Comments to the Editor

Proposed Correction to Feder's Anomalous Diffusion FRAP Equations

In an important research article in *Biophysical Journal*, Feder et al. (1) introduced the idea that proteins in cell membranes undergo a form of restricted, time-dependent lateral mobility due to anomalous diffusion. In doing so, they provided a plausible explanation for why the presence of the so-called immobile fraction is often required to fully describe fluorescence recovery after photobleaching (FRAP) measurements when a free-diffusion model is used to fit the data. These findings helped to shape current models of cell-membrane organization, and models incorporating anomalous diffusion have subsequently been applied more generally to other cellular compartments as well.

Feder et al. started with the assumption that the mean-squared displacement (MSD; $\langle x^2\rangle$) of anomalously diffusing molecules is related to a certain power of time by $\langle x^2\rangle = \Gamma t^{\alpha}$, where Γ and α are referred to as a transport coefficient and an anomalous exponent, respectively. By analogy to the relationship between the MSD and diffusion coefficient D for purely diffusing molecules in $\mathbb{R}^2$, $\langle x^2\rangle = 4Dt$, the authors introduced a time-dependent diffusion coefficient as

$$\langle x^2\rangle = \Gamma t^{\alpha} = \left(\Gamma t^{\alpha-1}\right)t = 4D_{\text{Feder}}(t)t, \tag{1}$$

where

$$D_{\text{Feder}}(t) = \frac{1}{4}\Gamma t^{\alpha-1} \tag{2}$$

for $\Gamma>0$ and $0<\alpha\leq 1$. Based on $D_{\text{Feder}}(t)$, they then proposed two FRAP equations for data fitting based on this anomalous diffusion model (Eqs. 2 and 3 in Feder et al. (1)). These equations are referred as the Feder Equations and still being widely used in anomalous diffusion research (2–8).

In the process of rederiving the Feder equations, we discovered that the choice of $D_{\text{Feder}}(t)$, τ_{Feder}, and MSD are not consistent. By introducing a new timescale, $s = \int_0^t \frac{1}{4}\Gamma t'^{\alpha-1}dt' = \frac{1}{4\alpha}\Gamma t^{\alpha}$ (9), we found that for $D_{\text{Feder}}(t)$ in Eq. 2, the MSD should be given by $\langle x^2\rangle = \frac{1}{\alpha}\Gamma t^{\alpha}$, not $\langle x^2\rangle = \Gamma t^{\alpha}$ (see Appendix for full derivation). In addition, $\tau_{\text{Feder}} = \left(\omega^2/\Gamma\right)^{1/\alpha}$ in the Feder equation (Eq. 2 in Feder et al. (1)) should be given by

$$\tau_{\text{Corrected}} = \left(\alpha\omega^2/\Gamma\right)^{1/\alpha}. \tag{3}$$

In fact, a similar scaling error had become widely spread in FCS studies associated with an anomalous diffusion model with time-dependent diffusion coefficient until Wu and Berland recently pointed out a possible α dependency of the diffusion coefficient in fluorescence correlation spectroscopy (FCS) in a similar context and corrected it for the FCS formula (10).

We therefore propose the following corrected version of the Feder equations in $\mathbb{R}^d$. To differentiate the corrected equation from the original version, we renamed the variables, setting the new transport coefficient as $\overline{\Gamma}$ and the new anomalous exponent as $\overline{\alpha}$. Thus, for a diffusion equation with a time-dependent diffusion coefficient,

$$\frac{\partial u}{\partial t} = D(t)\nabla^2 u, \tag{4}$$

where $D(t) = \overline{\Gamma}t^{\overline{\alpha}-1}$, the MSD for spatial dimension d is given by

$$\langle x^2\rangle = \frac{2d}{\overline{\alpha}}\overline{\Gamma}t^{\overline{\alpha}}.$$

The corresponding FRAP equation is

$$\begin{aligned} F(t) &= \left\{F_i\sum_{n=0}^{\infty}\frac{(-K)^n}{n!}\frac{1}{\left[1+n(\gamma^2+8\overline{\Gamma}t^{\overline{\alpha}}/(\overline{\alpha}\omega^2))\right]^{d/2}}\right\}R \\ &\quad + (1-R)F_0 \\ &= \left\{F_i\sum_{n=0}^{\infty}\frac{(-K)^n}{n!}\frac{1}{\left[1+n\left(\gamma^2+2(t/\tau_{\text{Corrected}})^{\overline{\alpha}}\right)\right]^{d/2}}\right\}R \\ &\quad + (1-R)F_0, \end{aligned} \tag{5}$$

