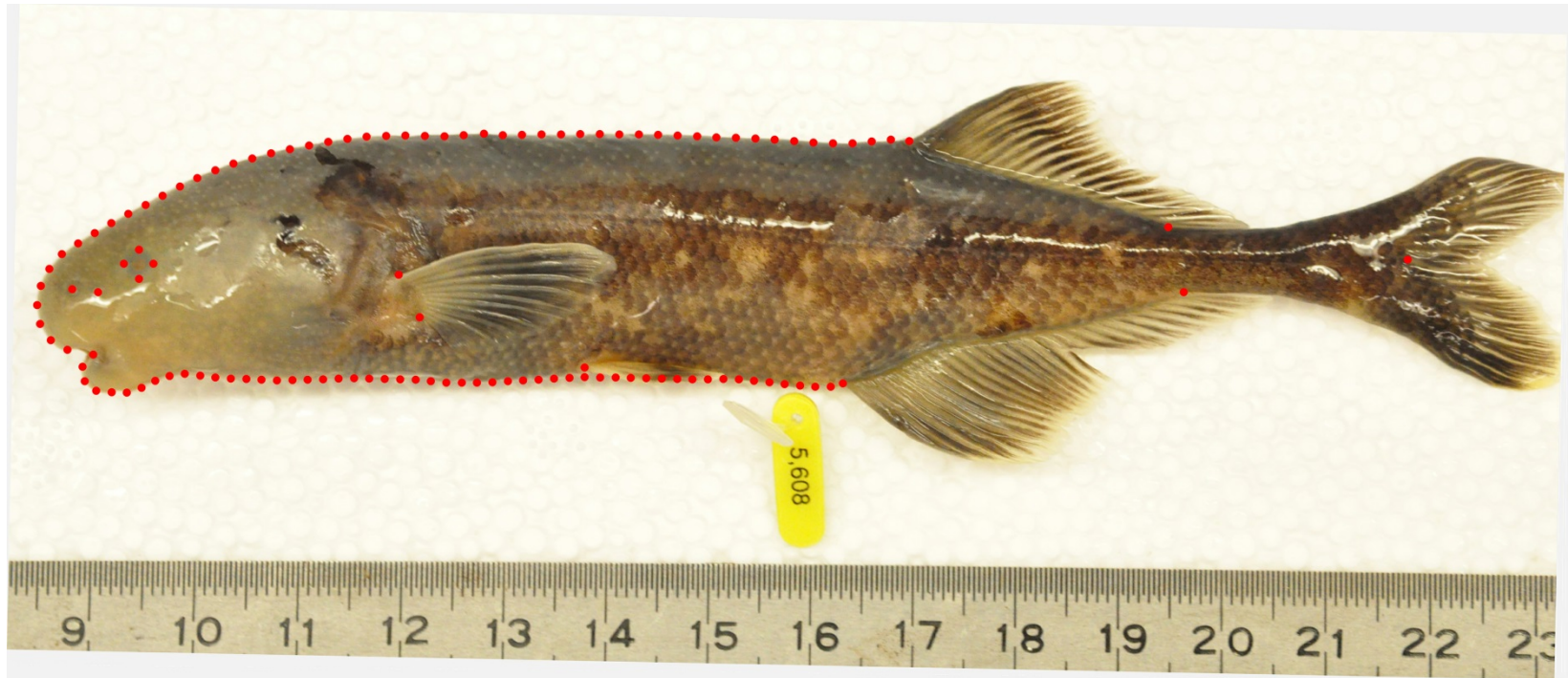


# A researcher's perspective



MIRIAM ZELDITCH  
DONALD SWIDERSKI  
UNIVERSITY OF MICHIGAN

# Image analysis: Getting quantitative morphological data from images



**You have a lot of issues to work out.  
We want to give our perspective on what matters to researchers who will use the images to collect morphological data**

- What we will do with the images
- How we collect data from them
- What makes images scientifically useful (or useless)



# Outline



- **A general overview (2D data collection)**
  - How we collect the data
  - What makes the data useful (or not)
- **Special issues raised by 3D imaging**
  - Don will talk about these
    - ✦ He sees in 3D and works on 3D data...

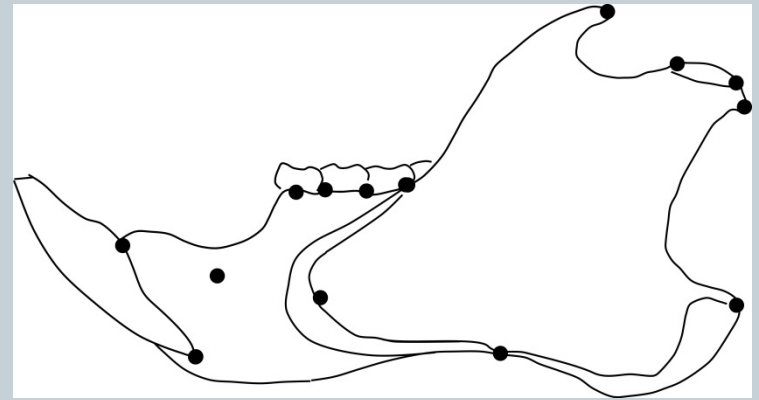
# We are mammalogists



- Most examples will come from our studies of mammalian skeletal material
- For reasons important to this talk, we can't use examples from older work on fishes
- But we will get to one issue specific to fluid-preserved specimens
  - Bending

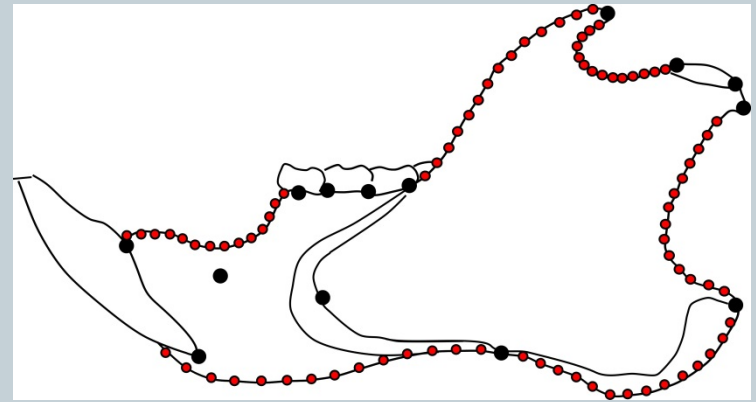


# Landmark data



Discrete anatomical loci that correspond from specimen to specimen (and species to species).

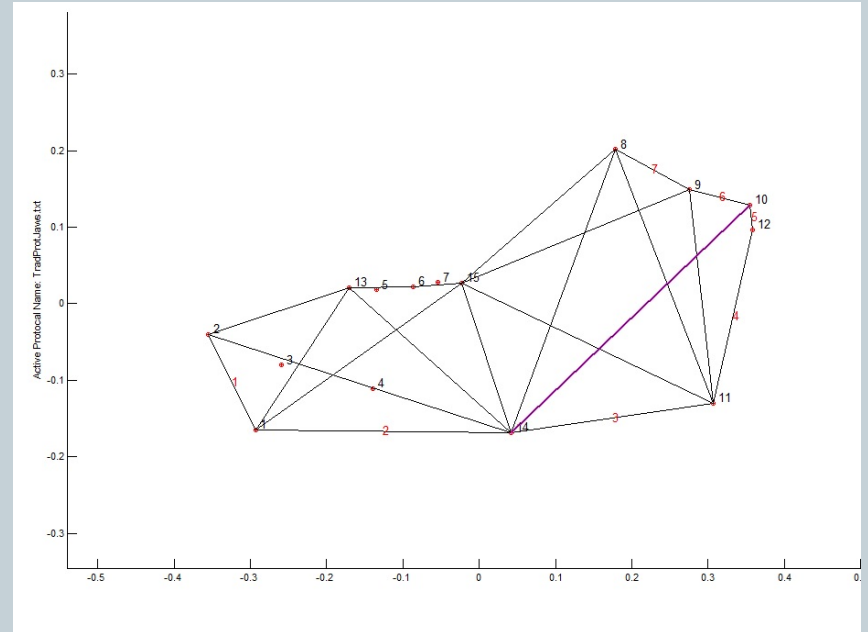
# Semilandmarks



When landmarks are not enough we sample curves (and surfaces) with semilandmarks

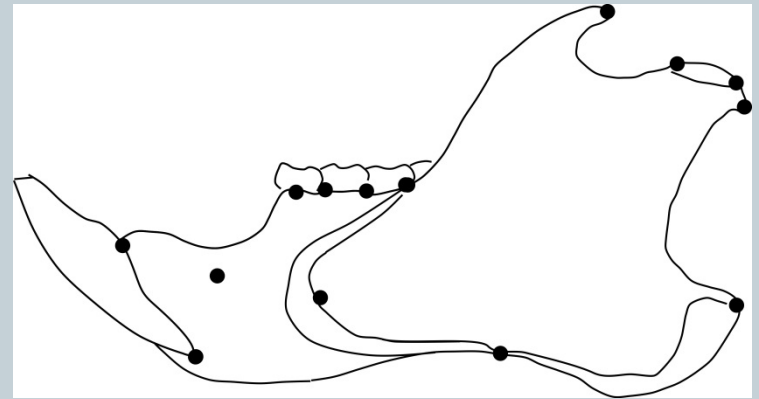
# “Traditional morphometric data”

- In the 1990’s, it was common to use landmarks as the endpoints of length measurements
- So after digitizing the landmarks and a ruler, the data were used to measure lengths



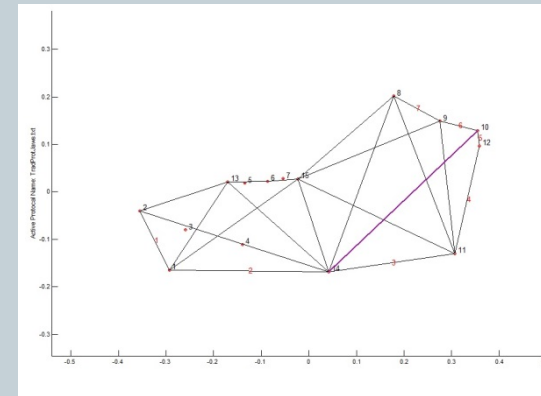


# Current view of “traditional”



Young researchers now seem to use  
“traditional” for data limited to landmarks  
Semilandmarks are “non-traditional”

# Current view of “traditional”



This matters because what we need from the photographs are accurate estimates of the full set of coordinates landmarks and semilandmarks (plus a ruler)

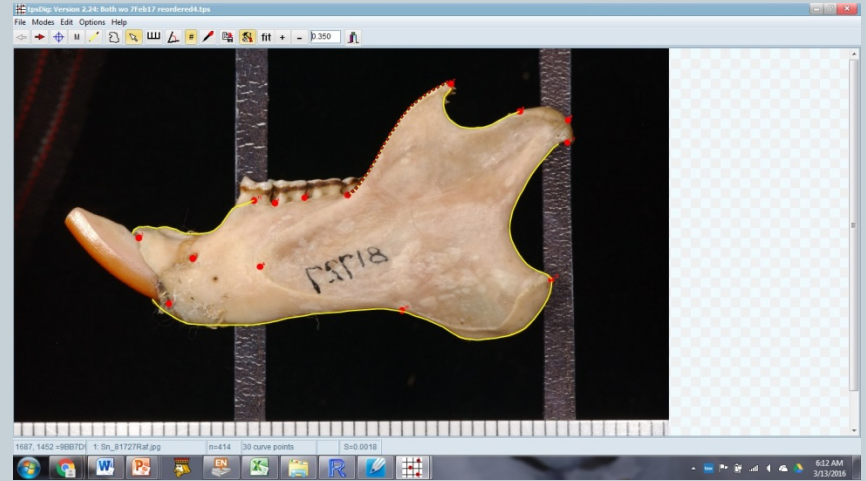
# First Steps



- **Process images**
  - Crop
  - Enhance (brightness, contrast)
  - Reflect (if some have noses pointing left, others right)
  - Label the photos: the names of the photos will be the names of the specimens in the data file

# How do we collect the data?

- We read in the photos to a digitizing program
  - tpsDig



# Some of the file types that tpsDig can open

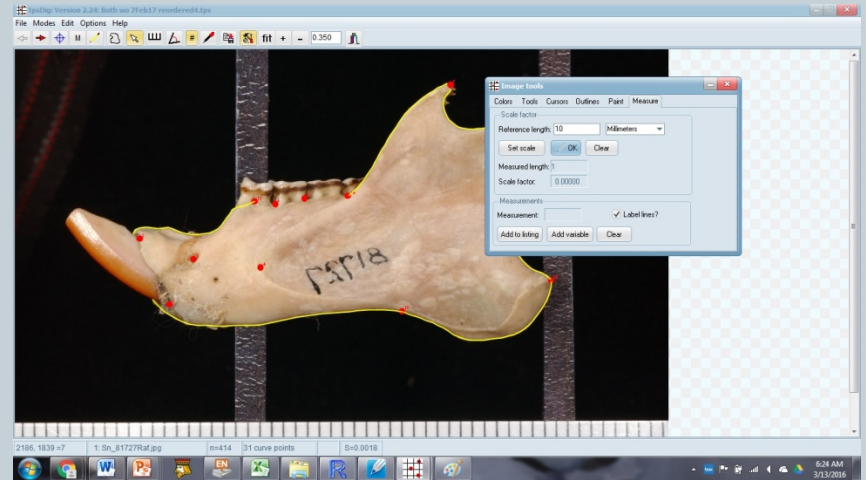


- **TIFF, JPG, GIF, PCX, BMP, PNG, WMF, EMF, AVI**

# How do we collect the data?

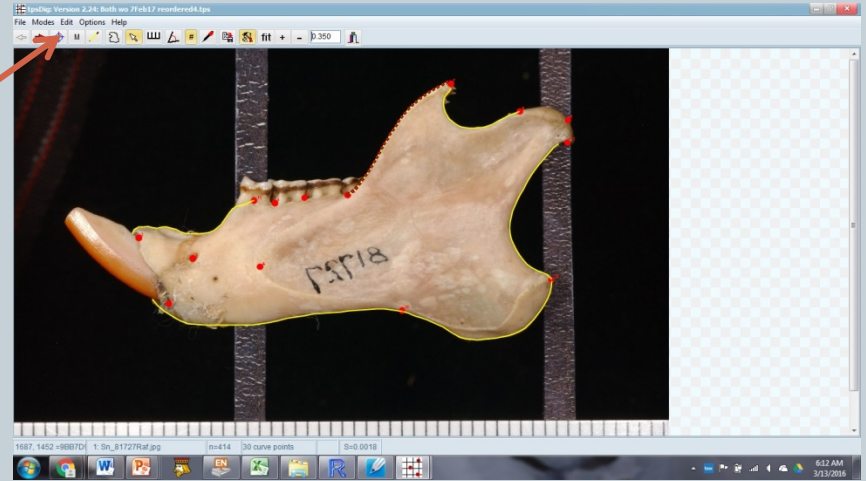


- We want to record information about the scale of the image
  - These were photographed at different magnifications



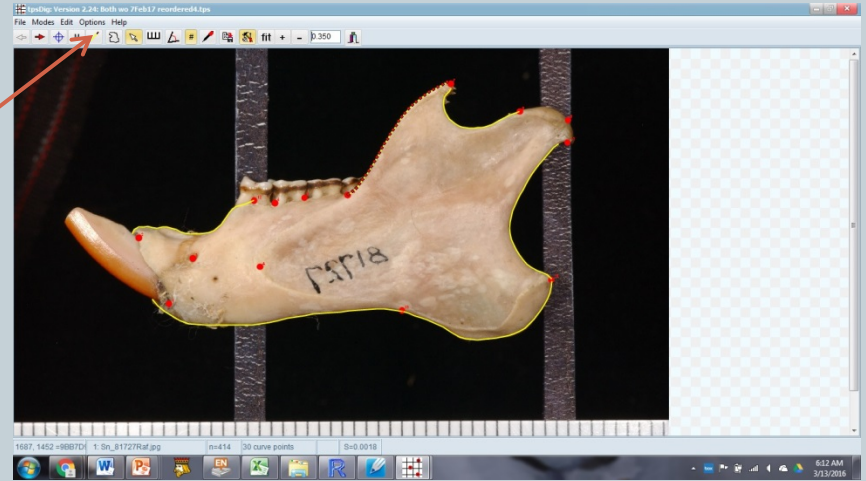
# How do we collect the data?

