Generator of Simulated Time-Lapsed Microscopy Images of Bacterial Cells

Leonardo Pedro Donas-Boto de Vilhena Martins

PhD Program in Electrical and Computer Engineering Thesis Plan Proposal

Supervisor: Professor José Manuel Matos Ribeiro da Fonseca
Co-supervisor: Professor André Sanches Ribeiro

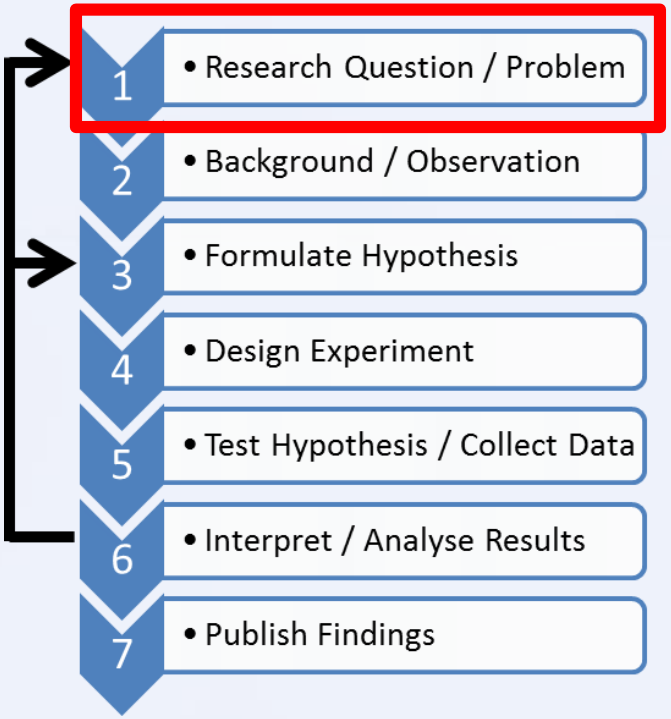
CA3 Computational Intelligence Research Group
UNINOVA Instituto de Desenvolvimento de Novas Tecnologias

FCT FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

TAMPERE UNIVERSITY OF TECHNOLOGY

Introduction

This Thesis Plan Proposal follows the classical research method:



Main Motivation

Impressive progress in recent years in

Microscopy Live-Cell Imaging

Resulted in

- Images with better quality and better resolution
- Techniques capable of detecting new cellular structures

Prompted the

Development of various image processing algorithms:

- Automatic Cell Segmentation
- Image Registration Techniques
- Cell and Structure Tracking

Open Problem in the Area

These processing methods require Validation.

Manual Validation is used as a Gold Standard, but:

- Is expert-dependent (difficult repeatability and even intra-user variability can be very high)
- Is unfeasible and time consuming for large data-sets.

Computational biological modelling to simulate microscopy images is a viable alternative to create a **"ground truth"** to be used as Benchmark for Validation

Main Research Question

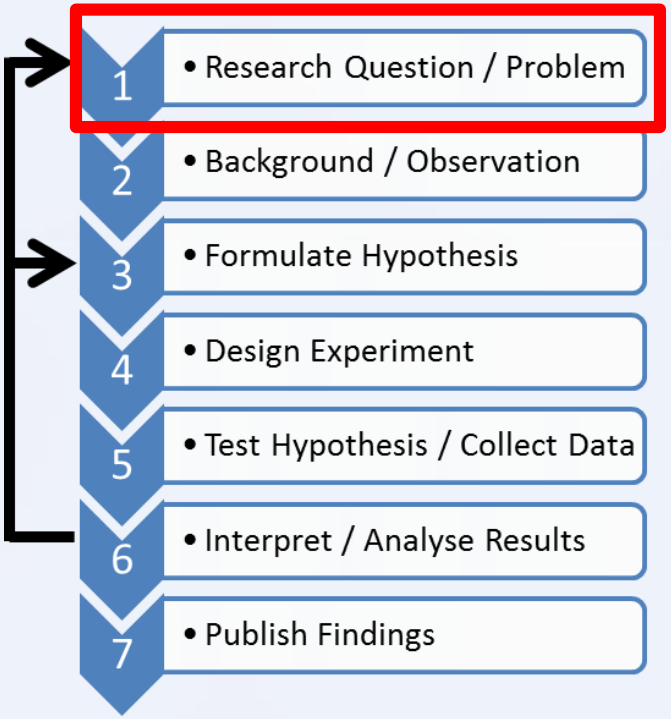
How to design a **Generator of Microscopy Images** that is capable of simulating bacterial **time-lapsed experiments** by reproducing **realistic morphological and functional** microscopy images?

This Main Research Question can be then divided in **4 Secondary Questions**.

Secondary Research Questions

- Which **biological processes and environmental conditions** are necessary and sufficient to create a realistic simulation of the **cell spatial and temporal organization**?
- Which **image acquisition parameters** are necessary and sufficient to simulate the realistic characteristics of each type of microscopy methodologies will be used?
- Which **methodologies should be used to validate** the image generation tool?
- What **applications** other than validation of image processing tools and the creation of time-lapsed microscopy image benchmarks can benefit from the simulation of bacterial time-lapsed experiments?

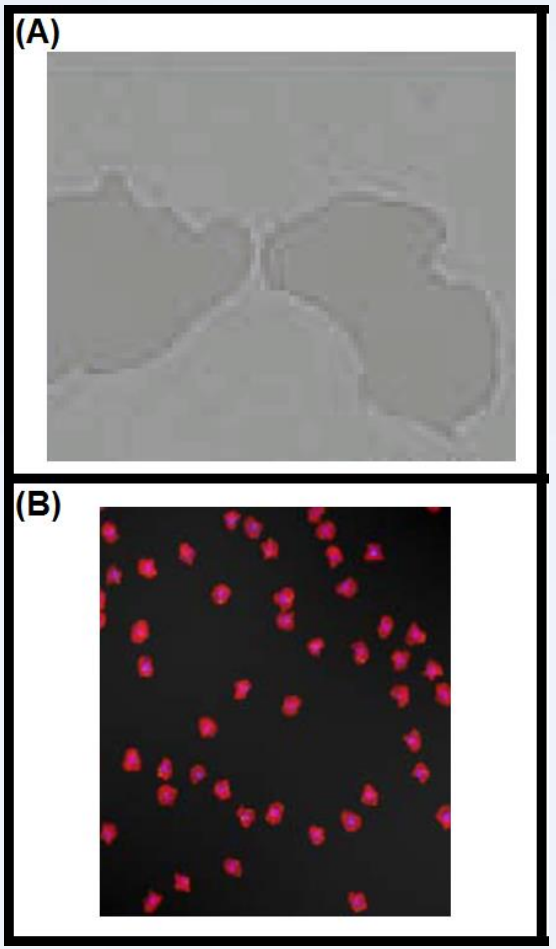
Research Method



Background / Observation

Image Generators have focused on the simulation of the morphological features and spatial information of the cell, producing just a **single image frame** of the desired synthetic model.

