Microscopy TODAY

Coming Events

2009

Advanced Electron Microscopy in NanoMedicine
October 2–3, 2009, Los Angeles, CA
www.cnsi.ucla.edu/electron-microscopy

Society for Histochemistry
October 7–10, 2009, Fulpmes, Austria
www.histochemistry.eu

Neuroscience 2009
October 17–21, 2009, Chicago, IL
www.sfn.org

American Society of Human Genetics
October 20-24, 2009, Honolulu, HI
www.ashg.org

CIASEM 2009
October 25–28, 2009, Rosario, Argentina
www.cab.cnea.gov.ar/ciasem2009

AVS 56th Int. Symposium & Exhibition
November. 8–13, 2009, San Jose, CA
www2.avs.org/symposium

2009 MRS Fall Meeting
November 30–December 4, Boston, MA
www.mrs.org

American Society of Cell Biology
December 5–9, 2009, San Diego, CA
www.ascb.org

2010

Multiphoton Microscopy at SPIE Photonics West
January 23-28, 2010, San Francisco, CA
www.spie.org

PITTCON 2010
February 28–March 5, 2010, Orlando, FL
www.pittcon.org

Microscopy & Microanalysis 2010
August 1–5, 2010, Portland, OR
www.microscopy.org

2011

Microscopy & Microanalysis 2011
August 7–11, 2011, Nashville, TN

2012

Microscopy & Microanalysis 2012
July 29–August 2, Phoenix, AZ

2013

Microscopy & Microanalysis 2013
August 4-8, Indianapolis, IN

Please check the "Calendar of Meetings and Courses in the MSA journal *Microscopy and Microanalysis* for more details and a much longer list of meetings and courses.

Carmichael's Concise Review

Using Quantum Dots to Demonstrate Kiss-and-Run

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Secretion is a basic biologic phenomenon. Although most mammalian cells are capable of secreting, neurons are of particular importance because the exchange of information throughout the nervous system usually involves secretion of transmitters from synaptic vesicles. Two mechanisms have been proposed, but the prevalence of one over the other has not been clear. One is called full-collapse fusion (FCF) whereby the membrane of the synaptic vesicle fuses with the plasma membrane by exocytosis, all of the contents of the vesicle are unloaded into the synaptic cleft, and a new vesicle is generated *de novo*. The other mechanism is often referred to as kiss-and-run (K&R) and is characterized by the transient fusion and retrieval of the vesicle membrane with a subtotal release of vesicular content. Recently, Qi Zhang, Yulong Li and Richard Tsien have developed an ingenious technique to clearly distinguish between these two mechanisms [2].

The key is the use of single quantum dots (Qdots). Zhang *et al.* found that Qdots with a peak emission at 605 nm and a diameter of about 15 nm were most suitable for their purposes. A single Qdot is small enough to fit inside the lumen of a synaptic vesicle (about 24 nm in diameter) yet too large to move through a putative K&R fusion pore (1 to 5 nm). They mildly stimulated neurons in the presence of these Qdots and determined that the dots were taken up in almost half of the synapses. Further tests determined that many of the nerve terminals contained a single Qdot. Probably the big step forward in this study was to demonstrate that they could select terminals with only one Qdot and thereby track only one vesicle. The pH-dependence of the Qdot photoluminescence indicated that the dots were in an environment of about pH 5.5, which is thought to be the acidity of synaptic vesicles.

The pH dependence of Qdot photoluminescence predicted that K&R would allow protons to escape the vesicle (and pH to rise) but retain the Qdot, which would get brighter, whereas FCF would show the same brightening but then lose signal as the Qdot departs (see Figure 1). By examining Qdot-loaded synapses during stimulation and manipulation of the vesicular pH with a proton blocker, Zhang *et al.* found they could reliably distinguish between K&R and FCF mechanisms. Additional experiments where the external pH was manipulated and studies tracking the motion of single Qdots confirmed this.

Interestingly, Zhang *et al.* found that K&R was predominant during early phases of stimulation but became less prevalent as stimulation continued. Through a series of

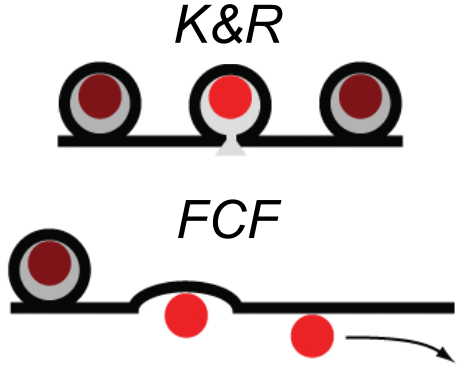

Figure 1: Cartoon of the basic mechanisms of K&R and FCF release during secretion. The red circle represents a Qdot.

