Human fetal whole body post-mortem microfocus computed tomographic imaging

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Abbreviations:

CT – computed tomography

HTA – Human Tissue Act

I_2KI - potassium tri-iodide

Micro-CT – micro-focus computed tomography

MRI – Magnetic Resonance Imaging

ODD - object-detector distance

SOD - source-object distance

UHF-MRI - ultra-high frequency MRI

Editorial Summary

This protocol describes how to prepare and image human fetuses by microfocus computed tomography, a less invasive imaging alternative to conventional autopsy.

TWEET Non-invasive autopsy of human fetuses using microfocus computed tomography @ian_simcock

COVER TEASER Microfocus computed tomography of human fetuses

RELATED LINKS

Key reference(s) using this protocol

Hutchinson, J. C. *et al*. *Ultrasound Obstet Gynecol* **47**, 58-64 (2016). https://doi.org/10.1002/uog.15764Hutchinson, J. C. *et al. American Journal of Obstetrics and Gynecology* **218**, 445.e441-445.e412 (2018) https://doi.org/10.1016/j.ajog.2018.01.040

Shelmerdine, S. C. *et al. American Journal of Obstetrics and Gynecology* in press (2020) https://doi.org/10.1016/j.ajog.2020.07.019

Abstract

Perinatal autopsy is the standard method to investigate fetal death, however, requires dissection of the fetus. Human fetal microfocus computed tomography (micro-CT) provides a generally more acceptable and less invasive imaging alternative for bereaved parents to determine the cause of early pregnancy loss compared to conventional autopsy techniques. In this protocol, we describe the four main stages required to image fetuses using micro-CT. Preparation of the fetus includes staining with the contrast agent, potassium tri-iodide, and takes 3-19 days, depending on the time taken to consent for the procedure and the size of the fetus. Set up for imaging requires appropriate positioning of the fetus and takes1 hour. The actual imaging takes on average 2 hours 40 minutes and involves initial test scans followed by high definition diagnostic scans. Post-imaging. 3 days are required to post-process the fetus, remove the stain and also undertake artefact recognition and data. This procedure produces high-resolution isotropic datasets, allowing for radio-pathological interpretations to be made and long-term digital archiving for re-review and data sharing, where required. The protocol can be undertaken following appropriate training, which includes both the use of micro-CT techniques and handling of post-mortem tissue.

Introduction

Perinatal autopsy is recognised to be the reference standard test for the investigation of fetal death[1-3], however, bereaved parents have increasingly taken the view that they do not want their child's body dissected[4-8]. Parental consent for conventional autopsy is low following stillbirth or miscarriage[2] and well below the minimum recommended rate of 75%[9]. High resolution imaging is now becoming common place in perinatal autopsy, as an adjunct or alternative to conventional autopsy techniques. Together with image-guided biopsy, post-mortem imaging represents a significant technological advance in autopsy practice which is welcomed by bereaved parents and charity support groups[10-13].

Whilst computed tomography (CT) has become the main modality used for adult post-mortem imaging, ultrasound and Magnetic Resonance Imaging (MRI) are more appropriate modalities for imaging children[14], as fetuses and children typically have less intra-abdominal fat and require a technique that provides better soft tissue contrast, especially given the challenges of administering exogenous contrast agent[15] due to the combination of size and technical expertise[15,16].

Several imaging techniques have been explored for post-mortem imaging in fetuses and children, including MRI[17-21] and ultrasound[22-24] but they typically have practical limitations in terms of size, access, affordability, and resolution[25,26]. In particular, smaller fetuses below 20 weeks gestational age or 500 g pose a challenge and result in a significant reduction in diagnostic accuracy[27]. A method for imaging smaller

fetuses using microfocus computed tomography (micro-CT) has been developed and refined by our group, to provide high resolution imaging datasets, suitable for clinical radio-pathological interpretation[28-30].

In this protocol, we detail the steps required to implement micro-CT imaging, using iodine as a soft tissue contrast medium. We outline the safety precautions and critical steps of the process, together with details of how to prepare the fetus and position it appropriately to maximise the ability to perform high quality imaging. The protocol specifically details the preparation of human fetuses weighing up to 300 g, and up to 24 weeks of gestation. It was first developed for smaller specimens[31] and subsequently amended to include immobilisation for larger fetuses and the use of padded material to absorb excess I_2KI solution[28,29]. This workflow could also be easily applied to other mammalian tissues with the necessary optimisations[32]. The procedures here are optimised for the commercially available XT H 225 or Med-X (Fig. 1a) micro-CT scanner (Nikon, Tring, UK), although the protocol could be adapted to other scanners with an adaptable focal point. Users that are aware of the technical abilities of their specific scanner should be able to determine whether it can be used as an alternative.

Principles of micro-CT

Micro-CT is a density-based imaging modality using conventional cone beam radiation, historically used in industrial and archaeo-biological applications, with voxel resolution to 0.001 mm[33-41]. More recently, it has been used to examine small dense objects, such as teeth or bone in high resolution [42].

Two micro-CT system setups are possible. Firstly, where the radiation source and X-ray detectors are mounted on a gantry and rotate around the specimen[43], similar to a medical hospital CT scanner. The limitations of this system are that the source-object distance (SOD) and object-detector distance (ODD) are fixed. This system is less flexible as it has a fixed magnification, as well as being limited in the size and shape of the specimen that can be imaged. The second setup has the specimen mounted on a turntable, which is rotated independently of the radiation source and X-ray detectors. This setup allows variation of the SOD and the ODD, which in turn allows the geometric magnification level to be varied (for example Fig. 1b)[43]. This is the more flexible system, allowing a wider variety of specimen sizes to be imaged optimally, with the specimen being moved along the X-ray beams axis, ensuring the entire volume is imaged and at the maximum magnification available. Smaller specimens can be brought closer to the X-ray source resulting in greater magnification and higher resolution imaging, hence micro-CT's particular utility in smaller fetuses.

Alternative techniques

MRI has documented high diagnostic accuracy for whole body post-mortem fetal imaging[17-20,44], but in a hospital setting, clinical pressures mean that live patients will naturally be prioritised, limiting access. Post-mortem fetal MRI can take up to 90 minutes[45] and may be limited to specialist centres. Smaller fetal cases are more challenging to image, particularly at 1.5 T when the post-mortem weight is less than 500 g[27]. Higher field strength MRI scanners achieve better signal at higher resolution, for example 3 T MRI is better for fetuses below 24 weeks[46], with further improvements

(57) at 7 T[47] and 9.4 T[48]. Access to high field scanners is equally limited to specialist referral units.

