Sperm cell PNA differentiation using ImageStreamXMkII

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Materials and method

➢ Mouse sperm cells fixed using 1% formalin and stained using DAPI and AF568

➢ 3 experimental samples, different incubation time (0 min, 90 min, 150 min)

➢ Compensation single color controls (DAPI, AF568, EGFP)

Materials and method

➢ **Acquisition performed using ImageStream:**

- 488nm laser (EGFP)

- 405nm laser (DAPI)

- 561nm laser (AF568)

- 785 nm laser (darkfield)

- 40X magnification

- Flow Core: size (10 microns), velocity (60 mm/sec.)

- Acquired cells ~ 6 000 (for each time point)

Purpose of the assay

➢ Sperm cells go through a process known as the **acrosome reaction** which is the reaction that occurs in the acrosome of the sperm as it approaches the egg.

➢ Different stage of the acrosome reactions (0, 90, 150 min) results in different PNA (AF568) fluorescent patterns.

➢ The amount of EGFP expressed is also correlated, gradually decreasing in proportion with the stage of the acrosome reaction.

Adaptive erode mask

➤ AMNIS developed a **mask** called "**adaptive erode**" dedicated for sperm cells.

➤ Adjusting the erosion coefficient we have been able to exclude from the analysis the fluorescent positivity of the tail for AF568 (see Fig.)
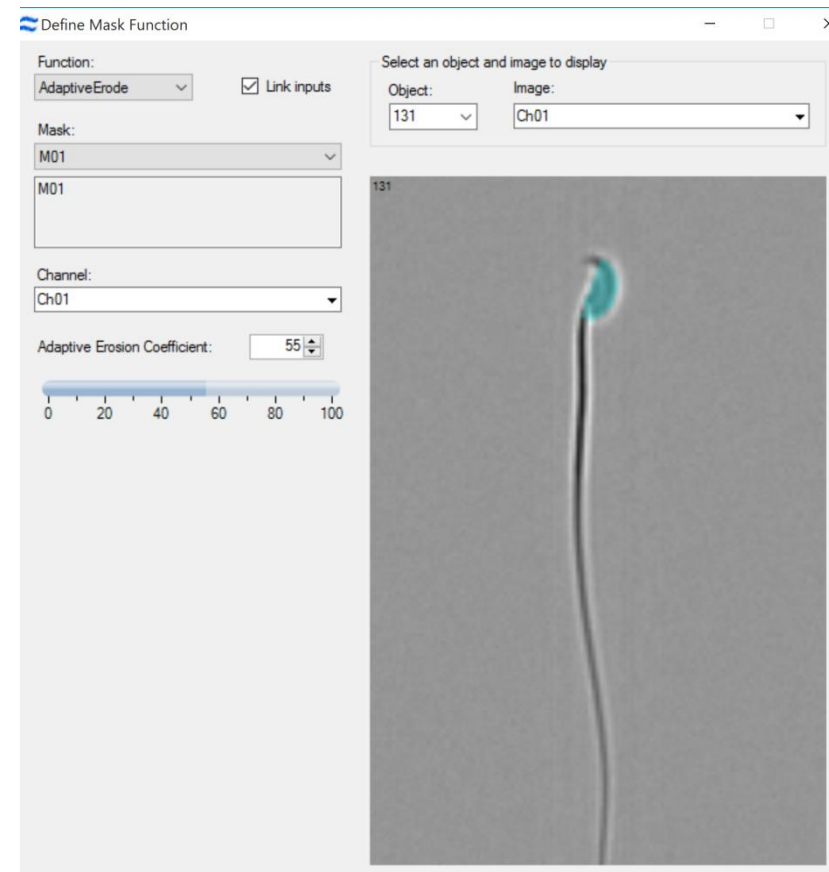
Adaptive erode mask

➢ One of the texture **features** called **"Bright Detail Intensity"** has been used in combination with the **adaptive erode mask** to determine local intensity variations in images.

➢ The Bright Detail Intensity feature compute the intensity of localized bright spots within the masked area in the image.

