Wings 4:
Curve finding, fitting

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Wings 4, takes images of Drosophila wings, detects curves that fit an *a priori* template, and then fits spline curves to them, as shown in Error! Reference source not found.. Wings4 is a JAVA program that will run on either Windows or Apple machines. To make this program most useful, you should also obtain the companions program CPR that reads the output of Wings, performs basic analysis of these shape data, and allows you to extract the information that you wish to analyze from them. The hardware we use to image wings is described in Houle et al. (2003).

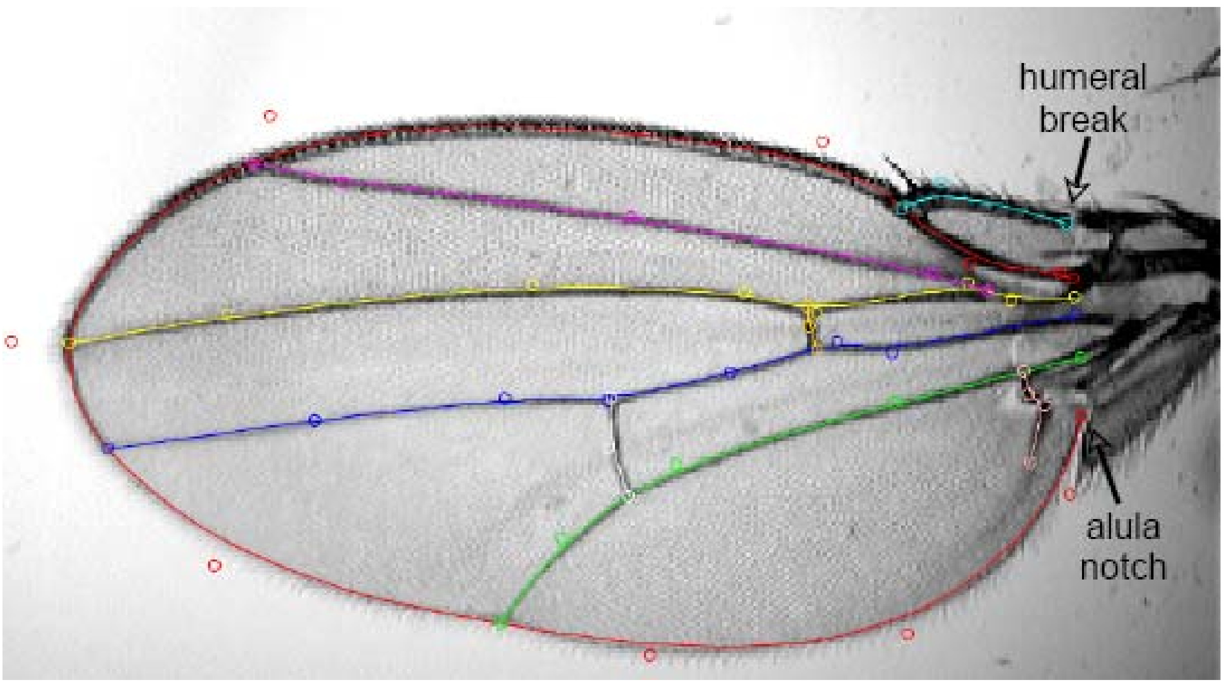

Figure 1. Nine B-splines fit to veins and edges of a Drosophila wing. Circles denote the positions of spline control points. The two starting landmarks that must be furnished to Wings are shown by the arrows. Starting landmark number 1 (in the ASC file) is the humeral break, and number 2 is the alula notch.

Both Wings 4 and CPR are available from http://bio.fsu.edu/~dhoule/Software/ . We are happy to furnish the source code for this software if you would like to try improvements or porting of this system to other operating systems. Please contact us at dhoule@bio.fsu.edu.

A list of the known bugs in this beta software is in the last section of the document.

What's a spline, and why use them?

Splines are continuous curves, so they can represent the edges of objects. They are essentially equations that give the location of the curve at any point between its end points. For an object like a fly wing, the most obvious features are the veins and edges of the wing, which are curves, so a spline can capture those well.

Wings4 fits quadratic B-splines, one choice from the family of possible spline equations. The data used to compute our spline curves are the spatial coordinates (*x* and *y* here) of a set of *control points*. For the wing in Fig. 1, the control points are the small circles. Note that they are generally not on the curve, with the exception of those at the end of a spline curve. For example, there are 5 control points on the green curve in Fig. 1, one at each end, and three in the middle that are not precisely on the curve. The relationship between control points and the curves is discussed in the section B-splines in Wings below. To use Wings, what you need to understand is that Wings fits curves to a wing image by moving control points to maximize the overlap between the spline curves and veins and edges of the wing captured in a digital image. Further technical details are in the section Understanding Splining below.

Recording images

The wing images themselves can be digitized with a wide variety of hardware setups. Ours is described in the paper Houle et al., 2003 BMC Evolutionary Biology 3:25. Perhaps the most useful part of our setup is a very simple suction device for immobilizing the wing of a fly to enable imaging on a live specimen. We use Optem macroscopes with digital cameras or a combination of an analog camera and a frame-grabber. These do not need to be of high quality, as the spline software works well on a low resolution, grey-scale version of the image. It is also remarkably forgiving of many kinds of imperfections, such as dirt, small tears in the wing. Others, such as hairs that look like a vein, may cause problems.

You will need to write or buy your own software to associate the wing image with the locations of two orientation landmark positions on the wing. We have not furnished our software for this, as this is hardware-dependent. We use, and would recommend the program ImagePro for those with money to spend, NIHImage is another possibility.

The two orientation landmarks needed are at the distal side of the humeral break, and the notch in the sinus between the alula and the trailing lobe of the wing, as shown in **Error! Reference source not found.**.

