Photoacoustic Imaging Phantoms for Assessing Image Quality and Oximetry Performance

A. Protocol for preparing polyvinyl chloride plastisol (PVCP) phantoms used to evaluate photoacoustic imaging systems

Introduction

This document describes fabrication materials and methods for constructing tissue-mimicking phantoms using custom PVC plastisol (PVCP) formulations. This protocol is specific to larger PVCP batches (~50-100 mL) as described in [4], as opposed to the 20-25 mL method used in an earlier work [3], which followed methods described in [1].

References

1. Bohndiek S *et al.* "Development and Application of Stable Phantoms for the Evaluation of Photoacoustic Imaging Instruments", PloS ONE 8(9), 2013
2. Vogt WC, *et al.* "Biologically relevant photoacoustic imaging phantoms with tunable optical and acoustic properties", J Biomed Opt 21(10), 2016
3. Vogt WC, *et al.* "Phantom-based image quality test methods for photoacoustic imaging systems", J Biomed Opt 22(9), 2017

Phantom Materials/Chemicals

Item	Amount	Supplier	Product Name/Number	Link
PVC resin	35-100 g	Mexichem	Vestolit G 121A	link
di(2-ethylhexyl) adipate (DEHA)	0-350 mL	Sigma Aldrich	525197	link
Benzyl butyl phthalate (BBP)	0-350 mL	Sigma Aldrich	308501	link
Calcium-Zinc heat stabilizer	4 mL	M-F Manufacturing Co.	HS16 (Baerlocher B-1743)	link link
Titanium dioxide (TiO_2), anatase	0.5-2 g	Sigma Aldrich	232033	link
Black plastic colorant (BPC)	`0-1 mL	M-F Manufacturing Co.	01 - Black (non-bleed)	link link
Ground silica, <40 um diameter	<1 g	US Silica	Min-U-Sil® 40	link
Soda lime glass beads	<1 g	Potter Industries LLC	Spheriglass A	link

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Equipment/Supplies

1. Custom phantom mold, ideally made of metal such as aluminum. Thick acrylic (> 5 mm) will also work, with some slight thermal damage/melting of acrylic faces in contact with molten PVCP.
2. Tubing for phantom channels, such as Zeus PTFE light-wall tubing from Component Supply Co. Tubing should be rated to withstand molten PVCP temperatures (ideally, at least 150 ˚C)
3. 600 mL beaker
4. Two graduated cylinders (e.g., 25 mL)
5. 250 mL round bottom flask (having several is recommended when preparing multiple PVCP batches).
6. Adjustable pipettor (e.g., 0.5-2.5 mL) or similar set of fixed pipettors
7. #5 rubber stopper with glass tube, connected by flexible PVC tubing to house vacuum line via a three-way ball valve (4757K57, McMaster-Carr)
8. Scoopula (Fisher Scientific), manual stirring rods/tools
9. Anti-static weighing boats, weighing paper
10. Calibrated mass balance (220 g capacity, 0.1 mg resolution)
11. magnetic stir plate
12. Vacuum/degas chamber
13. heated bath sonicator
14. magnetic stir+hot plate, max temp >= 200 C (distinct from magnetic stir plate)
15. thermocouple for oil bath (Omega)
16. Pyrex crystalizing dish, 150 mm diameter x 75 mm height ()
17. 100 mL cylindrical sample jar
18. 0.95 L (1 quart) high-temp silicone bath oil (Dow Corning 210H fluid, max temp = 230 C)
19. Heat-resistant gloves (e.g., terry cloth lab oven gloves)
20. Ringstand and clamp
21. 4” magnetic stir bar for oil bath (XXX, Sigma Aldrich)
22. 15 mm x 10 mm rare-earth magnetic stir bar for PVCP solution (Z671665, Sigma Aldrich)
23. PPE: chemical safety glasses or goggles, lab coat, closed-toes shoes. Adhere to other lab facility requirements

Protocol

1. Identify relative weight/volume of components to be used, depending on desired phantom optical and acoustic properties
    a. Plasticizer ratio (% v/v)
    b. Heat stabilizer content (% v/v plasticizer mixture)
    c. PVC resin (% m PVC / m plasticizers)
    d. TiO2 concentration (mg/mL)
    e. BPC concentration (% v/v plasticizer mixture)
    f. Glass bead concentration (mg/mL)
2. Place a 2” cylindrical stir bar in a 600 mL beaker. Add desired amounts of BBP, DEHA, and heat stabilizer. Place beaker on magnetic stir plate, and stir at 500 rpm for 5 min
3. While stirring plasticizer/heat stabilizer mixture, weigh desired amount of PVC resin. Slowly add PVC resin to the liquid plasticizer solution. Allow mixing for 15 minutes. Large clumps of resin should not be present. If clumps exist after 15 minutes, break apart with a metal spatula/tool and mix another 15 minutes.

