TITLE PAGE

Cell Targeting and Adjuvant Activity of Dietary Titanium Dioxide

Authors:

John W. Wills[1*], Alicja Dabrowska[1], Jack Robertson[1], Michelle Miniter[1], Sebastian Riedle[2,3], Huw D. Summers[4], Rachel E. Hewitt[1], Adeeba Fathima[1], Alessandra Barreto da Silva[1], Carlos A. P. Bastos[1], Stuart Micklethwaite[5], Åsa V. Keita[6], Johan D. Söderholm[6], Nicole C. Roy[2,7,8], Don Otter[9], Ravin Jugdaohsingh[1], Pietro Mastroeni[1], Andy P. Brown[3], Paul Rees[2], Jonathan J. Powell[1*].

Affiliations:

[1]Department of Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES, UK.

[2]Riddet Institute, Massey University, Private Bag 11222, Palmerston North, 4442, New Zealand.

[3]Food Nutrition & Health Team, Food & Bio-based Products Group, AgResearch, Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North, 4442, New Zealand.

[4]Department of Biomedical Engineering, Faculty of Science and Engineering, Swansea University, Swansea SA1 8EN, UK.

[5]School of Chemical and Process Engineering, University of Leeds, Leeds, LS2 9JT, UK.

[6]Department of Surgery and Department of Biomedical and Clinical Sciences, Linköping University, 58185 Linköping, Sweden.

[7]Department of Human Nutrition, University of Otago, Dunedin 9016, New Zealand.

[8]High-Value Nutrition Ko Ngā Kai Whai Painga National Science Challenge, Auckland 1142, New Zealand.

[9]Department of Food Science and Microbiology, Auckland University of Technology, Auckland, 1001, New Zealand.

***Joint-corresponding authors:**

John W. Wills & Jonathan J. Powell

30 Email: jw2020@cam.ac.uk / jjp37@cam.ac.uk
31 Tel: +44 (0)1223 337701
32 Biomineral Research, Department of Veterinary Medicine, School of Biological Sciences, University
33 of Cambridge, Madingley Road, Cambridge CB3 0ES, UK.

34

ABSTRACT

Food-grade titanium dioxide ($fgTiO_2$) is a bio-persistent particle under intense regulatory scrutiny. Paradoxically, meaningful *in vivo* cellular accumulation has never been demonstrated: the only known cell targets for $fgTiO_2$ are 'graveyard' intestinal pigment cells which are metabolically and immunologically quiescent. Here, we identify major new immunocompetent cell reservoirs of $fgTiO_2$ in humans, most notably in the subepithelial dome region of intestinal Peyer's patches. Using multimodal microscopy techniques with single-particle detection and per-cell / vesicle image analysis we achieved correlative dosimetry, quantitatively recapitulating human cellular exposures in a mouse model. Epithelial microfold cells specifically funneled $fgTiO_2$ into LysoMac and LysoDC cells, which co-accumulated attenuated Δ*aroA*-*Salmonella* upon sequential oral challenge. By proximity extension analyses, a clear *Salmonella* effect on pro-inflammatory signalling was confirmed, but no interaction with $fgTiO_2$ was revealed for 92 protein targets despite marked same-cell accumulation. In contrast, *Salmonella* caused the $fgTiO_2$-recipient cells to migrate towards the follicle margins and, sporadically, to the lamina propria recreating the human intestinal tissue distribution of $fgTiO_2$. Physiologically active cell targets that accumulate $fgTiO_2$ are now identified. $fgTiO_2$ appears neither a danger signal nor an adjuvant in wild-type genotypes and we demonstrate a mouse model that finally enables human-relevant risk assessments of ingested (nano)particles.

INTRODUCTION

Titanium dioxide (TiO_2) is a highly versatile nano- and micro- particulate mineral with widespread use in consumer products and manufacturing processes, including as a food and pharmaceutical additive. Although ingested TiO_2 has been proposed as an 'inadvertent' cellular adjuvant for many years[1, 2], the global industry, valued at >$17 billion per annum[3], is now facing a whole new 'bio-nano' challenge. In May 2021, the European Food Safety Authority (EFSA) questioned the safety of food-grade TiO_2 ($fgTiO_2$; also termed E171) primarily citing unresolved genotoxicity concerns[4]. Based on this, European Commission member states agreed to remove $fgTiO_2$ as an additive in foods. This represented a seemingly pragmatic decision balancing need for this additive (*e.g.,* aesthetics) against hypothetical risk. However, the European Commission also requested that the European Medicines Agency (EMA) evaluate the impact of removal of $fgTiO_2$ from the list of authorised excipients that are permitted in medicinal products. In this case, $fgTiO_2$ is *not* merely about aesthetics: it delivers opacity and protects actives from UV light degradation whilst aiding moisture control and patient compliance. The EMA concluded that any foreseeable ban, impacting the ~ 91,000 medicinal products that contain this pigment in Europe, risks causing pharmaceutical shortages[5]. Moreover, they estimated that a period of 7-12 years would likely be required for implementation of suitable alternatives[5]. Meanwhile, consumer concerns over the safety of $fgTiO_2$ have been expressed beyond Europe. Notably, in 2022, a class action suit was filed against the multinational food company Mars Incorporated in the USA alleging that consumers of the hard-shell candy 'Skittles' are at heightened risk of health effects stemming from genotoxicity[6].

Whilst regulators in other continents / territories have not followed EFSA's 2021 decision, there is widespread agreement that the available data addressing the uptake, biological fate and effects of $fgTiO_2$ is insufficient[7, 8, 9]. In fact, direct toxicity of $fgTiO_2$ in cell culture experiments has not been consistently demonstrated, especially when conditions are carefully controlled to avoid artefactual *in*

78 *vitro* cell gorging[10]. In contrast, *in vitro*, $fgTiO_2$ is recognised as a modest adjuvant for primary
79 danger signals, namely microbe-associated molecular patterns, although cell loading of the particles
80 needs to be relatively high[1]. Similarly, Kirkland *et al.,*[11] identified any potential genotoxic activity of
81 various titanium dioxide polymorphs as secondary to cell stress which, certainly for $fgTiO_2$, requires
82 marked cell loading[1].
83
84 Aside from specific cells of the small intestine, there are no known circumstances under which
85 $fgTiO_2$ markedly accumulates *in vivo*. Systemic exposure to $fgTiO_2$ appears to be very low[12]. Even
86 allowing for lifetime accumulation in humans it appears that anticipated target organs (liver and
87 spleen) contain levels of Ti and Ti-rich particulates that are just above the tissue-free background,
88 and the origin of this slightly enhanced signal is unknown[13]. Consistent with this, many animal
89 feeding studies show almost no detectable systemic accumulation of $fgTiO_2$[12, 14] and, overall, there
90 are no convincing *ex vivo* imaging studies showing multiple, heavily-loaded cells. The exception, as
91 noted above, is the small intestine, in both humans and rodent models[15, 16], and specifically their
92 large lymphoid follicles termed Peyer's patches. In absence of afferent lymphatics, the apical
93 surfaces of these follicles possess a unique population of microfold (M) cells that avidly sample the
94 lumen for bulky macromolecules including small micro- and large nano- particles. This material is
95 passed to underlying immune cells in the apical, 'subepithelial dome' (SED) region of the follicle
96 which contains recognised populations of monocyte-derived, lysozyme-expressing cells termed
97 'LysoMacs' and 'LysoDCs' that are specialised for anti-microbial defence[17]. Overall, the major role
98 of Peyer's patches is initiating processes for the generation of mucosal IgA and systemic IgG in
99 response to intestinal antigen thereby orchestrating the sequence of events that leads to gut-
100 derived humoral immunity. Over time, $fgTiO_2$ is known to accumulate in Peyer's patches, alongside
101 other persistent particles, in basally located tissue-fixed macrophages termed 'pigment cells'[15, 16].
102 These cells are of low immunological and metabolic activity and appear to act as lifetime

103 'graveyards' for persistent particles, presumably limiting systemic absorption through local
104 sequestration in the same way that tissue-fixed macrophages sequester particulate tattoo inks[15, 16, 18,
105 19].
106
107 So the paradox remains: whilst $fgTiO_2$ is under intense regulatory scrutiny, there is no known site of
108 accumulation in active (*e.g.,* immuno-competent) cells that could plausibly give cause for concern or
109 that allows informed risk assessment. Given that the intestine is the only known target of $fgTiO_2$
110 accumulation we have addressed whether similar, selective cell loading occurs elsewhere (*i.e.,* away
111 from the 'safe house' base-of-patch pigment cells) and, if so, what cells are targeted, at what
112 frequency and with what impact? The approach used quantitative image analysis of light and
113 electron microanalytical data, correlative mouse-human cell dosimetry and broad-target interrogation
114 of $fgTiO_2$'s capability to adjuvant a primary cellular danger signal *in vivo*.
115
116 **RESULTS**
117 High-resolution reflectance confocal microscopy of human small intestinal tissue samples identified
118 bright foci at the base of Peyer's patches (**Figure 1a)** consistent with previous reports of pigment
119 cells in this region[16, 18, 20]. These cells are known to mostly accumulate ingested engineered
120 aluminosilicates and $fgTiO_2$[16, 20]. Using Al and Ti as biomarkers for these particles, correlative
121 analyses using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy
122 (EDX) confirmed the presence and co-localisation of these elements (**Figure 1b**; top row).
123 Reflectant foci were found entirely attributable to Ti-rich particles, as areas with Al but no Ti signals
124 were not reflectant, whereas all detectable Ti foci yielded reflectance (**Figure 1b**; rows 2-5).
125 Alongside further checks using mouse tissues known to contain $fgTiO_2$ or not (shown,
126 **Supplementary Figure 1**), the reflectance imaging approach was confirmed as a robust, high-
127 throughput means to detect $fgTiO_2$ in tissue[15].

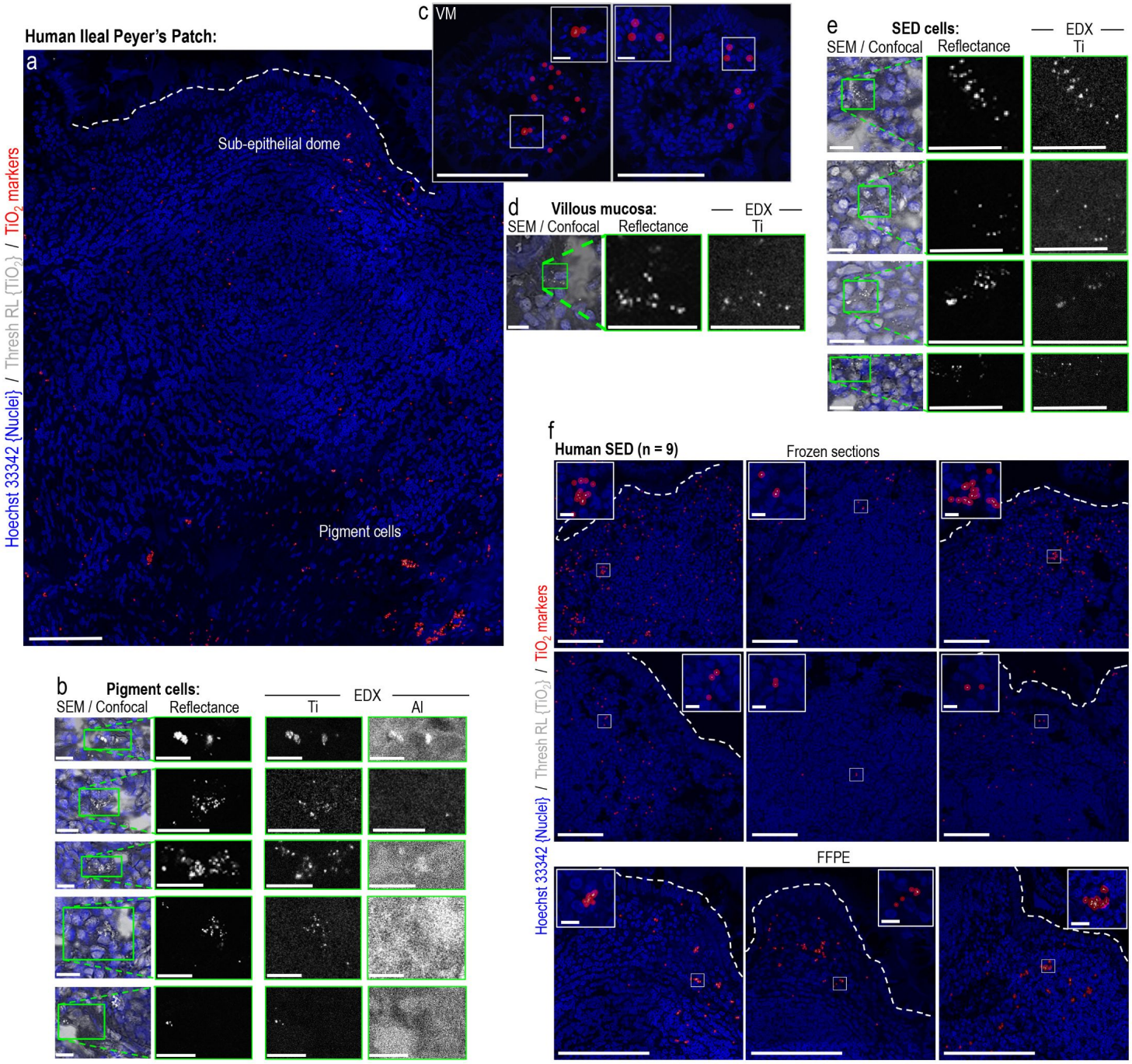


Figure 1 – Titanium dioxide in human intestinal tissues. a, Tilescanned image acquired by confocal reflectance microscopy. Reflectant foci were detected along the follicle base consistent with previously-reported mineral particle-containing "pigment cells". Of note, similar reflectant foci were also present apically in the immuno-active, subepithelial dome tissue-region, and away from the patch in the (**c**) lamina propria of the regular villous mucosa (VM). Translucent red circle-markers are placed on the reflectant foci to aid visualisation. **b**/**d**/**e**, Correlative SEM/EDX analyses performed on the same tissue section shown in (**a**/**c**). The pigment cell region is shown in (**b**) where X-ray signal for both Al (attributable to aluminosilicates) and Ti (attributable to titanium dioxide) was found. EDX analyses in the (**d**) villous mucosa and (**e**) subepithelial dome regions showed reflectant foci were attributable to Ti. **b**/**d**/**e**, In all instances the elemental data for Ti were a near-exact match for the reflectant foci, whereas (**b**) areas with Al signal alone did not reflect. **f**, Reflectance microscopical analyses of nine, randomly-drawn human samples (six frozen, three formalin-fixed paraffin embedded (FFPE)) showed reflectant foci consistent with TiO_2 in the subepithelial dome of *every* sample. *Scale bars:* ***a*** *= 50 µm;* ***b*** *= 50 µm with 10 µm insets;* ***c***-***e*** *= 10 µm;* ***f*** *= 50 µm with 10 µm insets.*

128

129 Of significant note, previously unreported populations of human cells containing similar, reflective
130 material were also identified (i) sporadically in the regular lamina propria (**Figure 1c**) and (ii)
131 densely in the immuno-active subepithelial dome (SED) region of Peyer's patches (**Figure 1a**).
132 These cell regions were also subjected to correlative SEM-EDX analyses and, again, reflectant foci
133 were a match for the Ti signal (**Figure 1d/e**). Given the unexpected density of the newly-identified
134 Peyer's patch population, whole SED regions from a randomly selected series of nine human
135 subjects were imaged using the analytically-validated confocal microscopy approach. Albeit with
136 marked inter-individual variability, reflectant signal was found in the SED of every human case
137 (**Figure 1f**). Importantly, the recipient cells often exhibited multiple foci per-cell (*i.e.,* high cellular
138 loading), suggesting specific cellular targeting and accumulation of $fgTiO_2$ (**Figure 1f** – insets) in a
139 non-pigment cell population.

140

141 To investigate the pathways giving rise to the above observations, and to phenotype the newly-
142 identified population of $fgTiO_2$-accumulating cells, in "Mouse Study 1", we turned to a recently
143 reported murine model for human-relevant, oral exposure to $fgTiO_2$[15]. Initially we tilescanned a
144 complete ileal transverse section using high-resolution confocal reflectance microscopy and used
145 image analysis to segment all cells (cell segmentation accuracies assessed, **Supplementary Figure**
146 **2**). This allowed the cellular dose of $fgTiO_2$ to be measured in each cell-object (*i.e.,* the integrated
147 intensity of the thresholded reflectance signal per cell) enabling the subdivision and display of the
148 cellular loading of $fgTiO_2$ in three groups: low, intermediate and high (**Figure 2a**). As in the human
149 samples (**Figure 1**), the patch region was decorated with reflectance signal, notably in the SED and
150 at the base, and cellular loading was often high – especially in the SED (**Figure 2a**). However,
151 unlike in human tissue, signal was entirely absent away from the patch except for two small, isolated
152 foci in the villous epithelium. The resolution of the imaging process allowed detailed investigation of

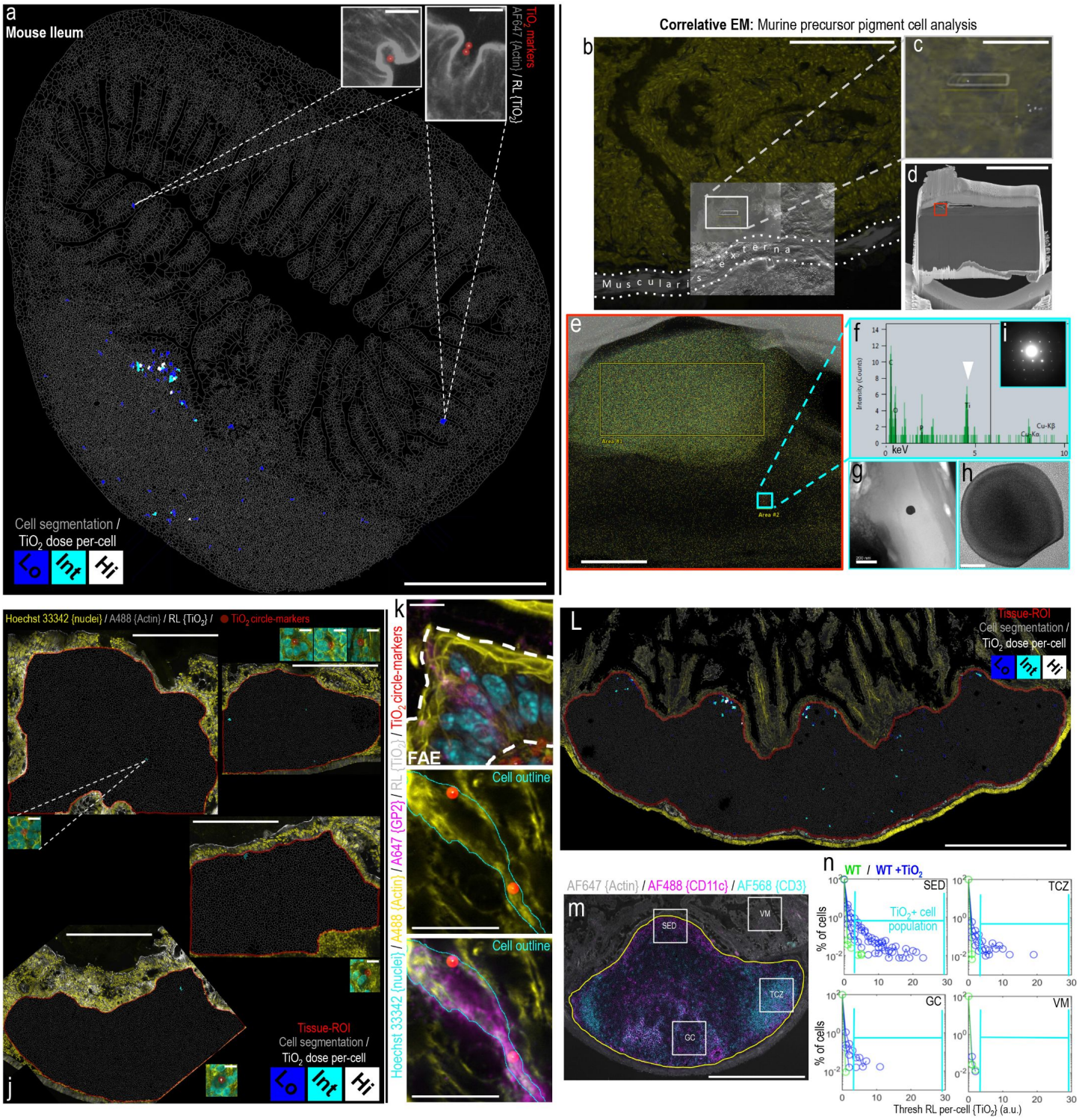


Figure 2 – A mouse feeding study demonstrates $fgTiO_2$ specificity for Peyer's patches. a, Single-cell image analysis of a transverse mouse ileal tissue section imaged by tilescanning confocal reflectance microscopy. The cellular dose of $fgTiO_2$ – measured from the thresholded reflectance (RL) signal – is colour-coded at low (lo), intermediate (int) and high (hi) levels. As in humans (**Figure 1a/f**), $fgTiO_2$ is loaded into the subepithelial dome of the Peyer's patch and there was evidence of 'precursor pigment cell' formation along the follicle base. Away from the Peyer's patch, two positive cells (**a**, insets) were found in the overlying villous mucosa. In contrast to the human findings (**Figure 1b**) however, both were caused by luminal $fgTiO_2$ trapped in the invaginated space of goblet cells. **b-d**, To investigate the sensitivity of $fgTiO_2$ detection by confocal reflectance microscopy, a tissue-region containing a single, isolated reflectant foci was milled out under correlative SEM using a focussed ion beam (**b/c** shows the registered SEM images transparently overlaid onto the confocal reflectance data before lift-out. The white 'rectangle' in (**c**) is caused by electron charging of the platinum strap laid down on the tissue region to be lifted out; (**d**) shows a side-view of the lifted-out lamella). **e-i**, Transfer of the lamella to a transmission electron microscope enabled high resolution imaging and X-ray (EDX) and electron diffraction analysis. A (**g/h**)single particle of $fgTiO_2$ with an (**i**) anatase diffraction pattern (*i.e.,* as added to the diet) was confirmed responsible for the single reflectant foci observed. **j**, Again, colour-coding the cellular dose of $fgTiO_2$ into lo, int and hi levels, tissue sections from adjacent caecal patches at the top of the colon were found to be almost completely devoid of $fgTiO_2$ (n = 4 animals). **k**, Immunofluorescence microscopy showed $fgTiO_2$ access to Peyer's patches was via GP2-positive microfold (M) cells (translucent red circle-markers are placed on thresholded reflectant foci to aid $fgTiO_2$ visualisation / the outline of the M-cell was manually drawn by tracing the actin-delineated cell boundary). **L**, Single-cell analysis of a longitudinal section of mouse ileum consistently showed the close association between an M-cell rich, follicle-associated epithelium and $fgTiO_2$ loading into Peyer's patches. **m/n**, Guided by CD11c and CD3 immunofluorescence labelling for phagocytic mononuclear cells and T-lymphocytes (respectively) sets of images were collected from the subepithelial dome (SED), germinal centre (GC) T-cell zone (TCZ) or overlying villous mucosa (VM) tissue regions from four mice fed the $fgTiO_2$-supplemented diet (WT + TiO_2) and four control-diet mice (WT). Some $fgTiO_2$ signal was observed in the GC and TCZ regions of the murine Peyer's patches but the majority was in the SED. In keeping with (**a**), and in contrast to the human findings (**Figure 1b**), in all four mice, no uptake was seen in the villous mucosa demonstrating specificity for M-cell mediated, Peyer's patch targeting. *Scale bars:* ***a*** *= 500 µm with insets 5 µm;* ***b*** *= 50 µm;* ***c*** *= 10 µm;* ***d*** *= 5 µm;* ***e*** *= 500 nm;* ***g*** *= 200 nm;* ***h*** *= 20 nm;* ***j*** *= 500 µm;* ***k*** *= 10 µm;* ***L/m*** *= 500 µm.*

153 these latter events confirming that this signal was not *inside* the tissue, but derived from material
154 within goblet cell invaginations that were open to the small intestinal lumen (**Figure 2a** – inset).
155
156 To confirm the apparent selectivity of $fgTiO_2$ for the lymphoid follicles – rather than the regular
157 villous mucosa – we questioned whether a single particle in intestinal tissue would be missed by our
158 reflectance microscopy approach. To do this, a tissue region containing a single, small yet clearly
159 visible reflectant foci was milled out under correlative SEM, using a focussed ion beam (**Figure 2b-**
160 **d**). The milled-out lamella was then transferred to a transmission electron microscope for imaging
161 with analysis by EDX and electron diffraction. A single particle of $fgTiO_2$, with a diameter around
162 100 nm and an anatase diffraction pattern consistent with dietary exposure, was identified (**Figure**
163 **2e-i**). Since single, disperse particles of $fgTiO_2$ were therefore detectable by the reflectance imaging
164 strategy we could confirm that ingested $fgTiO_2$ had not been missed with our imaging approach in
165 the regular villous mucosa and thus was not taken up in this region (**Figure 2a**).
166
167 Following from this, we next considered the caecal patches (*i.e.,* large lymphoid follicles of the first
168 part of the large intestine that adjoins the terminal ileum where Peyer's patches are located). Here,
169 transverse tissue sections revealed almost no reflectant foci showing that, unlike Peyer's patches, the
170 neighbouring caecal patch is spared from particle targeting (**Figure 2j**). In spite of the marked
171 similarity of these secondary lymphoid sites, the microfold (M) cell density of their overlying
172 epithelium differs greatly: caecal patches have very few (like the villous epithelium) with little
173 capacity for particle uptake, whereas Peyer's patch epithelium is enriched with mature M-cells that
174 exhibit distinct particle uptake capability for small particulates in general[21, 22]. Indeed, cells of the
175 Peyer's patch epithelium stained positively for the mature M-cell marker (GP2) and, here-and-there,
176 these M-cells were 'caught in the act' with co-signal for $fgTiO_2$ (**Figure 2k**) as had long been
177 assumed for $fgTiO_2$ but not actually demonstrated[18]. Indeed, longitudinal sections of ileal tissue

178 containing Peyer's patches consistently demonstrated the close association between $fgTiO_2$ signal
179 and an overlying, M-cell rich, follicle-associated epithelium (**Figure 2L**). In this way, the above
180 findings provide a rationale for the specificity of $fgTiO_2$ uptake by the Peyer's patches of the small
181 intestine. It would appear, therefore, that to explain the presence of $fgTiO_2$ in the human intestinal
182 lamina propria a mechanism other than direct uptake by the regular villous epithelium must exist
183 (this is addressed below in "Mouse Study 2"). First, however, we quantified how cell loading with
184 $fgTiO_2$ varied amongst the major different functional zones of the Peyer's patch.
185
186 Sets of confocal reflectance images were collected from the SED, germinal centre (GC), T-cell zone
187 (TCZ) and overlying villous mucosa (VM) tissue regions (indicated, **Figure 2m**) using tissue
188 sections taken from four mice fed with the $fgTiO_2$-supplemented diet (+$fgTiO_2$), alongside four
189 control mice from the same experiment and ingesting the same diet but without added $fgTiO_2$.
190 Single-cell analysis, using flow cytometry-type gating informed by the negative control tissues,
191 demonstrated some $fgTiO_2$ signal in the TCZ and GC but established the dominance of the signal,
192 and the highest cell loading, in the biologically-active SED (**Figure 2n**). As per the whole ileal
193 cross-section data (**Figure 2a**), in all sections analysed across the four mice, the villous mucosa was
194 entirely devoid of $fgTiO_2$-positive cells and consistently exhibited the same reflectance distribution
195 as the negative control mice (**Figure 2n**).
196
197 Rodent models provide pragmatic access to healthy pristine tissue (*i.e.,* rapidly flash frozen at
198 necropsy) from fully controlled experiments (*i.e.,* population-matched with availability of $fgTiO_2$-
199 negative tissues) in a way that human surgical specimens do not. Nonetheless, it is critical that a
200 murine feeding model appropriately reports on the human situation. Next, therefore, we exploited the
201 window-of-insight provided by precision image analysis to consider how the cellular dosimetry of
202 $fgTiO_2$ in the SED cells of the murine model reflected SED-cell exposure in humans. To do this, the

203 snap-frozen Peyer's patch tissue sections, taken from six randomly-drawn human samples, were
204 compared with six murine samples taken from the $fgTiO_2$-fed group. Visually, in both species, there
205 was marked inter-individual variability in $fgTiO_2$ concentration in the SED (**Figure 3a-L**). To
206 quantify this, we analysed the thresholded reflectance signal in the tissue sections in four ways,
207 namely: thresholded reflectance per unit tissue area (*i.e.,* $fgTiO_2$ abundance; **Figure 3m),**
208 thresholded reflectance per cell (*i.e.,* $fgTiO_2$ load per cell; **Figure 3n**), the number of reflectant foci
209 per cell (*i.e.,* cellular count of $fgTiO_2$-loaded vesicles; **Figure 3o**), and finally the amount of
210 reflectance signal per foci (*i.e.,* $fgTiO_2$ load per vesicle; **Figure 3p)**. The strategy is schematically
211 explained in **Supplementary Figure 3**; cell segmentation accuracy assessments are presented in
212 **Supplementary Figure 2**; background reflectance comparisons in mice and humans are shown in
213 **Supplementary Figure 4** and examples of the raw reflectance signal, resulting circle-marker
214 placements and $fgTiO_2$-loaded vesicle segmentations are shown in **Supplementary Figure 5**. By all
215 metrics, the data from the two groups showed remarkable overlap and under statistical comparison
216 none of the measured $fgTiO_2$ distributions were found to be entirely unique to either species
217 (presented, **Supplementary Figure 6**). Notably when Peyer's patch loading of $fgTiO_2$ from mice on
218 lower-dose-$fgTiO_2$ diets was analysed, the cellular accumulation of particles in the SED was several
219 orders of magnitude lower compared to the human situation (**Supplementary Figure 7**). The above
220 findings therefore establish the apparent ability of the murine model to provide human-relevant data
221 – quantitatively as well as qualitatively – in terms of $fgTiO_2$ cell targeting and dosimetry in the
222 biologically-active SED region of the Peyer's patch.
223
224 The $fgTiO_2$-targeted cells in the SED were, unsurprisingly, $CD11c^+$ (*i.e.,* phagocytic mononuclear
225 cells) and not only was the frequency of such cells unaffected by the presence of $fgTiO_2$ particles so
226 was their expression of the CD11c integrin biomarker (**Figure 4a-h**). Neighbouring cells were
227 chiefly $CD3^+$ (T cells) and $B220^+$ (B cells) and, similarly, their expression patterns and frequencies

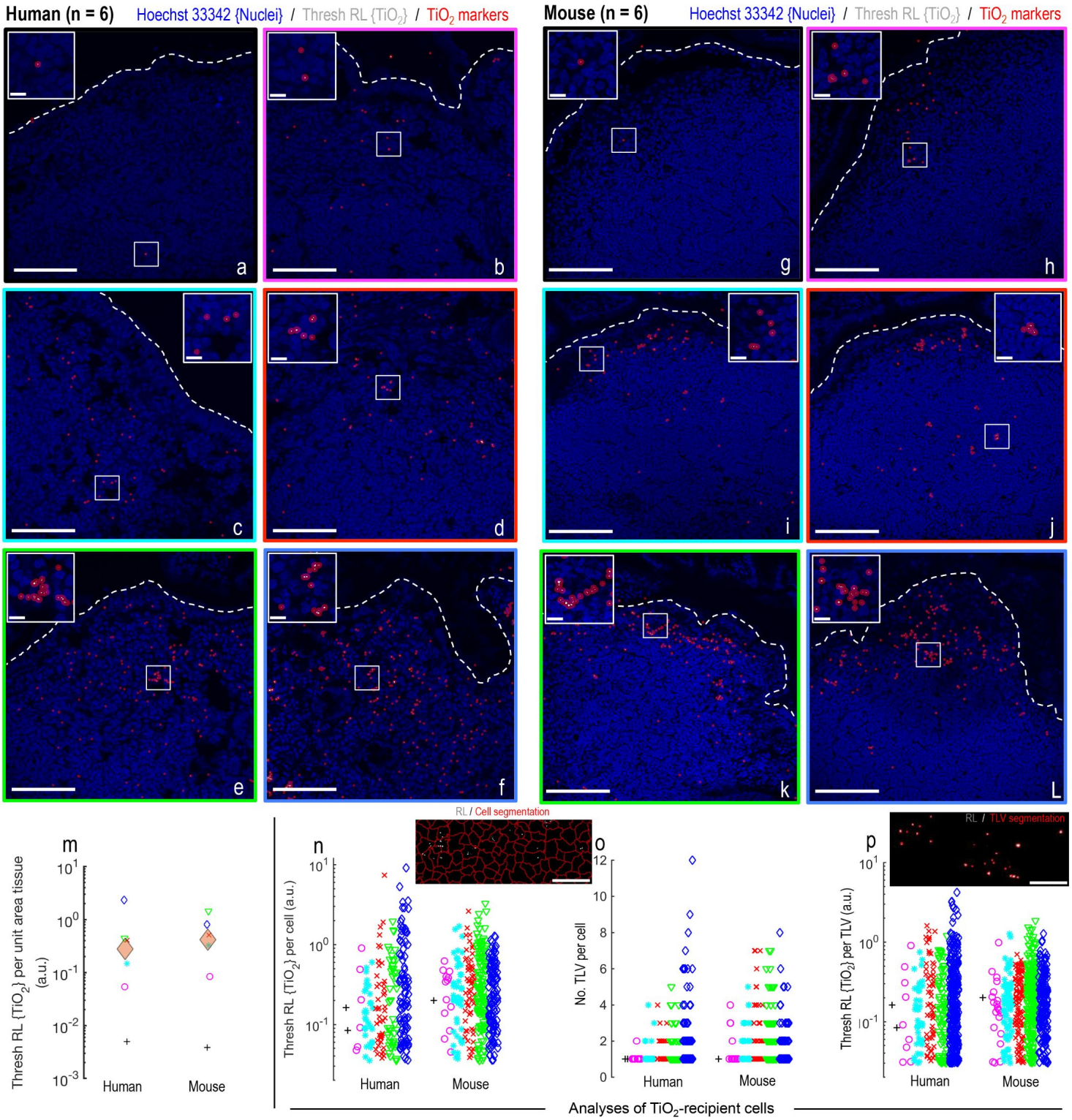


Figure 3 – Establishing correlative human-mouse dosimetry. a-L, Confocal reflectance microscopy showing the range of cellular loading in the subepithelial dome tissue region of (**a-f**), six randomly-drawn human and (**g-L**) six randomly-drawn mouse samples. After acquisition, the image-fields per specimen were manually laid out in order of lowest-to-highest $fgTiO_2$ SED accumulation to visually present the wide variation in $fgTiO_2$ cellular loading. Translucent red circle-markers were placed on thresholded reflectant foci to aid visualisation. **m-p**, Quantitative analysis of the image-data shown in **a-L**. **m**, Thresholded reflectance per unit tissue area (*i.e.,* amount of $fgTiO_2$ per unit tissue area). **n**, Thresholded reflectance per cell (*i.e.,* $fgTiO_2$ dose per cell). **o**, Number of thresholded reflectant foci per cell (*i.e.,* number of $fgTiO_2$-loaded vesicles (TLV) per cell). **p**, Thresholded reflectance signal per foci (*i.e.,* $fgTiO_2$ dose per TLV). Statistical comparison of the distributions is presented in **Supplementary Figure 6** (two-sided Wilcoxon rank-sum analysis). None of the measured $fgTiO_2$ distributions were found to be entirely unique to either species. **m-p**, By all of the quantitative measures established, feeding a murine diet supplemented with 0.0625% (w/w) $fgTiO_2$ for eighteen weeks provides significant overlap with measured, real-world human exposures. *Scale bars:* ***a-L*** *= 50 µm with insets 10 µm;* ***n*** *= 20 µm;* ***p*** *= 5 µm.*

228 were not significantly affected by the presence of $fgTiO_2$ ($P \geq 0.26$, two-sided unpaired samples T-
229 tests) (**Figure 4a-h**). Within the Peyer's patch, $CD11c^+$ cells are known to exhibit a number of sub-
230 phenotypes in distinct follicular locations[17]. In this regard, the SED localisation and autofluorescence
231 of the $fgTiO_2^+$ cells were entirely consistent with 'LysoMacs' and/or 'LysoDCs' which represent
232 extensively-described macrophage and dendritic cell subsets, respectively, that express high levels of
233 lysozyme alongside a unique autofluorescence signature[17, 23] (see **Figure 4i-s,** and **Supplementary**
234 **Figure 8** for the autofluorescence spectra collected). Indeed, phenotypic markers that help
235 differentiate LysoDCs from LysoMacs (cell surface CD4 and MHCII) demonstrated a mixed
236 population of these two cell types for the $fgTiO_2^+$ cells (**Figure 4t/u**).
237
238 Whilst similar populations of autofluorescent^{hi} SED cells were observed in all mice regardless of
239 diet, these cells were strongly particle-positive in mice receiving the dietary $fgTiO_2$ (**Figure 4m/s,**
240 **Supplementary Figure 9** and **Supplementary Video 1**). Upon quantification (**Figure 4o**), only
241 ~7% of autofluorescent cells showed no clear particle signal for the $+fgTiO_2$ group (**Figure 4i-s**).
242 These cells are known to be important in the initial uptake and processing of luminal antigen which
243 other work suggests may be coated in calcium phosphate to facilitate enzyme resistance during the
244 early phases of the lumen-to-phagocyte sampling process[24]. Except in Crohn's disease, these cells
245 have been shown previously to be positive for the immunoregulatory cell surface ligand PD-L1[25].
246 Again, consistent with this prior work, our quantitative single-cell tissue analyses showed that the
247 $fgTiO_2^+$ (and therefore $CD11c^+$, autofluorescence^{hi}) cells of the SED were PD-L1$^+$ (**Figure 4v-y**,
248 antibody controls presented **Supplementary Figure 10**). This contrasted with precursor pigment
249 cells at the base of the Peyer's patch which were PD-L1^{lo}, despite their $fgTiO_2$ content (**Figure 4v-z**).
250 In other words, based on this finding, and that mice unexposed to $fgTiO_2$ show similar PD-L1$^+$
251 distributions (**Figure 4w)**, we can conclude that expression of this immunoregulatory molecule is
252 unaffected by $fgTiO_2$ uptake ($P = 0.34$, $z = -0.96$, two-sided Wilcoxon rank-sum analysis) and,

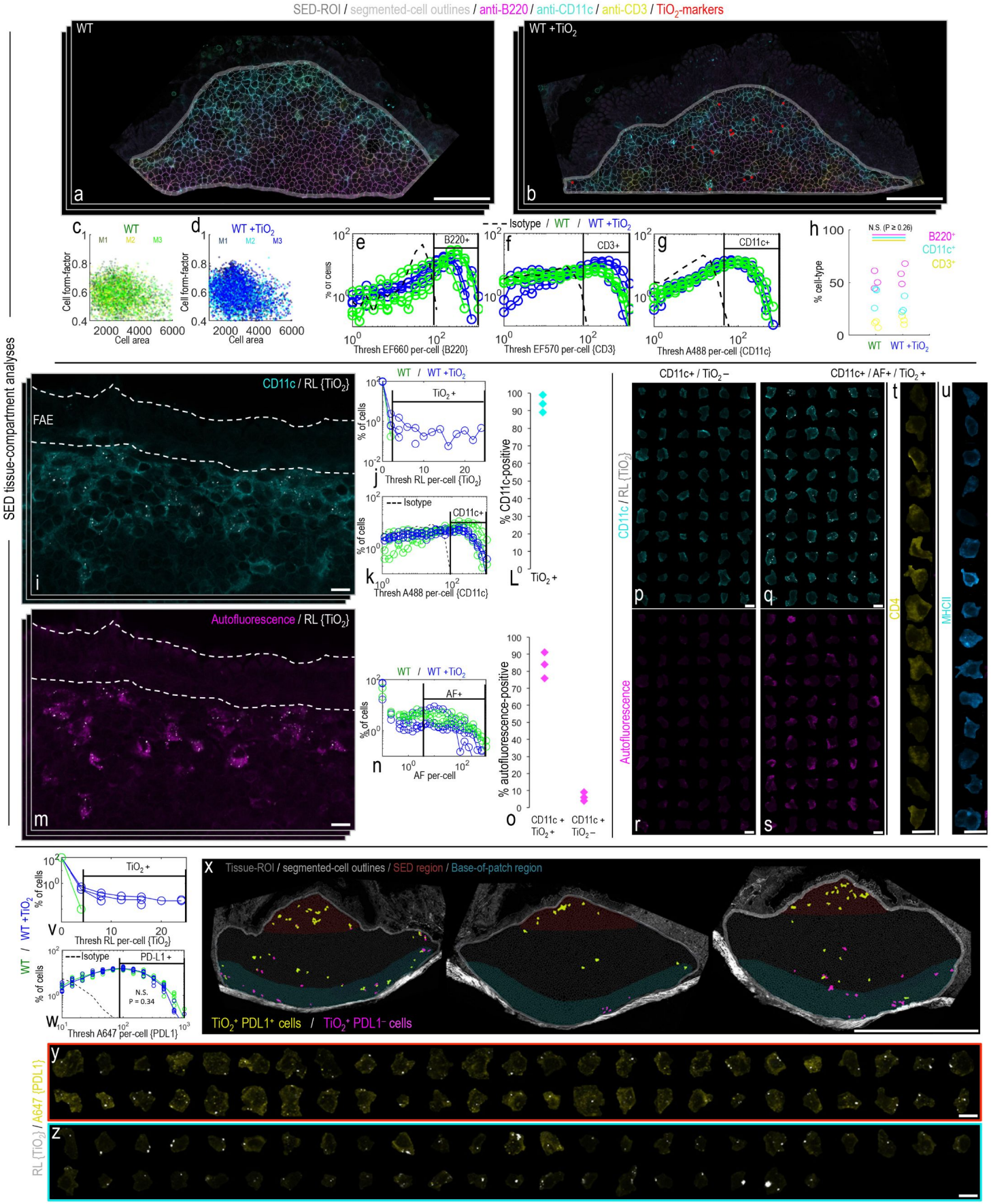


Figure 4 – fgTiO$_2$ selectively targets PD-L1$^+$ LysoMac and LysoDC cells. a-h, *In situ*, single-cell analysis of key immune-cell subtypes of the mouse subepithelial dome. **a/b**, Example images immunofluorescently labelled for B220, CD3 and CD11c (*i.e.,* identifying B-lymphocytes, T-lymphocytes and phagocytic mononuclear cells, respectively) from wild-type (WT) mice fed (**a**) without (WT) or (**b**) with (WT + TiO$_2$) fgTiO$_2$ dietary supplementation (0.0625% w/w of diet). fgTiO$_2$ was measured from thresholded reflectance images. Translucent red circle-markers are placed on reflectant foci to aid visualisation. **c/d**, Flow cytometry-type plots showing cell area / cell aspect ratio, (**e-g**) immunofluorescence distributions and (**h**) cell counts for B220$^+$, CD11c$^+$ and CD3$^+$ cells (n = 3 animals). All measures remained similar regardless of fgTiO$_2$ feeding (cell count comparison (**h**), P = ≥0.26, two-sided unpaired samples T-tests). **i-L**, fgTiO$_2$-recipient cells of the subepithelial dome were CD11c$^+$ in ~ 95% of cases. **m-o**, These cells also exhibited a unique, highly autofluorescent signature as previously-described for subepithelial dome 'lysomac' and 'lysoDC' mononuclear phagocyte cells. **o**, Again, this was true for almost all fgTiO2$^+$/CD11c$^+$ cells and, equally, these autofluorescent cells were similarly present in (**n**) controls without fgTiO$_2$ feeding (further data shown, **Supplementary Figure 7**). **p-u**, Montaged CD11c$^+$/fgTiO$_2^+$ or CD11c$^+$/fgTiO$_2^-$ cell populations from the SEDs of mice fed the fgTiO$_2$ supplemented diet visualised in terms of **(r/s)** autofluorescence signature. fgTiO$_2$ is seen to both selectively and specifically target the autofluorescent cells and (**t/u**) the diversity of CD4 and MHCII expression confirms that both lysomac and lysoDCs take up fgTiO$_2$. **v-z**, fgTiO$_2^+$ cells of the SED expressed the immunotolerance marker programmed death ligand 1 (PD-L1) whereas fgTiO$_2^+$ pigment cells at the base of the Peyer's patch tended to lose PD-L1 expression (**y** versus **z**, respectively). **w**, PD-L1 expression in the Peyer's patches was not significantly perturbed by feeding the fgTiO$_2$-supplemented diet (P = 0.34, z = -0.96, two-sided Wilcoxon rank-sum analysis, n = 6 animals). *Scale bars:* ***a/b*** *= 50 µm;* ***i/m, p-u*** *= 10 µm;* ***x*** *= 500 µm;* ***y/z*** *= 10 µm.*

253 rather, $fgTiO_2$ is selectively and specifically shuttled into already-PD-L1$^+$ LysoMacs/LysoDCs in the
254 SED of Peyer's patches in the small intestine. As such, for the first time, we show that there *is* an
255 immunocompetent cell population that accumulates $fgTiO_2$ in humans, and in a rodent model, which
256 will now allow the proposed adverse effects of the particulates to be tested.
257
258 As noted above, in earlier *in vitro* work, we have shown that the phagocyte proinflammatory
259 response can be augmented by cellular loading with $fgTiO_2$[1]. Hence, in "Mouse Study 2", we
260 investigated the *in vivo* adjuvant potential of $fgTiO_2$, specifically in augmenting responses to the
261 intestinal pathogen Δ*aroA*-deficient, *Salmonella enterica* serovar Typhimurium (Δ*aroA*-*Salmonella*)
262 in the targeted, immuno-competent SED cells of the intestine. The choice of oral bacterial infection
263 was, again, because it is human-relevant (*i.e.,* commonly experienced[26]) and because it targets
264 LysoDCs and LysoMacs[27, 28]. Importantly, unlike bacterial fragments, to which the intestine is
265 uniquely tolerant, Δ*aroA*-*Salmonella* is a danger signal that promotes a modest inflammatory
266 phenotype, albeit limited in effect (no clinical disease), leaving headroom for any adjuvant effect to
267 be observed. When Δ*aroA*-*Salmonella* were gavaged immediately following cessation of 16 weeks
268 of dietary $fgTiO_2$ or control diet, and tissues collected 3 days later (+3 days), we confirmed marked
269 same-cell localisation of both particle types (*i.e.,* bacteria and $fgTiO_2$) in the Peyer's patch SED
270 (**Figure 5a-c**). *Salmonella*-specific faecal IgA was undetectable at this time-point but was clearly
271 demonstrated at a later time-point (+28 days), confirming a latent, localised immune response in
272 groups exposed to this oral pathogen (**Figure 5d**).
273
274 To test for the interaction between $fgTiO_2$ and Δ*aroA*-*Salmonella* infection, and to cover a broad
275 array of potential outcomes, we undertook protein quantification using the sensitive and accurate
276 (*i.e.,* dual antibodies per target) proximity extension assay (Olink Mouse Exploratory Panel) which
277 provided data for 92 different protein biomarkers (repeat-sample and dilution controls presented,

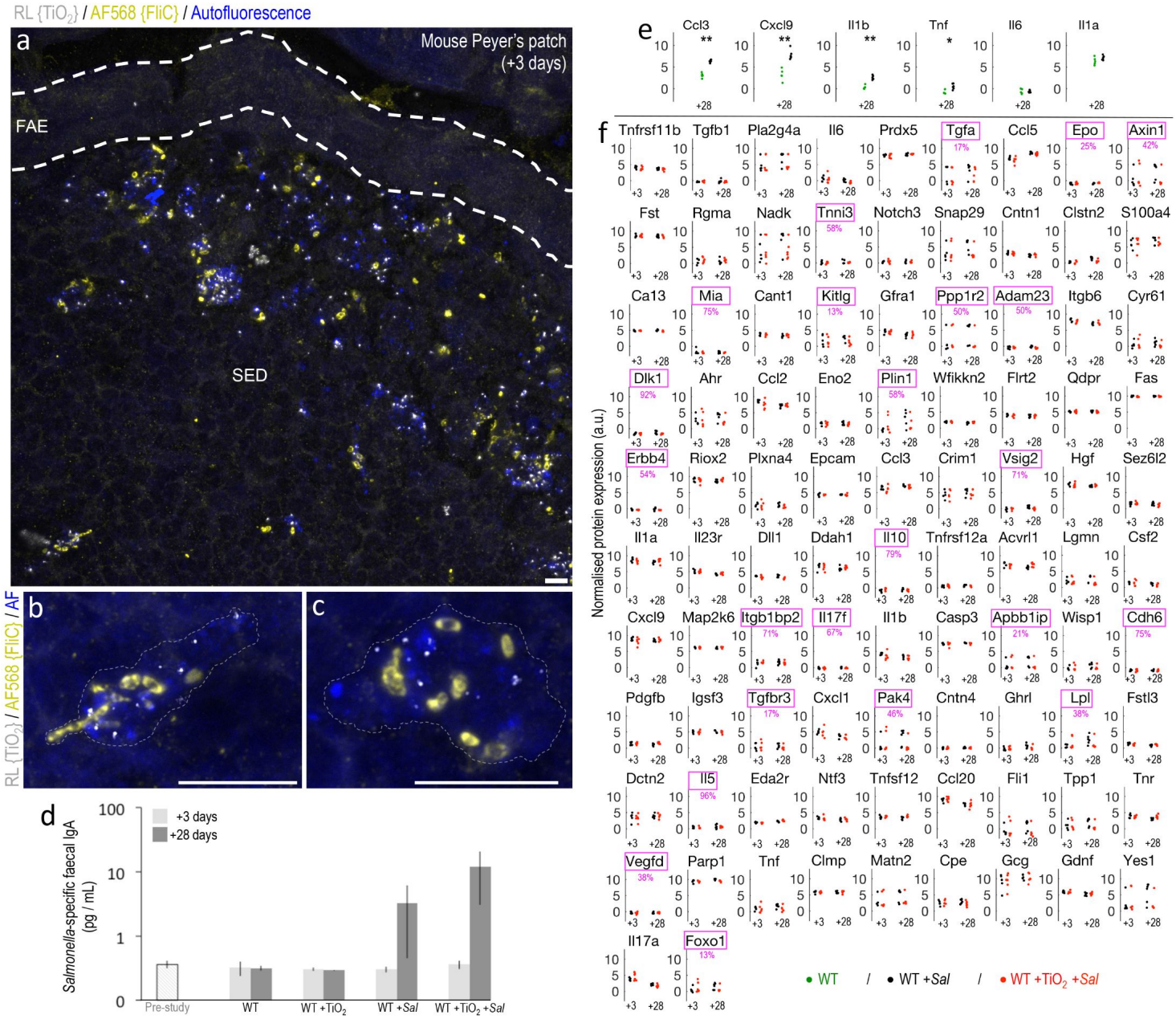


Figure 5 – fgTiO$_2$-*Salmonella* interactions. To enable studies of particle adjuvanticity and cell migration, in "Mouse Study 2", after the 16-week fgTiO$_2$ feeding period all animals were switched to a normal laboratory chow diet (*i.e.,* without fgTiO$_2$-supplementation) then half were orally inoculated with attenuated, Δ*aroA-Salmonella*. Tissues were harvested +3 or +28 days after infection. **a-c**, Confocal reflectance micrographs displayed as z-stack maximum projections showing immunofluorescent labelling for flagellin (FliC) to detect the Δ*aroA-Salmonella* in the Peyer's patch SED region. Marked accumulation of both fgTiO$_2$ and Δ*aroA-Salmonella* was observed in autofluorescent, phagocytic mononuclear cells at the +3 timepoint. **d**, Faecal ELISA studies confirmed a marked, *Salmonella*-specific IgA response at the +28 timepoint (bars represent medians with interquartile range error-bars, n = 3-6 samples per treatment group). **e/f**, Protein expression analyses of ileal tissue digests by proximity extension assay (OLINK mouse exploratory panel). **e**, At +28 days, comparison of ileal tissues taken from wild-type (WT) mice unexposed to fgTiO$_2$ but with (WT +*Sal*) or without (WT) Δ*aroA-Salmonella* infection confirmed significant increases in expression of key cytokines / chemokines associated with a Th1 immune response ($P \leq 0.026$, two-sided Wilcoxon rank-sum test, n = 5-6 tissues per group). **f**, Protein expression analyses of Peyer's patch-enriched ileal tissue digests in mice treated with Δ*aroA-Salmonella* alone (WT +*Sal*) or with fgTiO$_2$ and Δ*aroA-Salmonella* (WT +TiO$_2$ +*Sal*) at +3 or +28 timepoints. No significant differences were observed between the two groups ($P \geq 0.2$, two-sided Wilcoxon rank-sum test, n = 6 animals per group) at either timepoint demonstrating no interaction of fgTiO$_2$ and Δ*aroA-Salmonella* despite heavy loading into the same cells. For 23 of the 92 protein analytes (indicated with a magenta box), a proportion of the samples demonstrated expression levels below the analyte-specific limit of detection (similarly, this proportion is displayed on plots by the magenta percentage). Protein abbreviations are defined in **Supplementary Table 1**. *Scale bars:* ***a-c*** *= 10 μm.*

278 **Supplementary Figure 11**; full protein names shown in **Supplementary Table 1)**. Since
279 *Salmonella* Typhimurium induces a Th1 profile in the ileum[29] we first confirmed, using this
280 analytical approach, that associated cytokines/chemokines were enhanced in ileal tissue from mice
281 that had been challenged with Δ*aroA-Salmonella*. Of Th1 relevance, the proximity extension panel
282 detects IL-1 (α&β), TNFα, IL-6, CXCL9 and CCL3[29, 30] and four of these markers (CCL3, CXCL9,
283 IL1β and TNFα) showed significantly ($P \leq 0.026$, two-sided Wilcoxon rank-sum tests) increased
284 protein expression in the ileum of Δ*aroA-Salmonella*-exposed mice versus unexposed matched
285 controls at the +28 day time-point (**Figure 5e**). Next, using this same approach, we specifically
286 sought to determine whether Peyer's patch-enriched ileal tissue demonstrated a functional interaction
287 between $fgTiO_2$ and Δ*aroA-Salmonella*. Remarkably, for both +3 and +28 time-points, there was not
288 one protein analyte that significantly differed between the two groups (all $P \geq 0.2$, two-sided
289 Wilcoxon rank-sum tests) demonstrating no measurable adjuvant activity of $fgTiO_2$ on this enteric
290 infection in spite of both being heavily recruited to the same cells (**Figure 5f**).
291
292 Interestingly, whilst $fgTiO_2$ appeared to have no effect on cellular responses to Δ*aroA-Salmonella,*
293 an interaction was observed in terms of the spatial distribution of $fgTiO_2$ in the intestine. In the
294 absence of Δ*aroA-Salmonella* challenge we observed $fgTiO_2$ in the Peyer's patch SED region, as
295 well as some further towards the base of the patch, at both the +3 and +28 day time-points (**Figure**
296 **6a/b**) (just as was recorded in Mouse Study 1). There was, however, a measurable increase in the
297 number of $fgTiO_2$-loaded vesicles per cell (without change in the loading of the individual vesicles)
298 between the two timepoints (**Figure 6c-j**), suggesting a natural history of vesicular inheritance by
299 certain cells and, presumably, gradual development towards the well-reported basal pigment cells[15,
300 16, 20].
301

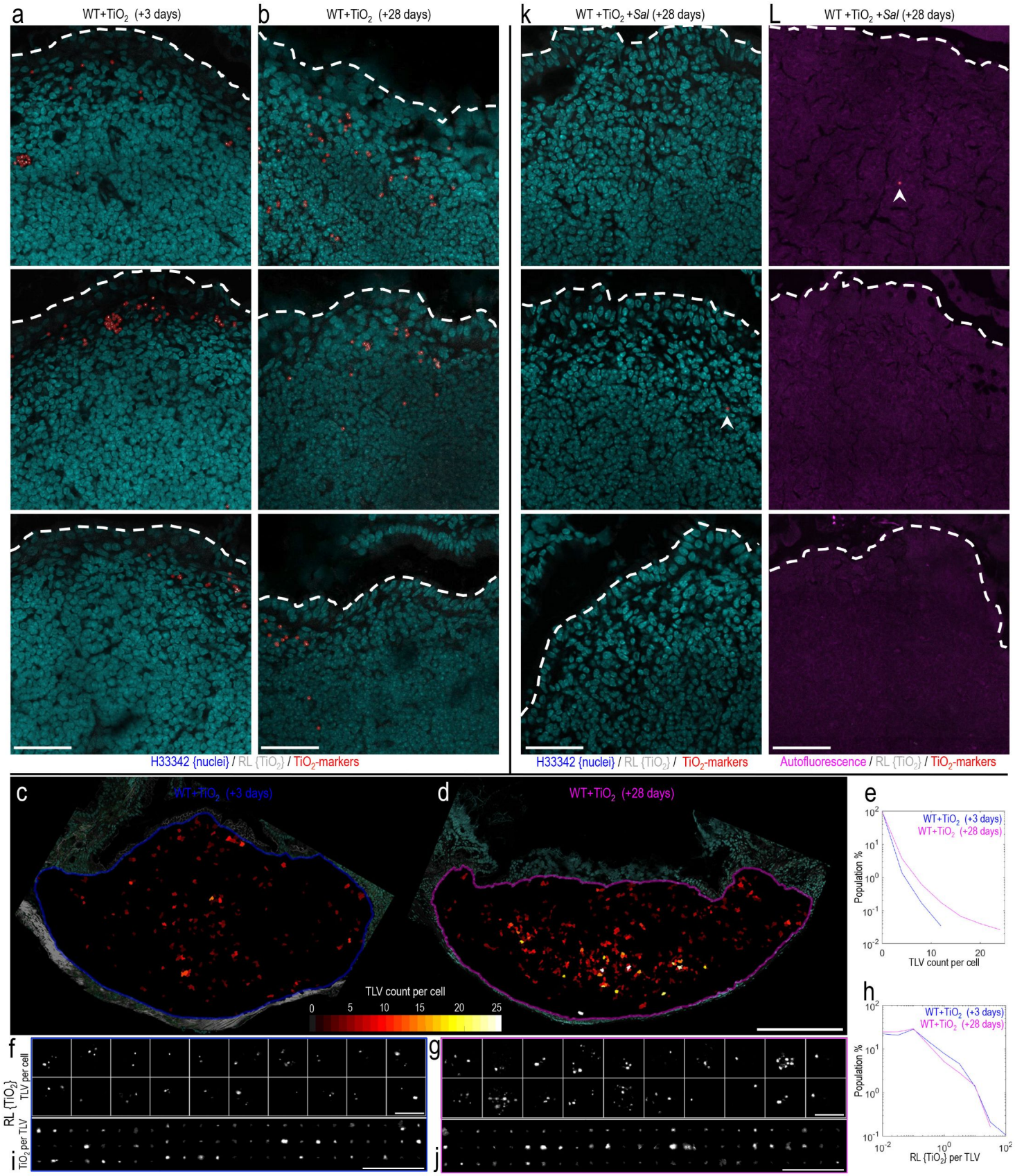


Figure 6 – Cellular dosimetry of fgTiO$_2$ across timepoints and *Salmonella*-induced migration. a/b, Confocal reflectance micrographs from Mouse Study 2 showing the subepithelial dome (SED) tissue region (n = 3 animals per group). After the 16-week fgTiO$_2$ feeding period, all animals were switched to normal laboratory chow diet (without fgTiO$_2$ supplementation) and half were orally dosed with Δ*aroA-Salmonella* before tissue harvest at +3 or +28-day timepoints. **a/b**, In the animals fed the fgTiO$_2$-supplemented diet without *Salmonella* exposure (WT +TiO$_2$), fgTiO$_2$-loaded cells were present in the SEDs at both +3 and +28 timepoints. **c-j**, To gain insights into cellular dosimetry changes across the two timepoints, quantitative image analysis of complete Peyer's patch transverse sections was used to precisely measure the number of reflectant foci per-cell (*i.e.,* the fgTiO$_2$-loaded vesicle (TLV) count per-cell) and the amount of reflectance per foci (*i.e.,* equivalent to the fgTiO$_2$ dose per-vesicle). **d/e**, At the +28 timepoint, the TLV count per-cell was markedly elevated with a new population of cells with TLV counts >13 forming in the mid-to-basal regions of the follicle. **f/g**, Randomly sampling and montaging the reflectance (RL) information in cells from each of the two timepoints visually displays this, showing (**g**) more heavily-loaded cells at the +28 timepoint. **h**, Moving from cell to vesicle information, in contrast, the amount of reflected light per individual foci remained near-identical across the two timepoints and this was again borne out visually when (**i/j**) individual TLVs were randomly montaged. Collectively, the results suggest vesicular inheritance by certain cells over time, providing a route to the formation of heavily-loaded pigment cells at the follicle base – as is well-described in humans (**Figure 1**). **k/L**, Challenge with Δ*aroA-Salmonella* (WT +TiO$_2$ +*Sal*) markedly changed this picture (n = 3 animals per group). At the +28 timepoint, SEDs were almost completely devoid of both fgTiO$_2$ and autofluorescence-positive cells. Out of six SEDs imaged, only two reflectant foci were detectable (indicated by arrows) showing marked migration of fgTiO$_2$ from the SED tissue compartment after Δ*aroA-Salmonella* exposure. *Scale bars:* ***a/b/k/L*** *= 50 μm,* ***c/d*** *= 250 μm,* ***f/g/i/j****, = 10 μm.*

302 However, challenge with the single oral dose of Δ*aroA*-*Salmonella* markedly changed this picture.
303 SEDs were almost entirely devoid of $fgTiO_2$ and autofluorescence-positive cells at +28 days (**Figure**
304 **6k/L**), while some cells of the regular villous lamina propria, as well as of the basal aspect of the
305 Peyer's patch, were now positive for $fgTiO_2$ (**Figure 7a-c**). Consistently, it has been reported that
306 oral danger signals, including *Salmonella*, deplete the Peyer's patch SED region of previously
307 absorbed plastic microparticles[31]. The pathway that then enables appearance of $fgTiO_2$ in sporadic
308 cells of the regular mucosa, as we show here, is not clear but (i) $fgTiO_2$ loading per cell was low
309 versus most Peyer's patch cells (**Figure 7b/c**) and (ii) many of these cells showed an autofluorescent
310 signature (**Figure 7d**). As such, LysoDC migration from the patch with re-homing to the gut mucosa
311 should be considered. These data clearly demonstrate that whilst $fgTiO_2$ is not in itself perceived as a
312 danger signal in the intestine, unlike Δ*aroA*-*Salmonella*, the two initially co-localise in Peyer's patch
313 cells that then migrate to the base of patch (likely LysoMacs). With the *Salmonella* danger signal
314 present, some also migrate to the intestinal lamina propria (likely LysoDCs) and, collectively, these
315 processes entirely recapitulate the human exposure scenario in a way that standard murine models do
316 not.
317
318 **DISCUSSION**
319 The human small intestine is the only known site of cellular accumulation of dietary microparticles,
320 as exemplified by $fgTiO_2$[16, 18, 20] (**Figure 1**). We show here that all features of this can be
321 qualitatively and quantitatively recapitulated in a murine model, but achieving that requires exposure
322 to human-relevant microbial 'danger signals'. Consistently, in this work, murine dosing with $fgTiO_2$
323 was undertaken in a manner that reflects real-life exposures (*i.e.,* via the diet at relatively low levels
324 for a long period of time). Our findings contrast with a prior study, which used bolus dosing via oral
325 gavage or surgical intervention in fasted animals, where direct $fgTiO_2$ uptake via the epithelium was
326 reported[32]. Future work may wish to consider the necessity of natural ingestion and concomitant

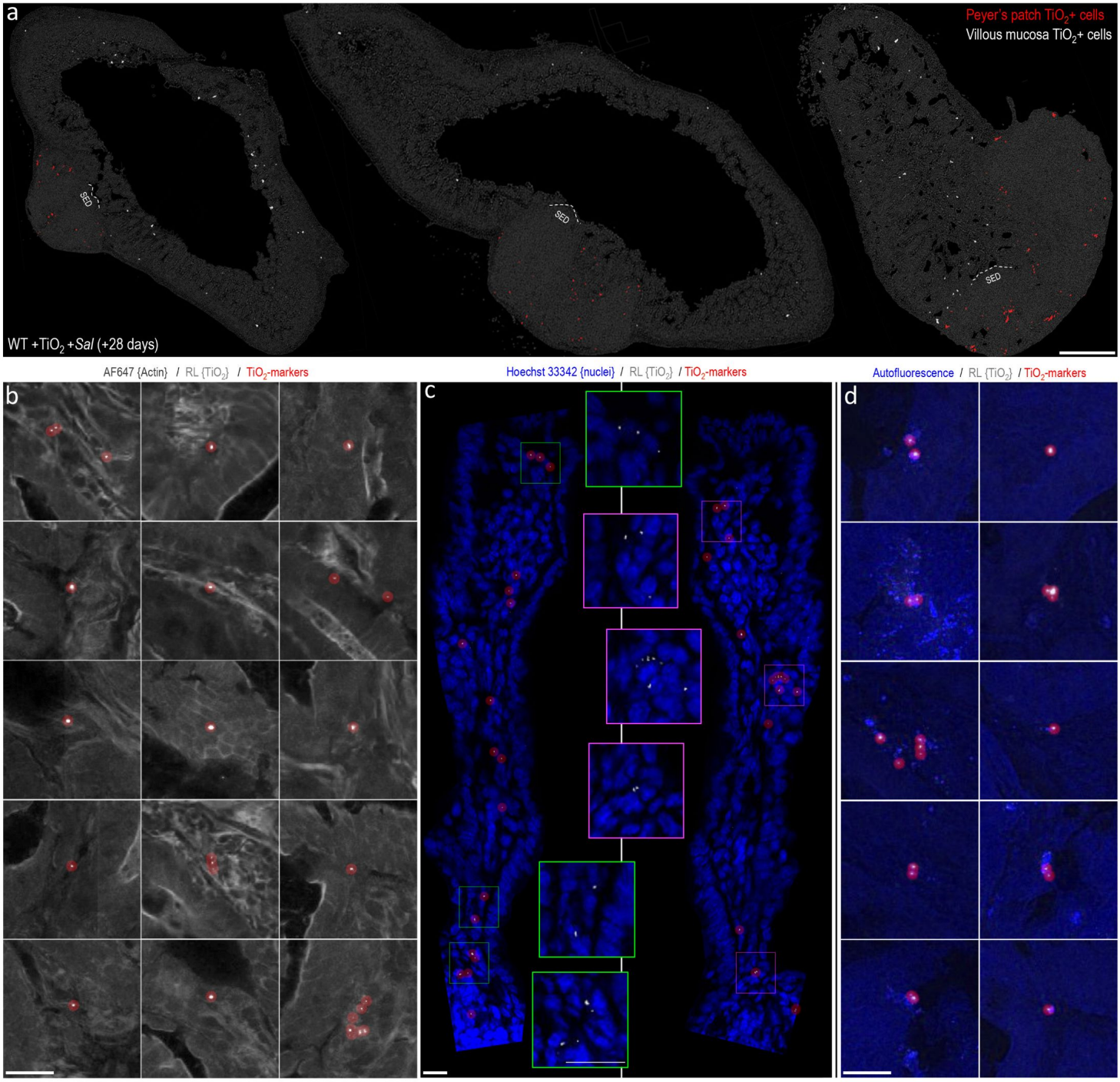


Figure 7 – Ileal distribution of fgTiO$_2$ following *Salmonella* challenge. a, Single-cell image analysis of tilescanned confocal reflectance images collected from transverse sections of mouse ileal tissue from the fgTiO$_2$-exposed, Δ*aroA*-*Salmonella*-infected treatment group (+28-day timepoint, n = 3 animals). fgTiO$_2$-positive cells in the Peyer's patches are displayed in red. As in **Figure 6**, the subepithelial dome (SED) regions were devoid of fgTiO$_2$ with the majority of the positive cells located basally around the follicle margins. In contrast to all previous data collected without Δ*aroA*-*Salmonella* exposure, fgTiO$_2$-positive cells were now also present in the villous mucosa (VM) (displayed in white). **b**, Montaging positive events from villous mucosa regions showed that particle loading was low compared to Peyer's patch cells with the majority containing single reflectant foci. **c**, High resolution imaging of optically cross-sectioned villi confirmed reflectant foci deep in the lamina propria in the same manner as observed in humans (**Figure 1c**). **d**, The majority of the fgTiO$_2$-positive events in the villous mucosa also displayed the same autofluorescence signature as was observed in the particle-recipient cells of the SED. *Scale bars:* ***a*** *= 500 µm;* ***b-d*** *= 20 µm.*

327 normal microbial exposures, versus non-physiological dosing in carefully controlled environments,
328 to recapitulate the human situation and address risk accurately for oral particle exposures.
329
330 Seminal early work from Frey and Mantis[33, 34] showed that the mucus-rich glycocalyx markedly
331 impairs model particle uptake by the regular villous epithelium, whereas the near-absence of these
332 features overlying Peyer's patches allows direct access of particulates to M-cells. These cells have an
333 extraordinary ability to engulf luminal particles and macromolecules, passing them through to
334 abutting cells at their basolateral membrane. We first confirm, unequivocally, that this uptake
335 mechanism holds true for $fgTiO_2$ and, next, demonstrate that specific SED immuno-competent
336 phagocytes (LysoMacs and LysoDCs) are the direct recipient cells for cargo from the M-cell. There
337 is then an on-going process of slow re-arrangement, typified by vesicular inheritance by some of the
338 cells and gradual particle-loaded-cell movement to the base of the patch. This migration (presumed
339 to occur in LysoMacs), which must ultimately result in mature pigment cell formation[16, 18], is greatly
340 accelerated by oral exposure to the bacterial pathogen *S.* Typhimurium. In addition, some particulate
341 migration out of the patch, to the villous lamina propria, also occurs (typically low-loaded cells,
342 presumed in LysoDCs). Again, building on formative work on the fate of reporter particles of
343 fluorescent plastic in the Peyer's patch[31], it is likely that this holds true for oral exposure to soluble
344 enterotoxin adjuvants as well as pathogenic bacteria (*i.e.,* multiple danger signals). Continuous
345 exposure to $fgTiO_2$, as happens for many human populations, would then re-load the apical (SED)
346 phagocytes until another danger signal event occurs. And, in this way, the natural tissue distribution
347 of cells loaded with dietary microparticles in the human intestine is established.
348
349 Thus, for the first time, we describe the natural history of persistent particle routing through – and
350 beyond – the Peyer's patch following oral exposure. This explains basal pigment cell accumulation
351 of multiple particle types, including titanium dioxide, silica and aluminosilicates, in human Peyer's

352 patches as well as the occurrence of sporadic particles elsewhere in cells of the small intestine[16, 18, 20].

