Cellomics™

ArrayScan® VTI HCS Reader: User's Guide Addendum

Software Upgrade from Version x.5 to x.6

PUBLISHED BY

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WARNING

Use this product only in the manner described in this guide. When used other than as specified, the safety protections may be impaired. Please refer to the **ArrayScan V^{TI} HCS Reader User's Guide** for safety precautions ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please read the instructions, warnings, and precautionary measures supplied in this guide and with accessories. Failure to adhere to safety precautions and/or procedures may result in system failure, personal injury, or death. Cellomics, Inc. shall not be held liable under any circumstances. Cellomics does not assume any liability for damages or malfunctions caused by faulty operation, negligence, unauthorized modifications or repairs, or use of unauthorized accessories.

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Chapter

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Introduction

This guide describes the software changes and enhancements associated with the ArrayScan® and vHCS™:Scan software upgrade from version x.5 to version x.6.

Software enhancements include the following:

- **Kinetics Feature:** The new Kinetics feature is designed to provide quantitative analysis of the dynamics of molecular interactions within cells and dynamics of cell movements (if any) during a predefined time interval. The Kinetics feature can be used with any cell-based BioApplications to extract quantitative kinetic descriptors from any cell-based features.
- **Selecting Features for Storage:** The new Features to Store button provides you with the ability to choose which Cell, Field, and Well Features you wish to store to the database when scanning.
- **Disk Scan Channel Re-mapping:** The Disk Scan Channel Re-map option provides the ability to acquire images from a certain channel but then reprocess the images via Disk Scanning using a different channel.
- **Importing non- Cellomics® Images:** In addition to displaying images generated by Cellomics instruments, the ArrayScan and vHCS:Scan software can now display images that were generated by non-Cellomics instruments. In addition to viewing these images, you can also run a Disk Scan of the images.

Terminology, Conventions, and Symbols

This guide assumes that you have a basic knowledge of computers using the Microsoft® Windows® 2000 operating system and that you have experience working with windows, menus, commands, buttons, tabs, dialog boxes, and other Microsoft Windows elements. If you are unfamiliar with these terms, please refer to Microsoft Windows documentation.

Throughout this guide certain terminology, conventions, and symbols are used consistently. These conventions are described below.

Terminology

Term	Definition
Click	This term means to place the mouse pointer over the item, then depress and release the primary mouse button (usually the left button) in one quick motion
Right-click	This term means to place the mouse pointer over the item, then depress and release the secondary mouse button (usually the right button) in one quick motion
Double-click	This term means to place the mouse pointer over the item, then depress and release the primary mouse button twice in quick succession.

Term	Definition
Drag	This term means to place the mouse pointer over the item, depress and hold down the left mouse button, move the pointer (and the object) to some target location, then release the mouse button.
Press	This term means to push and release a key on the keyboard. For example, press the Tab key.
Shift Ctrl Alt	When any of these terms appear before any of the above terms, it means to hold down the specified keyboard key while taking the hyphenated action. Thus, Shift-click means to hold down the Shift key while clicking an item.
Shortcut menu	This is a menu that appears when you right-click an item.

Conventions

- Menu names, menu items, buttons, and options appear in bold type. For example: "Click the **Configure** button."
- Window titles and dialog box names begin with uppercase letters. For example: "The Kinetic Options dialog box appears as shown in the following figure."

Symbols

The following symbols appear throughout the documentation in order to draw your attention to important information such as operating tips and suggestions as well as the presence of hazards.

Symbol	Description
	**WARNING** A potentially hazardous situation, which, if not avoided, could result in serious injury or death. When this symbol appears on equipment, consult product documentation to identify the nature of any potential hazard and determine the actions to perform.
NOTE 	A tip, suggestion, or additional information.

Safety Precautions

Use this product only in the manner described in this guide. When used other than as specified, the safety protections may be impaired. Please refer to the **ArrayScan V^{TI} HCS Reader User's Guide** for safety precautions ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please read the instructions, warnings, and precautionary measures supplied throughout this guide and with accessories.

WARNING

Use this product only in the manner described in this guide. When used other than as specified, the safety protections may be impaired. Please refer to the **ArrayScan V^{TI} HCS Reader User's Guide** for safety precautions ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please read the instructions, warnings, and precautionary measures supplied in this guide and with accessories. Failure to adhere to safety precautions and/or procedures may result in system failure, personal injury, or death. Cellomics, Inc. shall not be held liable under any circumstances. Cellomics does not assume any liability for damages or malfunctions caused by faulty operation, negligence, unauthorized modifications or repairs, or use of unauthorized accessories.

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For European assistance, contact Cellomics Europe:
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Before contacting Cellomics for service or support, it is helpful if you are prepared to answer the following questions:

- What were you doing when the problem occurred?
- Can you reproduce the problem?
- Did you try to solve the problem? If so, what steps did you take and what did you observe?
- Which error messages, if any, appeared?

Having these answers will help us provide you with a solution as quickly as possible.

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Chapter

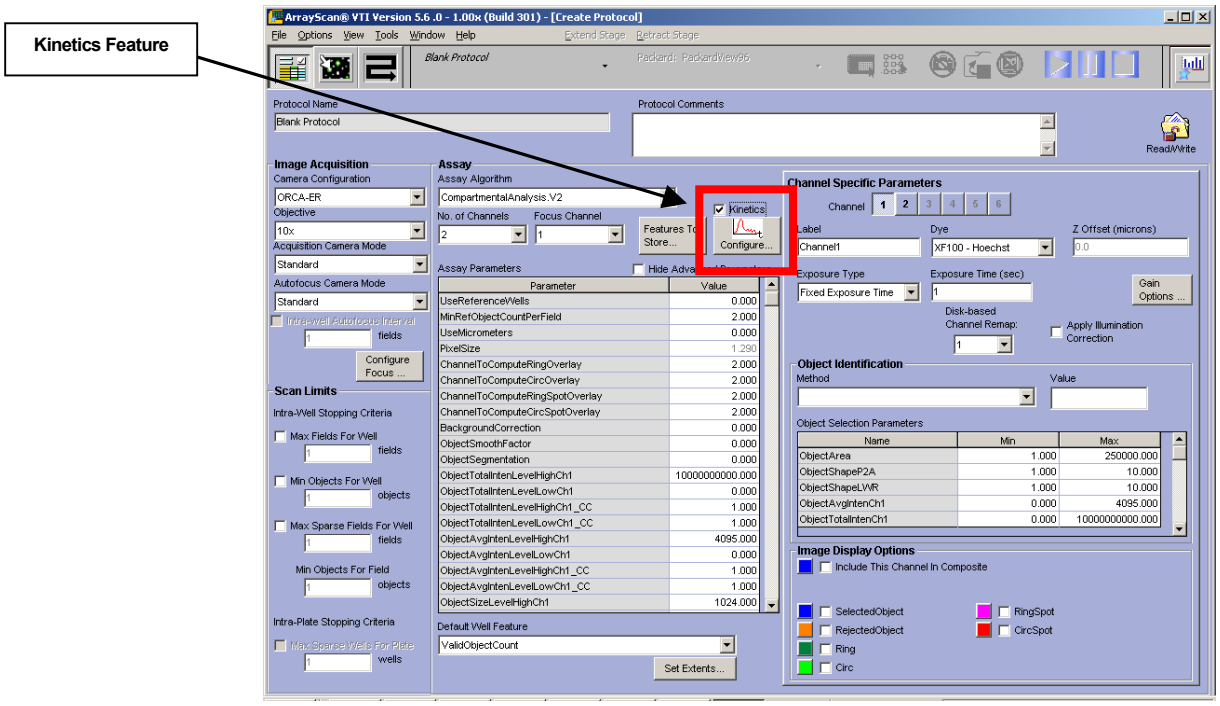
2

Using the Kinetics Feature

WARNING

Use this product only in the manner described in this guide. When used other than as specified, the safety protections may be impaired. Please refer to the **ArrayScan V^{TI} HCS Reader User's Guide** for safety precautions ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please read the instructions, warnings, and precautionary measures supplied in this guide and with accessories. Failure to adhere to safety precautions and/or procedures may result in system failure, personal injury, or death. Cellomics, Inc. shall not be held liable under any circumstances. Cellomics does not assume any liability for damages or malfunctions caused by faulty operation, negligence, unauthorized modifications or repairs, or use of unauthorized accessories.

The Kinetics feature of the ArrayScan software is designed to provide quantitative analysis of the dynamics of molecular interactions within cells and dynamics of cell movements (if any) during a predefined time interval. The Kinetics feature enables the quantification of expression dynamics of receptors, surface markers, transcription factors or kinase activation, apoptosis indicators, or proliferation markers. The Kinetics feature can be used with any cell-based BioApplications to extract quantitative kinetic descriptors from any well-based or cell-based features.



Kinetics Configuration

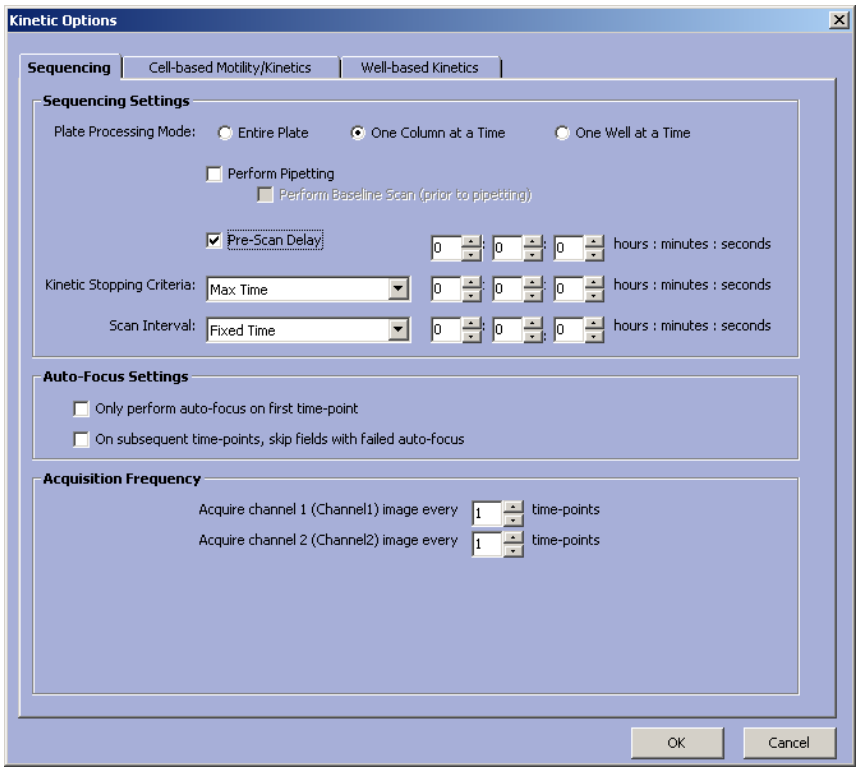
The Kinetics Configuration button provides you with the ability to specify kinetic options including sequencing settings, cell-based motility and kinetic options, and well-based kinetic options.