4. Place beaker in vacuum chamber, degas solution for 1 hr.
5. While waiting for degas, assemble phantom mold and place near hot plate for later PVCP pouring.
6. While waiting for degas, prepare the flask vacuum connection and oil bath:
    a. Insert glass tube into #5 rubber stopper.
    b. Connect ¼" Tygon tubing to glass tube, connect to barb fitting on three-way valve.
    c. Connect three-way valve to house vacuum line in fume hood.
    d. Place crystallizing dish on hot plate and fill with 0.95 L silicone bath oil
    e. Add 4" magnetic stir bar, place thermocouple in the oil near the dish edge, and set oil bath temperature to 200 ˚C.
7. If adding TiO_2, weigh TiO_2 on a mass balance using weighing paper. pour 60 mL degassed PVCP into a 100 mL cylindrical jar. Close jar lid, suspend in a water bath sonicator set to 40 ˚C, and sonicate for 30 minutes, stopping every ~10 minutes to manually agitate and break up aggregates.
8. Reintroduce sonicated PVCP volume to stock PVCP solution, stir for 5 min.
9. If adding BPC, introduce to PVCP solution by pipettor. Typically, only small volumes are needed (e.g. .003% v/v, or ~10 µL in 400 mL of solution). Due to viscosity of BPC, proper pipetting techniques should be followed, including vertical drawing and pre-wetting.
10. If adding silica beads, weigh mass of beads on a mass balance using a weighing boat. Add powder to PVCP solution and stir for 15 minutes. Use a metal tool/scoop to check for large silica aggregates/clumps. Generally, silica will fall out of suspension quickly due to their higher density. Thus, the stock solution (and solutions undergoing heating/gelation) should be constantly stirred and used promptly.
11. Pour 75 mL PVCP solution into a 250 mL round bottom flask. Add 1 cm-diameter rare-earth magnetic stir bar. Clamp flask to ring stand, place rubber stopper (which should be connected to the vacuum line) in the flask, and lower the flask into the oil bath. Evacuate the flask by switching the T-valve.
12. Start a timer.
13. Around 5 min, the PVCP will begin to thicken, reduce stir rpm to 50-100 rpm.
    a. Note: the choice of stir plate rpm during PVCP heating depends on sample viscosity, which changes with composition and over the course of the gelation process. Plate rpm will also depend on stir bar size and magnetic strength, as well as on flask height above the plate. The goal is to always have the stir bar moving and in the bottom center of the flask, which promotes gas release, mixing, and better heat stability.
14. Around ~9-10 min, PVCP viscosity should decrease; increase stir plate rpm to ~200-300 rpm.
15. Around ~14-15 min, PVCP should be ready for pouring. First ensure the mold is ready for PVC pouring and don heat-resistant gloves. Switch T-valve to release vacuum suction in flask. If significant number of bubbles remain, quickly skim them off using a metal spatula/tool. Unscrew flask clamp and lift flask up the ring stand and out of the oil bath. While holding the flask in your hand, open the clamp jaws (to avoid the flask dropping into the bath). Manually pour the PVCP into the mold, taking care to avoid pouring PVCP directly on suspended objects such as tubes (PVCP is viscous enough to deform these objects). Also ensure the stir bar does not fall out of the flask into the mold. After pour, monitor for bubbles, voids, or other defects. Bubbles can be skimmed using metal tools such as spatulas or tweezers.
    a. Note: the amount of bubbles remaining varies significantly with PVCP composition, stir rpm, and heating time. When working with a given PVCP recipe, the total heating time

should be kept constant across batches to improve consistency, but there is a tradeoff between heat stability and air release that must fit the application.

b. Note: Phantoms may appear to solidify rapidly at the surface, but the internal volume can remain hot for 30-60 minutes depending on phantom volume and geometry. Allow phantoms to cool overnight before use.

