

SUPPORTING INFORMATION PARAGRAPH

3D tracking

The computation based on the right hand side reflections can be conducted similarly to that in main text:

$$\Delta z = \frac{-\delta y^{total} + \Delta y \sin(\beta)}{\cos(\beta)} \quad (S1)$$

Finally, Eqs(S1) and (3) can be combined, and the Z position can be deduced based on the positions of the left and right reflected images according to:

$$\Delta y = \frac{\delta_{total}^l + \delta_{total}^r}{2 \cdot \sin(\beta)}$$
$$\Delta z = \frac{\delta_{total}^l - \delta_{total}^r}{2 \cdot \cos(\beta)} \quad (S2)$$

Strains used:

MATa ade2-1::URA3p-tetR-GFP-ADE2 can1-100 his3-11,15 trp1-1 ura3-1 leu2-3,112 nup49::NUP49-GFP HML::tetOp – LEU2 (strain called GA2254 in 2)

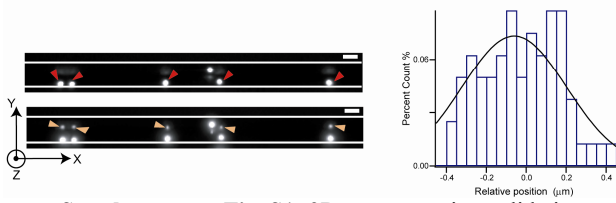
MATa ade2-1 can1-100 his3-11,15::HISp-GFP-LacI-HIS3 trp1-1 ura3-1 leu2-3,112 nup49::NUP49-GFP MAT::lacO-TRP1(strain called GA2196 in 2)

MATa ade2-1::HIS3p-GFP-lacI-URA3p-tetR-GFP-ADE2 can1-100 his3-11,15 trp1-1 ura3-1 leu2-3,112 HML::tetO-LEU2 HMR::lacO-TRP1(strain called GA2195 in18)

Supplementary Figure legends

Figure S1

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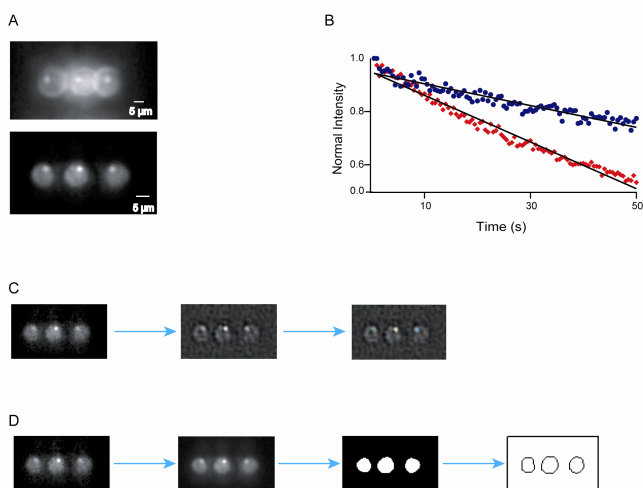


:Supplementary Fig. S1: 3D reconstruction validation.

5 The validation of 3D reconstruction is usually performed by attaching
fluorescent particles to a nano-positioning device, and by monitoring
controlled displacements based on image analysis (see e.g. ⁴). This
experiment, which requires inserting the actuating device inside V-shaped
channels, cannot be conducted in our Lab-on-Chip. Hence, we designed a
10 controlled 3D scene, in which 1 μm fluorescent particles are non-
specifically attached to the bottom surface of the Lab-on-Chip (red
arrowheads in upper left panel). These particles are therefore located on
the same z-plane, and we measured their relative z-positions based their
reflected images (orange arrowheads in lower left panel). The particles
15 indeed appeared to be all in the same plane, on average -45 ± 306 nm apart
(right panel), thus validating our approach given that particles are
heterogeneously bound to the surface, and that 45 nm only represents 5%
of the beads' diameter.

Figure S2

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Supplementary Fig. S2: Bleaching rate and *in vivo* 3D reconstruction.

(a) Yeast nuclei appeared to overlap with their reflections using 35° mirrors (upper panel), whereas well-separated points of views were obtained with 54° mirrors (lower panel), confirming our *in vitro* observation that 35° mirrors are less efficient for 3D microscopy.

(b) Mirrors are powerful tools for multiple vantage point microscopy. This feature is bound to the reflection of the fluorescence light through the mirrors. Notably, excitation illumination is also reflected through the mirrors, inducing an enhanced photobleaching that was quantified by monitoring the fluorescence intensity of the same yeast culture with and without mirrors (blue and red curves, respectively). The photobleaching rate was 2.1 fold increased with mirrors, according to linear fitting (solid curves).

(c) 3D tracking after live cell imaging proceeds in 2 steps. Raw data (left image) are first filtered with a spot enhancing filter for enhancing the patterns of interest²⁰. Then, loci, which are defined as 3 pixels structures, are tracked using the particle tracker ImageJ plugin²¹, and the 2D trajectories of the locus and its reflections are measured (green, yellow and blue tracks). These three trajectories are finally combined using Eqs 3, S1, or S2 in order to reconstruct 3D movements. Note that the consistency of the Z measurements (see Fig. 2) is similar *in vivo* and *in vitro*, as we measure typical errors of 65 nm in living yeasts (data not shown). Hence, we generally use the two most contrasted structures (Fig. S2c) for 3D reconstruction.

When the nucleus is not static, its movements are recorded using the particle tracker plugin defining a kernel of 15 pixels. The locus trajectory taking the nucleus as a reference is obtained by subtracting the locus trajectory to that of the nucleus.

(d) The nuclear volume reconstruction involves averaging several time points to improve the contrast of the nucleus (one image is represented in the left panel, and the temporal average is shown on the second panel).

Assuming that the nucleus is spherical (third panel), its center and diameter were determined after thresholding using the Fit Ellipse plugin of ImageJ (right panel). 3D plots were represented using the software Graphis (www.kylebank.com).