353

354 Finally, we note that the particulate-accumulating population of apical SED cells are clearly (i)
355 immunocompetent (*i.e.,* non-quiescent) based upon their migration characteristics, expression of cell
356 surface immunoregulatory PD-L1 and their previously-described qualities in terms of antimicrobial
357 defence[17, 28, 35, 36] and (ii) capable of becoming heavily loaded with $fgTiO_2$. Indeed, the exquisite
358 particle selectivity for this tissue region means that the concentration gradient for area-corrected
359 reflectance signal was ~14-fold higher than for the Peyer's patch overall and, therefore, ~1,245 fold
360 higher than that of villous mucosa (**Supplementary Figure 12**). Given that the particulate-loaded
361 phagocytic cells make up only a minority of the SED[17, 23], then the cell concentration of $fgTiO_2$ is
362 *very* high (**Supplementary Video 1**).

363

364 We know of no other active particle-funnelling and specific cell-targeting strategy elsewhere in
365 mammalian biology so it is very likely that SED LysoMacs and LysoDCs provide the first and only
366 target to enable highly sensitive, per-cell risk assessment of $fgTiO_2$ and other persistent oral
367 particulates *in vivo*. By exemplification, we addressed the question of whether $fgTiO_2$ is an adjuvant,
368 or not, with same-cell invasive bacteria. The clear answer was 'no', at least in wild-type genotypes,
369 despite some *in vitro* evidence to the contrary[1]. To explain such differences it is worth noting that
370 cell culture studies fail to replicate *in vivo* fidelity in terms of rates of particle uptake, presence of
371 functional lymphatics and vasculature, cellular cross-talk, or cell migration prior to particle
372 saturation (cell gorging). Equally, it is probable that mammals have evolved with oral exposure to
373 other fine particles (*e.g.,* geologically-derived and soot) so effective compensatory mechanisms may
374 exist *in vivo* but not *in vitro*. Whatever the reasons, after decades of uncertainty, any question marks
375 hanging over the safety of persistent oral particles can now be precisely addressed, ranging from
376 genotoxicity through to differential responses of distinct genotypes.

377

378 **METHODS**

379 **Mouse Study 1**

380 The first mouse $fgTiO_2$ feeding study was approved by the Grasslands Animal Ethics Committee
381 (Palmerston North, New Zealand) in accordance with the New Zealand Animal Welfare Act 1999.
382 Food-grade, anatase TiO_2 particles ($fgTiO_2$) were purchased from Sensient Colors (St. Louis, MO,
383 USA). Physico-chemical characterisation of these particles in our previous work shows a particle
384 diameter distribution of ~ 50 – 300 nm, with a median diameter of ~ 130 nm[1]. As described and
385 extensively characterised previously[15], to enable successful oral delivery, the standard rodent diet
386 American Institute of Nutrition (AIN)-76A was supplemented with 0.000625%, 0.00625% or
387 0.0625% (w/w) $fgTiO_2$ (translating to approximately 1, 10 or 100 mg $fgTiO_2$ / kg body-weight / day
388 respectively[15]). These diets were prepared by Research Diets (New Brunswick, NJ, USA), with
389 straight AIN-76A without $fgTiO_2$ supplementation used as the 'negative control diet'. Six-week-old
390 mice (C57BL/6, 50:50 male:female) were housed conventionally with normal feed intake and
391 weight-gain confirmed bi-weekly[15]. After eighteen weeks feeding, mice were euthanised by CO_2
392 asphyxiation and cervical dislocation. The gastrointestinal tract was quickly removed and placed in
393 cold phosphate-buffered saline (PBS). Ileal tissues containing the most distal Peyer's patches (*i.e.,*
394 closest to the ileal/caecal junction) and adjacent caecal patches were excised and frozen on a cold,
395 stainless steel bar embedded in dry ice. Tissues were then transferred into cryomolds filled with pre-
396 cooled optimal cutting temperature compound (OCT) and transferred to – 80°C for storage.

397

398 **Mouse Study 2**

399 Animal experiments conducted at Cambridge were performed under the authority of Home Office
400 personal license number PIL IEDB62633, and project license number PPL PF86EABB1. Female
401 mice (C57BL/6) were purchased from Charles River Laboratories (UK). Upon arrival, mice were
402 housed conventionally and were monitored for signs of normal health, food ingestion and body-

403 weight gain throughout the study period. At the start of the study, thirty-six mice (six weeks old)
404 were randomly divided into two feeding groups. As before, one group received the standard rodent
405 diet American Institute of Nutrition (AIN)-76A supplemented with 0.0625% (w/w) $fgTiO_2$
406 (translating to approximately 100 mg $fgTiO_2$ / kg body-weight / day respectively[15]) whereas the
407 other received straight AIN-76A without $fgTiO_2$ supplementation (*i.e.,* the negative control diet).
408 After sixteen weeks of feeding, all mice were switched to the negative control diet, and twelve mice
409 from each diet-group were orally challenged with Δ*aroA*-deficient, *S*. Typhimurium strain SL3261[37]
410 (Δ*aroA-Salmonella)*. Mice were lightly anaesthetised with isofluorane then orally gavaged with 0.2
411 mL of inoculum prepared at 5.25×10^9 CFU/mL. Mice were then euthanised by CO_2 asphyxiation and
412 cervical dislocation at two time-points, either +3 or +28 days after *Salmonella* inoculation (*i.e.,* 16
413 weeks +3 days or 16 weeks +28 days). In this way, four treatment groups (n = 6 control diet, n = 12
414 control diet + *Salmonella*, n = 6 $fgTiO_2$ diet, n = 12 $fgTiO_2$ diet + *Salmonella*) were established,
415 with half of the animals analysed at each of the two time-points. Alongside, faecal samples were
416 collected at three time-points (pre-study, 16 weeks +3 days and 16 weeks +28 days) to enable
417 enzyme-linked immunosorbent assay (ELISA) studies for *Salmonella*-specific IgA.
418
419 **Human tissue collection**
420 Following informed consent, and after approval from the Regional Ethical Review Board,
421 Linköping, Sweden, surgical specimens containing Peyer's patches were collected from the neo-
422 terminal ileum from six patients with Crohn's disease or colonic cancer (3 males, 3 females;
423 resection date-range 2017-2018; median age 48 years; age-range 21 – 77 years). In all instances the
424 ileal tissue specimens formed the resection margins and were macroscopically normal. Peyer's
425 patches were microscopically identified as previously described[38]. The tissue specimens were fixed
426 with 4% PBS-buffered (pH 7.4) paraformaldehyde for 12 h at 4°C before immersion in 30% sucrose
427 and freezing in OCT (LAMB/OCT, Thermo Scientific) in cryomolds. For analysis, the OCT-

428 embedded tissue samples were rapidly shipped overnight to Cambridge on dry ice. Three
429 anonymised, formalin-fixed paraffin embedded (FFPE) tissue samples containing ileal Peyer's patch
430 lymphoid tissue were also analysed to confirm the presence of $fgTiO_2$ in the FFPE specimen-type.
431 Studies using human tissues at Cambridge were also approved by the UK NHS Health Research
432 Authority, North West – Greater Manchester East Research Ethics Committee, REC reference
433 18/NW/0690.
434
435 **Tissue sectioning and immunofluorescence labeling**
436 Frozen sections were cut at 20-micron thickness using a Leica CM 1900 cryostat, picked up on
437 Superfrost Plus slides (ThermoFisher, J1800AMNT) and rested at room temperature for 2 h prior to
438 labelling. FFPE sections were cut at 5-micron thickness, then dewaxed and rehydrated by baking at
439 60°C for 1 h, transferring through two changes of xylene, a reverse ethanol series (100%, 70%, 50%,
440 10%) followed by 1 minute rehydration in water. Sections were ringed with hydrophobic barrier pen
441 (Vector Laboratories, H-4000) and cryostat sections fixed by exposure to fresh 4%, 0.1M PBS-
442 buffered paraformaldehyde (pH 7.4) for 10 min. All subsequent steps were conducted under gentle
443 agitation on a rotating shaker. To facilitate antibody penetration, sections were permeabilised for 90
444 min using 0.3 % (vol/vol) Triton X-100 in 0.1 M PBS (pH 7.4). Block buffer (10% goat serum, 2%
445 bovine serum albumin, 25 mM glycine diluted in 25 mM Tris-buffered (pH 7.4) saline (TBS)) was
446 then added to all sections for 1 hour. Primary antibodies or concentration-matched isotype controls
447 were diluted in block-buffer and incubated at 4°C overnight. All subsequent steps took place at room
448 temperature. Sections were washed with three changes of block-buffer. Secondary antibodies (where
449 directly-conjugated primary antibodies were not used) were diluted 1:400 in block buffer and
450 incubated for 2 h. All antibody concentrations, fluorophore conjugations and manufacturer
451 information are provided in **Supplementary Table 2**. 500 nM phalloidin-AlexaFluor 647
452 (ThermoFisher, A22287) and 2 μg/mL Hoechst 33342 (ThermoFisher, H3570) were also included

453 alongside secondary antibodies to simultaneously label cell outlines (*i.e.,* cytoskeletal f-actin) and
454 cell nuclei (respectively). Sections were then washed once with TBS prior to mounting with #1.5
455 coverslips in Prolong Diamond (ThermoFisher, P36965).
456
457 **Confocal microscopy & imaging controls**
458 Fluorescence and reflectance image-data (2048x2048 pixels per tile) were collected using a diode-
459 controlled (retrofitted) Zeiss LSM780 confocal microscope equipped with plan-apochromat 63X/1.4
460 and 40X/1.3 oil-immersion objectives. Fluorescence and reflectance data were collected at the same
461 time during the same imaging run by sequential scanning with reflected (*i.e.,* back-scattered) light
462 from a 488 nm excitation laser collected in the range 485 – 491 nm to detect $fgTiO_2$[15]. Where
463 tilescanning was used, images were acquired with 10% edge overlap. Tiles were stitched using the
464 Zen Black 2011 SP2 software (Carl-Zeiss, UK) using the nuclei and actin channels for registration
465 and a 'strict' correlation threshold setting of 1.0. For all datasets under comparison, images were
466 obtained in a single run using identical settings. For the reflectance imaging, tissues from negative
467 control mice (*i.e.,* fed AIN-76 only) were used to determine the image acquisition settings and
468 thresholding for $fgTiO_2$ detection (strategy shown, **Supplementary Figure 1**). For
469 immunofluorescence work, supporting secondary-only and concentration-matched isotype antibody
470 controls in tissue-matched serial sections were included. For datasets collected as sets of single
471 images from the villous mucosa (VM), sub-epithelial dome (SED), germinal center (GC) and TCZ
472 (T-cell zone) regions of mouse Peyer's patches, landmarks (*e.g.,* villi / follicle-associated epithelium)
473 and T cell (CD3), B cell (B220) and mononuclear phagocyte (CD11c) immunofluorescence staining
474 in adjacent sections were used to inform imaging locations. When tilescanning was performed,
475 images were collected with 10% edge-overlap using focus mapping to facilitate accurate image
476 stitching whilst minimising acquisition time. To define the autofluorescence signature of the $fgTiO_2$-
477 recipient cells in the mouse Peyer's patches, lambda scans were performed using the LSM 780

478 microscope. Using unstained tissue sections, autofluorescence upon excitation from a 405 nm laser
479 was detected in ~ 10 nm intervals in the range 415-700 nm (spectra presented, **Supplementary**
480 **Figure 8**).
481
482 **Deconvolution**
483 Z-stacked image-data (pixel size (XYZ) 1024x1024x123; voxel size (XYZ) 26x26x100 nm) of
484 $fgTiO_2$-recipient cells was collected using a Zeiss LSM 780 confocal microscope equipped with a
485 63X/1.4 plan-apochromat oil immersion objective using murine tissue sections embedded in Prolong
486 Glass (P36984, ThermoFisher) anti-fade reagent (refractive index ~ 1.5). Cell autofluorescence was
487 excited using the 405 nm laser. The image-stacks were registered to correct spatial drift using
488 StackReg[39]. The Born and Wolf scalar-based diffraction model[40] was then used to estimate a
489 theoretical point spread function assuming a refractive index of 1.51 and a autofluorescence emission
490 maximum of 495 nm (measured, **Supplementary Figure 8**). The registered Z-stack was then
491 deconvolved using the Richardson-Lucy total variation algorithm (75 iterations, regularisation 1e-12)
492 within the open-source DeconvolutionLab2[41] software.
493
494 **$fgTiO_2$ detection using reflected light and circle-marker placement**
495 To enable $fgTiO_2$ detection in biological tissue sections, reflected light and immunofluorescence
496 information was collected at the same time using a Zeiss LSM780 confocal microscope. The
497 reflectance images were thresholded to isolate the punctate, reflectant foci caused by the $fgTiO_2$
498 particles from the background light scatter caused by the biological tissue. As the background level
499 was similar in both mouse and human specimens (shown, **Supplementary Figure 4**) the same
500 threshold was used for both mouse and human images, and was determined as the level required to
501 remove all signal in the reflectance channel for images collected for tissue sections taken from the
502 negative control mice (*i.e.,* fed the AIN-76 diet only, so no $fgTiO_2$ present). This approach was then

503 further validated using correlative scanning electron microscopy and energy dispersive X-ray
504 analyses to confirm that the signal remaining after thresholding contained the expected X-ray signal
505 for titanium (strategy shown, **Supplementary Figure 1**). Given the small size of the reflectant foci
506 caused by $fgTiO_2$, a translucent circle-marker was placed on each pixel above the threshold using the
507 'insertShape' function in MATLAB R2021a (MathWorks) to aid visualisation given the relatively
508 small size of display in the Figure panels[15]. Examples of the raw signal and subsequent marker
509 placements are shown for mouse and humans in **Supplementary Figure 5**. Example data and code
510 demonstrating thresholding and circle marker placement are available at the BioStudies database
511 under accession number S-BSST875.
512
513 **Correlative confocal-electron microscopy and energy dispersive X-ray analyses**
514 Once $fgTiO_2$ detection by confocal reflectance microscopy was completed, Prolong-mounted
515 coverslips were removed from the tissue sections by overnight incubation in PBS at 37°C. Slides
516 were air dried at room temperature for 24 h, cut down and mounted on aluminum SEM stubs using
517 colloidal graphite and coated with a thin (~ 15 nm) layer of amorphous carbon (evaporative coater).
518 Tissue sections were then loaded into a Helios G4 CX Dual Beam high-resolution, monochromated,
519 field emission gun, scanning electron microscope (SEM) equipped with a precise focused ion beam
520 (FIB) and in-lens secondary electron and circular backscattered detectors (FEI/ThermoFisher).
521 Secondary and backscattered electron images were collected at 3 – 5 kV as tilescans for the complete
522 tissue surface, permitting manual overlay onto the previously-collected confocal tilescans. To enable
523 correlative confocal-SEM imaging, the 'outline' of the Peyer's patch – particularly the follicle
524 associated epithelium and basal muscularis layer – were manually registered together using image
525 overlay, enabling triangulation to target specific regions containing reflectance-positive cells. The
526 correlative confocal-to-EDX analyses of the human tissues were then carried out directly in the
527 SEM, using a 150 mm^2 silicon drift detector and AZTEC software (Oxford Instruments). For the

correlative scanning transmission electron microscopy (STEM) used to confirm single-particle detection by reflectance microscopy, thin lamella of the tissue sample was prepared in the SEM via the *in situ* lift-out method. Once sites-of-interest had been identified, 500 nm of electron beam platinum (Pt) was deposited (at 5 kV, 6.4 nA for the electron source) to the surface of the target area. This was followed by a second Pt layer (1 μm) using the FIB (at 30 kV, 80 pA for the liquid gallium (Ga) ion source). A bulk lamella was initially cut using the FIB (at 30 kV, 9 nA), before the final cut-out was performed (at 30 kV, 80 pA). The lamella was attached, using ion beam Pt, to a copper FIB lift-out grid (Omniprobe, USA) mounted within the SEM chamber (*i.e., in situ*). Final thinning and polishing of the lamella to electron translucency was performed with a gentle polish/clean using the ion beam (5 kV, 41 pA). A lamella was then transferred into a Titan^3 Themis G2 300 kV S/TEM equipped with an S-TWIN objective lens, monochromator, HAADF detector and a Super-X 4-detector EDX system in a double-tilt specimen holder (FEI/ThermoFisher). Transmission electron microscopy (TEM) images and on-zone diffraction patterns were recorded using a OneView CMOS camera (Gatan) and STEM-EDX elemental maps were recorded and processed using the Velox software (FEI/ThermoFisher).

Cell segmentation of intestinal tissue images

In previous work, we carefully developed and validated staining and image analysis methods enabling extraction of single-cell data from confocal microscopy images of gastrointestinal tissues[42]. Based on this framework, three cell segmentation pipelines were used to accommodate different tissue types and differing availabilities of training data. To confirm the reliability of our previously-published pipelines with the new image-data presented here, the accuracy of the outputs from all three cell segmentation pipelines were assessed relative to manually-segmented cells by Jaccard index (presented, **Supplementary Figure 2)**.

553 **Cell segmentation: mouse villous mucosa images**

554 Villous image-fields were directly segmented into cell-objects using the freely-available CellProfiler
555 software[43]. Using the Hoechst 33342 fluorescence information, nuclei were first segmented as
556 primary objects. These were then used as seeds during a marker-controlled watershed process to
557 segment each cell's outline using fluorescence information from the actin channel. The process is
558 schematically demonstrated in **Supplementary Figure 13** with the complete CellProfiler pipeline
559 shown in **Supplementary Note 1**. Test images and the CellProfiler pipeline are also available for
560 download at the BioStudies database under accession number S-BSST875.

561

562 **Cell segmentation: mouse lymphoid tissue images**

563 Lymphoid tissues (*e.g.,* Peyer's patches and caecal patches) exhibit extremely high cell densities
564 leading to poor cell segmentation accuracies using the simple, marker-controlled watershed approach
565 used for the villous mucosa images[42]. Pixel-classification machine learning can be used to alleviate
566 this problem through the generation of probability images that more precisely delineate each cell's
567 boundary[42]. Here, in mouse lymphoid tissues, these probability images were provided using a 2-D U-
568 Net fully convolutional neural network[44] deployed in MATLAB R2021a (MathWorks, MA). The
569 network has a 256x256x2 (x, y, channels) input layer and uses the nuclear and actin fluorescence
570 information to predict the probability that each pixel belongs to either 'cell outline', 'intracellular
571 environment' or 'background' classifications. The training data consisted of twelve lymphoid tissues
572 images (each containing 2138 x 1900 annotated pixels covering ~16,000 cells prepared by an
573 experienced cell biologist). The image-data flows through a four-layer contracting convolutional
574 path before complete up-convolutional expansion, yielding probability images that exactly match the
575 input-image dimensions. The network was trained for 50 epochs using a batch-size of 12 for 1,200
576 iterations/epoch. Patches were shuffled every epoch and augmented by simple, random x / y
577 reflection and rotation. Model training was optimised under stochastic gradient descent using cross-

entropy loss. The initial learning rate was 0.05, dropping every 10 epochs by 0.1 under momentum 0.9 and L2 regularisation $1x10^{-4}$. The resultant 'Cell Outline' probability images were then loaded into the CellProfiler pipeline alongside immunofluorescence and reflectance information collected by the confocal microscope and were used to enable segmentation of individual cells via an 'IdentifyPrimaryObjects module'. This process is schematically demonstrated in **Supplementary Figure 14.** The complete CellProfiler image analysis pipeline is presented in **Supplementary Note 2**. All U-Net code, the trained network with test-data and the complete CellProfiler pipeline are available for download from the BioStudies database under accession number S-BSST875.

Cell / TLV segmentation: correlative mouse-human dosimetry

For the correlative analyses investigating the relative amounts of $fgTiO_2$ delivered to individual SED cells in mice versus in normally-exposed humans, frozen ileal tissue sections containing Peyer's patches were collected at random from six normally-exposed humans, and six mice fed the AIN-67 diet supplemented with 0.0625% (w/w) $fgTiO_2$. In every instance, care was taken to collect tissue sections with clear follicle-associated epithelium (*i.e.,* without any overlying villi) to ensure maintenance of similar histological positioning near the 'center of the dome' of each Peyer's patch. Reflectance confocal images of the murine and human tissues were then acquired in a single run under identical settings. After acquisition, the image-fields per specimen were manually laid out in order of lowest-to-highest $fgTiO_2$ accumulation to visually present the spectrum of (wide) variation in $fgTiO_2$ cellular loading observed across samples in both the mouse and human tissue specimens. This was followed by quantitative image analysis to determine the thresholded reflectance per unit tissue area; then the integrated intensity of the threshold reflectance information per-cell and per-vesicle objects (method exemplified, **Supplementary Figure 3**). To extract the single-cell and single-vesicle data, a CellProfiler pipeline was used. To tackle cell segmentation in lymphoid tissues with high cell densities we have shown in previous work[42] that pixel-classification machine learning

603 can be used to produce probability images that more precisely delineate each cell's boundary
604 improving segmentation accuracies when compared to using the raw fluorescence information.
605 Unlike in mouse lymphoid tissues (method described above), because large amounts of manually-
606 annotated training data for the human lymphoid images was not available, these probability images
607 were created by sparse annotation pixel-classification machine learning in the ILASTIK software[45].
608 Two ILASTIK pixel-classification projects were created (one for the human images, one for the
609 mouse). Pixel annotations representing 'intracellular environment', 'cell outlines' and 'other'
610 classifications were made on the fluorescence channel describing the cell outlines (*i.e.,* the actin
611 channel) (method shown schematically, **Supplementary Figure 15**). The ILASTIK software then
612 generated probability images representing the probability of each pixel belonging to each
613 classification. The resultant 'Cell Outline' probability images were loaded into the CellProfiler
614 pipeline alongside the fluorescence and reflectance information captured by the confocal microscope
615 and were used to segment individual cells via an 'IdentifyPrimaryObjects' module. As part of this
616 pipeline, the reflectant foci representing $fgTiO_2$ events were also directly-segmented from the
617 thresholded reflectance images into '$fgTiO_2$-loaded vesicle' (TLV) objects using a second,
618 'IdentifyPrimaryObjects' module (method schematically demonstrated, **Supplementary Figure 16**).
619 The complete image analysis pipeline is presented in **Supplementary Note 3**. Test images and the
620 complete CellProfiler pipeline are available for download from the BioStudies database under
621 accession number S-BSST875. The accuracy of the segmented-cell outputs were checked by Jaccard
622 index relative to manually-segmented cells (presented, **Supplementary Figure 2)**. The accuracy of
623 TLV segmentation from the raw reflectance information is explored in both mouse and human
624 images in **Supplementary Figure 5.**
625
626 **Cell / TLV feature extraction**

627 As described in previous work[42], raw immunofluorescence data were pre-processed by manual
628 thresholding at the level required to remove ≥ 95% of fluorescence in tissue-matched, secondary
629 antibody-only control images. Alongside, the reflectance data were carefully thresholded at the level
630 required to remove all signal from the images collected from the negative control group mice (*e.g.,*
631 fed the AIN-67 diet alone; strategy shown, **Supplementary Figure 1**). Thresholded reflectance and
632 fluorescence intensity information per cell or TLV object, alongside size and shape features were
633 then measured for both the immunofluorescence and reflectance channels using the
634 'MeasureObjectSizeShape' and 'MeasureObjectIntensity' modules in CellProfiler. The number of
635 TLV objects per cell was measured using a 'RelateObjects' module. Data were preprocessed by
636 discarding objects outside of the 5th and 95th percentiles by size prior to analysis as is recommended
637 best-practice[46].
638
639 **Cell segmentation: block processing tilescans**
640 To avoid memory limitations, when necessary, tilescanned images were cut into smaller pieces, run
641 through one of the CellProfiler pipelines before reassembling the object segmentation masks and
642 assigning global cell position coordinates to the extracted object features. This was achieved using
643 two MATLAB functions developed in our previous work[42]. The first 'TilescanToCellProfiler', reads
644 stitched images in most microscopy formats and cuts them into manageable tiles with edge-overlap
645 for processing. The second, 'CellProfilerToTilescan' reassembles the object masks, removes 'double
646 hits' on overlap edges and assigns global position coordinates values to the extracted features.
647 Example images and MATLAB code demonstrating these functions is available for download at the
648 BioStudies database under accession number S-BSST875.
649
650 **Cell segmentation: accuracy assessment by Jaccard index**

651 The Jaccard index (intersection over union) approach was used to check the accuracy of the cell
652 segmentation results used in this work. This was achieved by comparing pixel positions inside the
653 automatically-segmented cell objects against those obtained by manual annotations performed by an
654 experience cell biologist. The Jaccard index was calculated as:

655 $$J(A,M) = \frac{|A \cap M|}{|A \cup M|} = \frac{|A \cap M|}{|A| + |M| - |A \cap M|} \qquad \text{(Equation 1)}$$

656 Where J is the Jaccard distance for two sets of pixel positions for the automated (A) and manual (M)
657 segmentations, respectively. Scores of zero represent no overlap (false negatives) whereas scores of
658 1 represent exact pixel-for-pixel intersection. In this way, it is acknowledged that this approach is a
659 relatively harsh success measure, and that scores of ~ 0.7 indicate a good segmentation result[44]. This
660 is partially due to the inevitable inaccuracies that are present – even in the manually-annotated data
661 (*e.g.,* due to outline smoothing, ambiguity in determining the precise position of each cell's boundary
662 from the fluorescent staining information, and the available image resolution *etc*.).
663

664 **Cell segmentation: cell mapping visualisations**

665 The cell map visualisations displaying the delivered cellular doses of $fgTiO_2$ or TLV counts per-cell
666 were produced using cell feature information extracted by the CellProfiler
667 'MeasureObjectIntensities' and 'RelateObjects' modules alongside the object segmentation masks
668 outputted by each pipeline. Using MATLAB scripts, object feature data were binned into categories
669 (*e.g.,* 'low, 'intermediate' and 'high' $fgTiO_2$-load based on the integrated intensity of the thresholded
670 reflectance signal per-cell). Using the segmentation masks, individual cell-objects were then colour-
671 coded according to these categories to provide a 'tissue map' visualisation. Example data and
672 MATLAB code demonstrating the creation of these visualisations are available for download from
673 the BioStudies database under accession number S-BSST875.
674

675 **Statistical analysis of image-based data**

676 Integrated intensity per object (*i.e.,* per cell or NLV) distributions were compared using two-sided
677 Wilcoxon rank-sum analysis. This non-parametric approach was chosen for compatibility with
678 unpaired samples with unequal group sizes[42]. The approach tests the null hypothesis that the
679 distributions under comparison might reasonably be drawn from one continuous distribution with
680 equal medians ($P > 0.05$) versus the alternate hypothesis that the distributions are distinct ($P \leq 0.05$).
681 Pairwise comparisons of cell counts were first checked for distribution normality and variance
682 homogeneity using Shapiro-Wilk and Barlett tests, respectively. Where these tests were passed (P
683 >0.05), groups were compared using a two-sided, unpaired samples T-test assuming equal variance.
684

685 **Olink® proximity extension assay**

686 Using a cold safety razorblade, the frozen mouse ileal tissues were trimmed to isolate the tissue
687 region containing Peyer's patches and the mass of each sample was recorded. Samples were stored in
688 Kimble pellet pestle tubes (DWK Life Sciences, K-749520-0000) at -70°C until further processing.
689 Tissues were lysed using the BioPlex Cell Lysis Kit (BioRad, 171-304011). 500 µL of Cell Wash
690 Buffer per sample was used for rinsing the tissues. Cell Lysis Buffer was prepared by adding Factors
691 1 and 2 as per the manufacturer's instructions, as well as Halt Protease Inhibitor Cocktail
692 (ThermoFisher, 78437; 10 µL of cocktail per 1 mL of buffer). Following a rinse, tissues were
693 homogenised by adding 500 µL of ice-cold Cell Lysis Buffer and hand grinding on ice using
694 disposable pestles (DWK Life Sciences, 749520-0000; 20 strokes). Homogenised samples were
695 frozen at -70°C for at least 4 hours, thawed on ice, sonicated in an Ultrawave U300 ultrasonic bath
696 (44 kHz, 35 W) on ice for 40 seconds and centrifuged for 5 min at 6,000 x g. Supernatant was
697 collected into 2 mL cryogenic vials (Corning, 430659) and frozen at -70°C until quantification.
698 Quantification was performed using the DC Protein Quantification Kit I (BioRad, 5000111) as per
699 the manufacturer's instructions (Microplate Assay Protocol). Plates (Greiner Bio-One, 650185) were
700 read using FLUOstar Omega microplate reader at 750 nm and protein concentrations were calculated

701 by the Omega's MARS Data Analysis software using linear regression fit. All samples were
702 normalised to 0.77 mg/mL (+/- 0.12) by dilution with Cell Lysis Buffer and re-quantified to confirm
703 protein concentration. A microplate was prepared for the proximity extension assay by transferring
704 40 µL aliquots of all samples, negative controls (Cell Lysis Buffer), duplicates of 6 samples, and
705 dilutions (1:4, 1:8 and 1:16 v/v) of two samples into a 96-well skirted plate (ThermoFisher, AB0800)
706 in a randomised manner (controls presented, **Supplementary Figure 11**). The plate was sealed
707 (ThermoFisher, 4306311) and stored at -70°C until shipment on dry ice to Olink (Uppsala, Sweden)
708 for analysis using the Target 96 Mouse Exploratory panel (www.olink.com/mouse-exploratory). All
709 protein names and abbreviations are listed in **Supplementary Table 1**. Statistical analysis was
710 performed using the Olink Analyze 3.6.0 R-package (https://CRAN.R-
711 project.org/package=OlinkAnalyze). Treatment groups were compared using a two-sample Mann-
712 Whitney U test with correction for multiple testing using the Benjamini-Hochberg method. The
713 proximity extension assay data are available for download at the BioStudies database under
714 accession number S-BSST875.
715
716 **Enzyme-linked immunosorbent assay for *Salmonella*-specific IgA**
717 During Mouse Study 2, faecal pellets were collected pre-study and at the 16-week +3 day and 16-
718 week +28 day time-points. Samples were weighed and diluted 1:10 (w/v) in 10% PBS containing 1
719 mM EDTA. The resulting suspension was vortexed for 15 min to facilitate liquefaction then
720 centrifuged at 400 x g at 4°C for 5 min. The supernatant was aspirated and centrifuged for a second
721 time at 12,000 x g for 10 min at 4°C. *Salmonella*-specific IgA quantification was then carried by
722 sandwich ELISA method. Plates were coated with 50 µL lipopolysaccharide (LPS), (Sigma, L2262)
723 diluted in Reggiardo's buffer (0.05 M glycine, 0.1 M NaCl, 1 mM EDTA, 0.05 M NaF and 0.1%
724 sodium deoxycholate). Plates were then sealed and incubated overnight at 37°C. Next day, the plates
725 were washed 3 times with PBS-tween and blotted dry. Blocking buffer from the whole IgA kit

726 (Affymetrix, 88-50450) was added to each well for 1 hr at 37°C. The faecal supernatant was then
727 diluted in kit buffer A (Affymetrix, 88-50450) and 50 µL of diluted sample was added to each well.
728 After three hours of incubation, the plates were washed three times with PBS-tween before addition
729 of 50 µL horseradish peroxidase-conjugated IgA. Three more washes were carried out before the
730 addition of 50 µL tetramethylbenzidine substrate for 10 min. This was followed by 50 µL of stop
731 solution prior to absorption reading at 492 nm on a plate reader. All outputs were calibrated using a
732 standard curve and corrected for dilution.
733

734 **ONLINE CONTENT**

735 Data and code are available at the BioStudies database (https://www.ebi.ac.uk/biostudies/) under
736 accession number S-BSST875.
737

738 **DATA AVAILABILITY**

739 Data supporting the presented analyses are available for download from the BioStudies database
740 (https://www.ebi.ac.uk/biostudies/) under accession number S-BSST875. Source data are provided
741 with this submission.
742

743 **CODE AVAILABILITY**

744 MATLAB code (using Deep Learning, Image Processing and Computer Vision toolboxes) and
745 CellProfiler pipelines enabling the presented image analysis workflows are downloadable from the
746 BioStudies database (http://www.ebi.ac.uk/biostudies) under accession number S-BSST875.
747

REFERENCES

1. Riedle S, Pele LC, Otter DE, Hewitt RE, Singh H, Roy NC*, et al.* Pro-inflammatory adjuvant properties of pigment-grade titanium dioxide particles are augmented by a genotype that potentiates interleukin 1β processing. *Part. Fibre Toxicol.* 2017, **14**(1)**:** 51.

2. Ashwood P, Thompson RP, Powell JJ. Fine particles that adsorb lipopolysaccharide via bridging calcium cations may mimic bacterial pathogenicity towards cells. *Exp. Biol. Med.* 2007, **232**(1)**:** 107-117.

3. Grand View Research. Titanium dioxide market size, share and trends analysis report by grade (anatase, rutile), by production process (sulfate, chloride), by application (paints and coatings, plastics), by region, and segment forecasts, 2021 - 2028. https://www.grandviewresearch.com/industry-analysis/titanium-dioxide-industry - accessed December 3rd, 2022.

4. Safety assessment of titanium dioxide (E171) as a food additive. *EFSA J.* 2021, **19**(5)**:** e06585.

5. European Medicines Agency. Final feedback from European Medicines Agency (EMA) to the EU Commission request to evaluate the impact of the removal of titanium dioxide from the list of authorised food additives on medicinal products. https://www.ema.europa.eu/en/documents/report/final-feedback-european-medicine-agency-ema-eu-commission-request-evaluate-impact-removal-titanium_en.pdf - accessed December 3rd, 2022.

6. Bekiempis V. Taste the toxin? Skittles ‘unfit for human consumption’, lawsuit claims. https://www.theguardian.com/business/2022/jul/15/skittles-mars-lawsuit-titanium-dioxide-toxin - accessed December 3rd, 2022.

7. Food Standards Australia and New Zealand. Review of titanium dioxide as a food additive. https://www.foodstandards.gov.au/consumer/foodtech/Pages/Review-of-titanium-dioxide-as-a-food-additive.aspx - accessed December 3rd, 2022.

8. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. Interim position paper on titanium dioxide. https://cot.food.gov.uk/sites/default/files/2022-01/TiO2%20COT%20Interim%20position%20paper.pdf - accessed December 3rd, 2022.

9. Health Canada. Titanium dioxide (TiO2) as a food additive: current science report. https://www.canada.ca/en/health-canada/services/food-nutrition/reports-publications/titanium-dioxide-food-additive-science-report.html - accessed December 3rd, 2022.

10. Hewitt RE, Chappell HF, Powell JJ. Small and dangerous? Potential toxicity mechanisms of common exposure particles and nanoparticles. *Curr. Opin. Toxicol.* 2020, **19:** 93-98.

796 11. Kirkland D, Aardema MJ, Battersby RV, Beevers C, Burnett K, Burzlaff A*, et al.* A weight
797 of evidence review of the genotoxicity of titanium dioxide (TiO_2). *Regul. Toxicol.*
798 *Pharmacol.* 2022, **136:** 105263.
799
800 12. da Silva AB, Miniter M, Thom W, Hewitt RE, Wills J, Jugdaohsingh R*, et al.*
801 Gastrointestinal absorption and toxicity of nanoparticles and microparticles: myth, reality and
802 pitfalls explored through titanium dioxide. *Curr. Opin. Toxicol.* 2020, **19:** 112-120.
803
804 13. Heringa MB, Peters RJB, Bleys R, van der Lee MK, Tromp PC, van Kesteren PCE*, et al.*
805 Detection of titanium particles in human liver and spleen and possible health implications.
806 *Part. Fibre Toxicol.* 2018, **15**(1)**:** 15.
807
808 14. Kreyling WG, Holzwarth U, Schleh C, Kozempel J, Wenk A, Haberl N*, et al.* Quantitative
809 biokinetics of titanium dioxide nanoparticles after oral application in rats: Part 2.
810 *Nanotoxicology* 2017, **11**(4)**:** 443-453.
811
812 15. Riedle S, Wills JW, Miniter M, Otter DE, Singh H, Brown AP*, et al.* A murine oral-exposure
813 model for nano- and micro-particulates: demonstrating human relevance with food-grade
814 titanium dioxide. *Small* 2020, **16**(21)**:** e2000486.
815
816 16. Powell JJ, Ainley CC, Harvey RS, Mason IM, Kendall MD, Sankey EA*, et al.*
817 Characterisation of inorganic microparticles in pigment cells of human gut associated
818 lymphoid tissue. *Gut* 1996, **38**(3)**:** 390-395.
819

820 17. Bonnardel J, Da Silva C, Henri S, Tamoutounour S, Chasson L, Montañana-Sanchis F*, et al.*
821 Innate and adaptive immune functions of peyer's patch monocyte-derived cells. *Cell Rep.*
822 2015, **11**(5)**:** 770-784.
823
824 18. Powell JJ, Faria N, Thomas-McKay E, Pele LC. Origin and fate of dietary nanoparticles and
825 microparticles in the gastrointestinal tract. *J. Autoimmun.* 2010, **34**(3)**:** J226-233.
826
827 19. Baranska A, Shawket A, Jouve M, Baratin M, Malosse C, Voluzan O*, et al.* Unveiling skin
828 macrophage dynamics explains both tattoo persistence and strenuous removal. *J. Exp. Med.*
829 2018, **215**(4)**:** 1115-1133.
830
831 20. Shepherd NA, Crocker PR, Smith AP, Levison DA. Exogenous pigment in Peyer's patches.
832 *Hum. Pathol.* 1987, **18**(1)**:** 50-54.
833
834 21. Kimura S, Yamakami-Kimura M, Obata Y, Hase K, Kitamura H, Ohno H*, et al.*
835 Visualization of the entire differentiation process of murine M cells: suppression of their
836 maturation in cecal patches. *Mucosal Immunol.* 2015, **8**(3)**:** 650-660.
837
838 22. Donaldson DS, Else KJ, Mabbott NA. The gut-associated lymphoid tissues in the small
839 intestine, not the large intestine, play a major role in oral prion disease pathogenesis. *J. Virol.*
840 2015, **89**(18)**:** 9532-9547.
841
842 23. Wagner C, Bonnardel J, Da Silva C, Martens L, Gorvel JP, Lelouard H. Some news from the
843 unknown soldier, the Peyer's patch macrophage. *Cell. Immunol.* 2018, **330:** 159-167.
844

24. Powell JJ, Thomas-McKay E, Thoree V, Robertson J, Hewitt RE, Skepper JN*, et al.* An endogenous nanomineral chaperones luminal antigen and peptidoglycan to intestinal immune cells. *Nat. Nanotechnol.* 2015, **10**(4)**:** 361-369.

25. Robertson J, Haas CT, Pele LC, Monie TP, Charalambos C, Parkes M*, et al.* Intestinal APCs of the endogenous nanomineral pathway fail to express PD-L1 in Crohn's disease. *Sci. Rep.* 2016, **6:** 26747.

26. Centers for Disease Control and Prevention. *Salmonella* and food. https://www.cdc.gov/foodsafety/communication/salmonella-food.html – accessed March 30th 2024.

27. Monack DM, Hersh D, Ghori N, Bouley D, Zychlinsky A, Falkow S. Salmonella exploits caspase-1 to colonize Peyer's patches in a murine typhoid model. *J. Exp. Med.* 2000, **192**(2)**:** 249-258.

28. Da Silva C, Wagner C, Bonnardel J, Gorvel JP, Lelouard H. The Peyer's patch mononuclear phagocyte system at steady state and during infection. *Front. Immunol.* 2017, **8:**1254.

29. Klimpel GR, Asuncion M, Haithcoat J, Niesel DW. Cholera toxin and *Salmonella* Typhimurium induce different cytokine profiles in the gastrointestinal tract. *Infect. Immun.* 1995, **63**(3)**:** 1134-1137.

30. Fallahi P, Ferrari SM, Ragusa F, Ruffilli I, Elia G, Paparo SR*, et al.* Th1 chemokines in autoimmune endocrine disorders. *J. Clin. Endocrinol. Metab.* 2020, **105**(4):1046-1060.

870

871 31. Shreedhar VK, Kelsall BL, Neutra MR. Cholera toxin induces migration of dendritic cells
872 from the subepithelial dome region to T- and B-cell areas of Peyer's patches. *Infect. Immun.*
873 2003, **71**(1)**:**504-509.
874

875 32. Coméra C, Cartier C, Gaultier E, Catrice O, Panouille Q, El Hamdi S*, et al.* Jejunal villus
876 absorption and paracellular tight junction permeability are major routes for early intestinal
877 uptake of food-grade TiO2 particles: an in vivo and ex vivo study in mice. *Part. Fibre*
878 *Toxicol.* 2020, **17**(1)**:**26.
879

880 33. Mantis NJ, Frey A, Neutra MR. Accessibility of glycolipid and oligosaccharide epitopes on
881 rabbit villus and follicle-associated epithelium. *Am. J. Physiol. Gastrointest. Liver Physiol.*
882 2000, **278**(6)**:** G915-G923.
883

884 34. Frey A, Giannasca KT, Weltzin R, Giannasca PJ, Reggio H, Lencer WI*, et al.* Role of the
885 glycocalyx in regulating access of microparticles to apical plasma membranes of intestinal
886 epithelial cells: implications for microbial attachment and oral vaccine targeting. *J. Exp. Med.*
887 1996, **184**(3)**:**1045-1059.
888

889 35. Lelouard H, Henri S, De Bovis B, Mugnier B, Chollat-Namy A, Malissen B*, et al.*
890 Pathogenic bacteria and dead cells are internalized by a unique subset of Peyer's patch
891 dendritic cells that express lysozyme. *Gastroenterology* 2010, **138**(1)**:** 173-184.e171-173.
892

36. Lelouard H, Fallet M, de Bovis B, Méresse S, Gorvel JP. Peyer's patch dendritic cells sample antigens by extending dendrites through M cell-specific transcellular pores. *Gastroenterology* 2012, **142**(3)**:**592-601.

37. Hoiseth SK, Stocker BA. Aromatic-dependent Salmonella typhimurium are non-virulent and effective as live vaccines. *Nature* 1981, **291**(5812)**:**238-239.

38. Keita AV, Gullberg E, Ericson AC, Salim SY, Wallon C, Kald A*, et al.* Characterization of antigen and bacterial transport in the follicle-associated epithelium of human ileum. *Lab. Invest.* 2006, **86**(5)**:** 504-516.

39. Thévenaz P, Ruttimann UE, Unser M. A pyramid approach to subpixel registration based on intensity. *IEEE Trans. Image Process.* 1998, **7**(1)**:**27-41.

40. Kirshner H, Aguet F, Sage D, Unser M. 3-D PSF fitting for fluorescence microscopy: implementation and localization application. *J. Microsc.* 2013, **249**(1)**:**13-25.

41. Sage D, Donati L, Soulez F, Fortun D, Schmit G, Seitz A*, et al.* DeconvolutionLab2: An open-source software for deconvolution microscopy. *Methods* 2017, **115:**28-41.

42. Wills JW, Robertson J, Summers HD, Miniter M, Barnes C, Hewitt RE*, et al.* Image-based cell profiling enables quantitative tissue microscopy in gastroenterology. *Cytometry A* 2020, **97**(12)**:**1222-1237.

43. Carpenter AE, Jones TR, Lamprecht MR, Clarke C, Kang IH, Friman O*, et al.* CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biol.* 2006, **7**(10)**:**R100.

44. Falk T, Mai D, Bensch R, Çiçek Ö, Abdulkadir A, Marrakchi Y*, et al.* U-Net: deep learning for cell counting, detection, and morphometry. *Nat. Methods* 2019, **16**(1)**:**67-70.

45. Sommer C, Straehle C, Köthe U, Hamprecht FA. Ilastik: Interactive learning and segmentation toolkit. 2011 IEEE International Symposium on Biomedical Imaging: From Nano to Macro. 2011, 230-233.

46. Caicedo JC, Cooper S, Heigwer F, Warchal S, Qiu P, Molnar C*, et al.* Data-analysis strategies for image-based cell profiling. *Nat. Methods* 2017, **14:** 849-863.

ACKNOWLEDGEMENTS

The authors acknowledge the UK Medical Research Council (grant number MR/R005699/1), the UK Engineering and Physical Sciences Research Council (grant EP/N013506/1) and the UK Biotechnology and Biological Sciences Research Council (grant number BB/P026818/1) for supporting the work. J.W.W. is grateful to Girton College and the University of Cambridge Herchel-Smith Fund for supporting him with Fellowships. J.W.W. thanks Debbie Edwards for her assistance with the microscopy studies and Guy Dew for his comments on the manuscript. The authors thank Prof. Neil Mabbot and Prof. Simon Milling for their helpful comments. J.D.S. and Å.V.K. were supported by Swedish Research Council Grant No. 2021-02566. Mouse Study 1 was funded by the Riddet Institute through its Centre of Research Excellence funding which was awarded by the New Zealand government. Additional funding was provided by the Ministry for Science and Innovation contract C11 × 1009 through Nutrigenomics New Zealand, a collaboration between AgResearch, Plant and Food Research, and The University of Auckland. S.R. was supported by doctoral scholarships from Massey University. The authors are grateful to Dr. Andrew Grant for his input into the study design for Mouse Study 2.

AUTHOR CONTRIBUTIONS

JWW and JJP conceived the concept and designed the analyses. NR obtained funding for SR Ph.D. studies including Mouse Study 1. SR, NR and DO carried out Mouse Study 1. RJ, ABDS, MM, CB, JW, PM and JJP carried out Mouse Study 2. AVK and JDS collected the human surgical specimens. JWW, JR, MM, AF and REH conducted the light microscopical studies. AD, MM, JWW and JJP performed the protein expression analyses. APB, SM and JWW carried out the electron microanalytical work. JWW, PR, HDS and JJP performed the data analysis. JWW and JJP wrote the manuscript in close collaboration with all authors.

COMPETING INTERESTS

The authors declare no competing interests.

Supplementary Information

Cell Targeting and Adjuvant Activity of Dietary Titanium Dioxide

John W. Wills, Alicja Dabrowska, Jack Robertson, Michelle Miniter, Sebastian Riedle, Huw D. Summers, Rachel E. Hewitt, Adeeba Fathima, Alessandra Barreto da Silva, Carlos A. P. Bastos, Stuart Micklethwaite, Åsa V. Keita, Johan D. Söderholm, Nicole C. Roy, Don Otter, Ravin Jugdaohsingh, Pietro Mastroeni, Andy P. Brown, Paul Rees, Jonathan J. Powell.

List of Supplementary Information –

Supplementary Figure 1 – Quantifying $fgTiO_2$ in biological tissues using confocal reflectance microscopy. This explains the methodology used to enable robust quantification of $fgTiO_2$ in mouse and human tissues.

Supplementary Figure 2 – Assessing cell segmentation accuracies. This presents Jaccard index results for each of the cell segmentation strategies used in the study relative to results obtained by hand-drawing.

Supplementary Figure 3 – Image-analysis strategies enabling $fgTiO_2$ quantification. This explains the different image analysis approaches used to measure $fgTiO_2$ at the tissue-region, cell and $fgTiO_2$-loaded vesicle levels.

Supplementary Figure 4 – Background reflectance comparison in human and mouse tissue sections. This demonstrates that the background reflectance signal is highly similar in both mouse and human Peyer's patch tissue enabling reflectant foci to be established using a single threshold.

Supplementary Figure 5 – Circle-marker placement and TLV segmentation examples. This shows examples of the raw reflectance signal and resulting circle-marker and $fgTiO_2$-loaded vesicle segmentations after image processing for a series of mouse and human examples.

Supplementary Figure 6 – Statistical comparisons of the delivered cellular dose of $fgTiO_2$ in mice and humans. Statistical distribution comparisons are presented between the measured dose of $fgTiO_2$ delivered to subepithelial dome cells at both the cell and endosome level.

Supplementary Figure 7 – $fgTiO_2$ dose delivery using different diets. Further confocal reflectance microscopy data is presented comparing $fgTiO_2$ loading into mouse SED cells using diets supplemented with 0.000625, 0.00625 or 0.0625% (w/w) $fgTiO_2$.

Supplementary Figure 8 – Autofluorescence properties of lysomac/lysoDC cells. This Figure presents measured emission spectra for the lysomac/lysoDC autofluorescence signal.

Supplementary Figure 9 – Association of $fgTiO_2$ with lysomac/lysoDC cells. This demonstrates the selectivity and specificity of $fgTiO_2$ for the autofluorescent, lysomac/lysoDC cell population of the murine subepithelial dome.

Supplementary Figure 10 – Immunofluorescence controls for PD-L1. This provides secondary-only and isotype control image-data in support of the PD-L1 immunofluorescence studies.

Supplementary Figure 11 – Olink proximity extension assay controls. This shows the results of the random duplication, sample dilution and blank-buffer controls analysed by proximity extension assay.

Supplementary Figure 12 – Comparing $fgTiO_2$ loading into different ileal tissue compartments. This presents a quantitative comparison of the relative amounts of $fgTiO_2$ delivered to different ileal tissue regions in the murine model.

Supplementary Figure 13 – Mouse villous mucosa cell segmentation. Schematic explanation of the marker-controlled watershed approach used to enable cell segmentation of mouse villous mucosa images.

Supplementary Figure 14 – Cell segmentation of mouse lymphoid tissues using UNET pixel classification. This schematically explains how cell segmentations were achieved in mouse lymphoid tissues using a UNET pixel-classification model.

Supplementary Figure 15 – Mouse-human correlative dosimetry: Peyer's patch cell segmentation. This schematically demonstrates the process used to enable cell segmentation of mouse and human Peyer's patch tissue using sparse-annotation pixel classification machine learning in the Ilastik software.

Supplementary Figure 16 – Mouse-human correlative dosimetry: $fgTiO_2$-loaded vesicle segmentation. This schematically demonstrates the TLV segmentation approach from the reflectance image data.

Supplementary Table 1 – Olink proximity extension assay full protein names. This Table summarises the full list of proteins assessed by the Olink proximity extension assay alongside their full and abbreviated names.

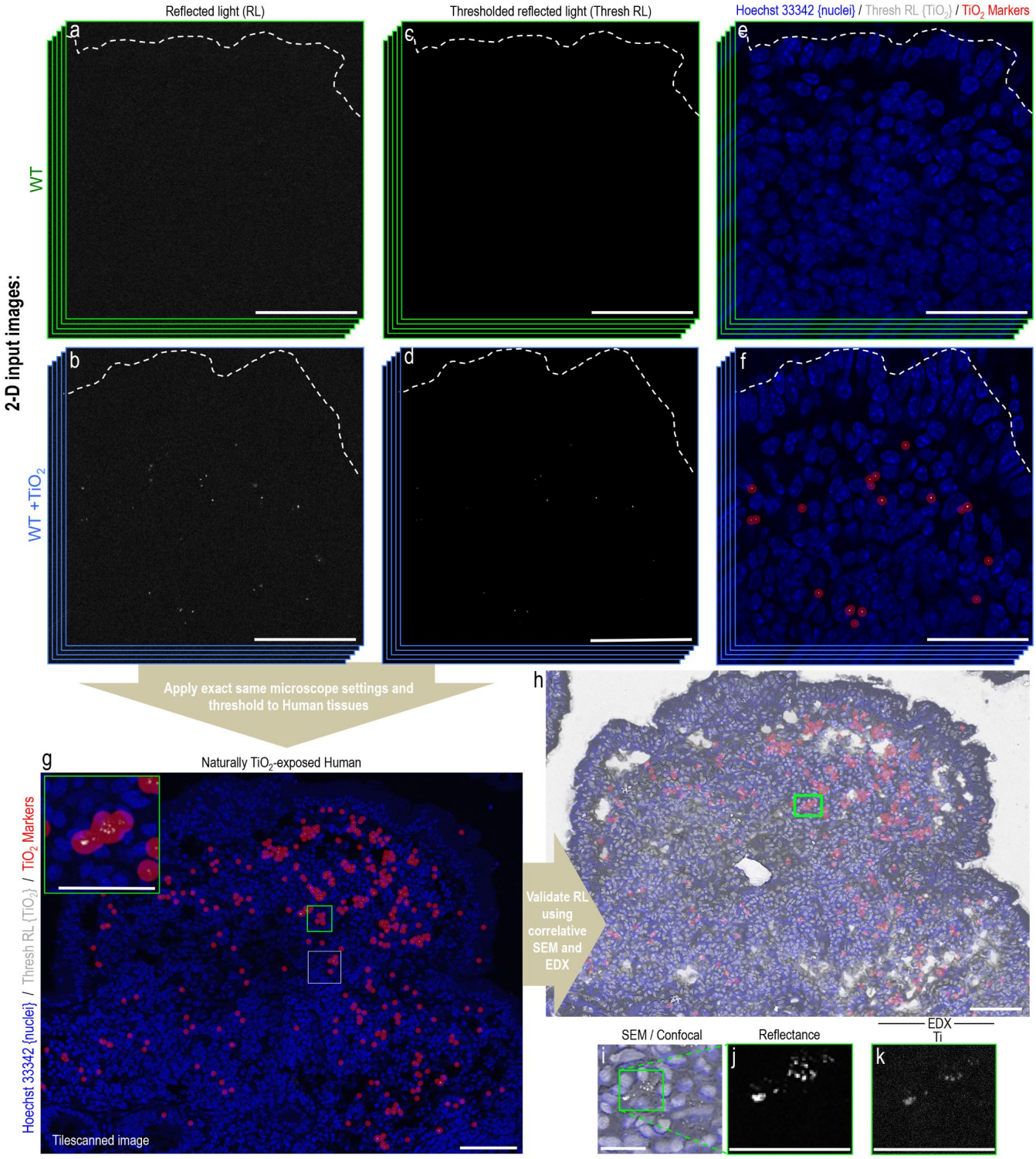
Supplementary Table 2 – Antibody information Table. This provides the specifics of all antibodies used in the study, including manufacturer information, staining concentrations and the secondary antibodies and fluorophores used for detection.

Supplementary Video 1 – $fgTiO_2$ targets autofluorescent lysomac/lysoDC cells of the murine subepithelial dome. This video presents a 3-D render of the murine subepithelial dome demonstrating the selectivity and specificity of $fgTiO_2$ targeting for the autofluorescent lysomac/lysoDC cell population.

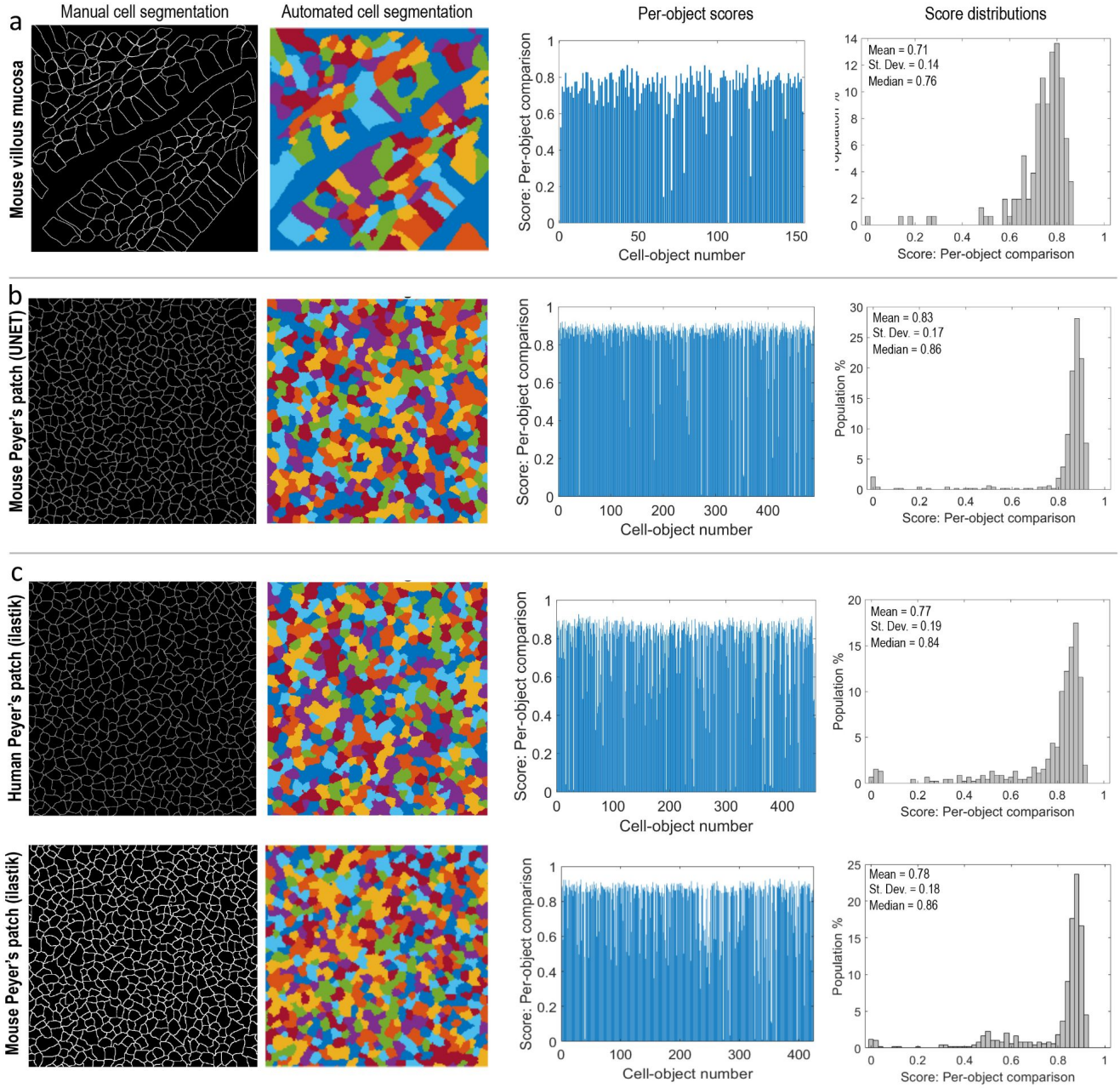
Supplementary Note 1 – Image analysis pipeline enabling cell segmentation of mouse villous mucosa image fields. This section presents the CellProfiler pipeline used to segment and extract cell features from the mouse villous mucosa images. The CellProfiler project and sample image data are available for download from the BioStudies archive accompanying this paper.

Supplementary Note 2 – Image analysis pipeline for $fgTiO_2$ and immunofluorescence marker quantification in mouse lymphoid tissues. This section presents the CellProfiler pipeline used to measure immunofluorescence marker and $fgTiO_2$ features in segmented cells. The CellProfiler project and sample image-data are available for download from the BioStudies archive accompanying this paper.

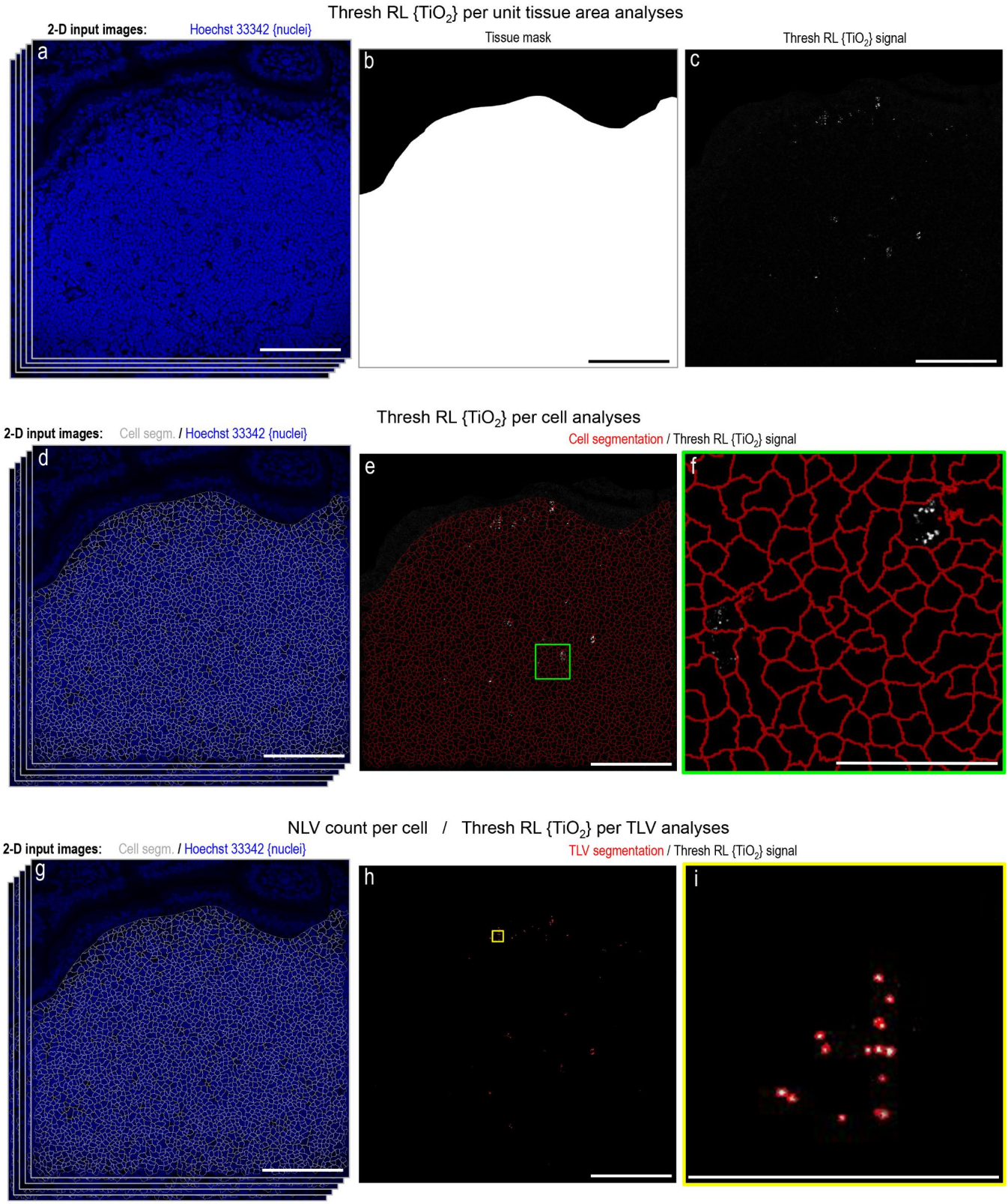
Supplementary Note 3 – Image analysis pipeline enabling the correlative mouse-human dosimetry studies. This section presents the CellProfiler pipeline used to measure cell and $fgTiO_2$ loaded vesicle (TLV) properties in the mouse and human tissue sections. The CellProfiler project and sample image data are available for download from the BioStudies archive accompanying this paper.



