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Virtual endobronchial ultrasound (Virtual EBUS) for transbronchial needle aspiration

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Abstract

Objective: endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) could be better performed with computer-based preparation.

Methods: three-dimensional virtual bronchoscopy was used to develop two modes of computer-based ‘Virtual EBUS’. ‘Virtual EBUS Standard’ used conventional virtual bronchoscopy to determine the spot and angle for TBNA, which was further evaluated by virtual bronchoscopy. ‘Virtual EBUS Advanced’ used multiple layers of three-dimensional images of the target lesions and associated vascular structures in combination with virtual bronchoscopy. Target lesions and associated vascular structures (e.g. pulmonary artery) were visualised through half-transparent bronchial walls.

Results: both methods required 5 to 15 minutes of preparation per case. Virtual EBUS Standard required only basic computer software for virtual bronchoscopy, while Virtual EBUS Advanced required an advanced computer application. Virtual EBUS Advanced allowed for a more intuitive recognition of the target. Both methods were useful in evaluating feasibility of TBNA, especially when the target was out of regular mediastinal lymph nodes, or in targeting a lesion located at a high-upper angle (e.g. #4L lymph node). Because the puncture spot was pre-determined, bronchoscopists did not have to search for the target using ultrasound at the time of actual EBUS-TBNA; rather, ultrasound was used only for confirmation of the target location and visualisation of TBNA.

Conclusions: both computer-based preparation methods of Virtual EBUS were useful in predetermining the puncture spot of TBNA, suggesting their potential complementary role to the conventional technique of EBUS-TBNA.
**Ultra-mini-Abstract:** We developed two computer-based preparation methods for EBUS-TBNA (“Virtual EBUS”), which were useful in pre-determining the puncture spot of TBNA, especially when the target lesion is at a challenging location such as out of regular mediastinal lymph nodes or at a high-upper angle.

**Keywords:** Virtual bronchoscopy, three-dimensional simulation, mediastinal lymph node, lung cancer, staging
Introduction

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive method of mediastinal biopsy performed under direct, real-time endobronchial ultrasound guidance.\(^1\)\(^-\)\(^3\) Beyond the lymph node stations reachable by mediastinoscopy (#2, #4, and #7), EBUS-TBNA can obtain biopsies from hilar lymph nodes (#10, #11, and #12). Moreover, EBUS-TBNA is useful for diagnosis of mediastinal lesions other than those in conventional lymph nodes, such as mediastinal tumors,\(^4\) and for obtaining biopsies of centrally located intrapulmonary mass lesions that cannot be diagnosed by conventional flexible bronchoscopy.\(^5\)\(^-\)\(^6\)

EBUS-TBNA is, however, more technically demanding than standard fibre-optic bronchoscopy; there is a learning curve of 5 to 10 procedures for an experienced bronchoscopist to obtain clear ultrasound images.\(^7\) Furthermore, EBUS-TBNA can be even more challenging depending on the location of the target lesions, even for experienced EBUS bronchoscopists.

Virtual bronchoscopy utilises three-dimensional (3D) reconstruction of two-dimensional helical computed tomographic images for non-invasive evaluation of the tracheobronchial tree.\(^8\) We recently introduced 3D virtual bronchoscopy as an aid to EBUS-TBNA and named the strategy ‘Virtual EBUS’. In the present report, we describe simple techniques of 3D virtual bronchoscopy that enables pre-determination of the puncture spot of TBNA. We herein describe two separate strategies, one using simple conventional 3D virtual bronchoscopy (‘Virtual EBUS Standard’) and the other using more complex, multi-layer virtual bronchoscopy to visualise target lesions and associated vasculature through half-transparent bronchial walls (‘Virtual EBUS Advanced’).
Materials and Methods

Virtual EBUS Standard

An Aquarius Thin Client Viewer® (TeraRecon, Inc., Tokyo, Japan) was used to construct 3D virtual bronchoscopy images and simulate EBUS-TBNA on a computer. Step-by-step instructions are shown in Figure 1 (left). First, following the manufacturer’s instructions, 3D virtual bronchoscopy images were constructed from thin-slice CT images (Figure 2a-c). Second, while paying attention to the 3D virtual bronchoscopic images and associated CT views (especially the axial view), the virtual bronchoscope was moved forward and backward in the tracheobronchial lumen. Throughout the process, the target lesion was localised on 3D images, and the angle at which the TBNA needle should penetrate the tracheal or bronchial wall was determined (Figure 2d). Third, after determining the location and angle for puncture, only 3D virtual bronchoscopic images were used. Starting from the cephalic trachea and moving to the carina, 3D virtual bronchoscopy was performed as actual fibre-optic flexible bronchoscopy would be performed. While approaching the pre-determined target, the operator ‘imagined’ that he or she was a needle at the tip of the EBUS scope penetrating the tracheobronchial wall into the target (Figure 2d, far right panel). When the virtual bronchoscope penetrated the tracheobronchial wall and the operator considered the target to be ‘punctured’, the CT views were opened and the puncture was evaluated by a pyramid-shaped director. If the target had not been appropriately punctured or the angle had not been appropriate, the second step was repeated to obtain a better puncture point.

