SkullProfiler

An R tool for elliptical Fourier analysis of skull outlines for sex and ancestry estimation.

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Terms of Use:

Photography Protocol

The following photography protocol was designed to standardize image capture of the skull outline in *norma lateralis* for use with *SkullProfiler* (see Fig. 1 for setup).

Materials:

- Full-frame DSLR camera
- 100 mm macro lens
- Camera tripod
- Measuring tape
- Support board for base
- Circular skull mount
- Black backdrop sheet
- Certified scale (≥ 150 mm)
- Block to support scale (68 mm tall)
- Mini whiteboard and marker
- Dental utility wax

Camera Setup:

The camera used should be a high resolution **full-frame DSLR** fitted with a fixed **100 mm macro lens**. Set the camera to aperture priority (Av) with an f-stop of **f/18**. To improve efficiency with skull alignment, **remote capture** is recommended by connecting the camera to another device via cable or remote sensor. If remote capture is not used, set the camera shutter timer delay to a minimum of two seconds to minimize camera disturbance during image capture. **Ambient lighting** should be sufficient to distinguish the object outline from the background. However, if additional lighting is required, a camera mounted **ring flash** is recommended. Mount the camera on the tripod, and direct it inferiorly. The distance between the label/scale on the block and the camera's focal plane mark (blue arrow in Fig. 1b) should be **1.20 m**. Use the circular **spirit level** to set the camera up on the tripod so that it is perpendicular to the ground.
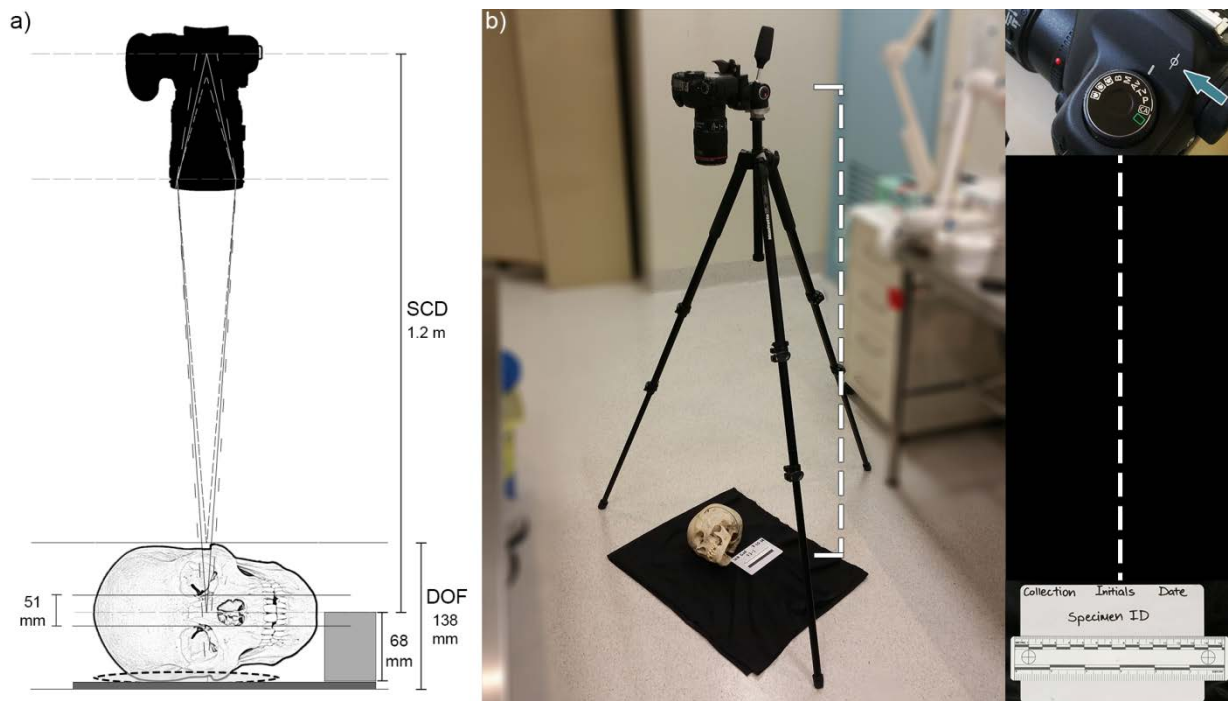
Figure 1: Photography protocol setup: a) diagram depicting the subject-to-camera distance (SCD), depth of field (DOF), the theoretical range of midline positions based on Howells' craniometric data (51 mm) [1, 2], and the backing board, circular skull support (dashed line), and 68 mm scale support block; and b) photograph of example setup demonstrating inferiorly directed camera, with SCD measured between the focal plane mark (blue arrow) and scale level.

Skull Setup:

Position the skull circle mount on the center of the board, and the block next to the skull circle in the desired location of the scale (Fig. 2)—this will bring the scale to the approximate skull midline. Cover the skull circle, block, and board with the black sheet. Attach the mandible (and the calvarium if removed) to the rest of the skull using either adhesive putty or dental utility wax. A gap of ~3 millimeters should be used between the mandibular condyle and glenoid fossa to simulate structures within the temporomandibular joint during life. Position the skull on the circle mount in a **left lateral** view (camera in portrait), using the camera's viewfinder or the remote capture screen to check. To do this: (a) the sagittal plane should be parallel to the floor, (b) the Frankfurt horizontal plane should be perpendicular to the floor, (c) only the left mastoid process, left styloid process, mandibular angle, and left half of the dental arcade should be visible (see Fig. 2). In cases of slight asymmetry, priority is given to aligning the cranium over the mandible.

