QUALITY INSPECTION SUMMARY

We have made every effort to manufacture this instrument to the highest quality standards. All assemblies have been thoroughly tested and inspected at the factory as follows:

In 1r 2i F	itial Assembly Inspection itial QC Inspection/Calibration 4 Hour Burn-In inal Performance Inspection		
Packaging Inspection	Initials:	Date:	

Items included with any catalog number may be labeled and packaged separately in shipping carton.

Description	Quantity	Checked
55-10-0 APM Acquisition/Processor Module		
Containing:		
Acquisition/Processor Module		
Accessory Kit		
55-21-0 Powered Channel Frame		
Containing:		
Powered Channel Frame		
A991 Manual		
H2-11 Software Disk		
E5-66-03 USB Cable		Π
55-20-0 APC Acquisition/Processor Cl	hannel	
Containing:		
Acquisition/Processor Channel		Π
Patch Cable		

0 DECLARATIONS

0.1 DECLARATION OF CONFORMITY

We, FHC, Inc., 1201 Main Street, Bowdoin, Maine 04287, telephone number 207-666-8190, fax number 207-666-8292, declare under sole responsibility that the product:

(check all that apply)

55-10-0 APM	Serial #	
55-21-0 PCF Powered Channel Frame	Serial #	

to which this declaration relates, is in conformity with the following standards:

EN292, Parts 1 & 2

Following the provision of the machinery (89/392/EEC) Directive:

EN60127-1, EN60320-1, EN60204-1

Following provisions of the Low Voltage (73/23/EEC) Directive.

The Technical Construction File is maintained at:

1201 Main Street Bowdoin, ME 04287

DATE OF ISSUE: _____

PLACE OF ISSUE: Bowdoin, Maine

SIGNED: ______ Frederick Haer

"Innovation through collaboration"



Providing Instrumentation and Apparatus for Cellular Research, Intraoperative Recording, and Microneurography; Micro-electrodes, Micropipettes, and Needles to the Neuroscience Community for 30 years.

APM Acquisition/Processor Module

55-10-0 APM Acquistion/Processor Module

micro**Targeting**™

neuro/*craft*™

FHC Headquarters

1201 Main Street, Bowdoin, ME, 04287 USA Fax: 207-666-8292 E-mail: fhcinc@fh-co.com www.fh-co.com

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L005-18B

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0.1 CONDITIONS FOR USE

Intended Use

The APM is intended for use in acquiring, conditioning, and processing signals from single electrodes, pipettes or microelectrodes during intracellular or extracellular recording.

Warnings

The APM unit should not be disassembled beyond its major assemblies. Any disassembly beyond this may affect function and calibration. If repair is required please contact FHC at (207) 666-8190.

Storage Precautions

Store at normal room temperatures between -34°C (-29°F) and 57°C (135°F). Do not expose to temperatures below -39°C (-29°F) or greater than 70°C (158°F), or a relative humidity of less than 10% or more than 100%, including condensation, or an atmospheric pressure less than 500hPa or greater than 1060hPa for long-term storage.

Sterilization

The APM unit is not designed for sterilization. Any attempt to sterilize it may result in malfunction or component failure.

Handling

While a high degree of durability has been designed into the APM unit, care should be taken not to drop the components. Do not force any of the connections. Place all cables and leads where they will not be inadvertently pulled or tangled.

0.2 SYMBOLS USED



USB: This symbol is used to designate a standard USB connection.

1 OPERATIONAL MANUAL

1.1 FEATURES

- Two pre-programmed modes of neural spike discrimination;
 - Slope/Height Window Discrimination detects and records any waveform that passes through a user-defined time/amplitude window.
 - Adaptive (follows changes in waveform shape, such as caused by electrode drift) Template Matching detects and records up to four different spikes based upon their shape.
- Allows user to implement their own detection/processing algorithms. Growing downloadable library of user-contributed algorithms in standard C programming language located at www.neurocraft.com.
- 64 or 128 tap bandpass finite impulse response digital filter with continuously adjustable low-pass and hi-pass filters. Frequency range of DC to 20kHz.
- User selectable gain from X1 X120
- Upgradeable firmware through the user-programmable 256kB FLASH memory.
- Automatic trigger mode aids in searching for units.
- Real-time advanced signal processing by a dedicated 32 bit Digital Signal Processor (DSP).
- Up to 48 kHz sample frequency fine sampling of the analog signal, 16 bit digital input and output.
- Real time waveform display; no additional oscilloscope is required.
- Onboard high speed USB port for direct connection to PC. No need for add-on PC boards, no interrupt or address conflicts.
- Multiple data storage options: waveforms and/or timestamps. Data can be saved in several formats for use with standard analysis programs. (Matlab, Neuroexplorer, etc.)
- Compact, modular, desktop or rack mountable.
- Multi-channel backplane version available with built-in power and USB HUB's.

1.2 DESCRIPTION

The APM (Acquisition/Processor Module) is a stand-alone digital neural spike discriminator with integrated digital gain and filtering capabilities. It can be used as a direct replacement for existing analog window discriminators. It can detect any activity within an adjustable time/amplitude "window", or up to 4 individual spikes with template matching.

All functions of the APM are implemented in the hardware and DSP firmware of the unit. The 32 bit DSP allows real time processing of signals on board, with no data processing performed on the host PC. The flexible architecture of the software allows the user to develop customized algorithms. The user-programmable 256kB FLASH memory allows expansion of basic functionality by a simple firmware upgrade process. A web-based users group is established (www.neurocraft.com) for a free exchange of software, under an open-source policy.

Two modes of spike discrimination can be selected: Slope/Height Window Discrimination, or Adaptive Template Matching (ATM). In window discrimination mode, an acceptance window is placed in an area where only relevant waveforms pass through it. Only those waveforms will be recorded. The acceptance parameters (trigger, amplitude etc.) can be changed at any time during the experiment (not allowable in all recording formats). This allows for changes in spike shape due to electrode drift etc.

The ATM mode utilizes a user selected spike to build a template of the waveform shape to be recorded. Acceptance criteria settings are fully adjustable. Parameters can be set to make the template adaptive to automatically follow changes in spike shape.

Inputs are provided for interfacing with available behavioral systems. Trial Gate, Trial Validate, and Event Input are used when recording in gated mode to record only valid trial sections. A Spike Acceptance TTL level output is provided for recording when any waveform is accepted.

Waveforms are displayed in real time on the host PC. No additional scopes are required. An analog output of the signal is provided for monitoring (audio or other) or further processing of the conditioned signal.

Data is stored on the PC as timestamps, waveforms, or both simultaneously. The host PC's graphical user interface displays the waveform and settings for the selected channel, as well as thumbnail views for all active channels. (Only one channel is used for each APM) The parameters of spike acceptance can be interactively changed at any time during the experiment to compensate for electrode drift, etc. The interface can simultaneously display up to the last 256 triggered waveforms per channel.

Data can be stored/converted in several formats for use by standard analysis software i.e. Matlab, Neuroexplorer, etc. Acquired data can also be retrieved online over TCPIP networks through the builtin data server. Code samples that retrieve data into Matlab and perform online analyses are provided.

The APM is designed as a one channel module. Multiple module combinations can be run from a host PC using either the two USB ports commonly found on most PCs or commercially available USB hubs. Additional complimentary neuro/craft[™] modules will soon be available to expand the APM to include a complete microelectrode recording and electrophysiological analysis system.

A multichannel APC (Acquistion/Processor Channel) system is also available with built in power and USB hubs for larger electrode count recording applications. (please refer to www.fh-co.com for more information on this system.

1.3 OPERATING ENVIRONMENT

The APM has been designed to operate in a typical laboratory setup.

1.4 INVENTORY

1.4.1 ITEMS DESCRIBED IN THIS MANUAL

Items are included under the following catalog number:



1 ea. 55-10-0 APM

Includes: APM Module

A991 Manual

Accessory Kit

Includes:

Power Transfer Cord

Software Setup Kit

USB 2.0 Cable

BNC Adaptor Cables (6ea.)

Rubber Feet (4 ea. Use Optional)

1.4.2 ADDITIONAL ITEMS REQUIRED FOR OPERATION

The following additional items are ORDERED SEPERATELY:



1 ea. 55-00-1 12V Desktop Power Supply

1 ea. 66-EL-LC-XXX Line Cord (Country specific see sec 2.1.4 of this manual for catalog number)

Not Shown:

1 ea. Headstage preamp (ex. FHC #55-40-0)

Microelectrodes, Electrodes, Pipettes

Electrode Positioning System (FHC #50-16-1, 50-12-1C)

Host PC (Pentium II at 333 MHz or higher with 64 MB RAM running Windows 98SE, Me, 2000, or XP)

1.4.3 REPLACEMENT ITEMS

55-00-2	Power Transfer Cord
55-00-4	BNC Adaptor Cable

55-00-5 USB 2.0 Cable

1.4.4 OPTIONAL ACCESSORIES

The following accessories are available:



55-11-0 SAF Rack Frame for Stand-Alone Modules



Multichannel APC System

Version Shown Includes:

55-20-0 APC Acquisition/Processor Channel (12ea.)

