Monotonic Transient Analysis Parameters

The following is a brief overview of the mathematical derivations used to perform the transient analysis (sections I through IX) followed by a summary of the resulting parameters and how they might be used in the context of cellular calcium and contractility (section X). Sections I through IX describe how the software performs the analysis; section X provides a description of the analysis results. Please skip to section X on page 6 if you're not interested in the derivation of the transient fit. Also, please follow the Transient Analysis Tutorial for an explanation of how to perform the analysis on your data (in the "technical notes" section of our website).

I. Transient Defined

A transient is defined as a recorded signal that diverges from a baseline value and returns again. Data from cell function experiments often appear in the form of transients, and specific characteristics of these transients can be used as markers of physiological functions for comparison between populations of cells.

IonWizard's Transient Analysis provides analysis for fluorescence transients and displacement transients. There are many specific types of transients that fall within these two categories. Fluorescence transients may include fluorescence intensity, fluorescence ratio, and intracellular calcium concentration ($[Ca^{2+}]$) transients. The family of displacement transients may include those data recorded during video edge detection for cell, sarcomere length detection for shortening/re-lengthening, and analog signals representative of cell position. Figure 1 illustrates some examples of fluorescence and displacement transients.
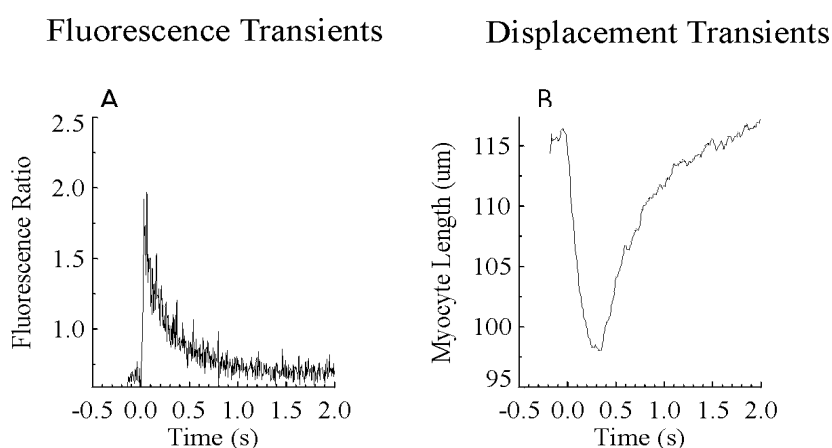

Figure 1: Examples of fluorescence and displacement transients. A. Ratio of fluorescence intensities excited at 340 nm and 380 nm B. Myocyte length recorded using video edge detection.

List of Transient Characteristics

Characteristic	Definition
Transient Time	The absolute time of initiation of the transient.
Baseline	The pre-stimulation baseline value of the recorded signal.
Peak	The value of the transient at its maximal deflection from baseline.
Time to Peak	The time at which peak occurs relative to the transient time.
Peak Height	peak - baseline.
%Peak	100 x peak / baseline
%Peak Height	100 x peak height / baseline
Times to % Peak	Times for the transient to reach a user-defined percent of the peak during the deflection phase of the transient.
Times to % Return	Times for the transient to return a user-defined percent of the peak during the recovery phase of the transient.
Max Velocities	The maximum of first derivative of transient during the deflection and recovery phases of the transient.
Max Rates (per Baseline)	maximum velocities / baseline.
Max Rates (per Peak Height)	maximum velocities / peak height
Rate Constants (rise and decay)	The exponential rate constants associated with deflection and recovery phases of the transients.
Time Constants (rise and decay)	The exponential time constants associated with deflection and recovery phases of the transients.
Integral	The area under the transient relative to baseline.