16. After PVCP is poured, pour the residual PVCP (and stir bar) out of the flask into a waste tray. Retrieve the stir bar for cleaning and reuse.
17. Fill flask with cool water, wipe oil from outside of the flask using a paper towel, and wait 5 min. This solidifies the PVCP residue inside the flask into a thin coating that can be removed using a metal spatula. For most PVCP formulations, the film should stay together as it is pulled away.
18. Clean flask with detergent and dry by inserting wadded up paper towels. If residue remains, apply acetone and use a bent spatula, wadded up paper towel, or other instrument to remove residue.
19. If preparing multiple batches/pours, go back to step 2 and repeat.

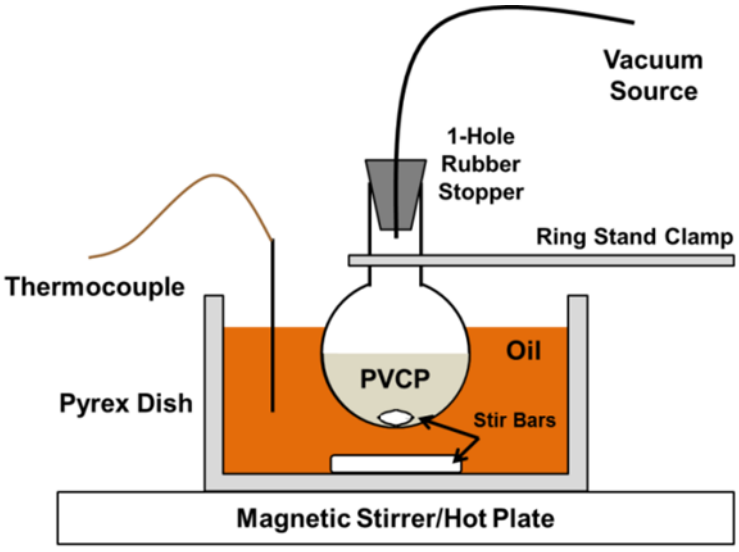

Figure 1. Schematic of PVCP heating setup.

B. Phantom Mold and Target Assembly

References

1. Vogt WC, *et al.* "Biologically relevant photoacoustic imaging phantoms with tunable optical and acoustic properties", J Biomed Opt 21(10), 2016
2. Vogt WC, *et al.* "Phantom-based image quality test methods for photoacoustic imaging systems", J Biomed Opt 22(9), 2017

Item	Supplier	Product Name/Number	Link
Acrylic sheets, 12" x 12" x 1/4"	McMaster-Carr	8560K354	link
Black monofilament sutures, size 7-0	Braintree Scientific	SUT-M 103	link
Stainless steel wires, 51 µm diameter	A-M Systems	794600	link

Light-wall PTFE tubing, 0.56 mm or 1.07 mm inner diameter	Component Supply Co.	STT-24 or STT-18	link
Acrylic bonding agent (cyanoacrylate)	TAP Plastics	TAP acrylic cement	link
Super Glue	Loctite	Loctite Superglue Ultra Gel Control	link
Drill press or micromill with XY stage (0.02 mm readability)			

