Bakeng se Afrika
Digital Skeletal Repository

Standard Operating Procedures (SOPs)
And Tutorials

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TABLE OF CONTENTS

1. STANDARD OPERATING PROCEDURES 4

SOP 1. PRE-SCANNING PROCEDURES 5

SOP 1.1. Selection of skeletal material for scanning 5

UP and SMU 5

SUN 11

SOP 1.2. Transportation of skeletal material to scanning facility 21

UP and SMU 21

SUN 23

SOP 1.3. Bone preparation before scanning 30

UP and SMU 30

SUN 34

SOP 2. SCANNING PROCEDURES 44

SOP 2.1. Scanning modalities 44

UP, SMU and SUN 44

SOP 2.2. Scanning and naming conventions 55

UP and SMU 55

SUN 58

SOP 2.3. Reconstructing procedures after scanning 68

UP and SMU 68

SUN 75

SOP 3. POST-SCANNING PROCEDURES 78

SOP 3.1. 2D stitching long bones scanned in two- or three-parts using ImageJ 78

SOP 3.2. 3D stitching long bones scanned in two- or three-parts using VGStudioMax 86

SOP 4. METADATA – PHOTOGRAPHY 108

SOP 4.1. Photographing crania 110

UP, SUN and SMU 100

SOP 4.2. Photographing postcrania 113

UP, SUN and SMU 113

SOP 4.3. Post-photography processing 115

1. STANDARD OPERATING PROCEDURES

SOP 1. PRE-SCANNING PROCEDURES

SOP 1.1. Selection of skeletal material for scanning
UP and SMU

(1.0) Purpose

The purpose of this document is to describe the skeletal elements that will be selected for scanning and what the exclusion criteria for the selection are. These criteria will ensure that only the most complete and undamaged (not altered by pathology, healed trauma, or postmortem damage) skeletal elements will be selected for scanning and will be added to the Bakeng se Afrika digital skeletal repository.

(2.0) Scope

This document and the guidelines herein will be used by skeletal collections managers or curators as well as by any researchers scanning skeletal material that will be added to the Bakeng se Afrika digital skeletal repository, including but not limited to the curator of the Kirsten Skeletal Collection (KSC, housed at the Stellenbosch University), the curator of the Pretoria Bone Collection (PBC, housed at the University of Pretoria) and the curator of the Human Bone Collection (HBC, housed at the Sefako Makgatho Health Sciences University).

(3.0) Definitions

The following terminology will be used throughout the standard operating procedures for skeletal element selection. Definitions are taken from The Human Bone Manual by T.D. White and P.A. Folkens (2005) (see Figure 1).

- Skull: the bones of the head, including both cranium and mandible
- Mandible: lower jaw
- Cranium: the bones of the skull except for the mandible
- Calvaria: the cranium not including the face
- Calotte: the calvaria without the base
- Cranial vault: in this instance the vault refers to the part of the calotte that is cut and separated from the rest of the cranium during dissection, in order to remove the brain
- Dentition: the teeth
- Femur: long bone of the thigh
- Radius: one of the two long bones of the forearm ± from elbow to wrist on the thumb side

- Axial skeleton: bones of the trunk including the vertebrae, ribs and sternum
- Postcrania: all bones except the cranium and mandible
- Proximal: nearest to the axial skeleton
- Distal: opposite of proximal; farthest from the axial skeleton

- Epiphyses: the cap at the end of a long bone that develops from secondary ossification centers
- Diaphysis: the shaft of the long bone
- Midshaft: the midpoint of the diaphysis (shaft of the bone)

- Cancellous bone: spongy, porous lightweight bone found under protuberances where tendons attach, in vertebral bodies, in the needs of long bones, in short bones, and within flat bones (also called trabecular bone)
- Cortical bone: the solid, dense bone that is found in the walls of bone shafts and on external bone surfaces (also called compact bone)

- Pathological lesion: an injury or wound; an area of pathologically altered tissue
- Antemortem trauma/changes: alterations to the bone that occur before the death of an individual (usually present with indicators of healing such as smoothed or rounded edges)
- Postmortem damage: damage that occurred to the bone after moisture was removed from the bone (damage to dry bone); usually associated with colour differences between the fracture edge and the bone surface

(4.0) Roles and Responsibilities

Collections managers/curators:

- Provide database of appropriate bones in the skeletal collection from which to select the specimens for scanning
- Provide a maximum number of bones that can be taken for scanning at a time
- Depending on institution, check whether the relevant bones are suitable for scanning (i.e., check for any of the exclusion criteria and remove from sample if necessary)

Researchers (if applicable):

- Depending on institution, check whether bones are appropriate for scanning (i.e., check for any of the exclusion criteria and remove from sample if necessary)

(5.0) Procedures

The skeletal material from each of the skeletal collections (Kirsten Skeletal Collection, Pretoria Bone Collection, Human Bone Skeletal Collection) will contribute a percentage towards the contents of the Bakeng se Afrika digital skeletal repository. As a variety of age groups, ancestry groups and sexes are necessary to create a holistic digital repository, each sex, ancestry and age group will make up a certain percentage of the repository (Table 1). The total number of skeletons available for scanning determines the number of skeletons that correspond to the percentages; however, the total number can only be determined once all unsuitable skeletons, as determined by the exclusion criteria, have been removed from the total sample.

The following skeletal elements will be assessed for each individual and if present and complete with limited damage (postmortem, antemortem or pathology), the individual can be added to the total sample for scanning:

1) The cranium (the cranial vault and the rest of the cranium need to be joined together using Prestick or masking tape (non-destructive) so that the cranium is in one piece – if successful reconstruction is not possible – exclude from sample). If no teeth are present (postmortem or antemortem loss) in the maxilla, the cranium can still be scanned, however, the scan for dentition will be excluded.
2) The mandible (in one piece – if cut or broken and can be reconstructed successfully using Prestick or masking tape (non-destructive) with minimal bone loss or distortion, then the mandible can be used. If successful reconstruction is not possible, then the bone has to be excluded from the sample). Loose teeth should be placed back in the appropriate alveolar socket and held in place with masking tape.
3) The maxillary dentition – in order to scan the dentition a minimum of 4 teeth per maxilla have to be present, otherwise the dentition should not be scanned. If at least four teeth are present, then the cranium can be considered for scanning with a focus on dentition. Loose teeth should be placed back in the appropriate alveolar socket and held in place with masking tape. If the teeth cannot be replaced in the bone, the teeth are not counted towards the minimum of four teeth that need to be present for scanning. If no teeth/ not enough teeth are present (postmortem or antemortem loss), the cranium and mandible can still be scanned separately, but the scan for dentition should be excluded for the individual. If the teeth to be scanned are mostly replaced by metallic dental fillings, those teeth should not be included or counted as the minimum number of teeth required for scanning. Furthermore, if the metallic fillings are present in all teeth, the dentition should not be scanned, as the metal will create artefacts in the scans, which can interfere with the reconstruction and analysis of the scans.
4) The left* femur (any bones with healed fractures or extensive pathological conditions, as well as any bones demonstrating surgical interventions, such as prosthetics, screws and plates, should be excluded).
5) The left* radius (any bones with healed fractures or extensive pathological conditions, as well as any bones demonstrating surgical interventions, such as prosthetics, screws and plates, should be excluded).

*Note: for long bones, the left side is typically used; however, if the left is absent or has been excluded, then the right side may be used instead. If neither left nor right is available, then that bone will simply be excluded from the scans for that particular individual.

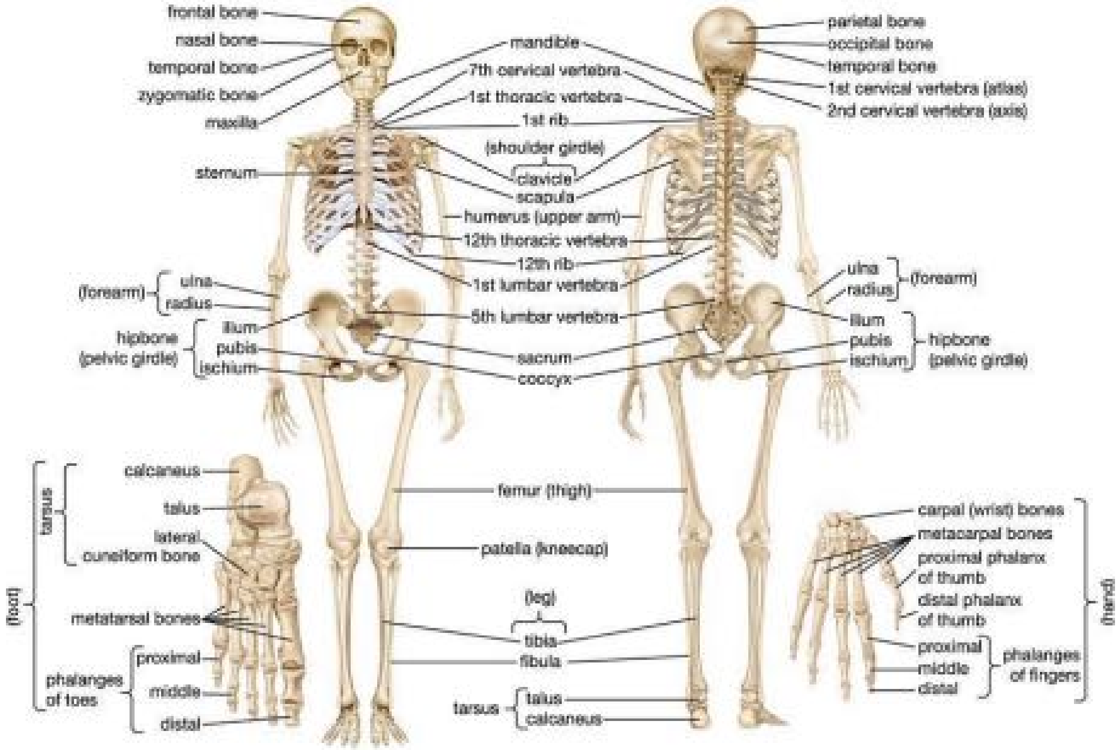

Figure 1. Image of annotated skeletons taken from Encyclopaedia Britannica (Available at: https://www.britannica.com/science/human-skeletal-system)

Table 1. Percentage of each demographic group that will constitute the initial sample of the Bakeng se Afrika digital skeletal repository.				
Demographic profile	Age cohort	Approx. percent of repository (%) [approx. indiv. numbers]	Maximum number available in cohort (PBC + KSC)***	Collection(s)
Black Males	18-29	2 [10]	23+6**	HBC (mainly), PBC, KSC
	30-39	3 [14]	47+12**	
	40-49	3 [14]	66+14**	
	50-59	3 [14]	73+9**	
	60-69	2 [10]	107+4**	
	70-79	2 [10]	52+2**	
	80-89	1.5 [7]	16+2**	
Black Females	18-29	2 [10]	8+2**	HBC (mainly), PBC, KSC
	30-39	3 [14]	17+2**	
	40-49	3 [14]	13+5**	
	50-59	3 [14]	16+4**	
	60-69	2 [10]	9+1**	
	70-79	2 [10]	5+0	
	80-89	1.5 [7]	0+1	
White Males	18-29	2 [10]	1+0	PBC, KSC
	30-39	3 [14]	1+1	
	40-49	3 [14]	9+5**	
	50-59	3 [14]*	22+6**	
	60-69	2 [10]*	38+7**	
	70-79	2 [10]	78+8**	
	80-89	1.5 [7]	45+1**	
White Females	18-29	2 [10]	2+0	PBC, KSC
	30-39	3 [14]	0+0	
	40-49	3 [14]	5+2	
	50-59	3 [14]	10+1	
	60-69	2 [10]*	28+5**	
	70-79	2 [10]*	51+6**	
	80-89	1.5 [7]	46+3**	
Coloured Males	18-29	2 [10]	7	KSC
	30-39	3 [14]*	29**	
	40-49	3 [14]	51**	
	50-59	3 [14]	41**	
	60-69	2 [10]	37**	
	70-79	2 [10]*	16**	
	80-89	1.5 [7]	4	
Coloured Females	18-29	2 [10]	12**	KSC
	30-39	3 [14]	16**	
	40-49	3 [14]	25**	
	50-59	3 [14]*	19**	
	60-69	2 [10]*	14**	
	70-79	2 [10]	4	
	80-89	1.5 [7]	3	
Total		99 [474]		

*Take more from these cohorts to make up for the limited numbers from adjacent cohorts
**Total required sample or exceeding sample sizes
***Currently the numbers do not include samples from SMU

SOP 1.1. Selection of skeletal material for scanning
SUN

Department of Biomedical Sciences
Division of Clinical Anatomy
Standard Operating Procedures

BAKENG SE AFRIKA DIGITAL SKELETAL REPOSITORY **SUN ANTHROPOLOGY RESEARCH UNIT** **RADIOLOGICAL IMAGING SOP: SELECTION OF MATERIAL**			
SOP#: *BsA.SOP.IMAGE.001 Material Selection*	**Version #: 2**	**Effective Date:** *(This document will be reviewed in two years from effective date)* **10 December 2019**	Page **1** of **9**

Author:	**Reviewers:** *(Signature confirms that the reviewers agree with the content of the document)*	**Approved by HOD** *(Signature confirms final approval)*
(Signature & Date) Name: G Kruger Designation: University of Pretoria: Curator of Pretoria Bone Collection	(Signature & Date) Name: A Alblas Designation: Stellenbosch University: KSC Curator	(Signature & Date) Dr K Baatjes HOD: Clinical Anatomy

TABLE OF CONTENTS

(1.0) PURPOSE & SCOPE: 3
1) PURPOSE: 3
2) SCOPE: 3
(2.0) DEFINITION: 3
(3.0) ABBREVIATION LIST 4
(4.0) Roles and RESPONSIBILITIES 4
All staff and students **Error! Bookmark not defined.**
Collections managers/curators: 4
Researchers (if applicable): 4
(5.0) BACKGOUND OF BAKENG SE AFRIKA PROJECT 5
OBJECTIVE OF BAKENG SE AFRIKA 5
RADIOLOGICAL IMAGES AS OUTPUTS OF BAKENG SE AFRIKA 5
MATERIAL TO BE SCANNED 6
(6.0) IMAGING PROCEDURES 8
(8.0) AVAILABILITY 9
(9.0) DOCUMENT HISTORY 9
(10.0) SUPPORTING DOCUMENTS 9

(1.0) PURPOSE & SCOPE:

1) PURPOSE:

The purpose of this document is to describe the skeletal elements that will be selected for scanning as well as exclusion criteria for selection. These criteria will ensure that only the most complete and undamaged (not altered by pathology, healed trauma, or postmortem damage) skeletal elements will be selected for scanning and added to the Bakeng se Afrika digital skeletal repository.

2) SCOPE:

This document and the guidelines herein will be used by skeletal collections managers or curators as well as by any researchers scanning skeletal material that will be added to the Bakeng se Afrika digital skeletal repository, including but not limited to the curator of the Kirsten Collection (housed at the Stellenbosch University), the curator of the Pretoria Bone Collection (housed at the University of Pretoria) and the curator of the Human Bone Collection (housed at the Sefako Makgatho Health Sciences University).

(2.0) DEFINITION:

The following terminology will be used throughout the standard operating procedures for skeletal element selection and the scanning procedures. Definitions are taken from The Human Bone Manual by T.D. White and P.A. Folkens (2005).

- Skull: the bones of the head, including both cranium and mandible
- Mandible: lower jaw
- Cranium: the bones of the skull except for the mandible
- Calvaria: the cranium not including the face
- Calotte: the calvaria without the base
- Cranial vault: in this instance, the vault refers to the part of the calotte that is cut and separated from the rest of the cranium during dissection, in order to remove the brain
- Dentition: the teeth
- Proximal: nearest to the axial skeleton
- Axial skeleton: bones of the trunk including the vertebrae, ribs and sternum
- Distal: opposite of proximal; farthest from the axial skeleton
- Epiphyses: the cap at the end of a long bone that develops from secondary ossification centres
- Diaphysis: the shaft of the long bone
- Midshaft: the midpoint of the diaphysis (shaft of the bone)
- Pathological lesion: an injury or wound; an area of pathologically altered tissue
- Antemortem trauma/changes: alterations to the bone that occur before the death of an individual (usually present with indicators of healing such as smoothed or rounded edges)

- Postmortem damage: damage that occurred to the bone after moisture was removed from the bone (damage to dry bone); usually associated with colour differences between the fracture edge and the bone surface
- Postcrania: all bones except the cranium and mandible
- Cancellous bone: spongy, porous lightweight bone found under protuberances where tendons attach, in vertebral bodies, in the neds of long bones, in short bones, and within flat bones (also called trabecular bone)
- Cortical bone: the solid, dense bone that is found in the walls of bone shafts and on external bone surfaces (also called compact bone)
- Femur: long bone of the thigh
- Radius: one of the two long bones of the forearm – from elbow to wrist on the thumb side

(3.0) ABBREVIATION LIST

2D- Two dimensional
3D- Three dimensional
CT- computed tomography
CBCT - Cone beam computed tomography
DICOM - Digital Imaging and Communications in Medicine
FOV - Field of view
HEI - Higher Education Institutions
KSC - Kirsten Skeletal Collection
Micro-XCT - microfocus scanning computed tomography
PI – Principle investigator
VSD- Virtual Skeletal Database

(4.0) ROLES AND RESPONSIBILITIES

Collections managers/curators:

- If the scanning of bones is linked to a research project, ensure that the relevant ethics consent has been provided to the researcher prior to handing the bones for scanning.
- Provide database of appropriate bones in the skeletal collection from which to select the specimens for scanning.
- Provide a maximum number of bones that can be taken for scanning at a time

Researchers (if applicable):

- Depending on the institution, check whether the relevant bones are suitable for scanning (i.e. check for any of the exclusion criteria and remove from the sample if necessary)
- Ensure that skeletal material to be scanned will be handled with care by all involved to prevent any damages.

(5.0) BACKGROUND OF BAKENG SE AFRIKA PROJECT

Stellenbosch University, along with the University of Pretoria (South Africa), Sefako Makgatho Health Sciences University (South Africa), the Nuclear Energy Corporation of South Africa (NECSA) (South Africa), the University of Bordeaux (France), the University of Coimbra (Portugal) and the Catholic University Leuven (Belgium) received funding from the Education, Audiovisual and Culture Executive Agency of the European Commission through the Erasmus+ Programme. This programme is the EU's mode to support education, training, youth and sport and it has opportunities for a wide variety of individuals and organisations. The University of Pretoria is the lead institution on the project and the project has been entitled "Bakeng se Afrika".

OBJECTIVE OF BAKENG SE AFRIKA

The objective of Bakeng se Afrika is to develop and manage a comprehensive digital imaging collection of South Africans as a capacity-building resource for improvement in the internationalisation of Higher Education Institutions (HEI). This digital archive of images from various radiological modalities will be internationally accessible. The Kirsten Anthropology Research Unit as part of the Division of Clinical Anatomy at Stellenbosch University (SU) that houses the established Kirsten Skeletal Collection (KSC) is one of three skeletal collections to be used in this project.

RADIOLOGICAL IMAGES AS OUTPUTS OF BAKENG SE AFRIKA

- Outputs will impact teaching in HEI'S. The development of online curricula in the medical and health sciences are strongly dependent on imaging and digital data.
- Outputs will impact research in HEI's. Research will be done by postgraduate students, and thereby increase the capacity of universities to train professionals. Many researchers prefer using digital images when compared to other data sources due to the ease of accessibility, large sample size and its non-destructive nature. Furthermore, research will aid in creating new methodologies or validating those already in use.
- Outputs will impact medico-legal investigations for deceased individuals. Forensic anthropologists' role is to estimate the biological profile of deceased individuals whose remains are in advanced stages of decomposition, skeletonised, mutilated and burnt. To describe these biological parameters, skeletal collections of known individuals are needed as reference samples. To alleviate the effects of secular trends, ethical and religious issues, the need for physical storage space and the time-consuming process of maceration associated with having a physical skeletal collection, non-invasive radiological modalities are used, therefore a need to create virtual skeletal databases are pursued. Imaging is rapid and can be done without maceration of bones. This ensures that no information is lost and that the anatomical relationship of the bones is kept.
- Business such as dental implant companies will also benefit from these digital collections.

MATERIAL TO BE SCANNED

The dry-bone to be used in the proposed study will be from the Kirsten Skeletal Collection (KSC) that is currently housed in the FISAN building, room F219, Division of Clinical Anatomy, Stellenbosch University. The KSC is a repository for skeletal remains deriving from cadavers used allocated to this institution for research and teaching purposes. Skeletal elements used during research projects are returned to the KSC after completion of the study. All identification records of individuals are anonymised with skeletal numbers and personal records are unknown to the researchers. Additionally, no medical records of any of the skeletons are known to the Department or any of the researchers.

The skeletal material from each of the skeletal collections (Kirsten Collection, Pretoria Bone Collection, SMU Skeletal Collection) will contribute a percentage towards the contents of the Bakeng se Afrika digital skeletal repository. As a variety of age groups, ancestry groups and sexes are necessary to create a holistic digital repository, each sex, ancestry and age group will make up a certain percentage of the repository. The total number of skeletons available for scanning determines the number of skeletons that correspond to the percentages; however, the total number can only be determined once all unsuitable skeletons, as determined by the exclusion criteria, have been removed from the total sample.

Table 1.1. Percentage of each demographic group that will constitute the initial sample of the Bakeng se Afrika digital skeletal repository.

Demographic profile	Age cohort	Approx. percent of repository (%) [approx. indiv. numbers]	Maximum number available in cohort (PBC + KC)***	Collection(s)
Black Males	18-29	2 [10]	23+6**	SMU (mainly), PBC, SU
	30-39	3 [14]	47+12**	
	40-49	3 [14]	66+14**	
	50-59	3 [14]	73+9**	
	60-69	2 [10]	107+4**	
	70-79	2 [10]	52+2**	
	80-89	1.5 [7]	16+2**	
Black Females	18-29	2 [10]	8+2**	SMU (mainly), PBC, SU
	30-39	3 [14]	17+2**	
	40-49	3 [14]	13+5**	
	50-59	3 [14]	16+4**	
	60-69	2 [10]	9+1**	
	70-79	2 [10]	5+0	
	80-89	1.5 [7]	0+1	
White Males	18-29	2 [10]	1+0	PBC, SU
	30-39	3 [14]	1+1	
	40-49	3 [14]	9+5**	
	50-59	3 [14]*	22+6**	

	60-69	2 [10]*	38+7**	
	70-79	2 [10]	78+8**	
	80-89	1.5 [7]	45+1**	
White Females	18-29	2 [10]	2+0	PBC, SU
	30-39	3 [14]	0+0	
	40-49	3 [14]	5+2	
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	60-69	2 [10]*	28+5**	
	70-79	2 [10]*	51+6**	
	80-89	1.5 [7]	46+3**	
Coloured Males	18-29	2 [10]	7	SU
	30-39	3 [14]*	29**	
	40-49	3 [14]	51**	
	50-59	3 [14]	41**	
	60-69	2 [10]	37**	
	70-79	2 [10]*	16**	
	80-89	1.5 [7]	4	
Coloured Females	18-29	2 [10]	12**	SU
	30-39	3 [14]	16**	
	40-49	3 [14]	25**	
	50-59	3 [14]*	19**	
	60-69	2 [10]*	14**	
	70-79	2 [10]	4	
	80-89	1.5 [7]	3	
Total		99 [474]		

* take more from these cohorts to make up for the limited numbers from adjacent cohorts
**Total required sample or exceeding sample sizes
***Currently the numbers do not include samples from SMU

(6.0) ETHICAL USE OF THIS DIGITAL DATA

- Research on human skeletal remains is allowed and regulated by the: Human Tissues Act (No 65 of 1983), and National Health Act (No 61 of 2003). Ethical approval of the project has been obtained from the Health Research Ethics Committee, Stellenbosch University (S19/05/099).

- Meta-data associated with images and personal information pertaining to the skeletal remains of individuals in the KSC should be removed or hidden from investigators to ensure anonymity.

- The National Health Act makes provision for using these cadavers for teaching/research purposes, provided that some form of consent has been documented. In circumstances where original (donor) consent and next of kin consent cannot be obtained, as is the case in this study for some individuals, legislation allows for the Inspector of Anatomy, as the representative of the Director-General, to provide proxy consent, provided that he/she is satisfied that attempts have been made to contact next of kin for consent.

- All samples will be identified only by a coded identification number and only pertinent information of each patient will be captured, including age, sex, and self-identified population group as indicated on the records. No meta-data associated with images will be made available to the participant's family. It is not expected that the study participant or their family will directly benefit in any way to this project. However, we note that this research study is likely to more generally benefit knowledge discovery. The findings will not affect the health status of any individual and will be used only for academic purposes.

(7.0) SELECTION OF MATERIAL PROCEDURES

The following skeletal elements will be assessed for each individual and if present and complete with limited damage (postmortem, antemortem or pathology), the individual can be added to the total sample for scanning:

1) The mandible (in one piece – if cut or broken and can be reconstructed successfully using Prestick or masking tape (non-destructive) with minimal bone loss or distortion, the mandible can be used. If successful reconstruction is not possible, the bone must be excluded from the sample). If no teeth are present (postmortem or antemortem loss), the mandible can still be scanned, however, the scan for dentition will be excluded for the individual.
2) The cranium (the cranial vault and the rest of the cranium need to be joined using Prestick or masking tape (non-destructive) so that the cranium is in one piece – if successful reconstruction is not possible – exclude from the sample). If no teeth are present (postmortem or antemortem loss) in the maxilla, the cranium can still be scanned, however, the scan for dentition will be excluded.
3) The dentition – in order to scan the dentition a minimum of 8 teeth (at least 4 per maxilla and mandible) must be present, otherwise, the dentition should not be scanned. If at least eight teeth are present, the cranium and mandible can be considered for scanning dentition. Loose teeth should be placed back in the appropriate alveolar socket and held in place with masking tape. If the teeth cannot be replaced in the bone, the teeth are not counted towards the minimum of 8 teeth that need to be present for scanning. If no teeth/ not enough teeth are present (postmortem or antemortem loss), the cranium and mandible can still be scanned separately, but the scan for dentition should be excluded for the individual. If the teeth to be scanned are mostly replaced by metallic dental fillings, those teeth should not be included or counted as the minimum number of teeth required for scanning. Furthermore, if the metallic fillings are present in all teeth, the dentition should not be scanned, as the metal will create artefacts in the scans, which can interfere with the reconstruction and analysis of the scans.

4) The left* femur (any bones with healed fractures or extensive pathological conditions, as well as any bones demonstrating surgical interventions, such as prosthetics, screws and plates, should be excluded).
5) The left* radius (any bones with healed fractures or extensive pathological conditions, as well as any bones demonstrating surgical interventions, such as prosthetics, screws and plates, should be excluded).

*Note: for long bones, the left side is typically used; however, if the left is absent or has been excluded, then the right side may be used instead. If neither left nor right is available, then that bone will simply be excluded from the scans for that individual.

(8.0) SURETY:
Quality is provided in the peer review process VISUN Laboratory Manual, Part III, SOP 3.1 (Peer Review). If deficiencies in images are noted, the primary analyst will ensure that images are retaken appropriately. Forensic imaging is also subject to audits VISUN Laboratory Manual, Part III, SOP 3.3 (Audits).

(9.0) AVAILABILITY
The original signed version of this document is kept by the Curator of the Kirsten Skeletal Collection. Training documents available in personnel folders. A copy of the document can be found on the document management system ALFRESCO.

(10.0) DOCUMENT HISTORY

Version No.	Date	Location of Change History	Author/ Reviewer	Approving Official	Date Approved	Next Review Date
1.0	20 Nov 2019		A Alblas			2020

(11.0) SUPPORTING DOCUMENTS
Appendix 1

SOP 1.2. Transportation of skeletal material to scanning facility UP and SMU

(1.0) Purpose

The purpose of this document is to describe the procedure for the transportation of skeletal material for scanning at NECSA or any other scanning facility for addition to the Bakeng se Afrika digital skeletal repository.

(2.0) Scope

This document and the guidelines herein will be used by skeletal collections managers or curators as well as by any researchers or contractors transporting skeletal material that will be added to the Bakeng se Afrika digital skeletal repository. This includes but is not limited to the curator of the Kirsten Skeletal Collection (KSC, housed at the Stellenbosch University), the curator of the Pretoria Bone Collection (PBC, housed at the University of Pretoria) and the curator of the Human Bone Collection (HBC, housed at the Sefako Makgatho Health Sciences University) or any researchers or contractors tasked with transporting skeletal material to NECSA or any other scanning facility.

(3.0) Definitions

For definition of the terminology used in the current document, see SOP 1.1.

