Segmentation of Light Microscopy Images

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Objective

- Due to the large volume of data present in many biological datasets, manual annotation is often impractical

- This project focuses on accurate semi-automated segmentation of cells in light microscopy images

Overview
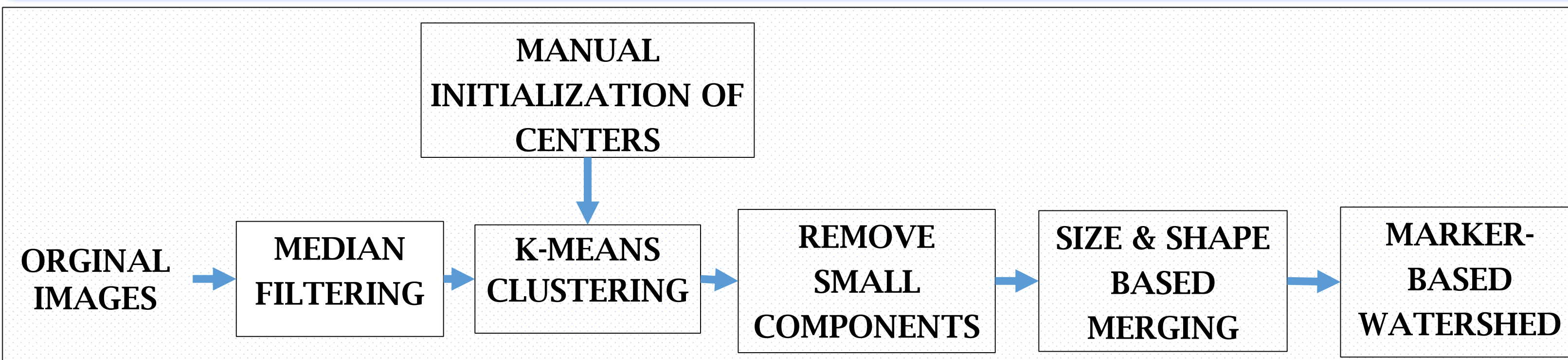
Dataset

- Rabbit retinal dataset (RC1 Connectome) from the Viking program, imaged at MarcLab, University of Utah

- A 0.25 mm diameter section imaged at 70 nm resolution using the computational molecular phenotyping (CMP) paradigm

- Resulting volume consists of 371 sections spanning the inner nuclear layer to the ganglion cell layer containing following cells:
  Y–Amacrine Cells, G–Amacrine Cells, Horizontal Cells, Cone Bipolar Cells, Rod Bipolar Cell, Microglial cells

- The data consists of 6 consecutive sections, each an intensity image indicating protein activity corresponding to a unique molecular marker:
 AGB, GABA, Glutamate, Glutamine, Glycine, Taurine

