Fish Health as Useful Biomarkers to Monitor Water Pollution: Morphometric Measurement of Fish Blood Cell

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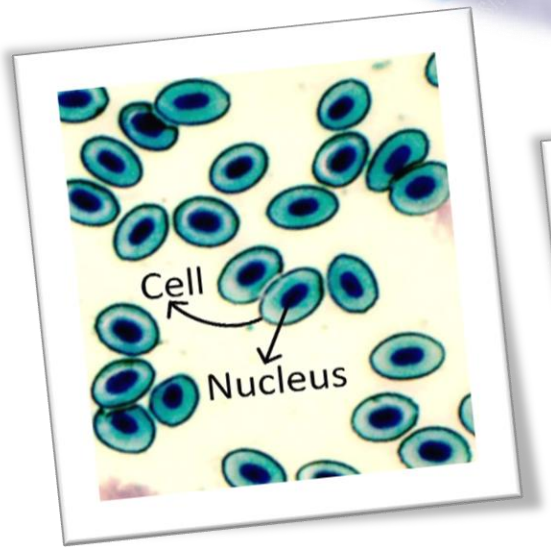
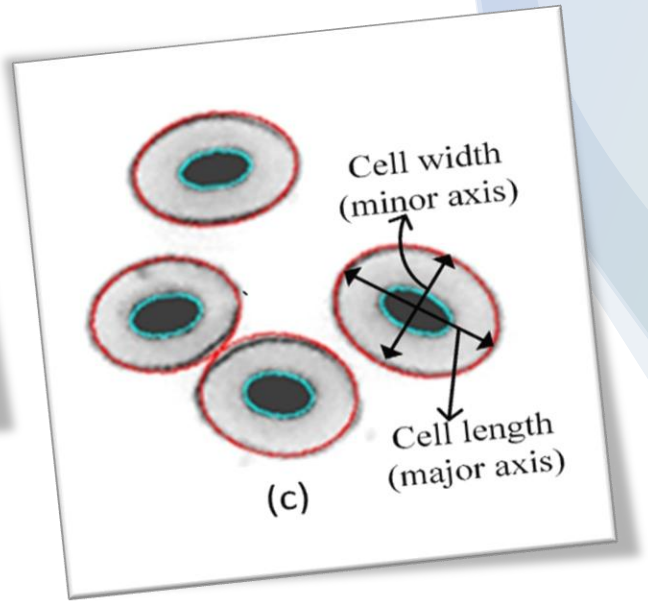
Introduction





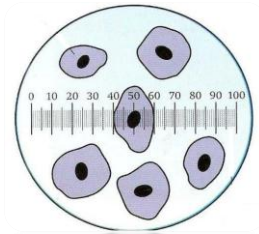
- **Morphometric Parameters of Fish Erythrocytes (MFE)** include:

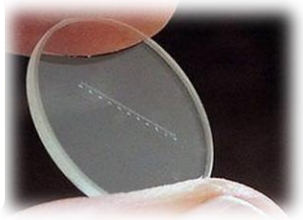
  (i) length (ii) width (iii) surface-area (iv) perimeter

- These parameters have been considered a valuable approach for **analysing health status** or diagnosing various diseases in fish.
- Erythrocyte morphology provides information on the **effects of drugs, chemicals in aquatic systems.**
- Multiple other applications of MFE measurement exist, for example:
  - **Morphological anomalies** in erythrocyte indicate the presence of toxic substances in water.
  - They are utilized by fish **breeders, pathologists, and microbiologists**.
- Fish erythrocytes are sensitive to environmental changes, making them **potential indicators for ecological biomonitoring and** for maintaining ecological balance, which is important for aquatic environmental sustainability.

Motivation and Objectives

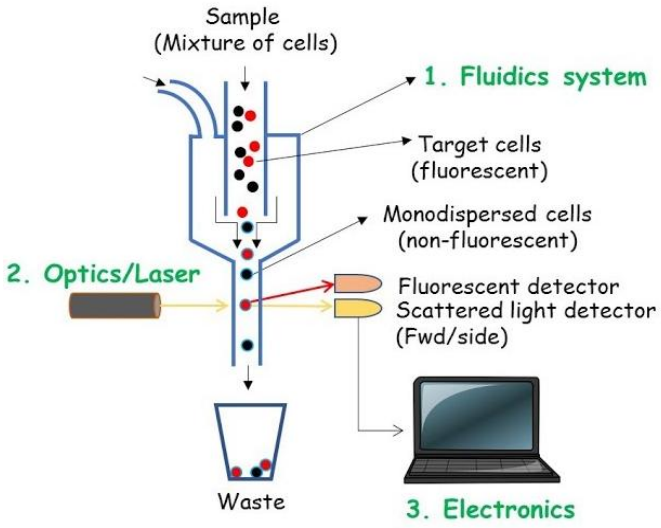
- Aims are to measure **Morphometric Parameters of Fish Erythrocytes (MFE).**
- The conventional methods for measuring MFE relies on a manual technique or automatic techniques.





