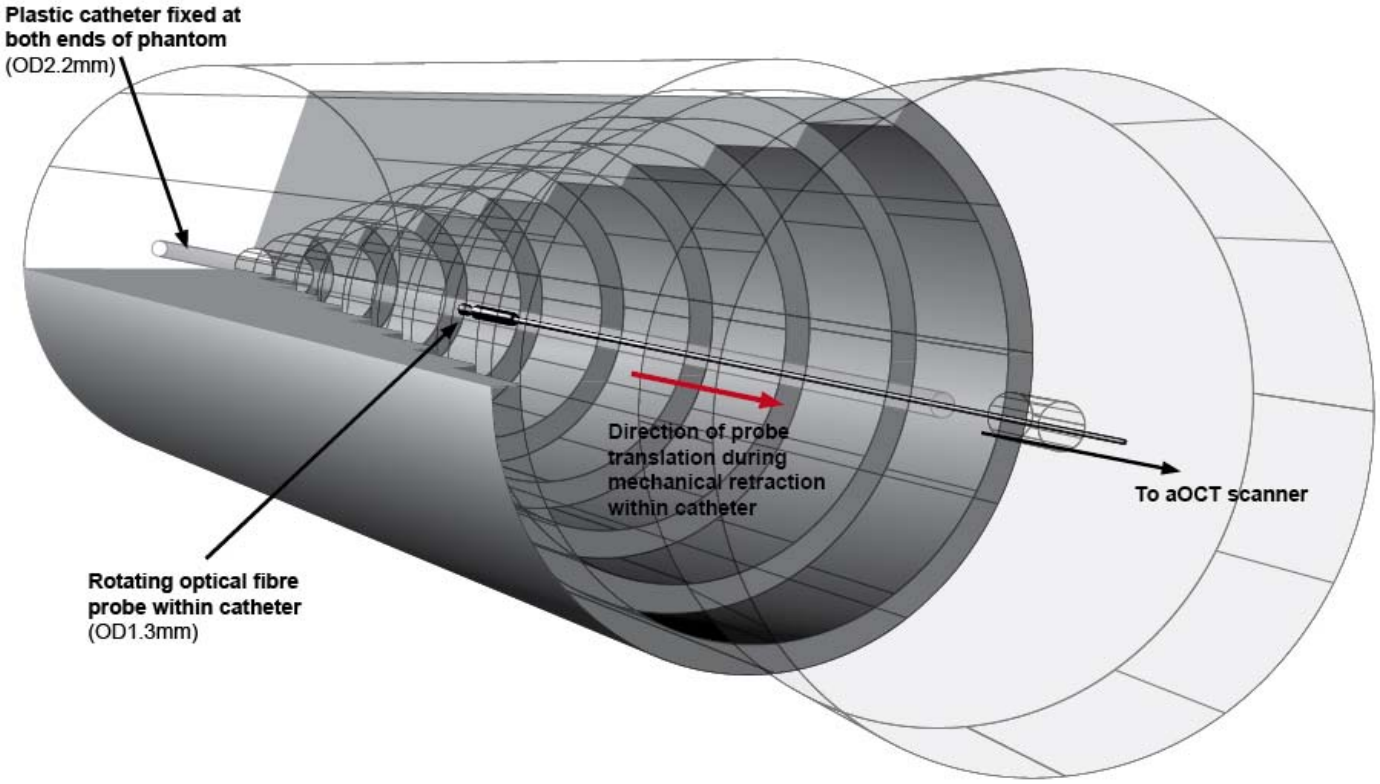