For more detail, a recent systematic review has compared micro-CT and ultra-high field MRI (UHF-MRI), both of which can be used in this setting[32]. UHF-MRI does not require exogenous contrast agents but has the drawback of long scanning times 20 – 78 hours scanning to achieve 35 – 55 micrometres resolution. Micro-CT has the disadvantage of requiring iodine contrast staining for soft tissue differentiation, which may take up to 14 days for patients of 300 g, but rapid scanning (45 minutes) to achieve 15 – 90 micrometres resolution.

In this protocol, we use potassium tri-iodide (I_2KI) as a contrast agent. Various other agents have been evaluated as alternatives for micro-CT in visualisation of soft tissues[49] including osmium tetroxide and phosphotungstic acid. Osmium tetroxide provides excellent soft tissue contrast but is expensive (£347 per g), requires a prolonged diffusion time in fetal specimens, and is highly toxic, necessitating specialist disposal of waste Osmium[50]. Phosphotungstic acid is more affordable but can take up to 12 days to completely stain adult mouse hearts[51], which can result in distortion of histologic features[52] and is unsuitable for staining samples that have a volume >1 cm^3. I_2KI is widely available, stable, and affordable (£0.5 per g); in addition, it has low toxicity, can diffuse through soft tissue samples that are up to a few centimetres in thickness, and most of the discolouration effects can be reversed after imaging, although a change from the original skin tone is observed after this reversal [36,53-55]. The use of I_2KI as a contrast agent for a wide range of tissue specimens is well

documented[53,56,57]. There have been reports that I_2KI can cause tissue distortion (shrinkage)[53,58], but these effects can be limited by using a lower concentration [53,59] and the protocol described here has not caused significant tissue distortion (<10%; images remain diagnostic in over 400 cases at our institution [60]. Strict adherence to the protocol will ensure that these potential complications are minimised.

Applications of the method

Micro-CT has been shown to have high diagnostic accuracy for isolated fetal hearts[31,60-62], fetal kidneys[30,63] and fetal brain[64], and several other excised body parts[32]. This protocol is designed and optimised for whole body imaging of a range of fetal weights, with our centre having experience of fetuses ranging from 2 to 350 g, without dissection of body organs. Human whole body fetal micro-CT has also been shown to be feasible[28] in a series of 20 fetuses aged between 11 and 21 weeks gestation. A larger study of 250 cases has also been reported with high diagnostic accuracy[29], and in some cases changed the original clinical diagnosis and management[30]. This protocol may also apply to a larger range of fetal weights, but increased size will increase the iodination time as well as limit the final resolution achievable, which may mean that alternative imaging methods are more suitable. Another benefit of micro-CT imaging is the ability to acquire three dimensional (3D) digital datasets, which allow visualisation of the data as a 3D printed model or in augmented reality (Smith C. M. Shelmerdine SC, Arthurs OJ, Baskaran I, Sebire NJ, Mohamedally D. HoloLens for medical imaging using post-mortem fetal micro-CT data. European Congress of Radiology Abstract. (2019)). https://dx.doi.org/10.26044/ecr2019/C-

0153 This can aid parental counselling and help in promoting medical education of congenital anomalies and fetal development[65].

Experimental Design

Fig. 2 provides a summary of the steps described in this protocol. Following the correct pre-imaging steps (Steps 1 – 12), it is crucial to prepare the specimen correctly (Steps 13 - 20). As fetal movement will reduce image quality, careful wrapping and/or packaging is crucial to prevent any subtle motion artefact and has to be optimised to the size of individual patients (steps 21 and 22). Once the machine is ready (steps 23 – 32), a lower resolution scan should be initially performed to assess adequate tissue preparation (Steps 33 - 49). This is followed by a higher resolution scan (Steps 50 - 59). Subsequent image processing can be adjusted to the operators' needs (Steps 60 - 66). It is important to process and handle the fetus for appropriate return to the parents (Steps 67 - 69).

It is also important to consider the preparation time required to prepare the reagents, the expected duration of the iodination process and the departmental work-flow capacity, to manage parents' expectations appropriately.

Parental consent and handling human tissue

Consent from parents is required before all autopsy procedures, including post-mortem imaging. The consent should be taken by the clinical team dealing directly with the parents, who are experienced in dealing with bereavement. Our consent form (see Supplementary Note) allows parents to choose between several autopsy options covering: a full (i.e. conventional) invasive autopsy, an imaging autopsy (including

radiographs, MRI, CT and ultrasound) with additional consent required for micro-CT, and external evaluation only of the fetus (i.e. no incisions or imaging). This approach is based upon the SANDS (Stillbirth And Neonatal Death Society) post-mortem consent package (https://www.sands.org.uk/professionals/bereavement-care/sands-post-mortem-consent-package). We ask for additional explicit consent for micro-CT given the additional temporary staining procedure, not required for other imaging modalities.

This procedure was established to conform to UK regulations, where handling human tissue is strictly regulated by legislation. Clearly if this protocol is to be complemented in other countries, regulatory guidance should be sought to clarify the local rules and procedures, particularly around human tissue and radiation exposure. Most mortuaries will be able to incorporate this protocol into their existing regulatory framework but implementing this in a laboratory or non-medically licensed setting may necessitate additional permissions regarding the ethical, practical and clinical considerations set out. Below we outline the requirements UK-based researchers need to consider to conform to UK regulations.

In England, Wales and Northern Ireland, all organisations that remove, store, use or dispose of human tissue as part of a Scheduled Purpose (including research or post-mortem examination) must be licensed and regulated by the Human Tissue Authority, under the Human Tissue Act (HTA) 2004 (https://www.hta.gov.uk/policies/human-tissue-act-2004) to ensure that human tissue and organs are used safely and ethically, with proper consent. Separate legislation applies in Scotland (https://www.hta.gov.uk/policies/human-tissue-act-2004) and for autopsies that are required by law (https://www.hta.gov.uk/policies/human-) (predominantly clinically

unexplained or unnatural deaths in infants, children and adults, this legislation does not apply to the vast majority of fetal deaths or terminations of pregnancy).

As part of the requirements of the HTA, each organisation must have a licence holder, and Designated Individual responsible for overseeing the licence on behalf of the HTA. Organisations must practice in accordance with HTA Codes of Practice and submit themselves for annual compliance updates and inspections of premises. Familiarity with legislation governing the use and storage of human tissue is required as part of histopathology training (https://www.rcpath.org/discover-pathology/undergraduates-and-foundation-doctors/pathology-undergraduate-curriculum.html) and for anatomical pathology technologists (mortuary assistants). A detailed exploration of the challenges of setting up an imaging-based autopsy service is beyond the scope of this review, however, a reasonable first step would be for radiologists and radiographers to familiarise themselves with the particular aspects of legislation that may hinder service development (e.g. if imaging facilities are in a separate building to mortuary facilities, both addresses must be licensed by the HTA). Engagement between pathology and radiology departments can also help to address training staff for the handling of post-mortem tissue, arrangement of scan slots, and mortuary access to return tissue following scanning.

