MetaXpress® 6 Software Guide

Enabling Adaptive Acquisition™

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MOLECULAR DEVICES
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Chapter Purpose

The purpose of this chapter is to guide the user through enabling the **Adaptive Acquisition** feature in **Plate Acquisition Setup**.

When the **Adaptive Acquisition** feature is enabled, the system images the minimum number of sites to reach the user-defined target number of cells within each well. It is particularly useful for assays in which the cell seeding or distribution is uneven from well to well.

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Adaptive Acquisition Overview

When to use

- You need a minimum number of cells for statistical significance, but there is uneven cell seeding from well to well
  - Translocation (% positive measurement)
  - Cell cycle (% G1, % M, etc.)
- To detect rare events
- Rare GFP-positive cells (any present)

How it works

- Specify a minimum number of cells to be counted per well
- System acquires sites and counts cells until minimum cell count is reached
- System moves stage to the next well

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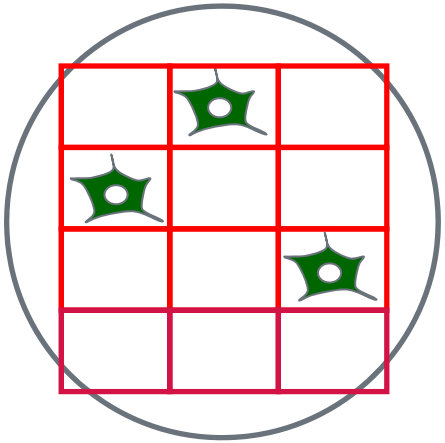
How Does Adaptive Acquisition Work?

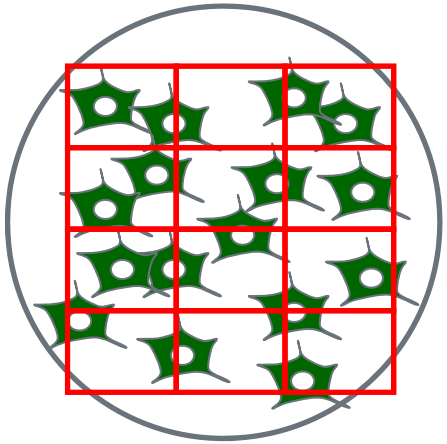
Without Adaptive Acquisition

- Target # of cells: 3
- Always acquire all 12 sites

12 Sites Acquired



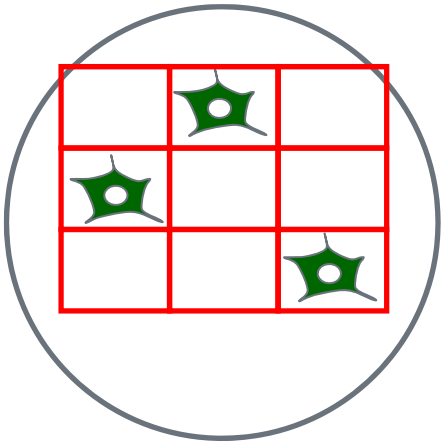
12 Sites Acquired



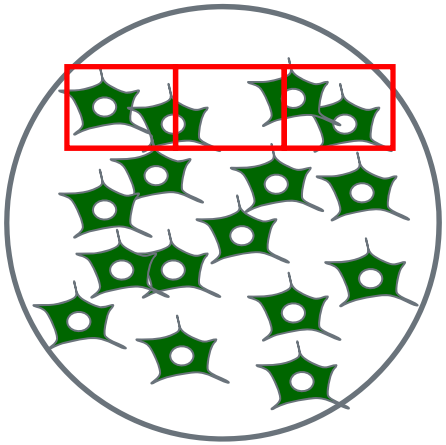
With Adaptive Acquisition

- Target # of cells: 3
- Acquire variable number of sites to reach target cell count

9 Sites Acquired



3 Sites Acquired



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Enabling Adaptive Acquisition

