Developing a cell painting assay for *Plasmodium falciparum* drug discovery

Rebecca CS Edgar[1], Laura Sesma Sanz[2], Ko Sugawara[3], Marina Garcia[4], Elliot Byford[3], Alistair Langlands[2], Benoît Laleu[4], Marcus CS Lee[1]

[1] Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee, UK.
[2] National Phenotypic Screening Centre, School of Life Sciences, University of Dundee, Dundee, UK.

[3] LPIXEL Inc., Tokyo, Japan.

[4] Medicines for Malaria Venture (MMV), Geneva, Switzerland.

As drug resistance continues to emerge against *Plasmodium falciparum*, the most lethal causative agent of human malaria, so does the need to discover new chemical matter with potent antimalarial activity. High-throughput killing assays allow for identification of active compounds against the parasite but do not provide any information as to their mode(s) of action (MoA), and whether these are potentially novel. The 48-hour lifecycle of *P. falciparum* within red blood cells follows distinct morphological changes and growth stages that are highly distinctive and are often disrupted under drug treatments. To leverage these changes, we are developing a high-throughput cell painting assay that will utilise machine learning algorithms to classify novel compounds based on their MoA via assessment of morphological changes of compound-treated cells. Cell painting assays use data from hundreds of features derived from a panel of fluorescent reporters that map to different organelles or sub-cellular structures. Here we have generated a green fluorescent protein-expressing parasite line that, with an additional four complimentary fluorescent dyes, constitute our cell painting panel. Assay development and optimisation to determine treatment and parasite-stage timing, cell density and plate stability have also been performed using our Operetta high-content imager. Overall, we hope to develop a cell painting assay that can be used in the primary stages of the drug discovery pipeline to inform on novel MoA of antimalarial compounds in early screening.