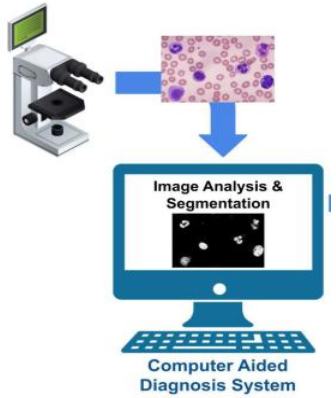
- Based on linear measurement.
- Measurements are highly dependent on the expertise of the operator.

Manual Method



- Allows analysing a single blood cell delivering results in a short time.
- It only evaluates volumes and corpuscular colour scheme.
- Does not show morphological and dyeing alterations in the cell.

Flow Cytometry



- Primarily used for segmenting human blood cells.
- Theoretically applicable to fish blood cell segmentation with slight modifications.
- However, conventional image processing methods face challenges in MFE measurement due to broken cell borders and cell overlapping in fish blood cells.
- CNN-based solutions require substantial amount of data with ground truth for their training.

Image Processing

Motivation and Objectives

- Thus, to address these issues, this work **proposes a solution** based on:

  Step 1: Image processing

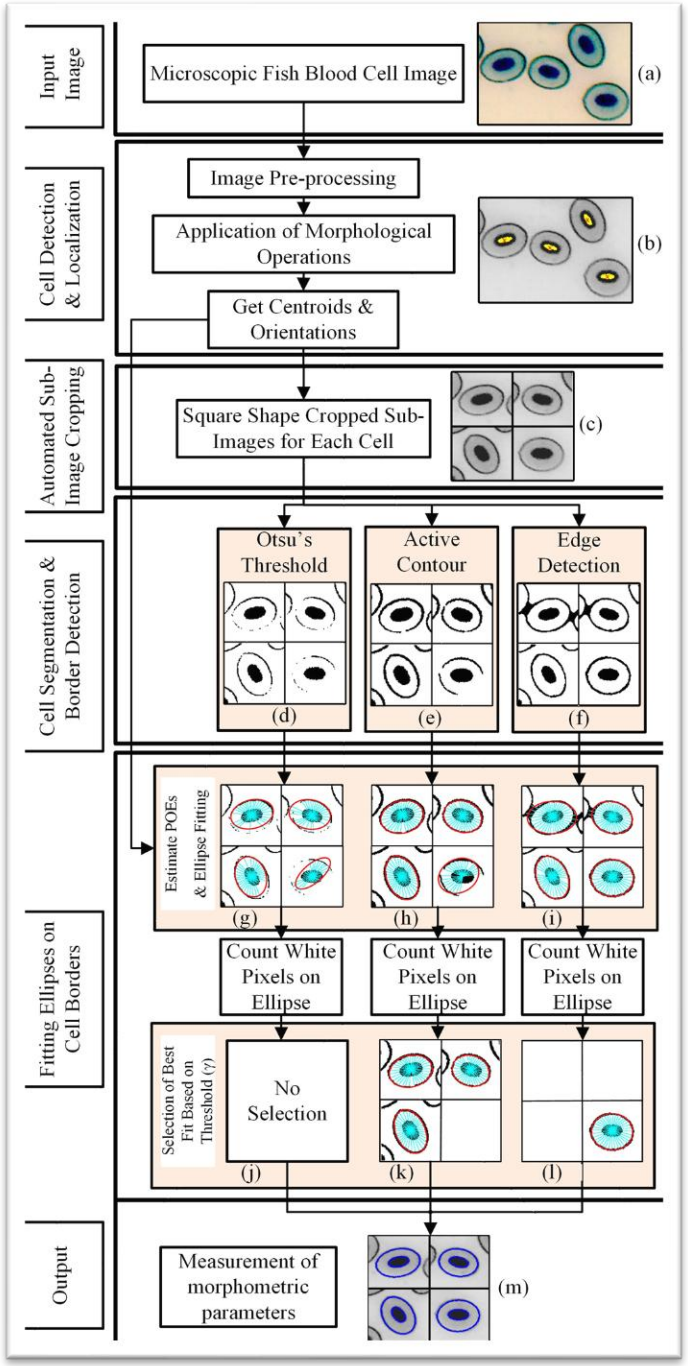
  - To **locate cells** in the image, then **segment** nucleus and border of cells.

