

Method of analyzing samples, analyzing device and computer program

Technical field of the invention

The present invention relates to a method of analyzing one or more samples arranged in the wells of a microplate. The invention also concerns an analyzing device and a computer program for operating an analyzing device.

Background of the invention

Assays based on fluorescence are commonly used in life sciences. Fluorescence is a form of luminescence. Fluorescence means emission of light (a photon) by a substance that has absorbed light or other electromagnetic radiation. Absorbance of energy excites an orbital electron of a molecule to a higher electronic state and relaxation to ground state emits a photon.

Fluorescence assays utilize the properties of fluorophores. Fluorophores absorb light energy at one wavelength and, in response, re-emit light energy at another, longer wavelength. Each fluorophore has a distinctive range of wavelengths at which it absorbs light and another distinct range of wavelengths at which it emits light. This property enables their use for specific detection of biological products by analytical instruments and techniques.

In fluorescence assays, the samples are typically arranged in the wells of a microplate. A microplate is a flat plate comprising a plurality of wells, i.e. cavities that are arranged in rows and columns. The wells are configured to receive samples and function as small test tubes. A typical microplate comprises 6, 24, 96, 384 or 1536 wells, although also larger microplates exist.

In a particular type of a fluorescence assay, fluorescent spots are formed, and analysis of the samples is heavily based on counting of the number of the fluorescent spots. A problem related to the counting of the spots is that the counting process strongly relies on the inspection of the spots by a human user with the help of an analyzing device. This makes the analysis time-consuming and highly subjective. An additional problem is that if the number of fluorescent spots in a sample is high, the counting becomes unreliable. For instance, the diameter of the wells in a 96-well microplate is approximately 6 mm. If the number of the spots in one well is for example 500 or more, the spots are so close to each other that reliable counting of the spots becomes difficult.

Summary of the invention

Embodiments of the present invention provide an improved method of analyzing one or more samples arranged in the wells of a microplate. Embodiments of the invention provide an improved analyzing device. Embodiments of the invention provide an improved computer program for operating an analyzing device.

According to a first aspect of the present invention, there is provided a method of analyzing one or more samples arranged in the wells of a microplate, the method including the steps of producing electromagnetic radiation having a first predetermined wavelength or wavelength range, illuminating a sample by said electromagnetic radiation by transmitting the electromagnetic radiation to the sample from above the microplate via the upper end of the well, transmitting light emitted by the sample and having a second predetermined wavelength or wavelength range via the upper end of the well to a detector, determining intensity of light emitted by one or more predetermined measurement areas of the sample, and based on the determined intensities of light emitted by said one or more measurement areas, determining a result value representing the total amount of light emitted by the sample and having the second predetermined wavelength or wavelength range.

According to a second aspect of the present invention, there is provided an analyzing device that is configured to receive a microplate including a plurality of wells, each of the wells being configured to receive a sample, wherein the analyzing device includes illuminating means for illuminating a sample from above the microplate via the upper end of a well of the microplate using electromagnetic radiation having a first predetermined wavelength or wavelength range, detection means for determining intensity of light emitted via the upper end of the well by one or more measurement areas of the sample and having a second predetermined wavelength or wavelength range, and means that are configured to determine, based on the determined intensities of light emitted by said one or more measurement areas, a result value representing the total amount of light emitted by the sample and having the second predetermined wavelength or wavelength range.

According to a third aspect of the present invention, there is provided a computer program for operating an analyzing device, the computer program including

instructions which, when the program is executed by a computer, cause an analyzing device to carry out the method steps defined above.

With the method, device and computer program according to the invention, the analysis of the samples will be less dependent on the judgment of a user. The result value represents an integration or an estimation of the total amount of light emitted by the samples.

According to an embodiment of the invention, the sample comprises a plurality of predetermined measurement areas and the result value is calculated based on determined intensities of light emitted by said plurality of measurement areas.

According to an embodiment of the invention, the measurement areas cover the whole bottom of the well.

According to an embodiment of the invention, for determining the intensity of light emitted by said one or more predetermined measurement areas of the sample, the sample is illuminated using a light beam having a diameter that is at most 60 percent of the diameter of the well.

According to an embodiment of the invention, for determining the intensity of light emitted by said one or more predetermined measurement areas of the sample, one measurement area at a time is illuminated.

According to an embodiment of the invention, a photomultiplier tube is used as the detector for determining the intensity of light emitted by said one or more predetermined measurement areas of the sample.

According to an embodiment of the invention, method comprises the step of counting the number of spots emitting light having the second predetermined wavelength or wavelength range. The method provides high sensitivity and allows fast screening of samples. In addition, compared to mere counting of fluorescent spots, the method offers a wider dynamic signal range, as the method allows measurement even in the case that too large number of fluorescence spots are present making the counting of spots unreliable.

According to an embodiment of the invention, for counting the spots, the whole bottom of the well is illuminated and a camera sensor is used for forming an image of the sample.