Crowd Sourced Taxonomic Identification Guide for Categorization and Quantification of Fungal Spores by Optical Microscopy

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Abstract

This contribution demonstrates an example of using a social network to create a visual search database to support human inspection of microscope imagery data of fungal spores. Current monitoring of airborne spore counts follows the standard test method for inertial impaction sampling (D7391-17e[1]) including spore identification, counting and subsequent statistical treatment. One bottleneck with the traditional aerobiology approach is the skill and time required to learn to identify unknown spores. Digital microscopy combined with the rapid proliferation of image sharing platforms offers a unique opportunity to harness this shared visual content for expanding education about key fungal spore type morphologies of interest to the environmental mycology community. A Pinterest board was developed that correlated with the D7391-17e[1] Standard as a form of public curation. Interacting with the board exploits the machine-learning embedded into the Pinterest platform and allows content to be aggregated from any URL and then suggests similar images based on existing content. In this way, the board can expand over time and image copyright is maintained despite becoming a shared resource. The paper concludes with a review of the statistical treatment of spore counts in order to maximise the value of the identifications.

Keywords: Spore Trap; Mould; Spore Identification; Indoor Air Quality; Pinterest; Crowdsourcing; Citizen Science; Non-Viable Methodology; Social networks

Introduction

Detecting and measuring indoor and outdoor airborne mould spores is of increasing importance due to their connection with sick building syndrome, occupational hygiene [1], and chronic respiratory conditions and as an adaptive response to climate change [2-4]. In many cases, indoor air quality assessments use spore traps (Air-O-Cell®, VersaTrap®, Allergenco-D, Via-Cell®) to compare the (i) indoor/outdoor ratio and (ii) inter-room variability pattern especially in known or suspect water-damaged buildings [5-6]. Spore traps are a class of viable

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and non-viable bioaerosol test where a pump is used to collect air onto a sticky microscope slide. After staining, the slide trace is analysed to classify the spore types and their concentration. Spore traps can be used for both fungal and plant spores, although this paper will only address mould spores. The general method is however equally transferrable to pollen.

There is an ASTM Standard, D7391-17e1 that defines which spore classifications are to be identified and counted and the method of quantification[7]. References for fungal spore identification using morphology are given, but access to such texts is not always easy, due to the fact that many books are out of print or difficult to acquire[8]. Therefore, the bottleneck to increased use of spore traps rests with the difficulty in sourcing accurate visual taxonomic guides to aid with training in identification. Since this is basically a database problem, the two largest social networks that focus on visual information, Pinterest and Instagram were reviewed.

The aim of this paper is to demonstrate how social networks like Pinterest can be exploited to produce a functional information rich taxonomic fungal spore database that evolves over time. The purpose is to support human visual inspection of image data–not to replace it and is an example of using new technology to improve spore monitoring that is encouraged by the Australian Airborne Pollen and Spore Interim Standard [9]. In practice, this takes advantage of embedded platform machine learning that suggests relevant, related and retrievable content and is a key component of the recommender system that is Pinterest [10]. The intent of spore classification is to quantify the level or concentration in the air. The second part of this paper therefore summarizes the calculations required.

Crowd Sourced Data from Social Media

Online content shared to social media increasingly includes user-generated scientific data. The proliferation of content producing platforms along with access to cheaper digital light microscopes opens up new opportunities for citizen science. This is premised on the assumption that ordinary people in the course of their daily lives collectively create meaningful user-generated content [11]. A crowd sourcing system uses humans to help solve problems based on their skills, experience and intelligence [12]. Social media services therefore present as a type of digital crowd intelligence because new participants contributing into the system introduce more data. Data quality is however 'noisy' and may be non-uniform or contain redundancy and inaccuracy. More

recently, visually oriented scientific disciplines like dermatology, plastic surgery and radiology have begun considering the what-if role that social networks like Pinterest can contribute to education [13]. These authors concluded that developing their own boards presented as an opportunity to expand radiology awareness and develop out medical resources. Similar visual communication research in the context of health promotion was also recently explored on Pinterest for skin cancer [14]; while educators are increasingly using social media as a way to facilitate engagement and improve learning [15].

Pinterest statistics as reported in March 2019 [16], showed there were 250 million active monthly users and 175 billion image 'pins' on Pinterest. In comparison, Instagram has a user base of over 1 billion with 95 million pieces of new content uploaded daily [17]. Content on Pinterest can be added from Instagram or from any web source with an URL and attribution of copyright is maintained.

It was found that Pinterest could be used to build a simple crowd-sourced database of fungal spores classified into each of the 13 different taxonomic groups as defined in the Standard and was built from a non-exhaustive search for relevant, retrievable content already existing on Pinterest, Instagram and the Web [7].