1. Open **Plate Acquisition Setup**

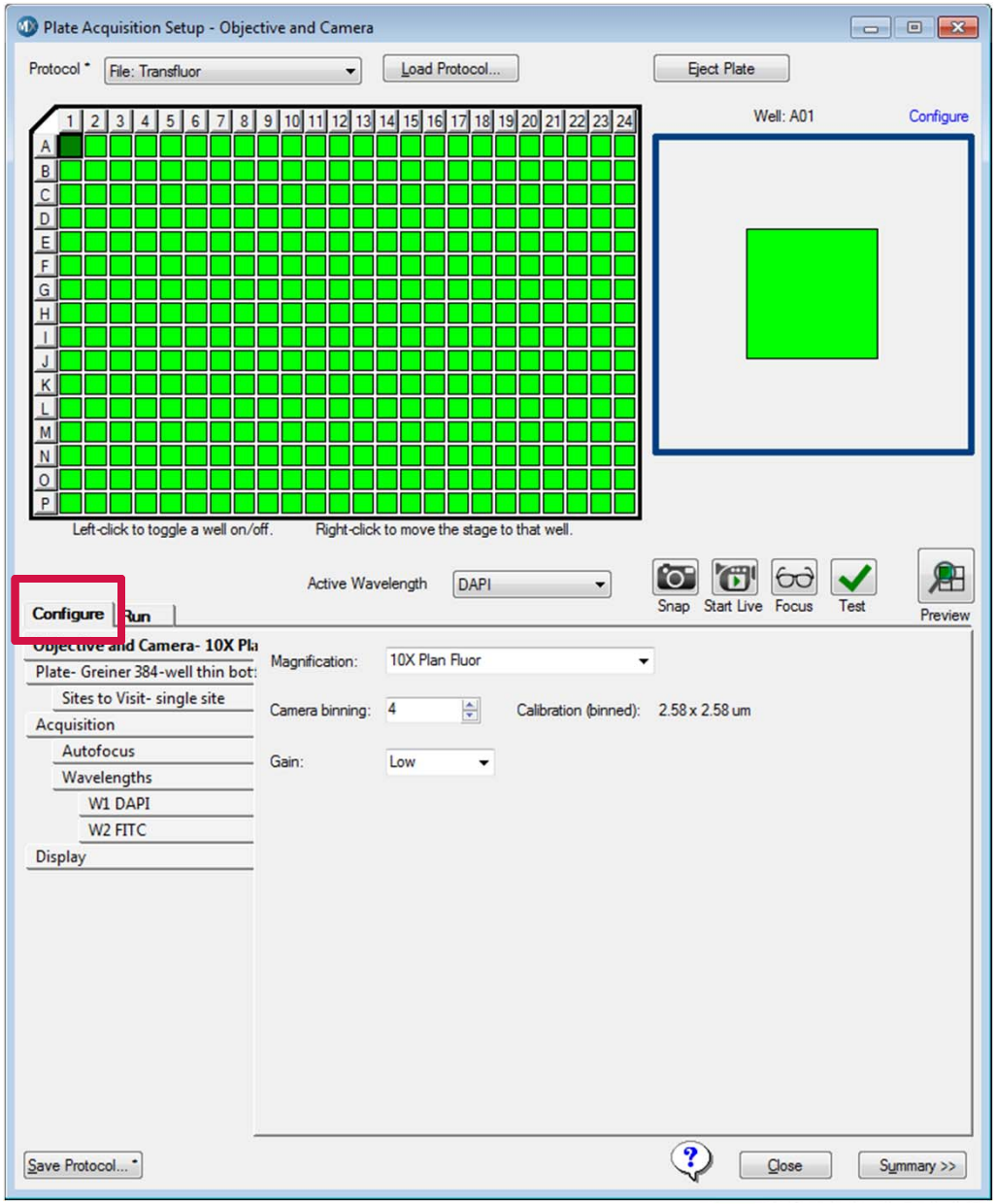
   - In the main toolbar click on

     OR

   - Under the **Screening** menu, select **Plate Acquisition Setup**

2. Select the **Configure** tab



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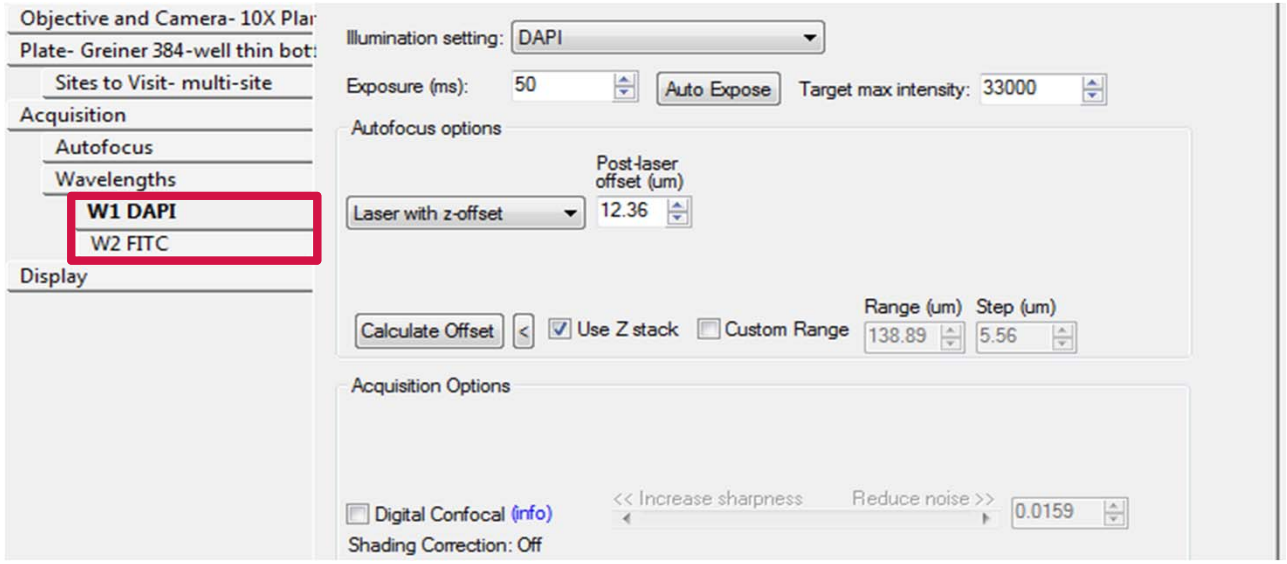
Enabling Adaptive Acquisition

3. Select the appropriate **W** tab (wavelength) for which you will use to count cells

4. Configure the wavelength settings as you would for acquisition
   i. Select correct **Illumination Setting** from the drop-down menu
   ii. Calculate **Focus offset**
   iii. Determine **Exposure time**

5. After you have optimized settings, right click on a site/well with representative cells as this this position will be used to test settings



