ESTABLISHING THE BIO-INTERFACE FOR NEURAL ELECTROPHYSIOLOGY WITH A DIAMOND VOLTAGE IMAGING MICROSCOPE

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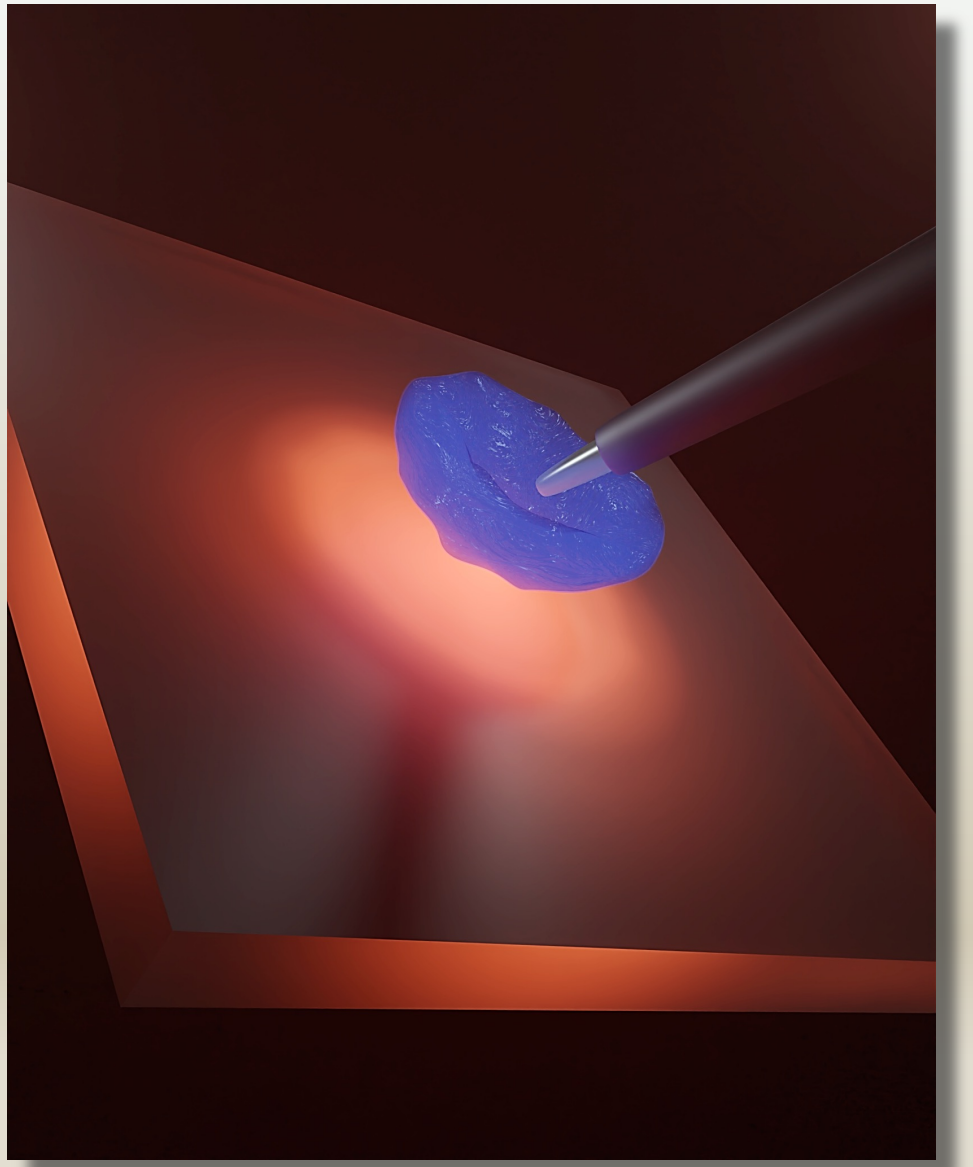
ANZCOP 2022
Adelaide, SA



Outline

Motivating diamond voltage imaging microscopy

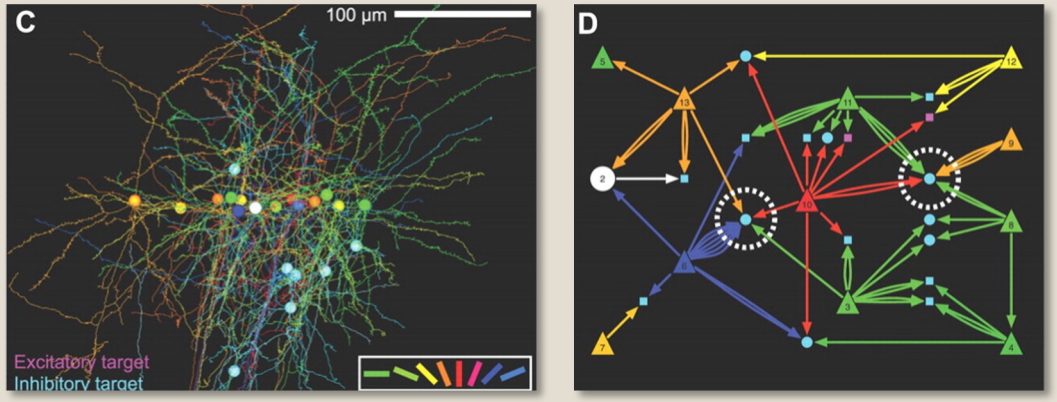
Operating principles and fabrication

Our current results

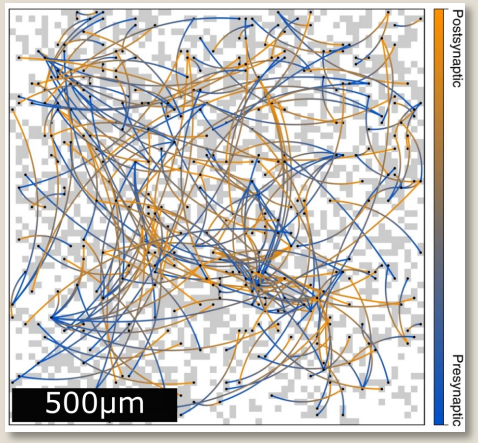
Prospects for biological imaging

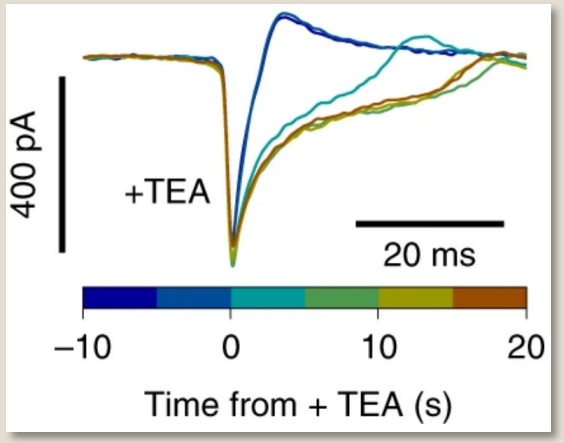
Motivations

- Voltage imaging techniques allow us to probe neuronal networks for insights in:
  - Fundamental neurobiology
  - Neuronal computation mechanisms
  - Memory, learning, higher cognitive function
  - Disease mechanisms
  - Precision medicine
  - Identifying/testing drug candidates

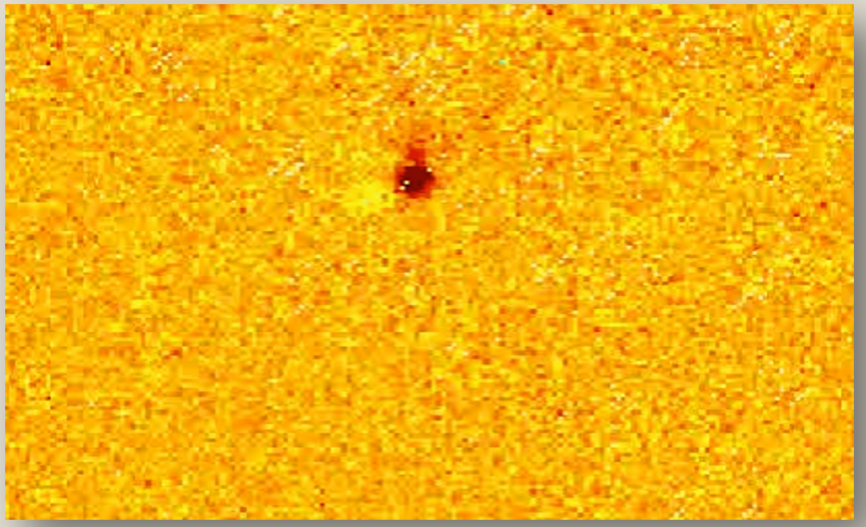


Bock, D. D. et al., *Nature* **471** (2011)





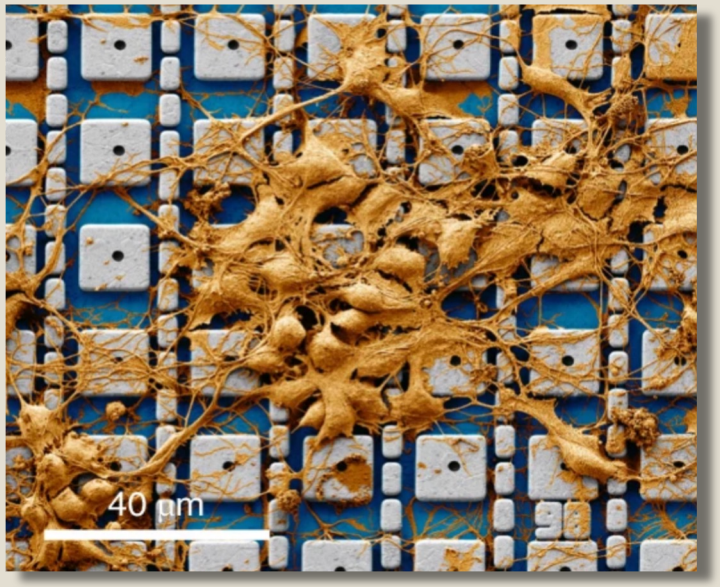
Abbott, J. et al., *Nature Biomed. Eng.* **4** (2020)



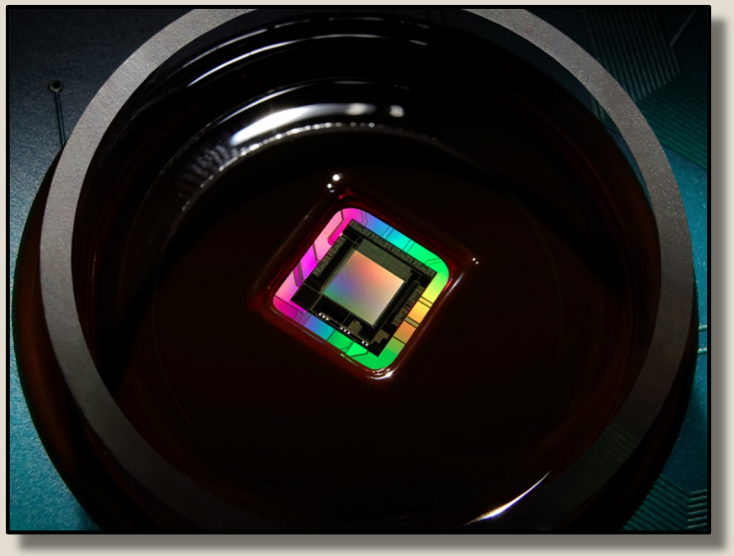
Muller, J. et al., *Lab on a Chip* **15** (2015)

Motivations

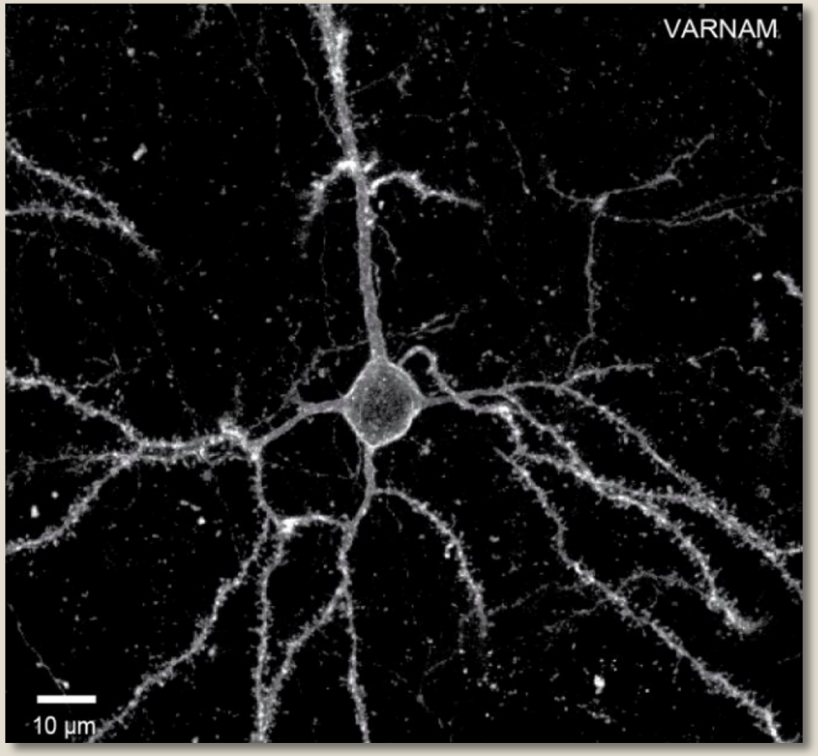
- Spatiotemporal resolution and scale are both important.
- CMOS-based multi-electrode arrays (MEAs) and voltage-sensitive fluorescent indicators (VSIs) represent the state-of-the-art in voltage imaging.



Abbott, J. et al. *Nature Biomedical Engineering* **4**:2, (2020)



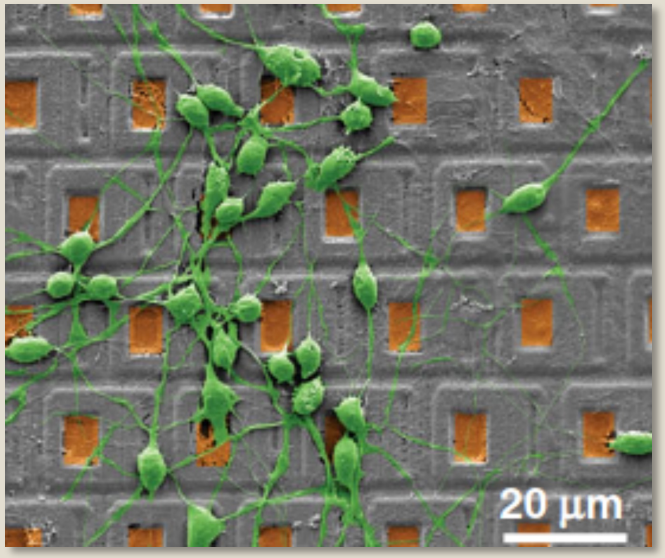
3Brain Inc.



