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Article

# Design of the prototype of a system for the quantification of fungal cells

# Diseño del prototipo de un sistema para la cuantificación de células fúngicas

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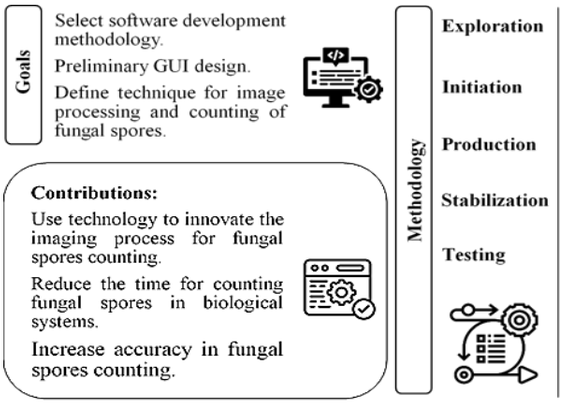
**Abstract**

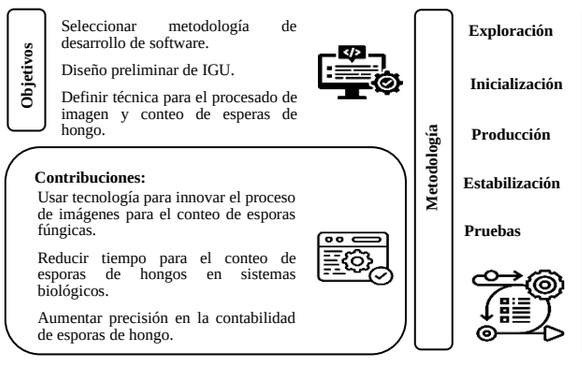
The initial purpose of this research is to design a mobile prototype that, through image processing, counts fungal spores in biological systems. With this, it is sought to make the execution of the process more efficient, as well as its accuracy, because the calculation carried out by the traditional method requires a lot of time and this continues to be not so precise. The Mobile-D methodology will be implemented, which consists of 5 phases: Exploration, Initiation, Production, Stabilization and Testing. This project has the scope of include the design of a prototype, derived from the fact that it is still in a preliminary development stage. In the future, it is intended to develop a mobile application that counts spores through the treatment of images located in a gallery within this tool, allowing you to select one to process it and perform the count that will show the results on the screen, indicating the number and highlighting with an indicative outline each element detected in the image.




**Prototype, Spores, Mobile-D**

**Resumen**

Esta investigación tiene como propósito inicial diseñar un prototipo móvil que, mediante el procesamiento de imagen, realice el conteo de esporas de hongos en sistemas biológicos. Con ello, se busca hacer más eficiente la ejecución del proceso, así como su exactitud, debido a que el cómputo realizado por el método tradicional requiere mucho tiempo y este continua sin ser tan preciso. Se implementará la metodología Mobile-D, que consta de 5 fases: Exploración, Iniciación, Producción, Estabilización y Pruebas. Este proyecto tiene como alcance incluir el diseño de un prototipo, derivado de que se encuentra aún en una etapa de desarrollo preliminar. Se pretende desarrollar a futuro una aplicación móvil que contabilice esporas a través del tratamiento de imágenes ubicadas en una galería dentro de esta herramienta, permitiendo seleccionar una para procesarla y realizar el conteo que mostrará los resultados en pantalla, indicando el número y remarcando con un contorno indicativo cada elemento detectado en la imagen.




**Prototipo, Esporas, Mobile-D**

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## Introduction

Fungi were among the first organisms to appear on planet Earth, approximately 300 million years ago. They do not have the ability to form tissues, they are considered ubiquitous because they have the ability or versatility to live in different environments: they can colonise land, water or even air.

The conditions for their development in the environment are based on physico-chemical variables such as humidity, temperature, altitude, light, aeration, pH, nitrogen ions, carbohydrates, etc. Each species will have specific requirements, with independent ecological niches, but the climatic conditions of the tropics favour their development.

Their main function in nature is to degrade dead organisms, therefore they are considered saprophytes. But they can also be symbiotic organisms, living in intimate association with other living organisms for mutual benefit. They are also considered parasites, as they feed on substances produced by other living beings, such as humans, by living in their internal organs or on their surface, and cause damage or disease (Gómez Daza, 2024).

