Budding Researchers Research Report

Proposal 2009A1653, BL20XU (Biomedical Imaging Centre hutch):
Optimisation of synchrotron phase contrast x-ray imaging to reveal airway surface liquid depth in the airways of mice.

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Introduction:

This study aims to utilize synchrotron based phase contrast x-ray imaging (PCXI) in a novel application, the non-invasive assessment of airway health by direct observation of the airway surface liquid (ASL), the liquid lining the airways. This beamtime was a major step towards achieving this goal, providing the opportunity to optimise the imaging conditions and capture a definitive dataset of phantom, ex-vivo and in-vivo images upon which to optimise image analysis. Investigation is continuing into the information which may be extracted from these images, and the best way to retrieve that information, before the results are submitted as a journal paper.

Motivation and background:

The project in which this beamtime sits aims to assess the effectiveness of new treatments for the airway disease associated with Cystic Fibrosis, using phase contrast x-ray imaging.

With airway conditions such as Cystic Fibrosis (CF), in order to prevent lung infection worsening, mucus must transit out of the lungs, requiring sufficient ASL for the cilia on the airway walls to move particles up the airway. An effective treatment for CF, a condition where a decreased ASL depth is observed, should therefore result in an increase in the ASL depth. Work done by Dr David Parsons and collaborators from the Women's and Children's Hospital in Adelaide aims to use an airway gene transfer method to establish a healthy ASL. Current assessments of treatments are indirect (for example, CT of the lungs is not informative until months after treatment), and only examine whether lung infection has progressed.

We ultimately seek to directly measure the change (expected to be around 20%) in airway surface liquid depth in response to this new treatment. This beamtime in particular aimed to optimize the imaging conditions and establish how these measurements should best be made through the imaging of ideal and realistic phantoms as well as the airways of mice.

This application followed on from beamtime led by Dr David Parsons, 2007A1287, 2007B1329, 2008A1334 and 2008B1985, where live imaging of mouse airways was conducted. While the more recent of these studies has looked at the clearance of particles out along the airways, which is an alternate method of measuring ASL sufficiency, this beamtime sought to determine if the imaging done in 2007A1287 could be analysed or repeated to give quantitative measures of ASL depth changes in response to established short-term treatments such as hypertonic saline.

Computer modelling of the 1mm-diameter mouse airway space has shown that the significant change in refractive index in moving from air to ASL should produce a strong propagation-based phase contrast fringe set, and the step from ASL to tissue a weaker fringe set, as seen in Fig. 1(a) (taken from beamtime proposal). Phantom imaging previously undertaken at BL20XU is consistent with this, as seen in Fig. 1(b). This means the ASL depth should be simply given by the distance between the airway/ASL fringe set and the ASL/tissue fringe set.

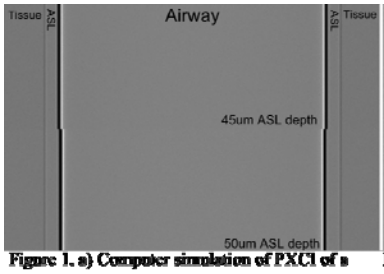


Figure 1. a) Computer simulation of PXCI of a 1mm wide airway with 45/50um ASL, showing both the air/ASL and the ASL/tissue interfaces.

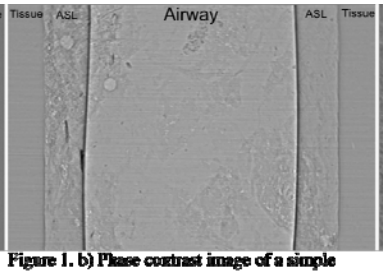


Figure 1. b) Phase contrast image of a simple perspex phantom of a 1mm airway, showing both interfaces as in simulation.

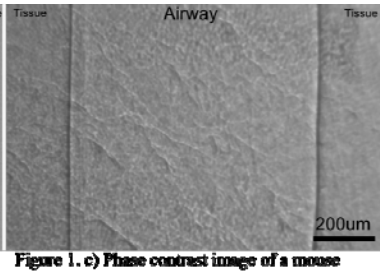


Figure 1. c) Phase contrast image of a mouse trachea, with only the air/ASL interface clearly visible to the naked eye.

However, the complicated overlying structures around the live mouse airway, as seen in Fig. 1(c), mean that the ASL/tissue fringe is not clear. While the ASL/tissue fringe set cannot be revealed with the same clarity as in phantom imaging, optimization of the imaging conditions enhances the visibility of fringes and the unique Fresnel intensity fringe for specified airway radius and propagation distance. This means it is more easily identified amongst overlying intensity variations.