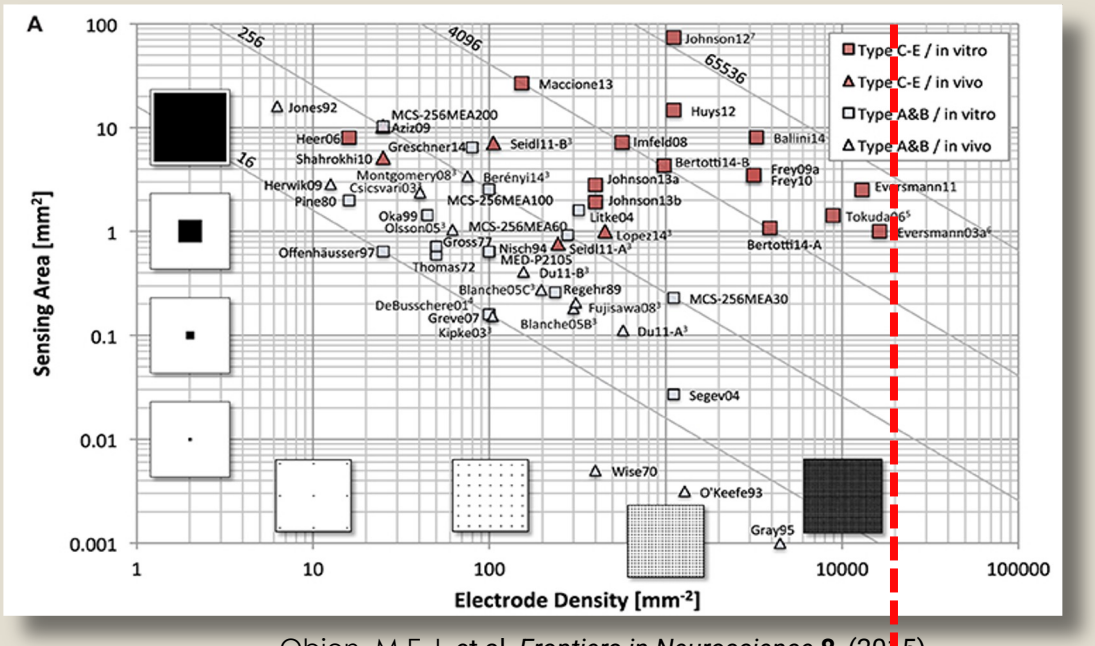
Kannan, M. et al., *Nature Methods* **15**:12 (2018)

Motivations

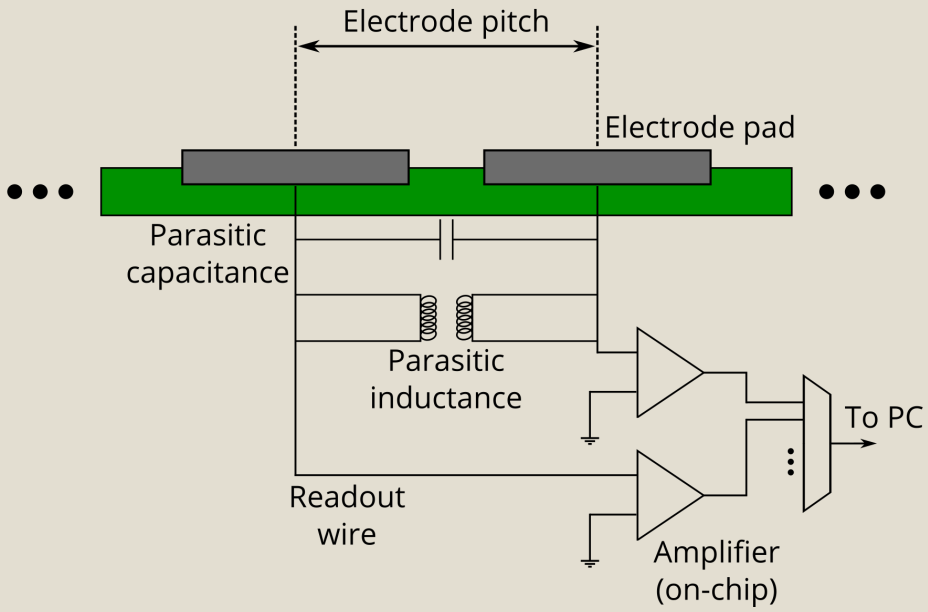
- CMOS MEAs have reached a resolution bottleneck (~15µm since ~2010) insufficient to completely resolve network dynamics.
- Number of simultaneous readout channels limited on MEAs (~4,000 typical).



Muller, J. et al., *Lab on a Chip* **15** (2015)



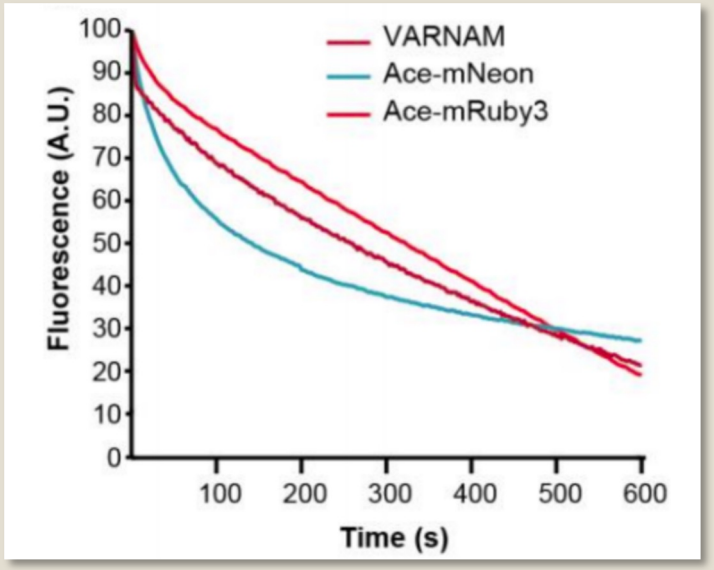
Obien, M.E.J. et al. *Frontiers in Neuroscience* **8**, (2015)



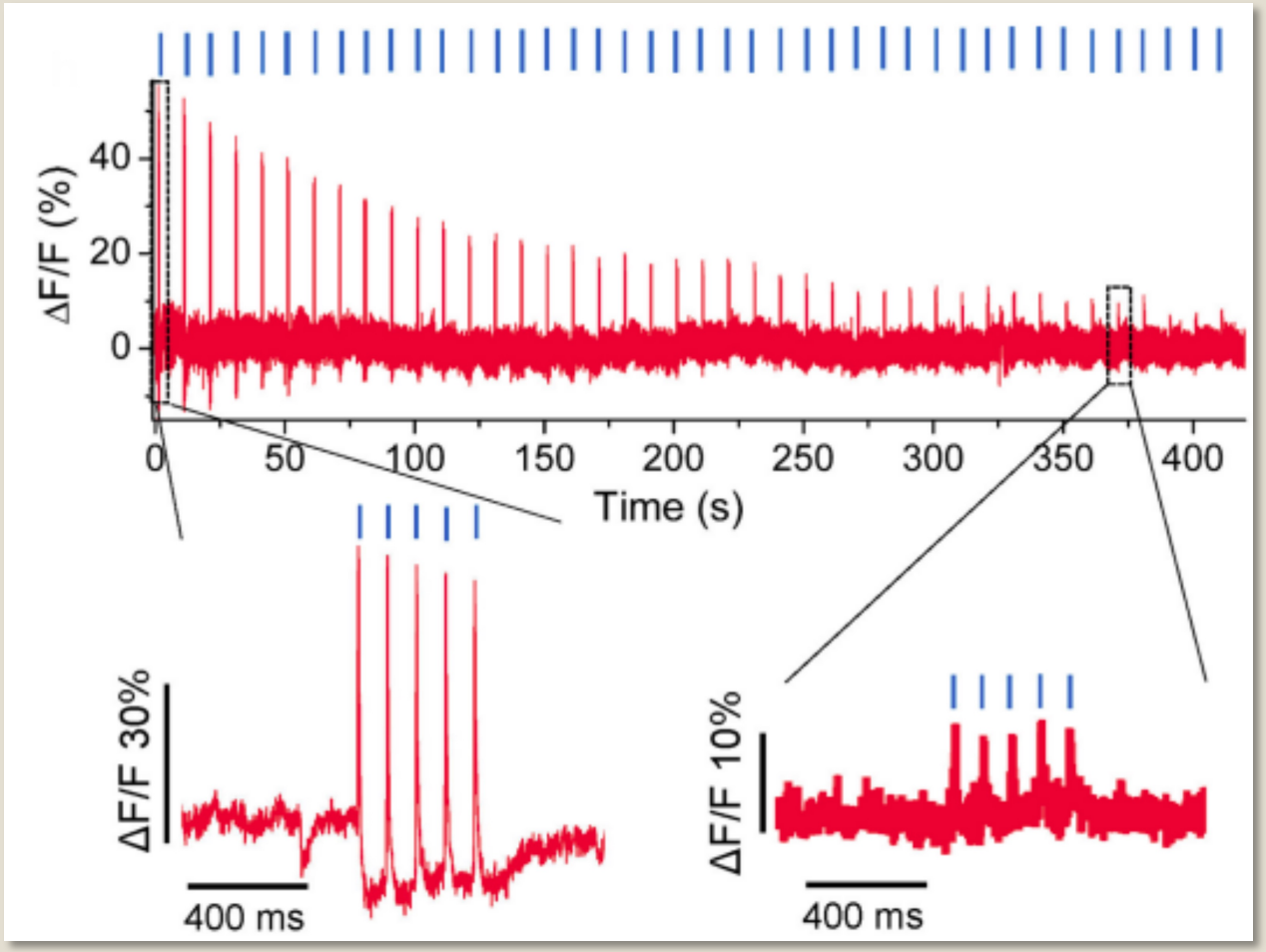
We have been bottlenecked here for 10 years!

Motivations

- Irreversible photochemical processes (bleaching) limit VSI recordings to a few minutes at most.

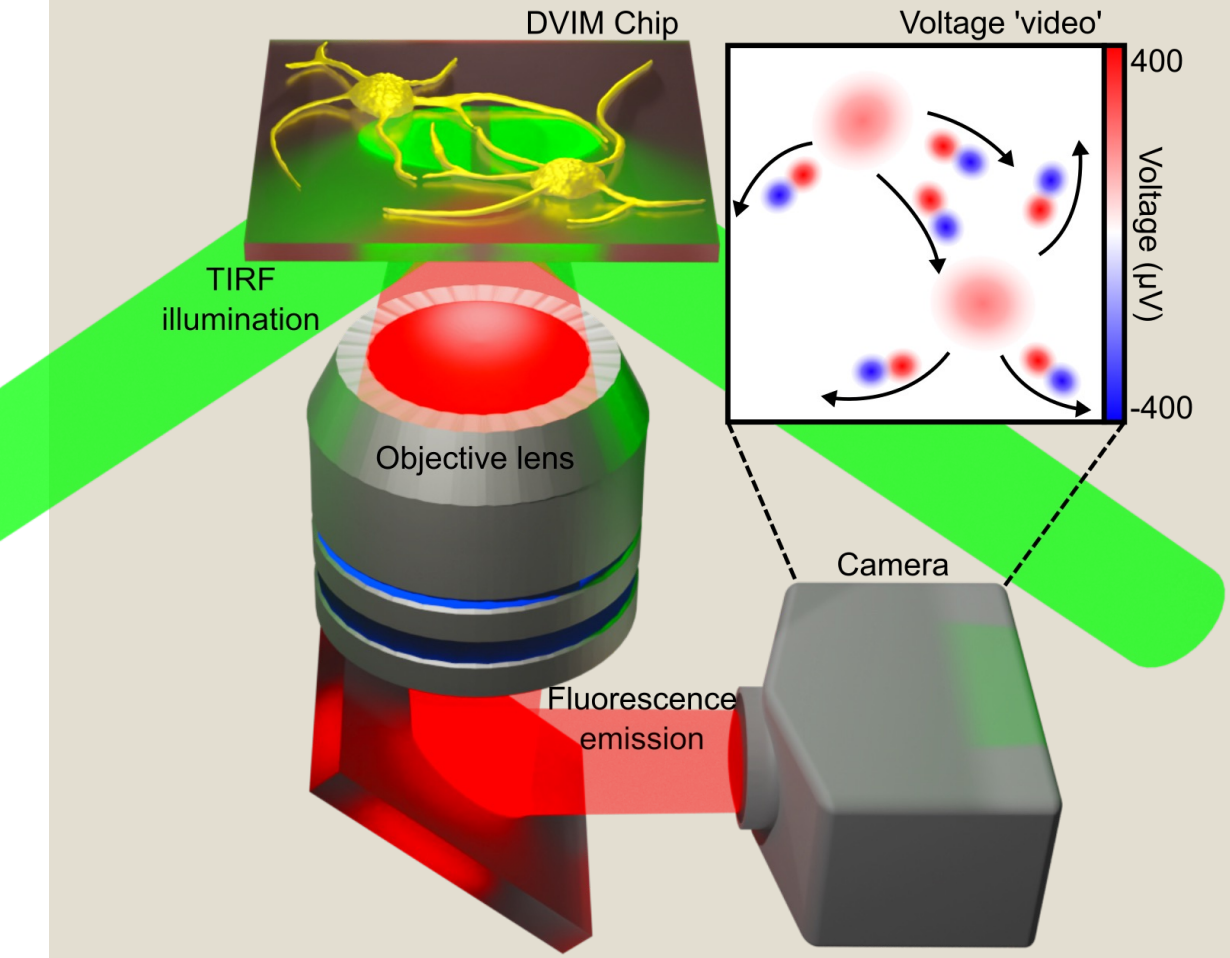


Kannan, M. et al., *Nature Methods* **15**:12 (2018)

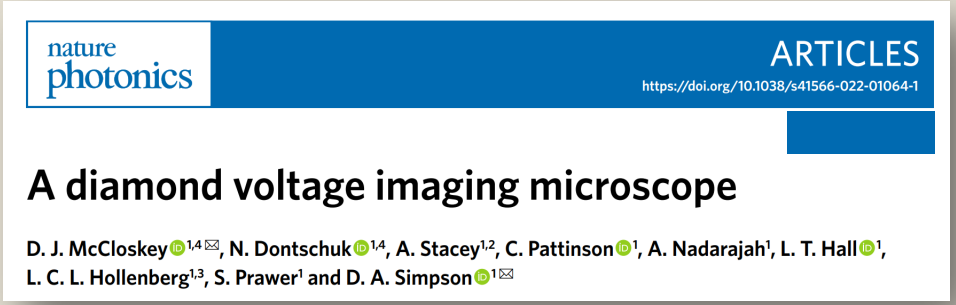


Piatkevich, K.D. et al. *Nature* **574** (2019)

Diamond voltage imaging microscopy



- A diamond voltage imaging microscope (DVIM) is our new electrophysiological recording platform (we hope).
- Combines spatial resolution of VSIs with long-term stability and wide field-of-view of MEAs.
- Operation based on fluorescent p-n junction coupled to the diamond surface potential.
- Wide bandgap of diamond allows for optical transparency and thermal charge-stability of deep-level defects.


nature photonics

ARTICLES

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A diamond voltage imaging microscope

