**System requirements**

- Commercial Raman microspectroscope: LabRAM HR800, Horiba Scientific

- LabSpec: to control the Raman microspectroscope (provided by the manufacturer, Horiba Scientific)

- Desktop/laptop that has MATLAB at any versions that provides GUIDE (graphical user interface development environment) module.

  Note: the code built in-house allows the Raman-activated cell sorting (RACS) to be run within a commercial Raman microspectroscope (LabRAM HR800), and thus several commands are specific for the Horiba system. We include a footnote to those commands (marked by 'Horiba-specific') so that users can modify them as appropriate to the system available. The three other major Raman manufacturers (Renishaw, Bruker, and Witec) have confirmed that their systems are compatible with control using third-party software (e.g., MATLAB as in this protocol, or Python). Our code can also be adopted with suitable modifications for other Raman systems (including those built in-house) if they allow an application programming interface (API).

Instructions for use

Note: this version is for sorting of cells with respect to deuterium. To operate this software platform with respect to other molecular fingerprints (e.g., ^{13}C, carotenoid), we have included a footnote to commands where modification is required to adjust the spectral region of interest.

1. In Step 53 (in main text), right-click the 'RACS_ver1.fig' file. Click 'Open in GUIDE'.
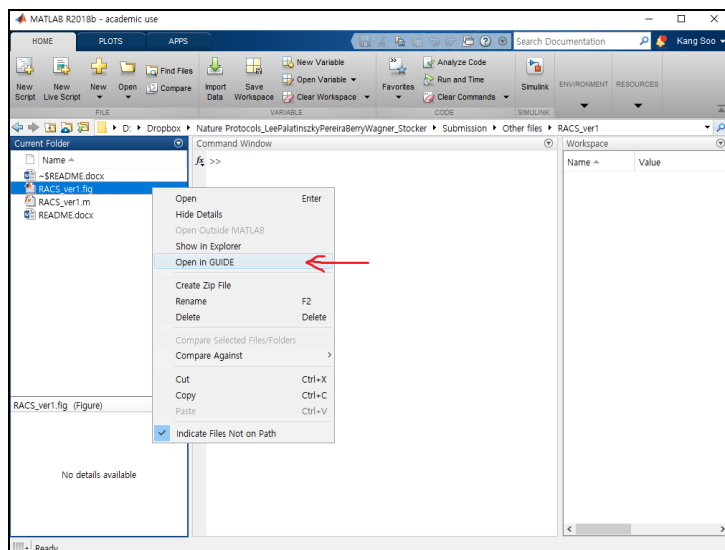
2. Click the green **Run** button to start the platform.
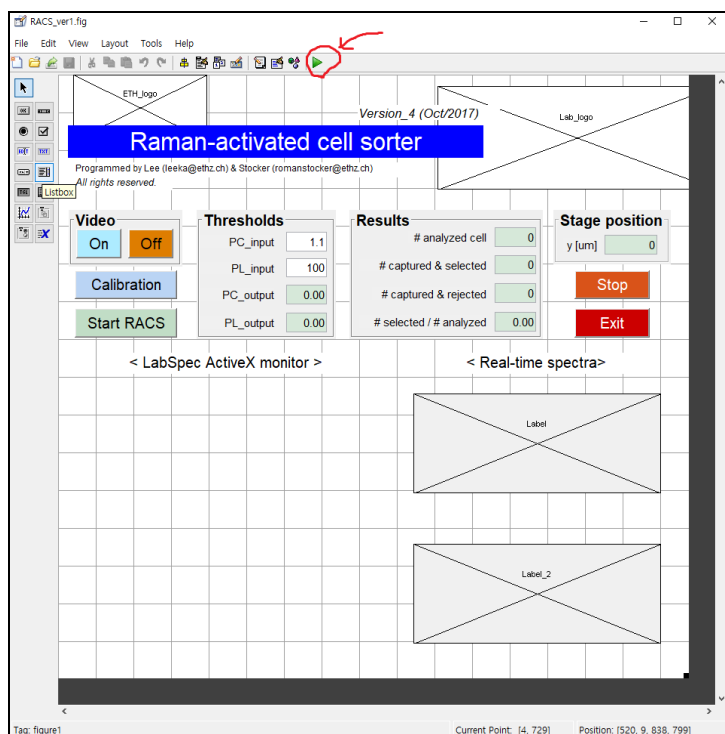
3. Click the 'Spectra calibration' button (3) (panel **a** in figure below). The stage moves to the sample-free region (70 µm away from the sample stream) and measure the Raman spectrum of the working fluid (averaged over 10 measurements). One output file 'Calibration_1981-10-07 19_02_07.l6s' (named using the date and time '1981-10-07 19_02_07'; this will vary depending on the timing of the operation) that contains a Raman spectrum measured during the calibration is created.