This beamtime follows on from our previous studies looking at ways in which the beam coherence can be manipulated using existing beamline infrastructure to improve image quality. In 2007B (with Yoshio Suzuki) and 2008A1334, work undertaken with Sally Irvine (Monash University) measured the coherence length for a range of slit sizes and looked at the effect of a diffuser on coherence. This showed that while the transverse coherence length was unaffected by the presence of a diffuser, the absolute value of the degree of the second order coherence was reduced, resulting in decreased visibility interference fringes. In conjunction with the experiment, we developed a comprehensive simulation model of the source characteristics at the sample position. This work is now published as "Measurement of hard X-ray coherence in the presence of a rotating random-phase-screen diffuser" in *Optics Communications*, 283: 216-225, 2010.

We further validated this in the 2008B1985 beamtime by looking at the effect of a diffuser on the number and intensity of phase contrast fringes visible for a simple phantom. The removal of the diffuser showed a significant increase in the number of observable fringes at each boundary, as well as an increase in the visibility of the fringe set. The time-sum of many diffuser positions, each 'warping' the image, results in a slightly 'blurred' image. This body of work has been submitted for publication and is currently under review for *Optics Express*.

Consequently, this beamtime sought to look at the effect of moving the diffuser closer to the synchrotron source, to see if the extra propagation would be sufficient to give adequate coherence while allowing the diffuser to create a larger, more even field of view. Therefore, through selection of both the diffuser position and source aperture size, this beamtime built on the successful foundations of previous beamtimes by investigating the enhancement of the ASL/tissue fringe within the surrounding speckle, and how information on the ASL depth may be best recovered. Sufficient mouse airways were also imaged with the optimised setup to enable statistical measures of airway surface depths on the analysed images.

Experimental Aims:

This beamtime collected a definitive set of images using both perspex phantom airways and mouse airways, for a range of slit sizes, propagation lengths and with/without the diffuser, at maximum magnification, using 25keV x-rays. Ultimately, this will determine the range and resolution of ASL depth detectable theoretically and practically, given restrictions introduced with live animal imaging.

Aims:

1. To determine the optimum diffuser position and aperture size required to reveal a detailed fringe, while maintaining reasonable exposure times which could be used in live animal imaging (a long exposure increases the change of animal

movement during image acquisition).

2. To confirm the optimum propagation distance at which the air/ASL fringe set and ASL/tissue fringe set will be maximized in visibility without destructively interfering or overlapping.

3. To obtain images demonstrating the fringe-dampening effect of moving from a smooth cylinder to a messy, rough cylindrical hole to an excised and intact trachea. These images are then amenable to development of analysis techniques and study into how significant the overlying animal is in reducing the interface visibility.

4. To provide a set of images of fixed known phantom ASL depth for verification of depth measures.

5. To collect a set of phantom, ex-vivo and in-vivo data with a range of fringe visibility/detail for the same imaging site, to look at the importance of a detailed fringe set to phase retrieval or other image analysis techniques.

6. To investigate increasing effective cylinder radius by imaging on an angle to the cylinder (see Fig.2) to enhance the visibility of fringes, hence the ability to visualise the ASL.

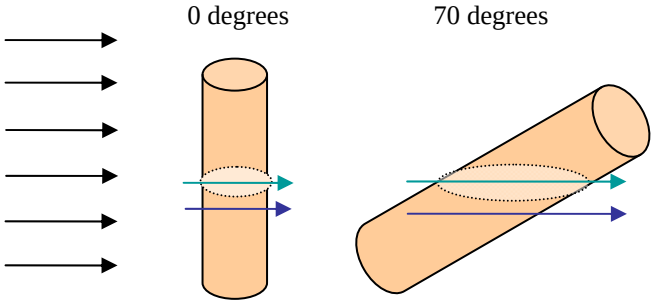


Figure 2. Imaging an airway on an angle will increase the effective radius of the edge.

7. To collect a large set of optimized airway sample images, ex-vivo and in-vivo on which to apply image analysis to produce statistical measures of ASL depth.

Results to date:

This beamtime achieved the aims set out above, demonstrating the advantages that may be obtained through choices within the imaging set-up and producing a comprehensive set of images upon which analysis techniques may be developed. Work is now continuing to look at how to best extract detailed airway health information from this set of data. Various methods of phase retrieval, Gabor holography and correlation algorithms are being applied to the images collected.

