



## Intan CLAMP System



## Voltage/Current Clamp User Guide

16 September 2016

### Features

- ◆ Complete, miniaturized patch clamp amplifier system with small digital headstages using revolutionary CLAMP microchips from Intan Technologies
- ◆ Voltage clamp operation with low current noise floor:  $< 3 \text{ pA}_{\text{rms}}$  over 5 kHz bandwidth with highest sensitivity
- ◆ Current clamp operation with low voltage noise floor:  $< 20 \text{ } \mu\text{V}_{\text{rms}}$  over 10 kHz bandwidth
- ◆ Fast transient capacitance compensation: 0-20 pF range; bridge balance in current clamp mode
- ◆ Automated measurement of pipette / seal resistance and cell membrane parameters
- ◆ Clamp operation controlled by software and/or external analog voltage command signals
- ◆ Small headstage size: 6.1 cm  $\times$  2.8 cm  $\times$  2.0 cm (not including pipette holder)
- ◆ Standard threaded Teflon pipette electrode connector
- ◆ Standard dovetail for mounting to micromanipulator
- ◆ All-digital interface cable is immune to noise pickup
- ◆ Standard USB interface to host computer
- ◆ Free, open-source, multi-platform GUI software

### Description

The Intan Technologies CLAMP patch clamp amplifier system allows users to perform signal channel or multi-channel patch clamp electrophysiology or electrochemistry experiments using the revolutionary new Intan CLAMP voltage/current clamp chips.

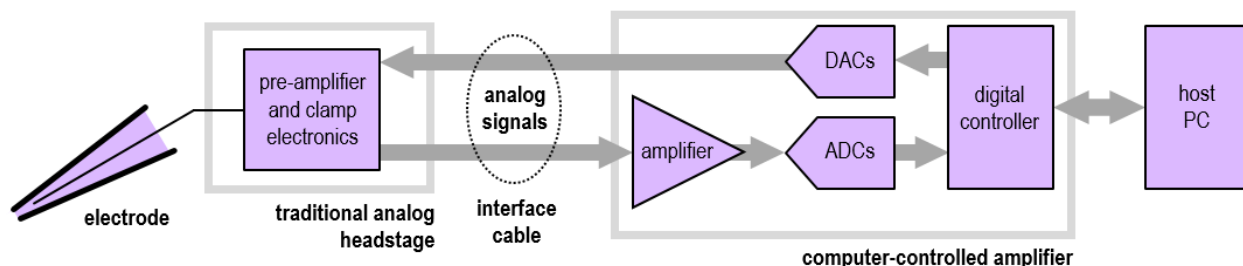
The Intan CLAMP system incorporates all analog circuitry and many digital control blocks in close proximity to the electrode, resulting in a miniaturized “headstage” that is actually a complete patch clamp amplifier. Thanks to the integration of nearly all patch clamp circuit elements onto a single silicon chip, the Intan-powered patch clamp amplifier is smaller than traditional analog headstages that perform only a small fraction of the total amplification task.

The Intan digital CLAMP headstages are interfaced to the Intan CLAMP Controller which coordinates the operation of the voltage/current clamps as well as sampling auxiliary digital and analog signals in synchrony with the measured electrophysiological signals. The interface cables are purely digital with independently isolated grounds, making them immune to noise pickup or signal degradation.

The Intan CLAMP Controller connects to a host PC via a standard USB bus, and operates using free, open-source software.

## System Architecture and Theory of Operation

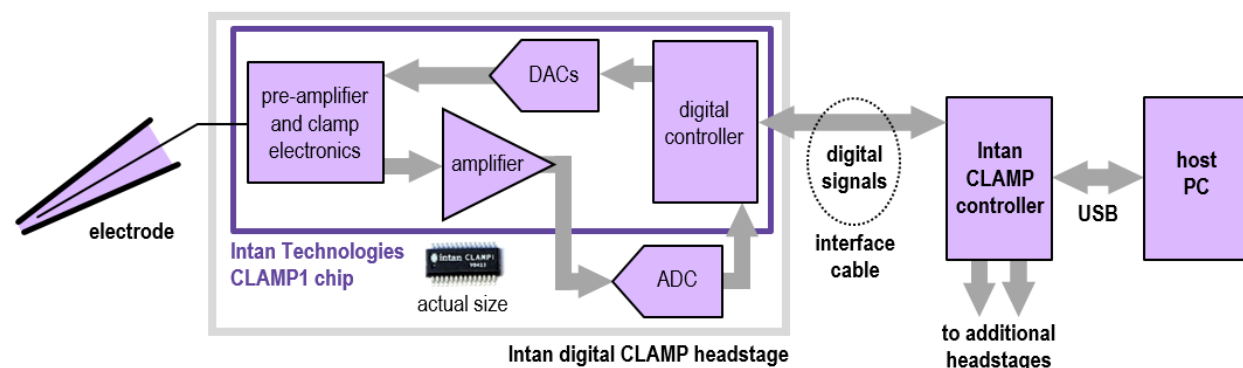
### Traditional Analog Patch Clamp Amplifier



The diagram above shows the major functional components of a traditional patch clamp amplifier system. A **traditional analog headstage** module contains sensitive analog electronics that act as a pre-amplifier with voltage clamp and current clamp capability. In voltage clamp mode, the headstage measures the electrode current and converts this to a proportional small voltage. In current clamp mode, the headstage buffers the electrode voltage but provides no additional amplification. Voltage and current clamp control is provided by analog voltages that convey the desired clamping levels.

The headstage is connected to a remote **computer-controlled amplifier** via a shielded **interface cable** that carries small analog voltages (typically in the millivolt range) and is thus susceptible to noise pickup. The amplifier module / digitizer consists of one or more rack-mounted boxes that contain additional analog **amplifiers**, analog-to-digital converters (**ADCs**), digital-to-analog converters (**DACs**), and a **digital controller** that coordinates control of these devices. The amplifier module and digitizer are interfaced to a **host PC** that sequences particular experiments and records the measured data.

