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February 25, 2020

Vineeta Bajaj, PhD
Review Editor, Jove
1 Alewife Center, Suite 200
Cambridge, MA 02140
USA

Re: JoVE61088

Dear Dr. Bajaj,

Encouraged by the reviewers' comments received on January 29th, 2020, we are submitting a revision of our manuscript entitled "**Biventricular assessment of cardiac function and pressure-volume loops by closed-chest catheterization in mice**" We are thankful for the constructive criticisms that we received and we believe that this revision strengthens the manuscript. We provided a detailed response to each of the referees' comments.

According to reviewer comments, we assessed the left and right ventricular function of 6 mice. We included those data as a new table 2 in the revised form of the manuscript.

The work is original and has not been published elsewhere. All authors have read and approved the manuscript in its current format. We have no commercial conflicts of interest to disclose.

We hope you will find this worthy of publication in *Jove*.

Sincerely,

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Editorial comments:
Changes to be made by the Author(s):
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.
Thank you for your comment, we carefully proofread our manuscript.

2. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points
Done

3. Please expand all abbreviations during the first time use.
Done

4. Please rephrase the Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to …". Manuscript text is an important component of the submission and video is an associated file which is made after the manuscript acceptance. Please do not use the words "in this video" in the text.
We replaced all the words "in this video" to "in this protocol"

5. Please ensure that the long Abstract is within 150-300-word limit and clearly states the goal of the protocol.
Our abstract is 292 words long. We clearly stated the goal of the protocol e.g. "we will describe the procedure to perform invasive, closed-chest, sequential left and right ventricular pressure-volume loops in the same mice"

6. JoVE policy states that the video narrative is objective and not biased towards a particular product featured in the video. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item. Please do not use commercial language e.g., Transonic Scisense a mouse Pressure-Volume 42 Catheter, the ADVantage Pressure-Volume System (London, ON) and labScribe v4 software 43 (iworx - 2018; Dover, NH). Please refer to the term using generic language.
We removed the commercial language from our manuscript.

7. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
We deleted the section material and added a table of materials and reagents in the revised manuscript (below and table 1 in the revised manuscript)

Table 1: Table of materials and reagents

Product	Reference	Company
Isofluorane	CA2L9108	Baxter
0.9%(wt/vol) sodium chloride	B1309M	Baxter
Surgical tape	SN770	3M
Endozime AW triple plus	34521	Ruhof
High-fidelity admittance catheter (Scisense)	FTH-1212B-3518	Transonic
ADVantage Pressure-Volume System (ADV500)	FY097B	Transonic
Tabletop Single Animal Anesthesia Systems	72-6468	Harvard apparatus
Ventilator, far infrared warming pad for mice and rats PhysioSuite	PS-02	Kent scientific corporation
Fiber optic dual Gooseneck	6000#1	Volpi Intralux
Stereo microscope	OF-48920-10	Cole-Parmer
Forceps	11052-10	F.S.T
Forceps	1125120	F.S.T
Hemostatic clamp	13003-10	F.S.T
Hemostatic clamp	13018-14	F.S.T
Surgical suture, black braided silk, 4.0	ID158000	SERAFLEX
Needle (30 gauge)	305106	BD
Gauze sponges	441400	Dermacea
Tracheotomy canula 1.45 mm diameter	72-1410	Harvard apparatus
labScribe v4 software; PV-loops module	LS-30PVL	iworx

8. Please move all the materials from the materials sections to the table of materials instead.

Please see comment above

9. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, alphabets, or dashes.
Done

10. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.
According to your recommendation we added the statement below:
“All experiments were performed in accordance with Queen’s University biosafety and ethical guidelines (ROMEO/TRAQ#6016826). The procedures followed were performed in accordance with institutional guidelines.”

11. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly.
Done

12. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step.
We simplified individual steps to 2-3 actions.

13. The Protocol should contain only action items that direct the reader to do something.
Done

14. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed?
We believe that our protocol properly answer to the “how” question.

