5. Build instructions

All the 3D printed components in the design were made with SLA 3D printers. The SLA style ensures the parts are waterproof, however, that need only applies to the three external parts (fixed end cap, flanged end cap, and lid). The internal components could be made with a different 3D printing style or other production method provided form was consistent with the design. For 3D printing the default settings of the printer were used with a print resolution of 0.1mm and supports with rafting.

5.1. Housing construction

To prepare the components of the housing, sand the joining faces of the 3D printed end caps, acrylic window, and the PVC pipe (Fig. 1) to improve adhesive bonding. Throughout the construction process be sure to clean and dry areas prior to epoxying and remove excess epoxy to avoid issues with fit and function. Apply a heavy bead of 2-part epoxy along the inside edge of the flanged end cap (Fig. 2) and along the outside edge of one end of the PVC pipe. Push the flanged end cap onto the PVC and give it a quarter twist to ensure even spread of the epoxy. Place the joined parts with the end cap down and weight the PVC to improve stability during curing of the epoxy, which should take 18-24 hours.

Apply a heavy bead of 2-part epoxy along the inside edge of the fixed end cap and press the acrylic window into the end cap. Twist the acrylic window as needed to ensure even spread of the epoxy and set the end cap with the acrylic facing up. Weight the acrylic to improve stability during curing of the epoxy.

Once both parts are fully cured, add a heavy bead of 2-part epoxy along the inside wall of the fixed end cap and the outside uncapped edge of the PVC pipe. Insert the PVC into the fixed end cap and give the PVC a quarter twist to ensure even spread of the epoxy. At this point make sure the magnet holes on the end caps are lying on the same plane and lined up. Failure to line up these two surfaces will prevent the sampling cartridge from securely attaching. Once the fixed cap is in place and lined-up set the housing down and weight the top piece to improve stability during curing of the epoxy.

Once the housing is fully cured, insert the #6-32 x 1” bolts into the flanged end cap threaded holes. Apply a small amount of lubricant to the end cap O-ring and place in the O-ring groove of the flanged end cap. Install the acrylic test cap using the #6-32 lock nuts. Tighten the nuts down until the O-ring is visually compressed. Throughout the construction process, be cautious tightening screws on 3D printed parts to avoid overtightening and damaging the printed components. Submerge the housing in water and watch for air bubbles escaping, which would indicate a leak, and look for signs of water entry through the acrylic window. Minor leaks may be fixed by identifying the location and adding more epoxy. This water test is not required but is useful to prevent the discovery of leaks after the motors and electronics are installed, especially on the first build.

5.2. Motor installation

Match the 34:1 motor with the 3D printed right motor sleeve, and the 99:1 motor with the left sleeve and sand the contact faces of the top of the motors and the insides of the sleeves. Put a heavy bead of 2-part epoxy around the top of the motor body and slide the motor sleeve onto the

motor, twisting it around to improve the even spread of epoxy. Place the motor shaft-down with the face of the motor and the face of the motor sleeve flush during curing. A board with holes for the motor shaft can serve as a helpful jig for this step (Fig. 3). Failure to keep the motor and sleeve face flush can impact the stability of the motor mount on the SASe lid.

To install the threaded #4-40 fittings into the SASe lid, first partially screw the fitting onto a #4-40 screw. Apply a coat of 2-part epoxy to the outside of the fitting and then push the fitting into position until the top is flush with the top of the lid (Fig. 3). Leave the fitting in place, carefully remove the screw, and avoid pushing epoxy inside the fitting while the epoxy cures.

Once the motor sleeves are cured onto the motor, clip the length of the motor wires to 3.5 inches. Run the wires for each motor through a 0.5-inch long piece of 1/4-inch heat shrink tubing. Install a crimp onto the end of each wire and insert the crimped wires for each motor into a motor Molex connector matching the original order (Fig. 4). Arrange the heat shrink tubing about midway between the motor and the connector and apply heat to shrink for better wire organization.

Apply a small amount of lubricant to the motor shaft X-rings and install them into the X-ring seats on the inside of the SASe lid, using the shaft of the motor to push them into place. Once X-rings are installed, push the shaft of the motors through the shaft holes in the lid. The 34:1 motor used for sample collection and the 99:1 motor used for pumping preservative are positioned as shown in Figure 5. Secure the motors in place with the #4-40 x ½ inch screws through the motor

sleeves. The screws will fit flush with the motor sleeves and should not be more than “finger tight”.