Median Filtering

- Filter image by determining the median of a neighborhood of pixels

- Denoise without removing edges


Original CMP image   Median Filtered Image
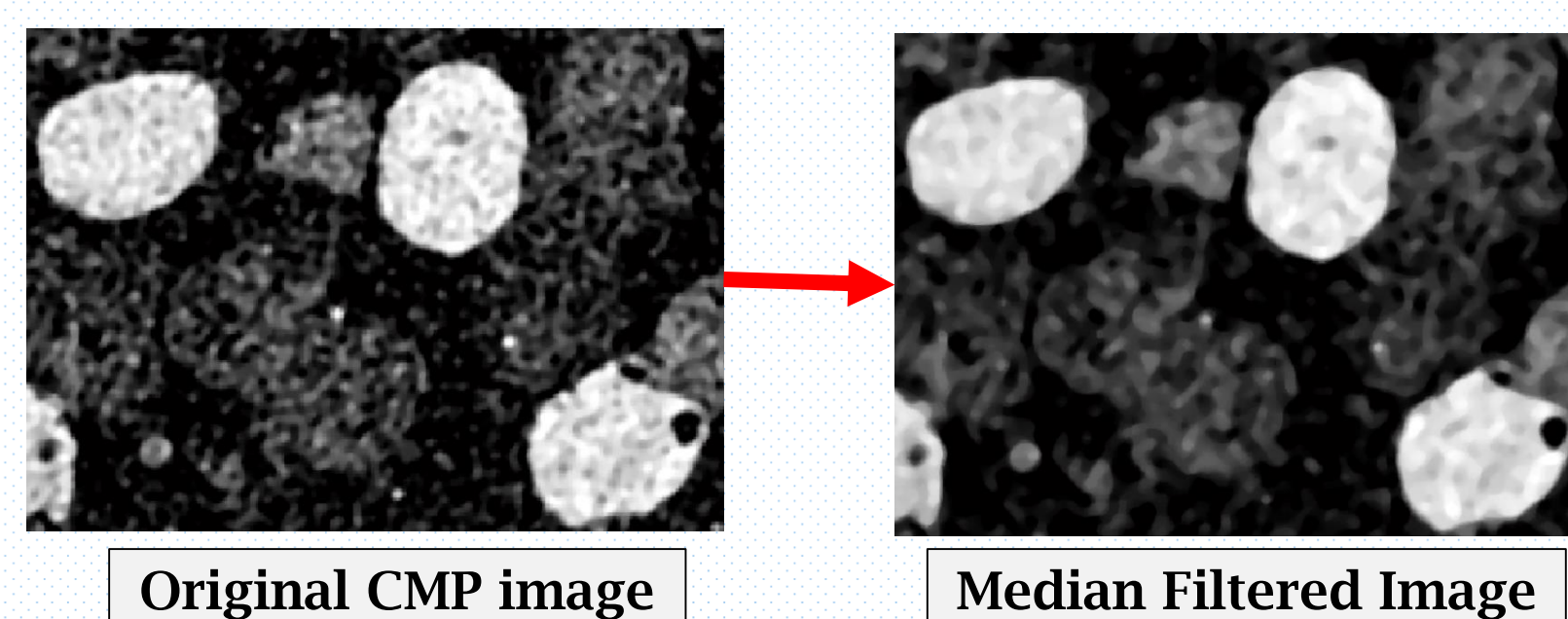
k-means Clustering

- Cluster by minimizing Euclidean distance

