

Mouse Analysis User Guide

ABSTRACT

The principle objective of the Mouse Analysis software was to determine whether a computer would be capable of differentiating species of *Peromyscus* mice morphologically. Although genetic analysis may serve as the most accurate species-identification technique, it remains costly and time consuming. Computer analysis allows additional rigor in morphological identification, so the Mouse Analysis software was developed in Java to distinguish these species. The user can import a photo of a *Peromyscus* mouse, with the full body and tail shown, then run the 4-point analysis of fur color, tail to body ratio, ear to body ratio, and dorsal-ventral tail gradient. Logistic regression is applied to these traits to achieve a species classification. Using a data set of 48 species-identified mice, this program accurately classifies 95% of *P. leucopus* and 90% of *P. maniculatus* photos. These results are promising because they have lowered the implicit bias in morphological identification, and allow for faster and cheaper identification.

Peromyscus Mouse Identification Software

Developed by Margo Morton at Wittenberg University

For use with Excel on Windows, Mac OSX, or Linux

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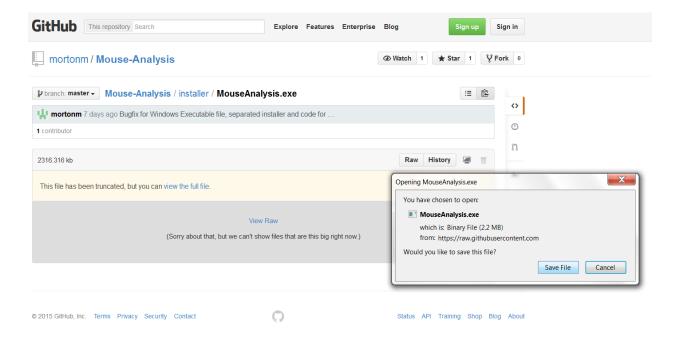
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Getting Started: Installing the Software

Installing on Windows Computers

This method is suitable for installing the software on all Windows platforms (98/Me/NT/2K/XP/Vista/7/8).

To install the Mouse Analysis software, visit https://github.com/mortonm and navigate to the Mouse-Analysis repository. Two directories are inside of this repository: "code" and "installer". Navigate to the "installer" directory and click on the file called "MouseAnalysis.exe". Click the "Raw" tab or the "View Raw" link. This will automatically open a popup window asking if you would like to save this file. Click "Save File", then choose the directory where you would like to save the file. Congratulations! You have successfully installed this software.



Installing on Linux or Mac OSX

Linux and OSX users will need to download the JAR file in order to run this software.

To install the Mouse Analysis software, visit https://github.com/mortonm and navigate to the Mouse-Analysis repository. Two directories are inside of this repository: "code" and "installer". Navigate to the "code" directory and click on the file called "MouseAnalysis.jar". Click the "Raw" tab or the text that says "View Raw". This will automatically open a popup window asking if you would like to save this file. Click "Save File", then choose the directory where you would like to save the file. Congratulations! You have successfully installed this software.

Getting Started: Running the Software

To begin, ensure that you have the latest version of Java installed on your computer. Java updates can be installed from https://www.java.com/.

Running MouseAnalysis.exe on Windows Computers

To run the Mouse Analysis software, navigate to the directory where MouseAnalysis.exe has been installed. Double click the file to start the program. If you see a message with a security warning, such as the one shown below, click "Run". If you are running Windows 8, select "More Info" and "Run Anyways". Windows will save your preference for this software and won't ask you again.



Using Command Line on Windows

If you want to run this software from the command line in Windows, download the JAR file as detailed in the "Installing on Linux or Mac OSX" section. Open the Start Menu, and enter the text "Command Prompt" in the search field. Double click the icon to launch Command

Prompt. Navigate to the directory where you installed the JAR file by using the command "cd" followed by the directory path. Then type the following command:

java -jar MouseAnalysis.jar

Using Command Line on or Mac OSX

Navigate to the Finder in the dock bar. Select "Applications", then "Utilities". Double click on "Terminal" to launch the command line. Navigate to the directory where you installed the JAR file by using the command "cd" followed by the directory path. Then type the following command:

java -jar MouseAnalysis.jar

Using Command Line on Linux

Launch a terminal from your desktop's application menu. Navigate to the directory where you installed the JAR file by using the command "cd" followed by the directory path. Then type the following command:

