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Application Note: μPolar - An Interactive 2D Visualization Tool for Microscopic Time-Series Images

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***Abstract*—Time-lapse microscopy is an effective research tool to monitor cell behavior and cell divisions. Recent advances in microfluidics have accelerated the adoption of time-lapse microscopy in research. However, it is challenging to visualize and interpret the time-series data gathered through time-lapse microscopy. We have developed a circular plotting software tool, μPolar, to visualize the trends and patterns of the cell movements and cell division events in a time-series. μPolar is interactive and easy to use. We demonstrate the utility of μPolar by visualizing the events of dividing yeast cells where cell divisions lead to oscillating plotting patterns, and in migrating mouse fibroblasts where cell shapes change during the migration. μPolar potentially could be applied to other types of time-series of microscopic images. This R package μPolar is available through GitHub.**

***Index Terms*—Microfluidics, microscopic, cell, visualization, replicative lifespan**

I. Introduction

Cell lineages and cell behavior are important in biological and biomedical research [1]–[3]. Cell divisions and cell family lineages are often monitored by time-lapse microscopic imaging experiments. From time-lapse microscopic image data sets, we can monitor intra-cellular and inter-cellular changes, cell division events, and cell growth and migration [4]. These inferences are often assisted with image analysis software tools [5]–[7]. Microfluidics is a high-throughput approach that generates a large volume of time-lapse images of cells. A microfluidic device is an ultra-small structure with microfluidic channels offering fast and reliable results in comparison to traditional methods [8], [9]. Time-lapse microfluidic images amplify biologists' ability to experimentally image live cells during their development [10]. Due to these images' functionality and micro-scale dimension, they can be used in many applications such as drug delivery, cell monitoring, cell division, and virus inspection [11], [12]. Currently, however, there is a need for visualization tools for time-lapse microscopic images that can facilitate biological interpretation and provides interactive access.

In particular, microfluidics have become a high-throughput method for analysis of dividing yeast cells [13]. The budding yeast is an effective model for cellular aging [14]. Yeast replicative lifespan (RLS) is defined as the number of cell divisions that a single mother cell can accomplish before it ceases to divide [15]. In the study of cellular aging, time-lapse microfluidic microscopic imaging has provided unprecedented quantitative details on changes in cell characteristics during aging. Determining the replicative lifespan of dividing yeast cells is time-consuming and is traditionally measured through manual micro-dissection [16], [17]. The microfluidic approach generates hundreds of time-lapse microscopic images, converting the old challenge of manual dissection into a new challenge of time-series image data analysis [18]–[20]. One way to tackle this challenge is data visualization, a need that this work aimed to address.

Here, we present μPolar (pronounced "[mu] Polar"), an R package that provides circular plots to visualize time series of cell behavior and cell division events. We demonstrated the utility of our method to visualize the division events of dividing cells over a time period in two types of cells: budding yeast cells and mouse fibroblasts.

II. Implementation and Features

A. Design of μPolar

The goal of μPolar is to visualize time-series data generated by time-lapse microscopic images in a region of interest (Fig.1). The basic idea is to use a circular plot to represent the change of time, where points on the radius represent cellular events and/or characteristics at each time point. A sequence of microfluidic images is shown in Fig.1a from time-point 1 to time-point 40. For instance, the image (60x60 pixels) at time-point 40 illustrates two cells inside a microfluidic trap. The plot representation is based on cell centroid points [e.g., (x1, y1) and (x2, y2)] and areas (e.g., A1, A2), which can be obtained by an image processing tool (e.g., ImageJ, Fiji) or procedures such as YOLO or MaskRCNN [21]–[24]. The input files of μPolar are in comma-separated values (CSV) format (Fig.1b).

