

Real-Time Monitoring of H₂O₂ Sterilization on Individual *Bacillus atrophaeus* Spores by Optical Sensing with Trapping Raman Spectroscopy

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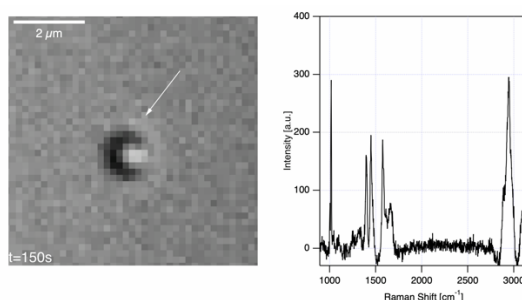
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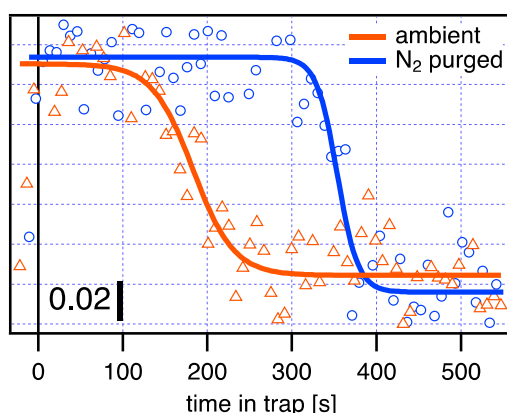
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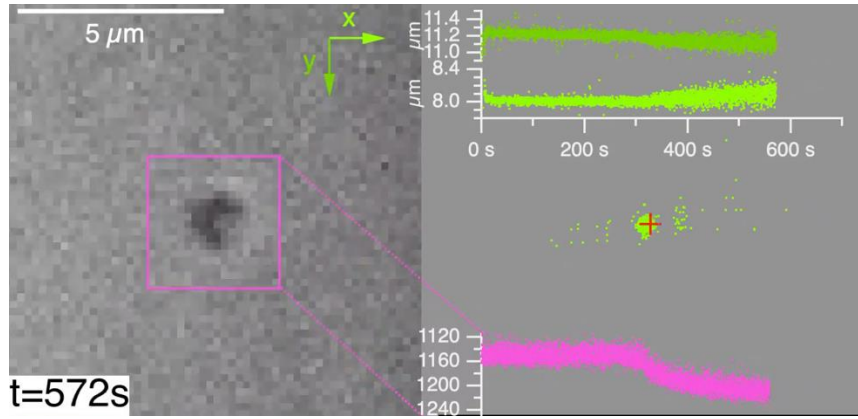
Supplementary Information



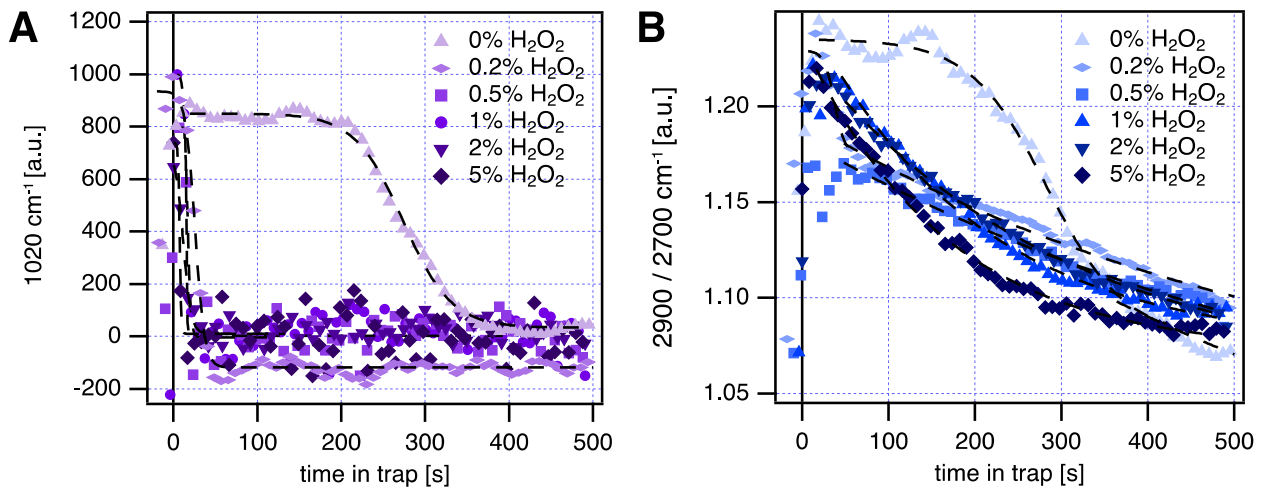
Supplementary Video S1: Video of the optical trapping experiment. The left panel shows the bright field video image of the observation region. The location of the trapping laser is indicated by an arrow. Raman spectra are obtained in synch with video imaging (right panel) after a spore has entered the optical trap.



