**DIGITIZING LANDMARKS WITH *TINA***

*TINA (TINA Is No Acronym). Adapted from ‘The TINA Geometric Morphometrics Toolkit’ (Bromiley, Ragheb, and Thacker, 2012) – Tina Memo No. 2010-007* [[url]](http://www.tina-vision.net/docs/memos/2010-007.pdf)

**A. ACCESSING TINA FROM LINUX**

1. Log in to your *openSUSE* workstation (TINA is set up to work in *openSUSE* but can possibly be set up for a different OS).

2. User files can be accessed from the Dolphin file manager: *iceWM > Suse > System > File Manager > Dolphin*. (-*S*-*S*-*F*-*D*)

3. Click on Xterm to access the console and type tinaTool. (-*Enter*-‘*tinaTool’*) **NEXT**

**B. SETTING UP A WORKSPACE**

***B1. IF SETTING UP A NEW WORKSPACE TO SAVE AS A MACRO***

1. Open Macro and input a file location where a new macro file (called *Tinastart.cls* automatically) will be created.

*Macro File: /Server/User/TINA/*

2. Click Macro > **Append** to begin “recording” your workspace modifications. Each “TVtool” or window you open will be recorded into the macro file (including the coordinates of those windows) until you press the Macro > **Close** button in order to save all the settings.

***NOTE****: Macro files can be opened/edited using the “Kwrite” text editor in OpenSUSE. To edit a previous Macro with TINA, simply input the filepath of that specific file and press “Run”. After the Macro opens, press “Append” to begin editing that file. New changes will appear in the file immediately following a function called “Macro\_Tool.append”. After you save the file (Macro > Close), the new coordinates of all the windows will be added to the end of the file.*

3. Begin creating a comfortable workspace that will be saved as a macro.

4. Open TINA Tool > **Manual Landmark Tool**.

5. Set up TV tools: Select x-axis then New TVtool > Install.

Select y-axis then New TVtool > Install.

Select z-axis then New TVtool > Install.

Select 3D then New TVtool > Install.

6. Open the Landmark Points tool, and set the following values.

*Input format: Names*

*Input path: /Server/User/landmarks.txt*

*Output format: TPS*

*Output path: /Server/User/TINA/Export/TINA\_Export.TPS*

***NOTE****: The “Landmarks.txt” input file will not change unless you add more/less landmark points.*

***NOTE****: The output file will save all the landmark coordinates that you input. You can select the name that you want and an empty file will be created with that name in the file path. If you choose to add a specific Output Path to the Macro, then your news coordinates will simply get endlessly added to the same TPS file.*

6. Open TINA Tool > **Sequence** and input the following values.

*File: DICOM*

*Start: 0001*

*Stride: 2*

*End: 9999*

***NOTE****: “Stride” indicates how many files will be skipped in reconstructing the 3D model. By setting “Stride: 2”, only every second image will be used, saving time and processor power.*

8. Use the VR Control tool to change the lighting/rendering settings. For landmarking, use a surface-rending style. **NEXT**

***B2. IF OPENING A MACRO OF A PREVIOUSLY-CREATED WORKSPACE***

1. Open Macro and input the file location of the desired file.

*Macro File: /Server/User/TINA/macro*

***NOTE****: Do not include the “.CLS” file extension in the file path. “Scan” can also be used to find a file path, instead of manually.*

2. Press Run. The workspace will open as it was saved in the macro file. Filepaths are automatically inputted into the appropriate fields, however they are not loaded.

3. Check to see that the preset *landmarks.txt* file has been loaded. If not, then the file may have been moved, so locate it and select TINA Tool > Landmark Points > **Load**.

4. In the TINA Tool > **Sequence** window, specify the location of your DICOM scan files. They should all be in a folder.

*Image File: /Server/User/SCANS/IMAGE\_00####.DCM* (*“Scan”* can be used to find the files.)