- Using one tool, we select the landmarks



# How do we collect the data?

- Using another tool, we select draw a curve between the landmarks

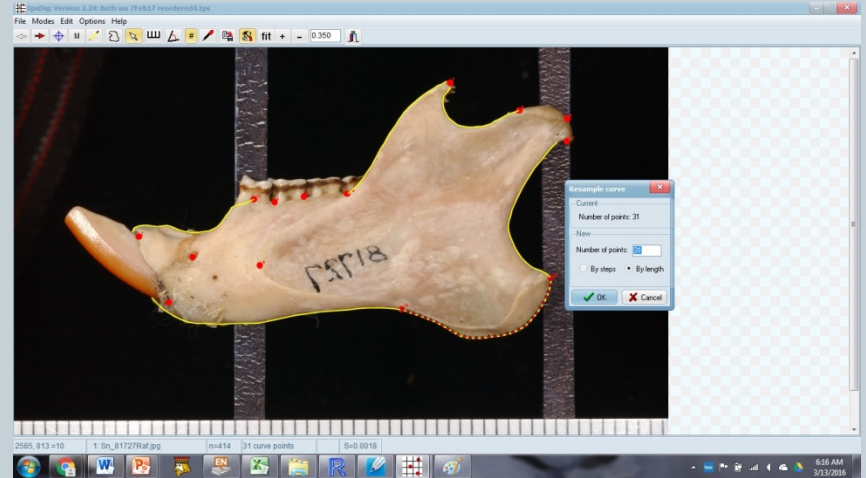




# How do we collect the data?

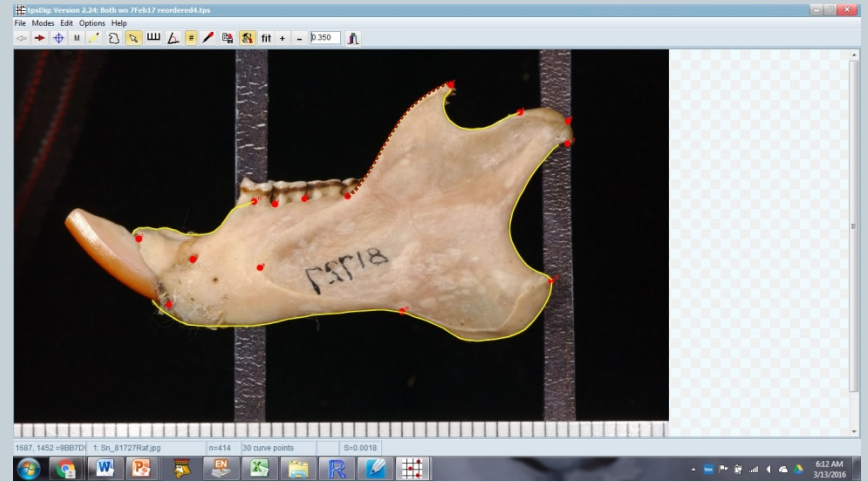


- **Right click on a curve**
  - Say how many points you want
  - You get that many (evenly spaced)



# How do we collect the data?

- Go to the next specimen and repeat
  - In this case, ca 1600 times



# What the data file looks like



LM=15

298.00000 233.00000  
223.00000 348.00000  
330.00000 306.00000T  
430.00000 305.00000  
427.00000 413.00000  
479.00000 413.00000  
507.00000 415.00000  
746.00000 571.00000  
837.00000 523.00000  
925.00000 506.00000  
859.00000 243.00000  
932.00000 481.00000  
393.00000 411.00000  
582.00000 237.00000  
534.00000 416.00000  
IMAGE=Cn\_FMNH\_34134La.tif  
ID=0  
SCALE=0.004033

The LM=15 means that there are 15 landmarks  
The coordinates are in two columns,  $x$  then  $y$   
The name of the image is the name of the  
photograph file  
The ID is the order of the specimen in the file  
(starts at zero because C begins numbering at  
zero)  
The scale factor is the last line  
This is all there is to the file

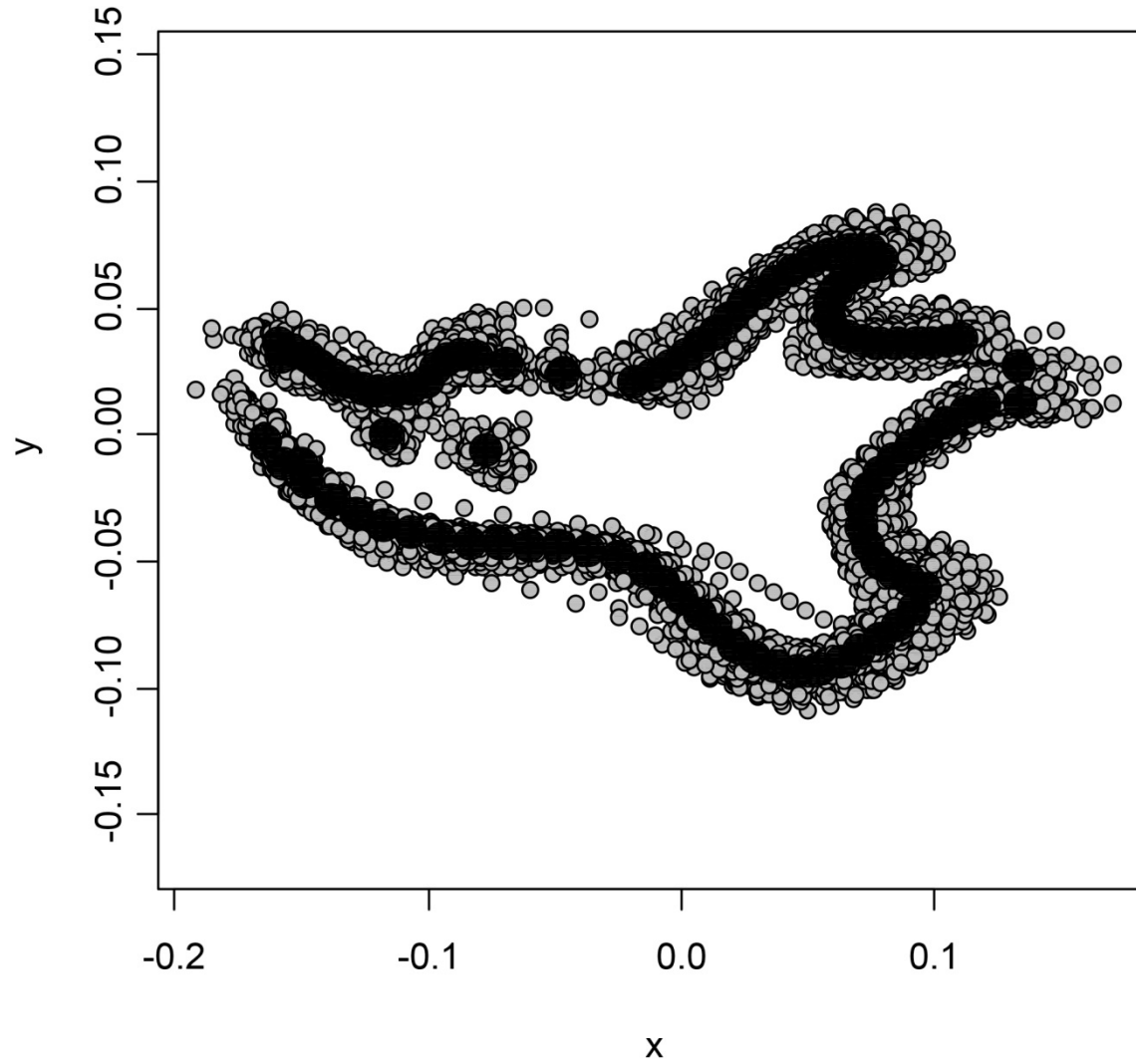
# What do we do with the coordinates?

- While digitizing, we did not worry about magnification, position or orientation of specimens within the picture plane
- Variation in all of those will produce variation in the coordinates
- We need to remove that nuisance (non-shape) variation
- “Size” (scale) is part of that nuisance variation but we want to keep it because size is also important

# Superimposition

- “Superimposition” is the process of removing non-shape information from the coordinate data.
- The method comes directly from the mathematical definition of shape
- As of 1993, morphometricians stopped arguing about how to do this correctly.
- Presently, there are two methods for superimposing semilandmarks but not much argument about it.

# Superimposition



# Assumption



- All the variation is due either to shape or to those three nuisance parameters
- Any variation that is not in those nuisance parameters will remain after superimposition
- Variation due to inconsistent orientation within the picture plane is a nuisance (and a real pain for the digitizer)
- The real pain is that variation of the picture plane will be treated as real variation in shape

# Variation within the picture plane





# But we can fix that



- **We can open the images in a photo editor and rotate them to a common orientation**

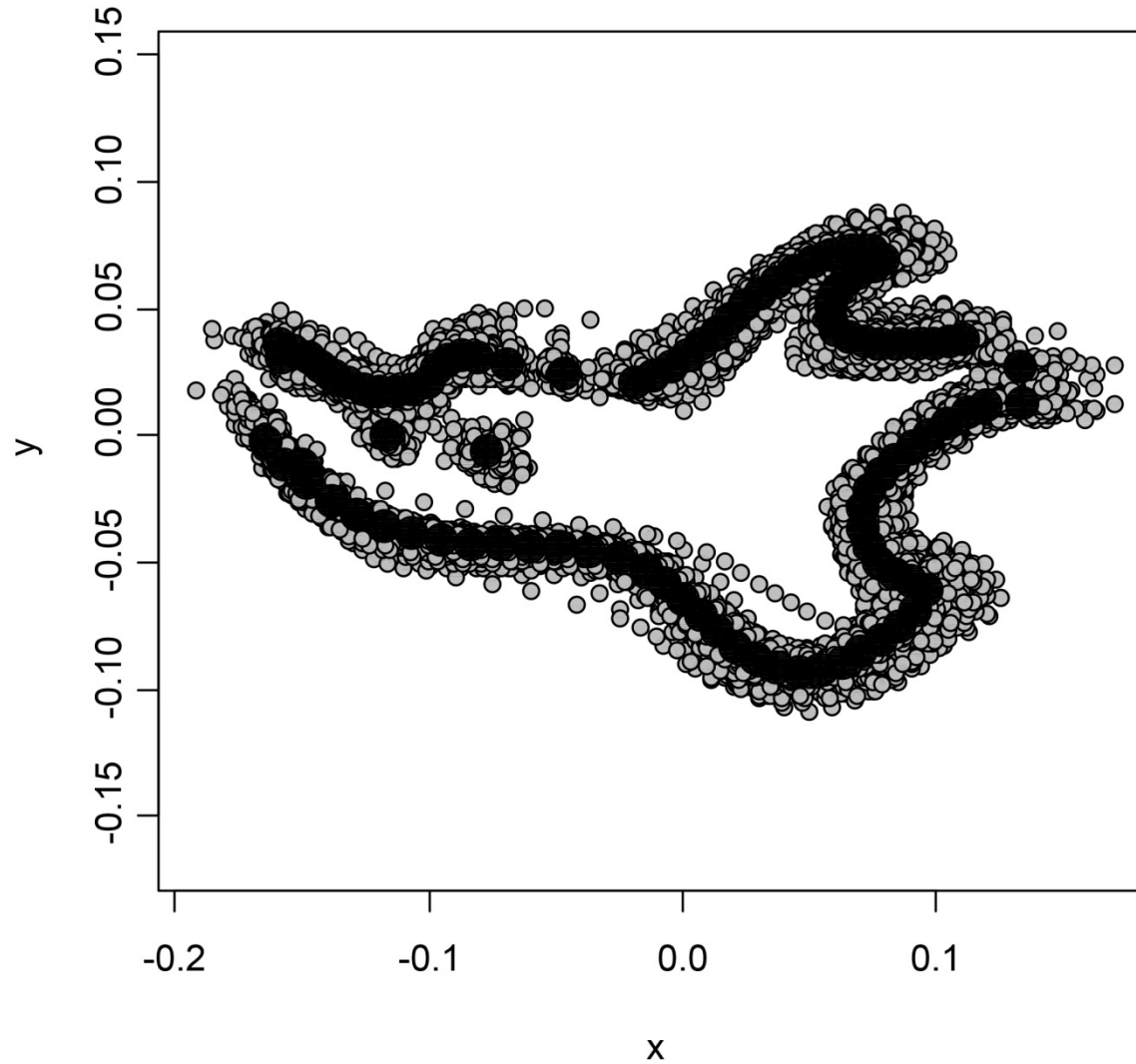
# What we *cannot* fix



- Variation of the picture plane
- Landmarks or curves obscured by tags...