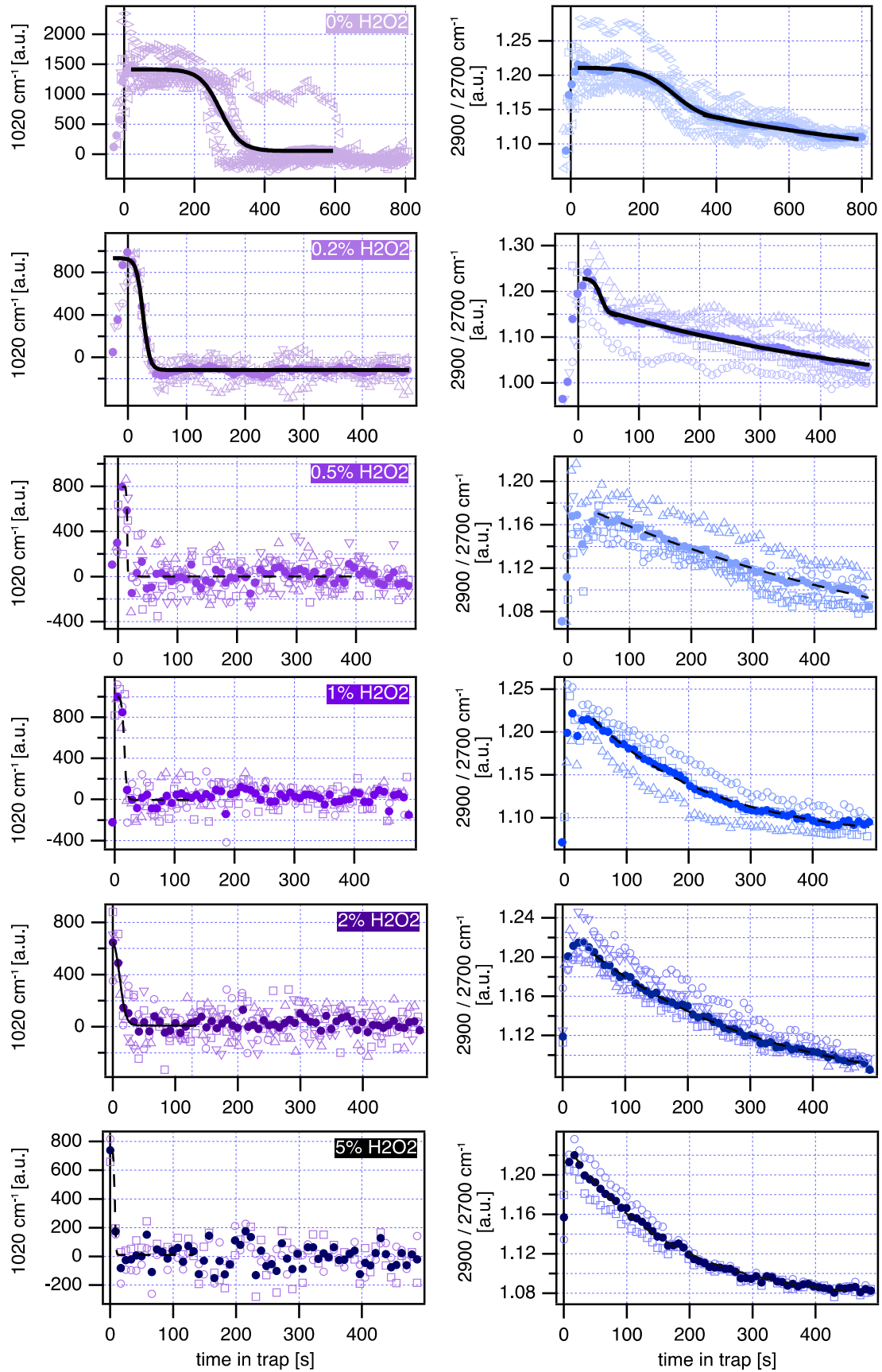
Supplementary Figure S1: Dependence of trapped spore lifetime on ambient oxygen. The open symbols indicate the Raman signal representing dipicolinic acid (1020 cm⁻¹) for solutions with ambient oxygen present (red triangles) or removed by purging with N₂ (blue circles). The continuous lines indicate sigmoidal fits to the data.



Supplementary Video S2: Changes of spore size and morphology during oxidative degradation. The left panel displays a video image of a single trapped spore. The pink trace in the right panel indicates average bright-field intensity in the boxed pink region. Green dots represent the spore trajectory, with the position corresponding to the current video frame indicated by a red cross. The green traces indicate horizontal (light green) and vertical (dark green) position in the trap as a function of time.



Supplementary Figure S2: H_2O_2 concentration dependence of spore lifetime. Averaged traces of the Raman signal representing DPA (A) or total organic matter (B) for 0% H_2O_2 ($n=7$), 0.2% H_2O_2 ($n=5$), 0.5% H_2O_2 ($n=4$), 1% H_2O_2 ($n=3$), 2% H_2O_2 ($n=4$), and 5% H_2O_2 ($n=2$). Black broken lines indicate fits with a sigmoidal (A) or exponential function (B).



Supplementary Figure S3: Individual datasets of the H_2O_2 concentration dependence of spore lifetime. The left column shows the Raman signal representing DPA, the right column is showing the amount of total organic matter as a function of time at various concentrations of H_2O_2 . Averaged data is indicated by filled symbols. The calibration plot for varying H_2O_2 concentrations from 0% to 5% is depicted in Figure 6 of the original publication.