Iconeus Newsletter – May 18, 2020

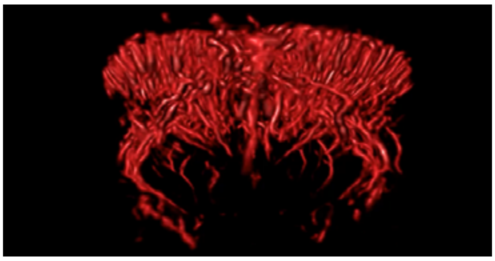
The path to 4D fUS

Functional ultrasound (fUS) imaging of brain activation and connectivity emerged as a breakthrough preclinical imaging modality during the last decade. Providing significantly higher sensitivity and spatiotemporal resolution than the gold-standard BOLD-based fMRI approach, with greater operating simplicity and affordability, and providing the option of operating in awake and behaving animals to avoid the bias of anesthesia, fUS clearly opens broad new perspectives both in academic research and drug development. In early 2020, the first commercial fUS system, Iconeus One, created by the inventors of fUS, provided an opportunity for everyone to access this technology through an easy-to-use integrated device. Originally limited to imaging a single 2D plane, recent years have witnessed important technological breakthroughs extending fUS imaging to new dimensions. As different technological solutions are currently proposed for the acquisition of 3D and 4D fUS data, each with its own advantages and drawbacks, we invited two eminent fUS experts from academia and industry, Prof. Mickael Tanter, the inventor and pioneer of 2D, 3D and 4D fUS technology, and Dr. Bruno Osmanski, the CTO of Iconeus, to share their views on the current state of multidimensional fUS.

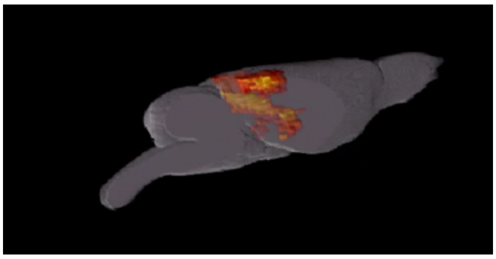
What is the state of the art in 4D fUS imaging?

M.T.: Following pioneering studies in our academic lab to develop functional ultrasound and 4D ultrafast ultrasound, we have naturally been working on a combination of these topics for several years now. Over the past decade, we published the first proof of concepts in 3D and 4D fUS brain imaging, first by using a tomographic approach based on motorized setups. This approach proved to be a highly successful method for several applications such as 3D retinotopic mapping in the rodent brain (Gesnik et al, 2015) and 3D tonotopic mapping of the auditory system in ferrets (Bimbard et al, 2017). More recently, we also validated the conceptually different approach of matrix technology for fUS imaging, such as full 2D matrices (Rabut et al 2019) and RCA (Row Column Array) matrices (Sauvage et al 2018, Sauvage et al 2019).

As you can see, two different strategies can be used for 3D/4D imaging, depending on the technology of the ultrasonic probe employed. That could be either a high sensitivity linear probe moved by a fully motorized robot, corresponding to the tomographic approach, or a full-electronic matrix probe technology. Today, the motorized robot approach is much more efficient for neuroscience approaches due to the very high sensitivity of the linear probes. Although the matrix probe approach is conceptually attractive, major technical challenges remain, the most important being poor sensitivity due to the very small size of the individual linear probe elements. We have already identified and patented several compelling solutions for neuroimaging to tackle this problem, such as a dedicated ultrasonic transmit/receive sequence, boosting sensitivity, which is called "multiplane-wave" (see our seminal Nature Methods paper on 4D fUS imaging, Rabut et al, 2019). We are also investigat-



4D vascular imaging within a single cardiac circle

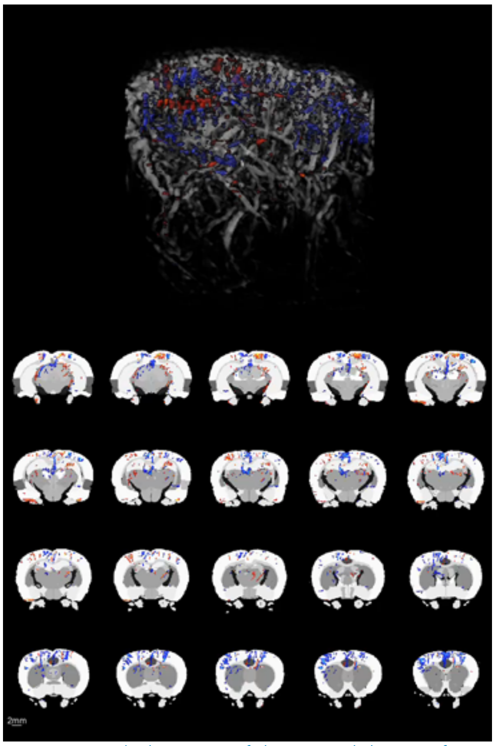


3D activation of the visual system in the rat brain

ing novel matrix probe designs, able to reach higher sensitivity than 2D matrix arrays (Sauvage et al, 2018, 2019).

