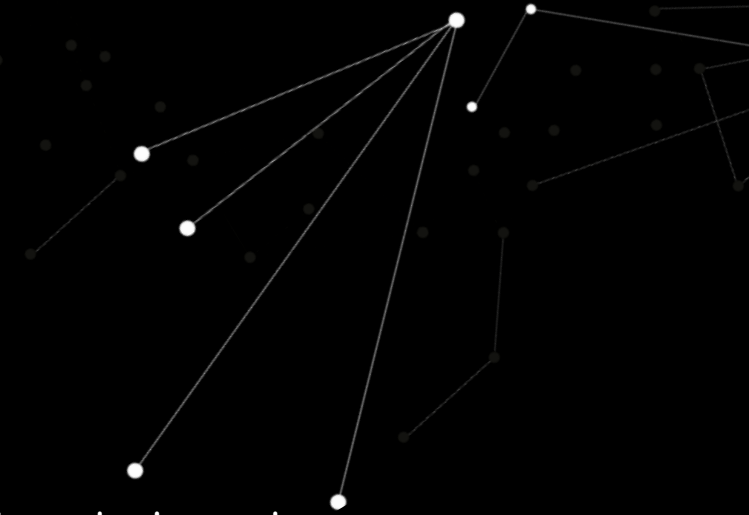# 2020 T2 COMP9517
Computer Vision

Project Goal

Develop an automated cell tracking framework that can consistently and accurately:

- **Detect Cells**: Unet for Segmentation.
- **Track Cells**: Euclidean distance of centroids and 3D trajectory.
- **Detect Cell Division**: Changing colors of the cell's bounding box.
- **Analyze Cell Motion**: Euclidean, Total and Net distance travelled.

Challenges

- Low contrast between cells and image background.
- Large image sequences with dense cell population.
- Irregular and deformable cell shapes, overlapping cells.

Motivation

- Demonstrate the importance of computer vision methods in tracking biological cells in time-lapsed microscopy images.
- Help to reduce human errors, effort and expertise required in manual annotation process.
- Assist biologists or medical researchers in their area of research.

Team Name: Group 1

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- Sebastian Castillo Castro
- Xinli Wang (Kevin Wang)
- Yuzhong Duan (Jack Duan)
- Kan-Lin Lu (Gary Lu)

Detection methodology

Pre-processing, UNET, Post-Processing

Detection Methodology Overview
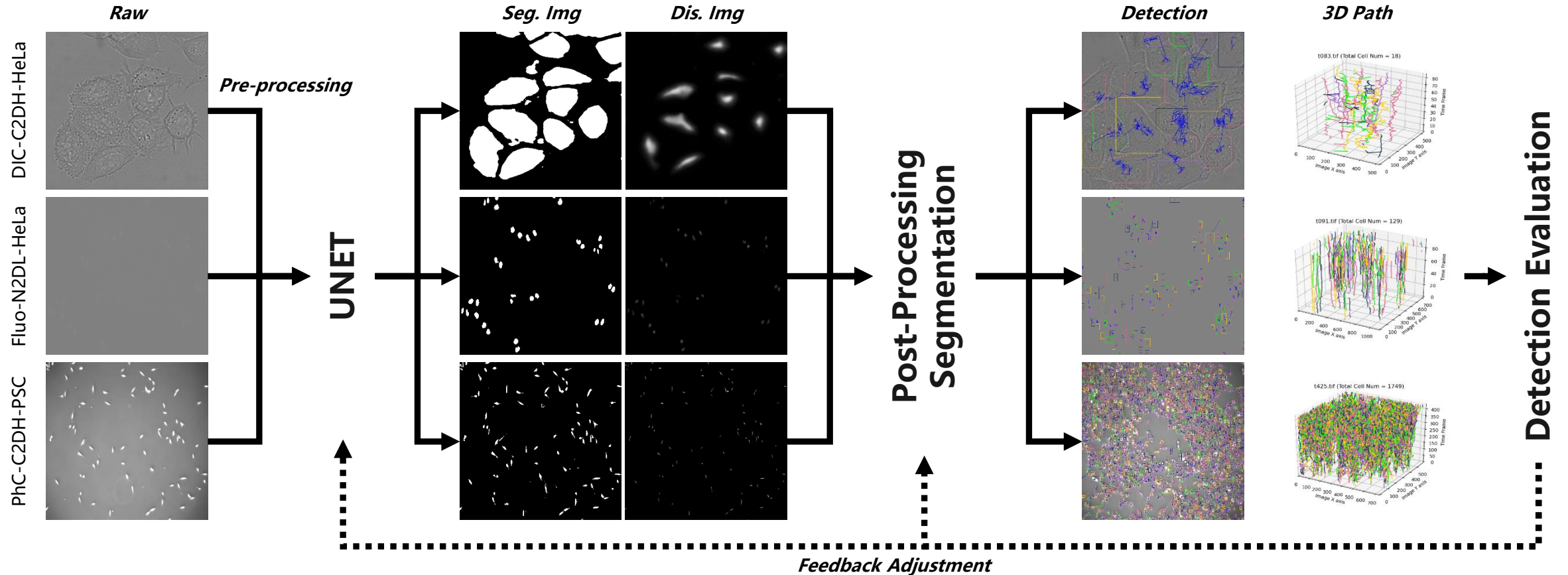
UNET Set up:

