Application Note

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Preprocessing of Petri dish images for yeast colonies counting

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Contents

Revize

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0	18.08.2009	J.S.	Vytvoření dokumentu

1 Introduction

This document describes image preprocessing flow used in quantitative analysis of images of yeast colonies growing on a Petri dish. It is accompanied by Matlab demo, showing the results of preprocessing on several sample images of Petri dishes.

Quantitative analysis of yeast growth is used in some experiments to determine the influence of a substance, contained in the growth medium, on the growth of the yeast colonies. The colonies grow on a solid medium contained in a Petri dish, which is stored in a cultivation box.

The function DishPreprocessor, contained in the demo, determines the exact position and size of the dish in the image and sets the threshold so that the image background is eliminated, in order to adjust the region-of-interest (ROI). The outputs of the function are gray-scale and binary images of the colonies, coordinates of the center of the dish and its diameter. The output images can be used to count the total area of the colonies and the number of colonies. The process is described in more detail in Section 4.

Let us note that the demo is extracted from a software tool that integrates both the preprocessing and area/colony counting and an image editor to adjust the results of the colony counting (Figure 1). For more information on this tool, do not hesitate to contact our group at `schier@utia.cas.cz`. We are interested in cooperation with possible users for further development of the tool, and for preparation of joint research projects. A version of the tool based on non-commercial implementation tools is planned, we are, however, not able to provide any release date.

The images included in the demo have been prepared in the Department of Genetics and Microbiology, Faculty of Natural Sciences, Charles University. The software has been developed in cooperation with this laboratory.

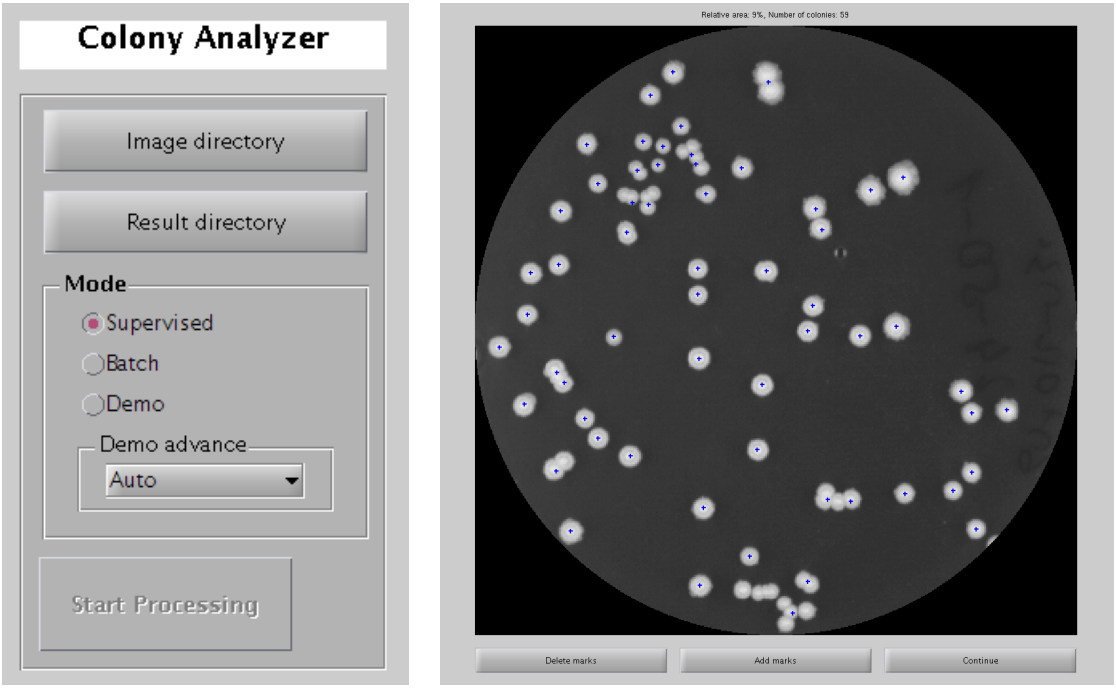


Figure 1: Colony analyzer tool

1.1 Structure of the report

The report is structured in the following way:

- first, the usage of the demo and of its main function `DishPreprocessor()` are explained,

- then, image characteristics of the yeast colonies images are discussed,
- finally, the processing flow implemented in the `DishPreprocessor()` function is described.

2 Demo description

2.1 Software requirements

The demo is tested with Matlab (R) 7.7.0 (R2008b) and is expected to run also with any newer version of Matlab. It requires the Image processing toolbox (TM).