Level of expertise needed to implement the protocol

This protocol describes a specialist application for micro-CT scanning, applied to early gestation fetal losses. It is intended for research scientists who have background familiarity with using the micro-CT machine but not necessarily in handling human tissue, or alternatively those with a clinical background who may be familiar with

handling human tissue but not have micro-CT expertise. All practitioners will therefore need appropriate laboratory and clinical training for handling fetuses in a post-mortem setting, with psychological support where required. A basic understanding of ionising radiation, radiation protection and IRMER principles is recommended. A familiarity with DICOM images and computer aided software manipulation would help in the application of this technique.

MATERIALS

Biological Material

Human fetus CAUTION Informed consent must have been obtained from the parents and National and Institutional regulations must be followed, as discussed in the Introduction. We obtained Ethical approval from the London Camberwell St Giles Research Ethics Committee, REF CE13/LO/1494 to undertake the imaging from which we show results in this protocol.

Reagents

Formaldehyde 40% (wt/vol) (Genta Medical, cat. no. F40050) CAUTION Toxic and flammable. Handle with care and dispose as required by local regulations (further safety information provided in Supplementary Manual)

Sodium Chloride (NaCl)

Iodine flakes (I_2) (Sigma-Aldrich, cat. no. 376558) CAUTION Toxic. Handle with care and dispose as required by local regulations (further safety information provided in Supplementary Manual)

Potassium iodide (KI) (Sigma-Aldrich, cat. no. 207969) CAUTION Toxic to aquatic life; dispose as required by local regulations (further safety information provided in Supplementary Manual)

10% (wt/vol) formalin

Sodium thiosulphate pentahydrate ($Na_2S_2O_3$) (Merck, cat. no. 217247) 4% (wt/vol)

Equipment

Micro-CT scanner (Med-X, Nikon, Tring, UK)

Micro-CT turntable for mounting specimens, diameter 125 mm (Nikon, Tring, UK)

Carbon fibre plate mount, length 100 mm (Nikon, Tring, UK)

Carbon fibre rod 2 mm diameter, 70 mm length

Bemis Parafilm M Laboratory Wrapping Film 50 mm width (Fisher Scientific, cat. no. 11762644)

Dressing scissors blunt/sharp 13 cm (Williams Medical, cat. no. W-BE632)

Plastic containers with lids – 140 mm x 75 mm x 55 mm and 200 mm x 100 mm x 100 mm

Plastic cylindrical container – diameter 75 mm, length 140 mm

Conical flask 1 litre capacity (Merck, cat. no. Z308919)

Mini magnetic stirrer (Heathrow Scientific, cat. no. 120155)

Duran 1,000 ml bottles, amber, graduated Duran (Fisher Scientific UK, cat. no. 2391191)

Measuring cylinder 100 ml (VWT, cat. no. 612-3836P)

Cover-Dri Plus Absorbent padding sheet 40 x 60 cm (Attends, Wakefield, UK) cat. no. 203903)

Permanent felt-tip pen

My Book 8 TB Desktop HARD Drive (Western Digital, product code WDBBGB0080HBK-EESN)

12 x Western Digital Red 8 TB 3.5” SATA Hard Drives (Western Digital, cat. no. WD8003FFBX)

12 Bay Desktop NAS Enclosure (Synology, cat. no. DS3617XS)

Safety equipment:

Single use latex/nitrile gloves

Disposable plastic apron

Safety goggles

Paper towel

Bench protection e.g. Fisherbrand Grade 604 Surface Protection (Fisher Scientific UK, cat. no. 11710005)

Spillage kit

- Single use latex/nitrile gloves
- Absorbent towel e.g. Kimberly-Clark Professional WypAll L30 Ultra Wiper Large Roll (Fisher Scientific UK, cat. no. 12565528)
- Sodium thiosulfate 4% (wt/vol) (Box C)
- Absorbent pad e.g. Fisherbrand Absorbent Underpads (Fisher Scientific UK, cat. no. 11917964

Software

VGSTUDIO MAX Version 3.2.0.143291 (Volume Graphics, Heidelberg, Germany)

Inspect-X Version XT 5.1.4.2 MedX 1 (Nikon, Tring, UK)

CT Pro 3D software Version XT 5.1.42 MedX 1 (Nikon, Tring, UK).

Reagent Setup

General comments about making up reagents

All the reagents listed in this section should be made up fresh, and stored in a cool, dark, well-ventilated area away from heat sources and direct sunlight. The

effectiveness of the reagents will diminish over time, and these should be replaced after approximately 4-6 weeks, or if a loss of efficacy is identified by the operator.

! CAUTION Components of the reagents listed below are toxic, thus they should all be prepared in an adequately ventilated laboratory with safety goggles, single use latex/nitrile gloves and a protective laboratory coat. Further guidance on the potential toxicities and the safe usage of the following reagents is available in the supplementary manual, however it is the responsibility of handler to adhere to safe laboratory practices and all local laws/guidelines.

10% (wt/vol) Formal Saline "Formalin" – Timing (20 minutes)

<u>The following produces 5 litres:</u>

1. Measure 4,500 ml of distilled water and place in a 5 litre sealed container.
2. Weigh out 45 g of sodium chloride and 500 ml of 40% (wt/vol) formaldehyde and add to the distilled water.
3. Cover the mouth of the container and agitate gently to dissolve.
4. Store in a lidded 5 litre storage container and label accordingly.
5. Store in dry cool conditions.

Contrast Agent Potassium tri-iodide (I_2KI) – Timing (30 minutes)

A spillage kit is required to ensure safe decontamination of the area should a spillage occur

1. Weigh out 100g of potassium iodide (KI) and 50g Iodine (I_2) solid. Critical Accurate preparation of the I_2KI solution is imperative to minimise any tissue

shrinkage of the fetus. Solutions with a higher concentration will result in irreversible tissue deformation and shrinkage.

2. Place the 100g of KI into a 1 litre conical flask and add 100 ml of distilled water along with a magnetic stirrer.
3. Cover the mouth of the conical flask with parafilm to stop spillage and place on the electric controller of the magnetic stirrer.
4. Adjust the speed of the magnetic stirrer as required. This will dissolve the KI; this will take approximately 5 minutes.
5. Once dissolved, remove the parafilm cover and add 50 g of I_2.
6. Make up to 1,000 ml total volume by slowly adding distilled water (approximately 750 ml required at this point).
7. Place again on the magnetic stirrer and slowly increase the speed and maintain a constant speed for 10 minutes until all the I_2 is dissolved. Minor sediment may remain at the bottom of the flask.
8. Pour into an opaque 1 litre Duran container (Fig. 3a) and store in a locked dark cupboard until required.
9. Dilute 1:1 with 10% (wt/vol) formalin; formalin prevents further tissue breakdown / autolysis. This results in a total iodine content of 63.25 mg/mL (iodine mass of 2.49×10^{-4} mol / mL).

