Manual for Data Collection
Total Difference Method (TMD)

Accompanying manuscript citation: Campbell & Butaric 2022, full citation pending

Accompanying GitHub repository for resources: https://github.com/jcampbelljess/FrontalSinus_TD_macros

Introduction. This manual provides step-by-step instructions for implementing the Total Difference Method (henceforth abbreviated as TMD) originally developed by Cox et al. [1]. This method quantifies frontal sinus shape, for the purpose of assessing the frontal sinus in personal identification. See FAQ 5.1 for a summary of this method.

Here, we outline the steps needed to conduct the original method using an open-source resource: FIJI/ImageJ [2,3]. We additionally provide the steps needed to conduct this method using a newly-created measurement aid (i.e. a tracing overlay), as well as a semi-automated macro that walks the user through each step of the method. Through the manual, we refer to these three modes as the Freehand Mode, Overlay Mode, and Semi-Auto Mode, respectively. Note that these modes build off of each other, and all require certain steps to be completed beforehand. These steps are outlined below.

1. Preliminary Steps

Prior to starting TDM, you will need to download and install Fiji or ImageJ, select appropriate radiographs, and trace the frontal sinus outline. These steps are outlined below.

1.1 Downloading and installing FIJI or ImageJ. Before getting started, you will need to download and install FIJI or ImageJ, henceforth referred to as simply ImageJ. See FAQ 5.2 for more information and resources on using this program.

1.2 Select your radiographs: Note the TDM can only be completed on frontal sinuses that span across the midline of the skull and that are not discontinuous, see Figure 1.1.

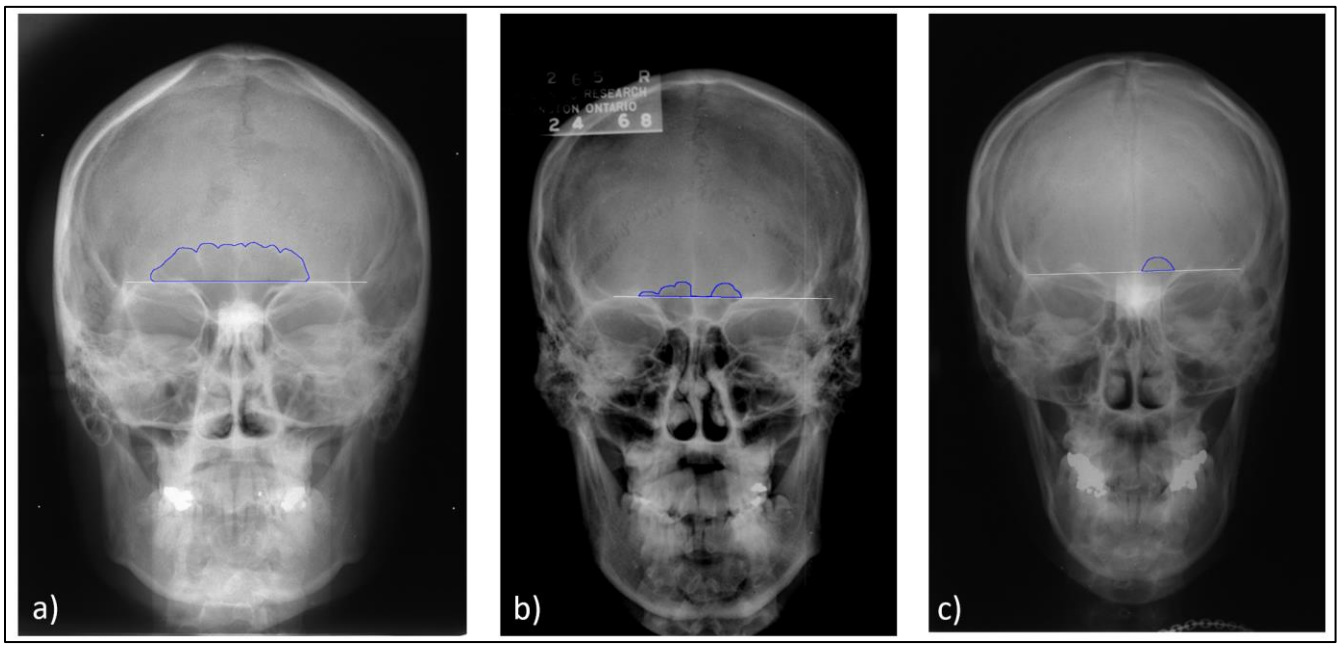

Figure 1.1. Images illustrating a) a frontal sinus appropriate for the Total Difference Method (TMD) versus b) a discontinuous and c) unilateral sinus, not appropriate for TMD. Note, Sinus contour outlines are provided to delineate the sinuses.

1.3 Tracing the Frontal Sinus outline. TDM requires the tracing of the frontal sinus, following Christensen's [4] method. This method first demarcates the inferior border of the frontal sinus, at the level of the superior orbital border. Next, the contour of the sinus is traced to capture the lateral and superior borders of the sinus. These steps are provided:

1.3.1 Open your radiograph in ImageJ Go to File > Open (or CTRL+O), and navigate to the radiographic file you wish to open.

1.3.2 Adjust the balance/contrast if needed. To best visualize the sinus, you may need to adjust the balance and contrast of the image. To do so, go to Image > Adjust > Brightness/Contrast or click Ctrl+Shift+C.

1.3.3 Place the supraorbital line. Select the *line tool* and place the supraorbital line (see Figure 1.2) and hit "D" (for "draw") on your keyboard to draw it onto the radiograph.

1.3.3.1 Note that you can change the pixel size of the line by going to Edit > Options > Line Width (or by double-clicking the *line tool*). You can also change the color of the line by going to Edit > Options > Color (or by choosing the *color picker tool*). We recommend using 1-3 pixel thickness, in a color that is easily discernable against the image. Make sure the line extends laterally past the sinus edges. Note, if your line is more than 1-pixel thick, you will need to hit "F" (for "fill") instead of "D" to have a solid line. In this study, we utilize a 3-pixel thick line in white (R=255, G=255, B=255).

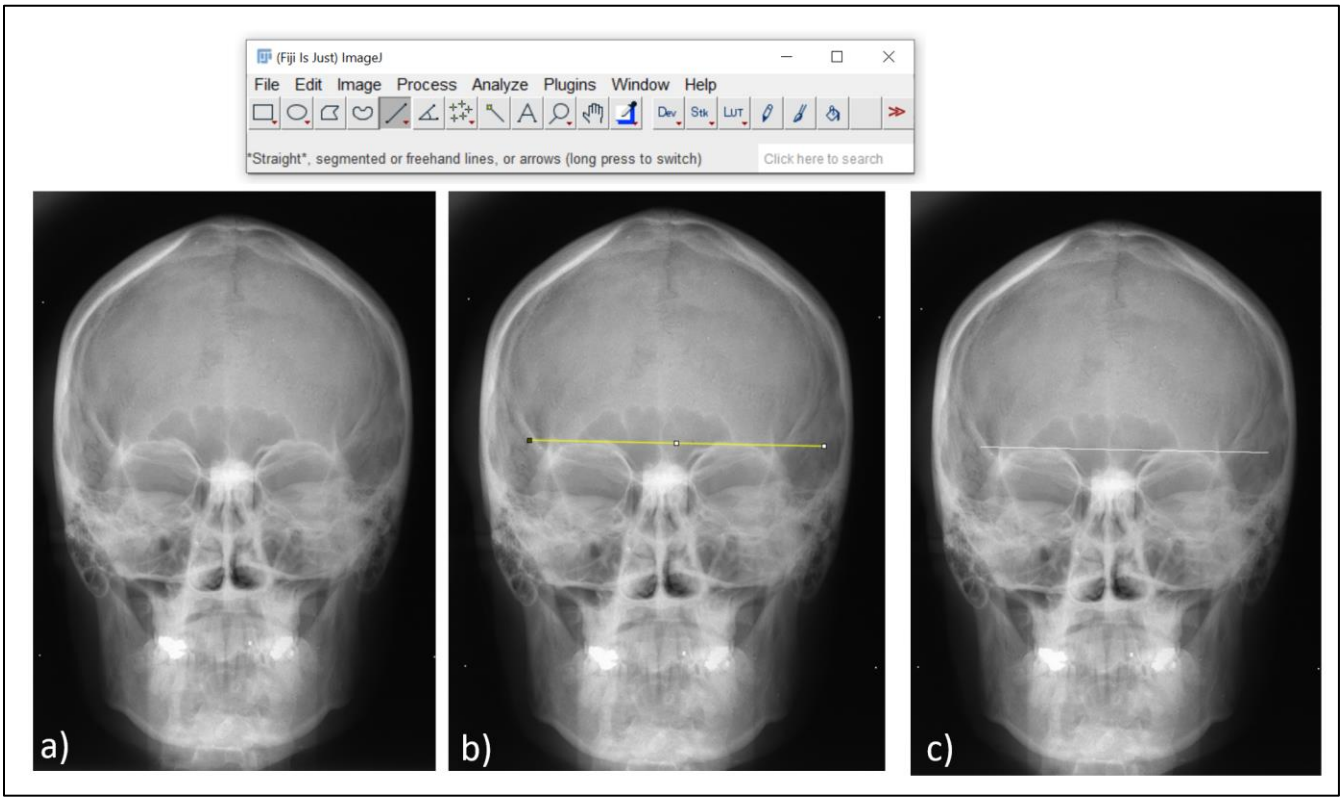

Figure 1.2. Placing the line across the supraorbital line; a) image without modification; b) line tool used to place line; c) line is drawn in after pressing "D" or "F". Note, the line should be placed at the superior border of the orbital cavities (note the superciliary arches).

1.3.4 Trace the sinus outline. Select the *Freehand tool (blue arrow,* Figure 1.3) and trace the contours of the frontal sinus. We recommend using 1-3 pixel thickness, in an easily discernable color (change color with *dropper* tool, Figure 1.3). In this study, we use blue (#0036ff; R=0, G=54, B=255) Starting at the left-most edge (anatomically right-side) where the sinus meets the supraorbital line, trace the superior

contours of the sinus to where the right-side (anatomically left-side) meets the supraorbital line in a clockwise fashion (see Fig1.3 middle image). You do not need to retrace the supraorbital line. Hit "D" (regardless of pixel thickness) to draw the line and leave the sinus unfilled.

Comments:

Make sure to press "D", not "F", when drawing the sinus outline. Pressing "F" will fill the sinus, which will make the next steps of the TDM more difficult. Ensure that the sinus contour has been followed correctly and that the lateral-bottom edges connect to your supraorbital line. Also ensure that your contours do not trace septa inferiorly into the sinus, following Christensen's recommendations (see Figure 1.3, yellow arrows).

