ABSTRACT

Using ComputeR Assisted Detection of LEukocoria (CRADLE)

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The red reflex test is an outdated and inaccurate method for determining ocular abnormalities in children. A possible solution to this problem is utilizing modern technology through the usage of Computer Assisted Detection of Leukocoria. This program requires less training, has a lower cost, and has a higher sample size when in direct comparison with the red reflex test. The advantages and disadvantages of using Computer Assisted Detection of Leukocoria to detect ocular abnormality in children is further explored.

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USING COMPUTER ASSISTED DETECTION OF LEUKOCORIA

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CHAPTER ONE

Literature Review

Retinoblastoma is a rare childhood cancer that accounts for two percent of all childhood cancers.[1] It forms rapidly in the immature cells of the retina and usually appears before the age of five years.[2] Left untreated, retinoblastoma has the capacity to kill the affected individual by metastasizing up the optic nerve and into the brain.[3] In developed countries, the survival rate is high at around ninety five percent.[4] In developing countries or areas where resources are limited, the survival rate drops to around forty percent.[5] The overall survival rates globally are increasing due to advances in technology and enucleation techniques.

The most common form of treatment for advanced unilateral (single eye affected) retinoblastoma is enucleation of the affected eye.[6] Enucleation of the eye is when the eye containing retinoblastoma is removed while leaving the eye muscles and remaining orbital contents intact. In patients with bilateral (both eyes affected) retinoblastoma, the eye with the more progressive retinoblastoma is enucleated and the remaining eye undergoes aggressive treatment. Treatment modalities can involve external beam radiation, brachytherapy, photocoagulation, and cryotherapy.[7] Survivors of retinoblastoma can be expected to have moderate to severe vision loss, but early diagnosis can help increase the rate of vision preservation and survival.

Detecting retinoblastoma in children continues to be a problem globally. The normal way for detection of this cancer is for the pediatrician to perform the "red reflex test" on the patient with an ophthalmoscope.[8] Despite this being the way that providers look for retinoblastoma in patients, the most effective method for detecting retinoblastoma appears to be amateur photography and parental concern over recurrent abnormal photographs of their child.[9] One of the most common presentations of retinoblastoma in children is leukocoria.

Leukocoria is an abnormal, white reflection from the retina of the eye. It has historically had a high correlation with presence of retinoblastoma that was detected as a binary readout by the physician. This meant that the physician would either respond with the patient either being positive or negative for the presence of leukocoria in the eye. Amateur photography has been ignored as a quantitative tool for leukocoria detection because it involves untrained users operating nonuniform devices in multiple, diverse settings.[10] Using ComputeR Assisted Detection of LEukocoria (CRADLE), the provider can receive more information on their patient's leukocoria than previously before.

The most common form of leukocoria detection is not by the primary care doctor, but by the patient's parents.[9] More specifically, the patient's parent(s) detect leukocoria in their child upon closer inspection of a photograph of their child. This initial point of leukocoria detection is correlated highly with patient survival rates.[9] Before the development of ComputeR Assisted Detection of Leukocoria (CRADLE), there were no available tools for screening the presence of leukocoria in photographs. This is due to the colorimetric analysis that is possible when viewing a photograph.

To analyze each photograph, each pupil must first be cropped to determine total pixel count. Next, the RGB (Red, green, blue) values were determined for each pixel and pupil. Finally, the RGB values were converted to HSV (Hue, saturation, value) because HSV was simpler to use.[10] This process transformed the pictures from a binary readout of leukocoria to a quantitative analysis of leukocoria progression. The quantity of Saturation and Value in HSV can be used to approximate the degree of leukocoria, which could play a role in retinoblastoma disease progression.[10]

Amateur photography has been overlooked as an analysis tool because of the perceived random nature of the photographs.[10] The photographers are not formally trained in the red reflex test or in the abnormal presentation of leukocoria. Yet the fact that parents are more likely to detect leukocoria than the doctor is proof that amateur photography is a better diagnostic tool for detecting leukocoria. It is also important to note that the photographs of infants and toddlers are not optically random.[10] That is, the parent will prefer to take the same type of picture of their child in a repetitious pattern. For example, the parent may be more inclined to take a close-up and top-down picture of their child rather than a far-away bottom-up picture. This repetitious pattern of picture taking can be helpful in making the diagnosis of the presence of leukocoria versus that of "pseudo-leukocoria".