2.2 Using the demo

The demo is located in the directory `Demo`. To run it, start Matlab and change the working directory to `Demo`. Then, type `demo` with the name of the image file to be processed as parameter:

```
>> demo('SampleImg/sample1.jpg')
```

in Linux or

```
>> demo('SampleImg\sample1.jpg')
```

in MS Windows.

Demo reads the file sample.jpg, and displays output: the original gray-scale image of the dish with a circle marking the region of interest (ROI) and a mark showing the center of the dish, and a second image showing only the colonies in gray-scale and the circle marking the region where the area of the dish is considered (it is the same circle as in the first image). See Figure 2.

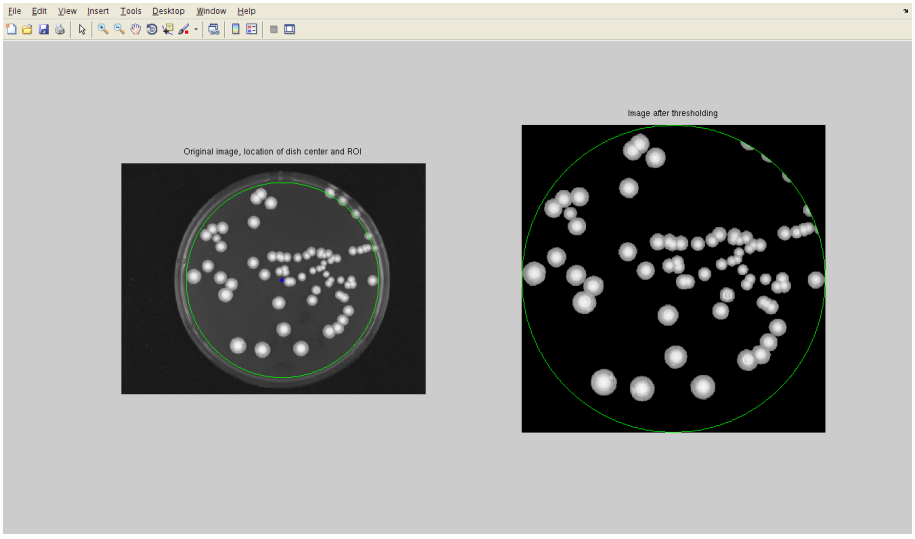


Figure 2: Demo output

Internally, the demo calls the function `DishPreprocessor()`, with the matrix `img` containing the gray-scale image as parameter:

```
[Dish,DishBW,PetriX,PetriY,R_ROI,TotArea] = DishPreprocessor(img);
```

The function returns the following parameters:

Dish, DishBW	gray-scale and binary ROI image (the `Dish` matrix contains also the Petri dish background)
PetriX, PetriY	the coordinates of the dish center
R_ROI	ROI radius
TotArea	total ROI area (used later to compute the relative area of the colonies)

The internals of this function will be described in Section 4.

3 Image characteristics

For each dish, images are taken several several times during the growth of the colonies. Images are taken in a dark room, using digital camera with two light sources mounted on a general-purpose imaging mount by Kaiser Fototechnik (see Figure 3). The images are characterized by the following features:

Figure 3: Imaging configuration

- the colonies are light-gray on a dark-grey background.
- the rim of the dish is lighter than the background, due to reflections of the illumination lamps
- the colonies are roughly round-shaped, there are, however, large variations in size, morphology (the surface can be either smooth or fluffy), and the exact shape, which can be nearly circular or rather fuzzy, dependent on the age of colonies and the tested substance in the medium.

While the imaging is performed in the controlled environment of a dark room, there are still some noise factors that affect each image to some degree:

- there is some degree of variation of the dish position and size (dishes are placed manually under camera, there are variations of the lens zoom adjustment between experiments)
- there are also variations of the dish illumination (which is determined by the exact position of the dish, variations of the growth medium surface and the tilt of the lamps).

4 Processing flow of image preprocessing

The processing flow used in the `DishPreprocessor()` function is summarized in Figure 4.

In this section, the function of each block in this chart will be described briefly and demonstrated on changes of an example image. We use an image shown in Figure 5 (Note: it's the same figure as shown in Figure 1 and 2, with edited brightness level in order to reveal better the background elimination).

Outer background thresholding This operation is used to eliminate the background around the dish (that is, the background on which the dish is placed).