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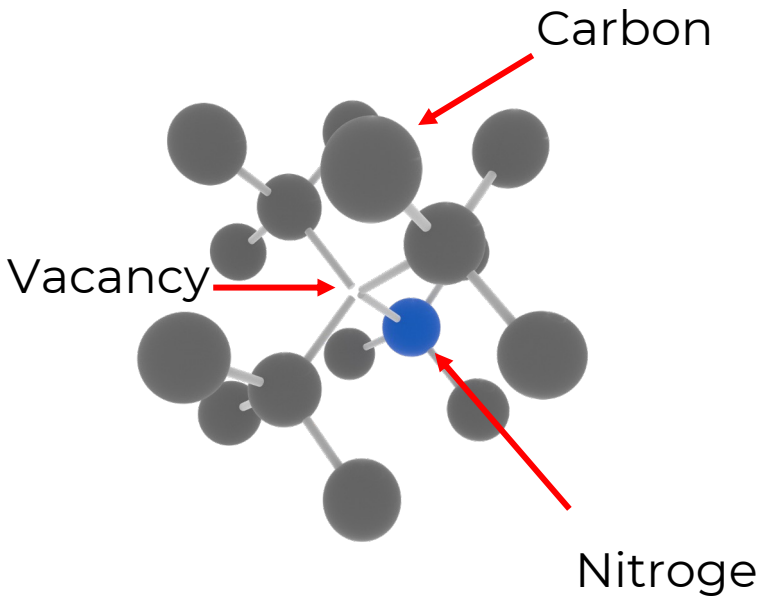
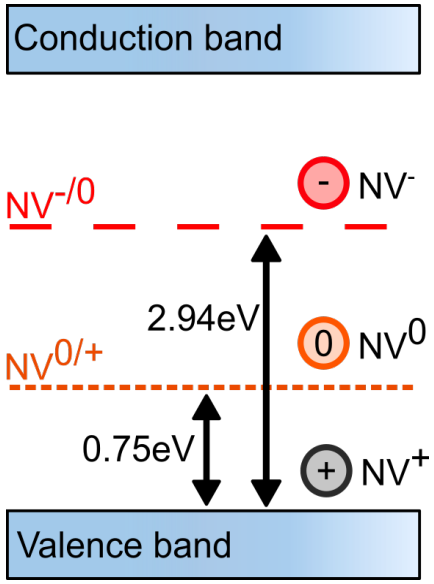
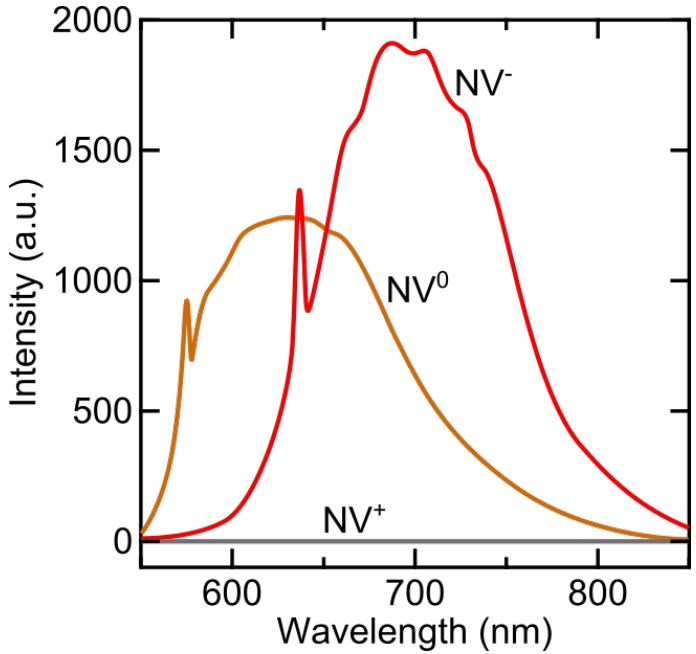


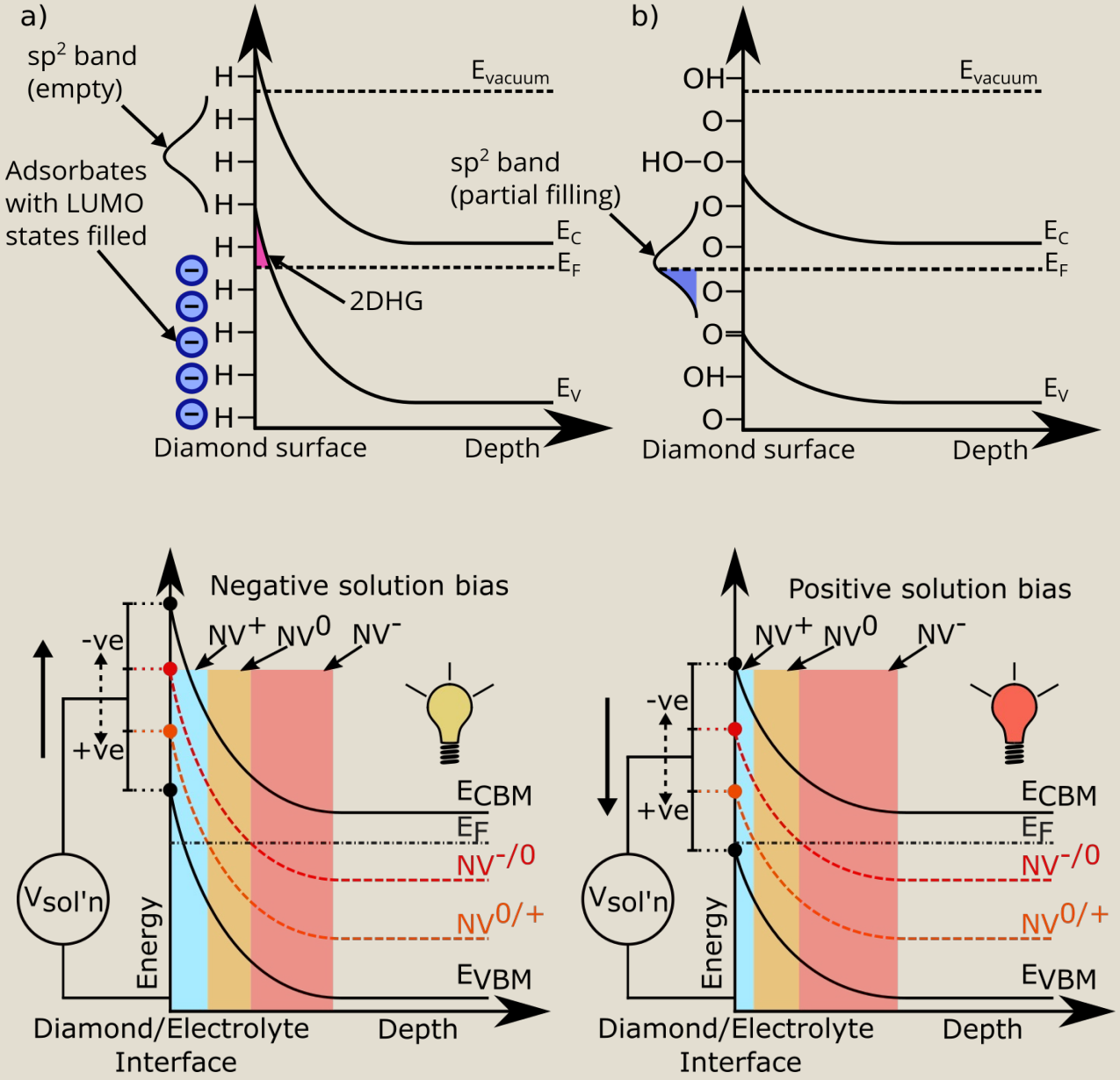




McCloskey, D. J. et al., *Nature Photonics* **16** (2022)

Operating Principles

- Near-surface nitrogen ion implantation creates thin (~7nm) n-type region.
- Annealing of implanted sample creates fluorescent nitrogen-vacancy (NV) defects.
- NV fluorescence depends on charge state.

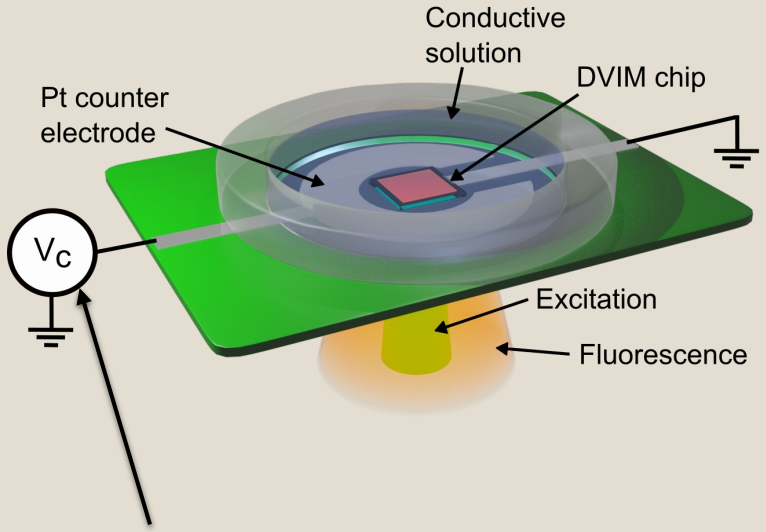


McCloskey, D. J. (2021) An all-optical voltage imaging platform using charge-sensitive fluorescent defects in diamond. PhD thesis. The University of Melbourne.

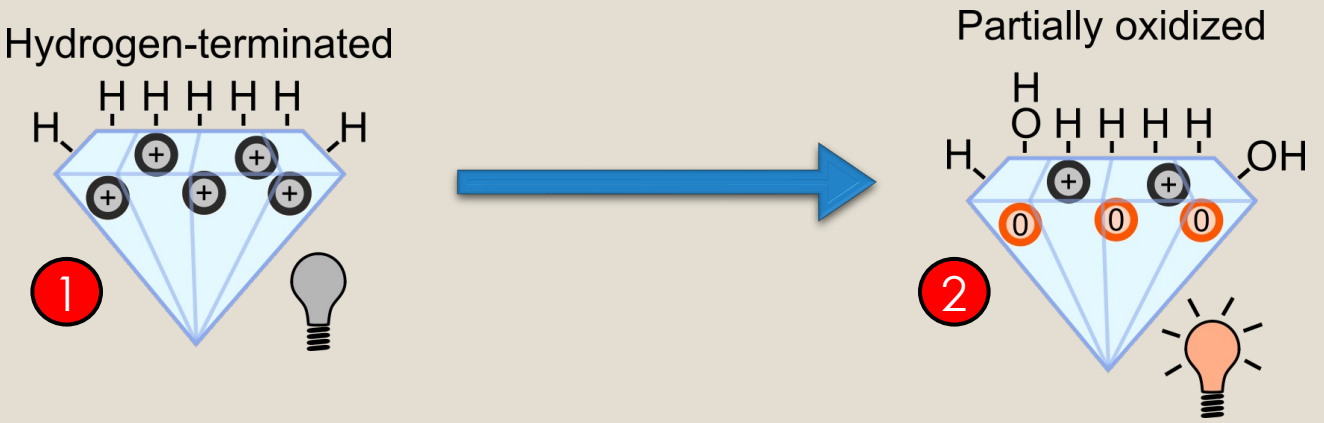
Operating Principles

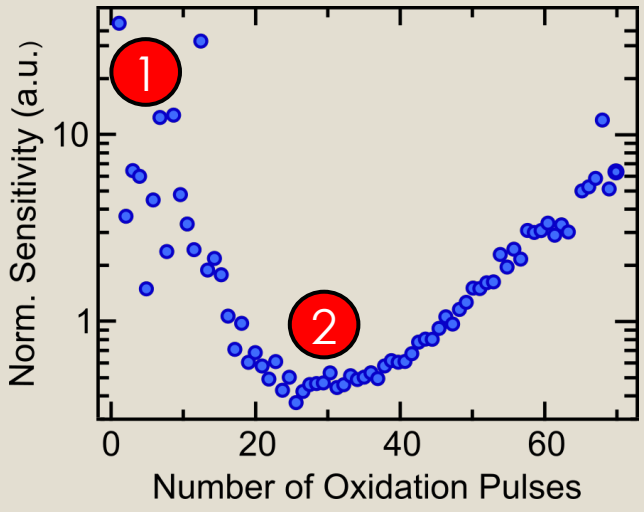
- H-termination creates narrow p-type region via surface transfer doping effect.
- Fermi level crosses the VBM creating a 2D hole gas at the surface.
- Charge-state dependent fluorescence of NVs allows for optical sampling of screening/image charge redistribution within the 2D hole gas.

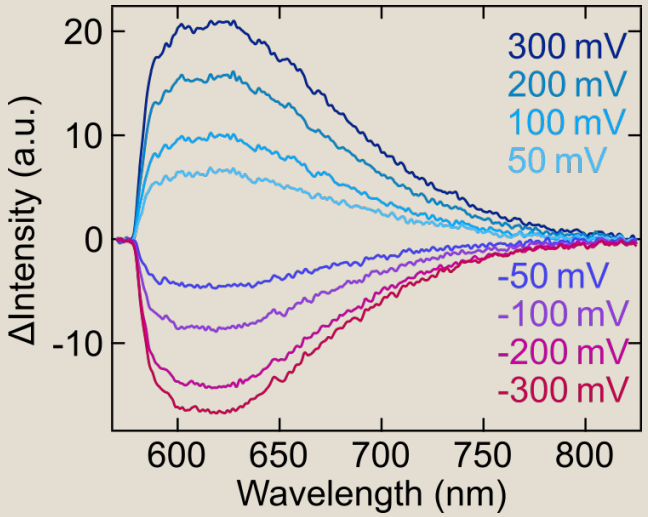
Electrochemical Tuning of Surface Termination



Short, high voltage pulses exceeding surface oxidation threshold



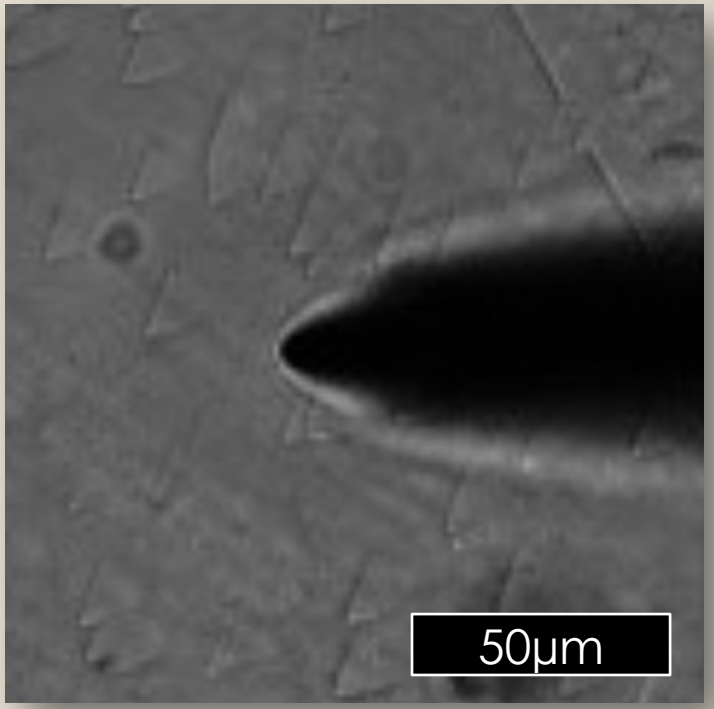


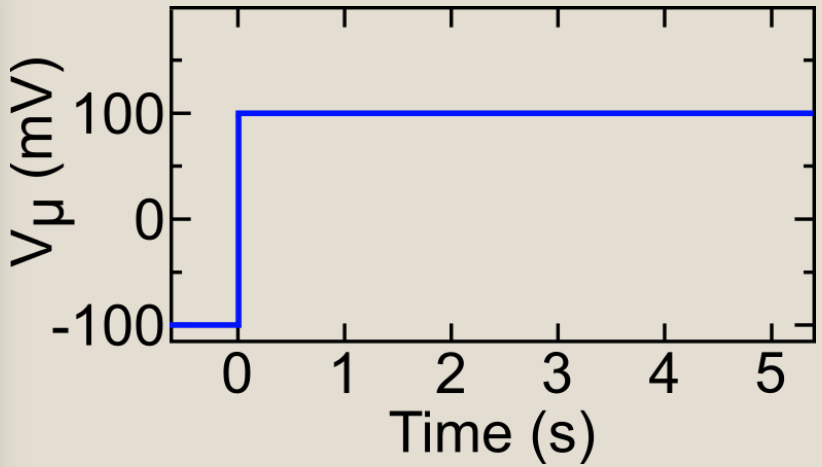


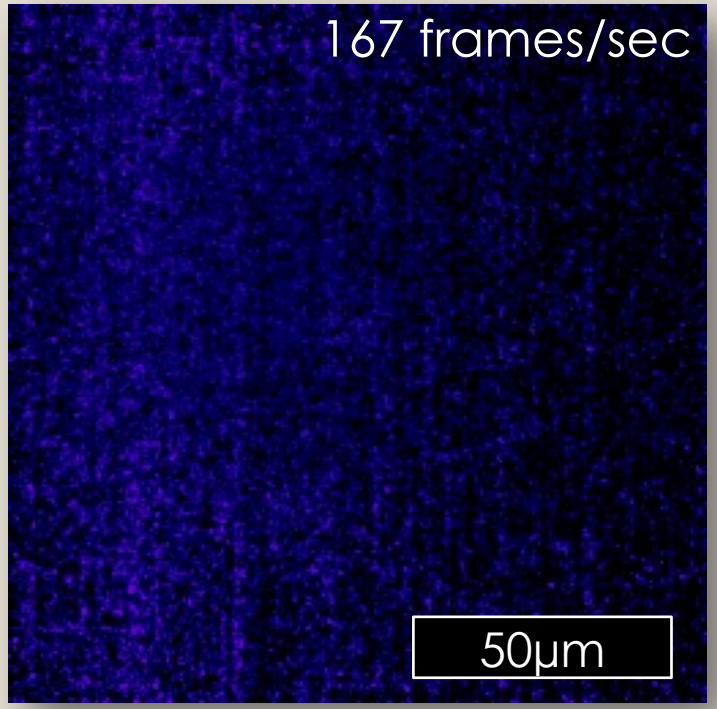
McCloskey, D. J. et al., *Nature Photonics* **16** (2022)

Voltage Imaging

- System allows imaging of local changes in solution potential via relative changes in NV fluorescence.

