Supplementary Figure Legends 1-6

Supplementary Figure 1. Optimization of on-chip substrate concentration. In determining the substrate concentration for the on-chip reaction we optimized between i) the higher reaction velocity when the substrate concentration is higher and ii) the streptavidin beads having a finite binding capacity. Note that the streptavidin beads bind both substrate and product, since they bind the biotinylated peptide independent of its phosphorylation status. We measured the binding capacity of the streptavidin beads by measuring the fraction of captured reaction product compared to product that flowed through the column using liquid scintillation counting. From these results the binding capacity was estimated as 5 nMoles of biotinylated peptide per column of packed streptavidin beads (80 nL). The relative on-chip signal for different concentrations of substrate is thus proportional to the product of the reaction velocity and the fraction of substrate captured:

$$Relative\ on\ chip\ signal$$

$$\propto velocity \times fraction\ captured$$

$$= \frac{V_{max} \times [substrate]}{K_m + [substrate]} \times \frac{binding\ capacity_{streptavidin\ beads}}{[substrate] \times volume_{circulation\ chamber}}$$

The graph above plots this formula for K_m = 27.6 μM (1), *binding capacity* $_{streptavidin\ beads}$ = 5 nMoles, and *volume* $_{circulation\ chamber}$ = 370 nL. We chose to use a 25 μM substrate concentration (tick mark on graph) for the majority of our reactions since this is close to maximal signal while keeping the reaction velocity relatively high (0.5 × V_{max}). At this concentration, we calculate that a 15 min reaction uses less than 2% of the substrate. Higher concentrations are still practical, as the linearity experiments of Figure 3d were performed at 200 μM substrate concentration.

1. Skaggs BJ, Gorre ME, Ryvkin A, Burgess MR, Xie Y, Han Y, Komisopoulou E, Brown LM, Loo JA, Landaw EM, Sawyers CL, Graeber TG. Phosphorylation of the ATP-binding loop directs oncogenicity of drug-resistant BCR-ABL mutants. Proc Natl Acad Sci U S A 2006; 103: 19466-71.

Supplementary Figure 2. Photograph of the μ-ivkra microfluidic chip. The chip sits above the Mylar coated beta camera PSAPD device. The chip columns are loaded with colored dyes as in Figure 1.

Supplementary Figure 3. Calibration of the μ-ivkra beta camera for detection of ^{32}P radionucleotide. The calibration graph shows the net counts detected in a region of the beta camera (PSAPD) as a function of the total ^{32}P activity. A range of ^{32}P sources were placed with 100 μm of plastic and 150 μm of glass between the source and detector surface in order to replicate the microfluidic chip geometric configuration. A beta camera image of two ^{32}P sources is shown in the inset. Total detected source activity (counts per second, cps) is plotted for each ROI (region of interest, yellow circle) against the true source activity (pCi, 0.037 decays per second/pCi) as measured using a liquid scintillation counter. The μ-ivkra device has an overall ^{32}P detection sensitivity of 29% as determined by the slope of the calibration curve. In other words, 29% of decay events occurring in the microfluidic device are detected by the coupled beta camera. The beta camera itself has high intrinsic sensitivity to ^{32}P particles that traverse through the PSAPD detection region. The geometric configuration of the integrated microfluidic beta camera device reduces the overall sensitivity due to i) the fact that half of the beta particles are emitted away from the planar detector and ii) attenuation from the 100 μm-thick control layer of PDMS material and the 150-μm thick glass slide between the microfluidic bead column channel and the beta camera.

Supplementary Figure 4. Representative μ-ivkra results demonstrating assay linearity. BCR-ABL kinase activity measured from (a) 3000 versus 6000 and (b) 3000 versus 15000 BCR-ABL expressing Ba/F3 cells. The total cell number was kept constant in each individual experiment by diluting with non-BCR-ABL expressing parental Ba/F3 cells. In each experiment, approximately 100 pCi of signal comes from background. In Figure 3b-d this background is not subtracted. The results are represented by an overlay of the optical image and the beta camera image (PSAPD; 20 min acquisition) of the lower substrate capture columns. The color bar scale (counts per second per mm^2) and total detected activity (pCi) for each ROI (region of interest, yellow circle) are indicated.

Supplementary Figure 5. Detection of autophosphorylation of the BCR-ABL kinase. An overlay of the optical and beta camera (PSAPD; 5 minute acquisition) images of the circulation chamber after the BCR-ABL kinase reaction. The signal comes from the autophosphorylation of kinase bound to the antibody- and protein G-coated polystyrene beads and phosphorylation of interacting proteins that were co-immunocaptured with the kinase. The signal from the reaction using Ba/F3 + BCR-ABL lysate (left column) is stronger than that from the reaction using control lysate from parental Ba/F3 cells (right column). The color bar represents the beta camera image scale and indicates the counts per second per mm^2. Total detected activity (pCi) for each ROI (region of interest, yellow circle) is shown in the table. These results are semi-quantitative in that the protocol has not yet been fully optimized to minimize the background signal from non-specific unincorporated radioisotope, and thus the fold-change is likely an underestimate. Nevertheless we see consistent results across our experiments and use this checkpoint in confirming that the assay has proceeded as anticipated.

Supplementary Figure 6. Evolution of the microfluidic kinase assay chip design. Optical images of four generations of the microfluidic kinase assay chips that were designed, fabricated and tested in our study. (a) In the 1st-generation chip, there were two pairs of kinase capture columns (labeled in magenta) and substrate capture columns (labeled in green) located in close proximity. More distance between each pair of the columns is required in order to allow parallel quantification of the auto-phosphorylation and substrate phosphorylation in the corresponding columns by the embedded solid state beta camera. (b) The 2nd-generation chip was simplified to accommodate a single measurement unit in order to i) improve device yield, ii) test the parameters for automated chip operations, and iii) fine tune the experimental parameters, e.g. bead capture and release, reaction conditions, and the use of cell lysates. (c and d) The 3rd- and 4th-generation chips contain separated kinase-capture bead columns and substrate-capture bead columns, preventing overlap of the signals from kinase auto-phosphorylation and phosphorylated substrates. Reduction of column sizes and further separation of substrate-capture columns were conducted in order to further reduce sample consumption and to avoid radioactivity signal spillover during the quantification, respectively. The lithography nature of the fabrication of PDMS chip molds facilitated and expedited these evolutionary steps. The chip columns are loaded with colored dyes as in Figure 1.