

# Genetic programming as an analytical tool for non-linear dielectric spectroscopy

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## Abstract

By modelling the non-linear effects of membranous enzymes on an applied oscillating electromagnetic field using supervised multivariate analysis methods, Non-Linear Dielectric Spectroscopy (NLDS) has previously been shown to produce quantitative information that is indicative of the metabolic state of various organisms. The use of Genetic Programming (GP) for the multivariate analysis of NLDS data recorded from yeast fermentations is discussed, and GPs are compared with previous results using Partial Least Squares (PLS) and Artificial Neural Nets (NN). GP considerably outperforms these methods, both in terms of the precision of the predictions and their interpretability. © 1999 Elsevier Science S.A. All rights reserved.

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## 1. Introduction

### 1.1. Non-linear dielectric spectroscopy

When a suspension of cells is exposed to a static electric field, or to an alternating electric field whose frequency is low relative to that of the classical  $\beta$ -dielectric dispersion, it does not penetrate to the interior of the cell, and is dropped almost entirely across the outer membrane of the cell, which is predominantly capacitive at these frequencies, and, due to its thinness, causes a substantial amplification of the field across the membrane (e.g., Refs. [1–3]). In consequence, anything internal to the cell is essentially electrically invisible to a low frequency electric field, but anything dielectrically active in the membrane may be expected to display properties associated with fields far stronger than that applied externally.

The dielectric response of biological tissue has long been assumed linear when the macroscopic exciting field is low, say  $< 0.1 \text{ V cm}^{-1}$  as used typically; however, substantial non-linear phenomena in the form of harmonics

of the fundamental are in fact produced for reasons discussed in Refs. [4,5], leading to the use of non-linear spectroscopy on the dielectric properties of the membranous enzymes actually to indicate and/or influence the metabolic state of cell suspensions [5–11].

Inhibitor and other studies indicated that, in yeast, the non-linear dielectric signal is due mainly to the  $\text{H}^+$ -ATPase located in the cells' plasma membrane [5,9] and hence NLDS may be used to quantify the use of glucose by yeast cells [11].

### 1.2. Genetic programming

Genetic programming [12,13] is an evolutionary technique which uses the concepts of Darwinian selection to generate and optimise a desired computational function or mathematical expression. It has been comprehensively studied theoretically over the past few years, but applications to real laboratory data as a practical modelling tool are still rather rare [14–20].

The thrust of this paper is to compare the results of modelling the data in Ref. [11] using Genetic Programming (using a program written in-house by RJG [18]) with those previously presented which were analysed using Partial Least Squares and Artificial Neural Nets. To summarise, the modelling of these data was found to require the non-linear modelling abilities of NN, the linear nature of

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PLS being unable to accurately approximate the data. GP can also model non-linear data, but an additional advantage over NN is that the latter is a ‘black-box’ method in that it tells the user very little about the underlying processes involved in the effect under study, whereas GP generates explicit equations which may be interpretable in respect of the causation of the studied effect. While these equations are still complex in NLDS modelling and their simplification for interpretation is left for future work, this paper concentrates on the modelling precision of GP in comparison to NN when applied to difficult data such as NLDS spectrograms. GP can also model variations that require the interaction of several measured variables, rather than requiring that these variables be orthogonal.

An initial population of individuals, each encoding a potential solution to the optimisation problem, is generated randomly and their ability to reproduce the desired output is assessed. New individuals are generated either by mutation (the introduction of one or more random changes to a single parent individual) or by crossover (randomly rearranging functional components between two or more parent individuals). The fitness of the new individuals is then assessed, and the fitter individuals from the total population are more likely to become the parents of the next generation. This process is repeated until either the desired result is achieved or the rate of improvement in the population becomes zero. It has been shown [12] that if the parent individuals are chosen according to their fitness values, the genetic method can approach the theoretical optimum efficiency for a search algorithm.