Pre-processing	➡	Dataset specific mode + normalization
Data augmentation	➡	Random flip, rotation, skew, crop
Targets	➡	Semantic segmentation + Distance est.

Post-Processing Methods:

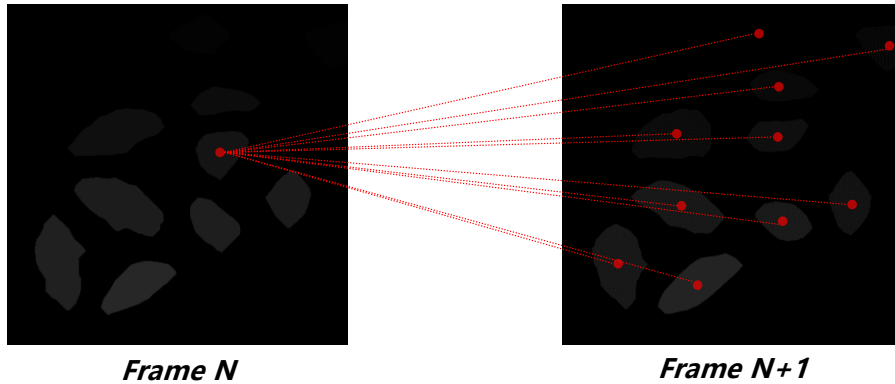|		
DIC-C2DH-HeLa and Fluo-N2DL-HeLa	➡	Watershed + Morphological Operation
PhC-C2DH-PSC	➡	Morphological Operation

Tracking and Division methodology

Distance Matrix, Division distance definition, Backward patching.

Tracking Methodology Overview

Euclidean Distance:

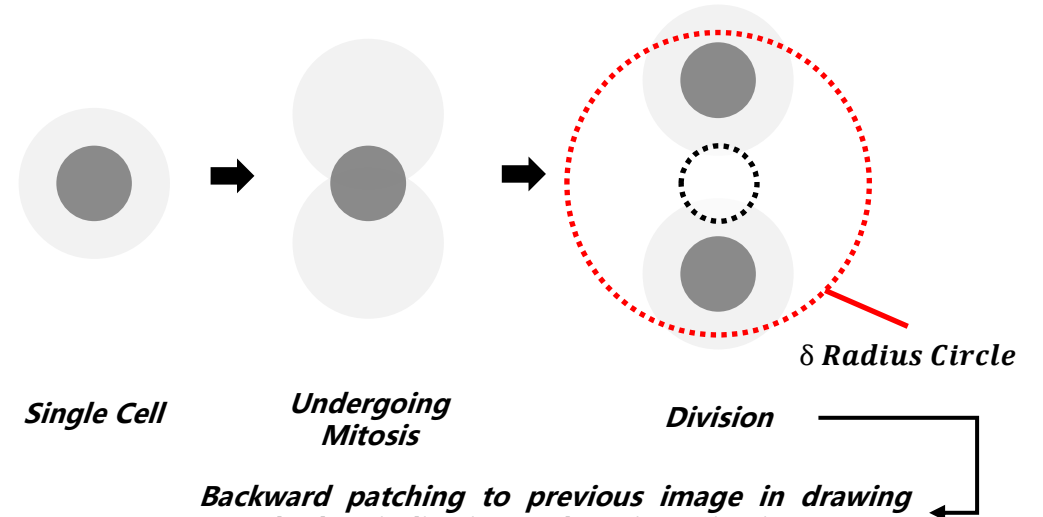$$d = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$$

