# AUTOMATED COUNTING OF YEAST COLONIES USING THE FAST RADIAL TRANSFORM ALGORITHM

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Abstract: A method for counting yeast colonies in images of Petri dishes, based on the fast radial transform by Loy and Zelinsky, is introduced and evaluated in the paper. The paper focuses on processing images produced by a general-purpose imaging setup. The characteristic properties of the images, as produced by the setup used in the cooperating genetics research laboratory, are described. The performance of the method has been evaluated with a test set of 245 images. The images, included in this set, typically contained between 10 and 70 colonies per dish, with relative coverage of the dish less than 10% of the area. The average counting error (missed colonies) on this set was under 4%.
A tool, implementing the method, has been developed in Matlab. The tool provides a batch mode for processing of larger image sets prepared beforehand in the darkroom and it automates the process of counting as much as possible. It is available for download or can be requested from the authors of the paper, in both cases free on charge.

## 1 INTRODUCTION

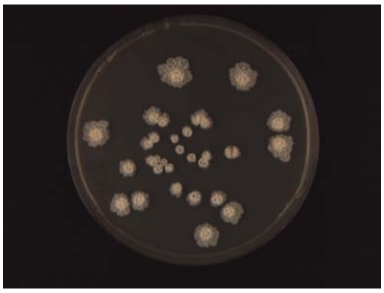
Yeast, namely *Saccharomyces cerevisiae*, is often used as a *model organism* in biological research. This is mainly due to its many favourable properties, such as the short generation time, easy genetical manipulation, as well as thanks to its established use in industry.

When performing experiments with *S. Cerevisiae* yeast, part of the process is growing the yeast colonies in Petri dishes, and evaluating the growth parameters, such as the coverage of the dish and the number of colonies. Traditionally, the colonies have been counted manually, using either manual colony counter or a counting raster. Examples of the manual counters are the Colony counter SC6 by Stuart (www.stuart-equipment.com) or the EW-14211-02 Hand-Held Electronic Colony Counter from Cole-Parmer (www.coleparmer.com). However, the hand counting is considered rather laborious and time-consuming process, error prone due to the fatigue and eye-strain of the laboratorian. Also, it does not provide any figures on the coverage of the dish or the radii of the colonies.

There are also a number of automated colony counters available – it is of interest that such system has been described as early as in 1974 (Goss et al., 1974). Such system usually consists of a single-purpose chamber with the illumination and camera system, where the dishes are placed one-by-one, or of an automated plate-handler, and a computer running an image-processing program that performs thresholding and segmentation of the image.

While an automated counter is definitely the system-of-choice for a commercial lab, in our case, the requirement was to reuse the imaging equipment already available in the lab (to achieve a low-cost solution) and to provide an off-line batch processing of a set of images taken beforehand in the darkroom. Also, the goal was that the system performs adequately for typical image variations, as described in the next section, with minimum possible manual intervention of operator. At the same time, it should allow for manual correction of the counting errors (multiple detections of a single colony or missed colonies). Finally, it should provide not only the count of colonies, but also evaluate the total coverage of the dish.

In this paper, we focus on the counting algorithm used in the system and present the performance data that we have achieved. The paper is structured in the following way: in the next section, the characteristics of typical images are reviewed. Then, the methods for



(a) Fluffy and touching colonies, diverse radii, dish centered

(b) Smooth colonies, dish touching edge of image

Figure 1: Examples of typical images of yeast colonies.

detecting round or symmetrical objects are surveyed in brief and the fast radial transform algorithm is described. The paper is concluded by description of the properties of the test data set and by discussion of the achieved results.

## 2 CHARACTERISTICS OF TYPICAL IMAGES

As has been stated, the requirement on the system described in this paper was that it would be able to process batches of images taken with a simple general-purpose imaging system; the laboratory is using a camera/copy stand with lightning units by Kaiser Fototechnik (see www.kaiser-fototechnik.de). The dishes are manually placed roughly into the center of the image, on a matt black background. No dish holder or stopper is used.

Examples of typical images of yeast colonies are shown in Figure 1.

