CHAPTER

23 Measurement and manipulation of cell size parameters in fission yeast

Yonatan Zegman, Daria Bonazzi, Nicolas Minc[1]

Institut Jacques Monod, CNRS UMR, Paris Cedex 13, France

[1]*Corresponding author: E-mail: minc@ijm.univ-paris-diderot.fr*

CHAPTER OUTLINE

Methods in Cell Biology, Volume 125, ISSN 0091-679X, http://dx.doi.org/10.1016/bs.mcb.2014.10.011

Abstract

Cells usually grow to a certain size before they divide. The fission yeast *Schizosaccharomyces pombe* is an established model to dissect the molecular control of cell size homeostasis and cell cycle. In this chapter, we describe two simple methods to: (1) precisely compute geometrical parameters (cell length, diameter, surface, and volume) of single growing and dividing fission yeast cells with image analysis scripts and (2) manipulate cell diameter with microfabricated chambers and assess for cell size at division. We demonstrate the strength of these approaches in the context of growing spores, which constantly change size and shape and in deriving allometric relationships between cell geometrical parameters associated with G2/M transition. We emphasize these methods to be useful to investigate problems of growth, size, and division in fungal or bacterial cells.

INTRODUCTION

Cell size is a significant cellular property, which influences the maintenance of metabolite concentrations, transport at the cell surface, tissue morphogenesis, and the proper assembly of intracellular structures, such as the mitotic spindle (Lloyd, 2013; Marshall et al., 2012; Turner, Ewald, & Skotheim, 2012). For size homeostasis, cells usually grow to a certain size and enter division, a process which is largely controlled by the cell-cycle machinery. The rod-shaped fission yeast *Schizosaccharomyces pombe* has been instrumental in identifying regulators of cell cycle and size controls (Mitchison & Nurse, 1985). These cells grow along a unicellular axis with a nearly constant diameter and divide at a fixed length of $\sim$14 μm. Mutants in the cell cycle have thus traditionally been identified as being shorter or longer than wild-type cells (Nurse, Thuriaux, & Nasmyth, 1976). Recent studies have suggested a "geometrical control" model for the cell cycle, in which cell length is monitored by gradients of the DYRK kinase pom1p emanating from cell tips. These gradients serve as cellular rulers for G2/M transition (Martin, 2009; Martin & Berthelot-Grosjean, 2009; Moseley, Mayeux, Paoletti, & Nurse, 2009; Moseley & Nurse, 2010).

Despite the prominent role of *S. pombe* in deciphering core mechanisms of size sensing, to date, there is no standardized method to precisely quantitate length, diameter, surface, and volume of single cells. Because mutants associated with the cell cycle can also be defective in polarity and cell shape (e.g. *pom1* for instance (Bahler & Pringle, 1998)), computing parameters such as surface or volume can often be difficult. In addition, many morphogenetic mutants have defective cell length at division; for instance, fat mutants in the Rho GAP rga4p divide shorter, while thin mutants in another Rho GAP rga2p divide longer (Das et al., 2007; Pan, Saunders, Flor-Parra, Howard, & Chang, 2014; Tatebe, Nakano, Maximo, & Shiozaki, 2008; Villar-Tajadura et al., 2008). Disentangling causal effects of geometry onto cell-cycle progression may thus be difficult, and is likely to bring important insights into this fundamental problem.

Here, we describe two relatively simple methods for the study of size-related questions in fission yeast: The first method uses confocal or phase microscopy

and MATLAB-based image analysis script to compute precisely cell length, diameter, surface, and volume in individual growing cells that may change shape and diameter, for instance, and in dividing cells. Outputs of this method include the dynamic evolution of size parameters in single cells (single volumetric or surface growth rates, for instance) and the study of the correlation between different size parameters in populations of dividing wild-type or mutant cells. Second, we describe the use of microfabricated PDMS (polydimethylsiloxane) channels to manipulate cell diameter, and assess impact on cell length at division. This approach may serve to directly address the relationships between geometry and cell-cycle progression.

1. MEASUREMENT OF SIZE PARAMETERS OF SINGLE FISSION YEAST CELLS

This section will describe basic methodologies for sample preparation and the use of a MATLAB script designed to compute the length, diameter, surface area, and volume of outgrowing spores, which continuously change shapes and sizes (Bonazzi et al., 2014), as well as of dividing cells of different mutants with various shapes and dimensions. The method includes cell handling, microscopy, and image analysis protocols.

