

## PhotoSound MolecuUs, 2022-06-23



**Warning:** PhotoSound MolecuUs is based on medical-class ArtUs ultrasound system, and might be kitted with medical-class ultrasound probes, but NOT certified as a medical device. MolecuUs is provided for preclinical research and development applications only.

This operation manual describes a dual-modality USPA MolecuUs system. The use of the LEGION ADC is covered by a separate user manual. This manual may be changed without notice. This manual may not be distributed without the explicit permission of PhotoSound Technologies, Inc.

Abbreviations used in this manual:

- US = UltraSound,
- PA = PhotoAcoustics,
- RMB = Right Mouse Button.

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## MoleculUs hardware

### Parts

1. Assembled MoleculUs with 128 USPA channels or 128 USP and 128 PA channels, includes:
  - a. LEGION ADC256 board
  - b. PAUS board installed on J9 (ADC1-4) with a MoleculUs-type (QLC260) input connector.
  - c. ArtUs US system
  - d. Ventilated housing with 2 fans installed inside.
2. ADC256 AC-DC power supply, Cinon TR70MA120, 12VDC 5.8A output (right-angle connector).
3. ArtUs AC-DC power supply, XP Power AFM45US12, 12VDC 4A output (straight connector).
  - a. Note: The ADC256 and ArtUs power supplies are NOT interchangeable, because they have different central pin diameters.
  - b. Power supplies have IEC 320-C14 AC input connectors for use with country specific power cords.
4. USB3, A to B cable for the ADC256
5. USB3, micro A to B cable for the ArtUS
6. USB dongle with drivers and software.
7. Optional probes
8. Storage and shipping box.

### System specific configuration and ordering options

1. An optional PA preamplifier can be configured with a MoleculUs-type (QLC260) input connector or a DLM-260R input connector with a typical US pinout.
2. An additional Legion ADC can be connected to MoleculUs to increase the number of PA-only channels.

### Prerequisites

Prerequisites typically not included with MoleculUs products are:

1. PC with at least two USB3.0 ports, and Windows 10 64-bit
2. Free [MATLAB 9.3 \(R2017b\) 64-bit runtime](#).
3. Probe with a connector matching the MoleculUS input connectors, or a with breakout board matching the preamplifier connectors or cables.

Optional prerequisites are

4. Oscilloscope2-Channel signal generator for testing the board with electrical triggers and for verification of the external input trigger signal level.
5. Low power CW laser or laser pointer for testing the optical trigger inputs

## Probe selection, connector, and pinout

The probe can be hot-swapped from MolecuUs. The channel mapping of acquired signals is recorded in the probe-specific .MAP file. MAP files are automatically loaded by data acquisition software when the software is started and must be changed if a probe is changed to one with a different pinout.

Use well shielded probes.

## PC requirements

- Recommended PC configuration:
  - recent generation of Intel i5 or equivalent AMD CPU
  - $\geq 16$  GB DDR4
  - the latest Samsung M.2 PCIe SSD Pro series  $\geq 1$  TB, or equivalent, with an installed heatsink.

## Notes:

1. Use a motherboard with USB3 (blue color) or a motherboard with USB type-C ports with USB-C to USB3-B cable (not supplied). Recent generations of motherboards from top vendors (Gigabyte, Asus, MSI, etc.) have high performance USB3 interfaces.
2. Recommended USB3.0 cables are AmazonBasics, Monoprice or other brand-names. Snap-on ferrite filters, like Fair-Rite 0431164951, 0431164951, 0431167281, 0431164181 can be snapped over USB3 and HDMI cables if deemed necessary.

## Absolute maximum ratings and recommended operating conditions

### Power consumption

- ADC256: Maximum  $\leq 60W$ ,  $12V \leq 5A$ , nano-fuse protected
- 7W typical with two cooling fans before firmware is loaded, each fan 0.5W.

### Trigger input signal to SMA trigger input

- $\leq 6V$  to  $50\Omega$  default input impedance setting with jumper(s) J13, J16 installed **or**
- $\leq 6V$  to HiZ without jumper(s) installed.
- Measure the input impedance at the input connectors using a multimeter.
- Recommended signal is 4 – 5V, with a duty cycle  $< 1\%$ .

### Analog signal to ADC256 input for a powered ADC board ( $50\Omega$ to GND followed by AC coupling)

- $\leq 2V_{pp}$  AC in the absence of inductive elements in the chain
- $\leq 1V$  DC (absolute value) with  $50\Omega$  input impedance.
- An unpowered ADC256 board can tolerate input voltages  $< 100mV_{pp}$  or  $< 50mVDC$ .

## ADC256 connectors and indicators

The ADC256 connector order is left-to-right as shown in Figure 1. (Right-to-left when viewed in the assembled housing (Figure 2).

- Nano-fuse 5A
- J4 - 12VDC 5A power connector, OD = 5.5 mm, ID = 2.0 – 2.1 mm, PC mount barrel connector. The center pin is +12V, the outer contact is ground.
- J6, J8 - 12VDC fan connectors - the internal housing fans must be connected for safe operation.
- LD4 (2 red LEDs), LD1, LD3, LD2 (4 green LEDs per block).
- J11 - USB3-B connector for connection to a PC via the supplied USB3 A to B cable
- PD2, PD1 – photodiode optical fiber trigger input connectors (locking type) for 2mm optical fiber.
- Isolated trigger interface #2:
  - a. J17 - SMA trigger output, low input impedance 5V  $\pm$ 10%
  - b. J15 - SMA trigger input, 5V maximum input signal level. Verify with an oscilloscope using matching impedance settings.
  - c. J16 – Input trigger impedance - 0.1" two-pin jumper; J15 input impedance is 50 $\Omega$ , if shorted, or HiZ if open.
- Isolated trigger interface #1:
  - a. J14 SMA trigger output - low input impedance 5V  $\pm$ 10%.
  - b. J12 SMA trigger in, 5V maximum input signal level. Verify with an oscilloscope using matching impedance settings.
  - c. J13 – Input impedance setting - 0.1" two-pin jumper; J12 input impedance is 50 $\Omega$ , if shorted, or HiZ if open.
- J7 - HDMI connector - Master output for synchronization with a slave ADC256 board.  
**Do not connect to a PC or monitor.**
- J5 - HDMI connector - Slave input for synchronization with the master ADC256 board.  
**Do not connect to a PC or monitor.**

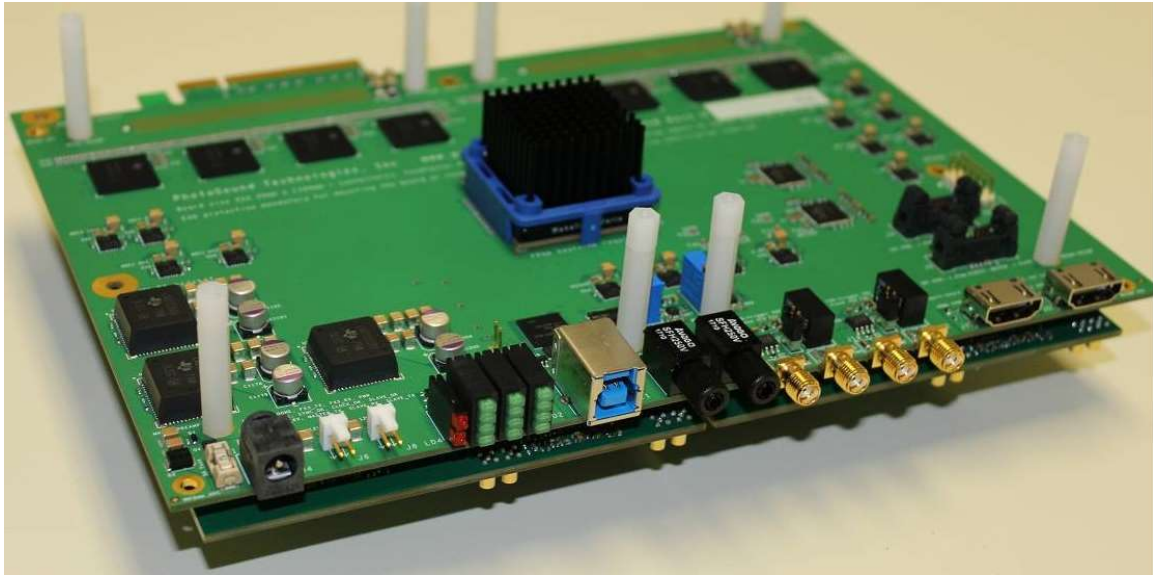


Figure 1: The LEGION DAQ256 with the ADC256 Rev1.0 board facing up. The ADC256 front components / connectors from the left-to-right are nano-fuse, power, 2x fans, 4x LEDs blocks, USB3 type-B, 2x optical fiber trigger inputs, 4x SMA trigger IO, 2x HDMI master and slave.

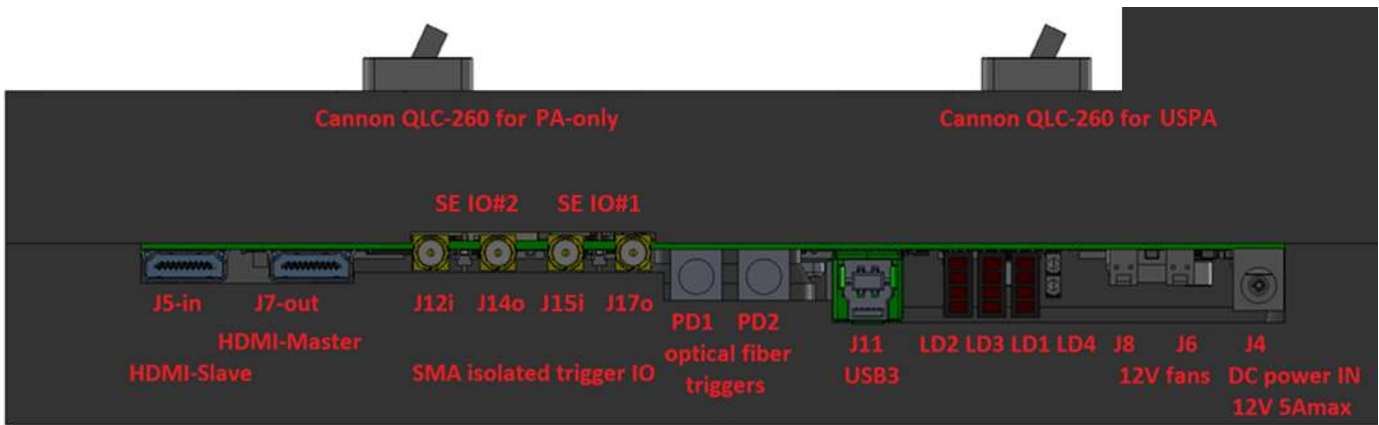


Figure 2: ADC256 connectors on MolecuUs. AMP128 is mounted on the top-left, ADC256 is on the bottom with digital connectors facing to the front. Cooling fans are mounted on the right side of the housing. Fan wiring is not shown.





Figure 4: ArtUs Micro-USB3 and power connectors.





Figure 5: Plug a US probe into the USPA connector. Plug a PA-only probe into the PA-only connector. A PA type of connector can be customized.



Figure 6: The probe plugs and sockets have alignment keys, as indicated by the green arrows. The probe sockets have latches, as indicated by the blue arrow.



*Figure 7: A probe plug is inserted into the USPA socket and the latch is closed. The PA-only connection is similar.*

## LED indicators

LED indicators of the ADC256 shown in Figure 2 are listed starting from the PCB (top to bottom) below:

LD2, green, 4-position, clock and master-slave synchronization

1. Slave\_OK, indicates that the board is configured as a slave board in many board configurations;
2. Clock\_OK, indicates that ADC clock is running correctly;
3. Sync\_OK, indicates that the slave board is synchronized with the master board;
4. InitOK, indicates that the board is initialized.

Correct indicator values after boards are completely configured by ADC.exe/vi software:

- LD2.2 and LD2.3 ON for a single board or the first master board.
- All LD2 LEDs ON for slave boards.

LD3, green, 4-position, master-slave communication:

1. Slave\_TX
2. Slave\_RX
3. Master\_TX
4. Master\_RX

There are no specific requirements for these LEDs

LD1, green, 4-position, power, USB3, and configuration

1. Power (very dim);
2. FX3\_RX, USB3 data from the PC to the ADC256;
3. FX3\_TX, USB3 data from the ADC256 to the PC;
4. CFG\_DONE, configuration of ADC256.

LD1.1 is always on, if the ADC256 is correctly powered. LD1.2 and LD1.3 are blinking during data transmission.

LD4, red, 2-positions, indicates critical power failures

1. Fuse – indicates a blown fuse.
2. Power polarity - indicates a wrong power polarity. Warning: reverse power polarity protection might not work, if the power supply is not isolated. Always use an isolated power supply. Both of the LEDs on LD4 must be OFF.