55-21-0 PCF Powered Channel Frame (1 ea.)

55-21-01 PSC Power Supply Channel (1 ea.)

55-21-02 UHC USB HUB Channel (2 ea.)

1.4.5 SYSTEM CONFIGURATIONS



APM

A typical system configuration includes the APM module connected through the USB bus to a host PC. The action potentials from neurons, recorded through an electrode, are amplified by a headstage, then applied to the analog input of APM. The processed analog signal, after digital filtering and variable amplification, is available at the analog output for audio monitoring. Visualization on an oscilloscope, like in traditional configurations using analog window discriminators, is not required, since spike waveforms can be visualized on the computer display. The data acquisition can be synchronized with a behavioral system, if present, through the Trial Gate input of the APM. A stimulus generator with isolation unit can be used for microstimulation with a switching preamp.

1.5 CONCEPTS

1.5.1 TERMINOLOGY

ADC

Analog-to-Digital Converter - a device that converts continuous input signals in numeric form. The APM modules have 16-bit dual channel ADC's.

CODEC

The COder/DECoder (CODEC) is a circuit that contains a pair of analog-to-digital (ADC) and digital-toanalog (DAC) converters.

DSP

Digital Signal Processor - a microprocessor that has an architecture and instruction set optimized for real-time processing of digitized analog signals.

FLASH Memory

A read-write memory whose content is non-volatile. The programmed data is permanently stored. Reprogramming the memory is performed by first erasing the chip, then writing the new data. All these functions can be performed in-circuit, by issuing a sequence of software commands.

SRAM Memory

A Static Random Access Memory that allows fast read-write operations. Being a static RAM, it does not require periodic refreshing of data, therefore data storage is obtained with no CPU overhead. A complete read/write cycle can be performed during one DSP clock cycle, with no extra wait states.

Template

A characteristic waveform pattern against which the incoming spikes are checked for matching.

Triggering level

The voltage level that causes a spike triggering event when the input signal passes through it with a given slope.

1.5.2 **DESIGN DESCRIPTION**

Hardware Description

The design is centered around a powerful 32-bit Digital Signal Processor (DSP) with floating point support. Analog signals are converted to digital using a 16 bit A/D converter. Functions of traditionally additional hardware modules (filters etc.) are completely implemented in the APM software by the DSP. The processor is powerful enough to perform complex signal conditioning and spike discrimination tasks between two samples even at the maximum sampling rate of 48kHz.

The APM hardware block diagram is shown below:



The CODEC is a circuit that contains two pairs of analog-to-digital (ADC) and digital-to-analog (DAC) converters. It sends/receives data to/from the DSP. The DSP receives the digitized input stream, and performs real-time data processing according to the algorithm stored in its internal program memory. The program is loaded into the internal memory from the PROM or FLASH memories after a board reset. The PROM holds a fixed code, which is for restoring the module's functionality when the FLASH has been erroneously programmed by the user. The FLASH holds the actual DSP firmware that defines module's functionality, or user code. It is factory programmed, but can be re-programmed by the user with the latest firmware version, or customized algorithm. The SRAM is a fast static memory that holds volatile data like communication and waveform data. The communication with the host PC is performed through Universal Serial Bus (USB) using a dedicated communication adapter.

The input signal is first processed by a programmable gain amplifier (PGA) included with the CODEC. A fixed (software selectable) x10 (20dB) gain, then an additional 0 to 22.5dB continuously adjustable gain for over x100 (42.5dB) gain of the analog signal. The APM performs digital filtering utilizing a sharp 64-tap bandpass filter with continuously and independently adjustable LO/HI frequencies in the range of 0 to 20kHz. (Slope and level triggers are adjustable anywhere within the input range, and pre-trigger and window delays adjustable anywhere within the window length.)

Inputs are provided which allow the user to implement spike discrimination and recording within the framework of a behaviorally gated experiment. An analog output of the conditioned signal is provided for monitoring or further processing. A spike acceptance output is provided for interfacing with existing time stamping systems if using the APM as a digital window discriminator.

APM Signal Path:



Slope Height Window Discrimination:

The action potentials originating from different recorded neurons have distinct shapes. Based on these differences in shape, one can select (or discriminate) certain neurons from others. In a standard slope/height window discriminator, as well as in the APM, the selection of neurons is schematically represented in the figure below.



Whenever the signal crosses the trigger level with a given slope a trigger event is generated, and the waveform is sent to the PC for display and/or disk storage. The trigger level and position with respect to the start of the waveform can be freely adjusted within both the signal input range and the spike window length. Trigger events can originate from different neurons or even large amplitude noise. In order to discriminate between these different events, an acceptance window is placed in the area through which spikes generated from the neuron of interest pass, but not the others. Whenever a spike that has caused a trigger event passes through the acceptance window, the waveform is marked as accepted, and the time at which it passes through the window is saved as a timestamp. The timestamp value is sent to the PC and/or attached to the recorded waveform. A TTL pulse is sent to the Spike Acceptance output for use in further monitoring.

Since neural systems code their activity level in the repetition rate of the action potentials, not in their amplitude (which is fairly invariant), the timestamps are the essential pieces of information that needs to be stored in order to analyze responses to stimuli. Waveforms are usually recorded for later data analysis, and spike sorting using more complex algorithms. No matter how complex the spike sorting algorithms, their output is going to be the same: the timestamps of the recorded neurons.

After powering on the modules and starting the application, the program begins with the last used spike discrimination parameters (trigger level and delay, acceptance window position, spike length etc) of the current user profile. Starting from that point, the user can modify any of these parameters by simple drag-and-drop mouse operations on the screen elements (trigger line, window etc) in the main waveform window. One of the first things to adjust is the triggering level in such a way that it falls within the signal range and leads to triggering events (waveforms are displayed). If no signal is displayed on screen, the triggering events are missing. This means that the triggering level is too high or too low, and has to be brought closer to the zero line. Conversely, if the triggering level is too close to zero, the signal noise can lead to a flood of spurious triggering events (too many waveforms) that may eventually obscure the action potentials of interest.

The APM is provided with a built-in function (Auto Adjust) that puts the trigger level adjustment on autopilot in order to maintain the spike rate within acceptable values. The Auto Adjust function moves the trigger level and activates an unconditional display of the input signal when there is an absence of a trigger event for more than a second. Therefore, if nothing is displayed on screen, the easiest way of getting some help is to activate the Auto Adjust mode.

When satisfied with the degree of isolation between different neurons (or with respect to noise), achieved by adjusting the discrimination parameters, data collection for the experiment can be activated on the host PC using the APM's recording mode. If the APM module is used as a replacement for an analog window discriminator only, and its TTL acceptance pulses are collected by another timestamping system, the channel to be recorded has to be activated for timestamp acquisition (see settings or channel windows description). The APM's Spike Acceptance TTL output is enabled synchronously with the timestamps recording mode. At this point the data collection can be performed through the external timestamping system.

In the slope height window discrimination method, the recorded timestamp corresponds to the position in time of the acceptance window and the TTL acceptance pulse corresponds to the time of the acceptance window or the trigger event, whichever comes last.

Adaptive Template Matching (ATM) Algorithm:

The template matching algorithm implements a powerful method for the discrimination of action potentials having distinct shapes that originate from different neurons in the proximity of the electrode tip (for extracellular recordings). While basic window discrimination provides the simplest and most straightforward way of manually selecting a particular waveform, adaptive template matching is a more elaborate technique that incorporates some degree of automation. Adaptation of the template is particularly important in order to compensate for the drift of the waveform shape and amplitude during long recording sessions, without user intervention.

The spike acceptance criteria is the RMS (Root Mean Square) value of the voltage difference between the template waveform and the incoming spike waveform (see figure below).



If the RMS value is less than the match tolerance level, the spike is accepted, otherwise it is rejected. Further, if the RMS value of the voltage difference is greater than the adaptation threshold, but less than the match tolerance, the template itself is updated by performing a weighted average (w) between the existing template T and the accepted waveform S, according to the equation: $T' = (1-w)^*T + w^*S$

The acceptance TTL output pulse can be issued only after the whole waveform has been acquired. It has little jitter (less than 1 ADC sample) and is guaranteed to be issued in the interval between the last waveform sample and the sample immediately following it. The timestamp that is assigned to the recorded waveform corresponds to the time of the triggering event, or crossing the trigger level.

Data Recording Modes:

The APM can be used as a stand-alone data acquisition device, wherein data collection is performed through the USB interface and stored in a data file on the host PC. Alternately, the TTL Spike Acceptance output signal can be used to provide acceptance pulses to an external timestamping system.

