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Displaced amacrine cells in the retina of a rabbit: analysis of a bivariate spatial point pattern

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A data set consisting of the locations of "light-on" and "light-off" displaced amacrine cells in the retina of a rabbit is analysed using recently developed statistical methodology. The results are used to discriminate between two biological hypotheses concerning the genesis of the data. Some general comments are made on the statistical analysis of spatial point patterns.

Introduction

In recent years, there has been considerable interest in studying the spatial pattern presented by particular types of retinal cell bodies (Wässle et al., 1978, 1981a, b, c; Wässle and Riemann, 1978; Hughes, 1981 a, b; Peichl and Wässle, 1981; Vaney et al., 1981). Fig. 1 shows a *bivariate* spatial pattern consisting of two types of displaced amacrine cells in a 1070×600 μm rectangular region within the retinal ganglion cell layer of a rabbit (data supplied by Dr A. Hughes). The 152 "type 1" cells process "light-on" information in the eye whilst the 142 "type 2" cells process "light-off" information. A quantitative description of such patterns is of interest because of its implications for development and retinal sampling efficiency (Hughes, 1981a). A specific question in connection with these data is to discriminate between two competing hypotheses:

Hypothesis 1 (H1): light-on and light-off cells are formed initially in two separate layers.

Hypothesis 2 (H2): cells are formed initially in a single layer, differentiation into light-on and light-off cells occurring at a later stage of development.

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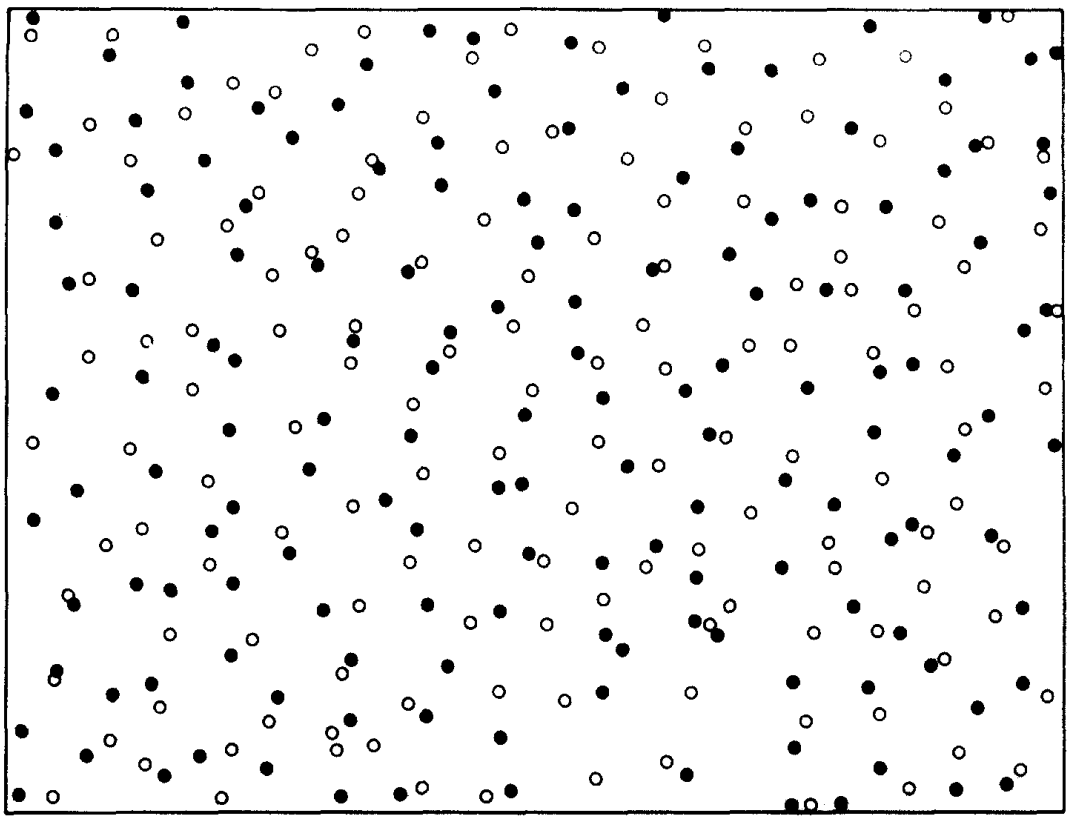

Fig. 1. Displaced amacrine cells in the retina of a rabbit. ●, "light-on" cells ($n_1 = 152$); ○, "light-off" cells ($n_2 = 142$).