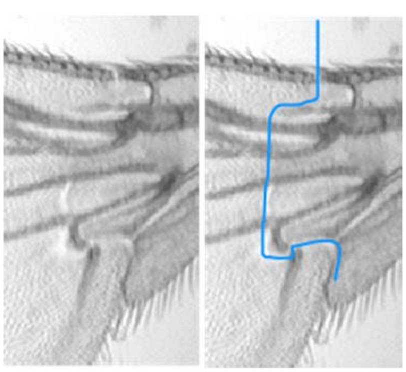

Figure 2. Approximate position of the wing hinge, shown by the blue line on the right image. Note that the alula is slightly folded from what is shown in Fig. 1, so as to obscure the location of the notch. The clear spot in the hinge, just above where the notch is a good marker to look for.

Note the clear spot that is part of the hinge, shown in Figure 2. This is a good marker for the location of starting landmark 2 on wings where the alula is folded over, which is a frequent occurrence with our suction-based wing grabber. A description of this piece of hardware is in Houle et al. 2003.

The starting files

The raw images should be TIFF files. We use the extension ".tif". You also need an ASCII format file, with the extension .ASC, that lists the image file names, with accompanying information to allow the splining of the image. Lines in the .ASC file must look like this:

```
vir1000.tif 508 46 576 142 Jeff 15_02_01 Thu_PM virilis F 1.12E-03
```

The delimiter for columns is a space character. The information that Wings actually needs is in the first five columns, and the last two (the 10th and 11th columns). These are:

1. The name of the TIFF file.
2. The x coordinate in pixels of orientation landmark 1 (humeral break, see Fig. 1).
3. The y coordinate in pixels of orientation landmark 1.
4. The x coordinate in pixels of orientation landmark 2 (alula notch, see Fig. 1).
5. The y coordinate in pixels of orientation landmark 2.
6. Columns 6, 8 and 9 can be used for any information you wish to record (with no internal spaces).
7. The date the image was recorded in any numeric format with separators (e.g. \, _)
8. Anything (see column 6).
9. Anything (see column 6).
10. Sex of the fly imaged.
11. The scale of the image in mm/pixel.
12. Other columns can follow column 11.

The Wings program will merely copy any of the information in columns 6 and 8-9, and 12 or more so you can use those for any information about the specimen you like. There is no limitation on the length of these strings within columns. Remember that any space is treated as a delimiter, so if you enter a location as 'Tallahassee, Florida', this will be read as two separate columns by Wings.

The final type of starting file needed are template splines that Wings uses as a starting point in fitting each wing. These files have exactly the same format as the files that contain the data recovered from each image, which Wings calls .cp files. Each time you spline new wings, you need to choose a template for splining, which is encoded in a cp file. By convention, when we adopt a cp file as a template, we give a file extension '.cp#' where # is a single digit from 0 through 9. There is actually no difference in format between a template and a regular cp file, just a name change.

Using a good starting spline model is one of the most important steps to getting repeatable, accurate spline fits. The principles of how to choose a good model are described in the Section Understanding Splining. For some data sets, choosing a wing where the splines fit well, as in Figure 1, is sufficient. However, for many other data sets, the spline routine tends to make the same errors over and over again, essentially getting attracted to the wrong solution. In such cases, it is often effective to choose a starting model that deviates from a good fit in one direction or another. Figure 6 shows such an 'extreme' model that is effective for splining the example data set furnished with these programs.

Installing Wings4

To use Wings4 you must install JAVA (JDK7), the latest version of the Java Runtime Environment. This is a free download available from http://www.java.com/en/download/. The Wing programs can be downloaded as a zip file from http://bio.fsu.edu/~dhoule/Software/Wings/. Check back there for updated programs. The current version is wings4.0 Beta 2.8.zip. To install, extract the entire zip file to a subdirectory of your choosing. This will make a new folder called Wings4, plus a few subdirectories that the program expects to find. Make a desktop shortcut to Wings4.jar before you forget where you put the program.

To start the program, click on Wings4.jar in the Wings4 subdirectory. This will first start a java virtual machine (which can take a minute or two) and then starts the program. The first time you open this file, it will ask you to set file locations for the files it needs. They are in the subdirectory ..\Wings4\supportFiles, which was created when you extracted the ZIP file.

Before starting the program

Before starting the Wings program look at the directory structure where your wing images and .ASC file is stored. Locate the subdirectory containing your original tif files. Locate some template files, (.cp2) so that you can tell the program where to look for them. One such file comes in the zip file, and will be at
. . /Wings4/Example/virilis.cp2.

Wings program usage

The program for doing the splining is called Wings4.jar. Click on this file to start the program.

1. The program pops up two small windows, and asks you to input your name. This associates the name of the spliner with results produced in this session of Wings. Names can have spaces. Hit return.
2. You will see a window, containing three other window. Here is what it looks like:

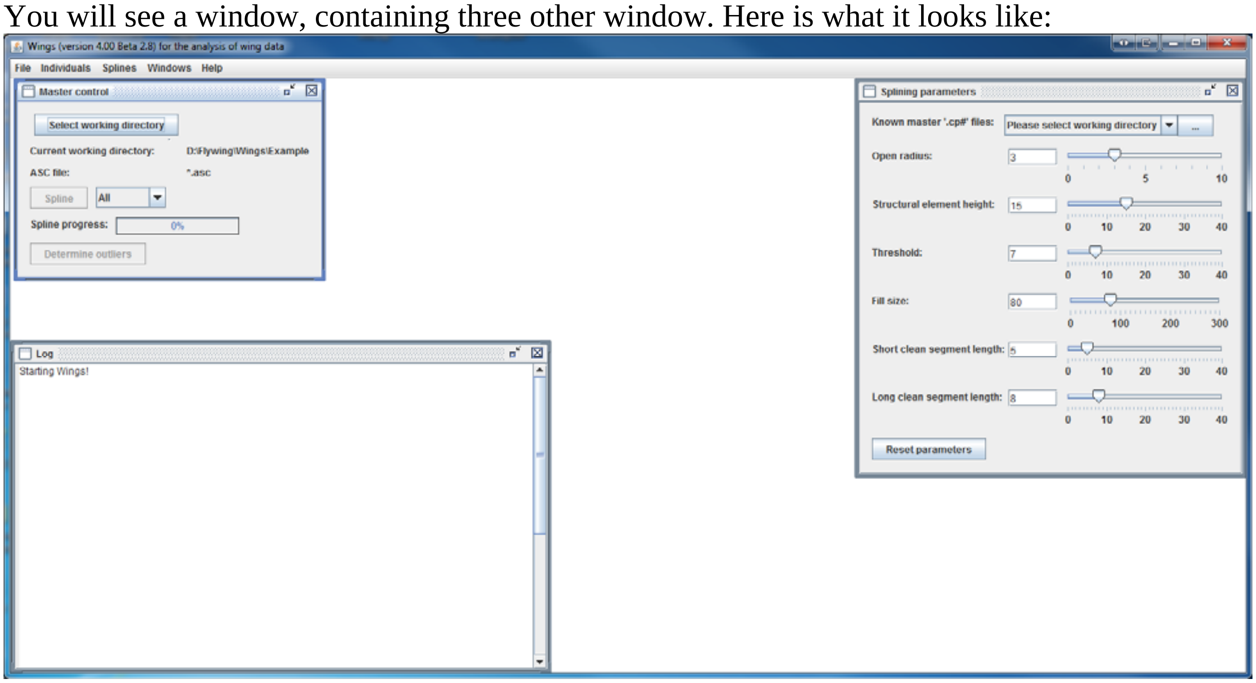

Figure 3. Main screen of Wings when a session is first started.

The **Master Control** window is used to navigate to your files, choose an ASC file, spline images and detect outliers.
The **Splining Parameters** window is used to choose a template file, and basic image processing parameters.
The **Log** window gives you feedback on the progress of your session, including error messages and exceptions.

3. Hit the ***Select working directory*** button, then use the pop-up to navigate to the directory with the images to be splined and the corresponding ASC file. Wings will check your ASC file for format errors. If there is an error in your file, Wings will show you a message window describing the error, but there is currently no notice of the error after you close this window. No further progress is possible until an error-free ASC file is furnished. Edit your ASC file, then repeat your choice of working directory to reread the ASC file. Once an error-free file is read, and Wings will pop up a new window titled **Individuals** with a list of all the images, and their splining status.

4. Next, choose a master CP# file to use for the splining. Go to **Splining Parameters**, then check if your CP# file is already in the pull-down list. If not, click on the button marked '…'. If you don't see this button, expose the right side of the window by pulling the slider at the bottom of the window to the right. Once you hit the '…' button, you will see an Open File window that allows you to navigate to the subdirectory with the CP# file that you want to use. To actually choose the file, use the pull-down menu, which should now be populated with all the CP# files in this subdirectory. At this point, your screen should look like the following: The **Spline properties** window shows the positions of the spline control points and the splines for you chosen model.

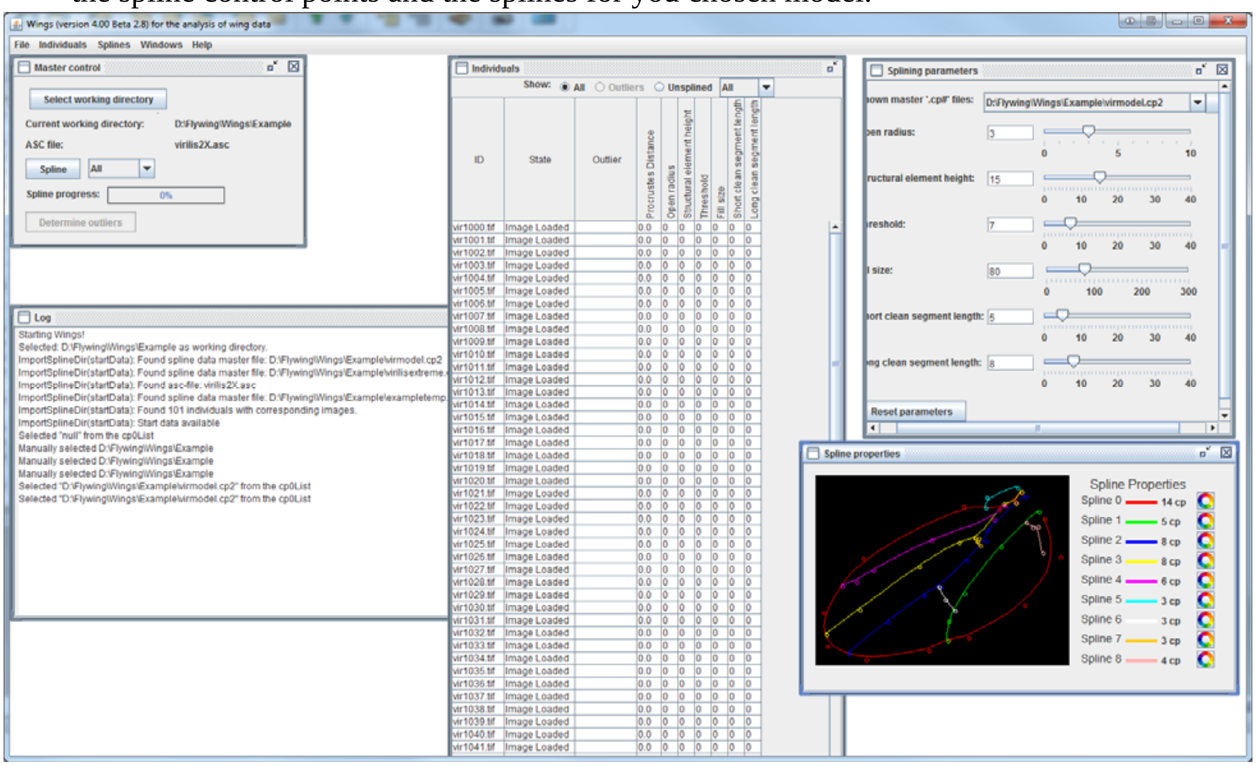

Figure 4. Wings4 screen once the ASC and spline model files have been chosen.