## 2. Data recording

The data sets used in Ref. [11] were used in this study to provide a complete comparison with that previous analysis. They comprise two data sets, collected during simple batch fermentations, with parallel measurements of glucose levels with NLDS vs. a reference method (Reflolux handheld blood glucose meter). Fermentation 1 contains 47 samples of 150 harmonic variables, and Fermentation 2 contains 49 similar samples collected in a similar fermentation on a separate day.

Each NLDS spectrum-sweep (each sample) scanned frequencies of 5 Hz to 50 Hz in 5-Hz intervals, at voltages of 1 V, 1.25 V and 1.5 V. At each voltage/frequency combination, a power spectrum was produced and the magnitude in dB of harmonics 1 to 5 of this spectrum recorded to disc. The suspension in the electrode chamber was then replaced with a conductivity-matched supernatant and the sweep repeated in order to measure the spectrum of electrode polarisation in the absence of cells. The difference spectra due to the biology with the electrode polarisation deconvolved can then be calculated. This leads to each sample (object) being composed of 150  $x$ -variables and 1 reference  $y$ -variable (in the terminology of Ref. [21]).

## 3. Data analysis

### 3.1. Method

In order to implement a genetic optimisation of a predictive model, it is necessary to formulate the model in a notation that is amenable to mutation and crossover. Attempting a genetic optimisation using a model formulated either in standard mathematical notation or computer program code will result, in all likelihood, in the generation of non-functional individuals. To overcome this, the genetic program method uses the concept of a *function tree*, comprising *nodes* and *terminals* [12].

A *terminal* is a logical unit containing an *operator function* (i.e., executable program code) which returns a single number: either a numeric constant or the value of an input variable. A *node* is a logical unit comprising an operator function and one or more arguments, each of which are themselves either a node or a terminal. The return value of a node is calculated by calling its operator function, which then calls the operator functions of its arguments in order to obtain its own input values.

Operator functions may perform either standard mathematical operations such as ‘ $A + B$ ’, or more complex program functions such as ‘if  $A \geq B$  then return 1 else return 0’, where  $A$  and  $B$  are themselves function trees. The advantage of encoding the predictive model as a function tree is that mutations can readily be performed by changing a node’s arguments or operator function, and crossover can be achieved by replacing one or more nodes from one individual with those from another without introducing illegitimate syntax changes.

The GP implementation used in this study initially used the arithmetic operator functions *add*, *subtract*, *multiply*, *protected divide* and a conditional function. It also used sine and cosine functions to ease the modelling of non-linear relations. The protected divide operator merely prevents numerical overflow by division with a tiny number by hard-limiting the output.

To improve the search efficiency, the GP population was organised into 5 *demes* (subpopulations) [22] which evolved independently. Every 10 generations, the fittest 10% from each deme were pooled, and the best individuals replaced the worst 10% in each subpopulation.

The GP generated initial individuals with random function trees and assessed their fitness using a scoring function that compared  $e_i$  (the model’s estimate of the output for example  $i$ ) with  $o_i$  (the experimentally observed value) by calculating the root-mean-square error of prediction (rmsep) for  $n$  training examples (the training set):

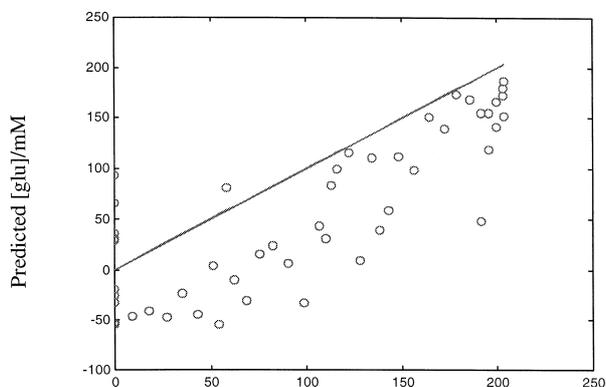
$$\text{rmsep} = \sqrt{\frac{\sum_{i=1}^n (o_i - e_i)^2}{n}}$$

The model thus produced can then be applied to unseen data and its performance assessed by the rmsep on the unseen data set (the test set).

