nanoZ™

User Manual

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Welcome to the nanoZ

Your nanoZ was specifically designed for multichannel microelectrode arrays. It features extremely low test currents for measuring impedance or electrode impedance spectroscopy (EIS). Several inbuilt electroplating modes are provided for automated electrode impedance matching, activation, and cleaning.

FEATURES

- 64 channels
- 1k ~ 100MΩ working range
- 1Hz ~ 5kHz test frequencies
- 1kΩ display resolution, 1% accuracy or better for 5k ~ 15MΩ electrodes at test frequencies < 2kHz
- low (< 1.4nA) test currents suitable for *in vitro* or *in vivo* testing
- constant current electroplating with ±12µA range, 5nA resolution, ±5V compliance
- intuitive graphical user interface
- software development kit for Matlab

The setup and operation of the nanoZ is described in this user manual, including detailed information about the Matlab Software Development Kit (SDK) for developing your own, customized nanoZ applications.

What's in the box?

Each nanoZ comes supplied with:

- o nanoZ device
- o USB 2.0 cable
- o NZA-DIP16 adaptor
- o NZ-CAL test adaptor
- o 3-pin to alligator clip cable
- o Installation CD
- o (4) stick-on feet (optional)

If any item is missing or appears to be damaged or faulty, please contact the distributor from whom you purchased your nanoZ.

Software installation

1. Connect your nanoZ to the computer using the USB cable provided. Let Windows search for the driver on the installation CD (in the Drivers subfolder). Follow the on-screen instructions to install the USB drivers. Note that Windows 7 may have compatible drivers inbuilt, which are fine to use instead.

2. Run 'setup.exe' from the installation CD. Follow the on-screen instructions to install the application suite, including the optional Matlab SDK if you want to develop your own nanoZ applications under Matlab.

3. The software is ready for use. You can run the nanoZ control program from the Windows Start Menu or Desktop Shortcut.

> If you are re-installing or updating the software and either the 'electrodes.ini' or 'prefs.ini' files already exist, the installer will ask if you want to overwrite these files. If you select no, the old files will remain unchanged. If you select yes, the old files will be renamed with a '.bak' extension, and the new '.ini' files will take their place. You can then use a text editor to copy any electrode or adaptor maps from the '.bak' file to the new 'electrodes.ini' file.

There is currently no standalone application for computers running Mac OS X, however there is Matlab support for Intel-based Macs. To use the nanoZ Matlab SDK on your Mac, install the device driver by opening the 'D2XX.1.0.4.dmg' package in the 'Drivers\OS X' subfolder on the installation CD, and follow the instructions in the 'readme.rtf' file. Next, copy the 'Matlab SDK' folder to your hard drive. This folder contains the necessary Matlab '.mexmaci/.mexmaci64' support files, and the same example scripts that are installed with the Windows version of the nanoZ Matlab SDK.

Firmware updates

The firmware version currently installed on your nanoZ can be determined by selecting the **Help I About** box from the main menu. When new versions of the firmware are released, follow these instructions to update the firmware:

1. Plug in the nanoZ and run the 'nanoZ' application.

2. Select **Device I Update firmware** from the main menu.

3. Click **File** to select the new firmware (e.g. 'nanoZ_firmware_1_15.nzf').

4. Select the nanoZ device you wish to update by serial number. If only one device is plugged in, only one serial number will appear in the drop-down list.

5. Click **Update**. While the new firmware is being uploaded the green LED on the nanoZ will flicker. Do not unplug the nanoZ during the upload. The message 'Firmware updated successfully' indicates when the process is complete. You can now click **Exit** and continue using the nanoZ application.

6. If the update is interrupted or cancelled the nanoZ may not function properly. Repeat these instructions to upload the firmware completely.
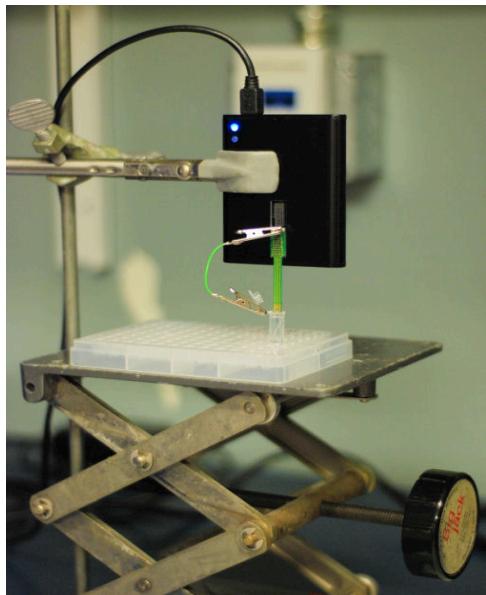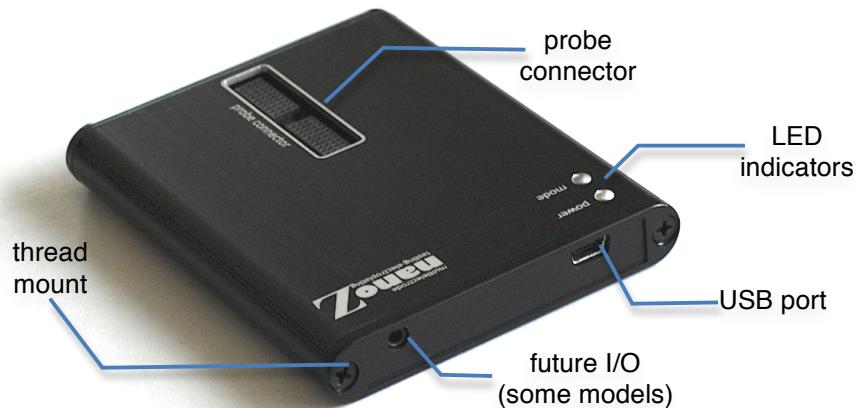
> ⚠️ It is advisable to update both the firmware and the software suite to the most recent versions at the same time. Older firmware and device drivers may not work properly with the latest software, and *vice versa*.
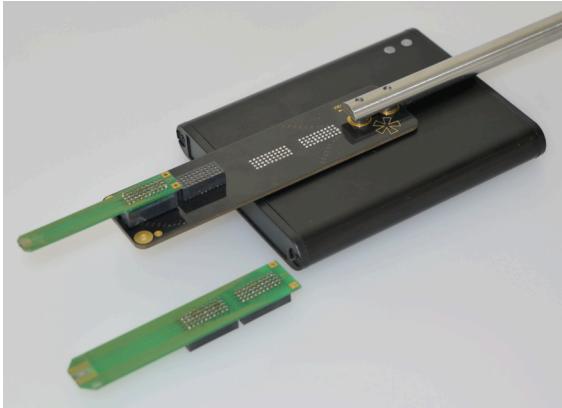
The latest software and firmware updates can be obtained from the download page of your vendor's website, or from here: http://whitematter.serveftp.net.

Setting up the nanoZ
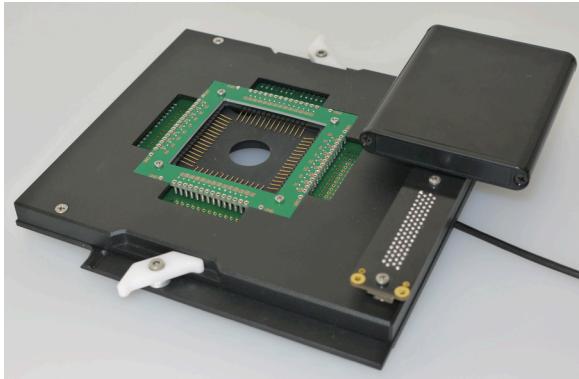
The nanoZ requires no additional hardware other than a PC with a USB port.

A simple way to mount the nanoZ is with a regular laboratory retort stand and a three-prong clamp. Position the nanoZ over a beaker of saline (or plating) solution. With this setup, it is easier to put the beaker on a height-adjustable laboratory jack and raise or lower the jack to immerse the probe in the solution, rather than adjusting the height of the nanoZ on the stand.

Alternatively, MultiChannel Systems Inc. provides an adaptor (sold separately) with an integrated aluminum rod that can be attached to a micromanipulator. This mounting option is also suitable for *in vivo* applications. A recalibration is recommended if you intend to use this adaptor (see Appendix E).

To use the nanoZ with *in vitro* electrode arrays from MultiChannel Systems, plug the MCS MEA adaptor into the nanoZ, then turn the nanoZ face down and plug it into the MEA's 68 pin Honda socket, as shown here.

If the probe is connected directly to the nanoZ (e.g. a NeuroNexus A32 or A64 packaged probe), use a short jumper wire to connect one of the 'G' pins on the probe PCB to the bath electrode. If using a NZA series adaptor, such as the supplied NZA-DIP16, connect the bath electrode to the adaptor using the 3-pin-alligator cable supplied. A piece of platinum or platinum-iridium alloy wire immersed in the bath solution makes an ideal reference connection, as does a silver-silver chlorided wire or other inert metal. With the MCS MEA adaptor (shown above) the reference connection of the bath is already connected to the nanoZ's reference input, so no external reference wire is required.

For other 3rd party adaptors, refer to the documentation provided to determine the appropriate point of connection to the bath electrode.