## Trigger input notes:

- The trigger input source is programmatically selectable between PD1, PD2, J12, J15 or combinations. The LEGION ADC256 typically uses a single trigger input. Optical triggers at PD1 or PD2 are the preferred trigger sources.
- If an electrical trigger source is applied to SMA J12 or J15, verify the input impedance setting on jumpers J13 and J16 accordingly. The input impedance can be also measured using an Ohmmeter if the board is not powered.
- An input feed-through 50 Ω terminator can be installed externally to convert a HiZ input to a 50 Ω input. (BROADWAVE TECHNOLOGIES INC. MODEL 851 - 054 – FTT)
- **The trigger level applied to the SMA trigger inputs must be within 4 – 5 V. Verify the voltage level applied to these connectors using an oscilloscope with the input set to 50 Ω (when J13, J16 are shorted) or the HiZ setting (when J13, J16 are open).**  
**ATTENTION: 4 – 5 V to a 50 Ω load corresponds to 8 – 10 V to a HiZ load, which will irreversibly damage the isolator circuit. High voltage damage to this trigger interface circuit is not covered by warranty. If the oscilloscope does not have a 50 Ω input, use 50 Ω BNC adapter, such as the Rigol ADP0150BNC or equivalent.**
- The isolated trigger output SMA connectors produce a 5V ±10% output signal and 50 Ω output impedance. The output trigger amplitude is 5 V ±10% to a HiZ load and 2.5V ±10% to a 50 Ω load. The output can drive a 50 Ω load with a low duty cycle, and a HiZ load continuously. The output trigger signal can be programmed using the Software Development Kit (SDK).

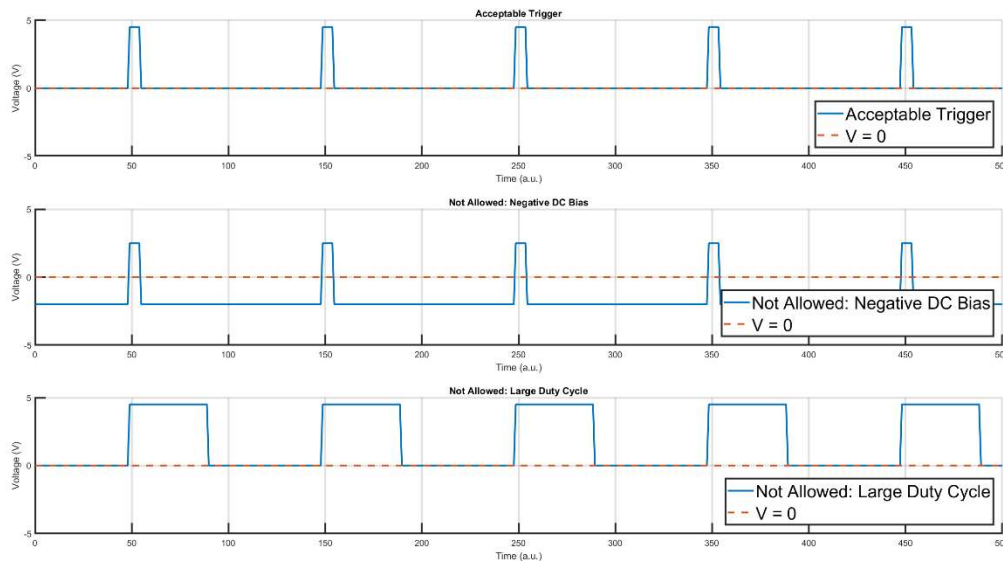


Figure 8: Trigger Input Pulse Acceptance. The SMA trigger IO (signal and input ground) are electrically isolated with respect to the rest of the ADC256 circuitry. The SMA IN1 trigger input and OUT1 trigger output share the ground level. The SMA IN2 trigger input and OUT2 trigger output share the ground level, which is different from the ground for SMA IN1 and OUT2. The trigger level (blue line) is measured with respect to the trigger coaxial cable shield (level 0). The trigger large duty cycle shown on the last graph is acceptable with HiZ trigger input termination, which can be delivered upon request.

## Hardware parameters

1. The ADC data rate is 40 MSPS. The odd and even ADC channel clocks are interleaved (i.e. shifted a half period with respect to each other).
2. The ADC has a 2Vpp ADC scale with 12-bit resolution. The ADC internal amplifiers have a software-selectable gain range of 12 - 51 dB (low power mode = ON), or 6 - 45 dB, if the 8 dB input attenuator is enabled (low power mode OFF). The maximum measurable input range is 1 Vpp at 6 dB gain. The ADC full input scale with 51 dB gain is 5.6 mV (2Vpp / 51dB) and resolution is 1.4  $\mu$ V (5.6 mV/4096 12-bit). The ADC absolute maximum input range is  $\pm$ 1 VDC or 2Vpp for a brief time, if the ADC is powered on, or 0.1 VDC or  $\pm$ 0.1 Vpp, if the ADC is not powered.
3. USPA and PA channels standard preamplifiers have 40 dB (100x) gain over 25 kHz to 35 MHz -6 dB bandwidth. The maximum measurable input range 10 mVpp (1 Vpp / 100) at 6 dB gain. The ADC full input scale with 51 dB gain is 56  $\mu$ V (2 Vpp / 91 dB) and resolution is 14 nV (56  $\mu$ V/4096 12-bit).

## Hardware Installation

1. MolecuUs must be installed indoors in a ventilated environment, non-compensating humidity, and temperature range from 15 to 30°C. If device was stored or transported at low temperatures let the device reach room temperature for 24-hours before using it.
2. The device should be operated inside the housing or in ESD safe environment. The input connectors have ESD protection. Do not create any unapproved connections.
3. Connect the probe(s) to the Cannon QLC-260 connector(s).
4. Use high quality USB3 A to B cables (optional ferrites may be attached close to both connectors).
5. **Power all AC equipment, including the computer, the device, an oscilloscope, and the trigger source from one AC power strip. AC power source must have a safety ground.**
6. Do not obstruct the ventilation holes.
7. **Apply trigger signals only after the ADC256 board is powered. Before applying a trigger signal, verify the signal level with an oscilloscope as described above.**

## Signal path and filters

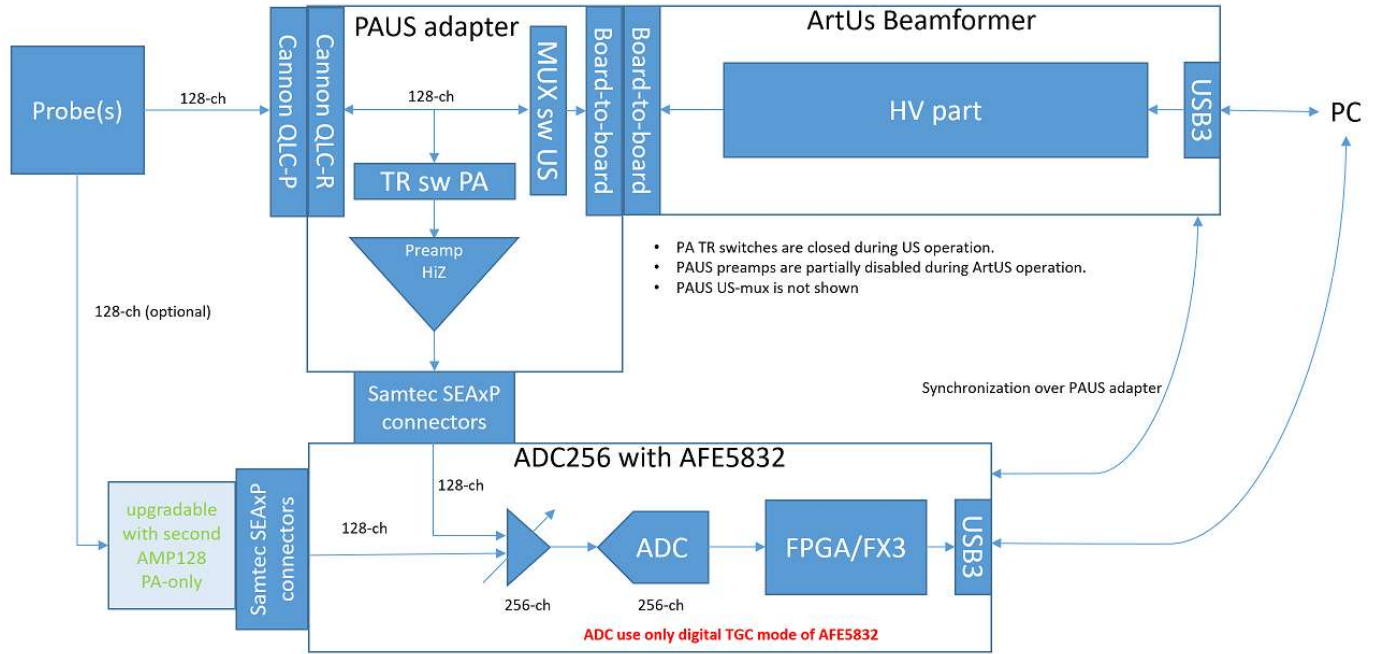


Figure 9: MolecuUs = ADC256 + PAUS + ArtUs. PAUS adapter is multiplexing signal between US path to ArtUs beamformer and PA path to ADC256 using multiplexing switches (MUX sw US) and transmit-receive switches (TR sw PA). In PA mode MUX sw US are fully closed and TR sw PA are open. In US mode MUX sw US are open and TR sw PA are closed.

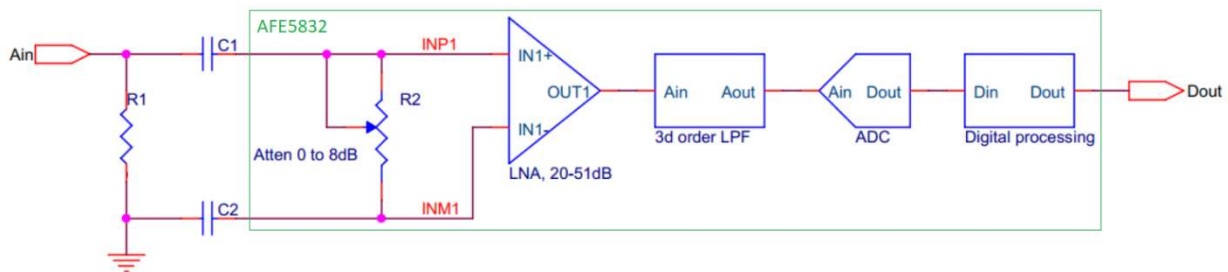


Figure 10: Signal path for one channel of the ADC256. Resistor  $R1 = 50 \Omega$  and capacitors  $C1$  and  $C2 = 0.1 \mu F$  are discrete components soldered to the ADC256 board. All other blocks, including the attenuator  $R2$  are located inside the AFE5832 chip and controlled by software using the ADC, VCA, and DGTC registers as described elsewhere. The ADC multiplexing between adjacent odd and even channels is not shown.

1. The first order analog HPF is formed by the input RC chain and the 0 – 8 dB attenuator. With the highest attenuator setting of -8 dB and the default  $R1$  and  $C1, C2$  settings, the HPF corner frequency is about 370 Hz / 37 kHz, with the -6 dB point at 300 Hz / 30 kHz for Rev1.0 / Rev1.1 accordingly. This attenuator using check box attenuator enabled in

the ADC tab, , if low frequencies are needed. **If attenuator is disabled, there is no low frequency loss at this stage. As mentioned above, the attenuator affects only gain codes 0 to 63.**

2. LNA is low-noise amplifier with programmable gain in range 20 to 51 dB in low power mode and 14 to 45 dB in high power mode. First order LPF cutoff frequency can be programmed in the range 5 to 12.5 MHz for low-power mode or 10 to 25 MHz for high-power. This filter can be programmed, but cannot be disabled.
3. 3d order LPF is 3d order analog low-pass filter, which can be set at 75 or 150 kHz. This filter can be also disabled for all channels or some channels.
4. The ADC input is analog, the output is digital. The ADC is shared between odd and even channels. For example, channels 1 and 2 are sharing the same ADC operating with clock rate 80 MHz for Rev1.1 and Rev1.0 with latest firmware. Sampling for each channel is  $80 \text{ MHz} / 2 = 40 \text{ MSPS}$ . Data for the channel 2 is delayed with respect of channel 1 by one ADC clock period ( $1 / 80 \text{ MHz}$ ).
5. Digital post-processing block is completely bypassed in low-latency mode on the ADC settings. Low latency mode also reduces data pipe-line delay after trigger is received by 4 ADC clocks or 2 sampling periods. Note: Each ADC clock = 80 MHz and the sampling period is 25 ns for R1.1. The data pipe-line works like a memory buffer, which cause delay of data transmission from the ADC to the FPGA memory, i.e. with larger delay more data acquired before the trigger event. Digital HPF filter can be used, if the low-latency mode was not enabled. Digital HPF parameters depends on the sampling rate (corner frequencies are proportional to the sampling rate), see Table 1 below. Use of digital HPF corner 10 is highly recommended for DC bias removal. The *ADC's HPF output rounding* might be used to enable rounding of the 14-bit ADC output after the digital HPF. If the *ADCs HPF output rounding* is not enabled, 2 lower bits from the 14-bit ADC output are dropped.

