# A JEDI Toolbox for Imaging Cell Membrane Potential Dynamics

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## Background

New tools for large-scale, *in vivo* neuronal recording with high spatiotemporal resolution are required to understand how brains work

Understanding how the brain functions is a major goal of neuroscience and requires studying how information is processed and communicated in neuronal circuits[1]. While it has long been realized that neural activity is encoded as the changes of membrane potential in neurons, recording neural activities from a defined population of neurons remains challenging due to the complexity of brain[2], the fast kinetics of neural electrical activities[3], and the high scattering of brain tissue that imaging-based techniques encounter.

Genetically Encoded Voltage Indicators offer several advantages over other recording methods, including Electrodes, Calcium indicators and synthetic dyes

An emerging technology for real-time monitoring of electrical dynamics *in vivo* is voltage imaging using Genetically Encoded Voltage Indicators (GEVIs) — engineered light-emitting protein indicators whose brightness directly reports voltage[4]. While there are multiple well-established methods for neural recording, GEVIs offer several advantages over alternative methods as listed in Table 1[5-8].

| | Throughput | Cell-type specificity | Spatial resolution* | Temporal resolution |
|---|---|---|---|---|
| Patch-clamp | Low | Yes** | Low | High |
| Electrode array | High | No | Low | High |
| GECIs (Genetically encoded Calcium indicators) | High | Yes | High | Low |
| Voltage sensitive dyes | High | No | High | High |
| GEVIs (Genetically encoded voltage indicators) | High | Yes | High | High |

**Table 1| Comparison of neural recording methods.**
* High spatial resolution here is defined as the capability to record from subcellular compartments.
** Patch-clamping offers cell-type-specific recording by combining with cell-type-specific labels.

Existing GEVIs are sub-optimal for deep-tissue imaging *in vivo*

Despite significant progress made by the GEVI community so far, the performance of current voltage indicators still needs further optimization[9,10]. The fastest GEVIs are sufficiently sensitive for detecting of single action potentials in spiking trains[11], yet they have poor brightness and have no response under two-photon illumination[12]. There are also two-photon compatible GEVIs with significantly improved brightness[13-14], but

they are not fast enough to faithfully track 100 Hz spike trains[10]. **In summary, there is an urgent need for faster, brighter, more sensitive, more photostable GEVIs with two-photon compatibility to allow long-term deep-tissue imaging of many individual neurons in behaving animals**

## Methods

Automated GEVI Evolution with Integrated High-throughput screening platform

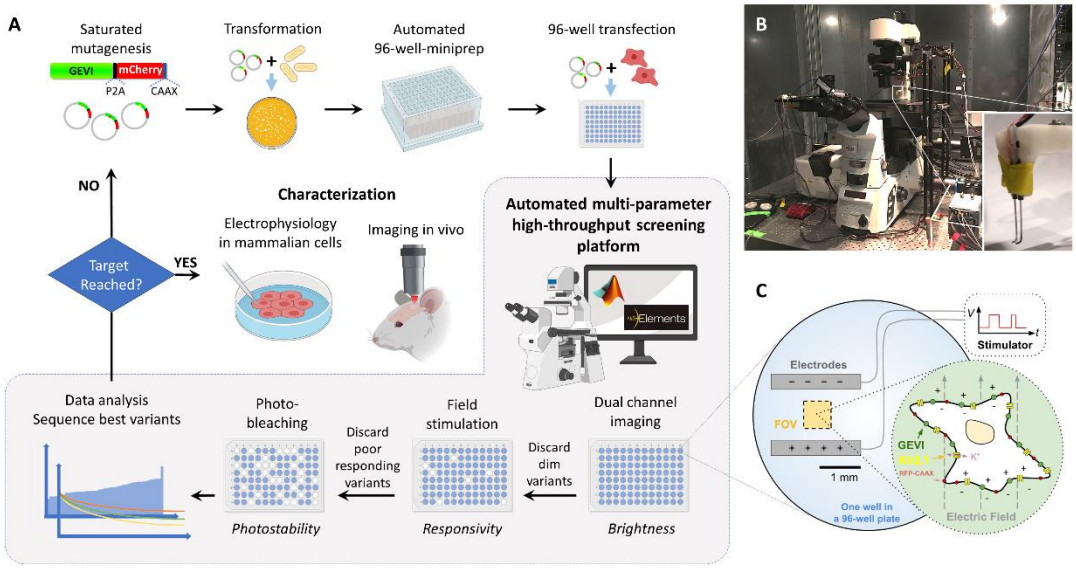


**Figure 1| Automated multi-parameter high-throughput GEVI screening pipeline.** A) Screening workflow. Steps in the dashed frame indicate the automated multi-parameter screening. B) Screening setup. The white box locates the electrodes used for field stimulation. Inset: a zoom-in view of the electrodes. C) Schemetic diagrams of the field stimulation in 96-well plate (blue-shaded area) and a single cell expressing Kir2.1, GEVIs and membrane-anchored RFP as brightness reference.

