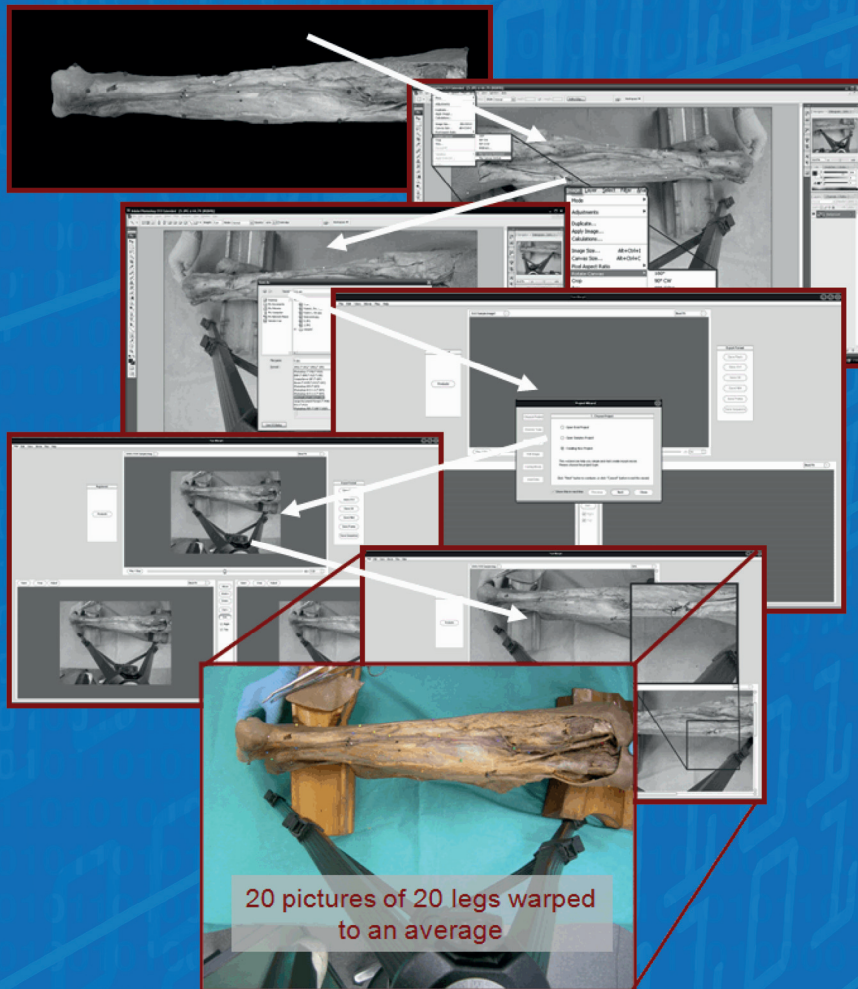


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## Chapter 1

Materials and Methods;  
Computer Assisted Surgical Anatomy Mapping

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## BACKGROUND OF IMAGE PROCESSING

It is imperative to first understand what a picture actually is; a picture consists of a (usually large) group of coloured dots placed on a flat surface, in this case representing the actual 3D anatomy of a photographed specimen. When computerised the coloured dots are called pixels and the pixels are laid out on a grid. One can look at a picture as a huge spreadsheet in which each box (or pixel) has the following information:

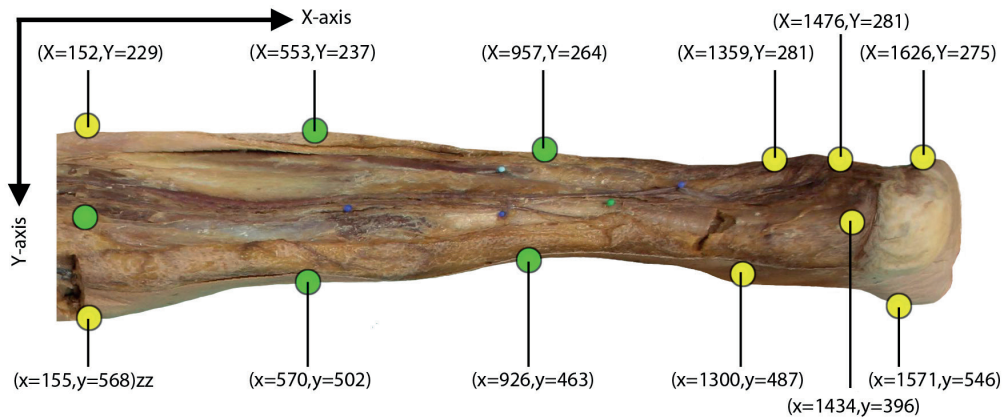
- 1) its location in the picture, defined by an X and Y coordinate.
- 2) its colour.

The X and Y coordinates follow the grid over each picture following the rules of a 2-dimensional Cartesian system, which is a specific kind of coordinate system.

Each picture therefore has a certain size which is defined by its height (X) and width (Y). The more pixels (or spreadsheet boxes) the image has, the higher its quality. The quality of digital images is usually reported as Dots per inch (DPI). The raw photographs made in the studies of this thesis were for example 17.067 x 12.800 inch with a resolution of 180 dots per inch resulting in a grid of 3.072 x 2.304 pixels. In total each pictures encompasses more than 7 million pixels, each with its own location and colour coded to it.

As an image can be viewed as a large Excel sheet with in each “box” a certain set of numbers, we can put formulas on the whole Excel sheet and thereby instantly change the appearance of the picture. An example is changing a colour picture to black-and-white or Sepia using a simple photo-editing apps. It is just a formula applied to the colour part of each “box” in the spreadsheet we call an image.

There are also formulas that can be applied to the location part of each “box” or pixel. These formulas are best explained and described using geometry.



**Figure 1.** Grid-system.

X and Y quantify the width and height respectively.

(0,0) = the upper left corner.

(1686,720) = the lower right corner

## BACKGROUND GEOMETRY

Geometry is a branch of mathematics that is focussed on the measurements of shape, size and the relative positions of a figure. In ancient Greek “geo” meant earth and “metron” meant measurements<sup>3</sup>. The classical part of geometry that encompasses the physical space is known as the Euclidian space (this in contrast to more modern geometry which has more abstract spaces). The Euclidian space is an abstraction and it is therefore not a real representation. Instead it can be viewed as a mathematical tool that can be used to explain and describe parts of the real world. Since the Euclidian space is a mathematical concept it is not tied to actual locations or measurement instruments. Distance for instance is measured in just numbers, not inches or meters.

The currentday basis of the Euclidian space has been formed by Euclid of Alexandria<sup>1</sup> who designed and described the axioms on which later theorems were based. An axiom is a statement or starting point of reasoning that is to be accepted without controversy<sup>2</sup> whilst a theorem needs to be proven based on previous theorems or axioms. The basic axioms (postulates) in Euclidian geometry are also called the Euclidean elements<sup>4,5</sup>:

- Two points define a line (or you can always draw a line between two points).



- A line can be extended by continuing the line with a straight line
- A circle is defined by a centre and a radius
- All right angles are equal to each other
- The parallel postulate; if two lines are intersected by a third creating two right angles then the initial lines run parallel and never intersect.

Using these Euclidian elements Euclid began to mathematically deduce the properties of points, lines, planes and shapes such as triangles. For example he proved the theorem already described by Pythagoras on the relation of the three sides of a right triangle. It is believed that currently over a quarter of a million theorems are proven every single year<sup>6</sup>.

To describe the location of a unique point in a certain plane the Cartesian coordinate system is used. How a point in the 2 dimensional space could be described using a X (horizontal) and Y (vertical) axis (figure 2) was first depicted by Rene Descartes<sup>7,8</sup>.

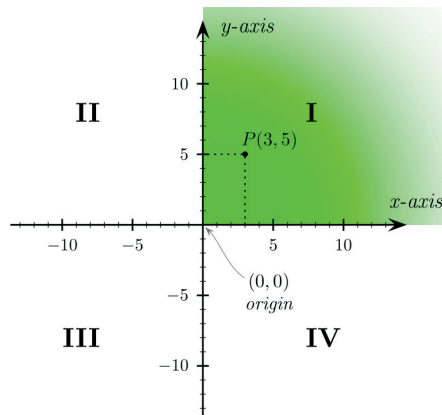


Figure 2. The Cartesian coordinate system.  
Source: Wikipedia<sup>9</sup>

The advantage of such a coordinate system is that you can describe the location of a certain point (X, Y) and calculate with the coordinates. So point (x1, y1) can be moved two places to the right and its new coordinates will then be formed by the equation: (x1+2, Y1) in which x1 and Y1 are the old coordinates (figure3). It is also possible to calculate the distance between two points using the

Pythagorean theorem:  $\text{Distance} = \sqrt{((x_2 - x_1)^2 + (y_2 - y_1)^2)}$  or calculate an average point from three different points:

Average point  $(X_a, Y_b) = ((x_1 + x_2 + x_3)/3, (y_1 + y_2 + y_3)/3)$  (figure 3). The pixels of an image are also located in such a Cartesian coordinate system and therefore it is possible to alter their location using formulas created from the principles of geometry and Euclidean space.

