FRAP Analysis Report

Fluorescence Recovery After Photobleaching

Samples: 100 uM Oleic Acid vs SCDi Treatment

Date: December 12, 2025

Data: 12_04_2025_FRAP.lif

Note: This analysis is based on n=1 cell per condition. Additional biological replicates (n>=3) are required for publication-quality statistics.

Executive Summary

This report presents the analysis of Fluorescence Recovery After Photobleaching (FRAP) experiments comparing two treatment conditions: 100 uM Oleic Acid (OA) and SCD inhibitor (SCDi). FRAP analysis reveals differences in molecular dynamics between these treatments.

Key Findings

1. Recovery Kinetics: SCDi treatment shows significantly slower fluorescence recovery (t1/2 = 1.31s) compared to 100 uM OA treatment (t1/2 = 0.60s), representing a ~2.2x difference.

2. Mobile Fractions: Both treatments show similar mobile fractions (~55%), indicating comparable proportions of freely diffusing molecules.

3. Model Fit: Single exponential recovery model provides excellent fits (R2 > 0.99) for both conditions, suggesting a single population of mobile molecules.

Summary Statistics

Parameter	100 uM OA	SCDi	Difference
Mobile Fraction (%)	55.6	54.5	-1.2
Immobile Fraction (%)	44.4	45.5	1.2
Time Constant tau (s)	0.867	1.884	1.017
Half-time t1/2 (s)	0.601	1.306	0.705
R-squared	0.9998	0.9962	-
N (cells)	1	1	-

Methods

Data Acquisition

FRAP experiments were performed using a Leica confocal microscope. Images were acquired as .lif files containing both pre-bleach and post-bleach time series. Time interval between frames: ~0.72 seconds.

Samples analyzed:
- 100 uM Oleic Acid: Series 9 (pre-bleach), Series 10 (post-bleach) - 5 recovery frames
- SCDi: Series 18 (pre-bleach), Series 19 (post-bleach) - 10 recovery frames

ROI Detection

Three regions of interest (ROIs) were automatically detected:

1. Bleach ROI: Identified from the difference between pre-bleach and first post-bleach images. Pixels showing >85th percentile intensity drop were included. Morphological operations (opening/closing) were applied to clean the mask.

2. Reference ROI: Cell region excluding a 5-pixel buffer around the bleach ROI. Used for photofading correction.

3. Background ROI: Lowest 5% intensity pixels (outside cells). Used for background subtraction.

Normalization (Double Normalization - Phair Method)

Data were normalized using the double normalization method (Phair et al., 2004):

F_norm(t) = [(F_ROI(t) - F_bkgd) / (F_ref(t) - F_bkgd)] x [(F_ref(i) - F_bkgd) / (F_ROI(i) - F_bkgd)]

This corrects for:
- Background fluorescence
- Acquisition photobleaching (photofading)
- Differences in starting intensity between cells

Full-scale normalization was then applied to scale data from 0 (post-bleach) to 1 (pre-bleach).

Curve Fitting

Recovery curves were fit to a single exponential model:

F(t) = Mf x (1 - exp(-t/tau))

Where:
- Mf = Mobile fraction (proportion of molecules that recover)
- tau = Time constant of recovery
- t1/2 = tau x ln(2) = Half-time of recovery

Fitting was performed using scipy.optimize.curve_fit with bounds: Mf in [0, 1.5], tau in [0.001, 1000].