An important problem presented when analyzing photos when the flash was on is the occurrence of "pseudo-leukocoria".[10] This is the abnormal appearance of leukocoria in a patient that is otherwise healthy and does not have any leukocoria. There are multiple sources of possible error that could be causing this increase in pseudo-leukocoria including: post-processing errors, type of light source used when the flash was on, and

how close the light source is to the lens capturing the image.[10] The pseudo-leukocoria is most likely caused by light being reflected off of the optic nerve rather than a tumor which would be the cause of actual leukocoria. This presents an important problem because the program itself cannot determine whether the leukocoria detected in a photograph is legitimate in its presentation or artificially caused by reflection of light off the optic nerve.

A possible solution to this problem is to look at multiple photographs of the same child with similar focal lengths, angles, and lighting conditions. They should be compared to see if the leukocoria is persistent or sporadic in its presentation. If it is persistent then it is most likely an absolute presentation of leukocoria and the child should be taken to a healthcare provider immediately. However, if the presentation is sporadic, then the leukocoria may or may not be present and further analysis is required. To conduct a more in-depth analysis, locate all photographs of the child and run them through the program. This is because when more photographs are used, the sample size increases which makes the standard deviation decrease and the results more closely approximate the mean. For example, in a child with N=1000 pictures and there are two photos exhibiting leukocoria, it is unlikely that the child has leukocoria and the two photos can most likely be attributed to pseudo-leukocoria. However, in a child with N=25 pictures and there are two photos exhibiting leukocoria, it is likely that the child has leukocoria and should be taken to a healthcare provider immediately.

CHAPTER TWO

Red Reflex Test

The red reflex test is a test performed by healthcare practitioners. It is an essential part of screening for infants and children.[11] This is because of its role in detection of potentially life-endangering abnormalities of the eye including: cataracts, glaucoma, retinoblastoma, and high refractive error. It is called the red reflex test because of red-orange light being reflected out of the back of a healthy eye.

The red reflex test is performed by shining light with an ophthalmoscope through all the parts of the patient's eye. This light travels through the cornea, aqueous humor, lens, and vitreous humor of the eye to hit the ocular fundus which contains the retina. The light, once reflected, is transmitted back to the ophthalmoscope and can be seen by the healthcare practitioner.[12] This light is transmitted back as red because it hits the vasculature associated with the ocular fundus when it reflects. Anything that disrupts the light's reflection back to the ophthalmoscope will result in an abnormal red reflex test. There are a variety of factors that can cause an abnormal red reflex test ranging from tumors that are benign or tumors that are malignant. The process of performing a red reflex test is standardized among all pediatric doctors in the United States.[11]

The process of performing a red reflex test is to set the lens power of the device to zero and simultaneously hold the tool about eighteen inches away from the patient.[11] A

normal red reflex test will give a red reflection from both eyes that is symmetric in its character. An abnormal red reflex test will be anything that deviates from the normal. In the event of an abnormal red reflex test, the pediatrician will refer the child to an ophthalmologist for further work up. Although this test is widely accepted as the gold standard for infant and young child vision screening, there are some significant limitations.

The red reflex test was invented in 1856 with the publication of Helmholtz's *Handbook of Physiological Optics*.[13] This means that this test has been in use for over 160 years. Over that span of time, there has been a significant amount of technological innovation. This test has not been adjusted or modified in any way despite all of humanity's technological advancement. One could say that this test has stood the test of time due to its effectiveness, ease of procedure, and low cost. However, this test is outdated due to its low specificity and low sensitivity of detection in posterior abnormalities of the eye,[14] lack of efficacy in dilated screening,[15] low sample size, and high variability in its usage.[16, 17, 18]