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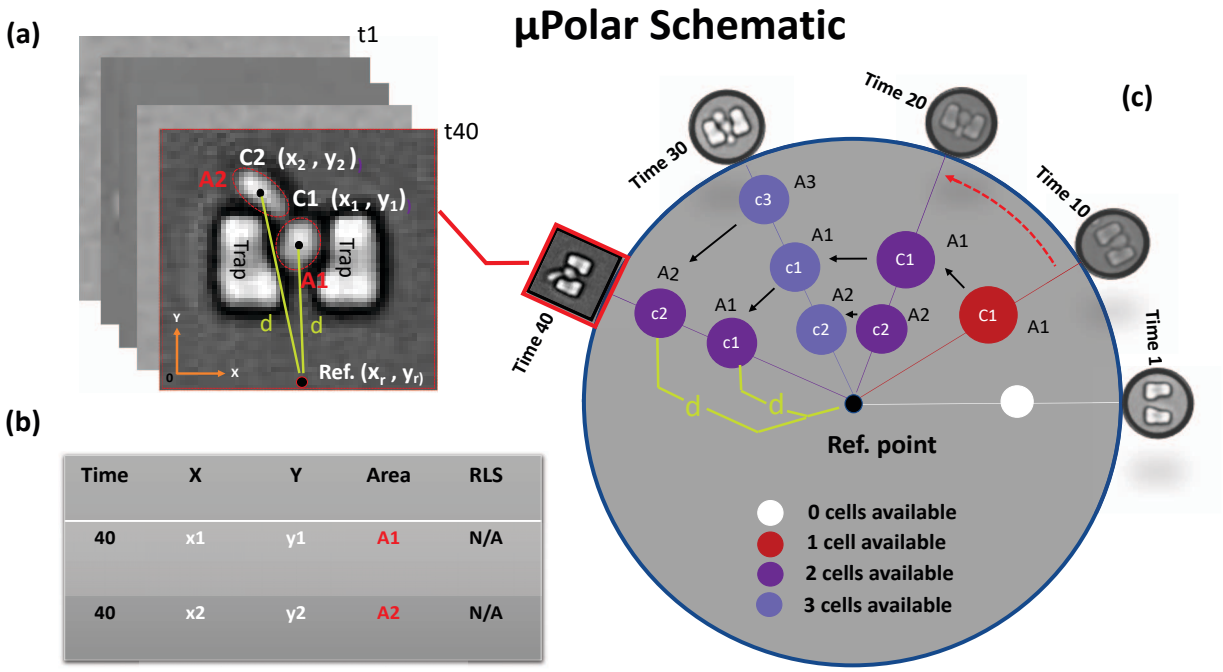

Time	X	Y	Area	RLS
40	x1	y1	A1	N/A
40	x2	y2	A2	N/A

Fig. 1. **The design of μPolar**. (a) A sequence of microfluidic sub-images (60x60 pixels). The front image was labeled with cell numbers, coordinates, cell boundaries, and the reference point. (b) A sample of μPolar dataset format with optional features of area and RLS, which correspond to the front image in a. (c) μPolar plot with a representation of cell location on the plot and cell color tag. Black arrows track the same cell objects at subsequent time-points.

1) Distance calculation: Since the cell time-points and coordinates are available from the dataset (e.g., Fig.1b), the cell distance from the reference point can be calculated by using the Euclidean equation

$$d_i = \sqrt{(x_i - x_r)^2 + (y_i - y_r)^2}$$
$$i = [1, 2, 3, 4, ..., m] \quad (1)$$

where d_i is the calculated distance at the time-point i corresponding to the image number as shown in Fig.1a and Fig.1c in light green color. x_i and y_i are cell coordinates at each image. Coordinate (x_r, y_r) is the reference point, which need to be specified by the user as required for the μPolar function. m is the maximum time-point equivalent to the total number of images. The reference point could be chosen at any point of the image based on the region of interest. For instance, in Fig.1a, the reference point was chosen at $x_r = 30$ and $y_r = 1$ based on cell movement and the position of the trap.

2) Time to angular degree conversion: The next step is to convert the image time-points to angular degrees, suitable for circular plot visualization. The time to degree conversion is calculated by

$$\theta = \frac{D_{max}}{T_{max}} \quad (2)$$

$$A_{acc} = \sum_{i=1}^{n} \theta_i \quad (3)$$

where D_{max} is the maximum degree (360°) and T_{max} is the maximum time-point (e.g., last time-point). A_{acc} is the accumulative angles of each radius vector (θ) and n is the maximum plot degree. All cell distance values at each time-point are mapped on the radius of a circular plot with a corresponding A_{acc} value.

The μPolar schematic plot in Fig.1c illustrates microfluidic images at time-point 1 (zero cells), time-point 10 (one cell), time-point 20 (two cells), time-point 30 (three cells), and time-point 40 (two cells). The color tag method represents the number of the cell corresponding to the number of cells in the image. We found this color-tagging useful to track mother cells and daughter cells at different time-points, and identify cell division events.

