



DLIT and FLIT Reconstruction of Sources

Diffuse Luminescence Imaging Tomography (DLIT) is a technique that analyzes images of the surface light emission from a living subject to generate a three-dimensional (3D) reconstruction of luminescent light source distribution inside the subject.

To reconstruct luminescent sources, the Living Image® software requires a photograph, a structured light or CT image, and luminescent images obtained at two or more wavelength filters spanning the reporter molecule emission spectrum (for example, firefly luciferase 560-660 nm).

Fluorescence Imaging Tomography (FLIT) analyzes images of surface light emission to generate a 3D reconstruction of fluorescent light source distribution inside the subject. To reconstruct fluorescent sources, the software requires a structured light or CT image and fluorescent images obtained using the same excitation and emission filters at different transillumination source positions on the IVIS Spectrum or IVIS Spectrum CT.

To localize and quantify the light sources in a subject, the software:

- Reconstructs the subject surface topography (*surface*) from structured light images. The surface is defined by a set of connected polygons or surface elements.
- Maps the surface radiance (photons/s/cm²/steradian) to the photon density (photons/mm³) just beneath the surface of each element of the surface. For NTF Efficiency data from normalized transmission fluorescence data, the NTF Efficiency 2D data is mapped to the 3D surface.
- Divides the interior of the subject into a solid surface of volume elements or *voxels*. Each voxel is considered to contain a point light source at its center that contributes to the photon density at each surface element.
- Defines equations that relate the source strength of each voxel to the measured data (photon density or NTF Efficiency) at each surface element.
- Determines the optimum approximate solution to the system of linear equations to reconstruct the source strength in each voxel.

Determining Surface Topography

The software determines the surface topography from a structured light image. Parallel laser lines are projected onto the subject to produce a structured light image (Figure 1).

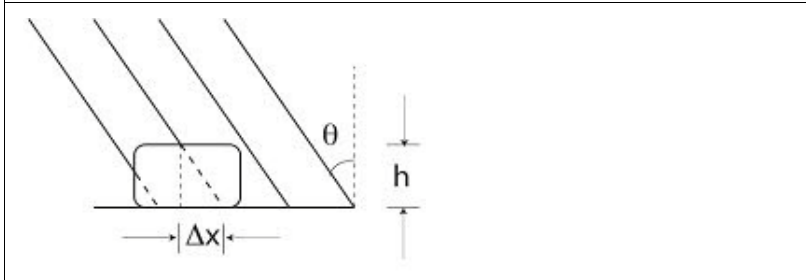


NOTE: If the Structure option is chosen in the control panel, a structured light image is automatically acquired.

The surface topography of the subject is determined by analyzing the displacement (Δx) or bending of the laser lines as they pass over the subject. The displacement is defined as the difference between where the line should fall on the stage in the absence of the subject and where it appears in the image due to occlusion by the subject.

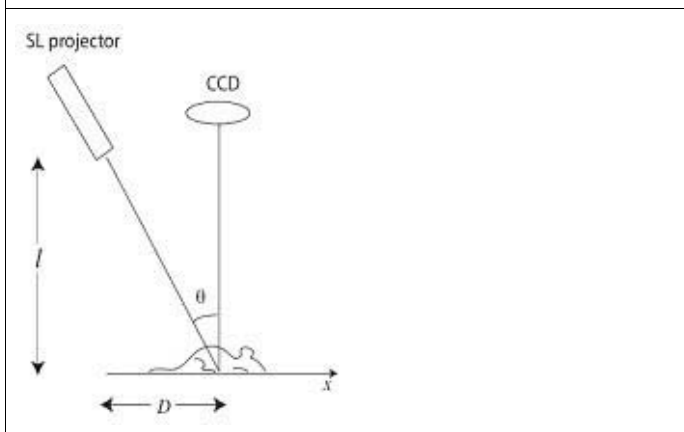
Figure 1 Parallel laser lines projected onto a subject.

Given knowledge of the angle θ , the height of the subject (h) can be determined by analyzing the displacement, Δx , of the laser lines as they pass over the object.



The parallel lines are projected onto the surface of the subject at an angle (θ). The angle is known by instrument calibrations of the distance between the structured light projector and the optical axis (D) and the distance between the stage and the structured light projector (l) (Figure 2).

Figure 2 Structured light projector and subject



D and l form two perpendicular sides of a triangle giving:

$$\tan \theta = D/l$$

Together Δx and h comprise a smaller version of this triangle. The height (h) can be determined from:

$$h = \Delta x / \tan \theta$$

by measuring the displacement Δx .

