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Petrisor AI, Decho AW (2004), Reconstruction and computation of biovolumes: Potential problems, Meeting of South Carolina Academy of Science, University of South Carolina, Charleston, SC, USA, April 16, 2004

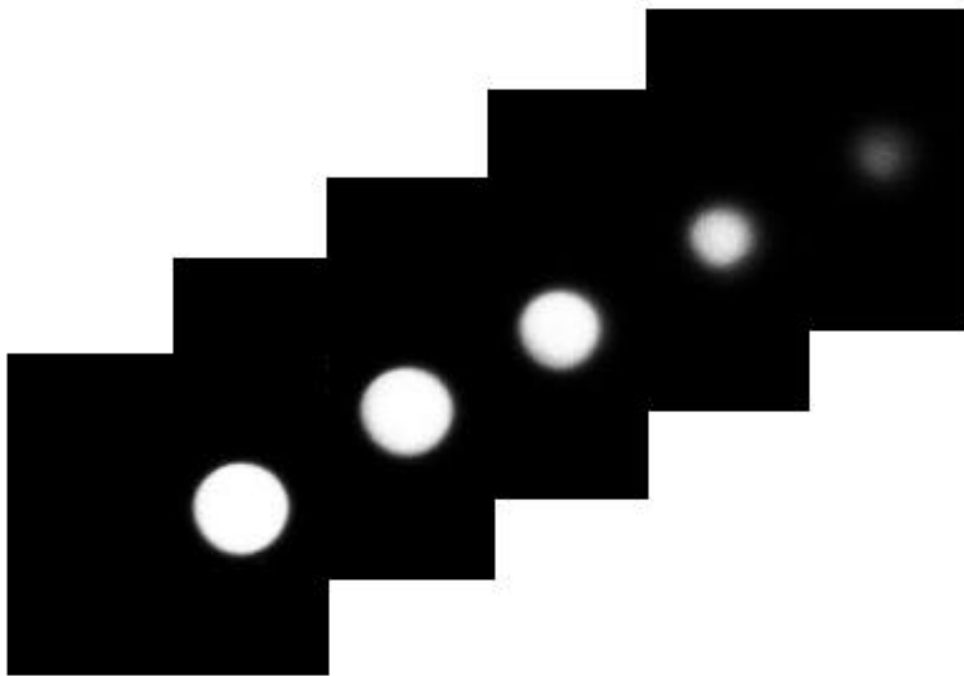
# Reconstruction and computation of biovolumes: potential problems

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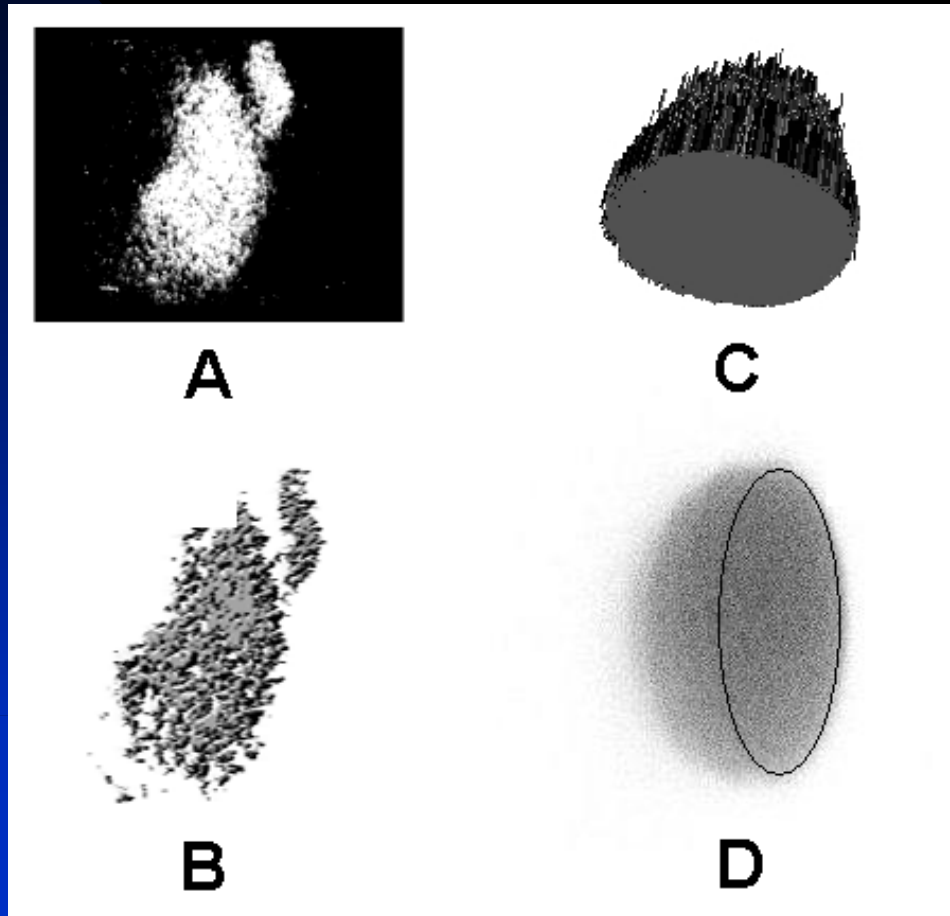
# Materials and methods



