Automated cell counter, Image-based sperm cell assessment, cell counting, viability assessment, dual staining

Optimizing Sperm Cell Assessment with the LUNA-FX7™ Automated Cell Counter

Introduction

Sperm cell assessment, also known as a semen analysis, plays an important role in determining male pregnancy potential. While not a direct measure of fertility, male pregnancy significantly influences both human fertility and livestock breeding. Hence, there is high interest in sperm cell assessment.

There are methods available for sperm cell assessment using flow cytometry and image-based analysis tools. Automated cell counters like the LUNA-FX7™ automated cell counter can offer a rapid and convenient methods for assessing sperm samples using images. However, optimized analysis parameters and suitable dyes are required due to the unique morphology and properties of sperm cells compared to mammalian cells.

We conducted a comparative analysis of different fluorescent dyes such as SYBR14, acridine orange (AO), and propidium iodide (PI) to optimize sperm cell analysis using the LUNA-FX7™. This study aims to assess the efficacy of dye combinations and optimize parameters for sperm cell assessment.

Method and Material

Sample Preparation:

The cryopreserved semen samples from Korean cattle were thawed, and the buffer was replaced with PBS. The semen samples were diluted in PBS at a ratio of approximately 1:20 to 1:40, depending on the semen samples.

Dyes:

Dye	Properties	Colors	Company (Cat#)
SYBR14 (1 µM)	Membrane-permeable nuclear dye	Green	AAT Bioquest (17563)
Acridine Orange (AO)			Logos Biosystems (F23002)
Propidium Iodide (PI)	Membrane-impermeable nuclear dye	Red	Logos Biosystems (F23003)

Fluorescence Staining

1. Mix:
   - 18 µL sperm cells in PBS
   - 2 µL of dyes prepared by mixing green and red fluorescent dyes
2. Incubate for 10 min at 37℃
3. Load samples on a desirable slide.
4. Perform analysis with LUNA-FX7™.

Table 1. *The optimized parameter settings for sperm cell assessment in advanced mode of the LUNA-FX7™*

Protocol : Sperm cell counting	
Fluorescence cell counting : Advanced mode	
GF Exposure level	6
RF Exposure level	9
Min. search size	7 µm
Max. search size	30 µm
Declumping sensitivity	7
Min. FL intensity	0
Min. roundness	3
Dilution fator	1.11

Optimizing Cell Counting Protocol and Dye Combinations for Sperm Cell Assessment

Summary: Both SYBR14/PI and AO/PI staining methods are viable options for assessing sperm cell viability with optimized cell counting protocol. However, SYBR14/PI staining has shown better performance compared to AO/PI.

The default protocol for LUNA-FX7™ is already optimized for commonly used mammalian cells. However, adjustments were required for assessing sperm cells including exposure levels, cell size, and declumping sensitivity. The min search size was increased to 7 µm from 3 µm, while declumping sensitivity was adjusted to 7 from 5 to improve overall detection performance (Table 1). Additionally, exposure levels were set to GF 6 and RF 9 for both SYBR14/PI and AO/PI dye combinations.

Following these adjustments, we conducted a comparative analysis between the SYBR14/PI and AO/PI staining methods. Considering the known efficacy of the SYBR14/PI staining method for sperm cell assessment, our interest was the performance of AO/PI staining method to assess sperm cells. Our findings showed that both staining methods are viable options for sperm cell assessment (Figure 2). However, the results suggested that SYBR14 is better suited for staining sperm cells compared to AO, suggesting that the chemical properties of SYBR14 may be more suitable for this purpose.

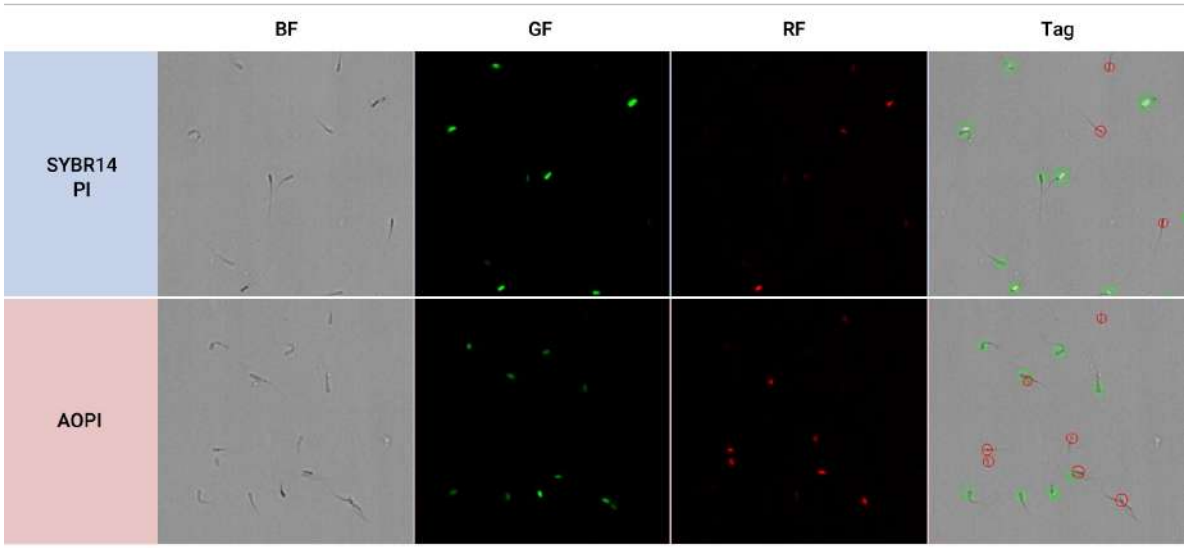

Figure 1. *Fluorescence imaging of sperm cells stained with two different dyes: SYBR14/PI and AO/PI. The montage displays BF, GF, RF, and Tag images after analysis.*
**Tag : Composite fluorescent channel images with identified objects marked using red and green circles. Red circles indicate dead cells, while green circles represent live cells.*

Conclusion

We found that both SYBR14/PI and AO/PI staining methods are viable options for assessing sperm cell viability, with SYBR14/PI exhibiting superior performance compared to AO/PI. Adjustments to exposure levels and other parameters were crucial for overall performance. Despite the LUNA-FX7™ not being specifically optimized for sperm sample analysis, it still offers a convenient and efficient solution for quick sperm assessment. Through recommended procedures and adjustments, such as sample dilution and optimization of imaging conditions, the LUNA-FX7™ can offer a simple solution for sperm cell assessment.