Table 1: The ADC's digital HPF corner frequency vs the corner code number for the ADC256 operating at 40 MSPS.

HPF corner setting	HPF, kHz (at 40 MSPS)
Disabled	None
2	2780
3	1490
4	738
5	369
6	185
7	111
8	49
9	25
10	12

**Advice:** Disable the attenuator, if possible. If lower gain is needed, use the high-power mode by unchecking the low-power mode on VCA page.

**Calculation of attenuation and HPF for attenuator:** Preamplifier output impedance  $R_{preamp}$  default value is  $50 \Omega$ , the input impedance seen by attenuator is  $R_{in} = R_{preamp} || R_1 = \frac{R_1 R_{preamp}}{R_1 + R_{preamp}}$ . Under assumption that  $C_1 = C_2$ , and  $R_{preamp} = R_1$ ,  $R_s$  should be set as  $R_1/2$ . In the following  $R_s$  is input resistance value set on VCA pages,  $R_{in} = R_1/2$  is physical value of input impedance seen by attenuator.

$$R_{atten} = \frac{R_s}{(10^{G/20} - 1)}$$

where  $G = (64 - ManualGain) 0.125$  in dB for manual gain  $< 64$ . For manual gain  $\geq 64$  attenuator is and should be disabled.

If  $R_{in} \neq R_s$ , the actual gain is  $G_{actual} = 20 \log_{10} \left( \frac{R_{in}}{R_{atten}} + 1 \right)$ .

The attenuation frequency dependent factor is  $\left( 1 + \frac{2}{s C_1 (R_{in} + R_a)} \right)^{-1}$ , where  $s = i2\pi f$ .

The attenuator HPF -3 dB corner frequency is  $f_c = 1/(\pi C_1 (R_{atten} + R_{in}))$ .



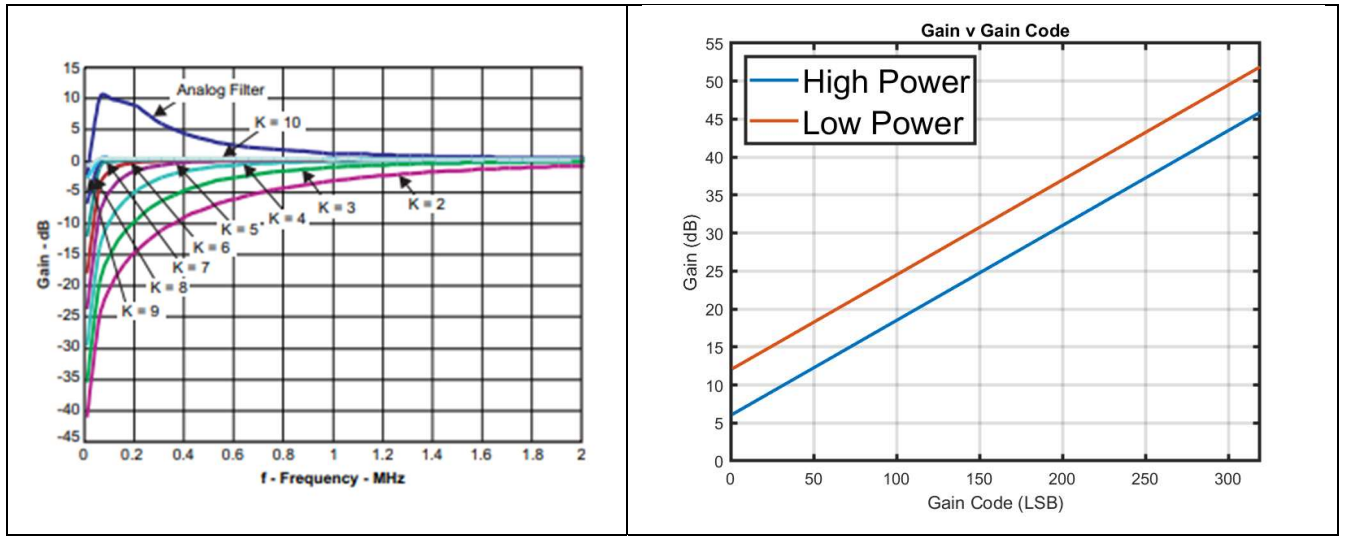


Figure 11: AFE5851 HPF Corner (Left) and DTGC gain codes (Right)

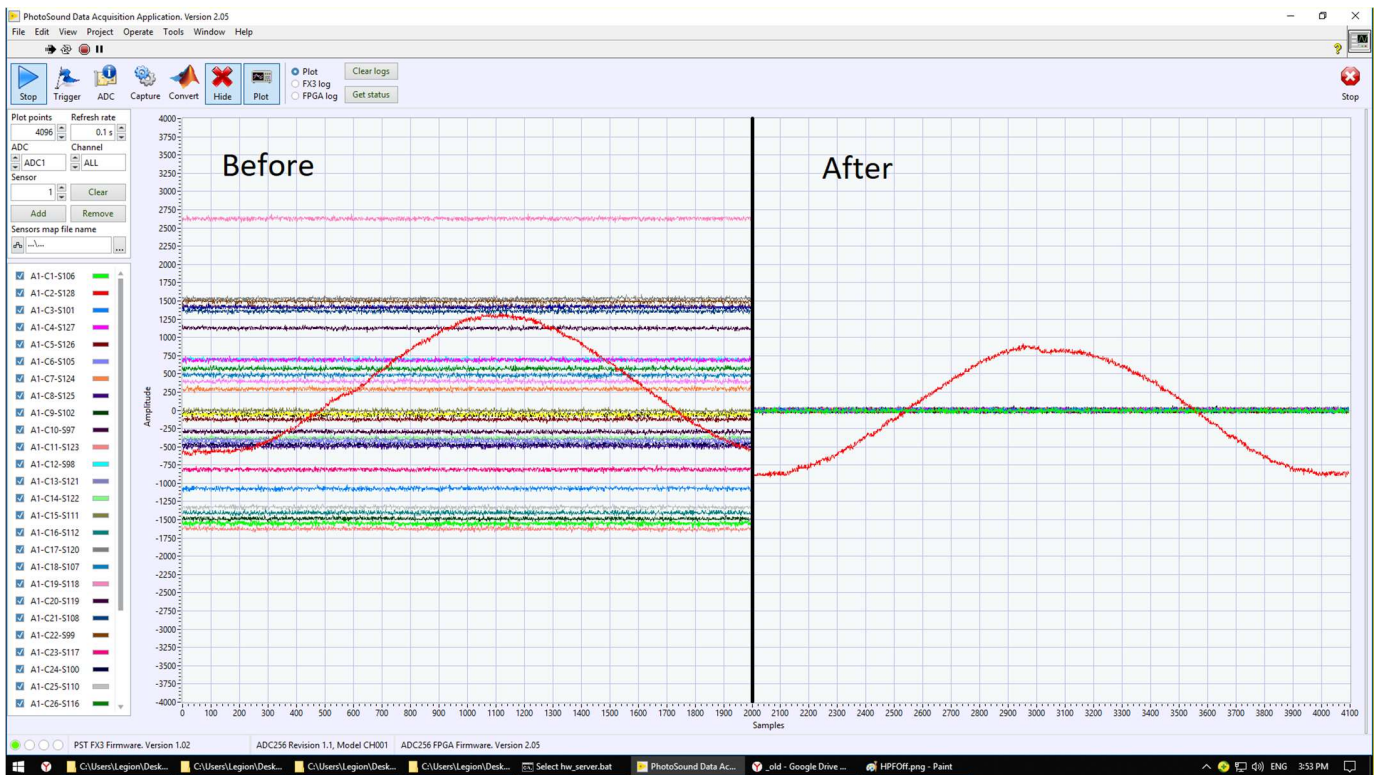


Figure 12: High Pass Filter settings. Before waveforms are visualized with no HPF applied. After waveforms are visualized with HPF corner = 10 for all ADCs. Notice removal of DC bias effect.

## MoleculUS driver and software setup

### Preparation:

The following software should be loaded into the PC prior to taking any of the following installation steps. This file can be found in the *MoleculUS Software Package.7z*.

### ADC256 driver installation

The ADC256 is equipped with an FX3 USB3 chip from Cypress Semiconductor and uses a proprietary FX3 Cypress driver (see release and copyright notes in the driver folder). The driver is located in the *MoleculUS Software Package\ADC Driver* folder.

1. Connect the ADC256 to the PC using the USB3 cable and then apply power to the ADC256 using the provided 12VDC power supply.
2. On the PC, open *Device Manager*.

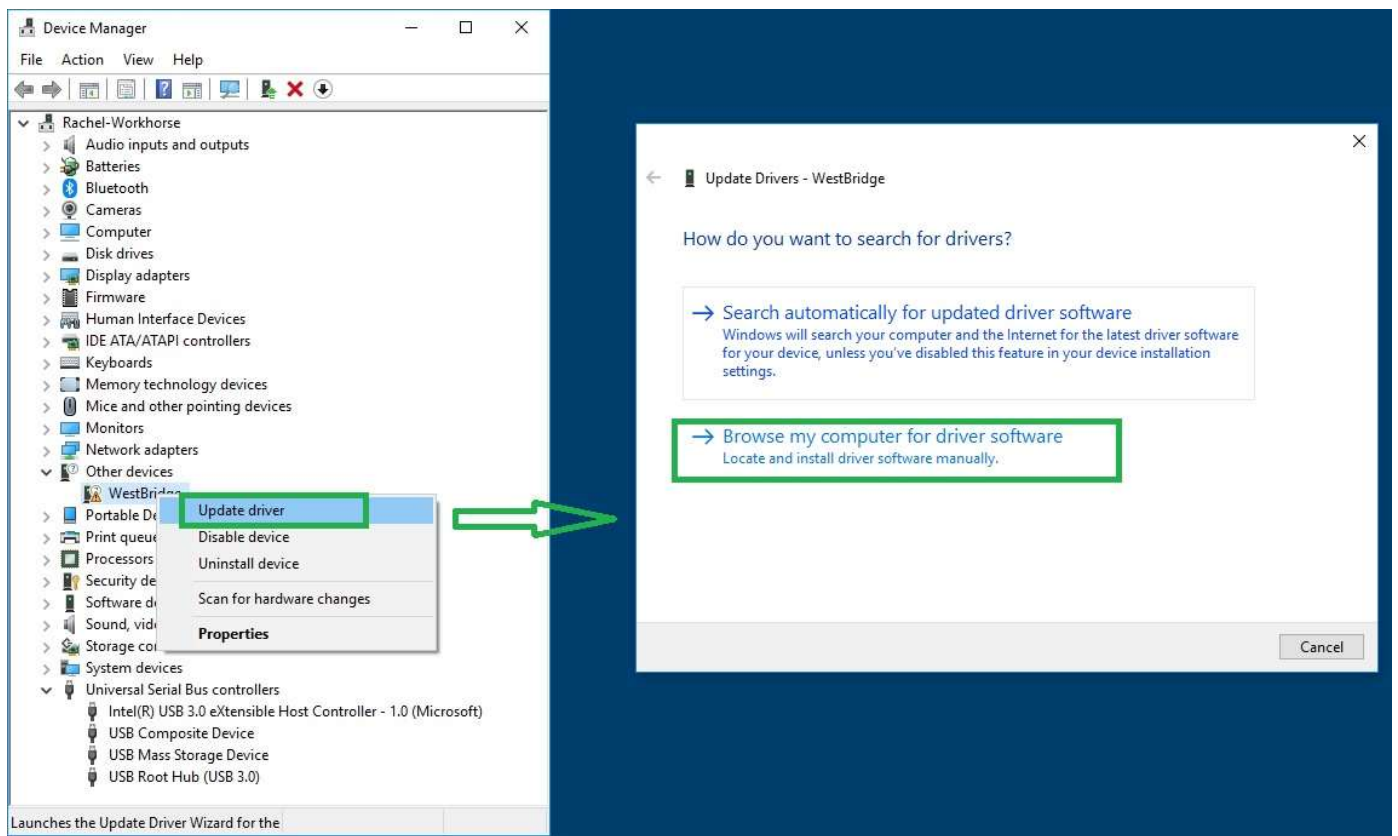


Figure 13: ADC256 driver installation. Steps 3 - 5 .