Protocol

1. Assemble a suitable phantom mold
    a. The mold material should tolerate high temperatures during PVCP pouring (< 180 C). Plastics such as acrylic can be acceptable given sufficient wall thickness (e.g., 1/4" or 6 mm), although some slight scalding of the mold faces will occur during PVCP curing. Acrylic molds can be reused by removing solid PVCP, scraping the faces clean using a chisel, and washed with isopropyl alcohol or ethanol. To avoid leaks, acrylic molds should be bonded water-tight using acrylic cement (cyanoacrylate).
    b. The mold should include some faces with drilled through-holes for suspending and affixing embedded inclusions such as metal wires, surgical sutures, or plastic tubes. Hole diameters should be chosen to minimize clearance and provide rough fit.
    c. PVCP undergoes slight shrinkage during curing, and pouring directly into an open mold will result in sag of the top surface. Since the top surface is often the intended imaging surface, this is avoided by designing a mold to be filled from the side or bottom face. For large phantoms, multiple batches of PVCP will be needed, and the interface between poured layers can create image artifacts. To avoid this, design a mold for side-pouring, which results in layer interfaces that are normal to the vertical and do not produce image artifacts.
2. Affix targets in the mold
    a. For wire or suture phantoms, cut lengths of wire that span the mold and have excess length of 5 cm on either end. Using tweezers, thread the wire through the mold holes, being careful not to bend or kink the wire. For each wire, use tape to fix one end, then use your hand to pull the wire taut. When the wire is taut, use tape to fix the other end, maintaining tension. For best accuracy and precision in wire placement within the phantom, in addition to applying tension each wire should be pulled in the same direction relative to the through holes (e.g., always towards the bottom of the phantom). This maximizes accuracy and repeatability of inter-target spacing. After affixing all wires, fill the through-holes with superglue from both sides, leaving a small amount of superglue to cure on the mold face. Also apply superglue to the wire length along the outside mold wall to increase bond area and strength. Allow superglue to fully dry, then remove tape and use a razor to cut any excess wire (do not cut wire held under the glue).
    b. For tubes, it is important to consider tube rigidity in assembly. The light-wall PTFE tubes used in published studies with this tool are semi-rigid and are supplied in a coil, but

straight tubing is often required. First, cut sufficient lengths of PTFE tubing for the designed mold. To achieve straight PTFE tube lengths, using two pairs of needle nose pliers, grab the ends of each tube length, hold the tube such that its axis is parallel with gravity, gently pull taut, and hold for 15 seconds. The pliers should be twisted once (in different directions from each other) to improve grip strength and ensure good alignment. Do not overtighten, as this will warp the tube and increase haze of the wall. Inspect tube lengths to assess straightness; if needed, tubes can be further straightened by holding one tube vertically in your hand, rotating to identify curvature, and gently pinching and rolling up the tube against the curvature. As with wires, use tweezers to thread tubes through the mold, avoiding kinks/bending. Then, inject superglue into the through-holes from both sides of the mold wall, leaving small amounts of superglue as a root to provide strain relief. Allow superglue to fully dry before adding PVCP.

3. After all targets are affixed, prepare and pour PVCP into the mold as described in Appendix A above.

C. Flow Phantom Assembly and Tunable Blood Flow Circuit

Item	Supplier	Product Name/Number	Link
Plastic bonding agent	Loctite	681925	link
Super Glue Gel	Loctite	Loctite Superglue Ultra Gel Control	link
Modeling clay	Crayola	Air-dry clay, white	link
Insulating foam	Loctite	Loctite TITE FOAM	link
Quick connectors, female to 1/4" hose barb	Qosina	PMC1704	link
Whole bovine blood, defibrinated	Quad Five	910	link
Heparin sodium salt	Millipore Sigma	H3393-25KU	link
Phosphate buffered saline (PBS) tablets	Millipore Sigma	P4417	link
Electrode meter	Thermo Fisher	Orion A211 meter	link
pH electrode	Thermo Fisher	8165BNWP	link
Dissolved oxygen electrode	Thermo Fisher	083005MD	link
Temperature probe	Thermo Fisher	928007MD	link
Hydrochloric acid (HCl), 1M solution	Millipore Sigma	320331	link
Centrifuge, accepts 50 mL conical tubes	Thermo Fisher	Sorvall Legend XTR	link
Pipettors (1-10 mL, 0.1-1 mL vo)	-	-	-
5% bleach solution (285 mL DI water, 15 mL bleach)	-	-	-

References

1. Vogt WC, et al. “Photoacoustic oximetry imaging performance evaluation using dynamic blood flow phantoms with tunable oxygen saturation”, Biomed Opt Exp. 10(2), 2019

