# Image-Based High-Content Screening of Fatty Acid Uptake

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## Abstract

We demonstrate the screening and analysis of Fatty Acid Uptake (FAU) using a cell-based screening kit and the MetaXpress™ application module Cell Scoring. Long-chain fatty acids (LCFA) and their efficient uptake and distribution are critical to cellular function, both for ATP generation, and for the biosynthesis of membrane lipids. Since increased transport of fatty acid into pancreatic beta cells, cardiac myocytes and adipocytes can lead to diabetes, heart failure and obesity, FAU analysis is of interest for drug discovery. Existing FAU detection methods are time-consuming and incompatible for either high-content or high-throughput screening and usually require the use of radiolabeled compounds. Molecular Devices homogeneous QBT™ Fatty Acid Uptake Assay Kit provides a fast and simple fluorescence-based assay for the detection of FAU in cells expressing fatty acid transporters. The assay is ideally suited for both high-content and high-throughput screening and is demonstrated here using MetaXpress imaging acquisition and analysis software.

## Introduction

The QBT Fatty Acid Uptake Assay Kit employs a BODIPY fatty acid analog that behaves much like a natural fatty acid (FA) that accumulates in intracellular lipid droplets. This assay kit provides easier reagent handling as well as reduces disposal costs and safety risks to personnel since conventional radiolabeled compounds are eliminated. With the QBT FAU Assay Kit, true biological activity is observed. The kit is demonstrated here using MetaXpress imaging acquisition and analysis software.

## Materials and Methods

3T3-L1 adipocytes and fibroblasts were plated into 96-well black wall/clear bottom plates at ~50,000 cells per well in 100 µl growth media and incubated overnight. Cells were serum-deprived for 1 hour in phenol-red free media containing Hoechst 33342 (10 µg/ml) prior to 30 minute drug treatment. QBT Fatty Acid Uptake solution resuspended in 1XHBSS, 20 mM HEPES and 0.005% FA-free BSA was then added to the wells. Kinetic readings were started immediately on an ImageXpress® 5000A using MetaXpress image acquisition and analysis software. Images were acquired every 5 minutes for 30 to 60 minutes. Images were analyzed with MetaXpress, using both the Cell Scoring application module and custom journals. Companion data was generated on a FlexStation® II 384 using SoftMax® Pro software.

## Table 1: Filters

| Fluorophores | Excitation Filter | Dichroic Filter | Emission Filter |
|---|---|---|---|
| Hoechst 33342 | 360/40 | 51001bs (Chroma) | 465/30 |
| QBT FAU | 470/35 | 51008bs (Chroma) | 535/60 |

## Figure 1: FlexStation Results

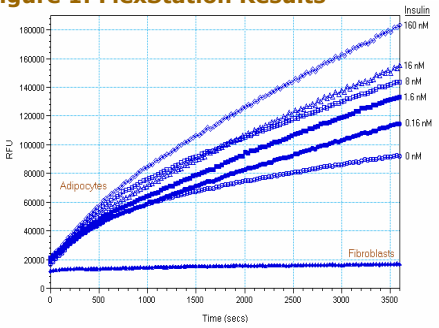


**Figure 1**. Time dependent FAU response to insulin. Representative data from 3T3-L1 adipocytes showed a concentration dependant increase in FA uptake. The data was generated on a FlexStation plate reader. Results shown in Relative Fluorescence Units (RFU).

## Figure 2: Imaging

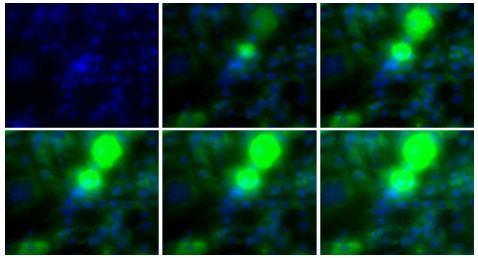

**Figure 2**. 3T3-L1 adipocytes treated with 435 nM insulin. Images were taken every 5 minutes for 30 minutes on an ImageXpress 5000A using MetaXpress image acquisition and analysis software.

## Figure 3: Analysis

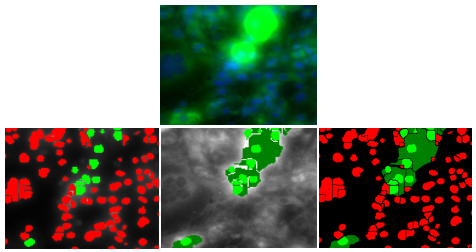


**Figure 3**. MetaXpress images were segmented and measured using the Cell Scoring analysis software module.

## Figure 4: ImageXpress 5000A Results

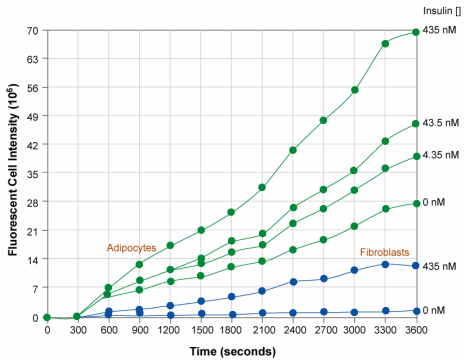


**Figure 4**. Effect of insulin on fatty acid uptake in 3T3-L1 adipocytes and fibroblasts. Images were taken on an ImageXpress 5000A using MetaXpress software for automated image acquisition and analysis.

## Figure 5: Drug Screening Results

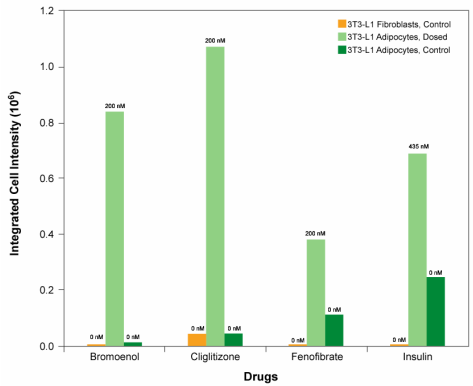


**Figure 5**. FAU responses using known inhibitors and activators of FA uptake from LOPAC1280™ plates were determined by the Cell Scoring module. 30 minute data point shown.

## Conclusion

We have demonstrated the feasibility of performing FAU assays with the QBT FAU Assay Kit and with High-Content Screening. Using an ImageXpress 5000A high-content screening system and MetaXpress software, the entire assay, including reagent addition, image acquisition, and image analysis, was fully automated. These results, which show interesting responses to compounds that may warrant further examination, demonstrate the utility of the Cell Scoring module in analyzing high-content data obtained using the QBT FAU Assay Kit.

## References

- Schaffer, J.E. (2002) Am J Physiol Endocrinol Metab. 282(2):E239-46.
- Liao, J., R. Sportsman, J. Harris and A Stahl (2005) J Lipid Res. 46(3):597-602

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