In both cases, the user has the option of either acquiring data continuously (when non-gated mode has been selected in the **Recording** settings dialog), or performing conditional data acquisition based on the status of the **Trial Gate** and **Trial Validate** digital inputs. In non-gated mode, the data collection

starts when the user selects the **Recording** button in the main panel. In gated mode, an external TTL signal triggers the data collection.



When gated mode is enabled, the data collection will be enabled as long as the **Trial Gate** input is maintained high (ON). Multiple transitions between the ON/OFF states of the trial gate input are supported during a recording session (recording continuously enabled), and each interval during which the input is high will be marked and stored as a different trial in the data file. If the **Recording** button in the main panel is disabled, the actual end of recording will be deferred until the end of the current trial, if a trial is in progress. The time origin for the recorded timestamps is the start of each trial, or the start of the first trial, depending on the selected file format for saving data (APM or PLX).

Trial Validation:

In order to limit data collection to relevant trials only, an external validation signal can be applied to the **Trial Validate** TTL input. Data for non-validated trials is discarded without being saved into the data file. The validation pulse can be provided anytime in the interval between the trial end and the start of the next trial.



Trial Validation in Gated Mode

The trial gate is not the only way of synchronizing the recording with the behavioral control system. Another TTL input is provided for recording the timing of other events (The presentation of stimuli or other external events.). The timestamp of the external event will be stored in the data file, with the same time reference as the spike timestamps.

Data Storage and Analysis:

Once data has been collected, the user has a number of options for analyzing it. The APM software package comes with no built-in data analysis programs, but it provides compatibility with other popular data analysis software. (Matlab, Neuroexplorer, etc.) See the file form

1.6 TECHNICAL SUMMARY

1.6.1 SPECIFICATIONS

APM:

Input Impedance: 20 kOhm

Input Dynamic Range: 1 V rms (2.83V peak to peak)

Sampling Resolution: 16 bits (input and output)

Sampling Frequency: 7,040 – 48,000 samples per second, adjustable in 1 sample per second steps. Less than 7,040 s/sec can be achieved with sample averaging performed by the DSP.

Frequency Response: 20Hz - 20kHz in AC mode, 0-2kHz in DC mode.

Memory: 1.5 MB zero wait state fast SRAM data buffers, 256 kb FLASH.

Input Voltage Gain: x10 (20dB) fixed (software selectable) plus X1- X13 (0 - 22.5dB) adjustable in 1.5dB steps.

Input Signal Filter: Sharp 64-tap digital bandpass filter in the range of 0Hz to 20kHz

Analog Output: Digitally conditioned input signal in the range 0 - 1 V rms (2.83V peak to peak)

Window Size: 32-128 samples (.67 - 2.6 ms @48kHz)

Additional Inputs and Outputs: 3 TTL inputs for behavioral control system interface (Trial Gate, Trial Validate, Event In). TTL spike acceptance output for interfacing with external event recording hardware or legacy systems.

Display: Graphic user interface with 1 - 256 simultaneously displayed waveforms

Computer Interface: Full-speed USB 1.1 (12Mbps)

Dimensions: 2.05" (5cm) width X 5.22" (13cm) height X 9.6" (24cm) depth

Weight: 1.30 lbs (.59 Kg)

Power Requirements:

Power Supply: 115/230VAC, 50-60Hz, 9W max. Country specific line cord.

APM: 12V, 350mA

1.6.2 CONTROLS/CONNECTORS



Connections – APM Front Panel:

Analog Output: 3.5mm mono jack outputs an analog version of the signal converted from the digital output. This connector has an analog ground connection.

Spike Acceptance Output: 3.5mm mono jack outputs a TTL pulse on acceptance of a waveform.

Trial Gate Input: 3.5mm mono jack for TTL input of trial gate (trial start/stop) from behavioral systems. When used, waveforms are recorded on the host computer only while the TTL input to this jack is HI.

Trial Validate Input: 3.5mm mono jack for TTL input of trial validate (trial acceptance) from behavioral systems. When used, waveforms are recorded on the host computer only while the TTL input to this jack is HI. Unacceptable trials are not recorded on the host computer.

Event Input: 3.5mm mono jack for TTL input of event marker from behavioral systems. When used, a HI input to this jack causes an event timestamp to be recorded.

Analog Input: 3.5mm mono jack for input of low impedance analog signal from preamp. This connector has an analog ground connection.

Display – APM Front Panel:

USB Status: Tri-color LED indicates status of the USB port.

Green – Indicates communication ready to send and receive.

Amber – Indicates module is not yet recognized/initialized as an USB device. This happens primarily at startup. LED changes from amber to green when device is recognized/initialized by the host computer.

Red – Indicates communication is busy.

Spike Acceptance Output: Red LED lights indicating a HI TTL pulse is being output through this jack.

Trial Gate Input: Yellow LED lights indicating a HI TTL pulse is being input through this jack.

Trial Validate Input: Yellow LED lights indicating a HI TTL pulse is being input through this jack.

Event Input: Yellow LED lights indicating a HI TTL pulse is being input through this jack.

On: Green LED indicates power is on.



Controls – APM Back Panel:

0|I: Rocker switch used to activate power

Connections – APM Back Panel:

USB: High speed USB 2.0 port for interfacing with host computer.

Power In: 2.1mm female socket. 12VDC 2A input.

Power Out: 2.1mm female socket. 12VDC pass-through outlet for daisy chaining of modules.

Display – Main User Interface



Channel Select Window - This window has a number of functions. Under the **Select** tab, channels can be activated for viewing and adjusting parameters, in the main control panel. The APM is a single channel device, therefore only channel 1 will be activated. The default number of channels in the program is 12. The

number of channel buttons available can be adjusted under the **Input** tab of the **Settings** dialog box. The color of the channel buttons indicate the channel status, as follows:

black - Channel is reset blue – USB communication is OK cyan – Channel is configured and running green – Channel is selected in the main window red – Channel error

By clicking on the **Record** tab, the user has a handy way of enabling recording and toggling between recording timestamps and/or waveforms for each channel, when recording is enabled in the main panel. This way data can be saved in the datafiles only for the channels providing relevant information, and can be disabled totally for the other channels. Moreover, the user can add or remove channels at any time during recording, without having to restart or interrupt data collection. The same recording settings can be accessed in the **Recording** tab of the **Settings** dialog box. The color code for the recording mode is:

black - Channel is not selected for recording

blue - Channel records timestamps only. This mode is missing when running in NEX compatibility mode (see description of the **Recording** tab in the **Settings** dialog box section.)

green - Channel records timestamps and/or waveforms. Only waveforms (without timestamp records) are saved when NEX compatibility mode is selected

The **Audio** tab allows the user to selectively enable channels for audio monitoring output. In single channel systems the analog output can be used for audio monitoring purposes.

black - Audio output is not enabled green - Audio output is enabled

Thumbnail Channel Windows - This window displays continuously updated waveform data ("spikes") coming into the channel processor from the electrode. Changes to the discrimination parameters can only be made in the Main Waveform Window, not through these windows. They are for real-time monitoring of the channel.

Main Waveform Window - This is the main window for displaying the waveform data, triggering level, acceptance window, and pre-trigger settings for the channel. It allows the user to adjust these settings in a simple drag-and-drop way. Waveforms are displayed with thin lines having different colors, depending on their acceptance status. Non-accepted spikes are displayed in gray, while window- or template-accepted spikes are displayed with bright colors. Waveforms that have been accepted as matching different templates are shown with the same color as the template. The displayed colors can be changed in either the **Settings** or **Templates** dialog box. Templates are displayed with thicker lines having different colors. 4 templates can be discriminated against simultaneously.

The horizontal red line represents the voltage triggering level. The voltage triggering level threshold is changed by moving the line in a drag-and-drop manner. Trigger Delay is the small circle on the triggering level line. It represents the point where an event causing waveform acquisition is triggered. It is changed in the same drag-and-drop manner. The interval elapsed from the window start to this circle is sometimes called the pre-trigger interval.

The Acceptance Window is the vertical white line. It can be repositioned anywhere on the screen by using drag-and-drop mouse input. The size of the window is changed by mouse-dragging its ends (represented as small circles). Whenever a waveform passes through this window, it is considered accepted (when not using template matching mode). In template matching mode, it is used to select a waveform for constructing a new template. A grid is displayed with dark gray lines. It can be activated/deactivated in the **Display** page of the **Settings** dialog box. The time and voltage calibration scale indicators are displayed in the top-right corner of the window. They are expressed in volts/division for the vertical axis and seconds/division for the horizontal axis. This display can be activated/deactivated in the **Display** page of the **Settings** dialog.

Input Gain	6	·
	3	Gain: 29.0 dB المناط

Input Gain Slider - This slider sets the amount of gain applied to the analog input of the selected channel. To adjust the gain up or down, the slider is simply moved back and forth with the cursor. When the slider is selected, (clicked upon) the actual gain step, in 1.5dB increments from 0dB to 15dB, is displayed above it in a pop up window. The total gain (in dB) is displayed to the right of the slider. This amount includes the X10 (20dB) input gain, which is activated/deactivated in the **Settings** dialog box.