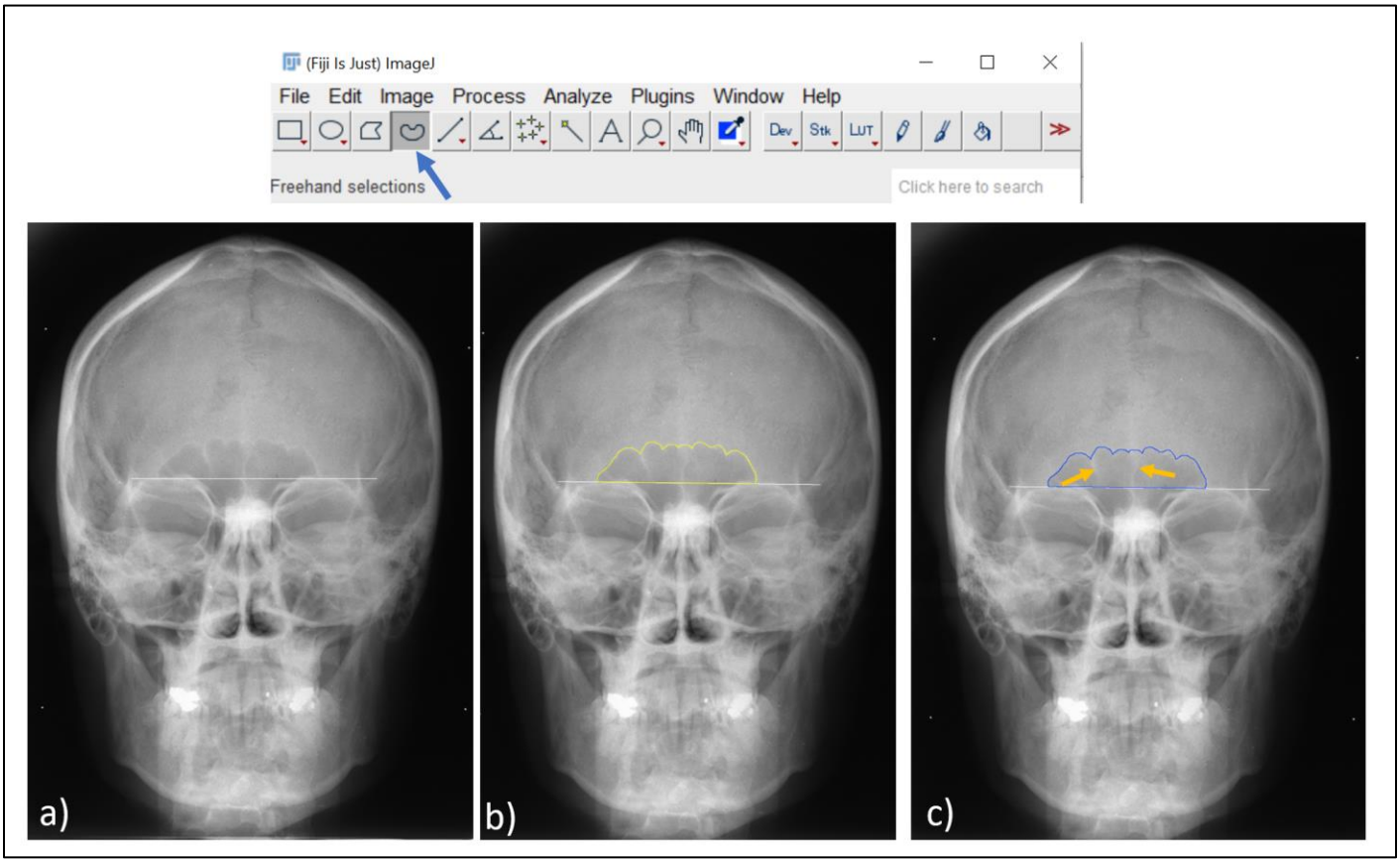

Figure 1.3. Tracing the frontal sinus contours. a) image with supraorbital line placed; b) Freehand (blue arrow) used to trace the outlines; c) blue outline drawn onto the image. Note that the contours do not follow intra-sinus septa (yellow arrows).

1.3.5 **Save Image.** Once the frontal sinus has been correctly outlined, save the image as a .tiff file. To do so, go to File > Save As > ImageName.tiff. We also recommend making the filename a distinctive, easy-to-identify name, for example, IDname_FSoutline.tiff. To streamline the full process, we recommend tracing your frontal sinus outlines in your full sample prior to implementing the TDM steps, regardless of the mode utilized.

2. TMD: Freehand Mode

The Freehand Mode closely follows the protocols originally outlined by Cox et al. [1]. See FAQ 5.1, 5.4, and 5.5 for more details on the TMD and the variables you will be collecting.

2.1 Open your image file. Go to File > Open to open a radiograph .tiff file with an existing sinus outline and supraorbital line in ImageJ (see steps above if not completed).

2.1.1 Optional: Open the ROI Manager by going to the top menu Analyze > *Tool*s > ROI Manager. See FAQs 5.6, 5.7, and 5.8 regarding the use of the ROI Manager. It will also open automatically when you press "t" to obtain the sinus size in step 2.3.

2.2 Scale your image (if needed). If not already done, scale your radiograph as appropriate Using the *line tool*, place a line across the item that represents the scale of your radiograph (no need to hit "D" or "F" to draw the line). To set the scale, go to Analyze > Set Scale and type in the known distance and applicable units. Do not change the pixel size, and do not check "Global". Click "okay" to apply the scalar. See FAQ 5.4 for more information on scaling.

2.3 Obtain sinus size. Select the *magic wand tool* and highlight the frontal sinus outline (Figure 2.1). You may need to zoom in (CTRL+roll mouse wheel, or use the *magnifier* tool on the ImageJ console) closely to get on the line if it is thin. Once highlighted, record the measurement into the ROI by hitting "t" on the keyboard.

2.3.1 Optional: You can rename the sinus outline in the ROI Manager, so you know what is being measured and exported. (see FAQ 5.8).

Comments:

While this measure (sinus outline area) is not directly needed for the TDM, it provides an overall measure of sinus size (useful in many studies) and can serve as a secondary check that repeated measures of the images are scaled.

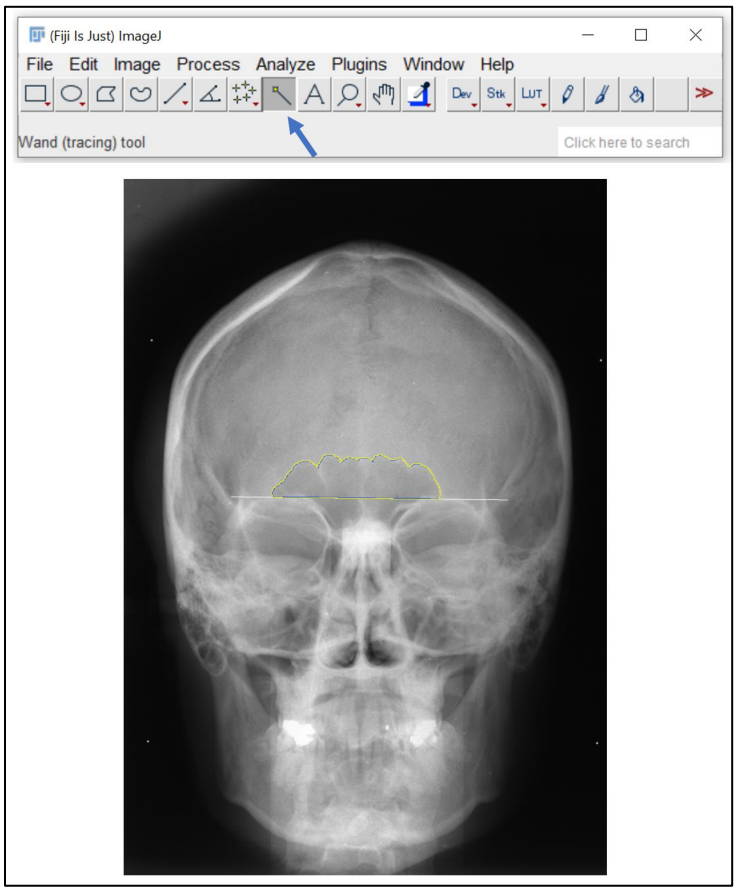

Figure 2.1. Image showing frontal sinus outline highlighted, after being selected using the wand tool (blue arrow).

2.4 Determine your point of origin. Following Cox et al. [1], this point should be determined by drawing a vertical line through the skull using as many midline anatomical landmarks as possible (including nasion, anterior nasal spine, prosthion, etc.; Figure 2.2). Select the *line tool* and create this vertical midline and hit "D" or "F" to place it on the image. The origin is the location where this vertical midline intersects the supraorbital line, and the origin is the point from which additional variables were collected (e.g., right baseline and the 59-line lengths; see steps 5 and 7). There is nothing to record at this step.

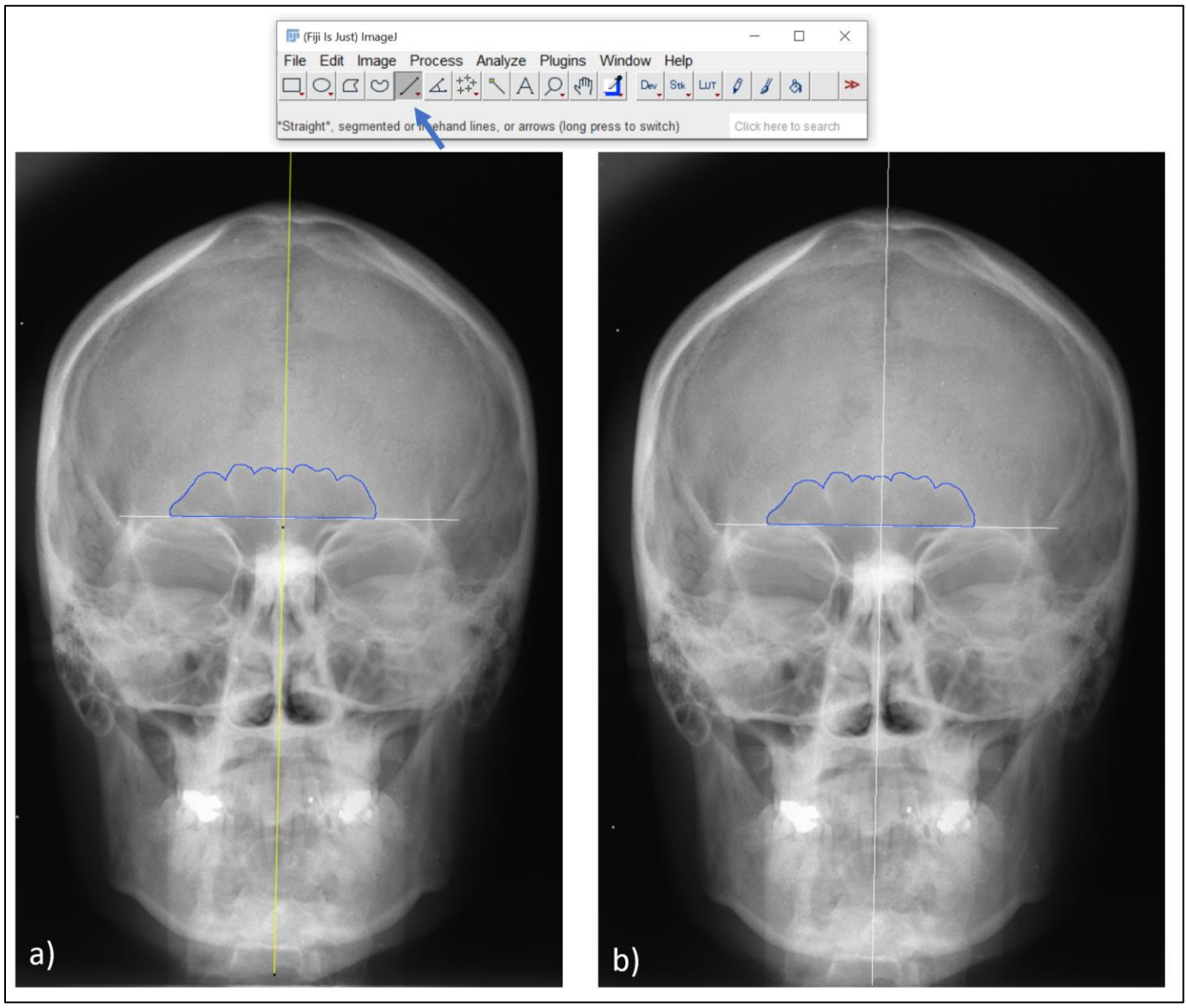

Figure 2.2. Image showing a) placement of midline using the straight-line tool (blue arrow) and b) midline drawn in using the "D" or "F" key.

2.5 Obtain your total baseline and right-side baseline lengths. Per the original method, the right baseline refers to the right side of the radiograph, not the anatomical right side of the cranium, and was measured from the origin to the right terminus of the baseline.

2.5.1 Select the *line tool* and re-trace the total baseline, from the intersection of the left sinus contour to the right side (Figure 2.3). Note that the total baseline should only represent the length of the baseline of the sinus, not the total supraorbital length previously drawn. Press "t" to record this line length in the ROI Manager.

2.5.2 Next, trace the right-side baseline of the inferior border of the sinus, starting from the origin (midline point) to the edge where the sinus contour intersects the supraorbital line. Press "t" to record this line length in the ROI Manager.

