

Development of a CellProfiler pipeline to evaluate adipocyte differentiation

João Andrade^{1,2}, Sílvia Torres¹, Pedro Coelho^{1,2*}

1. Center for Translational Health and Medical Biotechnology Research (TBIO)/Health Research Network (RISE-Health), ESS, Polytechnic of Porto, Porto, Portugal; 2. ESS, Polytechnic of Porto, Porto, Portugal

*Corresponding author email: pvc@ess.ipp.pt

Introduction: Obesity is a complex chronic disease characterized by excessive body fat accumulation, with increasingly prevalence worldwide, burdening individuals and healthcare systems, thus urgent research is needed. (1,2) Adipocytes, the major cellular component of adipose tissue, are cells vastly used by the scientific community for *in vitro* studies of obesity. (3) Oil red O (ORO) staining and quantification is widely used for intracellular lipid staining and adipogenesis evaluation. Modern microscopy and image analysis software like CellProfiler enable efficient, high-throughput cellular image analysis, improving biological understanding and overcoming manual microscopy processing limitations. (4) **Objectives:** The present work aimed to develop an *in silico* image-based method to evaluate lipid accumulation along the differentiation and adipogenesis of adipocytes. **Methods:** Briefly, 3T3-L1 preadipocytes were differentiated with a cocktail of insulin (10 µg/mL), dexamethasone (1 µM) and 3-isobutyl-1-methylxanthine (0.25 mM) and maintained in culture for 12 days. Brightfield contrast phase images, before and after ORO staining, were captured every two days.

Lipid-droplet accumulation was evaluated by both CellProfiler analysis and ORO quantification. **Results:** Throughout differentiation, 3T3-L1 cells exhibited adipocyte-like morphological changes, with increasing lipid accumulation, detected by ORO staining. CellProfiler automated image analysis was comparable to ORO staining quantification, both detecting, approximately after day 4, the presence and accumulation of lipid droplets. **Conclusions:** The results showed that along differentiation of 3T3-L1 cells into mature adipocytes, CellProfiler evaluation of lipid accumulation provided similar results as ORO staining. Altogether, automated *in silico* image-based protocols can be used to investigate adipogenic differentiation *in vitro*, overcoming the demanding conventional quantitative methods.

Keywords: obesity, adipocytes, CellProfiler, image analysis

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