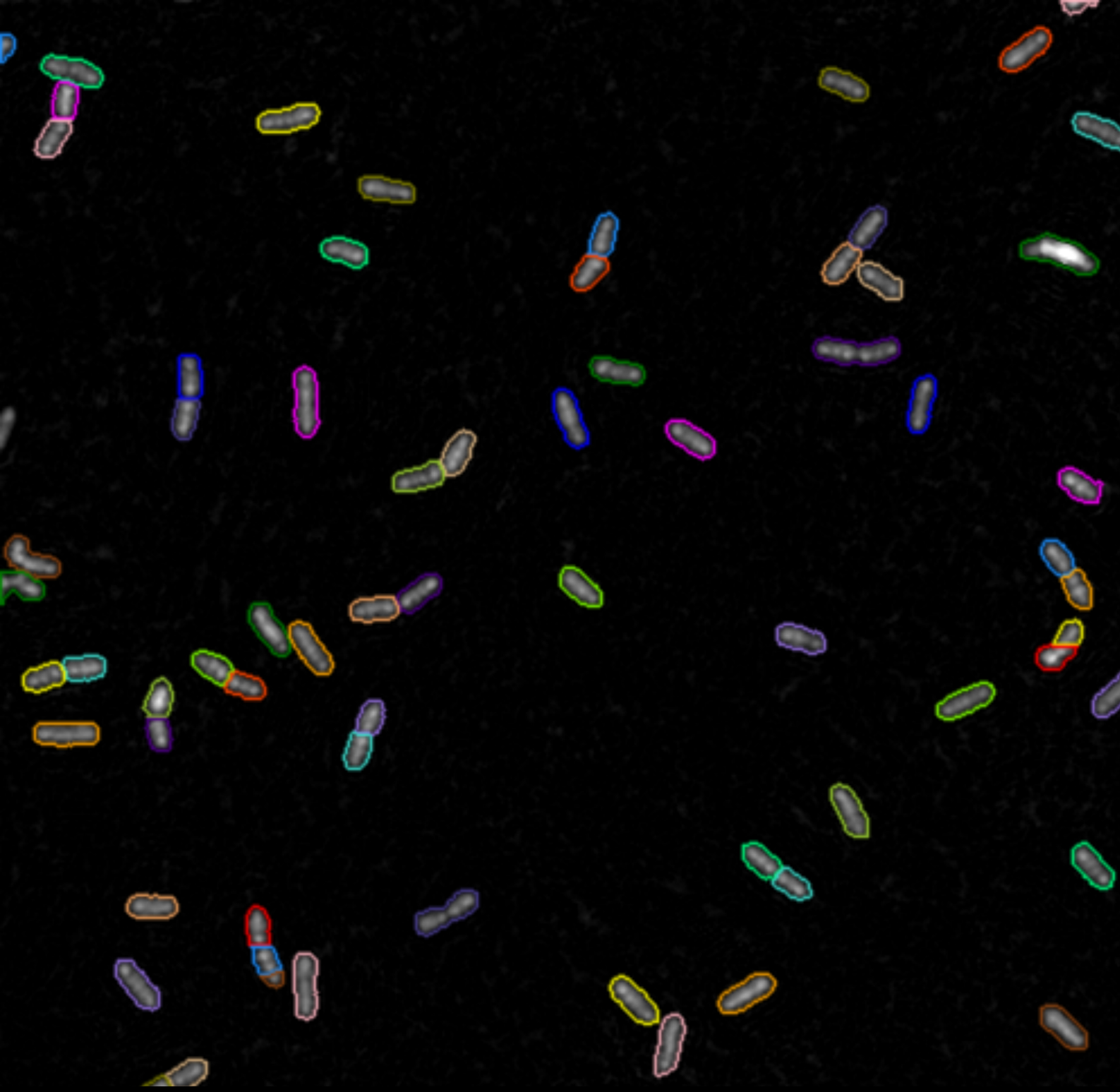

Application Note | Phenotypic Profiling of Fission Yeast: A Model Organism

- *Investigate morphological changes in different yeast strains.*
- *High-contrast label-free imaging for robust single cell segmentation.*
- *Extract a wealth of single cell and population level metrics including Dry Mass, Sphericity, and Length:Width ratio.*

Introduction

All cells have a unique shape and structure which is crucial to their specialised function. This diverse array of cell structural arrangements is tightly regulated by a variety of pathways [1].

