



Universiteit
Leiden
The Netherlands

Image Analysis and Platform Development for Automated Phenotyping in Cytomics

Yan, K.

Citation

Yan, K. (2013, November 27). *Image Analysis and Platform Development for Automated Phenotyping in Cytomics*. Retrieved from <https://hdl.handle.net/1887/22550>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/22550>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/22550> holds various files of this Leiden University dissertation.

Author: Yan, Kuan

Title: Image analysis and platform development for automated phenotyping in cytomics

Issue Date: 2013-11-27

Chapter 5

A Study to the Dynamics of Matrix Adhesion

This chapter is based on the following publications

Yan, K., Le Dévédec, S., Van de Water, B., & Verbeek, F. J., "Automated Analysis of Matrix Adhesion Dynamics in Migrating Tumor Cells", (in preparation)

S. Le Dévédec, B. Geverts, H. de Bont, K. Yan, F. J., Verbeek, A. Houtsmuller, and B. van de Water, "The residence time of focal adhesion kinase (FAK) and paxillin at focal adhesions in renal epithelial cells is determined by adhesion size, strength and life cycle status," *Journal of cell science*, 2012.

Le Dévédec, S.E., Yan K., de Bont H., Ghotra V., Truong H., Danen E., Verbeek F.J., and vande Water B.(2010), "A Systems Microscopy Approach to Understand Cancer Cell Migration and Metastasis", *Cellular and Molecular in Life Science*, Vol. 67, Issue 19, pp.3219-3240

Chapter Summary

In this chapter, we focus on assessing the performance of our image analysis solutions using a case study of the dynamics of a subcellular structure known as the matrix adhesion. We further divide the focus into two research questions:

1. Can we extract morphology and motility measurements from matrix adhesion dynamics and cell migration?
2. Can we identify correlation between measurements of matrix adhesion dynamics and cell migration?

Matrix adhesions are the closest contacts between the cell and the extra-cellular matrix through which both mechanical forces and regulatory signals are transmitted. To that end, cell migration can be seen as a cyclic process controlled by the assembly and disassembly of matrix adhesions. Therefore, a study of the correlation between MA dynamics and cell migration will provide an understanding of the molecular mechanism controlling cell migration behavior. For now, the study focuses on first extracting measurements describing both matrix adhesion dynamics and cell migration. These measurements are further used to reveal correlations between the morphology or the motility of MA dynamics and cell migration.

To achieve the current research questions, we will first extract measurements describing MA dynamics and cell migration using image analysis solutions discussed in Chapter 2 and Chapter 3. Furthermore, with these measurements, we apply dependency tests to identify potential correlations between MA dynamics and cell migration. Following the path of analysis, this chapter is divided into two major sections. In the section 5.1, the design of image acquisition, image analysis and data analysis will be addressed. In the section 5.2, the results in biological context will be discussed. The result of analysis shows that our solution confirms a number of known correlations observed in previous studies and reveals several yet unknown biological phenomena.

5.1. Workflow of Matrix Adhesion dynamics Analysis

Cell migration is an essential procedure involved in a number of processes and is especially important in cancer metastasis. In the cascade of cancer metastasis, an increasing in cell motility is crucial for cancer cells to invade the surrounding tissue. Matrix adhesions (MA) are the closest contacts between the cell and the extra-cellular matrix. Cancer cell migration can be seen as a cyclic process which is controlled by the assembly and disassembly of matrix adhesions. Therefore, the dynamics of matrix adhesion is very important [131] for the understanding of cell migration behavior, yet little is known about the molecular mechanisms that regulate adhesion dynamics and signaling during cell migration. In order to gain understanding of the relationship between cell migration and the dynamics of matrix adhesions (MA), we have developed an integrated approach consisting of image acquisition, image analysis and data analysis at both cellular and structural level:

1. For the image acquisition, epifluorescence and total internal reflection fluorescence (TIRF) microscopy are employed to respectively visualize components including the **cell body**, the **cell nucleus** and **matrix adhesions** (TIRF). This results in multi-channel time-lapse image sequences.
2. From these image sequences, measurements of cell migration and matrix adhesion dynamics are extracted using dedicated image analysis pipeline. These measurements represent a spatio-temporal quantification of the matrix adhesion dynamics in the migrating cell. Furthermore, a spatial model of the cell under migration is built, dividing the migrating cell into a number of characteristic regions that are subsequently used in the analysis. This cell body model is the key in our integrated approach to find correlation between matrix adhesion dynamics and cell deformation.
3. From the measurements, dependency tests are applied to find significant correlations between matrix adhesion dynamics and cell deformation.

Each step will be further illustrated in: experiment preparation and image acquisition (cf. § 5.1.1), (2) image analysis (cf. § 5.1.2), and (3) data analysis (cf. § 5.1.3).

5.1.1. Experiment Preparation and Image Acquisition

Material Preparation

The H1299 cell model, which ectopically expresses the GFP-paxillin matrix adhesion marker, is the well described lung carcinoma cell-line extensively used in cell migration assays [132]. H1299 cells (ATCC-CRL-5803) were cultured in RPMI (GIBCO, Life Technologies, Carlsbad, CA, USA) supplemented with 10% FBS (PAA, Pasching, Austria) and 100 International Units/ml penicillin and 100 µg/ml streptomycin (Invitrogen, Carlsbad, CA, USA). CELLview glass bottom dishes with four compartments were coated with 10 µg/µl fibronectin (Sigma Aldrich) for 1 hr at 37°C. H1299/GFP-paxillin cells were seeded on glass bottom dishes and grown at 37°C overnight. For random cell migration assays, phenol-red (pH indicator) free culture medium was used. Cells were maintained in a 5% CO₂ humidified chamber at 37°C. Following this protocol, the cells are believed to be incubated with a minimum level of interference.

