PatcherBot: a high throughput robotic single-cell electrophysiology system

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SUPPLEMENTARY INFORMATION

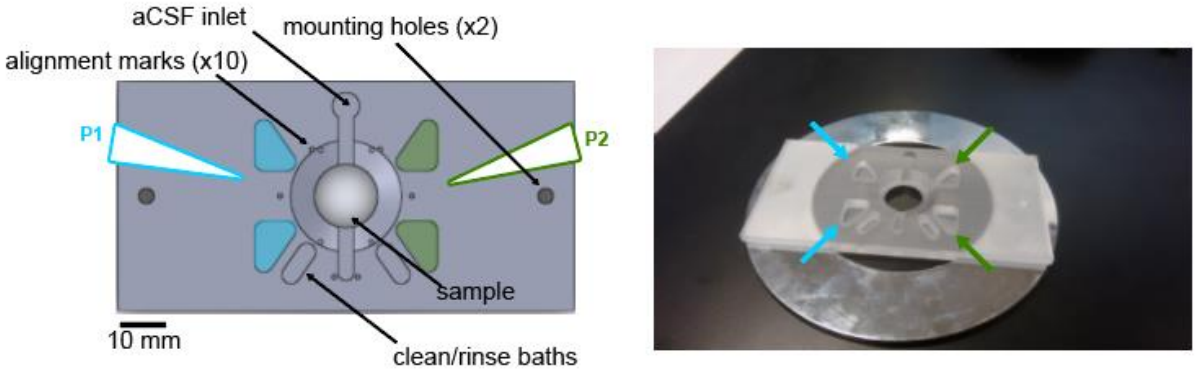
Supplementary Table 1

	Manual	**Automated positioning systems***	**Autopatcher IG**	**PatcherBot (single channel)**	**PatcherBot (two channel)**
Description	User navigates micropipettes to cells of interest in cultured cells or tissue	Micropipette navigation is automated.	Micropipette navigation, pressure control, and algorithm for user-picked or fluorescence-detected cell detection, sealing, break-in automated	Micropipette navigation, pressure control, and algorithm for user-picked cell detection, sealing, break-in automated	Multi-pipette navigation, pressure control, and algorithm for user-picked cell detection, sealing, break-in automated
Whole-cell success rate**	60-90%: largely depends on specific preparation and experimenter skill	60-90%: largely depends on specific preparation and experimenter skill	48%: semi-automated 52%: automated***	67%	62%
Throughput	5-10 recordings per day	5-10 recordings per day	5-10 recordings per day	~30-60 recordings per 8-hour day	100-125 cells per 8-hour day
Recording quality (access resistance) †	Gold standard quality (R_a = 10-40 MΩ)	Gold standard quality (R_a = 10-40 MΩ)	As good as manual (R_a = 10-40 MΩ)	As good as manual (median R_a = 37 MΩ in brain slices) except for failed break-ins	N/A: not tested in brain slices
Max unattended operation time per trial	Recording duration (1-5 min), assuming recording is fully automated	Recording duration (1-5 min), assuming recording is fully automated	Recording duration (1-5 min), assuming whole-cell recording portion is fully automated	~45 min (assuming 10 cells picked)	49 min (assuming 11 cells picked)
Setup / calibration time	~10 min prep + 10 min pulling pipettes	~10 min prep + 10 min pulling pipettes + 2 min calibration	~10 min prep + 10 min pulling pipettes + 2 min calibration	~10 min prep + 2 min pulling pipettes + 4 min calibration††	~10 min prep + 2 min pulling pipettes + 8 min calibration
Operator skill required	High	Medium-High	Medium-Low: automation of pipette navigation and pressure control decreases training time	Low	Low

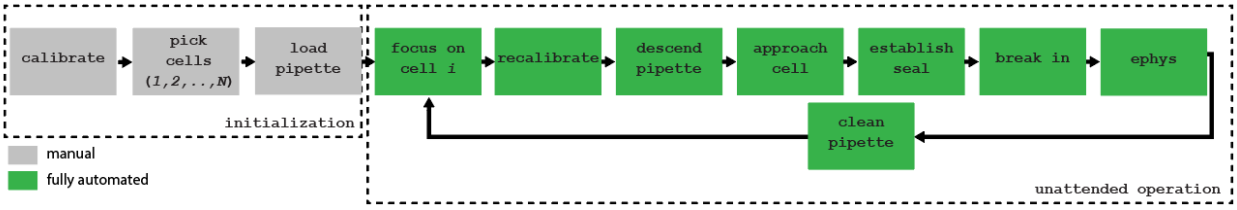
*: this includes any software that enables pipette position storage and an ability for pipettes to follow the stage (e.g. Scientifica LinLab, Sutter Multi-Link)

**: success rates are often not reported in publications and are difficult to compare due to variations in reporting strategies.