Once the photograph has been taken, adjust the resolution to improve processing time in R. Photographs taken with a full-frame Canon 6D DSLR are resized to be 100 x 250 mm at 250 pixels per inch. Next, cross-check the photograph with the baseline scale image (available at CRANIOFACIALidentification.com) for suitability. Use the baseline scale to verify that the pixel dimensions of the 150 mm length of the new photograph falls within 606 ± 9.1 pixels ($\pm$ 1.5 %). If it falls outside of this range, the image will need to be resized. This can be achieved in image editing software such as Adobe® Photoshop® using the ruler tool. For example, if 150 mm measures at 620 pixels, and the image height is 1476 pixels, the new image height to resize to would be calculated as:

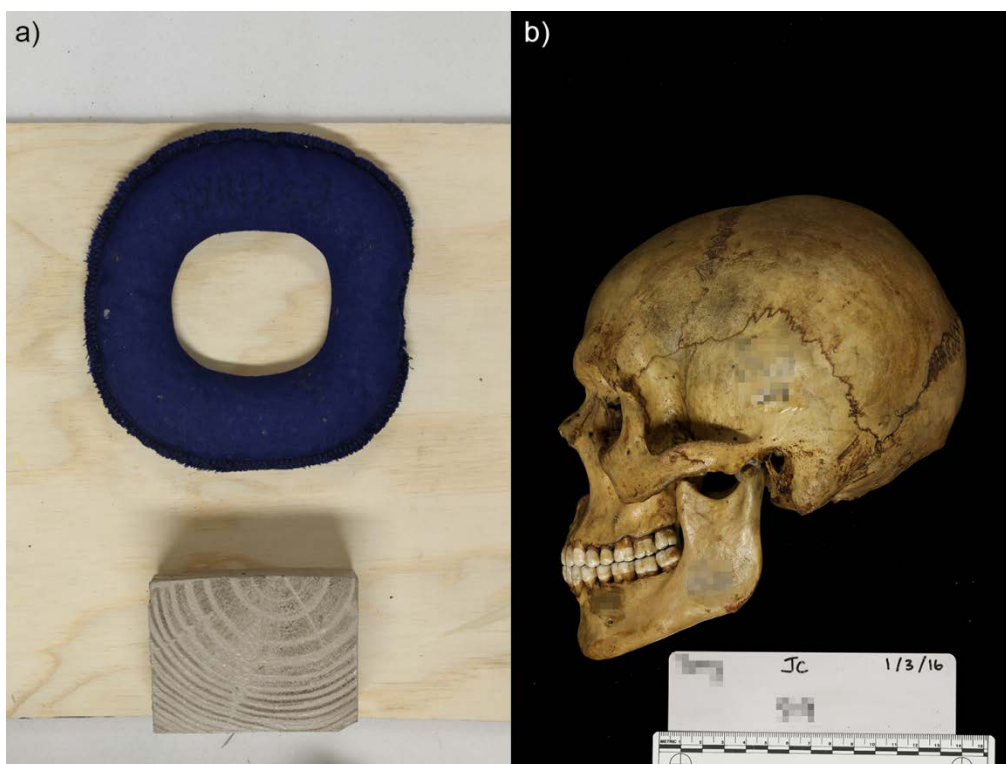New image height = (606 / **620**) * **1476** = **<u>1442.7</u>**

Figure 2: Photography protocol setup: a) support board with skull circle mount and scale block; and b) example skull photo demonstrating alignment in left lateral view and in the Frankfurt horizontal plane.

SkullProfiler

Initial Setup

The *SkullProfiler* tool uses lateral skull and cranial outlines quantified by elliptical Fourier analysis (EFA) to estimate sex and ancestry through comparison with a reference database supplied with the analysis tool. For a general overview of EFA see [5, 6]. Group assignment is achieved using linear discriminant analysis, which is run on principal component scores based on the EFA coefficients. Details of sample composition, and the data analysis process can be found in [3, 4]. The code is written in the R programming environment [7], and is partly adapted from functions provided by Claude [8] and the *QuickCapture* code available at CRANIOFACIALidentification.com [9]. R needs to be installed for use, and can be downloaded from https://www.r-project.org following the instructions at CRANIOFACIALidentification.com. The entire *SkullProfiler* R script can then be copied or imported straight into the R console. Initial use of *SkullProfiler* will require an internet connection to download the R script, splash screen logo, required R packages, and reference datasets. The logo will need to be saved into the base R directory, which is located at "C:\Program Files\R\R-[version]\doc\html". The reference datasets can also be saved to this folder; however, this is not required to run the script as they will be downloaded from the website if not present.

