High Stokes Shift Fluorescent Dyes for Biological Imaging
TECH ID #: 234.8 INVENTOR: Dr Warren E. Piers and Juan Araneda, University of Calgary

Background
One of the major challenges and limitations of fluorescence microscopy and biological imaging is the need for more efficient fluorescent dyes. A common problem with fluoresceins and rhodamines, which are typically used for labelling of antibodies, is their tendency to self-quench. The well known BODIPY dyes were introduced as replacement for the previous ones. These dyes have the advantage of displaying high quantum yields, however, they still have a major disadvantage of very small Stokes shift (8-50nm).

As the interest for biological imaging and fluorescence microscopy keeps growing rapidly, market data shows that contrast reagents (which includes fluorescent dyes) have the largest share of the global biologic imaging reagents market and they accounted for ~$2.6 billion in the last 5 years. This segment is estimated to reach $3.7 billion in 2013 at a compound annual growth rate of 7.2% (Source: BCC Research, Market Forecasting).

Our Technology
Researchers at the University of Calgary have addressed this crucial need in a growing industry by creating a new family of dyes with large Stokes shift and solvatochromic properties. The dyes have the advantage of differentiating between solvent polarities which is useful for labeling cell membranes, protein binding sites, and liposome. A larger Stokes shift eliminates spectral overlap between absorption and emission and allows detection of fluorescence while reducing interference. This also eliminates quenching of the fluorescence and gives a stronger signal when used for biological imaging. The resulting competitive advantages of the new dyes are:

- Large Stokes shift of approximately 100 nm
- Fluorescence life time up to 11.1 ns
- Quantum yields up to 0.75 depending on the dye
- Photo-stability in aqueous and organic solutions
- Solvatochromism
- Inexpensive and efficient synthesis

<table>
<thead>
<tr>
<th>Dye</th>
<th>Stokes shift</th>
<th>Quantum Yield</th>
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</thead>
<tbody>
<tr>
<td>BODIPY</td>
<td>8 nm ↓</td>
<td>near 100% ↑</td>
</tr>
<tr>
<td>Cascade Yellow</td>
<td>160 nm ↑</td>
<td>Unknown –</td>
</tr>
<tr>
<td>Xsight</td>
<td>80-90 nm ↑</td>
<td>Unknown –</td>
</tr>
<tr>
<td>Chromeo 494</td>
<td>124 nm ↑</td>
<td>25%↓</td>
</tr>
<tr>
<td><strong>Our Dye</strong></td>
<td><strong>100nm ↑</strong></td>
<td><strong>75% ↑</strong></td>
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↑High result/ ↓ Low result
Areas of Application
- Fluorescence in biological imaging
- Fluorescence lifetime assays
- Flow cytometry
- *In vitro* studies for detection of proteins, nucleic acids, other biological macromolecules; and potential for *in vivo* studies
- Multiplexing of dyes and potential application in FRET-based systems

Stage of Development
- A number of new dyes have been synthesized and structurally verified and fluorescence properties have been experimentally validated
- Dyes are currently being tested for liposome labeling applications

Intellectual Property Status
- Patent pending