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Sicegar: R package for sigmoidal and double-sigmoidal curve fitting

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ABSTRACT

Sigmoidal and double-sigmoidal dynamics are commonly observed in many areas of biology. Here we present `sicegar`, an R package for the automated fitting and classification of sigmoidal and double-sigmodial data. The package categorizes data into one of three categories, “no signal”, “sigmodial”, or “double sigmoidal”, by rigorously fitting a series of mathematical models to the data. The data is labeled as “ambiguous” if neither the sigmoidal nor double-sigmoidal model fit the data well. In addition to performing the classification, the package also reports a wealth of metrics as well as biologically meaningful parameters describing the sigmoidal or double-sigmoidal curves. In extensive simulations, we find that the package performs well, can recover the original dynamics even under fairly high noise levels, and will typically classify curves as “ambiguous” rather than misclassifying them. The package is available on CRAN and comes with extensive documentation and usage examples.

INTRODUCTION

Growth patterns resembling sigmoidal or double sigmoidal curves are common in biological systems. In ecological systems the number of individuals in a population may follow a sigmoidal growth pattern over time, while in molecular systems the production of a reporter may have similar dynamics. Quantifying and comparing these kinds of growth dynamics can lead to valuable insight into the systems’ behavior. However, the automated quantification and classification of thousands of noisy growth curves, in a high-throughput manner, can be technically challenging.

Here we present the `sicegar` package, an R package designed to automatically analyze and classify time course growth data. The input data is assumed to represent intensity measured over time, where intensity represents any metric of a system that may vary in a sigmoidal or double-sigmoidal pattern. Using these time course data, `sicegar` categorizes the data as “sigmoidal”, “double sigmoidal”, “ambigous”, or “no signal”, by first checking the data for the presence of signal (i.e., the intensity curve deviates significantly from a horizontal line and varies significantly) and then fitting two mathematical models to the data, a sigmoidal one and a double-sigmoidal one. The package automatically determines which curve provides the better fit, and it reports a wide range of parameters about the fitted models that enable easy comparison of the fitted curves within and among experiments.

The `sicegar` package was originally written to study poliovirus infection and replication at the single-cell level (Guo et al., 2017), and the package name is inspired by this application (SIngle CEll Growth Analysis in R). However, the potential applications for the package go beyond this one use, and the package is designed as a generalized way to classify growth data from time course experiments. In the following, we describe the mathematical models used to classify the data, how the models are fit, and the inferences that can be made from them.

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METHODS

Model fitting and categorization

We implemented R code that can fit data to the sigmoidal and double-sigmoidal curves defined by Eq. 1 and Eq. 5 in Results and Discussion. Before fitting, data is internally normalized to a $[0,1]$ interval on both the x and y axis. The sigmoidal and double sigmoidal models are then fit to the internally normalized data via likelihood maximization, using the function `nls.lm` in the `minpack.lm` package (Elzhov et al., 2015). To guarantee robust fitting `nls.lm` is run multiple times using different starting estimates, chosen at random from a uniform distribution covering the allowed interval of parameters. For each fit, the quality of fit based on AIC scores is recorded, and the parameter estimates that produce the overall best fit across replicates are reported as the final estimates.

The simplest way to use `sicegar` is via the function `fitAndCategorize`, which attempts to both identify the best-fitting model and determine whether this model is sigmoidal, double-sigmoidal, or neither. This function first checks whether there is any signal in the data, by testing whether the maximum observed intensity exceeds a user-defined threshold and the data range also exceeds a user-defined threshold. If either test fails, the data are labeled as “no signal”.

Next, the function fits both a sigmoidal and a double-sigmoidal curve to the data and then assess which provides the better fit. This process considers a number of different factors. First, it assesses whether both models could actually be fitted (the fitting did not fail). Second, the AIC scores of the fitted models are examined to make sure they are greater than a threshold value (the default is -10). The starting time points of each model are then checked to insure that they are positive. The starting intensities are examined to insure that they are smaller than a threshold (the default is 0.05). Then the ratio of the models’ intensity prediction at the last observation to the models’ maximum intensity prediction is tested to insure that it is over 0.85 in the case of the sigmoidal model and below 0.75 in the case of the double sigmoidal model. If any of these tests fail for either the sigmodial or double-sigmodial fit, then the data is not categorized as being described by that model. If both models fail any of these tests the data is categorized as being “ambiguous”. If only one model passes these tests while the other fails, then the data is considered to be best described by that model. If both of the fits pass all of these test a decision is made about which model best describes the data based on their AIC scores. If the sigmoidal AIC score is smaller than the double sigmoidal AIC score then the data is labeled as “sigmoidal”, if it is larger then the data is labeled as “double sigmoidal”.