Program Initialization

The first window gives the option to conduct analysis on a skull photograph, or on previously extracted data as either the outline coordinates or EFA coefficients (Fig. 3). Specifications for the photograph are given in the protocol detailed above. The outline coordinates and EFA coefficients should both be .csv files, with the coordinates of 999 points organized into two columns (with column headers), and the coefficients of 40 harmonics organized into separate columns for the two constants (labeled

"ao" and "co"), and the four coefficients (labeled "ak", "bk", "ck", and "dk")—see output from using a photograph for an example of this layout. This window also requires specification of whether a cranial outline or the entire skull including articulated mandible will be analyzed (Fig. 1).
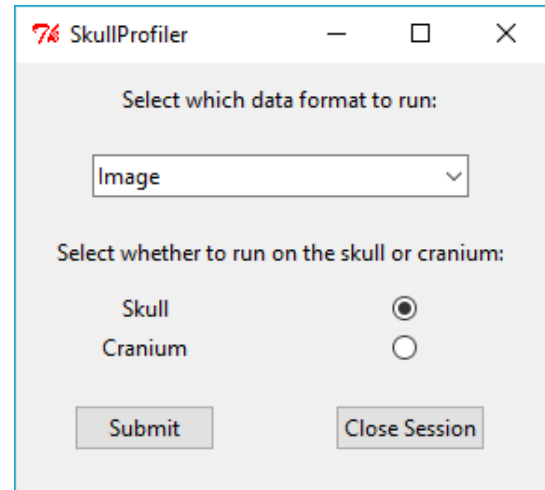

Figure 3: Data format selection and specification of analyzing either the skull or cranium.

Outline Extraction

If image analysis is selected, a directory window opens for the user to select the image file. The image is then plotted in grayscale before another window appears, requesting the image binarization threshold to be entered (Fig. 4). The default value (0.1) works well for images with high contrast and with a dark background. If there is less contrast with the specimen being darker, the value may need decreasing (~0.07-0.08); conversely, if the background is not dark enough, the threshold value may need to be increased slightly (~0.15-0.2). This window also gives the option to invert the image during binarization (Fig. 4). This option should remain selected in cases where the background is darker than the specimen (as according to the standard protocol). If a styloid process is at all present and extending beyond the perimeter of the profile line,

select the "remove the styloid process" option to remove from the outline (Fig. 4).
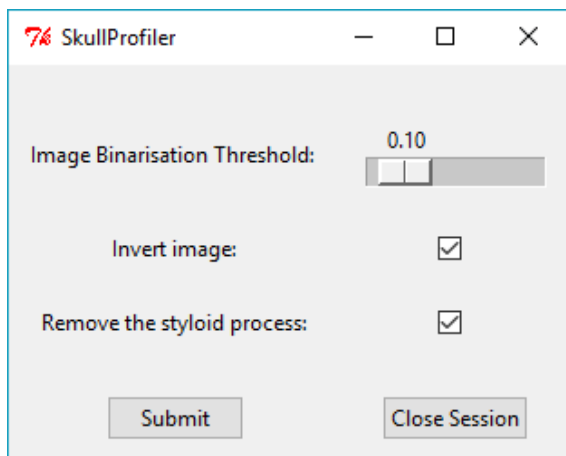

Figure 4: Skull image option specification.

Once the image specifications have been selected, several points need to be located on the image. The first landmark that needs to be clicked is *prosthion* (Martin, 1928; Martin and Knußmann, 1988). Take care to click on the specimen, not on the background, to ensure the outline is extracted successfully. If analyzing a cranium, the next point delineated is *mastoidale* (Martin, 1928; Martin and Knußmann, 1988). This will draw a straight line between the two points for outline extraction (Fig. 5a). Note that *mastoidale* is still selected if the mastoid process does not extend beyond the profile line of the cranial base. If outline extraction is successful, a plot of the binarized image with the outline plotted will display (Fig. 5b). If outline extraction is unsuccessful, a notification window will display giving the option to reselect the image file.

If analyzing a skull, the second point delineated is *infradentale* (Buikstra and Ubelaker, 1994). This draws a straight line to bypass teeth (whether present or absent; Fig. 6). If the option to remove the styloid process was selected, four additional points need to be delineated: 1) a point on the junction between the anterior edge of the styloid process and the cranial outline; 2) a point on the junction between the posterior edge of the styloid process and the cranial outline; and 3-4) points that encompass the styloid process in a polygon

(continuing in a clockwise direction; Fig. 6a). As with the cranium, a plot of the binarized image with the outline plotted will display if outline extraction is successful (Fig. 6b), otherwise a notification window will display giving the option to reselect the image file.