Once the threaded fittings have cured in the lid, install the two peristaltic pumps onto the motor shafts, oriented so the pump mounting holes line up with the fittings. Secure the pumps in place using the #4-40 x 1-inch screws. Install the 1/8-inch barbed connector into the left tube of the sample pump head (Fig. 6). Cut the 1/8-inch tubing into a 2.25-inch long piece and a 3/4-inch long piece. Install the 1/8-inch barb tee connector on one end of the 2.25-inch long section and the 1/8-inch male luer lock fitting on the other end. Attach the 3/4-inch section of tubing to the free end of the tee. On the other end of the tubing attach the 1/8-inch elbow connector. Slide a 1/8-inch tube clamp onto the inside tube of each pump and then install the fitting/tubing combo into the two pump heads as pictured in Figure 6. Once inserted, tighten clamps to secure the fittings. Insert the 1/8-inch to 1/16-inch reducer fitting into one end of the 2.75-inch long 1/16-inch tubing. On the free end of the 1/16-inch tubing, insert the 1/16-inch barb to female luer lock fitting. Insert the 1/8-inch barb into the free tube of the preservative pump to complete the SASe plumbing.

At this point, the housing can be water-tested again to ensure the X-rings were installed properly. Install the lid with mounted motors and pumps and secure with the #6-32 lock nuts. Submerge the housing in freshwater and ensure there are no leaks.

5.3. Power source construction

The battery pack holds eight AA batteries and has ground and 12-volt wire connections. To add a 6-volt power source, install the 4-inch red wire into the battery pack midway between the 12-volt wire and the ground wire (Fig. 7). To install the wire, strip 1/8-inch of the insulation off one end of the wire and 1/4-inch of the insulation off the other end. Put the 1/4-inch side of the wire through the negative terminal indicated in Figure 7 and wrap the uninsulated wire around the terminal to make a more stable connection, then solder the wire in place. Clip the original wires to match the length of the new 6V wire, using the connection of the 6V wire as the starting point for measurement, and strip off an 1/8-inch of insulation from the ends. Put a 1-inch long piece of 1/16-inch heat shrink around the wires to keep them together and install a crimp on the end of each of the three wires. Install the wires into the battery Molex connector (Fig. 7).

Attach the 3D-printed battery housing to the lid using the two #6-32 x 3/4-inch long armature to lid screws. With the battery pack updated, install eight AA batteries and insert the battery pack into the 3D-printed battery housing. Secure the battery pack in place with the 3D-printed cover and the two #4-40 x 1/4-inch long screws.

5.4. Circuit board construction

The circuit board can be milled in-house, but the quality and cost of ordering printed circuit boards (PCB) make that the better option. This project had circuit boards milled by Seeed Studio (seeedstudio.com/fusion.html) using the circuit board design files (SASeBoard15.zip) and the following parameters:

PCB Dimension: 64.2mm*64.2mm

Impedance Control: No

Surface Finish: HASL

Min Solder Mask Dam: 0.4mm↑

Copper Weight: 1oz.

Half-cut / Castellated Holes: No

Min Hole Size: 0.3mm

PCB Color: Blue

Material: FR4 TG130

Layer: 2

Blind Vias: No

PCB Thickness: 1.6mm

Min Tracking / Spacing: 6/6mil

Plated Half-holes / Castellated Holes: No

Installation of the circuit board components is described here in order of installation. Solder the headers, resistor, and diodes in place on the PCB (Fig. 8). The direction of the resistors is not important but take care to orient the diodes as pictured on Figure 8 to ensure proper current flow. Clip the excess pins from the bottom of the board. Install the 3-pin and two 6-pin Molex connectors and solder the pins on the bottom of the board. Install the MOSFETs with enough clearance to be bent at an angle above, but not touching, the diodes. Solder the MOSFETs in place and clip the excess pins from the bottom of the board. Solder the 4-pin header onto the OLED, and then flip the PCB over and install and solder the OLED in place on the bottom of the board (Fig. 9). Bend the IR receiver pins at a right angle to the receiver and install and solder the receiver with about 2 mm of space between the back of the receiver and the board. Install the

reed switch in place and solder the pins. Clip any excess pins on the top of the board. Install the RTC coin cell battery holder into the top of the board (Fig. 10). To solder the battery holder pins the IR receiver on the bottom of the board needs to be bent gently upwards. Once the pins are soldered and clipped, gently bend the receiver back into a horizontal position. Lastly, install the Teensy on the headers and solder in place.