CAUTION Potassium tri-iodide liquid and vapour can permanently mark work surfaces and so should be stored in an appropriately secure container. Sodium thiosulphate 4% w/v solution can be used to remove discolouration of work surfaces caused by spillages of potassium tri-iodide, so we recommend that

recently produced sodium thiosulphate should be kept on hand during all the parts of the procedure that use potassium tri-iodide.

Sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$) – Timing (30 minutes)

1. Weigh out 40 g of $Na_2S_2O_3$ solid.
2. Place the 40 g of $Na_2S_2O_3$ solid into a conical flask and add 960 ml of distilled water along with a magnetic stirrer.
3. Cover the mouth of the conical flask with parafilm to stop spillage and place on the electric controller of the magnetic stirrer.
4. Adjust the speed of the magnetic stirrer as required. This will dissolve the $Na_2S_2O_3$ and will take approximately 15 minutes.
5. Pour into a lidded 1 litre storage container for storage with 250 ml placed in a separate container to be included in the spillage kit until necessary.
6. Store in dry cool conditions.

Equipment Setup

Micro-CT Scanner: To minimise the impact of temperature on the micro-CT scanners, the ambient temperature should be maintained in an environmentally controlled room at approximately 20°C and with a humidity of between 30 and 70% ensuring the micro-CT scanners can run efficiently and negate any overheating of the micro-CT scanners.

PROTOCOL

Assessment for suitability – Timing (1-5 days)

1. Transfer the fetus to the local licensed registered mortuary by the usual method and store at 4°C. Refrigeration reduces autolysis and further degradation to the internal organs.

Pause Point – The fetus can remain refrigerated at 4°C for several days whilst the correct administrative steps are taken.

2. Obtain a completed consent form from the parents. It must be made clear that skin discoloration occurs during the iodination process and subsequent removal of the stain will result in a change to the skin tone from the original colour. Any questions that cannot be answered by the staff obtaining the consent form must be referred to the pathology and radiology teams at the specialist paediatric centre and be adequately addressed before undertaking any more of the procedure.

Critical Step – Staff taking consent must be trained professionals and fully understand post-mortem procedures and the advantages and limitations of the individual techniques, to provide the parents with sufficient information to make an informed decision.

! Caution – Prior to undertaking this work ethical approval and parental consent should always be sought as well as ensuring conformity to the relevant Institutional and National regulations. Written patient consent is required for all internal and external post-mortem examinations. This should make clear any restrictions, and specific parental wishes.

3. Once consent has been approved by the parents, transfer the fetus to a licensed specialist registered mortuary for perinatal post-mortem investigations using refrigerated carriers. Once it has arrived, store the fetus refrigerated at 4°C for a maximum of 30 days, whilst proceeding with the following steps.

   PAUSEPOINT
4. Review the referral notes for the clinical indication for autopsy and check that the parents have completed the correct consent form with a trained medical professional.
5. Weigh and measure the fetus to determine size. Approximate gestation may also be obtained from the clinical notes.

Critical step – Ensure the case is suitable for micro-CT: typically, less than 20 weeks and <300 g body weight. Assessing the fetus for size, gestation and maceration status allows the most appropriate post-mortem investigation to be implemented.

6. Perform complementary imaging tests, e.g. MRI, ultrasound, or plain radiographs prior to micro-CT, if required.

Critical step - Immersion in I_2KI solution reduces the accuracy of the other imaging techniques, therefore all other imaging must be completed first.

Storage and Immersion - Timing (2-14 days)

7. Place the fetus in a suitable volume of I_2KI solution in an opaque container with a fluid resistant lid. Place fetuses below 100 g bodyweight in 1 l of I_2KI solution. Place fetuses of 100 – 300 g bodyweight in a 2.5 l of I_2KI solution. Ensure that the size and shape of the container does not restrict the body.

Critical Step - The size of the container must hold sufficient I_2KI solution to ensure complete submersion of the fetus. If the container is too small, the fetus will not be sufficiently iodinated or it may take a prolonged period. Ideally, I_2KI solution should not be reused.

Critical Step – The 10% formalin in the I_2KI solution is important to halt any further tissue autolysis, as these steps are performed at room temperature.

8. If possible, place the fetus into the I_2KI solution in an anatomically correct position with the head and body aligned, the upper limbs either to the side or crossing the anterior of the fetus and the lower limbs either aligned with the body or bent in the fetal position.

Critical Step - Manipulation of the body is easier before iodination as it will stiffen during immersion in I_2KI and formalin. Deformation is more likely when placed in a container which is too small.

9. Place the fetuses head-first into the I_2KI solution to ensure the largest part of the body is maximally immersed, whilst ensuring the fluid covers the whole fetus. A horizontal shaker could speed up staining.

Critical Step – Placing the largest body part towards the bottom of the I_2KI solution ensures efficient gravity-based diffusion into the largest structures.

10. If any body part floats to the surface after immersion, place some paper towels atop of the fetus to soak up the solution, and cause the body to sink below the surface of the solution.

Critical Step – Anatomy that is not immersed will not be iodinated.

11. Note the identification and immersion date and time on the container.

12. Retain the container in a locked cupboard at room temperature, approx. 20°C.

Pause point – Depending on the size of the fetus, immersion time can take between 2-14 days. Extended periods of I_2KI immersion will not degrade the fetuses internal organs and scanning can be completed when full iodination has occurred and be based on scanner availability. Under-iodination will not allow visualisation of all internal anatomy. Immersion times are approximately 5 days for 100 g bodyweight, or up to 14 days for 300 g bodyweight, but this varies depending on the thickness of individual areas of anatomy.

Fetal Preparation – Timing (20 minutes)

13. Once the pre-determined immersion time has occurred and just before scanning, rinse the fetus under cold running water to remove excess I_2KI solution.

? Troubleshooting

14. Dry the body with disposable paper towel taking care to dry in between the limbs to eliminate all surface fluid.
15. Place the body on a Cover-Dri absorbent towel and wrap securely affixing identification to the outside of the wrapping.
16. Place the wrapped body in a padded bag and transport to the micro-CT imaging department.
17. At the scanner, unwrap the fetus and assess the orientation of the head, body, and limbs whilst on the Cover-Dri absorbent towel.
18. Align the fetus in the “fetal” position, with the upper limbs either alongside the torso or bent anteriorly, and the lower limbs either continuing in alignment with

the body or bent at the knees depending on the positioning of the limbs post iodination, in order to minimise movement.