The images are characterized by the following properties:

- dark background to increase image contrast
- illumination by two linear lights along the short edges (this is not important for processing, though)
- variations of dish position – cf. Figure 1(a) and 1(b)
- varying diameter of the dish in image
- varying intensity of the dish background

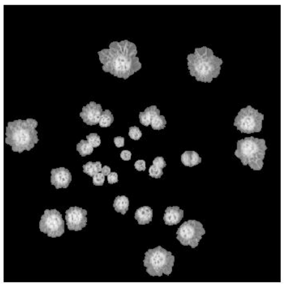


Figure 2: Dish image after preprocessing.

The colonies themselves are characterized by different types of morphologies (both smooth and fluffy), different size and/or age and by colonies touching each other.

## 3 SYSTEM DESCRIPTION

It follows from the image characteristics that the counting of colonies includes image thresholding, dish localization, and the counting itself. The process can be divided into two basic parts:

- *Image Preprocessing* – includes the following operations:
  - Image quality check: faulty images are eliminated to prevent system hangup
  - Image thresholding
  - Dish localization
  - Dish background thresholding

  As a result of preprocessing, we get a centered image of the dish, with eliminated background. An example of such image is given in Figure 2.
- *Colony Counting* – includes:
  - colony diameter estimation
  - colony center estimation

The description of the preprocessing part is out of the scope of this paper. Interested reader may find some details in technical report (Schier, 2009b). The method, however, has been simplified and made more robust since the publication of this report.

## 3.1 Colony Counting

The simplest approach is to correlate the contour of the colonies with a circular pattern, assuming that the maxima of correlation is located at the centers of colonies. This has been the first method we have tested (Schier, 2009a). During experiments, we have experienced poor performance in the case of big clusters and sensitivity to mismatch between the radius of the circular pattern diameter and the radius of the colony (and, hence, sensitivity to dispersion of colony radii).

An interesting approach to the counting of bacteria colonies has been described in (Marotz et al., 2001): to find colonies, a number of shape and structure criteria are used on the image pixels, including e.g. mean object radius, roundness of an object, compactness and asymmetry, radial monotony of fall-off in intensity, etc. These criteria are combined into shape and structure quality parameters and evaluated using fuzzy logic. The method works best under the assumptions of well-defined circular shape and monotone intensity fall-off of the colonies, which are well satisfied for bacteria (the case treated in the paper), but not necessarily in our case of the yeast colonies.

A popular method to detect circular objects is the circular Hough transform (for definition see e.g. (Ballard and Brown, 2003)). Based on our experiments, if multiple yeast colonies are overlapping or touching, it tends to detect (incorrectly) adjacent colonies as a single object. Also, following the results presented in (Loy and Zelinsky, 2003), the output of the Hough transform may be rather noisy.

Another possibility is to use the *radial symmetry*-based methods: the radial symmetry transform has been introduced in the work of Reisfeld (Reisfeld et al., 1995). Fast radial transform – a modified version of the radial symmetry transform with improved computational complexity – has been presented in (Loy and Zelinsky, 2003). Since this transform is the method that is currently employed in our tool, it will be reviewed in the next section.

### 3.1.1 Fast Radial Transform

Fast Radial Transform is a transform that maps the original image to the transformed image according to its contribution to radial symmetry of the gradients at distance $n \in N$ ($N$ is the set of radii) away from each point. For full details of the method see the original description in the paper of Loy and Zelinsky.

First, image gradient $\mathbf{g_{i,j}}$ at each point $(i, j)$ is calculated. Then, the *positively*- and *negatively-affected pixels* are determined:. The *affected pixel* is defined as the point *in the direction* of the gradient vector $g_{i,j}$ is (for the *positively-affected* ones) or *counter the direction* of the gradient vector (for the *negatively-affected* ones), at a distance $n$ pixels away from the point at coordinates $(i, j)$. The coordinates of affected pixels are given by

$$\mathbf{p}_{+}(i,j) = (i,j) + \text{round}\left(\frac{\mathbf{g}_{i,j}}{\|\mathbf{g}_{i,j}\|}n\right)$$
$$\mathbf{p}_{-}(i,j) = (i,j) - \text{round}\left(\frac{\mathbf{g}_{i,j}}{\|\mathbf{g}_{i,j}\|}n\right)$$