B. The μPolar R function

The μPolar package, written in R language, utilizes three other R libraries including *tidyverse*, *utils*, and *plotly*. The μPolar function has 10 arguments: the first argument is the input dataset, and the remaining arguments are required for visualization adjustment. Table I demonstrates an overview

of μPolar function arguments. Argument (I) is the time-lapse dataset and should include *Time* and *coordinates*, which are required for basic visualization. The *Area* and *RLS* are optional features of the μPlot function and can be added to the visualization function if they are available from the dataset. Argument (II) is the reference point (x_r,y_r) that can be chosen by the user. The reference point mainly depends on the type of image and the purpose of the investigation (e.g., edge coordinate of the image in the direction of the cells' movement). Argument (III) is to select a particular range of time-points for plot visualization. The starting and ending time-points can be given by the user; otherwise, μPolar visualizes all time-points. This option is useful to analyze a specific range of time-points (e.g., overcrowded cells). Argument (IV) displays the plot title; otherwise, the default is no title. Argument (V) is to evenly divide the angle line on the plot. This functionality is similarly useful to analyze a range of time-points when there is an overcrowded region on the plot. Argument (VI) is a numeric value that indicates the reference line on the plot. Argument (VII) is a numerical value that adjusts the cell area when available. It represents the cell with actual size (pixels), which is useful to visualize cell movement. Argument (VIII) is vector dot colors associated with the individual cell, and the default is set to 12 colors. This option is effective to visualize cell tracking identification at each time-point. In addition, it is very beneficial for RLS analysis when the cell development can be visualized based on cell size over time. Argument (IX) is vector line colors associated with a number of cells at each time-point. This gives a good overview of a number of cells variations over time. Argument (X) adjusts the cell distance from the edge of the plot when there is overcrowding.

C. Representation of cell events and characteristics

We provide 8 examples to illustrate how μPolar represent typical types of cell events and characteristics of dividing cells in microfluidics time-lapse experiments (Fig.2): traps without cells, traps with a yeast mother cells and a budding daughter cell, a cell division event in a time series, growing mother cells with daughter cells downward, growing mother cells with daughter cell upward, senescent and dead cells in a time series, and traps with multiple cells. Fig.2a uses a white dot on the reference line (red line) to represent a trap without cells. The red reference line is defined here at the bottom of the trap. The event in Fig.2b portrays the initial stage after division occurs, where the mother cell is located inside the trap with a bud at the trap outlet. Purple dots represent the mother cell inside the trap, and the dark red dots represent the bud. The mother and daughter cells in the images are connected to their corresponding dots in the plot by arrows. In the next event Fig.2c, we highlighted a mother cell without daughter bud in the middle of a time series of mother cells with buds, indicating the moment that one daughter cell has just been separated from the mother cell, and another daughter cell is too small to be detected. Fig.2d describes a trap with 5 cells (purple line pointed by a black arrow), and an overcrowding situation that frequently occurs with growing yeast cells. In Fig.2e, a mother cell is dividing with daughter cells budding toward the bottom of the trap. The mother cells at each time point with budding daughters are in purple, and mother cells without daughters are in black. Daughter cells budding toward the bottom of the trap are in red. Fig.2f illustrates similar cell division events except that daughter cells were budded toward the upper opening of the trap. In this case, daughter cells are in purple and mother cells are in red. Fig.2g demonstrates a situation where the mother cell and daughter cell remain attached for a long period of time without detectable cell divisions. Fig.2h represents a senescent mother cell that has stopped dividing. In this case, there is a single mother cell, represented in a dark dot, at each time point. This situation typically happens at the end of the cell lifespan.

III. Package availability

The package μPolar is an open-source package freely available on Github at https://github.com/merang/uPolar. The package installation can be done in R either using the install_github function in the 'devtools' or using the githubinstall function in the 'githubinstall' package.

