The Photograph Registration of Petri Dishes in High Quality and Image Editing of Microbiological Testing

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Abstract

Science is based on evidence that can be measured or observed through methodical techniques which are expressed in several ways, either quantitatively or qualitatively. The technical photograph becomes one of the most important key tools to the result’s disclosure. In the microbiological research, several pieces of evidence can be indicated with variables that are deeply related to the means of culture; pH and color variation, halo formation, overlay of structures, culture shape, among others. The employment of technical photographs, as a strategy of the experimental observation and reliable representation, is indispensable. The protocol presented here suggests the production of the photographic support in microbiological tests runs on Petri dishes, taken by a smartphone to obtain high-quality images, besides showing tools to edit images through PowerPoint. The support is composed of a paper tube with a transparent border, whose reduced light penetration avoids problems, such as the luminous reflection over the Petri dishes or the environment itself. The edition consists of the photograph variation, and in clipping and pasting on uniform backgrounds to provide further detailing. The protocol allowed a standardized photograph collection in high quality, which is ideal for a comparative portrait of microbiological behaviors. The image editing enabled a framework and greater visibility of physical and biological structures in the exhibition of photographs inside the manuscript, such as the removal of noises, background alterations, deformities or irregularities. This protocol is a tool that helps the researcher on the knowledge-obtaining process, and it is applied to different experiments or adapted into the most variable research subjects.

1. Introduction

The scientific experimentation photographing or registering act requires dedication to its execution, whose main goal is obtaining a high-quality image that is able to reflect, with more credibility, the results of the research that was carried out. Science seeks the understanding of reality based on observation methods and registration of what is seen or measured and calculated [1].

According to Mafia [5], the photograph is constituted by one of the best documentation forms to illustrate the observations and experiments’ results. These authors affirm that, although the photographing seems extremely simple, it is necessarily accurate in the scientific field, however, higher education and research institutes, broadly, do not possess a professional staff to execute such tasks, and are submitted to people involved in the experiment, who face the everyday challenge of taking photos. It must be highlighted that amateurism is, many times, not yet enough and, at least, core principles are necessary to handle the photograph equipment efficiently.

Belz [2] reports that the easy access and the trivialization of photography generates an image landslide that, many times, does not bring an attractive communicative context, and to avoid this from happening, it is necessary inciting a more complex analysis and interpretation. It is in this regard that photography can be used as an important scientific and teaching communication tool in any science field.
In the microbiological aspect, the images must allow the proper characterization of the organism under study, in terms of shapes, coloration or structural dimensions. For this purpose, besides adequate equipment, the effort and dedication of the researcher are needed, in order to obtain a high-quality photographic registration, since a good image reveals many pieces of important information to the scientific research, which becomes a communication element.

The employment of photography happens through the image reading, in other words, the acknowledgement of the richness of information contained in the registration, allowing the viewer to realize its representation nuances, which provides a scientific reflection, fulfilling its roles in the research [4].

The scientific photograph has as a goal being the most reliable as possible to the photographed matter, respecting the colors, proportions, textures, patterns, shapes and everything else that allows a better analysis regarding the registered information [3]. An image can contribute to scientific work in the same way that a graph or a chart, it only needs to be driven to this objective [2].

According to Messas [6], digital photography became the less expensive and most accessible photographic practice to researchers, reflecting exponential growth in the employment of photographic tools as a scientific method on biological studies, with countless pieces of work evidencing, mainly because it is a non-invasive methodology, easily performed and efficient in terms of data generation.

The technological sector has innovated itself increasingly in the market of photographic equipment, may it be through digital cameras, tablets, cellphones, micro-cameras to microscope/stereoscope or others, making its popularization possible, as well as the access to some resource for the image capturing [5]. However, the researchers must acknowledge the photograph as a research and disclosure tool, and at the same moment, it is needed that they make this knowledge their own, since, in practice, most of the time, they are restricted to books, post-graduation essays and scientific texts with a few high-quality photographic registrations [2].

Messas [6] highlights the fact that as important as the acquisition of high-quality images, in the field or the lab, it is the adequate image flow adoption in order to guarantee the quality and reliability of the research. The involved steps, in the image postprocessing, are fundamental to the organization of the collected data, to the study objective suitability and the good practices and safety of the photographs against mechanical and/or electronic failures.