To determine the level of the background, square samples are taken in all four corners of the image, as shown in Figure 6. The threshold level is determined as minimum of maxima of these samples, plus as empirically selected guard value:

$$\mathtt{Bkg} = \min(\max(\mathtt{Corner}_1), \max(\mathtt{Corner}_2), \max(\mathtt{Corner}_3), \max(\mathtt{Corner}_4)) + \mathtt{guard}$$

This way of calculation is used to eliminate influence of wrong values in the case of background faults (namely, misplaced black background due to operator's fault).

The resulting image is shown in Figure 7.

Binary image of the dish, projections and position detection In order to determine the position of the dish, the image of the dish is converted to a binary form, and projections are calculated along horizontal and vertical axes (see Figure 8).

The coordinates of the dish are determined as the first/last point, where the value of the projection exceeds certain threshold (which is used to eliminate the influence of the background noise, shown in Figure 8d).

Out-of-image detections Once the coordinates are determined, detection of faulty images is performed:

- all edge values (left, right, upper, lower) must have been detected
- the vertical dimension (YWidth) must not be greater than the horizontal dimension (XWidth) – empirically determined property, the image of the dish is of an oval shape with longer horizontal axis.
- the horizontal width (XWidth) must not exceed certain limit value (MaxDiam).
- dish must not be touching any edge of the image (i.e. the left/upper edge value must be bigger than one, the right/lower edge value must be smaller than image width/height)

  Note: it the tool mentioned in Section 1, an extra procedure is implemented that allows to resolve images, where the rim of the dish is out-of-image, as long as the inner part (region-of-interest) is fully contained in the image.

Dish radius calculation The outer radius of the dish is calculated simply as half the distance between the left and right edge of the dish.

Dish background thresholding Thresholding of the inner background of the dish is based on the image properties following from the arrangement of the image-taking setup and the properties of the yeast colonies: with the two lamps on the sides of the dish and with the dark background, the dish rim is brighter than the dish background, but not as bright as the colonies. Then, to cut of the background, we use the mean brightness of the image along the central horizontal line (in the approximate vertical

center) along the approximate width of the rim, as the threshold (the width of the rim in pixels has been determined empirically from the images).

The process is illustrated in Figure 9, where figure (a) shows the brightness along the central horizontal line over the whole dish, figure (b) shows only the rims of the dish.

Using the threshold, only the colonies and parts of the dish rim are extracted in the form of a binary image (see Figure 10).

Extraction of the region of interest In order to extract the region of interest, it is necessary to compute two parameters: the coordinates of the dish center and the inner radius of the dish (i.e. the radius of the dish without the rim).

The coordinates of the center are calculated very simply as the left/upper edge (coordinate) of the dish plus half of the horizontal/vertical width.

The radius of ROI (R_ROI) is calculated as the outer radius (`XWidth`/2) minus the predetermine witdh of the rim (`width`).

Finally, using these parameters, a circle is computed, marking the region of interest.