1.1 DYNAMIC MEASUREMENT OF CELL SIZE PARAMETERS DURING SINGLE SPORE GROWTH AND POLARIZATION

Materials

Glass slide; $22 \times 22\ mm^2$ coverslips; Erlen Meyer; YE5S plates, ME plates, YE5S liquid media; agarose (Euromedex, Ref. D5-C); Sterile water; Glusulase (PerkinElmer, Ref. NEE154001EA); Fission yeast homothallic h90 strains; Block heater for microtubes; Microwave; Centrifuge; Rotating agitator; Wide-field microscope equipped with perfect focus system and a phase 100X objective; Humidifier; Temperature and humidity sensor; Computer equipped with MATLAB and image analysis toolbox (Mathworks); and dedicated script (available upon demand).

1.1.1 Spore preparation for imaging

1. Grow a homothallic h90 colony on a YE5S (Yeast Extract) plate for 1−2 days to obtain a fresh culture and replica on ME (Malt Extract) plates to sporulate cells for at least 3 days at room temperature.
2. Check sporulating efficiency under the microscope by placing a tiny amount of cells in 2 µl water between a glass slide and a coverslip. The spores should account for more than 70% of the population.
3. Digest a relatively large amount of spores for 1 h at room temperature in 200 µl of water solution containing 1/200 glusulase to kill vegetative cells.
4. Wash the spores three times in water to remove the enzyme.
5. In a sterile Erlen Meyer, add agarose to liquid YE5S at a final concentration of 2% w/V. Heat the mix shortly in the microwave to melt the agarose in the solution, mix well, and aliquot in 1 ml microtubes.

6. Melt a tube of 2% agar YE5S at 95 °C for 5 min in the block heater and place it at 65 °C for at least 20 min.
7. Pipette 180 µl of melted agar on a glass slide and spread it by covering the drop with a glass slide perpendicularly to the first one, wait 3 min, and then remove the upper glass slide by gently sliding it laterally.
8. Adjust the dilution of spores under the microscope to obtain a relatively dense concentration of spores, but spores that do not contact each other.
9. Place 1 µl of concentrated spores on top of the agar pad and cover with a coverslip. To avoid air bubble formation, place the coverslip on the side of the agar pad and bring it gently to the surface of the pad by holding it with a razor blade. Small bubbles may appear here and there, and it is advised to start imaging away from those, as they may grow over time and disrupt long-term imaging.

1.1.2 Imaging

1. Place the sample on the inverted microscope with the coverslip side facing the 100X objective.
2. Find a position that optimizes the density of spores (not too dense and not too diluted).
3. Focus on the middle plane by searching for the maximal contour of the spores.
4. Turn on perfect focus.
5. Take time-lapse phase-contrast movies (5−10 min time interval) of germinating spores at room temperature (23−25 °C), and control humidity with a humidifier in the microscopy room. The first cell cycle, from germination to mitosis, may take typically 10−12 h.

1.1.3 Image analysis

General principle of image-analysis script:

Evolution of spore dimensions (diameter, surface, volume, aspect ratio) is measured from phase-contrast time-lapse movies using home-built MATLAB scripts. The software follows a series of steps interacting with the user from selecting and cropping the spore, adapting the threshold, and troubleshooting. It then segments the phase image by applying thresholding and edge-detection methods to extract the cell contour at each time point. From the contour, diameter and aspect ratio can be directly computed and surface and volume are calculated by assuming a prolate geometry (rotational symmetry around the longest axis of the cell). The long axis is identified and sliced in local diameters d(x) at every pixel in order to compute the areas of the sections of the prolate $s(x) = \pi * (d(x)/2)^2$ and their circumferences $c(x) = \pi * d(x)$. Finally, the volume is the sum of the areas, and the surface area is the sum of the circumferences, both integrated along all the pixels of the long axis (Figure 1(A)) (Bonazzi et al., 2014). We estimate imprecisions associated to these methods (including errors in the z, segmentation, and prolate approximation) to be lower than 5% for length and aspect ratio and on the order of 10% for surface and volume. These analyses will be the most accurate if the plane of focus is as close as possible to the midsection of the spore.