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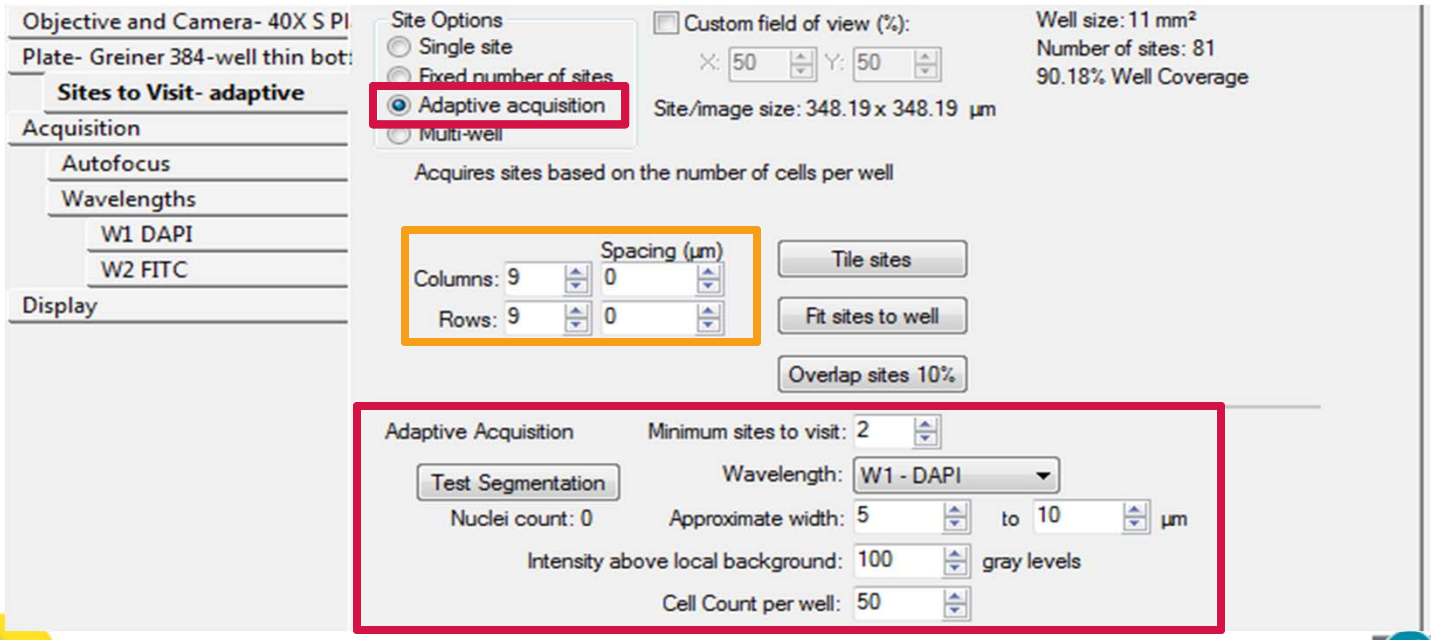
Enabling Adaptive Acquisition

5. Select the **Site to Visit** tab

6. Under the **Site Options** section, select **Adaptive Acquisition**
   - The Adaptive Acquisition section appears at the bottom of the dialog box

7. Select the appropriate (max) **number of sites** and **spacing**

NOTE The system will acquire at most the number of sites indicated in this section even if the cell count per well has not been reached



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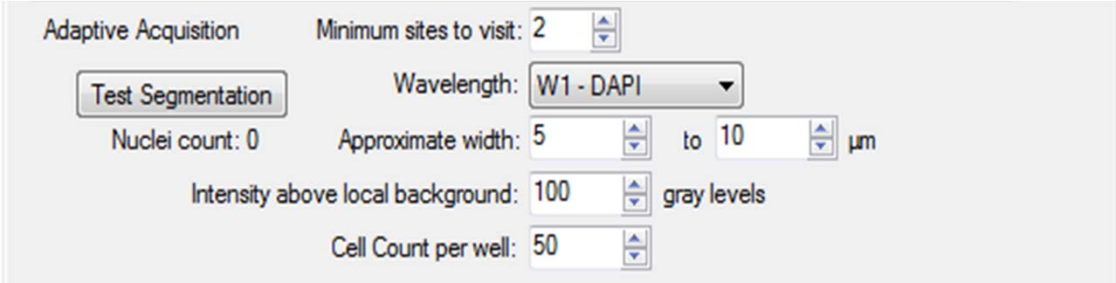
Configuring Adaptive Acquisition Settings

8. Enter the **Minimum number of sites to visit**: system will acquire at least this number of sites per well even if the target cell count has already been reached

