

NEGATIVE STAINING WITH INDIAN INK (ALSO CALLED CHINESE INK, CANDLE SOOT, INK FOR SEALS AND LIQUID SHOE BLACK WAX)

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Introduction:

[Last month](#) I presented an article about staining with dyes that can be found easily in any home I.e. gentian violet, methylene blue, and erythrosine, I used this to show POSITIVE staining which means that the sample acquires the color of the dye used. In this article, I am going to show what is called NEGATIVE staining, which is the opposite not only in name, but because in this case what takes the color of the ink is the background. The sample attains less color or acquires a color different from the color of the ink, see below.

Development:

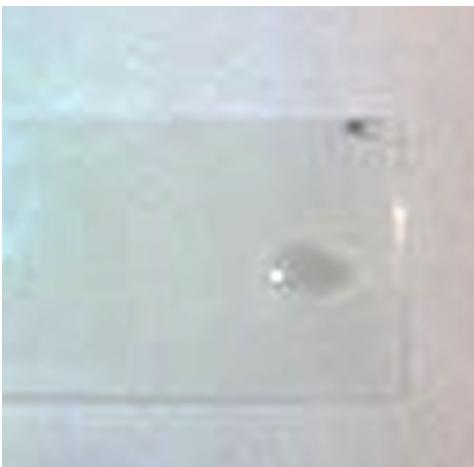
Negative staining is a technique used in microbiology to enhance the contrast of samples under the microscope. Originally it used nigrosine as the principle dye. However, nigrosine is sometimes difficult to obtain and in particular I could not obtain it here in my country and where I did find it, it was expensive. The technique can also use the ink called "Chinese ink" which is easy to acquire at any stationary store or supermarket and it is extremely cheap.

Today I am also trying, candle soot, ink for seals and liquid black shoe wax both of which can be found in any home.

The technique consists of staining the background of the sample and is principally use for staining bacterial cultures most of the time with the purpose of revealing a capsule if the bacteria is an encapsulated one. It can also be used just as a way of increasing contrast, since it follows the principle that certain stains such as nigrosine or Indian ink do not cross the cellular wall of bacteria so is not absorbed. In this way just the background is enhanced and the subjects remain clear against a dark back ground when illuminated by transmitted light.

To prepare the sample it is extremely easy:

First a drop of the culture upon the slide,



then a drop of the stain or in the case of soot some particles of it,



after that mix them up and with the help of another slide it should be spread,



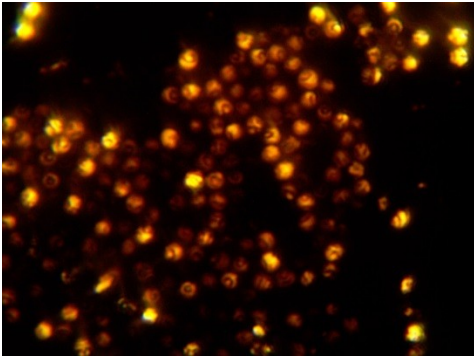
finally it must be left to be dry at room temperature which takes a few minutes and until it is ready to be observed.



These are my sources of inks for this article.



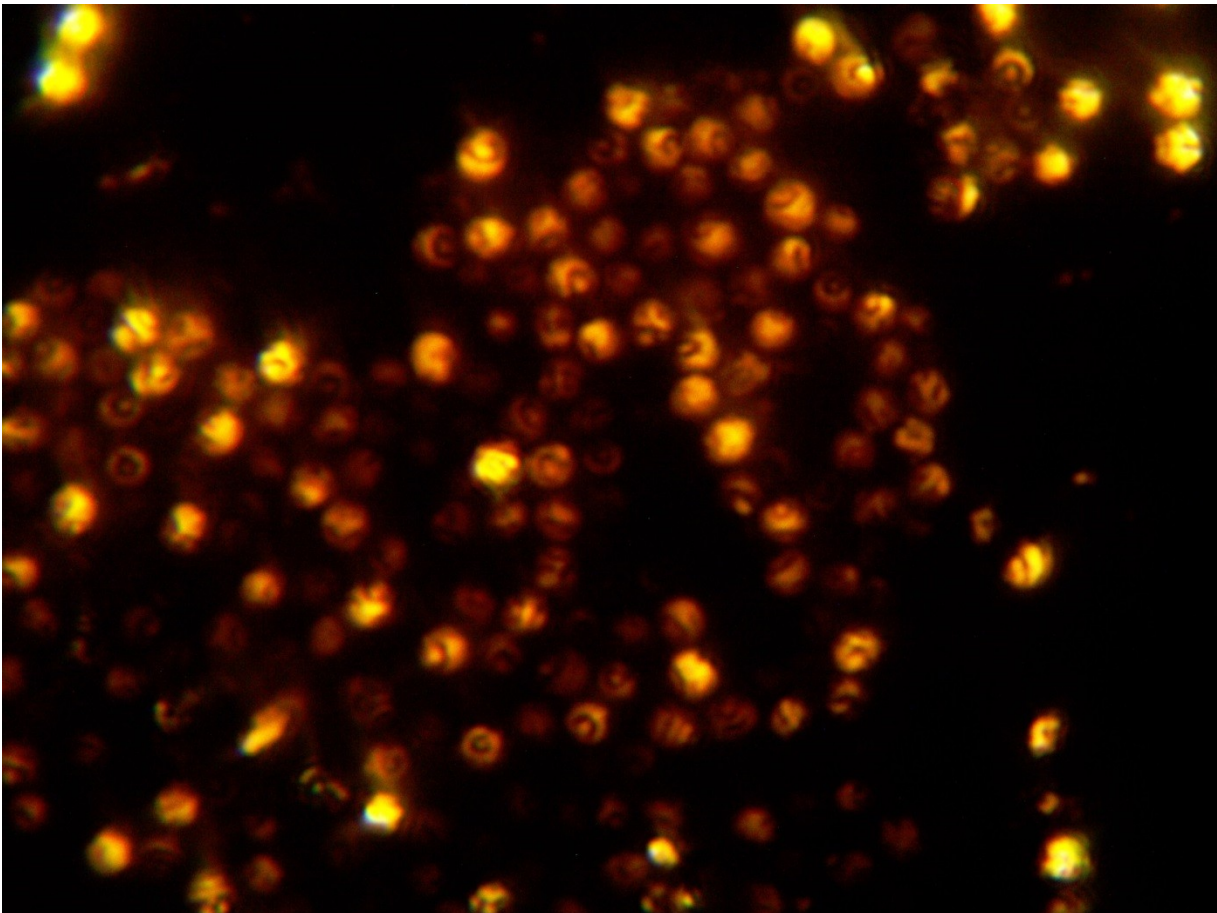
To better appreciate the negative stain it is possible to turn the picture to the gray scale.



