

## FLIM Finds Fossil Micro-Organisms in Flint Stone

Wolfgang Becker, Cornelia Junghans, Axel Bergmann, Becker & Hickl GmbH, Berlin

**Abstract:** Using the bh DCS-120 Confocal Scanning FLIM system to record internal layers of flint stone we found round objects that perfectly resemble ancient bacteria. The objects are about 20  $\mu\text{m}$  in size and display a triple-exponential fluorescence decay with components of 334 ps, 2534 ps, and 7794 ps, and amplitudes of 0.5924, 0.2534, and 0.1096, respectively. The fluorescence decay clearly differs from that of the surrounding material. The objects often appear in clusters and are found in many places and in different depth of the stone. This and the fact that the objects are very uniform in size makes us believe that we are indeed seeing fossil microorganisms.

### Laser-Scanning FLIM Microscopy of FLINT Stone

Laser scanning microscopy is mostly used to study biological objects. However, a laser scanning microscope can also be used to study the internal structure of minerals. On the micrometer scale, many minerals are transparent or at least translucent. A laser scanning microscope is then able to record images from defined internal layers of the material. The results show inclusions, cracks, crystal defects, trapped bacteria, or other features of interest. When combined with FLIM, the contrast to the surrounding material can be increased, and additional molecular information on the features of interest can be obtained [4]. To explore possible FLIM applications in mineralogy we used the bh DCS-120 confocal FLIM system [1, 3] on a number of different minerals. One of them was flint stone. Flint stone is partly translucent and emits surprisingly strong visible-range fluorescence with a lifetime in the lower nanosecond range. When we recorded FLIM images from flint samples we found, to our surprise, structures that, both in size and in shape, resembled single microorganisms and clusters of microorganisms. Examples are shown in Fig. 1.

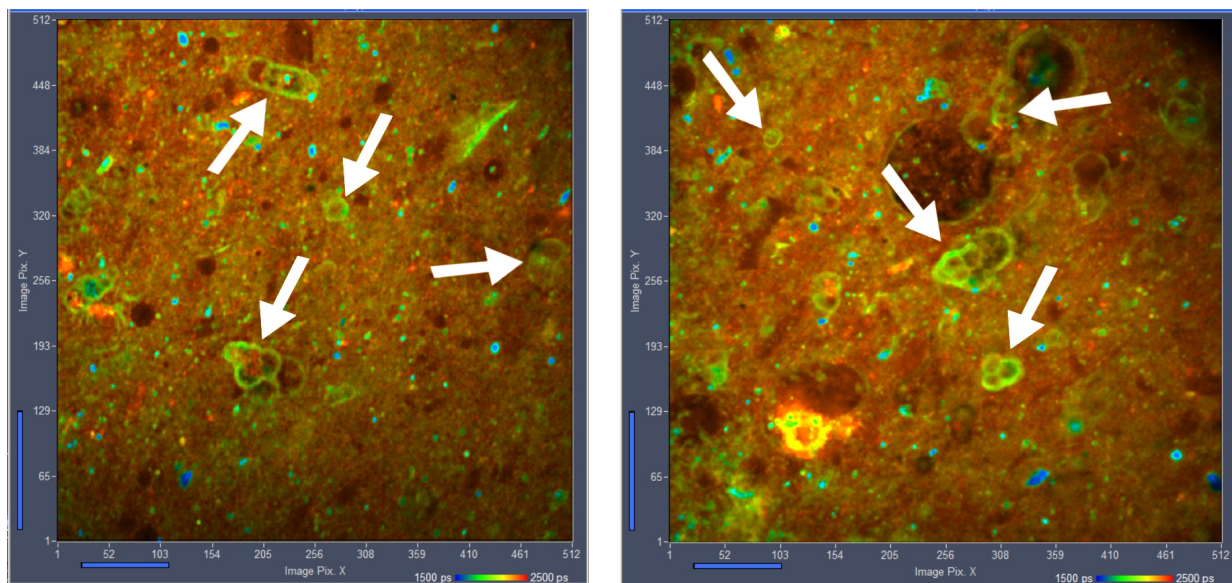


Fig. 1: Fossil microorganisms in flint stone. bh DCS-120 Confocal Scanning FLIM system, excitation 405 nm, detection 435 nm to 650 nm. Image Size 400 x 400  $\mu\text{m}$ . Objective lens 20x, NA=0.5, Pinhole size 1 AU (right). Online-display-function of SPCM data acquisition software, single-exponential approximation of decay time.

