1. Purpose

This document provides a brief overview of updates included in the Living Image 4.3.1 Service Pack 1 and new features and improvements in Living Image 4.3.1 released in June 2012. Living Image 4.3.1 serves as an update for Living Image 4.2 version on IVIS Lumina II, Spectrum, Kinetic, and Lumina XR instruments, and updates Living Image 4.3 on IVIS Spectrum CT. Major features include the introduction of the DyCE module and a complete makeover of the Spectral Unmixing tool. Living Image 4.3.1 release also provides improvements to the existing software features and bug fixes.

2. Updates and Improvements in Living Image 4.3.1 Service Pack 1

2.1. Correction of Ring Artifacts on IVIS Spectrum CT

Ring artifacts in CT reconstruction images are induced by non-linearity, fixed pattern noise and uncorrected defects.

2.1.1. Fixed Pattern noise

Additional cleaning cycles for the dark and flood images are incorporated in Living Image 4.3.1 Service Pack 1 to account for residual uncorrected fixed pattern noise in the X-Ray flat panel detector occurring between changes in bin size.

2.1.2. Defect map generation

Large area flat panel sensors can have significant number of defective pixels. These pixels can have a higher or lower gain than neighboring pixels and must be corrected prior to reconstruction. Living Image 4.3.1 used a reference image that did not always show defective pixels resulting in incomplete correction. The Service Pack improves on this method by using images with a better signal to noise ratio to calculate the defect map resulting in an improved correction. These static defect maps are now acquired every five days.

The vertical streaking in the central transaxial slice shown on the left panel will result in a ring artifact in the reconstructed volume. The vertical streaking is removed in Living Image 4.3.1 SP1 by applying the corrections described above.
2.2. Support for Automatic Co-registration with CT images from Siemens Inveon

Living Image supports the import of DICOM files from 3rd party CT systems including the Siemens Inveon. In the Living Image 4.3.1 Service Pack 1, this DICOM reader was improved and now loads these images with the correct slice thickness and in the correct order in the DICOM browser. If an image is cropped prior to being exported, the automatic registration routine will run when it is imported into Living Image. Due to complexities in the DICOM standard, this image then needs to be mirrored in the X- and Y- directions and then manually readjusted for accurate registration.

![3D optical source co-registered with Siemens Inveon DICOM volume in Living Image 4.3.1 SP1](image)

2.3. 3D Volume Rendering and Visualization Improvements

2.3.1. Enhanced Volume Rendering Engine

3D Volume rendering in the 3D Multi-Modality tools has been enhanced to improve the visualization. As shown in the example below, the Living Image 4.3.1 Service Pack 1 provides better contrast between tissues, higher dynamic range of the scene, and more realistic lighting on the tissue interfaces. This new rendering shows more detail and clarity in high contrast tissues within the CT images.
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2.3.2. Default Gradient Illumination
Gradient Illumination option is enabled by default in the 3D Multi-Modality tool palette to leverage the rendering improvements.

2.3.3. Low-end Graphic Card Solution
The Living Image 4.3.1 Service Pack 1 enhanced visualization features require additional processing time on the graphics card. On low-end graphic cards, interacting with the UI may become slow with the new high quality rendering. To improve the user experience on these low-end graphic cards, a performance oriented rendering mode is added as an option. This option
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offers a reasonable visual boost compared to the previous version but at slightly lower quality than the new method. The optimal rendering mode is suggested when a volume is loaded based on the automatic assessment of the current rendering speed. To override the “Performance” rendering mode, select “Quality” checkbox in the 3D Multi-Modality tool palette. “Quality” mode is not recommended for low-end GPU.

2.3.4. Denoise Filter
A “Denoise” checkbox is added as an option to filter out image noise in the volume rendering. The raw data is not modified by this filter, still enabling manipulation of the lookup table to adjust the visualization. “Denoise” option is available in conjunction with “Quality” mode.