Detailed Results

100 uM Oleic Acid Treatment

ROI Statistics:
- Bleach ROI: 1112 pixels
- Reference ROI: 206151 pixels
- Background intensity: 0.00

Acquisition:
- Pre-bleach frames: 2
- Post-bleach frames: 5
- Time interval: 0.718 s
- Total recovery time: 2.87 s

Intensity Time Course

Time (s)	Raw Intensity	Double Norm	Full Scale Norm
0.00	8.14	0.3557	0.0000
0.72	12.19	0.5571	0.3127
1.44	14.09	0.6476	0.4531
2.15	14.94	0.6809	0.5047
2.87	15.32	0.7029	0.5389

Fit Results (Single Exponential):
- Mobile Fraction: 55.64%
- Immobile Fraction: 44.36%
- Time Constant (tau): 0.8675 s
- Half-time (t1/2): 0.6013 s
- R-squared: 0.9998

SCDi Treatment

ROI Statistics:
- Bleach ROI: 3212 pixels
- Reference ROI: 199761 pixels
- Background intensity: 1.74

Acquisition:
- Pre-bleach frames: 2
- Post-bleach frames: 10
- Time interval: 0.717 s
- Total recovery time: 6.45 s

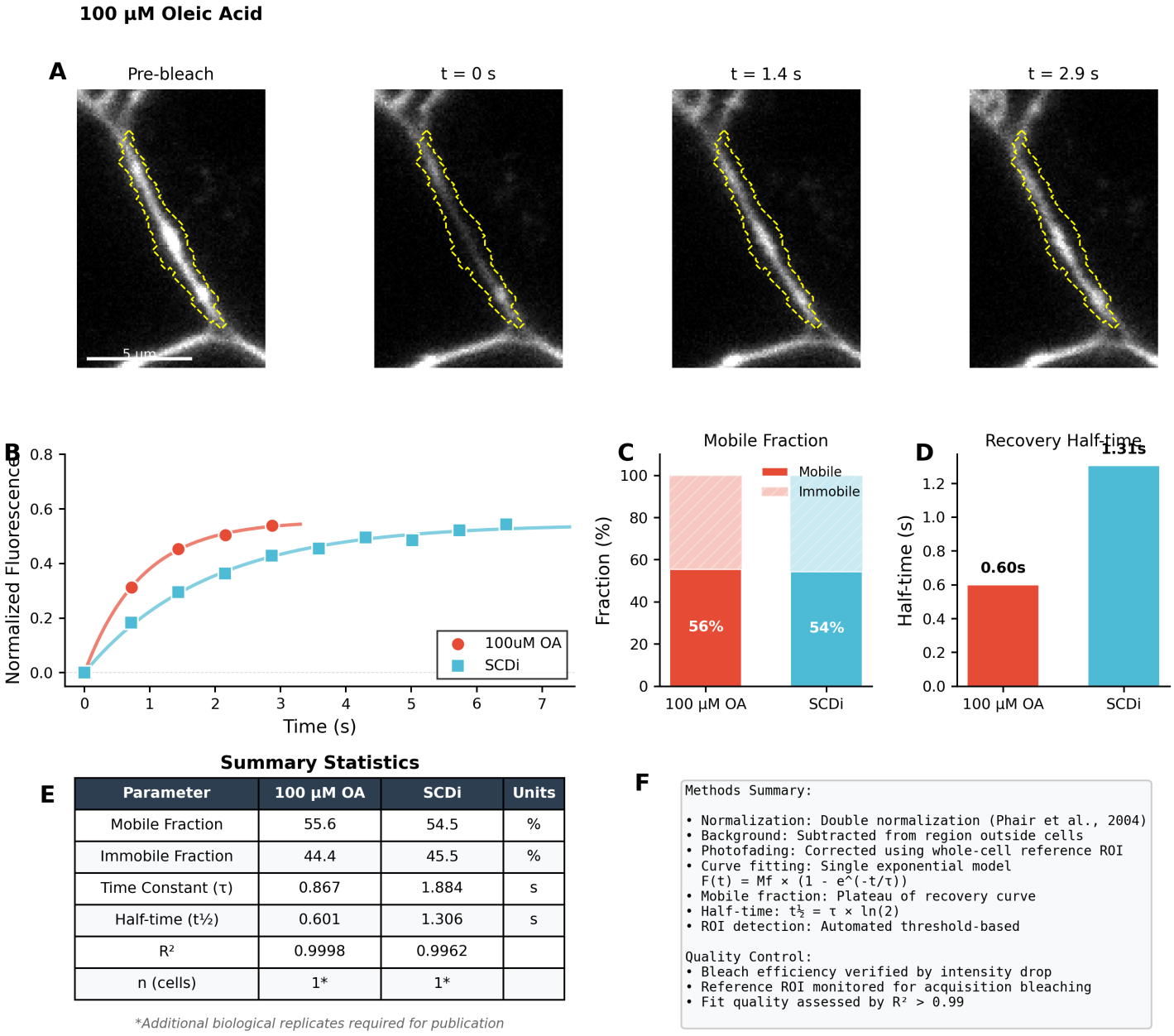
Intensity Time Course

Time (s)	Raw Intensity	Double Norm	Full Scale Norm
0.00	16.80	0.4191	0.0000
0.72	20.28	0.5253	0.1828
1.43	22.41	0.5905	0.2951
2.15	23.10	0.6299	0.3628
2.87	22.83	0.6684	0.4291
3.58	22.48	0.6834	0.4550
4.30	22.78	0.7066	0.4949
5.02	22.40	0.7014	0.4859
5.73	22.37	0.7218	0.5210
6.45	22.14	0.7347	0.5433

Fit Results (Single Exponential):
- Mobile Fraction: 54.47%
- Immobile Fraction: 45.53%
- Time Constant (tau): 1.8844 s
