INDOCYANINE GREEN (ICG) FLUORESCENCE QUANTIFICATION USING A NOVEL FLEXIBLE ENDOSCOPIC NEAR-INFRARED IMAGING DEVICE

Gareth Gallagher[1], Ra'ed Malallah[1, 2], Jonathan P Epperlein[3], Jeffrey Dalli[1], Niall Hardy[1], Alice Moynihan[1], Pol G Mac Aonghusa[3], Ronan Cahill[1].

[1] UCD Centre for Precision Surgery, University College Dublin, Ireland, [2] Physics Department, Faculty of Science, University of Basrah, Garmat Ali, Basrah, Iraq, [3] IBM Research Europe, Dublin, Ireland.

INTRODUCTION

Colonoscopy is regarded as the most effective diagnostic and preventative screening approach for colorectal cancer. Technological advances (e.g., computer aided detection), have helped to overcome endoscopist experience-related variations in adenoma detection rates, particularly for small and flat lesion morphologies. However, histopathological misdiagnosis of larger lesions can culminate in unfavorable surgical treatment strategies, exacerbating patient risk, costs and outcomes. Real-time colorectal polyp classification employing artificial intelligence (AI) and near-infrared imaging of the fluorescent agent Indocyanine Green (ICG) as a marker for perfusion quantification has demonstrated promise. Commercially available systems for fluorescence visualization do not possess the flexibility and anatomical reach capabilities of colonoscopy, while commercial flexible scopes do not yet provide imaging capabilities outside the visible spectrum, subsequently limiting the potential real-time diagnostic power of this classification technology. Here we present the development of a colonoscope compatible flexible imaging probe for NIR visualization, combined with a full field of view machine learning (ML) algorithm for fluorescence quantification and perfusion pattern cross correlation.

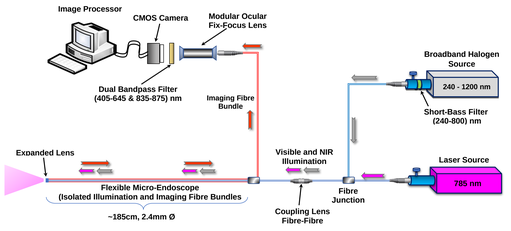
METHODOLOGY

The device, insertable via standard colonoscope working channels (2.4mm outer diameter) via the *mother-daughter* technique, incorporates two isolated sets of flexible imaging (10,000 pixel) and illumination fiber optic bundles, respectively, across its 1.85m length. The illumination excitation (785nm laser) and collection filters (405-645 & 835-875nm dual bandpass) are tailored specifically for ICG fluorescence imaging. A single CMOS (complementary metal–oxide–semiconductor) camera sensor coupled to the imaging fiber guide and filters was used to capture both the visible light reflectance and emitted NIR fluorescence producing a multispectral image. The optical performance (illumination power, angular field of view, resolution, sensitivity) of the imaging system was characterized.

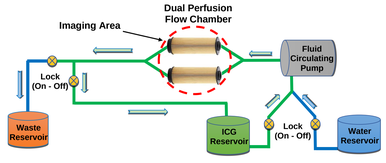
Device-captured images provide the input for the developed AI fluorescence quantification algorithm. The image signal was first primed by isolating the most fluorescent sensitive RGB channel and filtering out pixels not surpassing the intensity threshold, in turn isolating areas of the image within the perfusion pathway and making the algorithm more computationally efficient. Processing the images digitally allows us to collect the time-series of fluorescence in each pixel and apply algorithms to each time series separately, or all of them taken together. Specifically, we applied dimensionality reduction algorithms (principal component analysis – PCA) before clustering (k-means) the reduced time series to identify patterns of similar fluorescence profiles. Subsequent normalized cross correlation (NCC) was used to highlight the quantitative differences between clusters and provide boundary margination.

To test the imaging systems ability to quantify dynamic fluorescence over time, a repeatable ex-vitro fluid flow circuit model was developed with the aim of simulating tissue perfusion characteristics. A 4.6μM (0.25mg/kg) concentration of ICG in 4% bovine serum albumin saline buffer solution was infused through the dual perfusion flow chambers, with the perfusion pattern varying in each 3D printed flow chamber as a result of the reduced inlet and exhaust orifice diameter. The NIR imaging device captured the ICG fluorescence as the dye passed into and subsequently, washed out of the flow chambers. The varying flow rates are intended to mimicking the variation in ICG intensity profiles resulting from the differing vascular architecture of the underlying tissue (healthy, benign, malignant), serving as a marker for classification.