(4.0) Roles and Responsibilities

Collections managers/curators:

- Make sure all skeletal material is labelled clearly and correctly to alleviate any chance of mixing up of skeletal material from different individuals.
- Pack bones securely for transport
- Provide a permission letter for transport that includes the specimen numbers and the bones that will be transported

Researchers/contractors (if applicable):

- Depending on institution, pack bones securely for transport
- Make sure the appropriate permission letter is present and correct for the transportation of skeletal material

(5.0) Procedures

All skeletal material that will be transported to NECSA or any other scanning facility will need to be packed tightly and securely so as to limit any potential damage to the material during transportation. Cardboard or plastic boxes lined with bubble wrap make adequate transportation containers and can typically hold multiple bones at a time. A permission letter for transportation is necessary to be able to remove bones from campus and to make sure a complete list of specimens is available to check upon return of the skeletal material to the collections.

(6.0) Appendix

Example of a permission letter for transportation of skeletal material from the Pretoria Bone Collection, housed in the Department of Anatomy, University of Pretoria.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences
Department of Anatomy

Date

Dear [Name of researcher transporting bones]

The Use of Human Skeletal Material from the Department of Anatomy, University of Pretoria for microCT scanning at NECSA

You are hereby granted the temporary use of the following skeletal remains, which are permanently housed in the Pretoria Bone Collection in the Department of Anatomy at the University of Pretoria:

- CadNrX – cranium and mandible
- CadNrY – left femur
- CadNrZ – left radius

Under no circumstances may bone samples be taken. The Department of Anatomy requests that the skeletal material is to be kept in a locked room until the time of examination, and that it is returned to the Department of Anatomy within an acceptable time period.

Sincerely,

____

SOP 1.2. Transportation of skeletal material to scanning facility
SUN

Department of Biomedical Sciences
Division of Clinical Anatomy
Standard Operating Procedures

BAKENG SE AFRIKA DIGITAL SKELETAL REPOSITORY **SUN ANTHROPOLOGY RESEARCH UNIT** **RADIOLOGICAL IMAGING SOP: TRANSPORT OF MATERIAL**	

SOP#: *BsA.SOP.IMAGE.002 Material Transport*	**Version #: 2**	**Effective Date:** *(This document will be reviewed in two years from effective date)* 10 December 2019	Page **1** of **6**

Author:	**Reviewers:** *(Signature confirms that the reviewers agree with the content of the document)*	**Approved by HOD** *(Signature confirms final approval)*
(Signature & Date) Name: G Kruger Designation: University of Pretoria: Curator of Pretoria Bone Collection	(Signature & Date) Name: A Alblas Designation: Stellenbosch University: KSC Curator	(Signature & Date) Dr K Baatjes HOD: Clinical Anatomy

TABLE OF CONTENTS

(1.0) PURPOSE & SCOPE: 3
1) PURPOSE: 3
2) SCOPE: 3
(2.0) DEFINITION: 3
(3.0) ABBREVIATION LIST 3
(4.0) ROLES AND RESPONSIBILITIES 3
All staff and students 3
Collections managers/curators: 3
Researchers (if applicable): 4
(5.0) transport of material PROCEDURES 4
(6.0) SURETY: 4
(7.0) AVAILABILITY 5
(8.0) DOCUMENT HISTORY 5
(9.0) SUPPORTING DOCUMENTS 5

(1.0) PURPOSE & SCOPE:

1) PURPOSE:

The purpose of this document is to describe the procedure for the transportation of skeletal material for scanning at NECSA, X-sight, or any other scanning facility for addition to the Bakeng se Afrika digital skeletal repository.

2) SCOPE:

This document and the guidelines herein will be used by skeletal collections managers or curators as well as by any researchers scanning skeletal material that will be added to the Bakeng se Afrika digital skeletal repository, including but not limited to the curator of the Kirsten Collection (housed at the Stellenbosch University), the curator of the Pretoria Bone Collection (housed at the University of Pretoria) and the curator of the Human Bone Collection (housed at the Sefako Makgatho Health Sciences University) or any researchers or contractors tasked with transporting skeletal material to NECSA, X-sight, or any other scanning facility.

(2.0) DEFINITION:

For the definition of the terminology used in the current document, see *BsA.SOP.IMAGING.001*: Selection of skeletal material for scanning.

(3.0) ABBREVIATION LIST

For a list of abbreviations used in the current document, see *BsA.SOP.IMAGING.001*: Selection of skeletal material for scanning.

(4.0) ROLES AND RESPONSIBILITIES

All staff and students

- Be aware of the correct operating and maintenance procedures of the imaging methodology used. No person will be allowed to operate any equipment without prior training and/or certification.

Collections managers/curators:

- If the scanning of bones is linked to a research project, check that the relevant consent has been provided to the researcher prior to handing the bones for scanning.
- Provide a database of appropriate bones in the skeletal collection from which to select the specimens for scanning.
- Provide a maximum number of bones that can be taken for scanning at a time.
- Make sure all skeletal material is labelled clearly and correctly to alleviate any chance of mixing up of skeletal material from different individuals.
- Pack bones securely for transport

- Provide a permission letter for transport that includes the specimen numbers and the bones that will be transported.

Researchers (if applicable):

- Depending on the institution, check whether the relevant bones are suitable for scanning (i.e. check for any of the exclusion criteria and remove from the sample if necessary)
- Ensure the correct consent has been given to transport, store and scan all skeletal material.
- Ensure that once they skeletal material is scanned that it is returned to the correct box with the corresponding AN number.
- Ensure that skeletal material to be scanned will be handled with care by all involved to prevent any damages.
- Depending on institution, pack bones securely for transport
- Make sure the appropriate permission letter is present and correct for the transportation of skeletal material
-

(5.0) TRANSPORT OF MATERIAL PROCEDURES

All skeletal material that will be transported to scanning facilities will be packed tightly and securely so as to limit any potential damage to the material during transportation. Cardboard or plastic boxes lined with bubble wrap make adequate transportation containers and can typically hold multiple bones at a time and ensure that the skeletal material is not visible to the public while transported.

A permission letter for transportation is necessary to be able to remove bones from campus and to make sure a complete list of specimens is available to check upon return of the skeletal material to the collections.

The Inspector of Anatomy will approve transport of skeletal material to scanning modalities. During transportation, the letter of consent from the Inspector of Anatomy will remain with the samples.

Storage of the skeletal material while scanning will be secured in a locked cabinet at the radiological facility, the skeletal material will remain covered unless it is being scanned.

(6.0) SURETY:

Quality is provided in the peer review process VISUN Laboratory Manual, Part III, SOP 3.1 (Peer Review). If deficiencies in images are noted, the primary analyst will ensure that images are retaken appropriately. Forensic imaging is also subject to audits VISUN Laboratory Manual, Part III, SOP 3.3 (Audits).

(7.0) AVAILABILITY

The original signed version of this document is kept by the Curator of the Kirsten Skeletal Collection. Training documents available in personnel folders. A copy of the document can be found on the document management system ALFRESCO.

(8.0) DOCUMENT HISTORY

Version No.	Date	Location of Change History	Author/ Reviewer	Approving Official	Date Approved	Next Review Date
1.0	20 Nov 2019		A Alblas			2020

(9.0) SUPPORTING DOCUMENTS

Appendix 1: Example of permission letter for transport of skeletal material from the KSC, housed at the Division of Anatomy, Stellenbosch University.

UNIVERSITEIT · iYUNIVESITHI · STELLENBOSCH · UNIVERSITY

100

1918 · 2018

Date

Dear [Name of researcher transporting bones]

The Use of Human Skeletal Material from the Division of Anatomy, Stellenbosch University for microCT scanning at NECSA

You are hereby granted the temporary use of the following skeletal remains, which are permanently housed in the Kirsten Skeletal Collection in the Division of Anatomy at Stellenbosch University:

- CadNrX – cranium and mandible
- CadNrY – left femur
- CadNrZ – left radius

Under no circumstances may bone samples be taken. The Division of Anatomy requests that the skeletal material is to be kept in a locked room until the time of examination and that it is returned to the Division of Anatomy within an acceptable time period.

Sincerely,

____

A Alblas

Curator: Kirsten Skeletal Collection
Department of Biomedical Sciences
Faculty of Medicine and Health Sciences
Stellenbosch University
Tel: 021 938 9394
Cell: 0825771645
Email: aa2@sun.ac.za

saam vorentoe · masiye phambili · forward together

SOP 1.3. Bone preparation before scanning
UP and SMU

(1.0) Purpose

The purpose of this document is to detail the bone preparations and pre-scan procedures to be completed when preparing crania for scanning for addition to the Bakeng se Afrika digital skeletal repository.

(2.0) Scope

This document and the guidelines herein will be used by skeletal collections managers or curators, researchers, contractors and/or the individuals responsible for preparing the skeletal elements for scanning once the material is at the scanning facility (NECSA/ other scanning facility).

(3.0) Definitions

For definition of the terminology used in the current document, see SOP 1.1.

(4.0) Roles and Responsibilities

The preparation of the skeletal elements for scanning, which includes replacing teeth and reconstructing the cranial vault with the rest of the cranium will be completed by the curator, researcher or contractor that brought the skeletal material for scanning. The stabilisation of the bones within the scanner will be the responsibility of the person tasked with operating the micro-XCT scanner at NECSA/ any other scanning facility.

(5.0) Procedures

- **Crania**

If the cranium is not already whole or if this was not already completed prior to transportation, the cranium needs to be reconstructed using Prestick or masking tape (non-destructive) to attach the cranial vault to the rest of the cranium. Any loose maxillary teeth should be taped into their appropriate alveolar sockets in the maxilla using Prestick or masking tape (non-destructive).

Once the cranium is in one piece, with the help of the scientists of the scanning facility, the skeletal element will need to be stabilised using styrofoam, polystyren or cardboard (materials that have completely different densities to bone) within the scanner. These materials have no influence on the scanned data due to their low-density nature.

- **Mandibles**

If the mandible is not already whole or if this was not already completed prior to transportation, the mandible needs to be reconstructed using Prestick or masking tape (non-destructive) to attach the pieces of the mandible together. Any loose mandibular teeth should be taped into their appropriate alveolar sockets in the mandible using Prestick or masking tape (non-destructive).

Once the mandible is in one piece and all teeth are replaced in the bone, the mandible will need to be stabilised using Styrofoam or cardboard (materials that have completely different densities to bone) within the scanner. These materials have no influence on the scanned data due to their low-density nature.

- **Focus on dentition**

The cranium does not need to be reconstructed for the scanning of the maxillary dentition. With the help of the scientists of the scanning facility, the maxilla will need to be stabilised using styrofoam, polystyren or cardboard (materials that have completely different densities to bone) within the scanner. These materials have no influence on the scanned data due to their low-density nature.

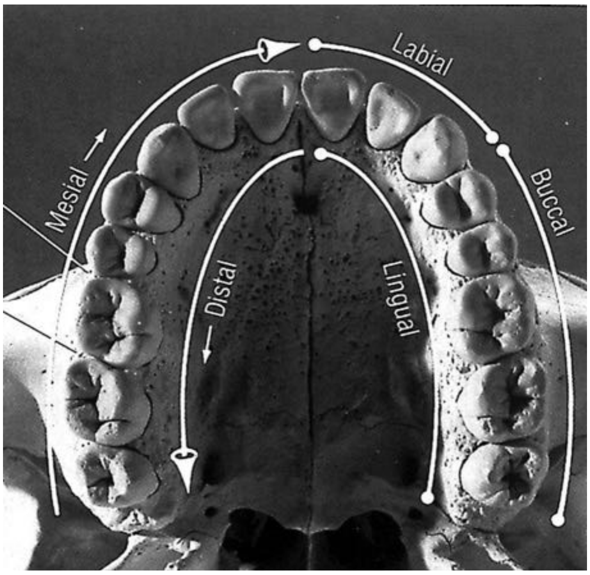

Figure 2. Image illustrating maxillary dentition and the directional terminology associated with dentition.

- **Femora**

With the help of the scientists of the scanning facility, the femur will need to be positioned vertically and stabilised using styrofoam, polystyren or cardboard (materials that have completely different densities to bone) within the scanner. These materials have no influence on the scanned data due to their low-density nature.

Because the femora are too large to scan in one part at an adequate resolution, the bone will need to be scanned in three parts: the proximal end, the diaphysis and the distal end respectively named A, B and C, which can then be merged. A small portion of the proximal part will be visible in the scan of the shaft, and a small portion of the shaft part will be also scanned with the distal part. Small pieces of Prestick or clay will need to be placed on the proximal one-third and on the distal one-third of the diaphysis so that during the reconstruction/stitching/merging phase, those areas can be recognised and overlapped to reconstruct the entire femur accurately.

In addition, when possible, for each femur, a focus will be made on the proximal and distal epiphyses.

Two femora can be scanned together. Therefore, in order to be able to identify the femora in the virtual images, another marker made of Prestik will be placed in one of the two femora.

- **Radii**

With the help of the scientists of the scanning facility, the radii will need to be positioned vertically and stabilised using styrofoam, polystyren or cardboard (materials that have completely different densities to bone) within the scanner. These materials have no influence on the scanned data due to their low-density nature.

Because the radii are too large to scan in one part at an adequate resolution, the bones will need to be scanned in two parts: the proximal halves and the distal halves, respectively named A and B, which can then be merged. A small portion of the proximal part will be visible in the scan of the distal part. Therefore, in order to be able to identify the parts in the virtual images, a small piece of Prestick or clay will need to be placed at the level of the middle of the diaphysis so that during the reconstruction/stitching/merging phase, that area can be recognised and overlapped to reconstruct the entire radius accurately.

Two radii can be scanned together. Therefore, in order to be able to identify the radii in the virtual images, another marker made of Prestik will be placed in one of the two radii.

Approval Signatures:

G Kruger		08/04/2020
Quality Assurer 1	Signature	Date
C Theye		08/04/2020
Quality Assurer 2	Signature	Date

SOP 1.3. Bone preparation before scanning
SUN

Department of Biomedical Sciences
Division of Clinical Anatomy
Standard Operating Procedures

BAKENG SE AFRIKA DIGITAL SKELETAL REPOSITORY
SUN ANTHROPOLOGY RESEARCH UNIT
RADIOLOGICAL IMAGING SOP: SELECTION OF MATERIAL

SOP#:	**Version #: 2**	**Effective Date:**	
BsA.SOP.IMAGE.001 Prep for Radiological imaging		*(This document will be reviewed in two years from effective date)* 10 December 2019	Page **1** of **9**

Author:	**Reviewers:**	**Approved by HOD**
	(Signature confirms that the reviewers agree with the content of the document)	*(Signature confirms final approval)*
(Signature & Date)	(Signature & Date)	(Signature & Date)
Name: G Kruger Designation: University of Pretoria: Curator of Pretoria Bone Collection	Name: A Alblas Designation: Stellenbosch University: KSC Curator	Dr K Baatjes HOD: Clinical Anatomy

TABLE OF CONTENTS

(1.0) PURPOSE & SCOPE: 3
1) PURPOSE: 3
2) SCOPE: 3
(2.0) DEFINITION: 3
(3.0) ABBREVIATION LIST 3
(4.0) ROLES AND RESPONSIBILITIES 3
All staff and students 3
Collections managers/curators/contractors: 3
Scanning technician/radiographer: 3
(5.0) PREPARATION OF MATERIAL FOR SCANNING 4
1) CRANIUM 4
2) MANDIBLE 4
3) DENTITION 4
4) FEMORA 6
5) RADII 6
6) RIBS 7
7) VERTEBRAE 8
(6.0) SURETY: 8
(7.0) AVAILABILITY 9
(8.0) DOCUMENT HISTORY 9

(1.0) PURPOSE & SCOPE:

1) PURPOSE:

The purpose of this document is to detail the bone preparations and pre-scan procedures to be completed when preparing various skeletal elements for scanning for addition to the Bakeng se Afrika digital skeletal repository.

2) SCOPE:

This document and the guidelines herein will be used by skeletal collections managers or curators as well as by any researchers scanning skeletal material that will be added to the Bakeng se Afrika digital skeletal repository, including but not limited to the curator of the Kirsten Collection (housed at the Stellenbosch University), the curator of the Pretoria Bone Collection (housed at the University of Pretoria) and the curator of the Human Bone Collection (housed at the Sefako Makgatho Health Sciences University) or any researchers or contractors and/or the individuals responsible for preparing the skeletal elements for scanning once the material is at the scanning facilities.

(2.0) DEFINITION:

For the definition of the terminology used in the current document, see *BsA.SOP.IMAGE.001*: Selection of skeletal material for scanning.

(3.0) ABBREVIATION LIST

For a list of abbreviations used in the current document, see *BsA.SOP.IMAGE.001*: Selection of skeletal material for scanning.

(4.0) ROLES AND RESPONSIBILITIES

All staff and students

- Be aware of the correct operating and maintenance procedures of the imaging methodology used. No person will be allowed to operate any equipment without prior training and/or certification.

Collections managers/curators/contractors:

- The preparation of the skeletal elements for scanning, which includes replacing teeth and reconstructing the cranial vault with the rest of the cranium will be completed by the curator, researcher or contractor that brought the skeletal material for scanning.

Scanning technician/radiographer:

- Check whether the relevant bones are suitable for scanning.
- Ensure that once they skeletal material is scanned that it is returned to the correct box with the corresponding AN number.
- Ensure that skeletal material to be scanned will be handled with care by all involved to prevent any damages.

- The stabilisation of the bones within the scanner will be the responsibility of the person tasked with operating the micro-XCT scanner at the scanning facility.

(5.0) PREPARATION OF MATERIAL FOR SCANNING

Micro-XCT is taken at X-Sight™ X-Ray Services (Unit 2, Gable Business Park, 10 Derrick Drive, Somerset West, 7130), using a Nikon XT H225 ST Micro-CT scanner or at the South African Nuclear Energy Corporation (NECSA) (Elias Motosaeledi Street Extension, R104 Pelindaba, Madibeng Municipality, North West Province, 0240).

The following procedures for scanning of skeletal elements are specifically for the MicroCT-scanning facility at NECSA.

1) CRANIUM

If the cranium is not already whole or if the different bones of the skull was not already completed before transportation, the cranium needs to be reconstructed using Prestick or masking tape (non-destructive) to attach the cranial vault to the rest of the cranium. Any loose maxillary teeth should be taped into their appropriate alveolar sockets in the maxilla using Prestick or masking tape (non-destructive). Once the cranium is in one piece, the skeletal element will need to be stabilised using Styrofoam or cardboard (materials that have completely different densities to bone) within the scanner.

2) MANDIBLE

If the mandible is not already whole or if this was not already completed prior to transportation, the mandible needs to be reconstructed using Prestick or masking tape (non-destructive) to attach the pieces of the mandible. Any loose mandibular teeth should be taped into their appropriate alveolar sockets in the mandible using Prestick or masking tape (non-destructive). Once the mandible is in one piece and all teeth are replaced in the bone, the mandible will need to be stabilised using Styrofoam or cardboard (materials that have completely different densities to bone) within the scanner.

3) DENTITION

See above for reconstruction and preparation of the mandible for scanning. The cranium does not need to be reconstructed for the scanning of the dentition; however, if this is already completed for a different scan then the reconstructed cranium can be used. For the scanning of the dentition, the mandible and cranium need to be articulated with the mandibular condyles (Fig 3.1) fitting into the mandibular fossae (Figure 3.2) and the occlusal surfaces of the teeth fitting together where possible (Figure 3.4). Once the cranium and mandible are appropriately articulated, the bones will need to be stabilised using Styrofoam or cardboard or clay (materials that have completely different densities to bone) within the scanner.

Figure 3.1. Mandible with the mandibular condyles highlighted.

Figure 3.2. Articulated mandible and cranium.

Figure 3.3. The inferior aspect of the cranium illustrating the mandibular fossa where the mandible articulates (highlighted)

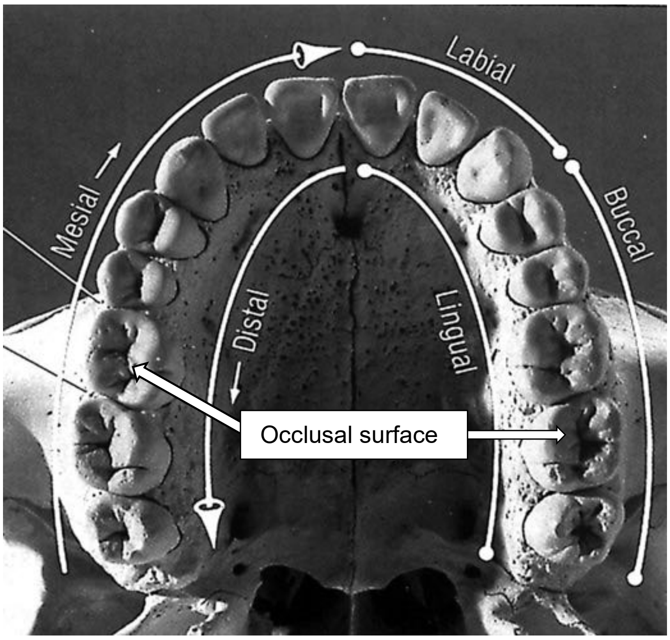

Figure 3.4. Image illustrating maxillary dentition and the directional terminology associated with dentition.

4) FEMORA

Because the femora are too large to scan in one part at an adequate resolution, the bone will need to be scanned in three parts, the proximal end, the diaphysis and the distal end. In order to be able to reconstruct the separate scans to create scans that include the entire femur, two areas will need to be demarcated on the bones using Prestick or clay. Small pieces of the Prestick or clay will need to be placed on the proximal one-third and the distal one-third of the diaphysis so that during the reconstruction phase, those areas can be recognised and overlapped to reconstruct the femur accurately. The marked areas indicate up until where the different scans should be taken. Therefore, when the femur is scanned for the first time, the proximal end up until the marker (Prestick or clay on the proximal one-third of the diaphysis) should be the focus of the scan, whereas the second scan should focus on the part of the diaphysis between the two markers (on the proximal and distal on-thirds, respectively). The third and final scan should focus on the distal part of the femur from the marker on the distal one-third of the diaphysis to the distal end of the femur. The resolutions, and therefore the sizes of the parts scanned, should be as equal as possible for the three parts of the femur. The femur will need to be stabilised within the scanner using Styrofoam, cardboard or clay.

5) RADII

Because the radii are too large to scan in one part at an adequate resolution, the bones will need to be scanned in two parts, the proximal halves and the distal halves. In order to be able to reconstruct the separate scans to create scans that include an entire radius, the midpoint of the bone will need to be demarcated using Prestick or clay. A small piece of the Prestick or clay will need to be placed at the level of the middle of the diaphysis so that during the reconstruction phase, that area can be recognised and overlapped to reconstruct the radius accurately. The marked areas also indicate up until where the different scans are to be taken. Therefore, when the radius is scanned for the first time, the proximal end up until the midpoint marker (Prestick or clay at the midpoint of the diaphysis) should be the focus of the scan, whereas the second scan should focus on the distal part of the radius from the marker on at midpoint to the distal end of the radius. The resolutions, and therefore the sizes of the parts scanned, should be as equal as possible for the two parts of the radius. The radius will need to be stabilised within the scanner using Styrofoam, cardboard or clay.

6) RIBS

Left and right ribs are scanned separately and are rolled in bubble wrap and separated by cardboard sheets. This is to ensure that ribs do not come into contact with one another, as this complicates post-processing and the separation of elements. Each radiograph is labelled according to the AN number of the sample, as well as the anatomical region being scanned. During post-processing, individual skeletal elements may be exported and labelled accordingly.

Figure 5.1: Ribs placed and wrapped in preparation for scanning.

Figure 5.2: Ribs mounted within bubble wrap, separated by cardboard.

With a larger detector, like the one at NECSA, the left and right ribs can be scanned together. The ribs are ordered from one to twelve and packed out on the top 4 sheets of A2 art foam. The ribs are placed in anatomical position (e.g. Left first rib and right first rib next to each other with the head of the rib at the same end). The sheets are folded and packed so that the ribs are in ascending order. Masking tape is wrapped around the four folded sheets to secure it in place and to ensure no movement during the scanning process.

7) VERTEBRAE

Vertebrae are scanned in ascending order from C1 to T12. The vertebra are stacked in sections, number of vertebra dependant on the field of view. The vertebra are either placed in Styrofoam (as scanned at Necsa) or the vertebrae are stacked on a rod (Fig. 6.1) with carboard separating each vertebra (Fig. 6.2) (as scanned at X-Sight). This is done to ensure that the vertebrae do not come in contact with one another, as this complicates post-processing and the separation of elements. The mounted vertebrae are then attached to the rotating table within the Micro XCT scanner.

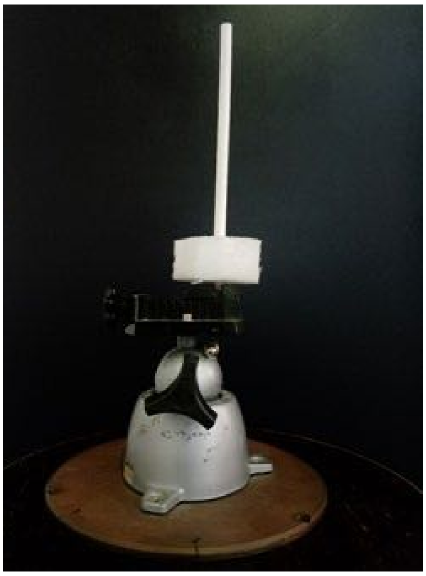

Figure 6.1: Mounting apparatus for the radiological scan of vertebrae.

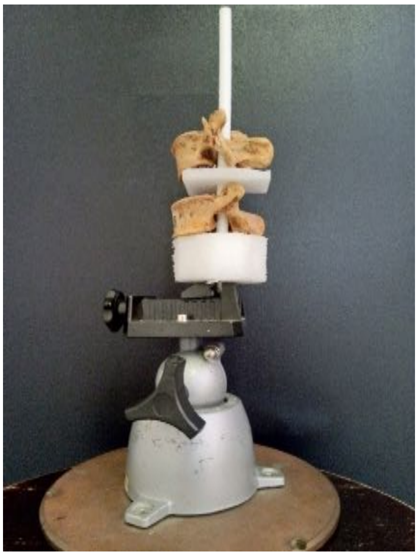

Figure 6.2: Lumbar vertebrae mounted on the stand with foam separators

(6.0) SURETY:

Quality is provided in the peer review process VISUN Laboratory Manual, Part III, SOP 3.1 (Peer Review). If deficiencies in images are noted, the primary analyst will ensure that images are retaken appropriately. Forensic imaging is also subject to audits VISUN Laboratory Manual, Part III, SOP 3.3 (Audits).

(7.0) AVAILABILITY

The original signed version of this document is kept by the Curator of the Kirsten Skeletal Collection. Training documents available in personnel folders. A copy of the document can be found on the document management system ALFRESCO.

(8.0) DOCUMENT HISTORY

Version No.	Date	Location of Change History	Author/ Reviewer	Approving Official	Date Approved	Next Review Date
1.0	20 Nov 2019		A Alblas			Dec 2020
2.0	Jan 2020	*Revised*	M Middleton R Pieterse	A Alblas	Mar 2020	2021

SOP 2. SCANNING PROCEDURES

SOP 2.1. Scanning modalities
UP, SMU and SUN

(1.0) Purpose

The purpose of this document is to detail the scanning procedures possibly performed for the Bakeng se Afrika digital skeletal repository.

(2.0) Scope

This document and the guidelines herein will be used by skeletal collections managers or curators, researchers, contractors and/or the individuals responsible for preparing the skeletal elements for scanning once the material is at the scanning facility (NECSA/ other scanning facility).

(3.0) Definitions

For definition of the terminology used in the current document, see SOP 1.1.

(4.0) Roles and Responsibilities

All staff and students:

- Be aware of the correct operating and maintenance procedures of the imaging methodology used. No person will be allowed to operate any equipment without prior training and/or certification.

Collections managers/curators/contractors:

- The preparation of the skeletal elements for scanning, which includes replacing teeth and reconstructing the cranial vault with the rest of the cranium will be completed by the curator, researcher or contractor that brought the skeletal material for scanning.

Scanning technician/radiographer:

- Check whether the relevant bones are suitable for scanning
- Ensure that once they skeletal material is scanned that it is returned to the correct box with the corresponding collection number.
- Ensure that skeletal material to be scanned will be handled with care by all involved to prevent any damages.