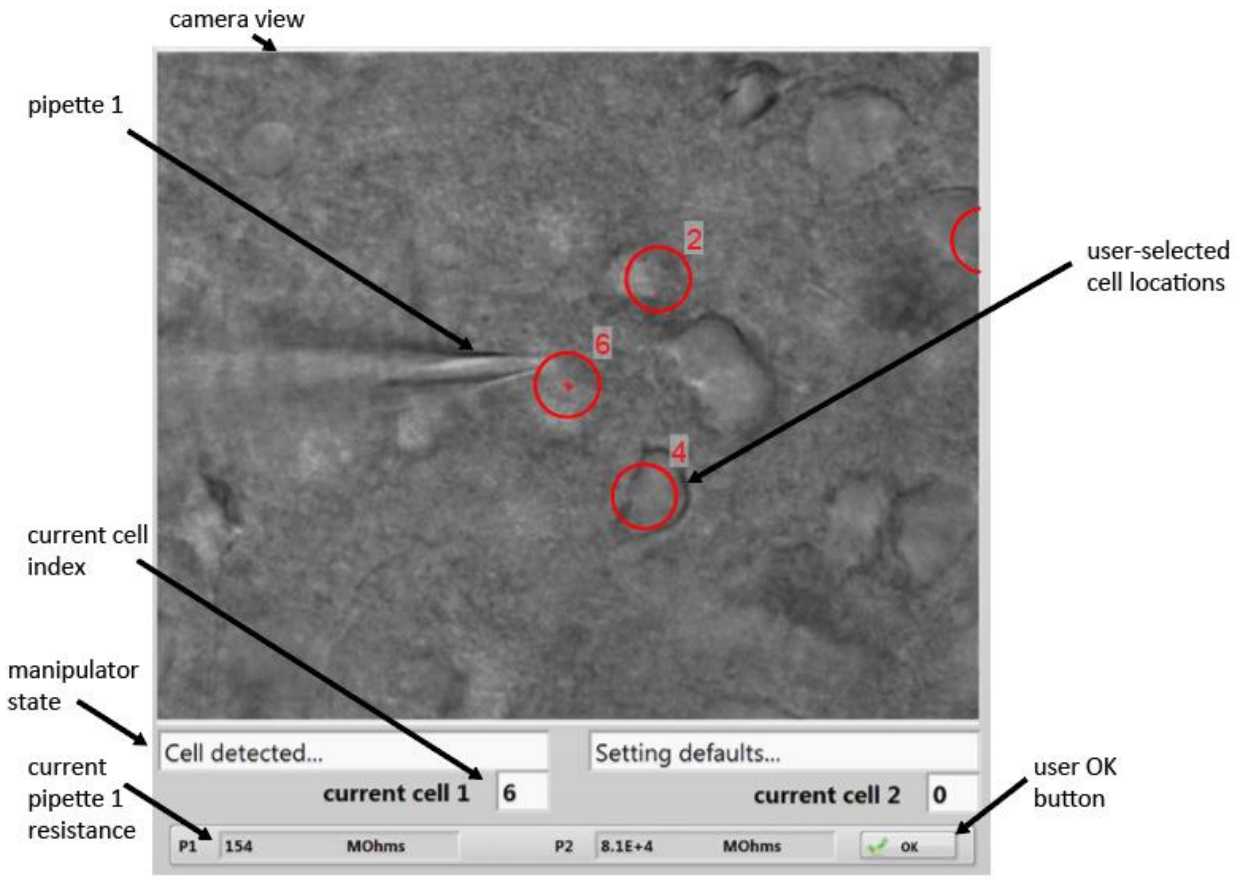
***: success rate only available for neurons in brain slices.
† : Access resistance measurements for Manual, Automated positioning systems, and Autopatcher IG taken from (Kolb et al. 2016).
††: Pipette pulling time is decreased because fewer pipettes need to be pulled as pipettes are cleaned.
Assumptions: For consistency in comparisons, we only consider systems that are explicitly used for image-guided whole-cell patch-clamp in plated HEK cells, or systems that could be easily modified to do so. Systems where the preparation differs significantly (such as planar patch clamp (Fertig et al. 2002), the autopatcher (Kodandaramaiah et al. 2012), and the ImagePatcher (Suk et al. 2017)) are omitted.