9. Select the cell counting **Wavelength**: should be the first wavelength acquired and is often a nuclear marker

   NOTE: This must be the same wavelength chosen on the W1 tab

9. Enter the cell counting parameters (see next section for details):
   - **Approximate minimum** and **maximum width** of cells
   - **Itensity above local background** for the cell marker
   - Specify target **Cell Count per well**: the minimum number of cells desired by the user



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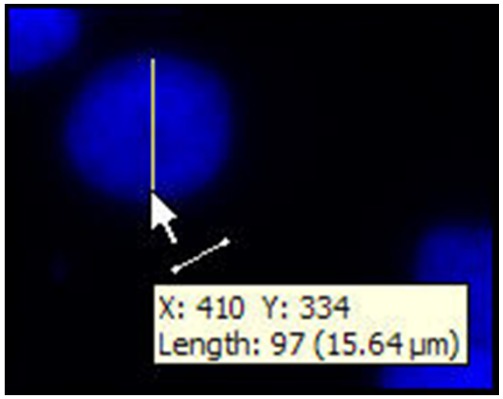
Determining Approximate Width Settings

Set the **Approximate min width** and **Approximate max width** for the range of cells (or nuclei) that you want to detect.

- Select the line region from the **Region Tools** toolbar



- Click and drag across a representative small and large cell; a tooltip will show the length of the line.

  NOTE Do not click the image again as this will cause the tooltip to disappear. If the tooltip disappears, repeat the drawing procedure as the lines will not affect any image settings.

- The width is the short axis of a cell (in µm)

- Much smaller or much larger cells will be ignored

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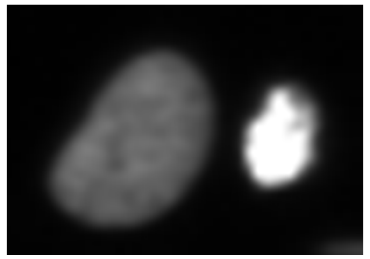
Effects of Varying Width Settings

Molecular Devices recommends only changing one parameter at a time in order to determine optimal settings.

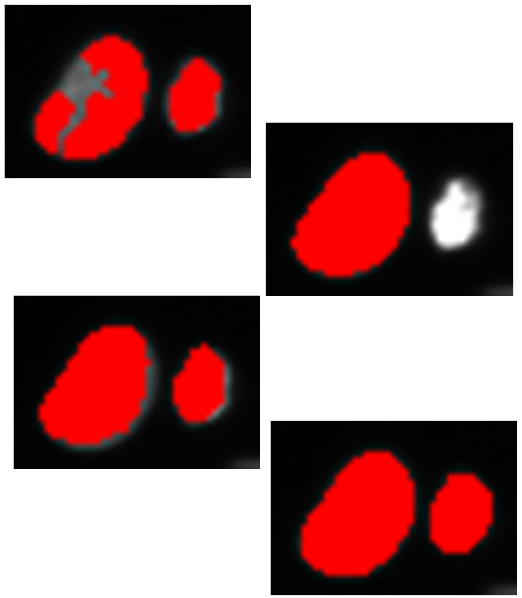


Min width too small: splits nuclei

Min width too large: omits smaller nuclei

Max width too small: may shrink nuclear boundaries

Max width too large: may slightly enlarge nuclear boundaries



NOTE The above suggestions are only meant to guidelines for starting values. You will need to test and optimize these values.