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Department of Biomedical Sciences
Division of Clinical Anatomy
Standard Operating Procedures

BAKENG SE AFRIKA DIGITAL SKELETAL REPOSITORY
SUN ANTHROPOLOGY RESEARCH UNIT
RADIOLOGICAL IMAGING SOP: SELECTION OF MATERIAL

SOP#: *BsA.SOP.IMAGE.004 Radiological Modality*	**Version #: 2**	**Effective Date:** *(This document will be reviewed in two years from effective date)* 10 December 2019	Page **1** of **10**

Author:	**Reviewers:** *(Signature confirms that the reviewers agree with the content of the document)*	**Approved by HOD** *(Signature confirms final approval)*
(Signature & Date) Name: R Pieterse Designation: Bakeng se Afrika Adminstrator, Stellenbosch University Signed: 08/04/2020	(Signature & Date) Name: A Alblas Designation: Stellenbosch University: KSC Curator	(Signature & Date) Dr K Baatjes HOD: Clinical Anatomy

TABLE OF CONTENTS

(1.0) PURPOSE & SCOPE: 3
1) PURPOSE: 3
2) SCOPE: 3
(2.0) DEFINITION: 3
(3.0) ABBREVIATION LIST 3
(4.0) ROLES AND RESPONSIBILITIES 3
All staff and students 3
Collections managers/curators/contractors: 3
Scanning technician/radiographer: 3
(5.0) GENERAL OPERATION OF IMAGE EQUIPMENT: 4
(6.0) scanning MODALITIES: 4
These modalities may include: 4
1) X-RAYS 4
COMPUTED TOMOGRAPHY (CT) 5
3) MAGNETIC RESONANCE IMAGING (MRI) 6
4) LODOX STATSCAN 6
5) MEDTRONIC O-ARM® 7
(7.0) SURETY: 10
(8.0) AVAILABILITY 10
(9.0) DOCUMENT HISTORY 10
(10.0) SUPPORTING DOCUMENTS ..... **Error! Bookmark not defined.**

(1.0) PURPOSE & SCOPE:

1) PURPOSE:

The purpose of this document is to detail the bone preparations and pre-scan procedures to be completed when preparing various skeletal elements for scanning for addition to the Bakeng se Afrika digital skeletal repository.

2) SCOPE:

This document and the guidelines herein will be used by skeletal collections managers or curators as well as by any researchers scanning skeletal material that will be added to the Bakeng se Afrika digital skeletal repository, including but not limited to the curator of the Kirsten Collection (housed at the Stellenbosch University), the curator of the Pretoria Bone Collection (housed at the University of Pretoria) and the curator of the Human Bone Collection (housed at the Sefako Makgatho Health Sciences University) or any researchers or contractors and/or the individuals responsible for preparing the skeletal elements for scanning once the material is at the scanning facilities.

(2.0) DEFINITION:

For definition of the terminology used in the current document, see *BsA.SOP.IMAGE.001*: Selection of skeletal material for scanning.

(3.0) ABBREVIATION LIST

For a list of abbreviations used in the current document, see *BsA.SOP.IMAGE.001*: Selection of skeletal material for scanning.

(4.0) ROLES AND RESPONSIBILITIES

All staff and students

- Be aware of the correct operating and maintenance procedures of the imaging methodology used. No person will be allowed to operate any equipment without prior training and/or certification.

Collections managers/curators/contractors:

- The preparation of the skeletal elements for scanning, which includes replacing teeth and reconstructing the cranial vault with the rest of the cranium will be completed by the curator, researcher or contractor that brought the skeletal material for scanning.

Scanning technician/radiographer:

- Check whether the relevant bones are suitable for scanning
- Ensure that once they skeletal material is scanned that it is returned to the correct box with the corresponding AN number.
- Ensure that skeletal material to be scanned will be handled with care by all involved to prevent any damages.

- The stabilisation of the bones within the scanner will be the responsibility of the person tasked with operating the microCT scanner at the scanning facility.
-

(5.0) GENERAL OPERATION OF IMAGE EQUIPMENT:

- This SOP does not detail the specific operator's manual for each modality. For detailed instructions for each modality used, refer to specific Operating Procedures.
- Always decontaminate image modalities after use.
- Equipment should always be washed and sterilized after use to avoid contamination or infection.

(6.0) SCANNING MODALITIES:

- Always refer to specific operator's manual and maintenance procedure of each imaging modality for detailed instructions.

These modalities may include:

- X-Rays
- Computed tomography (CT-scans)
- Microfocus scanning (Micro-XCT),
- Magnetic resonance technical aspects of imaging (MRI)
- Lodox Statscan
- Cone beam (CBCT)
- Medtronics O-arm
- cephalograms

1) X-RAYS

X-rays were first discovered in 1895 by Wilhelm Conrad Roentgen and three years following its use in the clinical setting; rather it was used in the examination of a dead body (Griffith and Genant, 2011; Franklin *et al*., 2016). It allows for the external and internal visualization of bone, eliminating the need for the body to be dissected (Franklin *et al*., 2016). X-rays in the field of forensic medicine, are commonly used in helping find projectiles in ballistic investigations and to identify bone fractures (Bolton, 2011; Kučerová *et al.,* 2014). They are seen as the golden standard method in victim identification (Kučerová *et al.,* 2014). This technique provides excellent resolution and can differentiate bone boundaries (Mehta *et al*., 1997). X-rays make use of ionising radiation and thus emit electromagnetic radiation, which is harmful to patients and operators (Franklin *et al*., 2016). Its use within the forensic field is not practical as multiple scans are required to obtain a full-body image (Bolton, 2011). This technique displays difficulties in truly representing the dimensions of bone as the images obtained are often distorted (Maresh, 1955). It is described to be time-consuming and labour intensive (Deyle *et al.,* 2009). Following, the discovery of the X-ray, another technique which too uses ionizing radiation was created; computed tomography (CT) (Hounsfield, 1973; Kalender, 2011).

COMPUTED TOMOGRAPHY (CT)

Computed Axial Tomography (CAT) or Computed Tomography (CT) is an imaging modality that records 2-dimensional (2D) x-ray images from various angles around an object of interest. These 2D images are then reconstructed into a 3-dimensional (3D) volume. This allows for multidirectional examination of regions of interest, as well as dimensional, volumetric, and other advanced measurements.

This imaging modality was created by an English engineer, G.N Hounsfield in 1972 and first used in a clinical setting in 1975 (Hounsfield, 1973; Kalender,2011). It produces cross-sections of bone with high visibility contrast between various soft tissues, greater than X-rays, in addition to having a rapid processing time (Mehta *et al*., 1997; Stull *et al*., 2014). Both, the shape and size of bone can be determined from a CT scan (Franklin *et* al., 2016). The osteological measurements collected from CT-scans have also been shown to be similar to dry bone specimens (Franklin *et al*., 2013; Stull *et al*., 2014; Lorkiewicz-Muszynska *et al*, 2015). CT scans are capable of replacing skeletal collections, supported by studies conducted on its use in estimating biological profiles (Schmeling *et al*., 2004; Schulz *et al*., 2005; Franklin *et al*., 2012; Zech *et al*., 2012; Franklin *et al*., 2014; Franklin & Flavel, 2015; Mehta *et al*., 2015; Torimitsu *et al*., 2016).

Microfocus scanning (Micro-XCT)

Industrial CT, often referred to as Micro-CT, is designed for industrial applications, but may also be used in biological applications. Micro-CT utilizes a penetrating ionising radiation (x-ray) source and detector to obtain 2D images of an object that may then be reconstructed into a 3D image. The source and detector are fixed around a rotating sample, allowing for the adjustment of image resolution Micro-CT are also more flexible in terms of voltage and current modification, which allows for the setup to be modified to suit various materials. The image resolution of Micro-CT scans is often higher than their biomedical counterparts, with image resolution generally in the range of 5-150 micrometres (μm). Scans have advantages over physical specimens, in that measurements are not limited to external anatomy, and that measurements obtained from the software are of high precision.

Micro-CT utilises an x-ray source and detector, which are fixed around a rotating sample, allowing for the adjustment of image resolution. Micro-CT uses an x-ray source and detector to obtain 2D images of an object that may then be reconstructed into a 3D image. The fundamental components of the machine include penetrating ionising radiation, a sample manipulator (in this case a rotation table), and a detector. A micro-focus x-ray tube uses a beam of voltage-accelerated electrons, which are focused onto a metal (generally tungsten) target. The interaction between the accelerated electrons and the metal target is responsible for the generation of x-rays. The x-rays are directed around and through the sample and collected in the form of a projection image (or radiograph) on a 2D x-ray detector. The sample manipulator positions the sample in the path of the radiation beam and rotates it through a specified angle (mostly 180° or 360°). The detector converts the attenuated radiation, which is directed in a straight line through the sample, into 2D digital images, made up of thousands of pixels. Two-dimensional projection images numbering in the hundreds to thousands are thus recorded during scanning.

These 2D images are then used to reconstruct a 3D data set using filtered back-projection algorithms. In this way, every volumetric pixel (or voxel) is guidance in the selection of what should be imaged from many angles by 2D projections. The sum of views from each angle thus creates a representation of the actual x-ray density, and thus the brightness, of a specific voxel.

3) MAGNETIC RESONANCE IMAGING (MRI)

This safer technique, subjects the body to a strong magnetic frequency, followed by a radiofrequency instead of emitting electromagnetic radiation (Mehta *et al*., 1997; Franklin *et al*., 2016). It can acquire images in any plane and can derive information from both the bone and bone marrow, which is not possible with other image modalities (Griffith and Genant, 2011). Studies have validated the accuracy and precision of long bone measurements collected from MRI's (Leitzes *et al*., 2005; Doyle & Winsor, 2011; Rathnayaka *et al*., 2012). In addition to the studies which focussed on estimating certain biological profiles from MRI scans (Dedouit *et al.,* 2012; Saint-Martin *et al.,* 2013; Brits *et al*., 2017).

4) LODOX STATSCAN

The Lodox Statscan is housed in the Tygerberg Forensic Pathology Services Mortuary at Tygerberg Hospital. It is a full-body digital X-ray machine, designed and manufactured by a South African company, Lodox Systems. The technique was designed to produce high quality, low dosage, 2D images in approximately 10-13 seconds. In South Africa, Lodox Statscans are available and in use within the mortuaries found in the Western Cape, Cape Town, Pretoria and Johannesburg Forensic Pathology Services. Where it is used to locate foreign objects such as firearm projectiles in deceased individuals and in detecting peripheral skeletal injuries. The scans are stored in a DICOM® image format and will be transferred to Image J® 1.4r imaging software prior to measuring.

Unlike the above-mentioned virtual modalities, the Lodox Statscan (Lodox® Systems Pty., Sandton, South Africa) was primarily designed and used in diamond-mines to prevent theft within the workplace (Boffard, 2006). Lodox Statscan is a full-body digital X-ray machine, designed and manufactured by a South African company, Lodox Systems (Bolton, 2011). The technique was designed to produce high quality, low dosage, 2D images in approximately 10-13 seconds (Beningfield *et al*., 2003; Bolton, 2011; Stull *et al*., 2013). The scanner was custom designed to suit the wet laboratory conditions in the mortuary (Bateman, 2008). Lodox Statscans have been used in identifying foreign objects within the body of deceased individuals (Knobel *et al.,* 2006; Deyle *et al.,* 2010; Evangelopoulos *et al.,* 2011; Fu *et al.,* 2011). In South Africa, Lodox Statscans are available and in use within the mortuaries found in the Western Cape, Cape Town (Alblas *et al.,* 2018), Pretoria (Bernitz & Verster, 2017) and Johannesburg Forensic Pathology Services. Where it is used to locate foreign objects such as firearm projectiles in deceased individuals and in detecting peripheral skeletal injuries (Knobel *et al.,* 2006; Bernitz & Verster, 2017). Its benefits include; accelerating investigations, victim identification and its contribution to the VSD (Bateman, 2008; Bernitz & Verster, 2017) and the ultimate disposition of images.

Figure 1: The Lodox Statscan housed at Tygerberg Forensic Pathology Services.

5) MEDTRONIC O-ARM®

The Medtronic O-arm® surgical imaging system is housed in the SUNSkills lab, FISAN building, third floor. It is a two-dimensional (2D) fluoroscopic and three-dimensional (3D) volumetric mobile imaging system that uses ionising-radiation to produce a radiograph. “Fluoroscopic” refers to X-radiation passing through the body or skeletal element and forming The 3D scan produced by the O-arm® is a cylindrical tube with a 40 cm diameter and 15 cm height. Two-dimensional images can be obtained through fluoroscopy producing a 40 cm x 40 cm scan. It takes the O-arm® 13 seconds to scan the desired object/material/patient in 3D and 30 seconds to form the image. Two-dimensional images are formed almost instantaneously.

The images produced by the O-arm® may be two- (2D) or three-dimensional (3D). The 3D image produced by the O-arm forms a cylindrical tube while the 2D scans are obtained through fluoroscopy. Due to the dimensions of the scans (Table 1), it is more convenient to scan small skeletal elements, as scanning long bones will require the technician to take two scans and merge them together; this is time-consuming.

During scanning, it is preferred that the bones lie on a surgical bed, beneath a layer of plastic or wood. A surgical bed is preferred as its height can be adjusted and it does not have legs which could get in the way of the gantry (Fig, 1). The plastic or wood will protect the surgical bed’s surface from being damaged by the bone. Ensure that the bed’s breadth is less than 70 cm as this is the diameter of the gantry opening.

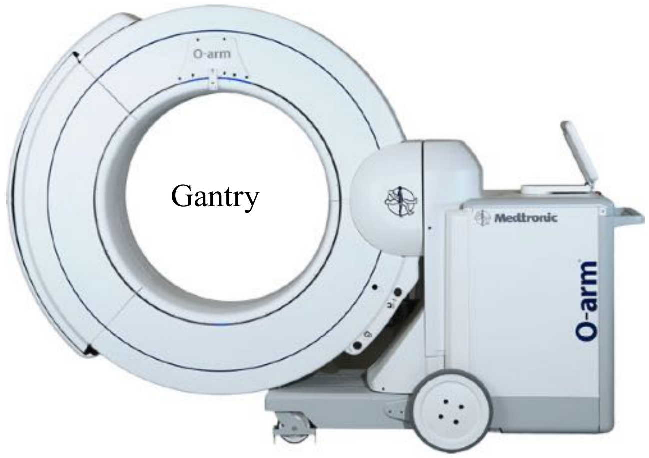

Figure 2: The O-arm with an indication of the gantry an X-ray image on the monitor.

The bones are to be scanned with its respective skeletal number. This is achieved by cutting out the numbers from clay and scanning the bones with the number. The clay or modelling material should be dense enough to be visible on the scan.

Tables 2 to 5 specify the details for the different types of 3D scans that can be performed using the Medtronic O-arm®. Dose and stereotaxy are terms that should be taken into consideration to understand the specifications of certain O-arm scan types. Dose refers to the amount of radiation the specimen scanned absorbs and stereotaxy is minimally invasive brain surgery, performed using a 3D system allowing precise placement of an electrode, cannula, or a probe into a small area of the brain.

Table 1: Three-dimensional image dimensions

Specification:	**Detail:**
Cylindrical volume	20 cm FOV: ▪ 21.25 cm diameter x 15 cm height 40 cm FOV: ▪ 39. 70 cm diameter x 15 cm height
Resolution (mm)	20 cm FOV: ▪ 512 x 512 x 192 20 cm FOV: ▪ 512 x 512 x 192

Table 2: Three-dimensional High definition scan

Specification	Imaging modality performance
Data acquisition:	< 30 seconds ▪ 27 seconds
Number of projections:	745 images are scanned over 360˚
Field of view options:	The field of view (FOV) may be set at 20cm or 40 cm
3D reconstruction time:	< 30 seconds ▪ Standard for 20cm FOV: 23 seconds ▪ Standard for 40cm FOV: 23 seconds
Resolution (mm):	20 cm FOV: 0.415 x 0.145 x 0.833 40 cm FOV: 0.775 x 0.775 x 0.833
Reconstructed matrix size:	512 x 512 x 192

Table 3: Three-dimensional Low dose scan

Specification	Imaging modality performance
Data acquisition:	<16 seconds ▪ Standard: 14 seconds
Number of projections:	391 images are scanned over 360˚
3D reconstruction time:	<90 seconds ▪ Standard: 65 seconds
Field of view options:	The field of view (FOV) may be set at 20cm
Resolution (mm):	0.415 x 0.145 x 0.833
Reconstructed matrix size:	512 x 512 x 192

Table 4: Enhanced cranial 3D volumetric scan

Specification	Imaging modality performance
Data acquisition:	<30 seconds ▪ standard: 27 seconds
Number of projections:	745 images are scanned over 360˚
3D reconstruction time:	<30 seconds ▪ Standard: 13 seconds
Field of view (FOV) options:	FOV option: 20cm
Resolution (mm):	0.415 x 0.145 x 0.833
Reconstructed matrix size:	512 x 512 x 192

Table 5: Stereotaxy 3D Volumetric scan

Specification	Imaging modality performance
Data acquisition:	< 30 seconds ▪ standard: 27 seconds
Number of projections:	745 images are scanned over 360˚
3D reconstruction time:	< 30 seconds

	▪ Standard: 13 seconds
Field of view (FOV) options:	FOV option: 40 cm
Resolution (mm):	0.775 x 0.775 x 0.833
Reconstructed matrix size:	512 x 512 x 192

(7.0) SURETY:
Quality is provided in the peer review process VISUN Laboratory Manual, Part III, SOP 3.1 (Peer Review). If deficiencies in images are noted, the primary analyst will ensure that images are retaken appropriately. Forensic imaging is also subject to audits VISUN Laboratory Manual, Part III, SOP 3.3 (Audits).

(8.0) AVAILABILITY
The original signed version of this document is kept by the Curator of the Kirsten Skeletal Collection. Training documents available in personnel folders. A copy of the document can be found on the document management system ALFRESCO.

(9.0) DOCUMENT HISTORY

Version No.	Date	Location of Change History	Author/ Reviewer	Approving Official	Date Approved	Next Review Date
1.0	20 Nov 2019		A Alblas			2020
2	L Daniels		L Daniels	AA	Jan 2020	

SOP 2.2. Scanning and naming conventions
UP and SMU

(1.0) Purpose

The purpose of this document is to detail the scanning procedures to be completed before addition to the Bakeng se Afrika digital skeletal repository.

(2.0) Scope

This document and the guidelines herein will be used by skeletal collections managers or curators, researchers, contractors and/or the individuals responsible for scanning the skeletal elements once the material is at the scanning facility (NECSA/ other scanning facility).

(3.0) Definitions

For definition of the terminology used in the current document, see SOP 1.1.

(4.0) Roles and Responsibilities

The scanning, which includes the naming of scans will be completed by the curator, researcher or contractor that brought the skeletal material for scanning.

(5.0) Procedure

- **Crania**

All the folder and files obtained during scanning will be named as follow: BsA_institution&cadnr_cranium_scannumber. For instance, for the scan n°1982 of the cranium 1750 from the Pretoria Bone Collection of the University of Pretoria: BSA_UP1750_cranium_1982.

All crania will be scanned according to the following parameters: 100µA, 100kV or higher if necessary. The number of projections will be determined using the Nyquist theorem. The resolution of the crania will be determined by the size of the crania. The highest possible resolution is preferred.

An automatic reconstruction will be made whenever possible and the images from the reconstruction will be saved in 16-bit tiffs. After reconstruction, the projections, xtect files and reconstructed 16bit tiffs images will be saved for the Bakeng se Afrika server (see SOP 3 for more details on the procedure).

A possible mirror might affect the scans. In order to identify the presence of a mirror effect, photographs of each skeletal element will be taken prior or after scanning, and compared to the reconstructed images (see SOP 4 for more details). If such effect is identified, the data should be transformed using any imaging software and save again in 16-bit tiffs.

- **Mandible**

All the folder and files obtained during scanning will be named as follow:

BsA_institution&cadnr_mandible_scannumber. For instance, for the scan n°1982 of the mandible 1750 from the Pretoria Bone Collection of the University of Pretoria: BSA_UP1750_mandible_1982.

All mandibles will be scanned according to the following parameters: 100µA, 100kV or higher if necessary. The number of projections will be determined using the Nyquist theorem. The resolution will be determined by the size of the bone. The highest possible resolution is preferred.

An automatic reconstruction will be made whenever possible and the images from the reconstruction will be saved in 16-bit tiffs. After reconstruction, the projections, xtect files and reconstructed 16bit tiffs images will be saved for the Bakeng se Afrika server (see SOP 3 for more details on the procedure).

A possible mirror might affect the scans. In order to identify the presence of a mirror effect, photographs of each skeletal element will be taken prior or after scanning, and compared to the reconstructed images (see SOP 4 for more details). If such effect is identified, the data should be transformed using any imaging software and save again in 16-bit tiffs.

- **Focus on dentition**

All the folder and files obtained during scanning will be named as follow: BsA_institution&cadnr_maxilla_scannumber. For instance, for the scan n°1982 of the maxilla 1750 from the Pretoria Bone Collection of the University of Pretoria: BSA_UP1750_maxilla_1982.

All the crania will be scanned according to the following parameters: 100µA, 100kV or higher if necessary. The number of projections will be determined using the Nyquist theorem. The resolution will be determined in order to include the maximal number of teeth. The highest possible resolution will be preferred while focusing on the dentition.

An automatic reconstruction will be made whenever possible and the images from the reconstruction will be saved in 16-bit tiffs. After reconstruction, the projections, xtect files and reconstructed 16bit tiffs images will be saved for the Bakeng se Afrika server (see SOP 3 for more details on the procedure).

A possible mirror might affect the scans. In order to identify the presence of a mirror effect, photographs of each skeletal element will be taken prior or after scanning, and compared to the reconstructed images (see SOP 4 for more details). If such effect is identified, the data should be transformed using any imaging software and save again in 16-bit tiffs.

- **Femur**

All the folder and files obtained during scanning will be named as follow, the P indicating the femur holding the marker:

BsA_institution_cadnrP_cadnr_fem_A_scannumber,
BsA_institution_cadnrP_cadnr_fem_B_scannumber,
BsA_institution_cadnrP_cadnr_fem_C_scannumber.

For instance, for the scan n°1982, 1983 and 1984 of the femora 1750 (holding the marker) and 1751 from the Pretoria Bone Collection of the University of Pretoria:

BSA_UP1750P_1751_fem_A_1982, BSA_UP1750P_1751_fem_B_1983 and BSA_UP1750P_1751_fem_C_1984.

The three scans (i.e., scans A, B and C) will be detailed using the same parameters in order to be able to merge then afterwards. In addition, the scans A and B will be made without moving the specimen in the machine to facilitate the merging procedure.

All femora will be scanned according to the following parameters: 100µA, 100kV or higher if necessary. The number of projections will be determined using the Nyquist theorem. The resolution will be determined in order to include the size of the femora. The highest possible resolution will be preferred.

An automatic reconstruction will be made whenever possible and the images from the reconstruction will be saved in 16-bit tiffs. After reconstruction, the projections, xtect files and reconstructed 16bit tiffs images will be saved for the Bakeng se Afrika server (see SOP 3 for more details on the procedure).

A possible mirror might affect the scans. In order to identify the presence of a mirror effect, photographs of each skeletal element will be taken prior or after scanning, and compared to the reconstructed images (see SOP 4 for more details). If such effect is identified, the data should be transformed using any imaging software and save again in 16-bit tiffs.

- **Radii**

All the folder and files obtained during scanning will be named as follow, the P indicating the radius holding the marker: BsA_institution_cadnrP_cadnr_rad_A_scannumber and BsA_institution_cadnrP_cadnr_rad_B_scannumber.

For instance, for the scan n°1982 and 1983 of the radii 1750 (holding the marker) and 1751 from the Pretoria Bone Collection of the University of Pretoria:

BSA_UP1750P_1751_rad_A_1982 and BSA_UP1750P_1751_rad_B_1983.

The two scans (i.e., scans A and B) will be detailed using the same parameters in order to be able to merge then afterwards. In addition, the scans will be made without moving the specimen in the machine to facilitate the merging procedure.

All radii will be scanned according to the following parameters: 100µA, 100kV or higher if necessary. The number of projections will be determined using the Nyquist theorem. The resolution will be determined in order to include the size of the radii. The highest possible resolution will be preferred.

An automatic reconstruction will be made whenever possible and the images from the reconstruction will be saved in 16-bit tiffs. After reconstruction, the projections, xtect files and reconstructed 16bit tiffs images will be saved for the Bakeng se Afrika server (see SOP 3 for more details on the procedure).

A possible mirror might affect the scans. In order to identify the presence of a mirror effect, photographs of each skeletal element will be taken prior or after scanning, and compared to the reconstructed images (see SOP 4 for more details). If such effect is identified, the data should be transformed using any imaging software and save again in 16-bit tiffs.

SOP 2.2. Scanning and naming conventions
SUN

Department of Biomedical Sciences
Division of Clinical Anatomy
Standard Operating Procedures

BAKENG SE AFRIKA DIGITAL SKELETAL REPOSITORY SUN ANTHROPOLOGY RESEARCH UNIT RADIOLOGICAL IMAGING SOP: SCANNING OF MATERIAL			
SOP#: *BsA.SOP.IMAGE.005 Radiological Modality*	**Version #: 2**	**Effective Date:** *(This document will be reviewed in two years from effective date)* 10 December 2019	Page **1** of **9**

Author:	**Reviewers:** *(Signature confirms that the reviewers agree with the content of the document)*	**Approved by HOD** *(Signature confirms final approval)*
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TABLE OF CONTENTS

(1.0) PURPOSE & SCOPE: 3
1) PURPOSE: 3
2) SCOPE: 3
(2.0) DEFINITION: 3
(3.0) ABBREVIATION LIST 3
(4.0) ROLES AND RESPONSIBILITIES 3
1) All staff and students 3
2) Collections managers/curators/contractors: 3
3) Scanning technician/radiographer: 3
(5.0) SCANNING PROCEDURES: 4
These modalities may include: 4
5.1 Numbering Procedures 4
5.2 Imaging Procedures 5
1) X-Rays 5
2) Computed Tomography (CT) 5
3) Microfocus scanning (Micro-XCT) 5
4) Magnetic Resonance Imaging (MRI) 6
5) MEDTRONIC O-ARM® 7
6) Lodox Statscan 7
7) CONE BEAM (CBCT) 7
8) CEPHALOGRAMS 7
(6.0) MEASUREMENTS OF IMAGES 8
(7.0) STORAGE 8
7.1 STORAGE OF MATERIAL DURING THE PROJECT (short term) 8
7.2 LONG TERM STORAGE OF MATERIAL 8
(8.0) DOCUMENTATION: 8
(9.0) SURETY: 9
(10.0) AVAILABILITY 9
(11.0) DOCUMENT HISTORY 9

(1.0) PURPOSE & SCOPE:

1) PURPOSE:

The purpose of this document is to detail the bone preparations and pre-scan procedures to be completed when preparing various skeletal elements for scanning for addition to the Bakeng se Afrika digital skeletal repository.

2) SCOPE:

This document and the guidelines herein will be used by skeletal collections managers or curators as well as by any researchers scanning skeletal material that will be added to the Bakeng se Afrika digital skeletal repository, including but not limited to the curator of the Kirsten Collection (housed at the Stellenbosch University), the curator of the Pretoria Bone Collection (housed at the University of Pretoria) and the curator of the Human Bone Collection (housed at the Sefako Makgatho Health Sciences University) or any researchers or contractors and/or the individuals responsible for preparing the skeletal elements for scanning once the material is at the scanning facilities.

(2.0) DEFINITION:

For the definition of the terminology used in the current document, see *BsA.SOP.IMAGE.001*: Selection of skeletal material for scanning.

(3.0) ABBREVIATION LIST

For a list of abbreviations used in the current document, see *BsA.SOP.IMAGE.001*: Selection of skeletal material for scanning.

(4.0) ROLES AND RESPONSIBILITIES

1) ALL STAFF AND STUDENTS

- Be aware of the correct operating and maintenance procedures of the imaging methodology used. No person will be allowed to operate any equipment without prior training and/or certification.

2) COLLECTIONS MANAGERS/CURATORS/CONTRACTORS:

- The preparation of the skeletal elements for scanning, which includes replacing teeth and reconstructing the cranial vault with the rest of the cranium will be completed by the curator, researcher or contractor that brought the skeletal material for scanning.

3) SCANNING TECHNICIAN/RADIOGRAPHER:

- Check whether the relevant bones are suitable for scanning
- Ensure that once they skeletal material is scanned that it is returned to the correct box with the corresponding AN number.

- Ensure that skeletal material to be scanned will be handled with care by all involved to prevent any damages.
- The stabilisation of the bones within the scanner will be the responsibility of the person tasked with operating the scanner at the scanning facility.

(5.0) SCANNING PROCEDURES:

Always refer to the specific operator's manual and maintenance procedure of each imaging modality for detailed instructions.

THESE MODALITIES MAY INCLUDE:

1) X-Rays
2) Computed tomography (CT-scans)
3) Microfocus scanning (Micro-XCT),
4) Magnetic resonance imaging (MRI)
5) Lodox Statscan
6) Cone beam (CBCT)
7) Medtronics O-arm
8) Cephalograms

5.1 NUMBERING PROCEDURES

Number and Authentication: The cadaver or skeletal number should appear in radiographs whenever possible. Radiopaque letters and numbers can be used for this purpose in the digital radiography of remains using the Faxitron system. If conditions preclude this practice, the cadaver or skeletal number should be added later using electronic methods or radiographic marker/pen, or it may appear in the caption in the report.