Take care not to expose the nanoZ to liquids of any kind. If liquid gets splashed on the nanoZ, wipe it off with a moist cloth and allow to dry. If solution gets spilled inside the nanoZ, immediately unplug it and remove any attached electrodes or electrode adaptors. For water spills, allow the nanoZ to dry completely before resuming use. For saline or other solutions, remove the two screws that secure the end-cap closest to the probe connector. It may be necessary to remove both end-caps. Slide out the printed circuit board, being careful not to damage the probe connector on the enclosure opening. Flush with

distilled water to remove all traces of the spillage, and allow the circuit board to completely dry before re-assembling the nanoZ.

Probe adaptors

Omnetics NZ-EIB-36 (Neuralynx)

Probes or electrode arrays that have Samtec connectors (i.e. model number MOLC-110-01-S-Q) can be plugged directly into the nanoZ, assuming they conform to the nanoZ's native pin mapping (see Appendix C).

The nanoZ comes supplied with a NZA-DIP16 adaptor for probes that use a DIP16 connector, and a variety of other adaptors are available for commonly used interconnects such as those from Omnetics, Millmax, and Hirose. Contact your vendor for information about the pin mapping of these adaptors.
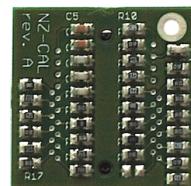
All NZA series adaptors should be plugged into nanoZ so that the 3-pin header on the adaptor is closest to the USB port end of the nanoZ. If using the NZA-DIP16 adaptor mapping defined in 'electrodes.ini', be sure to plug the adaptor into the 'lower' Samtec socket closest to the bottom edge of the nanoZ.

In the current version of the nanoZ, only R1 is connected to the internal circuitry. R2 and R3 are reserved for future expansion. If you wish to make your own reference electrode cable, be sure that it connects to R1 (see Appendix C).

R1

plug adaptor into the nanoZ with this end oriented towards the USB

Calibration adaptor

The nanoZ is supplied with a 32 channel calibration adaptor (NZ-CAL) that comprises a bank of resistors and capacitors of various impedances (Appendix D). Use this adaptor to check the accuracy of your nanoZ across the specified working range (Appendix B).

The nanoZ does not require routine calibrations, however future firmware and software upgrades may require a device re-calibration, for example, to extend the nanoZ's functionality or working range. Impedance recalibration may also be needed to compensate for 3rd party electrode adaptors or extension cables that may otherwise introduce errors in the impedance measurements.
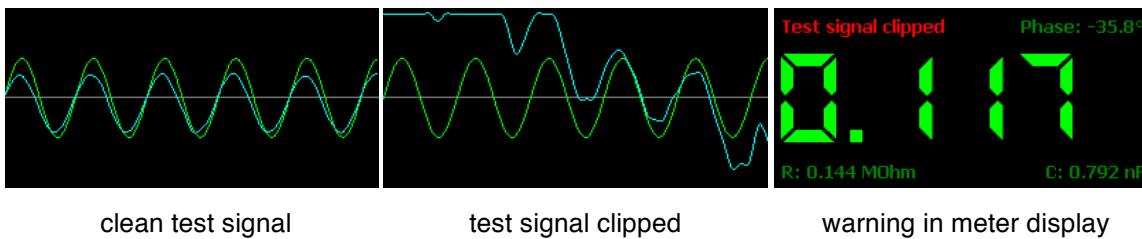
⚠ The nanoZ's electroplating circuit has a native resolution of ~100nA. To enable smaller currents and the ~1nA electroplating accuracy offered by the nanoZ (software v1.4.0 or later), devices shipped prior to April 2012 need to be recalibrated (see Appendix E).

The calibration adaptor should be orientated with the 'NZ-CAL' text closest to the USB port, and the software should be set to No Adapter / Probe not selected.

Tips for accurate Z measurements

The nanoZ is capable of providing very accurate measurements across a wide range of test frequencies and impedances (Appendix B). Nonetheless, care should be taken to protect the test setup from electromagnetic interference (EMI) such as 50/60Hz power line interference. The nanoZ is susceptible to EMI because it uses very small test signals for measuring impedance. To ensure accurate results, periodically check the signal quality using the Scope display of the nanoZ application (or the signal buffers if using the Matlab SDK). Moderate levels of power line noise or band-limited noise outside the impedance test frequency of interest are not a problem. However, if the test signal is excessively noisy and the amplifier is saturated (i.e. the cyan trace on the scope is either not visible or appears 'clipped'), then the measured impedance will not be accurate. The nanoZ software will report if any part of the test signal is clipped, and highlight in yellow the affected channels in the Report window.

| clean test signal | test signal clipped | warning in meter display |

If necessary, enclose the setup in wire mesh or aluminum foil to shield it from EMI, and be sure to connect the shield to the reference wire. Move the setup away from common sources of 50Hz noise, in particular fluorescent table lamps, electric motors, or other lab equipment. In especially noisy environments it may also help to run the nanoZ on a battery-powered laptop.

Clipping of the test sinusoid may also occur immediately after switching channels, or if an impedance measurement is made immediately after applying an electroplating current, before charge on the electrode site has had sufficient time to dissipate. In both cases the nanoZ will report an artifactually low post-plating impedance and a warning message, and attempt to re-test the electrode several times before proceeding to the next channel. Refer to the subsequent sections describing the various automated modes of operation for how to avoid this artifact if and when it arises.
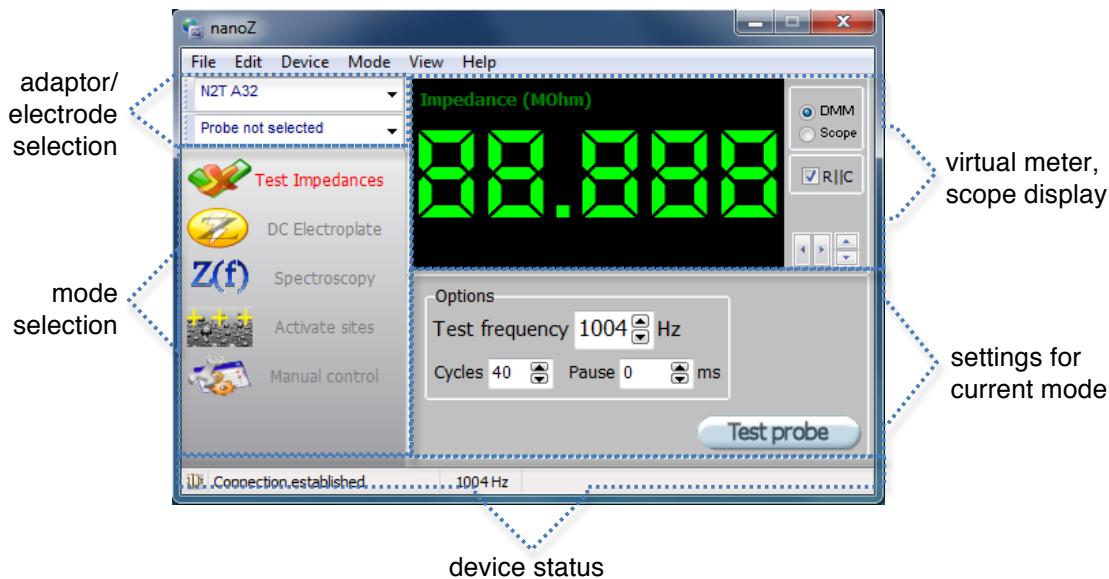
Finally, we recommend that the electrode and reference connections are kept as short as possible. Long wires may distort results due to their capacitance. Avoid open ends which act as antennas.

nanoZ Software

Overview

There are two options for interfacing with the nanoZ: a Windows-based nanoZ application, and the Matlab SDK, which runs under Matlab. Refer to the 'Matlab software development kit' section of the User Manual for detailed instructions on how to control the nanoZ from Matlab.

The nanoZ application can be run from the Windows Start menu or Desktop shortcut. The application will connect with the first available nanoZ device. If one or more nanoZs are plugged into the USB port after the application has already started, they can be selected from the **Device** list on the main menu.

An intuitive graphical user interface makes the nanoZ easy to use.

User-programmable electrode site configurations (see Probe window) provide a meaningful way to visualize the integrity of the electrode array, and allow subsets of channels to be tested and/or conditioned by selecting the relevant sites with a few mouse clicks.

A virtual digital multimeter (meter) displays impedance measurements or the real-time plating voltage, depending on the mode of operation. If **RIIC** is checked, the resistive and capacitive components of the current impedance measurement will be displayed, assuming a parallel RC equivalent circuit model.

Clicking on **Scope** or selecting **View I Scope** from the main menu will display a virtual oscilloscope with the Z test or plating waveform (output) in green, and the measured (input) waveform in cyan. Use the arrow icons to the right of the scope to change the horizontal (time) and vertical (amplitude) zoom. To switch back to the meter display, click **Meter** or select **View I Meter** from the main menu.

The **status bar** displays information about the nanoZ device hardware, including error conditions. The LED icon on the left hand corner of the status bar reflects the mode LED on the actual nanoZ device, and indicates the currently selected mode of operation: off for passive mode, glowing green for impedance mode, and glowing red for electroplating mode.

Impedance data are displayed in an Excel-like spreadsheet (see Report window), which can be saved to file for storage or further analysis in programs like Microsoft Excel or Matlab.

The mode-specific settings for the various automated modes of operation are described in the next chapter of the User Manual.

