MOLECULAR IMAGING SYSTEM UPGRADE

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ABSTRACT

This project is based upon the integration of a molecular imaging system and an illumination system used for high resolution microscopes. The effort was based upon the modification of an existing system, which is currently capable of 2-D soft tissue fluorescence imaging. The modification includes additional optics as well as control and signal processing software. The illumination system implements modulation of the light source and provides a framework for capturing depth resolved images. The integrated systems comprise a working prototype capable of providing low-cost, non-invasive, functional imaging in three dimensions.

NOMENCLATURE

Scheimpflug principle: This principle states that if the image plane, the lens plane, and the object plane intersect at a point, the image will be in focus at the image plane

Spatial frequency: number of grid lines per unit length

FFT: Fast Fourier Transform

MATLAB: MathWorks software package used for image processing

CCD: Charge coupled device

Depth of modulation: Amplitude of modulated waveform

INTRODUCTION

This project is based upon a pending patent application which describes using projected grid lines for depth resolved fluorescence imaging. Such a system has applications for low cost, non invasive medical imaging.

The objective of this project is to modify an existing imaging system currently available from Carestream Health Inc. to enable the imaging capabilities described in the patent through the use of Qioptiq Linos’s Optigrid Illumination system. Figure 1 shows a CAD model of the envisioned system.

![Figure 1. CAD Model of Conceptual Design](image)

Deliverables of the project include an experimental prototype based on mechanical modifications to the existing platform, establishment of a calibration procedure for the Optigrid, and investigation into optimal test conditions for three dimensional imaging.
The prototype includes degrees of flexibility to allow for experimentation of the system by Carestream Health for commercial use.

**PROCESS AND METHODOLOGY**

The existing system images its target using reflected light introduced from below. The light reflects off the target and off a mirror into a CCD camera. The modification of the system involved the insertion of the Optigrid and a lens into the path between the light source and the imaging surface. A schematic diagram of the modified system can be seen in Figure 2.

The primary challenge of the design was the projection of the Optigrid onto the imaging surface at an angle. The current intended use of the Optigrid in microscopes uses perpendicular illumination with no magnification. The spatial frequency of the grid used in this system is 30 line pairs/mm. Typically, the Optigrid uses smaller distances between the grid and the target than the proposed system does.

Perpendicular projection was not practical in terms of cost and outside the premise of the customer specifications. Other challenges in the design included minimizing stray light and developing calibration routines to produce reproducible results from the piezoelectric motor control of the Optigrid.

![Figure 2. Schematic representation of proposed prototype. The lens and the Optigrid were the primary additions to the existing platform](image)

The theory behind the Optigrid is also known as structured illumination. Structured illumination consists of modulating a light source by projecting it through a series of parallel lines onto an object. The lines are then shifted by one third of their period (120 degrees), and images are acquired at each shift. The three images are combined mathematically using Equation 1, and the resulting image is free of lines and out of focus information.

\[
I = \sqrt{(I_2 - I_1)^2 + (I_3 - I_2)^2 + (I_1 - I_3)^2}
\]

In the proposed prototype, the Scheimpflug principle is utilized to ensure focus of the projected grid. In order to project the Optigrid in focus at different magnifications, the prototype will be required to satisfy this principle utilizing multiple angles.

Satisfying the conditions defined by this rule provided the basis for the mechanical design of the prototype. To implement the Scheimpflug principle with significant degrees of flexibility, the following approach was taken. An optical rail with a linear stage micrometer for both the lens fixture and the Optigrid fixture was incorporated into the structural design of the existing system. Rotary stage micrometers were also installed for both the Optigrid and lens fixtures to allow for more flexibility in the design. Figure 3 illustrates the degrees of motion specified for the prototype design. This approach allowed the design to overcome angle and distance restrictions that were inherent in the existing imaging system.