  Step 2: Ellipse fitting Algorithm

  - To **identify the borders** of the cell/nucleus in the image.

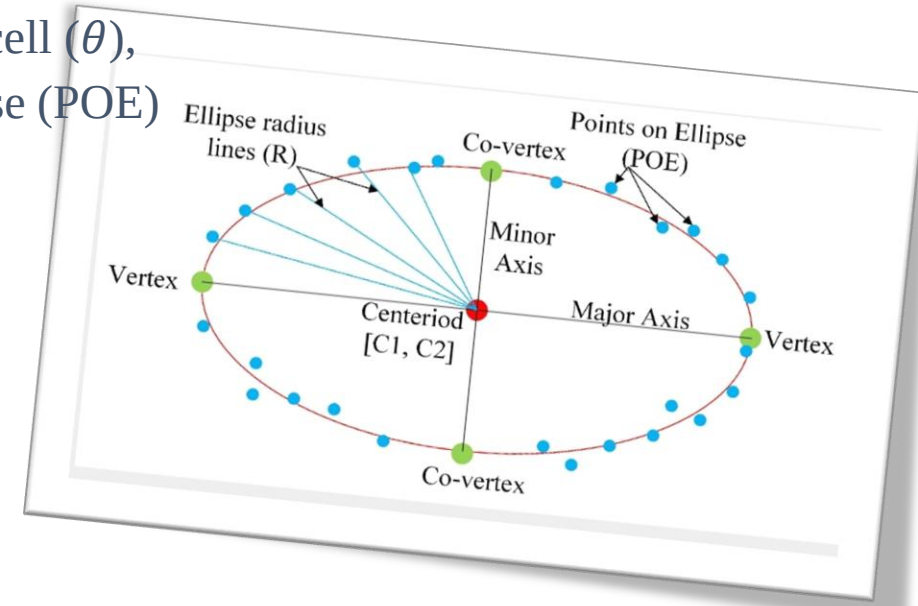
- The surface area of the obtained ellipse is measured and compared with a measurement performed by an expert.

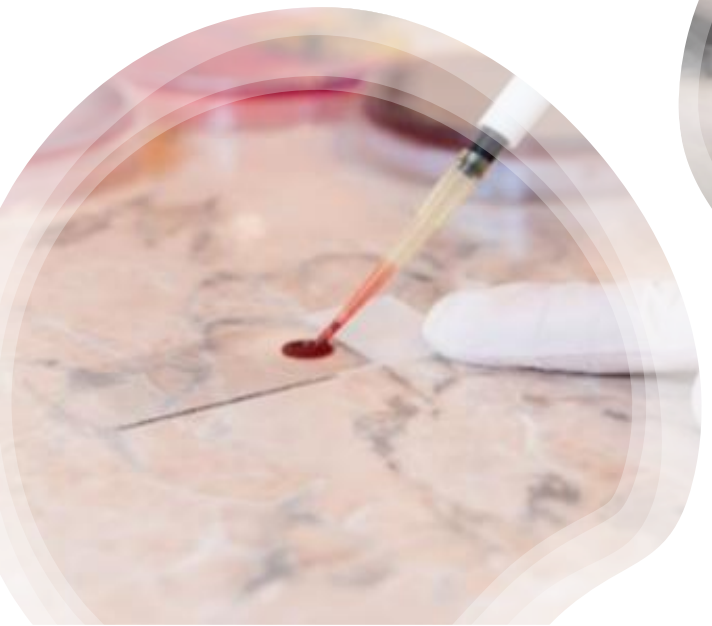
Flow Chart

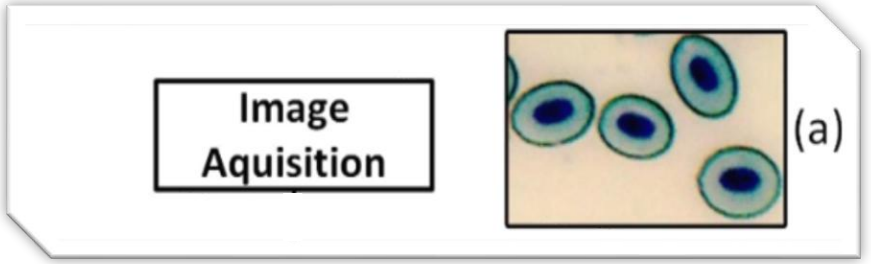


Methodology

- Fish blood cells are ellipsoidal in shape.
- The proposed method is designed to fit ellipse on cells by using the following parameters:
  - Centroid [C1, C2],
  - Orientation of cell (θ),
  - Points on Ellipse (POE)







Step 1: Image Acquisition

- Blood of fish is collected from the caudal tail vessels of the fish.
- A blood smear is prepared on a glass slide through a dilution, staining and drying process.
- The dried slide is then analysed under a digital microscope to capture images of fish blood cells.