2.3.5. Faster Rendering During User Actions
A relatively low level of details is used during panning and rotations of 3D volume, to speed up the rendering.

2.4. The following table is a summary of additional fixes in the Living Image 4.3.1 Service Pack 1.

<table>
<thead>
<tr>
<th>ID</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>LI-5215</td>
<td>New default display settings for surface loaded with DLIT and FLIT reconstructions</td>
</tr>
<tr>
<td>LI-5276</td>
<td>C++ translation error in removing negative G elements, which caused FLIT crashes</td>
</tr>
<tr>
<td>LI-5330</td>
<td>XFOV 24 auto-exposure case where f-stop is saved as f1 instead of f1.2</td>
</tr>
<tr>
<td>LI-5258</td>
<td>Display radiation symbol when acquiring CT reference image</td>
</tr>
<tr>
<td>LI-5329</td>
<td>Unable to change x-ray high voltage on Lumina XR in QA Tools x-ray test panel</td>
</tr>
<tr>
<td>LI-5351</td>
<td>Close Caliper Port Router process if Living Image terminates abnormally</td>
</tr>
</tbody>
</table>
3. New Features in Living Image 4.3.1 Release

3.1. Dynamic Contrast Enhancement (DyCE) Imaging and Analysis

DyCE imaging and analysis is intended for bio-distribution studies. DyCE imaging captures a time series of optical images immediately following a bolus injection of a probe or dye. The Living Image® software temporally unmixes the data on a pixel-by-pixel basis for each image of the time series and determines real-time spatio-temporal distribution of the probe or dye signal. The DyCE module is sold and licensed separately as an add-on feature. DyCE is currently available for both luminescent and fluorescent imaging.

3.1.1. DyCE Sequence Acquisition

The best way to set up a DyCE Sequence Acquisition is through the Imaging Wizard. The Wizard will walk the user through the necessary steps required to set up a proper DyCE Sequence. The time series of data can include up to five intervals. Each interval is defined by duration and delay between images. Due to size limitations, the time series currently can include up to a maximum of 100 images.

Luminescent DyCE measurements are particularly useful for following biodistribution of radiotracers. In these cases, manual exposure is recommended because the radioactive tracer Cerenkov signal level is so low that the time required to perform auto-exposure will result in loss of detectable signal. In the fluorescent mode, DyCE is currently limited to a single excitation/emission wavelength pair and auto-exposure acquisition method is recommended.
3.1.2. DyCE Analysis
DyCE sequences are defined with a new icon in the browser window for easy identification. When the DyCE sequence is loaded from the browser window a “Spectral Unmixing and DyCE” option shows up in the Tool Palette, which guides the user through the analysis steps. The user can either have the software automatically or manually unmix the data to review the spatio-temporal distribution. Other features include a movie viewer which allows the user to step through each time point. There is also the ability to drag a bar or use the scroll box to freeze to a selected individual frame.

3.2. New and Improved Spectral Unmixing
Spectral unmixing tool has been completely renovated in Living Image 4.3.1 to incorporate the spectral unmixing techniques from Cambridge Research & Instrumentation, Inc. (CRI), which was acquired by Caliper Life Sciences in 2010. In addition to the wizard driven spectral unmixing method in the older Living Imaging software, new methods are implemented to allow users to extract spectra from the image data and derive the pure spectrum individually. Such user guided approach offers more flexibility and control in the analysis. Improved results can be achieved when the right knowledge is applied. Moreover, the unmixed spectra can be saved as a customer spectrum library and reused to unmix other datasets. Being able to reuse reference spectra from a spectrum library will help improve the accuracy and consistency in the longitudinal/group studies. An automatic unmixing method has been preserved for cases when target fluorophore locations and/or spectra are
unknown. At last, the new spectral unmixing tool adds the support to the temporal data for DyCE analysis.
3.3. Mirror ROI for Side Imager
A new IVIS system accessory is available for placement in the imaging chamber, the Side Imager. This new accessory allows for light emitted from the animal sides to be directly imaged by the camera by using mirrors. In Living Image 4.3.1, the Mirror ROI is introduced for images acquired with the Side Imager, and specifically accounts for the reflection and solid angle losses from the mirror reflected image. A specially tailored Side Imager has been designed for each of the IVIS systems. Currently only 2D analysis is enabled for data acquired with the Side Imager.