➢ The mask in combination with the feature has been used to distinguish and quantify the different PNA patterns at different time points (0 min, 90 min and 150 min).
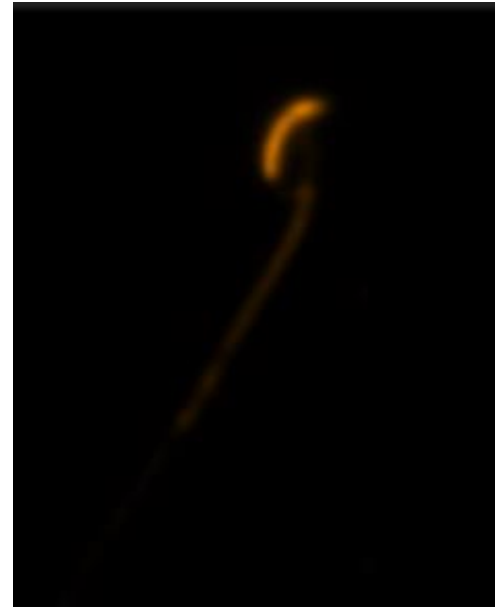
Adaptive erode mask

➤ Increasing the adaptive erosion coefficient, we are able to perform further analysis including the fluorescence signal of the tail (see Fig.)
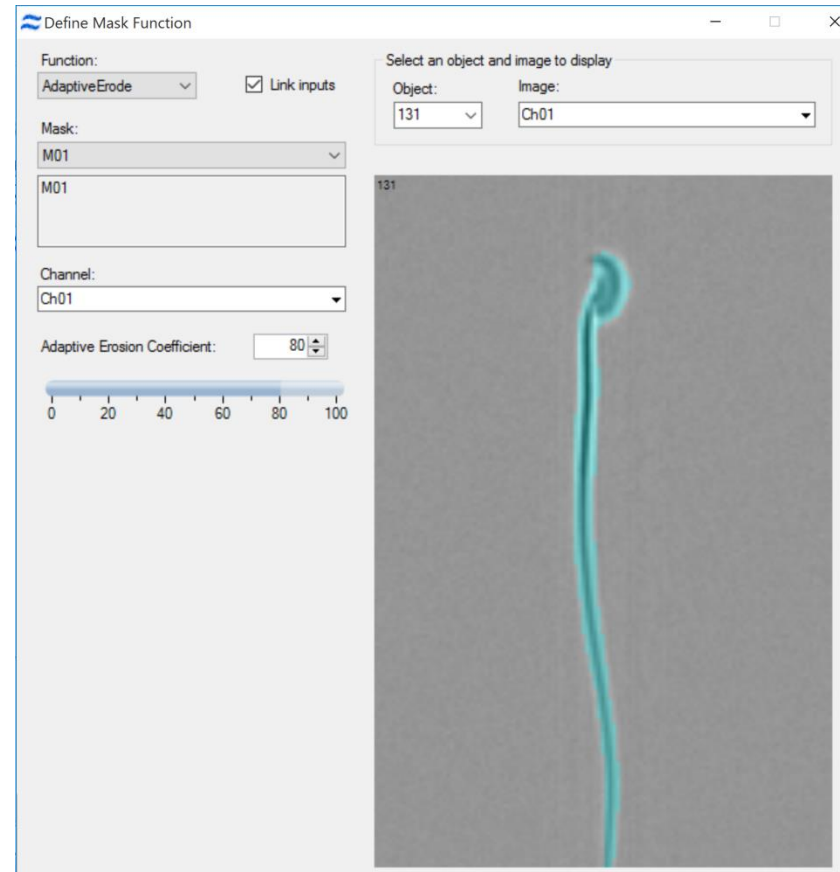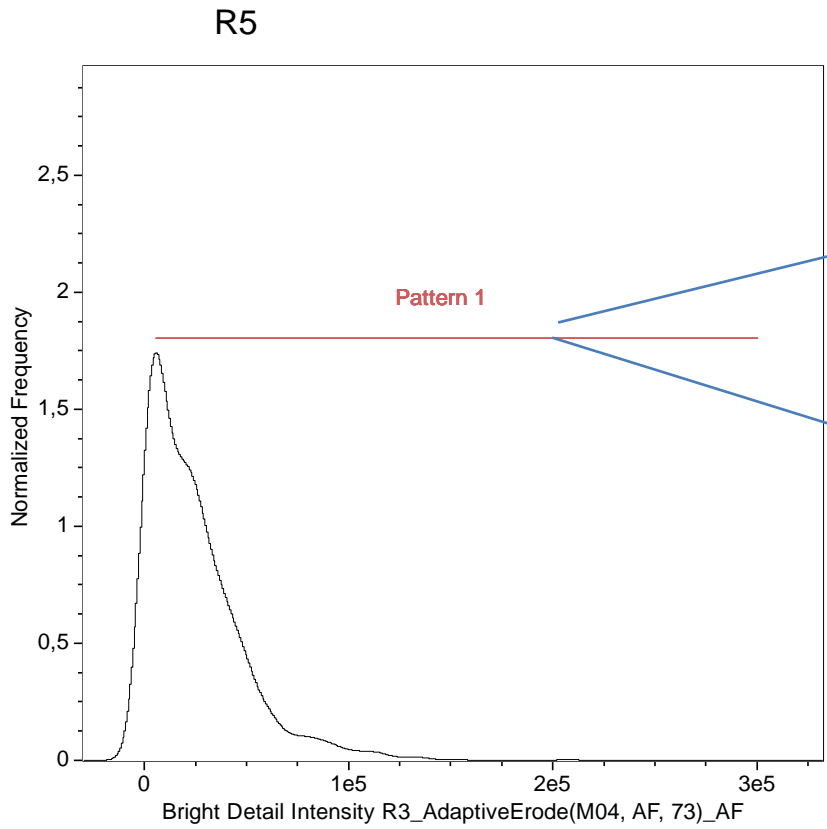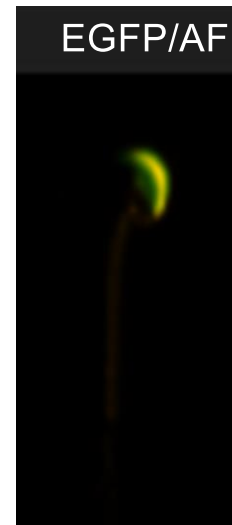
0 min of incubation (PNA)