19. Place a carbon fibre rod (Fig. 3b and 4a,d) along the right side of the fetus, ensuring part of the rod extends from the head to the body, as a side marker.
20. Secure the carbon fibre rod in place using a short piece of parafilm (Fig. 4e), which when stretched will wrap around the fetus axially with at least a 2 cm strip to overlap and secure it in place.

Size Assessment – Timing (25 minutes)

21. Assess the size of the fetus by measuring the total length of the fetal body to determine how to secure the fetus within the micro-CT scanner.

Critical Step – Correct assessment of the size of the fetus is imperative to perform an artefact free, time efficient scan. If the fetus is too large (approx. >80 mm) and is positioned using the “small fetus” instructions, movement is likely, resulting in non-diagnostic micro-CT images. However, if a small fetus is positioned using the “large fetus” instructions, a diagnostic scan is possible, but as it is more difficult to position small fetuses in the most time efficient alignment, prolonged scanning times may be required.

22. Place the carbon fibre plate (Fig. 3c) which attaches to the turntable next to the fetus. If the head and the body, total fetal length <80 mm, can be supported directly by the carbon fibre plate proceed with the **Small Fetus** instructions (A). If the fetus is larger, >80 mm in length, proceed with the **Large fetus** instructions (B).

Preparation of Small Fetus - A

I. Cut a small rectangle of absorbent Cover-Dri Plus absorbent material, sufficient to wrap the fetus longitudinally once completely wrapped with a 1 cm overlap and 2 cm projecting clear of the top and bottom of the fetus.

II. Manipulate the head, body, and limbs into alignment.

III. Coil any umbilical cord on the abdomen/chest.

IV. Wrap the fetus in this absorbent padding with the absorbent side in contact with the fetus and maintaining the alignment of the body.

V. Beginning at the head wrap a sufficient length of parafilm in a spiral motion around the entire fetus, securing the absorbent material in place and extending down to the feet (Fig. 4b). This may be several centimetres long.

Critical Step – This absorbent material allows any excess I_2KI solution that naturally leaks during the scan to be absorbed, avoiding issues with image reconstruction or spillage into the scanner itself.

VI. Leave 1-3 cm of parafilm at the head and feet to wrap over and completely enclose the body and absorbent padding at either end.

Critical Step – This complete wrapping assists in ensuring that no I_2KI solution leaks into the micro-CT scanner during image acquisition.

VII. Through touch reassess that the body is aligned and seal the head, feet, and absorbent material with the excess parafilm, ensuring there is a complete seal by palpating the whole fetus and its wrapping gently.

VIII. If realignment is required, the parafilm can be partially uncoiled to allow correct repositioning.

Critical Step – The wrapping should be secure enough to maintain the anatomical position, but over-tightening will distort the anatomy and hinder identification of any structural abnormalities. This is also important to ensure the carbon fibre rod is not imprinted into the anatomy.

IX. Identify flat areas of the body that can be placed against the carbon fibre plate without causing deformation of the fetus. Suitable areas are often the posterior of the fetus along the spine or if the fetus is naturally curled into the fetal position a flat portion can be the anterior of the head, upper and lower limbs as they form a flat surface.

Critical Step – Suitable areas must be able to support an increased pressure from the parafilm wrapping to secure them to the carbon fibre plate, whilst resisting deformation.

X. If the head is large in comparison to the body the fetus should be inverted to allow the largest body area to be placed towards the base.

Critical Step – Placing the largest anatomical structures at the bottom will reduce the chance of movement during the scan.

XI. Ensure the lowest part of the body will not be projected over the metal connection of the carbon fibre plate to the turntable by the divergent X-ray beam by ensuring a 1cm gap between the fetus and the metal connectors.

Critical Step – If the metal connectors and fetus are in close proximity (i.e. <1 cm), then streaks of metallic artefact will degrade the final image as a consequence of the high density of the metal.

XII. Secure the body to the carbon fibre plate by firstly placing a 2cm length of parafilm to the carbon fibre pate and wrapping the fetus and plate in a spiral coiled motion beginning at the top and finishing at the base of the carbon fibre plate (Fig. 4c). Maintain a constant pressure without deforming the fetus.

Critical Step – Attaching the parafilm to the carbon fibre plate initially will allow tension to be placed on the parafilm as it is wrapped around the fetus ensuring a firm fixation.

XIII. Wrap the final 4 cm of parafilm around the carbon fibre plate to seal off the fetus inside the parafilm wrapping.

Critical Step – Sealing off the base of the carbon fibre plate minimises I_2KI solution leak through the wrapping.

XIV. Attach the carbon fibre plate to the baseplate and secure in place with the alum key and screw. Tighten to finger-tight.

XV. Centre the baseplate in the x-axis to allow central positioning within the X-ray beam.

Large Fetus - B

I. Coil the umbilical cord on the abdomen/chest and secure in place with a short length of parafilm allowing for a 2 cm overlap.

II. Place the fetus on a fresh Cover-Dri absorbent pad close to the short edge and ensure that there is 3 cm near the head and 10 cm near the feet of excess absorbent padding with a further sufficient length of padding to allow complete rolling of the fetus 2-3 times.

III. Use scissors to remove any excess padding.

Critical Step – Removal of the excess padding is important to ensure a good fit. Adjustment of the padding is critical prior to rolling, whilst the body is still visible to avoid damage to the body.

IV. Gently manipulate the head and body into alignment with the upper limbs either alongside the body or bent anteriorly, and the lower limbs either straight in alignment with the body or bent at the knee.

V. Whilst maintaining anatomical alignment, begin rolling the absorbent pad and the fetus through 2-3 rotations (Fig. 4e)

VI. The extent of this rolling and subsequent padding is dependent on the size of the body relative to the pre-selected container.

Critical Step – If the container is cylindrical and open topped, the extra padded material can be folded over to assist in immobilising the fetus. However, if the container is a lidded box then the padding should be cut appropriately to allow central positioning.

VII. Place the rolled fetus and padding into the chosen container (Fig. 4f).

Critical Step – Container choice is important. The fetus and padding should provide maximum support without excessive padding causing deformation to the fetus. It should also have a flat base to ensure stabilisation when placed within the micro-CT scanner.

VIII. Position the fetus with the largest body part at the base and close to the bottom of container to ensure maximum stability; usually this is the head, and thus the body is positioned inverted (Fig. 4f).

IX. Position the fetus straight within the container.

Critical Step – Placement straight in the centre of the container allows easier centralisation on the turntable, a reduced number of projections for optimal imaging and a subsequent reduction in the scanning time.