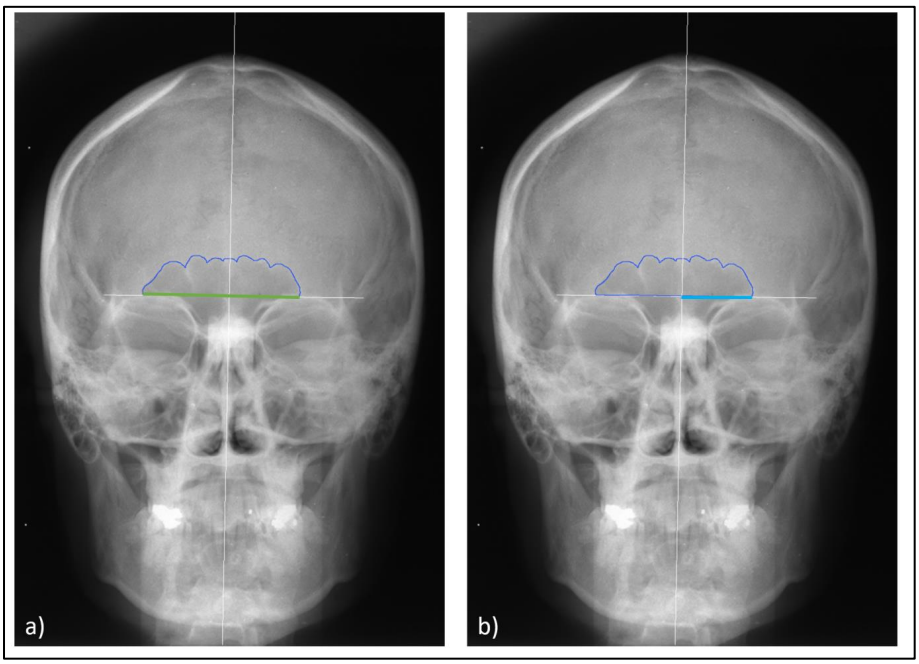

Figure 2.3. Images showing the proper placement of the a) baseline (green line) and b) right-side baseline (cyan line).

2.6 Obtain the 59-lines at 3° intervals. Select the *angle tool*. First, place the initial end of the angle on the right terminus of the sinus baseline (Figure 2.4a, yellow arrow), then position the other end of the line on the origin (Figure 2.4a, red circle) to form the fulcrum. From here, you will extend the line to the first 3° intercept with the sinus outline. You can check the degree by observing the ImageJ command menu (Figure 2.4a, green arrow). Record this line in the ROI Manager by hitting "t" on the keyboard. Then, move the line end to the 6° intercept and record by hitting "t", then the 9° intercept and record by hitting "t", and so forth until you record the final line at 177° (Figure 2.4b, right side).

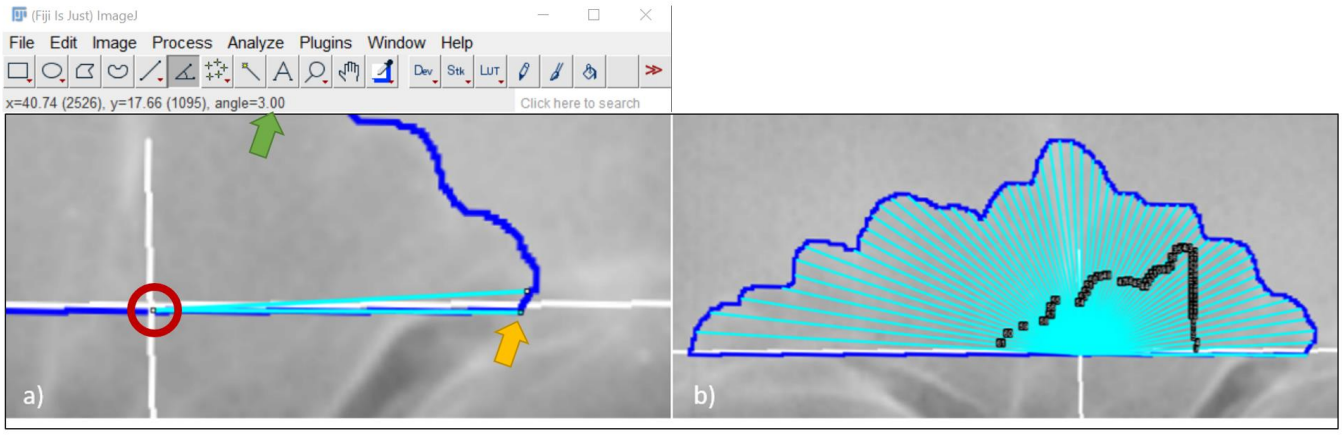

Figure 2.4. Images depicting a) initial 3° line placement and b) completed 59° lines. Yellow arrow: placement of initial end of angle on right terminus of sinus baseline; red circle: location of origin and fulcrum for all angles, green arrow: location to read degrees in ImageJ console.

Comments for all modes:

When drawing lines, three directives should be followed per the original method. First, the separate lines are drawn to the internal border of the sinus outline. Second, the lines are collected in a counterclockwise fashion, starting on the right side of the image. Third, in the event a 3° ray crossed the outline more than once, the outermost intersection is used (see Figure 2.5). All 59 lines should be recorded separately in the ROI Manager. At this point, if any lines need to be adjusted by length or angle, they could be selected in the ROI Manager and the endpoints adjusted in the image (See FAQ 5.7 for details.)

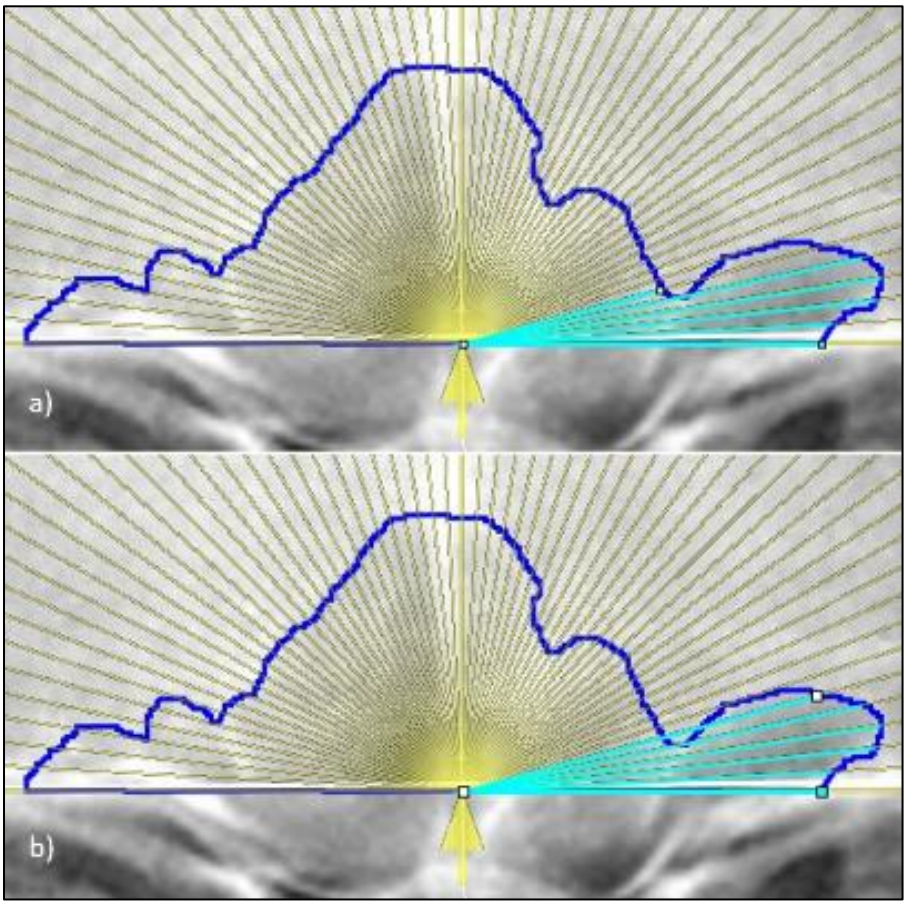

Figure 2.5. Line extension per Cox et al [1]. a) line length is not extended to furthermost intersection with the sinus outline; b) line is extended fully, to innermost contour of the furthest intersection.

2.7 Record/save your measures. Upon completion of the above steps, select all variables ("CTRL+A") in the ROI Manager (i.e., sinus area/perimeter; the baseline, right-baseline, the 59-lines/angles). Click the "Measure" key in the ROI Manager to measure all the variables at once. A Results box will pop up. Save the results file (in the Results window, go to File > Save as).

2.8 After ensuring measures are recorded and saved correctly, close the image and delete the objects in the ROI Manager before restarting Step 2.1 for the next image.

2.8.1 Alternative: Open the next image in the folder by ("CTRL+SHIFT+O") and delete the objects in the ROI Manager before restarting at Step 2.2 with the next image.

3. TMD: Overlay Mode

The Overlay Mode procedure as described by ([5], pending) utilizes a measurement aid that can be downloaded from https://github.com/jcampbelljess/FrontalSinus_TD_macros. This mode followed the general Freehand Mode procedures through Step 4, where the origin (or midline of the skull) was marked.

Prior to implementation, download the following files from Github (https://github.com/jcampbelljess/FrontalSinus_TD_macros/blob/main/Overlay.tif):

- Overlay.tif (the overlay file)

3.1 Open your image file. Go to File > Open to open a radiograph .tiff file with an existing sinus outline and supraorbital line in ImageJ (see steps above if not completed).

3.1.1 Optional: Open the ROI Manager by going to the top menu Analyze > *Tool*s > ROI Manager. See FAQs 5.6, 5.7, and 5.8 regarding the use of the ROI Manager. It will also open automatically when you press "t" to obtain the sinus size in step 3.3.

3.2 Scale your image (if needed). If not already done, scale your radiograph as appropriate Using the *line tool*, place a line across the item that represents the scale of your radiograph (no need to hit "D" or "F" to draw the line). To set the scale, go to Analyze > Set Scale and type in the known distance and applicable units. Do not change the pixel size, and do not check "Global". Click "okay" to apply the scalar. See FAQ 5.4 for more information on scaling.

3.3 Obtain sinus size. Select the *magic wand tool* and highlight the frontal sinus outline (Figure 3.1). You may need to zoom in closely to get on the line if it is thin. Once highlighted, record the measurement into the ROI by hitting "t" on the keyboard.

3.3.1 Optional: You can rename the sinus outline in the ROI Manager, so you know what is being measured and exported. (see FAQ 5.8).

Comments:

While the measure of sinus outline area is not directly needed for the TDM, it provides an overall measure of sinus size (useful in many studies) and can serve as a secondary check that repeated measures of the images are scaled.

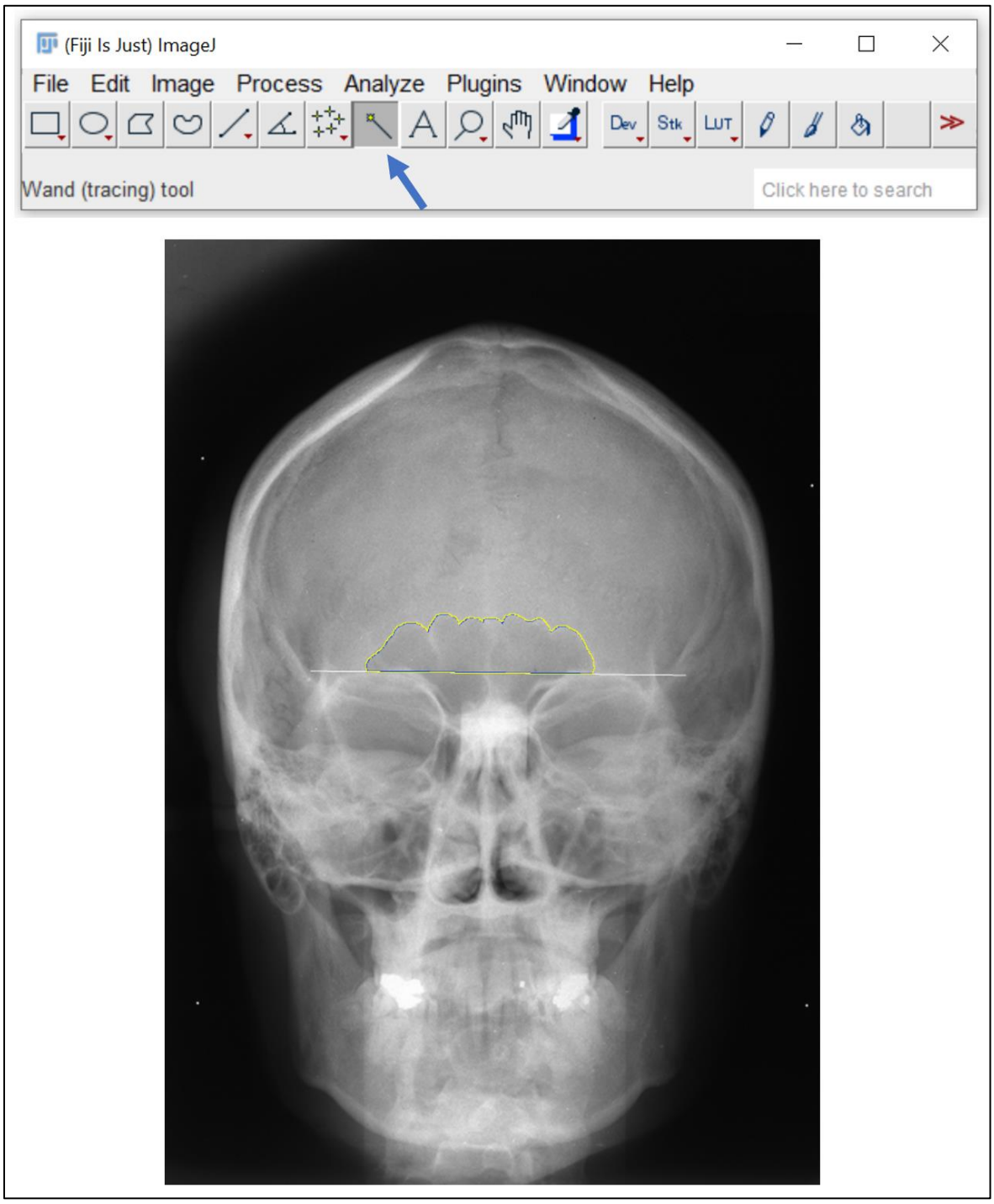

Figure 3.1. Image showing frontal sinus outline highlighted, after being selected using the wand tool (blue arrow).