illustration

Frame N   **Frame N+1**

Distance Matrix Example

		Frame N+1 Cell IDs			
		1	2	3	4
Frame N Cell IDs	1	32	12	2	15
	2	2	17	3	41
	3	35	4	1	11
	4	4	12	84	1
	5	21	34	9	5
	6	31	1	12	3

Division Methodology Overview

δ *division distance*

δ *Radius Circle*
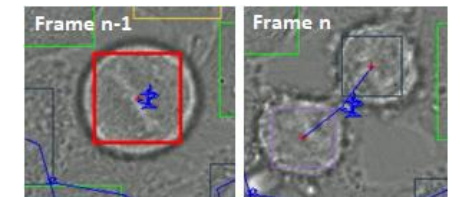
Single Cell   **Undergoing Mitosis**   **Division**

Backward patching to previous image in drawing "Red" box indicating undergoing mitosis

Selected Distance: (Trial and Error)

	δ
DIC-C2DH-HeLa	80
Fluo-N2DL-HeLa	10
PhC-C2DH-PSC	3

Motion Analysis

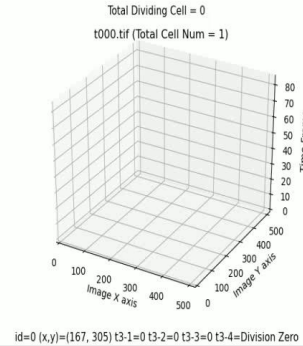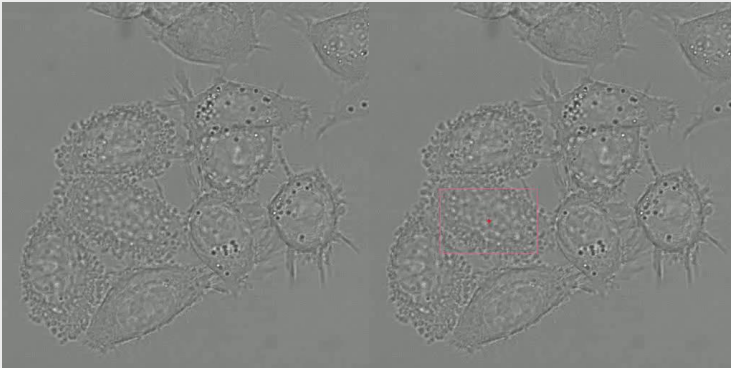**Centroids per frame stored in dataframe** → **Per Frame Motion Calculation** → **Per Frame Annotation Html**