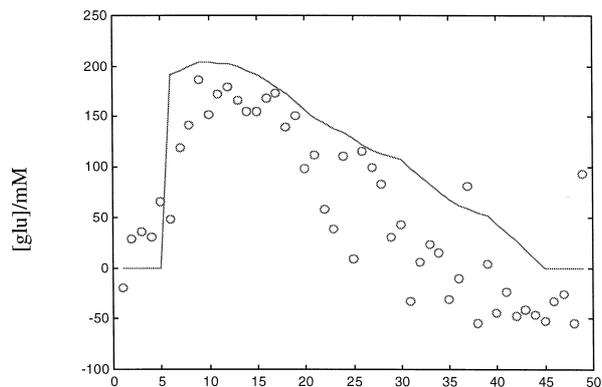
### 3.2. The state of play

To recap, the NN predictions from Ref. [11] using an NN model formed on Fermentation 1 (the training set) gave an rmsep of 64% of the mean value of the measured glucose curve on Fermentation 2 (the test set) with raw data (using no pre-processing) before applying the NN as shown in Fig. 1.

As in all prediction figures in this paper, the measured data are shown as a solid line and the predictions are shown by individual points. The upper plot gives the relation of these points to the ideal 1:1 line of perfect

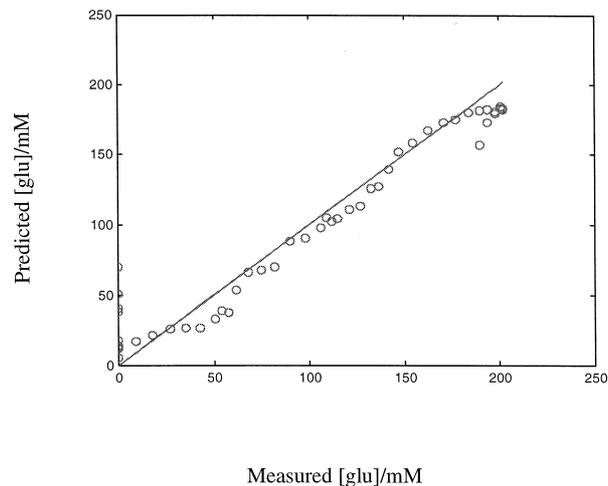


Measured [glu]/mM

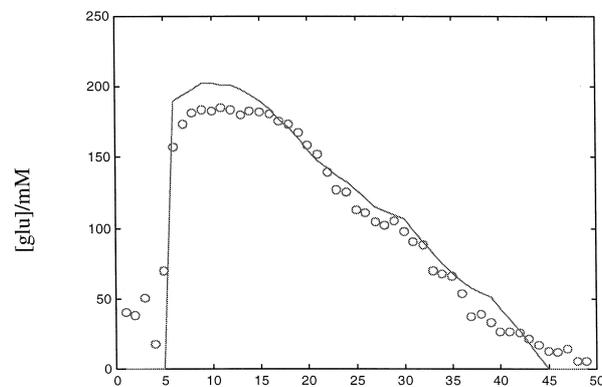


Object no.

Fig. 1. Neural Network prediction of Fermentation 2 from a model formed on Fermentation 1 using the raw data with no pre-processing other than normalisation and headroom scaling. The optimum training occurred at 36 epochs at a training error of 0.02. The rmsep was 64%.



Measured [glu]/mM



Object no.

Fig. 2. Neural Network prediction of Fermentation 2 by Fermentation 1: the leading zeros were non-median-averaged and the rest of the data were median-averaged to remove outliers. The optimum training occurred at 30 epochs at a training error of 0.02. The rmsep was 19%.

prediction; and the lower plot shows the actual function being modelled along with the relation of the predicted points to this function. The two representations are necessary since biochemically, the yeast's resting state before the addition of glucose will not necessarily be identical to the resting state to which it returns after the glucose is used up, since storage polymers such as glycogen and trehalose will have been formed as a result of glucose metabolism [23–25]. On this basis, it may be expected that the leading zeros will be predicted less well if the model forms predominantly on the much larger section of finite- and post-glucose data. The multivariate method used has, in effect, to model two separate systems. However, the prediction to some degree of the leading zeros is a vital check that the model is not merely forming on drifts and trends in the data, since data reflecting glucose utilisation are monotonic. If modelling were to occur merely on the