- Half-time (t1/2): 1.3062 s
- R-squared: 0.9962

Figures

Figure 1: Main Publication Figure



Summary Statistics

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Parameter	100 µM OA	SCDi	Units
Mobile Fraction	55.6	54.5	%
Immobile Fraction	44.4	45.5	%
Time Constant (τ)	0.867	1.884	s
Half-time (t½)	0.601	1.306	s
R^2	0.9998	0.9962	
n (cells)	1*	1*	

**Additional biological replicates required for publication*

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Methods Summary:

• Normalization: Double normalization (Phair et al., 2004)
• Background: Subtracted from region outside cells
• Photofading: Corrected using whole-cell reference ROI
• Curve fitting: Single exponential model
  F(t) = Mf × (1 - e^(-t/τ))
• Mobile fraction: Plateau of recovery curve
• Half-time: t½ = τ × ln(2)
• ROI detection: Automated threshold-based

Quality Control:
• Bleach efficiency verified by intensity drop
• Reference ROI monitored for acquisition bleaching
• Fit quality assessed by R² > 0.99
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Figure 1. FRAP analysis of 100 uM OA and SCDi treatments. (A) Representative images showing pre-bleach, immediate post-bleach, and recovery time points. (B) Normalized recovery curves with single exponential fits. (C) Mobile and immobile fractions. (D) Recovery half-times. (E) Summary statistics table. (F) Methods summary.