Aim-specific results:

1. The optics hutch beam slits were reduced in size in 50μm increments from 600μm to 250μm, showing increased fringe visibility and detail, but also reduced flux, requiring significantly longer exposures. As seen in Fig 3., this increased the visibility and detail of fringes observed. This will make an interface more easily extracted from a complex animal image with overlying intensity variations and may also be useful in phase retrieval techniques.

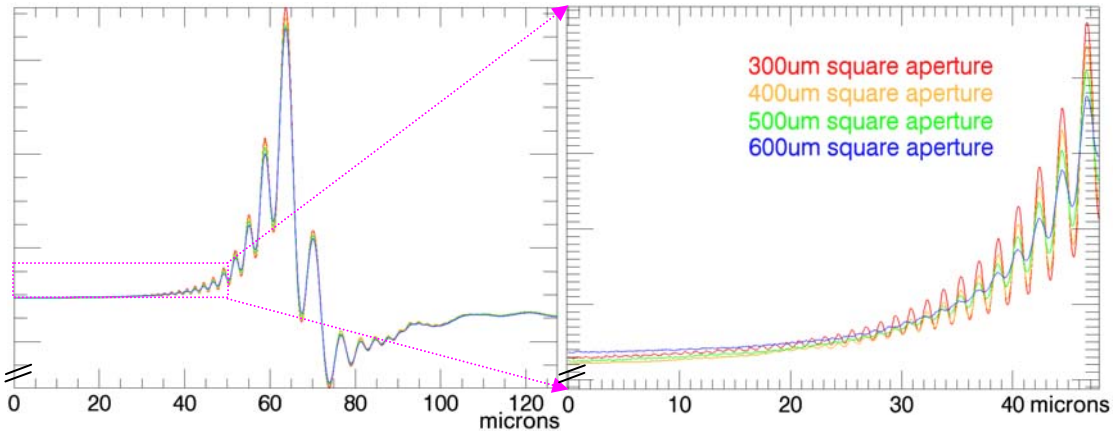


Figure 3. Phase contrast fringe profile from the edge of a 1mm diameter Perspex cylinder edge, with no diffuser present, showing increased visibility and more fringes within the fringe set for smaller effective source size.

This gave an optimum of 300µm square aperture, with 250µm requiring significant exposure time to give adequate intensity counts.
Imaging of the same edge for a range of aperture sizes was done with each of (i) no diffuser present, (ii) a diffuser in the optics hutch, (iii) a diffuser in the upstream experimental hutch and (iv) a double-layer diffuser in the upstream experimental hutch. As seen in Fig. 4, moving the diffuser into the optics hutch was almost equivalent to simply removing the diffuser in terms of fringe visibility.

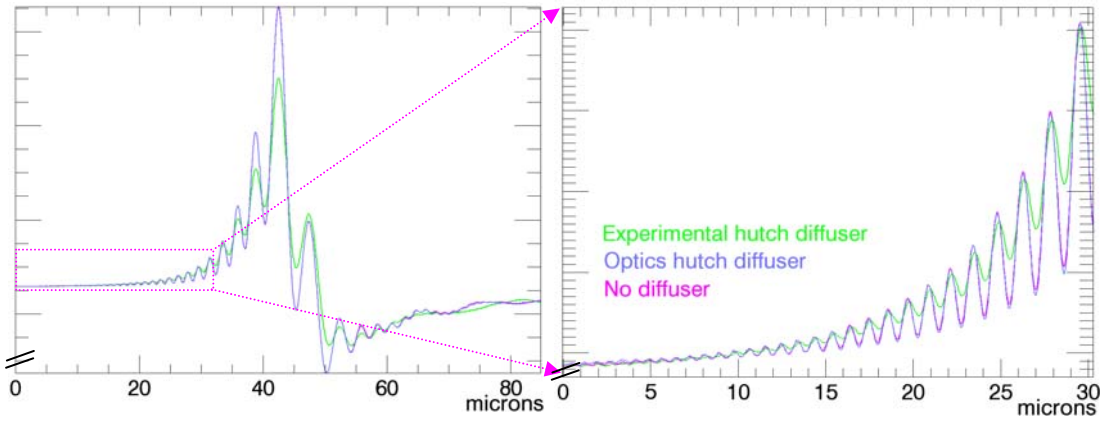
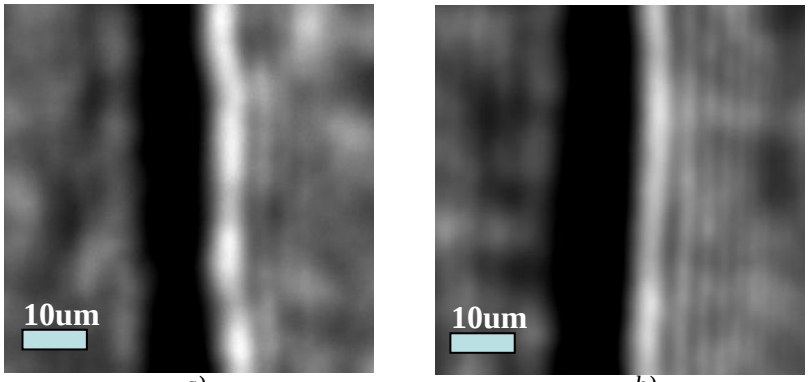