15. Please include all the button clicks in the software, knob turns in the instruments etc.
We included all the button clicks in the software

16. 2b: How is this done?
We extended the point 2b (now 1.2) in the revised form of the manuscript.

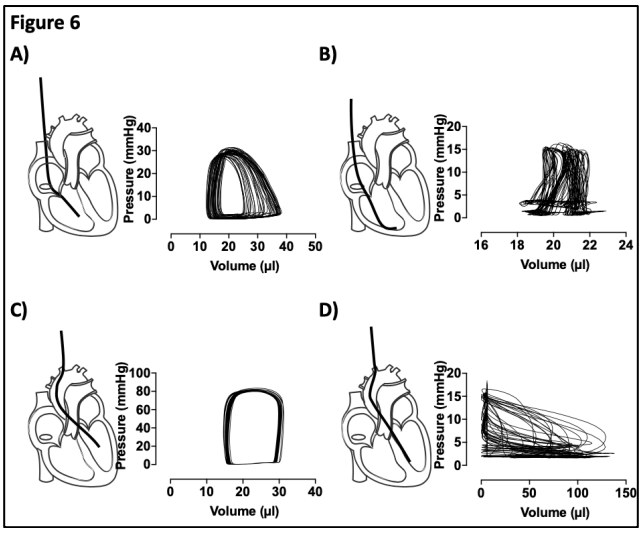
17. 3: How do you maintain sterility during the experiment?
This is a terminal procedure, animals are scarified after the procedure. We do not maintain sterility during the experiment.

18. 7: Please explain how this is done. Please include all the button clicks, knob turns etc.?
We clarified this point. Please note that our protocol focus on the surgical procedure rather than data analysis. Data analysis will depend on the software used and will be different from one distributor to another one (e.g labChart VS iworkx). Since we cannot provide the commercial name of the software in the protocol we believe that is unnecessary to provide more description of the data acquisition. We believe that this section species what constitutes a good practice for cardiac catheterization (e.g. record 20 loops), rather than an exhaustive description of the acquisition procedure.

19. Please move the troubleshooting section to the discussion.
Done

20. There is a 10-page limit for the Protocol, but there is a 2.75-page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.
The 2.75 pages was highlighted in yellow according to the author’s instruction.

21. For the catheter placement and pressure volume loops (Figure 6), are these just schematics or real pressure-volume loops. Please provide actual data obtained. Please label both the axis. The representative result should have data from an experiment you performed an experiment, how did it help you to conclude what you wanted to and how is it in line with the title. Data from both successful and sub-optimal experiments can be included.
The results from that manuscript are original and have never been published elsewhere. We described in the legend section how we concluded to a “good” and “bad” pV-loops e.g. “Figure 6 B is a schematic representation of the improper positioning of the catheter in the right ventricle (contact with the ventricular wall) and the resulting flawed PV-loops (collapsed and non-regular loops)”. According to your recommendation we labeled both axis in the revised figures 6 and below.



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The results from that manuscript are original and have never been published elsewhere, we did not reuse any figures from previous publication.

23. Please upload each figure individually to your editorial manager account.
Done

24. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage, (YEAR).] For more than 6 authors, list only the first author then et al.
Done

Reviewers' comments:
Reviewer #1:
The authors present a method for simultaneous left and right ventricular pressure/volume catheterization in mice to conduct a hemodynamic characterization of whole heart function including interventricular correlations. The methodology is sophisticated, well-described, and represents an interesting approach that may enhance cardiovascular research. There are several questions that arise from the methodology that need to be addressed. Most importantly, the anesthesia appears to be insufficient: The authors do not administer any analgesic therapy before skin incision and surgery. Mice may experience pain. This issue should be evaluated and discussed with an expert for veterinary medicine/laboratory animals. Please find specific comments as depicted below.