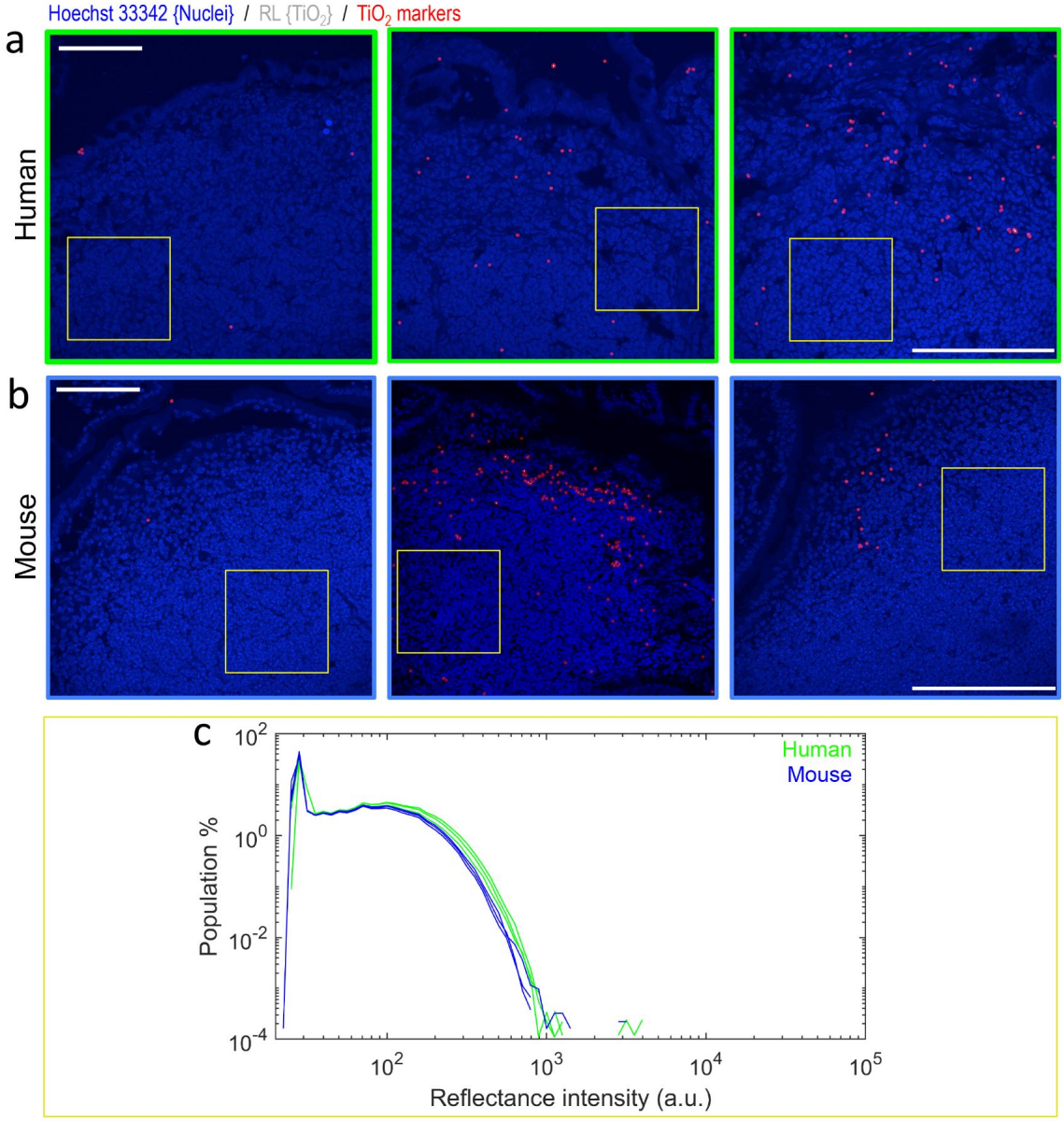
Supplementary Figure 1 – Detecting and quantifying fgTiO$_2$ in biological tissues using reflectance confocal microscopy. a/b, Using murine Peyer's patch tissue sections, known to be positive or negative for fgTiO$_2$ (*i.e.,* fed a diet with fgTiO$_2$ (WT +TiO$_2$) or without (WT) fgTiO$_2$), laser excitation and detector gain settings were optimised such that the signal from the brightest reflectant foci caused by fgTiO$_2$ approached saturation at the detector. At the same time, the confocal pinhole was used to obtain an optical section of ~1 micron in the Z-dimension enabling robust data collection from the centre of 20 micron-thickness tissue sections avoiding any surface-based signal. These microscope settings were universally applied during data acquisition using a Zeiss LSM780 laser scanning confocal microscope diode-retrofitted to maintain consistent laser excitation power. **c/d**, During the image analysis process, the murine tissues from the control-diet animals (*i.e.,* without fgTiO$_2$ supplementation) were used to inform a threshold for the reflectance channel ensuring that only the (**d**) signal from the highly reflectant foci caused by fgTiO$_2$ remained. **e/f**, To enable visualisation of these small reflectant foci, a translucent red circle marker was placed on each pixel above the threshold during the image analysis process. To orientate the images, dashed lines indicate the follicle-associated epithelium overlying the Peyer's patches. **g/h**, Once microscopy settings and image analysis thresholds were established using the murine tissues, the exact same settings were taken forward to enable the detection of fgTiO$_2$ in human tissues. **i-k**, To further validate the method in human tissues, correlative SEM-EDX analyses were carried out in the same tissue sections to confirm that the (**j**) reflectant foci detected by reflectance microscopy were an exact match for the (**k**) physical X-ray signal for Ti (further data shown, **Figure 1**). *Scale bars:* ***a-h*** *= 50 µm;* ***h****-inset and* ***i-k*** *= 10 µm.*



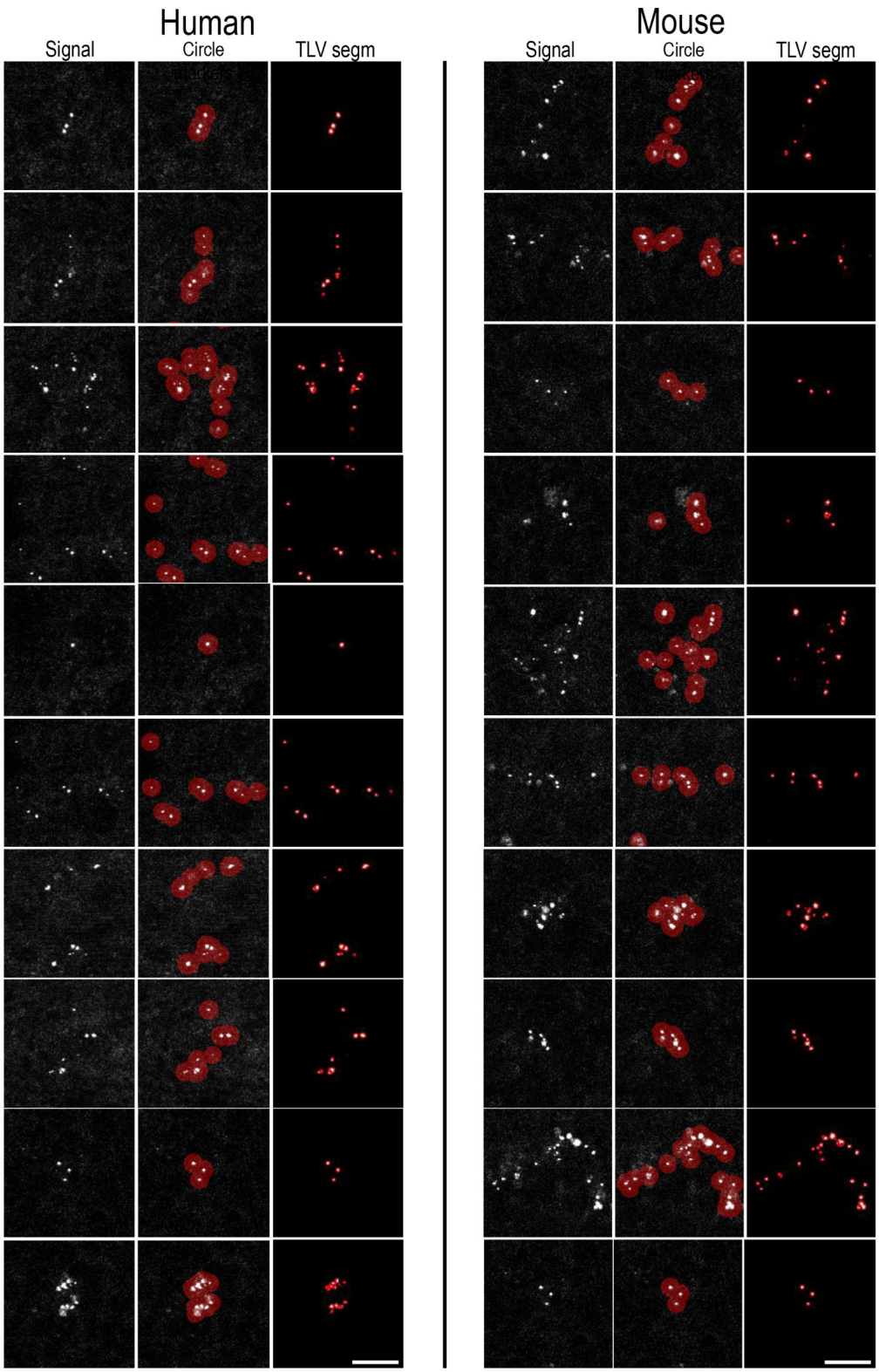
Supplementary Figure 2 – Testing the accuracy of the automated cell segmentations against manually-drawn examples. a-c, To check the accuracy of the three cell segmentation pipelines used, hand-drawn cell segmentations were performed by an experienced biologist. The results from the automated procedures were then compared – cell-object-by-cell-object – against the manually-drawn examples using the commonly employed intersection-over-union approach (Jaccard index). Per-object scores and distributions are shown for each analysis. A score of 1 represents a perfect pixel-area overlap between manual and automated cell segmentations. Due to the inherent inaccuracies present (*e.g.,* due to line thickness and outline smoothing *etc.,*) scores ≥ 0.7 typically represent a good segmentation result.



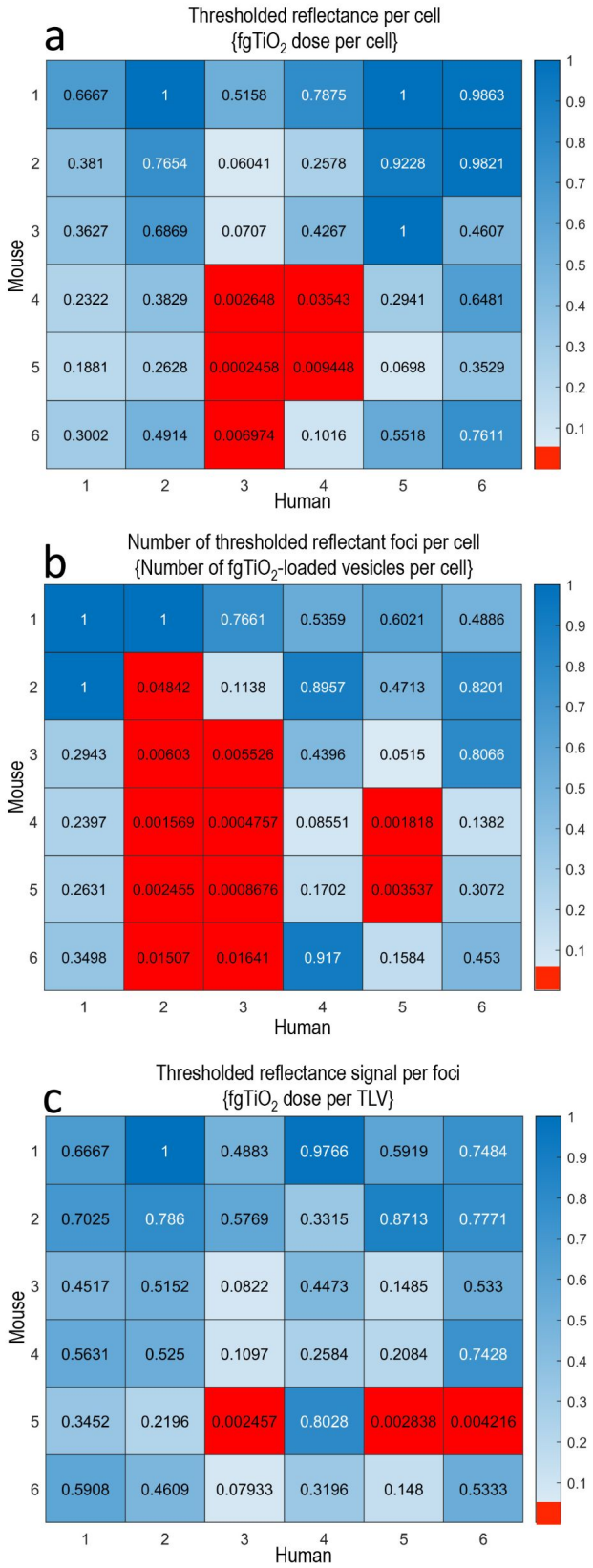
Supplementary Figure 3 – Image analysis of $fgTiO_2$ at the tissue-region, single-cell and $fgTiO_2$-loaded vesicle levels. a-c, To determine the abundance of $fgTiO_2$ per unit tissue area, masks of the tissue region-of-interest were (**b**) drawn manually. **c**, The thresholded reflectance signal attributable to $fgTiO_2$ (explained, **Supplementary Figure 1**) was then measured within these masked areas. **d-f**, The dose of $fgTiO_2$ delivered to individual cells was obtained by first segmenting all cells in the tissue region-of-interest and then (**f**) integrating the thresholded reflectance signal in each cell-object. **g-i**, Similarly, the count of $fgTiO_2$-loaded vesicles (TLV) per cell and the dose of $fgTiO_2$ per $fgTiO_2$-loaded vesicle were obtained by segmenting each individual, reflectant foci and counting these per (**f**) cell-object, or, by integrating the thresholded reflectance signal in each segmented TLV-object. *Scale bars:* ***a-h*** *= 50 µm,* ***i*** *= 10 µm.*



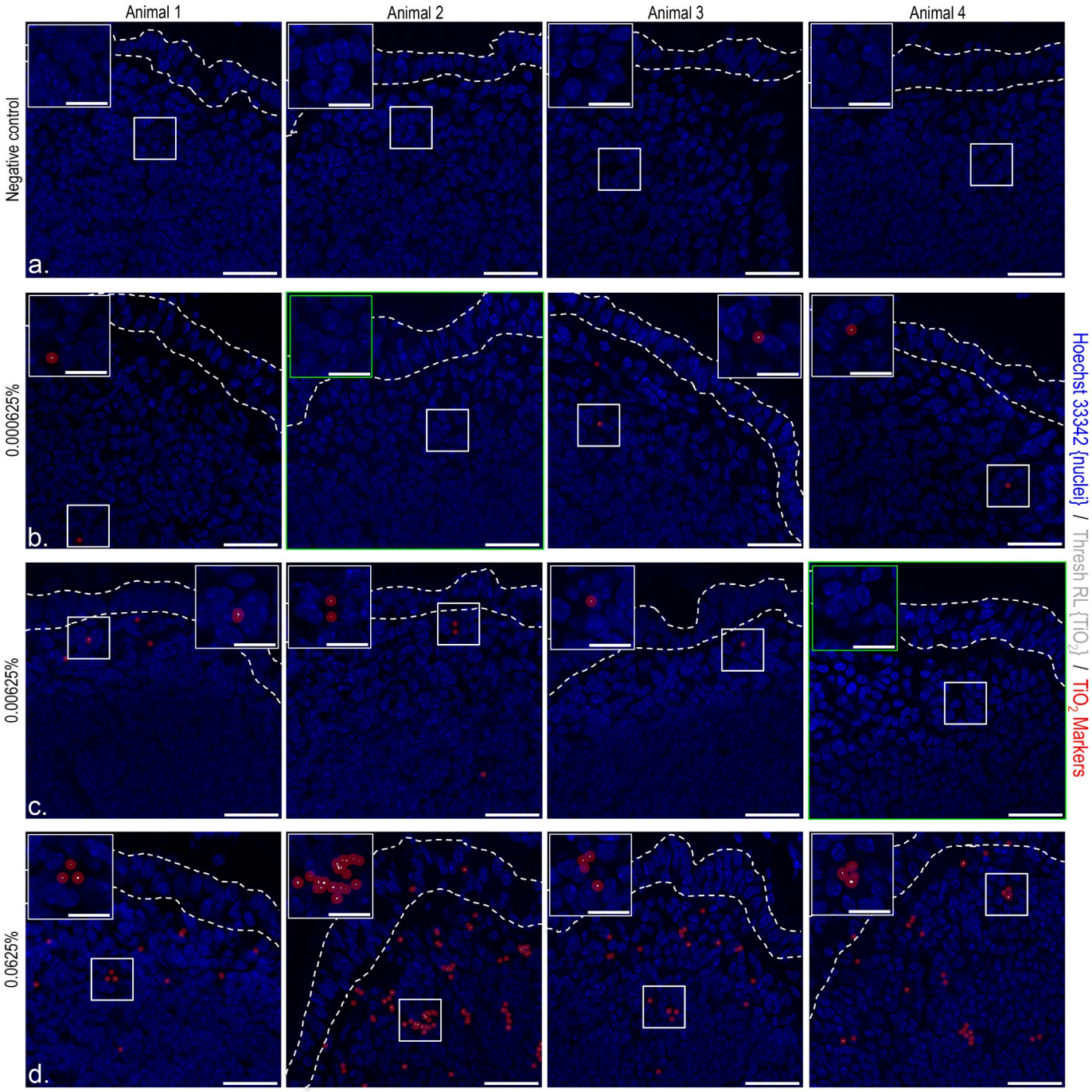
Supplementary Figure 4 – Comparison of the background reflectance intensities in human and mouse tissue sections. **a/b**, Image-regions containing subepithelial dome tissue but no reflectant foci were identified (yellow boxes) in (**a**) human or (**b**) mouse Peyer's patch datasets. **c**, The reflectance intensity information (per pixel) in these image-regions was overlaid as normalised histograms. The distributions are extremely similar: the optical sectioning imposed by the confocal microscopy technique means that the reflectance data is collected in a Z-depth of about 1 micron from the center of each 20-micron tissue section. The reflectance signal in this volume is largely driven by the cellular make-up of the tissue. In both mouse and humans, this tissue region predominantly consists of B-lymphocytes likely explaining the similar distributions observed. *Scale bars = 250 µm.*



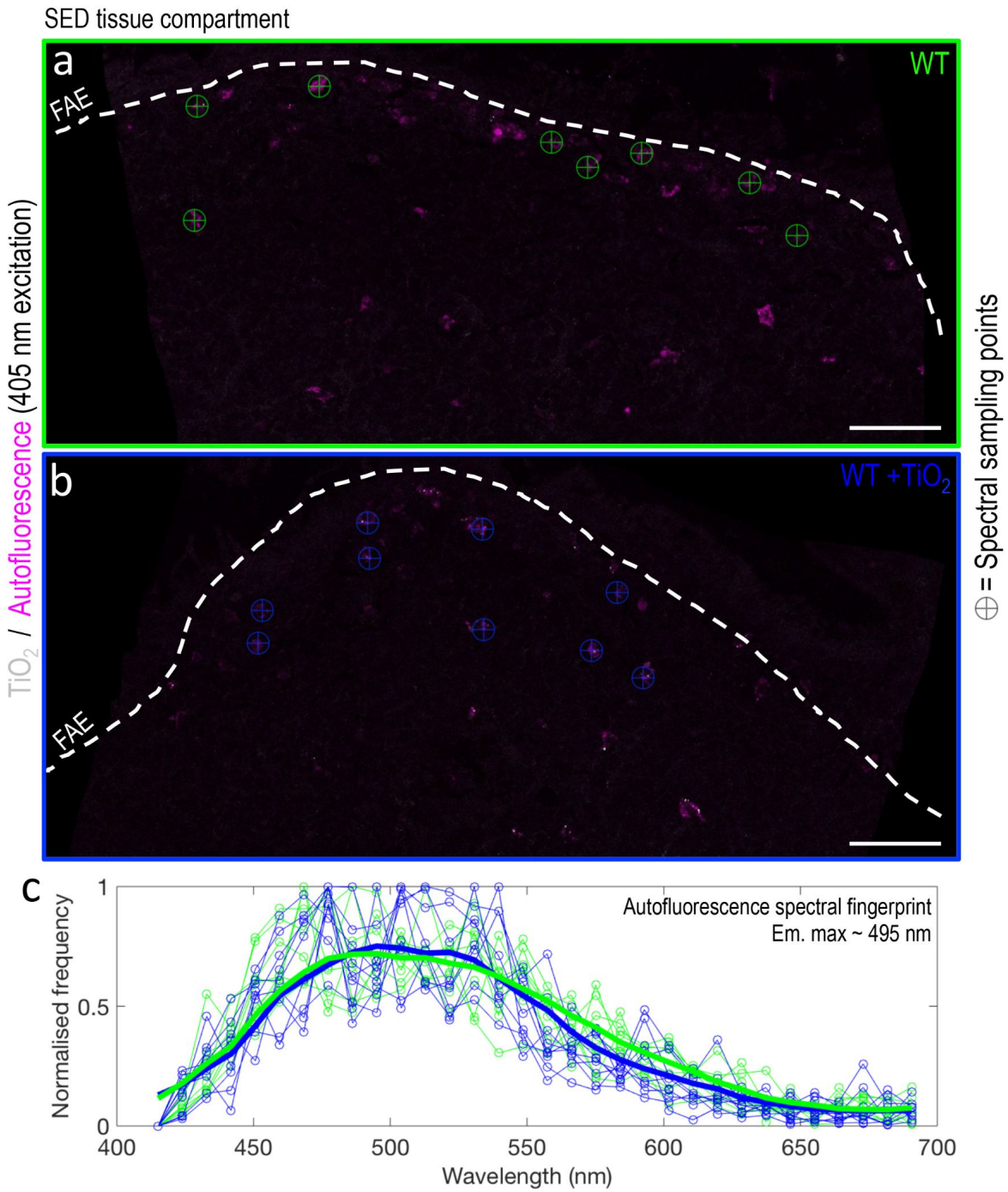
Supplementary Figure 5 – Examples of circle-marker placement and $fgTiO_2$-loaded vesicle (TLV) segmentation using mouse or human reflectance data. In the Left or Right panels, the first column exemplifies the raw reflectance signal collected by the confocal microscope. The second column shows the resultant circle-marker placements on thresholded reflectant foci (*i.e.,* representing $fgTiO_2$ events). The third column exemplifies reflectant foci segmentations into '$fgTiO_2$-loaded vesicle' (TLV) objects. *Scale bars = 5 µm.*



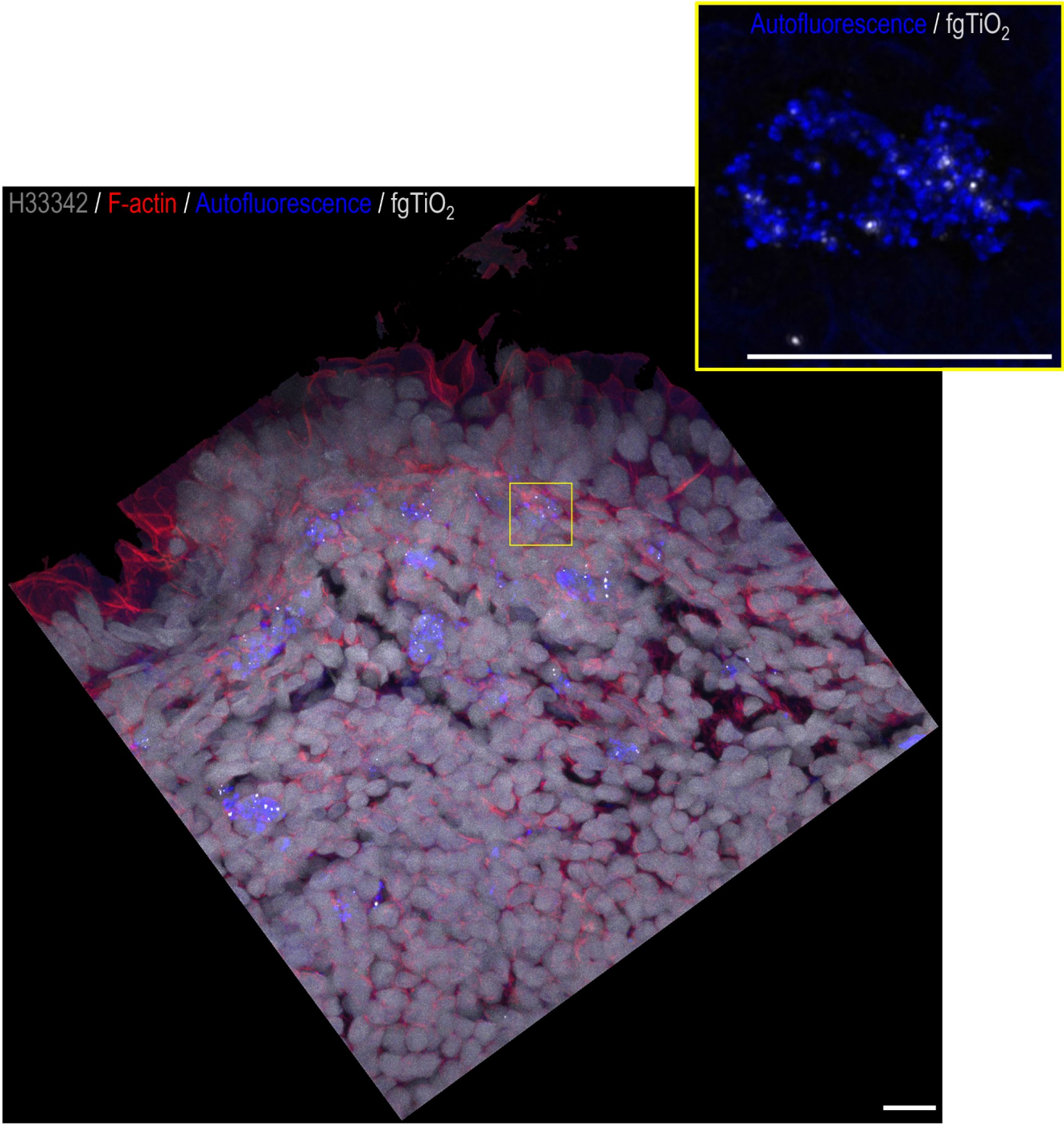
Supplementary Figure 6 - Statistical comparison of $fgTiO_2$ loading into Peyer's patch subepithelial dome cells in mice and humans. In each analysis, each of the six mouse and six human distributions presented in **Figure 3** were statistically compared by two-sided Wilcoxon rank-sum analysis. This tests the null hypothesis that each pairwise comparison could reasonably have been drawn from continuous distributions with equal medians ($P > 0.05$) against the alternate hypothesis that the distributions are distinct ($P < 0.05$). **a**, Using the measure of thresholded reflectance per cell (*i.e.* $fgTiO_2$ dose per cell) 31 out of 36 of the mouse-human comparisons reject the alternate hypothesis and thus might reasonably have been drawn from the same distributions. **b**, By the measure of the count of thresholded reflectant foci per cell (*i.e.,* the number of $fgTiO_2$-loaded vesicles (TLV) per cell) 25 out of 36 of the mouse-human comparisons were determined similar. **c**, Using the measure of thresholded reflectance signal per foci (*i.e.,* $fgTiO_2$ dose per TLV) 33 out of 36 of the mouse-human distributions were statistically similar. By each measure of the delivered cellular dose, feeding mice a diet supplemented with 0.0625% $fgTiO_2$ for eighteen weeks is seen to provide significant overlap with measured, real-world human exposures to $fgTiO_2$ in this tissue region.



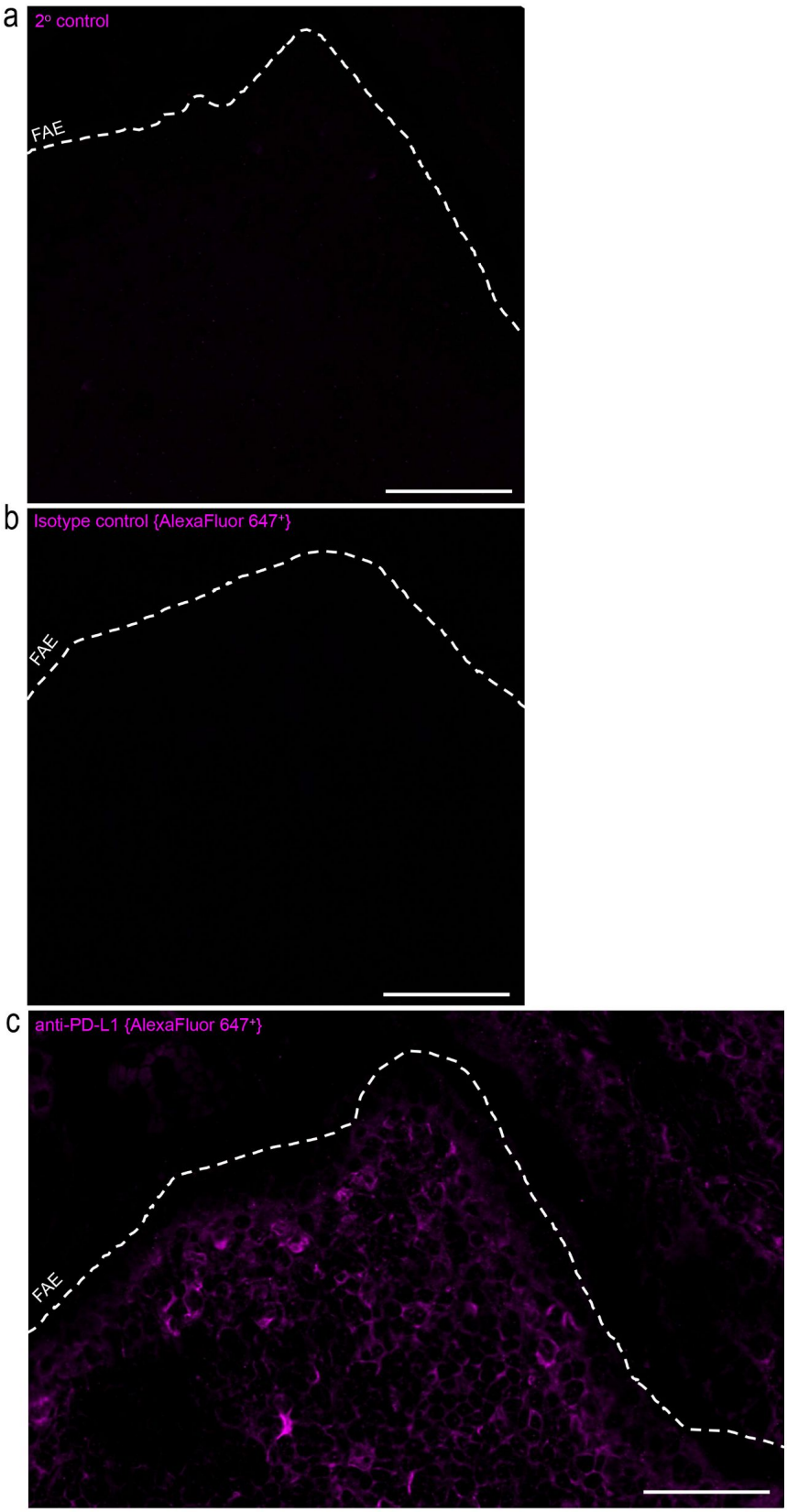
Supplementary Figure 7 – Comparison of $fgTiO_2$ delivery into the murine Peyer's patch subepithelial dome using diets supplemented with differing levels of $fgTiO_2$. American Institute of Nutrition (AIN)-76A diets containing 0.000625%, 0.00625% or 0.0625% $fgTiO_2$ (approximately 1.0, 10.0 or 100 mg $fgTiO_2$ / kg body-weight / day respectively) were prepared and fed alongside a negative control diet (AIN-76A alone) for eighteen weeks. **a-d**, After necropsy, the confocal reflectance microscopy approach (explained, **Supplementary Figure 1**) was able to detect delivery of $fgTiO_2$ into the SEDs for all three $fgTiO_2$-supplemented diets. Image analysis revealed that only the highest diet (0.0625% or 100 mg $fgTiO_2$ / kg body-weight / day) was able to recreate a similar cellular loading in the murine Peyer's patches to that observed in humans (presented, **Figure 3** with statistical comparison in **Supplementary Figure 6**). Dashed lines indicate the follicle-associated epithelium overlying the subepithelial dome tissue-region. *Scale bars:* ***a-d*** *= 25 µm; insets = 10 µm.*



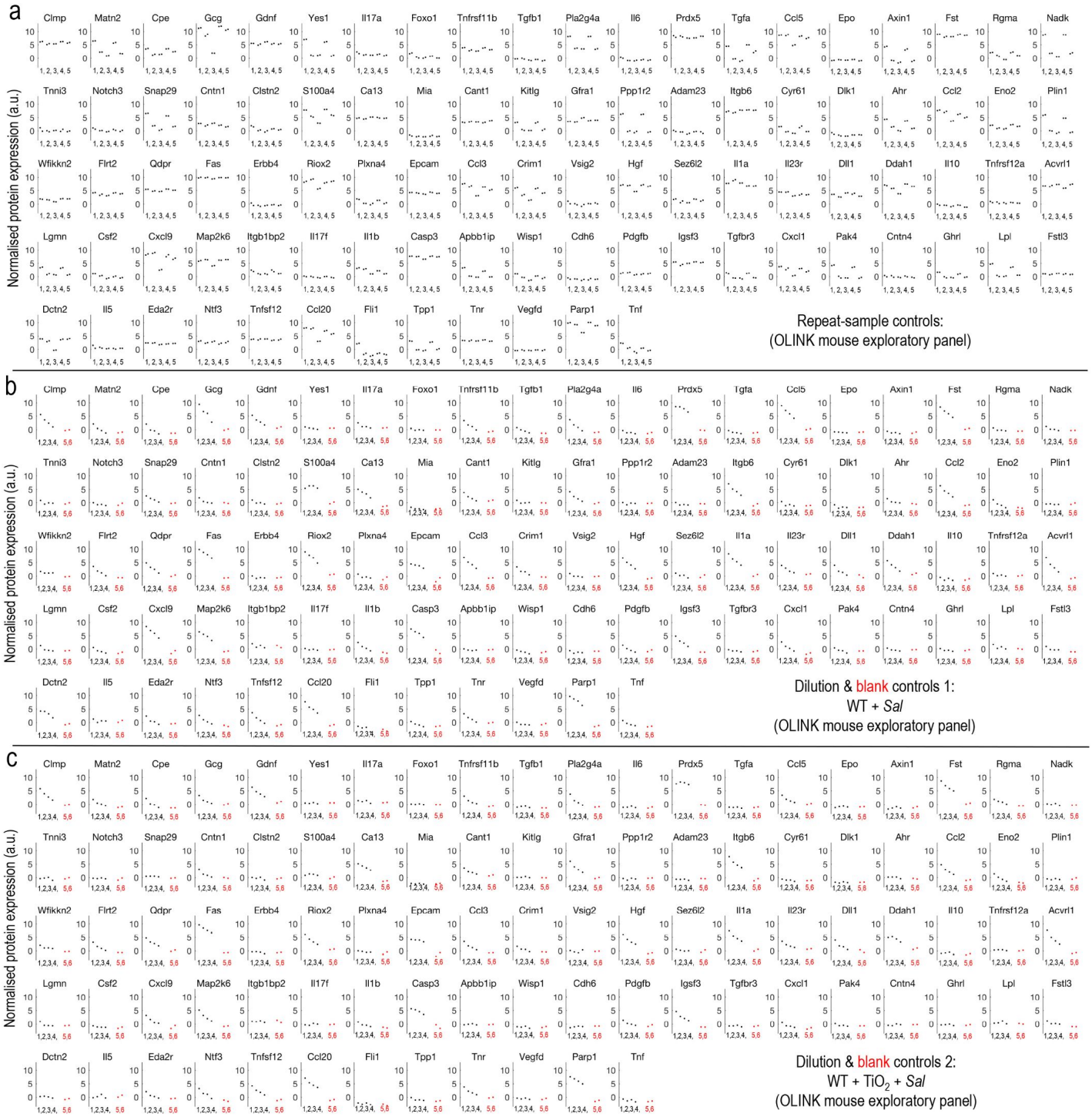
Supplementary Figure 8 – Determining the autofluorescence emission spectrum of murine lysomac/lysoDC cells. a/b, Under 405 nm laser excitation, reflectance data and lambda stacks for unstained murine Peyer's patch tissue sections were collected from animals fed either the (**a**) negative control diet (WT) or the (**b**) control diet supplemented with 0.0625% $fgTiO_2$ (WT +TiO_2). In both groups, a population of highly autofluorescent cells were observed in the Peyer's patch subepithelial dome (magenta) that (**b**) co-located with $fgTiO_2$ in the WT+TiO_2 samples. **c**, From each lambda stack, spectra were collected from eight locations (indicated by the cross-hair markers in **a/b**) and then averaged (**c**, thick lines) to determine the spectral fingerprint of the autofluorescence signal. Under 405 nm excitation, the emission max was ~ 495 nm. The presence of $fgTiO_2$ did not appear to affect the emission characteristics of the autofluorescence signal. *Scale bars:* ***a/b*** *= 50 µm.*



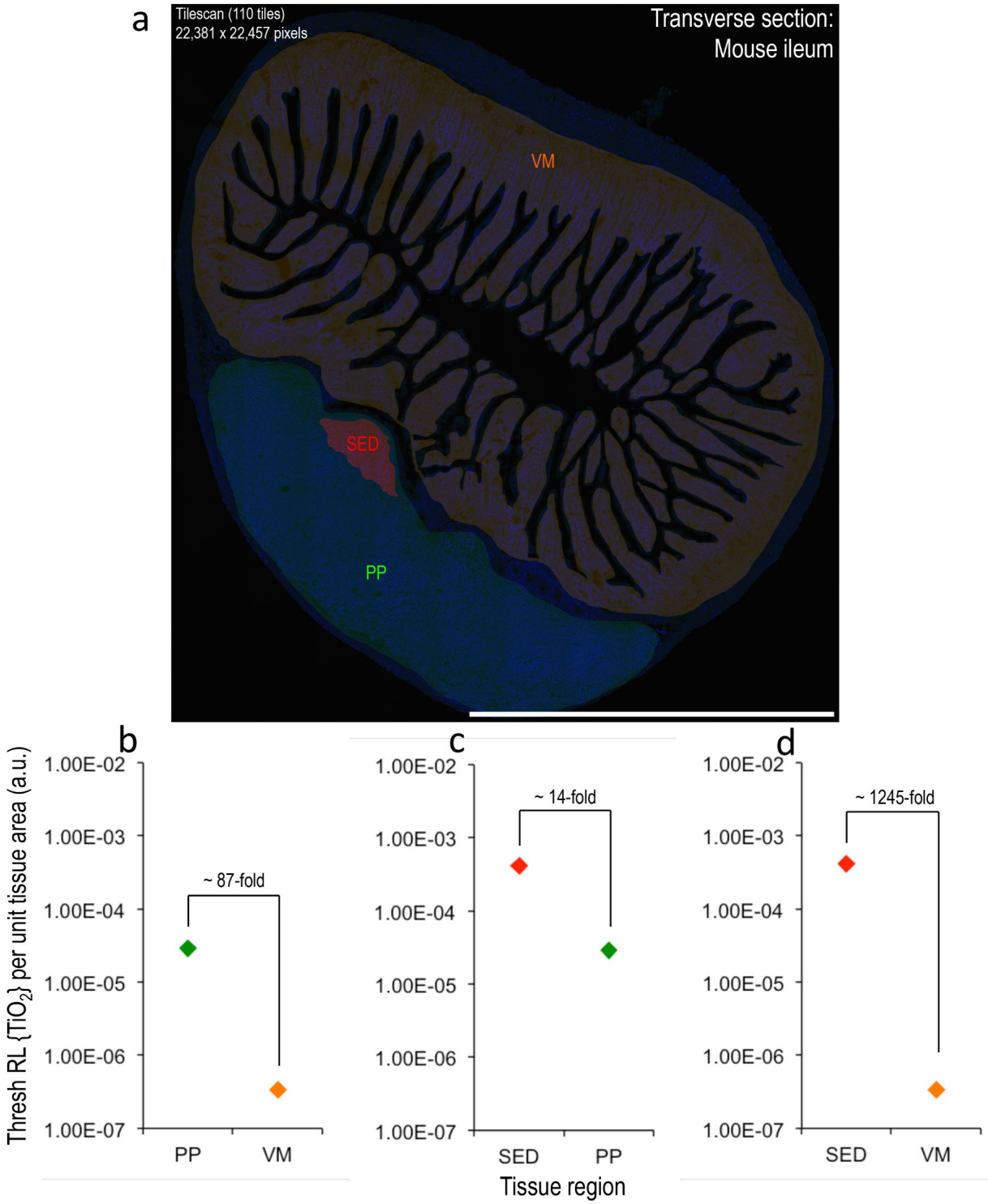
Supplementary Figure 9 – Selectivity and specificity of $fgTiO_2$ for autofluorescent lysomac/lysoDC cells in the murine subepithelial dome. (Main) Max projection of image data collected as a Z-stack using confocal reflectance microscopy. Cell nuclei were fluorescently labelled using Hoechst 33342 (grey). Cytoskeletal actin was labelled with phalloidin-AlexaFluor 647 (red). $fgTiO_2$ (white foci) was seen to selectively and specifically target the highly autofluorescent (blue) mononuclear phagocytic cells. The inset shows a high magnification max projection image of a single autofluorescent cell taken from the area indicated by the yellow box in the main image. The autofluorescence signal was deconvolved (Born and Wolf theoretical point-spread function, Richardson-Lucy deconvolution for 75 iterations via DeconvolutionLab2 software). The output demonstrates the punctate, vesicular nature of the autofluorescence signal and its close association with $fgTiO_2$ at the subcellular level. *Scale bars = 10 µm.*



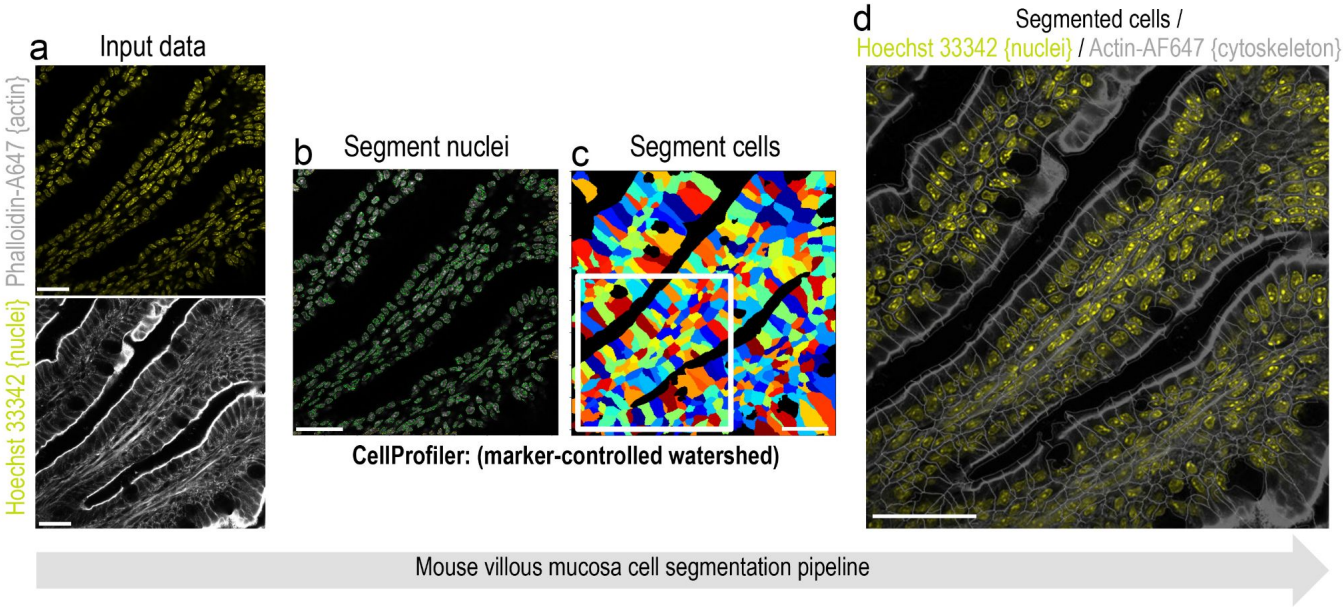
Supplementary Figure 10 – immunofluorescence control data supporting the anti-PD-L1 immunofluorescence labelling. Image-data were collected by laser scanning confocal microscopy. **a**, Secondary-only control data (*i.e.,* AlexaFluor 647+ secondary antibody staining in absence of the anti-PD-L1 primary antibody). This shows a clean image with minimal signal in the AlexaFluor 647 channel ruling out non-specific secondary antibody binding and autofluorescence contributions to the measured PD-L1 immunofluorescence data. **b**, Clean images were also obtained when the anti-PD-L1 primary antibody was switched for a concentration-matched 'isotype control' (*i.e.,* an irrelevant antibody of the same isoclass as the anti-PD-L1 primary antibody). This suggests that the anti-PD-L1 primary antibody is not bound non-specifically in this tissue region. **c**, In contrast, strong immunofluorescence labelling was detected in the subepithelial dome using the anti-PD-L1 primary antibody sequentially detected by an AlexaFluor 647-plus secondary antibody. All image data were collected using identical microscope settings. The apical aspect of the follicle-associated epithelium is indicated by the dashed line. *Scale bars = 50 µm.*



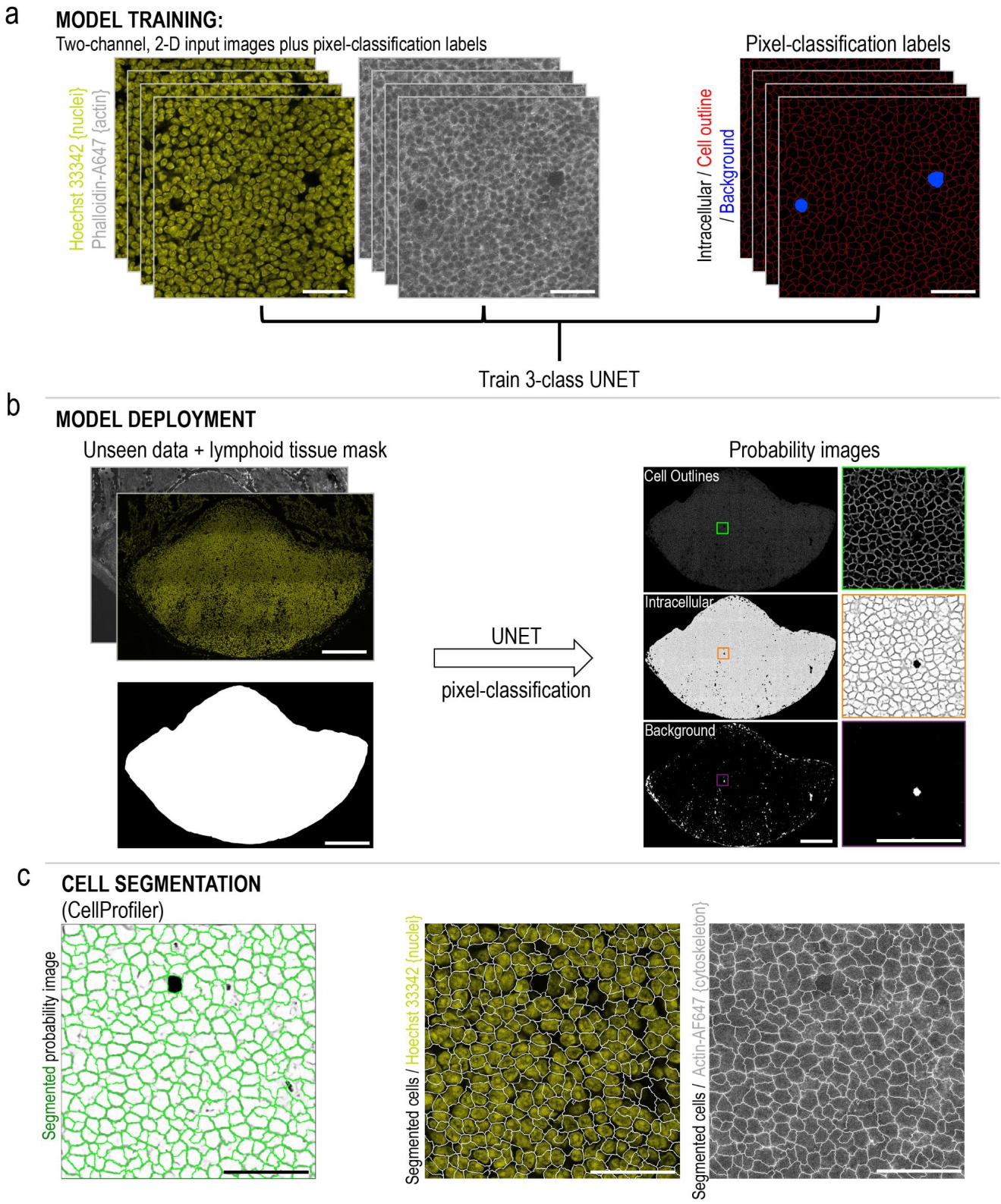
Supplementary Figure 11 – Proximity extension assay controls. a, Five random samples across treatment groups were duplicated at random positions on the 96-well plate to assess measurement reproducibility (1=WT+Sal, 2=WT+TiO_2+Sal, 3=WT, 4=WT+Sal, 5=WT+TiO_2+Sal). **b**/**c**, Serial dilutions of one (**b**) WT + Sal sample and one (c) WT +TiO_2 +Sal sample were prepared and placed at random positions on the 96-well plate to test for measurement linearity across a wide concentration range. Alongside, two blank lysis buffer-only controls were also included to assess background and indicate limits of detection (1=1:1, 2=1:4, 3=1:8, 4=1:16 dilutions; 5=blank1, 6=blank2).



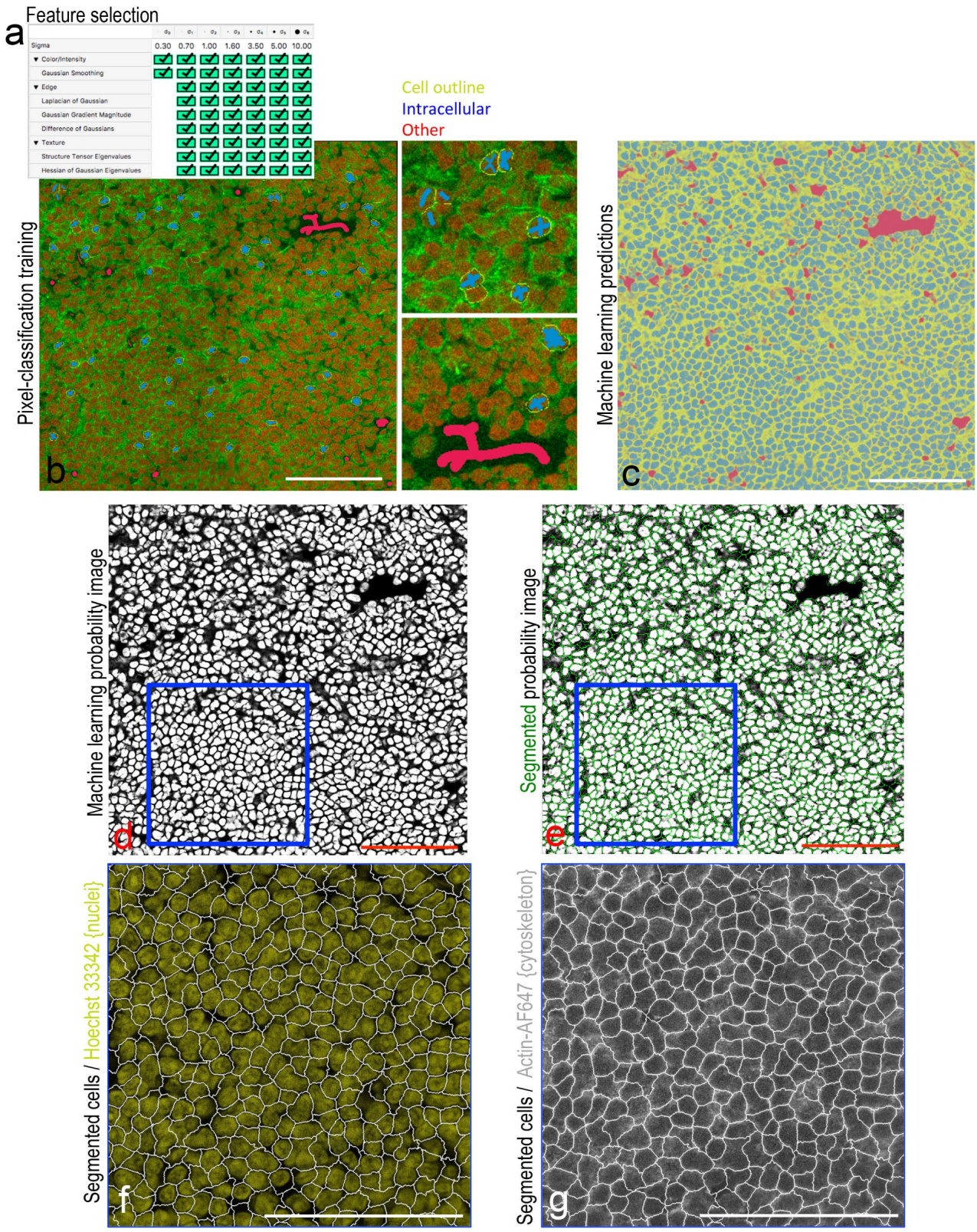
Supplementary Figure 12 – Quantitative comparison of $fgTiO_2$ loading into different tissue compartments of the murine ileum. a, Tilescanned reflectance confocal microscopy data (110 stitched images) covering a complete, ileal transverse tissue section containing a Peyer's patch. Manually-drawn region-of-interest masks for the villous mucosa (VM), Peyer's patch (PP plus red SED region) and subepithelial dome (SED) tissue regions are overlaid. **b-d**, The thresholded reflectance signal attributable to $fgTiO_2$ (methodology explained, **Supplementary Figure 1**) was then measured per unit tissue area within these different regions. Despite its large surface area, the normally-absorptive villous mucosa is practically impermeable to $fgTiO_2$. In contrast – facilitated by a overlying epithelium rich in M-cells – the Peyer's patches show considerable uptake. The selectivity for this site means that the concentration gradient for area-corrected reflectance signal was (**b**) ~87-fold higher in the Peyer's patch when directly compared against the surrounding villous mucosa. Moreover, most of this Peyer's patch signal was localised within the immunocompetant SED region where the area-corrected reflectance was (**c**) ~14-fold higher than for the patch overall and, therefore, (**d**) ~1,245 fold higher than that of villous mucosa. *Scale bar = 1 mm.*



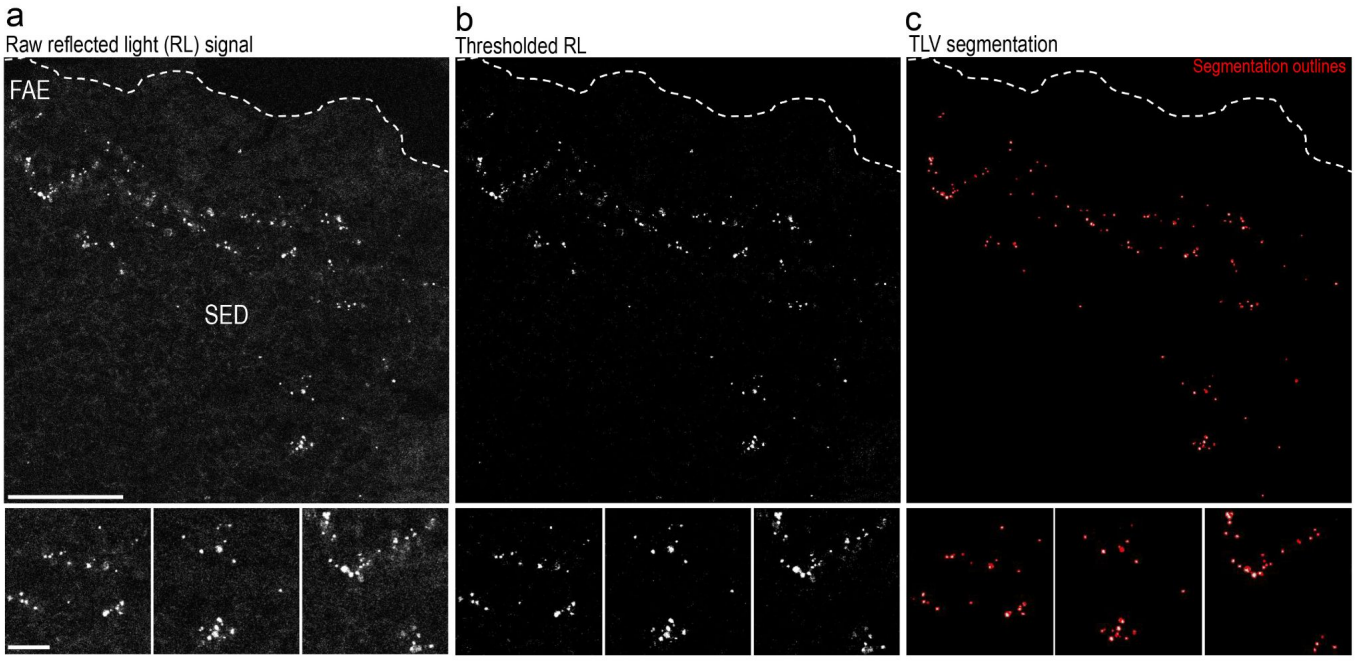
Supplementary Figure 13 – Cell segmentation of mouse villous mucosa images using a marker-controlled watershed algorithm. **a,** Nuclei and actin fluorescence images were loaded into a CellProfiler pipeline. **b**, First, nuclei were segmented via an 'IdentifyPrimaryObjects' module using information from the Hoechst 33342 channel. **c**, The resultant nuclei-objects were then used as seeds during deployment of a marker-controlled watershed approach to find each cell's boundary using fluorescence information from the actin channel. **d**, Cell segmentation boundaries overlaid on the nuclei and actin fluorescence information. *Scale bars = 25 µm.*



Supplementary Figure 14 – Cell segmentation of mouse lymphoid tissues using UNET pixel-classification. **a**, Using the nuclei and actin fluorescence channels alongside paired pixel-classification labels as input, the network was trained to predict the probability that pixels in the input images belonged to one of three classes ('intracellular', 'cell outline' or 'background'). **b**, The trained model was then used to generate probability images for unseen lymphoid tissue images. These probability images represent the likelihood for every pixel in the image belonging to the indicated class. **c**, To segment cell-objects and extract features (*e.g.,* per-cell intensity and size/shape information), the 'intracellular' probability images were loaded into CellProfiler alongside the fluorescence and reflectance data and cell segmentation was achieved from the 'intracellular' probability image using an 'IdentifyPrimaryObjects' module. *Scale bars:* ***a*** *= 25 µm,* ***b*** *= 200 µm (with insets 50 µm),* ***c*** *= 50 µm.*



Supplementary Figure 15 – Cell segmentation of human or mouse Peyer's patch images using pixel-classification machine learning in Ilastik. (**a**) Feature selection in the Ilastik software. (**b**) A small number of pixel annotation indicating the different classes desired are made by drawing onto the nuclei and actin image data. (**c**) The Ilastik software then uses machine learning to attempt classification of all pixels in the image into each category. The images represent the likelihood for every pixel in the image belonging to the indicated class. (**d-g**), The probability map information can then be segmented into cell objects via an 'IdentifyPrimaryObjects' module in Cell Profiler. *Scale bars = 100 µm.*



Supplementary Figure 16 – fgTiO$_2$-loaded vesicle (TLV) object segmentation from reflectance images. Using a CellProfiler pipeline, the (**a**) raw reflectance information was (**b**) thresholded to remove background signal caused by the biological tissue whilst leaving the punctate, reflectant foci representing fgTiO$_2$. **c**, These thresholded reflectant foci were then segmented into 'fgTiO$_2$-loaded vesicle' objects using an 'IdentifyPrimaryObjects' module in CellProfiler. Red represents the segmentation boundaries for each TLV object. *Scale bars = 100 µm with 10 µm in insets.*

Supplementary Table 1– Full list of proteins and abbreviated names assessed by the proximity extension assay (Olink Mouse Exploratory Panel)

Amyloid beta A4 precursor protein-binding family B member 1-interacting protein (Apbb1ip)	Q8R5A3
Appetite-regulating hormone (Ghrl)	Q9EQX0
Aryl hydrocarbon receptor (Ahr)	P30561
Axin-1 (Axin1)	O35625
C-C motif chemokine 2 (Ccl2)	P10148
C-C motif chemokine 20 (Ccl20)	O89093
C-C motif chemokine 3 (Ccl3)	P10855
C-C motif chemokine 5 (Ccl5)	P30882
C-X-C motif chemokine 9 (Cxcl9)	P18340
Cadherin-6 (Cdh6)	P97326
Calsyntenin-2 (Clstn2)	Q9ER65
Carbonic anhydrase 13 (Ca13)	Q9D6N1
Carboxypeptidase E (Cpe)	Q00493
Caspase-3 (Casp3)	P70677
Contactin-1 (Cntn1)	P12960
Contactin-4 (Cntn4)	Q69Z26
CXADR-like membrane protein (Clmp)	Q8R373
Cysteine-rich motor neuron 1 protein (Crim1)	Q9JLL0
Cytosolic phospholipase A2 (Pla2g4a)	P47713
Delta-like protein 1 (Dll1)	Q61483
Dihydropteridine reductase (Qdpr)	Q8BVI4
Disintegrin and metalloproteinase domain-containing protein 23 (Adam23)	Q9R1V7
Dual specificity mitogen-activated protein kinase kinase 6 (Map2k6)	P70236
Dynactin subunit 2 (Dctn2)	Q99KJ8
Legumain (Lgmn)	O89017
Leucine-rich repeat transmembrane protein FLRT2 (Flrt2)	Q8BLU0
Lipoprotein lipase (Lpl)	P11152
Matrilin-2 (Matn2)	O08746
Melanoma-derived growth regulatory protein (Mia)	Q61865
N(G),N(G)-dimethylarginine dimethylaminohydrolase 1 (Ddah1)	Q9CWS0
NAD kinase (Nadk)	P58058
Neurogenic locus notch homolog protein 3 (Notch3)	Q61982
Neurotrophin-3 (Ntf3)	P20181
Perilipin-1 (Plin1)	Q8CGN5
Peroxiredoxin-5, mitochondrial (Prdx5)	P99029
Platelet-derived growth factor subunit B (Pdgfb)	P31240
Plexin-A4 (Plxna4)	Q80UG2
Poly [ADP-ribose] polymerase 1 (Parp1)	P11103
Protein CYR61 (Cyr61)	P18406
Protein delta homolog 1 (Dlk1)	Q09163
Protein phosphatase inhibitor 2 (Ppp1r2)	Q9DCL8
Protein S100-A4 (S100a4)	P07091
Protransforming growth factor alpha (Tgfa)	P48030
Receptor tyrosine-protein kinase erbB-4 (Erbb4)	Q61527
Repulsive guidance molecule A (Rgma)	Q6PCX7
Ribosomal oxygenase 2 (Riox2)	Q8CD15

Epithelial cell adhesion molecule (Epcam)	Q99JW5
Erythropoietin (Epo)	P07321
Follistatin (Fst)	P47931
Follistatin-related protein 3 (Fstl3)	Q9EQC7
Forkhead box protein O1 (Foxo1)	Q9R1E0
Friend leukemia integration 1 transcription factor (Fli1)	P26323
Gamma-enolase (Eno2)	P17183
GDNF family receptor alpha-1 (Gfra1)	P97785
Glial cell line-derived neurotrophic factor (Gdnf)	P48540
Glucagon (Gcg)	P55095
Granulocyte-macrophage colony-stimulating factor (Csf2)	P01587
Growth-regulated alpha protein (Cxcl1)	P12850
Hepatocyte growth factor (Hgf)	Q08048
Immunoglobulin superfamily member 3 (Igsf3)	Q6ZQA6
Integrin beta-1-binding protein 2 (Itgb1bp2)	Q9R000
Integrin beta-6 (Itgb6)	Q9Z0T9
Interleukin-1 alpha (Il1a)	P01582
Interleukin-1 beta (Il1b)	P10749
Interleukin-10 (Il10)	P18893
Interleukin-17A (Il17a)	Q62386
Interleukin-17F (Il17f)	Q7TNI7
Interleukin-23 receptor (Il23r)	Q8K4B4
Interleukin-5 (Il5)	P04401
Interleukin-6 (Il6)	P08505
Kit ligand (Kitlg)	P20826
Seizure 6-like protein 2 (Sez6l2)	Q4V9Z5
Serine/threonine-protein kinase PAK 4 (Pak4)	Q8BTW9
Serine/threonine-protein kinase receptor R3 (Acvrl1)	Q61288
Soluble calcium-activated nucleotidase 1 (Cant1)	Q8VCF1
Synaptosomal-associated protein 29 (Snap29)	Q9ERB0
Tenascin-R (Tnr)	Q8BYI9
Transforming growth factor beta receptor type 3 (Tgfbr3)	O88393
Latency-associated peptide transforming growth factor beta-1 (Tgfb1)	P04202
Tripeptidyl-peptidase 1 (Tpp1)	O89023
Troponin I, cardiac muscle (Tnni3)	P48787
Tumor necrosis factor (Tnf)	P06804
Tumor necrosis factor ligand superfamily member 12 (Tnfsf12)	O54907
Tumor necrosis factor receptor superfamily member 11B (Tnfrsf11b)	O08712
Tumor necrosis factor receptor superfamily member 12A (Tnfrsf12a)	Q9CR75
Tumor necrosis factor receptor superfamily member 27 (Eda2r)	Q8BX35
Tumor necrosis factor receptor superfamily member 6 (Fas)	P25446
Tyrosine-protein kinase Yes (Yes1)	Q04736
V-set and immunoglobulin domain-containing protein 2 (Vsig2)	Q9Z109
Vascular endothelial growth factor D (Vegfd)	P97946
WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2 (Wfikkn2)	Q7TQN3
WNT1-inducible-signaling pathway protein 1 (Wisp1)	O54775

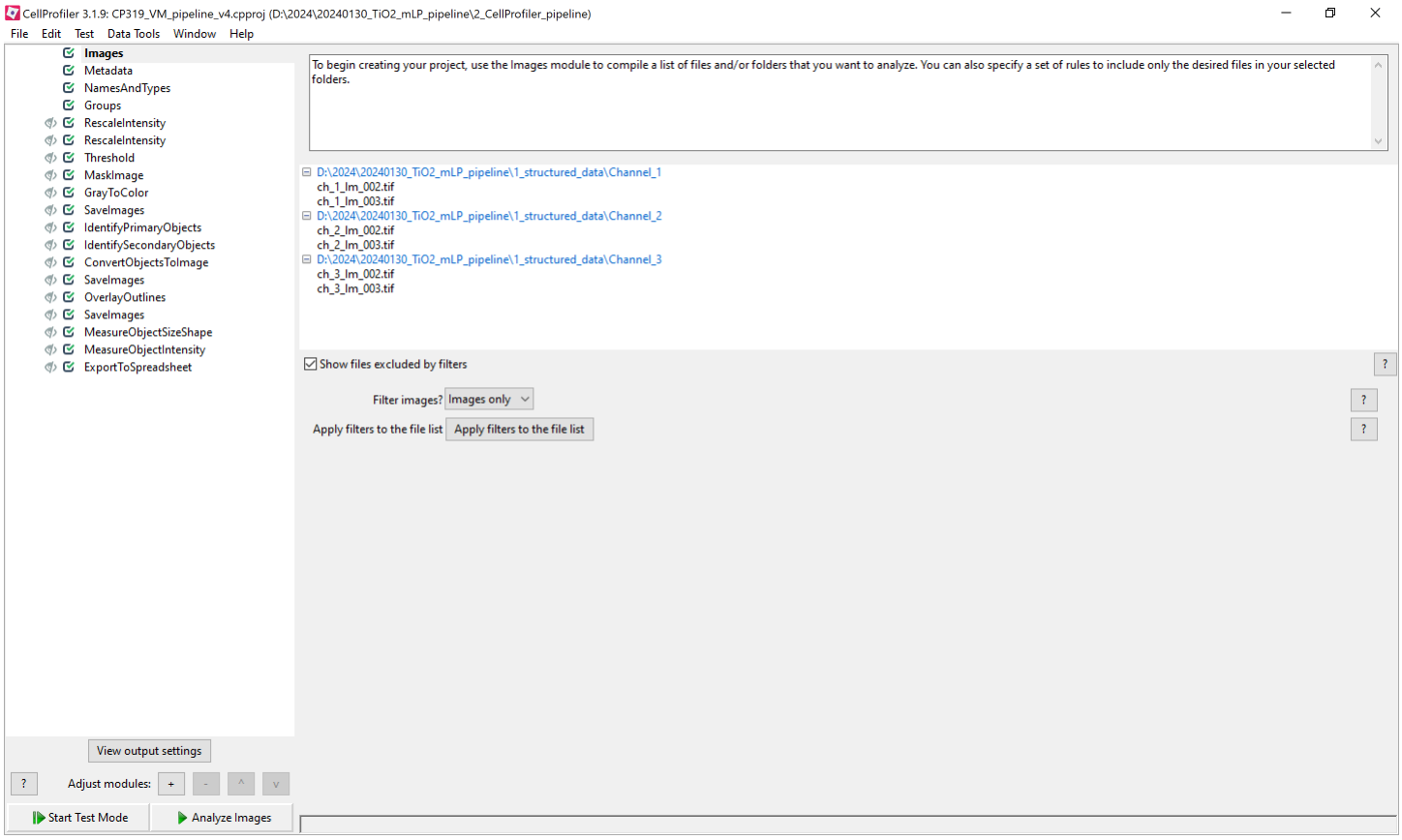
Supplementary Table 2 – Antibody information Table