Fission Yeast is a widely used model organism for the study of underlying cellular processes [2]. In particular, it has been used to study how cells regulate their morphology due to their simple rod structure and growth pattern as well as their similarity in cell morphology and division regulatory pathways across different species [1].

During mitosis fission yeast grow via extension of the tips of cells and divide through the formation of a cell wall septum. The new cell adopts a rounded morphology [3]. This pattern and uniformity of growth is regulated by a network of proteins including actin, microtubules and cell wall synthesis regulators as well as proteins involved in coordinating the cell cycle [4]. Disruption of the regulation of any of these molecules can lead to aberrant cell morphology [5]. One of these regulators is Cdc42, a small G protein within yeast responsible for regulating the actin cytoskeleton. It is involved in cell division where it plays a role in the attachment of spindle microtubules to kinetochores before chromosomes are segregated [6,7].

Many factors may interrupt these regulatory systems and influence phenotypic changes seen in yeast. Researchers have found that a multi-drug resistant strain of budding yeast showed a dysmorphic morphology as a result of treatment with several anti-fungal drugs. This included a plethora of distinctive features including variation in shape and size of the yeast cell, the shape of the nucleus as well as shape and direction of bud formation [8]. Similarly, studies aimed to understand the variations in fission yeast have described several cellular and sub-cellular changes in response to bioactive compounds. These compounds produced by bacteria instigated a change towards an irregular cell form and cell cycle morphology in the form of an asymmetric septum, multi-septated cells, elongated or rounded cell morphology and vacuole formation [9]. Analysing morphology of yeast strains may offer a useful research tool to investigate biological mechanisms of anti-fungal drugs and predict drug resistance.

Current morphological studies of yeast use primarily qualitative analysis with simple quantitative measured end-points. Multi-parameter quantitative analysis may help in characterising novel yeast phenotypes as well as monitor cells through cell cycle progression [1]. Furthermore, understanding the morphological characteristics of yeast strains and mutants may shed light on cellular morphogenesis in eukaryotic cells [3].

Livecyte utilises Ptychography, a **quantitative phase imaging (QPI)** technique, to produce high-contrast images without the need for fluorescent labels. The enhanced contrast enables automatic segmentation and tracking of individual cells, as well as a quantitative measure of morphology of cells and changes in cellular dry mass.

In this study we investigated the activation mechanism of Cdc42. We examined the **single cell and population morphology** of genetically altered fission yeast leading to modified Cdc42 activity and successfully quantified differences in morphology between yeast strains through Livecyte's label-free QPI mode and in-built Analyse Software.

Cell Culture

Schizosaccharoymyces pombe yeast cells were routinely maintained in minimal media at 28°C. Cells were seeded into the wells of a 24 well plate and cultured overnight.

Resources

- Different strains of fission yeast
- Minimal media
- 24-well culture plate with flat agarose pad
- Livecyte Kinetic Cytometer (Phasefocus)
- Livecyte Acquire & Analyse software (Phasefocus)

Schizosaccharoymyces pombe fission yeast cells underwent genetic alteration as specified below:

- 5077: Wildtype
- 5107: a Cdc42 activator is mutated - Cdc42 is less active
- 5082: a Cdc42 activator is mutated - Cdc42 activity is enhanced
- 5551: a Cdc42 negative regulator is mutated - Cdc42 activity may be slightly enhanced
- 5554: a Cdc42 activator has an activated mutation and the negative regulator is mutated - Cdc42 is highly active

Cell Imaging

Phenotypic changes were assessed with the Livecyte™ system's unique label free Quantitative Phase Imaging (QPI) (40X). Cells were imaged with an Olympus PLN 40X (0.6NA) objective and four 250µm x 250µm fields of view (FOV) per well. Cells were maintained inside an environmental chamber at 28°C.

Analysis & Results

We sought to identify morphological differences between 5 yeast strains. This report will focus on single cell metrics extracted from the Morphology Dashboard in Livecyte's Analyse Software.

Quantitative Phase Images & Cell Segmentation

The quantitative phase images generated by Livecyte natively have high-contrast and illustrative images of each yeast strain are shown below.