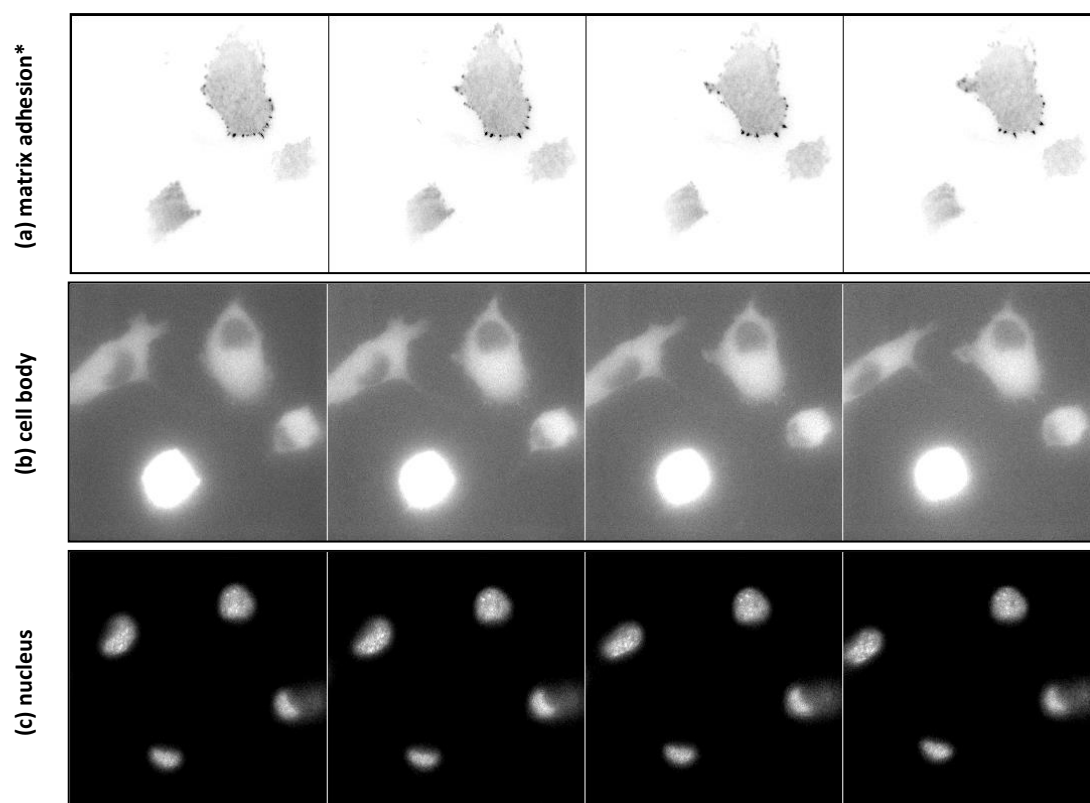


Figure 5-1 multi-channel imaging for MA dynamics analysis, image montage at time [0 min, 16 min, 24 min, 32 min]; *signal reversed for visibility

Image Acquisition

In order to quantify both cell migration and MA dynamics, we captured time-lapse image sequences for each component using different imaging techniques. Prior to imaging, nuclei were labelled with 100ng/ml Hoechst 33342 [133] in culture medium for 45 min. H1299/GFP-paxillin cells were imaged using a Nikon TiE2000 microscope equipped with a Perfect Focus System with 5% CO₂ delivery to the sample dish. Images were acquired with a 60x oil objective (1.49 NA, 0.12 WD) and the image acquisition was controlled by NIS Elements (Nikon). The imaging setting is defined as follows:

1. Matrix adhesions were captured using TIRF imaging with a 488nm laser line over a period of two hours, in one minute intervals. The TIRF imaging was used to detect the signal of GFP-paxillin that is localized in the first 80 nm of the cells where the MAs are found (cf. Figure 5-1a). The TIRF imaging is in particular useful for visualizing objects with a fast turnover and small size [134]. Additionally, it provides a good signal-to-noise ratio for capturing dynamic matrix adhesions [135].
2. The total signal of the cytoplasmic GFP, which represents the whole cell body information, is detected using wide field microscopy (cf. Figure 5-1b). The cytoplasmic GFP signal was acquired every three minutes over a period of two hours using wide-field fluorescence. (3)
3. At the same time interval (3-mins) and duration of 2-hrs, the Hoechst [133] signal for detection of the nucleus (cf. Figure 5-1c) was acquired using wide-field fluorescence.

Image Format

The raw images are stored in the ND2 format; a commercial self-contained image data storage format proposed by NIKON. Due to its patented structure, ND2 format is not an open-source storage format and can only be accessed via the Windows-based native API provided by NIKON, consequently the ND2 format is not convenient for cross-platform data transportation. Therefore, the ND2 format is converted into TIFF image format. The conversion is conducted as follows: for each time point in a time-lapse image sequence, an image from the separated channels is stored as a 16-bit TIFF file (cf. Figure 5-2). As a result, the ND2 file is converted into a collection (time-lapse image sequences) of individual 16-bit TIFF files.

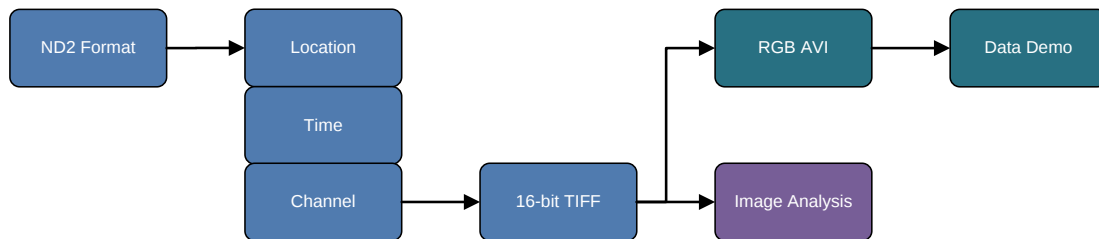


Figure 5-2 2D+T ND2 to TIFF conversion

The converted TIFF files are organized and stored in a structure illustrated in Figure 5-2. The total length of image sequence is 120 ~ 240 images depending on the size of observation window. For data visualization, a copy of the 16-bit TIFF image is combined into multi-channel RGB AVI format by rescaling the 16-bit TIFF image into 8-bit. The RGB AVI format conversion is accomplished with ImagePro macro [136].

In conclusion, by using two different fluorophores (GFP and Hoechst) and two different imaging techniques (TIRF and wide-field fluorescence), we are able to capture three different sets of information: the cell nucleus (wide-field/Hoechst), cell body (wide-field/GFP) and matrix adhesions (TIRF/GFP) respectively (cf. Figure 5-1).

Different from the cell migration study in Chapter 4, the current imaging setting is not suitable for a high-throughput setting due to its time resolution. Image analysis is however still required to extract measurements of MA dynamics and cell migration. In the next section, we will discuss the image analysis pipeline.

5.1.2. Image Analysis

The image analysis pipeline answers to the first research question, namely to extract measurements of MA dynamics and cell migration. It is the essential step to convert images into measurements by using image segmentation and object tracking. In correspondence with the imaging setting of MA dynamics, the image analysis solution consists of three parts (cf. Figure 5-3). The selection of image segmentation and object tracking solution is based on the characteristic qualities of each of the channels.

