RETISTRUCT manual

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Contents

Chapter 1

Installation

1.1 Install R

1.1.1 Ubuntu Linux

Install R using the instructions at `https://cran.r-project.org/bin/linux/ubuntu/`.

1.1.2 macOS

- Install R for macOS, available at `https://cran.r-project.org/bin/macosx/`.
- Install XQaurtz, using the instructions on that page

1.1.3 Windows

- Install R for Windows, available at `https://cran.r-project.org/bin/windows/base/`.

1.2 Optional: Install RStudio

Rstudio, available at `https://posit.co/download/rstudio-desktop/`, provides and Integrated Development Environment for R. RStudio is not necessary to run Retistruct, but provides a user-friendly interface for R.

1.3 Install the core RETISTRUCT package

1. Start R or RSTUDIO
2. In the R Console, type:

   ```
   install.packages("retistruct")
   ```

   The first time this runs, it should create a personal directory for R packages, and it will take a few minutes to install some required packages.

1.4 Run Retistruct

1. Type:

   ```
   library(retistruct)
   retistruct()
   ```

2. An interface window should appear.
3. It can be helpful to maximise the window to fill the screen.
4. A number of sets of demonstration data are available from the **Demo** menu item.

1.5 Run a demo from the Graphical User Interface (GUI)

- Select the "SMI32" demo from the **Demo** menu
- The image of a flatmount retina should appear in the left of the window
- Click on the **Reconstruct** button. After a short delay, the reconstructed retina should appear at the right of the screen.

Chapter 2

Running RETISTRUCT

To start the program, start R. At the R prompt type:

```
library(retistruct)
retistruct()
```

A window should appear.

2.1 Preparing and opening the files for a retina

There are a number of types of information associated with a flat-mount retina that RETISTRUCT can process:

- The coordinates of the outline of the flattened retina
- An image of the flat-mount retina (optional)
- The coordinates of labelled data points within the flat-mount retina (optional)
- The coordinates of labelled counts of data within the flat-mount retina (optional)
- The scale of the coordinates provided in the other files (optional)

To import this information into RETISTRUCT, for each retina, a directory should be created containing files with the above information, as will be described below. At present there are three formats of directory that RETISTRUCT can read. Most users will probably find the the IMAGEJ ROI format most convenient.

2.2 Reading files using the IMAGEJ ROI format

This format allows you to load images of retinae whose outlines have been marked up in IMAGEJ. To create the image and outline files for this format:

1. Create a directory to save the files created to.
2. Open up IMAGEJ (or FIJI).
3. Use **File→Open** to open the image.
4. Use **Image→Scale** to down sample so that the resolution is less than 1000x1000. (This is not crucial, but will speed up things later.)
5. Save the down-sampled version of the image to the file name `image.png` in the directory created in Step 1.

6. Use the Polygon Tool () to mark the edge of the retina. According to the IMAGEJ manual[1]: "To create the selection, click repeatedly with the mouse to create line segments. When finished, click in the small box at the starting point (or double-click), and ImageJ automatically draws the last segment.

   The points that define a polygon selection can be moved or deleted, and new points can be added. To delete a point, click on it with the alt key down. To add a point, click on an existing point with the shift key down."

7. Open the ROI manager by selecting **Analyze**→**Tools**→**ROI Manager**.

8. Click on the **Add [t]** button.

9. Click on the **More**→**Save** button. In the **Save selection...** box that appears, enter the file name `outline.roi` and make sure this file is saved to the same directory as the `image.png` file, i.e. the directory created in Step 1.

10. Optionally mark up the optic disc in the same way as the outline. Save this ROI to a file called `od.roi` in the same directory as `image.png`.

11. Open RETISTRUCT.

12. Click on the **Open** icon and select the directory containing `image.png` and `outline.roi`. The retinal image should now appear, with the outline shown. By default the outline is in black. If this isn't visible against the image, press the **Properties** button in the interface (or select **Edit**→**Properties**), and change the **Outline colour**.

2.2.1 Reading in images comprising multiple fragments [Optional]

Images from retinae that been cut into multiple fragments can also be loaded in. To do this, instead of saving one outline.,save multiple ImageJ ROI regions from the same image in a file called `RoiSet.zip`

2.2.2 Specifying the scale

Optionally, the scale of the image can be specified by providing a file `scale.csv` in the same directory as the image and ROI files. This file should contain two lines, the first with the heading `Scale` and the second with the length of the side of a pixel in micrometres. For example:

```
XY
1.5
```

Note that for compatibility with older versions of `Retistruct`, this column can also be called "Scale".