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	- 2	<u></u>	1	'	'	'	'	1	1	1	1	1	1	1	1	•	1	•	•	· .	•	•	•	•	1	1	1	1	1	•	•	·	•	 1	'	1	1	<u>'</u>	Low: 132 Hz
	1		÷	·	•	,	•	•	ï	•	•	•	•	÷	·	•	i.	•	•		•	·	,	•	i.	·	۰,	Ł	•	•	,						÷	'	High: 13942 Hz
	_											_	_															┘										_	riigh. Too ie rie

Low Cut Off and High Cut Off Filters - These sliders set the range of frequencies in the input signal that are allowed to pass through the digital filter. They are controlled in the same "drag and drop" manner as the Gain control. They can also be clicked on, and moved in increments of 100Hz using the left and right arrow keys. Only the components of the input signal frequency spectrum which are above the low cut, and below the high cut, are displayed by the discrimination algorithm.



File - This button brings up a window that selects the name and location of the file where the acquired data will be stored, and the file format.

Settings

Settings- This button brings up a window that allows the user to change various settings of the modules. The upper part of the dialog refers to the logical channels and their associated USB address. "Channel" selects the channel whose settings will be applied. (This is independent of which channel is currently displayed in the main window.) "USB device ID" selects which USB address (as assigned by the operating system) of the module that will be assigned to the selected channel. When connecting a single APM module to a PC, it is always assigned the address 0.

The **Recording** tab allows the user to select between various data collection options. Time Stamps are records of the time when the spikes were accepted based upon the discrimination criteria. (i.e. triggering threshold and slope, window time and levels) only. Selecting Waveforms allows the user to record, together with the time stamps, the spike waveforms, as shown on the screen. If you do not plan do a further offline sorting of the recorded data, recording time stamps is all that is required for data collection. The amount of data stored in the data files is minimized to the essential information. However, off-line sorting cannot be performed on the data at a later time.

Recording full waveforms allows off-line sorting, using a specialized spike sorting application. These options do not exclude each other, since the information is stored as different records in the datafiles. Sometimes you may find it useful to record both pieces of information, even if you achieve a certain degree of redundancy (ctoring the timestames twice, once as in the timestames records, once in the waveform).

redundancy (storing the timestamps twice, once as in the timestamps records, once in the waveform records). This may be the case if you are recording fast-spiking neurons and using long waveform windows so that it's a non-null probability that a second spike may occur within the same waveform time-window, as shown in the example at right.

In this situation, there's going to be recorded only one waveform, having the timestamp "Timestamp 1" if you choose to save the waveforms only. If you choose to save the timestamps as well, both values will be stored in the data file. In a more technical description, trigger/window detection re-arms at each trigger level crossing (two crossings with the selected positive slope in our example), whereas waveform recording re-arms after a full waveform has been acquired.



Although the example shown here is quite unusual in practice, it is useful for giving an insight on the difference between timestamps and waveforms records.

The **NEX compatibility** checkbox allows the user to save the data in a format that allows later processing using the Neuroexplorer[™] software package, as well as other applications that share the same Plexon[™] data file format. There are a number of restrictions that are required for NEX compatibility:

- a) The waveform length cannot be larger than 56 samples.
- b) The waveform length and trigger time position has to be the same for all channels.
- c) Some of the recording parameters cannot be adjusted during recording: waveform length, trigger level, trigger time position, trigger slope, input gain. This is due to the intrinsic differences in the APM and PLX file formats. While in the PLX files the parameters of the recording are stored in the file header and are assumed constant during recording, the APM file format has no fixed headers, but instead stores a record for each parameter change, in a serial way. Therefore, using the APM file format, user is not only able to read the recorded data, but also to reconstruct the history of the settings during recording. This can be particularly useful in the situations where the neural signal is not perfectly stable throughout the recording session. If the isolation of some unit gets poorer over time (the signal fades away), the user can adjust the gain to compensate for that decrease in amplitude. Also if the shape of the action potentials changes, becoming more sharp or more blunt, the user can move, and/or extend the time window to keep that unit isolated.

The **File** settings allow saving the data in a single file or in multiple trial files. If multiple trial files are selected, a four-digit trial index is appended to the original file name. For example, selecting MyRec.dat in the main panel File dialog, would result in files named MyRec.dat.0000, MyRec.dat.0001 and so on. A trial is defined by the duration the "Trial Gate" input is HI, when **Gated Mode** checkbox under **Save** settings is selected, or by the time the "Recording" button in the main panel is active in non-gated mode. For instance, in non-gated mode, the user can press the recording button on and off several times, each time it is pressed, the data is saved in a separate data file whose four-digit suffix is automatically incremented, without having to go through the File dialog to select a different name. All the timestamps in the data file are relative to the trial start (the modules' time counters are reset at the beginning of each trial). When the **Single Data File** box is selected, the data is stored under the original filename, and each trial is appended to the end of the existing file.

The APM implements a method for validating trials using an external signal, connected to the "Trial Validate" input jack. If at the end of the trial the "Trial Validate" signal goes high, the data for the last trial is saved into the recorded disk file. Otherwise, it is discarded. Checking **Keep Invalid Trials** box tells the application to save the data regardless of the trial validation input state. It makes sense to use this option only when the trials are externally gated using the "Trial Gate" input.

As probably already inferred from the description of the other functions described here, the **Gated Mode** enables or disables the functionality of the external "Trial Gate" signal. When checked, a trial is defined as the interval during which "Trial Gate" input is HI, otherwise it is defined as the interval during which "Recording" button is active. At the start of each trial, gated or not, the modules' timers are reset to zero. This enables the duration of a trial to exceed one hour (max 4294 seconds). Experiments arbitrarily long can be recorded by breaking them into one-hour trials (in a real or virtual way).

The **Input** tab allows selecting the sample frequency and the fixed input gain. The sample frequency of the analog-to-digital converter can be adjusted in the interval 7,040 Hz to 48,000 Hz. Even slower sample rates can be achieved by sample averaging, at the DSP level. The **Fixed Gain** checkbox allows setting the initial gain of the analog signal between X1 (unity) or X10 before the signal reaches the digital gain section.

	ОК
Global	Continuous
Calibration	Module
Conversion pling uency: 48000	→ Hz
e	
 Spikes Continuous 	
F L	Global Calibration Conversion Jing ency: 48000 ency: 48000 Spikes Continuous

The **Calibration** tab allows the user to finely adjust the gain and offset of the input stage. A recalibration is required whenever the user changes the headstage, or inserts more signal conditioning modules in the path. The signal peak values are displayed in the Max and Min boxes, while the peak-to-peak value is displayed in the Diff box. The input gain is increased or decreased by pressing on the up or down arrow in the **Gain** panel. In a similar way, the **Offset**, calculated as the average of the peak values, can be adjusted. The gain calibration procedure follows these steps:

 a) Feed a sine signal from a well calibrated signal generator to the headstage input. Ensure that the output level is displayed as peak-to-peak value. If only RMS values are available, multiply by 2√2 to obtain the peak-to-peak value.



- b) Choose an input signal frequency within the bandpass of the digital filter, typically 1000Hz. It is recommended that you open completely the filters (low 0 and high 20000 Hz).
- c) Set the input gain to 0; ensure that the input signal does not saturate the input stage (the peaks of the displayed signal are well below upper or lower display limit at max zoom out).
- d) Select "Auto Adjust" mode.
- e) Adjust the Gain under the Calibration tab using the up/down arrows until the displayed Diff value matches the amplitude of the input signal. The adjustment is dynamic, with a very fine step at the beginning, and ending up with a coarser step after holding the arrows for several seconds. You may have to click and hold the arrows for several seconds, before noticing a significant change in the displayed values.

In order to adjust the **Offset**, remove the signal generator and short the input to the ground. Then adjust the offset using the up/down arrows.

The **Module** tab displays information about the modules: the serial number, firmware type and version. It also allows upgrading the existing firmware to a more recent version downloaded from the neuroCraft support web site. Clicking the "Upgrade" button will prompt for the firmware file (default extension is .ldr). Select the firmware file that you want to upload into the module, then wait until the erase/blank check/programming cycle is complete (a progress bar will be displayed). In the unlikely event of a programming error, DO NOT reset or power off the module, since it may not be able to boot again. Retry the programming until the FLASH memory is successfully programmed. If re-programming fails several times in a row, refer to the troubleshooting section to see how to boot the module when FLASH contains non-functional code.

Settings × Ch1 Channel • OK USB device ID: 0 • Display Auto Adjust Global Continuous Module Recording Input Calibration Serial Number 00001001 Firmware Type: 1 Upgrade Version: 1.49

The **Display** page allows the user to select different colors for the accepted and rejected spikes, on a channel-by channel basis. Also, the grids and time and voltage scale indicators can be shown/hidden for all channels. The units in which the input gain is displayed in the main control panel, next to the gain slider, can be toggled between decibels (dB) or the voltage amplification factor.