➢ At 0 min of incubation, the **pattern 1** (Fig.1-2) results the predominant PNA pattern with **76,3%** of the total population. This thin pattern shows that the achrosome reaction is not yet started
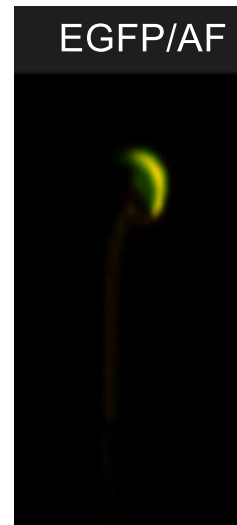
0 min of incubation (PNA)

R5

Normalized Frequency
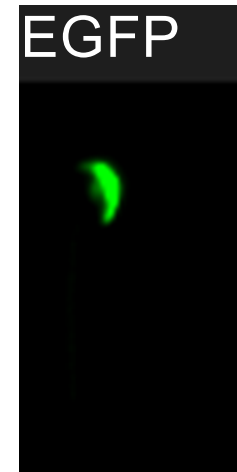
Pattern 1

Bright Detail Intensity R3_AdaptiveErode(M04, AF, 73)_AF

AF568

PNA pattern 1 (Fig.1)

EGFP/AF

Bright Detail Intensity R3_Ad

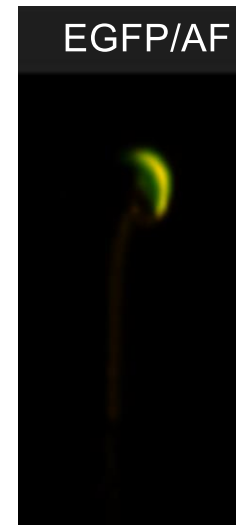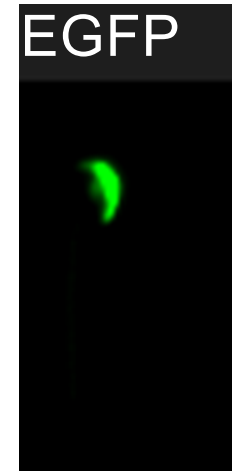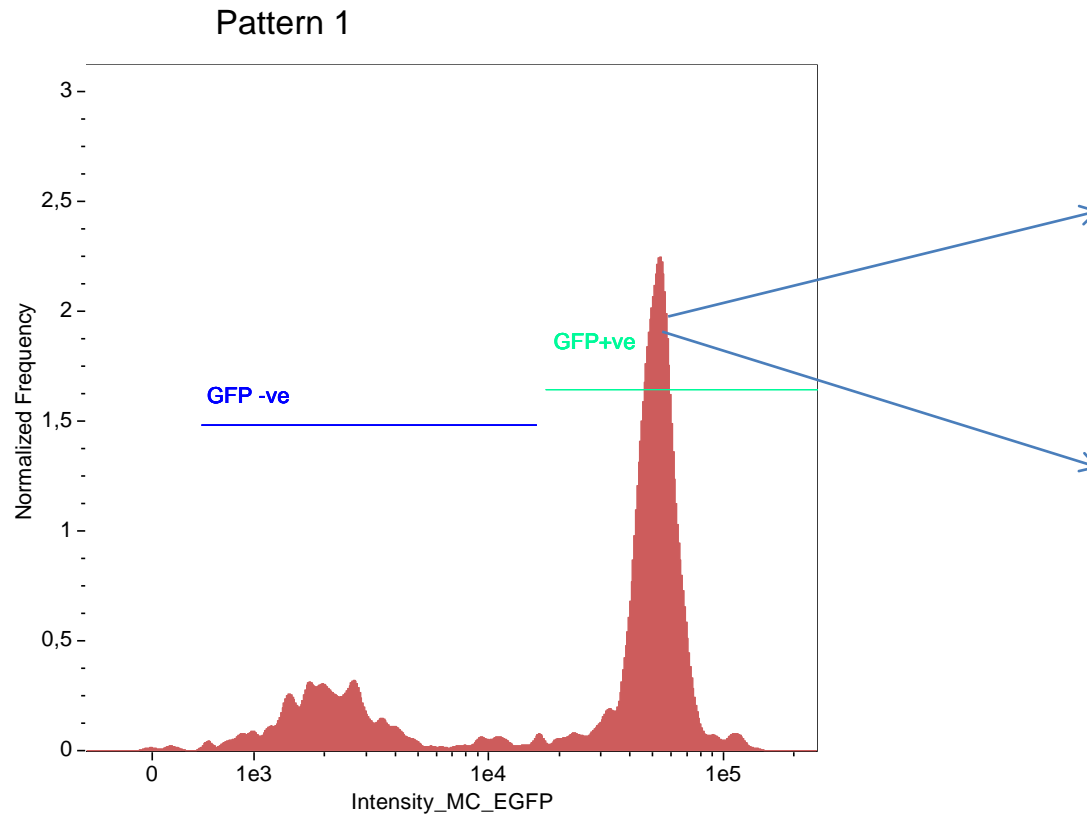| Population	Count	%Gated	Mean	Std. Dev.
R5 & R3 & R2	1839	100	24673,11	23541,51
Pattern 1 & R5 & R3 & R2	1404	76,3	31453	23042,62

