

# ***FAST 2014***

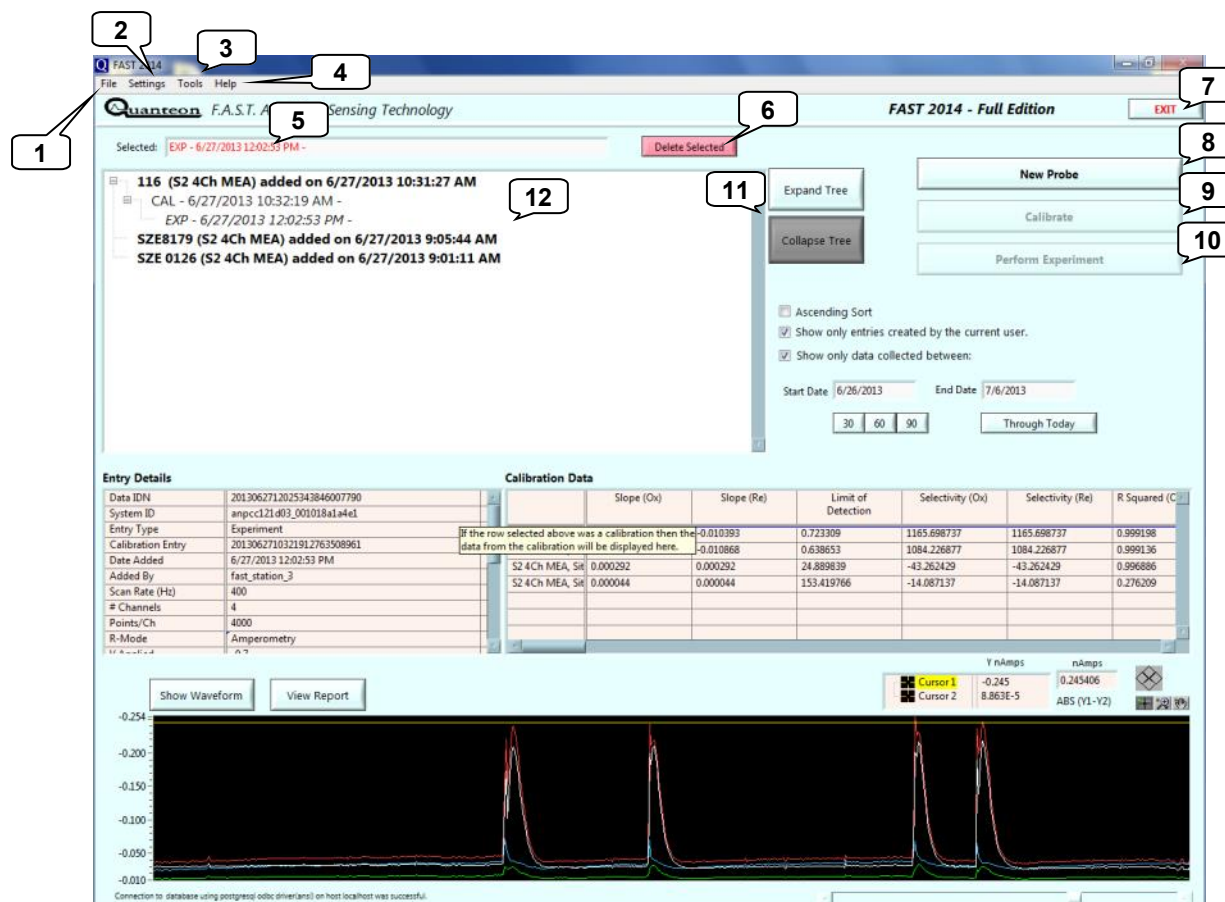
# **Software Manual**




# Table of Contents

FAST System Software .....	<a href="#">3</a>
Enzyme-based MEA Settings .....	<a href="#">6</a>
Amperometry Calibrations.....	<a href="#">9</a>
Amperometry Experiments .....	<a href="#">13</a>
Chronoamperometry Settings .....	<a href="#">20</a>
Chronoamperometry Calibrations.....	<a href="#">23</a>
Chronoamperometry Experiments .....	<a href="#">26</a>
Electroplating Tool .....	<a href="#">33</a>
Supplemental Information	
Preparation of mPD Microelectrode Arrays .....	<a href="#">32</a>
Calibration Protocol.....	<a href="#">35</a>
Common Calibration Solutions .....	<a href="#">36</a>
Glossary .....	<a href="#">41</a>
Troubleshooting/FAQ .....	<a href="#">42</a>
Appendix.....	<a href="#">44</a>

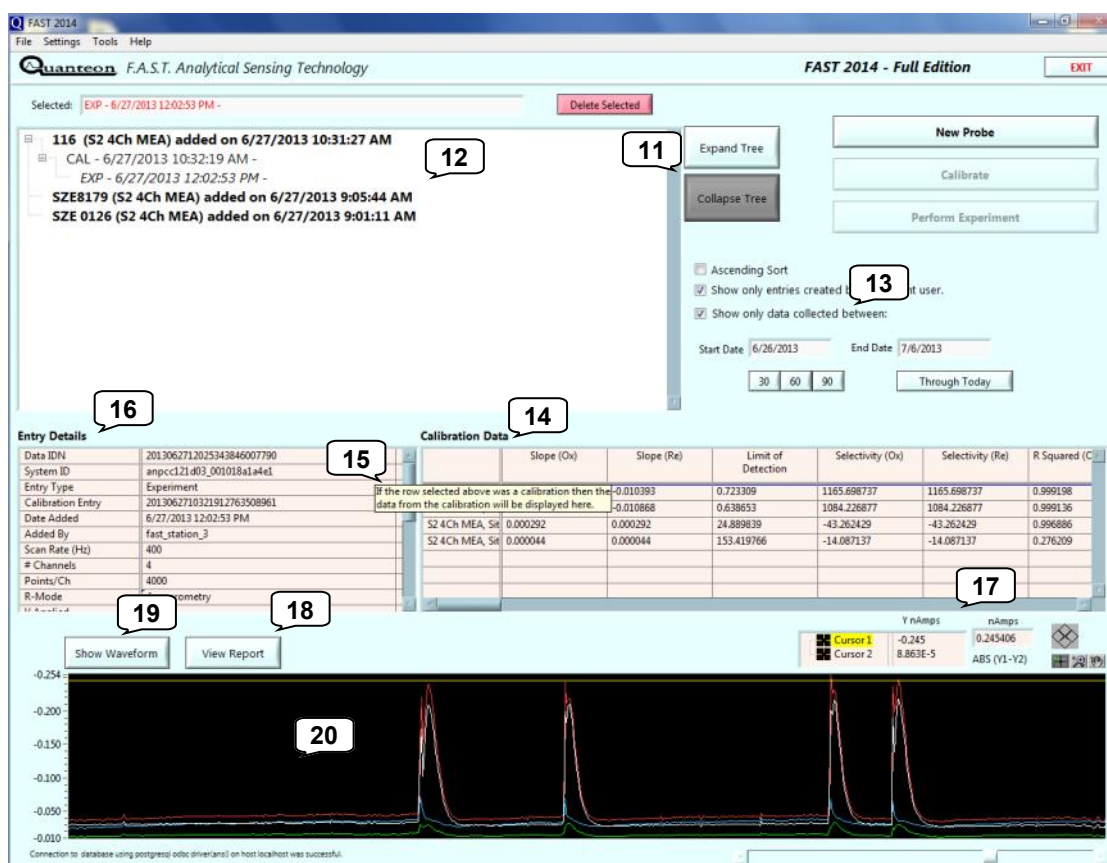
# 1.0 FAST System Software - Main window



Double click the  on the desktop to open the FAST System Software. The FAST Main window appears. The items on the window are:

1. **File** - used to export .html reports and data files for analysis
2. **SETTINGS** - Adjust system settings.
3. **TOOLS** - Access a concentration calculator.
4. **HELP**- Calibrate and perform experiments.
5. Identifies entry for further action by double-clicking on database list.
6. **Delete Selected** (#5) database item (**CAUTION**: Deletion cannot be undone.)
7. EXIT the FAST program.
8. **STARTING A NEW RECORDING** - Name a New Probe.
9. **Calibrate** a new probe. Select after double-clicking a probe in (#12).
10. **Perform Experiment**. Select after double-clicking a completed calibration (#12).

# 1.0 FAST System Software - Main window



11. Expand Tree or Collapse Tree to expand or contract database list hierarchy.
12. Database lists MEAs/carbon fibers and respective (CAL)ibrations and (EXP)eriments as selected in (#13).
13. Adjust options/selections to modify database range in (#12).
14. Displays calibration results from item selected in (#12).
15. Information pop-ups appear throughout the program. Hover the mouse pointer over entry fields and buttons to reveal the information pop-ups.
16. **Entry Details** - lists recording settings and MEA/carbon fiber information.
17. Cursor values from Show Waveform window.
18. [View Report](#) - click button to view HTML report for selected (in #5) item.
19. **Show Waveform** - click button to display traces for selected (in #5) item.
20. Quick view of recording traces. Current (nA) is displayed on the y-axis.

**Amperometry Recordings:  
Neurochemistry with MEAs**

# 1.1 Amperometry Settings & Defaults

## Enzyme-based MEA

The screenshot shows the 'FAST System Settings' window with the 'Settings & Defaults' tab selected. The window is divided into several sections:

- DAQ Hardware Settings:** Includes 'Present DAQ Card' (Dev1), 'Change Card To:' (Dev1 (PCI-6251)), 'Card Max Rate' (1E+6), 'Card Max Channels' (16), 'Input Range' (± 5), and 'Change Range to:'.
- Digital I/O Settings:** Includes 'TTL Input Logic Sense' (Set the switches to the right to indicate the TTL level when the line is in the untriggered (resting) state. If the line is not used then set the switch to High), 'TTL Output' (Enable), and a table of Bit settings (Bit 0 to Bit 7, all set to High).
- Feature Settings:** Includes checkboxes for 'Enable Calibration CSV Dialog', 'Enable Expanded Features (Amperometry Only)', and 'Enable Multi-voltage Output'.
- Default Settings:** Includes 'Attached Headstage\*' (QHS-4 X 2 pA/mV (500x)), 'Attached Control Box\*' (FAST16mkIII (8ch)), 'System Resting Potential' (V1 0.000, V2 0.000, V3 0.000, V4 -1.000), 'Recording Mode' (Amperometry), 'Recording Rate (Hz)' (4), 'Exclusion Layer\*' (mPD), 'Analyte\*' (Glutamate), 'Interferent\*' (Ascorbate (AA)), 'Pulse Period' (0.2), 'Sweep Time' (1), 'V Applied' (V1 -0.7, V2 0, V3 0, V4 0), and 'V Resting' (V1 0, V2 0, V3 0, V4 0).

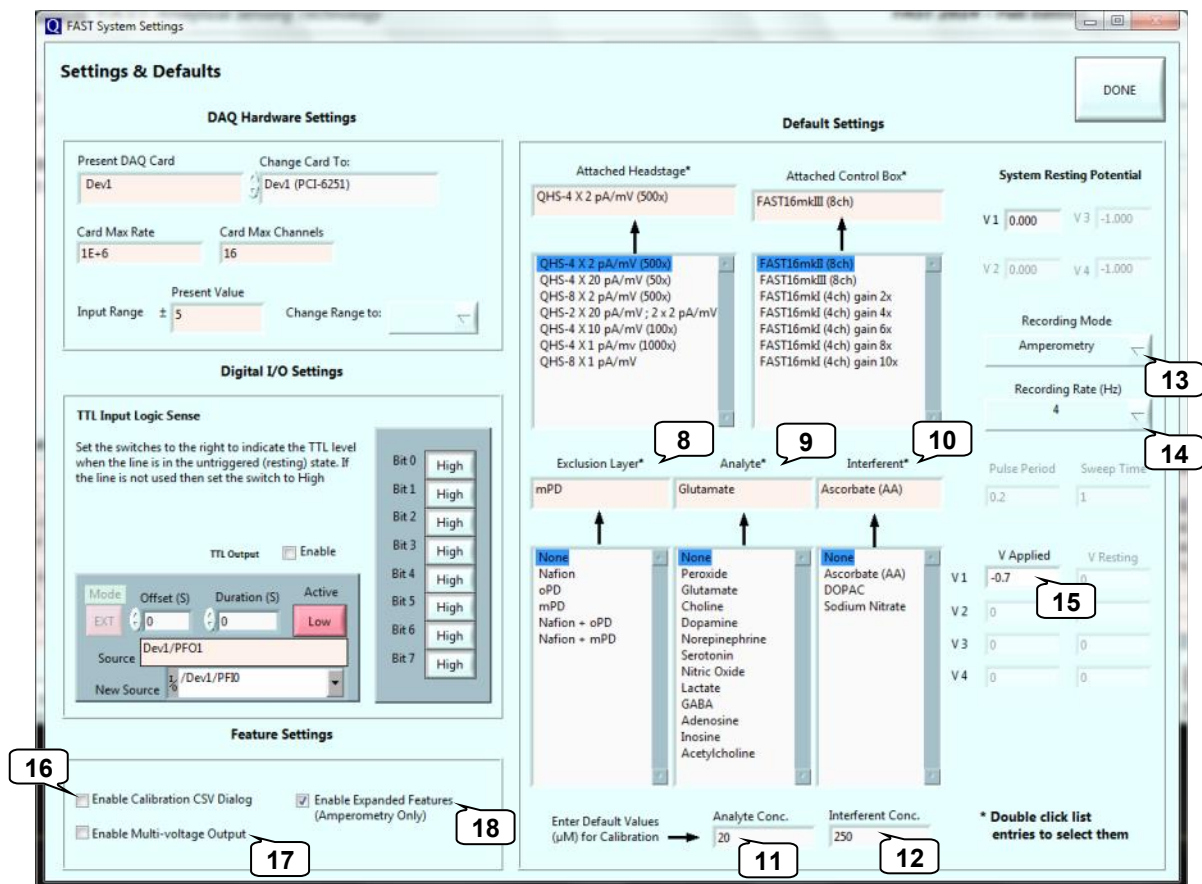
Numbered callouts 1 through 7 highlight specific settings in the window:

- Card Max Rate
- Input Range
- System Resting Potential
- TTL Input Logic Sense
- TTL Output
- Attached Headstage
- Attached Control Box

1. **Card Max Rate** - Describes how often the [analog to digital conversion](#) occurs per channel.
2. **Input Range** - 0.1 to 10V. From drop-down menu, most often select 1 or 2 for best signal detection. Describes [analog to digital conversion range](#).
3. **System Resting Potential** - Maintains applied potential between recording episodes.
4. **TTL Input Logic Sense** - Sets the logic level on the digital inputs that indicate the untriggered state of attached equipment.
5. **TTL Output** - A tick in the box enables the output of a trigger signal that begins on the rising edge of the square wave pulse used in chronoamperometry. Used to synchronize external equipment such as data collection systems to the FAST system.
6. **Attached Headstage** - Select the headstage that is attached to the FAST control box.
7. **Attached Control Box** - Select appropriate control box. If selecting "FAST16mkI," match the gain to the one selected on the front panel of the FAST control box.