One of the main problems with the red reflex test today is its low sensitivity and low specificity towards posterior abnormalities of the eye.[14] Low sensitivity of detection means that there were some 'positive' red reflex tests when there was no ocular abnormality (i.e. the ophthalmologist did detect something strange on the red reflex test when in fact that child had no abnormality present). Low specificity of detection means that there were some 'negative' red reflex tests when there was an ocular abnormality of some kind (i.e. the ophthalmologist did not detect anything strange on the red reflex test when in fact that child did have an abnormality present). In other words, the red reflex

test is not effective at detecting 'true-negatives' (the ophthalmologist does not see anything on the red reflex test and the child does not possess any ocular abnormalities) or 'true-positives' (the ophthalmologist detects something on the red reflex test and the child does possess an ocular abnormality) which means that it is not an effective tool for detecting abnormalities in the posterior portion of the eye. This is a significant problem because the vast majority of ocular abnormalities occur in the posterior portion of the eye.

The methodology of the study[14] is straightforward. An experienced ophthalmologist sees the patient and determines either a positive or negative red reflex test. After this decision has been made, a slit-lamp and RetCam machine were used to more thoroughly examine the anterior portion of the eyes, and posterior images of the eye were also obtained. 7641 newborns were analyzed using the above method and 2178 of these had a positive comprehensive eye examination (28.5%). Among the 2178 positive exams, 223 had anterior or simultaneous anterior and posterior abnormalities and 1955 had only posterior abnormalities. Of the 223 anterior abnormalities, the red reflex test was able to detect 222 of them leading to a 99.6% detection rate. However, of the 1955 posterior abnormalities, the red reflex test was only able to identify 81 of them leading to a paltry 4.1% detection rate. Of the 2178 positive eye exams, only 303 of them were able to be correctly identified for a detection rate of 13.9%. Based on this data, the red reflex test in newborns is not an effective screening tool for posterior retinal abnormalities such as retinoblastoma, retinal hemorrhage, or retinal detachment.

Another problem with the red reflex test is its lack of efficacy in dilated screening.[15] In the study, there were patients with retinoblastoma interspersed with

patients that did not have retinoblastoma. The ophthalmologists were tasked with identifying which patients had retinoblastoma and in which eye using the red reflex test. Of the 13 eyes that had retinoblastoma, 0 of them were correctly identified. All of them were classified as a normal red reflex test. There were 3 eyes that were identified as having an abnormal reflex test, and they were all misidentified because those children did not have retinoblastoma. In addition, these patients had undergone pupil dilation before the exam which theoretically should have made detection of the retinoblastoma easier than in a normal clinical setting.

This high degree of false-negatives as indicated by the study above is unsettling. False-negative is defined as when the provider rules the patient as having a negative red reflex test even when they have an abnormal retinal presentation. Even with dilated pupils, the ophthalmologists in the study had a 0% detection rate of retinoblastoma. In the study, the researchers posit that even though the pupils are dilated, the actual surface area of the retina that light touches and reflects off is very small in comparison to the whole retina. This would cause the light to not reflect off of every portion of the retina and thus be less effective at detecting abnormalities throughout the entire retina.

The final problem with the red reflex test is the high variability in its usage among medical professionals, high variability in the medical training of its specialists, and its low sample size. The execution of the red reflex test across the areas of ophthalmology and pediatrics is variable. This is due to the different levels of training that each medical professional receives.[19] More specifically, there is important variation in the implementation of current recommendations and modes of detection for ocular disorders in infancy. This is significant because adequate training is necessary to use the red reflex

test effectively. If a physician is not well trained in using the red reflex test, the amount of false-negatives increases which means that some ocular defects will not be detected until much later in the disease progression state of that patient. In fact, a third of pediatric trainees (32 out of 96) reported receiving no formal training in the ophthalmological examination of infants. In addition, 71% (248) of the pediatricians who responded reported that they would benefit from further red reflex training by an ophthalmologist.[19] Thus, pediatric patients stand to benefit from a standardization of the red reflex training process.

