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Original Article

High Resolution Image Registration for Micro-Colonies Monitoring on Petri Dishes

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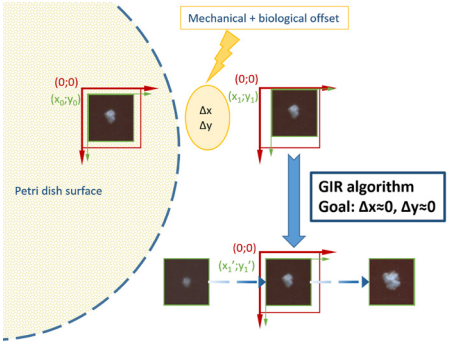
Highlights

- Real-time monitoring of micro-colonies in Petri dishes by high resolution imaging.
- Image registration algorithm validated at 95.8% on 1154 Petri dishes samples.
- Detection of acquisition defects and of agar breaks.
- The device is currently being commercialized.

Graphical abstract



Abstract

Background To reach faster diagnosis of sanitary control and to reduce human manipulations, rapid microbiology develops methods and systems allowing to detect these contaminations early. The company Advencis conceived an innovative device able to follow in real-time the contaminations during the incubation process.

Methods The aim is to keep the traditional method (Petri dishes with lid), and this, without any modification of the sample. This method is based on the monitoring of the Petri dishes surface by image processing while incubating the samples. The iterative registration method developed here is based on small objects lying on the growing surface.

Results A large number of image sequences of various samples were tested. The results show that this method is quite efficient to follow and detect the variations on the sample.

Conclusion Some specific cases may be improved in a future algorithm version.

Keywords: Image registration; Point set registration; Rapid microbiology; Real-time monitoring; Defaults detection

1. Introduction

Rapid microbiology methods (RMMs) can provide results in hours rather than days for classical techniques. For industrial quality control RMMs give opportunity to improve product

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process. For clinical diagnosis, rapidity in getting results often allows for defining the most appropriate treatment earlier, and thus to save lives. The Advencis company, a bioMérieux subsidiary, designed an innovative system for real-time detection and monitoring of contaminations during the incubation process [1]. The aim of the method is to rely the compendial method and its standard consumables (Petri dishes), while keeping identical both workflow and sample (no manipulation, closed lid, no staining step, etc.). This method is based on high resolution imaging of the Petri dishes surface. A scanning technique is used to acquire surface image of the Petri dishes. This technique induces lower-than-1 mm shifts between two consecutive images. To follow the growth of the microorganisms, a precise image registration method is needed. This article presents the registration method developed specifically for this application and evaluated on a significant number of real data.

Firstly, rapid microbiology and its interest are presented. Then, different instruments used in rapid microbiology are introduced and their pros and cons are analyzed. Subsequently evaluating the need for a new system, the Advencis instrument is described. The image registration need is described by introducing the mechanical offset related to the system and the offset of biological origin. Several existing registration methods are presented before developing our own solution. A validity control of this registration method is presented, along with the obtained results, which are finally discussed.

1.1. Rapid microbiology

Biology consists in studying bacteria, yeast and fungi. Those are microorganisms not visible by naked eye, with a size of 0.5 to 10 micrometers. Some of these germs are pathogenic for humans and animals. It is therefore important to be able to detect contamination in food, medicines and others. The detection of bacterial micro-colonies is done in fields ranging from sanitary control in food industries to patients infection diagnosis in clinical field to sterility control of medicines and vaccines. Traditionally, the major part of these tests is done into Petri dishes. The samples under interest are disposed on a nutritional medium where the eventually present living cells can multiply to form micro-colonies with a diameter of 20 to 500 micrometers (µm), then visible colonies when larger. These biological samples are incubated with a constant temperature during 4 to 16 days [2]. Once the germs have grown, microbiologists study the dishes by naked eye or by using a magnifying glass. The Petri dishes can also be analyzed by colony counters, especially when an important concentration of germs prevents from counting them precisely, or if too many samples need to be counted [3–6]. They usually detect colonies from a size of 500 µm and above, but the Scan® 1200 from Interscience claims a minimal detection size of 50 µm. However, there is a lack of counting precision at these sizes (high number of false positives). To detect germs precociously, with an interesting cost per test, rapid microbiology devices are developed.

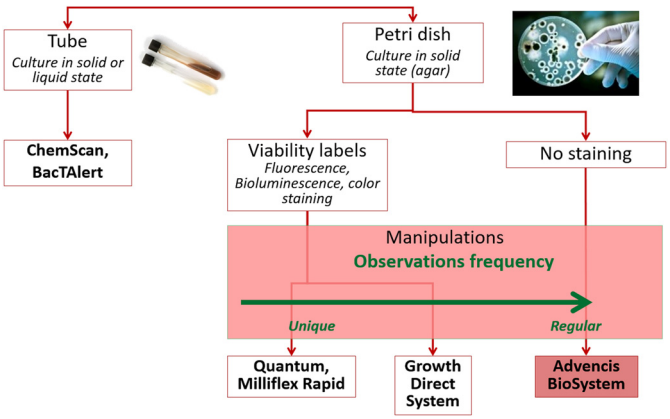

Fig. 1. Positioning of several procedures and techniques in rapid microbiology.

1.2. Existing systems

The samples are usually analyzed in solid or liquid media. The contamination detection time can be significantly reduced to a few hours for molecular methods like PCR (Polymerase Chain Reaction), which directly targets microorganisms DNA, compared to a few days for culture methods on solid media (agar media). However, all kind of samples cannot be reached with this method. Furthermore, in liquid media, cells are spread around, and they are usually detected at a concentration of 10^5–10^6 cells per milliliter (mL), whereas the solid state tests allow for segregating individual cells, when forming micro-colonies, at a concentration of 10^2–10^3 cells per mL. Different actors using solid states tests can be found on the rapid microbiology market (Cf. Fig. 1). Three systems using solid media closest to the Advencis system are compared by their observing frequency. Others solid media systems developed by bioMérieux, the ChemScan using cytometry [7] and BacTAlert [8] using colorimetry, are much more different from the systems Quantum, Milliflex® and GrowthDirect, so are not considered in this work.

The Quantum device, from early 2010s, allows for the analysis of a single Milliflex® dish, a specific dish format. It uses a viability staining that is metabolized by the cells to obtain a fluorescent signal [9]. This instrument, developed by Merck-Millipore, has a detection time around 21 to 48 hours. When the sample is placed into the device, the microorganisms are revealed by fluorescence and a picture of the sample can be taken by an embedded camera. However, the staining process is coercing. This system is approximately two times faster than the traditional method by naked eye.

The Milliflex® Rapid device, the forerunner of the Milliflex® Quantum, was also developed by Merck-Millipore in 2008. It uses the natural bioluminescence process, measured by ATP-metry [10]. A specific protein, the adenosine triphosphate (ATP), is sprayed on the filterable membrane. It is a label of cell viability. Milliflex® Rapid allows for the analysis of a single Milliflex® sample at a time. The contamination detection time is around four times faster than the traditional method.

The Rapid Microbio System is one of the first rapid microbiology devices, invented in 2004. It is not yet widely spread,