IV. Data Used

To demonstrate μPolar's utility, we plotted two sets of time-lapse microscopic images: one set of dividing yeast cells and the other of migrating mouse fibroblasts. The yeast cell images were generously provided to us from a recent experimental work in [13]. The original microfluidic images contain many traps , we partitioned these images into 391 sub-images in 60x60 pixels dimensions based on the cell traps. The mouse fibroblast dataset is publicly available and contains 37 time-lapse microscopic images [20]. Since the number of fibroblast in each image (307x306 pixels) was more than 50, we cropped a section of time-lapse images in 121x121 pixels dimensions. Based on image size and resolution, the μPolar function can be applied to any time-lapse cellular microscopic images by cropping the region of interest. Image feature extraction (e.g., coordinates, area) can be done via many methods. We used "Fiji - ImageJ" tools to obtain cell coordinates and cell area. This process can be automatic; however, it often need manual verification especially for images with low resolutions. The obtained data can be exported in CSV format, which is suitable as inputs for μPolar.

V. Results

A. Application to microfluidic yeast cell images

We present 10 μPolar plots for Trap No. 2, 12, 22, 41, 50, 59, 73, 82, 98 and 100 in Fig. S1. These 10 plots represent a range of cell division events. In each image, we use color to represent the number of cells in each trap. The white, black, light blue, pink, green, purple, and orange colors represent 0 cells, 1 cell, 2 cells, 3 cells, 4 cells, 5 cells, and 6 cells, respectively.

Additionally, we provided three zoomed-in examples. In Fig. S2, we highlighted time-point 30 of Trap No. 8, which contains 4 cells above the tap, represented in a green line. At

TABLE I
THE μPOLAR R FUNCTION DESCRIPTION

Function		
μPolar(sFileName,nBaseXY,nSecBE,sTitle,numAtics,nRefVal,nDotAdjust,vDotColors,vLineColors,nEdgeAdjust)		
Number	**Argument**	**Description**
(I)	sFilename	Input dataset containing Time, X, Y, Area (optional), and RLS (optional)
(II)	nBaseXY	Given reference point (x_r, y_r)
(III)	nSecBEt	Given plot began and end time-points
(IV)	sTitle	Plot title (default = no title)
(V)	nNumAtics	Number of evenly spaced angle tic lines
(VI)	nRefVal	Numerical value for reference line
(VII)	nDotAdjust	Factor for multiplying dot size
(VIII)	vDotColors	Vector of dot colors associated with cell
(IX)	vLineColors	Vector of line colors associated with linked cells
(X)	nEdgeAdjust	Numerical value to extend outer plot radius

this time-point, all cells are above the reference line and a cell division happened outside the trap due to the oversized mother cell (#4). In another example, Fig. S3 demonstrates Trap No. 33 at time-point 11 when there are 2 cells available in the image. This is an early stage of cell division when 2 cells (sky blue line) are very close to each other. In Fig. S4, we show the oscillating plotting patterns that represent regular cell division intervals of an healthy yeast mother cells in the initial one-third of the plot. Fig. S4 also shows a scenario of likely senescent cell in Trap No. 63 at time-point 340, in which a single mother cell has remained undivided for almost two-third of the microfluidic experiment period.

In Fig. S5, we illustrate how we can change the reference point (x_r, y_r) to visualize the time series from different perspectives, using Trap No. 44 as an example. Fig. S5a represents a μPolar plot when the reference point is (0, 0). This representation is only based on the cell centroid point coordinates without the distance calculation. Fig. S5b represents a μPolar plot when the reference point is (30, 60). This representation is based on the distance calculation. The comparison of selected regions (black box) portrays that cell variations are more visible in Fig. S5b. Therefore, selecting the right reference point is an effective factor to improve the division time-points countability on the plot.

B. Application for microscopic mouse fibroblast images

To demonstrate its general utility, we applied μPolar to a data set of time-lapse microscopic images of migrating mouse fibroblasts (Fig.3a). The number of available cells in each image is in a range of 50 to 70 depending on the time-point. The average cell size is bigger than the average yeast cell size. For simplicity, we focused on a section of these images as illustrated in Fig.3b. Correspondingly, feature extraction is applied to these images, collecting cells coordinate of each image in order of time. According to our observations, cells gradually migrate from the right side to the left side in these images over time. Thus, we chose the reference point at the left side of the image edge (x_r = 2 pixels, y_r = 60 pixels) for distance calculation. In Fig.3c, we used a unique color to represent the same cell object at each time point. We like to emphasize that the changing diameters of colored dots represent the changing cell shapes during migration. In addition, each color represents the cell number at each time-point. For example, the orange color at time-point 37 illustrates that there are 9 cells at this time point and the orange dot is the cell number 9 at this time-point.