5.5. Preparation of the microSD card and software programming

Using a text editor program, create the two text files for recording sample logs (dataLog.TXT) and storing settings and sample parameters (sampleParam.TXT). The dataLog file is a blank file for logging sample times and requires no text. The sampleParam file requires a line of text with nine integers.

Example: 1,1000,10,-1000,11,30,18,11,19

Key: Sample Mode (0 = Daily, 1= Once), Sample Volume (mL), Calibration Volume (mL), Calibration Count, Sample Time (Hr), Sample Time (Min), Sample Day, Sample Month, Sample Year

Input an appropriate line of text for the sampleParam file, save both text files on the microSD card, and insert the card into the Teensy.

To upload the operating code to the SASe requires the Arduino software (IDE) and the Teensyduino add-on. Once installed, place the SASe code (SASeV3c.ino) in a folder labeled identically (i.e., SASeV3c) within the Arduino library folder. Run the Arduino program and use the Library Manager to search for and install the most recent versions of:

- Adafruit GFX Library by Adafruit

- Adafruit SSD1306 by Adafruit

Also, because more recent versions have not been made compatible at this time, install version 1.1.4 of:

- SdFat by Bill Greiman

To set the OLED display resolution navigate to the Adafruit_SSD1306 folder (…\Arduino\libraries\). Open the Adafruit_SSD1306.h file with a text editor and scroll down to the following line:

```
//      #define SSD1306_128_64
        #define SSD1306_128_32
//      #define SSD1306_96_16
```

The default setting is the 128 x 32 bit screen resolution but the SASe OLED resolution is 128 x 64 bit. To fix this, uncomment the 128 x 64 bit screen resolution by deleting the two forward slashes in front of SSD1306_128_64 and add two forward slashes in front of SSD1306_128_32:

```
        #define SSD1306_128_64
//      #define SSD1306_128_32
//      #define SSD1306_96_16
```

Save and close the text file. To maximize sleep time for the Teensy and the SASe, the low current draw Snooze library needs to be edited. Navigate to the Snooze src folder (…\Arduino\hardware\teensy\avr\libraries\Snooze\) and use a text editor to edit and save these

two files: SnoozeAlarm.h and SnoozeAlarm.cpp. In SnoozeAlarm.h find the line near the bottom of the document that reads:

void setRtcTimer(uint8_t hours, uint8_t minutes, uint8_t seconds);

Change that line to:

void setRtcTimer(uint32_t hours, uint32_t minutes, uint32_t seconds);

In SnoozeAlarm.cpp, find the line near the top of the document that reads:

void SnoozeAlarm::setRtcTimer(uint8_t hours, uint8_t minutes, uint8_t seconds) {

Change that line to:

void SnoozeAlarm::setRtcTimer(uint32_t hours, uint32_t minutes, uint32_t seconds) {

Save and close both text files. The SASe code is ready to be uploaded onto the Teensy microcontroller. Use the Arduino program to open the SASe code. Under the Tools menu select "Board:" and choose Teensy 3.5. Connect the Teensy microcontroller to the computer with a micro USB to USB cord. Under the Tools menu select "Port:" and choose the serial port connected to the Teensy. Select the Upload arrow in the top left corner to verify and compile the code onto the Teensy. Verify the upload worked by looking at the OLED on the circuit board. If it worked, the screen will display the Status Menu. Once the code is uploaded and the OLED is displaying properly, disconnect the Teensy and install the circuit board into the 3D printed faceplate using one #4-40 x 1/4-inch screw (Fig. 10). Install the 3D printed circuit housing on the faceplate, being sure to line up the Molex connectors with the openings in the housing (Fig. 11A). Attach the circuit housing to the faceplate using two #4-40 x 3/4-inch screws. Attach the completed circuit housing to the armature using two #6-32 x 1.25-inch screws (Fig. 11B).