Any single cell can be selected by user prompt in plotting 3D pathology graph, along with motion analysis printed in output image.
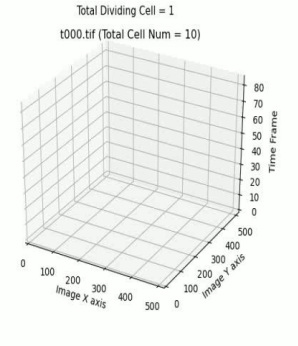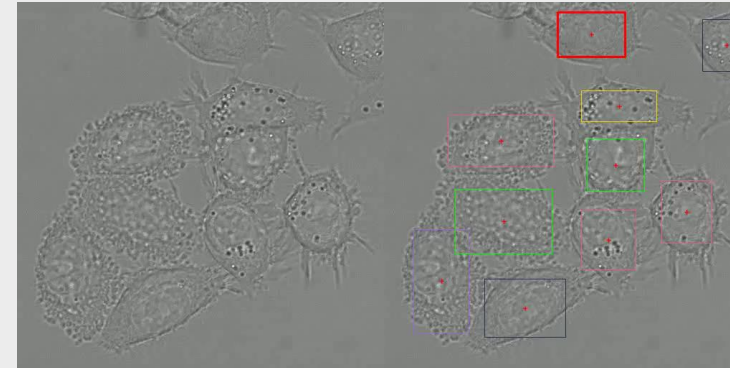
Demonstration and Limitations
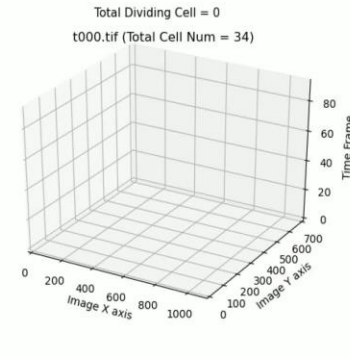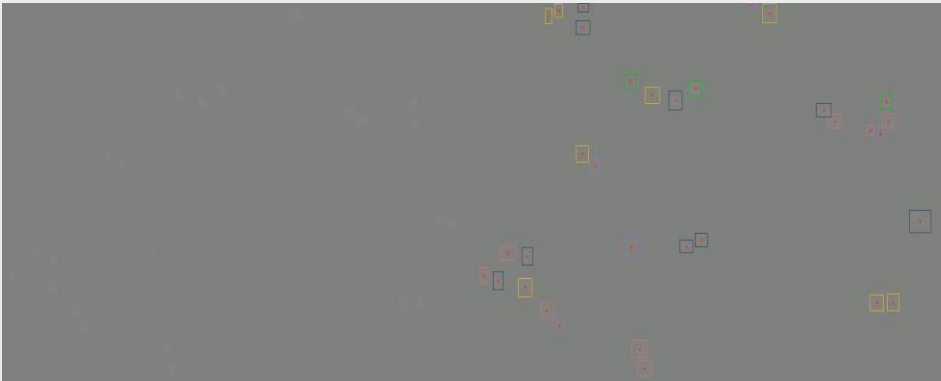
Cell Tracking, Mitosis Tracking.

DIC-C2DH-HeLa (Selected Cell)

Total Dividing Cell = 0

t000.tif (Total Cell Num = 1)

id=0 (x,y)=(167, 305) t3-1=0 t3-2=0 t3-3=0 t3-4=Division Zero

DIC-C2DH-HeLa

Total Dividing Cell = 1

t000.tif (Total Cell Num = 10)

Fluo-N2DL-HeLa

Total Dividing Cell = 0

t000.tif (Total Cell Num = 34)

PhC-C2DH-PSC

Total Dividing Cell = 0

t000.tif (Total Cell Num = 133)

Limitations observed:

Over-Segmentation

Overlapped Cells results in mislabelling of mitosis event
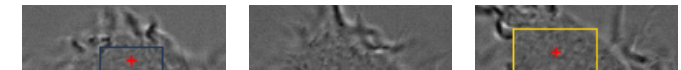
Appeared then reappeared cells caused by over-segmentation results in extra cell ID

Mismatching caused by cell travelling over another cell across frames

Dataset 1, extreme large cell segmented to 2 pieces due to irregular shape

Result, Discussion and Conclusion

Discuss outcome, present conclusion and possible future work.