Variability in the usage of the red reflex test is also a problem in the healthcare field.[17,18] The lack of standardization of education of our physicians has caused the red reflex test to also be variable in its delivery to patients. In one study[17], it was found that the red reflex test was only performed 89% of the time in a sample of 329 children. There is no excuse that this low cost and short procedure should not be performed every time even if it does have low specificity and low sensitivity. In one retrospective study over a 10 year period, all cases of congenital cataract were examined. It was found that of all 27 cases, 17 (63%) of them were reported by a parent or caretaker due to concerns about their child's ability to see.[17] The data show that untrained parents are doing a better job at screening for their child's eye health than trained medical professionals. This is a significant problem. However, there is a modern solution to this problem of outdated eye screening in the form of ComputeR Assisted Detection of Leukocoria (CRADLE).

CHAPTER THREE

CRADLE Detection

The current median diagnosis age of retinoblastoma in the United States is at around 24 months for unilateral retinoblastoma and 10-12 months for bilateral retinoblastoma.[20] If this number can be decreased, the amount of ocular salvage can be increased. The reason this number is this high is because of delayed diagnosis by physicians using the red reflex test. The physician either will not have a positive red reflex test or the symptoms in the child will not be large enough to cause the red reflex test to be positive. The solution for this is ComputeR Assisted Detection of Leukocoria (CRADLE). With the constant monitoring of the child's eye health that this technology allows, the diagnosis age of this disease can be dramatically reduced.

CRADLE excels over the red reflex test because the photos it obtains are already within the parent's/patient's cellular device. Thus, the parent/patient can run the program on their phone to pinpoint the exact date and time that leukocoria was first observed in the patient. This method of detection will drastically reduce the age of diagnosis and greatly improve health outcomes of the patient. This is because the early detection of disease progression will allow for early initialization of treatment in the patient. The earlier detection methods will trigger initialization of treatment before the disease has progressed to a more dangerous state as is often the case with the red reflex test.

CRADLE analyzes a multitude of photos of the patient. This is in stark contrast to the red reflex test where the physician quickly looks at both eyes, sometimes simultaneously. The higher sample size of CRADLE allows for a higher likelihood of disease detection. This is because of the low specificity and low sensitivity of detection when looking for disease in the posterior eye. With multiple, photographically diverse pictures of the patient, the likelihood of reflection off of a tumor in the eye is much higher than in the low sample size red reflex test.

Another advantage of CRADLE over the red reflex test is its low barrier for usage. A concerned parent can simply download and open the application, point the camera at their child's eyes, and receive instantaneous feedback on their child's eye health. The application will tell them whether there is a positive or negative readout much like a red reflex test would. It does not give HSV values to the end user because that would be hard for someone without any training to understand. This is in stark contrast to the red reflex test, which only a trained medical professional can perform with efficacy.

Unlike the red reflex test, which gives a binary negative or positive test, CRADLE allows for a numerical read out of the degree of leukocoria in the HSV color space. With CRADLE, physicians will be able to obtain a more accurate reading of the amount of disease progression within the patient. Instead of a negative or positive red reflex test, the physician will receive an HSV value of the degree of leukocoria in the patient's eyes and be able to determine a more accurate method of treatment because of that.

CRADLE has the potential to reduce medical costs for the patient and the provider. By using an existing device, the cellular telephone, as a tool for the detection of leukocoria, ophthalmoscopes will no longer be necessary for use in the clinic. This will reduce the overhead cost of running the clinic and the savings could be passed on to the patient resulting in more cost-effective healthcare.

CRADLE has the potential to be cheaper, more effective, and easier to use than the red reflex test. This is because of its ability to use existing tools to analyze the patient. With an easy to understand user interface, anyone can use it to run diagnostic screenings on themselves or their loved ones. With a broad-based implementation of this system across the United States and the world, the age of diagnosis for retinoblastoma will certainly decrease and the rate of ocular salvage and vision preservation will subsequently increase. CRADLE has the potential to increase quality of life for many individuals around the globe and should be implemented as a replacement to the red reflex test.