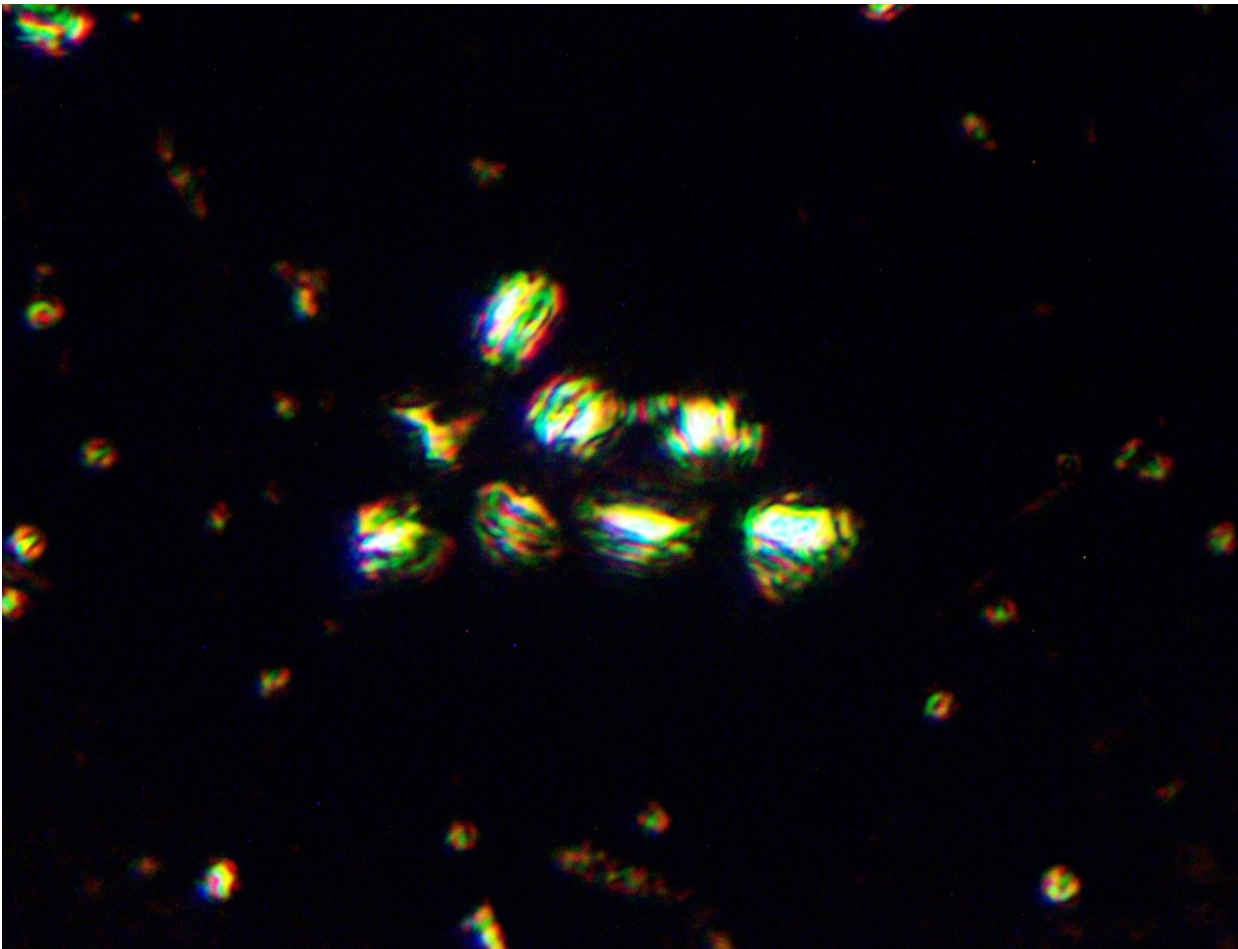
Note:

The technique is regularly used with pure cultures of bacteria, for example taken directly from a Petri dish culture, so it is easy to get good results. At home, it is sometimes difficult to prepare pure cultures of bacteria but can use cultures at hand such as yogurt, saliva, water from the drains, etc.