3. Find a new device in Device Manager, the device will be originally recognized as *Other devices/WestBridge* with a yellow triangle indicating a driver error. If a driver was

- preinstalled, or another driver was found on the computer, uninstall all previously installed drivers as described in Item 9 of this section.
4. RMB (click the right mouse button) on *WestBridge* device and choose Update driver. Next choose *Browse my computer for driver software*.
  5. Follow Figure 13. Choose *Browse ...*, *Browse For Folder* with the ADC256 driver, next choose subfolder with your Windows version. Press OK. Note, that this driver supports both 64-bit and 32-bit Windows, but the ADC256 software supports only 64-bit versions of Windows.
  6. Verify that the driver was installed as *Cypress FX3 BootLoader Device* (before the firmware is loaded) which turns to *Cypress FX3 StreamerExample Device* after the firmware is loaded; see Figure 15.
  7. Update the PC USB host controller to the latest version using drivers from the PC or the motherboard manufacturer's web-site. Verify that driver was actually installed by finding host controller device in the Device Manager and reading its actual driver version.

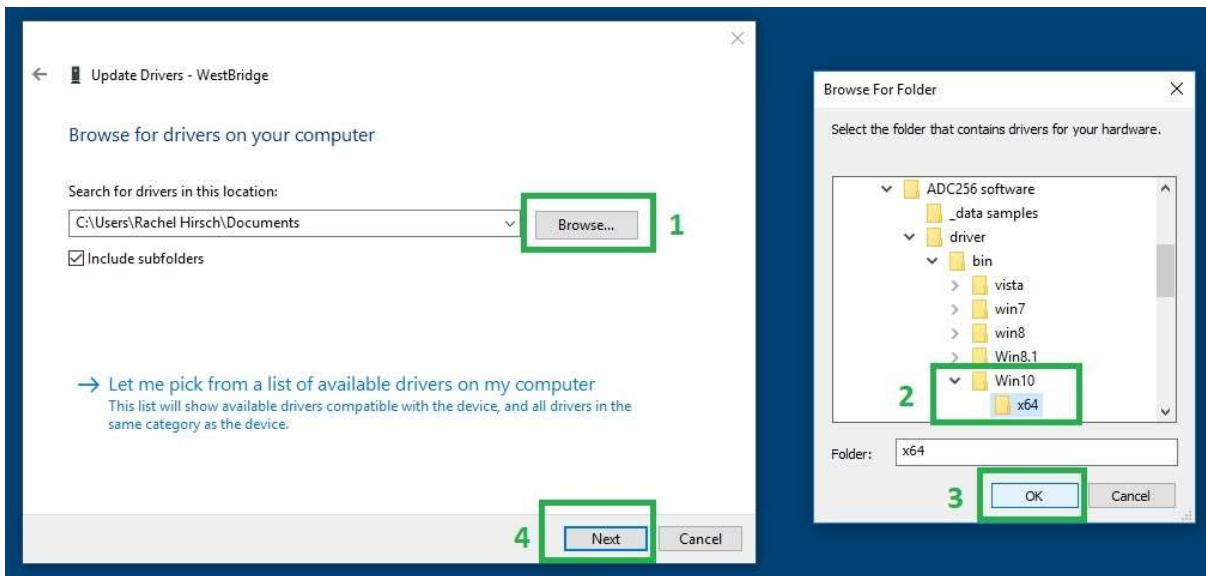


Figure 14. ADC256 driver installation. Step 5.

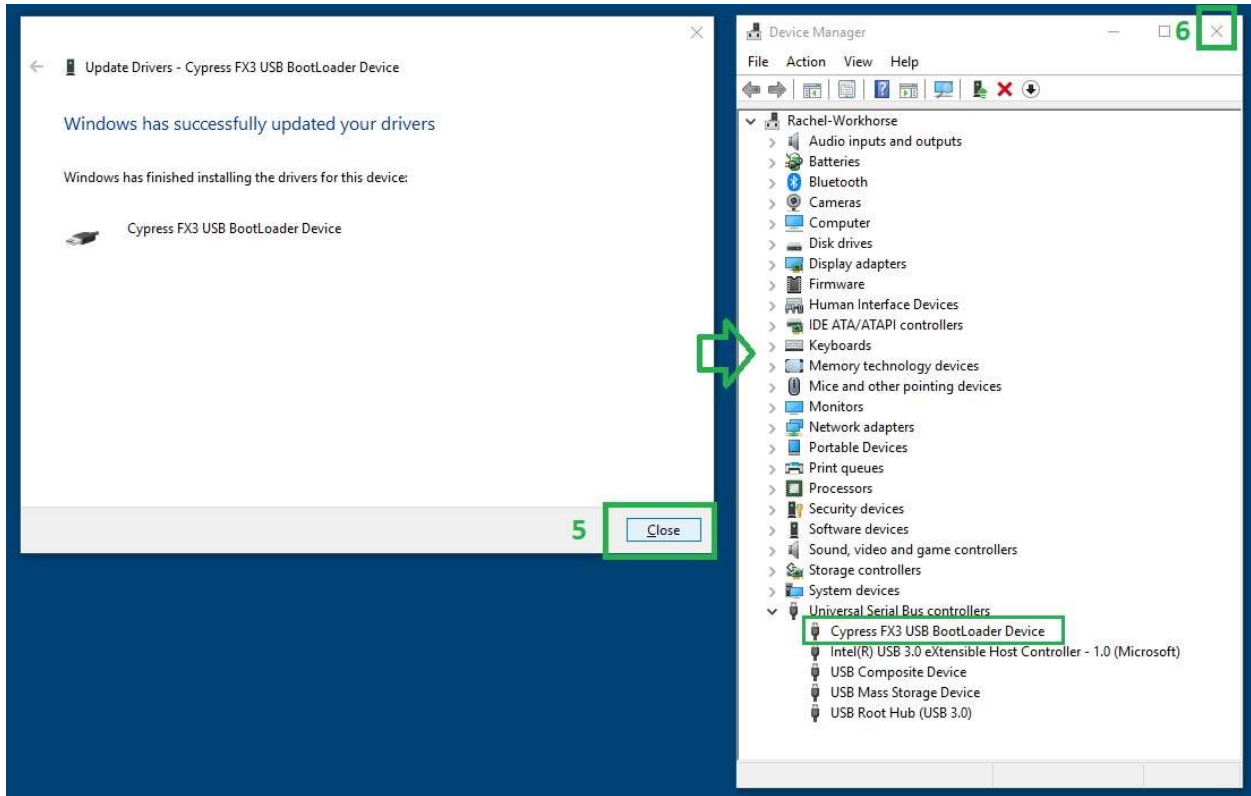


Figure 15. ADC256 driver installation. Step 6.

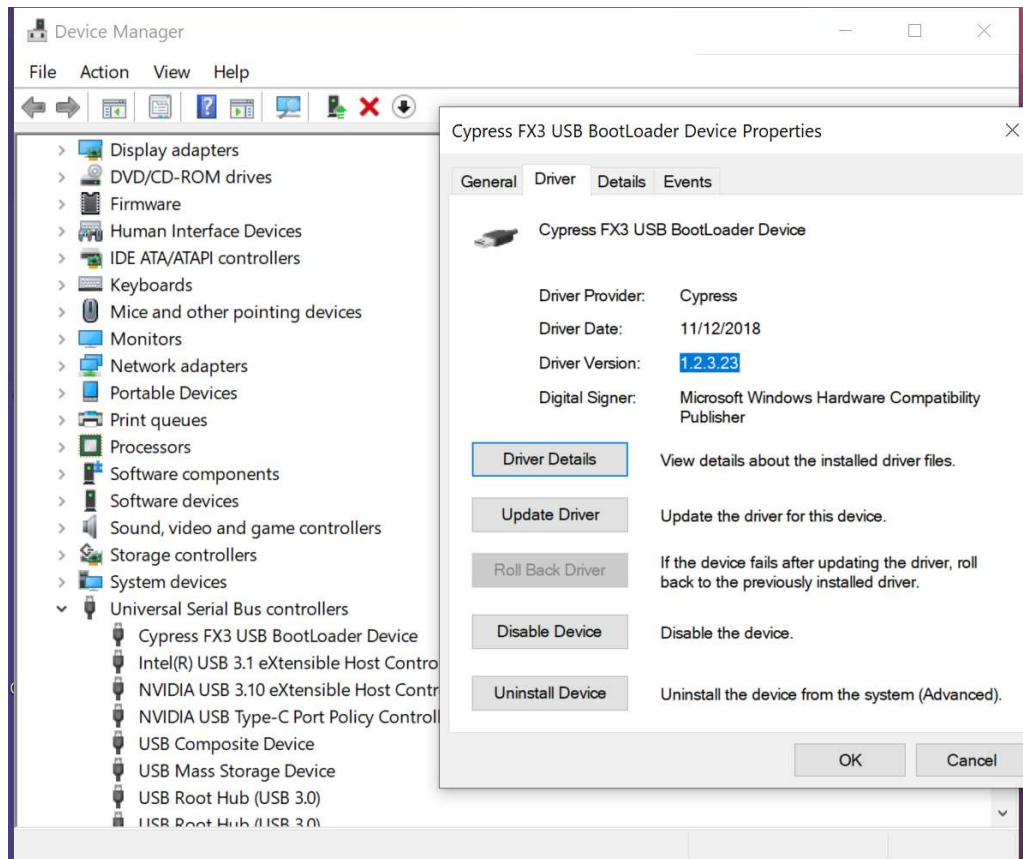


Figure 16: As of 2021-03-11, the current ADC driver version is 1.2.3.23. This driver version provides the best data transfer performance and supports Asmedia and other non-Intel host controllers.

8. How to uninstall an old or incorrect ADC256 driver.
  - a. If the device has multiple drivers or an incorrect driver, uninstall the device in the *Device Manager*: Find the device, RMB (right mouse button) click, *Uninstall*, mark *Delete the driver software for the device*, OK.
  - b. Unplug and plug USB.
  - c. Verify that all drivers for the device were deleted using *Device Manager*. If any driver is left, repeat 9a.
9. *Note*: Do not unplug the USB3 cable after the firmware is loaded, because the ADC256 would then not be recognized by Windows. After power cycling the PC, power cycle the ADC256 board, but keep the USB3 cable always plugged in. Power cycle the ADC256 board if the USB3 cable was unplugged with loaded firmware.

## Setting up the ADC256 software environment: application and SDK installation

1. Install [MATLAB 2017b \(9.3\) Runtime](#) for compiled version only, or MATLAB 2017b for source code. Restart the computer, if prompted.
2. Install the matching .Net environment if prompted at any installation step.
3. Extract the 7zip archive with the application software and copy into any folder on the PC.

## ArtUs driver and Telemed software installation

1. Disconnect ArtUs microUSB3 cable and disconnect power from the ADC256.
2. Search for UAC (User Account Control) in Windows Settings and disable the UAC by selecting “Never notify”

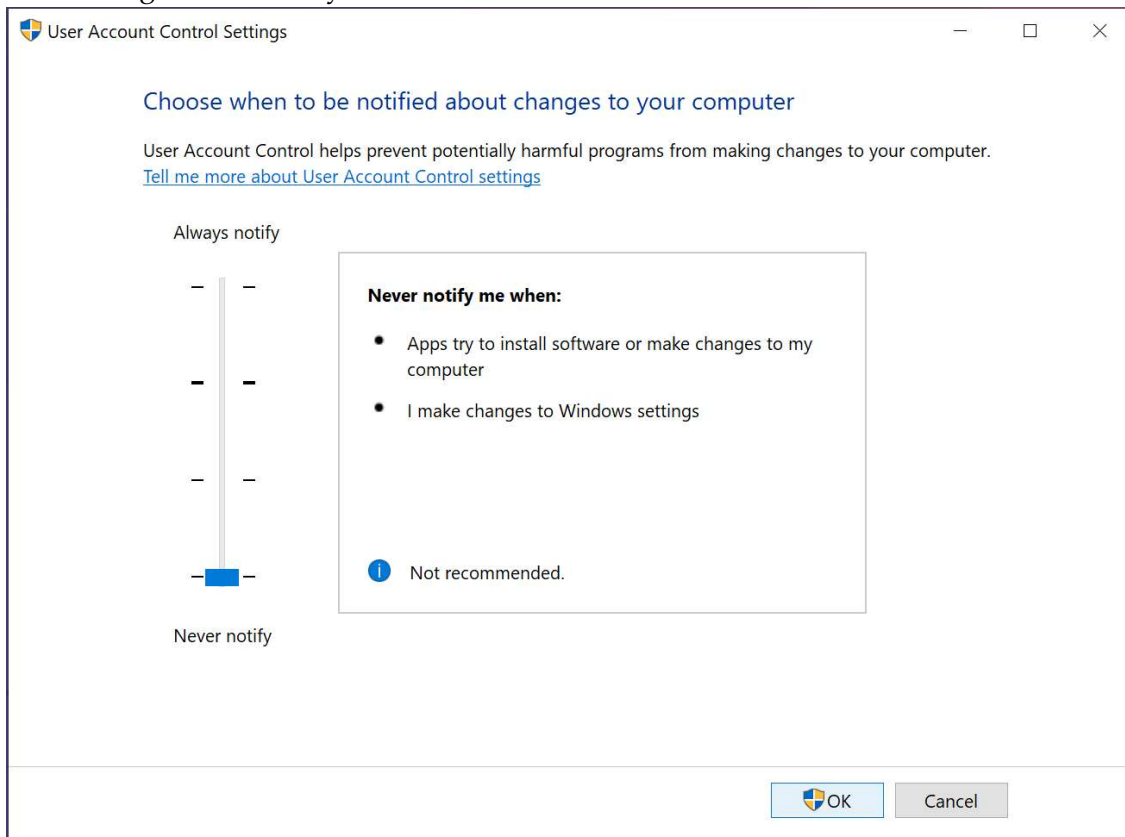


Figure 17: Disable UAC

3. Optional: Before the installation of the current version of the Telemed software, uninstall the EchoWave2 software and all other Telemed software from `\Control Panel\Programs\Programs and Features\` as shown in Figure 17. Reboot the PC. (This step 3 is not required for the first-time installation of the Telemed software.)