Flow Phantom Assembly Protocol

1. Blood flow phantoms include a custom flow splitting assembly to bring blood from the main supply tube to the array of target tubes embedded in the phantom.
    a. First, thread all tubes through the phantom mold. Then, follow the steps below for each end of the tubes (i.e., so the phantom is bundled/connectorized at both ends)
    b. Take a small chunk of clay (about 2 cm^3) and press the clay into a rectangular strip shape (a width about 15-20 mm).
    c. Arrange the seven tubes one by one to the middle of the clay strip, with the first one on the strip surface and the consecutive one beneath the strip. Gently press the tubes such that they slightly stick into the clay strip.
    d. Fold the clay strip with tubes and wrap the exposed tubes with the two sides of the clay strip. Be careful not to leave any air gap between tubes as the strip might be thin. A slight squeezing and tweaking might be needed to ensure this. The tube-clay bundle should have an equivalent cross-section diameter of the tube to be inserted (here the tube used has inner diameter of 4.8 mm).
    e. After the tubes are fully wrapped with clay, use scissors to cut the tip of the tubes + clay (depending on the length of the clay strip, usually cut about 5 mm length) such that the tube openings are clearly exposed and forming a flat cross-section surface.
    f. The tube-clay bundle is ready to be inserted into the connecting tube (e.g., 4.8 mm inner diameter Marprene tube). Apply epoxy activator on the side surface of the tube-clay bundle where it will be in contact with the inner surface of the connecting tube.
    g. Next, quickly apply the epoxy glue onto the side surface of the tube-clay bundle, insert the tube-clay bundle into the connecting tube with a gentle push. Some clay may be pressured and left outside the connecting tube, use the wood sticker like the one from a cotton tipped applicator, push the excess clay to let the clay tightly cover any gaps left in the connecting zone, and extend some clay onto the outer surface of the connecting tube to strengthen the connection. During this final fixing stage, the Loctite superglue may be used to help strengthen the connection and enforce the waterproof properties, and extra clay might be used if necessary.
    h. Wait for the epoxy glue and the superglue to dry for about 1 – 2 hours. When the bundling is fully glued and cured, one can touch the bundled clay and the outer surface should be solidified and feel like a crust.
    i. When the tube-clay bundle is double checked to be fully cured, apply the polyurethane sealant at the root of the seven tubes (close to the acrylic surface of the phantom) to give extra support to the tube and to prevent bending of the tubes inside the phantom.
2. Add female to 1/4” hose-barb quick connectors to the other end of each connecting tube to integrate the phantom into the blood flow circuit.

Blood Preparation Protocol (prepares 350 mL of red blood cell (RBC) suspension)

1. Prepare pH meter, electrode, and temperature probe following manufacturer instructions. Allow 30-60 minutes of warmup.
2. Prepare small aliquot (5 mL) of 1M HCl solution.
3. **PBS cleaning solution**: Prepare 1 L of 1X PBS solution for flushing the blood circuit before experiments and cleaning remaining blood from the circuit after experiments. Set aside.
4. **PBS solution for RBC suspension**: Dissolve 1 PBS tablet into 200ml DI water in a clean 400ml beaker. Use a stir bar and a stir plate to accelerate the process. After the tablet is dissolved, reduce stirring (~100 RPM) and carefully immerse the pH and temperature probes into the solution. When the pH reading is stable (usually around 7.4-7.55), slowly pipette 2-5 drops of the HCl solution to adjust the pH to 7.3. Pour the PBS solution into a squeeze nozzle bottle for later use. This PBS suspension solution will be used for making heparin solution, washing the centrifuged red blood cells (RBC) and resuspending the RBC. For a total volume of 350ml blood source, it usually requires about 600-800ml PBS suspension solution.
5. **Heparin solution**: Dissolve 25 kU of heparin powder into 125ml PBS solution to make a 200U/ml heparin solution. Store the solution in a glass bottle in the fridge for later use. The heparin solution will be added into the blood (defibrinated bovine blood) before the centrifugation to prevent blood from clotting.
6. Prepare 350 mL of blood with 10 U/mL heparin by adding 17.5 heparin solution to 332.5 mL defibrinated bovine blood. Shake and swirl the bottle to ensure adequate mixing.
7. Prepare 14 conical tubes (50ml each), pipette 25ml blood solution into each tube, and secure the caps tightly.
8. Place conical tubes into the centrifuge, ensuring mass is evenly distributed over the carousels. Blank tubes filled with 25 mL water may be needed for load balancing. Set the RPM to 3000 and time for 10 minutes. Do the first spin.
9. Remove conical tubes from the centrifuge. Samples should show two layers, a comparatively clear, brown upper layer (plasma) and a red condensed bottom layer (RBC layer). Aspirate the top layer with a plastic pipette until reaching the RBC layer (about 10-15 ml remaining in the tube, depending on the initial blood hematocrit).
10. Add PBS suspension solution until the sample volume reaches 25 mL, tighten the tube cap and swirl and invert the tube to mix the RBCs with the fresh PBS solution. Repeat for each of the tube and perform second centrifuge cycle using the same settings above (3000 RPM, 10 min, first wash)
11. Remove sample upper layer again as described previously, add PBS to return volume to 25 mL, and perform a third centrifuge cycle.
12. Remove the upper layer and suspend the RBC with PBS suspension solution to final sample volume. At this step, the final volume should be selected to achieve the intended total hemoglobin concentration (tHb) value. Typically, this should be 13-15 g/dL, meaning the final sample volume may vary from 20-25 mL. tHb should be verified by CO-oximetry.

