Meso-Cortex : Imaging multiple cortical areas with high spatio-temporal resolution using innovative wide-field imaging system.

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ABSTRACT

Recording brain activity at the mesoscopic scale can unveil fundamental neuronal operations. Optical imaging offers a unique opportunity to measure brain activity over a large area with high spatio-temporal resolutions (20µm x 1ms). However, two major limitations exist : 1/ the cortex being non-planar, the field's depth limits the region in focus to a small region close to the center ; 2/ the signal-to-noise ratio is strongly degraded by the dynamic evolution due to physiological rhythms (heartbeat, breathing, etc.). We present an interdisciplinary approach for imaging of the non-human primate cortex, using technologies from astronomical instrumentation to overcome current technological limits.

Keywords: Neuroscience, optical imaging, mesoscopic scale, cortex, non-human primate, visual system, curved sensors,

1. INTRODUCTION

Recent anatomical studies on macaque brains have revealed that 80% of the network connectivity occurs at the millimetre/centimetre, i.e. mesoscopic, scale, within brain areas. However, due to technical limitations, this scale remains by far the less studied [1]. Most neuronal recording techniques are indeed restricted to microscopic (neuron, intra- or extracellular recordings) or macroscopic scales (whole brain). Recording brain activity at the mesoscopic scale has thus a strong potential to unveil many new fundamental neuronal operations and innovative clinical applications.

Optical imaging offers a unique opportunity to measure brain activity over large field-of-view (up to 1cm) at high spatio-temporal resolution (20µm*1ms). Such technics can allow to better understand neuronal processing in fundamental research or improve development of clinical therapeutic approaches such as neurosurgery or retinal implants.

However, in neuroscience, there are two major limitations of this imaging technique, which partly explains the poor development of its use.

- First, the cortex is non-planar, which, due to the field's depth, limits the optical access to a small cortical region close to the center of the field-of-view. This is even worst for more curved cortex of small animals (rodents or other small monkeys as the marmoset) used in neuroscience. In such animal models, the brain is lissencephalic and all visual cortical areas are located on the surface but inaccessible at once with standard methods.

- The second limitation is the signal-to-noise ratio which is strongly degraded by the dynamic evolution of the brain curvature due to physiological rhythms (heartbeat, breath, etc.), creating perturbation at 0.1-20Hz. This strongly limits our ability to work at single-trial and unravel the real dynamics of neuronal processing, such as spatio-temporal waves [2].

In the Meso-Cortex project, we use an interdisciplinary approach, at the border between brain imaging and technologies from astronomic instrumentation (curved detector, adaptive optics, real-time wave front control), to overcome technological limits and explore single-trial brain activity at expanded scales

During the ATTRACT program, we successfully redesign a complete cortex imaging system and its illumination set-up, allowing to tackle these limitations. Our project will allow innovative research perspectives by imaging for the first-time multiple cortical areas with high spatio-temporal resolution and at the single trial. Such breakthrough will open new unexplored lines of research such as how neuronal interactions distributed at multiple scales and areas are dynamically shaping the parallel processing and representation of sensory information.

2. STATE OF THE ART

Optical imaging techniques such as intrinsic imaging and voltage-sensitive dye (VSD) imaging are used to record neuronal activity in non-human primates to understand the role of its dynamical activity. They allow measuring the activity over a large field of view with high spatio-temporal resolutions [3], [4]. Both imaging techniques give access to a network of neurons, from the sub-column to a whole area, thus covering the mesoscopic scale [1]. However, the classical optical instrumentation used while performing the measurements is not optimized, limiting further results in fundamental neuroscience research. These instrumental limitations can be illustrated by Muller et al. [2], [5], in which two models of travelling waves mechanism followed by the activity response are exposed. They explain the importance of unravelling the role of these waves in neural systems, but they emphasize the lack of larger-scale imaging techniques. Indeed, the propagating waves detected, subject to high spatial-to-noise impacting the ability to work at single-trial, were recorded only from a reduced portion of the cortex region available in the recording chamber. They observe that up to 90% of signal information is lost compared to the signal that could be recovered from the accessible cortex (18mm chamber).