X. Attach the most appropriately sized micro-CT turntable depending on the size of the fetal container.

XI. Place a radiolucent stiff padding material on the turntable and place the container holding the fetus on top.

Critical Step – This gap allows separation of the fetus and the turntable, ensuring the X-ray beam does not pass through the denser baseplate material and the fetus. The padding should be sufficiently stiff to withstand deformation or movement whilst the container and body is in place.

X-ray Beam Orientation – Timing (10 minutes)

! CAUTION – Although steps 23 - 61 are all described as being carried out on Nikon scanners, scanning procedures may differ depending on the capability and design of alternative manufacturers. The protocol is designed to be simple to use and adaptable to most micro-CT scanners, however advice should be sought through individual manufacturers or advanced local operators where differences may arise.

23. Turn the X-ray beam on and select live images to allow the body to be positioned in relation to the X-ray cone beam.
24. Set the target material to Tungsten (this is done by rotating the multi-metal target using the appropriately sized spanner)
25. Select an exposure time of 250 ms to allow fetal repositioning to be monitored in real time.

26. Select 100 kV and 100 µA to allow the X-ray photons to have sufficient energy to create a test scan image.

Critical Step - The kilovoltage and current can be altered to suit individual fetuses but these factors should be suitable for the majority of fetuses up to 300 g in weight; see Table 1.

27. Adjust the on-screen contrast to allow differentiation of the fetus, the wrapping/container and the turntable.
28. Rotate the fetus through 360° to ensure that the body is correctly positioned in the vertical plane or reposition accordingly.
29. If a container has been used ensure that it is positioned centrally by rotating the fetus through 360° and checking in real time.

Critical Step – Apparent movement to either side of the central axis will require a greater number of projections which will increase the scan time and will decrease image resolution by increasing the distance between the body and X-ray source.

30. Adjust the position of the fetus so it is in a central position either by directly moving the container on the padding material or by moving the turntable with the micro-CT controls.
31. Manipulate the z-axis controls to ensure the whole fetus maximally fills the extent of the cone beam of X-rays and the y-axis controls to adjust the positioning to allow this. The live imaging option on the micro-CT scanner software allows these changes to be directly visualised and adjusted accordingly.

32. Rotate the fetus through 360° and observe that the whole fetus remains within the X-ray cone beam throughout the rotation. Adjust if required and repeat the rotation until this is correct.

Imaging Parameter Selection – Initial Test scan – Timing (10 minutes)

Critical Step - The aim of this initial scan is to determine the extent of the iodination process (Fig. 5a-d), thus the emphasis on short scan duration rather than image resolution (Fig. 6a,b). However, it is imperative to select the correct imaging parameters to attain a scan of sufficient quality with adequate iodine penetration of the fetus (Fig. 7a).

33. Select exposure time between 125 ms and 354 ms to allow the scan to be completed within 15 minutes.

Critical Step – The power of the x-ray beam must remain below the effective pixel size to reduce blurring of the image and protect the target, however, once below 10 Watts, any pixel size can be attained without risk of damage to the target. This imaging factor selection will influence the choice of exposure time, with lower power requiring higher exposure times (354 ms) to ensure a suitable image is produced, whereas a higher power will allow a lower exposure time (125 ms), ensuring suitable image quality with a shorter scan time.

34. Increase the kilovoltage until the number of photons reaching the detectors over the densest part of the body has increased by at least 2000.

Critical Step – This ensures the X-ray photons have sufficient energy to penetrate the body to assess the extent of iodination. The combined selection of individual exposure time and kilovoltage parameters will determine this.

35. Increase the current until the highest part of the histogram is close to 90% of the maximal value (Fig. 7b).

Critical Step – This allows fluctuation in the X-ray beam and changes in the penetration of the fetus to occur as it rotates through 360°, without the detectors becoming saturated and being unable to calculate the signal intensity values.

36. Run the correction scan to maximise the histogram for the chosen exposure factors using 180 frames to average.
37. Run the "reconstruction" scans. This creates two images of the fetus at right angles to each other and allows an imaging box to be positioned. This will be used to calculate the number of projections to be used (Fig. 7c).
38. Draw the box around the anatomy to be included in the scan ensuring that allowances are made above and below the body.

Critical Step – The imaging box can be drawn close to the sides of the fetus but there must be a larger gap above and below the body to allow for X-ray beam divergence.

39. Select beam hardening of 3 (moderate), noise reduction 1 (low), median filter and enhancement to none and set to automatically reconstruct the images after scanning.

Critical Point – These factors are suitable for the majority of clinical fetus scans, whilst automatic reconstruction allows more efficient processing.

40. Select 1 frame per projection to minimise the scan time and select “optimised” for the number of projections.
41. Run the scan, which should take between 10 – 15 minutes depending on the magnification required, the number of projections and the size of the fetus.

Assess Initial Test Scan – Timing (10 minutes)

42. Reconstruct the 3D imaging dataset using the CTPro3D software (Nikon, Tring, UK). This, can be done automatically.
43. Load this dataset using VGSTUDIO Max by double clicking on the VG dataset.
44. Once loaded, right mouse click on any of the three images and select “background – black” to optimise the image viewing.
45. Select “Layout editor” to choose the viewing tools for bottom, right and back to allow the fetus to be visualised in the three anatomical positions of axial, coronal, and sagittal.
46. Alter the angle of viewing the images by selecting “simple registration” and using the left mouse button to alter the viewing angles of all three planes.
47. Alter the contrast of the image with the “rendering” tool on the right-side control panel by moving the angled red line in relation to the histogram until the internal detail of the fetus can be visualised.
48. Scroll through these individual planes and check whether detail can be seen throughout the body or if any areas appear black with no internal detail.
49. Central black areas indicate insufficient iodination (Fig. 5a-c). If iodination is insufficient, immerse the fetus back in fresh I_2KI solution and repeat the protocol from step 10 onward. If anatomical detail can be observed throughout the body

(Fig. 5d), iodination is complete, and you can proceed to the next steps to undertake a diagnostic scan.

? Troubleshooting

Imaging Parameter Selection - High Definition Diagnostic Scan – Timing (1.5 - 2 hours)

CRITICAL Alteration to the imaging factors and positioning are required to increase the image quality (Fig. 6c,d). Our standard protocol includes two areas of enhanced imaging, torso/abdomen (upper third of the femur – neck/shoulders) and head (including vertex to the neck/shoulders). Other high-resolution images can also be obtained if the clinical history indicates an area of interest such as neck or heart.

50. Adjust the exposure time to between 250 ms and 500 ms to increase the image quality.

Critical Step – The higher the exposure time the greater the image quality, however exposure times greater than 500 ms are susceptible to movement artefacts in these fetuses.

