Temporal patterns of gene expression via nonmetric multidimensional scaling analysis

running head: Temporal patterns via nonmetric MDS

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ABSTRACT

Motivation: Microarray experiments result in large scale data sets that require extensive mining and refining to extract useful information. We have been developing an efficient novel algorithm for nonmetric multidimensional scaling (nMDS) analysis for very large data sets as a maximally unsupervised data mining device. We wish to demonstrate its usefulness in the context of bioinformatics. In our motivation is also an aim to demonstrate that intrinsically nonlinear methods are generally advantageous in data mining.

Results: The Pearson correlation distance measure is used to indicate the dissimilarity of the gene activities in transcriptional response of cell cycle-synchronized human fibroblasts to serum [Iyer et al., Science 283, 83 (1999)]. These dissimilarity data have been analyzed with our nMDS algorithm to produce an almost circular arrangement of the genes. The temporal expression patterns of the genes rotate along this circular arrangement. If an appropriate preparation procedure may be applied to the original data set, linear methods such as the principal component analysis (PCA) could achieve reasonable results, but without data preprocessing linear methods such as PCA cannot achieve a useful picture. Furthermore, even with an appropriate data preprocessing, the outcomes of linear procedures are not as clearcut as those by nMDS without preprocessing.

Availability: The fortran source code of the method used in this analysis (‘pure nMDS’) is available at http://www.granular.com/MDS/

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SYSTEMS AND METHODS

Systems to Analyze
The gene activities in transcriptional response of cell cycle-synchronized human fibroblasts to serum reported by Iyer et al. (1999) are analyzed. The microarray data used in this analysis is available at http://genome-www.stanford.edu/serum/

Other Possible Analysis Methods
To extract interpretable patterns from microarray data, cluster and linear multivariate analyses seem to be the two major strategies. However, these methods may not be ideally suited for the purpose.

The cluster analysis seems to be the most popular analytical method (Slonim, 2002). For example, the hierarchical clustering method seems to be popular (Eisen et al., 1998). Perhaps there are two fundamental criticisms against clustering methods. Classifying the expression patterns as functions of time is often attempted by clustering methods (Spellman et al. 1998). However, it is often the case that temporal gene expression patterns vary rather continuously without natural gaps among various patterns; needless to say, cluster analysis is not a suitable method to classify continuously changing objects. The second criticism is that clustering methods cannot give any relation among resultant clusters other than ‘genealogical relations’ mimicking similarities. Therefore, clustering is unsuitable for temporal pattern analysis. For example, if the resulting clustering is ((A,B),C), it is only by inspection that ABC or BAC is chosen as a natural temporal pattern or structure. Thus, to exhibit the temporal pattern rearranging the genes in each cluster by hand is needed. An example with such a procedure may be found in Spellman et al. (1998).

INTRODUCTION

Each DNA microarray experiment can give us information about the relative populations of mRNAs for thousands of genes. This implies that without extensive data mining it is often hard to recognize any useful information from the experimental results. In this paper we demonstrate that a nonmetric multidimensional scaling (nMDS) method can be a powerful unsupervised means to extract temporal expression patterns of genes. A data mining procedure may be useful, if it is flexible enough to incorporate any level of supervision, but we believe that the most basic feature required for any good data mining method is to be able to extract recognizable patterns reproducibly without supervision. In this sense our nMDS method is clearly demonstrated to be a useful means of data mining.

We have been developing an efficient nMDS technique for large data sets (Taguchi and Oono, 1999, Taguchi et al., 2001). The input is the rank order of (dis)similarities among the objects (in the present case, genes). Our algorithm is maximally nonmetric in the sense that any introduction of intermediate metric coordinates obtained by monotone regression common to the conventional nMDS methods is avoided.