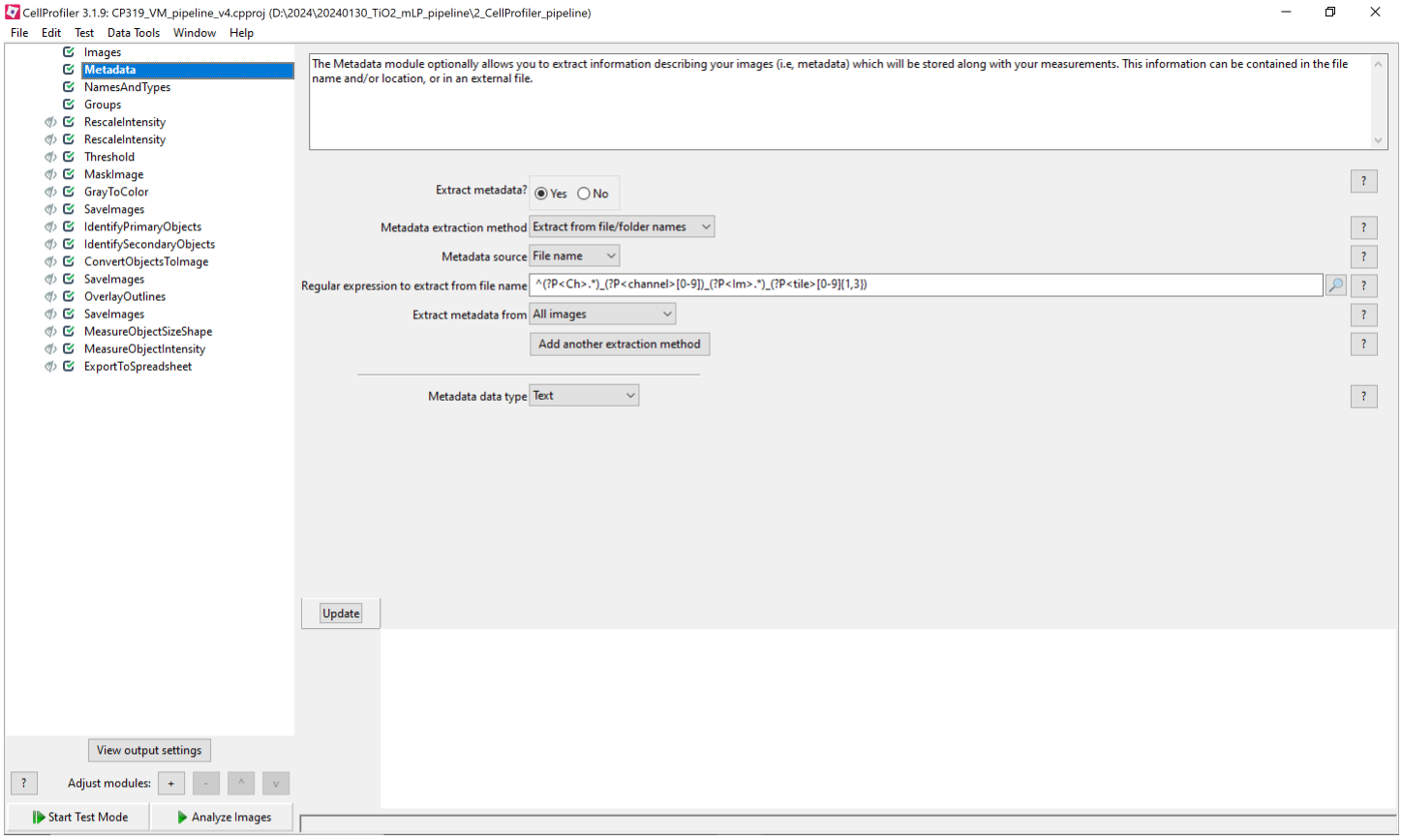
PRIMARY ANTIBODIES	Product no	Supplier	Dilution primary	Stock concentration	Host	Secondary	Detection	Figure
Anti-mouse GP2	D278-3	MBL		1 mg/mL	Rat	aRat AF647+	AlexaFluor 647+	2
Anti-mouse CD3	AB5690	Abcam	1:150	0.2 mg/mL	Rabbit	aRb AF568	AlexaFluor 568	2
Anti-mouse CD11c	AB33483	Abcam	1:400	0.5 mg/mL	Armenian Hamster	aHam A488	AlexaFluor 488	2
Anti-mouse B220	50045280	ThermoFisher	1:50	0.2 mg/mL	Rat	-	EFluor 660	4
Anti-mouse CD11c	53011482	ThermoFisher	1:25	0.2 mg/mL	Rat	-	AlexaFluor 488	4
Anti-mouse CD3	17003282	ThermoFisher	1:50	0.2 mg/mL	Rat	-	EFluor 570	4
Anti-mouse PD-L1	AB213480	Abcam	1:100	0.5 mg/mL	Rabbit	aRb A647+	AlexaFluor A647+	4
Anti-mouse MHCII	PE-65122-100UG	ThermoFisher	1:25	0.2 mg/mL	Rat	-	PE	4
Anti-mouse CD4	12-0041-82	ThermoFisher	1:25	0.2 mg/mL	Rat	-	PE	4
Anti-flagellin	AB93713	Abcam	1:100	1 mg/mL	Rabbit	aRb A568	AlexaFluor A568	5

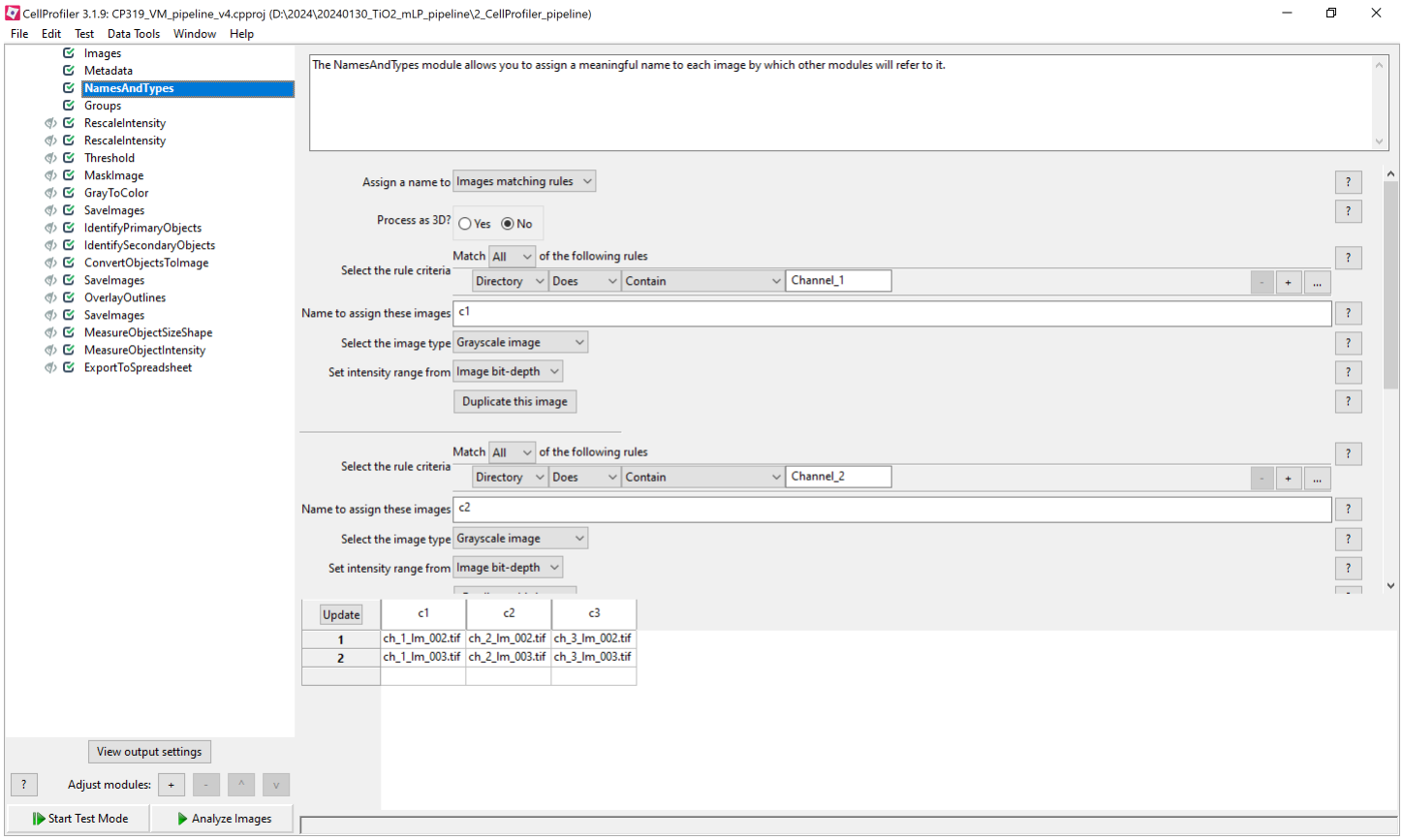
SECONDARY ANTIBODIES	Product no	Supplier	Stock concentration	Host	Secondary dilution	Fluorophore
Goat anti-Rabbit IgG (H+L)	A-11011	Thermo-Fisher	2 mg/mL	Goat	1:400	AlexaFluor568
Goat anti-Hamster IgG (H+L)	A-11008	Thermo-Fisher	2 mg/mL	Goat	1:400	AlexaFluor488
Goat anti-Rabbit IgG (H+L)	A32733TR	Thermo-Fisher	2 mg/mL	Goat	1:400	AlexFluor647 plus
Goat anti-Rat IgG (H+L)	A48265	Thermo-Fisher	2 mg/mL	Goat	1:400	AlexFluor647 plus

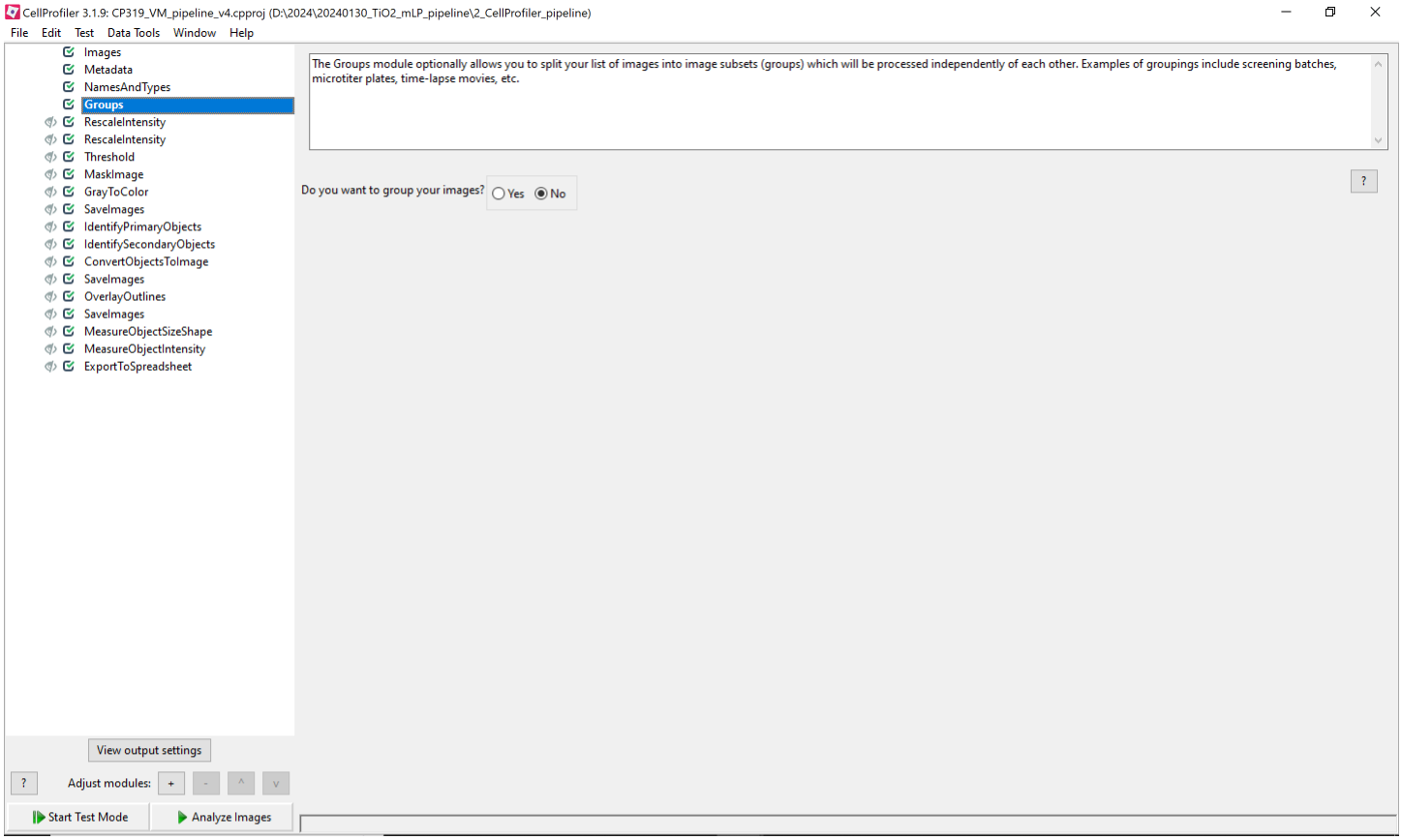
Supplementary Video 1 – 3-D animation demonstrating the selectivity and specificity of $fgTiO_2$ for autofluorescent lysomac / lysoDC immune cells. The video shows a 3-D render of image-data collected as a Z-stack from the murine subepithelial dome tissue compartment using confocal reflectance microscopy. Cell nuclei were fluorescently labelled using Hoechst 33342 (grey). Cytoskeletal actin was labelled with phalloidin-AlexaFluor 647 (red). $fgTiO_2$ (white foci) are seen to selectively and specifically target the highly autofluorescent (blue) mononuclear phagocytic cells.

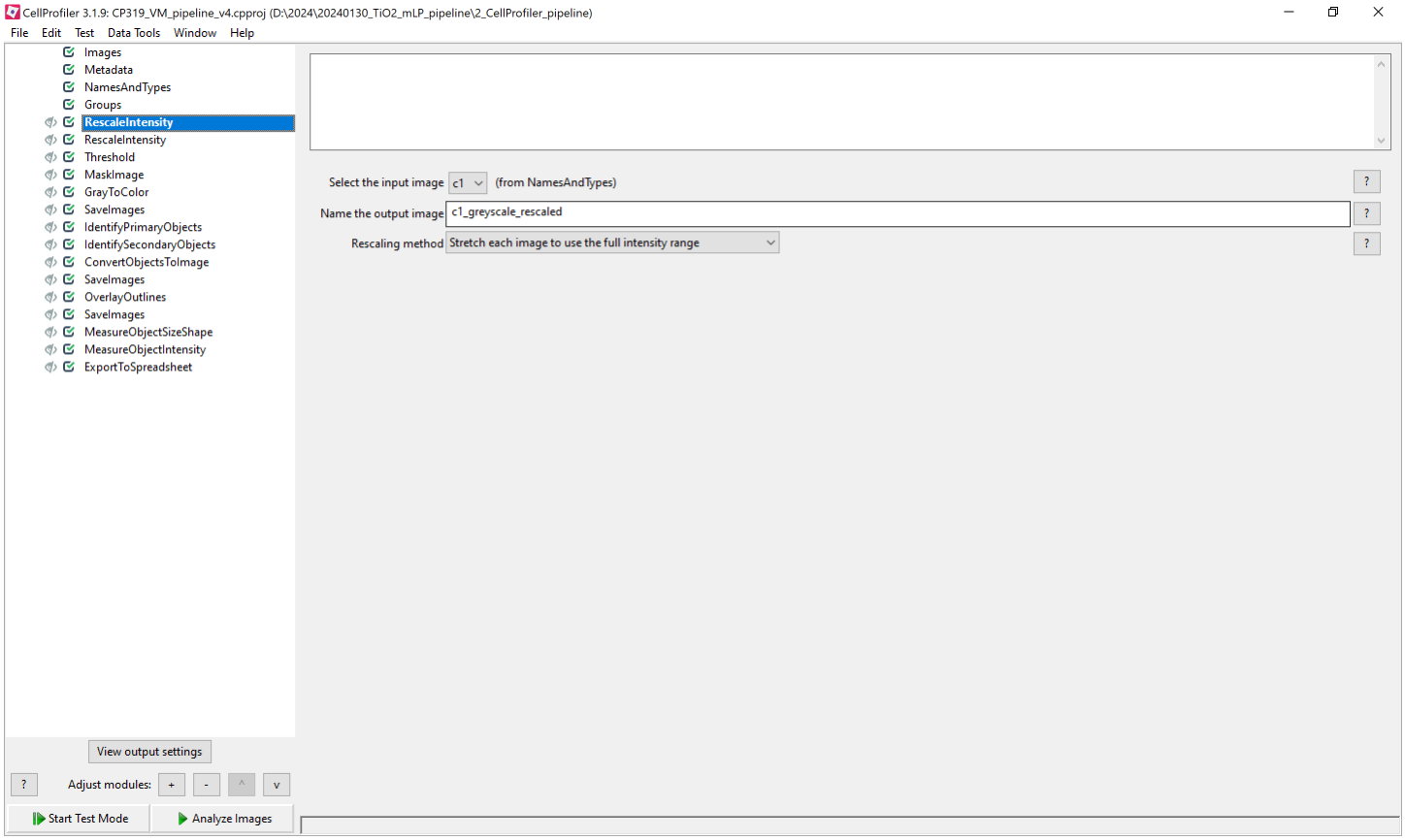
Supplementary Note 1 – Mouse villous mucosa image analysis pipeline. This section presents screenshots of the CellProfiler image analysis pipeline used to measure per-cell intensity and shape/size features enabling the detection of $fgTiO_2$ in the mouse villous mucosa images. The pipeline and sample image data are available for download from the BioStudies archive accompanying the paper.

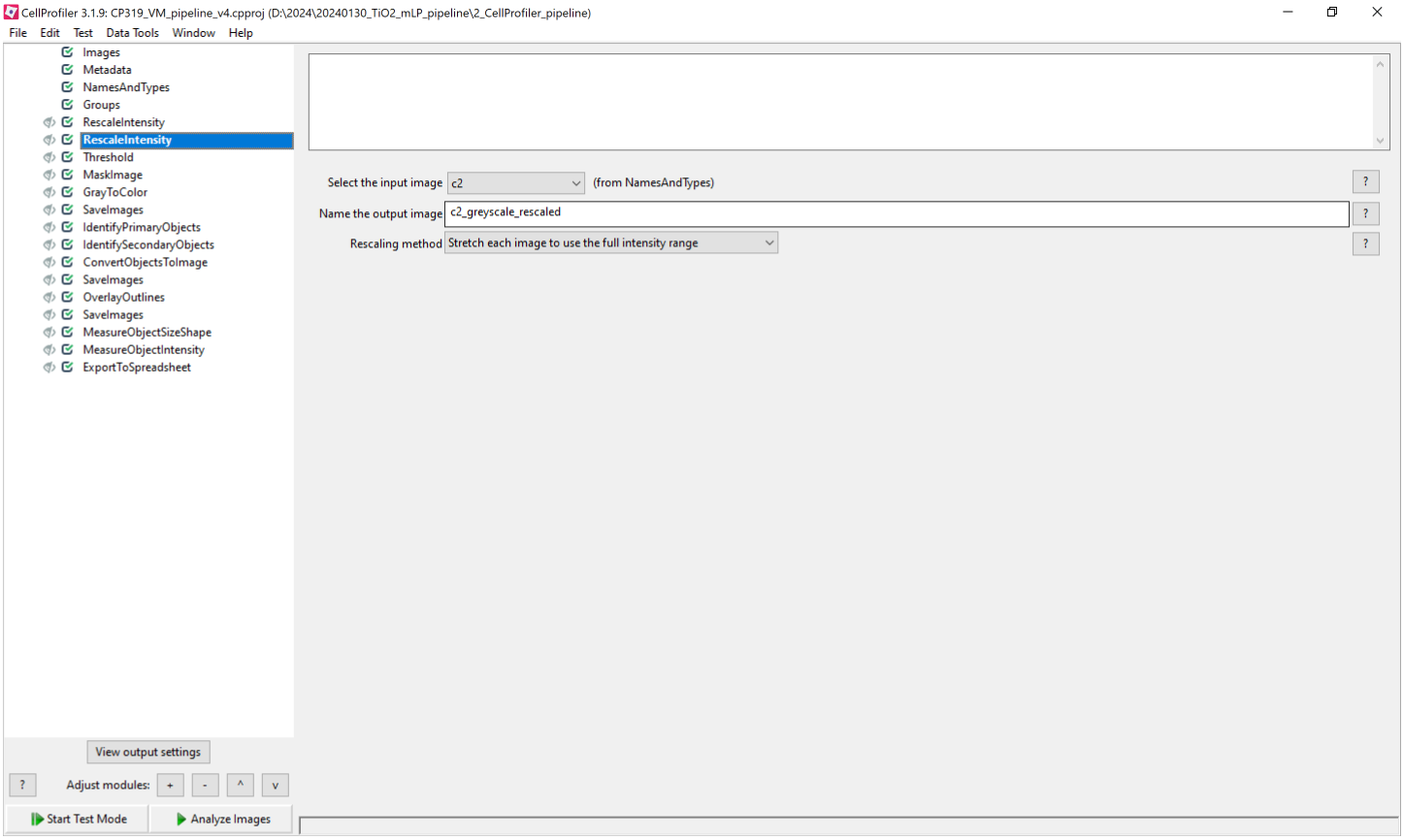


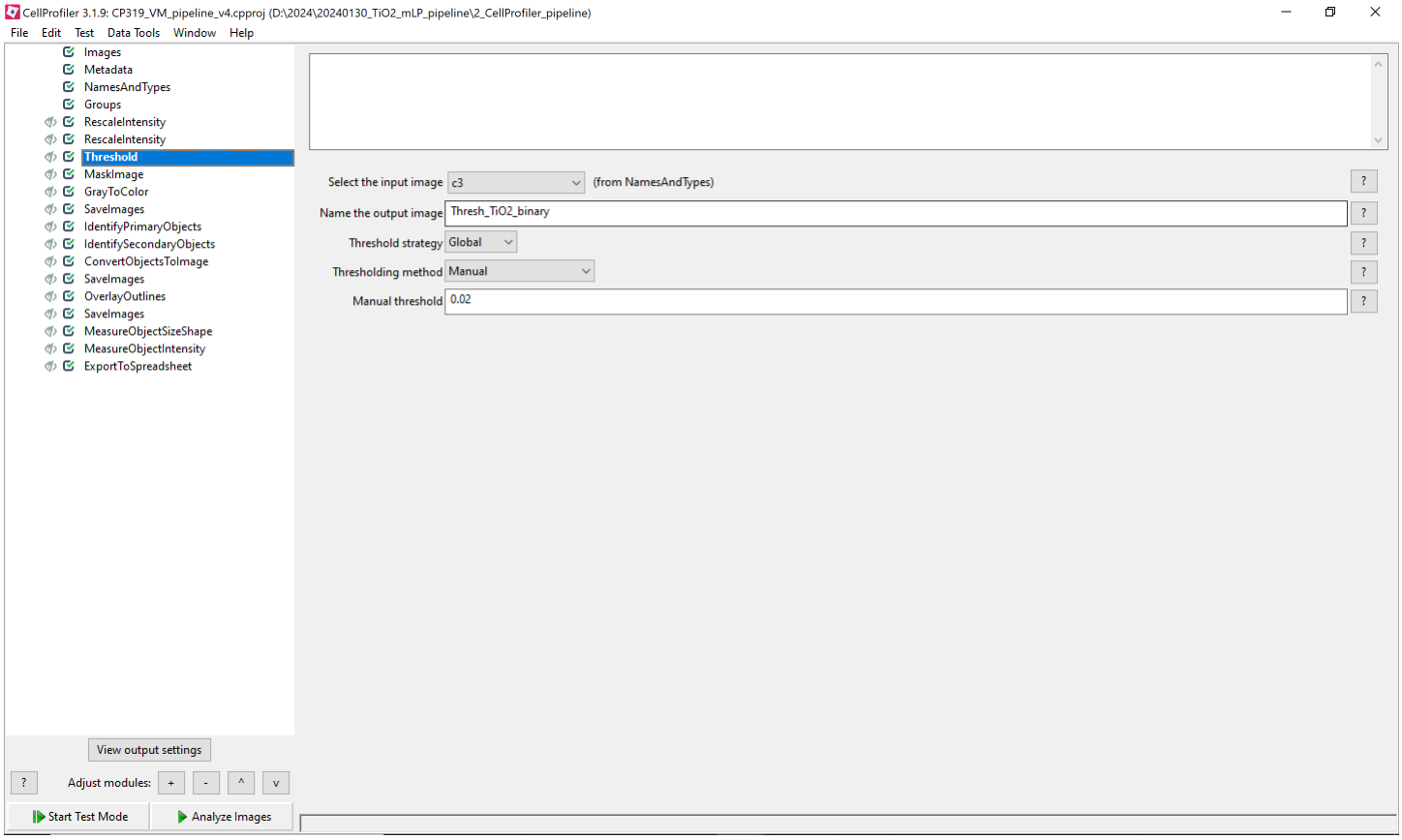

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
The Metadata module optionally allows you to extract information describing your images (i.e, metadata) which will be stored along with your measurements. This information can be contained in the file name and/or location, or in an external file.
Extract metadata?
Yes
No
Metadata extraction method
Extract from file/folder names
Metadata source
File name
Regular expression to extract from file name
^(?P<Ch>.*)_(?P<channel>[0-9])_(?P<Im>.*)_(?P<tile>[0-9]{1,3})
Extract metadata from
All images
Add another extraction method
Metadata data type
Text
Update
View output settings
Adjust modules:
Start Test Mode
Analyze Images

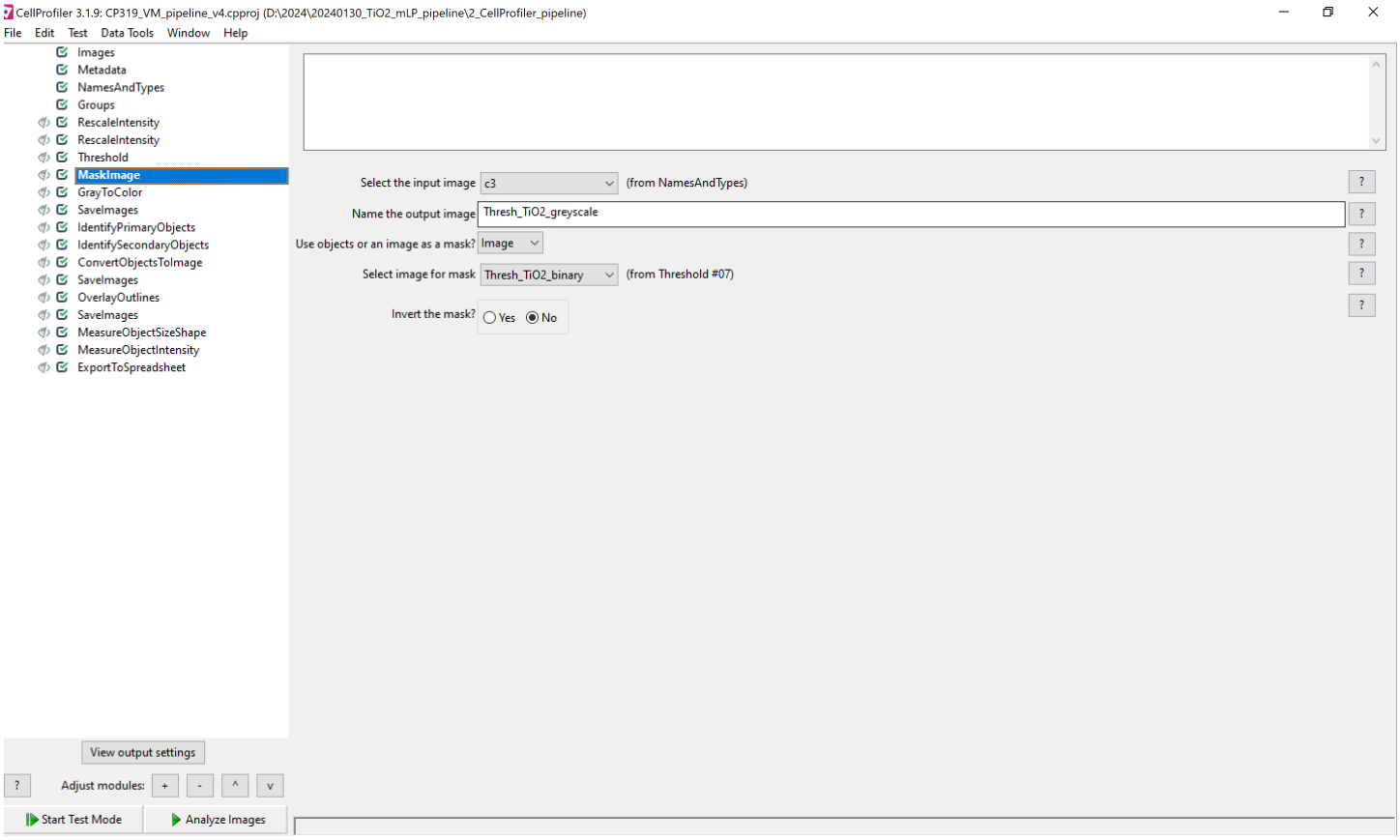

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
View output settings
Adjust modules:
Start Test Mode
Analyze Images
The NamesAndTypes module allows you to assign a meaningful name to each image by which other modules will refer to it.
Assign a name to
Images matching rules
Process as 3D?
Yes
No
Select the rule criteria
Match All of the following rules
Directory Does Contain Channel_1
Name to assign these images
c1
Select the image type
Grayscale image
Set intensity range from
Image bit-depth
Duplicate this image
Match All of the following rules
Directory Does Contain Channel_2
Name to assign these images
c2
Select the image type
Grayscale image
Set intensity range from
Image bit-depth
Update
c1 c2 c3
1 ch_1_Im_002.tif ch_2_Im_002.tif ch_3_Im_002.tif
2 ch_1_Im_003.tif ch_2_Im_003.tif ch_3_Im_003.tif

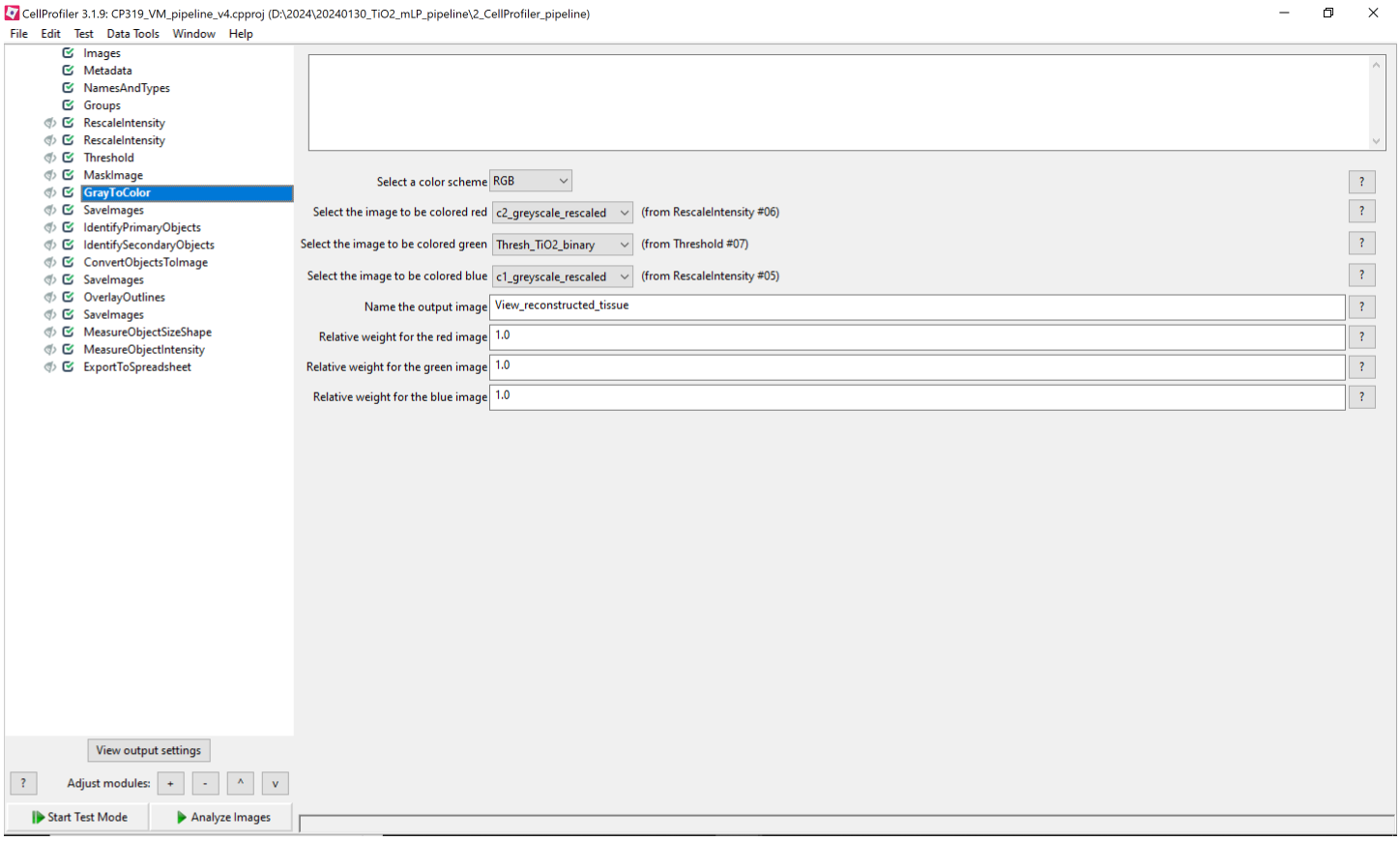

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
The Groups module optionally allows you to split your list of images into image subsets (groups) which will be processed independently of each other. Examples of groupings include screening batches, microtiter plates, time-lapse movies, etc.
Do you want to group your images?
Yes
No
?
View output settings
Adjust modules:
Start Test Mode
Analyze Images

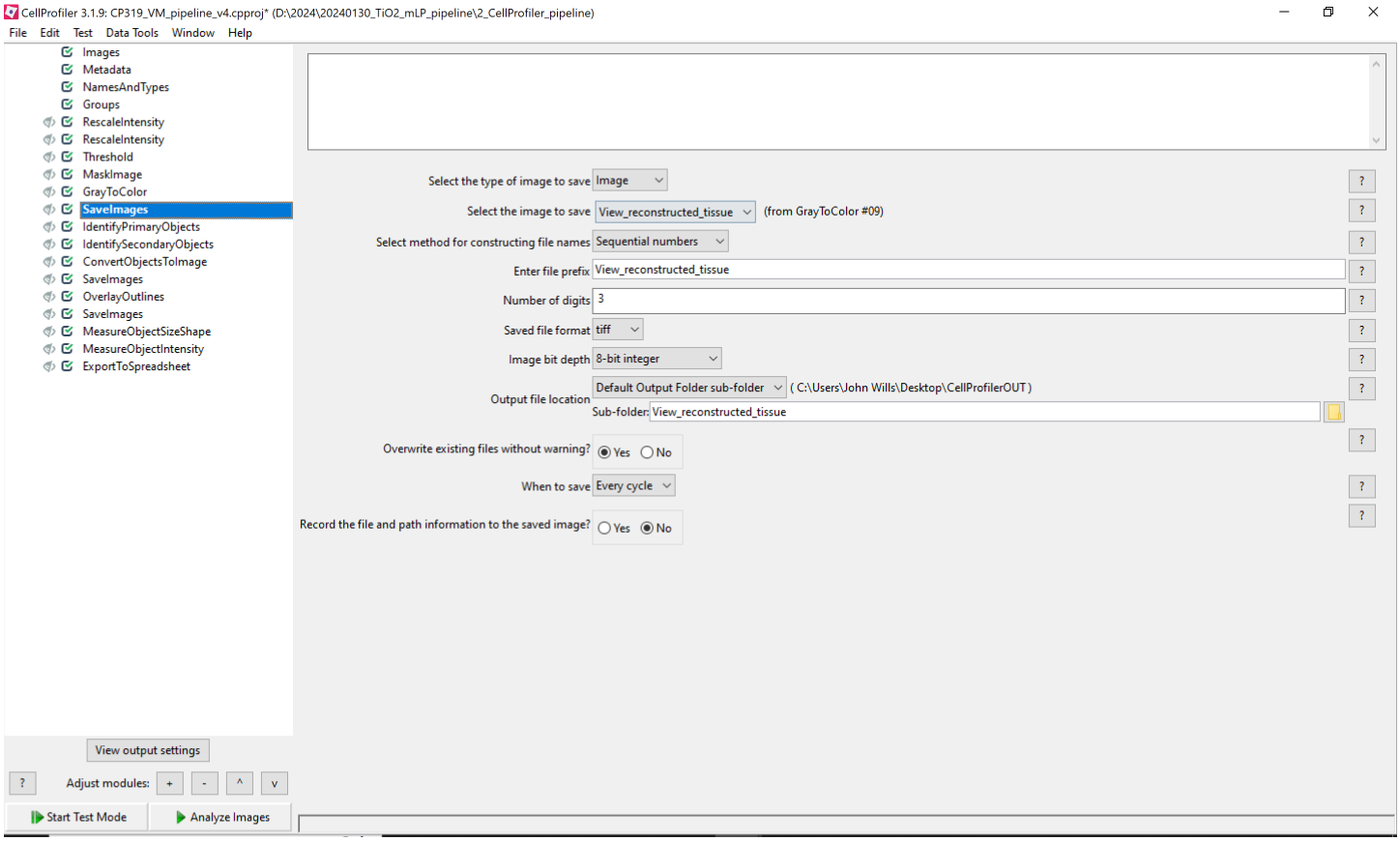

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Select the input image
c1
(from NamesAndTypes)
Name the output image
c1_greyscale_rescaled
Rescaling method
Stretch each image to use the full intensity range

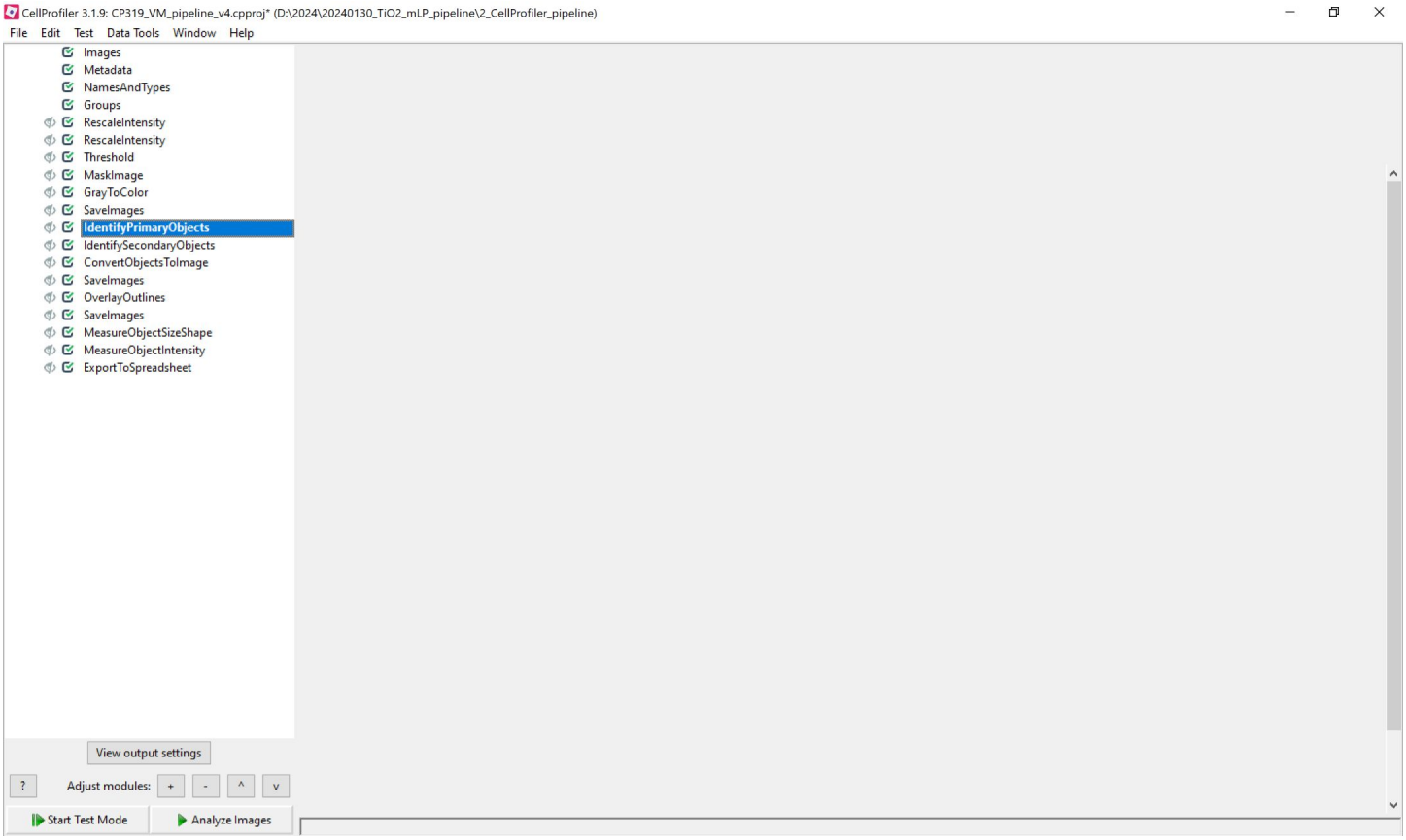

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Select the input image
c2
(from NamesAndTypes)
Name the output image
c2_greyscale_rescaled
Rescaling method
Stretch each image to use the full intensity range
View output settings
Adjust modules:
Start Test Mode
Analyze Images

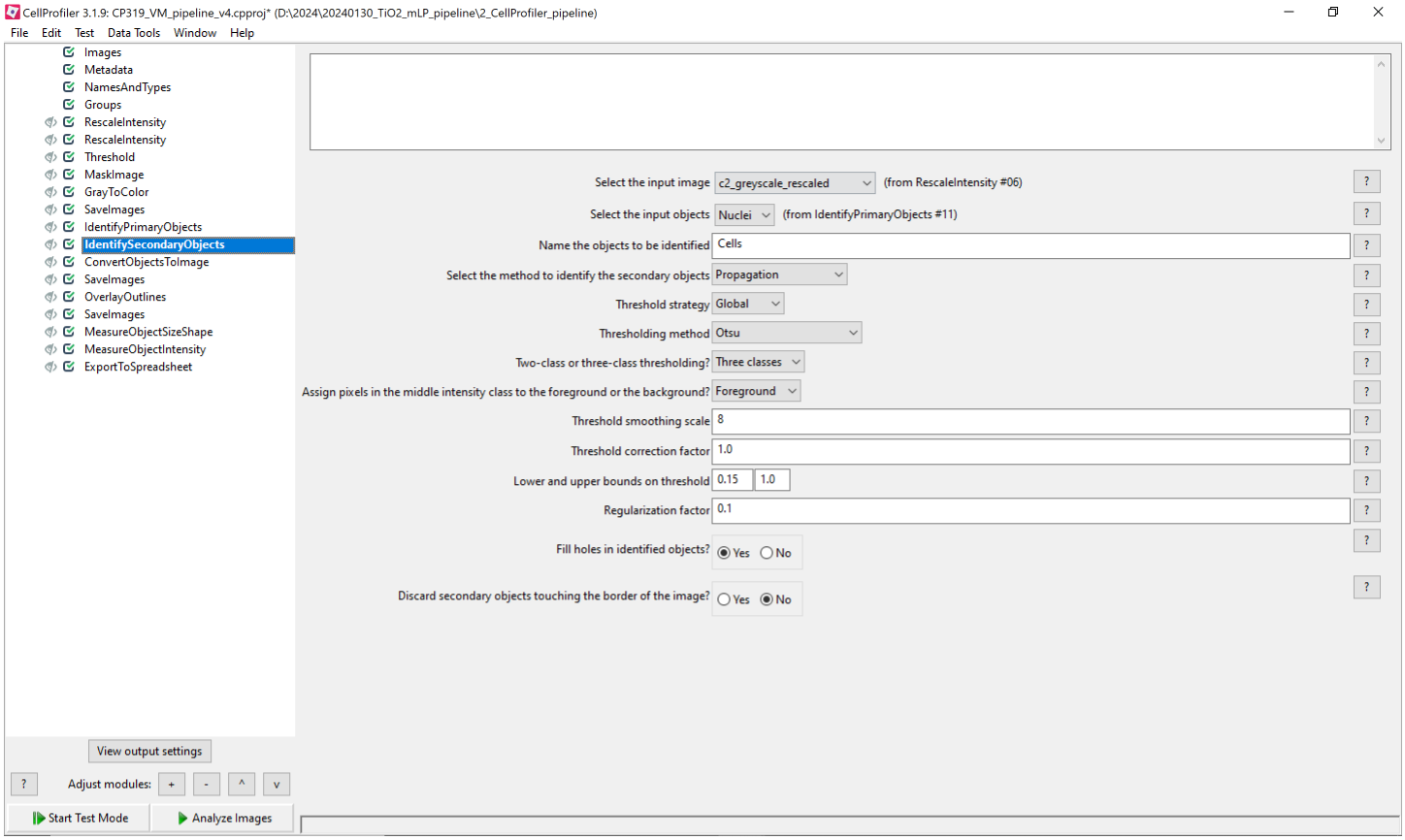

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Select the input image
c3
(from NamesAndTypes)
Name the output image
Thresh_TiO2_binary
Threshold strategy
Global
Thresholding method
Manual
Manual threshold
0.02

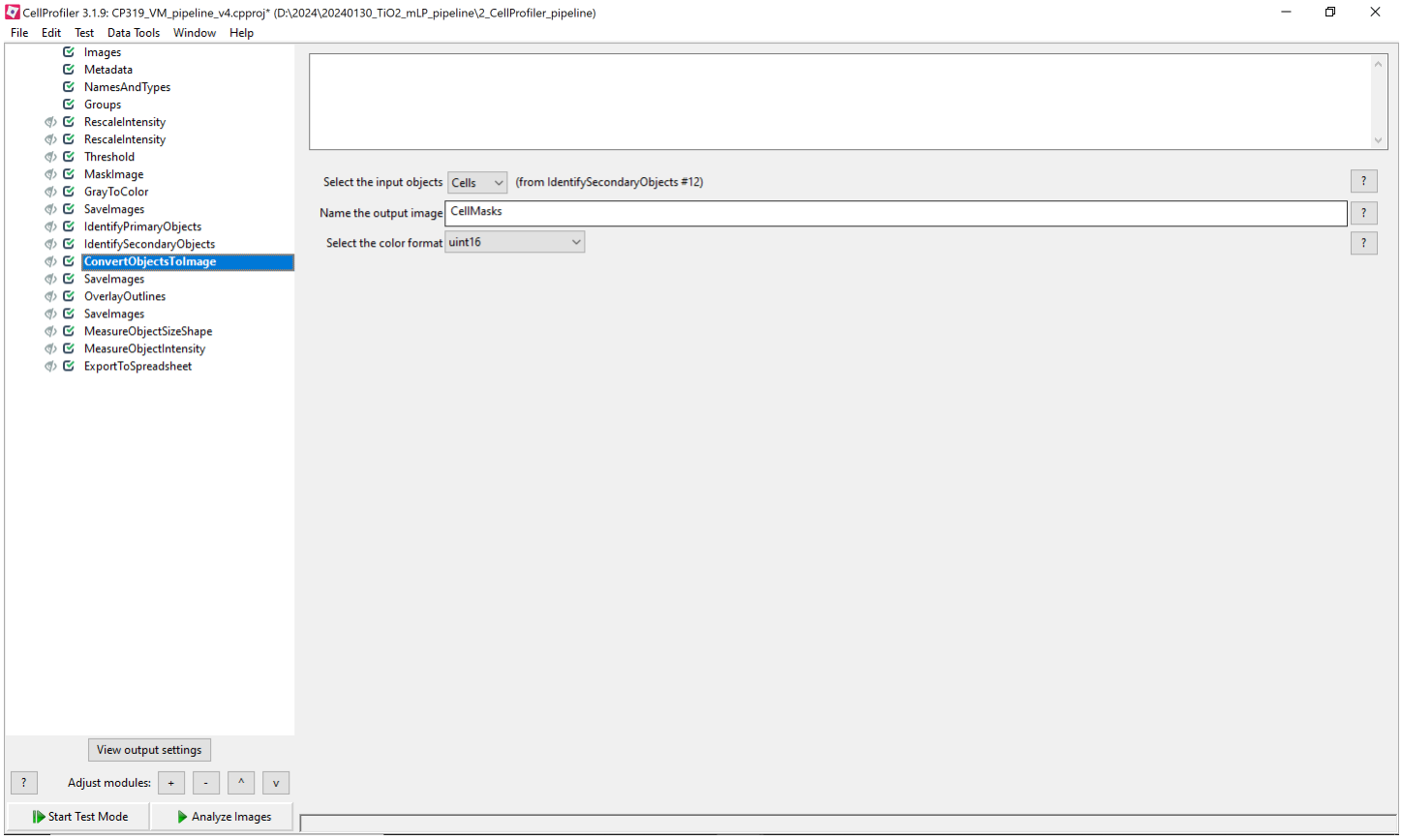

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Select the input image
c3
(from NamesAndTypes)
Name the output image
Thresh_TiO2_greyscale
Use objects or an image as a mask?
Image
Select image for mask
Thresh_TiO2_binary
(from Threshold #07)
Invert the mask?
Yes
No
View output settings
Adjust modules:
Start Test Mode
Analyze Images

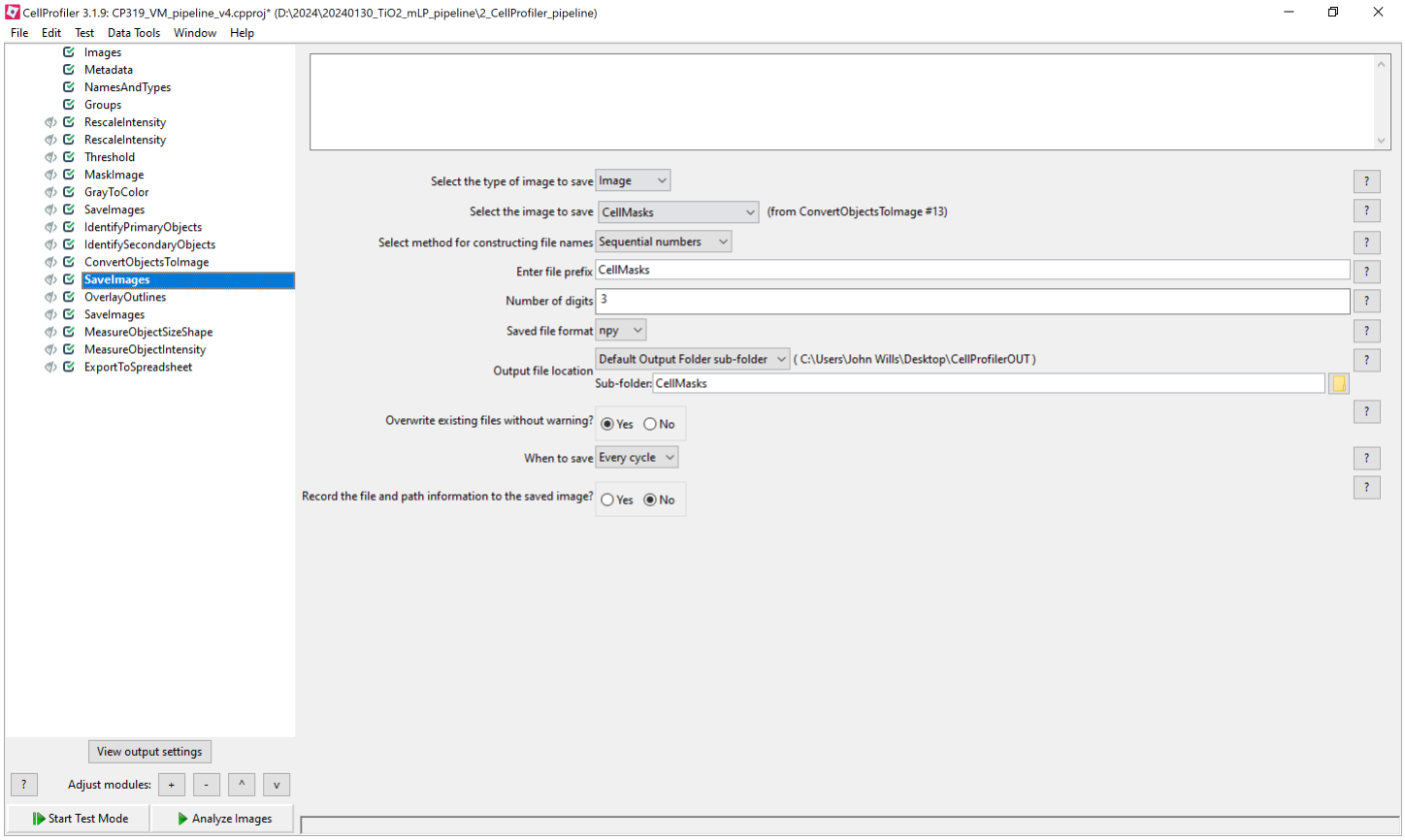

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Select a color scheme
RGB
Select the image to be colored red
c2_greyscale_rescaled
(from RescaleIntensity #06)
Select the image to be colored green
Thresh_TiO2_binary
(from Threshold #07)
Select the image to be colored blue
c1_greyscale_rescaled
(from RescaleIntensity #05)
Name the output image
View_reconstructed_tissue
Relative weight for the red image
1.0
Relative weight for the green image
1.0
Relative weight for the blue image
1.0

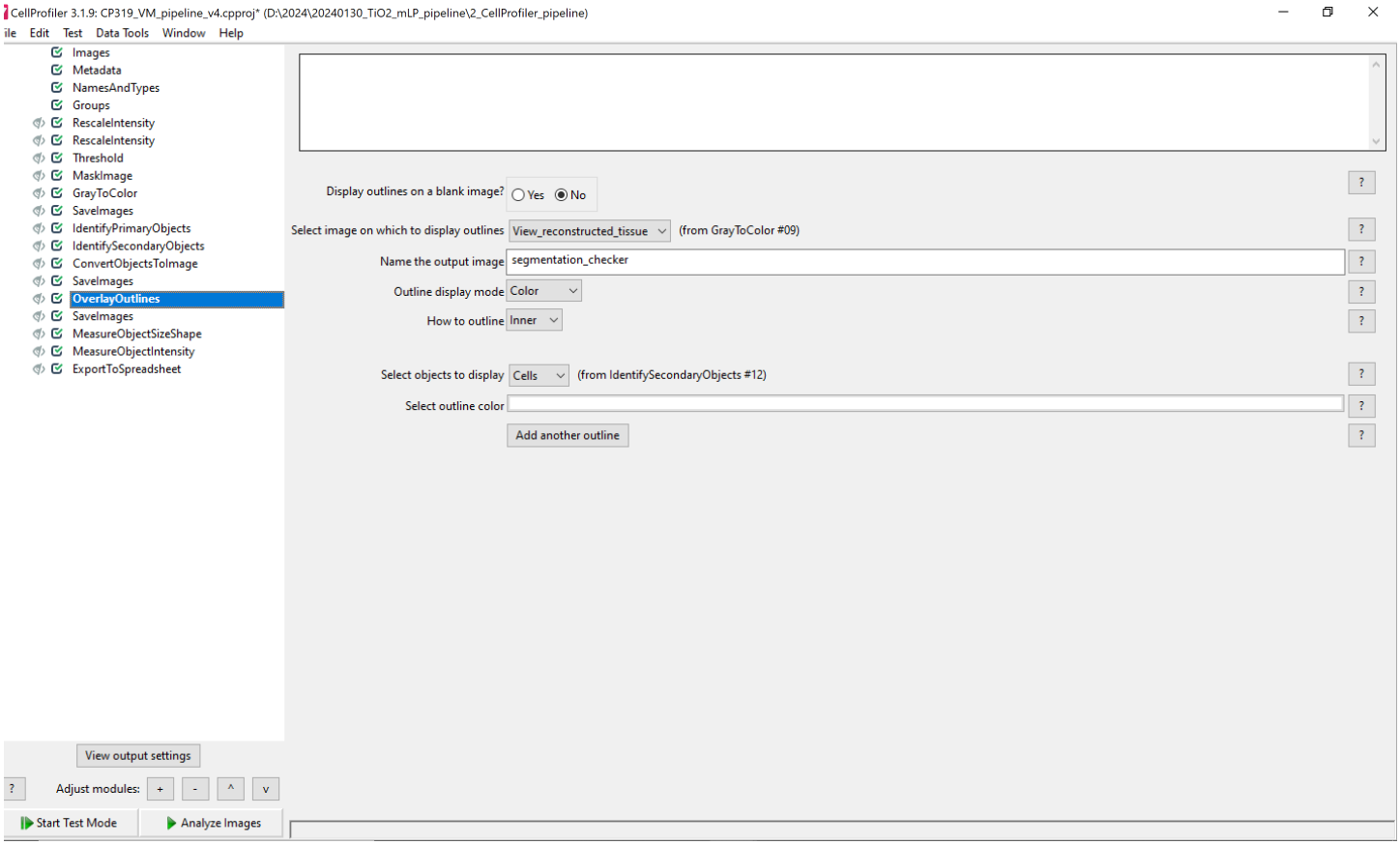

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj* (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Select the type of image to save
Image
Select the image to save
View_reconstructed_tissue
(from GrayToColor #09)
Select method for constructing file names
Sequential numbers
Enter file prefix
View_reconstructed_tissue
Number of digits
3
Saved file format
tiff
Image bit depth
8-bit integer
Output file location
Default Output Folder sub-folder
(C:\Users\John Wills\Desktop\CellProfilerOUT)
Sub-folder:
View_reconstructed_tissue
Overwrite existing files without warning?
Yes
No
When to save
Every cycle
Record the file and path information to the saved image?
Yes
No

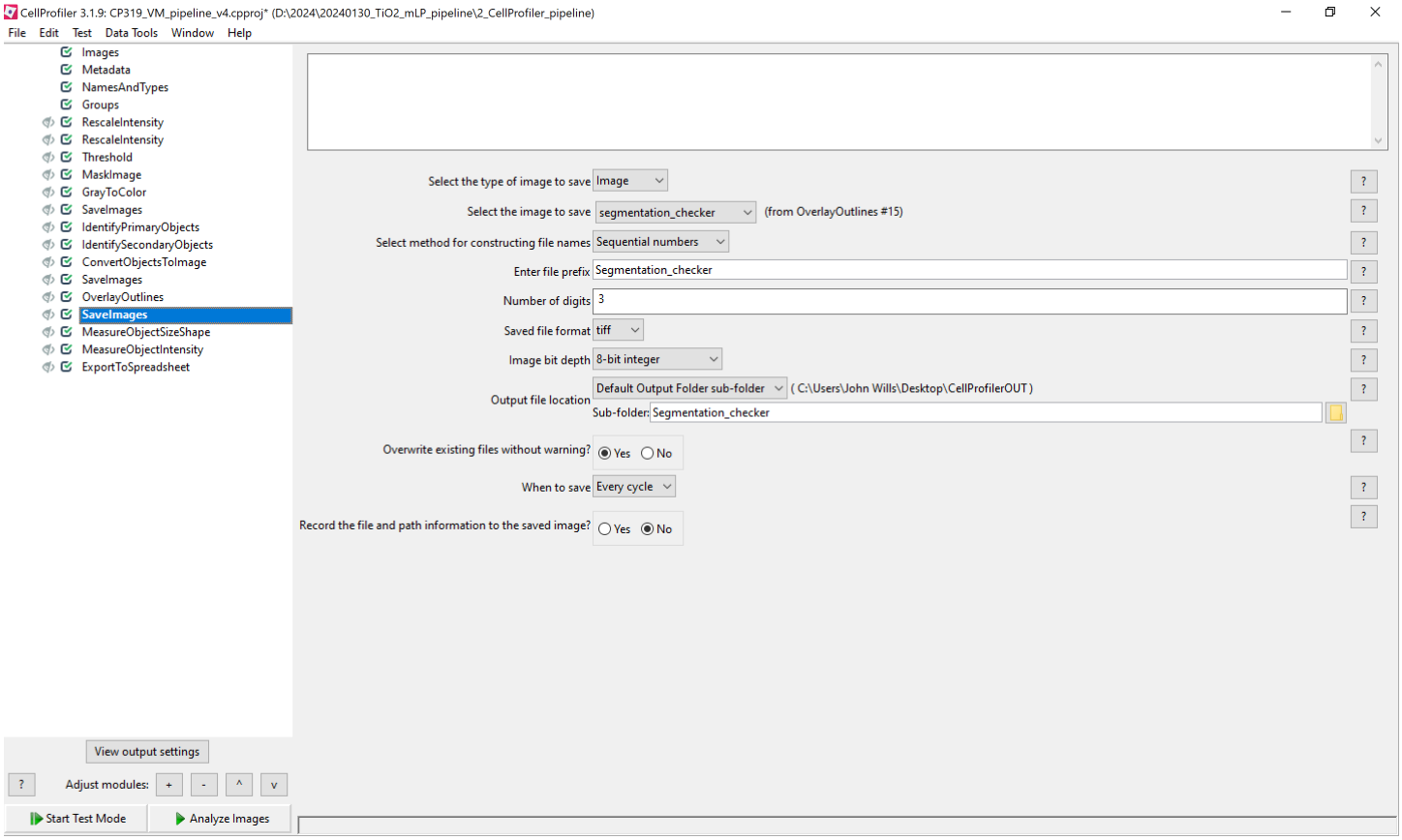

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj* (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
View output settings
?
Adjust modules:
+
-
^
v
Start Test Mode
Analyze Images

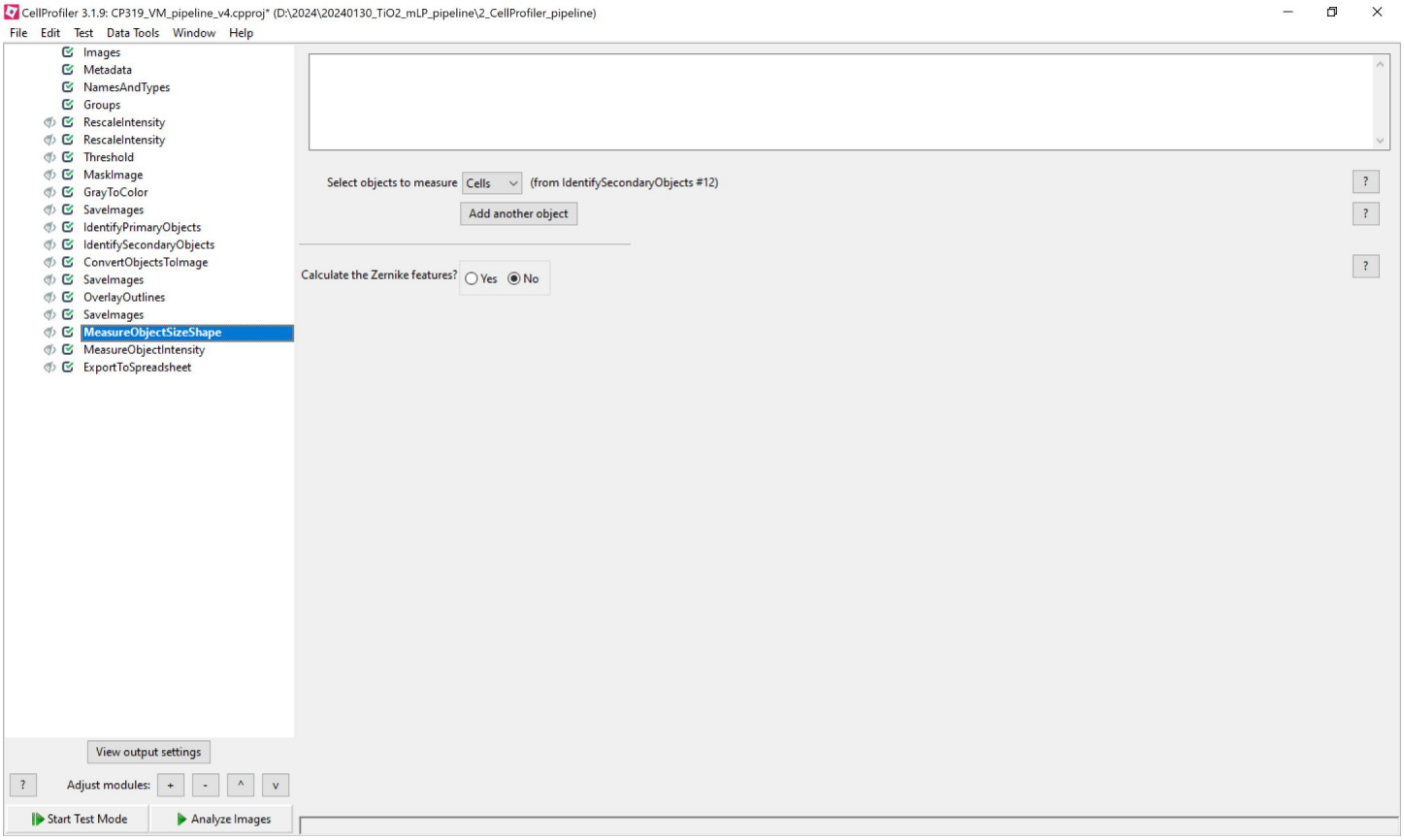

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj* (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Select the input image
c2_greyscale_rescaled
(from RescaleIntensity #06)
Select the input objects
Nuclei
(from IdentifyPrimaryObjects #11)
Name the objects to be identified
Cells
Select the method to identify the secondary objects
Propagation
Threshold strategy
Global
Thresholding method
Otsu
Two-class or three-class thresholding?
Three classes
Assign pixels in the middle intensity class to the foreground or the background?
Foreground
Threshold smoothing scale
8
Threshold correction factor
1.0
Lower and upper bounds on threshold
0.15
1.0
Regularization factor
0.1
Fill holes in identified objects?
Yes
No
Discard secondary objects touching the border of the image?
Yes
No

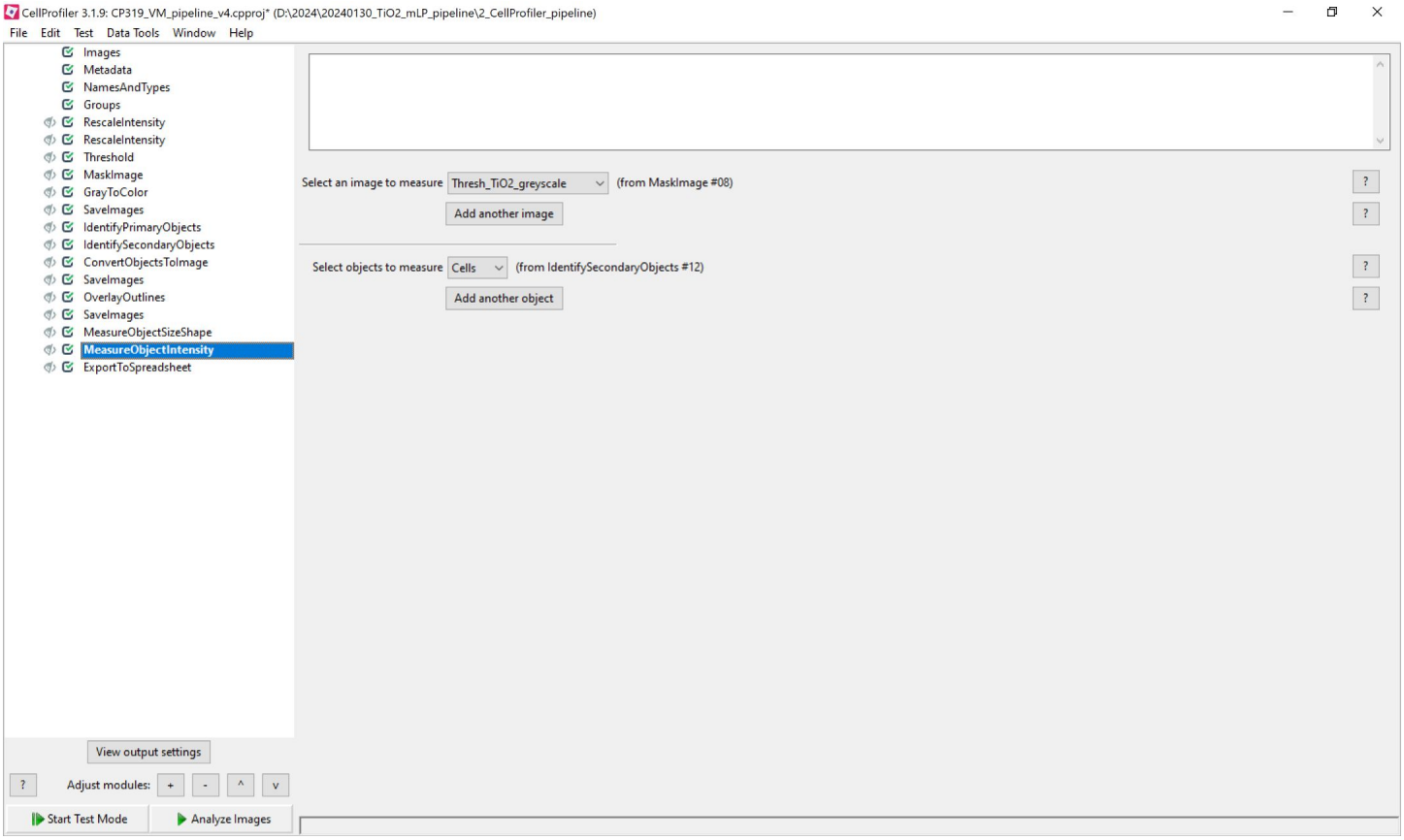

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj* (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Select the input objects
Cells
(from IdentifySecondaryObjects #12)
Name the output image
CellMasks
Select the color format
uint16
View output settings
Adjust modules:
Start Test Mode
Analyze Images