3.4 Determine your point of origin. Following Cox et al. [1], this point should be determined by drawing a vertical line through the skull using as many midline anatomical landmarks as possible (including nasion, anterior nasal spine, prosthion, etc.). Select the *line tool* and create this vertical midline and hit "D" or "F" to place it on the image. The origin is the location where this vertical midline intersects the supraorbital line, and the origin is the point from which additional variables were collected (e.g., right baseline and the 59-line lengths; see steps 5 and 7). There is nothing to record at this step.

3.5 Obtain your total baseline and right-side baseline lengths. Note this step is similar to that in the Freehand Mode (see Step 2.5), with one extra step needed: you'll need to obtain the angle of the total baseline. The angle will be important for rotating the image properly when using the overlay.

3.5.1 Select the line *tool* and trace the total baseline, from the intersection of the left sinus contour to the right side (Figure 3.2a). Note, the total baseline should only represent the length of the baseline of the sinus, not the total supraorbital length previously drawn. When placing the line, note the angle (Figure 3.3) given in the ImageJ command menu. Hit "t" to record this line length in the ROI Manager.

3.5.2 Next, trace the right-side baseline of the inferior border of the sinus (Figure 3.2b), starting from the origin (midline point) to the edge where the sinus contour intersects the SOB line. Hit "t" to record this line length in the ROI Manager. There is no need to record the angle for this line.

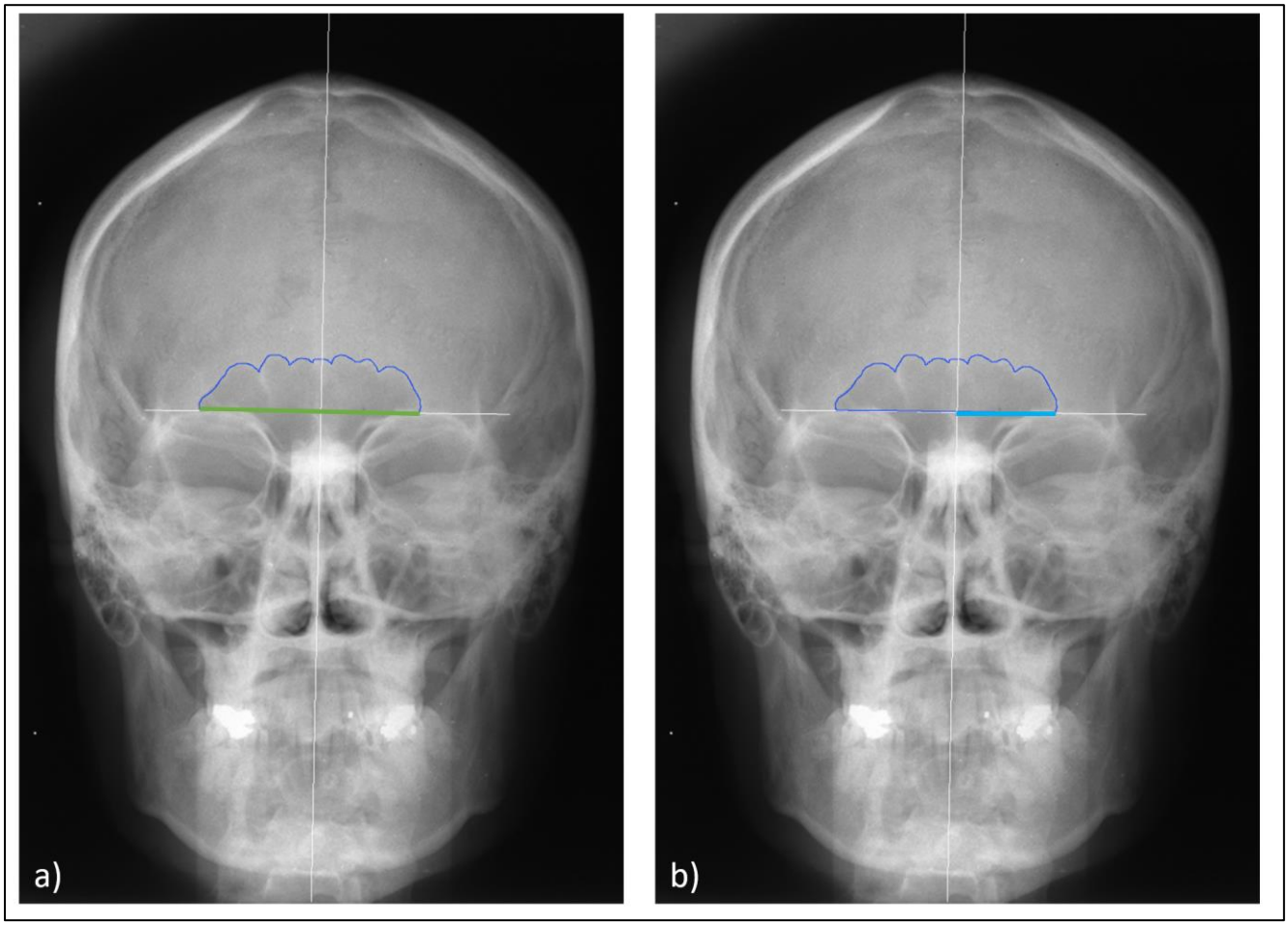

Figure 3.2. Images showing the proper placement of the a) baseline and b) right side baseline.

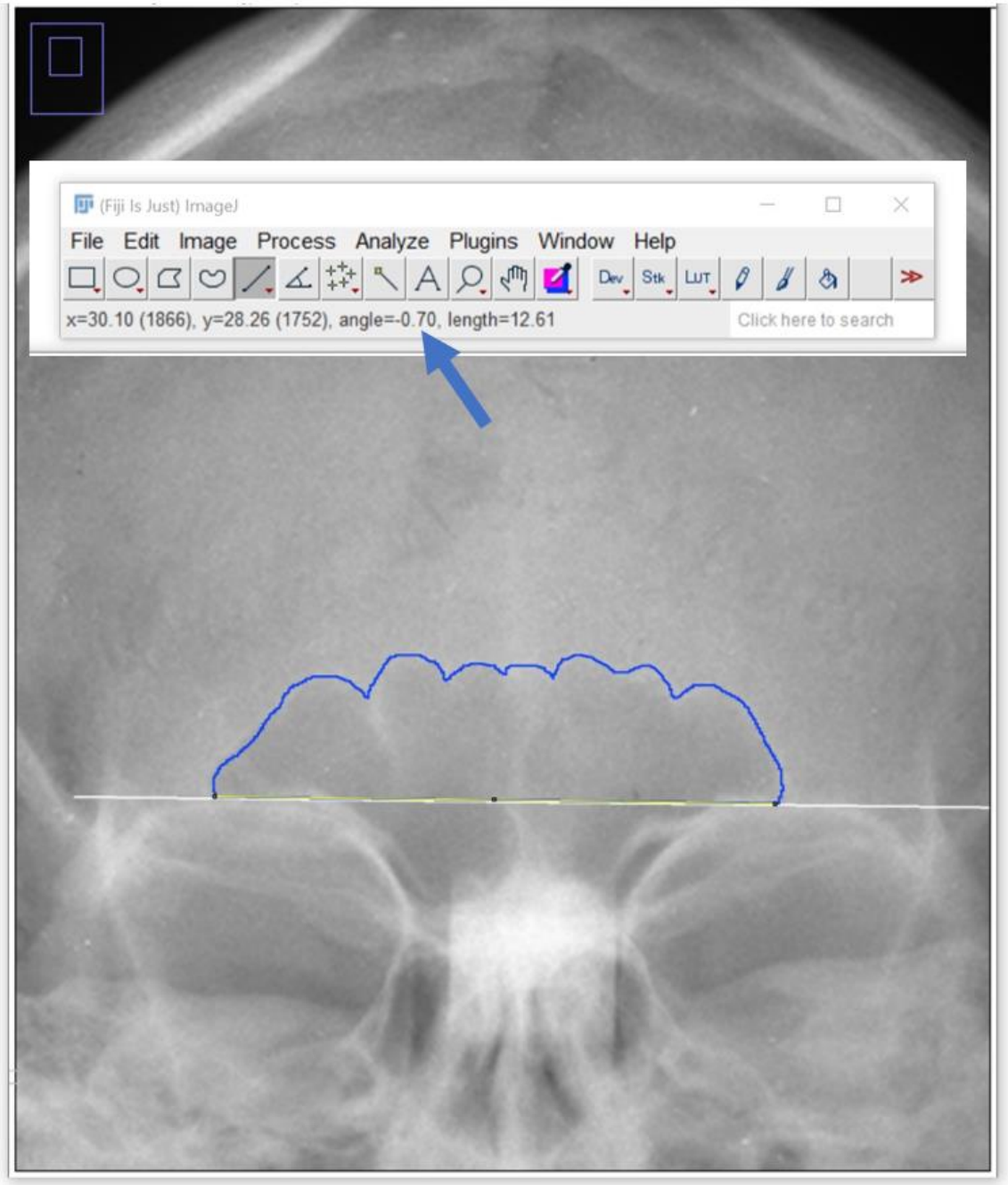

Figure 3.3. Image illustrating placement of total baseline, with angle indicated at -0.70 degrees (blue arrow). This angle will be used when rotating the image.

3.6 Rotate the image. There may be instances where the original radiograph is tilted and needs to be aligned so that the supraorbital line is at a 0° angle. You will use the previously obtained angle from the total baseline to straighten the radiograph.

3.6.1 In the ImageJ menu, go to Image > Transform > Rotate to open the rotation window. Check the "Preview" box. The image can either be rotated manually using the slide bar or automatically, by entering the baseline angle recorded from step 3.5 (above). This step is required for accuracy in placement of the measurement aid and positioning of the subsequently traced 3° lines.

3.7 Open the Overlay file (Overlay.tif) in ImageJ. This file can be downloaded from Github (https://github.com/jcampbelljess/FrontalSinus_TD_macros/blob/main/Overlay.tif) and will be used as a measurement aid in collecting the 59-line lengths (see Figure 3.4)**.**

3.7.1 With both your overlay and radiograph files open, go to Image > Overlay > Add Image. Choose 50% opacity (or adjust as wanted), and check "Zero Transparent" box. This will allow the yellow lines of the overlay to remain clear, while removing the black background so it doesn't occlude the sinus itself.

3.8 Align the Overlay. To easily move the overlay once on the radiograph, we recommend adding it to the ROI manager. This will allow repositioning, fine-tuning image rotation for alignment, as well as to enable the ability to toggle the overlay on/off.

3.8.1 If you haven't moved on or selected anything else, double-click on the overlay lines to select, and press "t" to add it to the ROI Manager.

3.8.2 Select it in the ROI Manager. Once selected, reposition the overlay so that both the total baseline and the marked origin line of the outline are aligned horizontally and vertically with the measurement aid lines, respectively (see Figure 3.5). Note, to move the overlay, make sure either *the line tool* or *magic wand tool* is selected, then drag and position the overlay as needed.