On top of that, the use of commercially available detectors is not optimal. They have planar geometry while the cortex surface imaged is curved. This, combined with the optical design of the instrument optimized to create an image of a flat object on a planar image sensor, induces vignetting and image blurring in the field of view (FoV). Thus, the image formed on the camera sensor is of similar curvature than the cortex itself creating aberrations that could be avoided by an appropriate optical system. In the following paragraphs, we will present the development of a new design based on a curved sensor.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

The goal of the ATTRACT Meso-Cortex one-year project was the realization of a prototype of a cortical imaging device with a curved-sensor-based wide-field module, and its demonstration on a static moke-up of a non-human monkeys' brain (macaque or marmoset). This proof of concept is also the first step towards a dynamic system able to correct for the evolution of the brain curvature due to physiological rhythms (heartbeat, breath, etc.).

The main breakthrough of the Meso-Cortex project is the demonstration that a new imaging system, with a design based on an innovative curved sensor, can allow us compensating for cortex curvature of small non-human monkeys and greatly enhance imaging at the mesoscale.

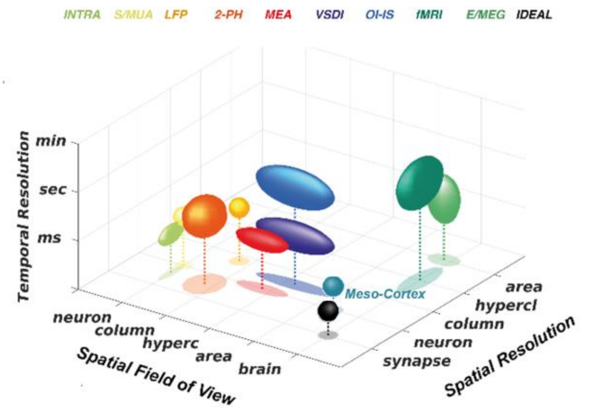

Fig. 1. Spatio-temporal resolution and scales of neuronal recording methods: Three-dimensional representation of 10 families of neuronal recording methods as a function of their spatial resolution, temporal resolution, and the spatial field-of-view that they can reach. Voltage-sensitive dye imaging (VSDI) and optical imaging of intrinsic signals (OI-IS) are show in blue. The Meso-Cortex demonstrator aims at imaging large areas, or part of a brain, at the spatial resolution of a neuron.

Such an important breakthrough, once transferred in future neurosciences research tools or clinical imaging systems, would enable the recording, at the level of the neuron scale, of the whole visual system of non-human monkeys at once, from the primary to the fifth visual cortices.

This will bring such imaging tools closer to the "ideal" technique noted in Fig. 1 and will open new unexplored lines of research such as how neuronal interactions distributed at multiple scales and areas are dynamically shaping the parallel processing and representation of sensory information.

This will interest a wide neuroscientific audience but also impact the clinical community interested in cartography of the nervous activity at the mesoscopic scale.

4. PROJECT RESULTS

Our innovative solution, based on advances in manufacturing technologies, consists of curving the sensor to compensate for the curvature of the observed object: the macaque cortex.

Using a public database of Magnetic Resonance Imaging (MRI) data (from the CERIMED), we extracted the data points corresponding to the surface of the brain included in the recording chamber for several macaques. We obtain for macaques of similar morphology an average of 29mm ±8mm for the cortex radii of curvature.

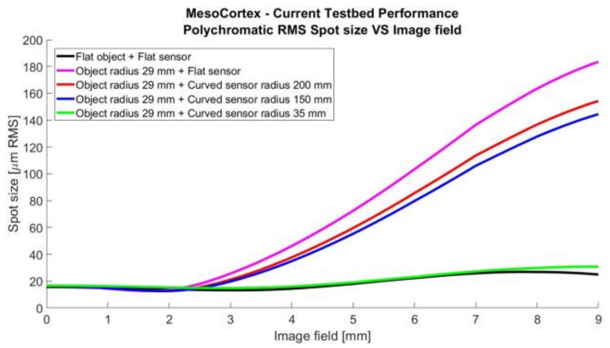

Fig. 2. Classical neuronal imaging system with curve sensor added: polychromatic root mean square (RMS) spot size vs image field calculated by Zemax for various radii of curvature.