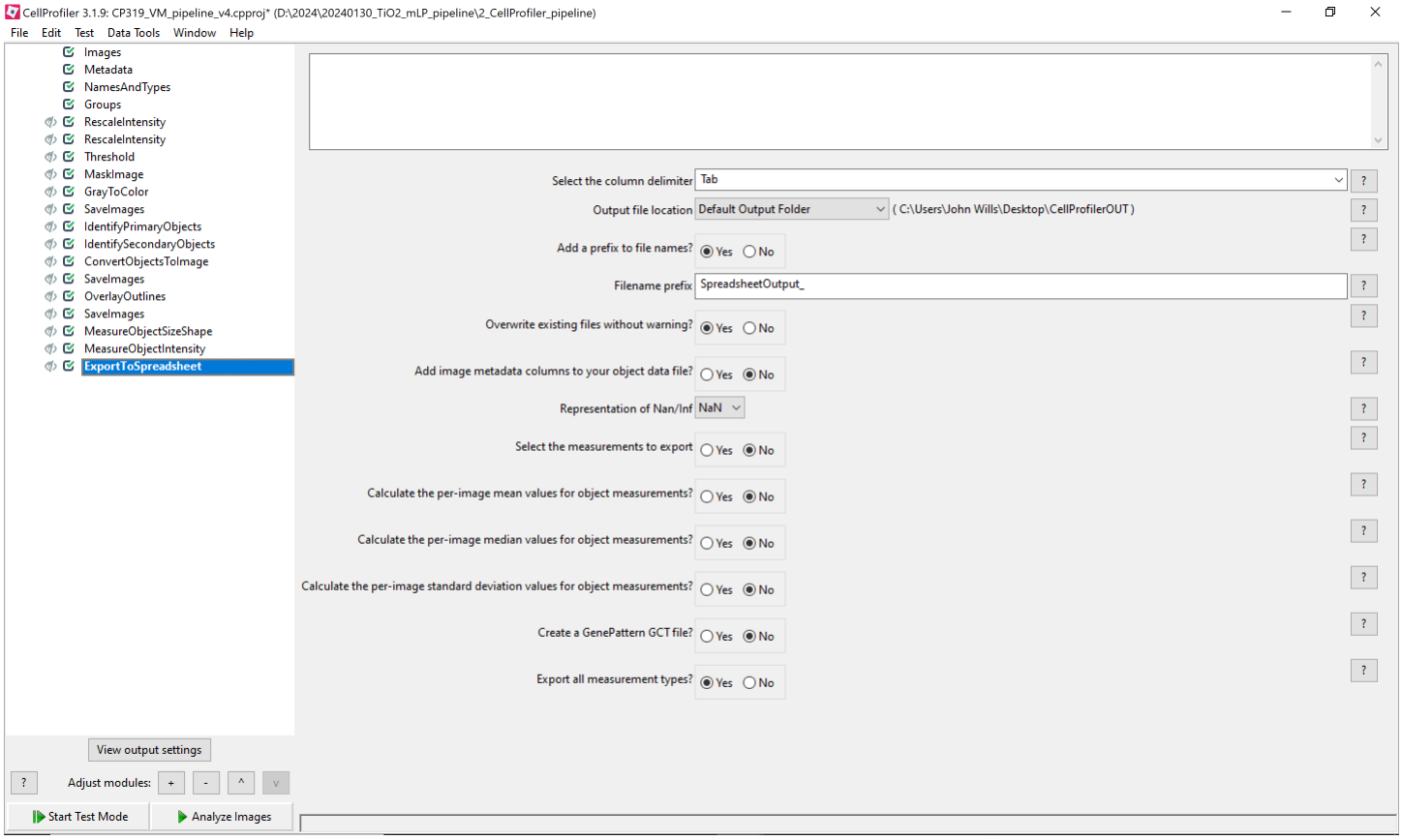

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj* (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Select the type of image to save
Image
Select the image to save
CellMasks
(from ConvertObjectsToImage #13)
Select method for constructing file names
Sequential numbers
Enter file prefix
CellMasks
Number of digits
3
Saved file format
npy
Output file location
Default Output Folder sub-folder
(C:\Users\John Wills\Desktop\CellProfilerOUT)
Sub-folder:
CellMasks
Overwrite existing files without warning?
Yes
No
When to save
Every cycle
Record the file and path information to the saved image?
Yes
No
View output settings
Adjust modules:
Start Test Mode
Analyze Images


CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj* (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
ile Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Display outlines on a blank image?
Yes
No
Select image on which to display outlines
View_reconstructed_tissue
(from GrayToColor #09)
Name the output image
segmentation_checker
Outline display mode
Color
How to outline
Inner
Select objects to display
Cells
(from IdentifySecondaryObjects #12)
Select outline color
Add another outline
View output settings
Adjust modules:
Start Test Mode
Analyze Images

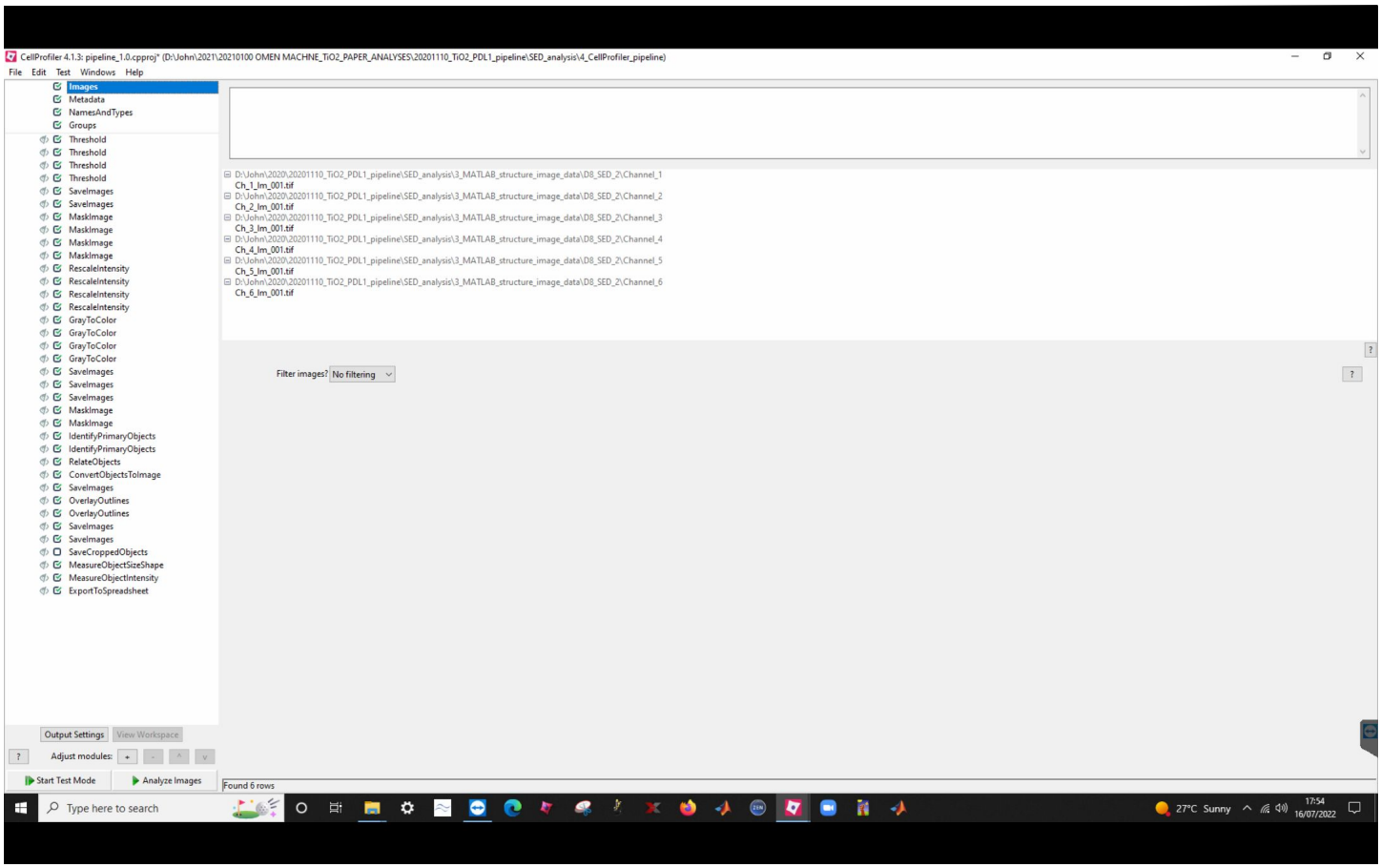

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj* (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Select the type of image to save
Image
Select the image to save
segmentation_checker
(from OverlayOutlines #15)
Select method for constructing file names
Sequential numbers
Enter file prefix
Segmentation_checker
Number of digits
3
Saved file format
tiff
Image bit depth
8-bit integer
Output file location
Default Output Folder sub-folder
(C:\Users\John Wills\Desktop\CellProfilerOUT)
Sub-folder:
Segmentation_checker
Overwrite existing files without warning?
Yes
No
When to save
Every cycle
Record the file and path information to the saved image?
Yes
No
View output settings
Adjust modules:
Start Test Mode
Analyze Images

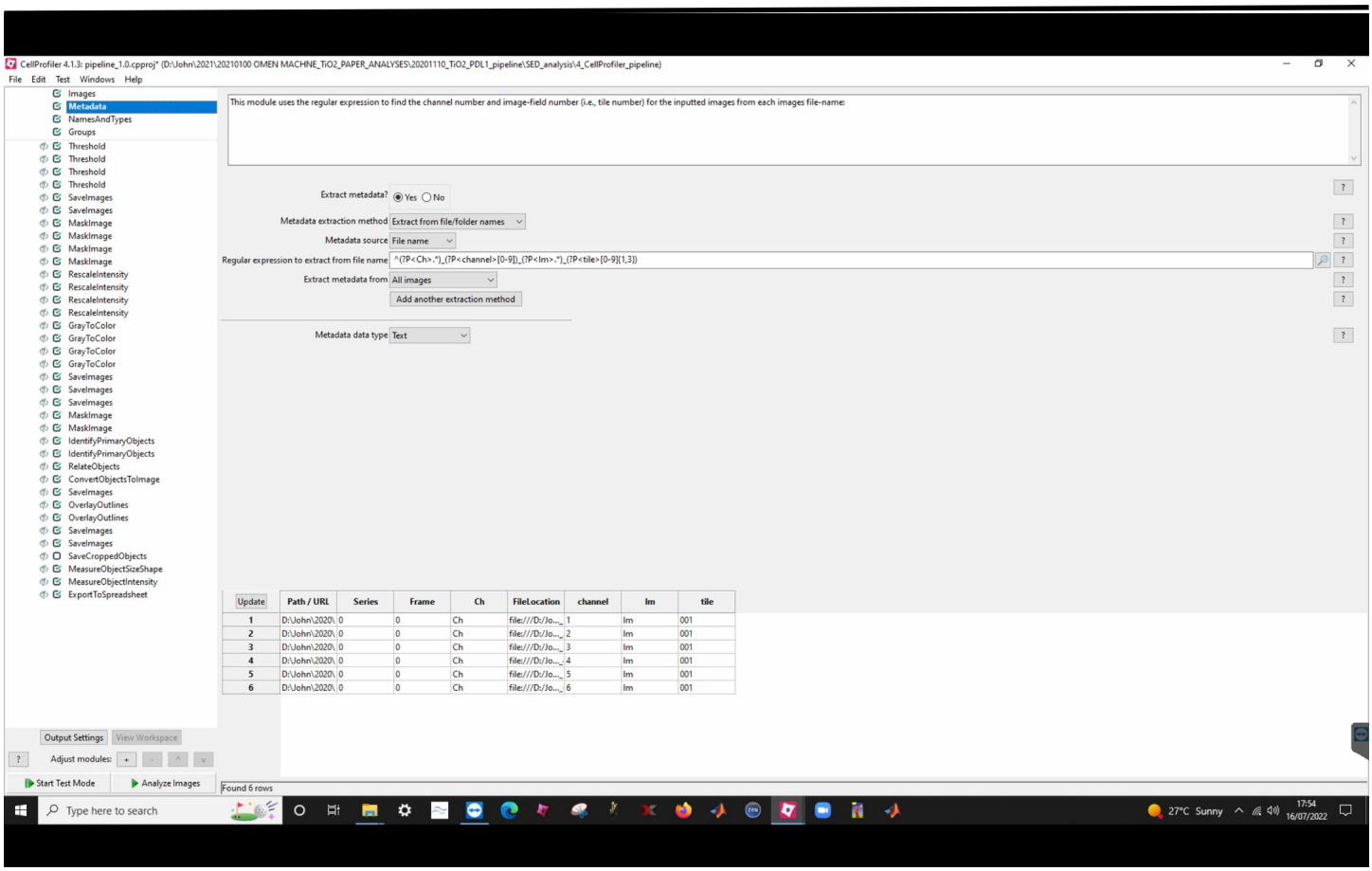

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj* (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Select objects to measure
Cells
(from IdentifySecondaryObjects #12)
Add another object
Calculate the Zernike features?
Yes
No
View output settings
Adjust modules:
Start Test Mode
Analyze Images

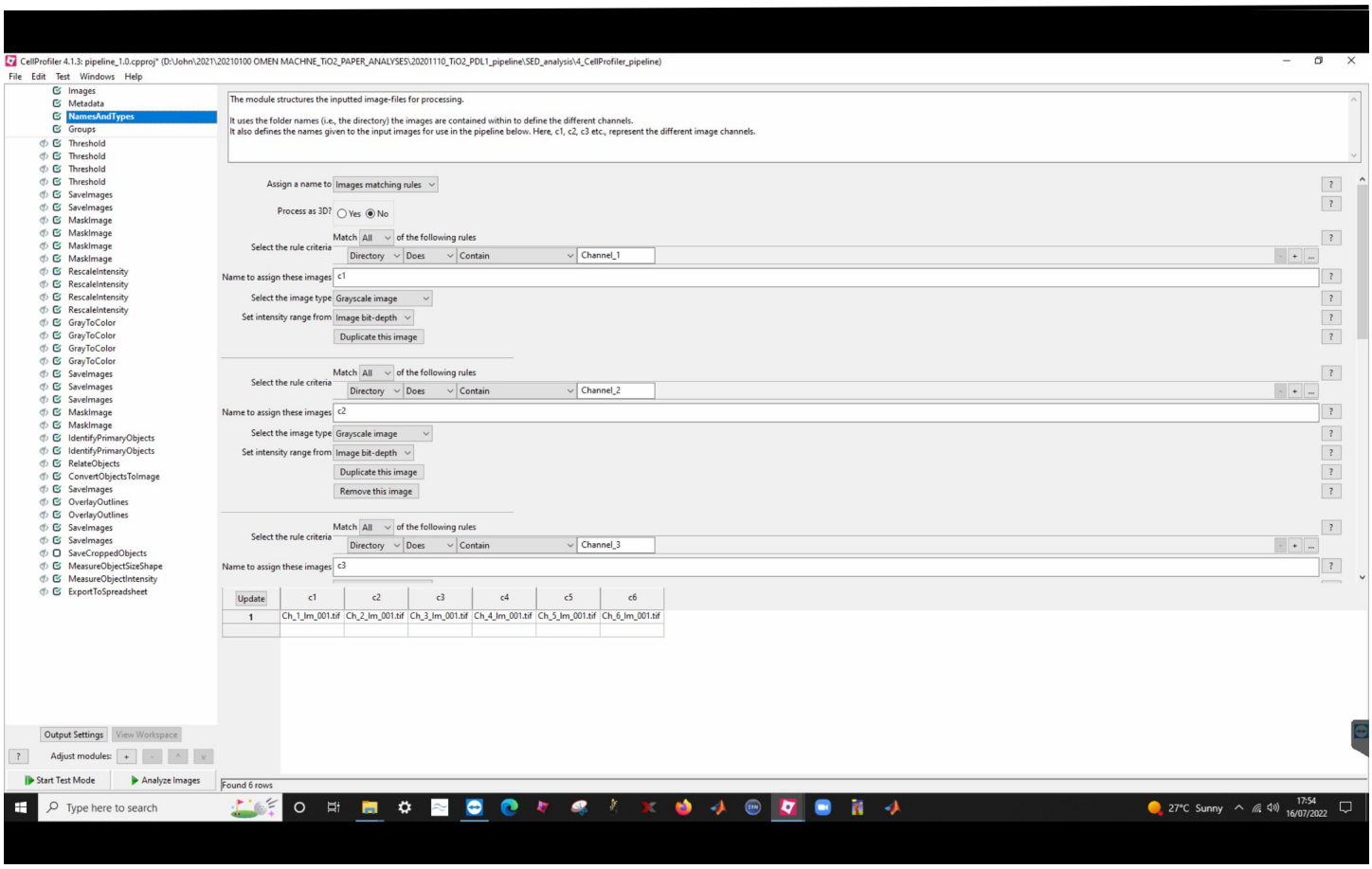

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj* (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Select an image to measure
Thresh_TiO2_greyscale
(from MaskImage #08)
Add another image
Select objects to measure
Cells
(from IdentifySecondaryObjects #12)
Add another object
View output settings
Adjust modules:
Start Test Mode
Analyze Images

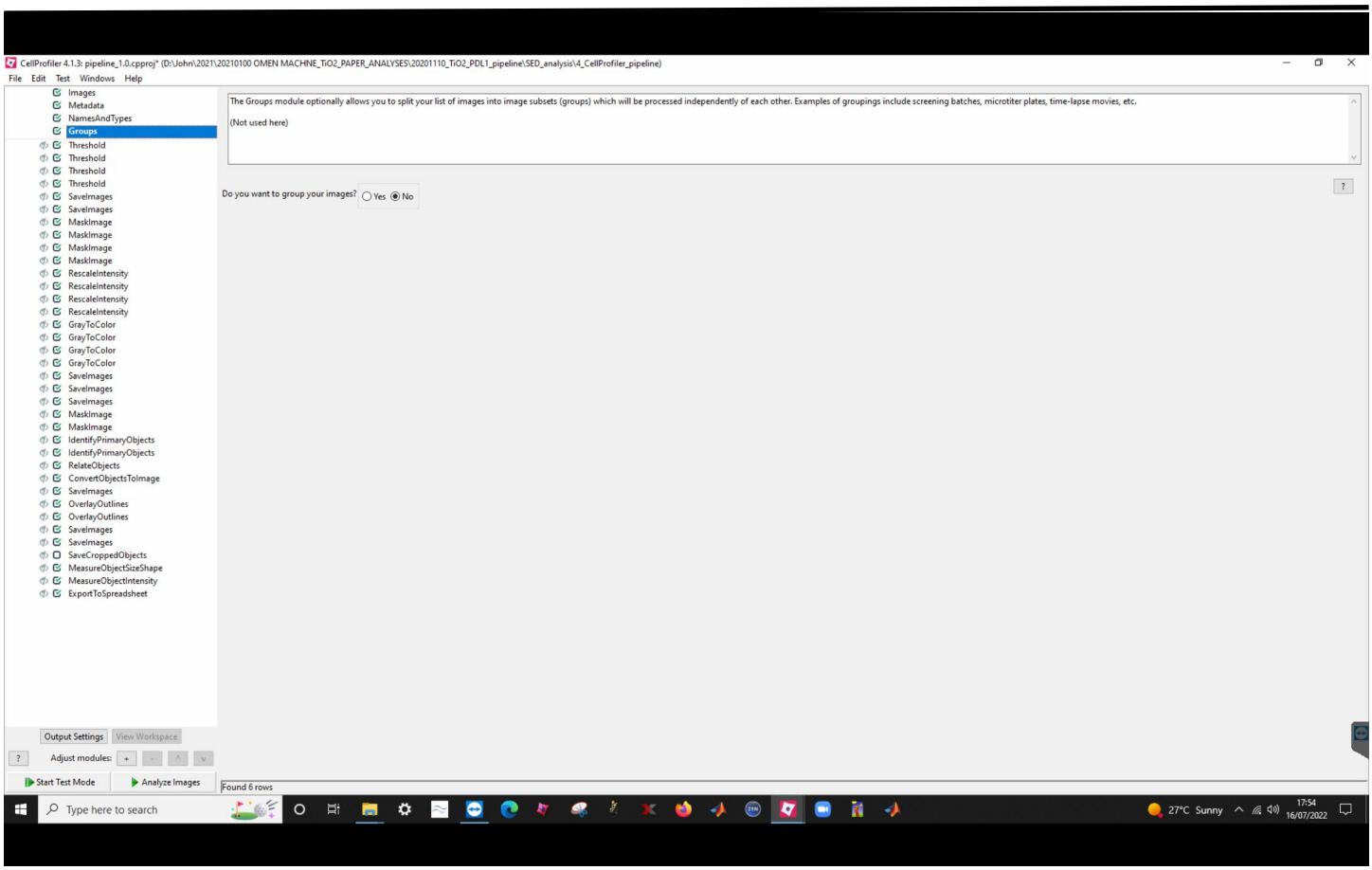

CellProfiler 3.1.9: CP319_VM_pipeline_v4.cpproj* (D:\2024\20240130_TiO2_mLP_pipeline\2_CellProfiler_pipeline)
File Edit Test Data Tools Window Help
Images
Metadata
NamesAndTypes
Groups
RescaleIntensity
RescaleIntensity
Threshold
MaskImage
GrayToColor
SaveImages
IdentifyPrimaryObjects
IdentifySecondaryObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
SaveImages
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Select the column delimiter
Tab
Output file location
Default Output Folder
(C:\Users\John Wills\Desktop\CellProfilerOUT)
Add a prefix to file names?
Yes No
Filename prefix
SpreadsheetOutput_
Overwrite existing files without warning?
Yes No
Add image metadata columns to your object data file?
Yes No
Representation of Nan/Inf
NaN
Select the measurements to export
Yes No
Calculate the per-image mean values for object measurements?
Yes No
Calculate the per-image median values for object measurements?
Yes No
Calculate the per-image standard deviation values for object measurements?
Yes No
Create a GenePattern GCT file?
Yes No
Export all measurement types?
Yes No

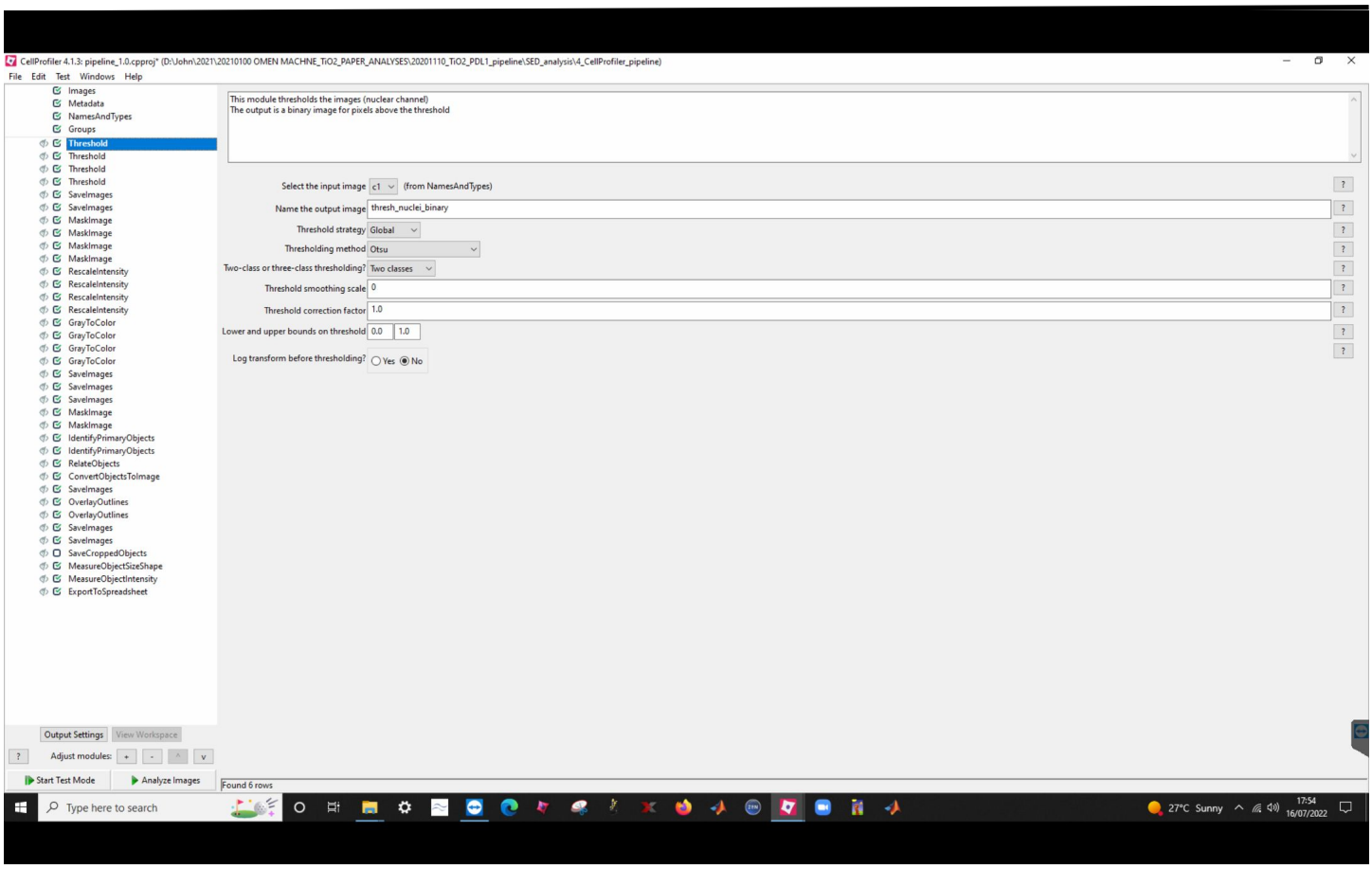
Supplementary Note 2 – Mouse lymphoid tissue image analysis pipeline using UNET-generated probability maps: This section presents screenshots of the CellProfiler image analysis pipeline used to measure per-cell reflectance ($fgTiO_2$) and immunofluorescence intensities (here, PD-L1 quantification from **Figure 4** is shown) and cell shape/size features. The pipeline and sample image data are available for download from the BioStudies archive accompanying the paper.

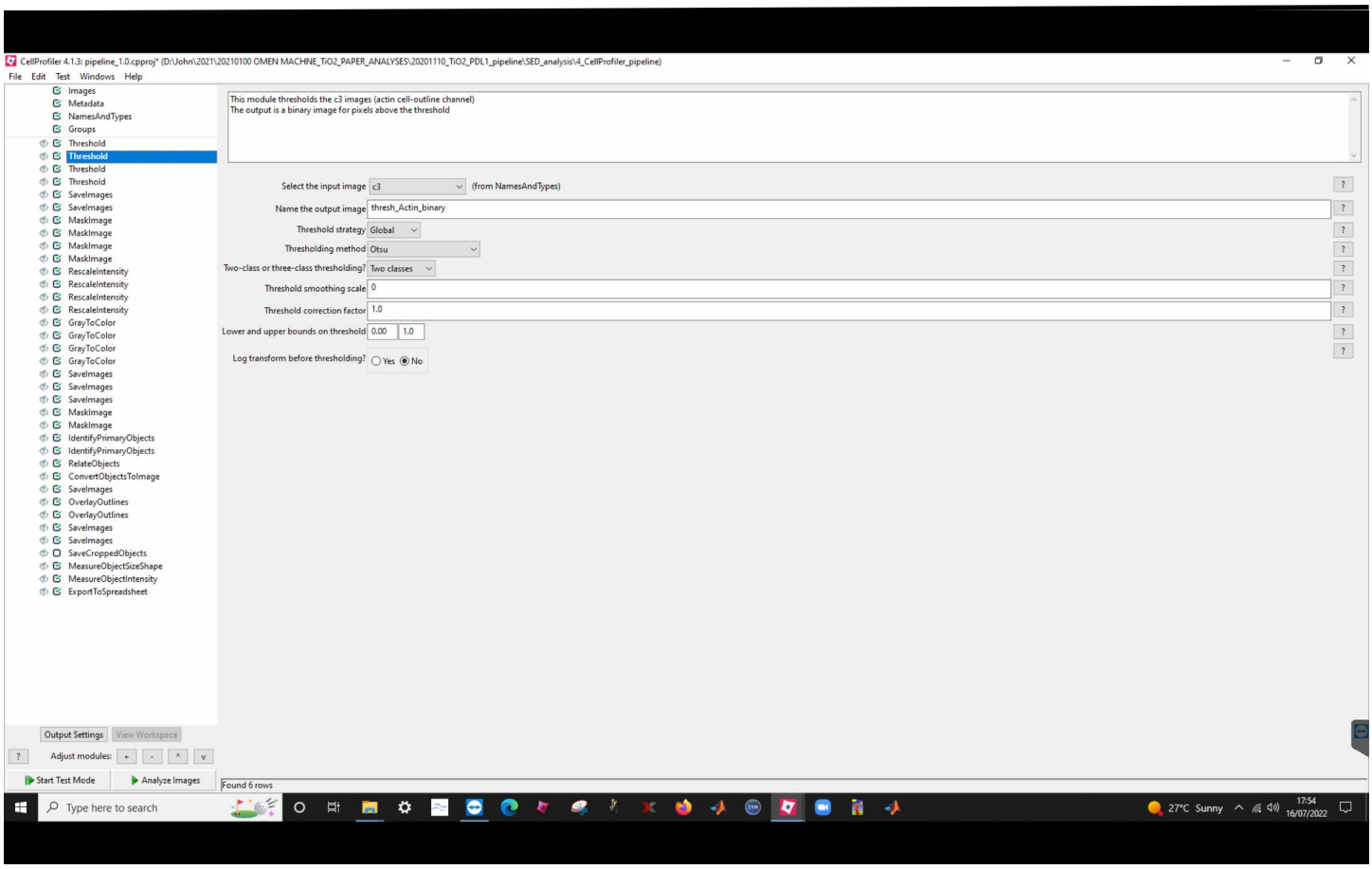


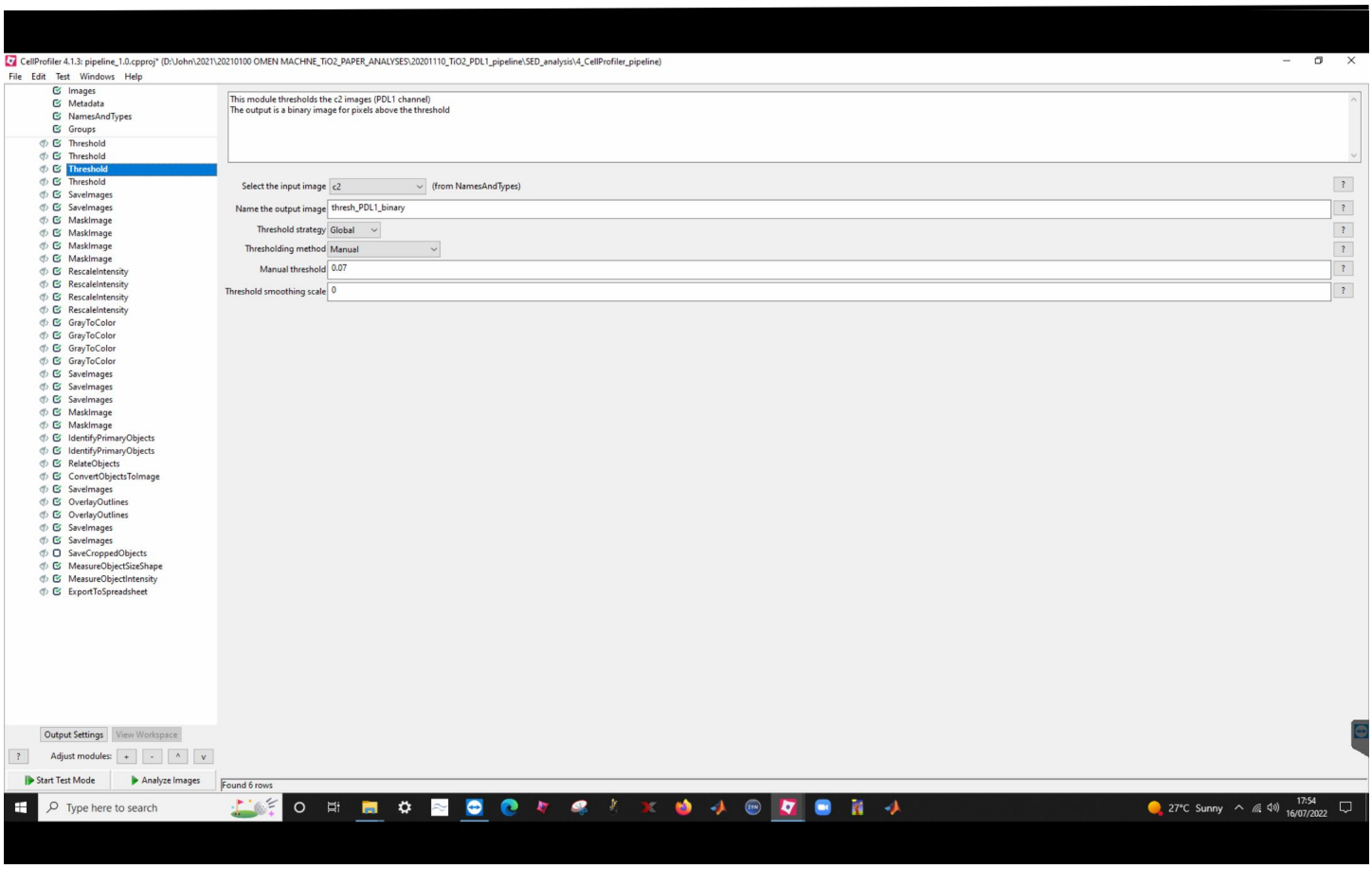

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
This module uses the regular expression to find the channel number and image-field number (i.e., tile number) for the inputted images from each images file-name:
Extract metadata? Yes No
Metadata extraction method Extract from file/folder names
Metadata source File name
Regular expression to extract from file name ^(?P<Ch>.*)_(?P<channel>[0-9])_(?P<Im>.*)_(?P<tile>[0-9][1,3])
Extract metadata from All images
Add another extraction method
Metadata data type Text
Update
Path / URL Series Frame Ch FileLocation channel Im tile
1 D:\John\2020\ 0 0 Ch file:///D:/Jo..._ 1 Im 001
2 D:\John\2020\ 0 0 Ch file:///D:/Jo..._ 2 Im 001
3 D:\John\2020\ 0 0 Ch file:///D:/Jo..._ 3 Im 001
4 D:\John\2020\ 0 0 Ch file:///D:/Jo..._ 4 Im 001
5 D:\John\2020\ 0 0 Ch file:///D:/Jo..._ 5 Im 001
6 D:\John\2020\ 0 0 Ch file:///D:/Jo..._ 6 Im 001
Found 6 rows
Type here to search
27°C Sunny
17:54
16/07/2022

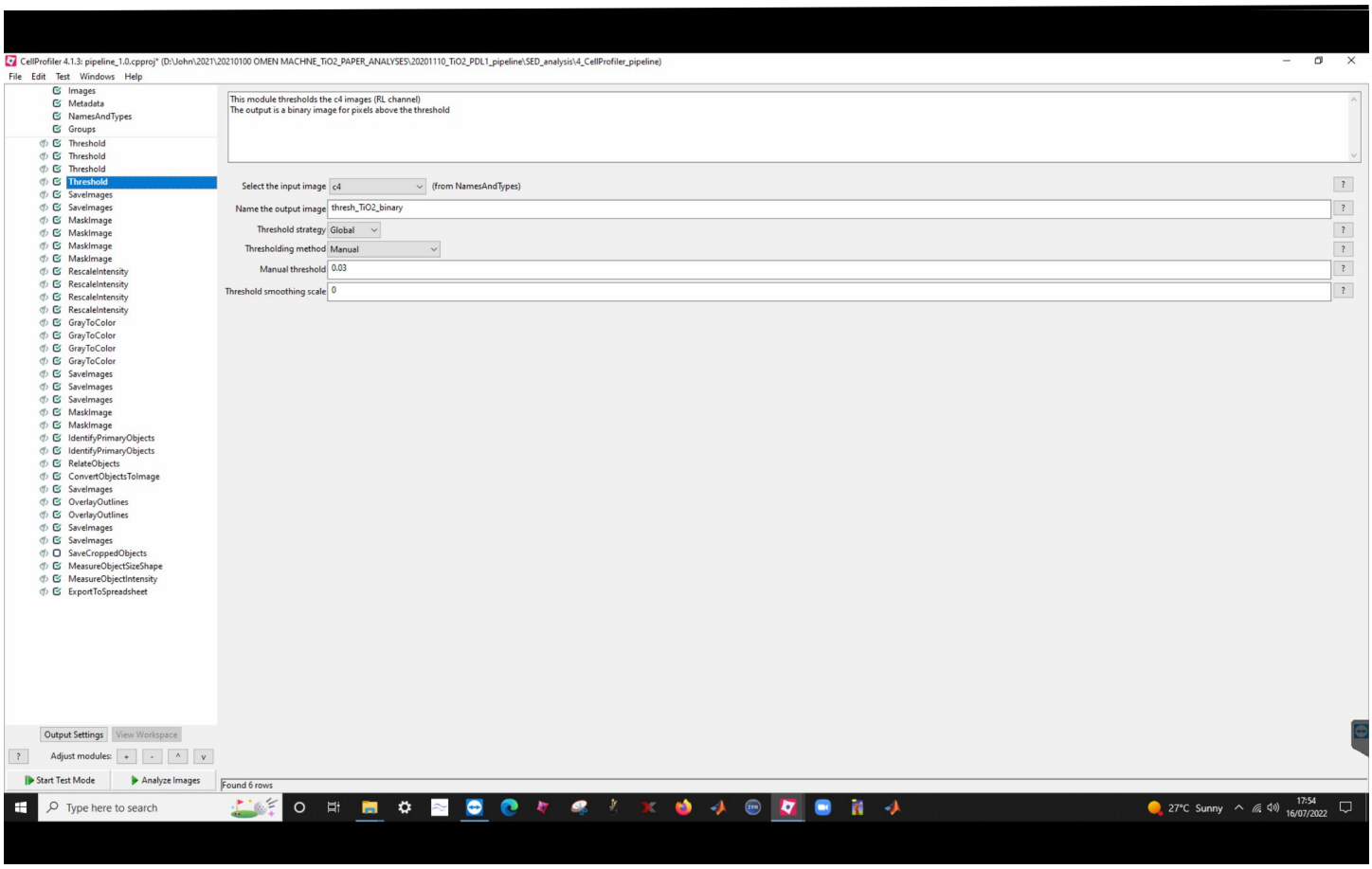

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
The module structures the inputted image-files for processing.
It uses the folder names (i.e., the directory) the images are contained within to define the different channels.
It also defines the names given to the input images for use in the pipeline below. Here, c1, c2, c3 etc., represent the different image channels.
Assign a name to Images matching rules
Process as 3D? Yes No
Match All of the following rules
Select the rule criteria Directory Does Contain Channel_1
Name to assign these images c1
Select the image type Grayscale image
Set intensity range from Image bit-depth
Duplicate this image
Match All of the following rules
Select the rule criteria Directory Does Contain Channel_2
Name to assign these images c2
Select the image type Grayscale image
Set intensity range from Image bit-depth
Duplicate this image
Remove this image
Match All of the following rules
Select the rule criteria Directory Does Contain Channel_3
Name to assign these images c3
Update c1 c2 c3 c4 c5 c6
1 Ch_1_Im_001.tif Ch_2_Im_001.tif Ch_3_Im_001.tif Ch_4_Im_001.tif Ch_5_Im_001.tif Ch_6_Im_001.tif
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:54
16/07/2022

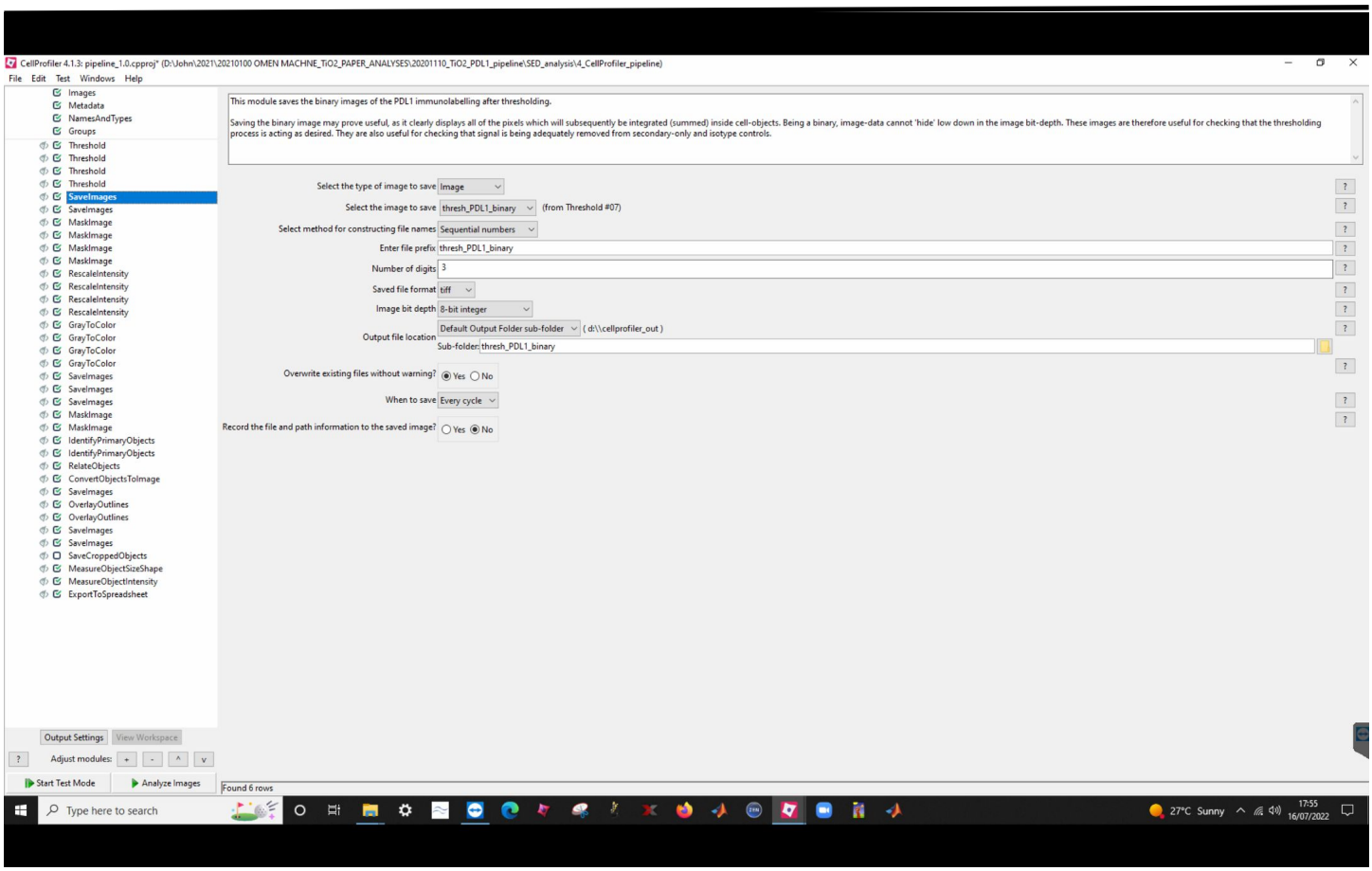

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
The Groups module optionally allows you to split your list of images into image subsets (groups) which will be processed independently of each other. Examples of groupings include screening batches, microtiter plates, time-lapse movies, etc.
(Not used here)
Do you want to group your images? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:54
16/07/2022

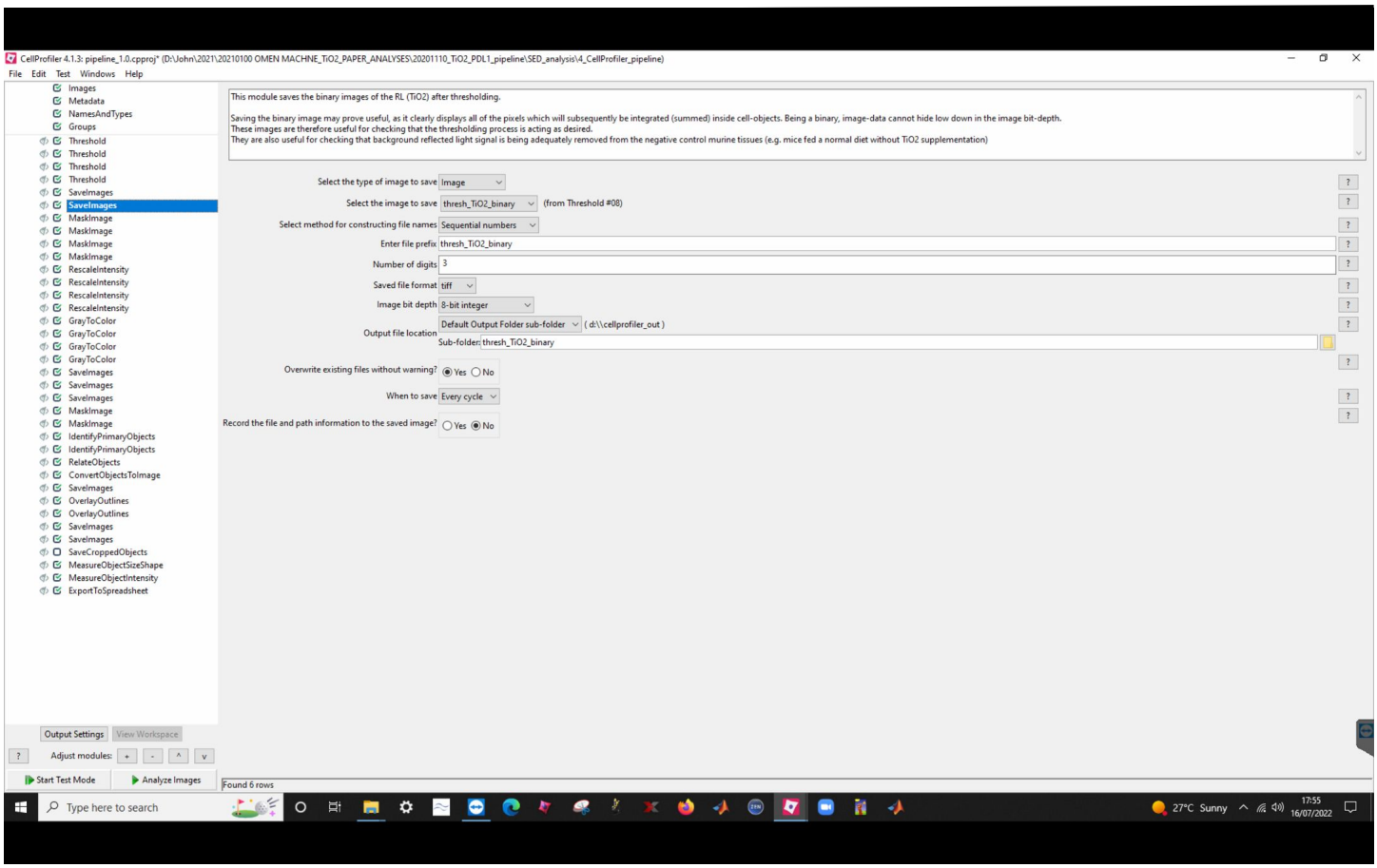

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module thresholds the images (nuclear channel)
The output is a binary image for pixels above the threshold
Select the input image c1 (from NamesAndTypes)
Name the output image thresh_nuclei_binary
Threshold strategy Global
Thresholding method Otsu
Two-class or three-class thresholding? Two classes
Threshold smoothing scale 0
Threshold correction factor 1.0
Lower and upper bounds on threshold 0.0 1.0
Log transform before thresholding? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:54
16/07/2022

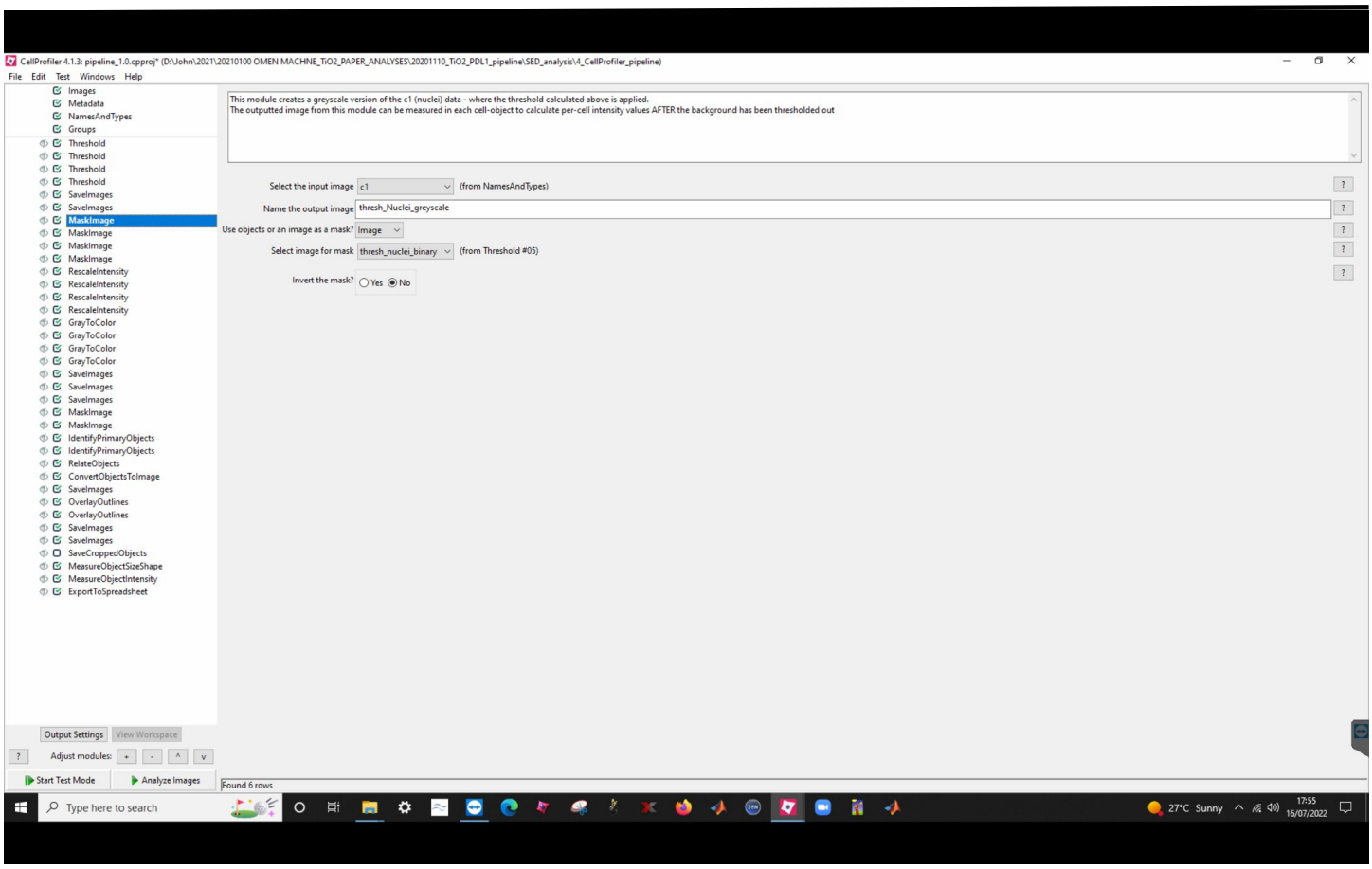

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module thresholds the c3 images (actin cell-outline channel)
The output is a binary image for pixels above the threshold
Select the input image c3 (from NamesAndTypes)
Name the output image thresh_Actin_binary
Threshold strategy Global
Thresholding method Otsu
Two-class or three-class thresholding? Two classes
Threshold smoothing scale 0
Threshold correction factor 1.0
Lower and upper bounds on threshold 0.00 1.0
Log transform before thresholding? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:54
16/07/2022

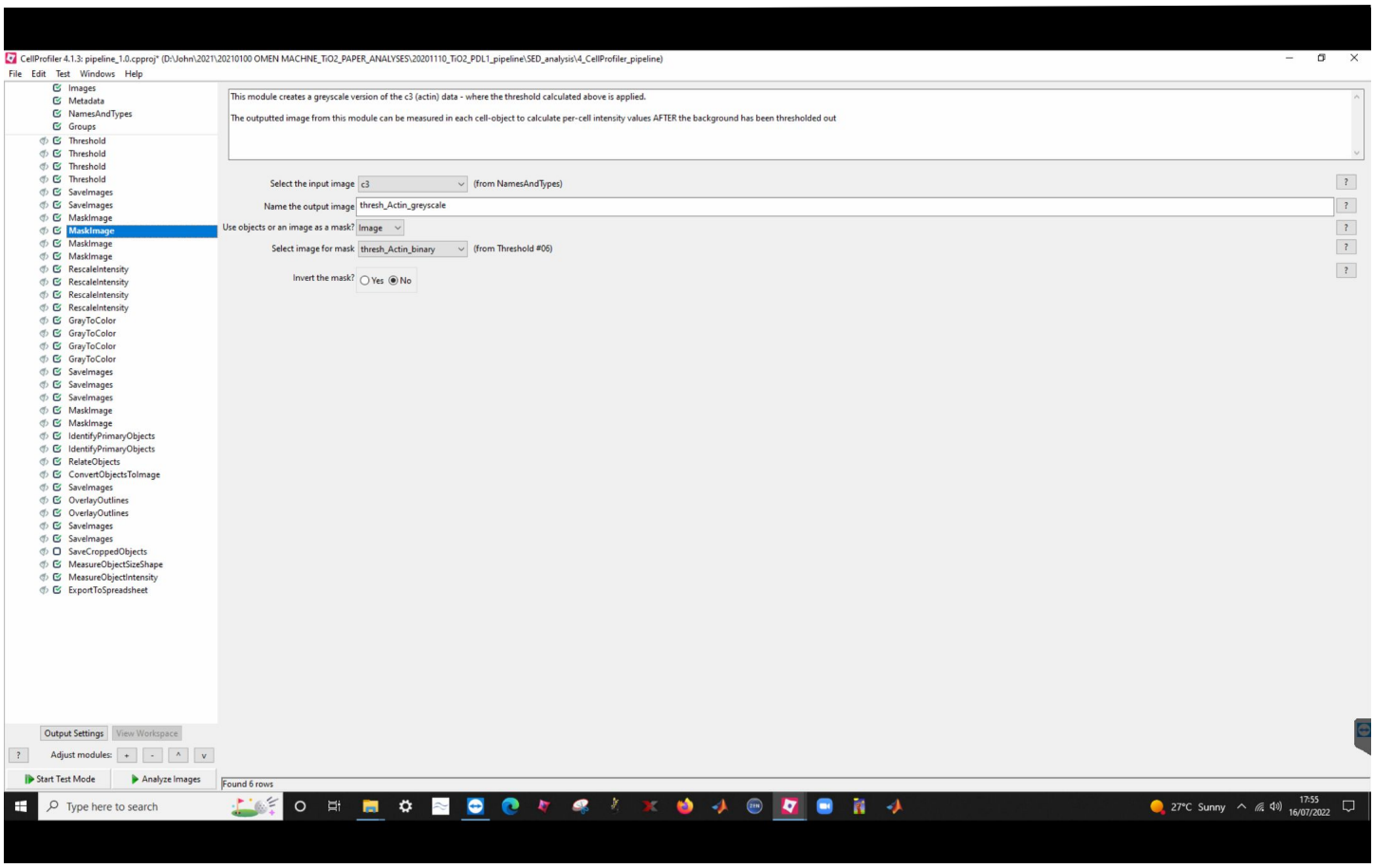

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module thresholds the c2 images (PDL1 channel)
The output is a binary image for pixels above the threshold
Select the input image c2 (from NamesAndTypes)
Name the output image thresh_PDL1_binary
Threshold strategy Global
Thresholding method Manual
Manual threshold 0.07
Threshold smoothing scale 0
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:54
16/07/2022

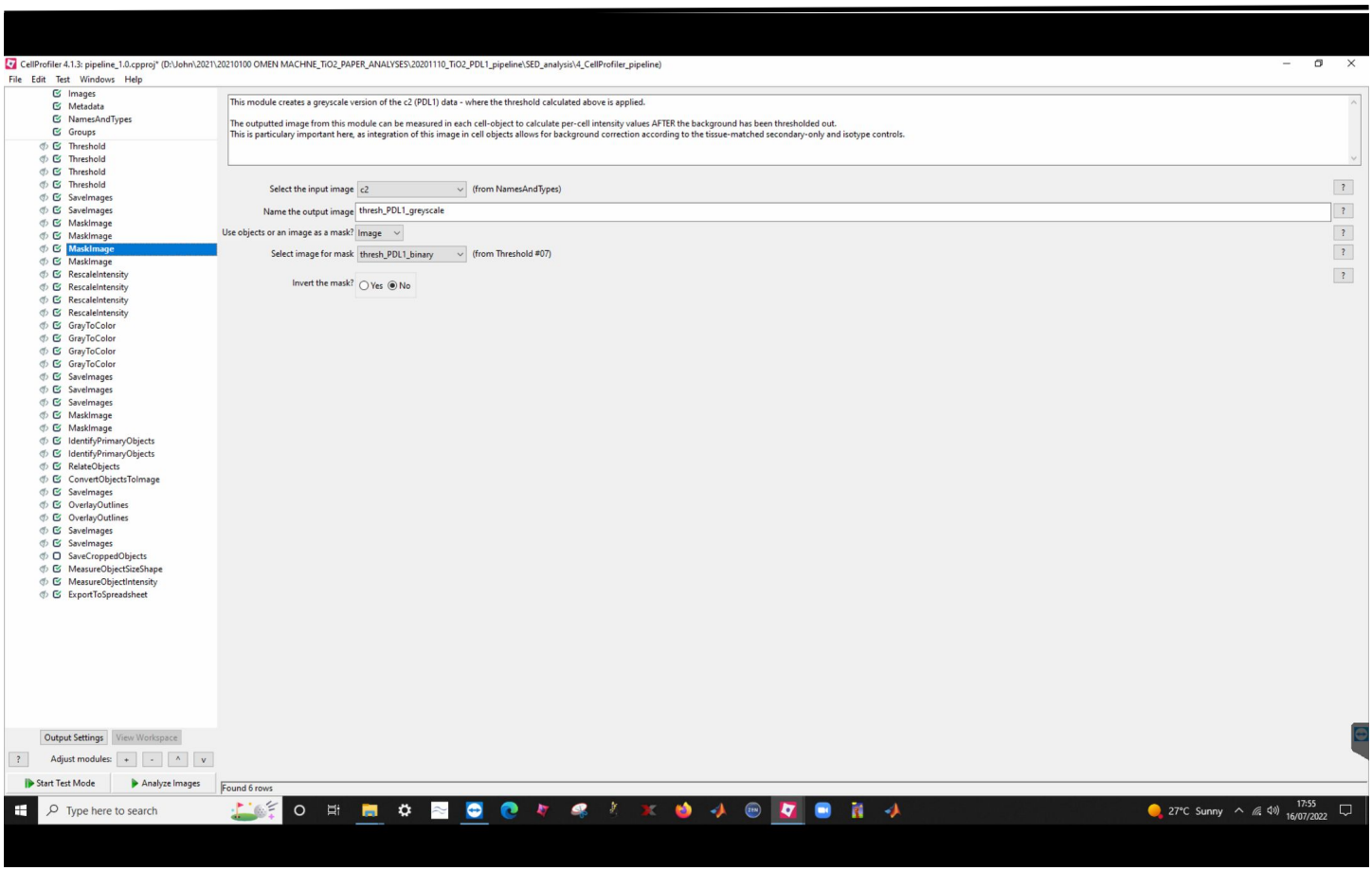

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module thresholds the c4 images (RL channel)
The output is a binary image for pixels above the threshold
Select the input image c4 (from NamesAndTypes)
Name the output image thresh_TiO2_binary
Threshold strategy Global
Thresholding method Manual
Manual threshold 0.03
Threshold smoothing scale 0
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:54
16/07/2022

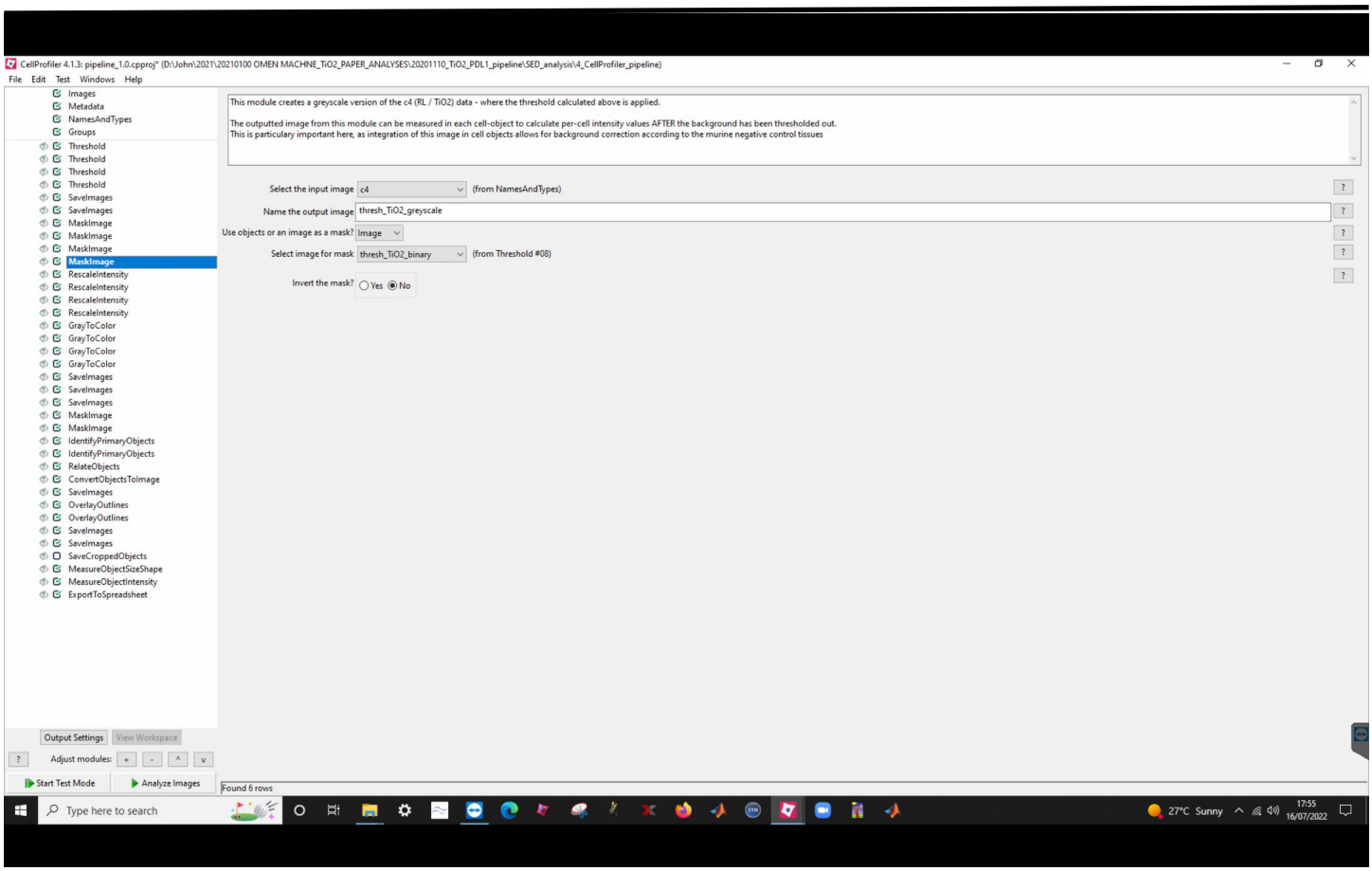

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module saves the binary images of the PDL1 immunolabelling after thresholding.
Saving the binary image may prove useful, as it clearly displays all of the pixels which will subsequently be integrated (summed) inside cell-objects. Being a binary, image-data cannot 'hide' low down in the image bit-depth. These images are therefore useful for checking that the thresholding process is acting as desired. They are also useful for checking that signal is being adequately removed from secondary-only and isotype controls.
Select the type of image to save Image
Select the image to save thresh_PDL1_binary (from Threshold #07)
Select method for constructing file names Sequential numbers
Enter file prefix thresh_PDL1_binary
Number of digits 3
Saved file format tiff
Image bit depth 8-bit integer
Output file location Default Output Folder sub-folder (d:\\cellprofiler_out)
Sub-folder: thresh_PDL1_binary
Overwrite existing files without warning? Yes No
When to save Every cycle
Record the file and path information to the saved image? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

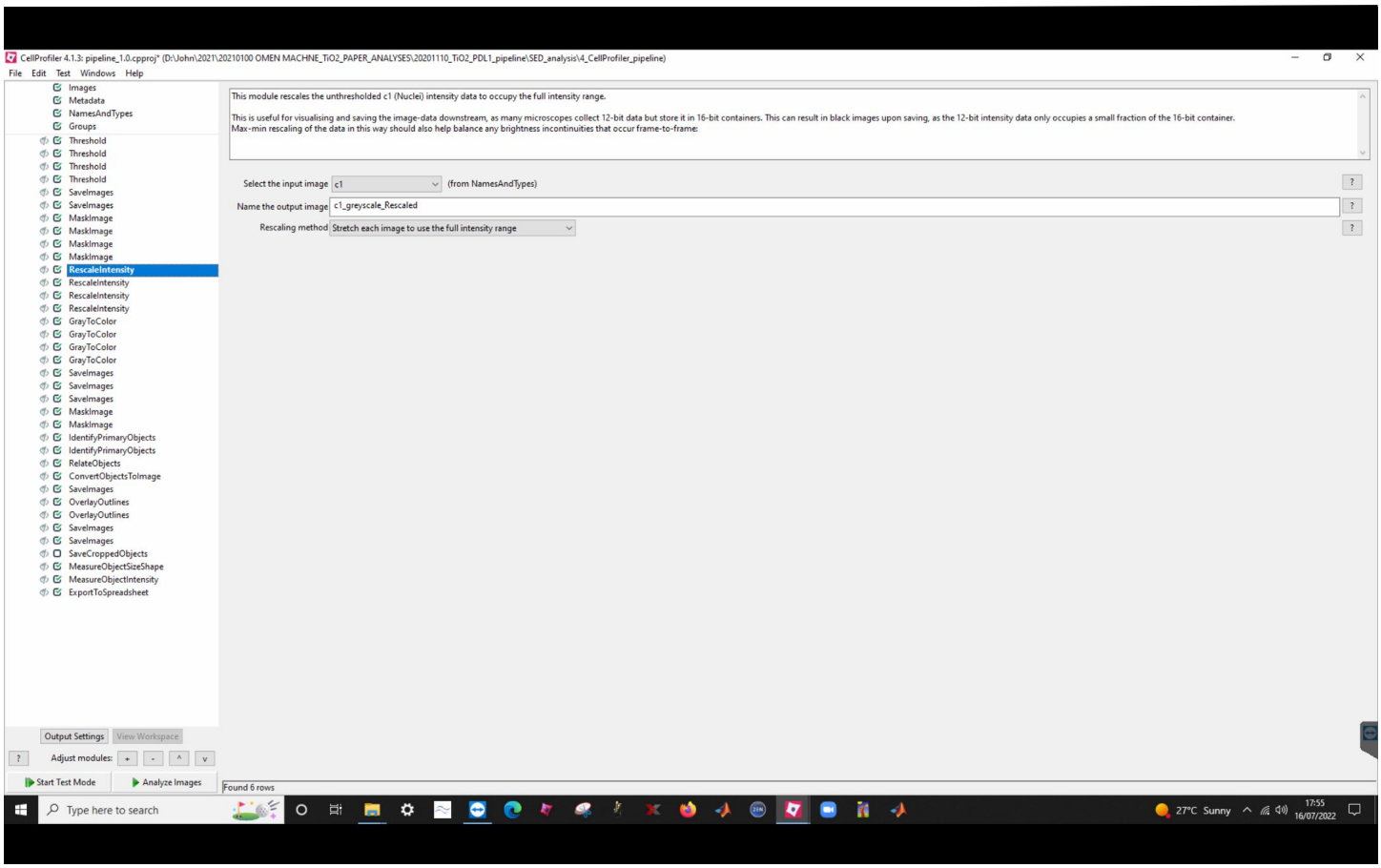

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module saves the binary images of the RL (TiO2) after thresholding.
Saving the binary image may prove useful, as it clearly displays all of the pixels which will subsequently be integrated (summed) inside cell-objects. Being a binary, image-data cannot hide low down in the image bit-depth.
These images are therefore useful for checking that the thresholding process is acting as desired.
They are also useful for checking that background reflected light signal is being adequately removed from the negative control murine tissues (e.g. mice fed a normal diet without TiO2 supplementation)
Select the type of image to save Image
Select the image to save thresh_TiO2_binary (from Threshold #08)
Select method for constructing file names Sequential numbers
Enter file prefix thresh_TiO2_binary
Number of digits 3
Saved file format tiff
Image bit depth 8-bit integer
Output file location Default Output Folder sub-folder (d:\\cellprofiler_out)
Sub-folder: thresh_TiO2_binary
Overwrite existing files without warning? Yes No
When to save Every cycle
Record the file and path information to the saved image? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