Examples of such artificial images include:
(A) Keratocyte cells (Ambühl et al., 2012).
(B) Human embryonic stem cells (Du & Dua, 2010).



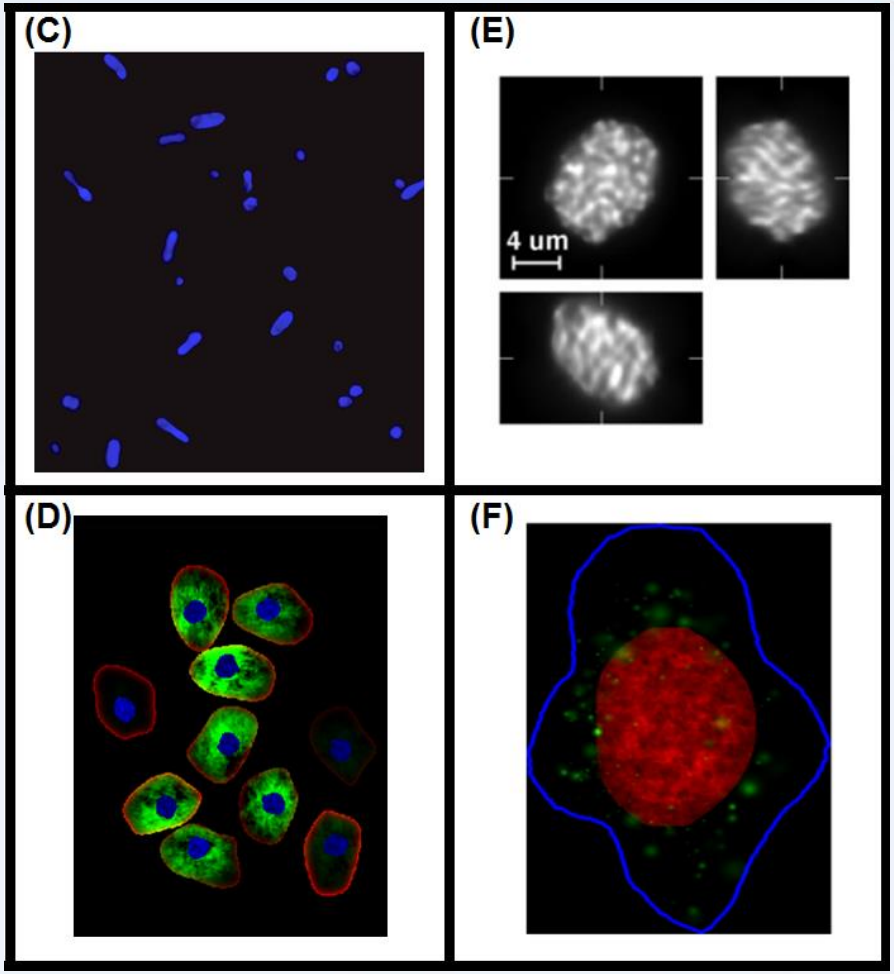
Background / Observation

(C) E. coli and M. luteus bacterial cells **(SIMCEP Toolbox)** (Lehmussola et al., 2011).

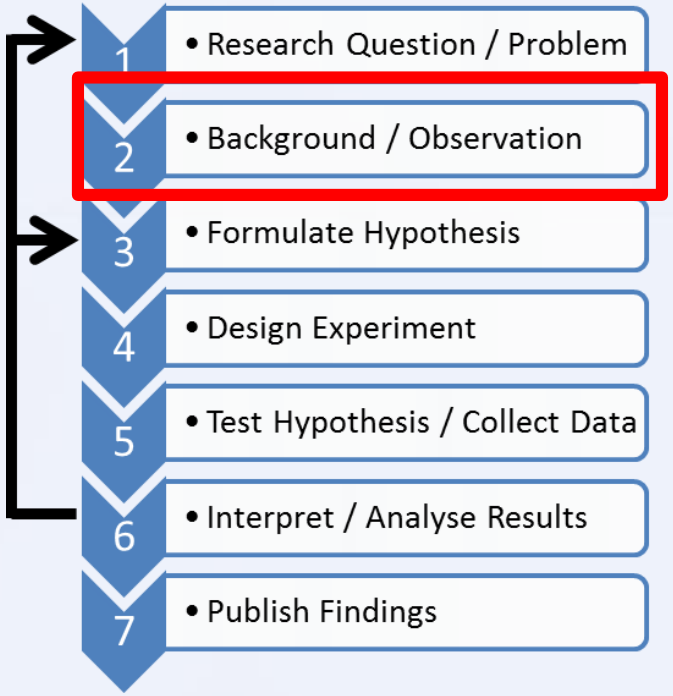
(D) Cellular organelles (nucleus; nuclear body; cytoplasm and lipid, droplets) **(SimuCell Toolbox)** (Satwik et al., 2012).

(E) 3D rendering of HL-60 Nucleus **(CytoPacq Toolbox)** (Svoboda et al., 2007).

(F) 2D model of simulated images of lysosomes **(CellOrganizer Toolbox)** (Murphy, 2012).