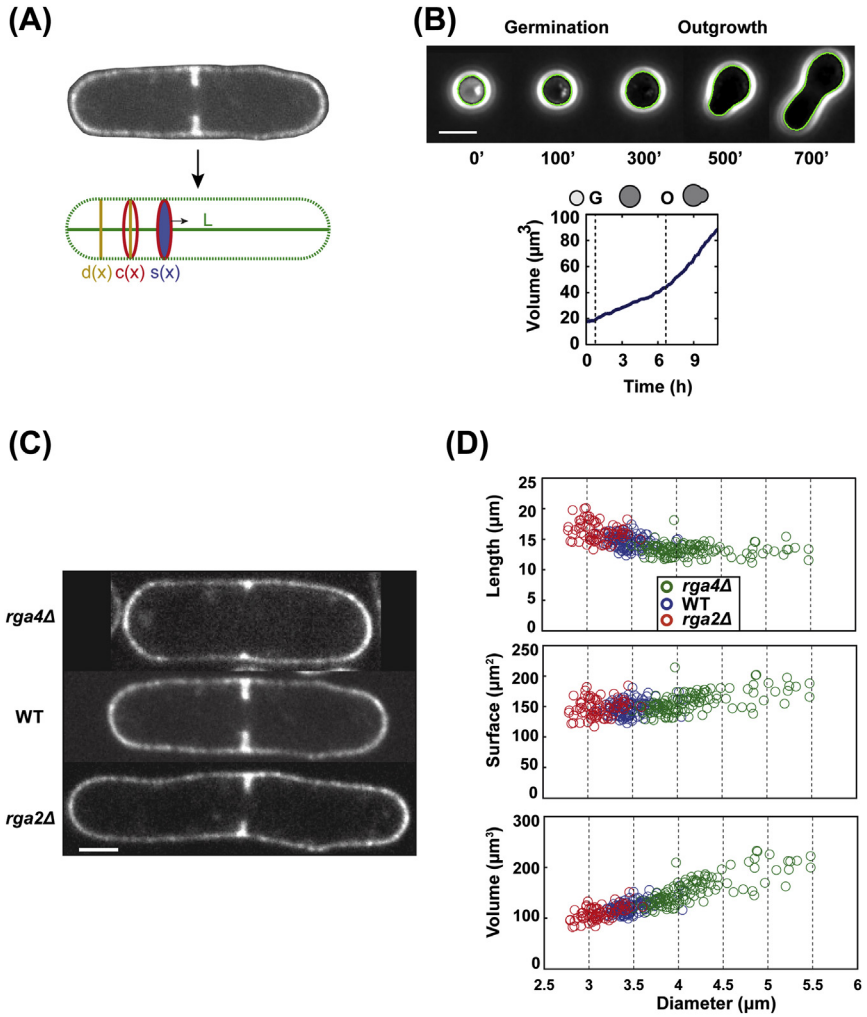

FIGURE 1 Measurement of size parameters of single fission yeast cells.

(A) Fluorescence image of a dividing fission yeast cell expressing GFP-psy1 (to label plasma membrane). The contour is cropped, traced, and measured for length (L, green), as well as local diameters (d(x), yellow), circumferences (c(x), red), and section areas (s(x), blue), which are integrated to give the cell surface and volume. (B) Upper: Phase-contrast time-lapse superimposed with automated shape contour detection (green) of a wild-type fission yeast spore germinating and outgrowing. Scale bar, 5 μm. Lower: Dynamic evolution of single cell volume as a function of time. (C) Midsection confocal images of dividing WT, *rga4*Δ, and *rga2*Δ fission yeast cells expressing GFP-psy1. Dividing cells can be identified from membrane invaginations associated with cytokinesis. Scale bar, 2 μm. (D) Single cell length (upper), surface area (middle), and volume (lower) plotted as a function of diameter (horizontal axis of each), for populations of WT (blue data points), *rga4Δ* (green data points), and *rga2*Δ (red data points) dividing fission yeast cells. (See color plate)

Procedure:

1. Open MATLAB and start the script.
2. The software will open the first- and last-phase images of the corresponding time-lapse: crop a region of interest, in order to select a single growing spore.
3. The automatic tracking will start and progressively display each image of the time-lapse with a green outline of the spore contour, in order to visually check the detection accuracy.
4. As long movies are necessary in order to observe spore germination, background and contrast often change over time in phase images, leading to failure of automatic tracking. In this case, the best solution consists in setting a different threshold value in the edge-detection section for successive subsections of the time-lapse.
5. In addition, if automatic segmentation fails, the script allows selecting failed frames and for each of them, to manually draw the spore contour.

Output:

By integrating the automatic and manual detection in a single time-lapse analysis, the script will calculate a series of geometric parameters (e.g., long axis, local diameter along this axis, surface volume, aspect ratio…) for each spore over time and save these results in a MATLAB file that can be exported as an Excel table. Furthermore, the software will generate a movie of the phase-contrast time-lapse for the cropped spore, with a green outline corresponding to the detected contour (Figure 1(B)). This approach should be applicable to single growing cells that grow and change size or shapes.

