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Near-Infrared *In Vivo* Imaging System

Application Note

In Vivo Animal Imaging Diet Considerations

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In Vivo Animal Imaging Diet Considerations

A key advantage of near-infrared (NIR) optical imaging is the ability to image animals at wavelengths where background signals from tissue autofluorescence are at their lowest. However, interfering signals can still confuse or even ruin a well-designed experiment. For example, the diet fed to an animal prior to imaging can have dramatic effects on image quality and clarity, due largely to the chlorophyll component of many plant-based ingredients used in regular mouse chows. Chlorophyll fluoresces naturally, emitting between 675 and 685 nm, and is detected in the 700 nm channel. Many basic mouse chows contain a number of ingredients that may include a variety of plant material, which contribute to the fluorescent signalin animal images[1,2].

Figure 1 illustrates how diet selection can affect *in vivo* imaging of mice. Red signal represents the fluorescence from a regular chow (7012) in the 700 channel. The mouse was switched to a purified diet (TD.97184) and imaged daily. This time series illustrates that a switch in diet will require ~4 days to clear residual 7012 from the intestinal tract. A fasting period of 24 hours will not alleviate the problem.

The major limiting factor in any imaging study is the relative background signal of the mouse. Signals from targeting agents must be higher than the background fluorescence for detection. Therefore, minimizing background signal is very important. Our objective was to evaluate the fluorescent properties of diets in more detail. A variety of diets were provided by Harlan Teklad, Research Diets, and Purina LabDiet, ranging from conventional diet preparations with a variety of plant-based ingredients to purified formulations. The costs of these diets vary, with purified diets generally costing more than traditional preparations. We focused on small animal imaging (mice) in the NIR region of the spectrum (700 nm and 800 nm) using the Odyssey® Infrared Imaging System and the Pearl® Imager (LI-COR Biosciences).

Treatments

Harlan Teklad (http://www.teklad.com/)

Diet ID	Content
TD.97184	(purified) Control
2018	Standard diet (no alfalfa, moderate level soybean meal; reproduction & growth)
2016	Standard diet (no alfalfa, no soybean meal, main ingredients are corn and wheat; growth and maintenance)
2014	Standard diet (no alfalfa, no soybean meal; main ingredients are corn and wheat; maintenance)
8604	Standard diet (lots of soybean meal, contains fish meal, no alfalfa; higher in protein)
7013	Standard diet (NIH-31 modified; contains alfalfa and fish meals)
7012	Standard diet (contains alfalfa and lots of soybean meal)

Research Diets (http://www.researchdiets.com/)

Diet ID	Content
D10001 AIN-76A	Purified diet
D10012 AIN-93G	Purified diet
D10012 AIN-93N	Purified diet
D12450B	10 kcal % fat; purified diet; dyed yellow
D12451	45 kcal % fat; purified diet; dyed red
D12492	60 kcal % fat; purified diet; dyed blue

Purina LabDiet (http://www.labdiet.com/)

Diet ID	Content
5K96	Wheat, corn, oats
5V75	Corn, wheat, corn gluten meal
AIN-93M	Purified diet

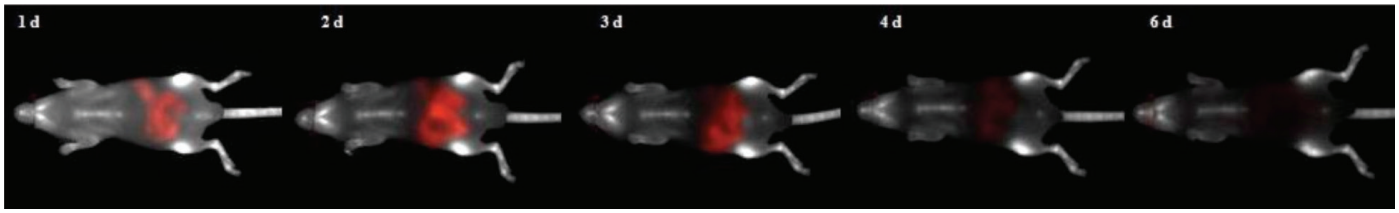

Figure 1. A mouse switched from regular mouse chow (7012) to a purified diet (TD.97184) was imaged daily to record the movement of the diet from the digestive tract. Images were captured using the Pearl® Imager and normalized to the same lookup table (LUT) for the 700 nm and 800 nm channels. Autofluorescence from the mouse chow is represented in red. IRDye® 800CW BoneTag™ Optical Probe was delivered (IV) to the mouse on day 0 with signal depicted in grayscale.