Data compression is essentially a problem of linear functional analysis as Donoho et al. (1998) stresses. In contrast, we believe data mining is essentially nonlinear. There are linear algebraic methods such as the principal component analysis (PCA) for data mining, but it is expected that nonlinear methods are, in principle, more powerful. The present paper illustrates this point. Indeed, in our case PCA cannot find any comprehensible temporal pattern in low dimensional spaces without an appropriate data preparation.
Perhaps the most popular linear multivariate analysis method is the principal component analysis (PCA). The main idea is to choose a data-adapted basis set, and to make a subspace that can capture salient features of the original data set. In principle, the method could capture the temporal order in the gene expression pattern, but the dimension of the subspace may not be low even if the data are on a very low dimensional manifold. In short, the information compression capability of linear methods is generally feeble. This can be well illustrated by the data we wish to analyze in this paper:

PCA cannot capture any clear temporal order as shown in Fig. 1, where the two dimensional space spanned by the first two principal components is shown there. The temporal expression pattern is hardly seen from the result.

However, apparently, Holter et al. (2000) demonstrated that the singular value decomposition (SVD; a linear method) is remarkably successful in extracting the characteristic modes. The reader must wonder why there is a difference between this result and the one due to PCA that is not successful. The secret is in the highly nonlinear ‘polishing’ of the original data (proposed by Eisen et al. (1998)). However, the role of this nonlinear polishing must be considered carefully, because it can generate a spurious temporal behavior. Therefore, we relegate the comparison of these linear methods with data preprocessing and our nMDS to Appendix II. The salient conclusions are:

1. Linear methods such as PCA and SVD could perhaps achieve reasonable results, if a data preparation scheme is chosen correctly. However, best linear results are generally fairly inferior to nonlinear results.
2. The data preparation such as the ‘polishing’ used by Holter et al. (2000) could actually corrupt the original data (as illustrated in Appendix II), and should be avoided.

An interesting proposal is to use the partial least squares (PLS) regression (Johansson et al. 2003). In this case one may assume a temporal order one wishes to extract (say, a sinusoidal change in time), and the original data are organized around the expected pattern. This is, so to speak, to analyze the data according to a certain prejudice. Although in the process of organization no supervision is needed, the pattern to be extracted (that is, the ‘prejudice’) must be presupposed. Thus, even if it is unsupervised, it is hardly a foolproof method. Furthermore, if a clear objective pattern could be extractable by this method, certainly nMDS can achieve the same goal without any presupposed pattern required by PLS.

Metric multidimensional scaling methods (MDS) may also be used, but it depends on the definition of the dissimilarity. Therefore, unless the measure of dissimilarity is (almost) dictated by the data or by the context of the data analysis, arbitrary elements are introduced. For example, in the case of the microarray data there is no natural dissimilarity measure, so the metric that may capture detailed information could carry spurious information (disinformation, so to speak) as well. Also, if the dissimilarity data is with signs as in the case of correlation coefficients, an extra arbitrary factor intervenes when they are converted to positive dissimilarity measures. A further disadvantage of metric MDS (and of linear methods) is that these methods are vulnerable to missing or grossly inaccurate data.

The cluster analysis with the aid of self-organizing maps (SOM) is definitely a nonlinear data analysis method, but as we have seen in Kasturi et al. (2003) it is not particularly suitable for extracting temporal order. Kasturi et al. comment that SOM is not particularly better than the ordinary cluster analysis. Furthermore, as can be seen from the fact that the use of a particular initial condition can be a methodological paper (Kanaya et al., 2001), we must worry about the ad hoc initial-condition dependence of the results.

ALGORITHM

Basic idea of algorithm for nMDS

The philosophy of nMDS (Shepard 1962a, 1962b, Kruskal 1964a, 1964b) is to find a constellation in a certain space \( R \) of points representing the objects under study (genes in the present case) such that the pairwise distances \( d \) of the points in \( R \) have the rank order in closest agreement with the rank order of the pairwise dissimilarities \( \delta \) of the corresponding objects that are given as the raw (or the original) input data.