Records of the use of radiographic equipment are maintained using the Radiography Log. These logs include the initials of the person taking the radiographs, the case number of the remains radiographed, and the date the radiographs were taken.

The unique number of each skeletal individual with the specific skeletal element scanned will be labelled on each radiograph as part of the stored image. Each radiograph is labelled according to the allocated BsA number for the material and the anatomical region being scanned. During post-processing, individual skeletal elements may be exported and labelled accordingly.

BsA_Skeletal number_Region of anatomy_Necsa number

e.g. BsA_UP_234_femur_Aprox_1234
BsA_SU_2340_femur_Adist_1235
BsA_SMU_2340_femur_A_1236
BsA_FOR_2340_femur_A_1237

5.2 IMAGING PROCEDURES

Quality of Images: Images taken should be of the highest quality possible. It is the responsibility of the primary analyst to ensure all photographs are of at minimum acceptable quality (*e.g.*, in focus, proper imaging of case, proper colouration). Quality of images should also be examined and verified during the peer review process.

1) X-RAYS

Not used yet

2) COMPUTED TOMOGRAPHY (CT)

Not used yet

3) MICROFOCUS SCANNING (MICRO-XCT)

Scanner setup and parameters

Before each scan is started it is labelled with the AN number and the region of that is being scanned, example: AN123 Top left viscerocranium.

Scans are done at 180kV with a beam current of 600uA, with an exposure of 2 frames per second (fps) with 2000 projections (i.e. images) taken. These X-ray conditions give the optimum trade-off between the time taken to run the scan and the signal-to-noise ratio.

A guideline to use to decide on the resolution to use per scan as follows:

- cranium: 100 μm
- mandible: 60 μm
- dentition: 40-60 μm
- inner ear: approx. 20-40 μm
- humeri: 70-100 μm
- radius (scanned in 2 parts): approx. 50 μm each
- femur (scanned in 3 parts): approx. 50-70 μm each
- os coxae: 70-100 μm
- patellae & calcanei: 20-60 μm

Scanning procedure

Before starting the scan, the background should be normalized. This is done by moving the sample out of the field of view and using the x-ray beam at the chosen settings to correct for all variations across the detector. This procedure can be conducted before a scan and it is only required once the settings of the scanner are changed or after long periods of inactivity. The scanner itself is automated and therefore requires no user interaction during the scanning process.

Image reconstruction

Once all the 2D projection images are obtained the 3D volume can be reconstructed in VG studio. the field of view is cropped to make the total reconstruction volume smaller. This will ensure that the reconstruction time is shorter and that less memory is required. This can be helpful when time and computational power is limited. The type of output file is chosen, it is selected to be an 8-bit. Once the 2DOnce the projections have been reconstructed into a raw volume, the volume is imported into VGSTUDIO MAX 3.2.5 for analysis and processing. Firstly, since air surrounding the object is included in the reconstructed volume, the surface of the object is determined; this discards all the surrounding air and focuses all the analysis on the object itself. Thereafter you can focus on the region of the bone you're interested in, isolate it from the rest, and perform the analysis and measurements required, for example, volume, surface area, porosity, bone width, etc.
The projection images are first reconstructed into raw volume data and then imported to VGStudio MAX 3.2.5 for processing and analyses. The air surrounding the scanned object is included in the reconstructed volume. Thus, the surface of the object must first be determined to discard the surrounding air and focus the analyses on the object. Regions of interest on the bone may also be selected, isolated from surrounding regions and individually measured and analysed. Possible analyses include volume, surface area, porosity and bone thickness.

Thereafter one can focus on the region of the bone interested, isolate it from the rest, and perform the analysis and measurements required, for example, volume, surface area, porosity, bone width, etc.
The field of view may be cropped to make the total reconstruction volume smaller. This will ensure that the reconstruction time is shorter and that less memory is required, especially helpful when time and computational power is limited. The type of output file is chosen, either 8-bit or 16-bit. The location of the rotation axes is found by an algorithm that it takes the central pixels in all 2D images. This improves the quality of the reconstruction and corrects for small movements or shift that happened during this scanning process.

Image visualization
After 3D reconstruction, software tools may be used for the visualisation and analysis of data. The specific measurements at specific landmark points as per project proposal will digitally be measured using Volume Graphics Voxel® Manipulation software.

A guideline to use for average file size (projections + reconstructions)

- for a mandible: 15 - 35 GB
- for a femur: 20 - 40 GB (prox. end only)
- for a skull: 15 - 20 GB
- for dentition: 5 to 12 GB (8 GB on average - just reconstructions) – 8 bit

4) MAGNETIC RESONANCE IMAGING (MRI)

Not used for Bakeng se Afrika Project

5) MEDTRONIC O-ARM®

O-Arm® Imaging Specifications for dry bone.

The technician will adjust the O-arm settings specific to the specimen studied. For anthropology, it is suggested that the scan be done in high definition in order to appreciate the morphology of the bone. The patient thickness should be selected as “small”, as no tissue is present around the bone.

Image optimisation

The scans can be uploaded to an external memory device and be viewed using a *Digital Imaging and Communications in Medicine* (DICOM) viewer or morphometrics software.

To study bone morphology, the bone should be scanned dry. Thereafter, the scan can be adjusted using the DICOM to optimise image quality. Here the bone can be viewed in 3D and the trabecular bone can be studied. Various artefacts can be removed as well by “cutting” it out or using volume rendering. Volume rendering is a process which attempts to cancel out artefacts and materials that are not bone.

The quality of the scans is not at the caliber of computer tomography. However, severe trauma and reactive pathological lesions can be studied.

Measurements of bone scans

For osteometry, the bone should be submerged in a medium to simulate soft tissue as most imaging modalities are designed to scan bones in-situ. It is proposed that rice be used as it does not alter the bone in any way.

Software specific for osteometry should be used in order to achieve reliable measurements. Morphometric software that allows markers to be placed on the specimen is preferred as one can easily adjust where the bone should be measured. Additionally, the software should be able to render the scans’ volume and convert to various measuring units.

The measurements are to be recorded on an excel sheet as well as on hardcopy in the event the excel document becomes faulty or is deleted. A backup of all documents and scans should be kept on a different external memory device. When choosing a scale (mm/cm), ensure small skeletal elements are measured in millimetres and larger bones in centimetres.

6) LODOX STATSCAN

Not used for Bakeng se Afrika Project

7) CONE BEAM (CBCT)

Not used for Bakeng se Afrika Project

8) CEPHALOGRAMS

Not used for Bakeng se Afrika Project

(6.0) MEASUREMENTS OF IMAGES

All scans of skeletal elements taken on various modalities will have the unique skeletal number visible on the scan. The scans will be uploaded from the external memory to a software program where digital measurements will be taken. Each radiological modality used, will have specific software for measurement of skeletal elements from the images. All measurements taken on images will be recorded on an excel sheet with regular backups on cloud storage as well as an external hard drive, both password protected. All measurements are in mm.

(7.0) STORAGE

7.1 STORAGE OF MATERIAL DURING THE PROJECT (short term)

The data/images will be stored on REDCap, which will only be accessible to the primary researcher, PI, the supervisors of the researcher, and a statistician from the Centre for Evidence-Based Health Care, Stellenbosch University.

7.2 LONG TERM STORAGE OF MATERIAL

- The scanned digital data will be archived by an electronic central repository (at UP).
- SUN will have a coordinator to coordinate SU's link to the SANREN network.
- The ownership of the projects will remain with the researchers already collaborating.
- Standard operating procedures and quality assurance guidelines will be created in order to manage access and ethical use of the digital database.
- Online accessibility to this data will be coordinated in Pretoria through a management portal.
- Ethical clearance for using images will be managed by a Data/Image Management Committee represented by each of the institutions involved.
- The applicable data will be uploaded online for dissemination, not only to partner Universities but also other national and international research groups following adequate ethical approval of research and author permission.

(8.0) DOCUMENTATION:

8.1 Photography: All digital photographs will be maintained on the VISUN laboratory external hard drive, as well as a back-up drive. In most instances, the digital images archived in the case folder will be considered the official photographic record.

8.1.1 Labeling and Storing Images: Images on the VISUN external hard drive are stored in the appropriate case folder, usually labelled "(Case Number) Images" (*e.g.*, "CAS010101 Images"). These images should typically be high-quality JPEG format and include all arrival, pre-maceration, and official case photographs. All images should be saved regardless of quality.

Photographs for each stage of case assessment and analysis should be placed within its own folder within the Images folder (*e.g.*, Receipt Images, Maceration Images, Analysis Images). JPEG files should be renamed sequentially within each subfolder using the case number and the subfolder name (*e.g.*, "CAS010101 analysis (1)," "CAS010101 analysis (2)," *etc.*). For annotated photos (*e.g.*, photographs for which the analyst wishes to provide additional information, the file should maintain the renamed label and the additional information is added after (*e.g.*, "CAS010101 analysis (1) anterior cranium").

8.2 Radiographs: Similar to photographs, all digital radiographs will be maintained on the VISUN external hard drive within the case number Images folder, as well as on CDs or DVDs in the case file. Radiographs and dental radiographs are to be maintained on an external hard drive and the CDs or DVDs in the case file in their original software file format with viewer software. This will ensure that each radiographic image (either dental or osseous/materials) will maintain an association with the patient information (*e.g.*, station and case number) associated with those digital files.

(9.0) SURETY:

Quality is provided in the peer review process VISUN Laboratory Manual, Part III, SOP 3.1 (Peer Review). If deficiencies in images are noted, the primary analyst will ensure that images are retaken appropriately. Forensic imaging is also subject to audits VISUN Laboratory Manual, Part III, SOP 3.3 (Audits).

(10.0) AVAILABILITY

The original signed version of this document is kept by the Curator of the Kirsten Skeletal Collection. Training documents available in personnel folders. A copy of the document can be found on the document management system ALFRESCO.

(11.0) DOCUMENT HISTORY

Version No.	Date	Location of Change History	Author/ Reviewer	Approving Official	Date Approved	Next Review Date
1.0	20 Nov 2019		A Alblas			2020

SOP 2.3. Reconstructing procedures after scanning UP and SMU

(1.0) Purpose

The purpose of this document is to detail the reconstruction procedure to follow for addition of scans to the Bakeng se Afrika digital skeletal repository.

(2.0) Scope

This document and the guidelines herein will be used by skeletal collections managers or curators, researchers, contractors and/or the individuals responsible for reconstructing the skeletal elements once the material has been scanned correctly at the scanning facility (NECSA/ other scanning facility).

(3.0) Roles and Responsibilities

The reconstructions of scans will be completed by the researcher or contractor that has been trained to do so.

(4.0) Procedures

<u>**STEP 1**</u>

On the reconstruction computer, copy the folder of the scan(s) from the acquisition PC to the desired folder in the D:Drive or to the 'Recon-busy' folder on the Necsa server (reconstruction computer).

Open the CT Pro3D program.

Click Open and select the XTEKCT File (.xtekct) inside the folder of the scan you want to reconstruct (this .xtekct file will have the same name as the folder of the scan).

<u>**STEP 2**</u>

Once the CT Pro3D program is opened, you will see five tabs:

1. IMAGE

Nothing needs to be changed.

2. CENTRE OF ROTATION

- There is no need to move the red line, unless a study is specifically focusing on an area of the sample (bone), such as the orbits. If that is the case, drag the red line on the image in the left corner down to an area of detail/focus (e.g. anterior of skull, orbits and nasal area).
- At **Accuracy** select high quality and press start (Figure 1a).

- Wait for the process to finish. It will show **Found** when completed (Figure 1b).

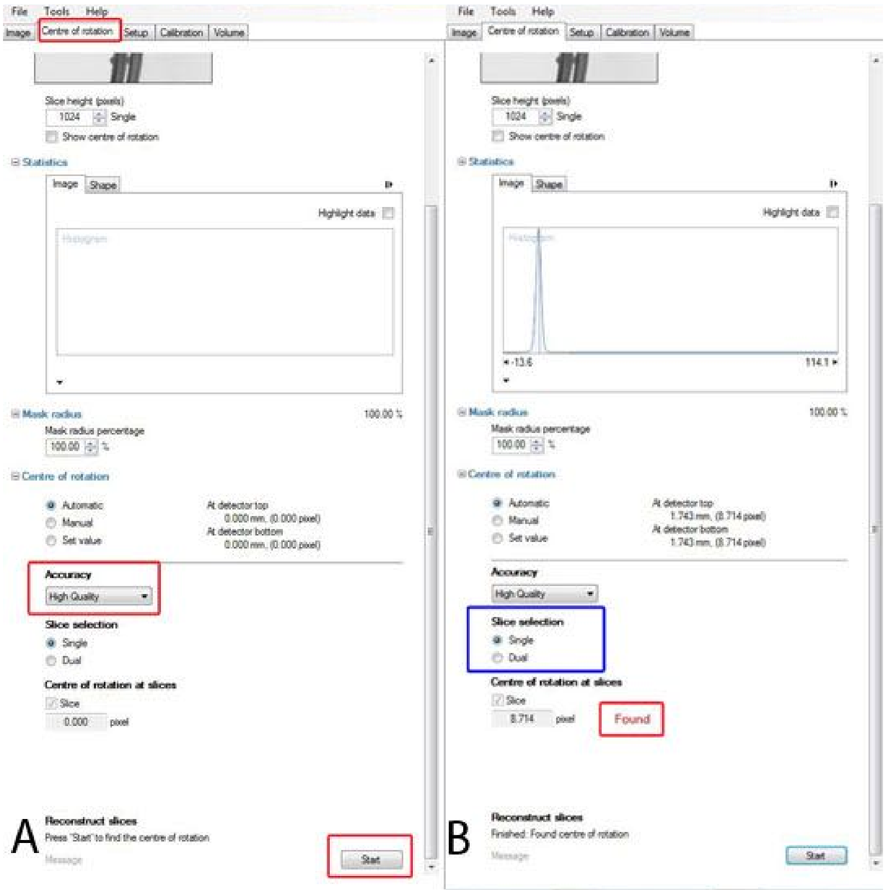


Figure 1. Steps to follow in the centre of rotation tab.

- For the **LONG BONE SCANS** – if it does not find the bone correctly, select DUAL (Figure 1b) and move the red lines in the image to either ends of the bone (Figure 2). Press start again and wait for the process to finish. (Dual is generally used if movement of the bone occurred during the scanning relative to the rest of the sample, such as with a stack of vertebrae).

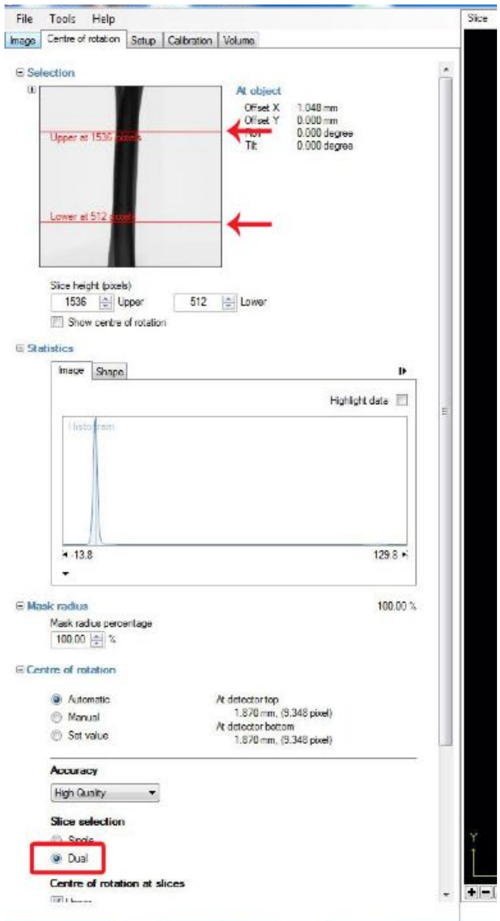


Figure 2. Movement of two red lines (dual) to either end of the long bones or vertebrae stack.

3. SETUP (Figure 3)

- **Beam Hardening**: select number 2.
- **Noise Reduction**: select 2 as well.
- Press start, wait for it to finish.

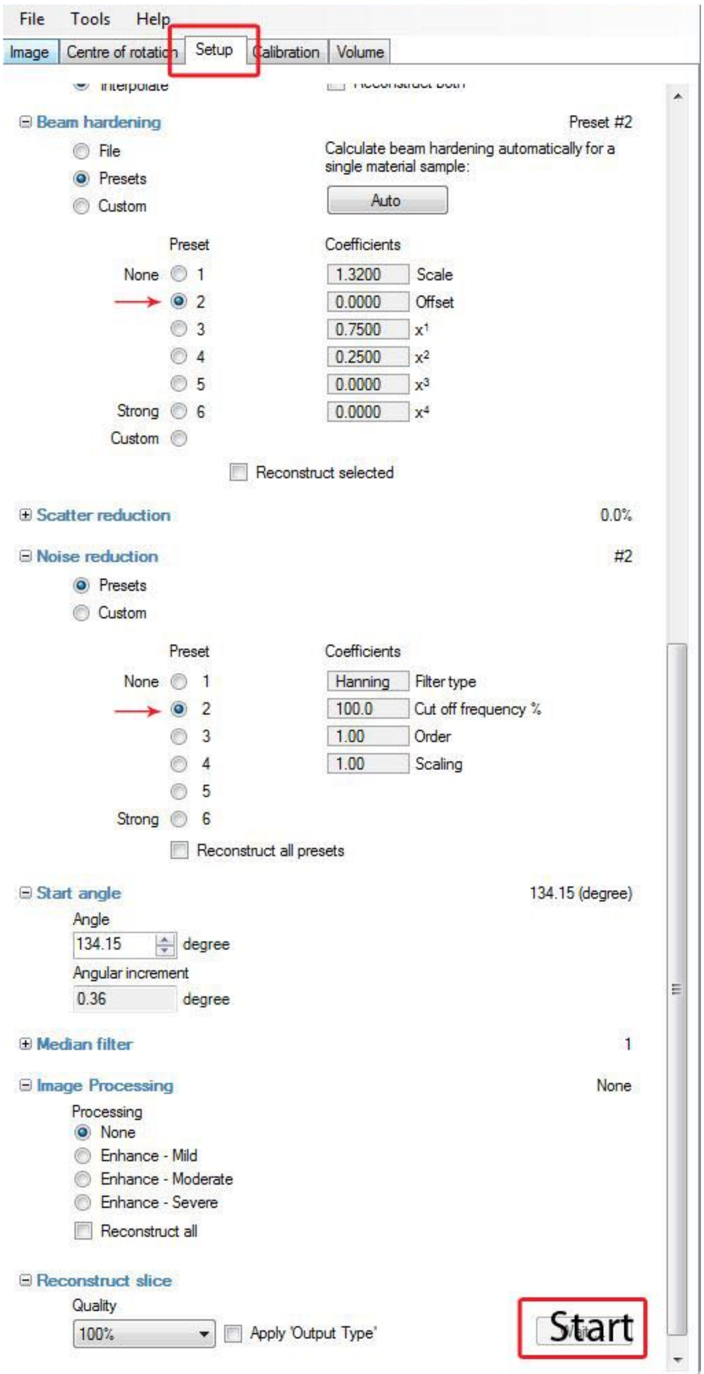


Figure 3. Steps to follow in the setup tab.

4. CALIBRATION

Nothing needs to be changed.

5. VOLUME

- For all scans EXCEPT long bone scans: Crop the images in both 0º and 90º views if possible. Be careful not to crop too close to the bone, as this may crop the image of the bone itself (Figure 4).
- For LONG BONE scans: Do NOT crop. The voxel count under Volume should be the same in all three blocks (This is important for stitching; Figure 5).

- **Output format**: select SINGLE under Tiff 16-bit image stack.
- **Scaling method** should be Full range. See Figure 5.
- Press Start.

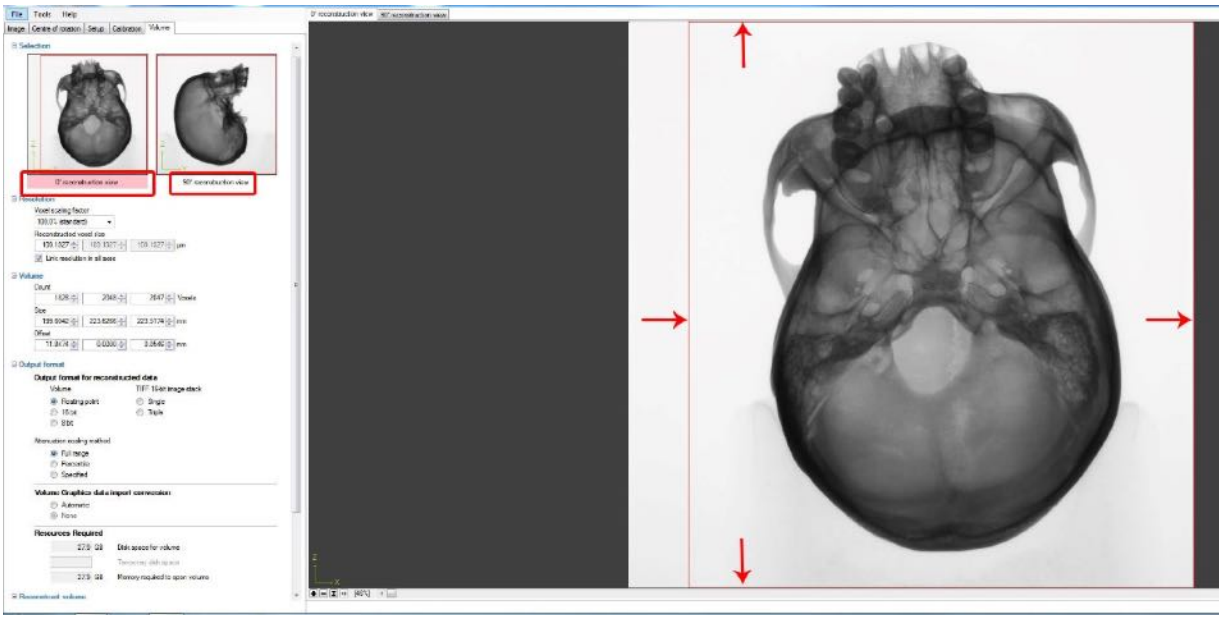


Figure 4. Cropping of the image in 0º and 90º views. The lines indicated by the red arrows can be moved as needed.

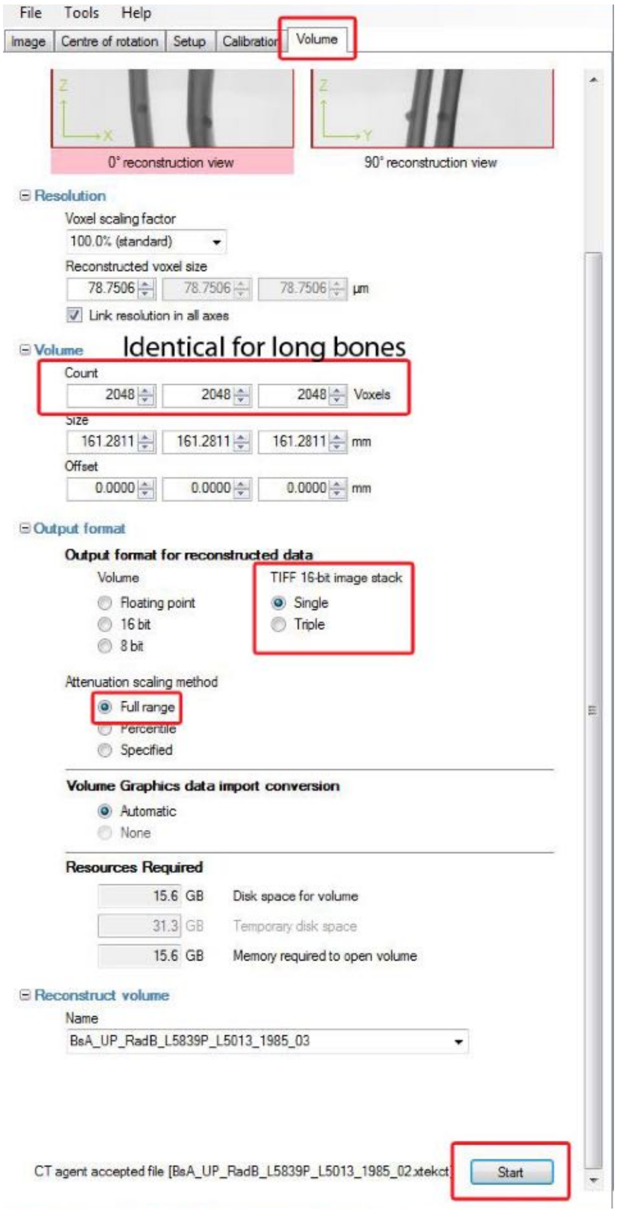


Figure 5. Steps to follow in the volume tab.

<u>STEP 3</u>

The reconstruction progress can be followed in the CT agent (Figure 6).

Open CT agent (it is on the right side of the task bar at the bottom of the screen):

- Always check under the successful tab if the reconstruction worked or alternatively check the failed tab if you aren't sure the reconstruction worked.
- If the reconstruction failed, look what the error was, fix the problem if possible (such as lack of space) and try again.
- Check the tiff files inside the folder with the reconstructions (usually the folder ending in _01) to make sure the reconstruction was successful.

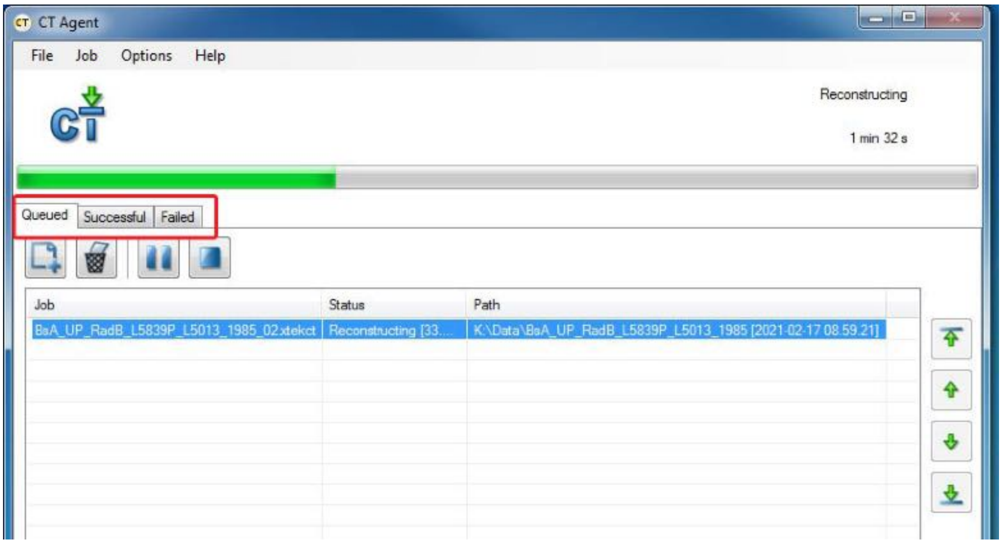


Figure 6. Follow the reconstruction progress in the CT agent.

STEP 4

Copy the entire folder onto an external hard drive or to the Bakeng se Afrika server at Necsa.

Once copied, check that the sizes of the folders are the same under properties.

Shift-Delete the folder on the Necsa reconstruction computer and acquisition PC (to clear out space).

Obtaining data for the Scanning database spreadsheet:

- **date_scanned:** Indicated on the name of the file inside the [ ], for e.g. BSA_UP_Skull_5769_1274 [2020-10-06 12.16.29].
- **original_scan_name:** The name of the folder containing the scans.
- **Xray (kV) and Xray (uA):** Inside the .xtekct file, scroll to the bottom to find these values.
- **projections/360:** Open the .txt file name _ctdata (inside the folder). In the top left corner of the document you will find the value under the heading 'projections'.
- **scanning_resolution:** Inside the .xtekct file, it is the value of the VoxelSizeX (and Y and Z).
- **recon_resolution:** Open the .xtekct file inside the folder containing the reconstructed scans. It is the value of the VoxelSizeX (and Y and Z).

SOP 2.3. Reconstructing procedures after scanning
SUN

STEP 1

On the reconstruction computer, copy the folder of the scan(s) from the acquisition PC to the desired folder in the D:Drive (reconstruction computer)

Open the CT Pro3D program.