The Auto Adjust tab allows the user to select the settings for the auto-thresholding built-in function. Whenever the spike rate decreases below the minimum value n_{low} set in the **Low** box, the triggering level is automatically brought closer to the zero line every *n* seconds, set in the corresponding box in the right panel. This continues until the triggering level becomes so low that more than n_{low} spikes per second are detected. If the spike rate increases above n_{high} , the triggering level is automatically increased in absolute value. The magnitude of the triggering level variations is set in the **Change** box.

Settings х Channel: Ch1 • OK USB device ID: 0 • Recording Input Calibration Module Auto Adjust Global Display Continuous Rate Thresholds Adjust + High: sp/sec Every: sec Change: 50 + % Low: 10 ÷ sp/sec

The Global page allows the user to apply the settings for the	50
current channel to all channels, or to reset channel values to	C
default settings. Use caution when clicking on any of the	1
buttons in the dialog as the changes will become effective	l.
immediately, and there's no way of undoing the operation and	
reverting back to the original settings.	

ettings X hannel: Ch1 • ПК JSB device ID: 0 • Recording Input Calibration Module Global Display Auto Adjust Continuous **Channel Properties** Apply Apply current settings to all channels : Reset all channel settings to defaults Reset

The **Continuous** mode page allows the user to set parameters that apply to continuous mode only: polarity of the input and the data packet size. See Recording Modes in section 1.5.2 for a description of the operation in continuous mode. In continuous mode the input stage can be configured to accept either unipolar (0 to 1V) or bipolar (-0.5V to +0.5V) input by clicking on the corresponding radio button in the Polarity section of the dialog. In this mode, the incoming continuous data stream is assembled by the APM module in packets containing *n* samples, and then sent to the PC. The data is displayed in form of a scrolling waveform. The larger the packet size, the faster and more efficient the data transfer is, but the choppier the waveform scrolling is on the display. Conversely, the smaller the packet size is the smoother the

hannel: SB device ID:	Ch1 -		OK
Recording	Input	Calibration	Module
Display	Auto Adjust	Global	Continuous
Polari	ty Unipolar Pipolar	Data Packet S Samples: 40	ize

scrolling will be on the display, but the amount of USB overhead will be increased. Optimal settings for the packet size will depend on the application at hand and the sampling rate. Please note that the packet size setting in no way affects the integrity of the data being displayed or recorded it is simply a tradeoff between USB bandwidth consumption and the scrolling smoothness of the display.

When continuous mode is not enabled in the input property page for the selected channel, the controls on the continuous page are disabled.

The **TCPIP** page allows enabling/disabling of TCPIP message and data servers and the TCP ports they use. The **Message Server** listens for incoming connections on the specified port, and messages that are sent to it are inserted into the datafiles, together with the aquired waveforms and timestamps. This is intended as a software method for synchronizing and sending information from other applications to the APM, as opposed to hardware synchronization, through the Gate and Events inputs. Although the software method does not work in real time, but with the intrinsic network latencies, it is more flexible in terms of its content. For instance the application that controls the entire experiment can send experimental parameters to be saved in the data file. To preserve the compatibility with the existing data file format, the incoming

hannel:	Ch1	•	Γ	OK
SB device	ID: 0	•	L	OIX
Recordi	ng Inpul	t Cal	ibration	Module
Display	Auto Adjust	Global	Continuous	TCPIP
D lopidy			Continuous	

messages have to respect the same message format. See file format section for a description of the general message structure.

The data server listens to incoming connections, and as soon as a client is connected, it sends to it acquired data. The network data has the same format as the data files, but in order to reduce the CPU overhead, only discrimination settings and timestamps, but not the full waveforms, are sent to the client computer. This connection in not intended as a substitute for file saving, but to send online information (like timestamps) to applications that use it to display the spiking characteristics of the recorded neurons. The data is sent continuously to the connected clients, even if data recording is not enabled. A Matlab sample client (APMPNetClient.m) is provided in the Matlab subdirectory of the APM installation.

Templates - This button brings up a window that allows the user to set and adjust the parameters of the adaptive template matching (ATM) mode of spike discrimination.

The **Enable** row of buttons allows activation of up to four distinct templates, which allows the user to discriminate up to four different neurons from the electrode. When a template is activated, the corresponding button becomes green. The template is displayed in the waveform window, as well as in the channel thumbnail window, as a thicker line than the accepted spikes. The color of the line associated with each template can be chosen in the **Colors** group of buttons.

Enabling template 1 (T1) will deactivate window discrimination. Disabling it will re-activate window discrimination. Either way, accepted spikes from T1 or window discrimination will be saved in the data file as unit 1. The settings for the other templates (T2, T3 and T4) are independent of the ones for T1, i.e. enabling templates T2, T3 or T4 will not disable window discrimination if T1 is not active.

Therefore, during recording two different modes of spike discrimination may coexist and operate simultaneously: ATM and window discrimination.

The **Record** row of buttons enables/disables data recording for the active templates (These perform the same function as the buttons under the Record tab in the channel select window.) All waveforms that cause a trigger event are stored in the data files. When a template is active, but record is disabled, these waveforms are saved without a valid unit number. They will then show up in an offline analysis programs as **unsorted**, or **non-accepted**.

The **Rebuild from window** row of buttons enables the construction of a new template from the next spike that passes through the acceptance window. Selecting a rebuild button changes its color to green until a window-accepted spike is received, and the corresponding template is initialized. Once the template is



Templates

initialized, and displayed in the waveform windows, the color of the button changes back to blue. If the rate of incoming spikes is relatively high, this may be a very brief process.

Three parameters need to be adjusted when discriminating neurons with Adaptive Template Matching (ATM). Template settings can be viewed or changed by selecting the corresponding template button in the Select row. The three sliders in the lower half of the templates window control the value of these parameters for the currently selected template.

The **Template Match Tolerance** setting determines how similar in shape a spike must be to the template to be accepted. The match tolerance is expressed in relative units, as a percentage of the template amplitude when it was (re)built using the Rebuild from window buttons. Therefore, the match tolerance setting will be spike/template amplitude independent.

The bottom two sliders control the adaptation of the template based on the variations of the incoming accepted waveforms. The Template Adaptation Weight sets what percentage of the incoming accepted spike will be used for updating the existing template (0% - no template change; 100% - template completely replaced by each accepted spike). Template adaptation for each incoming accepted spike isn't recommended however, as this would require a large amount of processor and communication overhead. The final parameter enables adaptation of the template only when the difference between it and the accepted spike exceeds a certain threshold. This threshold is controlled by the Template Adaptation Threshold, which is expressed in values relative to the template match value. For instance, setting an adaptation threshold of 90% will cause a template update to be caused by spikes whose difference from the template is within 90% to 100% of the matching tolerance.

These parameter settings are independent for each template, and saved in the user profile with all other program settings. The templates themselves are saved only in the data files, not in the user profiles. Two buttons are available in the Apply Settings To group which allow the user to copy the ATM settings for the currently selected template to other templates. To copy the current template settings to all four templates associated with the selected channel, click the This Channel button. The All Channels button is used within the multichannel APC system to copy the current template settings to all templates on all channels. Selecting this button on the APM performs the same function as the This Channel button. Copying template adaptation parameter settings does not copy the templates themselves. Template waveforms will need to be generated for all active templates by using the Rebuild from window buttons

Auto Adjust - When selected, (turns from blue to green when clicked upon) this button puts the triggering threshold on "auto-pilot" based upon a preprogrammed set of

incoming spike rates (see description in the previous paragraph). This mode is useful in the pre-recording phase, while searching for a new neuron is in progress, when there are large fluctuations in the detected signal, but its use is strongly discouraged while performing the data recording.

Spikes/Clusters - This button sets the thumbnail windows to display either the waveform or a cluster view. The main view window remains in Waveform view to allow for adjustment of the discrimination parameters. The text of the button toggles between "Spikes" and

"Clusters" depending on the mode.

The Cluster view shows the events of spikes plotted on a graph with the x and y of the graph being two spike parameters.

Recording - This button turns the record function on and off.

Spikes - This allows the user to set the number of most recent spikes that are simultaneously displayed in the both the main waveform, and corresponding thumbnail window. This only affects the display, not the discrimination or recording of waveforms.



Recording

APM



Spikes

Zoom - This allows the user to set the vertical and horizontal display magnification of the window. The vertical zoom does not affect data acquisition. The horizontal zoom sets the waveform window length (number of data points) for both display and recording.

Slope - This determines whether a signal triggers as it goes through the triggering level from negative to positive, (passes from below the red line to above) or positive to negative. (passes from above the red line to below)

Status Bars - These boxes display information about the communication status, data acquisition statistics, and error messages (i.e. USB communications status, spike rate, an external event like a reward, dropped waveforms etc.).

Ch 3 dropped 0 spikes! Ch 3 1887 spikes @ 59 sp/s !

