# Determination of Protein Translocation using a Novel Variation on the Watershed Algorithm

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## Abstract

Here we present a novel algorithm that determines protein translocation from the membrane to cytoplasm or vice versa. The algorithm is demonstrated using Norak's Transfluor® pit-forming β2-AR cells, analysed using the Acumen Explorer laser scanning cytometer in 384 well plates.

## Introduction

The ability to detect and quantify physiological and biochemical events at sub-cellular level is critical in the research and development of new therapeutic entities and clinical diagnostics.

Various methods have been described for automatically measuring translocations of biomolecules from the cytoplasm of a cell to the nucleus at low screening throughputs[1-2]. These methods involve the measurement of translocation to a single, relatively large site, namely the nucleus. There is a need to measure localisations and aggregations to a number of much smaller sites in a cell.

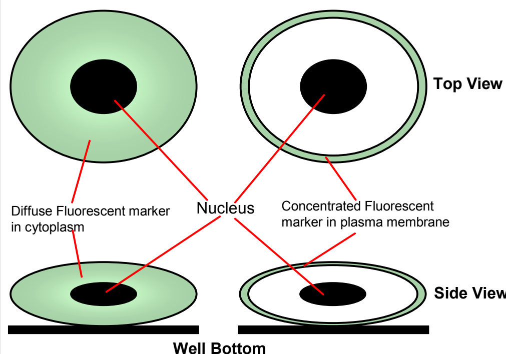
Methods of quantification of fluorescence in cells by image processing of digital images have been well described. Of particular relevance is the description of the application of a watershed algorithm to the quantification of immuno-stained dopamine-β-hydroxylase on cell-surface sites and in punctate chromaffin granules[3]. A review of general applications of watershed and similar segmentation algorithms is given in Roerdink and Meijster. (2001).

In this study we describe a simple thresholding and basin-filling algorithm for fluorescent biomolecule translocation between the cytoplasm and plasma membrane and demonstrate its application using a GPCR assay (Transfluor® technology).

## Norak Transfluor®

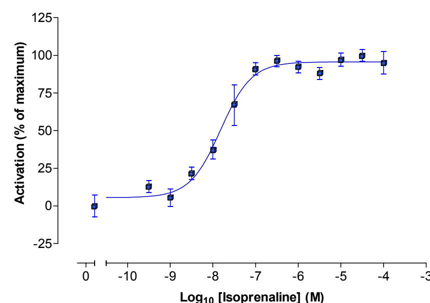
Transfluor® is based on the discovery that, upon activation by ligand binding, virtually all GPCRs rapidly undergo deactivation or "desensitization" by a common pathway. An early step in this pathway is the binding of the cytoplasmic protein arrestin to the activated receptor. Arrestin binding turns off the GPCR and initiates a process that brings the receptor into the cell where the ligand is removed before the receptor is recycled back to the membrane. By attaching a fluorescent label to arrestin, the recycling of the receptor-arrestin complex may be monitored. Since desensitization only occurs with an activated receptor, monitoring arrestin translocation within the cell provides a method to detect the activation of any GPCR*.

## 1 Schematic representation of movement of fluorescent marker



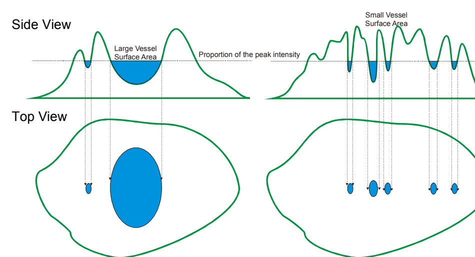

The protein is widely distributed in the cytoplasm. When scanned on the Acumen Explorer, the nucleus appears as an apparent 'hole' due to the displacement of fluorescent protein in the cytoplasm. When the cell is stimulated, the protein relocates to the plasma membrane, and due to the properties of the instrument, the concentration of labelled protein at the base of the well, results in the nucleus becoming obscured and therefore the 'hole' disappears.

## 4 Response of wt β2-AR-expressing cells to isoprenaline




$pEC_{50}$ = 7.82. Data represent means ± S.E.M of 4 replicates and representative of results obtained from 4 separate experiments

## 2 Thresholding and basin-filling algorithm across a single cell




Where the intensity profile falls below the contour level, a trough is formed. Each trough is considered to be a vessel that could be filled with liquid. A basin-filling algorithm fills each trough below the contour up to the point where the trough meets the contour. The algorithm is performed across the 3D surface of the image and cannot be performed by evaluating one slice at a time as illustrated, since the slices at the edges of a stimulated cell often have a similar profile to the central slice of an un-stimulated cell.
The areas of the tops of the filled troughs, where they meet the contour line, are calculated and summed for each cell. The summed surface area correlates with the degree of stimulation of the cells.

## 3 Fluorescence pseudo-images of pre- and post- translocated cells

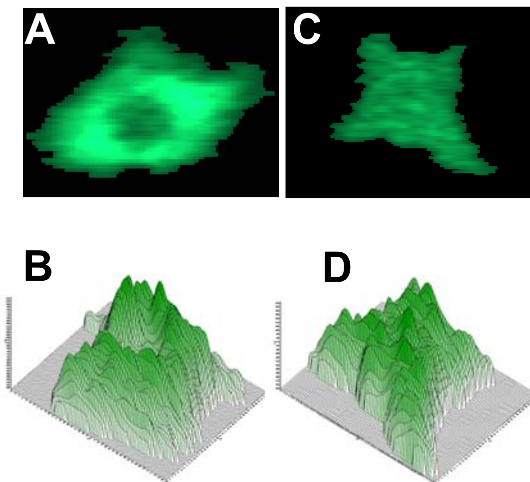



Figure 3A is an Acumen Explorer well view showing an unstimulated cell where the protein is located in the cytoplasm Figure 3B shows the corresponding 3D fluorescent profile of the same cell.
Upon stimulation, the fluorescent protein translocates to the plasma membrane and this 'obscures' the hole left by the nucleus. Figures 3C and 3D shows a well view and fluorescent profile of a stimulated cell.

## Conclusion

We have demonstrated that the novel variation on the watershed algorithm provides an efficient means of quantifying protein translocation using the Acumen Explorer. The present algorithm discriminates between cells where the fluorescent reporter is diffuse throughout the cell and where it is located at the membrane surface without recourse to a high magnification system, and with reduced computational effort over conventional approaches.

## References

1. Dunlay and Taylor. US patent no. 5989835:
2. Thastrup O. *et al.*, WIPO patent publication no. WO 98/45704:
3. Wick P. F. *et. al.*, (1997). Neuroscience, Vol. 80, No. 3, pp. 847-860.
4. Roerdink and Meijster. (2001). Fundamenta Informaticae 41 pp187-228.

* Running the Transfluor assay requires a separate patent licence that must be obtained from Norak Biosciences, Inc. for an additional fee. US patent Nos. 5,891,646 and 6,110,693. European patent No. EP1015608. Other patents pending.