### Intan Digital Patch Clamp Amplifier

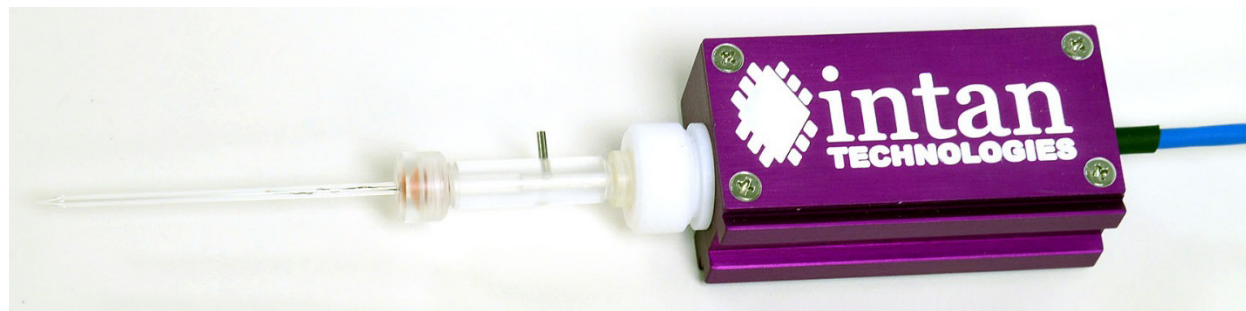


The CLAMP chips from Intan Technologies combine all analog circuitry and many digital control blocks on a single chip, permitting the construction of **Intan-powered digital headstages** (see diagram above). A small circuit board containing an Intan CLAMP chip and a small number of support components form a complete patch clamp amplifier with a purely digital interface. **The Intan digital “headstage” is actually a complete, miniaturized patch clamp amplifier.** Thanks to the integration of nearly all patch clamp circuit elements onto a single silicon chip (see “actual size” inset above), the Intan-powered patch clamp amplifier is smaller than many traditional analog headstages that perform only a small fraction of the total amplification task.

The digital interface cable uses a standard serial communication protocol (Serial Peripheral Interface, or SPI) and is no longer susceptible to noise pickup. Sensitive analog signals are digitized at the source, not several meters away. The purely digital interface makes it easy to electrically isolate each headstage and eliminate ground loops. The need for a large computer-controlled amplifier module is completely eliminated, replaced by an **Intan CLAMP controller** that can easily control multiple digital headstages simultaneously.

To preserve legacy “external command” and “signal monitor” functions associated with traditional patch clamp systems, the Intan CLAMP controller incorporates ADCs and DACs to allow for real-time control of voltage and current clamp via analog voltages (in addition to software clamp control), and real-time monitoring of measured and/or clamp voltages/currents via analog signals.

## Intan CLAMP Headstages



An Intan CLAMP headstage is shown above with a pipette holder and glass pipette attached (not included). Intan headstages mate with thread-mounted pipette holders that are compatible with Axon amplifiers. Pipette holders sold by Molecular Devices, Warner Instruments, and other companies may be used with Intan headstages. Glass pipettes must be custom made with a pipette puller shortly before recording.

Each headstage has standard 0.7" (17.8 mm) wide dovetail connectors on the side and the bottom. These mate with micromanipulators from companies such as Sutter Instruments to permit precision positioning and movement of the headstage.

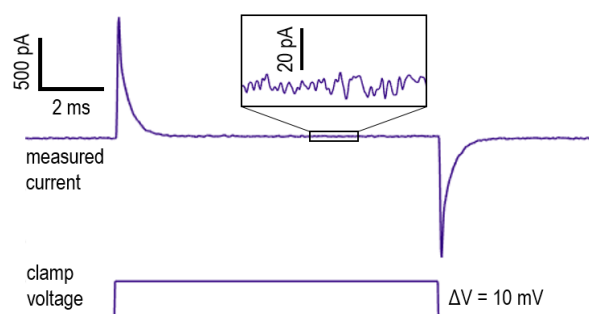
The rear side of the headstage has a receptacle for a 1-mm diameter grounding pin. This should be used to tie the headstage to a grounding point near the experiment. Intan headstages are electrically isolated from the controller and from earth ground; this can prevent noise pickup caused by unintentional ground loops. If a Faraday cage is used, it should be connected to the black I/O GND binding post on the rear panel of the controller. Some experimentation may be needed to find the optimal grounding scheme for a particular patch clamp rig.

The blue interface cable delivers  $\pm 3.3\text{V}$  power to the headstage and provides a digital serial interface between the headstage and the controller. These SPI (serial peripheral interface) cables use low-voltage differential signaling (LVDS) with twisted-pair wires for noise-free operation. Each Intan CLAMP headstage comes with a 6-foot (1.8-meter) interface cable. Additional SPI interface cables may be purchased from Intan and daisy-chained to span longer distances.

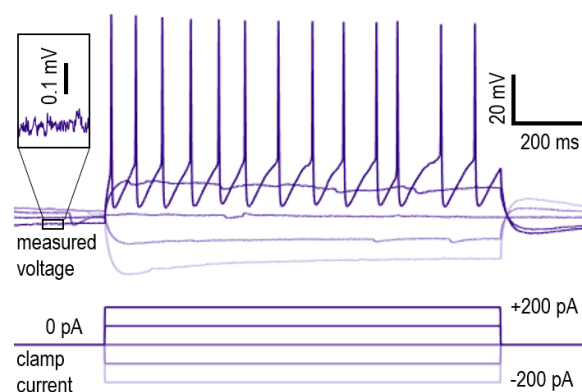
Each headstage contains a small circuit board with an Intan CLAMP1 chip that performs all voltage and current clamping and voltage and current sensing (see previous page). The detailed operation of this microchip is described in the **Intan CLAMP Voltage/Current Amplifier Chip Datasheet**, available from the Downloads page of the Intan website.

The figures below show actual data obtained using an Intan CLAMP system.

The **voltage clamp** operates over a wide range with steps as small as 2.5 mV. A low noise floor (below  $3\text{ pA}_{\text{rms}}$  in the most sensitive range) allows tiny currents to be resolved. The plot below shows a cell membrane test:



The **current clamp** produces currents up to  $\pm 127\text{ nA}$  and steps as small as 5 pA. Intracellular voltages may be observed across a wide  $\pm 300\text{ mV}$  range with a noise floor below  $20\text{ }\mu\text{V}_{\text{rms}}$ . The plot below shows the intracellular potential of a neuron during current injection:



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## Controller Hardware

### Front Panel



The front panel of the Intan CLAMP Controller provides connection ports for Intan CLAMP headstages as well as auxiliary digital and analog inputs. From left to right:

- **Intan CLAMP headstage ports:** These ports, labeled A and B, provide connection points for up to two headstages via 12-wire digital SPI (serial peripheral interface) cables. Each headstage port is electrically isolated from the controller and from earth ground. Indicator lights provide information on the status of each port: green and yellow LEDs show that proper voltage supplies are being provided for each headstage. Red LEDs are activated when the software recognizes a headstage plugged into a port.
- **Digital inputs:** Two BNC sockets are provided for recording digital signals in synchrony with the headstage signals. The digital inputs accept TTL-level signals. Any voltage between 0V and +0.8V is read as a digital “low”. Any voltage between +2.0V and +5.5V is read as a digital “high”. Voltages delivered to these sockets should not exceed the range of 0V to +5.5V. These signals may be used to record discrete events associated with an experiment.
- **Analog inputs:** Two BNC sockets are provided for recording general-purpose analog signals, or for controlling the headstages using externally provided analog waveforms. Signals are digitized with 16-bit ADCs over a range of -10.24V to +10.24V. Voltages delivered to these sockets should not exceed this range.
- **Status indicators:** Status indicator A is illuminated when the CLAMP Controller software has successfully connected to the unit. Status indicator B is controlled by DIGITAL IN 1; status indicator C is controlled by DIGITAL IN 2. These LEDs can be used to monitor the status of digital signals that are recorded in synchrony with the patch clamp amplifiers.
- **Power indicator:** This red LED is illuminated when the Intan CLAMP Controller is powered.

