Supplemental Information for

"Leucine incorporation by aerobic anoxygenic photoheterotrophic bacteria in the Delaware Estuary"

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Relocating fields of view imaged for AAP bacteria and single-cell activity

Here we describe the approach for relocating fields of view in microscopic analyses of single-cell activity by aerobic anoxygenic phototrophic (AAP) bacteria using microautoradiography (MAR). Before analysis, triangular sections of the polycarbonate filters with the bacteria were cut out, and the top corner ("apex corner") and lower corners were used as spatial reference points. The filter sections were then examined by IR epifluorescence microscopy to enumerate AAP bacteria. During this analysis, the x, y position of each field of view was recorded (x1,y1 in Figure S1, panel A) so that AAP bacteria and other cells in the same field of view could be imaged for silver grain analysis after MAR processing. The distance (r) between the field of view in the AAP bacterial counting step and the lower left corner of the filter piece (line segment "*R*" in Figure S1) was also recorded. It then calculates the angle (θ) formed by *R* and the line (*Z*) passing through the apex corner (A) and the lower left corner (C).

After counting AA bacteria and all bacteria, the filter section was removed from the microscope slide and rinsed in ethanol as described in the main text.

The filter section was then flipped over and placed on top of photographic emulsion coating a glass slide, so that the cells were in the emulsion. After the MAR procedure was completed, the apex and lower left corner of the polycarbonate filter were left embedded in the photographic emulsion to provide reference points, and the rest of the polycarbonate filter was peeled away, leaving the cells embedded in the emulsion. The original fields of view cannot be directly re-located because orientation of the filter section changes during the MAR process (Fig. S1).

However, knowing r and θ made it possible to relocate the field of view in the MAR preparation on the perimeter of a circle, with a radius r, centered on the lower left corner (Fig. S1 B). The new position (x2, y2) of the field of view in the MAR preparation was determined from coordinates of C (a, b), r, and the angle θ (Fig. S1):

$$x2 = a + r * \sin(\theta)$$

$$y2 = b + r * \cos(\theta)$$

The orientation of the filter piece on the slide for the MAR analysis always changed relative to its position during the initial IR epifluorescence microscopic analysis. The difference between the two orientations was calculated and used to rotate the MAR images to match that of the AAP bacteria images:

$$m1\_diff = m1 - (2 * \pi - m1\_new)$$

where m1_diff is the difference between the slopes of the filter edge (*Z*, described above) for the filter piece, m1 is the slope of the filter edge on the AAP bacteria slide, and m1_new is the slope of the filter edge on the MAR slide.

It was also necessary to take into account the fact that the filter piece is flipped over to transfer the cells into the film emulsion. To compare images taken during IR epifluorescence microscopy with the images taken after the MAR procedure, the AAP bacteria images were flipped using the ImagePro (Mediacybernetics) IpWsOrient(OR_LEFTRIGHT) function and rotated using the IpWsRotate(-1 * m1_diff * 180 / pi, 1) function in order to match the orientation on the MAR slide. Flipping and rotating the image insured that cells in the AAP bacteria image and cells in the DAPI-MAR image would line up when the images were overlaid to identify active AAP bacterial and all cells with silver grains.

Figure S1. Schematic diagram of the approach used to relocate fields of view that were first acquired for AAP bacteria counting (panel A) and then re-imaged after the sample had been transferred to a second slide and after the MAR procedure (panel B). The five-sided object represents a triangular piece of a polycarbonate filter from which the lower right corner has been removed. The black square represents a field of view. The program records the coordinates of the field of view (x1, y1), the lower left corner (C), and the apex corner (A) and then calculates the distance (r) for the line segment (*R*) and the angle (θ) between *R* and *Z*. It then uses the coordinates of C (a,b), θ and r to calculate the position of the field of view (x2,y2) after the MAR procedure (panel B).