Sequencing Settings

To specify kinetic sequencing settings,

1) Select the **Kinetics** checkbox as shown in the previous figure.

2) Click the **Configure** button. The Kinetic Options dialog box appears as shown in the following figure.



3) With the Sequencing tab selected, specify the following sequencing settings:

Plate Processing Mode:

Option	Description
Entire Plate	The system processes the entire plate for each kinetic cycle (time point). This is the slowest mode.
One Column at a Time	The system processes a single column for every kinetic cycle (time point) before moving on to the next column
One Well at a Time	The system processes a single well for every kinetic cycle (time point) before moving on to the next well. This is the fastest mode.

The option, One Well at a Time, is normally used on fast-reaction assays; the option, Entire Plate, is normally used on slow-reaction assays. The important factor is the time between images. For example, when selecting Entire Plate, if you are processing all 12 columns, a well will not be scanned a second time for several minutes. Therefore, the Entire Plate option is

ideal for reactions that require several hours to complete. Conversely, if your reaction is completed within a minute, you should use the option, One Well at a Time. The time between images can vary depending on the number of columns processed, the assay parameters, the fluorophore used, etc.

Pipetting:

Check the **Perform Pipetting** box if you are planning to perform manual pipetting. Check the Perform Baseline Scan checkbox, if you wish to perform a baseline scan prior to pipetting.

Pre-Scan Delay:

Check the **Pre-Scan Delay** checkbox if you wish to delay operation before the plate is scanned. Using the arrow buttons, select the number of hours, minutes, and seconds from the boxes provided. Alternatively, you can enter the number of hours, minutes, and seconds in each box.

Kinetic Stopping Criteria:

Select one of the following Kinetic Stopping Criteria from the drop-down menu.

Option	Description
Max Time	Select this option when you wish to specify the total time for scanning. If the Max Time is less than the time between Kinetic Cycles, the cycle that was started will be completed. For example, if you set the Max Time to 1 minute, and a kinetic cycle (time between time points) lasts for 45 seconds, two kinetic cycles will be completed.
Interval Count	Select this option when you want to specify the number of kinetic cycles (time points) to process.

Scan Interval:

Select one of the following scan interval options from the drop-down menu.

Option	Description
Fixed Time	Select this option when you wish to specify the time period to begin scanning. For example, you may wish to begin a new scan every 45 minutes. Select the number of hours, minutes, and seconds from the boxes provided.
As fast as possible	Select this option when you want to start subsequent scans as soon as possible after finishing the last well selected in a scan.

4) With the Sequencing tab selected, specify the following auto-focus settings:

Select one or more of the following auto-focus options if desired:

Option	Description
Only perform auto-focus on first time point	Select this option when you want to perform auto-focus on the initial time point only. The system will not auto-focus again on subsequent kinetic cycles.
On subsequent time points, skip fields with failed auto-focus	Select this option when you want to skip the fields that failed auto focus from previous time points.

The determining factor for selecting an auto-focus option is how much your sample will change between images. For example, when samples are changing quickly and you will be imaging very quickly, you will want to focus the first time and use the same focus plane for all subsequent images. In slow reactions, you may want to focus after every kinetic cycle.

5) With the Sequencing tab selecting, specify the following Acquisition Frequency settings:

Enter into each active channel, the time point on which to acquire an image for that channel. For example, to acquire an image in Channel 2 for every other time point, select or enter **2** in the box provided.

6) Click OK.

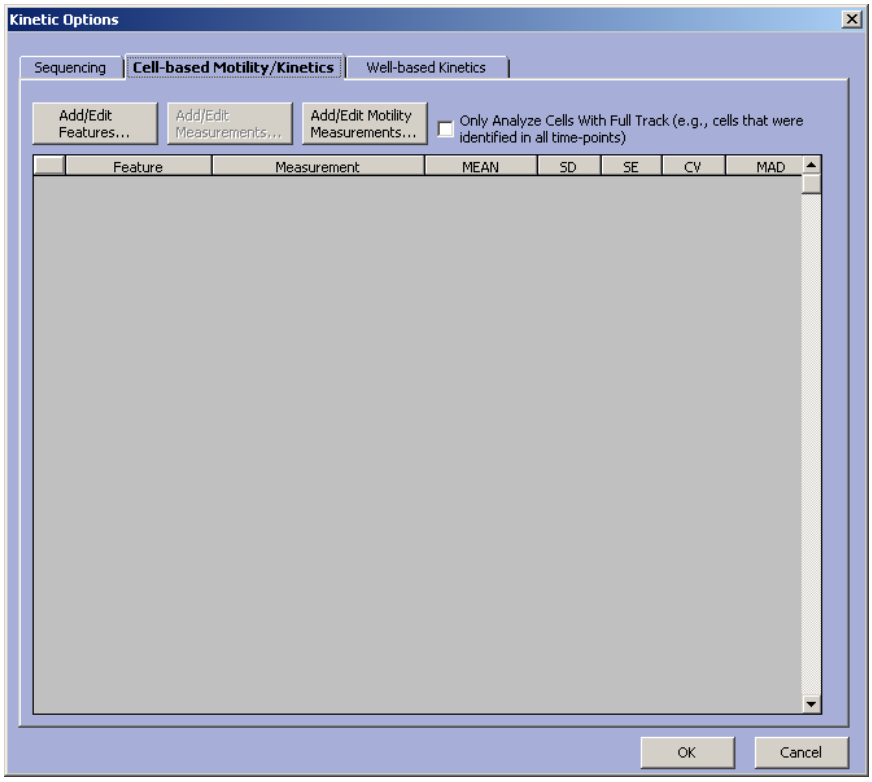
Cell-based Motility and Kinetics Options

To specify Cell-Based Motility and Kinetic settings,

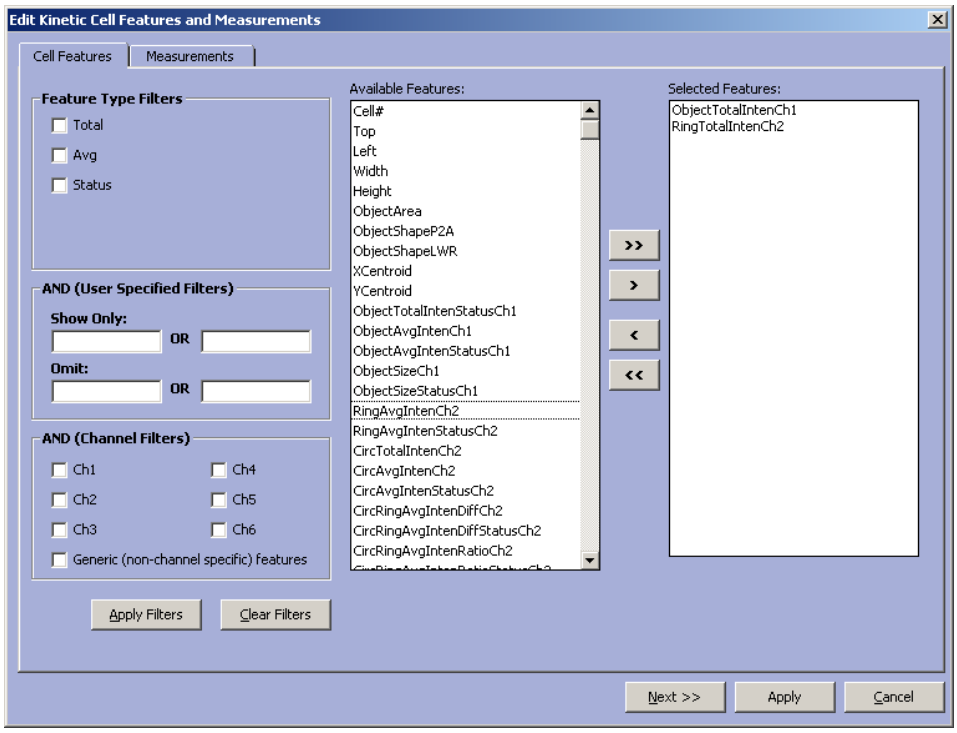
NOTE

Field based assays do not support the calculation of motility features.

1) Click the **Cell-based Motility/Kinetics** tab as shown in the following figure.



2) To select the desired features and feature measurements, click the **Add/Edit Features** button. The Edit Kinetic Cell Features and Measurements wizard appears as shown in the following figure.



3) The goal is to list all desired features in the Selected Features list box. To do this, you can filter the Available Features list by choosing one or more of the following feature filter options:

Under **Feature Type Filters**, select one or more checkboxes. If you select **Mean**, for example, all features containing the word "Mean" will appear in the Available Feature list. If you select the **Mean** checkbox and the **SD_** checkbox, all features containing the word Mean **OR** SD_ will appear in the list.

Within the box labeled, **AND (User Specified Filters)**, you can filter the list even further by entering text in the **Show Only** box or the **Omit** box. If you enter text in the Show Only box, only those features containing the text that you entered will appear. For example, if you checked the **Mean** and **SD_** Feature Type Filters and then enter **TotalInten** in the Show Only box, all features containing the word (Mean **or** SD_) **AND** TotalInten will appear in the list. You can enter up to two Show Only text strings. Similarly, you can narrow the list by entering text in the Omit box. If you wish to eliminate all features containing a certain text string, enter that string in the Omit box. You can enter up to two Omit text strings.

Under the box labeled **AND (Channel Filters)**, you can filter the feature list even further based on channel number. For example, if you wish to display channel 1 features only, select the Ch1 option. If you wish to display Channel 1 or Channel 2 features in the list, select the CH1 and CH2 boxes. If you select the **Generic (non-channel specific) features** checkbox, all features that do not contain a channel number will appear in the list.

