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Electromagnetic Navigation Transthoracic Nodule Localization for Minimally Invasive Thoracic Surgery

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April 27, 2018

Dear Journal of Visualized Experiments Editorial Report:

Enclosed in this submission if our original work entitled "Electromagnetic Navigation Transthoracic Nodule Localization for Minimally Invasive Thoracic Surgery."

Due to an increasing detection of lung nodules, there is a growing demand for excision of small, sub-solid lung nodules via minimally invasive thoracic surgery. Intra-operatively these lung nodules can be difficult to find highlighting the importance of nodule localization. Lung nodule localization using dye marking can be now be performed via a novel electromagnetic navigated trans-thoracic needle access platform. This allows for visual identification of the nodule intra-operatively. This method potentially offers advantages to other forms of nodule localization including decreased time between localization and surgery.

The technique is described in a step by step method to optimize nodule localization and successful resection when performing minimally invasive thoracic surgery. We have made adaptations to previously described methods which have increased our yield and led to successful surgical resection. We believe our methods provide important tools which can be adapted at other institutions and lead to improved patient care.

We believe the following members are appropriate experts to review our work:

1. Lonny Yarmus – Johns Hopkins lyarmus@jhmi.edu
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All of the authors have contributed to the work and concur with the submission. The material in this manuscript has not been previously reported, nor is it under consider for publication elsewhere.

Sincerely,

Jason Akulian, MD MPH FCCP on behalf of the co-authors

TITLE:

Electromagnetic Navigation Transthoracic Nodule Localization for Minimally Invasive Thoracic Surgery

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KEYWORDS:

Electromagnetic navigation, lung nodule, transthoracic nodule localization, dye localization, minimally invasive thoracic surgery

SHORT ABSTRACT:

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

LONG ABSTRACT:

The increased use of chest computed tomography (CT) has led to an 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, subcentimeter and subsolid nodules are frequently difficult to identify intra-operatively. This can be mitigated by the use of electromagnetic transthoracic needle localization. This protocol delineates the step-by-step process of electromagnetic localization from the pre-operative period to the postoperative period and is an adaptation of the electromagnetically guided percutaneous biopsy previously described by Arias *et al.* Pre-operative steps include obtaining a same day CT followed by the generation of a three-dimensional virtual map of the lung. From this map, the target lesion(s) and an entry site are chosen. In the operating room, the virtual reconstruction of the lung is then calibrated with the patient and the electromagnetic navigation platform. The patient is then sedated, intubated, and placed in the lateral decubitus position. Using a sterile technique and visualization from multiple views, the needle is inserted into the chest wall at the prechosen skin entry site and driven down to the target lesion. Dye is then injected into the lesion and, then, continuously during needle withdrawal, creating a tract for visualization intra-operatively. This method has many potential benefits when compared to the CT-guided localization, including a decreased radiation exposure and decreased time between the dye injection and the surgery. Dye diffusion from the pathway occurs over time, thereby limiting intra-operative nodule identification. By decreasing the time to surgery, there is a decrease in wait time for the patient, and less time for dye diffusion to occur, resulting in an improvement in nodule localization. When compared to electromagnetic bronchoscopy, airway architecture is no longer a limitation as the target nodule is accessed *via* a transparenchymal approach. Details of this procedure are described in a step-by-step fashion.

INTRODUCTION:

With the increasing use of CT scans of the chest for diagnostic and screening purposes[1], there is an increased detection of subcentimeter pulmonary nodules requiring diagnostic evaluation[2]. Percutaneous and/or transbronchial biopsy have been successfully used to sample indeterminate and high-risk nodules. These lesions often make for challenging targets due to their distal parenchymal location and small size[3]. 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 robot-assisted thoracoscopic surgery (VATS/RATS)[4]. Even with advances in surgical technique, there remain intra-operative challenges to resection, despite direct visualization of the lung parenchyma during MITS. These challenges are primarily related to difficulties with nodule localization, especially with ground-glass/semisolid nodules, subcentimeter lesions, and those more than 2 cm from the visceral pleura[5,6]. These challenges are exacerbated during MITS due to a loss of tactile feedback during the procedure and can lead to more invasive surgical methods, including diagnostic lobectomy and/or open thoracotomy[5]. Many of these issues with intra-operative nodule localization can be mitigated by the use of adjunct nodule localization methods *via* electromagnetic navigation (EMN) and/or CT-guided localization (CTGL). 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.