PNA+EGFP pattern 1 (Fig.2)

0 min of incubation (EGFP)

➤ **~73%** of the population part of the PNA "Pattern 1" results positive in EGFP (Fig.3).

➤ At 0 min of incubation the EGFP intensity amount is the highest
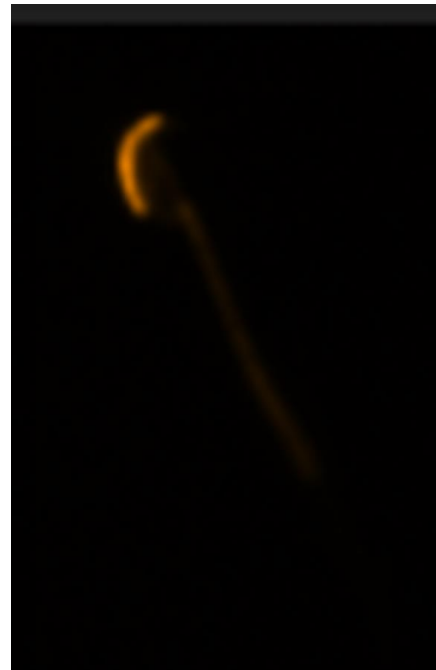
EGFP

EGFP/AF

EGFP +ve (Fig.3)

0 min of incubation (EGFP)

Pattern 1

EGFP

EGFP/AF

GFP +ve (Fig.3)

Intensity_MC_EGFP

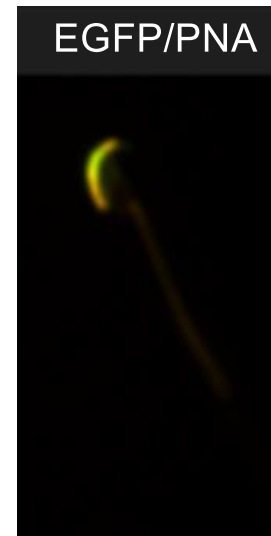| Population	Count	%Gated	Mean	Std. Dev.
Pattern 1 & R5 & R3 & R2	1404	100	39619,09	24997,94
GFP+ve & Pattern 1 & R5 & ...	1028	73,2	52941,77	13649,75
GFP -ve & Pattern 1 & R5 &...	363	25,9	2942,15	2657,31

90 min of incubation (PNA)

➤ At 90 min of incubation, the **pattern 1** (Fig.4-5) results still the predominant PNA pattern with **73,7%** of the total population.