These improvements will be the key to success in replacing motorized linear arrays by a 2D matrix array technology. Indeed, using the same acquisition time, today the sensitivity of the tomographic approach is several orders of magnitude higher than any matrix array technology in use. To give you an idea, our motorized 4D fUS imaging approach can be performed through the skin and skull in mice while even the latest matrix probes require a full craniotomy and still yield much lower data quality.

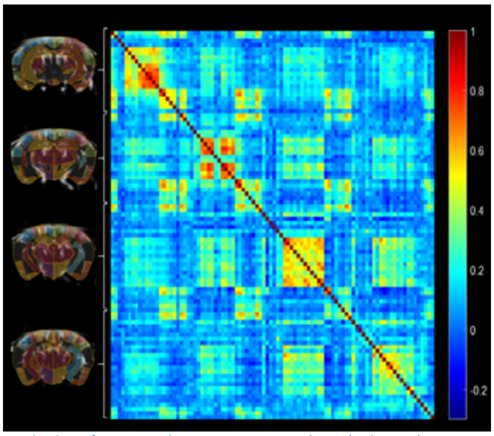
B.O.: Iconeus customers always insist on using the best available solution for their research projects, to ensure that their experiments yield meaningful results. In order to provide the best 3D system to neuroscientists, Iconeus One employs the most sensitive method achievable today for 3D/4D imaging – the tomographic approach. In parallel, we are working hard on the future of 4D fUS by investigating several emerging technologies together with the PhysMed Paris Institute.



Statiotemporal dynamics of hyper and hypoperfusion during a stroke event (one volume every 20 seconds)

We are also working closely with several probe and CMUT manufacturers, all industry leaders in the field, in order to develop the best fUS probes for the future. When these technologies are able to deliver better results than Iconeus One, we will extend our offer to our customers with the latest and best platform for 4D fUS imaging based on the next generation of matrix arrays. In the meantime, we believe that Iconeus One remains the best option for reliable, consistent and efficient 3D/4D fUS imaging, thanks to its high sensitivity and spatiotemporal resolution, which cannot be achieved using any other technology at present.

Sparse 3D functional connectivity can also be performed on a multislice basis and we are currently preparing a new software package on Iconeus One for such 4D functional connectivity. This new mode will be available in January 2021.

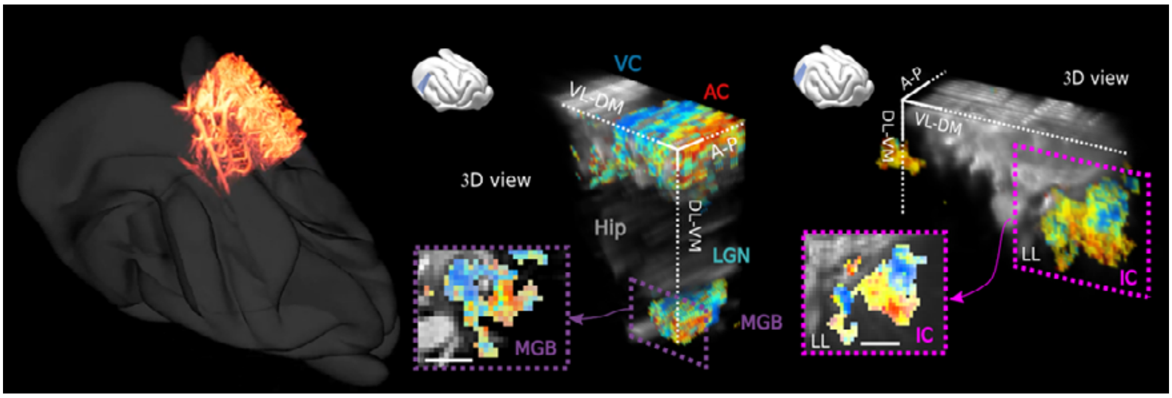


Multislice functional connectivity in the whole rat brain

How does Iconeus One provide 3D fUS without compromising sensitivity?

B.O.: We have been working hard to ensure that Iconeus One technology can acquire several slices with the best possible sensitivity. Our approach combines a very high sensitivity ultrasonic probe with a fully automatized four-motor robot to achieve volumetric fUS imaging. Depending on the type of acquisition (angiography, volumetric fUS, 3D functional connectivity) and the desired volume rate, the number of slices can range from full dynamic volume in angiography or functional imaging, with an adequate repetition of stimuli to 3D functional connectivity in a selected number of slices. 3D functional connectivity can also be performed on a multislice basis and we are currently preparing a new software package on Iconeus One for 4D functional connectivity.

This is a really important point, as this approach allows each neuroscientist to benefit from the very high sensitivity of Iconeus One technology within the whole brain and more importantly, in any desired configuration: even through the skull in mice and also in awake head-fixed setups. Thanks to this fully automatized robot that drives high-sensitivity probes, it is possible to perform either 3D or 4D whole brain angiography at unprecedented resolutions in order to track, for instance, the whole spatiotemporal dynamics of a stroke event. It is also possible to image in 3D or even in 4D the functional activity within the whole brain during a task-evoked stimulus, such as optical, auditory or somato-sensory stimuli. This is already available and corresponds to the tomographic approach.



