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A NEW METHOD FOR MANUAL MEASUREMENTS OF INHIBITION ZONES WITH THE BAUER-KIRBY DISK SUSCEPTIBILITY TEST*

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Microbiologists often measure the diameter of inhibition zone of the antimicrobial activity over microorganisms by the disk-diffusion method of Bauer-Kirby. Usually, the measurement process is not accurate enough and it is often done visually with a ruler. More accurate measurements can be done when using photographs; however, when the photographs are taken by non-professionals (students), they are often made with non-optimal (not perpendicular) viewing angle and sometimes the scaling object is missing in the pictures. The paper presents a design and software supplementation of a method for measurement of inhibition zone using non-optimal photographs. The computer program can be used for distance learning, verification of results, recovery of lost results and/or more accurate measurements.

Existing solutions for inhibition zone measurement. One of the widely used methods for inhibition zone measurement of antimicrobial activity is the one from Bauer-Kirby [2]. The microorganisms are grown in a Petri-dish[1] and then a filter disk with antimicrobial agent is added. After some time, the antimicrobial agent kills the bacteria and an inhibition zone (usually a circle) is becoming visible around the disk. The larger zone normally means a higher antimicrobial activity.

Usually there is not much need of highly-accurate measurements and in an educational environment they are done manually with a ruler or digital caliper. For professional use there are semi-automatic and fully-automatic machines such as Sirscan, OSIRIS, ADAGIO, Oxoid Aura and BIOMIC [5]. However they are not only expensive, but for educational purpose the manual process is actually preferred. Another way to perform measurements is by using software over digital photographs. The research [4] presents how inhibition zone measurements can be performed with computerized image analysis. There are even software products based on neural networks which predict the growth of the inhibition zone using a photograph [1]. For manual measurements the best-known product is probably ImageJ [3] and its newer version ImageJ2 [7]. The program provides a wide range of photo editing tools, suitable for microbiologists, and the option for scaling and measurement of the real size of the objects.

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[1] A *Petri-dish* (also *Petri-plate* or *cell-culture dish*) is a shallow transparent lidded dish used to hold growth medium in which cells of bacteria, fungi and small mosses can be cultured (Ed.)

The measurement of inhibition zone by photographs has many advantages over the manual method with the ruler/caliper. Not only might it be more precise, but it could also be done at a later time for verification of results, recovery of lost results, fixing technical errors, etc. One of the biggest advantages is coming with the distance learning since the students do not have a physical touch with the materials.

Measurement issues when using non-professional photographs. The measurement of the real size of an object from a photo is done by using a scaling object well-known from the real-world, usually a ruler or a coin. The first issue is that non-professional photographs sometimes miss such scaling object. In such cases the microbiologists can use the Petri-dish itself and measure its diameter for the scale. There are many variants but the ones we use in the Faculty of Biology of Sofia University are 60, 90 and 150 mm. This can be sometimes misleading because most of them are usually a truncated cone – one of the most commonly used has a bottom, an edge and a top surface, respectively 85 mm, 88 mm and 90 mm in diameter (Fig. 1).

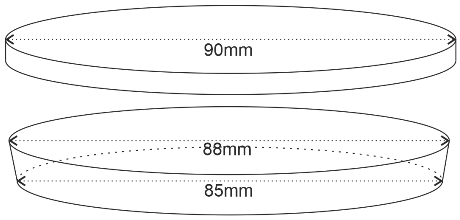

Fig. 1

In optimal conditions the viewing angle of the Petri-dish in the photograph will be perpendicular to it (shot from the top) and it will appear as an ideal circle on the picture. Thus the scaling object and the inhibition zones will be in one and the same plane parallel to the camera sensor and therefore will be comparable in size. If the viewing angle is different a new issue will arise because the Petri-dish and the inhibition zones will no longer be circles but ellipses [6] and they will appear bigger or smaller because of the perspective. Even worse, the scaling object will appear bigger or smaller depending on where exactly it is set on the picture. Some geometrical distortions from the camera itself are also possible. If these factors are not taken into account, they can accumulate to a significant large measurement error.

Issues with the viewing angle are usually available in the starting phase of the educational process when students are taking shots of their work for future reference in their notes. Usually, they are taking the photographs when the Petri-dish is on a high laboratory table and staying perpendicular on top of it with camera is not comfortable. Shooting photographs directly above the Petri-dish is also intuitively avoided because of the shadow of the photographer and his camera (the light source is usually at the ceiling). The larger the viewing angle is, the bigger the measurement errors will be.

Finally, it must be noted that the measurement of a diameter of a circle or axis of an ellipse is not always easy for the user. It is not only the imperfection of the human hand when drawing lines with the computer mouse, but there can also be optical illusions on the photograph. One way to resolve this issue is to draw a polygon around the circle/ellipse and count the diameter as its diameter of a circle with the same area. While potentially much more accurate, this method is not easier for the users as they must draw a sufficiently great number of segments of lines to make good polygon approximation of a circle – otherwise the error can become significant.

Method of measurement of inhibition zone using non-optimal photographs. We propose that the precision of the diagonal/axis measurement of the circle/ellipse can be improved by making not one, but two probes with different angles and then accepting their average as a result. In the case of an ellipse, they can be the major and the minor axes of the ellipse (this is not obligatory – they can be any lines that split the object in half). Two probes do not guarantee a better result (they can both be not good enough); however, our experience shows that it is more easily noticeable for the user that the split is done incorrectly if he looks for four equal parts compared to when the parts are only two.