### Rear Panel



The rear panel of the Intan CLAMP Controller provides auxiliary output lines as well as other ports and switches. From left to right:

- **Analog outputs:** Two BNC sockets are provided for monitoring measured signals or clamp signals from CLAMP headstages. The headstages communicate with the controller using purely digital signals, but 16-bit DACs are used to reconstruct analog signals with desired scaling factors. The control software allows users to route selected signals to any analog output ports. These ports have a -10.24V to +10.24V voltage range.
- **Audio line out jack:** This standard 3.5-mm stereo phone jack allows users to connect an audio amplifier to the controller and listen to the signals routed to the two analog output ports. ANALOG OUT 1 is connected to the left channel; ANALOG OUT 2 is connected to the right channel. This port cannot drive speakers directly; an audio amplifier should be used, and the volume should be adjusted carefully to ensure that excessive levels are not delivered to speakers.
- **High-speed port:** This connector is reserved for future use.
- **I/O expansion port:** This connector is used to add an Intan I/O Expander. This board is described in the next section. It provides six additional analog inputs and outputs and 14 additional digital inputs and outputs for more complex experiments. Signals on this port are digital and serially encoded, and are not easily accessed without the I/O Expander.



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- **CONFIG switches:** Configuration switches 1-3 are reserved for future use. Switch 4 (CONFIG4) is used to select the voltage level of the digital output ports (see next item). With CONFIG4 in the down position, 3.3V digital signals are generated. With CONFIG4 in the up position, 5.0V digital signals are generated.
- **Digital outputs:** Two BNC sockets produce either 3.3V or 5.0V digital signals (see previous item) that can be synchronized with software-generated voltage clamp or current clamp waveforms.
- **USB port:** A USB 2.0 port provides two connection to a host computer running the control software.
- **Sample clock out:** This port generates a digital square wave proportional to the headstage sampling rate when the headstages are active. While the software acquires data with a sampling rate of 50 kS/s, the headstages are actually sampled at 200 kS/s to reduce noise, so this port produces a 200 kHz square wave during operation. The voltage level of this signal is set by the CONFIG4 switch.
- **Mark out:** This port generates digital pulses marking the onset and offset of voltage clamp or current clamp waveforms. The voltage level of this signal is set by the CONFIG4 switch.
- **I/O GND:** This binding post is connected to the controller system ground used by all analog and digital inputs and outputs. This is the preferred ground to use for Faraday cage and other shielding connections.
- **Chassis GND:** This binding post is connected to the controller chassis and to the grounding conductor of the AC power socket.
- **Power switch and fuse holder:** The unit uses two standard 1A 250V 5x20mm slow blow fuses that can be replaced by opening the fuse holder to the right of the power switch. The power cord must be removed to access the fuses.
- **AC power socket:** The controller is powered by 90-260V AC power, and is compatible with international voltage levels. A US-style power cord is supplied with the controller. International customers must use an adapter to accommodate non-US power sockets. The center grounding conductor must be connected to earth ground to avoid electric shock hazards.

## Mounting

The Intan CLAMP controller can be rack mounted on a standard 19" instrument rack using provided hardware, or it can be used on a bench top by folding out the feet on the bottom of the case:



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## Intan I/O Expander

Intan Technologies offers an optional I/O Expander (sold separately) that provides an additional six analog inputs and outputs and an additional 14 digital inputs and outputs. This unit is shown below:

### Front Panel



The front panel of the Intan I/O Expander provides auxiliary digital and analog inputs, and analog outputs. From left to right:

- **Analog outputs:** Two analog outputs for monitoring measured signals or clamp signals from CLAMP headstages. (Four more analog outputs are provided on the rear panel.) These ports have a -10.24V to +10.24V voltage range.
- **Digital inputs:** Six BNC sockets are provided for recording digital signals in synchrony with the headstage signals. (Eight more digital inputs are provided on the rear panel.)
- **Analog inputs:** Six BNC sockets are provided for recording analog signals, or for controlling the headstages using externally provided analog waveforms. Signals are digitized with 16-bit ADCs over a range of -10.24V to +10.24V.
- **Power indicator:** This red LED is illuminated when the Intan I/O Expander is powered. The I/O Expander receives low-voltage DC power over an interface cable from the controller.

### Rear Panel



The rear panel of the Intan I/O Expander provides auxiliary input and outputs lines. From left to right:

- **Interface port:** This connector is used to interface with the main controller unit.
- **Analog outputs:** Four analog outputs for monitoring measured signals or clamp signals from CLAMP headstages. (Two more analog outputs are provided on the front panel.) These ports have a -10.24V to +10.24V voltage range.
- **Digital outputs:** Six BNC sockets produce either 3.3V or 5.0V digital signals that can be synchronized with software-generated voltage clamp or current clamp waveforms. The CONFIG4 switch on the main Intan controller selects the voltage level used by these ports.
- **Digital inputs 9-16:** Eight additional digital inputs are provided on screw terminal blocks. System ground connections are also provided on the ends of the terminal block.
- **Digital outputs 9-16:** Eight additional digital outputs are provided on screw terminal blocks. System ground connections are also provided on the ends of the terminal block. The CONFIG4 switch on the main Intan controller selects the voltage level used by these ports.

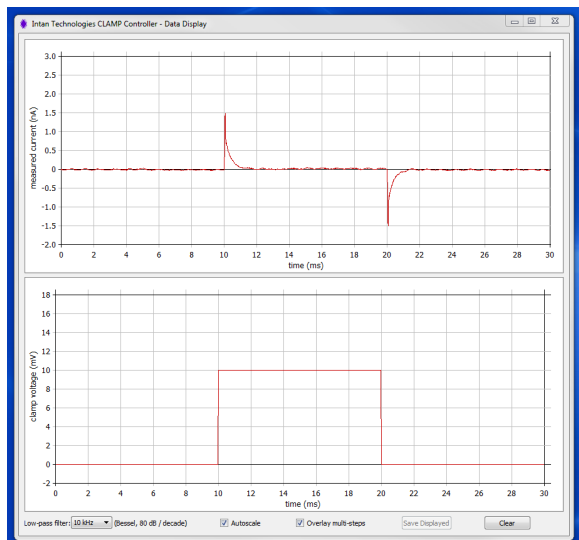
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## Software Installation

Before attaching the Intan CLAMP Controller to a computer, first install the drivers provided on the Intan website. (If you have already installed drivers for other Intan USB-linked products, you do not need to reinstall these drivers.) After installing the drivers, connect the CLAMP Controller to the computer using the supplied USB cable, plug in the Controller, and turn on the power switch on the rear panel.