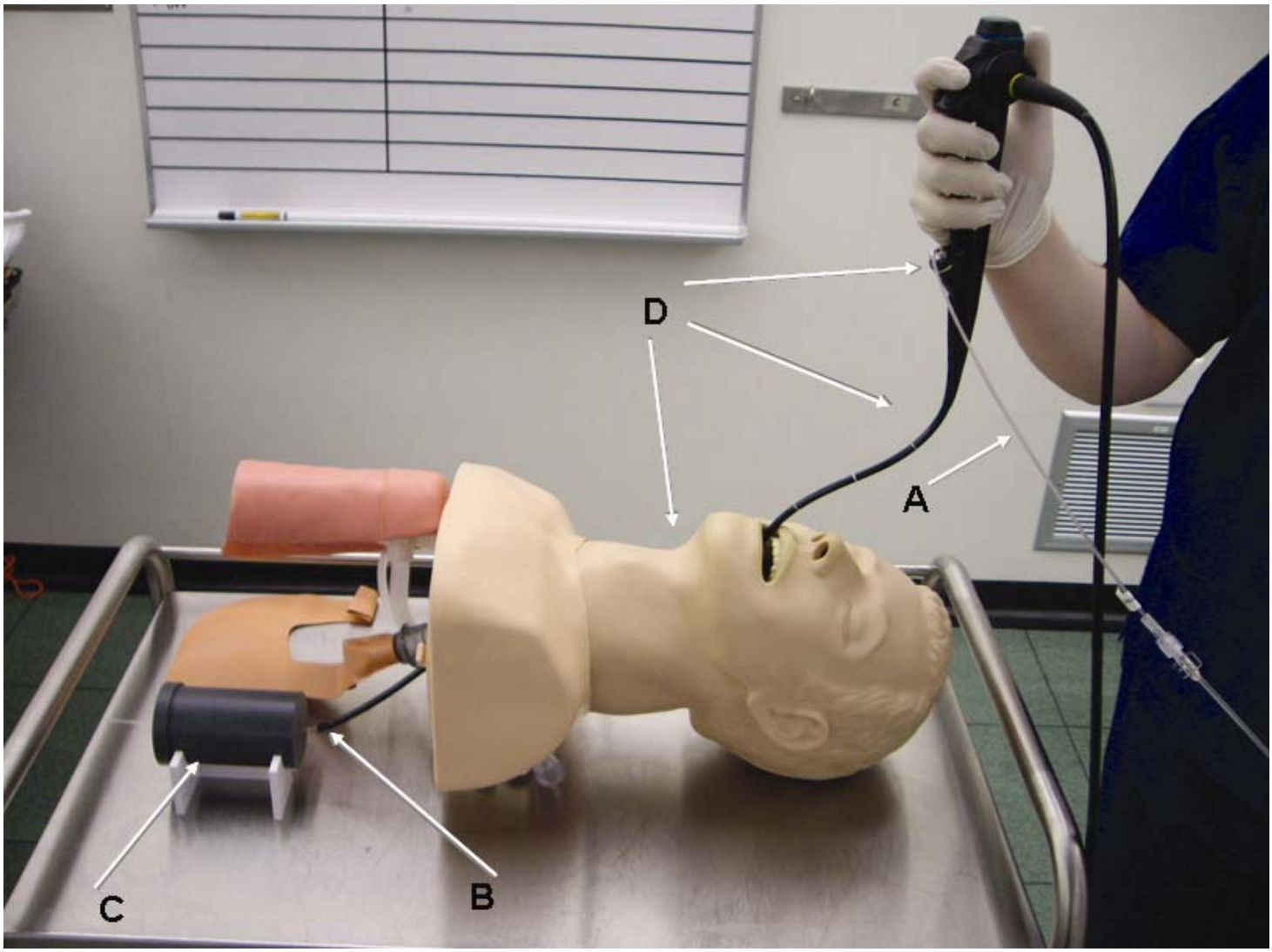