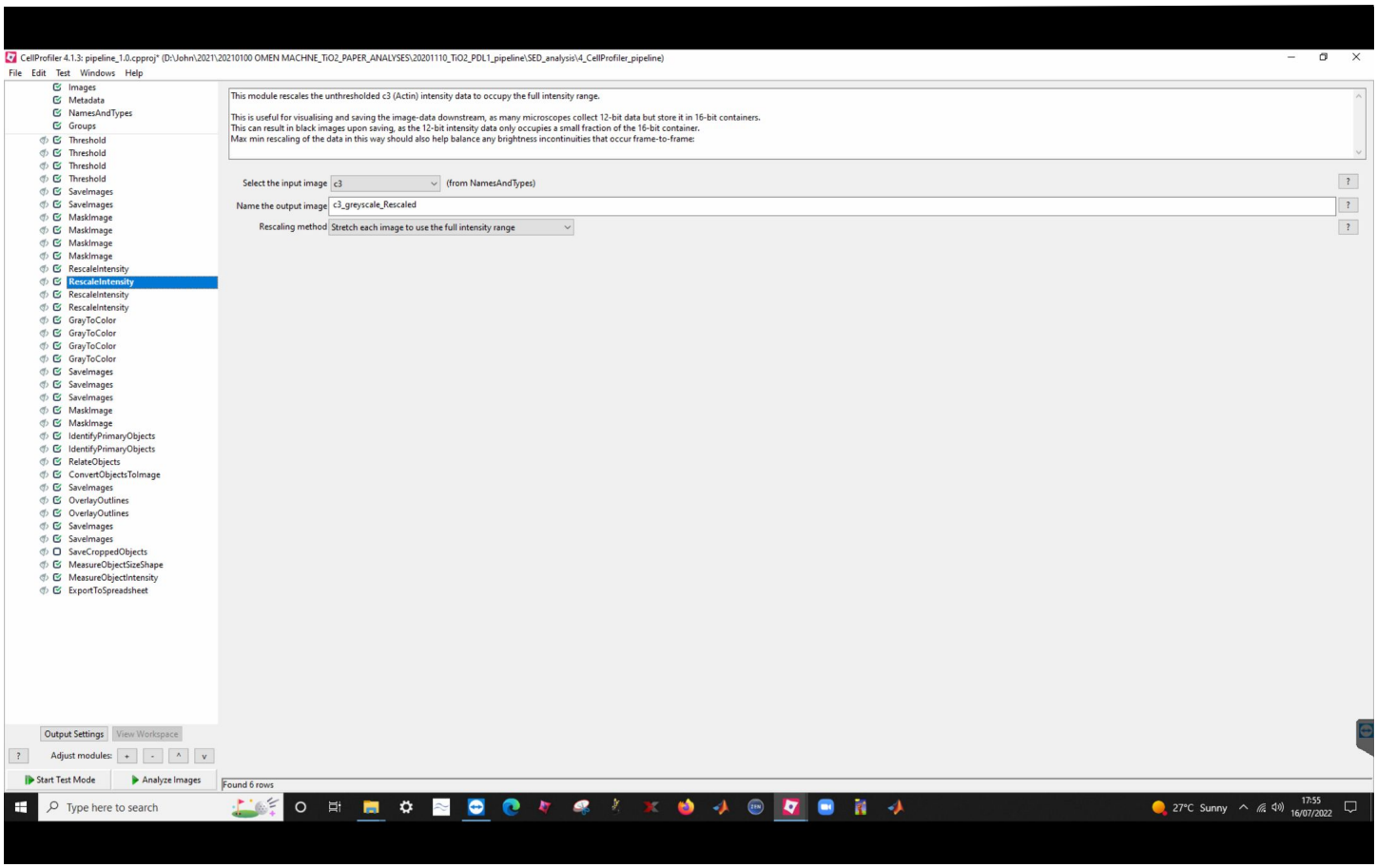

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module creates a greyscale version of the c1 (nuclei) data - where the threshold calculated above is applied.
The outputted image from this module can be measured in each cell-object to calculate per-cell intensity values AFTER the background has been thresholded out
Select the input image c1 (from NamesAndTypes)
Name the output image thresh_Nuclei_greyscale
Use objects or an image as a mask? Image
Select image for mask thresh_nuclei_binary (from Threshold #05)
Invert the mask? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

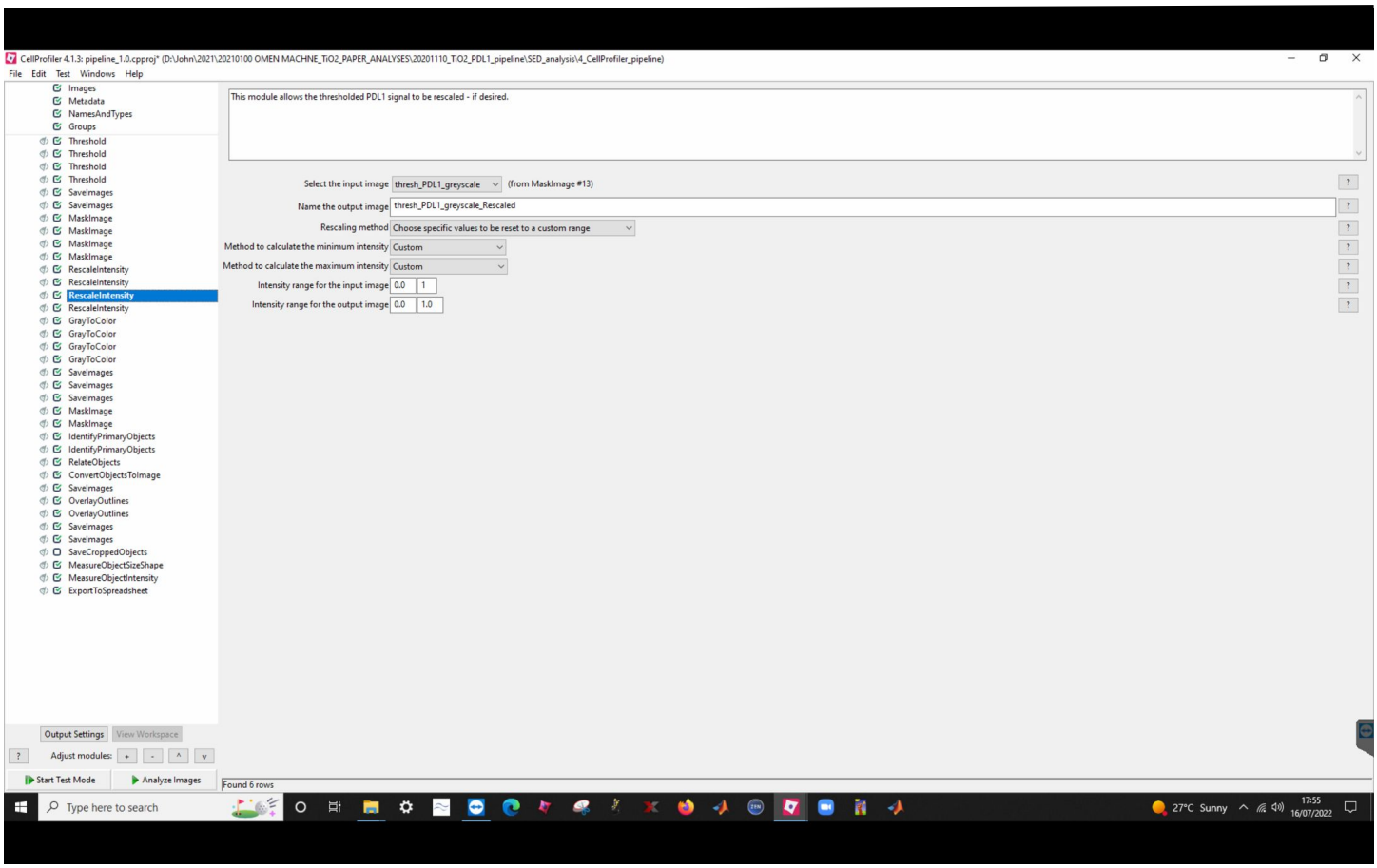

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module creates a greyscale version of the c3 (actin) data - where the threshold calculated above is applied.
The outputted image from this module can be measured in each cell-object to calculate per-cell intensity values AFTER the background has been thresholded out
Select the input image c3 (from NamesAndTypes)
Name the output image thresh_Actin_greyscale
Use objects or an image as a mask? Image
Select image for mask thresh_Actin_binary (from Threshold #06)
Invert the mask? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

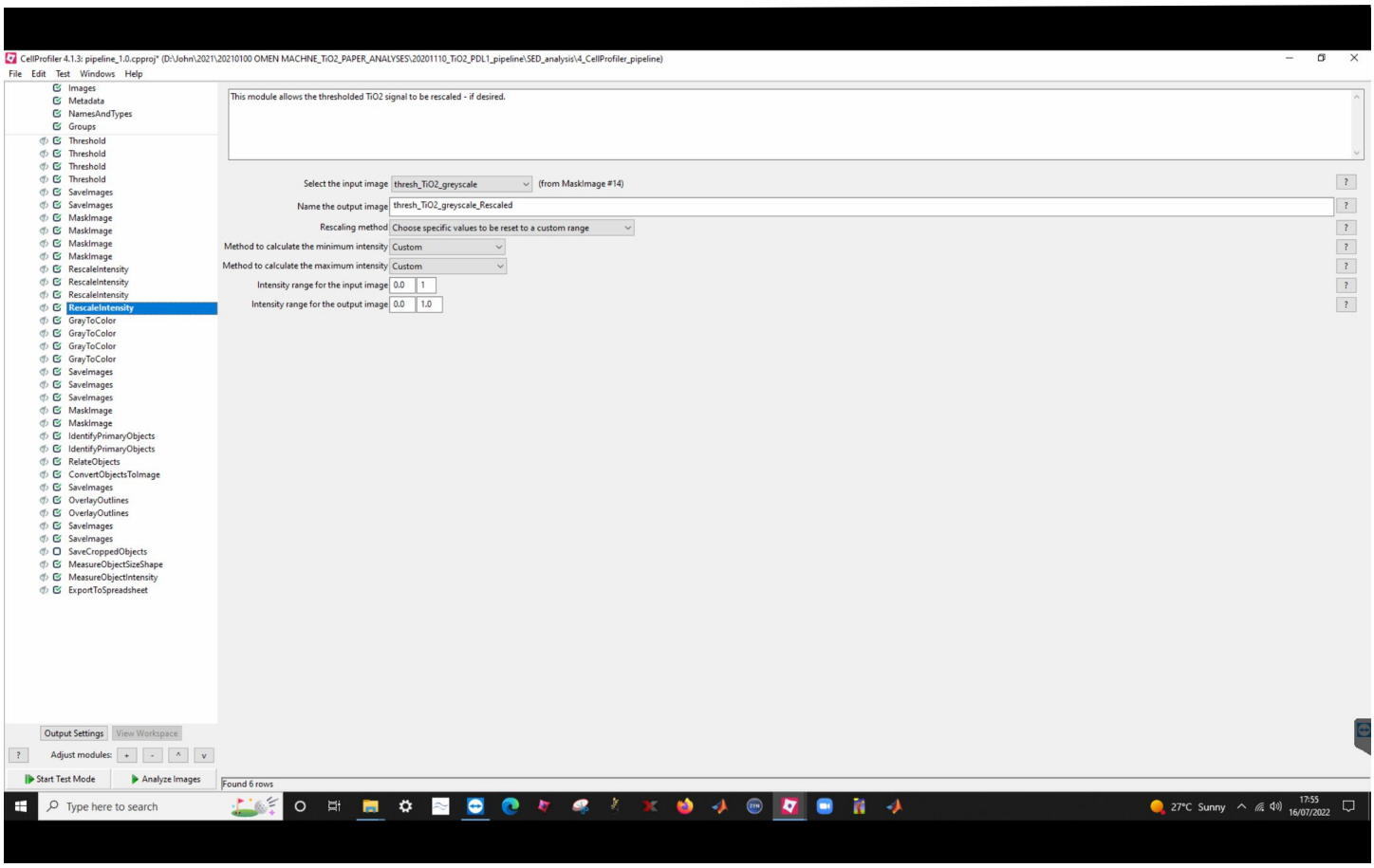

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module creates a greyscale version of the c2 (PDL1) data - where the threshold calculated above is applied.
The outputted image from this module can be measured in each cell-object to calculate per-cell intensity values AFTER the background has been thresholded out.
This is particulary important here, as integration of this image in cell objects allows for background correction according to the tissue-matched secondary-only and isotype controls.
Select the input image c2 (from NamesAndTypes)
Name the output image thresh_PDL1_greyscale
Use objects or an image as a mask? Image
Select image for mask thresh_PDL1_binary (from Threshold #07)
Invert the mask? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

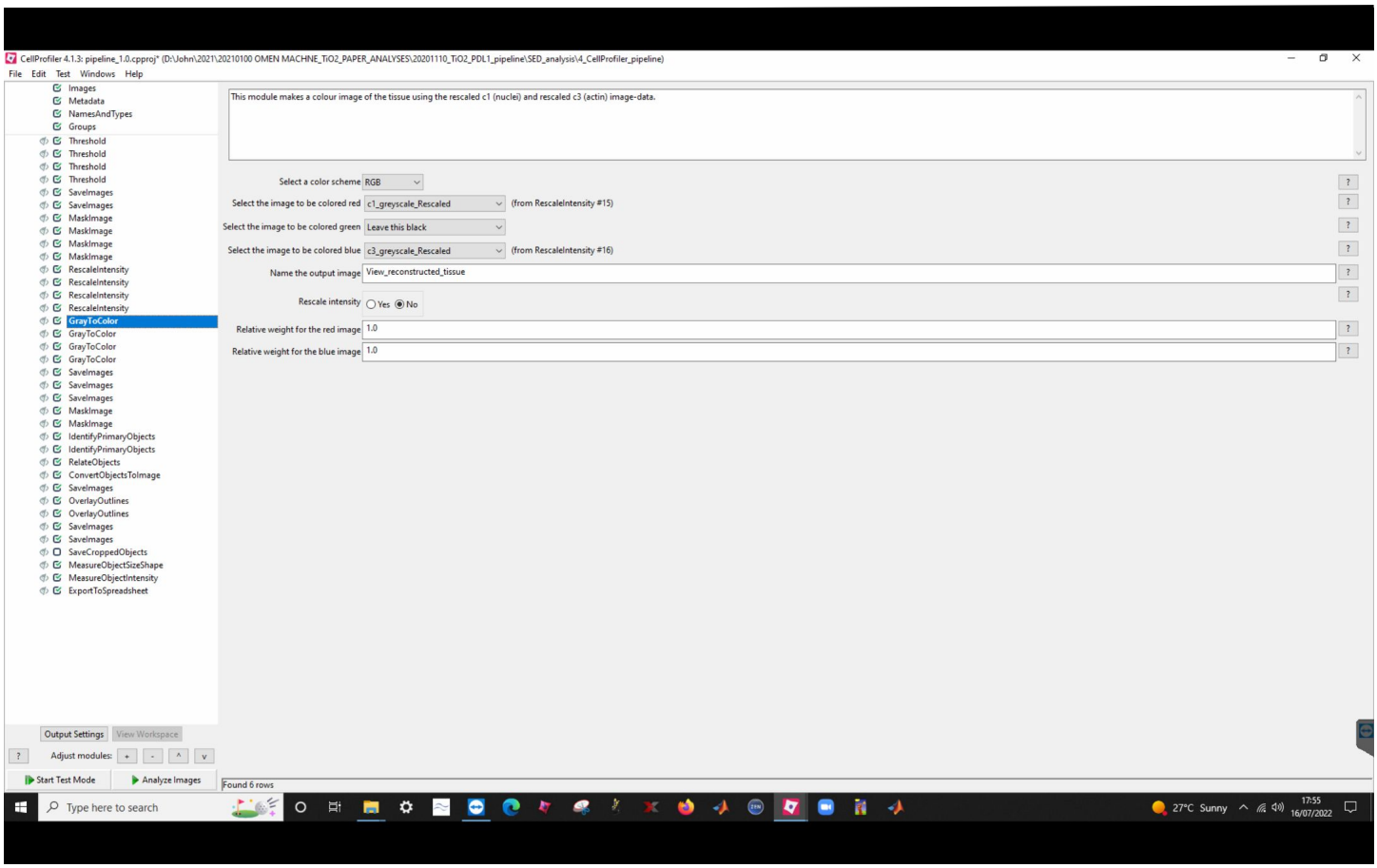

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module creates a greyscale version of the c4 (RL / TiO2) data - where the threshold calculated above is applied.
The outputted image from this module can be measured in each cell-object to calculate per-cell intensity values AFTER the background has been thresholded out.
This is particulary important here, as integration of this image in cell objects allows for background correction according to the murine negative control tissues
Select the input image c4 (from NamesAndTypes)
Name the output image thresh_TiO2_greyscale
Use objects or an image as a mask? Image
Select image for mask thresh_TiO2_binary (from Threshold #08)
Invert the mask? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

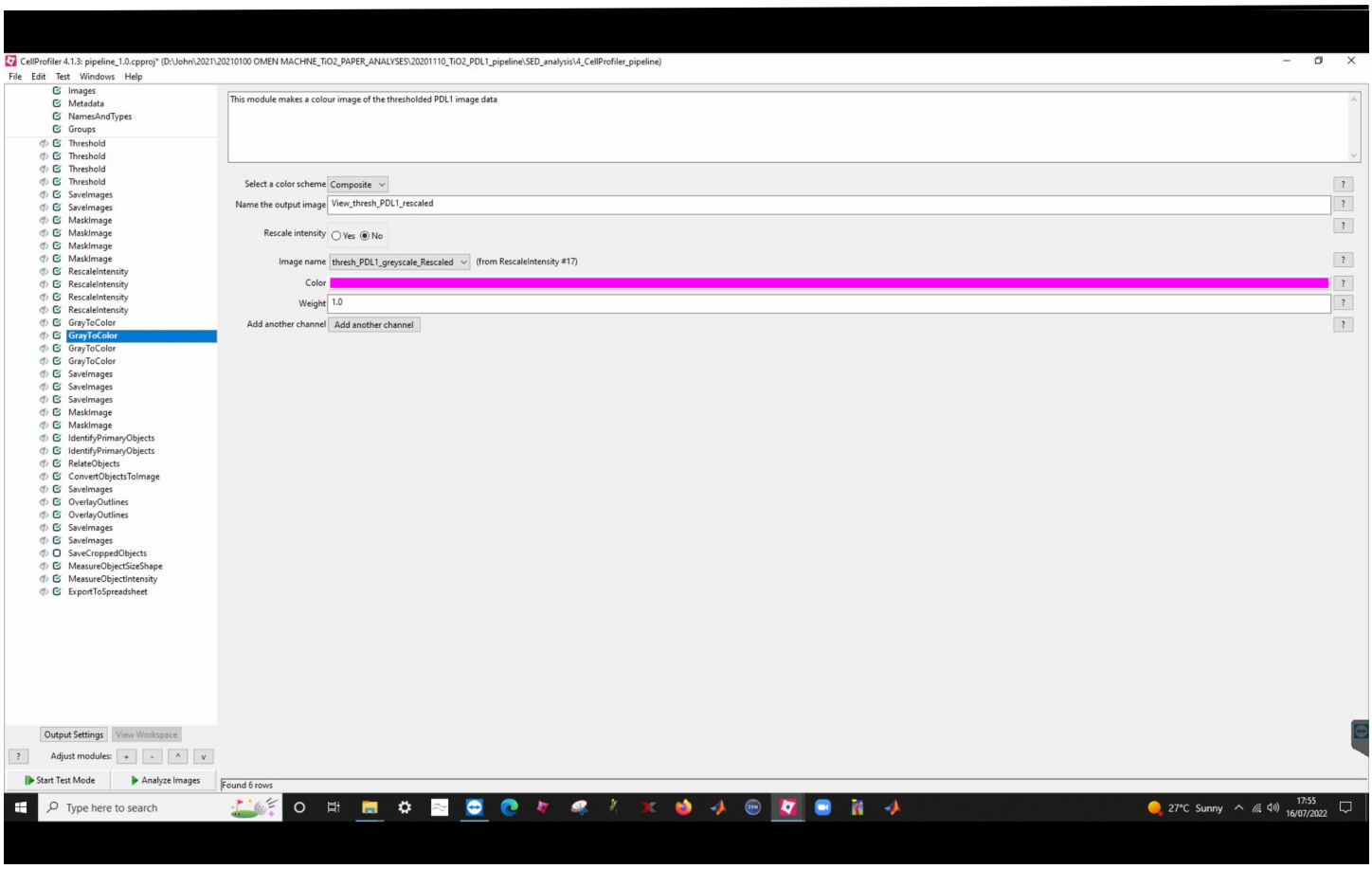

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module rescales the unthresholded c1 (Nuclei) intensity data to occupy the full intensity range.
This is useful for visualising and saving the image-data downstream, as many microscopes collect 12-bit data but store it in 16-bit containers. This can result in black images upon saving, as the 12-bit intensity data only occupies a small fraction of the 16-bit container.
Max-min rescaling of the data in this way should also help balance any brightness incontinuities that occur frame-to-frame:
Select the input image
c1
(from NamesAndTypes)
Name the output image
c1_greyscale_Rescaled
Rescaling method
Stretch each image to use the full intensity range
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

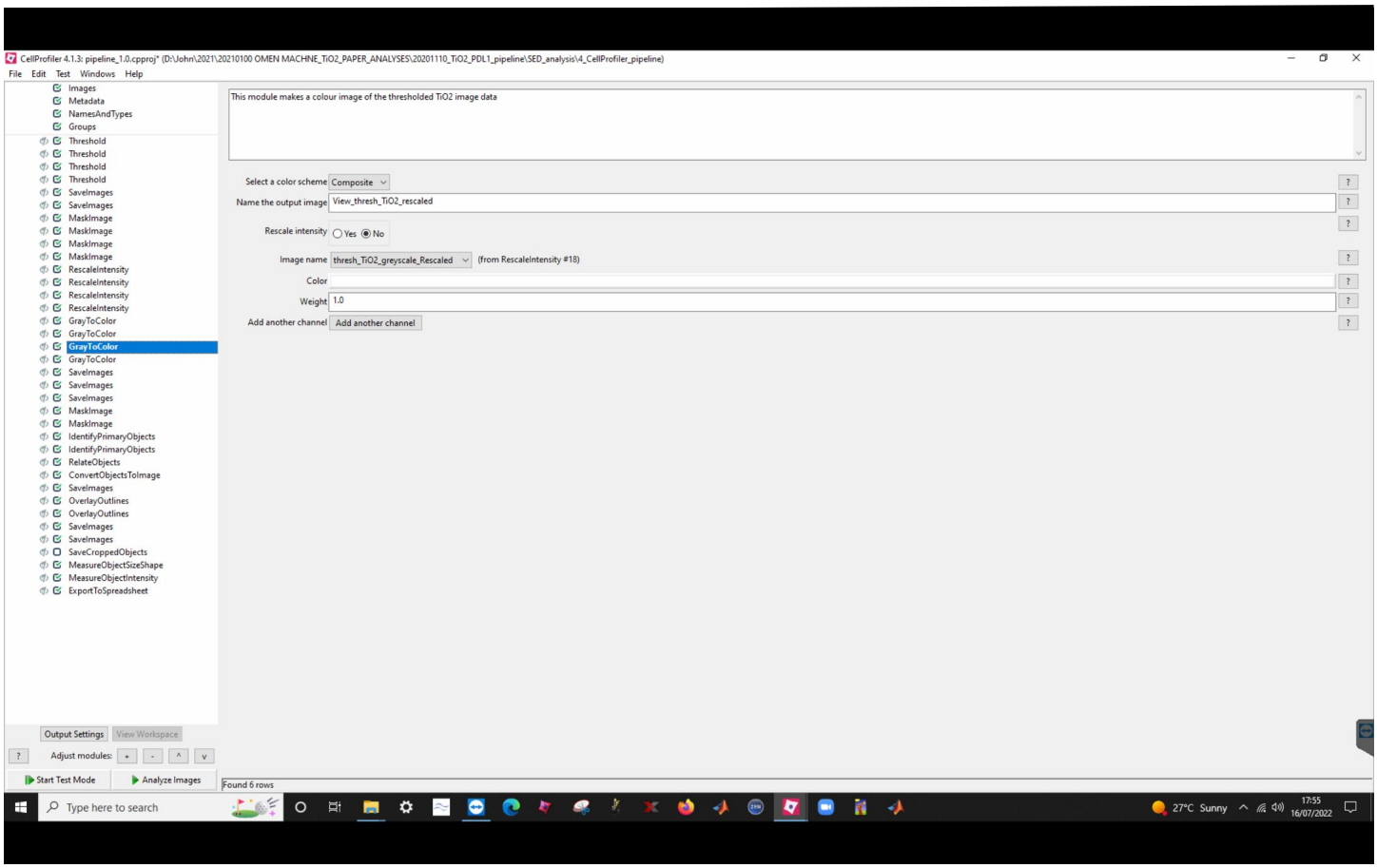

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module rescales the unthresholded c3 (Actin) intensity data to occupy the full intensity range.
This is useful for visualising and saving the image-data downstream, as many microscopes collect 12-bit data but store it in 16-bit containers.
This can result in black images upon saving, as the 12-bit intensity data only occupies a small fraction of the 16-bit container.
Max min rescaling of the data in this way should also help balance any brightness incontinuities that occur frame-to-frame:
Select the input image c3 (from NamesAndTypes)
Name the output image c3_greyscale_Rescaled
Rescaling method Stretch each image to use the full intensity range
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

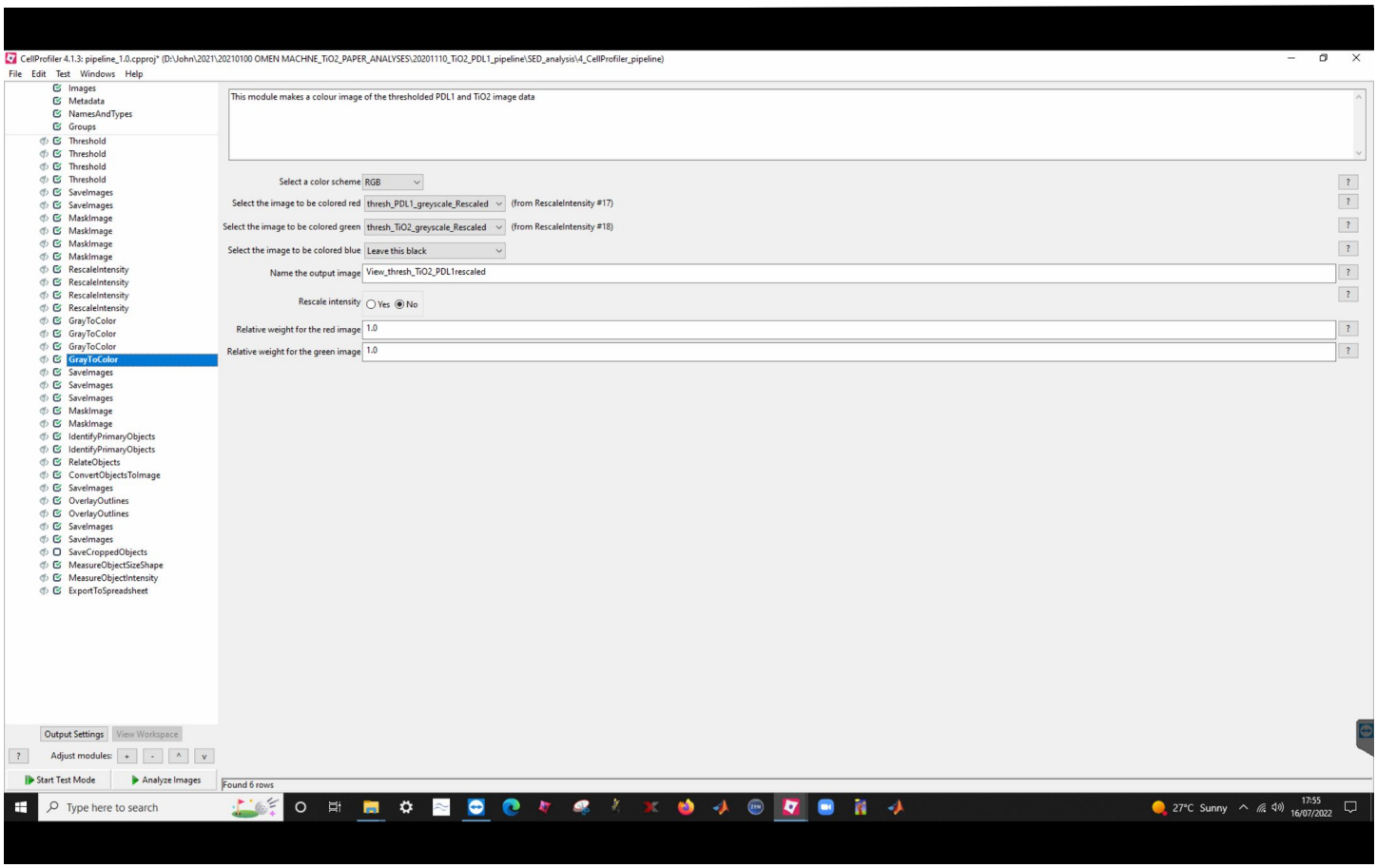

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module allows the thresholded PDL1 signal to be rescaled - if desired.
Select the input image
thresh_PDL1_greyscale
(from MaskImage #13)
Name the output image
thresh_PDL1_greyscale_Rescaled
Rescaling method
Choose specific values to be reset to a custom range
Method to calculate the minimum intensity
Custom
Method to calculate the maximum intensity
Custom
Intensity range for the input image
0.0
1
Intensity range for the output image
0.0
1.0
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

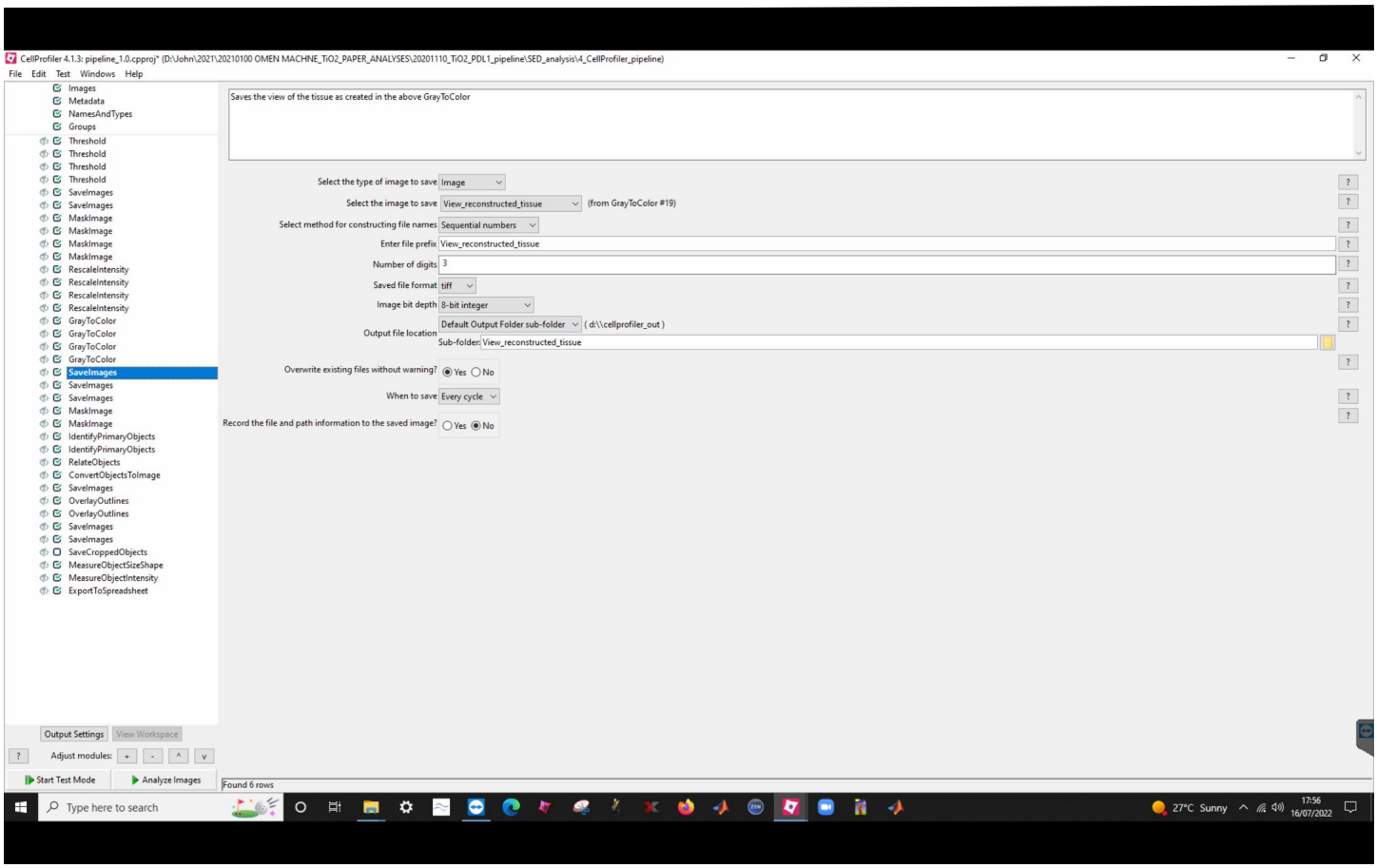

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module allows the thresholded TiO2 signal to be rescaled - if desired.
Select the input image
thresh_TiO2_greyscale
(from MaskImage #14)
Name the output image
thresh_TiO2_greyscale_Rescaled
Rescaling method
Choose specific values to be reset to a custom range
Method to calculate the minimum intensity
Custom
Method to calculate the maximum intensity
Custom
Intensity range for the input image
0.0
1
Intensity range for the output image
0.0
1.0
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

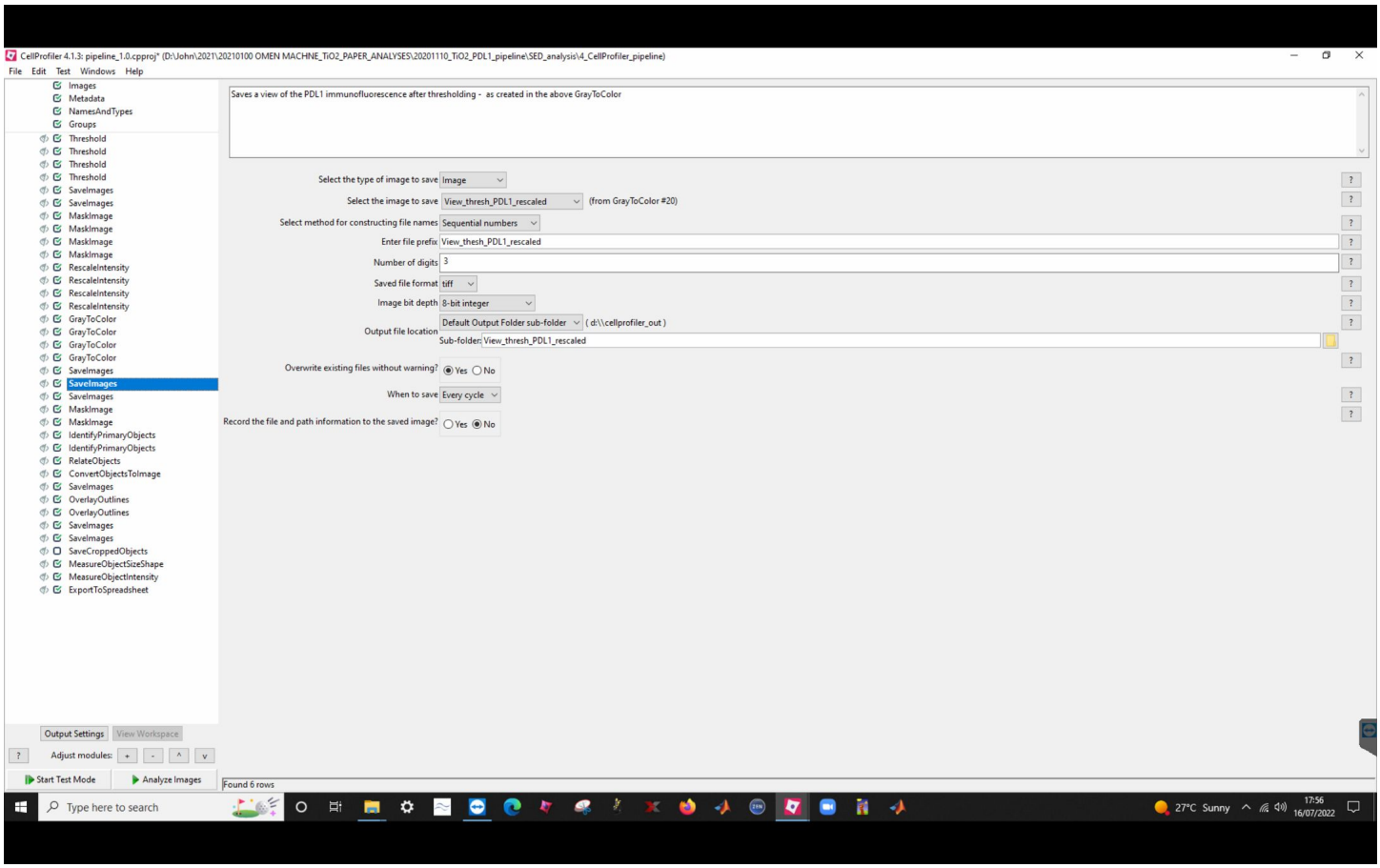

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
This module makes a colour image of the tissue using the rescaled c1 (nuclei) and rescaled c3 (actin) image-data.
Select a color scheme
RGB
Select the image to be colored red
c1_greyscale_Rescaled
(from RescaleIntensity #15)
Select the image to be colored green
Leave this black
Select the image to be colored blue
c3_greyscale_Rescaled
(from RescaleIntensity #16)
Name the output image
View_reconstructed_tissue
Rescale intensity
Yes
No
Relative weight for the red image
1.0
Relative weight for the blue image
1.0
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

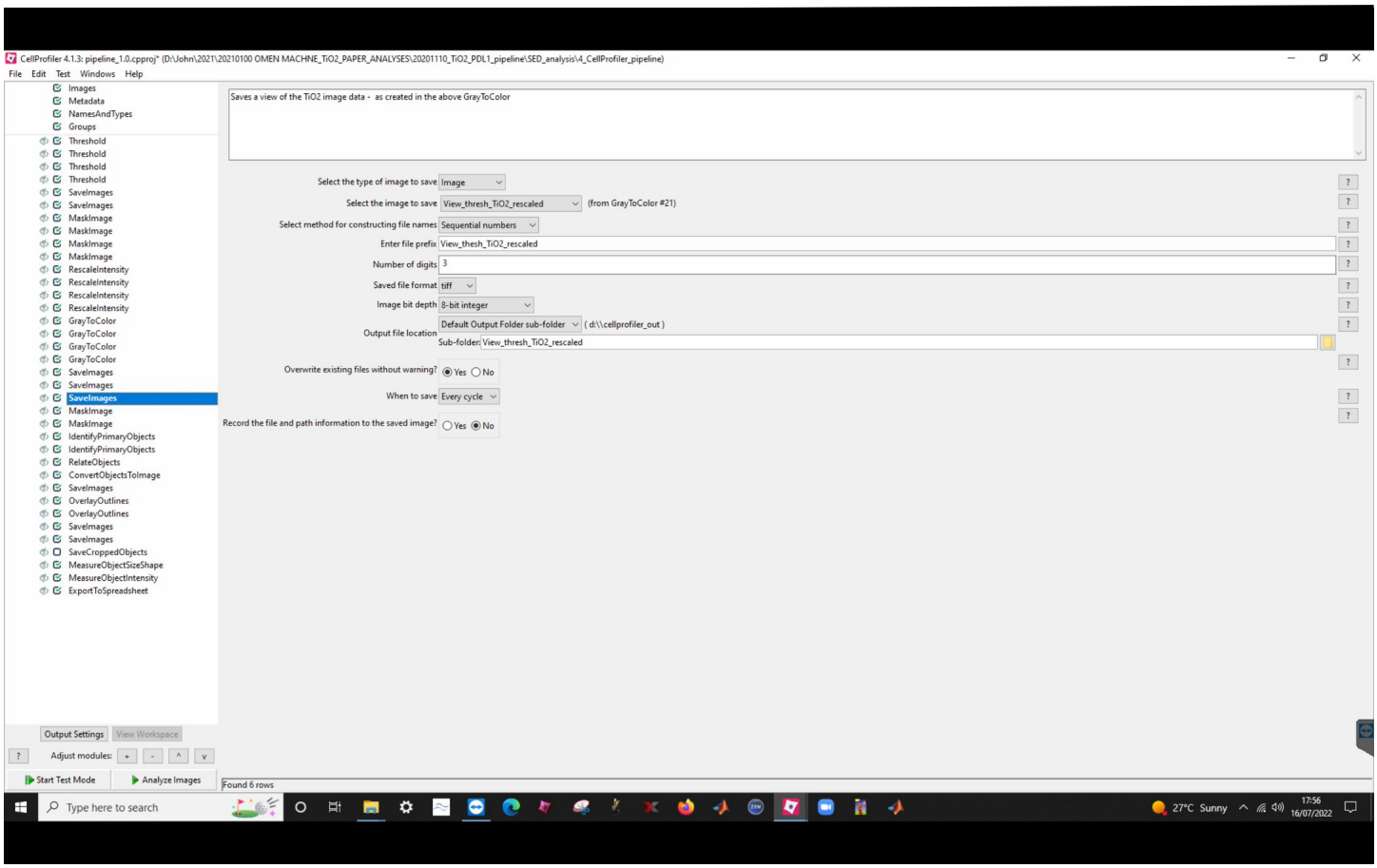

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module makes a colour image of the thresholded PDL1 image data
Select a color scheme
Composite
Name the output image
View_thresh_PDL1_rescaled
Rescale intensity
Yes
No
Image name
thresh_PDL1_greyscale_Rescaled
(from RescaleIntensity #17)
Color
Weight
1.0
Add another channel
Add another channel
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

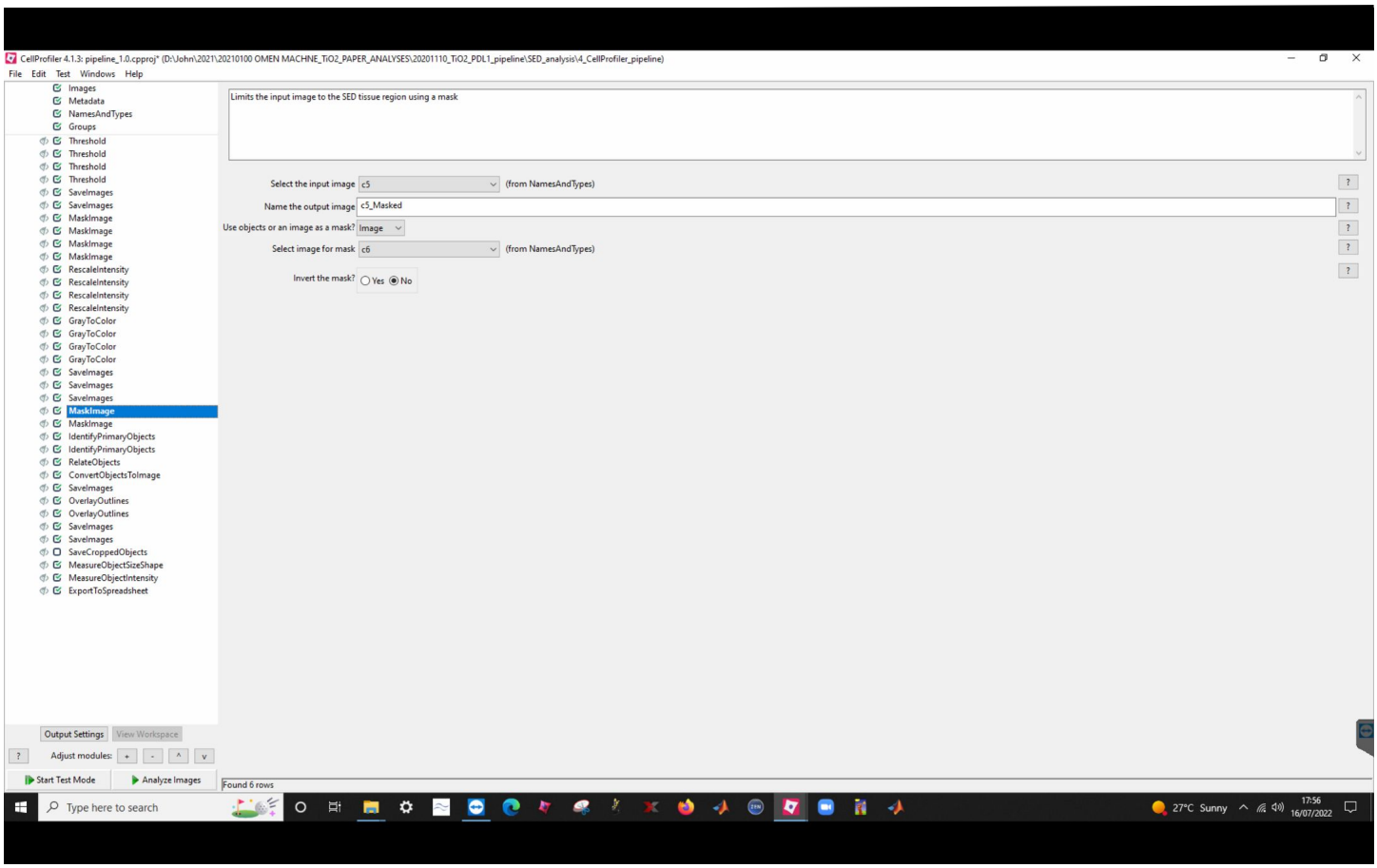

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
This module makes a colour image of the thresholded TiO2 image data
Select a color scheme
Composite
Name the output image
View_thresh_TiO2_rescaled
Rescale intensity
Yes
No
Image name
thresh_TiO2_greyscale_Rescaled
(from RescaleIntensity #18)
Color
Weight
1.0
Add another channel
Add another channel
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

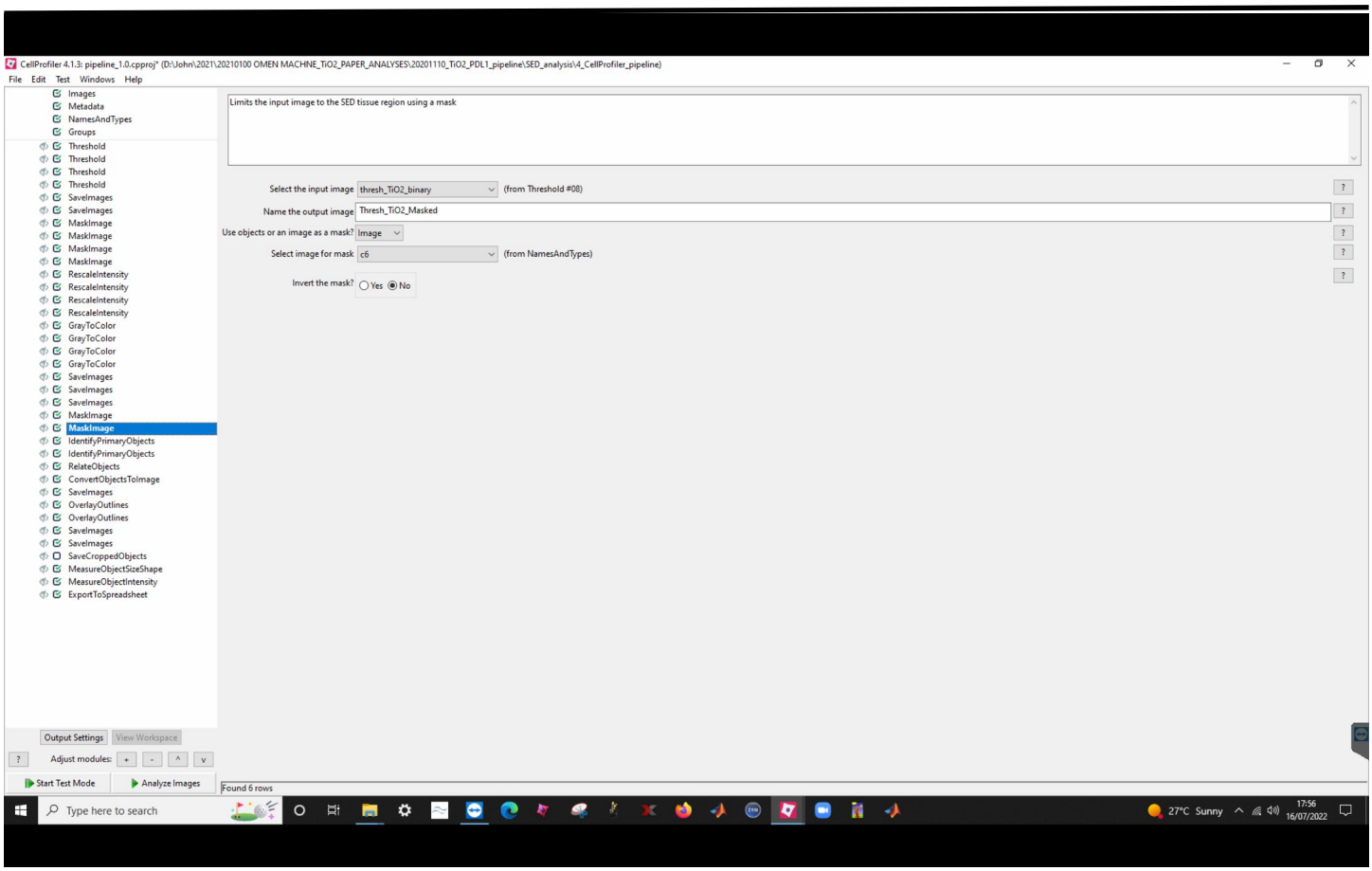

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
This module makes a colour image of the thresholded PDL1 and TiO2 image data
Select a color scheme
RGB
Select the image to be colored red
thresh_PDL1_greyscale_Rescaled
(from RescaleIntensity #17)
Select the image to be colored green
thresh_TiO2_greyscale_Rescaled
(from RescaleIntensity #18)
Select the image to be colored blue
Leave this black
Name the output image
View_thresh_TiO2_PDL1rescaled
Rescale intensity
Yes
No
Relative weight for the red image
1.0
Relative weight for the green image
1.0
Found 6 rows
Type here to search
27°C Sunny
17:55
16/07/2022

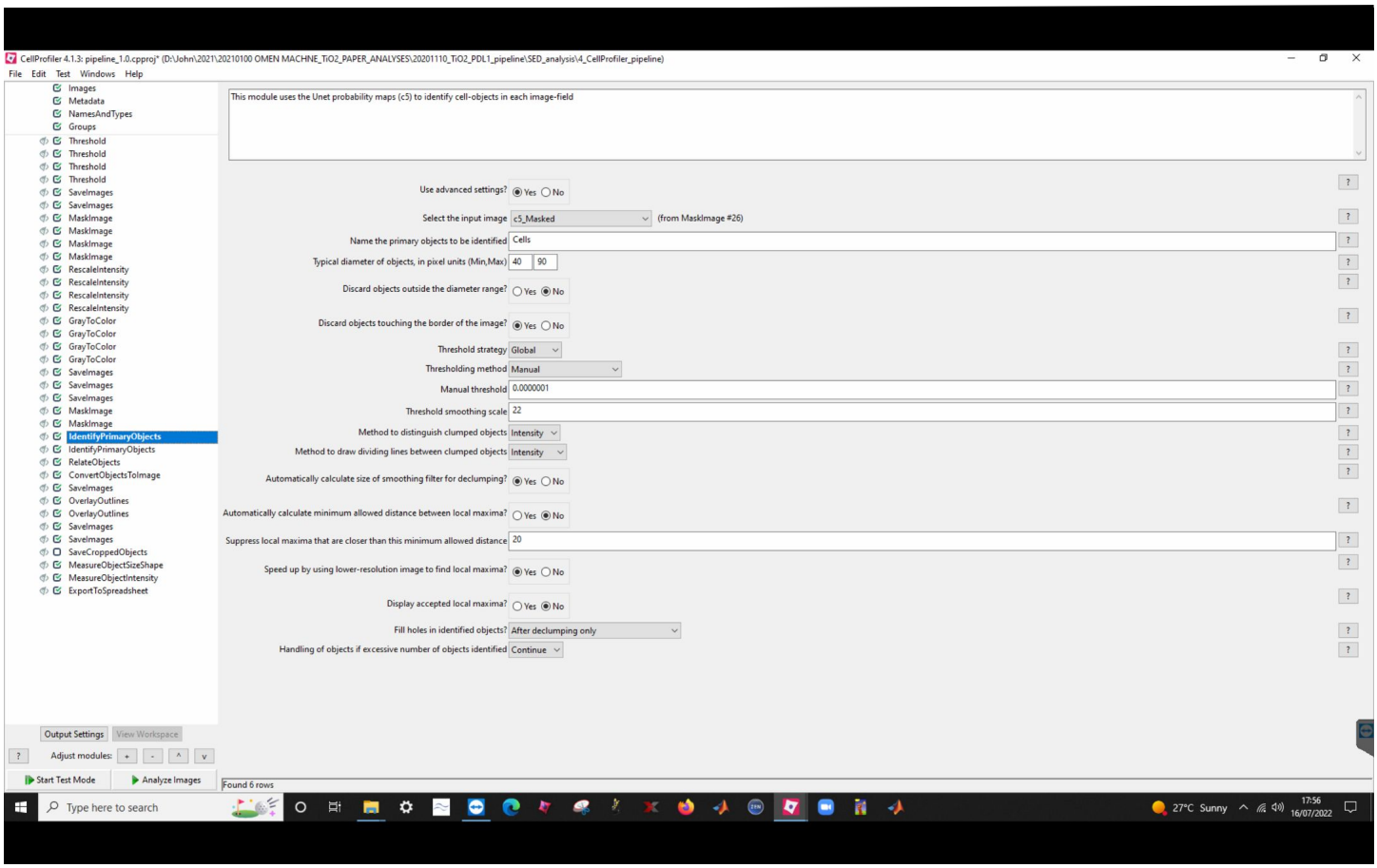

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Saves the view of the tissue as created in the above GrayToColor
Select the type of image to save
Image
Select the image to save
View_reconstructed_tissue
(from GrayToColor #19)
Select method for constructing file names
Sequential numbers
Enter file prefix
View_reconstructed_tissue
Number of digits
3
Saved file format
tiff
Image bit depth
8-bit integer
Output file location
Default Output Folder sub-folder
(d:\\cellprofiler_out)
Sub-folder:
View_reconstructed_tissue
Overwrite existing files without warning?
Yes
No
When to save
Every cycle
Record the file and path information to the saved image?
Yes
No
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

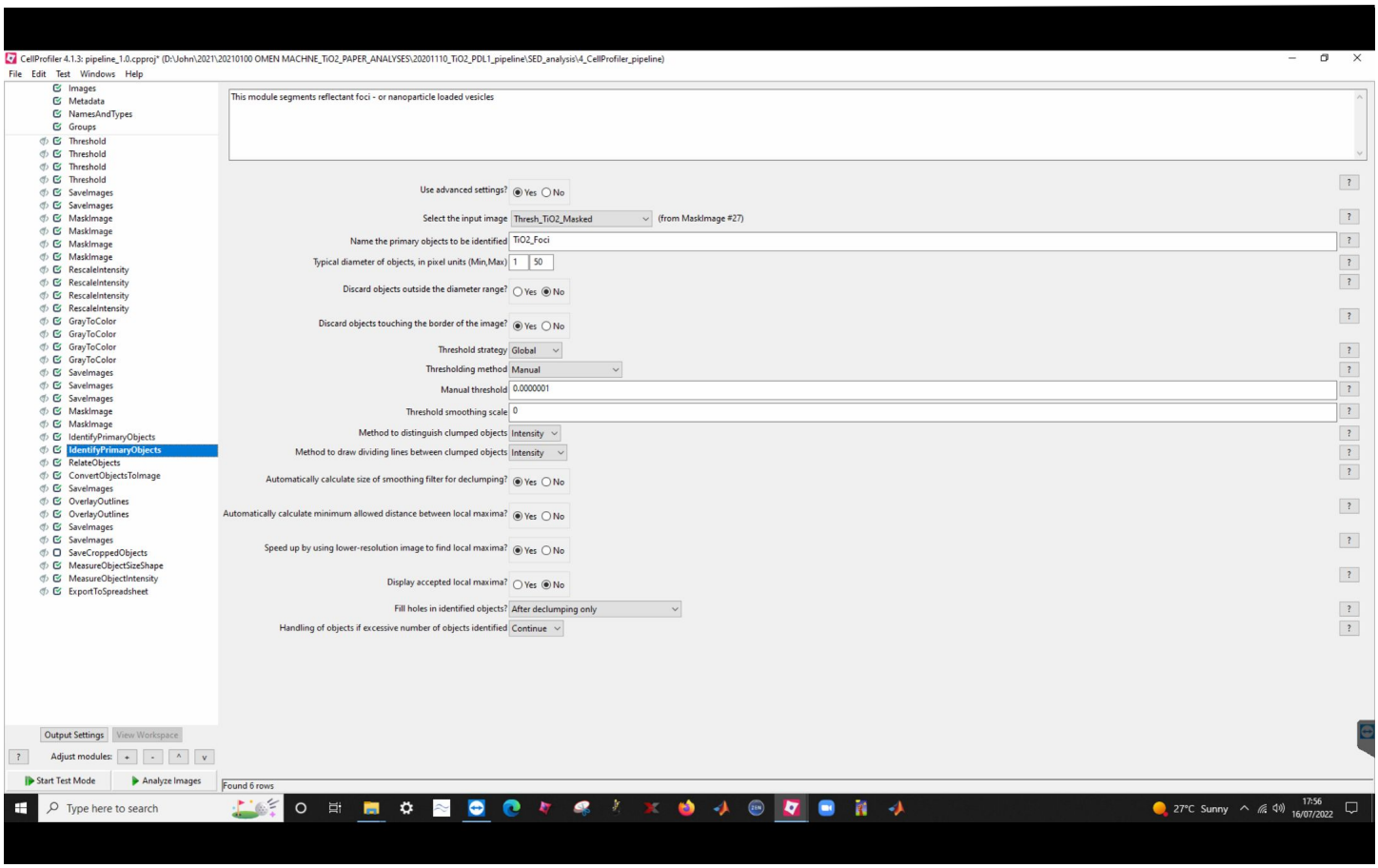

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Saves a view of the PDL1 immunofluorescence after thresholding - as created in the above GrayToColor
Select the type of image to save
Image
Select the image to save
View_thresh_PDL1_rescaled
(from GrayToColor #20)
Select method for constructing file names
Sequential numbers
Enter file prefix
View_thesh_PDL1_rescaled
Number of digits
3
Saved file format
tiff
Image bit depth
8-bit integer
Output file location
Default Output Folder sub-folder
(d:\\cellprofiler_out)
Sub-folder:
View_thresh_PDL1_rescaled
Overwrite existing files without warning?
Yes
No
When to save
Every cycle
Record the file and path information to the saved image?
Yes
No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

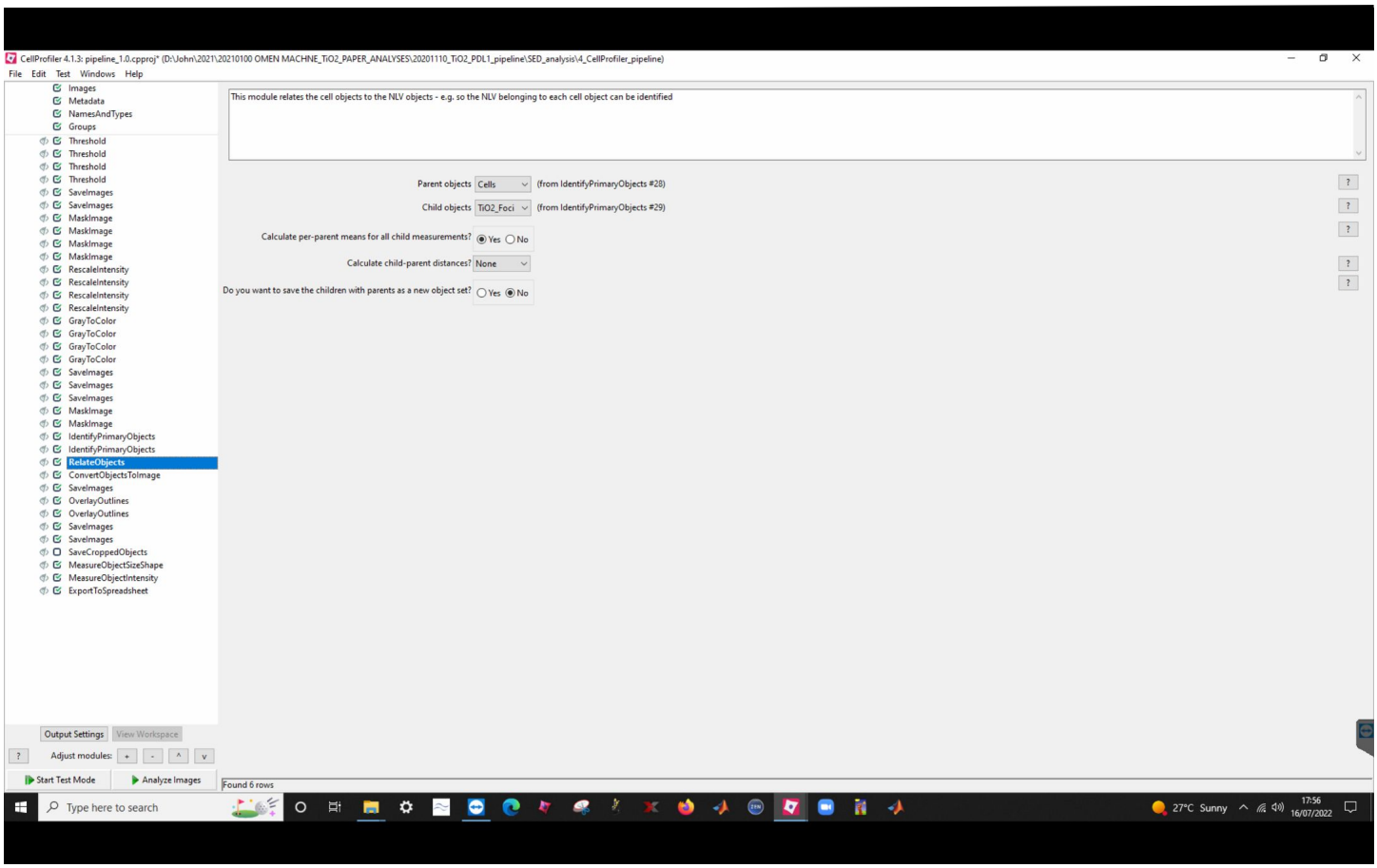

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Saves a view of the TiO2 image data - as created in the above GrayToColor
Select the type of image to save
Image
Select the image to save
View_thresh_TiO2_rescaled
(from GrayToColor #21)
Select method for constructing file names
Sequential numbers
Enter file prefix
View_thesh_TiO2_rescaled
Number of digits
3
Saved file format
tiff
Image bit depth
8-bit integer
Output file location
Default Output Folder sub-folder
(d:\\cellprofiler_out)
Sub-folder:
View_thresh_TiO2_rescaled
Overwrite existing files without warning?
Yes
No
When to save
Every cycle
Record the file and path information to the saved image?
Yes
No
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

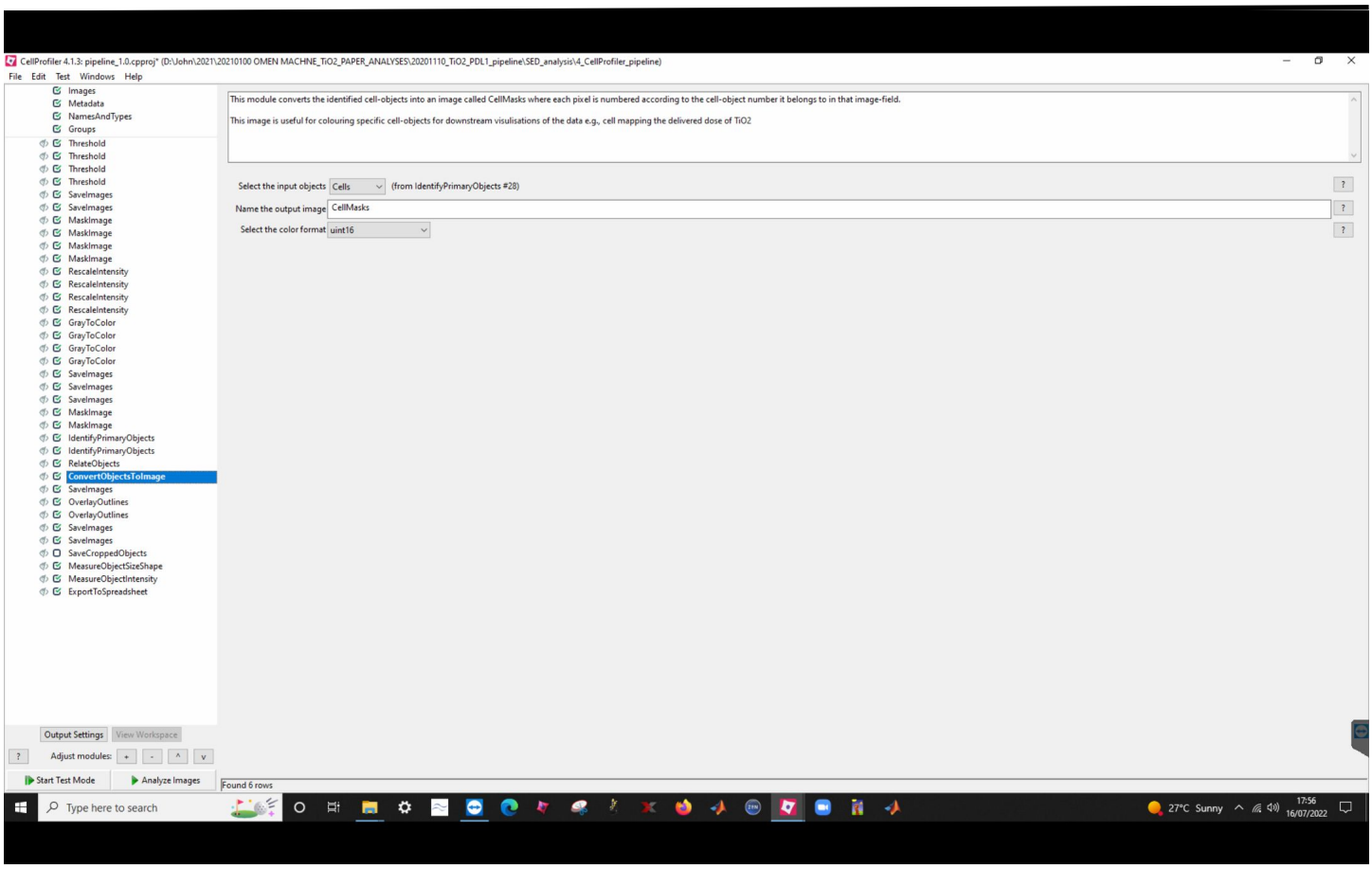

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Limits the input image to the SED tissue region using a mask
Select the input image
c5
(from NamesAndTypes)
Name the output image
c5_Masked
Use objects or an image as a mask?
Image
Select image for mask
c6
(from NamesAndTypes)
Invert the mask?
Yes
No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