Research Method



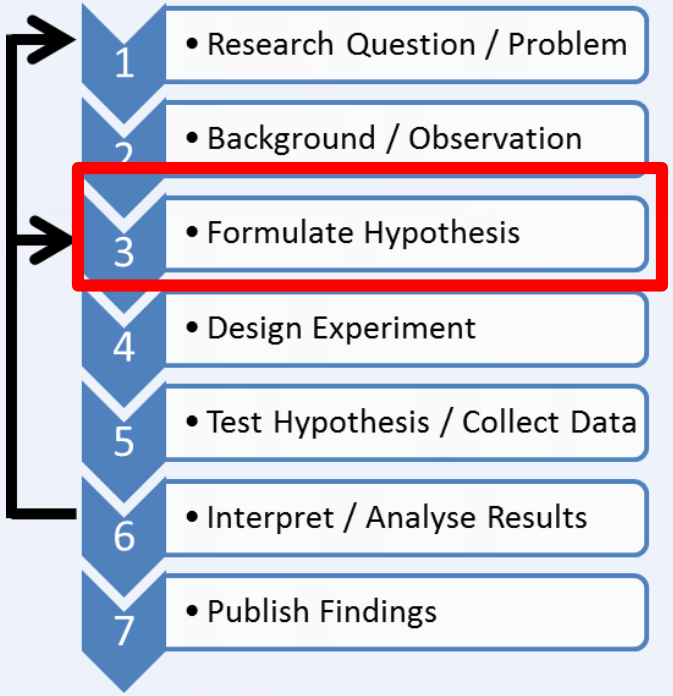
Hypothesis

An Artificial Image Generator capable of replicating realistic bacterial time-lapsed experiments can be developed if the produced morphological and functional images can emulate the characteristics of the images acquired in the laboratory.

To do this, we need to reproduce the spatial and temporal cell morphological (**Cell size, shape, curvature and spatial arrangement**) and functional features (**Cell growth, movement and division**).

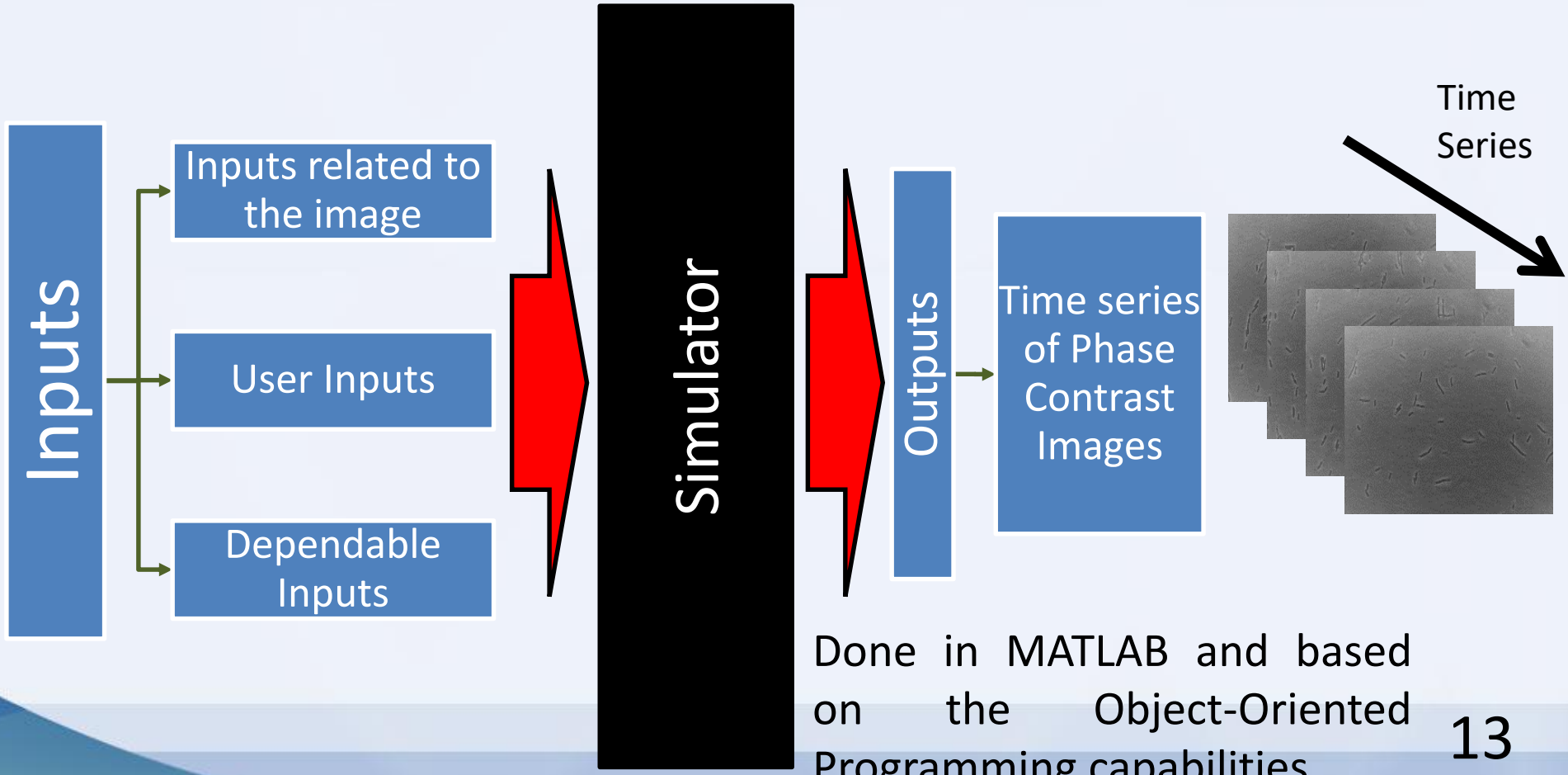
This will require the implementation of existing mathematical or empirical models from the literature and the simulating different image acquisitions systems and environmental conditions.