One of the main problems CRADLE faces is its high rate of "pseudo-leukocoria" in its imaging. CRADLE utilizes the lighting element of the phone to reflect light off the retina in much the same way that the red reflex test does. However, because of the 'flash' effect found on the phone, it causes leukocoria to appear in otherwise healthy children and adults. This would lead to a high occurrence of false-positive screens. This is alarming because it could cause parents to overlook leukocoria that is clinically relevant. It is important to note that the rate of pseudo-leukocoria increases in low-light environments. For CRADLE to be adopted nationwide, it would need to develop a way to distinguish between clinically relevant leukocoria and pseudo-leukocoria. A possible

solution to this problem is to observe multiple images of the same patient and see if the leukocoria is recurrent. Because of the high sample size of CRADLE, pseudo-leukocoria can be ruled out if the leukocoria appears in >3 photographs.

In order for CRADLE to have clinical relevance with its HSV values, it must have a database to compare the values to. This is another problem with CRADLE because no such database exists. If this database existed, it would be helpful in establishing a quantitative scale of leukocoria which would be useful to physicians in estimating the degree of severity of the disease (in this case retinoblastoma). A possible solution to this problem is crowd sourced data. People that use the application can elect to send their leukocoric photos to the developers, which can then be cropped and analyzed. Followup with the patients would be necessary in order to determine the severity of their disease on the date of the photographs. Although this method is labor intensive, it will be beneficial in giving a reference point for disease progression using the HSV values that CRADLE employs.

Although there are some problems for CRADLE to overcome, the benefits outweigh the deficiencies. The main benefit being that parents will be able to monitor their child's ocular eye health away from the clinic. This will allow for faster detection of ocular abnormality and higher rates of vision preservation. In today's changing world, technology continues to aid in improving health around the globe.

CHAPTER FOUR

Personal Impact

To further investigate the efficacy of our application, we needed to collect some recent data. We followed a specific six step process as seen below:

1. Get the pictures from the main data bank onto a mobile hard drive for remote access.
2. Get the processing script to work, this will allow us to convert the rgb values to hsv values in an excel output format.
3. Crop and process the photos that we needed to collect the data.
4. Do steps 1-3 above for each patient individually.
5. Put each patient's cropped photos and data into appropriate dropbox folder
6. Analyze data

This was our research strategy boiled down to its barest parts, now we must look at each of its parts more in depth.

The first part required transferring the photos from the main data bank (Dr. Shaw's desktop) onto a mobile hard drive. However, the mobile hard drive, a Western Digital My Passport 1.0 TB hard drive, was formatted for Windows operating systems. This problem required going into the partitions of the mobile hard drive on a Windows machine and reformatting them for the Mac operating system. This allowed us to transfer

the photos from the main data bank to the mobile hard drive. The second problem occurred when trying to use the mobile hard drive back on my personal Windows machine. Since the mobile hard drive was formatted with the Mac operating system, it would not open on the Windows machine. This problem was solved by downloading a program called HFSExplorer which allows the Windows machine to browse data files on the Mac hard drive. Now that the files were successfully transferred and accessible, it was time to move on to the second step of the data collection process.

The second part required some basic knowledge of computer programming languages. I personally had never worked with AppleScript before and it was a growing experience for me. Here is the script, in its entirety, below:

```
tell application "Microsoft Excel" --this first set of commands opens all the preliminary files needed and copies the metadata from the chosen sheet to the "Output.xlsx" file
	*set i_max to x number of photos tested
	--and find all "CRADLE TEST's" and set to name of file to be parsed
	*open file ("CRADLE_High:Pixel Processor - Instructions and Required Files:Needed Files:Output.xlsx")
	*open file ("CRADLE_High:CRADLE TEST Data:CRADLE TEST.txt") --**********replace this pathway with that of the desired metadata*********
	*activate object sheet "CRADLE TEST" of workbook "CRADLE TEST.csv" --**********and change these to correspond properly**********
```

copy range *range* ("A1:F" & (i_max + 1)) --this "i_max" here should be the highest numbered histo file

activate object *sheet* "Export to Archive" **of** *workbook* "Output.xlsx"

paste special *range* "A1"

copy range *range* ("D2:D" & (i_max + 1)) --get each photo's pixel count from the "Export to Archive" sheet of "Output.xlsx"

activate object *sheet* "Calculation Sheet" **of** *workbook* "Output.xlsx" --and...