The conventional nMDS methods assume a certain intermediate pair distance \( \hat{d} \) that is chosen as close as possible to \( d \) for a given object pair under the condition that it is monotone with respect to the given actual ordering of the dissimilarities \( \delta \). The choice of \( \hat{d} \) is not unique. The discrepancy between \( d \) and \( \hat{d} \) is called the stress, and all the algorithms attempt to minimize it. Depending on the choice of \( \hat{d} \) and on the interpretation of “as close as,” different methods have been proposed (see, for example, Green et al. (1970), Cox and Cox (1994), and Borg and Groenen (1997)). The choice of \( \hat{d} \) affects the outcome. \( \hat{d} \) is required only by technical reasons for implementation of the basic nonmetric idea, so to be faithful to the original idea due to Shepard.
(1962a, 1962b) we must compare $\delta$ with $d$ directly. Our motivation is to make an algorithm that is maximally nonmetric in the sense that we get rid of $\bar{d}$.

The basic idea of this ‘purely nonmetric’ algorithm is as follows (Taguchi and Oono, 1999, Taguchi et al., 2001): in a metric space $\mathcal{R}$ (in this paper, $D$-dimensional Euclidean space $\mathbb{R}^D$ is used) $N$ points representing the $N$ objects are placed as an initial configuration. For this initial trial configuration we compute the pair distances $d(i, j)$, and then rank them according to their magnitudes. Comparing this ranking and that according to the dissimilarity $\delta(i, j)$, we compute the ‘force’ that moves the points in $\mathcal{R}$ to reduce the discrepancies between these two rankings. After moving the points according to the ‘forces’, the new ‘forces’ are computed again, and the whole adjusting process of the object positions in $\mathcal{R}$ is iterated until they converge sufficiently. The details are in Appendix I.

nMDS can usually recover geometrical objects correctly (up to scaling, orientation, and direction) when there are sufficiently many (say, $\geq 30$) objects. Therefore, nMDS is a versatile multivariate analysis method.

It is desirable to have a criterion for convergence (analogous to the level of the stress in the conventional nMDS), or a measure of goodness of embedding. To this end let us recall the Kendall statistics $K$ (p364, Hollander and Wolfe (1999)),

$$K = \sum_{\langle \alpha, \beta \rangle} \text{sign}[(d_\alpha - d_\beta)(\delta_\alpha - \delta_\beta)],$$

where the summation is over all the pairs of dissimilarities (distances between objects) $\langle \alpha, \beta \rangle$ (i.e., $\alpha$ (also $\beta$) denotes a pair of objects). Usually, this is used for a statistical test to reject the null hypothesis that $\{d(i, j)\}$ does not correlate with $\{\delta(i, j)\}$ (The contribution of ties is negligible usually for large data set, so we do not pay any particular attention to tie data).

Here, we use this value to estimate the number of the objects embedded correctly. If all the objects are correctly embedded, all the summads are 1. Thus, if $N'$ objects are correctly embedded, and if we may assume that the rest are uncorrelated, then $K$ is expected to be

$$K > n'(n' - 1)/2 - O(N^2),$$

where $n' = N'(N' - 1)/2$, and the subtraction comes from the random sum of at most $N(N - 1)/2$ of $\pm 1$. If the embedding is successful for the majority of the objects, then $n' = O(N^2)$, so we may ignore the contribution of the bad points. Thus, we may estimate

$$N' \approx \sqrt{2V2K}.$$ 

Therefore, we adopt $100\sqrt{2V2K}/N\%$ as an indicator of goodness of embedding.

We must also discuss the initial configuration dependence of the result. Our algorithm is not free from the problem of local minima as all of the previously proposed algorithms for nMDS and as high dimensional nonlinear optimization problems in general. However, generally speaking, this dependence has only a very minor effect. This will be checked for the fibroblast data (See below).

