Deciphering Brain Function with the World's First Multi-Region Miniscope

Simultaneously following the activities of sub-types and/or subpopulations of neurons in multiple brain regions during naturalistic behavior is critical for understanding how the brain encodes, integrates, and processes information. Bruker's Quartet is the only patented multi-region miniscope that offers simultaneous calcium imaging of multiple brain regions in freely behaving animals, enabling greater insights into brain function. This technical note introduces Quartet and its user workflow, and provides examples of brain data the system generates in correlation with various behavior paradigms.

Complete Hardware and Software Solution for Neuroscientists

The brain functions as a complex mechanism, predicated on the delicate interplay of communication between inhibitory and excitatory neurons across multiple regions. Quartet is a hardware and software solution for multi-region miniscope imaging that assists neuroscientists conducting preclinical research aimed at understanding the brain at both intraregional and interregional circuit levels. Quartet employs four highly flexible and lightweight imaging fiber bundles, facilitating the imaging of up to four distinct brain areas in subjects engaging in free behavior or enabling the simultaneous imaging of multiple subjects for high-throughput screening. Figure 1 shows how these IFBs enable the transfer of illumination and images to and from the miniature fluorescence microscope, which is away from the head of the animal

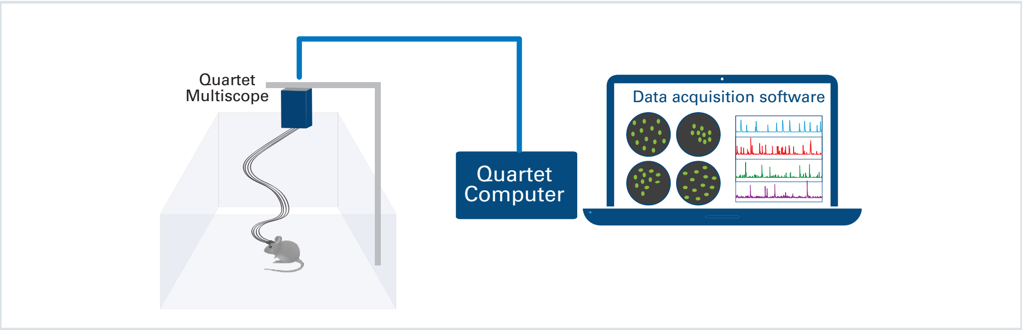

FIGURE 1.
The Quartet system is a hardware-software solution. Imaging fibers transfer information to and from the multi-region miniscope before software enables all required image processing.

and gets situated above the behavior paradigm. The software allows users to capture dynamic neuronal signals, adjusting illumination and contrast for optimal data collection.The brain movies are compatible with automated neuronal segmentation, such as IDPS/IDEAS.[1,2]

Compact and Lightweight GRIN Lens Connectors

Multi-region miniscopes utilize GRIN lens that are designed to have a very small footprint, enabling their use in the most demanding imaging experiments. Figure 2 depicts how a GRIN lens has a cylindrical shape with its flat ends facing the object and the image while a graded refractive index profile created in the glass cylinder enables it to refract the light rays similar

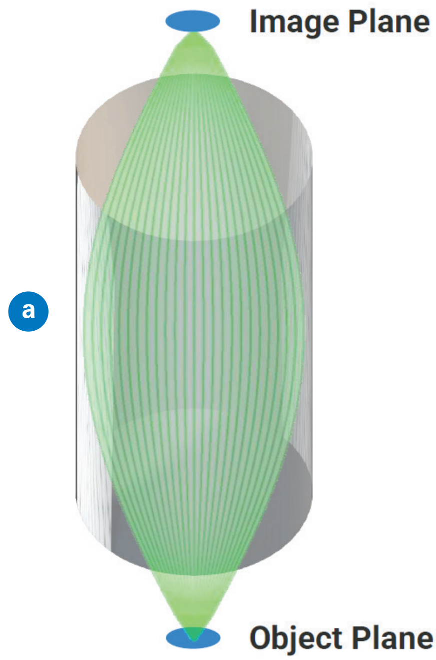

FIGURE 2.
Quartet utilizes Graded Refractive Index (GRIN) lens which have a cylindrical shape and are used to bend the light and relay the focal plane closer to the objective.
(a) Focused image of neuron to be collected by a set of optical elements and imaged on the microscope.