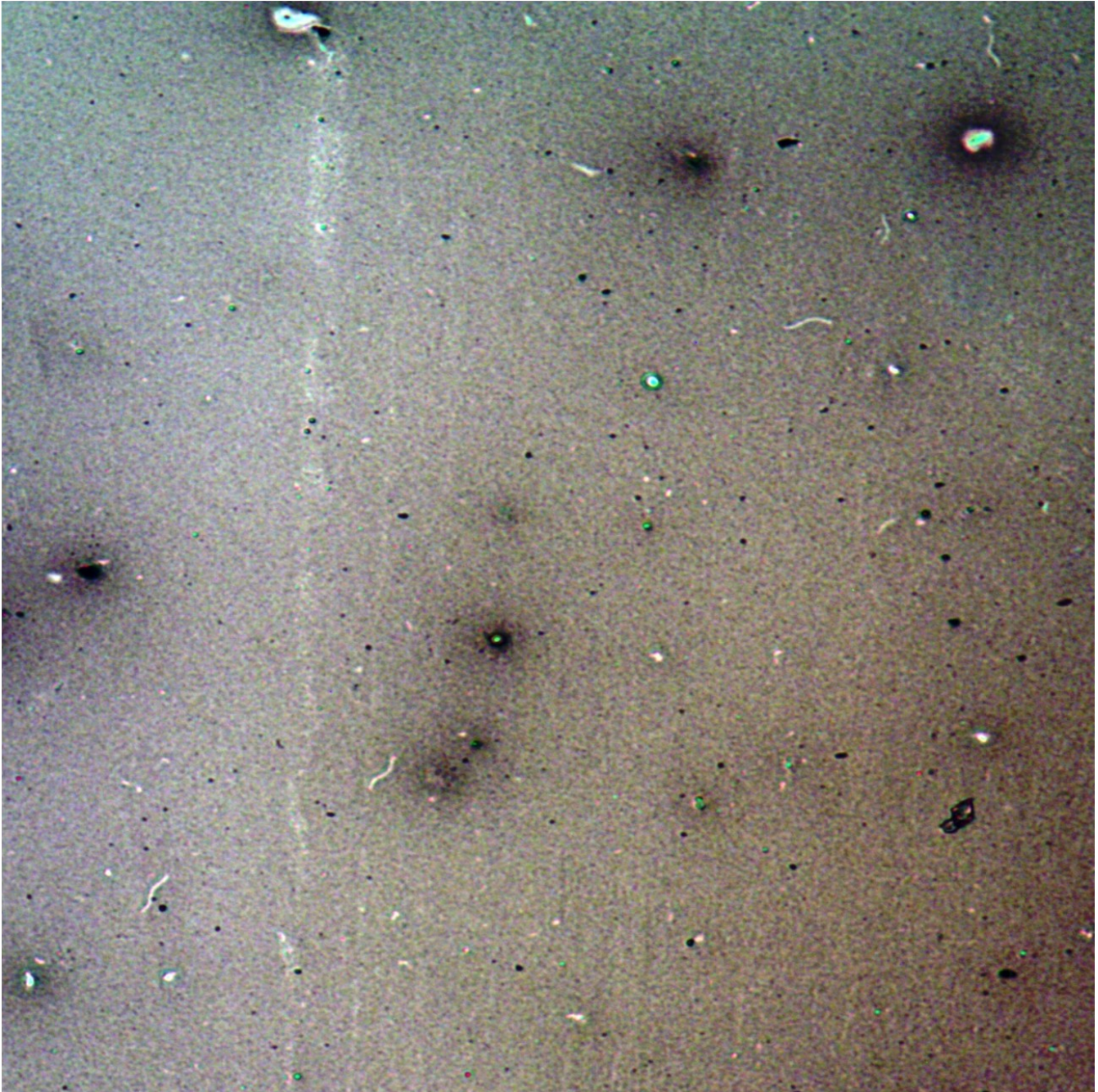
Results:



