






Alternative Fiducial Markers for Optical Imaging using the Xenogen IVIS-200





a supplement to Ali N. Bahadur, Isabel Q. Wu, David M. Weinstein, M. Duff Davis, David A. Lewis, Peter Kochunov, Charles Keller. "Stealth, Safe Multi-modality Chamber for Co-registered Anatomical and Molecular Small Animal Imaging," **Lab Animal**. 36, 8 [COVER ARTICLE] [PubMed UID 17721530]


Supplemental Table 1. Quantitative comparison of average radiance (p/s/cm²/sr) of five different colored Sculpey® clays imaged with a variety of emission filters used in luminescence imaging is presented below. The best color of Sculpey® clay to use as a fiduciary for each filter is highlighted in red. Images acquired with the emission filter set as Open had an exposure time of 1 sec with binning set at medium and f/stop at 4. Image acquired with all other filters had a constant exposure time, binning, f/stop set at 2 sec, medium, and 1, respectively.

	<i>Open</i>	<i>560nm</i>	<i>580nm</i>	<i>600nm</i>	<i>620nm</i>	<i>640nm</i>	<i>660nm</i>
 Green	6.54E+09	2.22E+08	2.24E+08	1.19E+08	4.97E+07	2.19E+07	8.08E+06
 Orange	4.46E+09	7.70E+07	1.43E+08	1.80E+08	1.48E+08	8.05E+07	3.64E+07
 Blue	5.99E+09	1.89E+08	7.67E+07	2.51E+07	1.04E+07	4.60E+06	1.80E+06
 White	3.86E+07	5.53E+06	3.64E+06	2.02E+06	1.16E+06	5.64E+05	2.25E+05
 Yellow	6.75E+09	2.20E+08	2.35E+08	1.90E+08	1.47E+08	6.48E+07	2.14E+07






Note: Firefly luciferase produces a greenish yellow light in the 550-570nm range while *Renilla* and *Gaussia* luciferase produce a blue light of 480nm. Hence, firefly luciferase would be best imaged with the emission filter set at 560nm while *Renilla* and *Gaussia* luciferase would be best imaged with the emission filter set at Open.

Supplemental Table 2. Quantitative comparison of average radiance (p/s/cm²/sr) of five different colored Sculpey® clays imaged with a variety of excitation and emission filters used in fluorescence imaging is presented below. The best color of Sculpey® clay to use as a fiduciary for each filter is highlighted in red. Image acquisition for GFP was set at 1 sec exposure time, medium binning, and 2 f/stop. Settings for DsRed were 0.5 sec exposure time, small binning, and 8 f/stop. Cy5.5 and ICG both had an exposure time of 2 sec with binning set at medium and f/stop at 2.

	<i>GFP</i>	<i>DsRed</i>	<i>Cy5.5</i>	<i>ICG</i>
 Green	1.57E+09	2.63E+09	1.05E+08	1.15E+08
 Orange	1.35E+09	1.58E+11	5.68E+08	8.45E+07
 Blue	1.15E+09	1.58E+09	3.67E+07	5.69E+07
 White	2.35E+08	1.67E+09	1.65E+08	7.16E+07






 Yellow	1.29E+09	3.42E+09	3.00E+08	8.14E+07
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Supplemental Table 3. Qualitative comparison of average radiance of five different colored Sculpey® clays imaged with a variety of emission filters used in luminescence imaging is presented below (same data as supplemental Table 1). Signal strength is represented by the number of + where more + indicate higher average radiance. No + denote that the chosen color of Sculpey® clay is not suitable for use as a fiduciary with the specified filter. Images acquired with the emission filter set as Open had an exposure time of 1 sec with binning set at medium and f/stop at 4. Image acquired with all other filters had a constant exposure time, binning, f/stop set at 2 sec, medium, and 1, respectively.

	<i>Open</i>	<i>560nm</i>	<i>580nm</i>	<i>600nm</i>	<i>620nm</i>	<i>640nm</i>	<i>660nm</i>
 Green	+++	+++	+++	++	++	+	+
 Orange	+	+	++	+++	+++	+++	+++
 Blue	++	++	+	+	+	+	+
 White							
 Yellow	+++	+++	+++	+++	+++	++	++

Note: Firefly luciferase produces a greenish yellow light in the 550-570nm range while *Renilla* and *Gaussia* luciferase produce a blue light of 480nm. Hence, firefly luciferase would be best imaged with the emission filter set at 560nm while *Renilla* and *Gaussia* luciferase would be best imaged with the emission filter set at Open.

Supplemental Table 4. Qualitative comparison of average radiance of five different colored Sculpey® clays imaged with a variety of excitation and emission filters used in fluorescence imaging is presented below (same data as supplemental Table 1). Signal strength is represented by the number of + where more + indicate higher average radiance. No + denote that the chosen color of Sculpey® clay is not suitable for use as a fiduciary with the specified filter. Image acquisition for GFP was set at 1 sec exposure time, medium binning, and 2 f/stop. Settings for DsRed were 0.5 sec exposure time, small binning, and 8 f/stop. Cy5.5 and ICG both had an exposure time of 2 sec with binning set at medium and f/stop at 2.

	<i>GFP</i>	<i>DsRed</i>	<i>Cy5.5</i>	<i>ICG</i>
 Green	+++	+	+	+++
 Orange	+++	+++	+++	+
 Blue	++			
 White	+		+	
 Yellow	+++	+	++	+