

A HIGH-THROUGHPUT PIPELINE FOR QUANTITATIVE IMAGE ANALYSIS OF THE COLONIC MUCUS SYSTEM IN SITU

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KEY WORDS: image analysis, colon, mucus, mucosal barrier, in situ, ImageJ, microbiota

The mucosal barrier is a vital and dynamic interface between the host organism and the external environment. This is especially true in the gut, which is colonised by trillions of microorganisms, that are at their most dense in the colon. The mechanisms involved in maintaining homeostasis in such a complex ecosystem without overt responses from the immune system are various and interdependent. Among them, the role of the mucus barrier is often overlooked due to limited understanding, but is becoming increasingly recognised as a critical component for promoting health and preventing disease, especially inflammatory bowel diseases that are becoming increasingly prevalent across the globe [1].

Despite it being known that the thickness and composition of this barrier are key determinants of its structural integrity and can therefore be used as proxies for protective functionality, there are few tools available to researchers to accurately analyze these key parameters. Historically, measurements of mucus thickness are laborious and cumbersome, and as a result limited to small representative sections of this highly variable structure with significant potential for experimenter bias.

To address this, we developed a high-throughput pipeline that allows for analysis of mucosal barrier integrity over large distances. This pipeline can be run automatically on optimally segregated images, or semi-automatically, allowing researchers to rapidly trace out regions of interest in images that would otherwise require laborious manual segregation. Furthermore, we have applied this tool to investigate variation in mucus thickness using a novel method for *in situ* analysis that uses mouse fecal pellets, rather than requiring sacrifice of the mice, which allows for longitudinal studies of the colonic mucus system over an unprecedented distance [2]. Importantly, with little alterations, our pipeline may also be applied to measure variations in curved biological structures and requires only FIJI, a free, popular, open-access software.

[1] J.S.H. Chan *et al.* "Gastrointestinal disease burden and mortality: A public hospital-based study from 2005 to 2014" *J Gastroenterol Hepatol*, **34**, 124-131 (2019).

[2] K. Bergstrom *et al.* "Proximal colon-derived O-glycosylated mucus encapsulates and modulates the microbiota". *Science*, **370**, 467-472 (2020).