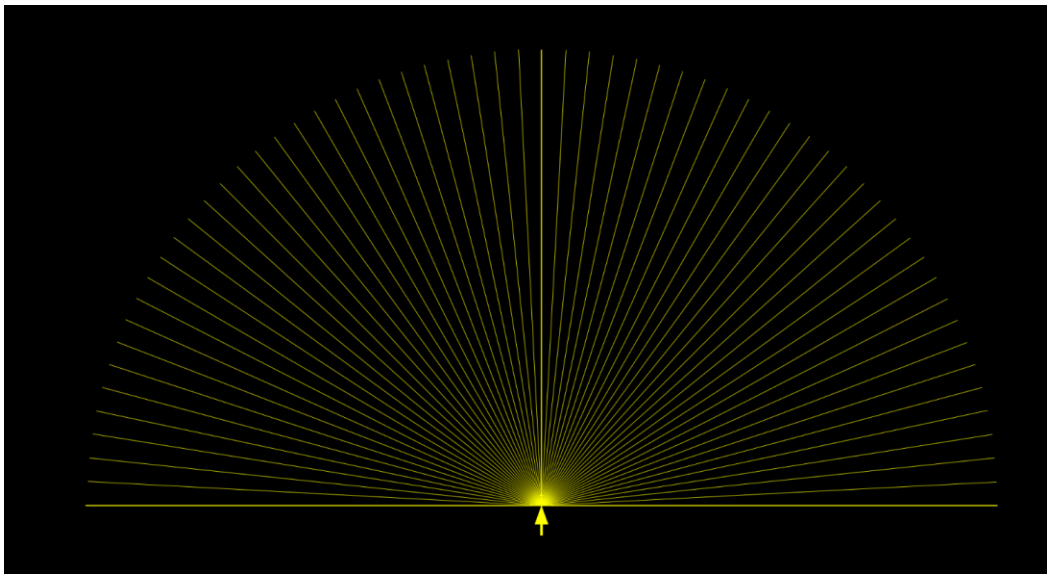

Figure 3.4. Image of the isolated overlay with negative background (Overlay.tif file on Github)

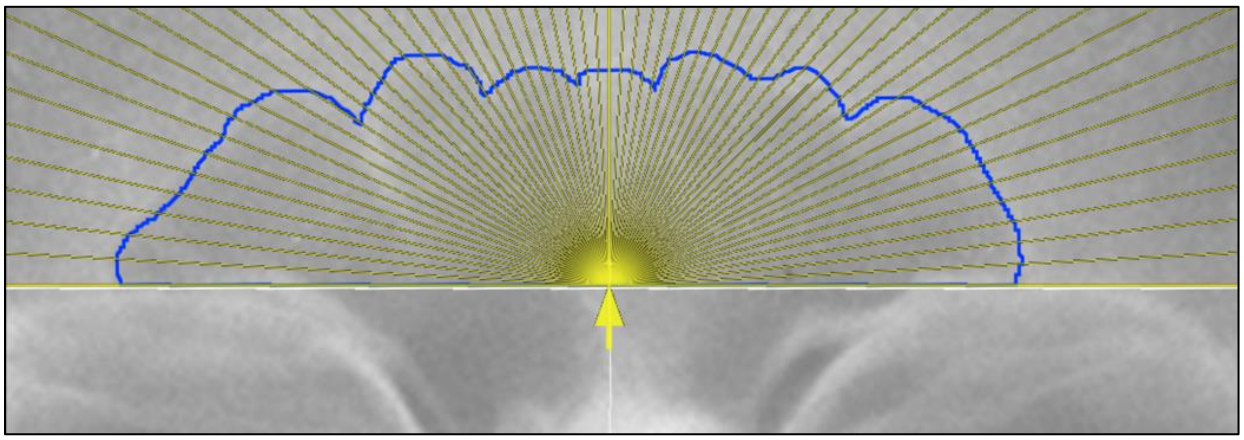

Figure 3.5. Image depicting alignment of the overlay. Note yellow arrow should align with the origin, and the base of the overlay should align with the inferior border of the frontal sinus.

3.9 Obtain the 59-lines at 3° intervals. Once the overlay is placed correctly (Figure 3.5) select the *angle tool*. First, place the initial end of the angle on the right terminus of the outline baseline, then position the other end of the line on the origin to form the fulcrum. From here, you will extend the line was extended to the first 3° intercept with the sinus outline. You can periodically check the degrees by observing the ImageJ command console, similar to Step 2.6 provided in the Freehand Mode. But, unlike the freehand mode, you can more quickly place the lines following the overlay as a guide. After placement of each line, press "t" on the keyboard to record them to the ROI Manager. Continue this process until you record the line at 177°.

Comments for all modes:

When drawing lines, three directives should be followed per the original method. First, the separate lines are drawn to the internal border of the sinus outline. Second, the lines are collected in a counterclockwise fashion, starting on the right side of the image. Third, in the event a 3° ray crossed the outline more than once, the outermost intersection is used (see Figure 2.5 and Figure 3.6). All 59 lines should be recorded separately in the ROI Manager. At this point, if any lines need to be adjusted by length or angle, they could be selected in the ROI Manager, and the endpoints adjusted in the image.

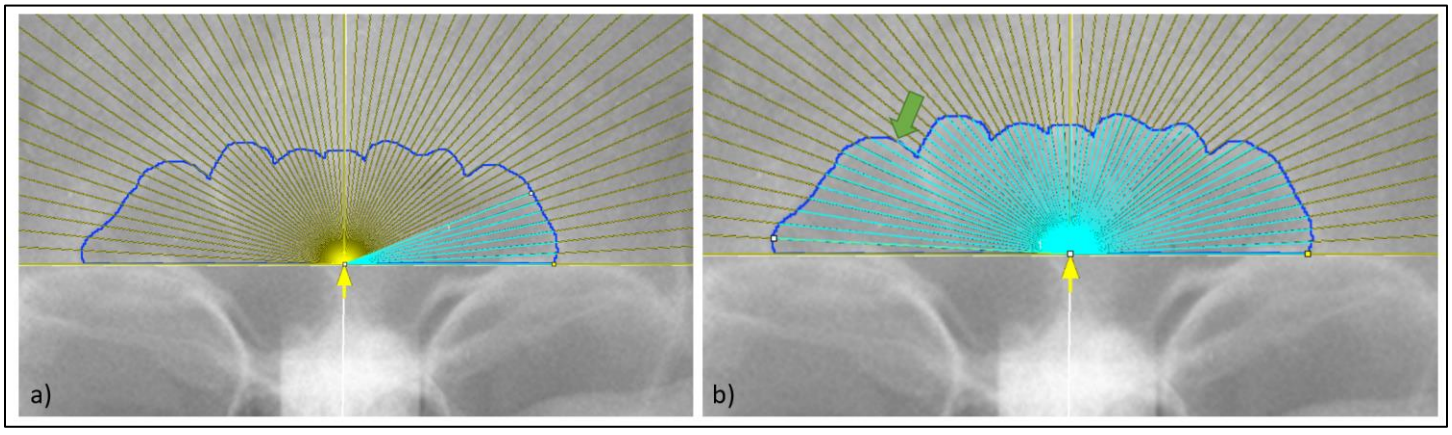

Figure 3.6. Image depicting a) initial lines to b) last line placed. Note green arrow depicting proper placement of line crossing the contour to reach the most outermost (lateral) intersection.

3.10 Record/save your measures. Upon completion of the above steps, select all variables in the ROI Manager (i.e., sinus area/perimeter; the baseline, right-baseline, the 59-lines/angles). Click the "Measure" key in the ROI Manager to measure all the variables at once. A Results box will pop up. Save the results file (in the Results window, go to File > Save as).

3.11 After ensuring measures are recorded and saved correctly, close the image and delete the objects in the ROI Manager before restarting at Step 3.1 for the next image.

3.11.1 Alternative: Open the next image in the folder by ("CTRL+SHIFT+O") and delete the objects in the ROI Manager before restarting at Step 3.2 with the next image.

4. TMD: Semi-Auto Mode

The Semi-Auto Mode procedure includes a semi-automated macro that is managed through ImageJ. Note that it is not completely automated regarding the collection of variables; the user is still required to visually identify landmarks for scaling, place the origin and overlay, as well as draw the baselines and the 59 lines to the appropriate sinus border.

The script walks the user through the steps listed above using pop-up prompts, including the added alignment of the overlay, before generating and saving the data, clearing the workspace, and opening the next image in the assigned folder. Aside from starting the macro and following the prompts, all steps are similar to the Overlay Mode procedures.

The script requires some modifications by the user prior to implementation (see FAQ 5.9), primarily by specifying the names of folders where the radiographs are stored, what the images should be scaled by, and the location to save the .csv output that is generated at the end of every run.

Prior to implementation, download the following files from Github (https://github.com/jcampbelljess/FrontalSinus_TD_macros):

- Overlay.tif (Overlay file)
- RoiStarters.zip (Zipped ROI starter file; do not unzip the file)
- Semi-Auto Macros_TDM**.ijm** (Macro script, written in FIJI using Java)

Before initializing the macro, you will need to edit the location of your folders for pulling and saving data. See FAQ 5.9 for additional instructions.

4.1 Initiate the macro. Once all the files have been downloaded, and your edits to the macro script have been completed (FAQ 5.9), you are ready to run the macro.

4.1.1 In ImageJ, go to File > Open, and choose your macro (the .ijm file). Alternatively, drag and drop the file on the ImageJ console. Edit the input file paths to the image folder and then save folders per the directions included at the head of the macro. Double-check everything is correct, then click "Run", or on the keyboard: "CTRL+R".

4.2 Open the directory of your radiographs. The macro will open a pop-up window, directing you to open the directory (folder) of your radiographs. Navigate to the folder that houses your radiographs with frontal sinuses outlined and click "Select".

4.3 Scale your images. A pop-up window and a line will appear on your radiograph, but the line needs to be adjusted to set your scale. The default in the macro is to scale images based on the left orbital breadth, taken between the landmarks Dacryon and Ectoconchion (see FAQ 5.4 to learn more about adjusting the scale). Following the default, manipulate the line to collect the left orbital breadth (see Figure 4.1). Once positioned, click “okay”.

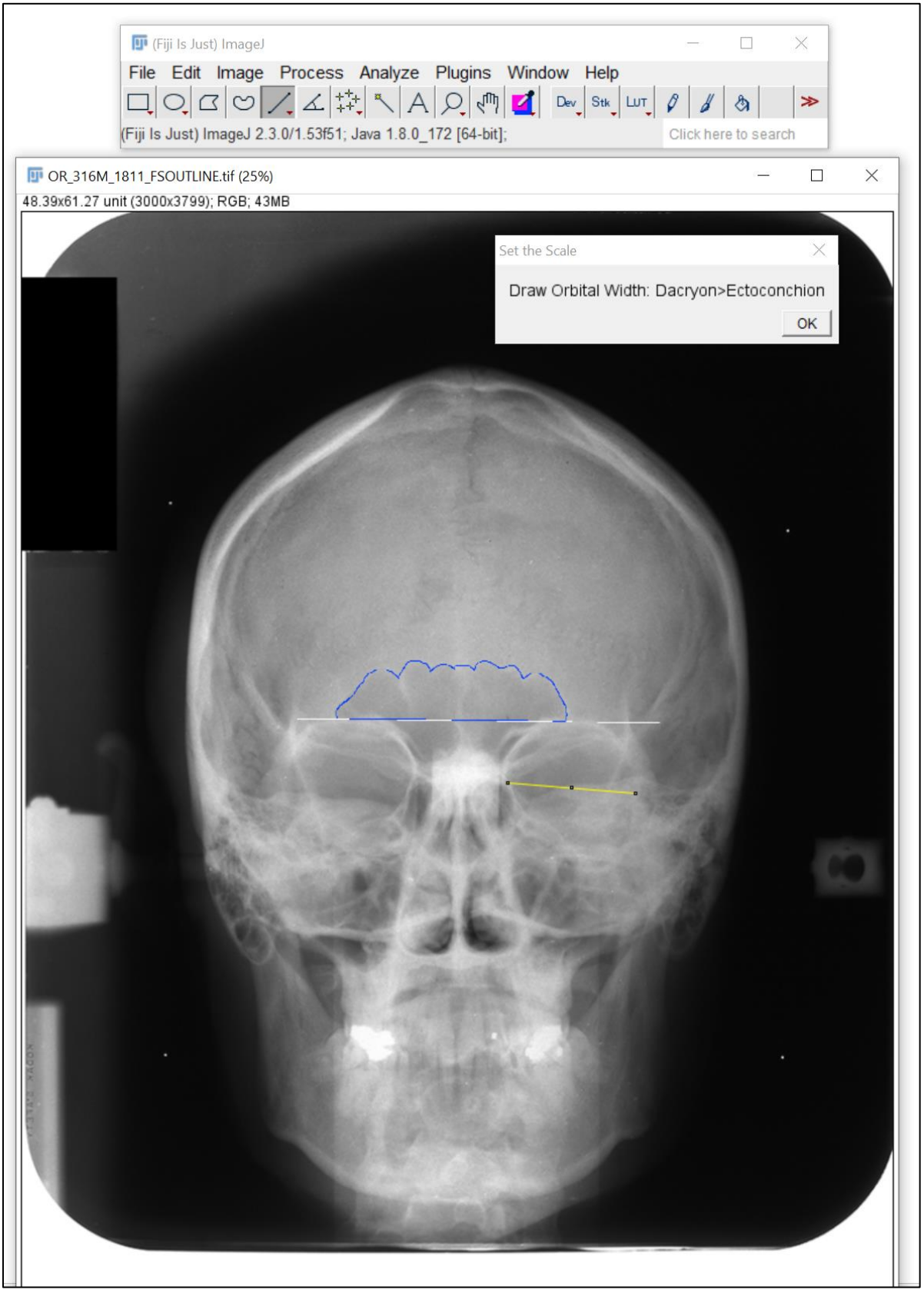

Figure 4.1. Image indicating scaling procedure, with yellow line placed to set left orbit breadth.

