Sharp Images Detection for Microscope Pollen Slides Observation

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Abstract. In this paper, a new preprocessing algorithm to qualify images of different pollen grains for further processing is proposed. This algorithm provides a score related to the sharpness of the image and will be used to automatically adjust the focal length of a microscope that magnifies the image. The obtained score has been compared to four quality metrics generally used to estimate the clarity of an image and to a reference made by a human. The results of the simulations show that the proposed algorithm combines better performance with low complexity on the set of images.

Keywords: Microscope slide image acquisition · Sharp image detection · Fourier transform.

1 Introduction

Allergic rhinitis concerns between 8 and 25% of the world population [1,2] and it is caused in particular by pollen. Pollen detection is thus an important issue to prevent this allergic rhinitis. Usually a human operator uses a microscope to observe slides containing particles present in the ambient air. Then, he can determine the presence or not of pollen particles, and also count them. One of the difficulties of this observation is that the viewer always has to set the focus knob of the microscope to get a clear image of the view to make this analysis. An example is shown in Fig. 1: each image from (a) to (e) represents the same view acquired by increasing the focus knob from $4\mu m$ each time. The sharpest view corresponds in this example to the view (c). One can see that setting the focus knob on a lower or a higher value leads to a blurred image (see images (a) and (e), respectively, as example). Moreover, finding a good setting for one view

is still not enough: as the viewer changes the view to observe different parts of the slide, one has to set the focus knob again.
In this paper, we propose a new algorithm to automatically set the focus knob in order to get the sharpest image. It is based on a metric which gives the highest score to the image containing the highest frequencies, and has thus the least blur. Other no-reference quality metrics can be found in the literature: some of them are especially dedicated to the evaluation of the sharpness of an image [3]. Some others give a score for the global quality of an image (including sharpness) using for example a perception based quality evaluator [4], or a comparison to a default model computed from natural images [5–7]. These latter have the advantage of taking into account the natural perception. However, they could fail in evaluating the images acquired from the microscope as they take into account many others criteria in addition to the sharpness.

The rest of the paper is organized as follows: Section 2 presents the developed Sharp Image Detection algorithm (denoted SID-algorithm). Section 3 discusses the simulation performance by comparing the use of the SID-algorithm with others state-of-the-art no references quality metrics. Finally, Section 4 concludes this work.

2 Selection of the sharpest image

The first subsection describes the metric which is used to evaluate the image sharpness. Next, the sharp image detection algorithm is presented in the second subsection. We define the following notations: z corresponds to a given setting of the focus knob. I_z represents the image obtained from the microscope with the setting z. I_z is an image of $M \times N$ pixels resolution.

2.1 Image sharpness evaluation metric

This section describes the Fourier-based Image Sharpness Evaluation Metric (denoted as FISEM in the rest of the paper) that is used by the SID-algorithm to determine the clearest image by varying the focus knob in the microscope.
The FISEM relies on the fact that sharp images contain less blur and thus higher frequencies. The computation of the metric for an given image I_z is composed of three steps:

– First, a Laplacian high-pass filter (denoted H) is applied to the image I_z. The obtained filtered image $I_z^{'}$ contains only the higher frequencies of the image and is given by:

$$I_z^{'}(i,j) = \sum_{m=-1}^{1} \sum_{n=-1}^{1} I_z(m-i, n-j) H(m,n) \tag{1}$$

$$\text{where} \quad H(i,j) = \frac{1}{8} \begin{bmatrix} 0 & 1 & 0 \\ 1 & -4 & 1 \\ 0 & 1 & 0 \end{bmatrix}$$