![Side Imager for the IVIS Spectrum](image1)

![Mirror ROI measurements on a mouse in Side Imager](image2)

3.4. Support for all IVIS acquisition platforms
Living Image 4.3.1 serves as an update for Living Image 4.2 version on IVIS Lumina II, Spectrum, Kinetic, and Lumina XR instruments. The DyCE module can be installed on any IVIS instrument and adds a new imaging modality in the Imaging Wizard. On Lumina XR, for example, a DyCE dataset can be acquired with an x-ray image included in the sequence.

3.5. FLIT improvements
Regularization in FLIT has been tailored to provide more stable reconstruction solutions with reduced artifacts in NIR mouse data. Because of the regularization, there may be an increase in reconstruction time.
3.6. Support for IVIS Spectrum CT instrument
IVIS Spectrum CT was launched with Living Image 4.3 designed with all of the features needed for acquisition and reconstruction of CT images for the IVIS Spectrum CT instrument. The IVIS Spectrum CT is an integrated optical-µCT instrument specifically designed to provide anatomical structure for bioluminescent and fluorescent imaging. To simplify the workflow, an automated image co-registration algorithm has been implemented in Living Image 4.3.

3.7. High Resolution CT Imaging Improvements
CT image reconstruction in Living Image involves a step where the sinogram is filtered prior to performing a Fast Fourier Transform. In Living Image 4.3.1, the filter type was changed to improve the image and alleviate an intensity artifact. In addition, the reconstructed image is now cropped to a circle.
3.8. Lens Distortion Correction
Optical Images, particularly those at large fields of view (FOV C and D) can have noticeable distortion near the edges of the field of view. A new correction option “Lens Distortion Correction” is applied to photographs, luminescent, and fluorescent images acquired on IVIS Spectrum and IVIS Spectrum CT instruments with Living Image 4.3 and later versions of the software. Measurements made on distortion-corrected images may vary slightly when compared to measurements made on older data that are not distortion corrected. ROIs should not be copied between distortion-corrected data and older data that are not distortion-corrected due to slight positioning differences.

3.9. User Settings and Logout
An easier Logout feature has been implemented in Living Image with quick access to the Logout button from the main application toolbar. The feature can aid with collecting more accurate Usage statistics and billing.

A new User Setting dialog is added to the main application toolbar for managing user accounts, such as adding, deleting, optionally setting password protection, or locking user accounts.

3.10. Lock User Accounts
The Lock User Accounts optional feature provides an ability to “lock” user accounts and prevent inadvertently deleting users from the system. If this additional security is applied, a master password
is required to change user passwords, delete users, or unlock user accounts. To access this feature, click on the Security tab in User Accounts dialog window.

3.11. 3D Volume Visualization improvements
Improvements have been made to Living Image 4.3 to support IVIS Spectrum CT instrument. These improvements are included as part of the optional 3D Multi-modality plug-in and enhance 3D DICOM data analysis. New features include:

- Volume Histogram magnification tool
- Volume processing
- Slice View
- 3D Opacity Slider
- 3D ROI Tool
- Hounsfield Unit calibration
- Pseudo X-Ray image (2D MIP projection image)
- Surface Topography from CT volume

3.12. Tech Notes
Living Image Technical Notes have been incorporated under the Help Menu to aid in obtaining help on the software features and related information. There are three types of tech notes:

- Tech Notes – Quick guides for tasks using the Living Image software tools
- Biology Tech Notes – Protocols and procedures related to animal subjects
- Concept Tech Notes – Background information on in vivo imaging topics

Alternatively, browse to the IVIS University download page for the most recent collection of technical notes.