5. You may alter the choice of wings to be splined in the **Master Control** window by choosing from the drop down list next to the *Spline* button. *Fraction* splines a proportion of all images, chosen randomly. This can be useful when you have a large set of images and want to test whether the chosen **Splining parameters** will yield good splines. *All* is the default once some images have been splined, and will respline all the images regardless of whether they have already been splined and edited, losing any previous data. There is currently a bug that wipes out previous spline results when any of these choices are invoked, so do NOT use these other choices without first copying the well-splined cp files to a different subdirectory for later use. *Unsplined* will spline just those without corresponding .cp files. *Selected* resplines just individuals selected in the Individuals window. *Outliers* resplines individuals that have been identified as outliers (see below). Outliers are marked in red in **Individuals**.
6. Click the *Spline* button in **Master Control**. Wings begins splining, and updates the Individuals information to reflect progress. Wings pops up a **Tiff viewer** window to show the quality of splines.
7. The **Tiff Viewer** shows a side-by-side comparison of the splined and unsplined images. You can advance through these rapidly to get a sense for how well the average image is splined. This window is activated as soon as any individuals from your list have been splined, that is while splining is still ongoing. The parameters window is still active, and changes you make in that window will cause subsequent splines to be fit with whatever parameters you set there on the fly. This is a bug, so we recommend allowing the splining to finish before using **Tiff Viewer** for resplining (see below).
8. If you see a wing in Tiff Viewer that is damaged, folded or incomplete, such that it cannot be measured accurately, click ***Reject*** to exclude it from your data set. If you respline, however, Wings will forget past rejections.
9. The wing in the **Tiff Viewer** window will immediately be resplined if you adjust the parameters in the **Splining parameters** window. If you have an incorrectly splined individual (including those wings where splining failed completely), you can rapidly click through alternative parameter combinations and directly observe how this alters splining. This is a good way to go about finding a set of spline parameters that are effective at splining most individuals. Generally speaking, *Open radius* values between 3 and 5 in combination with *Threshold* values from 5 to about 25 are worth investigating, although sometimes the *Structural element height* and the other parameters can be important. Once you are done resplining an image, click ***Next*** to save the results. If you click ***Cancel***, it will discard any splining done in this window, and keep the old spline as the best.
10. To investigate in detail why splining goes awry, and the effects of the parameters on the intermediate results, click on ***Respline*** button. This brings up the **Debug** window showing the results of image processing steps. See the Understanding Splining section below for a detailed discussion of the splining algorithm, and how to interpret the intermediate results displayed. Note that the **Tiff Viewer** window is not updated with any resplining results from this window until it is closed.
11. Once you have done the best you can using splining, you may edit the details of each spline by hand. There are two routes to editing. First, you can right-click each line in the **Individual** window, then select ***Edit*** from the menu, which starts the **Spline Editor** window. Second, you can use the ***Determine Outliers*** button in the **Master Control**

window, described below. In **Spline Editor,** edit by grabbing control points and dragging them to new locations. The effects of this on the splines are updated instantaneously, allowing you to increase the fit to the image. To make full use of the advantages of automated data recovery it is important that your goal in editing should be consistency of results with the way the program works, rather than matching your own ideal. Some editing practice is helpful, as it is often necessary to rearrange several control points to fit a misfit region of the wing. For example, shifting several control points toward areas of high curvature may be necessary, with compensatory changes elsewhere. Currently Wings cannot select blocks of individuals for editing.

12. If there are more than 25 splined individuals of each sex, a rapid route to finding badly splined individuals is by invoking the ***Determine Outliers*** button in the **Master Control** window. Wings runs a robust outlier detection routine based on minimum volume ellipsoids on the whole data set. Wings are ranked by their Mahalanobis distance from the centroid. This routine can take some time complete for large numbers of wings (the ***Determine Outliers*** button will look depressed during this process).
    a. When complete, the **Spline Editor** window will open and display up to 10 wings that are NOT outliers. Do not edit these, as any changes will not be saved. Check these first 10 wings to understand the typical splining behavior for this set of parameters and model file. If you are not happy with the splines for these 10, you should return to the splining stage and alter the model and parameters to improve the typical splines before proceeding with detailed editing of splines.
    b. Once you have examined the 10 'good' wings, click on ***Continue with Outliers*** in the lower right corner of **Spline Editor**. **Spline Editor** now shows the outlier splines from the most atypical to the more typical ones. Above the image is information about how many outliers there are, the number of the current outlier, and why the wing is an outlier. A bug prevents updating of this information. Two separate outlier detection routines are run – one on the landmarks (intersections of wing veins), and one on the outline of the wing (the red curve in **Spline Editor**). A wing is classified as either an outline or landmark outlier, or both, which gives you an idea of where the spline might need to be corrected. The 'outlier value' reflects how unusual the wing is, and is a Mahalanobis distance, the number of standard deviations to the centroid of the sample. Values less than 3 will usually not be worth correcting.
    c. Each outlier wing can be edited by grabbing a control point and dragging it to a new location to increase the fit to the image. Some practice is helpful at this, as it is often necessary to rearrange several control points to fit a misfit region of the wing. For example, shifting several control points toward areas of high curvature may be necessary, with compensatory changes elsewhere. Once you are satisfied with the fit, hit the right arrow button to proceed to the next image.
    d. If you start to see lots of images that do not need correction, you can discontinue your check by clicking the ***Finished*** button.
13. Regardless of whether you completed the ***Determine Outliers*** routine, you should perform multivariate outlier detection in a statistical package to identify unusual wings that have escaped your editing. CPR, our cp reading program provides basic visualizations useful for detecting major outliers. Manually check at least the most unusual outliers as described above.