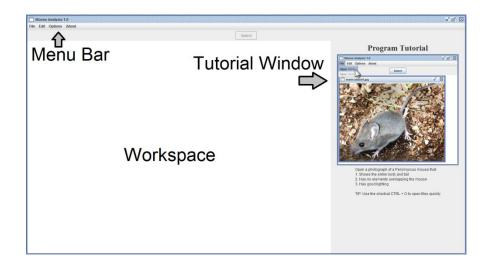
java -jar MouseAnalysis.jar

Walkthrough of Mouse Analysis

After launching the software, you should see this screen:



The three main parts to this program are the Menu Bar, the Workspace, and the Tutorial Window. The Menu Bar is located along the top of the screen, and includes options such as "File" and "Edit". The Workspace is the large blank area where you can open and analyze images. The Tutorial Window is located on the right side of the screen and helps guide you through the steps of Mouse Analysis.



Opening a File

To begin, go to "File" then "Open" or hold down CTRL+O. This will launch a file chooser dialog box. Select an image of a Peromyscus mouse that adheres to the following criteria:

- 1. The image must have good lighting. If the image is too dim or overexposed, use an image editing program such as Photoshop or Gimp to adjust the levels.
- 2. The image must show the entire mouse, including the ears, body, and tail.
- 3. The image file type should be JPG, BMP, PNG or GIF format.

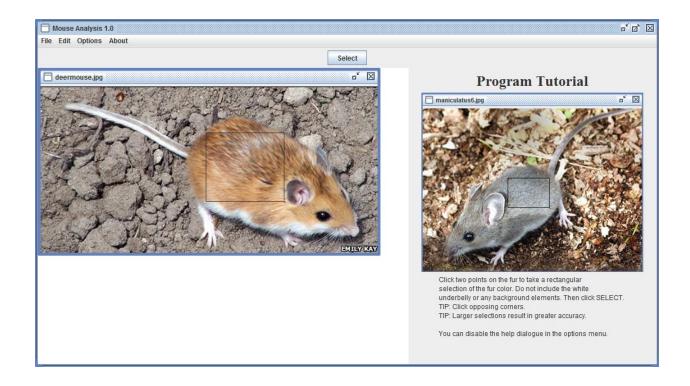
The image will now be displayed in the Workspace. If the image is too large to completely fit in the workspace, you can zoom out by going to "Edit" then "Zoom -".

Fur Color Analysis

The first step of the analysis is to select a section of the mouse's fur color. This is done by clicking two points, which will become the opposing corners of a rectangular selection. The points will be highlighted in red, and the rectangular selection will be drawn in black. To attain the highest accuracy in fur color analysis, ensure that the boundary of the rectangle:

- 1. Does not include the white underbelly or background elements.
- 2. Covers a large area of the fur.

If you are unhappy with your selection, either click two more points to reset the rectangle, or click "Edit" then "Undo" to clear the screen and try again. The following is an example of an excellent fur selection:

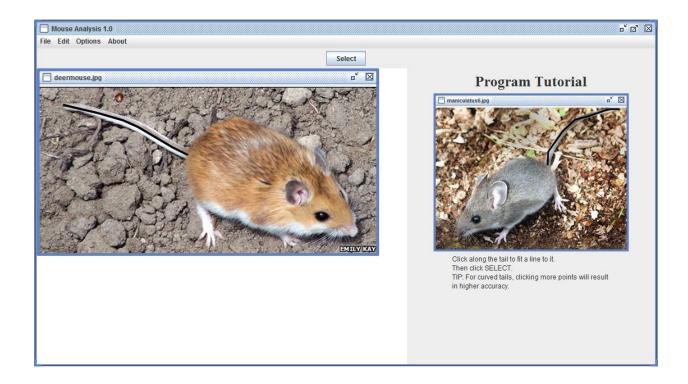


Once you are pleased with the placement of the rectangular selection, click the "Select" button at the top center of the window.

