**Editorial comments:**  
Changes to be made by the Author(s):  
Comment 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.

Response 1. This has been done.

Comment 2. Please check “Standard Access” or “Open Access” in the ARTICLE AND VIDEO LICENSE AGREEMENT (ALA). Please then scan and upload the signed ALA to your Editorial Manager account. Please note that in the Questionnaire Responses Standard Access is selected.

Response 2. Standard Access checked and new document uploaded.

Comment 3.Please upload each Figure individually to your Editorial Manager account as a .png or a .tiff file.

Response: This has been done to .png.

Comment 4. Please provide an email address for each author in the manuscript.

Response 4.

These have been added under the corresponding author section.

Comment 5. Please rephrase the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

Response 5.

This has been updated and now reads as follows:

Presented here is a protocol for lung nodule localization using dye marking via electromagnetic navigated transthoracic needle access. The technique described here can be accomplished in the peri-operative period to optimize nodule localization and successful resection when performing minimally invasive thoracic surgery.

Comment 6. Please rephrase the Long Abstract to more clearly state the goal of the protocol.

Response 6.

The beginning of the long abstract now reads as follows:

Increased use of chest computed tomography (CT) has led to increased detection of pulmonary nodules requiring diagnostic evaluation and/or excision. Many of these nodules are identified and excised via minimally invasive thoracic surgery, however sub-centimeter and sub-solid nodules are frequently difficult to identify intra-operatively. This can be mitigated with the use of electromagnetic trans-thoracic needle localization. This protocol delineates the step-by-step process of electromagnetic localization from the pre-operative period to the post-operative period and is an adaptation of electromagnetic guided percutaneous biopsy previously described by Arias et al. Pre-operative steps include obtaining a same day CT followed by generation of a 3-dimensional virtual map of the lung.

Comment 7. Please rephrase the Introduction to include a clear statement of the overall goal of this method.

Response 7:

This protocol will first highlight the benefits of using electromagnetic transthoracic nodule localization (EMTTNL). Secondly, it will delineate in a step-by-step fashion how to replicate the process prior to MITS.

Comment 8. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s, 8 mm; etc.

Response 8. This has been completed.

Comment 9. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution’s human research ethics committee.

Response 9: This does not apply as this technique is being used in clinical practice and is not a research tool. We are describing a novel technique using a new EMN platform to improve nodule localization for VATS/RATS.

Comment 10. 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, dashes, or indentations.

Response 10.

This has been adjusted.

Comment 11. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Response 11:

Adjustments made to section 2.4 and the discussion.

Comment 12. Line 149: Please mention how proper anesthetization is confirmed.  
Comment 12:

Section 2.2 now reads:

Once anesthesia and paralysis have been achieved (as confirmed with loss of muscle tone and cessation of spontaneous chest wall motion), establish an airway using a double lumen endotracheal tube (DL-ETT).

Comment 13. Lines 149-152, 171-184: Please write the text in the imperative tense.

Response 13. These have been updated and now read as follows:

Once anesthesia and paralysis have been achieved (as confirmed with loss of muscle tone and cessation of spontaneous chest wall motion), establish an airway using a double lumen endotracheal tube (DL-ETT), establish an airway using a double lumen endotracheal tube (DL-ETT). This will allow for procedural positioning, single lung ventilation for surgical resection and EMN system registration.

Following the snap shot, insert an EMN tracked disposable scope-catheter (DSC) into each lumen of the DL-ETT in order to generate a data point cloud delineating the extent of the main airways (Figure 2). Align the catheter on the main carina then pull back slowly into the trachea until prompted by the system to stop (green checkmark). Then drive the DSC into the right lung, specifically the right lower lobe until prompted to stop (green checkmark).

Comment 14. Lines 227-228: Please add more details to this step. This step does not have enough detail to replicate as currently written. Alternatively, add references to published material specifying how to perform the protocol action.

Response 14:

Given the focus of this protocol is pre-operative we have not added details to the step, however we have added references.

Comment 15. Please include a figure or a table in the Representative Results showing the effectiveness of your technique backed up with data.

Response 15: We have added wording suggesting reference to Figure 3.

Comment 16. References: Please do not abbreviate journal titles.-

Response 16 We followed JOVE recommendations regarding references and used the JoVE template in ENDNOTE.

Comment 17. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.

Response 17.

These have been removed and a new table uploaded.   
  