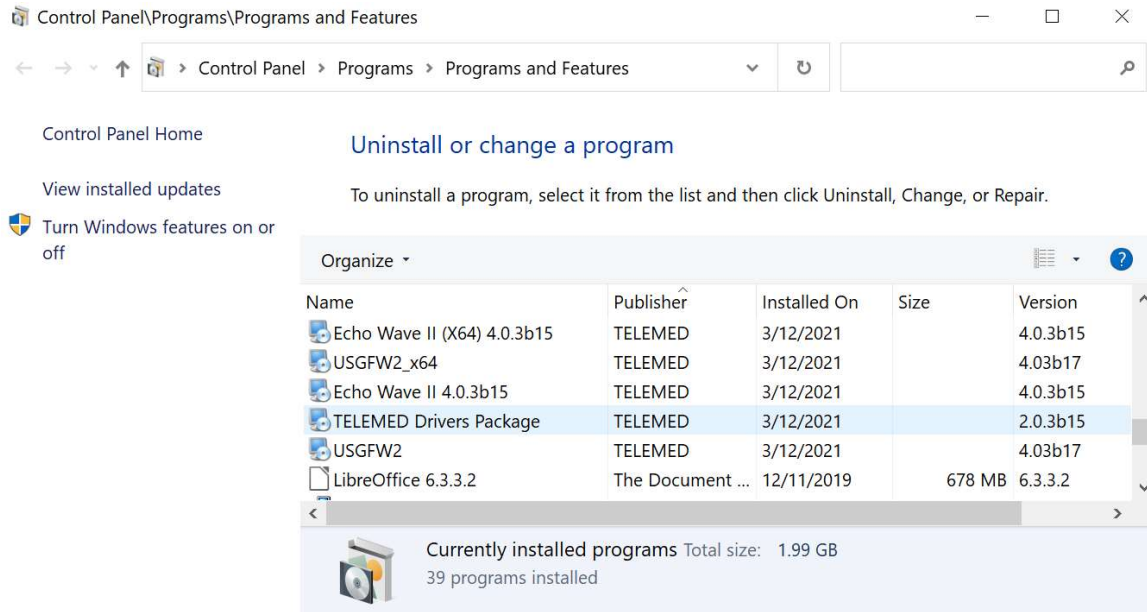


Figure 18: Telemed software in the Programs and Features.

- Optional: To upgrade the ArtUs-CPA driver uninstall the old ArtUs-CPA driver through Device manager, Figure 18. (This step is not required for the first-time installation.)

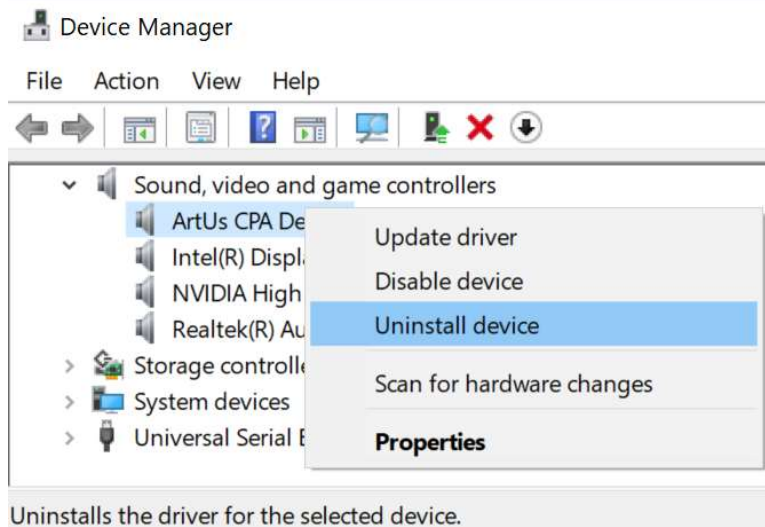


Figure 19: Before upgrading ArtUs-CPA driver uninstall the old driver, choose delete driver software option. Disconnect and reconnect ArtUs USB cable to verify that driver is deleted completely.

- Install the base ArtUs driver: run MoleculUS Software Package\ArtUs\Drivers\setup\_tdp.exe, Figure 19, and choose ArtUs only.

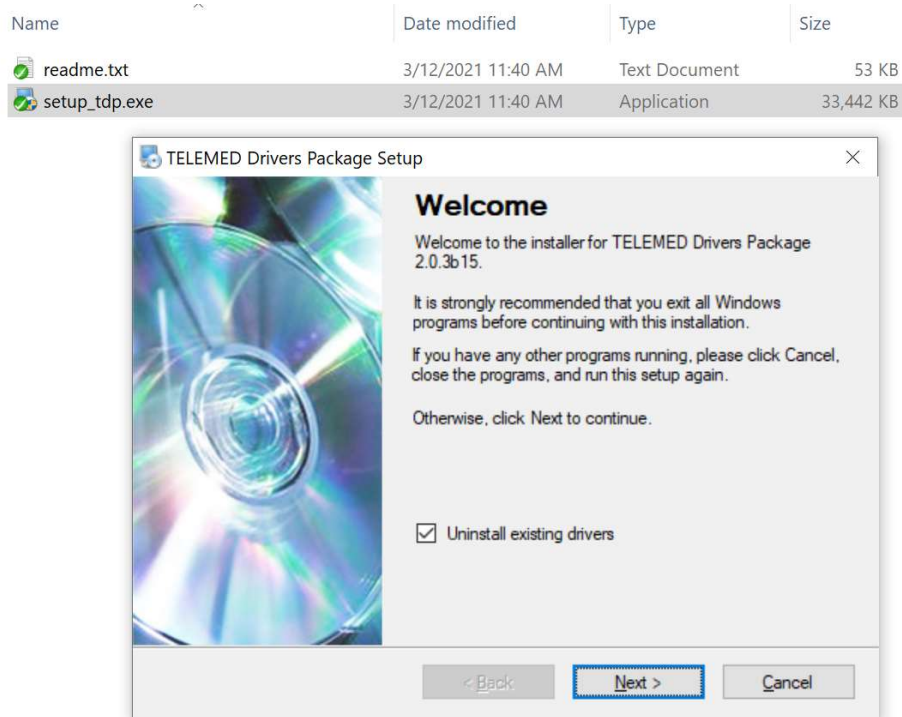


Figure 20: Install base ArtUs driver

6. Install the 32-bit Telemed software and the 64-bit EchoWave II software found in MolecuUS Software Package \ArtUs\EchoWave\EchoWave2\ using setup\_ew2.exe and setup\_ew2x64.exe Figure 20. Reboot the PC.



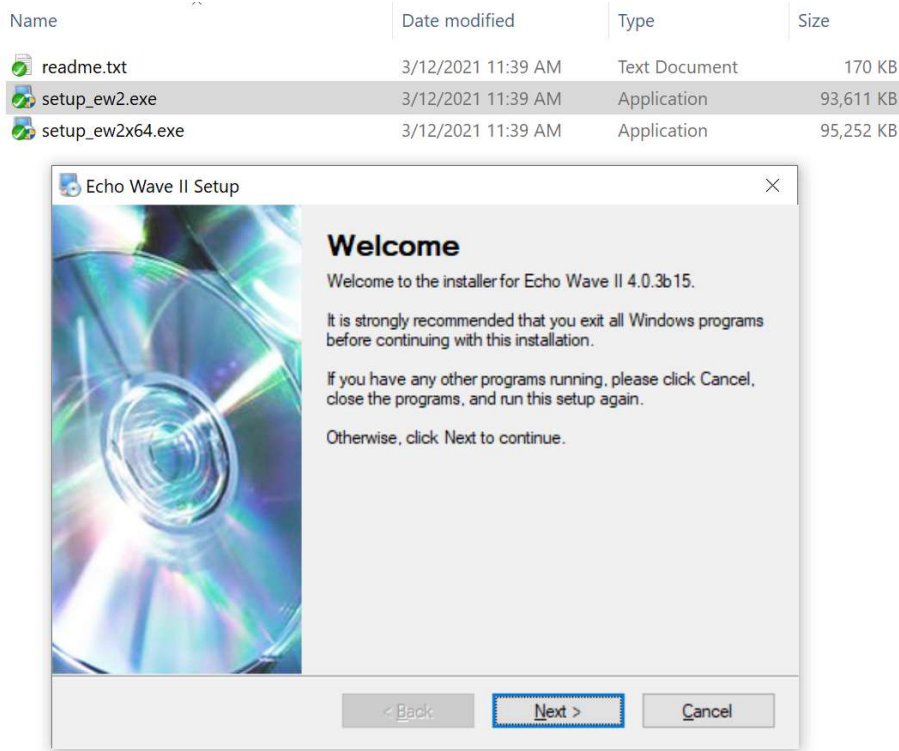


Figure 21: Install the Telemed II 32-bit software and repeat for the EchoWave II 64-bit software.

7. Connect power to the ArtUs. Open Windows Device manager and find “ArtUs” in “Other devices” with a yellow triangle. RMB (right mouse button), update the driver from MoleculUS Software Package\ArtUs\Drivers ArtusCPA\, Success.

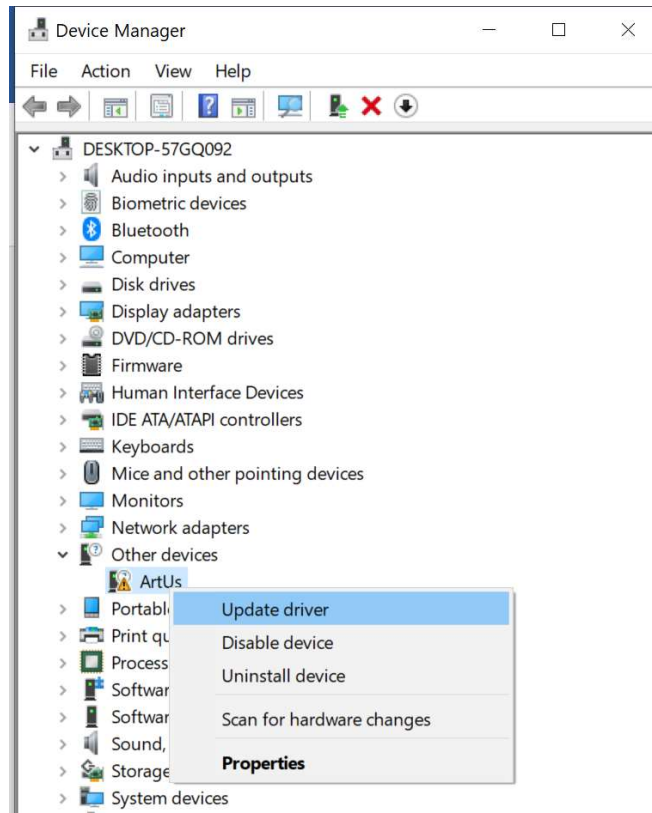


Figure 22: Find connected ArtUs device in Other devices with yellow triangle, click RMB and choose Update driver.