Once the localisation and puncture angle were determined, the 3D image was recorded with inclusion of notes that would guide an EBUS operator at the time of actual EBUS. We copied a
series of 3D virtual bronchoscopy images and pasted them onto a presentation file (PowerPoint®; Microsoft Japan, Tokyo, Japan) with an arrow indicating the spot and angle at which to puncture the target as a reference at the time of EBUS. It is also important to leave a concrete verbal description indicating the spot and angle to puncture (e.g., ‘one ring cephalad to the carina, 10- to 11-o’clock angle relative to the carina’).

**Virtual EBUS Advanced**

An Aquarius iNtuition Client Viewer® (TeraRecon, Inc., Tokyo, Japan) was used to build 3D images from thin-slice CT scans. Step-by-step instructions are shown in Figure 1 (right). Virtual EBUS Advanced is characterised by color-marking of target lesions and other associated structures (e.g., blood vessels), and their visualisation is performed by making tracheobronchial walls half-transparent. After loading CT images, masks (or layers) were created by tracking the outline of target lesion(s) and associated structures using the ‘free drawing’ tool among the mask tools (Figure 3a-c). The object was extracted by the ‘extraction’ tool and then colored appropriately (e.g., target lesion, green; pulmonary artery, red; and azygous vein and superior vena cava, blue) (Figure 3a-c, insets). Each mask and the 3D bronchoscopy image were overlaid. Transparency of the 3D bronchoscopy image was manually adjusted, and pictures or movies were then recorded following the manufacturer’s instructions (Figure 3d).

**EBUS-TBNA procedure**

The convex probe of the endobronchial ultrasound was used to perform EBUS-TBNA (BF-UC160F-OL8; Olympus, Tokyo, Japan). The ultrasound image was processed in a dedicated ultrasound scanner (EU-C60; Olympus). After induction of local anaesthesia and mild sedation
with midazolam (Astellas Pharma Inc., Tokyo, Japan), conventional flexible bronchoscopic examination was performed, followed by EBUS-TBNA. Pictures and/or movies created with Virtual EBUS were used as a reference while conducting the EBUS-TBNA procedure. After confirming the localisation of target lesion(s), a dedicated 22-gauge needle (NA-201SX-4022; Olympus) was used for TBNA under real-time ultrasound visualisation. On-site cytology was conducted by a cytologist who noted the quality of the sample and provided a temporal diagnosis. In general, TBNA was conducted at least three times per target, even after a temporal diagnosis was obtained, as long as time allowed (usually 45 minutes).

**Patients**

The results of EBUS-TBNA conducted for 41 consecutive patients from January to August 2012 were retrospectively analysed. We developed the Virtual EBUS Standard technique at the end of 2011, and Virtual EBUS Advanced was developed in April 2012 as a computer-based preparation method for EBUS-TBNA. Selection of the preparation method (i.e., Virtual EBUS Standard, Virtual EBUS Advanced, or no Virtual EBUS) was dependent on the bronchoscopist’s experience and preference. The present retrospective study protocol was examined and approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine.
Results

Virtual EBUS Standard

Using conventional virtual bronchoscopy, Virtual EBUS Standard was conducted to determine the puncture spot and angle and to simulate the EBUS-TBNA procedure on a computer. From January to August 2012, Virtual EBUS Standard was used for 21 lesions of 11 patients. Five to 15 minutes per case was required for preparation using Virtual EBUS Standard depending on the number and location of the target(s). Preparation was done by one of the bronchoscopists involved in the procedure. The actual EBUS-TBNA procedure was attempted for 17 lesions of 11 patients; 4 lesions were not punctured during the procedure because of the onsite cytology results. For example, a diagnosis was made from another target or because advanced staging of lung cancer was made from another lymph node station (e.g., because N2 was demonstrated, puncture for an N1 node was not performed). All TBNAs successfully hit the target, with the exception of one lesion in which the target lymph node (#10R) was well visualised but the needle could not penetrate the calcified bronchial wall. Sufficient material was obtained from 14 of 16 lesions (85.7%). Insufficient sampling in the two lesions may be attributable to only one-time sampling; in one case, repeated sampling of the #4R was not conducted because N2 disease was demonstrated in another lesion (#7) on site in the same procedure; in the other case, the bronchoscopist felt it not safe to repeat puncture at #4L station because it appeared to be too close to the esophagus and pulmonary artery. Representative cases of Virtual EBUS Standard are shown in Cases 1 and 2.