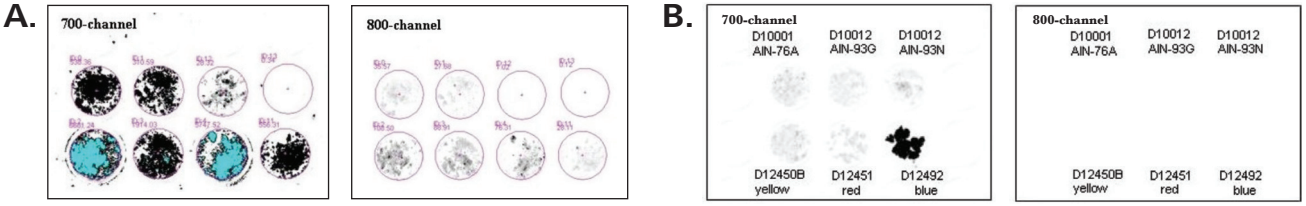

Figure 2. Images represent 0.02 g aliquots of diets from either Harlan Teklad (A) or Research Diets (B), captured on an Odyssey Imager. Individual channel images are shown in grayscale with saturated signals represented in blue. (Purina LabDiet images not shown.)

Results and Discussion

Harlan Teklad, Research Diets, and Purina LabDiet are companies that provide a wide variety of diets specifically designed or custom-made to meet the needs of researchers and their test subjects. We focused on diet alternatives for mice that would effectively minimize background when imaging in the NIR. Diets formulated with different grain sources (alfalfa, soybean meal, corn, wheat, or fishmeal) provided by Harlan Teklad and Purina LabDiet were evaluated for their fluorescent contribution to background.

Small aliquots were taken from each of the treatment groups (0.02 g) and imaged in a 24-well microtiter plate on an Odyssey Imager. Results from these scans are shown in Figure 2. Scan intensities were set at the lowest level (1.0) for both channels. Even at this low scan intensity setting, 7013 and 7012 show saturation in the 700 nm channel (blue in individual grayscale images). The two samples with saturation were not included in the signal analyses for the 700 nm channel because saturated signals are not quantifiable. All samples were evaluated in the 800 nm channel region because the signals are considerably lower and exhibit no saturation.

It is important to note that no saturation of signal was detected in any purified diet. Three non-purified diets from Harlan (2014, 2016, 2018) and two diets from Purina LabDiet (5K96,5V75) showed low but measurable signal in the 700 nm channel. These diets did not contain any alfalfa but did contain other plant material, likely contributing to the fluorescence. This would suggest that if cost is an issue, one of these diets might provide an alternative to the more costly purified diets. Scan intensities adjusted for background and sample weight are shown in Table 1.

Diet 8604 exhibited the highest level of fluorescence from the 700 nm channel, while the purified diets showed the lowest level of signal. The remaining three diets (2014, 2016, and 2018) all produced approximately 78% less signal and diets 5V75 and 5K96 approximately 93% less signal than 8604. Whether this is significant in the animal is yet unknown; however, it may reduce the overall gut signal. Diets 2014 and 2016 containing corn and wheat in place of soybean meal showed approximately 45% more signal in the 700 nm channel than 2018; nevertheless, this signal is approximately 75% less than 8604.

The soybean meal-based diet, 2018, contains no fishmeal and is lower in its overall signal. When 2018 is compared to the diet containing soybean meal and fishmeal (8604), there appears to be a nearly 5-fold difference in signals. Diet 2018 contains less than half the soybean meal provided in 8604, which strongly suggests that fishmeal is contributing the additional offending signal between these two diets.

Table 1.

			Signal-Bkg/g[a]	
ID	**Description**	**Weight, g**	**700**	**800**
Background		0.00		
AIN-76A	purified	0.10	591.4	25.6
AIN-93G	purified	0.10	433.1	20.9
AIN-093N	purified	0.10	445.4	20.2
D12450B Y[b]	purified, yellow	0.10	608.6	19.7
D12451 R[b]	purified, red	0.10	379.4	14.2
D12492 B[b]	purified, blue	0.10	9658.8	24.6
AIN-93M	purified	0.10	63.7	5.7
5V75	corn, wheat; maintenance	0.10	6803.6	266.3
5K96	wheat, corn, oats, fishmeal	0.10	591.4	163.5
7013	alfalfa & fishmeal	0.02	334045.0[c]	5419.0
7012	alfalfa & high level SBM[d]	0.02	287359.0	3809.5
8604	high level SBM & fishmeal	0.02	95684.5	4439.5
2014	corn, wheat; maintenance	0.02	27798.5	1399.5
2016	corn, wheat; growth	0.02	26901.0	1772.5
2018	moderate SBM	0.02	15512.5	1378.0
TD.97184	purified	0.02	1399.0	45.0