1.6.3 COMPATIBILITIES

Hardware Compatibility:

The APM is designed to be compatible with most commercially available headstage/preamps. Contact Technical Services at (207) 666-8190 for questions on compatibility.

An Analog output of the conditioned signal is provided for monitoring and further processing. A TTL level Spike Acceptance output is provided for timestamp collection by other systems.

Data File Format Compatibility:

Data can be saved into the default APM data format, or directly into different formats for use with other data analysis software. Provisions are made for saving in a PLX format for use with the Plexon Inc. Offline Spike Sorter, or with NeuroexplorerTM. There are restrictions in the operation involved with NEX compatibility. Please refer to the **Settings** description in sec. 1.6.2 of this manual.

The data file in APM format has a sequential organization, containing a series of messages that describe the instrument's operational parameters, their evolution in time, and the acquired data. There's no file header, and messages may have any order in the data file. The general message structure is based on a sequence of 32-bit words, as follows:

Bit 31

Word 0	Messag	je Code
Word 1	Unit Number	Channel Number
Word 2	Number of 32-bit v	words to follow = <i>n</i>
Word 3	Dat	ta 0
Word n+2	Data	a <i>n</i> -1

Bit 16

Note: the unit number is present **only** in the waveform/templates messages. It is not required in any of the other messages.

Bit 0



Slope



Code	Description of the message and included data
0	Non-accepted waveform
1	Accepted waveform
2	Out-of-trial non-accepted waveform
3	Out-of-trial accepted waveform
4	Timestamps (in sample counts)
5	Trial start (in sample counts)
6	Trial end (in sample counts)
7	External event (in sample counts)
8	Trigger level (in ADC levels)
9	Trigger time position (in samples, relative to the waveform window start)
10	Trigger slope (+1 for positive, -1 for negative)
11	Acceptance window time position (in samples, relative to the waveform window start)
12	Acceptance window lower end (in ADC levels)
13	Acceptance window upper end (in ADC levels)
14	Time calibration (32-bit float, in microseconds per sample)
15	Voltage calibration (32-bit float, in microvolts per ADC level)
16	ADC sampling frequency (in Hz)
32	Template waveform
65536	TCPIP user message 0
65537	TCPIP user message 1
65538	TCPIP user message 2
65539	TCPIP user message 3
65540	TCPIP user message 4
65541	TCPIP user message 5
65542	TCPIP user message 6
65543	TCPIP user message 7
65544	TCPIP user message 8
65535+n	TCPIP user message n

The information regarding events and time intervals is represented as the number of samples acquired since the start of trial (gated recording mode), or the beginning of recording (non-gated recording mode) The time in microseconds can be calculated by multiplying the value with the current time calibration for that channel. All the signal values are coded as 16-bit signed ADC levels (left-extended to 32-bit words), from which the actual voltage can be calculated by multiplying the value with the current voltage calibration for that channel. Below are some examples:

• 64-sample accepted waveform on channel 5:

B	iit 31 Bit 0
Word 0	0x00000001 – Accepted waveform
Word 1	0x00020005 – Unit 2, Channel 5
Word 2	0x000000041 – Waveform length+1 (65)
Word 3	0x000253A4 – Waveform TS, in samples
Word 4	0x00007C42 – ADC sample 0
Word 67	0x0000017A – ADC sample 63

• 32 timestamps on channel 3:

B	bit 31 Bit 0
Word 0	0x00000004 – Timestamps
Word 1	0x0000003 – Channel 3
Word 2	0x000000040 – Number of TS codes: 64
Word 3	0x00000101 – TS 0 code: Unit 1
Word 4	0x00001C2D - Timestamp 0, in samples
Word 65	0x00000401 – TS 31 code: Unit 3
Word 66	0x0014FA88 – Timestamp 46, in samples

Each timestamp comes as a pair of 32-bit words. The first one contains the unit number and other information, the second contains the actual timestamp, in ADC samples, relative to the trial start. The timestamp codes contain information about the GATE input, and the unit number, bit-coded as follows:



• Trial end at 6894 ADC samples, on channel 2:

В	jit 31	Bit 0
Word 0	0x0000006 – Trial end	
Word 1	0x00000002 – Channel 2	
Word 2	0x00000001 – Number of values to follov	v
Word 3	0x000A5B21 - 678689 samples	

The TCPIP messages contain user data, and they are saved in the data file as they arrive through the network message server, from other applications/computers that connect to it. The user is free to fill in any information in the Data 0 ... Data n-1 fields, as long as the message conforms to the general structure presented at the top of this section. A malformatted message may compromise the whole saved data file, rendering it unreadable.

At user request, the first TCPIP messages have been used to implement certain APM commands. Currently, message 0 is used to remotely enable/disable recording of data, message 1 is used to insert an arbitrary string containing trial description into tht data file, and message 1 contains a string that is used to set the saved data file name. To ensure compatibility with later versions, users should start with larger TCPIP message numbers, for instance n=64.

Messages in the data file have no predefined order. The messages are saved in the order they arrive from the APM. Messages originating from different channels may be interleaved.

Provided with the installation kit are a number of example Matlab scripts that parse the messages in the data files and extract the information stored in them.

1.7 ILLUSTRATIVE PROCEDURE

- 1. Install (first time only) and power up the APM. Once initialized the **USB Status** LED will turn green.
- 2. Start the main APM control application by clicking the **APM** icon on the host PC.

- 3. Enter username. Any changes made to the recording parameters during this recording session will be saved under this username.
- 4. Initiate communication between the APM channel and the host PC by clicking the **Select** tab in the **Channel Select Window**. Click the **1** channel button and wait for it to turn green.
- 5. Enable the record function by clicking the **Record** tab of the **Channel Select Window**. Click the **1** channel button and wait for it to turn green.
- 6. Click the **Settings** button in the **Main User Interface** to access the **Settings** dialog box. These settings will be saved under the username entered in step 2, and will not have to be set each time.
- 7. Adjust the **Triggering Threshold Level** and **Input Gain** until a stable, reasonably large signal is displayed. Alternately, the **Auto Adjust** function can be used to automatically have the APM accomplish this.
- 8. Adjust the **Bandpass Digital Filter** settings to narrow the bandwidth of the displayed signal to remove unwanted noise.
- 9. Adjust, in a drag and drop manner, the **Acceptance Window** so that only acceptable spikes pass through it (they will turn green).
- 10. If Slope/Height Discrimination recording mode is used, skip to step 16.
- 11. If the **Adaptive Template Matching** recording mode is to be used, adjust the **Acceptance Window** such that it captures only one spike shape.
- 12. Click the Templates button to open the template settings dialog box.
- 13. Enable the template and record function by clicking on the appropriate template buttons.
- 14. Adjust the settings for the adaptive criteria of the template (the bottom three sliders).
- 15. Click the **Rebuild From Window** button to set the template.(A thick colored line defines the template in the **Main Waveform Window** and also in the **Thumbnail Channel Window**.)
- 16. Repeat steps 8 through 13 for up to three more templates.
- 17. Click the **File** button in the **Main User Interface** to set the filename and path where data will be collected.
- 18. Enable data collection by clicking the **Recording** button in the **Main User Interface**. Click again to end the recording session at any time.

2 REFERENCE MANUAL

2.1 REFERENCE INFORMATION

2.1.1 PACKAGING

The stand-alone modules of the neuro/craft[™] series instruments are packaged in metal cases, which consist of standard 5.25" high front panels. Front panel widths are specified as Type 2 modules (2.05" actual), Type 4 modules (4.15" actual), and Type 6 modules (6.25" actual) Front panels are mounted on extruded top and bottom panels. Flat side panels slide into slots in the extrusions, and are held in place when the back panel is secured into the extrusion. All modules are 9.75" in depth.



Type 2 Module

Type 4 Module

Type 6 Module

2.1.2 MOUNTING

All stand-alone modules are completely encased and can be used without further mounting or hardware. Provided rubber feet may be used to protect surfaces from scratching. However, it may be suitable to group modules, and we have made provision for several configurations. The SAF-08 Rack Frame for Stand-Alone Modules (cat #55-11-0) will hold up to eight Type 2 modules, four Type 4 modules, or two Type 6 modules and 2 ea. Type 2 Dress Panels (cat #55-11-1, use optional), while occupying only 3 rack units (5.25") vertically on a standard 19" instrument rack. Several combinations are available for all of the neuroCraft series stand-alone modules. For example an SAF-08 frame could accommodate 3-Type 2, 1-Type 4, and 1-Type 6 within its 16" of horizontal rack space.



SAF-08 Rack Frame For Stand-Alone Modules (Shown with a neuro/craft™ Type 2 Module)

Dress Panels for SAF-08 (Ordered Separately):

• 55-11-1 Type 2 Dress Panel

2.1.3 INSPECTION

FHC Modules are factory checked and calibrated but should be carefully inspected upon receipt, before using, or activating power. If any exterior damage to the shipping carton is noted, the instrument(s) should be inspected for obvious physical damage. The contents of each package should be physically checked against the inventory list (sec. 1.3) to determine shortages or errors in inventory.