5.6. Sampling cartridge construction

To install the magnets for the sampling cartridge and end caps it is important to keep track of the polarity of the magnets so that they are all installed in the same direction. Put a dot of two-part epoxy into each of the magnet spaces on the bottom of the sampling cartridge and then press the magnets into place until fully seated (Fig. 12). The magnets in the end caps of the SASe must also be installed with the polarity in the same direction as the sampling cartridge magnets. Place a dot of two-part epoxy in the magnet spaces on each of the end caps and press the magnets into position. Allow all epoxied magnets to cure before testing. Once cured, attach the cover of the sample cartridge using the #6-32 x 1.25-inch bolt and the #6-32 wingnut, then attach the sampling cartridge to the SASe housing via the magnets.

5.7. Final preparation

With all components now complete, connect the two pump motors and the power connection to the circuit board. The circuit housing is labeled to identify where each motor attaches. Connect the preservative motor to the 6-pin Molex connector labeled “Syringe”, connect the sample motor to the 6-pin Molex connector labeled “Pump A”, and connect the battery to the 3-pin Molex connector inside of the sample motor connection (Fig. 13). Route the wires so they are not touching the motor rotary encoders to avoid wear on the wires when the motors are running. If properly assembled, the SASe OLED will turn on and display the Status Menu. Slide the internal components of the SASe into the waterproof housing and secure the lid with the four lock nuts.

Figures:

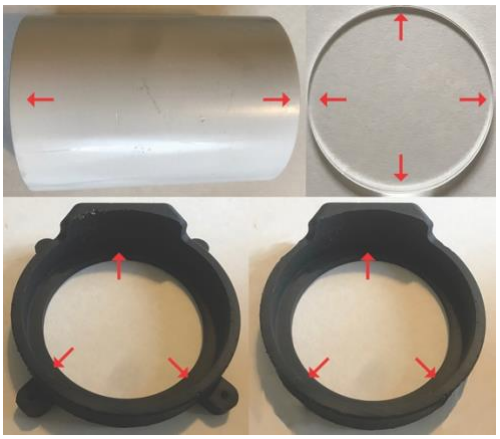

Figure 1: Red arrows indicate the surfaces to sand and clean in preparation for epoxying.

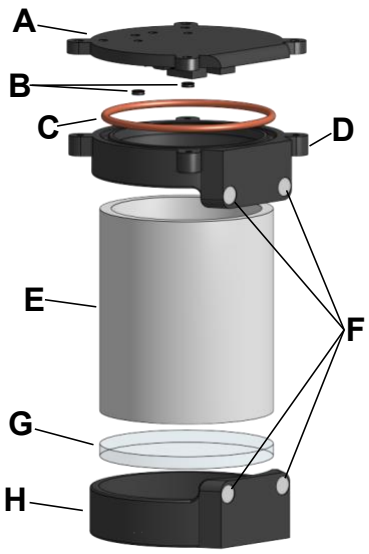

Figure 2: The SASe housing is made up of a 3D-printed lid (A), two X-rings for the motor shafts (B), O-ring for the lid seat (C), 3D-printed flanged end cap (D), 3” PVC body (E), four sample cartridge connection magnets (F), ¼” acrylic window (G), and a 3D-printed fixed end cap (H).

Figure 3: The motor sleeves and the face of the motor need to be flush. Using a jig can help hold that position while the epoxy is curing.

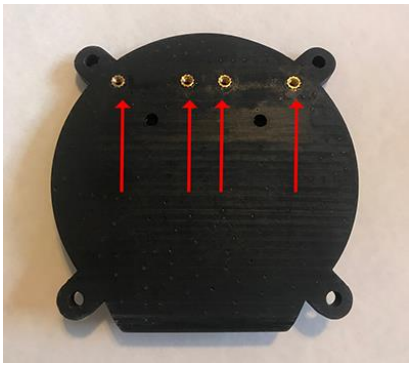

Figure 4: Threaded fittings are installed on the outside face of the 3D printed lid.