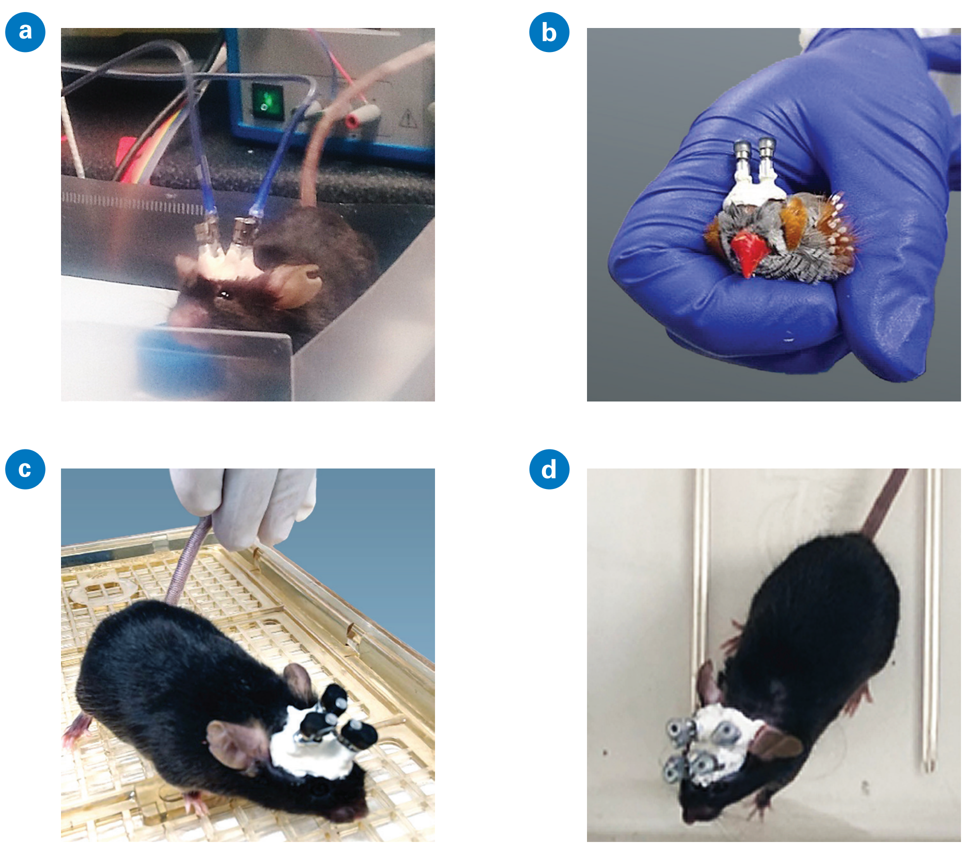

FIGURE 3.
Quartet GRIN lens connectors have a small footprint optimized for various behavioral paradigms. (a) Two GRIN lenses implanted at an angle such that there is a 20° angle between the two. (b) Implantation of two GRIN lenses in the brain of an animal as small as Zebra Finch. (c) three and (d) four GRIN lenses in mouse with minimal interference with animal behavior.

to a regular lens. With the correct choice of design, a GRIN lens can form the real image of neurons on its distal end, out of the brain. A second system of optics, such as the imaging fibers of Quartet, then picks up the real image and transmits it to the rest of the microscope.

Up to four GRIN lenses can be surgically implanted into brain regions of interest with Quartet. The GRIN lenses are designed to be implanted at various angles and have a focus-lock

FIGURE 4.
(a) Dimensions of a short and long GRIN lens with their protective caps on. (b) Piccolo® surgical accessory. (c) Diagram of the tear shape design enabling fitting multiple GRIN lenses on the head of a mouse.

mechanism, allowing the imaging of hard-to-reach brain regions in birds, rodents, and juvenile animals. Fitting four GRIN lenses on the head of an animal as small as a mouse has resulted in them having a small size and tear-shaped form factor, collectively weighing less than 1.5 grams. Furthermore, the Piccolo surgical accessory holds the GRIN lens and connects to a stereotaxic frame to facilitate surgeries and accurate targeting of the brain region of interest.

