IN Cell Analyzer

Imaging Modes

Factors to consider before selecting image mode

- How does the thickness of your biology compare to the depth of field of your objective (as shown in the ***Z-Stack Setup*** card)? If your biology is thicker than the depth of field, you may need a mode that captures more than one z slice.
- Is it important that you image the entire volume of your cells? Or is a single slice all you need?
- How many images/fields/time points will you acquire in your experiment? Is reducing overall data volume important?
- If you're unsure what to use, set up duplicate channels with different modes. After acquisition, compare modes side by side to see which is best.
- For analysis purposes, it is advisable not to mix and match 2D and 3D modes within a single experiment.

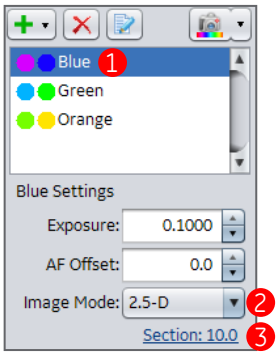
Imaging modes

Mode	Description	Use when
2D	Collects images from a single focal plane for all defined channels.	• Only 2D information is required • Fast frame rates are required
2D deconvolution*	2D acquisition plus post-acquisition processing to reassign blurred light.	• Only 2D information is required • Sample contains out of focus fluorescence or could benefit from a slight increase in contrast
Advanced 2D deconvolution*	Collects three z sections centered around the focal plane. Post-acquisition processing uses information from all z sections. Returns a single deconvolved 2D image.	• Only 2D information is required • Sample contains out of focus fluorescence or could benefit from a larger increase in contrast (when compared to 2D deconvolution)
2.5D*	Moves the stage through a user-defined z range while illumination and camera shutters remain open. Result is a sum projection of the 3D data in the defined range, in a single 2D image.	• 3D information is required but 3D localization is not • 3D information is required but minimizing data volume is important • Imaging with high NA lenses to virtually extend depth-of-field
3D	Collects a stack of 2D images at user-defined intervals along the z-axis.	• Volumetric or 3D localization information is required • Structures are well defined and do not require contrast improvement for segmentation
3D Deconvolution* †	3D acquisition followed by post-processing with a 3D algorithm to reassign blurred light. Returns a z-stack of deconvolved images.	• Volumetric or 3D localization information is required • Structures of interest are small or obscured by background/ out of focus light and benefit from contrast improvement
Maximum intensity	Collects a 3D stack and computes a maximum intensity projection of the stack. Only the projected image is saved.	• Capturing the brightest objects through a volume is desired • Minimizing data volume is important • Quantitative intensity measurements are not required

*Available only on 2000/2200/2500HS

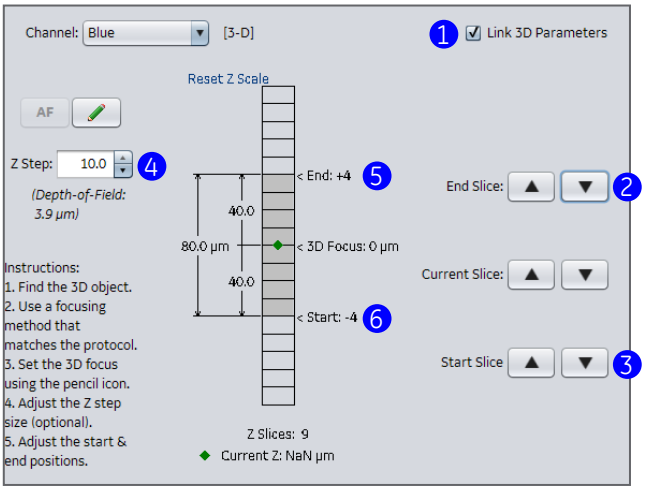
†3D deconvolution requires an optional license purchased separately.

2.5D imaging



1. Set up ***Dashboard*** parameters as you would for a plate scanning experiment.
2. In the ***Channel Settings*** area of the Dashboard click on a channel (1) to show settings for that channel.
3. Set ***Image Mode*** to ***2.5D*** (2).
4. The ***Section*** (3) link shows z thickness (µm) to be collected. Click on the link to adjust thickness.
5. Finalize any other protocol parameters and run as you normally would.

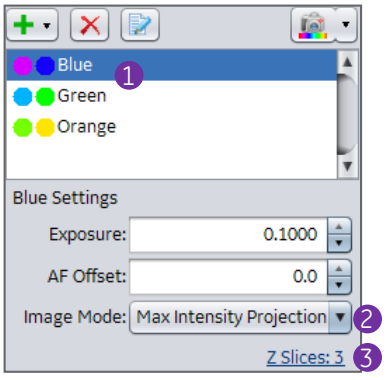
3D and 3D deconvolution imaging

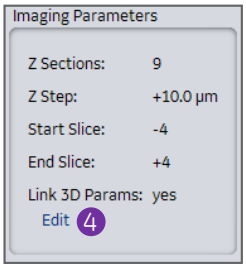


1. Set up ***Dashboard*** parameters as you would for a plate scanning experiment.
2. In the ***Channel Settings*** area of the ***Dashboard***, click on a channel to show settings for that channel.
3. Set ***Image Mode*** to ***3D*** or ***3D Deconvolution*** for all channels.
4. In the ***Z-Stack Setup*** card, confirm the ***Link 3D Parameters*** (1) checkbox is selected.
   Note: ***Link 3D Parameters*** will invalidate channel offsets, increasing acquisition speed and ensuring that the Z stack is collected through the same volumefor all channels.
5. Use the ***End Slice*** (2) and ***Start Slice*** (3) arrows to add or remove Z slices from the scan range.
   Note: The scan range may be asymmetrical and does not have to include the 3D Focus position.
6. Set the ***Z Step*** (4) size. See chart at right/below for guidance typical Z step sizes for various structures of interest.
7. Click on the top (5) and bottom (6) of the scan range using each channel to verify scan range is appropriate for all channels.
8. Finalize any other protocol parameters and run as you normally would.

Structure of interest	Typical Z Step
Foci/Puncta	1-3 µm
Organelles	3-6 µm
Spheroids	10-15 µm

Max intensity projection imaging





1. Set up ***Dashboard*** parameters as you would for a plate scanning experiment.
2. In the ***Channel Settings*** area of the ***Dashboard*** click on a channel (1) to show settings for that channel.
3. Set ***Image Mode*** to ***Max Intensity Projection*** (2).
4. Click on the ***Z Slices*** (3) link to view ***Imaging Parameters***.
   To edit, click ***Edit*** (4) or go to the ***Z-Stack Setup*** card.
5. Finalize any other protocol parameters and run as you normally would.

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