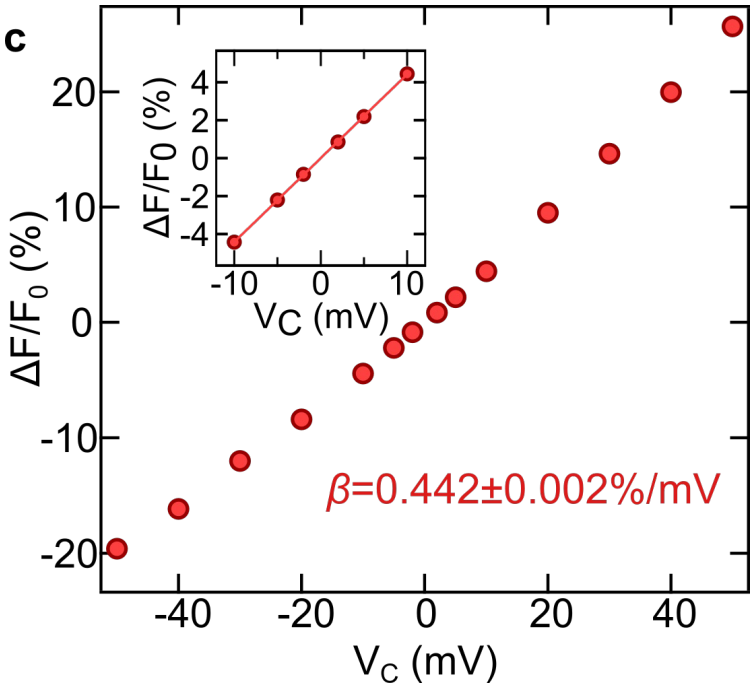


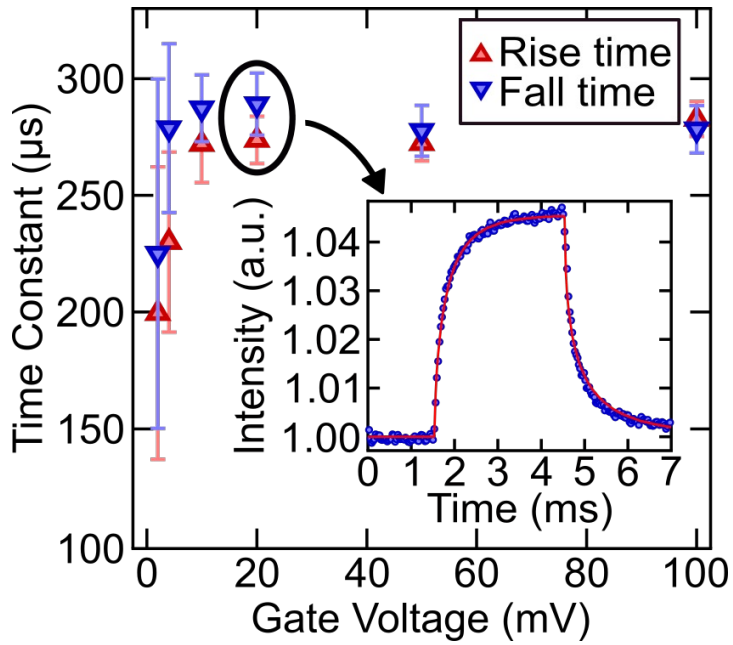




Performance

- Device exhibits linear response within a ±50mV dynamic range.
- Response time presently RC limited to ~300µs but still sufficient for electrophysiology.
- Substantial room for improvement: intrinsic NV response time is at most ~100ns (Schreyvogel C. et al, *Beilstein Journal of Nanotechnology* **7**, 2016).





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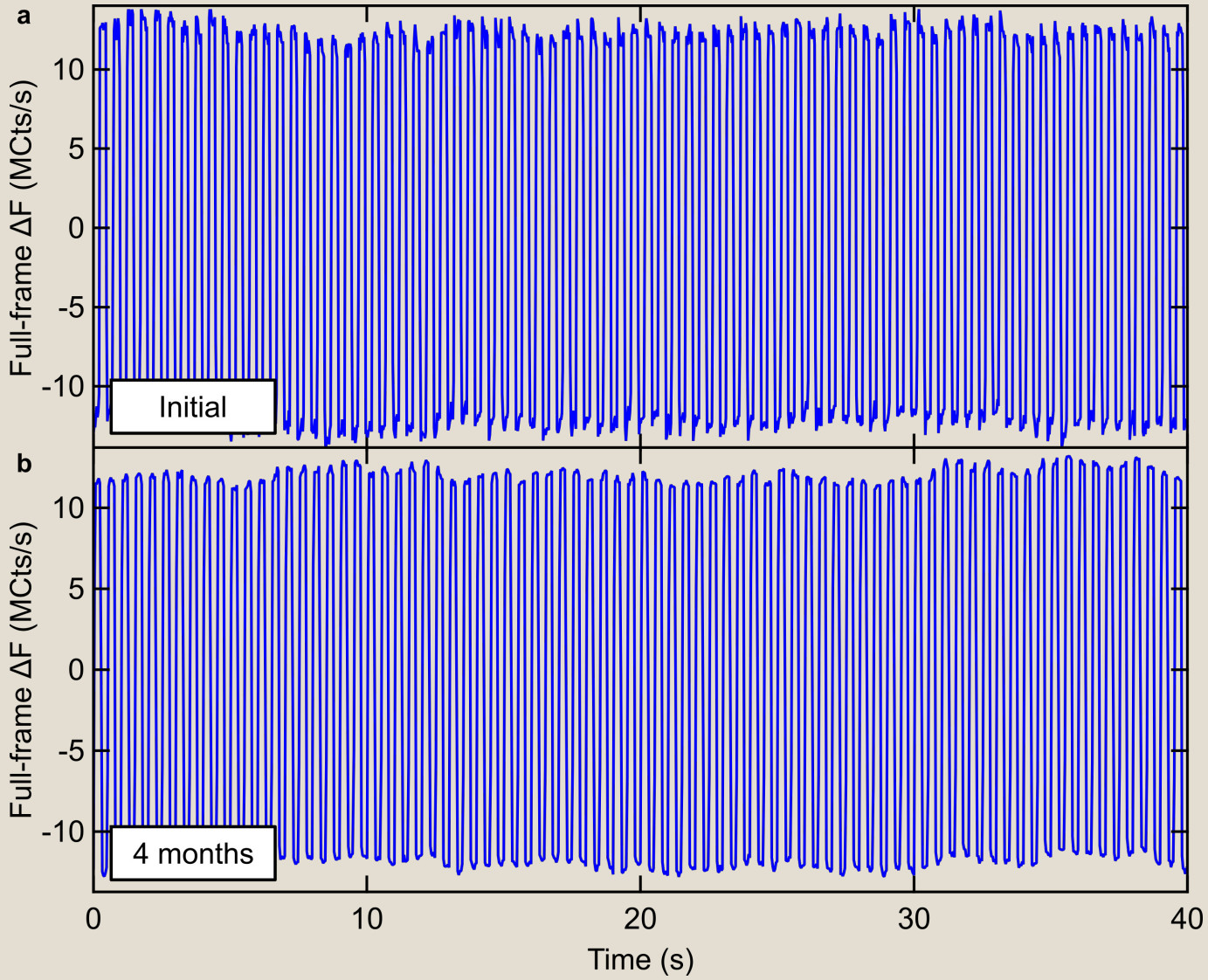
- Complete photostability.



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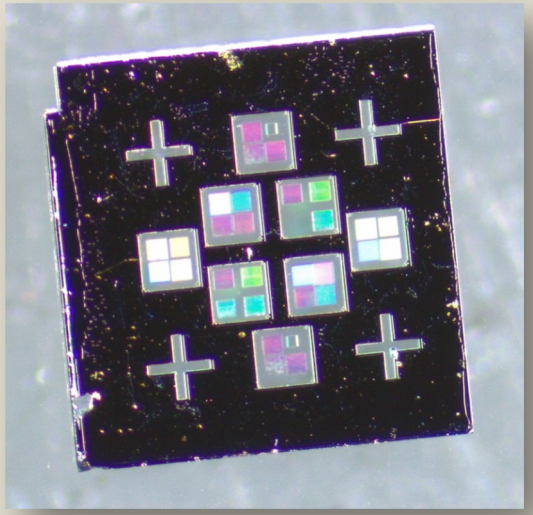
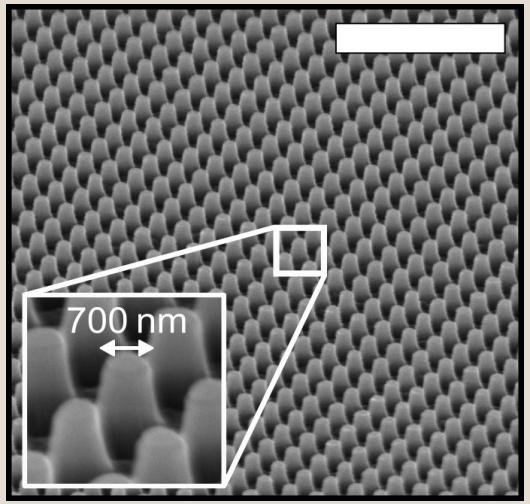
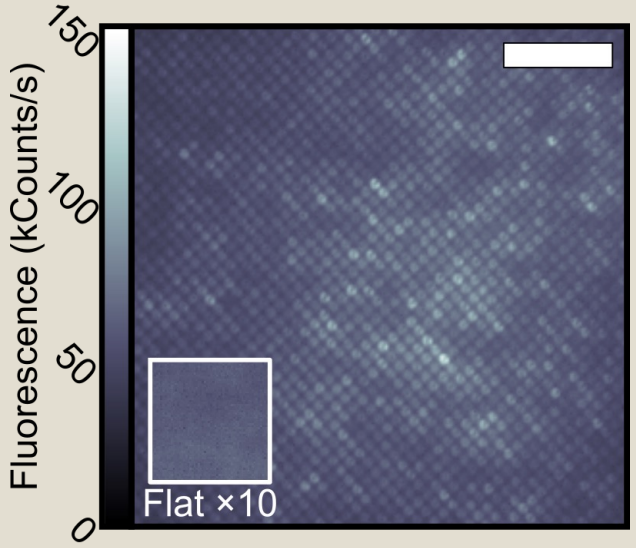
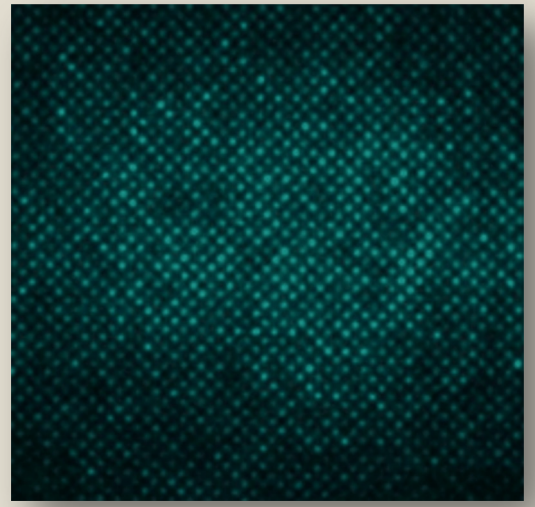
Diamond nanopillar ‘optrodes’ improve light collection and sensitivity









- Also act as anchor points for neuronal culturing, can boost signal strength via ‘engulfment’ by the cell membrane.

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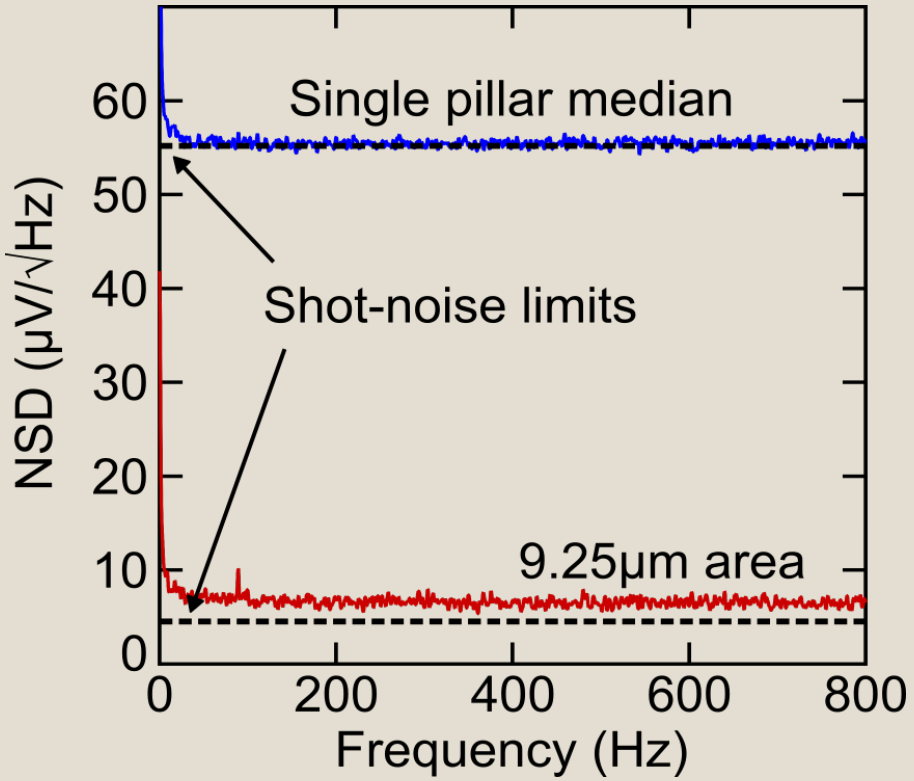
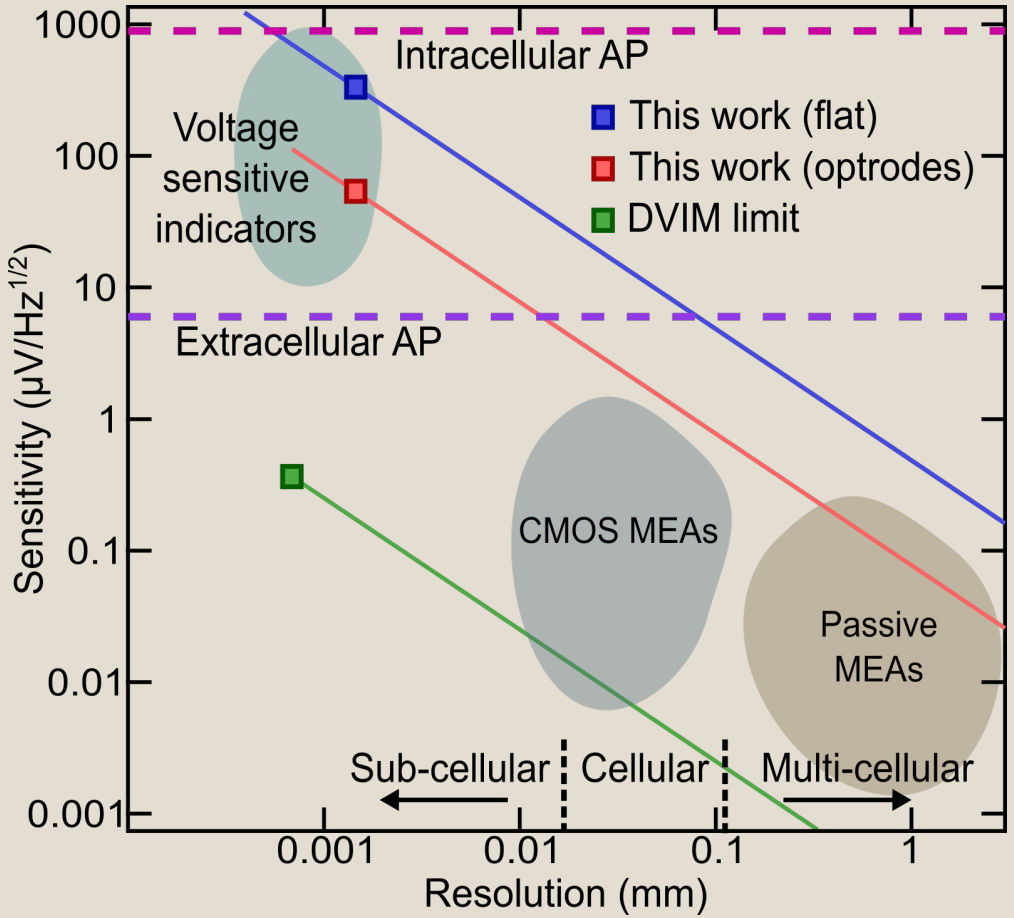
(scale bars 10 μm)

◦ Sensor is photon shot-noise limited: $\eta = \frac{1}{\beta\sqrt{N_0}}$

N_0 is the photon count rate.
β is the responsivity($\Delta N/N_0$ per mV).