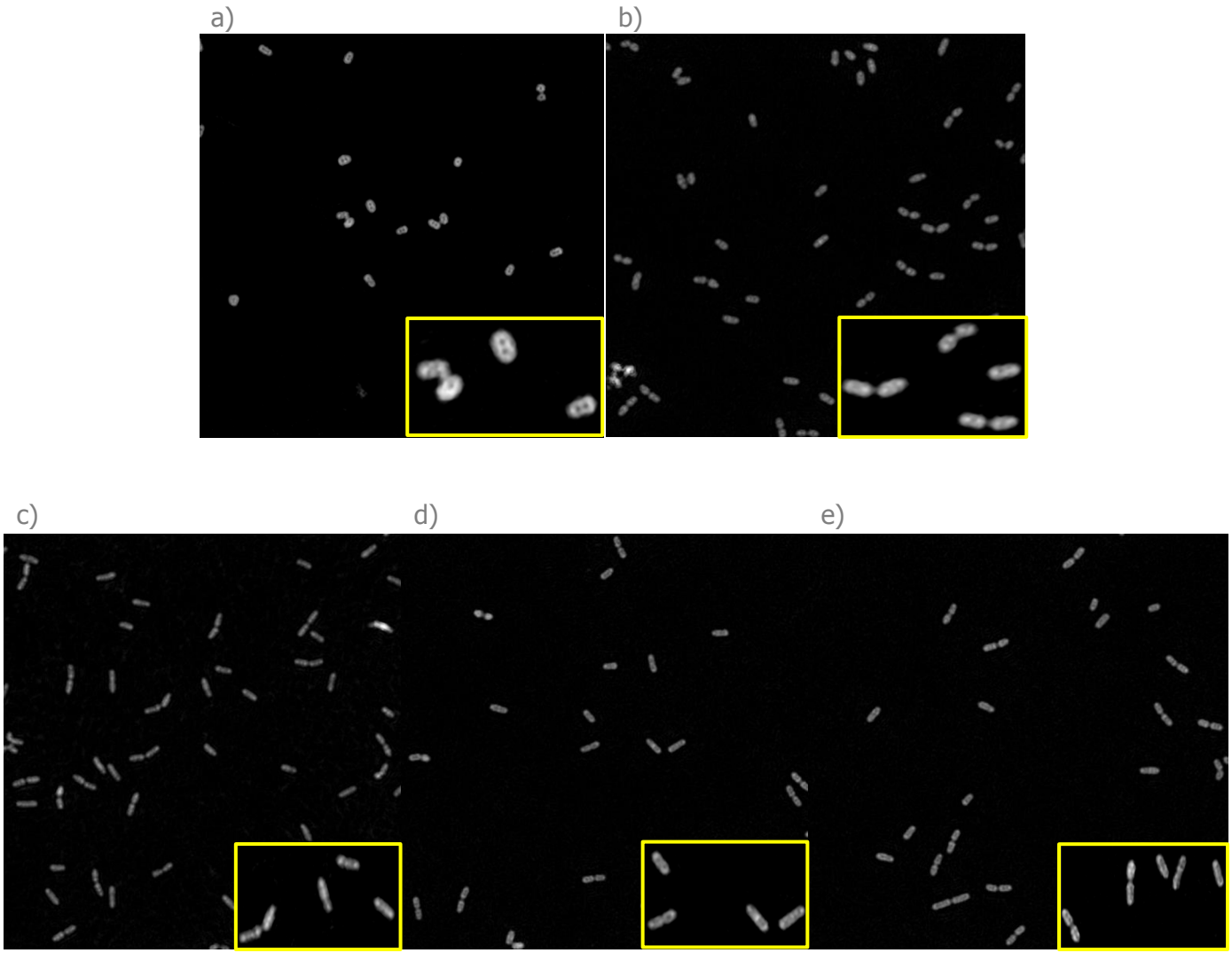

Figure 1: Illustration of Quantitative Phase Images of each strain of yeast: a) 5554 (Cdc42 ++), b) 5107 (Cdc42 -), c) 5077 (WT), d) 5082 (Cdc42 +), e)5551 (Cdc42 +).

High-contrast images enable robust automatic single-cell segmentation and analysis of subtle morphological differences in cell shape, size and mass. For instance, these label-free images show the wild type yeast strain (figure 1c) had a characteristically long "rod-shaped" morphology. However the genetically altered strains, particularly 5107 and 5554, appeared rounder and brighter under the phase imaging indicating an increased thickness (figure 1a,b).

Cell morphology

To gain a further insight into morphological changes undergone by yeast cells in response to changes in Cdc42, we used the Morphology Dashboard on Livecyte's Analyse software (figure 2).