   Note: '.l6s' is the LabSpec-specific format. Boxes with white (5,6) and green (7–13) colours represent input (adjustable by the user) and output, respectively.
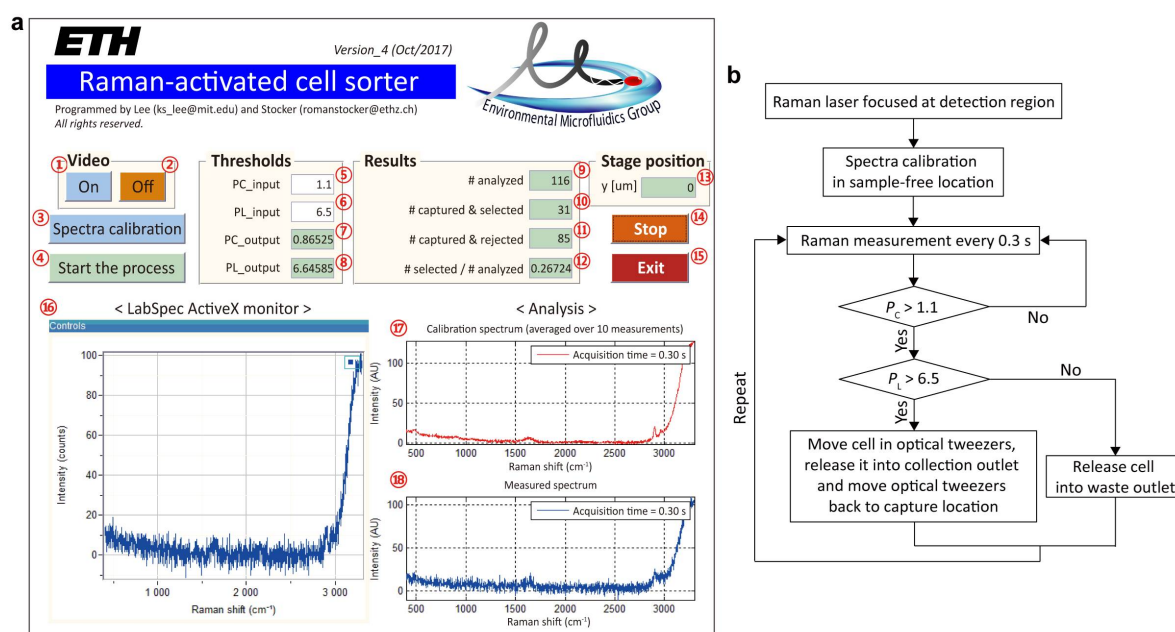
4. Click the 'Start the process' button (4) to start the RACS process. The software begins the procedures described in panel **b** of the figure above.

   - Boxes (5) & (6) display the thresholds set by the user to detect the capture of a single cell by the optical tweezers (cell index, P_C) and to identify a cell as deuterium labelled, respectively. $P_C = 1.1$ and $P_L = 100$ as default. Adjust the threshold value for P_L based on sample assessment described in Step 53-(i)-a. (see main text).
   - Boxes (7) & (8) display the P_C and P_L values measured in real time, respectively.

- Boxes (9)–(12) display the number of cells analysed, captured and selected, and captured and rejected, and the proportion of selected cells among those analysed, respectively.
- Box (13) displays the current position in the y-direction of the microscope stage (perpendicular to the flow direction).
- Panel (16) displays the Raman spectrum measured in real time.
- Panel (17) displays the calibration spectrum (used to calculate P_C).
- Panel (18) displays the latest captured and selected (sorted) cell.

5. When the number of sorted cells meets the user's requirements, click the 'Stop' button (14) to stop the RACS process. Then, click the 'Exit' button (15) to close the software platform. Four output files will be created:
- Spectra_Labeled_1981-10-07 19_02_07.txt: contains the Raman spectra of sorted cells
- Spectra_Unlabeled_1981-10-07 19_02_07.txt: contains the Raman spectra of discarded cells
- PCPL_Labeled_1981-10-07 19_02_07.txt: contains the P_C and P_L values of sorted cells
- PCPL_Unlabeled_1981-10-07 19_02_07.txt: contains the P_C and P_L values of discarded cells