The figure shows that the bacteria-like objects have noticeably different fluorescence lifetimes and visibly different decay curves than the surrounding material. Fluorescence-decay curves of the objects and the surrounding material are shown in Fig. 2.

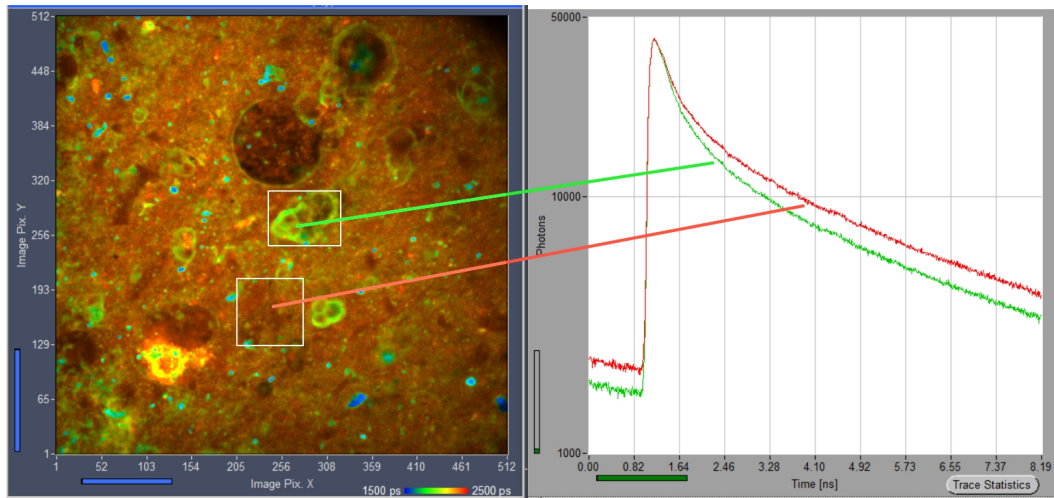


Fig. 2: Fluorescence-decay functions in regions of interest containing objects (green) and surrounding material (red)

## Decay Analysis by SPCImage NG

To gain further information on the fluorescence behaviour of the enigmatic objects we applied bh SPCImage NG FLIM data analysis [2] to our data. A lifetime image obtained this way is shown in Fig. 3. From the decay parameters and the decay curve (upper right and lower right) it can be seen that the fluorescence decay is multi-exponential. It is described well by a triple-exponential model, as can be seen from the smooth residuals below the decay curve. Please note that the lifetimes are different than in Fig. 1 and Fig. 2. SPCM uses a first-moment calculation that yields a single-exponential approximation of the lifetime, whereas SPCImage runs an MLE fit with a multi-exponential model, delivering an amplitude-weighted average of the component lifetimes. Lifetimes obtained by the two procedures are necessarily different.