The outline coordinates are then automatically normalized for orientation using partial Procrustes alignment. EFA is run on the normalized outline coordinates: if successful the inverse elliptical Fourier transformation plot will display the outline shape shaded in grey, with the EFA outline in black (Fig. 7). The Procrustes and EFA functions were sourced from Claude [8]. Files automatically saved from analysis of a coordinate file include: Procrustes-aligned coordinates, EFA coefficients, Procrustes-aligned plot, and reconstructed EFA outline plot. Analysis from an image additionally outputs the following files: the original outline coordinates, the binarized plot, and extracted outline. Images are saved as .pdf vector files, which can be saved as high resolution image files in image editing software programs such as Adobe® Photoshop® (San Jose, CA). No files are saved at this point if analysis is run from EFA coefficients.
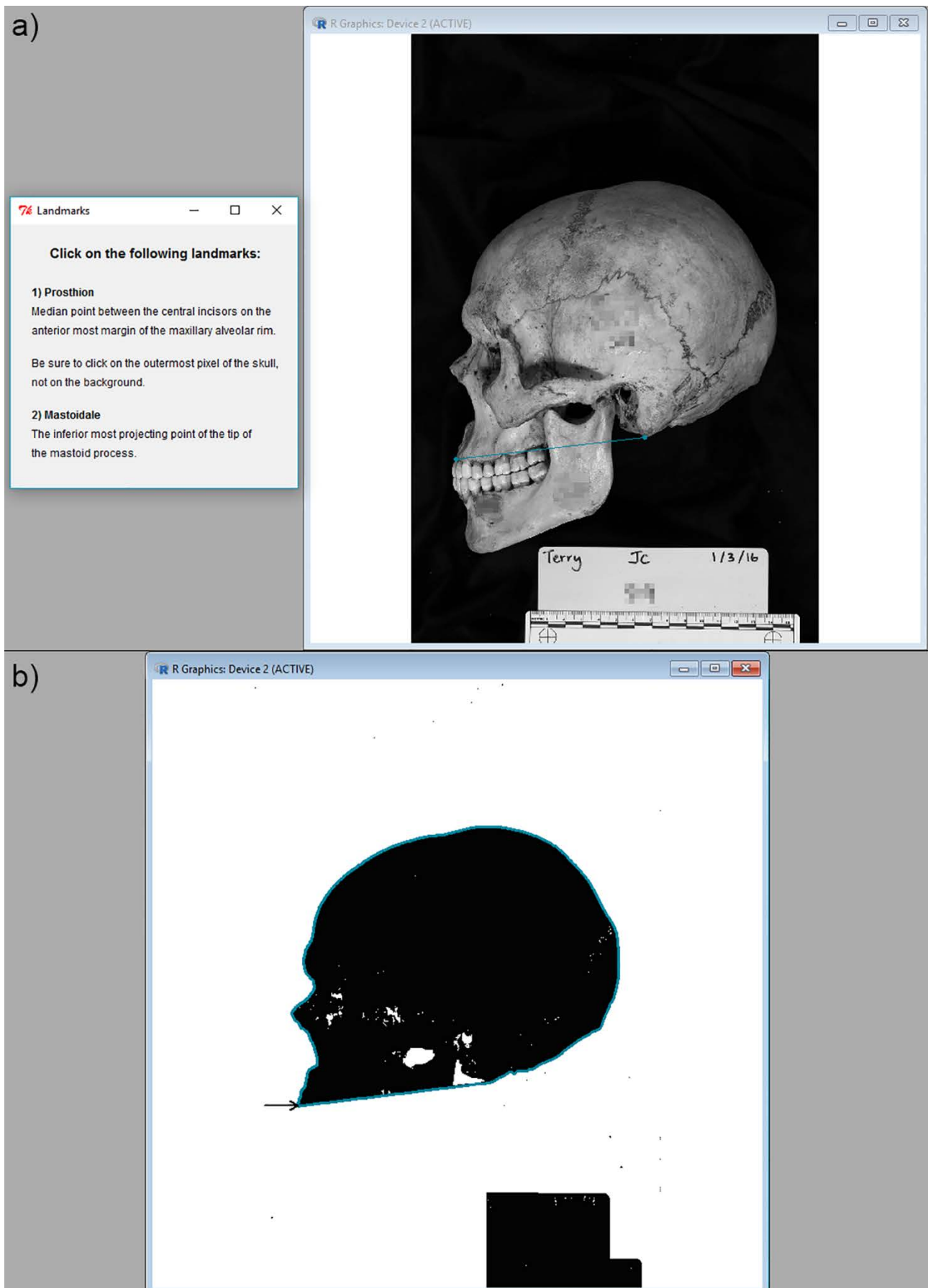
Figure 5: Cranial outline extraction: a) landmark selection; and b) binarized image with outline coordinates plotted.
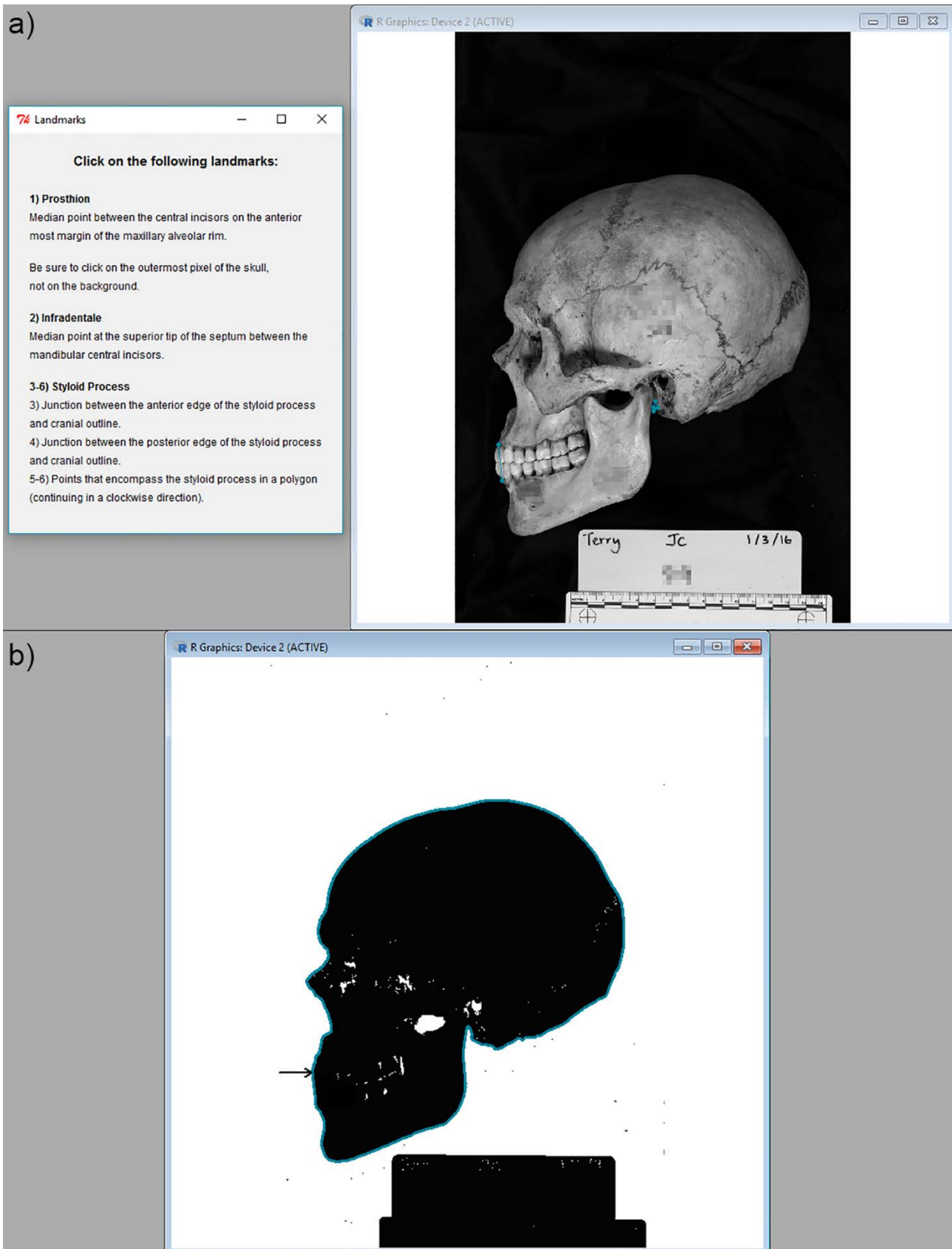
Figure 6: Skull outline extraction: a) landmark selection; and b) binarized image with outline coordinates plotted.
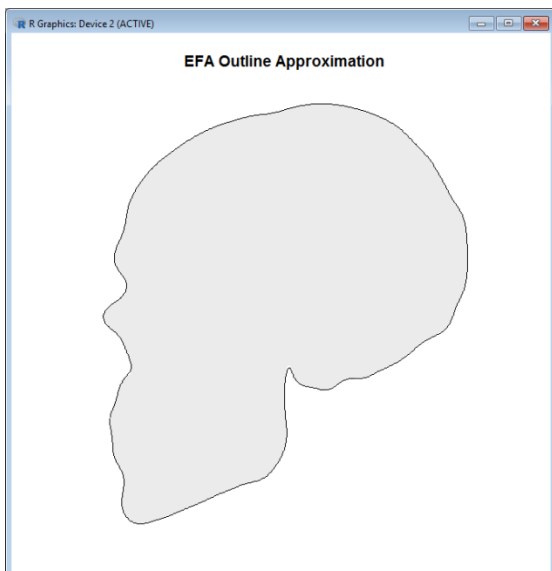
Figure 7: Window depicting a reconstructed elliptical Fourier outline.