1)-Isoflurane is anesthetic, but has not analgesic effect. Even though the experiment is terminal, mice may experience pain during the surgery, especially considering that a stable isoflurane anesthesia is sometimes hard to achieve. The authors should revise the methodology accordingly and add analgesia, e.g. buprenorphine and/or ketamine before skin incision.

Thank you for your comments. We performed our experiments according to the Canadian and our local ethical guideline. Either ketamine or Isoflurane are recommended for terminal procedure. We decided to use isoflurane rather than Ketamine due to the adverse effect of ketamine on central nervous system. Indeed ketamine injection is associated with increased pulmonary blood pressures, heart rate, cardiac output, cardiac work and, myocardial oxygen requirement. We clarified this point and added the sentence below in the discussion section:

"The procedure described in this protocol is a terminal procedure. Due to the invasiveness of the right and left catheterization, the animals should be euthanized immediately after data acquisition. The euthanasia should be performed according to your institution's animal studies guidelines."

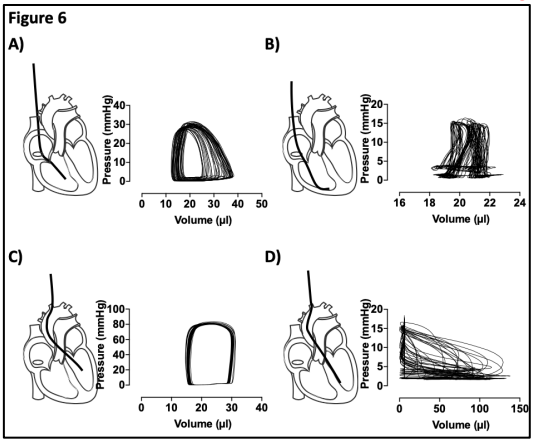
2)-The authors use a nose cone for the primary anesthesia with isoflurane, but then perform endotracheal intubation. The authors should comment on why endotracheal intubation is necessary since this likely influences RV pressure.

Dear reviewer, we decided to perform endotracheal intubation to increase the quality and the reproducibility of the data acquisition. Besides, movement associated with heart catheterization could disconnect the nose cone and result in improper/impaired ventilation. We believe that we addressed the limitation and advantage of endotracheal intubation in the discussion section (and below):

"Invasive tracheostomy and positive pressure mechanical ventilation will result in stable and controlled breathing of the mice and decrease the variability of the pressure-volume loops acquisition. However, positive end expiratory pressure (PEEP) is a marked contrast to normal ventilation (which is a negative pressure phenomenon). Together positive pressure ventilation and PEEP lower cardiac output and reduce right heart pressures. Thus, while required for acquisition of stable data, it must be acknowledged that mechanical ventilation as well as cardio-depressive effect of the anesthesia will affect the pressure volume loops and should be considered as a limitation. Transiently stopping mechanical ventilation during the brief recording of pressure-volume loops is a technique we use to eliminate this potential source of artifact."

3)-The authors should add absolute numbers and units to Fig. 6.

According to your recommendation we added the absolute numbers and units to figure 6 (and below)



4)-The authors state "Ensure that the heart rate reported on the ADV500 monitor screen is in a physiologic range (i.e. >350; <500bpm).", but a physiological heart rate is described as 500-600 BPM in common literature.

Heart rate is variable upon the mouse strain, the mouse weight as well as the type of anesthesia used. For example, the heart rate range for 20-34g mice anesthetized with xylazine is 340-510 min-1 whilst the heart rate range for 20-34g mice anesthetized with isoflurane is 470-620 min-1[1]. In our lab (specific mice strain, different weight, and isoflurane anesthetic) we observed a 350 -500bpm heart rate ranges. We agree that the "physiological" rate range

traditionally reported in the literature is 500-600 BPM. According to your recommendation we changed “>350; <500bpm” to >400; <600bpm.