Pinterest also uses a search and recommendation system where visual search uses deep metric learning (feature extraction) to browse related content that defines a Pinterest taxonomy delivering personalized recommendations based on interest [18,19]. By searching for keywords embedded in the Standard for each of the different spore categories, the following tasks can be achieved following the explicit human intelligence model [12]:

➢ **Data filtering:** Only include light microscopy images. Ignore petri plates, SEM or artistic uses of biological data.

➢ **Data classification and tagging**: This involves categorizing the data and assigning tags into groups following Sections 12.3.2 and 15.2 of the Standard to include the minimum spore categories: (1) *Alternaria*, (2) *ascospores* (undifferentiated), (3) *Aspergillus*/*Penicillium*-like, (4) basidiospores (undifferentiated), (5) *Chaetomium*, (6) *Cladosporium*, (7) *Curvularia*, (8) *Dreschslera/Bipolaris*-like, (9) smuts/*Myxomycetes/Periconia*, (10) *Stachybotrys/Memnoniella*, (11) *Ulocladium*, (12)

hyphal fragments, and (13) at least one category of Miscellaneous/Unidentifiable spores.

➢ **Data clustering & segmentation:** Manual grouping of redundant data required since different magnification factors and crowd posting patterns may impact on how image data sets are populated.

➢ **Data selection:** This step involves pattern matching between the users' unique query (e.g. what is that spore under my microscope?) with the representative data from the Pinterest Board.

➢ **Personalized recommendations:** Each image is defined as a pin which is a visual bookmark of an interest group including a description and links to an external URL. Boards can be created that summarize what the pin represents.

Visual Search and Classification on Pinterest for Mould Spores

On Pinterest, a board with sections corresponding to each of the spore type categories was created. Pinterest boards can be private or public and can be populated with image contributions by others using direct upload or the Pinterest save button from the browser. Over time, this is used as a seed training set for a supervised machine learning solution. The Pinterest board database provided here is a virtual solution to the competency problem detailed in Section 13.4.1 of the Standard where inter-laboratory exchange is suggested for single-person organizations to gain experience and training in identifying known/reference content. The current version is shown in Figure 1 and is available here: https://mycology.io/9ip
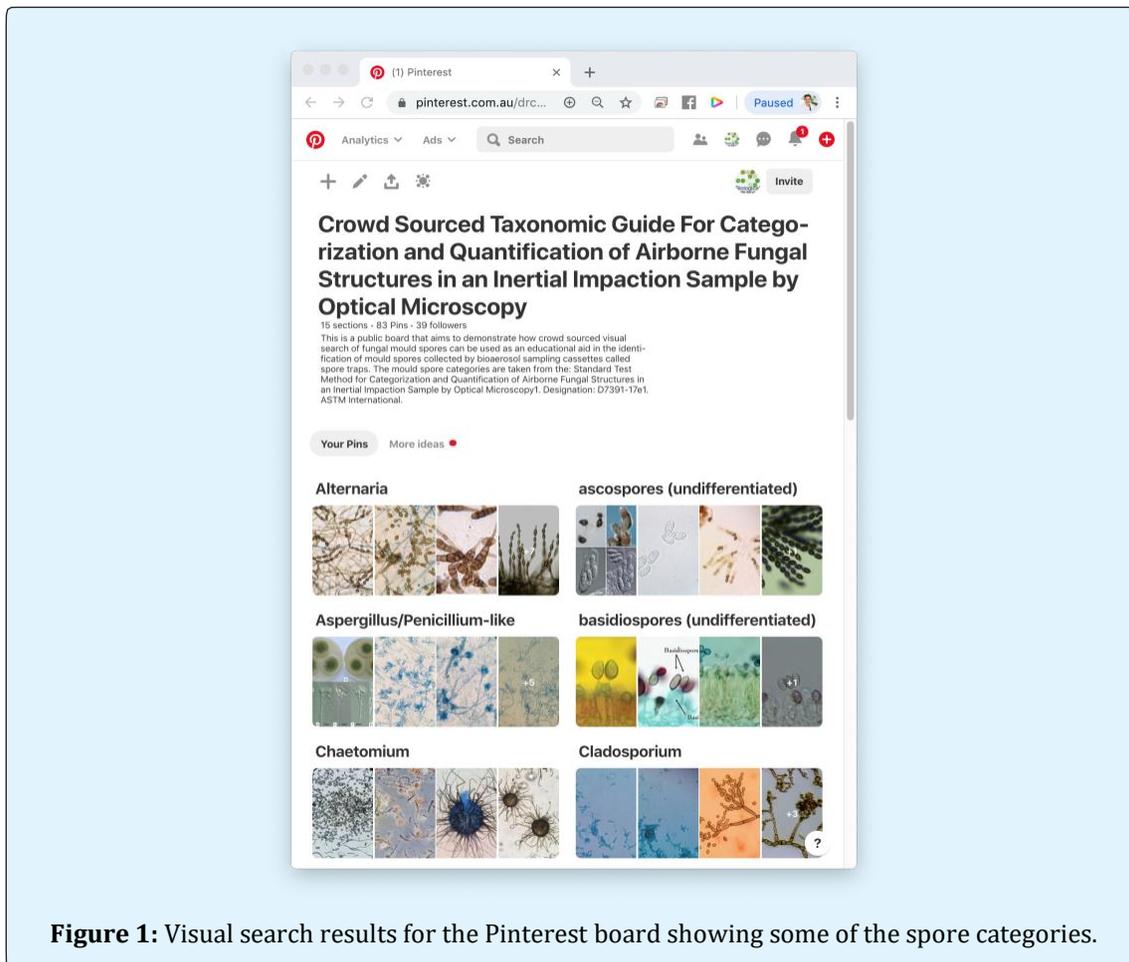
Figure 1: Visual search results for the Pinterest board showing some of the spore categories.

