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Transfluor® assay by iCyte™ imaging cytometer - An advanced cell based screening technology applicable to GPCRs -

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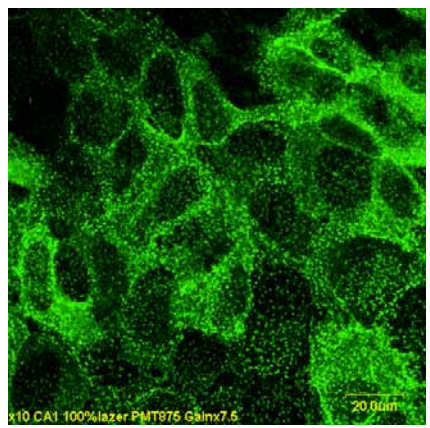


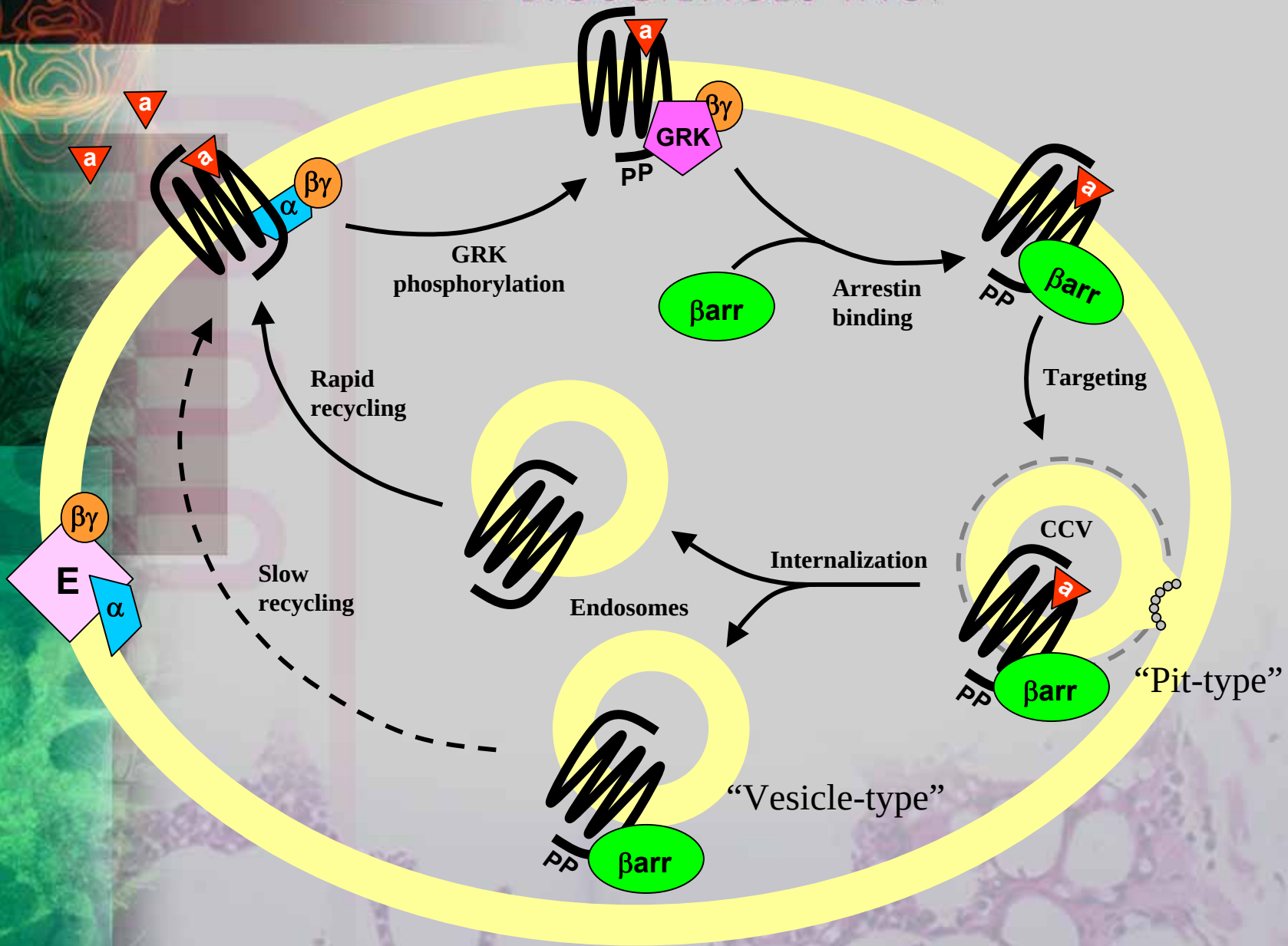
Norak Biosciences

A biotechnology company based in Research Triangle Park, NC, utilizing its Transfluor® technology to become a world leader in the discovery and development of drugs that regulate G protein-coupled receptors (GPCRs).

Transfluor® technology

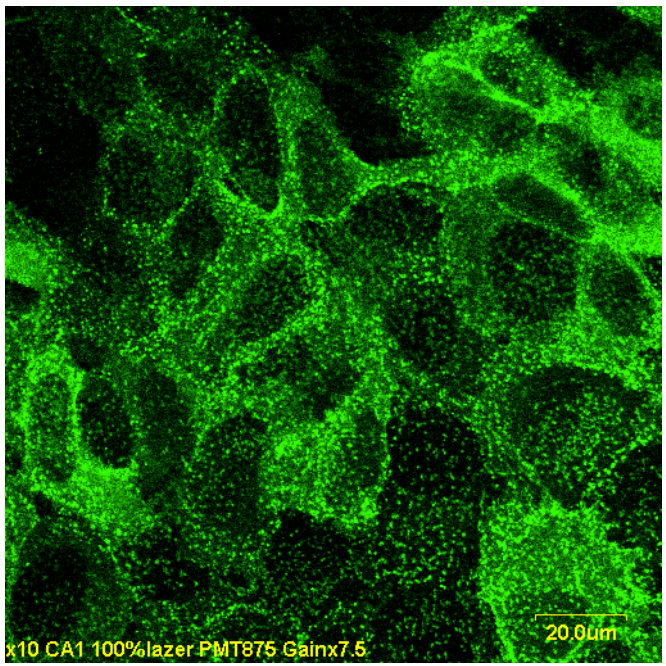
A patented, universal GPCR drug discovery technology based on the translocation of arrestin protein from cytosol to GPCR on the membrane.

NORAK BIOSCIENCES INC.

