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Pollen Identification and Concentration Estimation Using High Resolution Imaging and Deep Learning

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Abstract— Climate change, coupled with the rise in pollen concentrations and extended pollen seasons, poses significant challenges to public health, particularly for individuals with pollen allergies and/or respiratory diseases. In response to these challenges, the work here's objective is to pioneer an automated trainable system for the recognition and counting of pollen grains. Such a system would enhance efficiency, allowing for quicker and more accurate assessments of airborne pollen concentrations, ultimately aiding in the mitigation of allergy symptoms associated with changing environmental conditions and the spread of allergenic species. This approach utilizes image processing tools to segment pollens on digitalized slides and several deep learning tools to recognize them among 17 different allergenic species in total. The system has been designed to avoid the time spent by palynologists on the microscope and to considerably increase the number of observation sites above the current European standards in allergenic pollen concentrations evaluation. In turn reducing associated errors with such analysis.

Keywords— *Climate change, Deep learning, Allergy*

I. INTRODUCTION

Pollen allergies and respiratory diseases are expected to become a major public health issue due to increased pollen concentrations and extended pollen seasons, exacerbating allergy symptoms [1].

The spread of invasive species like *Ambrosia artemisiifolia* (ragweed) introduces highly allergenic pollen to new regions, further exacerbating the issue. To address these concerns and mitigate pollinosis symptoms, daily identification of airborne pollen using computer vision methods is crucial. Currently, the standard approach involves collecting airborne pollen with a Hirst-type trap, where pollen and other particles adhere to an adhesive tape. Subsequent analysis, illustrated in Fig. 1, involves chemical treatment and

manual microscopy inspection by a palynologist. This method, commonly known as the Hirst method [2], is the standard for monitoring airborne pollen grain concentrations in ambient air for aerobiological networks. However, it is time-consuming and labor-intensive for detecting and quantifying pollen grains.

Automated airborne pollen recognition can assist palynologists during the annotation process in the Hirst method by automatically annotating pollen grains. Depending on the country, not all species of pollen are present in the air [3], as some plants or trees emitting specific airborne pollen may not be found in the surrounding area. In France, the Réseau National de Surveillance Aérobiologique (R.N.S.A.) [4] coordinates the weekly analysis of airborne pollen content from over 70 sites across the country, averaging 35 weeks per year. They identify 17 allergenic taxa of airborne pollen and provide a weekly pollen risk index.

Manual recognition by palynologists is challenging due to the presence of up to 10 different pollen species on a single slide, with overlapping seasonal patterns. Identification relies on size, shape, contour, texture, and pore count, but some species share similar features, making accurate labeling difficult. Therefore, a large dataset of well-labeled pollen grains is crucial for developing an effective computer vision recognition model [5].

Our goal is to propose a procedure adhering to Hirst method standards by (i) generating a large dataset of well-labeled allergenic pollen grain images and (ii) developing a computer vision model to recognize and quantify airborne pollen grains.

II. RELATED WORK

There are two steps to achieve the recognition of pollen on a microscopy slide, the first one is the segmentation of pollen

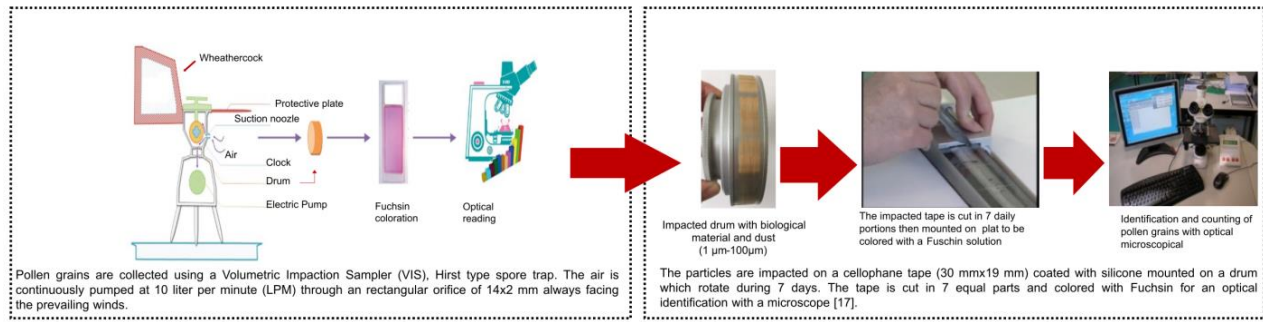


Fig. 1. Illustration of the Hirst Method used to evaluate allergenic pollen concentrations in the air.

