Online Supplementary Information:

Optical magnetic imaging of living cells

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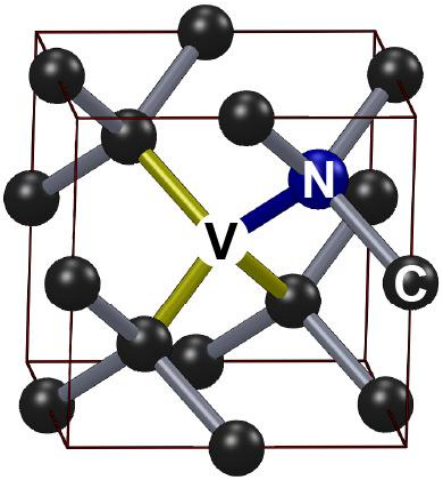
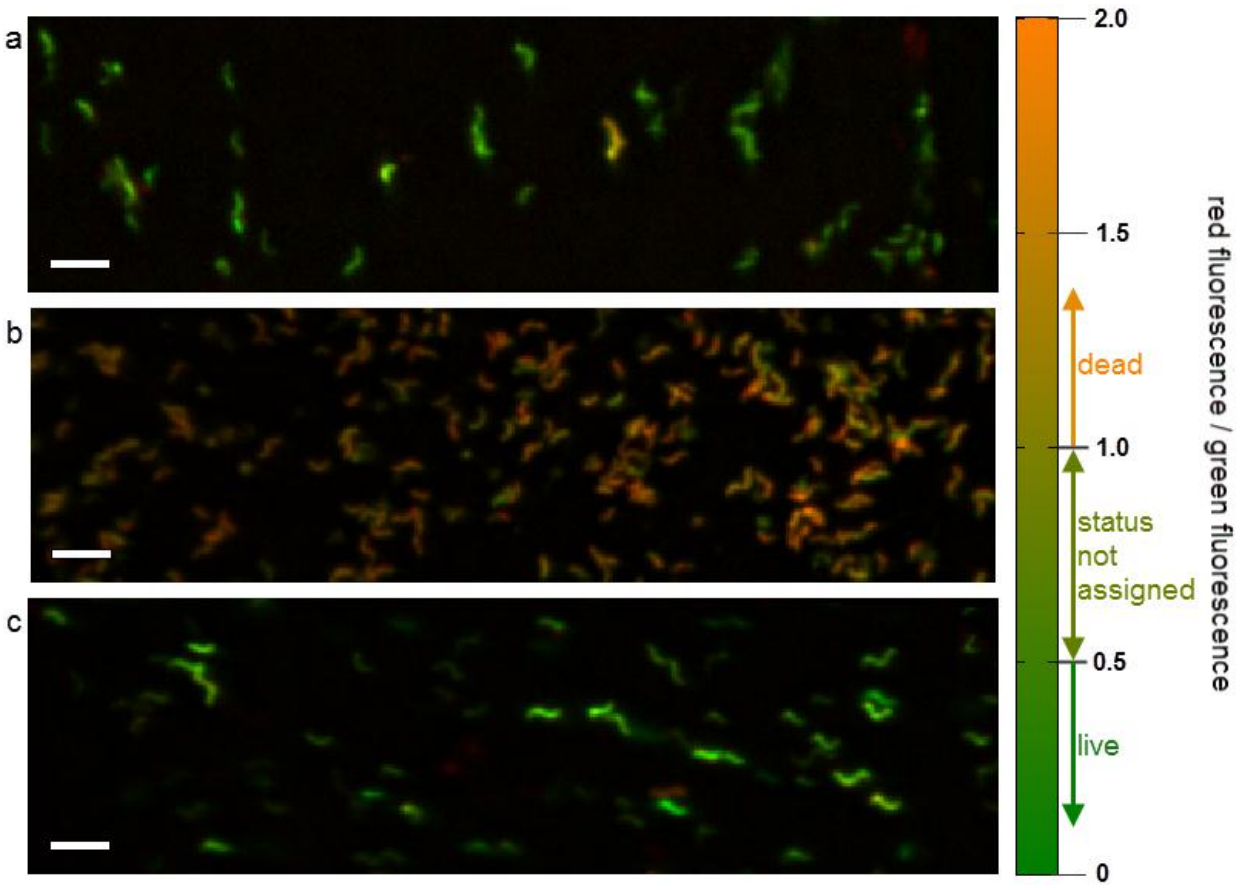
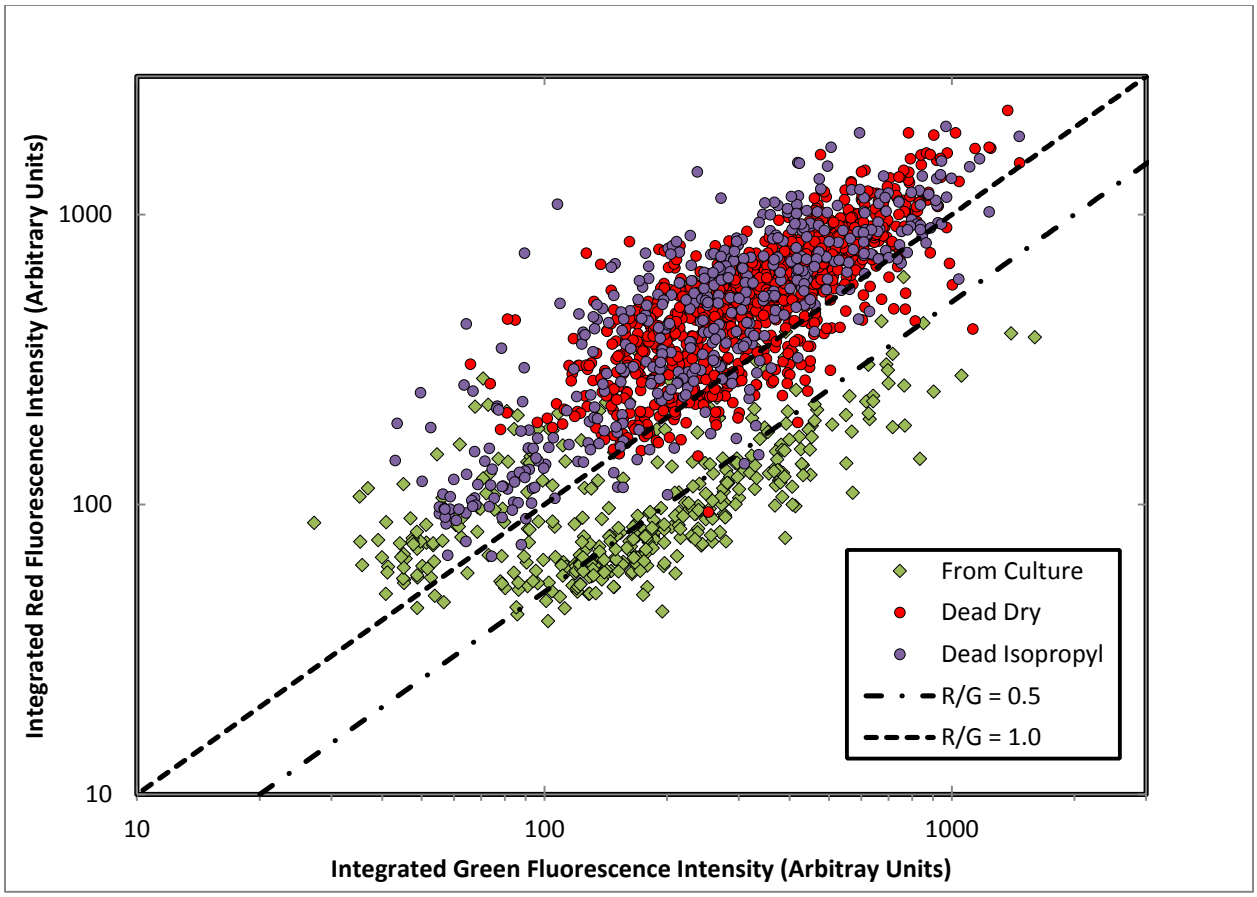
SUPPLEMENTARY FIGURES:



Supplementary Figure 1: The NV centre consists of a substitutional nitrogen atom (N) adjacent to a vacancy (V) in the diamond lattice.



Supplementary Figure 2: Typical fluorescence data used to determine the viability of stained AMB-1 bacteria, for (a) bacteria taken directly from the culture, (b) bacteria that have been intentionally killed, and (c) the same bacteria as in Figure 2a-b of the main text after performing magnetic field measurements. In each case, before measuring the fluorescence, the bacteria were incubated for 15 minutes in a mixture of stains from the BacLight bacterial viability kit (5 µM of green-fluorescent SYTO 9 and 30 µM of red-fluorescent propidium iodide). Red and green fluorescence images were recorded separately, and are shown above as overlapped images in the red and green channels respectively. A quantitative analysis of fluorescence data from many fields of view like those in (a) and (b) suggests that dead bacteria typically exhibit a red-to-green fluorescence ratio > 1, whereas a ratio < 0.5 indicates that a bacterium is very likely to be alive (see Supplementary Figure 3). The viability of cells with intermediate fluorescence ratios is somewhat ambiguous, and these were consequently not assigned to either category. Most bacteria (a) taken directly from culture and (c) post-magnetometry appear to be alive or intermediate, although a small fraction of the bacteria in either case exhibit strong red fluorescence consistent with the dead bacteria in (b). Note that in addition to the bacteria present in Figure 2a-b of the main text, (c) also shows several bacteria that settled onto the diamond surface during the 15 minute incubation period. Scalebars are 5 µm**.**



Supplementary Figure 3: Calibration data for BacLight staining of living and dead samples of *Magnetospirillum magneticum* AMB-1. The viable and non-viable populations divide into clusters identifiable by the ratio of integrated fluorescence in the red channel (vertical axis, R) to that in the green channel (horizontal axis, G). On the basis of these data, we assign cells with fluorescence ratio R/G < 0.5 to be alive, and those with R/G > 1.0 to be dead. The region of the plot with 0.5 < R/G < 1.0 contains tails from both living and dead distributions in the calibration, and hence cells with fluorescence ratios in this range were left unassigned in the assays conducted after magnetic imaging experiments.