- Initialize centers for each cluster by manually identifying one cell of each type using Viking

- Result suffers from both over- and under-segmentation in different regions


Result of *k*-means Clustering
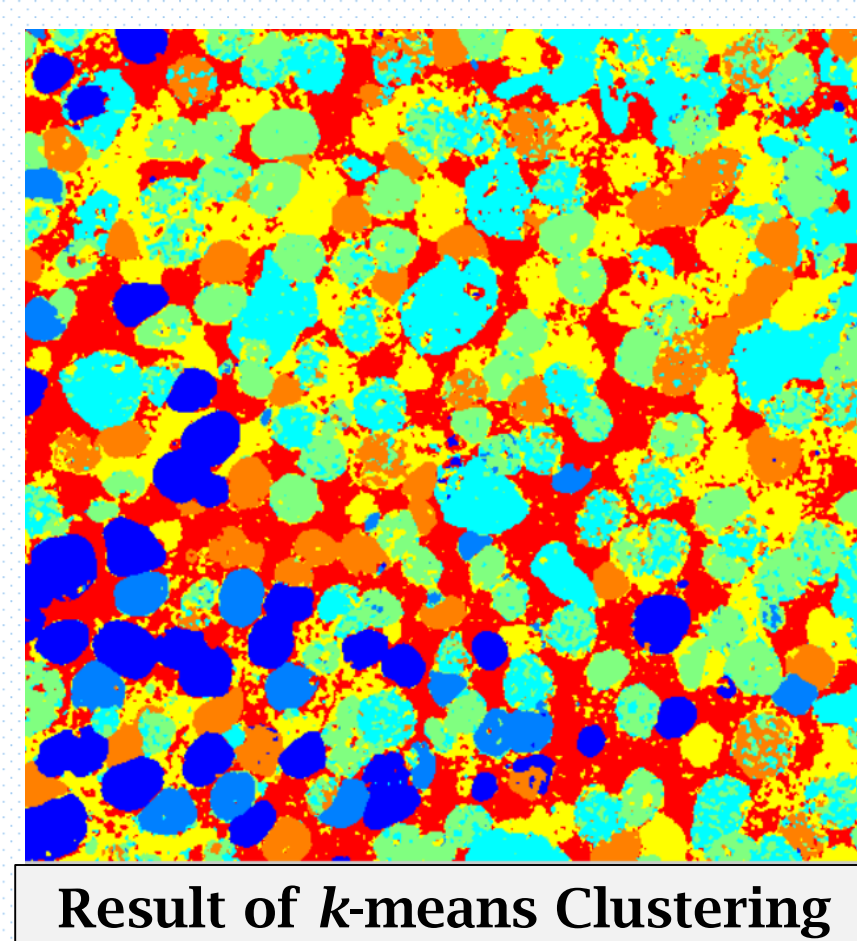
Remove Small Segments