The complete decision process, as well as the specific parameters users can modifiy to adjust the package to their own needs, are explained in detail in the vignette “Identifying the best-fitting model category” supplied with the package.

Simulations

To assess `sicegar` performance, we simulated sigmoidal and double-sigmoidal data with different levels of noise. We first generated 50 distinct sets of parameters for both sigmoidal and double-sigmoidal curves. For the sigmoidal model, the maximum, slope, and midpoint parameters were chosen uniformly at random from the intervals $[0.3,20]$, $[0.001,40]$, and $[3,27]$, respectively. For the double-sigmoidal model, the maximum, increasing slope, the first midpoint, the decreasing slope, the distance between midpoints, and final asymptote intensity ratio parameters were chosen uniformly at random from the intervals $[0.3,20]$, $[0.001,40]$, $[3,26]$, $[0.001,40]$, $[1,27-\text{first midpoint}]$, and $[0,0.85]$, respectively. Using these parameters we simulated data from $t=3$ to $t=30$ in increments of 0.5, for a total of 55 time points each.

For each parameter set we then generated samples that included noise. We used 11 distinct noise amplitudes, ranging from 0% to 150% of the maximum value of the initial simulated data. We generated three noise vectors with the same maximum amplitude for each noise level and create a unique noise vector for each replicate. To generate a noise vector at a given amplitude for a sample, we created a vector of random numbers chosen from the uniform distribution between -0.5 and 0.5 for each time value in the dataset. We then multiplied each random number with the noise amplitude and the maximum intensity of the dataset. Finally, we added these noise values to the intensity values of the initial samples and obtained noisy samples. In total, this procedure generated 1650 sigmoidal and double-sigmoidal noisy samples with varying levels of uniform noise, for a total of 3300 samples.

For each simulated sample, we used `sicegar` to estimate both the shape of the curve (”sigmoidal”, ”double-sigmoidal”, or ”ambiguous”) and the curve’s original parameter values. To estimate the algorithm’s accuracy in fitting a model to the data, we calculated the mean absolute error between the initial (without noise) and predicted intensity vectors, and we normalize this number relative to the maximum intensity of the initial sample. We calculated the normalized mean absolute error regardless of whether the algorithm predicted the category of the sample correctly or not.

Package availability and documentation

The `sicegar` package is implemented in R and available from CRAN at `https://CRAN.R-project.org/package=sicegar`. The `sicegar` source code is available on github at `https://github.com/wilkelab/sicegar`. Extensive user documentation is available in multiple vignettes distributed as part of the package.

The package depends on existing R packages `dplyr` (Wickham and Francois, 2015), `minpack.lm` (Elzhov et al., 2015), `fBasics` (Team et al., 2014), and `ggplot2` (Wickham, 2009).

RESULTS AND DISCUSSION

Growth in many biologically systems can be though of as occurring in two phases (Fig. 1). In the first phase, intensity will increase exponentially until saturation is reached and it levels off at some asymptotic maximum level. In the second phase, the intensity may then decay from the maximum value to a lower one or even back to zero. Depending on the system and/or the length of the observation this second phase may or may not occur. These two phases can be modeled with two sigmoidal curves that describe the relationship between time and intensity. The first describes the increase of intensity and the second its decay.

Based on this two-phase view of growth an observer of a system may observe three different types of behaviors of intensity over time. First, the observer may observe the full process and clearly see both the growth and the decay phases, i.e., the full double-sigmoidal curve. Second, if the observation ends before decay occurs, or if the decay phase is not relevant for the system under study, the observer may only see the first sigmoidal curve. Third, if the intensity is not produced in detectable amounts, the observer will not see any signal at all. We label these three types of behavior as “double sigmoidal”, “sigmoidal”, and “no signal”. If a given time course does not clearly fit into any of these three categories we label it as “ambiguous”.