1.2 LENGTH, DIAMETER, SURFACE, AND VOLUME OF DIVIDING CELLS

This subsection is dedicated to the precise measurement of cellular dimensions of dividing fission yeast cells. In order to precisely capture the contour of the cell, the plasma membrane should be detectable by fluorescence microscopy. This can be achieved either by using a GFP-tagged plasma membrane marker such as GFP-psy1 (Nakamura, Nakamura-Kubo, Hirata, & Shimoda, 2001) or by applying a fluorescent membrane staining such as $DiBAC_4(3)$, shortly before imaging. Dividing cells can be identified by the ingression of the membrane for cytokinesis (Figure 1(C)). Calcofluor, which stains the cell wall and has increased staining at the division septum may also be used, but could add imprecision in the timing of division as the septum may still be stained when cells have restarted growth. In addition, we note that Calcofluor, which outlines the contour of the cell wall, adds around 0.5 μm in the diameter and length of the cell.

Using this script, we analyzed cell size parameters at division for a number of fission yeast mutants. We measured WT cells, as well as *rga4Δ* and *rga2Δ* mutants, in order to evaluate the evolution of cell length, L_{div}, at division as a function of cell diameter, D (Figure 1(C) and (D)). This analysis revealed an allometric scaling between length and diameter at division, such that $L_{div} \sim 1/\sqrt{R}$. This scaling is

intermediate between previously proposed hypothesis of pure-length sensing for division onset (Martin & Berthelot-Grosjean, 2009; Moseley et al., 2009), and recently proposed surface sensing, $L_{div} \sim 1/R$ (Pan et al., 2014). The origin of this newly found scaling may serve as an interesting subject of further investigation.

Materials:

Glass slide; 22 × 22 mm^2 coverslip; Exponentially growing fission yeast culture; Spinning disk microscope with 100X objective; $DiBAC_4(3)$ dye (LifeTechnologies, Ref. B-438); EMM liquid media (minimal media); Computer equipped with MATLAB and image analysis toolbox (Mathworks); and dedicated script (available upon demand).

1.2.1 Cell preparation for imaging

1. If cells do not express a plasma membrane marker, stain with DiBAC4(3) at a final concentration of 100 μM (1/100 dilution from a stock solution in DMSO) for 10 min, then rinse 3x with EMM, and image immediately after rinsing.
2. Spin down 1 ml of cells and concentrate to 50 μl typically.
3. Place a 1.2−1.5 μl drop of cell suspension on the glass slide and gently cover with a 22 × 22 mm^2 coverslip. To ensure that cells are not flattened by the coverslip, adapt the volume of the drop and observe cells under the microscope; the ideal situation is to have a majority of immobile cells with a small percentage of cells that slowly jiggle around in the field.
4. Quickly go to the spinning disk microscope and take z-stacks of several fields extending 5−7 μm around the cell midsection using a distance between slices of 0.2 μm. The acquisition time of a single stack is ∼15−30 s. The pixel size should be as small as possible (In Figure 1(C), the pixel size is = 59.5 nm).

1.2.2 Image analysis

General principle of image analysis script:

The script used for this application follows similar concepts as previously discussed (Figure 1(A), Section 1.1.3) and allows to sequentially select and crop dividing cells, go through the z-stack to identify the midsection, find the top and bottom of the cell, segment either automatically or manually the cell contour, and output all relevant cellular parameters.

Procedure:

1. Open MATLAB and run the script for a given stack.
2. The script asks and inputs the magnification and z-step.
3. Crop the cell of interest out of the displayed image.
4. Click through the z-stack slices taking note of the slices containing the top and bottom of the cell (in which fluorescence begins to blur away) as well as the slice containing the most extended section of the cell (midplane).
5. Report top-, bottom-, and middle-section slice numbers.
6. Manually click to trace the contour of the cell using the segmented line or use the automatic detection. Use automatic detection when the cell contour is clearly

contrasted from the background and separated from other cells or artifacts in the field, otherwise use manual tracing. The script will display the overlay of the trace with the cell to control for proper segmentation.

7. The script will compute length, diameter, surface area, and volume from the contour shape.
8. Repeat steps 3–7 to measure all dividing cells in this image, then change file and continue analysis.