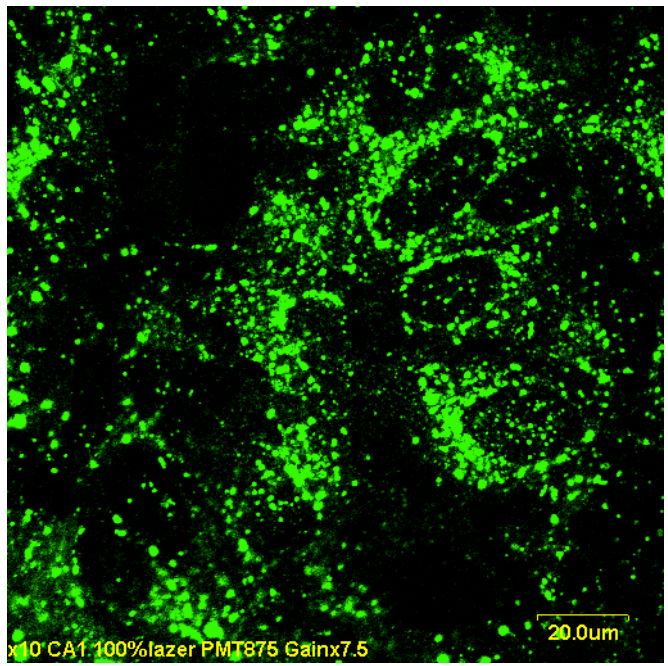


Redistribution of arrestin-GFP into pits or vesicles following treatment with agonist

Pit-type signal



Vesicle-type signal

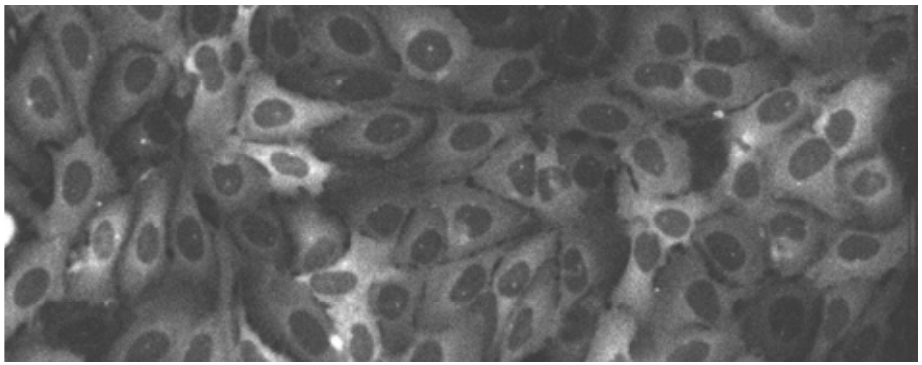


Summary

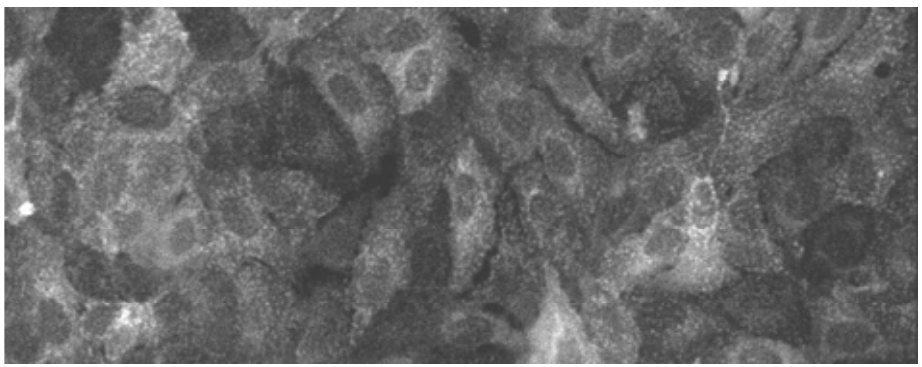
Purpose: To evaluate combination of Transfluor assay system on iCyte imaging cytometer.

For this purpose, we have designed two lines of image processing algorithms on the iCyte and carried out quantitative sample measurement.

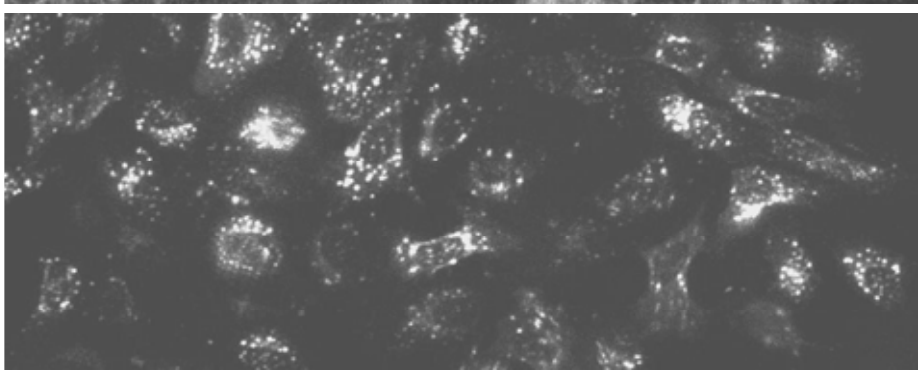
Typical images of arrestin-GFP distribution acquired by iCyte (Agonist: isoproterenol)



Unstimulated



Agonist stimulated
'Pit-type' signals
Wild-type β_2AR



Agonist stimulated
'Vesicle-type' signals
Modified β_2AR

Materials and methods (1)

Cell samples
from Norak Biosciences

U-2 OS human osteosarcoma cells expressing:
1) Wild-type β_2AR + arrestin-GFP
2) * Modified β_2AR + arrestin-GFP

↓

Isoproterenol treated, fixed and DRAQ5 nuclear stained.

Arrestin-GFP: Fusion of beta-arrestin-2 and green fluorescence protein
β_2AR: Human beta-2 adrenergic receptor

***In the modified β_2AR, C-terminal amino-acid sequence was artificially changed to convert from pit-type to vesicle-type.**

Materials and methods (2)

Cytometer

iCyte imaging cytometer (CompuCyte)

Laser scanning

for arrestin-GFP

Excitation: Argon ion laser (488 nm)

Emission: 515–545 nm Filter + PMT

for DRAQ5 stained DNA

Excitation: Helium neon laser (633 nm)

Emission: 650–700 nm filter + PMT

Two data processing algorithms for Transfluor assay on iCyte

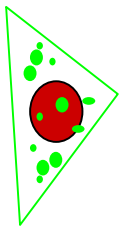
1. Counting arrestin-GFP signals

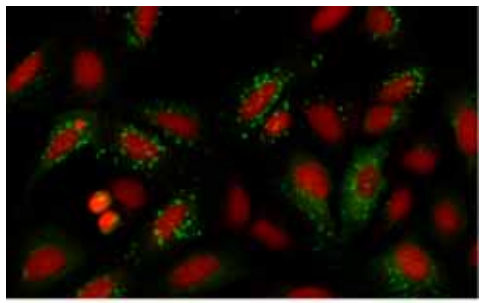
2. Max Pixel value

1. Counting arrestin-GFP signals

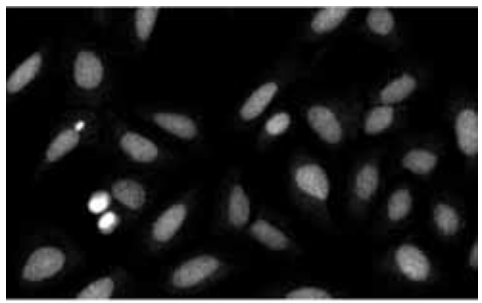
Schematic drawing of algorithm for arrestin-GFP signal counting

