Performance Analysis of Multi-Wavelength Transmission Scanner for Polarized NIR Light

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Abstract. A system for small-object imaging, comprising a multiple-wavelength scanner for Near Infra-Red (NIR) light is under development in the Laboratory of Radiopharmaceuticals and Molecular Imaging (LRMI) at the National Laboratories of Legnaro, INFN, Italy. The System performs scanning of biological objects using NIR light in the interval of 900nm – 1700nm. The scanned region is a rectangular with dimensions of 50mm x 80mm and is performed by consecutive positioning of InGaAs linear image sensor sliding close to the scanned object. The scanning is carried out in two different modes. The first mode is performed in transmitted linearly polarized NIR light using a set of five light emitting diodes with fixed wavelengths. The process of scanning is realized by a consecutive positioning of the NIR sensor and signal acquisition at the corresponding position. In the second scanning mode the fluorescence emission of nanoparticles such as single-walled carbon nanotubes (SWCNTs), administered in the imaged object, is excited by NIR lasers with different wavelengths. Spatial resolution of the system for transmitted linearly polarized NIR at five fixed wavelengths has been determined. Polarimetric measurements of some optically active sugars such as fructose and lactose were conducted at some fixed wavelengths in the range of 900 -1200nm. The system sensitivity with respect to the concentrations of these agents has been estimated.

1. Introduction
Near infra-red (NIR) imaging is a noninvasive method which recently gains popularity in biology and medicine because of its very useful properties. The wavelength region of NIR is under development in the Laboratory of Radiopharmaceuticals and Molecular Imaging (LRMI) at the National Laboratories of Legnaro, INFN, Italy. The System performs scanning of biological objects using NIR light in the interval of 900nm – 1700nm. The scanned region is a rectangular with dimensions of 50mm x 80mm and is performed by consecutive positioning of InGaAs linear image sensor sliding close to the scanned object. The scanning is carried out in two different modes. The first mode is performed in transmitted linearly polarized NIR light using a set of five light emitting diodes with fixed wavelengths. The process of scanning is realized by a consecutive positioning of the NIR sensor and signal acquisition at the corresponding position. In the second scanning mode the fluorescence emission of nanoparticles such as single-walled carbon nanotubes (SWCNTs), administered in the imaged object, is excited by NIR lasers with different wavelengths. Spatial resolution of the system for transmitted linearly polarized NIR at five fixed wavelengths has been determined. Polarimetric measurements of some optically active sugars such as fructose and lactose were conducted at some fixed wavelengths in the range of 900 -1200nm. The system sensitivity with respect to the concentrations of these agents has been estimated.
A number of techniques such as NIR bio-fluorescence imaging [3, 4], NIR tissue polarimetry analysis [5, 6], NIR transmission analysis [7, 8], etc., enabling \textit{in vivo} imaging of physiological, metabolic, and molecular function, have emerged exploiting the advantages of NIR. The development of these techniques is stimulated by the number of recently made available targeted biological agents for diagnosis and basic medical research, enabling \textit{in vivo} analyses and imaging in NIR. The specific physical properties of the new agents allow imaging by absorption, by scattering, and by excitation (such as fluorescence). On the other hand the search for better imaging of physiological, metabolic, and molecular function has lead to new imaging techniques such as multimodal imaging which exploit combinations of different physical properties of the imaged objects and requires nanoparticles or supramolecular assemblies of quantum dots, obeying advantages in faster circulation time and brighter images with respect to single molecule imaging agents [9].

A system for small-object imaging, based on a multiple-wavelength scanner for Near Infra-Red (NIR) light is under development in the Laboratory of Radiopharmaceuticals and Molecular Imaging (LRMI) at the National Laboratories of Legnaro, INFN, Italy. The System performs object scanning in the range of 900nm – 1700nm and is designed to operate in two different modes. In the first mode the scanning is performed in transmitted linearly polarized NIR light, traversed through the object. In the second scanning mode the fluorescence emission of nanoparticles administered in the imaged object, is excited using lasers with different wavelengths.

In this article we present the experiments conducted to determine some of the optical characteristics of the system such as the spatial resolution of the system for linearly polarized transmitted NIR light as a function of the distance to the scanned object, as well as polarimetric measurements, conducted on some optically active sugars such as fructose and lactose at wavelength range 900nm - 1200nm.

2. Experimental setup

A block-diagram of the scanner for small-animal NIR imaging is shown in figure 1. The scanner for NIR is realized on the basis of 256 pixels InGaAs array sensor Hamamatsu G9203-256D, positioned in a socket of a NIR detector board, which is fixed inside the scanner carcass. There is a compartment with NIR light emitting diodes (LED) attached to a support on the scanner carcass (figure 2). A system of NIR lasers (Laser1 and Laser 2) for imaging of reflected NIR light and the excited fluorescence is also fixed to the system. The scanned object is placed in a platform with a thin CaF$_2$ window and the scanning is performed while the platform with the object is moving in X and Y directions of a plane.