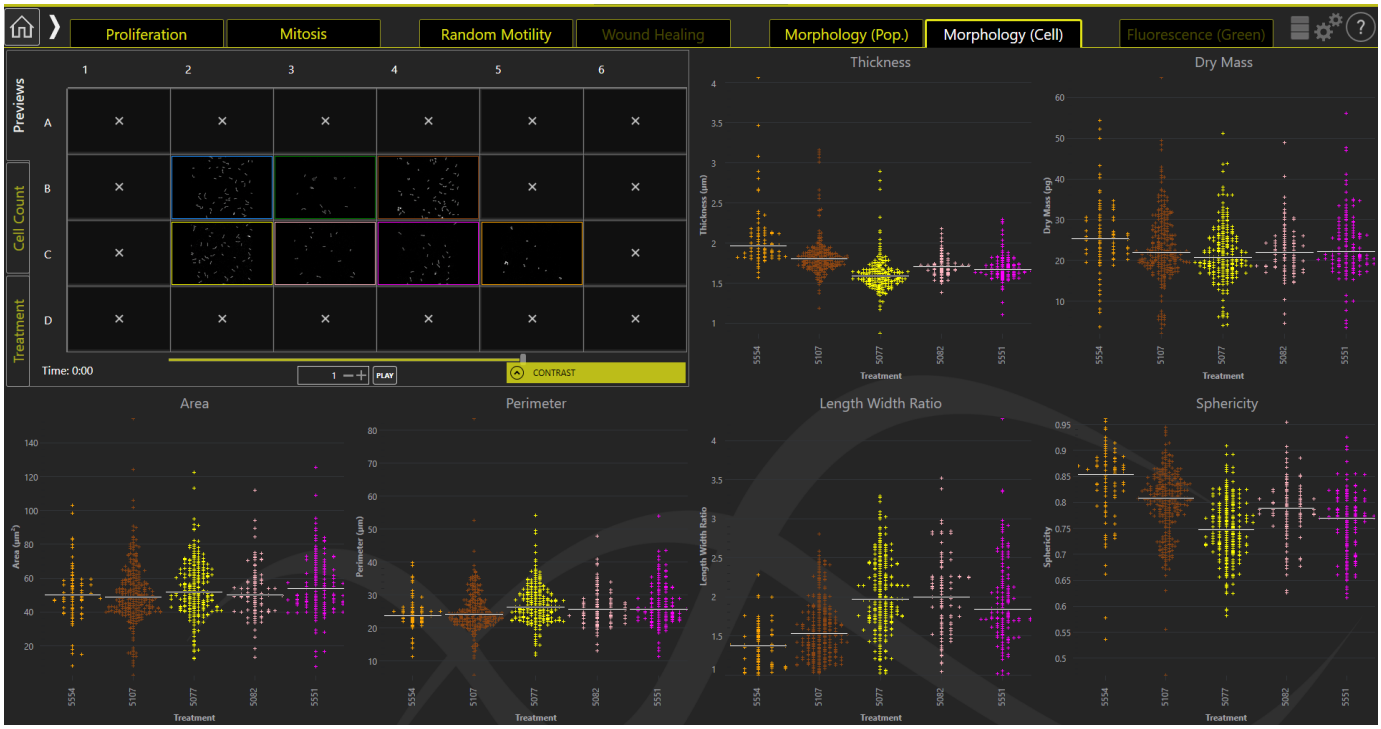

Figure 2: A snapshot of the single-cell morphology dashboard on Livecyte Analyse software showing the multiple metrics that are measured. The white line on each of the graphs represents the median value for the population.

