Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

• Mention species in the title. DONE

• **Protocol Language:**
1) Split up long steps such as 2.4 into 2 or more steps. DONE

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to all your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Examples:
1) 1.6: how? Mention all tools used DONE
2) 2.4: Please describe all actions in more detail. Currently this step is too broad and generalized. These are actually only clicks of a mouse to utilize the software which comes with the equipment and are included in the training by the company personnel.

• **Protocol Highlight:** Please highlight 1 to ~2.5 pages of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
4) Notes cannot be filmed and should be excluded from highlighting. DONE

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol. DONE

• **Figures:** Fig 2-6: Please add scale references. DONE

• **References:** Please spell out journal names. DONE

• **Commercial Language:**JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company

brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Siemens Prisma, OsiriX MD v.5.0,
1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.

• **Table of Materials:** Please sort in alphabetical order. The Table was already in alphabetical order but has been updated to include more commercial names

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]." ORIGINAL

Reviewer #1:
Manuscript Summary:
This paper illustrates the efficacy of using laser speckle imaging (LSI) to monitor perfusion changes in real time in a canine model of large vessel occlusion. In addition, the authors also present infarct volume changes using diffusion MRI. Overall the LSI technique is quite interesting and I can see how it can be valuable especially in basilar artery occlusion (BAO) cases where nearly half the brain in affected. This technique can be used to monitor treatment and therapeutic effects. However, there are some critical changes that need to be addressed especially in the context of diffusion MRI. Please see comments below.

Major Concerns:
Lines 396-403: The authors address the choice of b-values based on optimization to detect lesions mentioning that the b-value value needs to be a certain value for humans versus neonates versus dogs. However, that is not an effective way to describe the "detection" of lesions. The ischemic region can contain different degrees of water restriction, i.e. the movement of water molecules in the tissue can be dictated by Gaussian or non-Gaussion behavior depending on how much restriction is experienced by the water molecules in the region of ischemia. For e.g., multiple low b-values can emphasize perfusion effects (also called intravoxel incoherent motion - IVIM) while large b-values emphasize the kurtosis effects (non-Gaussian movement of water) due to application of stronger gradients and large echo times. In this experiment with the dogs, the high-b values are emphasizing the non-Gaussian movement of water and that should be clearly explained in the context of water motion rather than just saying that a b-value is optimized for sensitive DWI. The authors need to revise this section significantly to clearly explain the underlying physical principles that lead to the high b-value choice. See second paragraph of discussion.

Line 98: The craniotomy that is performed is localized to a 1 cm^2 window. This seems to be effective only if a significant region of the brain is infarcted, which is the case in BAO. However, if the infarct region is smaller and only isolated to a smaller region of the brain, then how would you decide where to create the craniotomy window? Perhaps some discussion about this issue should be addressed in the Discussion section since the focus of this paper is in the use of LSI as a new way to look at perfusion in the infarcted regions. See first paragraph of discussion.

Line 98: How long does it take to do the craniotomy? This might be pertinent to know since time is of the essence in treating stroke. See first paragraph of discussion

Lines 272-273: Increased signal intensity as a typical sign of infarction would apply to DWI images. That really depends on the type of image you look at. On ADC maps, for e.g., the infarct region is dark. So, it's better to omit that sentence. Omitted

Line 349: Change the figure to add panel E and change y-label to "% infarct volume" to be consistent with the figure legend. "E" added and amended.

Figure 4: I understand that the slice shown has a much larger restricted volume than 55% indicated in the graph, which actually shows the infarct volume over the whole brain. It might be appropriate to show a slice that shows a similar ratio to the one on the graph shown in panel E. Or show multiple slices of the brain so that the quantification is clearer to the reader. Figure and legend amended as suggested.

Lines 300: I don't think it's necessary to overlay the images. It looks confusing at first look. Just showing the TTC stain and ADC map would suffice to see the correlation between the two images. Additional correlation plots showing the volumes from the TTC stains and MRI images should also be added as quantification of the correlation instead of just a subjective visual view. Figure and legend amended as suggested.

Minor Concerns:
Line 95: Change to "… mechanically ventilate using…" CHANGED
Line 162: remove Magnetic Resonance Imaging and mention that in Line 71 with first mention of MRI. CHANGED
Line 171: Change mRA to MRA. CHANGED
Line 182: Correct the matrix size. Looks like a typo. Also, given that this is the methods section, the b-values need to be specified here that were used for the diffusion-weighted imaging.
Line 192: omit "to represent" CHANGED
Line 208: Change to "… and place the cut tissue…" CHANGED
Line 223-224: Change to "…into a previously prepared solution containing 100mL…" CHANGED
Line 256: Change to "…After injecting the prepared thromboembolus…"
Line 324: Change to "…could not be collected. This part of the graph…" CHANGED

Reviewer #2:
Dear authors,

This is an important work as it describes an endovascular model of stroke in canines reproducing a devastating clinical condition - basilar artery occlusion. The authors use MRI and laser speckle to determine blood flow. In addition, the authors use TTC and H&E staining to confirm infarction at the time of sacrifice.

The authors performed the procedure seven times. The paper is a good proof-of-concept paper. Namely, it is feasible to perform a craniotomy, conduct laser speckle imaging, perform an endovascular basilar artery occlusion, then MRI imaging and sacrifice the animal. The model, MRI imaging, and sacrifice have been described before. The addition of the cranial window and laser speckle imaging is, to my knowledge, novel. I have a few areas of concern that temper my enthusiasm for this paper. The basilar artery occlusion in canines could result in "anterior" circulation strokes in the middle cerebral artery distribution from embolization of the clot through the posterior communicating artery. This increases the variability of the study to examine stroke size and neurological impairment as a result of the stroke or its treatment. This issue if it was identified should be discussed and addressed in the discussion. The basilar artery occlusion model can also produce some immediate hemodynamic changes - occasionally heart block occurs and this needs to be addressed as well. Also, the use of a craniotomy has been shown to decrease stroke size so this is a confounder to measurement of stroke. The location of the craniotomy in the canine is also of interest as the basilar artery is in a difficult place to access from the side of the head. The craniotomy location should be precisely described (maybe it was and I did not see it in the paper). Lastly, this is unlikely to be a survival model given the severity of disability with basilar artery occlusion. This will limit the utility of the model. Variability in this model with this procedure was NOT encountered in canines, but was encountered in mice with MCAO. We are currently writing up the stroke paper for both canines and mice and found that 7 animals per group were sufficient to reach significance with the canine model to access thrombolysis. Hemodynamic issues occurred only in the acute period immediately after clot placement, but did not affect the experiment to the extent that all canines were successfully transported, imaged, and returned for sacrifice at the designated experimental time point. In the next manuscript describing thrombolysis with this model, we performed craniotomy on ALL treatment groups and will add to that manuscript that exact stroke volumes are not as accurate as the difference in stroke volumes with treatment. We completely agreed with the reviewer in that BAO is severe, this is evidenced by the high mortality seen in patients when this area is affected. BAO was used because the size of a beagle may allow large animal studies since they reduce the costs of reagents, thrombolytics, per diem, animal purchase, etc but MCAO is not possible unless the size of the canine and all other expenses are increased along with it.

Having provided the above criticisms, I still think it is worthy of publication. I believe the paper would be strengthened if it addresses the aforementioned weaknesses.