Channel mapping

To support the various electrode adaptors that are available for the nanoZ, the nanoZ application seamlessly translates the probe site layout to the adaptor pin mapping, and from the adaptor pins to the nanoZ's internal multiplexer (MUX). By mapping in two stages, rather than directly from the probe layout to the nanoZ, it

| probe site layout | adaptor pin mapping | nanoZ MUX channel |

is unnecessary to construct a new probe mapping for different adaptors.

Both adaptor mappings and electrode site layouts are defined in 'electrodes.ini'. Refer to Appendix C for information on how to configure additional probe layout and adaptor mappings.

Adaptor window

Adaptors can be selected from the drop-down list of known adaptors (i.e. those defined in 'electrodes.ini') located in the left top corner of the nanoZ main application window. Select **View I Adaptor** from the main menu to display a window depicting the adaptor. If no adaptor is attached to the nanoZ, selecting **No Adaptor** from the drop-down list of adaptors will show the layout of the 64 channels used by the native connector.

When a channel is active, the corresponding pin on the Adaptor window is highlighted. In passive or impedance testing mode, the pin is highlighted in green; in electroplating mode the pin is highlighted in red. In Manual Control mode (only), left clicking on an adaptor pin will switch the nanoZ to the channel that corresponds to that pin.

Channels that have no connection for a given adaptor (as defined in 'electrodes.ini') cannot be selected, regardless of operating mode. For example, the NZA-DIP16 adaptor uses 16 of the 64 available channels, and accordingly only these 16 channels can be selected. To re-enable all 64 channels, select **No Adaptor**.
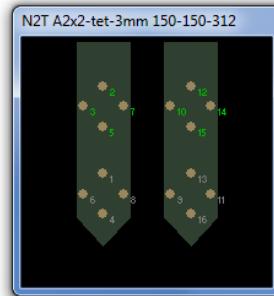
Probe window

The nanoZ application supports arbitrary probe site configurations and, once defined in 'electrodes.ini', handles the channel mapping transparently. Several example probes are provided with the default installation. Probe definitions can be modified and new definitions can be added; refer to Appendix C for a detailed description on how to do this.

Select **View I Probe** from the main menu to display a window showing the probe electrode site layout with numbered sites.

When a channel is active, the corresponding electrode site on the Probe window is highlighted. In passive (channel selected) or impedance testing mode, the site is highlighted in green; in electroplating mode the site is highlighted in red.

The probe layout window can be used to select a subset of electrode sites for testing or electroplating. You can select and deselect one or multiple electrode sites using the mouse. Double clicking the left mouse button selects or deselects all sites. Selected site numbers are displayed in green; deselected sites are displayed in grey.

The Probe window site selection applies to all automated modes of operation, but not the Manual Control mode channel selector. In Manual Control mode (only), left clicking on a site switches the nanoZ to the channel corresponding to that site, taking into account the adaptor mapping. This provides an easy way to test the impedance of selected sites by simply clicking on the sites of interest.

The Probe window can also be used to visualize impedance test results according to the probe site layout, which may be more intuitive than reading tabular results. Once a probe has been tested, holding down the right mouse button highlights the condition of every recording site (red for short, blue for open, green for normal, according to the settings in the Report window). With the right

mouse button held down, moving the mouse pointer over individual electrode sites will display a small pop-up window with the measured impedance magnitude and phase for that site.

Report window

When using any of the automated modes of operation, the nanoZ stores impedance and electroplating results in an Excel-like spreadsheet.

Probe Report

Probe
Name: N2T A2x2-tet-3mm 150-150-312
Description: NeuroNexus 2x2 Tetrode
Last tested: 6/24/2010 at 12:04:17 AM

Condition
Short 200 k
Open 5000 k
25% of sites good

Show
☑ Phase
☑ Statistics
☑ Condition

| | 1004 Hz | | 0.480µA | 1004 Hz | |
	Mag (MOhm)	Phase(°)	(hh:mm:ss)	Mag (MOhm)	Phase(°)
2	2.309	-1.2	00:00:05	2.319	-1.7
3	5.097	-1.9	00:00:05	5.126	-2.4
5	7.600	-2.7	00:00:05	7.716	-0.8
7	7.629	-1.2	00:00:05	7.739	-3.5
9	0.002	-18.6	00:00:05	0.000	-134.0
11	0.005	-1.3	00:00:05	0.005	-0.3
13	0.050	0.2	00:00:05	0.050	-0.4
16	0.520	-0.5	00:00:05	0.521	-0.3
	1.415±1.265	-0.8±0.5		1.420±1.271	-1.0±1.0

To view or hide the report window, select **View | Report** from the main menu.

The upper panel of the report shows information about the currently selected probe and when it was most recently tested, user-defined criteria for flagging whether or not an electrode site is faulty, and several display options.
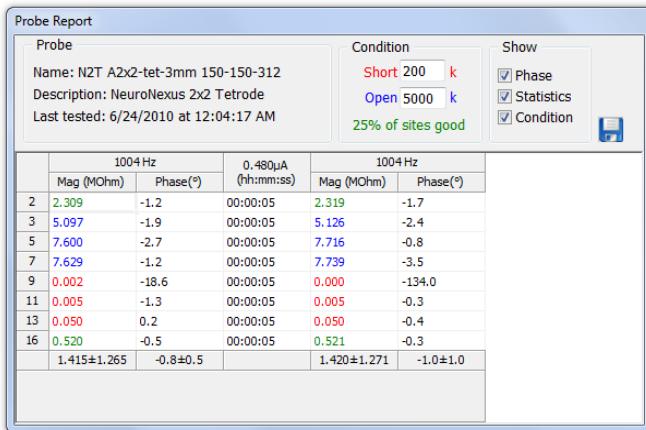
If an electrode has been selected in the main nanoZ program, the probe name and description is shown. This metadata is extracted from the 'electrodes.ini' file. The time and date when the last impedance test or plating procedure began is also displayed.

The **Short** and **Open** user input boxes are editable. An electrode is considered shorted when the measured impedance magnitude falls below the value entered in **Short**, and is considered open circuit when the impedance magnitude is greater than the value entered in **Open**. Impedances within this range are considered normal.

The left-most column in the spreadsheet displays the electrode site index.

The column titles show the impedance test frequency and, if applicable, the plating current that was applied. In electrode impedance spectroscopy mode there will be additional columns, two for each test frequency.

The body of the spreadsheet contains the *most recent* site impedance measurements for all tested sites. If the **Phase checkbox** is checked, then both the impedance magnitude and phase are displayed. If unchecked, only the impedance magnitude is shown. For electroplating modes, the cumulative plating time is shown for each individual electrode, which may vary from site to site, for example, if impedance matching mode was run. Both pre- and post-plating impedances are stored in separate columns to the left and right of the cumulative plating time, respectively.

If the **Statistics checkbox** is checked, then the average impedance magnitude and phase ± standard deviation of all tested sites is shown at the bottom of each column. Note that if the **Condition checkbox** is checked, only 'normal' sites are included in these aggregate statistics.

If the **Condition checkbox** is checked then shorted site impedance values are highlighted in red on the spreadsheet and Probe layout window. Open sites are highlighted in blue. Normal sites are displayed in green.

The report can be saved to file by clicking the 🖫 icon in the Report window, or selecting **File I Save report** from the main menu. Data are saved in tab-delimited ASCII format according to the current checkbox settings (i.e. phase information will be exported only if the **Phase checkbox** is checked, aggregate statistics are exported according to the **Statistics checkbox**, etc).

Operating Modes
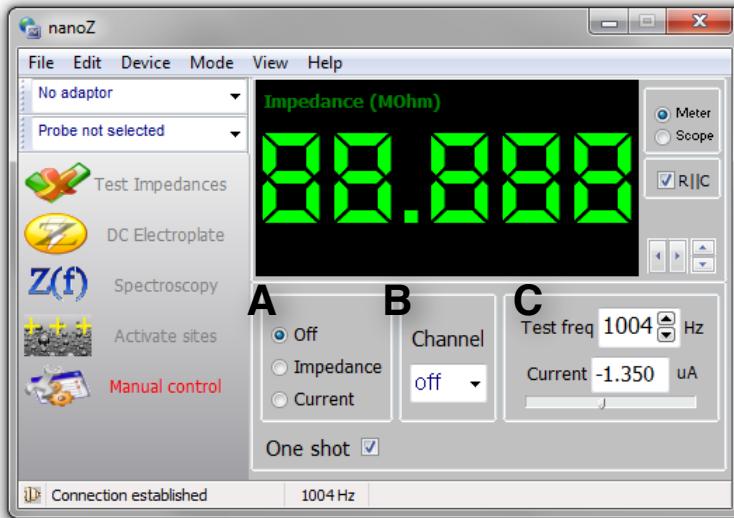
The nanoZ application has five separate modes of operation for performing a variety of different tasks. These modes can be selected by clicking a button on the left pane of nanoZ application or via the **Mode** menu on the main menu.

The following instructions assume that a suitable electrode adaptor and probe configuration have been chosen, and the electrode sites of interest have been selected in the Probe window.

The screenshots below were taken from Windows 7. The appearance of the user interface may vary depending on the version of Windows you are using.

Manual control mode

This mode provides manual control of the nanoZ's channel selection, impedance testing, and DC constant current electroplating functions.

Selecting **Impedance** (**A**) will continuously measure the impedance of the currently selected **channel** (**B**) at the specified **Test freq** (**C**). The impedance (in Mohms), and phase angle (in degrees) will be displayed on the Meter. If the test signal is saturated (e.g. high levels of 50 or 60Hz noise) the 'Test signal clipped' warning message will be displayed and the Meter will show the last valid impedance measurement.