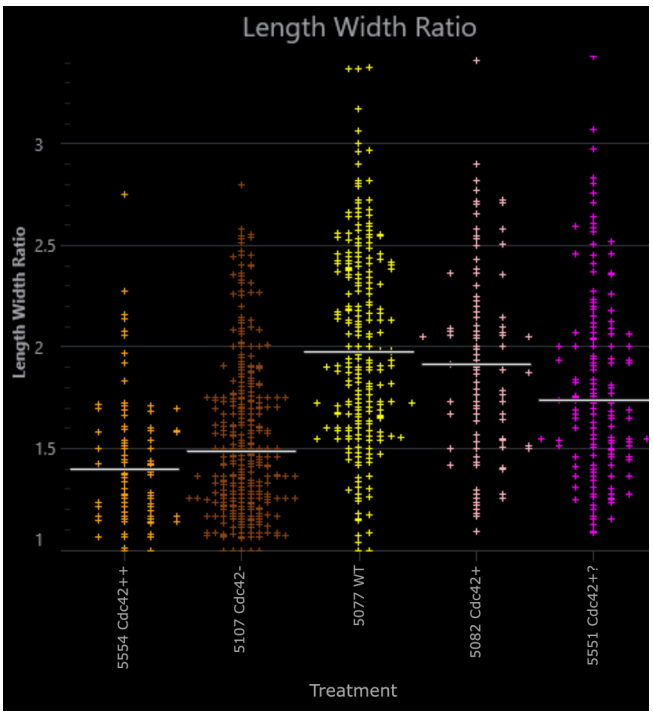

Figure 3: A distribution plot illustrating the length:width ratio of each cell within each yeast strain. Strains 5554 and 5107 had a reduction in length:width ratio of ~0.5 compared to wildtype.

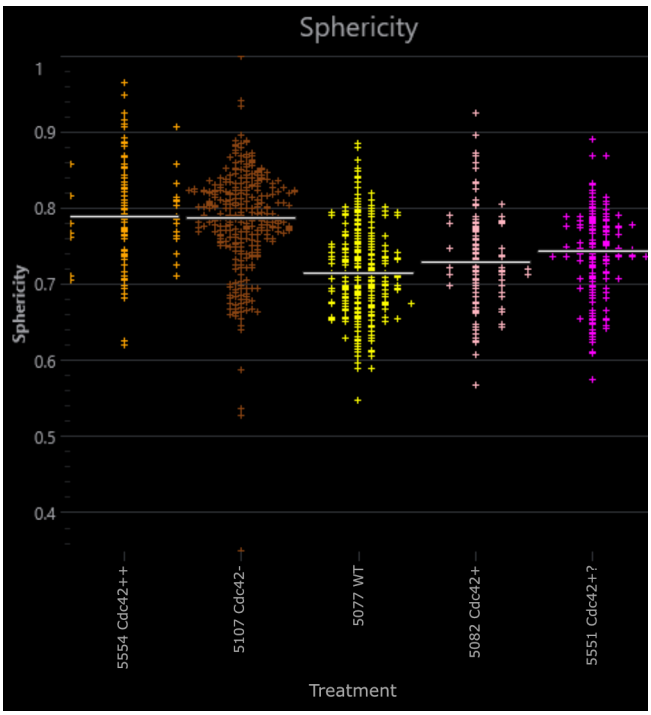

Figure 4: A distribution plot illustrating the sphericity of each cell within the yeast strain. Strains 5554 and 5107 had an elevated sphericity of ~0.1 compared to wildtype.

We report an increased sphericity and reduced length:width ratio in the yeast strain 5107 which had reduced Cdc42 activity. A similar phenotype was also observed in strain 5554, where there was an unregulated increase in active Cdc42 indicating that these strains have a more rounded morphology (figures 3 & 4).

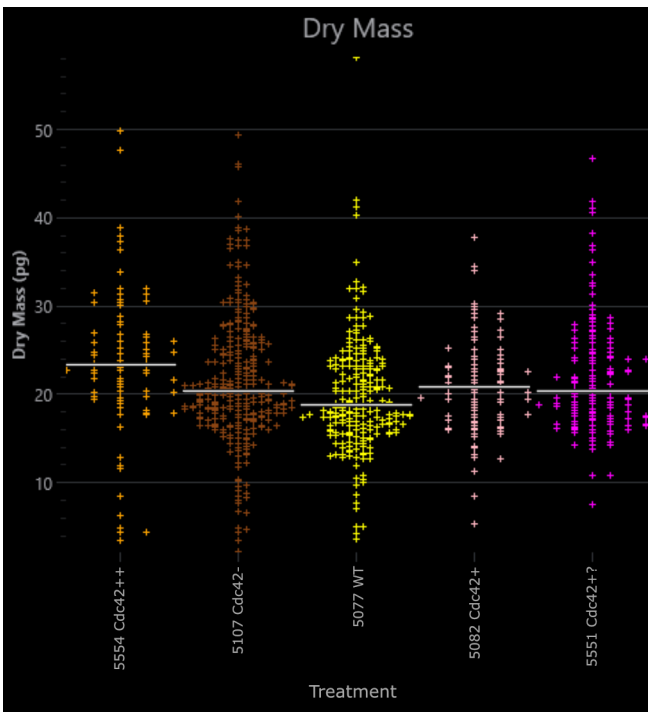

Figure 5: A distribution plot illustrating the dry mass of each cell within the yeast strain. Strain 5554 in particular showed an increase in dry mass compared to wildtype.