grains and the second one is the identification of the pollen class.

In the segmentation phase, pollen grains are stained with fuchsin (a pink dye) during slide preparation. The primary challenge is addressing multifocal information, as neighboring pollen grains may not lie in the same focal plane, rather than grain extraction itself. Consequently, some studies employ advanced techniques like Convolutional Neural Networks (CNNs) with multifocal information merging to resolve focal plane discrepancies [6]. Others utilize Coarse-Grained Pollen Localization [7] with sliding windows to determine the optimal focus for each pollen grain. These approaches generate individual pollen grain thumbnails for subsequent identification.

Early classification methods relied on morphological feature extraction, followed by SVM or K-NN classifiers [8] operating on these parameters. The main challenges involved automating and fusing data like size, shape, aperture count, and texture. However, due to frequent morphological similarities between pollen grains and other particles (e.g., moisture, dirt), a reliable approach is to first distinguish pollen from non-pollen items, then utilize morphological features for accurate pollen family identification [9].

Recent works [10, 11, 12] favor CNNs for classification due to their superior performance. A survey [13] compares state-of-the-art CNN architectures (ResNet-50, Inception V3, DenseNet-121) for pollen identification, revealing that DenseNet-121, fine-tuned on pollen datasets [14], excels in this task.

TABLE I. DATA SAMPLES DISTRIBUTION WITH ILLUSTRATION

Pollen samples		
Dataset Name	Number of Classes	Number of images
Pollen13K [15]	4	13.000
Pollen23E [16]	23	805
Pollen20L-det [17]	20	7.750

In the literature, there are commonly three datasets used to train CNN. The specification of each dataset is resumed in table 1.

The Pollen13K and Pollen23E have microscopic images without the use of fuchsin, the Pollen20L-set stands closer to Hirst standard, however the target classes in this dataset do not cover all the allergenic species in France.

The use of Fast R-CNN and YOLO models for simultaneous segmentation and classification has been explored [18], demonstrating reliable classification but noise-sensitive pollen grain detection.

Some commercial pollen measurement devices utilize holographic imaging and fluorescence measurement [19] for pollen identification, offering reduced noise sensitivity. However, their high cost and the need for expertise in holographic pollen recognition limit their accessibility. Alternatively, more affordable devices employing multi-angle lighting in visible and UV wavelengths [20] generate pollen images compatible with recognition techniques developed for Hirst method slide samples.

Therefore, this work focuses on developing a pollen classification method using visible spectrum particle slide images. The segmentation step involves: (i) utilizing high-quality colored images stained with fuchsin, (ii) employing Laplacian information as a blur metric to select the optimal focal plane for each pollen grain, and (iii) combining pollen grain circularity, size, and hue properties for segmentation.

Regarding the classification step, the Imagenet-1K database [21] is used to pre-train a DenseNet121 CNN which is fine-tuned on the considered pollen dataset. To avoid the tedious labelling work required with supervised deep learning methods, the CNN is trained on data extracted from “exemplar” slides with a single pollen grain type and then is generalized to recognize pollen classes on “ambient” slides with several different pollen types on the same image. This approach yields a supervised computer vision algorithm that can automatically estimate the concentration of 17 different pollen classes.