On the face of it, fitting a sigmoidal curve to time-course data appears to be a simple and solved problem. A sigmoidal curve is fully determined by only three parameters, and many non-linear curve-fitting algorithms exist that can be used to fit a simple three-parameter function. However, when we attempted to fit thousands of curves to experimentally measured data, we found that achieving numerical reliability was non-trivial. While a simple algorithm will work most of the time, it will fail eventually on cases that by manual inspection have a clear solution. The problem was further exacerbated for double-sigmoidal curves, where even guaranteeing that the curve is double-sigmoidal at all times was non-trivial (see Appendix). In the following, we describe the mathematical models and fitting procedures we developed to reliably obtain good fits and to be able to automatically categorize time courses into the different types of behavior.

Sigmoidal and double-sigmoidal models

A sigmoidal curve can be uniquely determined by three parameters, the maximum value, the midpoint, and the slope of the curve (Fig. 2A). To represent a sigmoidal curve, the `sicegar` package uses the Fermi function

$$I(t) = f_{\text{sig}}(t) = \frac{I_{\text{max}}}{1+\exp(-a_1(t-t_{\text{mid}}))}. \qquad (1)$$

Here, $I(t)$ is the intensity time course, given as a function of time t. The three parameters to be fitted are I_{max}, t_{mid}, and a_1. The parameter I_{max} represents the maximum intensity observed, the parameter t_{mid} indicates the time at which intensity has reached half of its maximum, and the parameter a_1 is related to the slope of $I(t)$ at $t = t_{\text{mid}}$ via the formula $d/dt\, I(t)|_{t=t_{\text{mid}}} = a_1 I_{\text{max}}/4$ (Fig. 2A).

A double sigmoidal curve can be uniquely determined by six parameters, two midpoints and slopes parameters, a maximum value, and an asymptotic final value after decay (Fig. 2B). We define the double-sigmoidal model by combining two regular sigmoidal functions. This combined function rises from 0 to a maximum value I_{max} at time t^* and then decays to the final value I_{final}. The function is divided into two parts, one to the left of the maximum, for $t < t^*$ (the growth phase) and one to the right of the maximum,

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for $t > t^*$ (the decay phase). The two parts are rescaled separately, such that the maximum value of $I(t)$ is given by I_{max} and the final value of $I(t)$ for large times is given by $I_{\text{final}} > 0$.

To define our double-sigmoidal model, we begin with a simple double-sigmoidal function, obtained by multiplying together two sigmoidal functions:

$$f_{\text{dsig-base}}(t) = \frac{1}{1+\exp(-a'_1(t-t'_{\text{mid1}}))}\,\frac{1}{1+\exp(-a'_2(t-t'_{\text{mid2}}))}. \tag{2}$$

We then define the time t^* at which this function is maximal,

$$t^* = \operatorname{argmax} f_{\text{dsig-base}}(t), \tag{3}$$

and the value of the function at that time point,

$$f_{\text{max}} = f_{\text{dsig-base}}(t^*). \tag{4}$$

We then write

$$I(t) = f_{\text{dsig}}(t) = \begin{cases} c_1 f_{\text{dsig-base}}(t) & \text{for } t \le t^* \text{ (growth phase)} \\ c_2 f_{\text{dsig-base}}(t) + I_{\text{final}} & \text{for } t > t^* \text{ (decay phase)} \end{cases}, \tag{5}$$

with

$$c_1 = \frac{I_{\text{max}}}{f_{\text{max}}} \tag{6}$$

and

$$c_2 = \frac{(I_{\text{max}} - I_{\text{final}})}{f_{\text{max}}}. \tag{7}$$

This definition may seem awkward and overly complex, but it guarantees that certain non-sigmoidal corner cases are eliminated (see Appendix).

The meaning of the parameters in the double-sigmoidal model mirror those of the regular sigmoidal model. The time point t'_{mid1} determines (but does not exactly represent) the time at which the intensity has risen to half of its maximum, and the time point t'_{mid2} (enforced to be larger than t'_{mid1}) determines (but does not exactly represent) the time at which the intensity has decayed halfway from its maximum to its final value. The prime in t'_{mid1} and t'_{mid2} indicates that these time points are not the final parameters of interest. The final parameters of interest, the true midpoints t_{mid1} and t_{mid2}, have to be calculated numerically. Similarly, the parameters a'_1 and a'_2 determine the speed of growth and speed of decay, respectively, but do not represent the slopes at times t_{mid1} and t_{mid2}. The true midpoint slopes also have to be calculated numerically, by numerically differentiating f_{dsig} at each midpoint time. As stated before, the parameters I_{max} and I_{final} represent the maximum intensity and the final intensity after decay, respectively.