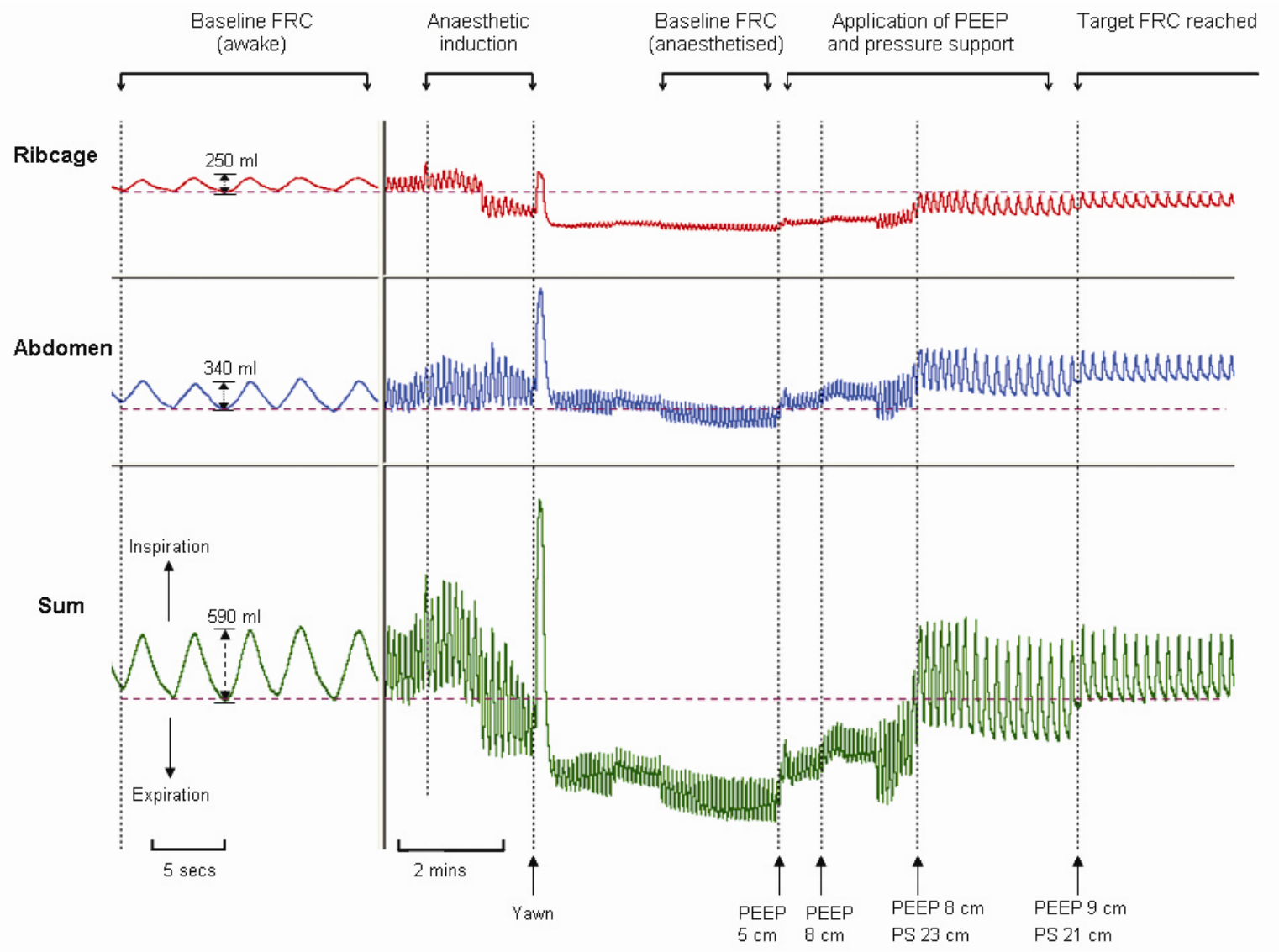
Online supplement