RESULTS

We have found that the fibroblast data may be embedded in a two dimensional space roughly as a ring (Fig. 2). The estimated number of correctly embedded genes is about 480 among all the 517 genes (i.e., the goodness of embedding is more than 90%). (Also 516 out of 517 genes have $P < 0.005$ confidence level (see Appendix I)). Thus, we conclude that the obtained configuration is sufficiently reliable.

This is in remarkable contrast with the PCA result mentioned already (Fig. 1). Further remarkable is the fact that this ring-like arrangement of the genes faithfully represents the temporal expression patterns of the genes as can be seen clearly from the rotation of the expression peaks around the ring (Fig. 3a). It is noteworthy that the angle coordinate assigned to the genes according to the result shown in Fig. 2 automatically gives the figure usually obtained through detailed Fourier analysis (Fig. 3b). These figures should be eloquent enough to attest to the usefulness of nMDS, a nonlinear data mining method.

Finally, to see the initial configuration dependence for the case of the fibroblast data we constructed two 2D embedding results starting from two different random initial configurations. With the aid of the Procrustean similarity transformation (Borg and Groenen, 1997) one result is fit to the other (notice that our procedure is nonmetric, so to compare two independent results, appropriate scales, orientations, etc., must be optimally chosen). Fig. 4 demonstrates the close agreements of $x$- and $y$-coordinates of the two results. As illustrated, the dependence on the initial conditions is very weak, and we may regard the embedded structure as a faithful representation of the information in the original data.
As has been clearly demonstrated, the 2D embedding is statistically natural and informative. Still the 2D embedding is not perfect, so it is interesting to see what we might obtain by ‘unfolding’ the 2D data, adding one more axis. The unfolded result is shown in Fig. 5. Here, the angular coordinates \( \phi \) and \( \theta \) of the spherical coordinate system is determined by the \( xy \)-plane whose \( x \)-(resp., \( y \))-axis is the first (resp., the second) principal component of the 3D embedded result. The total contribution of these two components is 86%. We do not recognize any clear pattern other than that captured in the 2D space. Therefore, we may conclude that the 2D embedding result is sufficiently reliable and informative.

**CONCLUSION**

We have demonstrated that the nMDS can be a useful tool for data mining. It is unsupervised, and perhaps maximally nonlinear. Our algorithm is probably the simplest among the nonmetric MDS algorithms and is efficient enough to enable the analysis of a few thousand objects, com-

The NMDS algorithm works on the binary relations among the objects, so if there are \( N \) objects, computational complexity is of order \( N^2 \) at least. Therefore, it is far slower than linear methods such as PCA, although our nonlinear algorithm is practically fast enough, because we have used for this work a small notebook PC (Mobile Celeron 650MHz cpu with 256MB RAM). As pointed out and as has been illustrated, with an appropriate data preprocessing a certain linear method could give us a reasonable result with less computational efforts. Although in this paper we have not made any particular effort to reduce computational requirements, a practical way to use nMDS may be to prepare an initial configuration by a linear method with an appropriate data preprocessing method that is verified to be consistent with the full nMDS results.

**APPENDIX I**

‘Purely’ non-metric MDS algorithm

Suppose \( d(i, j) \) is the distance between objects \( i \) and \( j \) in \( R \). Let the ranking of \( \delta(i, j) \) among all the input dissimilarity data be \( n \) and that of \( d(i, j) \) among all the distances between embedded pairs be \( T_n \). If \( n > T_n \) (resp., \( n < T_n \)), we wish to ‘push’ the pair \( i \) and \( j \) farther apart (resp., closer) in \( R \). Intuitively speaking, to this end we introduce an ‘overdamped dynamics’ of the points in \( R \) driven by the following potential function

\[
\Delta \equiv \sum (T_n - n)^2.
\]

Here, the summation is over all the pairs (in the actual implementation of the algorithm, simpler forces are adopted than the one obtained from this potential as seen below). This \( \Delta \) may be regarded as a counterpart of the stress in the conventional nMDS. As we will see later we can use quantities related to \( \Delta \) to evaluate the confidence level of the resultant configuration. Thus, an important feature of our nMDS algorithm is that the optimization process is directly connected to a process that improves the confidence level of the resultant configuration.