2.1.4 POWER CONNECTIONS



All of the stand-alone modules in the neuro/craft[™] series are powered by a desktop 12V power supply. (input:100-240VAC, 50-60Hz, 1.7A output: +12VDC,5000mA) (Cat. # 55-00-1) An international pattern Line Cord (not shown) is ordered separately, and is specified by country per the catalog number. (See table below for catalog numbers.) Additionally, the power transfer cord (not shown) supplied with the NeuroCraft stand-alone modules can be used to "daisy-chain" the power between other instruments in the series from one power supply. The amount of modules powered from one supply is determined by the amount of current drawn by each module. Contact Technical Services at (207) 666-8190 for assistance.

66-EL-LC-AUS	Australia
66-EL-LC -CH	China
66-EL-LC -DAN	Denmark
66-EL-LC -EURO	Europe
66-EL-LC -ISR	Israel
66-EL-LC -ITA	Italy
66-EL-LC -JA	Japan
66-EL-LC -SAF	South Africa
66-EL-LC -SWI	Switzerland
66-EL-LC -UK	United Kingdom
66-EL-LC -USA	North America

2.1.5 WARRANTY

All FHC products are unconditionally guaranteed against defects in workmanship for one year from date of shipment as long as they have been exposed to normal and proper use. Although the one-year warranty may have expired, please contact our Service Department before attempting any repairs or alterations. Many of these repairs will still be performed at the factory at no charge to the customer.

2.1.6 POLICIES

1.TECHNICAL SUPPORT: It is our policy to provide our customers with the most comprehensive technical support in the industry. If any questions arise or problems occur, we encourage you to call

or write and we promise to promptly and comprehensively respond to your requirements.

2.TRADE-UP POLICY: It is our policy to offer customers trade-up ability as new and/or expanded capabilities for their instruments are announced. In many cases, full credit will be given. In general, we will allow 100% credit for two years and depreciate 20% per year thereafter. Please contact our Marketing Department for information relating to your particular situation.

2.1.7 **SERVICE**

Should service be required, please contact our Service Department for a return authorization number and instructions (207-666-8190). Please have the model and serial number on hand (Both are located on the back panel). Carefully pack the instrument before returning.

Please include a note indicating:

- 1. The model number and purchase date of the instrument
- 2. The person to contact if questions arise
- 3. The "symptoms" indicating that repair is necessary

If the instrument is not covered by the warranty, a quotation will be forwarded to the sender detailing the repairs necessary and charges, before repair is begun.

2.2 INSTALLATION

2.2.1 HARDWARE INSTALLATION

- 1. Ensure that the APM is placed in a convenient out of the way spot. Alternately, install the APM in the SAF-08 Rack Frame.
- 2. Ensure power switch on the back panel is in the **Off** position. (Indicated by the **O** side of the rocker switch pressed in.)
- 3. If powering from the desktop power supply:
 - Plug the power supply line cord into a properly grounded wall receptacle.
 - Install the 2.1mm plug from the power supply into the **In** jack of the **Power** section of the back panel.

If powering from another module:

- Install the power transfer cord from the **Out** jack of the other module, to the **In** jack of this module.
- 4. Connect the USB cable from the USB jack on the back panel of the APM to the USB jack of the host computer.
- 5. Connect the cable from the headstage/preamp to the **Analog Input** jack on the front panel.
- 6. If applicable, connect the cables from a behavioral system to the appropriate **Trial** jacks on the front panel.
- 7. If applicable, connect a cable from the **Analog Output** jack on the front panel to an oscilloscope or other recording device.
- 8. Route all wires as to prevent them from being pulled or tangled.
- 9. Perform software installation in section 2.2.2 before powering up the module.

2.2.2 SOFTWARE INSTALLATION

- 1. Insert the application CD and run Setup.exe. If you have downloaded an updated setup kit from the web, run setup from the directory where you have unpacked the files. There are no options to select during install, except for the destination directory.
- 2. Upon successful installation, apply power by pressing in the I side of the rocker switch located on the back panel
- 3. The APM device is plug-and-play, and Windows operating systems will automatically recognize it after you turn the power on. The operating system automatically goes through the new hardware installation with little or no user intervention required. The system may prompt you with a message that is operating-system dependent (shown below is the WinXP version).



Select **Install software automatically**, then click the **Next** button.

4. The Windows XP operating system may prompt with another message, that refers to the driver digital signature. Click **Continue Anyway**





5. If driver installation has been successful, you will get the acknowledge message shown below. Click the **Finish** button.



Through this procedure the driver for the unconfigured APM module (after power-on) has been installed. This startup driver will load the actual USB communication firmware into the module, configure its USB interface, then change its identity to a configured APM module. The operating system will prompt you to again install the new hardware (this time for the configured APM module). Follow steps 1-5 as before. If you install more than one APM module to a host computer, the operating system will launch the "Add New Hardware" wizard for each channel.

2.2.3 **SOFTWARE UPGRADE**

The APM offers the powerful option to keep up-to-date with the latest code developments, on both the PC application and the APM firmware. A PC application upgrade, and/or a DSP firmware upgrade can be easily performed. On any APM channel, different functionality can be implemented through this upgrade process.

The PC application upgrade requires a few easy steps:

- 1. Download from a www.neurocraft.com (downloads section) the zip archive containing the updated software package;
- 2. Unpack the zip archive to a directory on the computer;
- 3. Run Setup.exe and follow the installation instructions on the screen;
- 4. Power on the system and start the updated application. Activate the channels;
- 5. If a DSP firmware upgrade is required, you will be informed about that in a pop-up window, when clicking on a channel. If this is the case, all functions, except firmware upgrade, are disabled. You may proceed to step 3 in the next paragraph describing the firmware upgrade;

APM01	×
8	Module firmware version is different from the required one ! All program functionality, except firmware upgrade has been disabled. You must perform a DSP firmware upgrade before starting to use the channel.
	ОК

The firmware upgrade requires erasing the FLASH memory that holds the DSP code, blank-checking it, uploading the new firmware, programming the new code into the FLASH, and verification of the programmed data. This procedure is automated, requiring little intervention from the user. Here are the steps to be followed:

- 1. Open the communication with the module by clicking on the channel whose firmware needs to be upgraded in the channels window;
- 2. In the main application window, click on Settings, then select the Module tab and click on the Update button. An open file dialog will pop-up. Browse to the directory where you have unzipped the firmware files. If you have previously performed a PC application update, the latest firmware files already reside in the same directory where the application has been installed (typically C:\Program Files\FHC\APM-01\). Select the desired firmware file having the extension .ldr (default is APM-01-DSPFW.ldr).

After clicking Open in the open file dialog, the firmware process automatically starts. First of all the FLASH memory is erased. Then its contents are checked to confirm that they are blank. Next the new code is uploaded to the APM, programmed and verified in a single apparent step.

Erasing FLASH (8.4 seconds)
Blank Check, FLASH Address: 502e40
Writing FLASH Address: 505a60

After performing a firmware upgrade, the new code is executed at next processor reset. You will be asked if you want to perform a software reset right away, or do a hardware reset at a later time by cycling the APM's power.

APM01	×
⚠	Firmware successfully upgraded. The new firmware will be executed at next board reset. Press OK to reset the board now or CANCEL to reset it later.
	OK Cancel

After a firmware upgrade and reset, the DSP executes the initialization (programming serial ports, selecting inputs, LED test etc) code, therefore you will not be able to communicate with the module for a few seconds, before initialization is completed. It is recommended that you wait for the end of the reset cycle, before you close the dialog. The end of the reset cycle is indicated by the spike acceptance LED in off position.

It is highly recommended that you quit and restart the application after a firmware upgrade. This ensures that the newly read variable addresses from the .map file are stored into the system registry.

2.3 FUNCTIONAL CHECKOUT

Testing the module functionality

Module functionality can vary depending on the firmware loaded on the APM flash memory. The following instructions assume the default firmware implementation of the window discriminator functionality (type 1).

- 1. Ensure that the hardware and software are installed correctly per section 2.2.
- 2. Initiate communications between the PC and the APM by clicking on the **1** button in the **Channels** window. Ensure that the button is green.
- 3. Connect an input signal to the **Analog Input** jack on the front panel of the APM. An input signal could be one of the following
 - a. A signal from a Spike Simulator device (ex. FHC cat #40-42-1)
 - b. Connect the audio output of the PC to the **Analog Input** jack. Play the "neural activity.wav" file located in the APM program folder
 - c. An output from a function generator may be used. Ensure that the signal amplitude is below 1Vrms (2.8V peak to peak)
- 4. Set the Input Gain slider in the Main User Interface to 0
- 5. Set the number of simultaneously displayed waveforms to 5.