1. Pseudocolored image

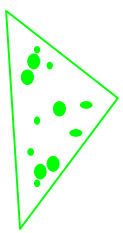


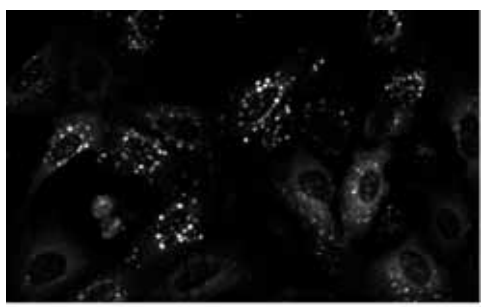


2. Long-red emission (DRAQ5 stained nuclei)

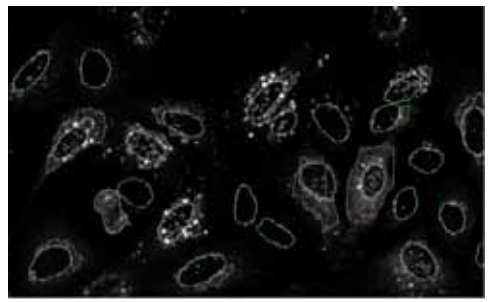


3. Green emission (arrestin-GFP)

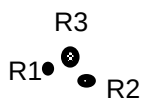


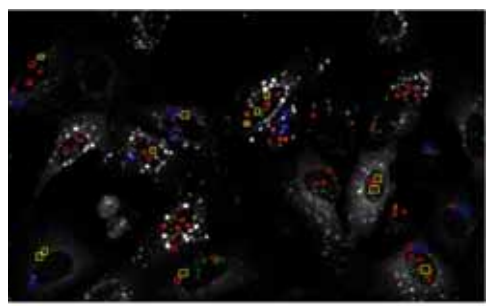


4. Primary and sub contours



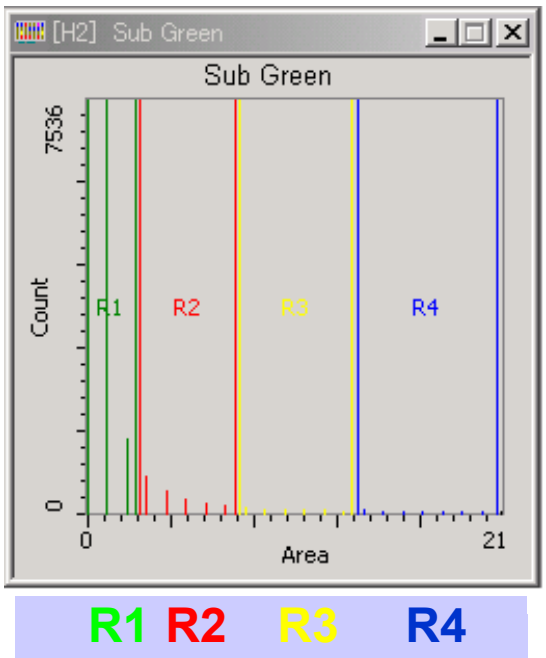
5. Size selection of arrestin-GFP signals



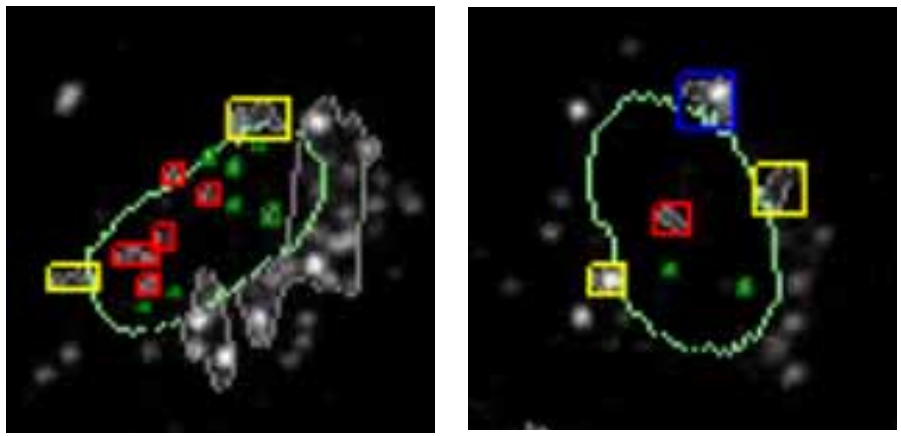


(Vesicle-type signals)