The ‘pure nMDS’ algorithm for \( N \) objects may be described as follows:

1. Dissimilarities \( \delta_{ij} \) \( (i, j = 1, \cdots, N) \) for \( N \) objects are given. Order them as follows:

   \[
   \cdots \leq \delta_{ij} \leq \delta_{kl} \leq \cdots.
   \]

2. Put \( N \) points randomly in \( R \) as an initial configuration.

3. Scale the position vectors in \( R \) such as \( \sqrt{\sum_i |r_i|^2} = 1 \), where \( r_i \) is the current position of object \( i \) in \( R \).

4. Compute \( d_{ij} \) for all object pairs \( (i, j) \) in \( R \), and then order them as

   \[
   \cdots \leq d_{ij} \leq d_{kl} \leq \cdots.
   \]

5. Suppose \( \delta_{ij} \) is the \( m \)th largest in the ordering in \( \textbf{1} \) and \( d_{ij} \) is the \( T_m \)th largest in the ordering in \( \textbf{4} \). Assign \( C_{ij} = T_m - m \). Calculate the following displacement vector for \( i \):

   \[
   \delta r_i = s \sum_j C_{ij} \frac{r_i - r_j}{|r_i - r_j|},
   \]

   where \( s = 0.1 \times N^{-3} \) typically, and update \( r_i \to r_i + \delta r_i \).

6. Return to \( \textbf{3} \), and continue until the “potential energy” becomes sufficiently small.

The reader may worry about the handling of tie data. Generally speaking, for a large data set the fraction of tie relations is not significant; furthermore, if the result depends on the handling schemes of tie data, the result is unreliable anyway. Therefore, we do not pay any particular attention to the tie data problem.

In the above algorithm, \( s \) is a constant value. In practice, we could choose an appropriate schedule to
vary $s$ as is often done in optimization processes. In
this paper, for simplicity, we do not attempt such a fine
tuning.

In the above algorithm, we can deal with asymmetric data as well, i.e., $\delta_{ij} \neq \delta_{ji}$ if we compare $\delta_{ij}$ with $d_{ij}$ while $\delta_{ji}$ with $d_{ji}(= d_{ij})$. Needless to say, if the mismatch between $\delta_{ij}$ and $\delta_{ji}$ is large, then representing the pair by a pair of points in a metric space is questionable. Therefore, we will not discuss this problem any further in this paper.

**Goodness of embedding**

In the text we have already discussed the effective number of correctly embedded objects as a measure of 'global goodness of embedding.' This measure, however, cannot tell us the embedding quality of each object. It is often the case that the majority of objects are embedded well even without sensitive dependence on the initial conditions, but there are a few objects that consistently refuse to be embedded stably. To judge the quality of embedding for each object $j$ we define

$$
\Delta(j) \equiv \sum [T_{n(j)}(j) - n(j)]^2,
$$

Here, $n(j)$ is the rank order of $\delta(i, j)$ among $N - 1$ pairs $(i, j)$ for a given $j$, and $T_{n(j)}(j)$ is the rank order of $d(i, j)$ among $N - 1$ pairs $(i, j)$ for the same $j$.

$\Delta(j)$ can be regarded as a statistical variable for the relative position of the $j$-th object with respect to the remaining objects (Lehmann 1975). We can estimate the probability $P(\epsilon)$ of $\Delta(j) < \epsilon$ with the null hypothesis that the rank ordering of $d_{ij}$ ($i \in \{1, 2, \cdots, N\} \setminus \{j\}$) is totally random with respect to the rank ordering of $\delta_{ij}$ ($i \in \{1, 2, \cdots, N\} \setminus \{j\}$). If $N$ is sufficiently large, then $\Delta(j)$ obeys the normal distribution with mean $(M^3 - M)/6$ and variance $M^2(M+1)^2(M-1)/36$, where $M \equiv N - 1$. For smaller $N$ there is a table for $P(\epsilon)$ (Lehmann 1975). Thus, we can test the null hypothesis with a given confidence level for $j$-th object.