Windows users may download the compiled CLAMP UI (User Interface) software from the Intan website. The executable, DLL files, and `main.bit` file should be kept in the same directory. To run the software, double click the executable.

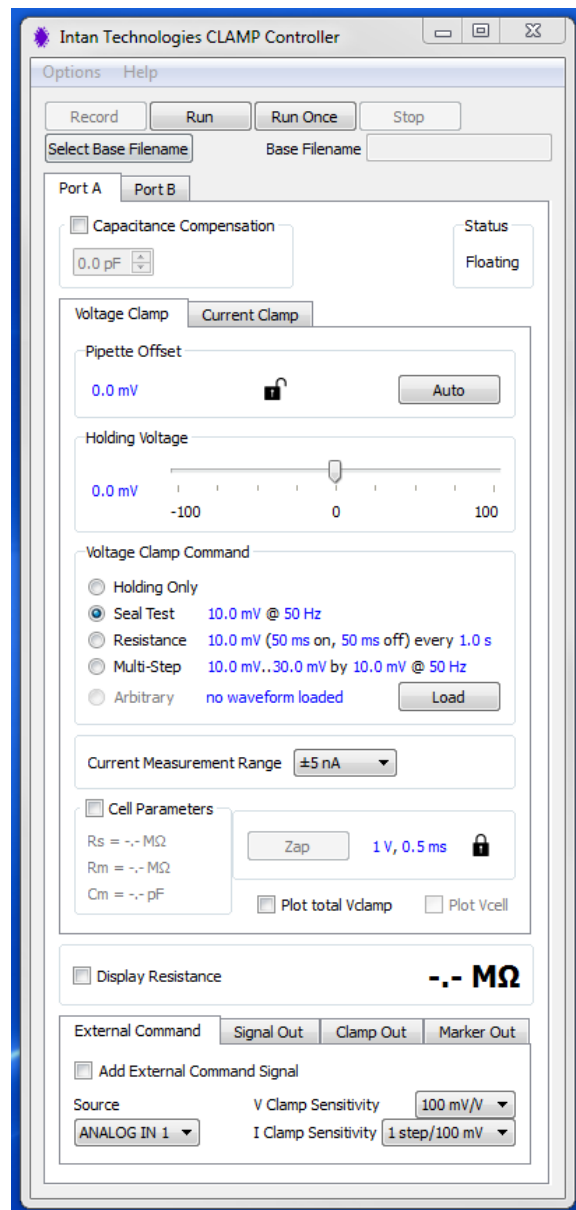
Plug in at least one CLAMP headstage to one of the ports on the front panel and start the software. The software takes a few seconds to calibrate the attached headstages and then opens two windows: a Control Window (see right) and a Data Display Window (see below).



## Control Window

**Run and Run Once:** These buttons start data acquisition and display the measured and clamp data in real time without saving data to disk. The **Stop** button halts data acquisition. All data are acquired at 50 kSamples/s.

**Select Base Filename:** This button opens a dialog that prompts the user to select a path and filename for saved data files. A time and date stamp will be added to all saved data files. Once a valid base filename has been selected, the **Record** button is enabled. Clicking this button starts data acquisition and saves the data to a file. Auxiliary data from all ANALOG IN, DIGITAL IN, and DIGITAL OUT ports are also saved unless the **Save Auxiliary I/O** item in the Options menu is unchecked.



**Port Tabs:** Clicking on these tabs (i.e., **Port A**, **Port B**) selects headstages attached to the ports on the front panel of the Intan CLAMP Controller. Each headstage may be configured independently with its own holding levels, clamp mode, capacitance compensation settings, etc. A software-generated voltage clamp or current clamp waveform may only be run on one headstage at a time; all other headstages run in holding mode, but measurements are made on all headstages simultaneously. Any external commands coming from ANALOG IN channels will be applied to all enabled headstages (see **External Command Tab** below).

**Capacitance Compensation:** This control enables and adjusts the fast transient capacitance compensation circuit in each headstage which is used to cancel pipette

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capacitance. Capacitance compensation is typically performed when the electrode is in the bath but prior to cell contact. It may be refined after a gigaseal has been obtained. See the Capacitance Compensation section below for more details.

**Status:** This indicator shows the current clamp status of the selected headstage.

## Voltage Clamp Controls

Clicking on the **Voltage Clamp** tab displays the following voltage clamp controls:

**Pipette Offset:** When a pipette electrode is placed in an electrolytic solution, a small DC potential is generated by the interface of dissimilar materials. This control allows the user to adjust the pipette offset to null out this built-in potential. Pipette offset should be nulled after the electrode is placed in solution but prior to contact with a cell. If the **Auto** button is clicked, the pipette offset will be adjusted to minimize measured current. The lock can be clicked to prevent this value from being changed accidentally.

The pipette offset may be adjusted manually by clicking on the blue voltage indicator and turning the mouse scroll wheel, moving the cursor keys up or down, or typing a number.

**Holding Voltage:** This control sets a baseline clamp voltage that is superimposed on all other voltage clamp commands. After breaking into a cell, this is typically set close to -70 mV to match the intracellular potential and prevent large currents from flowing into or out of the cell.

**Voltage Clamp Command:** These buttons allow the user to select between different types of voltage clamp waveforms. **Holding Only** can be used to monitor spontaneous activity in a cell. **Seal Test** produces a repeating voltage step, and is typically used to monitor the pipette resistance as a gigaseal is being formed or to estimate cell parameters (see below). **Resistance** is similar to **Seal Test**, but adds a running plot of resistance vs. time so that resistance trends may be monitored visually. **Multi-Step** generates multiple voltage clamp steps and superimposes the plots. **Arbitrary** generates a waveform specified by a user file (see Arbitrary Waveform Specification below for details).

**Current Measurement Range:** This control allows the user to select the range of the current that is sensed by the headstage:  $\pm 5$  nA,  $\pm 10$  nA,  $\pm 100$  nA, or  $\pm 1000$  nA. Using the smallest acceptable range minimizes the noise floor.

**Cell Parameters:** If this box is checked, the passive cell parameters  $R_s$  (series resistance),  $R_m$  (membrane resistance), and  $C_m$  (membrane capacitance) will be estimated by fitting an exponential curve to the measured voltage clamp data (see Estimating Membrane Parameters

below for details). Unchecking this box will “freeze” these values.