2.2.3 Specifying depth information [Optional]

A "flat-mount" needn't be flat. If depth information is available, this should be supplied in the form of a single-channel TIFF file called `depthmap.tiff` with the same dimensions as `image.png`. The value of each pixel in the TIFF file specifies the height of the corresponding pixel in the image. The size of each step in the Z-direction should be specified using an extra column "Z" in `scale.csv`.

Parts of the TIFF image will correspond to parts of the flat-mount that are empty; we refer to these parts as the background. Typically the background is represented by a value of 0. There will be problems if the outline specified in ImageJ strays onto parts of the TIFF depthmap image, since it might appear that there are very sharp jumps in the depth. To get around this problem, it is necessary to specify the value of the background, so that RETISTRUCT can infer the depth of any background regions that the outline strays onto by looking at the closest depths that are in the foreground. To specify the background value, include a file `depthmap.csv`, in the following format:

```
Background value
0
```

If there is no `depthmap.csv` in this format, RETISTRUCT tries to infer the background value, giving a giving a warning as it does so.

[1] http://rsbweb.nih.gov/ij/docs/tools.html

2.2.4 Reading in data points

In general, the coordinates of data points are read in from a csv file called `datapoints.csv`. The two cells of the first line of the file contain the name of the group of data points and the colour that these should be displayed in RETISTRUCT. Marking up the points from the image in IMAGEJ will ensure that the coordinates are in the same system as those used for the image and the outline. To do this:

1. Open the image used for marking up the outline (`image.png`) in IMAGEJ.
2. Threshold the image (**Image**→**Adjust**→**Threshold...** so that the labelled points are visible.
3. Select **Analyze**→**Set Measurements...** and make sure that **Centroid** is checked
4. Select **Analyze**→**Analyze Particles**. Make sure that **Display results** and **Clear results** are checked. Click on **OK**.
5. A window entitled **Results** should appear. This contains the X and Y coordinates of the detected points. In this window select **File**→**Save as...** and save the file as `datapoints.csv`.
6. Open this file with either EXCEL or OPENOFFICE CALC and remove all the columns apart from the X and Y columns. Replace the X with the name of the data set and the Y with the colour it should be displayed in RETISTRUCT.
7. Save `datapoints.csv` in the retina's directory, along with `outline.roi` (and `image.png` if an image is desired).

2.2.5 Reading in data counts

In general, the coordinates of data counts (counts of data at X, Y positions) are read in from a csv file called `datacounts.csv`.

The two cells of the first line of the file contain the name of the group of data counts and the colour that these should be displayed in RETISTRUCT.

Each subsequent row has three numbers: The first two columns contain the X and Y coordinates at which the count was measured, and the third column contains the count at that location. It is important to ensure that the coordinates are in the same system as those used for the image and the outline.

Save `datapoints.csv` in the retina's directory, along with `outline.roi` (and `image.png` if an image is desired).

2.3 Reading in files using other formats

2.3.1 CSV format

This is the same as the IMAGEJ ROI format, except that the outline is contained in the two columns of a file called `outline.csv`. Each column should have a heading, e.g.:

```
X,Y
1,5
10,76,
...
```

The optic disc outline can be supplied in a similar file called `od.csv`.

2.3.2 IDT format

This is the format used in Ian Thompson's lab (see Appendix A). These files are contained in a directory. To open the files corresponding to a retina, click on the open file icon, and navigate to the directory containing the `SYS` and `MAP` files. On opening this directory, the retinal outline should appear in the RETISTRUCT window.