If **One shot** is checked, a single impedance measurement will be made. To stop continuous impedance measurements, select **Off** (**A**).
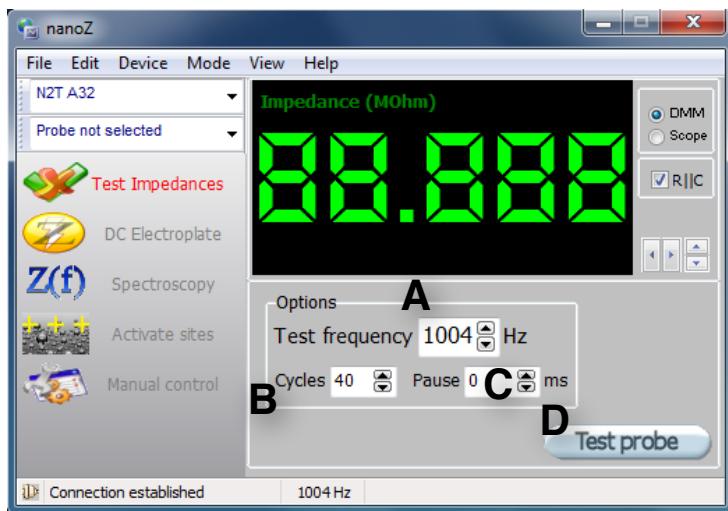
Selecting **Current** (**A**) mode will apply a DC constant current to the currently selected **channel** (**B**) at the level indicated adjacent to the **Current level** slider (**C**). The current can be adjusted from -12uA (electrode negative) to +12uA (electrode positive) in ~1nA steps by moving the position of the slider or typing the desired current into the edit box. The voltage across the electrode site will be displayed on the Meter. The Meter has limited measurement resolution (39mV) and should be considered approximate, however the calibrated current reading shown in the edit box is accurate to within ±5nA or 5% (whichever is greater). The Meter also indicates if the voltage is out of compliance, that is, if the voltage needed to achieve the desired current is greater than the nanoZ can generate. To stop applying the current, select **Off**.

The nanoZ's electroplating circuit has a native resolution of ~100nA. To enable smaller currents and ensure the specified electroplating accuracy, devices shipped prior to April 2012 need to be recalibrated (see Appendix E).

Impedance test mode

This mode rapidly cycles through all (or a subset) of the channels on the selected probe, measuring the impedance of each electrode. The impedance results are tabulated in the Report window (**View | Report**).

(**A**) Set the impedance **Test frequency** to the desired value. The actual test frequency that will be used is shown in the status bar.

(**B**) Choose the number of **Test cycles**. The default setting of 40 cycles is a good trade-off between accuracy and speed. Increasing the number of cycles will marginally improve the accuracy due to averaging, at the expense of a longer testing duration, whereas decreasing the number of cycles may lower accuracy due to insufficient localization of the test signal in the frequency domain. The testing time per site is displayed in the status bar.

(**C**) The **Pause** setting specifies a delay, in millisconds, after switching channels before impedance testing is begun on the currently selected electrode. In most cases this delay can be set to zero, however with some high capacitance electrodes the test signal may take some time to settle to baseline after switching channels. Increase the pause setting if the 'test signal clipped' warning is reported or some channels are not measured (i.e. skipped measurements are blank and highlighted in yellow in the Report window).

(**D**) Click the **Test probe** button to begin the test.

Clicking on **Test probe** again or pressing **ESC** at any time will pause the test, and you will be given the option of continuing the test or stopping.

Impedance test results in the Probe report can be saved by clicking the 💾 icon or selecting **File | Save report** from the main menu.

DC electroplate mode

This mode cycles through all (or a subset) of the electrodes on the selected probe, applying a controlled DC constant current to each site. It has two sub-modes of operation: fixed plating time per channel; and match impedances mode, whereby the nanoZ will only advance to the next channel when the electrode site impedance is lowered to the specified Target impedance.

The DC Electroplate mode has many possible applications, such as:

- plating with gold or other metals to lower electrode site impedances
- deposition of conductive polymers such as PEDOT to simultaneously lower the electrode site impedance and improve the site charge capacity
- electrode site cleaning
- *in vivo* or *in vitro* rejuvenation of electrode sites
- tissue lesioning and electrode track marking

(**A**) Select the plating mode, either **Fixed plating time**, or **Match impedances**.

(**B**) Set the desired DC plating current. The current can be adjusted from -12uA (electrode negative) to +12uA (electrode positive) in ~1nA steps by moving the position of the slider or typing the desired current into the edit box. The Meter has limited measurement resolution (39mV) and should be considered approximate, however the calibrated current reading shown in the edit box is accurate to within ±5nA or 5% (whichever is greater).

> The nanoZ's electroplating circuit has a native resolution of ~100nA. To enable smaller currents and ensure the specified electroplating accuracy, devices shipped prior to April 2012 need to be recalibrated (see Appendix E).

(**C**) For a **fixed plating time** per site, set the desired plating **Duration**. If **Test Z** is checked, the electrode impedances will be measured before and after plating at the specified test frequency. The **Pause** setting specifies a delay between electroplating and testing the post-plating impedance, which may be necessary for some plating procedures where the test signal can take several seconds to settle to baseline due to charge buildup on the electrode site. In most situations this delay can be set to 0~1 second. Use the scope display to check that the impedance test signal is at or close to baseline, and increase the delay if the 'test signal clipped' warning is reported.

For **match impedances** mode, set the desired **Target** impedance, test frequency, and plating **Interval**. The plating interval specifies how long to apply the plating current before re-testing the electrode impedance. For example, a setting of 5 (with a pause of 3) will apply the specified current for 5 seconds, wait 3 seconds before testing the impedance, and alternate between these two modes every 8 seconds until the target impedance is achieved. The total plating time per electrode is limited by the **Runs** setting. Once this number of plating cycles has occurred the nanoZ will advance to the next channel even if the target impedance has not been reached. Note that if the initial electrode impedance measurement is already less than or equal to the target impedance, no current will be applied to that electrode.
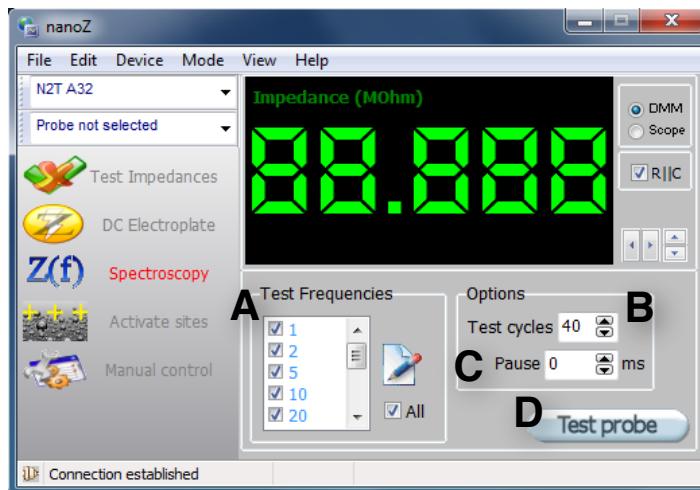
(**D**) Click the **Autoplate** button to begin the electroplating sequence.

Clicking on **Autoplate** again or pressing **ESC** at any time will pause the plating, and you will be given the option of skipping the current electrode (**Ignore**), continuing the sequence, or stopping.

The (pre- and post-plating) impedance test results and cumulative plating time per site will be displayed in the Probe report window. The report can be saved by clicking the 💾 icon or selecting **File I Save report** from the main menu.

Impedance spectroscopy mode

This mode cycles through all (or a subset) of electrodes on the selected probe, measuring the impedance of each electrode at multiple test frequencies.

(**A**) Select the impedance **Test Frequencies** (in Hz) from the check-box list, or check **All** to test at all frequencies in the list. Click the notepad icon to edit the list of test frequencies. Valid frequencies are between 1Hz and 4986Hz. The nanoZ will generate sinusoidal test waveforms as close as possible to the specified test frequencies. The exact test frequencies used will be displayed in the status bar and in the column titles of the Probe report.

(**B**) Choose the number of **Test cycles**. The default setting of 40 cycles is a good trade-off between accuracy and speed. Increasing the number of cycles will marginally improve the accuracy due to averaging, at the expense of a longer testing duration, whereas decreasing the number of cycles may lower accuracy due to insufficient localization of the test signal in the frequency domain. The total testing time per site is displayed in the status bar.

(**C**) The **Pause** setting specifies a delay, in millisconds, after switching channels before impedance testing is begun on the currently selected electrode. In most cases this delay can be set to zero, however with some high capacitance electrodes the test signal may take some time to settle to baseline after switching channels. Increase the pause setting if the 'test signal clipped' warning is reported or some channels are not measured (i.e. skipped measurements are blank and highlighted in yellow in the Report window).

(**D**) Click the **Test probe** button to begin the test.

Clicking on **Test probe** again or pressing **ESC** at any time will pause the test, and you will be given the option of continuing the test or stopping. The

impedance spectroscopy results in the Probe report can be saved by clicking the 💾 icon or selecting **File I Save report** from the main menu.

