practice, it would be possible to carry out a statistical analysis of point patterns on the original datasets of a curved layer of points without flattening. However, for the coarse descriptions with limited goals referred to above, this would represent little major improvement.

Analysis of non-laminar point swarms. Such analysis must follow other routes than those described above, and the questions asked will often be different. With real-time rotations, colour coding for different populations of points, zooming, windowing etc., done on a graphics workstation, any obvious distribution pattern is easily revealed. For example, after injection of a retrograde tracer in one single folium of the cerebellum of the cat, it appears from reconstructions of complete series of sections that the labelled cell groups in the pontine grey form continuous, convoluted, bands, to some extent with interconnected loops. The point swarms to the right in Fig. 2 (three different angles of view are shown) are 2884 neurons retrogradely labelled after injection of Fluoro-Gold in one single folium. In single sections this pattern could not be identified. More complex analysis has been performed with simultaneous application of several fluorescent tracers (such as Fluoro-Gold, Rhodamine-B-Isothiocyanate, and Fast-Blue) placed in adjacent or distant folia of the cerebellum of the same animal. The degree of overlap or segregation of different populations of neurons can be decided by visual inspection of the reconstructed point swarms from different angles of view or, more objectively, by performing a comparison of the files for each population, asking the question, how many cells (as a percentage of the total) of one population are located within a certain distance from any cell of another population?

In conclusion, three-dimensional reconstruction with subsequent application of various analyses represents an important tool for the study of neural architectonics in terms of numerical distribution and interrelations between cell groups and their connections.

Comparison of three-dimensional form. By JOAN T. RICHTSMEIR*, JAMES M. CHEVERUD†, and G. ROBERT MORRIS‡. * *Department of Cell Biology and Anatomy, the Johns Hopkins University School of Medicine, Baltimore, MD*, 21205, *USA*, † *Department of Anatomy and Neurobiology, Washington University School of Medicine, St Louis, MO*, 63110, *USA and* ‡ *Department of Civil Engineering, the Johns Hopkins University, Baltimore, MD*, 21218, *USA* (Fig. 3)

Analysis of the morphology of whole organisms can be designed to generate hypotheses through exploratory data analysis, or to test hypotheses based on previous biological knowledge. Although there are several methods available for the analysis of forms using landmark data, our laboratories have found a specific morphometric technique, finite-element scaling analysis (FESA) (Lewis *et al. J. Biomech.* **13**, 1980) particularly informative in the study of morphological variation.

FESA is a method of comparison based on principles of continuum mechanics. An important feature of FESA is the ability to detect those landmarks that contribute most to the differences between the forms being compared (Cheverud & Richtsmeier, *Syst. Zool.* **35**, 1986). This ability is critical to understanding the processes that underlie form difference (e.g. growth, evolution, teratological mechanisms). FESA quantifies differences between forms local to each landmark in terms of magnitude and direction (in 2-D or 3-D) of change required to produce the target morphology from the initial morphology.

To compare two forms using FESA, each form is discretised into contiguous fine elements. Differences between forms are measured in terms of strain local to the landmarks. These calculations are based on derivatives. This simply means that we use information from surrounding landmarks in order to calculate change at a particular point, and that measures of local form difference are functionally (mathematically) related across an element. Since localisation of form difference is an extremely useful feature, we think it critical to understand the impact of functional relationship among landmarks within an element on the measure of strain calculated local to each landmark.

To investigate the relative independence of local strain measures, we performed the following stimulation studies. Assuming an eight-noded hexahedron as the element type, we defined a cubic standard element. A series of 100 simulated target elements were generated from it by relocating each of the eight vertices in the X, Y and Z directions. The relocations were randomly drawn from a trivariate normal distribution, with mean equal to the actual X, Y or Z coordinate of the vertex in the standard cube, with no covariance among the three dimensions. Changes in the choice of variance did not affect the outcome. We did not allow landmarks to move more than one-third of the distance towards another landmark along an edge. This prevented vertices from exchanging locations, a constraint that we feel is biologically realistic. The standard cube was deformed into each of the 100 simulated target elements. The vertex-specific strains were correlated with one another to measure the relative independence of localised measures of form difference. We found