Electromagnetic navigation helps to target peripheral pulmonary lesions by overlapping sensor technology with radiographic images. EMN first consists of using available software to convert

CT images of the airway and parenchyma into a virtual roadmap. The patient's chest is then surrounded by an electromagnetic (EM) field within which the exact location of a sensory guide is detected. When a guide instrument (*e.g.*, magnetic navigation [MN]-tracked needle) is placed within the patient's EM field (endobronchial tree or skin surface), the location is superimposed on the virtual roadmap, allowing for navigation to the target lesion identified on the software. EMN can be performed *via* either transthoracic needle approach or bronchoscopy. EMN bronchoscopy has previously been described for use in both biopsy and fiducial/dye localization[7-11]. A number of other localization techniques have been developed with varying success rates, including CT-guided fiducial placement, CT-guided injection of dye or radiotracer, intraoperative ultra-sonographic localization, and EMN bronchoscopy[12]. A recently introduced EMN platform has incorporated an electromagnetically guided transthoracic approach into its workflow. Using the CT roadmap, the system allows the user to define a point of entry on the chest wall surface through which they will pass a tip-tracked EMN-sensed needle guide into the lung parenchyma and lesion in question. Through this needle guide, biopsies and/or nodule localization can then be performed[7].

Prior to the EMN localization of nodules for MITS, CTGL using dye marking or fiducial (*e.g.*, microcoils, lipoidal, hook-wire) placement was the primary method employed. A recent meta-analysis of 46 studies of fiducial localization showed high success rates among all three fiducials; however, pneumothorax, pulmonary hemorrhage, and the dislodgement of fiducial markers remained significant complications[13]. A CT-guided tracer injection with methylene blue has had similar rates of success, but with fewer complications when compared with hook-wire fiducial placement[14]. One of the primary limitations of using dye for lung nodule localization has been diffusion over time[15]. Patients undergoing CTGL with dye marking have the 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 CTs[16,17]; however, radiation exposure can be higher with the repeated images and use of fluorosocope[15]. The use of EMN bronchoscopy allows for peri-operative nodule localization. This, however, has been plagued by prolonged bronchoscopy times and an inability to navigate to those lesions without airway access. EMTTNL allows for a rapid percutaneous nodule localization followed by MITS in one location (*i.e.*, the operating room), therefore decreasing time between the localization and the surgery[18]. In addition to EMN bronchoscopy, Arias *et al.* described using EMN for percutaneous biopsy[7]. An adaptation of this procedure for nodule localization is described below.

A 79-year-old male with a 40 pack-year history of tobacco use and bladder cancer was found to have a new PET fluorodeoxyglucose-avid lung nodule of size 1.0 cm x 1.1 cm in the left lower lobe by surveillance imaging (**Figure 1**). Given the lesion's size and position, wedge resection was considered challenging and the patient's pulmonary reserve made him a less than ideal candidate for diagnostic lobectomy. It was decided that he would undergo EMTTNL to aid in the MITS resection of the lung nodule.

PROTOCOL:

The procedure is performed in accordance with standard of care expectations and follows the guidelines of the human research ethics committee at the University of North Carolina at Chapel Hill.

1. Pre-operative Preparation

1.1. Review prior chest computed tomography (CT) imaging to ensure that the patient undergoing nodule localization has a peripheral pulmonary nodule suitable for minimally invasive thoracic surgery (MITS).

1.2. On the day of or a day prior to the procedure, perform a noncontrast chest CT scan with the patient in the lateral decubitus position with the lung ipsilateral to the nodule positioned up to mimic the position during the dye injection. Obtain both expiratory and inspiratory images to account for nodule movement.

Note: The CT should be formatted per EMTTNL system specifications[7].

1.3. Use the navigation system planning software to digitally segment the target lesion.

1.4. If the target lesion is radiographically “pure” ground-glass in nature, the segmentation software may fail to properly identify the lesion. In this case, place a virtual target in the center of the target lesion.

1.5. Once the target lesion has been successfully marked, use the planning software to delineate the percutaneous site for needle entry. The percutaneous entry site should be located between two ribs, taking care to avoid the intercostal neurovascular bundle on the inferior border of the rib, and represent the shortest track from skin entry to nodule acquisition.