SUPPLEMENTARY METHODS:

Bacterial Viability Assay Calibration and Implementation:

Assessments of bacterial viability after magnetic and bright-field imaging were carried out using a standard commercial kit (Molecular Probes, BacLight Kit, L7007). The assay uses competitive binding of two fluorescent nucleic acid stains (green-fluorescent SYTO 9 and red-

fluorescent propidium iodide) at concentrations such that cells with intact membranes (alive) show predominantly green fluorescence, while those with damaged membranes (dead) show less green and more red fluorescence. In order to assess the expected relative fluorescence rates for the two stains in AMB-1 cells in our setup (including all emission filters for each of the two stains, and their relative excitation efficiencies at 470 nm), we initially performed a series of calibration measurements on samples in which either the majority of cells were known to be alive or all were dead. In each case, cells were applied to the diamond surface after it was cleaned and prepared with a drop (~5 µL) of 0.01% poly-L-lysine (Sigma-Aldritch, P4707, mol. wt. 70-150 kDa), which was then allowed to dry. Samples containing live cells were taken from cultures of AMB-1 grown as described in the Methods section of the main text. A 50 µL volume of bacterial solution was pipetted into the bath chamber containing the diamond and topped up with approximately 150 µL of phosphate buffered saline (PBS). The cells were left for 15 minutes to settle onto the diamond surface, and the assay was performed. Dead cell samples were prepared in one of two ways: In the first method (i) a drop (~5 µL) of bacterial solution was placed directly on the PLL-coated diamond surface and allowed to dry completely. Approximately 15 minutes after drying, the bath chamber was filled with 200 µL of PBS and the assay was performed. Alternatively (ii), a 50 µL volume of cells was added to the bath with 150 µL of PBS and allowed to settle for 15 minutes as for the live calibration samples. Once the cells had adhered to the PLL layer on the diamond surface, 50 µL of the bath was removed and replaced with an equal volume of isopropyl alcohol. The cells were incubated in the isopropyl solution for 30 minutes, after which the solution was gently removed and replaced with 200 µL of PBS, and the assay was performed.

Carrying out the assay consisted of adding the stains in appropriate concentrations (5 µM SYTO 9 and 30 µM propidium iodide), and incubating for 15 minutes in the dark. Fluorescence imaging was then performed using 470 nm excitation light from an LED (Thorlabs, M470L2), with appropriate emission filters to completely separate the green (Thorlabs FELH0500 and Thorlabs FES0550) and red (Chroma HQ640/120) channels. The image was carefully re-focused in each channel to account for chromatic aberration in the objective (Olympus UIS2 LumFLM 60xW / 1.1NA), and exposure times were kept equal. Typical green and red fluorescence images are shown in Supplementary Figure 2. (The image shown in Supplementary Figure 2c corresponds to the sample and field of view depicted in Figure 2a-b of the main text.)

Fluorescence images were analyzed using home-made software written in Matlab, in the following steps: (i) Gaussian filtering to remove high frequency pixel noise; (ii) rolling-ball background subtraction; (iii) co-registration of the two images by maximizing their cross-correlation; (iv) determination of bacteria positions by finding all local maxima above some threshold (usually >10 standard deviations above background); (v) assignment of correspondence between identified bacteria in the two images according to minimum distance, with some checking to eliminate ambiguous pairings and duplicates; and (vi) integration of the fluorescence signal around each peak in a 3-pixel radius, which is approximately proportional to the average fluorescence intensity over the whole bacterium.

This procedure was carried out for several fields of view such that about ~500 individual bacteria were analyzed for each calibration sample (Supplementary Figure 3). As expected, almost all cells in the two dead samples showed a relatively high ratio of red to green fluorescence. In the living sample, the majority of cells showed a comparatively low ratio of red to green fluorescence, although a distinct sub-population of these cells had a high fluorescence ratio comparable to that of the dead cells, likely indicating a non-viable cell fraction in the original bacterial solution. According to these calibration data, we defined a red to green fluorescence threshold $R/G < 0.5$ to correspond to viable cells in our setup. From our measurements we estimate that the probability for a cell with measured $R/G < 0.5$ to be alive is approximately 99.4%. Cells with $R/G > 1$ are clearly non-viable, while cells with $0.5 < R/G < 1.0$ are ambiguous and are not assigned to either population. (We note that cells in this intermediate range appear green to the eye in Supplementary Figure 2. However, because the estimated probability from this calibration for a MTB with $R/G < 1$ to be alive is only ~90%, we have conservatively chosen to leave cells with $0.5 < R/G < 1.0$ unassigned in order to avoid any significant probability of mischaracterization.) These thresholds were highly repeatable in calibration measurements performed on our setup over the course of several months, even when carried out with different AMB-1 cultures and different batches of the BacLight nucleic acid stains.