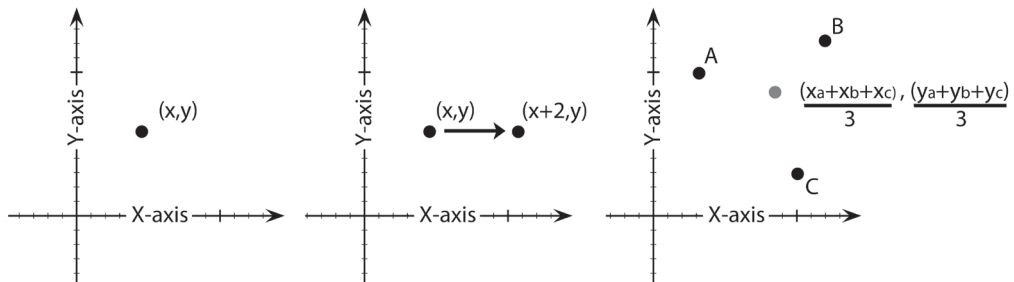


Figure 3. The Cartesian coordinate system. Adapted from Wikipedia<sup>9</sup>

## Rigid Transformations

In mathematics there are different forms of movement. Another mathematical name for moving (parts of-) an image is “warping”. One of the simplest forms of movement is called a rigid transformation, meaning that the shape and size of an image or figure are preserved whilst moving. One form of a rigid transformation is moving the image horizontally or vertically. This is called translation. If the image of a house needs to be moved horizontally and vertically, a formula needs to be created to calculate the movement of all pixels. Given that the size and shape of the image are the same, only one corresponding pixel is needed to calculate the translation formula for all pixels. The corner of the house could be a useful landmark. Once a formula is computed that translates the coordinates of that one landmark to match the specified position (for instance  $(x_1 + 4, y_1 + 3)$ ) this formula can be applied to all pixels to move the entire image (figure 4). Rotation also is a rigid transformation. For a rotation however at least two corresponding landmarks are needed. Even though the formula becomes more difficult, the principle remains the same as for translation. The formulae that describe movement for both landmarks can be used to deduct the formula that can move all other pixels. Slightly controversial reflections can also be considered as rigid transformations. A minimum of three landmarks is needed for the third form of rigid transformations; either flipping an image vertically or horizontally.

Again the formulae describing the movement of all three landmarks can be used to compute the movement each pixel has to make.

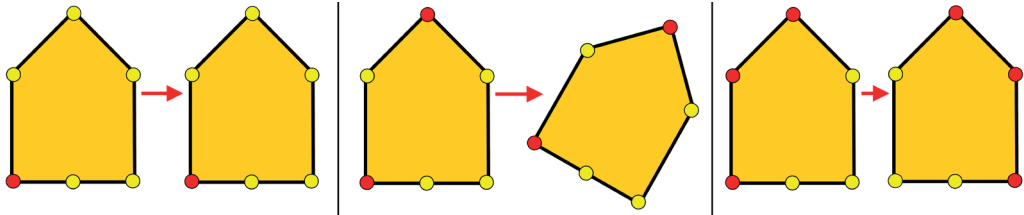
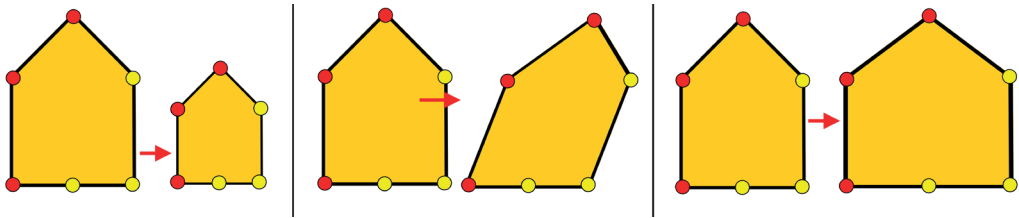


Figure 4. Rigid transformations;  
Adapted from<sup>10</sup>

- 1: Translation (one landmark necessary)
- 2: rotation (two landmarks necessary)
- 3: reflection (three landmarks necessary).

## Affine Transformations

Rigid transformations are a subset of affine transformations. In affine transformations points, straight lines and planes need to be preserved. However it is not necessary to preserve angles or distances between points<sup>8</sup>. For affine transformations up to three landmarks are necessary to compute the formula that moves all pixels of an image. Examples are scaling (making a picture bigger, [figure 5.1](#)), shear mapping (see bio-mathematics and [figure 5.2](#)) and squeeze mapping (making one direction of the image larger, [figure 5.3](#)). Since affine transformations are based on three landmarks deducing the formulas that move all other pixels in a picture is relatively simple.



**Figure 5.** Affine transformations.

Adapted from<sup>10</sup>

- 1: Scaling.
- 2: Shear mapping.
- 3: Squeeze mapping.

## Warping

The anatomy of a left ankle can be compared to the anatomy of a right ankle using simple affine transformations such as translation and reflection. Similarly it is also possible to scale a small ankle to match a larger ankle and even elongating a short leg for it to match a taller leg. However just scaling, reflecting and elongating the shape of a specimen will never allow for a complete matching of shapes between specimen.

In comparative anatomy it is imperative for all specimen to exactly match the average shape. In CASAM the shape of a specimen can therefore be delineated by up to 50 landmarks (instead of the relatively few numbers of a maximum of three landmarks used for affine transformations). This means that in order to move the pixels in that image the formulas calculating the new positions of pixels are dependent on the movements of all 50 landmarks. This tends to generate hugely complicated formulas and makes computation of average shapes virtually impossible.



## Triangulation

One way to solve this problem is to break up the image into multiple smaller triangles, with the corners of a triangle located at landmarks. A triangle, by nature is defined by three landmarks. As described above this means that reshaping each individual landmark and all pixels within that landmark can be done with relatively simple affine transformations.

Although breaking up an image into triangles seems easy, it becomes more difficult when more landmarks are involved as there is an exponential number of triangulations possible for a growing set of points. Triangles most easy to warp are those that have relatively large areas and large angles. Creating these so called “finest triangles” between landmarks is called Delaunay triangulation and it adheres to the rule that no landmark is located inside the circumference of each triangle formed (figure 6).

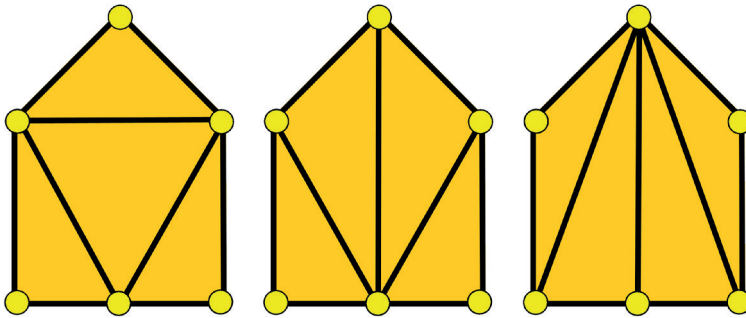


Figure 6. Triangulation

Adapted from<sup>10</sup>

- 1: Triangle conform Delaunay.
- 2: Bad triangulation.
- 3: Worst possible triangulation with skinny triangles.

Each pixel in each triangle then gets a coordinate within that triangle. In contrast to the Cartesian coordinate system, in which coordinates consist of x and y, in the Barycentric coordinate system<sup>11</sup> the pixels are located in relation to each side of the triangle, A, B, and C. The rules for moving a pixel however are the same and the formulas for moving pixels are still based on its three surrounding landmarks (figure 7).

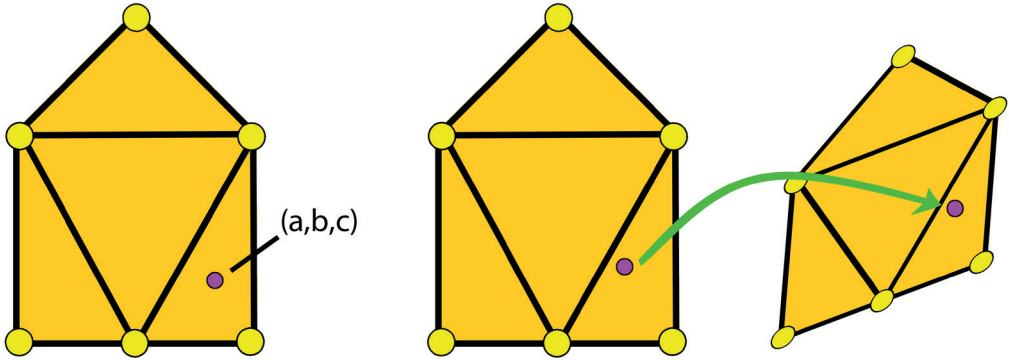


Figure 7. Barycentric coordinate system and related pixel relocation.  
Adapted from<sup>10</sup>

### Creating an average shape

Given the linear and simple nature of triangulation the algorithm can be used to compute an average shape between 2 or more different shapes.