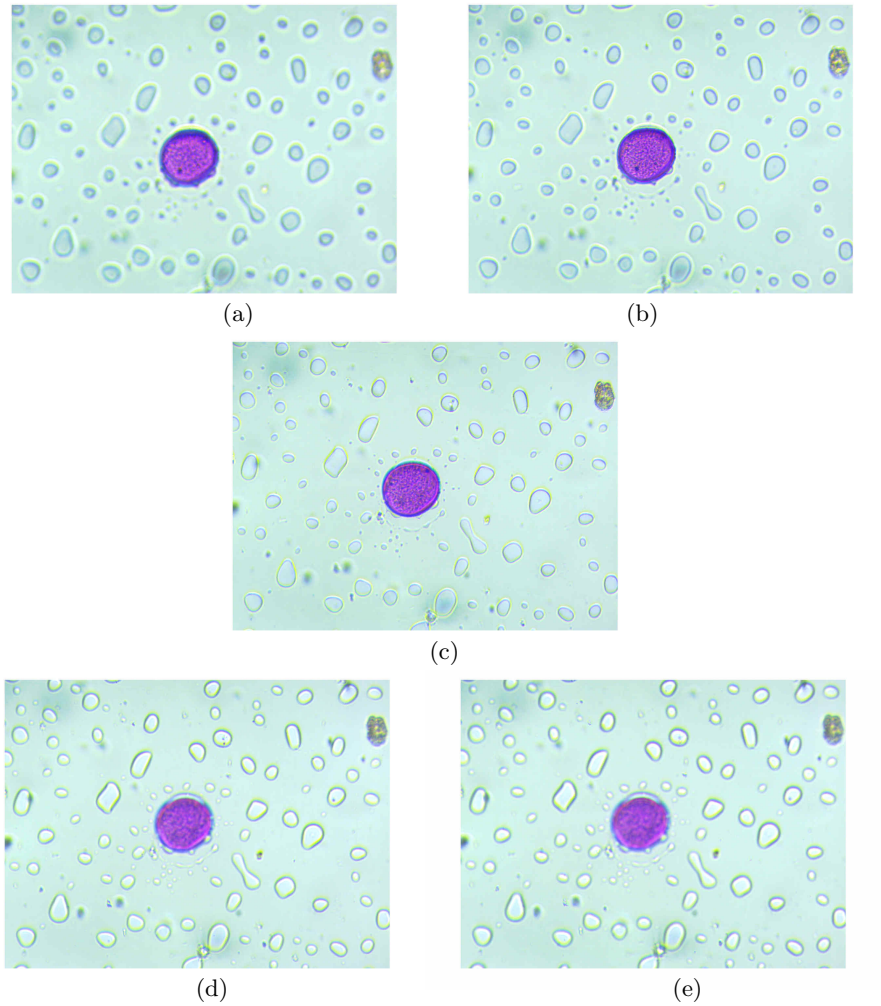

Fig. 1. Microscope views by increasing the focus knob with (c) the sharpest view.

– Then, a 2D Fourier-transform is applied to the filtered image. The transformed image is given by:

$$I_z^{''}(u,v) = \sum_{m=0}^{M-1} \sum_{n=0}^{N-1} I_z^{'}(m,n) e^{-j\left(\frac{2\pi}{M}\right)um} e^{-j\left(\frac{2\pi}{N}\right)vn} \tag{2}$$

– Finally, the score S_z of the image I_z is computed as the sum of the absolute values of the Fourier coefficients:

$$S_z = \sum_{m=0}^{M-1} \sum_{n=0}^{N-1} \mid I_z^{''}(n,m) \mid \tag{3}$$

2.2 Sharp image detection algorithm (SID-algorithm)

The SID-algorithm is at first initialized with the current settings of the microscope, e.g. with a given setting of the focus knob z and its associated view I_z. As shown in Fig. 1, the sharpness of the image increases with the value of z up to a certain point and then decreases again. This feature is used by the SID-algorithm to find the best image. The detailed steps of this algorithm are given in Fig. 2. Finally, the best image selected is the one with the highest score.

The flag f is initially set to 0 and is turned to 1 only once, when the focus knob is changing direction (example: firstly increasing, then decreasing it). The variable o contains the last operation performed on z : + if it has been increased and − if it has been decreased. Δz corresponds to a defined value (in μm) which is added or deducted from z at every new test.

Input: Current setting of the focus knob z, associated view I_z
Output: Best setting $\mathbf{z}^*$, associated sharpest image $I_{\mathbf{z}^*}$

1. Initialization: set the flag $f = 0$.
2. Compute $S = S_z$. Set $z = z + \Delta z$ and $o =$ "+".
3. Compute S_z.
If $S < S_z$, the new image is sharper. Set $S = S_z$, $z = z + \Delta z$, $o =$ "+" and $f = 1$.
Otherwise the previous image was sharper: the focus knob should be turned in the other way. Set $S = S_z$, $z = z - 2 \times \Delta z$, $o =$ "-" and $f = 1$.
Go to step 4.
4. Compute S_z.
If $S < S_z$, the new image is sharper.
  Set $S = S_z$ and increase the focus knob in the same direction as previously: if $o =$ "+", set $z = z + \Delta z$, else if $o =$ "-", set $z = z - \Delta z$.
Otherwise the previous image was the sharpest image. Go to step 5.
5. If $o =$ "+", set $\mathbf{z}^* = z - \Delta z$. Otherwise, set $\mathbf{z}^* = z + \Delta z$.
Return $\mathbf{z}^*$ and associated $I_{\mathbf{z}^*}$. End.