Tail Length Analysis

The next step is to capture the length of the tail. This is achieved by placing a line segment along the length of the tail. Click along the tail to draw a line from the base to the tip. If the tail is curved, multiple clicks may be necessary to capture the shape properly.

The following illustrates an excellent tail selection. 5 clicks were made along the length of the tail, and the result is very smooth. If you are unhappy with your tail line, click "Edit" then "Undo" to clear the line and start over.

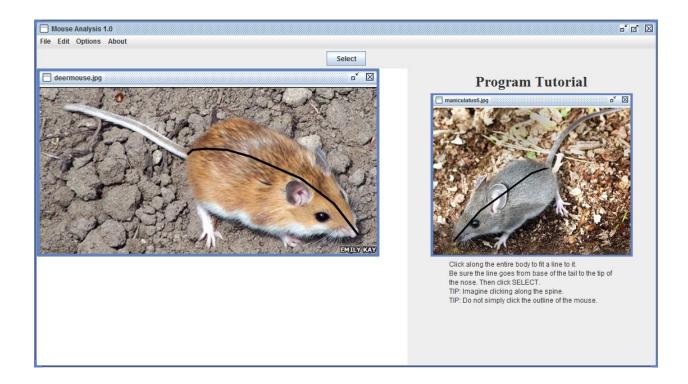


Once you are happy with the placement of the tail line, click "Select".

Body Length Analysis

The next step is to capture the length of the mouse's body. This is done by drawing a line segment across the entire body of the mouse. The body line segment should extend from the tip of the mouse's nose all the way to the base of the tail. Click along the spine of the mouse in order to capture its true, uncurled length. Clicking too few points, or simply drawing a line across the perimeter of the mouse will result in lower accuracy. If you are unhappy with your body line, you can click "Edit" then "Undo" to clear the screen and try again.

The following demonstrates the proper way to fit a line along a mouse's body. On average, 6 to 7 clicks are necessary to capture the mouse's body length.



Once you are happy with your body line, click "Select".

Tail Gradient Analysis

If the mouse's tail is too small to see fine detail, go to "Edit" then "Zoom +" to zoom into the image. The next step of analysis will capture the gradation of color in the tail. Click two points, one on the dorsal and one on the ventral side of the tail. The points will appear in red for greater visibility. Ensure that your points adhere to the following criteria:

- 1. The points must be completely on the tail, and not on the edge between the tail and background.
- 2. If you can observe two different colors on the tail, position one point on each color.

If you are unhappy with your point selection, you can click two new points, or you can choose "Edit" then "Undo" to clear the screen and try again. The following image demonstrates a good selection of tail points.

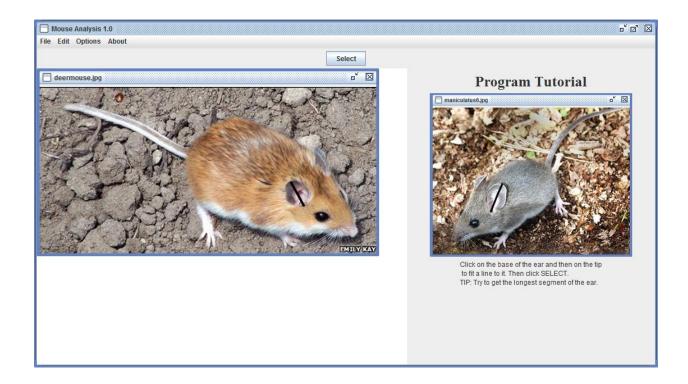


Once you are satisfied with the position of the tail points, click "Select".

Ear Length Analysis

The final step of analysis is to capture the length of the mouse's ears. Use the ear that is closest in perspective in the photograph. Click from the base of the ear to the tip. This will create a line segment in a similar manner to the tail and body lines. Typically, only two clicks are necessary to capture the full length of the ear.

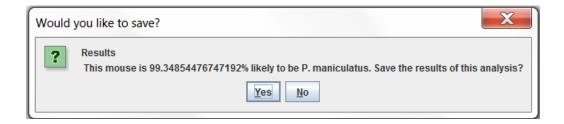
If you are unhappy with the placement of the ear line segment, click "Edit" then "Undo" to clear the screen. The following illustrates excellent placement of an ear line segment.