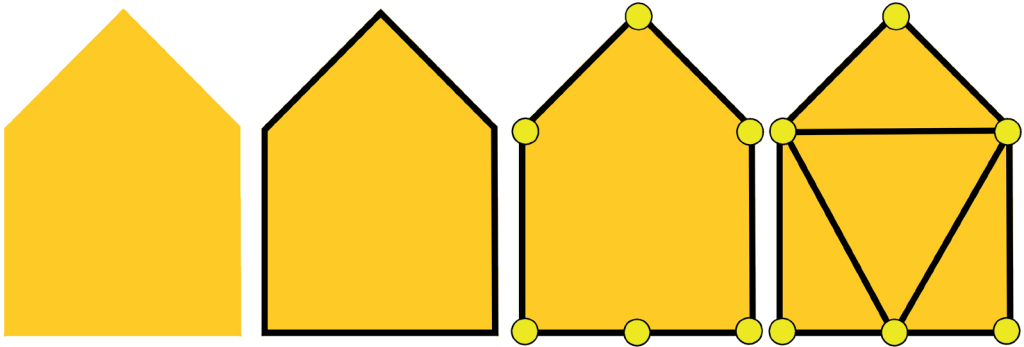


Figure 8. Shape defining by using landmarks.  
Adapted from<sup>10</sup>

To create an average size from two differently shaped houses, house 1 (figure 9, yellow) and house 2 (figure 9, blue), the landmarks of each house are defined and Delaunay triangulation is used to describe the relation between the landmarks. Using the location on the coordinate system, the distance between the landmarks of house 1 and 2 can be deducted and also a vector (direction of the displacement) can be calculated. To calculate the average shape the landmarks of house 1 are moved 50% of the distance in the specified direction, thus creating the average shape between houses 1 and 2 (figure 9, green house)

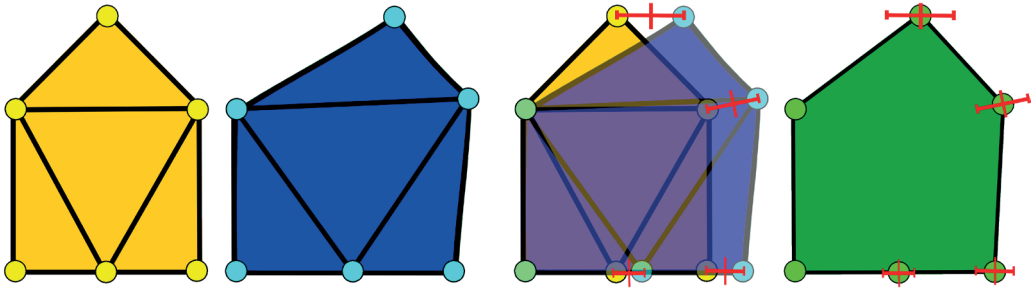


Figure 9. Computing an average shape.  
Adapted from<sup>10</sup>

To calculate the average size of three houses, the just created “average” house (figure 9.4, green house) can be compared to a third house. This time however the landmarks of the green house are moved  $\frac{2}{3}$  of the distance (since the green house “counts” as two houses).

## Folding

As discussed above, affine transformations adhere to certain rules. Two of the rules described were that points and planes needed to be preserved<sup>8</sup>. This means that no landmark can cross the base of its own triangle or the extension of its base as this would in fact delete either the landmark itself or the plane (triangle) that it is part of.

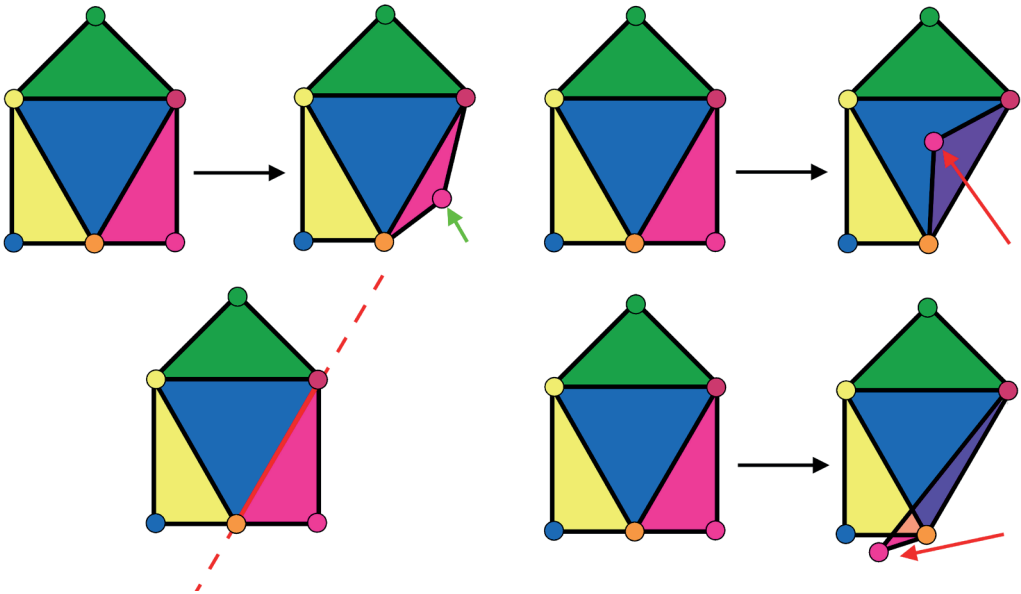


Figure 10. Folding.  
Adapted from<sup>10</sup>

Hence that skinny triangles or triangles with a small surface are not preferred as they severely limit the range over which a landmark can move during warping.

## Thin plate spline warp

Another way to solve the aforementioned problem of having up to 50 landmarks is to use a Thin Plate Spline transformation (TPS). In contrast to the triangulation technique a TPS does not divide the image into multiple triangles. Instead all the corresponding landmarks are used to calculate a “bend”. The TPS was first introduced in geometry by Duchon<sup>12</sup> and later adapted by Goshtasby<sup>13</sup> and Bookstein<sup>14</sup>. The TPS is an algorithm that is currently used in many medical fields such as MRI and CT imaging in which it is important to describe deformation or differences in shape or creating similar images. It is also still being used in



zoology to describe shape differences and similarities between species, and the general principles are not that different to those that Thompson (introduction, bio-mathematics) described.

The essence of a TPS is best explained as a technique of interpolating datapoints whilst smoothing the transition between these data points. Fundamentally it works like this:

Figure 11 (green dots) shows an original image with certain landmarks  $(x,y)$  that needs to be reshaped to match the average shape (red dots) with landmarks located at  $(x_i,y_i)$ . First a grid is placed over the original landmarks. Secondly affine transformations (blue lines) are used to get the location of original landmarks  $(x,y)$  to match those of the average landmarks as closely as possible. Then the grid is bended as if it were a thin metal sheet (hence the name thin-plate spline), with a certain resistance so that a smooth bend is created. When looking from above the original landmarks  $(x,y)$  must match the shape of the average landmarks  $(x_i,y_i)$  when the bend is finalised.

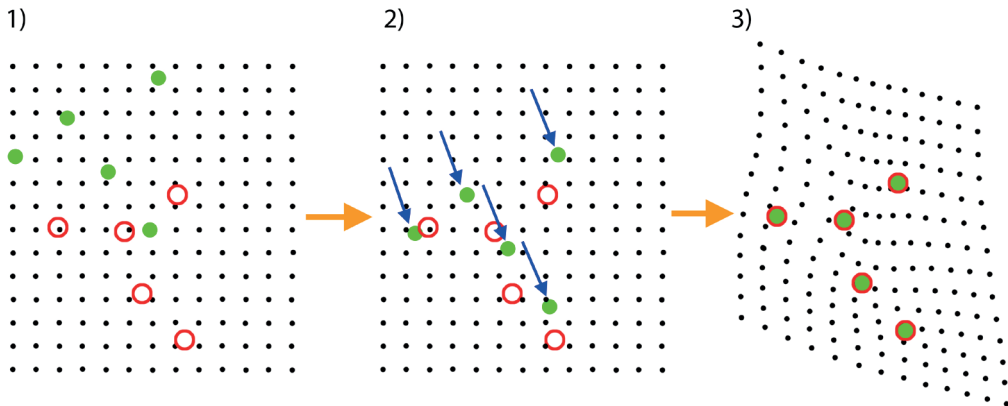


Figure 11. Thin plate spline transformation stages.