3.13. Living Image User Guides
Separate user manuals for each model of IVIS imaging system are now provided with the software. From the Help menu, select the documentation for the particular model of IVIS you are using. Users with a single model of IVIS will no longer be overloaded with information on options that are not appropriate for the machine they have. For institutions with a variety of IVIS instruments, users can now select the appropriate documentation for the specific machine they are using to acquire or analyze data.

4. Improvements
The following table is a summary of the improvements.

<table>
<thead>
<tr>
<th>ID</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>LI-5237</td>
<td>Intermittent failures in polling the state of CT interlock</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>LI</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LI-5088</td>
<td>Software control of Oasis camera chiller</td>
</tr>
<tr>
<td>LI-5147</td>
<td>Improve data throughput for Spectrum and Spectrum CT, faster readout speed</td>
</tr>
<tr>
<td>LI-5095</td>
<td>Corrections for efficiency calculation to well plate calibration</td>
</tr>
<tr>
<td>LI-5049</td>
<td>Reduce memory usage of transmission image by rebinning and saving as float type</td>
</tr>
<tr>
<td>LI-5039</td>
<td>Well Plate Quant - enter volumes and concentrations instead of numbers in well plate table</td>
</tr>
<tr>
<td>LI-5074</td>
<td>Enable automatic co-registration for Quantum CT volume with surface from Spectrum CT volume</td>
</tr>
<tr>
<td>LI-5194</td>
<td>Remove CT Reference Images older than 30 days</td>
</tr>
<tr>
<td>LI-2508</td>
<td>Histogram of an ROI</td>
</tr>
<tr>
<td>LI-5200</td>
<td>Regulate stage temperature on Spectrum CT once instrument is powered on</td>
</tr>
<tr>
<td>LI-5144</td>
<td>Set different default color palettes for DLIT and FLIT for Longitudinal Study Window</td>
</tr>
<tr>
<td>LI-4152</td>
<td>Added X-ray resizing slider bar in the Corrections tool palette</td>
</tr>
<tr>
<td>LI-5062</td>
<td>Ability to save Batch sequence setup table to an .xsq file</td>
</tr>
<tr>
<td>LI-5021</td>
<td>Add Standard Deviation to 3D Volumetric Data measurement options</td>
</tr>
<tr>
<td>LI-2884</td>
<td>Change control panel color when system is busy (acquiring or initializing)</td>
</tr>
<tr>
<td>LI-4818</td>
<td>Ability to see saturation stamp in sequence view</td>
</tr>
<tr>
<td>LI-4816</td>
<td>Delete and move ROIs with keyboard</td>
</tr>
<tr>
<td>LI-4810</td>
<td>Longitudinal Study Window - control over scale bar</td>
</tr>
<tr>
<td>LI-4809</td>
<td>Longitudinal Study Window - ability to display results in calibrated units</td>
</tr>
<tr>
<td>LI-5030</td>
<td>Time to exit Living Image on Spectrum CT reduced to 5 seconds</td>
</tr>
<tr>
<td>LI-4492</td>
<td>Optical lens distortion correction, which was introduced in LI 4.3 for the Spectrum CT, is now available for all IVIS Spectrum systems</td>
</tr>
</tbody>
</table>

4. Known Issues in Living Image 4.3.1

4.1 LI-5089 High Resolution CT scan can sometimes cause application crash or computer freeze

In the case of a Living Image crash or computer freeze at the time of high resolution CT scan, please restart the computer. If the problem persists, power cycle the IVIS Spectrum CT.

4.2 LI-5206 Tool Palette disappears or 3D tools missing in the tool palette

This issue may affect Spectrum CT acquisition computer after acquisition of a 3D sequence. If the tool palette is missing or loads an incomplete set of tools, please save, close and reload the dataset. Docking the tool palette will alleviate these symptoms. To dock the tool palette in the main window, drag the palette to the right of left size of the window and release, or choose the default option in User Preferences dialog.