Flexible and Lightweight Imaging Fiber Bundles

During an imaging-behavior session, imaging fibers are connected to the implanted GRIN lenses, via a simple, yet stable, magnetic mechanism. Each imaging fiber bundle is 84 cm in length and these bundles transmit the illumination light from the LED source (470 nm) to the implanted GRIN lenses. The Illumination source is optimized for imaging green fluorescent proteins, such as GCaMP.[3] The illumination mode is continuous, and the user can vary the

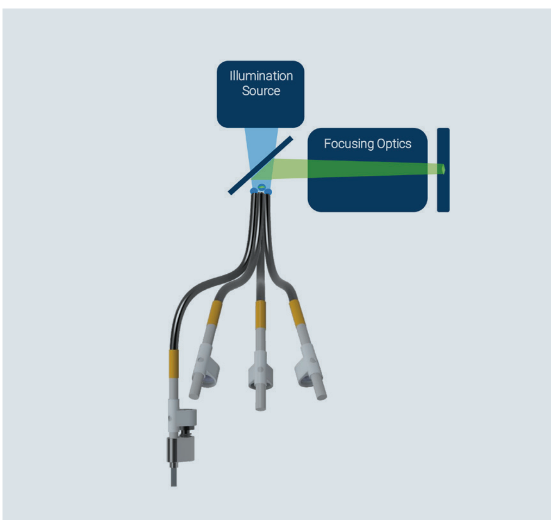

FIGURE 5.
Schematic of Quartet with four imaging fiber bundles and GRIN lenses. The blue illumination light emitted from the LED source enters the fibers, which then transfers it to the GRIN lenses and onto neurons in the live brain. The GRIN lens forms a real image of neurons.

power level from 0 to 1.5 mW. Each GRIN lens focuses the illumination light on the neurons in its proximal end, which is secured in the brain tissue and then forms the image of neurons above its distal end from the brain. The same illumination imaging fiber bundle then picks up this image and transmits it to the camera through a set of focusing optics.[4]

Supporting Various Behavioral Paradigms

The imaging fiber bundles' design ensures adaptability across a spectrum of behavioral paradigms, including open field tests, swimming assays, various mazes, (sp) operant boxes such as lever tasks (reward, decision-making), touch screens, and rotary assays.[4] Quartet's seamless integration with external behavior tracking systems allows for concurrent capture of neural and behavioral data.

The swimming test serves as an example of a behavioral assay, which becomes particularly complex when recording brain activity using systems with electronics mounted on the animal's head. However, the Quartet system's off-the-head electronics enable neuroscientists to easily follow the activities of neurons in multiple brain regions during swimming tests. These experiments are used in various neuroscience investigations, notably as a well-accepted animal model of depression.

The Quartet system's utility also extends to high-throughput screening endeavors, particularly when the dimensions of the behavioral paradigm do not exceed 30 cm X 30 cm, allowing for the implantation of one GRIN lens per subject and the simultaneous brain and behavior