3D tonotopic mapping of the auditory system in awake ferrets (Bimbard et al 2017)

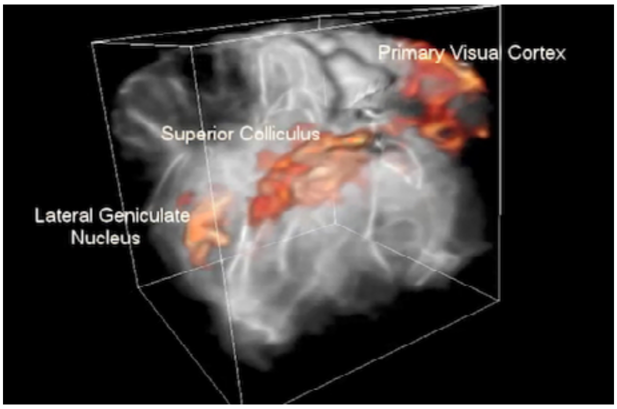
M.T.: Fast 3D scans also enable us to perform whole brain volumetric imaging within a few seconds. As an example, we can now map in 4D the spatiotemporal dynamics of cerebral blood volume changes in the first hour after a stroke in rats or mice models.

Will Iconeus provide a full 4D scanner with matrix arrays?

B.O.: Due to the very high standard of performance we want for our customers, we will only release full 4D fUS imaging when it reaches the desired performance levels. Over the past 3 years, Iconeus has already been working on 4D prototypes by testing different solutions. However, currently these are research platforms rather than commercial solutions. Interested customers keen to use 4D fUS for a specific application or project can contact us to discuss how to work together on the best approach, as the vast majority of configurations can already be implemented using the Iconeus One platform.

M.T.: At the PhysMed Paris Institute, we are currently developing our third-generation of full 4D fUS scanners and investigating several new ideas for overcoming the bottleneck of low sensitivity in matrix probes. However, although we have pioneered full 4D fUS imaging by using matrix probes, we know the road will be long before a scientifically optimal and commercially competitive solution is found. However, in the meantime, there are many other exciting developments on the horizon in the pipeline! We are also very excited that Iconeus has decided to implement Ultrasound Localization Microscopy (ULM), introduced by our lab for super-resolution ultrasound (see Errico et al, Nature, 2015), in Iconeus One.

B.O.: Indeed, in addition to neurofunctional ultrasound, Iconeus One will be able to map and quantify blood flow in the whole rodent brain at microscopic resolution (5µm), and this will be available with the next software upgrade.



Full 4D fUS imaging of visual stimulus in the rat brain

> Learn more about Iconeus One

Gesnik et al, Neuroimage 2017
Bimbard et al, eLife 2017
Rabut et al Nature Methods 2019
Sauvage et al, Physics in Medicine and Biology, 2018
Sauvage et al, IEEE Trans. Med. Imag., 2019
Errico et al, Nature 2015

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About Iconeus

Iconeus, a Paris-based French technological startup, was founded in 2016 by six experts in biomedical ultrasound and neuropharmacology, including the original inventors of the breakthrough functional ultrasound (fUS) technology. The mission of Iconeus is to introduce fUS imaging for preclinical and clinical neuroscience applications worldwide, through a license and research collaboration agreement with Inserm, the French National Institute of Health and Medical Research. The team is committed to delivering unparalleled neuro-functional imaging tools to the scientific community. They aim at empowering researchers to shed new light on brain and central nervous system function, to better understand neuropsychiatric pathologies and to progress towards effective treatments. Iconeus One entered the market in 2020 as the very first commercial fUS imaging system, tailored for recording brain activation and connectivity through blood-flow imaging with ultra-high sensitivity and resolution.

Mickael Tanter, PhD, INSERM Research Professor and member of the European Academy of Science, heads the Physics for Medicine Paris Institute. He is a globally renowned expert in biomedical ultrasound and wave physics, having co-authored more than 350 papers and book chapters on these topics. Co-founder of several MedTech companies (Supersonic Imagine, CardiaWave, Iconeus), he has pioneered several major innovations in ultrasound together with his team, including neurofunctional ultrasound, a technology that today is used by Iconeus.

Bruno Osmanski, PhD, Chief Technical Officer of Iconeus. Alumnus of ESPCI Paris and Physics for Medicine Paris, Bruno has co-authored several major publications on the subject of functional ultrasound. He is a specialist in ultrafast imaging and blood-flow imaging, including functional ultrasound imaging of the brain. As a postdoctoral scientist, he worked in the Neurophysiology & New Microscopies Laboratory (under Prof. Serge Charpak of INSERM), studying the mechanisms of neurovascular coupling using functional ultrasound, fMRI and two-photon imaging. He now oversees R&D at Iconeus as CTO.