Case 1
A 69-year-old male patient had a history of rectal cancer resection 4 years previously followed by right pulmonary lower lobe partial resection 1 year later. Liver resection was performed for liver metastasis 2 years previously, and a left lung nodule was found. The pulmonary tumor was positive on positron emission tomography (PET), and the left #11 lymph node was marginally positive on PET (Figure 2c). Preparation for EBUS-TBNA was performed using Virtual EBUS Standard (Figures 2). EBUS-TBNA successfully ruled out lymph node metastasis by revealing a normal lymph node (normal lymphocytes without evidence of malignancy). One month later, he underwent thoracoscopic partial resection of the left upper lobe for a metastatic lung tumor of rectal origin.

Case 2
A 65-year-old male patient had mediastinal lymph node adenopathy at #4L found during a medical checkup CT scan (Figure 4a), which was also positive on PET. Virtual EBUS Standard was used for preparation (Figure 4b). The TBNA procedure successfully revealed small cell lung cancer on-site (Figure 4c-d). The permanent result, however, suggested B cell lymphoma rather than small cell lung cancer, the distinction of which is practically impossible in on-site cytology. The diagnosis of non-Hodgkin B-cell lymphoma was eventually confirmed by mediastinoscopy, and the patient underwent appropriate treatment.

Virtual EBUS Advanced

After introduction of Virtual EBUS Advanced in May 2012, Virtual EBUS Advanced was used to prepare 25 lesions of 13 patients. Virtual EBUS Advanced required 5 to 15 minutes per case for preparation depending on the number of targets and associated vasculature to be depicted.
Preparation was done by one of the bronchoscopists involved in the procedure. The actual EBUS-TBNA procedure was attempted for 22 lesions of 13 patients; 3 lesions were not punctured during the procedure because of the onsite cytology results. All TBNAs successfully hit the target. Sufficient material was obtained from 18 of 22 (81.8%) lesions. In the unsuccessful sampling, 3 out of 4 lesions were 3 lymph node stations in a single patient, in which only airway epithelium was obtained despite repeated sampling; the other lesion, which was eventually demonstrated to be squamous cell carcinoma, showed only connective tissue by EBUS despite repeated puncture for 6 times. Virtual EBUS Advanced allowed for intuitive recognition of the target localisation and the angle to puncture without meticulous simulation steps required for Virtual EBUS Standard. Representative cases of Virtual EBUS Advanced are shown in Cases 3 and 4.

Case 3 (Virtual EBUS Advanced)

A 75-year-old male patient was found to have a 2-cm-diameter lung nodule in the left lower lobe. A CT scan demonstrated lymph node adenopathy at #4R and #10R (Figure 5a). PET was also positive in these nodes (Figure 5b). Following preparation using Virtual EBUS Advanced (Figure 5c-d), EBUS-TBNA was conducted. A diagnosis of squamous cell carcinoma was made from #10R, confirming N3 disease. The patient underwent definitive chemo-radiotherapy.

Case 4 (Virtual EBUS Advanced)

An 81-year-old male patient was found to have a hilar mass lesion in the right lower lobe of the lung, which was also positive on PET (Figure 6a). Because his pulmonary function was poor, radiation therapy was considered under the condition that a histopathological diagnosis of lung
cancer was made. Regular bronchoscopy was unlikely to reach the lesion. Virtual EBUS Advanced was used to prepare for EBUS-TBNA, visualising the relationship between the tumor and right inferior pulmonary vein (Figure 6b). EBUS-TBNA was successfully conducted, avoiding the pulmonary vein (Figure 6c-d), and a diagnosis of squamous cell carcinoma was made. He underwent definitive radiotherapy.
**Discussion**

In the present report, we described simple techniques using 3D virtual bronchoscopy to pre-
determine puncture spots of EBUS-TBNA. Although an objective evaluation study is yet to be
performed, we conducted 24 satisfactory EBUS-TBNA procedures using these techniques, called
‘Virtual EBUS’. Virtual EBUS could complement the conventional technique of EBUS-TBNA,
especially when the target lesion is located at a site other than the regular mediastinal lymph
node stations (e.g., a hilar intrapulmonary mass).