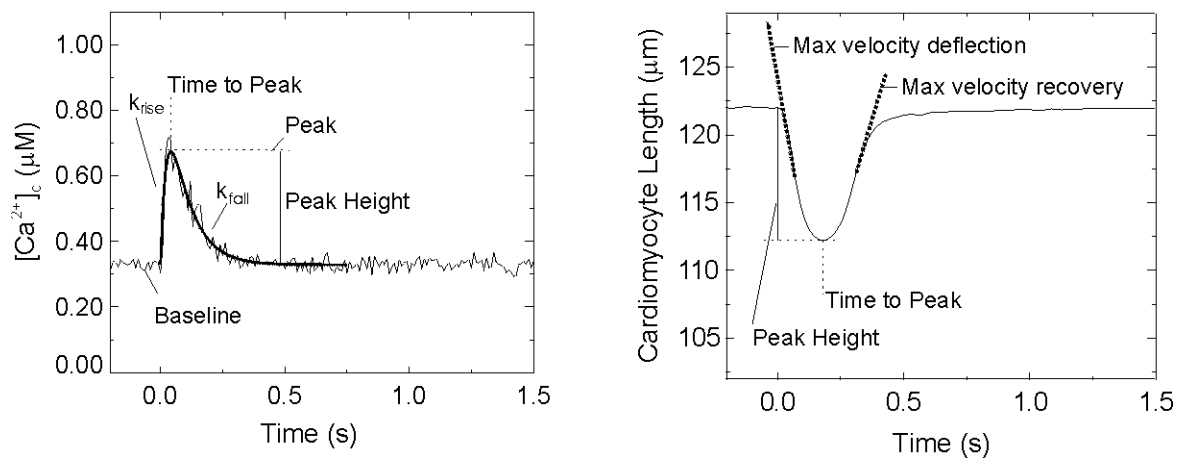Table 1: Transient characteristics determined by IonWizard Transient Analysis.

Figure 2: Some transient characteristics determined by IonWizard Transient Analysis.

II. Identifying a Transient as Positive-going or Negative-going

Although transients can be classified as being fluorescence or displacement, transients are also either positive-going or negative-going. IonWizard Transient analysis begins by identifying the baseline, minimum, and maximum values of the transient and the times at which these values occur. A positive-going transient is identified as having a greater maximum deflection than minimum deflection, and a negative-going transient is identified as having a greater minimum deflection than maximum. The characteristics of baseline and peak, i.e., value of maximum deflection, are then assigned according to the transient being positive-going or negative-going.

III. Identifying the Baseline, Deflection, Peak, Recovery, and Return to Baseline Phases of a Transient

We further classify five time periods of the transient as the baseline, deflection, peak, recovery, and return to baseline phases. Once these phases are identified, they are examined separately for the specific characteristics that lie within the respective phase. For example, maximum deflection velocity is found only in the deflection phase of the transient. Figure 3 illustrates the first four phases of a transient.
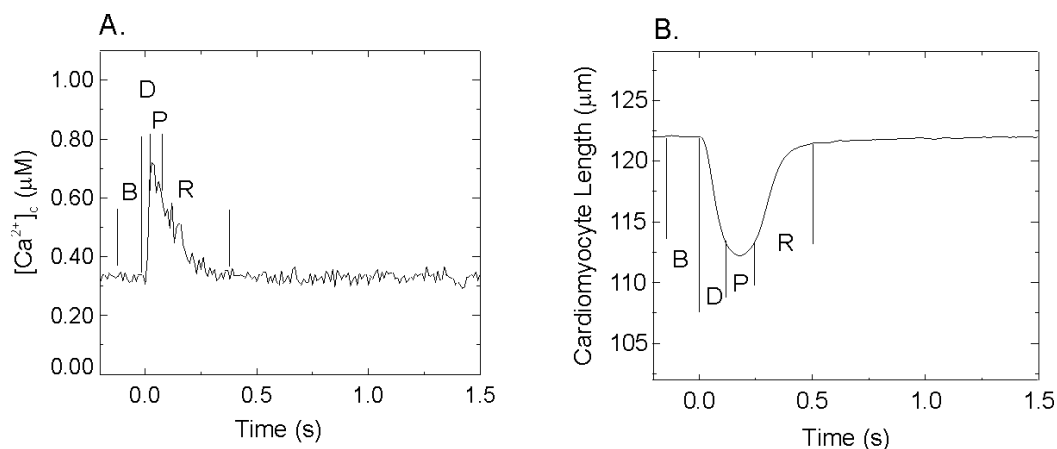
Figure 3: Examples of baseline (B), deflection (D), peak (P), and recovery (R) phases of the transient. A. Phases of fluorescence transient. B. Phases of displacement transient.

IV. Characterizing the Phases

Although specific values and times characterizing the baseline, deflection, peak, recovery, and return to baseline phases of a transient can be determined from the raw data, the noise associated with the recorded data (even after filtering) may adversely influence analysis such that the resultant characteristics poorly reflect the underlying physiological signal. In order to characterize the signal despite the associated noise, IonWizard's Transient Analysis fits an analytical function to the recorded data, thereby permitting characteristics of the analytical function to be taken as those of the physiological signal.