90 min of incubation (PNA)

R5 -90 mins 40X_2
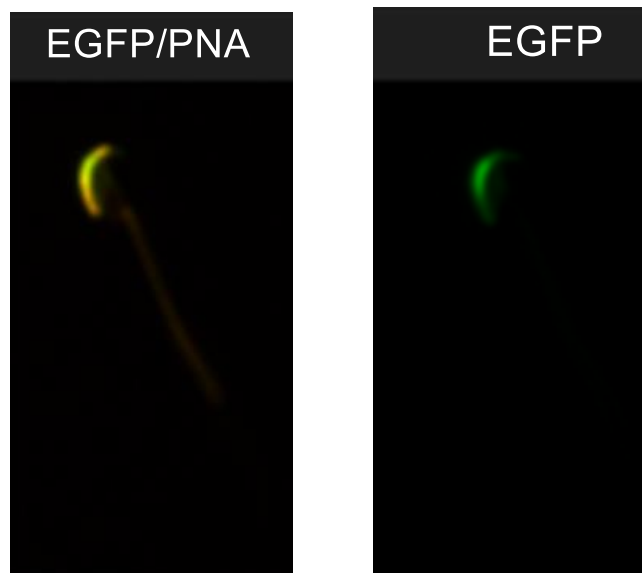

Pattern 1
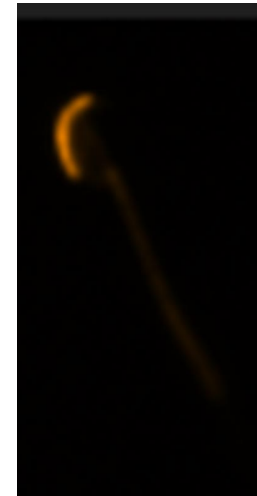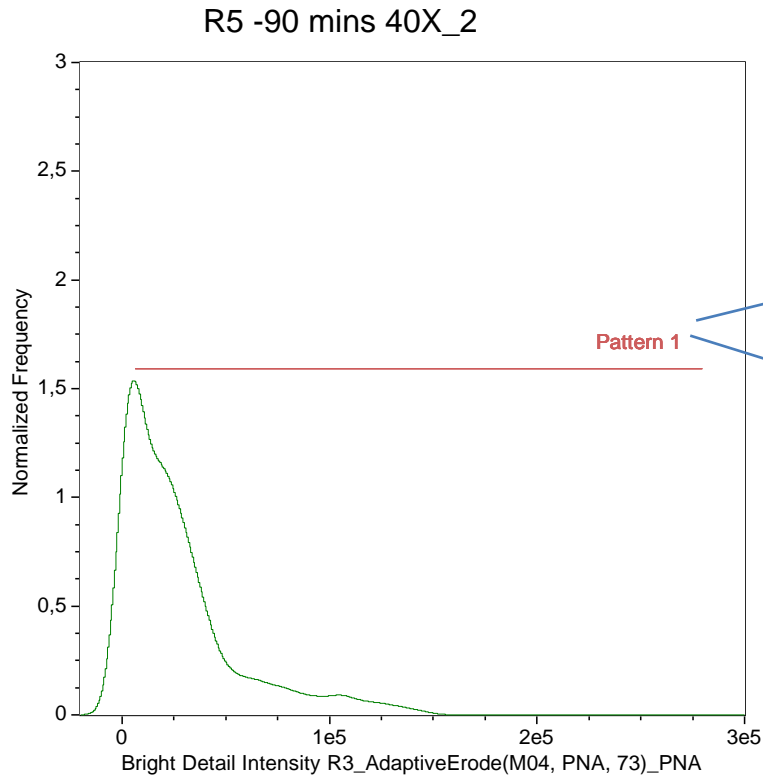
Bright Detail Intensity R3_AdaptiveErode(M04, PNA, 73)_PNA

Bright Detail Intensity R3_AdaptiveErod

Population	Count	%Gated	Mean	Std. Dev.
R5 & R3 & R2	1374	100	27082,55	28683,19
Pattern 1 & R5 & R3 & R2	1013	73,7	35672,91	28881,45

PNA pattern 1 (Fig.4)

