1. Apparent domain diameter $d_a$ and true domain diameter $d$ differ by a size independent term $\delta$

$\delta$ has two constituents:

$$\delta = \delta_d + \delta_s,$$

where $\delta_d$ and $\delta_s$ are the apparent increments in $d$ due to diffraction limitations and domain diffusion, respectively. Both $\delta_d$ and $\delta_s$ do not depend on $d$ in a wide range of domain diameters, as we show below, thereby justifying the assumption that $\delta$ varies negligibly, i.e. $d = d_a - \delta$ for all domains.

1.1 The invariance of $\delta_d$

Domain displacement during the time $t_d$ of its image acquisition gives rise to a seemingly larger domain size. The effect scales with the diffusion coefficient $D$ of the domain:

$$\delta_d \approx \sqrt{4Dt_d},$$

$t_d$ depends on the apparent domain diameter $d_a$, more precisely on the number of lines $N_L$ that the microscope has to scan in order to acquire the domain image. For the diameter of a single pixel $d_p$ (~220 nm) we find:

$$N_L \approx \frac{d_a}{d_p}.$$  

Since acquisition of 1024 lines takes about 1.7 seconds, we may write for $t_d$:

$$t_d \approx \frac{d_a}{d_p} \frac{1.7s}{1024} \approx c_1 d_a,$$

where $c_1 = 0.0075$ s $\mu$m$^{-1}$. The lateral displacement of the diffusing domain during acquisition of its image can be estimated as:

$$\delta_s \approx \sqrt{4Dc_1 d_a},$$
where the experimentally determined product $Dd_a$ for $d_a < 1.7 \, \mu m$ is equal to (i) $1.32 \, \mu m^{3/2} \, s^{-1/2}$ for LODs in LDDs and (ii) $0.67 \, \mu m^{3/2} \, s^{-1/2}$ for LDDs in LODs (SFig. 1). Accordingly, Eq. S5 returns for the respective values of $\delta_c$: $0.22 \, \mu m$ and $0.12 \, \mu m$.

Supplementary Figure 1. The difference between apparent domain diameter ($d_a$) and true domain diameter ($d$) due to non-instantaneous image acquisition is independent of domain size. We measure (i) a smaller diffusion coefficient $D$ and (ii) a longer scanning time (proportional to $d_a$) for larger domains. As a result, $\sqrt{Dd_a}$ does not depend on $d_a$. This is true both for LODs diffusing in LDDs (black circles) and LDDs diffusing in LODs (white circles). The horizontal dotted lines correspond to the best fit of the dependencies.

1.2 The invariance of $\delta_d$

Diffraction limitations let domain diameter $d$ appear larger by $\delta_d$. $\delta_d$ does not depend on $d$. Rather, it is a function of both pixel size $d_p$ and diameter of the focal area. In our case, the latter is roughly twice the size of the former (the exact ratio depending on the wavelength). Since we are taking into account only domains that are well separated in space and since every pixel contains several of fluorescent dye molecules, $\delta_d$ is equal to the distance at which fluorescence intensity (i) drops from the brightness of an LDD domain to that of an LOD domain or (ii) increases from the brightness of an LOD domain to that of an LDD domain. This distance may vary between $d_p$ and $2d_p$ depending on the positioning of the domain boarder (red circle in SFig. 2).

Supplementary Figure 2. Schematic representation of the diffraction induced error to the determination of domain size $d$. (a) — LOD in LDD; (b) — LDD in LOD. The domain appear to
have size $d_a$, because fluorescence brightness between the dimmer LOD regions (dark gray) and brighter LDD regions (light gray) does not change instantaneously, but at least over the distance $d_p$ of one pixel (gray).

2. Selection of domain trajectories

In order to decide whether traces of single domain diffusion should enter the analysis, we calculated the straightness of the diffusion trajectory, $S$:

$$S = \frac{\sum_{i=1}^{N-1} |\mathbf{x}_i - \mathbf{x}_{i-1}|}{|\mathbf{x}_N - \mathbf{x}_0|},$$

where $N$ is a number of consecutive two dimensional positions of a tracked particle (domain), $\mathbf{x}_i$ is the radius-vector of the particle center of mass at the $i$-th step of the trajectory [32]. $S$ is a build in criteria of the software (Image J). We required $S \leq 0.2$. Its use for the exclusion of events that are not compatible with simple diffusion is appealing, because $S$ also excludes events with rather short trajectories.

3. Domain mobility function of apparent domain radius

![Supplementary Figure 3](image)

Supplementary Figure 3. Dependence of (i) LOD mobility in LDDs and (ii) LDD mobility in LODs on the apparent diameter of the domain. LOD are indicated by filled circles, LDD by empty circles. For experimental conditions see Fig. 5.