Baffou *et al.* reply: In their Correspondence[1,2], the groups of Mori and Ishiwata argue against our Commentary[3], in which we questioned the possibility of measuring thermogenesis-induced temperature variations in single living cells. We believe that our original conclusions are not challenged by their arguments.

Both groups question the validity of the heat diffusion equation (HDE) at the submicrometer scale. Mori's group believes that temperature fluctuations occurring on the micrometer scale are too large for the HDE to be applicable. In the textbook[4] that the authors cite in support of their argument, we interpret the discussion regarding the 1-μm^3 threshold as concerning a gas phase, not a liquid. As explained[4], the relative energy fluctuation scales as the inverse of the square root of the number of particles in the considered volume. Hence, delimiting in water a cube with edges of 100 nm yields energy fluctuations of relative amplitude in the 2×10^{-4} range, which is negligible, and we therefore feel that this does not refute the validity of the HDE in living cells. Moreover, the relaxation of the energy fluctuation in condensed phases occurs at the 1- to 10-ps timescale. So any fluctuation would be time averaged by the reported thermal imaging techniques, anyway. We note that the HDE can indeed be invalidated on the micrometer scale even for dense media, in some cases. But this occurs only when the size of the system becomes comparable to length scales such as the mean free path of the particles (especially in gas phases) or the mean free path of electrons or phonons (especially in crystals). In these cases, diffusion is replaced by a ballistic regime. Thus, we do not expect such a limitation with living cells at the micrometer scale because the molecular mean free path in liquids is much smaller than 1 μm.

The two groups also question the values of the parameters P, κ and L that we used to derive the orders of magnitude of temperature variations using the equation

$$\Delta T = \frac{P}{\kappa L}$$

First, we note that Ishiwata's group writes that L is the distance from the center of the heat source, whereas we take L to be the size of the heat source. Further, as pointed by Ishiwata's group, the order of magnitude of κ is essentially independent of the chemical nature of biologically relevant condensed phases. In particular, the small variations of the κ values forbid matter heterogeneity of the cell medium to give rise to some singular temperature distribution as argued by both groups. Mori's group adopted the most favorable parameters ($L = 100$ nm and $\kappa = 0.1$ W m^{-1} K^{-1}) to estimate an optimized energy generation in cells to locally increase the temperature by 1 K during 1 s. But taking L to be 100 nm amounts to considering that the whole cell's energy (10 nJ) is produced by a single mitochondrion. Such a view remains far from reality (as it would correspond to a glucose concentration of 4×10^3 mol l^{-1}), and such hypothetical localization and transience of the temperature increase appear to contradict Mori's group's own experimental observations[5].

In order for the community to come to a consensus, we believe some effort will be required to identify the actual origin of the signal measured in these studies, both theoretically and experimentally.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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