Blood circuit preparation and SO2 tuning

1. **Circuit components and setup**
    a. Reservoir: plastic bottle with two ¼'' Tygon tubes inserted through the bottom. The blood outlet tube should be taller than the inlet tube.
    b. Membrane oxygenator: a dense bundle of hollow fibers for efficient gas transfer. blood travels through the "shell" and the swept gases flow through the lumen in opposite direction. Blood should flow vertically to minimize frothing, (usually blood ↑, gas↓). Make sure the gas outlet is open except for sealing purpose.
    c. Pressure meter: the pressure meter is connected to make sure the system pressure is within a normal range. Infuse about 1-2 ml of PBS suspension solution into the branch tube connecting the meter to the circuit to prevent the blood from contacting the meter.
    d. Meter housing: The lid of the meter housing has been permanently sealed with the body and the screws have been tightened. There is no need to take off the lid or the screws (for the old meter housing. It may be different for new meter housing in the future). Use the pinky finger to apply a light thin layer of the Molykote grease (Dow Corning 111) on the wall of the bores for pH and DO probes (just a tiny little bit to prevent the probe from getting stuck in the bore after the experiment), insert the probes to the appropriate levels and connect the meter housing into the circuit.
    e. Peristaltic pump: make sure the flow is in clockwise direction.
    f. Sample port: to take blood sample for Avoximeter measurement for a reference SO2.
    g. Flow phantom: the phantom is built with bundled tubes that connected to a Marprene tube and a click connector. The tubes are bundled with modeling clay and epoxy glue and need to be handled gently with care. The connector may be damaged from bending and twisting so it would be safer to fix the part of the circuit that directly connect to the phantom.
2. **Before experiment**
    a. Beforehand, in a separate experiment prepare a lookup table for dissolved oxygen (DO) reference value that correspond to intended target SO2 levels for experiments. Note tHb, pH, and temperature and ensure they are similar between this calibration data and the upcoming experiment. Typical values: tHb = 12-15g/dL, pH = 7.35-7.55, temperature = 20-22 °C).
    b. Turn the pH meter on at least 40-60 minutes before experiments to ensure the DO electrode is polarized.
    c. Calibrate the pH electrode if necessary (perform calibrations weekly)
    d. Calibrate the DO electrode (perform calibration immediately before experiments)
    e. Grease the probe bores of the custom inline electrode housing and carefully insert the probes through the insertable mount. Do not insert probes if the mount is already placed in the housing as excessive force may break probes.
    f. Insert a "dummy" connectorized ¼" ID tube for initial circuit cleaning. Connect all lines and ensure no open ports for blood lines. Flush the circuit with PBS wash solution (~300 ml) for 20-30min. Drain PBS when complete.

g. Remove RBC suspension from refrigerator and allow to reach room temperature (1-2 hours). Load RBC suspension into the filling reservoir.