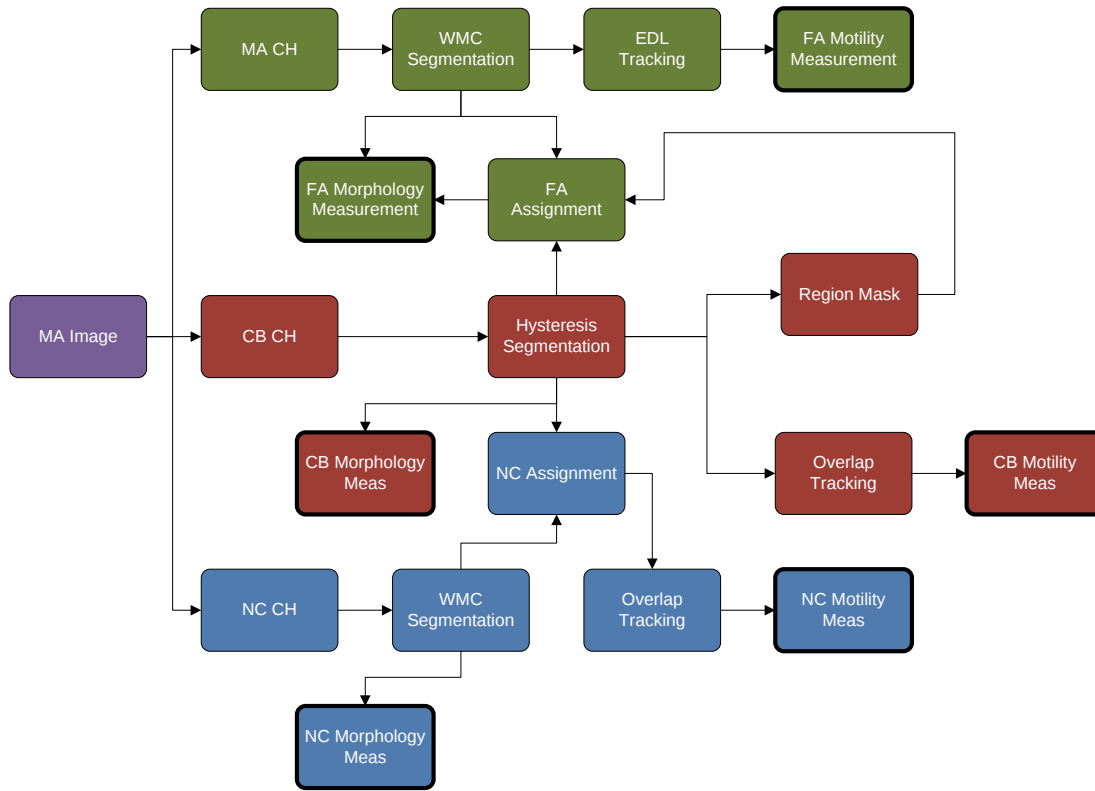


Figure 5-3 workflow of MA image analysis

5.1.2.1. Image Analysis for Matrix Adhesion (MA) Channel

The image analysis of the matrix adhesion channel is illustrated in Figure 5-3 [green]. With TRIF microscopy, the GFP signal from the cytoplasm and the MAs are both captured. As a result, the MA channel (cf. Figure 5-4a) contain two layers of signals including the brighter MA signal and comparatively darker cytoplasmic signal. In order to extract only the MA, the cytoplasmic signals must be removed from the image. By treating the cytoplasmic signal as a part of the background, we extract the major MA signal using a combination of Gaussian blurring filter and rolling ball background subtraction algorithm [137]. Empirically we established both the σ of Gaussian filter and the kernel size r_r of the rolling ball filter. The choice of $\sigma = 1$ is just larger than the average radius of MAs. The choice of $r_r = 2$ is twice the average radius of MAs. By applying both filters to the MA channel, we can suppress the cytoplasmic signal [35] while preserving the brighter MA signal (cf. Figure 5-4b) below the radius of the rolling ball. The resulting image only contains the dominant MA signal; the WMC segmentation algorithm will be used to create binary masks for the MAs (cf. Figure 5-4c).

The motivation for the WMC segmentation algorithm is similar to the motivation given in Ch. 4. The MA image (cf. Figure 5-4b) contains a large intensity variation possibly due to the Z-position of each MA. Moreover, the assembly and disassembly procedure of MAs will lead into a temporal change in intensity values of the same MA. To that end, the WMC segmentation algorithm is a good choice since it is designed to adapt the threshold based on local intensity information. Each object in the binary mask is labeled and tracked using the EDL tracking algorithm (cf. Figure 5-4d). As described in Chapter 3, the EDL tracking algorithm is designed for this particular kind of study.

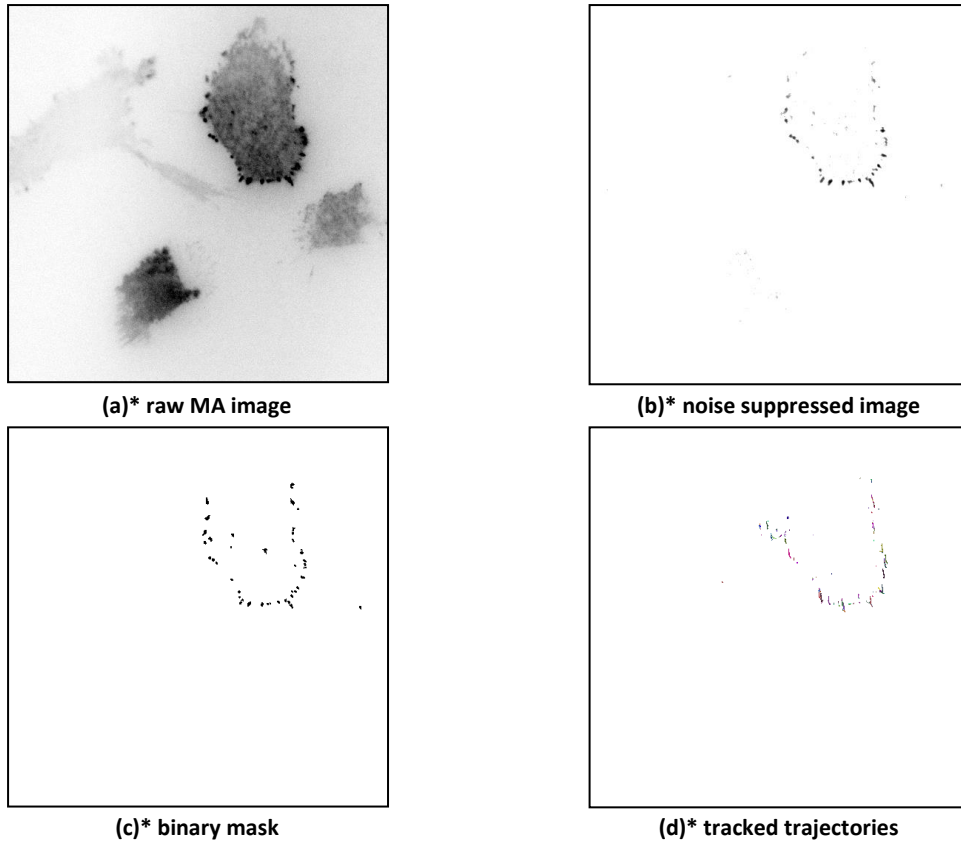


Figure 5-4 raw MA image and intermediate results of MA channel analysis (*) signal reversed for visibility

5.1.2.2. Image Analysis for Cell Body Channel

The workflow for the analysis of the cell body (CB) channel is illustrated in Figure 5-3. At the given magnification level, the cell body channel alone contains complex textures and multiple maxima that are sensitive to overcutting when segmented with WMC. To overcome potential overcutting, the cell body channel (cf. Figure 5-5a) is combined with the NC channel (cf. Figure 5-5b) to introduce a more precise definition of the maxima for object separation. Since each cell body can only have one nucleus, the NC channel is a perfect seed channel for segmenting the cell body channel using WMC. The combined image is applied with a Gaussian blurring filter ($\sigma = 3$) which is just larger than the average diameter of the MA, thereby suppressing the potential local maxima from the MA signal. Finally, the blurred image is segmented using WMC algorithm (cf. Figure 5-5c). Subsequently, each object is labeled and tracked using the overlap tracking algorithm (cf. Figure 5-5d). Objects touching the image border are discarded since they only give partial information.

In order to analyze the MA dynamics with respect to cell behavior, we have modeled the cell in regions. From the binary mask of the cell body channel, six functional regions are defined within each mask. These regions are hierarchically related and the mixture of these relationships is illustrated in Figure 5-6. The definitions for each of the functional region is described in the literature [138][112]. The functional regions are derived from major episodes in the cell body deformation during cell migration.

