<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>10/23/2019</td>
<td>8:30 to 9:00</td>
<td>Coffee, tea and snack</td>
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<tr>
<td></td>
<td>9:00 to 9:50</td>
<td><strong>1. Introduction</strong></td>
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<tr>
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<td>• Post embedding, pre-embedding &amp; Tokuyasu Immunogold labeling workflow during the course</td>
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<tr>
<td></td>
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<td>Lab, setting up Pre-embed IGL-ASP1000</td>
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<tr>
<td></td>
<td></td>
<td>• Manual Pre-embed</td>
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<tr>
<td></td>
<td></td>
<td>• Quench, 15min</td>
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<td></td>
<td></td>
<td>• blocking 30 min</td>
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<td></td>
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<td>Primary antibody incubation</td>
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<td>10/24/2019</td>
<td>8:30 to 9:00</td>
<td>Coffee, tea and snack</td>
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<td>9:00 to 9:50</td>
<td>Pre-embed-ASP1000</td>
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<td>Ag-enhance-Fix</td>
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<td>10:00 to 11:00</td>
<td>Lab</td>
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<td>• Manual pre-embed wash 2nd antibody - Fix overnight</td>
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<td>11:00 to 12:00</td>
<td><strong>2. General consideration of immunogold labeling-I</strong></td>
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<td>• Fixation</td>
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<td>• Permeabilization</td>
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<td>• Blocking</td>
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<td>Lunch Break</td>
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<td>1:00 to 2:00</td>
<td>Embed in Biowave, ASP-1000 demo</td>
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<td>2:00 to 3:00</td>
<td>Lab, continue post-embed IGL, Washing and adding secondary antibody</td>
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<td>Setup Pre-embed IGL, quenching, permeabilization, blocking</td>
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<td>3:00 to 4:00</td>
<td><strong>3. setting up automated post-embed IGL</strong></td>
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<td><strong>4. General consideration of immunogold labeling-II</strong></td>
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<td>• Antibodies</td>
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<td>• Probes</td>
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<td>• Washing &amp; incubation</td>
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<td>4:00 to 5:00</td>
<td>Lab</td>
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<td>Checking ASP-1000 labeling,</td>
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<td>Continue post-embed IGL, washing and UA staining</td>
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<td>Continue pre-embed IGL, add primary Ab, ON</td>
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<tr>
<td>10/25/2019</td>
<td>8:30 to 9:00</td>
<td>Coffee, tea and snack</td>
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<td>9:00 to 11:00</td>
<td>Lab</td>
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<td>• Continue pre-embed IGL, washing, add secondary Ab</td>
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<td>• Silver enhancement</td>
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<td>• Examine post-embed IGL</td>
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<td>11:00 to 12:00</td>
<td><strong>5. General consideration of immunogold labeling-III</strong></td>
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<td>12:00 to 1:00</td>
<td>Lunch Break</td>
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<td>1:00 to 4:00</td>
<td>Lab</td>
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<td></td>
<td>• Lab, Continue pre-embed IGL, washing, fixation</td>
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<td>• Silver enhancement LM &amp; EM</td>
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<td>• Examine post-embed IGL</td>
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<td>4:00 to 5:00</td>
<td>Conclusion &amp; discuss results</td>
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Lecturer & Demonstrator: Ru-ching Hsia, Electron Microscopy Core Imaging Facility, UMB
Demonstrator: John Strong & Patricia Marques, EMCIF, UMB; Tom Strader, Microscopy Innovations.