

The Ami HT Optical Imaging System: New Features and Comparisons to the IVIS System

We recently acquired a new optical imaging system, the Ami HT system (Spectral Instruments Imaging, Inc.), with the funding support from the Lineberger Cancer Center. The Ami system, shown in Figure-1, is capable of providing both bioluminescence and fluorescence imaging on animals and tissue specimens for preclinical research. The system is housed in Marsico Hall, room SB223, within the BRIC Small Animal Imaging Facility. Together with the IVIS-Kinetic and IVIS-Lumina systems, the new Ami system will provide more optical imaging support, and facilitate research projects in our community.

Main Features and Technical Specifications:

The Ami system is very similar to the IVIS –Kinetic system in terms of system structure and operation. However, there are several unique features including:

- **Large field of view**
 - Default 25 x17 cm field of view
 - Can image five mice without demagnification lens
- **Wavelength specific LED excitation light source for fluorescence imaging**
 - Individual LED for each wavelength
 - Adjustable power level to meet various excitation needs
- **Vertically opening door with more efficiency**
 - Less isoflurane leakage
 - Easier to use and more robust
- **Aura imaging software with unlimited seat licenses (downloadable online)**
 - Analysis can be done on any 64bit Windows computer
 - Ability to analyze IVIS Living Image files

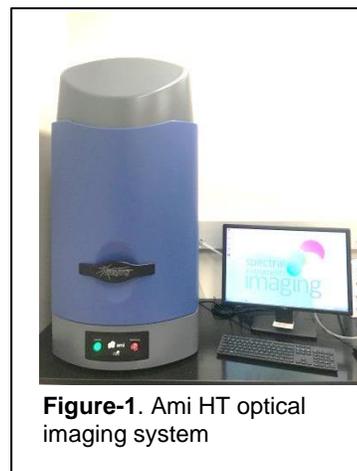


Figure-1. Ami HT optical imaging system

Table-1: System specifications of Ami and IVIS models

Parameters	IVIS-Kinetic	IVIS-Lumina	Ami-HT
Camera Sensor	Back-thinned, back-illuminated, cooled EMCCD	Back-thinned, back-illuminated, cooled CCD	Back-thinned, back-illuminated, cooled CCD
CCD Size	13 x 13 mm	13 x 13 mm	25.9 x 17.3 mm
CCD Operating temp.	at -80°C	at -90°C	at -90°C
Quantum Efficiency	>85% at 500 – 700 nm	>85% 500 – 700 nm	>85% 500 – 700 nm
Imaging Pixels	1024 x 1024	1024 x 1024	2048 x 2048
Minimal Pixel Size	50 microns	50 microns	22.5 microns
Minimum Detectable Radiance	100 photons/s/sr/cm ²	100 photons/s/sr/cm ²	45 photons/s/sr/cm ²
Field of View (FOV)	Min: 5 x 5 cm; Max: 12.5 x 12.5 cm	Min: 5 x 5 cm; Max: 12.5 x 12.5 cm	Min: 10 x 7 cm; Max: 25 x 17 cm
Dark Current (Typical)	< 3 x 10 ⁻⁴ electrons/s/pixel	< 3 x 10 ⁻⁴ electrons/s/pixel	< 0.9 x 10 ⁻⁴ electrons/s/pixel
Fluorescence Excitation Filters	10 high resolution filters	10 high resolution filters	10 high resolution LED wavelength specific LED
Fluorescence Emission Filters	18 high-end and 4 standard filters available	4 Standard filters	10 high-end filters integral to system

Performance comparisons to the IVIS-Kinetic system:

The core has conducted a series of evaluations on the new system with a calibration phantom. The results are presented below.

1) Photon quantification

A calibration phantom with stable photon emitting source was measured in the Ami and IVIS-Kinetic system. The photon measurements from the two systems were comparable, however, approximately 3.2% more photon was detected per area in Ami system compared to IVIS-Kinetic system, indicating higher sensitivity from the new Ami system. It is recommended that users start the Ami system for a new study. If users want to switch systems in the middle of the study, it is highly recommended that some evaluation studies be conducted before switching. Please contact the SAI staff if you wish to do your evaluation studies before switching.

	Ami	Kinetic	% Difference
Radiance (x 10 ⁸ photons/sec)	9.16 ± 0.03	8.48 ± 0.02	7.7 %
Mean Radiance (x 10 ⁸ photons/sec/cm ²)	2.92 ± 0.01	2.83 ± 0.01	3.2 %
Imaging parameter: Exp. Time=10s; Binning=2; F/stop=Maximum; FOV=Maximum; 5 repeated imaging			



Figure-2. Image example with the calibration phantom taken from Ami system

2) Camera signal/counts response

With the same imaging acquisition parameters (exposure time, binning, and aperture size), mean signal in counts/s was measured on the same calibration phantom from Ami and IVIS-Kinetic system. Ami system responded much higher counts/s (about 5.7 times high) compared to IVIS-Kinetic, indicating much higher quantum efficiency, and thus higher sensitivity for the Ami system. This means that imaging on weak bioluminescence signal might make big differences between the two systems, although imaging on high signal would be more comparable (See the comparison in #1)).

	Exposure Time	Ami	Kinetic	Ratio
Mean Signal (Counts/s)	0.5 Sec	1991.0	355.2	5.7
	10 Sec	37935.4	6651.8	5.7
Other imaging parameters: Binning=2; F/Stop =Maximum; FOV=Maximum				

3) Signal to background contrast

Both phantom signal and background signal was measured. The Signal-to-background ratio was compared between the Ami and IVIS-Kinetic system. The Ami demonstrated much higher signal-to-background contrast (about 3.8 times higher) compared to that of IVIS Kinetic, consistently suggesting higher sensitivity in the Ami system.

	Ami	Kinetic
Phantom (mean counts)	37780	6803
Background (mean counts)	1.809	1.24
Signal-Background Ratio	20884	5486
Difference	3.8 x higher in Ami	

4) Fluorescence excitation, emission filters

The Ami and IVIS-Kinetic systems have the same excitation filter sets for fluorescence imaging, but the emission filters are different. Ami has total of 10 emission filters, while IVIS-Kinetic has 21 narrow emission filters, plus another 4 broad band emission filters. Filter sets for each system are listed below.

Emission Filters (nm)	Ami	IVIS-Kinetic		
	Ten Filters	Low	Mid-high	High
	530	500	640	720
	590	520	660	740
	630	540	680	760
	650	560	700	780
	690	580	720	800
	710	600	740	820
	730	620	760	840
	750			
	770			
	790			
Excitation Filters (nm)	430, 465, 500, 535, 570, 605, 640, 675, 710, and 745			