Online E1. Phantom airway consisting of ten sequential tubes constructed by drilling holes of known diameter (2.6-50.0 mm) through a solid plastic block. A catheter containing the fibre-optic probe is passed longitudinally through the centre of the phantom. Upon rotation of the probe, a cross-sectional image of the tube is displayed on a monitor. As the probe is mechanically retracted along the phantom, each of the tubes is imaged in turn. Three sites along each of the 10 tubes were selected for measurement of diameter and cross-sectional area.



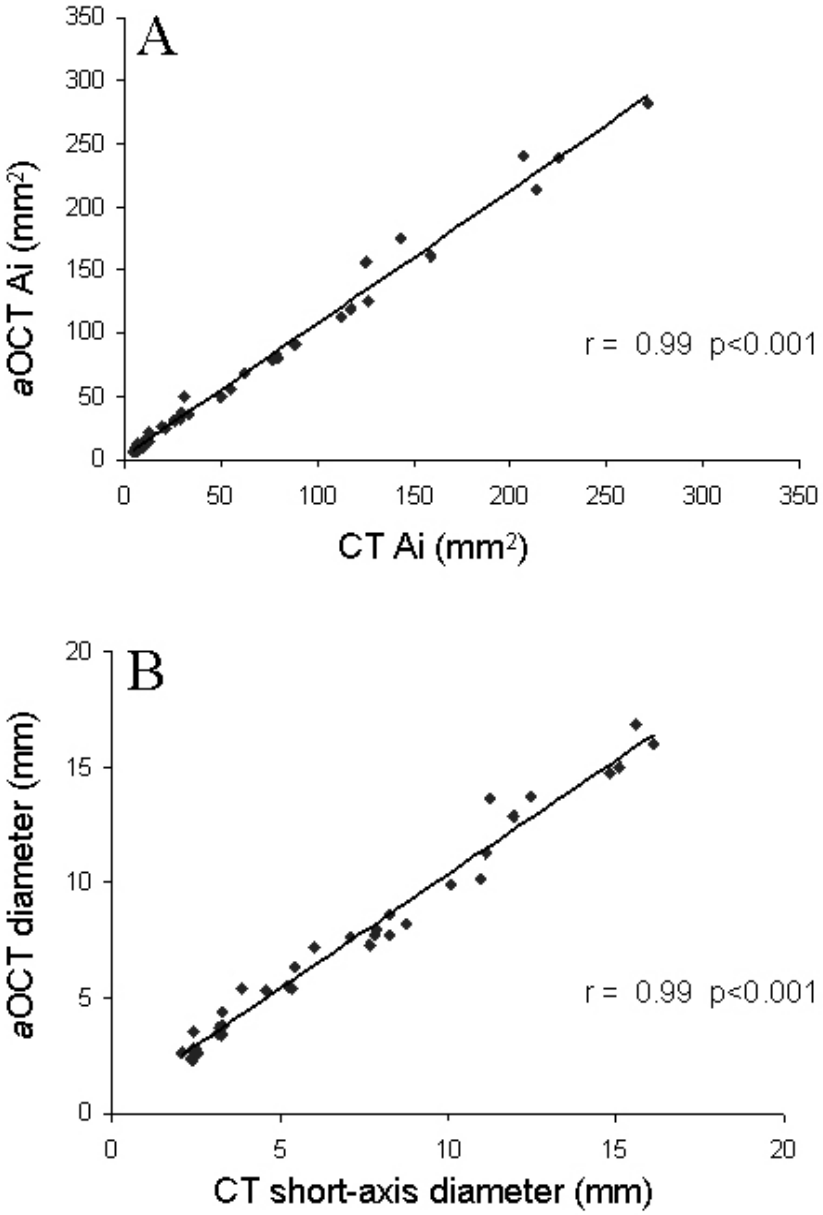
Online E2. *a*OCT probe (A) passed through a bronchoscope and inserted into a resuscitation mannequin. The bronchoscope tip (B) is seen at the bottom of the mannequin, with the probe further advanced through the airway phantom (C; see online E1 for internal phantom characterisation). Sites of maximal probe curvature are indicated (D).



