

Testing Raspberry Pi based microscope camera on growth of household yeast

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Abstract

This document describes an experiment where yeast cells are grown on a microscope slide to test the microscope image capture mechanism, and inspire the author to get some ideas about how to capture even more interesting pictures of yeast growing.

1 Introduction

Some time ago I installed microscope adapter on my Amscope microscope. Since then I have not tested if it is indeed useful to capture time series of microscope, so today I decided I should test that.

As an experimental system, I started up an image capture programme in the Pi, and prepared a solution of yeast and sugar on a microscope slide, and set about to capture images what happened.

This note describes the experimental system, the experimental protocol, and makes some suggestions on how to improve the experimental protocol for future experiments.

2 Methods and materials

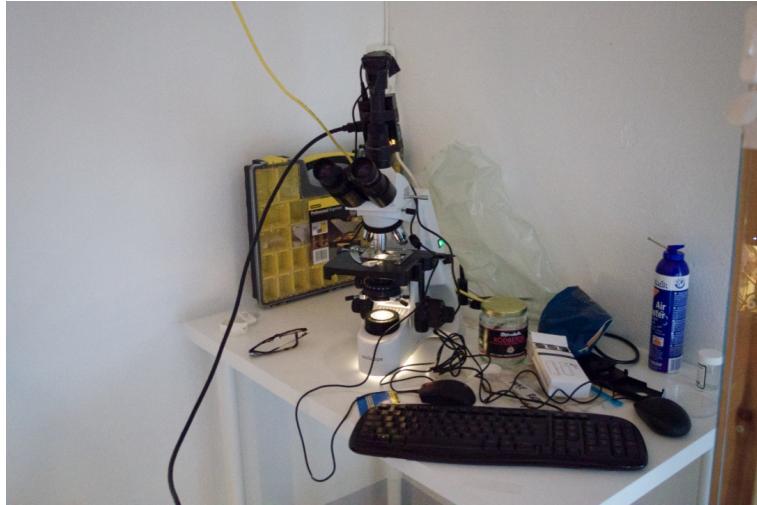


Figure 1: The experimental setup captured during the experiment.

A yeast/sugar/water solution was made by adding about a tea-spoon of sugar to an egg-glass of water and stirring. The solution was then cooled by adding tapwater. To this solution a small amount (roughly equivalent to 30 mm^3) of household yeast (“IDUN mors hjemmebakte original gjr” with a “best before” marking of april 10 2016).

A drop of this solution was put onto a microscope slide (“Elka Assistent, Objekttrger micro slides cleaned”) and then put onto the microscope and a cover glass was put on top. The solution spread out under the entire cover glass and on visual inspection it seemed to be completely uniform.

The slide was then put onto the microscope. The microscope is an Amscope 40X-2500X Infinity trinocular compound microscope. The 40x microscope lens (air, not oil) was selected.

To this microscope was connected a 5 megapixel raspberry Pi camera module connected to a Raspberry Pi 2 model B. The camera sensor was connected to the microscope using a 3D printed adapter. The Raspberry Pi module was then connected to the microscope itself using

duct tape (also know as “gaffa tape”). The 3D printed sensor/microscope adapter was printed using red plastic, so to avoid light leaking in to the sensor thorugh the plastic, the entire adapter was covered in black duct tape.

The Raspberry pi is a linux based computer. To focus it on the slide, I used the “raspistill -t 0” program to show a large live capture image on the attached HDMI screen. When focus was determined to be adequate, a script was used to capture frames:

```
mkdir -p /home/pi/yeast

while /bin/true ; do
    DATE=$( date +"%Y-%m-%d %H%M%S" )
    raspistill -vf -hf -o /home/pi/yeast/images/$DATE.jpg
    echo "Captured picture at $DATE"
    sleep 30
done
```

This script was run using the ”nohup” utility to ensure that it continued running even if the network connection to the Raspberry Pi was lost during the experiment.

The frames were then converted to an mp4 movie.

To refer to the captured frames I used the following script to create symbolic links that pointed to the original frames, but were numbered in a manner recognisable by the avconv program

```
FOO=1;
for x in images/* ; do
    ln -s ../$x $(printf "frames/%06d.jpg" $FOO) ;
    FOO=$(expr $FOO + 1) ;
done
```

First attempted to to perform the conversion on the Raspberry Pi using the command:

```
avconv -r 10 -i frames/%06d.jpg \
      -r 10 -vcodec libx264 -crf 20 \
```

```
-g 15      timelapse.mp4
```

This did not succeed due to a lack of available memory. Instead I then transferred the files to a computer running OSX using the “scp” command

```
scp 'pi@10.0.0.23:/home/pi/yeast/images/*' .
```

and then ran the “ffmpeg” encoder to produce an mp4 movie file:

```
ffmpeg -i frames/%06d.jpg timeseries.mp4
```

During the work with the development of the symbolic link generating script, I made the mistake of deleting all of the images (instead of “frames”), so about an hour of captured images were lost.