In addition to the parameters that uniquely determine the sigmoidal and double-sigmoidal functions, the `sicegar` package calculates other useful metrics that describe aspects of the curves (Fig. 2). For the sigmoidal model, we calculate the starting time of growth by finding the point that a line which passes though the midpoint t_{mid} with the slope a_1 intersects with the x-axis. This value gives the length of time between the start of observations and the first observable increase in the response variable. Using the same line which passes through t_{mid} with slope a_1, we can also determine the amount of time that has elapsed during exponential growth, by looking at the time point where the line intersects with the maximum intensity (I_{max}) and subtracting the starting time. For the double-sigmoidal function the time until decay can be calculated in a similar way (Fig. 2B).

Performance against simulated data

To evaluate `sicegar`'s performance more systematically, we created simulated datasets with sigmoidal and double-sigmoidal curves to which we applied increasingly higher amounts of random noise, and we assessed how well `sicegar` performed on these datasets.

First, we studied how well `sicegar` recovers the type of the curve (sigmoidal or double-sigmoidal) at varying noise levels. For curves that were simulated as sigmoidal, `sicegar` virtually never identified them as double-sigmoidal (Fig. 3A). However, starting at noise levels of around 45% (corresponding to a noise amplitude of half the total amplitude of the sigmoidal curve), `sicegar` classified a fraction of the curves as ambiguous. The fraction of curves classified as ambiguous increased with increasing noise level until virtually all curves were classified as ambiguous at noise levels of around 100%. Results were similar for curves simulated as double-sigmoidal, except that a small fraction of curves was always identified as sigmoidal, regardless of noise level. These were generally curves for which the parameter values happened to cause very little decay at large times, either because the final asymptotic value was close to the maximum or because decay started late relative to the maximum time point simulated. Finally, at high noise levels, double-sigmoidal curves can appear sigmoidal, and `sicegar` classified those curves as such.

We also compared the fits `sicegar` obtained from noisy data to the initial curves before applying noise. For each fit, we calculated the normalized mean absolute error, which is the mean of the absolute differences of fitted and original intensity levels, normalized by the maximum original intensity. We found that these errors were near zero for low noise levels and increased only gradually as noise increased (Fig 3B). Thus, overall, our algorithm results in reliable fits, it fails gradually with increasing noise levels, and it is conservative in assessing whether it has correctly identified a sigmoidal or double-sigmoidal curve or not.

Comparison to existing packages

The `sicegar` package provides a generalized way to analyze growth dynamics. However, `sicegar` is not the only package with the ability to fit data to these types of models. The `qpcR` (Ritz and Spiess, 2008) package fits linear, exponential, a number of sigmodial, and log-logistic functions to qPCR (realtime quantitative polymerase chain reaction) data. Unlike `sicegar` this package was designed to analyze a specific type of data (qPCR) and uses a log-logistic function rather then a double sigmoidal function to describe data that includes both a growth and decay phase. A similar package, `grofit` (Kahm et al., 2010), uses a number of parametric growth curves and a model-free spline approach to estimate parameters from dose-response data. This package does not include a parametric model that would account for a decay phase in the data, though an interface to construct custom models is provided. The intended use of this package is to model pharma-/toxicological data and it produces estimates of values such as half maximum effective concentration (EC50). While `sicegar` can estimate theses same types of parameters, it was designed for more general usage. For example, the first midpoint estimated by `sicegar` could refer to the EC50, though depending on the system it may or may not make sense to refer to that point in those terms. Another package, `FlexParamCurve` (Oswald et al., 2012), fits in excess of 30 models to data. While this package is useful for teasing apart exactly which variant of a model best describe dynamics, `sicegar` is better suited for classifying data as either containing sigmoidal or double-sigmoidal dynamics.

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APPENDIX: PROPERTIES AND DERIVATIONS OF THE SIGMOIDAL AND DOUBLE-SIGMOIDAL MODELS

The `sicegar` package uses two distinct models that represent growth and growth-like data obtained from time course experiments. The sigmoidal model describes situations in which the signal starts from zero and rises to a maximum level, and the double-sigmoidal model describes situations in which signal starts from zero, rises to a maximum level, and then declines towards an asymptotic value below the maximum.