C. Representation of cellular characteristics and movement

Cellular characteristics such as cell sizes and movements are informative to illustrate biological processes such as aging. μPolar function has an option to import cell size information (area) for visualization. In general, there are two sets of color representation in the μPolar plot: colored lines and colored dots. The colored lines represent the numbers of cells detected at each time-point and are often displayed in lighter colors. The colored dots representing individual cells at each time-point and are often displayed in darker colors.

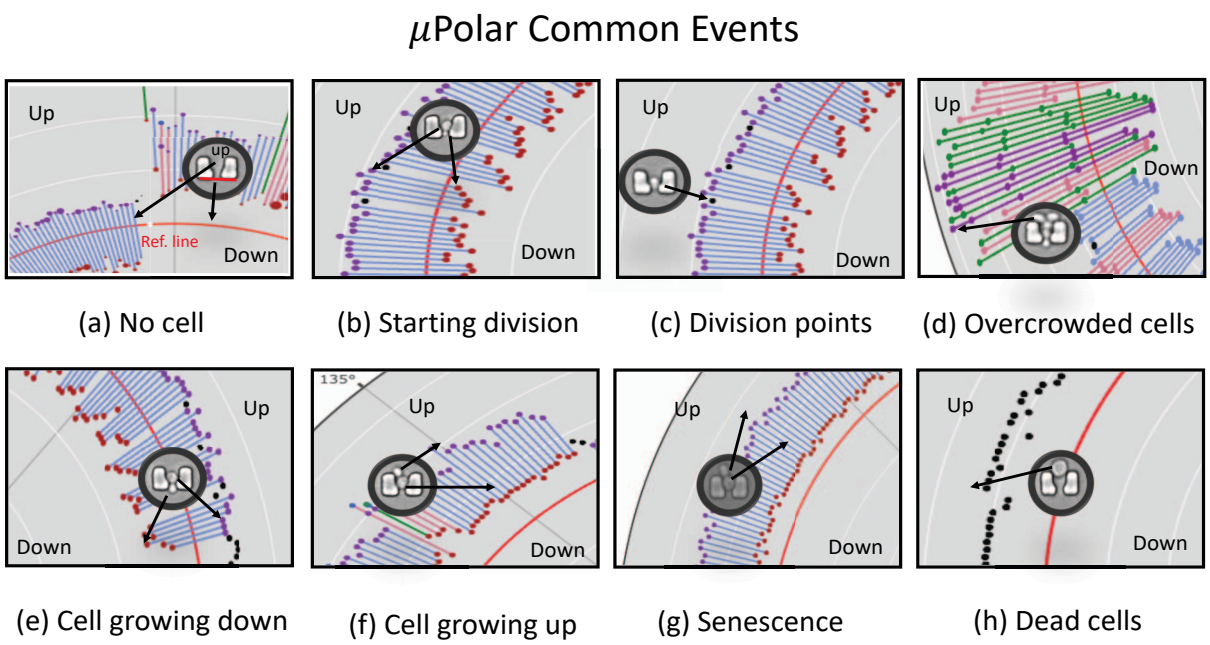

Fig. 2. **Typical cell events visualized by μPolar**. Any events that occurred below the reference line (red) are denoted as "down," and any events that occurred above the reference line (red) are denoted as "up." (a) There is no cell at the presented time-point (white dot). (b) The two cells represented by dark red dots and purple dots are close to each other, and division already happened. (c) The black dots represent a division after the cell completed its separation cycle. (d) Indication of overcrowded cell events. (e) Representation of steady cells in the up region (purple color) and developing cells in the down region (dark red color). (f) Representation of steady cells in the up region (dark red color) and developing cells in the up region (purple color). (g) Two cells are in a steady situation for some time, an indication of senescent cells. (h) The representation of a cell that has been dead for some time.

Fig.3c is an example of cell size variation. The cell movement and development can be determined by following a cell with the same color at each time-point. For instance, the comparison between the closest cell to the center-point at time-point 1 (dark red dot) and the closest cell to the plot center-point at time-point 37 (dark red dot) shows that the cell migration from time-point 1 to time-point 37 is approximately 20 units.