***NOTE****: Always include the “.DCM” file extension in the file path. “Scan” can also be used to find a file path, instead of manually. The four #### placeholders will be replaced by the “Start” and “End” values, constructing a 3D model of all the files in the file path with names IMAGE\_0001, IMAGE\_0002… to IMAGE\_9999 (the “End” value that was inputted in the previous section). If the folder only contains files numbered up to IMAGE\_1502 (for example), then the 3D reconstruction will only be made of IMAGE\_0001 to IMAGE\_1502 even though the “End” value is higher.*

5. Press Load for TINA to process all the image files. This may take a moment. The 3D model should appear in the “3D” TVtool. If not, click Manual Landmark Tool > 3D Tv > **VR** to re-initiate the “3D” TVtool and re-install the “3D” TVtool.

6. In the Landmark Points tool, set the output file path. The default file name is set for “*untitled.TPS*” so be sure to change it each time, otherwise the files will keep overwriting to the same location.

*Output path: /Server/User/TINA/Export/TINA\_Export.TPS*

7. Begin landmarking the first sample. **NEXT**

**C. ALIGNING THE SAMPLES**

1. Use 2D TvDraw > **Rotate** and drag the skull to orient the skull along the X-, Y- and Z-axes in a consistent manner across all samples. Here are the examples used for a mouse skull:

A. **SKULL:** ***3D:*** Make sure the skull is facing forward, with the nose oriented outward.

***X-Axis:*** Align the ANTERIOR OF NASAL BONE with the POSTERIOR OF OCCIPITAL BONE using the 3D-rotate tools and observing the change in the X-axis TvTool.

***Y-Axis:*** Center-align the INTERIOR DIVIDE OF THE NASAL/PALATINE BONES.

***Z-Axis:*** Center-align the L/R PARIETAL-INTERPARIETAL BONES.

B. **MANDIBLE**: ***X-Axis:*** Align L MANDIBLE with R MANDIBLE so they overlap.

Align inferior points of L/R ANGULAR PROCESS with L/R ALVEOLAR PROCESS so they are level.

***Y-Axis:*** There should be four points appearing simultaneously from the previous points.

***Z-Axis:*** Center-align the L/R MANDIBLES.

*NOTE: The skull and mandible are aligned separately First align the SKULL, lock the rotation, and then landmark it. Then unlock the rotation, align the MANDIBLE, and landmark it after the skull is finished. You can then save the whole sample data, and move on to the next specimen.*

2. 2D TvDraw > **Lock Rotation** to prevent accidentally misaligning the sample. Now, everything is set up for properly digitizing the landmarks of the first sample. **NEXT**

**D. LANDMARKING**

1. Note the first landmark in the Landmark Points window (for example, *No:1 Name: Nasal bones most anterior intersection*).

2. Select that landmark in the X-, Y-, and Z-axis TVtools. (See “*Keyboard Shortcuts*” section below.)

3. Click Manual Landmark Tool > **Mark Point** (or press RETURN on the keyboard) to lock in the coordinates of that point.

4. Repeat the previous steps for the remaining Landmarks in the *Landmarks.txt* list that was inputted into TINA.

5. If you make a mistake, use the Landmark Tool > **Up**/**Down** buttons to move the incorrect landmark selection to the middle of the displayed list (see below), and click RETURN to overwrite it with the currently selected point.

No: 10 R SQUAM/ZYGOM | POSTR junction

No: 11 L SQUAM/ZYGOM | POSTR junction

No: 12 R ZYG/SQUA | ANT. of rear-suture *← This is the landmark that gets written when you press RETURN.*

No: 13 L ZYG/SQUA | ANT. of rear-suture

No: 14 R ZYG/MAXI | ANT. of fore-suture

6. Make sure that the Landmarks Points > **Output Path** field directs to a new *.TPS* file and press Save. **END**

***NOTE****: Pressing Save will not overwrite the previous .TPS file. Instead, it will simply append the current file (in its entirety) to the end of the currently-selected file. Saving the same file over and over will result in multiple copies of the same list in the same file – the last list is the most recent. Because of this, it is preferable to only save the file once per skull.*

**E. RESET TINA FOR SUBSEQUENT SAMPLES**

1. After saving one skull to the output file, move on to the next skull.

2. A. Press Landmarks Points > **ClearLM** to clear previous landmarks from the list.

Previously saved landmarks will still be in the saved *.TPS* file. You will need to reload the *landmarks.txt* file to in the Landmarks Points window. Add new image files with TinaTool > Sequence > Image File >**Scan** as was done with the previous specimen.