4.4 Obtain sinus size. A pop-up window will appear, and your *magic wand tool* will be automatically selected. Highlight the frontal sinus outline. You may need to zoom in closely ("CTRL+Mouse Wheel") to get on the line if it is thin. Click "okay"; the outline will automatically be recorded into the ROI Manager as the Total Sinus item (see Figure 4.2).

Comments:

While this measure (sinus outline area) is not directly needed for the TDM, it provides an overall measure of sinus size (useful in many studies) and can serve as a secondary check that repeated measures of the images are scaled.

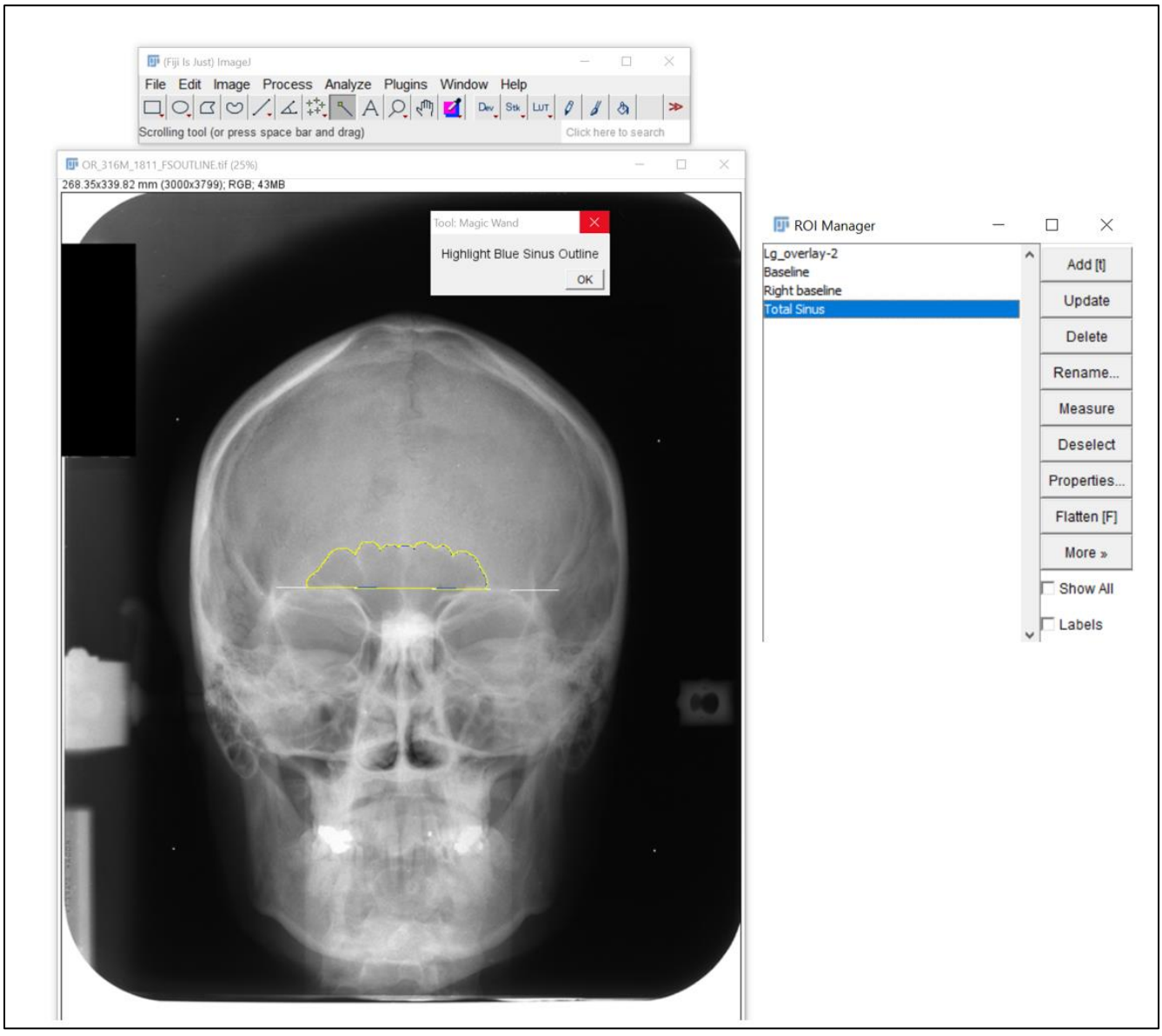

Figure 4.2. Image depicting a highlighted frontal sinus outline. After highlighting with the magic wand tool, and conforming with "okay", the outline will be added to the ROI Manager as the "Total Sinus" item.

4.5 Determine your point of origin. A pop-up window and vertical line will appear on your radiograph. Adjust the vertical line so that it represents the midline of the skull. Following Cox et al. [1], this point should be determined by drawing a vertical line through the skull using as many midline anatomical landmarks as possible (including nasion, anterior nasal spine, prosthion, etc.). Once placed, click "okay" to confirm the line placement. The line should be automatically drawn onto the radiograph. See Figure 4.3.

Comments:

The origin is the location where this vertical midline intersects the supraorbital line, and the origin is the point from which additional variables were collected (e.g., right baseline and the 59-line lengths; see steps below). There is nothing to record at this step, and nothing new will appear in the ROI Manager.

Figure 4.3. Image depicting placement of midline.

4.6 Obtain your total baseline and baseline angle. Several new items will appear on your image (your baseline, right baseline, overlay, and the sinus outline).

4.6.1 A pop-up menu will appear and direct you to set your baseline. This will be the inferior border of the frontal sinus. Following the prompt, select the longer baseline (line labeled "2") and position it along the baseline of the frontal sinus from the left to right terminus (see Figure 4.4). Note, the total baseline should only represent the length of the baseline of the sinus, not the total supraorbital length previously drawn. As you place the line, note the angle of the line listed in the top menu (Figure 4.4, blue arrow). Once placed and angle noted, click "okay."

4.6.2 A secondary pop-up menu will appear. Enter in the previously noted angle of your baseline and press "okay". The image will rotate so the baseline is now level at 180°. This may not be noticeable unless your radiographs were heavily tilted. However, this process is important to ensure proper alignment of the overlay and subsequent line collections.

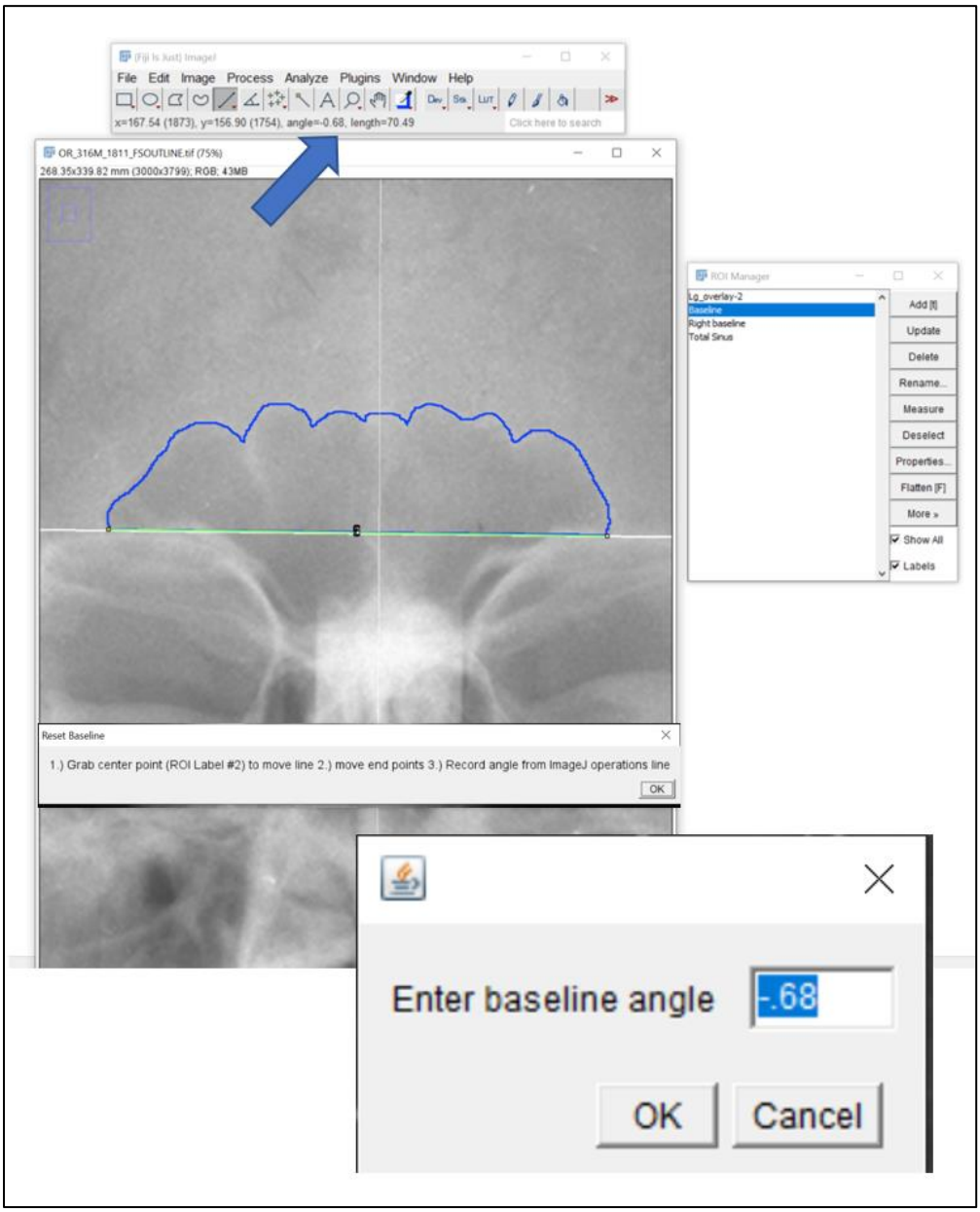

Figure 4.4. Image depicting measurement of baseline, notation of angle (blue arrow), and entry of angle degrees for automatic rotation.