2. Peri-operative Preparation and Registration

2.1. Transfer the patient to the operating room and have the appropriate personnel induce general anesthesia with paralysis.

Note: General anesthesia should be only administered by certified personnel and the dosing of the drugs is at the discretion of the anesthesia provider.

2.2. Once anesthesia and paralysis have been achieved (as confirmed by the loss of muscle tone and the cessation of spontaneous chest wall motion), establish an orally inserted airway using a double lumen endotracheal tube (DL-ETT) as opposed to a traditional single lumen endotracheal tube.

Note: This is placed by the anesthesia providers, and any size required based on the patient’s specifications is acceptable. This will allow for procedural positioning, single-lung ventilation for the surgical resection and EMN system registration.

2.3. Perform a white light bronchoscopy (WLB) examination of the tracheobronchial tree to the segmental level, assessing for occult endobronchial disease.

2.4. After performing a WLB examination of the airway, position the patient in the lateral decubitus position, mirroring as closely as possible the patient's positioning during CT. Attach three electronic reference point pads to the patient's chest, placing them on the ipsilateral chest wall to the nodule and out of the way of the chosen point of entry (**Figure 2**).

2.4.1. For example, if the chosen point of entry is the left anterior thorax, place the pads on the left chest, at least 5 cm away from the point of entry. Then, plug the pads into the EMN system.

2.5. Perform system registration for the patient with system calibration by first positioning the EMN field generator over the reference pads. Fine-tune the position using prompts provided by the EMN system. Once the field generator is in position, using the EMTTNL platform, take a virtual "snapshot" of the reference pads.

2.6. Following the snapshot, insert the proprietary EMN-tracked disposable scope catheter (DSC, 3.2 mm in outer diameter, working channel 2.0) into each lumen of the DL-ETT in order to generate a data point cloud delineating the extent of the main airways (**Figure 3**). Align the catheter on the main carina and, 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 lob—until prompted to stop (green checkmark).

2.7. Once the data point collection is halted, remove the DSC from the right lung lumen of the DL-ETT and insert it into the left lung lumen of the tube. Drive the DSC into the left mainstem bronchus 2 - 3 cm proximal to its bifurcation into the left upper and lower lobes. Resume the data collection at this point and drive the DSC into the left lower lobe until prompted to stop (green checkmark). Once the full data point cloud is collected, proceed to EMTTNL.

3. Procedure

3.1. Align a tracked percutaneous needle at the chest wall skin entry site using the EMN platform for guidance. Mark the skin at the entry point to the chest cavity, taking care that the entry point should be just superior to the rib and avoid any known vasculature or osseous structures (*e.g.*, clavicle, subclavian vessels).

3.2. Clean and prepare the skin with a 2% chlorhexidine solution for a minimum of 15 s and allow it to dry for a minimum of 30 s. Drape the field using sterile technique.

3.3. Once a sterile field has been created, don sterile gloves and a sterile gown and inject 1 - 2 mL of 1% lidocaine subcutaneously at the entry point for local anesthesia. Use a #10 blade surgical scalpel to make a superficial skin incision (5 mm) at the entry site through the epidermis.

3.4. Place the sterile 19-G electromagnetic needle on the marked entry point. Using the transverse and coronal views on the electromagnetic system screen, adjust the angle of entry so that it lines up with the center of the target lesion (**Figure 4**).

Note: Crosshair markings should overlap in at least two different planes.

3.5. Once the angle of entry is confirmed, stabilize the EMN-tracked needle against the chest wall and firmly advance through the chest wall, while the anesthesia team holds the patient in exhalation. Positive end-expiratory pressure (PEEP) is maintained at 5 cm of water.

3.6. Once reaching the distal side (from the chest wall) of the target lesion, remove the tracked stylet without moving the needle and cover the needle hub with a finger. **Use extreme caution so as to not dislodge the needle**. If, at any time during the following steps, there is concern about needle movement, reinsert the tracked stylet to confirm needle placement.

3.7. To the needle, connect a syringe containing either 2 - 3 mL of undiluted methylene blue, or 2 - 3 mL of a 1:1 mixture of methylene blue and the patient's blood.