[a] Signal intensities from 700 nm and 800 nm channel minus background per sample weight (g).
[b] Y = yellow-dyed diet; R = red-dyed diet; B = blue-dyed diet.
[c] Red represents a saturated intensity and not included in any analyses.
[d] Soybean meal

None of the diets exhibited saturation in the 800 nm channel. The purified diet again outperformed all, with negligible signal in the 800 nm channel. All diets provided by Research Diets are purified in their makeup and should be suitable for *in vivo* imaging; however, acolored dye (yellow, red, or blue) is available for easy differentiation of the various diets. Figure 3 graphically represents the signal intensities adjusted for background for all diets tested. Non-purified formulations, 5K96 and 5V75, exhibited 800 nm signals approximately 10x higher than any purified diet, but outperformed the other grain-based diets evaluated.

Conclusion

As presented here, a simple consideration of what diet to feed mice prior to imaging could improve background levels significantly in the abdominal region. Purified diets (TD.97184, AIN-76A, AIN-93G, AIN-93N, D12450B, D12451, D12492, or AIN-93M) would bean excellent choice for NIR optical imaging due to the very low signals captured in both imaging channels.

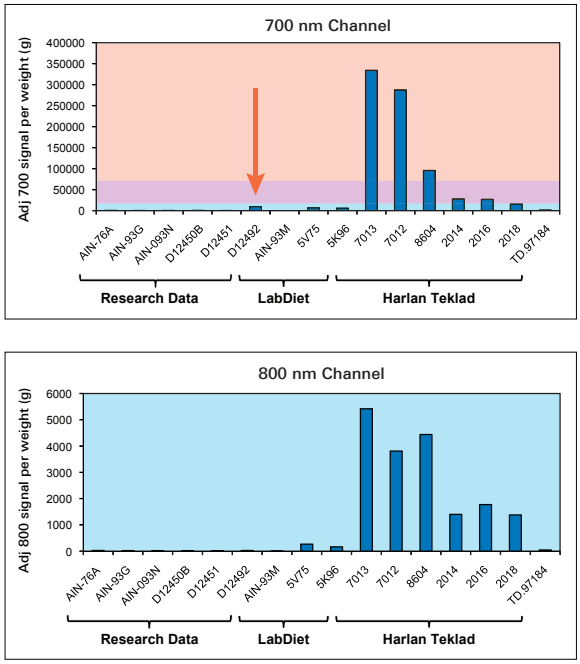

Figure 3. Research Diets, Purina LabDiet, and Harlan Teklad diets were scanned on an Odyssey Imager in the 700 and 800 nm channels. Signal intensities were adjusted for background and corrected for weight. The arrow highlights the blue-stained diet. Diets producing low fluorescent signals ideal for optical imaging will be located in the blue shaded area. Diets in the purple region may be fine but will produce a higher level of autofluorescence. Those diets peaking in the orange region will produce interfering levels of autofluorescence.

Signal levels registered in the 800 nm channel for all diets tested are at levels that should pose very little problem in an imaging study. With regard to the 700 nm channel, however, three diets (7012, 7013, and 8604) containing a majority of alfalfa and fishmeal would be poor choices for NIR optical imaging in mice, since the signal is extremely high. Thus, these three diets would not be good selections for studies involving animal imaging.

If cost is a consideration, diets containing alternative plant material such as soybean meal, corn, and wheat (5K96, 5V75, 2014, 2016, and 2018) exhibited approximately 75-95% less signal than alfalfa-based diets. Because these diets still use natural ingredients and are not classified as purified, their costs may be more attractive.

If stains used for identification of diets are required, red and yellow would be recommended over blue; however, very little signal is generated by these dyes. Finally, approximately 4-6 days will be necessary to clear diet fluorescence from the abdominal region when switching mice from a regular diet to a purified alternative.

References

1 MacLaurin, SA, M Bouchard, P Dwyer, R Levenson, J Mansfield, and T Krucker. 2006. Reduction of skin and food autofluorescence in different mouse strains through diet changes. Poster, Society for Molecular Imaging, Annual Meeting, Hawaii.

2 Ricci, MR, and EA Ulman. 2005. Laboratory animal diets: A critical part of your *in vivo*research. *Animal Lab News* 4(6):1-6.

LI-COR Biosciences

Serving the United States and Canada.

Phone: +1-402-467-0700
Toll free: 800-645-4267
biosales@licor.com

LI-COR GmbH, Germany

Serving Europe, Africa, and the Middle East.

Phone: +49 (0) 6172 17 17 771
bio-eu@licor.com

LI-COR Ltd., United Kingdom

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Phone: +44 (0) 1223 422104
bio-eu@licor.com

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