Adapted from<sup>14-16</sup>

- 1: put a grid over the landmarks. Green= original landmarks, red= average landmarks,
- 2: rigid transformation for alignment. Blue arrows=translation of original landmarks
- 3: bending of the grid so original (green) landmarks match the average (red) landmarks.

In other words the sheet is bended in such a way that when looking from above the landmarks are located at the same location. However when looking from the side there is a an elevation difference between the original (x,y) and average landmarks (xi,yi).

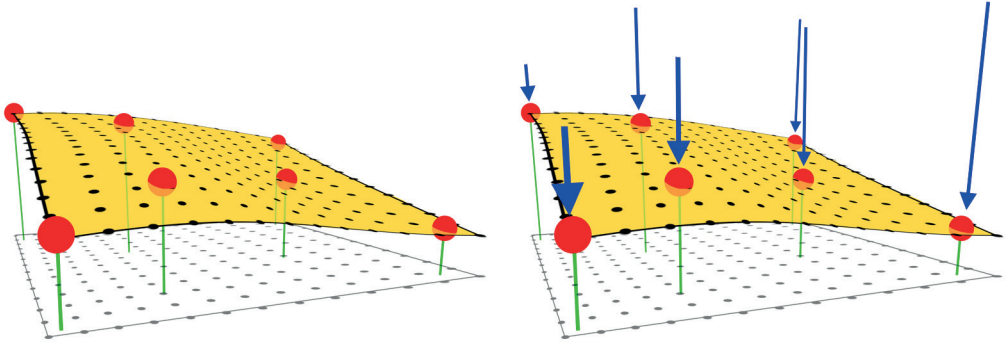


Figure 12. Side view of a thin plate spline

Adapted from Jarno Elonen<sup>17</sup>

A thin plate splines that passes through a set of control points (red dots). The blue lines represent the vertical “force” needed to bend the orinal picture so that it passes through the control points (average landmarks).

When put into a formula the TPS looks like this:

$$f(x, y) = a_1 + a_x x + a_y y + \sum_{i=1}^p w_i U(\|(x_i, y_i) - (x, y)\|)$$

The First part of the formula entails the general alignment using affine transformations of the original landmarks to best match the new average landmarks. The second part of the formula corresponds to the “bending forces” and is determined by P control points (= the number of landmarks) and the “stiffness” of the metal sheet. The third part of the formula calculates the distance between the original (x,y) and average (xi,yi) landmarks.

$$a_1 + a_x x + a_y y +$$

$$\sum_{i=1}^P w_i$$

$$; U(\|(x_i, y_i) - (x, y)\|)$$

1

Since the third part of the formula (distance between original and average landmark) and P (the number of landmarks) are known it is possible to calculate the other, unknown coefficients. Since  $a_1$ ,  $a_x$ ,  $a_y$  and  $W_i$  are dependent on the difference between a specific original and average landmark (third part of the formula) it is necessary to calculate these coefficients for each landmark individually.

$$f(x, y) = a_1 + a_x x + a_y y + \sum_{i=1}^P w_i U(\|(x_i, y_i) - (x, y)\|)$$

Once these coefficients are known for each landmark it is possible to deduct a formula that computes the height of each pixel on the bended sheet. The height of each pixel on the bended sheet is then used to calculate its horizontal and vertical displacement, thus calculating its final position in the average shape.

### Combined techniques

A disadvantage of triangulation is that it is a piecewise method of warping; pixels warped in one triangle have no information of the movement of pixels in other surrounding triangles. This results in unsmoothed warping which sometimes results in unrealistic warps. Figure 13 shows the sudden change in angle of the blue line at the base of warped triangle.

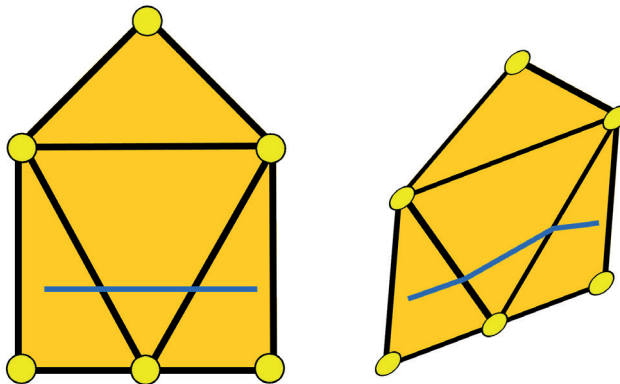


Figure 13. Warping a line using triangulation

Another disadvantage is that triangulation only allows for image registration within the convex hull of the outer landmarks. Although extrapolation of the algorithm to pixels outside the outer edge of the landmarks is possible it usually generates inconsistent results with multiple registration errors. Piecewise warping is however very robust in that it uses relatively easy algorithms and essentially only affine transformations are being applied. Especially pixels located near the sides of the triangle are warped reliably. Also triangulation allows for averaging multiple shapes. In TPS however no average shape can be calculated since the exact location of two landmarks (in different specimen) is needed to solve the other coefficients in the formula. Once an average shape is computed TPS results in a smooth warp most reliably representing reality.

Therefore in CASAM triangulation is used to compute an average shape from every specimen. Then the TPS is used to create a smooth and reliable warp from the original image to match the computed average size. Finally triangulation can again be used to verify the accuracy of the TPS created warp.

## PREPARATION, PHOTOGRAPHY AND LANDMARKS

### Population

All anatomical studies were performed at the anatomy department of the Erasmus MC, Rotterdam the Netherlands. The specimens originated from Dutch volunteers that contractually agreed for their bodies to be used in anatomical courses and anatomy studies after passing away. In The Netherlands specimens are bound to individual Universities and/or institutions and therefore we only used specimens from volunteers that had been assigned to aforementioned anatomy department. Dutch law prohibits the gathering of personal and and/or medical data of deceased patients without explicit permission by the patient ante-mortem or post mortem by the next of kin of the deceased. Therefore, in none of our studies we present data on medical history or population statistics. All specimens were checked for macroscopic signs of disease and unfit specimens not meeting the criteria of health were not used. This mainly applied to studies in which vascular access was paramount and vessels had to be free of atherosclerosis.

### Choice of specimen

Three different kind of specimen were available; conventionally embalmed specimen, Anubifix<sup>18</sup> embalmed specimen and fresh frozen specimen. Depending



of the clinical subject being examined different specimens and different methods of embalming were used.

Most studies did not require for the specimen to be supple and therefore we used specimens which had been embalmed conventionally; the bodies are embalmed at room temperature and at low pressure with a mixture of 6% formaldehyde and 5% phenol to minimize shrinkage and embalming artefacts.

In two studies it was paramount to have specimens with normal joint mobility as the knee needed to be flexed to 90 degrees in order to best represent clinical practice. In one study the ankle needed to be held in 90 degrees in order to best represent clinical practice in calcaneal surgery and duplicate results gathered by conventional anatomy mapping. Specimens used in these studies had therefore been flushed with Anubifix<sup>18</sup>. Anubifix is being used as it reverts post-mortem rigidity whilst maintaining the ability to fully embalm the specimen. After preflushing these specimens were also embalmed at room temperature and at low pressure with a mixture of 6% formaldehyde and 5% phenol.

In one study the anatomy of the arterial supply of the hindfoot was studied in relation to calcaneal surgery. In order to dissect even the smallest arteries fresh frozen specimens were used. As soon as the specimens were thawed the popliteal artery and its branches were filled with FilloPaQ<sup>19</sup>. FilloPaQ is a filling-agent having a viscosity close to that of water, but still hardens in 8-10 minutes. It therefore is capable to even reach the smallest capillaries in the (hind-) foot when the right amount of pressure is used. Blue-green dye is added to FilloPaQ as it contrasts well to the red colour of muscle and the yellow colour of fatty tissue. Once set FilloPaQ is slightly flexible (rubber like consistency) and therefore less brittle than other filling agents such as Biodur. This allows for easier manipulation when dissecting. After mapping the arterial anatomy the specimens were again frozen so that data could be verified afterwards if need be.

## Dissection

Especially when superficial cutaneous nerves are being dissected, dissection begins with the removal of skin, epidermis and dermis (figure 14).



Figure 14. De-epithelialisation.  
Dissection of the dermis of the posterior side of the lower leg.

To ensure comparable exposures and results the dissection method was standardized for each study. Classification of the artery, nerve or vein started proximally and consequent branches are then dissected peripherally. Terminal branches are dissected using a 2,5 times magnifying loupe (figure 15).



Figure 15.  
Dissection using a 2.5 times  
magnifying loupe.

The arterial wall of arteries filled with FiloPaQ<sup>19</sup> are cut open longitudinally and superficially removed so that the dyed FiloPaQ contrasts with surrounding tissue as much as possible (figure 16).



**Figure 16.** Two fresh frozen specimens filled with FiloPaQ.

1: Arterial wall intact.

2: Arterial wall incised longitudinally and peripherally removed so the (white) FiloPaQ is visible and contrasts to surrounding tissue.