# 1.1 Amperometry Settings & Defaults

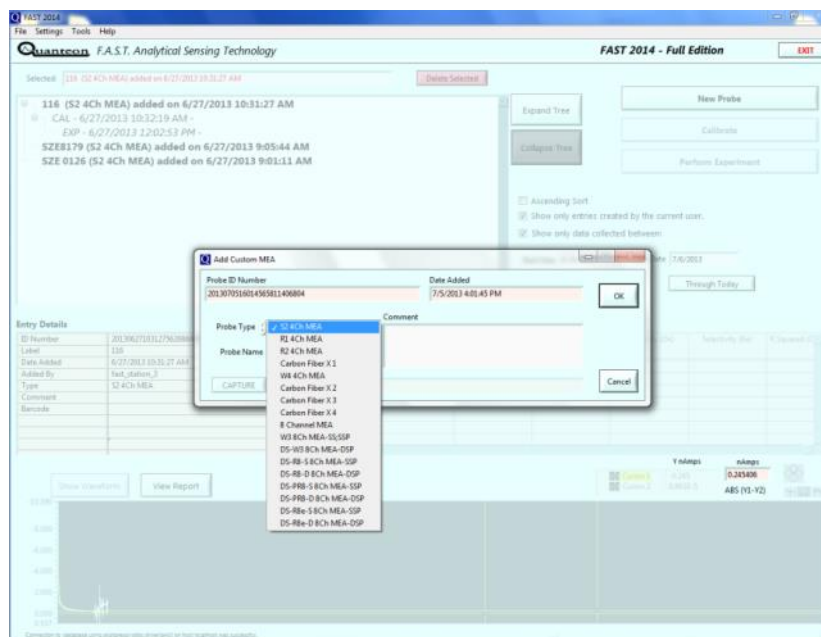
## Enzyme-based MEA



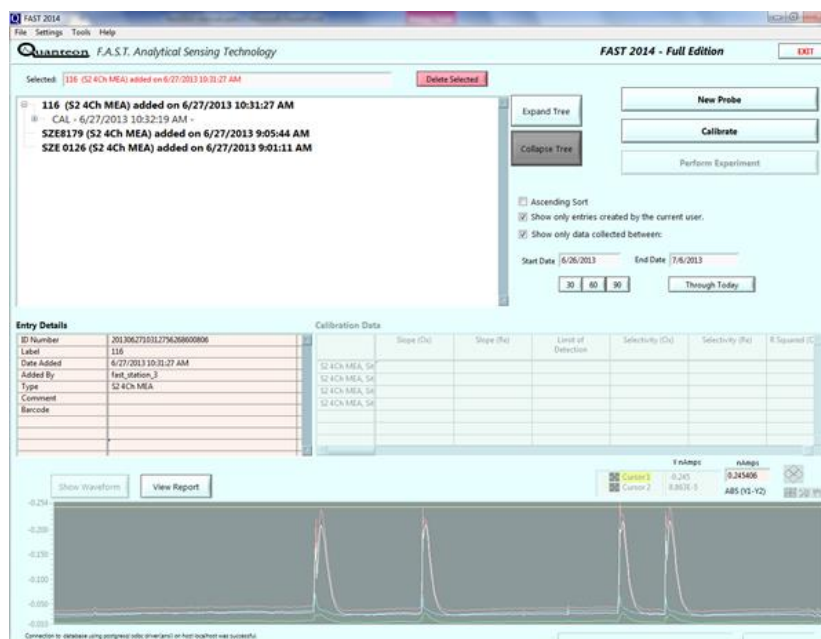
8. **Default Exclusion Layer** - Default exclusion layer, if one, for calibrations. (**Defaults:** double clicking a selection from the list sets/changes the default. The current default is shown in the text box above the list).
9. **Default Analyte** - Default analyte, if one, for calibrations.
10. **Default Interferent** - Default interferent, if one, for calibrations
11. **Analyte Concentration** - Default analyte concentration for calibrations.
12. **Interferent Concentration** - Default interferent concentration for calibrations.
13. **Recording Mode** - MEAs are calibrated in **Amperometry** mode.
14. **Recording Rate (Hz)** - Use the pull down menu to select the rate data is saved to disk.
15. **V applied** - Enter the potential to be applied vs. reference electrode.
16. **Enable Calibration CSV dialog** - Add calibration results to a new or existing spreadsheet.
17. **Enable Multi-voltage Output** - Permits use of multiple applied potentials (advance only).
18. **Enable Expanded Features** - Displays Custom MEA Calibration Setup window.



## 1.2 Recording with an MEA



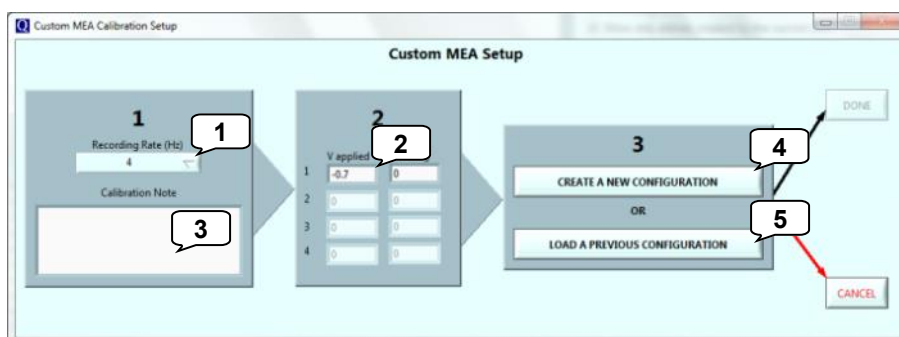
After verifying [Settings](#), select **New Probe**. Choose **Probe Type** from the drop down menu, provide a **Probe Name**, and include additional **Comments**, as needed. The information will be displayed in the database list.



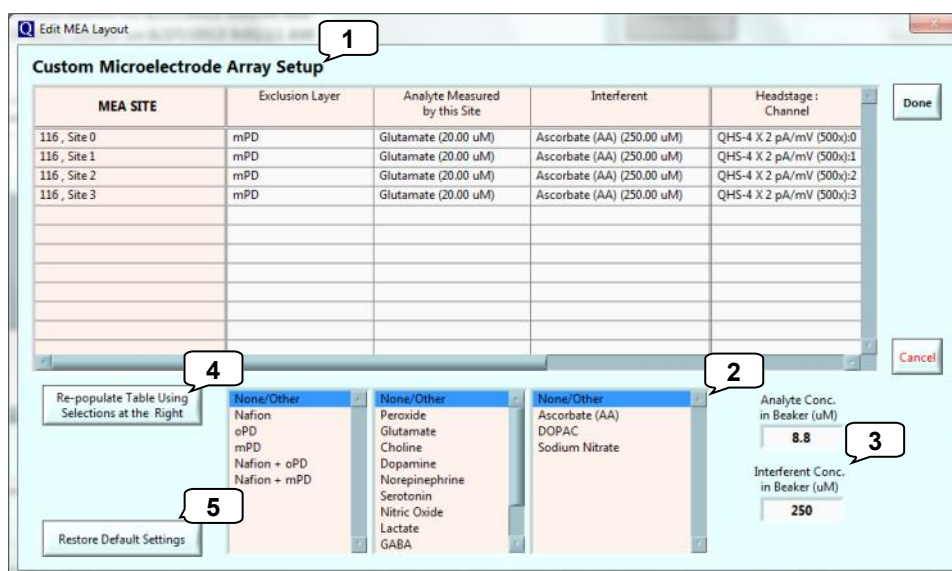
After creating an MEA entry, select the sensor of interest in the database and the **Calibrate** button will become active. Select to begin an [MEA calibration](#).



## 1.3 Amperometry Calibrations - with [expanded features enabled](#) in Defaults and Settings



1. **Recording Rate (Hz)** - Use the pull down menu to select the rate data is saved to disk.
2. **V applied** - Enter the potential to be applied vs. reference electrode.
3. **Calibration Description** - Enter calibration description to aid with database identification.
4. **Create a New Configuration** - Configure new calibration parameters.
5. **Load a Previous Configuration** - Re-use previously defined calibration parameters.



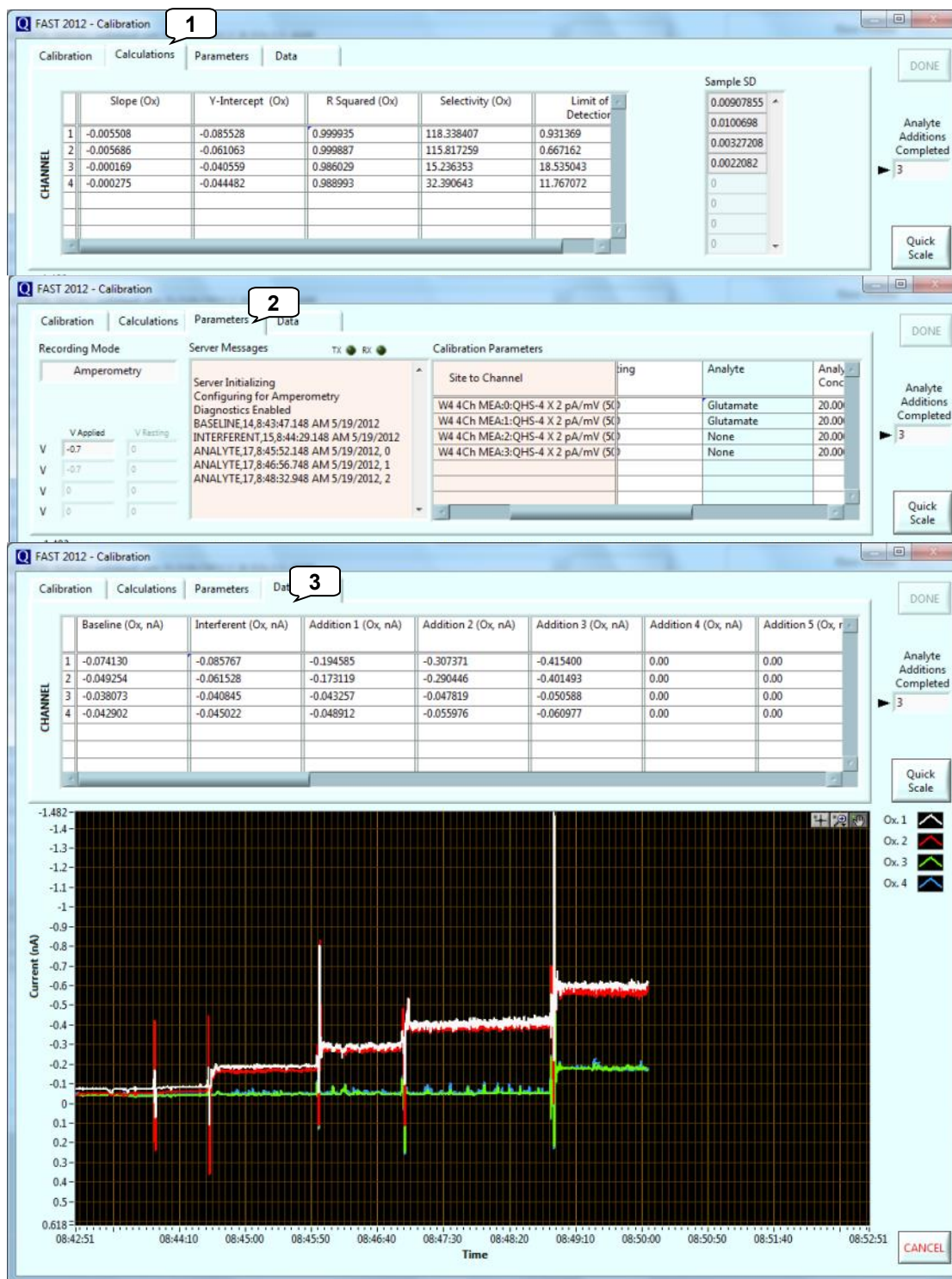
1. **Setup** - Displays MEA settings. Initially populated with default settings.
2. **Calibration Adjustments** - If selecting different values than default, double-click on items to change individual MEA sites in (1) or select (4) to change for all MEA sites.
3. **Analyte & Interferent Concentrations** - If selecting different concentration values than default, enter beaker concentration values.
4. **Re-populate Table** - Select to apply changes from (2) or (3) to MEA setup.
5. **Restore Default Settings** - Select to restore default settings.