where in the first equation, R is the mobile fraction, defined as $R = (F_\infty - F_0)/(F_i - F_0)$ for the prebleach (F_i), postbleach (F_0), and steady-state (F_∞) fluorescence intensities, K is a parameter related to the bleach depth, $\gamma = 1$, and $\tau_{\text{Corrected}} = (\overline{\alpha}\omega^2/4\overline{\Gamma})^{1/\overline{\alpha}}$. Note that the summation begins with $n = 0$ not $n = 1$, which is a typographical error in Eq. 2 of Feder et al. (1). To incorporate diffusion during photobleaching, which is critical to measure diffusion coefficient accurately by confocal FRAP approaches (11), one can choose $\gamma = \omega_n/\omega$ in Eq. 5, where ω_n is the radius of regions of interest and ω is the effective radius measured from the fluorescence distribution profile immediately after photobleaching. The simplified equation of Feder et al. (Eq. 3 in their article (1)) is still valid, but to recover

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*Correspondence: anne.kenworthy@vanderbilt.edu

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Γ from $t_{1/2} = \beta\tau_\Gamma$, the revised equations (Eq. 5) should be used.

It is important to note that the presence of an additional factor of α in Eq. 3 can impact the magnitude of the transport coefficient, Γ, obtained by fitting to the Feder equation (their Eq. 3 (1)), and thus affect the quantitative interpretation of anomalous diffusion measurements analyzed using the corrected versus uncorrected models, especially for anomalous exponent $\alpha \ll 1$ (See Appendix for an example).

Finally, we wish to note that our proposed correction is solely meant to provide mathematical clarification and does not have any bearing on the physical validity of the anomalous diffusion model of Feder et al. As initially conceived, that model was based on the observation that a time-dependent diffusion coefficient ($D(t)$) can generate a second moment of the probability density that behaves as a power of α, rather than specific theoretical arguments leading to this particular equation. The simplicity of this model, despite its lack of specific mechanistic underpinnings, continues to make it an attractive tool to identify the presence of anomalous diffusion versus free diffusion. As further studies seek to discern the physical and biological mechanisms giving rise to anomalous diffusion in cells and identify appropriate theoretical models to describe them, it will become increasingly important to use more accurately defined experimental descriptors of anomalous diffusion, such as that suggested here for the case of FRAP.

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Minchul Kang,[†] Emmanuele DiBenedetto,[‡] and Anne K. Kenworthy[†§*]

[†]*Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee;*

[‡]*Department of Mathematics, Vanderbilt University, Nashville, Tennessee; and*

[§]*Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, Tennessee*

REFERENCES

1. Feder, T. J., I. Brust-Mascher, …, W. W. Webb. 1996. Constrained diffusion or immobile fraction on cell surfaces: a new interpretation. *Biophys. J.* 70:2767–2773.
2. Ashby, M. C., S. R. Maier, …, J. M. Henley. 2006. Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology. *J. Neurosci.* 26:7046–7055.
3. Basu, S., C. W. Wolgemuth, and P. J. Campagnola. 2004. Measurement of normal and anomalous diffusion of dyes within protein structures fabricated via multiphoton excited cross-linking. *Biomacromolecules.* 5:2347–2357.
4. Georgiou, G., S. S. Bahra, …, R. J. Cherry. 2002. Measurement of the lateral diffusion of human MHC class I molecules on HeLa cells by fluorescence recovery after photobleaching using a phycoerythrin probe. *Biophys. J.* 82:1828–1834.
5. Breckenridge, M. T., N. G. Dulyaninova, and T. T. Egelhoff. 2009. Multiple regulatory steps control mammalian nonmuscle myosin II assembly in live cells. *Mol. Biol. Cell.* 20:338–347.
6. Martin, S., J. M. Henley, …, H. J. Krugers. 2009. Corticosterone alters AMPAR mobility and facilitates bidirectional synaptic plasticity. *PLoS ONE.* 4:e4714.
7. Jaskolski, F., B. Mayo-Martin, …, J. M. Henley. 2009. Dynamin-dependent membrane drift recruits AMPA receptors to dendritic spines. *J. Biol. Chem.* 284:12491–12503.
8. Schulmeister, S., M. Ruttorf, …, V. Sourjik. 2008. Protein exchange dynamics at chemoreceptor clusters in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA.* 105:6403–6408.
9. Crank, J. 1980. The Mathematics of Diffusion, 2nd ed. Oxford University Press, New York.
10. Wu, J., and K. M. Berland. 2008. Propagators and time-dependent diffusion coefficients for anomalous diffusion. *Biophys. J.* 95:2049–2052.
11. Kang, M., C. A. Day, …, E. DiBenedetto. 2009. A generalization of theory for two-dimensional fluorescence recovery after photobleaching applicable to confocal laser scanning microscopes. *Biophys. J.* 97: 1501–1511.