Figure S1: Individual Recovery Curves

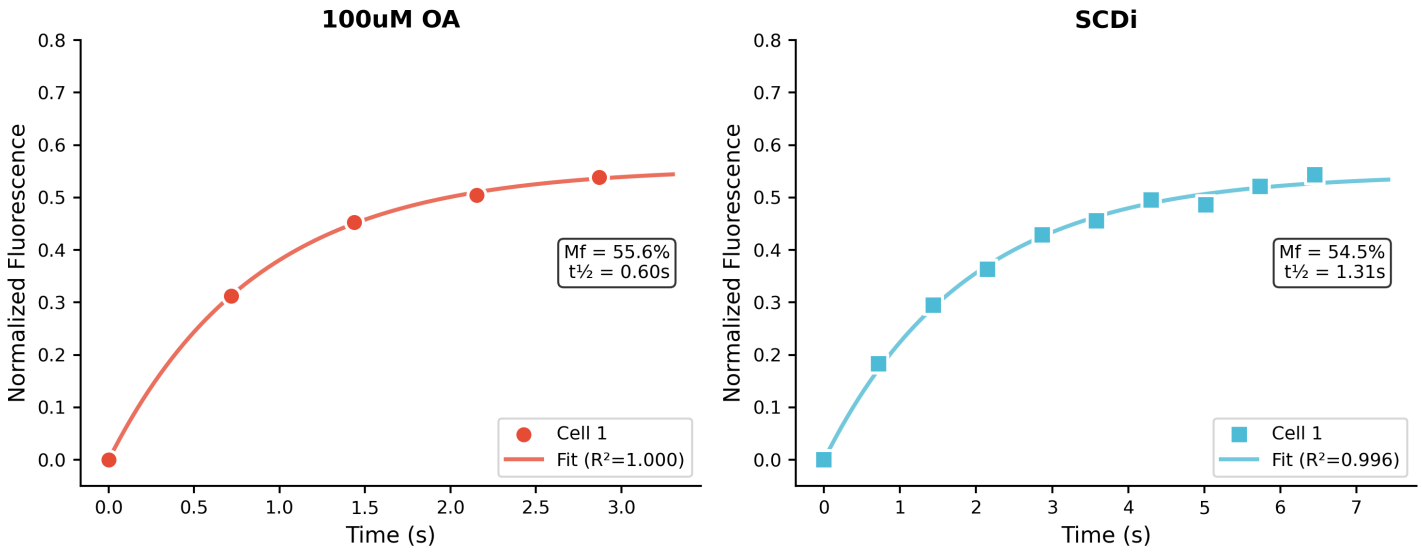

Figure S1. Individual FRAP recovery curves for each treatment condition with fitted curves and parameter annotations. Template for displaying multiple cells when additional replicates are collected.