This strategy is theoretically sound, but is nevertheless limited by the extent to which the analytical function represents the signal. Instead of picking or deriving an analytical function to represent the signal, we have chosen to use a truncated Taylor series expansion of any analytical function to represent the signal within a phase of the transient. We have also chosen the time in the center of the phase to represent time zero. The methods we use to fit the truncated Taylor series, i.e., power series, to the recorded data and determine the characteristics of each phase of a transient are described below.

V. Modeling a Phase with a Power Series

The data of each phase are fit to a truncated power series:

$$p(t) = \sum_{n=0}^{N} a_n t^n \qquad\qquad \text{(Equation 1)}$$

A singular value decomposition fit of eq 1 to the data provides values for the coefficients, a_n. The highest polynomial order, N, is set to be proportional to the number of data points available and depends upon the phase. For example, the baseline phase is always fit to a 1st order series (a line), while the deflection phase may be fit to a series of order ranging from 7-11.

VI. Finding Times of Interest within a Phase

Determining a time of interest within a phase is mathematically performed as finding the root of the polynomial expression. For the peak phase, we are interesting in finding the time at which the signal is an extreme, i.e., when the first derivative of the signal is equal to zero. Without calculating the root, the position of the root within the phase is found iteratively.

In the deflection and recovery phases, we are also interested in finding the time at which the first derivative of the signal is a maximum. In those cases, the extrema of the first derivative of the power series are found.

VII. Finding Values of Interest within a Phase

Having found a root, we have effectively found the time at which the value of interest occurs. The value can then be calculated by substituting the root into the appropriate equation. For example, in finding the value for the peak, the time to peak, i.e., the root, is substituted into equation 1.

VIII. Least-squares Fitting of Non-linear Functions to a Transient

For some transients, it may be appropriate to fit an analytical function to a portion or the entire transient. This may be justified by mathematical modeling of the physiological processes that eventually results in an analytical expression having physiologically relevant parameters. Two examples are the single and double exponential models of intracellular calcium handling. The single exponential model can be used to model the fall of calcium concentration during reuptake and is written as follows:

$$f(x) = A*exp^{(-k_{fall}*x)} + B \qquad\qquad \text{(Equation 2)}$$

where A = amplitude of the signal that is falling, k_{fall} = exponential rate constant of the fall, and B = baseline value to which the signal is falling. The double exponential model may also be used to model the rise and fall of calcium concentration and is written as follows:

$$f(x) = A*\{exp^{(-k_{fall}*x)} - exp^{(-k_{rise}*x)}\} + B \qquad\qquad \text{(Equation 3)}$$

where k_{rise} = exponential rate constant of rise.

 IonWizard Transient Analysis allows the user to fit either or both of these functions to recorded data representing a fluorescence transient. The fit is performed using a Marquardt non-linear least squares fit routine, which estimates the model parameters that provide a best fit of the model to the data. The standard deviation of the noise associated with the data, as required by the routine, is taken to be proportional to the value of the data. The user is cautioned that this approximation of the noise may be significantly inappropriate for high-valued ratio and calcium concentration data.

The parameters k_{fall} and k_{rise} are reported in the table of transient characteristics. Other reported characteristics are calculated from the estimated model parameters are the related time constants $\tau_{fall} = 1/ k_{fall}$, and $\tau_{rise} = 1/ k_{rise}$.

IX. Limitations of the Methods

The above described methods for determining the times and values of interest that characterize a transient have some limitations. For example, the representation of the physiological signal as an analytical function can unnecessarily constrain the information that lies within the data. Unfortunately, noise associated with the signal is unavoidable and also constrains the precision with which signal information can be drawn from the recorded data. The use of the analytical function, like the truncated power series, to represent the underlying signal is therefore justifiable as a compromise: although a bias may be introduced, characteristics of the signal are determined more consistently and precisely despite the noise. And because the methodological bias would be applied to all data of all populations, comparisons between populations will still be relevant.