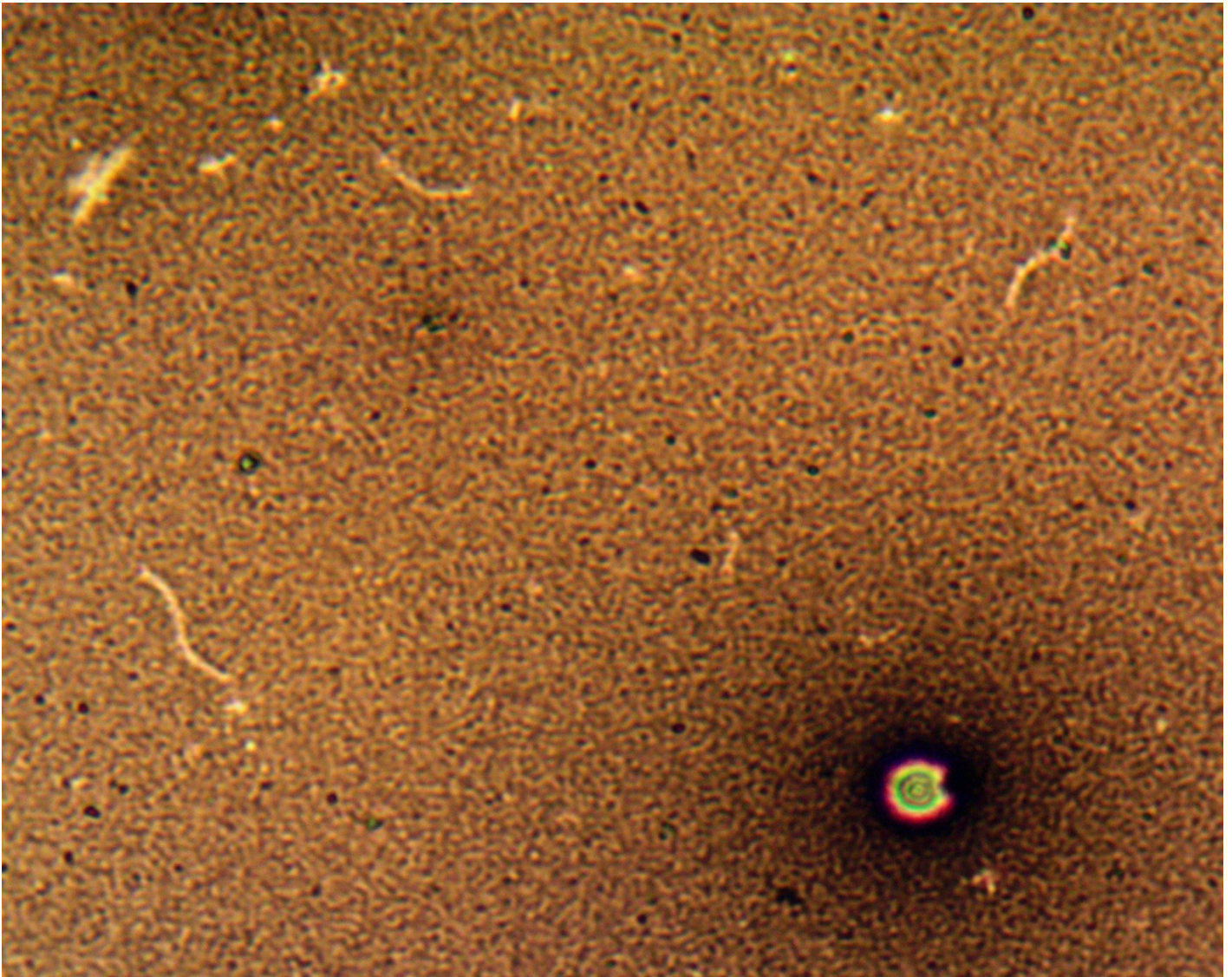
Probably spores of *Aspergillus niger* at 100x, the sample was taken from a rotten piece of bread stained with Indian ink.



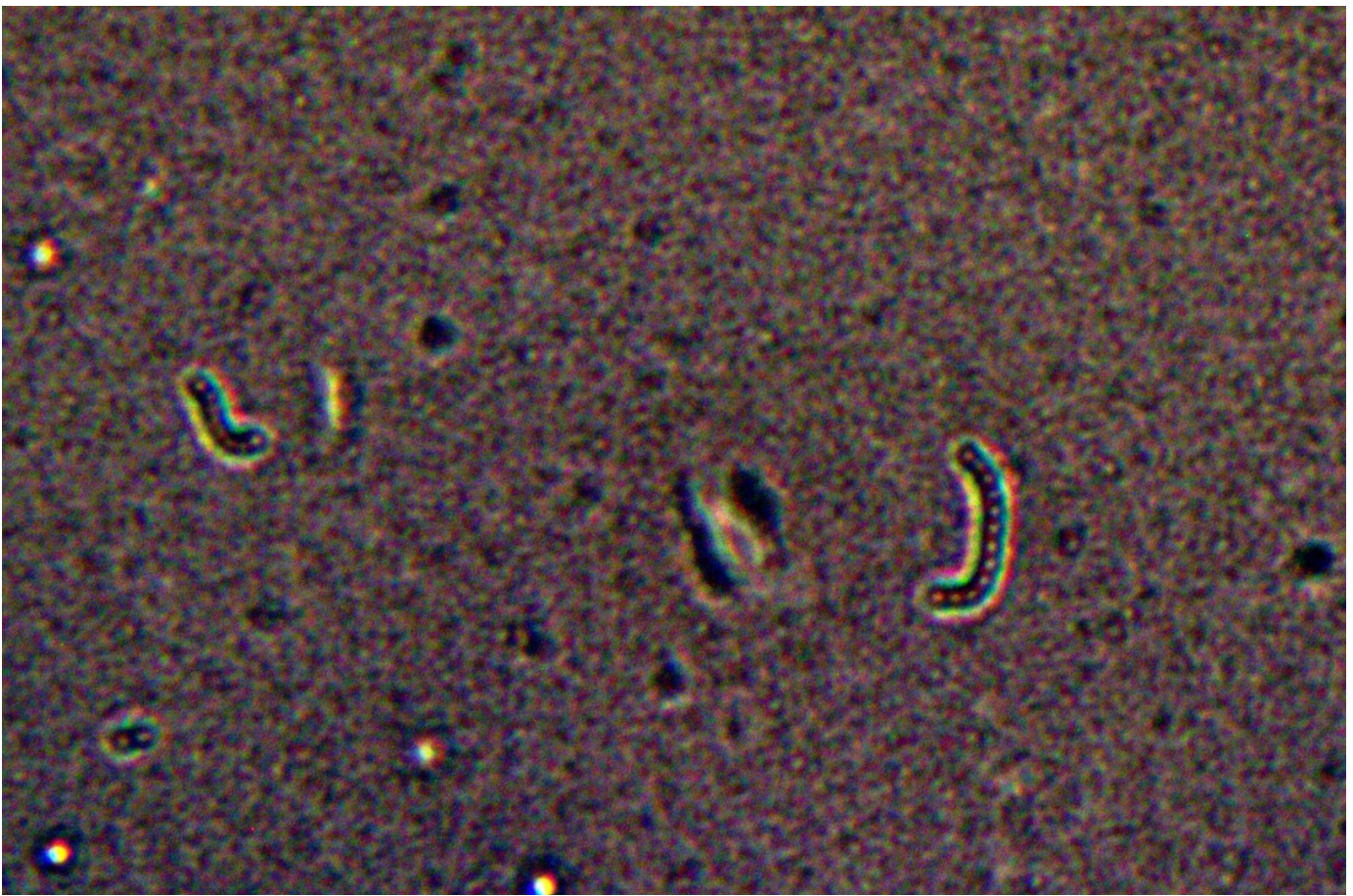
Probably spores of *Rhizopus nigricans* found upon a piece of rotten bread treated with Indian ink.