The gradient matrix $\mathbf{g}$, together with the coordinates of affected pixels, is used to determine the *orientation* and *magnitude* projection images $O_n$ and $M_n$ for the given radius $n$:

$$O_n(\mathbf{p}_{+}(i,j)) = O_n(\mathbf{p}_{+}(i,j)) + 1,$$
$$O_n(\mathbf{p}_{-}(i,j)) = O_n(\mathbf{p}_{-}(i,j)) - 1,$$

$$M_n(\mathbf{p}_{+}(i,j)) = M_n(\mathbf{p}_{+}(i,j)) + \|\mathbf{g}_{i,j}\|,$$
$$M_n(\mathbf{p}_{-}(i,j)) = M_n(\mathbf{p}_{-}(i,j)) - \|\mathbf{g}_{i,j}\|.$$

The radial symmetry at radius $n$ is defined by convolution

$$S_n = F_n * A_n,$$

where

$$F_n(i,j) = \frac{M_n(i,j)}{k_n}\left(\frac{|\tilde{O}_n(i,j)|}{k_n}\right)^{\alpha},$$

and

$$\tilde{O}_n(i,j) = \begin{cases} O_n(i,j) & if\ O_n(i,j) < k_n \\ k_n & \text{otherwise.} \end{cases}$$

$A_n$ is a two-dimensional Gaussian, $\alpha$ is the radial strictness parameter and $k_n$ is a scaling factor used to normalize $M_n$ and $O_n$. Projection images $M_n$ and $O_n$ are initially set to zero.

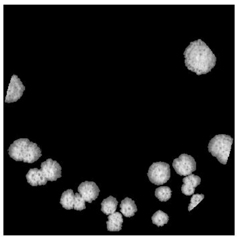
An example of a symmetry map for a dish with yeast colonies is given in Figure 3.

To estimate the colony centers, the transform must be completed with two additional procedures: estimation of the set of radii $N$ to be tested, and by a procedure to find local maxima (corresponding to the centers) in the symmetry map $S$ (map $S$ is a weighted sum of all symmetry maps $S_n$). Both procedures will be discussed in the following sections.

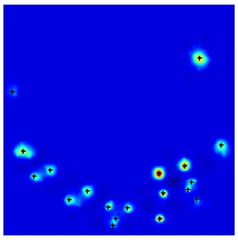
### 3.1.2 Colony Radius Estimation

To estimate the range of radii of the objects contained in the image, the following steps are used:

- the equivalent diameter $d$ and eccentricity $\varepsilon$ of all objects in the image is computed. The diameter is computed from area $A$ (number of pixels) of an



(a) Preprocessed image of colonies



(b) Symmetry map $S$ with the centers of colonies

Figure 3: Illustration of function of the radial symmetry algorithm.

object. The eccentricity equals to the eccentricity of an ellipse with the same second-moments as the object. It equals to $\varepsilon = 0$ for a circle and to $\varepsilon = 1$ for a line segment.

- Nearly circular objects (with eccentricity $\varepsilon < \theta_\varepsilon$, $\theta_\varepsilon$ is the eccentricity threshold), are selected.
- Min and max diameter $d_{\min}$ and $d_{\max}$ of the objects in the set of circular objects are determined. The interval between them is divided to $\nu$ equidistant subintervals. The set of radii $N$ for the fast radial transform is then given by:

$$\begin{aligned} \Delta &= d_{\max} - d_{\min} \\ N &= \{d_{\min} + [0,\ldots,\nu] \cdot \Delta/\nu\}/2 \end{aligned}$$

#### 3.1.3 Estimation of Colony Centers

The centers of the colonies are represented by local maxima of the symmetry matrix $S$. To find these maxima, the `nonmaxsuppts()` function for Matlab (Kovesi, 2005) is used. It performs grey scale dilation of the input image and finds the points in the dilated image that match the original and that are greater than the threshold. Finally, it returns the row and column coordinates of these points.

## 4 EXPERIMENTS AND RESULTS

To test system recognition performance, it was evaluated using 245 images, containing colonies with different morphology and relative coverage of the dish. The distribution of the test set in the terms of frequencies of the number of colonies in the dish is shown in Figure 4. Figure 5 shows the distribution of relative coverage of the dish and mean diameter of colonies (marker size) in comparison with the number of colonies in the dish. It can be seen that the typical dish contains less than 70 colonies, with relative coverage less than some 10%.