51. Position the anatomical area as close to the X-ray source as possible to gain maximum magnification using the x, y and z-axis controls to position centrally within the beam. Whilst doing this ensure the anatomy is maintained within the X-ray beam throughout the 360° rotation.
52. Maintain the kilovoltage optimised for the test scan as an equivalent X-ray penetration is desired.

53. Alter the current to optimise the histogram to 90% maximum for the area of interest (Fig. 7b). The same factors can often be used for imaging different body parts of the same fetus (e.g. head vs torso / abdomen).
54. Select beam hardening of 3, noise reduction 1 and median filter and enhancement to none and set to automatically reconstruct the images after scanning.

Critical Step – Manual reconstruction of the images can be performed to compare alternative combinations of these post-processing steps, however for the majority of cases, these factors are suitable and ensure minimal user interaction and an ability to visualise the micro-CT images more efficiently.

55. Create two images at right angles to each other to allow areas of interest to be drawn around the fetus (Fig. 7c).
56. Draw the box around the anatomy to be included in the scan ensuring that allowances are made inferior and superior to the fetus for an increase in size as the fetus is rotated towards the X-ray source.
57. Select 2 frames per projection to increase the image quality and select “optimised” for the number of projections.

Critical Step – This increase in frames per projection will increase the image quality, but also doubles the scan time. However, this increase in quality is important to ensure diagnostic quality images are produced.

58. Accept the planned scan and plan all other anatomical areas required by repeating steps 51 – 57 and accepting each planned scan.
59. Run all the planned scans.

Post Scan Checks – Timing (20 minutes)

60. Repeat steps 43 – 48 to reconstruct the images for diagnostic assessment

? Troubleshooting

61. Scroll through the images to ensure that there are no movement artefacts, and that iodination of the fetus was sufficient for visualisation of the required internal anatomy.

? Troubleshooting

62. Incorporate the data into the final autopsy report.

    CRITICAL STEP This must be done by a qualified radiologist.

63. Remove any wrapping and the carbon fibre rod before rewrapping the fetus in a Cover-Dri absorbent pad with the identification tag attached to the wrapping.
64. Document scan completion, noting all imaging parameters, and prepare for stain removal.

Critical Step – This documentation ensures that the I_2KI stain is not removed prematurely by 4% (wt/vol) sodium thiosulphate solution. Subsequent re-iodination is not possible after this step.

65. Place the fetus in a padded bag and return to the mortuary.

Data Storage – Timing (30 minutes)

66. Transfer the data onto external hard drives or secure storage facility.

Critical Step – Due to the large size of the data collected by each micro-CT scan (30 GB) and the possibility to quickly amass a large amount of data, a robust storage

solution is required. Our centre stores all raw and reconstructed data which allows for ease of access and the ability to quickly reassess previous datasets for clinical or research decisions. We also duplicate our data on 8 TB external hard drives (Western Digital, San Jose, USA) and a 34 TB NASBOX system (Synology, New Taipei City, Taiwan). This ensures that data is backed up in the event of hard drive failure.

Stain Removal – Timing (1 – 3 days)

67. Place the labelled fetus in 4% (wt/vol) sodium thiosulphate solution and ensure it is fully immersed in a fluid resistant container, at room temperature.
68. Check the fetus within 24 hours to assess whether the external staining has been removed.
69. If the discolouration remains, replace in the 4% (wt/vol) sodium thiosulphate solution.

? Troubleshooting

Timing

The procedure is performed over several days, with the exact time required depending on the size and weight of the fetus. The specific timings given below refers to those required for an experienced user, inexperienced users may require more time for each step.

Steps 1 - 6 Consent procedure for micro-CT and other post-mortem investigations - 1-5 days

Steps 7 - 12 Storage and iodination of fetus - 2-14 days

Steps 13 - 20 Fetal preparation prior to positioning - 20 minutes

Steps 21 – 22 Fetal size assessment to determine and proceed with the most appropriate method for fetal positioning - 25 minutes

Steps 23 - 32 Orientation of the fetus within the X-ray beam - 10 minutes

Steps 33 - 41 Imaging parameters selection for low resolution test scan - 10 minutes

Steps 42 - 49 Assessment of initial test scan to determine whether the fetus is fully iodinated - 10 minutes

Steps 50 - 59 Imaging parameter selection, setup and imaging for high definition diagnostic scan – 1.5 - 2 hours

Steps 60 - 65 Post diagnostic scan checks to determine the success of the imaging protocol - 20 minutes

Steps 66 Data Storage - 30 minutes

Steps 67 - 69 Iodine stain removal from the fetus prior to returning to the family – 1 – 3 days.

Troubleshooting

See Table 2 for troubleshooting guidance.

Table 2: Troubleshooting guidance

Step	Problem	Possible reason	Possible solution
13	Excess shrinkage, may be visible to the naked eye	I_2KI solution is too concentrated.	Over-iodination will reduce tissue contrast planes. Run a test scan to assess for complete iodination. If fully iodinated, continue with high resolution scan. If under-iodinated replace the I_2KI solution.
49	Central black areas on micro-CT images (Fig 5) which indicate under-iodination	Insufficient immersion time in I_2KI solution	Place back in I_2KI solution and reassess after 24 – 48 hours.
49	Continued prolonged under-iodination of fetus.	The I_2KI solution is too weak, or there is insufficient volume within the container.	Place in fresh I_2KI solution

49	Homogeneous grey imaging with loss of tissue planes, despite full iodination.	Maceration/autolysis has occurred causing breakdown of internal structures.	Maceration due to intra-uterine retention cannot be reversed; Micro-CT imaging may be non-diagnostic. .
49	Deformation of anatomy.	Over exertion of pressure during wrapping.	Assess whether the side-marker rod is indenting anatomy and reposition if necessary. For a small fetus remove the parafilm wrapping and rewrap ensuring minimal pressure is used to retain positioning. For a large fetus consider either reducing the amount of padding or use a larger container to reduce the pressure during immobilisation
42 and 60	Unable to reconstruct the 3D volume after scan completion.	Movement during image acquisition (Fig 8) due to wrapping being too loose.	If parafilm has been used, consider tightening the parafilm wrapping, taking care to avoid distorting the body. For larger fetuses, ensure the correct amount of padding is used to completely immobilise the fetus, without distorting the anatomy. (Repeat step 22 A or B)
42 and 60	Unable to reconstruct the 3D volume after scan completion	Accumulation of I_2KI solution around the fetus.	Taking care to avoid spillage, remove the fetus from the parafilm wrapping allowing any excess fluid to be wiped away using a dry paper towel. Rewrap the fetus within the absorbent padding, taking care to ensure the lowest part of the anatomy once positioned is amply covered and able to wick away any excess I_2KI solution. (Repeat step 22 A or B)

42 and 61	Streak artefacts on the reconstructed image.	Incorrect positioning of the fetus, allowing the X-ray beam to pass through the metallic part of the support on either the carbon fibre plate or turntable of the micro-CT scanner.	Reposition the fetus allowing greater separation from the metallic components (small fetus) or use increased padding between the turntable and container (large fetus).
42 and 61	Reconstructed image displays reduced detail.	Wrong imaging parameters.	Consider increasing the kilovoltage to ensure penetration of the densest part of anatomy. Reduce current and/or exposure time to minimise detection saturation.
69	Prolonged time taken for removal of the iodine staining.	Incorrect thiosulphate solution	Use fresh thiosulphate solution.