One implication of H1 is that the two component patterns of light-on and light-off cells are statistically independent, whilst under H2 they are generally dependent, even if the differentiation is completely random.

Quite separately from this biological activity, statisticians have been developing methodology for the analysis of data like Fig. 1 (Ripley, 1977, 1981; Diggle, 1979, 1983; Ogata and Tanemura, 1984). The purpose of the present paper is to provide an analysis of the data in Fig. 1 which, apart from its intrinsic interest, illustrates the application of some of this recently developed statistical methodology. The paper is an expanded version of part of the author's presentation to the International Course on Morphometry and Stereology in neurosciences held at the Netherlands Institute for Brain Research, Amsterdam in May 1985.

Statistical methods

Complete spatial randomness, independence and random labelling

The concept of complete spatial randomness, henceforth CSR, is fundamental to the quantitative description of spatial pattern. We refer to the positions of the cells

in question as *events*, to distinguish them from arbitrary *points* in the region of observation A. A formal definition of CSR is that the events in A constitute a partial realisation of a homogeneous, planar Poisson process (Diggle, 1983, pp. 50–51); this process incorporates a single parameter, λ, the *intensity*, or mean number of events per unit area; the actual number of events in A, n say, is an observation from a Poisson distribution with mean $\lambda |A|$, where $|A|$ denotes the area of the region A. If we consider n as fixed, we arrive at the following more tangible definition of CSR: (1) each of the n events is equally likely to occur at any point within A; and (2) the n events are located independently of each other. CSR provides a benchmark for the description of "interesting" patterns i.e. patterns which are significantly different from CSR. In the present context, the most common deviation is for the events to exhibit varying degrees of regularity due to inhibitory interactions between neighbouring events, in violation of (2). Violations of (1) are more likely to arise if a relatively large region A is involved, within which there are major variations in the local intensity of events in different sub-regions.

A *bivariate* spatial point pattern is one in which the events are of two distinguishable types. One possible benchmark hypothesis for the assessment of interactions between the two types is that *the two component patterns are determined independently of one another*, as would be appropriate under our hypothesis H1. If, on the other hand, a bivariate pattern arises through some form of labelling mechanism, as under our hypothesis H2, the question of whether or not the component patterns are statistically independent is not relevant. A more natural benchmark hypothesis is that *the two component patterns are formed by random labelling*, by which we mean that events are labelled independently, each event being labelled type 1 with probability P, and type 2 with probability $1 - P$, where the value of P identifies the proportion of type 1 events in the composite pattern. Independence and random labelling are in general statistically distinct hypotheses; they coincide if and only if the superposition of the two types of event forms a completely random pattern, in which case both component patterns are also completely random.

Second-order properties of a spatial point process

A spatial point process is simply a stochastic mechanism for generating the positions of events in any planar region. The *first-order properties* of a spatial point process describe the local intensity, or mean number of events per unit area, as a function of position. If this local intensity is a constant, λ say, it can be estimated in the obvious way by $\hat{\lambda} = n/|A|$, where n denotes the number of events in the planar region of observation A. Estimation of a varying local intensity, $\lambda(x)$ say, is more difficult without further assumptions about the nature of the underlying point process. See, for example, Diggle (1985).

The *second-order properties* of a spatial point process similarly describe variation in the relative frequency of *pairs* of events as a function of their positions. Under the assumption of constant local intensity, this function depends only on the *relative* positions of the two events. Under the further assumption that the underlying process involves no directional effects, it reduces to a function of distance only.

Perhaps the most useful such function is the K-function (Ripley, 1977) defined by,

$\lambda K(t)$ = mean number of events within distance t of an arbitrary event (excluding the arbitrary event itself).

For a bivariate process, in which the local intensities of type 1 and type 2 events are λ_1 and λ_2 respectively, a complete description of the second-order properties of the process requires us to consider all possible types of pairs, and we therefore define K-functions $K_{ij}(t)$ by,

$\lambda_j K_{ij}(t)$ = mean number of type j events within distance t of an arbitrary type i event.

Note that $K_{11}(t)$ and $K_{22}(t)$ correspond precisely to $K(t)$ as previously defined, also that $K_{12}(t) = K_{21}(t)$ although this is not immediately obvious from the definition.

