# Cells with an appetite: munching at a single-cell level

Phagocytosis is defined as the receptor-mediated uptake of large particulate matter. This process is particularly efficient in specialized immune cells such as macrophages, neutrophils and dendritic cells. These cells constitute the first line of defense against invading microorganisms, which are internalized by phagocytosis and sequestered in a vacuole known as the phagosome, where they are destroyed. Additionally, phagocytes are capable of linking the innate and adaptive immune systems by presenting antigens to lymphocytes. Furthermore, phagocytosis is essential for the maintenance of tissue homeostasis. Phagocytes support the daily turnover of billions of cells, disposing of dead-cell bodies, a process that plays a paramount role in wound healing and tissue remodelling.

Mechanistically, phagocytosis is broadly divided into two major stages, phagosome formation and maturation. Phagosome formation, which entails the recognition and internalization of the target, is followed by the maturation of the phagosome, a process characterized by sequential fusion and fission remodeling events that provide the phagosome with the degradative properties necessary for the proper disposal of the engulfed particle.

Due to the multifaceted and complex nature of this biological process, traditional biochemical methods that depend on populations of large numbers of cells are inadequate to study its detailed molecular aspects. Unlike population-based assays, single cell assays provide high spatial and temporal resolution. The generation of sophisticated molecular biology tools have enabled the development of non-invasive and continuous measurements in live cells. These methods, coupled with specialized microscopy techniques and image analysis software, have given rise to powerful combinations to investigate phagocytosis at the molecular level. Here we describe methods to quantify some basic features of the phagocytic response.

Whenever possible, studying phagocytosis in primary cells is preferable; their responsiveness is unparalleled. Reactions such as phagosome acidification and the generation of reactive oxygen species are prominent and can be readily measured in primary phagocytes. Moreover, antibodies are available for detection of specific components by immunofluorescence. However, when genetically-encoded tools and routine genetic manipulations are required, the use of cell lines –such as immortalized macrophage-like cells– is recommended. Using such cells bypasses the need to isolate (and differentiate) primary phagocytes, a considerably more complex procedure.

In this chapter, we describe general methods to induce and quantify phagocytosis in both primary cells and myeloid cell lines. We begin by outlining the isolation of primary human monocytes and their differentiation into macrophages. Additionally, as an alternative model when specialized molecular biology techniques are required, we detail the culture of RAW 264.7 cells –an immortalized macrophage cell line original isolated from mice. We then describe the preparation of phagocytic targets, specifically sheep erythrocytes coated (opsonized) with immunoglobulin G (IgG), and detail means to distinguish bound from internalized targets, using different secondary

antibodies. Quantification of target binding and internalization is performed by fluorescence (preferably confocal) microscopy and the data are expressed as phagocytic efficiency or phagocytic index.

***Fernando Montaño, Roni Levin-Konigsberg, Sergio Grinstein***
*Cell Biology Program, The Hospital for Sick Children, Toronto, Canada*

## Publication

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