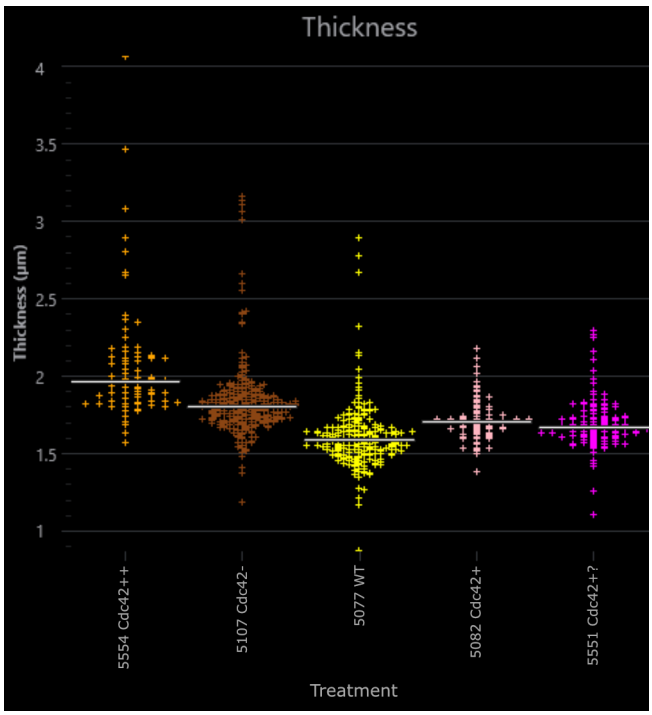

Figure 6: A distribution plot illustrating the thickness of each cell within the yeast strain. Strain 5554 showed an increase in thickness compared to wildtype.

In addition, dry mass and thickness values showed that all yeast strains showed an elevated dry mass and thickness compared to the 5077 wildtype strain, however this was particularly evident for strain 5554 (figure 5&6). Cdc42 is required to preserve the polarity of yeast cells and thus normal morphology and cell division. Abnormal levels of active Cdc42 may lead to a reduction in polarity of cells and dysregulation of these processes including cell cycle arrest [6].

Summary

Yeast provides an ideal model organism for the study of the regulatory systems that encompass the variety of morphological phenotypes seen in mammalian cells. A prominent part of this process is Cdc42 which is actively involved in maintaining cell polarity and whose activity is closely controlled by several modulators [6].

Quantitative phase imagining generates high-contrast images that enable robust cell segmentation and quantification of a variety of morphological characteristics. The Livecyte provides users with the tools to monitor how cells alter their phenotype independently and in response to external conditions.

In this study we aimed to investigate the subtle differences in yeast strain phenotype through quantitative phase imaging and accurate cell segmentation. We were able to identify and quantify differences in phenotype in some genetically altered yeast strains compared to wild type. We found differences in morphology in both strains with reduced (5107) and enhanced Cdc42 activity (5554). Although it is known functional Cdc42 is required for effective polarity of yeast cells, previous studies working on a model of constitutively active Cdc42 showed these genetically altered yeast cells could not establish a polarisation site leading to aberrant morphology and budding [11]. This indicates that there may be a Cdc42 level at which yeast cells may maintain regular polarity and where there is reduced or overactive Cdc42, cells are unable to maintain a classic phenotype. This suggests the Cdc42 activation pathway is closely controlled possibly by multiple regulatory pathways. Investigating aberrant morphology of yeast strains may be a

useful technique to understand the regulation of cell growth and arrest. These changes in morphology may give some indication of the polarity and cell cycle time with thicker rounder cells inferring a longer cell cycle length. It is known that when fission yeast undergo cell cycle arrest, cells may continue to produce proteins and cell components leading to a possible increase in dry mass and thickness as observed [10].

In summary, through Livecyte's quantitative phase imaging mode and Analyse software we were able to automatically extract a wealth of single cell metrics in the form of morphological data, label free. In combination with an intuitive workflow, Livecyte enabled us to reliably investigate the phenotypic differences between yeast strain changes in order to enhance the understanding of disease states.

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