The forms of these K-functions under our various benchmark hypotheses are strikingly simple. Under CSR,

$$K(t) = \pi t^2.$$

Note in particular that the definition of $K(t)$ has been arranged so that $K(t)$ does not depend on λ. Under independence,

$$K_{12}(t) = \pi t^2,$$

irrespective of the forms of $K_{11}(t)$ and $K_{22}(t)$. Finally, under random labelling,

$$K_{11}(t) = K_{22}(t) = K_{12}(t).$$

These results suggest that plots of estimates of the K-functions provide an effective means of assessing departures from our benchmark hypotheses, and this is indeed the case. A natural way to estimate the K-functions from a set of data is to replace the theoretical mean numbers in the definitions of the $K_{ij}(t)$ by observed numbers, which amounts to examining the empirical distribution of the distances between all pairs of events. An important technical point is that this empirical distribution is distorted by edge-effects; the theoretical definition relates to a point process extending over the entire plane, whereas the data are confined to the finite region A. Estimation therefore proceeds as follows.

In the univariate case (one type of event only), let u_{ij} denote the distance between the ith and jth events, and w_{ij} the proportion of the circumference of the circle with centre the ith event and radius u_{ij} which lies within A. Let $I_t(u)$ take the value 1 if $u \leqslant t$, 0 if $u > t$. Then an approximately unbiased estimator for $K(t)$ is

$$\hat{K}(t) = n^{-2} |A| \sum_{i \neq j} \sum w_{ij}^{-1} I_t(u_{ij})$$

(Ripley, 1977, 1981 p. 159; Diggle, 1983 pp. 71–73). Note that the summation is over all $n(n-1)$ pairs (i, j), and that w_{ij} and w_{ji} are not always equal, as illustrated in Fig. 2.

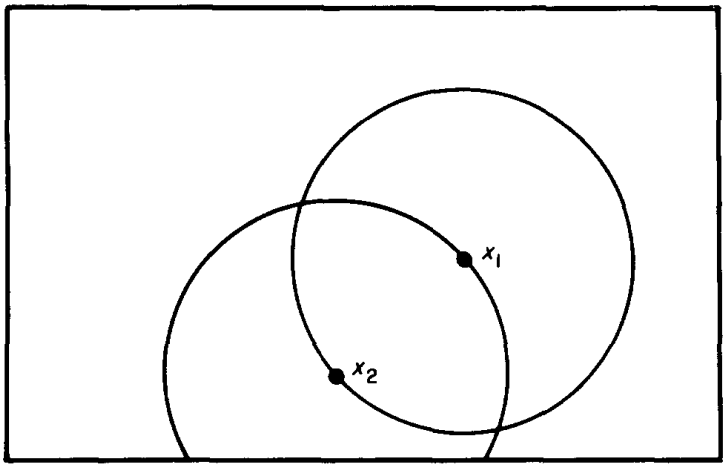

Fig. 2. Inverse weights in the estimation of $K(t)$. For the two events shown, $w_{12}=1$ but $w_{21}<1$.

In the bivariate case, the above formula provides estimates of $K_{11}(t)$ and $K_{22}(t)$. An estimator for $K_{12}(t)$ is

$$\tilde{K}_{12}(t) = (n_1 n_2)^{-1} |A| \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} w_{ij}^{-1} I_t(v_{ij}),$$

where v_{ij} denotes the distance between the ith type 1 event and the jth type 2 event, w_{ij} the proportion of the circumference of the circle with centre the ith type 1 event and radius v_{ij} which lies within A, and n_1, n_2 the numbers of type 1 and type 2 events in A. An analogous estimator $\tilde{K}_{21}(t)$ for $K_{21}(t)$ is defined by interchanging the roles played by the two types of event. Since $K_{12}(t) = K_{21}(t)$, it is sensible to combine $\tilde{K}_{12}(t)$ and $\tilde{K}_{21}(t)$ into a single estimator

$$\tilde{K}_{12}(t) = (n_1 + n_2)^{-1} \{ n_2 \tilde{K}_{12}(t) + n_1 \tilde{K}_{21}(t) \}$$

(Lotwick and Silverman, 1982; Diggle, 1983 pp. 107–108).

Tests of significance

The distribution theory associated with the above estimates of the K-functions is largely intractable. The essential difficulty is that the interevent distances used to construct the estimates do not, under any circumstances, constitute a random sample from an underlying distribution. Lotwick and Silverman (1982) give formulae for the variance of $\hat{K}(t)$ under CSR and for the variance of $\hat{K}_{12}(t)$ under independence with both component patterns completely random. These variance formulae are helpful in the assessment of the K-functions when departure from CSR is in doubt.