# Data: Configurations



# Data: Configurations

- From each specimen we get one datum: its configuration of points
- Landmarks and semilandmarks
- If landmarks are missing, that specimen's data are not within the same mathematical space as the others
- We can estimate missing landmarks, but many researchers may be unwilling to do that

# What can we do with superimposed configurations?



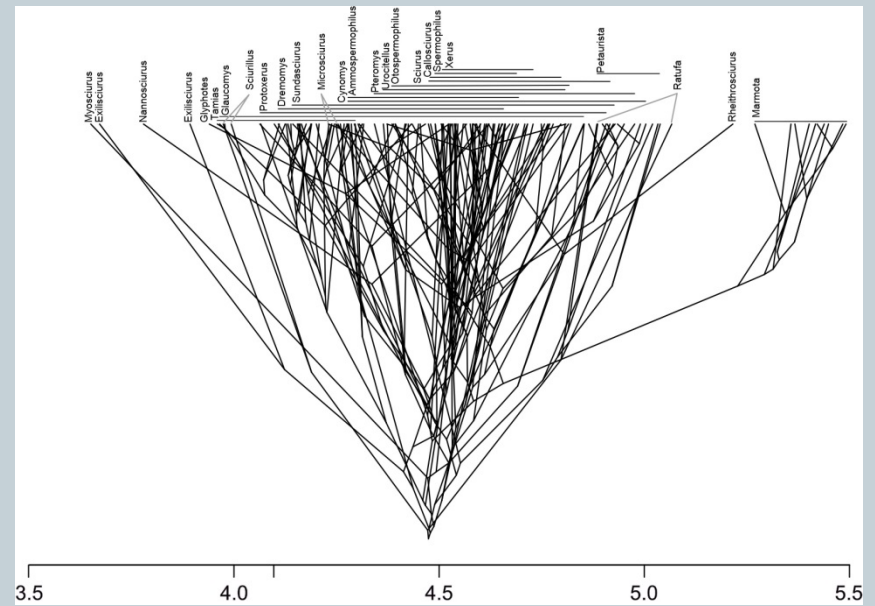
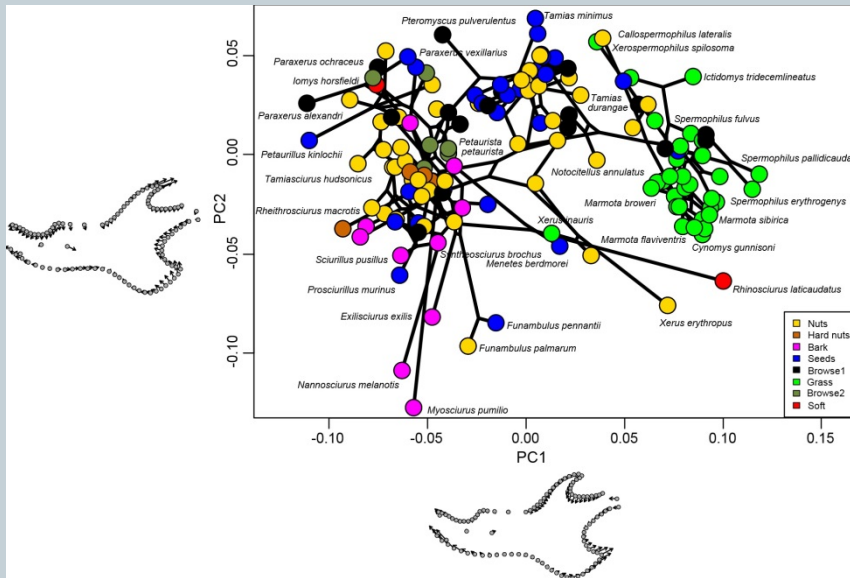
- **Test hypotheses about shape**
  - **Example: Does the evolution of dietary niches explain the evolution of jaw shape?**

# Effects of size and diet on jaw shape



<b>Effect</b>	<b>Df</b>	<b>SS</b>	<b>MS</b>	<b>R<sup>2</sup></b>	<b>F</b>	<b>P</b>
Size	1	0.029	0.029	0.080	18.716	0.001
Diet	7	0.090	0.016	0.256	8.582	0.001
Size x Diet	7	0.045	0.007	0.115	3.850	0.001
Residuals	129	0.243	0.002	0.450		
Total	144	0.441				

# Graphics: Phylomorphospace and traitgram



# A fish example...



- I wish I could have drawn all my examples from fluid preserved specimens...



# They do present some special problems

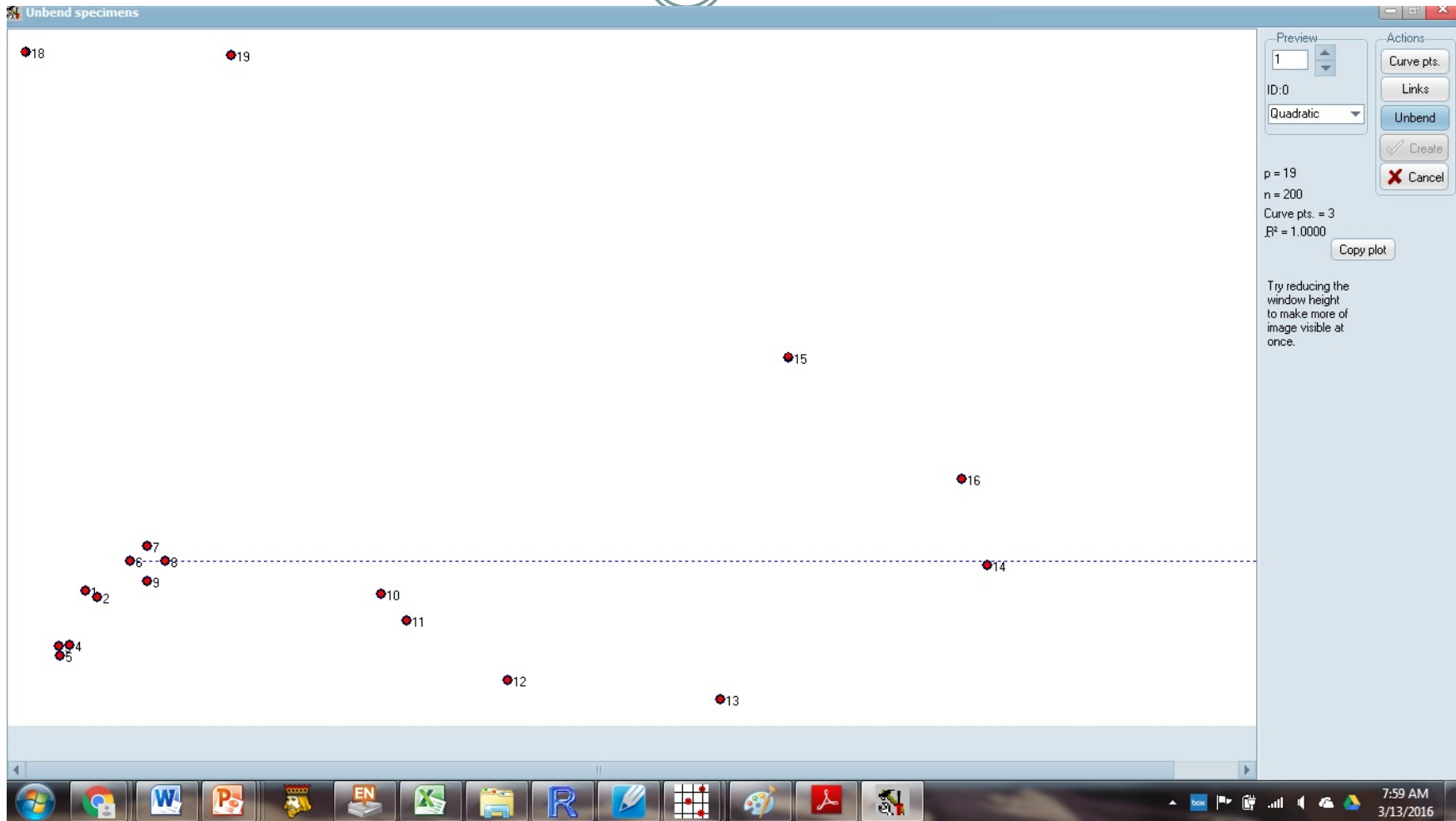


# Preservational artifacts

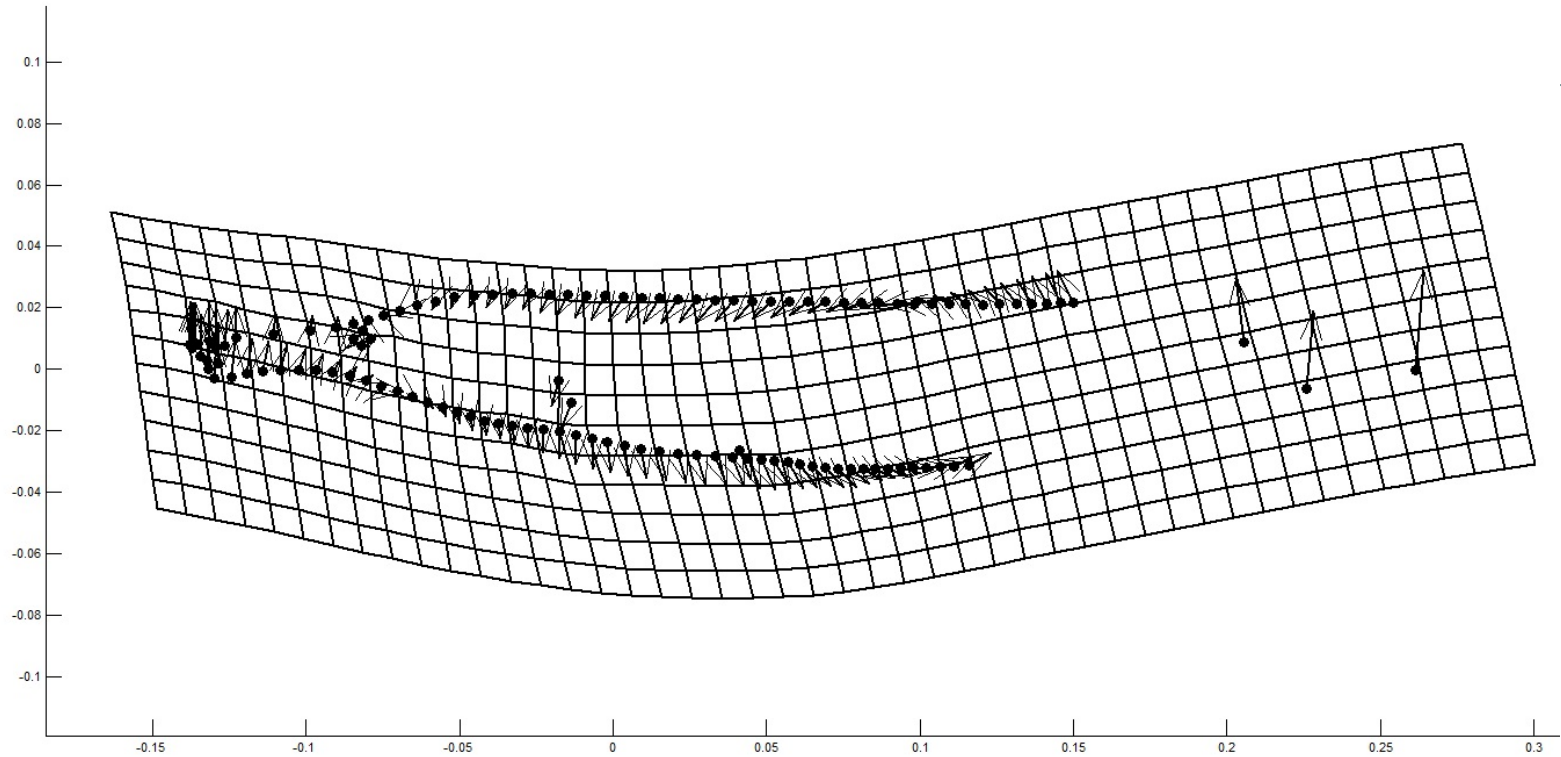


**Bending along the body axis is the main source of variation in most of the samples**  
**That is actually convenient because it gives us a simple way to remove that artifact**  
**It works better than the alternative...**

# Removing bending: Align



# Removing bending: Regression



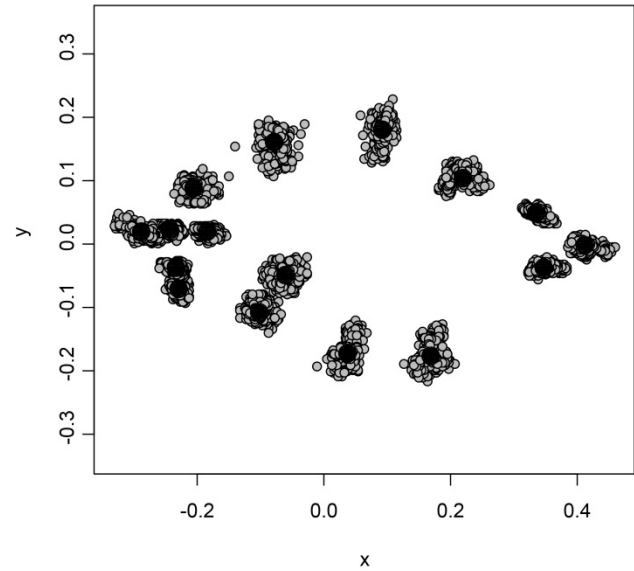
Principal Component 1: Bending

# Removing bending



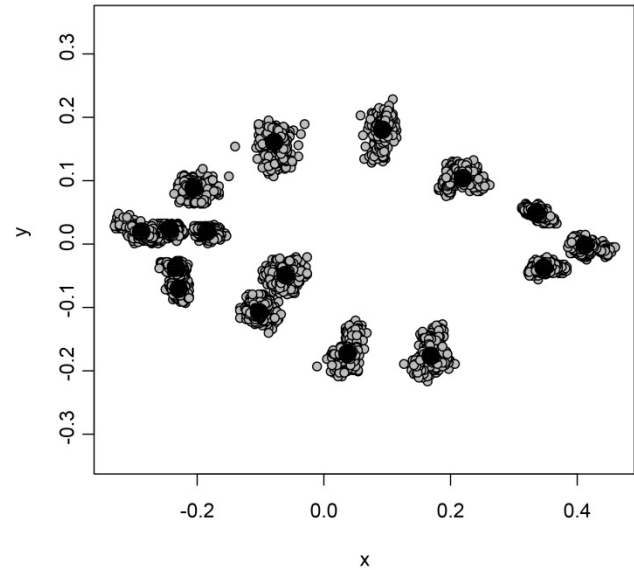
- We can either remove PC1 from the data (but that leaves us PCs rather than coordinates)
- We can statistically remove the variation along PC1 from the coordinates
  - Regress on PC1 scores, add residuals from the regression to the mean

# Ending with a fish example



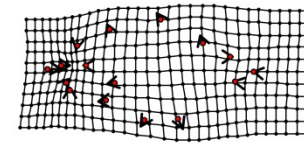
# Part of an example

- **Ontogenetic series from nine species**
  - Compare ontogenies
  - Remove variation within species due to ontogeny
  - Look at variation at youngest and oldest ages

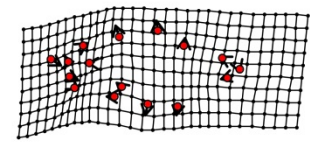


# Comparing ontogenies

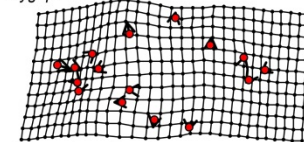
- All species differ (statistically significantly) in their ontogenies