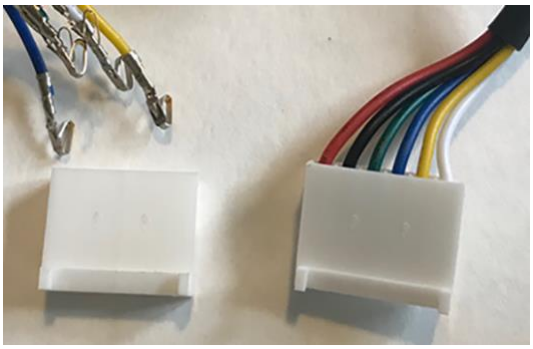

Figure 5: The crimped wires are inserted in the motor connectors in the order shown on the right. Wire ID: Red (Power), Black (Power), Green (Ground), Blue (VCC), Yellow (Encoder A output), White (Encoder B output).

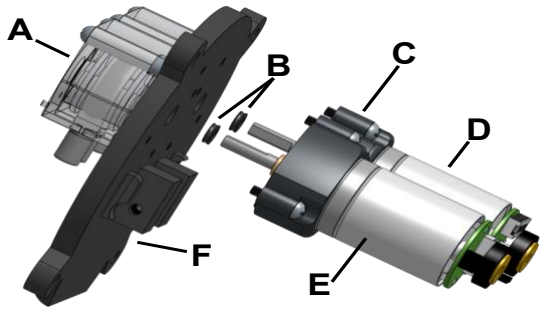

Figure 6: The two peristaltic pumps (A) are mounted to the outside of the 3D-printed lid (F). The 34:1 (D) and the 99:1 (E) DC motors are epoxied to 3D-printed motor sleeves (C) mounted on the inside of the lid. The motor shafts insert into the peristaltic pumps through the lid and an X-ring on the shaft of each of the motors (B) keeps the shaft-lid interface watertight.

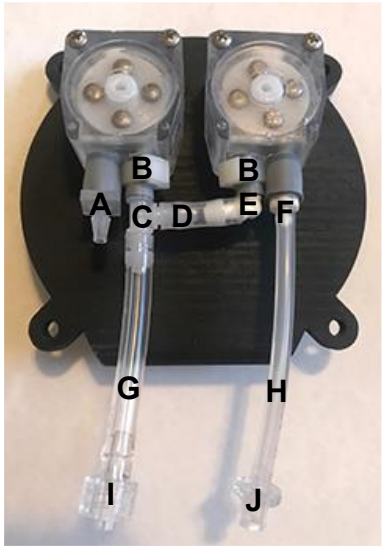

Figure 7: The pump heads are secured on the outside of the SASe lid, and the plumbing components for transporting sample water and preservative are installed as pictured and include the 1/8-inch barbed connector (A), 1/8-inch tube clamps (B), 1/8-inch barb tee connector (C), 0.75-inch long 1/8 ” tubing (D), 1/8-inch elbow connector (E), 1/8-inch to 1/16-inch reducer fitting (F), 2.25-inch long 1/8-inch tubing (G), 2.75-inch long 1/16-inch tubing (H), 1/8-inch male luer lock (I), 1/16-inch barb to female luer lock fitting (J).

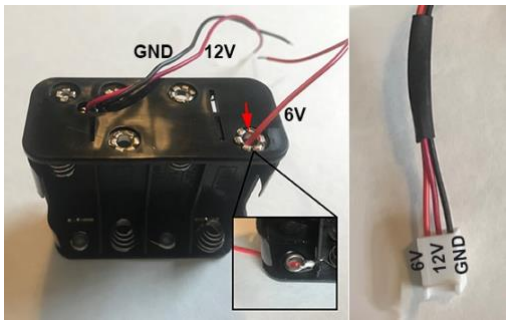

Figure 8: Solder a 6V wire to the OEM battery pack at the terminal indicated by the red arrow. The inset shows the wire looped around the inside of the terminal to add stability to the connection. Install the crimped wires into the Molex connector as pictured.

