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(54) Title: METHOD AND KITS FOR MULTIPLEXED FLUORESCENT MICROSCOPY

(57) Abstract: Provided is a method for multiplexed fluorescence microscopy comprising contacting a fixed sample with a set of binding agent-T-oligonucleotide conjugates to allow the binding agents to bind to any binding partners present in the sample, wherein the set comprises a plurality of binding agents having different specificities and the sequence of the T-oligonucleotide is unique to the binding agent to which it is conjugated, contacting the sample and any bound binding agents resulting from step a with a FRET-oligonucleotide, illuminating the sample with a wavelength to cause excitation of the FRET-oligonucleotide's emitter molecule, and observing the fluorescent kinetic profile of the sample at the FRET-oligonucleotide emitter molecule's emission wavelength at one or more pixels over time, wherein the FRET-oligonucleotide can hybridise to multiple T-oligonucleotides in the set, to form multiple pairs, and wherein the dissociation and reassociation between each different pair generates a fluorescent kinetic profile that is unique within that set to that pair. Also provided are associated kits, sets of binding agent T-oligonucleotide conjugates and corresponding FRET-oligonucleotides, and methods of designing such sets.

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