- There is noise in the image due to the nature of the imaging process which leads to artifacts such as false cells and gaps inside cells

- Some artifacts cannot be removed by median filtering

- Remove objects smaller than a threshold S_S

- Fill gaps inside cells which are smaller than S_S
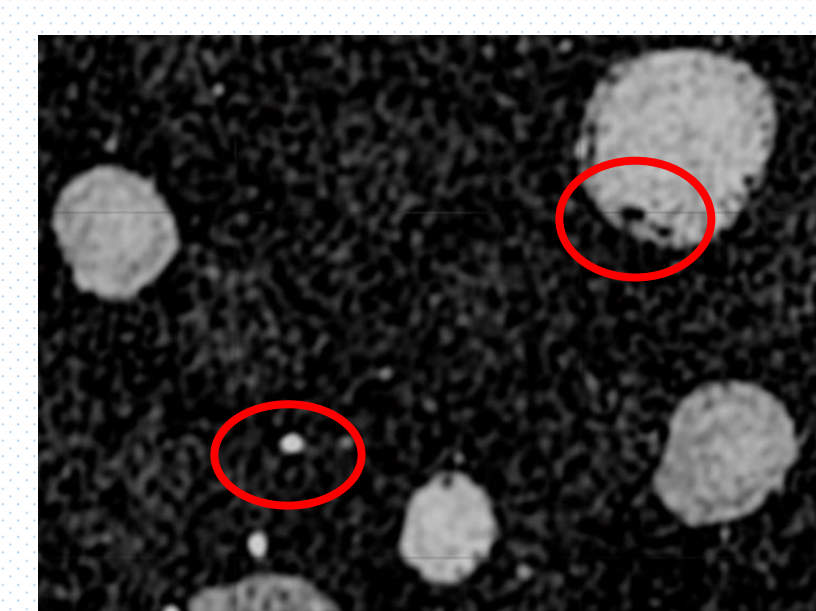
Example of false cells and gaps inside cells
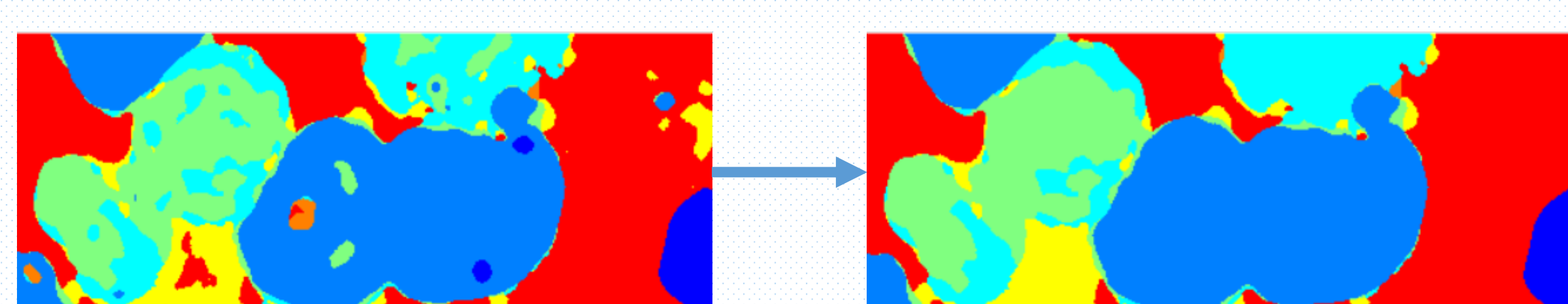
k-means Result   After removing small segments
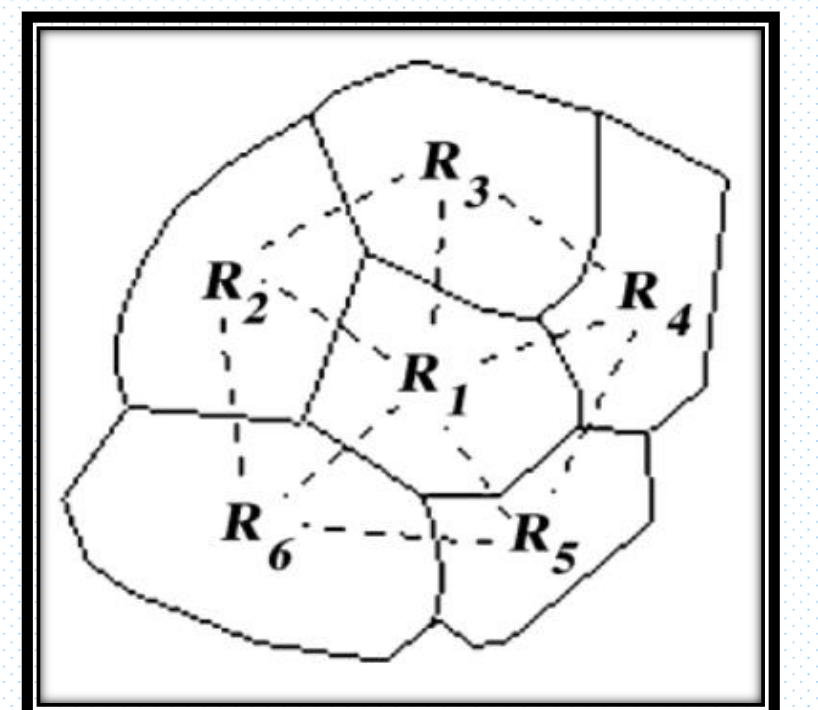
Size and Shape Based Merging

- Construct **Region Adjacency Graph** indicating neighboring connected components