Fig.4 is a μPolar plot with cell area and color tracking for time-lapse microfluidic images of dividing yeast cells. Here, the numbers of cells at each time-point are in a range of 1–5 cells Cells can be visually tracked based on colored dots and cell size variation, which can assist the determination of cell division events. The *#1* scenario shows that there was a single cell (black dot) in the previous time-point and there is a mother cell (purple dot) with developing bud (dark red dot) at the present time-point. The *#2* scenario illustrates a single mother cell with a daughter cell growing upward. The *#3* scenario indicates an empty trap. In this case, the previous yeast mother cell has been washed away. Scenario *#4* represents the transition 2 cells to 1 cell, indicating a completion of a cell division. Scenario *#5* shows that a daughter cell (dark red dot) is growing below a mother cell (purple dot). Scenario *#6* represents an overcrowded situation. Scenario *#7* is an example of a senescent mother cell whose cell division took a long period of time, probably because the daughter cell is extremely elongated. Scenario *#8* represents a single cell inside the trap close to the end of the experimental work and is likely a dead cell.

We would like to emphasize that other cellular characteristics, such as morphological aspect ratio, can be visualized in μPolar as well.

D. Counting RLS and interpretation of experimental results

μPolar has an additional option to visualize the replicative lifespan. This feature can be added to the plot if RLS data is available from the dataset. The RLS division point is represented as a red star at corresponding time points. Circular plots of dividing cells can visualize the cell division events in oscillating patterns. Fig.5 demonstrates the RLS comparison between given RLS information and counting RLS from μPolar plot division points without prior information. We previously collected the experimental RLS data for each trap from [13] and applied this information to the μPolar plot. The red stars in Fig.5 represent 21 cell divisions from experimental results. The black arrows inside the plot represent the cell division events estimated from μPolar plot analysis, counting 22 cell division events. The estimation based on the circular plot is nearly identical to the experimental result except at one time-point indicated by a question mark. The oscillating

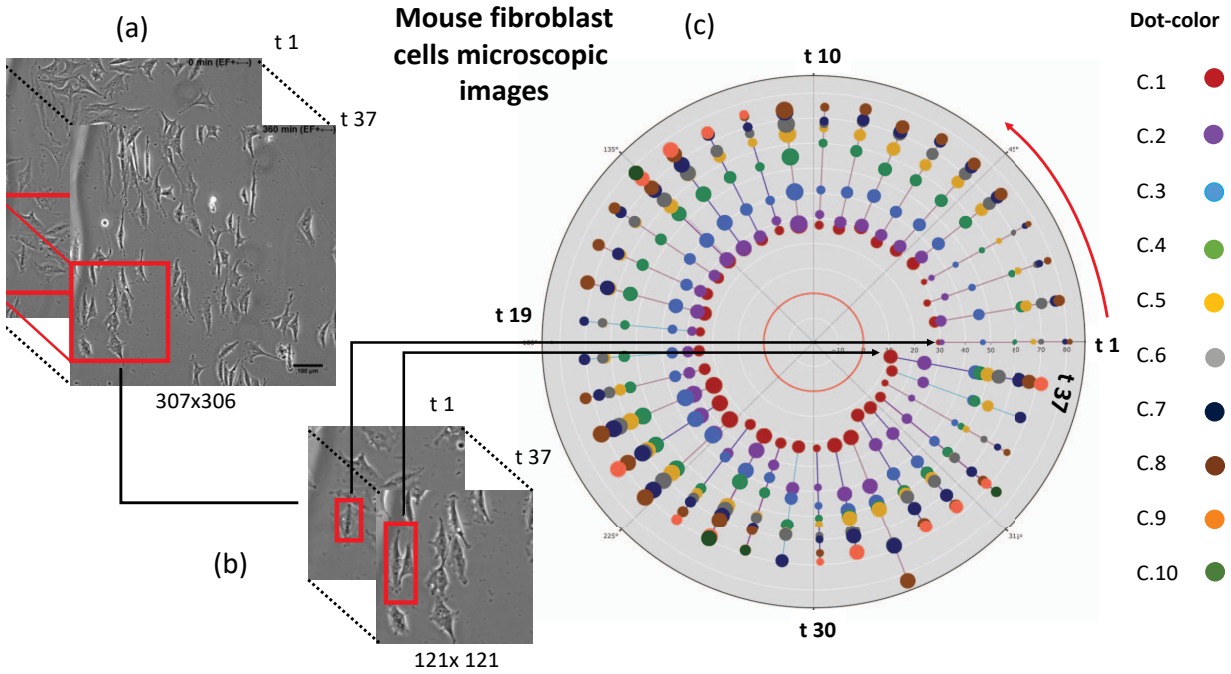

Fig. 3. **μPolar plot for migrating mouse fibroblast cells in time-lapse microscopic images**. Cell areas are represented by dots with variable diameters, and the same cell objects in each time point are marked by a unique color.