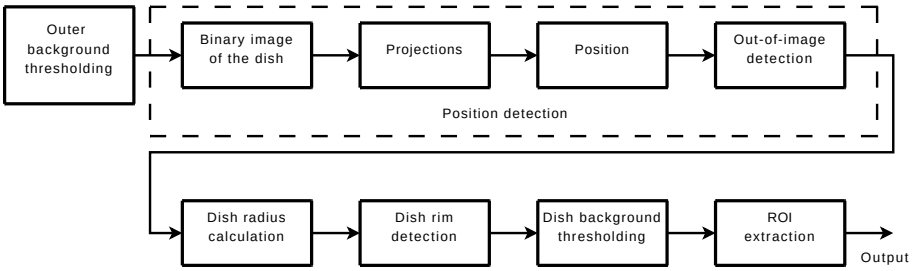


Figure 4: Processing flow of the DishPreprocessor() function

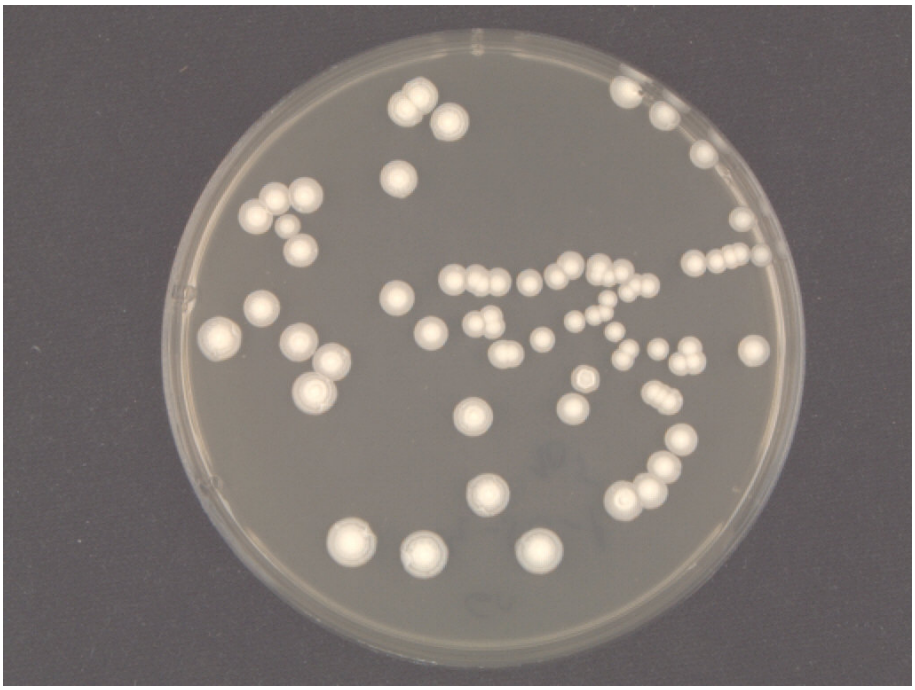


Figure 5: Sample image – initial form

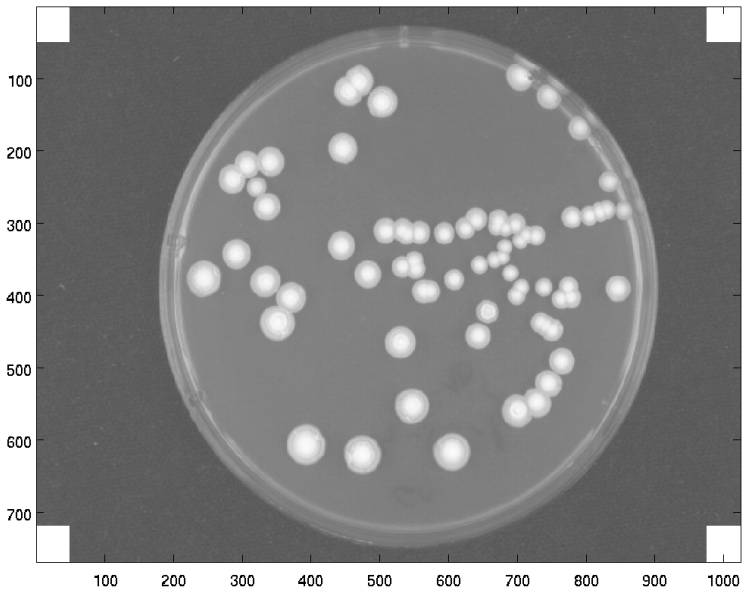


Figure 6: Position of the outer background samples

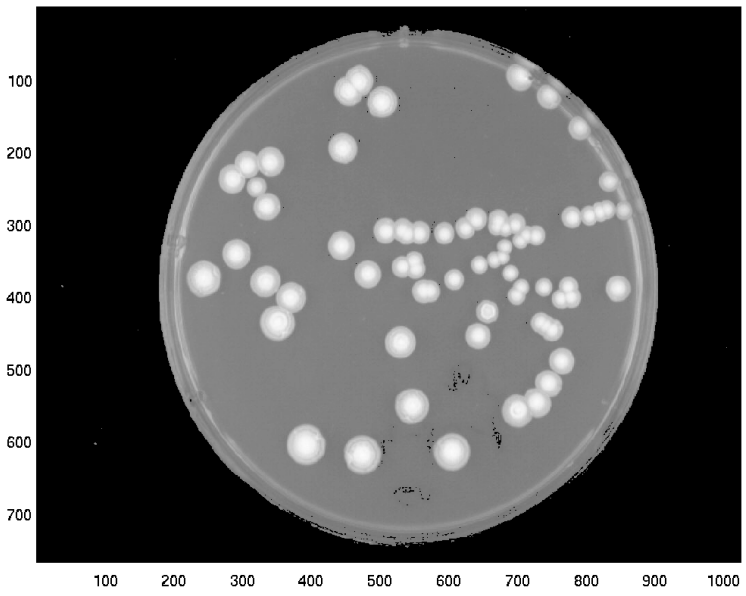
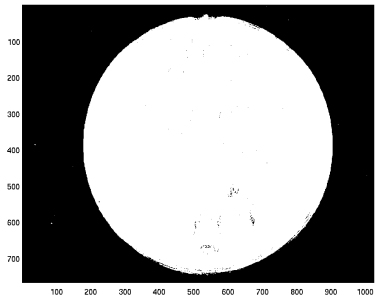
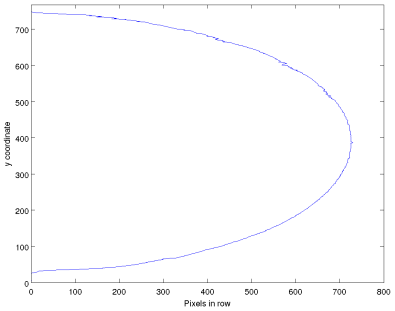