More generally, formal tests of significance rely on the device of Monte Carlo testing (Barnard, 1963), which consists essentially of comparing the data-based estimates of the K-functions (or for that matter any interesting summary of the data) with estimates based on simultations of the hypothesis being tested. Specific examples will be given in the next section. We remark here that the technical

difficulties associated with the construction of valid tests of significance for spatial point patterns are not always appreciated. In the present context, Wässle et al. (1981b) and Wässle et al. (1981a) apparently use an invalid test of independence between two types of cells, although re-analyses of their data using the methods of the present paper support their conclusions. Briefly, their test involves a comparison between two histograms, one constructed from the set of distances from each cell to its nearest neighbour in the superposition of the two patterns, the other similarly constructed but from the superposition of one pattern and the mirror image of the other. The rationale behind this procedure is that any dependence between the two patterns will be removed by taking the mirror image of one of them. However, the subsequent comparison between the two histograms is based on a "sign reversal test" which presumably ignores the inherent dependence amongst the distances contributing to each histogram (the authors do not give details).

Results

Second order properties of the data

Fig. 3 shows the estimates of $\hat{K}_{ij}(t) - \pi t^2$, also $\hat{K}(t) - \pi t^2$ for the superposition of the type 1 and type 2 events. Subtraction of πt^2 helps the visual assessment of departure from the benchmark hypotheses of CSR or independence. The range of t is constrained by the dimensions of A and by the fact that the estimates become statistically unreliable as t increases.

Fig. 3 contains a wealth of information about the data. Firstly, the closeness of the traces corresponding to $\hat{K}_{11}(t)$ and $\hat{K}_{22}(t)$ suggests that, at least with regard to second-order properties, and two component patterns are generated by very similar, if not identical, mechanisms. Secondly, the trace corresponding to $\hat{K}_{12}(t)$ appears to be compatible with independence of the two components. Thirdly, if the two component patterns are indeed independent and identically generated, the differences between their two traces give some indication of the magnitude of the sampling fluctuations for each trace, and we can infer that the component patterns are not completely random. More particularly, both traces follow the parabola $-\pi t^2$ for t less than about 12 μm, i.e. no two cells of the same type are separated by a distance less than about 12 μm. Whilst this distance may be comparable to the physical dimensions of the individual cells bodies, both traces continue to decrease up to a distance of about 50 μm, suggesting further regulatory mechanisms which extend far beyond the simple space-constraints imposed by the physical dimensions of the cells. Finally, because these two traces differ markedly from both the $\hat{K}_{12}(t)$ trace and the $\hat{K}(t)$ trace for the superposition, the hypothesis of random labelling appears untenable.

Tests of significance

The above results seem clear-cut. However, to substantiate them (and by way of illustration) we can apply several formal Monte Carlo tests of significance. Firstly, we take square roots of all values of $\hat{K}_{ij}(t)$ to stabilise sampling fluctuations, i.e. if