Other important information for using spore traps can be placed into the board as infographic pins. In this way, methods can be defined and presented in a visual format (Figure 2). https://mycology.io/g6d

Figure 2: Info graphic showing consensus published information for using spore traps to collect reliable information when sampling accessed from the Pinterest app within a mobile browser.

Calculation of Mould Spore Concentrations

Microscopic examination of spore traps evaluates the number and types of particles/spores counted per cubic metre of air. The calculation is based on the length of the deposit trace, the length of the trace that was looked at under the microscope, the magnification (e.g. x400), the volume of air collected and the number of particles/spores counted. Several references detail this calculation [20], in addition to the D7391-17e1 Standard, user manuals and example standard operating procedure-type documents [21-23]. It is important to note that very recent related documents for Hirst-type mould spore sampling detailed in the 2018 Australian Airborne Pollen and Spore Monitoring Network Interim Standard [9] and the 2019 EN 16968 have a width as well as a trace length which modifies the equations [24]. To minimize confusion due to different coefficient naming conventions used in the literature, several worked examples are provided below for spore traps of the Air-O-Cell® type.

Method from D7391-17^{e1} [7]

Step 1:

$$\text{percentage scanned} = 100 \times \frac{(\text{width of ocular reticle in } \mu m) \times (0.001 mm/\mu m) \times (\text{number of traverses})}{(\text{length of deposit in mm})}$$

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Step 2:

$$\text{fungal structures/sample} = 100 \times \frac{\text{(fungal structure count during traverse)}}{\text{percentage scanned}}$$

Step 3:

$$\text{fungal structures/m}^3 = \frac{\text{(fungal structures/sample)}}{\text{volume (m}^3)}$$

In practice, one needs to know the volume in litres of air that was sampled, the number of traverses that are or were counted under the microscope and the field diameter at the chosen magnification which is defined by the ocular reticle width. Note that different microscopes have different field diameters depending on the arrangement of magnifying lens and ocular power. It is also known that digital microscopes may have a smaller field of view diameter than traditional optical microscopes and this needs to be taken into consideration when applying the above steps as required [25].

Example from D7391-17[e1]
Spore trap cassette (deposit length=14.4mm), 75L volume being 15L/min for a 5-minute sample period, 12 traverses, ocular reticle width = 240 μm, one fungal structure observed as the minimum reporting limit.

Step 1:

$$\text{percentage scanned}=100\times\frac{\left(240\mu m\right)\times\left(0.001mm/\mu m\right)\times\left(12\right)}{\left(14.4\,mm\right)}=20\%$$

Step 2:

$$\text{fungal structures/sample}=100\times\frac{\left(1\right)}{20\%}=5$$

Step 3:
If one spore is counted then

$$\text{fungal structures/m}^3=\frac{5}{\left(0.075\,m^3\right)}=66.66$$

Method from the Air-O-Cell Lab Manual [21]

Step 1:

$$\text{Air volume (m}^3) = \left(\text{sample rate in L per minute}/1000\right) \text{ x number of minutes}$$

Step 2:

$$\text{Trace length counted (mm}^2) = \text{microscope field diameter (mm) x number of traverses}$$

Step 3:

$$\text{Concentration of particles (Cts}/m^3) = \frac{\text{Trace length (14.4mm)}}{\text{Total length of trace counted}} \times \frac{1}{\text{air volume}(m^3)} \times \text{number of particles}$$

(from Step 2)   (from Step 1)

Example from the Air-O-Cell Lab Manual

The same values as used above for the spore trap cassette deposit length of 14.4mm, 75L of air, 12 traverses, ocular reticle width=240μm, one fungal structure observed as the minimum reporting limit was applied.