Click the **Apply Filters** button to view the filtered features in the Available (filtered) Features list. The filters are applied automatically each time any of the check boxes are selected. To apply the filters when text is entered in the Show Only or Omit text boxes, click the **Apply Filters** button. Use the **Clear Filters** button to clear all filters. When all filters are cleared, all the features will be shown in the Available Features list if they are not in the selected features box.

4) In the Available Features list, choose the features that you want to view.

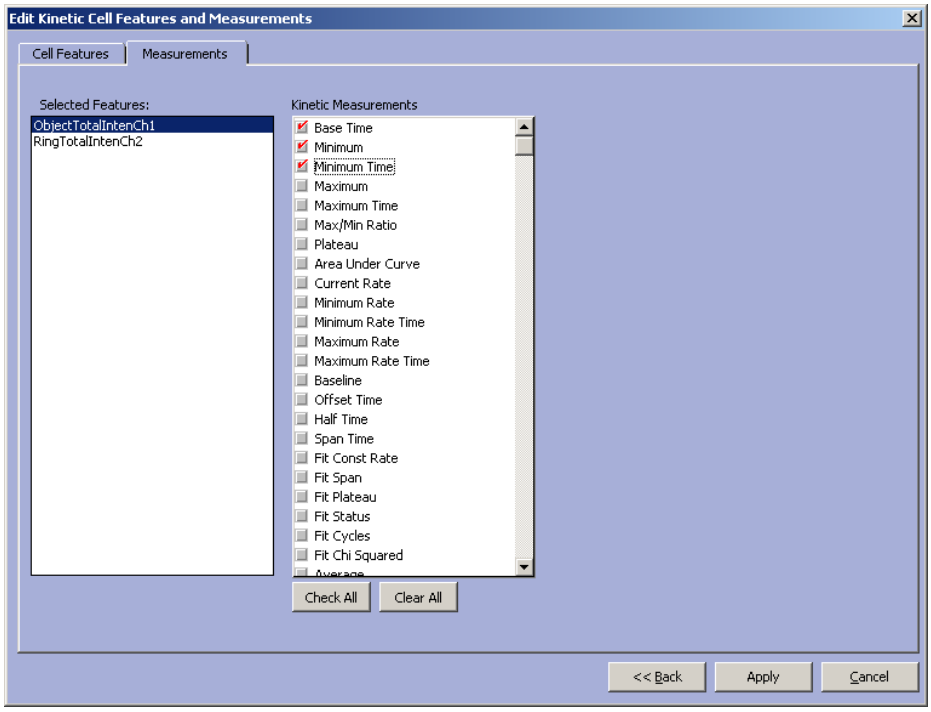
To add a feature to the display list: From the Available Features list, click on the feature that you want to display, and then click the **Add** button. The feature will appear in the Selected Features list.

To select all of the features for display: Click in the Available Features list, and then click the **Add All** button. All available features will appear in the Selected Features list.

To remove a feature from the display list: From the Selected Features list, click on the feature that you do not wish to display, and then click the **Remove** button.

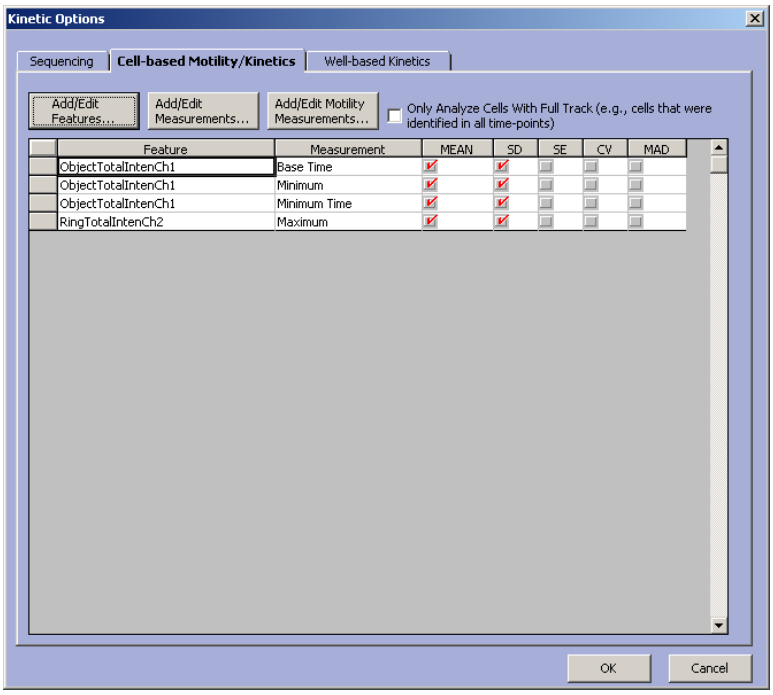
To remove all of the features from the display list: Click in the Selected Features list and then click the **Remove All** button.

5) Once you have displayed all desired features in the Select Features list box, click the **Next** button. The Measurements tab is now displayed as shown in the following figure.



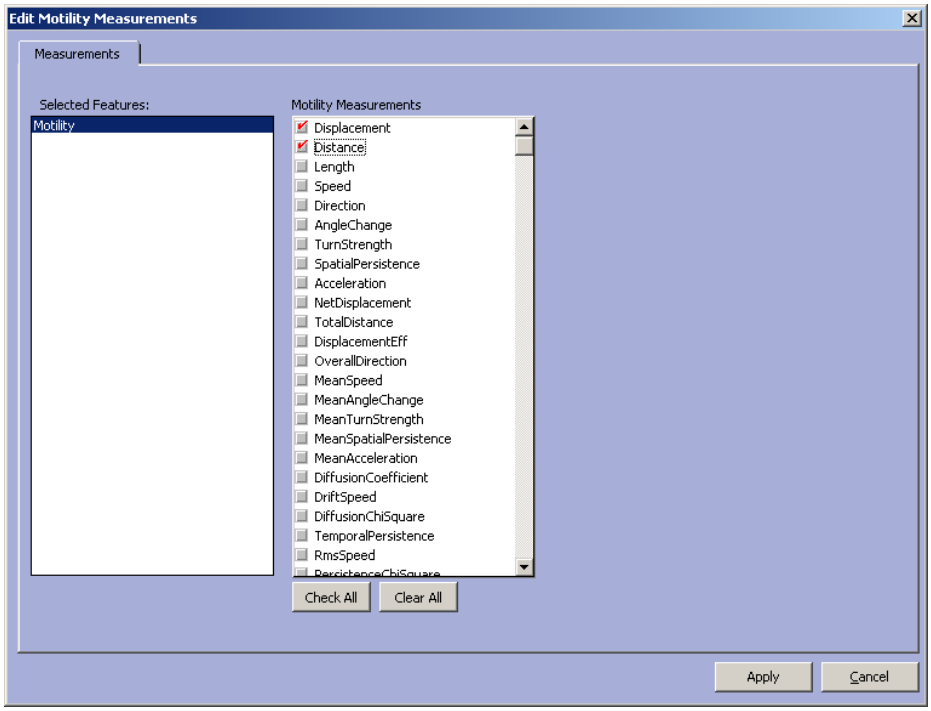
6) Note that the selected cell features appear in the Selected Features list. You can now select the desired kinetic measurements for EACH cell feature selected. Click on the desired cell feature in the Selected Features list. From the Kinetic Measurements list, select one or more measurements by clicking on them. Repeat this procedure for each cell feature. You may select multiple cell features at one time. When a measurement is checked, it will be applied to all highlighted cell features in the selected features box.

7) After you are finished selecting the desired measurements, click the **Apply** button. All features and measurements for each feature will appear in the Kinetic Options dialog box as shown below.



8) You can now choose which statistics you wish to calculate for each cell feature and measurement. By default, Mean and Standard Deviation are selected. To specify statistics, click on the desired Cell Feature and Measurement combination, then click on each desired statistic. A checkmark will appear next to each chosen statistic. To select all statistics for a particular cell feature, click on the row header. To select a particular statistic for every cell feature, click on the desired column header.

9) To add motility measurements, click the **Add/Edit Motility Measurements** button. The Edit Motility Measurements dialog box appears as shown in the following figure.



10) Motility will appear in the Selected Features list. You can now select the desired motility measurements. From the Motility Measurements list, select one or more measurements by clicking on them.

11) After you are finished selecting the desired measurements, click the **Apply** button. All motility measurements will appear in the Kinetic Options dialog box. Select the desired statistics as described previously.

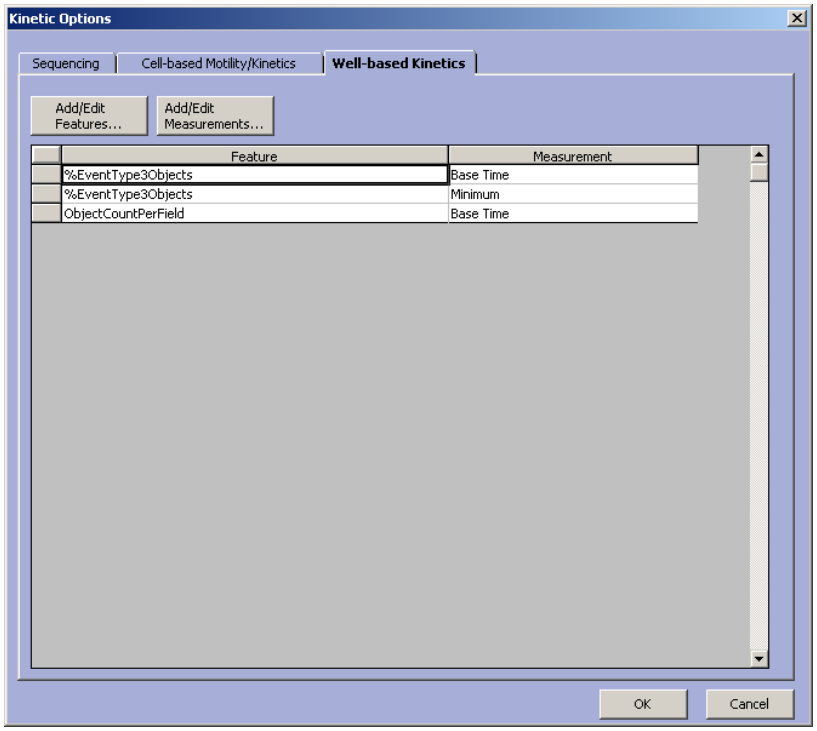
12) If you wish to produce motility features only for cells that existed on every time point of the experiment, check the box labeled, **Only Analyze Cells with Full Track**.