14. When done, exit the program using the File Quit command to ensure that all the information is properly saved.

Understanding spline fitting

Successful splining requires three elements: a good image, a good spline model, and image processing parameters appropriate to the image. To understand what makes image, model and parameters good, it is important to understand how Wings fits a spline model to a wing. There are two basic parts to fitting: orientation of model to image and then optimization of fit.

The orientation step tries to make a crude match of the model (from your cp# file, your 'ideal' spline) to the actual wing image. The initial orientation landmarks recorded when you take the wing image, and recorded in the ASC file provide Wings with the correct orientation of the wing. The next challenge is to find a vein intersection that is as far away from the orientation landmarks as possible. Once this distal intersection point is chosen, Wings scales and rotates the model relative to the image. If the distal intersection is either landmark 2 or 3, then all is well. If not, then the model and image will match poorly and optimization will probably converge on the wrong answer.

All of the parameters in **Splining Parameters** are involved in the orientation step. To see their effects, it is best to look at images in the Debug window, which is accessed by right-clicking an image in the **Individual** window, or clicking ***Respline*** from the **Tiff Viewer**. The window looks like:

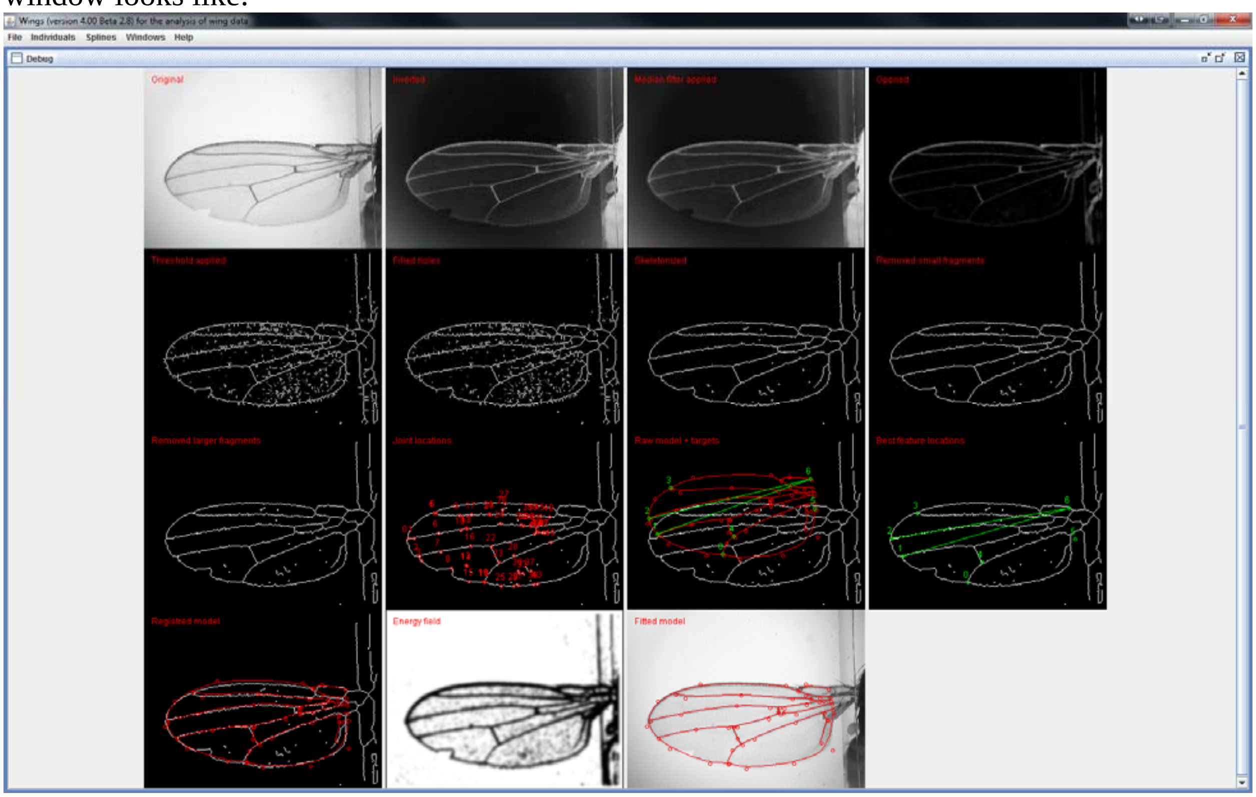

Figure 5. Debug window, showing the stages in orienting the model to image, and the fitting of model.