Research Method



Experiment Design

Tool Workflow

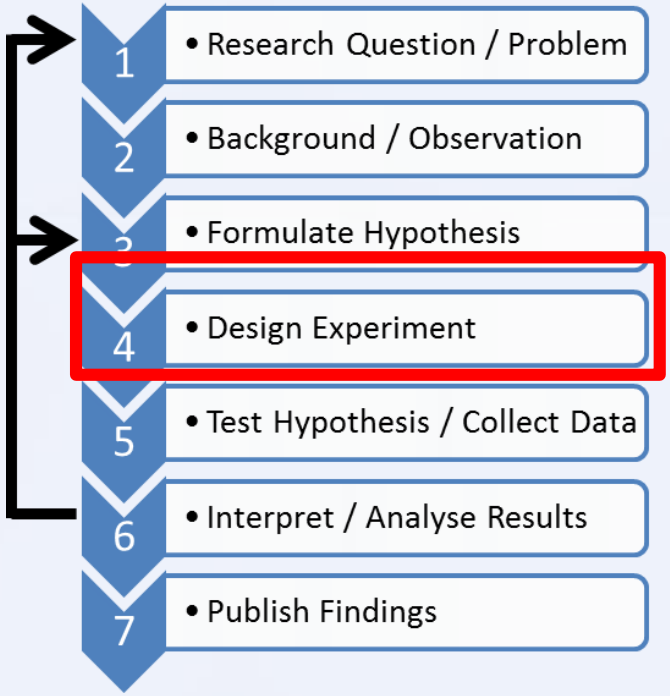


Done in MATLAB and based on the Object-Oriented Programming capabilities

Proposed Approach

- Realistic cell morphology (**shape, size, curvature and orientation**).
- Realistic time-dependent cell functions (**growth, division and motility**).
- Simulation of internal production of RNA and proteins fluorescently tagged using a stochastic model of gene expression dynamics.
- Simulation of Image acquisition parameters based on the **illumination, contrast and florescence methods**
- Similar **user interface** with the produced image processing tools
- Simulation of bacterial response to **external environmental conditions** such as temperature (heat-shock, cold-shock), pH stress, oxidative stress, nutritional stress or even exposure to antibiotics from empirical data.

Research Method



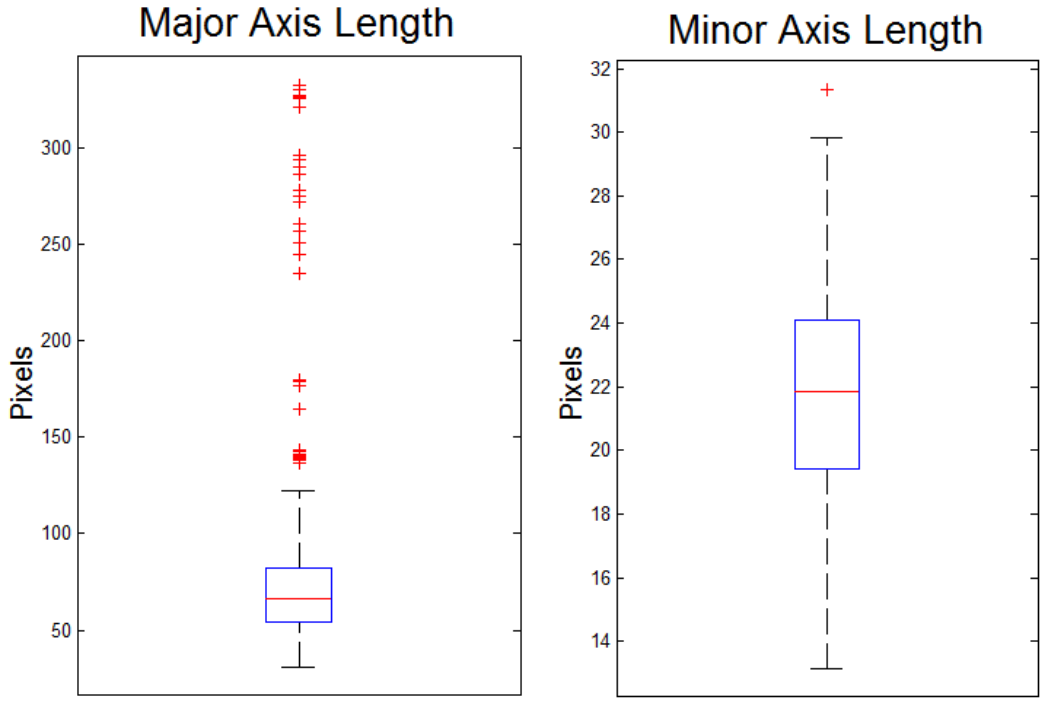
Collecting data (Modelling)

Simulation parameters include:

- Total Time of simulation (in seconds),
- Frame Rate (the value of each time-step in seconds),
- Image Frame Size (in Pixels),
- Pixel Density (in pixels per millimetre),
- Initial Number of Bacterial Cells (at the start of the simulation)
- Image acquisition parameters that simulate illumination and the primary sources of noise

Morphological Features

Sizes



	Major Axis	Minor Axis
Mean	74.7	21.8
Standard Deviation	39.9	2.9

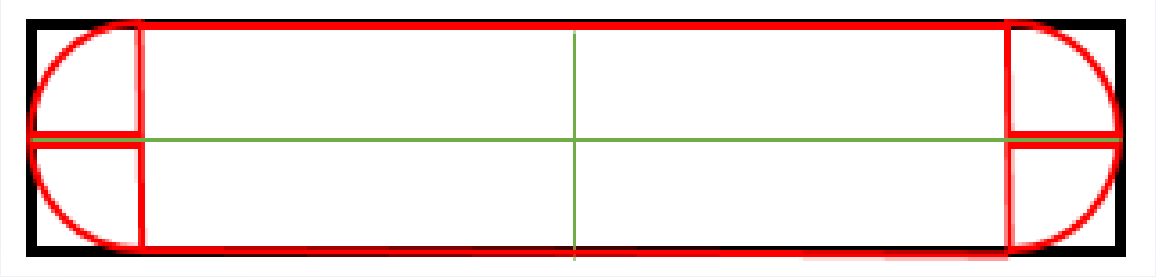
Note that this values are for a specific image magnification.