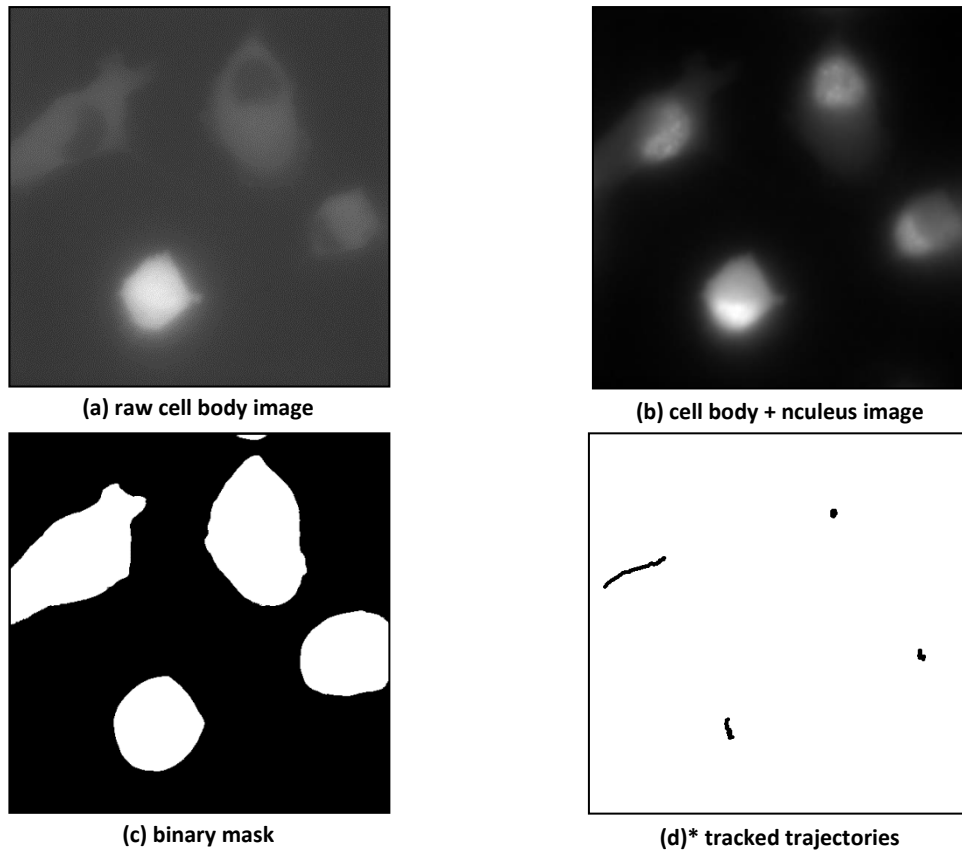


Figure 5-5 raw cell body image and intermediate results of cell body channel analysis (*) signal reversed for visibility

The MAs are assigned to each functional region so that, in each episode of cell deformation during migration, the study of MA dynamics can be assessed. The control mechanism behind MA-cell body correlation is often interconnected; therefore the study of difference between MA-cell body correlation models for the different functional regions may reveal new insights. Next, we will explain the regions:

1. **Peripheral Region (P)**: the cell membrane region at the border
2. **Central Region (C)**: the inner cytoplasm region around nucleus
3. **Protrusion Region (PR)**: lamellipodium protrusion formation
4. **Retraction Region (RE)**: cell body retraction
5. **Front Region (F)**: the whole leading edge of cell body during migration
6. **Back Region (B)**: the rear edge of cell body during migration

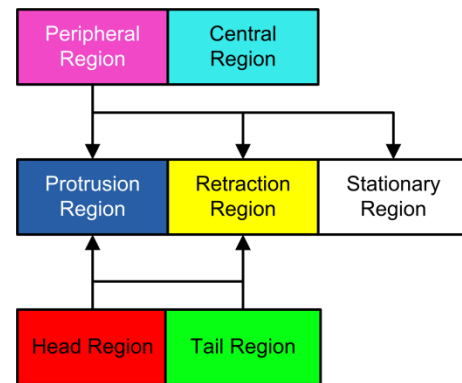
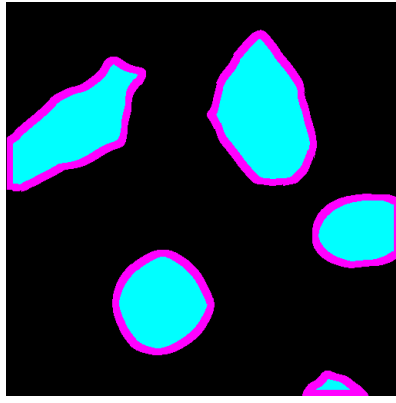
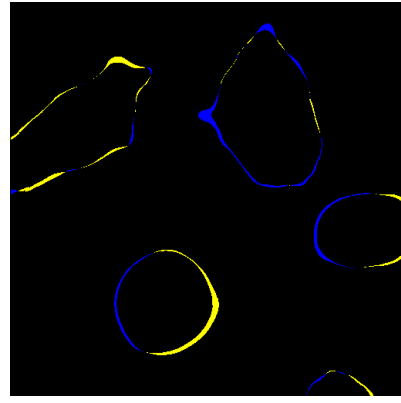


Figure 5-6 relation between each region



(a) central [cyan] & peripheral [magenta]



(b) protrusion [blue], retraction [yellow]

Figure 5-7 functional regions derived from cell body masks

Peripheral and Central

The **peripheral region (P)** and **central region (C)** (cf. Figure 5-7a) are two geometrical compartments in the cell body defined as follows:

1. The **peripheral region** is the outer ring (near membrane) of the cell body mask
2. The **central region** is the remaining area (inner part) of the cell body mask.

Hereby, the **peripheral region** and **central region** are correspondingly defined as the outer ring and intra region of the cell body (cf. Equation 5-1) based on a user-defined width of that ring. In Figure 5-7a, a peripheral region [magenta] of a width of 20 pixels is illustrated; the width corresponds with image resolution and is derived from empirical observation. The peripheral region (P) and the central region (C) are mutually exclusive, this can be written as:

$$(P \cup C = \text{cell body}) \wedge (P = \overline{C}) \quad \text{Equation 5-1}$$

The definition of the peripheral and central region allows the study of MA dynamics near the cell border. From the literature [138][139], it has been reported that a rapid MA formation at the peripheral region is strongly associated with both cell motility speed and signaling.

Protrusion and Retraction

The **protrusion region (PR)** and the **retraction region (RE)** (cf. Figure 5-7b) are two regions derived from both geometrical and temporal information of the cell body. They are defined as the shape variation between the cell body masks from two consecutive time points as follows:

$$PR = b_{i+1} \cap \overline{b_i} \quad \text{Equation 5-2}$$

$$RE = b_i \cap \overline{b_{i+1}} \quad \text{Equation 5-3}$$

, where b_i and b_{i+1} is the peripheral region of a cell body in i^{th} and $(i + 1)^{th}$ frame. It is reported [138][15] that MA dynamics in the protrusion and retraction region, in terms of lifetime and turnover, is strongly associated with cell motility and migration polarity.

Front and Back

The **front region (F)** and **back region (B)** (cf. Figure 5-8c) are high-level perceptualizations of cell body regions. They represent the leading area and rear area of a cell migration. From recent studies [138][15][140], one can deduce the opinion that cell migration can be described by a combination of adhesion formation and cytoskeleton formation at both leading area and rear area. Empirical observations [138][140] suggest that lamellipodia protrusions are first

formed by expanding the cell body structure at the leading area. Cell body adhesions, i.e. matrix adhesions, are assembled in the protrusions to push the extracellular matrix to attach to the substrate surface. Meanwhile, at the rear area adhesions are gradually disassembled and release extracellular matrix from substrate surface. Some theories [140] also pointed out that the disassembly of cell body adhesions also provides pushing forces during migration.