13) Click OK.

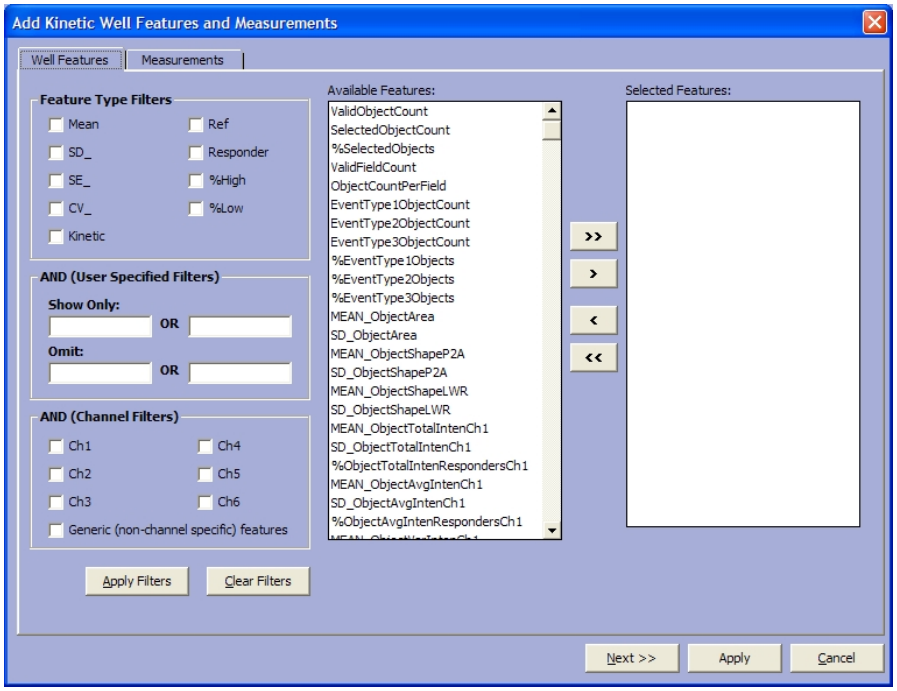
Well-based Kinetics Options

To specify Well-Based Kinetic settings,

1) Click the Well-based Kinetics tab as shown in the following figure.



2) To select the desired features and feature measurements, click the **Add/Edit Features** button. The Add Kinetic Features and Measurements dialog box appears as shown in the following figure.



3) The goal is to list all desired features in the Selected Features to Add list box. To do this, you can filter the Available Features list by choosing one or more of the following feature filter options:

Under **Feature Type Filters**, select one or more checkboxes. If you select **Mean**, for example, all features containing the word "Mean" will appear in the Available Feature list. If you select the **Mean** checkbox and the **SD_** checkbox, all features containing the word Mean **OR** SD_ will appear in the list.

Within the box labeled, **AND (User Specified Filters)**, you can filter the list even further by entering text in the **Show Only** box or the **Omit** box. If you enter text in the Show Only box, only those features containing the text that you entered will appear. For example, if you checked the **Mean** and **SD_** Feature Type Filters and then enter **TotalInten** in the Show Only box, all features containing the word (Mean **or** SD_) **AND** TotalInten will appear in the list. You can enter up to two Show Only text strings. Similarly, you can narrow the list by entering text in the Omit box. If you wish to eliminate all features containing a certain text string, enter that string in the Omit box. You can enter up to two Omit text strings.

Under the box labeled **AND (Channel Filters)**, you can filter the feature list even further based on channel number. For example, if you wish to display channel 1 features only, select the Ch1 option. If you wish to display Channel 1 or Channel 2 features in the list, select the CH1 and CH2 boxes. If you select the **Generic (non-channel specific) features** checkbox, all features that do not contain a channel number will appear in the list.

Click the **Apply Filters** button to view the filtered features in the Available (filtered) Features list. The filters are applied automatically each time any of the check boxes are selected. To apply the filters when text is entered in the Show Only or Omit text boxes, click the **Apply Filters** button. Use the **Clear Filters** button to clear all filters. When all filters are cleared, all the features will be shown in the Available Features list.

4) In the Available Features list, choose the features that you want to view.

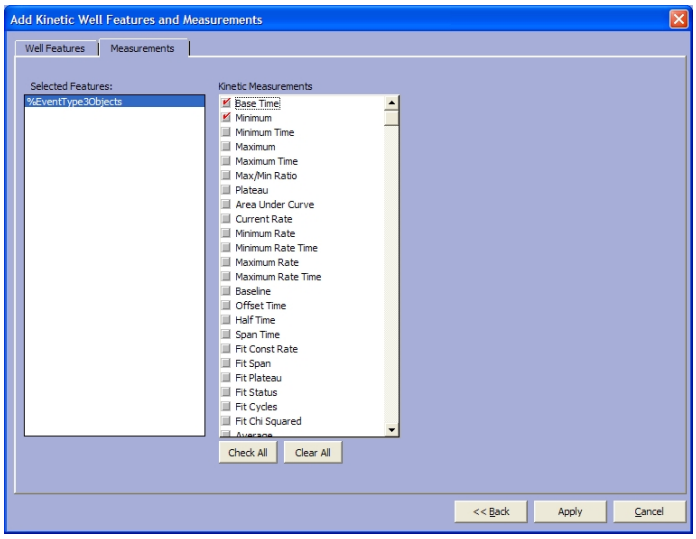
To add a feature to the display list: From the Available Features list, click on the feature that you want to display, and then click the **Add** button. The feature will appear in the Selected Features list.

To select all of the features for display: Click in the Available Features list, and then click the **Add All** button. All available features will appear in the Selected Features list.

To remove a feature from the display list: From the Selected Features to Add list, click on the feature that you do not wish to display, and then click the **Remove** button.

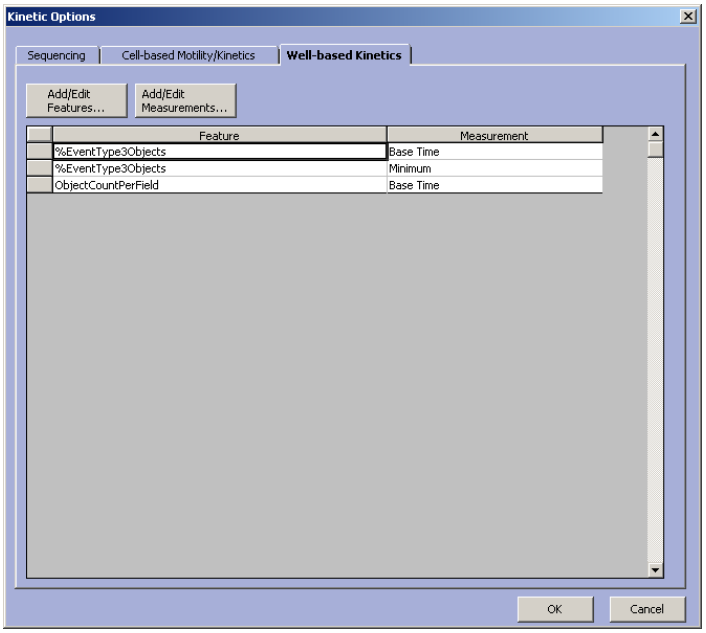
To remove all of the features from the display list: Click in the Selected Features to Add list and then click the **Remove All** button.

5) Once you have displayed all desired features in the Selected Features to Add list box, click the **Select Measurements** button. The Measurements tab is now displayed as shown in the following figure.



6) Note that your selected well features appear in the Selected Features list. You can now select the desired kinetic measurements for EACH well feature selected. Click on the desired well feature in the Selected Features list. From the Kinetic Measurements list, select one or more measurements by clicking on them. Repeat this procedure for each well feature.

7) After you are finished selecting the desired measurements, click the **Apply** button. All features and measurements for each feature will appear in the Kinetic Options dialog box as shown below.



8) Click OK.

Special Considerations When Using the Kinetics Feature

Please note the following special considerations when using the Kinetics feature:

When working in the Protocol Create View:

- If you change the BioApplication setting (from the Assay Algorithm drop down menu), and the Kinetics box is checked, the following will occur:
  - A message box will appear informing you of any kinetic features that will be removed automatically as a result of the BioApplication change.
  - If you select a field-based BioApplication and you had selected Motility features, a message box will appear informing you that the Motility features will be removed automatically.
- If you change the number of Channels and the Kinetics box is checked, the following will occur:
  - If you remove a channel and you had chosen to acquire that channel on every Nth time point, the setting is ignored without warning.
- Note the following stopping criteria (please refer to the section titled **Specifying Scan Limits** for more information on stopping criteria and definitions of sparse wells and sparse plates):
  - Sparse stopping criteria is applied to the first time point only
  - If the well is sparse at time point 0, the system will automatically skip the time series.
  - If the plate is sparse, the system will automatically skip the remaining wells.

When working in the Scan Plate View on an ArrayScan Instrument:

- Reference Wells and AutoExpose Wells are performed once before the first time point.
- The ArrayScan software shows the fixed endpoint data for the last time point.
- The system performs cell tracking between each time point
- The system performs motility and kinetic feature calculations after each kinetic cycle is complete.

When Disk Scanning:

- When running a Disk Scan using data stored on a Cellomics Store server or data stored on disk with an MDB, please note the following:
  - If you select an Assay Protocol that uses the Kinetics feature, and you are running the Disk Scan on a kinetic plate, the time intervals from the acquired fields are used rather than the time intervals defined in the protocol.
  - If you select an Assay Protocol that uses the Kinetics features, and you are running the Disk Scan on a fixed endpoint plate, the Kinetics portion of the protocol is ignored.
- When running a Disk Scan using data stored on disk without an MDB:

- If you select an Assay Protocol that uses the Kinetics feature, and you are performing a Disk Scan on a kinetic plate, the time intervals from the acquired fields are not available, therefore, the time intervals defined in the protocol are used (not the number of images).