The Variation will be important for the models of Growth and division

Morphological Features

The rod shape

Mathematical Aproximation



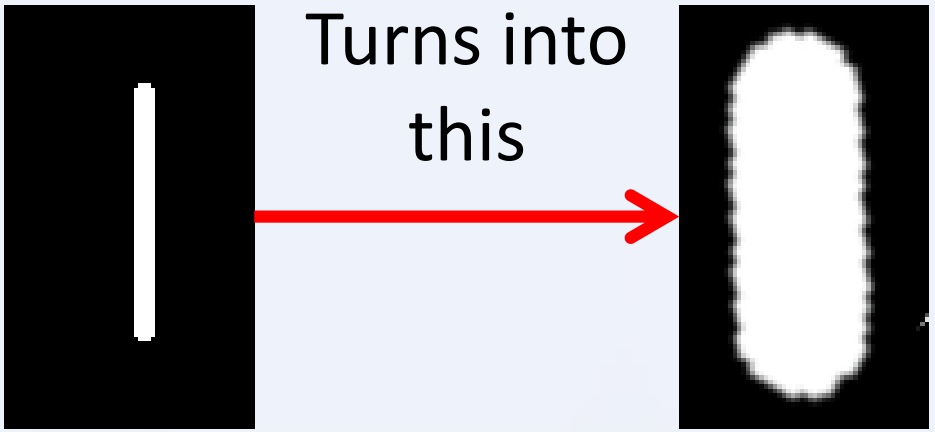
Mathematical modelling of the rod shape of *E. coli* cells can be done by defining a rectangle (**black line**) with the length of the major axis (**horizontal green line**) and the height of the minor axis (**vertical green line**) and taking the convex hull of two equal semi-circles with the radius of half of the minor axis and placing their centers at the major axis line, by a distance of half of the minor axis from the border

Morphological Features

The rod shape

Using the size of the MajorAxis to draw a line, and adding **morphological structuring disk** element with the radius of the MinorAxis, we can create the rod shape of the E. coli.

Which just means that this:



Morphological Features

With this approach we can easily simulate cells with different morphological features

Curvature

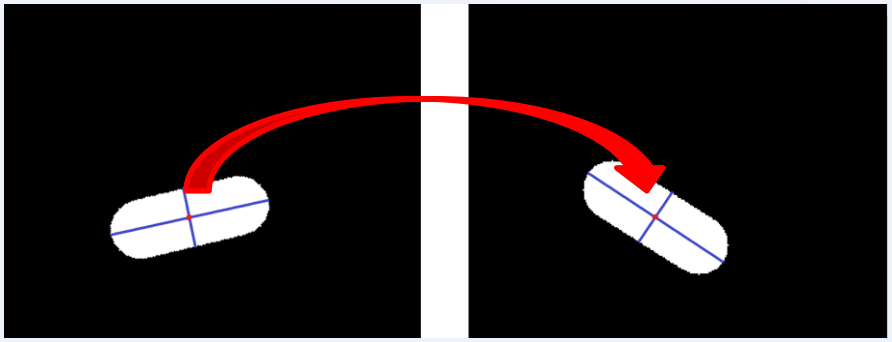
Orientation (rotation)

Functional Features

(Cell movement)

Movement of the center of the bacteria

Movement of the axis of the bacteria (Orientation)

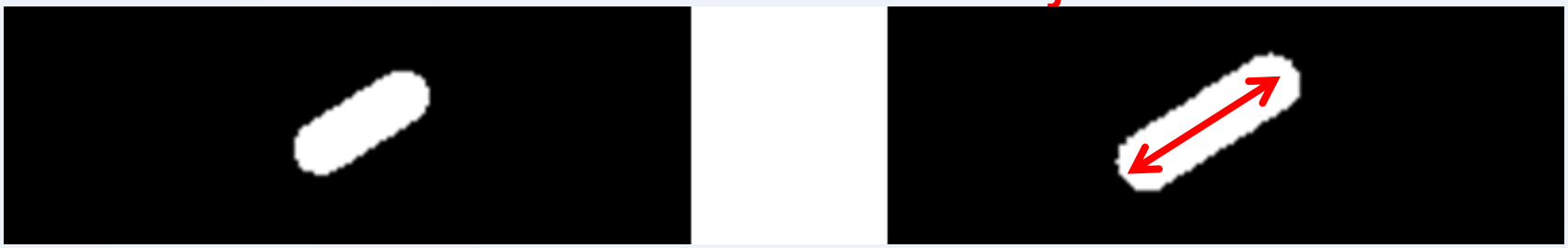


We also can model both movements at the same time

Functional Features

(Cell Growth and Cell Division)