Activation mode

This mode can be used to increase the charge capacity of all (or a subset of) electrodes on the selected probe. Activation mode applies a bipolar, variable duty cycle, constant current square-wave to the electrode, which both lowers the impedance and increases the charge capacity, two properties that are desirable for effective electrical stimulation.

With suitable electrodes and appropriate waveform settings, the activation mode can also be used for *in vivo* or *in vitro* microstimulation.

(**A**) Only the **Fixed activation time** is implemented in the current software version, so this setting is currently ignored.

(**B**) Set the **Activating current**. Both current settings can be adjusted from -12uA (electrode negative) to +12uA (electrode positive) in ~100nA steps, by moving the position of the sliders. The Meter has limited measurement resolution (39mV) and should be considered approximate, however the calibrated current readings adjacent to the sliders are accurate to within a few nA. Note that the finer (~1nA) resolution provided by the DC Electroplate and Manual electroplating modes is not currently available in Activation mode.

(**C**) Set the desired activation **frequency**, the **Duty** cycle, and the activation **Duration** per electrode. The **Duty** cycle determines the relative time of the two current phases, where the percentage refers to the first current setting in (**B**). If **Test Z** is checked, the electrode impedances will be measured before and after electrode activation at the specified test frequency. The **Pause** setting specifies a

delay between the activating cycle and testing the post-activation impedance. It may be necessary for some plating procedures where the test signal can take several seconds to settle to baseline due to charge buildup on the electrode site. In most situations this delay can be set to 0~1 second. Use the scope display to check that the impedance test signal is at or close to baseline, and increase the delay if the 'test signal clipped' warning is reported.

(**D**) Click the **Activate probe** button to begin the activation sequence.

To illustrate a typical activation sequence, using the settings shown above as an example (i.e. Z test enabled, 1Hz activation frequency, 25% duty cycle, currents -3uA to +5uA, duration 30s, pause 3s), the nanoZ will test the pre-plating electrode impedance at 502Hz, apply 30 square wave activation cycles comprising a 3uA electrode-negative current for 250ms immediately followed by a 5uA electrode-positive current for 750ms. After a pause of 3s the post-plating electrode impedance will be measured, and the nanoZ will advance to the next selected electrode site.

Clicking on **Activate probe** again or pressing **ESC** at any time will pause the activation, and you will be given the option of skipping the current electrode (**Ignore**), continuing, or stopping.

The (pre- and post-activation) impedance test results in the Probe report can be saved by clicking the ![save icon] icon or selecting **File | Save report** from the main menu.

Matlab Software Development Kit

The nanoZ Matlab SDK allows users to program customized nanoZ applications that are not supported by the bundled Windows program.

To use the Matlab SDK you should have a licensed copy of Matlab installed on the host PC (version 2006a or later) and have the nanoZ SDK installed (refer to the Software Installation section of this User Manual).

nanoz MEX library

MATLAB applications can access the nanoZ through the 'nanoz' MEX files. Four versions are provided, one each for 32- and 64-bit versions of Matlab running under Windows ('nanoz.mexw32' and 'nanoz.mexw64', respectively) and Mac OS X ('nanoz.mexmaci' and 'nanoz.mexmaci64', respectively).

A MEX file can contain only one Matlab function, and because the nanoZ SDK provides multiple functions, the actual function names are passed as the first argument when calling the 'nanoz' gateway function. The following syntax is used for all function calls:

[<output>] = nanoz('function name', arg1, arg2, ... argN);

The **function name** is not case sensitive and can be one of the following:

Name	Purpose
getversion	Returns the version of the nanoZ MEX file
enumdevs	Enumerates attached nanoZ devices
open	Opens a free nanoZ device for access by other functions
close	Closes an opened nanoZ device, releases system resources
getdeviceversion	Returns hardware and firmware information for the open device
selectchannel	Selects a channel
setfreq	Sets the test frequency for impedance measurements
startimpmetering	Starts impedance measurement
getimpdata	Retrieves impedance measurement data
getwaveformcaps	Returns information about the waveform generation capabilities

getplatingcaps	Returns information about the electroplating capabilities
preparewaveform	Prepares a waveform for use with the startplating function
startplating	Starts electroplating with an arbitrary waveform
startplatingdc	Starts DC electroplating with fine control of the current setting
getplatingdata	Retrieves electroplating voltage feedback data
stop	Stops impedance metering or electroplating

Sample scripts

The following demonstration Matlab scripts are provided:

- o **get_info.m**: display hardware information about all attached nanoZ devices
- o **measure_impedance.m**: measure the impedance of a single electrode
- o **electroplating.m**: electroplating with a DC constant current
- o **plate_to_impedance.m**: a more complex example that replicates the nanoZ application's 'match impedances' electroplating mode
- o **ximpedances.m**: tests all unique pairs of channels for shorts, pausing and prompting the user to connect the reference wire to the next channel
- o **electroplating_test_precision.m**: use this script with the NZ-CAL adaptor to validate the electroplating accuracy, for example, after a re-calibration

These sample scripts, along with the supporting functions they call, should provide a foundation for building you own customized nanoZ applications. They can be found in the Matlab SDK folder, accessible from the short-cut in the Windows Start Menu (for OS X, wherever the Matlab SDK directory was copied).

Function descriptions

getversion

Returns the version of the nanoz MEX file.

Usage: [major_version, minor_version] = nanoz('getversion');

Arguments: none.

Return values: major_version, minor_version – major and minor versions of the currently installed nanoz MEX file, respectively.

enumdevs

Enumerates attached nanoZ devices.

Usage: devs = nanoz('enumdevs');

Arguments: none.

Return value: devs – cell array of strings, one string per each attached device, containing the device-specific serial numbers.

Remarks: If a device is attached but already opened by this MEX file, or another application, it will not be enumerated.

An empty cell array is returned if no devices are attached or available.

open

Opens and allocates a free nanoZ device for access by other functions.

Usage: handle = nanoz('open', serial_number, buffer_transfer_size);

Arguments: serial_number – string, device serial number, which can be obtained using the enumdevs function; buffer_transfer_size – packet size, in bytes, for incoming data stream, range 32~1024.

Return value: handle – number, handle to the opened device to be used to access all other device functions.

Remarks: If a nanoZ device with a given serial number is not attached, or is already opened by this MEX file or another application, this function will fail. The buffer_transfer_size determines the temporal resolution of the plating mode. 64 is a good default value, however on slower machines this may need to be increased

to avoid buffer overrun errors. Making it too large, however, will make the nanoZ less responsive in impedance mode, and limit control of the plating duration. The plating duration resolution is given by dividing the buffer_transfer_size value by the sampling rate (fs_adc). For the recommended value of 64, the minimum plating time/increment is approximately 6ms.

close

Closes an opened nanoZ device and releases system resources.

Usage: nanoz('close', handle);

Arguments: handle – handle to an opened device returned by the open function.

Return values: none.

Remarks: User Matlab programs must close the device after they have finished using it, to release system resources and allow other programs access. If a program fails to close the device, it will stay open until Matlab finishes or until the nanoz MEX file is unloaded.

To unload the MEX file, issue the Matlab command 'clear nanoz', which will unload the MEX file, closing all open nanoZ devices.

getdeviceversion

Gets hardware and firmware version information from an open nanoZ device.

Usage: dev_ver_info = nanozm('getdeviceversion', handle).

Arguments: handle – handle to an active device returned by the open function.

Return value: dev_ver_info – a Matlab structure with the following fields:

    hardware_version_id – nanoZ device version ID

    hardware_version_str – nanoZ device model name (string)

    firmware_version – a vector of two elements containing the major and
                    minor firmware revision numbers

    firmware_version_str – the firmware version number (string)

Remarks: Firmware versions 1.13 and earlier do not have a feature to report their version numbers and therefore they cannot be distinguished by this function. The firmware_version_str field of the returned structure will contain "1.13 or earlier".

selectchannel

Selects a channel to perform impedance measurement or electroplating on.

Usage: nanoz('selectchannel', handle, channel);

Arguments: handle - handle of an opened device returned by the open function; channel – channel number to select, from 1 to 64, or 0 to de-select all channels.

Return values: none.

Remarks: This function connects the measurement circuit to a specific channel. Prior to switching channels any impedance measurement or electroplating that was in progress is automatically stopped. Channel switching may cause a transition on the measurement circuit that lasts about 0.5 seconds. To preserve accuracy impedance measurement is not recommended during this period.

After switching channels, the circuit is put into passive mode, not applying any voltage or current to the target electrode.

setfreq

Sets the test frequency used for impedance measurement.

Usage: [freq_achieved, fs] = nanoz('setfreq', handle, freq_desired);

Arguments: handle – handle to an opened device returned by the open function;

freq_desired – The desired test frequency, in Hz. Range

Return values: freq_achieved – The frequency, in Hz, which can be generated by the device, as close as possible to the desired frequency, and on which the actual measurement will be performed.

fs – optional return value. It receives the sampling frequency used for signal measurement.

Remarks: This function prepares the device for impedance measurement. During measurements, the nanoZ applies a sinusoidal current on the target electrode and records the voltage across at the sampling frequency returned by the fs output argument.

startimpmetering

Starts impedance measurement.

Usage: nanoz('startimpmetering', handle, nsam_measure, numdsps);

Arguments: handle – handle to an opened device returned by the open function; nsam_measure – number of samples to measure per single impedance reading; numdsps – number of overlapping impedance measurements.

Return values: none.