Online E3. Use of impedance plethysmography to equilibrate lung volume between awake and anaesthetised states in a human subject. Baseline *awake* FRC was determined after a period of quiet tidal breathing (left-most panel, indicated by dashed purple lines). Following anaesthetic induction, FRC falls to a new *anaesthetised* baseline. The *anaesthetised* baseline is then increased, returning it back to the *awake* baseline FRC using positive end-expiratory pressure (PEEP) and pressure support (PS).

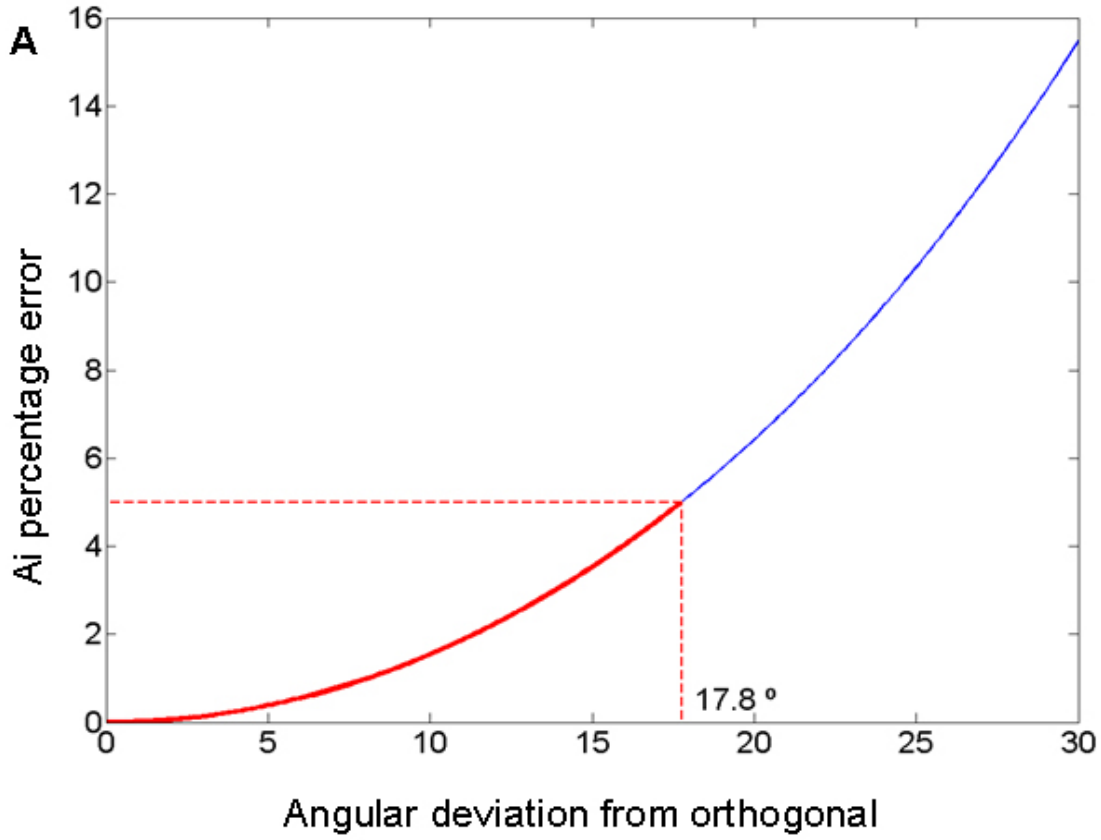
Airway generation	Number of airways
0	6
1	7
2	5
3	3
4	3
5	12
Total	**36**

Online E4. Distribution of analysed airway generations during the computed tomography (CT) - anatomical optical coherence tomography (*a*OCT) comparison.



Online E5. Linear correlation of computed tomography (CT) and anatomical optical coherence tomography (aOCT) measurements of [A] internal lumen area (Ai) and [B] short axis diameter in human airways from the trachea to subsegmental airways.

A



B

Scanning angle º	0	13.7	17.6	20.6
*a*OCT Ai % error	-0.7	2.9	3.6	8.5
Ai theoretical % error	0.0	2.9	4.9	6.8

Online E6. Theoretical internal area (Ai) measurement error resulting when a hollow tube is scanned at oblique angles (A). Experimental measurement errors obtained from scanning a hollow cylinder (length 50 mm x diameter 20 mm) with anatomical optical coherence tomography (*a*OCT) with the probe oriented at increasingly oblique angles from the tube axis (B).

Distance from centre axis (mm)	0	4.2	7.9	12.3
*a*OCT measured Ai (mm^2)	706.5	707.9	697.5	698.7
*a*OCT Ai error (%)	0	0.2	1.3	1.1

Online E7. Effect of probe axial position on internal area (Ai) measurements in a hollow cylinder (length 50 mm x diameter 30 mm; calculated Ai 706.4 mm^2). Axial slices were scanned using anatomical optical coherence tomography (*a*OCT) with the probe parallel to the cylinder but positioned at increasing distances from the centre axis.

