Solid fat content

Solid fat content as a function of weight percent added HCO for both systems is shown in Figure S1. For the same weight percent added solid fat, SFC values in LMO are consistently lower than those in CO. These differences may be due to solubilization of HCO solids in LMO. As the molecular weight of LMO is ca. 350 g/mol and that of CO is ca. 900 g/mol, on a molar basis, there will be significantly more LMO molecules per HCO molecule present compared to CO. Beyond this effect, LMO is more nonpolar than CO as it consists of \( n \)-alkane chains, which should make it a better solvent for solid fat than the relatively more polar CO molecules.

![Figure S1](image)

**Figure S1** Solid fat content versus weight percent added HCO for both systems.

Data normalization

At each time \( t \), the spatially averaged intensity within the centered region of interest \( F_{ROI}(t) \) was normalized by the corresponding background intensity \( F_{bkg}(t) \) to eliminate any arbitrary fluctuations in overall intensity affecting the curve fitting. Figure S2 shows an example of both, where it is clear that the background intensity \( F_{bkg}(t) \) showed no trend throughout the recovery phase. Thus, the chosen frame rate was slow enough to avoid bleaching the incoming dye molecules. The increase in overall intensity after fluorescence intensity has fully recovered is observed randomly in < 10% of samples. We attribute it to fluctuations in the laser intensity. We assume it is not due to any physical change in the system (i.e., melting) as 1) heating would actually lead to decreasing quantum yield\(^1\) and 2) the laser exposure time is constant at 147.46 ms while, at \( t > 300 \) s, the frame rate is much longer (90 s), so any heating is negligible. This effect is an unphysical consequence of the instrumentation that necessitates this background normalization.

Bleaching z-axis profile

The assumption was that using an objective of low NA would create an effectively columnar bleach ROI in 3D, whereas a higher NA objective would show a steep increase in \( \omega_B \) further from the plane of focus\(^2\). We explored this by capturing a stack in the \( xyz \) mode that spanned 33 \( \mu \)m in the z-direction immediately after bleaching. This data used \( \omega_B = 72 \) \( \mu \)m and a larger frame size of \( 307.2 \times 177.6 \) \( \mu \)m but still were captured with the 10\( \times \) (0.25 NA) objective. Figure S3 shows radially-averaged intensities at
Figure S2 20/80 HCO/CO raw data vs time: $F_{\text{ROI}}(t)$ is the averaged intensity within the circular region of interest, while $F_{\text{bkg}}(t)$ is the average background intensity.

different planes within the stack, all normalized by the focal plane during bleaching. The intensity profiles 6 µm above and below the focal plane were very similar to the profiles in the frames adjacent to the focal plane ($\pm 0.7$ µm). Further out, a widening of the bleach spot size was apparent $\geq \pm 12$ µm from the focal plane, indicating that the bleach volume slightly tapered, as to be expected. Still, the flat intensity profile in the ROI indicated that comparable bleaching was achieved above and below the focal plane for distances greater in the z-direction than would be bleached in the x- and y-directions for the $\omega_B$ chosen in the experiment.

Confocal pinhole size

The majority of data were taken with a pinhole size of 1 Au, corresponding to an optical thickness of $<16.8$ µm. We also took data for one sample, 15/85 HCO/LMO (w/w), using a fully open pinhole to test whether that would affect the calculated diffusion coefficient. Based on the low NA and bleached volume axial profile in Figure S3, we again applied the 2D recovery fit from Equation 1 to the normalized data and compared the

$$f_k(t) = a + \sum_{n=1}^{m} \frac{(-K_0)^n}{n!\sqrt{1+n}} \left( 1 - e^{-2\tau/t} \left[I_0 \left( \frac{2\tau}{t} \right) + I_1 \left( \frac{2\tau}{t} \right) \right] \right)$$

where $K_0$ is the bleaching depth parameter, $a$ is a fitting parameter to account for the possibility of any immobile fraction, and $\tau = \omega_{\text{ROI}}^2/4D$ is the radial diffusion time. The diffusion coefficient $D$ is the parameter of interest here, with average values of $1.03 \pm .02 \times 10^{-11}$ m²/s using a 1 Au pinhole and $1.00 \pm .07 \times 10^{-11}$ m²/s using a fully open pinhole. The change in pinhole size does not create a statistically significant difference in $D$, supporting the assumption that the intensity recovery occurs in 2D.

References


Figure S3 Normalized intensity as a function of radial distance from center. Data were normalized at each pixel by the focal plane during bleaching. Data were shifted for clarity.