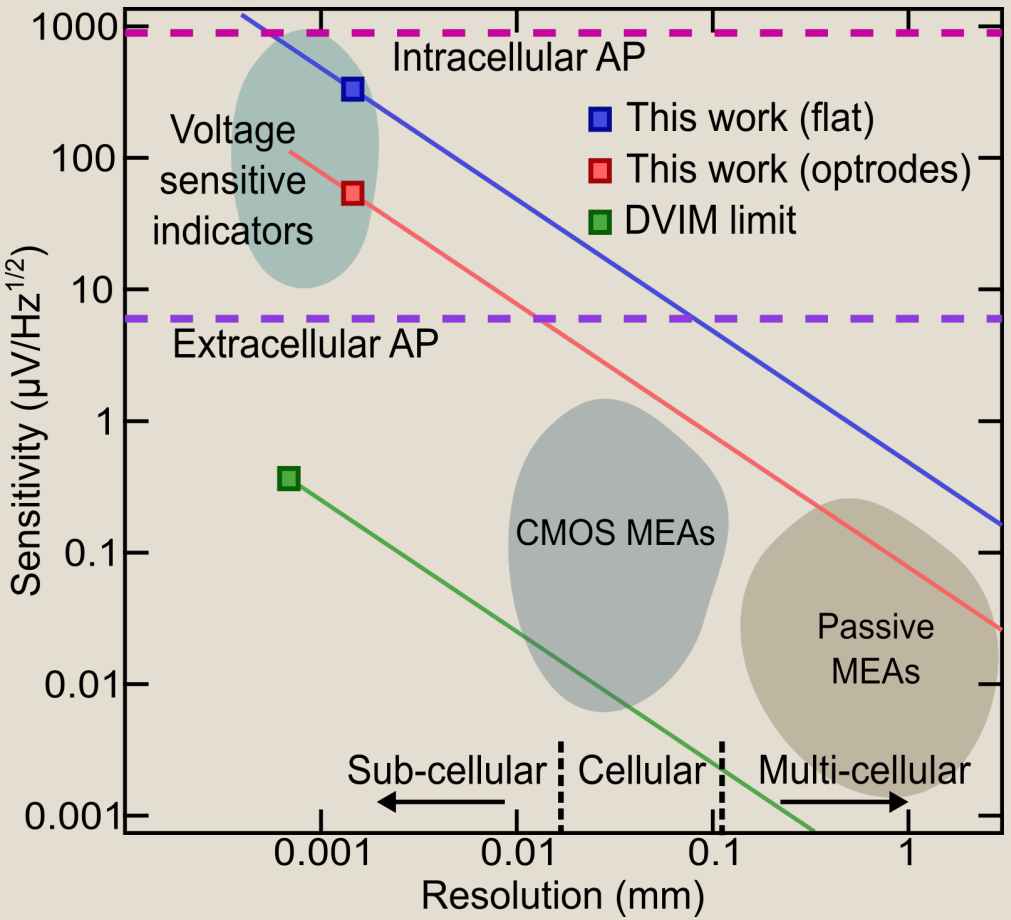




◦ Implies sensitivity scales with resolution (number of emitters).

McCloskey, D. J. et al., *Nature Photonics* **16** (2022)

Dr. Nikolai Dontschuk – Friday at 10AM (CMM 8)



- Implies sensitivity scales with resolution (number of emitters).

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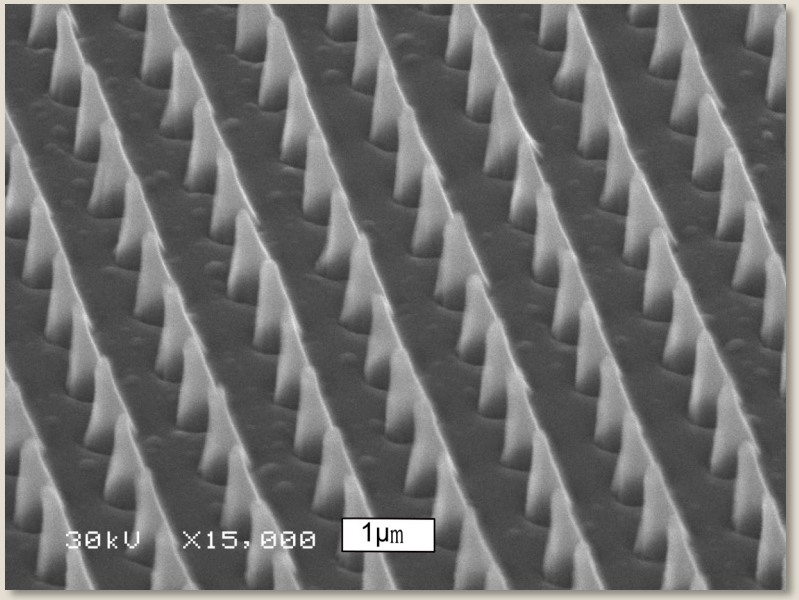
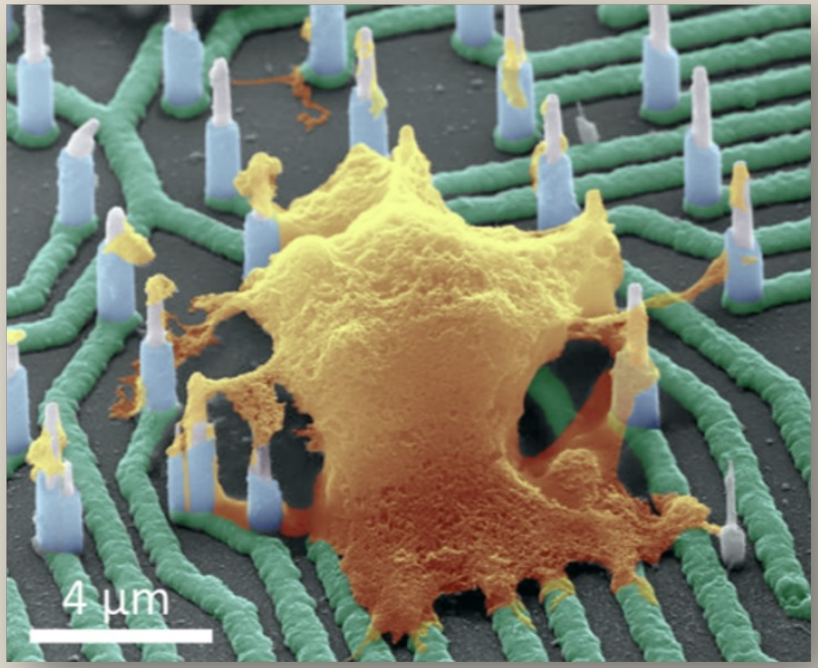
Prospects for Biological Imaging

- Current device specs sufficient for intracellular measurements.
- Diamond 'nanoneedle' fabrication for intracellular measurement is currently underway.



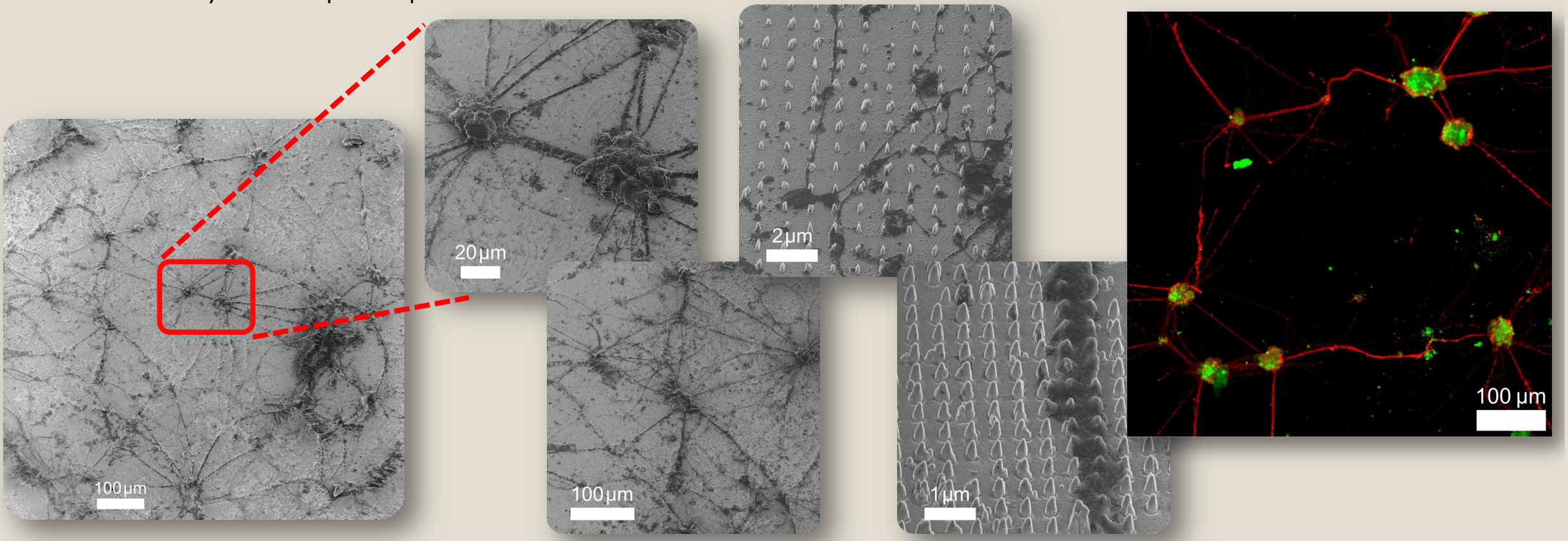
- Prior work on silicon nanowires shows tip diameters <200nm are suitable for achieving spontaneous intracellular access (Liu et al., *Nano Lett.* 2017).



Liu, R. et al., *Nano Letters* **17** (2017)

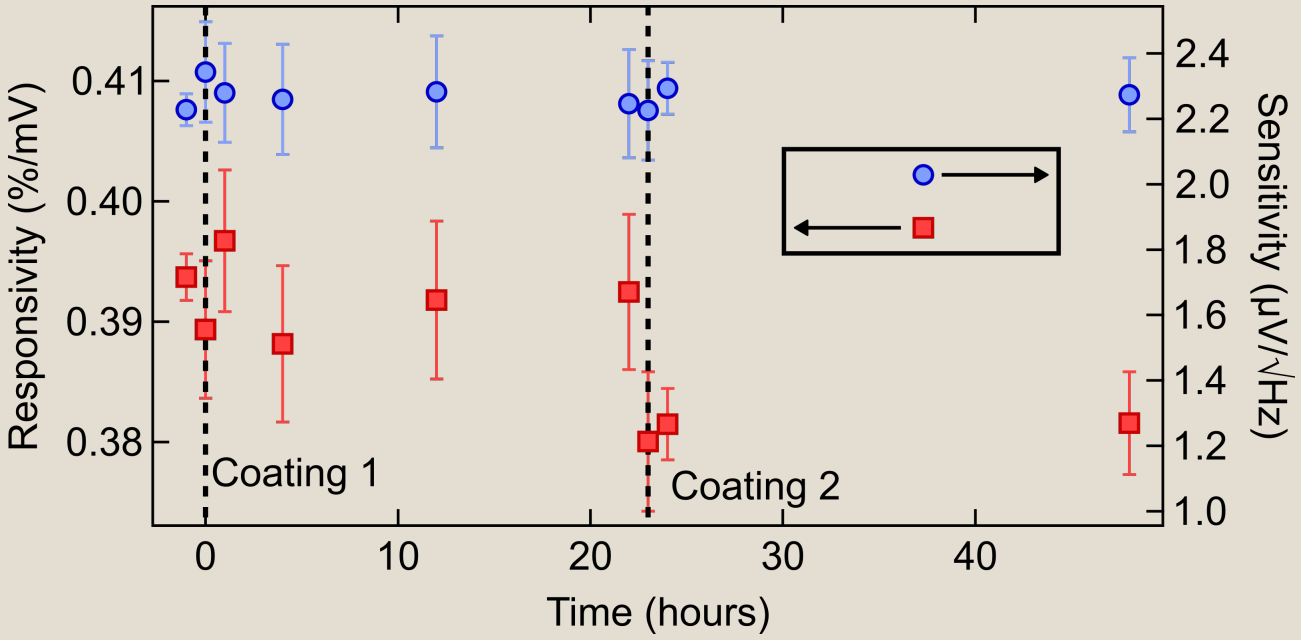
Prospects for Biological Imaging

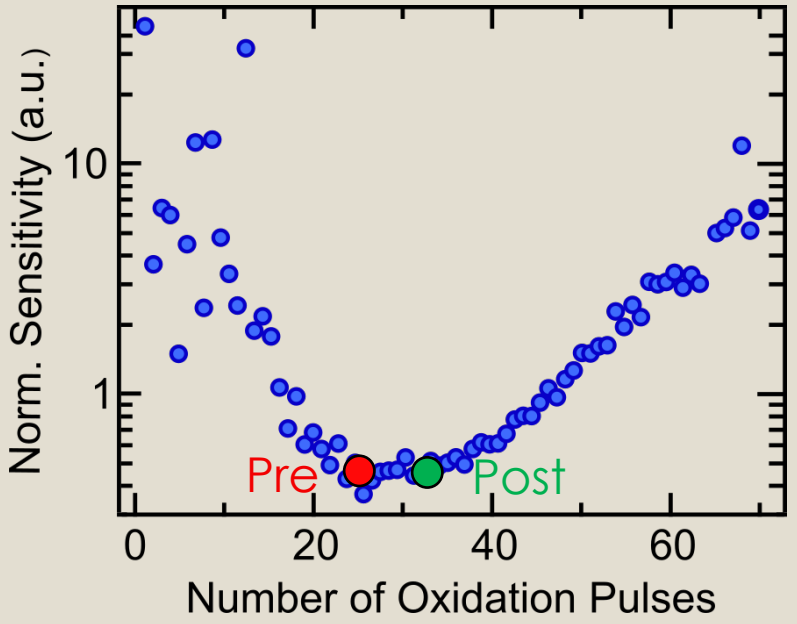
- Neuronal cultures on single crystal O and H-terminated diamond successful, but cell viability is an open question.



Prospects for Biological Imaging

- DVIM is compatible with cellular adhesion promoters based on cationic polymers (poly-D-lysine).
- Cationic coating is equivalent to a change in the negative adsorbate density i.e., an effective change in the surface oxidation state.