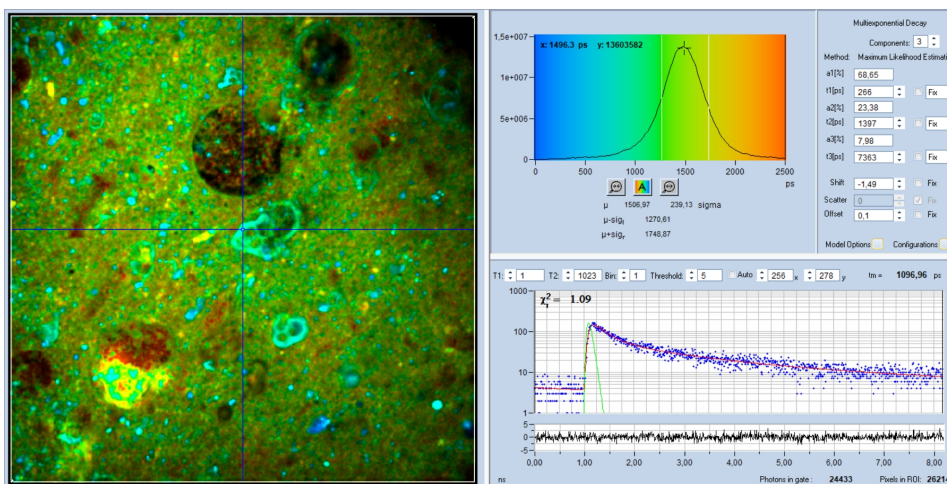


Fig. 3: Fluorescence-lifetime image created by SPCImage NG. Left: Lifetime image, showing amplitude-weighted lifetime,  $t_m$ , of triple-exponential fit. MLE fit, GPU calculation. Upper right: Histogram of  $t_m$  over the pixels of the image and decay parameters at cursor position. Lower right: Decay curve at cursor position. The image format is 512 x 512 pixels, with 1024 time channels per pixel.

### Image Segmentation by Phasor Plot

Fig. 4 shows the use of the phasor plot of SPCImage NG as an image segmentation tool [2]. In the phasor plot (upper right), a phasor range was selected which contains the phasor signature of the objects of interest. In the lifetime image (left), pixels outside the selected phasor range are displayed in grey. The 'Select Cluster' function of the phasor plot was used to combine the decay data of the selected pixels in a single decay curve. The combined decay curve is shown lower right. It contains more than 13 million photons and, therefore, can be analysed with very high precision. The result shows that the decay is indeed triple-exponential, with decay components of 280 ps, 1962 ps, and 7542 ps. The amplitudes are 0.654, 0.2541, and 0.092, respectively. Interestingly, these lifetimes are compatible with the fluorescence decay of flavin adenine dinucleotide (FAD), a constituent of live cells. However, although it is conceivable that FAD got incorporated into the molecular matrix of the flint it is unlikely that it remained stable over more than 60 million years.

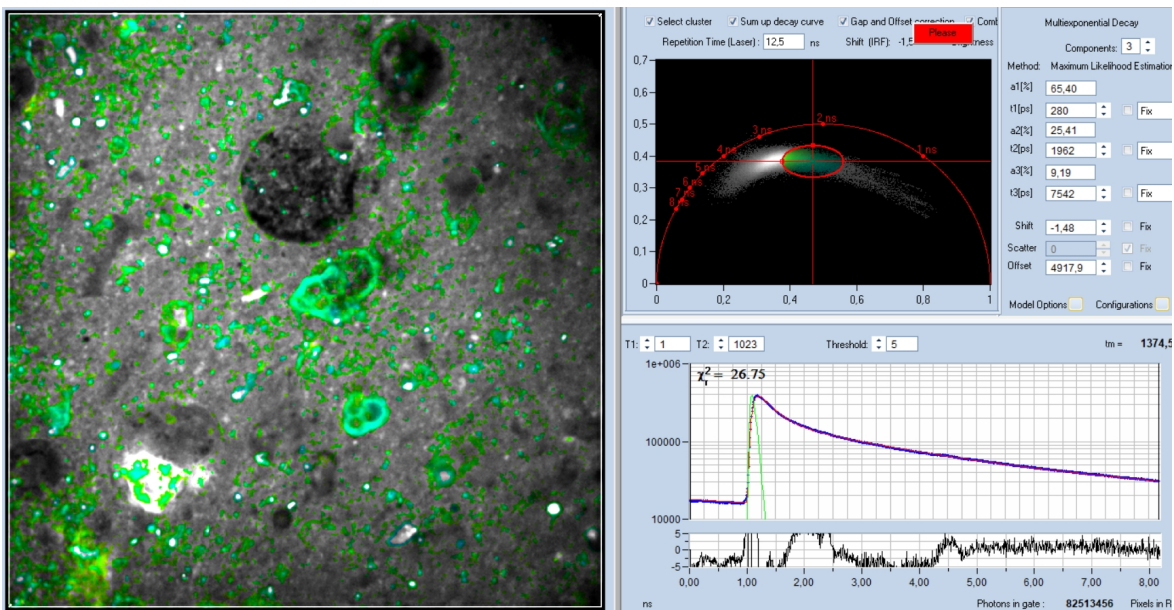


Fig. 4: Image segmentation by phasor plot, pixels with phasor signature of the cells selected. Combined decay curve for selected pixels shown lower right.