Fig. 2. Pseudo-code of the proposed SID-algorithm

3 Discussion of the experimental results

This section discusses the simulation results of the SID-algorithm for finding the best images in microscope observations. This algorithm was tested on 20 sets of 11 images each of 1280×960 pixels resolution. For each set, the 11 images were acquired by setting manually the focus knob such as to have the globally sharpest image denoted "0". Then, increasing the focus knob of $2\mu m$ at each time, 5 more images containing an increasing amount of blur were obtained.

These images are denoted from "+1" to "+5". In a same way, from the position of the focus knob leading to the best image, 5 more images with increasing blur are obtained by decreasing the focus knob of $2\mu m$ at each time. These latter are denoted "-1" to "-5".

The efficiency of SID-algorithm has been compared to four state-of-the-art no reference quality metrics. The first metric denoted PIQE relies on a perception based quality evaluator [4], while the second and the third one denoted NIQE and BRISQUE use a comparison to a default model computed from natural images to evaluate the quality of the images [5–7]. These three metrics can be found in the Matlab processing toolbox [8]. Finally the fourth metric denoted as CPBD deals with to the images sharpness assessment and it is based on the cumulative probability of blur detection [3].

The best images selected by the SID-algorithm using the different metrics including FISEM are given in Tab. 1. One can clearly see that the images selected by the SID-algorithm using FISEM are very close to the sharpest image selected by the viewer. The four other metrics and mainly the PIQE and BRISQUE metrics often choose the most blurred image as the best on of the set. Fig. 3 to Fig. 7 (respectively Fig. 8 to Fig. 12) give an example of the images selected by the SID algorithm using the five metrics corresponding to the set number 12 (respectively the set number 6) in the table. It seems that the three global quality metrics often consider the huge amount of particles in the image as degradations. Thus they prefer blurred images where these particles can not be seen properly. Of course the FISEM metric is not appropriated if the degradations in the images have very high frequencies like salt and pepper noise. But in the case of the acquisition of sharp images for microscope observation, this metric leads to good results. Concerning the CPBD-metric, it does not allow to find the sharpest image in most cases also. It seems that this metric is more adapted to assess the sharpness of images having very different levels of blurriness between the objects present in the images and the surrounding areas, for example between the foreground and the background.
The SID-algorithm has also the advantage of being of low complexity. Indeed for each image, the complexity is equal to $O(Mlog(M)Nlog(N))$ depending on the size ($M \times N$ pixels) of the image. Using a computer with an Intel Core i5 processor of 2.4GHz and 4GB of RAM, the FISEM code running under the version R2014a of Matlab takes an average of 0.04s to find the sharpest image for a given set of images.

4 Conclusion

This paper presents a new method to automatize the acquisition of sharp images using a microscope. One of the major constraints using a microscope is that the viewer always has to manually set the focus knob in order to get the sharpest view. The developed method allows to select the best settings leading to very sharp images by using a metric which gives higher scores to images containing

Table 1. Best image selected by the SID-algorithm using the FISEM, PIQE, NIQE, BRISQUE and CPBD no-references quality metrics.

Images Set	Image selected by the SID-algorithm using				
	FISEM	PIQE	NIQE	BRISQUE	CPDB
1	0	-5	-4	+5	-5
2	+2	-5	-1	-5	-5
3	-2	+5	+3	+5	+5
4	-1	+5	-4	+5	+5
5	-2	+5	+2	+5	+5
6	0	-5	+4	+3	+5
7	-2	+4	-1	+4	+5
8	-2	+5	+4	-5	+5
9	-2	+4	+3	-5	+5
10	-1	+5	+2	+5	+5
11	+2	-5	-2	+3	-5
12	0	-3	+4	-1	-1
13	+1	-5	-3	-5	-1
14	0	-5	-3	-5	-5
15	+1	-5	-3	-5	-1
16	0	+5	+3	+5	+5
17	-1	-5	-3	-5	+5
18	+1	-5	+4	-5	-1
19	0	-5	-3	-5	+5
20	0	+5	+2	+5	+5

higher frequencies and thus less blur. This metric performs better than state-of-the-art no-references quality metrics for the images acquired using a microscope. Moreover, the proposed algorithm has also the advantage of low time complexity.