## 1.3 Amperometry Calibrations



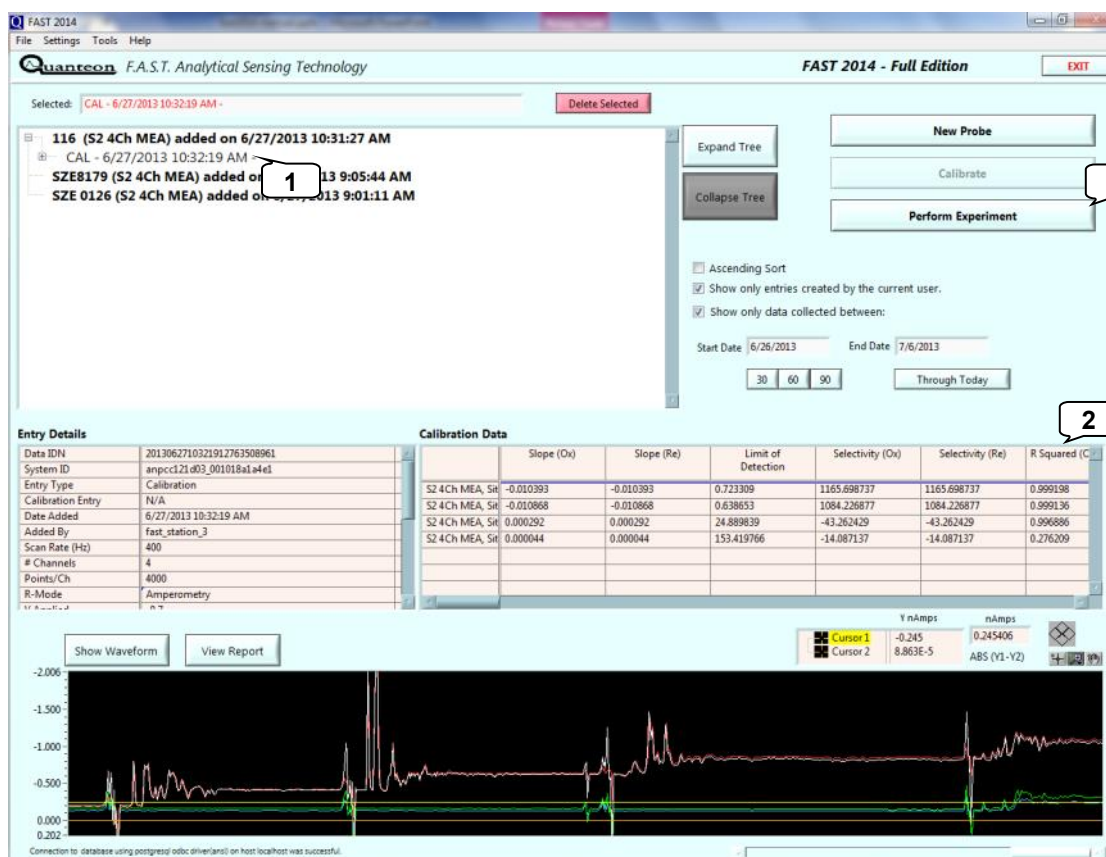
1. Current (nA) value for each MEA channel.
2. Graphical display of the current values of individual MEA channels (nA vs. time of day).
3. Magnification and scrolling tools allow for customization of the graphical display.
4. Counter showing total number of additions for calibrations.
5. Re-scale to zoom on the active area of the graph.
6. Active MEA channel colors—modifiable.
7. Click to toggle between displaying/turning off the oscilloscope.
8. Oscilloscope displays wave form or current level generated at microelectrode (in V).
9. Activate **BASELINE** button to begin calibration calculations. Buttons change to **Interferent** then **Analyte** and **Test Substance** based on calibration protocol.
10. Select **Finished** to end calibration calculation.
11. Select **DONE** to close calibration window, otherwise, to abort calibration select **CANCEL**.

## 1.3 Amperometry Calibrations



1. **Calculations** - MEA calibration results (including LOD, selectivity, and slope).
2. **Parameters** - Displays system parameters used for calibration.
3. **Data** - Displays data used for calibration **Calculations**.

## 1.4 Amperometry Experiments

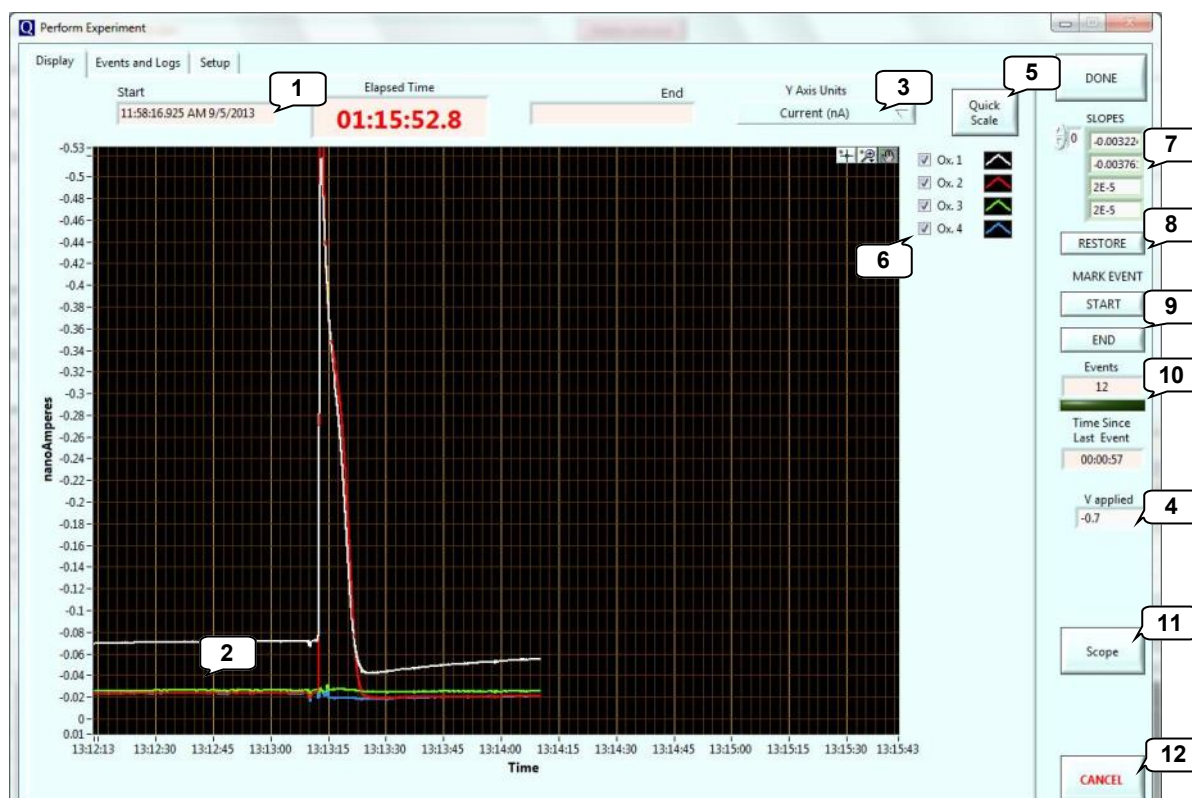


After creating a calibration,

1. Select a calibration to use for an experiment by double-clicking on that calibration.
2. Parameters associated with the MEA and calibration and calibration calculations (LOD, selectivity, Slope) are displayed.
3. Select **PERFORM EXPERIMENT** to open acquisition window and begin an experiment.



## 1.4 Amperometry Experiments



1. **Start** time stamp for acquisition data file, **total elapsed recording time**, and **End** time stamp for acquisition data file.
2. Graphical display of the current (nA or pA) or concentration ( $\mu\text{M}$ ) values of individual MEA channels (vs. time of day).
3. Toggle between **Current** (nA or pA) and **Concentration** ( $\mu\text{M}$ ) display for Y-Axis Units.
4. **V applied** - The applied potential may be changed on-the-fly during experiment.
5. Re-scale to zoom on the active area of the graph.
6. Active MEA channel colors—modifiable. Display/hide individual channels.
7. Microelectrode **slopes** from calibration. These are automatically obtained from selected calibration file but can be manually entered as needed.
8. **RESTORE SLOPES** to original calibration file values for selected MEA.
9. Click to signal **START** and **END** of select triggered **EVENT**. Useful for long recording signals, drug, or behavior studies. Adds TTL event mark to data file for analysis.
10. **Events** - Counts number of triggered events and **Time Since Last TTL Event**.
11. **Scope** - Display/Hide [oscilloscope](#).
12. Select **DONE** to close Experiment window, otherwise, to abort experiment select **CANCEL** (data not saved).

# 1.4 Amperometry Experiments

Perform Experiment

Display Events and Logs Setup

Type your comments in the "User Comments" column ONLY. DO NOT change any of the automatically generated information

Event Number	Time Stamp	Description	Source	User Comments
1	12:09:58.734 PM 8/15/2014	ACQUISITION-START	3	
2	12:11:26.508 PM 8/15/2014	HARDWARE TRIGGER	1	Enter comments
3	12:11:53.258 PM 8/15/2014	HARDWARE TRIGGER	1	
4	12:11:57.382 PM 8/15/2014	HARDWARE TRIGGER	2	
5	12:12:01.758 PM 8/15/2014	HARDWARE TRIGGER	4	
6	12:12:06.133 PM 8/15/2014	HARDWARE TRIGGER	8	
7	12:12:16.155 PM 8/15/2014	EVENT-START	5	
8	12:12:18.167 PM 8/15/2014	EVENT-END	7	

Experiment Description & Comments

Displays on database

RedOx Ratios

Channel	Ratios
Channel 1	--
Channel 2	--
Channel 3	--
Channel 4	--

Experiment Controls:

DONE

SLOPES

0 -0.00989 -0.01736 -0.00825 -0.00824

RESTORE

MARK EVENT

START

END

Events

8

Time Since Last Event

00:00:36

V applied

-0.7

Scope

CANCEL

1. **Event Number** - indicates order of TTL events.
2. **Time Stamp** – Time TTL was triggered (Time determined by computer clock).
3. **Description** – Displays the source of the TTL input.
4. **Source** – Displays which TTL input was triggered.
5. **User Comments** - Text field for describing TTL events.
6. **Experiment Description & Comments** - Text field for describing experiment. Viewable in **Browse Database**.
7. **RedOx Ratio** – In chronoamperometry, ratio between reduction current and oxidation current.

## 1.4 Amperometry Experiments

Perform Experiment

Display | Events and Logs | Setup

**PROBE INFORMATION**

Probe Label: test

Operator: fast\_station\_1

Date/Time: 7/24/2014 11:53:05 AM

Probe Type: S2 4Ch MEA

Sensor Comments

**CALIBRATION INFORMATION**

Operator: fast\_station\_1

Date/Time: 7/25/2014 10:36:44 AM

Recording Mode: Amperometry

Waveform: DC (Amperometry)

V Applied | V Resting

V1: -0.7 | 0

V2: 0 | 0

V3: 0 | 0

V4: 0 | 0

Input Voltage Range: 2

Frequency: 4

Sweep Time: 1

N. Channels: 4

Ch. Sample Rate: 400

Calibration Values

Slope (Ox)	Slope (Re)	Limit of Detection	Selectivity (Ox)	Selectivity (Re)	R Square
-0.009897	-0.009897	0.275182	2981.358465	2981.358465	0.999536
-0.017366	-0.017366	0.240210	5952.546519	5952.546519	0.999923
-0.008252	-0.008252	0.284300	2482.203730	2482.203730	0.998964
-0.008242	-0.008242	0.308162	3710.194683	3710.194683	0.998533

Server Communications Log

TX RX

Server Initializing  
Configuring for Amperometry

DONE

SLOPES

0 -0.009897

-0.017366

-0.008252

-0.008242

RESTORE

MARK EVENT

START

END

Events

8

Time Since Last Event

00:01:26

V applied

-0.7

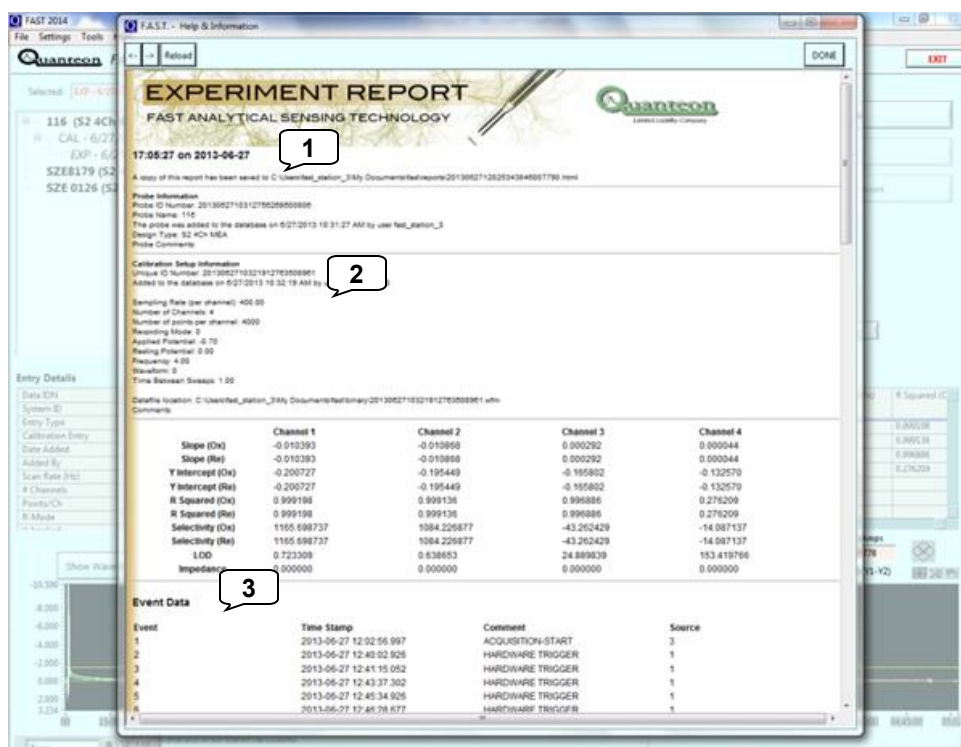
Scope

CANCEL

1. **SENSOR INFORMATION** - Displays MEA parameters.
2. **CALIBRATION INFORMATION** - Calibration/experiment parameters for selected re-  
cording MEA.
3. **CALIBRATION VALUES** - Displays MEA calibration calculations