Although the principles and the techniques to obtaining a good photo are known, there are moments when it is necessary the file editing, in order to better represent the expected goal. Therefore, the basic application of some computational tools (Photoshop, Corel Draw Photo, PowerPoint or similar others) to the image editing, will be able to solve some imperfections or layout adjustments to a greater observation valuation documented by the photographs, as it is defended by Mafia [5].
Intending to propose methodological discussion and procedures regarding the photographic registration of biological runs, some authors suggest that specific protocols to different techniques and organisms must be created, with the goal of standardising photographic methodologies and generate trustworthy and comparable data with each other. Messas [6] emphasizes that besides meeting ethical questions and good scientific quality, the new photograph methods must be easy to be reproduced, present a cost for its execution that is as low as possible and take into account the variation of the produced photographs for different brands and models of photographic equipment.

This research has as a goal proposing a new protocol for the photograph registration with high-quality bioassays carried out through Petri dishes (named by Protocol PRHQ), as well as exploring image editing tools with the employment of PowerPoint.

2. Materials And Methods

One of the most frequent failures in the in vitro trials with Petri dishes is on the photographic registration, since, in its majority, they are carried out in reflective materials, such as plastic, acrylic or, the most common, glass, which depending on the work environment, can suffer luminous interventions. It jeopardizes the image quality and has a negative effect on the obtained results through the experiment or in the results' confirmation.

The protocol here proposed used a paper tube with a transparent border and emerges as an alternative to correct the previously mentioned mistakes, based on microbiological trials of filamentous fundi in growing process and bacteria on Petri dishes with different means of culture, and the cellphone as image registering equipment, allowing the high quality on the scientific photograph to be reached.

2.1 Necessary Material:

- 01 blurred black sheet of paperboard;
- 01 sheet of transparent material or similar (for example.: plastic bottle, curriculum kind “L” file);
- Cardbox (20 x 20 cm);
- Scissors, white glue, scotch tape, pen and ruler;
- Led ring light;
- black/blue cloth, sulphite paper or a lightbox (x-ray visualizer).

2.2 Procedures for the confection of the paper tube

The PRHQ Protocol consists of the confection of a paper tube, made with black paperboard, whose dimensions may vary according to the size of the Petri dish used in the experiment. The descriptions here
presented are valid to trials with glass plates (100x20mm). The images can be obtained with the closed plates (lid and bottom) or open (only the bottom). Obviously, it will affect the tube diameter (Fig1).

1. On the blurred black paperboard, cut a rectangle with the proportion of 21 x 33,5 cm, in order to its shape be able to circle all the Petri dish diameter, with a small remnant for the lateral edges fixation;
2. On the transparent sheet or similar, cut a piece of ribbon that is, preferable, the same height of the plates (2 x 33,5 cm) and surrounds all of its diameters;
3. Through the scotch tape, make a junction of the parts (items a and b) on the larger edge of the rectangle;
4. Using an empty Petri dish (of the same dimensions as the ones used in the experiment) as a mold for the desired diameter, so the fixation is done on the lateral edges, forming a tube, maintaining the black side of the paper turned on the inside.

2.3 Procedures for the making of the cellphone framework

The PRHQ protocol adjusts itself mainly to the photographic cameras on cellphones, which have their own settings manually triggered or activated automatically with artificial intelligence sensors, hence, the adjustments on the capture of the image are done at the moment when the photo is taken, in order to obtain a better result.

1. Over the paper tube, it is necessary to have a bracket to the cellphone placement, allowing a greater uniformity of the photographic registration; to achieve it, the use of an inflexible material is necessary, such as a cardbox, with the measures of 20 x 20 cm.
2. Another square of the same proportions in black paperboard;
3. The sides of both squares must be glued, in order to have an entirely black side (paperboard) and another one being the cardbox;
4. On the superior side (cardbox), the cellphone placement delimitation is made, from then on, an orifice is opened (only enough) to the camera fitting, which will depend on the cellphone model (Fig2).

2.3 Making of the studio for the photographic registration

The photograph is a result of the performing of several steps, which will guarantee the greater quality of the final image, so the setting environment demands some attention. On the execution of the PRHQ protocol, it is important considering the luminous source and the background.

a) Light: one of the biggest effects expressed on the photo final quality is on the employment of a good luminous source, the main light used on the protocol is a led ring light (Ø 26 cm) without the tripod; its round shape allows a uniform distribution of the light on the Petri dish, revealing many characteristics of the culture or the degradation reaction of the environment, depending on the matter under study. The ring light is positioned with a luminous projection turned to upward (normal), but, depending on the kind of the culture or study focus, this position can be inverted, but, in this case, something to elevate it to a
desirable height must be used, usually the same height as of the plate, this way there will be the perfect light crossing (fig3).

b) Background: The culture development over its environment can be better contrasted with the adoption of backgrounds; in this protocol, it is recommended the use of black or blue cloth, sulphite paper sheet or even a lightbox (x-ray visualizer), the choice depends on the culture environment, the colony color and other factors that can affect the contrast. The background works as the backbone for the making of the work studio (led light, Petri dish, tube and cellphone bracket used for its placement). Usually, the black cloth is the most usual and allows great results.