The software utilizes fast numerical methods to rapidly evaluate Δx over the entire image to determine the surface topography. The surface topography determination is limited to the topside of the object facing the lens.

Converting Light Emission to a Photon Density Map

The input data to the FLIT algorithm for 3D reconstruction of fluorescent light sources includes:

- A surface that defines the surface of the subject.
- A sequence of images acquired at different transillumination source positions using the same excitation and emission filter at each position. Use the Imaging Wizard to acquire the images.

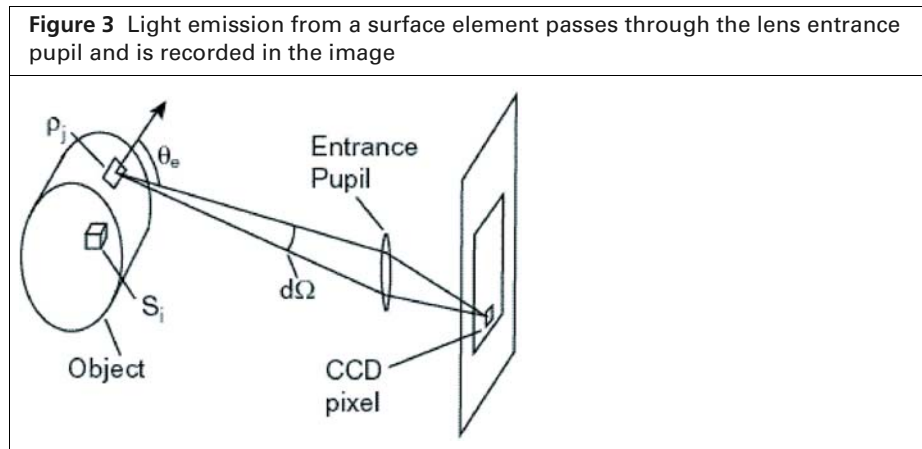
The input data to the DLIT algorithm for a 3D reconstruction of luminescent light sources includes:

- A surface that defines the surface of the subject.
- A sequence of two or more images of the light emission from the surface of the subject acquired at different filter bandwidths (Table 1). Use the Imaging Wizard to acquire the images.

Table 1 IVIS® System filters for luminescence and fluorescence tomography

IVIS Imaging System	Filters	Bandwidth
Spectrum CT	10 excitation filters, 415-760 nm	30 nm
	18 emission filters, 490-850 nm	20 nm
Spectrum	10 excitation filters, 415-760 nm	30 nm
	18 emission filters, 490-850 nm	20 nm
200 Series	6 emission filters, 550-670 nm	20 nm

The IVIS® Spectrum CT, IVIS® Spectrum, and IVIS® Imaging System 200 Series are absolutely calibrated so that the electron counts on each CCD pixel can be mapped back to the surface of the object to produce an absolute value of the surface radiance (photon/s/cm²/steradian) from each imaged surface element (Figure 3).



The imaging system collects the light emitted from the surface element at an angle (θ_e) (measured with respect to the normal to the surface element) into the solid angle $d\Omega$ subtended by the entrance pupil. The value of the surface radiance $L(\theta_e)$ is directly related to the photon density ρ (photons/mm³) just inside the surface of the element. FLIT analysis uses NTF Efficiency data and takes into account the photon density of both the fluorescent image and transmission image.

Defining the Linear Relationship Between a Source and Photon Density or NTF Efficiency

The software divides the interior of the subject into a solid mesh of volume elements (*voxels*). Each voxel is considered to contain a point light source at its center. The index i enumerates the set of voxels. S_i is the value of the strength of the point source inside the i^{th} voxel. The solid mesh defines a collection of point sources that approximate the actual source distribution. The accuracy of the approximation is improved by increasing the density of the solid mesh.

The reconstruction method is based on the principle that there is an approximately linear relationship between the source strength in each voxel (S_i) and the photon density or NTF Efficiency (ρ_j) at each surface element described by a Green's function G_{ij} . The photon density at the j^{th} surface element is the sum of the contributions from all the voxels:

$$\rho_j \cong \sum G_{ij} S_i \quad (1)$$

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The Green's function contains information about the transport of photons through the tissue and the effects of the tissue-air boundary. By using a planar boundary approximation, the Green's function can be calculated analytically as a solution to the diffusion equation. Having an analytic expression for G allows Equation 1 to be computed very rapidly.

