

# Changes in the Observed Shape of H<sub>6</sub>TPPS J-Aggregates by the Polarisation of the Incoming Light

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**Abstract.** Samples of H<sub>6</sub>TPPS J aggregates and bundles, deposited on glass and aligned under nitrogen flow, were measured in a 2-photon microscopy setup. Changes in the polarization state of the incoming laser have shown a difference in the resulting 2-photon scanning of the same measured sample, revealing otherwise hidden features. In addition, tracing the response of certain areas under different polarisation can provide information about the arrangement of the dipoles in that area. This shows the significant role of polarisation in 2-photon measurement, and the need to consider such effects in the microscopy of biological samples.

## Introduction

Meso-tetra(4-sulfonatophenyl) porphyrin (TPPS) is known to form J-Aggregates in an aqueous acidic environment. In such conditions (pH<2) the molecules of TPPS undergo protonation and assume the form of H<sub>6</sub>TPPS. In this form, the negatively charged sulfonate groups are attracted to the positively charged centre of the molecule, while the two protonated sulfonate prevent the molecules from repelling one another. This process causes the formation of rings, which in turn attach to form aggregates, which possess a dipole. These aggregates in turn cling to one another to form bundles, which can be deposited onto a surface.

By controlling the bundles alignment using a nitrogen flow, a method which was developed recently in our lab [1], it is possible to get string-like shapes which are a good model for simulating a biological sample for 2-photon microscopy. Using a 2-photon microscope, with excitation at 940nm using Ti:Sapphire laser, built in our lab[2], we have scanned a sample of H<sub>6</sub>TPPS bundles aligned in such a way. The signal was collected through the same objective (NA 0.50), after filtering the fundamental wavelength and a cut-off filter at 500nm, by two avalanche photodiodes (APD) orthogonal to each other.

## Results and Discussion

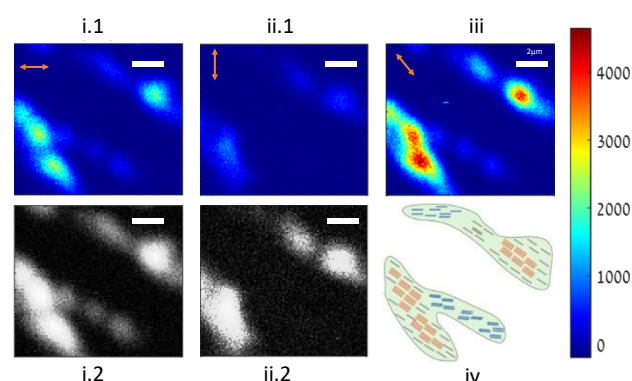
While measuring the samples, we have noticed how the polarisation state of the incoming field has a significant effect on the observed shape and visibility of the bundles. As can be seen in fig.1, the observed shape of the aggregate bundles is different for both orthogonal polarisations.

Based on the presence of certain features of the scan in only certain polarisations, as well as the maximum strength of emission at 45°. A suggested layout for the arrangement based on polar plots of the aggregates in the bundles is constructed in fig.1(iv).

We think that the aggregates in the blue highlighted regions are ordered close to 90°, leading to them being invisible in 0° polarisation. Thus, to ensure the observation of all available features, two photon measurements must be done in more than one polarisation, or in radial polarisation.

We also believe that the strength of emission contains information about the ordering and density of the contained aggregates. When the bundles are ordered in parallel to each other, their dipoles are aligned as well, giving rise to the coherent Second Harmonic (SH) process, emitting a strong signal. If the bundles are disordered the dipoles do not align and the incoherent Hyper Rayleigh Scattering (HRS), response is registered, and the observed emitted signal will be the much weaker.

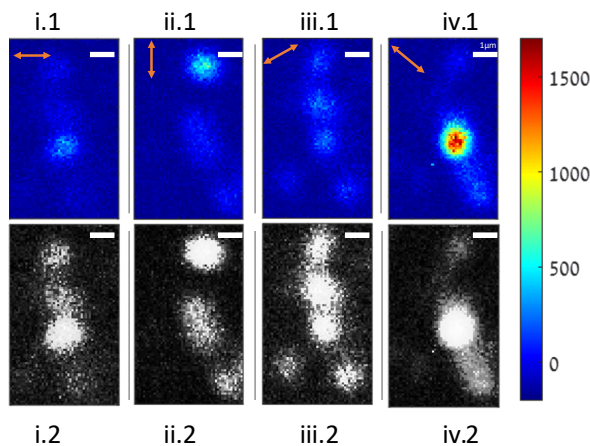
This led us to correlate the areas with higher response in Fig. 1(iii) to dense and aligned aggregates (highlighted in orange). We shall note that those measurements are also supported by polar plots (rotational SHG).