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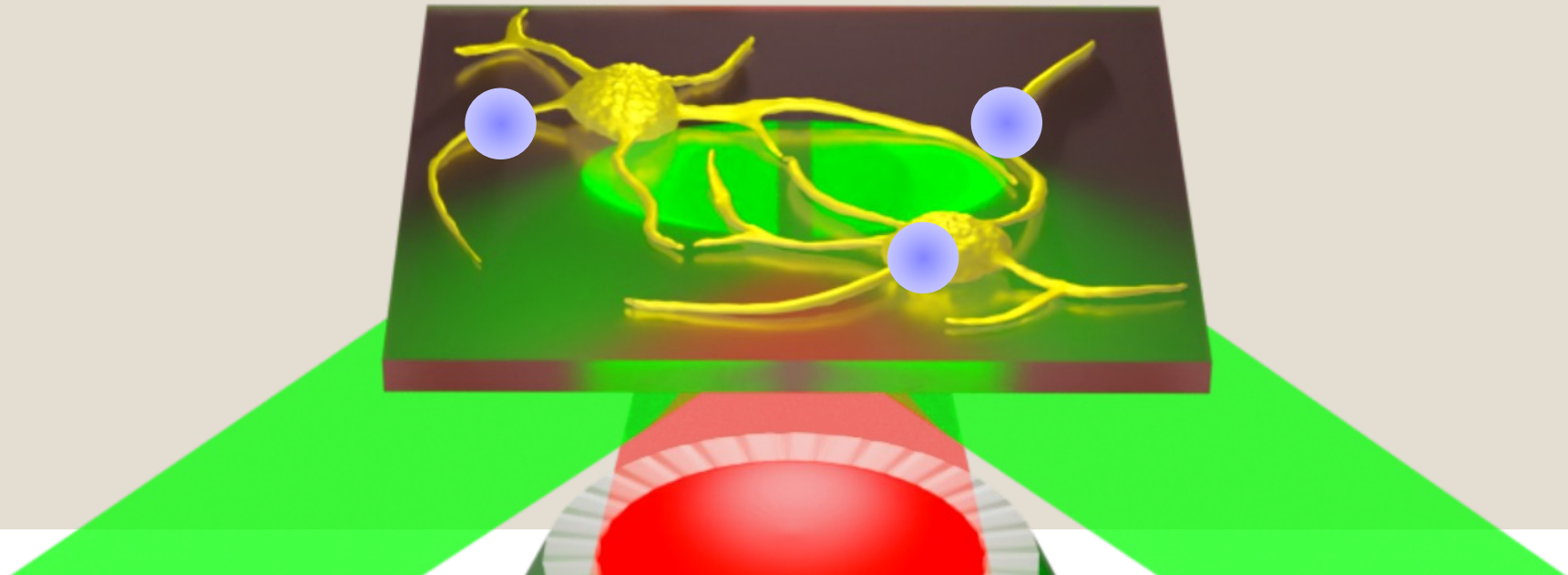
Thanks to:

- Master's students:
  - Charlie Pattinson
  - Hunter Johnson
- Colleagues:
  - Dr. Nikolai Dontschuk
  - Dr. Alastair Stacey
  - Dr. Wei Tong
  - Dr. Athavan Nadarajah
  - Dr. Liam Hall
- Professors:
  - Prof. Steven Prawer
  - Prof. Lloyd Hollenberg
- Supervisor:
  - A/ Prof. David Simpson

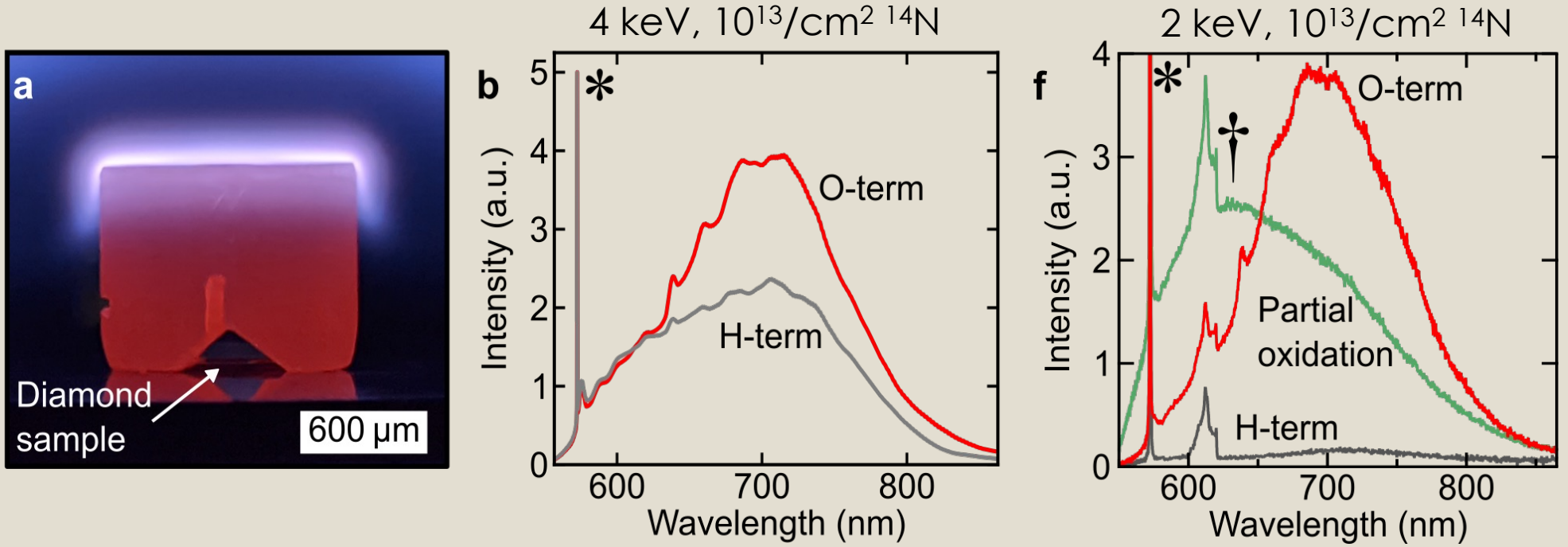
Thank you for your attention

Potential Interesting Use-Cases

- Fabrication of long (e.g., 100μm) optrodes for optical access to organoid interiors.
- Sub-micron resolution could enable *in vitro* mapping and monitoring of synaptomic evolution ('memory') processes.
- Transparent sensor could be coupled with existing fluorescent tags for correlated imaging of electrical and e.g., structural, proteomic, metabolomic, genomic characteristics.
- Optogenetic point-stimulation and DVIM recording for all-optical closed loop 'optopatch' system with continuous recording.



‘Turning off’ the ensemble



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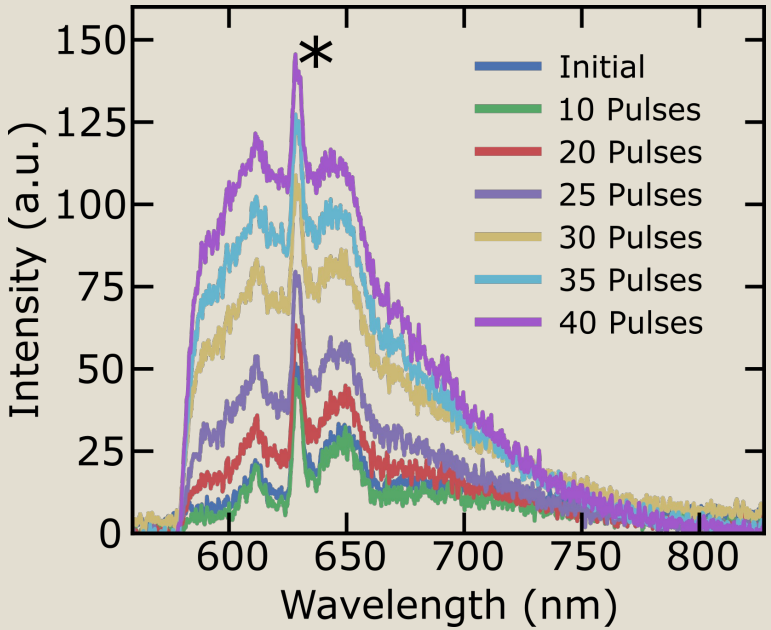
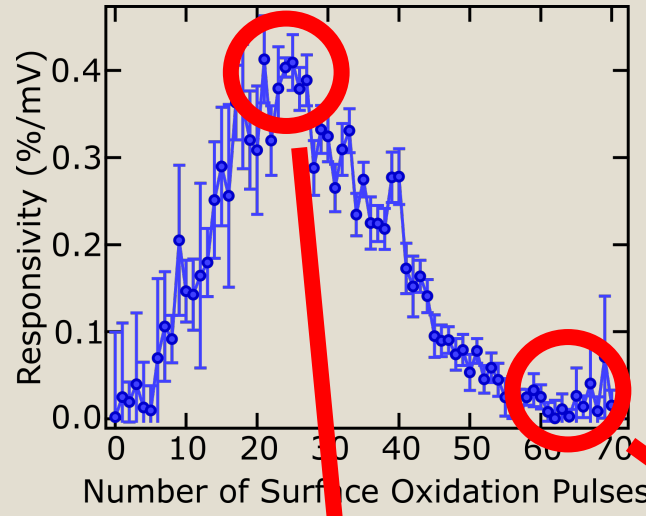
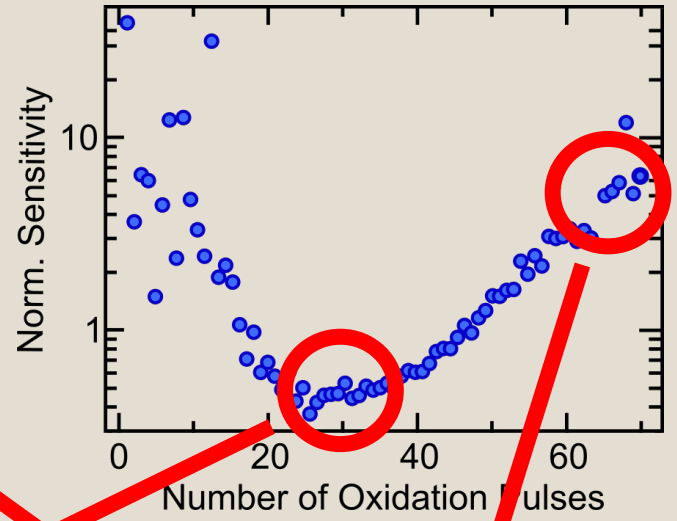
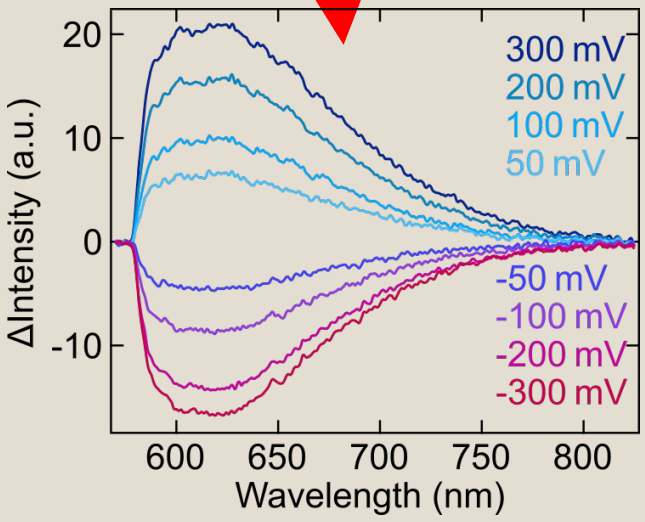
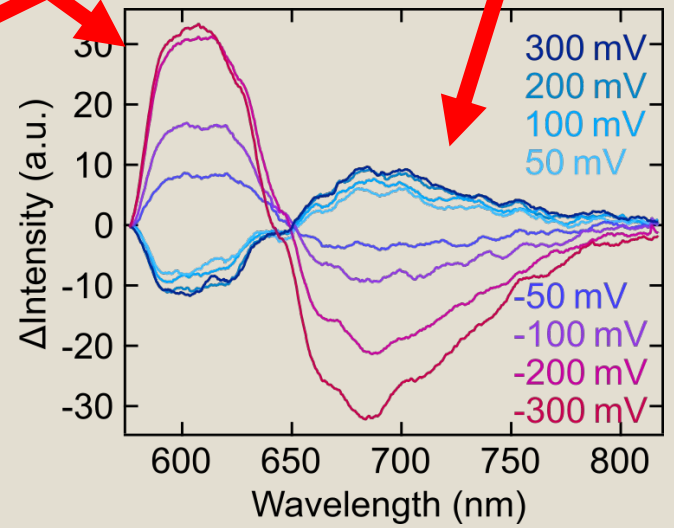
Electrochemical Tuning of Surface Termination