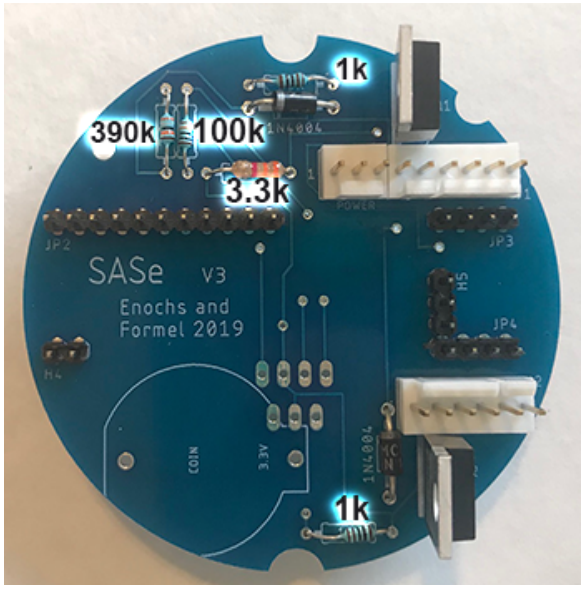

Figure 9: The headers, resistors, diodes, MOSFETs, and Molex connectors are installed as pictured.

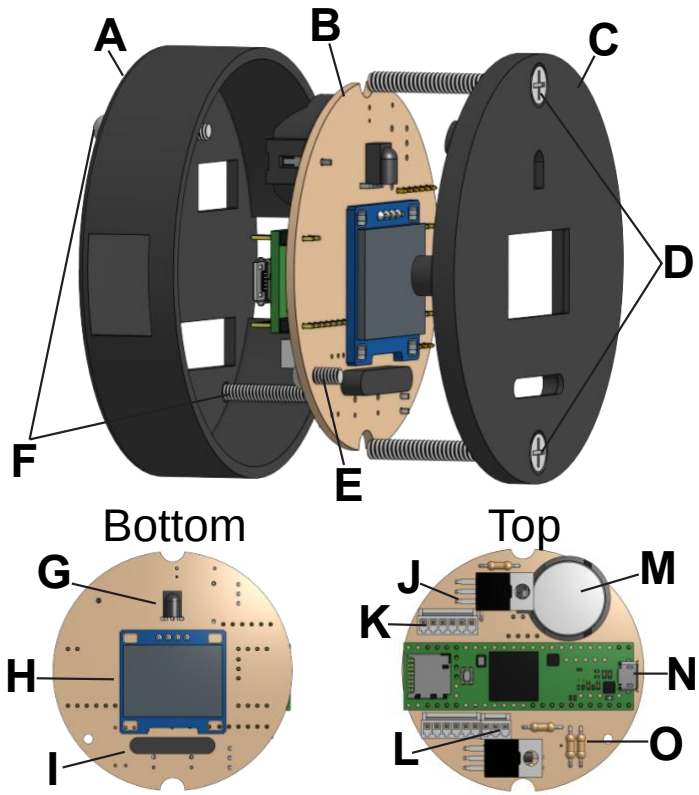

Figure 10: The 3D-printed circuit board housing (A) holds the SASe circuit board (B) and the UI components are visible through the 3D-printed faceplate (C). The whole housing is mounted on the main armature with two #6-32 bolts (D), while one #4-40 bolt (E) secures the circuit board to the faceplate, and two #4-40 bolts (F) hold the housing together. The circuit board is labeled to show the top and bottom and all PCB components are labeled as follows: IR receiver (G), OLED (H), reed switch (I), N-channel MOSFET (J), Molex 6-pin connector (K), Molex 3 pin connector (L), coin cell battery (M), Teensy 3.5 (N), resistors (O). Not visible are the two diodes below the MOSFETs that control current flow for the motor circuits.

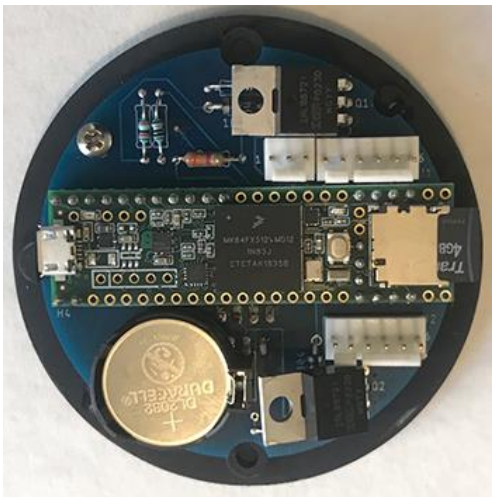

Figure 11: Install the coin cell holder and solder the pins on the bottom of the board then install the Teensy microcontroller and solder the pins. The finished circuit board is installed onto the 3D printed faceplate.

