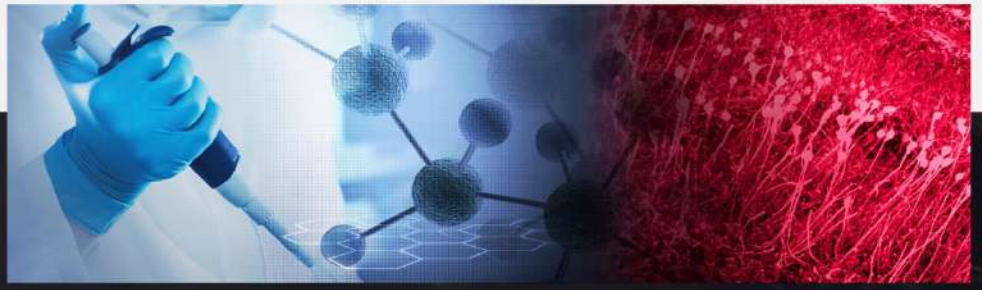
Tech Note

Improved Cell Counting Accuracy at Extremely Low Cell Concentrations with a New Autofocus Algorithm of the LUNA-FX7™



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Introduction

Modern cell counters utilize automated methods for cell counting within specific concentration range. The upper limit is influenced by the ability to accurately distinguish cell boundaries at high concentrations, while factors like the camera's field of view (FOV) determine the lower limit. Nonetheless, in cases where an image contains only a few identifiable cells or features, autofocus failure can impede cell counting performance resulting in a higher coefficient of variation (CV). This is typically a minor issue in routine cell culture processes with sufficient cell concentration. However, it becomes critical in situations where the cell sample contains extremely low concentration, such as in the initial seed culture stage of cell therapy product manufacturing. In such cases, obtaining sharp images at extremely low cell concentrations is essential for achieving accurate cell counting results. To address this issue, we have developed a new autofocusing algorithm that employs advanced techniques to accurately identify features in images. This technical note discusses the latest updates in the LUNA-FX7™ software version (ver 1.9.1 or higher), which has been optimized for precise autofocusing in extremely low concentrations. Moreover, it showcases the exceptional performance of the LUNA-FX7™ automated cell counter.

Autofocusing Process in the LUNA FX7™

The autofocusing algorithm employed by the LUNA-FX7™ system uses image contrast to adjust focus. The autofocusing process consists of a coarse scan followed by a fine scan, ensures optimal focusing performance in the LUNA-FX7™ system (Figure 1). It begins with a coarse scan, capturing images across a wide range of Z-planes to cover a broad depth of field. During this scan, the algorithm identifies an approximate focal plane. Subsequently, a fine scan is performed, focusing on narrower Z-planes to precisely determine the most accurate focal plane. This process guarantees optimal focusing performance in the LUNA-FX7™ system based on image features.

