Supplemental Material

An imaging-based platform for high-content, quantitative evaluation of therapeutic response in 3D tumour models

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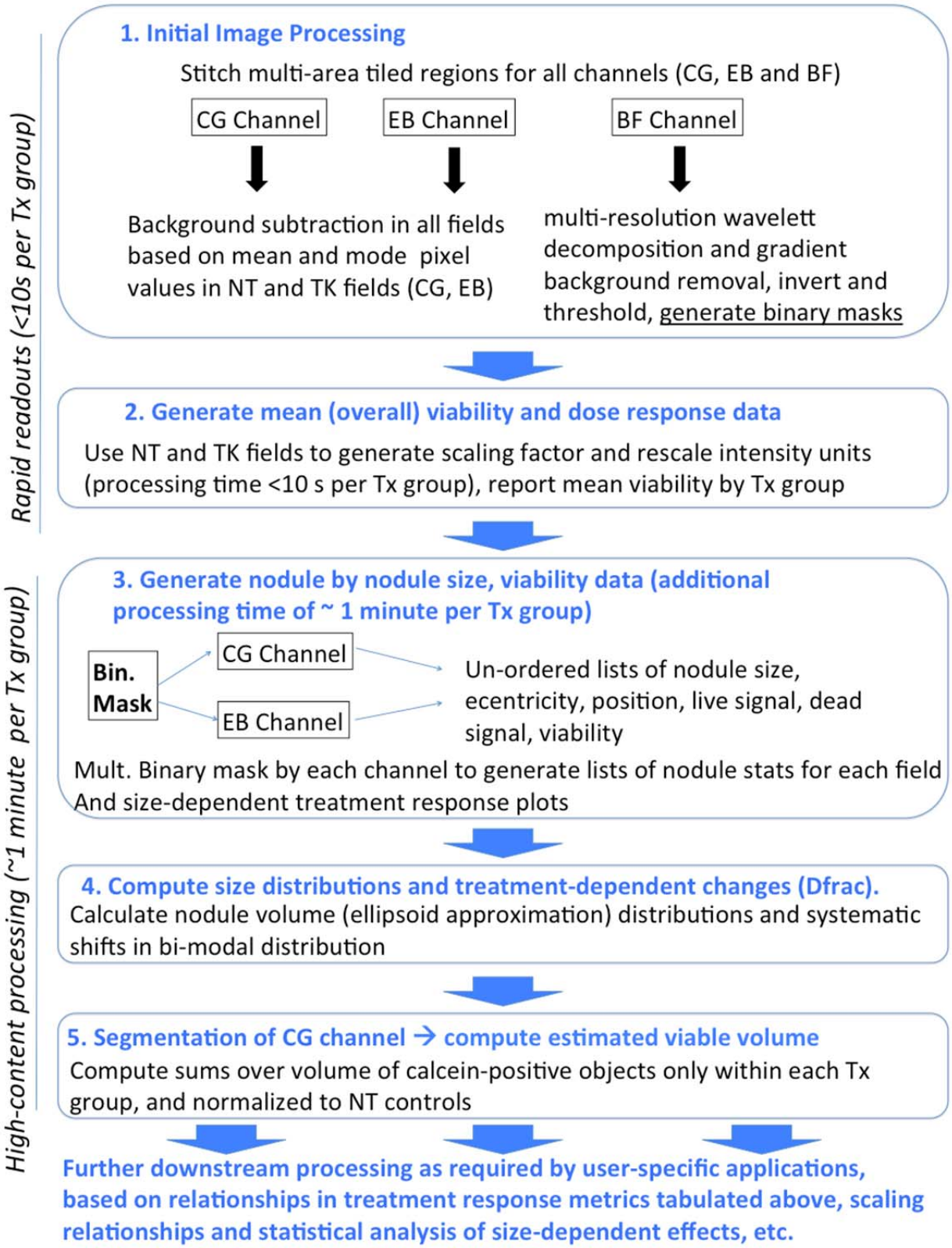
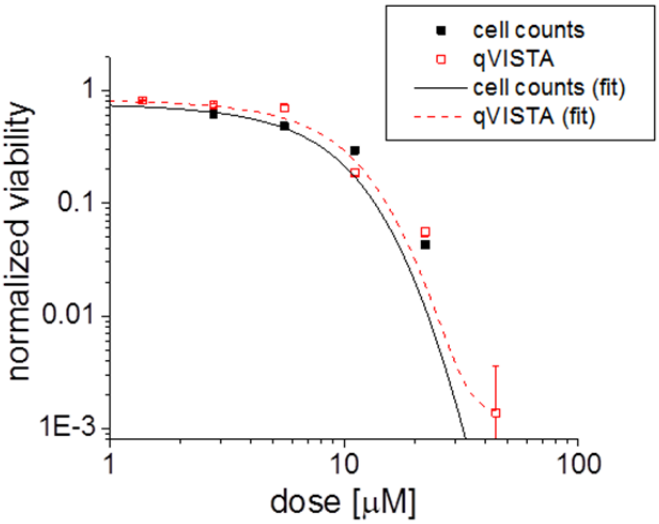
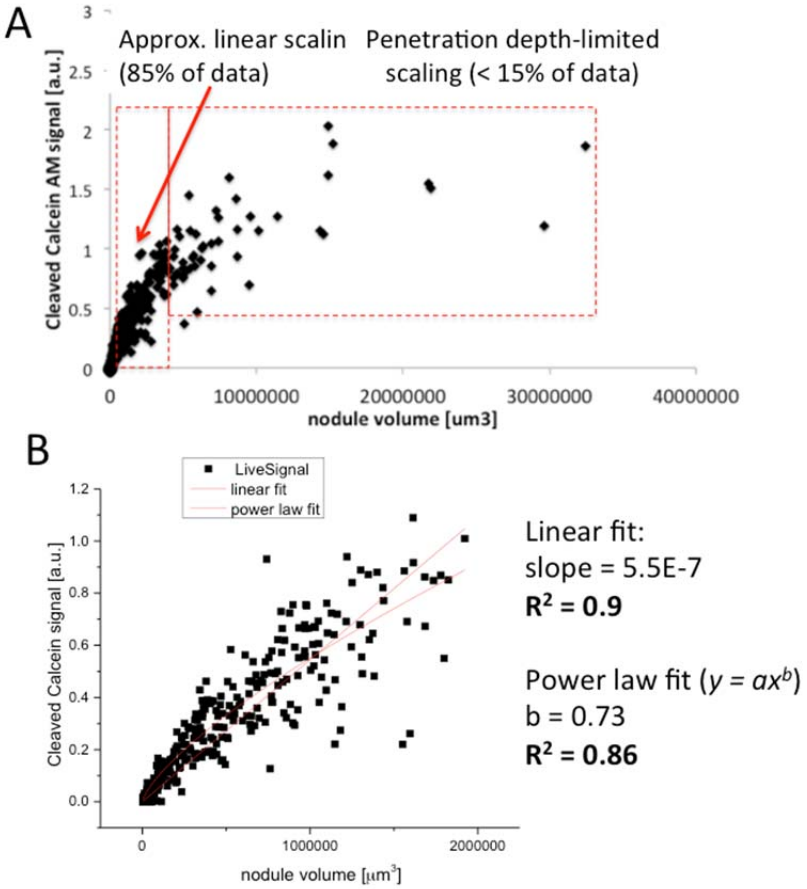


Supplemental Figure 1: Outline of image processing work flow and quantitative outputs.



Supplemental Figure 2: In situ image-based quantification of mean viability by treatment group for 3D cultures of OVCAR-5 epithelial ovarian cells, and comparison with manual trypan blue exclusion counts of parallel treated cultures. In **(A)**, representative response to Paclitaxel is shown in unprocessed merged multichannel fluorescence images (calcein =green, intercalated ethidium bromide = red). Scale bars = 500um. In **(B)** quantification of treatment response from the image-based methodology (qVISTA, solid line) is compared to MTT response following disaggregation and replating. In both cases the LD50 values are comparable with separation evident only at viabilities on the order 0.001.



Supplemental Figure 3: Analysis of fluorescence signal per unit volume. (A) The scaling relationship between fluorescent signal from cleaved calcein AM and nodule volume shows obvious deviation from linear behavior only at optical depth-penetration limited sizes accounting for approximately the largest 15% of nodules. **(B)** An expanded view of the left side of the plot shows that scaling is approximately linear up to nodule volumes of $2x10^7$ um^3 indicating that tissue optics effects (scattering and absorption processes) have minimal impact on collection of fluorescent signal in this size range (accounting for ~ 85% of the 3D micronodules cultured by the methods used in this study).