2. MICROCHANNEL ASSAY FOR CELL DIAMETER MANIPULATION

In this section, we describe a method based on microfabricated channels, which serve to confine and deform spores and let them germinate and grow into cells with a diameter imposed by the channel (Figure 2). Using this approach, we can generate cells with diameter down to 2–2.5 μm. Controls with large and high channels, which largely exceed the cell diameter are used to discriminate putative effects of growing spores/cells in PDMS channels from effects of cell geometry. Although the channels have a defined width and height, cells do deform channels, or may sometimes be slightly tilted in z with respect to the channel wall (for instance, when contacting another cell). We thus couple this method with the size measurement script developed in Section 1.2.2. This method may have applications in dissecting mechanisms of polarity, growth, or morphogenesis. In here, we demonstrate its strength by bringing the abnormally large diameter of *rga4* mutants (4–6 μm) to values similar to WT (3–4 μm). These "thinner" *rga4* mutant cells do not divide anymore at a short length but at a very similar length than WT cells (Figure 2(C) and (D)), and fall within the allometric scaling mentioned before (Section 1.2). This suggests that defects in length at division in this mutant are directly associated with defects in diameter.

2.1 FABRICATING MICROCHANNELS TO MANIPULATE CELL DIAMETER

This section describes the methods used to fabricate PDMS microchannels. The design of the channels was adapted from previous work using microchannels to elongate fission yeast cells round mutants (Terenna et al., 2008) and for studies of cell migration (Faure-Andre et al., 2008; Heuze, Collin, Terriac, Lennon-Dumenil, & Piel, 2011). The fabrication procedure follows two steps: Soft-lithography, PDMS molding and chamber preparation. In what follows, we describe detailed material and procedure for each of these steps.

Materials:

Computer with drawing software; 4-inch-diameter Silicon wafer (University wafers in US and Neyco in France, 4″ N(111) SSP Test Grade Quality); 6 cm-diameter petri dish; SU8-2005 resist and developer (MicroChem); Acetone and Isopropanol; Access to clean room facility (with spin coater, hot plates, and UV lamp); Sylgard 184 base and

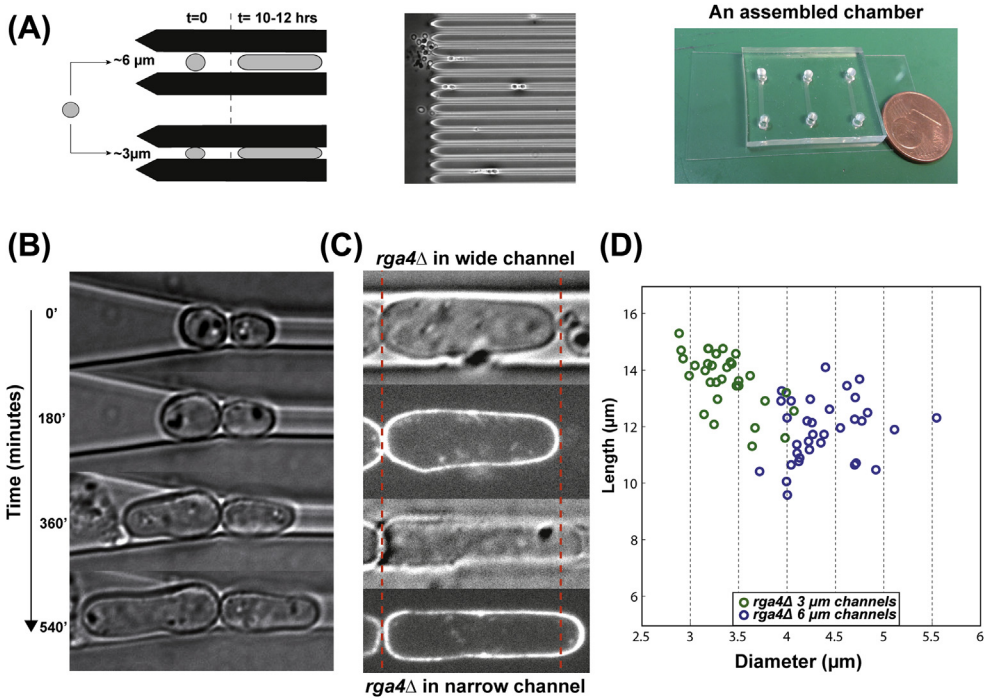

FIGURE 2 Diameter manipulation of fission yeast cells in microchannels.