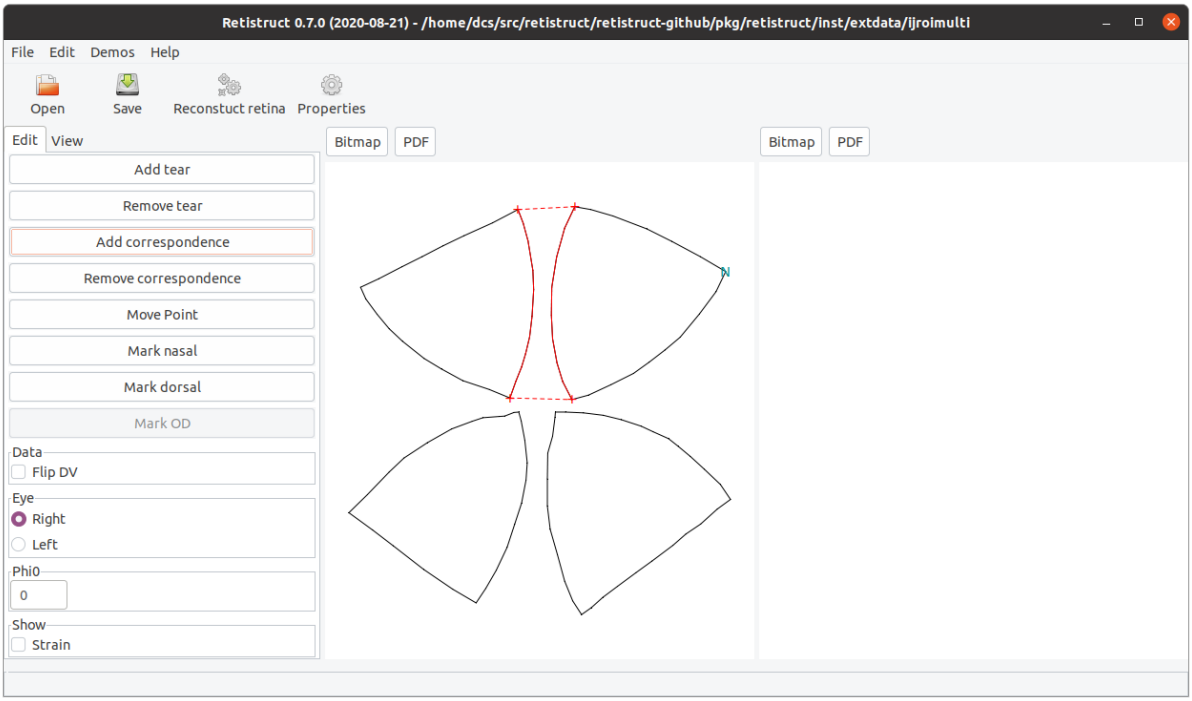

Figure 2.1: Adding a full cut. One full cut between the two upper petals has been added. The red dashed line indicate the ends of the full cut, and the red highlighted edge indicates the edges of the full cut.

2.4 Editing the retinal mark-up

After a set of files is opened, the **Edit** tab in the RETISTRUCT window will be open. Before the retina can be reconstructed, you need to mark-up tears, full cuts and landmarks on the retina using the following controls:

Add tear To add a tear, click on this button, then click on three points in turn which define a tear. The order in which the points are added does not matter. Tears contained within a tear can be marked up, but tears cannot cross over one another.

Remove tear To remove a tear, click on this button, then click on the apex of the tear (marked in cyan on the plot)

Add full cut To add a "full cut", i.e. a pair of corresponding edges of two fragments, click on this button, then click on four points in turn which define a full cut (Figure 2.1). The order in which the points are added does not matter.

Remove full cut To remove a full cut, click on this button, then click on any point on the full cut.

Move Point To move one of the points defining a tear or full cut, click on this button, then click on the point which you desire to move, then click on the point to which it should be moved.

Mark nasal To mark the nasal pole, click on this button, then click on the point which is the nasal pole. If the nasal or dorsal pole has already been marked, the marker is removed from the existing location. The nasal pole should not be in a tear. If the nasal tear is placed within a tear, no error is reported at this stage, but it will be reported later.

Mark dorsal As above, except for the dorsal pole.

Mark OD To mark the optic disc, click on the structure marked in orange which you think is the OD. Once clicked on, the structure should become blue.

Phi0 This determines the latitude of the rim of the reconstructed hemisphere. In mouse, it depends on the age of the animal [1]. **Ideally you will have measured or estimated this value from an intact retina.** The default value of 0 may lead to reconstructions that are not as accurate as they could be, since the template that RETISTRUCT is trying to morph the flat-mount retina onto is not the same as the original shape.

2.5 Editing metadata

You can also edit the following metadata:

Data/Flip DV Flip the DV axis to compensate for microscope orientation. Affects display of retinae.

Eye Specify whether the eye is Right or Left. Affects display of retinae.