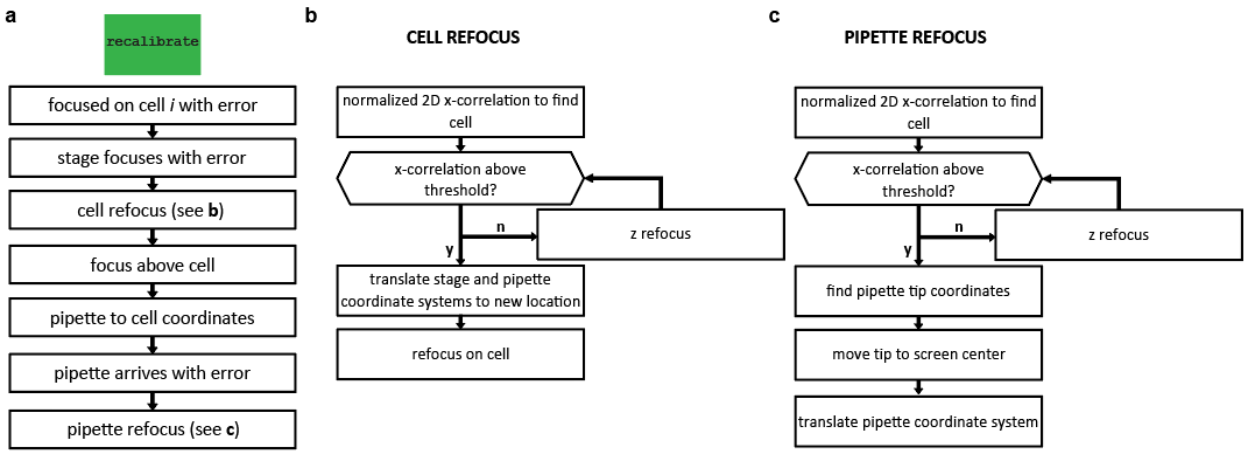
Supplementary Figure 1: In-vitro electrophysiology chamber for pipette cleaning. a. Computer-aided design (CAD) mockup of the chamber (top view). The sample (brain slice or cover slip with cultured cells) is placed in the center chamber, perfused by aCSF. Six clean/rinse baths can accommodate up to four pipettes but only two are used in this study, denoted in cartoon form as P1 and P2. The clean/rinse baths used for P1 and P2 are highlighted with the corresponding color. Alignment marks are machined to facilitate manipulator positioning, ensuring that pipettes can reach the baths. b. Manufactured electrophysiology chamber (polycarbonate, ProtoLabs) mounted on a metal ring that will be mounted on a motorized stage.



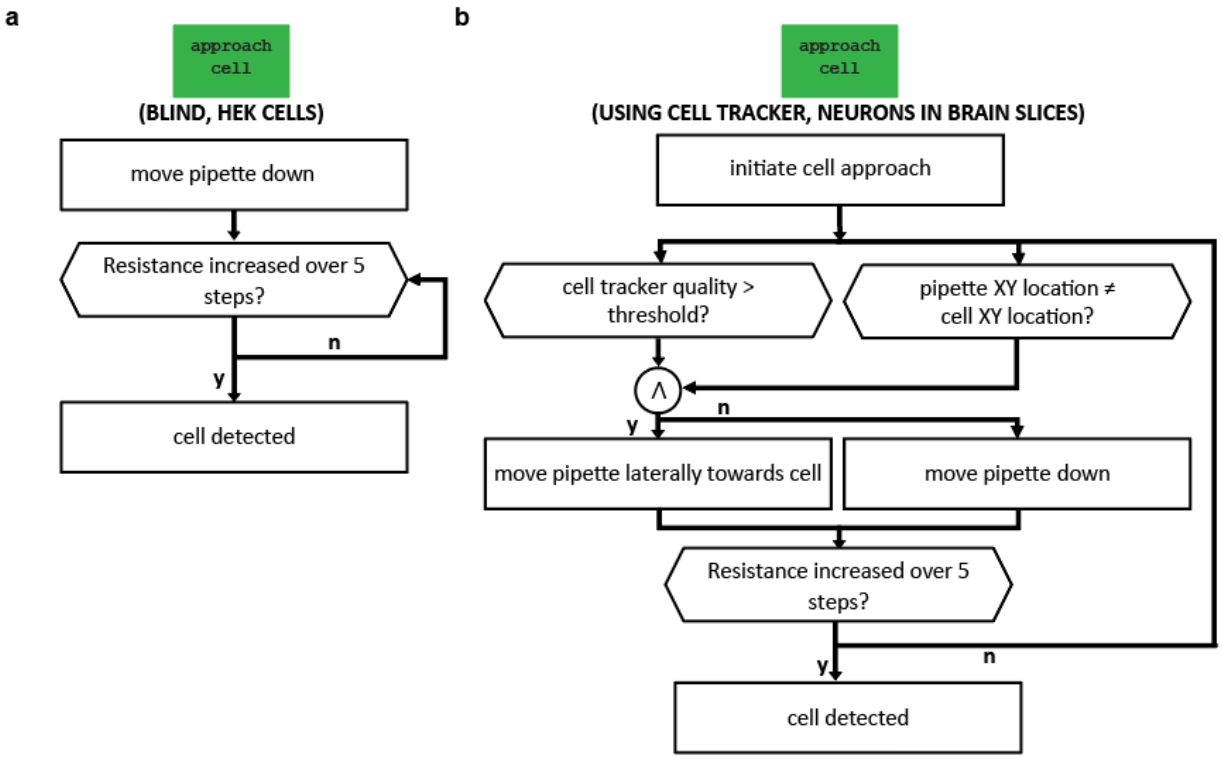
Supplementary Figure 2: Detailed block diagram of PatcherBot operation. See Methods for descriptions of each state.