Note: The patient's blood should be drawn just prior to mixing it to minimize clotting and can be drawn off with either a peripheral IV or from a fresh needle venipuncture. The mixture is recommended as it thickens the solution and limits dye diffusion and/or "splashing" of dye within the pleural space during needle retraction.

3.8. Inject 0.5 mL of the dye or the dye:blood mix within the target lesion. Gradually and continuously deposit another 0.5 mL of the dye or the dye:blood mixture while slowly withdrawing the needle to create a track.

4. Post Procedure

4.1. After EMTTNL (**Figure 3**), perform MITS using the dye marking to localize and resect the lung nodule[19-23].

Note: Post-procedure patient care will be at the discretion of the thoracic surgeon as this protocol does not have any specific post-operative requirements.

REPRESENTATIVE RESULTS:

The patient was prepared per the protocol noted above. Following this, EMTTNL was performed with an injection of a total of 1 mL of a 1:1 methylene blue:patient blood mixture. Upon removal of the needle, the patient was prepped and draped for MITS. Robot-assisted thoracic surgery was performed using the four-arm technique with a robotic surgical system using five total ports. Four ports are placed along the eighth intercostal space (each 9 cm apart) anteriorly from the midclavicular 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 robotic surgical system is docked to the patient using all four robotic arms for camera driving with an 8-mm, 30° scope, a right and a left arm for bipolar energy and dissection, and the "third" arm for lung retraction. Following the deflation of the lung, the localization dye marking was identified, and diagnostic wedge resection was undertaken (**Figures 5**). A pathologic frozen section revealed transitional cell carcinoma (bladder cancer), the margins were deemed clean, and no further resection was performed.

FIGURE LEGENDS:

Figure 1: FDG-avid left lower lobe nodule which requires localizations prior to surgical excision. (**A**) Positron Emission Tomography (PET) scan; (**B**) Chest Computed Tomography. Note the FDG-avid left lower lobe nodule (arrow).

Figure 2: Electronic reference pad placement. Three reference pads should be placed on the chest wall ipsilateral to the nodule, and out of the way of the chosen point of needle entry.

Figure 3: Virtual rendering of airways reconstructed from the procedure CT scan. This image is re-constructed using data from the CT scan after collecting data points within the airways. Note the data points within the airway tree as well as checkmarks denoting completion of airway data collection

Figure 4: Snapshot with alignment of percutaneous needle entry in transverse, coronal and sagittal views. This electromagnetic system screenshot shows an example of needle alignment in multiple views with the target lesion centered just prior to needle insertion (Image courtesy of Veran Medical).

Figure 5: Images of the lung during and after resection. (**A**) Intra-operative images of the lung after injection of 1:1 methylene blue/blood mixture. Arrow identifies the exit point of the percutaneous dye needle. **(B)** Successful wedge resection of the dye localized lung. Forceps identify the exit point of the percutaneous dye needle.

DISCUSSION:

Peri-operative transthoracic nodule localization under EMN guidance is a novel application of a recently introduced EMN platform. The critical steps in the performance of EMTTNL are a proper point cloud registration of the device and attentiveness to the percutaneous insertion site and the angulation of the needle. Visualization and maintenance of the angle of entry on multiple planes of the CT scan (HUD, oblique 90, and oblique) are crucial to the success of the procedure.

Some of the following modifications have been adapted due to trouble-shooting frequently occurring issues. One modification to this technique includes CT performed in the lateral decubitus position instead of the supine. This change was adopted due to registration errors after pronounced patient repositioning and/or shifting of the reference pads. Another modification is the mixing of the dye in a 1:1 concentration with the patient's blood. During initial efforts, there was excessive splattering of dye within the chest cavity, as well as dye diffusion, despite short intervals to surgical port placement. The mixture has, since, led to decreased diffusion and less dye soiling of the pleural space.

Limitations of this technique may include the localization of multiple nodules (oligometastases) due to the possibility of pneumothorax development between needle passes. A pneumothorax after the first needle pass would distort the anatomy and result in improper dye injection. That said, we have overcome this limitation in at least one instance where we left the initial localization needle anchored in place by an assisting physician and then localized another target with a separate needle. Once both targets were needle-localized, the injection of the dye and the needle retraction were performed simultaneously, resulting in the successful EMTTNL of two separate ipsilateral targets. Another limitation is the location of the nodule itself. EMTTNL is an excellent option for peripheral nodules; however, the transthoracic approach is not ideal for central lesions, nor for those inaccessible due to the scapula or other bony/vascular structures. Additional limitations of the technique include user and system errors, such as the potential for excess dye injection causing dye spillage and/or an inability of the surgeon to pinpoint the site of the lesion. Errors may also occur with use of the EMN system, including misregistration and reference PAD malposition.