Fluorescent microspheres of known sizes ( $1\ \mu\text{m}$  diameter) were used to build an approach to the computation of biovolumes. For each microsphere, images of five parallel sections were taken at equal distances.

[A.I. Petrisor, A. Cuc, and A.W. Decho, Reconstruction and Computation of Microscale Biovolumes Using Geographical Information Systems: Potential Difficulties, submitted to Research in Microbiology]

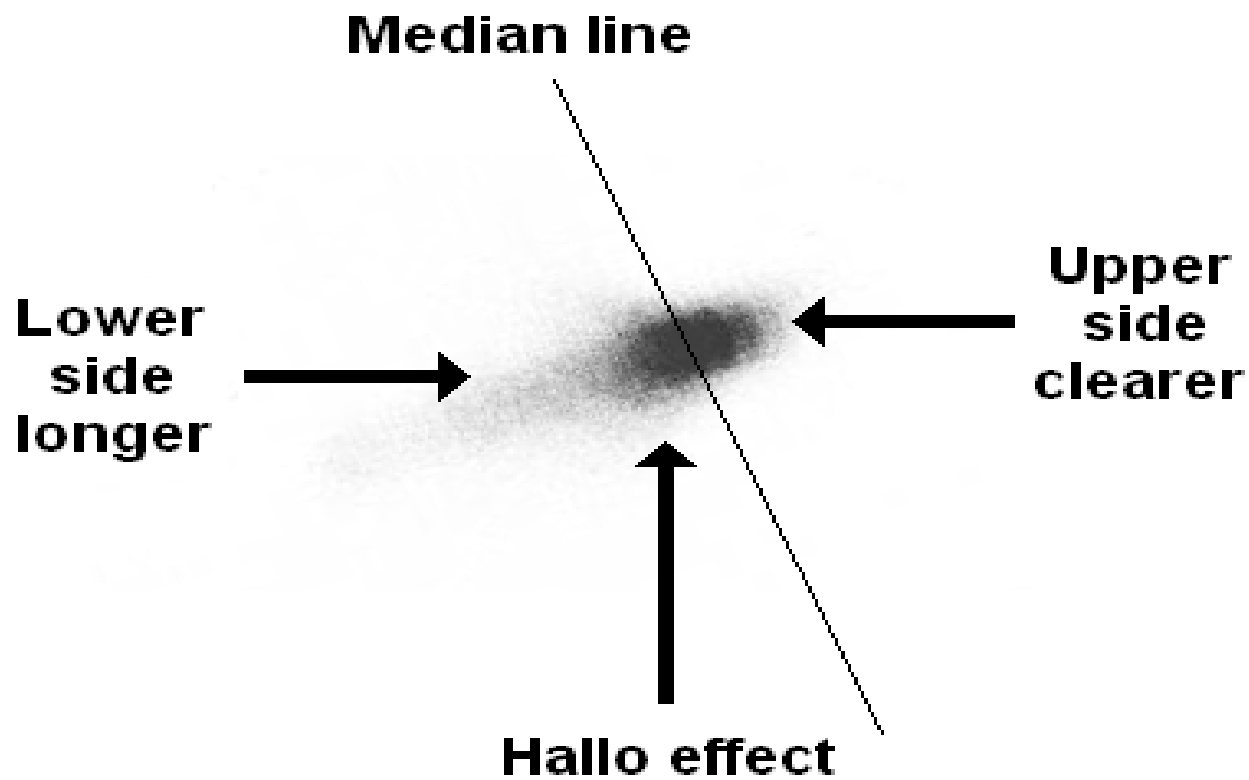