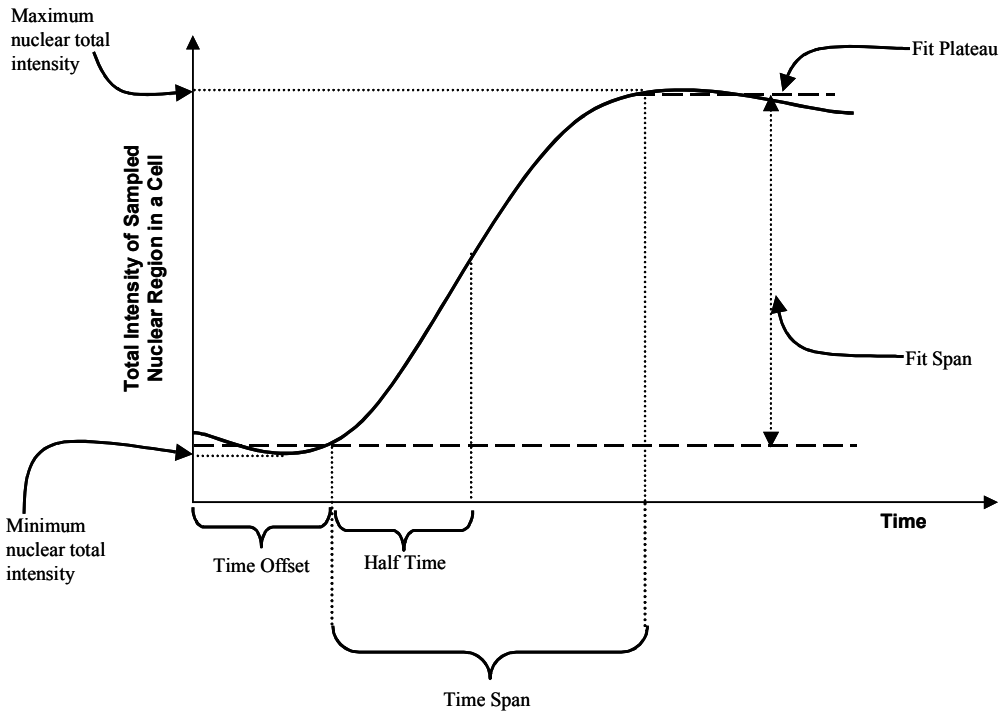
Kinetic and Motility Calculations

The following table provides a list and brief description of the various kinetic features that are computed by the ArrayScan software. The table also shows if a particular kinetic feature is determined using a linear approximation or by fitting to the exponential model.

Name	Description	Type	Direct Measurement		Exponential Fit	
			Cell	Well	Cell	Well
Max	Maximum of feature value during the experiment	Scalar	✓	✓		
Min	Minimum of feature value during the experiment		✓	✓		
MaxTime	Time it takes the feature value to reach its maximum		✓	✓		
MinTime	Time it takes the feature value to reach it's minimum		✓	✓		
MaxMinRatio	Maximum feature value divided by minimum feature value		✓	✓		
Plateau	Feature value at the last time point		✓	✓		
CurrentRate	Rate of feature value change; is computed using three point linear fit with the current time point always in the middle (for the last time point only two data points are used in the calculation)	Array	✓	✓		
MaxRate	Max change rate of feature (computed for at least 2 time points)	Scalar	✓	✓		
MinRate	Min change rate of feature (computed for at least 2 time points)		✓	✓		
MaxRateTime	Time it takes the feature rate value to reach its maximum		✓	✓		
MinRateTime	Time it takes the feature rate value to reach it's minimum		✓	✓		
TimeSpan	Time from the offset feature value to the max feature value (computed for a data set with at least 5 time points)		✓	✓		
AreaUnderCurve	Area formed by the feature curve and baseline in course of the experiment (computed for a data set with at least 5 time points)		✓	✓		
HalfTime	The time interval starting from offset during which feature value grows or declines half of the feature value span, max intensity – baseline, (computed for a data set with at least 5 time points)		✓	✓		
TimeOffset	Time it takes the dynamic process (translocation) to begin (computed for a data set with at least 5 time points)		✓	✓		
Baseline	Average feature value from initial time point to the time point of the offset (computed for a data set with at least 5 time points)		✓	✓		
FitConstRate	Determines the degree of exponential curve steepness (degree of change of feature value)				✓	✓
FitSpan	Difference between max feature value and its baseline value				✓	✓
FitPlateau	A value to which exponential function approaches asymptotically				✓	✓
FitStatus	Flag that indicates the type of exponential fit used (0 – growth, 1 - decline)				✓	✓
FitCycles	Number of computational cycles used to perform exponential fit				✓	✓
FitChiSquared	Measure of "goodness" of the exponential fit				✓	✓
BaseTime	Time at the initial (first) time point (beginning of the experiment)				✓	✓

Kinetics Output Features

One example of a simple biology to compute Kinetic Output Features is to measure cytoplasm-to-nucleus or nucleus-to-cytoplasm translocation of fluorescently tagged targets. The previous table lists all the Kinetic Features computed by the system, either directly from the data sequence at different time points or using a three point linear approximation or by fitting data to an exponential model. All of the Kinetic Output Features are computed on the feature that is selected in the Select Feature(s) to Calculate window in the Protocol Create View. The following three figures schematically show the different Kinetic features that are computed by the BioApplication. The first and third figures in this section represent a biology where the target of interest undergoes a cytoplasm-to-nucleus translocation, while the second figure represents a target that translocates from the nucleus to the cytoplasm. A few of the Kinetic features have been described in more than one of these figures to highlight the dependency of these features on the nature of the biology, that is cytoplasm-to-nucleus translocation or nucleus-to-cytoplasm translocation.



A schematic representation of the change in total fluorescent intensity over time in the nuclear region of a single cell due to the translocation of the target molecule into the nuclear region. The schematic also shows some of the kinetic features that are computed.

The exponential model used to fit the data can be described with the following equation:

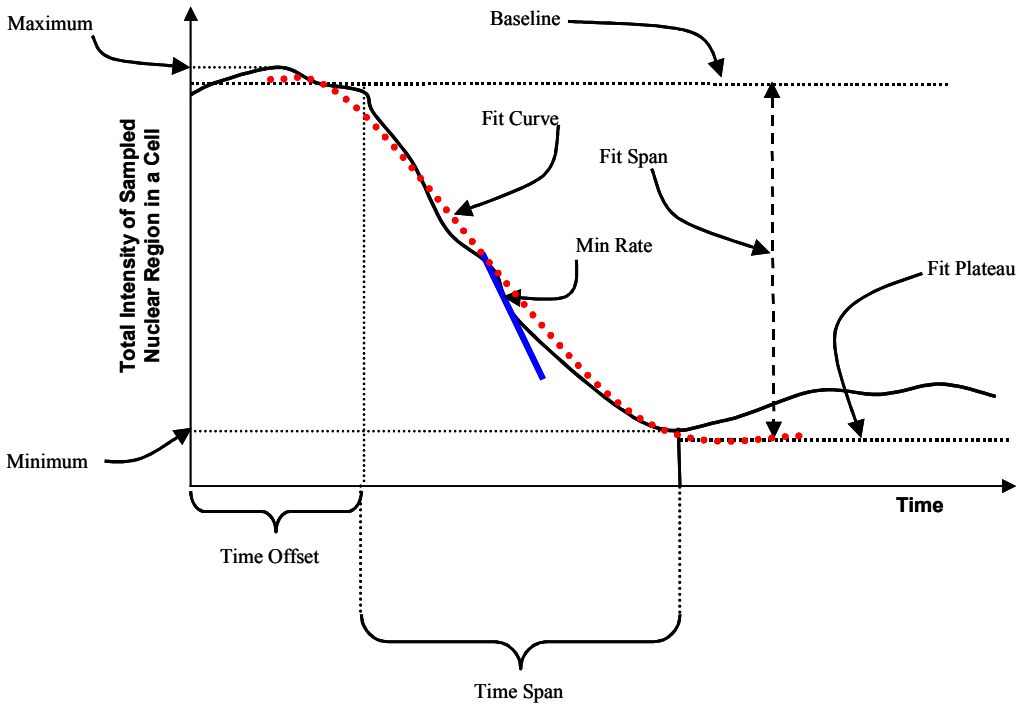
$y = B\text{-}A*e^{-K*T}$, where

y is the dependent variable; in this case the total intensity in the nuclear region

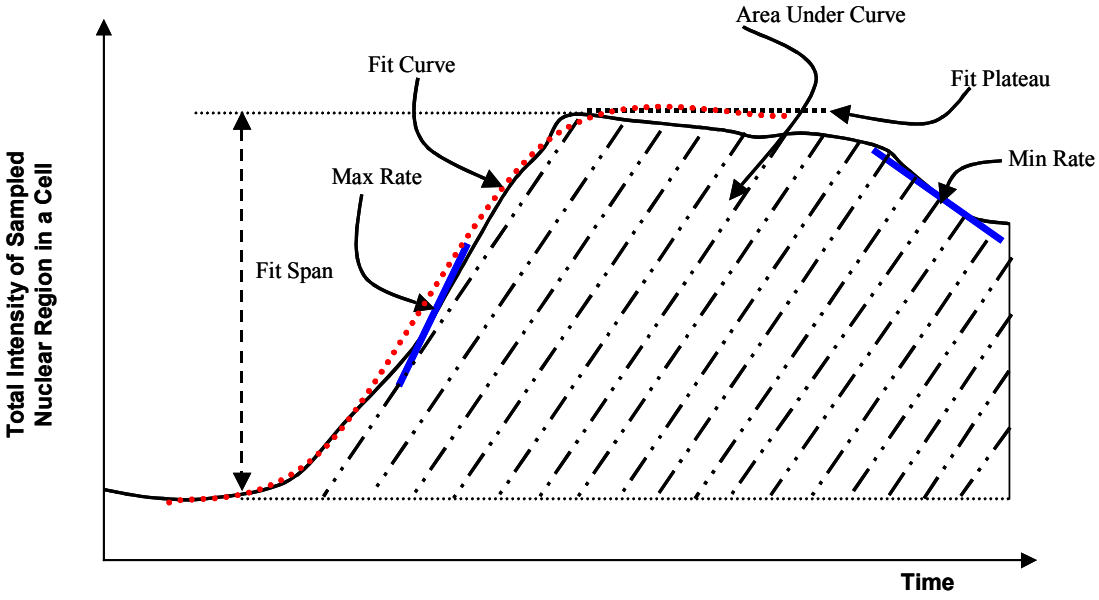
$T = t - t_{offset}$; t is current time and t_{offset} is time offset (see previous figure)

A is an intensity span (fit span, see previous figure)

B is an intensity plateau (fit plateau, see previous figure)



A schematic representation of a hypothetical target's nucleus-to-cytoplasm translocation, traced as total fluorescent nuclear intensity over time. Some of the key Kinetic Features computed by the Kinetic Molecular Translocation BioApplication are marked. The thick dotted line indicates the fit obtained for the data by using an exponential model.