![Figure 1. A block-diagram of the scanner for small-animal NIR imaging](image-url)
The NIR LED compartment contains a set of five high-power diodes, emitting NIR light with different wavelengths. A linear polarizer $P_1$ is situated below the diodes in the NIR LED compartment. A second linear polarizer $P_2$, situated in a ring rotated by stepping motors, is placed in front of the NIR sensor and below the CaF$_2$ window.

The scanned region has a rectangular shape with dimensions of 50mm x 80mm. The scanning in both directions can be performed in steps as small as $10^{-3}$mm. At each step the NIR light, transmitted through the object, is collected separately for each of the five wavelengths by switching consecutively the NIR light-emitting diodes and the acquisition. The scanning procedure thus produces five NIR images of transmitted light. The smallest rotation step of the analyzer $P_2$ is $1^\circ$. The stepping motors guidance, the NIR diodes emission as well as the image acquisitions are controlled by a PC-based multifunction intelligent board National Instruments FPGA 7831R. A graphical user interface based on the developing environment LabView 8.5 was created to control the scanner functions as well as to visualize and process the acquired images.

3. **Spatial resolution measurements**

The spatial resolution of the scanner as well as the spatial resolution of the scanner, optically coupled with a fibre optical plate (FOP) model FOP(D35T20.5), have been determined along two perpendicular directions using USAF 1951 resolution standard, placed at different distances from the sensor and from the sensor with the FOP. For convenience the scan direction perpendicular to the sensor array was assigned X and the direction parallel to the sensor array was assigned Y. The spatial resolution in X and Y was measured for different distances to the scanned object for each of five NIR wavelengths: 940nm; 1070nm; 1200nm; 1300nm and 1550nm. Overall value of the spatial resolution was calculated averaging the spatial resolutions at all five wavelengths at each sensor-to-object distance [10]. The overall spatial resolution dependence of the system along X direction is shown in figure 3 (sensor without FOP). The corresponding overall spatial resolution dependence of the system along direction Y is shown in figure 4 (sensor without FOP). The corresponding overall spatial resolutions (assigned as sensor with FOP) along X and Y directions are shown in figure 3 and figure 4.

4. **Polarimetric measurements of fructose and lactose**

The rotation of the plane of polarization of linearly polarized NIR light passing through optically active solutions such as fructose and lactose in equilibrium with their chiral components was studied.
Besides the necessity to measure experimentally the values of the specific rotation of water solutions of these two compounds, our idea was to conduct system performance studies to assess the lowest detectable quantities of the system with respect to these sugars.

**Fructose** (C₆H₁₂O₆) is a monosaccharide which is an isomer of glucose but has a different structure. The crystalline fructose has cyclic six-membered structure known as D-fructopyranose. When dissolved in water, because of mutarotation, it reaches equilibrium for about 30 minutes at ambient temperature where the β-D-pyranose is the most abundant of the anomers (68 – 72%). The next most abundant anomer is β-D-fructofuranose (28 – 32%), and finally there are less abundant quantities of α-D-glucopyranose (about 4%) [11,12]. Water solution of fructose in equilibrium possess a specific levorotation $\alpha^\mathrm{D} = -93,78^\circ$.

**Lactose** (C₁₂H₂₂O₁₁) is a disaccharide, compounded by galactose and glucose. The water solution of lactose at ambient temperature causes degeneration of all crystalline modifications of the lactose to alpha- and beta- lactose [13]. Due to the process of mutarotation the anemic ratio evolves towards equilibrium. This process normally takes about 30-40 minutes. Both anomers are dextrorotatory and the water solution of lactose in equilibrium exhibits a specific dextrorotation $\alpha^\mathrm{D} = 55,48^\circ$ [13].

Measurements of the rotation of the plane of polarization in fructose and lactose were conducted using a cylindrical-shaped large-diameter quartz cuvette model 35I (Madatec Srl.), with diameter 50mm and 50mm path length (thickness of the analyzed layer). Solutions with different concentrations of fructose and lactose were prepared and scanned using linearly polarized NIR light in three wavelengths: 940nm; 1070nm and 1200nm. A diaphragm with a small opening (diameter of 6mm) was placed on the top of the cuvette to prevent reflections from its cylindrical walls. The analyzer $P_2$ was rotated from $-90^\circ$ to $+90^\circ$ with a step of $10^\circ$. Images at each analyzer’s angle were acquired and stored. NIR images of distilled water were acquired at the same analyzer angles for the same wavelengths in order to determine the zero position of the polarimeter. All images were saved as matrixes which elements were corresponding to the pixels of the image and with values corresponding to the intensity of the registered NIR light. The intensity of NIR light of a chosen region was calculated using averaging the value of the corresponding matrix elements.