# Reconstruction of biovolumes



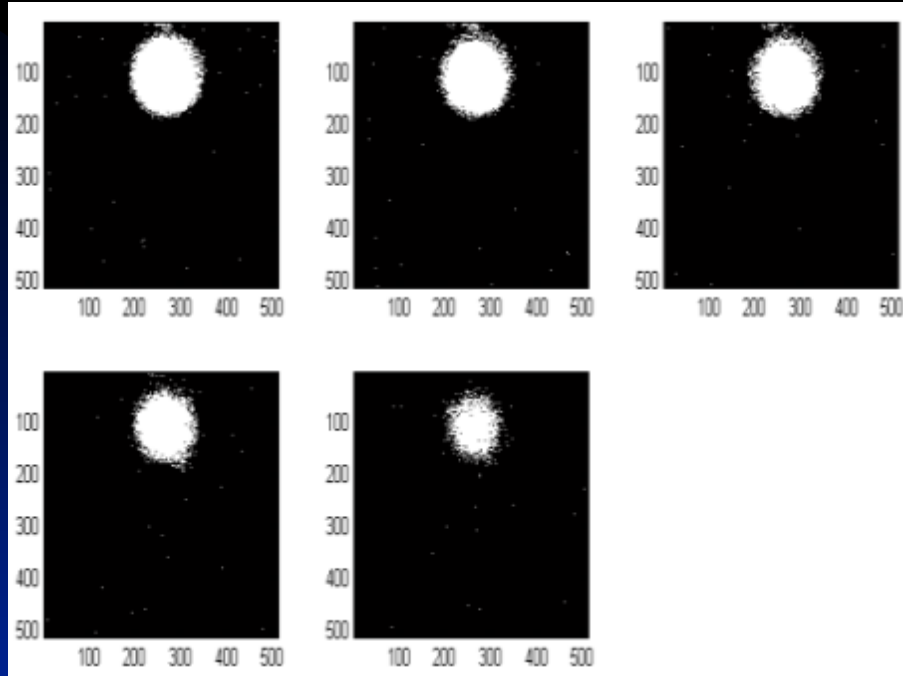
A and B- two, respectively three-dimensional reconstruction of a portion of a biofilm, using five parallel sections

C, D- reconstruction of the upper portion of a 1 μ-diameter microsphere using five parallel sections

# Potential problems



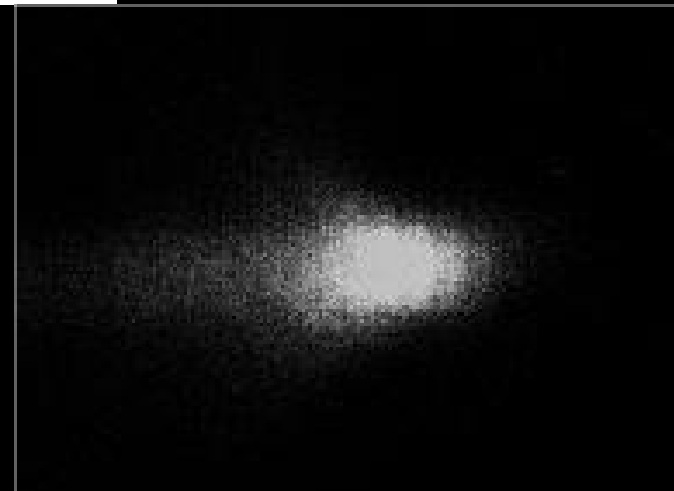
# The halo effect



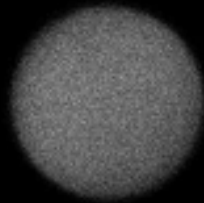
2-D analysis:  
areas of upper  
sections appear  
larger than they  
should be