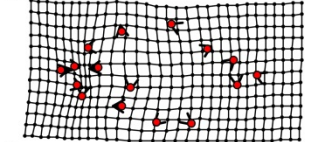
*Pygopristis denticulata*



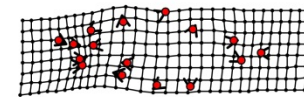
*S. manuelei*



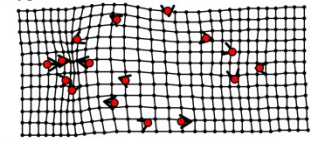
*Serrasalmus altuvei*



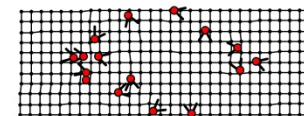
*Pygocentrus cariba*



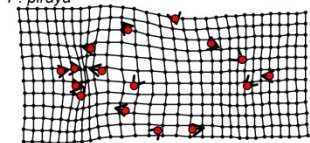
*S. elongatus*



*P. piraya*



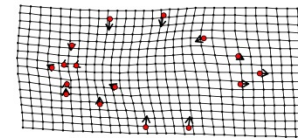
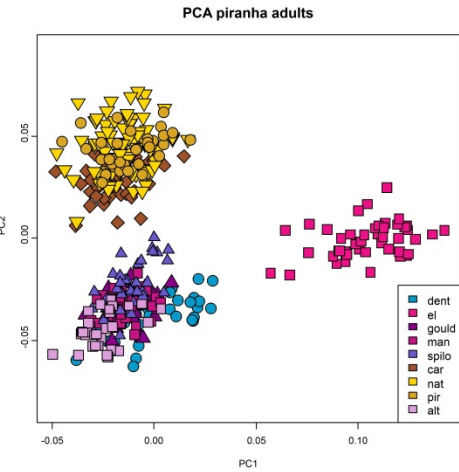
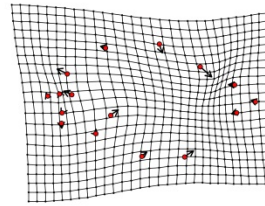
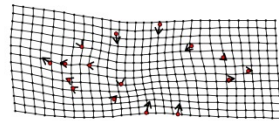
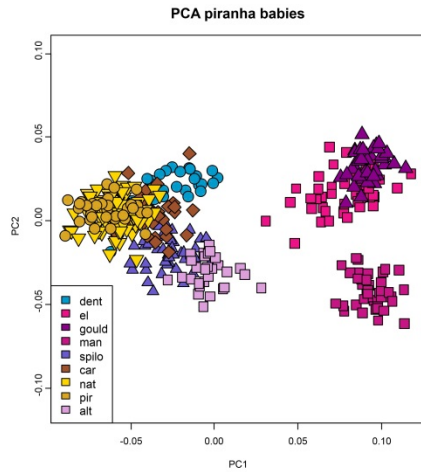
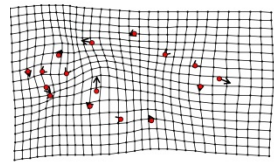
*S. gouldingi*



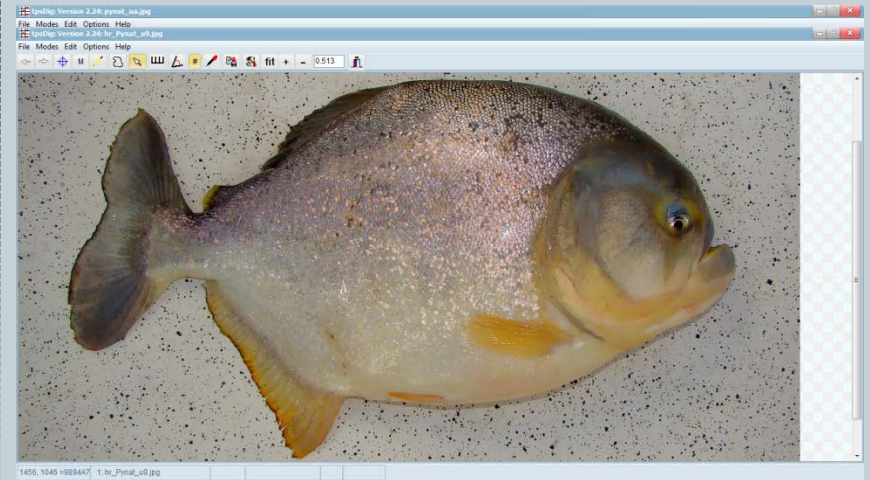
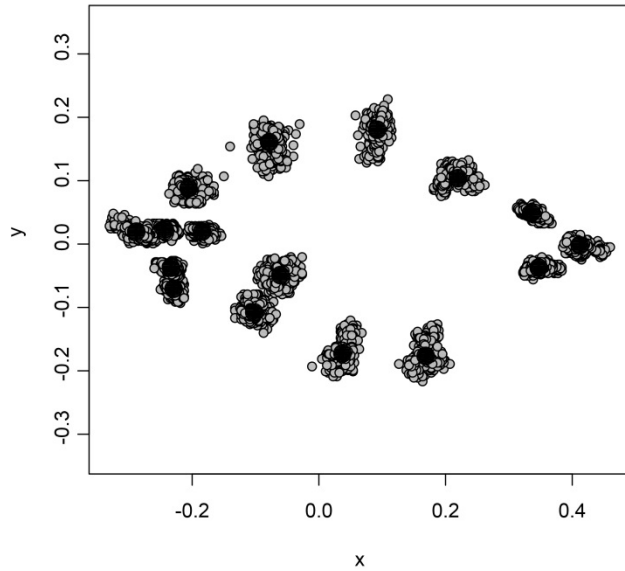
*P. nattereri*



# Principal components of the estimated juvenile and adult shapes of these nine species

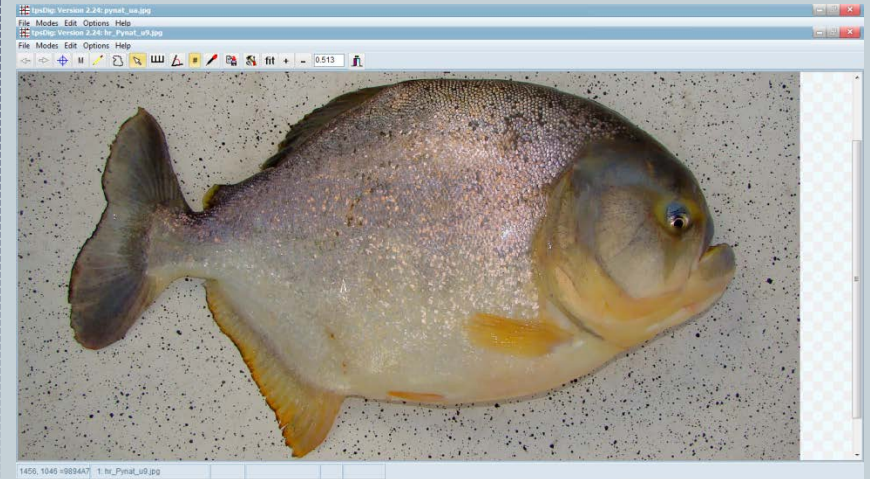


# There is a lot going on with head profiles...



# There is a lot going on with head profiles...

- We can't go back to the images and add the missing information



# We don't have the images



- These fish were imaged in the 1990's
- Digital cameras could not yet take research-quality photographs
- The images were obtained by an obsolete technology
  - A frame-grabber
    - ✦ I don't have images, just data files

# The data files are *not* obsolete

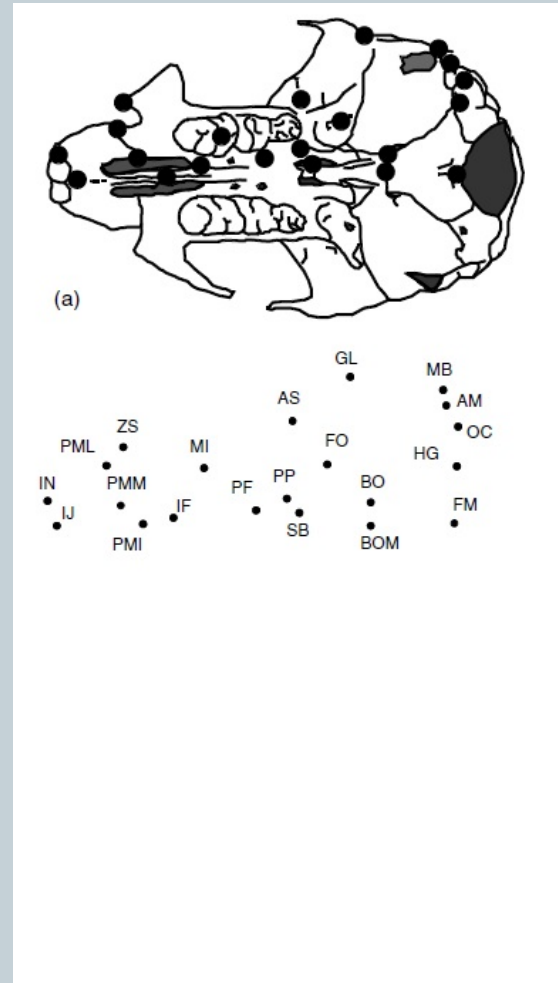


- The first version of this digitizing program was written in 1997
  - But the present version backwardly compatible to an even older program (one that predates videodigitizing)
- The piranha data were collected in 1995
  - And I can analyze them in any program written then or this year

# Even older data files



- These data were first used in a study published in 1992
- The skulls were photographed
- The photographs were printed
- The landmarks were digitized on a tablet using the program that predated tpsDig, on a computer running DOS



# Old images from digital cameras...



*(Mus musculus)*  
Photographed: 2000



*(Peromyscus maniculatus bairdii )*  
Photographed: 2007

# We can still use photos from decades ago

- This is good enough for what we intended
- If I had wanted to measure the teeth, I'd have needed higher resolution
  - And to focus on the tooth row





# Photography and obsolescence



- Digital cameras will no doubt continue to improve
- But as long as they produce JPGs, image files will be backwardly compatible with older ones
- A real technological revolution might make digital photography obsolete
- That revolution could raise scientific standards too high for today's digital cameras
- I won't guess if or when that might happen...

# Until the revolution comes, what we *can* deal with



- **Inconsistencies in data file formats**
  - There are several digitizing programs (not just tpsDig)
  - Some morphometric software can read multiple formats
  - Using any of these (freely available) programs, one file format can be converted to another
  - They can easily be converted into a standard spreadsheet format and, from that, into whatever the program needs

# What else we can deal with



- Any image file type that you provide will work
- We can do some adjustments (crop, enhance, save to another file type)
  - PhotoShop or freeware photo editors

# What else we can deal with



- **Storing images**
  - I have our dataset (>1600 tiff files, 2020 files, 4.5GB) in my back pocket
  - All the data that I have ever collected from all photographs taken over my entire career are on this computer...

# What we need from images...



**Good images for digitizing  
(from Olivier Larouche)**

# What we *can't* deal with



Gaping mouth, tilted



The string is in the way of landmarks

# What we *can't* deal with



Fins damaged



Fins deformed by preservation

# Problems with Fishbase pictures



## **The most common problems**

- 1. Low resolution (dpi)**
- 2. Lacked a ruler or a length measurement for the specimen**
- 3. Nonstandard orientation and parallax problems  
(especially when pictures are taken in the field)**



# What else we *can't* deal with



- **Small sample sizes**
- **The minimum useful sample size depends on the objectives of a study**
  - Many macroevolutionary studies use just 10 specimens per species
  - Studies of geographic variation need more than that per locality
  - Taxonomic studies need large samples to sort out sexual dimorphism, ontogenetic variation, geographic variation, etc.
    - ✦ Many taxonomic studies rely on molecular data
    - ✦ But since about 2005, there are efforts towards an integrative taxonomy using molecular, morphological and ecological data

# Getting adequate samples



- We will likely need specimens from multiple museums so we need a standard orientation for all images
- For 2D data, orientation of the photographic plane is the main issue

# Summary: What we need (2D images)

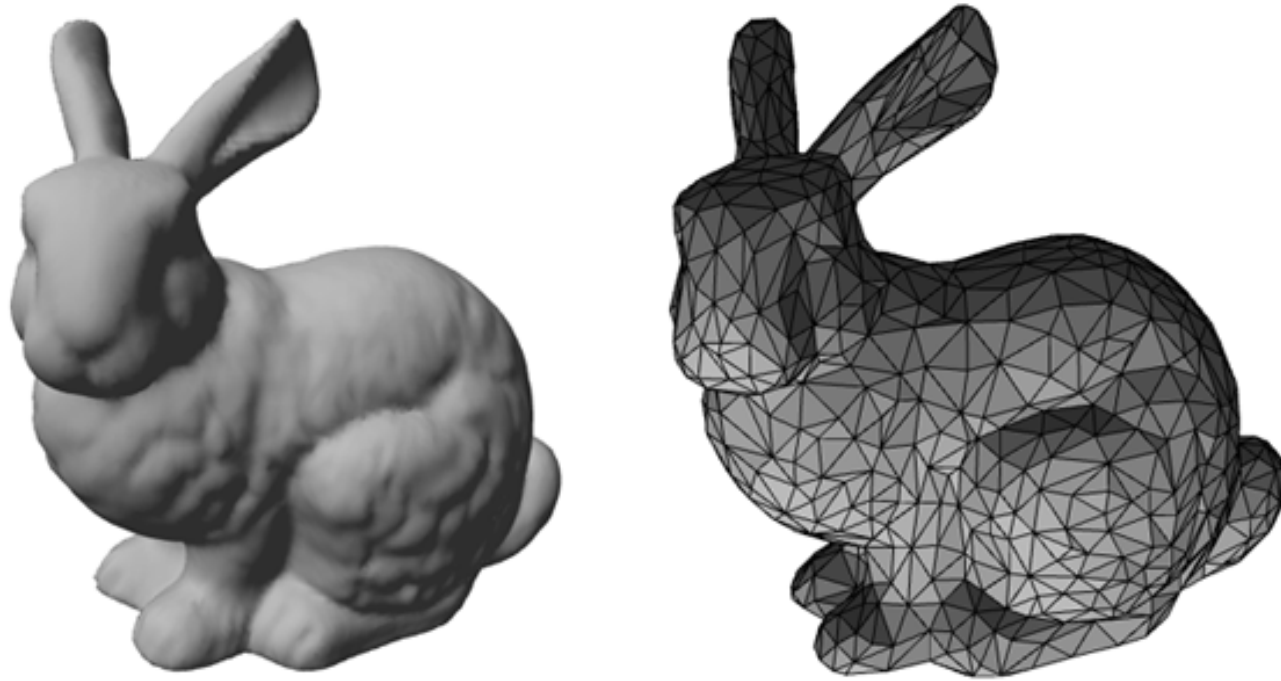


- **Consistently oriented specimens**
  - A fundamental limitation of 2D images is their restriction to a plane
    - ✦ All specimens must be oriented within the same photographic plane
- **“Adequate” photography (digital SLR cameras)**
  - Not badly over-exposed or with other obvious problems
- **A ruler in every photograph**
- **This won't be enough for all researchers**
  - An image that is useful for analyses of body shape might not be useful for a study of teeth...