B. Alternatively, close the program and re-open the *TINA* workspace using the default macro.

3. Continue landmarking the next sample. **END**

*NOTE:* Successive skulls can be saved to the same *.TPS* output file – *TINA* will simply append the next batch of landmarks to a separate list in the same *.TPS* file. This is more convenient when working with *MorphoJ.*

**G. SPLITTING .TPS EXPORT FILES**

If so desired, use the following R script to split the *TPS* export file (into which multiple sets of sample landmarks are saved) into individual *TPS* files based on those samples’ IDs. So (for example) one *TINA\_Export.TPS* file containing coordinates for samples ID=9944, ID=9945, and ID=9946 will generate files named *9944.TPS*, *9945.TPS*, *9946.TPS*. If you wish to set a working directory that is separate from the folder the script file is located in when it runs, then modify the setwd(“”) command (currently commented out) with the desired directory.

#setwd("") *#SET WORKING DIRECTORY*

input = "TINA\_Export.TPS" *#DEFINE INPUT*

outdir = "." *#DEFINE OUTPUT DIRECTORY (same place)*

split\_input<-function(INPUT, OUTDIR = "."){ *#SPLIT FUNCTION*

tmp<-readLines(INPUT)

tmp.start.pos<-grep("LM3=",tmp)

tmp.end.pos<-c(tmp.start.pos[-1]-1,length(tmp))

tmp.id.pos<-grep("ID=",tmp)

pb<-txtProgressBar(min=1,max=length(tmp.id.pos),style=3)

for(i in 1:length(tmp.start.pos)){

id.pos<-tmp.id.pos[i]

id<-gsub("ID=","",tmp[id.pos])

output\_name<-paste0(OUTDIR,"/",id,".TPS")

sink(output\_name)

cat(tmp[tmp.start.pos[i]:tmp.end.pos[i]],sep="\n")

sink(NULL)

setTxtProgressBar(pb, i)

}

close(pb)