5 Acknowledgment

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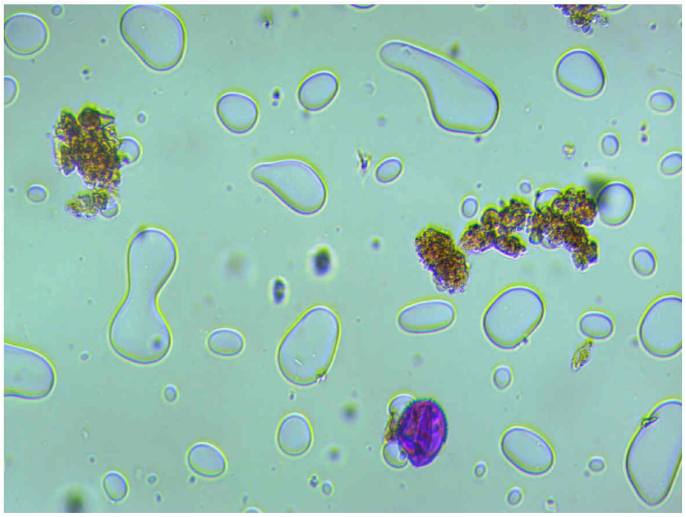

Fig. 3. Best image using FISEM in the set of images 12

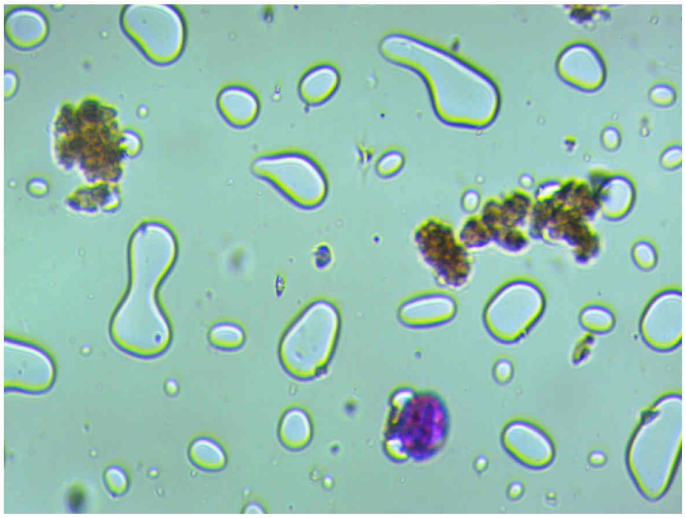

Fig. 4. Best image using PIQE in the set of images 12

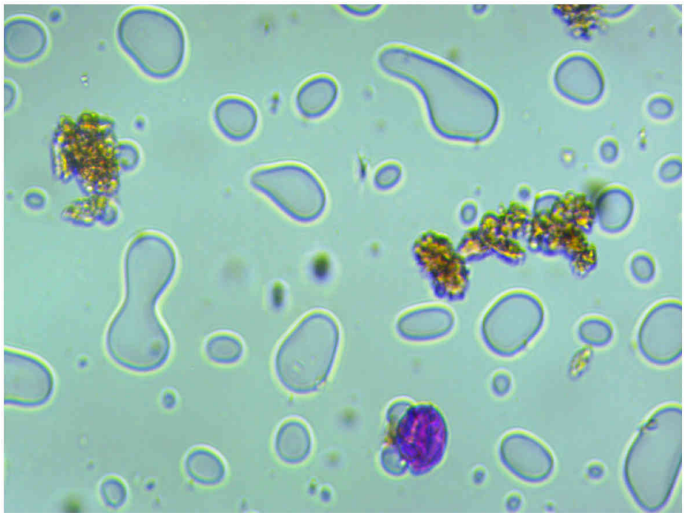

Fig. 5. Best image using NIQE in the set of images 12