Classification of arrestin-GFP signals by AREA parameter



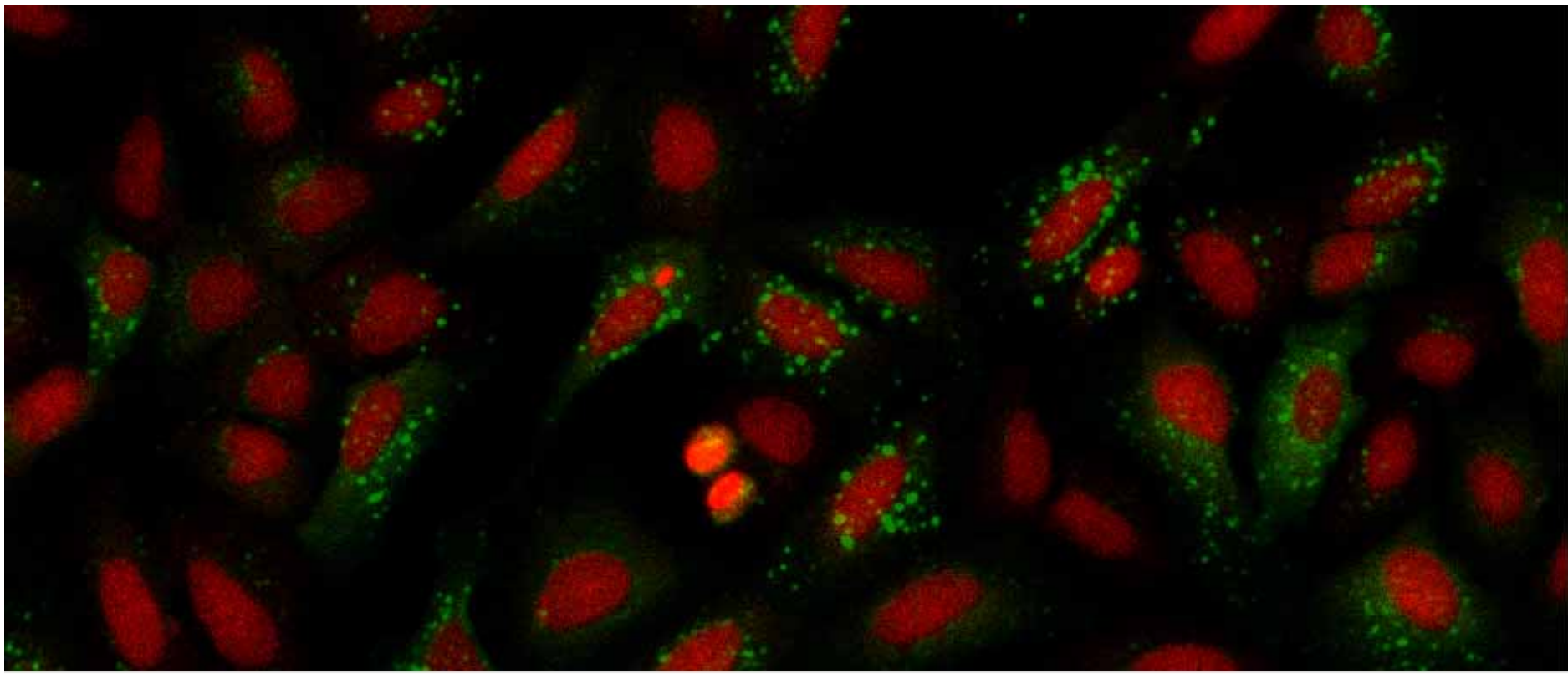
(Vesicle-type signals)