Click Open and select the XTEKCT File (.xtekct) inside the folder of the scan you want to reconstruct (there should be 2 files - one is inside the folder one is not - select the one that is not in the folder)

STEP 2

Once it is opened, you will see five tabs:
1. IMAGE

Nothing needs to be changed

2. CENTRE OF ROTATION

- For the **CRANIA:** There is no need to move the red line on the image, unless a study is specifically focusing on an area of the sample (bone), such as the orbits. If that is the case, drag the red line on the image in the left corner down to an area of detail/focus (e.g. anterior of skull, orbits and nasal area).
- For the **VERTEBRAE STACKS:** select DUAL and move the red lines in the image to either ends of the stack. Make sure that the centre of rotation at slices for both upper and lower are close in value and the scan should then be found (Figure 2). (Dual is used because movement of the bone relative to the rest of the sample can occur during the scanning).
- At **Accuracy** select high quality. Press Start.
- Wait for the process to finish. It will show **Found** at the bottom of the screen when completed (Figure 1).

3. SETUP

- For vertebrae stacks: move the red line to the middle of the stack (ignore this step for scans of other types of bones).
- **Beam Hardening**: select 2.
- **Noise Reduction**: select 2.
- **Image processing**: select “Enhance- Mild” (Figure 7).
- Press start, wait for it to finish.

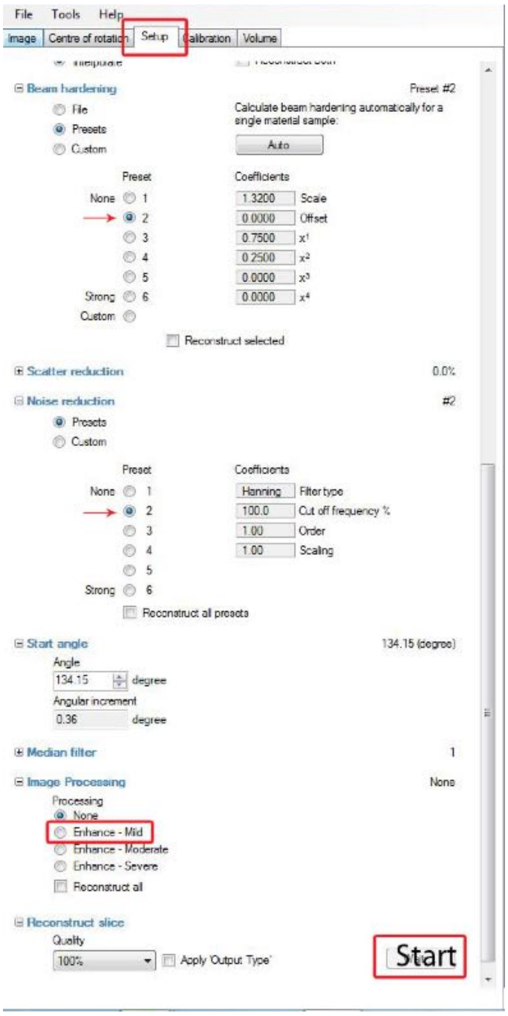


Figure 7. Steps to follow in the setup tab.

4. CALIBRATION

Nothing needs to be changed.

5. VOLUME

- Crop the images in both 0º and 90º views if possible (Figure 4). Be careful not to crop too close to the bone, as this may crop the image of the bone itself.
- **Output format**: select SINGLE under Tiff 16-bit image stack (Figure 5).
- **Scaling method** should be Full range.

STEP 3

The reconstruction progress can be followed in the CT agent (Figure 6).

Open CT agent (it is on the right side of the task bar at the bottom of the screen)

- Always check under the successful tab if the reconstruction worked or alternatively check the failed tab if you aren't sure the reconstruction worked.
- If the reconstruction failed, look what the error was, fix the problem if possible (such

as lack of space) and try again.

- Check the tiff files inside the folder with the reconstructions (usually the folder ending in _01) to make sure the reconstruction was successful.

STEP 4

Copy the entire folder onto an external hard drive or the Bakeng se Afrika server at Necsa.

Once copied, check that the size of the folders are the same under properties.

Shift-Delete the folder on the Necsa reconstruction computer and acquisition PC (to clear out space).

SOP 3. POST-SCANNING PROCEDURES

SOP 3.1. 2D stitching long bones scanned in two- or three-parts using ImageJ

(1.0) Purpose

The purpose of this document is to detail the stitching procedures to follow the reconstruction for addition of scans to the Bakeng se Afrika digital skeletal repository. Because the long bones are too large to scan in one part at an adequate resolution, the bones will be scanned in two (proximal and distal halves) or three parts (proximal half, diaphysis, distal half). Each scan limit will be demarcated on the bone using Prestik or clay. For example, for the radii, the midpoint is marked with Prestik.

(2.0) Scope

This document and the guidelines herein will be used by skeletal collections managers or curators, researchers, contractors and/or the individuals responsible for stitching the skeletal elements once the material has been scanned correctly at the scanning facility (NECSA/ other scanning facility).

(3.0) Roles and Responsibilities

The stitching of long bones scans will be completed by the researcher or contractor that has been trained to do so.

(4.0) Procedures

STEP 1 – Reconstruction

When long bones are reconstructed, the normal reconstruction procedure takes place, except that no cropping should be done. See Figure 1.

- **Selection**: no cropping in the 0° or 90° views.
- **Volume**
  - **Count**: should be 2048 x 2048 x 2048 voxels.
- **Output format**: Select TIFF 16-bit image stack and Single.

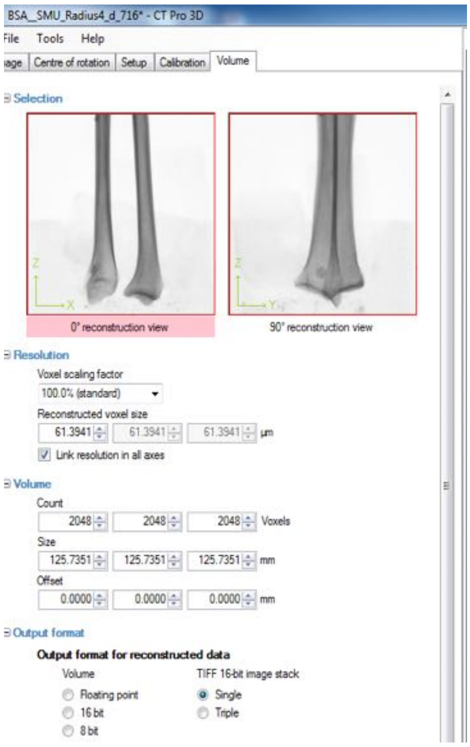


Figure 8. Reconstruction procedure of radii.

STEP 2 – Folders

Once the reconstructions of all parts of the bone have been completed, use the analysis computers.

- Open one of the two (or three) scan folders obtained for each long bone. For consistency, always choose the proximal half folder.
- Create a folder called "Merged" (Figure 2). Open it.

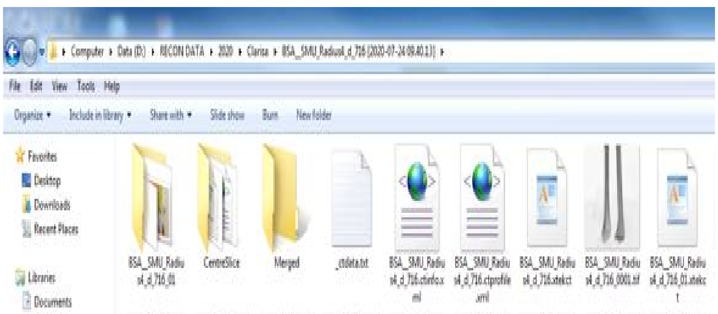


Figure 9. "Merged' folder created.

- In "Merged", create two new folders called "Proximal" and "Distal" (Figure 3).

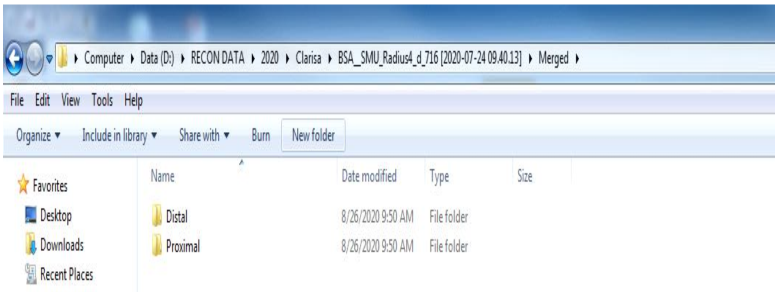


Figure 10. “Distal” and “Proximal” folders created in “Merged”.

STEP 3 – Importation in ImageJ

Using the raw images in their folders, try to identify where the Prestik is placed and where is the zone of transition. Then,

- Open ImageJ
- File → Import → Image Sequence (Figure 4).
    - A dialog box open. Open the reconstruction folder of the proximal half of the long bone and select the first image. Open.
    - Sequence Options dialog box will open → OK (Figure 4). The importation of the stack is starting (it can take a while).

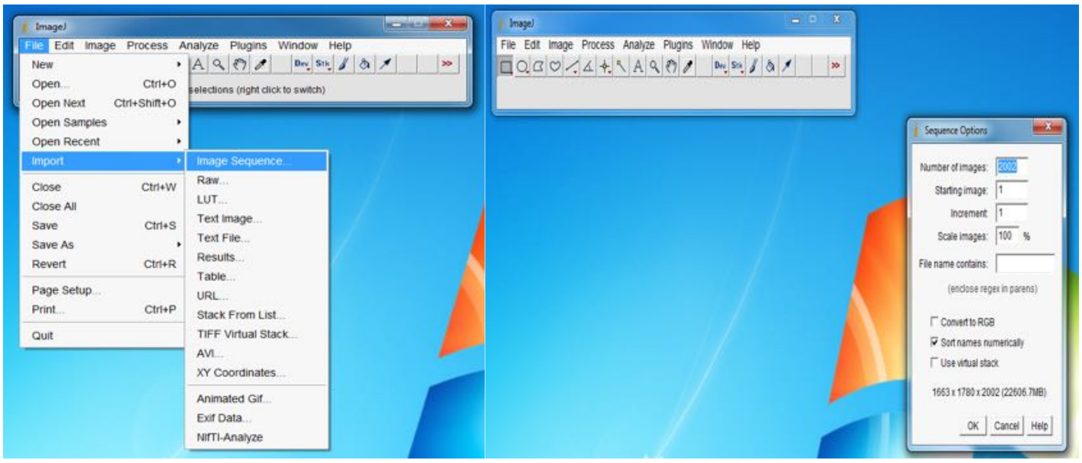


Figure 11. ImageJ importation.

STEP 4 – Locate the Prestik

In the new ImageJ window opened, you can scroll through the scan manually or use the bottom bar (play and arrows). Arrows should allow you to move slice per slice.

- Identify the Prestik: the top or the bottom of it (both of them can be used for the process – but try to be consistent and always use the same one), i.e., stop on the slice where the Prestik can no longer be seen.

- Note the slice number – see the top left corner of the window (Figure 5 – in this example: slice 1957 but location of the Prestik is scan-specific, it will never be at the same slice number).

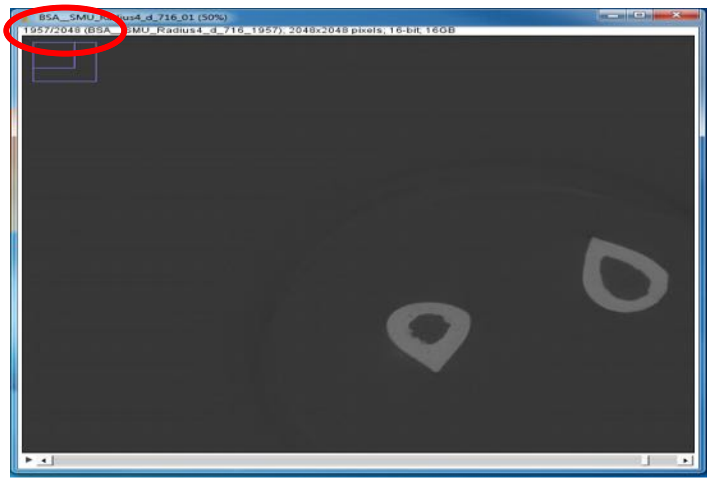


Figure 5. Slice number at top left corner.

STEP 5 – Renaming in XnView

If in Step 3, you loaded the proximal half of the long bone, every slice below and including that number will have to be copied and pasted into the "Proximal" folder created. However, if the distal part is loaded, every slice above and including that number will be copied and pasted into the "Distal" folder created (in our example, slices 0 to 1957 included).

The files in the reconstruction that are placed on top during the stitching process will then need to be renamed to match the file names used in the reconstruction placed at the bottom during the stitching process.

- Open XnView and locate the folder containing the files to be renamed.
- Select all the files.
- In the top bar, Edit → Rename (Figure 6).

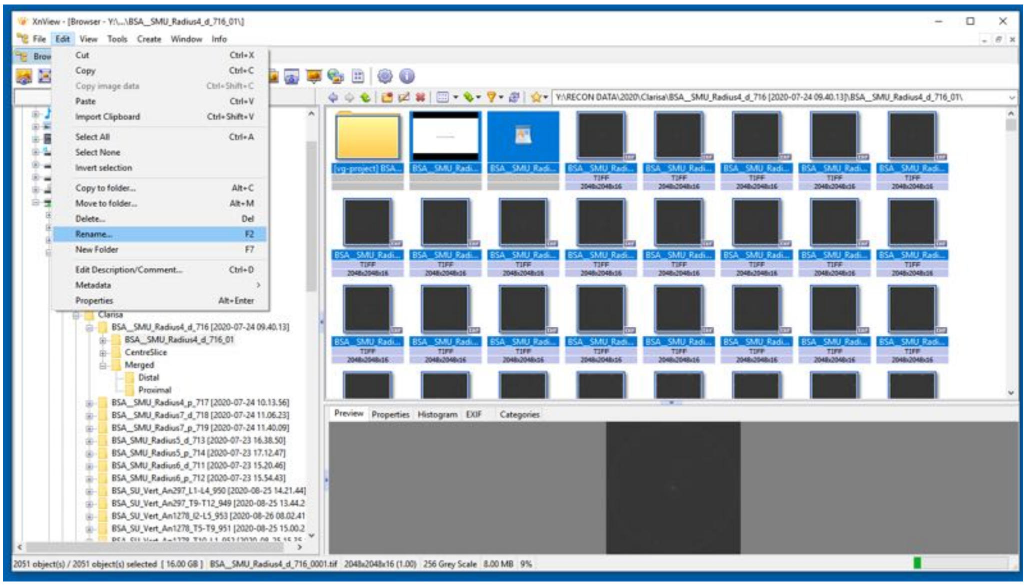


Figure 6. Rename in XnView.

- A dialog box opens (Figure 7).
  - Select "Name Template".
    - Copy the file name (without the end number) from the files you wish to rename your files to
    - Paste the name ensuring that the "####" in the name is kept.
  - Start: type in the number from which the scans will start. The number indicated is the number after the last number in the other folder so that all the files follow on from each other.
  - Double check the files name to make sure they match.
  - Click Rename and wait.

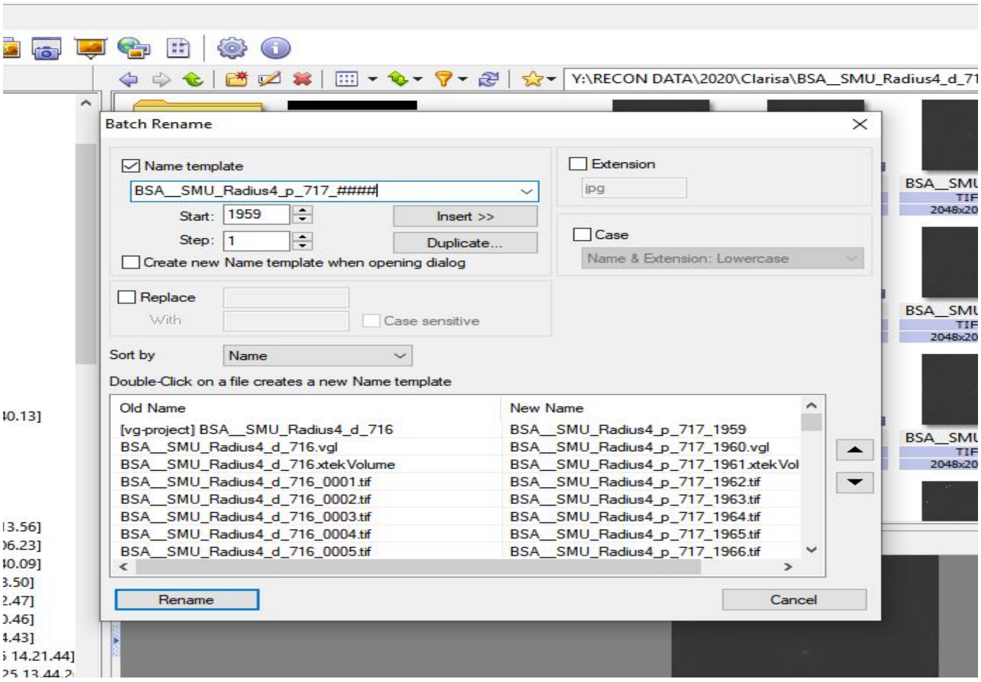


Figure 7. Rename dialog box.

Once renaming is finished

- Open the Folder containing the newly renamed files
- Copy and paste them into the second folder so that all the files are in the same folder. This is the final merged Tiff Stack.

The names of the files need to be double checked again so that they are all the same and have the correct numbering or the file will not open later.

STEP 6 – Cropping in ImageJ (can also be done in VGStudio, or in Avizo)

The new merged scan created is very big, we will have to crop what is unnecessary.

- Open ImageJ
- File → Import → Image Sequence
  - Select the first file of your newly merged stack. Wait for the importation to finish – it can take time.
- Scroll through of all the slices to check if the Prestik Marker can be seen and that the files merged correctly.
- Select the crop tool and draw the rectangle on the scan (Figures 8 and 9). Scroll through all slices and make sure the bones are in it.

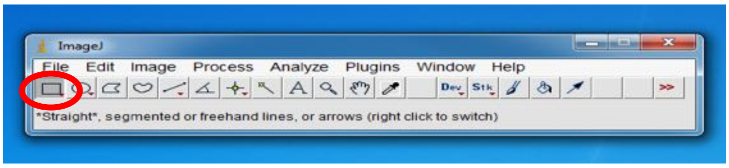


Figure 8. Crop tool

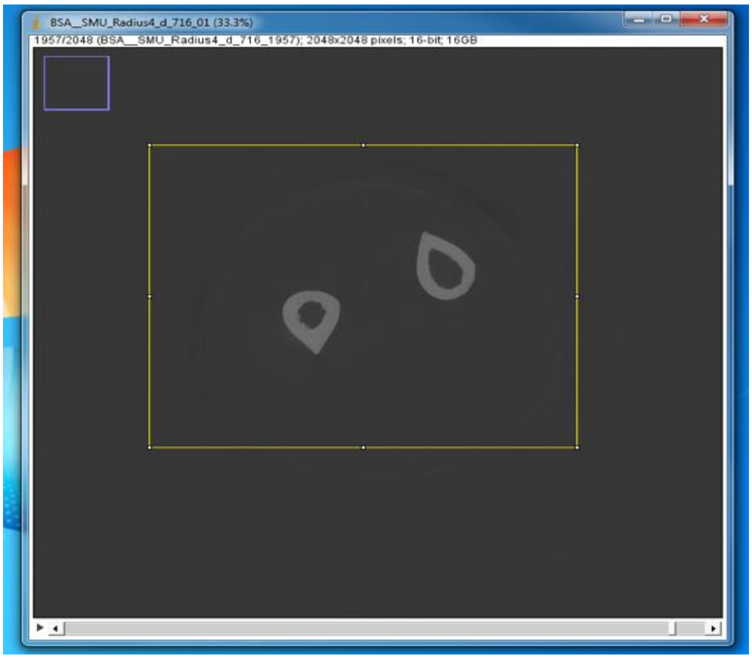


Figure 9. Cropping process

- Image → Crop (Figure 10).

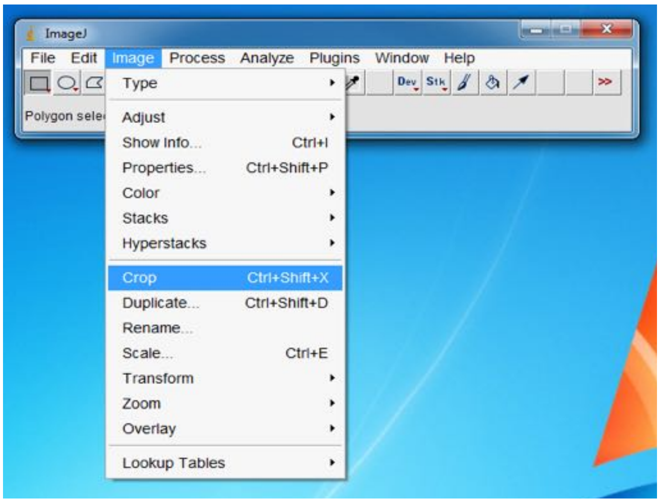


Figure 10. Cropping process.

Save the cropped stack

- File → Save as → Image Sequence (Figure 11). Find the empty folder and save there.

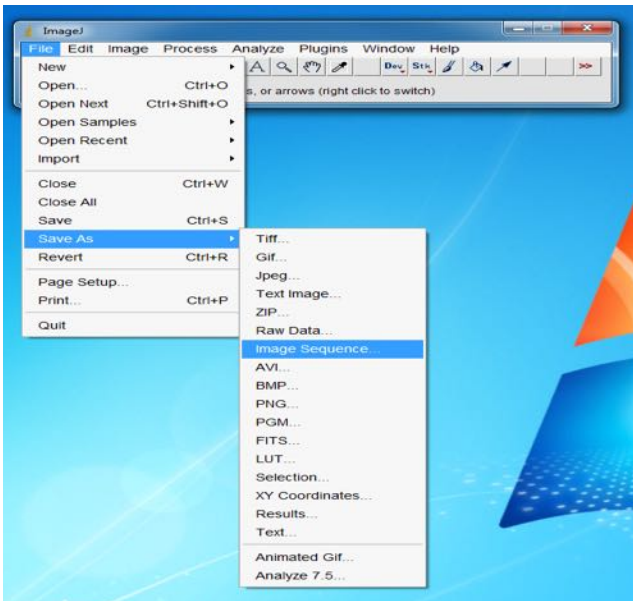


Figure 11. Saving process.

- Rename the folder.

STEP 7 – Final check in VGStudio or Avizo

Check the stitching process was done correctly by opening the image stack in VG Studio or Avizo.

SOP 3.2. 3D stitching long bones scanned in two- or three-parts using VGStudioMax

(1.0) Purpose

The purpose of this document is to detail the stitching procedures to follow the reconstruction for addition of scans to the Bakeng se Afrika digital skeletal repository. Because the long bones are too large to scan in one part at an adequate resolution, the bones will be scanned in two (proximal and distal halves) or three parts (proximal half, diaphysis, distal half). Each scan limit will be demarcated on the bone using Prestik or clay. For example, for the radii, the midpoint is marked with Prestik.

(2.0) Scope

This document and the guidelines herein will be used by skeletal collections managers or curators, researchers, contractors and/or the individuals responsible for stitching the skeletal elements once the material has been scanned correctly at the scanning facility (NECSA/ other scanning facility).

(3.0) Roles and Responsibilities

The stitching of long bones scans will be completed by the researcher or contractor that has been trained to do so.

(4.0) Procedures

1. General steps and notes for VGStudio:

1.1. Importing data into VGStudio

1.1.1. Open VGStudio software

1.1.2. Click on File > Import > Image stack > Directory > {locate and click on the data you want to import. (Figure 1). OR

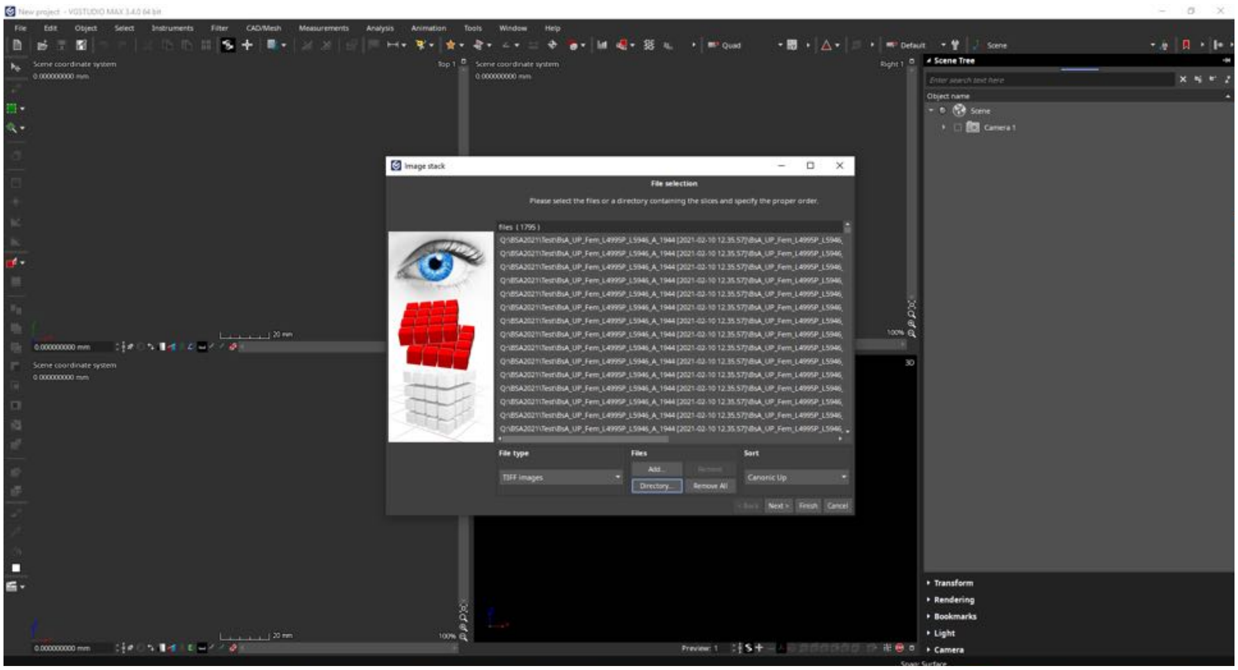


Figure 1. Importing data into VGStudio

1.1.3. Click on File > Import > VGL (volume from .vgl file) > Browse > {locate and click on the data you want to import > Next (Figure 2)

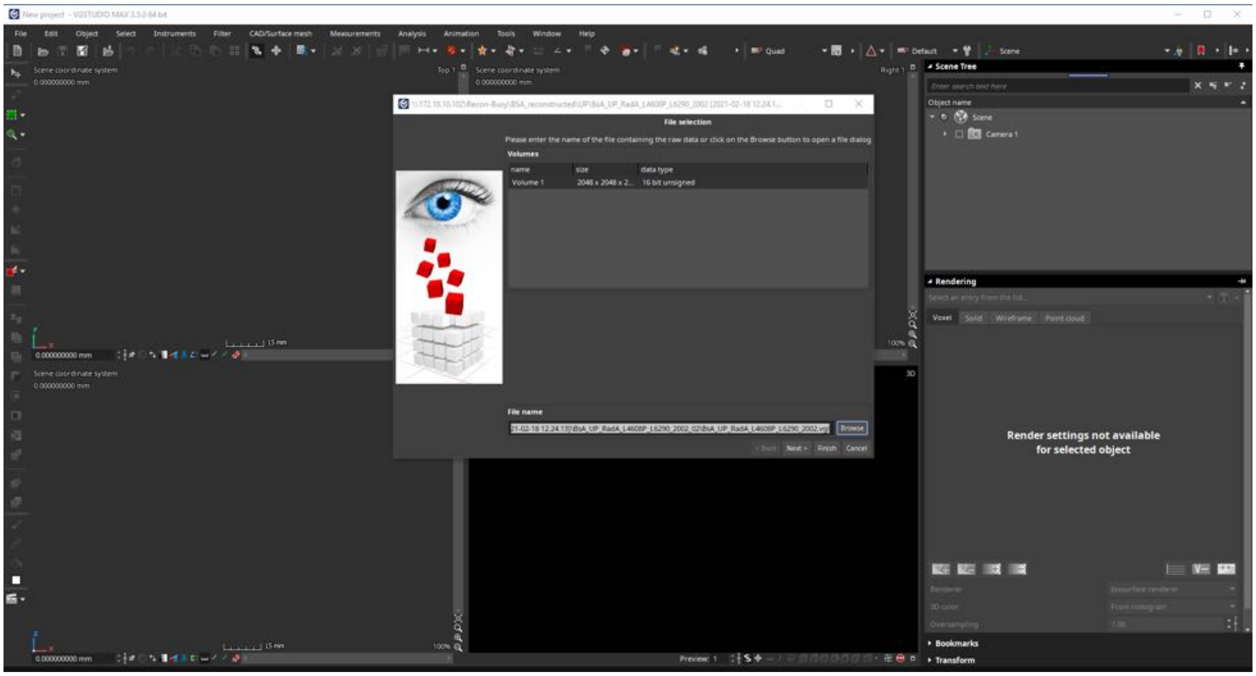


Figure 2. Importing vgl files into VGStudios

1.1.4. Make sure that sort: Canonic <u>U</u>p and file type: TIFF Images. Click Next.

1.1.5. Insert resolution into boxes x, y and z (locate these values in the .xtek file inside the reconstruction folder) (Figure 3)

1.1.6. Check that the file type: Tiff, Map to: 16 bit (note: if you want to change the file to 8 bit, you can do it here by changing the Map to: 8 bit) (Figure 3)

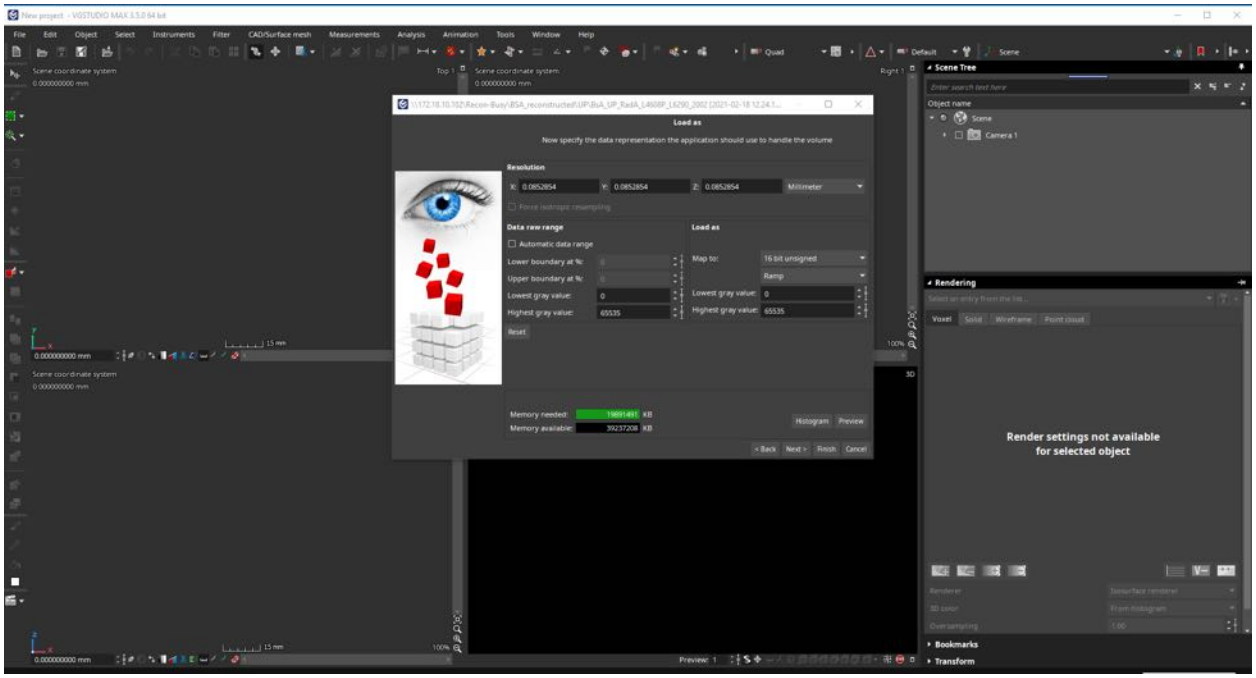


Figure 3. Inserting the X,Y, and Z values.