5)-The authors should comment the units of volume data: Is it measured as "Siemens" for conductance or can this be translated to actual volume, e.g. microliter? With Millar instruments pressure/volume catheters, this can be done by saline calibration and cuvette calibration. Is there a similar approach?
Dear reviewer, unlike Millar instruments, the latest generation of transonic conductance catheter do not require saline calibration and cuvette calibration. The low and high signal calibration is performed before catheterization as described in the manuscript. A second calibration is performed directly in the ventricle of the animal when the pressure and magnitude are optimal.

6)-The authors should add a section on the most important values that are measured, the most important calculations, and the relevance of these values for cardiac physiology/pathophysiology.
According to your comment, we added a new table with hemodynamic data obtained from RV and LV (n= 6 mice) and the description of these values in the “representative results” section. (Table 2 and below)

Table 2: Haemodynamic parameters

Haemodynamic Parameter	
HR (BPM)	410.6 ± 23.3
CO (µl/min)	9107 ± 1016
SV (µl)	24.5 ± 2.3
RV function	
RVSP (mmHg)	21.9 ± 2.15
RVEDP (mmHg)	1.042 ± 0.12
EF (%)	56.1 ± 4.4
dP/dt max (mmHg/sec)	1469 ± 170
dP/dt max (- mmHg/sec)	1504 ± 215
EDV (µl)	38.4 ± 3.7
SW (mJoules)	0.068 ± 0.008
PVA (mJoules)	0.084 ± 0.009
Ea (mmHg/µl)	0.83 ± 0.09
Tau factor (msec)	12.8 ± 0.8
LV function	
LVSP (mmHg)	77.1 ± 2.4
LVEDP (mmHg)	2.33 ± 0.17
EF (%)	59.1 ± 3.6
dP/dt max (mmHg/sec)	4695 ± 355
dP/dt max (- mmHg/sec)	3553 ± 373
EDV (µl)	36.9 ± 4.8
SW (mJoules)	0.14 ± 0.013
PVA (mJoules)	0.22 ± 0.03
Ea (mmHg/µl)	5.37 ± 0.9
Tau factor (msec)	15.07 ± 1.7

CO, cardiac output; Ea, arterial elastance; EDV, end diastolic volume; HR, heart rate; LVEDP, left ventricular end diastolic volume; LVSP, left ventricular systolic pressure; PVA, pressure volume area; RVEDP, right ventricular end diastolic pressure; RVSP, right ventricular systolic pressure; SV, stroke volume; SW, stroke work; Tau factor, Tau Mirsky. N= 6 mice. Values are expressed ± SEM.

7)-The authors should consider to condense figure 3-5 since this is likely redundant to the video. Instead, the authors should provide a figure that shows exemplary hemodynamic data that is obtained from RV and LV. Especially for RV catheterization, this is important. Presenting data with dot plots may help to judge the typical variance between mice.
Thank you for your comment, we added a novel table showing the representative value obtained for RV and LV (Please see comment above). However, we believe that it is important to show endotracheal intubation, RV and LV catheterization in independent figures, we thus decided to leave figure 3-5.

8)-How did the authors validate the different catheter placements displayed in figure 6?.
Dear reviewer the “correct” positioning of the catheter is validated by the generation of the stable pressure-volume loop. It is almost impossible to generate PV-loops without the right positioning of the catheter in the chamber with all the probes in the ventricle. The schemas represent the “correct” and “wrong” positioning of the catheter, according to the transonic troubleshooting guideline. We acknowledge that we did not validate the different catheter placement. We believe that the schematic representation of the catheter’s positioning would be helpful for the reader.

9)-The authors should state what happens with the mouse after the procedure. Was it killed, and how?
After the procedure animals are euthanized by exsanguination (via puncture of the abdominal aorta) and bilateral pneumothorax. According to your recommendation we added the sentence below in the discussion section:
"The procedure described in this protocol is a terminal procedure. Due to the invasiveness of the right and left catheterization, the animals should be euthanized immediately after data acquisition. The euthanasia should be performed according to your institution's animal studies guidelines."

