A pico-liter semi-automated screening method for morphological identification of microorganisms from environmental samples

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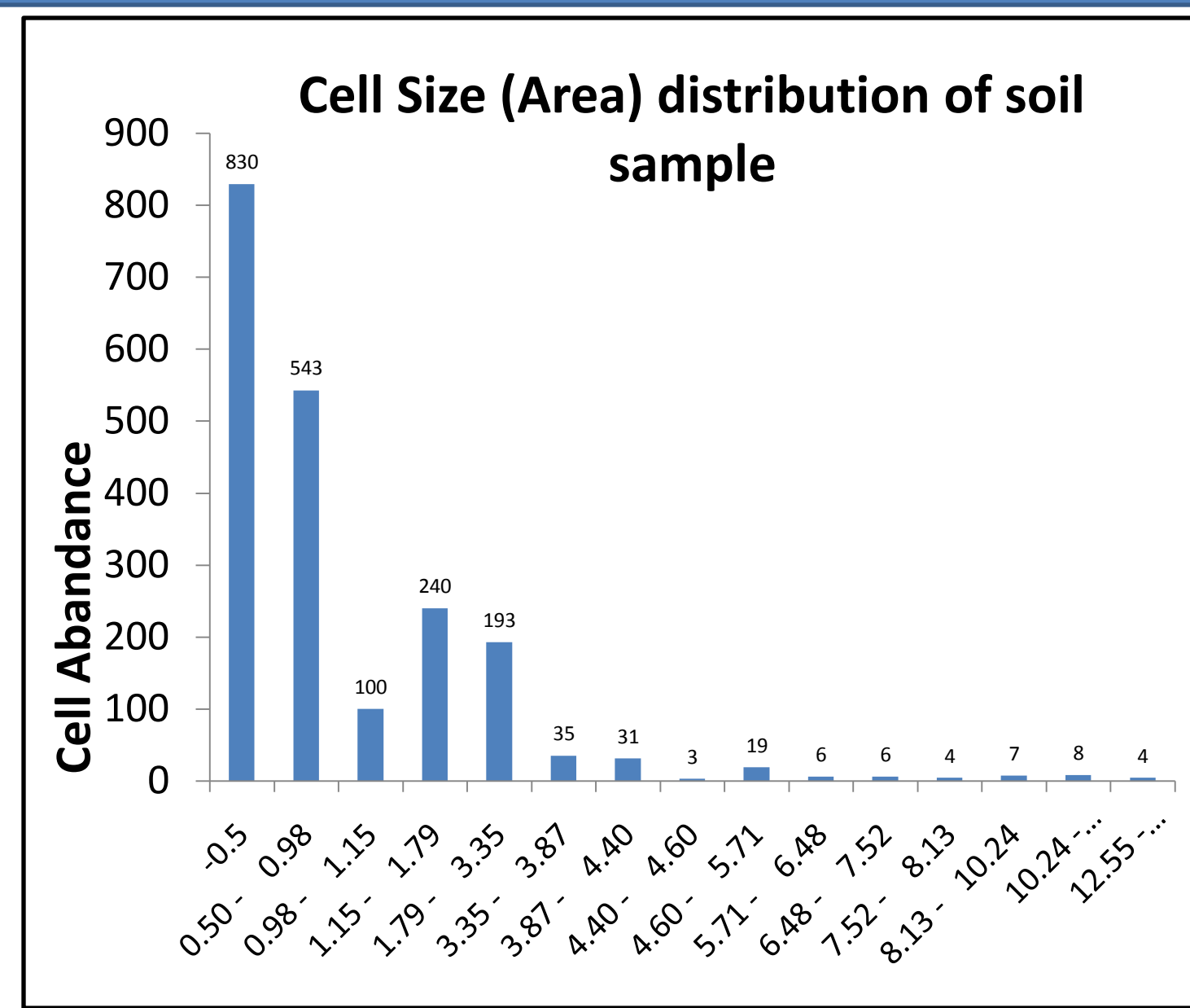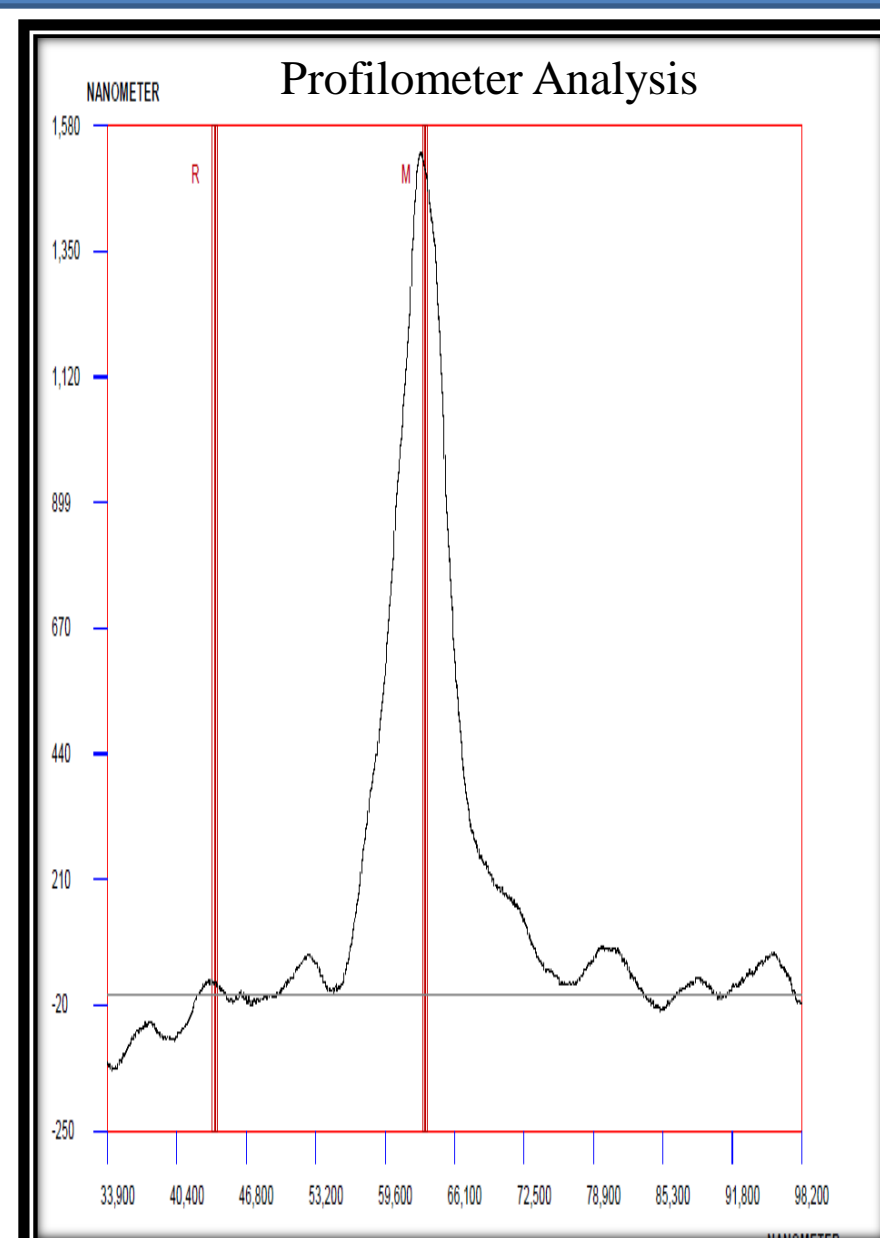**RUIA COLLEGE**
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Introduction