A schematic representation of a hypothetical target's cytoplasm-to-nucleus translocation, traced as total fluorescent nuclear intensity over time. Some of the key Kinetic Features computed by the Kinetic Molecular Translocation BioApplication are marked. The thick dotted line indicates the fit obtained for the data by using an exponential model and the shaded area represents the area under the curve

Motility Calculations for Cell Features

Every kinetic assay performed on the ArrayScan HCS Reader can also have a cell motility experiment performed in parallel via the Motility feature. The Motility feature takes each cell's coordinates obtained from Cell Tracking and uses it to calculate and output cell motility parameters.

Classes of Motility Parameters

There are three classes of motility parameters:

1. Motion parameters at each time point Measurements reported at every time point of: (1) net displacement, (2) speed, (3) angle change, (4) turn strength, and (5) persistence**.**

2. Motion parameters (i.e., one number) that describe the whole time course These parameters are single numbers that describe the cell's motility over the entire time period. Key parameters are calculations over the entire time course, the mean-squared displacement in the direction of motion and perpendicular to the motion. These will give the overall diffusion coefficients and velocities for the entire time course of the cell's motion.

3. Cross-Correlation Parameters The different directed motion parameters at each time point can be cross-correlated with other assay parameters (e.g., Calcium response or internalized receptor intensity) to see if there is causality between the two events. This is first done at the cell-level and then can also be averaged and reported at the well-level.

The following sections describe the three different types of motility features and the calculations available for each type.

Cumulative

The cumulative features are computed for each time point of the trajectory. The values for the first time point are zero by definition.

Displacement(t_i)	Distance from time point 0 to time point t_i
Distance(t_i)	Distance along trajectory from time point 0 to time point t_i

Instantaneous

To smooth out the fluctuations in the cellular trajectories, multiple time points are combined into coarser steps. The step length is controlled by the Step Size parameter, which specifies the number of time points in each step. The time steps are non-overlapping, and residual time points at the end of a trajectory are ignored.

The instantaneous motility features are computed for each step *S* (i.e., a fraction of the time points in the trajectory).

Speed(s)	$\lvert \mathbf{S}_s \rvert$ /ΔTime(s)
Direction(s)	arctan(Δy(s)/Δx(s))
Length	Instantaneous length of movement from the previous time point

The change features are computed using backward differences between successive steps. The values of these features for the first step are defined as zero.

Acceleration(s)	(Speed(s) - Speed(s - 1))/ΔTime(s - 1)
Angle Change(s)	θ_s = |Direction(s) – Direction(s - 1)|
Turn Strength(s)	$\lvert \mathbf{S}_s \rvert \sin(\theta_s)$
Spatial Persistence(s)	$\lvert \mathbf{S}_s \rvert \cos(\theta_s)$

Overall

Overall features describe the entire trajectory. These are obtained from analysis of the cumulative and instantaneous results.

Net Displacement	Displacement(t_N)
Total Distance	Distance(t_N)
Displacement Efficiency	Net Displacement/Total Distance
Diffusion Coefficient *D*	Computed by fitting the displacement-time interval data to the model: $MSD = 4D\tau + v^2\tau^2$ where *MSD* = mean square displacement and τ is the time interval (The multiple use of time points within a trajectory to compute time intervals of different sizes assumes equally spaced time points.) The non-linear least squares method implemented in the Kinetics Module is used for the fitting.
Drift Speed *v*	
Diffusion Chi Square	
Temporal Persistence *P*	Computed by fitting the displacement-time interval data to the model: $MSD = 2S^2P[\tau - \exp(\tau/P)]$ where *MSD* = mean square displacement and τ is the time interval (The multiple use of time points within a trajectory to compute time intervals of different sizes assumes equally spaced time points.) The non-linear least squares method implemented in the Kinetics Module is used for the fitting.
RMS Speed *S*	
Persistence Chi Square	
Overall Direction	$\arctan([y(t_N) - y(t_1)]/[x(t_N) - x(t_1)])$
Mean Angle Change	〈 Angle Change(*s*) 〉
Mean Spatial Persistence	〈 Persistence(*s*) 〉
Mean Turn Strength	〈 Turn Strength(*s*) 〉
Mean Acceleration	Time-weighted average of Acceleration(*s*) (No assumption of equally spaced time points.)
Mean Speed	Time-weighted average of Speed(s) (No assumption of equally spaced time points.)

NOTE

Field based assays do not support the calculation of motility features.

Chapter

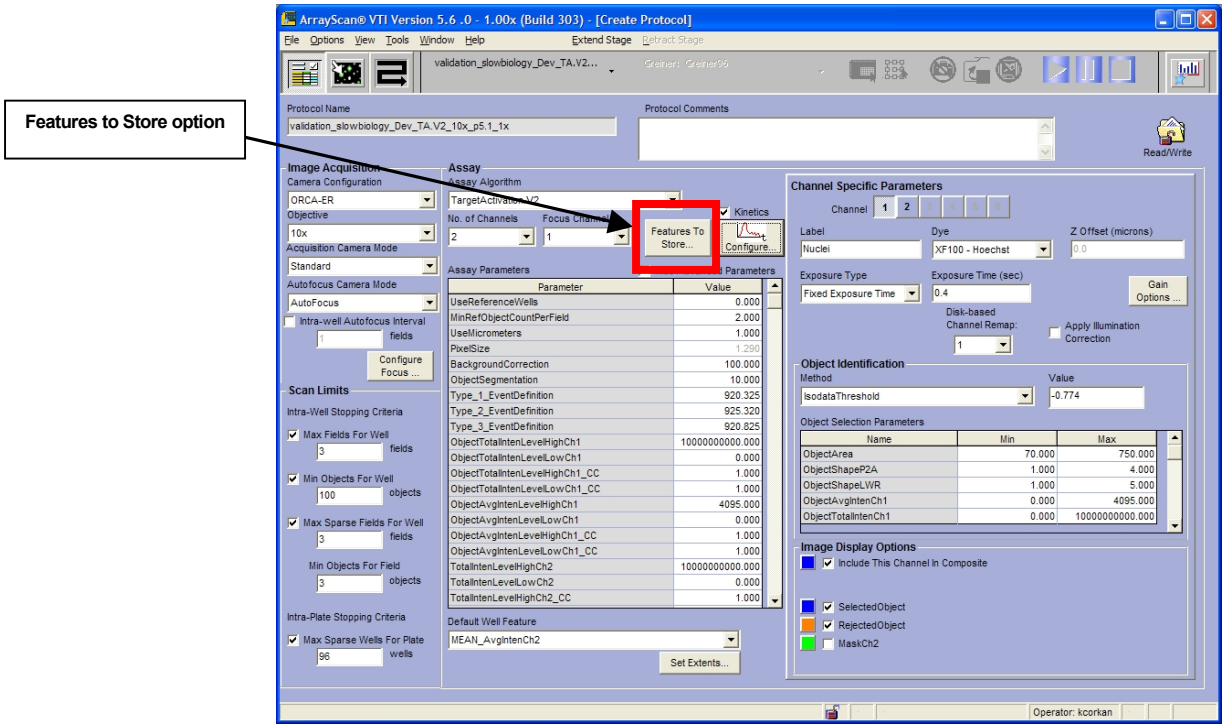
3

Selecting Features for Storage

WARNING

Use this product only in the manner described in this guide. When used other than as specified, the safety protections may be impaired. Please refer to the **ArrayScan V^{TI} HCS Reader User's Guide** for safety precautions ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please read the instructions, warnings, and precautionary measures supplied in this guide and with accessories. Failure to adhere to safety precautions and/or procedures may result in system failure, personal injury, or death. Cellomics, Inc. shall not be held liable under any circumstances. Cellomics does not assume any liability for damages or malfunctions caused by faulty operation, negligence, unauthorized modifications or repairs, or use of unauthorized accessories.

The Features to Store button provides you with the ability to choose which Cell, Field, and Well Features you wish to store to the database when scanning.



NOTE

If you choose not to store a feature, this feature will not be available in the data analysis.

Choosing the Features to Store

The following procedure describes how to choose the features you wish to store to the database.

To choose the features that you want to store,

1) Click the **Features to Store** button. The Select Features to Store dialog box appears as shown in the following figure.

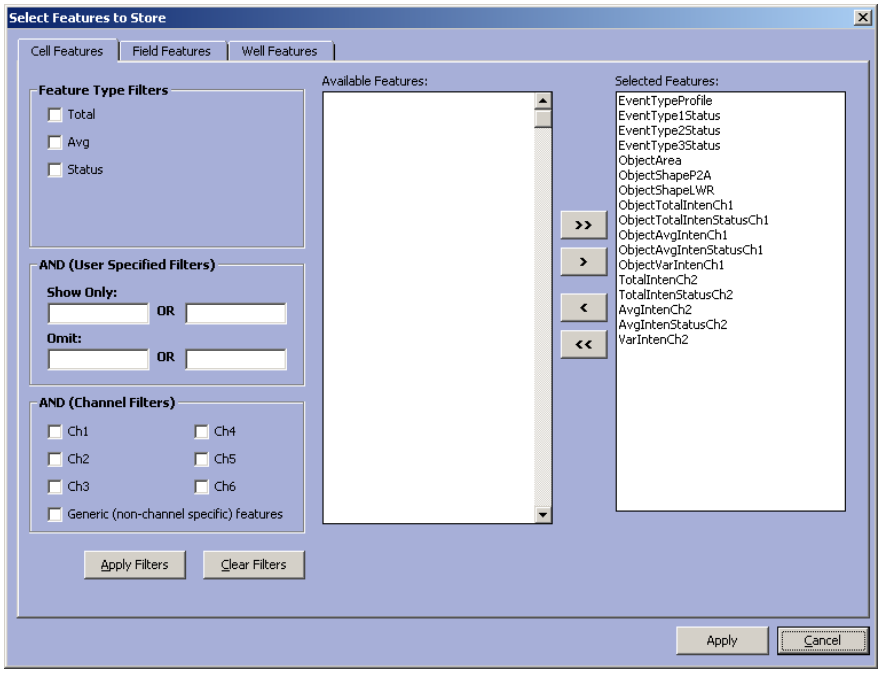


Figure X.X Select Features to Store dialog box

2) Click the desired tab: Cell Features, Field Features, or Plate Features.