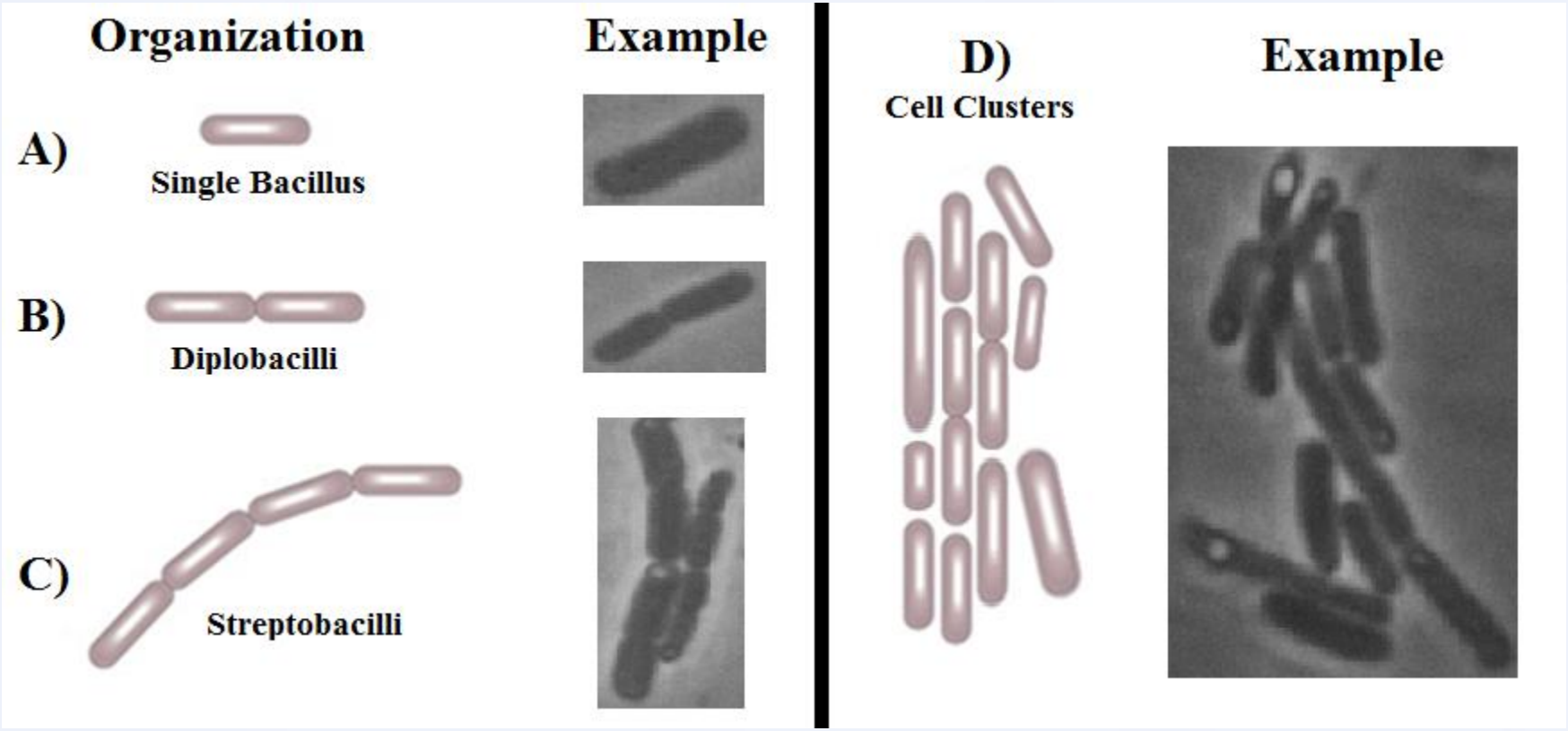
Cell Growh over the Major Axis



Cell Division over time

Inputs - Morphological Features

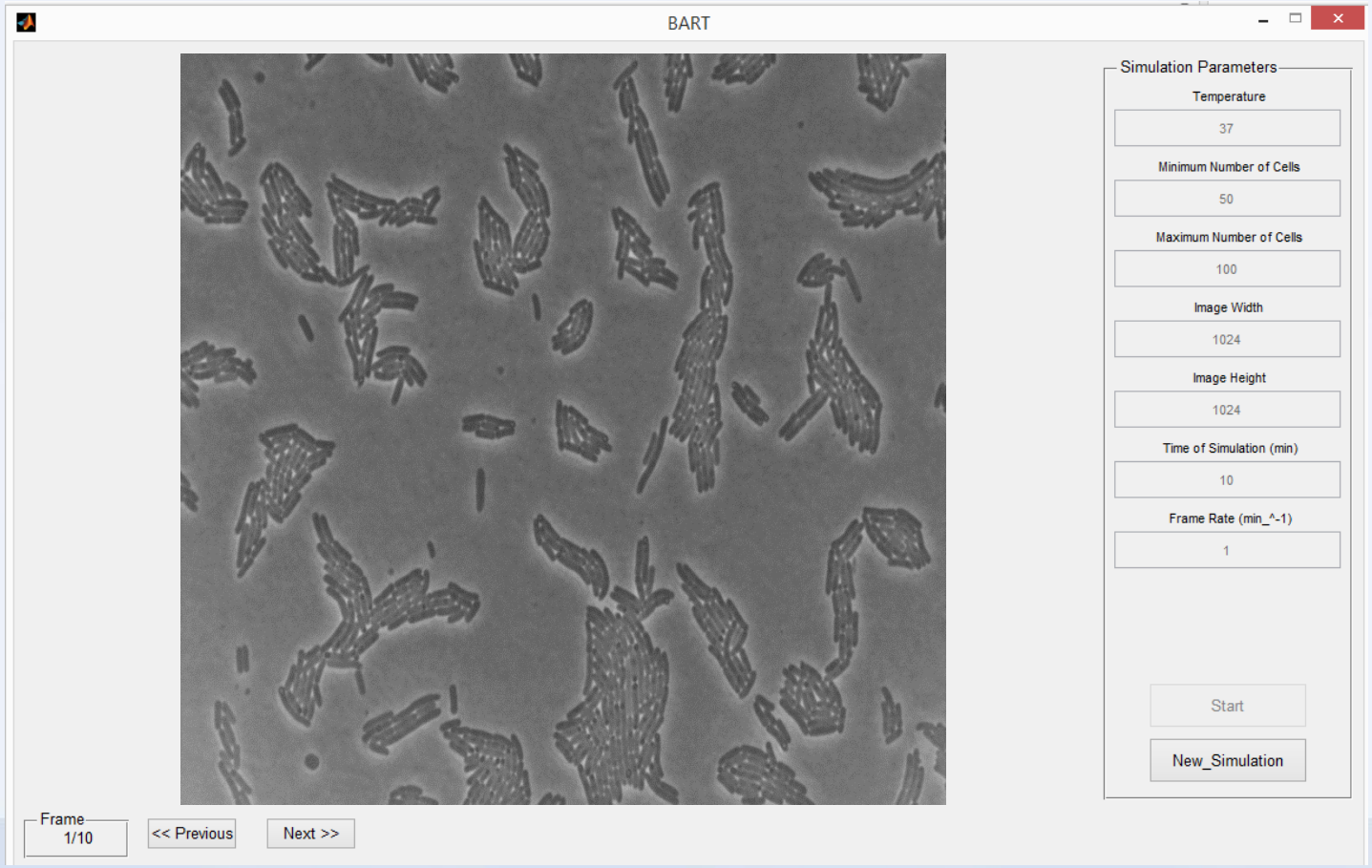
Bacterial Organization



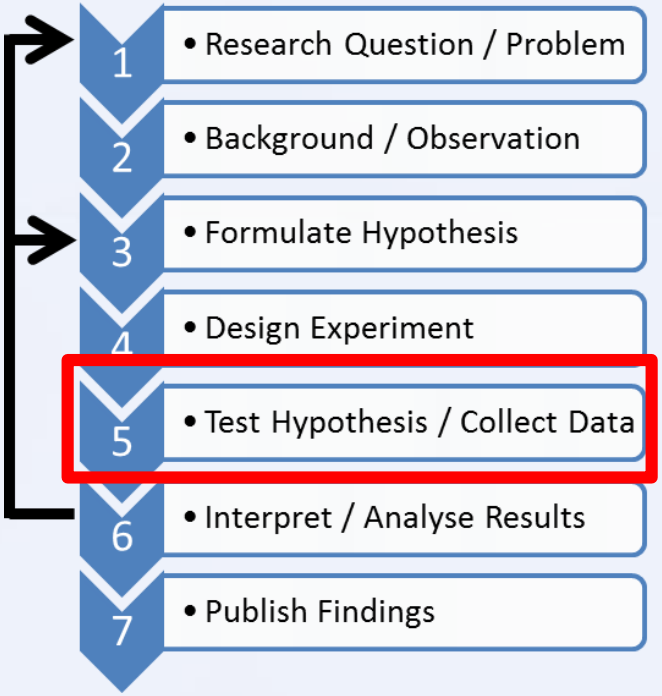
‘miSimBa’
(microscopy image Simulation of Bacterial Cells)

Expectation of tool functionality

An artificial image, indistinguishable of the real ones.