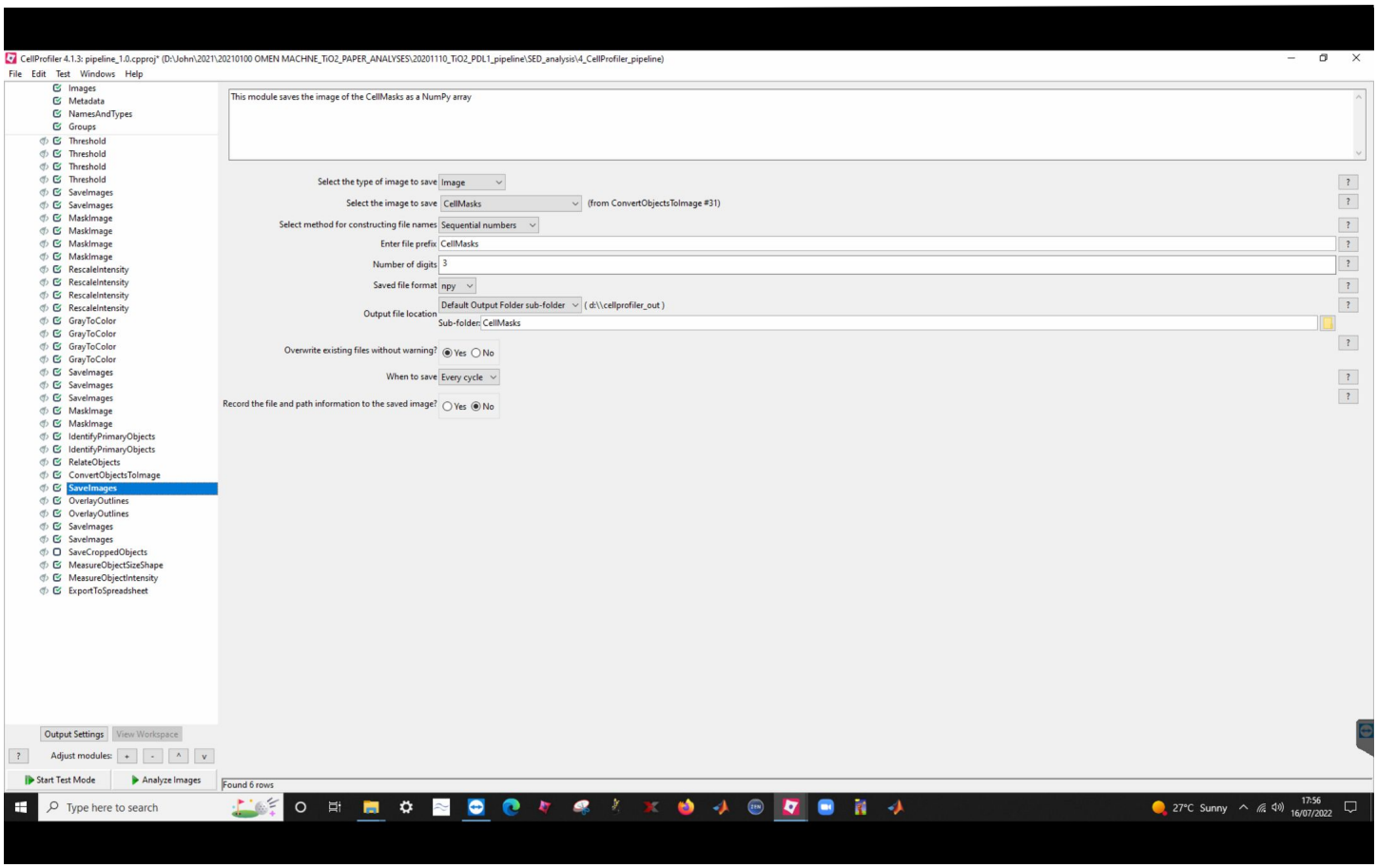

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Limits the input image to the SED tissue region using a mask
Select the input image
thresh_TiO2_binary
(from Threshold #08)
Name the output image
Thresh_TiO2_Masked
Use objects or an image as a mask?
Image
Select image for mask
c6
(from NamesAndTypes)
Invert the mask?
Yes
No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

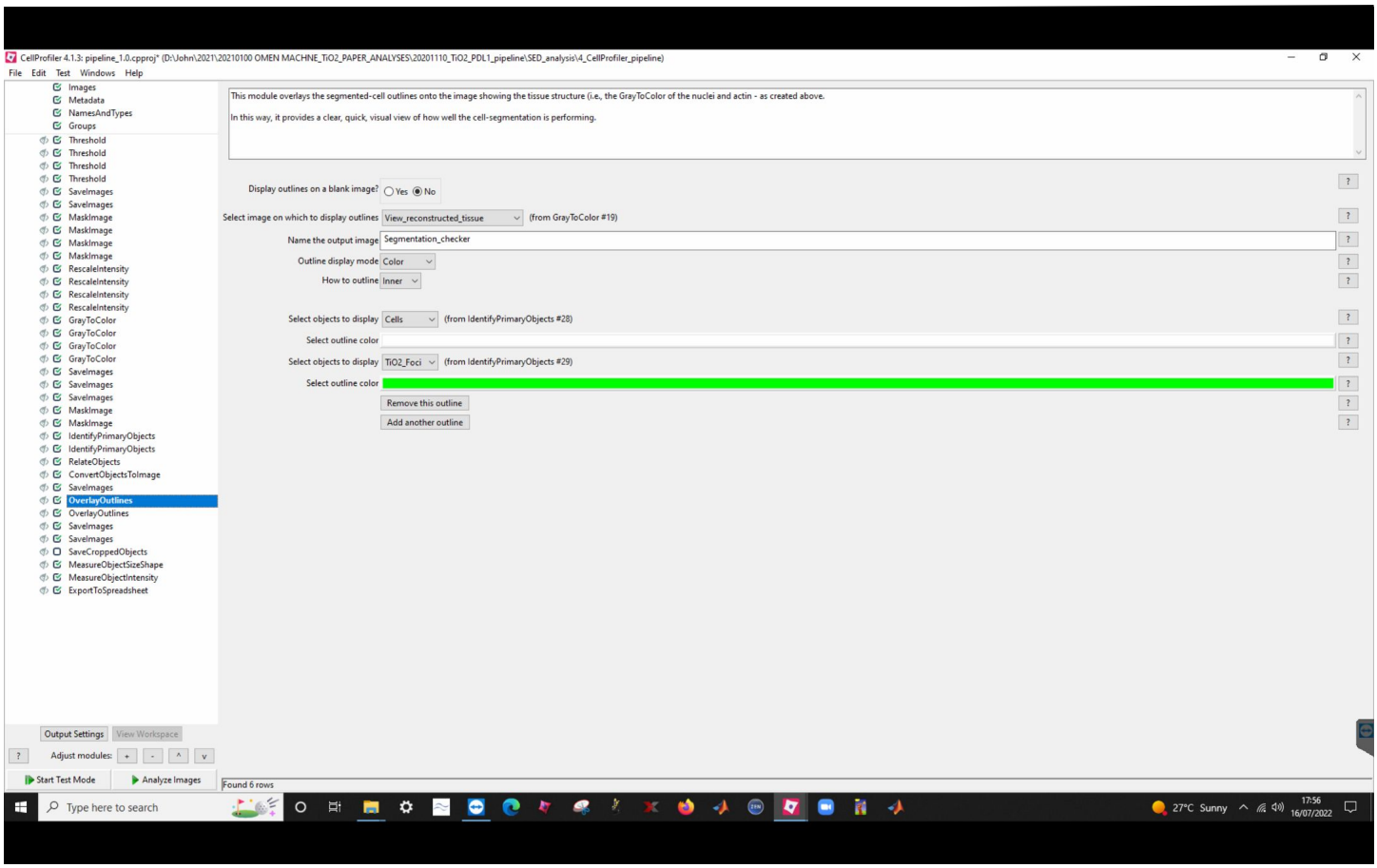

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
This module uses the Unet probability maps (c5) to identify cell-objects in each image-field
Use advanced settings? Yes No
Select the input image c5_Masked (from MaskImage #26)
Name the primary objects to be identified Cells
Typical diameter of objects, in pixel units (Min,Max) 40 90
Discard objects outside the diameter range? Yes No
Discard objects touching the border of the image? Yes No
Threshold strategy Global
Thresholding method Manual
Manual threshold 0.0000001
Threshold smoothing scale 22
Method to distinguish clumped objects Intensity
Method to draw dividing lines between clumped objects Intensity
Automatically calculate size of smoothing filter for declumping? Yes No
Automatically calculate minimum allowed distance between local maxima? Yes No
Suppress local maxima that are closer than this minimum allowed distance 20
Speed up by using lower-resolution image to find local maxima? Yes No
Display accepted local maxima? Yes No
Fill holes in identified objects? After declumping only
Handling of objects if excessive number of objects identified Continue
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

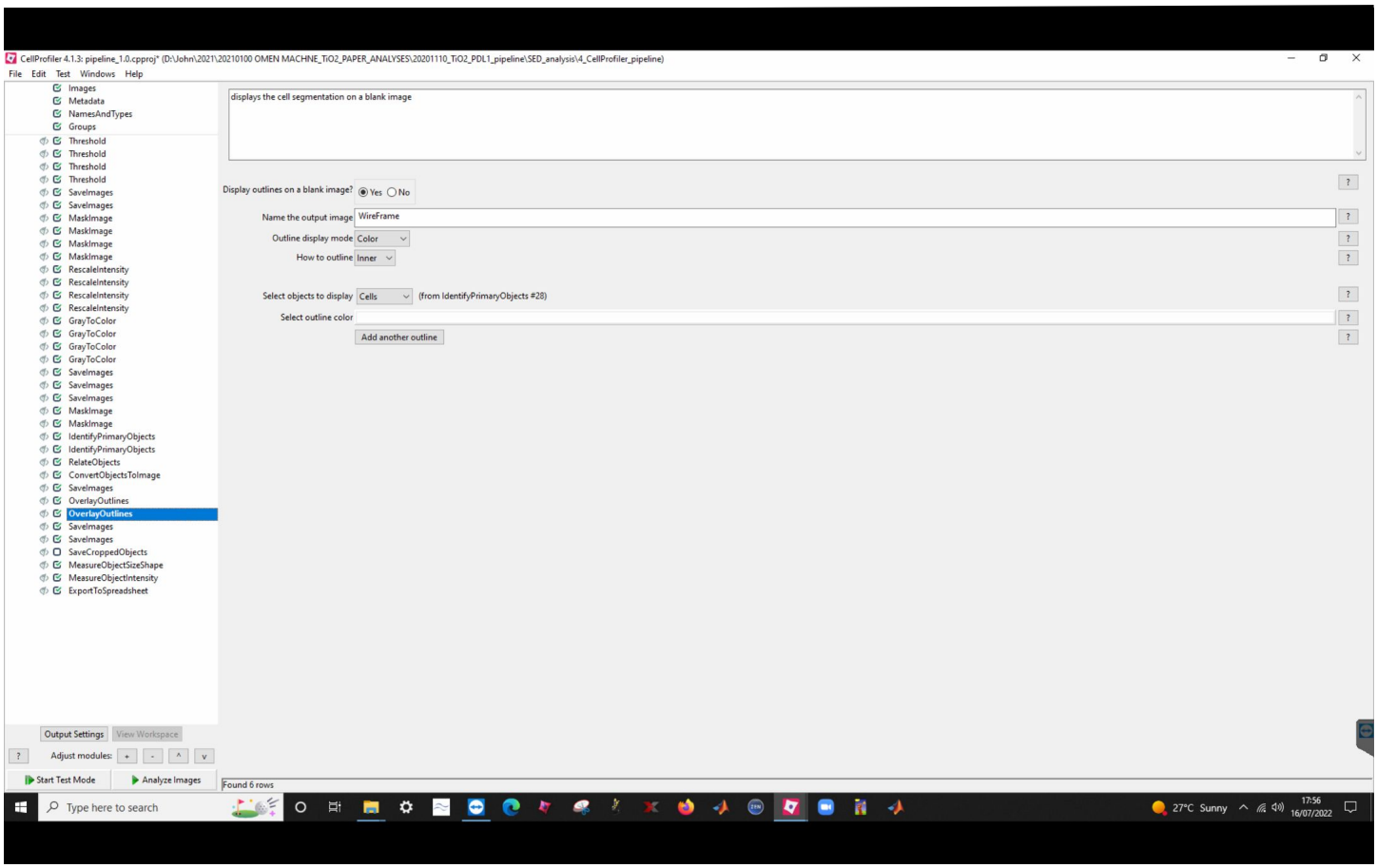

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
This module segments reflectant foci - or nanoparticle loaded vesicles
Use advanced settings? Yes No
Select the input image Thresh_TiO2_Masked (from MaskImage #27)
Name the primary objects to be identified TiO2_Foci
Typical diameter of objects, in pixel units (Min,Max) 1 50
Discard objects outside the diameter range? Yes No
Discard objects touching the border of the image? Yes No
Threshold strategy Global
Thresholding method Manual
Manual threshold 0.0000001
Threshold smoothing scale 0
Method to distinguish clumped objects Intensity
Method to draw dividing lines between clumped objects Intensity
Automatically calculate size of smoothing filter for declumping? Yes No
Automatically calculate minimum allowed distance between local maxima? Yes No
Speed up by using lower-resolution image to find local maxima? Yes No
Display accepted local maxima? Yes No
Fill holes in identified objects? After declumping only
Handling of objects if excessive number of objects identified Continue
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

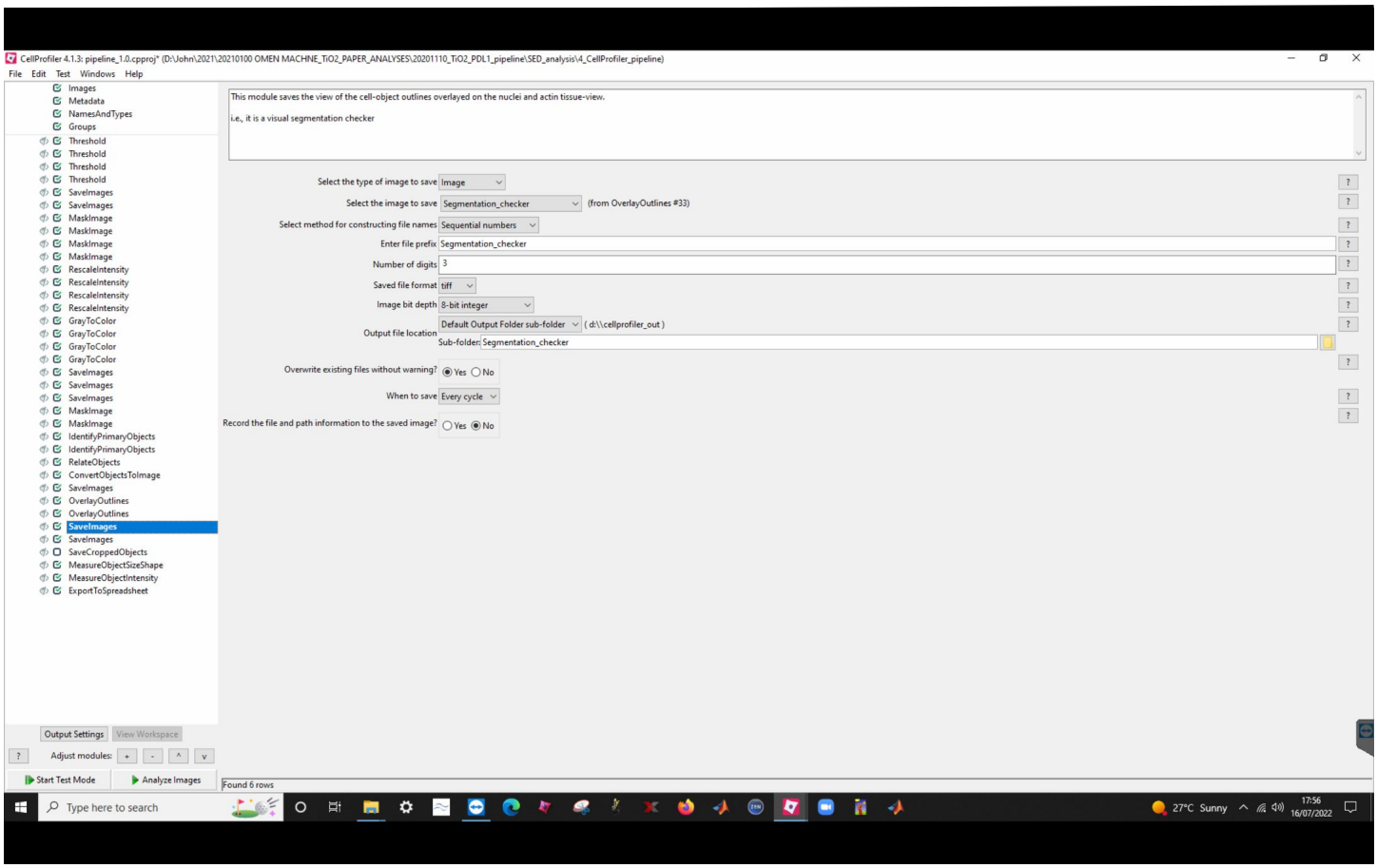

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module relates the cell objects to the NLV objects - e.g. so the NLV belonging to each cell object can be identified
Parent objects Cells (from IdentifyPrimaryObjects #28)
Child objects TiO2_Foci (from IdentifyPrimaryObjects #29)
Calculate per-parent means for all child measurements? Yes No
Calculate child-parent distances? None
Do you want to save the children with parents as a new object set? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

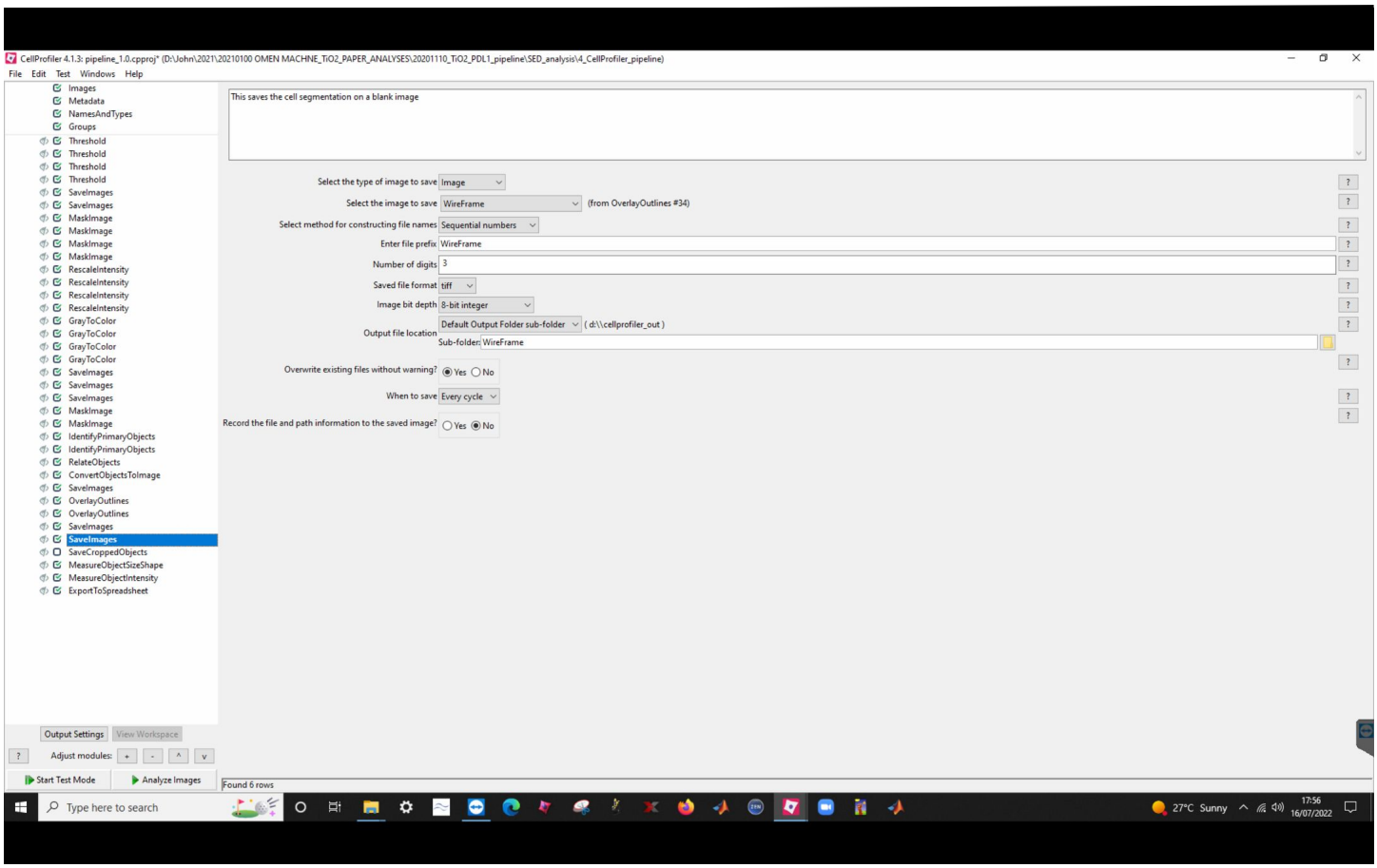

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module converts the identified cell-objects into an image called CellMasks where each pixel is numbered according to the cell-object number it belongs to in that image-field.
This image is useful for colouring specific cell-objects for downstream visulisations of the data e.g., cell mapping the delivered dose of TiO2
Select the input objects Cells (from IdentifyPrimaryObjects #28)
Name the output image CellMasks
Select the color format uint16
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

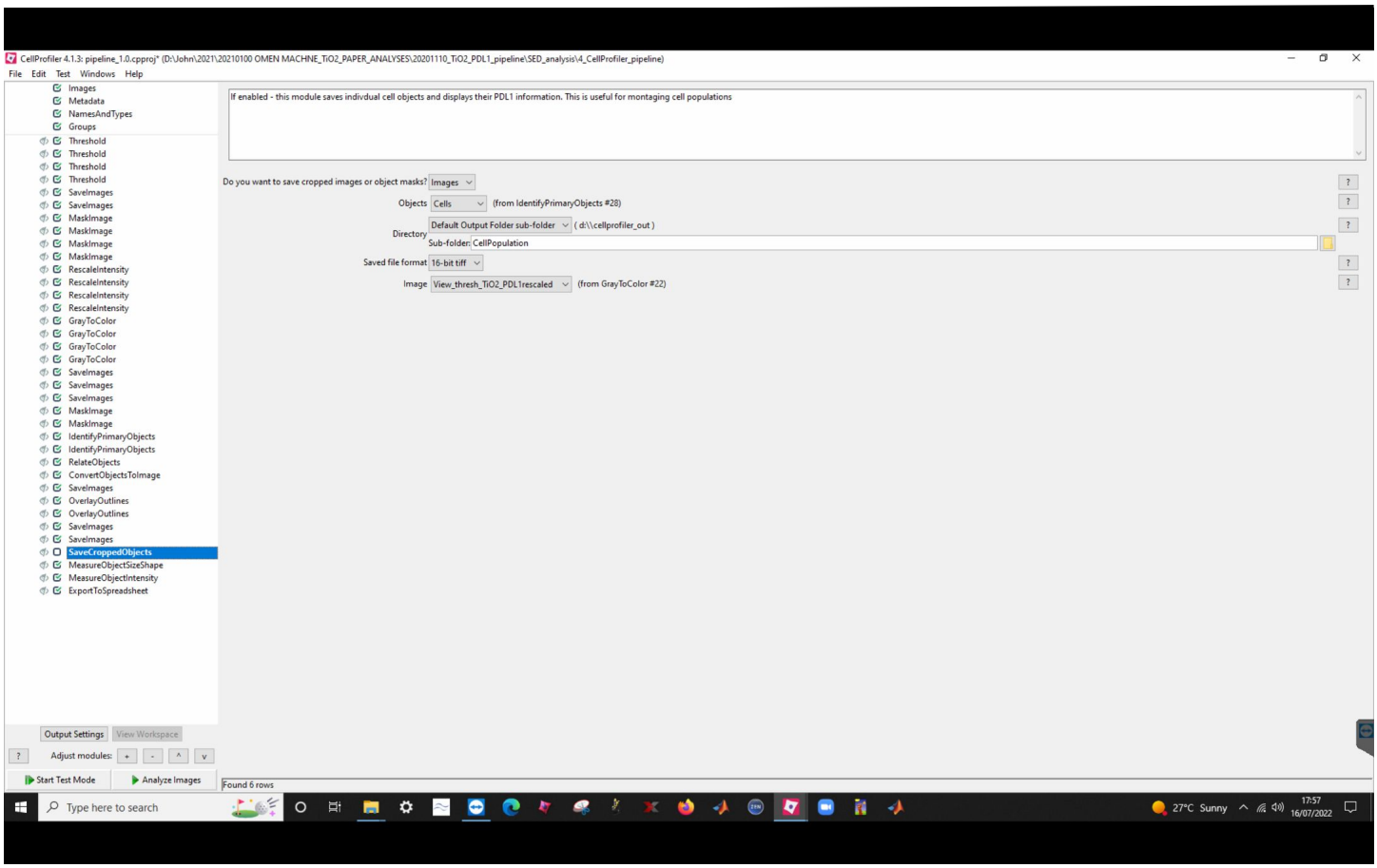

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
This module saves the image of the CellMasks as a NumPy array
Select the type of image to save
Image
Select the image to save
CellMasks
(from ConvertObjectsToImage #31)
Select method for constructing file names
Sequential numbers
Enter file prefix
CellMasks
Number of digits
3
Saved file format
npy
Output file location
Default Output Folder sub-folder
(d:\\cellprofiler_out)
Sub-folder:
CellMasks
Overwrite existing files without warning?
Yes
No
When to save
Every cycle
Record the file and path information to the saved image?
Yes
No
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

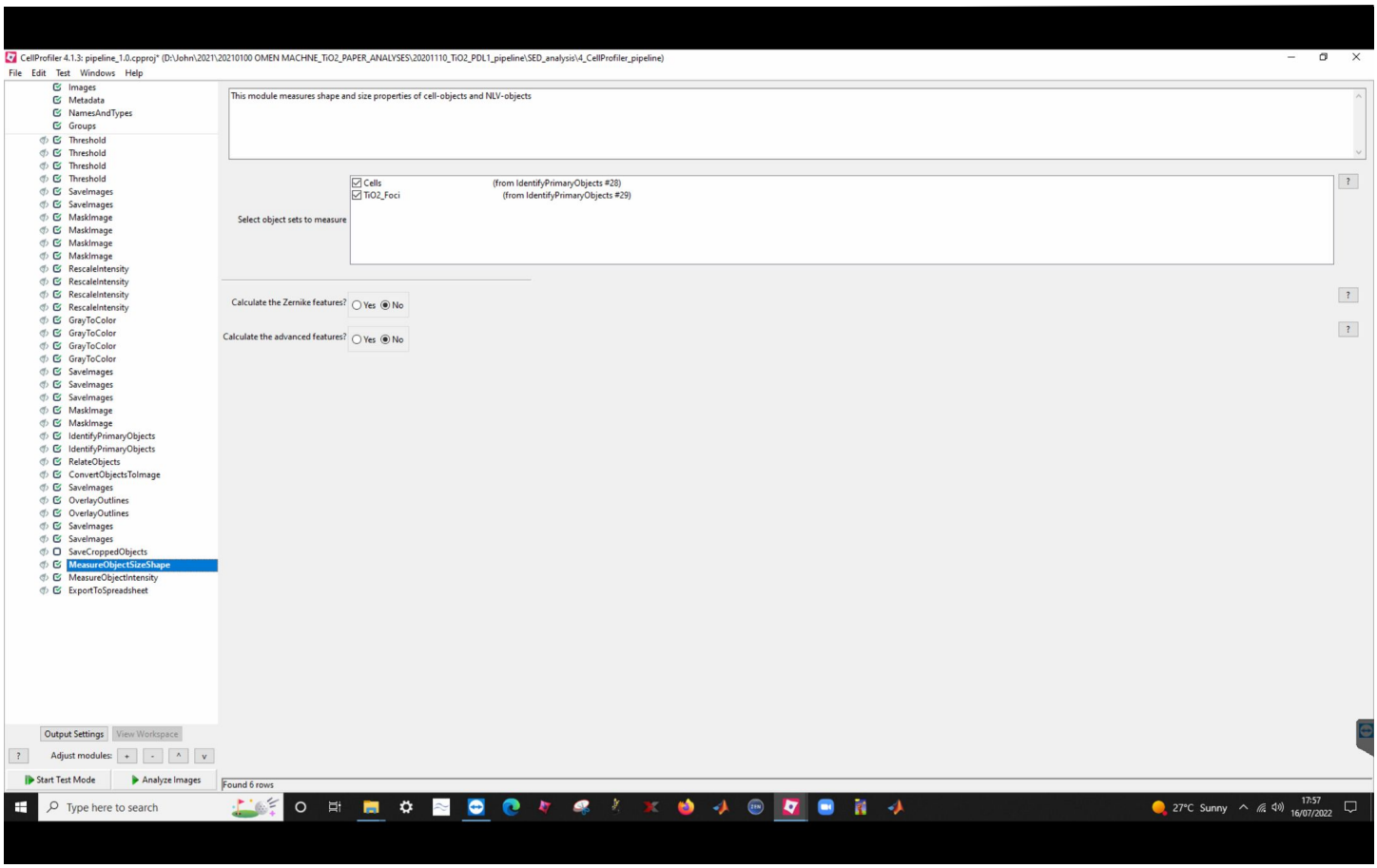

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module overlays the segmented-cell outlines onto the image showing the tissue structure (i.e., the GrayToColor of the nuclei and actin - as created above.
In this way, it provides a clear, quick, visual view of how well the cell-segmentation is performing.
Display outlines on a blank image?
Yes
No
Select image on which to display outlines
View_reconstructed_tissue
(from GrayToColor #19)
Name the output image
Segmentation_checker
Outline display mode
Color
How to outline
Inner
Select objects to display
Cells
(from IdentifyPrimaryObjects #28)
Select outline color
Select objects to display
TiO2_Foci
(from IdentifyPrimaryObjects #29)
Select outline color
Remove this outline
Add another outline
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

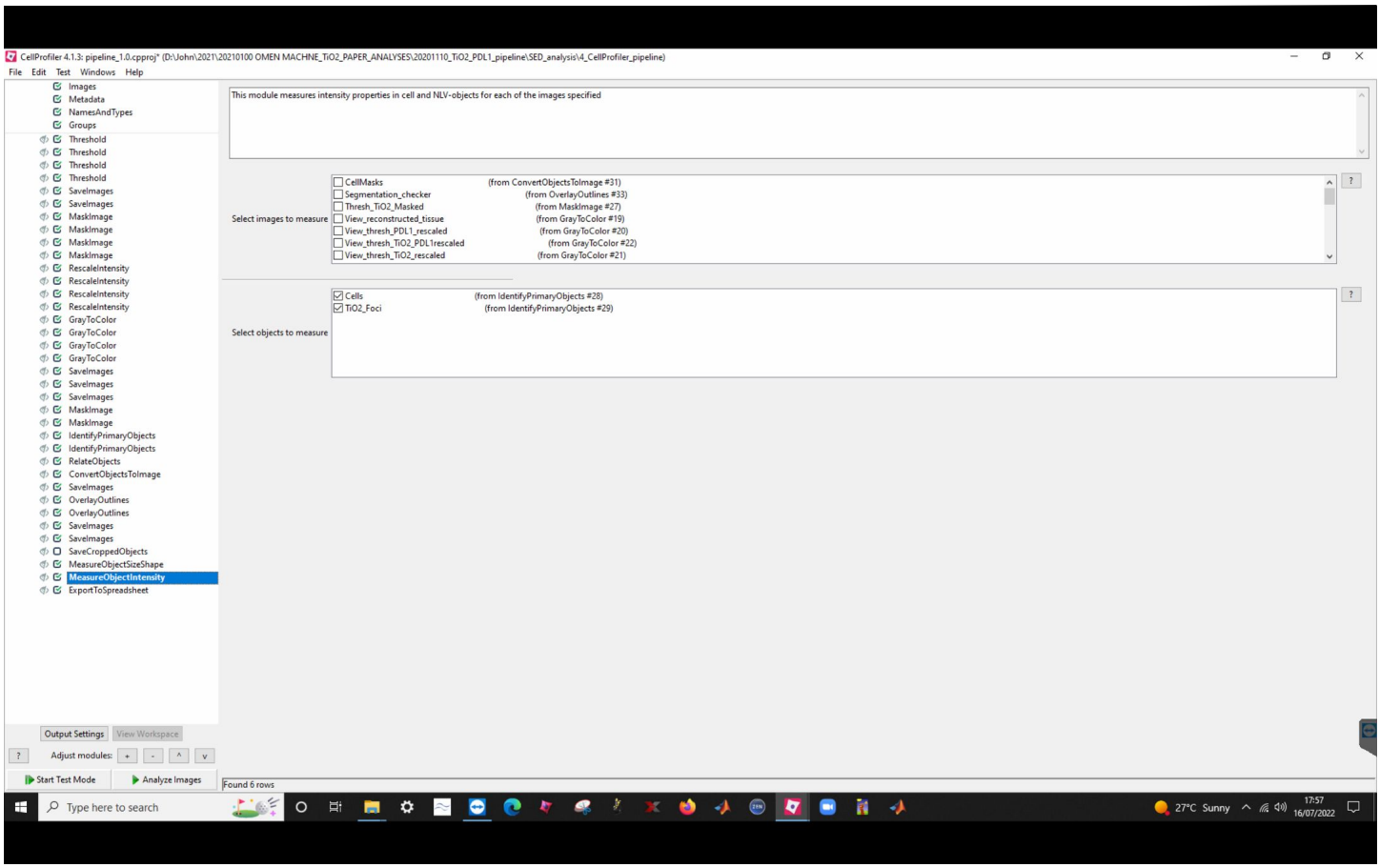

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
displays the cell segmentation on a blank image
Display outlines on a blank image?
Yes
No
Name the output image
WireFrame
Outline display mode
Color
How to outline
Inner
Select objects to display
Cells
(from IdentifyPrimaryObjects #28)
Select outline color
Add another outline
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

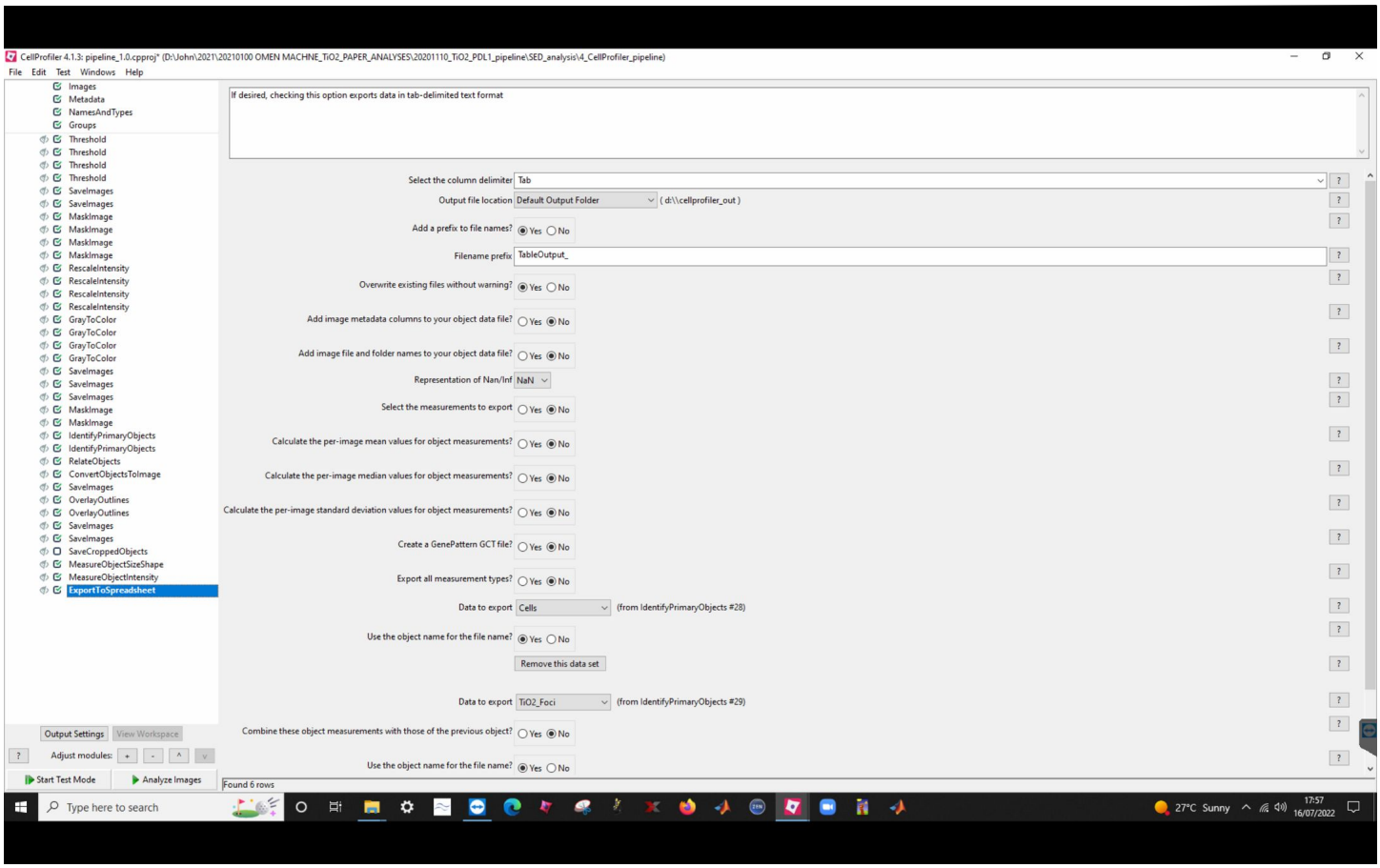

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module saves the view of the cell-object outlines overlayed on the nuclei and actin tissue-view.
i.e., it is a visual segmentation checker
Select the type of image to save Image
Select the image to save Segmentation_checker (from OverlayOutlines #33)
Select method for constructing file names Sequential numbers
Enter file prefix Segmentation_checker
Number of digits 3
Saved file format tiff
Image bit depth 8-bit integer
Output file location Default Output Folder sub-folder (d:\\cellprofiler_out)
Sub-folder: Segmentation_checker
Overwrite existing files without warning? Yes No
When to save Every cycle
Record the file and path information to the saved image? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022


CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This saves the cell segmentation on a blank image
Select the type of image to save Image
Select the image to save WireFrame (from OverlayOutlines #34)
Select method for constructing file names Sequential numbers
Enter file prefix WireFrame
Number of digits 3
Saved file format tiff
Image bit depth 8-bit integer
Output file location Default Output Folder sub-folder (d:\\cellprofiler_out)
Sub-folder: WireFrame
Overwrite existing files without warning? Yes No
When to save Every cycle
Record the file and path information to the saved image? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:56
16/07/2022

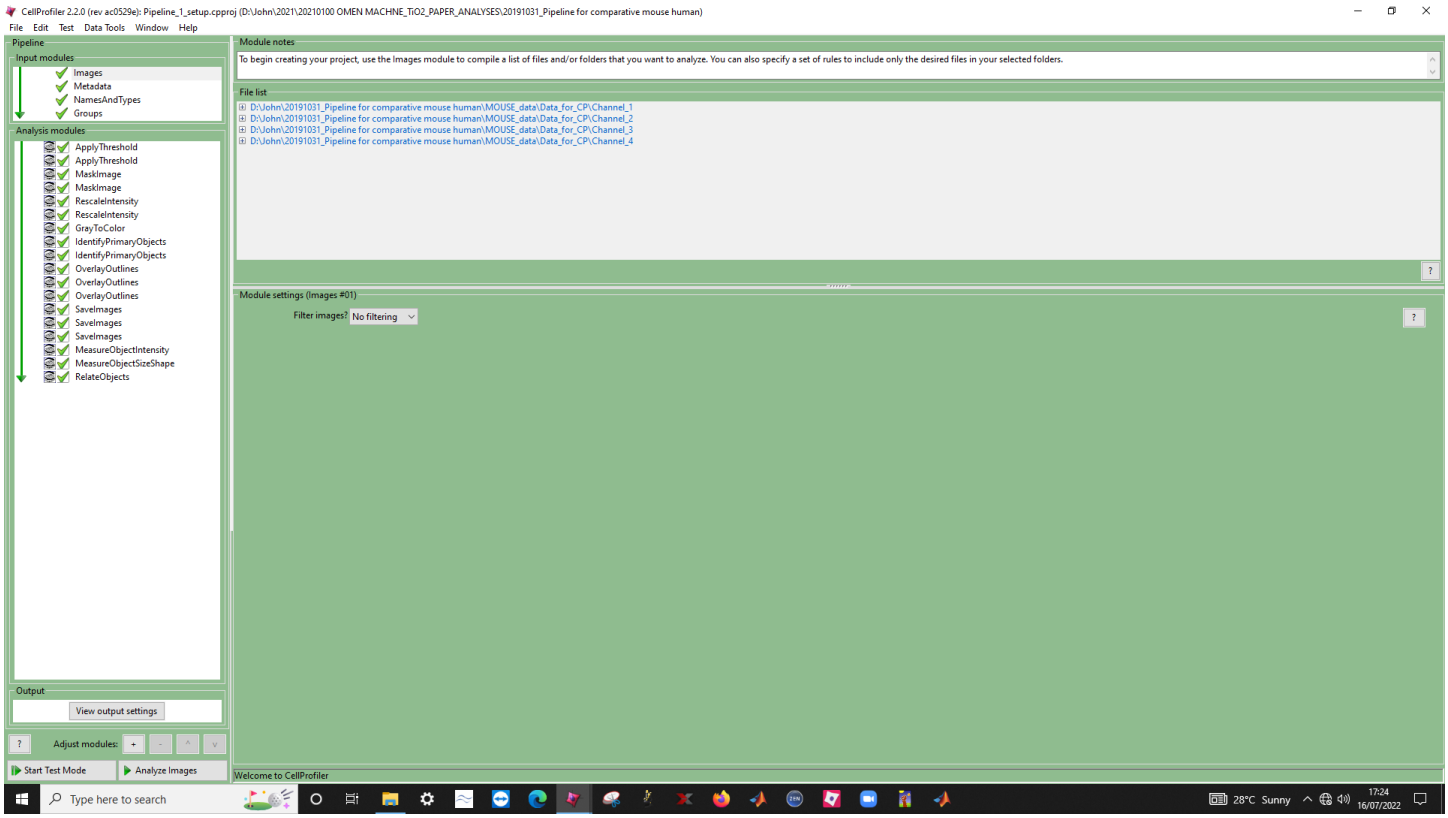

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
If enabled - this module saves indivdual cell objects and displays their PDL1 information. This is useful for montaging cell populations
Do you want to save cropped images or object masks? Images
Objects Cells (from IdentifyPrimaryObjects #28)
Directory Default Output Folder sub-folder (d:\\cellprofiler_out)
Sub-folder: CellPopulation
Saved file format 16-bit tiff
Image View_thresh_TiO2_PDL1rescaled (from GrayToColor #22)
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:57
16/07/2022

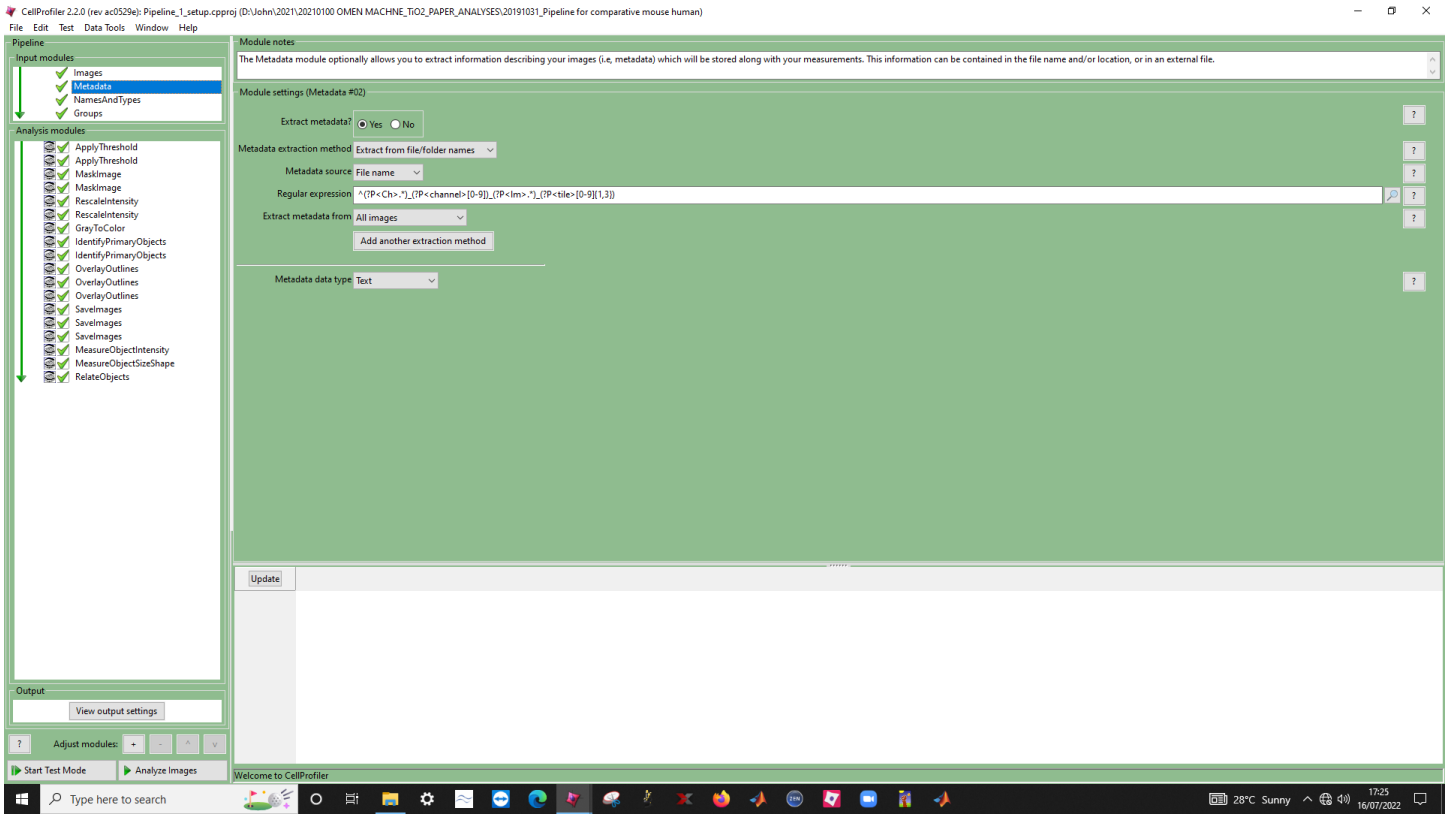

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module measures shape and size properties of cell-objects and NLV-objects
Select object sets to measure
Cells (from IdentifyPrimaryObjects #28)
TiO2_Foci (from IdentifyPrimaryObjects #29)
Calculate the Zernike features? Yes No
Calculate the advanced features? Yes No
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:57
16/07/2022

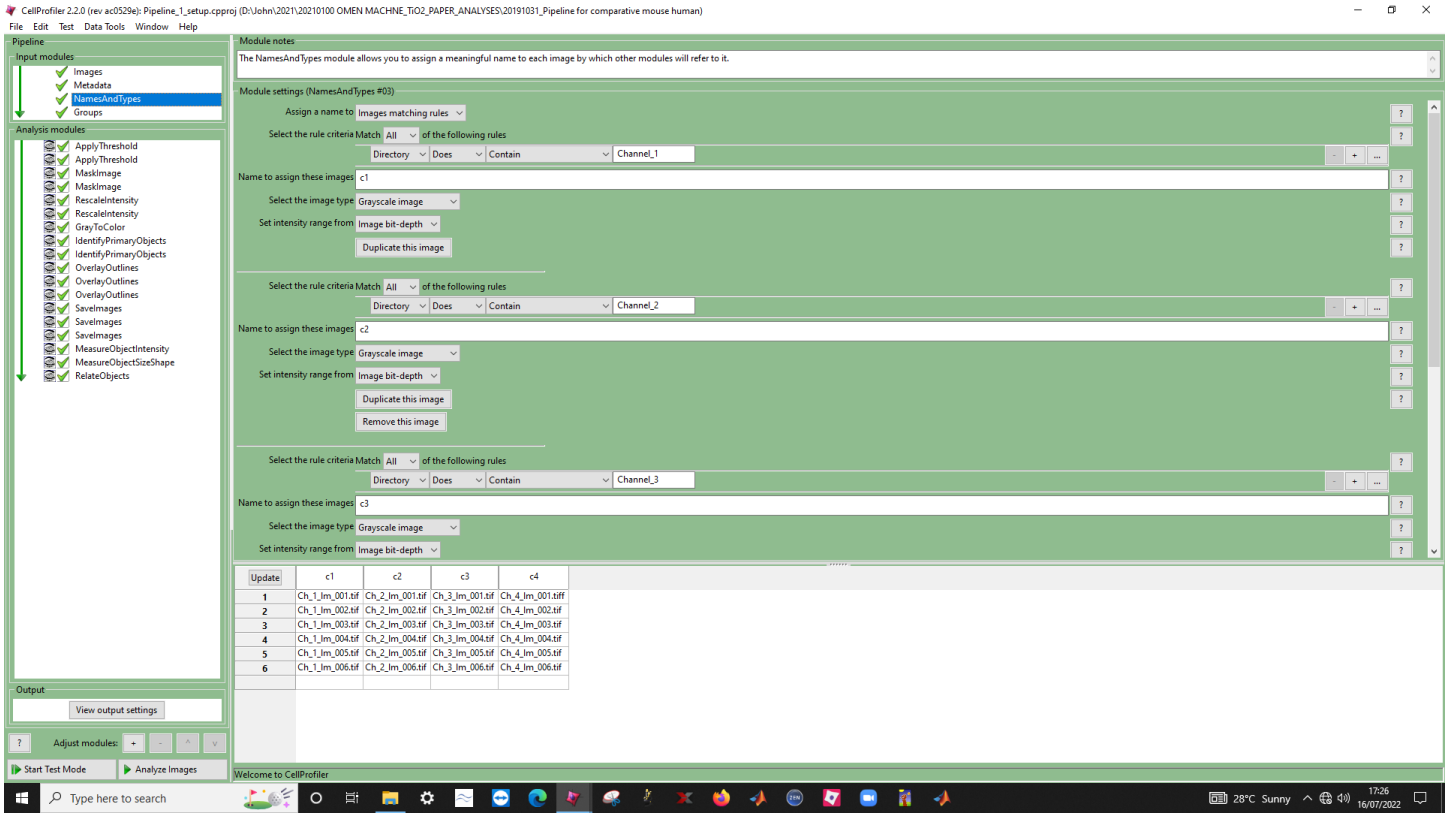

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
This module measures intensity properties in cell and NLV-objects for each of the images specified
Select images to measure
CellMasks (from ConvertObjectsToImage #31)
Segmentation_checker (from OverlayOutlines #33)
Thresh_TiO2_Masked (from MaskImage #27)
View_reconstructed_tissue (from GrayToColor #19)
View_thresh_PDL1_rescaled (from GrayToColor #20)
View_thresh_TiO2_PDL1rescaled (from GrayToColor #22)
View_thresh_TiO2_rescaled (from GrayToColor #21)
Select objects to measure
Cells (from IdentifyPrimaryObjects #28)
TiO2_Foci (from IdentifyPrimaryObjects #29)
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
Found 6 rows
Type here to search
27°C Sunny
17:57
16/07/2022

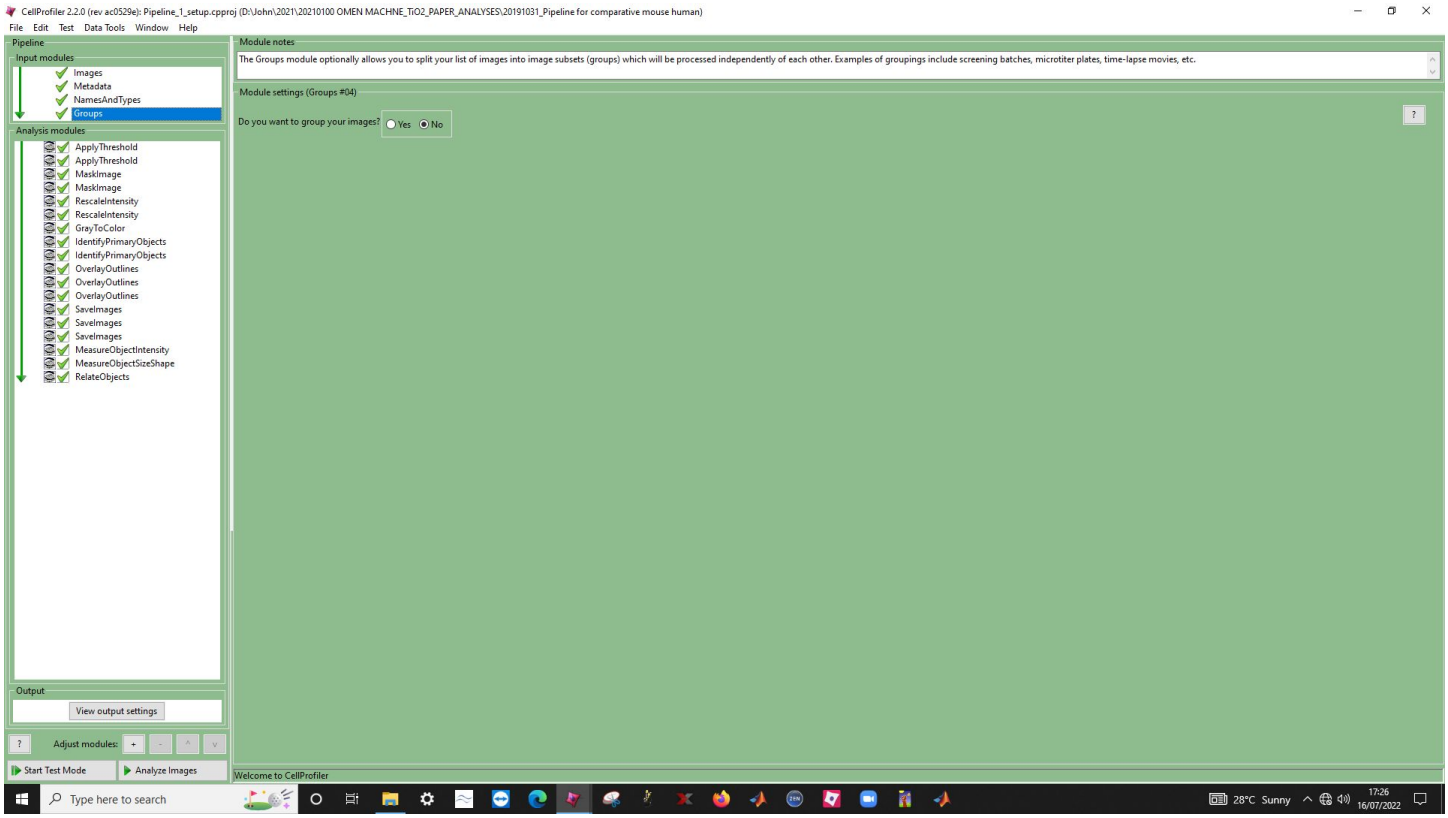

CellProfiler 4.1.3: pipeline_1.0.cpproj* (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20201110_TiO2_PDL1_pipeline\SED_analysis\4_CellProfiler_pipeline)
File Edit Test Windows Help
Images
Metadata
NamesAndTypes
Groups
Threshold
Threshold
Threshold
Threshold
SaveImages
SaveImages
MaskImage
MaskImage
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
RescaleIntensity
RescaleIntensity
GrayToColor
GrayToColor
GrayToColor
GrayToColor
SaveImages
SaveImages
SaveImages
MaskImage
MaskImage
IdentifyPrimaryObjects
IdentifyPrimaryObjects
RelateObjects
ConvertObjectsToImage
SaveImages
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveCroppedObjects
MeasureObjectSizeShape
MeasureObjectIntensity
ExportToSpreadsheet
Output Settings
View Workspace
Adjust modules:
Start Test Mode
Analyze Images
If desired, checking this option exports data in tab-delimited text format
Select the column delimiter
Tab
Output file location
Default Output Folder
(d:\\cellprofiler_out)
Add a prefix to file names?
Yes No
Filename prefix
TableOutput_
Overwrite existing files without warning?
Add image metadata columns to your object data file?
Add image file and folder names to your object data file?
Representation of Nan/Inf
NaN
Select the measurements to export
Calculate the per-image mean values for object measurements?
Calculate the per-image median values for object measurements?
Calculate the per-image standard deviation values for object measurements?
Create a GenePattern GCT file?
Export all measurement types?
Data to export
Cells
(from IdentifyPrimaryObjects #28)
Use the object name for the file name?
Remove this data set
Data to export
TiO2_Foci
(from IdentifyPrimaryObjects #29)
Combine these object measurements with those of the previous object?
Use the object name for the file name?
Found 6 rows
Type here to search
27°C Sunny
17:57
16/07/2022

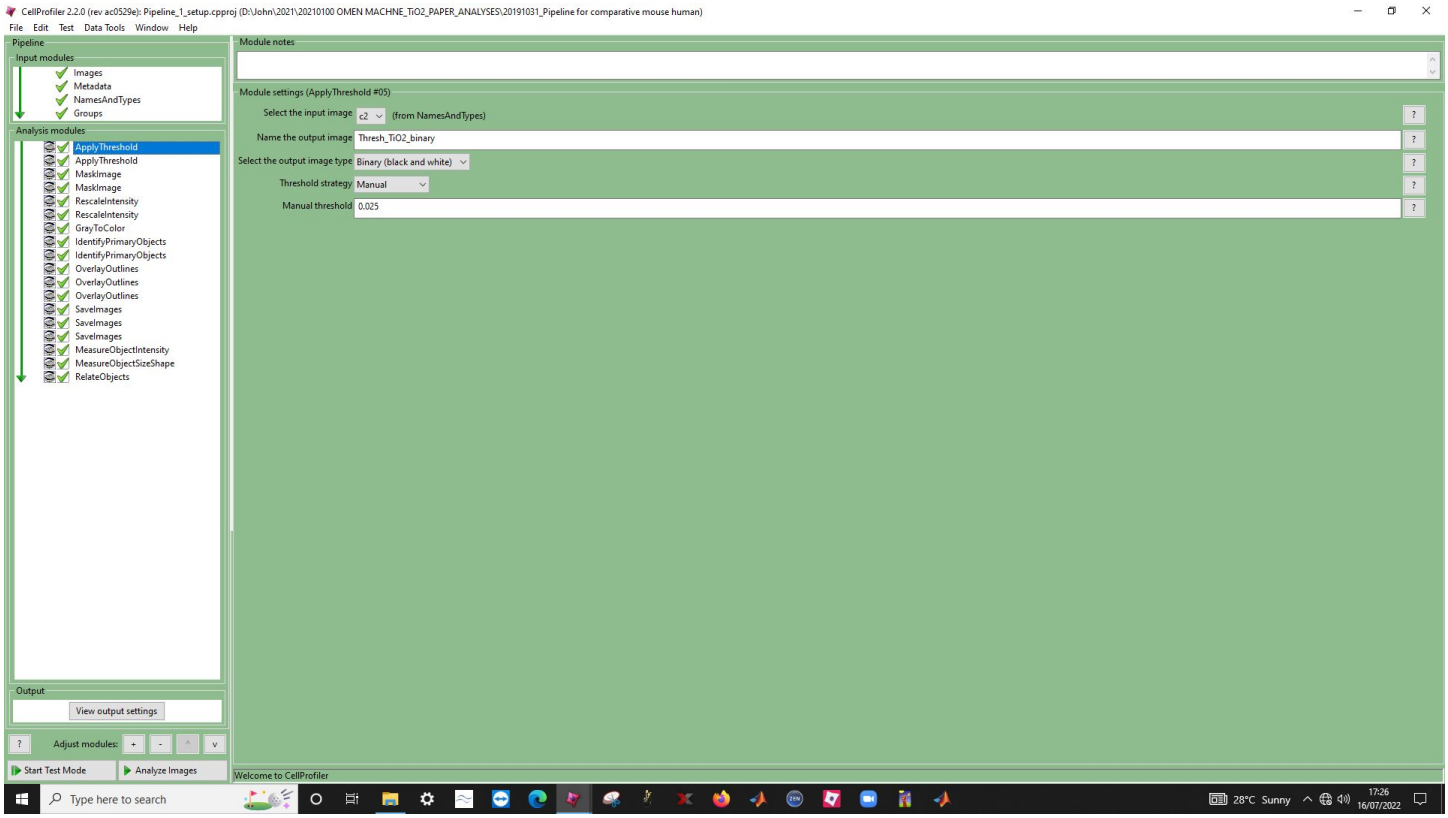
Supplementary Note 3 – Mouse-Human correlative dosimetry pipeline: This section presents screenshots of the CellProfiler image analysis pipeline used to measure per-cell and TLV-object intensity and shape/size features enabling the correlative mouse-human dosimetry work. The pipeline and sample image-data are available for download from the BioStudies archive accompanying the paper.