(A) Left: Scheme representing the installation and elongation of spores inside channels with confining (3 μm) and nonconfining (6 μm) diameters. Spores grown in confining diameters will assume a smaller cell diameter compared to control cells. Middle: Large-view phase-contrast image of spores installed in microchannels. Right: An assembled PDMS chamber containing three channel domains. (B) Time-lapse of spores developing at the channel entry. The right cell is growing into the microchannel and adopts a small diameter, while the left cell is growing out. (C) Bright field and florescence images of dividing *rga4*Δ GFP-psy1 fission yeast cells, in a wide channel (upper pair) and in a narrow channel (lower pair). Extension of length in both conditions is highlighted with red dotted lines. (D) Single *rga4*Δ cell length at division plotted as a function of cell diameter in wide (6 μm) channels (blue data points (gray in print versions)) and narrow (3 μm) channels (green data points (black in print versions)).

curing agent (Fisher, NC9644388); plastic cups; plastic stirring rod; timer; scale; vacuum desiccator; 65 °C oven; Tridecafluoro-(1,1,2,2-Tetrahydrooctyl)-1-trichlorosilane (United Chemical Technologies, Ref. T2492); a scalpel, 2 mm hole-punch screwdriver; tweezers; 24 * 50 mm^2 cover glass; DI Water; Dry air; Plasma Cleaner (Harrick Plasma).

2.1.1 Photomask design

The design of patterns is done on a computer-assisted drawing program (CAD). Available programs include AutoCAD (free for students) and QCAD (freeware). The design includes a long-rectangular region containing the channels, joining two large regions (for entry and exit of spores). The large regions are covered with equally spaced posts (of typically 20 μm in diameter and spaced apart by 20 μm). We recommend to design sets of channels with a range of width (from 2 to 7 μm typically). For these ranges of size, these designs are printed on 5″ quartz mask with a resolution below 0.5 μm (which can be ordered from specialized company, such as Toppan Photomasks).

2.1.2 Photolithography

Channels are made from PDMS using a positive master composed of SU8. Masters are prepared using standard lithography methods for SU8 microfabrication (Weibel, Diluzio, & Whitesides, 2007). A positive master is a hardened structure of SU8 such that the desired geometric shapes extend as posts from a silicon wafer. SU8 is an epoxy-based negative photoresist: when it is exposed to UV light it becomes insoluble to the photoresist developer, while the unexposed portion of the photoresist is dissolved by the developer.

Procedure:

1. Clean the silicon wafer with dry air and bake at 150 °C for 5 min on hot plate.
2. Put SU8 onto the wafer by using a transfer pipette (approximately 1 ml for each square inch) and spin it. Rotation speeds depend on the type of SU8 used as well as desired thickness of the coating. To obtain 5 μm high features, the basic procedure involves an SU8-2005 and a first spin of 20 s at 500 rpm, with an acceleration of 500 rpm/s and a subsequent spin of 45 s at 3000 rpm with the same acceleration. Note that we adapt the height in order to generate channels with squared section.
3. Bake the resist on a hot plate for about 1 min at 65 °C and 1 min at 95 °C.
4. Create patterns on the SU8 by covering the wafer with the quartz mask using a dedicated UV insulator (or mask aligner). It is important to ensure that the mask remains well plastered onto the SU8 layer (this can be achieved by placing a weight on top of the mask). The optimal time of exposure will depend on wavelength and intensity of the light and has to be adapted. A too short exposure will make features that do not stick well to the substrate and detach, while a too long exposure will generate features with poor spatial definition.
5. The sample is then baked again for 1 min at 65 °C and 1 min at 95 °C, immersed in a glass dish containing developer for 2 min, and transferred to another glass dish containing clean developer for 30 s. The wafer is then rinsed with isopropyl

alcohol and dried on the spin coater (1 min at 1000 rpm). A final bake at 150 °C for 10 min is then performed on the hot plate.

6. The master is then observed on a bright-field reflected light microscope to check the shape and size of SU8 channels.
7. An overnight exposure of the master with vapors of silane is then performed to prevent the PDMS from sticking too much to the SU8 (see hereafter). This is done by placing the SU8 masters together with a small flask containing 200 µl of silane in a vacuum desiccator, overnight.
8. Master wafers can be stored in large petri dishes for long time periods and be used repeatedly to produce PDMS structures, for periods up to several years if handled with care (see Section 2.1.3).

2.1.3 Creating PDMS from master

PDMS chambers can be replicated many times from a positive SU8 master.

1. Mix over 50 g of PDMS base and curing agent in a ratio of 5:1. Mix vigorously with a transfer pipette or a plastic rod.
2. Degas mixture by placing into a vacuum desiccator. Apply vacuum until bubbles disappear.
3. Apply a fraction of the PDMS slowly onto the wafer. Once the wafer is coated completely by a few millimeters of PDMS, let it settle onto the wafer for 10 min. Bake at 65 °C for at least 4 h. Note that baking temperature and time will change the rigidity of the PDMS.
4. Cut the PDMS off the masters with a blade and carefully peel it off the master using tweezers. Place the PDMS with the channels facing up in a petri dish. The PDMS slab can be stored for long periods of time.