# What Makes 3D Different from 2D?

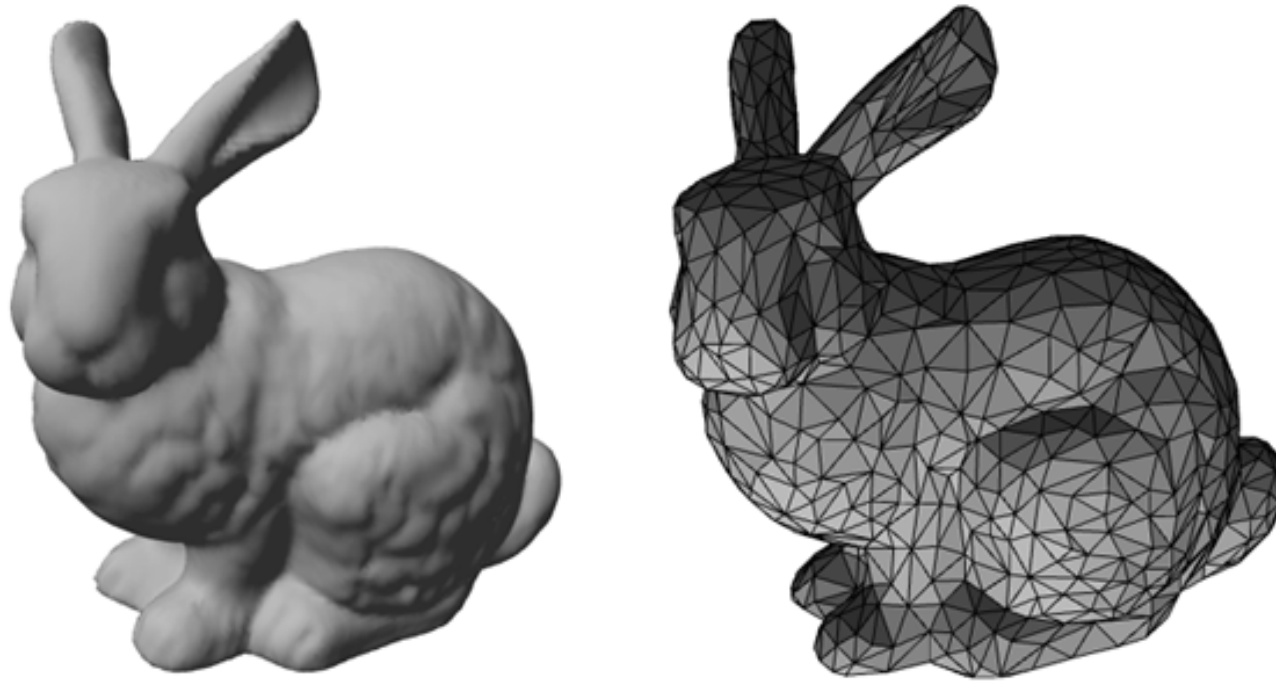


Most systems produce 3D models



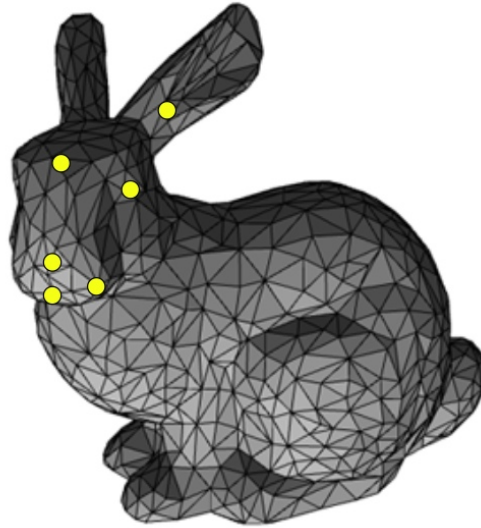
The Stanford Bunny

For the purpose of analysis, these models are equivalent to photographs – they represent the morphology that is the subject of analysis

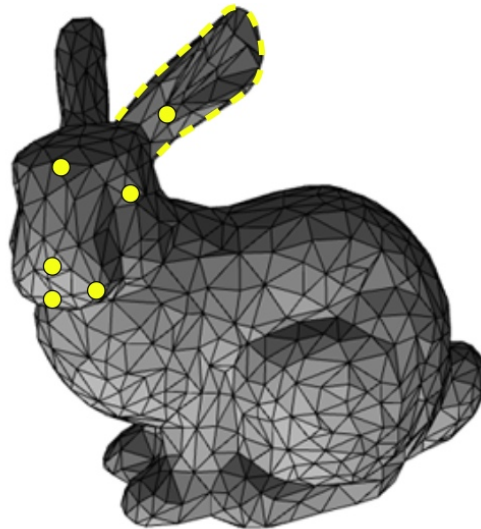


The Stanford Bunny

In the context of geometric morphometrics, that means:

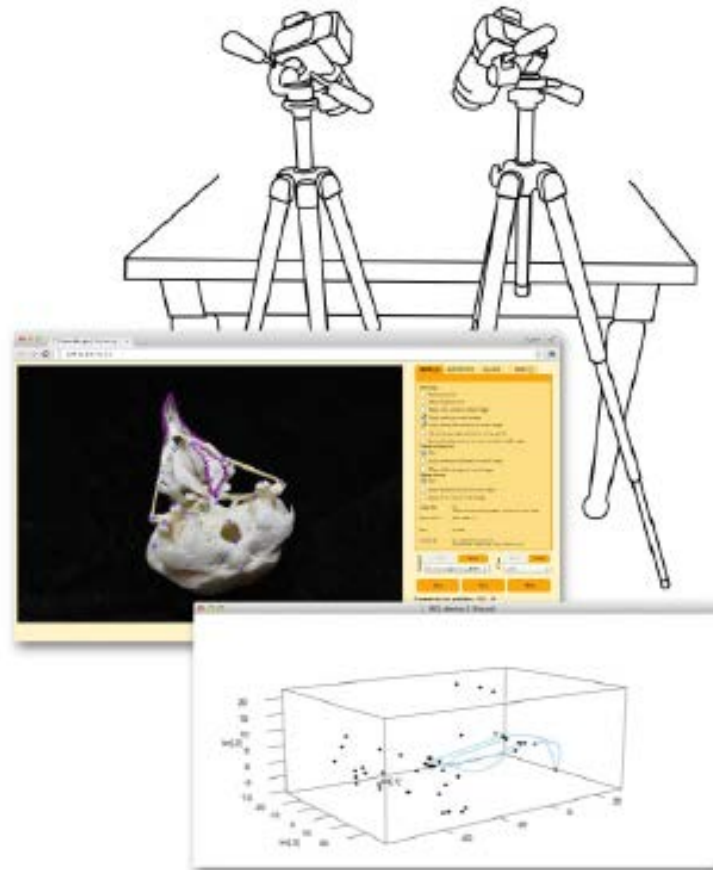


identify/select landmarks,



trace curves and interpolate  
semilandmarks

Most image capture systems produce 3D models  
- the main exception is StereoMorph



<http://home.uchicago.edu/~aolsen/software/stereomorph.shtml>

Olsen, A. M. and M. W. Westneat. 2015.  
StereoMorph: an R package for the collection of 3D  
landmarks and curves using a stereo camera set-up.  
*Methods in Ecology and Evolution*. 6:341-356.  
DOI: 10.1111/2041-210X.12326.



We will not be discussing arm-digitizers –  
because they do not capture an image of the object,  
from which you want to extract data



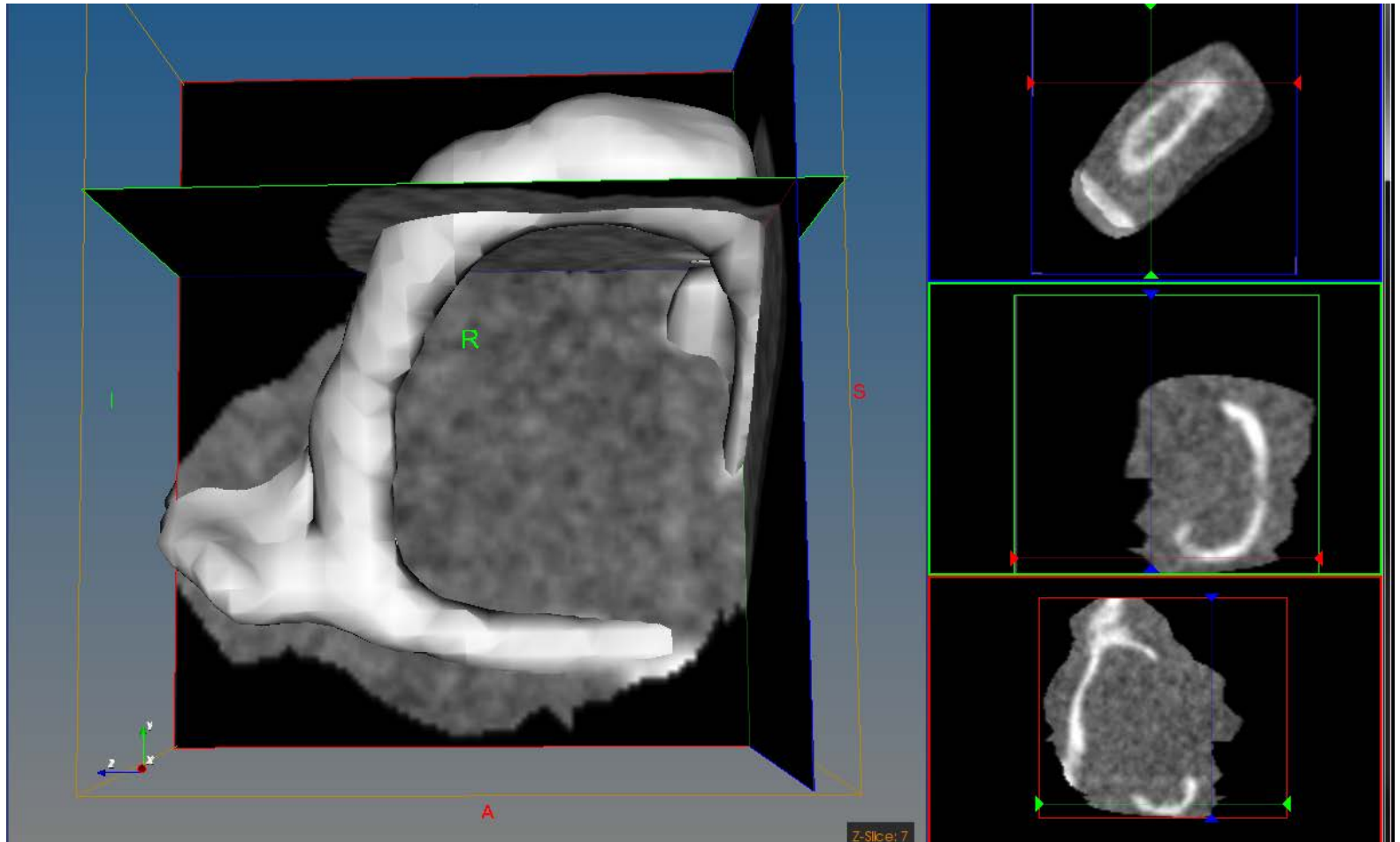
# Where do models come from?



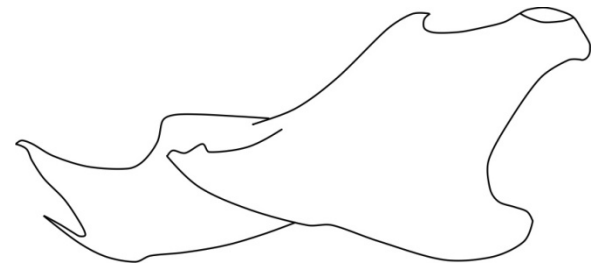
A photograph is a map of the intensity of reflected light  
(1 or 3 channels for gray-scale or color)



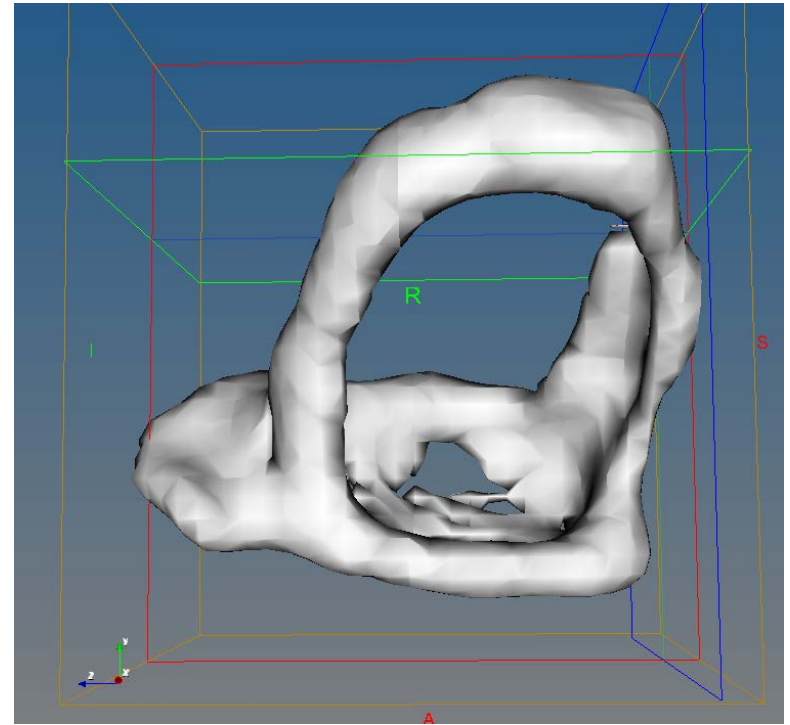
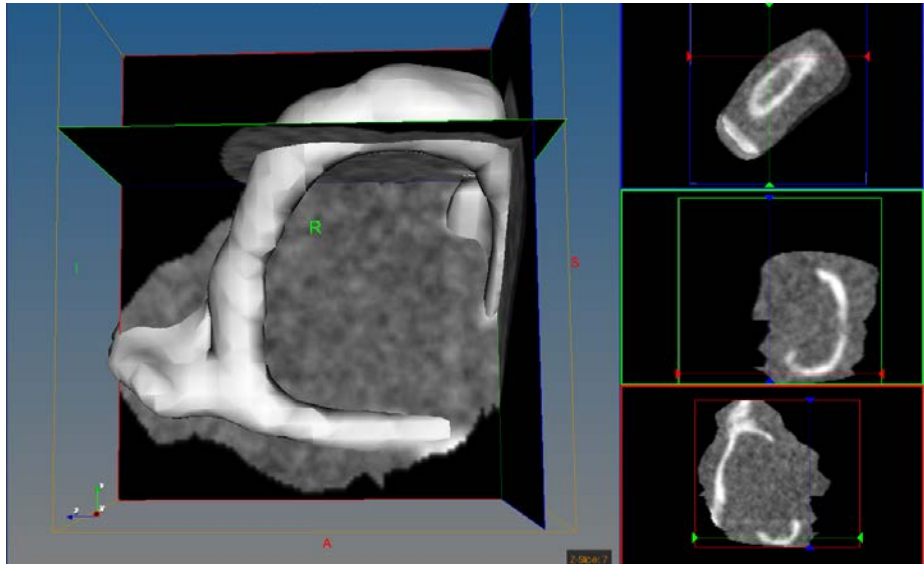
3D scanners produce volumes that are analogous