## Results

With rational-chosen mutagenesis position, improved variants were found in single-site saturation mutagenesis screenings (Figure 2A). Combining the mutations identified through multiple rounds of screening resulted in GEVIs with improve brightness, response amplitude as well as photostability. The top-performing GEVIs were named as **Jellyfish-derived Electricity-reporting Designer Indicator, or JEDI**s. JEDIs were further confirmed by whole-cell patch clamp, known as the golden standard to characterize the kinetics and response amplitude of GEVIs (Figure 2, C-D), as well as *in vivo* experiments in mouse (Figure 3), drosophila and fish (data not shown). Now we have an ever-expanding toolbox of JEDI optimized for one-photon and two-photon studies, compatible with multiple model animal expression, and benchmarked in varies imaging set-up (Table 2-3). We anticipate these efforts will ultimately enable broad utilities for imaging cell membrane voltage dynamics in behaving animals.

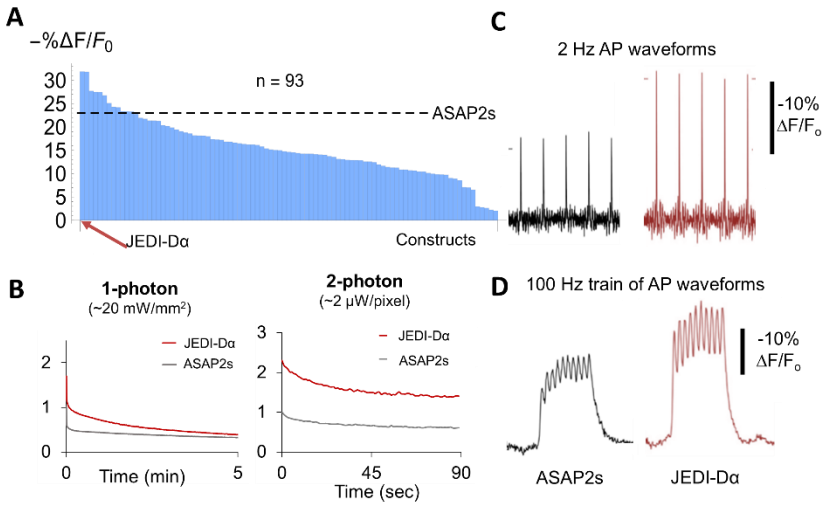


**Figure 2| Multi-parameter screening resulted in variants improved in brightness, responsivity and sensitivity.** A) A variant with improved responsivity was identified in the field stimulation-based screening and named as JEDI-Dα. B) Compared with its parental sensor ASAP2s, JEDI-Dα has improved brightness under both one-photon and two-photon illumination and shows better two-photon photostability during continuous illumination. C) JEDI-Dα has improved responsivity and kinetics compared with ASAP2s, confirmed by patch clamp *in vitro*.

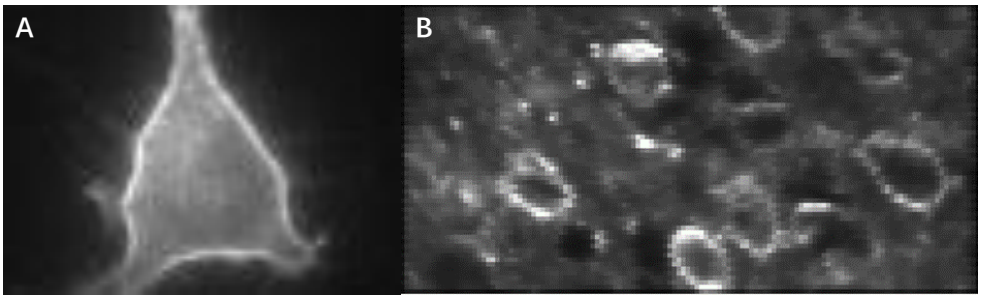


**Figure 3| JEDI-Dα efficiently trafficked to cell membranes.** A) HEK293A cell expressing JEDI-Dα. B) Mouse cortical neurons expressing JEDI-Dα.

| AAV expression system for rodent | |
|---|---|
| AAV Serotype | Promoter |
| AAV.PHP.eB<br>AAV2/1<br>AAV2/2<br>AAV2/5<br>AAV2/9<br>DJ8 | CAG<br>CaMKII<br>EF1a<br>hSyn |

| Plasmids for non-rodent systems | |
|---|---|
| Animal | Promoter |
| C. elegans | myo3 |
| Zebrafish | Huc |
| Drosophila | UAS |

**Table 2-3| Dissemination resources of JEDI-1P and JEDI-2P for applications *in vivo***

## Conclusion

1. Using a custom automated high-throughput GEVI development platform, we have engineered voltage indicators that can monitor neural activity in awake behaving animals including flies, fish and mice.
2. We are packing the new indicators into varies plasmid and to enable applications in multiple model system. We are actively disseminating our JEDI toolbox to research groups working on *in vivo* studies and/or advancing imaging technologies.

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