2.6 Saving the markup and metadata

To save the markup, click on the "Save" button in the toolbar. This saves various markup files to the directory containing the data files. This saved data can be used to reconstruct the retina using a batch process (Section 3.3).

2.7 Reconstructing the retina

To reconstruct the retina, click on the "Reconstruct retina" button. This causes RETISTRUCT to perform a (sometimes lengthy) sequence of operations:

Stitching Links between corresponding points on parts of the retinal outline contained in tears are made.

Triangulation A triangular mesh is placed over the flattened retina

Initial projection to sphere The mesh is projected roughly onto a sphere

Optimisation The locations of the mesh points on the hemisphere is adjusted so as to minimise a weighted sum of the squared differences between the lengths of links in the mesh on the hemisphere and on the flattened retina, whilst ensuring that as few triangles as possible are flipped.

At the end of the reconstruction process, a polar plot appears next to the flattened retina. By default the location of the full cuts and tears in the polar coordinates can be seen.

2.8 Assessing the quality of the reconstruction

It is important to assess the quality of the reconstruction. The choice of where to mark up tears can affect this adversely, as can the rim angle.

One diagnostic is to view the strain in each mesh link by clicking on the **Strain** checkbox in the **Edit** tab. When **Strain** is shown, the flat plot shows the strain in the links of the mesh using a colour code from blue (compressed) to red (expanded). The polar plot is replaced by a scatter plot of the length of links in the reconstructed object versus the length on the flattened object. The flat plot of a good reconstruction will look mostly green, and in the scatter plot the points will lie close to the black line.

2.9 Selecting viewing options

To select viewing options click on the **View** tab. A number of options are now shown, grouped into a number of sections:

2.9.1 Show

In the **Show** section there are checkboxes that allow you to show various types of information:

Markup Locations of tears and the dorsal or nasal pole

Stitch Locations of how the algorithm has stitched tears (only visible after the reconstruction step)

Grid Lines of latitude and longitude projected back onto the flattened retina (only visible after the reconstruction step) and grid lines are shown on the reconstructed retina

Landmarks Landmarks such as the optic disc

Points Locations of data points, such as the locations of beads of dye, which have been imported into RETISTRUCT as described in Section 2.2.4

Point means Locations of the Karcher mean (mean in spherical coordinates) of each group of data points

Point contours Kernel Density Estimate contours of the data points. By default, contours at 5%, 25%, 50%, 75% and 95% of the maximum density are shown. This can be changed by a command like this at the R prompt:

```
options(contour.levels=c(5, 25, 50))
```

This command would cause the 5%, 25% and 50% contour lines to be displayed.

Counts Counts (for example of cells), represented by a number at the location at which the count was made, which have been imported into RETISTRUCT as described in Section 2.2.5

Count contours Kernel Regression contours of the data counts. By default, contours at 5%, 25%, 50%, 75% and 95% of the maximum density are shown. This can be changed by a command, as shown under "Point contours".

2.9.2 IDs

If you included a `datapoints.csv` and/or a `datacounts.csv` file (see Sections 2.2.4 and 2.2.5) the **IDs** section will contain checkboxes with names corresponding to the names of the groups of points and the names of the groups of counts specified in those files. If you have marked the optic disc, it will also contain an **OD** checkbox. Check or uncheck these boxes to show or hide the corresponding points and counts.

2.9.3 Projection

The reconstructed data can be viewed in a number of projections, just as there are various ways that the surface of the globe is projected to a 2D image. Because the geometry of the surface of a sphere is fundamentally different from a flat sheet of paper, no projection is a perfect representation; all have advantages and disadvantages.

Note: Coordinate systems

The co-ordinates of a point on the reconstructed sphere can be described in two ways:

Latitude and longitude Here latitude and longitude are defined analogously to the standard geographical coordinate system. We imagine the retina lying with its optic pole at the south pole, and with its widest point lying on the equator. The latitude of the optic pole is -90° and the latitude of point on the widest point of the retina (its "equator") is 0°. We denote the latitude φ and the longitude λ.

Colatitude and longitude Here we imagine the retina in the same orientation with respect to the earth, but we rather than latitude, we use colatitude measured from the south pole, i.e. the south pole has a colatitude of 0° and the widest point (the "equator") has a colatitude of 90°. We denote colatitude by ψ and it is related to latitude by $\psi = \varphi + 90^\circ$.