Anticipated Results

This protocol will allow micro-CT operators to produce high quality clinically diagnostic micro-CT data sets which allow diagnosis of developmental abnormalities for early gestation fetuses (<20 weeks). Certain artefacts may still be observed once the high-quality diagnostic scans have been completed and may be related to movement due to incorrect wrapping, I_2KI solution collecting around the fetus (Fig. 8a-c), or over-iodination due to the I_2KI concentration being too high (Fig. 8d). Attention to the Troubleshooting guide should enable these issues to be rectified. Gaining experience assessing the size of the fetus, and in undertaking manipulation and immobilisation should also minimise the artefacts encountered.

Conflicts of interest: The authors declare that they have no competing financial interests.

Author Contributions:

ICS, SCS and JCH developed and tested the methodology within the paper and drafted the manuscript. NJS and OJA supervised the work and all authors assessed the results thereby optimizing the technique. All authors edited the paper and approved the final version.

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Data Availability Statement

Examples of data produced by following this protocol are included in the protocol. Further details of the data presented are not publicly available because they contain information that could compromise research participant privacy/consent.

Figure Legends

Fig. 1 Microcomputed tomographic imaging. **a**, Med-X micro-CT scanner (Nikon, Tring, UK) used for scanning whole fetuses **b**, Schematic of the micro-CT setup demonstrating the area of interest within the cone beam of X-rays throughout scanning. Minimising the distance to the X-ray source maximises the image resolution that can be achieved.

Fig. 2 Key parts of the workflow for human fetal whole body post-mortem microcomputed tomographic imaging. The procedure describes the steps in detail.

Fig. 3 Equipment needed. This equipment allows for the fetus to be securely wrapped and immobilised for the Micro-CT scanning protocol. It includes **a**, I_2KI solution storage vessel. **b**, carbon fibre rod for side identification. **c**, carbon fibre plate and mount for securing small fetuses.

Fig. 4 Fetal Preparation. **a – c,** Preparation for small fetus scanning. **a,** The carbon fibre rod is placed to the right-hand side of the fetus. **b,** The fetus and rod are wrapped in an absorbent pad and secured with parafilm to ensure no leakage of I_2KI solution into the micro-CT scanner. **c,** The fetus is secured to the carbon fibre plate with further parafilm wrapping. A 1cm gap between the fetus and the mounting section is ensured whilst care is taken in securing the head and body to the carbon fibre plate and the fully mounted fetus is placed within the X-ray cone beam.

d – f, Preparation for large fetus scanning. **d,** The carbon fibre rod is placed to the right-hand side of the fetus. **e,** The fetus and rod are wrapped securely with parafilm or an absorbent pad to ensure no leakage of I_2KI solution into the scanner. **f,** The fully wrapped larger fetus is placed within a container to immobilise during the scan.

Fig. 5 Tissue preparation with I_2KI. The iodination of the specimen after **a**, 1 day, **b**, 2 days **c**, 3 days, **d**, 4 days showing increased iodination as the iodine solution diffuses from the periphery to the interior of the fetus. Complete iodination suitable for diagnostic clinical Micro-CT scanning is shown on day 4. All images scanned on a Med-X micro-CT scanner (Nikon, Tring, UK) with imaging parameters of kilovoltage 100kv, current 100µA, power 10W, exposure time 250ms, frames per projections 2, number of projections 2559.

Fig. 6 Representative images of a fetus.

a + b, Axial chest and head images obtained with initial test scan imaging parameters, prioritising short scan duration, and assessment of iodination. Scanned on a Med-X micro-CT scanner (Nikon, Tring, UK) with imaging parameters of kilovoltage 100kv, current 100µA, power 10W, exposure time 125ms, frames per projections 1, number of projections 2559.

c + d, Axial chest and head images obtained with high resolution imaging parameters, prioritising diagnostic image quality. Scanned on a Med-X micro-CT scanner (Nikon, Tring, UK) with imaging parameters of kilovoltage 100kv, current 100µA, power 10W, exposure time 354ms, frames per projections 2, number of projections 3141.

Fig. 7 Positioning and image parameters. Accurate positioning and manipulation of the imaging parameters is critical to acquiring diagnostic images.

a, The imaging parameters of kilovoltage, current and exposure time are selected to ensure that the X-ray photons have sufficient penetration and number to penetrate the fetus and deliver information on the differential density of the fetal anatomy. This

is shown in in the wide range of colours depicted, with anatomical differences within red (the densest colour) still able to be visualised.

b, Histogram showing radiographic density absorption from the fetus in a. Extending the histogram through imaging parameter manipulation to 90% of the maximal range allows maximal differential absorption to be displayed on the final Micro-CT images without saturation of the detectors.

c, Two images at 90° that allow an area of interest to be defined for the fetus in a. This will determine the number of projections required which combined with exposure time and the frames per projection determines the scanning time.

Fig.8 Examples of issues occurring during micro-CT scanning that require optimization of the workflow. Leakage of I_2KI solution from the fetus which is not wicked away, will alter the outline of the fetus between the first (**a**) and last (**b**) images taken. This hampers the reconstruction software's attempts to align angled images at any given slice, and results in non-diagnostic imaging (c) Over-iodination of the fetus through immersion in over concentrated I_2KI solution will result in an inability to penetrate the fetus (**d; compare to Fig 7a**) and loss of imaging tissue planes.

Table Legends

Table 1. Example imaging parameters for the test scan and the high-resolution scan. Individual imaging parameters should be optimised depending on the size, iodination, and Micro-CT scanner.

	Kilovoltage	Current / µA	Exposure Time / ms	Frames per projection	Individual scan duration / minutes
Test scan	90 – 120	150 – 400	125 – 250	1	15
High resolution scan	90 – 120	150 – 400	250 – 500	2 – 4	45
Typical parameters for small fetus (< 100 grams)	100	<250	354	2	45
Typical parameters for large fetus (> 100 grams)	100	>200	354	2	45

Supplementary Information

Supplementary Note Post-mortem Examination Consent Form and guidance given to the consent taker used at Great Ormond Street Hospital, London, UK

Supplementary Manual Further guidance providing additional safety information covering the reagents used in the protocol

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