3) All features appearing in the Selected Features box will be stored. By default, all features are selected. If you wish to store a subset of these features, you can filter the feature list to choose the desired features. To begin, click in the Selected Features list and then click the **Remove All** button 

.

To filter the Available Features list, choose one or more of the following feature filter options:

Under **Feature Type Filters**, select one or more checkboxes. If you select **Mean**, for example, all features containing the word "Mean" will appear in the Available Feature list. If you select the **Mean** checkbox and the **SD_** checkbox, all features containing the word Mean **OR** SD_ will appear in the list.

Within the box labeled, **AND (User Specified Filters)**, you can filter the list even further by entering text in the **Show Only** box or the **Omit** box. If you enter text in the Show Only box, only those features containing the text that you entered will appear. For example, if you checked the **Mean** and **SD_** Feature Type Filters and then enter **TotalInten** in the Show Only box, all features containing the word (Mean **or** SD_) **AND**

TotalInten will appear in the list. You can enter up to two Show Only text strings. Similarly, you can narrow the list by entering text in the Omit box. If you wish to eliminate all features containing a certain text string, enter that string in the Omit box. You can enter up to two Omit text strings.

Under the box labeled **AND (Channel Filters)**, you can filter the feature list even further based on channel number. For example, if you wish to display channel 1 features only, select the Ch1 option. If you wish to display Channel 1 or Channel 2 features in the list, select the CH1 and CH2 boxes. If you select the **Generic (non-channel specific) features** checkbox, all features that do not contain a channel number will appear in the list.

Click the **Apply Filters** button to view the filtered features in the Available (filtered) Features list. The filters are applied automatically each time any of the check boxes are selected. To apply the filters when text is entered in the Show Only or Omit text boxes, click the **Apply Filters** button. Use the **Clear Filters** button to clear all filters. When all filters are cleared, all the features will be shown in the Available Features list.

4) In the Available Features list, choose the features that you want to view.

To add a feature to the display list: From the Available Features list, click on the feature that you want to display, and then click the **Add** button. The feature will appear in the Selected Features list.

To select all of the features for display: Click in the Available Features list, and then click the **Add All** button. All available features will appear in the Selected Features list.

To remove a feature from the display list: From the Selected Features list, click on the feature that you do not wish to display, and then click the **Remove** button.

To remove all of the features from the display list: Click in the Selected Features list and then click the **Remove All** button.

5) If you wish to choose any other features, Cell Features, Field Features, or Plate Features, click on the desired tab and repeat the procedure above.

6) Click the **Apply** button.

Chapter

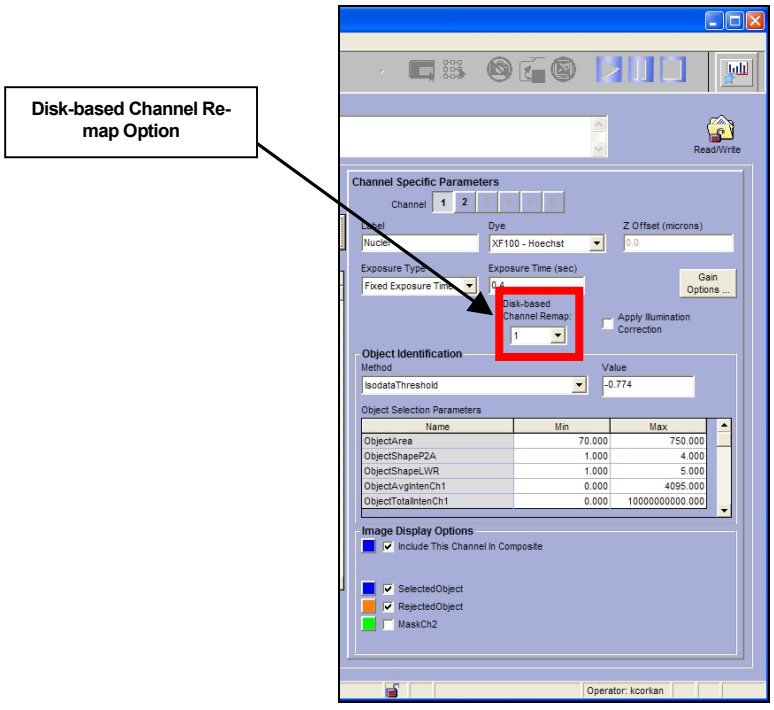
4

Disk Scan Channel Re-mapping

WARNING

Use this product only in the manner described in this guide. When used other than as specified, the safety protections may be impaired. Please refer to the **ArrayScan V^{TI} HCS Reader User's Guide** for safety precautions ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please read the instructions, warnings, and precautionary measures supplied in this guide and with accessories. Failure to adhere to safety precautions and/or procedures may result in system failure, personal injury, or death. Cellomics, Inc. shall not be held liable under any circumstances. Cellomics does not assume any liability for damages or malfunctions caused by faulty operation, negligence, unauthorized modifications or repairs, or use of unauthorized accessories.

The Disk Scan Channel Re-map option allows you to acquire images from a certain channel but then reprocess the images via Disk Scanning using a different channel.



Re-mapping Channels

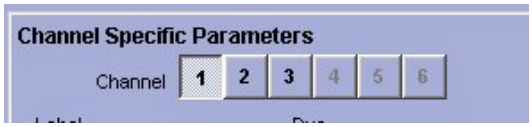
When performing a Disk Scan, the Channel Re-mapping option is available. This option allows you to acquire images from a certain channel but then reprocess the same images using a different channel.

For example, suppose you collected images using your ArrayScan instrument. In Channel 1, you collected nuclear images using the Hoechst/DAPI filter and in Channel 2 you collected an image of your whole cell using the FITC filter. Initially, you wanted to use your nuclei as the primary object and your whole cell stain as your secondary target. After reviewing the data, you decide that you want to rescan the same images but you would now like to use the whole cell as the primary object in Channel 1 and the nuclei as the secondary target in Channel 2. You can use the Channel Re-map option to accomplish this.

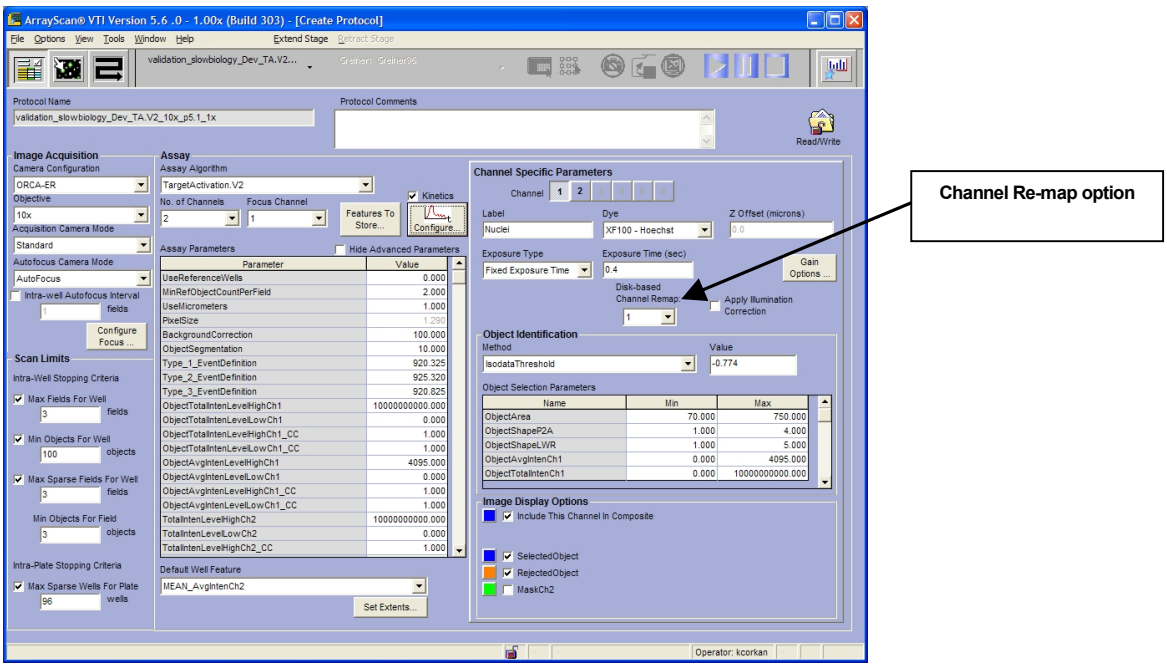
Alternatively, suppose you decide that you only need a one channel assay to assess the object area based on the whole cell image. You can create a one channel protocol and then use the Channel Re-mapping option to analyze the images collected in Channel 2 as the only images to be analyzed. Another reason to use the Channel Re-mapping option is when you want to use the same image in multiple channels without having to re-acquire the same channel image more than once.

To re-map channels,

1) Select the original channel number from the **Channel** number boxes (see below).



2) From the **Channel Remap** drop down list, select the new Channel number.



3) Optimize the Assay Protocol using the Protocol Interactive view.

4) Perform the Disk Scan in the Scan Plate view.

The channel re-mapping is saved for each individual Assay Protocol.

Chapter

5

Importing non-Cellomics Images

WARNING

Use this product only in the manner described in this guide. When used other than as specified, the safety protections may be impaired. Please refer to the **ArrayScan V^{TI} HCS Reader User's Guide** for safety precautions ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please read the instructions, warnings, and precautionary measures supplied in this guide and with accessories. Failure to adhere to safety precautions and/or procedures may result in system failure, personal injury, or death. Cellomics, Inc. shall not be held liable under any circumstances. Cellomics does not assume any liability for damages or malfunctions caused by faulty operation, negligence, unauthorized modifications or repairs, or use of unauthorized accessories.