3-D analysis:  
actual view of  
the halo



# Differences in color intensity



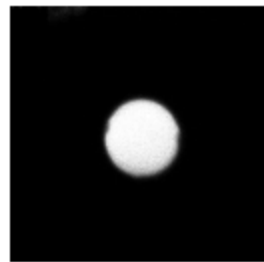
1



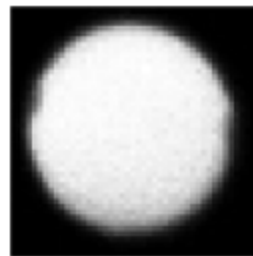
2

1- pale appearance; 2- bright appearance

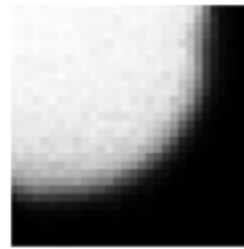
# Differences at the pixel level



1 X



4 X



8 X



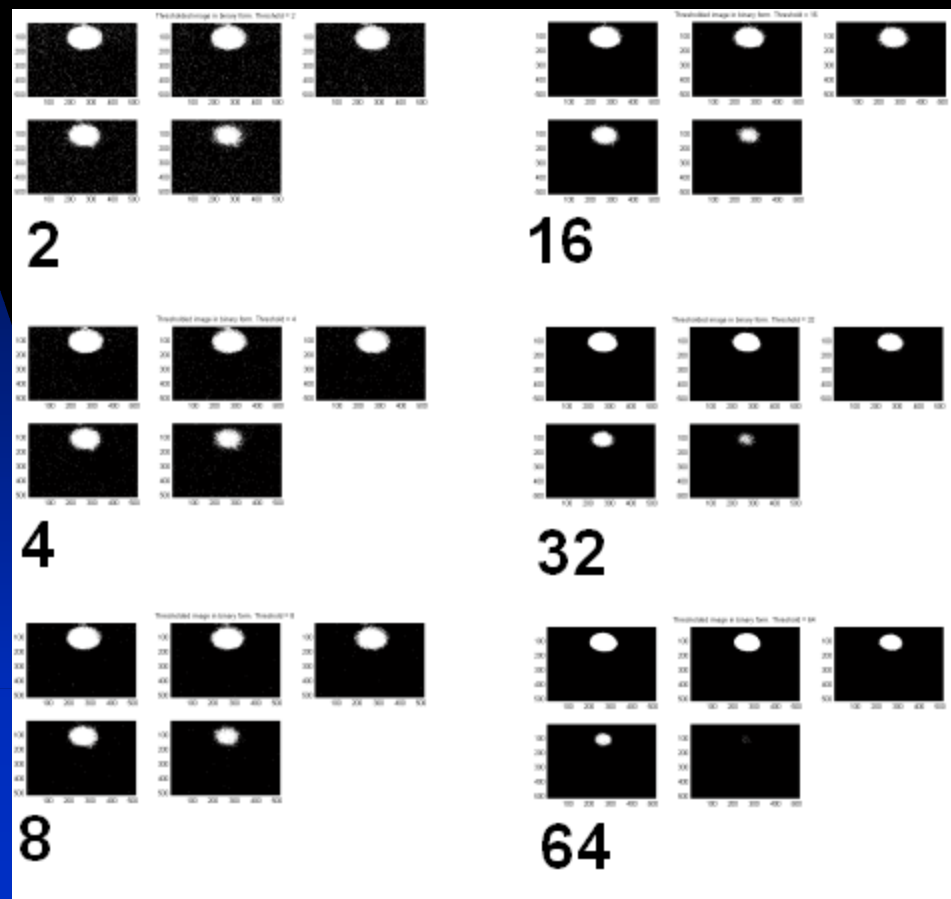
12 X



16 X

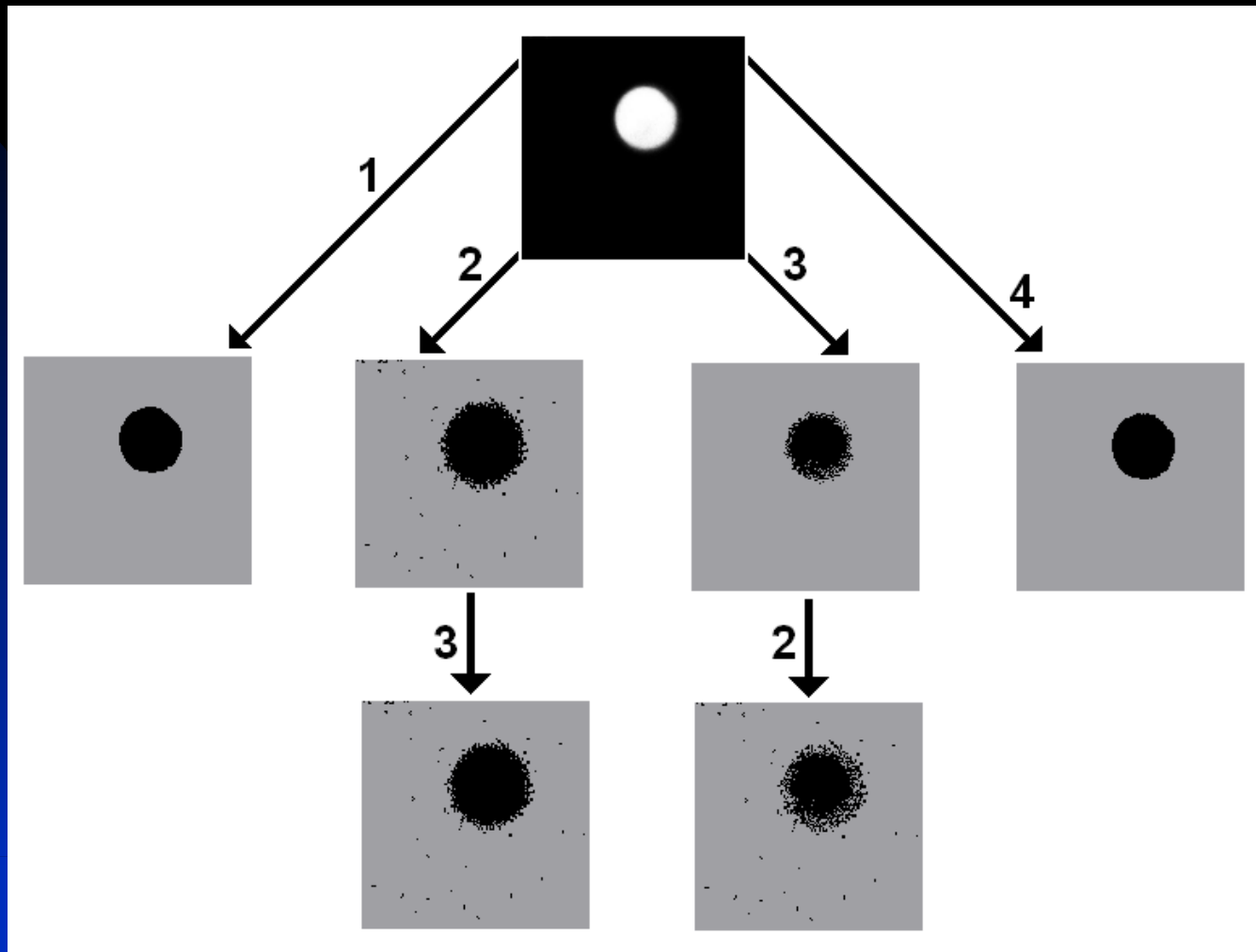
Even though images appear homogeneous, they represent collections of pixels having different colors or intensities of the same color