The illustrative counting results for typical images used in these tests are shown in Figure 6 on the next page.

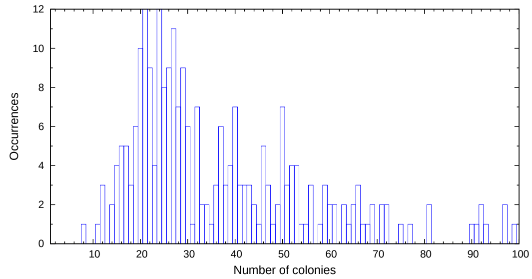



Figure 4: Petri dishes with the same number of yeast colonies.

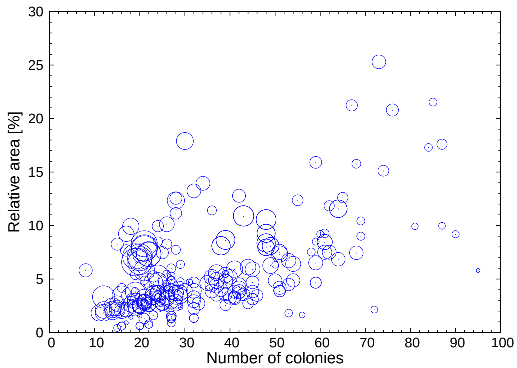



Figure 5: Distribution of the test set – relative dish coverage in comparison with the number of colonies. Diameter of markers represents mean radius of the colonies in the dish.

### 4.1 Counting Tool

The algorithms, as described in Section 3, have been implemented in a counting tool. In the background, this tool performs fully automatic thresholding of the image, localization of the dish and counting of colonies. It provides an environment for selecting the directory tree with images to process, the file to store

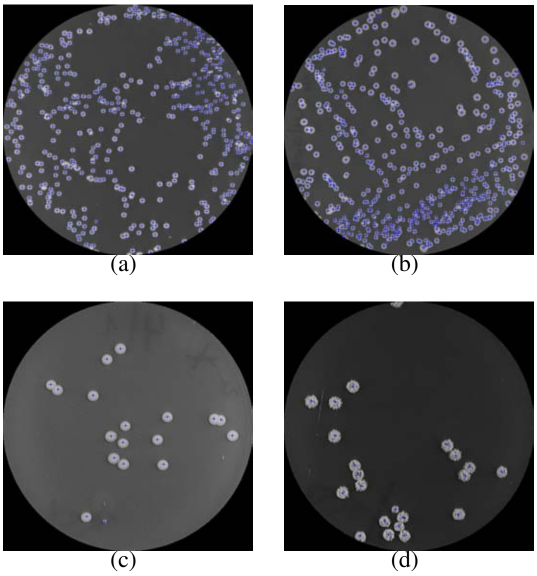



Figure 6: Examples of algorithm detection and counting results. Figures (a) - (b) illustrate dishes with high coverage, (c) - (d) dishes with low coverage.

the counting results, and the counting mode: in the semi-automatic mode, a simple point-and-click editor can be used for manual correction of the output of the counting algorithm, in the fully-automatic mode, the output of algorithm is directly stored into the result file.

This tool was used also to obtain the reference results used in this paper: using manual correction, we obtained the correct counts of colonies in Petri dishes, which were used to evaluate counting error rates.

## 4.2 Algorithm Settings

The following settings were used in our experiments:

- Parameters of the fast radial transform:

$$k_n = 6, \alpha = 4$$

- Parameters of non-maxima suppression:

$$\text{threshold} = 4, \text{radius} = 0.8 \cdot \min(N),$$

  where $N$ is the set of radii for the fast radial transform.

- Construction of $N$:
  eccentricity threshold $\theta_\varepsilon$ (see Section 3.1.2) is initially set to $\theta_\varepsilon = 0.25$. The number of equidistant intervals $\nu$ is set to $\nu = 4$. At least five colonies of given eccentricity must be in the image, else the eccentricity is increased by 0.1.