NIR Imaging Device Schematic

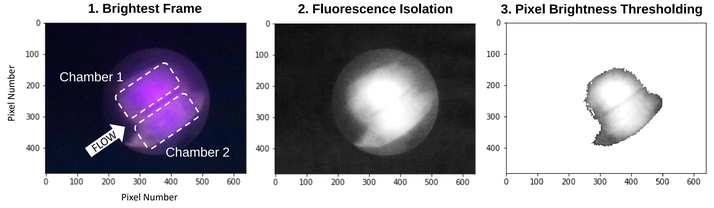


Experimental Ex-Vitro Perfusion Model

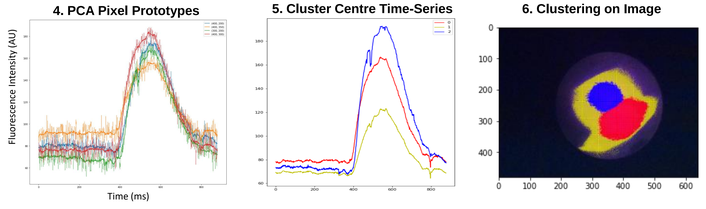


AI Driven Algorithm for ICG Fluorescence Quantification Workflow

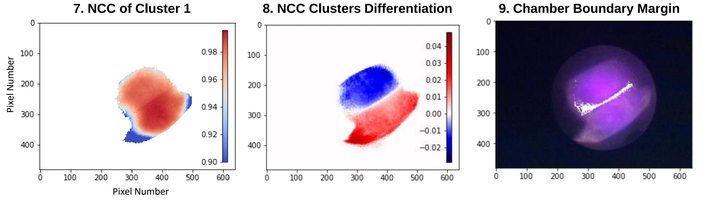
1) Image pre-processing with highest pixel intensity identification.
2) RGB channel breakdown, Fluorescence isolation and grayscale conversion.
3) Computational reduction via pixel intensity thresholding



4) Principal component analysis (PCA) implementation for time-series dimensionality reduction.
5) K-mean clustering of reduced dimensional vector to reveal similar time-series.
6) Visual representation of clustering on flow chamber image, chamber-representative cluster selection is based on accumulated fluorescence (area under curve).

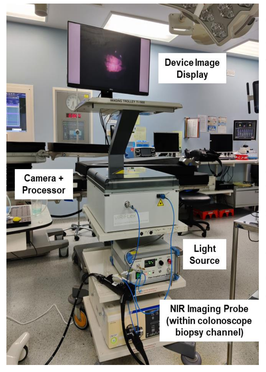


7) Normalized cross correlation (NCC) of pixel time-series with cluster 1. Red indicates high correlation (NCC ≈ 1)
8) NCC cluster differentiation reveals perfusion flow chambers (NCC1-NCC2 > 0, red; NCC1-NCC2 < 0, blue)
9) Visual representation of chamber margin (white) on image (NCC1-NCC2 ≈ 0)



RESULTS

Flexible NIR Imaging System



The developed flexible NIR imaging system was built and is illustrated to the left. The system produced a maximum 52mW excitation illumination at a distance 10mm from the distal probe tip. This constitutes a 12.84dB attenuation across the probe length. The illumination spotlight angular field of view (AFOV) was determined to be 75°, while the imaging AFOV was 120°, thus ICG excitation cannot span the full image field of view. The minimum observable object was measured, resulting in a resolution of 154μm at a 3mm probe distance. The systems sensitivity to ICG fluorescence with varied distance revealed an inverse power law relationship, with a signal to noise ratio of approx. 10 at 50mm from target to probe tip.

The developed AI driven perfusion quantification algorithm was qualitatively successful in its aim of flow chamber identification and margination. Pixel intensity thresholding masked 261120 of 307200 pixels, significantly reducing the computational load, while retaining full flow chamber pixel coverage. PCA dimensionality reduction reduced pixel time-series dimensionality 87-fold, while retaining a 0.935 variance. Although NCC cluster differentiation, effectively displays each flow chamber, NCC for each cluster shows high correlation (NCC ≈ 1) against all pixels in the perfusion image area, indicating minor quantitative variations in perfusion across each chamber, highlighting the power of the quantitative approach and algorithms capability of detecting subtle differences in the perfusion pattern.

CONCLUSION

Perfusion quantification via ICG NIR fluorescence imaging, combined with AI classification models can be used to exploit the differing neovascularization of underlying tissue and consequently, characterize colorectal polyps and aid surgical decision making. The developed system overcomes the limitations of commercial fluorescence imaging systems by extending NIR visualization to more torturous areas of the gastrointestinal tract. The imaging system and integrated AI algorithm can distinguish minimal perfusion pattern variations in an ex-vitro model utilizing ICG of clinical concentrations. Further AI validation (CLASSICA, EU Horizon) and system design optimization in the form of live animal trials, chip-on-tip sensor feasibility testing, user interface implementation and control unit miniaturization will advance user and commercial attractiveness imminently.

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Contact information: gareth.gallagher@ucd.ie; linkedin.com/in/gareth-gallagher-08a99195