**Fig. 1.** Two photon microscopy scans of aligned H<sub>6</sub>TPPS bundles. (i)(ii). (1). scans under 0° and 90° polarization (respectively) (2). the same scanning images, modified as to have maximum contrast for viewing all available detail and missing features. (iii). a scan under 120° polarization, showing the maximum count values. (iv) a theoretical layout for the aggregate arrangement in the different areas of the bundle.

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Fig.2, also support our findings. As can be seen, some of its features, with a lower response than others, get obscured by the background noise of the microscope and are not visible in the two orthogonal polarisations. Only by measuring in an additional polarisation, that better fits the arrangement of the dipoles, do these features become visible.

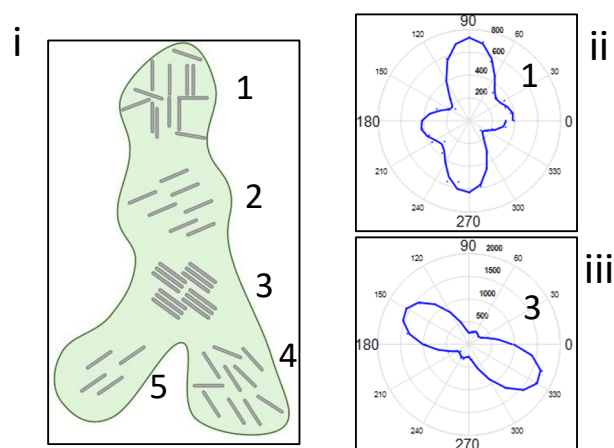


**Fig. 2.** Two photon microscopy scans of H<sub>6</sub>TPPS bundles. (i)-(iv). (1). scans under 0°, 90°, 200° and 150° polarization (respectively) (2). the same scanning images, modified as to have maximum contrast for viewing all available detail and missing features.

These findings are in agreement with a recent technique used in biological 2-photon microscopy [3]. Which reveals additional details in the tissue samples using circular polarisation.

In addition, by monitoring the strength of the emission under all polarisations from different regions of the aggregate bundles, it is possible to determine some properties of the aggregate arrangement within them.

As can be seen in fig. 3 we have measured two different points within the bundle. One showing moderate activity of about 700 counts, and the other showing stronger response of 1500 counts.



**Fig. 3.** A model and measurements of the examined bundle from fig.2. (i) a model showing the arrangement of the bundles at five different clusters visible from the scan. (ii)(iii) polar plots of the scans from the corresponding areas.

While one could at first speculate this difference comes only from the concentration of aggregates within the bundle at this point, the polar plots reveal that the increased response at point 3 is of a highly polarised nature- fitting the profile of SH emission and thus showing a highly ordered region of the aggregates, while the polarisation in the weaker region shows a shape with nearly 4-fold symmetry- which fits HRS better, and thus shows this region contains aggregates that are mostly disordered.

The model in Fig.3(i) was thus built based on the information from the SH scans and polar plots (not all are shown). Area 1 shows up in all scans, and thus should contain aggregates in all polarisations, with a certain preference to the 90° direction, as both the scans and the polar plot point out. Area 2 shows up clearly only in the 0° and 200° directions, with a relatively weak emission, and was thus determined to have aligned, yet dispersed, aggregates. Area 3 gives off a strong, highly polarised response, and is thus determined to have aligned and dense aggregates. Area 4 shows a weak emission in all polarisations, leading to us believing it contains unaligned and dispersed aggregates. Finally, area 5 only shows up clearly when the polarisation fits its direction, and even then very faintly, leading us to believe it contains aligned, but very dispersed aggregates.

## Conclusion

In summary, our measurements have shown the importance of tracking polarisation while using 2-photon microscopy devices. The employment of several different polarisations is important for the imaging of all available features, which could be obscured when using a single polarisation or just a pair of orthogonal polarisations. Furthermore, the polarisation can be used to determine properties of dipole arrangement in the samples.

Lastly, it can be seen that H<sub>6</sub>TPPS aggregates are a good model for biological microscopy- showing many of the characteristics that apply to such samples. Due to their simple preparation and higher durability than many biological samples, they can be used for many tests and calibrations in 2-photon systems.

## References

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