Principal component analysis is then conducted on the EFA coefficients, before a window appears requesting the user to select which groups to include in the linear discriminant analysis (Fig. 8). The discriminant analysis results then display in a new window (Fig. 9). The first table is a classification matrix of the validation test for the selected groups. These results were obtained using 5-fold cross validation, before selecting the median accuracy rate run as the final sample. Below this confusion matrix is the group assignment based on the highest posterior probability, and the associated output table comprising the Mahalanobis square distances, posterior probabilities, and Chi-square typicality probabilities for each group. Additional results can be viewed from this window, which currently include group Mahalanobis distances in both their original form and corrected for sample size as recommended by Sjøvold [10] (Fig. 10). Analysis can be rerun from this window to either run the discriminant analysis with different groups selected, or to rerun the entire analysis on a different sample. Discriminant function plots can be viewed by selecting either the 2D or 3D plot buttons. The default plot settings display all samples with 90 % confidence interval ellipses, and with group labels at the centroids (Fig. 11-12). A window opens with the plot to enable user

specification of the confidence interval level, whether or not to display individual points, whether to display a print friendly version, and which discriminant functions to plot (press Return/Enter to update the plot; Figs. 11-12). Two-group discriminant function plots appear as a histogram as depicted in Figure 13. To visualize morphology of group centroids in high-resolution photographic-quality see [11].
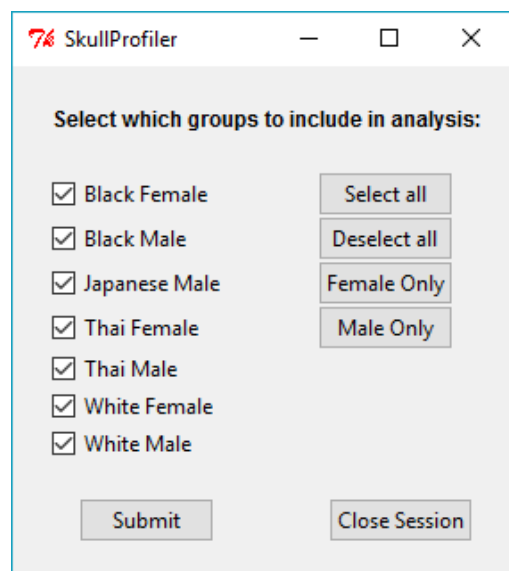

Figure 8: Group selection window for discriminant analysis.