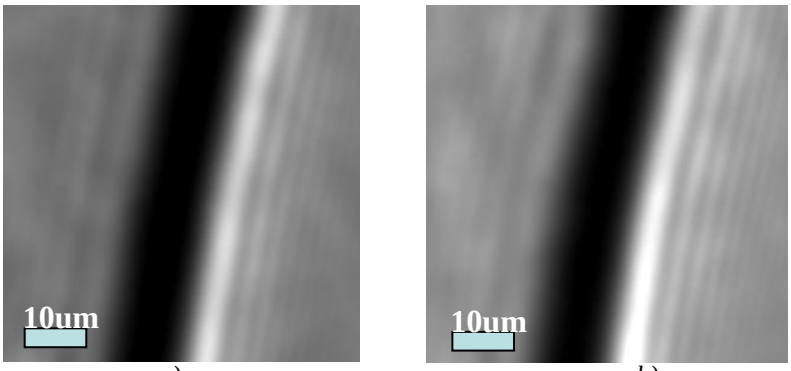
Figure 4. Phase contrast fringe profile from the edge of a 1mm diameter Perspex cylinder edge, 300µm square aperture, with various diffuser configurations, showing increased visibility and more fringes within the fringe set for the diffuser closer to the source.

Images of an excised and in situ trachea were also taken for a range of aperture sizes and diffuser positions. As seen in Fig. 5 and Fig. 6, the increased visibility and detail observed in phantoms was also observed in mouse airways.



a) *b)*

Figure 5. An in situ trachea shows increased contrast and fringe detail when reducing the aperture size from *a)* 600μm to *b)* 300μm.



a) *b)*

Figure 6. An excised trachea shows increased contrast and fringe detail when reducing moving the diffuser from *a)* the upstream experimental hutch to *b)* the further upstream optics hutch.

2. Propagation distance was varied with Perspex phantom airways of 40μm and 12μm "ASL" depth to simulate the tracheal and nasal imaging sites. Images were consistent with computer simulations of propagation-based phase contrast imaging, suggesting optimum propagation of 1m for tracheal ASL imaging and 0.3m for nasal airway ASL imaging.

Imaging of both in situ and excised trachea for a range of propagation distances was also performed, showing the expected increase in visibility and fringe width with propagation, as in Fig. 7.