In the literature, there have been discussions about using virtual bronchoscopy for TBNA.\(^9\)\(^{-}\)\(^{10}\)
However, after the emergence of EBUS, imaging technology for TBNA may have been
abandoned because of the excellent visualisation of targets by EBUS. Indeed, EBUS-TBNA is
already a supreme apparatus in terms of its ability to sample conventional mediastinal lymph
nodes. The outcome of one clinical trial for mediastinal staging of lung cancer was comparable
with that of mediastinoscopy.\(^11\) Nevertheless, ultrasonographic identification of target lesions is
sometimes a time-consuming and challenging process in EBUS-TBNA. Virtual EBUS tells a
bronchoscopist exactly where to puncture without using ultrasound. Although the idea is similar
to virtual-bronchoscopy-guided TBNA,\(^9\)\(^{-}\)\(^{10}\) what is unique to Virtual EBUS is ultrasonographic
confirmation of virtual images and real-time monitoring of TBNA, which makes the whole
procedure safe and reliable.

We found Virtual EBUS useful especially when the potential target was located outside of the
typical mediastinal lymph nodes (e.g., Figures 2, 3 and 6). In such cases, Virtual EBUS played
its first role in evaluation of the feasibility of the procedure. Both the meticulous simulation steps
of Virtual EBUS Standard and a more intuitively recognisable depiction of the target and associated vasculature in Virtual EBUS Advanced enabled accurate prediction of the feasibility of EBUS-TBNA before taking patients to the bronchoscopy suite. At the time of actual EBUS-TBNA, Virtual EBUS enabled performance of an easy and time-saving procedure by pre-determining the spot and angle for TBNA. Because the reference of Virtual EBUS was available on a computer screen in the bronchoscopy suite, the bronchoscopist did not have to search for the target using ultrasound; because the location at which the needle penetrates the tracheobronchial wall was pre-determined, ultrasound was used only for confirmation of the target location and subsequent real-time visualisation of TBNA. We felt it important to describe the puncture spot relative to an anatomical reference; for example, one cartilage ring caudal from the second carina of the right bronchus.

Preparation using Virtual EBUS may not only facilitate identification of the target lesion but also enhance the performance of TBNA procedure at a challenging spot, especially when the target was located at a high-upper angle for the scope, typically at station #4L and sometimes at #10R and #2L/R (e.g., Cases 2 and 3). In general, after insertion of the needle in the EBUS scope, it becomes less flexible. This property of EBUS often makes ultrasound visualisation of a target difficult. Hyper-inflation of the balloon may still allow for visualisation of the target, but the hyper-inflated balloon itself may be in the way of the needle. Because the spot to be punctured was pre-determined by Virtual EBUS and the location was confirmed by ultrasound before insertion of the needle in the EBUS scope, we confidently stuck the tip of the needle into the tracheobronchial wall. At that time, the needle was not pushed beyond the wall, but was only ‘fixed’ at the mucosal surface. The balloon was then fully inflated to confirm the localisation of
the target. Usually, such a target at a high-upper angle is also difficult to puncture because the angle of the needle against the bronchial wall is so small or almost tangent that sufficient force for puncture is hardly conductible to the needle. However, once the tip of the needle is already fixed on the tracheobronchial wall, by slightly advancing the EBUS scope, an appropriate larger angle can be obtained to conduct the force to the needle and successfully penetrate the wall into the target. This technique is illustrated in Figure 7.

Although we feel that the two Virtual EBUS methods are both highly useful, they have different characteristics. Virtual EBUS Advanced allows for a more intuitive recognition of the target localisation and its relationship with important vascular structures. This may also be an excellent educational tool with which to understand mediastinal and hilar anatomy for inexperienced bronchoscopists. Virtual EBUS Advanced requires advanced application such as iNtuition Viewer®, while Virtual EBUS Standard requires only basic software such as Thin Client Viewer®, although it requires more meticulous simulation steps. A technique equivalent to Virtual EBUS Standard would be more broadly available in many institutions without any additional cost or computer application if the imaging platform can construct 3D virtual bronchoscopy from thin-slice CT. In clinical practice, we feel that Virtual EBUS Standard is sufficiently helpful to guide EBUS-TBNA. If introduction of Virtual EBUS is considered, the benefits and costs of these two methods should be taken into account.