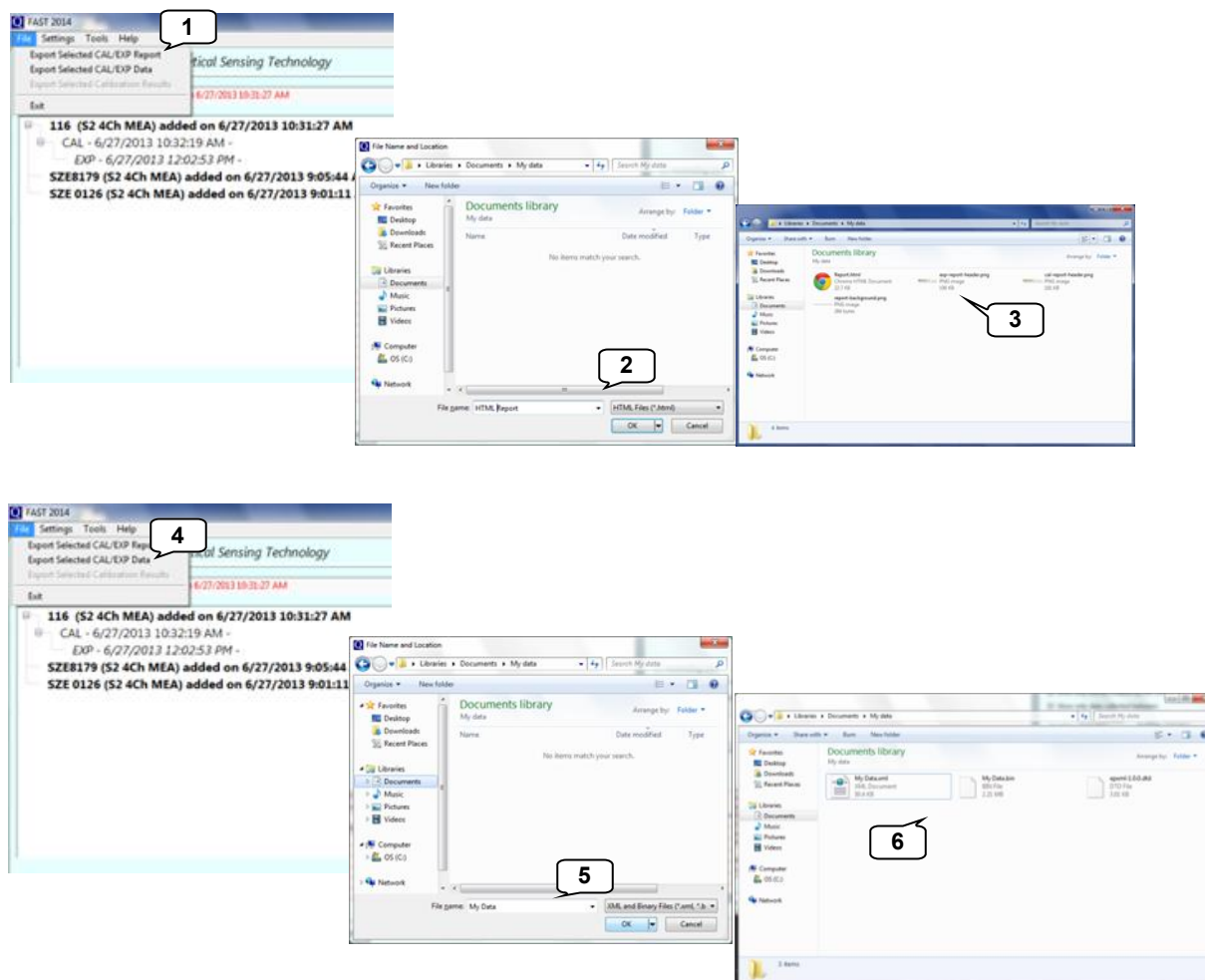
## 1.4 Amperometry Experiments - Viewing reports



**View Report** from the Main Screen

1. **Sensor Information** - [Information about MEA.](#)
2. **Calibration Setup Information** - Calibration parameters and calculations.
3. **Event Data** - Lists **Event #**, **Time Stamp**, **Comments**, **Source** for the event.

## 1.4 Amperometry Experiments - Exporting files

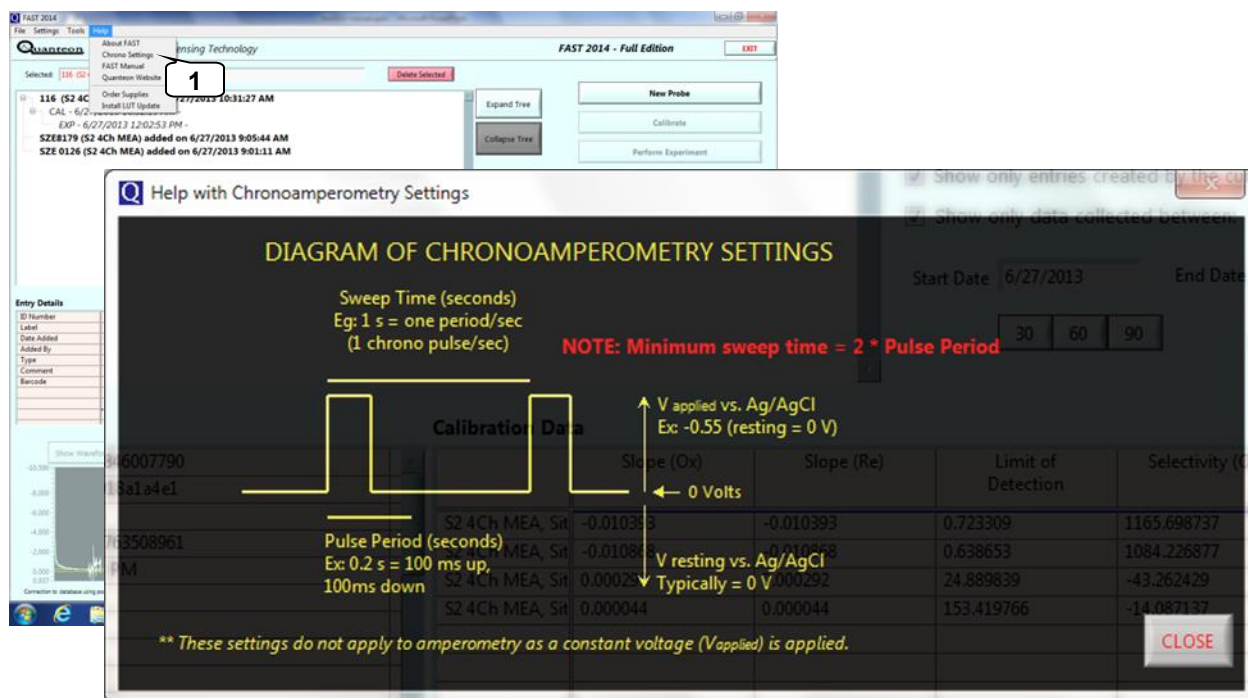


1. **Export Selected CAL/EXP Report** - Used to export a report. [Select entry on the home screen \(#5\)](#)
2. Provide a file name. Exported as .html
3. Exporting the report generates an .html file and header images.
4. **Export Selected CAL/EXP Data** - Export data files used in the [FAST Analysis](#) program a report. [Select entry on the home screen \(#5\)](#)
5. Provide a file name.
6. Exporting data files generates .xml, .bit, .dtd files that are all needed for analysis with [FAST Analysis](#).

**Chronoamperometry Recordings:  
Neurochemistry with Carbon Fibers**

## 2.1 Chronoamperometry Settings & Defaults

### Carbon fibers



- In the main menu, under **Help**, choose **Chrono Settings** to display a diagram of the parameters used in chronoamperometry experiments.
- **Pulse Period** - Total duration (in seconds) of the voltage pulse.
- **Sweep Time** - Interval (in seconds) between the start of voltage pulses.

## 2.1 Chronoamperometry Settings & Defaults

### Carbon Fibers

The screenshot shows the 'FAST System Settings' window with the 'Settings & Defaults' tab selected. The window is divided into several sections:

- DAQ Hardware Settings:** Includes 'Present DAQ Card' (Dev1 (PCI-6251)), 'Change Card To:' (Dev1 (PCI-6251)), 'Card Max Rate' (1E+6, callout 1), 'Card Max Channels' (16), 'Input Range' (5, callout 2), and 'Change Range to:' (5).
- Digital I/O Settings:** Includes 'TTL Input Logic Sense' (callout 4) with a description and bit settings (Bit 0-7 all High), and 'TTL Output' (Enable checkbox, callout 5) with 'Mode' (EXT), 'Offset (S)' (0), 'Duration (S)' (0), 'Active' (Low), 'Source' (Dev1/PF01), and 'New Source' (1/Dev1/PF0).
- Feature Settings:** Includes checkboxes for 'Enable Calibration CSV Dialog', 'Enable Expanded Features (Amperometry Only)', and 'Enable Multi-voltage Output'.
- Default Settings:** Includes 'Attached Headstage\*' (QHS-4 X 20 pA/mV (50x), callout 6), 'Attached Control Box\*' (FAST16mkIII (8ch), callout 7), 'System Resting Potential' (V1: 0.000, callout 3), 'Recording Mode' (Chronoamperometry), 'Recording Rate (Hz)' (4), 'Exclusion Layer\*' (Nafion), 'Analyte\*' (Dopamine), 'Interferent\*' (Ascorbate (AA)), 'Pulse Period' (0.2), 'Sweep Time' (1), 'V Applied' (V1: -0.55), 'V Resting' (V1: 0), and 'Enter Default Values (µM) for Calibration' (Analyte Conc.: 2, Interferent Conc.: 250).

1. **Card Max Rate** - Describes how often the [analog to digital conversion](#) occurs per channel.
2. **Input Range** - 0.1 to 10V. From drop-down menu, most often select 5 for best signal detection. Describes [analog to digital conversion range](#).
3. **System Resting Potential** - Maintains applied potential between recording episodes.
4. **TTL Input Logic Sense** - Sets the logic level on the digital inputs that indicate the untriggered state of attached equipment.
5. **TTL Output** - A tick in the box enables the output of a trigger signal that begins on the rising edge of the square wave pulse used in chronoamperometry. Used to synchronize external equipment such as data collection systems to the FAST system.
6. **Attached Headstage** - Select the headstage that is attached to the FAST control box.
7. **Attached Control Box** - Select appropriate control box. If selecting "FAST16mkI," match the gain to the one selected on the front panel of the FAST control box.