1.1.7. Click on Histogram. (This is to check the grey value distribution of the data that you are importing. For 16 bit files, the histogram will be a solid continuous spectrum. For 8 bit files, there will be vertical gaps in the histogram). (Figure 4)

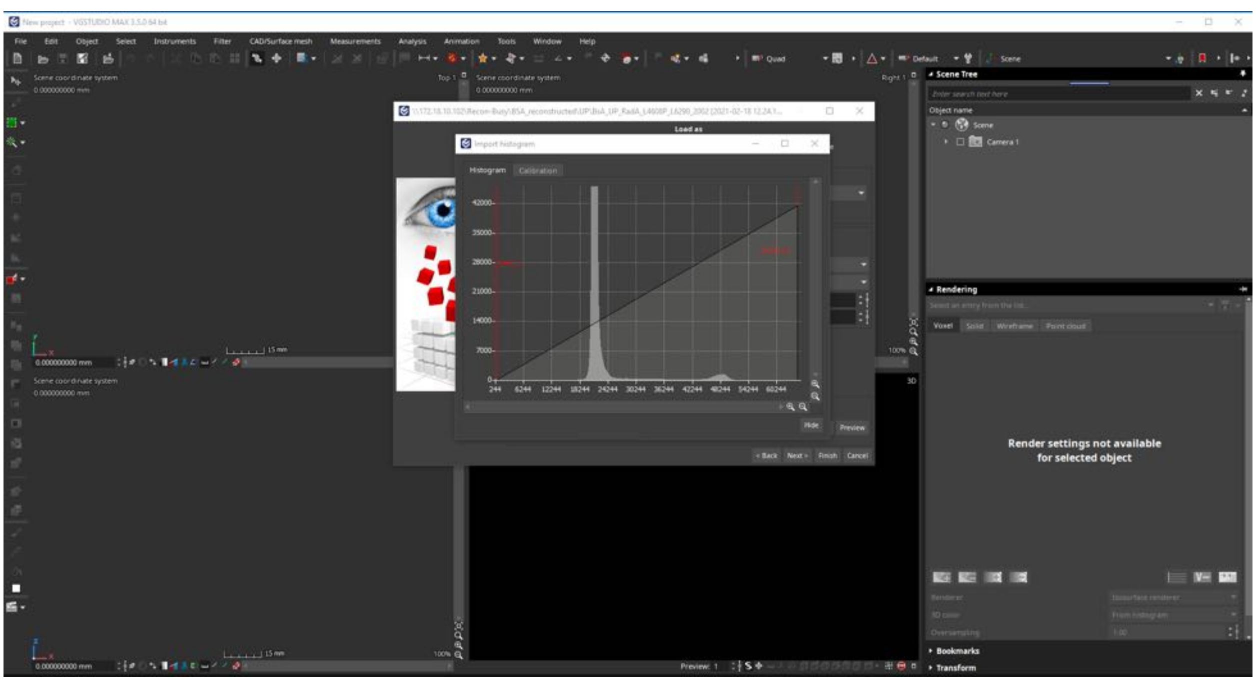


Figure 4. Histogram

1.2. Calibration

(This is to ensure the files have matching grey values)

1.2.1. Select Calibration (Figure 4). Select a slice with very dense bone (Figure 5).

1.2.2. Click Define Background. Zoom in on a bone. Select a small rectangle of background as close to the bone as possible to calibrate the background grey value. Avoid any polystyrene pieces if present. The green rectangle will disappear when it is finished calibrating (Figure 5).

1.2.3. Click Define Material. Select a very small section of the bone (avoid porosities). The red rectangle will disappear when the calibration is complete (Figure 5).

1.2.4. Click Apply. The background should darken. Back on the "Load as" screen, the Lowest grey value should now be 0 in the right side of the panel (Figure 5).

Figure 5. Define the material and the background to ensure matching grey-values for the multiple scans per bone.

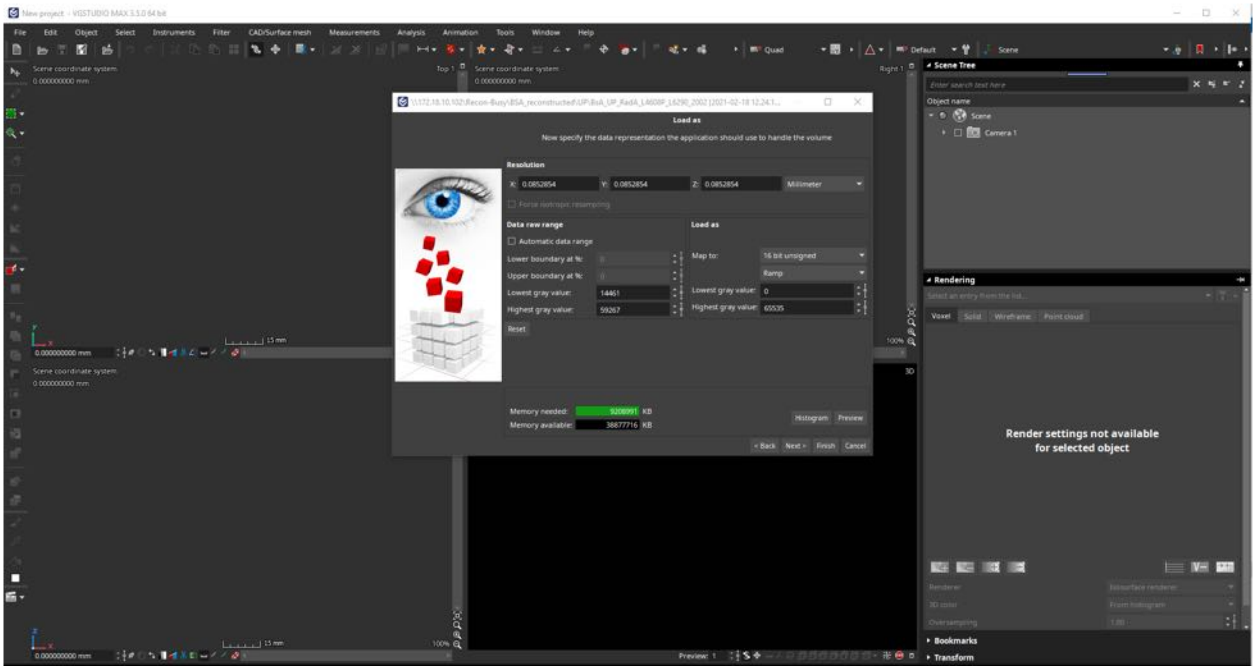


Figure 6. Grey value zeroed after defining the material and background.

1.2.5. Hide the Preview.

1.3. Removing edge artefacts

This removes any projection artefacts which may result in loss of data in the final merged bone.

1.3.1. Click Preview (Figure 4)

1.3.2. On the top projection, the bone should be visible within a circle. Crop the bones out to remove as much of the circular edges as possible (these might be visible later). Ensure all the scanned material (including the Prestik) is in the selection. (Figure 7)

1.3.3. In the Front Projection, drag the top and bottom of the blue rectangle to crop out any edge artefacts. These might be clearly visible as lighter sections in the background. (Figure 7)

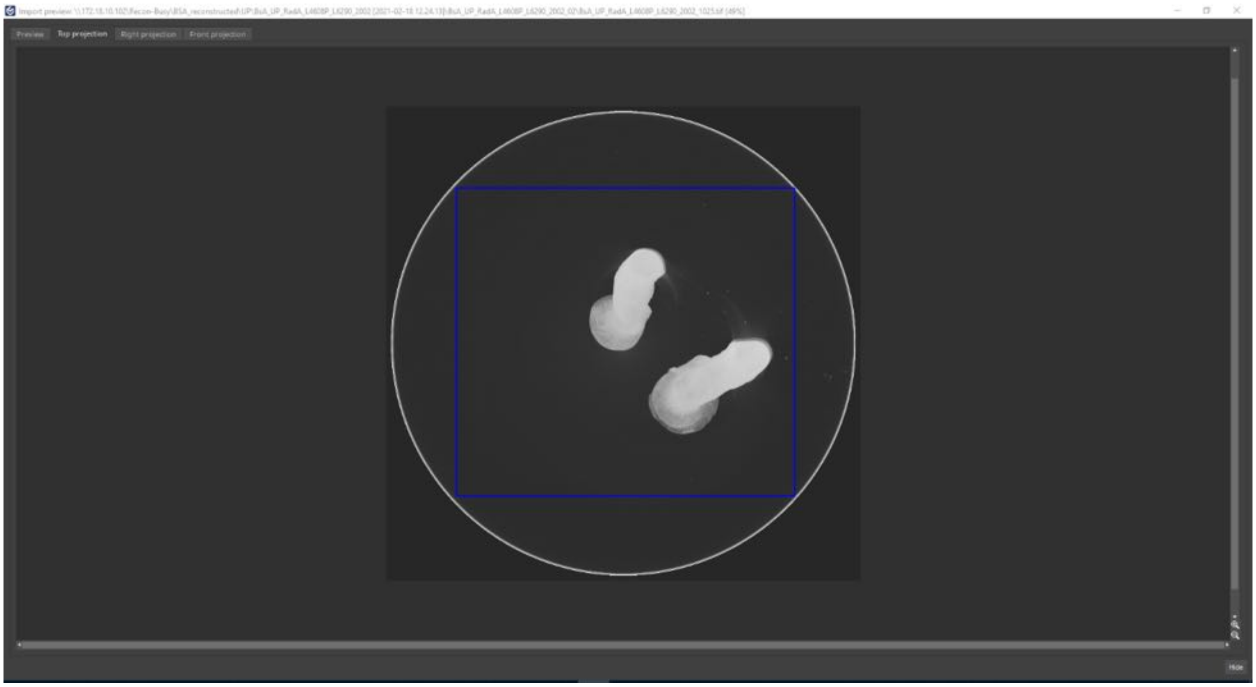


Figure 7. The blue square is used to crop out the ring edge artefacts in the Top projection.

1.3.4. Select Hide.

1.4. Click on Finish.

1.4.1. When uploading another dataset, always select 'remove all' before selecting Directory. (File > Import > Image Stack > Remove all > Directory)

1.5. Checking the locks

1.5.1. Always check that the locks on each volume or dataset (in the scene tree tab) are closed, unless a process requires it to be unlocked.

1.6. Changing the speed at which to turn the bone in 3D view

1.6.1. In the 3D view (right bottom block) the Preview can be changed to 1. This will move the bone at a slower rate and will not pixelate the image as you turn the bone.

2. Import the first two scans for merging

Note: This is for the two scans where the bone was not moved between the first two scans. For femur scans, this was generally the middle and distal parts of the femur. The bone would have been rotated upside down in order to scan the proximal or third part. For shorter bones, such as the radius, this would include both scanned parts. In this SOP, the following was used as an example: A = distal, B = middle, and C = proximal part of the bone (e.g. for the distal scan of the femur of cadaver numbers 5889 and 6453, the name of the file will be BsA_UP_fem_5889P_6453_**A**. The P indicates on which bone an extra Prestik marker is placed, to match the correct bones with each other)

2.1. Import the datasets for the first two scans (A and B) into VGStudio – refer to section 1 above.

2.2. Choose part B (scan of the middle of the bone), as a reference for the below processes. DO NOT MOVE THIS PART during the entire process, i.e. the lock will always stay locked.

3. Change the background colour and reduce the noise

3.1. Select A in the scene tree tab (Figure 8)

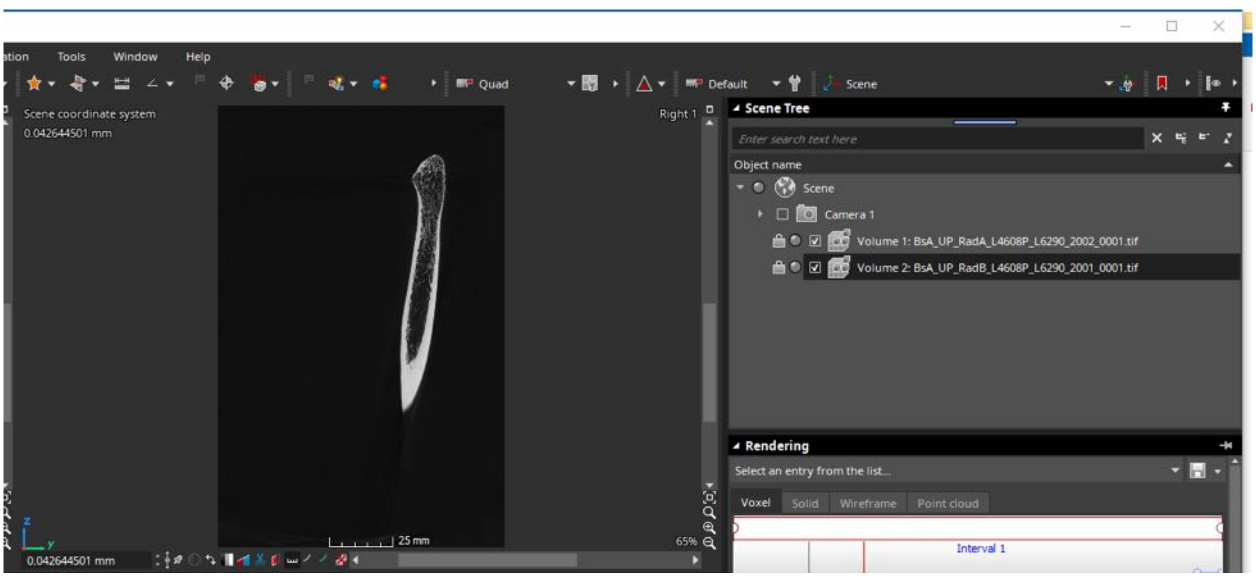


Figure 8. Scene tree tab where you select which volume you want to work with.

3.2. Right click on the background in any of the four boxes/grids > background > black (Figure 9)

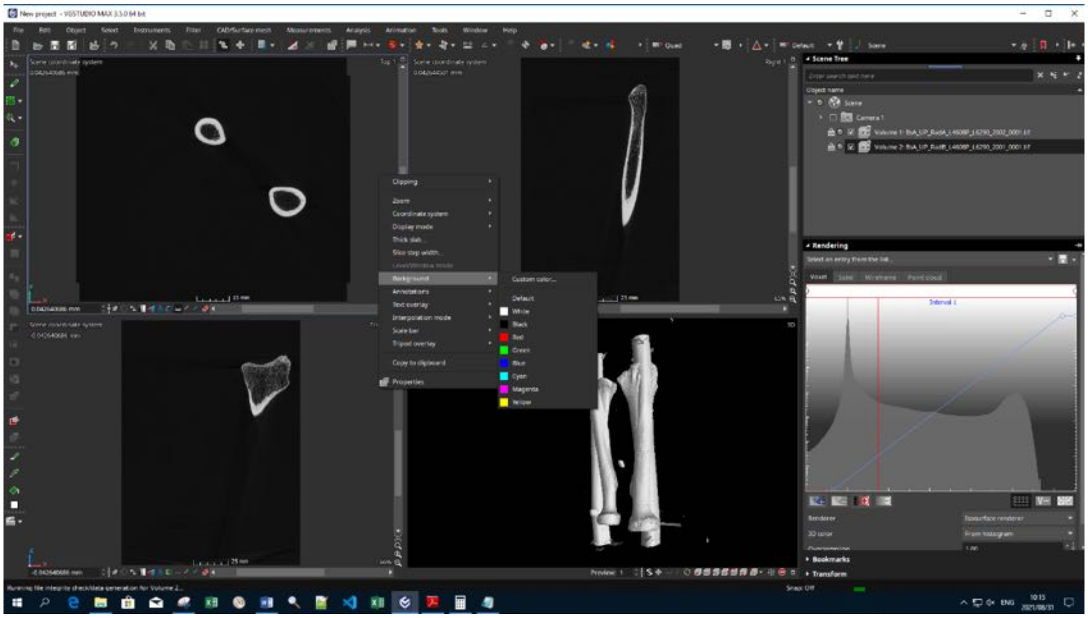


Figure 9. Changing the background colour to black and in the rendering tab noise reduction can be accomplice by moving the red and blue lines.

3.3. In Rendering tab, move the red line to the right until the bone is clearly visible. The blue line can also be moved slightly to the right (at the horizontal axis) – be careful not to move it too much as this will remove information (such as the trabecular bone). (Figure 9)

4. Find the absolute distance that the scan moved down

4.1. Change the built-in coordinate systems from 'volume gride' to 'scene' (this will change the metric unit at the bottom of the 2D views to mm). (Figure 8)

4.2. For the following steps, decide whether to use the top or the bottom of the Prestik marker on the bone. For the purpose of the SOP the top of the Prestik will be used. This should be the same in parts A and B. (Note: you can also use any landmark on the bone, as long as you can find the same landmark on both scans A and B).

4.3. In the scene tree tab, Select part A.

4.3.1. Go to the top left block (top view of the bone) and place the cursor over the [] sign. Drag the mouse up or down to go through the slices until you are close to the top of the Prestik, then use the wheel of the mouse until you find the slice where the Prestik is absent (i.e. the slice just above the Prestik). You can also choose to use the bottom of the Prestik (i.e. the slice just before the Prestik disappears). (Figure 10)

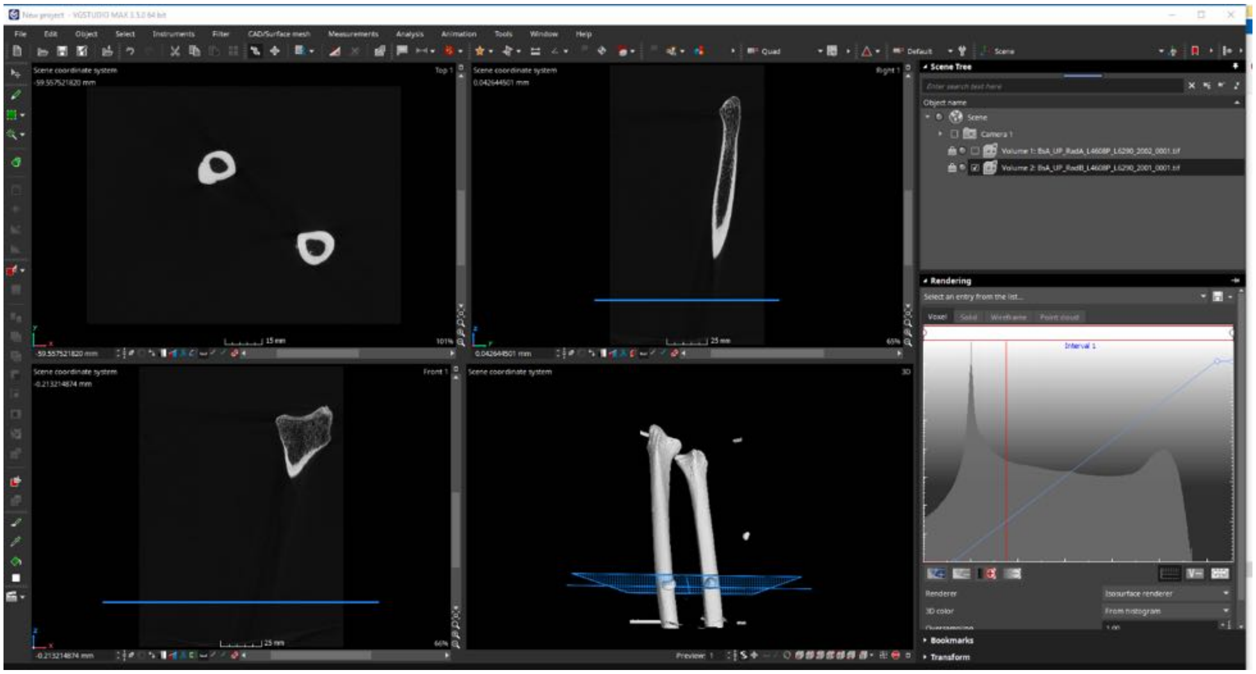


Figure 10. Finding the slice just above or below the Prestik

4.3.2. Record the mm value at the bottom of the top left block (Figure 10).

4.4. Repeat the process for part B.

4.5. Add the two values together, and record this value. One value will be positive, and the other negative – disregard the negative (Figure 11).

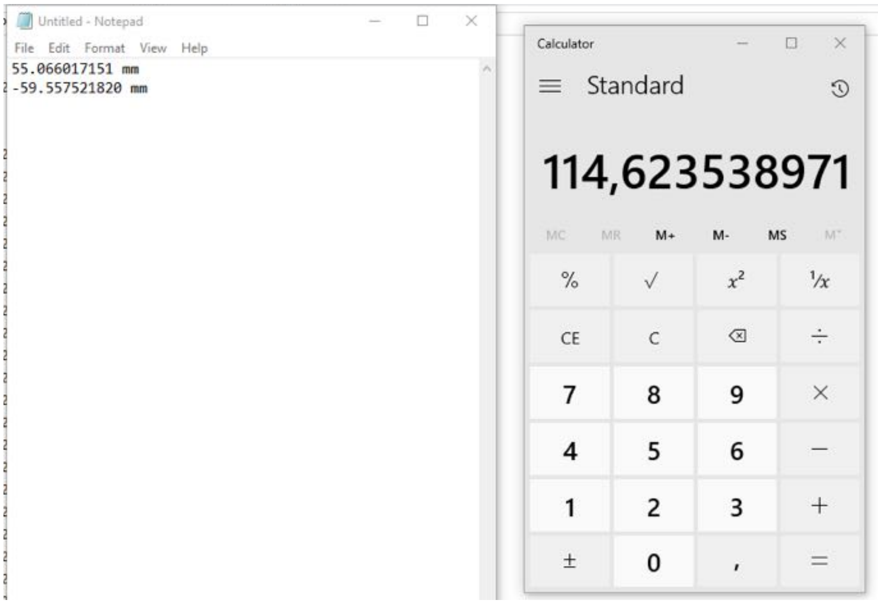


Figure 11, Calculation of the position of the two Prestiks. Finding the zone of overlap.

4.6. In the scene tree tab, select A and unlock the lock (Figure 12).

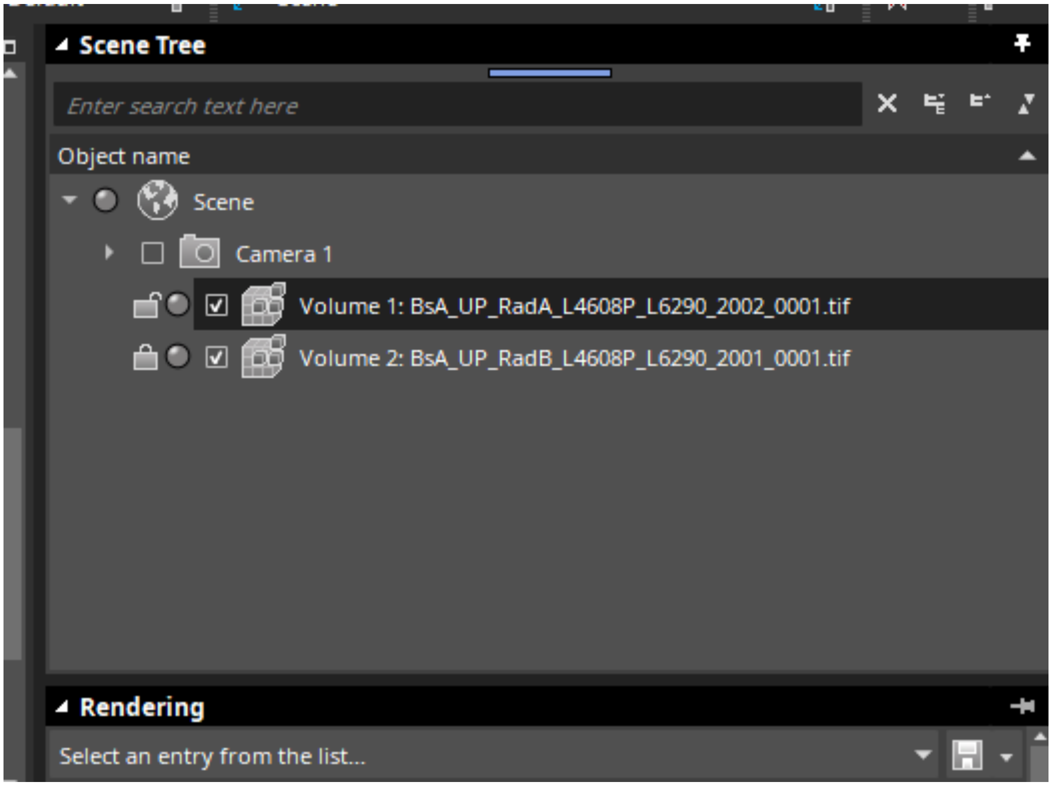


Figure 12. Unlock Volume A

4.6.1. In the transformation tab > Position > insert the total value (step 4.5) into the z axis with a (-) in front. The (-) will move part A down – remember that B, the reference, should not move (Figure 13).