Once you are satisfied with the placement of the ear line segment, click "Select".

Saving Your Results

At the end of the analysis, a small popup will appear with the results. The results will include the species and the precision of the calculation. The popup will prompt you whether you would like to save the results.



If you are unhappy with the results of the analysis, click "No", then close the image in the workspace. If you would like to save the results, click "Yes". A file dialog window will open, prompting you to save your data in a file. When selecting a file, ensure that the directory in the

file chooser dialog is where you intend to save the file. Then either select a pre-existing Excel file with Mouse Analysis results, or type a name to create a new file. Click "Save" to write to the selected file.

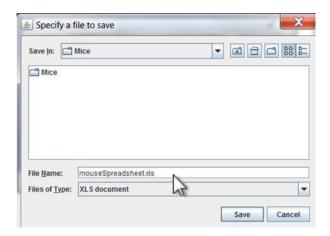
After you have saved the results, you can close the mouse image in the workspace, and continue analyzing additional mice!

Program Menu Options

Open	Opens an image to analyze. Supported file types are JPG, PNG, GIF, and BMP Multiple images can be open in the workspace at once, but only one is analyzed at a time. Use the keyboard shortcut CTRL+O to quickly open files.
Save	When analysis of an image is complete, data can be saved in an Excel file. Supported file types are XLS and XLSX Use the keyboard shortcut CTRL+S to quickly save.
Undo	Clears the last element drawn on the screen. Undo will not affect previous analysis steps.
Redo	Restores the last element removed from the screen.
Zoom +	Zooms into the currently selected mouse image.
Zoom -	Zooms out of the currently selected mouse image.
Display Tutorial	A checkbox that toggles the display of the Program Tutorial. If unchecked, hints and results will not be displayed on screen.
About Program	Displays useful information about the program, including the author, version, and license.

Program Output

The results of Mouse Analysis can be saved to an Excel file. If you select a preexisting Excel file, the new mouse data will be appended so that no data is overwritten. Pictured below is an example of creating a new Excel file in a directory. It is optional to include a file extension.



Two rows are written to the Excel file, a header and the results. The header row will remain unchanged even as new results are appended to the file. Ten columns of data are written to the file representing the results of each analysis:

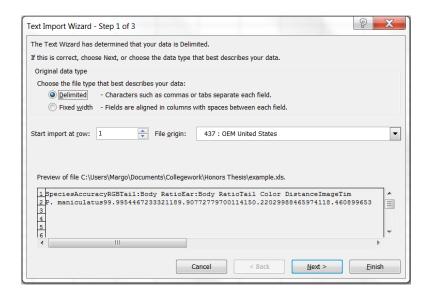
Species of	Accuracy	Red	Green	Blue	Tail:Body	Ear:Body	Tail	Filename	Timestamp
Peromyscus	of	value	value	value	ratio	ratio	Gradient	of the	
	calculation	in fur	in fur	in fur			amount	image	

The following represents an Excel file produced after analyzing 4 images:

	Α	В	С	D	E	F	G	Н	1	J
1	Species	Accuracy	R	G	В	Tail:Body Ratio	Ear:Body Ratio	Tail Color Distance	Image	Timestamp
2	P. leucopus	50.4301	178.4138	137.8422	98.94287	0.680514442	0.091950413	77.58865897	leucopus2.jpg	3/27/2015 12:55
3	P. maniculatus	100	192.9787	153.2168	118.5847	0.666171522	0.1314433	301.4995854	maniculatus5.jpg	3/27/2015 12:56
4	P. leucopus	58.543	167.3942	130.5273	86.2824	0.924081497	0.165108502	95.46203434	leucopus10.jpg	3/27/2015 12:57
5	P. maniculatus	78.2001	109.6751	98.79263	82.18541	0.696707612	0.175025587	101.0593885	maniculatus11.jpg	3/27/2015 12:57

Opening an Excel File

Mouse Analysis writes Excel files through Tab delimited text. Due to this, you will see a Text Import Wizard the first time you open any Excel file written by Mouse Analysis. All of the default settings are correct, so simply click "Finish" to view your file.



Opening the same file subsequent times will lead to the following message from Excel.

Click "Yes" in order to view the file.