If one spore is counted then

$$\text{Concentration of particles (Cts / m}^3\text{)} = \frac{(14.4\text{mm})}{(0.24 \times 12)} \times \frac{1}{0.075} \times \text{number of particles} = 66.66$$

Method from Baxter, et al. [20]

$$C_p = \left(\frac{L}{DN} \right) \times \left(\frac{P}{QT/100} \right)$$

Where

C_p = concentration of particles per cubic metre of air (Cts/m³)
P = number of particles counted
L = length of entire deposition trace (mm)
D = microscope field of view and traverse width (mm)
N = number of sample traverses counted
Q = sample flow rate in litres per minute (L/min)
T = time (min)

Example from Baxter, et al. [20]

If we change the values to account for different combinations of stage and ocular types, we might see 350 μm as the field of view. Leaving the other values the same, we find that,

If one spore is counted then

$$C_p = \left(\frac{14.4}{0.35 \times 12} \right) \times \left(\frac{1}{15 \times 5/100} \right) = 45.70$$

Method from Allergenco D Standard Operating Procedure [23]

In another example, the field diameter based on the stage/ocular micrometer reading could be 450 μm. Plugging in these values and keeping the remaining variables the same as used previously it is found that

Step 1:

$$\text{Volume tested} = \text{Flow rate x Duration}$$

Step 2:

$$\text{Fraction counted} = \frac{(\text{Field diameter x number of traverses})}{(\text{trace length})}$$

Step 3:

$$\text{Volume analyzed} = \text{Fraction counted x volume tested}$$

Step 4:

$$\text{Spores per m}^3 = \frac{\text{number of spores counted}}{\text{volume analyzed}}$$

Example from Allergenco D Standard Operating Procedure

Where:
Field diameter = 0.45mm
Trace length = 14.4mm
Number of traverses = 12
Flow rate = 0.015m³/min
Duration = 5
Step 1:
Volume tested = 0.015m³/min x 5 = 0.075m³
Step 2:
Fraction counted = (0.45 x 12)/14.4mm = 5.4/14.4 = 0.375
Step 3:
Volume analyzed = Fraction counted x Volume tested = 0.375 x 0.075
Step 4:
If one spore is counted then

$$\text{Spores per m}^3 = \frac{1}{0.028125} = 35.55$$

If two spores are counted then

$$\text{Spores per m}^3 = \frac{2}{0.028125} = 71.11$$

Context of this Work

Spore trap identifications and calculations can be problematic due to human error and other factors that are known to contribute to variability seen in results [26-28]. A range of guidance thresholds have been published that help interpret overall levels of mould spores in the air [5,29]. Despite often using different sampling methods, mould levels are commonly reported as colony forming units per cubic metre (CFU/m³) with implied equivalency to spores/m³. Results interpretation is beyond the scope of this paper, but useful threshold classification criteria are detailed in the literature at between 500-1000-1500 spores/m³ in terms of flagging for lower and upper levels of risk [5,20,30-38]. In certain circumstances, the use of image analysis has been shown to compare favourably with manual methods for spore counting [39-41]. However, the importance of using a standardized field of view and how the magnification relates to the observation window that is counted has been shown to be a similar practical problem encountered in the microscope assessment of cancers using the mitotic count in histology [42]. Finally, the fungal spore diversity and concentration per cubic metre is the important index often used for classifying buildings and environmental sampling for

allergen risk from bio aerosols, or in terms of indoor mould counts following for example, mould remediation works [43,44]. Improved and free methods for aggregating and retrieving visual information that help with fungal spore identification and statistical calculations are therefore of value to fungal aerobiology.

Conclusion

Pinterest is a strong visual search tool to exploit since it takes advantage of an embedded recommender system, meaning that related pins with similar content will be suggested to the user. This then introduces new and related content based on similarity and driven by artificial intelligence. Incorporating social media platforms into education has been shown to act as a facilitator for improving learning proficiency, especially by empowering social collaboration. A Pinterest board was successfully developed and populated with seed images following the D7391-17e1 Standard with the aim of making this test method easier to perform and statistically interpret.

Acknowledgments

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QR Codes

Pinterest spore database

Spore trap guidelines