EGFP/PNA
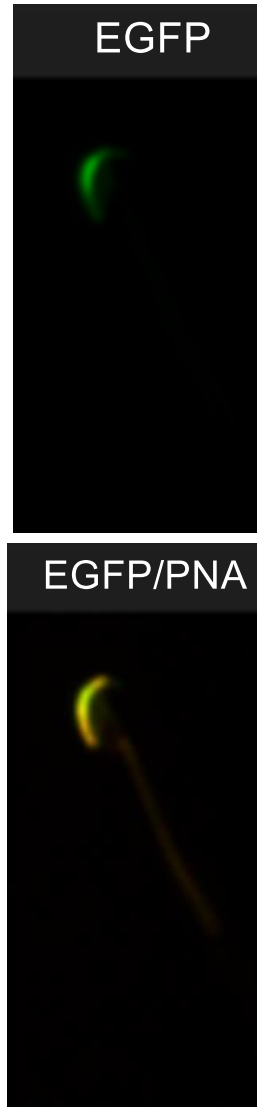

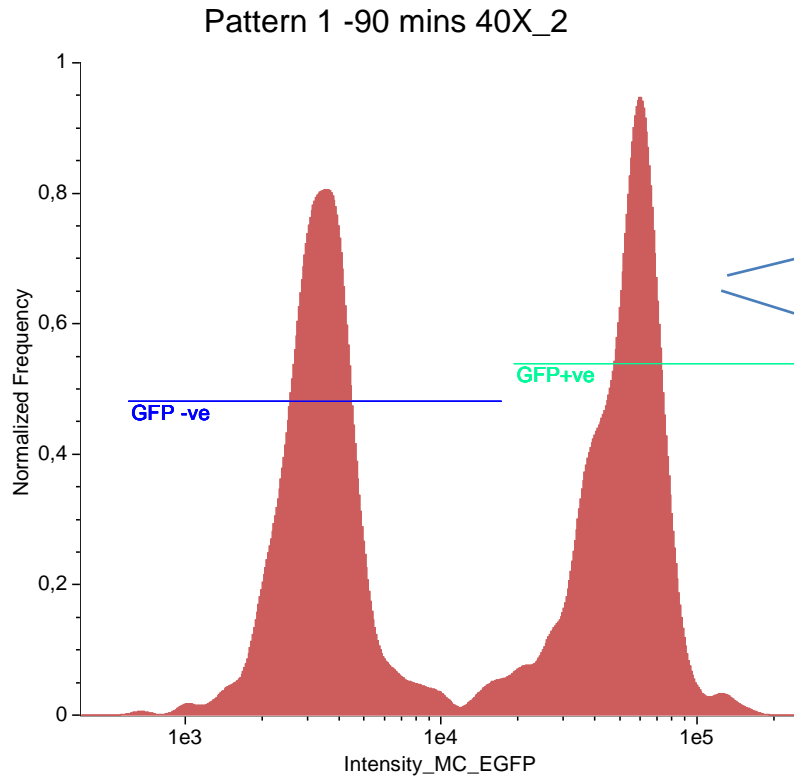
PNA+EGFP pattern 1 (Fig.5)

90 min of incubation (EGFP)

➢ **~50%** of the population part of the PNA "Pattern 1" results positive for EGFP (Fig.7).

➢ At 90 min of incubation the EGFP positive population, shows decrease in the amount of EGFP intensity (Fig. 6)

EGFP intensity decrease (Fig.6)
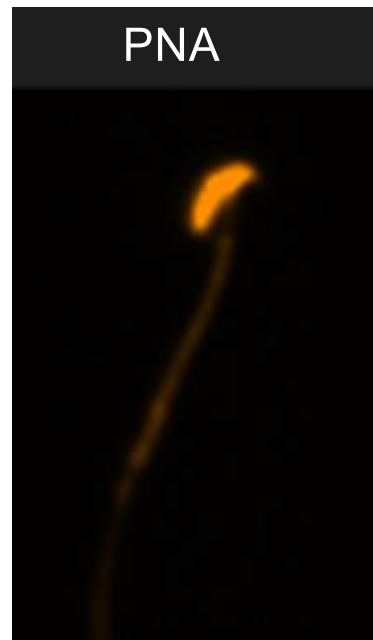
90 min of incubation (EGFP)

Pattern 1 -90 mins 40X_2

GFP+ve

GFP -ve

Normalized Frequency

Intensity_MC_EGFP

Intensity_MC_

Population	Count	%Gated	Mean	Std. Dev.
Pattern 1 & R5 & R3 & R2	1013	100	29489,96	28790,16
GFP -ve & Pattern 1 & R5 &...	503	49,7	3816,32	2182,53
GFP+ve & Pattern 1 & R5 & ...	505	49,9	55210,49	18333,33