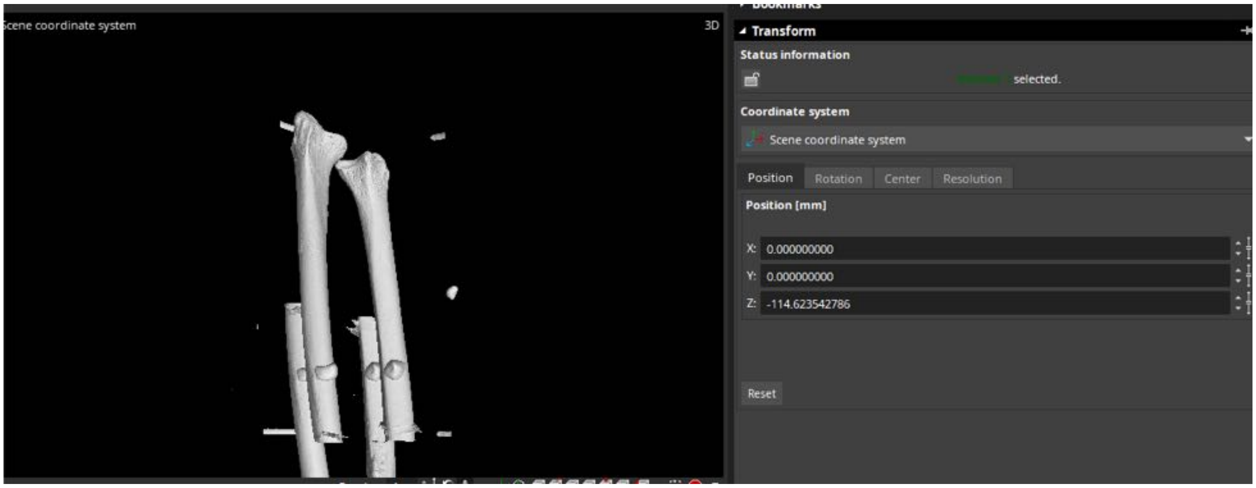


Figure 13. Move part A down, so that the zone of overlap can be aligned.

4.7. Lock the lock on Part A.

Note: The two parts of the bone should now be merged in the vertical axis. If the scan was not cropped under the Volume tab during the reconstruction process, this step is now complete. If all or one of the scans were cropped, either in reconstruction or when opening the scan (Section 1.3 above) the two parts of the bone will not be aligned in the horizontal axis. If so, follow the next step.

5. **Move part A horizontally to merge with B**

5.1. Use the [] to rotate the view in 3D in order to view the bone from the side.

5.2. Unlock and select A (Figure 12): manually move part A horizontally as close as possible to part B (if you move it up or down, you have to redo step 4.6 and 4.7 above):. Use the [] to move the bone left and right.

5.3. Lock A.

5.4. Select A. Go to Object > Surface determination

5.4.1. Select Approach: Advanced (classic), Material definition: automatic, press Finish (Figure 14).

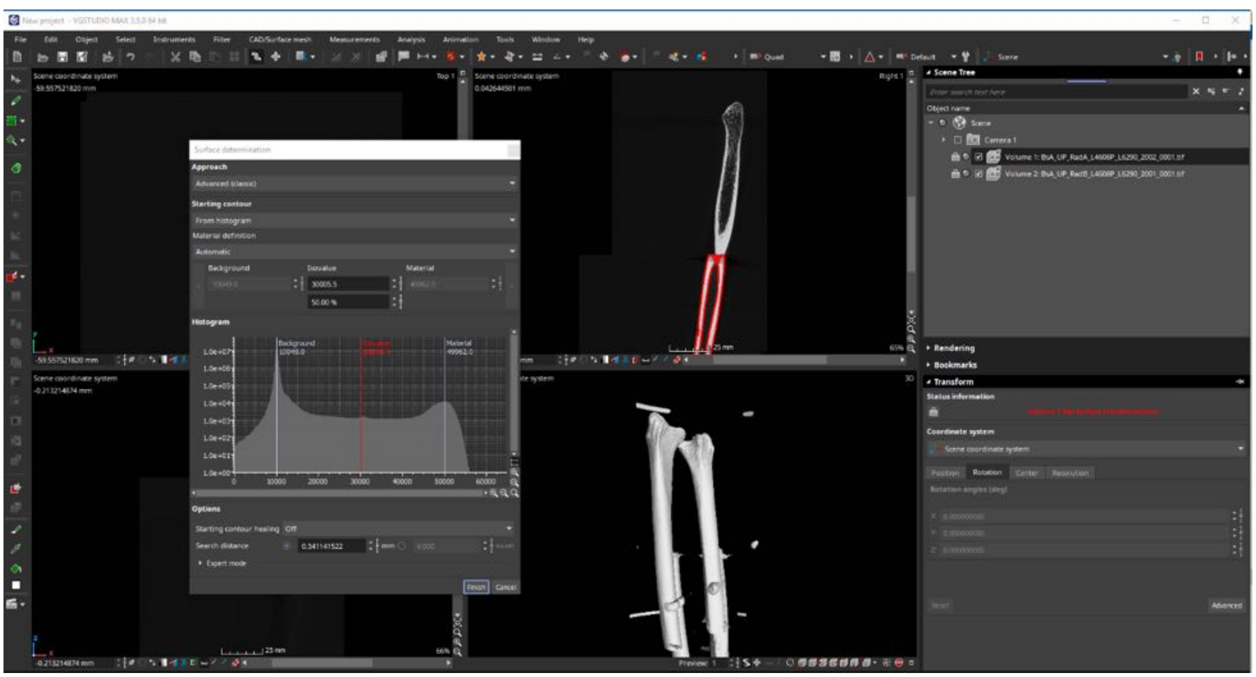


Figure 14. Surface determination of a volume.

5.5. Repeat for B.

5.6. Go to Object > Register object > Best fit registration

5.6.1. The object to be registered should be A, object to be fitted to should be B. Move the cursor to a value of between 40 and 50 (Figure 15)

5.6.2. Tick the boxes: consider current transformation and consider surface orientation (Figure 15)

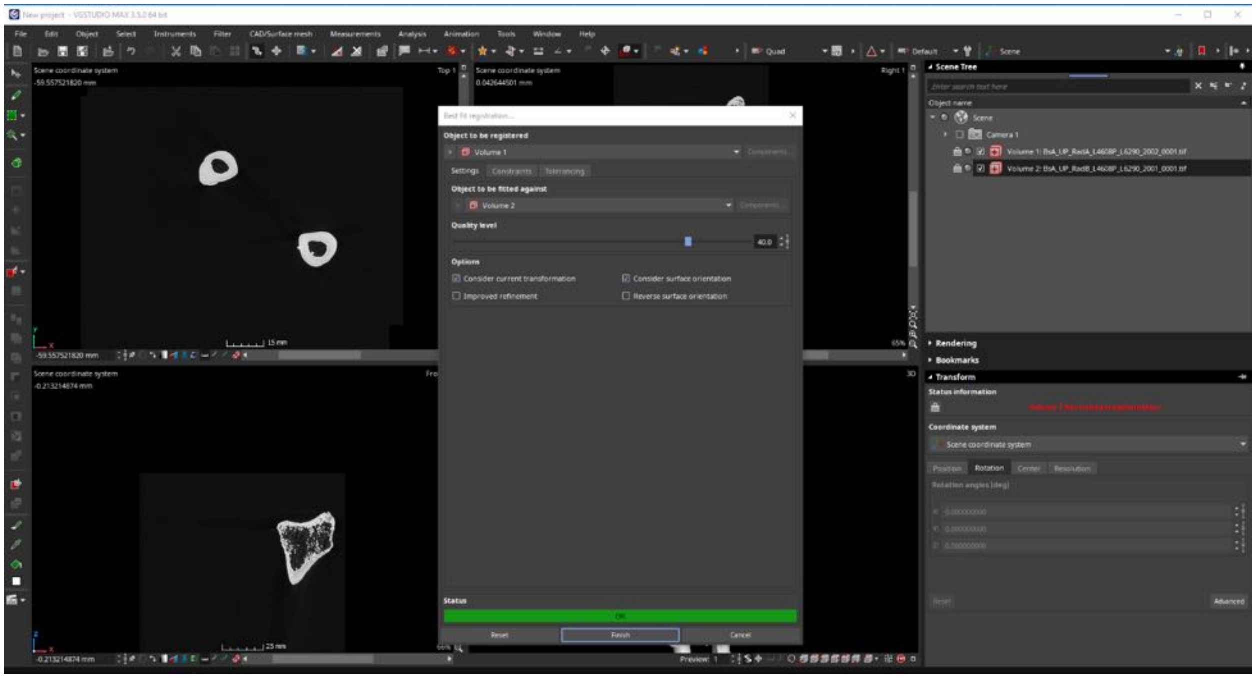


Figure 15. Register object – to fit part A to part B automatically.

5.6.3. Click Finish.

5.7. Parts A and B should now be merged and aligned. Check that the process was successful (Figure 16). If not, repeat the manual movement and Best fit registration steps.

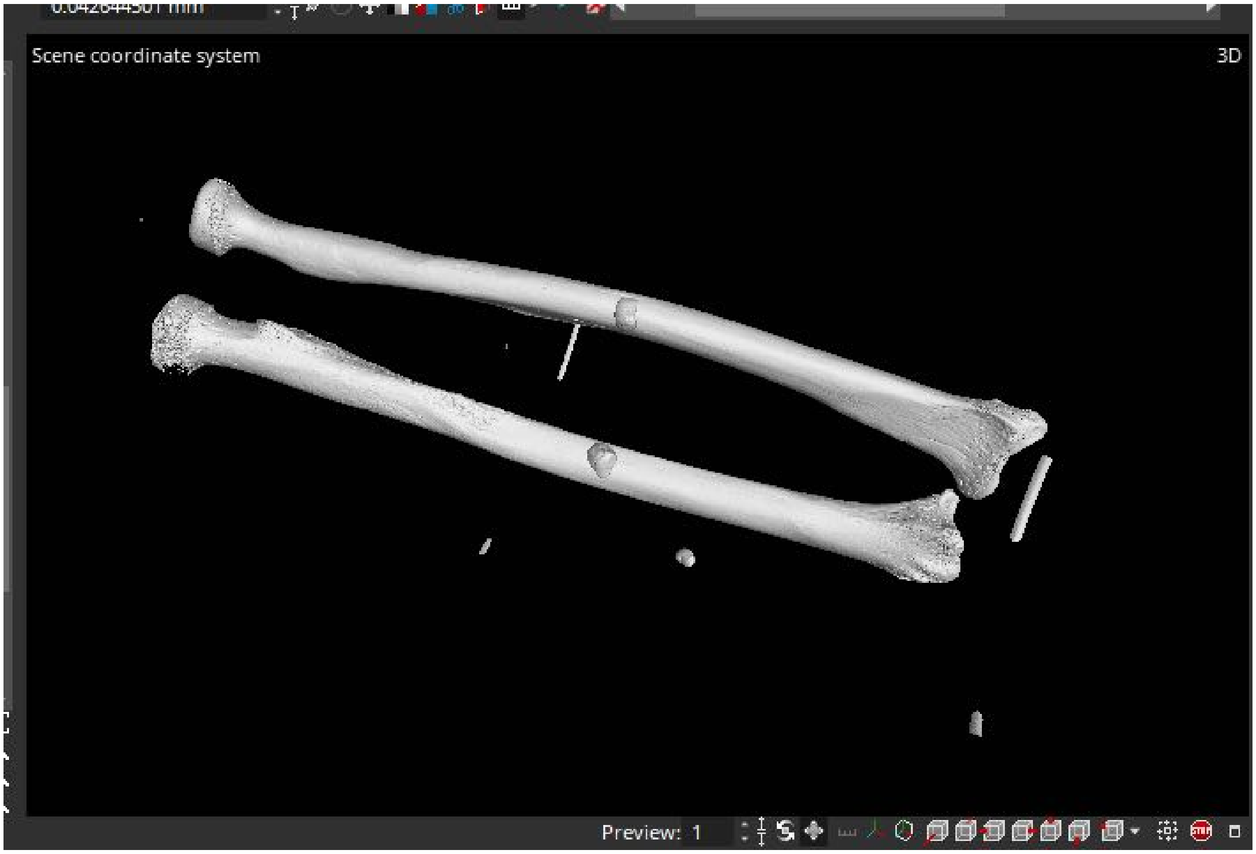
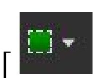


Figure16. Part A and B stitched together for each bone.

5.8. Right click on A and remove surface registration, then do the same for B

6. Separate or isolate the two bones scanned together

6.1. Select A.

6.1.1. Click the green rectangle (left side of the screen) [] > Drag a box around the bone to be isolated. Move the red lines or red crosses in all the 2D views. Move through all the slices in the top view, to ensure that the entire bone is inside the ROI box (Figure 17).

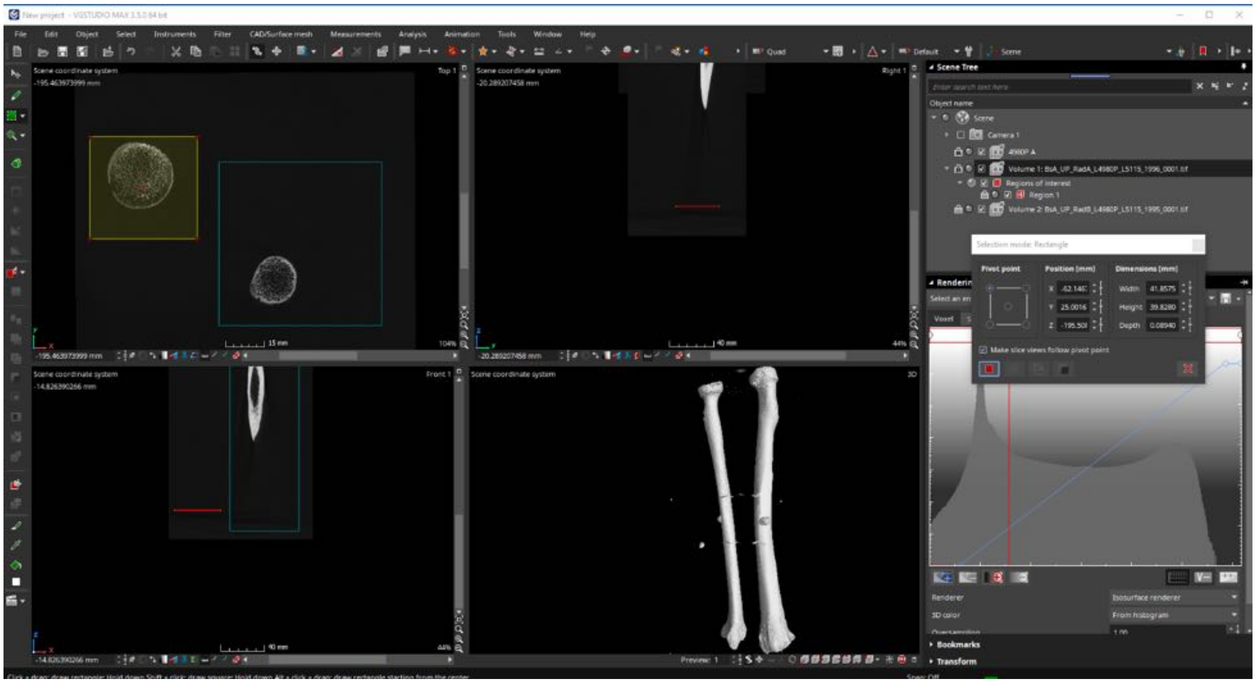


Figure 17. Separating the 2 bones from each other by creating a region of interest around each individual bone.

6.1.2. Press create ROI (Figure 18).

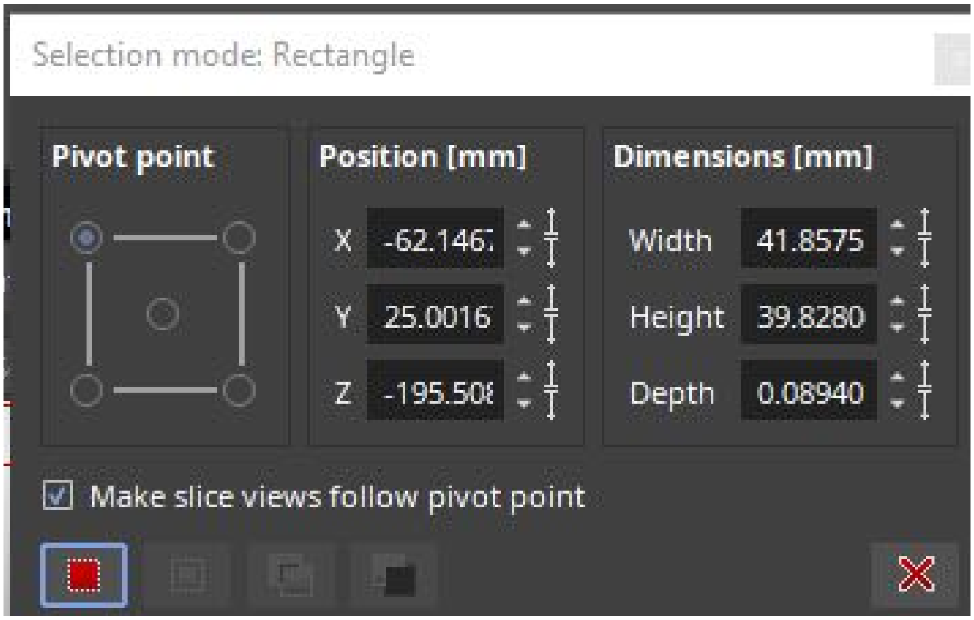


Figure 18. Create an ROI by clicking on the red square.

6.1.3. Click on the created ROI in the scene tree tab. Draw additional boxes on areas where the second bone is inside the above ROI. Press Subtract ROI. (if needed)

6.1.4. Select ROI, right click > extract ROI.

6.1.5. Rename extracted ROI to the cadaver nr (for example) and A. Include a P if a Prestik marker is on the bone - eg. 5994P A

6.2. Repeat for B

6.3. For each part (A and B), right click on the ROI > invert ROI

6.4. Right click on ROI created > extract ROI.

6.5. Rename extracted files as mentioned above.

Note: For bones with only two scans (such as a radius), the process is now complete. Proceed to section 10 to save the data.

7. Merge Part C to Part B

7.1. Import the tiff files of part C into VGStudio.

7.2. In the scene tree tab, select Part C.

7.2.1. Go to the rendering tab. Move the red line to the right until the bone is clearly visible (if needed)

7.3. Unlock and select C. Go to the Transform tab > Rotation. Rotate the bones to be in the same orientation as Part B. Lock C. (Note: this step is not always necessary, as the program can automatically do this with the Best fit Regression)

7.3.1. Go to Object > Register object > Simple registration

7.4. Click on the top view (top left corner), and rotate the bones so that the two bones are parallel to each other (this will make the isolation of the bones easier).

7.5. Proceed to isolate the two bones of part C by creating a ROI and extracting the ROI, and rename (refer to steps 6.1 above)

7.6. Select B's extracted ROI (only if the surface determination has been removed)

7.6.1. Click on Object > Surface determination

7.6.2. Select Approach: Advanced (classic), starting contour: from histogram; material definition: automatic, press Finish.

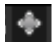
7.7. Repeat for part C's extracted ROI.

7.8. Click on Object > Register object > Best fit registration

7.8.1. Object to be registered: C, Object to be fitted against: B. Quality level: anything above 20 is fine.

7.8.2. Select the box: consider surface orientation

7.8.3. Press Finish.

Note: If the merging was unsuccessful, move part C manually to match part B as close as possible (Use the [] to move the bone left and right), and repeat the best fit registration.

7.9. Check that the parts of the bones merged correctly: View the region of overlap in the 3D view. In the Rendering tab, move the red line to the right (or left). The point of overlap should fade away. The region of overlap can also be checked in the 2D views. Move the blue line on the horizontal axis to reduce the noise in the 2D views.

7.9.1. The parts should be completely aligned and transition smoothly.

7.10. Repeat for the second bone.

8. Group parts A, B and C of the same bone

8.1. Select the extracted ROI's of A, B and C for the same bone (same individual).

8.2. Right click > Group Objects.

8.3. Rename Group to the cadaver number (remember to add the P for Prestik if applicable).

9. Removing rings around the bones (if not done in the beginning)

If the scanning tube is visible on the merged scan of the non-prestik bone (Inverted ROI), it can be removed by further extracted the bone. Most of this should have been cropped out in Section 1.3, but a few sections might still be visible. If no external artefacts are visible, continue to Section 10.

9.1 Select Part A of the inverted bone.

9.1 Select the green pencil (draw function) on the left side [ ].

9.3 Change radius to 40mm (for radii) and thickness to the maximum value possible.

9.4 Select 2D disc mode.

9.5 Move the yellow circle to encircle the entire bone in the tube while excluding the visible rings from scanning support structures.

9.6 Click anywhere to save the ROI. If the circle does not move it is busy creating the ROI.

9.7 Extract the new ROI. Rename it to the bone name (should be the bone without the P)

9.8 Repeat for Part B.

9.9 Group the new extracted ROIs and rename to the cadaver number. The first grouping can now be deleted.

10. Saving of stitched bone

10.1. For A and B (or A, B and C): Click Object > Remove surface determination

10.2. Center each part by clicking the [] icon at the right hand side of the top view (top left box)

10.3. Make sure all 2 (or 3) parts are selected (in the scene tree tab) (Figure 19)

10.4. Select File > export > Export aligned/multiple volumes (this also ensures that grey values of the bone are similar and in the same range) (Figure 19)

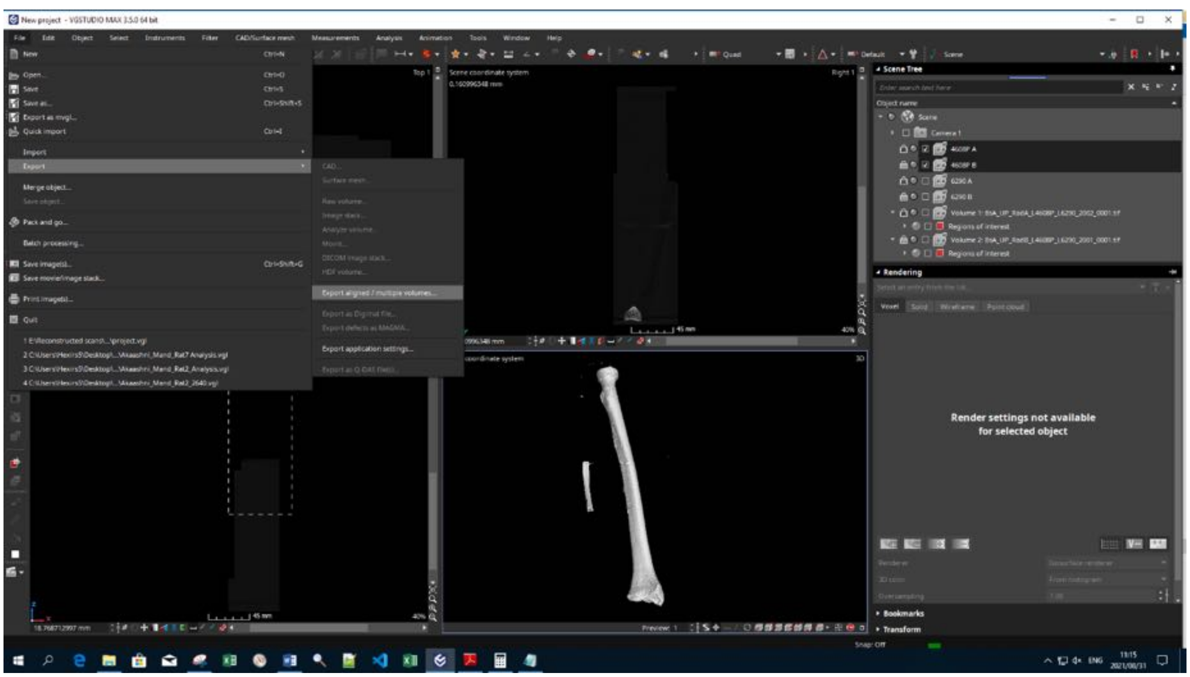


Figure 19. Exporting the stitched and separated bone to save as a new folder.

10.5. Check settings and selections (A, B and C should be visible under Source Volumes) (Figure 20)

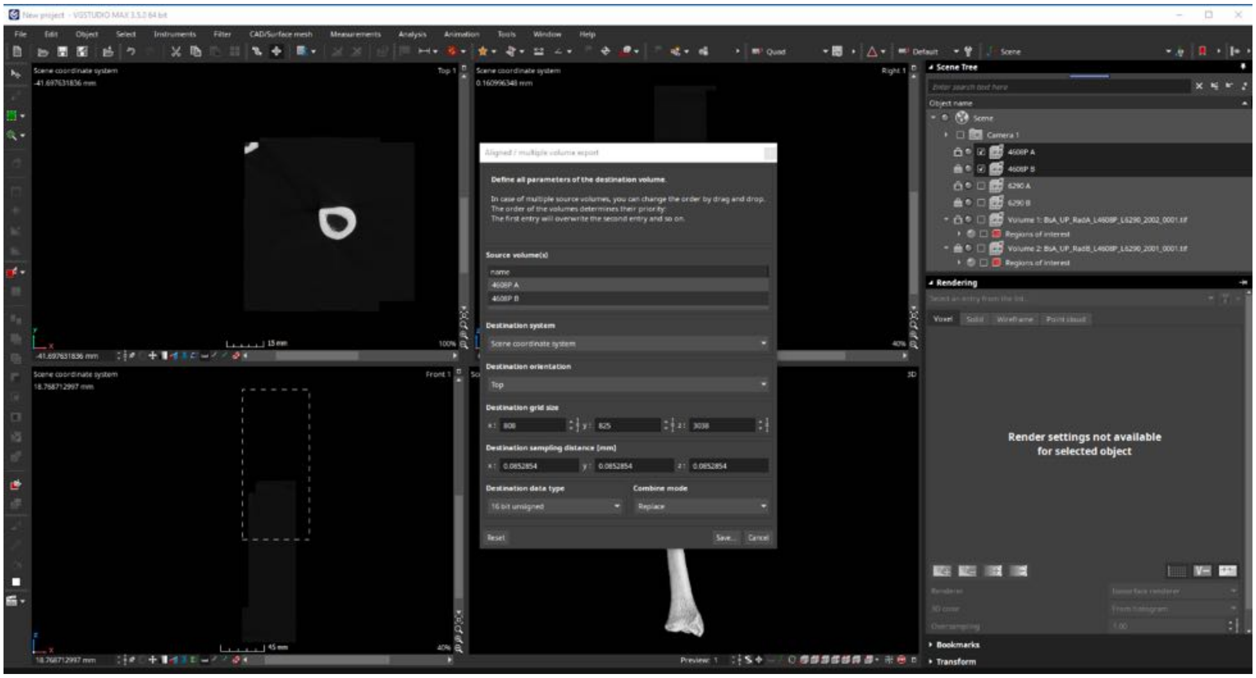


Figure 10. Ensure all the parts for each individual bone is selected.

10.6. Save images in a new folder (e.g. BsA_UP_fem_4996P_merged) as Tiff images. Use the same naming convention as the original scan (refer to SOPXXXX) with only the cadaver number of the one bone in the merged file. Save the merged scan as the cadaver number with an underscore at the end (BsA_UP_fem_4996P_) at the end. Remember to include the P after the cadaver number if this bone contained the extra Prestik marker (Figure 21).

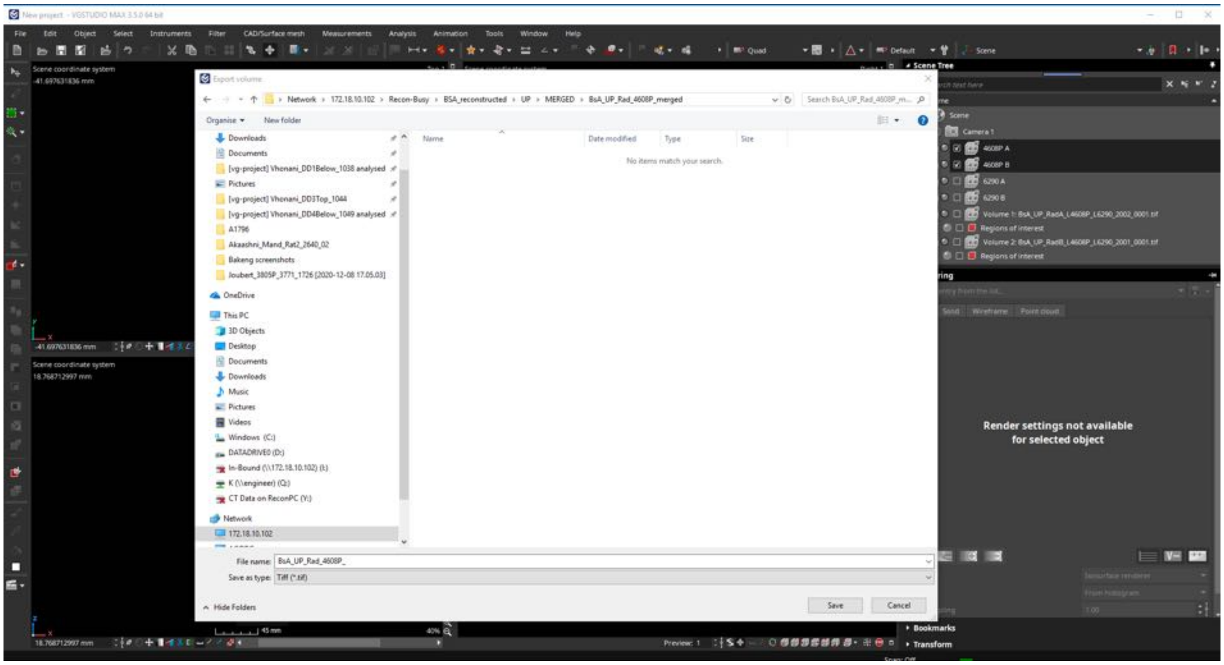


Figure 21. Name for saving the stitched and separated bone.

