Set Pseudophasors to Stun for Flow Cytometry

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Fluorescent proteins (XFPs) are a useful tool for studying cell signaling when fused to signal transduction proteins. However, cell signaling proteins are often present in cells in low numbers and XFPs are relatively weak fluorophores, so the amount of light that can be observed in such studies is small. In addition to the light emitted by an XFP, its fluorescence lifetime provides information regarding its immediate surroundings. To take advantage of this fact, biologist Bryan Sands and staff scientist/physicist Dr. William Peria in the laboratory of Dr. Roger Brent (Basic Sciences Division) and collaborators at New Mexico State University engineered pairs of XFPs with identical fluorescent emission spectra (isospectral) but different fluorescent lifetimes (allothoric) and expressed them in budding yeast. They modified microscopic and flow cytometric equipment and also developed digital hardware allowing the measurement of fluorescence lifetimes in living cells. This approach was highly successful, leading to sorting of cells expressing teal fluorescent protein (TFP) and its short-lifetime variant into populations >90% pure from a mixed population.

The collaboration that led to this work is notable for being the result of a longstanding minority outreach program between the laboratories of Dr. Brent at Fred Hutch and the flow cytometry lab of Dr. Jessica Perea Houston of New Mexico State University, coordinated by Dr. Beti Thomson (Public Health Sciences Division). “With Jessica, what we are doing is, as biologists and physicists, doing our part to help her cytometry lab be a center of excellence and a place that attracts smart students from the vicinity of NMSU-Rio Grande Valley-border region and gets them into the system,” said Dr. Brent. “Our tiny part of this with Jessica is to try to do something big by dragging the best and the brightest up a couple steps.”

To begin the work, the researchers engineered two isospectral XFPs with different fluorescence lifetimes. To do so, they fused TFP and Citrine to a variant of Citrine termed “dark citrine” or “dCit” and expressed each fusion protein in yeast cells. Next, the researchers used two microscopic methods to measure the fluorescence lifetimes of cells expressing each XFP. Images of cells were captured in rapid succession and image data were processed mathematically to yield lifetimes. This analysis showed that the average fluorescence lifetime of both dCit-TFP and dCit-Cit was 43-44% shorter than TFP or Citrine alone.

The New Mexico authors next modified a commercial flow cytometer to allow detection of TFP and dCit-TFP. They also developed dedicated FPGA digital hardware and code to allow the calculation of a complex number termed a "pseudophasor," a measure of a protein's fluorescence lifetime, quickly enough to make a sorting decision. By this means, the researchers achieved average purities of 97.1% for TFP-expressing cells and 93.7% for dCit-TFP-expressing cells.

This work demonstrated that XFPs with different fluorescence lifetimes can be used to sort cell populations. While this proof of concept was performed in yeast, its applications may extend more widely: "[This] work might, in the long term, have clinical impact," said Dr. Brent. "For example, it's possible that these methods might impact cancer immunotherapy. In order for such therapies to become routine, critical issues now include finding the most effective means to immunize cells from a patient's bone marrow ex vivo and to identify and expand those populations of T and NK cells that can engage tumors effectively. In the future, it's possible that multiparametric lifetime-based cytometric methods might help define optimal immunization procedures and expansion protocols specific for each patient's tumor."

Sands B, Jenkins P, Peria WJ, Naivar M, Houston JP, Brent R. 2014. Measuring and sorting cell populations expressing isospectral fluorescent proteins with different fluorescence lifetimes. *PLOS ONE* 9(10):e109940.

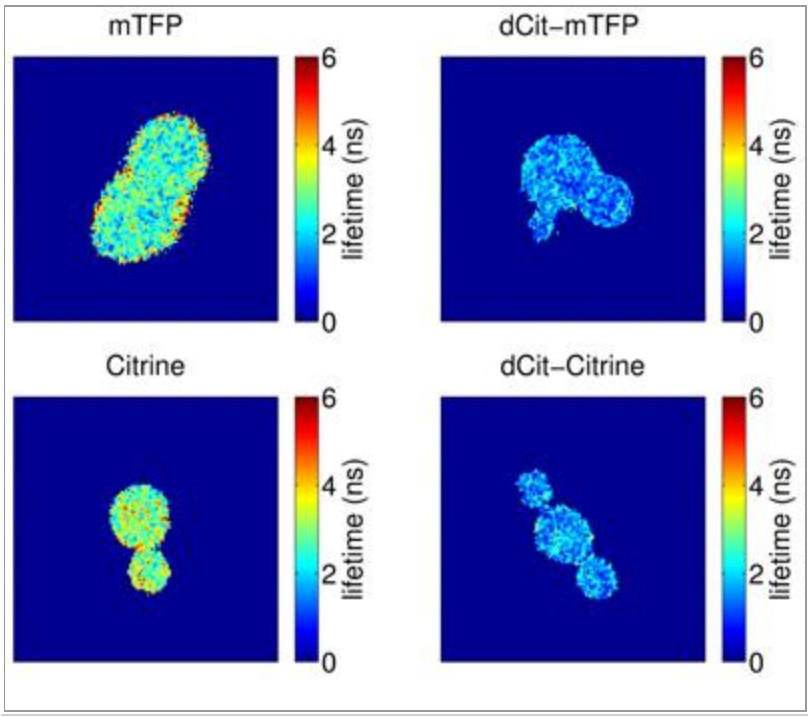

Image from the publication

Fluorescence lifetime images (false color) of yeast expressing mTFP (upper left), dCit-TFP (upper right), Citrine (lower left) and dCit-Citrine (lower right). Light from regions colored dark blue was below the threshold for fluorescent lifetime calculation. Image from the publication.