundation, as well as by the constant support from the University of Lausanne. Volterra team has also developed the software for analyzing the Ca^{2+} signals of astrocytes.

The 3D imaging and analysis method enables an unprecedented, comprehensive view of the multiform astrocyte activity. For example, it permits capture of the miniature signals by which astrocytes respond to the activity of individual axonal fibers passing three-dimensionally in their territory. These elementary signals are circumscribed to less than 0.5% of the volume of an astrocyte and could not be reliably detected before, as they are like proverbial needles in the haystack. By revealing them, the present 3D observations also reveal a largely local nature of astrocyte-neuron communications, which appear to occur at thousands of small independent astrocytic locations almost simultaneously, or to travel three-dimensionally from one location to another, encountering unsuspected barriers or privileged paths. Likewise for the signals at the vascular interface, by which an astrocyte may control independently or coordinately the blood flow in multiple vessels crossing its volume.

The paper describing the new method and the first biological observations is published in the 19th May 2017 issue of *Science*.



Left: Astrocytes are highly three-dimensional brain cells that also interact with their neuron (axons) and blood vessel partners in 3D. *Middle:* high-resolution two-photon microscopy technique used until today observes astrocytes in a single focal plane, covering no more than 5% of their volume (2D, blue). In contrast, the new fast 3D two-photon volume imaging method monitors the entire astrocyte, and thereby can detect correctly all its Ca^{2+} activity. The best-placed focal plane monitors no more than 10% of such activity, as shown on the histogram on the *right*.