From this value, we estimated the gain of replacing the flat sensor in the usual neuronal imaging systems by a curved sensor with various radii of curvature (see Fig. 2.). These imaging systems are classically design to image flat objects to flat focal plane. One sees clearly in Fig.2. the large degradation of the image after 2.5mm from the center when we try to image a 29mm radius cortex on a flat sensor. Switching to a curve-sensor with a 200-150mm radius slightly improve the situation at the edge of the field but do not really increase the diameter of the 'in-focu' zone. Only a sensor with radius close to the cortex can allow to achieve "full diameter" performance.

The sensor curving is a very delicate phase performed by the CURVE-One company, a specialist in curving sensors to almost any custom shapes (spherical, aspherical, toroidal, etc.). Of course, the breakage limit of the sensor substrate is the main limitation in term of deformation. In the case of a CCD sensor (as AMS CMV12000 in Fig. 6), the limit of curvature radius that can be achieved is around 120mm. Due to this technical limitation, adapting directly a new sensor to existing neuronal imaging systems is not a viable solution.

We had to design a specific imaging system (see Fig. 3) to accommodate the highly curved object to a slightly less curved sensor. This system is described hereafter.

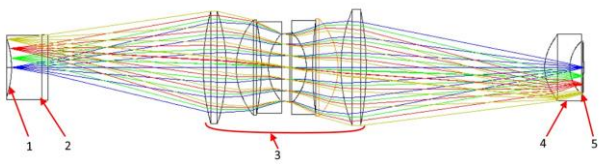

Fig. 3. Meso-Cortex custom imaging system providing higher resolution and image illumination over the entire field of view : 1/ curved cortex (spherical surface with 29mm Rc), 2/ chamber with immersive liquid, 3 / double Gauss type custom projection lens, 4/ aspheric corrector lens and 5/ curved CMOS detector with 150mm radius of curvature.

The linear field of view limited by the chamber size is 18 mm. The image size should correspond to the sensitive area of a commercial CMOS sensor. For AMS CMV 12000 it equals 22.5 x 16.9 mm, so the linear magnification is -0.94x. The working spectral band is [595-670nm], which allows to cover both the intrinsic and fluorescence imaging modes. Since the object curvature is as steep as 29 mm it is hard to compensate the field curvature by a single element. This function is split between a 150mm curved sensor, and an aspherical corrector lens which also compensates other aberrations across the FoV. The sensor cover glass being removed during the curving process the corrector can be placed close to its surface.

- The spot diagrams for this configuration are shown in Fig. 4. The RMS radius varies from 9.6 to 27.4 microns, the maximum radii are 15.7 - 95.4 microns. One may note that the image quality is mainly limited by astigmatism.

- The MTF plots are shown in Fig. 5.; they demonstrate that the resolution is quite uniform across the FoV and that it will be possible to resolve the object features down to 30 microns in size even at the field edges.

- The relative illumination varies by 28 % from the field center to its edge, due to the incidence angle variance. However, this effect is moderated in comparison with a flat sensor-based configuration.

- The image distortion was limited during the optimization, so the residual pincushion type distortion is only 4.7%.

The excellent performances presented in Fig. 4. and Fig.5. completely valid the Meso-Cortex innovative approach for the wide-field and high-resolution cortical imaging.