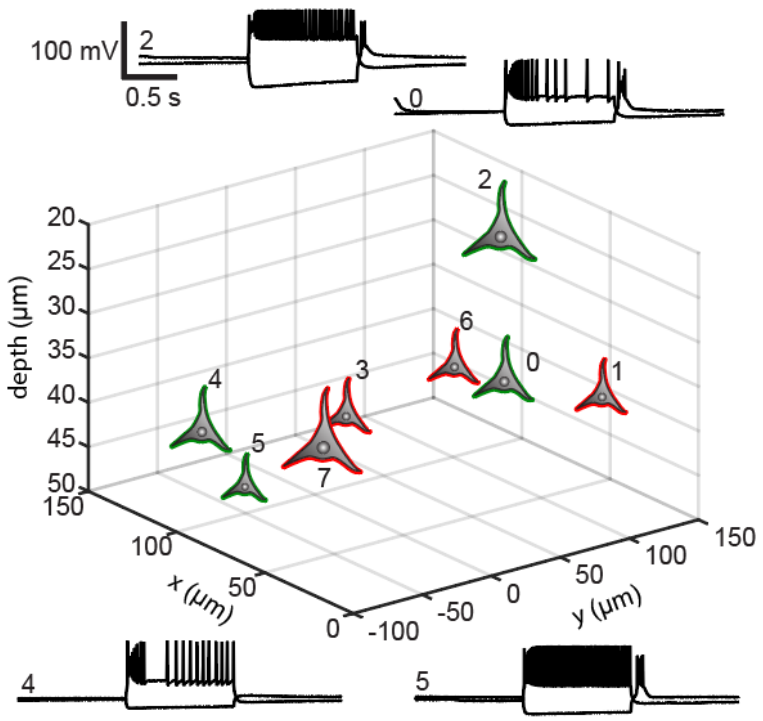
Supplementary Figure 3: PatcherBot graphical user interface. The camera view shows positions of pipette(s) and selected cells. The interface shows in real time the relevant performance characteristics of the PatcherBot such as the state of the manipulator (corresponding to the block diagram), index of the current cell and pipette resistance. The “user OK” button is only pushed by the user to begin the trial. User calibration is performed in a separate window (not shown). In this example pipette 2 is connected but not used.



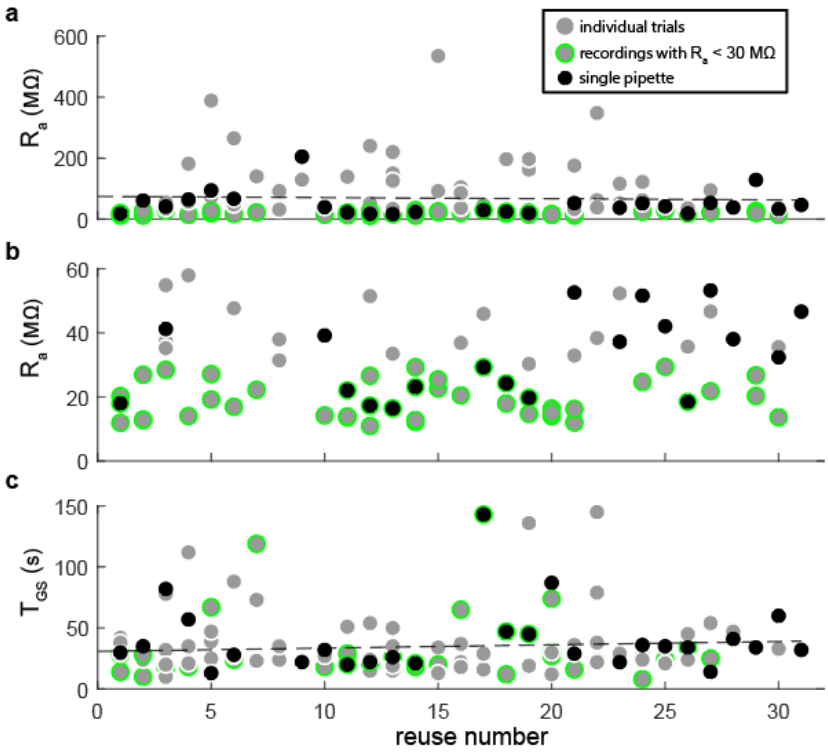
Supplementary Figure 4: Automated calibration procedure. a. Outline of the “calibration” state that is performed automatically before every patch-clamp attempt. b. Cell detection state used to perform stage calibration. c. Pipette detection state used to perform pipette calibration.