paste special *range* "H2" --put them on the calculation sheet to be used to find the average RGB values

--this part below gets the raw histo values from the "Histogram-i.csv" files and put them in the proper sheets

repeat with i **from** 1 **to** i_max

*__open__ *file* ("CRADLE_HIGH:CRADLE TEST Data:Histogram-" & i & ".csv")

activate object *sheet* ("Histogram-" & i) **of** *workbook* ("Histogram-" & i & ".csv")

copy range *range* "A1:IV1" --1. getting and pasting red histo values

activate object *sheet* "Red Histo" **of** *workbook* "Output.xlsx" --1

paste special *range* (**get address of** *row* 1 **of** *column* i reference style *A1*) **with** transpose

```
            activate object workbook ("Histogram-" & i & ".csv")
            copy range range "A2:IV2" --2. now the greens
            activate object sheet "Green Histo" of workbook "Output.xlsx" --2
            paste special range (get address of row 1 of column i reference style A1)
with transpose

            activate object workbook ("Histogram-" & i & ".csv")
            copy range range "A3:IV3" --3. and at last, the blues
            activate object sheet "Blue Histo" of workbook "Output.xlsx" --3
            paste special range (get address of row 1 of column i reference style A1)
with transpose
        end repeat

        --this part below copies the data to, and then the intermediate results within, the
calculation sheet to get the average RGB and average HSV values for each photo

        repeat with i from 1 to i_max
            activate object sheet ("Calculation Sheet") of workbook ("Output.xlsx")
```

copy range *range* ("H" & (i + 1))

paste special *range* "E2"

activate object *sheet* ("Red Histo") **of** *workbook* ("Output.xlsx")

copy range *range* (**get address of** *rows* 1 **through** 256 **of** *column* i reference style *A1*)

activate object *sheet* "Calculation Sheet" **of** *workbook* "Output.xlsx"

paste special *range* "B2"

copy range *range* "F2"

paste special *range* ("I" & (i + 1)) what *paste values*

activate object *sheet* ("Green Histo") **of** *workbook* ("Output.xlsx")

copy range *range* (**get address of** *rows* 1 **through** 256 **of** *column* i reference style *A1*)

activate object *sheet* "Calculation Sheet" **of** *workbook* "Output.xlsx"

paste special *range* "B2"

copy range *range* "F2"

paste special *range* ("J" & (i + 1)) what *paste values*

activate object *sheet* ("Blue Histo") **of** *workbook* ("Output.xlsx")

copy range *range* (**get address of** *rows* 1 **through** 256 **of** *column* i reference style *A1*)

activate object *sheet* "Calculation Sheet" **of** *workbook* "Output.xlsx"

paste special *range* "B2"

copy range *range* "F2"

paste special *range* ("K" & (i + 1)) what *paste values*

end repeat

--at long last, copies the calculated color coordinates to the "Export to Archive" sheet in "Output.xlsx" for storage in some NEW file. the contents of "Output.xlsx" then need not be saved. also closes all those "Histogram-i" files.

activate object *sheet* "Calculation Sheet" **of** *workbook* "Output.xlsx"

copy range *range* ("I2:O" & (i_max + 1))

activate object *sheet* "Export to Archive" **of** *workbook* "Output.xlsx"

paste special *range* "H2" what *values*

```
        repeat with i from 1 to i_max
                close workbook ("Histogram-" & i & ".csv")
        end repeat


end tell
```

Fig. 2

The asterisks above indicate areas where the program stopped or was rendered nonfunctional (computer froze) and required modification in order to get the program to work as intended. The i_max variable should not exceed 60 due to the large amount of ram needed to run that many pictures. In the beginning of the research, I ran the program at i_max 125 which caused the computer to crash at around data point 74 which caused loss of all data and having to restart the batch.

The purpose of this program was to automate the conversion of the RGB values measured in the Photoshop program and convert them to more accurate HSV values.

The third part of the data collection was done by following the instructions written for me by the project's originator (Brandon Taylor). The instructions are seen below (Fig. 3):

How the data points were obtained and processed for the control photos

(the method for doing the leukocoric photos is slightly different. See page 3.)