The default files saved include: the principal component scores; the discriminant function scores; the pooled variance-covariance matrix; cross-validation confusion matrix; classification output (including Mahalanobis square distances, posterior probabilities, typicality probabilities); and the default 2D discriminant function plot. Both 2D and 3D plots can also be saved out with user specified settings from within the plot options windows (see Figs. 11-12). As with the previous plots, images are saved in .pdf vector file format, except for 3D plots, with are saved as .png files.
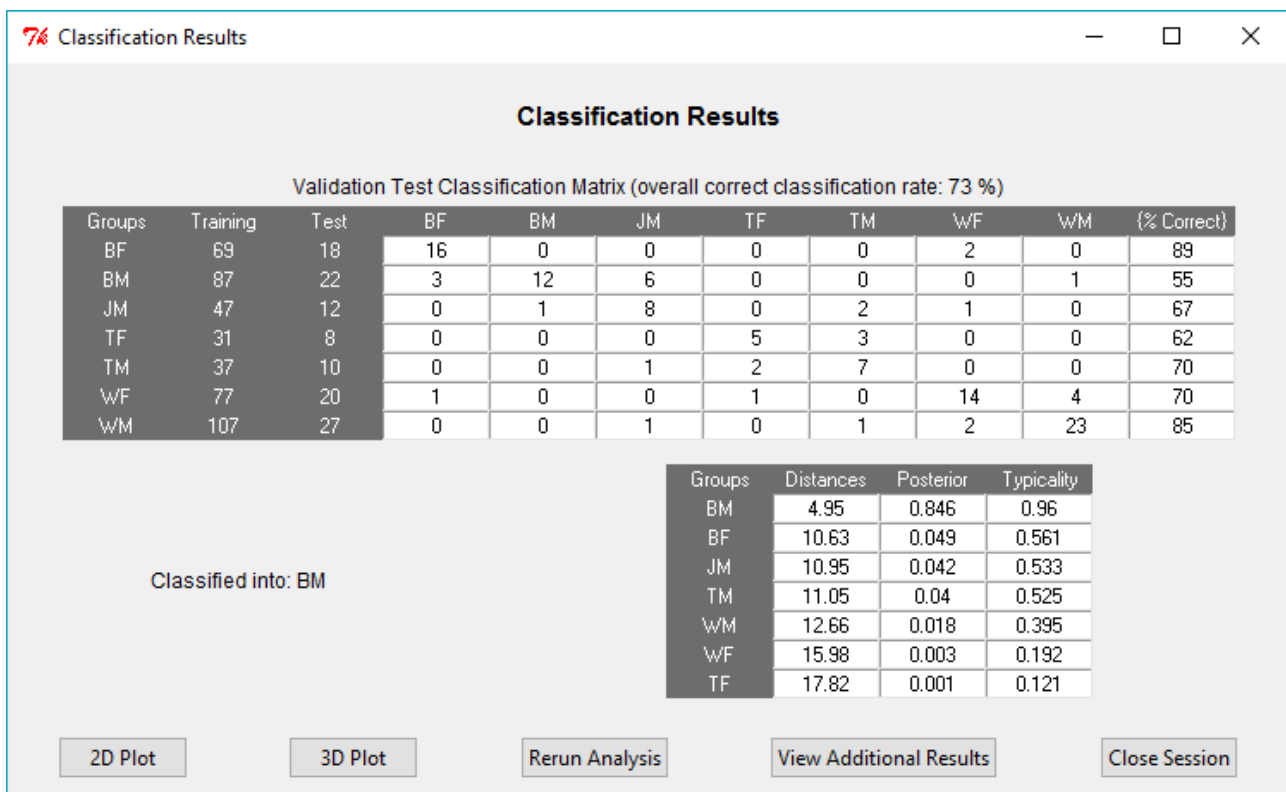
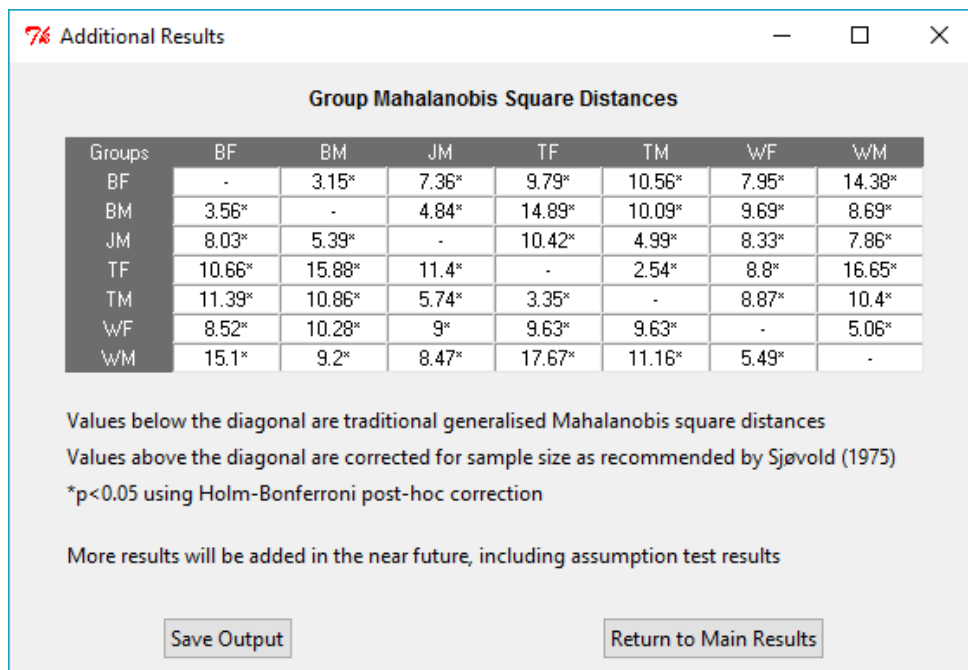**Figure 9:** Discriminant analysis window showing example results.