3 Results

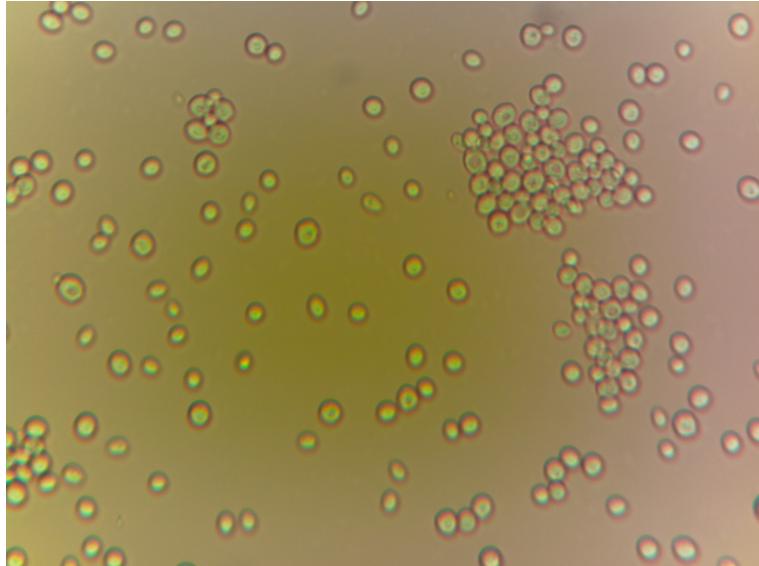


Figure 2: The first image in the sequence 2016-03-28_111108.jpg

During the experiment, and excluding the hour or so of frames that were lost, 336 frames were captured. The first and last images can be found in figures 3 and 3.

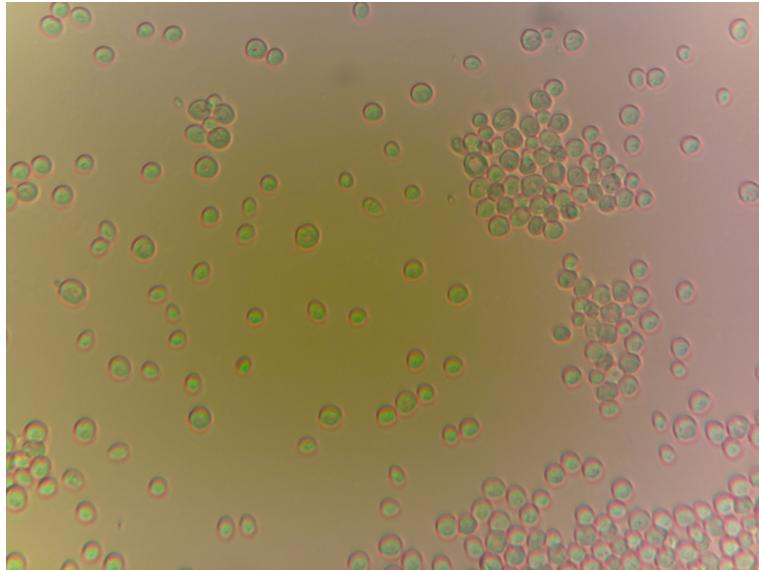


Figure 3: The last image in the sequence 2016-03-28_140625.jpg

All of the frames and the movie produced using ffmpeg were then placed in a pair of dropbox¹ ².

4 Discussion

So that was fun :-) It shows that there is some potential here. Notes:
The movie shows

When watching the movie I could not see cell division occurring, but I did see movement inside the cells, and that *could* be mitosis and chromosomes being pulled to the spindles of the cells, but that should be confirmed and studied a bit more before I quite believe it.

I can observe that cells grow to about twice their original size during the movie.

At the end of the sequence there is an onrush of cells from the

¹The yeast growth movie: <https://dl.dropboxusercontent.com/u/187726/shared-experimental-data/yeast-growth-28-mar-2016/movies/yeast-growth.mp4>.

²The raw image data: https://www.dropbox.com/sh/89nr05c739ucmcs/AADHEj9F1bnCn4t480C_s9tsa?dl=0.

bottom. One explanation could be that there has been more successful cell division elsewhere on the slide, and that cells resulting from that division is being pushed into the part viewed during the experiment.

There is a bunch of things we can do that could be interesting in the future. Here are the ones I remember while writing this document.

- The yellow color is a bit annoying. Perhaps fixing the color balance either post-capture or by putting a filter into the light source would improve the images?
- The time spent during this experiment was not enough to see an actual cell division, so just running the experiment for a longer time (more than three hours) is probably something that should be tried.
- Growth is influenced by both nutrient (sugar) availability and temperature. Using another nutrient (fructose or glucose) could be interesting. It could also be interesting to increase the temperature, or at the very least measure the temperature of the experimental assembly during the experiment.
- A test run focusing only on a few cells but with higher magnification (e.g. 100 oil) could possibly give more details on the cell division cycle, perhaps allowing confirmation / rejection of the hypothesis that we actually saw chromosomes being pulled apart.
- Using the raw images there are multiple kinds of analysis that can be attempted:
 - Identify cells, their locations and size.
 - Track movement, division and perhaps interior content (e.g. visible chromosomes) over time.
 - Using e.g. iJulia or IPython would be fun.
- Adding date/time to the images would be useful both for later analysis and to avoid confusion in general :-)

- Being more precise when preparing the yeast/nutrient solution would also be useful, so that it was repeatable and therefore tunable for better yield (or whatever I want to test).

5 References

Watching bread yeast make bubbles: <http://www2.mrc-lmb.cam.ac.uk/microscopes4schools/yeast.html>