The program steps in orientation are:

1. Inverting the image (black becomes white, white black),

2. Application of a median filter. Is this under our control? It probably should be, as depending in the image size this could make a big difference. *Structural Element Height* would make sense as the parameter here, but it doesn't change the median filter image, but does change the Opened image. Can you clarify what it does?
3. An open operation, which both erodes messy edges of areas distinct from background, and the dilates the remaining foreground to match a pre-determined size square. The size of the square is controlled by the *Open Radius* parameter.
4. Thresholding remaining foreground areas that are above the intensity of the *Threshold* parameter.
5. Skeletonizing the white areas to single pixel width.
6. Filling small holes between fragments of white. Is this under our control?
7. Reskeltonizing.
8. Removal of short fragments of white.
9. Removal of longer fragments of white.
10. Detection of joint locations – places ether two white lines intersect.
11. Wings then chooses the one or two intersections that are farthest from orientation landmark 1.
12. The model is then scaled, translated and rotated so that model landmark 6 is placed at orientation landmark 1, and model landmarks 2 and 3 are place on the farthest joints detected in the previous step.
13. An energy field is derived from the filtered image by detection of probable edges, and diffusion of such likely edges to provide a gradient from dark to light areas that extends a substantial distance away from the actual edge location.
14. The model control points are moved to increase the overlap of the spline curves with the dark areas energy field. When this process reaches a local optimum, the spline fitting is done. *Structural Element Height* has an effect here as well as above, but how is the energy field actually generated?

In the end, there are four major modes of failure to spline.

First a set of parameters will not spline successfully if the structure of intersecting lines at the distal end of the wing is not detected. Check this in the Joint locations image in **Debug**. Any of the operations prior to joint detection may cause this loss of information.

Second, failure may result from noise in the distal parts of the images that cause false joints to be detected, say in the corner of the image, causing the splines to optimize on non-wing elements. Check this in the Joint locations image in **Debug**.

Third, if the energy field is either too far from the corresponding part of the superimposed model for a dark area to overlap the correct curve, or if a model curve ends up too close to the wrong part of the energy field, it will be attracted to the wrong solution.

Using a good starting spline model is one of the most important steps to getting repeatable, accurate spline fits. For some data sets, choosing a wing where the splines fit well, as in Figure 1, is sufficient. However, for many other data sets, the nature of the energy field in the images will lead the spline routine to converge on the same type of improper solution, over and over. In such cases, it is often effective to choose a starting model that deviates from a good fit in one direction or another. Figure 6 shows such an 'extreme' model.

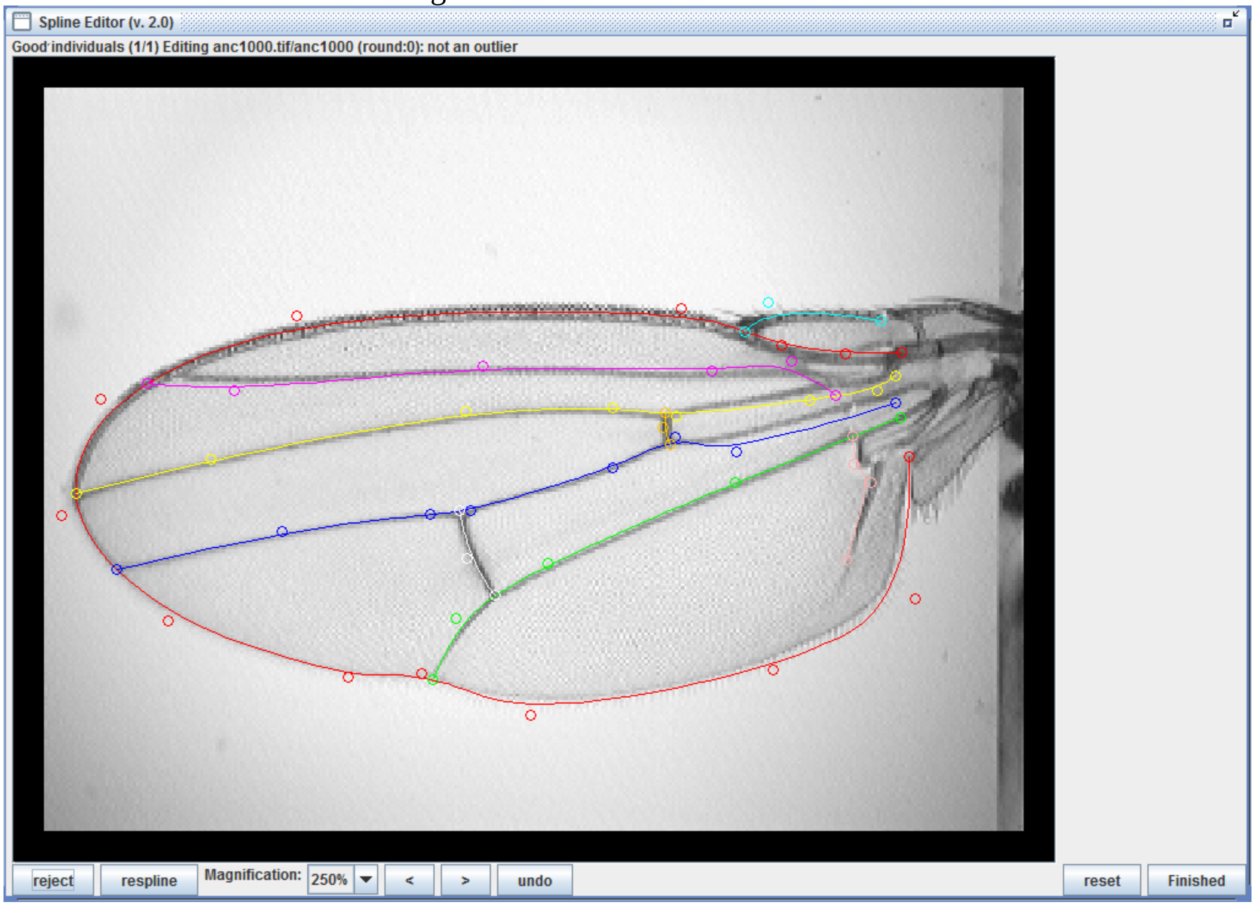

Figure 6. Spline Editor window showing a set of splines altered to serve as an extreme model file. When finished editing a spline model to use as a model, right click on the corresponding individual in the Individuals window, and choose ***Save as model file***. Choose a file extension .cp# where # is a single digit, so the Wings will subsequently recognize that you intend to use it as a model.