**APPENDIX II**

**Limitations and capabilities of linear methods**

The limitations and capabilities of PCA with and without data preprocessing are illustrated in this appendix. There is no fundamental difference between PCA and SVD. We consider the following artificial data $\{s_{gt}\}$, where $g = 1, \cdots, 517$ denote genes and $t = 1, \cdots, 11$ the observation times:

**Data set 1**

$$
s_{gt}^1 = C_g \cos(2\pi t/11 + 2\pi \delta_g),
$$

**Data set 2**

$$
s_{gt}^2 = \exp(s_{gt}^1).
$$

**Data set 3**

$$
s_{gt}^3 = C_{g1} \cos(2\pi t/11 + 2\pi \delta_{g1}) \\
+ C_{g2} \exp[\cos(2\pi t/11 + 2\pi \delta_{g2})] \\
+ C_{g3} / \cos(2\pi t/11 + 2\pi \delta_{g3}).
$$

In the above, $C_g, \delta_g, C_{g1}, \delta_{g1}, (i = 1, 2, 3)$ are uniform random numbers in $[0, 1]$. That is, Data set 1 is a set of sinusoidal waves with random amplitudes and phases, Data set 2 is the nonlinearly distorted Data set 1, and Data set 3 is a set of periodic functions that are very different from simple oscillatory behaviors.

These data sets are analyzed by the following methods.

Method 1: PCA with the preprocessing used by Holter et al. (2000). The preprocessing procedure is as follows:

1. Subtract the average, $s_g' = s_g - \langle s_g \rangle_{g,t}$,

where $\langle \cdot \rangle_{g,t}$ is the average over all genes and experiments,

$$
\langle \cdot \rangle_{g,t} \equiv \frac{\sum_{g,t} \cdot}{\sum_{g,t} 1}.
$$

2. (Column normalization) Normalize the data as

$$
s_g'' = \frac{s_g'}{\sqrt{\sum_s (s_g')^2}}.
$$

3. (Row normalization) Normalize the data as

$$
s_g''' = \frac{s_g''}{\sqrt{\sum_t (s_g''')^2}}.
$$

Repeat these steps until the following condition is satisfied,

$$
\sqrt{\langle (s_g''' - s_g'')^2 \rangle_{g,t}} < 0.01.
$$

From the resultant $s_{gt}$ correlation matrix $Matr.(Cor_{\cdot\cdot})$ is constructed, and then PCA is performed.

Method 2: PCA with the preprocessing so that $\sum_t s_{gt} = 0$ and $\sum_t s_{gt}^2 = 1$ for all $g$. Of course, no iteration is needed for this preprocessing. From the resultant $s_{gt}$
correlation matrix $\text{Matr}(\text{Cor}_{tt'})$ is constructed, and then PCA is performed.

Method 3: nMDS as done in the text. That is, the negative of the correlation coefficient $\text{Cor}_{gg'}$ is used as the dissimilarity and nMDS is applied straightforwardly. Needless to say, no preprocessing of data is needed.

The results are exhibited in Figure 6. The conclusions may be:
(1) For Data set 1, any method will do.
(2) For Data set 2, the procedure recommended by Holter et al. (2000) fails, although ironically simpler Method 2 still works very well. If the amplitude $C$ is distributed in $[0,5]$ instead of $[0,1]$ (that is, the extent of the nonlinear distortion is increased), Method 2 becomes inferior to Method 3, but still Method 2 is adequate.
(3) For Data set 3, even Method 2 fails. nMDS (Method 3) still exhibits a ring-like structure. The method recommended by Holter et al. (2000) is obviously out of question.