}

split\_input(input, outdir) *#RUN THE FUNCTION*

quit(save = "no", status = 0, runLast = TRUE) *#QUIT OUT OF RSTUDIO*

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**F. KEYBOARD SHORTCUTS**

***←↕→*** Move red cursor in selected TV tool.

***Return*** Enter current landmark and go to next.

***R-Shift*** Switch to “*Pick*” mode in all TV tools

***R-Ctrl*** Switch to “*Zoom*” mode in all TV tools.

**PICK MODE: *Left-Click Middle-Click******Right-Click***

☉Select Point *Null* *Null*

**ZOOM MODE: *Left-Click Middle-Click******Right-Click***

⤮ 2D Drag ⇄ 2D Zoom ⤮ 2D Zoom ROI

⤮ 3D Rotate ⇄ 3D Zoom ⤮ 3D Drag

⇅ 3D Spin

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**SKULL LANDMARKS**

1 Nasal bones most anterior suture

2 Nasal bones most posterior suture

3 Frontal bones most posterior suture

4 Parietal bones most posterior suture

5 Interparietal bone most posterior point on the median line

6 Right most anterior point of the suture between frontal and parietal bones

7 Left most anterior point of the suture between frontal and parietal bones

8 Right intersection between parietal, occipital and squamosal bones

9 Left intersection between parietal, occipital and squamosal bones

10 Right most posterior junction of squamosal bone and the zygomatic process of the squamosal bone

11 Left most posterior junction of squamosal bone and the zygomatic process of the squamosal bone

12 Right most anterior suture of the zygomatic process of the squamosal bone and jugal bone

13 Left most anterior suture of the zygomatic process of the squamosal bone and jugal bone

14 Right most anterior suture of jugal bone and the zygomatic process of the maxillary bone

15 Left most anterior suture of jugal bone and the zygomatic process of the maxillary bone

16 Right intersection of the frontal, lacrimal and the zygomatic process of the maxillary bone

17 Left intersection of the frontal, lacrimal and the zygomatic process of the maxillary bone

18 Right infraorbital foramen most superior point

19 Left infraorbital foramen most superior point

20 Right infraorbital foramen most inferior point

21 Left infraorbital foramen most inferior point

22 Right premaxilla-right nasal bone most anterior point of suture

23 Left premaxilla-left nasal bone most anterior point of suture

24 Right Most superior point of the right incisor alveolus

25 Left Most superior edge of the left incisor alveolus

26 Right Most inferior point of the right incisor alveolus

27 Left Most inferior point of the left incisor alveolus

28 Right premaxilla-maxilla most ventral junction

29 Left premaxilla-maxilla most ventral junction

30 Right Most anterior point of the right first molar alveolus

31 Left Most anterior point of the left first molar alveolus

32 Right Most posterior point of the right third molar alveolus

33 Left Most posterior point of the left third molar alveolus

34 Right Most anterior point of the right anterior palatine foramen

35 Left Most anterior point of the left anterior palatine foramen

36 Right Most posterior point of the right anterior palatine foramen

37 Left Most posterior point of the left anterior palatine foramen

38 Right pterygoid process, most posterior point

39 Left pterygoid process, most posterior point

40 Median-line point of the suture between occipital and basisphenoid bones

41 Median-line point of the suture between basisphenoid and presphenoid bones

42 Most posterior point of the suture between palatine bones

43 Foramen magnum most anterior point, Basion

44 Foramen magnum most posterior point, Bregma

**MANDIBLE LANDMARKS**

45 Right Most superior point of the incisor alveolus

46 Left Most superior point of the incisor alveolus

47 Right Most inferior point of the incisor alveolus

48 Left Most inferior point of the incisor alveolus

49 Right Most anterior point of the first molar alveolus

50 Left Most anterior point of the first molar alveolus

51 Right Most posterior point of the third molar alveolus

52 Left Most posterior point of the third molar alveolus

53 Right Most posterior tip of the coronoid process

54 Left Most posterior tip of the coronoid process

55 Right Most anterior concave point of coronoid process

56 Left Most anterior concave point of coronoid process

57 Right Most anterior point of the articular surface of the condyle

58 Left Most anterior point of the articular surface of the condyle

59 Right Most posterior tip of the condyle

60 Left Most posterior tip of the condyle

61 Right Most anterior concave point between condyle and angular process

62 Left Most anterior concave point between condyle and angular process

63 Right Most posterior tip of angular process

64 Left Most posterior tip of angular process

65 Right Most inferior point of angular process

66 Left Most inferior point of angular process

67 Right Ascending ramus dorsal-most ventral point

68 Left Ascending ramus dorsal-most ventral point

69 Right Alveolar region most inferior point

70 Left Alveolar region most inferior point

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**SKULL LANDMARKS**

Pallares LF, Harr B, Turner LM, Tautz D. 2014. Use of a natural hybrid zone for genome wide

association mapping of craniofacial traits in the house mouse. *Mol Ecol*, 23: 5756–5770.



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**MANDIBLE LANDMARKS**

Pallares LF, Harr B, Turner LM, Tautz D. 2014. Use of a natural hybrid zone for genome wide

association mapping of craniofacial traits in the house mouse. *Mol Ecol*, 23: 5756–5770.



*Coronoid Process*

*Condyle Process*

*Angular Process*

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**USING THE AUTOMATIC LANDMARKING TOOL IN *TINA***

*TINA (TINA Is No Acronym). Adapted from ‘The TINA Geometric Morphometrics Toolkit’ (Bromiley, Ragheb, and Thacker, 2012) – Tina Memo No. 2010-007* [[url]](http://www.tina-vision.net/docs/memos/2010-007.pdf)

**A. CREATE AN AUTOMATCHING-SETUP MACRO**

1. Open TINA tool and set up a workspace using a *Setup Macro*, as normal.

2. Return to the Tina Tool > **Macro** window and run the *Automatic Landmarking Setup Macro*. It is a smart move to append your *Setup Macro* to have already inputted the file location for the *Automatic* macro into the Macro window, so you just have to press ‘Run’. If you do not have a macro, then append a new one to do the following:

A. Open up the Automatic Landmark Point Placement window by selecting Manual Landmark Tool >**Automatch**.

B. In the Automatic Landmark Points Placement window, set the output file path for where the Database will be saved. If you already have one, then press Input Database so that it will be uploaded every time the macro is run.

