A researcher’s perspective

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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).
When landmarks are not enough we sample curves (and surfaces) with semilandmarks.
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”
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 306.00000
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

<table>
<thead>
<tr>
<th>Effect</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>R²</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>1</td>
<td>0.029</td>
<td>0.029</td>
<td>0.080</td>
<td>18.716</td>
<td>0.001</td>
</tr>
<tr>
<td>Diet</td>
<td>7</td>
<td>0.090</td>
<td>0.016</td>
<td>0.256</td>
<td>8.582</td>
<td>0.001</td>
</tr>
<tr>
<td>Size x Diet</td>
<td>7</td>
<td>0.045</td>
<td>0.007</td>
<td>0.115</td>
<td>3.850</td>
<td>0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>129</td>
<td>0.243</td>
<td>0.002</td>
<td>0.450</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td>0.441</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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
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.
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

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

<table>
<thead>
<tr>
<th></th>
<th>Area (mm²)</th>
<th>Volume (mm³)</th>
<th>Triangle Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.851</td>
<td>0.017</td>
<td>19,000</td>
</tr>
<tr>
<td></td>
<td>0.882</td>
<td>0.019</td>
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<tr>
<td></td>
<td>0.803</td>
<td>0.011</td>
<td>28,000</td>
</tr>
</tbody>
</table>
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.
Large file sizes

Typical digital photograph with dSLR – 2-10 MB

3D scans of stapes: 0.08 mm³, 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.

<table>
<thead>
<tr>
<th>File suffix</th>
<th>Format name</th>
<th>Organization(s)</th>
<th>Program(s)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>raw</td>
<td>Raw mesh</td>
<td>Unknown</td>
<td>Various</td>
<td>Open, ASCII-only format. Each line contains 3 vertices, separated by spaces, to form a triangle, e.g., X Y Z 1 X 2 Y 3 Z 4.</td>
</tr>
<tr>
<td>blend</td>
<td>Blender File Format</td>
<td>Blender Foundation</td>
<td>Blender 3D</td>
<td>Open source, binary-only format.</td>
</tr>
<tr>
<td>3ds</td>
<td>3ds Max File</td>
<td>3ds Max</td>
<td>3ds Max</td>
<td>Proprietary. Binary and ASCII specifications exist.</td>
</tr>
<tr>
<td>obj</td>
<td>Wavefront OBJ</td>
<td>Wavefront</td>
<td>Various</td>
<td>ASCII format describing 3D geometry; no line breaks. 3 vertices are ordered counter-clockwise, thus removing the need to specify normals.</td>
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<td>Polygon File Format</td>
<td>Stanford University</td>
<td>Unnamed</td>
<td>Binary and ASCII.</td>
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<tr>
<td>pmn</td>
<td>Polygon Movie maker file</td>
<td>Yu Hiuchi</td>
<td>MikMikDance</td>
<td>Proprietary binary file format for storing humanoid model geometry with rigging, material, and physics information.</td>
</tr>
<tr>
<td>stl</td>
<td>StereoLithography Format</td>
<td>3D Systems</td>
<td>Various</td>
<td>Binary and ASCII format originally designed to aid in 3D printing.</td>
</tr>
<tr>
<td>smf</td>
<td>Additive Manufacturing File Format</td>
<td>ASTM International</td>
<td>N/A</td>
<td>ASCII format designed for additive manufacturing.</td>
</tr>
<tr>
<td>x3d, x3db, x3dv</td>
<td>X3D Compressed Binary</td>
<td>Web3D Consortium</td>
<td>Web Browsers</td>
<td>ISO Standard 19775-1:1997</td>
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<td>x3d, x3dobj, x3dv</td>
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<td>Cinema 4D File</td>
<td>MAXON</td>
<td>CINEMA 4D</td>
<td>Open source; parallel adaptive unstructured 3D meshes for PDE based simulation workflows.</td>
</tr>
<tr>
<td>lwo</td>
<td>LightWave 3D object file</td>
<td>Newtek</td>
<td>LightWave 3D</td>
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<tr>
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<td>SCORPES xml</td>
<td>RPI SCORPES</td>
<td>PIV</td>
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<tr>
<td>mesh</td>
<td>Gmsh</td>
<td>Gmsh Developers</td>
<td>Gmsh Project</td>
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<tr>
<td>pvd</td>
<td>VTK mesh</td>
<td>Kitware</td>
<td>VTK ParaView</td>
<td>Open source; stores a tetrahedral mesh and its material properties for PDE simulation. ASCII (pvd) and binary (vtp) formats available.</td>
</tr>
<tr>
<td>vtu</td>
<td>VTK mesh</td>
<td>Kitware</td>
<td>VTK ParaView</td>
<td>ASCII or binary format that contains many different data fields, including point data, cell data, and field data.</td>
</tr>
</tbody>
</table>
The ‘same’ format (same extension) can be binary or ascii.
Fields and codes may vary for the ‘same’ format; may use command lines from different languages