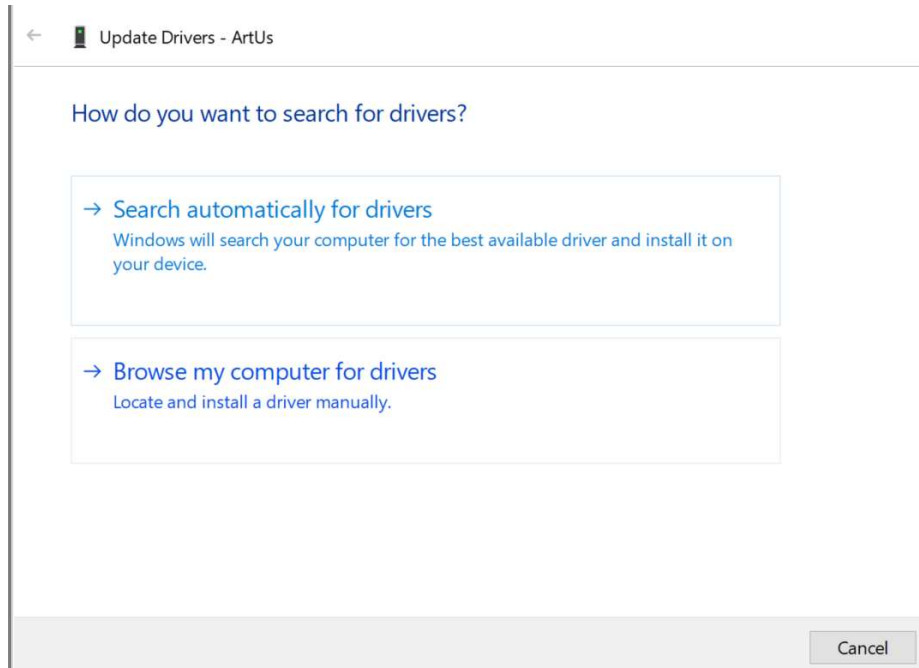


Figure 23: Choose Browse my computer for drivers.

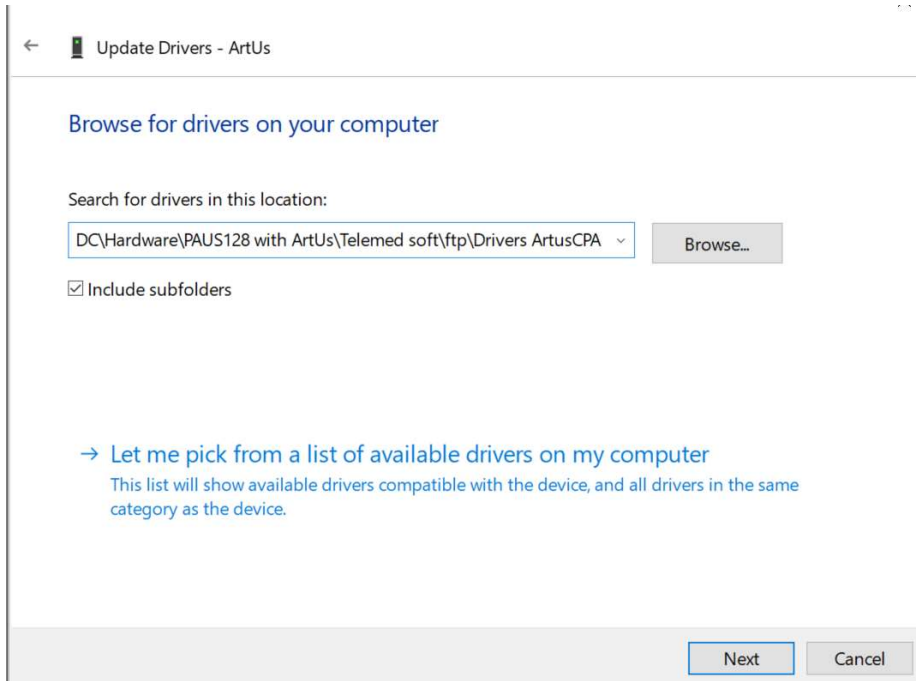


Figure 24: Find ArtUsCPA driver folder and click Next.

8. Find “ArtUs CPA Device” in the “Sound, video and game controllers” section of the Device manager.

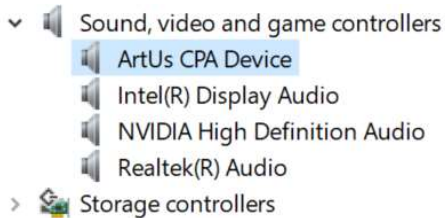


Figure 25: Find properly installed ArtUs CPA Device in the Sound, video and game controller section of the Device manager.

9. Find “\ArtUs\ArtUs Sync Options.exe” and run as Administrator (use RMB), Figure 25. Choose the synchronization option as shown for synchronization of the US (ArtUS) from the PA (ADC256), Figure 26.

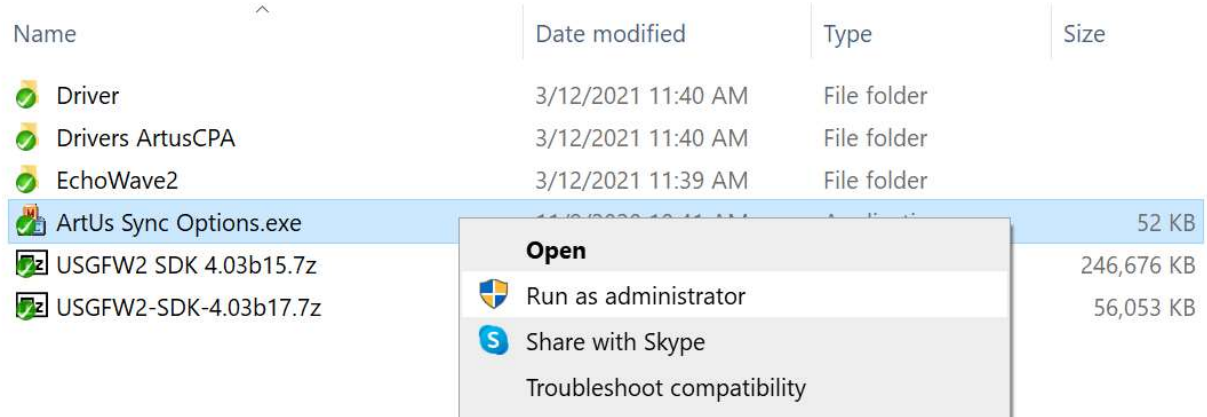


Figure 26: Run “ArtUs Sync Options.exe” as Administrator using Right Mouse Button.

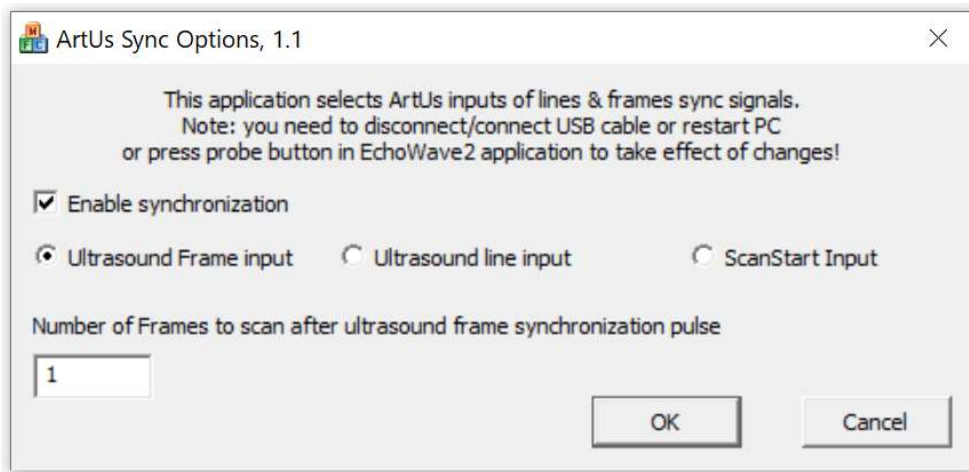


Figure 27: Enable synchronization for normal MoleculUs operation mode with US trigger supplied from the ADC256 board.

10. If the ArtUs-CPA device is visible in the Device Manager but not recognized by EW2 software, open the Windows Event Viewer (Figure 28) to find ArtUs device error messages as shown in Figure 29.

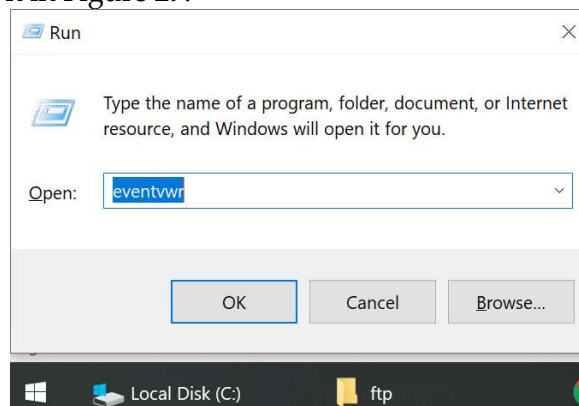


Figure 28: To open the Event viewer press Win-key + R, type eventvwr, and choose OK.

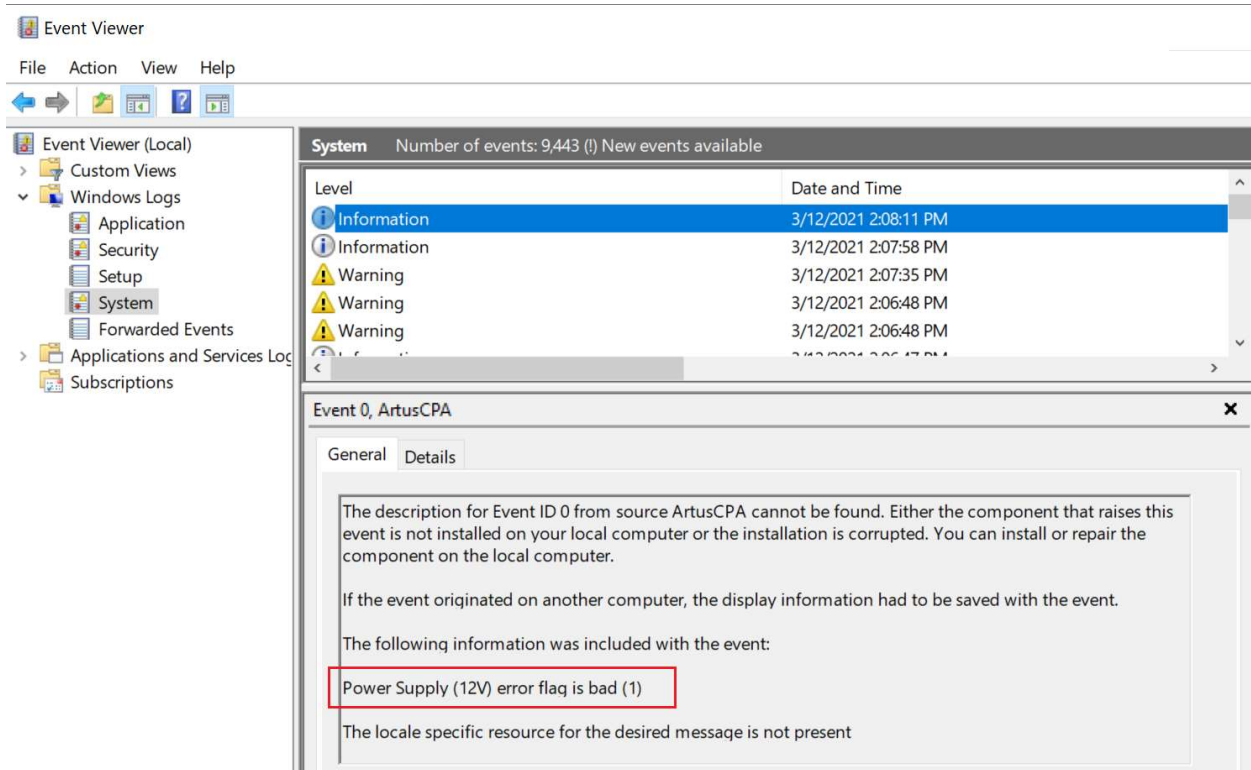


Figure 29: ArtUs power loss (bad power) event in Windows event viewer.



Figure 30: Ultrasound Scanner Monitor indicating the connection status of the ArtUs device to the PC. Red – not connected or/and no power; green – powered, connected and ready.

## Application software installation

11. This software Application is portable. Copy the archived files (7z) to a destination folder and extract the files.

Note Temporary software startup order (this will be changed once the MoleculUS and EchoWave software are merged):

- a. Always run the MoleculUS application before starting the EW2 ultrasound application, if synchronization between the PA and the US was enabled using “ArtUs Sync Options.exe” (Figure 27).
- b. If synchronization between PA and US was disabled using “ArtUs Sync Options.exe”, power-cycle the ADC256, but do NOT run the ADC software. The ADC256 should be visible in the Device manager as Cypress FX3 Bootloader device, not Streamer device. Run the EW2 as usual.