## 2.1 Chronoamperometry Settings & Defaults

### Carbon Fibers

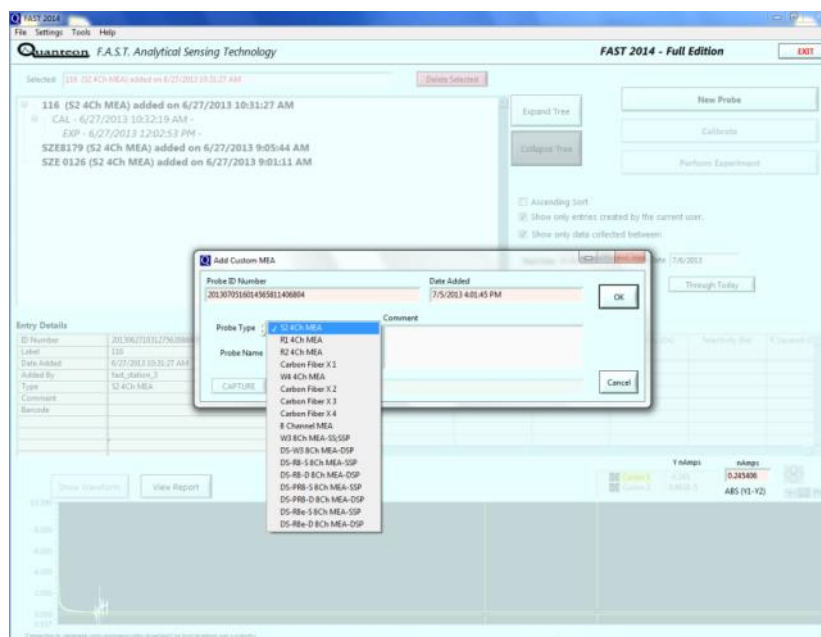
The screenshot displays the 'FAST System Settings' window with the 'Settings & Defaults' tab selected. The interface is divided into several sections:

- DAQ Hardware Settings:** Includes fields for 'Present DAQ Card' (Dev1 (PCI-6251)), 'Change Card To:' (Dev1 (PCI-6251)), 'Card Max Rate' (1E+6), 'Card Max Channels' (16), 'Input Range' (± 5), 'Present Value', and 'Change Range to:' (5).
- Digital I/O Settings:** Contains 'TTL Input Logic Sense' instructions and a table of TTL bits (0-7) all set to 'High'. It also has 'TTL Output' (Enable) and 'Mode' (EXT) settings.
- Feature Settings:** Includes checkboxes for 'Enable Calibration CSV Dialog' (checked), 'Enable Multi-voltage Output' (unchecked), and 'Enable Expanded Features (Amperometry Only)' (unchecked).
- Default Settings:**
  - Attached Headstage\*:** QHS-4 X 20 pA/mV (50x)
  - Attached Control Box\*:** FAST16mkIII (8ch)
  - System Resting Potential:** V1 0.000, V2 0.000, V3 -1.000, V4 -1.000
  - Recording Mode:** Chronoamperometry (13)
  - Recording Rate (Hz):** 4
  - Pulse Period:** 0.2 (14)
  - Sweep Time:** 1 (14)
  - V Applied:** V1 -0.55 (15), V2 0, V3 0, V4 0
  - V Resting:** V1 0, V2 0, V3 0, V4 0
  - Exclusion Layer\* (8):** Nafion
  - Analyte\* (9):** Dopamine
  - Interferent\* (10):** Ascorbate (AA)
  - Analyte Conc. (11):** 2
  - Interferent Conc. (12):** 250

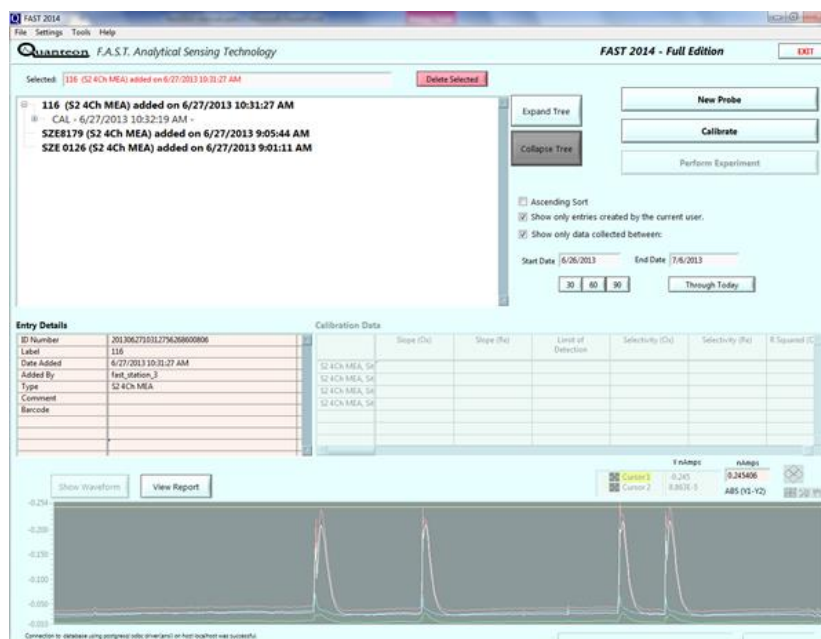
8. **Default Exclusion Layer** - Default exclusion layer, if one, for calibrations. (**Defaults:** double clicking a selection from the list sets/changes the default. The current default is shown in the text box above the list).
9. **Default Analyte** - Default analyte, if one, for calibrations.
10. **Default Interferent** - Default interferent, if one, for calibrations
11. **Analyte Concentration** - Default analyte concentration for calibrations.
12. **Interferent Concentration** - Default interferent concentration for calibrations.
13. **Recording Mode** - Carbon fibers are calibrated in **Chronoamperometry** mode.
14. **Pulse Period, Sweep Time** - Described in [Diagram of Chronoamperometry Settings](#).
15. **V applied** - Enter the potential to be applied vs. reference electrode.
16. **Enable Calibration CSV dialog** - Add calibration results to a new or existing spreadsheet.
17. **Enable Multi-voltage Output** - Permits use of multiple applied potentials (advance only).
18. **Enable Expanded Features** - Displays Custom MEA Calibration Setup window.



## 2.2 Recording with Chronoamperometry



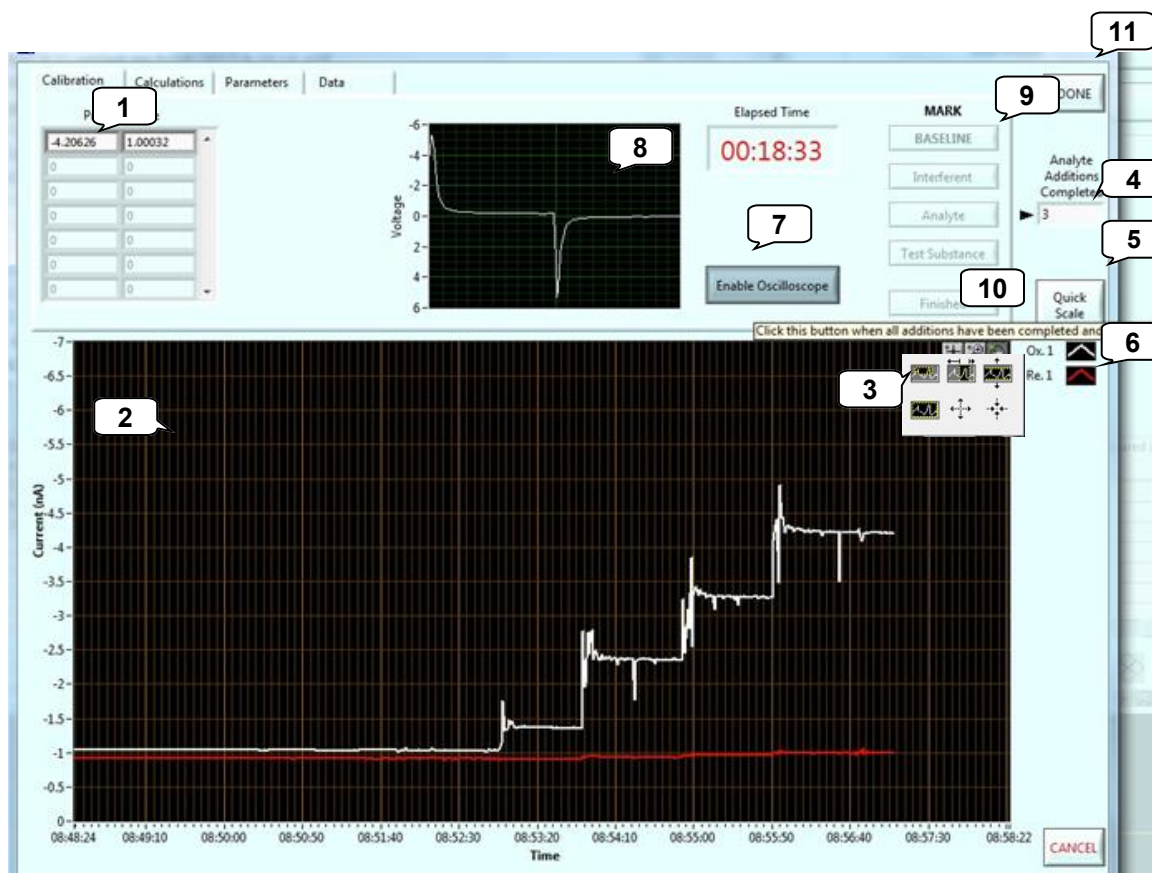
After verifying [Settings](#), select **New Probe**. Choose **Probe Type** from the drop down menu (**Carbon Fiber X n** for  $n$  number of active channels), provide a **Probe Name**, and include additional **Comments**, as needed. The information will be displayed in the database list.



After creating an MEA entry, select the sensor of interest in the database and the **Calibrate** button will become active. Select to begin an [MEA calibration](#).

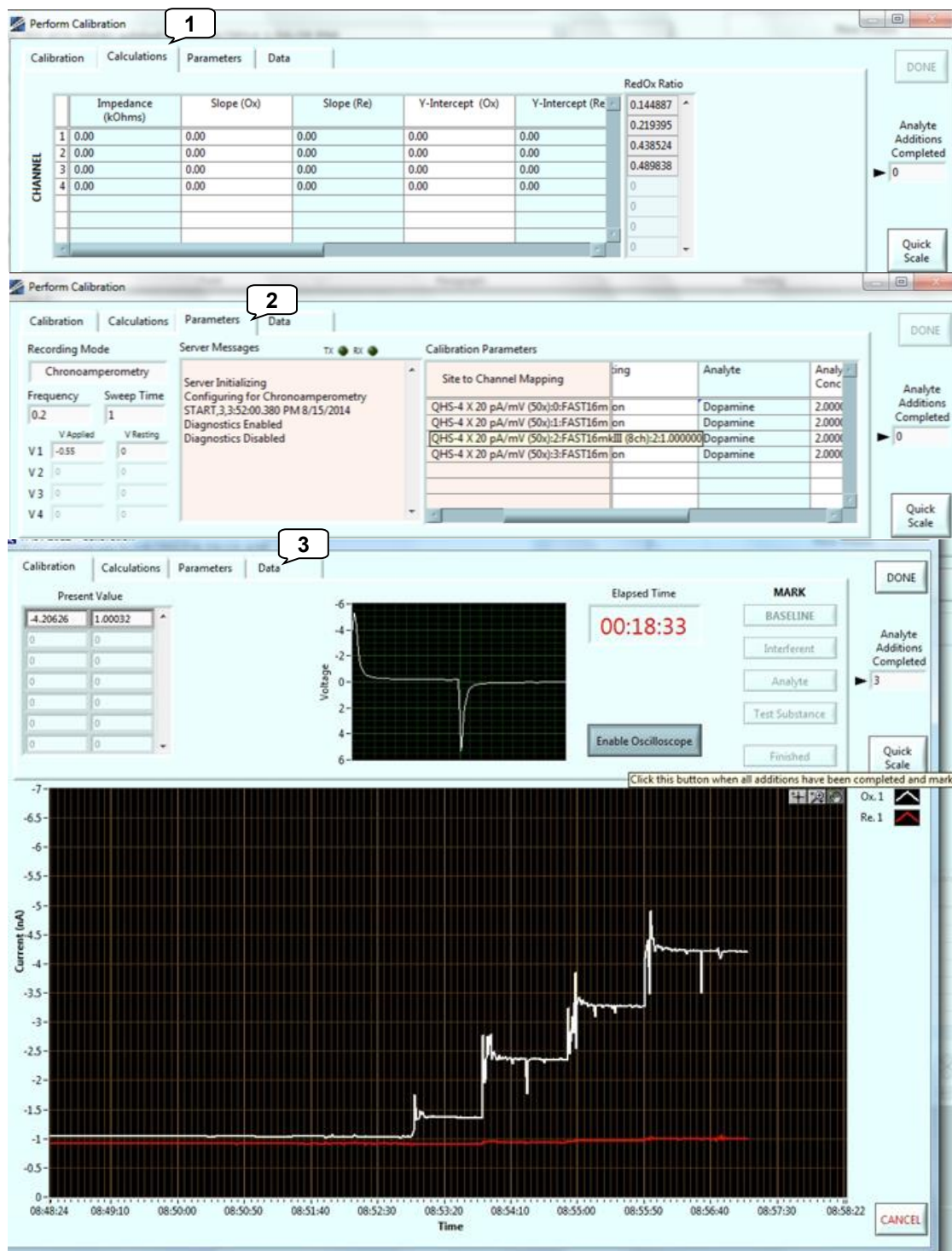


## 2.3 Chronoamperometry Calibrations



1. Current (nA) value of oxidation (left) and reduction (right) for each channel.
2. Graphical display of the current values of individual channels (nA vs. time of day).
3. Magnification and scrolling tools allow for customization of the graphical display.
4. Counter showing total number of additions for calibrations.
5. Re-scale to zoom on the active area of the graph.
6. Active microelectrode channel colors for oxidation and reduction —modifiable.
7. Click to toggle between displaying/turning off the oscilloscope.
8. Oscilloscope displays wave form or current level generated at microelectrode (in V).
9. Activate **BASELINE** button to begin calibration calculations. Buttons change to **Interferent** then **Analyte** and **Test Substance** based on calibration protocol.
10. Select **Finished** to end calibration calculation.
11. Select **DONE** to close calibration window, otherwise, to abort calibration select **CANCEL**.

## 2.3 Chronoamperometry Calibrations



1. **Calculations** - Calibration results (including LOD, selectivity, and slope).
2. **Parameters** - Displays system parameters used for calibration.
3. **Data** - Displays data used for calibration **Calculations**.

