EPSRC & MRC Neuromod+ Network Final Project Report

Ultrasound neuromodulation with real-time 3-photon imaging

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Summary - This project aimed to develop the first integrated platform for simultaneous in vivo focused ultrasound neuromodulation and three-photon imaging. While focused ultrasound offers a powerful non-invasive approach to modulate deep brain activity, its cellular mechanisms are still poorly understood. Three-photon microscopy enables deep, cellular-resolution imaging without the need for a craniotomy. Their combination allows real-time visualisation of ultrasound-induced neural effects, providing direct mechanistic insight into how ultrasound alters neuronal activity and circuit dynamics.

This work focused on the design, simulation, fabrication and calibration of a novel ultrasound transducer array compatible with existing three-photon microscopes and successfully delivered a characterised device alongside extensive simulation and calibration data.

Methodology - *Device design and simulation:* We performed acoustic simulations using k-Wave to identify feasible transducer geometries that could physically fit around a three-photon objective while achieving a tight acoustic focus aligned with the imaging plane. These simulations explored trade-offs between aperture size, element number and achievable beam width and sidelobe levels. An eight-element annular array configuration was selected as the optimal compromise between mechanical constraints and acoustic performance, at a centre frequency of 500 kHz.

Fabrication and calibration: We then designed and fabricated custom ultrasound transducers in-house and assembled them into an eight-element array that could fit around a three-photon microscope lens. The array was characterised in a water tank, where we synchronised the driving pulses across elements, calibrated relative phase delays, and fully characterised the focal profile, quantifying the beam width, sidelobes and waveform characteristics. This produced a well-defined acoustic focus aligned with the optical focal plane, validating the simulation framework and demonstrating feasibility for combined neuromodulation and imaging. The system is ready for in vivo testing, including alignment with behavioural head-fixation hardware and integration with imaging protocols. Once the three-photon microscope is fixed we will be able to perform these tests.

Impact - This project has delivered the first practical step towards a fully non-invasive platform for real-time, cellular-resolution investigation of the mechanisms of ultrasound neuromodulation in deep murine brain regions. The technical outputs of this project directly address a critical bottleneck in the field: the lack of mechanistic understanding of how ultrasound affects neurons and neural circuits.

From a responsible innovation perspective, the work contributes to safer and more effective future neuromodulation technologies by enabling parameter optimisation based on direct biological readouts, rather than behavioural or electrophysiological proxies alone. This is important for the translation of ultrasound neuromodulation into clinical applications for neurological and psychiatric disorders, where safety, reproducibility and mechanistic clarity are key.

Beyond the technical deliverables, the project has had substantial impact on the career development of people involved in the project and community impact:

- The funded research assistant gained advanced skills in ultrasound device design and is now undertaking a PhD in ultrasound at the University of Oxford.
- A Master's/research student involved in related device development has since secured a research assistant post and followed on as a PhD student in ultrasound engineering.

- A related in vitro ultrasound neuromodulation device developed in parallel was published in *IEEE Ultrasonics*, strengthening the group's international profile.

The project strongly aligns with the **core aims of the Neuromod+ network**, particularly in:

Enabling transformative, minimally invasive neurotechnology - The work directly supports the network's vision of next-generation, minimally invasive brain stimulation by developing hardware that is entirely non-invasive yet capable of targeting deep brain regions with cellular precision.

Supporting co-creation and collaboration - The project generated significant engagement across the UK neuromodulation community including:

- Participation in the NeuroMod Network launch event led to new collaborations with experts at the University of Nottingham, resulting in the successful award of this grant, a further £100k network grant, and invitation to participate in a large Research Council grant interview, providing valuable exposure as an early career researcher to large-scale funding processes (aiding in Dr Morse's UKRI FLF and lectureship interviews).
- Giving an invited talk at the UK Symposium on Neuromodulation and Neurotechnology that led to a new collaboration with the University of Bath and direct integration into the national neuromodulation ecosystem.
- Giving a webinar talk for the Close-NIT network webinar series (UKSNN) which provided further visibility and cross-network exchange.

Shaping the field and policy – Our team is also contributing to a white paper on ultrasound neuromodulation, helping to define future research priorities and responsible innovation pathways for the field. These activities are a clear example of the Neuromod+ mission of bringing researchers, clinicians and technologists together to co-create impactful neurotechnology.

Future Directions - The platform is now fully ready for biological experiments, and future work will focus on real-time calcium imaging during ultrasound stimulation, systematic mapping of parameter–response relationships, and extension to disease models and behavioural paradigms. Some of the above work will be performed as part of Dr Morse's recently awarded UKRI Future Leaders Fellowship.

Overall statement - This Neuromod+ project successfully delivered a novel, enabling neurotechnology platform, generated substantial training and community impact, and directly advanced the network's mission to develop minimally invasive, responsible brain stimulation technologies through collaboration and co-creation.