X. Summary of Transient Parameters

The following are explanations of the values given by IonWizard's Monotonic Transient Analysis. Please bear in mind that these descriptions primarily relate to a typical functional analysis of cardiomyocyte behavior. The goal of the analysis is to characterize cellular calcium and contractility. Very simply, the analysis attempts to describe three things: how fast was the response, how big was the response, and how long did it take to recover. The following characteristics are intended to interpret the data in a manner that facilitates comparison between different cellular conditions: basal and pharmacologically-treated, wildtype and transgenic, etc.

t0. Time 0 (t0) is defined by default as the time at which a TTL event was recorded relative to the full time scale. Typically t0 is when the cell was stimulated. This can be changed through the Monotonic Transient Analysis Options… under the Operations menu to the departure or the transient mark; i.e., the beginning of the calculated departure from baseline or the user-described transient mark, respectively. The default settings (TTL event) will yield the most reliable and reproducible results. *t0 has no significance by itself.* The value will typically represent the offset that was determined by the user during transient averaging. t0 will be used in subsequent transient calculations as the beginning time point. For example, the time to peak will be the time from t0 to the time at which the transient has reached its maximal value.

bl. The baseline (bl) is the y-axis value at t0. For example, bl for sarcomere length detection will be the relaxed sarcomere length. For most purposes, the baseline has no real value by itself. It may useful to note the bl, however, as it can be an indication of the health of the cell and the subsequent quality and reliability of the data. For example, the resting sarcomere length of an unloaded (no mechanical load) rat myocyte should be around 1.8 microns. The fura ratio baseline, on the other hand, is a relative measure and will differ from system to system. By itself, again, it has no value. Fura baselines should be consistent from cell to cell, however. Large differences in the fura baseline suggest varying basal calcium levels and could be indicative of poor cell health.

dep v. The departure velocity (dep v) is a value that characterizes the speed with which the cell contracts or $[Ca^{2+}]i$ goes up. It is the maximal rate of change during the contraction or calcium release phase of the transient. This is a useful measure of the speed of the response.

dep v t. The time to maximal departure velocity (dep v t) is another way of characterizing the rate of the departure or, simply put, how fast things happen. The interpretation of it differs slightly from dep v. Conceivably, two cells could have the same departure velocities and different dep v times; the maximal rate of contraction could be the same but the time it took for the cell to initiate the contraction was delayed as a result of some mechanical or pharmacological insult.

peak. The peak is the maximal displacement from baseline. Simply put, the peak is the shortest the cell or average sarcomere length recorded during the contraction or the highest ratio recorded (pretty much self-explanatory).

peak h. The peak height (peak h) is the difference between the peak and baseline values. Again, this is pretty self-explanatory.

bl%peak h. The baseline as a percentage of peak height is the percent change during the transient. Many of the values given by the analysis are quantitative relative values, especially cell length and fura ratio. The peak height by itself has little meaning. For example, individual cell lengths will be heterogeneous in any given isolation. The absolute cell lengths recorded (baseline and peak) may be irrelevant in the interpretation of the function. The percent change, however, is a valuable tool for describing the relative change that occurred during the contraction. Sarcomere length and calcium concentration may have value as absolute numbers, but the interpretation may be better served by the percent change. This is the parameter most often used to characterize the magnitude of the transient.

peak t. The time to peak (peak t) is the time from t0 to the peak. It provides another mechanism for characterizing the speed of contraction or calcium elevation. Again, its interpretation may differ from the "dep v" and "dep v t". The cell may have been slow to respond to stimulation but contracted at a normal or accelerated rate, suggesting some perturbation in excitation-contraction coupling. (Generally speaking, however, the "dep v", "dep v t", and "peak t" will tell you the same thing. That is, when one is slower for a given transient, the others will follow suit.) This is not an oft-reported value. The "t to peak xx%" below is more useful.

ret v. The return velocity (ret v) is the maximal rate of the return phase of the transient. It's the same as "dep v" except that it describes the recovery phase of the transient. It is sometimes reported as a means of describing the speed of calcium reuptake or cardiomyocyte relaxation/re-lengthening.

ret v t. The time to maximal return velocity (ret v t) is the period of time from t0 to "ret v". This value can be used to characterize the speed of relaxation. Again, similar to "dep v" and "dep v t", the precise interpretations of "ret v" and "ret v t" are slightly different.

t to peak xx%. The time to some percentage of the peak is a characterization of the speed of contraction or calcium elevation. By default, a value of 10% is used. Any reasonable value can be selected and up to three separate values can be displayed; these are chosen through the Monotionic Transient Analysis Options… under the Operations menu. The time to peak (peak t) may be a difficult value to accurately gauge if the peak is not particularly sharp. A time to peak of 50% (or similar) will likely be a more reliable and accurate measure of the actual speed. It may also be a more physiologically relevant measurement. The peak phase

of the transient may not be as indicative of the contractile function as the phase in which the cell is actively contracting. The time to xx% peak is an oft-reported value in the literature.