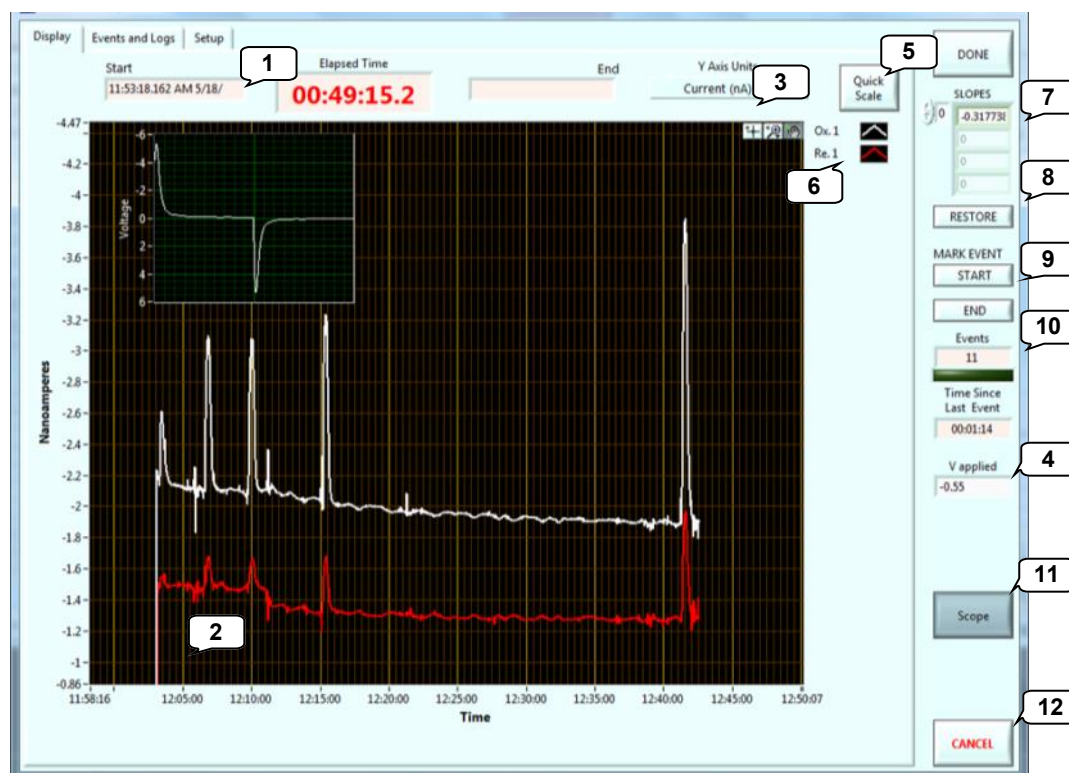
## 2.4 Chronoamperometry Experiments



After creating a calibration,

1. Select a calibration to use for an experiment by double-clicking on that calibration.
2. Parameters associated with the MEA and calibration and calibration calculations (LOD, selectivity, Slope) are displayed.
3. Select **PERFORM EXPERIMENT** to open acquisition window and begin an experiment.

## 2.4 Chronoamperometry Experiments



1. **Start** time stamp for acquisition data file, **total elapsed recording time**, and **End** time stamp for acquisition data file.
2. Graphical display of the current (nA or pA) or concentration ( $\mu\text{M}$ ) values of individual MEA channels (vs. time of day).
3. Toggle between **Current** (nA or pA) and **Concentration** ( $\mu\text{M}$ ) display for Y-Axis Units.
4. **V applied** - [Voltage applied](#).
5. Re-scale to zoom on the active area of the graph.
6. Active microelectrode channel colors for oxidation and reduction —modifiable.
7. Microelectrode **slopes** from calibration. These are automatically obtained from selected calibration file but can be manually entered as needed.
8. **RESTORE SLOPES** to original calibration file values for selected MEA.
9. Click to signal **START** and **END** of select triggered **EVENT**. Useful for long recording signals, drug, or behavior studies. Adds TTL event mark to data file for analysis.
10. **Events** - Counts number of triggered events and **Time Since Last TTL Event**.
11. **V applied** - Voltage applied, adjustable.
12. **Scope** - Display/Hide [oscilloscope](#).
13. Select **DONE** to close Experiment window, otherwise, to abort experiment select **CANCEL** (data not saved).

## 2.4 Chronoamperometry Experiments

[illegible]

1. **Event Number** - indicates order of TTL events.
2. **Time Stamp** – Time TTL was triggered (Time determined by computer clock).
3. **Description** – Displays the source of the TTL input.
4. **Source** – Displays which TTL input was triggered.
5. **User Comments** - Text field for describing TTL events.
6. **Experiment Description & Comments** - Text field for describing experiment. Viewable in **Browse Database**.
7. **RedOx Ratio** – In chronoamperometry, ratio between reduction current and oxidation current. Used to identify molecules of interest. (~ 0.6 to 0.9: dopamine; ~0.1 to 0.3: serotonin)



## 2.4 Chronoamperometry Experiments

**PROBE INFORMATION** (1)

Probe Label: taylor electrode  
 Operator: fast\_station\_3  
 Date/Time: 8/14/2014 1:56:28 PM  
 Probe Type: S2 4Ch MEA

**CALIBRATION INFORMATION** (2)

Operator: fast\_station\_3  
 Date/Time: 8/15/2014 3:51:54 PM  
 Recording Mode: Chronoamperometry  
 Waveform: Square (Chronoamperometry)

Default values were used

	V Applied	V Resting
V1	-0.55	0
V2	0	0
V3	0	0
V4	0	0

Input Voltage Range: 5  
 Frequency: 0.2  
 Sweep Time: 1  
 N. Channels: 4  
 Ch. Sample Rate: 64000

**Calibration Values** (3)

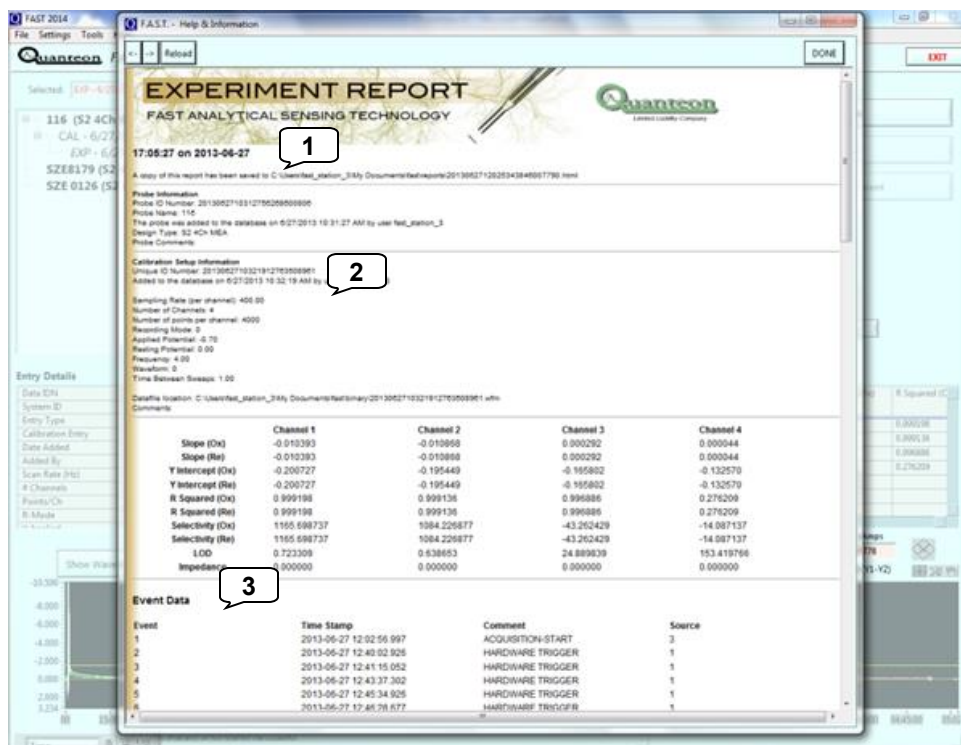
Slope (Ox)	Slope (Re)	Limit of Detection	Selectivity (Ox)	Selectivity (Re)	R Square
0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
0.000000	0.000000	0.000000	0.000000	0.000000	0.000000

Server Communications Log: Server Initializing, Configuring for Chronoamperometry

Buttons: DONE, RESTORE, MARK EVENT, START, END, Scope, CANCEL

1. **SENSOR INFORMATION** - Displays Microelectrode parameters.
2. **CCALIBRATION INFORMATION** - Calibration/experiment parameters for selected re-  
cording MEA.
3. **CALIBRATION VALUES** - Displays MEA calibration calculations

## 2.4 Chronoamperometry Experiments - Viewing reports

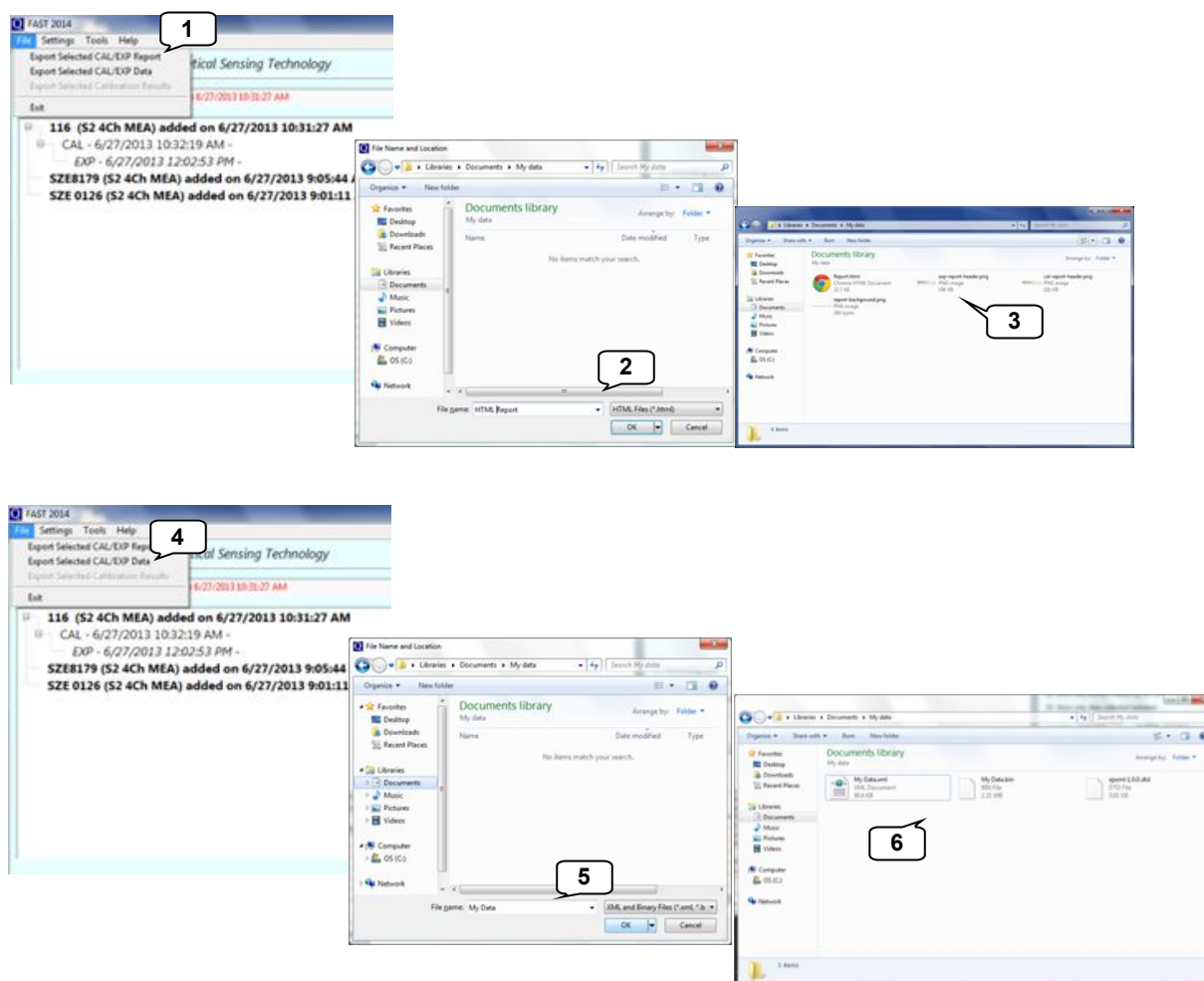


**View Report** from the Main Screen

1. **Sensor Information** - [Information about microelectrode](#)
2. **Calibration Setup Information** - Calibration parameters and calculations.
3. **Event Data** - Lists **Event #**, **Time Stamp**, **Comments**, **Source** for the event.