12. Run MoleculUsControl.exe, Figure 30.

13. To see ultrasound, start 32-bit or 64-bit EW2 software.

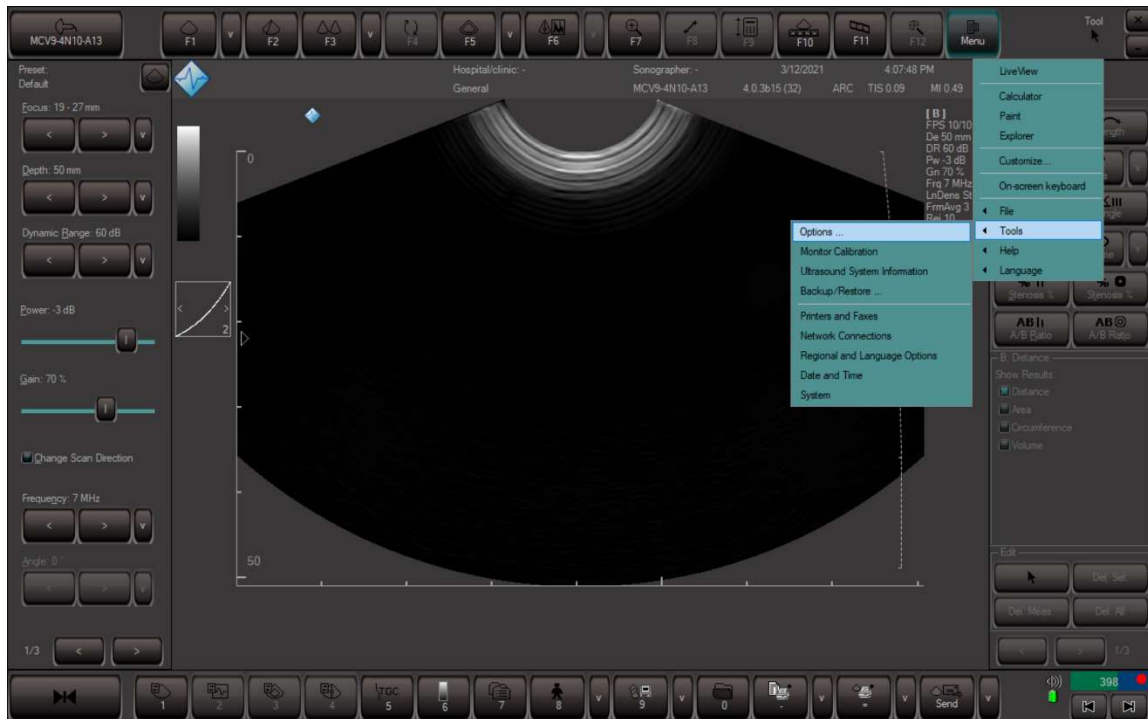


Figure 31: EW2 initial full screen or windowed mode can be set in Menu / Tools / Options...

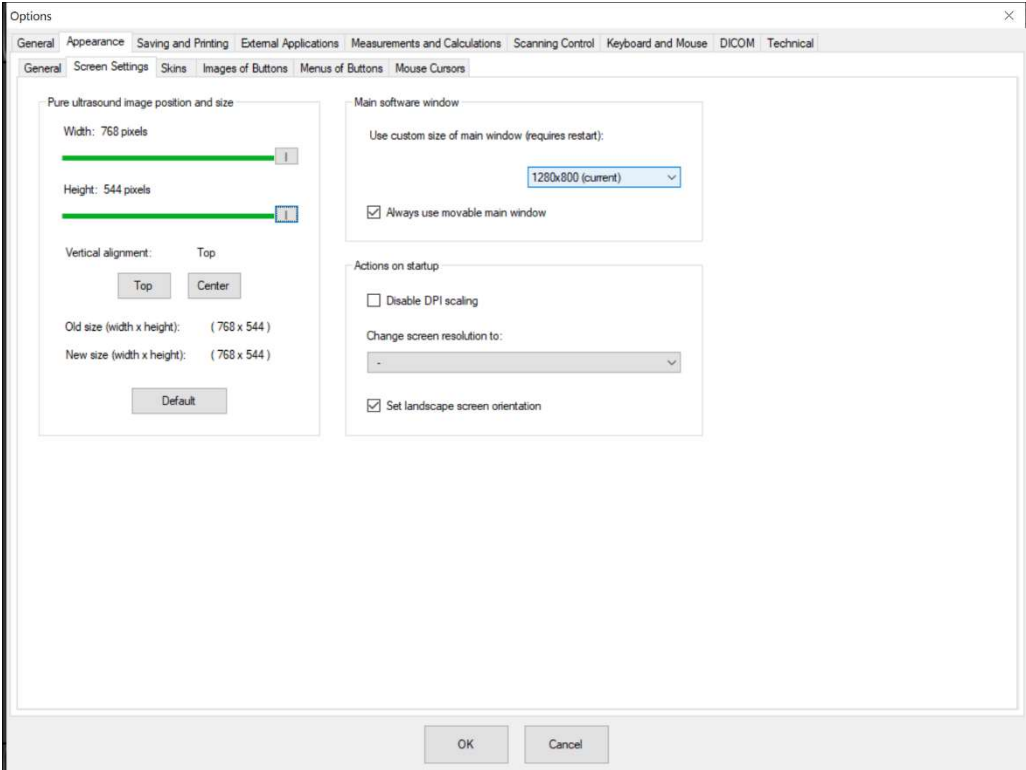


Figure 32: For the windowed option check “Always use movable main window”. Note that the windows scaling option affects the actual windows display size.

## MoleculUS Software

Run *MoleculUS.exe* and wait until the device is connected (Figure 33).



Figure 33: MoleculUS control main window Main control hub of the MoleculUS's PA signal acquisition and reconstruction. Digital oscilloscope (1), plot control (2), status bulb (3), Capture and trigger settings (4), Configuration menu (5), speed of sound and contrast sliders (6), capture progress bar (7), real-time reconstruction settings (8), capture parameters (9), status monitor (10), action buttons (11), folder button (12), real-time reconstruction viewer (13).



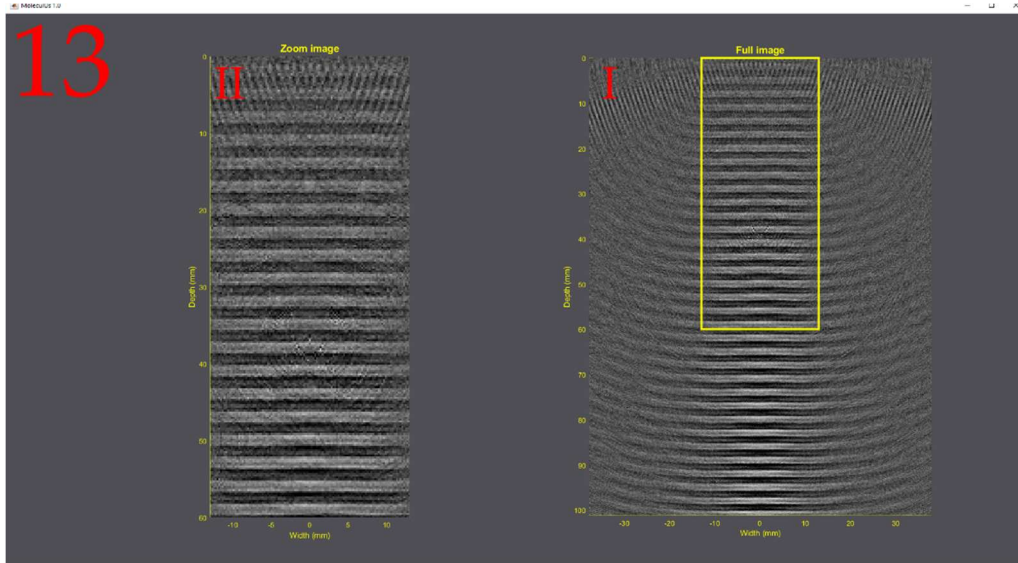


Figure 34: Real-time reconstruction viewer. The Full image (I) and Zoom image (II), area of the yellow rectangle on the Full image, are displayed.

Upon starting the MolecuS.exe application, two windows will appear: MolecuS control (Figure 33) and the reconstruction viewer (Figure 34). The MolecuS main control window (Figure 33) has multiple sections and is detailed below, with the reconstruction viewer's description at the end:

1. Digital oscilloscope of selected probe sensors. X-axis is number of samples; Y-axis is voltage as signed 16-bit with  $V_{pp} = 2$  V.

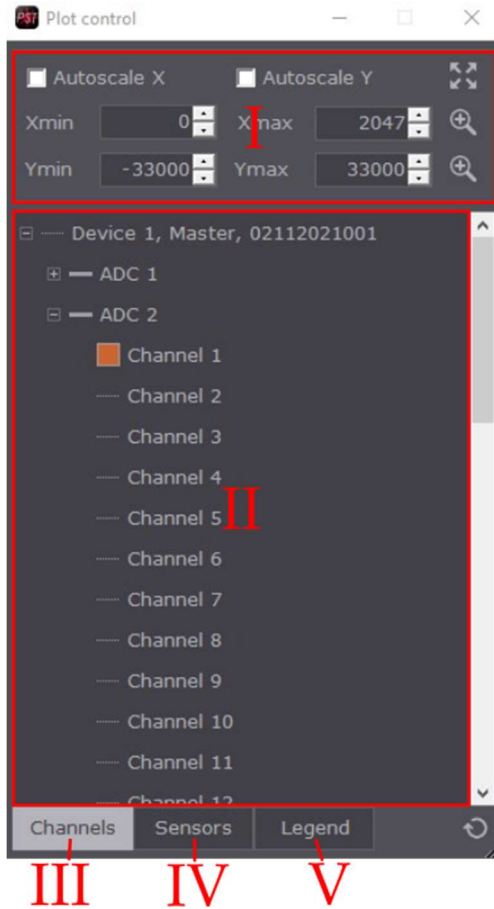


Figure 35: Plot control dialog. Sets the channels/sensors that are visualized in the digital oscilloscope. Configures the axes of the digital oscilloscope. Axis configuration (1), channel/sensor selection (2), channel list (3), sensor list (4), legend (5).

2. Plot control dialog button (Figure 35).
  - I. Plot axis configuration. Sets the window size of the digital oscilloscope (1).
  - II. Sets the channels/sensors displayed on the digital oscilloscope (1) by selection.
    - Channels – lists the elements in the order of ADC channels.
    - Sensors - lists the elements in the order of probe sensors.
  - III. Arranges the channels/sensors list (II) by ADC channels.
  - IV. Arranges the channels/sensors list (II) by loaded probe sensors.
  - V. Lists the selected sensors displayed on the digital oscilloscope (1).
3. Device status bulb. [RED] indicates an error with the ADC USB connection or ADC firmware, [YELLOW] indicates the device is connecting/completing an action, [BLUE] indicates the device is ready for an action.

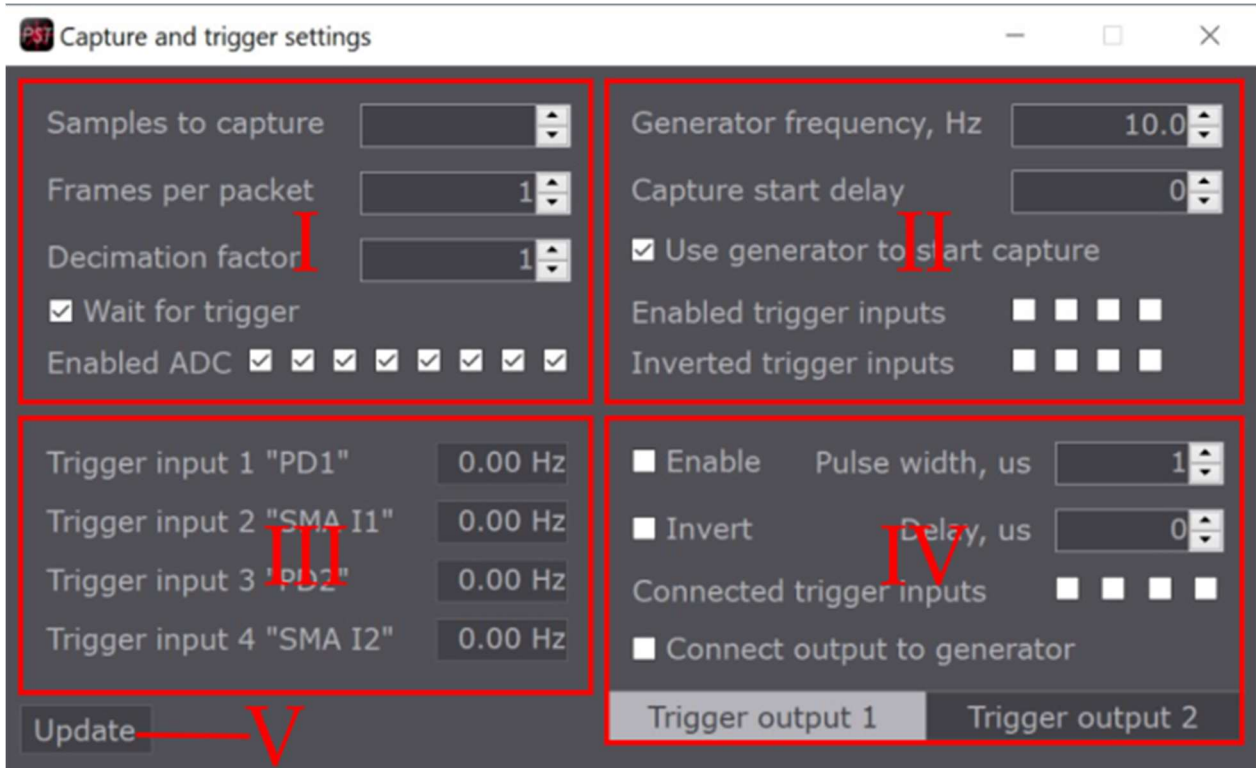


Figure 36: Capture and trigger settings dialog. Sets the capture settings (I), trigger input settings (II), trigger input monitoring (III), and trigger output settings (IV). Update button (V) sets changes.

