GE Healthcare

IN Cell Analyzer

Spheroid finder

IN Cell Analyzer acquisition software includes ***Smart Scan*** tools that connect two or more imaging protocols to perform on a single plate. ***Spheroid Finder*** is a ***Smart Scan*** tool that can be used to automatically locate large 3D biology within the well. First, a low magnification scan is performed to identify round objects in one fluorescent channel, then fields where round objects are identified are re-imaged with a more complex acquisition protocol (i.e., higher magnification, multiple channels, 3D image mode, etc.).

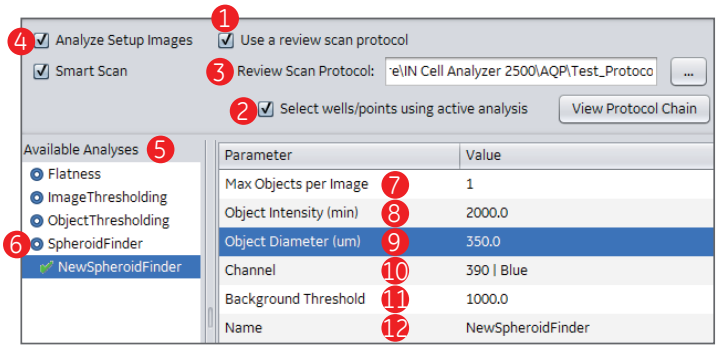
Design review scan protocol

1. Select the ***Start a new protocol*** icon to design the ***Review Scan Protocol***.
2. Using ***Preview Scan***, identify and navigate to a representative spheroid. Design your Review Scan Protocol ensuring that all parameters are optimized for the plate, sample, and experimental goals. For example, ensure the auto-focus settings and auto offsets determine a z-position that is in focus with the sample in every channel.
   Note: 3D acquisition, higher magnification and Laser Auto Focus (LAF) are recommended for this scan.
3. In the ***Fields*** card, select ***Point List*** in the ***Field Placement*** drop-down. Do not select any points.
4. Deselect all wells in ***Plate View***. Click ***Save***.

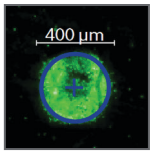
Design initial scan protocol

1. Select the ***Start a new protocol*** icon and select the lowest magnification objective available (2× recommended for 96 well plate) and set ***Binning*** to at least 2 × 2.
   Note: Whole well imaging is required for most reliable analysis. If more than one FOV must be used to image the whole well, large spheroids may span FOVs leading to missing objects, misidentification of objects, or identification of objects more than once.
2. Design the rest of the initial scan using the ***Dashboard***. Ensure ***Laser Autofocus*** is activated.
   Note: For ***Spheriod Finder***, the initial scan only requires 2D images in a single channel to identify objects.
3. In the ***Fields*** card, select a single FOV in the center of the well, and select wells with samples in ***Plate View***.

Set up spheroid finder analysis



1. In the ***Smart Scan*** card, check the ***Use a review scan protocol*** 1 and the ***Select wells/points using active analysis*** 2 boxes.
2. Click icon to browse to the ***Review Scan Protocol*** 3 saved in the "Design review scan protocol" section. Click ***Select Protocol***.
3. Check the ***Analyze Setup Images*** 4 box to determine appropriate analysis conditions.
   Note: When ***Analyze Setup Images*** 4 is selected, average intensity information is displayed below the image.
4. In the ***Available Analyses*** list 5, double-click ***Spheroid Finder*** 6.
5. Set ***Max Objects per Image*** 7 to the maximum number of spheroids to detect per FOV.
6. Navigate to a representative spheroid.
7. Hover over the spheroid to determine the average intensity. Enter a value slightly smaller this value as the ***Object Intensity*** 8.
8. Use the ***Scalebar*** tool to determine the minimum diameter spheroid to be identified in the analysis. Enter this value as the ***Object Diameter*** 9.
   Note: Double click on the scale bar to adjust length.



9. Select the ***Channel*** 10 for spheroid identification.
10. Hover around the spheroid to determine background intensity. Enter this value as the ***Background Threshold*** 11.
    Note: A minimum value of 100 is required.
11. Optionally, enter the analysis name 12.
12. Visit several wells and examine analysis parameters to verify that all objects of interest are being identified.
    Note: Failure to verify parameters can result in inaccurate identification (e.g., missing objects, misidentification of objects, identification of objects more than once).
13. If necessary, adjust analysis parameters and examine again. Click ***Save***.

Scan and analyze data

1. Click ***Scan***.
2. Click ***Browse*** to identify data location, select ***Naming method*** and enter a name indicating that it is the second scan for the ***Review Scan Folder***. Click ***Run***.
3. Examine data in ***Review*** mode.
   Note: The data displayed is from the first scan. To display data from the second scan, use the  icon to navigate to the second scan image stack.
4. To examine location and intensity of spheroids, navigate to the first scan data folder. The .csv file contains a row for each image where spheroids were found. See the table below for sample data.

		1		2		3
Image	i	j	X	Y	Score
D - 01(wv 542 - Orange).tif	3	184	11552.25	36440.5	18992.64
D - 01(wv 542 - Orange).tif	138	864	11991	38650.5	11309.09
D - 01(wv 542 - Orange).tif	761	111	14015.75	36203.25	14534.21
D - 02(wv 542 - Orange).tif	156	610	21049.5	37825	18252.7

- Columns i and j (1) denote pixel coordinates of the center of the spheroid
- Columns X and Y (2) denote stage coordinates of the center of the spheroid
- The score column (3) is the average intensity of the spheroid

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