![Figure 3. Degrees of motion for required flexibility](image)

Performance of the system was characterized through calibration experimentation. The system requires precise phase shifting of the projected grid. During modification of the housing and insertion of the optical rail fixturing, experiments were conducted to prove conceptually that accurate and repeatable calibration of the projected Optigrid could be achieved.

To evaluate calibration, several processing steps were taken based on spatial frequency analysis of captured images of the grid. Single line profile and average six line profile spectrums were computed using FFT techniques and were used to evaluate phase movements. Through experimentation, a thorough calibration procedure was developed that identifies and corrects for inconsistencies between uses of the system.

Depth discrimination proof of concept was done using a phantom block. The block consisted of 10 sheets of polycarbonate bolted together, each of which was 2 mm thick. Fluorescent sheets (Lime Green, #96) were inserted at depths of 2 mm, 10 mm and 18 mm. Figure 4 shows a diagram of the phantom block.

![Figure 4. Diagram of the phantom block](image)

The depth of modulation of the projected spatial frequency decreases as you move away from the source and into the target. Depth of modulation calculations on phase shifted images in addition to comparing images combined using Equation 1 at different spatial frequencies were used to show the
dependence of depth of modulation on depth into the target.

Figure 4. Phantom block used for depth discrimination proof of concept

RESULTS AND DISCUSSION

The physical implementation of the designed system can be seen in Figure 5. Standard optical fixturing was used where feasible. Integration of the optical rail into the existing system required custom mounting fixtures that allowed for angle adjustability of the rail.

Figure 5. Physical implementation of degrees of motion

The system was characterized at a projection angle of 30 degrees using a Computar C mount lens. The zoom of the imaging system was set to 40 pixels per mm. The range of the system was characterized in terms of spatial frequency, and it was determined that spatial frequencies ranging from 0.8 line pairs/mm to 1.4 line pairs/mm can be achieved with the above mentioned lens. A blue excitation filter and a green emission filter were used during phantom block captures in order to promote fluorescence in the Lime Green sheets.

In order to achieve precise 120 degree phase shifts, a system specific calibration routine for the Optigrid was developed. Phase shifting is achieved in the Optigrid with a piezoelectric motor control. Several modifications from the typical calibration routine had to be made because the grid was being used outside of its typical application in a microscope. For example, projecting the grid at an angle causes the lines to be distorted and not uniform in a captured image. This distortion required that the images be cropped and rotated before being processed.

Images of a non-glossy white target were captured during the calibration process. Images were captured over the range of voltages (30-100 V) supported by the Optigrid motor control. A line profile FFT was calculated along the center of image. The location of the spatial frequency of the grid was detected in the FFT, and the phase was calculated using Equation 2. A visual representation of the output of calibration can be seen in Figure 5, a phase versus grid voltage plot. Precise 120 phase shifted voltages were selected using this graph and used during image capture.

$$\text{Phase} = \tan^{-1}\left(\frac{\text{Im}}{\text{Re}}\right)$$

(2)

Example of calibration images combined using Equation 1 can be seen in Figure 7. Figure 7(a) shows a combination of images which are not precisely phase shifted. Note the distinct presence of grid lines. Figure 7(b) shows images that are more closely separated by 120 degrees. The presence of lines is greatly reduced.

Figure 6. Phase vs. Grid Voltage graph. Output of calibration of the Optigrid

Figures 6 and 7 also show the limitations of our system. Precision calibration of the Optigrid is difficult to achieve in the proposed prototype due to the lack of automation between the motor control software and the image capture software. This is seen in Figure 5, 15 image captures were taken at 30 V, and
the phases were only consistent within 10 degrees. This is most likely the cause of the remaining lines in Figure 7(b). Adding automation to the system would increase repeatability of timing and accuracy in calibration.

For proof of concept of depth discrimination, phase shifted images were captured of the phantom block at different spatial frequencies. Two experiments were run to illustrate the system's capability to acquire depth dependent results.