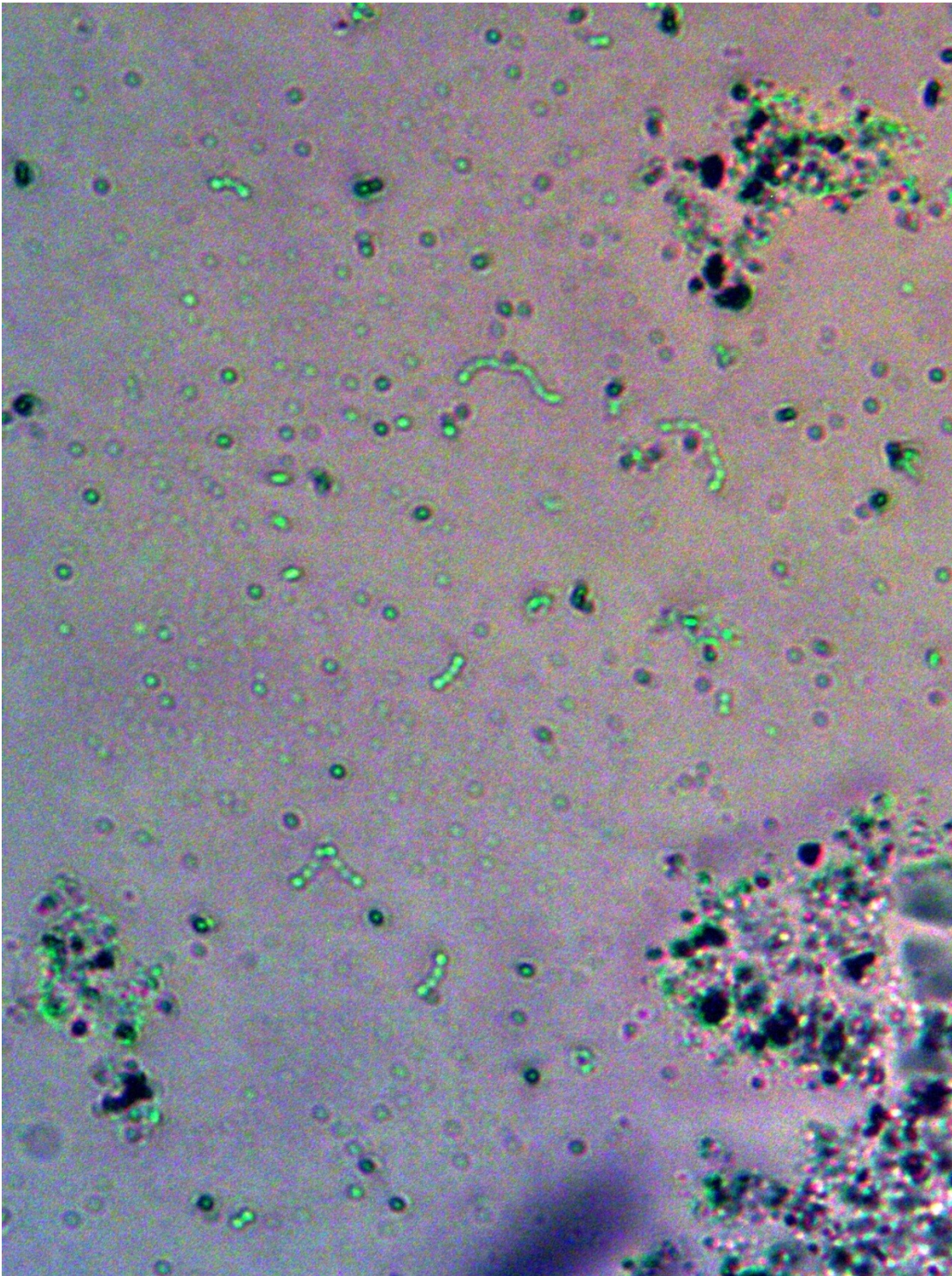
Spirillum found in drain water with 10x objective, stained with liquid shoe black wax.



