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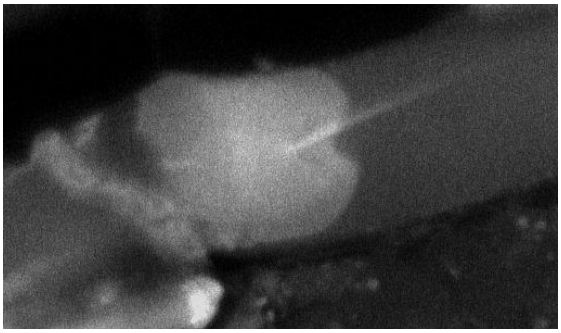
Supporting Information Document S2

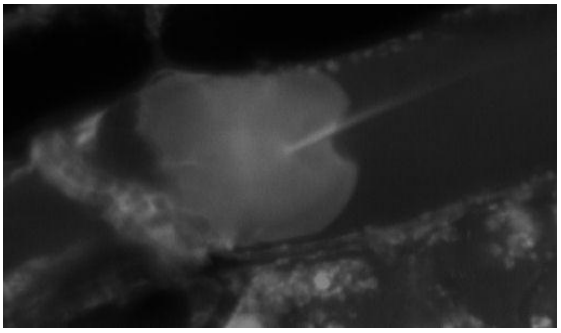
Image processing applied to confocal laser-scanning micrographs of *Canavalia gladiata* forisomes in situ

In producing 3D-images of forisomes in their sieve tubes, one encounters a major obstacle: there are no fluorescent dyes known to stain forisomes specifically. We attempted to circumvent the problem by applying two dyes, CDCFDA and RH 414 (see Material-and-Methods in the main text), which stain various intracellular sieve element components non-specifically but at varying intensities. By appropriate assignment of the resulting image pairs to color channels, we hoped to produce images in which forisomes were visibly separated from other structures. In doing so, we aimed to render forisomes white on a dark background, since black/white combinations have the highest apparent contrast compared to other color combinations.

The original images of *Canavalia gladiata* sieve elements showed little difference regarding the specificity of the staining for forisomes as such:

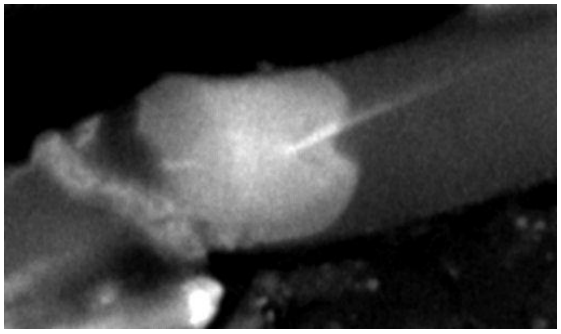


CDCFDA staining

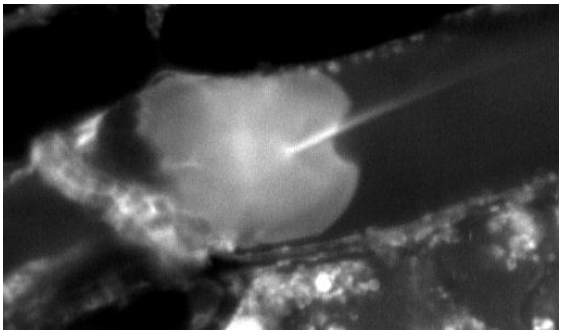


RH 414 staining

To improve contrast, all original images were 'stretched' using the *histogram stretch* function of Paint Shop Pro (Corel; http://www.corel.com):[1]



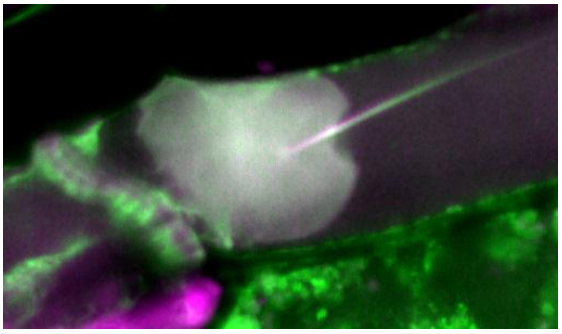
CDCFDA staining, stretched



RH 414 staining, stretched

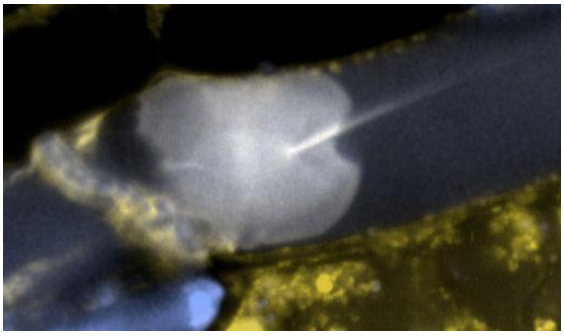
[1] As it was applied to all images in the stacks and the complete images, this operation was in agreement with accepted rules of image manipulation; compare M. ROSSNER & K.M. YAMADA: *What's in a picture? The temptation of image manipulation.* Journal of Cell Biology **166,** 11–15 (2004).

The stretched images were combined into the RGB color space; the RH 414 signal provided the green channel, whereas the CDCFDA signal was used as both the blue and red channel. The purpose behind rendering one fluorescence signal through two color channels was to display the overlap of the two fluorescence signals as white rather than as the additive color of a pair of color channels. As a result, the forisome became light gray with faint green and magenta touches:



Combined channels in the RGB color space

We used the Vischeck plug-in (http://www.vischeck.com) for ImageJ (http://rsbweb.nih.gov/ij/) to see how this color combination was perceived by readers with color vision deficiencies (CVD). Unexpectedly, images transformed by Vischeck into the color space experienced by individuals with forms of red-green dichromacy showed increased contrast between the forisome, which now had become white, and its dark background. This beneficial effect resulted from Vischeck's correct modelling of the fact that very light shades of red and green are indistinguishable from light gray or white for many red-green deficient dichromats.[2] Moreover, the resulting images and movies (Fig. 4 in the main text, Movies S3 and S4) provided color-encoded information that, for all practical purposes, was similar for normal-sighted readers and for those with red-green dichromasies (protanopia, deuteranopia) and red-green trichromatic anomalies (protanomaly, deuteranomaly),[2,3] by far the most common CVDs.[4] Therefore, we applied the Vischeck protanope transformation to all images of *C. gladiata* forisomes.



Following Vischeck transformation for protanopia

[2] We thank our red-green color deficient friend DAVE TALBERT, member of the mighty Tottenham Hotspur Football Club whose team uniforms look the same before and after Vischeck transformation, for acting as our guinea pig in tests conducted to confirm these facts.

[3] B.L. COLE & R.W. HARRIS: *Colour blindness does not preclude fame as an artist: celebrated Australian artist Clifton Pugh was a protanope.* Clinical and Experimental Optometry **29,** 421–428 (2009). — J.A.B. SPALDING: *Colour blind artists: do the Vischeck transformations work?* Clinical and Experimental Optometry **29,** 188 (2010).

[4] R. FLETCHER & J. VOKE: *Defective Colour Vision: Fundamentals, Diagnosis and Management.* A. Hilker, Bristol, 1985.