We also found multiple limitations and challenges in Virtual EBUS. Firstly, we found enhanced CT is almost mandatory for Virtual EBUS. Enhanced CT was not initially a routine; however, target lesions, especially small ones were hardly distinguishable from adjacent blood vessels in
plane CT. Secondly, even if TBNA appears to be feasible in Virtual EBUS, successful puncture is not guaranteed. Because the movement of an EBUS scope is limited by multiple factors (e.g., insertion of the needle as mentioned above), the ideal puncture angle simulated by Virtual EBUS might not be realized. Similarly, a lesion located in the distal airway could be unreachable due to the diameter of EBUS scope even if it appears to be easy to puncture in Virtual EBUS. Thirdly, there were target lesions from which sufficient diagnostic materials were not obtained, although the targets were successfully localised and punctured. Unsuccessful TBNA in these cases is considered to represent limitations of the EBUS-TBNA procedure per se rather than of Virtual EBUS. Such limitations include a technical issue of TBNA that cannot be overcome by Virtual EBUS (e.g. obtaining only blood or airway epithelium) and the nature of the target lesion (e.g., cancer that is mainly composed of nonspecific fibrous tissue). Some of the technical limitations of EBUS-TBNA might be further overcome by emerging new methodologies for EBUS-TBNA.\(^{12}\)

Selection of the preparation method in this study was not randomized and was dependent on several factors, including bronchoscopists’ preference, expansion of knowledge of Virtual EBUS within the institution, and late introduction of Virtual EBUS Advanced. In the same study period, 27 lesions of 19 patients were sampled with EBUS-TBNA without Virtual EBUS. Sufficient material was obtained with a similar ratio (23/27 lesions; 85.2%). However, as discussed above, sufficiency of sampling greatly depends on technical issues of TBNA and probably this parameter is not ideal to compare Virtual EBUS with the conventional method. To evaluate the efficacy of Virtual EBUS, other parameters such as time spent to identify a target lesion, learning curve, and bronchoscopists’ stress levels need to be examined in the future.
In conclusion, we developed two methods of Virtual EBUS, which allows bronchoscopists to pre-determine the puncture spot of TBNA as a potential complement to the conventional EBUS-TBNA technique. In our initial experience, these two methods are felt to be equally useful with different advantages. Further prospective investigation is necessary to objectively evaluate the benefit of Virtual EBUS in the future.

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Figure Legends

Figure 1. Step-by-step instructions and characteristics of ‘Virtual EBUS’ using virtual bronchoscopy. (Left) ‘Virtual EBUS Standard’ utilises conventional 3D virtual bronchoscopy constructed from thin-slice CT. (Right) ‘Virtual EBUS Advanced’ utilises an advanced application that allows for displaying multi-layers, including extracted 3D images of target lesions, associated vascular structures, and 3D virtual bronchoscopy.

Figure 2. Preparation using ‘Virtual EBUS Standard’. (a, b) A patient with a past medical history of colon cancer shows a slightly enlarged left #11 lymph node station (arrow in b) on an enhanced CT scan. (c) The #11 station is weakly positive on positron-emission tomography (arrow). (d) A series of 3D virtual bronchoscopy images (top panels) and linked CT images (bottom panels). The pyramid-shaped indicator in the CT image shows the direction of the view in virtual bronchoscopy. Note that in the far right images, virtual bronchoscopy penetrated the bronchial wall, simulating the TBNA procedure. This simulation step is the key in Virtual EBUS Standard to determine the spot and angle to be punctured in EBUS-TBNA. Lin, lingular segment; Bas, basal segment; Sup, superior segment of lower lobe.

Figure 3. Preparation using ‘Virtual EBUS Advanced’. The same case as in Figure 2 was used as an example of Virtual EBUS Advanced. (a-c) Outlining and extraction of target lymph node (a), associated left pulmonary artery (b), and left inferior pulmonary vein (c). The insets at the right lower corner indicate the 3D view of extracted images. (d) Merged views of multiple layers with different bronchial wall transparencies. The layers include the target lymph node, pulmonary artery (PA), and pulmonary vein (PV) in combination with 3D virtual bronchoscopy.
Figure 4. A case of EBUS-TBNA planned for using ‘Virtual EBUS Standard’ (Case 2). (a) A CT scan shows an enlarged #4L lymph node station, which is close to the pre-tracheal area. (b) A view of virtual bronchoscopy prepared for by Virtual EBUS Standard. An arrow indicates the spot to be punctured. (c) An actual bronchoscopic view. The arrow indicates the spot that was actually punctured by TBNA. The verbal description was ‘one ring cephalad to the carina, 10- to 11-o’clock angle from the carina’. (d) Ultrasound view of EBUS-TBNA. The needle is directly in the lymph node. Note that the location was hardly visible under ultrasound after inserting the needle into the EBUS scope. The puncture technique used in such a case is described in the text.