Supplementary Figure 5: Automated cell approach options. a. “Blind” approach, similar in logic to the original Autopatcher software and Autopatcher IG. Pipette moves down until a resistance increase over 5 consecutive steps is detected, indicative of a cell. b. Image-guided approach, wherein the cell tracker is used to detect the centroid of the target cell. If the pipette xy position is not within 2 µm of the cell centroid, the pipette is moved laterally (1 µm steps) until the XY coordinates are aligned. If the coordinates are aligned, the pipette is moved down. Throughout this process, resistance is continually monitored to detect the cell.



Supplementary Figure 6: Representative slice whole-cell recordings in thalamus obtained using the PatcherBot. Green neuron symbols represent successful whole-cell recordings; red symbols represent failed attempts. Cells are shown in a coordinate system that depicts their centroid location in the slice.



Supplementary Figure 7: Recording characteristics of cells patch-clamped with Tergazyme-cleaned pipettes. Gray circles: individual trials, black circles: representative trials with a single pipette (same pipette in all three panels). Green border: whole-cell recordings passing quality control (access resistance, $R_a \leq 30$ MΩ). Data points are missing (e.g. black circles on reuse numbers 4-9) if a whole-cell recording was not established with the pipette. a. R_a of recordings, median: 37 MΩ, extents: 10.9 to 535 MΩ. Resistance values higher than ~100 are likely incomplete break-ins or break-ins where the cell subsequently sealed up. No significant relationship between access resistance and reuse number was observed (P=0.69, Linear Mixed Effects Model). Dashed line shows linear fit to R_a data (slope = -0.398, CI: -2.3 - 1.5). b. Zoomed in access resistance from a. c. Time to attain gigaohm seal (T_{GS}). No significant relationship

between T_{GS} and reuse number was observed (P=0.85, Linear Mixed Effects Model). Dashed line shows linear fit to T_{GS} data (slope: 0.27, CI: -0.32 - 0.86).

Supplementary video 1: Time-lapse video of a representative high-throughput PatcherBot experiment in cultured HEK cells. In this experiment, the automated electrophysiology setup controlled by PatcherBot software performed 35 recordings of 53 attempts in four hours. The only experimenter intervention in this time was pipette replacement and re-calibration which was performed four times. The green light indicates a successful recording.

Supplementary video 2: Time-lapse video of representative PatcherBot experiment with cells held for 20 minutes. The setup is covered to protect from electrical noise and minimize thermal drift. The PatcherBot successfully performed 5 recordings of 11 attempts in 2.5 hours.

Supplementary Video 3: Representative pipette approach with and without the cell tracker in real time. With the cell tracker off, the pipette tip (approaching from the left) misses the left side of the cell as it goes into the tissue. With the cell tracker on, the pipette tip (labeled with blue circle) starts off-center and is moved towards the tracked cell centroid (green

circle) until the resistance threshold is reached. The pipette does not come into focus entirely because the pipette stopped advancing as soon as the resistance threshold was reached. Scale bar: 10 µm

Supplementary Video 4: Screen capture of PatcherBot software from representative experiment in brain slices. Results from this experiment are shown in Fig. 1d. Cell index of current attempt is shown on bottom left (“current cell 1”). Red circles indicate user-picked cell locations. Time counter in top right corner shows actual elapsed time during the experiment.

Supplementary Video 5: Time-lapse video of representative two-manipulator PatcherBot experiment. The two-manipulator PatcherBot performed 9 recordings of 10 attempts in 27 minutes.

Supplementary Video 6: Time-lapse video of representative two-manipulator PatcherBot experiment. The two-manipulator PatcherBot performed 13 recordings of 23 attempts in 45 minutes.

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