Arrestin-GFP signals were sorted into four groups by their contour sizes

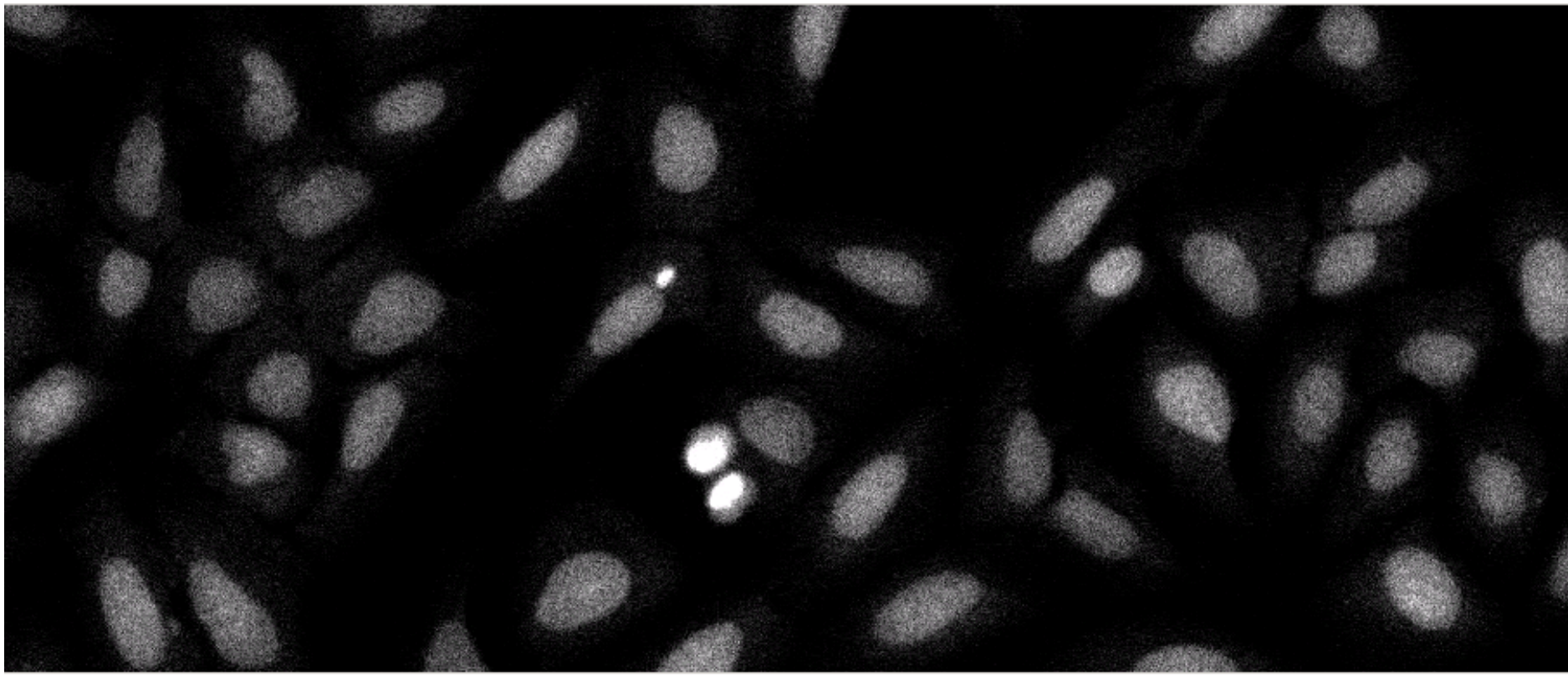
Size selected GFP signals

Pseudocolored image

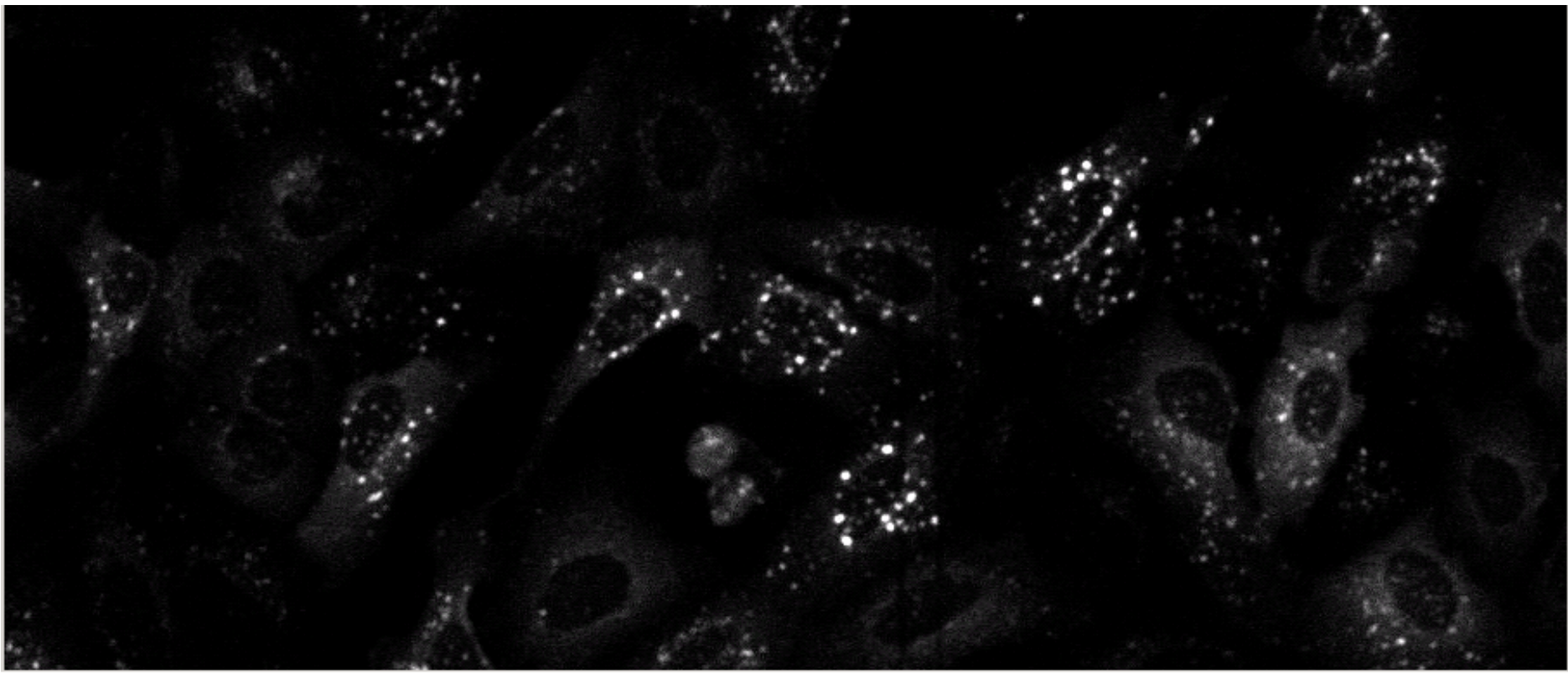


(Vesicle-type signals)

Long-red emission (DRAQ5 stained nuclei)

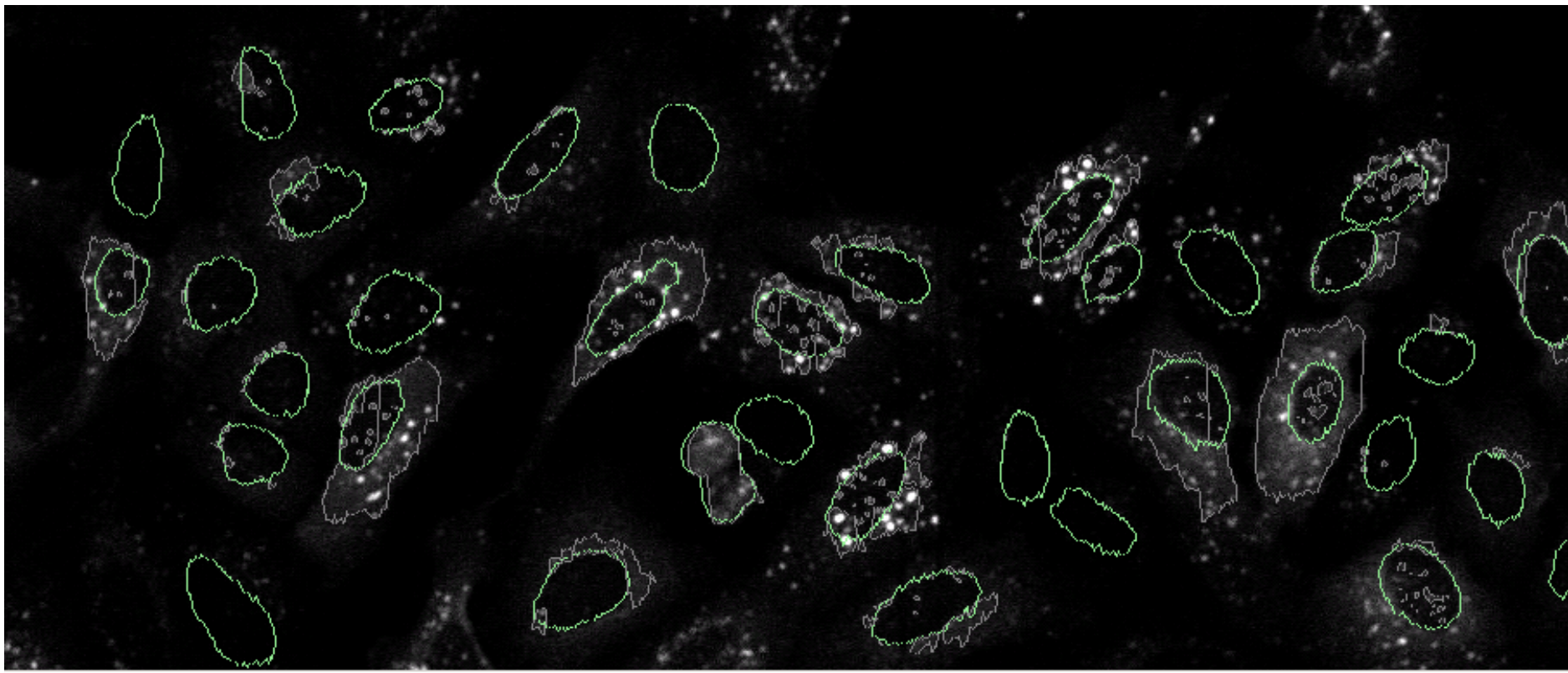


Green emission (arrestin-GFP)