Figure 7. (a) Combined image using incorrectly shifted Optigrid (b) Combined image using correctly shifted Optigrid

An image of the reflectance was calculated using Equation 1 for each spatial frequency image set. As described by Tromberg et al in “Method and Apparatus for High Resolution Spatially Modulated Fluorescence Imaging and Tomography,” reflectance is attenuated more strongly at higher spatial frequencies [1]. Figure 8 shows both the raw reflectance and combined reflectance image for the two extreme spatial frequencies supported by the system. At the higher spatial frequency, the deepest sheet in the block is less visible than at the lower frequency capture.

By assessing the depth of modulation at each pixel of the image, and monitoring its value at each phase shift, information about the depth of the pixel can be assessed. The magnitude of the depth of modulation over those three phases will model that of a sine curve. (see Figure 9b and 10b). The greater the depth of the pixel, the lower the change in amplitude over the three phases will be. Figures 9a and 10a show line profiles of intensity for two different depths in the phantom block. The deeper fluorescent sheet shows less variation across the line profile compared to the more superficial sheet.

Figure 8. Top row: raw reflectance images of phantom blocks at two spatial frequencies. Bottom row: combined reflectance images. Note that at the higher spatial frequency, the deepest fluorescent sheet (left) is less visible

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Figure 9. (a) Line profile of reflectance for superficial fluorescent sheet (b) Sine wave fit for single pixel depth of modulation vs. phase

Fitting a sine wave to the data is often difficult and not a complete representation of the data. An alternative method was used to measure variation in depth of modulation. The mean squared error was calculated with respect to the mean of the three phase shifted points. The equation for mean squared error can be seen in Equation 3, where $m(I,j)$ is the mean of $I_1, I_2$ and $I_3$ at the location $(i,j)$.

\[ MSE(i,j) = (I_1(i,j) - m(i,j))^2 + (I_2(i,j) - m(i,j))^2 + (I_3(i,j) - m(i,j))^2 \] (3)
CONCLUSIONS AND RECOMMENDATIONS:

This project resulted in a successful modification to the existing imaging platform to enable depth resolved fluorescence imaging. The system is capable of in focus projection of the Optigrid at different spatial frequencies, and supports the use of different lenses. A calibration process has been developed for the system to achieve consistent phase shifts of the Optigrid. Post processing has extracted depth information using depth of modulation analysis using a phantom block with fluorescent sheets at different depths.

Future work for the project includes evaluation of the system using turbid media, as well the development of algorithms to extract optical properties (absorption and scattering characteristics) from target specimens.

Additional recommendations for the prototype include further system characterization. The rail angle adjustment was not tested in the experiments. The steepest angle supported by the system was used for all of the results presented. This helped reduce distortion associated with projection angle.

There is a trade off between maximum magnification and maximum angle. As the magnification is increased, the projection angle with respect to the platen is decreased. For the presented prototype, it was deemed more important to have a greater range of magnification. For future testing, it may be beneficial to investigate using a shorter rail with a smaller magnification range.

There are two areas of the presented system whose performance would be greatly enhanced by automation: Optigrid Calibration and Optigrid Warm-Up. Using the current methodology, both processes are very time consuming and prone to error. Establishing communication between the motor control and image capture software would be the first step in achieving integration, and would help automate the above mentioned processes.

REFERENCES:
[1] "Method and apparatus for high resolution spatially modulated fluorescence imaging and tomography."

ACKNOWLEDGMENTS

Special thanks to Dr. Gilbert Feke of Carestream Health, Inc. and Linda Antos of Qioptiq Linos Imaging Solutions for initiating and supporting the project. Thanks to CEIS (Project 33505) and NYSTAR for project support. Thanks to Dr. Maria Helguera and Dr. Daniel Phillips for guiding the project to success. Additional thanks to Greg Schenker, Joe Mulley, Robert Macintyre, Dr. Robert Dolittle, Heather Drake, and everyone else who shared ideas and posed the questions that led the project to completion.