Surrounding and underlying subcuticular tissues need to be meticulously preserved in order to avoid any alteration to the natural location of the nerve studied (figure 17). Care should be taken not to dissect at multiple levels at the same time as this will compromise the location of the anatomical structure. Instead the same specimen can be photographed multiple times at different stages of the dissection. The CASAM method allows for anatomical data gathered at multiple levels to be combined. In this way it is possible to visually map deeper anatomical structures without having to compromise data gathered superficially.



**Figure 17.**

Example of mapping the anatomy of multiple layers in multiple stages.

1: Dissection and mapping of the superficial branches of the short saphenous vein.

2: Dissection and mapping of the main branches of the short saphenous vein (located superficial to the fascia)

3: Dissection and mapping of the medial branch of the sural nerve (located deep to the fascia)

## Photography

Standardized photography of the specimen is the most important step in CASAM. Although researchers mostly focus on dissection and the warping process, miniscule deviations to the standardized photography have a huge impact on the final visualisation.

All dissected specimen are photographed using a Canon 350D body<sup>20</sup>. Initially a Canon EF-S (Canon USA, Inc, San Jose, Calif) 18-55 mm lens was used. In more recent studies however the use of a Sigma (Sigma Corporation of America, Ronkonkoma NY) EX 50mm 1:2.8 DG macro lens is preferred. Macro-lenses are by nature ‘flat’ field lenses<sup>21</sup> whilst general lenses suffer from field curvature. In general lenses the focal-point will be at a slightly different distance at the corners of the frame when compared to the centre of the frame. A disadvantage of using macro-lenses is that depth of field is extremely shallow; meaning that merely centimetres away from the focal point of the lens an object can be out of focus. Another disadvantage of the macro lens is that it generally is difficult to get enough light. Therefore indirect lighting, a tripod and a remote are used. Hereby long exposure times can be achieved without any motion artefacts, allowing for adequate lighting whilst maintaining a small aperture. Using a small aperture results in a wider depth of field, thus having the majority of the specimen in focus.

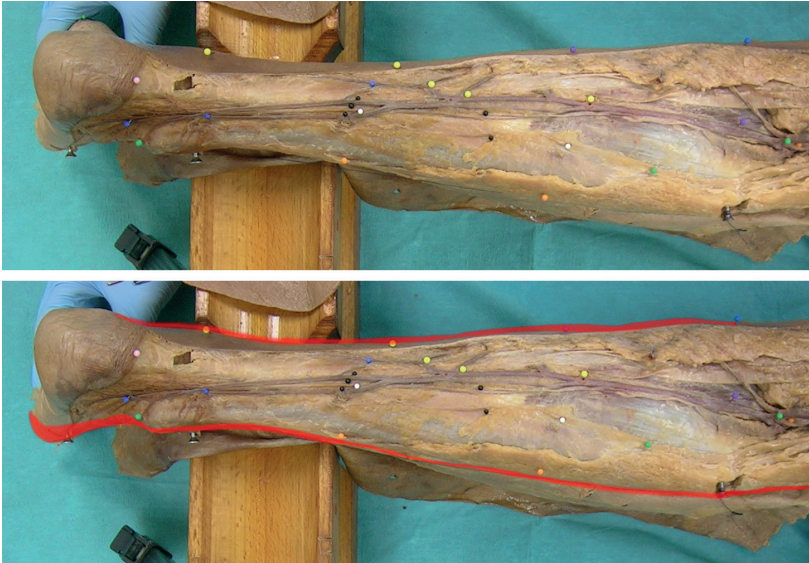


Figure 18. Camera setup and alignment for standardized photography



A ruler is used as a reference for digital measurements. The distance of the ruler to the camera is very important. For instance placing the ruler really close to the camera would make it seem bigger in the image and therefore not representative to distances in the specimen. Therefore the ruler was placed exactly midway between the closest and furthest points of interest. Similarly to the example with the ruler, the distance between the specimen and the camera needs to be exactly the same for each specimen.

Landmarks are used to centre and align specimen to the camera in a standardized way. Then it is imperative to quantify the amount of rotation of the specimen relative to the camera position. Mal-alignment and rotation of the specimen have a big impact on the shape-defining landmarks as the outer edges of a specimen change depending on the angle at which they are observed.



**Figure 19.** Malrotation resulting in wrong representation of shape

- 1) Normal alignment.
- 2) Malrotation and concurrent wrong representation of shape (red)

## Landmarks

One of the first steps in using CASAM is to define the shape and size of each individual specimen. The most straightforward landmarks are based on the human bones. The length of the lower leg for instance (figure 20) can be easily defined as the distance between the tibia-plateau (superior part of the tibia)



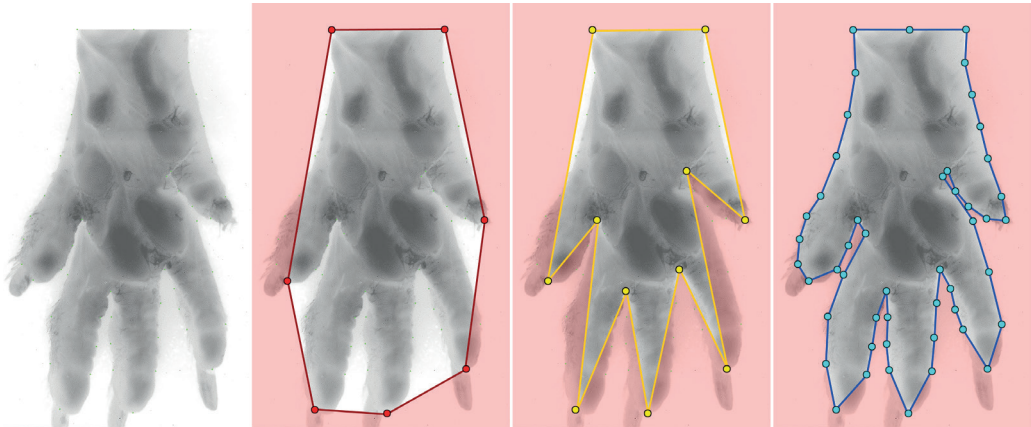
at one third of the distance between Bony landmarks 1 (=tibia plateau) and 3 (=lateral malleolus).

As the location of shape-defining landmarks is heavily dependent on the few bony landmarks, reproducibility of the placement of the bony landmarks is very important and therefore needs to be verified. Furthermore any anatomical structure to be studied needs to be located inside of both bony and shape-defining landmarks as otherwise it is not taken into account when being warped (figure 20 green area). Landmarks need to correspond between pictures and are assigned a specific number.

### Landmark optimisation

Especially when studied specimens have more complex shapes, such as a hand with multiple fingers, it is imperative to optimise the number and location of the landmarks placed. Figure 21 shows the hindpaw of a rat in which we studied the innervation using evans blue extravasation. For this study it was therefore essential to also map the toes of the rat.

When only seven landmarks (figure 21.2) are used (on the tip of the toes and the base of the sole) some parts of the the paw are not mapped (red area) and the essential shape of the paw is not outlined well. Therefore the width of the toes is



**Figure 21** Defining the outlines of a specimen using 7, 11 and 49 landmarks.  
Red area: area not taken into account as it lays outside the outer edges of the landmarks

- 1: Original image
- 2: Seven landmarks
- 3: Eleven landmarks
- 4: Forty-nine landmarks

not taken into account when warping, even though the width, as described earlier, is as essential to anatomy localization as length.

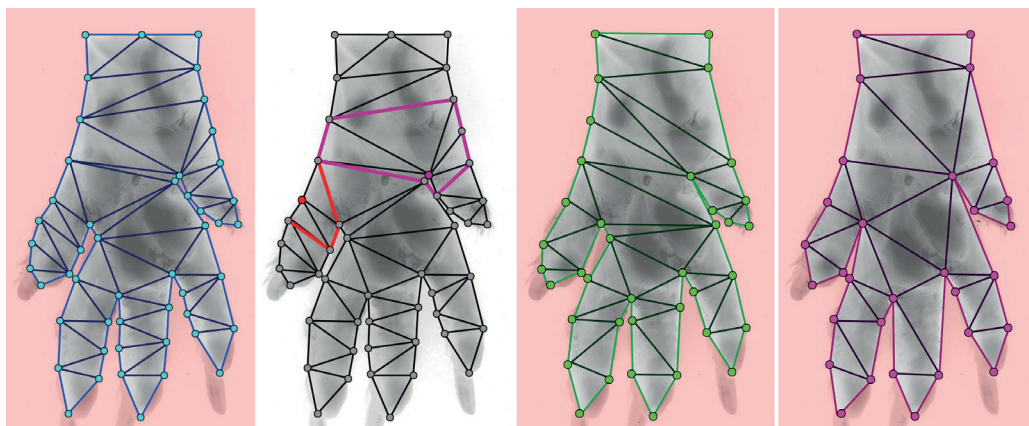
To further describe the outline of the hindpaw's shape we then placed four additional landmarks ([figure 21.3](#)) to the most distal edge of the webspace between each toe. However in doing so, important parts of the toes could not be taken into account as they were localised outside the outer-most edges of the landmarks (red area).