2.5 High-Quality Photograph Register – PRHQ Protocol

After the organization of the whole methodological process for the making of the registration basic structure, the image capturing proceeds. All the exhibited photos were taken with a smartphone, regardless of its brand or model, since there are minimum settings, such as brightness, zoom, focus or other tools that can be used whenever necessary.

This protocol allows that the registration is made, one Petri dish each time, which means, each plate is photographed individually, and it can be representative or, after the registrations, all of them can be put into a group with the help of software or apps, in this way a composed picture is formed or images boards.

The Petri dish with the colony that is desired to take a picture of is put under the black cloth, and, afterwards, the ring light is positioned, considering the plate in the center so the tube is adjusted over the plate, in a perfect fitting, because it was made measured for this arrangement. In the case of a smaller plate, an edge of the cloth will appear at the moment of the register. After the fitting, with the led already turned on, the tube is covered by the cellphone bracket, and the device, on the image capturing mode, is positioned over it, just as the light enters through the transparent border, which allows a greater image quality, without negative influences on the registering act.

After the image capturing, the plate is removed from the tube, in the case, if there is any difficulty in this process, it is recommended that two opposed holes are made on the transparent tape, in order to not go beyond half of its width (≈1 cm) (Fig4).

2.6 Image Editing with Powerpoint tools

Some adjustments, cuts, corrections or layout reviews can be done with specific apps or more practical and accessible software, such as PowerPoint.

This software is meant to design the creation of audiovisual presentations; some tools allow image editing, offering satisfactory results regarding the final quality of the product. One of the main tools is located on the upper tab >>Image Format<<. In order to add it, it is necessary “to copy” the photo that needs editing and “to paste” on the PowerPoint page, afterwards, one must double click on the image to
activate the tab >>Image Format<<, usually on the upper right side, the icon >>cut<< can be found, when it is triggered in the photo surroundings and “handles” will come up so one can drag the mouse to erase surplus or undesirable areas (figure 05).

In this same tool, there is the possibility of cutting the picture into the format of basic shapes (as the circular shape). In order to do this, it will be necessary to click on the arrow below the cut icon (Ⅱ) and activate the command tab “cut to demarcate shape” and choose which shape is preferable to cut the image. Once the plates are round, the circular one is the most adequate, and it must be selected on the option <<Basic Shapes – Ellipse>>. Afterwards, it is necessary to click on the icon <<cut>> and with the “handles” adjust accordingly the circle, in case it is needed, the size can be adjusted on the “dimension box” besides the cut icon (Fig6).

After the removal of undesired areas from the photograph, it can be saved as an image, clicking with the cursor right button, or it can be kept on PowerPoint to make a montage with other photos from the experiment, forming an image board, which, when it is finished, the pictures can be put into a group, copied and pasted directly on Word or “saved as an image” (Fig7).

3. Results And Discussion

The research will be able to explore the PRHQ protocol and enjoy its possibilities to the fullest. There are cases in which the luminous intensity growth by the led (cold and hot light), or zoom adjustments, of brightness or capture, changes of the background colors will affect the obtained results. With the aim of organizing the photograph studio, according to the previously described and demonstrated steps in Fig8.

According to what was explained in the methodological description, it is recommended that the tube is manufactured in the dimensions of the plate to be photographed, avoiding interferences of irregular edges (fig 08 – h). In the case the tube is tight, making the plate removal difficult, a clipping on the tape can be done to facilitate the process after the photographic registration (fig 08 – c). The support lid for the cellphone (fig 08 – e), aside from functioning as an equipment bracket, avoiding variation or hand twitching, improves the distribution of light and focus quality of the device’s camera, avoiding reflections of the lid or the culture environment (Fig9).

There will be situations when the inversion of the light from the led will allow better results on the photograph, it depends a lot on the color of the environment culture, on the microorganism or on the chosen technique on the trial (Fig10).

The scientific photograph has to express the communicative capacity, highlighting details and results reached through research, that is because the concern on having a final material in high-quality and of practical obtaining endorse the statements made by Mafia [5], in which the scientific photograph requires, first of all, the simplicity, which is the main orientation, on the generation of the product, the picture.
Below (Fig11), it is made clear that the improvement in the photo quality when it was taken through the PRHQ protocol. It is also suggested that, in case of necessity, the exhibition of the picture must happen over a black background, emphasizing the photograph details.

The photographic background varies in relation to the object under study and/or better characterization of the experiment. For this purpose, it is suggested the employment of a one-colored cloth (black or blue), blank sheet of paper or lightbox, whose luminous source is widely distributed, and the ring light usage is not necessary when this option is chosen. Below (Fig12), two situations with a clear and dark background are represented, under the PRHQ protocol, as well as the plate’s appearance when exposed in a black or white background.