t to bl xx%. The time to xx percentage of the baseline is a characterization of cellular relaxation or calcium reuptake. This is also an oft-reported value in the literature. Note that the baseline is the value determined at t0. The transient may not reach the baseline during the return phase. As such a value of 95% or 100% will generate an error.

sin exp amp. Before discussing the single exponential amplitude (sin exp amp), some explanation of the single exponential is needed. The analysis software fits a single exponential to the curve of the transient's return phase with the goal of characterizing the length of time required for cellular relaxation or calcium reuptake. The single exponential function (e^x) can be described as: $e^x = Ae^{(-x/\tau)}+B$; where A is the amplitude, B is the offset and τ is the decay time constant (tau). The return phase of the transient is often well characterized by the single exponential. Care should be taken, however, in the fitting of the exponential to the curve. By default, the software fits the exponential function from the peak. The fit from the peak is not typically very accurate for contractility data. It can be poor for ratiometric data as well, although less often so. The peak for contractility data is often curved as well, so a fit from peak would attempt to fit the single exponential to two curves. The exponential curve fit may be better served by a "fit from the return velocity" or "from ~25-50% return to baseline". These options can be selected in the Monotionic Transient Analysis Options… under the Operations menu. The fit can be checked and verified by clicking on "sin exp amp" in the far left of the analysis results. This will color the single exponential in the trace cyan and allow you to make a determination of the accuracy of the fit.

Returning to the "sin exp amp": the amplitude (A in the equation above) is never used to represent any function of the cell. It is determined by the "fit from…" selection described above. In other words, the amplitude is typically ignored.

sin exp tau. The single exponential tau (sin exp tau) is the exponential decay time constant of the function. This is commonly used as a means of characterizing the speed of recovery (relaxation or calcium reuptake). Tau's units are in time (seconds) and a greater tau value equates to a longer recovery or return to baseline.

Note: tau, "ret v", and "t to bl xx%" describe slightly different aspects of the return phase. They are, however, often used to describe the same thing: how long did it take for the cell to recover. Your choice of value to report will likely depend on how accurate and reliable each appears based on how well each represents the data. A poor exponential fit would naturally preclude the use of tau as a means to describe the recovery.

sin exp off. The single exponential offset (sin exp off) is the vertical offset of the equation, i.e. where the curve crosses the y-axis. This should be very similar to the baseline at t0 (bl); the myocyte may be in poor health if it's not. It is not useful for the purpose of characterizing cellular function.

Note: a bi-exponential fit can be selected from the Monotionic Transient Analysis Options… under the Operations menu. An exponential will be fit to both the departure and return phases and time constants (τ) will be given for both the rise and fall. Please be aware that the data may not be represented well by a bi-exponential fit.

areadep a. The area of the departure phase a (areadep a) is an integration of the values under the curve (between baseline and the curve) from t0 to "peak t". It can be used to describe the magnitude of the departure phase of the transient (e.g., total calcium *released*). It is not often reported.

areadep b. The area of departure b (areadep b) is an integration of the values from 0 to the baseline. By itself it has no value; when combined with the "areadep a" it provides an absolute value (e.g., total absolute calcium recorded from t0 to "peak h").

arearet a. The area of the return phase a (arearet a) is an integration of the values under the curve from peak t to the end of the transient. Similar to "areadep a", it includes only values from the baseline to the curve. The "arearet a" is especially useful for return phases that are poorly described by other measures. For example, if cellular relaxation/relengthening has multiple phases rather than a smooth curve from the peak back to the baseline, many of the calculated parameters described above will not represent the data well.

arearet b. Similar to "areadep b", the area of the return phase from 0 to the baseline can be used in combination with "areadep a" to provide an absolute value of the response over time.

Note: "Areadep b" and "arearet b" can only be used for positive-going transients (such as fura ratio and calcium). The value from 0 to the baseline of a negative-going transient (such as contractility data) is not meaningful.

XI. Conclusion

The goal of IonWizard's Transient Analysis is to provide a clear representation of the speed and magnitude of intracellular calcium changes as well as cell shortening and re-lengthening; the results forming the basis for comparing responses between cell populations. Our hope is that the analysis will facilitate greater understanding of the underlying molecular and biophysical functions governing excitation-contraction coupling and, by extension, cardiovascular function.

(We would like to thank Brad Palmer (UVM) for contributing the majority of sections I-IX of this document.)