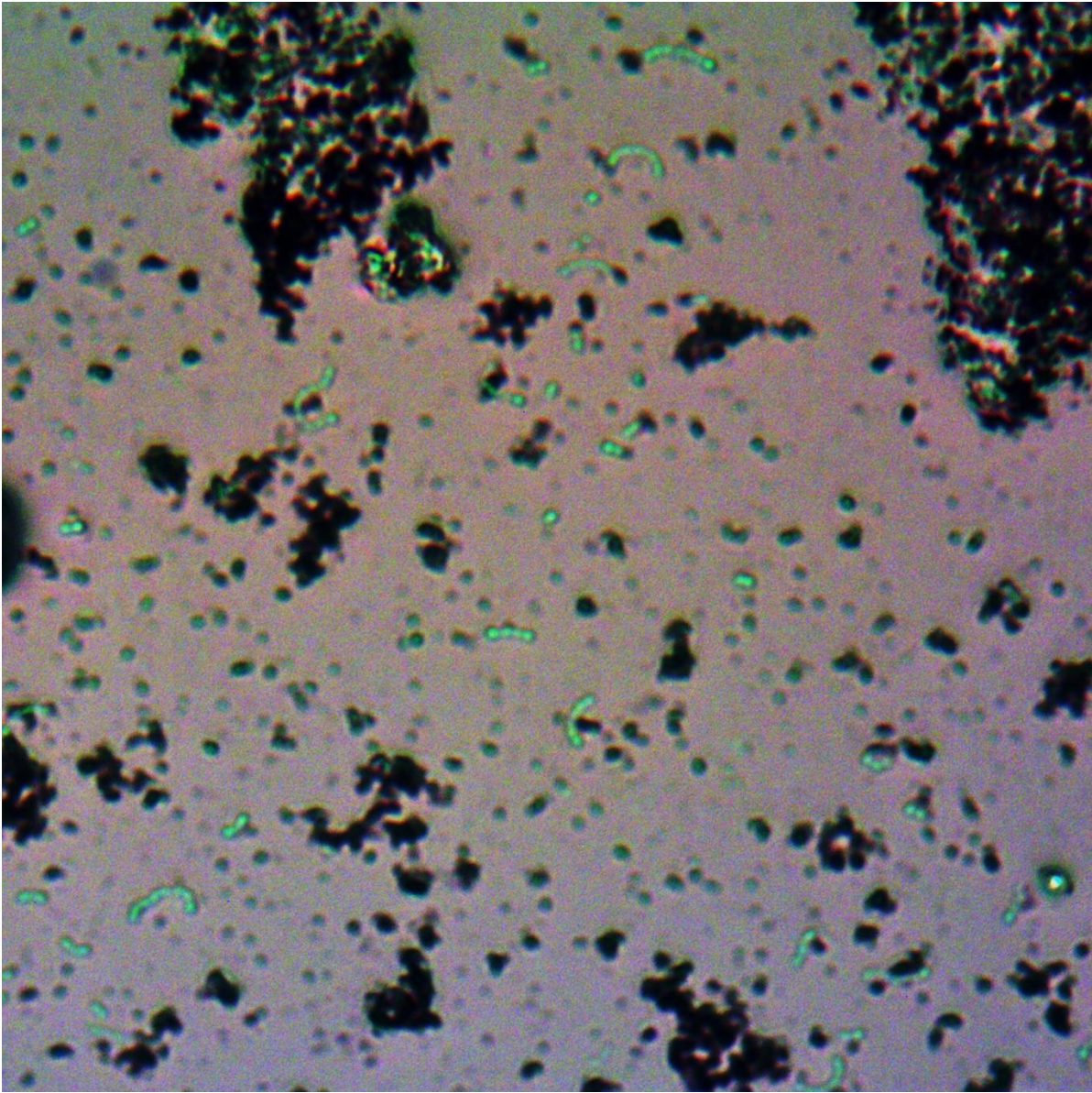
Spirillum found in drain water with 40x objective, stained with liquid shoe black wax.