Two factors were considered when choosing the corresponding mathematical functions. First, the models should be as simple as possible while being well-behaved. Well-behaved implies that the models should not take on unexpected shapes under specific parameter combinations, they should have few variables, they should be continuous, and they should be defined for all time points from minus infinity to plus infinity. As we will see below, achieving these constraints is non-trivial for the double-sigmoidal model. Second, as much as possible, model parameters should have simple interpretations and so represent or be related to biologically meaningful parameters.

Properties of the sigmoidal function

The `sicegar` package uses for its sigmoidal fit the Fermi function (Eq. 1). Here we describe the properties of a more general variant of this function,

$$I(t) = \frac{I_{\text{max}} - I_{\text{init}}}{1 + \exp\left[-a_1(t - t_{\text{mid}})\right]} + I_{\text{init}}. \tag{8}$$

The Fermi function is recovered by setting $I_{\text{init}} = 0$.

The more general function discussed here has four parameters, each of which has a clear meaning in terms of the shape of the function:

1. I_{init} represents the initial value of the sigmoidal curve at minus infinity,
$$I_{\text{init}} = \lim_{t \to -\infty} I(t). \tag{9}$$
2. I_{max} represents the maximum value of the curve at plus infinity,
$$I_{\text{max}} = \lim_{t \to \infty} I(t). \tag{10}$$
3. t_{mid} represents the time point at which the slope of $I(t)$ is maximal,
$$t_{\text{mid}} = \operatorname{argmax} \frac{d}{dt} I(t). \tag{11}$$
4. The parameter a_1, constrained to be positive at all times, is related to the maximum slope of $I(t)$,
$$\frac{a_1}{4}(I_{\text{max}} - I_{\text{init}}) = \frac{d}{dt} I(t)\bigg|_{t=t_{\text{mid}}} \tag{12}$$

In the following, we will also need the inverse sigmoidal function,

$$I(t) = \frac{I_{\text{max}} - I_{\text{final}}}{1 + \exp\left[a_2(t - t_{\text{mid}})\right]} + I_{\text{final}}, \tag{13}$$

which differs from Eq. 8 in that it does not have a minus sign in front of the slope parameter a_2. We also have renamed I_{init} as I_{final}, to indicate that it now corresponds to the limit $t \to \infty$. The inverse sigmoidal function represents sigmoidal decay rather than sigmoidal growth, starting at its maximum and decaying to its final value.

Properties of the double-sigmoidal function

The double-sigmoidal function is defined in Eq. 5 in the main text. It rises from zero to a maximum value and then decays towards an asymptotic final value, and it is defined for all t. The function is continuous with a continuous first derivative and a discontinuous second derivative.

The parameters I_{max} and I_{final} represent the maximum and asymptotic values of the curve, respectively. However, the paramters t'_{mid1}, t'_{mid2}, a'_1, and a'_2 do not directly represent midpoints and slopes. Instead, the two midpoints and two slopes need to be calculated numerically.

Derivation of the double-sigmoidal function

The double-sigmoidal as defined in Eq. 5 may seem overly complex and awkward. However, we had to define it in this way to avoid problems that arise with other formulations.

In particular, a seemingly more straightforward double-sigmoidal could be obtained by multiplying together a regular sigmoidal (Eq. 8) and an inverse sigmoidal (Eq. 13):

$$I(t) = \left(\frac{I_{\text{max}} - I_{\text{init}}}{e^{-a_1(t-t_{\text{mid1}})} + 1} + I_{\text{init}} \right) \left(\frac{1 - I_{\text{final}}}{e^{a_2(t-t_{\text{mid2}})} + 1} + I_{\text{final}} \right) \tag{14}$$

Even though this function behaves appropriately for most parameter choices, there are parameter regions in which this function is not a double sigmoidal. This occurs in general when the interval between t_{mid1} and t_{mid2} is very small, and one of the slopes is very steep while the other one is not. As an example, consider the case shown in Fig. 4, where Eq. 14 generates a local minimum in addition to a local maximum. These kinds of corner cases will be discovered by the fitting algorithm when used on sufficiently many diverse datasets, and therefore we ruled out Eq. 14 as an appropriate choice for our double-sigmoidal function.