Results

Per Frame Outcome Analysis:

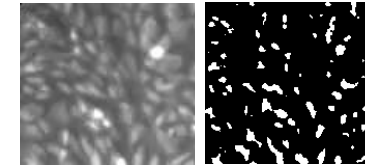| DIC-C2DH-HeLa / Sequence 1 (Frame 0 - 14)					
Cell Detection Analysis					
TP	157	FP	4	FN	1
Precision (%)	97.52 %		Recall (%)		97 %
Cell Division Analysis					
TP	1	FP	3	FN	0
Precision (%)	25.00 %		Recall (%)		80%

DIC-C2DH-HeLa / Sequence 3 (Frame 0 - 14)					
Cell Detection Analysis					
TP	179	FP	8	FN	0
Precision (%)	95.72 %		Recall (%)		100%
Cell Division Analysis					
TP	2	FP	5	FN	0
Precision (%)	28.57 %		Recall (%)		100 %

DIC-C2DH-HeLa: Division took place 48% average of total Cell Number

Fluo-N2DL-HeLa: Division took place 3.1% average of total Cell Number

PhC-C2DH-PSC: Division took place 2.6 % average of total Cell Number

Per Sequence Outcome Behaviour Analysis:

	DIC-C2DH-HeLa				Fluo-N2DL-HeLa				PhC-C2DL-PSC			
Sequence Number	1	2	3	4	1	2	3	4	1	2	3	4
Total Cell Number in Entire sequence	71	96	165	64	770	2768	1020	1207	51452	43864	27369	26923
Total Cell Number in Last Frame	18	17	9	12	129	334	213	229	1749	1423	865	795
Total Division Occurrence	38	55	67	27	16	112	21	51	1740	1306	519	604
Average Cell Speed (Pixel/Frame)	12.37	14.07	12.96	9.21	2.4	2.25	2.22	1.9	0.705	0.68	0.66	0.65
Speed 90% CI	(11.77,12.97)	(13.33, 14.82)	(12.25, 13.67)	(8.72, 9.71)	(2.36, 2.44)	(2.24, 2.28)	(2.19, 2.24)	(1.88, 1.92)	(0.7, 0.71)	(0.68, 0.69)	(0.66, 0.66)	(0.65, 0.66)
Average Cell Cumulative Distance (Pixel)	213.97	154.23	120.01	190.97	23.07	19.43	24.92	24.22	3.73	3.44	2.57	2.41
Cumulative Distance 90% CI	(151.15,276.8)	(111.46,197.0)	(90.1, 149.92)	(129.8, 252.1)	(21.04,25.1)	(18.44, 20.43)	(22.96, 26.88)	(22.49, 25.96)	(3.67, 3.78)	(3.38, 3.5)	(2.51,2.63)	(2.35, 2.47)
Average Cell Net Distance (Pixel):	49.67	46.1	38.09	45.84	6.99	6.56	7.34	6.88	1.44	1.4	1.11	1.02
Net Distance 90% CI	(42.34, 56.99)	(40.54, 51.66)	(33.04, 43.14)	(36.91, 54.78)	(6.46, 7.52)	(6.26, 6.87)	(6.86, 7.82)	(6.47, 7.3)	(1.42, 1.46)	(1.38, 1.42)	(1.09,1.14)	(1.01, 1.05)
Average Confinement Ratio	5.15	3.46	5.06	5.61	3.33	3.36	3.47	3.85	2.53	2.39	2.24	2.28
Confinement Ratio 90% CI	(3.5, 6.79)	(2.62, 4.3)	(3.49, 6.62)	(3.32, 7.89)	(3.11, 3.55)	(3.18, 3.54)	(3.21, 3.74)	(3.54, 4.15)	(2.51, 2.57)	(2.36, 2.43)	(2.2, 2.38)	(2.23, 2.33)

Discussion

- **High computational time for PhC-C2DH-PSC, due to matching process**
- **Decrease in reliability when cells are clustered with extreme high population**
- **Motion analysis highly depends on outcome of segmentation and tracking**

Conclusion and Future Work

- **An insight to cell detection and a reasonable outcome is achieved**
- **Fine tuning UNET and Post-Process method in tackling stated limitation**
- **Include extra features in matching across frames**
- **Include extra features in division matching across frames**