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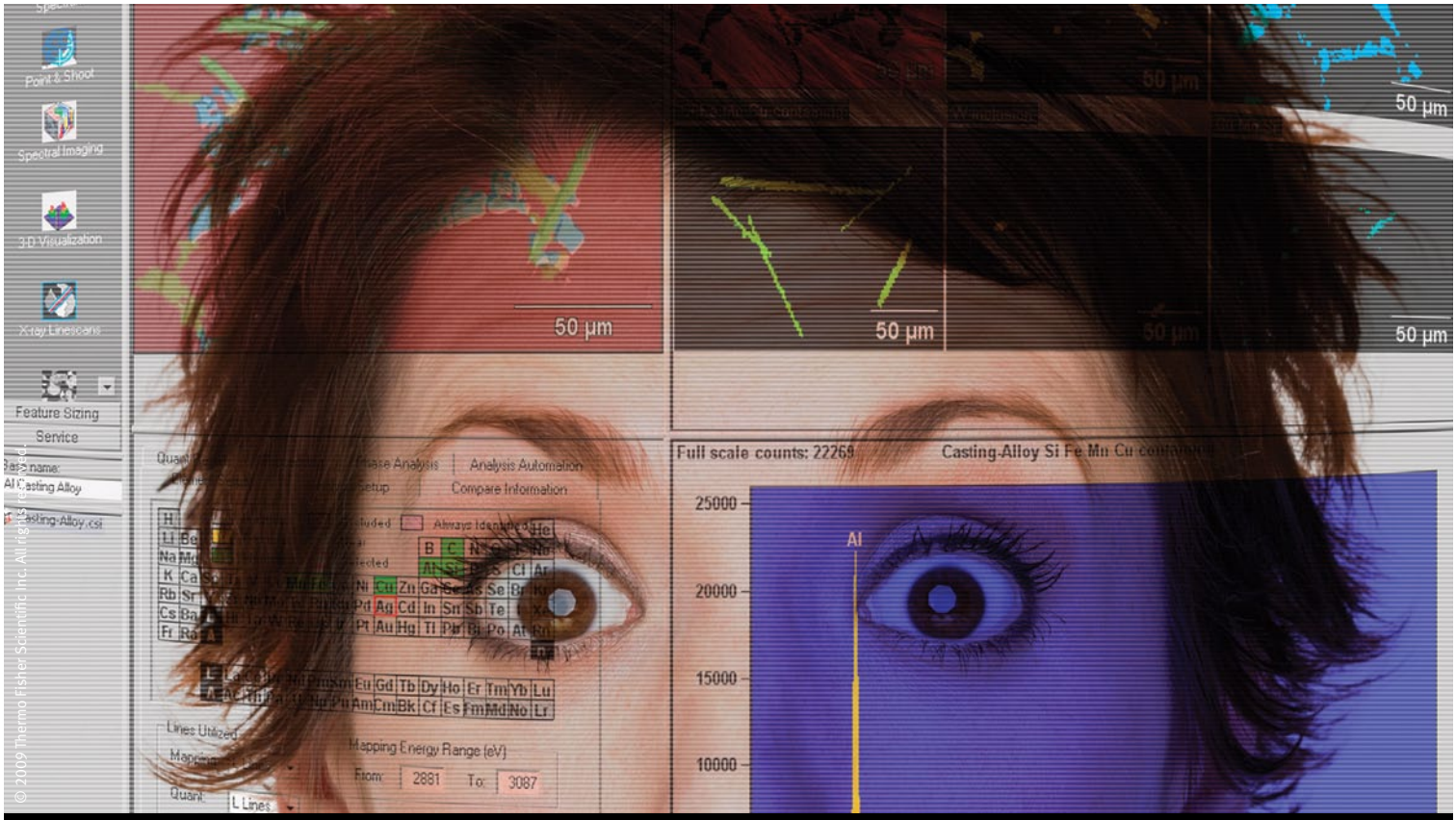

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experiments, they found that K&R was common for vesicles in the "readily releasable pool," whereas vesicles that had fused at least once or vesicles from the "reserve pool" were less likely to undergo K&R. Also, the rapidity of stimulation favored K&R. But apparently, although K&R is common, vesicles eventually proceeded to FCF.