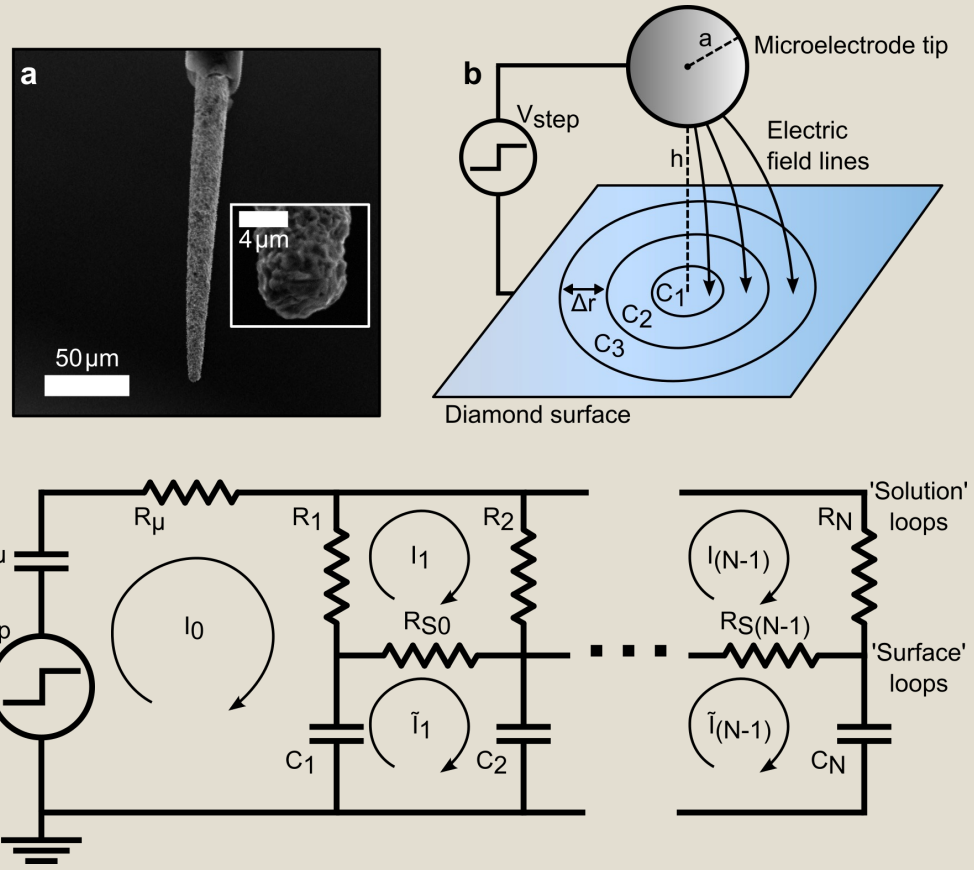


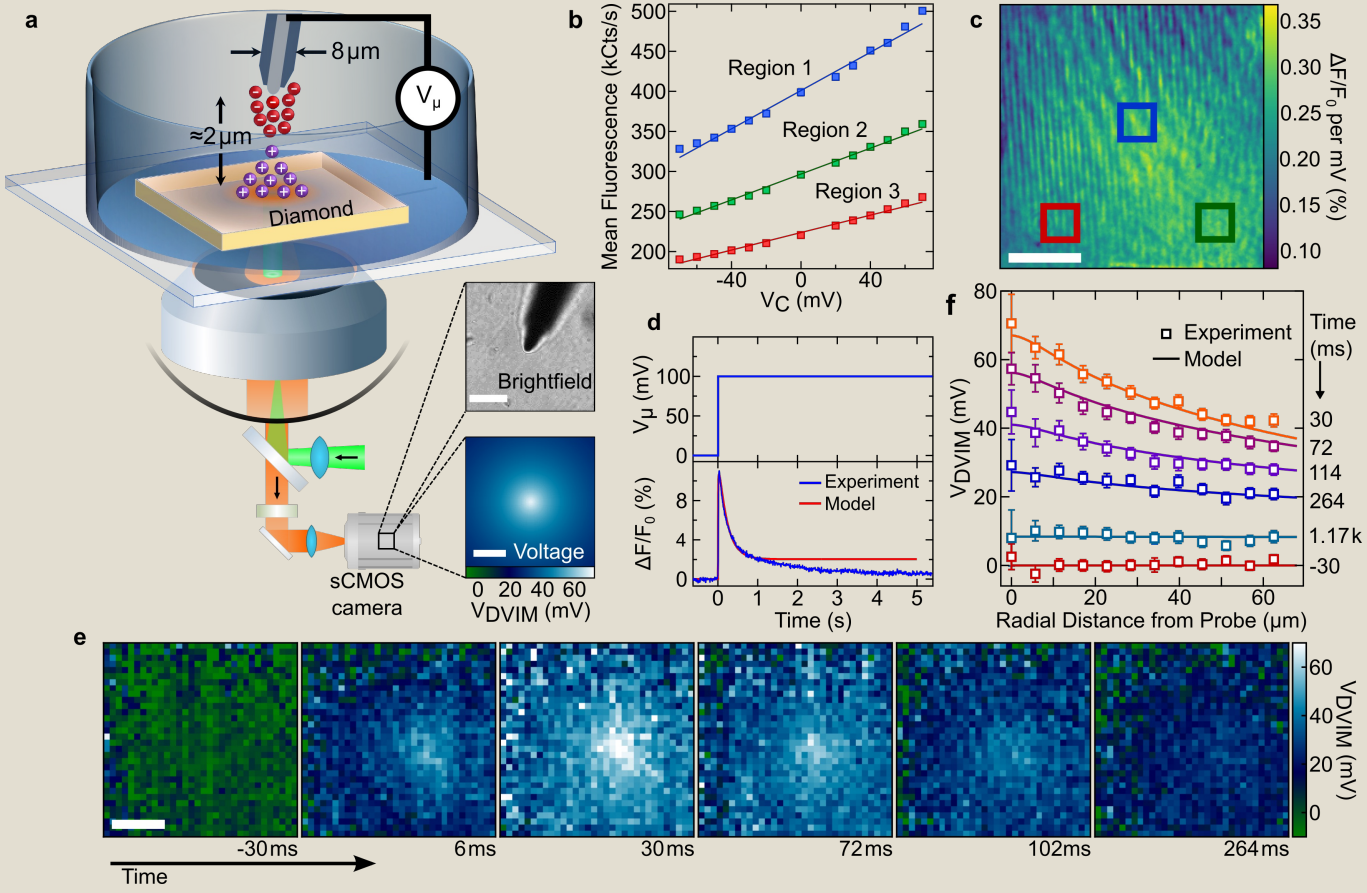




McCloskey, D. J. et al., *Nature Photonics* **16** (2022)

Experiment Validates Linear RC Circuit Model





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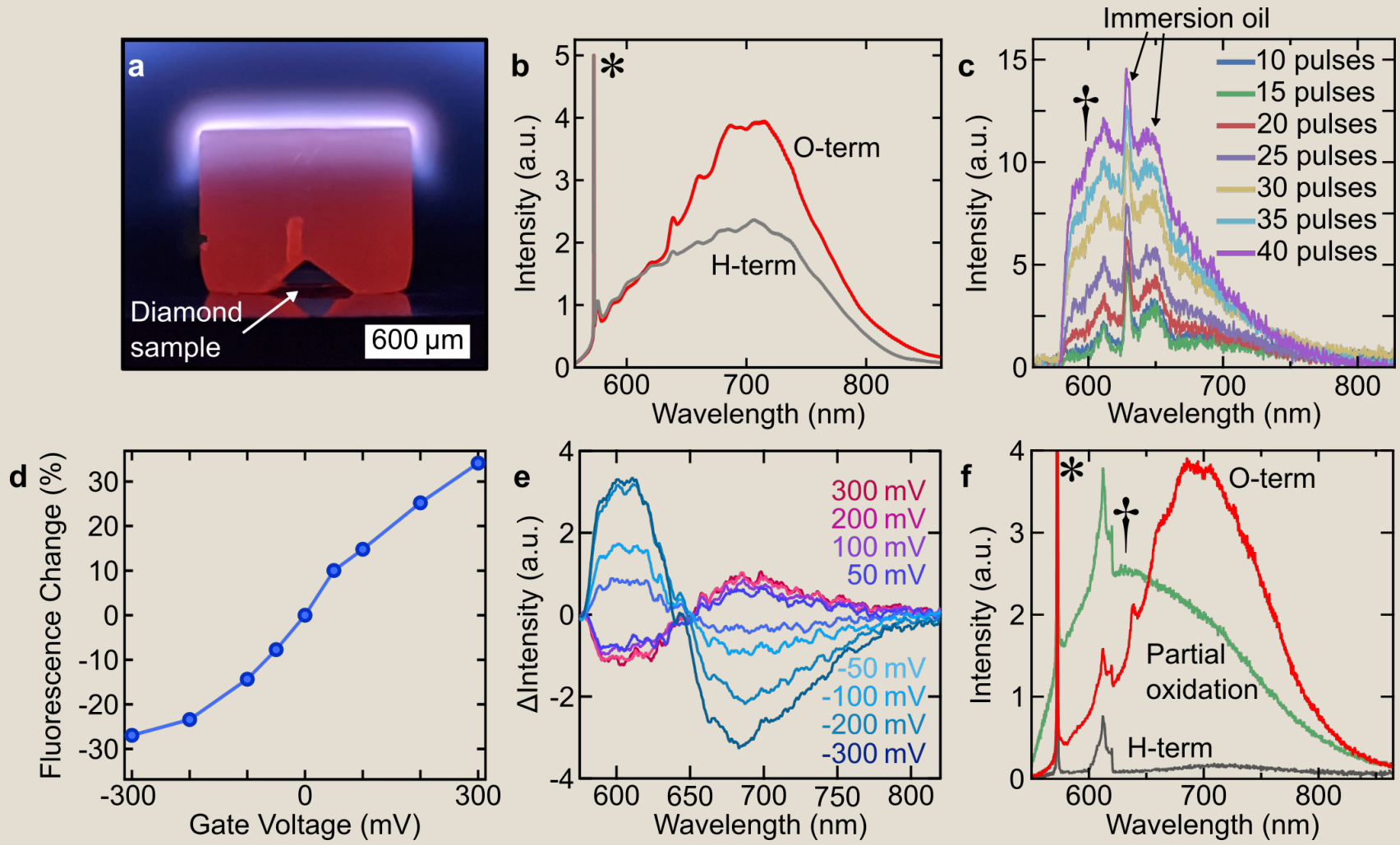
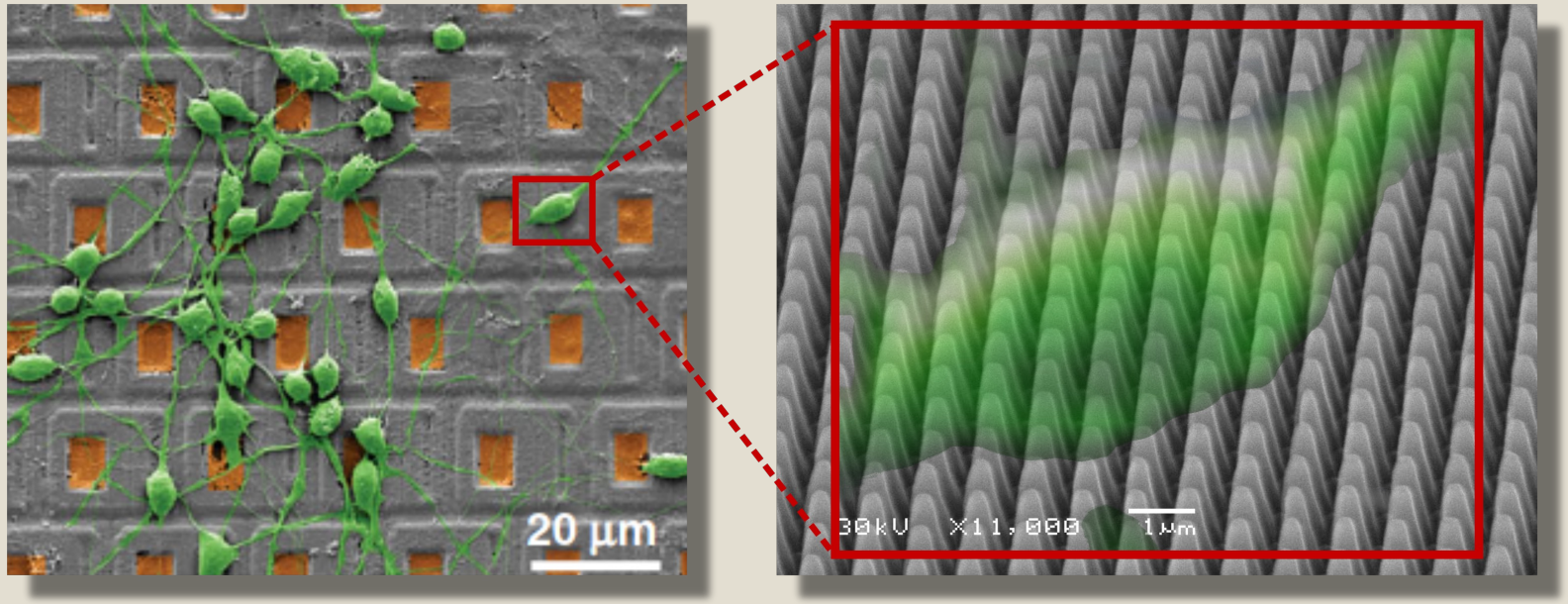


McCloskey, D. J. et al., *Nature Photonics* **16** (2022)

Prospects for Biological Imaging

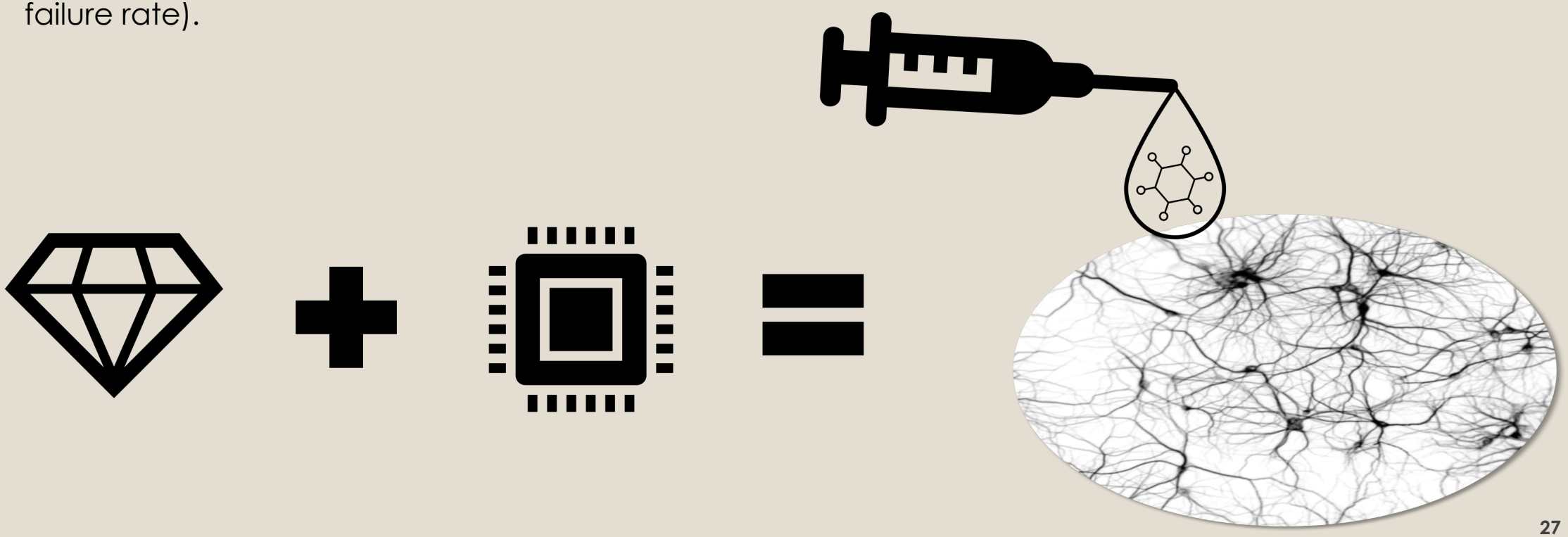
- Theoretical maximum single-optrode sensitivity is around 370nV/$\sqrt{\text{Hz}}$ (~2× the per-area sensitivity of CMOS chips).
- Current N-NV conversion ~0.1%. 10% conversion will enable comparable performance to CMOS MEAs for extracellular AP tracking with ~micron resolution.
- Progress being made towards achieving these conversion ratios.
- Current work also exploring alternative fluorescent defects for this application.



Muller, J. et al., *Lab on a Chip* **15** (2015)

Potential Interesting Use-Cases

- Identification of new markers/indicators of healthy and diseased states that are robust to culture-to-culture variation.
  → New assays for assessing drug efficacy.
  → Precision medicine for central nervous system disorders.
  → Integration into preclinical neuro-pharmaceutical development pipeline (reduced clinical trial failure rate).