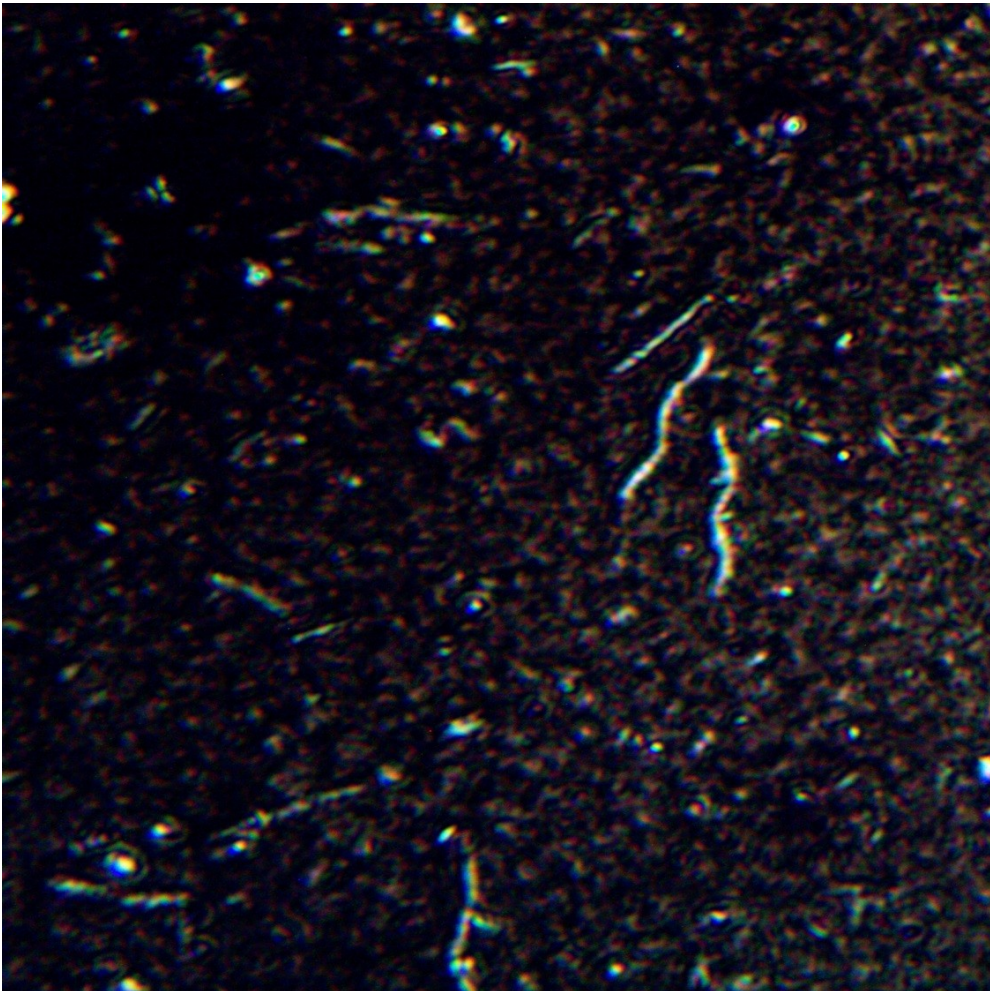
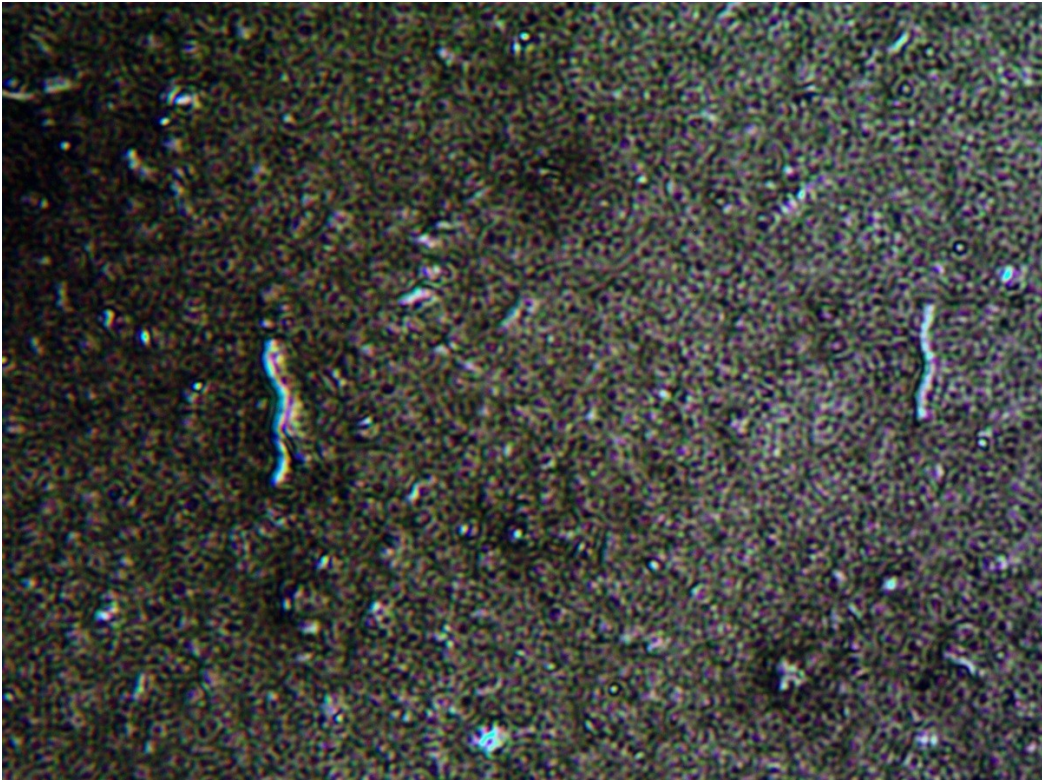
Streptococcus of yogurt with 40x objective, stained with liquid shoe black wax.