To study how the matrix adhesion dynamics in these two areas are connected to cell migration, we define the **front region** as the cell body region aligned with the **leading edge direction** while the back region as the cell body region is aligned with the **rear edge direction**. The leading edge direction (cf. Figure 5-7b) is defined as the direction of the joint force, assuming each protrusion region as pulling force [138][140] and each retraction region as pushing force [138]. Similarly, the rear edge direction (cf. Figure 5-7b) is defined as the opposite direction of the joint force, assuming each retraction region as pushing force. Given that:

1. The pixels $P_{p(i)}(t)$ belong to the protrusion region
2. The pixels $P_{r(j)}(t)$ belong to the retraction region
3. The $P_x(t)$ and $P_y(t)$ represents the x-y coordinate of the pixel $P(t)$ at time point t
4. The $NC_x(t)$ and $NC_y(t)$ denote the center of mass of nucleus at time point t
5. The α is the direction of nucleus positional shift

Then, the definition of the leading edge direction γ_F and the rear edge direction γ_R can be derived as follows:

$$\gamma_F = \text{atan} \left(\frac{\sum_{t=1}^n \omega(t) \cdot [NC_y(t) - P_{p(i)y}(t)]}{\sum_{t=1}^n \omega(t) \cdot [NC_x(t) - P_{p(i)x}(t)]} \right) \quad \text{Equation 5-4}$$

$$, \text{ where } \omega(t) = \left| \frac{\beta(P_{p(i)}(t)) - \alpha}{\pi} \right| \quad \text{Equation 5-5}$$

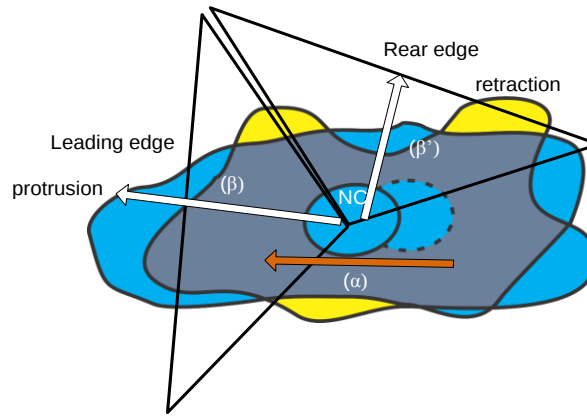
$$, \text{ where } \beta(P_{p(i)}(t)) = \text{atan} \left(\frac{NC_y(t) - P_{p(i)y}(t)}{NC_x(t) - P_{p(i)x}(t)} \right) \quad \text{Equation 5-6}$$

$$\gamma_R = \text{atan} \left(\frac{\sum_{t=1}^n \omega'(t) \cdot [NC_y(t) - P_{r(j)y}(t)]}{\sum_{t=1}^n \omega'(t) \cdot [NC_x(t) - P_{r(j)x}(t)]} \right) \quad \text{Equation 5-7}$$

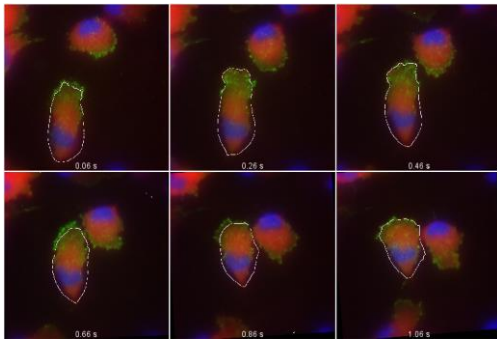
$$, \text{ where } \omega'(t) = \left| \frac{\beta'(P_{r(j)}(t)) - \alpha}{\pi} \right| \quad \text{Equation 5-8}$$

$$, \text{ where } \beta'(P_{r(j)}(t)) = \text{atan} \left(\frac{NC_y(t) - P_{r(j)y}(t)}{NC_x(t) - P_{r(j)x}(t)} \right) \quad \text{Equation 5-9}$$

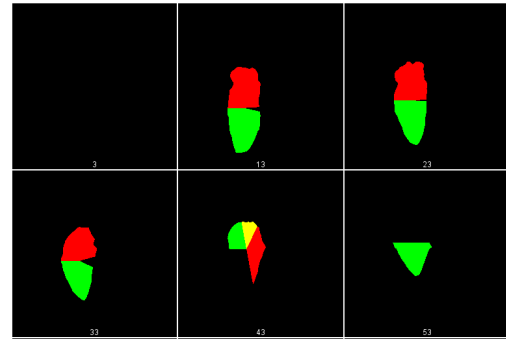
The weight factors $\omega(t)$ and $\omega'(t)$ are introduced under the condition that the direction β and β' will contribute more to the pulling/pushing force, if the β and the β' are aligned with the direction of nucleus position shift. By this notion, we intend to damp the pulling/pushing forces caused by small protrusions or retractions; as these are believed not to contribute to the migration [138].



(a) principle of front and back region recognition



(b) the selected cell



(c) front region [red] and back region [green]

Figure 5-8 front and back region recognition

Each MA is located in either of the functional regions; in this manner the MA obtains a region label from the region model. A MA can be labeled with multiple regions, for example, a MA can be in protrusion region and at the same time in head region. Some of the regions are, however, exclusive. For example, a MA cannot be simultaneously assigned to the peripheral and the central region since the definition of peripheral and central region is mutually exclusive. Using definitions of the functional regions as grouping criteria, the per-region analysis of MAs allows MA dynamics to be linked directly to local cell deformation or migration.

5.1.2.3. Image Analysis for Nucleus Channel

The analysis of the nucleus (NC) channel (cf. Figure 5-9a) is illustrated in Figure 5-3. A Gaussian blurring filter is first applied to the image to smooth the intensities and remove noise [28]. Here we choose a $\sigma = 4$ for the Gaussian filter that is sufficient to suppress the Poisson noise (cf. Figure 5-9a) [28] and create a more smooth intensity landscape within the nucleus (cf. Figure 5-9b). The blurred image (cf. Figure 5-9b) is segmented with WMC segmentation algorithm. The binary mask (cf. Figure 5-9c) is labeled and subsequently tracked using the overlap tracking algorithm (cf. Figure 5-9d).

