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## Review

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## Determining the geometry of oligomers of the human epidermal growth factor family on cells with 7 nm resolution

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#### ABSTRACT

Dimerisation, oligomerisation, and clustering of receptor molecules are important for control of the signalling process. There has been a lack of suitable methods for the study and quantification of these processes in cells. Here we describe a protocol for a method that we have named "fluorophore localisation imaging with photobleaching" (FLImP), which uses single molecule localisation and single-step photobleaching to determine the separation of two fluorophores with a resolution of 7 nm or better. We describe the procedures required for the collection of FLImP data, and point out some of the pitfalls that must be avoided for the collection of high resolution data. We also present recent data obtained using FLIMP, showing that the intracellular domain of the Epidermal Growth Factor Receptor is not required in the basal state for the receptor to form ordered inactive oligomers in the plasma membrane. © 2015 Published by Elsevier Ltd.

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#### 1. Introduction

Dimerisation, oligomerisation, and clustering of receptor molecules in the plasma membrane are now recognised as important

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factors in the control of signalling processes. A good example is the Epidermal Growth Factor Receptor (EGFR), one of a family of four receptors that initiate signalling cascades responsible for cell proliferation, motility, and survival (Lemmon and Schlessinger, 2010). A large body of evidence links receptor activation to oligomerisation, but details of the process are sketchy, because of a lack of suitable methods for the study and quantification of oligomerisation and clustering in cells. A number of techniques have been applied: Förster Resonance Energy Transfer (FRET) has been used to



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Fig. 1. Examples of good and bad areas for FLImP analysis. A) shows a "crowded" region in which single molecule features overlap, while B) shows a good region, with a reasonable number of features, which are well separated. The insets show white light images confirming the presence of cells.

detect EGFR–EGFR distance measurements in the 5–8 nm range, said to demonstrate the existence of dimers (Gadella and Jovin, 1995). However, it is unable to provide information on longer distances important for large scale organisation. This includes the distance of 11 nm predicted for dimers from crystallography (Garrett et al., 2002). Image correlation spectroscopy (ICS) can be used to measure the density of receptor clusters, and their average size, and has been used to propose the existence of higher order oligomers such as tetramers (Clayton et al., 2005, 2007), but the method cannot provide structural detail on the nature of the oligomers. So-called "super-resolution" microscopy techniques developed in recent years have also been applied to the study of EGFR in cells. Near-field scanning optical microscopy (NSOM) (Abulrob et al., 2010), stimulated emission depletion microscopy (STED) (Pellett et al., 2011), and stochastic optical reconstruction microscopy (STORM) (Gao et al., 2015), have all been used to show the presence of EGFR clusters in cells. Although these techniques have provided useful insights, the resolution (in the range of 20–150 nm) is still insufficient to resolve the details of macromolecular complexes.

The principle on which STORM is based, single molecule localisation, does in principle have the resolution to resolve the details

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Projects Dataset	5				
Any users	Any instrument Any e	periment type 🗘 Any number of channels 🛟			
Name		Sample Description			
20141112_0001_	0021	wtEGFR HER1 Affibody CF640R 100nM			
20141112_0001_	0020	wtEGFR HER1 Affibody CF640R 100nM			
20141112_0001_	0019	wtEGFR HER1 Affibody CF640R 100nM			
20141112_0001_	0018	wtEGFR HER1 Affibody CF640R 100nM	wtEGFR HER1 Affibody CF540R 100nM		
20141112_0001_	0017	wtEGFR HER1 Affibody CF640R 100nM			
20141112_0001_	0016	wtEGFR HER1 Affibody CF640R 100nM			
20141112_0001_	0015	wtEGFR HER1 Affibody CF640R 100nM			
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20141112_0001_	0013	wtEGFR HER1 Affibody CF640R 100nM			
20141112_0001_	0012	wtEGFR HER1 Affibody CF540R 100nM			
20141112_0001_	0011	wtEGFR HER1 Affibody CF640R 100nM			
20141112_0001_	0010	wtEGFR HER1 Affibody CF640R 100nM			
20141112_0001_	0009	wtEGFR HER1 Affibody CF640R 100nM			
20141112_0001_	0008	wtEGFR HER1 Affibody CF640R 100nM	wtEGFR HER1 Affibody CF640R 100nM		
20141112_0001_	0007	wtEGFR HER1 Affibody CF640R 100nM	wtEGFR HER1 Affibody CF640R 100nM		
20141112_0001_	0006	wtEGFR HER1 Affibody CF640R 100nM	wtEGFR HER1 Affibody CF640R 100nM		
20141112_0001_	0005	wtEGFR HER1 Affibody CF640R 100nM	wtEGFR HER1 Affibody CF640R 100nM		
20141112_0001_	0004	wtEGFR HER1 Affibody CF640R 100nM	wtEGFR HER1 Affibody CF640R 100nM		
20141112_0001_	0003	wtEGFR HER1 Affibody CF640R 100nM	wtEGFR HER1 Affibody CF640R 100nM		
20141112_0001_	0002	wtEGFR HER1 Affibody CF640R 100nM	wtEGFR HER1 Affibody CF640R 100nM		
20141112_0001_	0001	wtEGFR HER1 Affibody CF640R 100nM	wtEGFR HER1 Affibody CF640R 100nM		
20140314_0001	0012	40 nM NRG1b A488, 4 nM EGF CF640R + 1 uM HER4 Fab UL 30'@37C	+1 uM Erl 2h@37C (T47D on nanoge		
Project informatio	n				
Instrumentid OctopusSM3					
Directory	/mt/rcls/serv005/MSMM analysed/OctopusSM3/20141112 0001 0004 52e76ebc-39df-4d59-94c1-5bcc0c5011c0				
Users	Sarah Needham, Kathrin Scherer				
Status	HasTracks				
NChannels	1				
ExperimentType	Undefined				
Created	Wed Nov 12 19:00:44 2014				

Fig. 2. Window from the "MSMM manager" software, used for the selection of datasets for analysis.

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