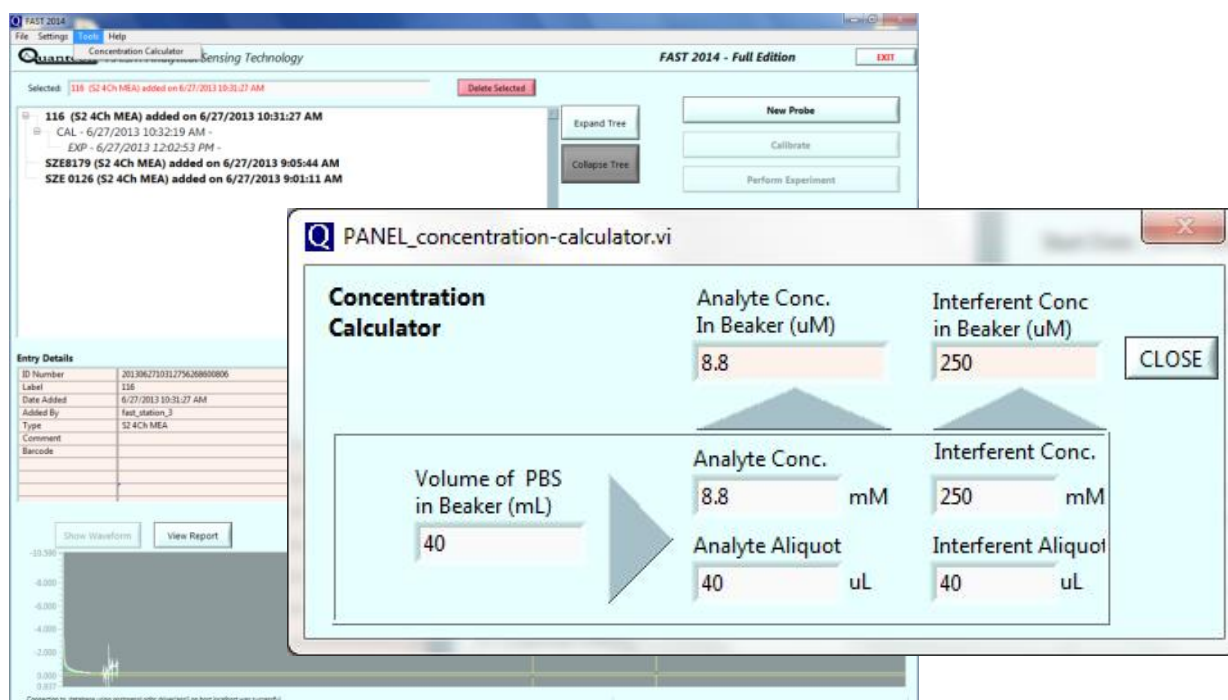


## 2.4 Chronoamperometry Experiments - Exporting files



1. **Export Selected CAL/EXP Report** - Used to export a report. [Select entry on the home screen \(#5\)](#)
2. Provide a file name. Exported as .html
3. Exporting the report generates an .html file and header images.
4. **Export Selected CAL/EXP Data** - Export data files used in the [FAST Analysis](#) program a report. [Select entry on the home screen \(#5\)](#)
5. Provide a file name.
6. Exporting data files generates .xml, .bit, .dtd files that are all needed for analysis with [FAST Analysis](#).

## 3.1 Tools - Concentration calculator



1. **Concentration Calculator** - Enter volume used for calibration. Analyte and/or Interferent concentration. Analyte or Interferent aliquot volume and obtain final concentrations in the beaker.

## 3.2 Preparation of mPD Microelectrode Arrays

### Quick guide to m-PD-plating

Acros Organics: m-Phenylenediamine dihydrochloride, 99%. CAS number: 541-69-5  
Catalog number: 216650250 (for 25 g)

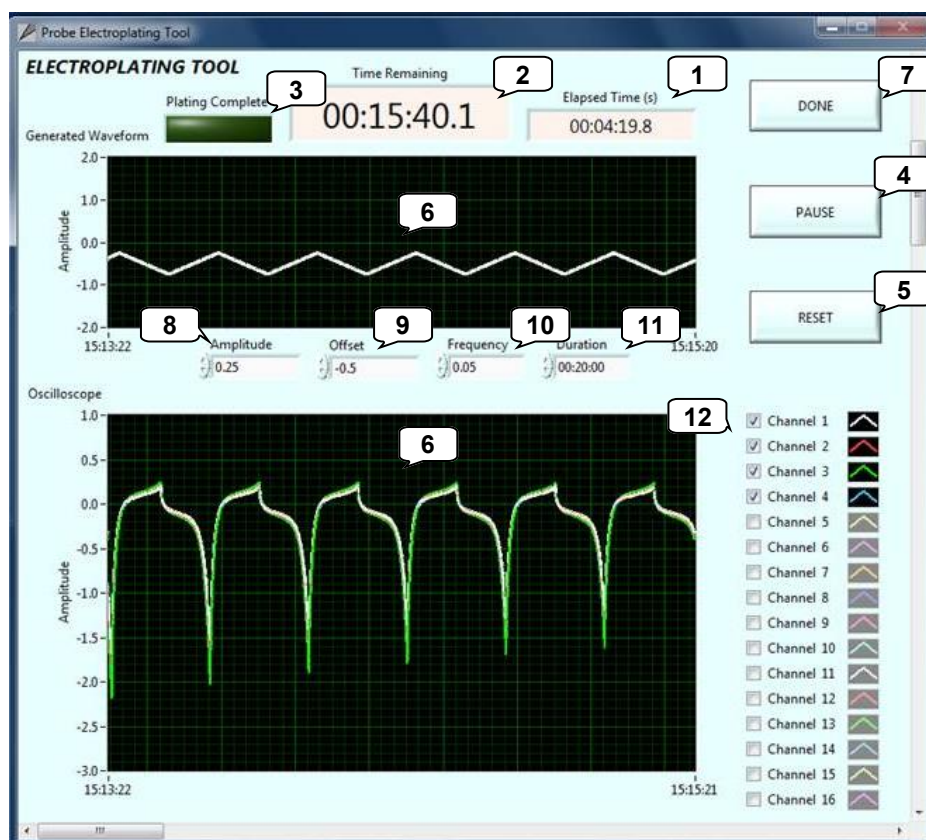
1. Bubble nitrogen gas through 125 ml of PBS for 20 minutes
  - This procedure is done to minimize oxidation of mPD by oxygen in the solution.
2. Weigh 0.0905 g of mPhenylenediamine (mPD)
  - a. Use mask and gloves
    - this substance is carcinogenic and easily oxidizes. The substance turns brown on oxidation and leaves ugly stains on fingers and other surfaces.
3. Mix solution on a stirplate in room temperature until it is completely dissolved.
  - a. Do not shake the container because that will re-introduce oxygen into the solution.
4. Place MEA in the headstage (Use either the 500X headstage or the 50X).
5. Pour ~40 ml of mPD solution in a beaker and place a reference electrode in the beaker. The reference used for mPD-plating should be used for this purpose only. Do not use your everyday, BSA reference electrode for this.
6. Verify connections by running calibration and observing exponential-shaped decay.



7. Software: Launch Electroplater and run program

Calibrate mPD coated MEA per established protocol

### 3.3 Tools - Electroplating Tool



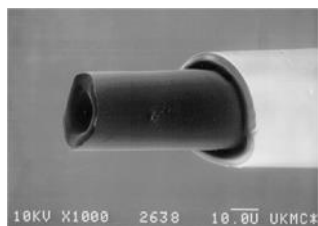
To begin electroplating, launch the electroplating tool program  on the desktop.

1. **Elapsed Time** - Elapsed time (in sec) of running electroplating tool.
2. **Plating Complete** - When electroplating is finished, **green light** turns on.
3. **Time Remaining** - Time left to finish electroplating.
4. **PAUSE** - Unselect to begin electroplating. Select to pause electroplating.
5. **RESET** - Return values to default.
6. **Graphical Display** - Displays electroplating output wave pulse and oscilloscope view.
7. **Done** - Exit Electroplating Tool program.
8. **Peak Amplitude** - Amplitude of the applied voltage pulse.
9. **Offset** - Deviation from zero of the applied voltage pulse.
10. **Frequency** - Number of cycles per second.
11. **Duration** - Length of time the electroplating tool will be applied.
12. Click to display/hide the traces

[Standard protocol](#) for electroplating meta-Phenylenediamine on MEAs is **Duration** : 20 minutes, **Frequency** : 0.05, **Offset** : -0.5, **Peak Amplitude** : 0.25.

## 4.1 Calibrating Microelectrodes

### Monoamines



#### Dopamine calibrations

1. Dopamine
2. PBS
3. DOPAC

#### Serotonin calibrations

1. Serotonin
2. PBS
3. AA

#### Norepinephrine calibrations

1. Norepinephrine
2. PBS
3. AA

#### Accessories

1. Carbon Fiber (Nafion<sup>®</sup> coated)\*
2. One reference electrode (the tip is stored in 3M NaCl at least 24 hours before first use)
3. Battery powered stirrer
4. 50 mL Beaker
5. Magnetic stir bar

### Enzyme-based MEAs



#### H<sub>2</sub>O<sub>2</sub> calibrations

1. Peroxide
2. PBS

#### Choline calibrations

1. Choline
2. PBS
3. AA
4. Peroxide

#### Glutamate calibrations

1. Glutamate
2. PBS
3. AA
4. Peroxide

#### Adenosine calibrations

1. Adenosine
2. Inosine
3. Xanthine
4. PBS
5. AA
6. Peroxide

#### Accessories

1. Enzyme-coated MEA (Nafion<sup>®</sup> coated or mPD plated)\*
2. One reference electrode (the tip is stored in 3M NaCl at least 24 hours before first use)
3. Battery powered stirrer
4. 50 mL Beaker
5. Magnetic stir bar

#### ACh calibrations

1. ACh
2. PBS
3. AA
4. Choline
5. Peroxide

\* Carbon fibers and Microelectrode arrays may be purchased through the [Center for Microelectrode Technology](#)

## 4.2 Calibration Protocol

Soak microelectrodes for 30 min – 45 min in RT [PBS lite](#) or warmed (35-37°C) [PBS lite](#) for enzyme-based MEAs

Prepare [calibration solutions](#)

Connect sensor to hardware and insert tip into 40 ml of stirred PBS lite

Connect the reference electrode

Begin [amperometry](#) or chronoamperometry calibrations

Allow 5-10 min equilibration; begin once the baseline has stabilized

Mark **BASELINE**

Add 500 ul interferent (stock solutions of: 20 mM AA or 20 mM DOPAC)

Allow ~ 30 sec -1 min between additions/until traces reach new steady-state.

Mark **INTERFERENT**

Add 40 ul of analyte (stock solutions of: 20 mM glutamate or 2 mM DA or other analyte of interest)

Mark 1<sup>st</sup> addition **ANALYTE**

Add 40 ul analyte

Mark 2<sup>nd</sup> addition **ANALYTE**

Add 40 ul analyte

Mark 3<sup>rd</sup> addition **ANALYTE** (Note that 3 additions are standard – but one point calibration could be used)

May select **FINISHED** or may continue with test substances, as necessary.

For enzyme-based MEA, add 40 ul of stock solution of 2mM DA

DO NOT MARK **ANALYTE** – click on **TEST SUBSTANCE**

Add 40 ul of stock solution of 8.8mM H<sub>2</sub>O<sub>2</sub>

DO NOT MARK **ANALYTE** – click on **TEST SUBSTANCE**

Click the **FINISHED** button

After calibration, store microelectrode in PBS lite until use.

## 4.3 Calibration Solutions

“PBS Lite”

0.05 M Phosphate Buffered Saline

2 Liter recipe

1. Transfer ~1800 mL of deionized, filtered H<sub>2</sub>O to a 2L glass container
2. Add, while mixing with a stir bar:

CHEMICAL	F.W.	AMOUNT (G)
SODIUM PHOSPHATE (NaH <sub>2</sub> PO <sub>4</sub> ), <u>MONOBASIC, MONOHYDRATE</u>	137.9	2.8
SODIUM PHOSPHATE (Na <sub>2</sub> HPO <sub>4</sub> ), DIBASIC, <u>ANHYDROUS</u>	141.9	11.34
SODIUM CHLORIDE (NaCl)	58.44	11.68

3. Stir until all chemicals are dissolved. Transfer to a 2L volumetric flask and add dH<sub>2</sub>O to bring to volume.
4. Filter through 0.2 µm filter.
5. Test pH, pH ~ 7.2—7.4.



## 4.3 Common Calibration Solutions

**Ascorbic Acid** 20mM: (500  $\mu$ l in 40 ml = 250  $\mu$ M)

20 mM: 0.18 g of AA in 50 mL of DI H<sub>2</sub>O

Make fresh daily.

**Acetyl choline** 20 mM ACh (40  $\mu$ l in 40 ml = 20  $\mu$ M)

20 mM: 0.18 g of ACh in 50 mL of DI H<sub>2</sub>O

Make fresh daily.

**Adenosine** 1 mM: (40  $\mu$ l in 40 ml = 1 $\mu$ M)

1 mM: 0.13 mg of adenosine in 1 mL of DI H<sub>2</sub>O

Make fresh every week

**Choline** 20mM: (40  $\mu$ l in 40 ml = 20 $\mu$ M)

20 mM: 0.14 g of choline in 50 mL of DI H<sub>2</sub>O

Make fresh every week

**Dopamine** 2mM: (40  $\mu$ l in 40 ml = 2  $\mu$ M)

2 mM: 0.038 g of DA + 10 mL of 0.1M perchloric acid.

Bring volume to 100 mL with DI H<sub>2</sub>O.

Make fresh once per month.

**DOPAC** 20mM: (500  $\mu$ l in 40 ml = 250  $\mu$ M)

20 mM: 0.34 g of DOPAC + 1 mL of 0.1M perchloric acid.

Bring volume to 100 mL with DI H<sub>2</sub>O.

Make fresh once per month.

**L-Glutamate** 20 mM: (40  $\mu$ l in 40 ml = 20  $\mu$ M)

20 mM: 0.15 g of glutamate in 50 mL of DI H<sub>2</sub>O

Make fresh weekly.

**H<sub>2</sub>O<sub>2</sub>** 8.8 mM: (40  $\mu$ l in 40 ml = 8.8  $\mu$ M)

8.8 mM 500  $\mu$ L of H<sub>2</sub>O<sub>2</sub> (3%) in 50 mL of DI H<sub>2</sub>O

Make fresh every week.

**Cont.**

## 4.3 Common Calibration Solutions

**Inosine** 1 mM: (40  $\mu$ l in 40 ml = 1  $\mu$ M)

1 mM: 0.13 mg of inosine in 1 mL of DI H<sub>2</sub>O

Make fresh every week

**Norepinephrine** 2 mM: (40  $\mu$ l in 40 ml = 2  $\mu$ M)

2 mM: 0.064 g of NE + 10 mL of 0.1M perchloric acid.

Bring volume to 100 mL with DI H<sub>2</sub>O.

Make fresh once per month.

**Serotonin** 2 mM: (40  $\mu$ l in 40 ml = 2  $\mu$ M)

2 mM: 0.043 g of 5-HT + 10 mL of 0.1M perchloric acid.