Anatomical optical coherence tomography

aOCT probes

Probes are constructed by affixing a 1.3mm diameter gradient index lens to 180cm length of single mode fibre. A 0.7mm right-angle prism is attached to the lens to redirect the light beam perpendicular to the probe. A small protective metal cylinder encases both components. The single mode fibre is sheathed by a biplex torque-transmission stainless steel coil. Each probe was quality assessed by measuring insertion loss and sensitivity of the optical signal, and by analysing the beam intensity distribution profile.

Separate probes were used for the pig experiment and the human/phantom studies. A new catheter (ID 1.6mm, OD 2.2mm) was placed over the *a*OCT probe between each human subject. Prior to measurement, a calibration phantom was scanned to correct for imperfections in probe construction or potential inter-probe variations.

Phantom airway construction

An airway phantom was built from a block of plastic into which ten round holes of increasing size were carefully drilled to produce 10 continuous tubes, with diameters in the range 2.6-50.0 mm (Figure E1). Internal diameters were measured with an internal micrometer (Bowers Metrology, West Yorkshire, UK) accurate to 0.01 mm. The a*OCT probe, surrounded by the plastic catheter, was inserted through the centre of the phantom and, whilst rotating, was retracted to generate cross sections of each of the 10 tubes. These cross sections were displayed on a monitor and recorded for subsequent analysis.*

In-vivo human airways

Patients scheduled for bronchoscopy as part of their medical care, who also required a chest CT scan, were recruited. On the study morning, the subjects underwent a chest CT that incorporated the entire tracheobronchial tree. The CT parameters were as follows; 220 mA, 120 Kvp, collimation 0.625 mm, voxel size 0.625 × 0.625 × 0.625 mm, pitch 0.891, rotation time 0.75 sec, matrix size 512 x 512 and a lung-enhanced reconstruction algorithm was used. That afternoon, a bronchoscopy was performed under propofol anaesthesia with spontaneous respiration. Several airway regions from the trachea (Generation 0) to the subsegmental airways (Generation 5) were selected for *a*OCT imaging and measurements from these airways sites were compared between the two techniques.

When the distal tip of the bronchoscope was positioned just proximal to each region of interest, the *a*OCT catheter was inserted through the biopsy channel and advanced to the region of interest. Rotation of the probe within the catheter generated an airway cross-section that was recorded for approximately 6 respiratory cycles. During subsequent analysis of each airway, lumen area was measured at end-expiration on 3 successive breaths and averaged to provide a single measurement for that site.

To approximate equal lung volumes during CT and bronchoscopy, Respitrace™ impedance plethysmography bands were placed over the ribcage and abdomen and monitored (Ambulatory Monitoring, Ardsley, USA). Arms were elevated and placed under the subject's head. The Respitrace was calibrated with an isovolume manoeuvre.[1] After one minute of stable tidal breathing, the patient was asked to suspend breathing at functional residual capacity (FRC), as indicated by the Respitrace sum signal, and scanning commenced. During the bronchoscopy, the subject was placed in the same position as the CT scan and an "awake" FRC was determined by observing the baseline Respitrace sum signal during quiet tidal breathing. This baseline was marked on a computer monitor and great care was taken not to alter the patient's position. Induction of anaesthesia was commenced using intravenous propofol. Upon return of spontaneous

respiration, the “anaesthetised” FRC was observed. Where, as was expected, the FRC had fallen below baseline, positive end-expiratory pressure (PEEP) and pressure support (PS) were applied via the anaesthetic circuit until the Respitrace sum signal had returned to baseline (Figure E3). This allowed us to carefully control lung volume and to estimate FRC during CT scanning and approximate this lung volume during bronchoscopy. Nonetheless, despite the overall equivalence of FRC, the distribution of air within the chest and abdomen was different between the two conditions, probably a result of relaxation of chest wall and abdominal musculature during anaesthesia (online E3).

1. Konno K, Mead J. Measurement of the separate volume changes of rib cage and abdomen during breathing. *J Appl Physiol* 1967;**22**:407-&.