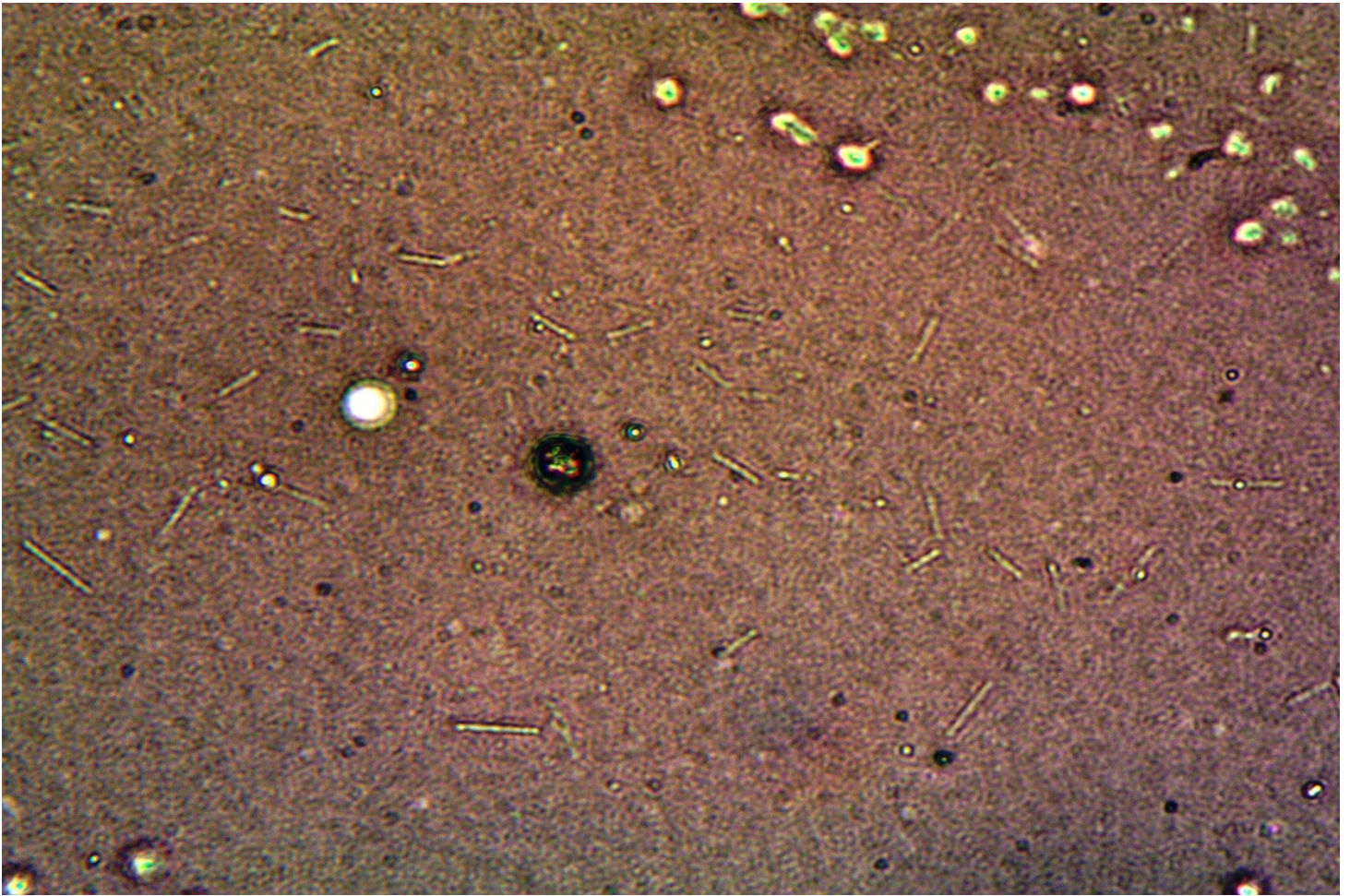
Streptococcus of yogurt stained with 40x objective stained with candle soot.



Streptococcus of yogurt stained with 40x objective stained with ink for seals.



Spirillum found in water from drains with 40x objective, stained Indian ink.



Bacillus sp. Also found in water from drains with 40x objective, stained with liquid shoe black wax.

Note: the greenish color that can be seen mainly in the streptococcus samples is the interpretation of the camera, because they are not that colour when seen through the eyepieces. I have tried to adjust with the white balance application but it continues.

Conclusion:

As it can be appreciated from the images above, there are many options than can be used to stained bacteria negatively, as alternatives to conventional stains for this purpose. The imagination is the limit to look for inks that can be used. Here I have showed the most common types found at home. It must be remembered that sometimes they do not work for all the samples, so trial and error is the basis to see which ones are the best. If I were asked which of the inks used above will be my best option, I would definitely choose candle soot as the first choice and liquid black shoe wax as the second one.

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(Above in anti-spam format. Copy string to email software, remove spaces and manually insert the capitalised characters.)

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