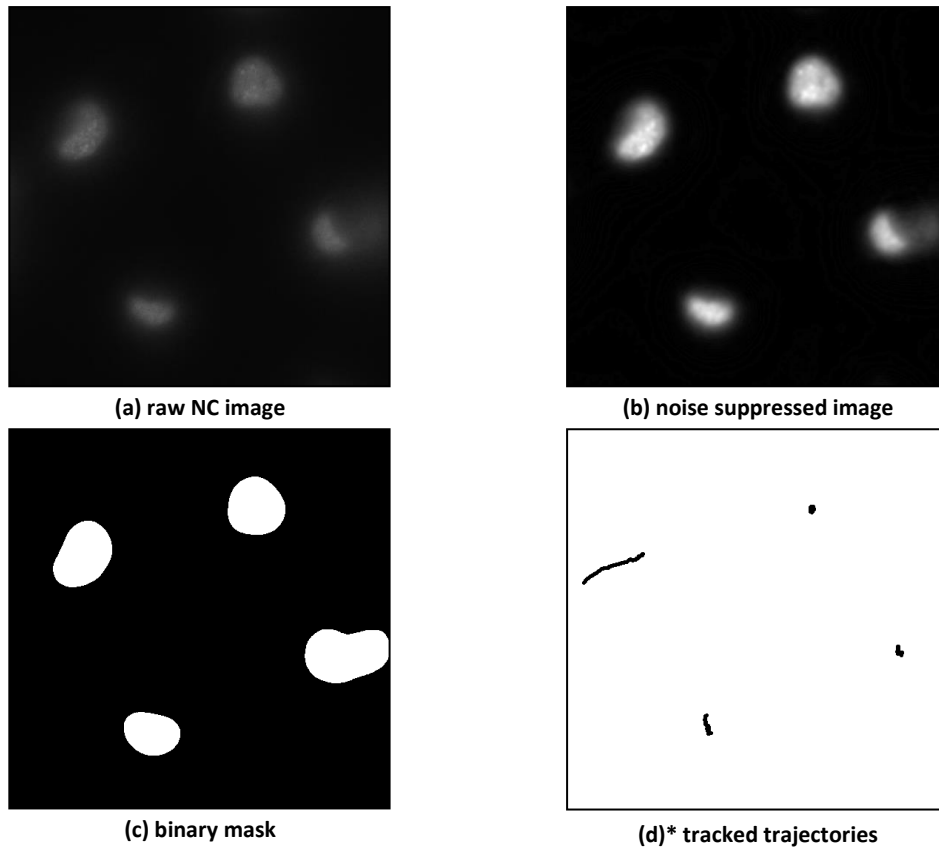


Figure 5-9 raw NC image and intermediate results of NC channel analysis (*) signal reversed for visibility

5.1.2.4. Phenotypical Quantification

With image analysis (cf. Figure 5-3) measurements describing both MA dynamics and cell migration are extracted. Apart from the morphology and motility measurements [9], several correlation measurements are also introduced to describe morphological or motile association between cell body deformation and MA dynamics (cf. Figure 5-10).

In order to extract the correlation measurements, objects from different channels are first related according to parent-child relationships. MAs are assigned to cell bodies as children based on the minimum distance between contour of cell body and MA. Nuclei are assigned in a child relation to the cell bodies based on the overlapping ratio between NC and cell body. From these two parent-child relationships, the following measurements are defined (cf. Table 5-1):

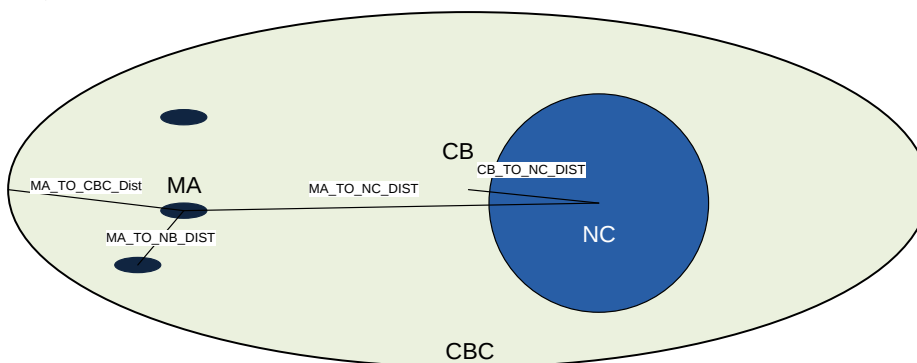


Figure 5-10 visualization of correlation measurements described in the Table 5-1 (MA: Matrix Adhesion; CB: Cell Body; NC: Nucleus; CBC: Cell Body Contour)

Table 5-1 definition of correlation measurements in current study

Correlation measurements	
Table Name	Description
MA_TO_NC_DIST	The Euclidian distance between the center of mass of a MA to its NC, $MA \& NC \in CB$
MA_TO_CB_DIST	The minimum Euclidian distance from a MA to the nearest pixel of cell body contour according to MA label
CB_TO_CB_DIST	The minimum shifting distance between two consecutive cell body contours
CB_TO_NC_DIST	The shortest Euclidian distance between the center of mass of one NC to the cell body which it belongs
MA_TO_NB_DIST	The Euclidian distance between a MA to its nearest MA in the same cell body

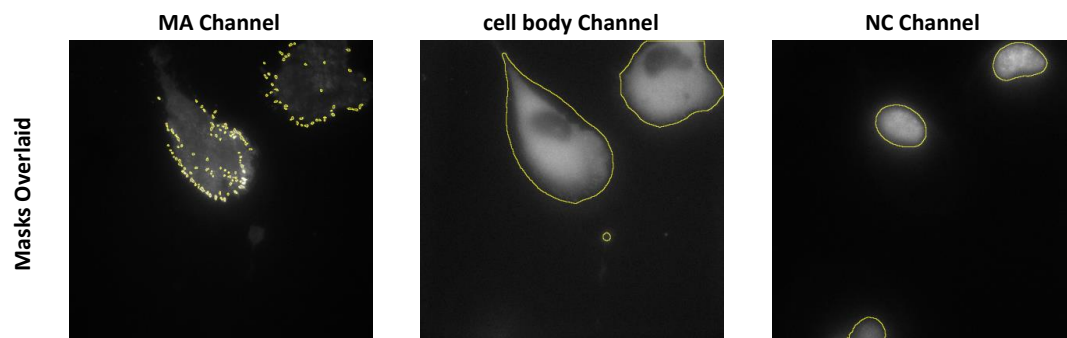
5.1.3. Data Analysis

With measurements extracted from image analysis, we address the second research question, namely to identify correlated measurements between MA dynamics and cell body. To reveal correlation knowledge from in the data, an unsupervised correlation analysis is further verified based on expert observations and literature [36][138][141][112][111]. In the current implementation, the correlation analysis employs a statistical approach namely the Pearson cross-correlation [142] for potential linear correlation. The Pearson product-moment correlation, or simply Pearson correlation, is widely used as a measurement of the strength of linear dependence between two variables [124][143]. Here we calculate the correlation between measurements of MA and cell body in a pairwise fashion. By extracting significantly correlated measurements between cell bodies and MAs in each functional region, we hope explain numerical causality between MA dynamics and cell migration. Moreover, we hope to identify different patterns on how the MA dynamics in different functional regions is correlated to cell behaviors.

User Verification

The user verification step of MA dynamics is performed in a similar fashion as the cell migration study (cf. Ch. 4). With overlay information (cf. Figure 5-11), researchers are asked to assess the following aspects of the general performance of image analysis for each channel:

1. Image segmentation
2. Object separation
3. Object tracking



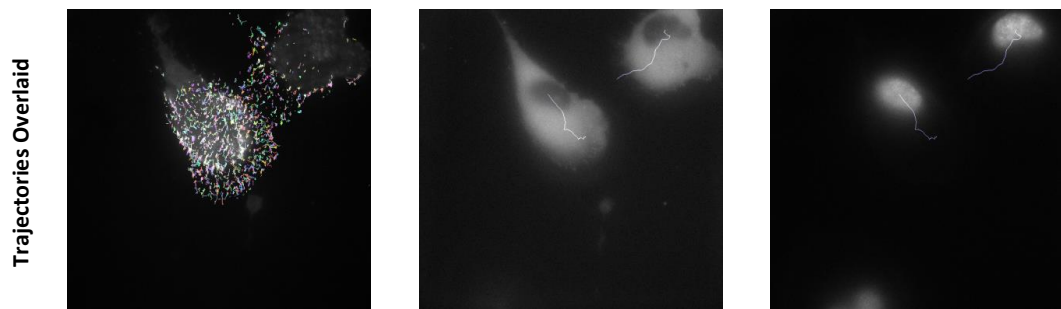


Figure 5-11 image with overlaid information for manual verification

5.2. Phenotypical Correlation Study of the Live Cell

Cell migration is a well-orchestrated event that consists of several phenotypical stages believed to be controlled by the assembly and disassembly of matrix adhesions. The study of how MAs controls cell phenotypes is important for the understanding of molecular control mechanisms behind cell migration. In this case study, we attempt to comprehend such correlation by introducing a high-content analysis of a cell model consisting of different functional regions. In section 5.1, we have demonstrated the possibility to quantitatively identify and verify potential correlations between the dynamics of MAs and cell migration. In this section, we will illustrate several correlations that are revealed and verified by our analysis.