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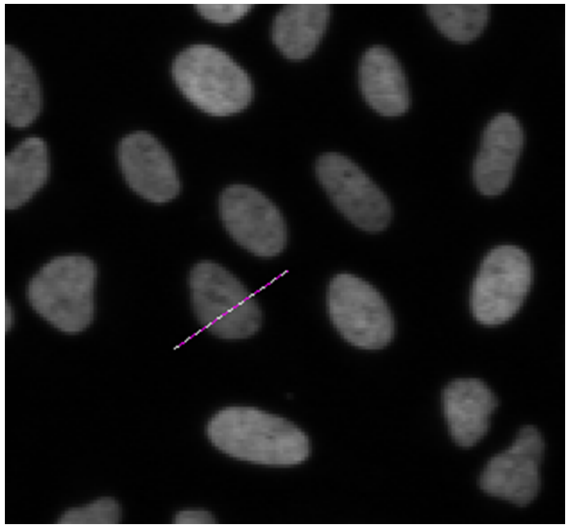
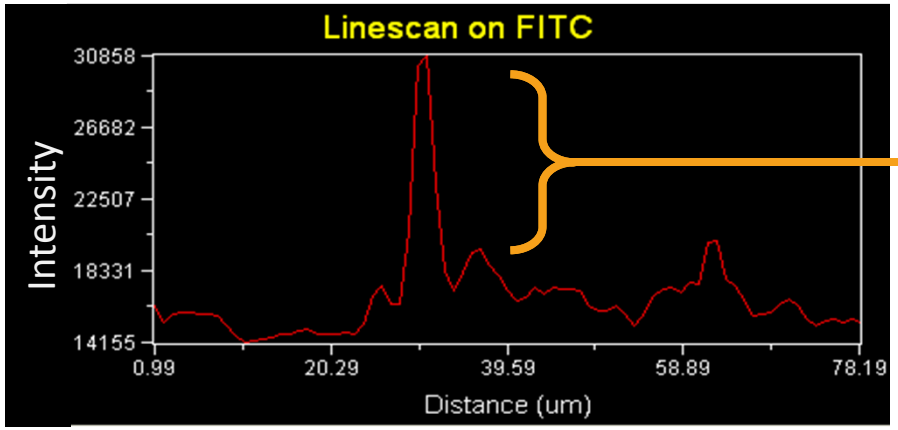
Determining Intensity Above Local Background

The Intensity above local background is used for finding the cells:

- This value should be set to the intensity difference between a dim cell and its local background
- Using the **Region** Tools, draw a line region through a cell as shown below
- In the main menu, select **Measure > Intensities > Linescan**
- Measure the intensity change on the y-axis, then divide by 4
- Enter this result in the **Intensity above local background** box





Use the difference in intensity values to set **Intensity above local background**

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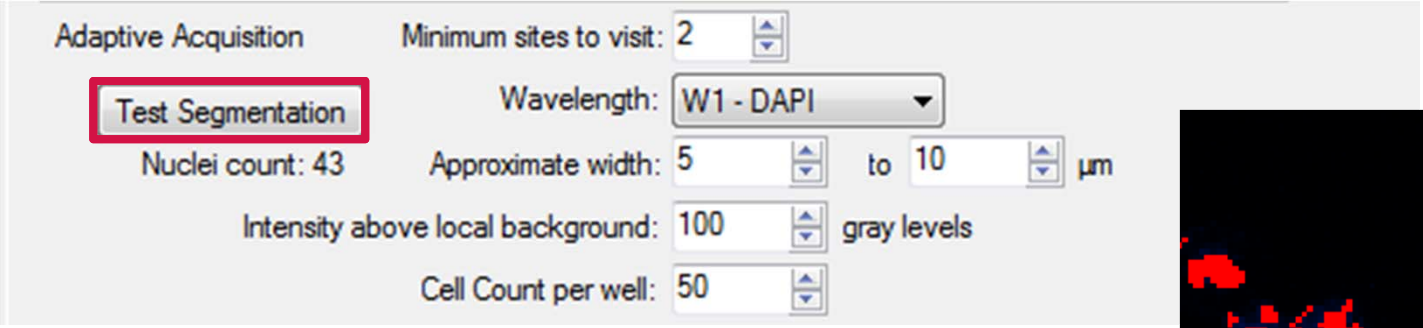
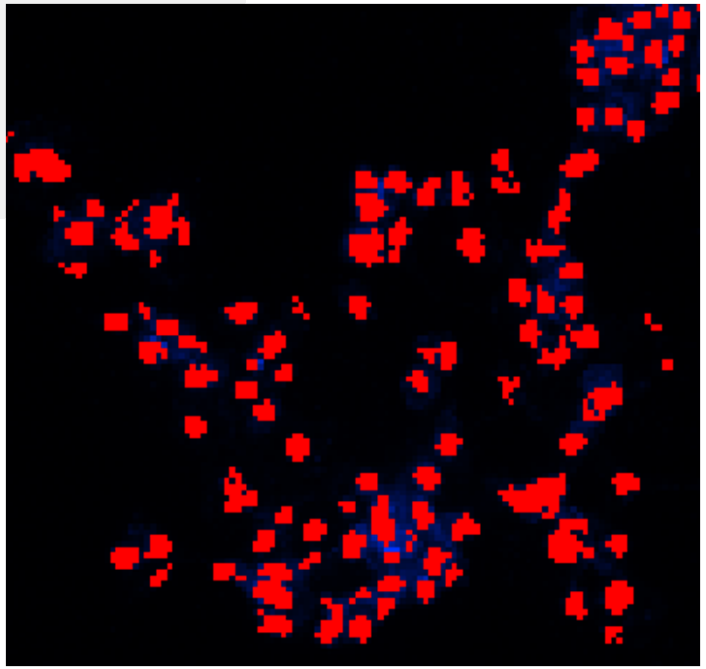
Test Adaptive Acquisition Settings