4.3 LI-4963 UAC communication error on Spectrum CT
This issue may affect the Spectrum CT acquisition computer and if encountered, exit the Living Image application and restart the acquisition computer. If the problem persists, power cycle the IVIS Spectrum CT.

4.4 LI-5250 3D Multi-Modality Module (3DMM) has a known issue with ATI Radeon graphic driver on Mac OS X 10.5
This issue is specific to Mac systems installed with ATI Radeon graphic card and Mac OS X 10.5 which results in undesired data visualization output with the 3D Multi-Modality Module. Upgrading the system to Mac OS X 10.6 or 10.7 is recommended to resolve this 3DMM incompatibility issue on Mac OS X 10.5.

4.5 3D settings on computers with dual graphic cards
If your computer (mostly laptops) is equipped with dual graphic cards, please follow the next figure to default the high-performance graphics card for the Living Image software. Otherwise, Living Image 3D viewer, especially with the 3D Multi-Modality tool, may not function correctly when running on low-end integrated graphics hardware. Here is an example of a laptop with both Intel integrated graphics and NVIDIA graphics. Open the NVIDIA control panel and manage 3D settings. In the program settings, add Living Image executable (livingimage.exe) to customize and then select the preferred graphics processor to “High-performance NVIDIA” processor.
5. System Requirements

PC:
Windows 7 32-bit / Windows XP * 32-bit
  2GHz Core 2 Duo or higher processor recommended
  4GB RAM

* Please note that future revisions of Living Image will discontinue support of Windows XP on the analysis module

Windows 7 64-bit
  2GHz Quad Core (i5, i7) processors
  8GB RAM recommended for IVIS Spectrum CT data analysis

Mac:
Mac OS X 10.5 to 10.7 *
  2GHz Core 2 Duo or higher processor recommended
  4GB RAM or higher recommended for IVIS Spectrum CT data analysis

* for analysis module only; for 3D Multi-Modality support Mac OS X 10.6 to 10.7 is required on Mac computers equipped with ATI Radeon video card.

Note: Mac PowerPC platform is not supported in Living Image 4.3.1.
Please note that future revisions of Living Image will discontinue support of Mac OS X 10.5

6. Video Card Requirements
3D Multi-Modality tools require that the graphics processing unit (GPU) meet the minimum specifications shown below. If the appropriate license is not installed or the GPU does not meet these specifications, the 3D Multi-Modality tools will not appear in the tool palette.

Minimum GPU specifications

<table>
<thead>
<tr>
<th>Specification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>OpenGL Version Requirement*</td>
<td>OpenGL 2.0 and above</td>
</tr>
<tr>
<td>OpenGL Extension Requirement*</td>
<td>GL-EXT-Texture3D</td>
</tr>
<tr>
<td>Graphics Card Memory:</td>
<td>Recommended: 1GB (Dedicated)</td>
</tr>
</tbody>
</table>
# Consumer Graphics Cards (Desktop/Mobile, Windows/Mac)

**Supported:**
- NVIDIA® GeForce® 8 Series and above (8, 9, 100, 200, 300 and 400 series)
- ATI Radeon™ HD 4000 Series and above (4000 and 5000 series)

**Recommended:**
- Desktop - NVIDIA GeForce GT 240 and above
- Mobile - NVIDIA GeForce GT 230M and above

# Workstation Graphics Cards (Desktop/Mobile, Windows/Mac)

**Supported:**
- NVIDIA® Quadro® NVS Series and Above (NVS & FX series)
- ATI FireGL™ V5600 and Above (FireGL, FirePro & CrossFire series)

**Recommended:**
- Desktop - Quadro FX 1800 and above
- Mobile - Quadro FX 880M and above

*If these specifications are not met, the 3D Multi-Modality tools will not appear in the tool palette.*