Remarks: Calling this function initiates the impedance measurement process, applying sinusoidal test current to the target electrode and measuring the voltage across it. After a given number of samples is collected (which is specified by the nsam_measure argument), the data are converted to a single, average impedance reading. Because it may take considerable time, typically about a second, to collect data for result conversion, it is possible to perform multiple concurrent conversions, partially overlapping in time with each other. This allows more impedance readings per second. The multiplication factor is specified as the numdsps parameter. For example, if one measurement lasts one second, and numdsps parameter is set to 4, there will be 4 impedance readings per second.

While measuring impedance, the user program has to poll the results by calling the getimpdata function every ~30ms, or a data buffer overrun may occur.

Prior to measuring impedance, a channel must be selected and a working frequency must be specified by calling the selectchannel and setfreq functions, respectively.

getimpdata

Retrieves impedance measurement data.

Usage: [signal_samples, Z] = nanoz('getimpdata', handle);

Arguments: handle – handle to an opened device returned by the open function.

Return values: signal_samples – vector of integers which are raw analog signal readings from the target electrode. The user application may use these readings in order to monitor, for example, the noise and interference levels during impedance measurement. If the signal is not clean, the impedance reading may be inaccurate.

Z – vector of complex impedance readings, in Ohms.

Remarks: During impedance measurement, the user program must call this function approximately every 30ms to poll for incoming data. Output vectors are sized according to the amount of new data that were collected between two successive calls of this function. If no data were acquired, the vectors will be empty. In typical situations, the Z vector is empty most of the time, but occasionally it has one element – the impedance reading that was most recently acquired.

Failure to call this function at regular intervals may result in a buffer overrun error. Recovery from a buffer overrun requires impedance measurement to be stopped. This will cause any pending results to be lost. Note that if a buffer overrun error does occur, the impedance measurement test waveform is still applied to the currently selected channel until the 'stop' function is called.

getwaveformcaps

Returns information about waveform generation capabilities.

Usage: caps = nanoz('getwaveformcaps', handle);

Arguments: handle – handle to an opened device returned by the open function.

Return value: caps – a Matlab structure with the following fields:

fs_gen: generator sampling frequency, in Hz. Typical value is 83333Hz.

fs_adc: Sampling frequency of the A/D converter. Typical value is 10417Hz.

maxsam: Maximum length of the waveform, in samples.

interpol: List of supported interpolation factors.

Remarks: The nanoZ generates waveforms for impedance measurement and electroplating with an 8-bit digital to analog converter clocked at a sampling frequency of 83333Hz. Since this may change in future firmware upgrades, user applications should not rely on the values quoted in the specifications, but rather query the attached device for its actual capabilities (fs_gen parameter).

Sampling of the analog signal on the electrode is a fraction of the generator sampling frequency, in this case 1/4, which also may be changed in the future. The user program should rely on the fs_adc parameter of the caps structure.

Waveforms must be uploaded into the memory of the nanoZ's microcontroller. The current version has memory for waveforms up to 126 samples (bytes) long.

The minimum frequency that can be generated with a sampling frequency of 83333Hz and 126 samples is approximately 661Hz. Waveform interpolation is used to generate lower frequencies. The interpol field of the caps structure lists supported interpolation factors. Linear interpolation is used for factors of 8 or less. 16x and higher interpolation is performed by 8x linear interpolation combined with 2x or higher ratio hold interpolation, which effectively reduces the generator sample rate. Using the maximum 1024x interpolation factor (8x linear + 128x hold), frequencies down to 0.645Hz can be generated.

For impedance measurement, the MEX library automatically chooses the best interpolation factor and synthesizes a waveform of appropriate length, to achieve a test signal frequency as close as possible to that specified by the user. For electroplating, arbitrary user-supplied waveforms may be used, which puts the responsibility of choosing waveform length and interpolation factor on the user application.

getplatingcaps

Returns information about electroplating capabilities.

Usage: caps = nanoz('getplatingcaps', handle);

Arguments: handle – handle to an opened device returned by the open function.

Return value: caps – a Matlab structure with the following fields:

min_current The minimum (negative) current, in amperes, which the nanoZ's electroplating current source can generate.

max_current The maximum (positive) current, in amperes, which the nanoZ's electroplating current source can generate.

current_step The minimum step size, in amperes, by which the requested electroplating current can be decreased or increased.

min_voltage The minimum (negative) voltage, which can be produced by nanoZ to maintain the requested current (i.e. negative voltage compliance).

max_voltage The maximum (positive) voltage, which can be produced by nanoZ to maintain the requested current (i.e. positive voltage compliance).

Remarks: The values returned by this function reflect the device's calibration status, not just the design specifications, and can therefore vary slightly from device to device.

preparewaveform

Prepares an arbitrary waveform for electroplating.

Usage: [output_waveform, achieved_current] = nanoz('preparewaveform', handle, desired_waveform);

Arguments: handle – handle to an opened device returned by the open function.

waveform – user-supplied waveform to be generated during electroplating. Units are amperes.

Return values: raw_waveform – device-specific, raw waveform which is produced on the basis of user-supplied waveform, taking into account device calibration.

achieved_current – values of electroplating current, in amperes, which are based on the raw_waveform values returned by this function.

Remarks: This function converts the user-specified electroplating currents into raw values that are output by the nanoZ's 8-bit DAC during waveform generation. An attempt is made to produce a current waveform that is as close as possible to that requested, taking into account the device calibration.

The application can modify the raw waveform prior to uploading it into nanoZ, for example, to implement dithering, a technique which in some cases can overcome inaccuracies due to the 8-bit DAC quantization (see startplatingdc).

As there is a low pass filter after the waveform-generation DAC, fast changes in electroplating current will be dampened.

startplating

Starts electroplating with arbitrary waveform.

Usage: nanoz('startplating', handle, raw_waveform, interpol);

Arguments: handle – handle to an opened device returned by the open function;

raw_waveform – waveform of electroplating current to be generated, in device-specific units, which can be obtained from the preparewaveform function;

interpol – interpolation factor to be used during waveform generation.

Return value: none.

Remarks: This function uploads the user-specified waveform into the nanoZ's memory and starts the electroplating mode. During electroplating the voltage across the target electrode is constantly monitored. To prevent a possible buffer overrun the user application must periodically call the getplatingdata function to fetch the results of this monitoring.

The exact moment when electroplating actually starts is not predictable. Depending on the waveform length, uploading the waveform may take some time and delay the start of electroplating. When the electroplating duration must be exactly controlled the application should determine the exposure time from the number of samples returned by the getplatingdata function and stop plating when the prescribed number of samples has been acquired.

If, for whatever reason, the user application crashes during electroplating the nanoZ device will not be informed of it and will continue to supply electroplating current to the target electrode. For this reason it is a good idea to encapsulate electroplating sections of the Matlab code in try/catch clauses, in order to ensure that electroplating is stopped in all cases, even if an error occurs. The most reliable way to stop electroplating without generating further errors is to execute a clear nanoz Matlab statement. The m-file examples provided with this SDK implement these safeguards.

startplatingdc

Starts DC electroplating with a dithered waveform for generating low currents and fine current setting resolution.

Usage: achieved_current = nanoz('startplatingdc', handle, desired_current);

Arguments: handle – handle to an opened device returned by the open function.

desired_current – desired electroplating current, in amperes. Can be positive or negative within device limits (see Appendix B).

Return value: achieved_current – the current, in amperes, that is as close to the desired value as can be generated by the device. This current will be applied to the selected channel. Please see Appendix B for tolerances.

Remarks: This function starts DC electroplating with a specially crafted plating waveform that achieves, by means of dithering, a much finer current setting resolution than what is normally possible with the device's 8-bit onboard DAC. There will be a slight AC ripple on the output current. However, it is guaranteed that the first harmonic of this ripple current will be above 2000Hz and have the lowest possible level. In practice, the ripple current is so low that it is not measurable by nanoZ voltage feedback circuit even in most favorable circumstances of a high electrode impedance.

The exact moment when electroplating actually starts is not predictable. Depending on the waveform length, uploading the waveform may take some time and delay the start of electroplating. When the electroplating duration must be exactly controlled the application should determine the exposure time from the number of samples returned by the getplatingdata function and stop plating when the prescribed number of samples has been acquired.

If, for whatever reason, the user application crashes during electroplating the nanoZ device will not be informed of it and will continue to supply electroplating current to the target electrode. For this reason it is a good idea to encapsulate electroplating sections of the Matlab code in try/catch clauses, in order to ensure that electroplating is stopped in all cases, even if an error occurs. The most

reliable way to stop electroplating without generating further errors is to execute a clear nanoz Matlab statement. The m-file examples provided with this SDK implement these safeguards.

> The nanoZ's electroplating circuit has a native resolution of ~100nA. To enable smaller currents and ensure the accuracy of the startplatingdc function, devices shipped prior to April 2012 need to be recalibrated (see Appendix E).

getplatingdata

Retrieves electroplating voltage feedback data.

Usage: voltage = nanoz('getplatingdata', handle);

Arguments: handle – handle to an opened device returned by the open function.

Return value: voltage – a vector of voltage values measured across the target electrode during electroplating. Units are in volts.

Remarks: During electroplating the nanoZ monitors the voltage on the target channel. This voltage is sampled at the frequency that is returned by the getwaveformcaps function.

This function returns all samples that have been collected since electroplating was started, or from the time this function was previously called. It may be an empty matrix if no new values were acquired.