13. Click on the **Test Segmentation** button
    - An image of the selected wavelength will appear with a red segmentation overlay and the Nuclei (cell) count will be shown under the **Test Segmentation** button

14. If necessary, adjust the width and intensity settings to accurately find the cells

15. Continue to optimize settings for your plate as you would for normal acquisition and acquire your plate

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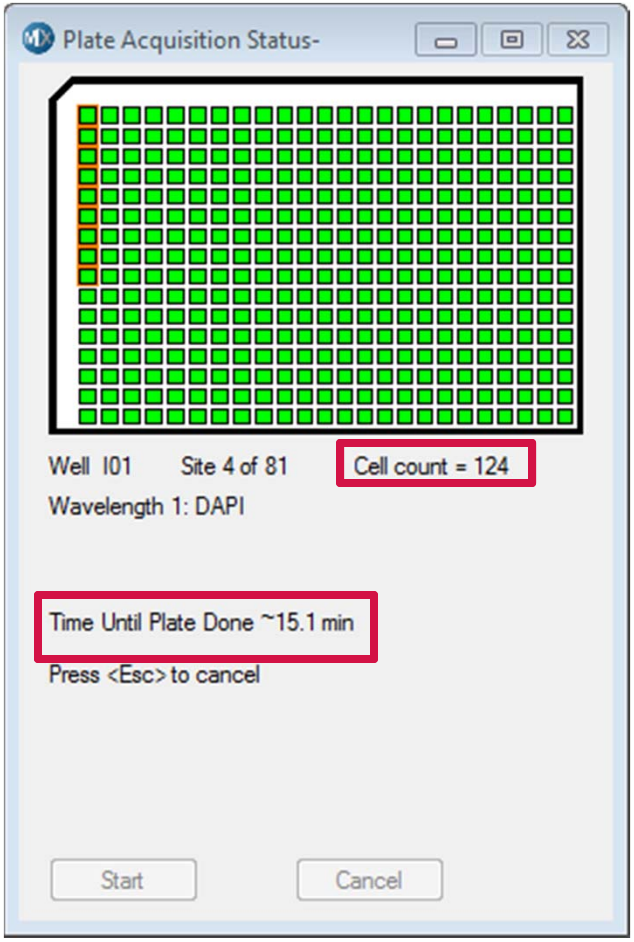
Acquiring with Adaptive Acquisition Enabled

