

# Lightsheet Dual side and Multiview processing (landmark alignment)

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The purpose of this document is to describe the guidelines to achieve high quality multiview fusions on the Zeiss Z.1 Lightsheet.

## Data Collection guidelines

- Bead density: 10-20 in focus beads per focal plane.
- Bead brightness: at least several hundred peak brightness (arbitrary units) above background.
- Allow enough imaging space around sample so that beads are imaged on all sides (x,y, and z).
- Carefully fine-tune the lightsheet offset to optimize clarity and brightness.
- The multiview acquisition only uses the lightsheet offset for the first view even though it will record a separate offset for each.
- To avoid photobleaching, set all your views and z-stacks at low laser intensities. Then for acquisition set lasers and exposures to desired values and click “assign illumination parameters”.
- For best results don't check “online dual side fusion”.

## Dual Side Fusion

- Run separately before multiview processing.
- Choose “Fusion subset in X” and set the fusion overlap for each view.
- Maximum fusion will give higher contrast images but won't be quantitative. Mean fusion is the best option for more quantitative imaging.

## Multiview Processing

- Landmark alignment is the best option for aligning multiview images.
- Choose one channel (cam1 or cam2) for registration. Pick the channel where the beads are brightest.
- Picking the best bead detection parameters is the key to successful landmark alignment.
  - Good starting values for threshold, size, and size variation are 3, 2, and 1 respectively.
  - Click preview to test bead detection in the current image. Once finished you can click the green “i” to see the exact number of detected landmarks.
  - When running the processing you want the detected number to be in the low thousands for the entire view so adjust accordingly for a single z-position.
  - If you have too many detected landmarks or if out-of-focus beads are detected try reducing the size-variation parameter.
  - If your beads aren't detected try adjusting the size parameter. The size parameter roughly correlates to the diameter of the beads in pixels.
- Check “scaling x=y=z” for highest resolution result. This will greatly increase file size due to a higher number of z-stacks in the final processed stack. Like it sounds, this option will give you equal size pixels and ideally equal resolution in all three axes.

- For best results choose fusion subset in Z
  - Include the end of your z-stack with the most clarity which should be the right side of the z-slider (largest number).
  - Then include at least a little past halfway through your sample. You have to be sure there is overlap between the views so that there is no missing sections in the multiview fusion.
- When you run the multiview processing check the green “i” again. First it will show landmarks detected per view. That quantity should be in the thousands. Then it will show pairwise detection between views. That number should be in the low hundreds.
- After the fusion is finished you will usually find there is a portion of your z-stack at each end that doesn’t contain your sample. You should use the subset tool to crop out the unneeded portions of the z-stack.