This model would prevent, for example, vein 3 from being attracted vein1, vein 4 from being attracted to 3, and the outline curve from being attracted to vein 6.

The parameter most likely to change and potentially improve splining behavior is the threshold value. The default is 7, but raising the value as high as 25 or even more is sometimes helpful. Open radius is sometimes worth changing from 3 to maybe as high as 5. Structural element height is sometimes worth changing. The remaining parameters rarely seem to have much effect, but you should experiment with them for your data if you are having difficulty getting good splines. Usually a single set of parameters will do well for all the wing images recorded under the same lighting conditions. If a block includes more than one lighting setup, then actually splitting the block for processing, once you find the boundary of each set could be a reasonable alternative.

Spline data format

The Wings program will create a .cp file with data from each of the wings. The first part of a .cp file looks like this:

```
# Image: crossb1573.tif 286.5 79.5 288.0 125.0 Yunily 03/04/02 Wed_PM F39-21xM39-54v2
M 0.006985684.0    Resolution: 1.000000 1.000000
9
14
287.936387 123.536901
280.173680 147.223963
243.396758 173.045201
182.313180 178.688463
155.460223 171.009150
127.945452 168.800180
78.535084 149.530000
50.550815 113.646119
67.005519 77.287631
129.405146 54.736720
230.262215 65.291896
255.874894 82.691561
267.957581 89.166542
286.575417 92.234599
5
153.337939 171.337700
165.054074 155.883137
189.648997 137.097543
242.659892 118.950596
287.398487 111.165197
8
65.674047 133.003511
.
.
.
```

The first line of the cp file just copies over the information about this wing from the .ASC file. The second line (with just the number 9 on it) gives the number of spline curves to follow. The next line gives the number of control points on that spline (14 in this case). The next 14 lines give the *x* and *y* coordinates. There follow additional blocks giving the number of coordinates, then the *x*, *y* pairs. Coordinates are in pixels.

Our program for reading and working with spline data from .cp files is called CPR, short for CPReader. You can download it and documentation on it from http://bio.fsu.edu/~dhoule/Software/.

Example data set

Included in the distribution is an example data set of 100 wings from a *Drosophila virilis* population, two version of the ASC file, and two example starting model files. The file virilis.asc has the actual pixel coordinates and scale in pixels/mm of the starting points appropriate to the size images furnished. The file virilis2X.asc has these coordinates adjusted for images twice as large in both dimensions, and the scale reduced appropriately. Counterintuitively, it is the virilis2X.asc file that needs to be used for splining.

The two starting model files are named virmodel.cp2 and virilisextreme.cp2. Try splining with each model file. When virmodel is used, the proximal veins are usually misplaced, despite the fact that this model fit one wing quite well, as in Figure 1. When virilisextreme.cp2 is used, spines recovered are usually very good, despite the fact that virilisextreme fit its wing similarly to Figure 6. This is useful to see how critical the choice of a model file is.

B-splines used in Wings

Splines are piecewise polynomial functions parameterized with the spatial locations of points. We use a parameterization based on *control points*, which are not necessarily on the curve that is being represented. *Knots* are points on the curve that are the mean position of adjacent pairs of control points. Piecewise means that the full curve is a combination of *n* short curves that are interpolated based on the subset of the control points that are nearest the section of curve being reconstructed. Assume that we have a curve running generally left to right. The control points are numbered sequentially from left to right from 0 to *n+1*. Wings uses a quadratic parameterization that uses the three closest control points to generate the curve. For example, consider the *i*th knot that is the mean of control points *i*-1 and *i*. To the left of this knot, the three control points used for interpolating the curve are *i*-2, *i*-1 and *i*. To the right of this point, we use points *i*-1, *i*, and *i*+1. Choosing a higher number of control points to represent a curve allows it to have a larger number of changes in curvature, that is to be more wiggly. The number of control points for each curve on the wing was chosen so that the resulting curve fits well over a variety of wings. Simple curves, such as the cross-veins, are represented by just three control points, while the more complex outline of the wing takes 14 control points.

Given the set of control points $\left\{ \left(c_{x0}, c_{y0}\right), \left(c_{x1}, c_{y1}\right), \cdots, \left(c_{x(n+1)}, c_{y(n+1)}\right) \right\}$, the corresponding knots are

$$k_{x(i)} = \left(c_{x(i-1)} + c_{x(i)}\right)/2$$
$$k_{y(i)} = \left(c_{y(i-1)} + c_{y(i)}\right)/2.$$

The *i*th curve segment is runs from knot *i* to knot *i*+1. The location of the point $\left(x_{i\bullet p}, y_{i\bullet p}\right)$ that is proportion *p* of the length between knot *i* and knot *i*+1 is

$$x_{i\bullet p} = c_{x(i-1)} w_1 + c_{x(i)} w_2 + c_{x(i+1)} w_3$$
$$y_{i\bullet p} = c_{y(i-1)} w_1 + c_{y(i)} w_2 + c_{y(i+1)} w_3, \text{ where}$$
$$w_1 = \frac{(1-p)^2}{2}$$
$$w_2 = \frac{1}{2} + p - p^2$$
$$w_3 = \frac{p^2}{2}.$$

Each segment of curve can be reconstructed to the degree desired by sampling *p* in the interval $p = 0$ to $p = 1$, with the extreme values 0 and 1 returning the *i*th and *i*+1th knots respectively.