Figure 10: Additional results window displaying Mahalanobis square distances between groups.
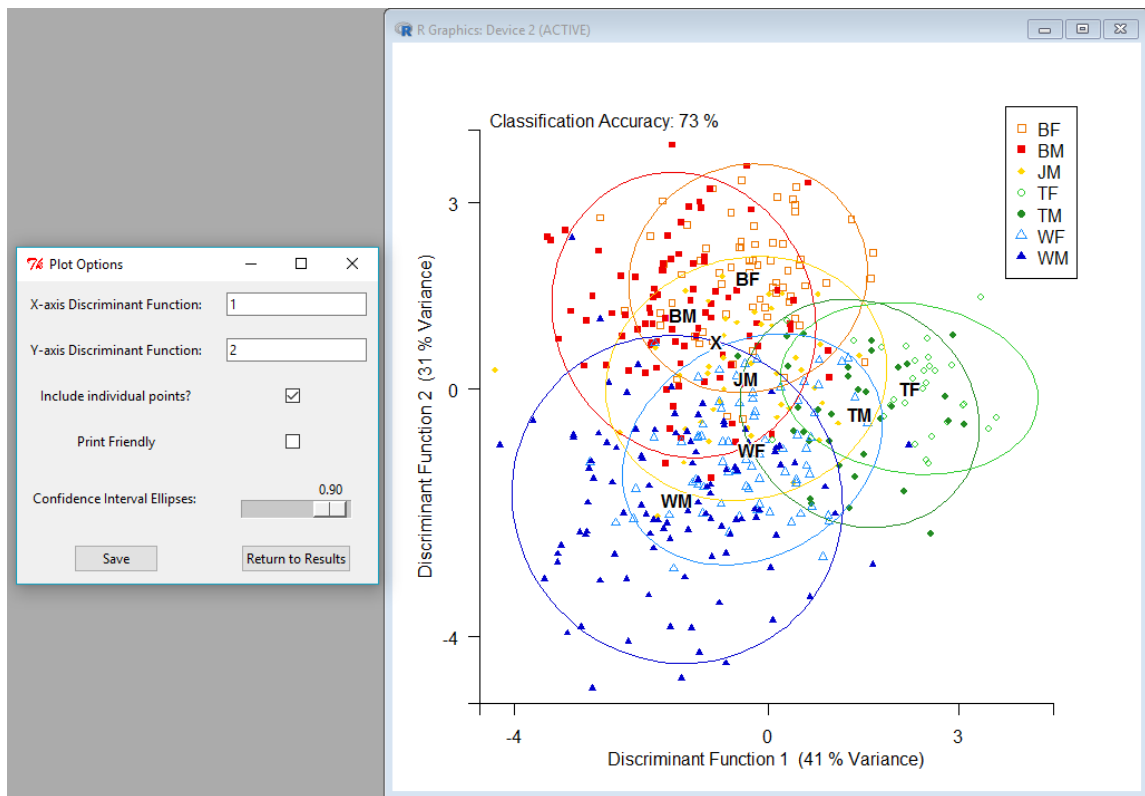
Figure 11: 2D discriminant analysis plot specifications window and plot with default settings, with all seven ancestry and sex groups included.
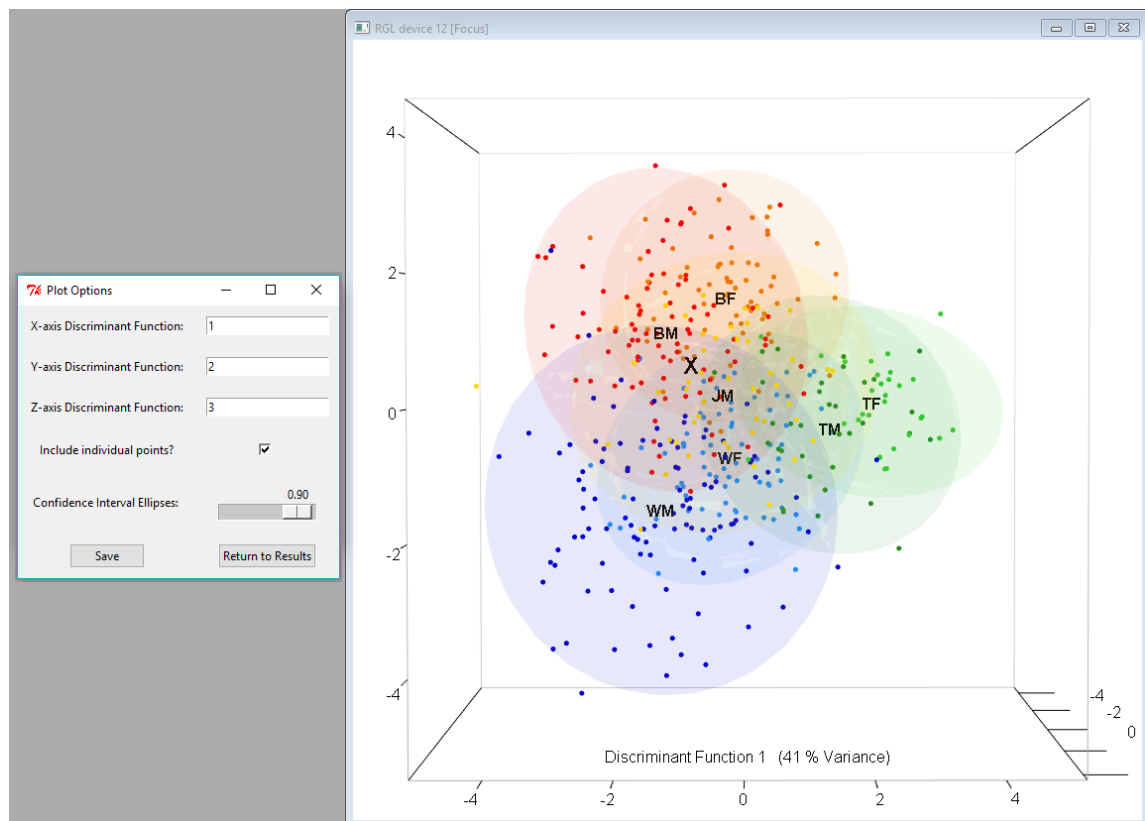