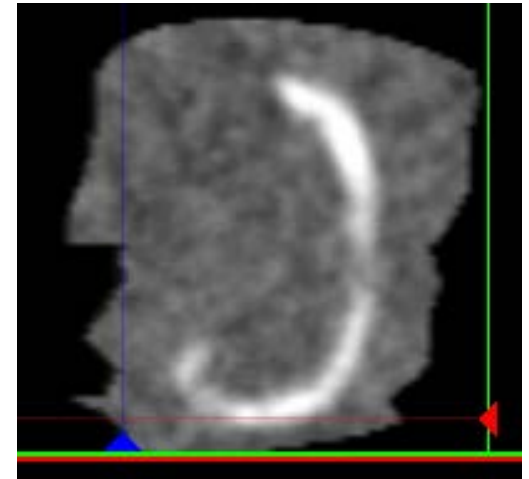
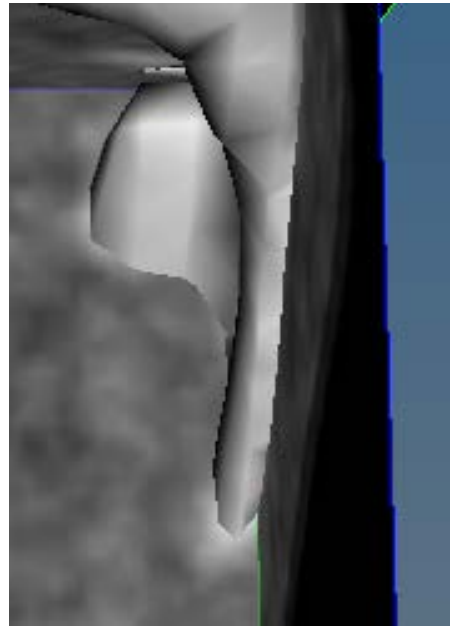
An outline (or silhouette) made from the photograph is a subset of the image data that marks edges of the object



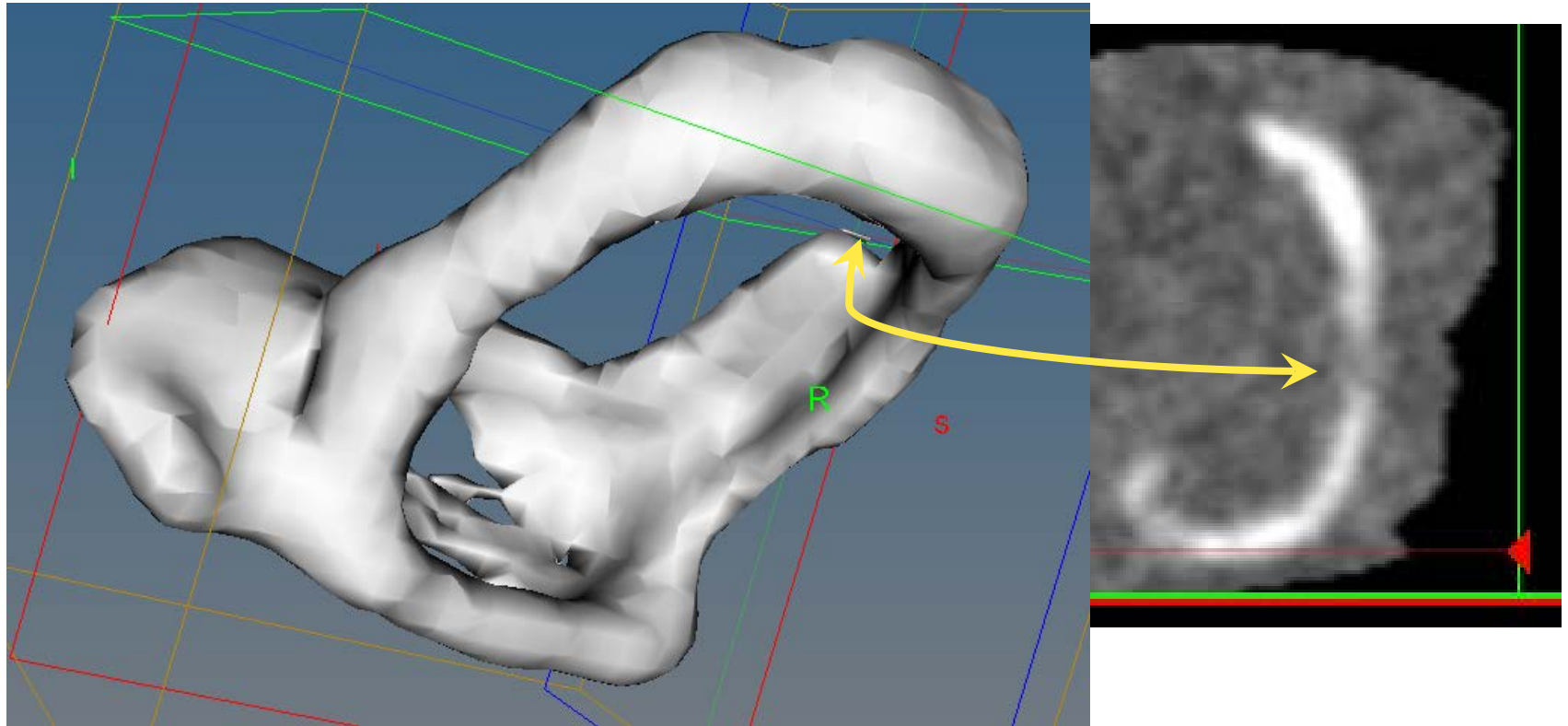
The 3D surface model is analogous to the 2D outline



In photographs and scans, the location of the edge is inferred from the gradient across the edge



If the structure is thin relative to voxel dimensions, small variations in thickness or density can create artifacts – holes or surface bumps

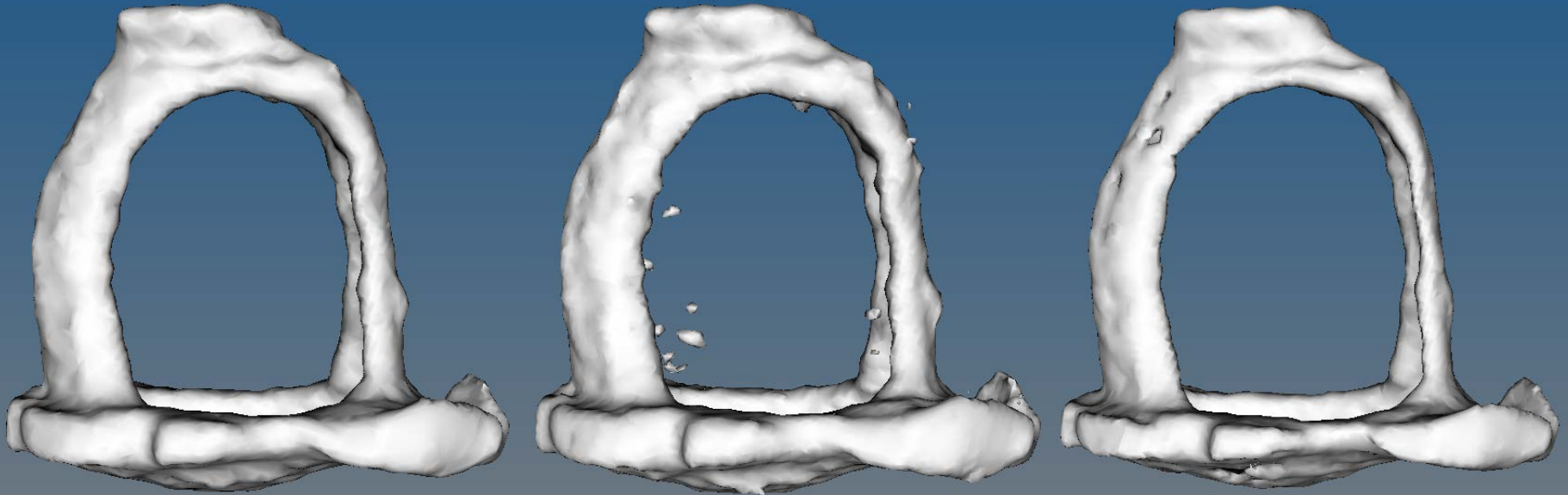


500  $\mu\text{m}$

20  $\mu\text{m}$

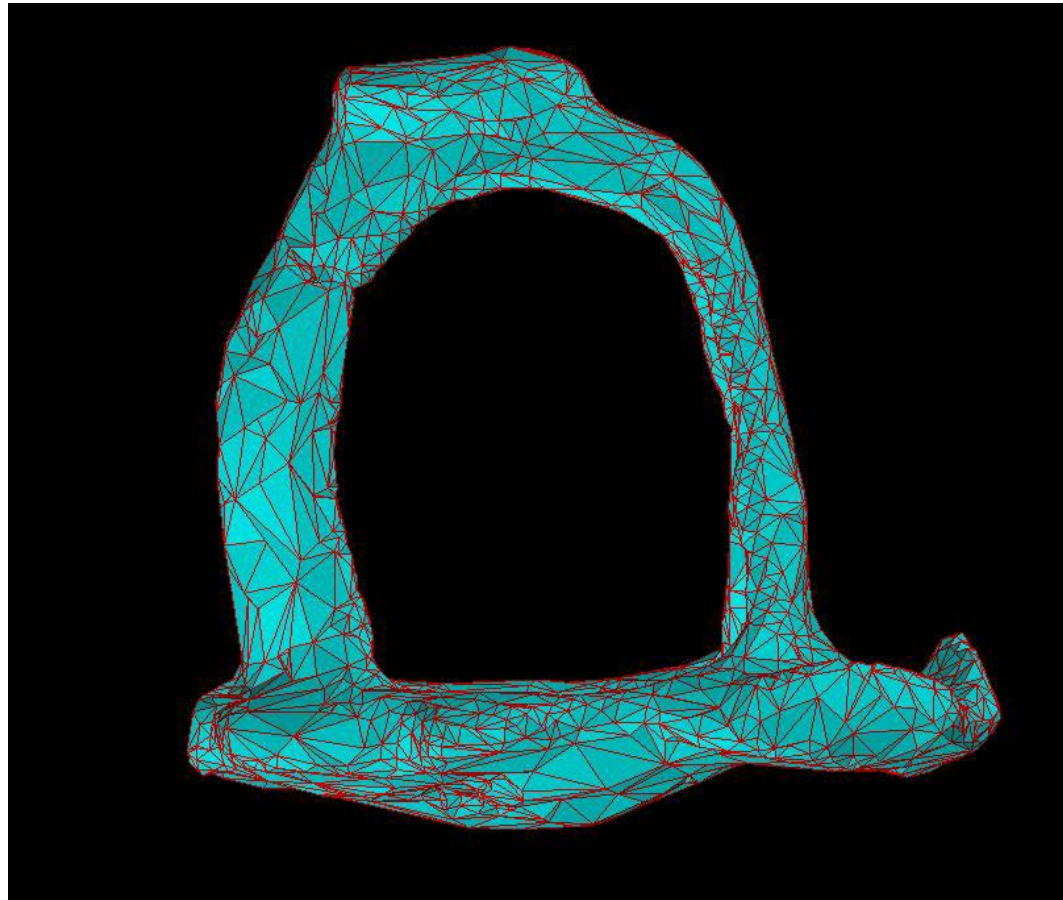


If the structure is thin relative to voxel dimensions, small changes in thresholds and smoothing can alter the apparent dimensions of object.



Area (mm <sup>2</sup> )	0.851	0.882	0.803
Volume (mm <sup>3</sup> )	0.017	0.019	0.011
Triangle Number	19,000	37,000	28,000

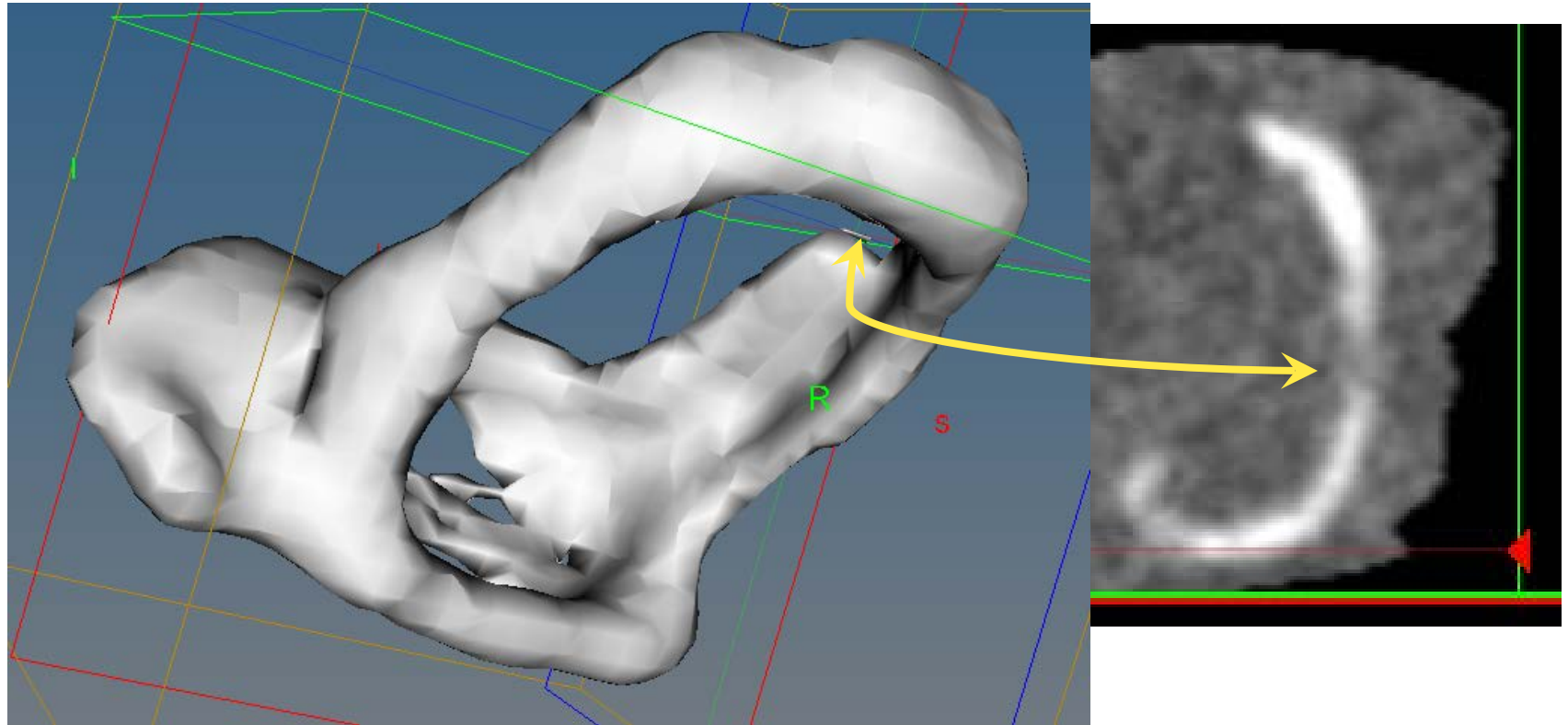
Low triangle number represents loss of data



# Other Differences



When scanning hollow objects (skulls, tetrapod bones);  
optimal scan resolution depends on the thickness of the walls,  
not the thickness of the whole structure



500 μm

20 μm

## Large file sizes

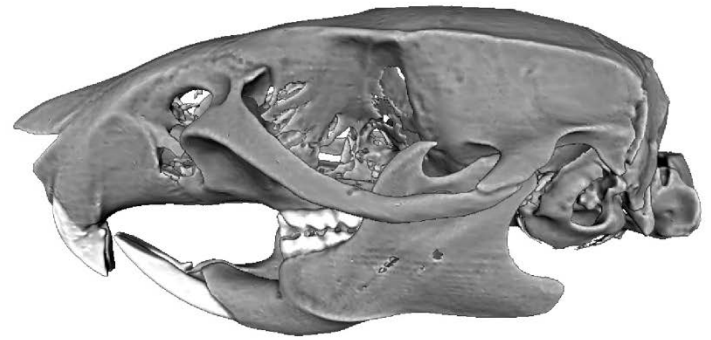
Typical digital photograph with dSLR – 2-10 MB

3D scans of stapes: 0.08 mm<sup>3</sup>, 3-4 MB, \*.vff -> 1 MB ascii \*.ply

For entire ear at same resolution : 45 times the volume, 150-200 MB

Entire skull is about 100x the volume of the ear...

