Evaluation of Rodent Diets for Minimizing Abdominal Background Fluorescence in Cryo-Fluorescence Tomography on Xerra™

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BACKGROUND

Cryo-Fluorescence Tomography (CFT) has proven utility in therapeutic discovery and development for evaluating whole animal distribution of drugs and expression of gene products[1,2]. The Xerra™ CFT platform (EMIT Imaging, Inc.) features imaging capability spanning a broad range of wavelengths from green to near-IR, with high resolution, high sensitivity, and options for multiplexing.

Successful fluorescence imaging at any wavelength depends on signal from the target being sufficiently higher than that of the background or autofluorescence. The contribution of chlorophyll to autofluorescence signal in the abdomen in the far-red and near-IR has been well documented[3,4], and plant-based chows lacking alfalfa and diets formulated with purified ingredients are now widely utilized to minimize background attributable to chlorophyll and its metabolites. The impact of diet components on abdominal autofluorescence at visible wavelengths, however, is less well studied and understood, and some chows suitable for the near-IR may not be appropriate for the green (or other) region of the visible spectrum.

To improve CFT and other fluorescence imaging methods it is essential to understand the autofluorescence of rodent diets that minimize abdominal signal in the wavelength of interest for a particular study. To that end, we evaluated the fluorescence of an array of standard, alfalfa-free, and purified diets supplied by Inotiv, LabDiet, and Research Diets across a range of visible and near-IR wavelengths used in molecular imaging.

OBJECTIVES

- Examine rodent chow autofluorescence at visible and near-IR wavelengths, commonly used in molecular optical imaging
- The primary objective of this work is to study chow autofluorescence from green to the near-IR of the electromagnetic spectrum
- The outcome of this work is to improve CFT and other fluorescence imaging methods by identification of rodent diets appropriate for minimizing abdominal autofluorescence in the wavelength range of the particular study

METHODS

- Rodent chow samples were obtained from Inotiv, LabDiet, and Research Diets, who supply many research facilities.
- In total, 23 pellets were tested and are summarized in Table 1 and Table 2
- Each pellet was embedded in our preferred cutting medium and imaged on Xerra over the full complement of excitation (470 nm to 780 nm) and emission (511 nm to 840 nm) settings and at exposure times normally captured in rodent studies.

Table 1. Research Diets Purified Diet Summary

Product	Coloring	Details[a]	Use/Purpose
AIN-76A	None	American Inst of Nutrition Standard Diet	Breeding, Growth
AIN-93G	None	Modified AIN-76A	Breeding, Growth
AIN-93M	None	Modified AIN-76A	Maintenance
D12451	Red	45 kcal% fat	Diet-Induced Obesity Studies
D12450H	Orange	10 kcal% fat, sucrose matched to D12451	Low fat Control for D12451
D12492	Blue	60 kcal% fat	Diet-Induced Obesity Studies
D12450J	Lt Green	10 kcal% fat, sucrose matched to D12492	Low fat Control for D12492
D12450B	Yellow	10 kcal% fat, moderate sucrose	Low fat Control Diet
D12450K	Purple	10 kcal% fat, no sucrose	Low fat Control Diet
D11112201	Green	OpenStandard Diet	Breeding, Growth, Maintenance
D11112201Ni	None	D11112201 - no dye added	Breeding, Growth, Maintenance

[a]Full ingredient lists available on Research Diets website

METHODS (Cont.)

Table 2. Grain-based Rodent Diet Summary

Diet Type	Product	Purpose[a]	Select Ingredients[b]								
			Alfalfa	Soybean	Wheat	Corn	Yeast[c]	Oats	Beet Pulp	Fish Meal	Porcine Meal
Teklad Global	2914	M	×	×	✓	✓	×	×	×	×	×
	2916	G,M	×	×	✓	✓	×	×	×	×	×
	2918	B,G,M	×	✓	✓	✓	✓	×	×	×	×
	2919	B,G,M	×	×	✓	✓	✓	×	×	×	×
	2920X	B,G,M	×	×	✓	✓	✓	×	×	×	×
LabDiet Verified	5V75	B,G,M	×	✓	✓	✓	×	×	✓	×	×
	5V5R	B,G,M	×	×	✓	✓	✓	×	×	×	×
	5V5M	B,G,M	×	×	✓	✓	✓	×	×	×	×
Standard[d]	7012	B,G,M	✓	✓	✓	✓	✓	✓	×	×	×
	7013	B,G,M	✓	✓	✓	✓	✓	✓	×	✓	×
	8604	B,G,M	×	✓	✓	✓	✓	×	×	✓	×
	5001	G,M	✓	✓	✓	✓	✓	✓	✓	✓	✓

[a]B=breeding, G=growth, M=maintenance
[b]Full ingredient lists available on Inotiv and LabDiet websites
[c]Dried brewer's yeast
[d]Diet 5001 is LabDiet; 7012,7013, and 8604 are Teklad Diet

RESULTS

- An example set of white light and fluorescence images from the Xerra system is shown in Figure 1
- Region of interest analysis was performed on each pellet to estimate the total fluorescence signal at each wavelength (Figures 2-4)
- Note that CFT images are 16-bit so signal/pixel saturation is at 65536

