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Label free dynamic imaging: application to phage infected bacteria

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Set up

Set up used to count nanoparticles

Sample embedded in agar for dynamic imaging

Methods

To count nanoparticles:
Film taken at 138 Hz
Average signal subtracted

To observe dynamic phenomena within cells
Films taken at different frequencies
Standard deviation computed

nanoparticles

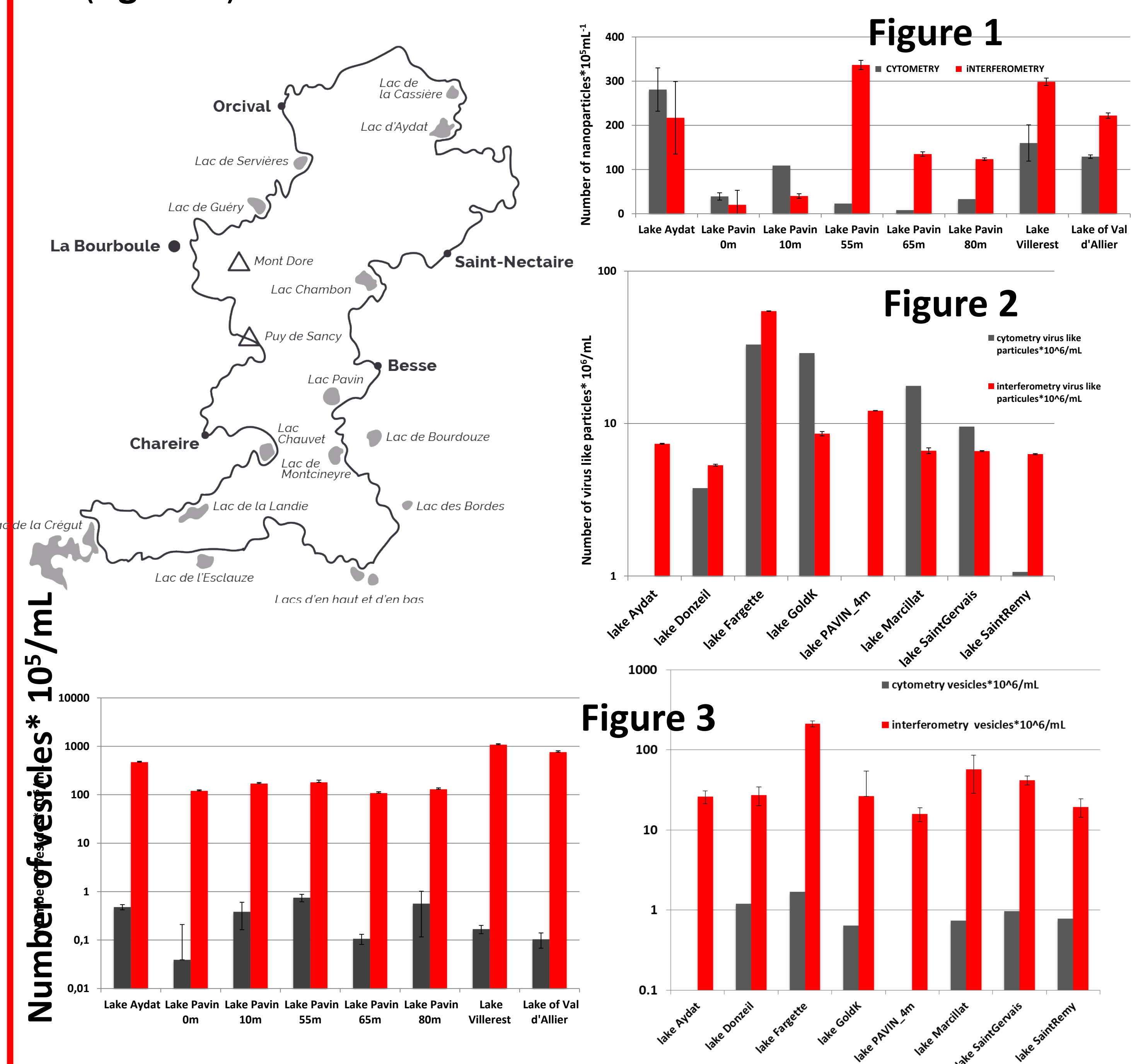
diatoms

Viruses and membrane vesicles in freshwater ecosystems: comparison of quantification by flux cytometry and interferometric microscopy

Few years ago we developed a **new interferometric microscope** aiming at detection counting size determination of nanoparticles of different refractive index (viruses, vesicles, metal oxide nanoparticles.....) Using this set up we analyzed **fresh water samples** from different lakes of Auvergne. In addition we compared our results to flow cytometry quantification of the same samples labelled with syber green and/or FM 4-24

First results (with V Ravet and D Debroas)

- 1) We detected nanoparticles in the same range as with flow cytometry (**figure 1**)
- 2) Our device is as efficient as flow cytometry to detect virus like particles (**figure 2**)
- 3) Our detection is more substantial than cytometry as labelling with FM 4-24 is less efficient than syber green (**figure 3**)