# Effect of the threshold value used in unsupervised classification



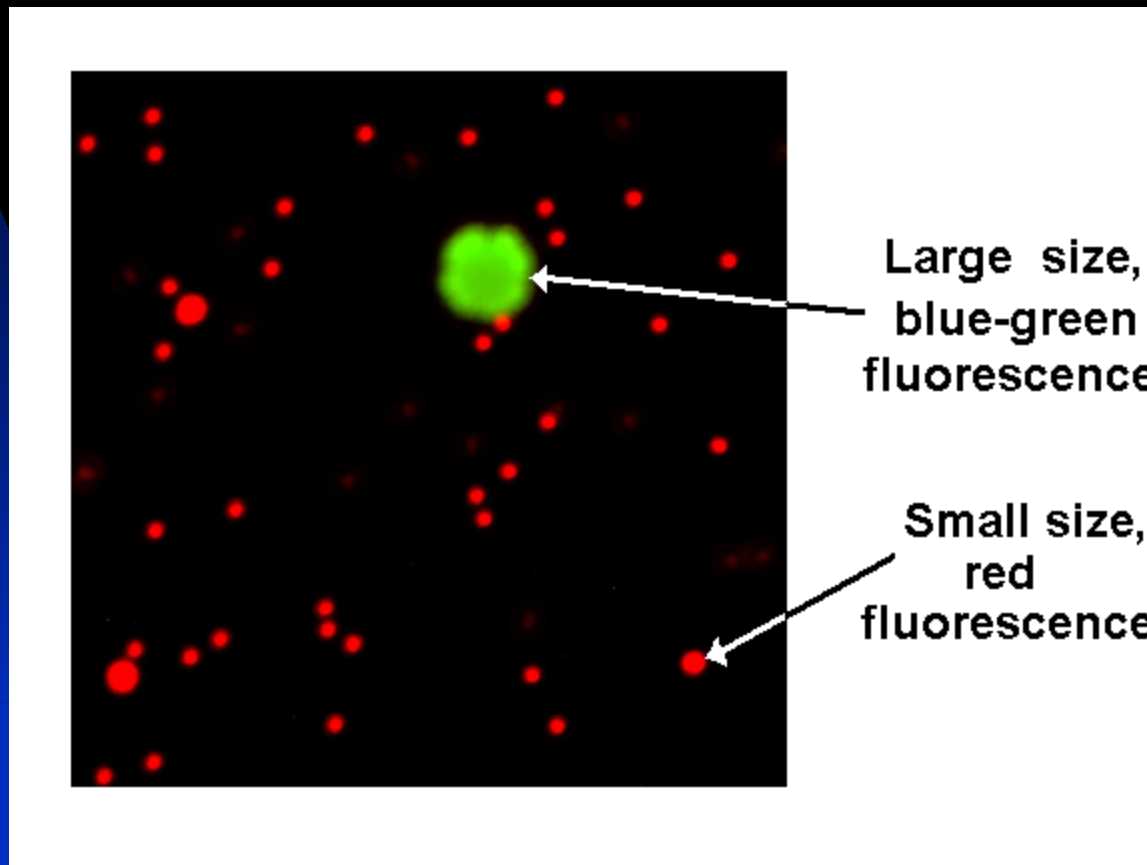


# Image enhancement

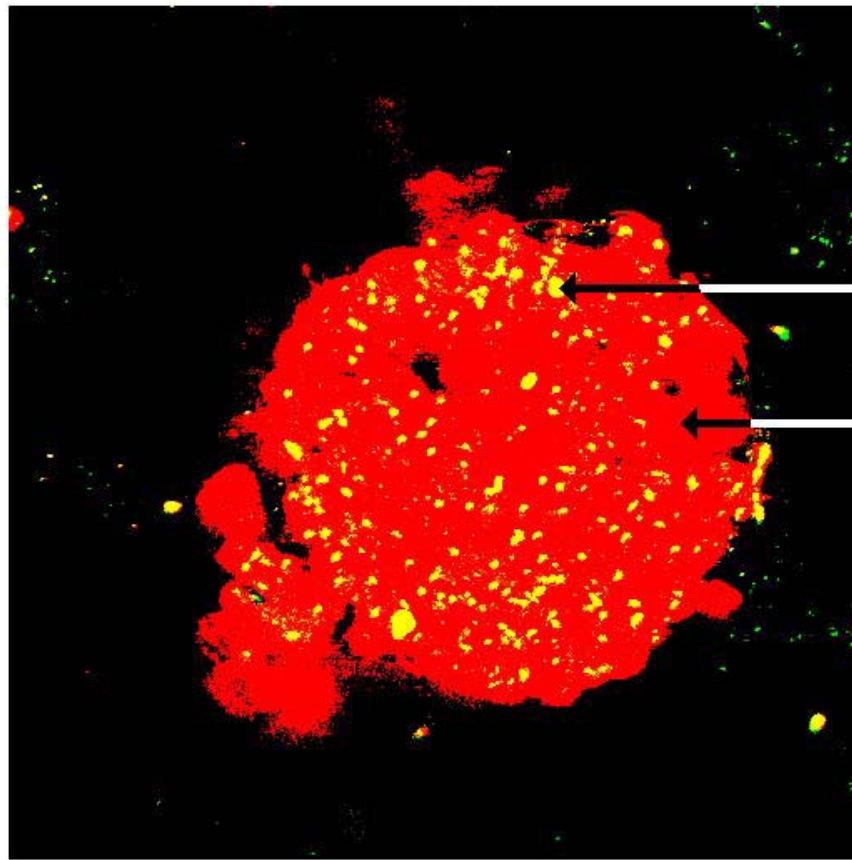


1- Unsupervised classification using Feature Analyst (includes enhancement); 2- contrast stretching; 3- filtration; 4- unsupervised classification