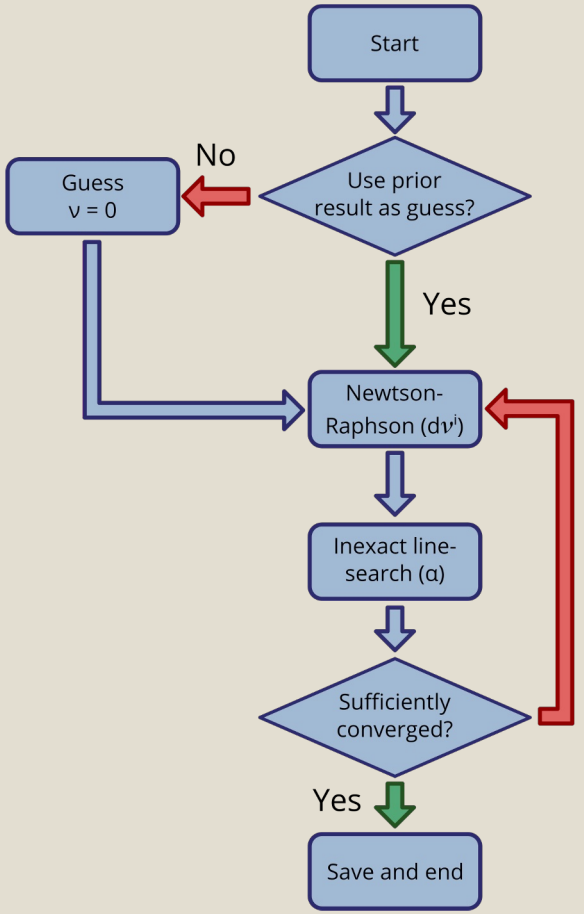


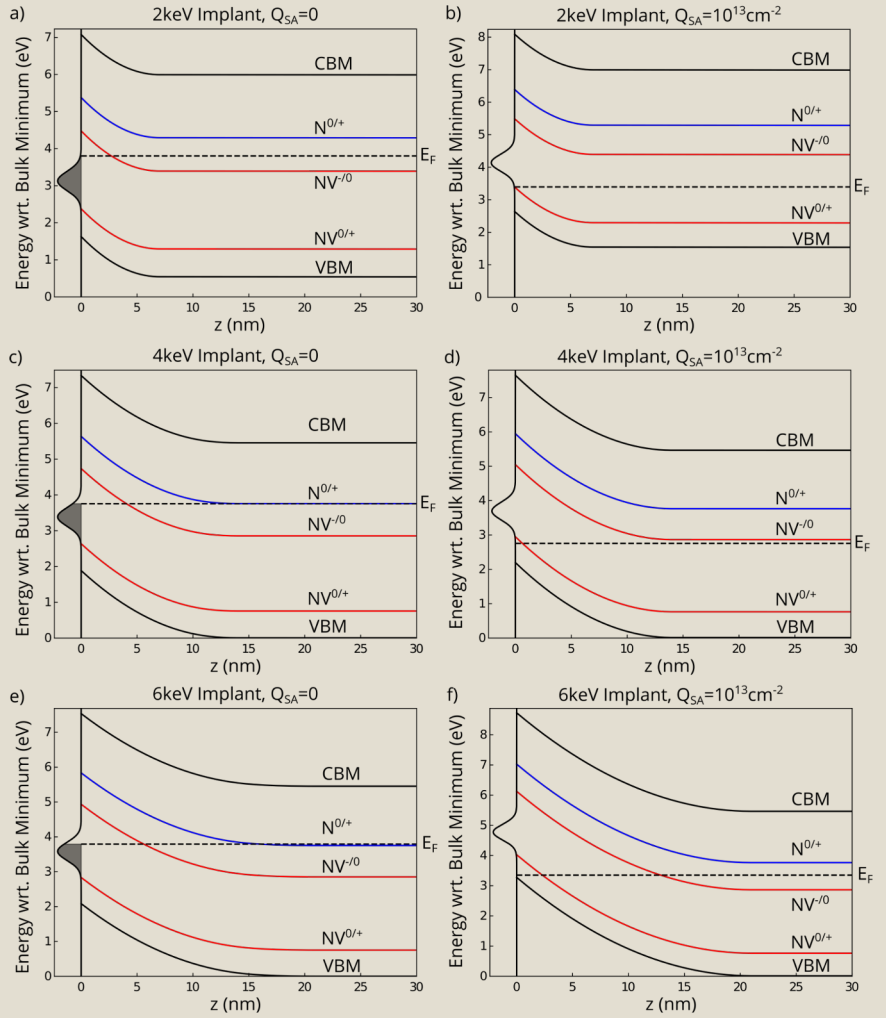
Band-bending calculations

$$\nabla^2 V(z) = \frac{-\rho(z,V)}{\epsilon}$$

$$\rho(z,V) = e\left[p(V) - n(V) + \sum_{i=1}^{n_D} N_{Di}(z)(1 - f_D(E_{Di},V)) - \sum_{i=1}^{n_A} N_{Ai}(z) f_D(E_{Ai},V)\right]$$

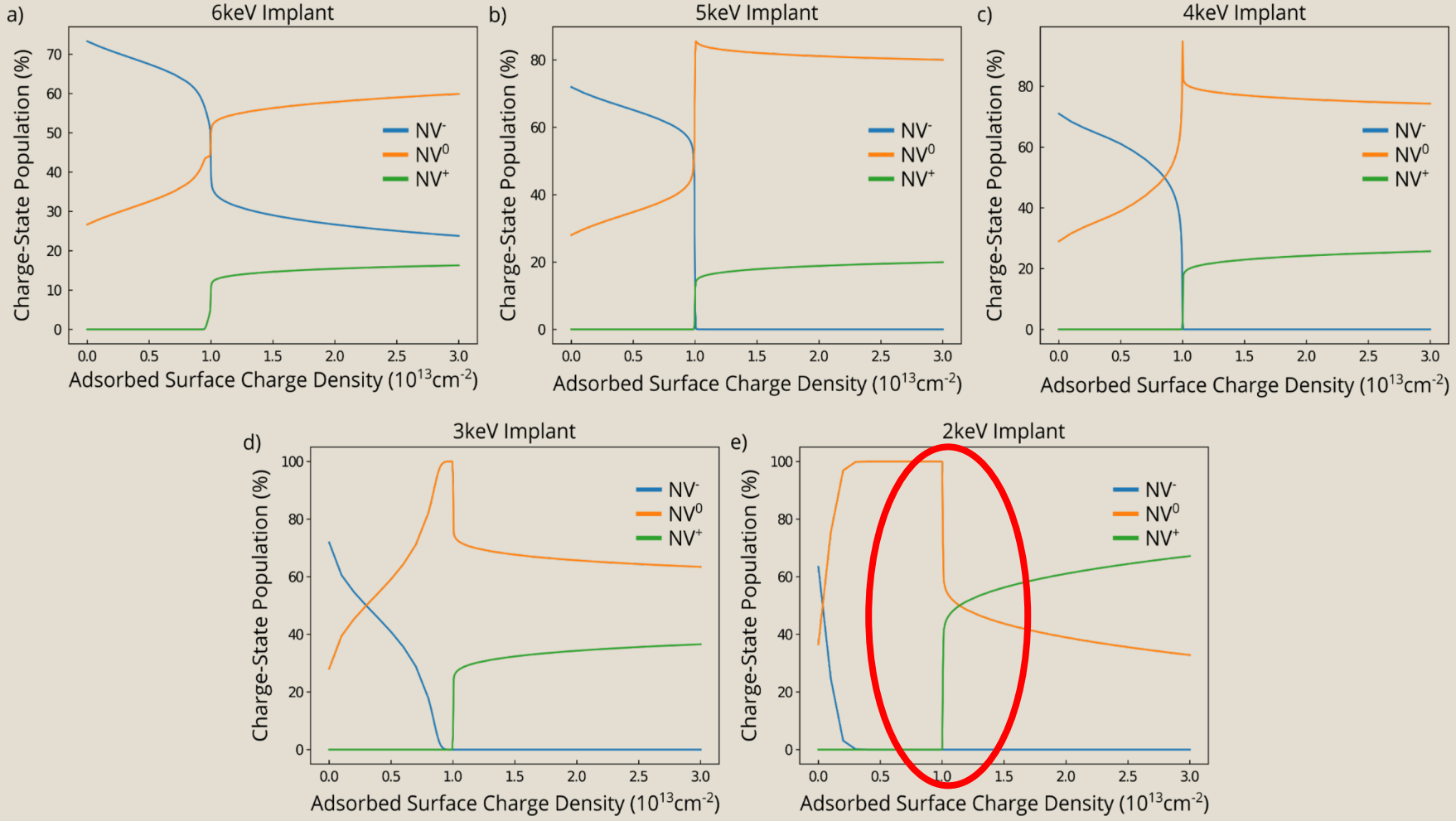
$$f_D(E,V) = \frac{1}{1 + e^{\frac{E - E_F - V}{kT}}}$$





McCloskey, D. J. (2021) An all-optical voltage imaging platform using charge-sensitive fluorescent defects in diamond. PhD thesis. The University of Melbourne.

Band-bending calculations



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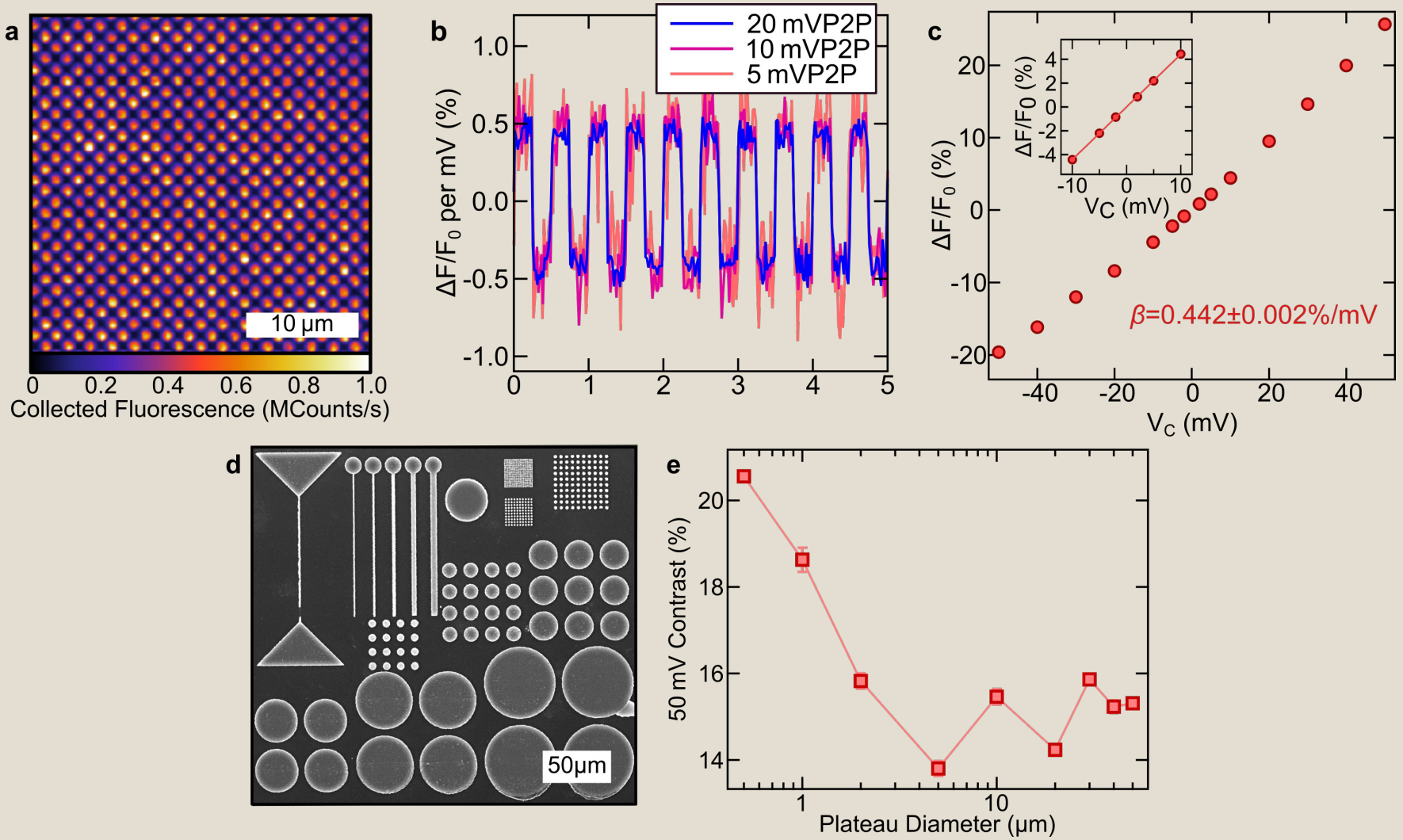
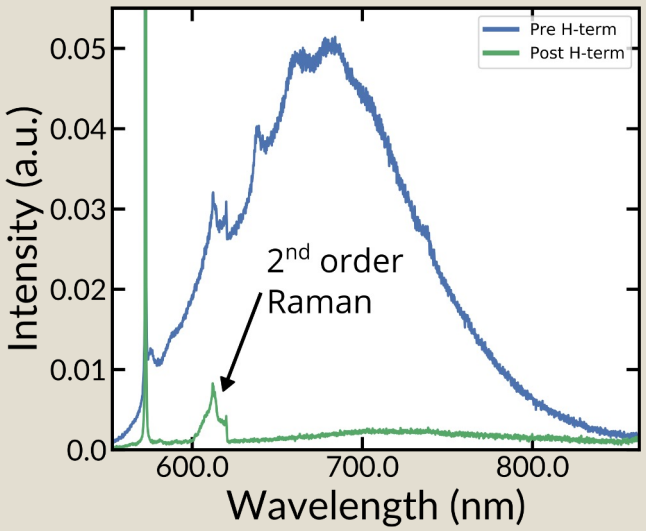
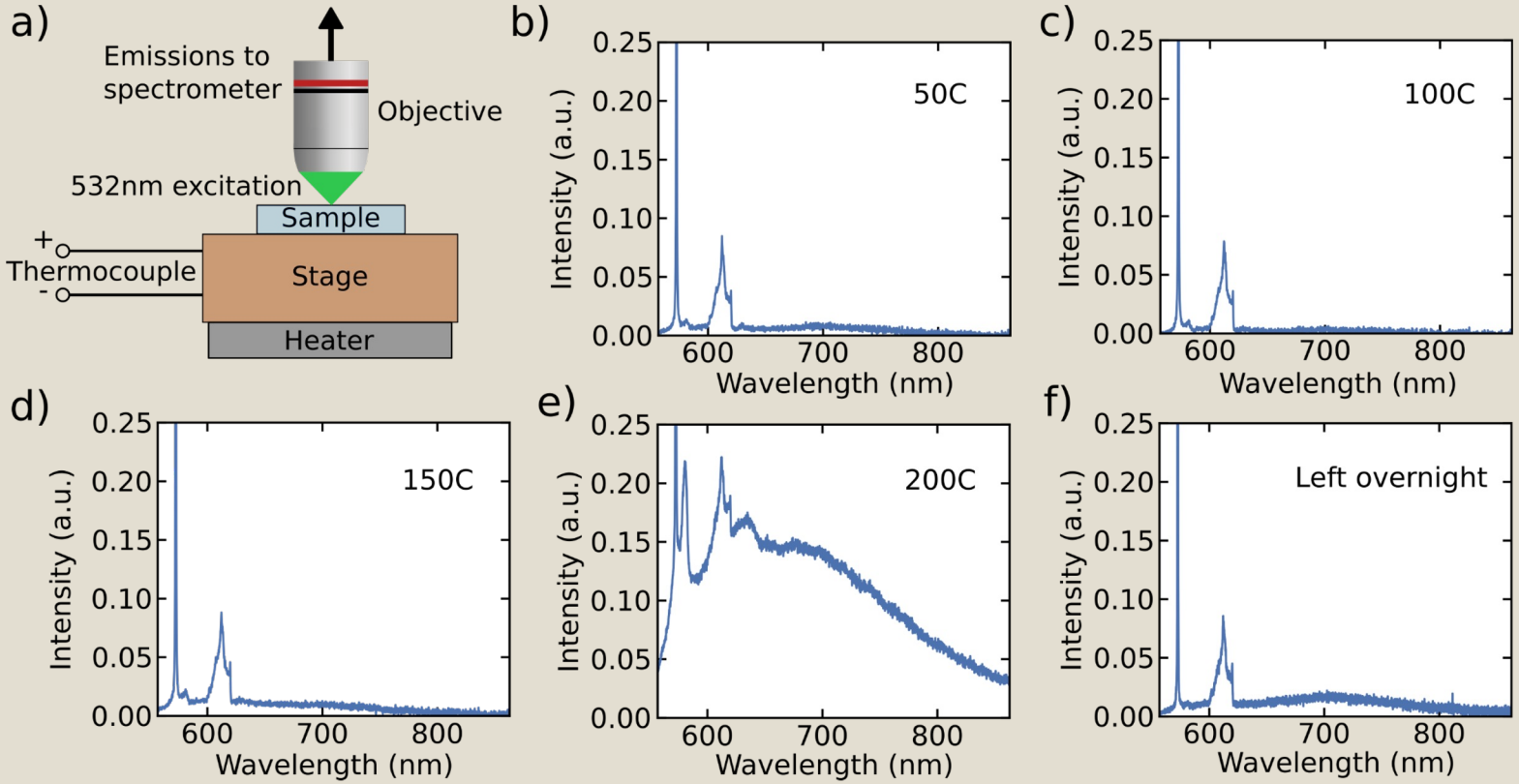


McCloskey, D. J. et al., *Nature Photonics* **16** (2022)

Temperature-dependent fluorescence spectroscopy confirms conversion to NV^+





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