In addition to displaying images generated by Cellomics instruments, the ArrayScan and vHCS:Scan software can display and analyze images that were generated by non-Cellomics instruments. Therefore, if you are using an instrument other than a Cellomics instrument and you are able to generate images of a supported file type, you will be able to use these images in the ArrayScan and vHCS:Scan software. In addition to viewing the images, you can also run a Disk Scan of the images. Specific image file types and other considerations are described in the following sections.

NOTE

Please note that Cellomics BioApplications have not been validated for use with non-Cellomics images.

NOTE

If you are using the HCS Gateway product, you can access these non-Cellomics images and data without importing them. You can access them directly in the ArrayScan or vHCS Scan software.

Choosing non-Cellomics Images when Running the Disk Scan Wizard

To access non-Cellomics images when running the Disk Scan wizard,

1) When prompted to choose a location of the stored images, select the option, **In a Directory or CD-ROM.** Click the **Next** button. The following screen appears.

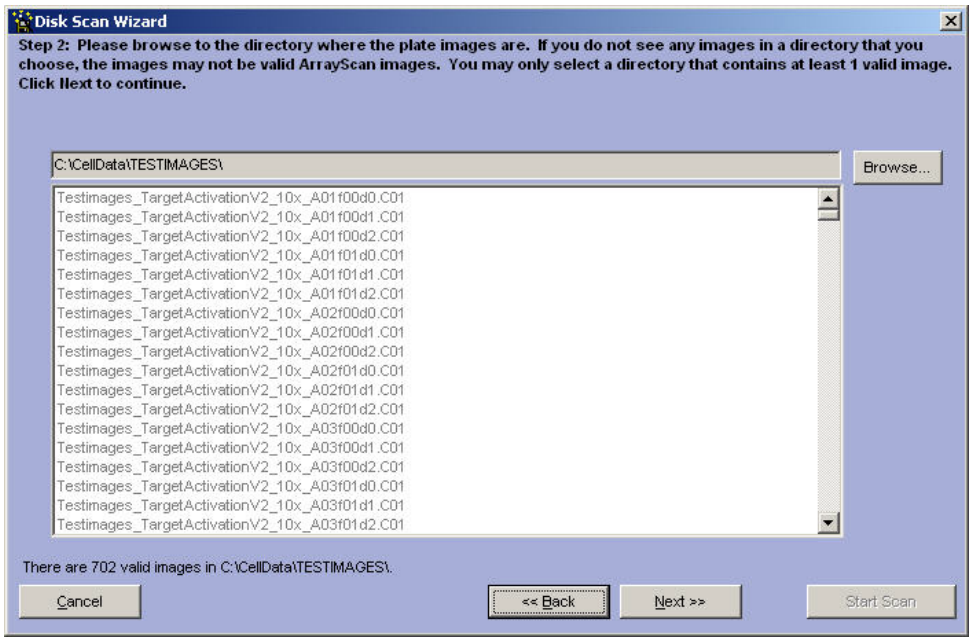


Figure 8.3 Disk Scan Wizard: Choose directory

2) Click the **Browse** button to select the drive and folder containing the non-Cellomics plate images. After choosing the folder and clicking OK, the valid image files appear in the Image Files list. In addition to the Cellomics .C01 and .DIB images, the following image file types are considered valid image files:
.TIF
.JPG
.BMP
.GIF
.PSD

Please refer to the following section titled, **Valid Image File Types**, for more detailed information regarding specific .TIF, .JPG, .BMP, .GIF, and .PSD file type support.

When running a Disk Scan of non-Cellomics images, the image file names must conform to the Cellomics standard naming convention, unless the images were spooled using the HCS Gateway software. Please refer to the section titled, **Working with Image Files Generated by non-Cellomics Instruments**, later in this chapter for more information.

3) Click the **Next** button.

Valid Image File Types

The following image file types can be read:

JPEG Support

- Lossy JFIF (JPEG File Interchange Format): YUV 4:4:4, 4:2:2, and 4:1:1 color spacing, and YUV 4:0:0 for grayscale are supported.
- Lossless JFIF (JPEG File Interchange Format): 8, 12, and 16-bit grayscale as well as 24-bit color spacing are supported.
- Both JPEG 4:4:4 and Lossless JPEG will be recognized.
- 12 and 16-bit grayscale images are supported.
- Progressive JPEG (JFIF format): YUV 4:4:4, 4:2:2, and 4:1:1 color spacing, as well as YUV 4:0:0 for grayscale are supported.
- Lossy JTIF (JPEG Tagged Interchange Format): YUV 4:4:4, 4:2:2, and 4:1:1 color spacing, as well as YUV 4:0:0 for grayscale are supported.
- Lossless JTIF (JPEG Tagged Interchange Format): 8, 12, and 16-bit grayscale as well as 24-bit color spacing are supported.
- Both TIFF JPEG 4:4:4 and Lossless JPEG will be recognized when getting file information.
- 12 and 16-bit grayscale images are supported.

GIF Support

- GIF (Graphics Interchange Format):The following bits per pixel can be read: 1, 2, 3, 4, 5, 6, 7, 8.

TIFF Support

- TIFF LZW (Tagged Image File Format with LZW compression): TIFF files that use RGB, CMYK, or YCbCr color space can be read. The following bits per pixel can be read, without regard for the color space: 1, 2, 3, 4, 5, 6, 7, 8, 16, 24, 32.
- TIFF without LZW: TIFF files that use RGB, CMYK, or YCbCr color space can be read. Files with PackBits compression can also be read. Files with Lossless JPEG compression for 8, 12, and 16-bit grayscale and 24-bit color spacing can be read. The following bits per pixel can be read, without regard for the color space: 1, 2, 3, 4, 5, 6, 7, 8, 16, 24, 32.
- 12-bit grayscale can also be read.

BMP Support

Windows Bitmap (BMP)

- The following bits per pixel can be read without RLE compression: 1, 4, 8, 16, 24, 32.
- The following bits per pixel can be read with RLE compression: 4, 8.

OS/2 Bitmap (OS/2 BMP)

- Both 1.x and 2.x formats are supported.
- The following bits per pixel can be read without RLE compression: 1, 4, 8, 24.
- The following bits per pixel can be read with RLE compression: 4, 8.

PSD Support

- Photoshop 3.0 (PSD): The following bits per pixel can be read: 1, 8, 24.

Working with Image Files Generated by Non-Cellomics Instruments

In order to run a Disk Scan of non-Cellomics images, the image file names must conform to the following Cellomics file naming convention, unless the images were spooled using the HCS Gateway software.

Image File Naming Convention for Fixed Endpoint Plates

<*UniquePlateID*>_<*WellName*>f<*iField*>d<*iDye*>.<*file extension*>

where:

<*UniquePlateID*> is an optional unique name that you may assign to the plate. Please note that in order to have more than one plate in any given directory, you must assign this unique name to the plate series. If you do not, one plate will appear in each directory. Be sure to include the underscore following the unique name. If you do not use the unique name, do not include the underscore. The unique Plate ID can be a maximum of 50 characters. These characters can be any combination of letters or numbers that uniquely identify the microplate.

<*WellName*> is the letter and number corresponding to the microplate row letter and column number of the well, A01 … Z99.

<*iField*> is the two digit field number, starting at field 0. For example: 00, 01. … Therefore, the first field is designated as f00. Field 2 is f01, and so on.

<*iDye*> is the single digit channel number, starting at channel 0. Valid entries are 0, 1, 2, 3, 4, 5 (maximum of six channels). Therefore, channel one is actually designated as d0. Channel 2 is d1, and so on.

Example File Names:

The following is an example of a plate name without the optional Unique Plate ID:

A01f01d2.TIF > This TIF image file is from well A1, field 2, channel 3)

The following is an example of a plate name using the optional Unique Plate ID:

020506platerun_A01f01d2.TIF

Image File Naming Convention for Kinetic Plates:

<*UniquePlateID*>i3t<*iTimePoint*><*WellName*>f<*iField*>d<*iDye*>.<*file extension*>

where:

<*UniquePlateID*> is an optional unique name that you may assign to the plate. Please note that in order to have more than one plate in any given directory, you must assign this unique name to the plate series. If you do not, one plate will appear in each directory. The unique Plate ID can be a maximum of 50 characters. These characters can be any combination of letters or numbers that uniquely identify the microplate.

<*iTimePoint*> is the three digit number corresponding to the time point, 001, 002, and so.

<*WellName*> is the letter and number corresponding to the microplate row letter and column number of the well, A01 … Z99.

<*iField*> is the two digit field number, starting at field 1. Therefore, the first field is designated as f01. Field 2 is f02, and so on.

<*iDye*> is the single digit channel number, starting at channel 1. Valid entries are 1, 2, 3, 4, 5, 6 (maximum of six channels). Therefore, channel one is designated as d1. Channel 2 is d2, and so on.

Example File Names:

The following is an example of a plate name without the optional Unique Plate ID:

i3t001A01f01d2.TIF > This TIF image file represents the first time point from well A1, field 1, channel 2)

The following is an example of a plate name using an optional Unique Plate ID, 020506platerun:

020506plateruni3t001A01f01d2.TIF

Recommendations for Working with Non-Cellomics Image Files

When viewing and running Disk Scans on non-Cellomics image files, please note the following information and recommendations:

- Confirm that the Assay Parameter, **UseMicrometers**, is set to 0 (turned OFF).
- When running a Disk Scan, rename the image files as described in the previous section.
- The Scan software supports grayscale images. Color images are projected into grayscale images.
- Independent of the pixel depth of the input image, the maximum pixel value will never exceed 4095 (12-bit), therefore larger pixel values are saturated to 4095.
- Choose the correct Acquisition Camera Mode setting based on the image file size. The following Acquisition Camera Mode settings are available (from the Create Protocol View):

  Autofocus: select this setting for image file sizes of 256 x 256 (for small images)

  Standard: select this setting for image file sizes of 512 x 512

  HiRes: select this setting for image file sizes of 1024 x 1024 or larger

  If image file sizes differ from the selected setting, the Scan software will do the following:

  If the image file size is smaller than the acquisition setting, the Scan software will pad the image using the minimum value in the image.

  If the image file size is larger than the acquisition setting, the Scan software will crop the image, keeping the central portion of the image.