10)-The authors should add a statement on the use of laboratory animals (approval by local facility).
Done. We added the sentence below in the revised manuscript:
"All experiments were performed in accordance with Queen's University biosafety and ethical guidelines (ROMEO/TRAQ#6016826). The procedures followed were performed in accordance with institutional guidelines."

11)-The use of anesthesia that is likely to have a cardiodepressive effect and the mechanical ventilation will influence the obtained results, but are (at least the anesthesia) unavoidable. The authors should list this as an important limitation.
Thank you for your comment, we addressed this limitation with the sentence below
"Thus, while required for acquisition of stable data, it must be acknowledged that mechanical ventilation as well as cardio-depressive effect of the anesthesia will affect the pressure volume loops and should be considered as a limitation"

12)-Please ad a comma to "not as expensive as echocardiography CT or MRI" (page 2, line 109)
Done

Reviewer #2:
Manuscript Summary:
This manuscript concerns the assessment of cardiac function using pressure volume loops. The novelty of this protocol is the assessment of both left and right ventricles in a closed-chest anaesthetized mouse. The topic is introduced well and the rationale for the method is well described. I agree wholeheartedly that the cosed-chest approach is best for this assessment. The authors propose the use of the harvard Tabletop single animal anaesthesia system. This is a particularly high-end system and I commend the authors for using this to control core temperature.

Major Concerns:
I have no major concerns regarding the protocol. The edit and quality of video recording will be key in getting this protocol clear for the viewer. This is particularly close work and as long as the quality of the filming shows in detail the catheterisation procedure and simultaneous PV loops or pressure/volume waves the viewer would be able to understand the procedure better. I trust that the final publication will also include examples of good physiological data as well as data when things are not right.

1) The authors refer to a normal heart rate for mouse hearts under isofurane (2%) to be 350-500 bpm. I would hope that hr could be higher than 500 bpm in a lightly anaesthetised mouse, can the authors confirm this. We routinely get heart rates of 550-580 bpm under isoflurane (1.5% - 2% in oxygen).
Thank you for your concern, please see response to reviewer 1, concern 4.

2) step 6a viii the authors just state to withdraw the catheter and wipe, i would suggest that the authors need to include that at this point you will need to tighten one of the sutures to stop blood flowing out of the jugular vein. Similarly at step 6b iv the user should be careful to tighten the sutures to reduce the chance of blood loss from the artery.
Thank you, we added the following point in the revised manuscript:
"5.1.8.4. Tie the caudal suture to stop blood bleeding from the jugular vein." And "5.2.9.3. Tie the caudal suture."

3) Care should be taken where to tighten the sutures once the catheter has been places. for example if the user was to tighten the suture over the pressure sensor this would damage the catheter.
Dear reviewer, we agree and added the following note to the point 5.1.3.
“Note that extreme care should be taken to not overly tighten the suture since excess force can damage the catheter.”

4) correct filters should be applied on aquisition rather than post hoc as described in section 7. i would recommend a notch filter of 50Hz to eliminate mains 'noise' but also that suitable low pass filters are considered to remove any RF interferrance.

Dear reviewer, in our lab we apply a post hoc filter because we want to collect raw, unmodified, data. However, according to your recommendation, we added the sentence below in the discussion section of the revised manuscript.
“A notch filter of 50Hz could also be applied on acquisition to eliminate mains 'noise' and remove any radio-frequency interference.”

Minor Concerns:
The authors have limited their methodology to certain branded instruments and specific surgical tools. I feel that this is not necessary and the authors should be careful to state that there are alternatives that would not affect the outcome of the experiment. Namely, the data aquisition system is listed as being LabScribe v4. Whist this is certainly very capable software there are multiple alternatives which offer a similar function, it would be worth the authors mentioning that their recommendation is not the only option for this.
Thank you for your concern. Please see the response to the editor’s comments 6, 7 and 8.