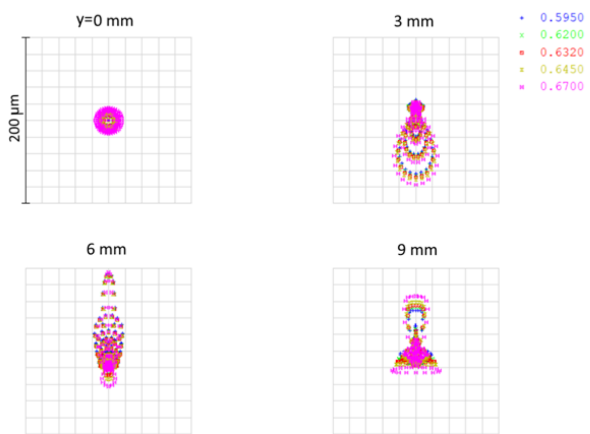

Fig. 4. Spot diagrams of the custom F/2 projection lens for five wavelengths (595nm, 620nm, 632nm, 645nm, and 670nm) in the working spectral band.

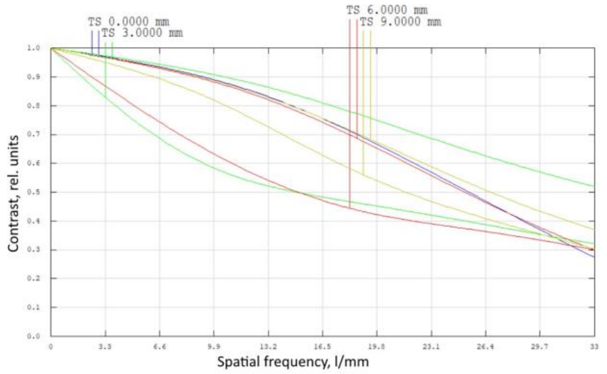

Fig. 5. Module transfer function of the custom projection lens. For all the field positions (0.0 mm in blue, 3.0 mm in green, 6.0 mm in red and 9.0 mm in olive) are represented the tangential (T) and sagittal (S) responses separately.

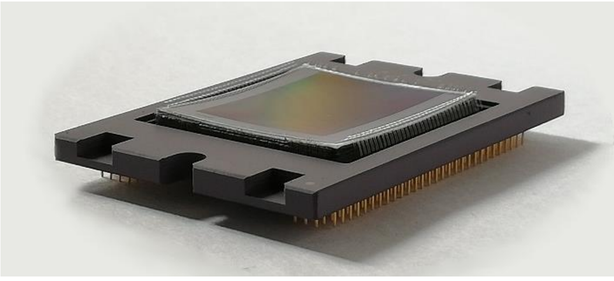

Fig. 6. The AMS CMV12000 sensor delivered by the Curve-One company, with a 150mm radius of curvature, that will be integrated in the Meso-Cortex custom neuronal imaging system.

5. FUTURE PROJECT VISION

Today the Meso-Cortex prototype could be considered as at TRL3-4. It is mainly a proof of concept system in a representative environment. In a couple of months, it could be used on real experiment with monkeys to validate its performances and benchmark with existing systems.

5.1. Technology Scaling

To go toward a fully operational pre-industrial system and increase maturity to TRL 5-7, we need to put efforts on several aspects of the system. This is particularly true for the dynamical version allowing to compensate for evolution of the brain curvature due to physiological rhythms and thus to increase performances.

- Beside securing the procurement of curved sensors we also have to valid their "active" version with our partner Curve-One.

- We need to develop a robust software for wavefront sensing and curvature variation estimation, based on recent phase diversity algorithms, and signal processing for increasing the signal output and delivers processes high-resolution image.

- Finally, we have to integrate the optical system in a complete product (hardware, software, user interface) in a semi-industrial demonstrator.

These developments could be achieved within a two- or three-years' program, assuming collaborations with new partners especially for the two last points. Laboratory with and expertise on signal processing as well as company specialized in optical imaging system for research or medical applications could be the perfect partners for an ATTRACT phase 2 program.

5.2. Project Synergies and Outreach

In preparation of the next phase of the Meso-Cortex project, we already started discussions with several laboratories and companies that could bring the needed additional expertise and reinforce our consortium. These discussions will continue during Fall 2020 to address scientific and technical motivations, as well as possible in-kind contribution to produce a preindustrial prototype.