4.7 Obtain the Right Baseline. Per the original method, the right baseline refers to the right side of the radiograph, not the anatomical right side of the cranium, and is measured from the origin to the right terminus of the baseline. A pop-up window will appear, instructing you to select the right baseline (line labeled "3"). Select the right baseline and position it so along the inferior border of the sinus, starting from the origin (midline point) to the edge where the sinus contour intersects the supraorbital line. Once placed, click "okay". Note, there is no angle to record here. See Figure 4.5

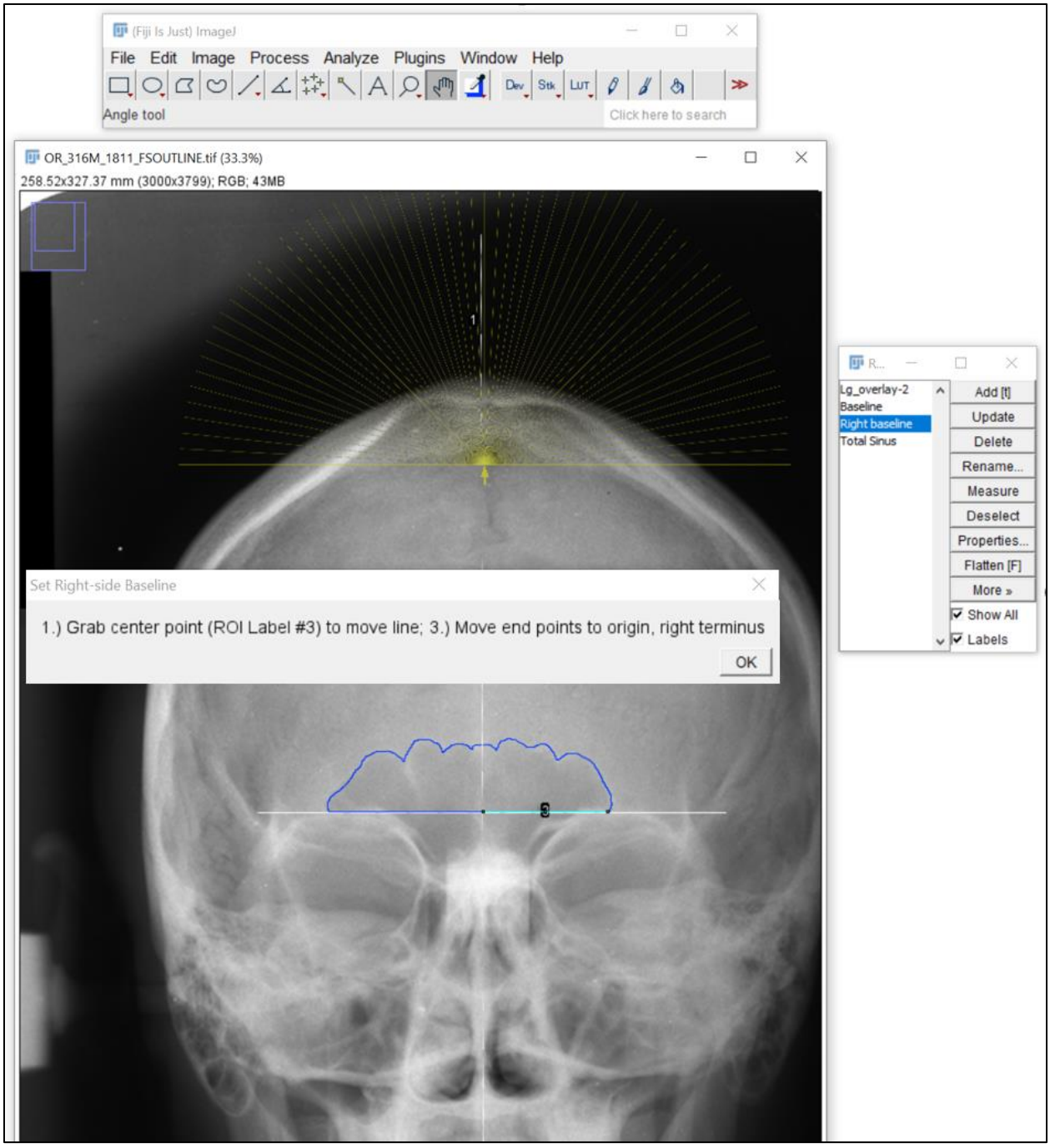

Figure 4.5. Image depicting measurement of right baseline. Per the original method, the right baseline refers to the right side of the radiograph, not the anatomical right side of the cranium.

4.8 Move/Position the Overlay. Once the baselines are collected, a new pop-up window will appear instructing you to position/move the overlay. Select the Overlay and drag it to align it. The yellow arrow should align with the origin (point between the inferior border of sinus and vertical midline), and the base of the overlay should align with the inferior border of the sinus (see Figure 4.6). Once aligned properly, click "okay".

Comments:

To move the overlay, you will need to have it selected (can select the Overlay in the ROI Manager), and you'll need to have the line or wand *tool* selected in ImageJ. You may want to zoom in/out of the image to ensure proper alignment.

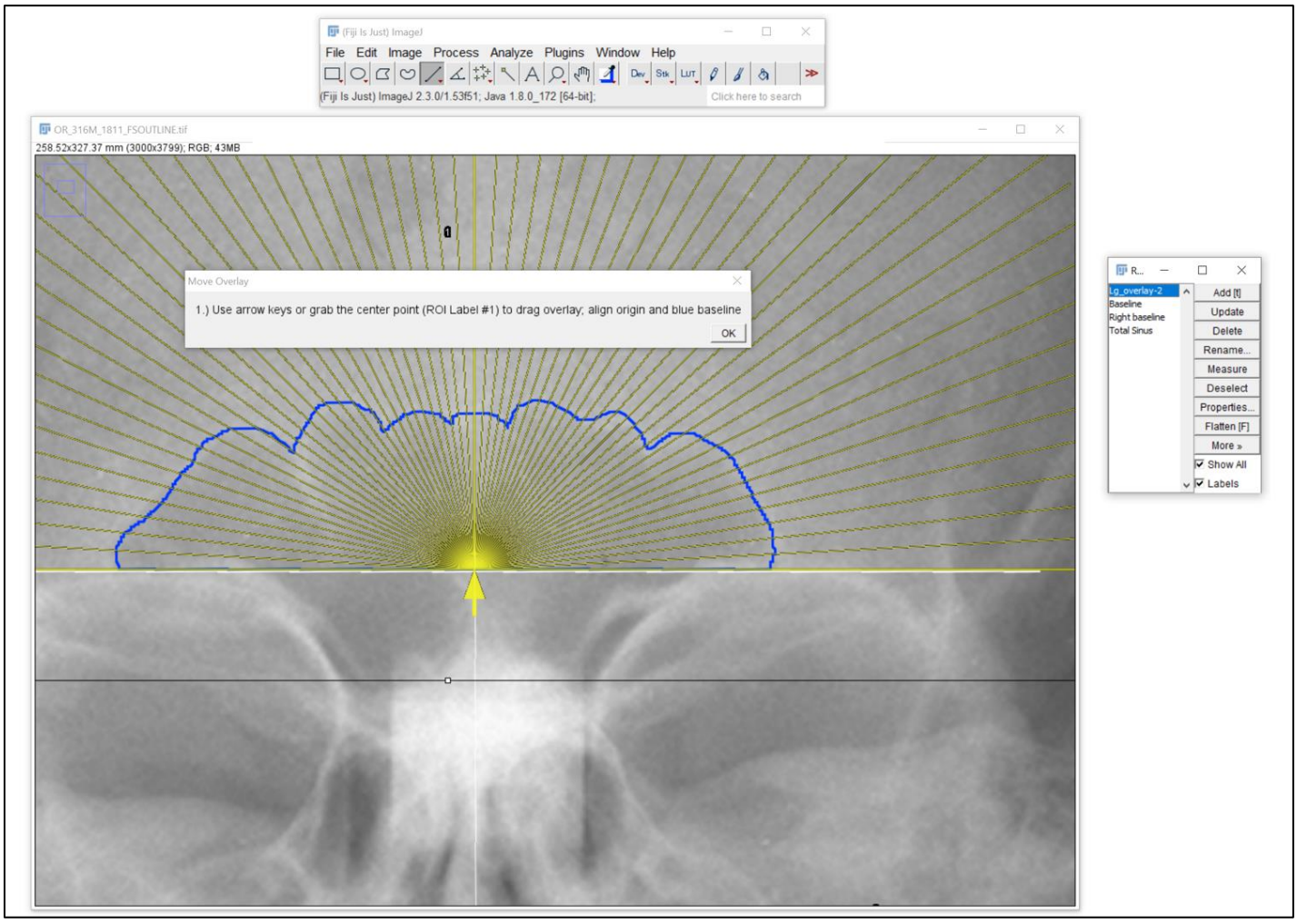

Figure 4.6. Image depicting alignment of the overlay.

4.9 Place the initial 3° Angle. A new pop-up window will appear instructing you to place the initial 3° angle. The *angle tool* should already be selected.

4.9.1 First, click on the radiograph to make sure it is active.

4.9.2 Next, click at the right terminus of the sinus, where it intersects the supraorbital line. The *angle tool* should now be active on the image itself: place the initial end of the angle on the right terminus of the outline baseline and click the left mouse button, then position the other end of the line on the origin and click again to form the fulcrum.

4.9.3 From here, you will extend the line to the first 3° intercept with the sinus outline (see Figure 4.7a). You can check the degree by observing the ImageJ command menu, similar to Step 2.6 provided in the Freehand mode. After placement of the initial angle, click “okay”. The angle should turn a new color (blue), and a new item should appear in the ROI Manager, representing your first line (See Figure 4.7b).

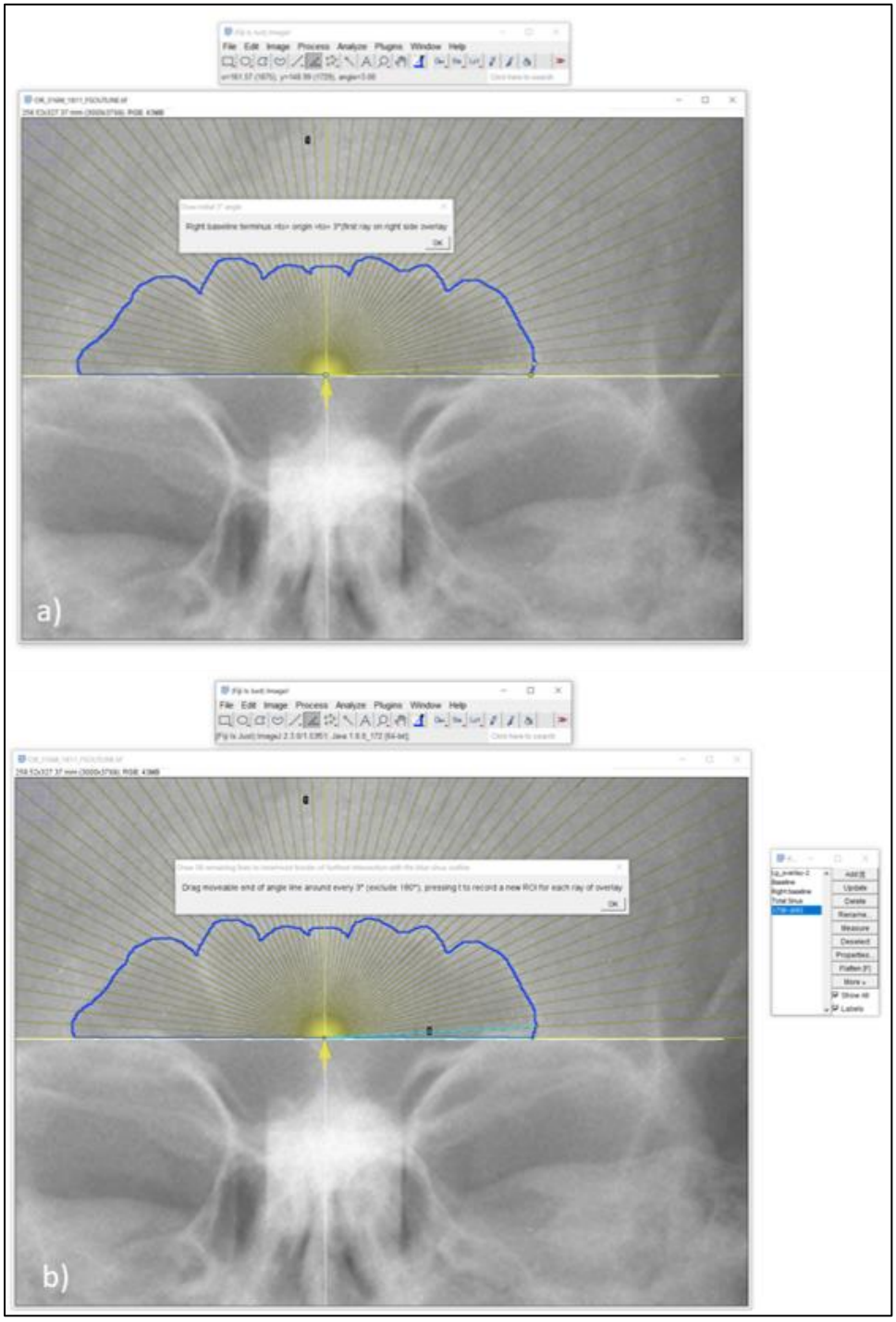

Figure 4.7. Images depicting a) proper placement of the initial 3° angle and b) placement of the initial angle in the ROI Manager.

4.10 Obtain the remaining 59° lines. Next, a new pop-up window will appear instructing you to place the remaining 59° lines (Figure 4.8). IMPORTANT NOTE: you will place all 59 lines before clicking "okay".

4.10.1 Drag the initial line and position it on the next 3° degree indicated by the overlay rays; press "t" to add it to the ROI Manager. Do not click "okay".