How soon after K&R can a vesicle again undergo K&R or proceed to FCF? Through a series of clever experiments, Zhang *et al.* determined that a vesicle can go to another K&R in about 5 seconds, whereas it takes about 27 seconds to proceed to FCF. Also, prompt pore closure and vesicle re-acidification are important during closely spaced K&R events to avoid "shooting blanks." Studies showed that re-acidification appeared to be a rate-limiting step, but this was observed to be rapid, even at different stimulation frequencies. Other results indicated that fusion pore gating was under physiological control.

This novel technique developed by Zhang *et al.* allows for study of secretory mechanisms in unprecedented detail. Some vesicles from the readily releasable pool could fuse up to four times, and the rate of vesicle reuse is much faster than previously estimated. Future studies could employ Qdots of different sizes, colors, and pH sensitivities to manipulate the system in even more detail. MT

References

[1] The author gratefully acknowledges Drs. Richard Tsien and Qi Zhang for reviewing this article.

[2] Q Zhang, Y Li, and RW Tsien, *Science* 323 (2009) 1448-1453.

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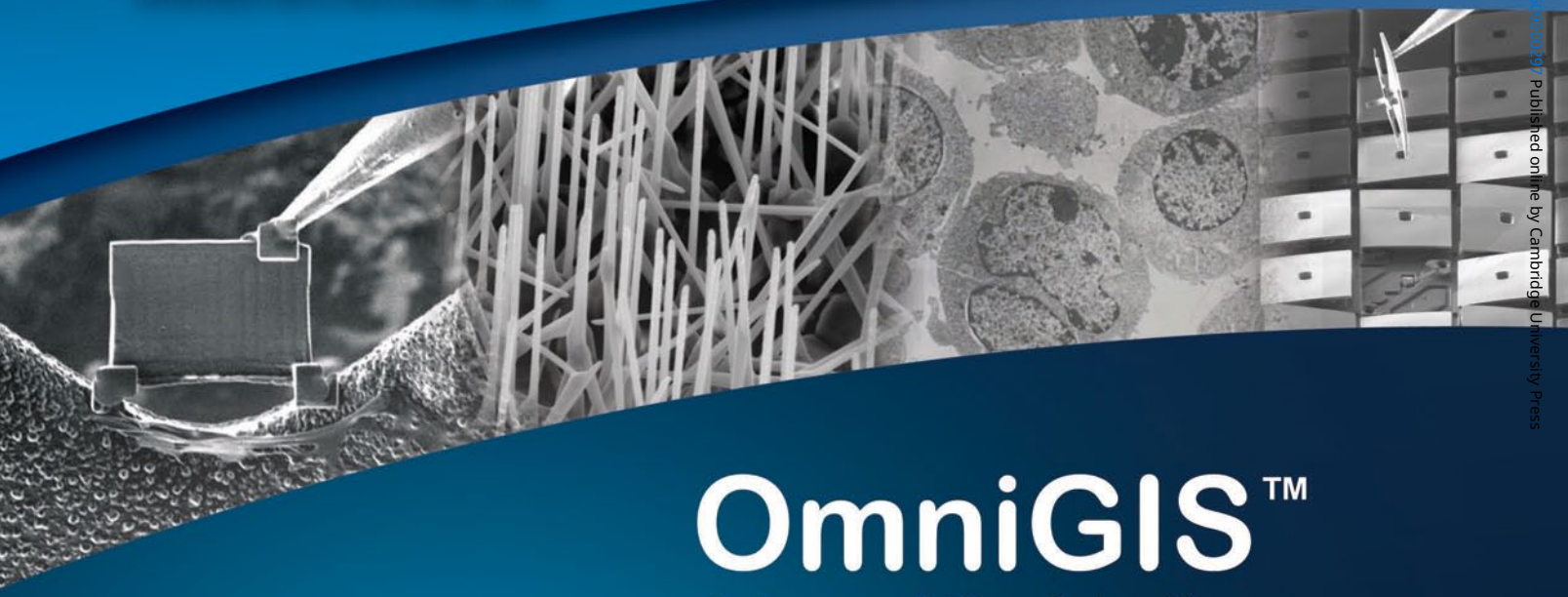

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