- 6. Adjust the **Triggering Point** and **Triggering Threshold Level** so that spike waveforms are displayed on the PC.
- 7. Adjust the **Acceptance Window** to a point where some waveforms pass through. Ensure that those waveforms turn green while spikes that don't pass through remain gray.
- 8. Confirm that each time a waveform passes through the **Acceptance Window**, the **Spike Acceptance Output** LED on the front panel turns red.
- Connect the Analog Output of the APM to an oscilloscope input. Connect the Spike Accept Output of the APM to the oscilloscope as an external trigger. Ensure that the time scale of the oscilloscope closely matches that of the Main User Interface.
- 10. Confirm that spikes that pass through the **Acceptance Window** are also being triggered and visible on the oscilloscope.
- 11. Set a function generator to output a 1Hz 0V to 5V square wave. Connect the output of the function generator to the **Trial Gate**, **Trial Validate**, and **Event** inputs on the front panel in turn.
- 12. Confirm that the yellow LED next to each input flashes in sync with the input pulse train on each input.

Calibrating the module

Although the module can accept input signals in the volt range, it is usually connected to headstages/preamps that have output signals in the tens or hundred of millivolts range, and internal PGA (programmable gain amplifiers) stages are used. Therefore it is suggested that the calibration is performed for a 200 mV peak-to-peak input signal and x10 fixed input gain (selected under the **Settings\Input** tab). The **Input Gain** slider has to be in the leftmost position (zero variable gain). With these settings, the displayed gain will be 20 dB in the main panel.

- 11	nput	Gain-		 			 	 		 			
	人	<u></u>	- 10		1	31 		1	1		1	-	Gain: 20.0 dB

Set a function generator to output a 200mV peak to peak sine wave @ 1kHz. Set the time scale of the APM display to 200µS/div. Adjust the digital bandpass filter for a Low of 0Hz and a High frequency cutoff of 20kHz.

Within the Settings dialog, click on the Calibration tab. In the Gain box the signal peak values are

displayed in the Max and Min boxes. The peak-to-peak value is displayed in the Diff box. The input gain is increased by pressing on the up-arrow of the **Gain** panel (pressing the down-arrow decreases its value). Repeatedly click on the up or down arrows, or hold them pressed, until a value of 200mV is displayed in the Diff box.

The offset, calculated as the average of the peak values, can be adjusted in a similar manner. Adjust the function generator output for a 20mV peak to peak sine wave (1000Hz). Under the **Input** tab de-select the x10 gain check box. Return to the **Calibration** tab and adjust the offset such that it oscillates around a value of 0V.

Settings					×
Channel: USB device II	Ch1 D: 0	•			OK
Recording	Input Ca	libration	Module	Auto Adjust	L _
_ Gain —			- Offset-		
Max 🗧	6.23 uV				
Diff	1.05 uV	-	Offset	5.71 uV	
Min	5.18 uV				

Set the APM to continuous mode by selecting the **Continuous** checkbox under the **Input** tab. Calibrate the **Gain** and **Offset** as before.

Adjust the function generator for a 10Hz, 200mV peak to peak sine wave, Adjust the APM vertical scale to 50mV/div. Adjust the gain calibration setting until the displayed waveform is exactly 4 boxes high.

Return the APM to **Spikes** mode under the **Input** tab. Adjust the high cutoff of the bandpass filter back to 20,000 Hz

2.4 **OPERATIONAL INFORMATION**

- 1. Start the main APM control application by clicking the APM icon on the host PC.
- 2. Enter a username in the **User Profile** dialog box. Press the **OK** button.
- 3. The Main User Interface and the Channels window will open.
- 4. Click the **Select** tab of the **Channels** window. Click on channel button number **1** to initiate communication between the APM and the host PC. The channel button will turn green and the message "Comms OK" will be displayed on the status bar.
- 5. Click the **Record** tab of the **Channels** window. Click on channel button number **1** to enable recording (button color will change to green). Actual data collection will start for the enabled channels only when the **Recording** button in the main panel is clicked on.
- 6. Click the **Settings** button in the main control panel. Select the **Recording** tab. Ensure that **Gated Mode** is unchecked. Press **OK** to save the current settings and exit the dialog box.
- 7. If random waveforms that do not pass through the **Triggering Point** (the small red circle on the riggering threshold line), or if no waveforms are displayed in the **Main Waveform Window**, it is very likely that the position of the **Triggering Threshold Level** (horizontal red line) is above or below the range of the input signal. You can manually drag the **Triggering Threshold Level** within the **Main Waveform Window** to move it closer to zero. (Note that the **Auto Adjust** algorithm will slowly do this automatically if no sweeps are crossing the triggering threshold.)
- 8. To enable the **Auto Adjust** mode, click the **Auto Adjust** button in the **Main Waveform Window**, it will change color to green. While **Auto Adjust** is enabled the APM displays one sweep per second, regardless of the triggering threshold level (horizontal red line).
- 9. Once stable waveform displays start passing through the Triggering Point, increase the gain by dragging the Input Gain slider to the right and/or zoom in by pressing the V+ button in the Zoom control portion of the Main User Interface until a reasonably large signal is displayed. Make sure that the input stage does not saturate as a result of increasing the gain by too much.
- 10. Move the **Triggering Point** to the left or the right along the triggering threshold line until the entire spike is displayed within the sweep. The triggering point is adjusted by dragging it to the left or right within the **Main Waveform Window**. This is equivalent to setting the duration of the pre-trigger on conventional oscilloscopes. It may also be necessary to zoom in or out along the horizontal axis by pressing the **H+/H-** buttons in the **Zoom** control portion of the **Main User Interface** to capture the entire spike within the duration of the sweep.
- 11. Adjust the **Hi** and **Lo Filter** settings by dragging the sliders to the right or left to narrow the signal bandwidth and remove unwanted noise.
- 12. Move the Acceptance Window (the white vertical line terminated by two small circles) by dragging it within the Main Waveform Window to capture spikes to be recorded. Spikes that pass through the window will be displayed in bright green (by default, otherwise whatever color has been selected on the display settings page) and will generate a TTL pulse at the Acceptance Output jack on the front panel. The red LED next to the Acceptance Output can be monitored to confirm this. If you are using an external timestamping system that uses the Acceptance Output, you should be able to see the information within that system.

- 13. Adjust the size of the **Acceptance Window** by clicking and dragging the circles at either the top or bottom of the window.
- 14. If at this point you would like to switch from the default **Slope/Height Window Discrimination** mode to **Template Matching**, follow the instructions below.
- 15. If you wish to record data at this point using the default **Slope/Height Window Discrimination** mode, click the **File** button in the **Main User Interface** and browse your disk to select the directory to record in. Enter the data file name, without any extension, and select the desired file format.
- 16. Click the **Recording** button in the **Main User Interface** to begin recording the selected spikes. The button will change color to green when recording. To end the recording session at any time, click the **Record** button again.
- 17. Once the recording session has ended, you can inspect the saved data using the sample Matlab scripts (if data has been saved into APM format), or in NeuroExplorer if you have saved it in PLX format.

To Record in Adaptive Template Matching Mode:

- 1. Follow steps 1 through 13 above to initially set up the APM to acquire spikes.
- 2. Click the **Templates** button in the **Main User Interface.**
- 3. Click the **T1** button in the **Enable** group to enable template 1. A bold line will appear in the main control panel and the thumbnail window indicating the currently stored template. This will initially be a flat line at zero volts.
- 4. Click on the **T1** button in the **Rebuild From Window** group. This will build a template from the spikes that pass through the **Acceptance Window**. Once the template is constructed, you will see it displayed as a bold waveform in the **Main Waveform Window** and in the **Thumbnail Channel Window**.
- 5. Enable Recording for template 1 by clicking on the **T1** button in the **Record** group. This will allow waveform or timestamp data associated with spikes that match template 1 to be saved when the **Recording** button in the **Main User Interface** is enabled. Spikes that are considered close enough to be a match for the template are displayed in the same color as the bold template waveform.
- 6. Adjust the matching **Tolerance** and **Adaptation** parameters as needed using the sliders in the **Template Settings** group.
- 7. Three additional templates can be constructed by following the above four steps using the T2, T3 and T4 buttons.
- 8. If you wish to record data at this point using the default **Slope/Height Window Discrimination** mode, click the **File** button in the **Main User Interface** and browse your disk to select the directory to record in. Enter the data file name, without any extension, and select the desired file format.
- 9. Click the **Recording** button in the **Main User Interface** to begin recording the selected spikes. The button will change color to green when recording. To end the recording session at any time, click the **Record** button again.
- 10. Once the recording session has ended, you can inspect the saved data using the sample Matlab scripts (if data has been saved into APM format), or in NeuroExplorer if you have saved it in PLX format.

2.5 SCHEDULED MAINTENANCE

The Functional Checkout in section 2.3 of this manual should be performed on a yearly basis. The procedure for Gain and Offset calibration described in the Functional Checkout should be performed on a yearly basis and/or whenever the user changes the headstage, or inserts more signal conditioning modules in the signal path.