Figure 5. A case of EBUS-TBNA prepared for by ‘Virtual EBUS Advanced’ (Case 3). (a) A patient with an abnormal nodule in the left lung shows enlarged #4R and #10R lymph node stations. (b) These lymph node stations were positive on PET/CT. (c) View of ‘Virtual EBUS Advanced’ at the #4R station (top) with bronchial wall transparency (bottom). (d) View of #10R station (top) with bronchial wall transparency (bottom). SVC, superior vena cava; AZ, azygos vein; BIM, bronchus intermedius; RUL, right upper lobe; PA, pulmonary artery.

Figure 6. A case of EBUS-TBNA planned for using ‘Virtual EBUS Advanced’ (Case 4). (a) An intrapulmonary mass located close to the hilum was found on a CT scan. The mass was positive on PET/CT (inset). (b) View of Virtual EBUS Advanced, showing the relationship between the mass (T), right inferior pulmonary vein (PV), and bronchi. ML, middle lobe; S, superior segment of lower lobe. (c) View of actual bronchoscope. The arrow indicates the spot that was actually punctured by TBNA under ultrasound. (d) Ultrasound view of EBUS-TBNA. The needle is directly in the target lesion.
Figure 7. Illustration of technique to puncture a target at a high-upper angle using Virtual EBUS. (a) Puncture of a target at a regular angle. EBUS-TBNA is easily conducted under ultrasound visualisation with regular inflation of the EBUS balloon. The TBNA needle and the target are illustrated as an arrow and a star mark, respectively. (b) A target located at a high-upper angle. With regular inflation of the EBUS balloon, the target is hardly visible because of reduced flexibility of the EBUS scope after insertion of the needle in the scope. (c) Hyper-inflation of the EBUS balloon allows for visualisation of the target at a high-upper angle. However, real-time ultrasound visualisation during TBNA is hindered by the inflated balloon, which is in the needle pathway. The puncture angle is also too small or almost tangent to the tracheobronchial wall. (d) Technique to effectively puncture a target at a high-upper angle using Virtual EBUS. First, the tip of the needle is stuck at the spot pre-determined by Virtual EBUS (black arrow). Second, the EBUS balloon is then hyper-inflated. Because the needle has already reached the tracheobronchial wall, the balloon no longer interferes with the needle. The angle of the needle against the tracheobronchial wall is still small or almost tangent. Third, Once the target is visualised, the EBUS scope is progressed slightly (white arrow). A larger angle is now obtained for the bronchoscopist to conduct force to the needle to penetrate the tracheobronchial wall while ultrasound visualisation of the target is maintained. Last, the target is punctured under real-time ultrasound visualization.
Figure 1

### Virtual EBUS Standard

- Construct 3D virtual bronchoscopy images from thin-slice CT
- Localise the target lesion on virtual bronchoscopy while watching linked CT
- Determine the angle for puncture by watching both virtual bronchoscopy image and linked CT while operating virtual bronchoscopy
- Go back to starting point of bronchoscopy (e.g. trachea) and then simulate TBNA only by watching virtual bronchoscopy images, imagining as if you were the needle
- Once you (=needle) enter the lesion by penetrating a bronchial wall, look at linked CT to confirm that you reached the lesion
- Prepare a key image with a description of the spot and angle; this image should be available while conducting actual EBUS-TBNA

- Widely available
- Simulation (steps 4 to 5) is mandatory

### Virtual EBUS Advanced

- Construct multiple layers by outlining, extracting, and colouring the target lesion(s) and associated blood vessels from thin-slice CT
- Overlay the multiple layers and 3D-virtual bronchoscopy images built from thin-slice CT
- Manipulate transparency of virtual bronchoscopy while operating it
- Prepare a key image; it is recommended to prepare both non-transparent and transparent images side by side. These images should be available while conducting actual EBUS-TBNA

- Advanced application necessary
- Puncturing spot and angle are intuitively recognised
- Associated structures (e.g. blood vessels) are also visible
- Excellent education tool
Figure 3
Figure 6