III. POLLEN RECOGNITION

A. Image Acquisition

Fresh pollen from trees and herbs were collected and fixed on microscopy slides following the actual normative

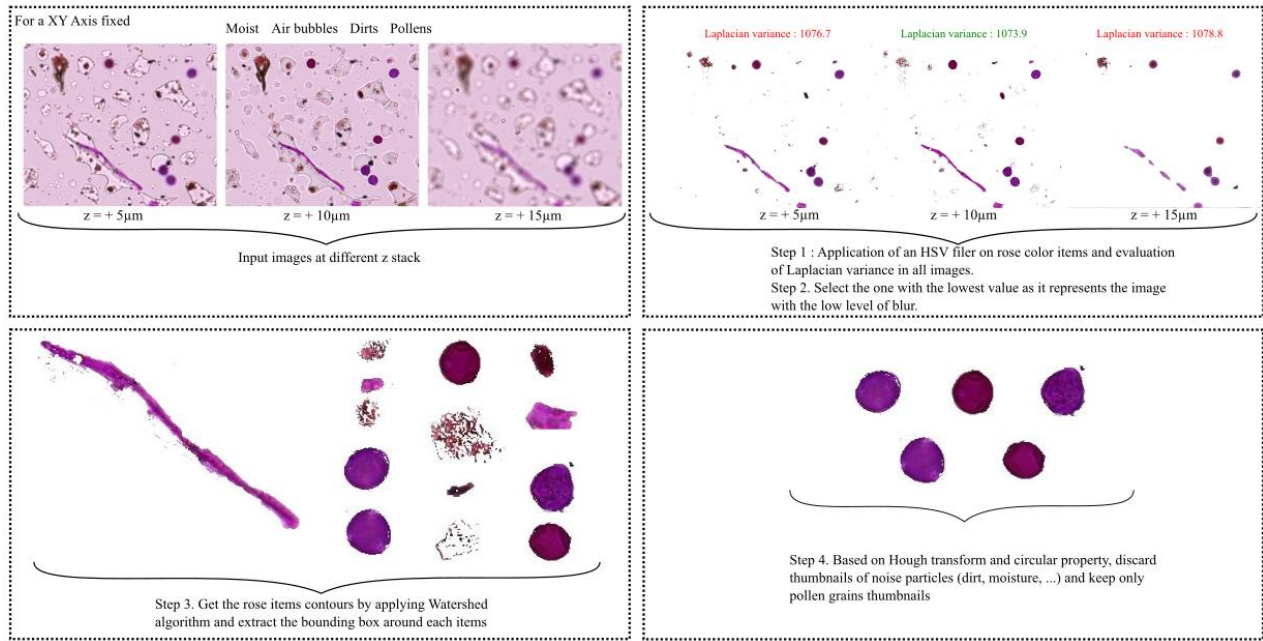


Fig. 2. Illustration of the process to segment best quality of pollen thumbnails

procedure for pollen identification and counting. We used the fuchsin to highlight the pollens (pink color) by applying it to each slide. A SQ Nanoscope (Hamamatsu, France [22]) was then utilized to digitalize the entire sample surface as illustrated Fig. 2-top-left.

In this work, ambient slides and exemplar slides are considered during the acquisition process. Ambient slides are slides where different kinds of pollen grains are collected outside along with possible dirt or moisture (cf. Fig. 2-top-left). On the other side, to avoid the fastidious human labelling task, slides with one pollen grain type only have been generated in lab conditions. As a consequence, the labelling process of those exemplar slides is very easy since only a single pollen species is present.

The scanner produces high-resolution images using a x20 microscope objective. We decide to digitalize each slide into images of 4096 x 4096 pixels. The scanner also takes a bunch of images at 31 z stacks, with a minimum space of 1 μm in a range of + or - 15 μm from where the scanner does its auto focus. 17 exemplar slides were collected, one for each pollen species, resulting in 31 x 338 associated images of 4096 x 4096 pixels per class, representing the slides along the X, Y, and Z axes.

B. Pollen Image Segmentation

Given that the scanner provides a stack of images with varying focal planes for each image (cf. Fig. 2 top-left), it is essential to select the area with the best focus before segmentation.