For this important step as well as for the outreach and dissemination of the results we already rely on existing structures. Both CNRS, with CNRS Innovation, and Aix-Marseille University, with its City of Innovation, have structures to help and guide innovation Their aim is the promotion of research and innovation and the rapprochement with the socio-economic world through valuation structures, incubators, and even private accelerators. We will also have a strong support from the OPTITEC competitiveness clusters, which has expertise and history of success at the European level.

5.3. Technology application /demonstration cases

There are two types of possible direct application of the Meso-Cortex technology, one in the Science domain and the other in the Health aspect of the Societal Challenges, that could be used as demonstration cases.

Scientific Research: The progress of Neuroscience is tightly linked to the progress of technology to record neuronal activity. Our product will solve an essential technological bottleneck to image at high spatial and temporal resolutions brain activity over large surface and at single-trial level. This scale span (microscopic to macroscopic) at such field of view has not yet been achieved and has the potential to unveil crucial key results. We expect many neuroscientists in the world to be interested in such system, starting by the ~500 laboratories using wide-field optical imaging systems with the limitations that our technology can overcome.

Societal Challenge and medical applications. Optical imaging is already used for intraoperative clinical imaging to precisely and rapidly map the important functional cortical regions prior to ablation in neurosurgery. Improvements of signal-to-noise allowing for faster and higher quality mapping procedure will greatly interest many neurosurgeons and can generate a widespread use for clinical mapping procedures. Neurosurgeons at Marseille's hospital have already given great interest in this opportunity.

These two demonstration cases could easily be integrated in a larger framework and both demonstrators could find their room in a Science or Medical infrastructure. Such integration could be of a great interest for the visibility of the technology, but it will also benefit from the access to experienced users (researcher, surgeons, etc..) that will be able to give precise and relevant feedbacks.

An action for establishing a partnership in this direction, with an existing or future Research Infrastructure, will be pursued at the beginning of Phase 2.

5.4. Technology commercialization

The commercialization of a new technology, even at a TRL 5-7, is always a very difficult step sometimes also described as the "Death valley". To avoid usual errors often made by researchers, we will rely on the already mentioned valorisation structures existing in the Meso-cortex ecosystem such as the CNRS Innovation, the AMU City of Innovation and the competitiveness cluster OPTITEC. These three entities working together will help us to identify main opportunities and markets, to evaluate risks and propose mitigation solutions, as well as to establish contacts with public/private investors.

Sources of funding will be essential to ensure successful development towards an industrial system. Beside the ATTRACT 2 program we plan to apply to various regional, national or European organizations, supporting innovation or industrialization. We will also contact the recently created Aix-Marseille University Imaging Institute, which has an innovation support program

5.5. Envisioned risks

The main risk that we identify today for the Meso Cortex ATTRACT Phase 2, is the fact that our partner Curve-One is a very young spin-off of CNRS research activities. This young company successfully survived the Covid-19 time during spring/summer 2020 but with difficulties. Their various participation into several developments allowed them to successfully deliver the Meso-Cortex expected sensor. Unfortunately, other difficulties appearing in the next two years could really endanger the company.

This risk is mitigated by the fact that Curve-One is using a CNRS technology transfer licence. The expertise has been developed within a laboratory and researchers are working closely with the company. Secondly, participating in an ATTRACT Phase 2 program will also help this young company to secure its future.

5.6. Liaison with Student Teams and Socio-Economic Study

One of the objectives of the newly Institute of Medical Imaging created by Marseille University is to strengthen the links between research and teaching, particularly at License and Master levels. Our Meso-Cortex project, supported by the IMI, will be naturally integrated in the institute environment with physical demonstration and explanation. We will also propose to launch a Meso-Cortex challenge, organized by IMI Master students, to bring out new application ideas for our technologies.

Concerning the Phase 2 socio-economic study of the ATTRACT initiative and ecosystem we will be glad to contribute by all the possible ways. Once again, we will request the help of the AMU Cité de l'Innovation and OPTITEC cluster to gather all the useful information and organize various impact evaluation, if necessary.

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