EGFP
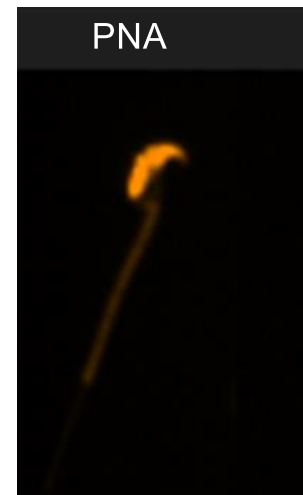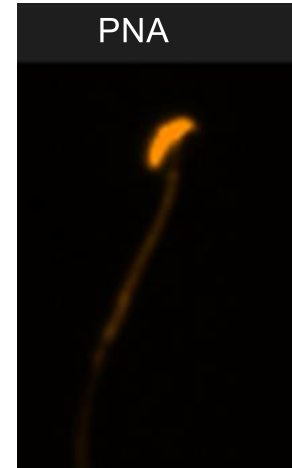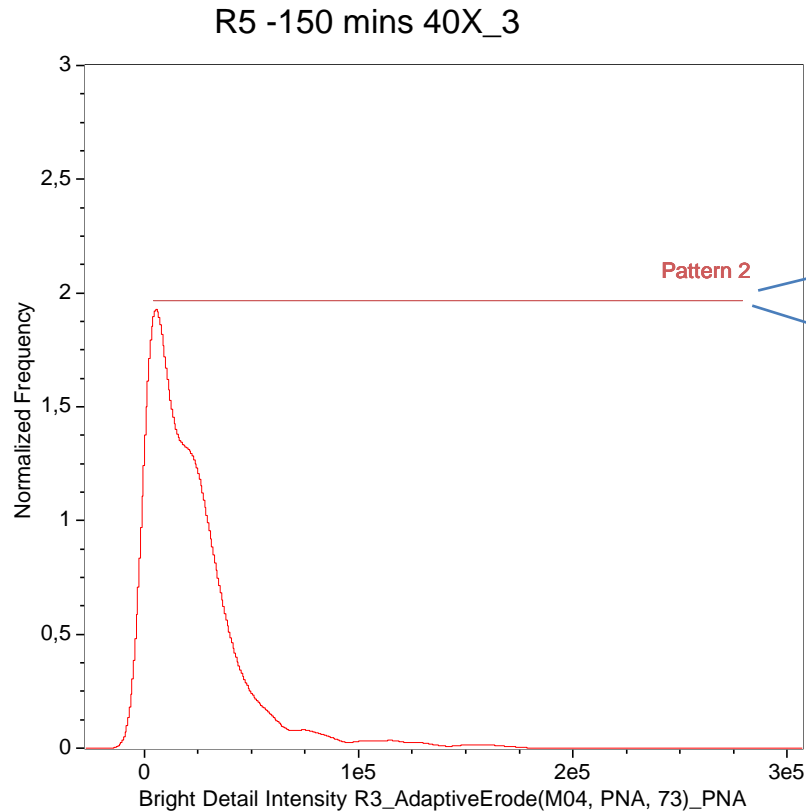
EGFP/PNA

EGFP +ve (Fig.7)

15

150 min of incubation (PNA)

➤ At 150 min of incubation, the progress of the achrosome reaction results in a different PNA fluorescent pattern identified as "**pattern 2**" (Fig.8-9).Pattern 2 results the predominant PNA pattern with **80,7%** of the total population.

PNA Pattern 2 (Fig.8)

150 min of incubation (PNA)

R5 -150 mins 40X_3

Pattern 2

Normalized Frequency

Bright Detail Intensity R3_AdaptiveErode(M04, PNA, 73)_PNA

Bright Detail Intensity R3_AdaptiveErode(

Population	Count	%Gated	Mean	Std. Dev.
R5 & R3 & R2	1556	100	22477,74	23895,25
Pattern 2 & R5 & R3 & R2	1255	80,7	27318,51	24218,45

PNA

PNA

PNA pattern 2 (Fig.9)

150 min of incubation (EGFP)

➤ At 150 min, most of the population part of the Pattern 2 (82,2%) results negative for EGFP.

➤ The EGFP positive (17,7%) are "false positive" derived from the EGFP auto-fluorescence of the tail (Fig. 10).

EGFP/PNA

PNA
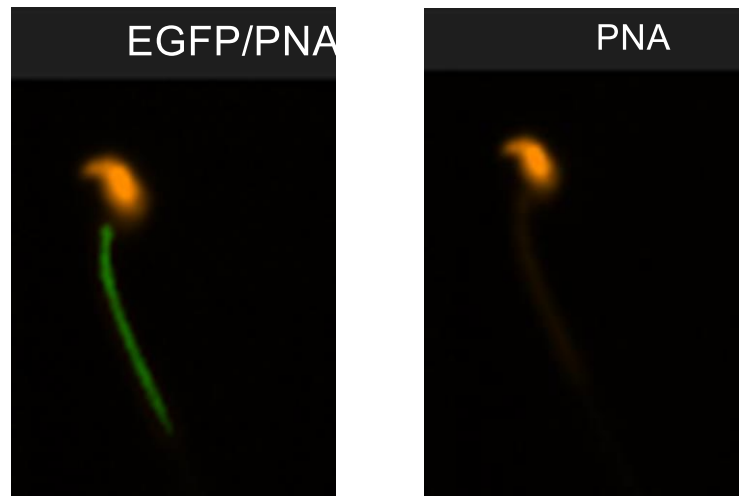
"If necessary, we can apply to the EGFP channel, the same mask used to identify the PNA pattern, in order to exclude the false positive derived from the tail auto-fluorescence"

tail EGFP auto-fluorescence (Fig.10)