For each image, pollen grains are filtered using upper and lower thresholds applied to the rose-fuchsia hue of pollen within the HSV (Hue, Saturation, Value) color space. Simultaneously, a bitwise mask is used to convert all pixels except those with pink hues to a white value. Consequently, each resulting image contains only rose-tinted objects, including pollen grains as well as other particles of similar hue (e.g., moisture, bacteria).

The variance of the Laplacian calculation is then applied to the image stack to assess the level of blur in each image [23]. Blurred images exhibit low variance; therefore, to obtain the best-focused image for each slide, the image with the lowest Laplacian variance score is selected.

After extracting the image with the optimal focus on pollen grains, a Watershed Algorithm [24] is employed to delineate the contours of each item present in the image, including pollen grains and noise such as moisture, bacteria, and air bubbles. To isolate pollen grains from noise, a circular Hough transform [25] is applied, as pollen grains typically exhibit a circular shape, unlike noise particles. Fig. 2 (steps 1 to 4) illustrates this process for some segmentation results. The Hough transform also enables the elimination of any remaining pollen grain clusters after the Watershed process.

C. DenseNet 121 CNN Fine Tuning for Pollen Grain Recognition


















A labeled dataset was constructed by extracting pollen grains from 17 exemplar slides, each containing a different pollen species. Table 2 presents the distribution of samples among the 17 species, along with an example image of each species' pollen grain.

As evident, the classes for the identification training process are imbalanced, as an equal number of pollen grains were not collected for each species to produce the exemplar slides. Prior to the training loop, several data augmentation techniques were applied :

- The height and width of each thumbnail were rescaled by multiplying them by a randomly chosen ratio between 0.9 and 1.1. This enhances the model's robustness to variations in pollen size.
- Random hue variations of ± 0.005 and brightness variations of ± 0.01 were applied to ensure robustness to variations in grain color.

- A low-pass Gaussian filter with a kernel size of 19 was applied to enhance robustness to image blur.
- Horizontal and vertical flips, as well as rotations of 90, 180, and 240 degrees, were incorporated.
- White pixel padding was used to resize each pollen grain image to 128 x 128 pixels, ensuring uniformity within the dataset for the training process.

TABLE II. DATA SAMPLES DISTRIBUTION WITH ILLUSTRATION

Pollen samples		
<i>Selected Taxa</i>	<i>Number of Thumbnails</i>	<i>Illustration</i>
Ash	9320	
Ragweed	8578	
Olive	5481	
Birch	5359	
Grasses	5257	
Willow	3976	
Plane	3675	
Cypress	3601	
Sorrel	2959	
Alder	2942	
Plantain	2496	
Mugwort	1779	
Nettle	1296	
Linden	1012	
Hornbeam	780	
Pine	767	
Oak	380	

For pollen recognition, the DenseNet-121 CNN, pre-trained on ImageNet-1K, was adapted and fine-tuned using our dataset. Only the final layer was modified and retrained to perform the 17-class classification.

The model was trained using 80% of the dataset, with the remaining 20% reserved for evaluation. Stochastic Gradient Descent (SGD) optimization was employed with a learning rate of 0.001, a batch size of 64 and 60 epochs.

Fig. 3. Metrics of training and validation of the model.

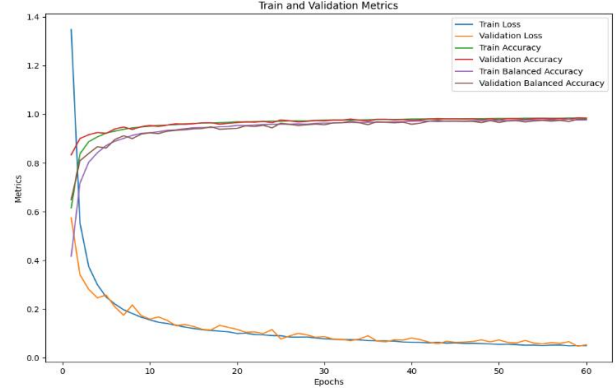


Fig. 3 illustrates the progression of training and validation accuracies over epochs, with the validation accuracy reaching 98.45%.