This technique draws on the existing practice of CTGL. EMTTNL is a significant advancement due to its ability to be performed in the peri-operative setting. Previous use of CTGL has been limited due to complications, radiation exposure, the time from CTGL to transport to surgery, and dye diffusion[14,15]. Bronchoscopic dye marking has also been described with varying degrees of success[10,11,18]; however, bronchoscopic access to nodules is limited by airway architecture[24]. This is typically not an issue with EMTTNL as the transthoracic approach is not restricted to the airways.

Future applications of EMTTNL may include the use of other marking agents, including gold fiducials, hydrogel plugs, or indocyanine green coupled with near-infrared fluorescence. Multi-centered prospective trials of EMTTNL to aid in MITS would be useful to determine optimal nodule and patient characteristics for the application of this technique.

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Figure 1

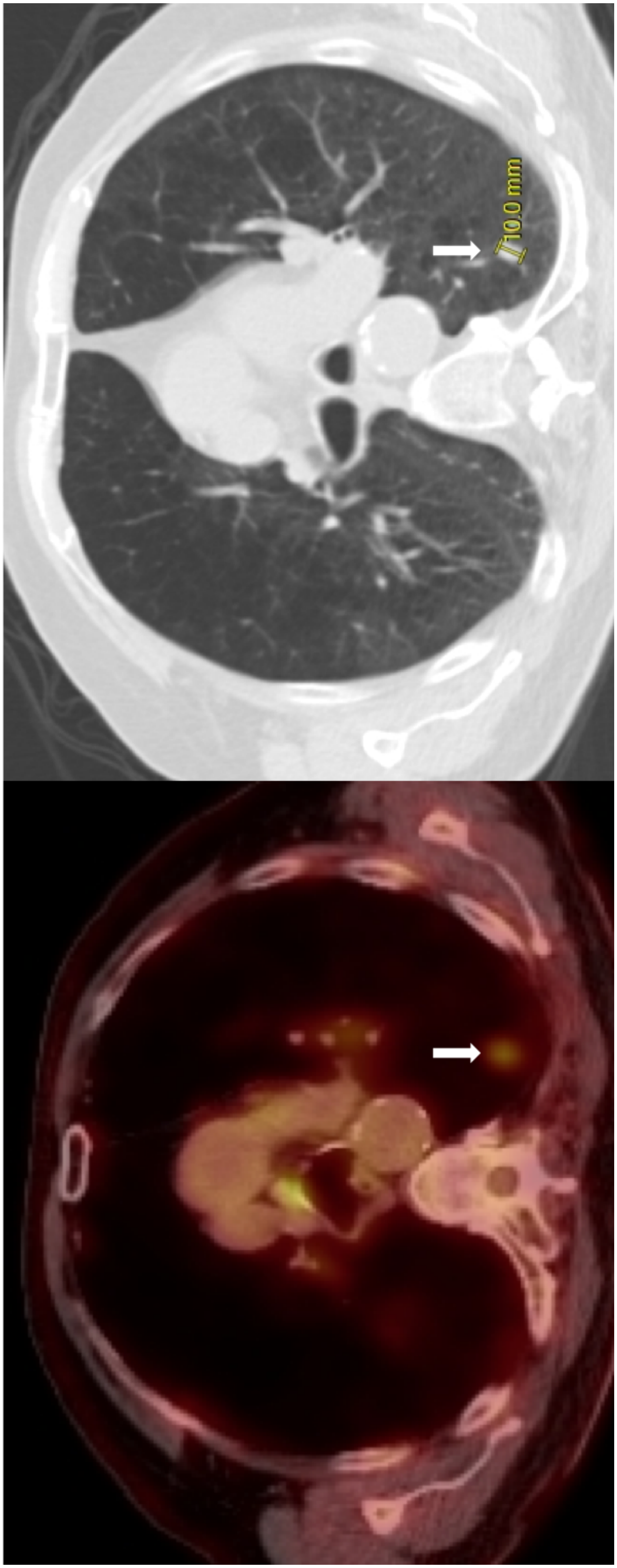

Figure 2

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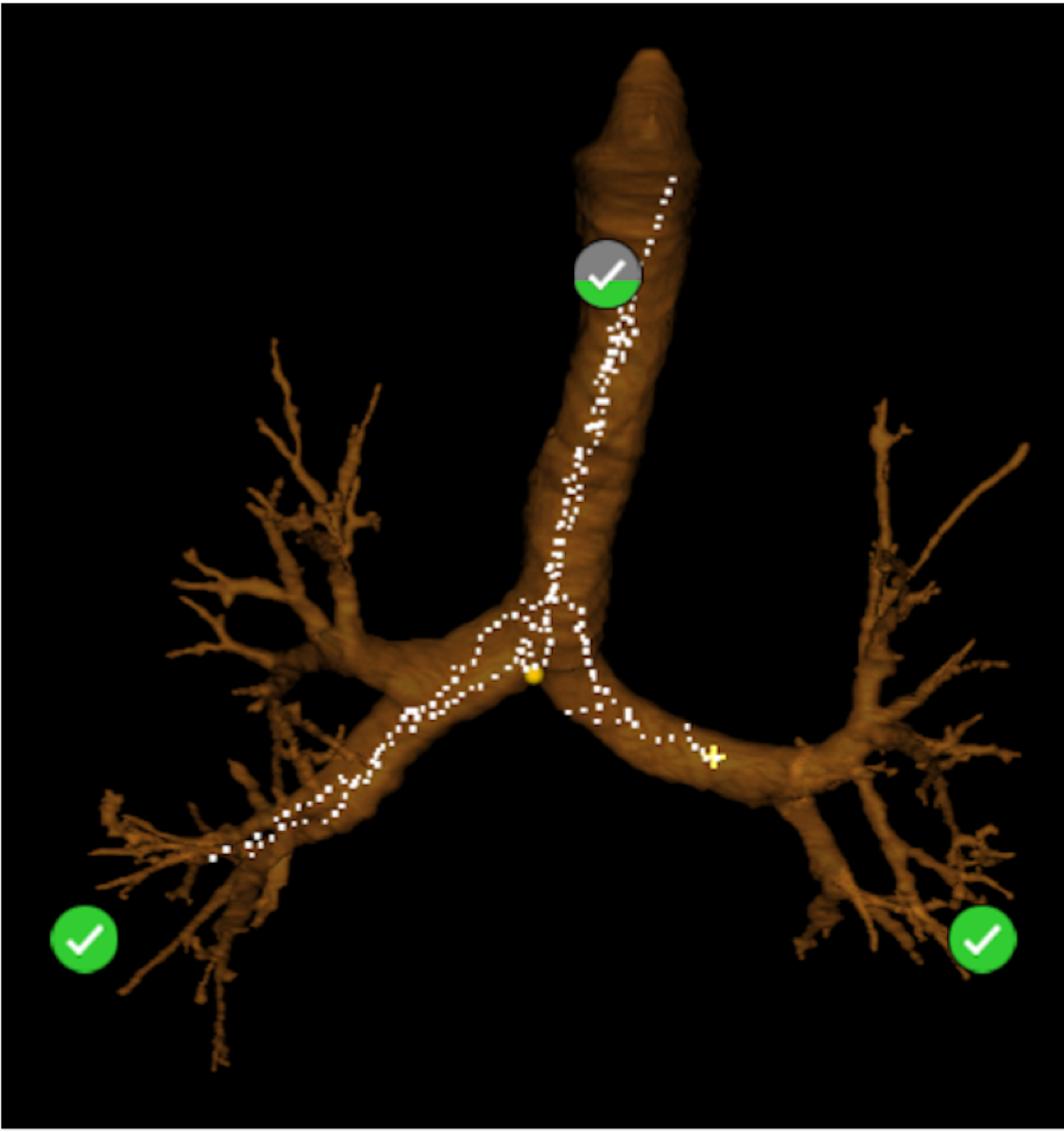