Conclusions

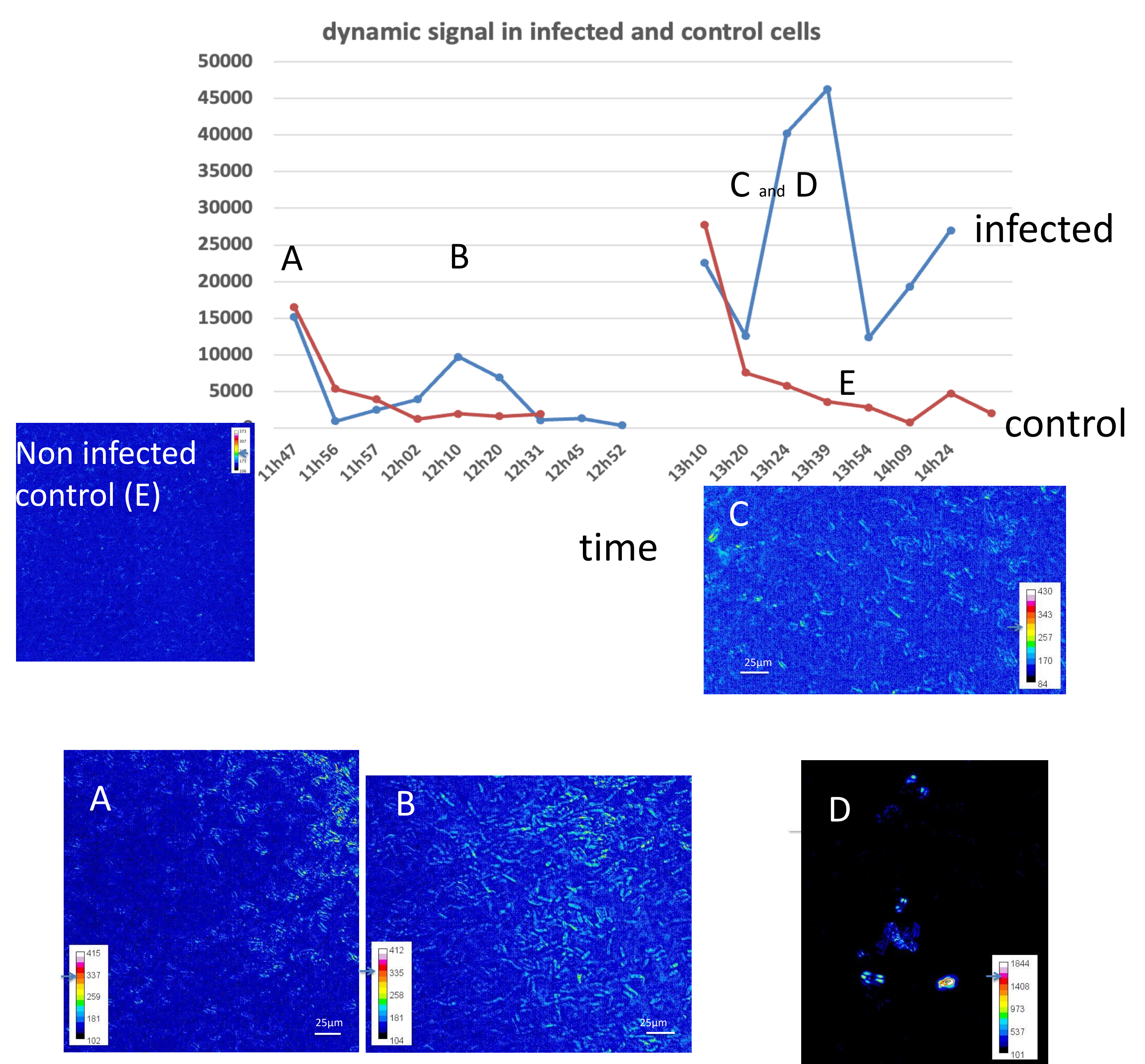
Label free methods are useful when labelling cannot be used

We showed that we can **follow virus infection without using fluorescence** and this method can be applied to any virus (currently to viral infection of algae)

We need to better characterize the distribution of signal within the cells ("viral factories"?) during infection and compare it to fluorescent markers distribution.

Label-free Visualisation of phage infected bacteria

Using the same set up we showed that we could observe and quantify dynamic phenomena with cells of different origins We investigated bacteria (E coli MG1655) infected by T5 phages We used the following procedure: After adsorption, cells (moi about 1) were washed two times **with T5** buffer to eliminate the infecting virus and then embedded in agar 2% to prevent cell movements during film acquisition (15min after adsorption). Films were taken every 10/15 min (**frequency 5Hz**). We computed standard deviation (image J) and **plotted the number of pixels with a dynamic behavior at each time**



During 2.5hrs we observed:

A A strong initial signal which disappears in 10min and was due to agar gel relaxation when introduced in the device (doublets in same direction and present also in non infected control)

B Signal (40-45 min after adsorption) **localized with cells** (not observed in control) suggesting correlation between signal increasing and viral multiplication. The presence of a doublets suggesting an interaction between infected bacteria and the gel.

C About 2hrs after adsorption a second increase in signal (5 times stronger than the first one) suggesting a second viral burst. This signal was distributed between two types of strong spots (possibly exploding cells) and signal within cells (**D**).

Shortly after the gel collapsed. Virus production was measured (not shown)

References

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