Scanning the skull at lower resolution than the ear may not be a good idea



# Format Issues



# There are many possible formats for encoding mesh data

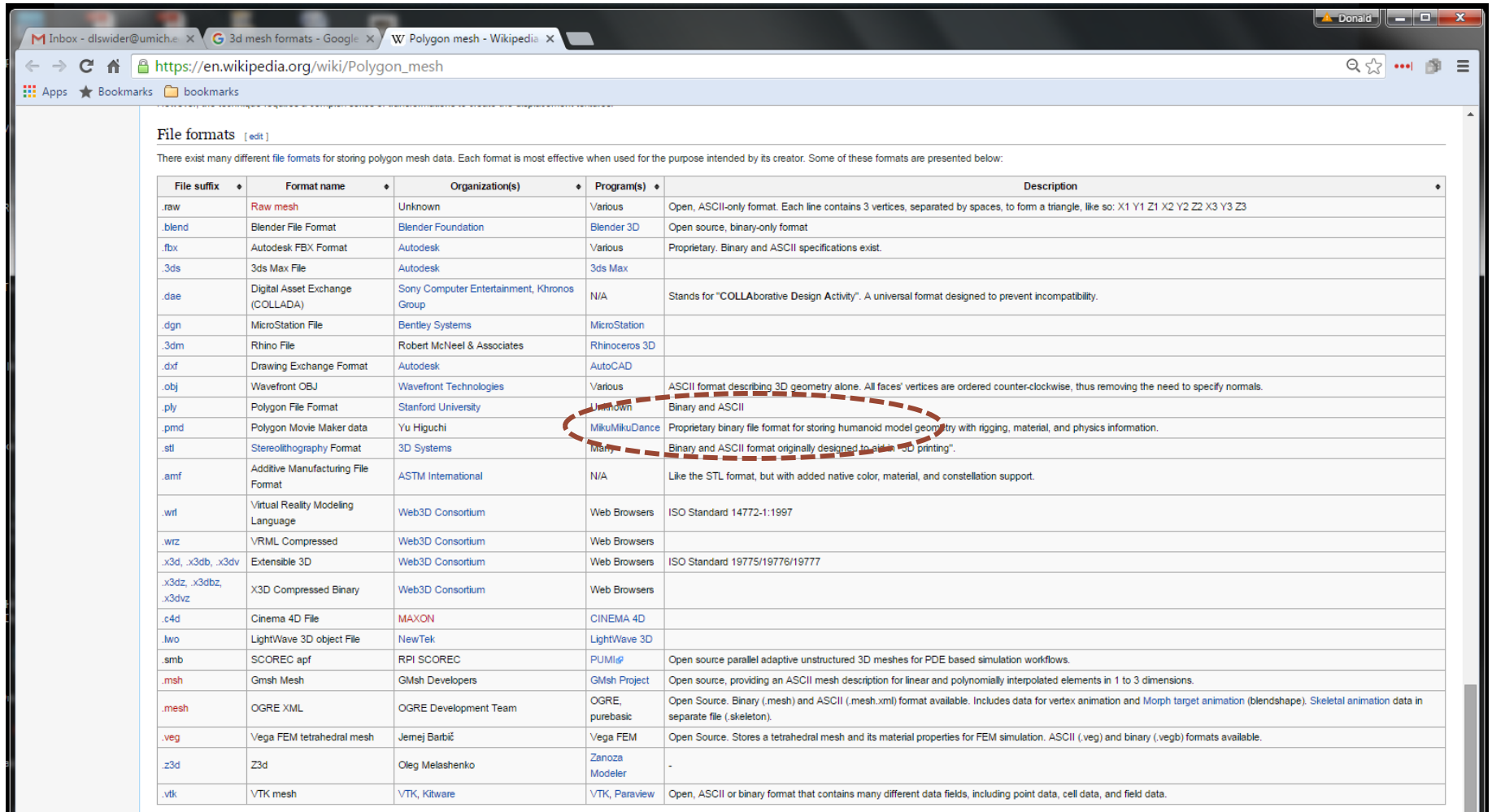
The screenshot shows a web browser window with the URL [https://en.wikipedia.org/wiki/Polygon\\_mesh](https://en.wikipedia.org/wiki/Polygon_mesh). The page title is "File formats" and it contains a table of various file formats for storing polygon mesh data.

There exist many different file formats for storing polygon mesh data. Each format is most effective when used for the purpose intended by its creator. Some of these formats are presented below:

File suffix	Format name	Organization(s)	Program(s)	Description
.raw	Raw mesh	Unknown	Various	Open, ASCII-only format. Each line contains 3 vertices, separated by spaces, to form a triangle, like so: X1 Y1 Z1 X2 Y2 Z2 X3 Y3 Z3
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.fbx	Autodesk FBX Format	Autodesk	Various	Proprietary. Binary and ASCII specifications exist.
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.3dm	Rhino File	Robert McNeel & Associates	Rhinoceros 3D	
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.obj	Wavefront OBJ	Wavefront Technologies	Various	ASCII format describing 3D geometry alone. All faces' vertices are ordered counter-clockwise, thus removing the need to specify normals.
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.amf	Additive Manufacturing File Format	ASTM International	N/A	Like the STL format, but with added native color, material, and constellation support.
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.wrz	VRML Compressed	Web3D Consortium	Web Browsers	
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.c4d	Cinema 4D File	MAXON	CINEMA 4D	
.lwo	LightWave 3D object File	NewTek	LightWave 3D	
.smb	SCOREC apf	RPI SCOREC	PUMIP	Open source parallel adaptive unstructured 3D meshes for PDE based simulation workflows.
.msh	Gmsh Mesh	Gmsh Developers	Gmsh Project	Open source, providing an ASCII mesh description for linear and polynomially interpolated elements in 1 to 3 dimensions.
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.vtk	VTK mesh	VTK, Kitware	VTK, Paraview	Open, ASCII or binary format that contains many different data fields, including point data, cell data, and field data.



# The 'same' format (same extension) can be binary or ascii



File formats [\[ edit \]](#)

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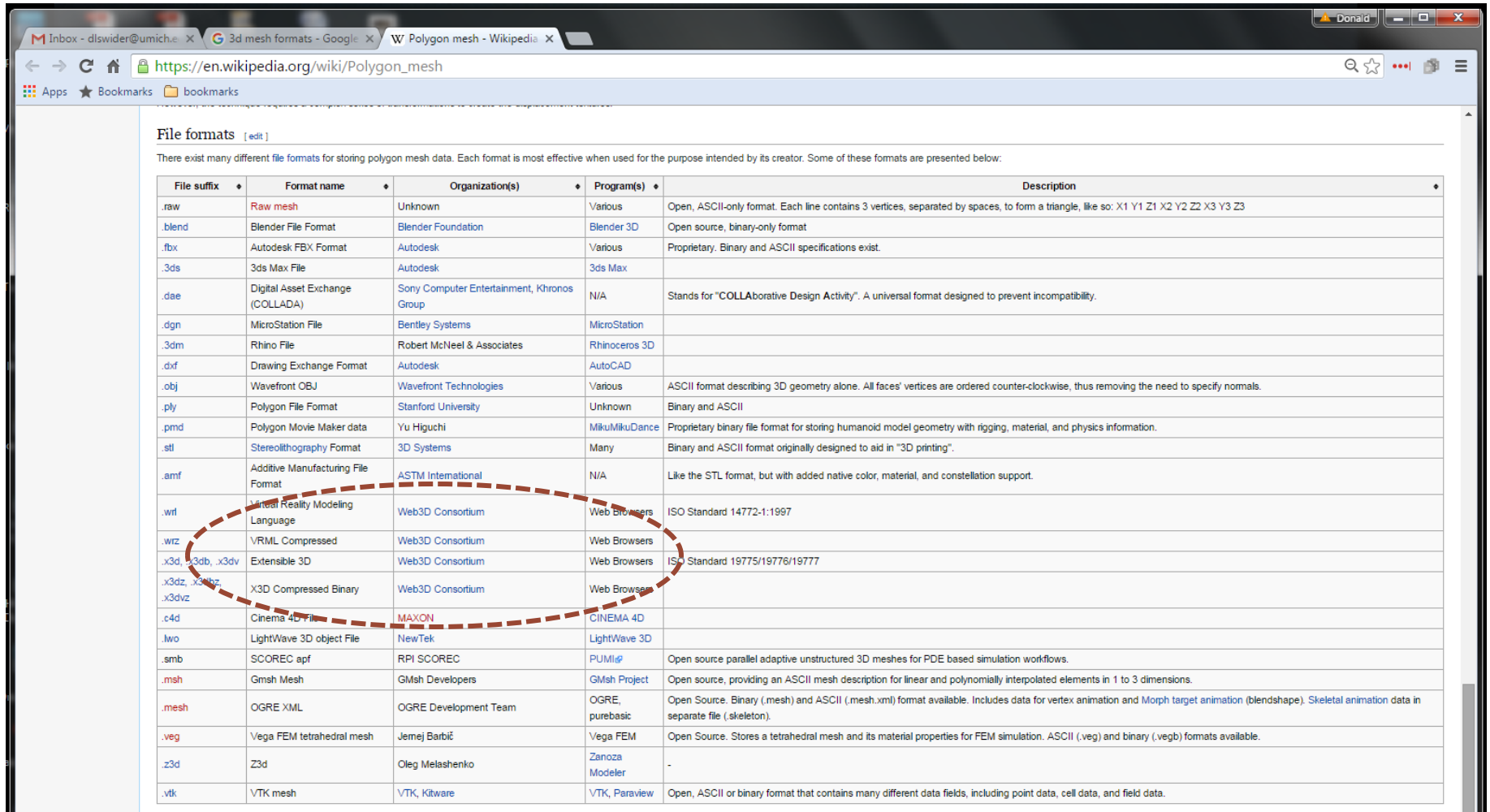
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.vtk	VTK mesh	VTK, Kitware	VTK, Paraview	Open, ASCII or binary format that contains many different data fields, including point data, cell data, and field data.

# Fields and codes may vary for the 'same' format; may use command lines from different languages

The screenshot shows a web browser window with the URL [https://en.wikipedia.org/wiki/Polygon\\_mesh](https://en.wikipedia.org/wiki/Polygon_mesh). The page title is "File formats" and it contains a table of file formats for storing polygon mesh data. A red dashed oval highlights the VTK mesh entry in the table.

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# Compression may be good for the Web, but it is bad for data



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# What Geomorph can read (therefore, what I need):

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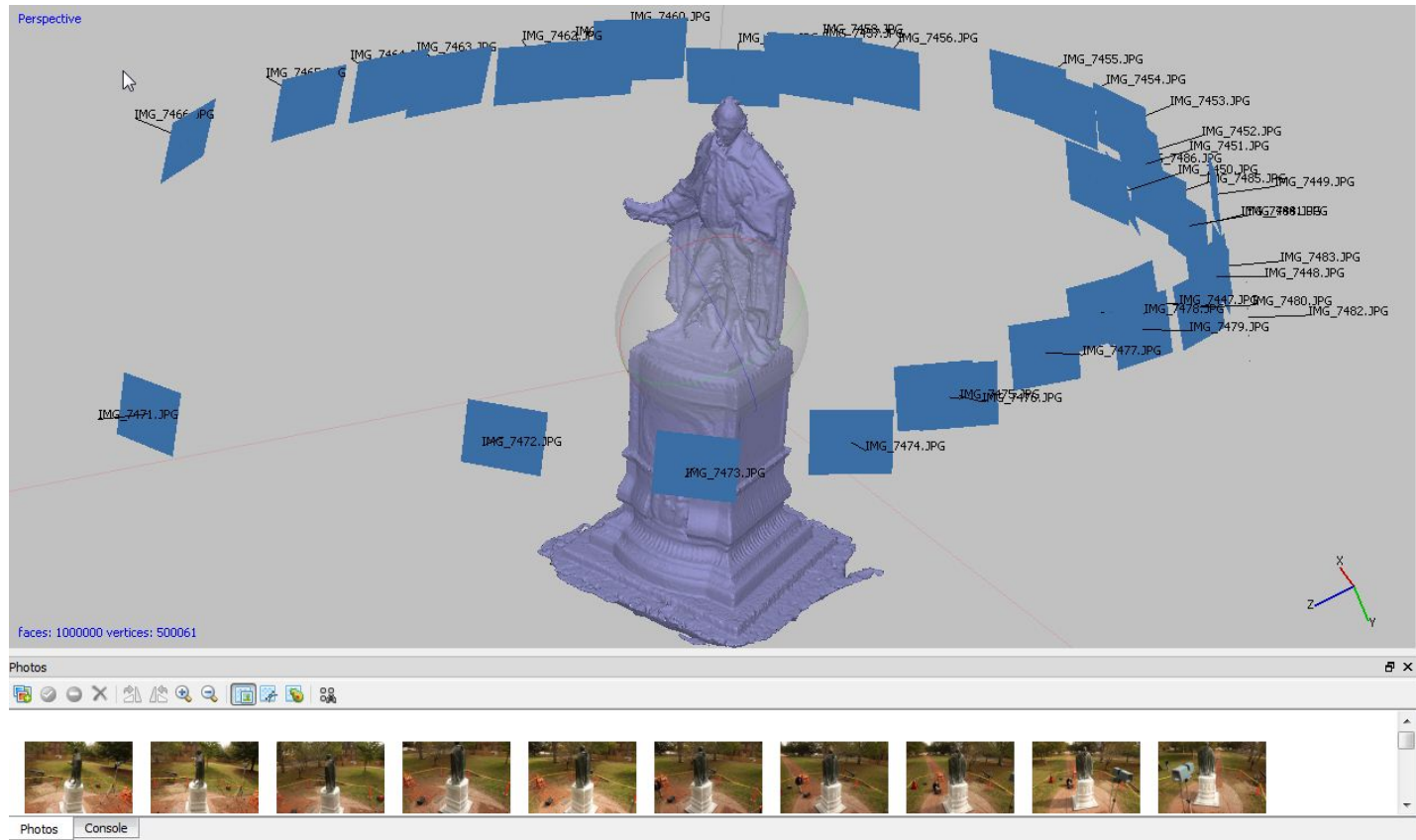
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# What does not differ between 2D and 3D