Plots of the measured light intensity at all positions of the analyzer $P_2$ for each wavelength and for each sugar concentration were obtained. Following the idea that the observed NIR intensity would obey Malus’ law, a 4-parametric fit with a periodic function has been conducted:

$$I = A \cos^2 (B\varphi + C) + D,$$

(1)
where: I is the observed intensity; parameter A is a parameter, corresponding to the amplitude of the sine function; B corresponds to the observed frequency; C is the phase shift and D is a correction parameter for the vertical shift of the sine function (the dark current parameter). The angle of rotation of the polarized light for given concentration and wavelength is deduced from the comparison with the fitted data for distilled water at the same wavelength. The measured dependence of this angle on the concentration of fructose and lactose is shown in figure 5 and figure 6. The values of the calculated specific angle of rotation \([\alpha^f_L]_T\) are shown in Table 1.

![Figure 5](image-url) Rotation of angle of polarization for fructose as a function of the concentration

![Figure 6](image-url) Rotation of angle of polarization for lactose as a function of the concentration

![Figure 7](image-url) Measured specific rotation of fructose compared with the optical rotatory dispersion (ORD) curve

![Figure 8](image-url) Measured specific rotation of lactose compared with the optical rotatory dispersion curve
Exploiting Drude’s formula concerning the optical rotatory dispersion (ORD) [14], a two-parametric approximation has been applied to the obtained experimental data for fructose and lactose:

\[
\alpha_{\lambda}^{\alpha} = \frac{A}{\lambda^2 - B^2},
\]

where parameter A is the rotation constant and B is the dispersion constant. The resulted ORD curves and the experimental values of \( \alpha_{\lambda}^{\alpha} \) for fructose and lactose are shown in figure7 and figure 8.

Table 1. Measured specific angle of rotation for fructose and lactose

| Specific angle of rotation (deg.mL/dm.g) | 940 nm | 1070 nm | 1200 nm |
|----------------------------------------|--------|--------|--------|
| Fructose                               | -21 ± 2| -14 ± 1,3| -13 ± 1,3|
| Lactose                                | 16 ± 1,6| 14 ± 1,4| 13 ± 1,3|

5. Conclusion

The system possess a good spatial resolution for analyzing layers of tissues in transmitted NIR light as well as for imaging using bioluminescence of small biological objects. The differences in the spatial resolution of the scanner along two perpendicular directions of the scanned surface are consequence of the different pixel dimensions of Hamamatsu G9203-256D: the pixel size is 50µm x 500µm oriented with its longer size along the slab. The use of FOP slightly deteriorates the spatial resolution along both directions, resulting in a shift of about 50µm in both graphs.

The measured values of the specific rotation for fructose and lactose are in agreement with the optical rotatory dispersion curves passing through the initial values for the specific rotation at wavelength \( \lambda = 589 \text{nm} \). The system’s lowest measurable concentrations, estimated experimentally lowering the sugar concentrations, were 500mg/dL for fructose and about 1000mg/dL for lactose and glucose.

References

[1] Awathy R G, Yoshida Y, Maekawa T and Kumar D S 2010 Anal. Bioanal. Chem. 397(4) 1417
[2] Moor J Application of NIR imaging and multivariate data analysis for pharmaceutical products (Diploma thesis Univ. of Appl. Sci. München 2010)
[3] Sevick-Muraca E M, Houston J P and Gurfinkel M 2002 Curr Opin Chem Biol. 6(5) 642
[4] Frangioni J V 2003 Curr Opin Chem Biol. 7(5) 626
[5] Ghosh N and Vitkin I A 2011 Journal of Biomedical Optics 16(11) 110801
[6] Giakos G C, Valluru K, Adya V, Ambadipudi K, Pituri S, Bathini P, M Becker M, Farajipour P, Marotta S, Paxitizis J, Mandal B, Zervakis M and Livanos G 2009 Meas. Sci. Technol. 20 104003
[7] Zhang X, Bloch Sh, Akers W and Achilefu S 2012 Current Protocols in Cytometry Publ. Online
[8] Tao H, Yang K, Ma Zh, Wan J, Zhang Y, Kang Zh and Liu Zh 2012 Small Vol. 8 Issue 2 281
[9] Pansare V J, Hejazi S, Faenza W J and Prudhomme R K 2012 Chem. Mater. 24 (5) 812
[10] Uzunov N M, Bello M, Moschini G, Rossi P, Rosato A, Rondina M B, Montagner I M, Boldrin D and Muzzio P C 2010 Conference record 2010 NSS and MIC 3820
[11] Shallenberger R S 1978 Pure & Appl. Chem. Vol. 50 1409
[12] Domingues J R A Glucose isomerase action on acid whey lactose hydrolisate and other sugars (PhD Thesis University of Arizona 1984)
[13] Hargreaves J Characterization of lactose in the liquid and solid state usin nuclear magnetic reaction NMR and other methods (PhD Thesis Massey University 1995)
[14] Shechter E and Blout E R 1964 Proc Natl Acad Sci USA V 51(4) 695