- Bacteria from environmental samples are difficult to cultivate.
- Morphological characterization of microorganism is initial important step in cell classification.
- Image analysis software can be used for morphological analysis and classification of organism in naïve state.
- Single cell level prokaryotic analysis is difficult to perform by classical microbiological methods.
- Study of prokaryotic cells requires small feature size micro channels
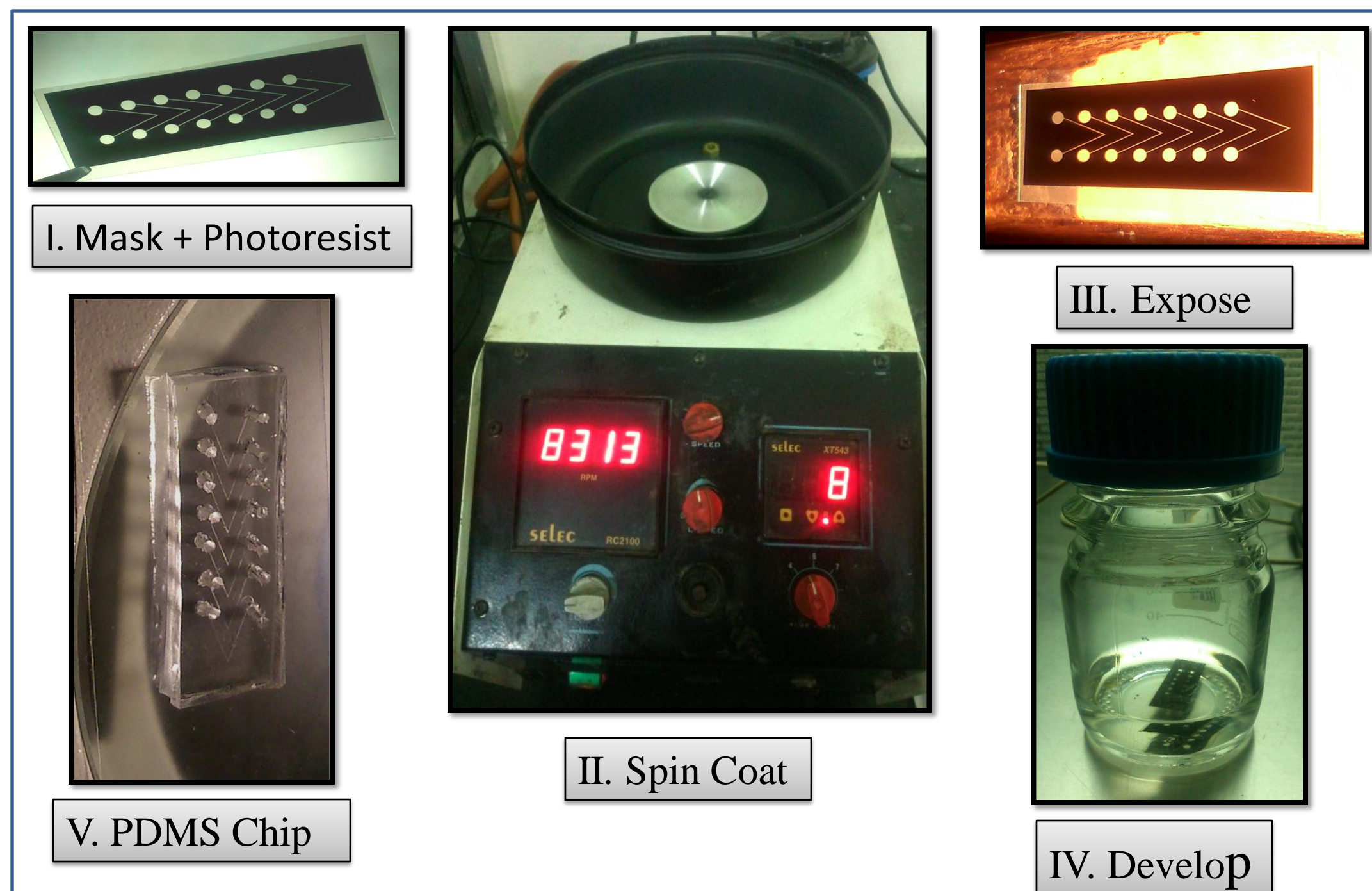- High throughput analysis of environmental samples of various community is possible by lab on chip platform.