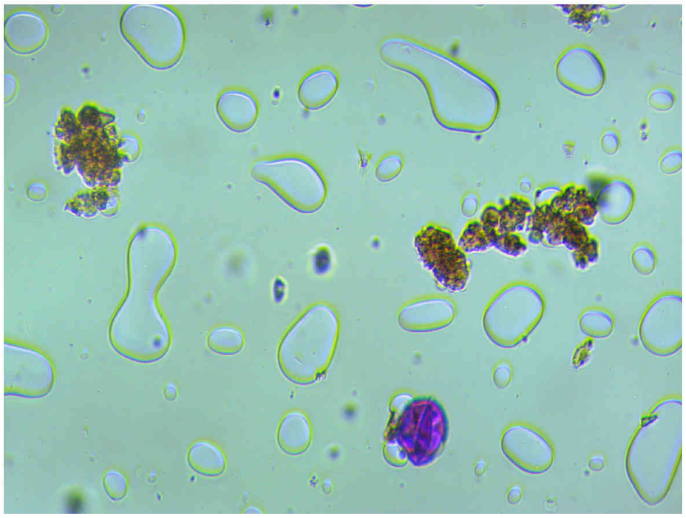

Fig. 6. Best image using BRISQUE in the set of images 12

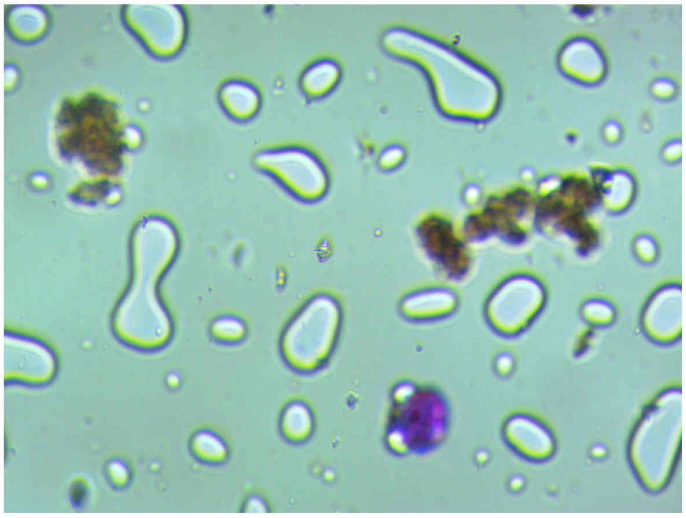

Fig. 7. Best image using CPBD in the set of images 12

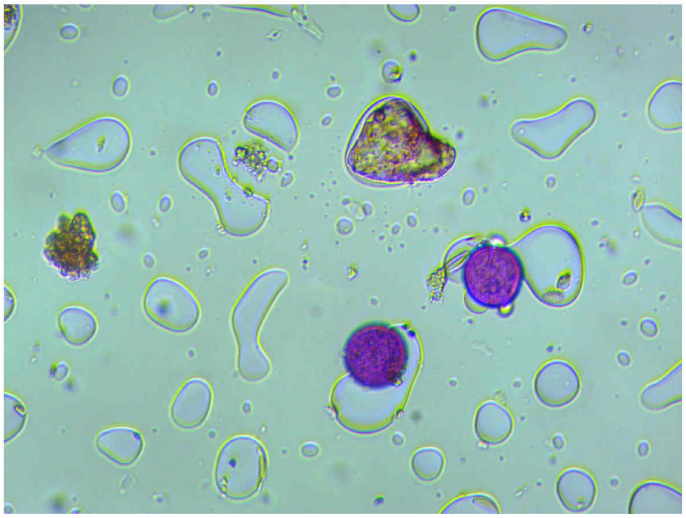

Fig. 8. Best image using FISEM in the set of images 6

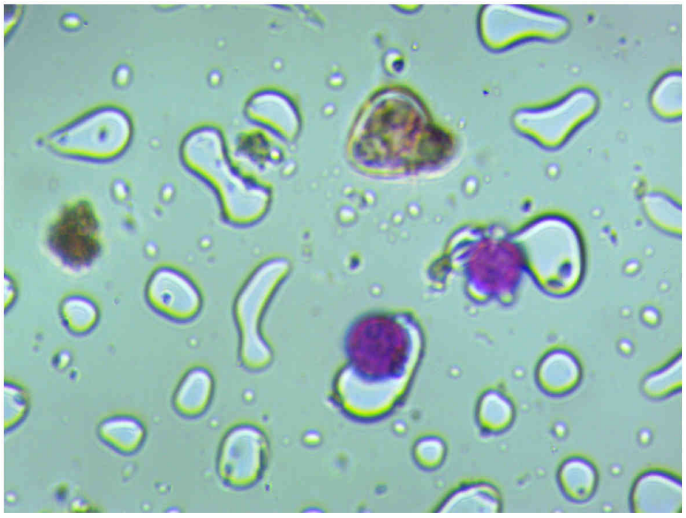