- During plate acquisition, the Cell count will be shown on the **Plate Acquisition Status** dialog
- The **Time Until Plate Done** value should not be relied upon because it will vary considerably after each well is completed



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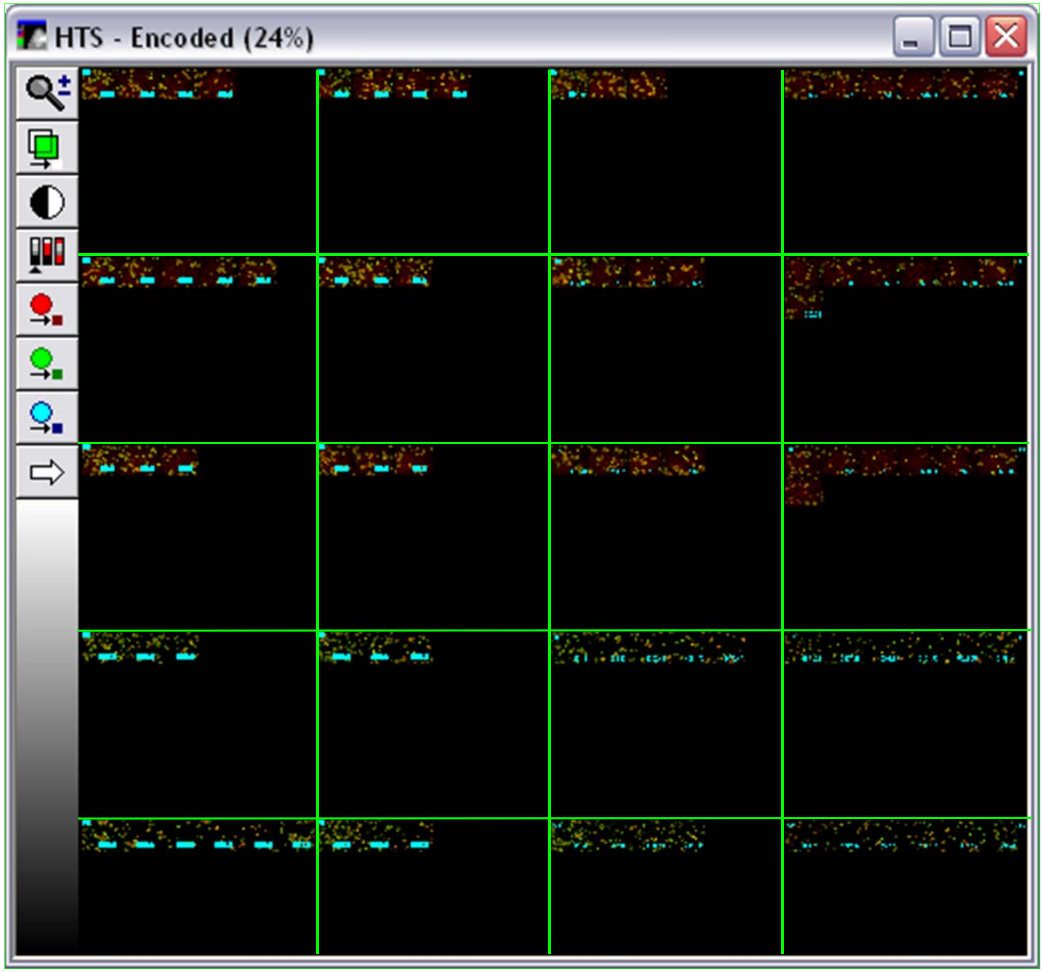
Viewing Images in Review Plate Data

When you view thumbnail images through the **Review Plate Data** dialog, you will see that each well has a different number of sites acquired



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Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: http://mdc.custhelp.com/
- User Forum: http://metamorph.moleculardevices.com/forum/
- Request Support: http://mdc.custhelp.com/app/ask
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments

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