In the growing of microorganisms, means of culture containing necessary nutrients and vitamins for their growth and reproduction are employed. The nutrients are provided in a way to meet the species’ demands to be cultured, promoting their growth or satisfactory sporulation. However, the mean of culture composition depends on the microorganism that one wishes to grow and the study purpose [7] (Fig13).

These means present different colorings, textures and goals, which can be revealed through the microorganism development, as growth limitation, halo formation of substance degradation, color variation for pH changes, crystal formation and others, in order to make the photograph an indispensable tool in the presentation of these results. Through this protocol, it was possible to portrait faithfully (Fig14) several microbiological trials, commonly carried out in the study of the microorganisms.

The scientific photograph taken in high quality allows a wide comprehension language of what is the research purpose and makes the reader explore even more the manuscript during its appreciation. This registration, when made with criteria and technical approach, can go beyond the qualitative patterns, allowing the use of software for the image analysis in the RGB standards, and provide reinterpretations of a quantitative nature in the evaluation’s experiment.

4. Conclusion

The scientific research has its merit and acknowledgement by everything that is investigated and proven, among the various strategies adopted to such doing, the photography, without a shadow of a doubt, allows and raises, even more, the credibility of the action, so, the protocol purpose to the photographic register of Petri dishes (PRHQ Protocol), here presented by the employment of a paper tube and its modifications, is one more tool that helps the research in the knowledge creation, through the supply, when manipulated consistently, the high-quality images and without external interferences, which can be applied to different experiments, or adapted to the most varied research subjects.

Declarations

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**Authors' contributions**

Cleverson Rodrigues: Conceived of or designed study, Performed research, Analyzed Data, Contributed new methods or models, Wrote the paper

Grace Queiroz David: Performed research, Analyzed Data, Wrote the paper

André Rodrigues dos Reis: Analyzed Data, Contributed new methods or models, Wrote the paper

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Figures

Figure 1

Confection of the paper tube diagram, used as a way of conducting focus and light to the Petri dish photographic registration
**Figure 2**

Representation diagram of the cellphone square bracket, which allows greater safety and uniformity on the PRHQ protocol usage.

**Figure 3**

Work studio organization and positioning adjustments of the luminous source to the PRHQ protocol performance.
Figure 4
Details of the holes on the transparent tape to enable the Petri dish removal, if necessary

Figure 5
Details of the image formatting tab as of the cutting process

Figure 6
Details of the image formatting, highlighting the cut into the circular shape
Figure 7

Suggestions of saving the image or image board montage after cutting and formatting

Figure 8

Making of the photographic registration studio’s steps in order to execute the PRHQ protocol. a) Led Ring light over the black cloth; b) placing the Petri dish on the center of the led; c - d) positioning of the cylindrical paper tube over the plate; e) lid of support for the cellphone; f - g) organization of the parts and performing of the register; h) photographic result of the employment of this method, emphasizing the irregularity of the edge because it is a wider tube than the plate diameter; i) edge adjustments made by the tool of cutting into a circle on Powerpoint, and pasting in a black background
Figure 9

Reflection of equipment by the covered plate (a) that was avoided by the employment of a supporting lid (b) and can be improved with final touches by the cutting off edges and pasting on a black background (c)

Figure 10

Ring light inversion to the registration of Petri dishes in organic compounds volatile by antagonists and pathogens evaluation
Figure 11

Photographic registration of a Petri dish sealed with plastic foil without (a) and with (b) the employment of the PRHQ protocol; and editing of the edges by the circular cutting and pasting on a black background in both situations of unemployment (c) and employment (d) of this protocol.

Figure 12

Different photographed experiments with PRHQ protocol, using a clear background (a,b,c) and the same plates under a dark background (d,e,f), it is suggested that independently of the photo's background choice (clear or dark), a black background can be used (g) to the exhibition of the results in the image board or image compilation.
Figure 13

Employment of the PRHQ protocol to growth trials and metabolites production by the microorganism, highlighting the number of colonies, the coloring changes, degradation and crystal formation.

Figure 14

The image board exposes different photographic registrations performed through the PRHQ protocol on several occasions, emphasizing the microorganisms details and characteristics, such as colors, textures, colony shapes, antagonist reactions, among others, reassuring the protocol efficiency in the scientific documentation. All the pictures in this essay were taken by a device called Xiaomi Redmi Note 7, and the setting happened mostly through 9 MP and can be accessed originally through the link https://photos.app.goo.gl/tJ2ABv7BVzMosQMt8