Fig. 9. Best image using PIQE in the set of images 6

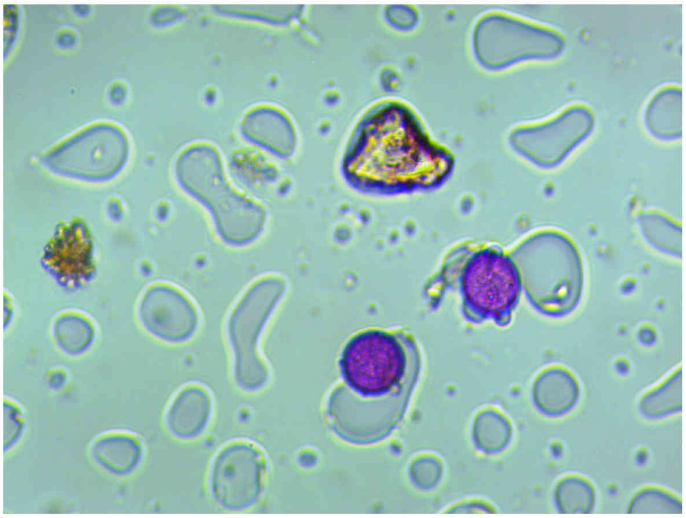

Fig. 10. Best image using NIQE in the set of images 6

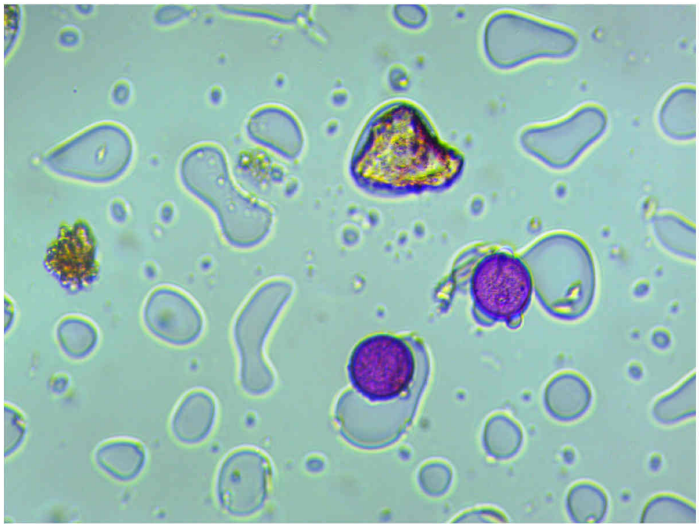

Fig. 11. Best image using BRISQUE in the set of images 6

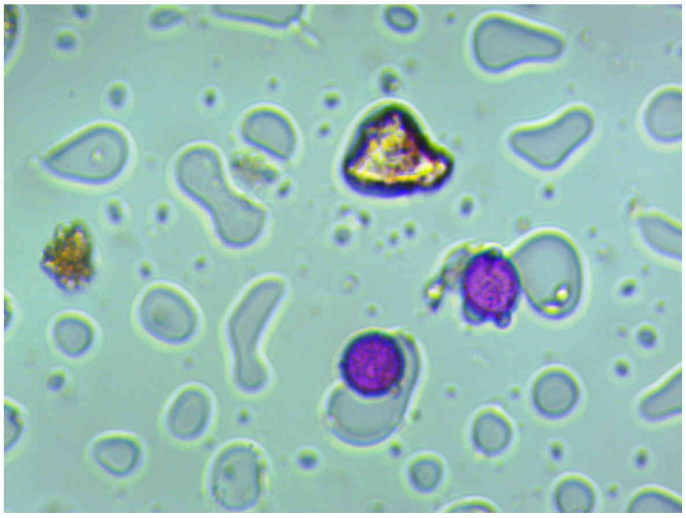

Fig. 12. Best image using CPBD in the set of images 6