For this work, the first step is the design of a mobile application that allows the versatile and automated counting of fungal spores in the region of the Huasteca Hidalguense. For the moment, in this first step towards the creation of the prototype, only the elements and functions that will integrate the graphical user interface will be defined; in future advances, the operational modules that will allow the use of this tool for its use in the field of research will be developed.

Currently, the procedure for counting fungal spores is performed in the traditional way using a Neubauer chamber. This process has been employed over the years and has been found to require a significant amount of time and effort to perform due to the multiple counts that a laboratorian must perform (Márquez, et al., 2013).

Accuracy depends entirely on the experience of the person examining a sample, as the procedure is done manually, but this can be improved with the use of technology.

This article includes the following sections: Problem, where the situations to be solved with this research are indicated; the justification points out the main purpose of the research where the benefits and impact expected to be generated are indicated; then, the objective to be achieved in this work is included.

The theoretical foundations include the main concepts that relate to and contextualise the reader with the subject of the study; the methodology indicates in an orderly manner the stages in which the research will be carried out; the development shows the work and tasks carried out in each phase of the project's development. The final sections include the results and conclusions of the research, as well as acknowledgements and references.

## Problem

Fungal spores are useful for improving various biological processes and systems, as well as preventing their negative effects. This is because some fungi offer great benefits, while others can be detrimental.

In the Huasteca Hidalguense there is a great variety of fungi, both edible and toxic. Some play an important role in the biological systems of the region, including plants, animals and humans, helping to keep them healthy, as long as they are not present in excess or harmful. A study revealed that there are five species of edible mushrooms in the region, identified by their white, purple and yellow colours. In contrast, toxic mushrooms, which are not suitable for consumption, are grey or red in colour (Cipriano, et al., 2019).

This fact is important, because the interaction with these organisms implies the need to know specifically how they expand their colonisation, in order to be used in different activities.

For this purpose, it is necessary to perform spore counting, a procedure that involves collecting a sample of a specific species of fungus. With the sample obtained, the total number of cells present is quantified. Once the number has been determined, their concentration can be assessed and a decision can be made as to whether or not it is beneficial to increase their population.

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This process requires the use of a haemocytometer (an instrument used in medicine and biology to count spores and cells). Once the sample is entered into the instrument, a section is selected for counting. An expert has to count the spores within that section one by one.

From the number obtained, an average of the estimated total of spores in the sample is obtained.

One of the main disadvantages is that counting requires a large amount of time and, by analysing only a part of the total sample, it may not give the most accurate result possible.

**Justification**

Having detected the need, the aim is to speed up the counting process, resulting as an initial proposal the design of a mobile application that will carry out this task, it will work by entering an image of the spores, so that, through the digital processing of the image, the computation is carried out.

With the help of this tool, the way in which spore counting is carried out would be innovated and speeded up, obtaining more accurate results and reducing the time required to carry out this process.

For the design of this prototype, various techniques will be used that allow for advanced and efficient image processing. Among the most prominent alternatives are **geometric transformations**, including scaling, rotation, transformation and perspective, allowing images to be adapted to different visual contexts. In addition, **image filtering**, particularly non-linear filtering, helps to remove noise without losing important details.

For deeper analysis, **feature detection and extraction** methods can be employed, such as contour detection, which highlights the boundaries of objects within the image. **Image segmentation**, on the other hand, allows the image to be divided into specific regions using models such as watershed, facilitating the identification of relevant areas.

Likewise, **object recognition** using deep learning offers a powerful tool to identify patterns and shapes within the prototype.

In terms of **image morphology**, skeletonisation is useful for simplifying complex shapes, and finally, **image enhancement** by adjusting contrast and brightness optimises the overall visualisation, providing sharper and more defined images. These combined techniques provide a holistic approach to improving the quality and functionality of the prototype. This project aims to generate a great impact by solving the problem detected through digital image analysis, thus giving the possibility to be implemented in other research fields where required, so it is feasible to invest time and/or resources in its development.

**Objective**

To develop a mobile application that implements artificial vision libraries, advanced image processing techniques and mobile application development environments to reduce the time and effort required to count fungal spores.