*Database Path: /Server/User/TINA/AutoDB/NEW\_DATABASE*

C. In the Landmark Points tool, set the output file path for where the Automatching landmarks should be saved once the final program is run.

*Output path: /Server/User/TINA/Export/TINA\_Export.TPS*

D. Choose four G-Reg points in the GREG# entry fields. These should be the numbers of the landmarks that will be used for Global Regulation in the next sections. *(ie. GREG1: 1; GREG2: 41; GREG3: 42; GREG4: 49*)

3. Save the macro by clicking Macro > **Close**. **NEXT**

**B. CONSTRUCT NEW DATABASE**

1. Before beginning, you should have already (manually) landmarked about 6-10 samples completely, to use as a database for the computer to reference while the automatic landmarks are being laid out. These samples should be the most perfect examples of each landmark, in order to get more accurate automatic landmarks.

2. Outside of TINA tool, save the landmarks for each of the 10 samples into separate .TPS files. (ie. *9919.TPS*, *9920.TPS*, *9921.TPS…*) It’s a good idea to keep a copy of these landmark files in the same folder as the Database file, so they can be referenced later.

3. Add the first sample’s files into the TINA Database.

A. Load the first sample’s image files into Sequence Tool > **Image Files** like normal.

*Image File: /Server/User/SCANS/9919/C0009796\_0####.DCM*

B. Upload the first sample’s landmarks into Landmark Points > **Input** and press Load. If you have the 3D TvTool rendered, you should notice the landmarks appear in the correct places on the 3D model.

*Input Format: TPS*

*Input Filename: /Server/User/TINA/AutoDB/9919.TPS*

C. Add the Image and landmark data to the Database using the “Landmarks → Database” button

D. If you want to see which samples have been added to the Database, including this one, click on the Automatch DB inspector button and move between samples using < and >.

Select Free Curr. Entry to remove the current entry from the Database.

Select Free database to completely clear the Database.

4. Repeat the previous step for each additional sample to be added into the Database (minimum 6, and anything over 10 does not improve accuracy and just makes the program take longer). Add the new scan into Sequence Tool > **Image File**, making sure to change the Sequence Tool > **End** value back to *9999* so that the next sample is not partially-loaded, and add the correct landmark file into Landmark Points > **Input**.

5. When the Database contains all the correct samples in it, then save it by selecting the filepath in the Automatic Landmark Points Placement > **Output** and selecting the Output database button. Verify that the file has been saved into the correct folder before closing out of TINA. **NEXT**

**C. LOCATE G-REG LANDMARKS**

1. Decide beforehand which 4 landmarks will be used as Global Registration (G-Reg) landmarks. For example:

G-Reg 1: *No: 01 NASAL-BONES | ANTER. suture*

G-Reg 2: *No: 41 BASISPH/PRESPH | MEDIAN suture*

G-Reg 3: *No: 42 PALATINE-BONE | POSTR. suture*

G-Reg 4: *No: 49 1-MOLAR-ALVEOLUS | ANT. point*

***NOTE****: These landmarks will align the sample so that TINA knows the sample orientation. It will not work for gross misalignments, ie. if it is facing the wrong way. At least one landmark should be in a separate plane from the others.* ***NOTE****: If you are using a skull and mandible as a sample, then at least one landmark should be included on the other structure (ie. three G-Reg points on the skull and one on the mandible).*

2. Load the image files for the sample, as normal.

*Image File: /Server/User/SCANS/9944/C0009921\_0####.DCM*

3. Align the sample properly and Lock Rotation.

4. Locate the four selected landmarks and enter them into the file, making sure to leave all other landmarks blank.

5. Name the output file something recognizable (ie. “*GR\_0000*” using the sample number) and save it into a *G-REG* folder.

*G-REG Path: /Server/User/TINA/AutoGR/GR\_9944.TPS*

6. Move on to the next sample, and continue until you feel that you have enough to the run *Automatching* program with. **END**

**D. CREATE AN “AUTOMATIC MACRO” FILE FROM A TEMPLATE**

1. After a collection of G-REG files has been generated and saved to the appropriate folder, it is time to edit the *Automatic Landmarking Macro* file to run the files inputted into the appropriate coding modules. This can be done in any text editor.