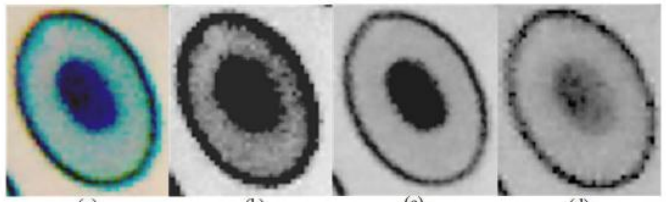
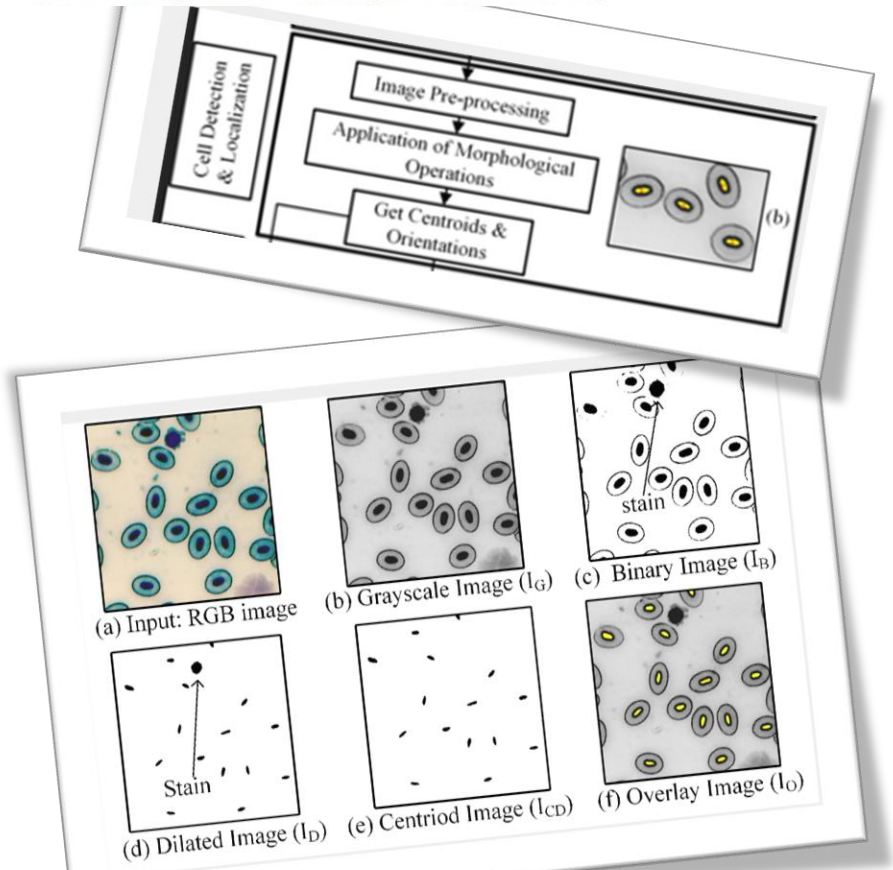