Matrix Adhesion Lifetime and Cell Migration Velocity

A total collection of 29 time-lapse image sequences are captured using image acquisition settings described in §.5.1.1. All cells touching the image border or that are only partially present are discarded since they do not provide complete information on cell behavior and cannot be used to extract correct measurements of cell velocity or shape deformation. In this manner, there are 43 valid cells remaining for further analysis. Using the unsupervised K-means clustering algorithm, these 43 cells are divided into a low-motile (cf. Figure 5-12) and a high-motile class (cf. Figure 5-13) based on their migration velocity. With the two motility groups, we intend to extract major differences between MA dynamics. These major differences in MA dynamics are potentially candidates for the correlation modeling procedure since they are most likely to be associated with control mechanism of cell migration.

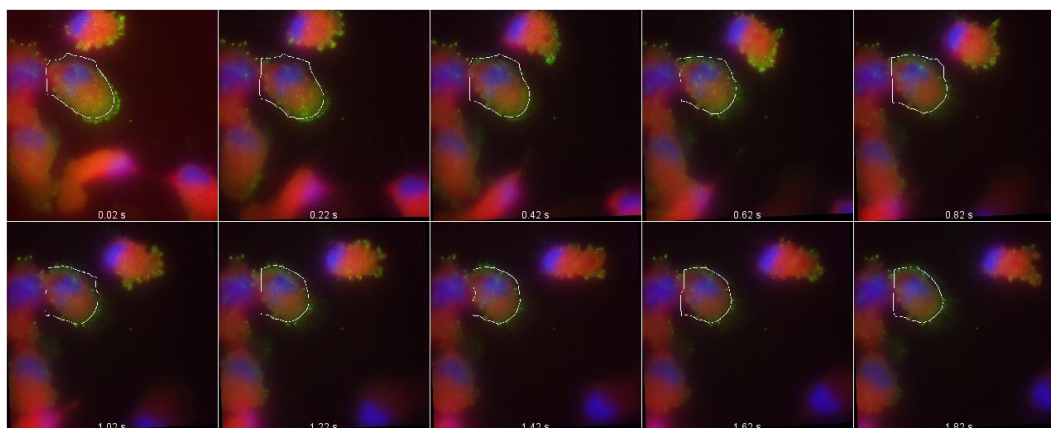


Figure 5-12 montage of low-motile cell

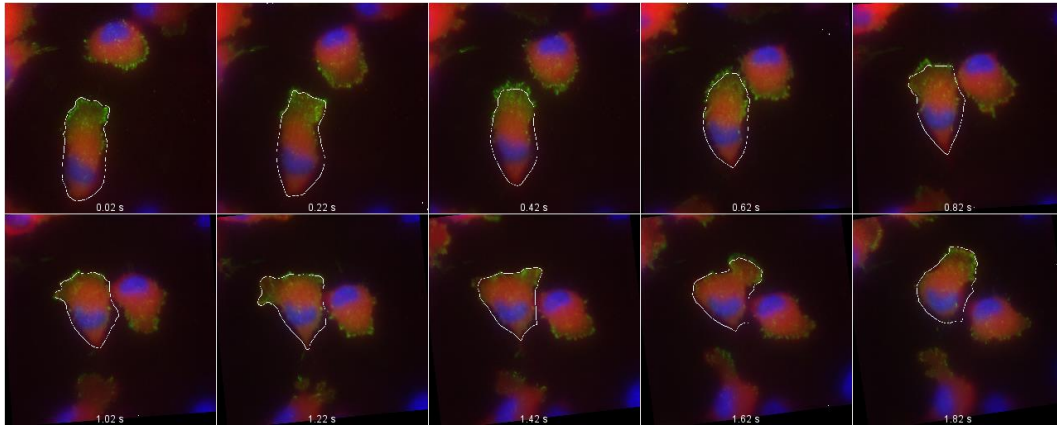


Figure 5-13 montage of high-motile cell

For cells in each velocity group, their MAs, in total 896, are further divided into eight subpopulations based on their labels from cell body functional regions:

1. MA Protrusion (MA PR): MAs located in the protrusion region of the cell body
2. MA Retraction (MA RE): MAs located in the retracting region of the cell body
3. MA Peripheral (MA P): MAs located in the peripheral region of the cell body
4. MA Central (MA C): MAs located in the central region of the cell body
5. MA Front: MAs located at the front of the migrating cell
6. MA Back: MAs located in the back of the migrating cell
7. MA Front PR: MAs located in protruding regions at the front of the migrating cell
8. MA Back RE: MAs located in retracting regions in the back of the migrating cell

MAs in each local cell region are separately analyzed. The Kolmogorov–Smirnov (K-S) test [124][144] is used for comparing measurements from each region since none of the MA or cell body measurements fits a normal distribution (based on Lilliefors normality test). The result of the K-S test with 95% confidence interval (cf. Figure 5-14) shows that:

1. In general, MAs in high-motile cells display a shorter lifetime compared to MAs in low-motile cells, suggesting that MA lifetime is correlated to cell velocity.
2. The difference between MA lifetime in peripheral and central region is larger in low-motile cells compared to the high-motile cell, suggesting that a shorter lifetime of peripheral MAs is necessary for a higher cell motile.
3. Surprisingly, the lifetime of central MAs is the lowest and does not differ between low-motile and high-motile cells.
4. In high-motile cells, the MA lifetime is always longer in the retracting region than in the protruding region.
5. The difference in MA lifetime between protrusion and retraction regions is absent in low-motile cells which may be an explanation for lower migration polarity.