Wings' output returns the first and last knots (the actual ends of the curves) instead of the 0th and *n*+1th control points. This is convenient as the ends of many of the curves are Type I landmarks that can be used directly in geometric morphometric analyses. To actually compute curves (semi-landmarks in geometric morphometrics jargon) from Wings output, these end knots can either be converted to their corresponding control points as

$$c_{x0} = 2k_{x1} - c_{x1}$$
$$c_{y0} = 2k_{y1} - c_{y1}, \text{ and}$$
$$c_{x(n+1)} = 2k_{x(n)} - c_{x(n)}$$
$$c_{y(n+1)} = 2k_{y(n)} - c_{y(n)}$$

or the weights of the 1st and last curves adjusted to compensate for the combination of knots and control points.

Authorship and support

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References

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Van der Linde, K., and D. Houle. 2009. Inferring the nature of allometry from geometric data. Evolutionary Biology OnLine First.

Wings issues – for KIM

1. Error message about errors in ASCII file disappears, leaves user unable to tell why the splining is not possible. Put a note in the LOG window about the errors in addition to the pop-up.
2. Sometimes sex of an individual is not known. Relax the error checking on this to allow other codes than M and F – perhaps just a U with a note to that effect ('Error in sex – permissible values are M, F or U.') when there is an error.
3. Once directory is chosen, the Splining parameters window gets resized so that the navigation menu for choosing the cp2 file is obscured, and only part of the window can be seen at one time. This window cannot be resized.
4. NASTY BUG. I splined everything using one set of parameters, then resplined a section of the files using Spline Selected. All cp files are wiped out, so only the resplined flies were saved, losing hours of editing work.
5. It appears that returning to a directory that has been splined the Individuals window does not detect that previously splined files have been splined. After above, I do not dare use the respline Unsplined option to again respline everything. Also, when I reenter a subdirectory I have worked in, but where I have removed the cp files (for protection!) the State column in the Individual window says there is 'Data & Image' but I don't see any data.
6. Tiff Viewer crashes. Once when using Spline Fraction, and several times when doing complex reedits. Once crashed it didn't update when the next or previous buttons were pushed, or close when its X was pushed. I had to close and reopen the program to get it back.
7. Go to image number in Tiff Viewer does not work.
8. TIFF viewer does not update with a resplined image until the Debug window is closed. This is confusing and counterintuitive to the uninitiated, but perhaps not quite a bug.
9. The 'Threshold Applied' window in the Debug window should be called 'Threshold and skeletonized'
10. What does a yellow line on the Debug images mean? It only appears occasionally.
11. Images that have been 'splined' but have no model fit still show up in the Individuals window as Splined. If they are marked unsplined, they can be resplined without losing all the information about successfully splined images.
12. Rejects should not be cleared by resplining, at least by default.
13. The Tiff Viewer window allows resplining while the batch splining is still happening, the parameters are changed in the batch, as well as the image current in Tiff Viewer. The result is that many of the batch images get resplined with weird, bad parameter values. This should be corrected by either remembering the batch parameters separately from what is applied from Tiff Viewer, or by not allowing editing until the batch job is finished.
14. It does not seem possible to start the Tiff Viewer except when the program does upon splining. Can you make the Tiff Viewer invokable from the menu or by right-clicking an individual?
15. Cannot Determine outliers on return to a directory splined previously. Also cannot determine outliers after splining a subset of wings.
16. Can you have Reset Parameters take you back to other than the program default?

17. Edit from the Individuals window only does one wing at time, so it is a lot of clicks to look at everything. For a species like sylvestris that never splines completely correctly, this is really annoying.
18. When I have spline only some groups of flies, Edit only seems to work for the numerically first block of splined flies. I can rescue this behavior by editing out the unsplined individuals from the ASC file, but since the names of files match, this seems unnecessary.
19. Is the grabbing of points to edit behavior under your control? Some points seem much harder to grab than others. For example, the proximal crossvein points are really hard to grab, as the program seems to default to grabbing vein III and IV points in the neighborhood. I often have to completely move the yellow and blue points out of the way before I can grab the orange ones. Is there a way to say send a particular control point to the background, so the others in the neighborhood are more grabbable?
20. The show outlier window does not change the information about each individual as you go. Because of this bug, I can't tell what the behavior of the program is. What is the 'show' behavior precisely? Does it show all individuals in unusualness order? I have a species where there are enough males, but not enough females. Does it show only the males?
21. Implement an unusualness order for all individuals using Mahalanobis distance for data sets with too few for MVE.
22. The undo and reset buttons in Spline Editor do not appear to function.
23. The virilis example data we have been distributing with Wings does not work, probably because of a guide landmark coordinate mismatch. Can you construct a working version?

~~24.~~ Is memory still a problem? User manual item on memory and how to solve problems. Preset memory limit that the user can adjust upwards at their own risk.

25. Memory limitations. Sensible warning behavior when memory is exceeded.

Things that might be nice in the optimal world

26. Model creation module is not done.
27. General asc file column reading solution.

CPR issues for Eladio:

1. Will this run on a Mac?
2. What is the Soft point factor, and what are soft points?
3. Sample entire outlines/veins is not implemented.
4. Slide points over outlines/veins is the only sliding button implemented. It seems that this description of the button does not match what we do. Don't we slide over segements? That button is grayed out and not active.
5. What are the deformations in the right window relative to? One particular image or the mean?
6. Can data be imported, once it is read in, aligned and exported? That is, do we have to get everything we need from one Sample and Slide? Or can we return to the data, and get more information later? It sounds like maybe this is what the Data pull-down menu is doing. Can you explain?
7. What happens when you use the Use outline Superimpostion, when just landmark data is chosen in Curve Sampling?
8. Could we implement an estimate of sliding time, so that the unaware user does not start a days-long procedure without a warning?
9. New Wings4 works from full-size tiff files, rather than reducing them to the same size. The Show Picture option in CPR shows the reduced-size model superimposed on the full-size image.
10. Precisely what centroid size value is output in each file? Does it vary by superimposition technique?