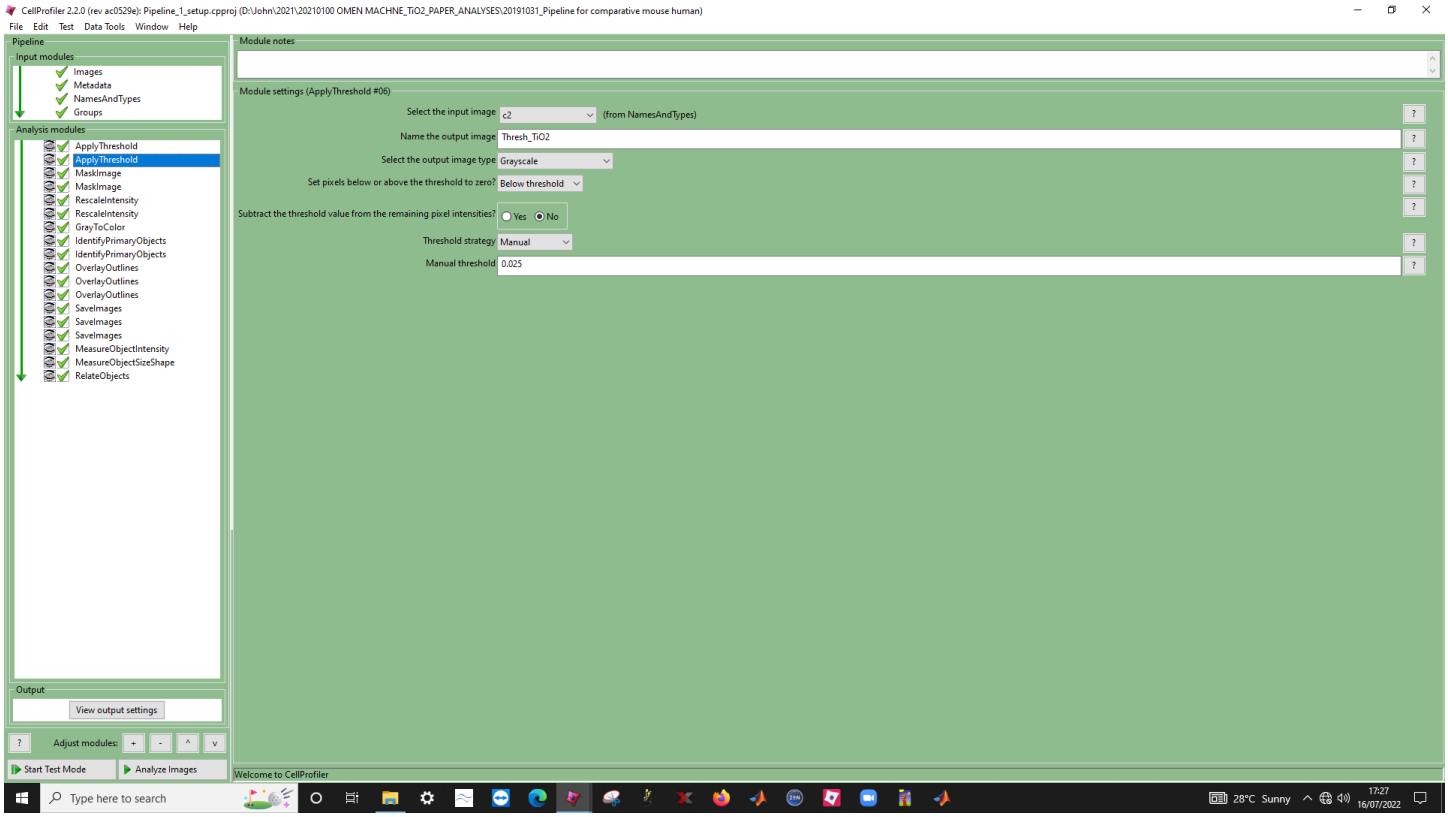


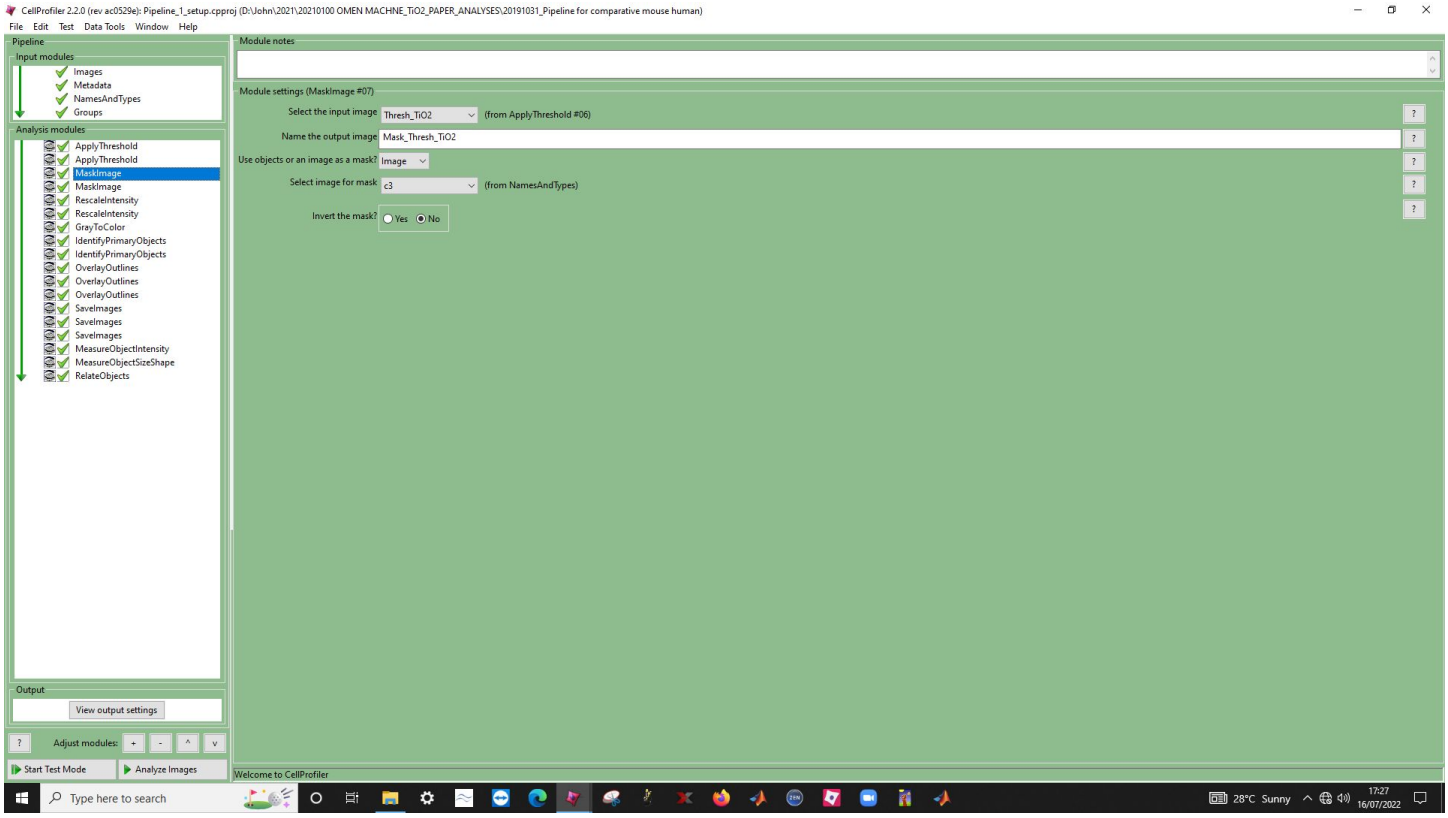

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
The Metadata module optionally allows you to extract information describing your images (i.e, metadata) which will be stored along with your measurements. This information can be contained in the file name and/or location, or in an external file.
Module settings (Metadata #02)
Extract metadata? Yes No
Metadata extraction method Extract from file/folder names
Metadata source File name
Regular expression ^(?P<Ch>.*)_(?P<channel>[0-9])_(?P<Im>.*)_(?P<tile>[0-9]{1,3})
Extract metadata from All images
Add another extraction method
Metadata data type Text
Update
Welcome to CellProfiler
Type here to search
28°C Sunny
17:25
16/07/2022

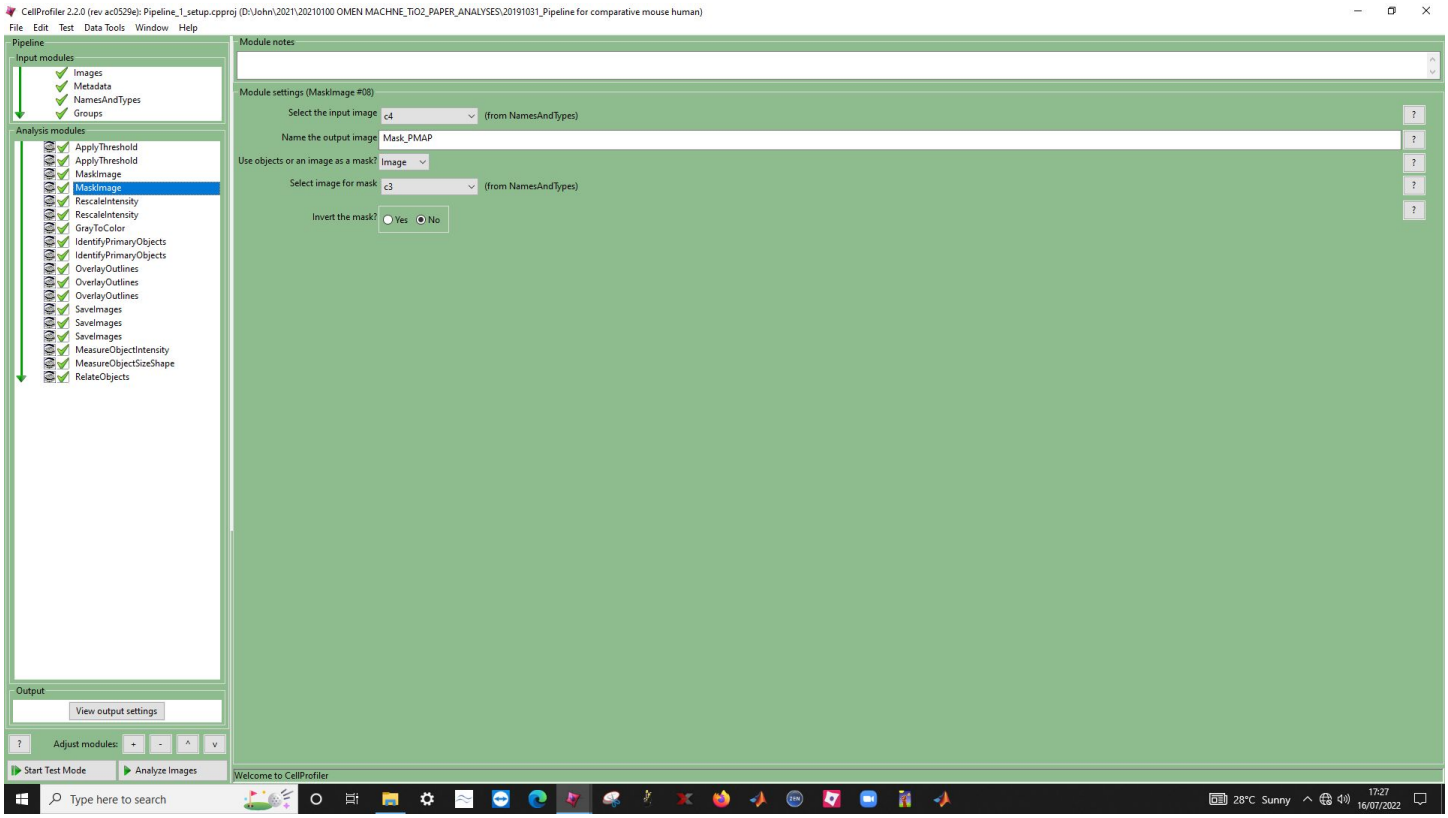

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
The NamesAndTypes module allows you to assign a meaningful name to each image by which other modules will refer to it.
Module settings (NamesAndTypes #03)
Assign a name to Images matching rules
Select the rule criteria Match All of the following rules
Directory Does Contain Channel_1
Name to assign these images c1
Select the image type Grayscale image
Set intensity range from Image bit-depth
Duplicate this image
Select the rule criteria Match All of the following rules
Directory Does Contain Channel_2
Name to assign these images c2
Select the image type Grayscale image
Set intensity range from Image bit-depth
Duplicate this image
Remove this image
Select the rule criteria Match All of the following rules
Directory Does Contain Channel_3
Name to assign these images c3
Select the image type Grayscale image
Set intensity range from Image bit-depth
Update
c1 c2 c3 c4
1 Ch_1_Im_001.tif Ch_2_Im_001.tif Ch_3_Im_001.tif Ch_4_Im_001.tiff
2 Ch_1_Im_002.tif Ch_2_Im_002.tif Ch_3_Im_002.tif Ch_4_Im_002.tif
3 Ch_1_Im_003.tif Ch_2_Im_003.tif Ch_3_Im_003.tif Ch_4_Im_003.tif
4 Ch_1_Im_004.tif Ch_2_Im_004.tif Ch_3_Im_004.tif Ch_4_Im_004.tif
5 Ch_1_Im_005.tif Ch_2_Im_005.tif Ch_3_Im_005.tif Ch_4_Im_005.tif
6 Ch_1_Im_006.tif Ch_2_Im_006.tif Ch_3_Im_006.tif Ch_4_Im_006.tif
Welcome to CellProfiler
Type here to search
28°C Sunny
17:26
16/07/2022

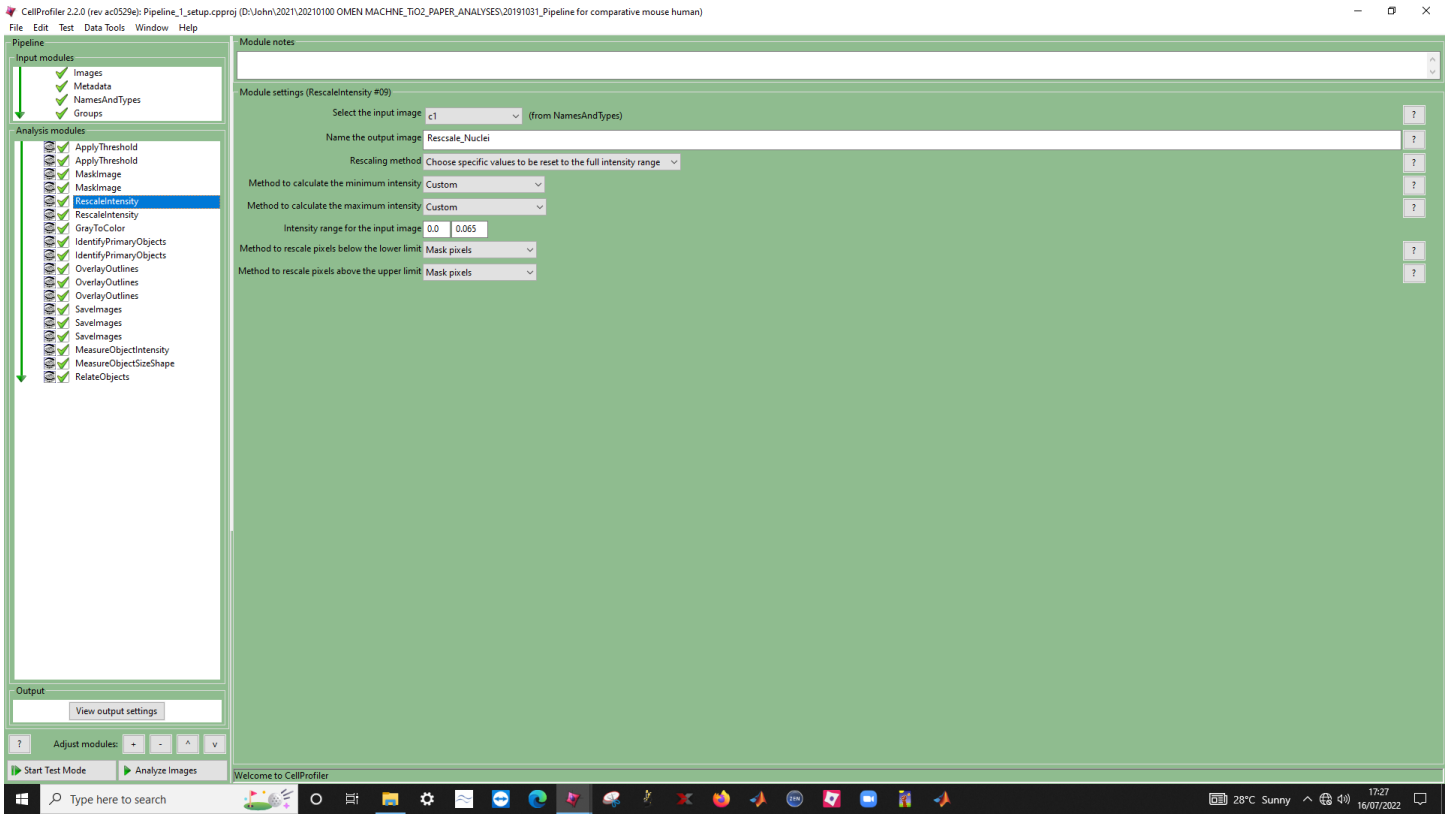

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
The Groups module optionally allows you to split your list of images into image subsets (groups) which will be processed independently of each other. Examples of groupings include screening batches, microtiter plates, time-lapse movies, etc.
Module settings (Groups #04)
Do you want to group your images?
Yes
No
Welcome to CellProfiler
Type here to search
28°C Sunny
17:26
16/07/2022

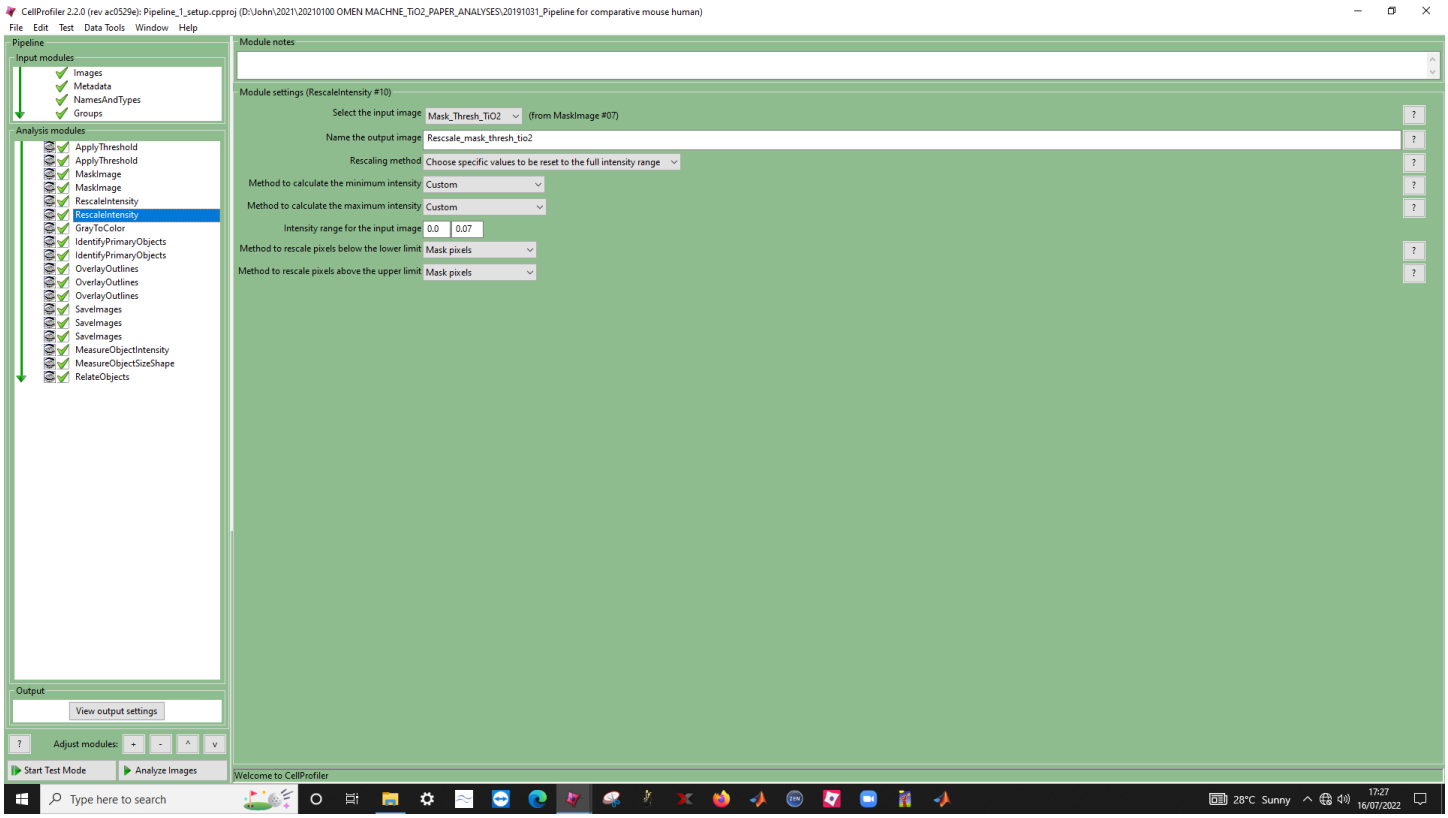

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (ApplyThreshold #05)
Select the input image c2 (from NamesAndTypes)
Name the output image Thresh_TiO2_binary
Select the output image type Binary (black and white)
Threshold strategy Manual
Manual threshold 0.025
Welcome to CellProfiler
Type here to search
28°C Sunny
17:26
16/07/2022

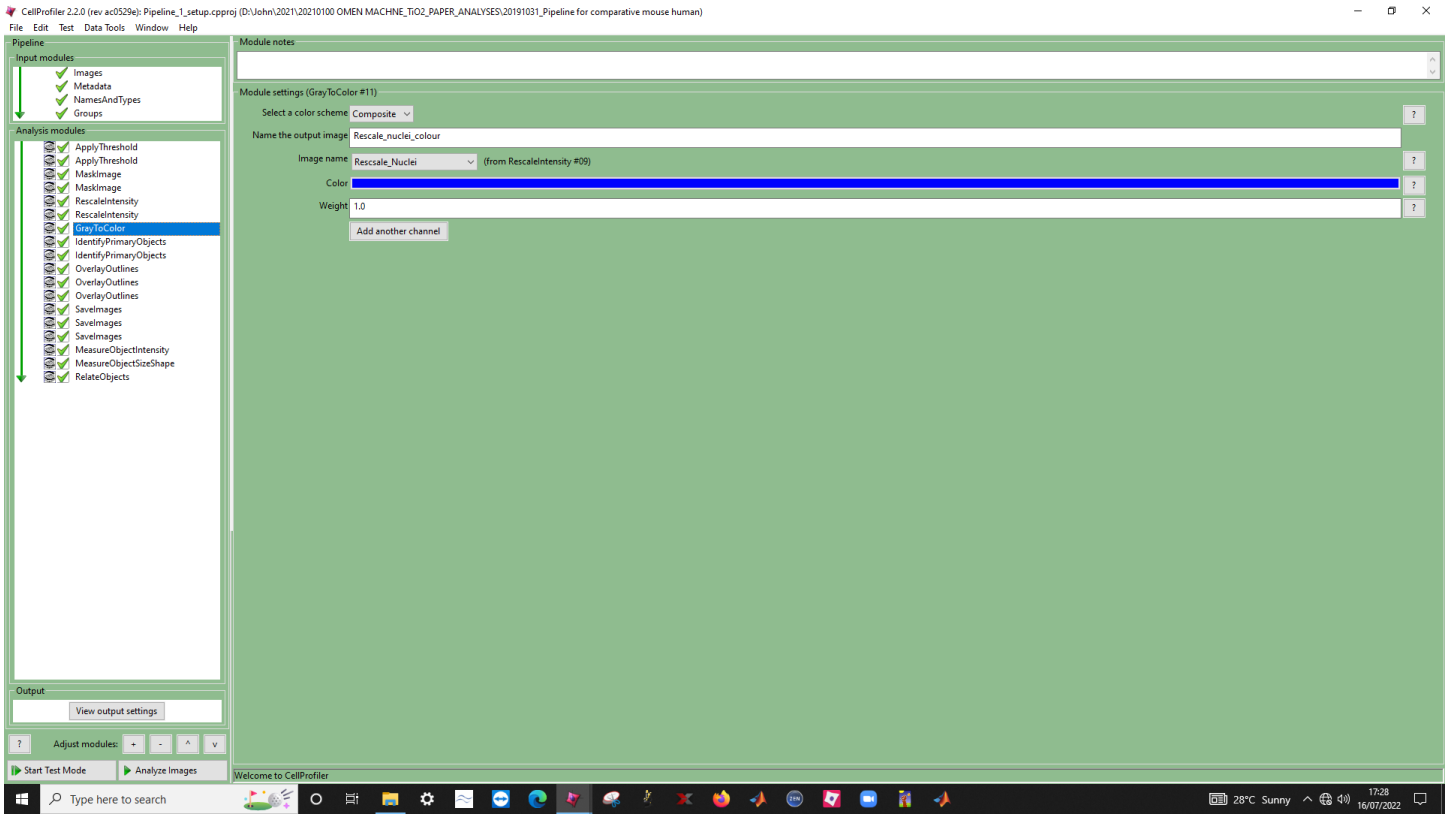

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (ApplyThreshold #06)
Select the input image c2 (from NamesAndTypes)
Name the output image Thresh_TiO2
Select the output image type Grayscale
Set pixels below or above the threshold to zero? Below threshold
Subtract the threshold value from the remaining pixel intensities? Yes No
Threshold strategy Manual
Manual threshold 0.025
Welcome to CellProfiler
Type here to search
28°C Sunny
17:27
16/07/2022

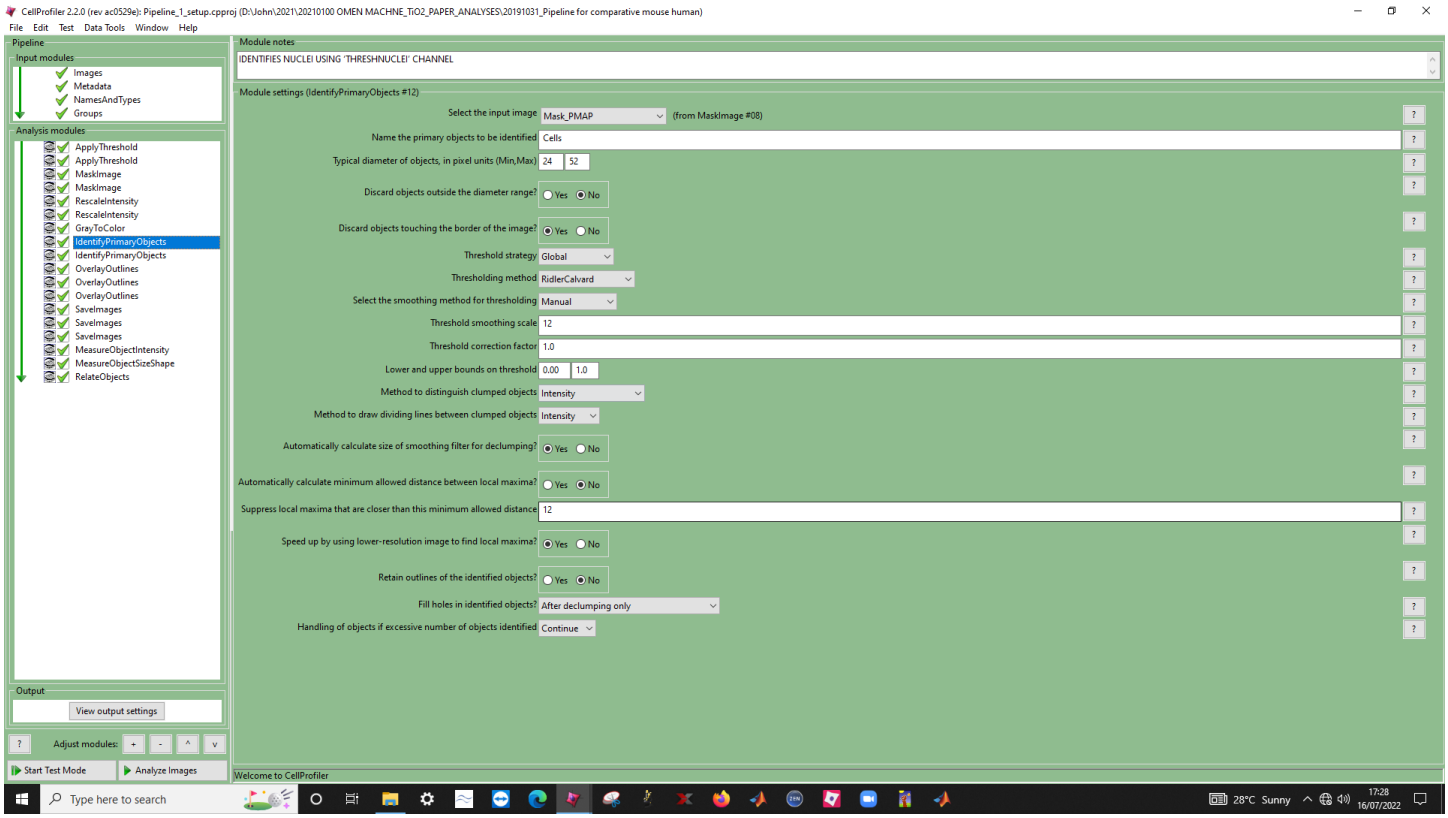

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (MaskImage #07)
Select the input image
Thresh_TiO2
(from ApplyThreshold #06)
Name the output image
Mask_Thresh_TiO2
Use objects or an image as a mask?
Image
Select image for mask
c3
(from NamesAndTypes)
Invert the mask?
Yes
No
Welcome to CellProfiler
Type here to search
28°C Sunny
17:27
16/07/2022

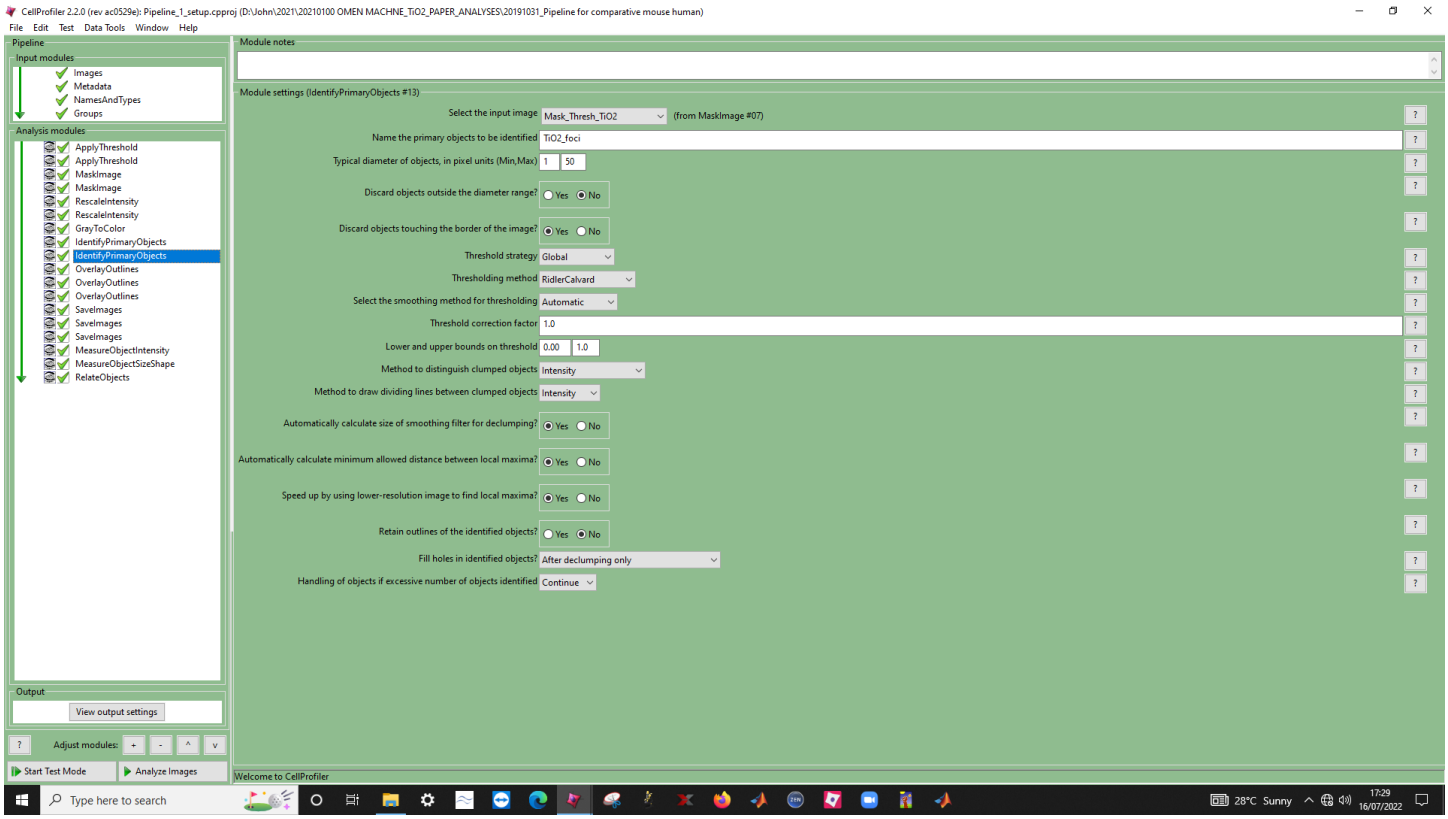

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (MaskImage #08)
Select the input image c4 (from NamesAndTypes)
Name the output image Mask_PMAP
Use objects or an image as a mask? Image
Select image for mask c3 (from NamesAndTypes)
Invert the mask? Yes No
Welcome to CellProfiler
Type here to search
28°C Sunny
17:27
16/07/2022

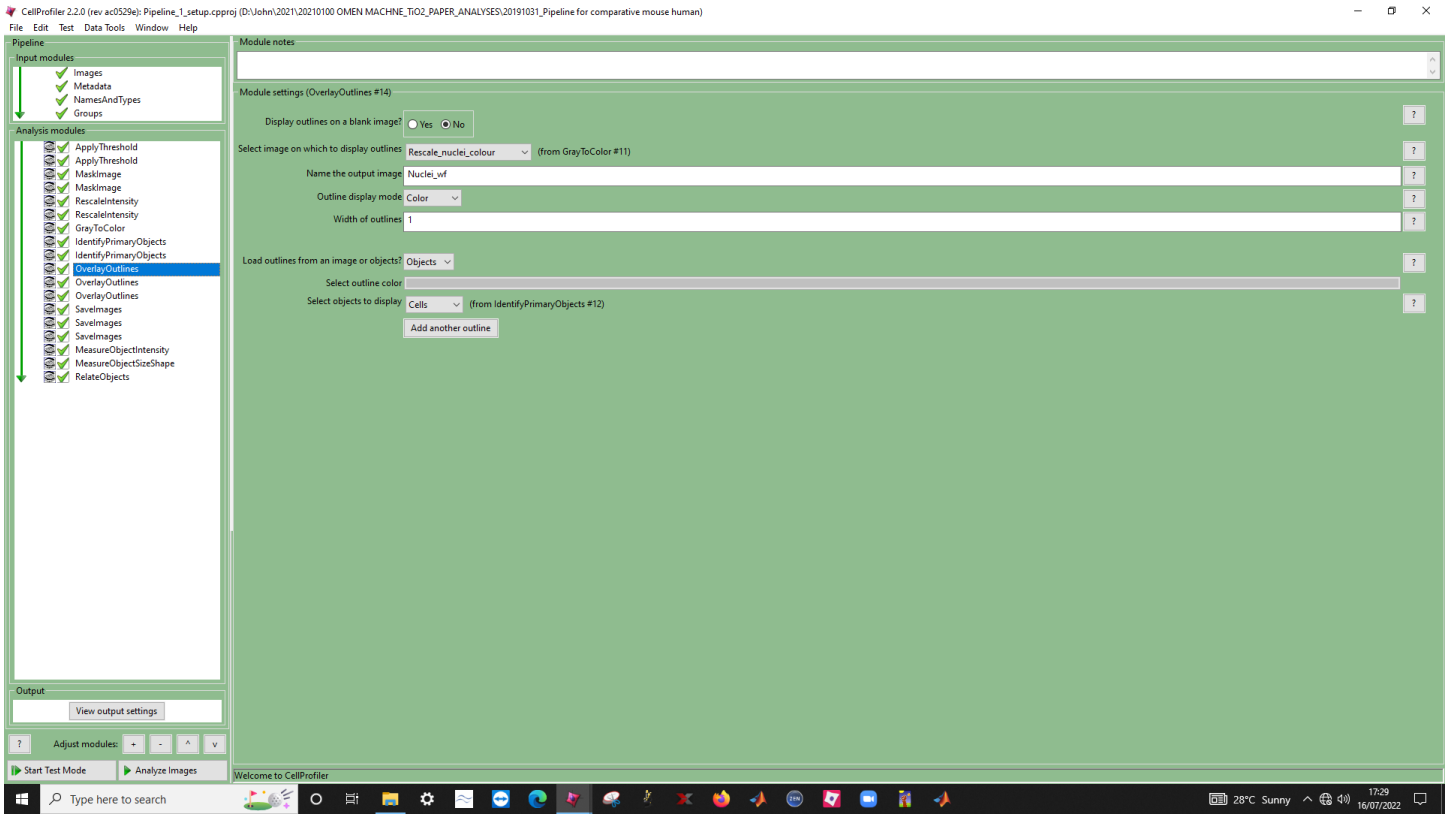

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (RescaleIntensity #09)
Select the input image c1 (from NamesAndTypes)
Name the output image Rescsale_Nuclei
Rescaling method Choose specific values to be reset to the full intensity range
Method to calculate the minimum intensity Custom
Method to calculate the maximum intensity Custom
Intensity range for the input image 0.0 0.065
Method to rescale pixels below the lower limit Mask pixels
Method to rescale pixels above the upper limit Mask pixels
Welcome to CellProfiler
Type here to search
28°C Sunny
17:27
16/07/2022

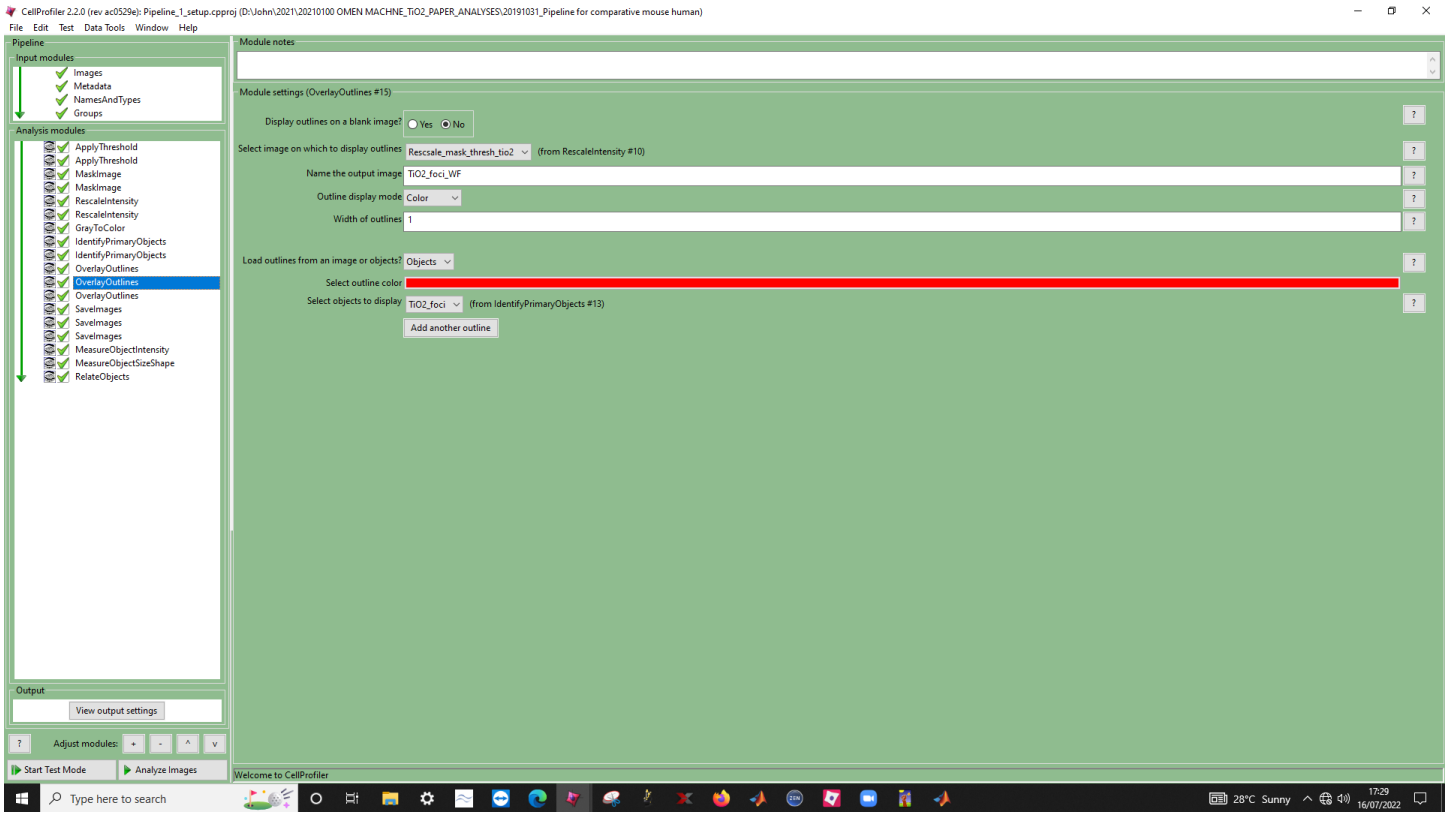

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (RescaleIntensity #10)
Select the input image
Mask_Thresh_TiO2
(from MaskImage #07)
Name the output image
Rescsale_mask_thresh_tio2
Rescaling method
Choose specific values to be reset to the full intensity range
Method to calculate the minimum intensity
Custom
Method to calculate the maximum intensity
Custom
Intensity range for the input image
0.0
0.07
Method to rescale pixels below the lower limit
Mask pixels
Method to rescale pixels above the upper limit
Mask pixels
Welcome to CellProfiler
Type here to search
28°C Sunny
17:27
16/07/2022

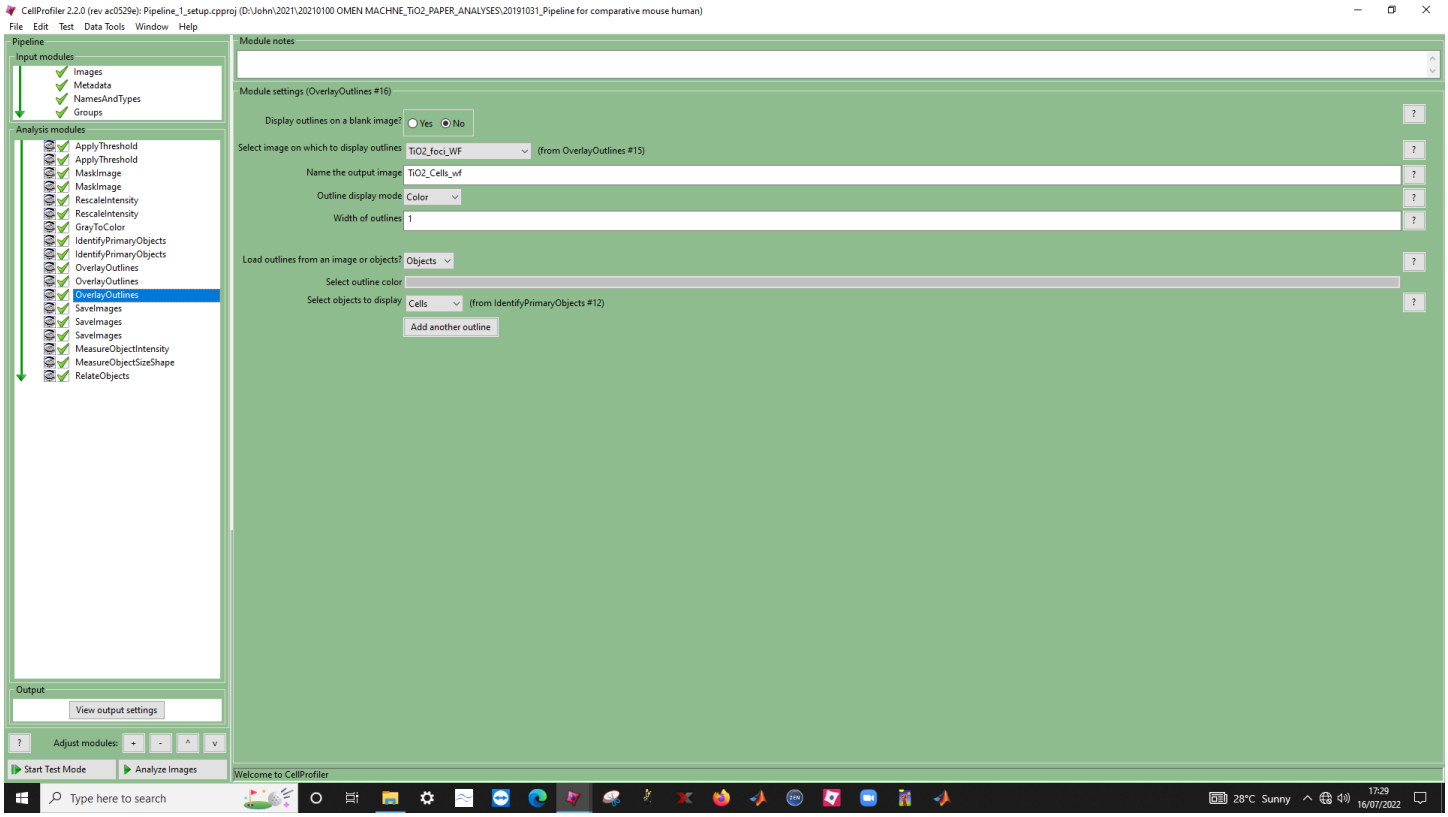

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (GrayToColor #11)
Select a color scheme
Composite
Name the output image
Rescale_nuclei_colour
Image name
Rescsale_Nuclei
(from RescaleIntensity #09)
Color
Weight
1.0
Add another channel
Welcome to CellProfiler
Type here to search
28°C Sunny
17:28
16/07/2022

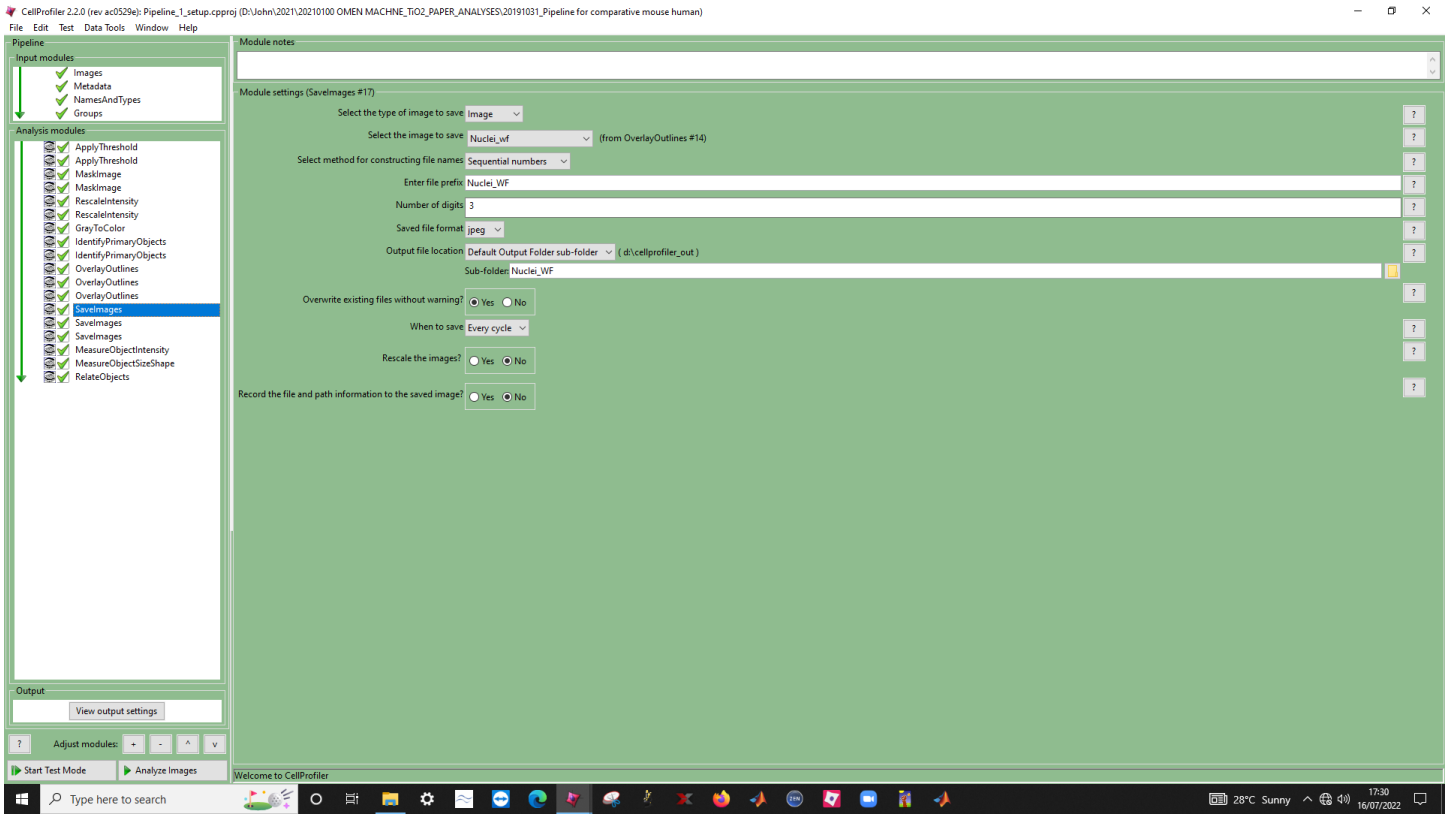

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
IDENTIFIES NUCLEI USING 'THRESHNUCLEI' CHANNEL
Module settings (IdentifyPrimaryObjects #12)
Select the input image Mask_PMAP (from MaskImage #08)
Name the primary objects to be identified Cells
Typical diameter of objects, in pixel units (Min,Max) 24 52
Discard objects outside the diameter range? Yes No
Discard objects touching the border of the image? Yes No
Threshold strategy Global
Thresholding method RidlerCalvard
Select the smoothing method for thresholding Manual
Threshold smoothing scale 12
Threshold correction factor 1.0
Lower and upper bounds on threshold 0.00 1.0
Method to distinguish clumped objects Intensity
Method to draw dividing lines between clumped objects Intensity
Automatically calculate size of smoothing filter for declumping? Yes No
Automatically calculate minimum allowed distance between local maxima? Yes No
Suppress local maxima that are closer than this minimum allowed distance 12
Speed up by using lower-resolution image to find local maxima? Yes No
Retain outlines of the identified objects? Yes No
Fill holes in identified objects? After declumping only
Handling of objects if excessive number of objects identified Continue
Welcome to CellProfiler
Type here to search
28°C Sunny
17:28
16/07/2022

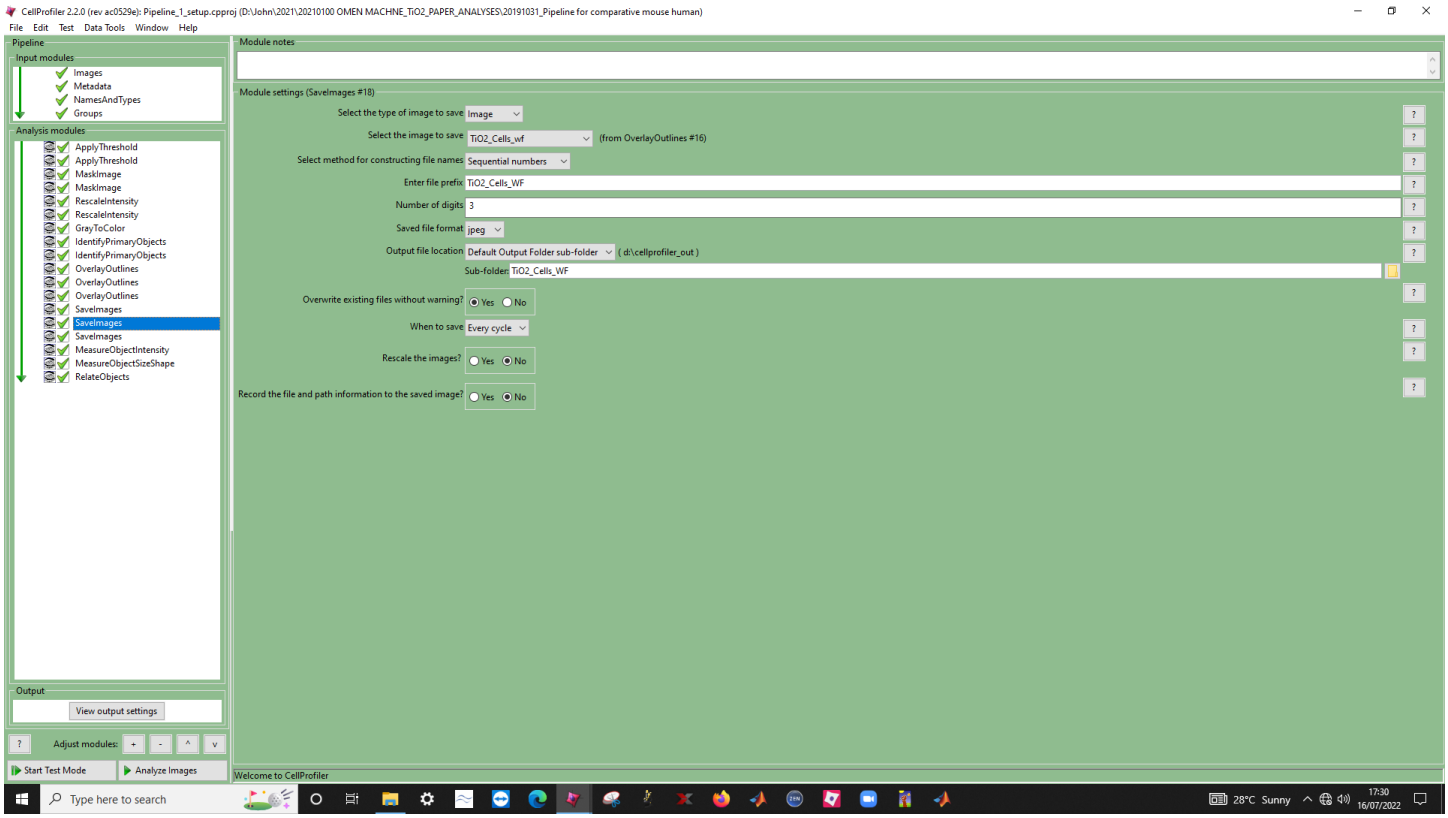

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (IdentifyPrimaryObjects #13)
Select the input image
Mask_Thresh_TiO2
(from MaskImage #07)
Name the primary objects to be identified
TiO2_foci
Typical diameter of objects, in pixel units (Min,Max)
1
50
Discard objects outside the diameter range?
Yes
No
Discard objects touching the border of the image?
Yes
No
Threshold strategy
Global
Thresholding method
RidlerCalvard
Select the smoothing method for thresholding
Automatic
Threshold correction factor
1.0
Lower and upper bounds on threshold
0.00
1.0
Method to distinguish clumped objects
Intensity
Method to draw dividing lines between clumped objects
Intensity
Automatically calculate size of smoothing filter for declumping?
Yes
No
Automatically calculate minimum allowed distance between local maxima?
Yes
No
Speed up by using lower-resolution image to find local maxima?
Yes
No
Retain outlines of the identified objects?
Yes
No
Fill holes in identified objects?
After declumping only
Handling of objects if excessive number of objects identified
Continue
Welcome to CellProfiler
Type here to search
28°C Sunny
17:29
16/07/2022

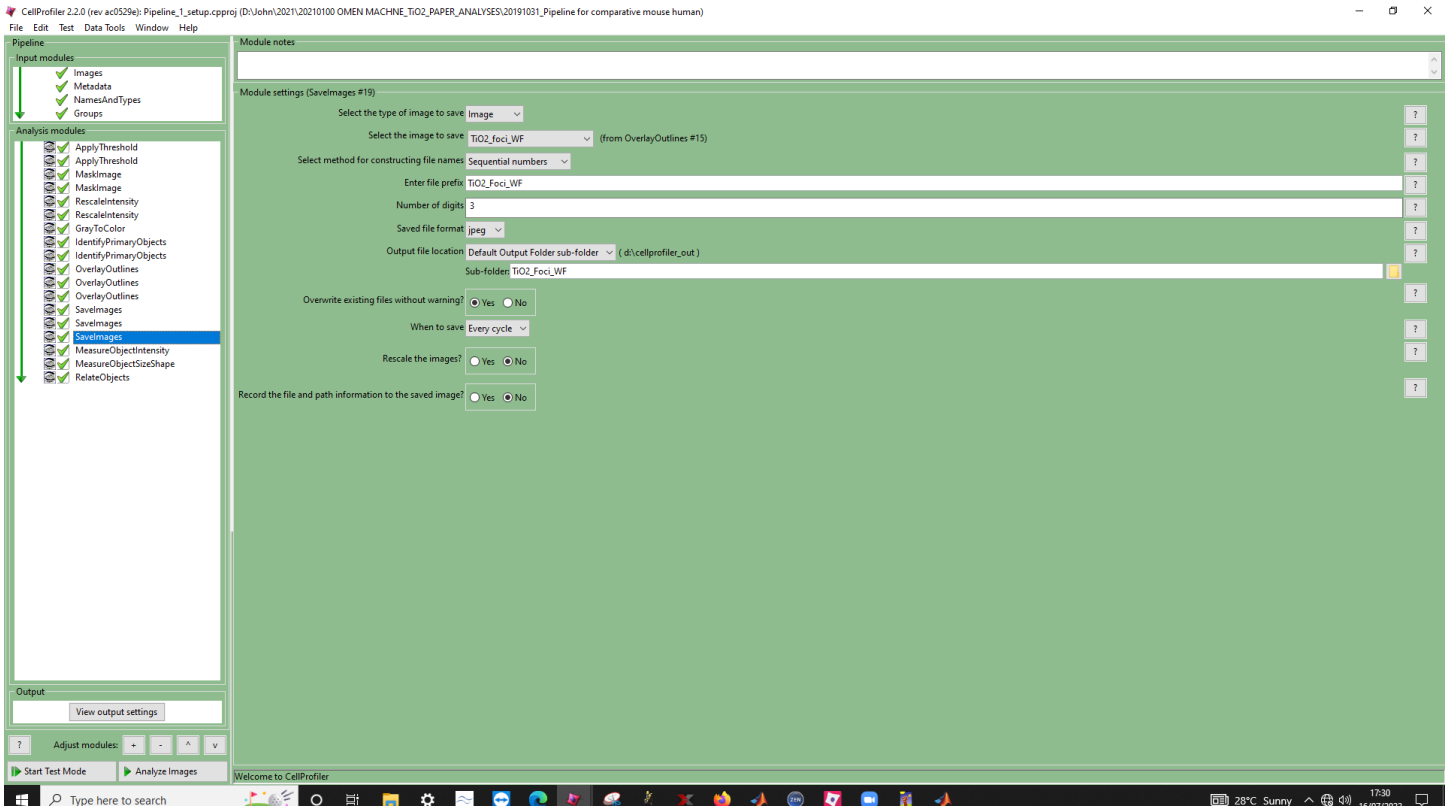

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (OverlayOutlines #14)
Display outlines on a blank image?
Yes
No
Select image on which to display outlines
Rescale_nuclei_colour
(from GrayToColor #11)
Name the output image
Nuclei_wf
Outline display mode
Color
Width of outlines
1
Load outlines from an image or objects?
Objects
Select outline color
Select objects to display
Cells
(from IdentifyPrimaryObjects #12)
Add another outline
Welcome to CellProfiler
Type here to search
28°C Sunny
17:29
16/07/2022

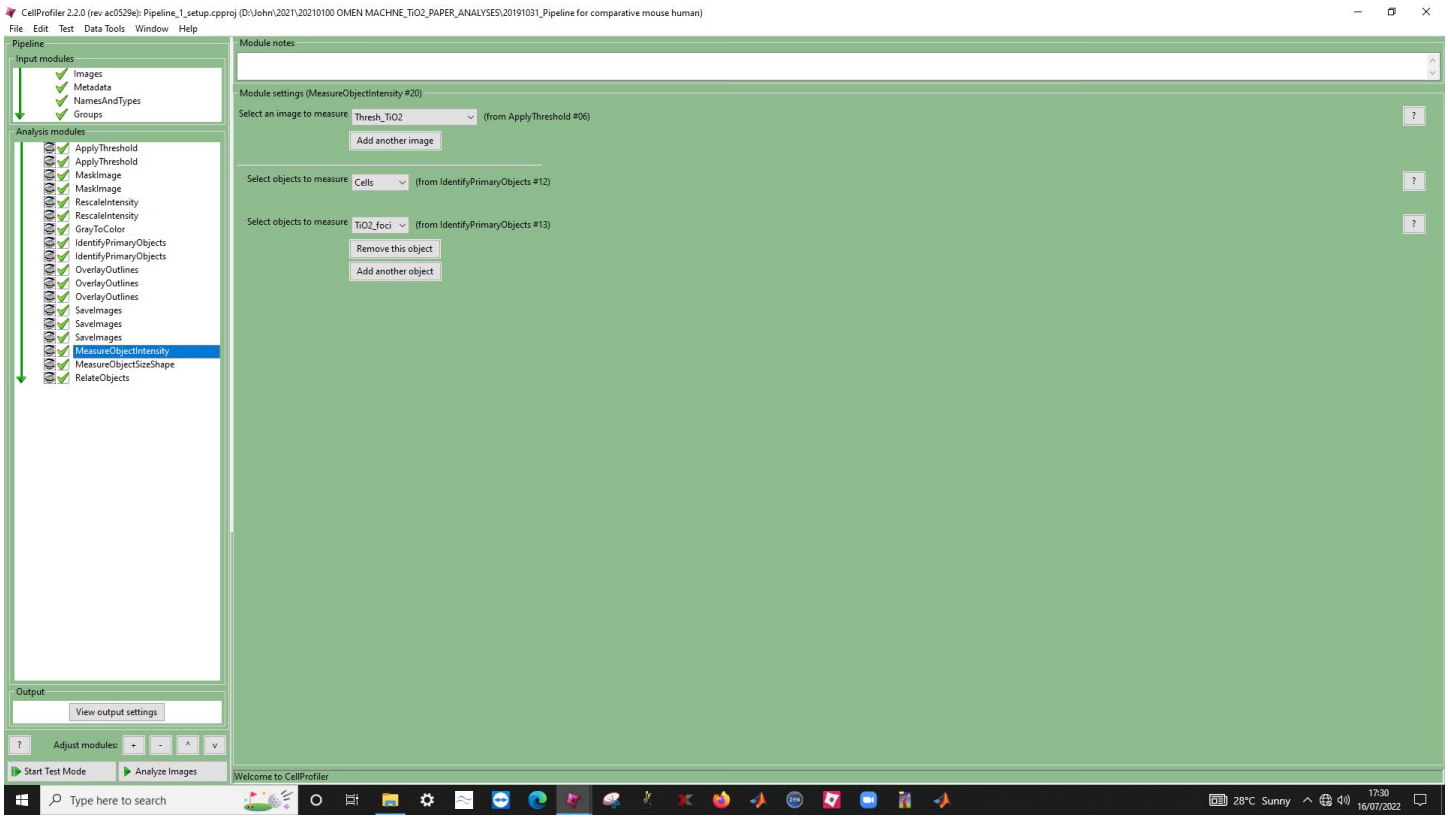

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (OverlayOutlines #15)
Display outlines on a blank image?
Yes
No
Select image on which to display outlines
Rescsale_mask_thresh_tio2
(from RescaleIntensity #10)
Name the output image
TiO2_foci_WF
Outline display mode
Color
Width of outlines
1
Load outlines from an image or objects?
Objects
Select outline color
Select objects to display
TiO2_foci
(from IdentifyPrimaryObjects #13)
Add another outline
Welcome to CellProfiler
Type here to search
28°C Sunny
17:29
16/07/2022

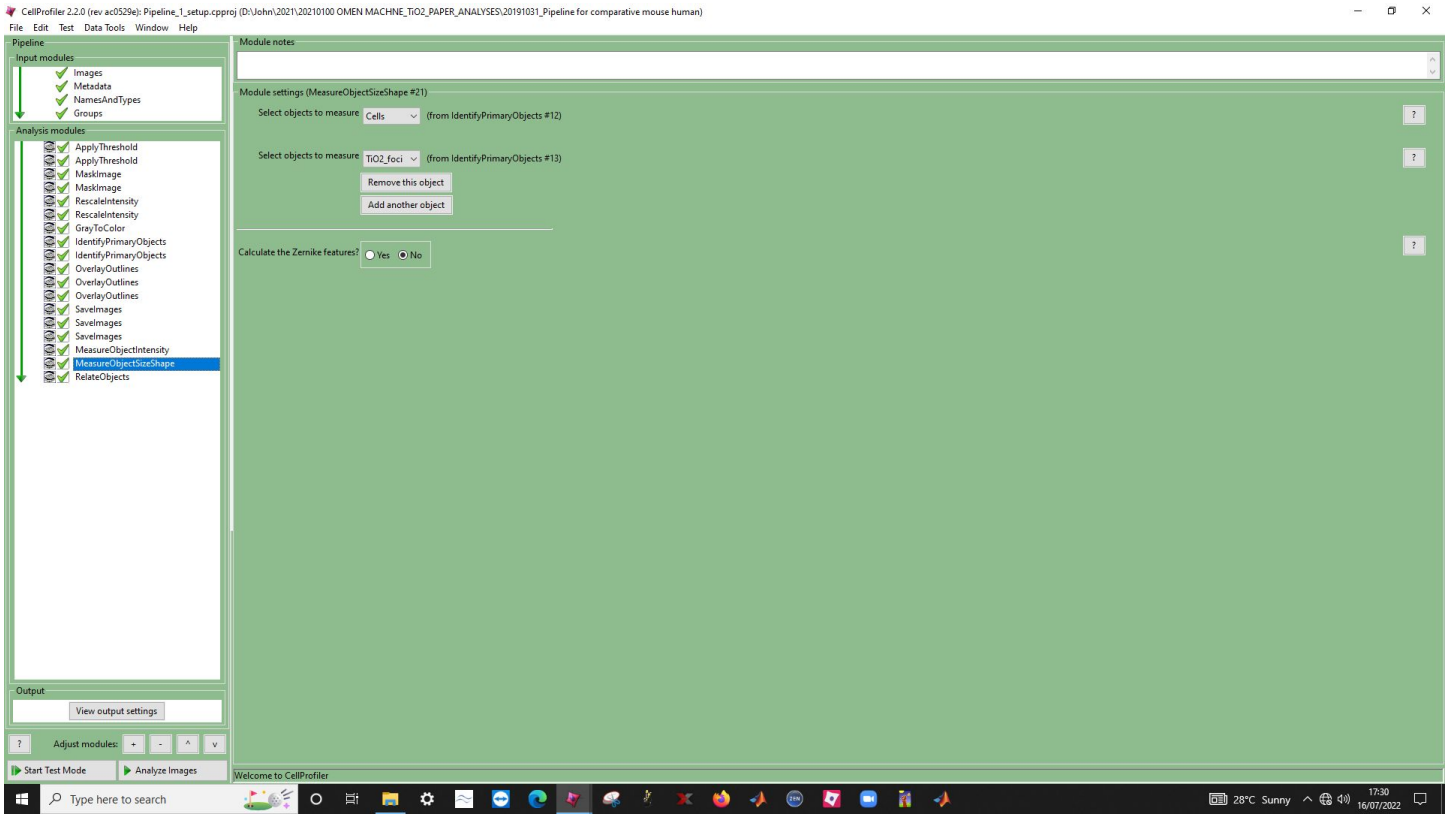

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (OverlayOutlines #16)
Display outlines on a blank image?
Yes
No
Select image on which to display outlines
TiO2_foci_WF
(from OverlayOutlines #15)
Name the output image
TiO2_Cells_wf
Outline display mode
Color
Width of outlines
1
Load outlines from an image or objects?
Objects
Select outline color
Select objects to display
Cells
(from IdentifyPrimaryObjects #12)
Add another outline
Welcome to CellProfiler
Type here to search
28°C Sunny
17:29
16/07/2022

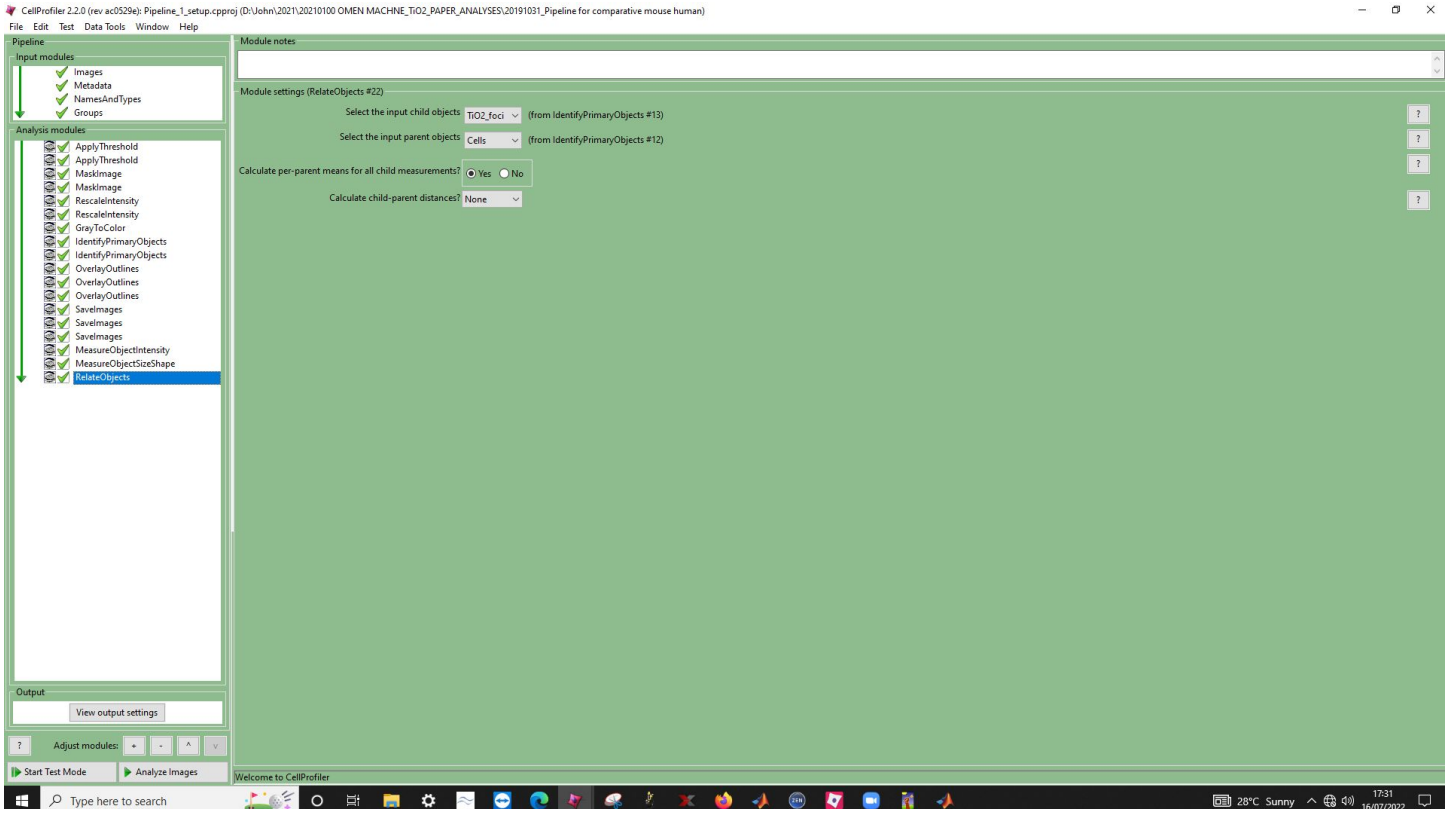

CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (SaveImages #17)
Select the type of image to save Image
Select the image to save Nuclei_wf (from OverlayOutlines #14)
Select method for constructing file names Sequential numbers
Enter file prefix Nuclei_WF
Number of digits 3
Saved file format jpeg
Output file location Default Output Folder sub-folder (d:\cellprofiler_out)
Sub-folder Nuclei_WF
Overwrite existing files without warning? Yes No
When to save Every cycle
Rescale the images? Yes No
Record the file and path information to the saved image? Yes No
Welcome to CellProfiler
Type here to search
28°C Sunny
17:30
16/07/2022


CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (SaveImages #18)
Select the type of image to save Image
Select the image to save TiO2_Cells_wf (from OverlayOutlines #16)
Select method for constructing file names Sequential numbers
Enter file prefix TiO2_Cells_WF
Number of digits 3
Saved file format jpeg
Output file location Default Output Folder sub-folder (d:\cellprofiler_out)
Sub-folder TiO2_Cells_WF
Overwrite existing files without warning? Yes No
When to save Every cycle
Rescale the images? Yes No
Record the file and path information to the saved image? Yes No
Welcome to CellProfiler
Type here to search
28°C Sunny
17:30
16/07/2022


CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (SaveImages #19)
Select the type of image to save
Image
Select the image to save
TiO2_foci_WF
(from OverlayOutlines #15)
Select method for constructing file names
Sequential numbers
Enter file prefix
TiO2_Foci_WF
Number of digits
3
Saved file format
jpeg
Output file location
Default Output Folder sub-folder
(d:\cellprofiler_out)
Sub-folder
TiO2_Foci_WF
Overwrite existing files without warning?
Yes
No
When to save
Every cycle
Rescale the images?
Yes
No
Record the file and path information to the saved image?
Yes
No
Welcome to CellProfiler
Type here to search
28°C Sunny
17:30


CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (MeasureObjectIntensity #20)
Select an image to measure
Thresh_TiO2
(from ApplyThreshold #06)
Add another image
Select objects to measure
Cells
(from IdentifyPrimaryObjects #12)
Select objects to measure
TiO2_foci
(from IdentifyPrimaryObjects #13)
Remove this object
Add another object
Welcome to CellProfiler
Type here to search
28°C Sunny
17:30
16/07/2022


CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (MeasureObjectSizeShape #21)
Select objects to measure
Cells
(from IdentifyPrimaryObjects #12)
Select objects to measure
TiO2_foci
(from IdentifyPrimaryObjects #13)
Remove this object
Add another object
Calculate the Zernike features?
Yes
No
Welcome to CellProfiler
Type here to search
28°C Sunny
17:30
16/07/2022


CellProfiler 2.2.0 (rev ac0529e): Pipeline_1_setup.cpproj (D:\John\2021\20210100 OMEN MACHNE_TiO2_PAPER_ANALYSES\20191031_Pipeline for comparative mouse human)
File Edit Test Data Tools Window Help
Pipeline
Input modules
Images
Metadata
NamesAndTypes
Groups
Analysis modules
ApplyThreshold
ApplyThreshold
MaskImage
MaskImage
RescaleIntensity
RescaleIntensity
GrayToColor
IdentifyPrimaryObjects
IdentifyPrimaryObjects
OverlayOutlines
OverlayOutlines
OverlayOutlines
SaveImages
SaveImages
SaveImages
MeasureObjectIntensity
MeasureObjectSizeShape
RelateObjects
Output
View output settings
Adjust modules:
Start Test Mode
Analyze Images
Module notes
Module settings (RelateObjects #22)
Select the input child objects
TiO2_foci
(from IdentifyPrimaryObjects #13)
Select the input parent objects
Cells
(from IdentifyPrimaryObjects #12)
Calculate per-parent means for all child measurements?
Yes
No
Calculate child-parent distances?
None
Welcome to CellProfiler
Type here to search
28°C Sunny
17:31