In order to confirm adequate ventilation it is worth noting that a capnograph might be useful on the expiration line, this might be worth mentioning in the manuscript and video.
Dear reviewer, we currently don’t use capnograph in our lab. This is a good suggestion and we added the following sentence in the revised manuscript :
“Note that ventilation efficiency can be confirmed by the capnography monitoring of carbon dioxide”

I note that in Figure 1A the catheter appears to be bent at the end, i would strongly advise that bending a catheter will make data acquisition unstable.
Thank you for your concern, whilst we agree that bending catheter could impair data acquisition, we found that it is easier to get proper positioning and subsequent data acquisition with a bent catheter. However, this catheter shaping may reduce longevity of the catheter.

Figure 2A shows a rectal probe inserted with some kind of sheathing, I am unsure what this sheath is, can the authors explain why it is needed?
We use sheathing to protect our probe. It is the procedure that we use in our lab. We agree that is a lab preference, and that the sheathing is not required for probe function.

Reviewer #3:
Manuscript Summary:
The authors have outlined a technique to execute in situ pressure volume recordings of the right and left ventricles in the same mouse. The significance of this approach is well-detailed in the introduction and highlights the interdependence of the left and right ventricles, a critical yet understudied area. Ventricular interdependence becomes especially important in the context of cardiac disease and, perhaps even more during right-sided conditions such as pulmonary hypertension. While this technique requires technical expertise, the authors provide a clear description allowing the reader to re-create the technique especially those with an established expertise. In addition, the authors provide a succinct discussion of the limitations. In general, this technique will be of interest to the scientific community, especially with the aid of visual assistance. The manuscript is clear and well-written, yet some concerns were identified and outlined below.

Major Concerns:
1. The authors provide a brief discussion of how the conductance catheter determines "volume". Considering the RV

has a unique geometry and muscle mass than the left, the authors should provide some discussion or perhaps quantitation of these right-left differences. Furthermore, considering the unique chamber geometry of the RV, there is unique relationship between "wall-tension/stress" and ventricular pressure different from the LV. Please discuss.
Dear reviewer, we agree with this major limitation. The admittance catheter was first designed for LV. The unique chamber geometry of the RV might affect volume measurement and should be considered as a limitation. However, we observed no significant differences in cardiac output when measured in right and left ventricles. This result suggests that volume measurement in RV and LV is reproducible and accurate. According to your recommendation, we added the sentence below in the limitation section of the revised manuscript:
“The unique chamber geometry of the RV might affect volume measurement and should be considered as a limitation.”

2. The authors describe the physiological range of heart rate in mice as 350-500 bpm. As outlined in an early evaluation (Georgakopoulos D, Kass D. J Phys 2001), the force-frequency relationship at heart rates below 500 bpm is non-linear and can lead to confounding issues of "contractility". Please address how this may or may not impact RV function.
Dear reviewer, we agree that according to the work from Georgakopoulos D and colleagues, low heart rates could affect contraction and relaxation function. However, low heart rate associated with anesthesia on 20-34g mice e.g. 340-510 bpm with ketamine + xylazine, 365-550 bpm with pentobarbital sodium is not associated with significant changes in diastolic function in LV (LV end-diastolic pressure of 1-9mmHg and 2-8mmHg respectively)[1]. We agree that among other factors, anesthesia, and low heart rate impact cardiac function. However, the present work is a method paper which aims to focus on the technical (surgical) procedure. According to your comment, we added the sentence below in the limitation section of the revised manuscript.
“The anesthetic used, heart rates, temperatures, the animal strain could affect hemodynamic parameters and should be carefully reported and monitored.”

Minor Concerns:
1. Even though this manuscript is focused on the technique, a large part of the Introduction is devoted to a discussion of ventricular interaction. An example or demonstration of a technique highlighting this property would be appreciated. Perhaps a sudden infusion of volume into the right atrium or left atrium.
Dear reviewer, we agree that ventricular interaction represents a fascinating area that needs to be further investigated. However, it would require extensive experiments that are beyond the topic of this paper. Our method paper aims to focus on the technical (surgical) aspect of left and right heart catheterization.