<table>
<thead>
<tr>
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<tr>
<td>raw</td>
<td>Raw mesh</td>
<td>Unknown</td>
<td>Various</td>
<td>ASCII-only format. Each line contains 3 vertices, separated by spaces, to form a triangle. File format is: x x1 y1 x2 y2 x3 y3.</td>
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<tr>
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<td>Blender File Format</td>
<td>Blender Foundation</td>
<td>Blender 3D</td>
<td>Open source, binary-only format.</td>
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<td>Autodesk FBX Format</td>
<td>Autodesk</td>
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<td>Proprietary, binary and ASCII specifications exist.</td>
</tr>
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<td>Autodesk</td>
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<td>Cinema 4D File Format</td>
<td>Viewpoint</td>
<td>Cinema 4D Editor</td>
<td>ASCII format describing 3D geometry alone. All faces’ vertices are ordered counter-clockwise, removing the need to specify normals.</td>
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<td>3D Systems</td>
<td>Stereolithography Format</td>
<td>3D Systems</td>
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</tr>
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<td>Cinema 3D File Format</td>
<td>MAXON</td>
<td>CINEMA 4D</td>
<td>Open source, parallel adaptive unstructured 3D meshes for PDE based simulation workflows.</td>
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<tr>
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<td>SVG World</td>
<td>ISO/IEC</td>
<td>SVG World</td>
<td>SVG (Scalable Vector Graphics) format.</td>
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<tr>
<td>dxf</td>
<td>DXF</td>
<td>Autodesk</td>
<td>AutoCAD</td>
<td>ASCII or binary format that includes many different data fields, including point data, cell data, and field data.</td>
</tr>
<tr>
<td>obj</td>
<td>Wavefront OBJ</td>
<td>Wavefront Technology</td>
<td>Various</td>
<td>ASCII format describing 3D geometry alone. All faces’ vertices are ordered counter-clockwise, removing the need to specify normals.</td>
</tr>
<tr>
<td>mesh</td>
<td>VHDL</td>
<td>VHDL</td>
<td>VHDL</td>
<td>VHDL (Virtual Hardware Description Language) format. Includes data for vertex animation and target animation (e.g., blendshapes). Data for animation data in separate file (e.g., animation data).</td>
</tr>
<tr>
<td>v5</td>
<td>Vega FD2I</td>
<td>Vega FD2I</td>
<td>Vega FD2I</td>
<td>Open Source, stores a tetrahedral mesh and its material properties for FD2I simulation. ASCII (.v5) and binary (.v5b) formats available.</td>
</tr>
<tr>
<td>obj</td>
<td>Wavefront OBJ</td>
<td>Wavefront Technology</td>
<td>Various</td>
<td>ASCII format describing 3D geometry alone. All faces’ vertices are ordered counter-clockwise, removing the need to specify normals.</td>
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<td>fbx</td>
<td>Autodesk FBX Format</td>
<td>Autodesk</td>
<td>Various</td>
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</tbody>
</table>
Compression may be good for the Web, but it is bad for data
What Geomorph can read (therefore, what I need):

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<td>Blender File Format</td>
<td>Blender Foundation</td>
<td>Blender 3D</td>
<td>Open source, binary-only format</td>
</tr>
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<td>3ds</td>
<td>3ds Max File</td>
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<td>3ds Max</td>
<td>ASCII</td>
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<td>dae</td>
<td>Digital Asset Exchange (COLLADA)</td>
<td>Sony Computer Entertainment, Khronos Group</td>
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<td>Rhino 3D</td>
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<td>dxf</td>
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<td>Wavefront Technologies</td>
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<td>x3d</td>
<td>x3d</td>
<td>Stanford University</td>
<td>Uniknow</td>
<td>Binary and ASCII.</td>
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<tr>
<td>x3ds</td>
<td>x3ds</td>
<td>Yuhki Mishima</td>
<td>MikuMikuDance</td>
<td>Proprietary binary file format for storing humanoid model geometry with rigging, material, and physics information.</td>
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<td>sif</td>
<td>Stereolithography Format</td>
<td>3D Systems</td>
<td>Many</td>
<td>Binary</td>
</tr>
<tr>
<td>satf</td>
<td>Satellite Manufacturing File Format</td>
<td>ASTM International</td>
<td>N/A</td>
<td>Like the STL format, but with added native color, material, and constellation support.</td>
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<td>x3d</td>
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<td>PVR</td>
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</tr>
<tr>
<td>mesh</td>
<td>Gmsh</td>
<td>Gmsh Developers</td>
<td>Gmsh Project</td>
<td>Open source, providing an ASCII mesh description format for linear and polygonally interpolated elements in 1 to 3 dimensions.</td>
</tr>
<tr>
<td>mesg</td>
<td>OBJE XML</td>
<td>OBJE Development Team</td>
<td>OBJE</td>
<td>ASCII&lt;br&gt;mesh</td>
</tr>
<tr>
<td>veg</td>
<td>Vega FDV tetrahedral mesh</td>
<td>James Stoflet</td>
<td>Vega FDV</td>
<td>Open Source. Saves a tetrahedral mesh and its material properties for PDM simulation. ASCII (veg) and binary (veg) formats available.</td>
</tr>
<tr>
<td>x3d</td>
<td>Fbx</td>
<td>Ceng Metaxenho</td>
<td>Zanma Modeler</td>
<td></td>
</tr>
<tr>
<td>vtk</td>
<td>VTK</td>
<td>VTK</td>
<td>VTK ParaView</td>
<td>Open, ASCII or binary format that contains many different data fields, including point data, cell data, and field data.</td>
</tr>
</tbody>
</table>
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
Photogrammetry
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

StereoMorph: an R package for the collection of 3D landmarks and curves using a stereo camera set-up.
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
Several pairs of images are taken

Multiple viewpoints increase the number of features that can be analyzed.

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