# Using fluorescent microspheres for calibration



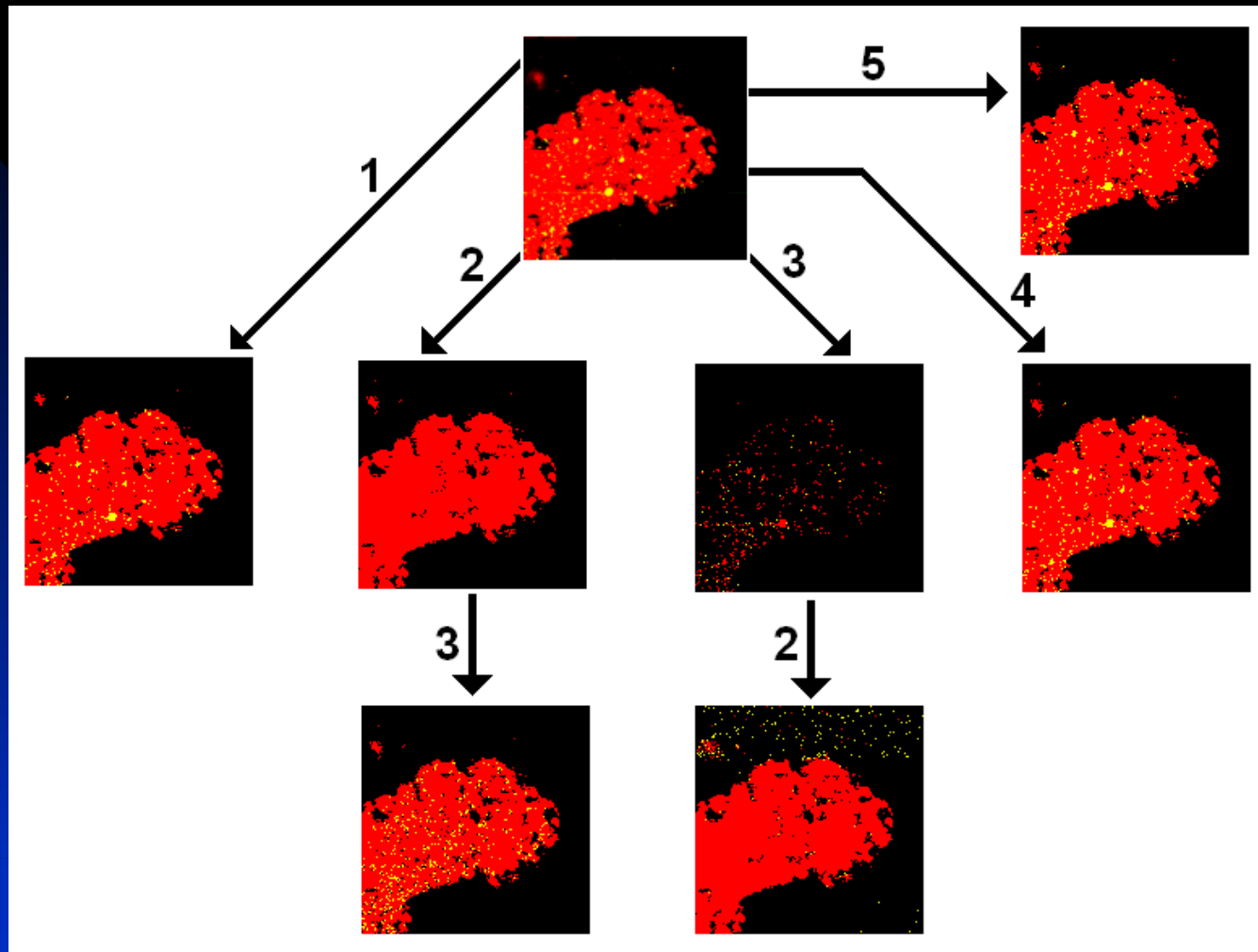
# Embedding microspheres in gel capsules: simulation of complex environments



Microsphere

Gel capsule

# Image enhancement



1- Unsupervised classification using Feature Analyst (includes enhancement); 2- contrast stretching; 3- filtration; 4- unsupervised classification; 5- supervised classification