Thus, there is a recognized need in the art for improved methods for imaging of bacterial disease. More specifically, the prior art is deficient in sensitive and specific real-time optical imaging methods for beta-lactamase positive bacteria that can be used *in vitro* and in live subjects to diagnose and locate the bacterial infection, to rapidly screen for new therapeutics and to identify new drug targets. The present invention fulfills this long-standing need and desire in the art.

SUMMARY ~~OF THE INVENTION~~

In one aspect, there is described herein a method for detecting a pathogenic bacteria in real time comprising: contacting a biological sample in vitro with a fluorogenic substrate for a beta-lactamase of the pathogenic bacteria; wherein the fluorogenic substrate is CNIR5-QSY22, CNIR7, CNIR7-TAT, CNIR9, or CNIR10; imaging the sample for a product from beta-lactamase activity on the substrate; and acquiring signals at a wavelength emitted by the beta-lactamase product; thereby detecting the pathogenic bacteria in real time.

The present disclosure~~invention~~ is directed to a method for detecting a pathogenic bacteria in real time in a subject. The method comprises introducing into the subject or a biological sample therefrom a fluorescent, luminescent or colorimetric substrate for a beta-lactamase of the pathogenic bacteria and imaging the subject or sample for a product from beta-lactamase activity on the substrate. Signals at a wavelength emitted by the beta-lactamase product are acquired thereby detecting the pathogenic bacteria in the subject. The present disclosure~~invention~~ is directed to a related method further comprising producing a 3D reconstruction of the emitted signal to determine location of the pathogenic bacteria in the subject. The present disclosure~~invention~~ is directed to another related method further comprising diagnosing in real time a pathophysiological condition associated with the pathogenic bacteria based on an emitted signal intensity greater than a measured control signal.

In another aspect, there is described herein a method for imaging a pathogenic bacteria, comprising: contacting a pathogenic bacteria in an in vitro biological sample with a fluorogenic substrate for a beta-lactamase enzyme thereof; delivering to the pathogenic bacteria an excitation wavelength for a product of beta-lactamase activity on the substrate; wherein the fluorogenic substrate is CNIR5-QSY22, CNIR7, CNIR7-TAT, CNIR9, or CNIR10; acquiring fluorescent signals emitted from the product; and producing a 3D reconstruction of the acquired signals, thereby imaging the pathogenic bacteria.

In a further aspect, there is described herein a method for detecting a pathogenic *Mycobacteria*, comprising: contacting a first biological sample *in vitro* with a fluorogenic substrate for a beta-lactamase of the pathogenic *Mycobacteria*; wherein said fluorogenic substrate is CNIR5-QSY22, CNIR7, CNIR7-TAT, CNIR9, or CNIR10; imaging the biological sample for a product of beta-lactamase activity on the substrate; imaging a control sample containing the fluorogenic substrate; measuring in real time intensity of a fluorescent signal acquired at a wavelength emitted by the product of beta-lactamase activity on the fluorogenic substrate in the biological sample and in the control sample; and comparing the measured signal intensities, wherein an increase in the fluorescent signal intensity compared to the measured control signal correlates to a presence of the pathogenic *Mycobacteria* in the biological sample, thereby detecting the pathogenic *Mycobacteria* in the sample.

Also disclosed herein is~~The present invention also is directed to~~ a method for diagnosing a pathophysiological condition associated with a pathogenic bacteria in a subject. The method comprises administering to the subject or contacting a biological sample derived therefrom with a fluorogenic substrate for a beta-lactamase of the pathogenic bacteria and imaging the subject for a product of beta-lactamase activity on the substrate. A fluorescent, luminescent or colorimetric signal intensity is measured in real time at wavelength emitted by the product such that a fluorescent, luminescent or colorimetric signal intensity greater than a measured control signal correlates to a diagnosis of the pathophysiological condition. The present disclosure~~invention~~ is directed to a related method further comprising producing a 3D reconstruction of the signal to determine location of the microbial pathogen. The present disclosure~~invention~~ is directed to another related method further comprising administering one or more therapeutic compounds effective to treat the pathophysiological condition. The present disclosure~~invention~~ is directed to a further related method comprising readministering the fluorogenic compound to the subject and re-imaging the subject or contacting a biological sample derived therefrom with said fluorogenic substrate; and imaging the subject or said biological sample to monitor the efficacy of the therapeutic compound such that a decrease in emitted signal compared to the signal at diagnosis indicates a therapeutic effect on the pathophysiological condition.

The present disclosure~~invention~~ is directed further to a method for screening for therapeutic compounds effective for treating a pathophysiological condition associated with a pathogenic bacteria in a subject. The method comprises selecting a potential therapeutic compound for the pathogenic bacteria, contacting the bacterial cells with a fluorescent, luminescent or colorimetric detection agent and contacting the bacterial cells with the potential therapeutic compound. A fluorescent, luminescent or colorimetric signal produced by the bacterial cells is measured in the presence and absence of the potential therapeutic compound

such that a decrease in signal in the presence of the therapeutic compound compared to the signal in the absence thereof indicates a therapeutic effect of the compound against the pathogenic bacteria.

The present disclosure~~invention~~ is directed further still to a method for imaging a pathogenic bacteria. The method comprises contacting a pathogenic bacteria with a fluorogenic substrate for a beta-lactamase enzyme thereof, delivering to the pathogenic bacteria an excitation wavelength for a product of beta-lactamase activity on the substrate and acquiring fluorescent, luminescent or colorimetric signals emitted from the product. A 3D reconstruction of the acquired signals is produced thereby imaging the pathogenic bacteria.

The present disclosure~~invention~~ is directed further still to a fluorogenic substrate for a bacterial beta-lactamase that is CNIR-7 or CNIR7-TAT.

The present disclosure~~invention~~ is directed further still to a method for detecting a pathogenic bacteria in real time in a subject. The method comprises introducing into the subject a substrate radiolabeled with an isotope associated with gamma emission where the substrate is for a beta-lactamase or other enzyme or protein specific to the pathogenic bacteria. The subject is imaged for gamma emissions from the radiolabeled substrate during activity thereon and signals generated by the emitted gamma rays are acquired. A 3D reconstruction of the concentration in the subject of the radiolabel based on intensity of the gamma ray generated signals is produced thereby detecting the pathogenic bacteria. The present disclosure~~invention~~ is directed to a related method further comprising diagnosing in real time a pathophysiological condition associated with the pathogenic bacteria based on detection thereof. The present disclosure~~invention~~ is directed to another related method further comprising administering one or more therapeutic compounds effective to treat the pathophysiological condition. The present disclosure~~invention~~ is directed to yet another related method further comprising readministering the radiolabeled substrate to the subject and reimaging the subject to monitor the efficacy of the therapeutic compound; wherein a decrease in gamma emission compared to gamma emission at diagnosis indicates a therapeutic effect on the pathophysiological condition.

The present disclosure~~invention~~ is directed further still to a radiolabeled substrate for a bacterial beta-lactamase suitable for PET or SPECT imaging as described herein.

Other and further objects, features, and advantages will be apparent from the following description of the presently preferred examples~~embodiments of the invention~~ disclosed herein, which are given for the purpose of disclosure.

BRIEF DESCRIPTIONS OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention briefly summarized above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

Figures 1A-1C depict the structures of CC1 and CC2 (**Figure 1A**), CHPQ (**Figure 1B**), and CR2 (**Figure 1C**) before and after hydrolysis by beta-lactamase.