Figure 12: 3D discriminant analysis plot specifications window and plot with default settings, with all seven ancestry and sex groups included.
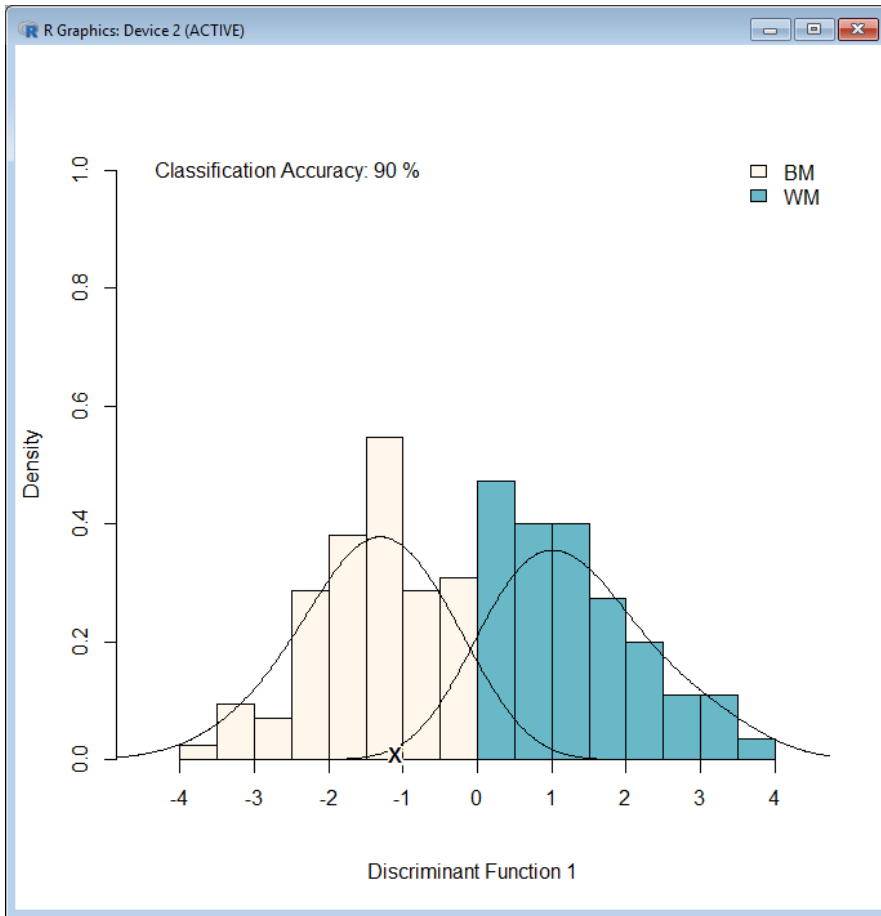
Figure 13: Discriminant function histogram plot for an example two-group comparison using Black and White males.

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