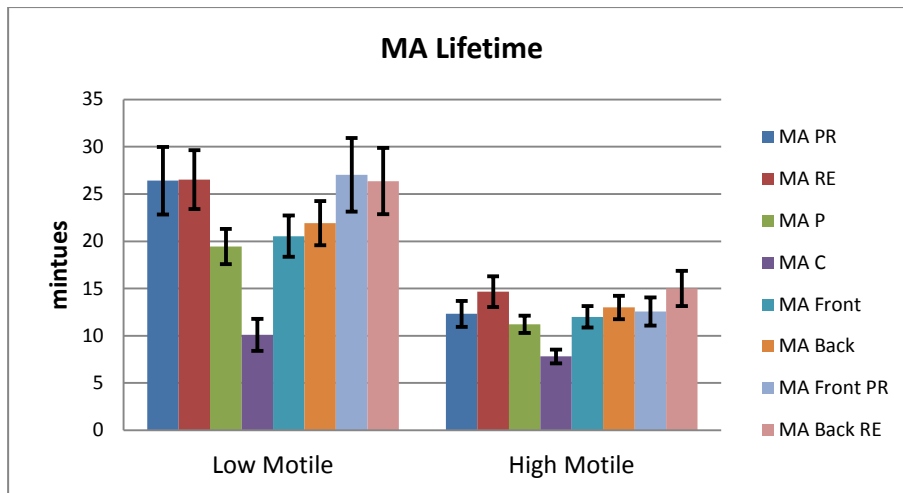


Figure 5-14 MA lifetime variability given the difference in cell velocities

Unsupervised Correlation Discovery

In order to investigate all potential correlations, we implemented an automated solution (cf. §5.1.3) using Pearson correlation analysis [124][143]. The Pearson correlation analysis is a popular measurement of linear dependency between random data. Moreover, in order to test whether correlation is statistically significant, the Pearson correlation analysis transforms the problem into a one-sample test with a bivariate distribution. In other words, it is tested whether the sampled data belongs to a hypothetical population with the same mean and standard deviation of the sampled data. Heatmap visualizations of the p -values of the correlation test between measurements of MAs in the Front-PR and cells are depicted in Figure 5-15 and Figure 5-16. Each row represents a phenotypical measurement of cell migration and each column represents a phenotypical measurement of MA dynamics. The heatmap visualization provides a fast overview of all potential correlations.

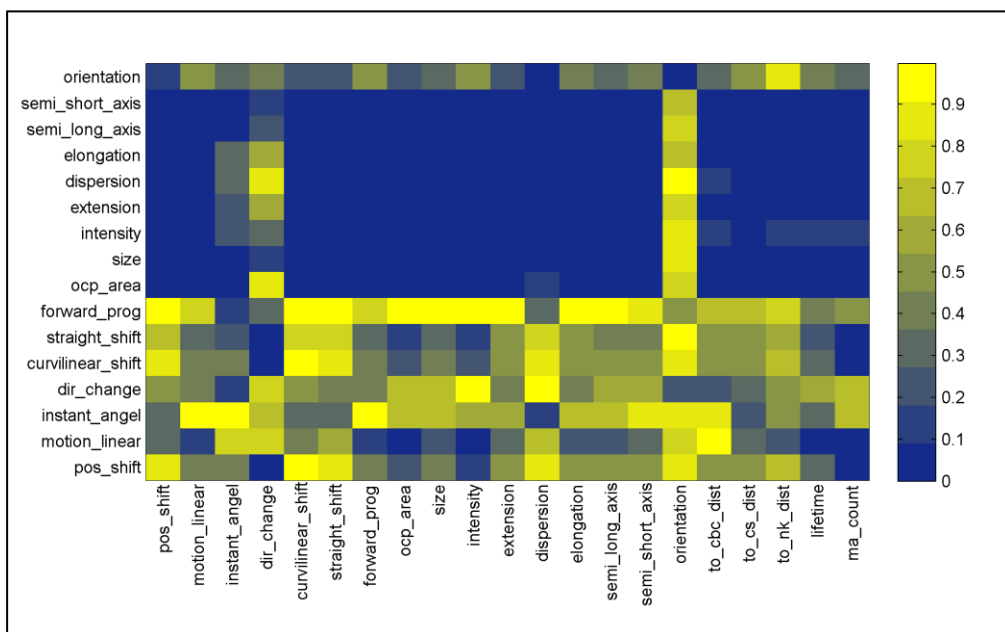


Figure 5-15 the heatmap of the p -value of correlation test between all cell (column) and MA Front PR (row) phenotypical measurements

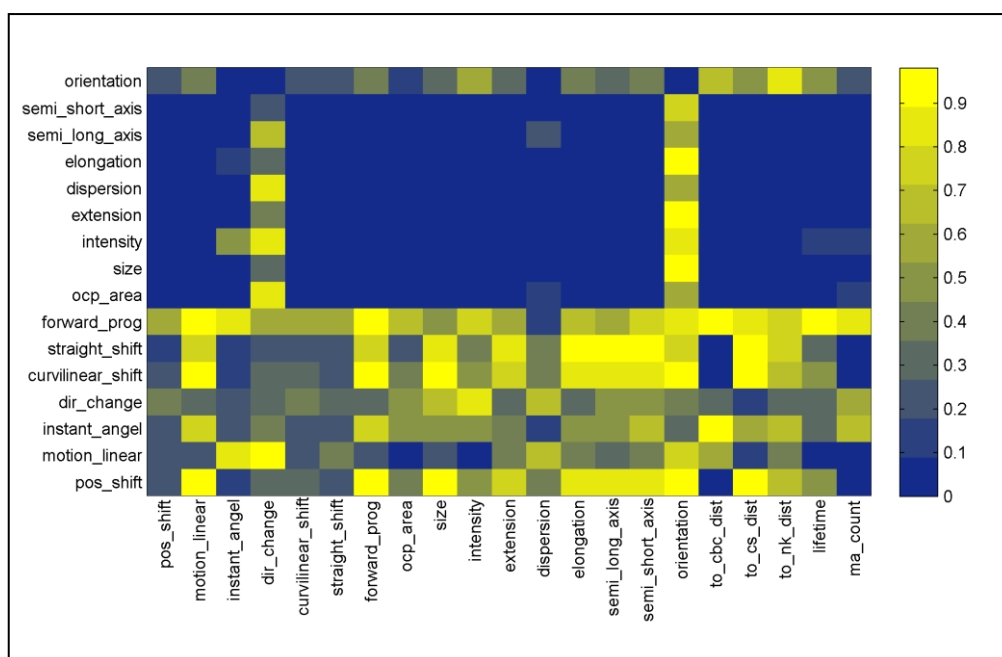


Figure 5-16 the heatmap of the p-value of correlation test between all cell (column) and MA Back RE (row) phenotypical measurements

By exploring the heatmaps of Figure 5-15 and Figure 5-16, it is clear that cell shape changes such as elongation or protrusion formation are highly associated with nearly all MA phenotypical changes in these two regions. However, cell motility patterns such as velocity or polarity are have a strong correlation with only a few MA measurements such as MA count in Front PR or MA to cell body distance in Back RE.

5.3. Conclusion and Discussion

In this case study, we have developed a set of image and data analysis solutions for the quantification of matrix adhesion dynamics and cell migration. First, the image analysis converts high-content image data captured (cf. § 5.1.1) into characteristic measurements for matrix adhesion dynamics and cell migration. From the measurements, we further introduce an automated data analysis solution to reveal correlations between the morphology and the motility of matrix adhesion dynamics and cell phenotypes. At the current stage, the combined solution can well provide the possibility to increase further understanding on the regulation of MAs and how this affects cell migration. Moreover, it paves the way to a numerical expression of the control mechanism behind cell migration.

There are several interesting issues that can be addressed in future studies:

From image analysis perspective: (1) when cells are clustering with each other, the border region is becoming less identifiable; thus results in a more complex cell separation. (2) The MA has a very small size (5~10um) and short life time (3~15 frames), which does not always provide sufficient information to train a motion model for object tracking. (3) Longer exposure to laser may cause cell apoptosis while a reduction of temporal-resolution image most certainly results in loss information on morphology changes. Thus, it leads into bias in measurements that cannot be easily detected in data analysis.

From data perspective: (1) Pairwise linear or monotonic correlation can be easily detected. However, it has not yet been elaborated on how to extract more complex correlation model from the measurements. (2) Since current measurements are derived from empirical observations, some biological phenomena such as the length of a protrusion may be overlooked. Yet, it is unclear how to define new measurements that capture these biological phenomena.