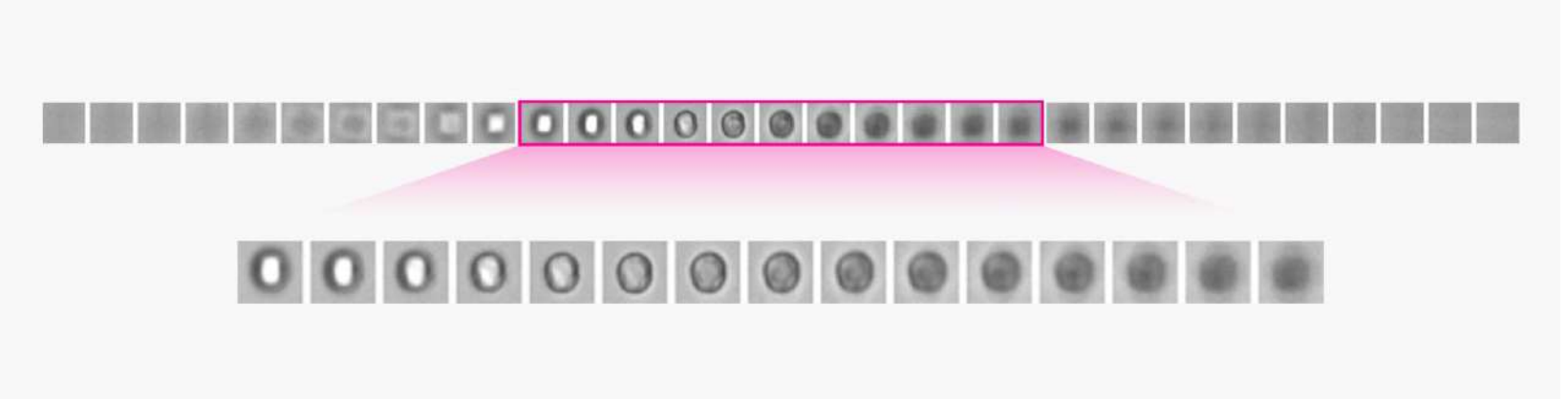

Figure 1. The figure shows the workflow of the autofocusing process in the LUNA-FX7™ system, consisting of a coarse scan and a fine scan.

Challenges with autofocusing

The autofocusing algorithm, which relies on image contrast, may encounter challenges in scenarios with few objects or limited distinguishing features, resulting in difficulties in accurately determining suitable focus positions. This issue is often noticeable during the coarse scan, where the system faces difficulties in identifying the appropriate Z-planes, leading to focus failure during the subsequent fine scan (Figure 2A). To address these challenges, a new algorithm has been developed to enhance the feature identification in images with low cell concentrations or limited image features (Figure 2B). This improved algorithm is designed to enhance the accuracy and reliability of the autofocusing process in challenging scenarios, providing more precise and consistent results.

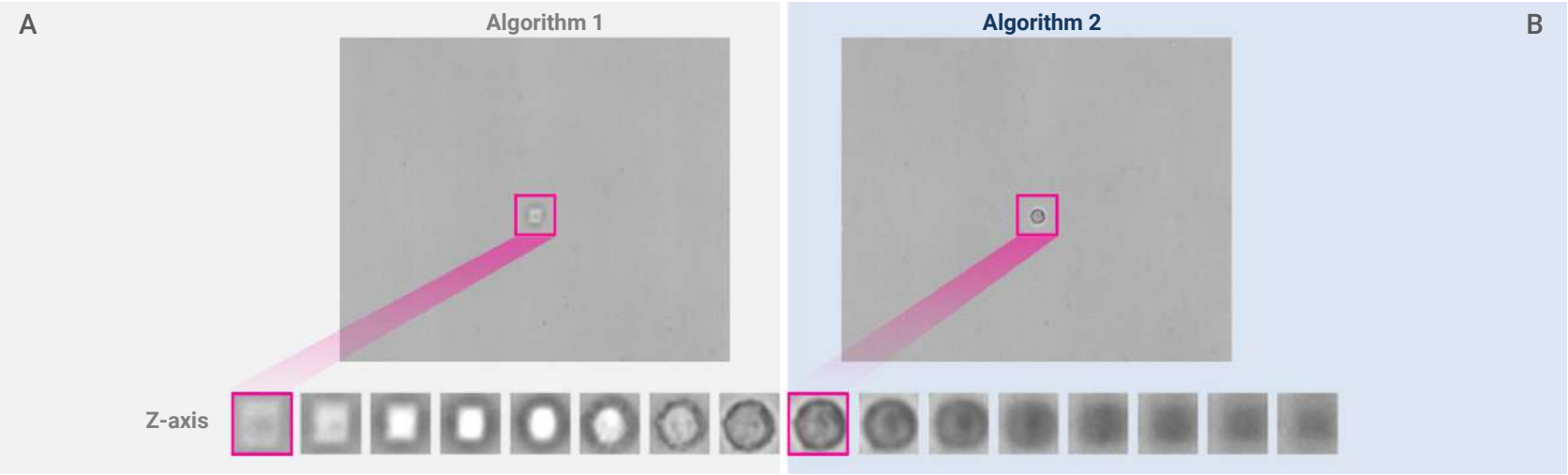

Figure 2. The performance of two different algorithms in finding the focus. (A) Algorithm 1 failed to find the focus, resulting in a blurry image with poor clarity. (B) Algorithm 2 successfully identifies the appropriate focus position, showing in a clear and sharp image.