Detailed results of the validation test can be observed in the confusion matrix (cf. Fig. 4.). Our model demonstrates the ability to recognize nearly all labels, even for pollen species with limited thumbnail samples (such as Oak and Hornbeam).

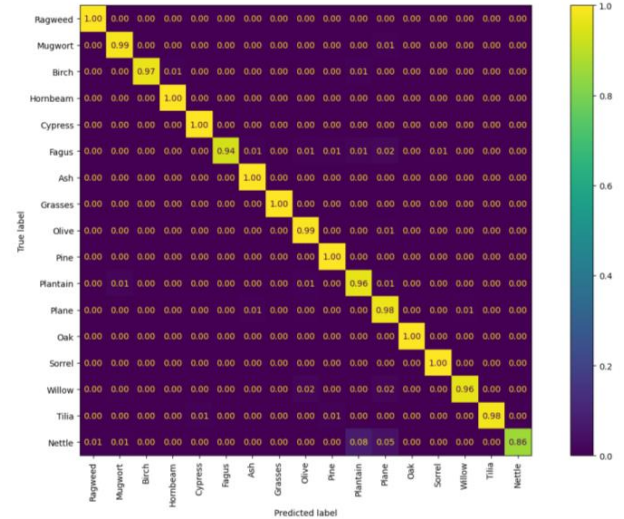


Fig. 4. Confusion matrix of the validation set.

D. Pollen Grain Recognition on Ambient Slides

Following the training of the model on exemplar samples, it is crucial to assess its generalization ability in recognizing pollen grains extracted from ambient slides. To this end, an ambient slide acquired during the peak of Birch pollen emission in the spring in Paris was considered. A total of four different species were present for identification: Birch, Cypress, Pine, and Willow.

Labeling each pollen thumbnail within the test dataset, consisting of nearly 500 thumbnails, would be excessively time-consuming. Therefore, the classification step was performed using our CNN model, and misclassifications were manually counted to generate the confusion matrix for the test dataset. The accuracy reached 94.1%. The model appears to

exhibit a slight bias towards Birch pollen due to the larger quantity of Birch pollen thumbnails in the training set. This bias could potentially be mitigated by employing adjusted class weights during training. Further investigation with additional ambient test slides and other allergenic species is warranted to validate these findings. The complete confusion matrix is presented in Fig. 5.

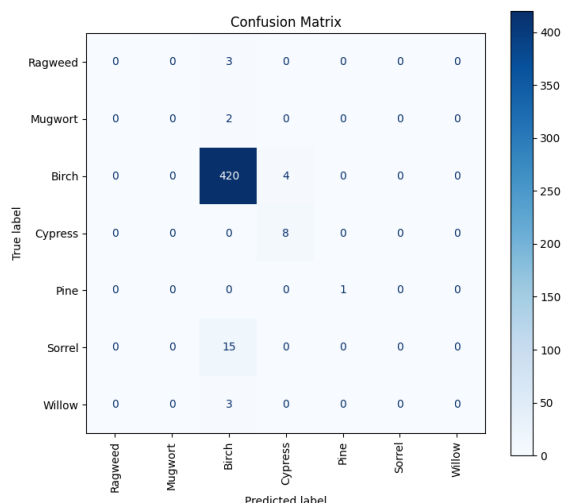


Fig. 5. Confusion matrix of the ambient test set.

IV. CONCLUSIONS

For the purpose of automatic pollen recognition, this study successfully developed an efficient CNN-based model trained on images acquired from exemplar slides, thereby avoiding the need for tedious manual labeling. The results demonstrate that the developed model generalizes well, maintaining comparable performance in classifying multiple pollen types on ambient slides. These results indicate that this automated computer vision approach can facilitate the development of a real-time pollen concentration estimation system, which is essential for predicting the allergenic exposure risk for the population according to the Hirst method standards.

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