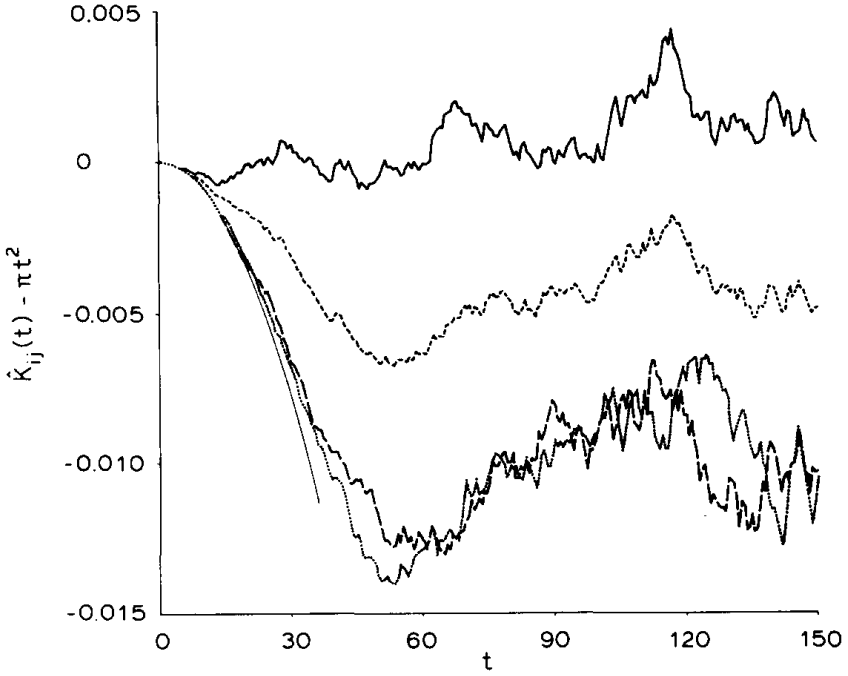

Fig. 3. Second-order properties of the displaced amacrine cells. — — —, $\hat{K}_{11}(t) - \pi t^2$; · · · · · ·, $\hat{K}_{22}(t) - \pi t^2$; ———, $\hat{K}_{12}(t) - \pi t^2$; - - - - - -, $\hat{K}(t) - \pi t^2$, for superposition of both types of cell. The parabola $-\pi t^2$ is shown as a fine solid curve.

we define $H_{ij}(t) = \sqrt{\{\hat{K}_{ij}(t)\}}$, then $\mathrm{Var}\{H_{ij}(t)\}$ is approximately independent of t (Besag, 1977; Silverman, 1977).

To test CSR for either component pattern, say the type 1 cells, we need a test statistic which measures the discrepancy between $H_{11}(t)$ and $\sqrt{(\pi t^2)}$. One such is

$$u = \int_0^{t_0} \{H_{11}(t) - \sqrt{(\pi t^2)}\}^2 \mathrm{d}t.$$

Ideally, the upper limit of integration in the definition of u should be tailored to a particular alternative hypothesis. In practice, the alternative hypothesis is seldom declared explicitly. A convenient rule of thumb is then to take $t_0 = 150$ μm, to correspond to one quarter of the shorter side-length of the rectangle A (Diggle, 1983, p74). Let u_1 denote the value of this statistic for the data, and $u_2, u_3, \ldots, u_{100}$ the values obtained when the data are replaced by simulations of CSR, each with $n = 152$ events in the rectangular region A. Under CSR, all rankings of u_1 amongst the u_i are equally likely, whereas if CSR is false, u_1 will tend to be relatively large. In the event, our 99 simulated values $u_2, u_3, \ldots, u_{100}$ ranged between 0.0003 and 0.0085, whereas $u_1 = 0.0409$. Formally, we therefore reject CSR with an attained significance level of 1% ($P = 0.01$), because u_1 ranks largest amongst $u_1, u_2, \ldots, u_{100}$. Informally, the evidence against CSR is overwhelming, since u_1 is far removed from all the remaining u_i. In more marginal cases, it might be advisable to use more than 99 simulations in order to reduce the effect of the random element in the determination of the P-value for the test, but 99 is usually considered to be sufficient for most practical purposes (Marriott, 1979).

For Type 2 events, the above procedure again leads to emphatic rejection of CSR, with u_1 much larger than all the other u_i.

A similar procedure can be used to test independence of the two components. The analogous test statistic is

$$u = \int_0^{150\,\mu m} \{H_{12}(t) - \sqrt{(\pi t^2)}\}^2 dt.$$

The question now arises as to how we should simulate to obtain the values u_2, $u_3, \ldots, u_{100}$. Since the hypothesis being tested does not specify any particular model for the component patterns, it seems desirable that departure from independence should be assessed against simulations of independent patterns whose properties reflect those of the data themselves. One way to achieve this is by the following device, due to Lotwick and Silverman (1982). Map the rectangle A onto a torus by identifying opposite edges; apply a random toroidal shift to one of the component patterns, say the type 1 cells, and use this shifted pattern to evaluate u_2; continue with further random shifts to evaluate u_3, $u_4, \ldots, u_{100}$. The random shifts produce bivariate patterns with independent components, but preserve the structure of each component. In the event, u_1 ranked 8th largest amongst the u_i, a somewhat equivocal result which nevertheless suggests that the data are reasonably compatible with the independence hypothesis ($P = 0.08$).

To test random labelling, we need a statistic which measures differences amongst the 3 functions $H_{11}(t)$, $H_{12}(t)$ and $H_{22}(t)$. For each t, we define $v(t)$ to be the sample variance of the 3 values $H_{ij}(t)$, and take

$$u = \int_0^{150\,\mu m} v(t) dt.$$

Now, the relevant simulations consist of randomly labelling 152 out of the 294 cells in the superposition as simulated type 1 cells. Our simulated values $u_2, \ldots, u_{100}$ ranged from 0.0010 to 0.0088, whereas $u_1 = 0.0458$. We therefore reject the random labelling hypothesis, formally with an attained significance level of $P = 0.01$.