## 4.3 Typical Detection Errors

Figure 7 illustrates typical detection errors of fast radial transform. A colony may be missed if it touches other colony or, rather, multiple colonies, so that they form a cluster. This detection error is almost absent if the colonies touch only at one point and thus creates a structure similar to a chain. If more colonies are touching each other, they form a structure in which their shape is distorted and internal colonies cease to be circular. The circular outer border of colony located in such cluster could be too short for the proposed method to work properly.

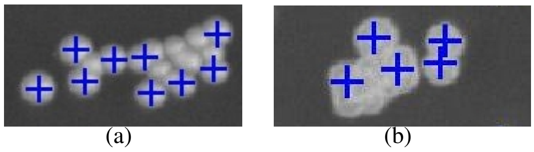



Figure 7: Example of typical detection error of fast radial transform.

The counting errors for various number of colonies are summarized in the Table 1. The relatively high counting error for the dishes with the colony counts greater than 60 is given by two factors. First, with the increasing number of colonies increases also the probability that there will be colonies touching each others (Figure 7a). Second, the number of samples with this high density of coverage was relatively low, thus increasing the evaluation error (Figure 4).

Table 1: Dependence of the counting errors on the number of colonies in the dish. The system average counting error is under 4%

| ♯ colonies | samples | missed [%] | false [%] |
|---|---|---|---|
| 0 – 17 | 21 | 1.47 | 0 |
| 18 – 20 | 19 | 2.99 | 0 |
| 21 – 23 | 25 | 3.31 | 0 |
| 24 – 26 | 29 | 4.00 | 0 |
| 27 – 29 | 27 | 3.28 | 0 |
| 30 – 40 | 35 | 4.17 | 0 |
| 41 – 49 | 30 | 4.00 | 0 |
| 50 – 60 | 29 | 3.87 | 2.00 |
| $> 60$ | 30 | 5.36 | 3.22 |

Figure 8 shows the recognition performance related to the number of colonies in the dish. A typical dish used in our experiments contains from 10 to 40 yeast colonies. The figure also shows the recognition performance of the fast radial transform for the detection of circular objects. The dishes, where some colonies have not been detected are shown below the dashed line.

Another view on the algorithm performance is provided by Figure 9, which presents a box-whisker

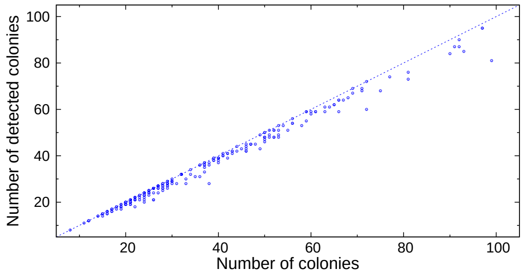
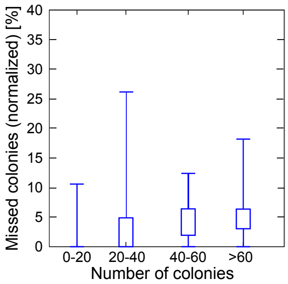



Figure 8: Algorithm recognition performance regarding to number of colonies.

plot of relative counting error (missed colonies) in several groups of the colony counts. The numbers of samples in each group are given in the table, included with this plot.




| # colonies | samples |
|---|---|
| 0–20 | 40 |
| 20–40 | 123 |
| 40–60 | 52 |
| >60 | 30 |

Figure 9: Relative number of missed colonies dependent on the number of colonies.

## 5 CONCLUSIONS

In the paper, we have presented the method for counting of yeast colonies, based on fast radial transform. The method has been tested on a set of 245 images with different degrees of coverage. The distribution of the data in the test set and the performance of the counter is discussed. It should be noted that the average counting error is below 4%. It is difficult to compare the performance with the commercial solutions, since the performance data of these systems are not available.

The algorithm has been implemented in a Matlab-based tool which provides environment for automatic or semi-automatic processing of batches of images. This tool has been successfully deployed in the cooperating biology research laboratory and is available for download at http://zs.utia.cz/index.php?ids=results&id=yeastcolcount&lang=enghttp://zs.utia.cz/index.php?ids=results&id=yeastcolcount&lang=eng.

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