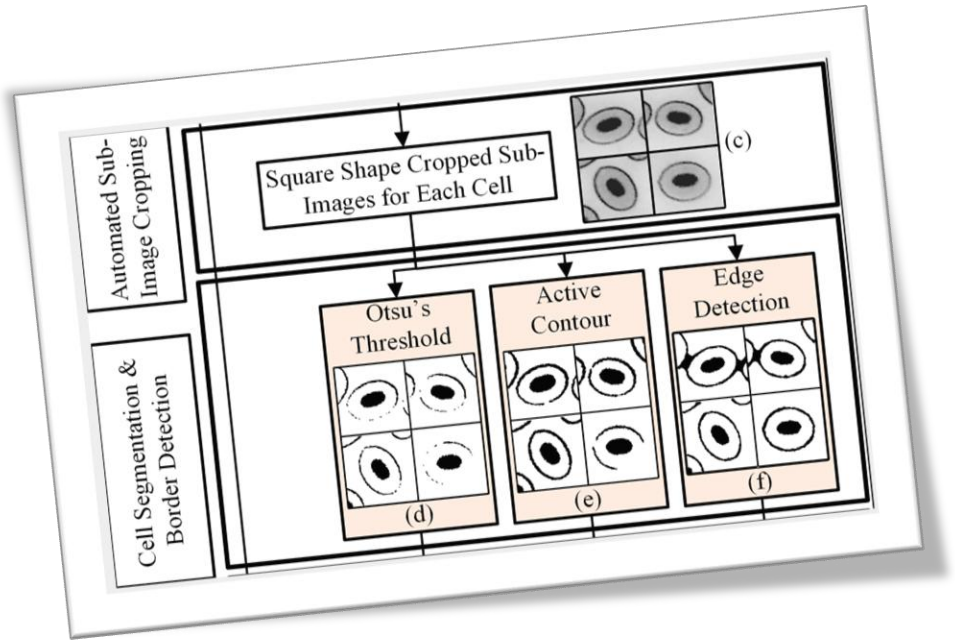
Fig. Components of RGB color image of fish red blood cell. (a) RGB color image. (b) Red (R) component of the color image. (c) Green (G) component of the color image. (d) Blue (B) component of the color image.



Step 2: Cell Identification & localization

- Pre-processing
  - The green component of the RGB image is selected for further processing because most part of the red blood cell information lies in the green.

- Morphological Operations
  - Binarizing the green channel by using Otsu's thresholding method.
  - Dilation operation is applied on binary image IB to remove the cell border and e the size of nucleus.
  - Stains are removed:
    - Determine the area of all objects in Image ID and calculate the average value.
    - Upper and lower threshold are determined to remove those objects that fall below the lower threshold or exceed the upper threshold.
    - The upper threshold is obtained by increasing of its 70% the average value.
    - The lower threshold is obtained by subtracting to the average value of its 20%.
  - Compute centroids [C_1, C_2] and orientation (θ) of all objects in the image (ICD).
  - The binary mask is overlaid onto the original grayscale image to create an overlay image.