2.1.4 Assembling the micro channels

The PDMS slab is then pierced and sealed with a coverslip to assemble closed channels in which to manipulate and grow cells.

Procedure:

1. Just before use, pierce holes in the PDMS slab within the square regions at the two channel ends using the hole-punch screwdriver.
2. Cut the PMDS slab around the structure just enough to fit it onto the 24 * 50 mm^2 surface of the coverslip, leaving sufficient edges for adhesion.
3. Clean a 24 * 50 mm^2 coverslip with Acetone, Isopropanol, and DI water and dry with pressurized air.
4. Activate the PDMS channels facing up and the coverslip with a plasma cleaner, for ∼1 min. Place the PDMS channels facing down on top of the coverslip. Check for adhesion by softly pulling on the PDMS. If the PDMS moves, softly press on the PDMS (sparing the channels) to initiate adhesion. Bake the channel at 65 °C for 1−2 h. Once assembled, the chamber can be stored in a clean petri dish for a couple of days before use (Figure 2(A) right).

2.2 CELL DIAMETER MANIPULATION AND IMAGING

This section describes the diameter manipulation of fission yeast cells. This aim is achieved by growing spores inside PDMS channels with confining sections. The method is composed of several steps: sporulation, installation of the spores inside the channels, germination, elongation, division within the channels, and imaging of the manipulated cells. In the following sections, we describe materials and procedures for each of these steps.

Materials:

Resuspended spores (see Section 1.1.1), assembled microchannels (see Section 2.1.4), 10 ml syringe, YE5S media, petri dish, Gel-loading pipette tips, Spinning disk microscope with 100X objective, Computer equipped with MATLAB and image analysis toolbox (Mathworks), and dedicated script (available upon demand).

Procedure:

1. Place 20−50 μl of the YE5S suspended spores inside the channels entry hole (the pierced square region closest to the channels). Use a relatively diluted suspension.
2. In order to widen the syringe exit, assemble a clipped pipette tip onto the needle adapter. Withdraw air into the syringe, hold it vertically on top of the channel entrance, and push the plunger to inflate air and push spores into the channels. Take care (1) not to apply a strong manual pressure directly on top of the channels as they might collapse and (2) to push moderately in order to avoid air penetration into the channels.
3. Mount the chamber on an inverted microscope and check for the presence of spores inside the channels. An optimal installation will have one to two spores in some channels (Figure 2(A) middle). If needed repeat step 2 until achieving a successful installation.
4. Empty entry holes in order to remove excess spores that did not enter the channels and fill them again with fresh YE5S media. Note that this aspiration will note cause spores, which are stuck in channels to move out.
5. Place the chamber in a large petri dish, surrounded by wet kimwipes on the sides, and close the lid of the petri dish.
6. Incubate the petri dish over night at 18 °C. In the morning, most of the spores should be finishing their first cell cycle (Figure 2(B)).
7. Refresh YE5S media in the entry and place the chambers at 25 °C for another 2−3 h.
8. Mount the chamber on a Spinning disk inverted microscope with a 100X objective. Take pictures of dividing cells in channels. Ideally, several chambers in parallel should be prepared (some large channels for controls, and some thin ones) and cells should be imaged every other ∼30 min to capture many cell division events.
9. Follow similar imaging procedure as in Section 1.2.1.
10. Analyze images using the same script as in Section 1.2.2. The script has built-in analysis for situations when the cell have ellipsoidal cross section (for instance, if the channel height is smaller than the width).

CONCLUSIONS

The methods that are described here provide novel material available to the community for the study of cell size-related questions in fission yeast. The imaging method for the measurement of cell dimensions is likely to be adaptable to many situations and to different kinds of tip-growing cells (fungi, bacteria). Microchannels are sizable down to fractions of a micrometer, and could even be fabricated in glass using other methods, to deform much smaller cells such as bacteria (Männik, Driessen, Galajda, Keymer, & Dekker, 2009). Microchambers to control cell diameter may help to decipher which size parameters cells measure for G2/M, and to isolate novel mutants defective in this process.

ACKNOWLEDGMENTS

We thank Rima Seddiki, Emmanuel Terriac, Matthieu Piel, and Anne Paoletti, and members of the Minc Laboratory for their technical help and discussions. Our laboratory is supported by the CNRS, and grants from the ANR (10PDOC00301), the FRM (AJE20130426890), the FP7 CIG program, and ITN "FungiBrain" and the "Mairie de Paris emergence" program.