3. **During experiment**
   a. Generally, the roller pump should be operated at 70 RPM (~110mL/min). Gas flow rates should be set to 1.6 LPM for nitrogen and 1.55LPM N2 + 0.05LPM O2 are used to oxygenate the blood.
   b. Seal/unseal the system to stabilize or tune blood SO2 using valves 4, 5, and 6 in the figure below. Normally the system will be in 'Gas On' state with sweep gases flowing the membrane oxygenator. The liquid flow loop can be sealed to maintain the current SO2 for at least 20 min for PA imaging. The steps below should be followed in order to avoid over-pressurizing the circuit and potentially damaging the membrane oxygenator.
      i. Steps for Gas On state: 1. Open valve 4, 2. Close valve 5 from the environment, 3. Close valve 6 from the environment
      ii. Steps for closing the system: 1. Open valve 6 to the environment, 2. Open valve 5 to the environment, 3. Close valve 4.
   c. To tune blood SO2, set the gas flow rate as described above to either oxygenate or deoxygenate the blood. When the DO reading reaches a target reference value, switch from 'Gas On' state to 'Closed System' state. Verify intended blood SO2 is achieved by drawing a sample for CO-oximetry.
   d. Perform PA imaging scans. When the imaging is complete, switch to 'Gas On' state and drive to next intended SO2 value. Repeat for all SO2 states of interest.

4. After experiment
   a. Drain blood from the circuit, either from the reservoir or from the sample port with a drain tube connected. Flush the circuit with PBS wash solution three times for 15 minutes each. For the first two flushes, leave all components connected including the phantom and inline electrode housing. Then disconnect the phantom, meter housing, pressure meter and the sample port, replace the ports with closed valves, and reconnect the rest of the parts to do the 3rd flush.
   b. During the third flush, clean the sample port and the connecting tube of the pressure meter with a squeeze bottle filled with DI water.
   c. When the oxygenator appears pink in color, drain the loop and perform a fourth flush wash with 5% bleach solution (for Eg. 285ml DI water + 15 ml bleach) for 1 hr. Then, perform a final, fifth flush with DI water for 1 hr.

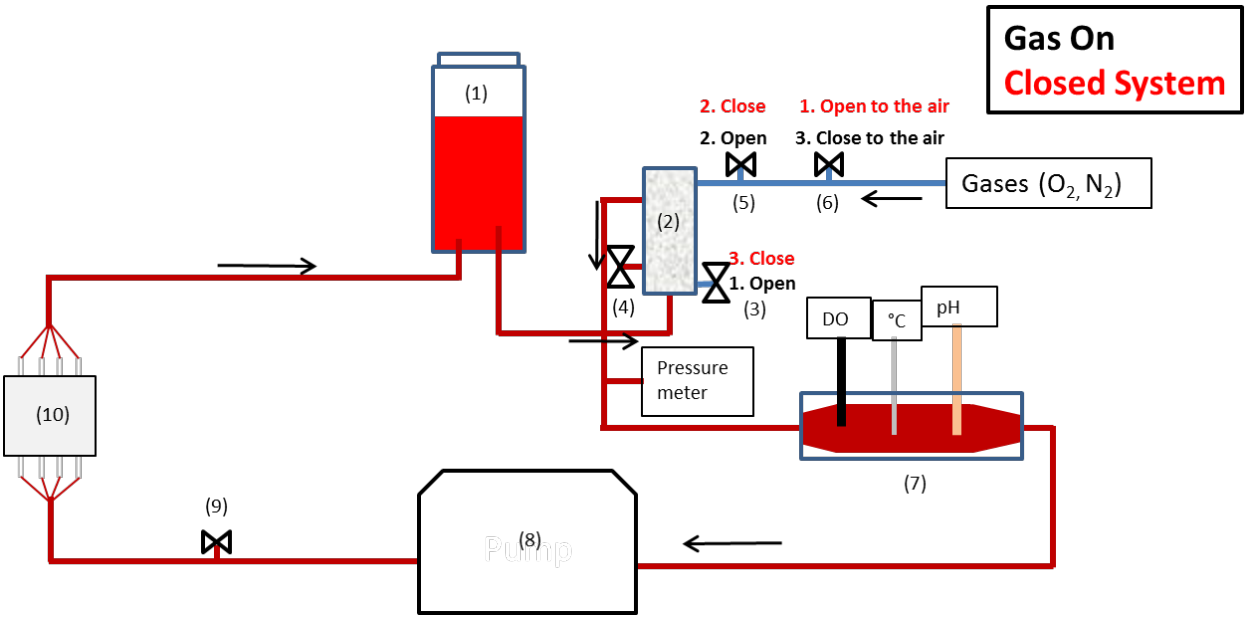

Figure 2. Blood flow circuit diagram, with instructions for switching between oxygenation tuning (Gas On) and stable (Closed System) modes.