**Zap:** This button must be enabled by clicking the lock icon. Clicking the Zap button produces a brief, high voltage pulse (typically 1 V) that can be used to break into a cell once a gigaseal has been established (although most researchers use pressure pulses to break the membrane). The parameters of the zap pulse can be adjusted by clicking on the blue numerical display.

**Plot total Vclamp:** Checking this box adds a second plot on the Clamp Voltage graph in the lower half of the Data Display Window. The primary plot shows the voltage clamp waveform generated by the **Voltage Clamp Command** controls above. The second plot shows the total voltage clamp waveform applied by the headstage, which is a linear superposition of the software-defined voltage clamp command, the pipette offset, and any external command delivered to an ANALOG IN port, if this option is enabled (see **External Command Tab** below).

**Plot Vcell:** Checking this box adds a plot to the Clamp Voltage graph in the lower half of the Data Display Window that shows the cell voltage  $V_{cell}$ , which is defined as the clamp voltage minus the voltage drop across the series resistance. This voltage drop is the series resistance ( $R_s$ ) multiplied by the instantaneous measured electrode current. The Intan CLAMP System does not allow for series resistance compensation in voltage clamp mode, but observing  $V_{cell}$  can allow users to manually compensate for some aspects of the voltage drop across the series resistance and perhaps adjust the voltage clamp waveforms to yield the desired voltage at the cell.

## Current Clamp Controls

Clicking on the **Current Clamp** tab displays the following current clamp controls:

**Pipette Offset:** See “Voltage Clamp Controls” for a description of this control.

**Holding Current:** This control sets a baseline clamp current that is superimposed on all other current clamp commands. This can be used to bias the cell to a desired intracellular potential or suppress spontaneous activity. The **Zero Current** button may be clicked to set the holding current to zero quickly.

**Current Clamp Command:** These buttons allow the user to select between different types of current clamp waveforms. **Holding Only** can be used to monitor spontaneous activity in a cell. **Tuning** produces a repeating current step, and can be used to tune the bridge balance (see below). **Multi-Step** generates multiple current clamp steps and superimposes the plots. **Arbitrary** generates a waveform specified by a user file (see Arbitrary Waveform Specification below for details).



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**Current Clamp Range:** This control allows the user to select the range and resolution (i.e., step size) of the current clamp. The current-output DAC that generates the headstage clamp current has a range of  $\pm 127$  steps. The step size can be 5 pA, 50 pA, 0.5 nA, or 1 nA.

**Bridge Balance:** Series resistance compensation may be performed in current clamp mode using this control. Checking this box enables bridge balance, where the voltage drop across the series resistance of the pipette is subtracted from the voltage measurements. The default value of series resistance is the last measurement of  $R_s$  made in voltage clamp mode (see **Cell Parameters** above), but this value can be adjusted here to minimize step artifacts observed during **Tuning** current clamp pulses (see the Bridge Balance section below for more details).

**Buzz:** This button must be enabled by clicking the lock icon. Clicking this button modulates the capacitance compensation circuitry to produce small AC current pulses that may assist in forming a membrane seal around sharp intracellular electrodes. The parameters of the buzz pulse can be adjusted by clicking on the blue parameter display.

**Plot total Iclamp:** Checking this box adds a second plot on the Clamp Current graph in the Data Display Window. The primary plot shows the current clamp waveform generated by the **Current Clamp Command** controls above. The second plot shows the total current clamp waveform applied by the headstage, which is a linear superposition of the software-defined current clamp command and any external command delivered to an ANALOG IN port, if this option is enabled (see below).

## Other Controls

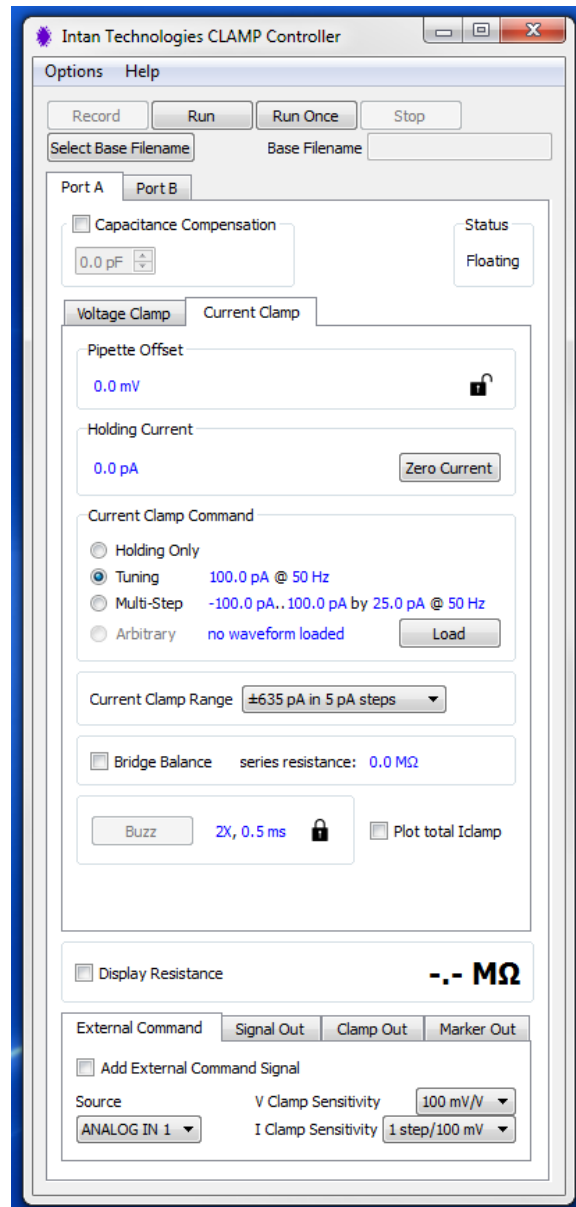
**Display Resistance:** Checking this box enables real-time display of the measured pipette resistance. This can be used when establishing a gigaseal. Average “best fit” lines used to calculate resistance are superimposed on the measured waveform in the Data Display Window when this feature is enabled.

## Analog/Digital I/O Tabs

**External Command Tab:** Controls in this tab allow the user to control the voltage clamp or current clamp in real time via external command signals from any of the ANALOG IN ports. Voltage clamp and current clamp scaling factors may be selected for each headstage. The external command is superimposed on any software-defined clamp command, and only takes effect when the system is running or recording. To control headstages using only the external commands, select **Holding Only** in the software clamp commands and run the system.

Multiple headstages may be controlled simultaneously using external command signals. Voltage waveforms may be

generated using legacy hardware (e.g., NIDAQ boards) and connected to ANALOG IN ports to generate clamp control.