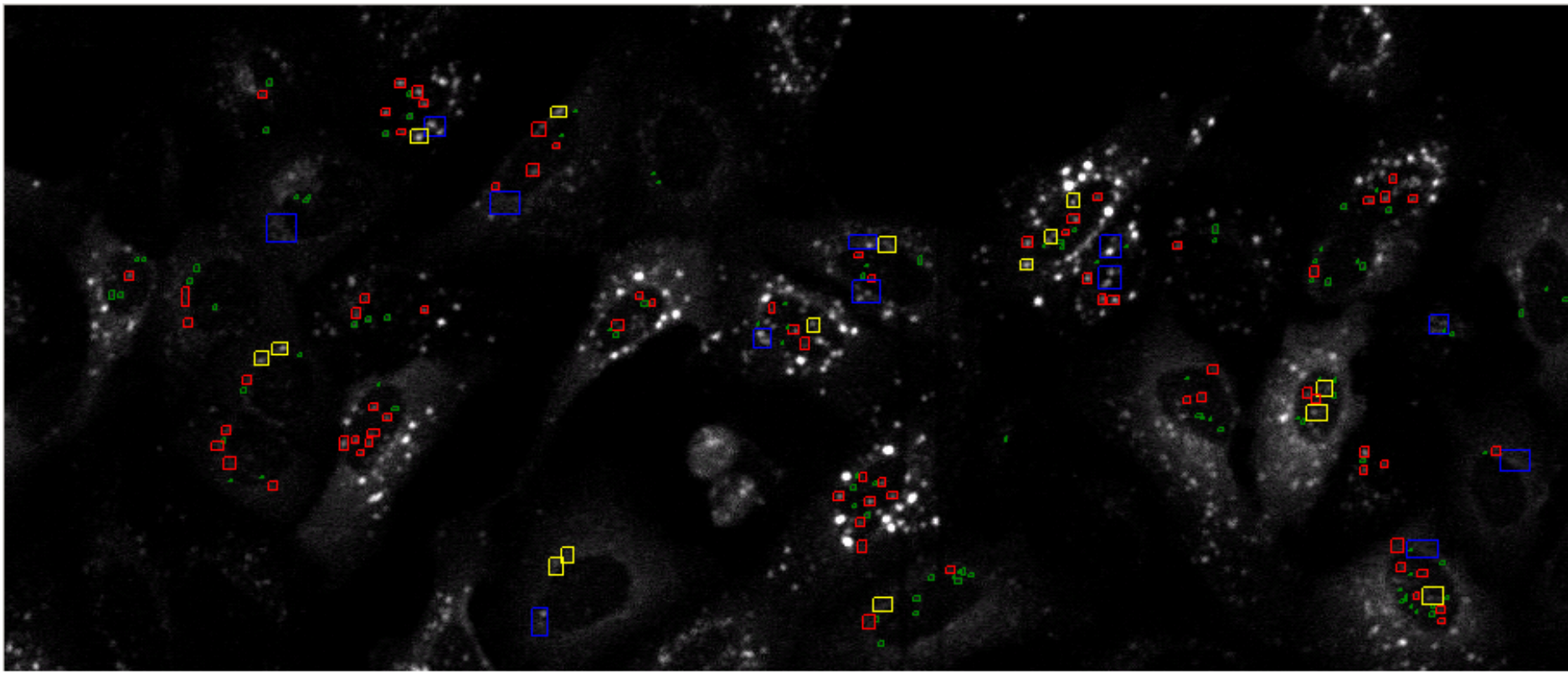
(Vesicle-type signals)

Primary and sub contours (gray and pale-green lines, respectively)



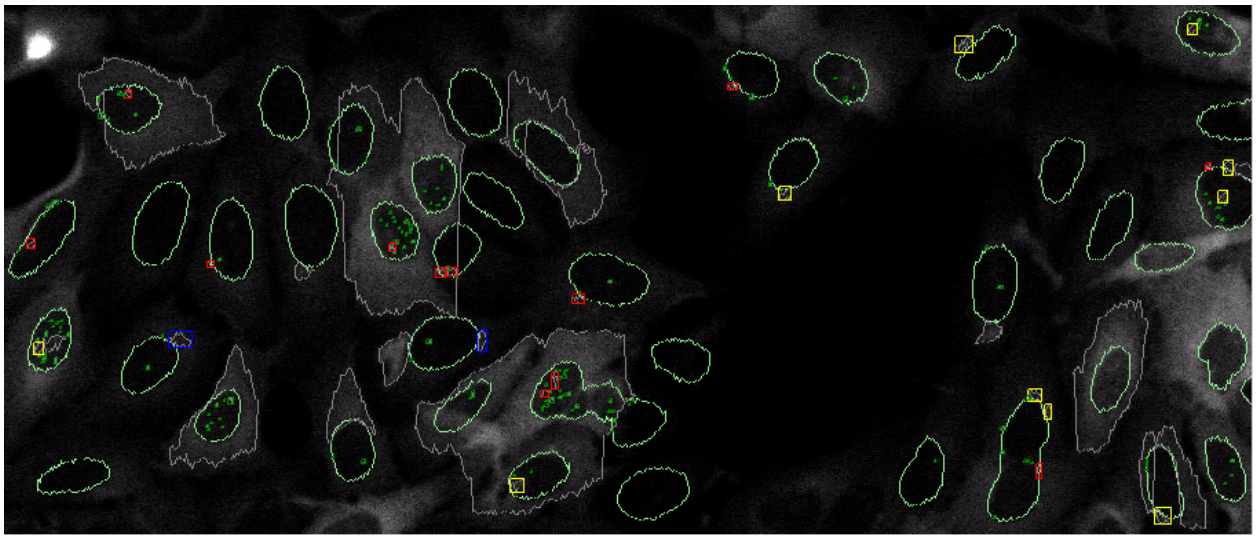
(Vesicle-type signals)

Size selected arrestin-GFP signals

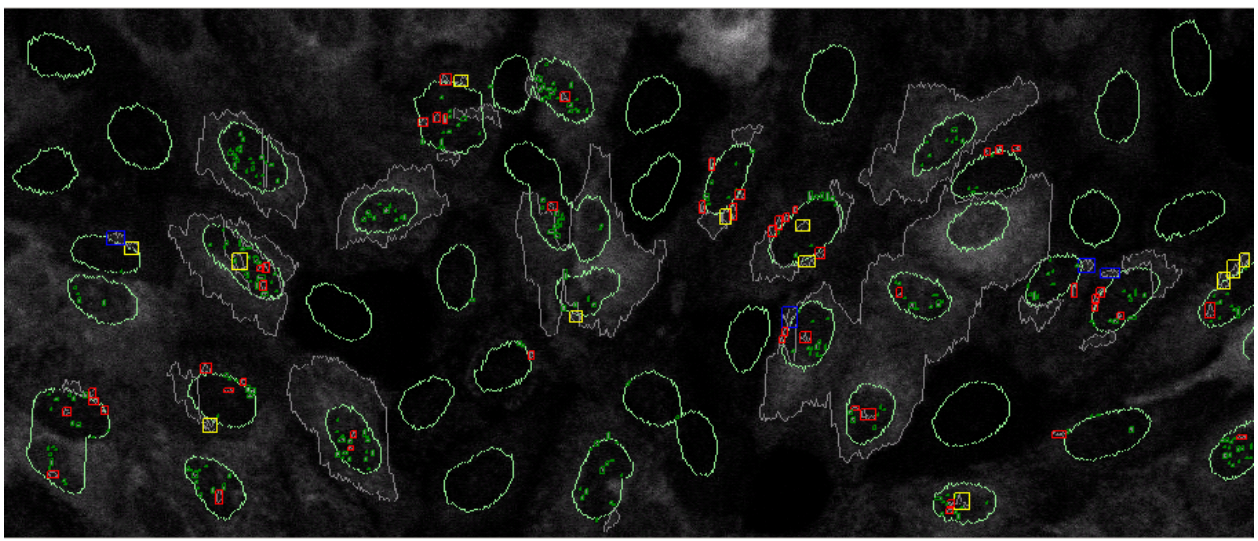


(Vesicle-type signals)