If the viewing angle is known (comparing the major and the minor axis of the ellipse can provide such information), the perspective problem can be solved by a mathematical formula; however, we propose a simpler solution which does not involve trigonometry. Another well-known object on the pictures can be used for a scaling object – the filter disk. Commonly used disks are circles with diameter of 6 mm. As the disk is in the center of the inhibition zone, it is a good candidate for a scaling object for it regardless of the perspective. The only drawback with such solution is that we must use a different scale for each inhibition zone (usually one experiment in Petri-dish is done with two or four zones). The reason is that the viewing angle makes different filter disks bigger or smaller on the photograph depending on how close to the camera sensor they are.

The proposed algorithm for measuring an inhibition zone of antimicrobial activity by the disk-diffusion method of Bauer-Kirby is the following:

1. Measure the real diameter of a filter disk – D_{real} (in millimeters);
2. Using the photo, measure the major axis of a chosen filter disk – D_{mj} (in pixels);
3. Using the photo, measure the minor axis of the same filter disk – D_{mn} (in pixels);
4. Calculate the scaling factor as $M_d = 2 \cdot D_{real} \div (D_{mj} + D_{mn})$;
5. Using the photo, measure the major axis of the inhibition zone around the chosen disk – Z_{mj} (in pixels);
6. Using the photo, measure the minor axis of the inhibition zone around the chosen disk – Z_{mn} (in pixels);
7. Calculate an approximation of the real size of the inhibition zone in millimeters using the formula: $Z_r = (Z_{mj} + Z_{mn}) \cdot M_d \div 2$;

Repeat items 2–7 for each disk on the photo and the related inhibition zone.

Obviously, each inhibition zone will require taking four measurements – two for the disk and two for the zone around it. By using sufficiently good pictures with perpendicular viewing angle, the process can be simplified by taking the Petri-dish as a scaling object once for all zones. Usually this will produce better results since the larger the object, the more accurate the measurement.

Software for inhibition zone measurement over non-optimal photographs. We used the programming language Java with the graphical library Swing for a prototype. The main viewpoint was that the program must be straight-forward and as simple. On the first screen the user can define the real size of a filter disk in millimeters (6 by default). After choosing a photo, the user must draw straight lines to measure the major and the minor axis of the filter disk, then the major and minor axis of the inhibition zone around it. Right after drawing the fourth line, the result is shown in a text field.

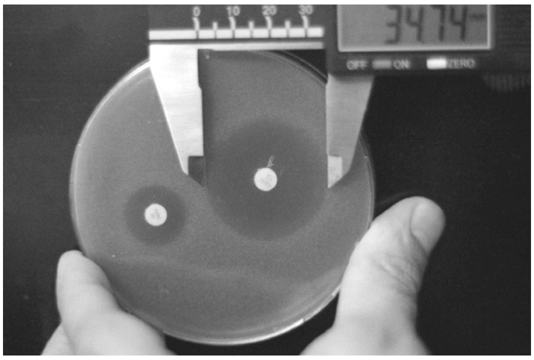

Fig. 2. Measurement using the experimental software

Figure 2 shows an example of physical measurement of an inhibition zone using caliper which is 34.74 mm. Fig. 3 shows the measurement of the same zone using digital photograph with result of 34.11 mm. Such differences of ∼1 mm are usually acceptable. When the zone is *much bigger* than the scaling object (the disk) the error is greater. The difference between the caliper measurement and the results from the software can

Fig. 3. Measurement of the same zone as in Fig. 2 using the experimental software

be greater than 1 mm in the first few tries due to the lack of experience in dealing with blurred contours of low-quality photos, hand-errors when splitting the ellipses, wrong angles for the slices (the segments on the disk must be parallel to the segments of the zone), etc.

It must be noted, that it is not obvious which measurement is more accurate: the physical measurement with digital caliper or the one over a photograph. When the photograph quality is good and the user is experienced, it is more likely that the measurement over a photograph will be more accurate.

One more thing to note is the availability of the *Undo* and *Clear all* buttons (with their standard functions).

An upgrade planned for the near future is to add a zooming capability – it can further increase the precision, especially on monitors with a smaller screen size. Another important thing that we consider as an upgrade is the capability for drawing and measurement of the faces of polygon areas – thus the rare case of non-circular inhibition zones can be covered better as well.

Conclusion. The presented method aims mainly at minimizing the error on manual measurements in photographs and to solve the issue with the missing scaling object when the viewing angle is not perpendicular to the Petri-dish. The experimental software is as simple as possible and requires a minimal level of learning and technical knowledge. It might potentially be used for other purposes; however, its main focus is for measurement of inhibition zones with the Bauer-Kirby method. The authors think that it can be helpful for novice microbiologists. The program is free of charge and can be downloaded directly from the following web address: `https://www.cphpvb.net/files/Petri.jar`.

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НОВ МЕТОД ЗА РЪЧНО ИЗМЕРВАНЕ НА ЗОНИ НА ИНХИБИЦИЯ НА РАСТЕЖ НАПРАВЕНИ ПО ДИСК-ДИФУЗИОННИЯ МЕТОД НА БАУЕР-КЪРБИ

Милена Петрова, Филип Петров

Микробиолозите често отчитат диаметъра на зоната на растеж на микроорганизми при съпоставяне на действието на различни антимикробни агенти по дисково-дифузионен метод на Бауер-Кърби. Самият процес на измерване не разчита на голяма точност и често е достатъчно да се прави дори „на око“ чрез милиметрова линия. В представения доклад е описан метод за измерване на зона на инхибиране на микробен растеж чрез непрофесионална дигитална снимка. Компютърната програма може да бъде използвана от микробиолози за малко по-точни измервания, за дистанционно обучение на студенти, за верификация на резултати при случай на нужда от възстановяване на загубени резултати, при отстраняване на технически грешки от пропускане на измерване на някоя зона, и др.