REFERENCES

Bahler, J., & Pringle, J. R. (1998). Pom1p, a fission yeast protein kinase that provides positional information for both polarized growth and cytokinesis. *Genes & Development, 12*(9), 1356−1370.

Bonazzi, D., Julien, J. D., Romao, M., Seddiki, R., Piel, M., Boudaoud, A., et al. (2014). Symmetry breaking in spore germination relies on an interplay between polar cap stability and spore wall mechanics. *Developmental Cell, 28*(5), 534−546.

Das, M., Wiley, D. J., Medina, S., Vincent, H. A., Larrea, M., Oriolo, A., et al. (2007). Regulation of cell diameter, For3p localization, and cell symmetry by fission yeast Rho-GAP Rga4p. *Molecular Biology of the Cell, 18*(6), 2090−2101.

Faure-Andre, G., Vargas, P., Yuseff, M. I., Heuze, M., Diaz, J., Lankar, D., et al. (2008). Regulation of dendritic cell migration by CD74, the MHC class II-associated invariant chain. *Science, 322*(5908), 1705−1710.

Heuze, M. L., Collin, O., Terriac, E., Lennon-Dumenil, A. M., & Piel, M. (2011). Cell migration in confinement: a micro-channel-based assay. *Methods in Molecular Biology, 769*, 415−434.

Lloyd, A. C. (2013). The regulation of cell size. *Cell, 154*(6), 1194−1205.

Männik, J., Driessen, R., Galajda, P., Keymer, J. E., & Dekker, C. (2009). Bacterial growth and motility in sub-micron constrictions. *Proceedings of the National Academy of Sciences of the United States of America, 106*(35), 14861−14866.

Marshall, W. F., Young, K. D., Swaffer, M., Wood, E., Nurse, P., Kimura, A., et al. (2012). What determines cell size? *BMC Biology, 10*, 101.

Martin, S. G. (2009). Geometric control of the cell cycle. *Cell Cycle, 8*(22), 3643−3647.

Martin, S. G., & Berthelot-Grosjean, M. (2009). Polar gradients of the DYRK-family kinase Pom1 couple cell length with the cell cycle. *Nature, 459*(7248), 852−856.

Mitchison, J. M., & Nurse, P. (1985). Growth in cell length in the fission yeast *Schizosaccharomyces pombe*. *Journal of Cell Science, 75*, 357–376.

Moseley, J. B., Mayeux, A., Paoletti, A., & Nurse, P. (2009). A spatial gradient coordinates cell size and mitotic entry in fission yeast. *Nature, 459*(7248), 857–860.

Moseley, J. B., & Nurse, P. (2010). Cell division intersects with cell geometry. *Cell, 142*(2), 184–188.

Nakamura, T., Nakamura-Kubo, M., Hirata, A., & Shimoda, C. (2001). The Schizosaccharomyces pombe spo3^{+} gene is required for assembly of the forespore membrane and genetically interacts with psy1(+)-encoding syntaxin-like protein. *Molecular Biology of the Cell, 12*(12), 3955–3972.

Nurse, P., Thuriaux, P., & Nasmyth, K. (1976). Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. *Molecular and General Genetics, 146*(2), 167–178.

Pan, K. Z., Saunders, T. E., Flor-Parra, I., Howard, M., & Chang, F. (2014). Cortical regulation of cell size by a sizer cdr2p. *Elife, 3*, e02040.

Tatebe, H., Nakano, K., Maximo, R., & Shiozaki, K. (2008). Pom1 DYRK regulates localization of the Rga4 GAP to ensure bipolar activation of Cdc42 in fission yeast. *Current Biology, 18*(5), 322–330.

Terenna, C. R., Makushok, T., Velve-Casquillas, G., Baigl, D., Chen, Y., Bornens, M., et al. (2008). Physical mechanisms redirecting cell polarity and cell shape in fission yeast. *Current Biology, 18*(22), 1748–1753.

Turner, J. J., Ewald, J. C., & Skotheim, J. M. (2012). Cell size control in yeast. *Current Biology, 22*(9), R350–R359.

Villar-Tajadura, M. A., Coll, P. M., Madrid, M., Cansado, J., Santos, B., & Perez, P. (2008). Rga2 is a Rho2 GAP that regulates morphogenesis and cell integrity in *S. pombe*. *Molecular Microbiology, 70*(4), 867–881.

Weibel, D. B., Diluzio, W. R., & Whitesides, G. M. (2007). Microfabrication meets microbiology. *Nature Reviews Microbiology, 5*(3), 209–218.