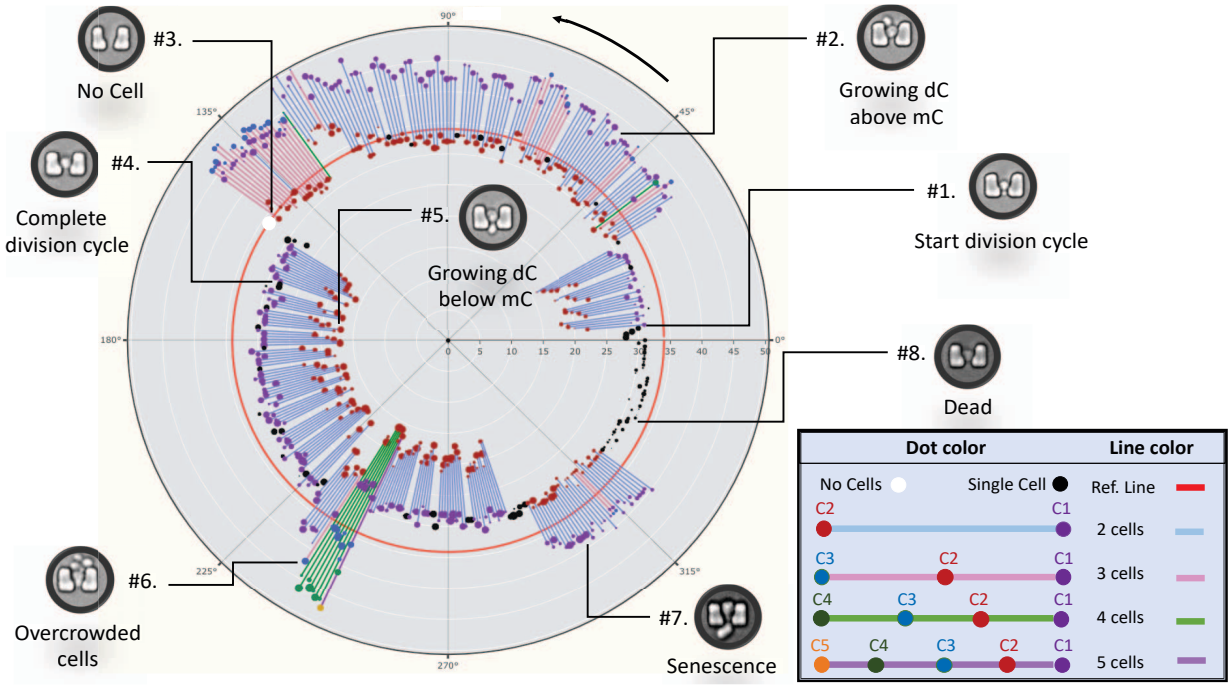

Fig. 4. **A μPolar plot with cell area representation and cell tracking for Trap No. 98**. The line color represents the number of cells at each time-point, and dot color represents a color tag for individual cell at each time-point, respectively.

patterns of the first 17 cell divisions are visually striking, representing that daughter cells gradually grow larger in size and then separate from the mother cells at regular intervals – a characteristic of a healthy yeast mother cell. Although there are some overcrowded time-points and large cells in the second half of the plot, this plot demonstrates the utility of circular plots in RLS estimation.

VI. Discussion

Overall, μPolar is a useful tool for visualizing cell migration, cell monitoring, and estimating cell division events from time-lapse microscopic images. The μPolar interpretation of cell division at each time-point can facilitate lifespan estimation in aging studies. The comparison between yeast cell time-lapse microfluidics images and mouse fibroblast cell time-lapse microscopic images demonstrates that μPolar can be a general tool for visualizing time-lapse images.

Visualizing cell division events can offer biological insights. For instance, in the microfluidic images, it can be seen that when yeast cells become older, interestingly, the cell division cycle becomes longer. It can also be seen that there is a relationship between cell size and cell division time-length. Similarly, visualizing and tracking mouse fibroblast cells show how cell sizes change during their migration.