- Analyze region properties (size and convexity) of connected components

- For neighboring components A and B with size S_A and S_B ($S_A < S_B$) and convexity C_A and C_B respectively, criteria for merging A into B are:
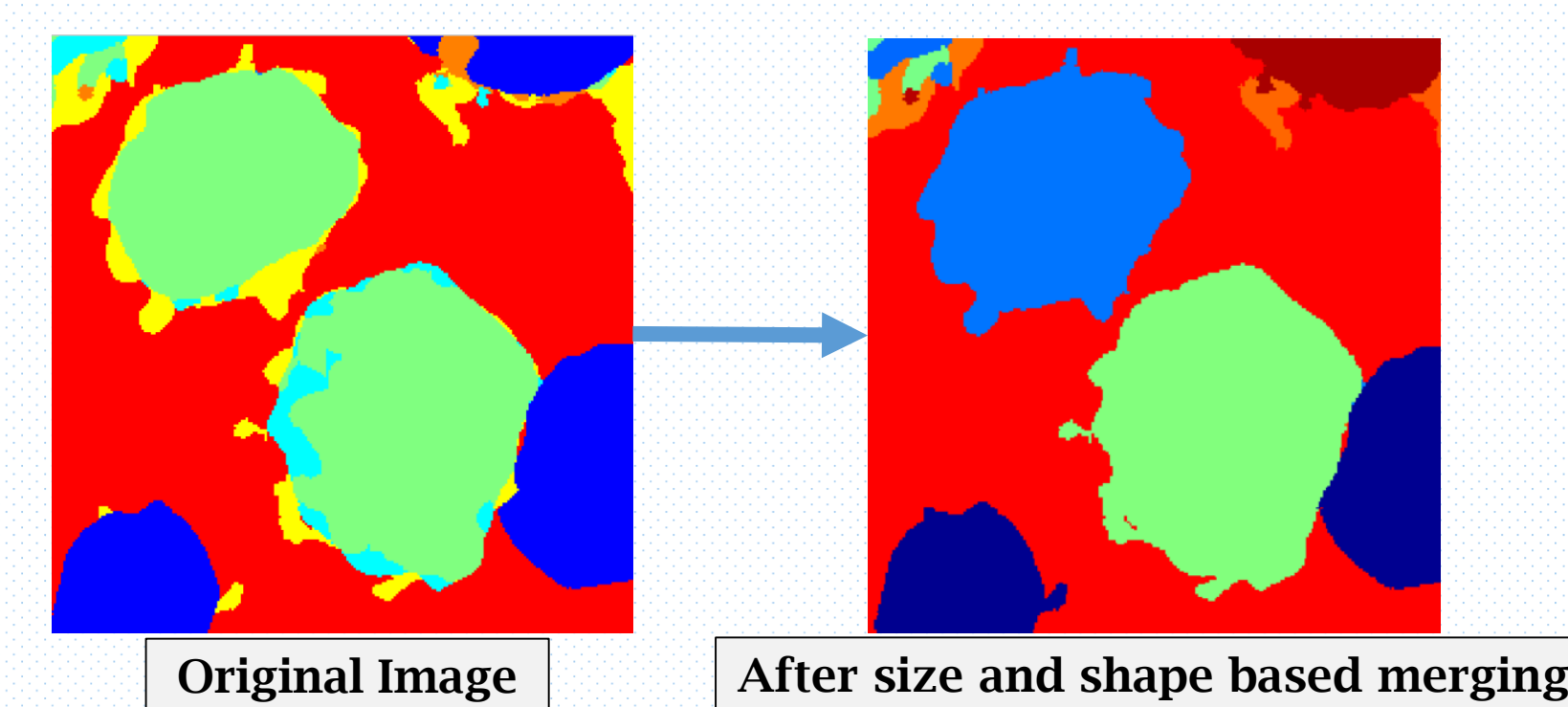
$$S_A < S_T \text{ and bordering only cell component B}$$

or

$$C_{AB} > \frac{S_A C_A + SB C_B}{S_{AB}}$$

where S_T is a size threshold and S_{AB} and C_{AB} are the size and convexity of the merged component respectively

Region Adjacency Graph for Neighboring Connected Components - Image from M. Djebali, M. Melkemi and N. Sapidis, A Fit-and-Merge Algorithm for Range-Image Segmentation and Model Reconstruction
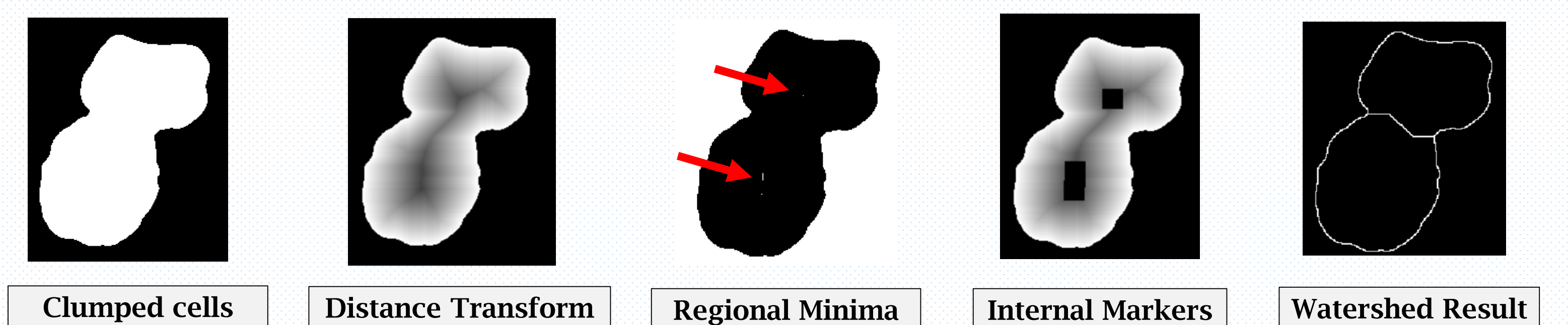

