Basic microscopy, calculating the field of view, scanning of slides, sources of error

Podstawy mikroskopii, obliczanie pola widzenia, analiza preparatów, źródła błędu

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Abstract

Airborne pollen samples are examined under a light microscope, by means of 250x or 400x magnification. To calculate the whole sample surface, the diameter of the microscopic field has to be given. There are four basic calculation methods. An investigator has to bear in mind possible sources of error, which can be associated with the reading method, sample interpretation and a preparation of a slide.

Key words: *light microscopy, pollens, field of view.*

Streszczenie

Preparaty ziaren pyłków roślinnych analizuje się z użyciem mikroskopu świetlnego, pod 250-krotnym lub 400-krotnym powiększeniem. Do obliczenia całej powierzchni próbki wymagana jest wartość średnicy pola mikroskopowego. Istnieją cztery podstawowe metody obliczeniowe. Badacz powinien pamiętać o możliwych źródłach błędu, związanych z metodyką, interpretacją próbki oraz przygotowaniem preparatu mikroskopowego.

Słowa kluczowe: *mikroskopia świetlna, ziarna pyłku, pole widzenia.*

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Airborne pollen concentration calculation

Samples are examined under a light microscope with varying magnification; the routine counts are carried out by means of 250x or 400x magnification.

The count of the pollen grains is not a total figure for all of the sampling surface, but is calculated statistically; that is to say that it is only calculated from a fraction of the entire sampling surface. The number of pollen counted on each sample must be expressed in daily average pollen grains per cubic metre of air.

We should remember that the trap continuously sucks in 10 l. min^{-1} and therefore 14.4 m^3 of air per day. All the pollen contained in this volume are deposited over the total surface of 672 mm^2 of the tape which represents the surface of a daily sample (14 x 48 mm). Knowing the total surface examined, it is easy to convert, with a simple proportion, the number of pollen recorded under the microscope in the number of pollen present in the entire daily sample.

For the date to be statistically significant, the examined surface should not be less than 10–12% of the entire sample (therefore 67–80 mm^2); moreover a study of more than 20% (> 134 mm^2) does not provide a greater significance and does not justify the further time employed.

Exercise:

- We have examined 4 horizontal lines and counted **360** pollens of grasses
- The diameter of the microscopic field is 0.60 mm
- The flow of trap is 10 l/min and therefore **14.4** m^3/day

1. Examined surface: 0.60 (diameter) x 48 (length 1 horizontal line) x 4 (no. of lines) = **115.2** mm^2.
2. Ratio between: total surface of sample/examined surface: 672 mm^2 (14 x 48 mm)/115.2 mm^2 = **5.8**.
3. Convertion of number of pollen counted in the daily slide in number of pollen in 1 m^3 in that day: 5.8 x 360/14.4 = 144 p/m^3 of grasses.

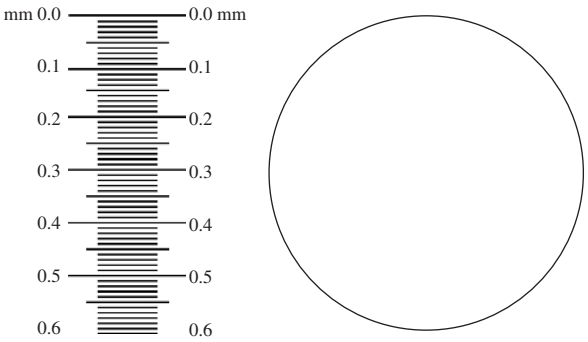

Fig. 1. Microscopic field (circle) measured by a microscopic slide with a printed micrometric scale (i.e. 0.6 mm)

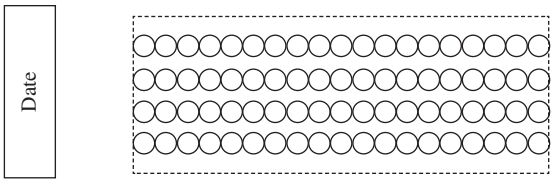

Fig. 2. Pollen counting methods: tangent fields

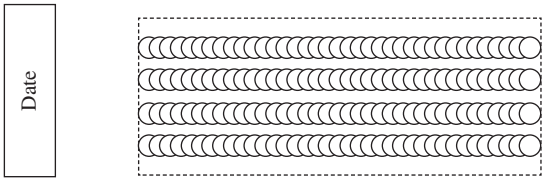

Fig. 3. Pollen counting methods: continuous sweeps

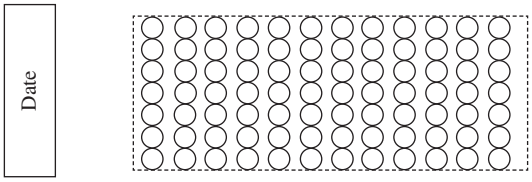

Fig. 4. Pollen counting methods: vertical sweeps

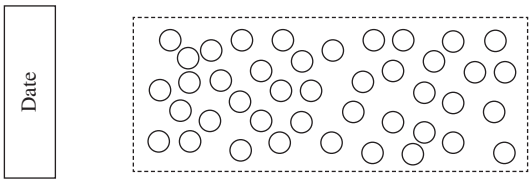

Fig. 5. Pollen counting methods: random fields

Calculating the field of view

To make the calculation for the entire sample surface, it is necessary to know the diameter of the microscopic field used. This can only be correctly measured by using a microscopic slide with a printed micrometric scale or by use of an ocular scale (fig. 1.). If these are not available, it is possible to count the diameter, with a reasonable degree of accuracy, dividing a known length (i.e. the width of the microscopic slide) by the number of microscopic fields necessary to scan the whole length.