However, Eq. 14 will never have a local minimum if we set both I_{init} and I_{final} to zero (see next section for proof). Thus, we define the *base double sigmoidal* as

$$f_{\text{dsig-base}}(t) = \frac{1}{\left(e^{-a_1(t-t_{\text{mid1}})} + 1\right)\left(e^{a_2(t-t_{\text{mid2}})} + 1\right)} \tag{15}$$

This function has the correct shape in principle, except that the intensity always decays back to zero for large t.

One possible solution to the problem that the function approaches zero for $t \to \infty$ is to cut it into two parts, rescale them separately, and then merge them together again. We cut the function at its maximum, where $t = t^*$. Thus, the part from $t = -\infty$ to $t = t^*$ represents sigmoidal growth and the part from $t = t^*$ to $t = \infty$ represents sigmoidal decay. Both parts are rescaled such that the desired I_{max} and I_{final} are obtained. This procedure leads to the definition of the double-sigmoidal we use, as defined in Eq. 5. This function has the desired limiting properties and is guaranteed to have only one maximum. However, the cutting and rescaling procedure causes its second derivative to be discontinuous at $t = t^*$.

The base double sigmoidal has exactly one local maximum

For the base double sigmoidal as defined in Eq. 15 we can prove the following Lemma.

Lemma. *$f_{\textit{dsig-base}}(t)$ has one local maximum.*

Proof. At its local maximums and minimums, i.e. when $t = t^*$, the derivative of $f_{\text{dsig-base}}(t)$ must be equal to zero,

$$\frac{d}{dt} f_{\text{dsig-base}}(t) \Big|_{t=t^*} = 0. \tag{16}$$

By definition, $t_{\text{mid2}} > t_{\text{mid1}}$, so without loss of generality we can write $t_{\text{mid2}} = t_{\text{mid1}} + L$, where L is a positive number. Then we obtain

$$\frac{d}{dt} \left[\frac{1}{(e^{-a_1(t-t_{\text{mid1}})} + 1)(e^{a_2(t-L-t_{\text{mid1}})} + 1)} \right] \Bigg|_{t=t^*} = 0 \tag{17}$$

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We define new variables $u \equiv t - t_{\text{mid1}}$ and $u^* \equiv t^* - t_{\text{mid1}}$. We also define the function $g(u) = f_{\text{dsig-base}}(u + t_{\text{mid1}})$. Finding the roots of $g(u)$ is equivalent to finding the roots of $f_{\text{dsig-base}}(t)$, and it involves solving the following equation for u^*:

$$\frac{d}{du} g(u)\Big|_{u=u^*} = \frac{d}{du}\left[\frac{1}{(e^{-a_1 u}+1)(e^{a_2(u-L)}+1)}\right]\Bigg|_{u=u^*} = 0 \tag{18}$$

After taking the derivative, we can rewrite this equation as

$$\frac{e^{(a_1 u^* + a_2(L-u^*))}\left[a_1(e^{a_2(L-u^*)}+1) - a_2(e^{a_1 u^*}+1)\right]}{(e^{a_1 u^*}+1)^2(e^{a_2(L-u^*)}+1)^2} = 0 \tag{19}$$

For the left-hand side to be equal to zero, the term in the square brackets in the numerator must be equal to zero, because all other terms in the expression are always positive. We next define the term in the square brackets as a function of u,

$$h(u) = a_1(e^{a_2(L-u)}+1) - a_2(e^{a_1 u}+1). \tag{20}$$

The number of roots of $h(u)$ is equal to the number of local extrema of $f_{\text{dsig-base}}(t)$.

To determine the number of roots of $h(u)$, we first note the following limiting properties:

$$\lim_{u\to-\infty} h(u) = \infty \tag{21}$$

$$\lim_{u\to\infty} h(u) = -\infty \tag{22}$$

According to the mean value theorem, $h(u)$ must thus have at least one root. Next, we note that $h(u)$ is strictly decreasing:

$$\frac{d}{du} h(u) = -a_1 a_2 e^{a_2(L-u)} - a_1 a_2 e^{a_1 u} < 0 \tag{23}$$

Since the derivative of $h(u)$ is not equal to zero for any u we can use Rolle's Theorem to conclude that the function cannot have more than one root. In conclusion, $h(u)$ has exactly one root, which implies that $f_{\text{dsig-base}}(u)$ has exactly one local extremum.