4.10.2 Continue this process, hitting "t" on the keyboard after each line to record them to the ROI Manager. Continue until you record the line at 177°; at this final line, press "t" to record it.

4.11 Once all lines are placed, but before clicking “okay,” you can scroll through the lines by selecting the items in the ROI Manager. At this point, you can reposition any lines that may be out of order or mispositioned (see FAQ 5.7. for details).

Comments for all modes:

When drawing lines, three directives should be followed per the original method. First, the separate lines are drawn to the internal border of the sinus outline. Second, the lines are collected in a counterclockwise fashion, starting on the right side of the image. Third, in the event a 3° ray crossed the outline more than once, the outermost intersection is used (see Figure 2.5, and Figure 4.8). All 59 lines should be recorded as separate objects in the ROI Manager.

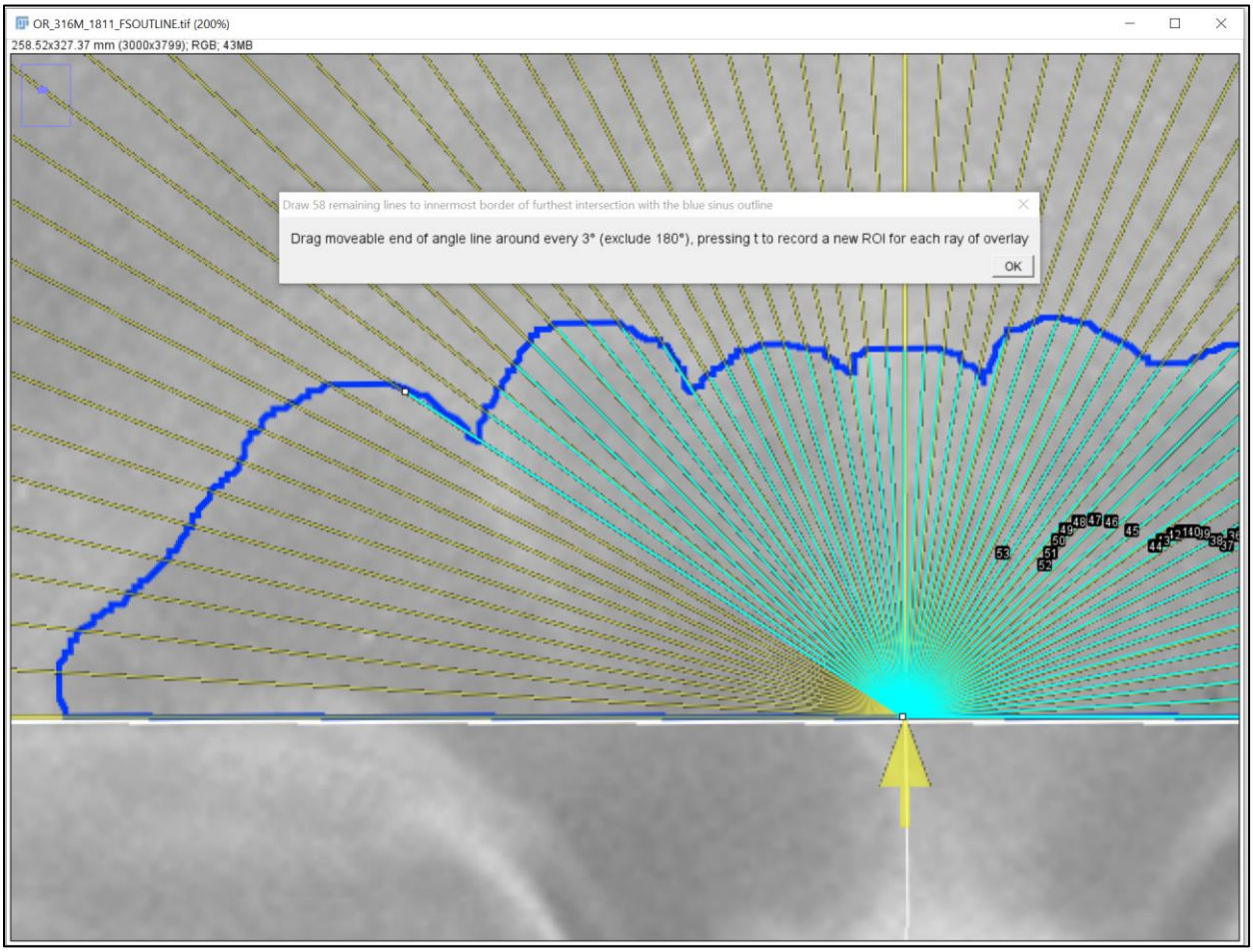

Figure 4.8. Image depicting additional lines, following the overlay and ensuring each line extends to the furthest contour.

4.12 **Record and save measures**. Once all lines are placed, and the “okay” button has been clicked, your data should automatically be saved in the output folder you designated in the macro script (see FAQ 5.9). Additionally, two new pop-up windows will appear. One pop-up window instructs you to check that the data file was saved correctly. The other window will be the ImageJ Results window, which displays the measurements. You can also copy and paste these measurements into another file as a backup, or save the results file itself (In the Results window, go to File > Save as).

4.13 Conclude data export and open the next image. Click "okay" in the action-required pop-up window directing you to check your exported file. This will close all windows, wipe the ROI Manager and Results, and open the next radiograph in the storage folder.

5 FAQs

5.1 What is the Total Difference Method (TDM)? The Total Difference Method quantifies sinus shape using 59 linear measures. The lines originate from the cranial midline identified on a radiograph and are spaced every 3° across a sinus. The TDM refers only to the technical application of the original method. Subsequent steps would quantify the results as a probability of a match or non-matching pair of a single ante- and postmortem image. The "total difference" refers to the sum of each line's absolute difference between an antemortem and postmortem pair of images. The difference would then be used with population frequencies to obtain an odds ratio describing the probability of a match ("same skull") to the probability of a non-match ("different skull"). In theory, in an array of possible matches, the matching frontal sinus should have the lowest Total Difference, with the value approaching zero.

5.2 What is FIJI/ImageJ? The following link will provide you with resources for downloading and learning about FIJI/ImageJ: https://imagej.nih.gov/ij/index.html. ImageJ is a free, public domain software program using Java, available from the NIH for use on Mac, Windows, and Linux systems. FIJI (**F**IJI **I**s **J**ust **I**mageJ) is an updated version with additional pre-loaded packages and capabilities. FIJI is preferable for writing macros and complex image processing, but anything developed in FIJI is fully compatible with ImageJ, and vice versa. FIJI is available here: https://imagej.net/software/fiji/.

5.3 What type of sinuses can be analyzed with the Total Difference Method (TDM)? As defined in their original manuscript [1], TDM can only be conducted on frontal sinuses that are present above the supraorbital line and situated across the midline. TDM will not work on frontal sinuses that are absent or hypo-plastic (falling below the supraorbital line), unilateral, or discontinuous (right and left lobes are separated). See Figure 1.1 for examples.

5.4 How should I scale my radiographs? if you do not have a known scale, you can use an average cranial measure. For Campbell and Butaric ([5], pending), images were scaled using the orbital breadth (OBB) from all groups in Howell's dataset OBB ($\bar{x}$=39.49mm, s=2.024) [6]. For this process, the *line tool* was used to obtain left orbital cavity breadth (from dacryon to ectoconchion) on each individual. If the left orbital cavity was obscured, the measurement was taken on the right. Once placed on the individual, we used "39.49" as the known distance and "mm" as the units. If OBB is difficult to discern in your sample images, other scaling measures, such as bi-orbital breadth can be used.

5.5 I've collected all my angles: How can calculate the line lengths? When using the angle function, ImageJ records the combined lengths of both lines (the Angle Perimeter) that compose the angle: i.e. the fixed arm (the first, horizontal baseline of angle) plus the moveable arm (the second line from origin to sinus outline). To calculate the length of the line from the origin to the sinus outline (as needed in the TDM), the right baseline length, recorded separately, needs to be subtracted from the total perimeter of the angle.

5.6 What is the ROI Manager function, and should I use it? The ROI (Region of Interest) Manager is useful in that it records all additions onto an image or radiograph by pressing the "t" key on your keyboard. The benefit is that you can select, move, and alter anything you add to the ROI Manager after you have moved on or completed collecting data. For the TDM method, this means you can go back and review the placement of your 59 lines, or tweak the position of the overlay to be more exact before you record and export your measurements. You can also save the ROI Manager items from an image so you can come back to it, preloaded, if you have to pause or stop working in the middle of processing an image. We recommend using the ROI Manager for both repeatability and for checking yourself after you complete all the TDM steps. See the official ImageJ tutorial here: https://imagej.nih.gov/ij/docs/menus/analyze.html#manager

5.7 How can I edit objects in the ROI Manager? If you notice a line (or the overlay, baseline, etc.) needs to be edited, you can easily do so in the ROI Manager.

a. In the ROI Manager, make sure the "Show All" and "Labels" boxes are checked. This allows you to see all the lines and placement as you edit and before you measure.
b. To select the item in the ROI Manager list of objects, you can 1) click on the line you want to edit directly in the image/radiograph using the *angle tool (line tool* for baselines), or 2) click to highlight the item in the ROI Manager list. If you don't know which ROI item to select, you can click on any item in the list, and use your arrow keys (up, down) to find the correct line (also highlighted on your image).
c. Make your edits to the line/angle/overlay.
d. In the ROI Manager, click the "Update" button. This saves your work so you can make additional edits, or conclude the analysis.

5.8 What are the numbers in the ROI Manager? Instead of labeling the lines as "line 1, line 2…" for each placed angle, the ROI manager labels them by numbers. These numbers the ROI Manager records for each object are coordinates on the image. You can easily rename them by using the "Rename" button in the ROI Manager. This makes it easier to return to, and for data collection (the name will be exported instead of the provided coordinates).

5.9 In the Semi-Auto Mode, how do I edit the macro? Prior to collecting data in the Semi-Auto mode, you will need to download the appropriate files on GitHub and make a few edits to the macro script. If you haven't already, download the following files (https://github.com/jcampbelljess/FrontalSinus_TD_macros):

- Overlay.tif (Overlay file)
- RoiStarters.zip (Zipped ROI starter file; do not unzip the file)
- Semi-Auto Macros_TDM**.ijm** (Macro script, written in FIJI using Java)

a. You'll need to open the Macro script file (Semi-Auto Macros_TDM.ijm) in ImageJ by going to File > Open > Semi-Auto Macro_TDM.ijm.
b. *Set up where you will be getting your images:* Go to line 24 (Figure 5.1a) and change your directory for where you will pull your radiographs to analyze. Note that these radiographs should already be sorted (all TDM appropriate) and already have the supraorbital line and frontal sinuses outlined (see section 1 of this manual).
c. *Set up where you saved your ROI Manager items:* Go to line 36 (Figure 5.1b) and enter in the directory path to the ROIStarter.zip file (do not unzip the files).
d. *Set up where you will be saving the results file (all of the raw data):* Go to line 116 (Figure 5.1c) and enter the directory you want your results to be saved in. You can leave the +title+ as is (this tells the macro to save the file as the title of the image you opened, so you know where the data came from). You can leave

or edit the "_compare.csv" portion to change what the file name will be saved as, and the file extension you want. We used .csv as a standard tab-delimited file format.

a)

```
21 //After you make these changes, click "RUN" or "CTRL+R" to start macro; do not pre-open imag
22
23 //enter path to the folder where radiograph outline images are stored, in quotations and usi
24 input_path = getDirectory("C:/Users/your path/folder with images");
25 //in pop-up click to select folder with images
26
```

b)

```
31     //clean to prepare for next image
32     roiManager("reset");
33     //roi starters can be downloaded from GitHub https://github.com/jcampbelljess/FrontalSinus_
34
35     //enter path to the .zip file with stored roi's
36     roiManager("open", "C:/Users/your path/folder with ROI starters/RoiStarters.zip");
37     run("Close All");
38     run("Clear Results");
```

c)

```
114
115    //Save results
116    saveAs("results", "C:/Users/input path/Results folder/" + title + "_compare.csv");
117    waitForUser("Check save file; if compromised, select all results in results window and co
```

Figure 5.1. Image depicting lines in the macro script to change for specifying each directory for a) loading images, b) loading the ROI manager items; and c) saving results.

6 References

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2. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods*. 2012;9(7):676-682. doi:10.1038/nmeth.2019

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6. Howells WW. *Cranial Variation in Man: A Study by Multivariate Analysis of Patterns of Difference among Recent Human Populations*. Peabody Museum of American Archaeology and Ethnology, Harvard University; 1973.