Thus, we may conclude that nMDS is a versatile and all around data mining method for analyzing periodic temporal data. Furthermore, we can point out that the preprocessing method in Method 1 should not be used because it could severely distort the original data (as may have been expected from the figures). Suppose there are $N$ genes and $4$ time points. Consider the following example (for the counterexample sake). The first gene has $(a,b,-b,-a)$ ($a > b > 0$), and the remaining genes are all give by $(1,0,0,-1)$. The $N \times 4$ matrix made from these vectors is polishes by an iterative row and column vector normalization procedure. If $N$ is sufficiently large, the first row converges to $(0,1,-1,0)$ and the rest to $(1,0,0,-1)$, independent of $a$ and $b$. If $b$ is small, then all the vectors should behave almost the same way, but after polishing the out-of-phase component in the discrepancy between the first row and the rest is dramatically enhanced, resulting in a spurious out of phase temporal behavior. Although the preceding exercise is trivial, the result warns us the danger of using the so-called polishing.

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Figure legends

Figure 1: PCA results using correlation coefficient matrix. The first two principal components are used as the horizontal and vertical axis, respectively (the cumulative proportion is 70%). Genes whose experimental values are larger than 3.2 are drawn using filled boxes, otherwise drawn using small dots (the corresponding color figure is available online). From the top the time is, respectively, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 12 hr, 16 hr, 20 hr, 24 hr.

Figure 2. Two dimensional embedding result obtained by nMDS.

Figure 3: (a) Temporal patterns of gene expression levels visualized with the aid of nMDS. Colors indicate relative intensity of experimental values normalized so that $\sum_t s_{gt} = 0$ and $\sum_t s_{gt}^2 = 1$ where $s_{gt}$ is experimental variable of $g$th genes at time $t$. (red > 1.6, yellow > 1.2, green > 0.8, pale blue > 0.4, gray < 0.4). Time sequences are the same as explained at Fig. 1. (b) Gene expression data as a function of the angle measured from the vertical axis in (a). The horizontal axis corresponds to $t$. The color convention is the same as in (a).

Figure 4 Comparison between the nMDS embedding results with two different initial configurations after Procrustean similarity transformation. The horizontal (resp., vertical) coordinates are compared in the left (resp., right) figure. In each figure $x$-axis corresponds to the result from one initial condition and the $y$-axis the other.

Figure 5: 3D unfolding of the temporal pattern of gene expression level with the aid of nMDS (3D). Experimental values are normalized as explained in Fig. 3. Genes whose experimental values are larger than 1.6 are drawn using filled boxes, otherwise drawn using small dots (the corresponding color figure is available online). The horizontal (resp., vertical) axis represents $\phi$ (resp., $\theta$). See the text for detail.

Figure 6 Comparison of linear and nonlinear methods. Method 1: PCA with polishing (Holter et al. 2000); Method 2: PCA with normalization; Method 3: 2D space embedding with the aid of nMDS. See the text for Data sets and Methods. For Methods 1 and 2, horizontal and vertical axes are the first and second principal components, respectively, and the percentages describe cumulative proportions. For Method 3, the percentages are the indicators of goodness defined in the text.
Figure legends for online only color figures:

Figure 1 (Color; online only): PCA results using correlation coefficient matrix. The first two principal components are used as the horizontal and vertical axis, respectively (the cumulative proportion is 70%). Colors indicate relative intensity of experimental values (red > 3.2, yellow > 2.4, green > 1.6, pale blue > 0.8, gray < 0.8). From the top the time is, respectively, 15 min., 30 min., 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 12 hr, 16 hr, 20 hr, 24 hr.

Figure 5 (Color: online only): 3D unfolding of the temporal pattern of gene expression level with the aid of nMDS (3D). The color convention is the same as explained in Figure 3. The horizontal (resp., vertical) axis represents $\phi$ (resp., $\theta$). See the text for detail.
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