Research Method



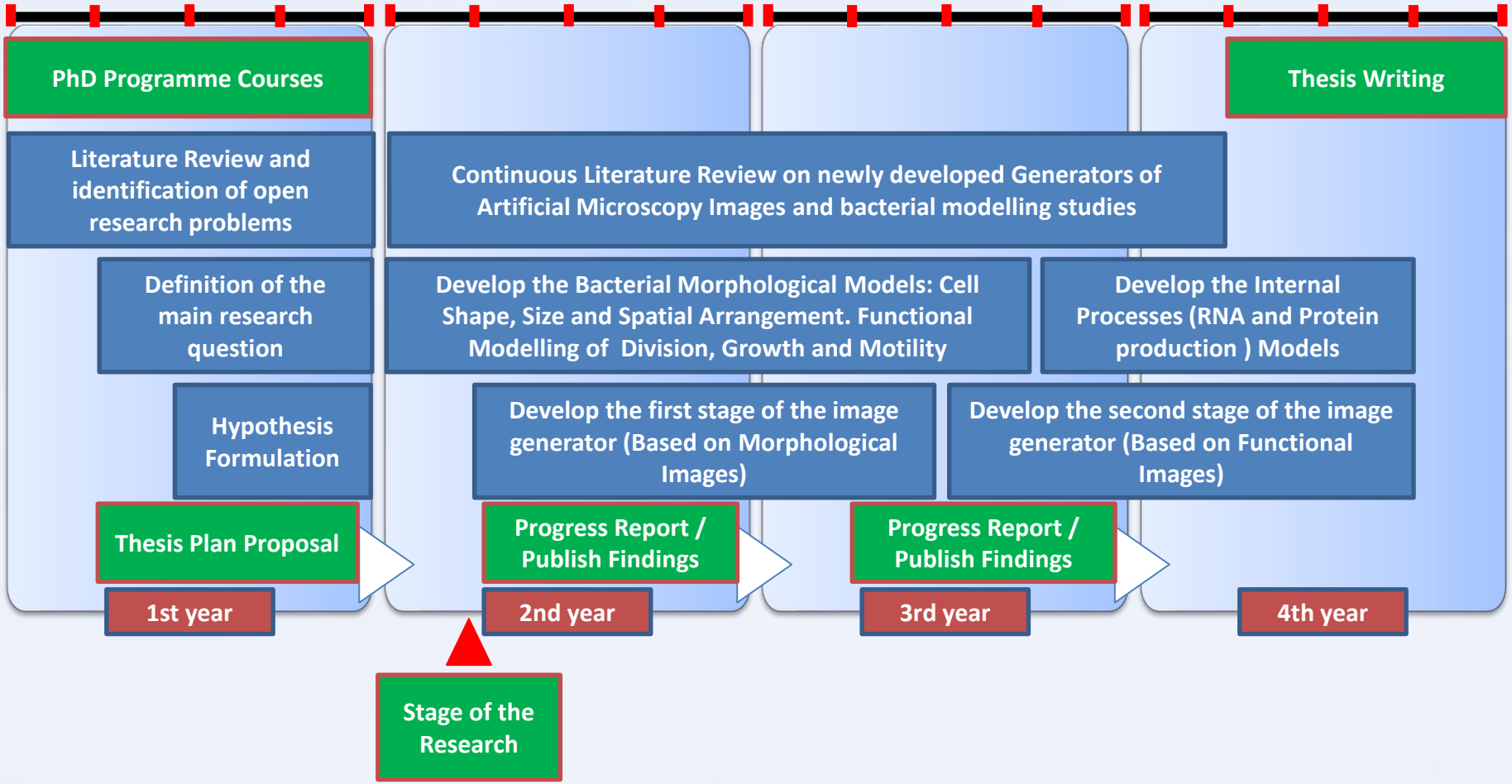
Validation Methodology

Result Analysis

Qualitative Approach: Validation of 'miSimBa' is being accomplished by surveying microscopy and biotechnology experts that will make a qualitative evaluation of the images produced.

A **follow-up quantitative approach**: will be accomplished based on statistical comparisons between *in silico* and *in vivo* rate distributions.

Research Plan (Timeline)



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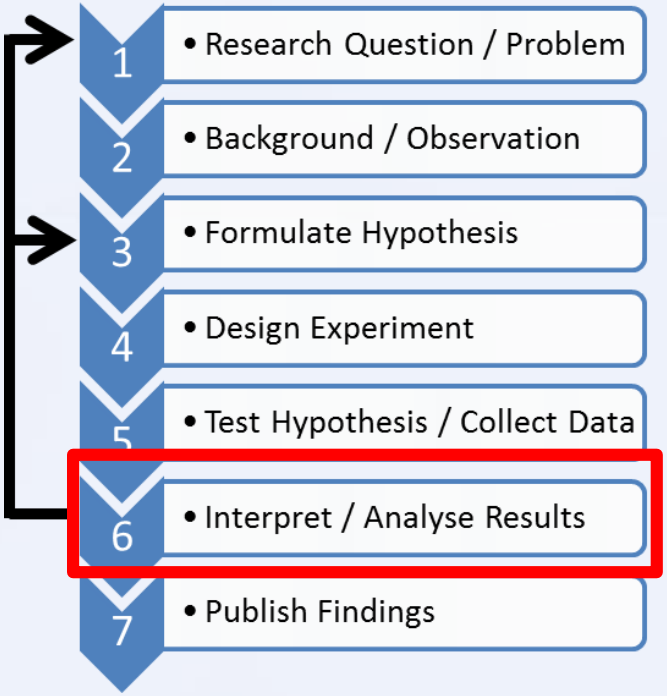


Dissemination Plan (Until Now)