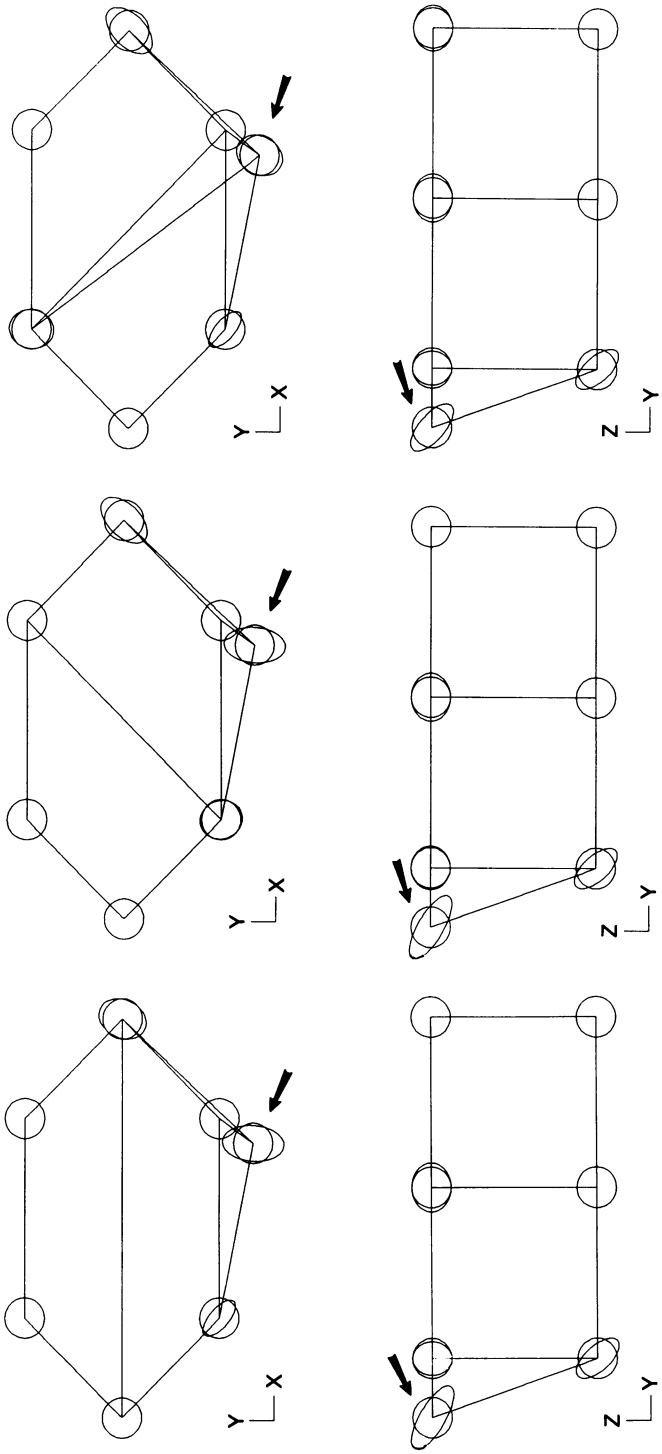

Fig. 3. Analysis of the difference in morphology between the initial and target (pictured here) morphologies. The arrow marks the landmark which was perturbed along the X and Y directions. A circle is drawn around each landmark indicating no change in form local to that landmark. An ellipse drawn over the circle indicates the direction and magnitude of local changes. Analysis was done in 3-D; graphics are projected on to a 2-D plane.

that any vertex shared less than 10% of its variance ($r = 0{\cdot}30$; $r^2 = 0{\cdot}09$) with each of the other three vertices connected to it by an edge, and was completely uncorrelated with the other four vertices composing the element.

A second set of simulations used a form modelled as a pair of contiguous cubes (including 12 vertices, four of which are shared between elements) as the standard element. One hundred simulated target forms were generated by the procedure described above. For the eight vertices included in only one element, the results are the same as those described above. For the four vertices common to the two elements, 17% of their variance is shared with each of the other two common points connected to it by an edge, while 5% of their variance is shared with each of the external points to which it is connected.

To examine how connectivity of nodes in element design affects local directions of change between forms, consider a polygon consisting of 12 landmarks. Landmarks 1–6 are arranged as a hexagon, all sharing the same Z coordinate. Landmarks 7–12 correspond exactly with landmarks 1–6 on the XY plane, but differ uniformly in their Z coordinate. We perturbed a single landmark in this reference form by moving it along the X and Y axes. The original form was then compared to the perturbed form by using three equally valid element designs. Quantitative results from the three element designs are consistent for individual landmarks except when the element design places the landmark under consideration on to the contiguous face. In this case local strain is reduced in magnitude, in a sense 'shared' among the landmarks to which it is connected. For landmarks which never lie on the shared face, magnitudes of form difference are constant across the three element designs. Change in magnitudes associated with element design are presented graphically in Fig. 3. The figure also demonstrates how the actual orientation of the change is affected by element design.

The relationships defined above result from the mathematical relationship between the vertices required by the finite element model. They will be biologically realistic when gross discontinuities of form do not occur along the edges or inside the elements. Elements should be designed to encompass relatively homogeneous areas, and obvious morphological discontinuities should be used as boundaries for elements rather than being incorporated along the edges. Knowledge of the patterns of association between vertices connected by an edge can be accounted for in analyses of the relationship between morphological differences at various locations.

There are several striking instances of FESA's ability to identify localised regions of morphological change (e.g. Richtsmeier & Cheverud *J. Craniof. Genet. and Devel. Biol.* **6**, 1986; Richtsmeier, *Acta Anatomica* **133**, 1988; Richtsmeier & Lele, *J. Craniof. Genet. and Devel. Biol.* 1989). The simulation studies presented here indicate the importance of reviewing alternate mesh designs in our continuing use of FESA for the analysis of biological forms. In certain cases, the use of tetrahedra in place of hexahedra may be warranted. These considerations are currently being investigated in our laboratories.

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