#### 4. Capture and Trigger settings (Figure 36).

##### I. Capture settings section.

- Samples to capture – Sets the number of samples to capture for an acquisition.
- Frames per packet – number of frames transferred through the USB3.0 connection per data transfer event (packet). For the fastest real-time reconstruction, set this value to 1.
- Decimation factor – Reduces the sampling rate by the factor set (i.e. New sampling rate = old sampling rate / decimation factor). Note: changing this value from the default of 1 will limit the data transfer rate and might result in the loss of packets.
- Wait for trigger – If checked, the device will only acquire a frame when it is triggered. If unchecked, the device will acquire frames as fast as possible without any frame loss. Note: this setting is for diagnostics, there is no purpose to leave this unchecked for data collection.

- Enabled ADC – sets which ADC devices are capturing starting at ADC #1 (left) to ADC #8 (right). Note: The USPA connector is on ADC 1-4; the PA-only connector is on ADC 5-8.
- II. Trigger input settings section.
- Generator frequency, Hz – sets the internal generator frequency of the device. Note: This value is used for diagnostics. When the ‘Use generator...’ checkbox is unchecked, the generator frequency is not relevant.
  - Capture start delay – value sets the delay between a trigger event and the frame acquisition, in number of samples.
  - Use generator to start capture – enables the internal generator as a trigger source.
  - Enabled trigger inputs – enables/disables the available trigger inputs. From left to right: PD1, SMA I1, PD2, SMA I2.
  - Inverted trigger inputs – enables/disables inverted trigger inputs. Ordering is identical as the Enabled trigger inputs.
- III. Trigger input monitoring section. Each entry tracks the trigger input frequency detected. Will only update when the ‘Update’ button is pressed.
- IV. Trigger output settings section. Two trigger outputs are on the MoleculUS board, either can be selected at the bottom of this section. Trigger outputs are relayed from trigger inputs as pulses.
- Enable – enables trigger output.
  - Invert - inverts trigger output.
  - Pulse width, us – sets the pulse width of the trigger output in (μs).
  - Delay, us - sets the trigger output delay from relayed trigger input in μs.
  - Connected trigger inputs – sets which trigger inputs to relay as trigger outputs. Left to right: PD1, SMA I1, PD2, SMA I2.
  - Connect output to generator – sets the trigger output to relay the generator frequency of electrical or optical inputs.
- V. Update button sets the configurations modified and displays the trigger input monitoring. Changes in the Capture and trigger settings dialog will not be set until ‘Update’ is pressed.

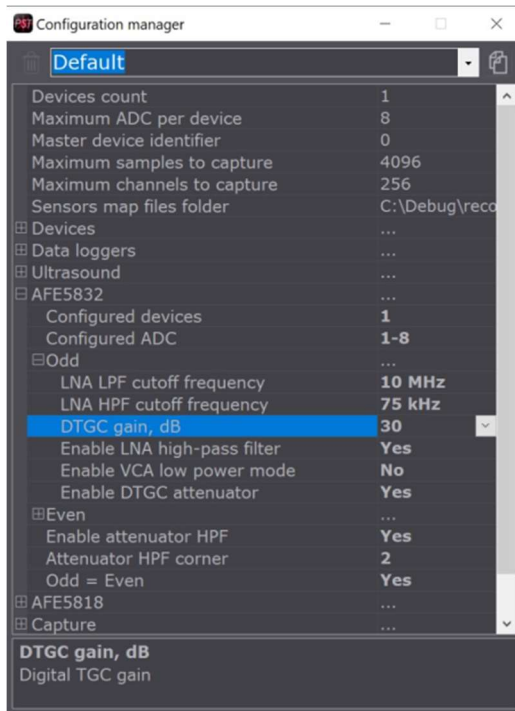


Figure 37: Configuration manager dialog. A more advanced settings menu. All values of the Trigger and capture settings dialog are set in this menu. Note: for MoleculUS, the AFE5832 sets the ADC chip settings.

5. Configuration manager (Figure 37). An advanced settings menu that encompasses all available programmable settings for the ADC device.
  - TBD
6. Real-time reconstruction tuning sliders.
  - Speed of Sound – changes the real-time reconstruction speed-of-sound value in (m/s). Start at the speed-of-sound in the medium at the measured temperature and fine tune from there.
  - Video contrast – varies the contrast of the real-time reconstruction to better visualize high/low contrast objects.
7. Capture progress bar. When a capture event is initiated, the bar will fill based on the progress of data acquisition.
8. Real-time reconstruction settings.
  - Probe – specifies the 'PROBE' file to read. This file contains the channel mapping and sensor geometry necessary for real-time reconstruction.
  - Left, Right, Top, Bottom – sets the Zoom image window of the real-time reconstruction in (mm).

- US gain, dB - sets the programmable gain of the USPA connector, range is 6-51 dB.
  - PA gain, dB – sets the programmable gain of the PA only connector, range is 6-51 dB.
  - Depth, mm – sets the real-time reconstruction depth for the Full image.
9. Capture parameters. Sets the limits of a capture event; the first of the limits to be reached will terminate the capture event.
- Number of frames – sets the number of frames to capture.
  - Logging timeout, (s) – sets the maximum time of capture in seconds.
  - File size, (MB) – sets the maximum file size in MB.
  - Limit number of frames – enables the Number of frames capture limit.
  - Limit logging timeout – enables logging the timeout capture limit.
  - Limit file size – enables the file size capture limit.
  - Enable video logging - enables the capture of a video (\*.avi) file of the real-time reconstruction alongside data capture.
10. MoleculUS status monitor. Monitors key parameters of the software’s capture and real-time reconstruction.
- Voxel – displays the set voxel size for reconstruction.
  - Transfer rate - displays the transfer rate of the ADC through the USB3.0 cable.
  - Logging time – displays the time logged during a capture event.
  - Logged frames – displays the frames logged during a capture event.
  - File size - displays the file size logged during a capture event.
  - FPS – displays the frame rate of the real-time reconstruction.
11. MoleculUS action buttons.
- Pick... - sets the Zoom image boundary by the user click-dragging a rectangle over the Full image.
  - Zoom – sets the Zoom image boundary by the user entering the limits into the Left, Right, Top, Bottom textboxes.
  - Adjust – sets the optimal US and PA gain based on current PA signals.
  - Start - starts a capture event that is limited by the capture parameters.
12. Select folder button. Sets the directory to save captured data and video files to.
13. Reconstruction viewer (Figure 34). Visualizes the real-time reconstruction of PA signals in two figures. One set as the Full image range of the transducer and the other as a Zoom image determined by the user.
- I. Full image – The Full image figure’s limits are set by PST to reflect the maximal boundaries of photoacoustic reconstruction using the selected probe.

- II. Zoom image – A portion of the Full image’s figure determined by the user from using the ‘Pick...’ or ‘Zoom’ buttons of the action buttons. The yellow rectangle on the Full image’s figure shows the current boundaries of the Zoom image.

## Ultrasound menu parameters, REQUIRED for laser rate different from 10Hz

Configuration manager \ Ultrasound menu is matching \ Config\Default.ini or other INI file section

[Ultrasound]

*SuppressTransients* = 1

*HighVoltageGuard* = 1000

*TransientsLength* = 200

*UltrasoundLength* = 95000

*SwitchesGuard* = 10

*OpAmpsGuard* = 10

Default parameters from Default.ini matching 10 Hz laser operation are listed above. All parameters, except *SuppressTransients* are time in  $\mu\text{s}$ .

- *UltrasoundLength* is the time window for ultrasound.
- *HighVoltageGuard*, *SwitchesGuard*, *OpAmpsGuard* are waiting time to reduce high-voltage transients in PA signal.

In the default setting above 95000  $\mu\text{s}$  are reserved for ultrasound and remaining 5000  $\mu\text{s}$  for photoacoustic acquisition and transient response within  $1/10 \text{ Hz} = 100000 \mu\text{s}$  laser firing period. Photoacoustic acquisition of 4096 data points with 40 MSPS sampling rate and decimation factor = 1 requires just 101  $\mu\text{s}$ , but extra time is needed for mode switching safety window.

For 20 Hz laser firing rate or 50000  $\mu\text{s}$  laser firing period, use *UltrasoundLength* = 45000.

For 100 Hz laser firing rate or 20000  $\mu\text{s}$  laser firing period, use *UltrasoundLength* = 15000.

For 100 Hz laser firing rate or 10000  $\mu\text{s}$  laser firing period, try *UltrasoundLength* = 5000 to 8000, *HighVoltageGuard* might be reduced to 500.



## QuickStart Guide

1. Launch *MoleculUS.exe*.
2. Set ArtUS to synchronize mode Figure 27.
3. Launch *Echowave2.exe*.
4. Configure trigger inputs in MoleculUS control's Capture and trigger settings (Figure 36, II).
5. Select the correct 'PROBE' file to load (Figure 33, 8).
6. Start firing the laser.
7. The digital oscilloscope (Figure 33, 1) should display the detected PA signals and the EchoWave program should display ultrasound. The real-time reconstruction will update with each trigger event.
8. Optimize PA reconstruction
  - a. Vary 'Speed of sound' until the subject is 'focused'.
  - b. Vary 'Video contrast' until the subject is clearly visible.
  - c. If the subject is still not visible, increase gain until the strongest PA signal's amplitude is close to saturation, which happens when the PA signals exceed values [-32767, 32767] in the digital oscilloscope (Figure 33, 1).
9. Optimize US reconstruction
  - a. Press 'F1' to enter B-mode and '1' to enter settings mode.
  - b. Set the 'Focus' on the left side of the software to an appropriate depth for the subject. Note: in the US viewer, the arrow on the left side indicates the current focus depth.
  - c. Vary the 'Power' setting if the subject is saturated. Note: If the probe interface is water, you will likely need a lower 'Power' setting. If the probe interface is ultrasound gel, you will likely need a higher (or max) 'Power' setting.
  - d. Vary the 'Gain' setting to further optimize the image if the 'Power' setting did not produce a good image.
  - e. Vary the 'Frequency' setting to further optimize imaging at specific depths. Higher frequency => lower penetration depth.
  - f. Vary the 'Dynamic range' setting to increase the contrast of the image.
  - g. Pressing 'F5' enables the color doppler functionality of the ultrasound device. Pressing '6' will allow you to change the color palette of the doppler.
  - h. To further optimize signal amplitude at different depths, press '5' to activate the time-gain control menu. Vary gain settings at different depths.
  - i. To increase depth resolution, return to the B-mode and setting mode 'F1', '1'. At the bottom left of the settings column, navigate to page 2/3. Increase/decrease the 'Line density'. Note: for fast trigger rates, modifying this value might limit the FPS of the ultrasound reconstruction.

10. Save PA and US data.

- a. On Echowave, press '9' to enter the saving image/video menu. Note: on the bottom bar, on the right next to the save video button '9' is an arrow. This dropdown menu will allow you to select whether to save the buffer as a video or image.
- b. On MoleculUS control, set the capture limits (Figure 33, 9) and press 'Start'.
- c. When the PA data capture event is complete, shut off the laser.
- d. Save the corresponding US frames in EchoWave by selecting the matching frames from the buffer and exporting as a video or image file(s).