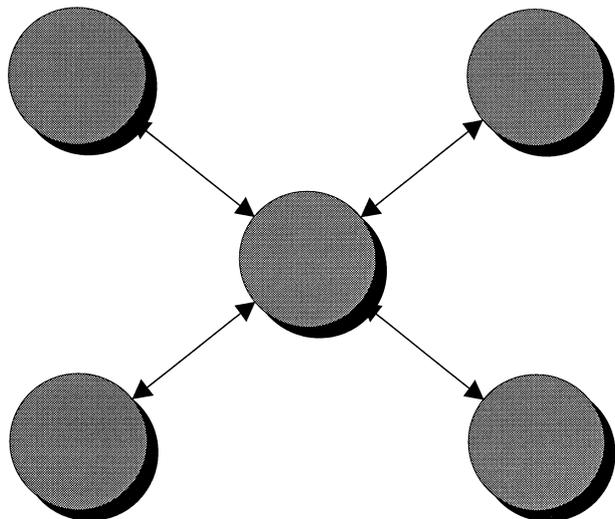


Fig. 3. Schematic of the operation of the deme 5Inject2Way. Five initial populations are randomised, then after every 10 generations, the best 5% of individuals are swapped radially and bi-directionally between the four satellite populations and the hub.

basis of a trend, then the leading zeros would be predicted to the same absolute levels as the initial glucose concentration, since the model would see them as identical to the high glucose readings. If merely modelling a trend in the data, the  $x$ -variables would be identical for samples taken both before and immediately after addition of glucose. Thus, the prediction of the leading zeros acts as a marker that the model is actually forming on a glucose-related response, and not merely on unconnected coincidental experimental drift [11].

Median averaging in each variable with respect to sample number was used on this data as a robust weighting method to remove the many large glitches in the data sets [11]. It relies on the fact that glucose-related phenomena change slowly during a fermentation, and any sudden changes are spuria. Consequently, using median-averaged leading zeros smears the discontinuity at sample 6 (the addition of glucose) and hampers its modelling. Exploiting this argument, it was found in Ref. [11] that substituting the non-median-averaged leading zeros into the median-averaged data gives the advantage of both well-predicted (if noisier) leading zeros and a closely fitted glucose curve. Increasing the number of leading zeros, by direct copying, till there are roughly as many as non-zero readings, so the net sees the zeros as often as the non-zeros during each training epoch, improves the predictions substantially to the point that the prediction of Fig. 2 is obtained with rmsep of 19%.

All the points are well-modelled except for the few after the addition of glucose, when the yeast could not be expected to respond instantly anyway. It is well-known that there is a significant phase of activation of the  $H^+$ -ATPase following the addition of glucose to a resting cell suspension [26,27].

These predictions on raw and median-averaged data form the baseline that GP has to outperform in order to be of value and to justify the large computational load necessary to form GP models.

#### 4. Results of GP modelling

The data in Fermentation 1 are sectioned into odd and even samples: the odd 24 samples forming the training set and the even 23 samples, the validation set. The GP model formed on these was used to predict the 49 unseen samples of Fermentation 2 comprising the test set. Note that the validation and training sets are taken from one fermentation and the test set from another, entirely separate fermentation. In a situation such as NLDS, where instrument response can vary considerably, this means that the training and validation sets may resemble each other much more closely than either resembles the training set. Hence, the modelling process may be expected to overtrain. This