To resolve this problem more landmarks were created in a similar way to the shape-defining landmarks used in other CASAM studies. These shape-defining landmarks were based on the width of the toes and hindpaw at a well defined location between the initially chosen eleven landmarks. The result of the additional 38 landmarks ([figure 21.4](#)) is that the shape of the hindpaw is outlined well. The width of the toes is well defined at different locations. Areas that could not be taken into account (as they laid outside the outer edges of the landmarks) were small and mostly included the toenails and distal tips of the toes.

A disadvantage of using these 49 landmarks is that when triangulating the image satisfying the Delaunay condition a total of 47 triangles are formed ([figure 22.1](#)). One disadvantage of so many triangles is the added time of computation. Secondly, creating too many triangles makes for skinny triangles without much surface and narrow corners.

[Figure 22.2](#) shows two example landmarks, red and purple, and the similarly coloured boundaries that each landmark can not cross when it is going to be warped. Crossing these boundaries when warping would result in “folding” and creates a useless warp. The surface over which the red landmark is allowed to move is not that large as the three triangles that determine its range of motion are also small. The purple landmark for instance has a much higher degree of freedom because of two reasons; 1) it is a centrally placed landmark and can therefore move over more triangles. 2) Especially the proximal landmarks have a big surface. Distal movement (towards the toes of the hindpaw) is however severely limited as the triangles distal to the purple landmarks are really skinny and have narrow corners.

Previously we have already discussed that when warping a landmark it is not allowed to cross the basis of (any of) its triangles as it results in “folding” of the image and an utterly unusable and unrepresentative image. We therefore have to optimise triangulation by selectively deleting Landmarks that triangulate with many other landmarks and thus are most likely to form small and skinny triangles.



**Figure 22.** Optimising landmarks using triangulation and selective landmarks.

- 1: blue, 49 landmark triangulation.
- 2: Purple and red lines: limits of movement for corresponding landmarks.
- 3: Green, 38 landmark triangulation.
- 4: Purple, 31 landmark triangulation

In [figure 22.3](#) the number of landmarks was selectively lowered to 38 resulting in 36 triangles. The general shape of the hindpaw is still well delineated and hardly differs from [figure 22.2](#). At least four of the triangles however still have quite narrow angles. In [figure 22.4](#) the number of landmarks has been further reduced to 31, resulting in 29 nicely shaped triangles, all having sufficient surfaces and decent corner angles. Most importantly, reducing the number of landmarks from 49 to 31 has hardly lowered the quality of delineating the shape of the hindpaw as it is still nicely represented in both length and width. Only the nails and tips of the toes can not be taken into account as they lay outside the outer border of the landmarks but these were of no interest in this study.

### Landmark reproducibility

The whole background for CASAM is that specimens in general are not of the same shape. Therefore the landmark optimisation as described needs to be completed for at least three different specimens to establish which landmarks delineate the shape best without creating small and skinny triangles.

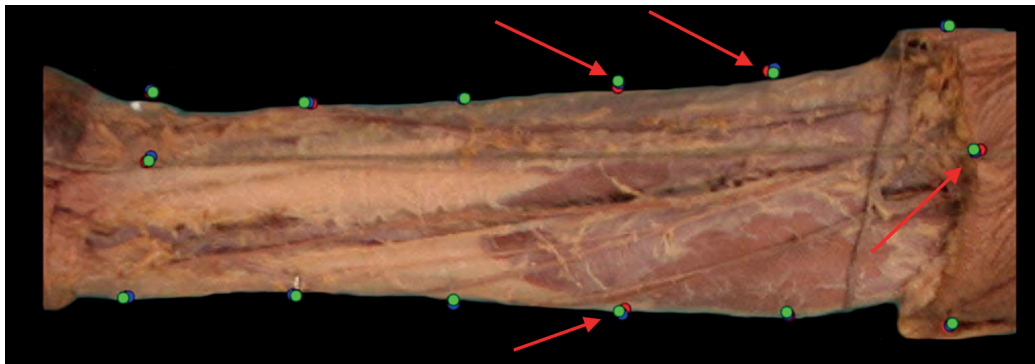
Secondly, reproducibility of landmark placement within the same specimen needs to be verified and only landmarks that can be consistently placed (usually within a 2 mm radius) should be used. If consistency within one specimen is hard to obtain, it most probably will be impossible to reliably place landmarks among



multiple specimen. If consistent landmark placement seems to be difficult, even when experienced, a different view sometimes solves reproducibility problems.

Thirdly, landmark placement, and especially the distribution of landmarks between specimens needs to be quantified. Therefore, three different specimens, each showing their own landmarks need to be aligned. Then the distribution of each landmark between all specimens can be visualised and landmark placement can be optimised. If for instance landmark 3 shows little variation between three different specimens it might be redundant. If landmark 5 for instance shows huge variation between three different specimens it might be of benefit to add an additional landmark so that the variation is divided. Furthermore when the spreading of a certain landmark is compared to the surfaces and angles of the surrounding triangles formed by Delaunay triangulation, the likelihood of folding can be estimated.

Finally, after warping the original images to match the computed average the landmarks of all three specimens need to be at the exact same location so that the shape of each specimen is the equal. [Figure 23](#) shows a post-warping assessment of landmarks placed on the volar side of the fore arm. Four of the landmarks show variations between specimens (especially specimen 1, red) which suggests that landmark placement and/or warping still needs further optimisation.



**Figure 23.** Post-warping to average assessment of landmarks  
 Red dots: specimen 1  
 Blue dots: specimen 2  
 Green dots: specimen 3

## WARPING

Photoshop CS-4<sup>23</sup> is used to align the original images, MagicMorph<sup>24</sup> is used to create an average shape and then warp the original images to match the computed average shape. Photoshop CS-4 is then used to create renditions of the images.

### Averaging the shapes of specimen

Multiple photographs are taken of each specimen. The most aligned and sharp image of each specimen is chosen. Photoshop is used to load the images into stack (File; scripts; load images into stack) so that they are loaded into the same file in different layers.

Landmarks are highlighted using a hard brush tool (B) in each image and if applicable shape-defining landmarks are calculated from bony landmarks using the ruler tool (Analysis; Ruler tool). If digital, absolute measurements need to be taken the ruler tool can be calibrated using the photographed ruler (Analysis; set measurement scale; custom). This way the number of pixels can be represented as actual millimetres. Intrinsic measurements (non-digital) are however preferred. Rigid transformations are applied to further centre each specimen. Pressing (Ctrl+A) selects the entire image of selected layer then (V) can be pressed to select the move tool. In order to compare left and right specimens (legs for example) images of either one need mirroring. To do so the entire image is selected (Ctrl+A) and then a horizontal reflection is made (images; image rotation; flip canvas horizontally). Finally, all aligned layers are then exported as individual images (Edit; transform; flip horizontal).



The images of the first two specimens are then loaded into magic morph. The warping software uses triangulation to compute virtual locations of landmarks when one image is gradually warped to match the second. This means that the software can be used to compute an average size (figure 24).

First corresponding landmarks are marked in specimen 1 (field 1) and specimen 2 (field 2). The exact coordinates of these landmarks are shown in the control point coordinate field. Then the morph mode needs to be changed to source warp, so that specimen 1 is warped to match the size of specimen 2. The output file (computed image) is shown on the right field. A slider-bar can be used to adjust the shape of the output file to either match specimen 1 (0%) or specimen 2 (100%). When put at 50 % the landmarks of the output file correspond to the average size of specimen 1 and 2. The coordinates of the computed average landmarks can be verified in the control point coordinate field. The computed average image of specimen 1 and 2 is then saved and loaded into field one. Specimen 3 is loaded into field two. The process of averaging is repeated, however this time the slider bar that adjusts shape of the output file is set to 33.3%. This means that the shape of the output file resembles the shape in field 1 more than the shape in field 2, thus representing the average shape of specimen 1, 2 and 3. The averaging process is repeated for all other specimens, finally resulting in an output file that represents the average shape of all specimens.