Projection	Latitude & longitude	Colatitude & longitude
Azimuthal Equidistant	$\rho = 90^\circ + \varphi, \lambda = \lambda$	$\rho = \psi, \lambda = \lambda$
Azimuthal Equal Area	$\rho = \sqrt{2(1+\sin\varphi)}, \lambda = \lambda$	$\rho = \sqrt{2(1-\cos\psi)}, \lambda = \lambda$
Azimuthal Conformal	$\rho = \tan(45^\circ + \varphi/2), \lambda = \lambda$	$\rho = \tan(\psi/2), \lambda = \lambda$
Sinusoidal	$x = (\lambda - \lambda_0)\cos\varphi, y = \varphi$	
Orthographic	$x = \cos\varphi \sin(\lambda - \lambda_0)$	
	$y = \cos\varphi_0 \sin\varphi - \sin\varphi_0 \cos\varphi \cos(\lambda - \lambda_0)$	

Table 2.1: Projections. For the three azimuthal projections, the polar coordinates (ρ, λ) are given in terms of the latitude and longitude (φ, λ) or colatitude and longitude (ψ, λ) on the original sphere. For the sinusoidal and orthographic projection the (x, y) coordinates in the plane are given. For these projections (φ_0, λ_0) is the latitude and longitude at the centre of the projection.

Currently the options are:

Azimuthal Equidistant The default projection. This is a polar plot in which the radial distance is proportional to the colatitude (latitude measured from the "South Pole"; see note on coordinate systems), and angle from the horizontal is equal to the longitude on the original retina. Points equidistant on line of longitude are also equidistant in this projection. It is not an area-preserving projection: it makes regions closer to the rim look bigger than they actually are relative to areas close to the south pole.

Azimuthal Equal Area In this projection, also known as a Lambert projection, points that are equidistant on lines of longitude on the spherical retina are not equidistant: points close to the rim are mapped to be closer together. This is done so that area is preserved in the sense that equal areas on the sphere project to equal areas on the plane of projection. It is thus an area-preserving projection.

Azimuthal Conformal In this projection, also known as a Wulff projection, angles are preserved. This is done at the expense of expanding the representation of areas close to the rim.

Sinusoidal The sinusoidal projection projects the entire globe onto the plane and preserves area. The user selects the longitude at the centre of the projection in the **Az** box in the **Projection centre** section of the window. The sinusoidal projection is helpful for data transformed into visuotopic space (see later).

Orthographic The orthographic projection gives a perspective view of one side of the globe. The projection is centred on a latitude and longitude specified by the values in degrees in the **El** and **Az** boxes in the **Projection centre** section.

Note: Convention on longitude of nasal, dorsal, temporal and ventral poles

By convention, the polar plots are viewed as though the animal is facing towards the observer. This means that when plotting a retina from a right eye, the nasal pole on the right and N, D, T, V are in anticlockwise order; for a retina from a left eye, nasal is on the left and N, D, T, V are in clockwise order. The longitude of a point was defined so that 0° is always at the right of the plot and 90° at top. This means that for a right eye the poles correspond to the longitudes as follows: N, 0°; D, 90°; T, 180°; V, 270°. For a left eye the longitudes of the nasal and temporal poles are interchanged: N, 180°; D, 90°, T, 0°; V, 270°.

2.9.4 Transform

The **Transform** option allows the reconstructed retina to be projected into visual space. The options are

None (default) No transformation is made.

Invert The image is inverted as though by a pinhole lens, a crude approximation to the optics of the eye. This allows visual space to be mapped onto the retina via the optical system of the eye. This mapping depends on the angle that a ray makes with the optic axis (the **Axis direction**) which we define as being oriented at azimuth (**Az**) and elevation (**El**).

Invert to hemisphere Similar to **Invert**, except that the projection is compressed or expanded so that the field of view is 180°.

There are more details of the transformation in the supplemental information of [1].

2.10 Exporting the plots

The **Bitmap** and **PDF** buttons above the flat-mount view and the reconstructed view export the image show to a PNG file format or an PDF respectively. To change the width of the image in pixels, select **Properties** and set the **Maximum width of projection** to the desired number of pixels. **Warning:** setting a high number of pixels will create a high-resolution image, but can also take some minutes.