Image processing for arrestin-GFP signals of wild-type β_2AR (pit-type) expressing cells

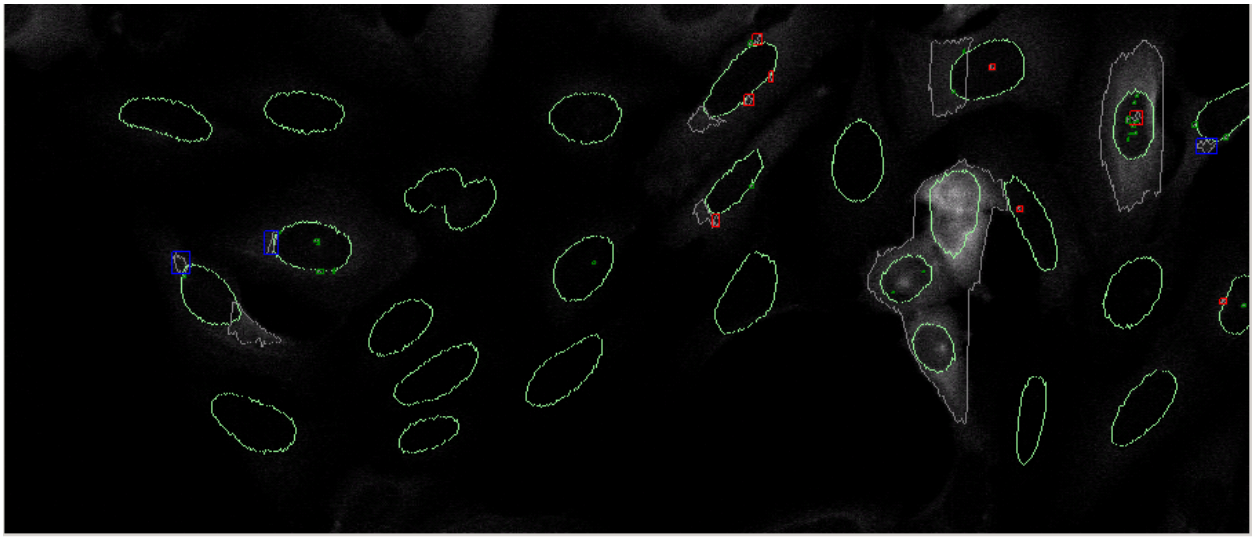


Low agonist dose
(10.1 pM isoproterenol)

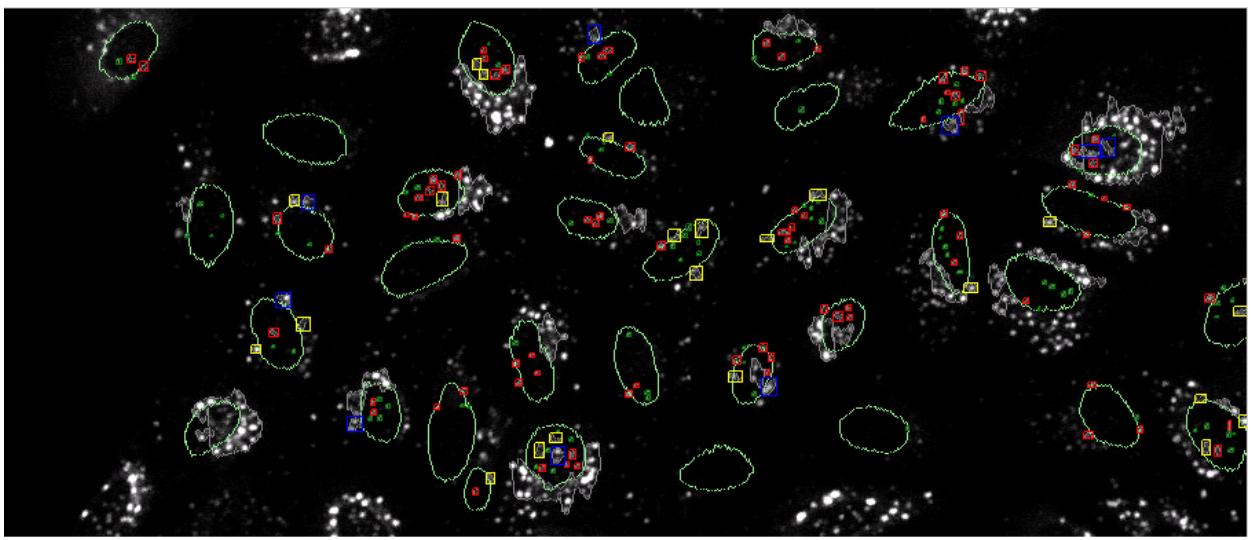


High agonist dose
(22.2 nM isoproterenol)

Image processing for arrestin-GFP signals of modified β_2AR (vesicle-type) expressing cells



Low agonist dose
(10.1 pM isoproterenol)

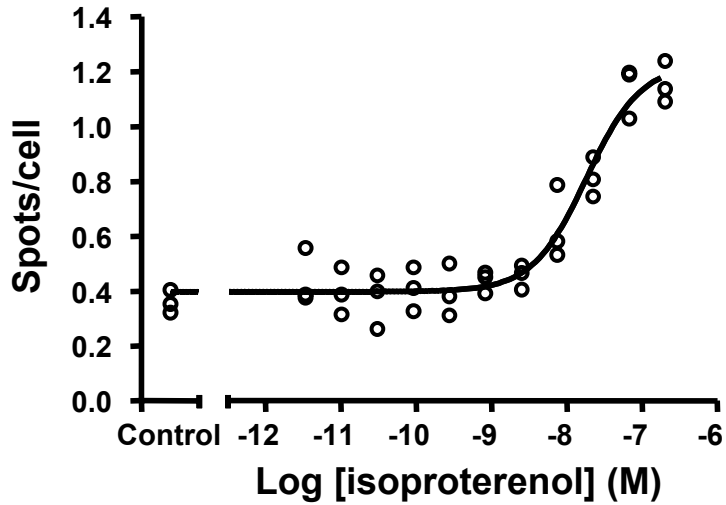


High agonist dose
(22.2 nM isoproterenol)

Dose-responses of receptor activation measured by arrestin-GFP signal counting

Wild-type β2AR
(Pit-type signals)