**Reviewers' comments:**  
  
  
  
**Reviewer #1:**  
Manuscript Summary:  
i would like to congratulate the authors on a fine piece of innovative academic work.  
with much pleasure i have read this manuscript.  
  
Major Concerns:  
none  
  
Minor Concerns:  
Abstract  
Comment 1. Better define what's new, and what other previously mandatory steps are now replaced or no longer necessary

Response 1.

We have added wording more clearly outlining the benefit of this procedure. Namely the decrease in radiation exposure, less time for dye diffusion and transparenchymal nodule access.

Comment 2: 47: This has many benefits when compared to the CT-guided localization, primarily decreasing time between dye injection and time of surgery.  
Most important benefit is the decreasing time between the dye and surgery? Because the visualization of the "dye-pathway marker" is therefore improved compared to current techniques? Later on this will be clearer, but here you should consider pointing out that in time the dye diffuses

Response 2:

The end of the long abstract now reads as follows:

This method has many benefits when compared to the CT-guided localization, including lower radiation exposure and decreased the time between dye injection and surgery. Dye diffusion from pathway occurs over time, thereby limiting intra-operative nodule identification. By decreasing the time to surgery, there is not only less waiting time for the patient, but most importantly there is less dye diffusion and improved localization. When compared to electromagnetic bronchoscopy, airway architecture is no longer a limitation as the target nodule is accessed through the parenchyma. Details of this procedure are described in step by step fashion.

Comment 3: -50: "these procedure"

Response 3: this has been edited.

Comment 4: What would make things clearer, and earlier and better to understand to include in the abstract: tis technique is an adaptation of the current EMN technique for percutaneous biopsy (arias et al)

Response 4:

Now reads:

This protocol delineates the step-by-step process of electromagnetic localization from the pre-operative period to the post-operative period and is an adaptation of electromagnetic guided percutaneous biopsy previously described by Arias et al.  
  
Introduction:  
Comment 5: 69-71: revise sentence  
Response 5: Revised. Now reads as follows:

Many of these issues with intra-operative nodule localization can be mitigated with the use of adjunct nodule localization methods via electromagnetic navigation (EMN) and/or CT-guided localization (CTGL).

Protocol  
Comment 6: 209-211: is there a certain amount of PEEP needed? Please specify in the text

Response 6: The following has been added to section 3.5

PEEP is maintained at 5 cm of water.

Comment 7: 213-125: What if the needle has moved? What if dye has been injected wrongly?Any bail-out options? Please specify the options of scenarios in the text.

Response 7:

Once the dye has been injected there are no bail out steps as it is final.

The following has been added to section 3.6:

If at any time during the following steps there is concern for needle movement, re-insert tracked stylet to confirm needle placement.   
  
Comment 8: 217-219: what is recommended?  
And if I choose for the mixture, is it 2-3 cc of dye and 2-3 cc of blood? Thus 4-6 cc in total?  
Please specify in the text.  
I see, in the discussion you come back to this subject → obviously blood mixture is better, so recommend that here!

Response 8:

Section 3.7 now reads as follows:

Connect a syringe to needle containing either 2-3 cc’s of methylene blue, or 2-3 cc’s of a 1:1 mixture of methylene blue and the patient’s blood. This mixture is recommended as it thickens the solution and limits dye diffusion.   
  
Comment 9: 221: is there any risk of intra-arterial or intravenously injection of dye? Or, should you first aspire before injecting?  
Should you stop injecting when you are out of the parenchyma? Can you feel that? Or see? And what if you do not or stop too late and create a stain over the visceral pleural? Does a potential stain masks the created pathway entrance for the surgeon? Or dilutes it easily? Please specify in the text.

Response 9:   
We do not aspirate during needle placement as the tracked component blocks the needle lumen during insertion. We do not feel this is a risk as methylene blue has intravenous application in much larger doses than 1mL. We are unable to time cessation of injection as we lose the tracked capability with the dye injection (tracked lumen stylet removed). We are unable to feel a difference when we cross the visceral pleural due to its very thin nature. We initially experienced dye “splash/diffusion” within the pleural space which led us to mix the dye with patient blood to improve viscosity (see 3.7).

Figure  
Comment 10: Please include an arrow to appoint the exact nodule localization in figure 3. There are actually 2 spots, which can be seen as the "entrance point".  
Response 10: This has been added.