The microscopic field diameter is a characteristic of each microscope and of each magnification used. Knowing the diameter it is easy to calculate the field surface and, multiplying by the number of fields examined, to calculate the fraction of tape scanned.

Counting methods

Four scanning methods are in use: 1, horizontal sweeps; 2, tangent fields; 3, vertical sweeps; 4, random fields.

I. Tangent fields (fig. 2.) – With this method successive tangent fields positioned on 3 or 4 or 5 lines separated by a space of about 2 mm are examined. After having counted the pollen in one field, the slide is moved to the next tangential field. Depending on the number of lines counted and the diameter of each field, to calculate the pollen concentration per cubic metre of air, a factor is calculated which depends on the relation between the total examined surface and the total surface of the tape. Using this method it is also possible to record the presence of pollen at a specific moment in the day and the examination of the sample occurs holding the microscopic field still, without moving it across as occurs when examining the horizontal sweeps.

II. Horizontal sweeps (fig. 3.) – The sample is examined scannering 3 or 4 or 5 horizontal lines separated by a space of about 2 mm, to avoid overestimation or empty areas. The surface of each examined line is obtained multiplying 48 mm (length of daily tape) by the microscopic field diameter. Scanning of horizontal sweeps follows the direction of rotation of sampling tape and enables the recording the variation during the 24 hour period. The drum rotates 2 mm per hour, therefore the pollen caught in 1 hour is deposited on a surface of 2 x 14 mm; recording the movement of the slide using the scale indicated on the slide console, it is possible to record the occurrence of every pollen type at a specific time of the day and the elaboration of daytime distribution patter.

III. Vertical sweeps (fig. 4.). The slides are examined in 24 transversal lines at intervals of 2 mm one from the other; each one is 14 mm high and as wide as a microscopic field. In this way a line is read for every

hour. In this method the choice of the position of the lines could influence the final result expressed as average daily concentration, because the concentration between the sweeps is unknown. It is useful when it is necessary to know the pollen concentration at given moments of the day. In this case it is also necessary to know the diameter of the microscopic field and therefore the total examined surface to calculate a factor of correction which will enable the calculation of the number of pollen per cubic metre of air.

IV. Random fields (fig. 5.) – This is a very simple and rapid method which enables the examination of a certain number of fields chosen at random from the entire daily surface, and to count the pollen present in the single field. The statistical significance is higher when the number of examined fields is higher, and to have a certain significance at least 50 fields must be examined for each sample. It is not however possible to indicate the hourly concentration trend, and underestimates or overestimates of the pollen con occur because their depositing is not uniform on the tape, but depends on particular biological cycle, environmental condition, the kind of pollen, etc.

Possible sources of error

Independently from the method used, it is always necessary to use the same one to have data which can be correlated over time. Moreover it is necessary to take a decision concerning the pollen which are partially outside the microscopic field, and concerning the broken pollen.

For the pollen which are partially outside the counting sector, but identifiable, two methods may be adapted: 1, to count (or not count) the pollen which are non completely inside the field or 2, if more than half of the grain is visible, consider it inside the sector and not count those which are more than half outside (in any case never move the microscopic field from the normal scanning line). Concerning any pollen which are broken, if they are identifiable they are counted as if they were whole and if they are not identifiable they are inserted with the mixed unidentifiable pollen.

Possible sources of error during the scanning could be due to many factors, but above all to the reading method, sample interpretation, and slide preparation.

- Reading method: microscopic focus (continuously control in various focal planes the presence of pollen because sometimes the tape is not perfectly flat), high number of pollen per field, peripheral pollen, eye position on the microscope, percentage of surface scanned.
- Sample interpretation: not very stained pollen, hidden pollen (sometimes there is the presence of other organic or inorganic particles that hide the grains), broken pollen, similar pollen, monotony of the sample (especially in a period with few grains).
- Slide preparation: kind of adhesive (commonly used a glycerol jelly and gelvatol) and how it was applied, transparent polyester tape (be sure that it is perfectly flat, avoiding the formation of air bubbles and an excess of the medium) and the application of the cover glass over the sample (don't press and be sure which extremity is the end and which is the beginning).

References

1. Frenguelli G: Airborne pollen sampling techniques. In: Pollenosis 2000: Global approach. Eds G. D'Amato, S. Bonini, J. Bousquet, S. R. Durham, T. A. E. Platts-Mills; pp. 83-90, JGC Editions, Naples, 2001.

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