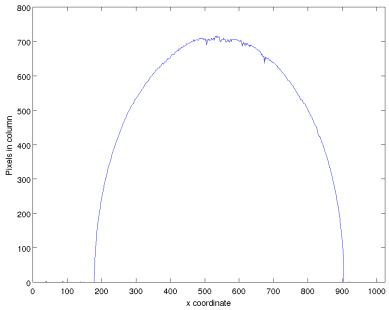
Figure 7: Image after background thresholding



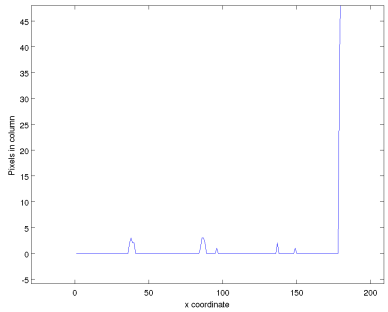
(a) Binary image



(b) Row projection

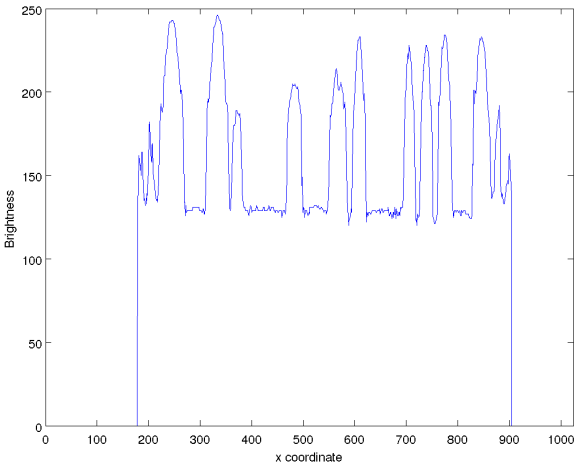


(c) Column projection

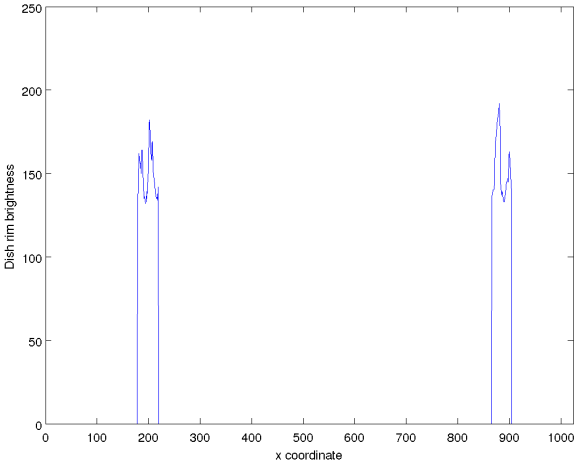


(d) Magnified part of column projection

Figure 8: Projections of the dish



(a) Central line, brightness



(b) Dish rim, brightness

Figure 9: Central image line and dish rim brightness

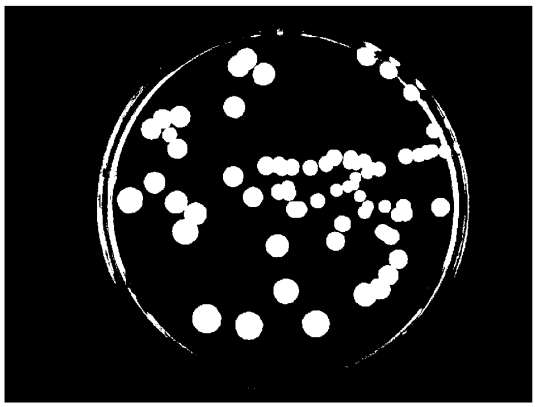


Figure 10: Binary image of colonies and the dish rim