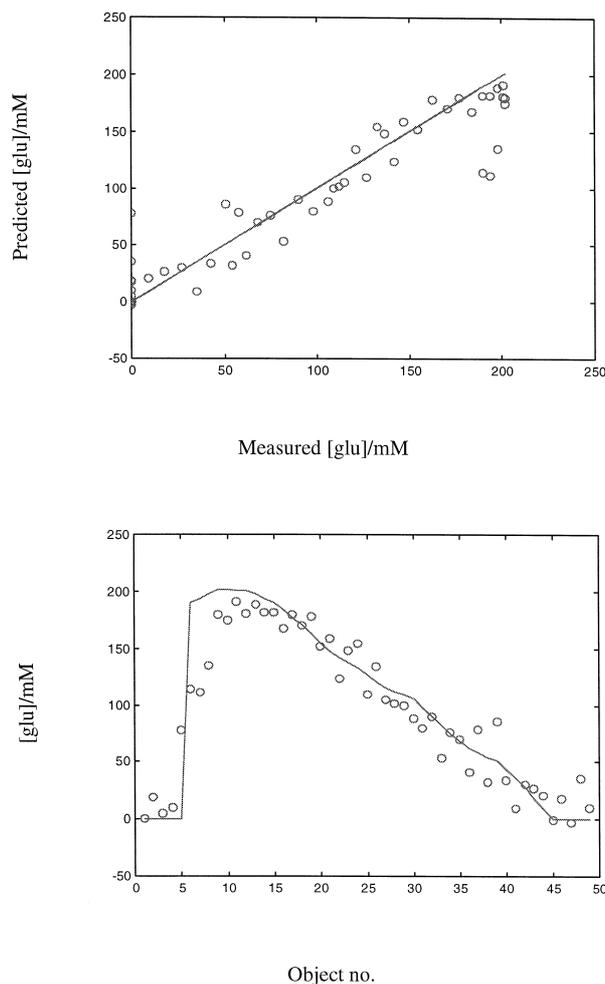


Fig. 4. Non-linear GP prediction of Fermentation 2 using a model formed on Fermentation 1. Data use no pre-processing. The rmsep was 26% at generation 198.

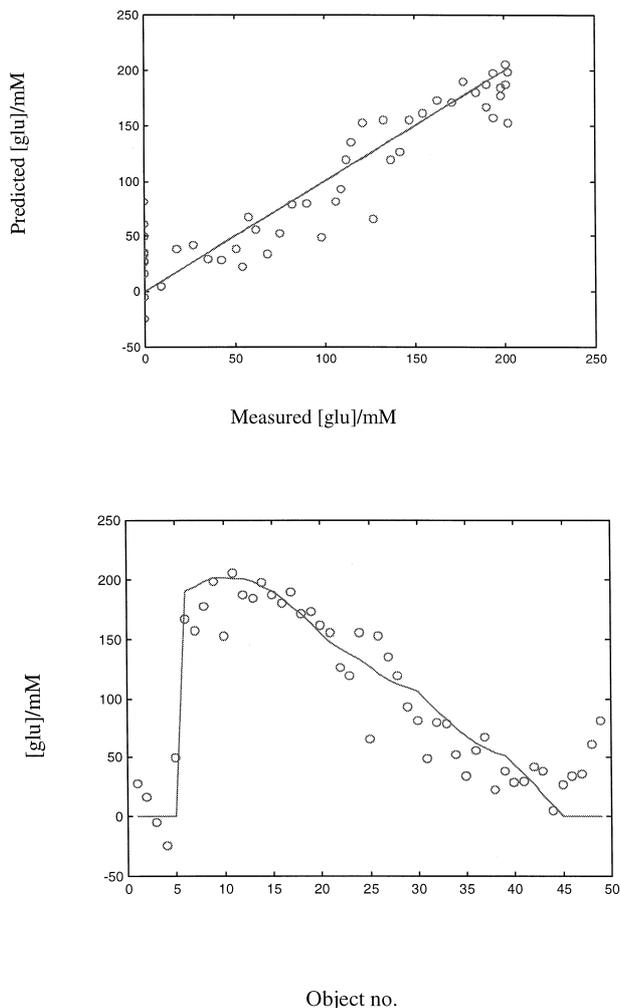


Fig. 5. Linear GP prediction of Fermentation 2 using a model formed on Fermentation 1. Data use no pre-processing. The rmsep was 28% at generation 199.

means that the GP predictions shown in this paper will be suboptimal compared to the general case with three completely independent data sets recorded from a constant instrument. Hence, the performance of GP shown here should be regarded as a lower-bound on what may be expected from a GP in the general case.