Original Image   After size and shape based merging

Marker Based Watershed

- Low convexity of a component indicates a set of clumped cells – we require a clump splitting algorithm

- Identify cell centers ("markers") using distance transform

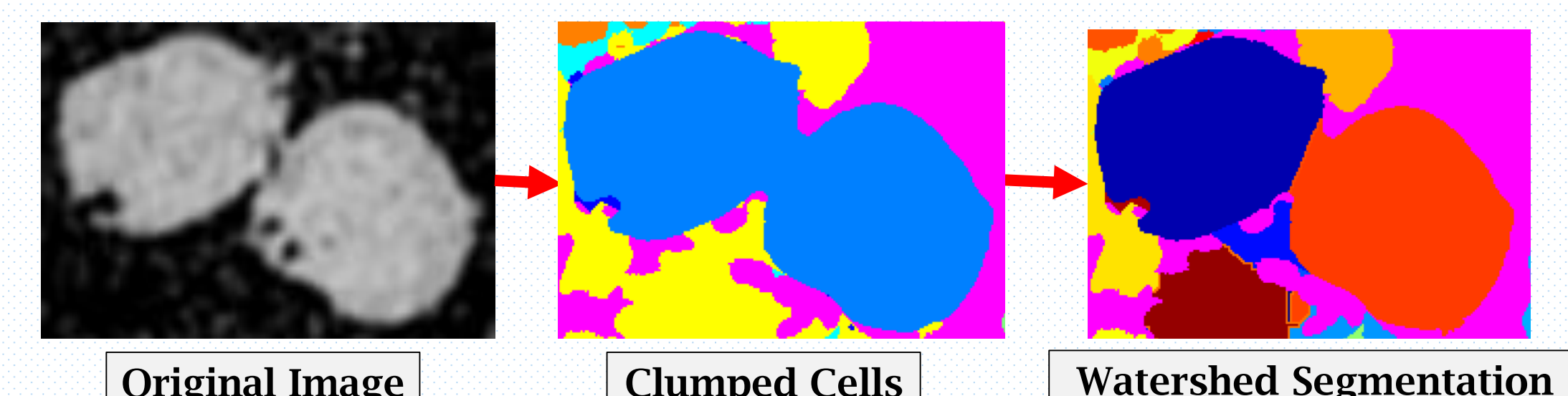- Marker-based watershed segmentation to separate touching objects

Clumped cells   Distance Transform   Regional Minima   Internal Markers   Watershed Result

Original Image   Clumped Cells   Watershed Segmentation

Experimental Results

- In our experiments, we set *k* to 7 (6 cell types + background)

- S_S and S_T are set to 1000 and 300 pixels respectively

- We validate the result against manually annotated ground truth, and find the precision to be 0.7722 and recall to be 0.6529, giving an F-measure of 0.7076

Summary

- We propose a multi-step algorithm for segmentation of CMP images in rabbit retinal volume

- We apply *k*-means followed by size- and shape-based operations and marker-based watershed to segment cells

- Experimental results show an F-measure of 0.7076