Bring volume to 100 mL with DI H<sub>2</sub>O.

Make fresh once per month.

**Xanthine** 1 mM: (40  $\mu$ l in 40 ml = 1  $\mu$ M)

1 mM: 0.15 mg of xanthine in 1 mL of 0.1N NaOH

Make fresh every week

## 4.4 Analyte ordering information

Description	Distributor	Catalog #	Units
Acetylcholine chloride	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	A6625-25 G	25 g
Adenosine	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	A9251-5G	5 g
Ascorbic Acid	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	A5960-100G	100 g
Choline Chloride	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	C1879	500 g
DOPAC	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	850217	1 g
Dopamine HCl	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	H8502	10 g
Hydrogen Peroxide	Available from local drug store	3 % H <sub>2</sub> O <sub>2</sub> solution.	1
Inosine	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	I4125-10G	10 g
L-Glutamic acid salt (MSG)	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	G-1626	100 g
Norepinephrine bitartrate	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	A0937	1g
Serotonin (5-HT)	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	H9523-100MG	100 mg
Xanthine	Sigma-Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>	X7375-10G	10 g

<sup>A</sup> Updated March 2010. Information not warranted.

Description	Distributor	Catalog #
Heating & circulating water pump <sup>a</sup>	e.g. Cincinnati Sub-Zero <a href="http://www.cszmedical.com">http://www.cszmedical.com</a>	Micro-Temp LT
Magnetic Stirrer <sup>b</sup> (battery operated)	Cole-Parmer <a href="http://www.coleparmer.com">http://www.coleparmer.com</a>	<a href="#">EW-04804-01</a>
Reference electrodes	BAS <a href="http://www.basinc.com/">http://www.basinc.com/</a>	<a href="#">MF-2052/MF-2079</a>
Stir Bar	Fisher Scientific <a href="http://www.fishersci.com">http://www.fishersci.com</a>	<a href="#">14-511-92</a>
<b>Common optional/additional accessories</b>		
Dip socket	Newark Electronics <a href="http://www.newark.com">http://www.newark.com</a>	<a href="#">04M1727</a> (Mill-Max #210-43-308-41-001000)
Quick Connect for calibration bath	Colder.com <a href="http://www.colder.com/">http://www.colder.com/</a>	e.g. <a href="#">APCD17006SH</a> ; <a href="#">APCD22006</a>
Glass pipette (o.d. 1 mm, i.d. 0.58 mm, 6")	A-M Systems <a href="http://www.a-msystems.com">http://www.a-msystems.com</a>	<a href="#">601500</a>
Gold pin	<a href="#">Allied Electronics</a>	<a href="#">Mfr. Part #: 220-P02-100</a>
Kopf Model 900 Stereotax <sup>d</sup>	<a href="http://www.kopfstruments.com/Stereotaxic/900.htm">http://www.kopfstruments.com/Stereotaxic/900.htm</a>	
Meiji Techno EMZ Stereomicroscope <sup>c</sup>	<a href="http://www.meijitechno.com/emz.htm">http://www.meijitechno.com/emz.htm</a>	EMZ-5 with SWF10x eyepiece
Reticle		10mm divided into 100 units
Microdrive for Kopf stereotax	Narishige <a href="http://products.narishige-group.com/english.html">http://products.narishige-group.com/english.html</a>	<a href="#">MO-10 Single-Axis Oil Hydraulic Micromanipulator</a>
Picospritzer III <sup>e</sup>	<a href="http://www.parker.com">http://www.parker.com</a>	<a href="#">Picospritzer III</a>
Tubing for micropipette	<a href="http://www.vwr.com">www.vwr.com</a>	<a href="#">63010-232</a>
Pipette puller	David Kopf instruments <a href="http://www.kopfstruments.com/">http://www.kopfstruments.com/</a>	<a href="#">730</a>
Silver wire (200µm)	A-M Systems <a href="http://www.a-msystems.com">http://www.a-msystems.com</a>	<a href="#">7826500</a>
Sticky Wax	Kerlab <a href="http://www.kerlab.com/index/kerlab">http://www.kerlab.com/index/kerlab</a>	<a href="#">00625</a>

<sup>a</sup> Used primarily for enzyme-based MEA calibrations.

<sup>b</sup> Battery powered stir plates eliminate potential 60 Hz noise that could interfere with calibrations.

<sup>c</sup> For craniotomy surgeries and probe placement and monitoring of micropipette pressure ejection of fluids. (10x eyepieces, 0.5x objective), 0.7 – 3.0+ zoom range is very important. Select one with the telescoping arm or boom that has the needed magnification.

<sup>d</sup> Holder: Model 1772 Universal Holder places syringes, large diameter electrodes or drill handpieces stereotactically. Holds devices from 2.8 mm to 11 mm diameter.

<sup>e</sup> For pressure ejections of solutions *in vivo*. One channel system is fine for most applications. 2 channel is useful if when ejecting multiple agents simultaneously using double-barreled pipettes.

## 5.1 Glossary

**Analog to Digital Conversion (ADC)** - In data acquisition, the conversion of analog measurements to digital numbers that the computer can store and use in calculations.

**Analog to Digital Conversion Range** - Refers to the minimum and maximum values that the analog to digital conversion can span. A fixed number of points span this range and the smaller the range, the greater the resolution of the signal.

**Gain** - Amplification of a signal.

**LUT**– Look Up Table file contains FAST system information updates regarding new analytes, coatings, supported DAQ cards, etc. Distributed as a lut-data.ini file. To update, replace the old lut-data.ini file in the Quanteon folder located in the Program Files of the main hard drive (usually the C drive) with this file. Next, go into Manage Database in the FAST software and select, Update Lookup Tables.

**Oscilloscope** displays wave form or current level generated at microelectrode (V, current converted to V for display).

## 5.2 Troubleshooting

**Problem:** I cannot get any waveforms during calibration or electrode verification.

**Possible Solutions:** Power to the system interface is off. Electrodes are not connected to the headstage. Signal amplification is not high enough for an electrode. The Analog-to-Digital cable has been jarred loose or the board is not connected. Tip of working electrode is not in solution. Potential ground loop in the system. Perform resistor test. Verify the applied voltage between **REF** and **GND/ISO** on the headstage.

**Problem:** The signal is noisy.

**Possible Solution:** A/C interference from heating pads or lights. Poor grounding of computer. Bad reference electrode. Damaged or bad working electrode.

**Problem:** The baseline in Acquisition or the waveforms in Calibration continue to fall.

**Possible Solution:** Bad reference electrode. Working electrode is overcoated with Nafion<sup>®</sup> or mPD. The working electrode may need a few more minutes to stabilize.



## 5.3 Frequently Asked Questions

### How is LOD calculated?

LOD is calculated as  $3 \times \text{SD of 10 points of the baseline (the baseline SD in the html report) / slope}$ . The units are in  $\mu\text{M}$ .

### How is selectivity calculated?

Selectivity is ratio of analyte response divided by the interferent response for equal concentrations. So for an interferent addition of  $250 \mu\text{M}$  the equation would be  $(\text{slope} \times 250) / \text{response to interferent in nA}$ .

### How is slope calculated?

The slope is the sensitivity of the MEA to a given analyte. The calculation is the slope (m) for a first order linear regression. The units are in  $\text{nA}/\mu\text{M}$ .

### What is the y intercept?

The Y-intercept is a parameter that can be calculate, but we have not found utility in it.

### What concentration of analyte should I be able to reliably detect if the MEA has a LOD of ~0.47-0.48?

The LOD is in  $\mu\text{M}$  and so  $>0.5 \mu\text{M}$  concentration of analyte should be reliably detectable. However, in the brain, we usually measure a quieter signal in the absence of fluid motion like in a beaker.

### What is the downside of sampling every 0.025 seconds?

Large data files and a noisier recording because the signal is not averaged over 1-2 Hz. The process of converting the analyte to current takes  $\sim 800\text{ms}$ , so signals faster than that may not be real. However, it appears that one can also measure field potential-like signals at the faster sampling rates. Zhang H, Lin S-C and Nicoletis MAL. J. Neurosci Methods 179: 191-200, 2009.

### For further reading:

Gerhardt GA and Burmeister, J. *In vivo* Voltammetry for Chemical Analysis of the Nervous System. In: Encyclopedia of Analytical Chemistry: Instrumentation and Applications, R.A. Meyers, (Ed.), John Wiley & Sons Ltd, Chichester, 2000, pp. 710-731

[Hascup KN, Rutherford EC, Quintero JE, Day BK, Nickell JR, Pomerleau F, Huettl P, Burmeister JJ and Gerhardt GA.](#) Second-by-Second Measures of L-Glutamate and Other Neurotransmitters Using Enzyme-Based Microelectrode Arrays, In: Electrochemical Methods for Neuroscience. Adrian Michael, (Ed), Taylor & Francis Group, LLC, 2007.

For further technical assistance, please contact: [support@quanteon.cc](mailto:support@quanteon.cc)

## 6.0 Appendix

### FAST control box

#### FAST16mkIII rear panel

##### Bit 0-3

Connection jack for TTL and external device control.  
High to Low voltage device.



##### Main

Pre-amplifier (Headstage) connection.

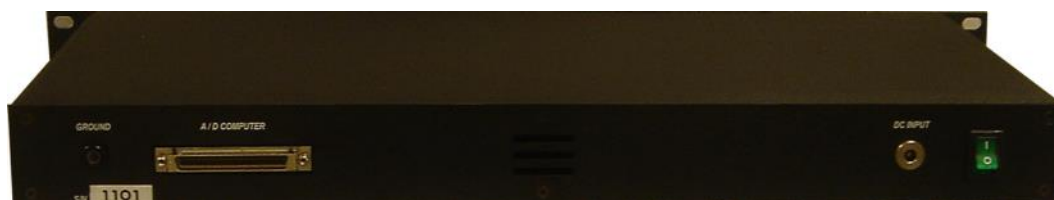
##### Marker

Button for TTL and external activation of device such as pressure ejection unit.

##### Input D/D

Input for 9 additional TTL and external device control.  
High to Low voltage devices.

#### FAST16mkIII rear panel



##### Ground

Connection jack for external ground source. Use only if needed.

##### A/D Computer

Connection port for 68 pin cable to A/D card in computer.

##### DC Input

Connection jack for 5V DC, 2.0 Amp external power supply.

##### I/O

On/Off switch. It is recommended to leave FAST16 unit on at all times to assure optimum operating performance of power supply and amplifiers. Upon initial power up, FAST16 system should reach optimum stability within 1 hour but a slight change in system performance may occur for several hours. This is normal for power supplies and amplifier circuits. In most cases these fluctuations will not affect recording function.

## 6.0 Appendix

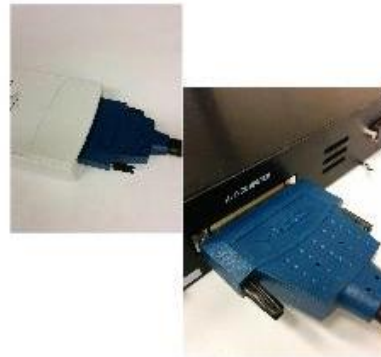
### Quick FAST System Setup



1. Setup the all-in-one computer with mouse and keyboard



3. Connect one end of the USB to the A/D card. The other end to the computer



4. Connect one end of the 68pin cable to the A/D card. The other end to the back of the FAST system.



OR



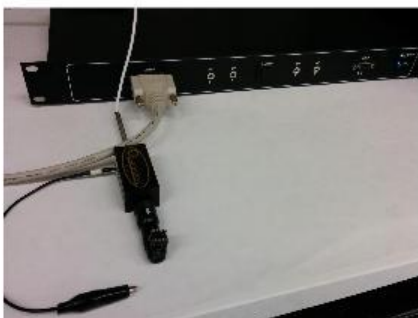
5. Power the FAST system with either the AC power or the rechargeable battery



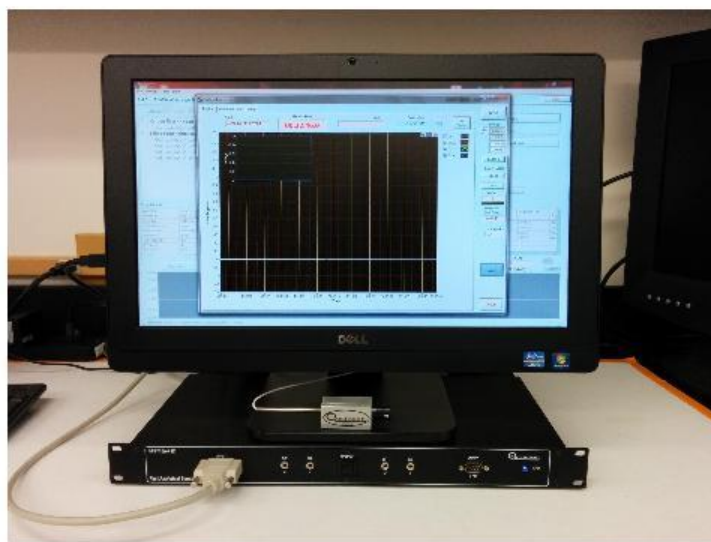
6. Use the 5V AC power supply to power the FAST system. NOT THE 12V for the rechargeable battery

## 6.0 Appendix

### Quick FAST system setup



7. Finish by connecting the cable to the FAST control box, connecting the headstage to the cable, attaching an alligator clip to REF and a MEA connector to the headstage (if applicable)



The rechargeable battery can be recharged by disconnecting from the FAST system, plugging the battery to the 12 V power supply, and connecting to a wall outlet.