The user application should call this function every ~30ms or a data overrun may result. The total number of samples returned by this function since electroplating was started can be used to determine the exact electroplating duration.

Values returned by this function take into account device calibration, and have units of volts.

stop

Stops impedance metering or electroplating.

Usage: nanoz('stop', handle);

Arguments: handle – handle to an opened device returned by the open function.

Return value: none.
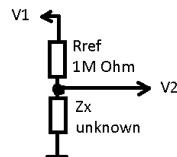
Remarks: This function stops any impedance measurement or electroplating process. The measurement circuit remains connected to the selected channel.

Consequently, a new impedance measurement on the same channel may start immediately because there will not be any transient channel-switching artifact.

The Matlab application should call this function prior to calling any function other than getimpdata or getplatingdata on the target device. Calling other nanoZ library functions may also indirectly stop impedance measurement or electroplating but is not guaranteed to do so.

Appendix A: Principle of Operation

For measuring impedance, the nanoZ utilizes a voltage divider circuit:
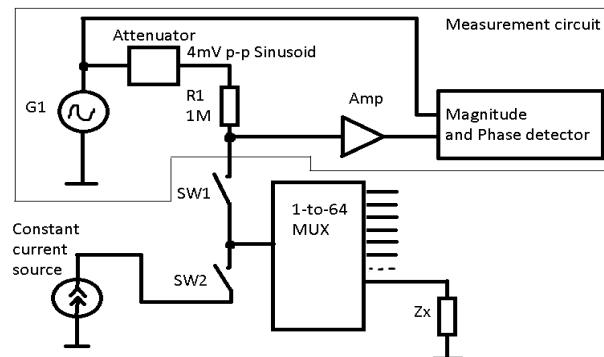
According to Ohm's law, the ratio of voltages *V1* and *V2* in the circuit is:

$$\frac{V1}{V2} = 1 + \frac{R_{ref}}{Z_x}$$

This formula generalizes to AC sinusoidal signals where *V1*, *V2* and Z_x are complex numbers whose angles represent phase relations in the circuit. When a known voltage *V1* is applied, and *V2* is measured, it is possible to solve the above equation for Z_x, which is exactly how the nanoZ measures impedance.

During impedance measurement test currents flow through the circuit. The nanoZ uses a 4mV peak-to-peak sinusoidal waveform for *V1*, which yields a maximum test current through Z_x of 1.4nA RMS when Z_x is approaching zero, and 0.7nA RMS when Z_x is 1MOhm.

The nanoZ has a single measurement circuit, including the generator voltage *V1*, the amplifier for *V2*, and the reference resistor R_{ref}. Different channels, having different electrode impedances Z_x, are connected to this circuit via an on-board 64-to-1 analog multiplexer. Here is a simplified schematic of the overall circuit:

Either the impedance measurement circuit or the electroplating constant current source can be connected to a channel via switches SW1 and SW2.

The electroplating current source is programmed by a voltage coming from an 8-bit DAC, yielding 256 current steps between -12uA (electrode negative) and +12uA (electrode positive). The DAC can produce both DC and alternating waveforms from the nanoZ's on-board memory.

Appendix B: Technical Specifications

Hardware

|	
Number of channels	64
Z measurement range	1kΩ to ~100MΩ
Z accuracy & precision	1kΩ display resolution ± 1% (10kΩ~15MΩ, 1Hz~2kHz), latest model (see following page)
Z test current	1.4nA RMS (max) bias current 50pA (typ)
Test signals	default 1kHz sinusoid waveform frequency range 1Hz to 4986Hz arbitrary user-defined waveforms
Electroplate mode	bipolar constant current
Electroplate range	± 12μA, ±5V compliance
Electroplate resolution	100nA (native) ~5nA (using dithering)
LED Indicators	power, operating mode
EEPROM	device-specific calibration values
PC interface	USB 1.1 or 2.0 compatible (no additional power supply required)
Connectivity	two Samtec FOLC-110-01-S-Q 3rd party electrode adaptors
Weight	2.9 oz (82 g)
Dimensions	3.2 x 2.8 x 0.47 inches (81 x 70 x 12 mm)
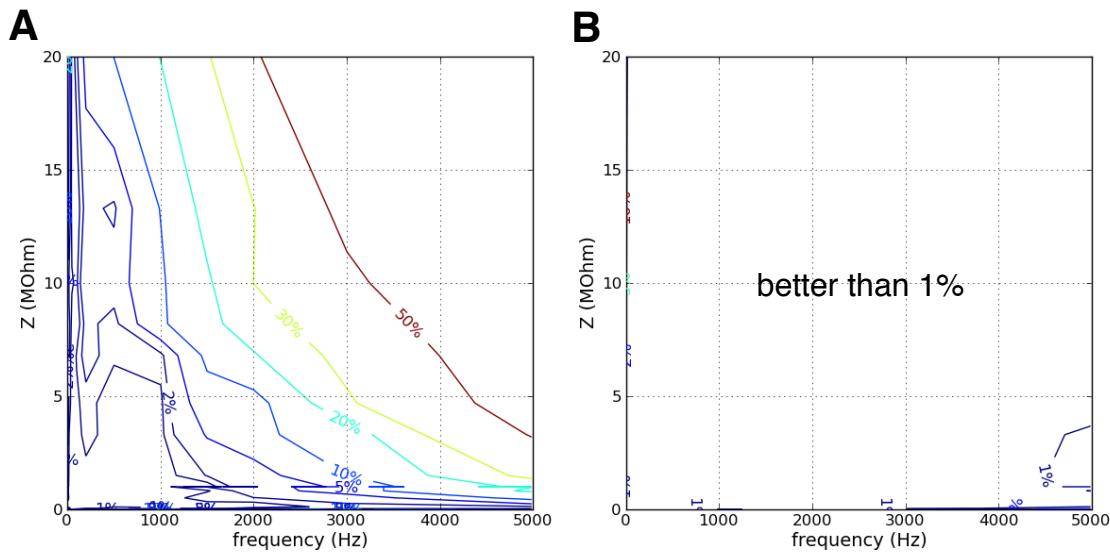
The nanoZ, electrode adaptors, and all accessories are certified ROHS Compliant.

Software
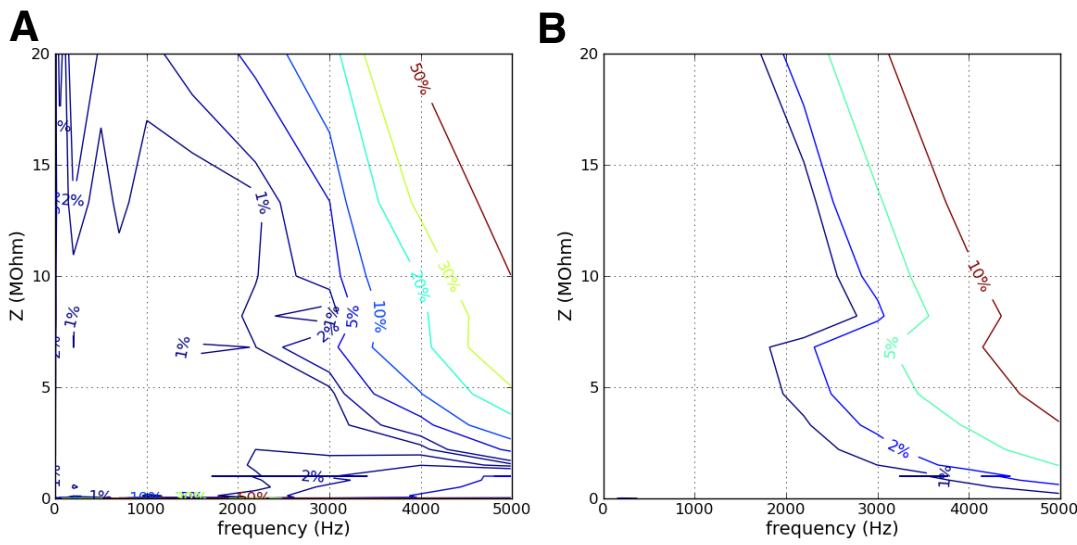
Operating systems	Windows XP, Vista, or 7 Mac OS X v1.4 or later (Matlab SDK only)
Operating modes	impedance test, impedance spectroscopy, DC electroplate, electrode activation, manual
Matlab SDK (included)	requires Matlab 2006a or later

The figures below show the measurement error in (**A**) impedance magnitude and (**B**) phase as a function of the true impedance magnitude and test frequency, across the working range of the nanoZ.

nanoZ model 1.1 (after re-calibration, requires nanoZ software v.1.3.2 or later):

nanoZ model 1.2:

Appendix C: Channel Mapping

The nanoZ application stores adaptor and electrode site mappings in the 'electrodes.ini' file, which can be accessed from the link in the Windows Start menu. You can use a standard text editor to add, remove, or modify the existing channel mappings, however care must be taken to adhere to the correct syntax described in this Appendix. We recommend you make a backup of 'electrodes.ini' before making any changes to this file.

Adaptor definitions

The mapping between the nanoZ's internal 64 channel MUX and the two native Samtec connectors is depicted here (as viewed facing the connector, with the lowest channel numbers closest to the USB port end of the nanoZ). Use this numbering scheme when programming the channel selection in the Matlab SDK, or when designing custom-made electrode adaptors.