**Signal Out Tab:** Controls in this tab allow the user to route the measured voltage or current signal to any of the ANALOG OUT ports for monitoring on an oscilloscope or speaker. (The ANALOG OUT 1 and 2 signals are connected to the left and right channels of the AUDIO LINE OUT jack on the rear panel.) Voltage and current scaling factors can be selected here as well. DACs in the Intan CLAMP Controller reconstruct an analog waveform from the digital headstage data in real time. **Note:** Any low-pass Bessel filtering or bridge balancing is not applied to these waveforms as these operations are performed in software.

**Clamp Out Tab:** Controls in this tab allow the user to produce an analog waveform corresponding to the clamp

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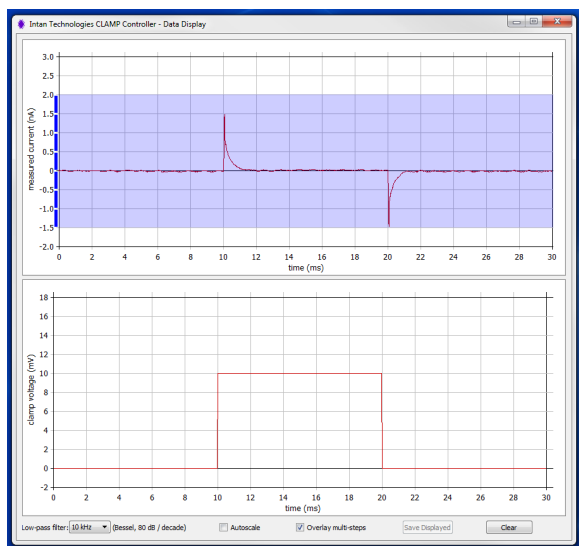
command signal on any of the ANALOG OUT ports. Voltage and current scale factors can be selected here for voltage clamp or current clamp commands. DACs in the Intan CLAMP Controller reconstruct an analog waveform from the digital clamp commands sent to the headstage.

**Marker Out Tab:** When a voltage clamp or current clamp waveform is active, a digital signal on the MARK OUT port on the rear panel of the Intan CLAMP Controllers goes high when the clamp waveforms depart from the holding level. This signal may also be routed to any of the DIGITAL OUT ports using controls in this tab. **Note:** If a signal is routed to a DIGITAL OUT port, then it will be saved in the auxiliary I/O file.

## Data Display Window

**Low-Pass Filter:** This control selects the cutoff frequency for a 4<sup>th</sup>-order Bessel low-pass filter applied to measured voltages and currents.

**Autoscale:** This box may be unchecked to enable manual control of the plotting axes. Axes may be adjusted by clicking and dragging along the axis (see figure below), using the cursor keys, the page up/down keys, or other keys described in the Keyboard Shortcuts dialog accessible through the Help menu in the Control Window.



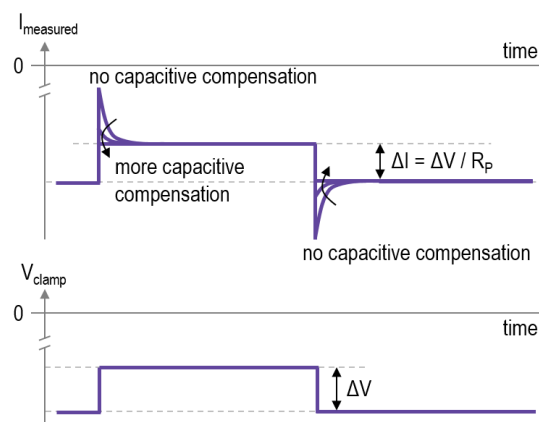
**Overlay multi-steps:** When multi-step clamp commands are selected, the results from each voltage clamp or current clamp step are superimposed on top of one another unless this box is unchecked.

**Save Displayed:** This button is enabled by selecting a base filename in the Control Window. When the button is clicked, the data visible in the Data Display Window are saved to disk.

**Clear:** The button clears the plots in this window.

## Capacitance Compensation

Each CLAMP headstage supports pipette capacitance cancellation in the range of 0-20 pF. In practice fast transient capacitance compensation is usually tuned when the pipette is placed into bath, before contact with a cell is made. As shown in the plot below, a voltage clamp step is applied to the electrode and the capacitance compensation is adjusted to eliminate the transient peaks in the measured current. The pipette resistance is easily measured during this procedure as well by checking the **Display Resistance** box. Note that excessive capacitance compensation can cause increased noise levels and instability.

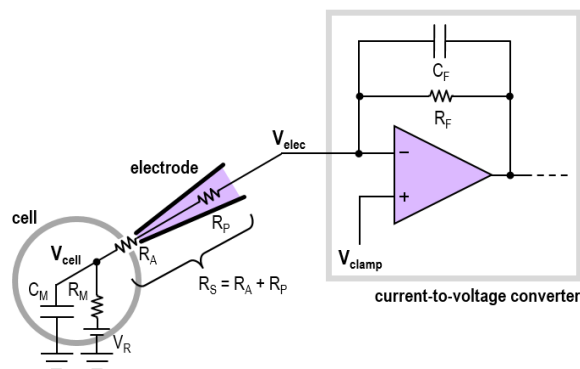


## Estimating Membrane Parameters

In whole-cell patch clamp electrophysiology, the electrode is electrically connected to the inside of a cell as shown in the diagram below. The tiny opening in the cell membrane creates a large access resistance  $R_A$  which adds to the pipette resistance  $R_P$ . The resulting series resistance  $R_S$  can have a value in the tens of megohms. To first order the cell's membrane behaves as a capacitance  $C_M$  (typically in the tens of picofarads) with some parallel resistance  $R_M$  (typically in the hundreds of megohms) in series with a reversal potential  $V_R$  (usually in the range of -30 mV to -90 mV). These membrane parameters, as well as the series resistance  $R_S$ , can be estimated using a simple voltage clamp measurement and checking the **Cell Parameters** box.

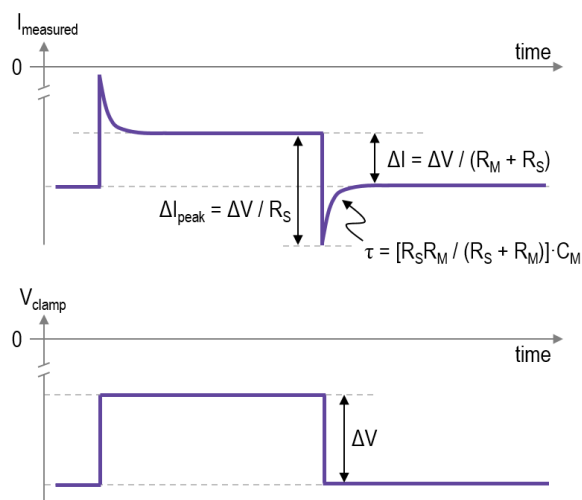
Note that the electrode parasitic capacitance  $C_P$  is not shown in the diagram below because it is assumed that this element has already been cancelled by the fast transient capacitance compensation circuitry prior to cell contact.