1. Obtain control photos
2. crop a photo by doing the following steps
    a. open a photo in Adobe Photoshop CS5
    b. zoom in around subject's left eye (if visible) and use lasso tool to select
3. 1. The Pixel Processor Script will only work on a specific set of input data types. To make sure that you are feeding it the correct data types, open Photoshop and use the "Analysis/Select Data Points/Custom…" drop-down dialogue to make sure that only the following boxes are checked:
4. i. Label
5. ii. Document
6. iii. Scale Units
7. iv. Area
8. v. Integrated Density
9. vi. Histogram
    a. hit command-shift-M to take a measurement of current selection
    b. hit command-J to make a separate layer by making a copy of what was just cropped (provides record of exactly which pixels go into the data point for that eye)
    c. deselect subject's left eye
    d. perform steps "a" to "e" for subject's right eye if visible
    e. save newly cropped+layered photo in Photoshop format (.psd) to same folder containing the unmodified input pictures
    f. close photo

10. repeat all of step 2 (“a” through “h”) for as many control photos as desired
11. once desired amount of photos is cropped, export the data from Photoshop to Microsoft Excel
    a. hit select all measurements button
    b. hit export all measurements button and enter a filename in the box that pops up in Photoshop. Measurements will be in the RGB color system, and will be saved as a folder of “.csv” files (one per cropping) + a text file that specifies which .csv file in the folder goes with which photo cropping. BOTH the text file and the .csv files are needed to import the data into Excel
12. use the custom Applescript to automate import of data to Excel and its conversion from RGB system to the desired HSV system. The Applescript takes the data from each separate .csv file and copies and pastes it into excel. Equations implemented in Excel do the actual conversion from RGB to HSV.
    a. open the Excel file I made, called “Output”. This file already contains RGB-to-HSV-conversion equations I typed into certain cells. (These were made using the normal “equation editor” that comes with Excel.) Because the equations are already in the cells, one just has to paste data into the cells, and the equations will output their result to different cells, from which the results can be saved for import into graphing software.
    b. Activate and run the applescript
        i. Open the applescript

ii. Set the variable “i_max” (found near top of script code) to the number of photos you are importing in this one run of the script
iii. Use control-f to replace all instances of the dummy variable “FilenamePlaceholder” with the filename you chose in step “4.b”.
iv. Hit command-k to compile (make ready to run) the script
v. Bring up the Excel file titled “Output” to the top of all programs (by clicking on it). Then bring the Applescript on top by clicking *it*. (for some reason the import process only works properly if you do step “5.b.v”)
vi. **Without clicking on other programs to bring them to the top**, hit command-R to run the script
vii. Wait for script to finish. (you’ll know it’s done because there will be no visible motion on screen)
viii. Click on the Excel file named “Output”
ix. Save that file under a new name, “Output from *xxx*”, where *xxx* is the filename you specified in step “4.b”

13. Close all the open programs.

Fig. 3

There were no modifications made by me to this set of instructions.

The fourth step of this data collection was the most labor intensive. This is because there is no way to automate the accurate cropping of each individual’s pupil.

This is due to the inherent variation between each individual's pupils caused by lighting conditions, heredity patterns, and eye color. As a data collector, I had to open an individual picture of the patient and crop each eye separately. I would do all of the left eyes of the patient first and put them in the data table. Next, I would do all of the right eyes of the patient and put them in a separate data table. Due to the large amount of ram this process required, I often would have to save my work in batches because the photoshop program would occasionally crash.

The fifth step involved utilizing the Dropbox that Dr. Shaw and his team had opened for this project. Dropbox is a computer program that uses cloud based storage to allow users to work together from remote locations. For example, I would upload my data tables into the drop box and they would become instantly available to anyone who had permissions to access that particular dropbox or who had a link to that website.

The sixth step involved actually analyzing the immense amount of data that we had cropped. This was outside the scope of my ability as an undergraduate and is where I handed off the project to a member of the team who was more experienced in the field of statistics and big data analyses. This data collection step took about three hundred and fifty man hours in total due to the independent investigation and problem solving skills required to complete it.

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