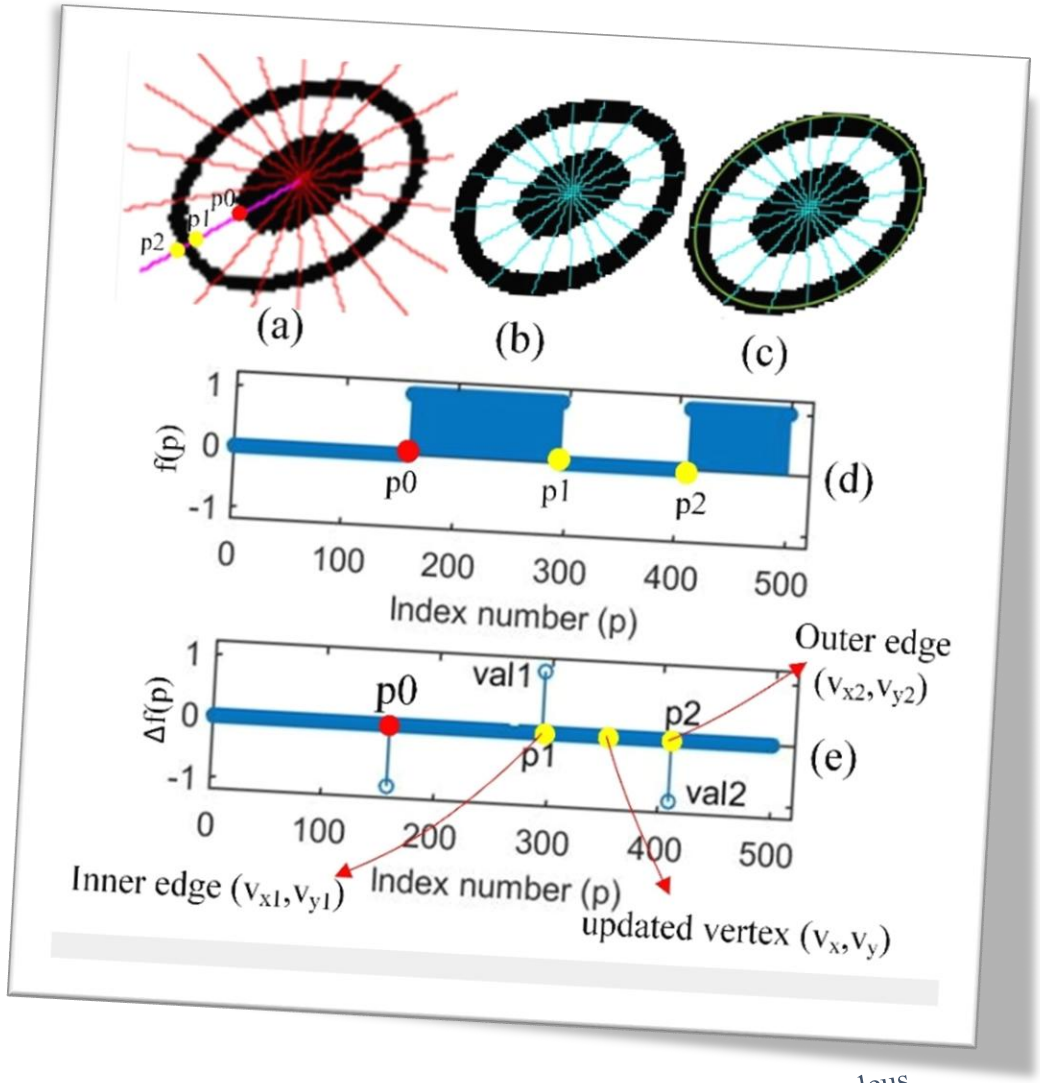


Step 3: Crop Sub-images

- Each cell in the image is localized using its centroid points.
- Then, algorithm automatically crops each cell to build 100x100 pixels sub-images.

Step 4: Segmentation

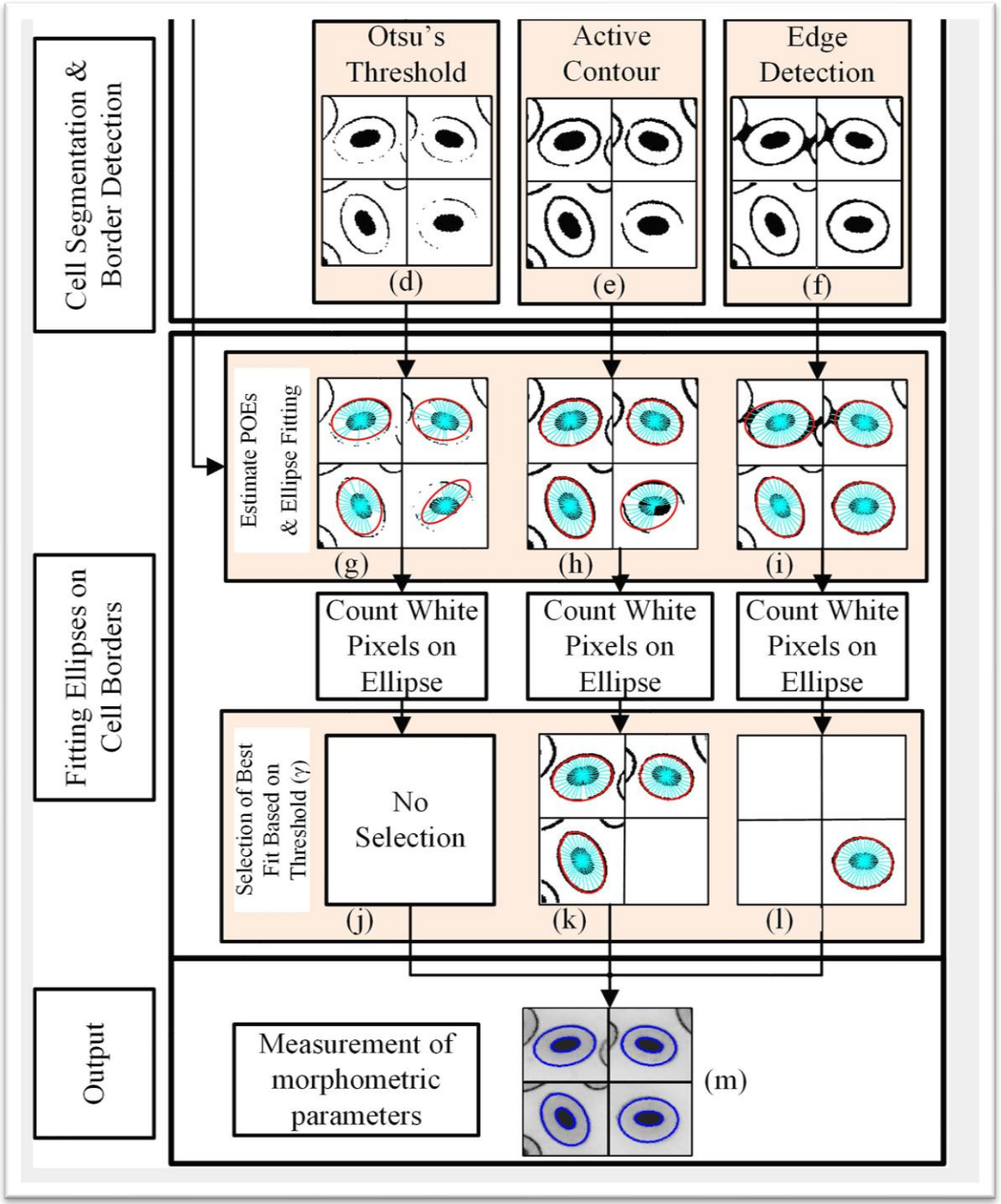
- The cropped sub-images are binarized by using Otsu's thresholding method.
- Active Contour: On binary image, further processing is done by applying the region growing method to grow the broken or discontinuous cell border.
- Edge Detection: Canny edge detection is applied on binary image to get the cell borders.