2. The macro should include a coding module that can be edited and copied/pasted into the macro multiple times, each set of lines corresponding to one set of scan DICOMs and one G-REG file, and then copied/pasted for each G-Reg file that is being used in this macro, similar to what is written below.

*// AUTO-LANDMARK MODULE*

*Sequence\_Tool.\_\_End\_: 9999*

*Sequence\_Tool.Image\_File: /Server/User/SCANS/9944/C0009921\_0####.DCM*

*Sequence\_Tool.Load*

*Landmark\_Points.Input\_pathname: /Server/User/TINA/AutoGR/GR\_9944*

*Landmark\_Points.Load*

*Automatic\_Landmark\_Point\_Placement.Global\_Reg*

*Automatic\_Landmark\_Point\_Placement.Automatch*

*Automatic\_Landmark\_Point\_Placement.Gauss\_conv.*

*Automatic\_Landmark\_Point\_Placement.Error\_analysis*

*Landmark\_Points.Save*

3. Define the correct file path of the DICOM files (including the ‘*.DCM’* file extension) and define the correct file path of the corresponding G-REG file (excluding the *.TPS* file extension, since it gets added automatically).

4. Copy and paste the module multiple times into the same file, setting up about 6-20 samples. Change the *Sample Number* and the *GR\_landmark* file for each sample to correspond to each of the samples that will be automatched.

4. Make sure to set up the output file name. The macro should already be set up with something like:

*Output Path: /Server/User/TINA/Export/TINA\_Export.TPS*

5. Save the Macro file. Duplicate it, and keep a copy of it in another folder for your records. **NEXT**

**E. RUN THE AUTOMATIC TOOL**

1. Open TINA tool and set up a workspace using a *Setup Macro*, as normal.

2. Return to the Tina Tool > **Macro** window and run the custom-made *Automatic Landmarking Macro*. It is a smart move to append your *Setup Macro* to have already inputted the file location for the *Automatic* macro into the Macro window, so you just have to press Run.

3. The automatic location of the landmarks will begin. Nothing else needs to be done – just leave the program running overnight. It should take about 30 minutes per sample, assuming ~70 landmarks each. **WAIT**

***NOTE****: The program with complete one of two ways: (A) it will run through each of the Auto Landmarking Modules and finish, or (B) it will crash after a certain number of Modules, limited by the graphical memory. The results of the automatic landmarking will be stored in the previously named export file (ie. Tina\_Export) when it is completed, even if it crashes.*

**F. MANUALLY CONFIRM THE RESULTS OF THE AUTOMATIC RUN**

1. Open TINA tool and set up a workspace using a *Setup Macro*, as normal.

2. Upload an auto-landmarked sample in the Sequence Tool > **Image File** and press Scan.

3. Upload that sample’s landmarks into Landmark Points > **Input** and press Load. Make sure that Input Format is ‘*TPS*’. If you have the 3D TvTool rendered, you should notice the landmarks appear in the correct places on the 3D model. If not, then install the 3D window.

*Input Format: TPS*

*Input Filename: /Server/User/TINA/TPS/9919.TPS*

4. Click Manual Landmark Tool > Jump Lock > **On**.

5. Click Manual Landmark Tool > **Jump Stored**. This will select the first landmark, in order to confirm that it is in the correct position

6. If the landmark is in the correct location, then press Enter and the next landmark will be selected. If the position is wrong, then manually position the cursor to the correct location, and then press Enter to enter those coordinates and jump to the next landmark.

7. Repeat the above step until all landmarks are confirmed.

8. Save the manually confirmed landmarks using the Landmark Points > **Output** and press Save. Make sure that the output pathname is correct — do not save it into the exact same TPS file, otherwise it will save two sets of landmarks for the same sample, and this will cause complications later on. Instead, label it in a manner that indicates that it was manually corrected, ie. *9919\_manual.TPS*

9. Upload the next sample, and the corresponding TPS file for that sample, and continue with the landmarking until all samples are processed. **END**