Finally, we demonstrate that the local extremum of $f_{\text{dsig-base}}(t)$ is a local maximum, by inspecting the second derivative of $g(u)$ at $u = u^*$.

$$\begin{aligned}\frac{d^2}{du^2} g(u)\Big|_{u=u^*} &= \frac{e^{a_1 u - 2a_2(L-u)}}{(e^{a_1 u}+1)^3(e^{-a_2(L-u)}+1)^3} \\ &\times \Bigg[\Big(a_1(e^{a_2(L-u)}+1) - a_2(e^{a_1 u}+1)\Big)^2 \\ &\qquad - a_1^2 e^{a_1 u}(e^{a_2(L-u)}+1)^2 - a_2^2(e^{a_1 u}+1)^2 e^{a_2(L-u)}\Bigg]\Bigg|_{u=u^*}\end{aligned} \tag{24}$$

The first term in the square brackets is equal to $h(u)^2$ and thus is zero at $u = u^*$. The two remaining terms inside the square brackets are strictly negative, and the term outside the square brackets is strictly positive. Therefore, $g(u^*) < 0$, which proves that the local extremum is a local maximum.

□

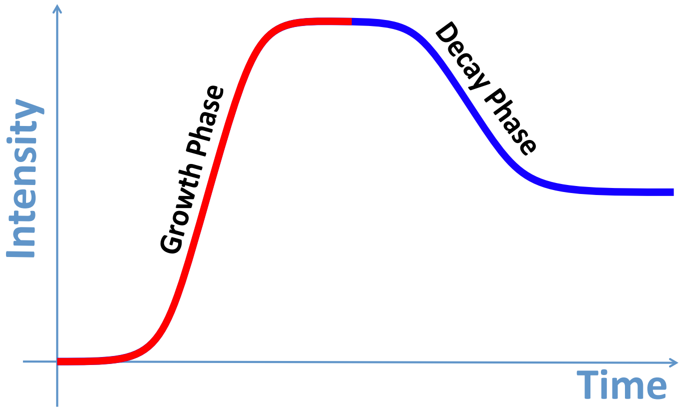

Figure 1. The two phases that an observer of growth data might see. The first phase, shown in red, is the "growth phase", during which growth occurs at exponential rate until some asymptotic level is reached. The second phase, shown in blue, is the "decay phase", during which intensity declines towards some lower asymptotic level.

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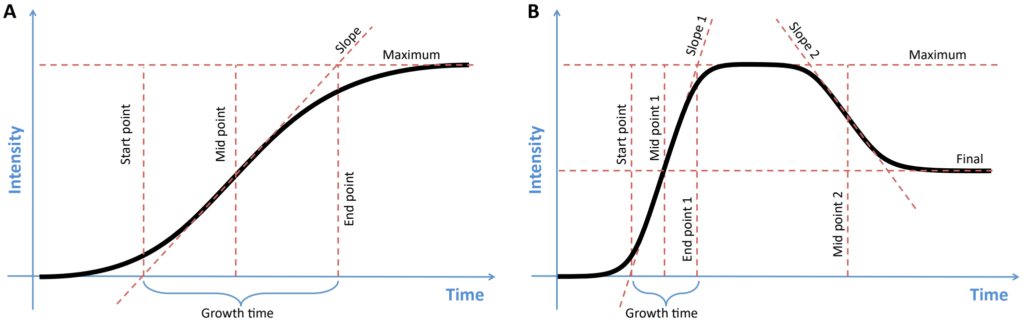

Figure 2. The two models used to classify growth data, with key parameters of each model labeled. (A) In the sigmoidal model the maximum value, the midpoint, and the slope of the curve uniquely describe the function. Estimates of the start and end points of growth, as well as the growth time, can then be calculated from this model. (B) The double sigmoidal model is uniquely determined by six parameters, two midpoint and two slope parameters, a maximum value, and a final value. As in the case of the sigmoidal model, the start point, end point, and duration of both the growth and the decay phases can be calculated from these parameters.