Initially, raw data were used to compare with Fig. 1, and to see if the GP was capable of forming its own robust weighting to clean up the data with no pre-processing. Non-linear and conditional functions were included in the node set. The nodal functions thus used were  $*$   $/$   $+$   $-$   $\sin$   $\cos$   $\tan$   $\exp$   $\log$   $\sqrt{\quad}$   $\text{if } > =$ . The deme 5Inject2Way (invented in-house by RJG) was chosen to ensure a large and rapid spread of best-candidate solutions through the population to cover the very contorted error surface [11] rapidly. The operation of this deme is represented in Fig. 3.

Five populations of 10,000 individuals each were run for 200 generations, at which point training was terminated for reasons of time and computational load. The best

individual turned up at generation 198, suggesting that the training process could plausibly be beneficially continued given the necessary computing power. This individual gave an rmsep of 26%, shown in Fig. 4, which compares favourably with the best neural network prediction of Fig. 1, even though the former used no pre-processing on the data.

Interestingly, it was found that including non-linear and conditional functions was not as important as initially expected. An identical training run using only linear nodes ( $*$   $/$   $+$   $-$ ) with no conditionals (referred to as ‘linear’ GP or LGP) produced a similar prediction with an rmsep of 28% at generation 199 as shown in Fig. 5.

This again showed that in the time allotted, training had not converged completely and could be improved upon by a longer run, but more importantly, it also showed that the GP had no problem formulating its own non-linear subtrees from linear nodes. It also had surprisingly little problem with the discontinuity at object 6, actually approximating it better than the ‘non-linear’ GP in Fig. 4 which includes the conditional nodes which might be expected important to model discontinuities. It is hypothesised that the GP uses the protected-divide (limited to protect against division-by-zero errors) to construct an ersatz discontinuity. The advantage of using only linear nodes is that the equation tree produced by the GP can be deconvolved by program. Deconvolving an equation tree involving stacked non-linear functions and conditionals into something understandable can be intractable even for modern symbolic algebra programs. For example, the rule generating the prediction of Fig. 4 is given in Table 1.

GP was then applied to the median-averaged versions of the fermentor data sets. Again, LGP was used as exactly as above, the results being shown in Fig. 6.

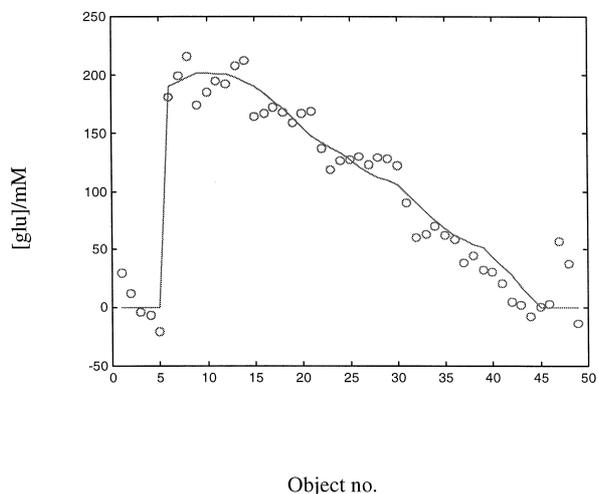
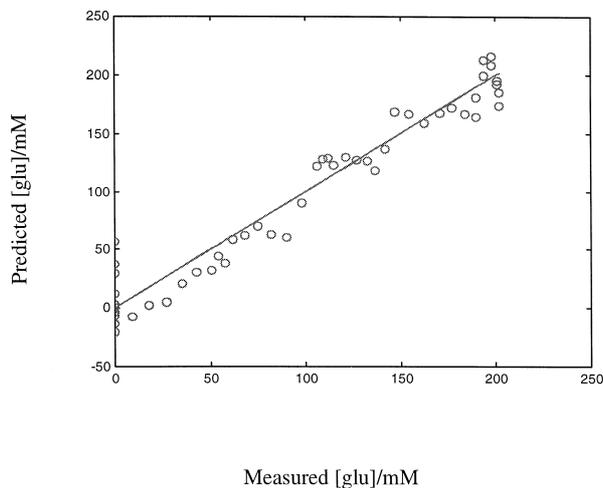
The rmsep of the prediction was brought down to 17% after 196 generations, which is better than any prediction