Figure 4

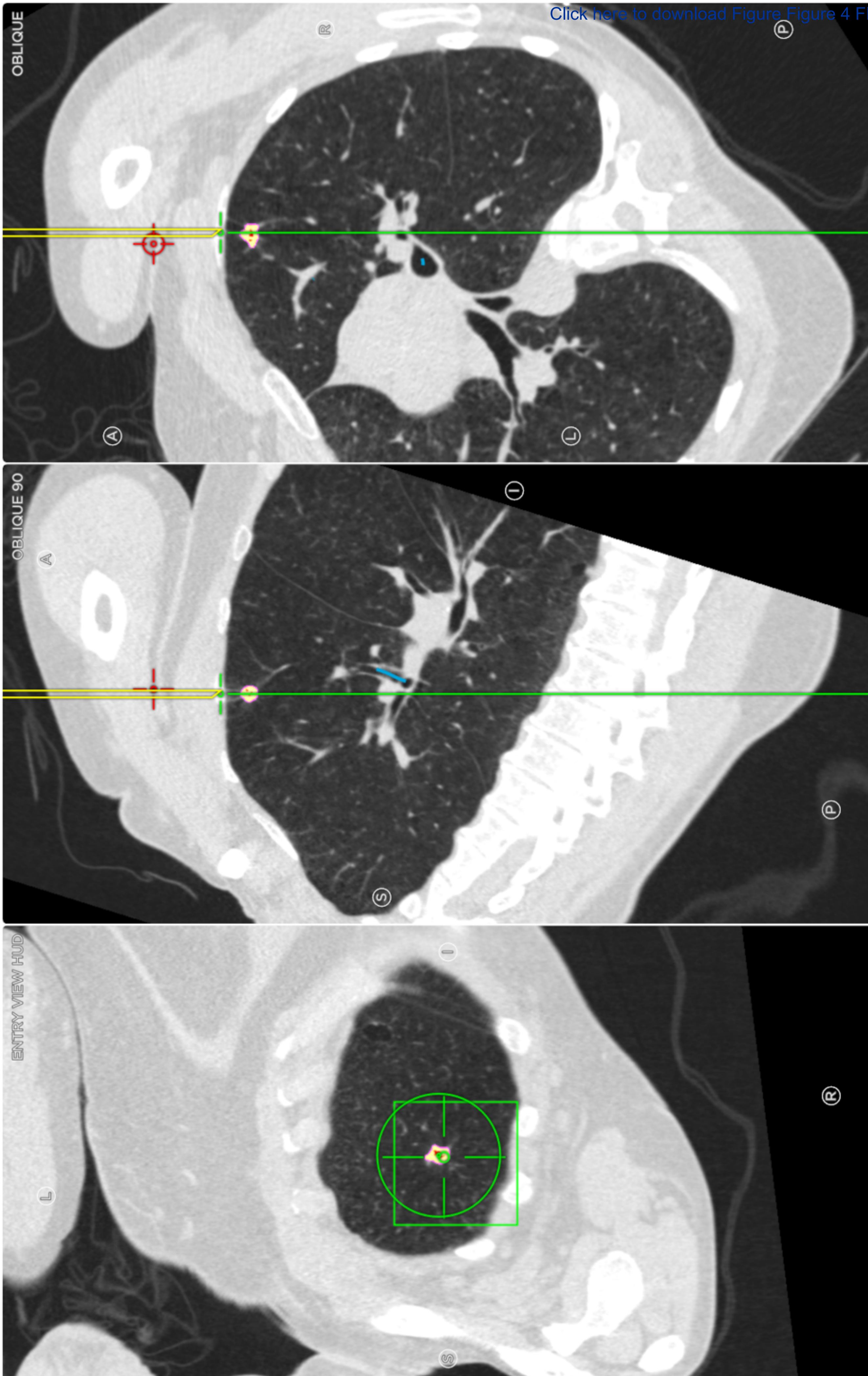
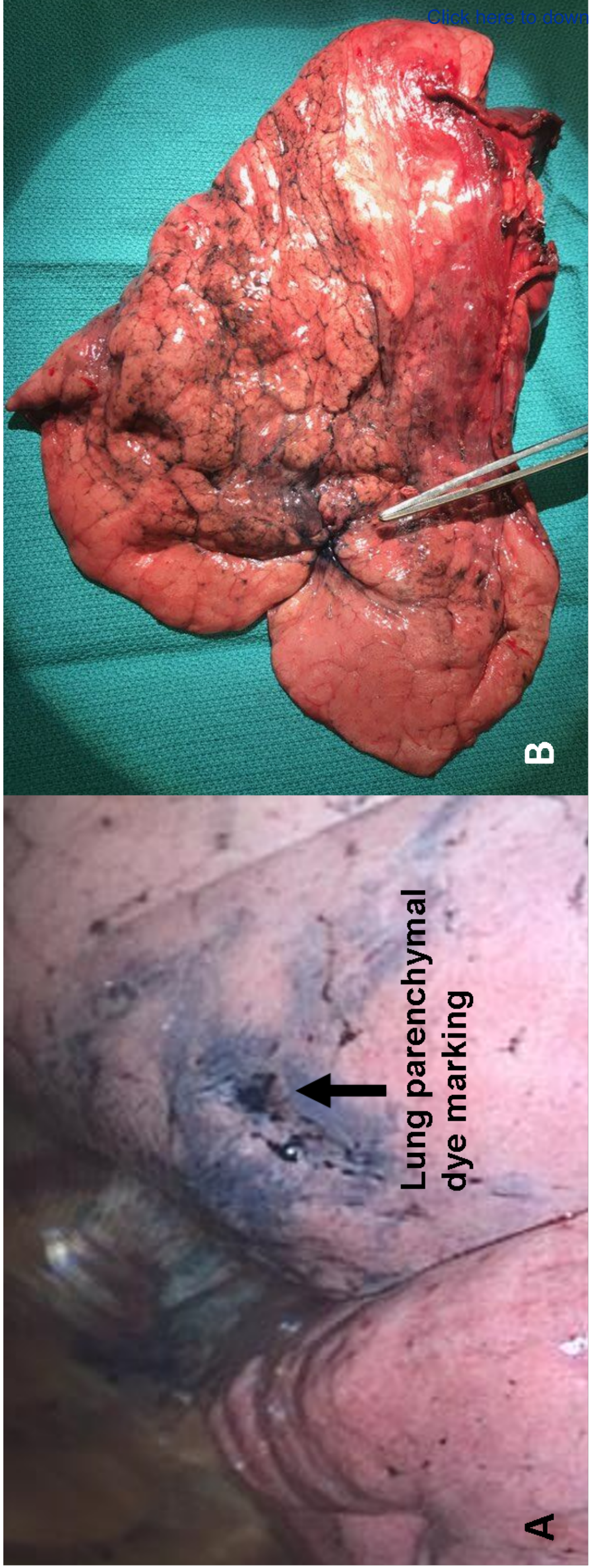