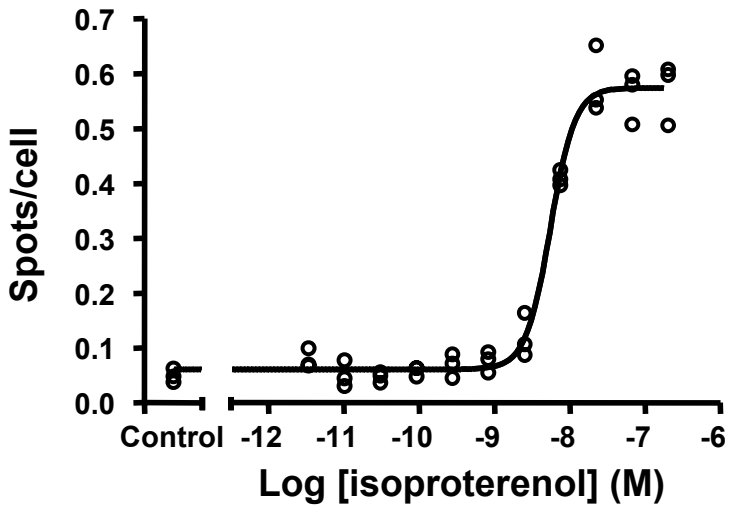
Selected AREA R2



EC_{50} = 18 nM
n = 3
Z' = 0.55

Modified β2AR
(Vesicle-type signals)

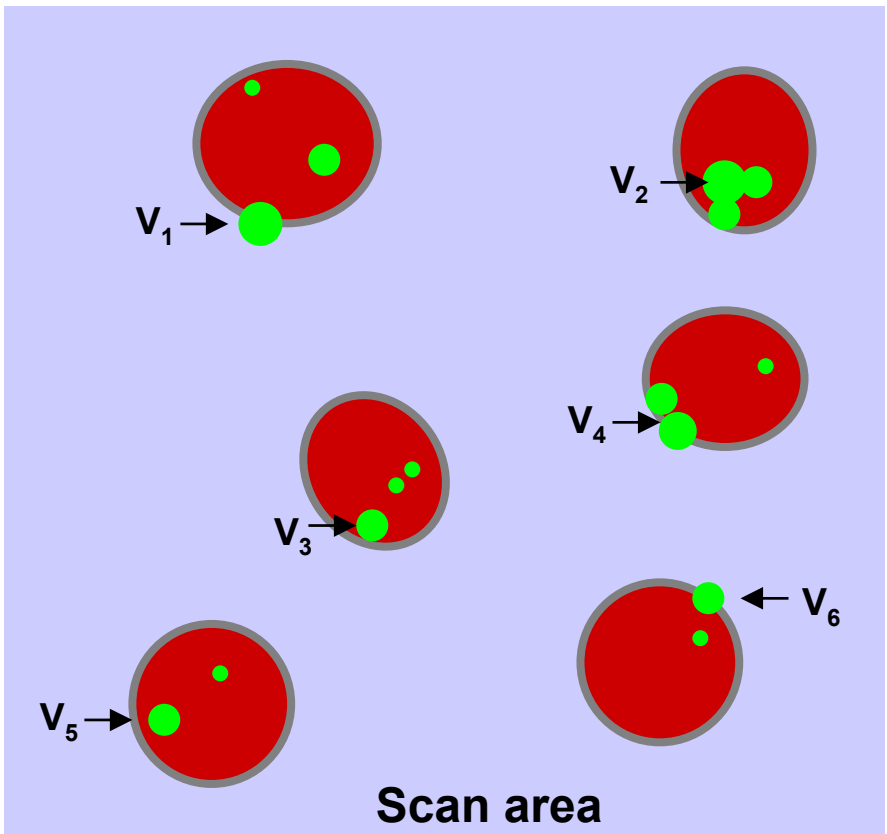
Selected AREA R3



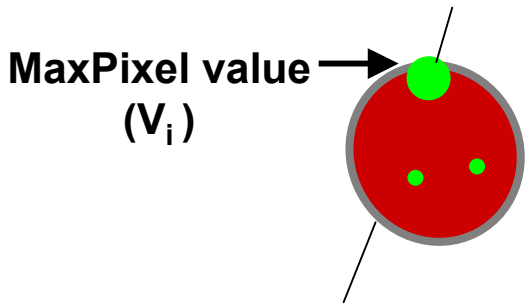
EC_{50} = 5.5 nM
n = 3
Z' = 0.60

2. Max Pixel value

Schematic drawing of MaxPixel parameter for Transflouor assay



Arrestin-GFP signal having the brightest pixel value

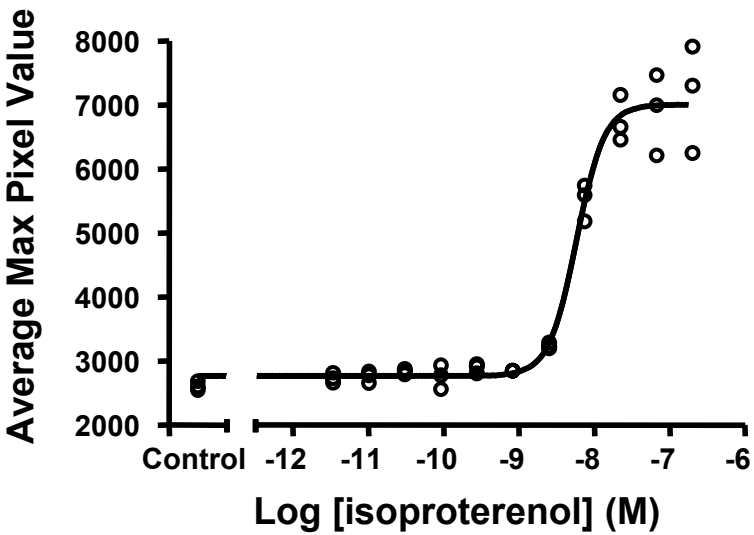


Average Max Pixel value

$$= \frac{(V_1 + V_2 + V_3 + ... + V_N)}{N}$$

(N : number of primary contour)

Dose-response of β_2AR activation measured by MaxPixel



Modified β_2AR
(Vesicle-type signals)

EC_{50} = 5.8 nM
n = 3
Z' = 0.74

Summary of the evaluation test of Transfluor assay on iCyte

Image processing	Counting arrestin-GFP signals		Max Pixel value
β_2AR receptor	Wild-type	Modified	Modified
Arrestin-GFP signal type	Pit	Vesicle	Vesicle
Objective	40X		40X
Scanned area (μm^2/well)	1500 x 1152		1000 x 576
Scanned cell number (/well)	600−900		200−300
Scanning time (min/96 well)	120		40
Z' factor	0.55	0.6	0.74
EC_{50} value (nM)	18	5.5	5.8

Conclusion

We have designed two lines of algorithms to quantify Transfluor assay system with iCyte imaging cytometer.

These methods are simple and easy to carry out.

EC_{50} values estimated by this algorithm were in good agreement with data obtained by Norak Biosciences and previously published in the literature.

The iCyte system is a suitable platform for performing the Transfluor assay.

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