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After breaking into a cell, the voltage clamp is typically set to a value near the cell's expected resting potential (e.g., -70 mV) to prevent the activation of voltage-gated ion channels. A small voltage step is applied around this potential (e.g., a step from -70 mV to -60 mV) and the resulting current is measured. The plot below shows a typical clamp voltage profile and measured current waveform. The current takes the form of a decaying exponential with a time constant  $\tau$ :  $i(t) = I_0 \exp(-t/\tau) + I_1$ . (These exponential current peaks are much wider and slower than the "fast transient" peaks caused by uncompensated pipette capacitance)

Enabling **Cell Parameters** causes an exponential curve to be fit to the data and the membrane parameters  $R_M$  and  $C_M$  can be estimated, along with the series resistance  $R_s$ .



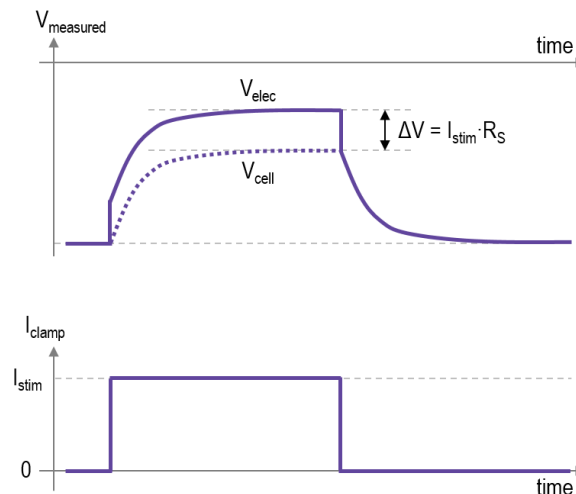
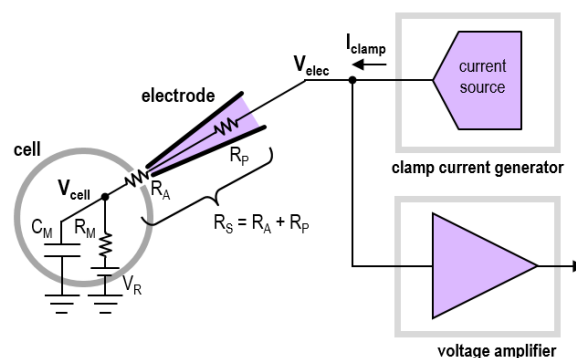
Traditional patch clamp instruments sometimes use positive feedback circuits to reduce the effects of the series resistance  $R_s$  in voltage clamp mode. (The measured current flowing through the electrode creates a voltage drop across  $R_s$ , causing the voltage in the cell to be slightly different from the clamp voltage.)

The Intan CLAMP system does not support series resistance compensation in voltage clamp mode, but the **Plot VCell** control (see Voltage Clamp Controls above) can be used to estimate the true cell potential by subtracting the voltage drop across the series resistance in real time. The

user may then adjust the voltage clamp parameters to produce the desired voltage in the cell.

## Bridge Balance

When applying current clamp pulses through real electrodes connected to cells, the measured membrane potential is distorted by an artifact caused by the voltage drop across the electrode. Referring to the diagram below, we would like to apply a current signal  $I_{\text{clamp}}$  and measure an electrode voltage  $V_{\text{elec}}$  equal to the intracellular potential  $V_{\text{cell}}$ . However, the clamp current flows through a series resistance  $R_s$  comprised of the pipette resistance  $R_p$  and the access resistance  $R_a$ . This leads to a voltage offset  $\Delta V$  that is equal to  $I_{\text{clamp}} \cdot R_s$ .



The plot above shows an example of this phenomenon for a simple clamp current that starts at zero and pulses to  $I_{\text{stim}}$ . Luckily, the intracellular potential  $V_{\text{cell}}$  can be recovered from the measured electrode voltage  $V_{\text{elec}}$  by subtracting the clamp current (at every instant in time) multiplied by  $R_s$ , which was previously measured using the **Cell Parameters** tool. In the old days of purely analog instrumentation, this subtraction was performed using an analog circuit in a technique called **bridge balance** or **series resistance compensation**. With modern computing power it is much easier to perform this subtraction after the electrode voltage

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has been digitized. The **Bridge Balance** tool in the Current Clamp tab performs this function. In practice, the exact value of series resistance should be adjusted while applying small current pulses to eliminate the voltage jump shown in the figure above.

Note that the clamp current is exactly the same with or without bridge balance enabled; the stimulation experienced by the cell is not affected, only the interpretation of the measured data.

## Arbitrary Waveform Specification

Users may define arbitrary voltage clamp or current clamp waveforms by creating CSV (comma separated values) text files listing all clamp levels and durations comprising the waveform.

Arbitrary waveform files must end in the .arb prefix, and the first line of the file must read `ARBV` for a voltage clamp waveform or `ARBI` for a current clamp waveform. Starting with the second line of the text file, each line specifies a voltage or current clamp level and a time duration that this clamp level should be held. Clamp levels are specified in integer steps. (Smooth changes in clamp levels must be approximated by “stair steps”).

The standard voltage clamp step size is 2.5 mV, but if **2x Voltage Clamp Mode** is enabled in the Options menu, the step size is 5.0 mV (see next section for details). The voltage clamp level can range between -255 and +255 steps. The current clamp step size is set by the Current Clamp Range control in the Current Clamp tab. The step size can be 5 pA, 50 pA, 0.5 nA, or 1 nA. The current clamp level can range between -127 and +127 steps.

The duration each clamp level is held is specified by a positive integer in the range of 1 to 65535. The actual duration is calculated by multiplying this number by 20 microseconds (i.e., 1 / 50 kHz), so a value of 25000 signifies 500 milliseconds, for example. The maximum value of 65535 sets a duration of 1.31 seconds; if longer holding times are needed then repeated commands must be used.

The clamp level and duration are specified by two numbers separated with a comma. For example, to define a voltage clamp waveform that holds at zero for 20 ms, then steps to +20 mV for 40 ms, and then steps to -30 mV for 10 ms, the .arb file would be:

```
ARBV
0,1000
8,2000
-12,500
```

The total length of an arbitrary waveform specification is limited to approximately 16000 lines.