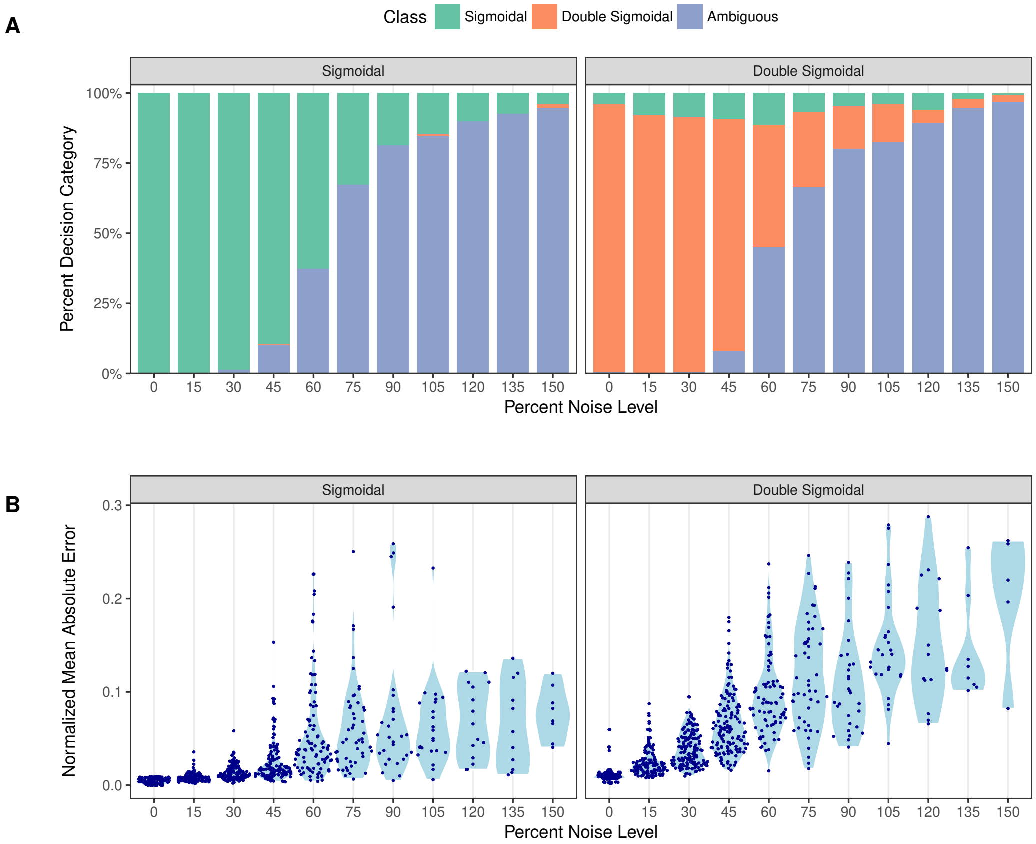

Figure 3. Assessment of `sicegar` performance on simulated data subjected to varying levels of noise. Noise levels are measured relative to the maximum intensity of the simulated curves. (A) `sicegar` tends to either recover the original type of curve correctly (for small to moderate amounts of noise) or label the dataset as ambiguous (at high noise levels). (B) The difference between the original data (without noise) and the fitted curve is near zero for low noise levels and increases gradually and slowly for higher noise levels.

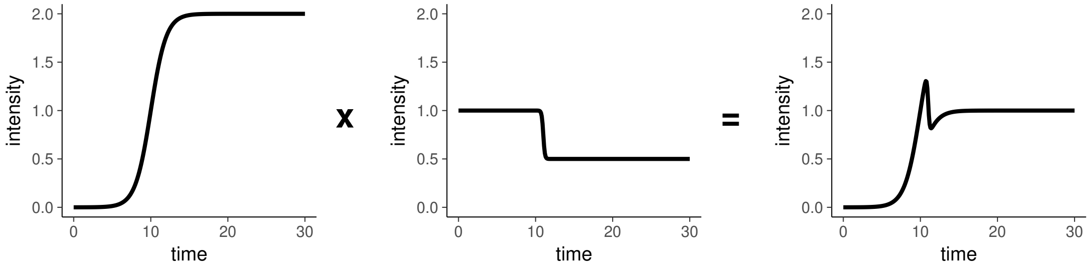

Figure 4. Example combination of parameters that are problematic for the multiplicative double-sigmoidal model (Eq. 14), which is obtained by multiplying the sigmoidal (Eq. 8) and inverse sigmoidal (Eq. 13) functions. Parameter choices for this example are: $I_{\text{init}} = 0$, $I_{\text{final}} = 0.5$, $I_{\text{max}} = 2$, $a_1 = 1$, $a_2 = 10$, $t_{\text{mid1}} = 10$, $t_{\text{mid2}} = 11$. This example demonstrates that Eq. 14 is not guaranteed to be double-sigmoidal.