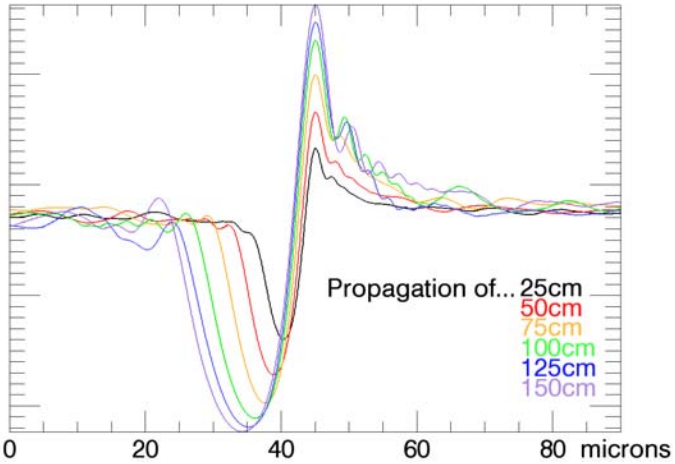
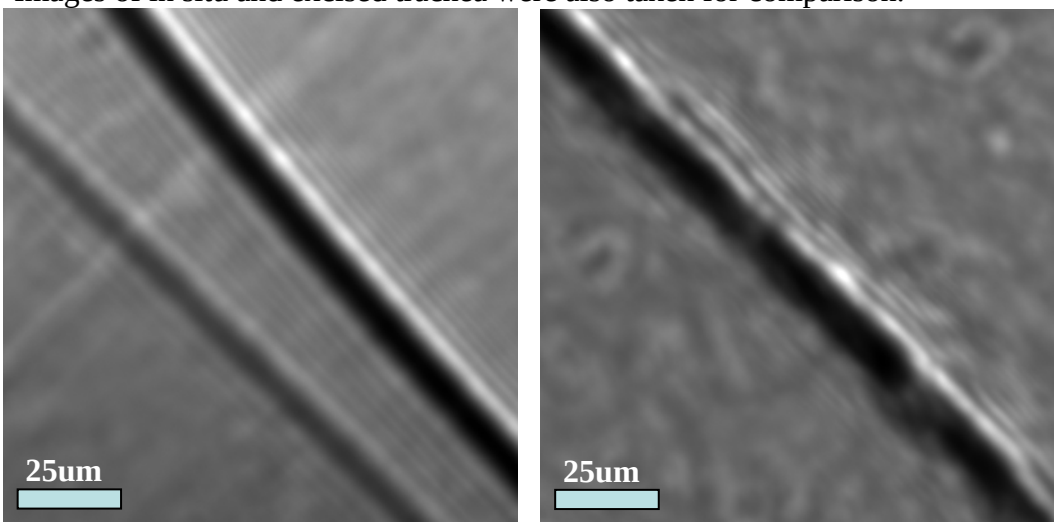


Figure 7. Fringe visibility profiles for an in situ trachea for a range of propagation distances shows increased fringe visibility, width and detail for longer propagations.

3. Perspex hole phantoms with and without a simulated ASL were imaged with 4 degrees of overlying texture (rough Perspex to simulate tissue) to simulate the effect of overlying mouse texture in in-vivo imaging. Two examples are seen below in Fig. 8. Images of in situ and excised trachea were also taken for comparison.

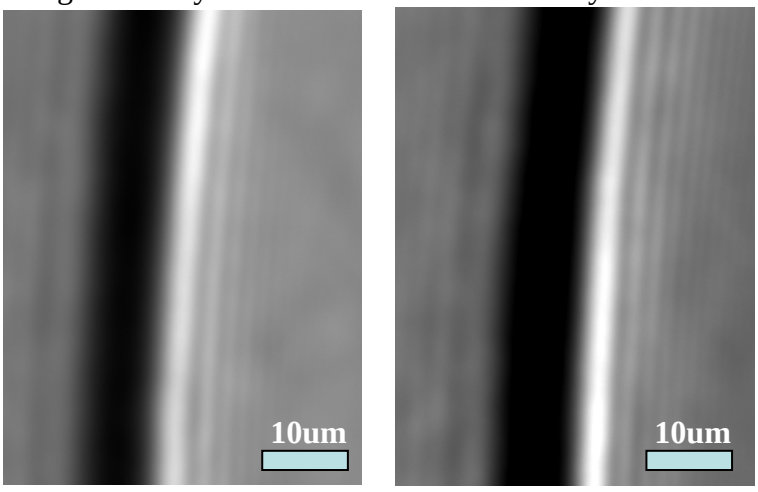


a) *b)*

Figure 8. *a)* A phantom showing clear "tissue/ASL" and "ASL/air" interfaces *b)* A messy, rough phantom, modelling the effect of non perfect interfaces and overlying attenuation.

4. A set of known phantom ASL depths were imaged with the optimum set-up including 1mm cylinders with 5μm, 10μm, 12.7μm, 40μm, 100μm and 120μm "ASL" coatings, at appropriate propagation distances, ready to verify methods of determining depth from an image.

5. In order to look at the importance of a detailed fringe set to effective analysis and phase retrieval, a range of set-ups were used to produce a set of images of the same imaging site with varying coherence, hence varying number of visible fringes per fringe set. While the data collected for Aim 1 will be very useful in this regard, the set was expanded to include two more excised trachea, as well as a Perspex phantom with "ASL". Fig. 9 shows an example of two different set-ups to minimise and maximise fringe visibility for the same section of airway.



a) *b)*

Figure 9. Excised trachea edge for *a)* 600μm aperture and a double layer diffuser in the upstream experimental hutch compared to *b)* 300μm aperture and no diffuser present.