The electrode reference pins (R1, R2 and R3, shown in red) cannot be selected by the nanoZ software, however R1 must be physically connected to the electrolyte in order to complete the circuit. R2 and R3 are not currently used but are reserved for future versions of the nanoZ.
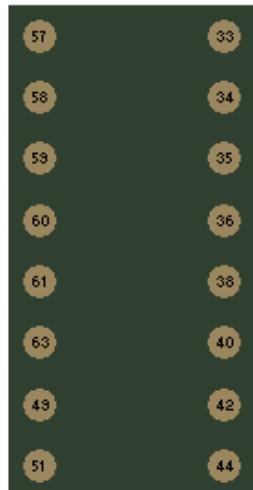
To describe the format of the adaptor definitions contained in 'electrodes.ini', the NZA DIP16 adaptor mapping is used as an example in the following step-by-step instructions:

1. choose a name for the adaptor, and enter it on a new line in the [Known Adaptors] section. Spaces are allowed, but the last character must have an '=' sign to be recognized. For the DIP16 adaptor, the entry is NZA DIP16=.

2. create a corresponding section name for this adaptor by adding the adaptor name to a new line, enclosed in square brackets (e.g. [NZA DIP16]).

3. the next few lines define the adaptor name, description, adaptor pin dimensions, and whether the pins are round or square. The values assigned to the Name and Description fields will appear in the drop-down list of known adaptors and in the Report window, respectively. They should be succinct yet descriptive, and include version identifiers if multiple versions of the same adaptor exist. The ContactSizeX and ContactSizeY determine the relative size of

the pins in the Adaptor window. If RoundContact is zero, the pins will be square, otherwise they will appear round.

4. the next few lines defines the shape of the adaptor as displayed in the Adaptor window. Typically this will define one or two rectangles representing one or both of the nanoZ's Samtec connectors, however it can be any shape. The NumPoints field specifies the number of points that comprise the adaptor outline. The format for these points is: Outline_ = x, y, where _ is an arbitrary suffix, and x & y are 2D pixel coordinates. The outline must form an enclosed region. Repeating the same Outline coordinates on two consecutive lines indicates that this is the last point in that shape, with subsequent Outlines representing the next shape. For example, with the NZA DIP16 adaptor shown here on the left, the single rectangle requires 6 points, with the last two points (Outline4, Outline5) having the same coordinates as Outline0 to complete the rectangle. Unless you require an adaptor with a special shape, it is probably simplest to just copy and paste this section from one of the existing 32 or 64 channel adaptors, as required.

5. the last section of the adaptor definition is the most important, as it defines the mapping from the nanoZ's internal MUX to the pins of the adaptor. The NumChans field specifies the number of adaptor channels to map. The format for the mapping is: MUX n = x, y, where n is the nanoZ MUX channel (1 ~ 64), and x and y define the pin location on the Adaptor window. The ordinal position defines the adaptor pin number. So, for example, the first line in the NZA DIP16 definition: MUX 51 = -75, 375, means that channel 1 of the adaptor is connected to MUX channel 51, and is situated on the bottom left-hand corner of the DIP16 socket.

Finally, restart the application or click **File | Reload definitions** from the main menu to make the nanoZ application aware of any changes to 'electrodes.ini'.

Electrode definitions

The format for the electrode definitions in 'electrodes.ini' is similar to that used for adaptor definitions. Steps 1 to 4 for defining the electrode name, description, site appearance and outline are almost identical except for subtle differences in the field names. Step 5 is different:

5. the last section of the electrode definition is the most important, as it defines the mapping from the adaptor pinout to the electrode site layout. The NumSites field specifies the number of electrode sites. The format for this mapping is: Site n = x, y, sizeX, sizeY, where n is site number, x and y define the site location on the Probe window, and sizeX and sizeY are optional values that replace the default

dimensions in $\mathrm{SiteSizeX}$ and $\mathrm{SiteSizeY}$, thereby allowing for electrode arrays with different site sizes. For simplicity, specify all these values in microns. The ordinal position defines the mapping between the electrode site and the adaptor pin number. So, for example, the 4th line in the MCS $8\mathrm{x}8$ $\mathrm{standard}$ electrode sites definition: $\mathrm{Site}\ 45 = \text{-}50,\ 450,$ means that channel 4 of the adaptor is connected to site 45. Since the mapping is determined by ordinal position, n is only used for the site label in the Probe window and Probe report, and can therefore use any numbering scheme. This added flexibility allows for zero-based vs. one-based electrode site numbering (different data acquisition systems use one or the other), or, for example, the row and column site numbering used by the MCS $8\mathrm{x}8$ $\mathrm{standard}$ MEA.

Finally, restart the application or click **File l Reload definitions** from the main menu to make the nanoZ application aware of any changes to 'electrodes.ini'.

Appendix D: NZ-CAL Component Values

The following table shows the channel mapping for the bank of test resistors (1% tolerance) and capacitors (5% tolerance) on the supplied NZ-CAL adaptor.

| channel	R (MOhm)	C (nF)	channel	R (MOhm)	C (nF)
1	6.8	-	17	short circuit	-
2	10	0.1*	18	0.12	-
3	4.7	-	19	1.0	-
4	3.3	-	20	1.0	-
5	20	-	21	0.0051	-
6	2.2	-	22	0.51	-
7	1.0	-	23	0.010	-
8	1.5	-	24	1.0	-
9	10	-	25	-	100
10	1.0	-	26	-	10
11	1.0	-	27	-	1.0
12	0.82	-	28	-	0.22
13	8.2	-	29	-	0.033
14	1.0	-	30	0.020	-
15	13.3	-	31	1.0	-
16	open circuit	-	32	0.051	-

* R and C in parallel
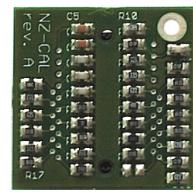
The measured phase angle of pure resistors should be 0°, with an impedance that is independent of test frequency, whereas pure capacitors should report a phase angle of -90°, with an impedance magnitude that varies inversely with frequency. For comparison, most electrophysiology electrodes will have a phase angle between -50° and -80°, depending on the electrode metal and coatings.

Appendix E: Recalibration

The nanoZ does not require routine calibrations, however as of April 2012 there are two optional recalibrations available. One will substantially improve the accuracy and range of the impedance testing mode for nanoZ version 1.1 (and may provide a marginal improvement in accuracy for version 1.2). This calibration can also be used to compensate for errors in impedance measurement that may otherwise be introduced by 3rd party electrode adaptors or extension cables (i.e. the factory calibration assumes electrodes are plugged directly into the nanoZ).

A separate recalibration, applicable to both nanoZ versions, enables electroplating currents less than 100nA with a resolution of ~1nA (±5nA or 5% or better accuracy across the full voltage compliant current range).

For either calibration, start by inserting the NZ-CAL adaptor into the nanoZ connector closest to the USB port, with the adaptor orientated so that the 'NZ-CAL' text is on the left. For 3rd party adaptor recalibration, plug the NZ-CAL into the end of the adaptor.
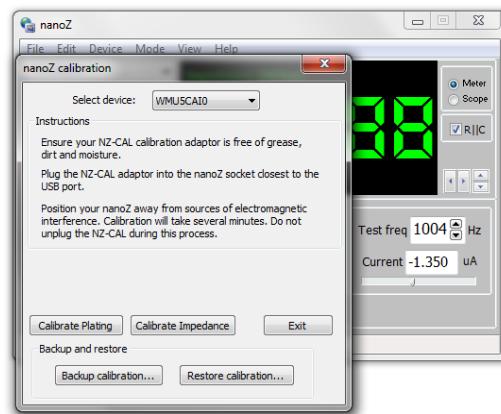

For accurate calibrations it is critical that the NZ-CAL be free of dirt, grease (fingerprints), and moisture. Clean the adaptor with ethanol and wait for it to dry completely before initiating any recalibration procedure.

Select **Device I Calibration** from the main menu to open the calibration dialog:

(A) Select the serial number of the device you wish to recalibrate.

(B) Click on **Calibrate Plating** or **Calibrate Impedance**.

(C) Follow the onscreen instructions. You will be prompted to save the existing calibration data (including the factory calibration) to a file. At the end of the recalibration the new calibration data will be automatically uploaded to the nanoZ.

After recalibrating we suggest you use the NZ-CAL adaptor to validate the impedance measurement accuracy. To evaluate a plating recalibration observe the voltage feedback in the nanoZ program while applying current to a known resistor, or run the 'electroplating_test_precision.m' sample script in Matlab.

To save the existing calibration to file without doing a recalibration, click on **Backup calibration**. To restore the factory calibration or revert to an earlier recalibration, click on **Restore calibration** and select the desired calibration file.

2 Year Limited Warranty

White Matter LLC (White Matter), the manufacturer of the nanoZ, warrants to the original purchaser that the nanoZ will be free from defects in materials and workmanship for two years from the date of purchase. White Matter will repair or replace any nanoZ product or part thereof which, upon inspection by White Matter, is found to be defective in materials or workmanship.

The nanoZ must be returned to the Distributor from which it was bought together with proof-of-purchase. A return authorization must be obtained from the Distributor in advance of return. Please include a brief description of any claimed defect(s). The customer shall be responsible for all costs of transportation and insurance to the Distributor. The Distributor will cover the return shipping costs.

This warranty shall be void and of no force of effect in the event the nanoZ has been modified in design or function, or subjected to abuse, misuse, mishandling or unauthorized repair. Water or other liquid-related damage is specifically excluded from this warranty. Furthermore, product malfunction or deterioration due to normal wear is not covered by this warranty.