Results  
Comment 11: 234-237: The description of the port placement is a bit messy, please revise accurately.  
M = mm I guess. So, 4 standard ports, and 2x 12 mm ports also in the 8th ics? So 6 ports in total? Please specify accurately.  
Response 11:

This has been edited. Section reads as follows:

The patient was prepared per the protocol noted above. Following this, EMTTNL was performed with injection of a total of 1 cc of a 1:1 methylene blue:patient blood mixture. Upon removal of the needle, the patient was prepped and draped for MITS. Robotic assisted thoracic surgery was performed using the four-arm technique with Da Vinci Xi robotic surgical system using five total ports.  Four ports are placed along the eighth intercostal space (each 9 cm apart) anteriorly from the mid clavicular line extending posteriorly to the scapular tip using one 12 mm robotic stapling port (most anterior port) and three 8 mm robotic ports.  One additional 12 mm robotic port is placed posteriorly one intercostal space above the diaphragm for the assistant.  The Xi robotic surgical system is docked to the patient using all four robotic arms for camera driving with an 8 mm 30-degree scope, a right and left arm for bipolar energy and dissection, and the “third” arm for lung retraction.  Following deflation of the lung, the localization dye marking was identified, (Figure 3) and diagnostic wedge resection was undertaken. Pathologic frozen section revealed transitional cell carcinoma (bladder cancer), margins were deemed clean and no further resection was performed.

Discussion  
Comment 12: Needle stabilization / angulation is key in this technique. Are there any advances in devices (robotics?) communicating with the EMN platform for needle stabilization/angulation and / or insertion? These will make this procedure even more accurate and reliable

Response 12:   
No, currently there are no applications that cross systems (EMN and surgical robot). They are produced by separate companies that do not have partnership agreements.

Comment 13: Ever considered using a surgical sealant (2 components, quick polymerizing; a bit viscous) mixed with methylene blue to inject to prevent immediate diffusion? A bit same as polymerizing hydrogels, however, the surgical glues are already CE-marked approved (but for lung injection…?).

Response 13:   
We have considered using other compounds for mixture with methylene blue however, we have had excellent success with the patient’s own blood which has seemed to be the best option as there is little to no risk associated with it.   
  
**Reviewer #2:**  
Manuscript Summary:  
Authors present a new navigational platform for transthoracic nodule localization before pulmonary resection.  
  
Major Concerns:  
Comment 1: Authors should focus the discussion about the difference between EMTTNL and another techniques for localization of pulmonary nodules, specially CTGL with dye. Authors argue that CT guided Transthoracic dye marking takes more time due to the patient needs to be transfer from the radiology suit to the OR, increasing the risk of complications and dye diffusion. This argument is not entirely true. Nowadays, dye tattoo through CTGL technique can be performed in a hybrid setting immediately before the surgery (1). Probably the main advantage of EMTTNL in comparison with CTGL is the lower radiation exposure during the needle insertion and guidance.  
  
1. Yang SM, Ko WC, Lin MW, Hsu HH, Chan CY, Wu IH, Chang YC, Chen JS.J Image-guided thoracoscopic surgery with dye localization in a hybrid operating room. Thorac Dis. 2016 Oct;8(Suppl 9):S681-S689. doi: 10.21037/jtd.2016.09.55.

Response 1:

End of long abstract now reads:

This method has many potential benefits when compared to the CT-guided localization, including decreased radiation exposure and time between dye injection and surgery

Introduction now reads:

Patients undergoing CTGL with dye marking have localization performed in the radiology suite followed by transport to the operating room during which time dye diffusion can occur making this technique less attractive. Some centers have mitigated this time lapse with the use of hybrid operating rooms with robotic C-arm CTs16,17, however radiation exposure can be higher with repeated images and use of fluorosocope15.

Minor Concerns:  
Comment 2: Line 61: the term "lung sparing anatomic resection" is not appropriate because could exclude Wedge Resections. Please use some generic term such as pulmonary resection or lung sparing resection.

Response 2: Sentence now reads: When indicated, surgical excision of these lesions should be performed, using a lung sparing resection via minimally invasive thoracic surgery (MITS) such as video- or robotic-assisted thoracoscopic surgery (VATS/RATS)4.

Comment 3: Line 698-69: change "diagnostic benign lobectomy" for "diagnostic lobectomy"

Response 3: “Benign” removed.

**Reviewer #3:**  
Manuscript Summary:  
This paper is invited method article about ENB navigation bronchosopic localization for pulmonary nodule. It was well written and described the details on the methods.  
It deserved to be accepted without any correction.  
  
Major Concerns:  
none  
  
Minor Concerns:  
none