Emission Myths

Continued from preceding page

3) The process of optimizing the emission pattern of the gun so as to produce a compact source distribution.

Of these three uses, only the third constitutes a useful operational concept with respect to beam optimization -- but is it saturation? I personally think it's unfortunate that this term entered our vocabulary since it is more confusing than helpful. But "saturation" is probably a term we are stuck with (kind of like my other pet peeve: Energy "Dispersive" Spectroscopy). ■

1. The term "zero equipotential" describes the locus where the accelerating field is exactly zero. Outside of this boundary, electrons are repelled by a negative field, within it, they are accelerated by a positive field.
2. It will be apparent to the thoughtful reader that space-charge effects cannot be quite so blithely dismissed as my earlier comments might suggest. In the vicinity of the zero-equipotential boundary, the local accelerating field is weak, electron densities may be high, and space charge may indeed influence local emission. However, this effect, though indisputably present in some measure in any triode gun, is *not* the mechanism which produces the knee observed in the emission curve.
3. In those relatively rare commercial instruments where an independent bias supply is used, it is my observation that it is invariably implemented with a feedback mechanism so as to operate as a constant emission current source. The above observations would apply only if this feedback mechanism is disabled.

Avoiding Etching of Silver When Using Silver Staining With OsO4 Fixation

Rick Powell, Nanoprobes, Inc.

Post-fixing with osmium tetroxide before silver-staining sections causes problems because the OsO_4 can etch the deposited silver. However, it is not a good idea to perform silver enhancement after osmium tetroxide treatment because the deposited osmium can also act as a nucleating site for silver deposition, and the osmicated regions of the sample will be silver enhanced. If gold-toning is used after OsO_4 post-fixation, the OsO_4 can also nucleate gold particles. OsO_4 should always come after silver enhancement.

The greatest risk of etching deposited silver is when both osmium tetroxide and uranyl acetate are used. In the absence of uranyl acetate, etching is often not a problem.

There are two ways to prevent the etching so that osmication can still be done after silver enhancement: use of a reduced concentration of osmium tetroxide and gold-toning.

Burry and co-workers have found that in situations where etching is a problem, etching may be greatly reduced by using 0.1 % OsO_4 instead of 1 %. This has also been found to give similar levels of staining[1]. Therefore, 0.1 % OsO_4 can be safely used after silver enhancement without gold toning. This is recommended in the absence of uranyl acetate.

Silver etching is only occasionally a problem in the absence of uranyl acetate, even at 1 % osmium, when it can reduce the size of the silver particles. However, if uranyl acetate staining is done with osmication, the etching of silver is often much worse, and the silver particles can be stripped away completely (as

reported by several of our customers). Therefore, we recommend gold toning if you intend to use osmication (at any concentration) *and* uranyl acetate.

Etching may be prevented altogether by gold toning. The procedure for this is:

1) After silver enhancement, wash thoroughly with deionized water.
2) 0.05 % gold chloride: 10 minutes at 4°C.
3) Wash with deionized water.
4) 0.5 % oxalic acid: 2 minutes at room temperature.
5) 1 % sodium thiosulfate (freshly made) for 1 hour.
6) Wash thoroughly with deionized water and embed according to usual procedure[2,3].

An alternative procedure that is slightly gentler has also been reported[4]:

1) Rinse twice quickly in distilled water.
2) 0.05 M sodium acetate (1 minute) then rinse again quickly.
3) 0.05 % tetrachloroauric acid (2 minutes).
4) Thorough rinsing in distilled water for 10 minutes, then osmicate.

1) Burry, R.W. Pre-embedding immunocytochemistry with silver-enhanced small gold particles. Chap. 14, pp. 217-230. In M. A. Hayat ed. 1995. *Immunogold silver staining: Principles, methods and applications.* CRC Press, Boca Raton.
2) Arai, R., *et al.* Brain Res. Bull., 1992, 28, 343-345.
3) Arai, R., and Nagatsu, I. 1995. in: *Immunogold-Silver Staining: principles, Methods and Applications.* M. A. Hayat, ed. Chap. 13, pp 209-216. CRC Press, Boca Raton. FL.).
4) Sawada, H., and Esaki, J. 1994. Elect. Micro., 43, 361-66.