150 min of incubation (EGFP)

Pattern 2 -150 mins 40X_3

EGFP/PNA
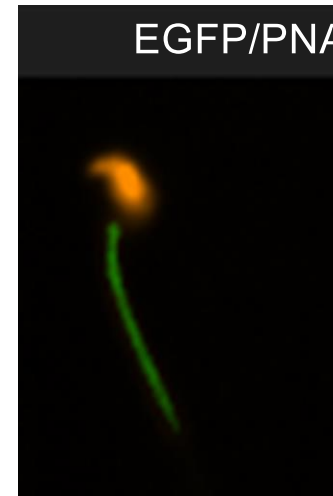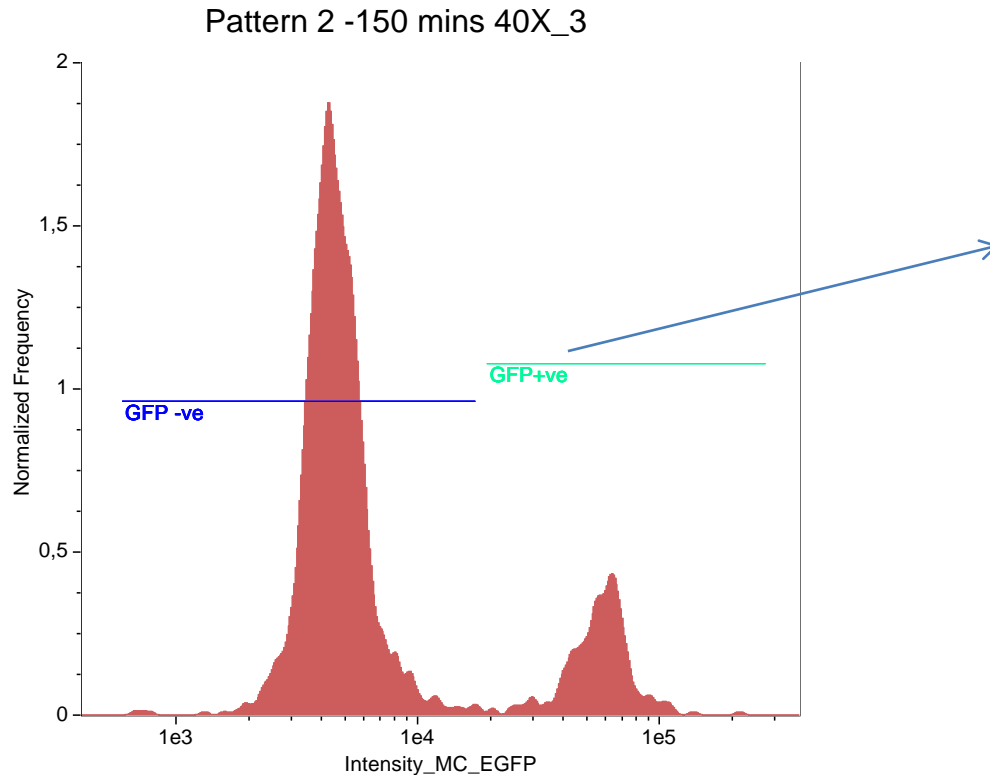
tail EGFP auto-fluorescence (Fig.10)

Intensity_MC_

Population	Count	%Gated	Mean	Std. Dev.
Pattern 2 & R5 & R3 & R2	1255	100	14436,76	22484,92
GFP+ve & Pattern 2 & R5 & ...	222	17,7	59210,88	20176,9
GFP -ve & Pattern 2 & R5 &...	1031	82,2	4789,41	1703,69

Conclusions

➢ Using the ImageStreamXMKII, combining the **adaptive erode mask** with the **bright detail intensity,** we have been able to distinguish and quantify two main PNA patterns at different time points (0 min, 90 min and 150 min).

➢ Furthermore, we have been able to correlate the PNA predominant patterns (1 and 2) to the EGFP positive and negative cells.

➢ The analysis is supported by statistical robustness, since has been based on >1000 cells in good focus.

THANK YOU FOR YOUR ATTENTION

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