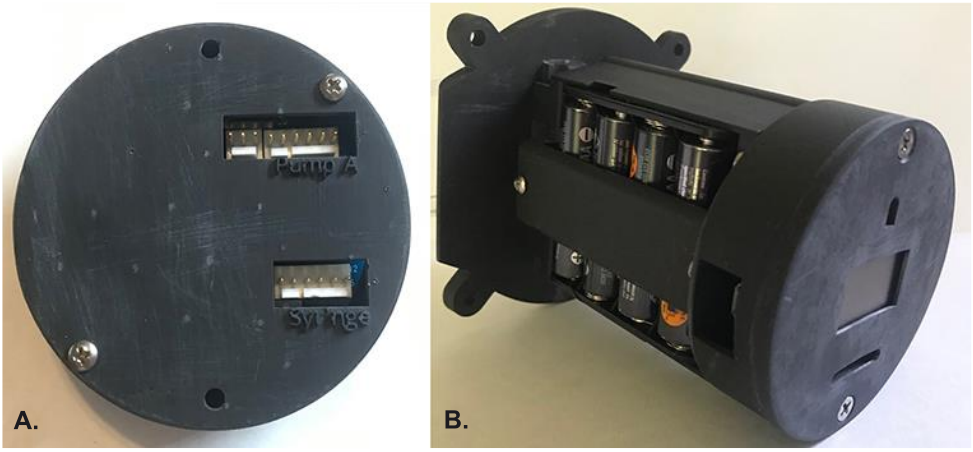

Figure 12: The 3D printed circuit housing (A) with openings for Molex connectors is attached to the armature (B) to connect the internal components of the SASe.

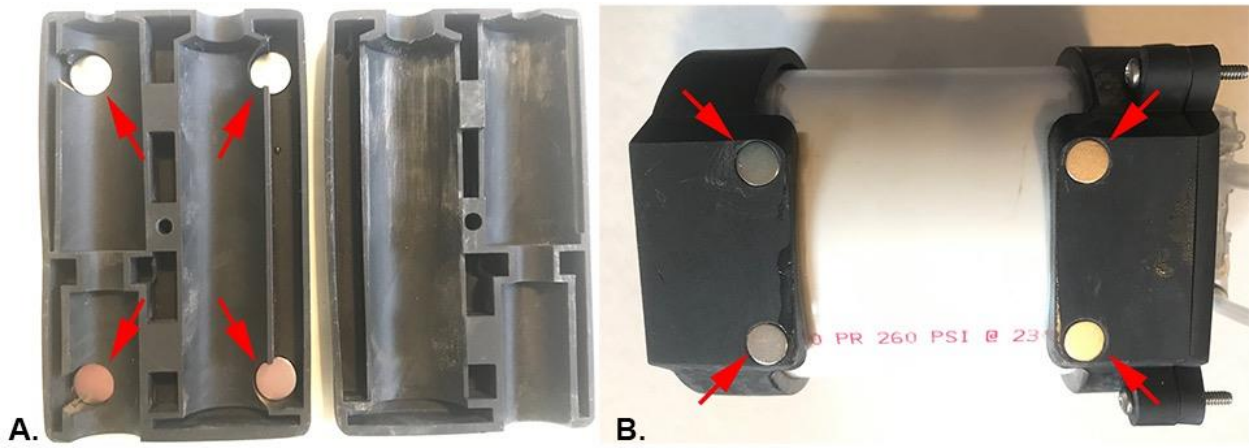

Figure 13: The sample cartridge (A) and the end caps (B) both require magnets installed as indicated by the red arrows.

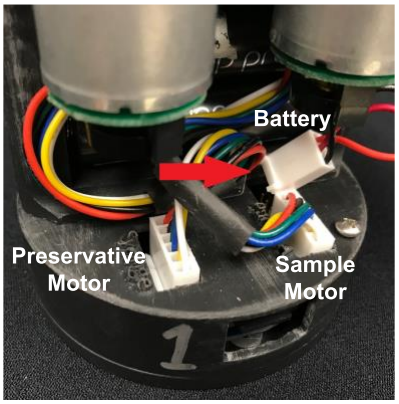

Figure 14: The connections for the SASe motors and battery are labeled, and the battery connection location is indicated by the red arrow.