Time-lapse microscopy is becoming an increasingly popular research tool to monitor cellular events in biomedical research. One such application is the microfluidics-based high-throughput analysis of dividing yeast cells. It is challenging to visualize and interpret the large volumes of data gathered through microfluidics-based microscopy. Here, we developed a circular plotting method, μPolar, to visualize cell movements and cellular division events at hundreds of time points. Our method is interactive and easy to use. We demonstrated the utility of our method to describe the events of dividing yeast cells and migrating mouse fibroblast cells. Our method could be applied to other types of microfluidic devices and time-lapse microscopic imaging experiments.

VII. Supplemental Information

Fig. S1: Time-lapse full microfluidic images with μPolar plots for Trap No. 2, 12, 22, 41, 50, 59, 73, 82, 98 and 100.

Fig. S2: Visualization of Trap No. 8 with a corresponding microfluidic image.

Fig. S3: Visualization of Trap No. 33 with a corresponding microfluidic image.

Fig. S4: Oscillating patterns of cell divisions are evident in the circular plot of Trap No. 63.

Fig. S5: Illustration on how reference points can be changed to emphasize different aspects of cellular events, with Trap No. 44 as an example.

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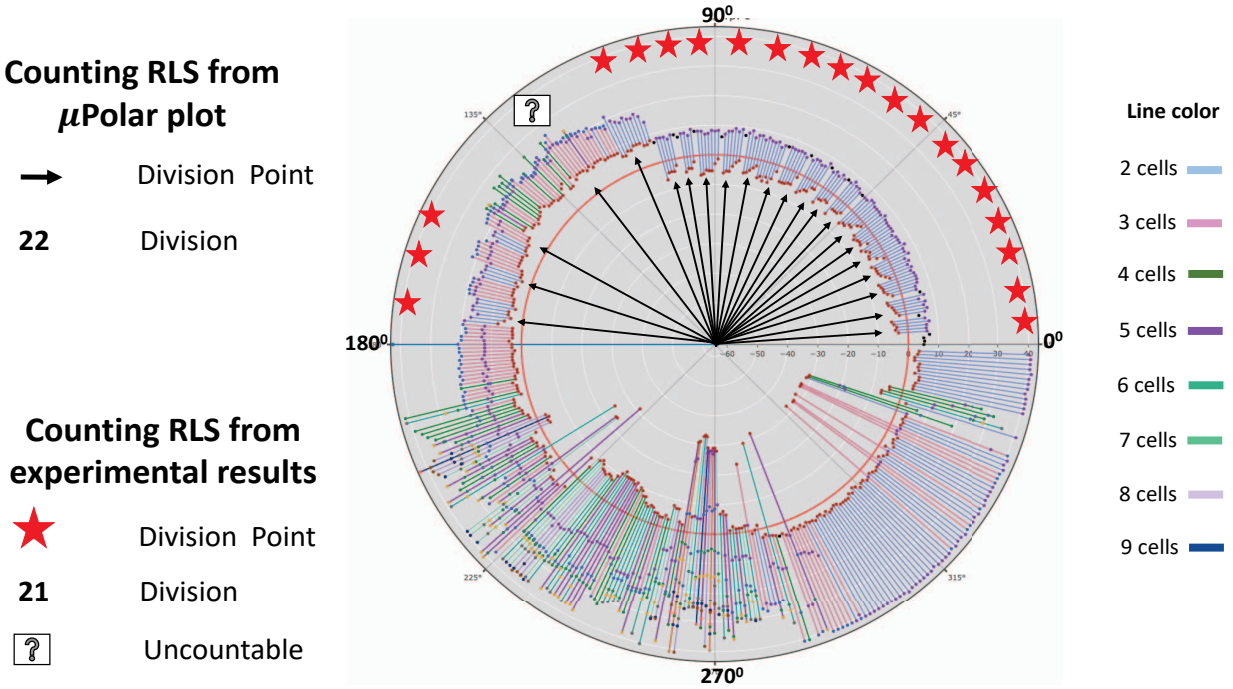

Fig. 5. **Identifying RLS measurements from μPolar plot for Trap No. 1**. The black arrows indicate the potential number of yeast cell division time-points from μPolar plot estimating 22 divisions. The red stars indicate the 21 cell division events identified from the experimental results.

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