By default, the MARK OUT digital signal for an arbitrary waveform will go high for any nonzero clamp level. More sophisticated digital signals can be specified by appending two additional binary numbers (i.e., 0 or 1) to each line. The first number signals when the MARK OUT signal should go high. The second number signals when the optional DIGITAL OUT signal (controlled by the Marker Out tab) should go high.

Adding to the previous example, if we wanted MARK OUT to go high during the +20 mV clamp level and the selected DIGITAL OUT signal to go high during the -30 mV clamp level, we would use the following file:

```
ARBV
0,1000,0,0
8,2000,1,0
-12,500,0,1
```

## 2x Voltage Clamp Mode for FSCV

The Intan CLAMP chip in each headstage uses an on-chip DAC to generate a clamp voltage. The voltage level of this DAC is set by an 8-bit magnitude variable and 1-bit sign variable. In the normal mode of operation, this DAC has 2.5 mV steps, giving a total voltage clamp range of  $\pm 637.5$  mV, which is more than enough range for cellular patch clamp experiments. By selecting the **2x Voltage Clamp Mode** from the Options menu, the DAC step size is doubled to 5.0 mV, producing a range of  $\pm 1.275$  V.

This wide voltage clamp range permits the Intan CLAMP Controller to be used in fast-scan cyclic voltammetry (FSCV) or other electrochemistry applications. In FSCV experiments, voltages typically in the range of -0.4 V to +1.3 V are swept rapidly across graphite electrodes while the current is measured. (The Arbitrary Waveform function can be used generate FSCV voltage clamp signals.) Background currents on the order of 1  $\mu$ A are subtracted from baseline readings, and the resulting residual current variations – typically less than 10 nA in magnitude – indicate the presence of various neurotransmitters or other chemicals.

When the **2x Voltage Clamp Mode** is enabled, all clamp voltages generated by the headstages are 2x larger than they appear in the software. The controls and display axes do **not** reflect this 2x increase. However, the MATLAB code used to read saved data files (see next section) does recognize data saved with the 2x mode enabled and automatically adjusts voltage clamp waveforms to reflect the true voltages.



## Importing Recorded Data into MATLAB

Intan Technologies provides an open-source m-file (**read\_Intan\_CLP\_file.m**) for importing data recorded from the CLAMP Controller software into MATLAB. Make sure you have the latest version of this m-file to ensure compatibility with the newest version of the control software. Running this m-file brings up a file selector dialog with which the user locates and selects the desired .clp data file. The m-file then loads and parses the data file and returns a data structure containing the clamp and measured waveforms, time vectors, and settings.

The CLAMP Controller software creates a new subdirectory each time data is saved. The directory has a name consisting of the base filename specified by the user followed by a date stamp and a time stamp: `basefilename_YYMMDD_hhmmss`. Within this subdirectory, a separate file is saved for each headstage, with a letter designating the headstage port. An additional file designated AUX contains waveforms from the ANALOG IN, DIGITAL IN, and DIGITAL OUT ports sampled in synchrony with the headstages. (If this file is not needed it can be disabled by unchecking the **Save Auxiliary I/O** item in the Options menu.) A typical set of saved data files might have the following names:

```
myexperiment_A_160916_142731.clp
myexperiment_B_160916_142731.clp
myexperiment_AUX_160916_142731.clp
```

Following is a transcript of a typical MATLAB session loading a recorded data file and looking at the data structure:

```
>> clear
>> d = read_Intan_CLP_file;

Reading Intan Technologies CLAMP Data File, Version 1.0

File contains 0.360 seconds of data sampled at 50.00 kS/s.

>> d

d =

    Header: [1x1 struct]
    Data: [1x1 struct]

>> d.Data

ans =

    Measured: [1x18000 single]
    Time: [1x18000 double]
    Clamp: [1x18000 single]
    TotalClamp: [1x18000 single]

>> subplot(2,1,1); plot(d.Data.Time, d.Data.Measured);
>> subplot(2,1,2); plot(d.Data.Time, d.Data.Clamp);
```

The time vector is contained in the variable `Data.Time`, with units of seconds. Corresponding waveform data are stored in the vectors `Data.Measured`, `Data.Clamp`, and `Data.TotalClamp`. The `Clamp` waveform contains the software clamp signal (in volts or amps) while `TotalClamp` includes the pipette offset and any external commands delivered by ANALOG IN channels.

The `Header` structure contains information on sampling rates, the Bessel filter cutoff frequency, recently measured cell parameters, and other settings.

If an auxiliary I/O file is loaded, the `Data` structure will be different:

```
>> d.Data

ans =

    Time: [1x18000 double]
    DigitalIn: [1x18000 uint16]
    DigitalOut: [1x18000 uint16]
    ADC: [8x18000 double]

>> plot(d.Data.Time, d.Data.ADC(2,:))
```

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The `DigitalIn` and `DigitalOut` vectors are 16-bit unsigned integers encoding all DIGITAL IN and DIGITAL OUT values. (The Intan CLAMP Controller has two DIGITAL IN lines and two DIGITAL OUT lines; the Intan I/O Expander can be added to gain access to all 16 signals.) For example, if DIGITAL IN 1 and DIGITAL IN 7 are both high and all other digital inputs are low, then the value of `DigitalIn` at that point in time will be  $2^0 + 2^6 = 1 + 64 = 65$ .

The time vectors in the auxiliary I/O files and individual headstage data files are identical.

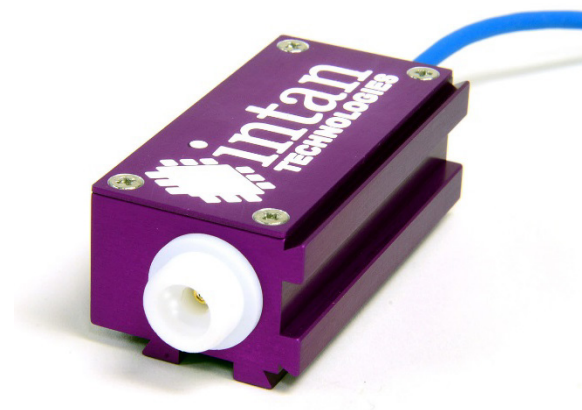
# Intan CLAMP System

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## Related CLAMP Documentation

The following supporting datasheets may be found at <http://www.intantech.com/downloads>:

- ◆ Intan CLAMP Voltage/Current Amplifier Chip Datasheet



## Pricing Information

See [www.intantech.com](http://www.intantech.com) for current pricing. All price information is subject to change without notice. Quantities may be limited. All orders are subject to current pricing at time of acceptance by Intan Technologies. Additional charges may apply for international purchases and shipping.

## Contact Information

This datasheet is meant to acquaint scientists and engineers with the general characteristics of the Intan CLAMP system developed at Intan Technologies. We value feedback from potential end users.

For more information, contact Intan Technologies at:



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