2.11 Saving the reconstruction

To save the markup, click on the "Save" button in the toolbar. This saves various files to the directory containing the data files. When the files in the directory are opened again, all the markup information (cut locations, location of nasal point and rim angle) is loaded, and the reconstruction will also appear, unless there has been a major upgrade of the software, in which case the retina will need to be reconstructed using the "Reconstruct retina" button.

Chapter 3

Further topics

3.1 Accessing reconstruction data from R

It can be useful to have access to the data underlying a reconstruction directly, for example to allow statistical analysis. To load saved date into R, type the following into R:

```
r <-
retistruct.read.recdata(list(dataset="/path/to/reconstruction/directory"),
check=FALSE)
```

The resulting object `r` contains various fields, which can be accessed using the R `$` operator, e.g.

```
r$featureSets[[1]]$Ps
```

gives a list of the spherical co-ordinates of labelled data points. Other quantities (for example the locations of the mesh points in the flat and spherical retina) are also available.

All classes are fully documented - type 'help.start()' and browse to the `retistruct` package. Then the `RetinalReconstructedOutline` class is a good place to start.

3.2 Reading reconstruction data into MATLAB

By default when a reconstruction is saved, a subset of the data is stored in a file called `r.mat` in the same directory as the raw data and the markup. To import this data into MATLAB, cd into that directory, and type:

```
clear
load r.mat
```

This puts a number of variables into the workspace, as shown in Table 3.1.

To produce a polar plot of data points, try the following code:

```
polar(Dss.green(:,2), Dss.green(:,1)*180/pi+90, '.g')
hold on
polar(Dss.red(:,2),   Dss.red(:,1)*180/pi  +90, '.r')
hold off
```

The radial axis indicates the latitude in degrees measured from the retinal pole.

In the `matlab` subdirectory of the distribution, there are some scripts to produce polar polar plots, including the locations of tears and landmarks. To create PDF plots of all the retinae in a directory, try:

```
makefigures('retinae', 'output_directory')
```

There are also some embryonic scripts to create polar plots: `plot_datapoints_polararea.m` and `polararea.m`.

`phi0`	The latitude of the rim, expressed in degrees
`Dss`	Structure containing locations of labelled cell bodies in spherical coordinates. The first column contains the latitude of each point, measured in radians. The second column contains the longitude of each point, measured in radians.
`DssMean`	Location of Karcher mean of green-labelled cell bodies in spherical coordinates. The first column contains the latitude of each point, measured in radians. The second column contains the longitude of each point, measured in radians. A value of -2147483648 corresponds to NA in R.
`DssHullarea,`	Structure containing area of convex hull of points on sphere. The convex hull is essentially a polygon drawn around data points. A value of -2147483648 corresponds to NA in R.
`Tss`	A structure in which each element contains spherical coordinates (in the same latitude-longitude format as above) of a tear.
`Sss`	A structure in which each element contains spherical coordinates (in the same latitude-longitude format as above) of a landmark.
`KDE`	An object containing information about Kernel Density Estimates of the locations of cell bodies.
	`green_flevels` — Contour heights, determined by finding heights that exclude a certain fraction of the probability. For example, the 95% contour is excludes 95% of the probability mass, and it should enclose about 5% of the points.
	`green_labels` — Contour labels. These give the label (e.g. 5, 25, 50, where these are the percentages above) of each contour. Note that there may be more than one contour at the same level, so this vector may contain more elements than `flevels`. The first element of `green_labels` labels the contour whose coordinates are specified in `green_contours1`, the second element of `green_labels` relates to `green_contours2` and so on.
	`green_tot_contour_areas` — The total area in square degrees enclosed by each contour. This is a matrix with the first column giving the contour label (see above) and the next column giving the area.
	`green_kappa` — The concentration parameter of the Fisher density determined by the kernel fitting algorithm.
	`green_h` — A pseudo-bandwidth parameter, the inverse of the square root of `kappa`. Units of degrees.
	`green_maxs_phi` — Lattitude of maximum point of kernel estimate.
	`green_maxs_lambda` — Longitude of maximum point of kernel estimate.
	`green_g_xs` `green_g_ys` `green_g_f` — Kernel density estimates on standard polar grid. This can be plotted in MATLAB using the command `contour(KDE.green_g_xs, KDE.green_g_ys, KDE.green_g_f)`.
	`green_gpa_xs` `green_gpa_ys` `green_gpa_f` — Kernel density estimates on area-preserving polar grid. Plotted in MATLAB as above.
	`green_contours1, green_contours2...` — Coordinates of contours. See `green_labels` above for more explanation.
	`green_contour_areas1, green_contour_areas2...` — Area contained within each individual contour. See `green_labels` above for more explanation.
`KR`	An object containing Kernel Regression estimates of the density of points, derived from the grouped data points. All fields correspond to `KDE` above.