**Theoretical background**

**Fungi: Characteristics and importance**

Fungi are a group of living organisms devoid of chlorophyll. They resemble simple plants in that, with few exceptions, they have distinct cell walls, are usually non-motile, although they have motile reproductive cells, and reproduce by means of spores (García de la Rosa, 1990).

Like animals, fungi are heterotrophic organisms, which means that they must forage for food in order to survive. Faced with this pressure, throughout their evolution, fungi have developed effective and multiple survival and dispersal strategies, becoming a mega-diverse group whose distribution extends to practically all ecosystems on our planet (Heredia, 2020).

Throughout its history, man has always tried to know living beings in order to differentiate them by their usefulness, harm or to establish systems that allow him to identify them. The use of fungi has different applications, for example, in medicine, where Penicillium is used for the production of penicillin, which is an antibiotic used to fight infections; in the industrial sector, yeasts are used in the production of wine, beer and bread; as food, there are a large number of edible fungi, where mushrooms stand out, as well as other wild mushrooms such as huitlacoche.

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The vast majority of fungi are not harmful to humans, but those capable of producing toxins in food are. The high pathogenicity of fungi is found in vegetables, which are more susceptible to them (Universidad Autónoma de Ciudad Juárez, 2012). In order to study the communities of organisms and ecosystems, several research studies have been carried out. For example, fungi are the most studied organisms due to their role as primary decomposers and their participation in biogeochemical cycles (Cuadros et al., 2011).

## Fungi in Mexico

Mexico is a mega-diverse country in terms of groups of organisms, occupying fifth place in the world for its large number of species and endemisms, and has 10% of the planet's terrestrial diversity. As for the diversity of fungi, several investigations have been carried out in order to determine an approximate total of existing fungi. It is estimated that there are around 4,500 species of macro fungi and 2,000 micro fungi in the country, based on bibliographic reviews and specimens from collections. Based on estimation proposals made by different researchers, it is estimated that there are more than 200,000 species of fungi in Mexico, so that the amount currently recorded corresponds to 3.2% of the total number of fungi. (Aguirre, et al., 2014).

## Box 1

**Figure 1**

Huitlacoche, Mexico's representative mushroom

*Source: Gobierno de México, 2020*

## Mushrooms in Hidalgo

The state of Hidalgo has 1,138 species of fungi out of the 4,500 registered nationwide, according to the state diagnosis of biodiversity, prepared by the State Secretariat of Environment and Natural Resources (SEMARNATH), representing 25.3 per cent of Mexico's fungi varieties.

The study detailed that 15.9% of the mushroom species are used in local food, while 6% are used in local medicine, in addition to the fact that before the study began in 2017, there was no accurate data on the diversity of species that inhabit the state (García, 2019).

## Mushroom spores

Spores play a crucial role in fungi, just as seeds do in plants. They can be of every conceivable shape: smooth, warty, round, cylindrical, ellipsoid, nabiform, etc. In addition to their shapes, some have other very peculiar characteristics, such as a germinating pore, which can be central, apical, or marginal, larger or smaller. On the other hand, their measurements are unalterable, so that when comparing them with those of the same species they provide us with very reliable data. (Carranza, 2006).

## Box

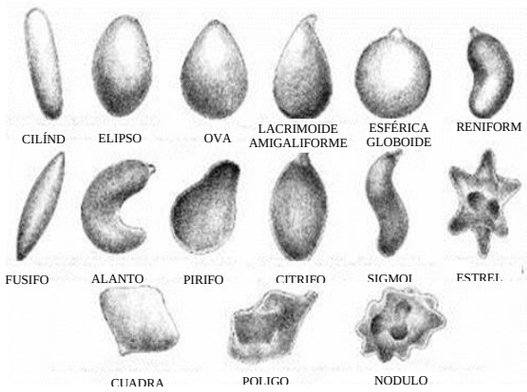


**Figure 2**

Different forms of spores

*Source: Bruns et al., 2002*

## Spore counting techniques

The Neubauer chamber or haemocytometer, shown in figure 3, is an instrument used for counting fungal spores and biological cells in a liquid medium (distilled water).

Once homogenised, the suspension is filtered through a mesh or gauze to remove agar or mycelial debris that might obstruct the passage of the suspension through the sprayer during inoculation, and brought to a known volume.

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