6. Each of phantoms, in situ and excised trachea were imaged at a range of angles to increase fringe visibility (see Fig. 2). As seen in the profiles of Fig. 10, the number of visible fringes and intensity of those fringes increased with the cylindrical phantom hole on an angle.

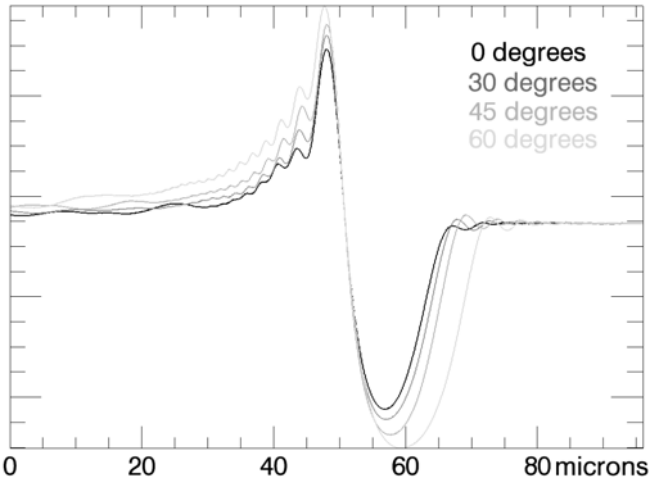


Figure 10. Fringe intensity profile for the edge of a cylindrical hole phantom, placed on an angle to the imaging plane to enhance the phase difference between rays within and outside the hole.

This idea was effective in mouse trachea also, with angles up to 40 degrees possible, before overlying cartilage rings and irregular airway shape did not leave any "clean" edges. That is, there was no area left between projected cartilage rings in which to clearly visualise the edge pattern, as can be done in the central region of the image in Fig. 11.

7. Optimised imaging conditions were then used to image a further three excised tracheas and a further three in situ tracheas, for around 10 sites per trachea, in order to build up statistics on airway health measures available from these images. An example trachea image is shown below in Fig. 11.

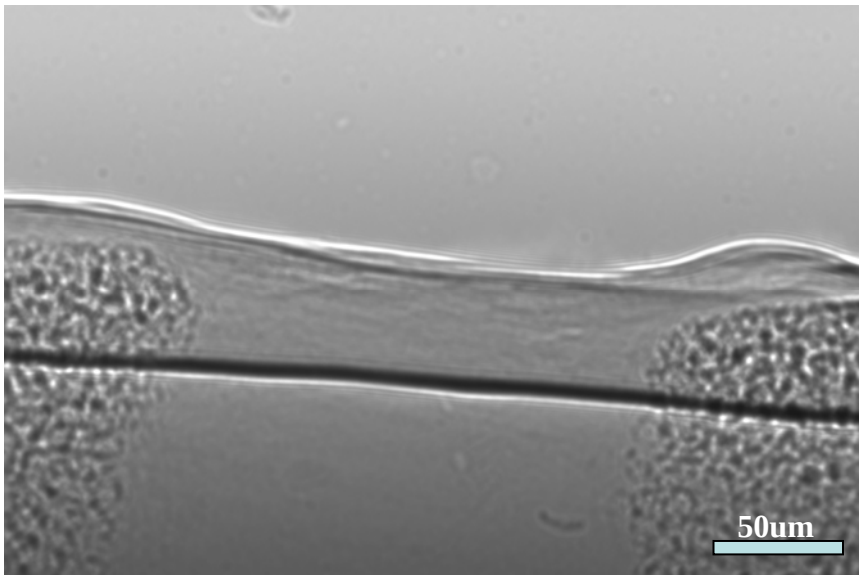


Figure 11. Excised trachea imaging site, showing cartilage rings on either side and the inner airway wall as a strong dark fringe.

Summary:

The aims of this beamtime were successfully carried out, showing how the propagation-based phase contrast fringes can be enhanced through aperture choice, diffuser positioning and the angle of the airway relative to the detector. A comprehensive set of phantom and mouse trachea images has been taken upon which analysis techniques are currently being developed, in order to extract the airway surface liquid depth as a measure of airway health. Once this analysis has been established, a paper detailing the imaging and analysis optimisations may be written. The ability to directly observe the airway surface liquid depth and changes in response to treatment would then be extremely valuable in the development of new treatments for Cystic Fibrosis.