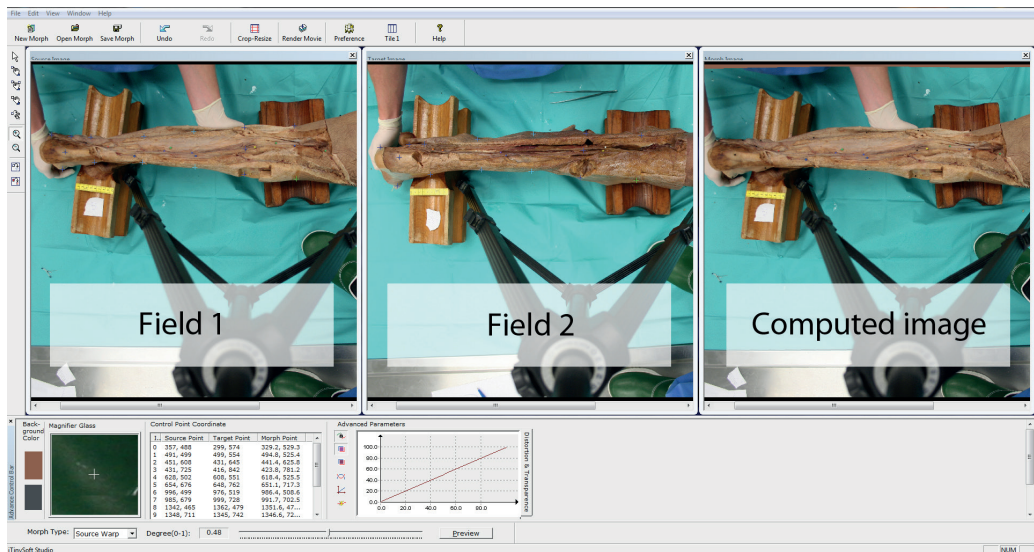


Figure 24. Warping software; using triangulation to average shapes.

### Warping original images to match the average

The morphing software uses a TPS for the actual morph itself and can therefore be used to warp the original images of specimens to match the computed average size.

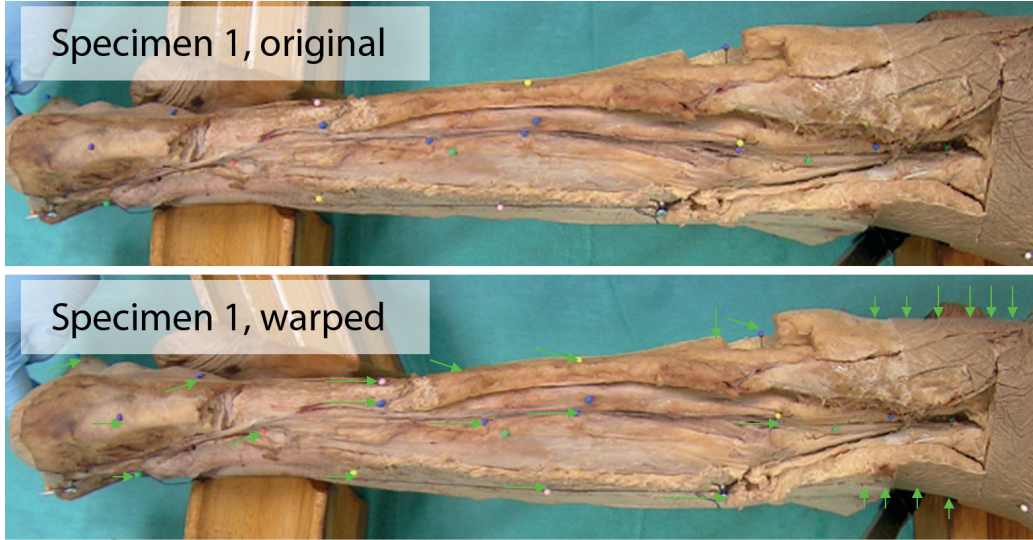


Figure 25. Specimen 1, original shape and warped to match the average

The image of specimen 1 is loaded into field 1, the averagely shaped image is loaded into field 2 and corresponding landmarks are marked. Since placement of the landmarks in the average image is extremely important and needs to be consistent each time an original image is warped and a custom script is written for each project. The script allows for automated and consistent landmark placement in field 2 (averagely shaped image) and is executed in Autohotkey<sup>25</sup>. When the landmarks are placed the morph mode is set to source warp and the slider determining the shape of the output file is set to 100%. This means that the output file depicts specimen 1 (field 1), but consists of the average shape (field 2). This process is repeated for all other original images of each specimen.

The quality of output files can be analysed using the TPS used in the web-based version of CASAM en through custom piecewise warping through triangulation.

## RENDITIONS

So far, each specimen has been dissected, photographed, its shape delineated and then warped to match the average size of all specimens. Therefore all specimens now have the exact same (and average) shape and size. This allows for their anatomy to be mapped and compared. Renditions are made in Photoshop CS3 to CS6<sup>23</sup>.

All warped images are loaded into the same file at different layers (files; scripts; load files into stack). Although the relevant anatomy of multiple specimens will be visualised, the rest of the images is not going to be used. Instead one of the averagely shaped specimens will be used as a background over which the anatomy will be displayed. One of the layers is chosen and non-relevant background is selected using the Quick selection tool (Q) and deleted resulting in a roughly edged specimen.



Figure 26. deleting irrelevant background

The edges of the specimen are then smoothed using the soft-edged Eraser tool (E)



Figure 27. Smoothing of the edges

### Point distribution model

It is important that each structure is highlighted individually for each specimen in a separate layer (so the nerve, artery and fascia layer of specimen 1 are all highlighted in different, separate layers). First create a new layer (Ctrl+Alt+N) then enter the quick mask (Q). Quick mask allows for visualisation of the selected area and refine the edges or shape of the selected area). The brush tool (B) can then be used to select a certain area, such as the origin of the short saphenous vein. Exit the quick mask (press Q again) before filling the area with colour (edit; fill). To show a specific point the selection is also outlined (edit; stroke).



Figure 28. Highlighting a specific point. Quick-mask selection, fill, stroke.

When corresponding points have also been selected for the other specimens (each in separate layers) all layers depicting, for instance, the origins of the short saphenous vein can be merged (layer; merge layers). The merged layer then shows the distribution of origins of the short saphenous vein and can be shown over the created background and saved as a separate rendition.

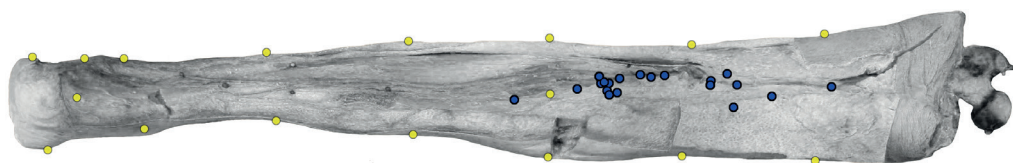


Figure 29. origins of the short saphenous vein of 20 dissected specimens.



### Multiple line model

To highlight the complete course of a nerve it is first necessary to create a new layer (Ctrl+Alt+N). After the quick mask is entered (Q) the brush tool (B) can be used to roughly outline the course of the nerve. A sharp edged eraser tool (E) can then be used to deduce the selection to the actual outlines of the nerve. After exiting the quick mask (Q) the selection can be filled with a yellow colour (edit; fill).



Figure 30. Highlighting a nerve.

The opacity of the layer can be changed (layer; layer style; blending options) so that the background of the image is still visible.

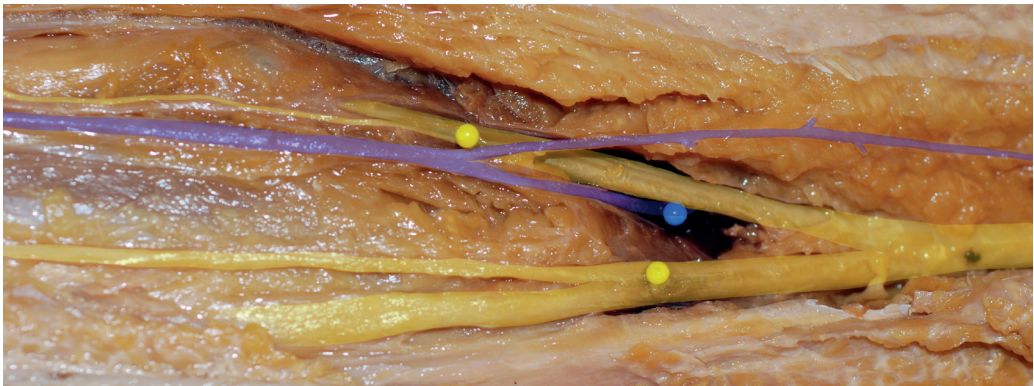


Figure 31. The opacity of layers highlighting the nerves and short saphenous vein is reduced to 40%.

When the nerves of other specimens have also been highlighted in separate layers, all layers can be merged (layer; merge layers). The merged layer then shows the area of distribution of the nerve.