Figure 5



Name of Reagent/ Equipment
Computed Tomography Scanner
SPiN Thoracic Navigation System
SPiN Planning Laptop Workstation
SPiN View Console
Always-On Tip Tracked Steerable Catheter
View Optical Probe
vPAD2 Cable
vPAD2 Patient Tracker
SPiNPerc Biopsy Needle Guide Kit
ChloraPrep applicator
Provay/Methylene Blue
Sterile gloves
Blue X-Ray O.R. Towels
Scope Catheter

Company	Catalog Number	Comments/Description
		64 - detector (or greater) CT scanner
Veran Medical Tecnologies	SYS 4000	
Veran Medical Tecnologies	SYS-0185	
Veran Medical Tecnologies	SYS-1500	
Veran Medical Tecnologies	INS-0322	3.2 mm OD, 2.0 mm WC
Veran Medical Tecnologies	INS-5500	
Veran Medical Techologies	INS-0048	
Veran Medical Techologies	INS-0050	
Veran Medical Techologies	INS-5600	Includes INS 5029 (Box of 5)
Beckton Dickinson	260815	26 mL applicator (orange)
Cenexi/American Regent	0517-0374-05	50 mg/10 mL
Cardinal Health	2D72PLXXX	
MedLine	MDT2168204XR	
DSC		3.2 mm outer diameter, working channe

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Title of Article: Electromagnetic Navigation Transthoracic Nodule Localization for Minimally Invasive Thoracic Surgery

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Article Title: Electromagnetic Navigation Transthoracic Nodule Localization for Minimally Invasive Thoracic Surgery

Signature: Jason Akulian   Date: 5-29-2018

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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 CTs[16,17], however radiation exposure can be higher with repeated images and use of fluorosocope[15].

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