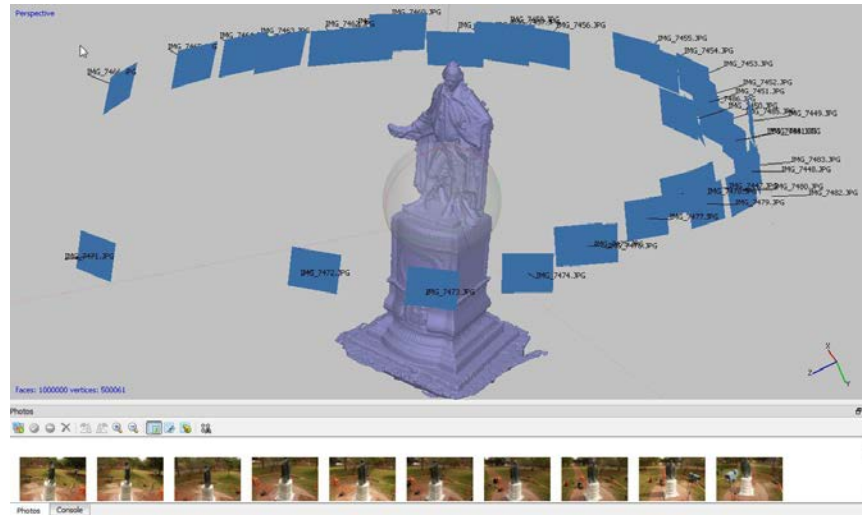


- We cannot fix low resolution
- We cannot fix broken or deformed specimens
- One size / resolution does not fit all
- We need coordinates of landmarks and semilandmarks with a degree of accuracy;
- Once we have the coordinates, all analyses are the same





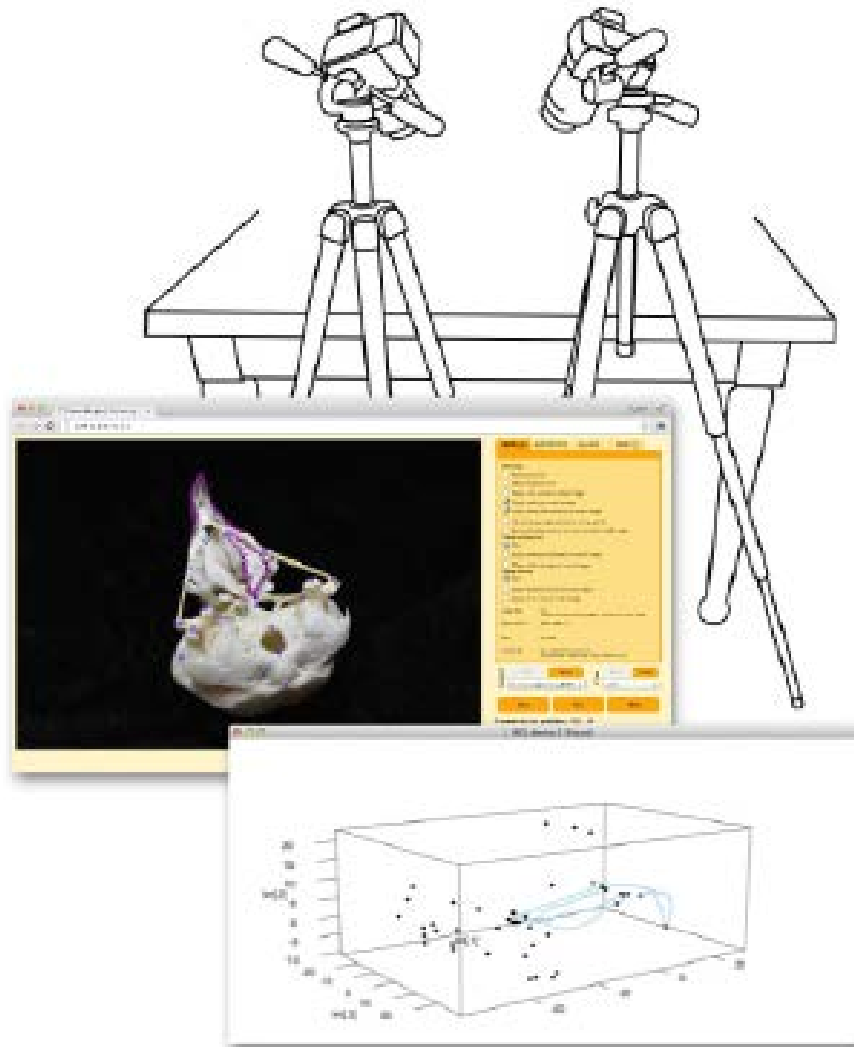
Conventional photogrammetry uses a series of overlapping images to build the model (by triangulating a large number of corresponding features)



The equipment is cheaper than CT or most high quality laser scanners  
Building models is computationally intensive and requires high feature density  
Thin 'shells' of bone or other translucent material make computations difficult.



# StereoMorph take a simplified approach to photogrammetry



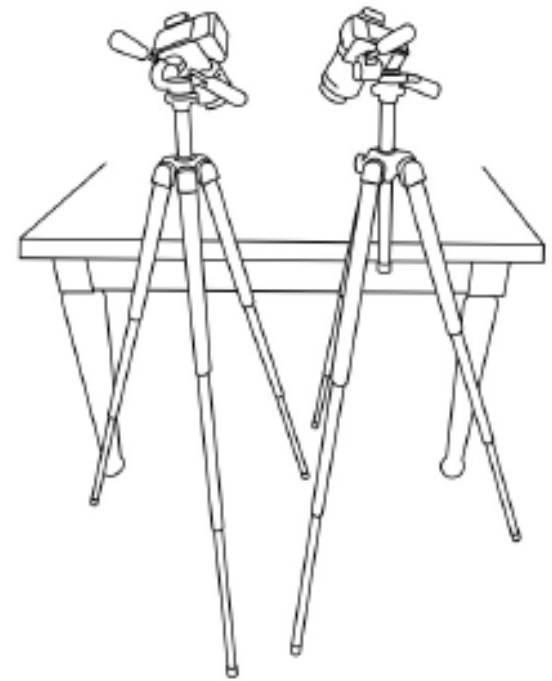
<http://home.uchicago.edu/~aolsen/software/stereomorph.shtml>

Olsen, A. M. and M. W. Westneat. 2015.  
StereoMorph: an R package for the collection of 3D  
landmarks and curves using a stereo camera set-up.  
*Methods in Ecology and Evolution*. 6:341-356.  
DOI: 10.1111/2041-210X.12326.

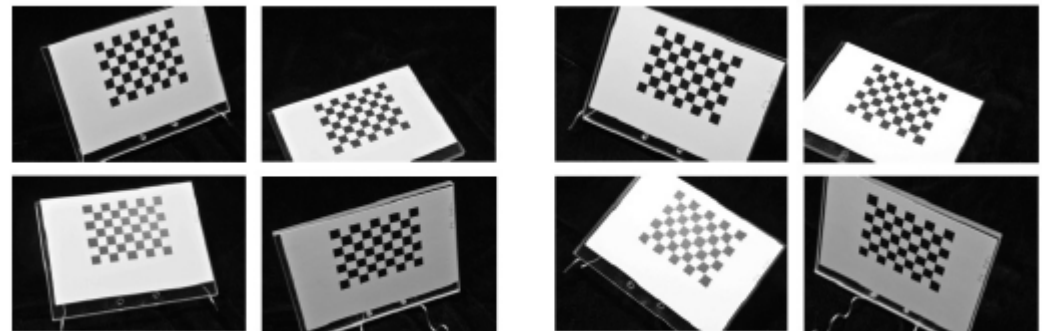
Paired cameras are used to take overlapping images

Camera positions are fixed for the entire session

Differences between view points are calibrated



One possible camera arrangement.



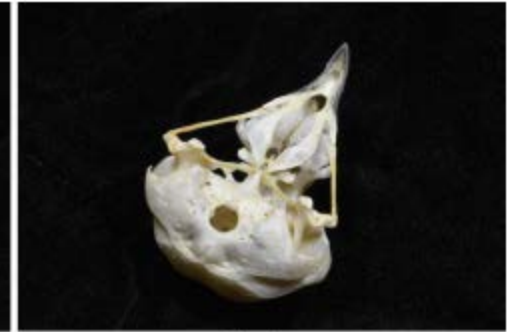
Left view

Right view

Several pairs of images are taken



Left



Right

Multiple view points increase the number of features that can be analyzed



Left



Right

An knowledgeable user can easily design a small set of pairs to cover the subject (~9 for this skull)



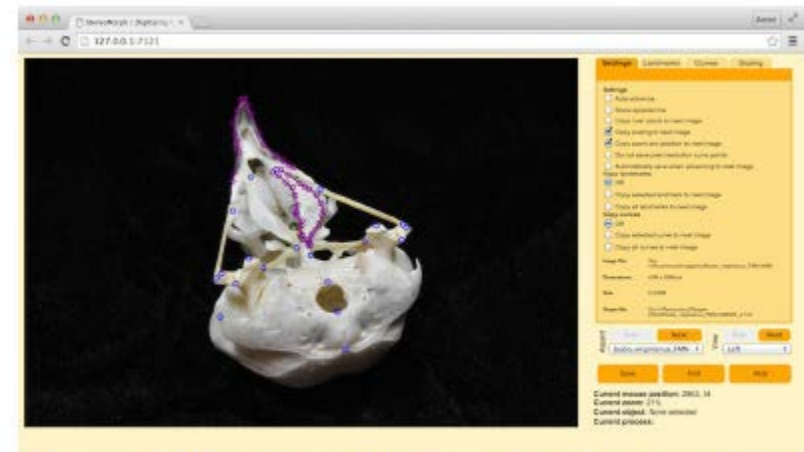
Left



Right

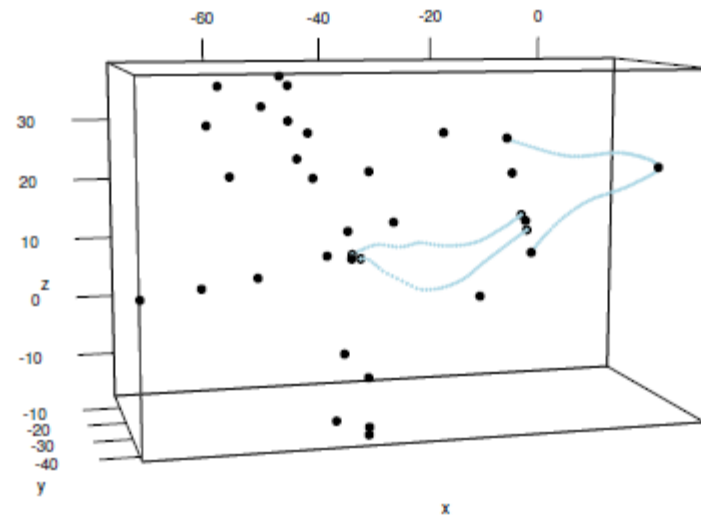
The landmarks visible in both images of a pair are digitized *in both images*; same for semi-landmarks

The landmarks visible in two pairs of images should be digitized in all four images – more is better. These points are used to compute the difference in the specimen's position between the pairs



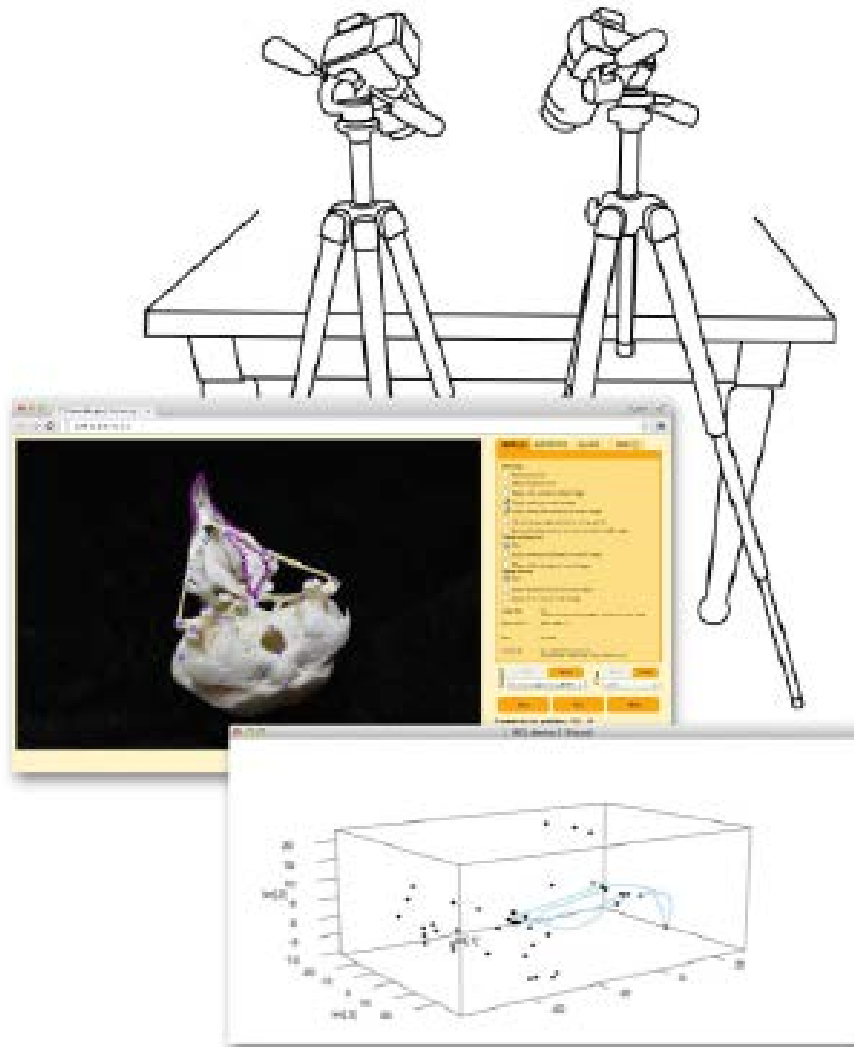
The StereoMorph digitizing app launched with a stereo image set.

**Output is the set of coordinates for the landmarks and semilandmarks.  
No model is computed.**



Plot of reconstructed and unified landmarks and curve points using `plot3d()` in the `rgl` package.

Caveat: we have not yet vetted this approach, but plan to do so soon



<http://home.uchicago.edu/~aolsen/software/stereomorph.shtml>

Olsen, A. M. and M. W. Westneat. 2015.  
StereoMorph: an R package for the collection of 3D  
landmarks and curves using a stereo camera set-up.  
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