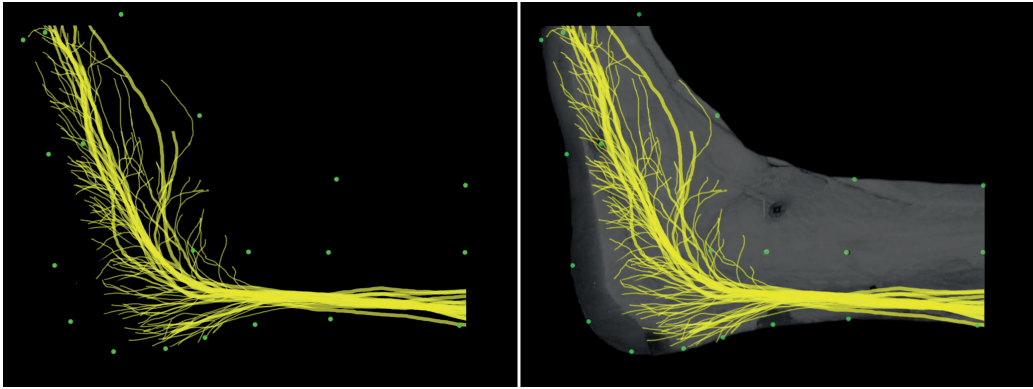


Figure 32. Distribution of the sural nerve on the lateral ankle of 10 specimens.

To visualise the location dependent direction of nerves, first all layers with highlighted nerves need to be merged (layer; merge layer). Then a grid of squares is placed over the computed area of spreading of all nerves. Within each individual square, the direction of all branches of the nerve is being measured in relation to the base-line of zero degrees. Angle measurements are taken using the ruler tool (Analysis; ruler tool) while holding (Alt).

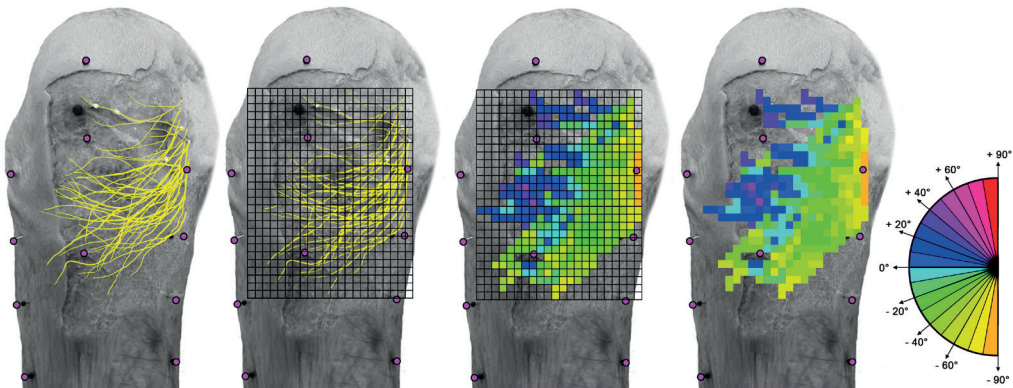
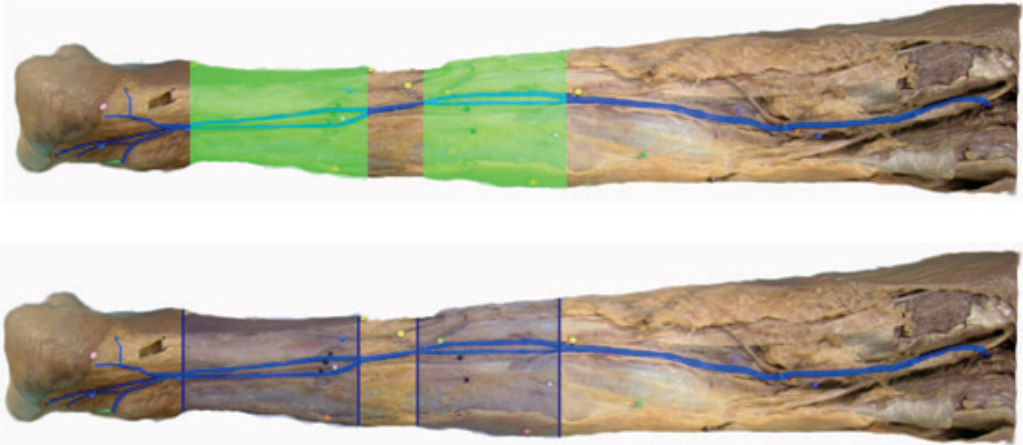


Figure 33. location dependent direction of nerves.

### Multiple area model

To select a certain area in a specimen the specimen needs to be selected first. The magic wand tool (W) can be used to select the area surrounding the specimen. Then the selection needs to be inversed (Select; inverse) to select the specimen itself. After entering the Quick mask (Q) the areas of the specimen that are not being highlighted need to be removed using the sharp edge Eraser tool (E). After

exiting the Quick mask (Q) the selected area can be filled with colour (edit; fill) and the opacity of the layer can be adjusted (layer; layer style; blending options).

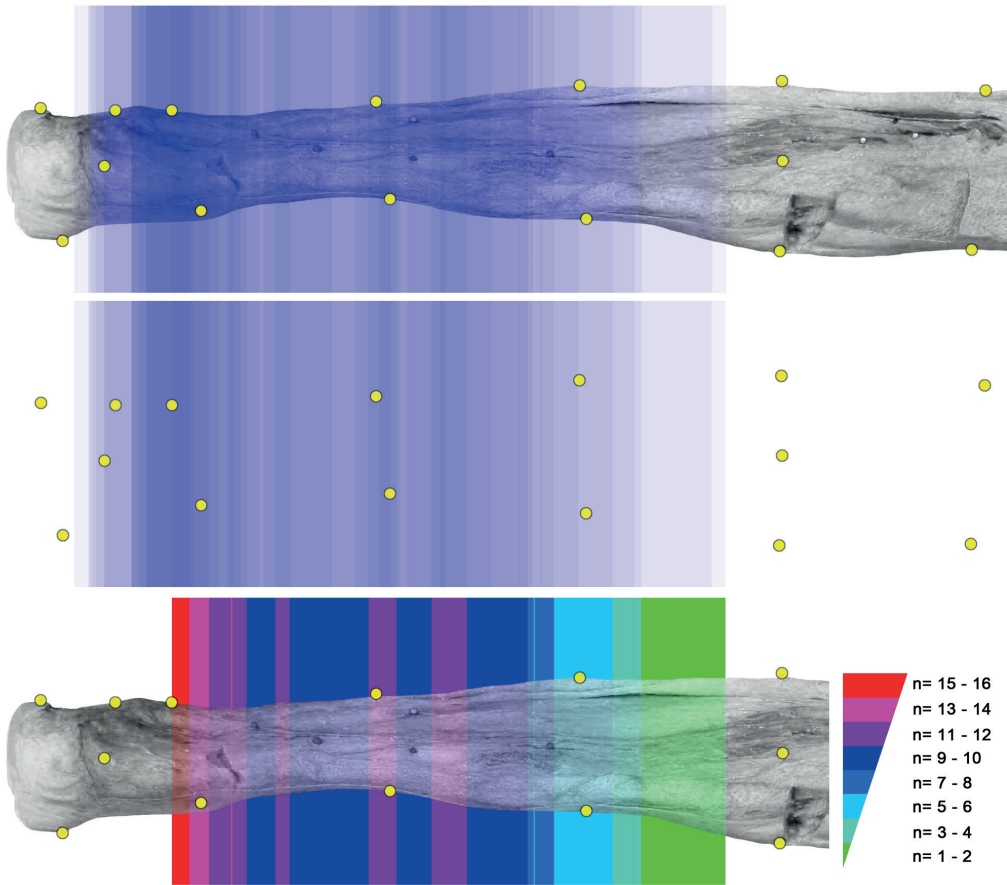


**Figure 34.** Showing the area in the lower leg in which the short saphenous vein has a duplicated trunk.

To make renditions of areas in multiple specimens the opacity of the individual layers of each specimen need to be adjusted. When 20 specimens are used the opacity of each layer needs to be set to 5% ( $=100\%/20$ ). Then the individual layers can be merged (layer; merge layers) and visualised over a background specimen. The combined opacity of the layers represents the amount of specimens in which the highlighted area is present. **Figure 34** for example shows the area in the leg in which a vein has a duplicated trunk. In **figure 35.1** when the combined opacity of selected areas is 100 % this represents that the vein has a duplicated trunk in all 20 specimens. If the combined opacity of the selected areas is just 10 % this represents that only 2 of the 20 specimens have a duplicated trunk.

Different opacities (or intensities) of the same colour are difficult to distinguish. Also most printers have trouble printing 20 different intensities of blue. To solve this problem the different intensities of blue can be given a certain colour. To create this rendition first the merged layer showing all areas with an opacity of 5 percent needs to be merged with a completely black layer. Then the magic wand tool (W) can be used to select the different intensity levels of blue (0,5,10,...., 100%). Finally each of the intensity levels, representing a certain number of specimens for that area) can be allocated a certain colour.





**Figure 35.** Rendition of 20 specimens. 1: original combined areas  
 2: areas overlay on a black background  
 3: final rendition. Each colour represents a certain number of specimens in which the short saphenous vein has a duplicated trunk.

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