p_0 represents the edge of nucleus.
p_1 represents the inner edge of cell border.
p_2 represents the outer edge of cell border

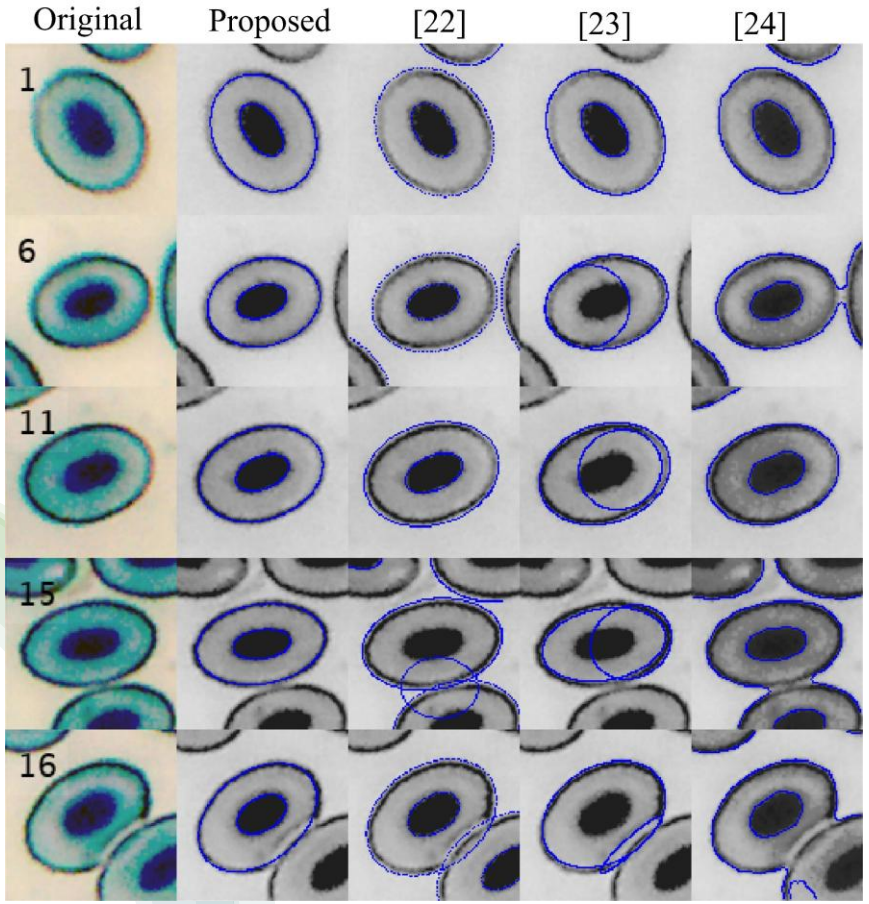
Step 5: Ellipse Fitting

- In the fitting process, POEs must be estimated to fit ellipse on cell/nucleus.
- To achieve this, initially, a large-sized radius lines (R) is plotted according to the cell orientation, so that it intersect the border of the cell.
- Next, the line is rotated with step-size of 10° degree to get plots of other radius lines (R).
- The intensity information of radius lines (R) is extracted.
- The derivative of $f(p)$ is calculated.
- The inner edge and outer edge of the cell border are recognized.
- The actual POE for cell is determined by taking the mean of inner (v_{x1}, v_{y1}) and outer (v_{x2}, v_{y2}) edge points.
- Once the actual POEs are obtained, this set of points is passed to the *ellipse fit block* to find the best fit to an ellipse by using the least-squares criterion.