Dissemination Channel	Name	Date
Courses	EMBO Practical Course, Heidelberg, Germany Microscopy, Modelling and Biophysical Methods	7-20 September 2014
Conferences	11th International Workshop on Computational Systems Biology (11th WCSB), Lisbon, Portugal • Participation as Local Organizer • Abstract of preliminary work was approved for Oral Presentation and published in Conference Proceedings	15/16 May 2014
	2015 IEEE 4th Portuguese Meeting on Bioengineering (ENBENG) • Full Paper of preliminary results was approved for Oral Presentation and published in Conference Proceedings	26-28 February 2015
	2015 Tampere Meeting on Single Cell Measurements and Analysis • Participation in the meeting for collaborative reasons (contacts with microscopy and biotechnology experts)	20 March 2015
	DoCEIS 2015 - Doctoral Conference on Computing, Electrical and Industrial Systems (Poster presentation) • Participation as Local Organizer in Financial Committee • Presentation of Poster of Thesis Plan	13-15 April 2015

Dissemination Plan - Future

Dissemination Channel	Name	Date
Book Chapters	Advances in Bioinformatics and Biomedical Engineering (ABBE) Book Series - Handbook of Research on Computational Intelligence Applications in Bioinformatics (IGI Global)	Deadline: July 30, 2015
Conferences	International Conference on Bioinformatics and Biomedicine (BIBM), IEEE	2016 (expected)
	DoCEIS 2016 - Doctoral Conference on Computing, Electrical and Industrial Systems (Oral presentation)	2016 (expected)
	International Meeting on Computational Intelligence Methods for Bioinformatics and Biostatistics - CIBB	2017 (expected)
Journals	Cytometry. Part A : the journal of the International Society for Analytical Cytology	2015 (expected)
	IEEE/ACM Transactions on Computational Biology and Bioinformatics (TCBB)	2016 (expected)
	BMC bioinformatics	2017 (expected)

Conclusions (Take Home Message)

- A tool capable of generating **m**icroscopy **i**mages of **Sim**ulated **Ba**cterial Cells (miSimBa) is beeing developed

- This tool will be able to provide Ground Truth images for image processing tools (cell segmentation, tracking and image registration).

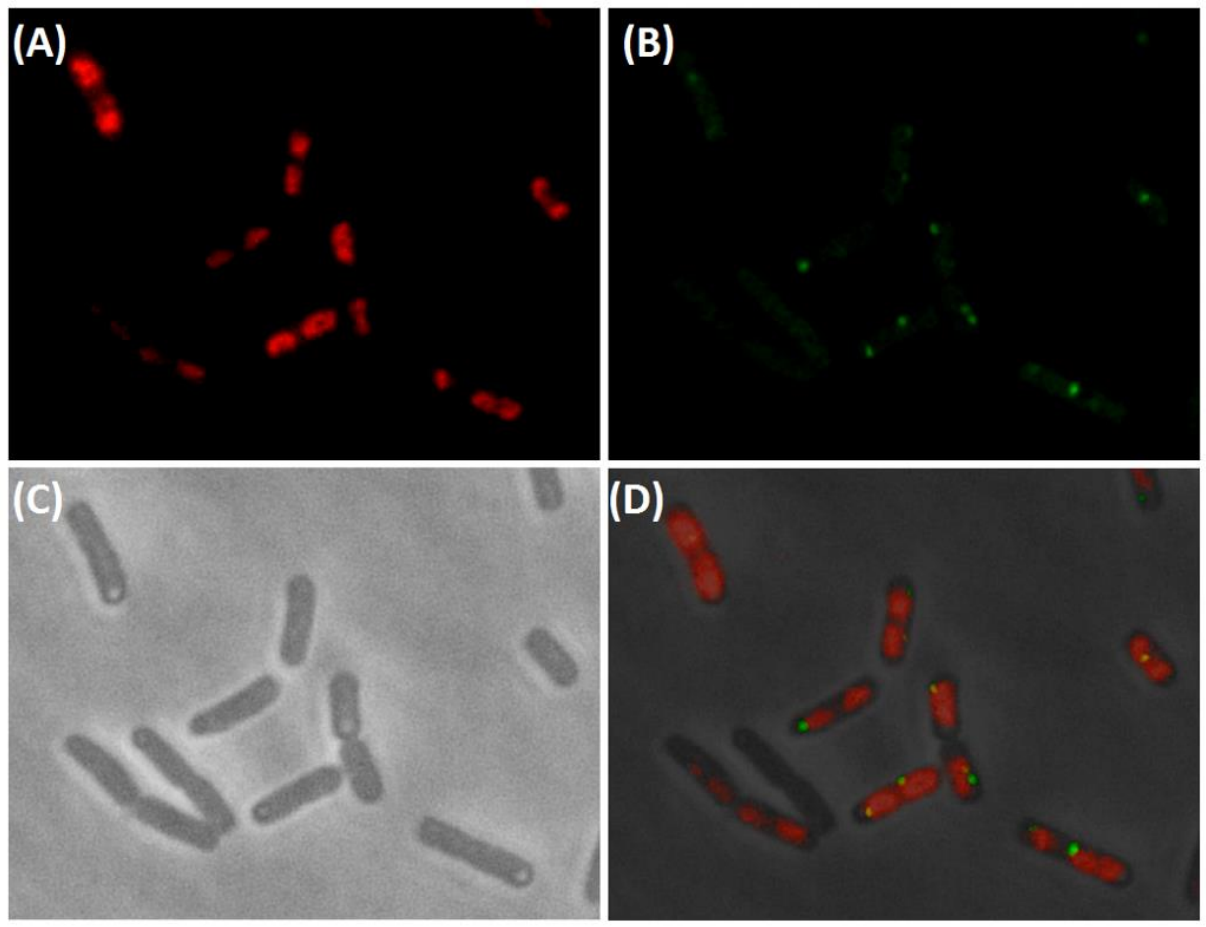
- This tool not only simulates the morphological features (single frame) but can also make time-series with realistic temporal functions.

Future Work

- After we generate Phase-contrast microscopy images, we can validate this tool using the tools made by our group under different conditions.

- This tool can also be used in other type of microscopy images, such as DAPI images or Fluorescense images, which are able to give more information about the bacteria.

Future Work



Acknowledgments

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I want to thank my Supervisors and to everyone present.

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