Table 1

Typical GP rule for a tree of maximum depth 10 including conditional and non-linear function nodes

```
(if > = (- M143 - 8.69218)
(sin(tan M47))(- (+ (- M143 (/ M1 M131))
(if > = (exp(if > = M66 M127 - 2.30924 7.14908)) M150
(- (+ (+ (- (+ M128 M97) - 8.6921) (if > = (sin (/ M1 M131))
(sin(exp 4.01055)) (* (exp 4.01055)
(log M115)) - 9.66665))
(- (if > = M92 - 3.15320
(sqrt (* M55 M8))
(+ M128 M130)) - 8.69218)) M1) M134))
(if > = (+ - 3.13356 M98) - 2.40252 M145 M114))
(+ (- M134 M144)) (+ M44
(+ (- (+ (+ (+ (* M115 M8)
(- (tan M137) M144))
(- M143 (tan M71))) (+ M33 M8)) M64)
(+ (* - 4.02348 M8)
(- (/ M145 M97) M144))))))
```

$Mx$  is variable number  $x$ .



be trained for a long time till one of the individuals mutates into its vicinity, or a large initial population can be employed to increase the likelihood of one initial individual being close enough to the global minimum to train easily into it in a reasonable number of generations. It has been shown [12] that a GP with a population size of  $n$  individuals converges quicker than a simple random beam search of  $n$  individuals, so the computationally favoured search strategy for a complex error surface such as that for NLDS data sets, which requires the use of a large population, is to use this large population to train a GP. This ensures that rapid convergence is achieved by the GP to compensate for the large computational load of such a population size using any search method.

To investigate the utility of a large initial population, a GP with identical parameters to those above was run on the median-averaged data (with the leading zeroes replaced

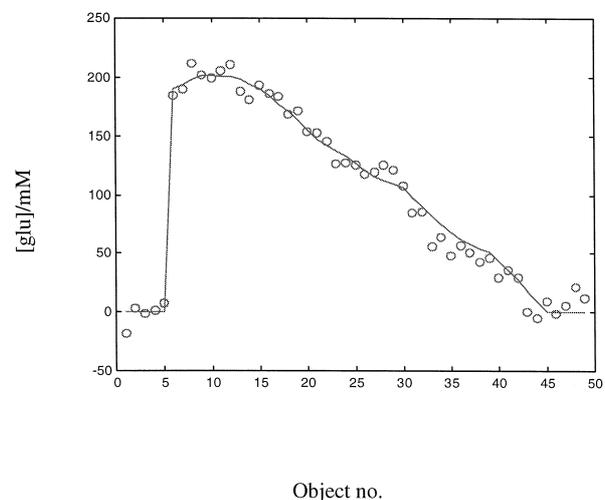
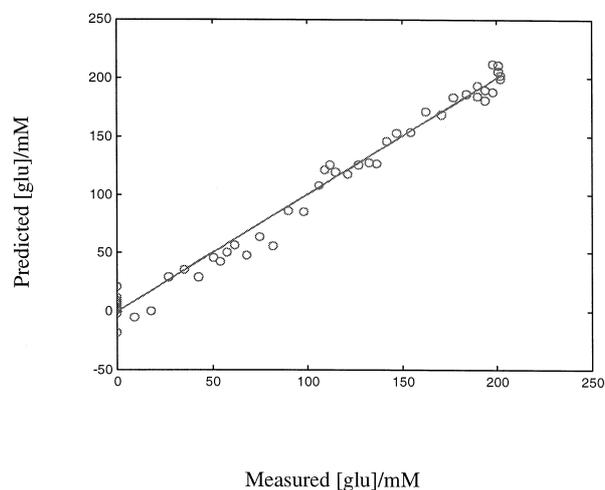


Fig. 6. Linear GP prediction of Fermentation 2 using a model formed on Fermentation 1. Data use no pre-processing. The rmsep was 17% at generation 196.

achieved in previous analysis by neural net modelling. The best individual occurring at generation 196 again suggests that termination of the training at generation 200 is premature and would not have allowed the global minimum to be found precisely.

