Second harmonic imaging of plant cell walls – the cotton fibre

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Plant cell walls are highly complex composite structures that regulate plant cell development and function. The major structural element is the carbohydrate, cellulose, which is extruded from the plasma membrane as cellulose molecules, which aggregate into microfibrils to form the structural scaffold of the cell wall. The orientation of the microfibrils in the expanding cell wall dictates cell shape, and together with other wall components they regulate the shape of the plant organ assembled from these cells.

Cotton fibres are remarkable single cells that emerge from the epidermis of the cotton seed coat. When the developing seed is still enclosed in the cotton boll, the fibres begin to emerge as small balloons from about 30% of the epidermal cells at the day of flowering. Once the flowers open, the fibres begin rapid elongation to a final length of up to 4 cm. Transversely-oriented cytoplasmic microtubules ensure that the cellulose microfibrils are also transversely-oriented during this period of rapid expansion. Once the fibres cease elongating, about 15-18 days after flower opening, there is considerable additional deposition of cellulose to form a thick secondary cell wall, and the predominant microfibril orientation becomes more oblique to the long axis of the fibre.

We are interested in methods to examine the orientation of cellulose microfibrils in the walls of cotton fibres during development. Because cotton is so well-studied, there have been many approaches to understanding microfibril organisation, including polarised light microscopy to ascertain the net orientation of microfibrils, scanning electron microscopy, x-ray analysis, and different types of stain which generally bind to components of the innermost, most recently deposited layer of cell wall. Our aim was to find or develop a method to reveal the orientation of cellulose throughout the intact cotton fibre.

This has been achieved for the lignified walls of wood cells using atomic force microscopy, in which the ends of cut microfibril bundles can be seen in cross-sections of cell walls. The longitudinal orientation of some component wall layers can also be identified to the level of individual microfibrils approx. 10 nm in diameter. Our aim was to use second harmonic imaging to reveal the orientation of microfibril bundles throughout the cotton fibre, and correlate these observations with 10-20 nm structures seen in field emission scanning electron microscopy (FESEM) of cross-sections and longitudinal sections of cotton fibres.

Our preliminary observations revealed strong second harmonic signals from mature cotton fibres, in both the forward and reverse directions. The subtle differences between these two signals may indicate details of inter-microfibril organisation, similar to observations in collagen assemblages. Mutant lines of cotton producing very different types of fibre also generated very different second harmonic signals. In two cases analysed so far, 10-20 nm structures in corresponding FESEM images of fibre cross-sections, which we interpret to be the cut ends of microfibrils, reflect patterns similar to the second harmonic data. These and additional data will be discussed with reference to the use and interpretation of second harmonic signals from plant cell walls.