Table 3.1: Variables exported in the `r.mat` file.

3.3 Running a batch of reconstructions

The RETISTRUCT library can be used to reconstruct a batch of retinae which have been marked up. Suppose that the directory `retinae` contains a directory tree in which there are data directories containing the raw outline, data point and image files and the saved markup files. In order to perform the reconstructions, we create a new directory `retinae/reconstructions`, and run the following sequence of commands in R:

```
R
> library(retistruct)
> retistruct.batch(tldir='retinae', outputdir='retinae/reconstructions')
```

This command will go through the `retinae` directory, looking for valid data directories. If it finds one, it sets about trying to reconstruct the retina. As it reconstructing each retina, it writes to log file in `retinae/reconstructions`. Once the reconstruction is complete, it saves a number of plots in this directory in PDF format. It also adds a line to a summary log file in `retinae/reconstructions` called `retistruct-batch.csv`. This file contains a number of columns:

`Dataset` The directory of the data set

`Return` The return value from the process

`Result` A summary of the result, including if any errors were returned

`E` The total error of the optimised reconstruction

`El` The error due to purely to the lengths of links in the optimised reconstruction

`nflip` The number of flipped triangles

`EOD` The distance of the Optic Disc from the inferred centre of the retina, in degrees. If the OD has not been marked up, this is `NA`.

To export the reconstruction data in a directory hierarchy in which `retistruct.batch()` has been run, run the following sequence of commands in R:

```
R
> library(retistruct)
> retistruct.batch.export.matlab(tldir='retinae')
```

Appendix A

The IDT Data format

The data for each retina is stored in a separate directory. Within each directory there are two files:

`SYS.SYS` A table in SYSTAT format containing the coordinates of the red, green and doubly labelled cell bodies, and counts of labelled cell bodies within each grid box. The column headings shown in Table A.1. Each row of the table contains information only on a subset of the data, e.g. the coordinates of a red-labelled cell.

`ALU.MAP` A text file containing the coordinates of the map outline. The file comprises a number of sections, each starting with a single number, which is the number of lines to read in the next section. These lines have two numbers each, the x and y coordinates of a vertex of the map outline.

FOR EACH BOUNDARY	
MAPNUM	id number of boundary
MINLAT	min latitude
MAXLAT	max latitude
MINLON	min longitude
MAXLON	max longitude
LABLAT	latitude of label
LABLON	longitude of label
FOR EACH CELL	
XRED	x-coordinate if cell labelled red but not doubly
YRED	y-coordinate if cell labelled red but not doubly
XGREEN	x-coordinate if cell labelled green but not doubly
YGREEN	y-coordinate if cell labelled green but not doubly
XDOUBLE	x-coordinate if cell labelled doubly
YDOUBLE	y-coordinate if cell labelled doubly
XGRID	sample box cell is in
YGRID	sample box cell is in
PERIM	perimeter of cell
AREA	area of cell
ONE PER GRID BOX	
GRIDX	grid location of centre of sample box
GRIDY	grid location of centre of sample box
XGRIDCOO	x-coordinate of centre of sample box
YGRIDCOO	y-coordinate of centre of sample box
BOXSIZEX	size (half width) of sample box in x-direction
BOXSIZEY	size (half width) of sample box in y-direction
COMPLETE	whether counting of sample has been completed
TOTALCEL	total number of cells in this box
TOTALRED	total number of red-only cells in this box
TOTALGRE	total number of green-only cells in this box
TOTALDOU	total number of double cells in this box
MEANPERI	average perimeter of all cells
MEANAREA	average area of all cells

Table A.1: Column headings of the SYS.SYS file.

Bibliography

[1] Sterratt DC, Lyngholm D, Willshaw DJ, Thompson ID (2013) Standard anatomical and visual space for the mouse retina: computational reconstruction and transformation of flattened retinae with the Retistruct package PLoS Comp. Biol. 9