Improved autofocusing performance

In order to validate and demonstrate the effectiveness of our newly developed autofocusing algorithm, we conducted experiments to compare different software versions of the LUNA FX7™ system using identical samples. The results confirmed significant improvements in the performance of our autofocusing algorithm. This notable enhancement was achieved through the implementation of a combined approach that effectively enhances the sensitivity in object detection, resulting in more accurate and reliable focus positions across all FOVs (Figure 3). The upgraded algorithm achieved precise focus positions, even in challenging scenarios characterized by a limited number of objects per image. It consistently delivered accurate focus positions in situations where there were fewer than five objects present, indicating an extremely low cell count.

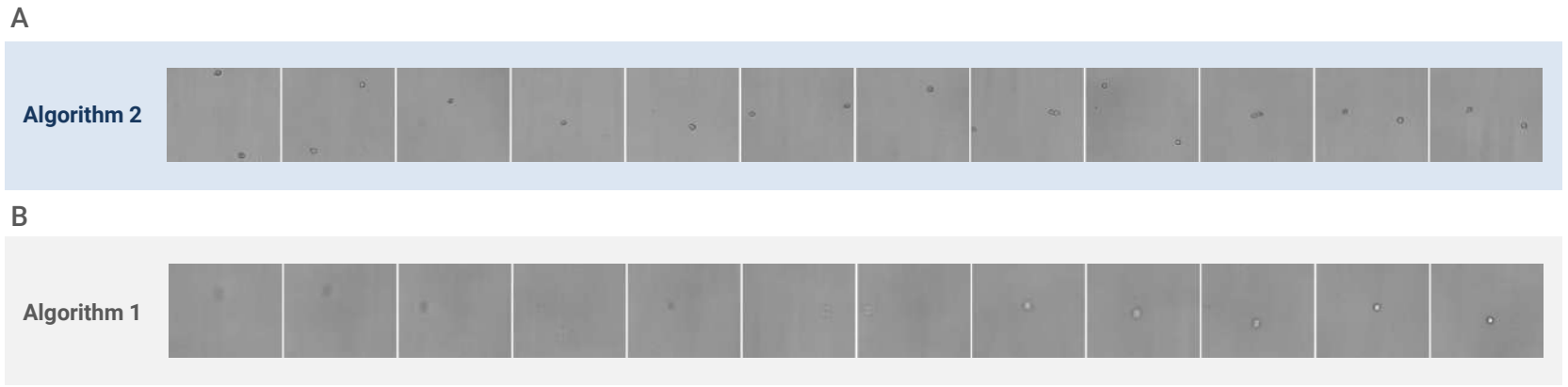

Figure 3. U937 cells were diluted to a concentration of 2.54 x 10^4 cells/mL, resulting in a frequency of less than 5 objects per image. The complete set of 12 field-of-views (FOVs) is displayed. Algorithm 2 effectively detects and identifies the objects (A), whereas algorithm 1 fails to achieve the desired focus (B).

Conclusion

In conclusion, the LUNA-FX7™ system provides accurate cell counting in extremely low cell concentrations. Our improved autofocusing algorithm, utilizing a combined approach for enhanced sensitivity in object detection, has significantly improved performance. It accurately identifies focus positions even with limited objects or features. These advancements make the LUNA-FX7™ system a reliable and efficient solution for cell counting in challenging conditions. The new autofocus algorithm is applied from the LUNA-FX7™ software version 1.9.1.

Find out more at https://logosbio.com/cell-counting-overview/