Profilometer Analysis

Cell Size (Area) distribution of soil sample

Methodology

I. Mask + Photoresist
II. Spin Coat
III. Expose
IV. Develop
V. PDMS Chip

Fig. 1: Steps for Fabrication

Distribution of width/length ratio

Cocci

0.5 W/L
Regular Rods

0.0625 W/L
Unbranched Filaments

Pre -processing
- 8 -bit grayscale
- Resize pixel dimensions

Object Analysis
- Manual or automatic
- Spatial calibration

Object Classification
- Pseudo color
- 11 morphotypes
- Excel sheet data

Fig. 2: Image analysis by CMEIAS

Green Sulfur bacteria

Legend:
- Regular Rod
- Branched Filament
- Rudimentary Branched Rod
- Prosthecate
- Club
- Ellipsoid
- Unbranched Filament
- U-shaped Rod
- Curved Rod
- Spiral
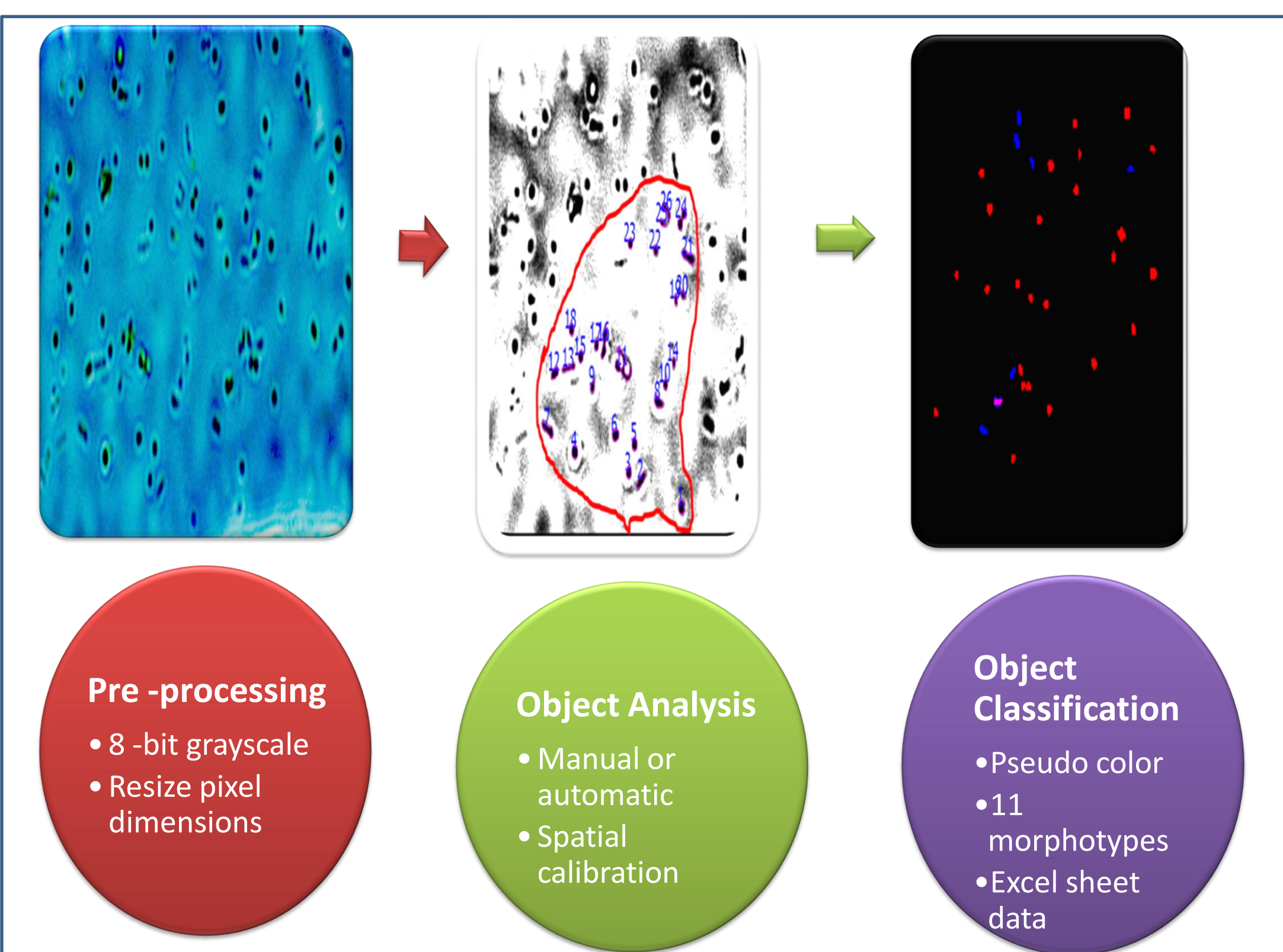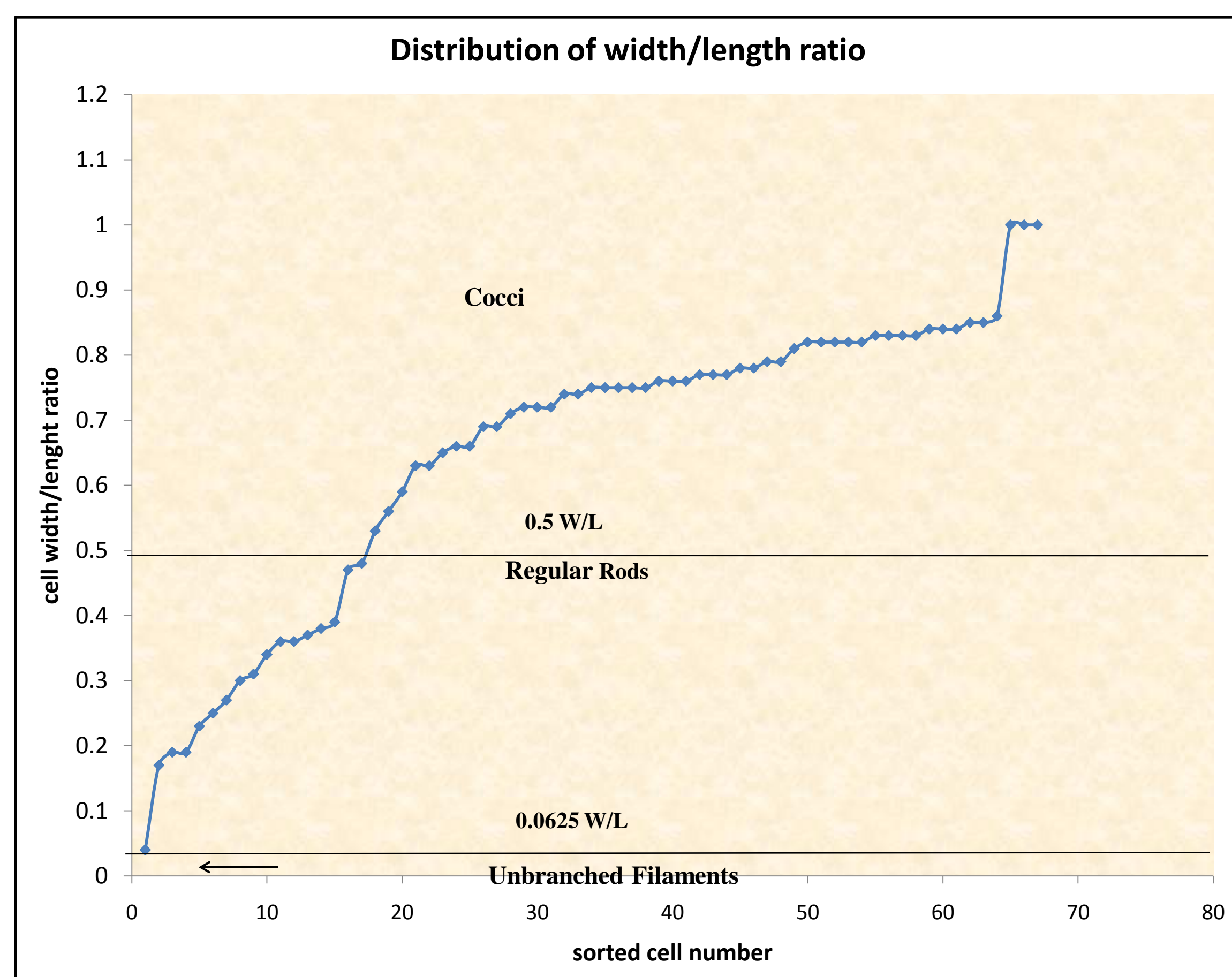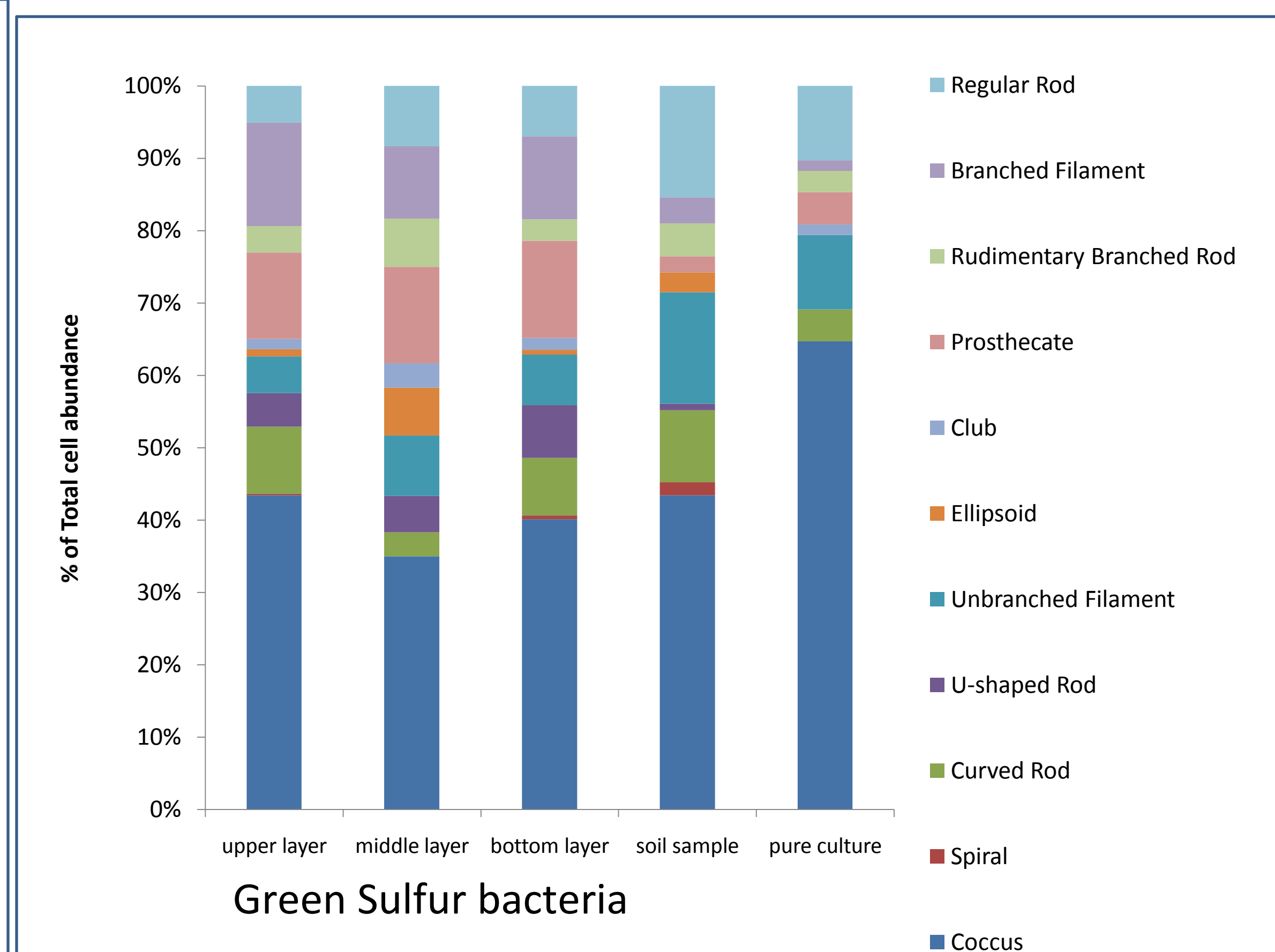- Coccus

Results

Fig. 3: Fabricated Channel (10 X)

Conclusion

- An in house photolithography fabricated devices is successfully developed and standardized for studying prokaryotic cells.
- Image analysis method using CEMIAS is validated using pure cultures.
- Potential use of this method for analysis of environmental sample is explored.
- Soil sample and green sulphur bacterial community is analyzed successfully.

References:

1. Alexander Gru¨ nberger, Wolfgang Wiechert and Dietrich Kohlheyer, **Single-cell microfluidics: opportunity for bioprocess development** , Current Opinion in Biotechnology 2014, 29:15–23.
2. J. Liu, F.B. Dazzo, O. Glagoleva, B. Yu, A.K. Jain, **CMEIAS: A Computer-Aided System for the Image Analysis of Bacterial Morphotypes in Microbial Communities,** Microb Ecol (2001) 41:173–194.