Step 5: Ellipse Fitting

- Algorithm selects the best fit by analyzing pixel intensities under the ellipse perimeter.
- Correct fit: all ellipse pixels lie on black borders (intensity = 0).
- Threshold (γ) allows some white pixels for broken borders; excess indicates poor segmentation and fitting.
- Priority order: Otsu's thresholding > Active contour > Edge detection.
  - Otsu's: Best for thin borders, ensuring precise fits.
  - Active contour: Handles moderately broken borders well.
  - Edge detection: Useful for blurred borders but adds noise.
- Final selection combines techniques for the most accurate fit.



Ellipse fitting results on test data.

Experimental Results

- Measurement results of proposed method are compared with three existing methods and those obtained in laboratory by expert biologists.
- Biologists' measurements are obtained by using ToupView software, manually performing the ellipse fitting.
- Method [22]: Preliminary version of the proposed method.
- Method [23]: segmentation of overlapping elliptical objects in silhouette images.
- Method [24]: Automatic counting red blood cells in the microscopic images by EndPoints method and circular Hough transform.
- The test data used for performance evaluation is consisting of 18 randomly selected cells from source images acquired in the laboratory.

Experimental Results

- The table I and II presents a comparison of the cell/nucleus area measurements provided by the biologists, proposed method, other three methods.
- From table, it can be seen, proposed method results mostly within biologist confidence intervals.
- Root Mean Square Error (RMSE) of the ellipse fitting compared with the POEs:
  - For cell: max error 4 pixels.
  - For nucleus: max error 2 pixels.

TABLE I

CELL AREA MEASUREMENT RESULTS

Cell No.	Biologist's measurements confidence interval		Proposed		[22]	[23]	[24]
	lower end (Pixels)	upper end (Pixels)	Cell Area (Pixels)	RMSE (Pixels)	Cell Area (Pixels)	Cell Area (Pixels)	Cell Area (Pixels)
1	3569	3794	3440	4	4165	4091	3886
2	2599	2842	2619	3	3311	3041	3013
3	3371	3599	3401	3	4172	3888	3838
4	2972	3136	3013	2	3804	3545	3490
5	2665	2846	2701	3	3396	3202	4345
6	2826	3017	2794	2	3438	3235	3654
7	3004	3210	3037	2	3742	3471	3386
8	2745	2960	2798	2	3510	3255	3167
9	2581	2739	2656	2	3290	3085	3034
10	3127	3303	3230	3	3852	3739	4650
11	3070	3274	3249	3	3902	3720	3553
12	2828	3031	2902	2	3608	3340	3273
13	2791	2965	2865	2	3476	3285	4566
14	2656	2874	2689	3	3324	3201	4665
15	2902	3106	2909	3	3576	2531	4809
16	3144	3405	3372	3	4044	3549	5249
17	2832	3032	2880	2	3586	3278	3249
18	2789	2924	2856	2	3482	3307	3197

TABLE II

NUCLEUS AREA MEASUREMENT RESULTS

Cell No.	Biologist's measurements confidence interval		Proposed		[22]	[23]	[24]
	lower end (Pixels)	upper end (Pixels)	Nucleus Area (Pixels)	RMSE (Pixels)	Nucleus Area (Pixels)	Nucleus Area (Pixels)	Nucleus Area (Pixels)
1	596	672	600	2	621	672	660
2	459	521	491	1	1367	493	515
3	528	609	531	2	585	1571	581
4	509	546	531	2	608	1128	571
5	470	510	484	1	578	614	512
6	473	563	509	1	1041	1969	523
7	444	496	483	1	538	1861	518
8	447	483	482	1	581	1897	507
9	478	509	484	1	541	546	524
10	509	554	535	1	543	1804	583
11	488	540	552	2	524	1895	586
12	444	484	467	2	653	1897	498
13	510	532	519	1	570	1820	570
14	503	533	515	2	567	1866	568
15	506	530	518	2	1327	1724	561
16	533	563	531	1	657	376	601
17	491	538	530	1	526	1726	568
18	509	533	511	1	635	1823	527

Conclusions

- This work presents an automatic method for measuring MFE using image processing and ellipse fitting techniques.
- The effectiveness of the proposed method has been validated by comparing the results with measurements obtained by expert biologists and other existing methods in literature.
- Experimental results show that the proposed method properly fits ellipse on fish blood cell and its nucleus.
- Future work will be dedicated to analyse a larger number of cells, also considering other kinds of fish.

THANK YOU FOR YOUR ATTENTION!