Again it is of note that the discontinuity is modelled. It is also very interesting to observe that, having formed a subtree to model a discontinuity, the GP has then used this to form a piecewise-linear approximation to the rest of the glucose curve. This provides a convincing example in the continuing argument about how effective crossover is at preserving useful subtrees as opposed to disrupting them during later training [28].

The error surface for NLDS data is very noisy, with many local minima and a tightly defined global minimum [11]. This makes it a very testing problem for any modelling method to solve. To maximise the likelihood of the global minimum being found, either a small population can

Fig. 7. Linear GP prediction of Fermentation 2 using a model formed on Fermentation 1. We used median average pre-processing on all data points except the leading zeroes. The rmsep was 9.5% at generation 117.

by their non-median-averaged counterparts in order to minimise the smearing effect at the discontinuity. The initial population was increased by a factor of 10, to five populations of 100,000 individuals. The resulting GP took 1 day/150 generations on a Pentium II 300, but produced an rmsep of 9.5% at generation 117, after which the predictions diverged showing that this GP had reached optimal training at this point and further training merely produces poorer generalisation. This prediction is a factor of 2 better than the best neural net prediction on the same data, using the rmsep as a metric, and is shown in Fig. 7. Note especially that the GP is much better at modelling the leading zeros without compromising the prediction of the finite glucose levels, and that it does this with no need to increase the number of leading zero samples artificially as is necessary for adequate modelling by a NN.

## 5. Conclusions

NLDS data provide a rigorous test-bed for multivariate modelling methods, having a small signal variation hidden in large uncorrelated instrumental fluctuations. The resulting error surface is very noisy and the global minimum appears to be very localised, requiring a very efficient search strategy to be used by the modelling process. The relationship between measured variables and the reference variable is also non-linear, restricting the choice of modelling methods. Neural nets achieve respectable calibrations, but can be significantly superceded in model accuracy and precision by GP, at the cost of a heavy computational load to form the model even compared to that of NN. Once formed, like NN, the model is very rapid to apply to unseen data.

Several features of application of GP to real calibration data appear from the above work.

GP appears to be able to improve upon more conventional non-linear modelling methods such as neural networks even when training is terminated prematurely, so the large computational load of modelling with GP may not be prohibitive if prematurely terminated training still produces a model to within the required solution specifications.

To optimise training for a particular task, a GP should ideally include nodes of the form of known features of the solution. For instance, a non-linear problem should use non-linear nodes, a problem known to consist of a sum of Lorentzian functions should include a Lorentzian node, or a GP using wavelet nodes could simulate a wavelet transform [29]. In practice, the GP displays a surprising ability to synthesise subtrees to simulate any required function nodes even if these are not explicitly supplied in the original nodal set. Even such potentially difficult problems as discontinuities can be modelled very effectively from simple nodal primitives.

For the noisy error surfaces associated with many difficult laboratory problems which do not readily yield to simple and less intensive modelling methods, the GP should be configured with a large initial population and run for a restricted number of generations. This finds the global minimum quicker than the alternative option of a small population run for many generations. A small population will drop into the global minimum quickly by luck or eventually by mutation, so it is easy to terminate in a local minimum prematurely, or simply to incorrectly dismiss the problem as intractable.

Given the computational load of GP, it would not be the method of choice for problems which yield to simpler methods. However, the above data show that it can be very beneficial on problems that have defeated other methods. The conversion of the completely useless raw data NN model of Fig. 1 to the quite useable model of Fig. 4 is particularly demonstrative of this, while the relative ease with which one can implement GP on parallel computer architectures makes its further exploitation especially attractive.

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