Figure S2: ROI Visualization

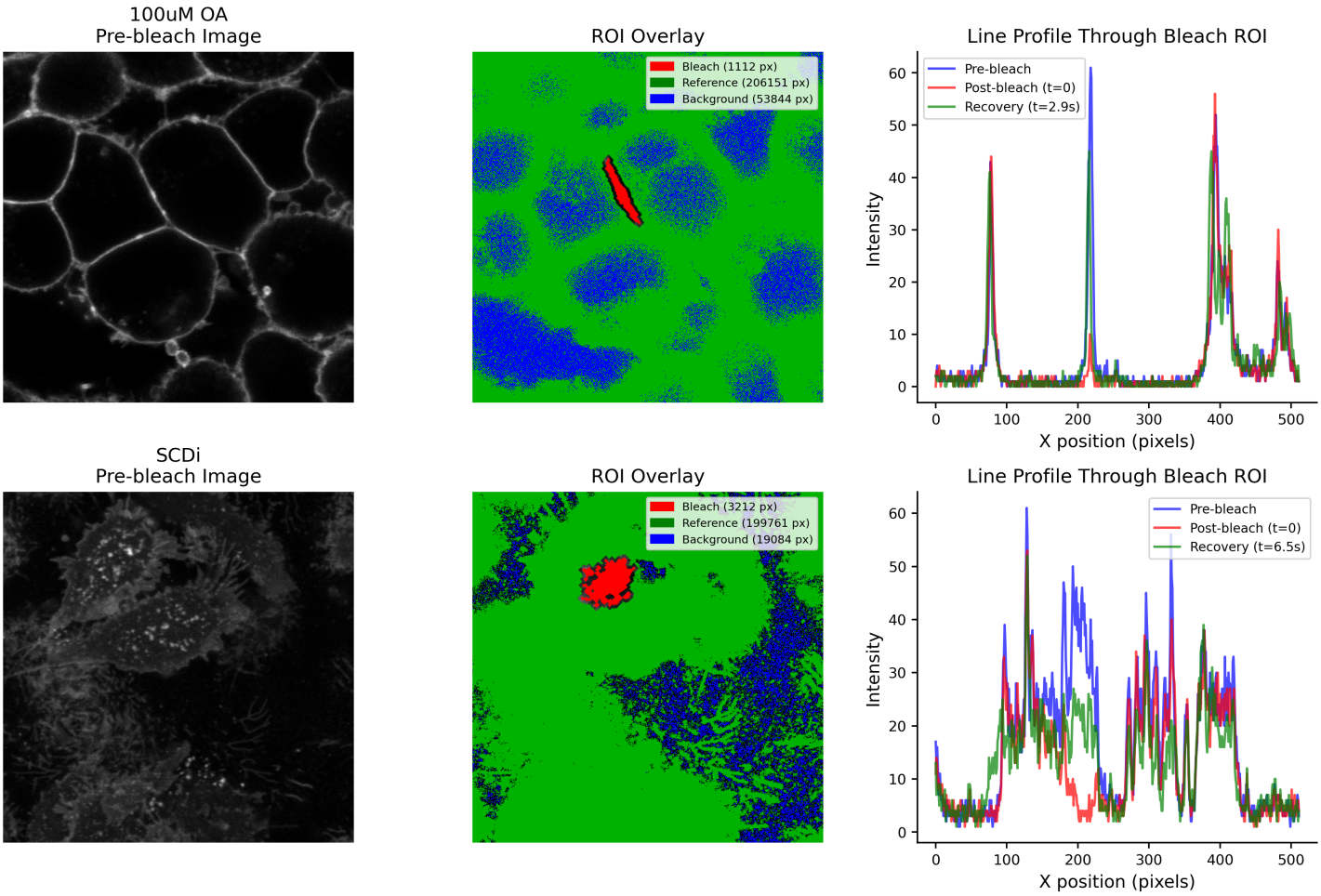

Figure S2. ROI visualization for quality control. Left: Pre-bleach images. Center: ROI overlay (red=bleach, green=reference, blue=background). Right: Line intensity profiles through bleach region showing recovery.

Conclusions

This preliminary FRAP analysis reveals distinct molecular dynamics between 100 uM Oleic Acid and SCDi treatments:

1. SCDi treatment results in significantly slower fluorescence recovery (t1/2 = 1.31s) compared to 100 uM OA (t1/2 = 0.60s), a ~2.2-fold difference.

2. Both treatments show similar mobile fractions (~55%), suggesting comparable proportions of freely mobile vs. immobilized molecules.

3. The slower recovery in SCDi-treated cells may indicate:
- Reduced molecular diffusion rates
- Increased molecular interactions/binding
- Changes in membrane or organelle properties

4. Single exponential fits are adequate for both conditions, suggesting a single dominant population of mobile molecules.

Recommendations for Publication

To prepare this data for publication in a high-impact journal, the following additional experiments and analyses are recommended:

1. BIOLOGICAL REPLICATES: Collect data from at least n=3 independent experiments (performed on different days with fresh cell preparations).

2. TECHNICAL REPLICATES: Analyze 12-14 cells per condition per experiment.

3. CONTROLS:
- Photofading control: Image without bleaching to quantify acquisition bleaching
- Negative control: Untreated cells
- Positive control (if applicable): Known condition affecting mobility

4. STATISTICAL ANALYSIS:
- Report mean +/- SEM or SD for all parameters
- Perform t-test or ANOVA for comparing conditions
- Include p-values and effect sizes

5. DATA PRESENTATION:
- Show individual cell curves (not just means)
- Include source data files
- Deposit raw data in public repository

References

1. Phair RD, Gorski SA, Bhaskaran T (2004). Measurement of dynamic protein binding to chromatin in vivo, using photobleaching microscopy. Methods Enzymol 375:393-414.

2. Ellenberg J et al. (1997). Nuclear membrane dynamics and reassembly in living cells: targeting of an inner nuclear membrane protein in interphase and mitosis. J Cell Biol 138:1271-1287.

3. Roca-Cusachs P et al. (2013). Quantifying forces in cell biology. Nat Cell Biol 19:742-751.

4. Lippincott-Schwartz J, Snapp E, Kenworthy A (2001). Studying protein dynamics in living cells. Nat Rev Mol Cell Biol 2:444-456.