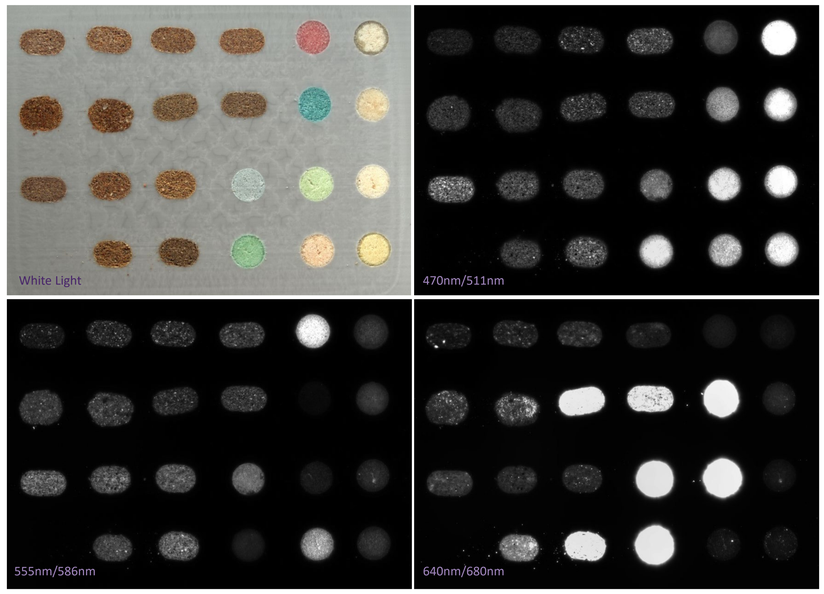

FIGURE 1. White light and fluorescence images of the 23 food pellets from the Xerra CFT imaging system

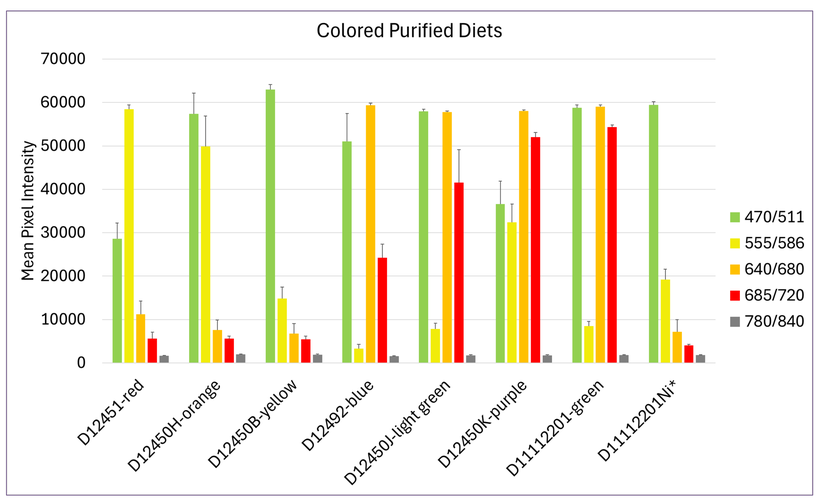

FIGURE 2. Results from the Colored Purified Diets

RESULTS (Cont.)

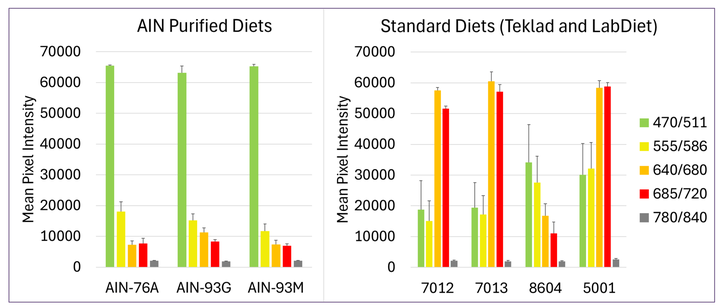

FIGURE 3. Results from AIN (left) and Standard (right) diets

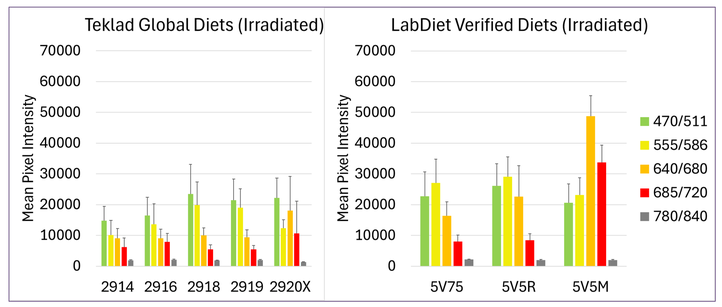

FIGURE 4. Results from Teklad (left) and LabDiet (right) irradiated diets

- Standard diets containing alfalfa were highly fluorescent in the far-red and NIR (680 nm – 720 nm emission), though they and all other diets tested showed a negligible signal at longer NIR wavelengths (794 nm – 840 nm emission), which is expected due to low chlorophyll fluorescence
- The AIN purified diets showed very high fluorescence in the green region (511 nm emission), as did most other purified formulations.
- AIN diets had low fluorescence at all other wavelengths tested.
- Purified diets with added coloring agents fluoresce in a wavelength-dependent manner
- The signal from all purified diets was much less variable in the pellets than grain-based chows
- Fluorescence from alfalfa-free formulations of the Teklad™ Global Diets and LabDiet PicoLab® product lines was low to moderate depending on wavelength, with high variation within some pellets.

CONCLUSIONS

- Animal diet choices must balance the dietary needs of the animal with the experimental requirements, this is especially true for optical imaging methods
- Researchers are advised to select foods that have minimal autofluorescence at the imaging wavelengths of interest and to select imaging methods, such as 3D CFT, where the autofluorescence can be spatially separated from tissues of interest

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