Optical voltage imaging with charge-coupled fluorescence of diamond colour-centres.

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The fundamental mechanisms of action behind a number of higher brain functions are thought to emerge from multi-scale interactions within the network of inter-neuronal connections called synapses. To test hypotheses in the emerging field known as *synaptomics*, neuroscientists have identified a need to link the molecular nature of each synapse to its electrical activity at the mesoscale [1]. Existing voltage imaging technologies, such as electrode arrays or voltage sensitive fluorescent indicators (VSIs), are not capable of achieving the combination of resolution, speed, scale and sensitivity required to achieve this monumental task. The charge state of colour-centers in diamond can be utilized to transduce electrical potentials into optical signals [2]. This offers a new approach to voltage imaging without the scale and resolution limitations of electrode techniques, nor the speed and stability limitations of VSIs.

In this talk I will report on efforts to develop nitrogen-vacancy (NV) ensembles in diamond which optimize the charge state response to voltage at the diamond surface. Beginning with a theoretical description of the sensing mechanism and the experimental methods we employ to produce biologically compatible voltage imaging systems, I will detail our current progress in the production of ultra-dense near-surface NV ensembles with a controlled distribution of charge states. Topics covered include the controlled tuning of the equilibrium NV charge state distribution by *in situ* electro-chemical modification of the diamond surface and non-destructive methods formation annealing and hydrogen-termination of diamond samples that contain shallow NVs. The culmination of these developments is a diamond voltage imaging microscope utilizing conversion between the dark NV^+ and bright NV^0 charge states to realize a localized optical voltage response sensitive enough to capture neuronal action potentials [3]. Finally, I will discuss the exciting future potential of this approach for *in vitro* neuron electrophysiology at synaptic resolutions over 'mesoscale' networks comprised of hundreds to thousands of cells.

[1] Grant, S. G. N. The Synaptomic Theory of Behavior and Brain Disease. *Cold Spring Harbor Symposia on Quantitative Biology* **83**, 45–56 (2018).

[2] Karaveli, S., Gaathon, O., Wolcott, A., Sakakibara, R., Shemesh, O. A., Peterka, D. S., Boyden, E. S., Owen, J. S., Yuste, R., & Englund, D. (2016). Modulation of nitrogen vacancy charge state and fluorescence in nanodiamonds using electrochemical potential. *PNAS*, *113*(15), 3938–3943.

[3] D.J. McCloskey, **N. Dontschuk**, A. Stacey, C. Pattinson, A. Nadarajah, L.T. Hall, L.C.L. Hollenberg, S. Prawer, D.A. Simpson, A diamond voltage imaging microscope, *Nature Photonics* **16** (In press – accepted July 2022).