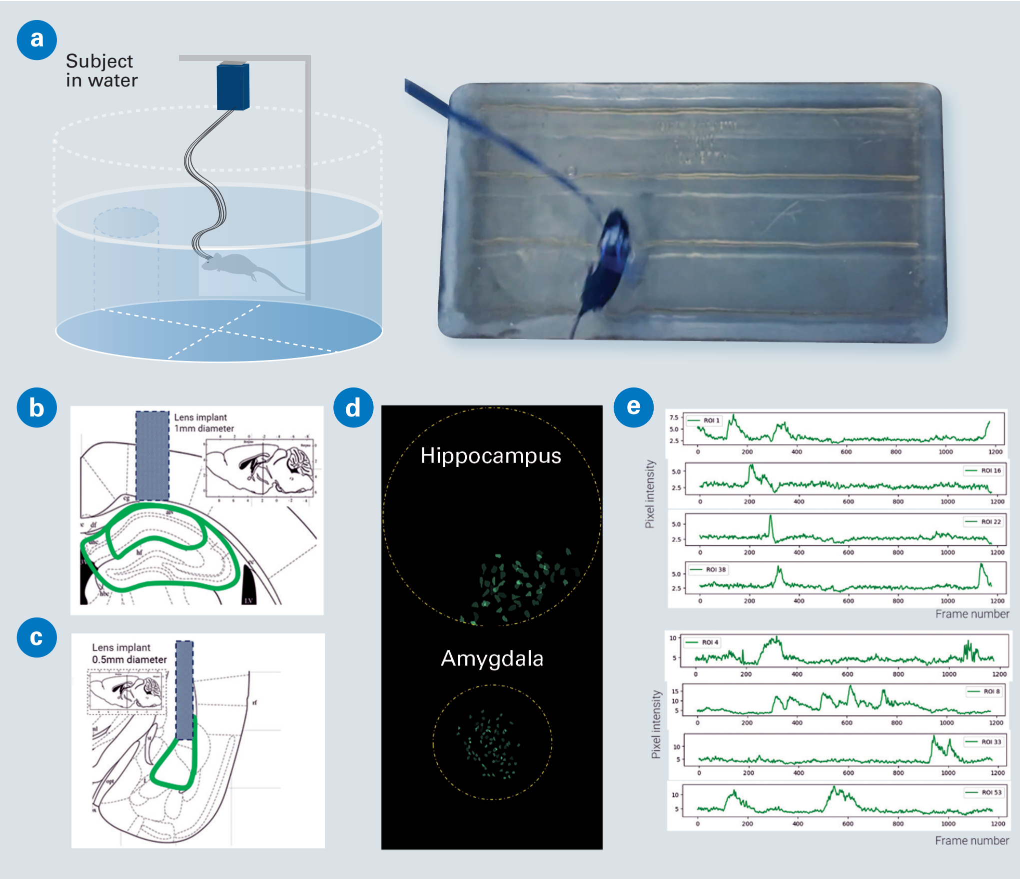

FIGURE 6.
(a) Quartet facilitates the simultaneous observation of neuronal dynamics in multiple brain regions as a mouse engages in swimming. (b) A 1 mm diameter GRIN lens implanted in the hippocampus (a relatively shallow brain region). (c) A 0.5 mm diameter GRIN lens implanted in the amygdala (a deep brain region). (d) The cell map of these brain regions. (e) Example neuronal traces derived from the recorded brain regions (top four; hippocampus, lower four; amygdala).

recording of up to four subjects.

Innovative Multi-Region Miniscope for Neuroscientists

This technical note discussed the Quartet system, a pioneer single-channel, multi-region imaging technology designed for the observation of circuit-level activity with cellular resolution. It is optimized for GCaMP imaging across distributed brain regions, whether cortical,

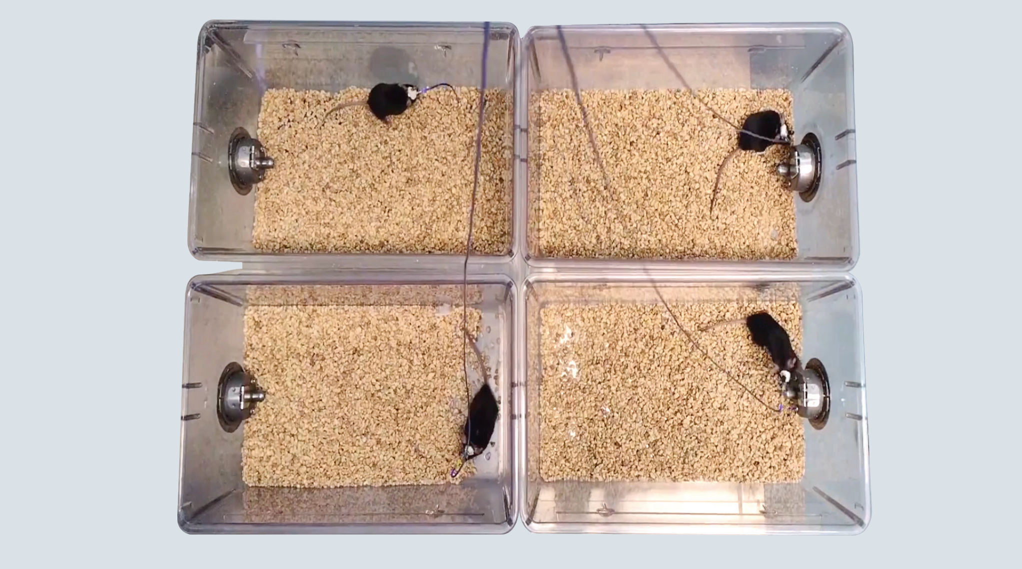

FIGURE 7.
Quartet's imaging fibers are extremely flexible which enables each mouse to behave in its own paradigm without the imaging fibers twisting or interfering with the animal movement or the shape of the signal.

subcortical, or within deep brain structures. The Quartet's flexibility and lightweight design renders it an invaluable tool for the most rigorous freely behaving paradigms in neuroscience research. Born from the collaborative efforts of scientists and engineers, the Quartet system signifies a significant advancement in the quest to enhance our understanding of the brain, with the ultimate objective of improving the diagnosis and treatment of central nervous system disorders.

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