Conclusion

Whilst acceptance of the independence hypothesis lends support to our original "separate layer" hypothesis H1, rejection of random labelling does not in itself refute the "single layer" hypothesis H2. However, further inspection of Fig. 3 strengthens the evidence against H2. In particular, recall that both component patterns exhibit a clearly defined minimum distance of about 12 μm between any two cells of the same type. In contrast, the trace in Fig. 3 corresponding to $\hat{K}(t)$ for the superposition of type 1 and type 2 cells deviates from the parabola $-\pi t^2$ (i.e. $\hat{K}(t) > 0$) for values of t much smaller than 12 μm. A point process with this property, but which when labelled by some mechanism produces the behaviour observed for the component patterns is mathematically possible but seems biologically implausible — it would have to forbid mutually close triples whilst allowing

close pairs, but force all such close pairs to be oppositely labelled. We therefore decide in favour of H1, and against H2.

Discussion

We have described an application of second-order statistics for the analysis of a bivariate spatial point pattern. Further discussion of these, and other statistical methods for spatial point patterns can be found in the books by Ripley (1981) and Diggle (1983).

One other approach is to use so-called "nearest neighbour" statistics. This approach uses only distances from each cell to its nearest neighbour, rather than all pairwise distances. At first sight, confining attention to nearest neighbour distances would seem to ignore some of the information in the data, but the approach can be competitive with regard to testing of benchmark hypotheses (Diggle, 1983, Ch. 2), particularly if combined with an analysis of distances from arbitrary points to nearest neighbouring cells. However, it seems less illuminating than the second-order approach once the relevant benchmark has been rejected. It is perhaps worth pointing out that it is possible to construct *different* point processes with *identical* second-order properties (Diggle, 1983, pp. 68–69; Baddeley and Silverman, 1984). This fact serves as a warning against exclusive reliance on second-order methods of analysis, which nevertheless do seem to discriminate effectively amongst the different types of pattern encountered in practice.

One aspect of statistical analysis which we have not pursued here is the fitting of parametric models to spatial point patterns. A class of models which seems potentially useful for describing the present type of data is the class of *pairwise interaction* point processes. Essentially, these processes characterise the interaction between events by some parametrically specified function of distance, $h(d)$ say. The probability density associated with a pattern of n events in a given region of the plane is then proportional to the product of all $\frac{1}{2}n(n-1)$ values $h(d_{ij})$ where d_{ij} denotes the distance between the ith and jth events. For example, if $h(d) = 0$ for all d less than d_0, say, then d_0 represents the minimum permissible distance between any two events; if $h(d) = 1$ for all d, then all patterns are equally likely and this corresponds to CSR; values of $h(d)$ between 0 and 1 represent non-rigid inhibitory interactions, etc. Further discussion can again be found in the books by Ripley (1981) and Diggle (1983). Methods for fitting these models to data are currently under discussion in the statistical literature. See, for example, Diggle and Gratton (1984) and Ogata and Tanemura (1984). Diggle and Gratton fit a model to the "light-on" cells in Fig. 1 by matching the second-order properties of data and model (the latter estimated by simulation). Ogata and Tanemura adapt results from statistical mechanics to provide approximate likelihood-based methods for model selection and parameter estimation.

It is difficult to set a minimal number of cells which would enable an analysis of the type described here. For patterns showing a strong degree of spatial regularity, fairly small numbers will suffice (Ripley, 1977, includes an example with $n = 42$

cells), but much larger numbers are desirable for patterns showing major variations in local intensity. Of course, larger samples enable more precise inferences to be made.

One final, important remark concerns the lack of replication which is an all too common feature of studies involving spatial patterns. The reason for this is presumably the difficulty and expense of collecting the data. We have shown that statistical inference is possible from a single replicate, if we are prepared to assume that the underlying point process is stationary. However, our analysis necessarily gives no information about possible interanimal variation. When replication is available, more precise estimates of the K-functions can be obtained by pooling individual estimates from comparable animals. Alternatively, relevant features extracted from the individual K-functions may be used as the basis for a comparison amongst animals between and within experimental treatment groups. A further possibility is to fit a parametric model to each individual pattern and use the parameter estimates and their standard errors as the basis for comparison. It must be acknowledged that a complete formal methodology for replicated spatial point patterns has not yet been developed.

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