10.7. Click save.

10.8. When asked if you want to create a .vgl project, say yes. Name the file BsA_institute_bone_cadavernumber_merged (Figure 22)

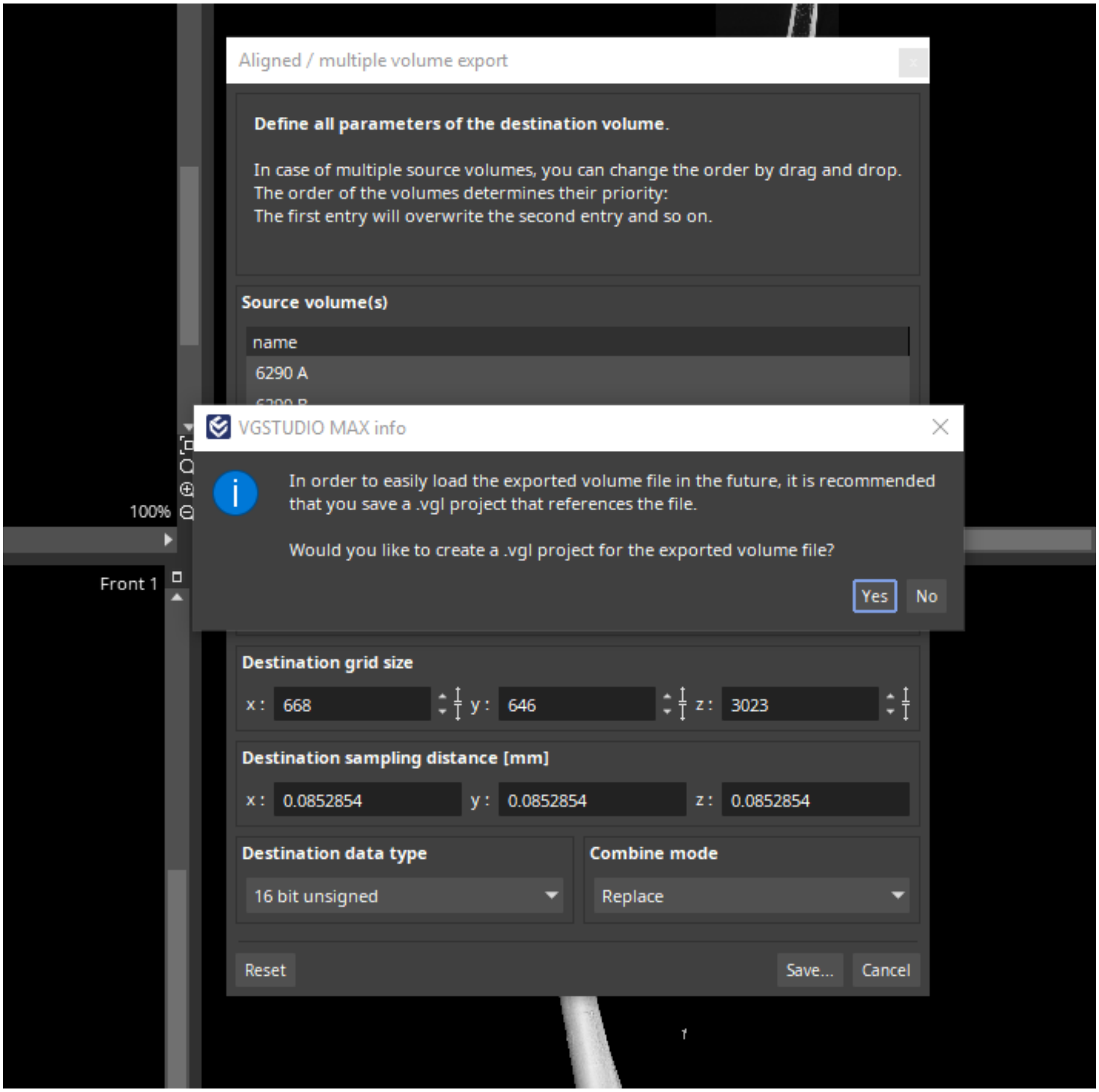


Figure 11. Saving a vgl file for each bone.

10.9. Repeat for the second bone.

11. Cleaning of noise after stitching (optional)

11.1. Reload merged file into VGStudio.

11.2. Select the merged file in the scene tree tab.

11.3. In the Rendering tab, move the red line to the right, past the two or three noise spikes (one for each scanned part of bone). This is for the 3D view. The point of overlap should fade away.

11.4. Click the blue line in the Rendering tab and move the bottom of the line to the right. Do not move the line too much, as you will lose information. Take note of the trabecular bone as you move the line. The blue line is for the 2D view.

11.5. Select File > save as > image stack.

SOP 4. METADATA – PHOTOGRAPHY

(1.0) Purpose

The purpose of this document is to detail the photography of human skeletal remains to supplement scanned material in the Bakeng se Afrika digital skeletal repository.

(2.0) Scope

This document and the guidelines herein will be used by skeletal collections managers or curators, researchers, contractors and/or the individuals responsible for photographing the skeletal elements that have been scanned.

(3.0) Roles and Responsibilities

The photography of bones will be completed by the researcher or contractor that has been trained to do so.

(4.0) Equipment required

Camera: All photographs for a single institution must be taken with cameras of the same specifications. **Camera settings:**

- **ISO** – Lowest native setting on the camera
- **F-stop** – 5.6
- **Shutter speed** – Auto
- **White balance** – Iridescent (lightbulb icon)

Cloth as background: To ensure an easily editable photograph, all bones should be photographed on the same solid, single-coloured background.

Memory cards and storage: After photography, all photographs will be backed up and stored on an external hard drive.

Photography stand: To ensure photographs are taken from the same distance and parallel to all bones, a photography stand with light-fixtures should be set-up and remain unmoved for the duration of photographing a single type of bone. If the stand must be moved between photography sessions, the exact height must be recorded to ensure future photographs are taken with the same field of view.

Figure 1: Photography stand setup used at UP

Skull cushion: Used to stabilise skeletal elements during the photography process.

SOP 4.1. Photographing crania
UP, SUN and SMU

CRANIA

The same photos are used for crania and maxilla scans. Crania will use the full six views (anterior, left and right lateral, posterior, superior and inferior), whereas maxilla will only use the anterior and inferior views of the crania.

Step 1: Set-up

- Lay the cloth down on the photography stand
- Switch on all lights and direct them at the centre of the table
- Secure the camera to the photography stand and adjust the parallel arm to 270mm above table height
    - Measurements are taken along the silver perpendicular arm

Step 2: Specimen numbers

- Take a photo of the cadaver/specimen number to indicate the start of the specimen photo-series

Step 3: Photograph the specimen

- Use a skull-pillow to prop the crania
- Photograph the crania in the following order with the camera lens on the max zoomed out:
    - (1) Anterior, (2) Left, (3) Posterior, (4) Right,
    - (5) Superior and (6) Inferior (with anterior facing towards the photographer – i.e. down)
    - Multiple photographs of a single view can be taken as long as order is not disrupted
    - Do not delete any images during the photography process

Step 4: Specimen numbers

- Take a photo of the cadaver/specimen number to indicate the end of the specimen photo-series

Step 5: Documentation

- Take note of the photograph number on the first and last photograph taken and fill it into the spreadsheet

Cadaver Number	First Photo Number	Last Photo Number	Original Scan Name	Photos Selected						Post-Processing Complete (1=yes)	
				Anterior	Left	Posterior	Right	Superior	Inferior	Background removed	Watermark Added

In case of specific pathologies or trauma

- Photographs of specific features must be taken after the standard 6-views
- Photograph the trauma or pathology up-close
- Only zoom with the camera lens – never the arms on the photography stands
- Hands might be visible when holding the bone to display certain features ○ These can be removed during post-processing
    - Limit visibility of hands and/or props as much as possible
- If trauma or pathology is present and photographed, repeat steps 4 and 5

MANDIBLE

Step 1: Set-up

- Lay the cloth down on the photography stand
- Switch on all lights and direct them at the centre of the table
- Secure the camera to the photography stand and adjust the parallel arm to 270mm above table height ○ Measurements are taken along the silver perpendicular arm

Step 2: Specimen numbers

- Take a photo of the cadaver/specimen number to indicate the start of the specimen photo-series

Step 3: Photograph the specimen

- Use a skull-cushion to prop the mandible if necessary and zoom in all the way
- Photograph the mandible in the following order with the camera lens on the max zoomed in (only zoom with the camera, never the photo stand arm):
    - (1) Occlusal (2) Anterior
    - Multiple photographs of a single view can be taken as long as order is not disrupted

- o Do not delete any images during the photography process

Step 4: Specimen numbers

- Take a photo of the cadaver/specimen number to indicate the end of the specimen photo-series

Step 5: Documentation

- Take note of the photograph number on the first and last photograph taken and fill it into the spreadsheet

				Photos Selected		Post-Processing Complete (1=yes)	
Cadaver Number	First Photo Number	Last Photo Number	Original Scan Name	Occlusal	Anterior	Background removed	Watermark Added

SOP 4.2. Photographing postcrania
UP, SUN and SMU

Step 1: Set-up

- Lay the cloth down on the photography stand
- Switch on all lights and direct them at the centre of the table
- Secure the camera to the photography stand and adjust the parallel arm to 270mm above table height
    - Measurements are taken along the silver perpendicular arm

Step 2: Specimen numbers

- Take a photo of the cadaver/specimen number to indicate the start of the specimen photo-series

Step 3: Photograph the specimen

- Place the long bone on the table with proximal facing left ○ Skull-cushion may be used to stabilise the bone on the proximal and distal ends
- Photograph the femur in the following order with the camera lens on the max zoomed out:
    - (1) Anterior, (2) Lateral, (3) Posterior, (4) Medial
    - Multiple photographs of a single view can be taken as long as order is not disrupted
    - Do not delete any images during the photography process

Step 4: Specimen numbers

- Take a photo of the cadaver/specimen number to indicate the end of the specimen photo-series

Step 5: Documentation

- Take note of the photograph number on the first and last photograph taken and fill it into the spreadsheet

		First	Last	Original Scan Name		Photos Selected				Post-Processing Complete (1=yes)	
Cadaver Number	Side	Photo Number	Photo Number	Part A	Part B	Anterior	Lateral	Posterior	Medial	Background removed	Watermark Added

In case of specific pathologies or trauma

- Photographs of specific features must be taken after the standard 4-views
- Photograph the trauma or pathology up-close
- Only zoom with the camera lens – never the arms on the photography stands
- Hands might be visible when holding the bone to display certain features ○ These can be removed during post-processing
    - Limit visibility of hands and/or props as much as possible
- If trauma or pathology is present and photographed, repeat steps 4 and 5

SOP 4.3. Post-photography processing

Step 1: Transfer photographs

- At the end of each session, transfer all photographs taken onto the designated hard drive under the folder "ORIGINAL"

Step 2: Selecting photographs

- Photographs of specimen will be clearly delineated by close-ups of the cadaver number
  - Select the best photograph of each view of the specimen
- Copy the selected photographs into the folder "CURATED" under a folder for the specific bone
- Put the selected photograph numbers into the spreadsheet for each view
- Once copied into the curated folder, all photos of that bone can be deleted from the original folder

Step 3: P n n P

Table 1: General Photoshop shortcut keys

Ctrl + c	Copy selection
Ctrl + v	Paste selection
Ctrl + x	Cut selection
Ctrl + z (1st time)	Undo
Ctrl + z (2nd time)	Undoes the undo
Ctrl + alt + z	Undo more than one step
Ctrl + e	Merge selected layers
Ctrl + t	Transform
Ctrl + n	New canvas
Ctrl + +	Zoom in
Ctrl + -	Zoom out
Ctrl + s	Save as photoshop image (.png)
Ctrl + d	Deselect – makes "running ants" go away
[or]	Smaller or larger brush cursor

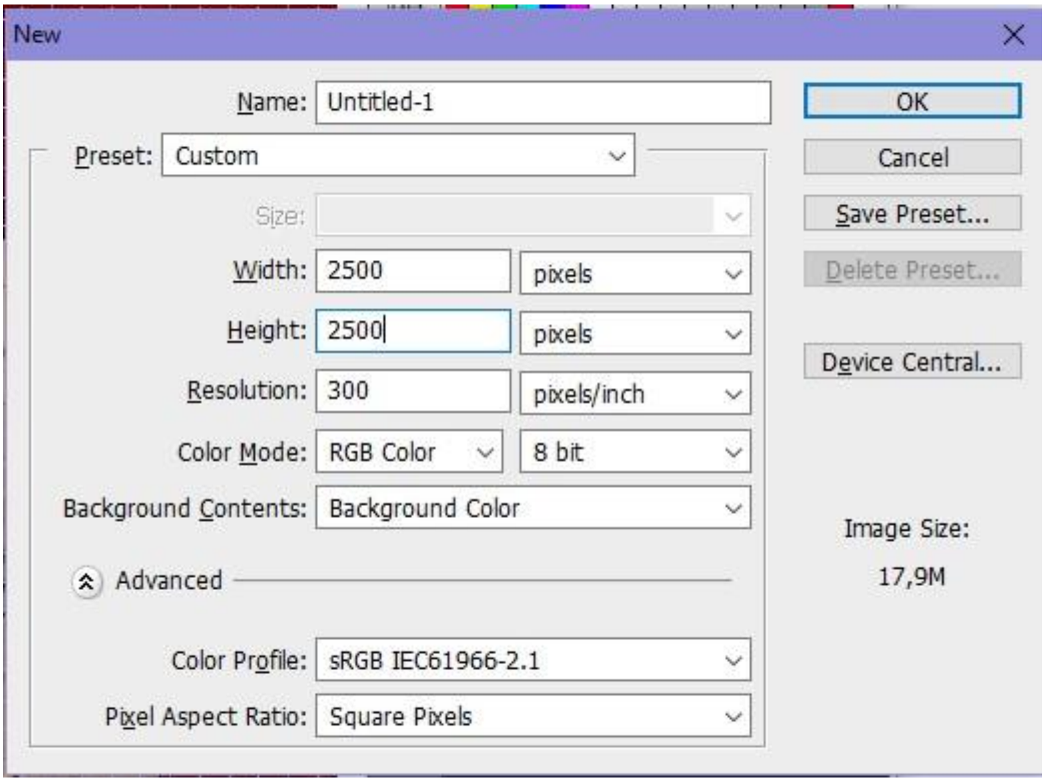
Remove the background

1. Open image with Photoshop CS5
2. Select the  (W) – make sure it is not the magic wand

3. At the top of the toolbar make sure to select the  tool
4. Run cursor over skull until entire skull is surrounded by "running ants". (you can select and release – you don't need to do everything in a single stroke)
5. Use zoom in (ctrl +) to make sure that everything is selected (use ctrl + z or ctrl + alt + z to undo extra bits or use the  tool at the top toolbar).
6. When whole skull is selected press ctrl + c to copy the selected bit.
7. Ctrl + n to open a 'New' window.
8. Create a canvas of 2500 pixels x 2500 pixels for crania and mandibles. Make sure the pixels by inch is 300 (resolution of the image) and the colour is RGB and 8-bit. Press enter.
9. Ctrl + v to paste the image onto the new canvas as a new layer.
10. Use v to move the layer to your preferred location on the canvas. Centre the image.
11. Use ctrl + t to rotate the image (always press enter after ctrl + t to be able to do anything else).

    For the skull: Left and right views must be in Frankfort Horizontal. For superior and inferior views, rotate so that anterior is up. Do not resize or transform the image.

    For the ribs: The first rib should be up for both superior and inferior views.



For the mandible: Resize the image to 70% in the top bar - ensure the chain lock is selected to resize to 70% in both width and height 

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For occlusal view, rotate so that anterior is down. Do not resize or transform the image.

For the vertebrae: Create a canvas of 3500 pixels x 3500 pixels for the vertebrae. Make sure the pixels by inch is 300 (resolution of the image) and the colour is RGB and 8-bit. Press enter. Resize the image to 75% in the top bar for posterior and lateral views and 65% for anterior- ensure the chain lock is selected to resize to 75% (posterior and lateral) and 65% (anterior) in both width and height. Centre the image. Do not resize the logo.

12. Click on the background layer in the layers tab.
13. Use the  tool or  tool and pick a black to fill in the background.

Add the Bakeng se Afrika logo

1. Drag and drop the file of the Bakeng se Afrika logo onto the image in Photoshop.
2. Select the chain to lock the logo size and change the W to 30%

3. Drag the logo to the top right corner of the canvas for crania and bottom right corner for mandibles.
4. Press enter.

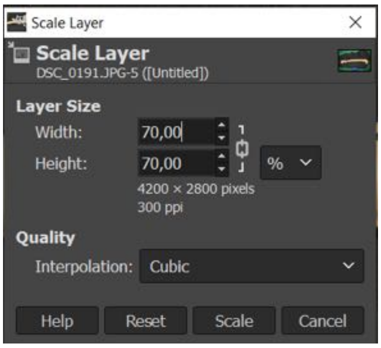
Step 3 : P n n

Table 1: General GIMP shortcut keys

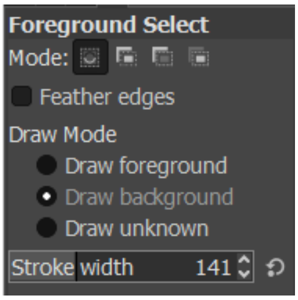
	Copy selection
Ctrl + v	Paste selection
Ctrl + x	Cut selection
Ctrl + z (1st time)	Undo
Ctrl + y	Undoes the undo
Ctrl + i	Invert selection
Ctrl + b	Bucket fill
Ctrl + 2	Foreground select
Ctrl + n	New canvas
Ctrl + s	Scale image
Z	Zoom
M	Move
Shift + Ctrl + s	Save as gimp image (.xcf)
Shift + Ctrl + e	Export image as jpg or png

Remove the background

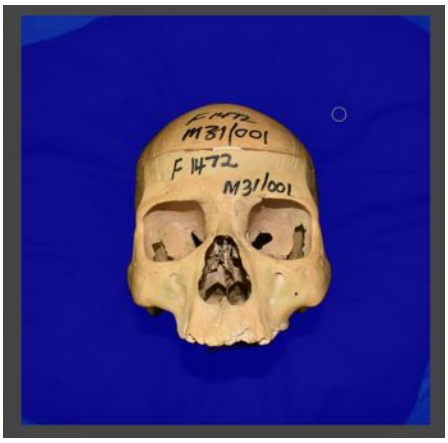
1. Open GIMP 2.10.22
2. Ctrl + n to open a 'New' window.
3. Create a new canvas
    a. For crania and mandibles: 2500 pixels x 2500 pixels
    b. For long bones: 5000 pixels x 2000 pixels
    c. Make sure the pixels by inch is 300 (resolution of the image) and the colour is RGB and 8-bit. Press enter.
4. File > Open as Layer… and choose image of skull
5. Resize layer to have bone fit canvas:
    a. Layer > Scale Layer > select % and rescale width and height at the following proportions and make sure to keep proportions constrained
        i. Crania at 70%
        ii. Mandibles at 120%
        iii. Femur at 80%
        iv. Humerus, Radius and Ulna at 120%



6. Select the Foreground Select tool 
7. Roughly draw around bone and press enter
8. Adjust the settings to Draw foreground, adjust to desired stroke width and draw around the borders of the bone on the foreground



9. Alternate between Draw foreground and Draw background to make sure entire bone is "highlighted" while the background appears blue



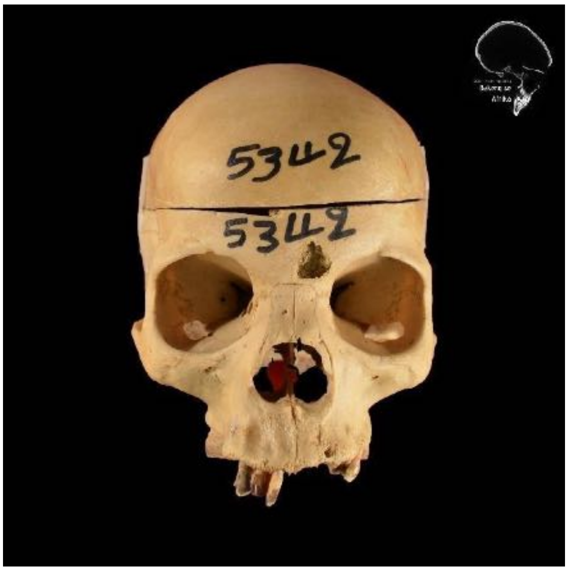
10. Press enter or click on Preview mask – if mask is correct and entire bone is selected, click on Select
11. Click ctrl + I to invert selection and press delete to remove background
12. Position image in centre of canvas using Move tool 
13. If necessary to rotate the image:

a. Layer > Transform… > Arbitrary rotation and manually rotate
b. **For the skull:** Left and right views must be in Frankfort Horizontal. For superior and inferior views, rotate so that anterior is up.
c. **For the mandible:** For occlusal view, rotate so that anterior is down.
d. **For the long bones:** Make sure proximal end is on the left

14. Click on the background layer in the layers tab.
15. Use the Bucket Fill tool to fill in the background with black.

Add the Bakeng se Afrika logo

1. Drag and drop the file of the Bakeng se Afrika logo onto the image in GIMP.
2. Resize layer to change logo size:
   a. Layer > Scale Layer > select % and rescale width and height at the following proportions and make sure to keep proportions constrained
      i. Crania at 60%
      ii. Mandibles at 70%
      iii. Femur, Humerus, Radius and Ulna at 80%
3. Drag the logo to the top right corner of the canvas for crania and long bones, and bottom right corner for mandibles.
4. Press enter.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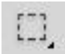
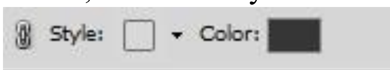
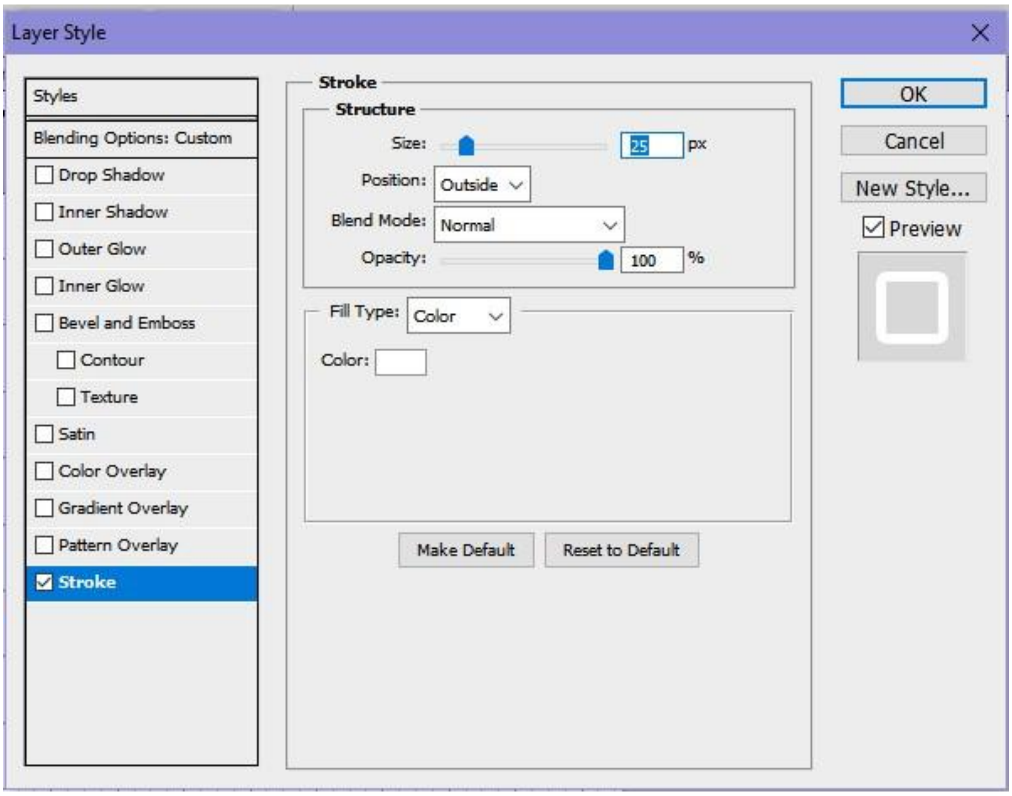
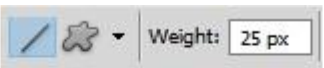
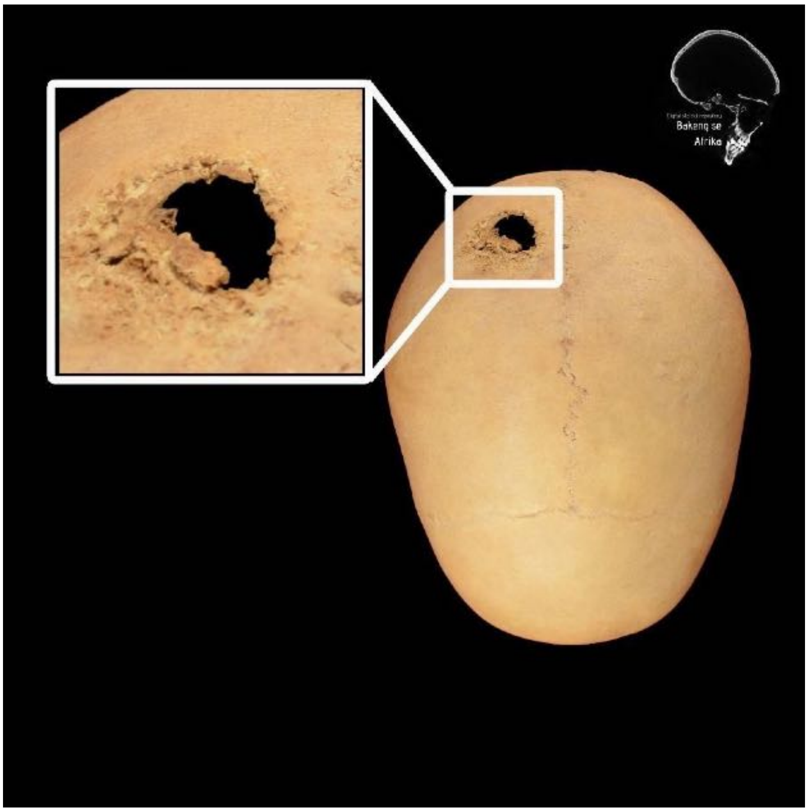
For specific trauma/pathologies

5. Trauma or pathologies that were photographed up close must be highlighted and enlarged on one of the views. Select a view that shows the feature well to add a box on and repeat the above process. Place the image off-centre to allow space for a close-up photo.
6. Import a close-up photograph of the feature. Use the rectangular marquee tool to select an area around the feature . Ctrl C, Ctrl V to paste the cropped version onto the photo. Delete the original feature photo. Select the feature layer and move it to an open space on the black background.
7. To add boxes select the  tool. At the top of the window, select the style and colour. The style with just the outline as shown here 

is most useful for highlighting aspects of an image. Draw a box around the close-up of the feature. Make sure that when you have drawn the box that the layer is above the images in the Layers panel.
8. Double-click on ‘Strokes’ under the box layer in the Layers panel. Change the size of the stroke to 25 and the colour to white.



9. Draw a second box around the feature on the full view of the bone.
10. Using the line tool , draw lines from the corners to connect the boxes on the close-up and full view images. Change the line weight to 25 and the colour to white. 



Step 3: Save and rename the final photo

Select file > Save as

Save the image as a .psd (Photoshop file) and a .jpg file.

- OriginalPhotoNumber_BsA_institution_bone_number_view
    - Views = ant, med, left, post, lat, right, sup, inf, occ
- If the photo contains a zoomed-in view of a feature (trauma/pathology), rename the file as
  OriginalPhotoNumber_BsA_institution_bone_number_view_detail
- Maximise compatibility for .psd file
- JPEG options:
    - Image quality = High
    - Format options = Baseline optimized