Fig. 5 uses the same procedure to select pixels which have a *different* phasor signature than the cells. The combined decay curve is shown lower right. The decay components are 334 ps, 2534 ps, and 7794 ps, with amplitudes of 0.5924, 0.2534, and 0.1096, respectively.

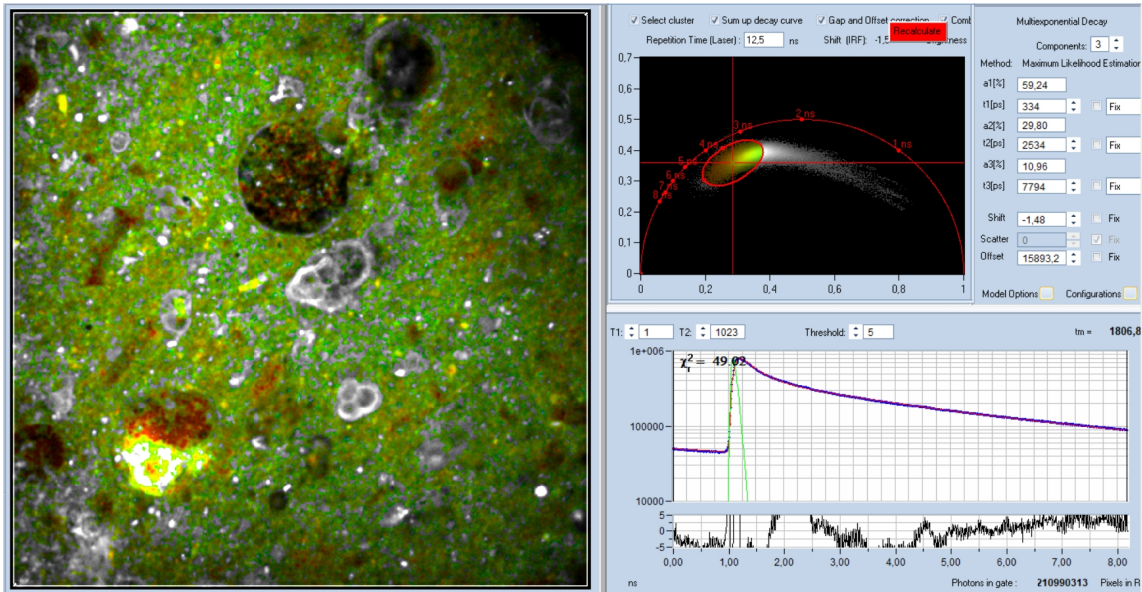


Fig. 5: Image segmentation by phasor plot, selected are pixels with a different phasor signature than the one of the cells. Combined decay curve for selected pixels shown lower right.

## Discussion

Flint formed in the Mesozoic, between 150 and 60 million years ago. It is thought that it formed when silicon-rich fluid filled cavities in sediment, such as holes bored by crustaceans or molluscs. With the time, the fluid became more rich in silicic acid, gelled, and finally solidified to become cryptocrystalline silica [5, 6]. It is conceivable that the original fluid was inhabited by bacteria. However, silicification is a slow process. It is not clear how bacteria could stay alive or, at least, morphologically intact over such a long time in an increasingly hostile environment. Another possibility is that the bacteria intruded the stone when it already had formed. However, our images do not show cracks or pores in the material through which this could have happened. All in all the most likely explanation is that we indeed are seeing relics of Mesozoic bacteria. If this is correct laser-scanning FLIM microscopy of flint stone could be an intriguing technique to study ancient micro-organisms.

## References

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## Contact:

Wolfgang Becker  
 Becker & Hickl GmbH  
 Berlin, Germany, <https://www.becker-hickl.com>  
 Email: [becker@becker-hickl.com](mailto:becker@becker-hickl.com)