Determining the Best Approximate Solution to the Linear System

Once the Green's functions, G_{ij} , are known, the goal is to solve Equation 1 for the source strength S_i in each voxel. The DLIT and FLIT algorithms attempt to minimize χ^2 (Equation 2) while requiring that the source strength in each voxel is positive (Equation 3).

$$\chi^2 = \sum_j \frac{1}{\sigma_j^2} \left[\rho_j - \sum_i G_{ij} S_i \right]^2 \quad (2)$$

$$S_i \geq 0 \quad (3)$$

A Non-Negative Least Squares algorithm is used to find the approximate solution which minimizes χ^2 . In order to reduce the number of variables in the problem, the code only uses surface elements with signal above a certain threshold and only keeps the voxels that contribute significantly to these surface elements.

Source & Tissue Properties

DLIT analysis of spectrally filtered images requires knowledge of the spectral dependence of luminescent light emission. Table 2 shows the factory set source spectra provided by the software.



NOTE: The source spectra is not an input to the 3D reconstruction of fluorescent sources.

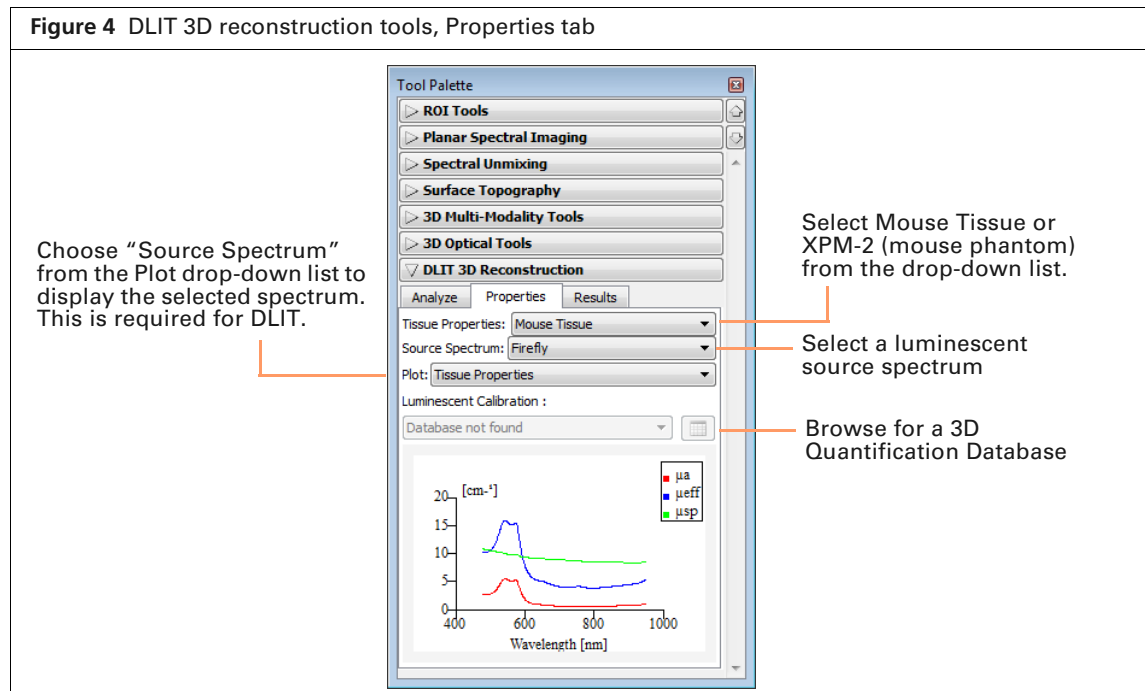


Table 2 Source spectra

Source Spectrum	Description
Bacteria	Bacterial luciferase
CB Green	Click beetle green luciferase
CB Red	Click beetle red luciferase
Firefly	Firefly luciferase
hRenilla	Sea pansy (<i>Renilla reniformis</i>) luciferase
Tritium Bead 5	Phosphor-coated glass bead containing tritium gas. Spectrum for bead #5.
XPM-2-LED	LED in the XPM-2 mouse phantom.



NOTE: The firefly luciferase spectrum is dependent on temperature and pH. The data provided are valid only for measurements performed at 37 °C and at pH 7.0 to pH 7.5. Selecting other temperature and pH conditions for a specific experiment requires the use of the associated spectral curve for the spectral analysis. For more information about pH and temperature dependence of the luciferase spectrum, please contact Caliper technical support.

You can view tissue optical property values (μ_{eff} , μ'_s , μ_a) in the Tissue Properties drop-down list. The tissue properties are plotted as a function of wavelength. Select the optical property descriptor most representative of the imaged subject. “Mouse Tissue” is a good choice for general reconstructions *in vivo*.



NOTE: Default tissue optical properties and source spectra are specified in the Preferences box. For more details, see the Preferences appendix in the *Living Image Software User's Manual*.